

Human Archaeogenomics in Southeast Asia: Long Distance Dispersals and Community Structure from the Holocene to Colonial Times

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
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Tübingen
2023

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der
Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:

10.07.2023

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Abbreviations

aDNA	ancient DNA
BCE	Before Common Era
BP	Before Present
bp	base pairs
C	cytosine
CE	Common Era
cM	centimorgan
DNA	deoxyribonucleic acid
IBD	identity-by-descent
ka	thousand years
ma	million years
MH	modern humans
NGS	next-generation sequencing
PCR	polymerase chain reaction
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
T	thymine
UDG	uracil-DNA glycosylase

Zusammenfassung

Das Feld der alten Erbinformationsforschung, auch bekannt als Archäogenetik, hat sich seit seinen Anfängen in den 1980ern stark vergrößert. Vorangetrieben durch technologische und methodische Innovationen können nun die menschliche Geschichte, die Entwicklung von Krankheitskeimen, Umwelt-DNS und viele weitere Forschungsfragen untersucht werden. Durch chemische und biologische Faktoren verändert, ist alte DNS kleinteilig, chemisch modifiziert und oft nur in kleinen Mengen vorhanden, weshalb spezielle Laborprotokolle zur Extraktion vonnöten sind. In tropischen Regionen ist die Extraktion noch anspruchsvoller, weshalb bisher nur wenige alte Genome aus Südostasien veröffentlicht wurden, einer geschichtsträchtigen Region mit hoher kultureller und genetischer Vielfalt. Als Teil dieser Dissertation wurden über hundert fossile Knochen aus Südostasien beprobt, die vom Mittelholozän bis in die Kolonialzeit datiert werden. Die extrahierte alte DNS war in Individuen von derselben und von unterschiedlichen Fundstätten sehr unterschiedlich gut erhalten, wobei das Felsenbein generell mehr DNS als beprobte Zähne enthielt. Mithilfe von Methoden zur genetischen Anreicherung wurden 68 alte Genome generiert, unter anderem das erste und älteste Genom aus Wallacea.

Während der Zeitpunkt der ersten Ankunft von modernen Menschen im östlichen Eurasien umstritten ist, wurde die erste Überquerung und Besiedelung von Wallacea, den Tiefseeinseln zwischen dem Festland und Papua-Neuguinea, verlässlich auf vor mindestens 50.000 Jahre datiert. Die genetische Analyse eines 7.200 Jahre alten Individuums von Sulawesi, beschrieben in Manuskript A, offenbarte genetischen Kontakt zwischen Wallacea und dem Festland vor oder während des Mittelholozäns. Dieses Individuum, das am besten als eine Vermischung von Abstammungslinien verwandt mit Menschen aus Ozeanien und Ostasien modelliert werden konnte, trug auch ca. 2,2% Erbgut von den Denisova-Menschen, was die Debatte über Denisova-Introgression jenseits der Wallace-Linie anheizt.

Mit der Entwicklung des Reis- und Hirseackerbaus vor 11.000 Jahren in den Flusstälern des Jangtsekiang und des Gelben Flusses im heutigen China wurden die Jäger-Sammler-Kulturen in Südostasien abgelöst. Der weitere Technologietransfer entlang Flussläufen nach Kontinental- und mit Booten ins insulare Südostasien bewirkte tiefgreifende kulturelle und demografische Veränderungen. Manuskript B beschreibt die Ergebnisse der genetischen Analyse von Individuen aus verschiedenen Teilen von Wallacea, datiert zwischen 2.600 und

250 Jahren, und belegt genetischen Austausch zwischen Austroasiatisch- und Austronesisch-assoziierten Gruppen in Süd- und Westwallacea.

Im Gegensatz zur Neolithisierung werden der Ursprung und die Ausbreitungsrouten der Metallurgie weiterhin kontrovers diskutiert. Dabei weisen die Entdeckung von Minen, Schmelzern und Artefakten die Verarbeitung von Bronze und kurz darauf von Eisen seit 3.000 Jahren nach. Im Nordwesten von Thailand markieren hölzerne, am Kopf- und Fußende geschnitzte Särge mehr als vierzig zwischen 2.300 und 1.000 Jahre alte Fundstätten. In Manuskript C wird dargestellt, dass die dazugehörigen Menschen Jäger-Sammler-, Jangtsekiang- und Gelber Fluss-assoziiertes Erbgut enthalten, was auf potenziell unterschiedliche Ausbreitungsrouten und Austausch der verschiedenen neolithischen Linien hindeutet. Die Analyse von 33 Genomen von mehreren Fundstätten ergab außerdem enge Verwandtschaftsbeziehungen zwischen Individuen derselben und entfernte Beziehungen zwischen Individuen unterschiedlicher Fundstätten.

Zunehmende Handelsbeziehungen und Verfügbarkeit von Gewürzen und Keramik führten ab dem 1. Jahrhundert nach Christus zunächst zu Staatenbildung in den Küstenstädten und später zu europäischem Interesse und Eroberung. Ab dem 16. Jahrhundert kolonialisierte Spanien erst Teile der Philippinen und dann Nordtaiwan. Bei archäologischen Ausgrabungen auf Heping Dao in Nordtaiwan wurden die Fundamente einer Kirche und ein angrenzender Friedhof aus der Kolonialzeit entdeckt und untersucht. Manuskript D beschreibt eine genetisch vielfältige Gemeinschaft, bei der die Individuen von europäischer Abstammung innerhalb der Kirchenmauern und solche von ost- und südostasiatischer Abstammung angrenzend an die Kirche begraben wurden. Dies deutet darauf hin, dass die soziale Hierarchie in der Anordnung der Gräber zum Ausdruck kommt, auch nach der Eroberung durch die Niederländer im Jahr 1642.

Die vorgelegten Manuskripte zeigen, dass es mithilfe von innovativen Verfahren möglich ist, alte DNS aus vielen Regionen von Südostasien zu gewinnen und analysieren, und veranschaulichen das Potenzial zur Untersuchung von genetischem Austausch über weite Entfernungen sowie der Struktur von lokalen Gemeinschaften. Um das technologische und finanzielle Ungleichgewicht zwischen westlichen und lokalen Forschern nicht weiter zu verstärken, sind gleichberechtigtes Zusammenarbeiten und die Einbindung der lokalen Bevölkerung wichtige Voraussetzungen, um für alle Beteiligten vorteilhafte archäogenetische Projekte zu entwickeln, mit denen die Geschichte Südostasien weiter erforscht werden kann.

Summary

Since its beginnings in the 1980s, the study of ancient genomic material, or archaeogenetics, has been growing rapidly. Fuelled by technological and methodological innovations, archaeogenetic studies now allow the study of human history, pathogen evolution, environmental DNA, and much more. Due to chemical and biological alteration, ancient DNA is fragmented, chemically modified, and often only present in very low quantities, which require specialised laboratory protocols. These challenges are exacerbated in tropical regions, which has led to a paucity of ancient genomes published from Southeast Asia, a region rich in human history, as well as present-day cultural and genetic diversity. For this thesis, we sampled over 100 archaeological individuals, dated from the middle Holocene to colonial times of Mainland and Island Southeast Asia. We observed highly variable ancient DNA preservation within and between sites, with the petrous portion of the temporal bone generally performing better than teeth. Utilising in-solution capture, we were able to generate and authenticate genome-wide data from 68 ancient humans, including the first and oldest sequenced individual from Wallacea.

While the timing of the first appearance of modern humans in Eastern Eurasia is debated, the first crossing and settlement of Wallacea, the oceanic islands between the mainland and Papua New Guinea, are securely dated to at least 50 ka ago. While the necessity of open-water crossings was already established, data from a 7.2-ka-old individual from Leang Panninge on Sulawesi, described in Manuscript A, also revealed contact with the mainland before or during the mid Holocene. Best modelled as an admixture between a more than 37-ka-old lineage related to Near Oceanians and deep East Asian-related ancestry, this individual also carried ca. 2.2% Denisovan-related ancestry, fuelling the debate of Denisovan introgression across the Wallace line.

The long persistence of hunter-gatherer-associated technocomplexes in the region was interrupted by the development of rice and millet agriculture in the Yangtze and Yellow River valleys, respectively, around 11 ka ago. The transmission of these techniques along river valleys into Mainland and by boats to Island Southeast Asia and the interactions with local hunter-gatherers had profound cultural and demographic effects. Manuscript B presented genomic data from different regions of Wallacea dated between 2,600 and 250 years ago, which demonstrated that interactions between the Austroasiatic- and Austronesian-associated dispersals extended into western and southern Wallacea.

In contrast, the origin and spread of metallurgy in Southeast Asia remain controversial. Mines, smelters, and artefacts demonstrated the presence of bronze and, shortly thereafter iron, throughout Mainland Southeast Asia from at least 3 ka ago. Wooden coffins with distinct head and foot end carvings distinguished more than 40 sites in northwestern Thailand, dated between 2.3 and 1 ka ago. In Manuscript C, we showed that the associated individuals harboured hunter-gatherer-, Yangtze River-, and Yellow River-related ancestry, potentially indicating different dispersal routes and interactions of the two Neolithic-related ancestries. With 33 genomes from several sites, we were also able to identify close genetic relatedness within and more distant relatedness between sites.

Increasing trade connections and availability of spices and ceramics led first to state formation in coastal polities from the 1st century CE and then to European interest and conquest. Beginning in the 16th century, Spain colonised areas of the Philippines and later northern Taiwan. Archaeological excavations on Heping Dao in northern Taiwan revealed the foundations of a colonial-era church and an adjacent graveyard. Manuscript D described a genetically diverse community, with individuals of European-related ancestry buried within and East and Southeast Asian-related individuals buried next to the church. This pointed towards the social hierarchy being expressed in the spatial arrangement of burials, likely also after the Dutch conquest in 1642.

The presented manuscripts demonstrate the possibility of ancient DNA retrieval from many regions of Southeast Asia and illustrate the potential to investigate long-distance dispersals and interactions, as well as regional connectivity and community structure. Since ancient DNA studies tend to amplify power asymmetries between western and local scholars, equitable collaborations and community engagement are great tools to develop mutually beneficial archaeogenetic projects investigating the history of Southeast Asia.

List of Publications and Manuscripts

Accepted manuscripts

MANUSCRIPT A: *Shared first authors.

S. Carlhoff*, A. Duli*, K. Nägele, M. Nur, L. Skov, I. Sumantri, A. A. Oktaviana, B. Hakim, B. Burhan, F. A. Syahdar, D. P. McGahan, D. Bulbeck, Y. L. Perston, K. Newman, A. M. Saiful, M. Ririmasse, S. Chia, Hasanuddin, D. A. T. Pulubuhu, Suryatman, Supriadi, C. Jeong, B. M. Peter, K. Prüfer, A. Powell, J. Krause, C. Posth & A. Brumm (2021) Genome of a middle Holocene hunter-gatherer from Wallacea. *Nature* **596**, 543-547.

MANUSCRIPT B: *Shared first authors.

S. Oliveira*, K. Nägele*, **S. Carlhoff***, I. Pugach, T. Koebardiaty, A. Hübner, M. Meyer, A. A. Oktaviana, M. Takenaka, C. Katagiri, D. B. Murti, R. S. Putri, Mahirta, F. Petchey, T. Higham, C. F. W. Higham, S. O'Connor, S. Hawkins, R. Kinaston, P. Bellwood, R. Ono, A. Powell, J. Krause, C. Posth & M. Stoneking (2022) Ancient genomes from the last three millennia support multiple human dispersals into Wallacea. *Nature Ecology & Evolution* **6**, 1024-1034.

Submitted manuscripts

MANUSCRIPT C:

S. Carlhoff, K. Nägele, A. B. Rohrlach, W. Kutanan, R. Shoocongdej, M. Stoneking, C. Posth & J. Krause. Genomic portrait and relatedness patterns of the Iron Age Log Coffin culture in northwestern Thailand.

Manuscripts ready for submission

MANUSCRIPT D:

S. Carlhoff, K. Nägele, E. Serrano Herrero, F. Valentin, E. Hsieh, C. Posth, C.-h. Tsang, M. Cruz Berrocal & J. Krause. Investigating the occupation history, social hierarchy, and life histories at an early colonial site in Taiwan through archaeology, archaeoethnology, and archaeogenetics.

Personal Contribution

MANUSCRIPT A:

I performed the lab work for the individual from Leang Panninge from sampling to sequencing. Under the supervision of C. Posth and K. Nägele, I conducted the genetic quality control and population genetic analyses, except for D-statistics and admixfrog, which were computed by K. Prüfer and B. M. Peter, respectively. I wrote the initial draft of the manuscript, which was then edited by K. Nägele, C. Posth, and A. Brumm and further critical input made by the remaining authors. I generated figures 2 and 3 of the main publication and prepared the archaeogenetic methods, results, and figures for the supplementary material.

MANUSCRIPT B:

I generated the genetic data in the lab and conducted initial quality control and exploratory analyses for the individuals from Nusa Tenggara. I analysed the mitochondrial sequences of the complete dataset and contributed the corresponding section to the supplementary material, as well as providing input through all stages of the manuscript.

MANUSCRIPT C:

I performed the lab work for 36 out of 42 petrous parts of the temporal bone and all teeth, except for sequencing. I conducted all quality control and population genetic analyses, plotted all figures, and wrote the manuscript and supplementary material with input from K. Nägele, C. Posth, R. Shoocongdej, and J. Krause.

MANUSCRIPT D:

I performed the quality control and population genetic analyses under supervision of K. Nägele and C. Posth, plotted all figures, and wrote the manuscript and supplementary material with input from K. Nägele, M. Cruz Berrocal, F. Valentin, and J. Krause.

1. An Introduction to Archaeogenetics and the History of Southeast Asia

1.1 The development of archaeogenetics and current methodologies

Built from the compounds ‘arkhaios’ (ancient) and ‘genetics’ (the study of heredity), archaeogenetics denote the study of ancient genetic material, also known as ancient DNA or aDNA. Since DNA encodes the genetic instructions for the development, functioning, growth, and reproduction of all known organisms and many viruses, archaeogenetics combines the knowledge of ancient and present-day genomics to understand the evolution and development of a species in a variety of contexts.

The first evidence of cell organelles persisting long after the death of an organism was published in the 1970s and early 1980s when analyses of insects and mollusc fossils showed intact peptide bonds, chromatin and ribosomes after tens of millions of years [1–3]. After the discovery that DNA may also be preserved in ancient specimens [4], DNA sequences extracted from a quagga, an extinct horse relative, allowed the phylogenetic placement within the *Equus* family [5] and was quickly succeeded by the molecular cloning of ancient human DNA from Egyptian mummies [6]. The invention of the Polymerase Chain Reaction (PCR) in 1983 [7] was a critical step for the study of aDNA, as it allowed targeted amplification of DNA sequences and ameliorated issues with low quantity and cloning efficiency of ancient DNA molecules [8]. Studies extracting DNA from tissue collections of extant (e.g. human brains preserved in peat bogs [9]) and extinct species (e.g. dry skins of the marsupial wolf and giant ground sloth [10]) flourished with these new advances [11].

However, only the extraction of DNA from bones [12,13] opened the doors for the analysis of genomic data from archaeological and forensic contexts [14–16]. Nevertheless, particularly studies of archaeological specimens were still facing the issue of distinguishing authentic ancient genetic material from extraneous material, such as recent contamination, and low sample size [17]. Controversial papers [18,19], reproducibility, authenticity, and even the viability of ancient DNA research as a whole were fiercely debated within the field [20–22]. In response, new studies suggested strict criteria for the authentication of ancient DNA, including spatial separation of ancient DNA laboratories, decontamination of equipment and reagents, ‘blank’ controls without any sample material, highly specific PCR primers, reproduction of results from the same and other DNA extracts of the specimen, replication in independent laboratories, and more (e.g. [17,23–25]). Among these controversies - and under

strict safeguards against contamination - the first genetic sequence of an archaic human, the Neandertal type specimen Neandertal 1 from western Germany, was recovered and placed Neandertals outside of present-day human mitochondrial variation [23].

Since PCR amplicon size is limited, the generation of ancient DNA sequences at the time required large amounts of fossil material, particularly for studying nuclear markers, which are present in lower frequency in the cell compared to mitochondria. In combination with the long duration of sequencing reactions, longer genome segments or even whole genomes would generate exorbitant operational costs and require astronomical amounts of archaeological material, reagents, and time (e.g. ca. 180 kg bone and 48,000 years of experimental work for the draft cave bear genome [26]). In the early 2000s, rapid developments in next-generation sequencing (NGS) technologies heralded a new era for archaeogenetics. Incremental technological improvements via shotgun sequencing after bacterial plasmid ligation [27], 454 sequencing [28,29], and the recognition of hair as a source of high-quality aDNA [30] finally led to the generation of the first ancient human genome produced with high-throughput ‘sequencing-by-synthesis’, or Illumina, sequencing [31]. Approximately 79% of the genome was recovered from 4,000-year-old hair belonging to a Saqqaq Culture-associated individual from Greenland [32].

The parallel sequencing of millions of loci with next-generation sequencing finally allowed for the study of whole genomes recovered from ultrashort DNA sequences [33]. To date, these technological advancements have led to the recovery of up to 560-780-ka-old genetic material from a horse [34] and the identification of a previously unknown extinct hominin relative from Siberia, the Denisovan [35,36]. NGS also facilitated the study of ancient bacterial pathogens of past epidemics, such as *Yersinia pestis* as the causative agent of the Black Death [37], the human-associated microbiome [38], and ancient epigenomes to understand past gene expression levels [39].

Despite improved techniques since the first recovery of ancient DNA four decades ago, archaeogenetic studies still face the same inherent challenges today. Compared to DNA extracted from living organisms, ancient DNA, irrespective of age, is degraded and chemically modified by various biological and chemical processes. As an essential part of organism function and carrier of the heritable genetic information of many living organisms, DNA is continuously repaired by enzymatic excision-repair processes, which cease when the organism dies [40]. The resulting reduced chemical stability is compounded by the

simultaneous degradation of the double-helix by endogenous nucleases upon death and autolysis by decomposition microbes [41–43]. After this initial rapid reduction of molecule length, fragment size does not decrease according to geological age but is hugely variable, potentially influenced by taphonomy, precipitation, and thermal fluctuation at the deposition site [42,44,45].

In addition to these biological and external influences, DNA is vulnerable to chemical processes, particularly hydrolysis. Due to the lack of the 2'-OH group of the ribose in the DNA helix, the *N*-glycosyl bond connecting the sugar backbone to the nucleobases is labile compared to RNA [43]. Since the nucleobases adenine and guanine are more readily released, this process is known as depurination and the resulting apurinic sites are susceptible to further degradation by β -elimination, which can lead to strand cleavage [46]. Consequently, purines are overrepresented in the immediate vicinity of 5' ends of strand breaks [47]. The nucleobase residues at uneven strand breaks, cytosines (C) in particular, are also vulnerable to hydrolytic deamination [43]. This process transforms the cytosine into deoxyuridine, which is a form of uracil, a nucleobase usually only found in RNA, and leads to the incorporation of adenine opposite to the deoxyuridine residues during the initial PCR replication [48]. In the final sequence, the original cytosine is read as a thymine (T), known as a C to T misincorporation, which accounts for the vast majority of miscoding modifications in ancient DNA sequences [48]. C to T substitutions occur predominantly at the 5' end of molecules and decrease rapidly in frequency over the first ten bases, whereas the complementary G to A substitutions mirror this pattern decreasing from the 3' end [47]. This distinct misincorporation pattern has become known as 'damage' and, as damage increases over time, is often sought as support that endogenous ancient DNA is present [45]. Additionally, temperature heavily influences cytosine deamination, suggesting that higher site mean temperature more often provides the activation energy necessary for hydrolysis [42]. Combined with short DNA fragments, C to T substitutions are key criteria for ancient DNA authentication [45,49].

The short and modified nature of its fragments also makes ancient DNA vulnerable to being out-competed by longer, exogenous molecules that were introduced to the sample after death [50]. Contamination from microbes or other ancient remains during interment, handling of remains after initial deposition or cross-contamination in the laboratory processing result in the endogenous proportion of a DNA sample often only accounting for 1% of a genetic library [51,52]. Therefore, the prevention of contamination and further DNA degradation is

essential during excavation, transport, storage, and laboratory processing [53]. This includes protective gear, decontamination of equipment, spatial separation of pre- and post-PCR processing, ‘blank’ laboratory controls, and cool, dry storage [24,53,54]. While contamination from other organisms is removed from genomic sequences through mapping to a reference genome [55], quantifying same organism contamination relies on sequence and ploidy differences or patterns of chemical damage to differentiate endogenous and contaminating genomes [56]. Haploid loci, such as the mitochondrial genome, can be leveraged to identify if more than one sequence is present in the sample [57,58], but extrapolation to the nuclear genome is only stable for samples with mitochondrial to nuclear ratio less than 200, often the case in petrous bones but not teeth [59]. Similarly, heterozygous sites on the X-chromosome can indicate contamination [60–62], but this is only applicable for XY individuals and may vary if contaminating and endogenous genome possess different karyotypes [63]. A third approach focuses on identifying and optionally isolating endogenous sequences by the characteristic C to T substitutions of ancient DNA [63,64].

While ancient DNA has been recovered from bones, teeth, dental calculus [38], skin and hair [32], sediment [65], and textiles [66], as well as coprolites, archaeobotanical remains, ceramics, shells, and specimen archives [67], the selection of sample material is crucial to accomplishing the determined research goals. Studies of human history often utilize the petrous part of the temporal bone, since the bony labyrinth has been shown to preserve ancient DNA particularly well [68,69]. However, the destruction of crania and petrous bones leads to a loss of morphological information [70] and differences in the treatment of skulls compared to post-cranial remains in many funerary contexts may bias the genetic results on top of ethical concerns [71]. Although sampling the petrous bone without opening the skull is possible [72] and auditory ossicles are an equally rich source of aDNA [73], in the absence of suitable cranial elements or to address aforementioned concerns, post-cranial elements, particularly phalanx, vertebra, and the talus bone, seem to provide viable alternative sampling sites [74]. Screening the bones to be sampled for sites of high-density during computer tomography [75] or preservation of collagen [76] can also help increase the success of destructive sampling. While teeth are also part of the skull, their abundance, resistance to degradation and potential to be sampled non-destructively make them valuable for many projects [77] and essential for investigations into ancient pathogens not present in the petrous bones [78]. Additionally, dental calculus (calcified dental plaque) is a rich source of metagenomic and paleoproteomic information, which can be extracted simultaneously [79], as

well as epigenomic information allowing inferences of the oral microbiome, diet, behaviour, pathogens, and health [38,80–82].

Recovering more, smaller fragments of authentic endogenous DNA has also driven the development of aDNA-specific laboratory protocols. After the first protocol exploiting that DNA is readily adsorbed to silicon dioxide particle [83], silica-based DNA extraction methods were established also for ancient DNA after decalcification of the bone with ethylenediaminetetraacetic acid and collagen digestion with proteinase K [84]. Modifications of the binding buffer composition adapted to around 35 bp long and damaged fragments were adopted as the field standard [85] with recent improvements for large-volume handling and integration of suspension-based extraction utilizing silica-coated magnetic particles [86]. After extraction from the bone matrix, DNA is bound and immortalised in a ‘library’, then able to be theoretically infinitely amplified without requiring access to the original sample. The adoption of Illumina-style libraries was first established for double-stranded [87] and shortly thereafter single-stranded DNA templates [88,89]. Since aDNA fragments are short, the ability to construct libraries from originally single- and originally double-, then heat-denatured into single-stranded molecules, minimises the loss of authentic segments [89]. In a step towards automation of laboratory methods, this protocol has been optimized for simultaneous processing of 96-well plates on automated liquid handling stations [90]. While incubation in sodium hypochlorite or selection of deoxyuracils effectively reduces contamination [91,92], uracil-DNA glycosylase (UDG) is often used to reduce the effect of DNA damage [93]. Part of the excision-repair pathway, this enzyme detects irregularities in the DNA structure and removes deoxyuridines resulting from cytosine deamination [43,93]. While UDG-treated libraries allow for analysis without DNA damage and not UDG-treated libraries are required for restriction to degraded fragments in the presence of contamination, partial-UDG treatment retains the damage signal at the terminal positions so that authenticity can be assessed on mostly non-damaged fragments [94].

Due to the high amounts of exogenous molecules present in the libraries, ancient samples are well-suited for enrichment methods that hybridise fragments to target sequences before amplification [95,96]. This so-called capture approach has been employed to recover, among others, the human mitochondrial genome [58,95], Y-chromosome [97], single-nucleotide polymorphisms (SNPs) informative for population genetic analyses (e.g. [98–100]), and immune-related genes [101], as well as bacterial and viral pathogens [102,103].

Their use for ancient DNA is only one of the many archaeological sciences through which human remains offer unique insights into the lives of past communities [104]. However, the inherent scarcity of human remains and their cultural significance to many groups and individuals make thorough evaluation of scientific potential by diverse stakeholders necessary. Ancient DNA is recovered through inherently destructive methods, and questioned projects employing the least invasive methods in consultation with multiple disciplines and communities are crucial to building trust between researchers and communities and developing sustainable stewardship of cultural heritage [105–107]. The current paucity of published ancient genomes from Southeast Asia demonstrates the timeliness of developing equitable partnerships with scholars and communities to develop ethical archaeogenetic research in the region.

1.2 Hominins and Humans in Southeast Asia

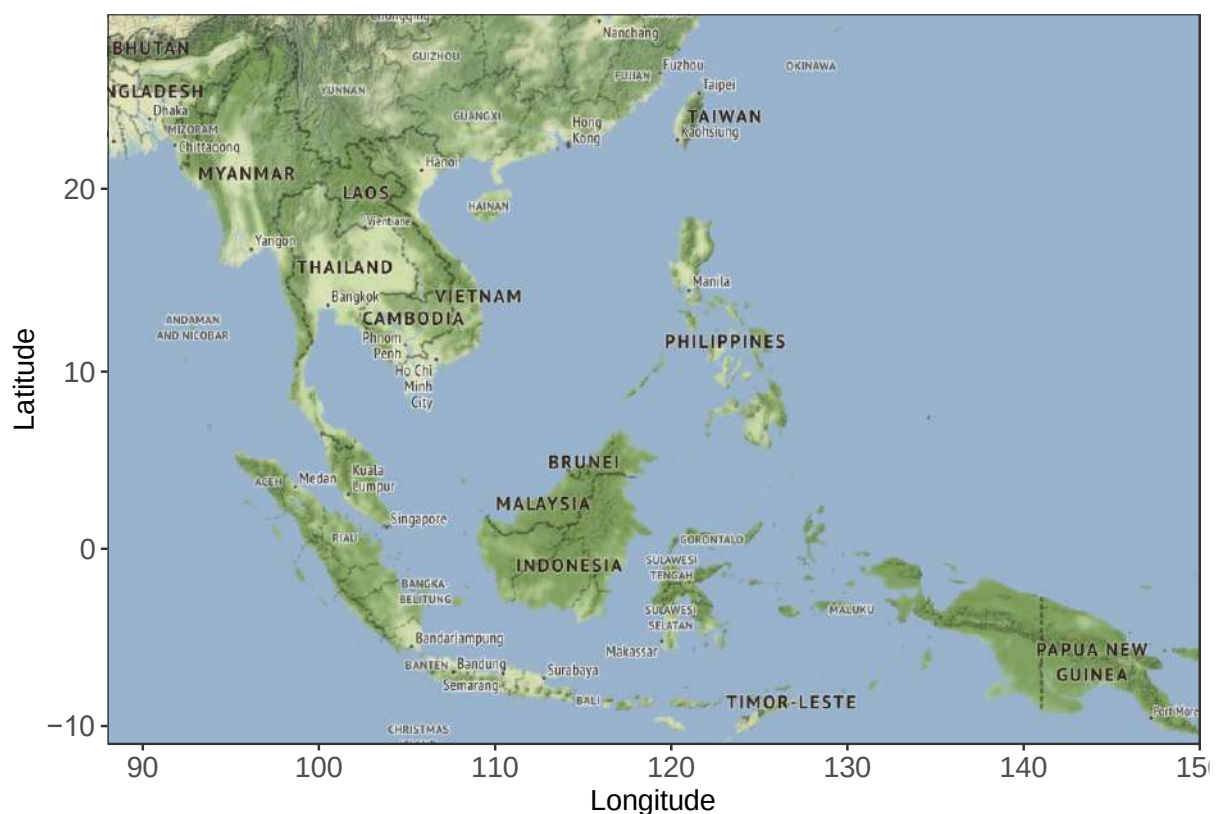


Figure 1. Map of Southeast Asia with present-day country borders, plotted with ggmap [108], map tiles by Stamen Design (CC BY 3.0), map data by OpenStreetMap (ODbL).

1.2.1 Archaic hominins

The first human lineage to disperse widely throughout Eurasia and the only hominin in Asia through much of the Pleistocene was *Homo erectus*. Known from sites in Java and northern China, detailed assessment of evolutionary relationships within this hominin is limited by a lack of fossil evidence from Southeast Asia [109]. On Java, individuals from Sangiran, Mojokerto, and Trinil dated from 1.5 to 0.8 ma ago represent an early phase of *H. erectus* [110], morphologically different from Late Middle and Late Pleistocene crania from Ngandong, Sambungmacan, and Ngawi [109,111], but if this represents local evolution due to genetic isolation or replacement by another lineage, remains uncertain [109,111,112]. Although initial dates of the ‘late *H. erectus*’ on Java of 70-30 ka ago [113,114] have been recently revised to 117 to 108 ka [115], the fossils at Ngandong still represent the most prolonged persistence of this archaic hominin.

Southeast Asia was also home to very geographically restricted hominin species. In 2004, the discovery of a partial skeleton of small stature with derived craniodental features at Liang Bua on Flores was announced as *Homo floresiensis* [116]. Despite the upper limb bones and additional cranial elements solidifying the assignment of a new species [117], the associated strata being dated to 95-12 ka ago and the small cranial capacity [117] fuelled the proposal that the holotype individual was *Homo sapiens* with a pathological condition or skeletal growth disorder instead [118–121]. However, direct comparisons showed that the skull morphology did not lie within modern human variation [122,123] and a revised stratigraphy dating the fossils between 100 and 60 ka ago and associated stone artefacts to 190-50 ka ago strengthened the position of *H. floresiensis* [124]. A *H. floresiensis*-like mandible and teeth from Mata Menge on Flores dating to 0.7 ma ago and stone artefacts from Wolo Sege older than 1.02 ma ago [125] underline the long hominin presence on Flores [126]. The announcement of another hominin from Island Southeast Asia several years later was not as controversial. Twelve skeletal elements excavated at Callao Cave showing both primitive and derived morphology were assigned *Homo luzonensis* in 2019 [127], and recent analyses suggest that both *H. floresiensis* and *H. luzonensis* evolved from a *H. erectus* group by separate insular isolation [128].

1.2.2 Early modern human settlement

The early archaeological record of modern humans (MH) in East and Southeast Asia is sparse and controversial. Several supposedly MH fossils have been dated to more than 100 ka ago

suggesting early dispersals not just into western [129] but also into eastern Eurasia. The oldest of such fossils are a mandible and two molars recovered from Zhirendong in southern China, which display a mixed morphology and were deposited between 190-130 ka ago based on *U*-series dating of speleotherms [130,131]. In Luna Cave, two molars have been dated to 127 and 70 ka ago based on associated flowstones [131], while 47 teeth of modern morphology were dated between 120 and 80 ka ago in Fuyan Cave [132]. Furthermore, seven teeth from Huanglong Cave and two from Longtanshan Cave have been associated with *U*-series dates of 101-81 and 83-64 ka ago, respectively [133,134]. However, reinvestigations of the sediment and flowstone ages compared to directly dated remains of the same context did not match for five supposedly early MH sites in southern China, including Huanglong, Luna and Fuyan Cave [135]. Direct ^{14}C dating of teeth from Fuyan Cave even showed dates as recent as 10-2 ka ago, despite associated sediment ages of 300-200 ka ago and flowstone ages of 180-80 ka ago [135]. Recent inspection of the C:N ratio and %C published in the aforementioned study suggest contamination of the analysed samples making them appear younger [136]. Similarly dated fossils have also been excavated in Island Southeast Asia, the oldest being a premolar recovered from Punung on Java associated with fauna dated to 143-118 ka ago [137,138], but both the assignment as MH and the date are controversial [139,140]. The identification as MH is also questionable for two teeth from Lida Ayer on Sumatra dating between 73 and 63 ka ago, which were recovered in the 1880s and first identified as MH in the 1940s [141]. Furthermore, a metatarsal from Callao in the Philippines dated to 67 ka ago has been assigned to both MH and *Homo floresiensis* [142], while a supposedly associated human tooth from the same site was dated to 50 ka ago [143]. Its extraordinary age compared to any other site on the continent, in combination with stratigraphic inconsistencies, has also raised scepticism of the 65 ka date of human artefacts found at Madjedbebe in northern Australia [144,145].

So far, securely dated and identified archaeological evidence suggests MH presence in Mainland, Island Southeast Asia, and Oceania from 50 ka ago [145,146]. Particularly the mandibular and cranial fragments of Tam Pà Ling Cave in Laos, dated to 46 ka ago [147], and the ‘Deep Skull’ from the Great Cave of Niah on Borneo, dated to 35 ka ago [139], are clear examples of modern humans without archaic features. However, concerns about *U-Th* dating of bone do apply [145]. Two additional individuals have been recovered from Tam Pà Ling Cave with both archaic and modern morphological features, indicating transitional morphological diversity within humans at the time [148–151]. Excavations at Tabon in the Philippines also revealed bone fragments dating to 47-16 ka ago and showed evidence of two

morphotypes present at the site [152]. One incisor from Nam Lot in Laos [153] and skull fragments from Laibin in China [154] further corroborate the arrival of MH in Southeast Asia between 50 and 40 ka ago, while three burials at Willandra Lakes in Australia establish not only the presence of MH, but also intricate mortuary rituals by 42-40 ka ago [155,156]. Forty-five-thousand-year-old cave art in Leang Tedongnge on Sulawesi [157], stone artefacts from Laili Cave in East Timor, dated to 44 ka ago [158], and evidence of the controlled use of fire at Liang Bua on Flores by 41 ka ago [159] also clearly demonstrate that MH rapidly spread from Mainland over Island Southeast Asia into Near Oceania.

On an archaeological timescale, this rapid settlement of a vast region of the globe is particularly remarkable due to the necessary open-water crossings. While Borneo, Sumatra, and Java were connected to the mainland as a continent called Sunda, Australia and New Guinea formed a vast landmass, Sahul. The Wallacean islands bridging the more than 1,000 km distance were always disconnected from the continental shelves by deep-water trenches [160]. Since the traversal of Wallacea required multiple sea crossings up to 70 km long against strong north-south currents, no other hominin, primate or large-bodied terrestrial mammal in general, had previously completed the journey [160,161]. Initial models assumed accidental drifting of small groups on natural rafts during storms [162], but the founding population requiring at least 1,300 to 1,500 individuals arriving within 700-900 years to avoid extinction [163], in combination with timed and directed movement drastically increasing likelihood of passage success, imply intentionality [164]. This is supported by several ‘distinctly human’ behaviours necessary to complete the journey, including complex conceptualization of tools, information exchange systems, and symbolic behaviour [161,165] in a natural progression from estuarine exploitation along the southern Asian coast [166]. Utilising bamboo floats [167], the consistent exploitation of high-ranking sea-shore resources could have driven early communities to move further along previously unoccupied coasts, as demonstrated by a large number of archaeological sites showing MH presence between 47-40 ka ago in Sahul and Wallacea [160,168]. Sea level rise after 65 ka ago might have provided a more productive coastline but also very variable tidal regimes and current systems, requiring long-term planning, knowledge, and adaptability from early voyagers [169].

However, whether a northern route through Sulawesi into New Guinea or a southern route through the southern islands onto the northwestern Australian shelf was taken remains disputed [170]. Fewer crossings and a total shorter distance would favour a northern crossing,

while the southern route seems parsimonious due to inter-island visibility and shorter voyages at the beginning [164]. Least-cost and inter-visibility analyses including bathymetry and currents have provided support for the northern [171,172] or the southern route [173] or have been inconclusive [164,174]. A recent stochastic-ecological model including population dynamics and carrying capacity suggests initial arrival at the northwestern Australian shelf 70-50 ka ago with a second entry through New Guinea [175].

Studies of present-day Near Oceanians utilising mitochondrial DNA, Y-chromosome and genome-wide data have also explored the genetic legacy of MH arrival in Sahul. The presence of all major non-African mitochondrial (M, N and R within N) and Y-chromosome (C, F) haplogroups in Australians and Papuans and their emergence around 50 ka ago strongly advocate for rapid dispersal of MH out of Africa along South and Southeast Asia into Oceania [176–178]. The subdivision of mitochondrial DNA subhaplogroups between Australia (M15, M16, M42, N13, O, P3, P5, P6, P8, P12, S*) [179,180] and New Guinea (e.g., P1, P2, P4a, M27, M28, Q*) [181] also suggests a very deep split between the two groups after the arrival of a common ancestor in Sahul [182]. Alternatively, ancestors of Australians and Papuans already arrived as two diverged groups at separate entry points [181,183], a view that is supported by Y-chromosome haplogroups [184]. Divergence estimates and geographic distribution of mitochondrial haplogroups within Australia also suggest fast spread along the east and west coast before 40 ka ago [179,183]. These analyses of a single genetic locus are well-supported by whole-genome studies, which conclude that Australians and Papuans diverged from Eurasians 72-51 ka ago before they split from one another 40-25 ka ago [185]. Present-day genomes also support the arrival of already differentiated groups in Sahul but suggest that interactions and gene flow between divergent groups shaped initial human presence in the region [186]. Studies of present-day Wallaceans are not as informative about the initial settlement of the region because the detected signals are dominated by demographic changes around the Last Glacial Maximum ca. 15 ka ago and in recent times ca. 3 ka ago [187].

The genetic snapshots provided by ancient DNA can help overcome some of the limitations of present-day data, but age and environmental conditions are unfavourable for DNA preservation in Southeast Asia and Oceania during this period. Ancient DNA recovered from 100-year-old Australian hair corroborates the direct connection to early MH dispersal, and also suggests the distinction of this human lineage from the ancestors of East Asians [60]. The

oldest genome recovered from East Asia, the 40 ka-year-old Tianyuan individual from northern China, represents a distinct lineage [188,189]. Connections of present-day East Asians to individuals from Bacho Kiro, Bulgaria dated to 46-53 ka ago [190], in combination with the 45-ka-old Ust'-Ishim individual from Siberia not contributing to neither western nor eastern Europeans [191], underline the potential of ancient DNA in illuminating the complex settlement patterns of Eastern Eurasia.

Ancient DNA also had a key role in characterising interactions between archaic humans and MH. Comparisons of the draft genome of Neandertals, an extinct hominin living in Western Eurasia until around 40 ka ago, identified gene flow into modern humans resulting in ca. 2% Neandertal-related ancestry in the genomes of present-day groups outside Africa [192,193]. The presence of the same introgressed segments in Europeans, Asians, Papuans, and Australians is compatible with admixture in the Middle East during the out-of-Africa migration around 60 ka ago [60,185,192]. Genetic information extracted from a finger bone and teeth excavated at Denisova Cave in the Altai mountains revealed a previously-unknown hominin population, then labelled Denisovans [35,36,194,195]. Despite the lack of morphological information and knowledge of their geographic distribution, it has been estimated that Denisovans diverged from Neandertals ca. 300 ka ago and potentially harboured genetic contribution from a more deeply divergent hominin group [193,196]. Further analyses showed that 2 to 5% Denisovan-related ancestry is present in groups in Oceania, eastern Indonesia, and the Philippines and at low levels in East Asian and Native American groups [88,197–199]. Comparisons of the Denisovan-related segments in these present-day groups indicated a deep split between a lineage more closely related to the Altai Denisovans, which introgressed into East Asians, and a more distantly related lineage admixing with the ancestors of both East Asians and Oceanians [200]. Recent analyses have even suggested two deeply divergent lineages admixing with the ancestors of Papuans, the first gene flow dating to 46 ka ago and the second to 25 ka ago, likely east of the Wallace line [201,202]. This opened the question of possible genetic contact with other hominins in Southeast Asia, e.g. *H. floresiensis* or *H. luzonensis*, but while an initial deep-learning study suggested the presence of a third archaic introgression into Asian and Oceanian groups [203], any additional super-archaic ancestry could not be confirmed recently [202,204,205]. While the discoveries of a Denisovan mandible from the Tibetan Plateau [206] and a molar from Tam Ngu Hao 2 in Laos [207] tentatively assigned to Denisovans offer potential insights into the Pleistocene distribution of the Denisovans in East Asia, direct genetic data has only been

obtained from sediment samples from Baishiya Karst Cave in Tibet [208]. Further genetic analyses of ancient MH from Southeast Asia and Wallacea are required to illuminate Denisovan distribution, as well as the location and timing of Denisova introgression events.

1.2.3 Holocene technocomplexes

The first humans in Southeast Asia were groups of hunter-gatherers, the worked stone of whom is a crucial feature of early modern human occupation and alteration of the environment. Most of the terminal Pleistocene to mid-Holocene lithic assemblages of the region have been assigned to the ‘Hòabínhian’ archaeological complex after its initial description from Hoa Binh Province in Vietnam [209]. Characterised by stone reduction sequences of river cobbles with overlapping, centripetal flake scars to ‘sumatraliths’ with flake scars on the face and edges, this assemblage is present at Xiaodong rock shelter in southern China by 43.5 ka ago and expanded southwards [210]. The Tham Lod rock shelter in northwestern Thailand has yielded the longest and oldest consistent ‘Hòabínhian’ stratigraphy from 34-12 ka ago, containing more than 100,000 artefacts [211–213]. Mainly used as a temporary settlement with areas for food preparation and lithic production from local sandstone, the site also included two burials and two fragmentary skeletons in the youngest Pleistocene layers associated with faunal remains, shells, flakes, and used cobble tools [212,214,215]. The Lang Kamnan site in western Thailand, dated between 30 and 11 ka ago, also featured ‘Hòabínhian’-like lithics [216]. Human occupation in Spirit Cave and Ban Rai in northwestern Thailand [212,217], Tham Ongbah, Khao Talu, Ment, and Heap caves in western Thailand [217], Tam Hang in northern Laos [218], and Laang Spean in western Cambodia [219] have been dated to 14-5 ka and Huai Hin in northwestern Thailand even to 3.7 ka ago [220]. The assemblages remain quite consistent with Tham Lod with only incremental changes over time, such as decreased use of sandstone, selection of smaller cobbles at Tam Hang, increased use of chert at Talu, and variation as a response to mobility, risk, and resource availability [217,218,221].

Such consistency in technology could have been facilitated by the extension of a forest-grassland mosaic ecosystem far into northwestern Thailand during the terminal Pleistocene, which could have resulted in favourable conditions for hunter-gatherers practising residential mobility in wet seasons and logistical mobility in dry seasons across the highlands of Mainland Southeast Asia [216,222,223]. Faunal and plant remains have yielded a vast range of exploited resources, including marine and freshwater shellfish, terrestrial snails, and

animals for marine sites and terrestrial fauna, freshwater shellfish, and snails for inland sites [224]. However, treating the 'Hòabìnhian' as a monolith has also been criticised for inhibiting studies of temporal and spatial variability as well as social patterns [216], for example, the appearance of coarse pottery within northern Vietnam, absence of cores from the assemblage on the Malaysian peninsula, and the inclusion of obsidian artefacts on Sumatra [209]. The deceased were placed in flexed positions, such as at Tham Lod, Ban Rai, Moh Kiew, Hang Cho, and Gua Cha [211,225–227], and rarely in extended positions, such as at Tham Lod [211], often marked by boulders on top of the skeleton. To date, detailed assessment of internal structure is limited by the low number of burials at 'Hòabìnhian'-associated sites.

Moving further into Island Southeast Asia, numerous other long-lasting assemblages are recognised, but with a more localised appearance. Groups associated with the 'Keplek' assemblage on Java occupied caves and rock shelters and subsisted on hunting and mollusc collection with tools made from bones, antlers, and shells between 12 and 4 ka ago [228]. Moreover, five assemblages persisting up to 15 ka ago at individual cave sites in East Kalimantan show the opportunistic reduction of flakes with a considerable use of ochre [209]. Cremations at Ille Cave on Palawan in the Philippines and a tightly flexed burial after the removal of hands and feet at Liang Lembudu on the Aru Islands also demonstrate the variability of burial rites during the early and middle Holocene, but the assessment of regional patterns is challenging [229–231]. On Sulawesi, the 'Toalean' assemblage exemplifies the limited knowledge available about humans during Holocene Southeast Asia. Characterised by osseous points, backed microliths, sawlettes, and serrated stone projectile points ('Maros points') fashioned from chert [232], this highly-localised group might have comprised of a substantially large population with complex social organisation that persisted between 8 and 1.5 ka ago [233,234]. The introduction of the endemic Celebes warty pig to Flores by around 7 ka ago and the dingo to Australia ca. 3.5 ka ago suggest that 'Toalean'-associated groups were managing a wild suid species and practised long-distance seafaring [235,236]. This, combined with ceramic sherds co-occurring with 'Toalean' artefacts in late strata dated to ca. 3.5 ka ago, challenge the traditional understanding of Holocene groups [234]. However, isolated teeth from poorly-provenienced excavations of Leang Codong and Batu Ejaya during the early 20th century are the only human remains recovered thus far, and connections to pre-, antecedent, and neighbouring groups remain unknown [233,234,237].

Dento- and craniometric analyses associate early Holocene individuals from all over Southeast Asia with present-day Indigenous Australians and Papuans. This includes Mesolithic Malaysia and Flores, early Holocene Gua Gunung Runtuh in Malaysia [238], 10.5-ka-old ‘Hòabìnhian’-associated individuals from Hang Cho in Vietnam [227], and Gaomiao hunter-gatherers from Hunan Province in China dated to 5.7 ka ago [239], suggesting common ancestry of early MH in Sunda with those in Sahul [240]. Similarly, genomic data of several physically distinct present-day groups from the Andaman Islands (Jarawa, Onge), Philippines (Agta, Aeta, Batak, Mamanwa), and Malaysia (Jehai, Kintak, Batek) indicate that their ancestors diverged from West Eurasians more than 38 ka ago and were basal to other East and Southeast Asians, potentially tracing their ancestry to the first MH in Sunda [241]. Craniometric analyses have recently also linked a 6-ka-old pre-ceramic individual from Xiaoma Caves in Taiwan with these groups [242]. In 2018, the recovery of aDNA from ‘Hòabìnhian’-associated individuals at Pha Faen in Laos and Gua Cha in Malaysia dated to 7.8 and 4.2 ka ago, respectively, provided a direct link by showing close genetic affinity between these ancient individuals and present-day Andamanese Islanders [243]. However, genetic analyses also showed that hunter-gatherers associated with Jōmon cord-marked pottery from Japan dated between 16-3 ka ago [244] were not part of the Australopapuan-related ‘first layer’ as suggested by craniometrics [245]. Instead, they diverge deeply on the East Asian lineage before the diversification of present-day groups with no evidence of gene flow with continental groups [243,246–248]. The 10.5-ka-old Longlin hunter-gatherer from Guangxi Province, southern China represents another deep East Asian lineage likely diverging from the ancestors of 9 to 4-ka-old individuals from Shandong and Fujian Province in China at a similar time as the Jōmon [249,250], emphasizing the potential of aDNA to illuminate the genetic diversity present in Holocene Eastern Eurasia.

1.2.4 Neolithic innovations

After such long persistence of well-established technocomplexes and subsistence strategies, communities in Southeast Asia transitioned rather rapidly to the domestication of plants and animals. Potentially facilitated by the use of knives for harvesting, the domestication of rice *Oryza sativa* was already in progress at Shangshan and Kuahuqiao in the Yangtze River Basin and spread northwards to the Yellow River region between 11 and 7 ka ago until it was intensively used from 7 ka ago, as demonstrated at Hemudu near the Yangtze River [251]. Simultaneously, foxtail millet *Setaria italica* underwent domestication at Nanzhuangtou and

Dongjulin in the Yellow River valley 11 to 9 ka ago and spread west- and southwards [252]. By 5 ka ago millet and by 4 ka ago rice farming reached Mainland Southeast Asia from coastal, southern, and southwest China [253], from where the transmission continued southwards along the coast and major rivers [254]. Dentometric similarities with Yangtze river delta groups suggested rapid movement along the coast to the Gulf of Thailand, where the site Khok Phanom Di in eastern central Thailand yielded rice husks dated to 5 ka ago alongside evidence for periodical estuarine gathering, fishing, and an influx of new residents between mortuary phases [255,256]. Mán Bạc on the Red River plains of Vietnam also shows direct connections to the Chinese Neolithic through nephrite bracelets and beads and pottery vessels similar to Sham Wan in Hong Kong [257]. Occupied from 4 ka ago, domesticated rice, pigs, and dogs, as well as hunted sharks, rays, deer, and rhinoceros were recovered at Mán Bạc in Vietnam, the type site of the Phung Nguyen culture [258]. The supine and flexed burials show two distinct morphologies similar to Neolithic Weidun from the Yangtze Valley and local hunter-gatherers, suggesting the integration of incoming farmers into local groups [255,259]. The supine burials at An Son near the Mekong delta, dated to 3.8-3 ka ago, even show cranial affinity to northern groups and dental affinity to hunter-gatherer groups. While the burial rites and grave goods are similar to Mán Bạc, the pottery style differs, potentially indicating a separate farmer source [254,257]. The pottery is more similar to Non Pa Wai in the Lopburi region of Thailand, where millet dominates the crop assemblage dated to 3.8 ka ago, which points towards influence from the Yellow River Basin [260]. Near the upper reaches of the Mekong on the Khorat Plateau in Thailand, contemporary supine and flexed burials with differences in rice consumption and distinct mortuary offerings at Ban Non Wat demonstrate the co-existence of hunter-gatherers and farmers around 3.5 ka ago [261]. The wide distribution of tripod vessels from Ban Kao in central Thailand to Jenderam Hilir in coastal Malaysia point towards another avenue into Southeast Asia along the Salween river [257]. However, despite the similarities in farmed crops, supine burial position, presence of incised pottery, domestication of pigs, cattle, and dogs, and ubiquitous contact with hunter-gatherers, agriculture was seemingly introduced along several routes at different times, and precise dating and potential interactions require further investigation [257].

The observed craniometric variation at early agricultural sites represents the second component of the ‘Two Layer’ hypothesis, where descendants of northeast Asians expanded southwards from 9 ka ago, interacted with the underlying Australopapuan-like ‘first layer’, and finally predominately occurred in the region with variable admixture degrees [245].

Archaeogenetic analyses have shown that the two agricultural centres in the Yangtze and Yellow River valleys are also associated with well-differentiated ancestries with an increasing admixture of northern ancestry into southern groups over time forming today's central Chinese gene pool [250]. Near the West Liao River in northern China, an increase in Yellow River-related ancestry coincides with the intensification of agriculture in the Late Neolithic [262], and the detection of Yellow River-related ancestry in groups from Tibet suggests that such farmers probably spread Sino-Tibetan languages southwards [263–265]. Due to cognate words for rice and rice cultivation in many Austroasiatic languages, the spread of agriculture from southern China into Southeast Asia has also been associated with the expansion of this language family [266,267]. Late Neolithic and early Bronze Age individuals from Vietnam, Laos, and Malaysia are indeed genetically most closely related to present-day Austroasiatic speakers and show evidence for admixture between Yangtze River farmer- and 'Hòabìnhian'-related groups [243,268], but more detailed local interactions remain understudied.

On the border between East and Island Southeast Asia, Neolithic-associated material first appeared in Taiwan during the Tapenkeng Period between 5.6 and 4.2 ka ago, characterised by ridge-rimmed, cord-marked, and comb-incised lined pottery, and is divided into Shuntanpu in northern, Niumatou in central, and Kuoyeh Culture in southern Taiwan [269]. These cultural spheres and trade networks extending to nearby Penghu, Orchid, and Green Island illustrate an important maritime component of this period and are associated with the first introduction of millet and rice farming as well as supine burials to the region [269,270]. While stone implements, pottery decorations, and jade slice-cutting techniques represent commonalities and potential origin in the Liangzhu, Keqiutou, Tanshishan, and Xiantouling-Tawan Cultures along China's southeast coast [269], the recovery of red-slipped and corded pottery, Taiwan-sourced nephrite, and domestic pigs at Nagsabaran on Luzon demonstrate the transmission of Fushan and Niuchoutsu Culture of eastern and southern Taiwan to the northern Philippines by 4 ka ago [271,272]. The red slip and distinctive shape of pottery in combination with rice phytoliths also appear at Minanga Sipakko in Western Sulawesi and the Mariana Islands by 3.5 ka ago [273,274] and at Bukit Tengkorak on Borneo and Pain Haka on Flores by 3 ka ago [275,276], suggesting a vast region of shared belief systems [276,277]. Around 3.3 ka ago, a distinct dentate-stamped pottery type, called Lapita [278], developed in the Bismarck Archipelago and is found on previously uninhabited areas on the Santa Cruz Islands, Vanuatu, and New Caledonia by 3 ka ago. Crossing over 800 km open ocean from

there, Fiji was settled by 2.85 ka ago and Hawai'i, Aotearoa, and Rapa Nui by ca. 800 ya [267,279].

This large area of shared cultural influence overlaps closely with that of present-day speakers of Austronesian languages, whose linguistic origins have also been traced to Taiwan around 5 ka ago before an expansion of the Malayo-Polynesian sub-branch into the Philippines and then Island Southeast Asia and Oceania in accordance with the 'pulse-pause' model [267,280,281]. Therefore, a unified model combining archaeology and linguistics refers to this process as the 'Austronesian expansion'. While the settlement of previously uninhabited islands in Remote Oceania must have included the movement of people, Austronesian languages and archaeological culture within Island Southeast Asia could have spread within pre-existing groups without human movement [282]. Initial single-locus genetic studies of present-day Austronesian speakers suggested haplotype connections between Indigenous Taiwanese and the Philippines [283], Polynesia and the B4a1a1 'Polynesian motif' [284–286], and mainland China [287,288], but some analyses indicated origins in eastern Indonesia [289] and no connection to Taiwan [290]. When using genome-wide data, however, all present-day Austronesian speaking groups from Island Southeast Asia were more closely related to Indigenous Taiwanese than mainland groups indicating that the 'Austronesian expansion' also had a genetic component [291]. Moreover, Lapita-associated individuals from Vanuatu and Tonga, dated to 2.9-2.6 ka, showed nearly exclusively Taiwanese-related ancestry, indicating rapid dispersal of people to Remote Oceania [292,293]. The Neolithic Yangtze River and Taiwanese Strait individuals likewise show a close relationship with Austronesian-speaking groups in Taiwan and Island Southeast Asia, corroborating the Austronesian origin on the mainland, where no Austronesian languages are spoken today [250,294]. Recent genomic data from the Philippines has complicated the picture by suggesting the Austronesian origin within the Kankanaey Igorots [295] and connecting this group to a pre-farming divergence from Indigenous Taiwanese and migration into the Philippines around 7 ka ago [199,202]. Despite being a central step of the Austronesian expansion, only one 1.8-ka-old low-coverage genome with exclusively Austronesian-related ancestry is published from the Philippines [243]. Conversely, at the crossroads of Island Southeast Asia, groups from the Indonesian islands show several different ancestries with distinct regional patterns. Eastern Indonesia has a high Papuan-related component [296], while Austronesian-related ancestry was suggested to have spread from non-Kankanaey Philippine sources over western into eastern Indonesia, associated with the spread of

Neolithic cultural features [296]. Western Indonesian groups additionally harbour ancestry from mainland Austroasiatic speakers, which is already present ca. 2 ka ago [243,291,296]. This potentially indicates interaction between the Neolithisations of Mainland and Island Southeast Asia [297], but direct insights into when these two streams reached western Indonesia, from where they originated and how far they dispersed are lacking.

1.2.5 The introduction of metallurgy

Shortly after their inception, the Neolithic settlements of Mainland Southeast Asia show evidence of bronze production, heralding another era of change. However, evaluating the impact of copper-based metallurgy has been limited by controversies about the source and timing of the necessary knowledge transmission [298]. The ‘long chronology’ is based on ^{14}C -dated crushed pottery from Ban Chiang in Thailand, which places bronze fragments and a copper-base spear point around 4-3.8 ka ago [299]. Without evidence of intermediate technologies, bronze appears as a fully developed technology supposedly transmitted from the Seima-Turbino phenomenon of the Urals via Gansu, Yunnan, and Laos to lowland Southeast Asia [299]. While the local selection of suitable technologies from a remotely developed ‘metallurgical package’ (prospecting, extraction, processing, smelting, casting) is agreed upon, the reliability of these ^{14}C dates has been questioned. Direct radiocarbon dates from human remains excavated at the Bronze Age cemeteries of Nong Nor, Ban Na Di, Ban Non Wat, and Ban Lum Khao instead suggest a ‘short chronology’ wherein metallurgy arrived virtually simultaneously between 3.2 and 3.1 ka ago [300,301]. Here copper and bronze smelting technologies were transmitted southwards from copper-rich sites in the Yangtze Valley or the Jiangchuan Basin dated to the later 2nd millennium BCE [300]. Centred around smelting in internally heated crucibles and the casting of socketed tools in ceramic bivalve moulds with suspended core, the ‘metallurgical package’ first appeared in the lower Song Hong River Valley of Vietnam [302]. Jade blades and bracelets already show direct connections to southern China and transmission point to Thailand from the late Neolithic onwards, which continued into the ‘classic Dong Dau phase’ between 3.2 and 2.7 ka ago by the introduction of sandstone moulds for axes and picks [302]. Mining technology and products also connect different regions, as demonstrated by the similarities in wood and rattan construction of the copper mining shafts at Vilabouly in Laos (2.5-1.5 ka) to Tonglushan in China, while isotope signatures reveal the presence of Vilabouly copper at Ban Chiang in Thailand [303,304]. Non Pa Wai, Nil Kham Haeng, and Non Mak La in the Khao Wong

Prachan Valley and the Phu Lon mining complex in northeast Thailand also represent large copper-producing communities where copper smelting was likely introduced by ‘founders’ around 3 ka ago and was traded to Myanmar and Ban Non Wat in Thailand [304,305]. While the analyses of early excavations suggested no social stratification or even heterarchies in Bronze Age Southeast Asia [306], grave good analyses from Khok Phanom Di, Ban Non Wat, and Ban Lum Khao in Thailand demonstrate a sharp rise in localised social inequality where valuables, such as copper-based ornaments, shell beads, and axes, were preferred and inherited by a temporary social elite [307,308]. On the Red River Plain of Vietnam, the Dong Son culture (2.5-1.8 ka) is associated with higher levels of status and wealth disparities between burials, larger habitation sites, and intensified agricultural production [309]. Highly sophisticated bronze tools suggest complex political relationships to procure raw materials and produce various agricultural tools, ritual artefacts, and weaponry [310]. As prestige objects, the well-known Dong Son drums were traded throughout Southeast Asia as far as southern Indonesia, New Guinea, and East Timor [311] and illustrate the increasing intra- and interregional cultural exchange [310].

Dong Son contexts also provide early evidence of iron objects, but these are more abundant in the Sa Huynh culture of southern Vietnam (2.3-1.9 ka), whose development through local innovation or immigration of Austronesian speakers from Malaysia or Borneo remains debated [312,313]. Grave goods interred alongside the distinct jar burials include carnelian, agate, and gold beads imported from India and signify the presence of a social elite [314]. In the highlands, wooden coffins appeared as a distinct mortuary practice with burials within hollowed tree logs placed in caves found from northwestern Thailand [315] to Myanmar [316] and India [317], as well as Laos [318], Cambodia [319], Malaysia [320], and the Philippines [321]. In Peninsular Thailand, burgeoning sea trade initiated the rapid growth of urban centres, such as Khao Sam Kaeo, where excavated Southeast Asian-style metallic, stone, and glass ornaments were manufactured by Indian craft persons using South Asian technologies by 2.4 ka ago [322,323]. The abundance of iron bloomery slag at the site lends support to a South Asian origin of iron technology in Southeast Asia, but a co-occurrence of bloomery smelting and cast iron making in China does not preclude knowledge or material transfer from East Asia [324]. Located on the Kra Isthmus, Khao Sam Kaeo sat on the intersection of the ‘Bay of Bengal network’ and the ‘South China Sea Interaction Sphere’ which distributed Dong Son drums, nephrite earrings (‘lingling-o’), and Sa Huynh-Kalanay ceramics to China, Taiwan, the Philippines, and Indonesia [322,325,326]. In Island Southeast

Asia, bronze and iron artefacts appear simultaneously with glass beads, distinctive earthenware, and, in some cases, Chinese trade ware and jar burials from 2.5 ka ago [327,328]. In Taiwan, this delay in the advent of the Metal Age at 1.8 ka ago compared to the mainland might result from the geographical isolation and independent nature of its Indigenous cultures [269]. The Metal Age in the Philippines began around 2.5 ka ago and saw the introduction of metalworking, glass beads, and high-fired ceramics from China and Mainland Southeast Asia [329]. While inter- and intra-continental interactions tracked by archaeology have evidently had significant impacts on the internal stratification of and cultural exchange between prehistoric societies all over Southeast Asia, the few Metal Age genomes available from individual sites in Myanmar, Thailand, Vietnam, Cambodia, and Indonesia currently do not allow for analysis of interactions or social structure from a genetic perspective [243,268].

1.2.6 Early historical states and European colonization

Large moated coastal polities, such as Co Loa in the Red River Delta and Angkor Borei in Cambodia, in combination with aspects of Indian administration and Hinduism, provided a fertile background for rapid early state formation. The best-documented polity is ‘Funan’ in the Mekong Delta, named after a state-like city described in numerous Chinese texts between the 1st and 6th century CE. The associated Oc Eo archaeological complex stretches over 2,500 ha and demonstrates the strong connection between port cities and agricultural hinterland by canals established pre-Indian influence. After its collapse and abandonment by the 7th century, the power centre moved successively inland with the Khmer-speaking Chenla or Zhenla kingdom and the later Greater Angkor lasting from the 9th to the 15th century [330]. In central Thailand, the dvāravatī complex appears in Chinese records from the 6th to 11th century, but some archaeological dates point towards an early phase starting from the 3rd century. Rather describing a style of monumental art and moated architecture mixing Hindu and Buddhist practices, the socio-political organisation of this polity centred around Nakhon Pathom in the western Chao Phraya Valley remains debated [331]. Based on rebellions against Angkor rule, the Tai kingdoms of Lanna in northern and Sukhothai in central Thailand were founded in the 13th century, of which the latter was integrated into the newly founded Ayutthaya kingdom in the 15th century. After the early adoption of Buddhism in the 5th and 6th centuries, the Srivijaya kingdom was founded in the Southeast of Sumatra in the late 7th century. Inscriptions document the establishment of a patrimonial government and a focus on

long-distance maritime trade as opposed to the 8th-century inland state of Mataram on Lombok [332]. In eighth-century Javanese polities, large Buddhist monuments in central locations, such as Borobudur, finished in 832, suggest popularity among the nobility, while Hinduism was widespread throughout the island. In the late 13th century, the Majapahit kingdom with its capital at Trowulan became influential until its demise in the 15th and 16th centuries [333]. The societal development on Bali seems to have been connected to Java, and the first king and fortified sites are mentioned in inscriptions from the 10th century [333]. However, the definite assignment of early state status there and in other parts of Southeast Asia is limited by the lack of historical and archaeological integration of inscriptions, isolated shrines, and historical records [332].

Capitalising on the pre-existing trade networks, the European desire for direct and permanent access to spices and ceramics from Southeast Asia led to the conquest of the Sultanate of Malacca in 1511 by Portugal and the creation of Portuguese Insulíndia (Indonesia). This was rapidly followed by the invasion of the Spanish East Indies (Philippines), Dutch East Indies (Indonesia), and Dutch Formosa (Taiwan). The later establishment of British Burma (Myanmar), British Malaya (Malaysia and Singapore), British Borneo (Malaysia and Brunei), and French Indochina (Laos, Cambodia and Vietnam) left only Siam (Thailand) without direct foreign rule. Previously only considered the receptor of northern European capitalist and globalising ideology, recent historical archaeological studies have illuminated pre-European continental networks and worldsystems, as well as the role of early colonial agents, such as China, Japan, Portugal, and Spain [334]. While the demographic dynamics, interactions between Indigenous groups and the colonisers, and their impact on culture and economy have been well-studied from historical documents of colonial capitals, such as Manila and the Philippines, [335,336], this remains underexplored during the early stages of European colonisation and in the colonial periphery. While the historical records are biased towards the colonial powers, ancient DNA, in collaboration with archaeology and anthropology, can provide a more comprehensive view of colonial settlements and help to understand the life there through their treatment of the deceased.

2. Aims and Objectives

The possibility of extracting DNA from archaeological material has given rise to archaeogenetics, a field combining archaeological and genetic analyses to understand the past. While Western Eurasia and other temperate regions have seen a large number of ancient genomes published, tropical regions, including Southeast Asia, a region of high present-day genomic and cultural diversity, have so far been understudied. This thesis aims to implement archaeogenetic approaches to gain insights into the population history of Southeast Asia by fully integrating multiple available lines of evidence and collaborating closely with local archaeologists.

At the crossroads between the Eurasian continent and Oceania, the Wallacean islands represent a crucial step in the early expansion of modern humans, necessarily requiring open-water crossings for the first time. In this thesis, I aim to illuminate the genetic make-up of Holocene modern humans on Sulawesi by investigating a ‘Toalean’-associated burial and the genetic relationships with Southeast Asian and Oceanian groups and archaic hominins.

Wallacea was later impacted by the Austronesian expansion, spreading farming, pottery, and sailing techniques southwards from Taiwan and the Philippines. My project aims to provide insights into the arrival of Austronesian-related ancestry and interactions with local groups by comparing the temporal variation of genetic ancestry between the northern Moluccas, Sulawesi, and the southern Indonesian island chain.

The introduction of bronze and, shortly thereafter, iron metallurgy initiated the development of complex craft-based settlements and trans-regional trade networks culminating in the formation of early states. I aim to explore the connectivity between regions and the social structure of local groups by studying the genetic ancestry profiles and genetic relatedness patterns of the Iron Age Log Coffin culture of northwestern Thailand.

Fuelled by the desire to control the valuable spice trade, from the 16th to the 20th century, European powers colonised progressively more regions of Southeast Asia. My project aims to help understand the social interactions and structure at early European settlements by examining the spatial organisation and genetic ancestries of a 17th-century cemetery associated with a Spanish church in northern Taiwan.

3. Manuscripts

3.1 Manuscript A: Genome of a middle Holocene hunter-gatherer from Wallacea

Selina Carlhoff*, Akin Duli*, Kathrin Nägele, Muhammad Nur, Laurits Skov, Iwan Sumantri, Adhi Agus Oktaviana, Budianto Hakim, Basran Burhan, Fardi Ali Syahdar, David P. McGahan, David Bulbeck, Yinika L. Perston, Kim Newman, Andi Muhammad Saiful, Marlon Ririmasse, Stephen Chia, Hasanuddin, Dwia Aries Tina Pulubuhu, Suryatman, Supriadi, Choongwon Jeong, Benjamin M. Peter, Kay Prüfer, Adam Powell, Johannes Krause, Cosimo Posth & Adam Brumm (2021) *Nature* **596**, 543-547.

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On the crossroads between the Pleistocene landmasses of Sunda (Mainland Southeast Asia and western Indonesia) and Sahul (Australia-New Guinea), Sulawesi and other Wallacean islands are crucial stepping stones in understanding early modern human settlement. In this paper, we describe the archaeological site of Leang Panninge in South Sulawesi, Indonesia and the recovered flexed burial. Covered with large cobbles and ochre after the burial around 7.2 ka ago, this 17-18-year-old anatomically female hunter-gatherer represents the first burial associated with the distinctive ‘Toalean’ technocomplex. We were able to extract ancient DNA from the petrous part of the temporal bone and used in-solution capture enrichment to produce the first genome-wide data from Wallacea. Comparisons with present-day groups show that this individual shows no direct affinity to any of the tested groups but indicate an equally close relationship with Papuans and Indigenous Australians. The Leang Panninge individual represents a previously undescribed genetic lineage, which branched off after Andamanese- and ‘Hòabìnhian’-related groups diverged but separated around the split of the ancestors of Papuan and Indigenous Australian groups around 37 ka ago. We also identify Denisovan-related alleles, which are reduced compared to Near Oceanians but likely originate from the same introgression event. This might indicate that the Denisovan-related gene flow into modern humans occurred before their arrival in Sahul, although additional genomic data is necessary to narrow down the location further. Through admixture modelling, we additionally determined a genetic contribution of around 50% from a ‘Deep Asian’-related lineage, which might result in the reduced Denisovan proportion.

Genome of a middle Holocene hunter-gatherer from Wallacea

<https://doi.org/10.1038/s41586-021-03823-6>

Received: 27 November 2020

Accepted: 13 July 2021

Published online: 25 August 2021

Open access

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Much remains unknown about the population history of early modern humans in southeast Asia, where the archaeological record is sparse and the tropical climate is inimical to the preservation of ancient human DNA¹. So far, only two low-coverage pre-Neolithic human genomes have been sequenced from this region. Both are from mainland Hòabinhian hunter-gatherer sites: Pha Faen in Laos, dated to 7939–7751 calibrated years before present (yr cal BP; present taken as AD 1950), and Gua Cha in Malaysia (4.4–4.2 kyr cal BP)¹. Here we report, to our knowledge, the first ancient human genome from Wallacea, the oceanic island zone between the Sunda Shelf (comprising mainland southeast Asia and the continental islands of western Indonesia) and Pleistocene Sahul (Australia–New Guinea). We extracted DNA from the petrous bone of a young female hunter-gatherer buried 7.3–7.2 kyr cal BP at the limestone cave of Leang Panninge² in South Sulawesi, Indonesia. Genetic analyses show that this pre-Neolithic forager, who is associated with the ‘Toalean’ technocomplex^{3,4}, shares most genetic drift and morphological similarities with present-day Papuan and Indigenous Australian groups, yet represents a previously unknown divergent human lineage that branched off around the time of the split between these populations approximately 37,000 years ago⁵. We also describe Denisovan and deep Asian-related ancestries in the Leang Panninge genome, and infer their large-scale displacement from the region today.

Modern humans crossed through Wallacea (Fig. 1a) to Sahul^{5–8} at least 50 thousand years ago (kya)⁹, and possibly by up to 65 kya¹⁰. Presently, however, the earliest archaeological evidence for our species in Wallacea dates to at least 45.5 kya for figurative art in Sulawesi¹¹, and 47–43 kyr cal BP for a behavioural shift at Liang Bua (Flores, Indonesia)¹². The oldest *Homo sapiens* skeletal remains date to 13 kya¹³. The route modern humans used to enter Sahul is not known¹⁴. Demographic models infer a population split between the ancestors of Oceanian and Eurasian groups approximately 58 kya, whereas Papuan and Aboriginal Australian groups separated around 37 kya⁵. Within this time interval, modern humans admixed multiple times with groups related to Denisovans^{15–23}, and potentially other unknown hominins^{24,25}. The genetic ancestry of the two Hòabinhian-associated foragers from Pha Faen and Gua Cha¹ shows the highest similarity to modern Andamanese peoples.

These ancient and present-day peoples lack substantial amounts of Denisovan-related ancestry, suggesting that the Hòabinhian-associated and Onge-related lineage diverged before the main archaic human introgression events¹. Current Wallacean individuals carry larger proportions of Denisovan-related ancestry, but at substantially lower frequencies than is the case in Papuan and Indigenous Australian individuals²⁰. This is probably due to admixture with the East Asian Neolithic farmers (‘Austronesian peoples’) who arrived in Wallacea around 4 kya^{20,26}.

The Toalean burial from Leang Panninge

The most distinctive archaeological assemblages associated with Holocene hunter-gatherers in Wallacea belong to the Toalean

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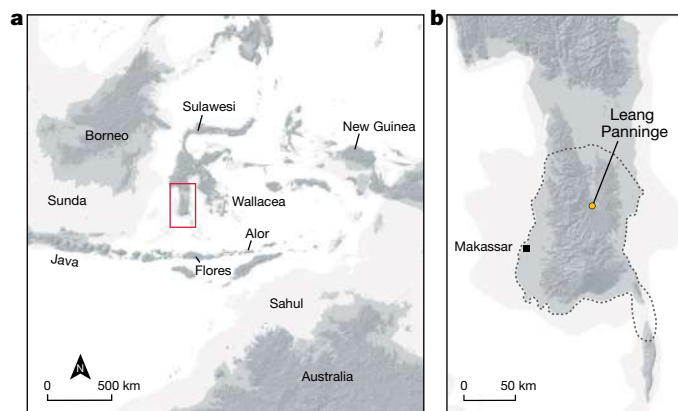


Fig. 1 | Study site location. **a**, Sulawesi and Wallacea. The red rectangle indicates the region shown in **b**. **b**, Leang Panninge. The dotted line indicates Toalean site distribution.

technocomplex (8–1.5 kya)^{3,4,27,28}. Found only in a 10,000 km² area of South Sulawesi³ (Fig. 1b), Toalean cultural assemblages are generally characterized by backed microliths and small stone projectiles ('Maros points')⁴ (Extended Data Fig. 1a–c). In 2015, excavations at Leang Panninge in the Mallawa district of Maros, South Sulawesi (Fig. 1b), uncovered the first relatively complete human burial from a secure Toalean context (Extended Data Figs. 1–5, Supplementary Information). The individual was interred in a flexed position²⁹ in a rich aceramic Toalean stratum. Exposed at a depth of around 190 cm, the burial has an inferred age of 7.3–7.2 kyr cal BP obtained from ¹⁴C dating of a *Canarium* sp. seed (Extended Data Figs. 2, 3, Supplementary Table 1). Morphological characters indicate that this Toalean forager was a 17–18-year-old female with a broadly Australo-Melanesian affinity, although the morphology does not fall outside the range of recent Southeast Asian variation (Supplementary Information).

Genomic analysis

We extracted ancient DNA from bone powder obtained from the petrous portion of the temporal bone of the Leang Panninge individual. After library preparation, we used a DNA hybridization capture approach to enrich for approximately 3 million single-nucleotide polymorphisms (SNPs) across the human genome (1240K and archaic admixture capture panels³⁰) as well as for the entire mitochondrial genome (mtDNA capture³¹). We retrieved 263,207 SNPs on the 1240K panel, 299,047 SNPs on the archaic admixture panel and the almost complete mtDNA sequence. Authenticity of the analysed ancient DNA was confirmed by short average fragment length, elevated damage patterns towards the molecule ends, and low autosomal and mtDNA contamination estimates (Supplementary Fig. 1). We confirmed that the individual was genetically of female sex. Analysis of the polymorphisms present in the reconstructed mtDNA sequence suggests a deeply divergent placement within mtDNA haplogroup M (Supplementary Table 17, Supplementary Fig. 2).

We initiated our genomic investigation by principal component analyses (PCAs), comparing the Leang Panninge genome with present-day individuals from East Asia, southeast Asia and Near Oceania (comprising Indigenous Australia, Papua New Guinea and Bougainville) genotyped on the Human Origins SNP panel^{18,32–34}. The newly generated genome and relevant published genomes from ancient individuals from eastern Eurasia were then projected on the PCA^{1,34–38}. Leang Panninge falls into PCA space not occupied by any present-day or ancient individuals, but is broadly located between Indigenous Australian peoples and the Onge (Fig. 2a, Extended Data Fig. 6). F_3 -statistics³³ of the form f_3 (Mbuti; Leang Panninge, X), where X is replaced with

present-day Asian-Pacific groups, indicated that the new genome shares most genetic drift with Near Oceanian individuals (Fig. 2b). We confirmed these results with f_4 -statistics³³, suggesting similar affinity of Leang Panninge and Papuan individuals to present-day Asian individuals, despite Near Oceanian groups forming a clade to the exclusion of Leang Panninge (Extended Data Fig. 7a, b). All present-day groups from the region, with the exception of the Mamanwa and the Lebbo²⁶, carry only a minor contribution of Papuan-related ancestry (Supplementary Fig. 4).

To investigate the presence and distribution of genetic contributions attributable to Denisovan-related groups³⁹, we calculated the statistic f_4 (Mbuti, Denisova; Leang Panninge, X), where X are groups from present-day Island Southeast Asia, Near Oceania and the Andamans, as well as ancient Asian-Pacific individuals^{1,37,38}. Positive values calculated for Near Oceanian groups suggest higher proportions of Denisovan-related ancestry than the Leang Panninge individual (z-scores of >3.19), while the Onge and the remaining ancient individuals returned negative values, indicating a lower proportion of Denisovan-related ancestry (Extended Data Fig. 7c, Supplementary Fig. 3). We also calculated f_4 -ratio statistics to estimate the Denisovan proportion using SNPs from the 1240K capture panel and Han individuals from East Asia as a baseline¹⁸. We confirmed that Indigenous Australian and Papuan individuals carry a similar amount of Denisovan ancestry (approximately 2.9%)^{18,21,40}, whereas the Leang Panninge individual has a lower value of approximately $2.2 \pm 0.5\%$ (Supplementary Tables 18–20). The Denisovan admixture proportion in the Leang Panninge individual is higher than in the Hòabinh individuals from Pha Faen and Gua Cha¹, suggesting that groups ancestral to hunter-gatherers from Wallacea and Sunda were involved in different introgression events with archaic hominins. In addition, we performed D -statistics on a set of SNPs designed to measure the contribution of archaic ancestry in modern humans (archaic admixture capture panel). The Leang Panninge individual shares fewer Denisovan-related alleles with Papuan individuals, but has more such alleles than most tested groups, including the Tianyuan individual from Late Pleistocene China³⁸. Neanderthal allele sharing is similar across all tested present-day non-African groups (Supplementary Tables 21–23). Finally, we ran admixfrog⁴¹ on the set of archaic admixture SNPs and measured 22.4 Mb (± 1.9 Mb) of Denisovan-related ancestry in 33 fragments distributed across the Leang Panninge genome. This contribution accounts for around half of what is found in Papuan groups, but there is a significant correlation between the Denisovan fragments in the Leang Panninge genome and those in present-day Near Oceanian groups, suggesting shared introgression events (Fig. 2c, Extended Data Fig. 8, Supplementary Fig. 5).

To investigate whether the apparent PCA shift of Leang Panninge away from Near Oceanian groups is due to genetic drift alone, we performed a multidimensional scaling plot based on genetic similarities measured as $1 - f_3$ (Mbuti; Leang Panninge, X). The multidimensional scaling positioning of the Leang Panninge individual recapitulates the PCA with an intermediate placement between Papuan and Asian individuals (Extended Data Fig. 9). We then used f_4 -statistics and qpWave³³ to formally test for the presence of additional genetic sources in Leang Panninge other than the Papuan-related ancestry. This identified a marginal affinity towards ancient Asian genomes (Extended Data Fig. 7d), and a minimum of two streams of ancestry when Denisova³⁷ and/or ancient Asian groups^{1,37,38} were included in the qpWave reference groups (Supplementary Table 24). On the basis of these results, we used qpAdm³³ to identify potential sources for an Asian-related ancestry in the genome alongside the Papuan-related component (Supplementary Table 25). Using a rotating approach among different Asian groups^{1,37,38}, we were able to model the Leang Panninge genome as a mixture between Papuan and Tianyuan ($51 \pm 11\%$) or Onge ($43 \pm 9\%$) (Fig. 3a, Supplementary Table 26). Further exploration with admixture graphs built in qpGraph³³ and TreeMix⁴², including present-day groups

and relevant ancient individuals^{37,38,40,43}, provided evidence for the presence of deep Asian ancestry (Fig. 3b, c, Supplementary Figs. 6–11). In TreeMix, the first admixture edge represents archaic introgression from Denisovan-related groups into the common ancestor of Leang Panninge and present-day Near Oceanian peoples. This is followed by an East Asian-related gene flow into Leang Panninge departing basally from the Qihe lineage, an early Neolithic genome from southeastern China³⁷ (Fig. 3b, Extended Data Fig. 10, Supplementary Fig. 6). The qpGraph analysis confirmed this branching pattern, with the Leang Panninge individual branching off from the Near Oceanian clade after the Denisovan gene flow, although with the most supported topology indicating around 50% of a basal East Asian component contributing to the Leang Panninge genome (Fig. 3c, Supplementary Figs. 7–11).

Discussion

Genome-wide analyses of the Leang Panninge individual show that most genetic drift is shared with present-day groups from New Guinea and Aboriginal Australia (Fig. 2b, Extended Data Fig. 7a). However, this Toalean-associated genome represents a previously undescribed ancestry profile, one that branched off after Onge-related and Hòabinhian-related lineages but around the time that Papuan and Indigenous Australian groups split (Fig. 3b, c, Extended Data Fig. 8, Supplementary Figs. 6–9). It is possible that this Toalean individual carries a local ancestry that was present in Sulawesi before the initial peopling of Sahul at least 50 kya⁹, although whether this population produced the Late Pleistocene rock art in the south of the island^{11,44,45} is unknown.

The Toalean individual carries substantial Denisovan-related ancestry, probably sharing the archaic admixture event with present-day Near Oceanian groups (Fig. 2c, Extended Data Figs. 7c, 10, Supplementary Fig. 5, Supplementary Tables 21–23). This provides strong support for the main Denisovan-related gene flow happening before modern humans reached Sahul, making both Wallacea and Sunda equally likely locations for this archaic introgression event. However, previously published hunter-gatherer genomes from Sunda carry almost no Denisovan-related ancestry (Supplementary Tables 18–20), suggesting either a Hòabinhian-related spread into southeast Asia after the aforementioned gene flow or that Wallacea was indeed the crucial meeting point between archaic and modern humans. The apparent presence of a long-established population of archaic hominins in southwestern Sulawesi⁴⁶ provides a possible source for the introgression event. Two previous studies have suggested that two deeply divergent Denisovan lineages admixed into the ancestors of Papuan individuals^{17,22}, but our genomic data currently do not have enough resolution to distinguish among one or multiple introgression pulses.

The lower amount of Denisovan ancestry in the Leang Panninge individual than in Papuan and Indigenous Australian individuals could result from: (1) an additional admixture with Denisovan ancestry into the common ancestors of Near Oceanian groups, or (2) a dilution of the Denisovan-related ancestry in the Leang Panninge genome through admixture with lineages carrying less or no such ancestry. Our allele frequency-based analyses do not support the first scenario (Supplementary Fig. 11), but they do favour the latter. The scarcity of pre-Neolithic genomes from across Asia prevents us from defining the exact source and admixture proportions of this gene flow event. It is noteworthy, however, that despite the reconstructed population trees (TreeMix and qpGraph) suggesting a genetic influence on middle Holocene Sulawesi from mainland East Asia, our qpAdm modelling cannot rule out a southeast Asian contribution from a group related to present-day Andamanese peoples (Fig. 3, Supplementary Figs. 6–11, Supplementary Table 26). This is consistent with a recent study that describes widespread admixtures across Asia between Onge-related and Tianyuan-related ancient populations⁴⁷. However, the presence of this type of ancestry in a middle Holocene forager from Wallacea

suggests that the Asian-related admixture could have taken place long before the expansion of Austronesian societies into the region.

We could not detect evidence for the Leang Panninge ancestry in any tested present-day groups (Supplementary Fig. 4). This could be owing to the overall limited proportion of Near Oceanian-related ancestry in Wallacea or large-scale genetic discontinuity between earlier hunter-gatherers and modern groups. The latter scenario would suggest that any genetic signal related to the Leang Panninge individual was obscured by later demographic processes, including the Austronesian expansion^{1,20,26,48}. Higher coverage genetic data from present-day populations in Sulawesi, and additional Toalean ancient genomes, are needed to further investigate this unique ancestry profile and the genetic diversity of hunter-gatherers from Wallacea more generally.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-021-03823-6>.

- McColl, H. et al. The prehistoric peopling of Southeast Asia. *Science* **361**, 88–92 (2018).
- Hasanuddin. Gua Panningge di Mallawa, Maros: kajian tentang gua hunian berdasarkan artefak batu dan sisa fauna. *Naditira Widya* **11**, 81–96 (2017).
- Bulbeck, D., Pasqua, M. & Di Lello, A. Culture history of the Toalean of South Sulawesi, Indonesia. *Asian Perspect.* **39**, 71–108 (2000).
- Perston, Y. L. et al. A standardised classification scheme for the mid-Holocene Toalean artefacts of South Sulawesi, Indonesia. *PLoS ONE* **16**, e0251138 (2021).
- Malaspinas, A.-S. et al. A genomic history of Aboriginal Australia. *Nature* **538**, 207–214 (2016).
- Pugach, I., Delfin, F., Gunnarsdóttir, E., Kayser, M. & Stoneking, M. Genome-wide data substantiate Holocene gene flow from India to Australia. *Proc. Natl Acad. Sci. USA* **110**, 1803–1808 (2013).
- Rasmussen, M. et al. An Aboriginal Australian genome reveals separate human dispersals into Asia. *Science* **334**, 94–98 (2011).
- Teixeira, J. C. & Cooper, A. Using hominin introgression to trace modern human dispersals. *Proc. Natl Acad. Sci. USA* **116**, 15327–15332 (2019).
- O'Connell, J. F. et al. When did *Homo sapiens* first reach Southeast Asia and Sahul? *Proc. Natl Acad. Sci. USA* **115**, 8482–8490 (2018).
- Clarkson, C. et al. Human occupation of northern Australia by 65,000 years ago. *Nature* **547**, 306–310 (2017).
- Brumm, A. et al. Oldest cave art found in Sulawesi. *Sci. Adv.* **7**, eabd4648 (2021).
- Sutikna, T. et al. The spatio-temporal distribution of archaeological and faunal finds at Liang Bua (Flores, Indonesia) in light of the revised chronology for *Homo floresiensis*. *J. Hum. Evol.* **124**, 52–74 (2018).
- Samper Carro, S. C. et al. Somewhere beyond the sea: human cranial remains from the Lesser Sunda Islands (Alor Island, Indonesia) provide insights on Late Pleistocene peopling of Island Southeast Asia. *J. Hum. Evol.* **134**, 102638 (2019).
- Bird, M. I. et al. Early human settlement of Sahul was not an accident. *Sci. Rep.* **9**, 8220 (2019).
- Browning, S. R., Browning, B. L., Zhou, Y., Tucci, S. & Akey, J. M. Analysis of human sequence data reveals two pulses of archaic Denisovan admixture. *Cell* **173**, 53–61.e9 (2018).
- GenomeAsia100K Consortium. The GenomeAsia 100K Project enables genetic discoveries across Asia. *Nature* **576**, 106–111 (2019).
- Jacobs, G. S. et al. Multiple deeply divergent Denisovan ancestries in Papuans. *Cell* **177**, 1010–1021.e32 (2019).
- Qin, P. & Stoneking, M. Denisovan ancestry in east Eurasian and Native American populations. *Mol. Biol. Evol.* **32**, 2665–2674 (2015).
- Reich, D. et al. Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* **468**, 1053–1060 (2010).
- Reich, D. et al. Denisova admixture and the first modern human dispersals into Southeast Asia and Oceania. *Am. J. Hum. Genet.* **89**, 516–528 (2011).
- Vernot, B. et al. Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals. *Science* **352**, 235–239 (2016).
- Choin, J. et al. Genomic insights into population history and biological adaptation in Oceania. *Nature* **592**, 583–589 (2021).
- Teixeira, J. C. et al. Widespread Denisovan ancestry in Island Southeast Asia but no evidence of substantial super-archaic hominin admixture. *Nat. Ecol. Evol.* **5**, 616–624 (2021).
- Mondal, M., Bertranpetit, J. & Lao, O. Approximate Bayesian computation with deep learning supports a third archaic introgression in Asia and Oceania. *Nat. Commun.* **10**, 246 (2019).
- Mondal, M. et al. Genomic analysis of Andamanese provides insights into ancient human migration into Asia and adaptation. *Nat. Genet.* **48**, 1066–1070 (2016).
- Lipson, M. et al. Reconstructing Austronesian population history in Island Southeast Asia. *Nat. Commun.* **5**, 4689 (2014).
- Suryatman, et al. Artefak batu preneolitik situs Leang Jarie: bukti teknologi Maros Point tertua di Kawasan budaya Toalean, Sulawesi Selatan. *AMERTA* **37**, 1–17 (2019).

28. Anggraeni, A., Simanjuntak, T., Bellwood, P. & Piper, P. Neolithic foundations in the Karama valley, West Sulawesi, Indonesia. *Antiquity* **88**, 740–756 (2014).
29. Pawlik, A., Croxier, R., Fuentes, R., Wood, R. & Piper, P. Burial traditions in early mid-Holocene Island Southeast Asia: new evidence from Bubog-1, Ilin Island, Mindoro Occidental. *Antiquity* **93**, 901–918 (2019).
30. Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
31. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
32. Lazaridis, I. et al. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
33. Patterson, N. et al. Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
34. Skoglund, P. et al. Genomic insights into the peopling of the Southwest Pacific. *Nature* **538**, 510–513 (2016).
35. Kanzawa-Kiriyama, H. et al. A partial nuclear genome of the Jomons who lived 3000 years ago in Fukushima, Japan. *J. Hum. Genet.* **62**, 213–221 (2017).
36. Sikora, M. et al. The population history of northeastern Siberia since the Pleistocene. *Nature* **570**, 182–188 (2019).
37. Yang, M. A. et al. Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* **369**, 282–288 (2020).
38. Yang, M. A. et al. 40,000-year-old individual from Asia provides insight into early population structure in Eurasia. *Curr. Biol.* **27**, 3202–3208.e9 (2017).
39. Meyer, M. et al. A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222–226 (2012).
40. Bergström, A. et al. Insights into human genetic variation and population history from 929 diverse genomes. *Science* **367**, eaay5012 (2020).
41. Peter, B. M. 100,000 years of gene flow between Neandertals and Denisovans in the Altai mountains. Preprint at <https://doi.org/10.1101/2020.03.13.990523> (2020).
42. Pickrell, J. K. & Pritchard, J. K. Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet.* **8**, e1002967 (2012).
43. Sikora, M. et al. Ancient genomes show social and reproductive behavior of early Upper Paleolithic foragers. *Science* **358**, 659–662 (2017).
44. Aubert, M. et al. Pleistocene cave art from Sulawesi, Indonesia. *Nature* **514**, 223–227 (2014).
45. Aubert, M. et al. Earliest hunting scene in prehistoric art. *Nature* **576**, 442–445 (2019).
46. van den Bergh, G. D. et al. Earliest hominin occupation of Sulawesi, Indonesia. *Nature* **529**, 208–211 (2016).
47. Wang, C.-C. et al. Genomic insights into the formation of human populations in East Asia. *Nature* **591**, 413–419 (2021).
48. Bellwood, P. *First Islanders: Prehistory and Human Migration in Island Southeast Asia* (Wiley Blackwell, 2017).
49. Mallick, S. et al. The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**, 201–206 (2016).
50. Fu, Q. et al. The genetic history of Ice Age Europe. *Nature* **534**, 200–205 (2016).

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Methods

Archaeology

Leang Panninge was first identified as a site with high archaeological potential during a 2013 survey by A.B., B.H. and B.B. Since this time, the limestone cave has been the focus of several excavations undertaken by different teams (Extended Data Fig. 2, Supplementary Information). The first, the excavation of a 1-m² test pit (labelled TP1), was conducted by Balai Arkeologi Sulawesi Selatan (Balar Sulsel) in 2014 (ref. ²). This was followed in 2015 by three excavations (of 1-m² test pits MLP/A.1'/13, MLP/A.2'/13 and MLP/B.3'/1) spread across the cave, including one just outside the mouth, by Balai Pelestarian Cagar Budaya (BPCB) Sulawesi Selatan. The purpose of these excavations was to assess the importance of the site (the resultant report concluded that it be listed on the BPCB cultural heritage database). Later the same year, Balar Sulsel returned in collaboration with Universitas Hasanuddin (UNHAS) and Universiti Sains Malaysia to excavate a trench in the northern end of the cave (contiguous units S8T5 and S8T6) and in the central floor area (contiguous units S16T6 and S17T6). Part of a human skull in a burial context was discovered towards the end of this excavation. Owing to time and financial constraints, the burial was covered with plastic sheets and the trench backfilled to protect it for subsequent excavations. Balar Sulsel continued work further into the cave in 2016 (excavation unit S30T9). Squares S16T6 and S17T6 were reopened in 2018 to retrieve the human skeleton encountered at the base of the 2015 excavation. In 2019, this trench was extended towards the back of the cave (forming contiguous units S16T7 and S17T7) by a joint Indonesian–Australian team from Griffith University and Pusat Penelitian Arkeologi Nasional (ARKENAS), UNHAS and Balar Sulsel. The primary objectives of the 2019 fieldwork were to assess these adjacent deposits for other human skeletal remains, as well as to obtain samples of plant carbon and other materials with which to more precisely determine the age of the human burial first exposed in 2015. The 2019 investigations were conducted under a foreign research permit issued by Indonesia's State Ministry of Research and Technology (permit no.: 154/SIP/FRP/E5/Dit.KI/VII/2017). The previous, Indonesian-led investigations at Leang Panninge were carried out under the terms of formal notifications to conduct research (*Surat Pemberitahuan* or *Surat Penyampaian*) lodged with local government authorities at various levels of administration, from regency/municipality (*kabupaten*) to district (*kecamatan*) to village (*desa*).

The 2015 excavations were conducted in arbitrary 10-cm-thick spits and wet-sieved through a 3-mm mesh, to a depth of approximately 190 cm, at which point the human skeletal remains were encountered in the southwestern corner of the excavation (spits 19 and 20, layer 4). In 2019, deposits were excavated using the same method, only this time in 5-cm spits; consequently, spit names in S16T7 or S17T7 originate from a depth half that of a spit with the same number in S16T6 or S17T6 (for example, spit 18 is 170–180 cm in the first case or 85–90 cm in the latter; see Extended Data Fig. 3). As noted, the skeleton was recovered from the site in 2018. Owing to the fragility of the skeletal remains, visible elements comprising the skull and pelvic areas were first consolidated with a hardening solution (Paraloid B72 acryl resin) and then removed from the deposit en bloc (Extended Data Fig. 4e). The 'skull block' and 'pelvic block' were both submitted to computer tomography (CT) at a hospital facility in Makassar, Indonesia (Balai Pengamanan Fasilitas Kesehatan Makassar), using the following CT parameters: collimation: 0.625 mm; pitch: 1/0.625; milliamperes and kilovolts: left alone; kernel: bone; retro reconstruction: 0.3-mm interslice. After CT scanning, the two sediment blocks were excavated under laboratory conditions to remove the skeletal remains. The sediment block containing the skull consisted of an intact portion of the original grave fill located immediately adjacent to and below the cranium, mandible and dental elements. The thickest part of this sediment block measured approximately 100 mm. During the 'skull block' excavation, we recovered the right petrous portion of the human temporal bone and thereafter submitted it for DNA analysis

at the Max Planck Institute for the Science of Human History (MPI-SHH) in Jena, Germany. We also recovered stone artefacts and faunal remains, as well as a burnt *Canarium* sp. seed located a few centimetres from the main cluster of cranial bones (Supplementary Information). This seed yielded an accelerator mass spectrometry (AMS) ¹⁴C age of 7264–7165 yr cal BP (Wk-48639) (Supplementary Table 1).

Morphological documentation

The Leang Panninge human remains (Supplementary Table 2) are stored at the Archaeology Laboratory of the Archaeology Department (Departemen Arkeologi Fakultas Ilmu Budaya) at UNHAS, Makassar, South Sulawesi, Indonesia. In 2019, D.B. reconstructed and described the human remains under the stewardship of M.N. and I.S. Joins were effected using Tarzan's Grip along with plasticine for missing bone. Skeletal weights were taken with a scale accurate to 1 g. Measurements were taken with a Kincrome electronic calliper accurate to 0.01 mm (generally rounded off to the closest tenth of a millimetre). Teeth were measured for their maximum mesiodistal and buccolingual diameters and also these diameters at the cemento-enamel junction. The dental morphological features recorded were those of the Arizona State University system⁵¹, including reference to standard plaques illustrated in that work, and in ref.⁵² for photographs of some other standard plaques. Other sources for recording measurements and anatomical characteristics are described in Supplementary Information.

Ancient DNA processing

Sampling, extraction, library preparation and indexing were performed in a dedicated clean room for ancient DNA at the MPI-SHH. We obtained bone powder from the right pars petrosa of the Leang Panninge individual by cutting along the margo superior and drilling near the cochlea⁵³. DNA was extracted using a modified version of the ancient DNA protocol described in ref.⁵⁴. From the extract, we built a double-stranded library after partial uracil-DNA glycosylase treatment⁵⁵ to reduce C>T transitions to the first two base pairs and a single-stranded library on an automated liquid handling system⁵⁶. After double indexing with unique index combinations⁵⁷, the libraries were shotgun-sequenced for a depth of approximately 4 million reads on an Illumina HiSeq 4000 at MPI-SHH using a 75-bp single-read configuration for initial quality assessments.

After further amplification, the libraries were hybridized in-solution to enrich for the complete mitogenome (mtDNA capture³¹) and twice for a targeted set of 2,986,592 SNPs across the human genome (two rounds of '1240K' and 'archaic ancestry'³⁰ captures). The capture products were then sequenced on an Illumina HiSeq 4000 at MPI-SHH using a 75-bp single-read configuration. After Adapter Removal as implemented in EAGER v.1.92.56⁵⁸, the mtDNA-enriched reads were aligned to the mitochondrial reference genome (rCRS) and the reads from the genome-wide captures to the human reference genome (hg19) using a mapping quality filter of 30 for the circularmapper v.1.93.5 and BWA⁵⁹ aligner, respectively. Duplicates were removed with DeDup v.0.12.2 (<https://github.com/apeltzer/DeDup>). Contamination of the single-stranded sequences was assessed with AuthenticCT v.1.0⁶⁰.

We reconstructed the mitochondrial consensus sequence and estimated mitochondrial contamination to $2 \pm 1\%$ using schmutzi⁶¹. The mitochondrial haplogroup was ascertained with Haplofind⁶². After merging with published data using mafft v.7.305⁶³, we constructed a maximum parsimony tree in MEGA X⁶⁴. On the basis of the misincorporation pattern determined by mapDamage v.2.0.9 as implemented in EAGER v.1.92.56⁵⁸, we trimmed 2 bp off the 1240K-captured double-stranded library data and genotyped the trimmed and untrimmed sequences individually for the 1240K panel using samtools v.1.3 (<https://github.com/samtools/samtools>) and pileupCaller v.1.4.0.2 (<https://github.com/stschiff/sequenceTools>), which randomly calls one allele per SNP site. The untrimmed and trimmed genotypes were then combined, retaining only transversions from the untrimmed genotype

and transitions from the trimmed genotype to maximize information from the trimmed ends. The single-stranded library data were instead genotyped using the single-stranded mode of pileupCaller and the two genotypes merged using a custom script. The resulting coverage was suitable for population genetics analyses with 263,207 SNPs on the 1240K and 135,432 SNPs on the Human Origins panel (HO). We also genotyped single-stranded and double-stranded data individually after filtering with PMDtools v.0.6⁶⁵.

Population genetic analyses

PCAs were performed using smartpca with shrinkmode and lsqmode enabled⁶⁶, calculating the principal components from present-day East and southeast Asian and Oceanian individuals genotyped on the Human Origins panel^{18,32–34} and projecting all ancient genomes.

All f_3 -statistics and f_4 -statistics were calculated using qp3pop v.420 (inbreed: YES) and qpDstat v.721, respectively³³. For f_3 -statistics, we used East and southeast Asian and Oceanian groups from the Human Origins dataset to include more comparative populations, whereas for f_4 -statistics, we used a more restricted dataset containing data from the Simons Genome Diversity Project (SGDP⁴⁹) genotyped on the 1240K panel to maximize the number of overlapping SNPs with the Leang Panninge individual. The results of f_3 -statistics were plotted in the geographical location of the test group using ggplot2 v.3.3.3 in RStudio v.1.2.1335. To investigate the proportion of Denisovan-related ancestry (α), we calculated f_4 -ratio statistics using qpF4Ratio^{18,20,33}, admixfrog⁴¹ and D -statistics³³ with a custom script. Using qpWave³³, we investigated whether we could distinguish between the Papuan-like ancestry present in the Leang Panninge individual compared with present-day Papuan individuals. Admixture proportions were estimated with qpAdm (all-nps: YES)³³. After file conversion with PLINK v.1.9⁶⁷, we ran TreeMix v.1.12⁴² setting the Denisovan genome³⁹ as the root and utilizing the parameters -k150 and -global. Models were plotted using RColorBrewer v.1.1.2 in RStudio v.1.2.1335 and the fit was assessed by residual inspection after each additional migration edge was added. Admixture graphs with qpGraph³³ were constructed (outpop: NULL, useallnps: YES, blsize: 0.05, forcezmode: YES, lsqmode: YES, diag: 0.0001, bigiter: 15, hires: YES, lambdascale: 1, initmix: 1,000, inbreed: YES) by adding one group after the other, moving from archaic humans over present-day groups to ancient samples and testing all possible one-way and two-way mixtures using a custom script. The decision on which model was chosen to progress with the addition of another group was made based on the lowest worst z-score calculated for each proposed tree. Admixture time estimation was calculated with DATES v.753⁶⁸.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The raw and aligned sequences are available at the European Nucleotide Archive under the accession number PRJEB43715.

Code availability

The custom scripts used in this study are available on request from the corresponding authors.

51. Scott, G. R. & Turner, C. G. II. *The Anthropology of Modern Human Teeth: Dental Morphology and its Variation in Recent Human Populations* (Cambridge Univ. Press, 1997).
52. Hillson, S. *Dental Anthropology* (Cambridge Univ. Press, 1996).
53. Pinhasi, R. et al. Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS ONE* **10**, e0129102 (2015).

54. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* **110**, 15758–15763 (2013).
55. Rohland, N., Harney, E., Mallick, S., Nordenfellt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Phil. Trans. R. Soc. Lond. B* **370**, 20130624 (2015).
56. Gansauge, M.-T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**, 2279–2300 (2020).
57. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
58. Peltzer, A. et al. EAGER: efficient ancient genome reconstruction. *Genome Biol.* **17**, 60 (2016).
59. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
60. Peyrégne, S. & Peter, B. M. AuthenticCT: a model of ancient DNA damage to estimate the proportion of present-day DNA contamination. *Genome Biol.* **21**, 246 (2020).
61. Renaud, G., Slon, V., Duggan, A. T. & Kelso, J. Schmutzi: estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA. *Genome Biol.* **16**, 224 (2015).
62. Vianello, D. et al. HAPLOFIND: a new method for high-throughput mtDNA haplogroup assignment. *Hum. Mutat.* **34**, 1189–1194 (2013).
63. Katoh, K., Misawa, K., Kuma, K. & Miyata, T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059–3066 (2002).
64. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 (2018).
65. Skoglund, P. et al. Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. *Proc. Natl Acad. Sci. USA* **111**, 2229–2234 (2014).
66. Patterson, N., Richter, D. J., Gnerre, S., Lander, E. S. & Reich, D. Genetic evidence for complex speciation of humans and chimpanzees. *Nature* **441**, 1103–1108 (2006).
67. Chang, C. C. et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, 7 (2015).
68. Narasimhan, V. M. et al. The formation of human populations in South and Central Asia. *Science* **365**, eaat7487 (2019).

Acknowledgements The 2019 research at Leang Panninge was funded by an Australian Research Council Future Fellowship awarded to A.B. (FT160100119), along with financial support from Griffith University. The research was authorized by the State Ministry of Research and Technology (RISTEK) and was conducted in collaboration with Pusat Penelitian Arkeologi Nasional (ARKENAS), Jakarta, Indonesia. Other key participating organizations in Indonesia include Departemen Arkeologi Fakultas Ilmu Budaya, Universitas Hasanuddin (UNHAS, Makassar), and Balai Arkeologi Sulawesi Selatan (Makassar). The following UNHAS archaeology students participated in the fieldwork: F. N. Shalawat, A. A. Qalam, K. M. Prayoga, M. Surra, M. N. Taufik and M. A. Oka. Local field assistants included Irwan, Amar and Hardin. The team assisting D.B.'s morphological analysis of the human skeletal remains included Fahkri (Balai Arkeologi Sulawesi Selatan), Cika and M. Hafdal. A. Crowther assisted with palaeobotanical identifications, and R. Wood advised on radiocarbon calibration. The 2015 excavation was undertaken by UNHAS in collaboration with the Centre for Global Archaeological Research, Universiti Sains Malaysia. We thank the computational and laboratory team at the Max Planck Institute for the Science of Human History (Jena), in particular C. Freund, R. Radzeviciute and A. Wissgott. Aspects of this research were also financially supported by S. Chia's Universiti Sains Malaysia Research University Grant (1001/ PARKEO/870002), the ERC Starting Grant 'Waves' (ERC758967) and the Max Planck Society. The digital elevation models in Fig. 1a, b were created in ArcGIS (QGIS) from Shuttle Radar Topography Mission (SRTM 1) Arc-Second Global data courtesy of the US Geological Survey.

Author contributions A.B., J.K., C.P. and A.D. conceived and led the study. The 2015 excavation was led by A.D. in collaboration with S. Chia and involved key contributions from M.N., I.S., Supriadi and D.A.T.P. A.B. directed the 2019 excavation, additional analysis and conceptualization in collaboration with A.A.O., M.R., B.H. and the senior Universitas Hasanuddin team (A.D., M.N. and I.S.). F.A.S. supervised the 2019 fieldwork activities with B.B., who recovered the skeletal remains from the site in 2018 with A.M.S. D.P.M. conducted the laboratory excavation of the human skeletal remains and provided key in-field and post-excavation observations and analyses. D.B. described and analysed the human skeletal remains. Y.L.P. and Suryatman analysed the stone artefacts. K. Newman conducted key post-excavation analyses, data processing and figure preparation with B.B. Hasanuddin conducted key early excavations at the site. S. Carlhoff generated genomic data. S. Carlhoff, L.S., B.M.P. and K.P. performed genetic analyses with critical input from C.P., K. Nägele, C.J. and J.K. S. Carlhoff, K. Nägele, C.P. and A.B. wrote the manuscript with critical input from J.K. and the remaining authors.

Funding Open access funding provided by Max Planck Society.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-021-03823-6>.

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Peer review information Nature thanks Richard Roberts, Martin Sikora and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

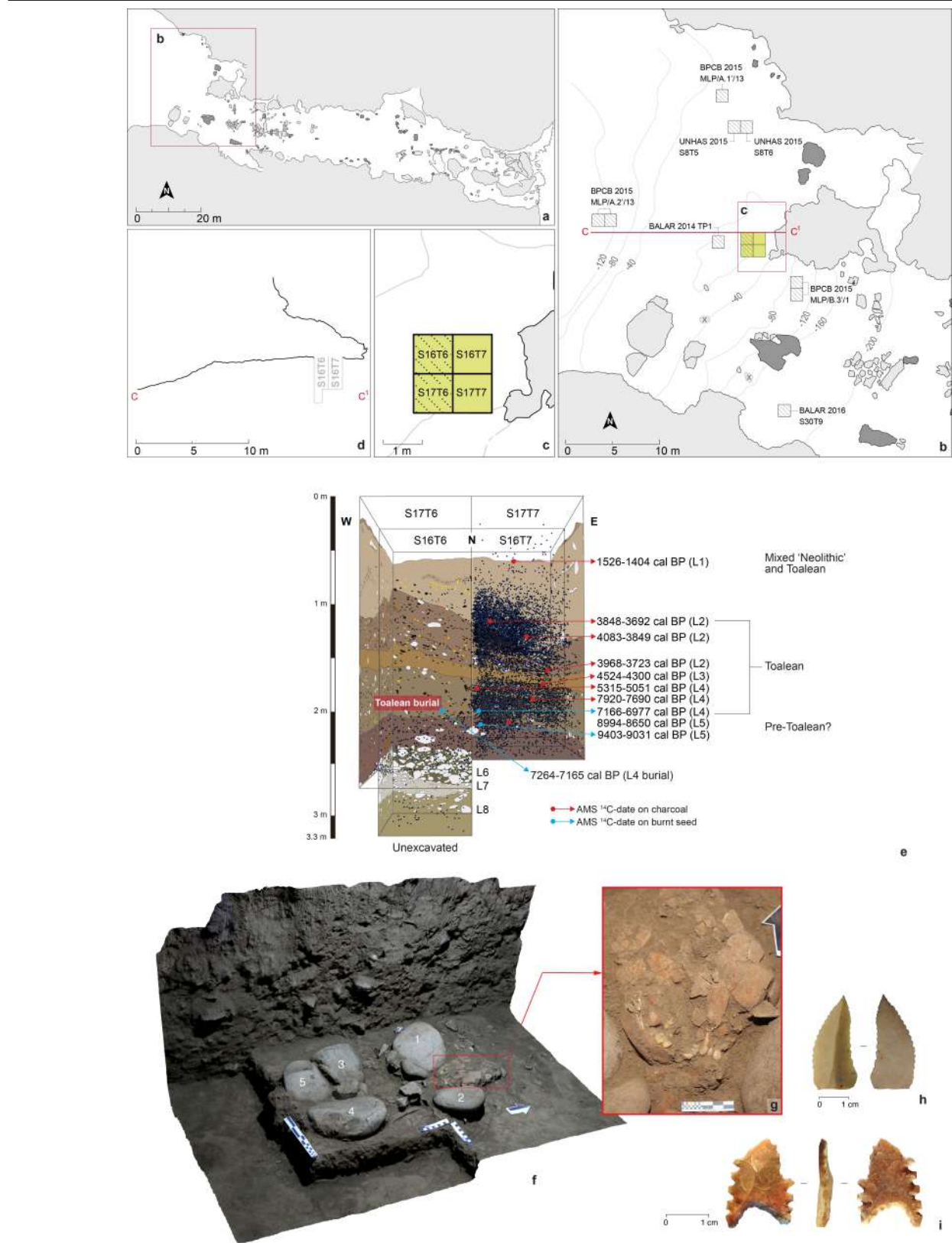
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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Toalean artefact types and excavations at Leang Panninge. a, b, Typical middle-to-late Holocene artefact types from Toalean assemblages in South Sulawesi; pressure-flaked stone 'Maros points' (both artefacts are from Leang Pajae, Maros) (**a**) and backed microliths (top: Leang Pajae; bottom: Leang Bulu Bettue, Maros) (**b**). **c,** Osseous points (left: from the layer 4 Toalean human burial, Leang Panninge; right: Leang Rakkoe, Maros). **d–i,** Excavation at Leang Panninge in 2019. Excavation of a trench placed adjacent to the rear wall in the west-facing cave entrance (**d, e**): viewed from northwest to southeast (note that the name of the cave site is incorrectly

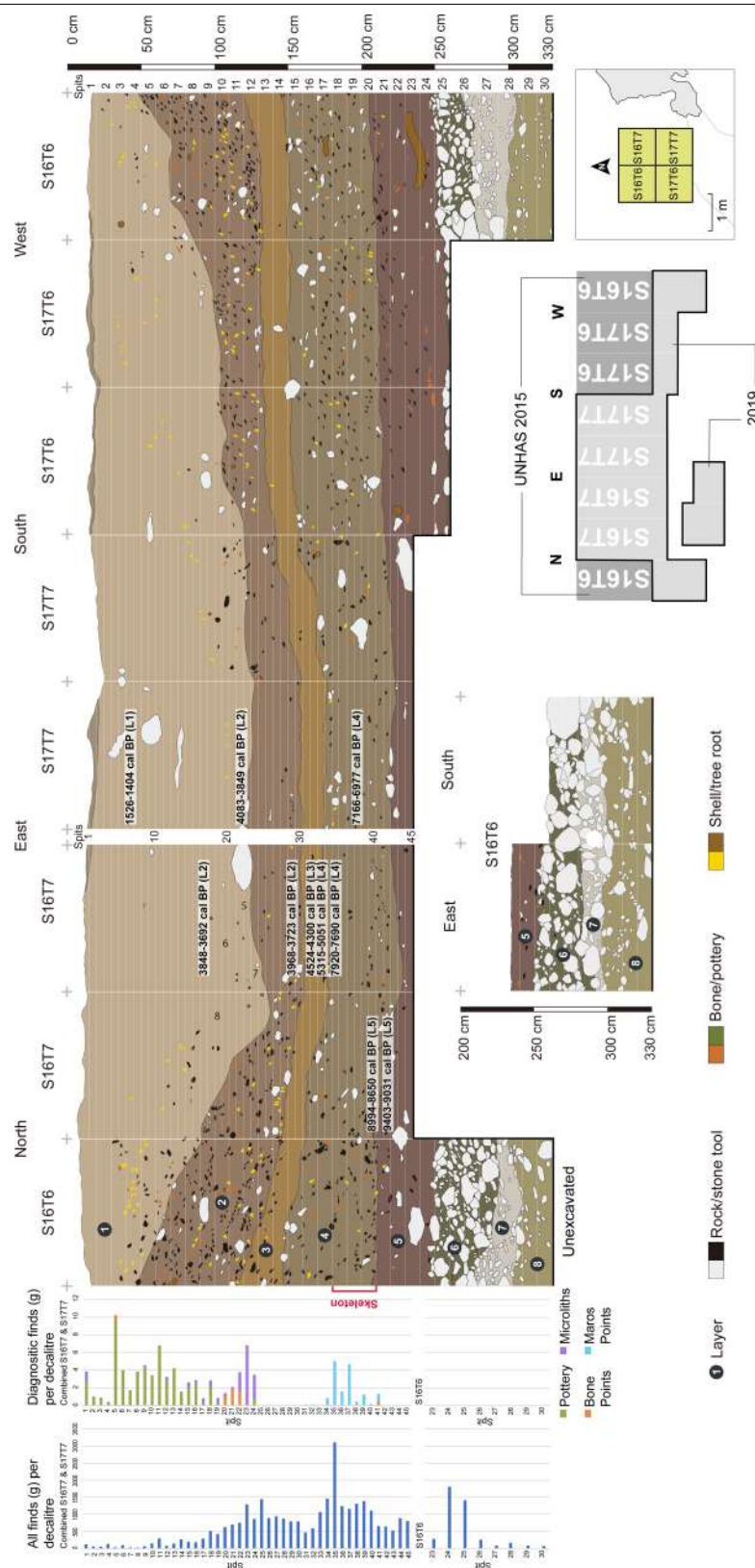
transcribed in this locally produced banner) (**d**); viewed from north to south (**e**); northwest to southeast (**f**); detail of the excavation work (**g**). Excavated trench faces at the end of the 2019 field season, following soil column-sampling (**h, i**): trench viewed from northeast to southwest, showing the intersection between the western walls of excavation squares S16T6 and S17T6 and southern walls of S17T6 and S17T7 (**h**); trench viewed from north to south, with the main stratigraphic section facing the viewer showing the northern walls of S16T6 and S16T7 (**i**). Image credits: Y. Perston (**a–c**); Leang Panninge research team (**d–i**).



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Site plan, stratigraphy and dating evidence at Leang Panninge. a–c, Plan views of the cave site showing the locations of previous excavation units and squares S16T6 and S17T6, where the Toalean burial was exposed in 2015. Excavations in the west-facing entrance (which is 10-m high and 28-m wide) yielded dense Toalean archaeological assemblages. Light grey, limestone bedrock; dark grey, speleothem column/pillar; grey with x, stalagmite. Contour levels are in centimetres. Acronyms and initialisms denoting Indonesian archaeological institutions and heritage departments are as follows: BALAR, Balai Arkeologi Sulawesi Selatan; BPCB, Balai Pelestarian Cagar Budaya Makassar; UNHAS, Universitas Hasanuddin. The 2015 trench was extended in 2019, forming squares S16T7 and S17T7. **d,** Section of the cave mouth. **e,** Stratigraphy and dating. L1, layer 1; L2, layer 2, and so on. Black dots denote the 3D-plotted positions of stone artefacts, faunal items (bone, teeth and shell), palaeobotanical remains, shells and other findings excavated in 2019. Eight distinct archaeological layers of human occupation are evident: the uppermost cultural stratum, layer 1 (approximately 1.5 kyr cal BP), contains ‘Neolithic’ pottery and Toalean backed microliths; layers 2–4 comprise aceramic Toalean occupation deposits. Maros points pre-date backed microliths and appear in the earliest Toalean level (see also ref.²⁷); layer 5

(approximately 9.4–8.7 kyr cal BP) and underlying strata (layers 6–8) are pre-Toalean. The deepest Toalean horizon, layer 4, a silty clay (7.9–5.1 kyr cal BP), contained the region’s only definite Toalean-associated burial. The burial was revealed in 2015 within the contiguous squares S16T6 and S17T6. In 2019, a 2 × 1 m trench excavated adjacent to these squares exposed undisturbed strata to a depth of 3 m without reaching bedrock (Extended Data Figs. 2, 3). Calibrated AMS ¹⁴C ages (95% probability) are shown on the right (Supplementary Information contains details on dating methods). Dated materials comprised samples of wood charcoal (*N* = 8) and charred *Canarium* sp. seeds (*N* = 3). The age inversion in layer 4 suggests a degree of post-depositional mixing that does not affect the inferred burial age. **f,** Toalean burial from layer 4 (3D image generated using PhotoScan). The individual was interred in a flexed position and covered with large cobbles (denoted 1–5). **g,** Fragmented cranium. **h, i,** Maros points recovered with the human remains. An atypical chert Maros point (**h**) was found just below the cranium. A classic chert Maros point (**i**) with a hollow base and serrated margins (tip is missing due to breakage) was found approximately 40 mm below the greater sciatic notch. Image credits: Leang Panninge research team (**a–g**); Y. Perston (**h, i**).



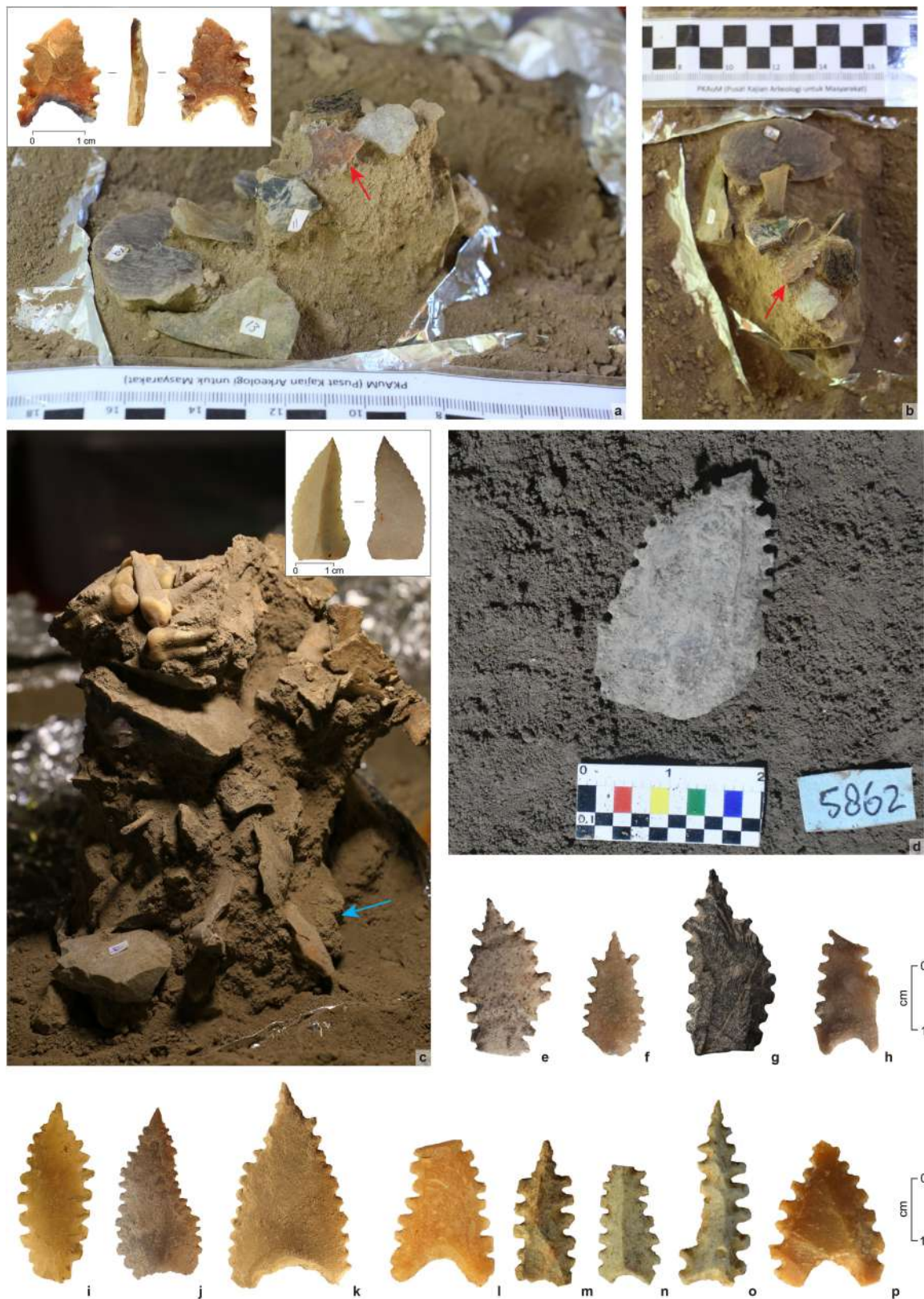
Extended Data Fig. 3 | Stratigraphic section at Leang Panninge. Data distributions presented in the tables on the left, and calibrated AMS radiocarbon (^{14}C) dates (shown projected onto the stratigraphic profile), are based on the results of the 2019 excavations. Calibrated AMS ^{14}C ages are

reported at 95% probability (Supplementary Information contains details on dating methods). The Toalean human skeleton was exposed in 2015 by a Universitas Hasanuddin (UNHAS) team in collaboration with Balar Sulsel and Universiti Sains Malaysia (see Supplementary Information).



Extended Data Fig. 4 | Toalean human burial from Leang Panninge. **a**, Single adult inhumation. The 17–18-year-old female was buried in a flexed position and partially covered with several large cobbles. **b**, Detail of the fragment clusters comprising the skull area. The skeleton is fragmentary (Supplementary Table 2), but the skull, although crushed post-mortem, is well represented by the mandible and mandibular dentition, maxilla and maxillary dentition, frontal bone, temporals, right parietal and occipital bone. The cranium was

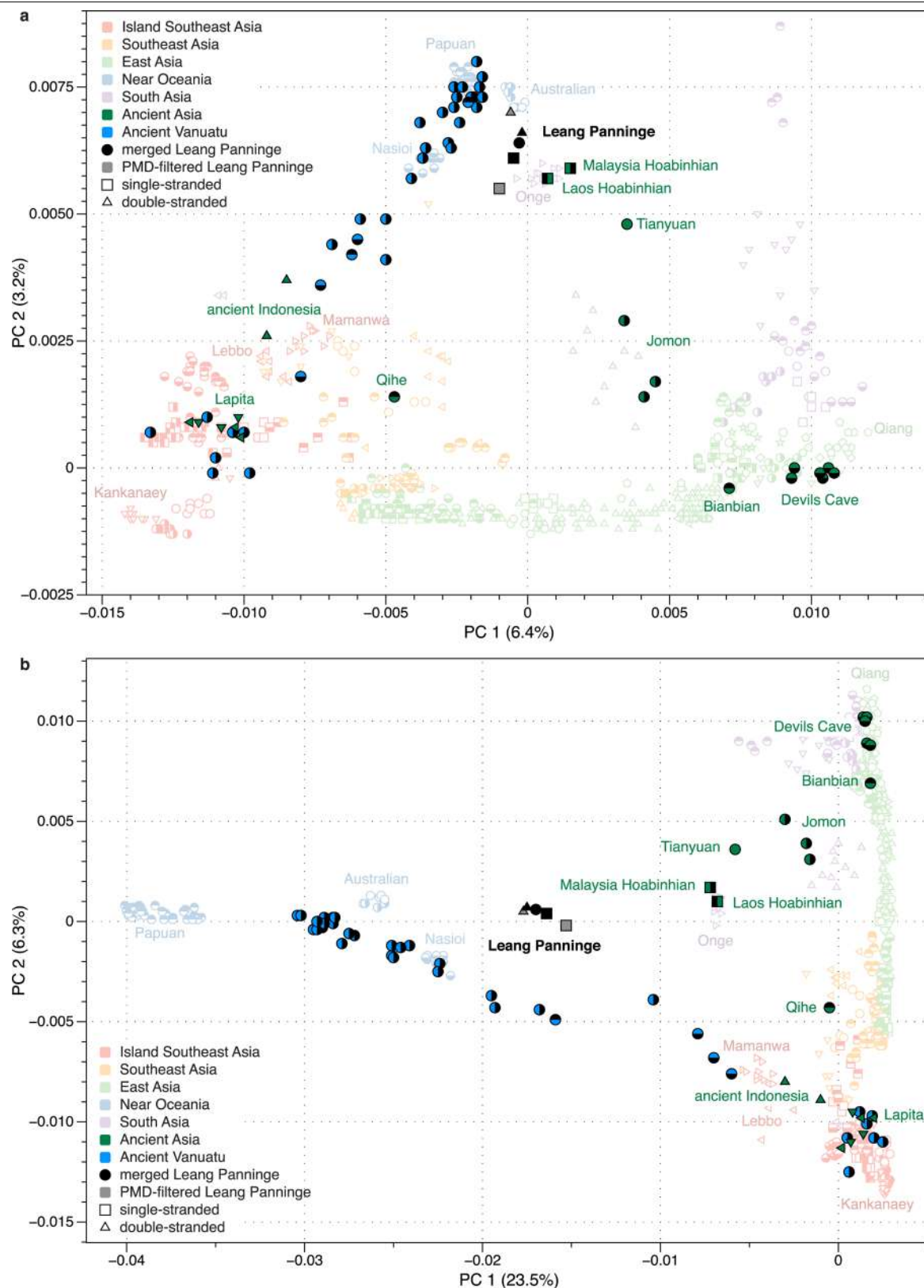
positioned between two large cobbles (denoted 1 and 2). **c**, Detail of the burial feature; cobble #1 was placed over one of the hands, cobble #4 was positioned over the left foot, and cobble #5 covered the pelvic region. **d**, Semi-complete pelvis and left foot (plantar surface facing up). **e**, Sediment block containing the cranium, following removal from the archaeological trench and before excavation in the laboratory. Image credits: Leang Panninge research team (**a–g**).



Extended Data Fig. 5 | See next page for caption.

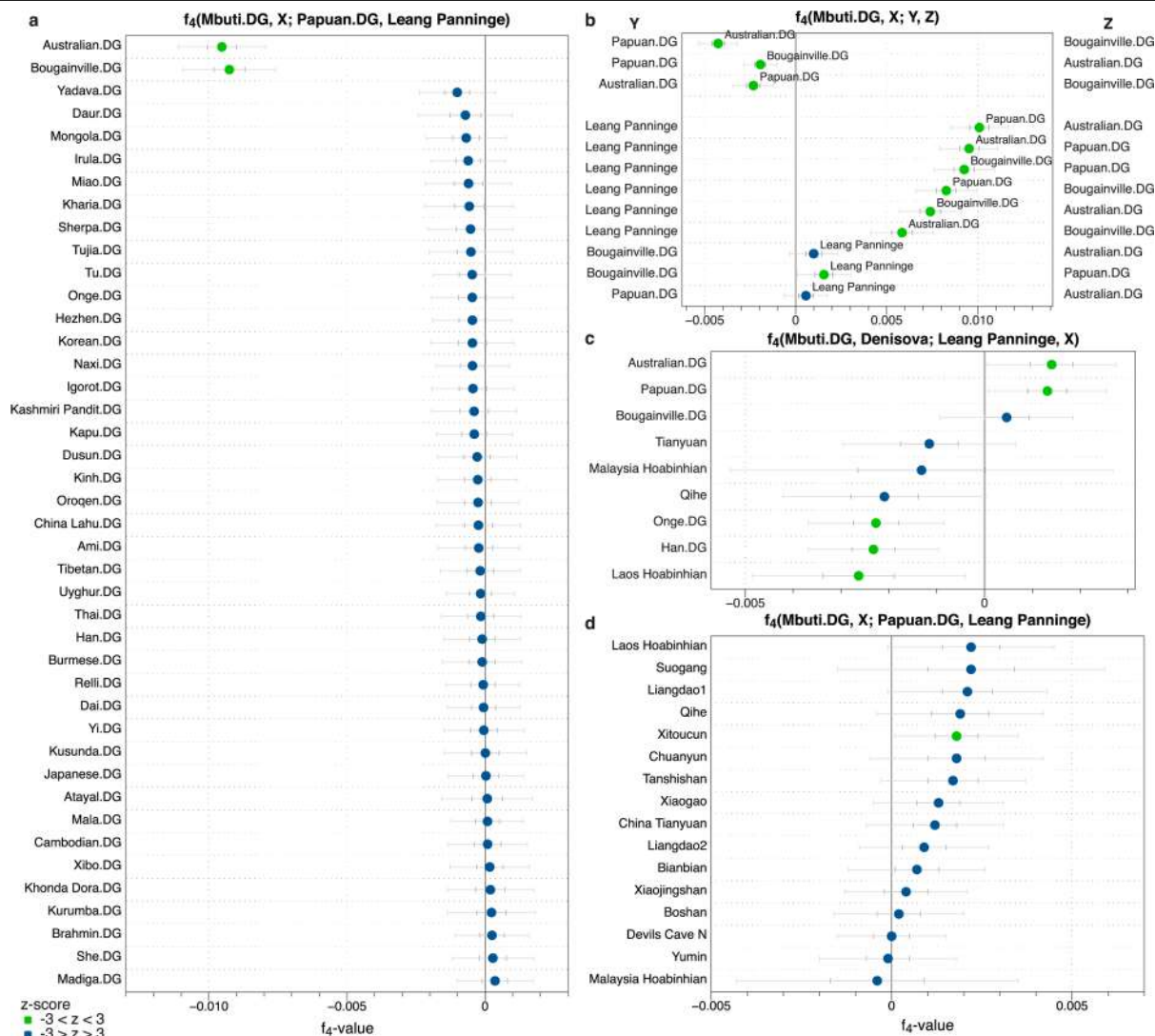
Extended Data Fig. 5 | Toalean stone tool types from the Toalean burial and associated contexts at Leang Panninge. a, b, Position of the chert Maros point (shown inset in **a**) found below the right pelvis during excavation of the sediment block containing the semi-complete pelves. The red arrows in **a** and **b** indicate the position of the Maros point amidst stone-flaking debris and other cultural artefacts (at the time these photographs were taken, the bones of the pelvic area had already been removed). **c,** Position of the non-classic chert Maros point (shown inset, original location highlighted by the blue arrow) found below the skull amidst other stone artefacts and cultural remains

(human teeth are still visible at the top of the sediment block under excavation). **d,** Maros point exposed during the excavation. **e–h,** Examples of Maros points from layer 4, above the level of the Toalean human burial: square S16T6, spit 18 (Find #62) (**e**); square S16T6, spit 18 (Find #77) (**f**); square S17T6, spit 18 (Find #54) (**g**); square S17T6, spit 16 (Find #95) (**h**). **i–k,** Examples of Maros points from spits 19 and 20 within squares S16T6 and S17T6, where the Toalean human burial was located. **l–p,** Examples of Maros points from other excavated squares at Leang Panninge. Image credits: Leang Panninge research team (**a–p**).



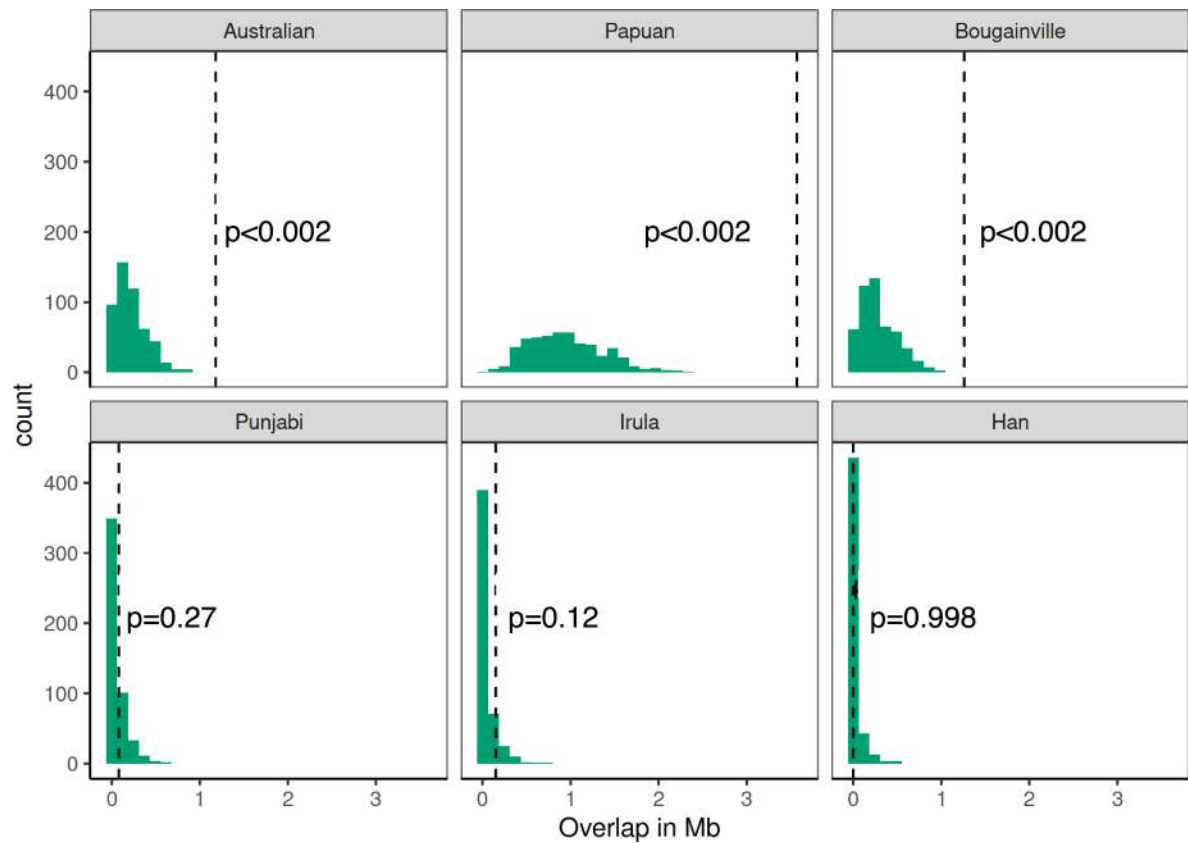
Extended Data Fig. 6 | Principal component analyses. Calculated on present-day groups^{18,32,33}, projecting all ancient genomes^{1,34–38} including either all available SNPs or only damaged DNA fragments after PMD filtering of the single-stranded and double-stranded libraries of the Leang Panninge

individual. **a**, Principal components calculated on individuals from East and southeast Asia where Papuan, Indigenous Australian and Nasioi (from Bougainville) groups are projected. **b**, Principal components calculated on individuals from East, southeast Asia and Near Oceania.



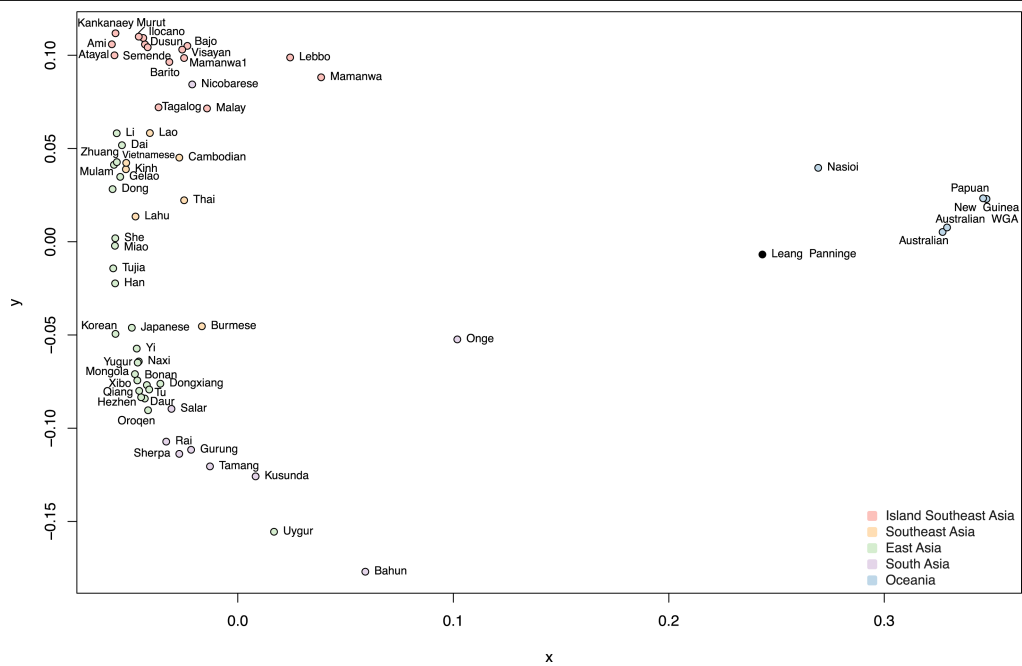
Extended Data Fig. 7 | The position of the Leang Panninge genome within the regional population genetic history. Tested with f_4 -statistics, where X denotes the tested group. **a**, The affinity of East Asian, South Asian, southeast Asian and Oceanian groups⁴⁹ to Leang Panninge and Papuan individuals. **b**, Affinities between present-day Near Oceanian groups and the Leang Panninge genome⁴⁹. **c**, The affinity of the Leang Panninge genome to the

Denisovan genome compared to present-day and ancient individuals from the region^{26,36-38}. **d**, The affinity of the Leang Panninge genome to ancient genomes from the region^{26,36-38} in comparison to Papuan groups⁴⁹. Data are presented as exact f_4 -values $\pm$ one and three standard errors indicated as dark and light grey lines, respectively; statistics with z-scores above |3| are displayed in green.

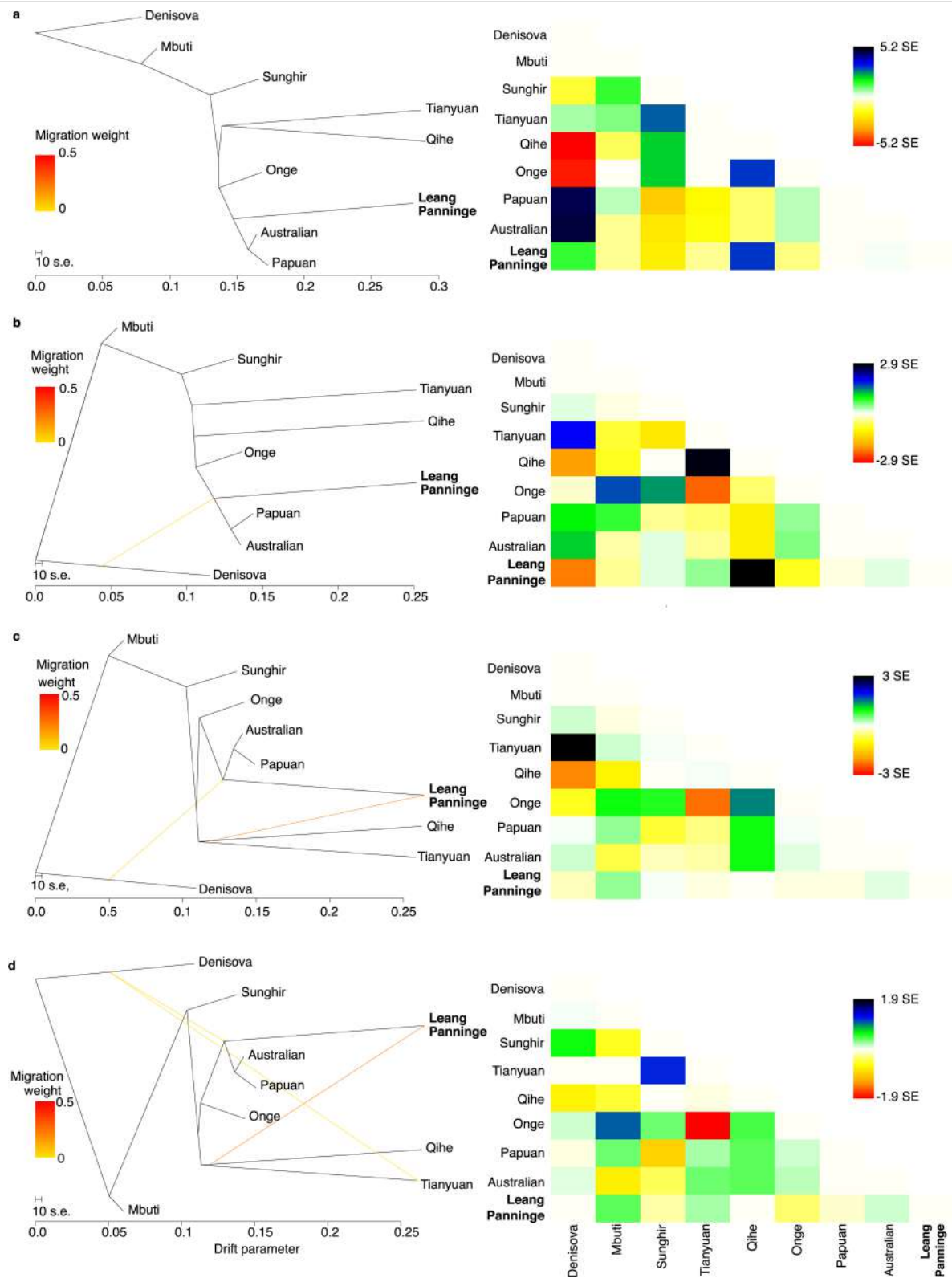


Extended Data Fig. 8 | Permutation test on the overlap of introgressed Denisovan sequence in the Leang Panninge individual and present-day groups. After 500 permutations where the Denisovan sequence is distributed randomly across the genome. The dotted vertical line is the observed overlap

in the non-permuted data. The empirical p-value indicates how many permutations show more overlap than the observed overlap in the non-permuted data.



Extended Data Fig. 9 | Multidimensional scaling plot. Visualizing the pairwise difference between Leang Panninge and East and southeast Asian and Near Oceanian groups^{18,32-34} as calculated from $1 - f_3$ (Mbuti; Leang Panninge, X) on the Human Origins dataset.



Extended Data Fig. 10 | The genetic relationship of Leang Panninge with other Asia-Pacific groups. Data for other Asia-Pacific groups from refs. ^{37–39,43,49}. **a–d**, Maximum likelihood trees after incrementally adding one

migration event (from zero to four) as inferred by TreeMix⁴²; corresponding residuals of individual models are presented on the right (Supplementary Fig. 6).

3.2 Manuscript B: Ancient genomes from the last three millennia support multiple human dispersals into Wallacea

Sandra Oliveira*, Kathrin Nägele*, **Selina Carlhoff***, Irina Pugach, Toetik Koesbardiati, Alexander Hübner, Matthias Meyer, Adhi Agus Oktaviana, Masami Takenaka, Chiaki Katagiri, Delta Bayu Murti, Rizky Sugianto Putri, Mahirta, Fiona Petchey, Thomas Higham, Charles F. W. Higham, Sue O'Connor, Stuart Hawkins, Rebecca Kinaston, Peter Bellwood, Rintaro Ono, Adam Powell, Johannes Krause, Cosimo Posth & Mark Stoneking (2022) *Nature Ecology & Evolution* **6**, 1024-1034.

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The present-day linguistic and genetic diversity of Wallacea has been shaped by the interactions of Papuan-related hunter-gatherers and Austronesian-related farmers arriving around 3.5 ka, as well as later trading networks during the Metal Age. In this study, we analyse genome-wide data from 8 archaeological sites in northern, central, and southern Wallacea dating between 2,600 and 250 years ago to better understand the timing of past demographic processes. We identify two different ancestry profiles, where individuals from the Northern Moluccas show both Austronesian- and Papuan-related ancestry, similar to groups from the Pacific, and those from Sulawesi and the southern Indonesian islands harbour an additional Mainland Southeast Asian-related component. In our admixture models, this ancestry is best represented by present-day Austroasiatic speakers and ancient individuals from Vietnam, Laos, and Thailand. In contrast, nowadays these three ancestry components are found in all regions of Wallacea in different proportions compared to the ancient individuals, indicating additional demographic changes over time. Estimating the admixture dates, we also observe several pulses of admixture or continuous gene flow. While the oldest individuals give similar dates around 3 ka ago, approaching the first appearance of Austronesian material culture in the region, younger individuals and present-day groups show more recent times. The identification of Papuan- and not Leang Panninge-related ancestry in these Metal Age individuals from Wallacea might also indicate re-introduction of Papuan-related ancestry after initial settlement. Our findings suggest a long history of genetic exchange in Wallacea with complex spatio-temporal patterns.



OPEN

Ancient genomes from the last three millennia support multiple human dispersals into Wallacea

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Previous research indicates that human genetic diversity in Wallacea— islands in present-day Eastern Indonesia and Timor-Leste that were never part of the Sunda or Sahul continental shelves— has been shaped by complex interactions between migrating Austronesian farmers and indigenous hunter-gatherer communities. Yet, inferences based on present-day groups proved insufficient to disentangle this region's demographic movements and admixture timings. Here, we investigate the spatio-temporal patterns of variation in Wallacea based on genome-wide data from 16 ancient individuals (2600–250 years BP) from the North Moluccas, Sulawesi and East Nusa Tenggara. While ancestry in the northern islands primarily reflects contact between Austronesian- and Papuan-related groups, ancestry in the southern islands reveals additional contributions from Mainland Southeast Asia that seem to predate the arrival of Austronesians. Admixture time estimates further support multiple and/or continuous admixture involving Papuan- and Asian-related groups throughout Wallacea. Our results clarify previously debated times of admixture and suggest that the Neolithic dispersals into Island Southeast Asia are associated with the spread of multiple genetic ancestries.

Wallacea (Fig. 1), a region of deep-sea islands located between the Sunda and Sahul continental shelves¹, has been both a bridge and a barrier for humans migrating from Asia to Oceania. Anatomically modern humans (AMHs) presumably first crossed Wallacea before reaching Sahul, for which the earliest unequivocal dates are approximately 47 ka^{2–5} (but see Clarkson et al.⁶). In Wallacea itself, the archaeological record indicates occupation by AMHs starting around 46 ka in the southern islands^{7–9}, 45.5 ka in Sulawesi¹⁰ and 36 ka in the northern islands (North Moluccas)¹¹. After a long period of isolation, the region was impacted by the Austronesian expansion. Equipped with new sailing and farming technologies, Austronesian-speaking groups likely

expanded out of Taiwan 4,000–4,500 ya^{12–14} and eventually settled in Island Southeast Asia (ISEA), Oceania and Madagascar. Their arrival is generally linked to the earliest appearance of pottery, which dates to approximately 3,500 ya in Wallacea^{11,15–18}. During the Late Neolithic and early Metal Age (2,300–2,000 ya), the maritime trade network intensified, with a movement of spices, bronze drums and glass beads connecting Wallacea to India and mainland SEA (MSEA)^{11,17,19–24}.

The contact between Austronesian-speaking farmers and hunter-gatherer communities is still reflected in the linguistic and biological diversity of Wallacea today. Austronesian languages of the Malayo-Polynesian subgroup are widespread throughout the

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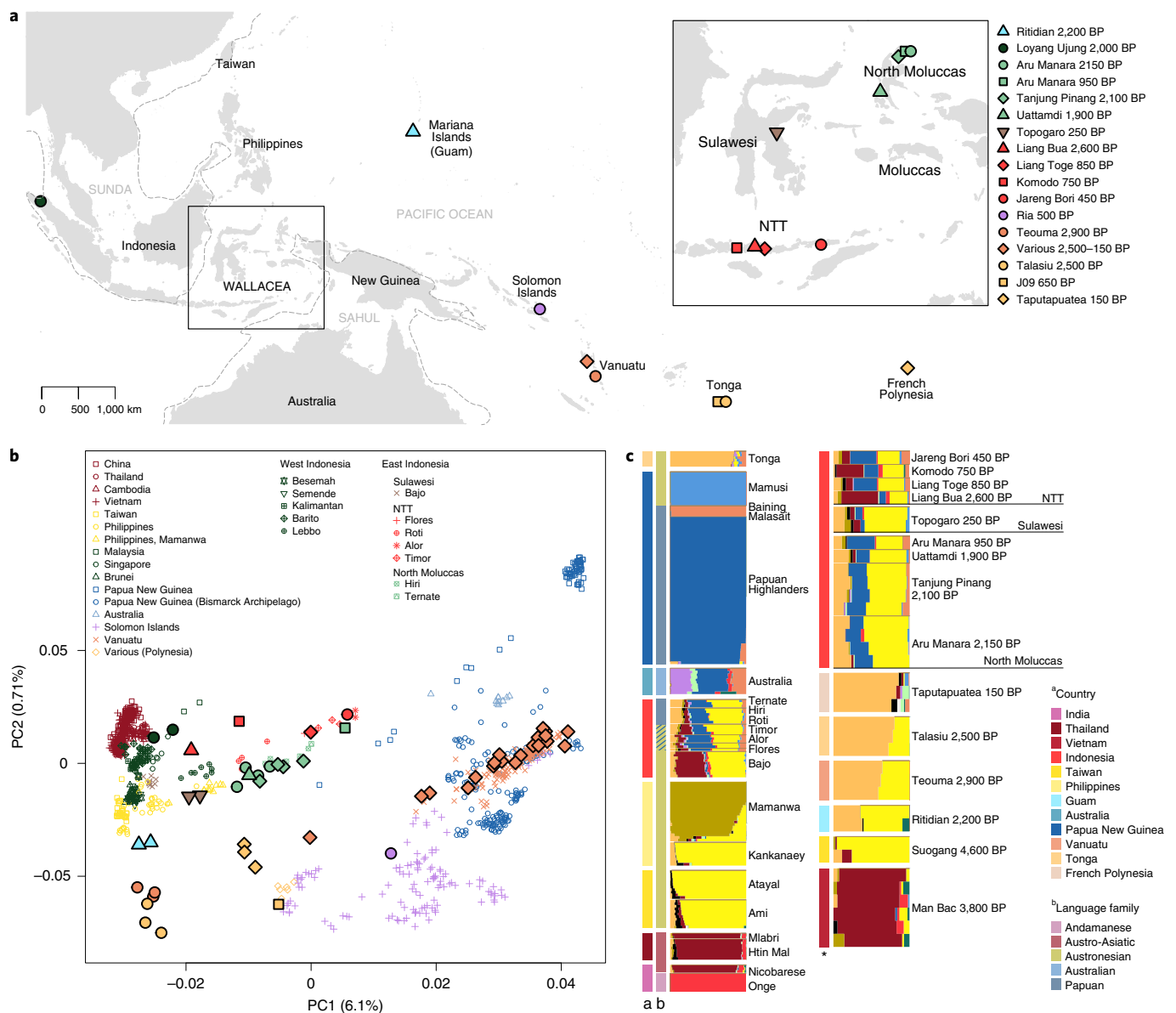


Fig. 1 | Sample provenance and the results of principal component and DyStruct analyses. a, Map showing the location of ancient individuals. **b**, PCA of publicly available whole-genome data merged with the Affymetrix Human Origins and Affymetrix 6.0 genotype data (dataset 1). Ancient individuals (shown with a black contour) are projected and their fill colour matches the colour of present-day individuals from the same geographical area. **c**, DyStruct results for dataset 1 displaying only a subset of the individuals included in the full analysis (Supplementary Fig. 1e). Newly generated individuals are highlighted in bold in the legend. Country and language information are displayed as colour bars to the left of the inferred ancestry components.

region²⁵ but a few dozen non-Austronesian (that is, Papuan) languages are also spoken in the North Moluccas, Timor, Alor and Pantar²⁶; some Austronesian languages show features acquired from Papuan languages²⁷.

The genomic composition of present-day Wallaceans shows signals of admixture between Papuan- and Asian-related ancestry most similar to that of present-day Austronesians^{28–30}. This dual ancestry is geographically distributed as a gradient of increasing Papuan-related ancestry from west to east^{28,30}. Previous studies have estimated admixture times based on present-day groups^{28–30}, providing the first inferences on the direction and rate of spread of genetic ancestry²⁸. However, the time estimates from different studies show discrepancies of more than 3,000 years (Supplementary Table 1) that cannot be solely attributed to ascertainment bias but also reflect limitations in admixture dating methods^{28,29}, which are

differentially affected by scenarios involving continuous or multiple pulses of gene flow from closely related sources³¹. Resolving the uncertainty in admixture dates has important implications for understanding the interactions between Austronesians and pre-Austronesian populations. Admixture dates close to the archaeological dates proposed for the Austronesian arrival would indicate that admixture occurred soon after contact, while more recent dates would imply that communities coexisted for some time before genetically mixing or were mixing for a prolonged period. Moreover, admixture dates predating the Austronesian arrival would suggest alternative explanations, such as genetic influences from other Asian-related groups³².

In this study, we leveraged the power of ancient DNA to investigate spatio-temporal patterns of variation within Wallacea during the last 2,500 years. We provide insights into the time of arrival of

Table 1 | Ancient samples from Wallacea included in this study

Sample name	Island, region	¹⁴ C date ± s.d. (BP)	Assigned group	Sex	mtDNA haplogroup	Y-chromosome haplogroup	Number of SNPs
AMA001	Morotai, North Moluccas	2,258 ± 30	Aru Manara 2,150 BP	M	B4a1a1	<i>C1b1a2b</i>	255,458
AMA003008	Morotai, North Moluccas	2,130 ± 24	Aru Manara 2,150 BP	F	<i>Q1d</i>	-	576,009
AMA004	Morotai, North Moluccas	2,009 ± 24	Aru Manara 2,150 BP	F	M73a	-	933,715
AMA005	Morotai, North Moluccas	n/a	Aru Manara 2,150 BP	F	B4a1a1	-	209,933
AMA009	Morotai, North Moluccas	968 ± 20	Aru Manara 950 BP	F	<i>Q1d</i>	-	935,157
TanjungPinang1	Morotai, North Moluccas	2,090 ± 180	Tanjung Pinang 2,100 BP	M	Q	<i>S1d1-</i>	870,652
TanjungPinang2	Morotai, North Moluccas	n/a	Tanjung Pinang 2,100 BP	M	<i>Q1</i>	O2a2b2a2b2	939,665
TanjungPinang4	Morotai, North Moluccas	n/a	Tanjung Pinang 2,100 BP	M	Q	<i>S1a1b1d2b-</i>	897,982
TanjungPinang6	Morotai, North Moluccas	n/a	Tanjung Pinang 2,100 BP	M	B4a1a	O2a2b2a2b2	1,028,190
Uattamdi1	Kayoa, North Moluccas	1,915 ± 27	Uattamdi 1,900 BP	M	E1a1a1	O1a2a1	895,300
TOP002	Sulawesi, central Sulawesi	211 ± 24	Topogaro 250 BP	M	E2a	O2a2a1a2a2	697,028
TOP004	Sulawesi, central Sulawesi	324 ± 24	Topogaro 250 BP	M	E2a	<i>M1a</i>	249,209
KMO001	Komodo, NTT	726 ± 19	Komodo 750 BP	F	B4a1a1	-	122,610
LIA001002	Flores, NTT	2,588 ± 23	Liang Bua 2,600 BP	F	M17a	-	873,614
LIT001	Flores, NTT	861 ± 20	Liang Toge 850 BP	F	E1a2	-	623,960
JAB001	Pantar, NTT	457 ± 19	Jareng Bori 450 BP	F	M7b1a2a1	-	848,849

The reported radiocarbon dates (¹⁴C) are uncalibrated. MtDNA and Y-chromosome haplogroups whose origins have been previously associated with Papuan groups are in italics; all other haplogroups are most likely of Asian-related origin.

the Austronesian-related ancestry, the temporal span of admixture and the relationship between the ancestry of incomers and that of other groups from Asia and Oceania. Additionally, we explore the impact and timing of an additional migration from MSEA to Wallacea.

Results

We extracted DNA from skeletal remains from 16 individuals dated to approximately 2,600–250 BP from 8 archaeological sites spanning the North Moluccas, Sulawesi and East Nusa Tenggara (for our purposes, East Nusa Tenggara has been abbreviated to NTT for Nusa Tenggara Timur) (Fig. 1a and Table 1). Sequencing libraries were then constructed and capture-enriched for approximately 1.2 million genome-wide single-nucleotide polymorphisms (SNPs)³³ and the complete mitochondrial DNA (mtDNA). The authenticity of ancient DNA was confirmed based on the elevated amounts of deaminated positions at the ends of reads and the short average fragment size (Supplementary Table 2). Contamination estimates were low (Supplementary Table 2).

The mtDNA and Y-chromosome haplogroups show that both Asian- and Papuan-related ancestries were already present in the North Moluccas approximately 2,150 BP (Table 1). Furthermore, 2 North Moluccas individuals dating to approximately 2,150–2,100 BP

carried mtDNA and Y-chromosome haplogroups associated with different ancestries, indicating that admixture started before then. In comparison to the individuals from NTT and Sulawesi, those from the North Moluccas showed a higher proportion of mtDNA lineages connecting them to Near Oceania, as attested by the Q haplogroups characteristic of Northern Sahul³⁴ and by the so-called ‘Polynesian pre-motif’ (B4a1a/B4a1a1) (ref. ³⁵). None of the individuals from Sulawesi or NTT carry Papuan-related mtDNA haplogroups, even though they are found there today^{36,37}.

To explore the genome-wide patterns of variation in ancient Wallaceans, we performed principal component analysis (PCA) based on different sets of present-day populations from Asia and Oceania and two combinations of SNP arrays (Methods). Ancient Wallaceans cluster between Papua New Guinea and Asia, together with present-day Wallaceans (Fig. 1b and Extended Data Fig. 1). However, the trajectory outlined by individuals from the northern (North Moluccas) versus southern (NTT) islands is slightly different, suggesting they may have distinct genetic histories. Ancient individuals from NTT cluster on a cline towards mainland Asians and some Western Indonesian groups, while ancient individuals from the North Moluccas align on a trajectory towards present-day Taiwanese/Philippine populations or even towards ancient individuals from Guam 2,200 BP, Vanuatu 2,900 BP and Tonga 2,500 BP

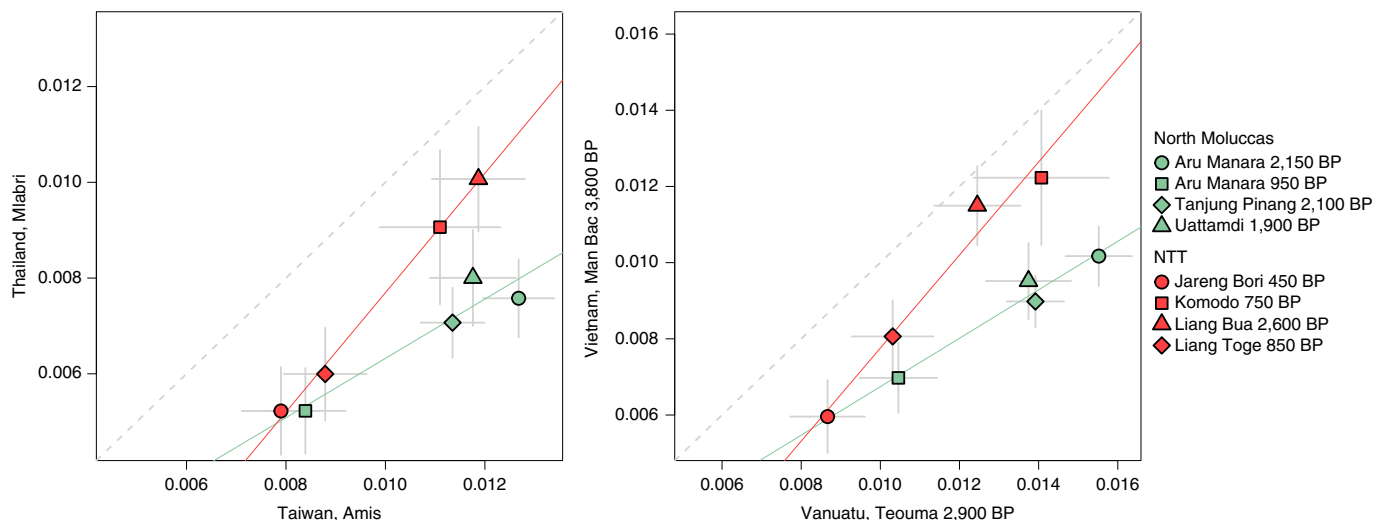


Fig. 2 | Biplots showing the results of two pairs of f_4 -statistics of the form $F_4(\text{Mbuti, test; New Guinea Highlanders, ancient Wallacea})$. The test groups are shown on the x and y axis labels. Data are presented as exact F_4 -values ± 2 s.e. indicated by the grey lines. Linear regression lines for the individuals from the North Moluccas and NTT are shown in green and red, respectively. The results for all tested pairs are shown in Supplementary Figs. 3 and 4; the results of their respective Bayesian models are shown in Supplementary Figs. 5–8.

(previously shown to have almost exclusively Austronesian-related ancestry)^{38,39}. The differences between the two Wallacean regions are more pronounced when projecting the ancient individuals into principal components that feature Asian-related variation (PC2 versus PC3; Extended Data Fig. 2).

We next used a model-based clustering method (DyStruct) to infer shared ancestry⁴⁰. The results for the best supported number of clusters in each of the tested datasets (Supplementary Fig. 1a,b) show that ancient Wallaceans shared ancestry with Papuan-speaking groups from New Guinea (dark blue component) and multiple Asian groups whose ancestry can be partitioned into three main components (Fig. 1c; see full results in Supplementary Fig. 1e,f). One component (yellow) is present at high frequencies in Austronesian-speaking groups from Taiwan, the Philippines and Indonesia, and ancient individuals from Taiwan; a second component (mango) is maximized in Polynesian-speaking groups from the Pacific and ancient individuals from the same region; and a third component (dark red) is widespread in present-day and ancient individuals from SEA. The most striking difference among ancient Wallaceans is the presence of the SEA component in ancient NTT and Sulawesi individuals but not in North Moluccan individuals. A more subtle difference occurs in the relative proportion of the two Austronesian-related components (Extended Data Fig. 3): ancient individuals from Sulawesi and NTT have a higher relative proportion of the Austronesian-related (yellow) component that predominates in Taiwan, compared to ancient individuals from the North Moluccas, who are more similar to groups from the Pacific.

To directly compare allele-sharing between ancient Wallaceans and different Asian-related groups, we used f_4 -statistics⁴¹. First, we computed an f_4 -statistic of the form $F_4(\text{Mbuti, ancient Wallacean; Amis, test})$, where the test group includes ancient and present-day groups from mainland Asia, ISEA and the Pacific who have no discernible Papuan-related ancestry (Supplementary Fig. 2 and Supplementary Table 3). Our results show that ancient individuals from the North Moluccas share more drift with ancient individuals from Vanuatu (2,900 BP) and Tonga (2,500 BP) than with Amis ($z > 2$). In contrast, ancient individuals from Sulawesi and NTT do not share additional drift with any tested groups. Nonetheless, the higher number of f_4 -statistics consistent with zero in tests involving ancient individuals from NTT (Komodo and Liang Bua)

indicates that they share as much drift with Amis as with several other groups, not only from Taiwan/Philippines but also SEA or Western Indonesia. This result, together with the identification of an ancestry component related to SEA (Supplementary Fig. 1e,f) in ancient NTT and Sulawesi individuals, supports a more complex admixture history in these parts of Wallacea.

We next analysed pairs of f_4 -statistics designed to capture any differences in Asian-related ancestry between individuals from the North Moluccas and NTT (Supplementary Figs. 3 and 4 and Supplementary Tables 4 and 5). All f_4 -statistics had the form $F_4(\text{Mbuti, test; New Guinea Highlanders, ancient Wallacean})$ and each pair compared the results for a fixed test group on the x axis (Amis or Vanuatu 2,900 BP for comparisons between modern or ancient test pairs, respectively) and various Asian-related test groups on the y axis. Since individuals from the North Moluccas lacked the SEA component (Fig. 1c), the best proxy for this component in individuals from NTT should maximize the differences in f_4 -statistics between regions. To estimate these differences, we used a Bayesian approach that accounts for measurement error in the f_4 -statistics (Supplementary Figs. 5 and 6). We concluded that the groups maximizing differences between ancient individuals from Wallacea are the present-day Mlabri or Nicobarese and ancient individuals from Vietnam (Mán Bạc 3,800 BP), Laos (Tam Pa Ping 3,000 BP) and Thailand (Ban Chiang 2,600 BP) (Fig. 2), but other related MSEA proxies could not be excluded (Supplementary Figs. 7 and 8). For several MSEA test groups, the 95% credible interval of the differences did not overlap zero within the range of F_4 values covering the Aru Manara 2,150 BP, Tanjung Pinang 2,100 BP, Uattamdi 1,900 BP, Liang Bua 2,600 BP and Komodo 750 BP, indicating strong support for the differences between NTT and the North Moluccas. Below this range (that is, for lower F_4 values), there is no support for regional differences, probably due to a decrease in power for differentiating Asian ancestries when the total Asian ancestry is low and the Papuan-related ancestry is high.

We also investigated the relationships between ancient Wallaceans and groups associated with the first colonization of Sahul or Wallacea using an f_4 -statistic of the form $F_4(\text{Mbuti, new ancient Wallacean; New Guinea Highlanders, test})$. Most ancient Wallacean individuals showed a significantly closer affinity to New Guinea Highlanders than to Australians, the Bismarck group or the

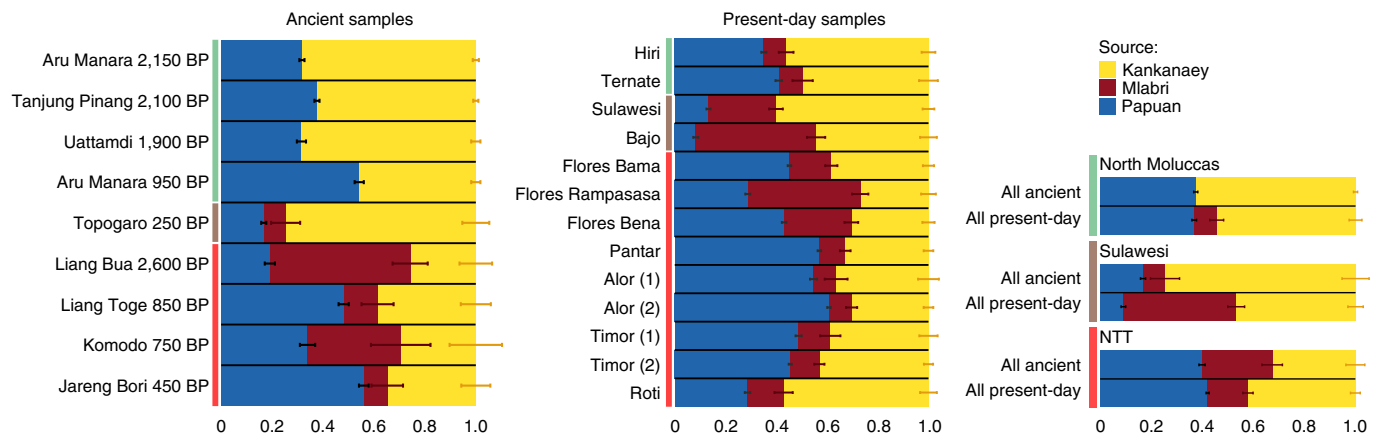


Fig. 3 | Ancestry proportions estimated with qpAdm for the model with the highest *P* value in each group. Individuals from the North Moluccas, Sulawesi and NTT are marked as green, brown and red vertical bars, respectively. Horizontal bars show the ancestry proportions ± 1 s.e. (calculated with block jackknife). The number after the name of present-day individuals indicates the genotyping array used: (1) Affymetrix 6.0; (2) Affymetrix Axiom Genome-Wide Human.

recently published pre-Neolithic individual from Sulawesi (Leang Panninge)⁴² (Supplementary Fig. 9 and Supplementary Table 6). The non-significant results are probably due to low amounts of data available for Leang Panninge and/or low amount of Papuan ancestry in Liang Bua and Topogaro (Supplementary Fig. 10). Nonetheless, tests involving Leang Panninge consistently exhibited the lowest F_4 values. Therefore, despite being from Wallacea, this ancient individual was not a good proxy for the Papuan-related ancestry of the newly reported ancient Wallaceans.

We further investigated potential differences in ancestry sources and proportions among ancient Wallaceans using the qpAdm software⁴¹. Our results indicate that whereas ancient individuals from the North Moluccas can be modelled as having both Papuan- and Austronesian-related ancestry, ancient individuals from NTT and Sulawesi were either consistent with or required a three-wave model, with additional SEA-related ancestry (Fig. 3 and Supplementary Table 7). Despite cases for which we identified more than 1 fitting model ($P > 0.01$), the estimated proportions under the model with the highest *P* value correlated with the proportions of Austronesian, Papuan and SEA ancestry inferred by DyStruct (Mantel statistic $r = 0.97$, $P < 0.001$). Ancient NTT individuals displayed more inter-island variance in their Papuan- and SEA-related ancestries ($s^2 = 0.026$ and 0.046 , respectively) compared to their Austronesian-related ancestry ($s^2 = 0.003$).

A comparison between the ancestry composition of ancient and present-day individuals from the same region (Fig. 3 and Supplementary Table 7) suggests that a small part (8%) of the Austronesian-related ancestry of ancient individuals from the North Moluccas was replaced by SEA ancestry in present-day groups, masking former differences between regions of Wallacea. The present-day groups from Sulawesi and NTT can be modelled by the same three ancestry components found in ancient individuals from those regions. However, the ancestry proportions of ancient and present-day groups showed some differences, which could indicate ancestry shifts over time or reflect the small sample sizes.

To gain insights into the relative order of admixture events between different ancestries in Wallacea, we used the admixture history graph (AHG) approach⁴³, which relies on differences in covariance between the components inferred by DyStruct (Supplementary Tables 8–10). The AHG, applied to both ancient and present-day data from NTT, suggests that the admixture of SEA- and Papuan-related ancestries occurred before the arrival of the Austronesian-related ancestry (Supplementary Table 10). An analogous test based on the three main ancestry components

observed in the North Moluccas (Papuan, Taiwan-Austronesian and Pacific-Austronesian) does not provide compelling evidence for backflow from the Pacific since the AHG inferred that Papuan ancestry was introduced into a population that already had both Austronesian-related components (Supplementary Table 10). This result suggests that drift had a more important role in the occurrence and distribution of the two Austronesian-related components.

Finally, we investigated the timing of admixture using the software DATES (Supplementary Table 11), applicable to ancient DNA from single individuals⁴⁴. With time series ancient data, we expected to reconcile previous admixture time estimates, despite gene flow complexity. Using Papuans and a pool of Asian groups as sources, we found that estimates for the oldest individuals from the Northern Moluccas (2,150 BP) and NTT (2,600 BP) are very similar (approximately 3,000 BP, adjusting for the archaeological age of each sample; Fig. 4), approaching archaeological dates for the arrival of the Austronesians in Wallacea. However, younger individuals displayed more recent estimates. This trend of decreasing admixture times extends to the present-day groups from the North Moluccas who show even younger admixture dates (approximately 1,400 BP) than ancient individuals from the same region. Admixture times for present-day and ancient samples from NTT overlapped. Our results indicate that both regions probably experienced multiple admixture pulses (and/or continuous gene flow), as suggested by the changes in ancestry proportions or composition over time (Fig. 3); however, the overall duration of admixture differed between regions.

Since we inferred that the Asian-related ancestry of ancient individuals from NTT was introduced by two Asian groups in separate events, we might expect admixture time estimates to differ using different proxies for this Asian ancestry. Therefore, we looked for differences in estimates using as sources Papuans and Austronesians (test a) versus Papuans and MSEA (test b). While the point estimates for the two NTT samples with the most MSEA ancestry were older in test b than test a, the confidence intervals overlapped (Extended Data Fig. 4). Either the different Asian ancestry sources were too similar or the admixture times were too close in time to reliably distinguish.

Discussion

This study greatly increases the amount of ancient genomic data from ISEA, a tropical region unsuitable for DNA preservation but particularly important for understanding human population interactions. The new data clarify Wallacea's admixture history and expose genetic relationships that were masked by recent demographic

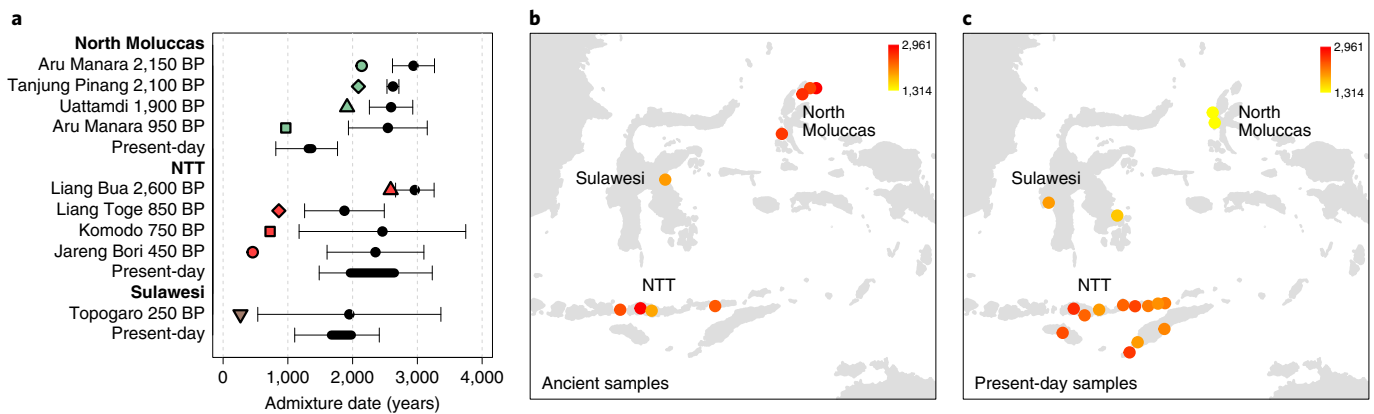


Fig. 4 | Admixture estimates. **a**, Admixture date point estimates ± 2 s.e. are shown in black. For the present-day groups, the bar represents the minimum and maximum point estimates (± 2 s.e.). The age of ancient individuals is indicated with filled symbols: green, North Moluccas; brown, Sulawesi; red, NTT. **b, c**, Spatial distribution of admixture date estimates for ancient and present-day individuals, with admixture dates depicted according to the heat plot.

processes in present-day populations. Our results reveal striking regional variation in Wallacea, some of which is found among the Austronesian-related ancestry of ancient individuals. The most remarkable differences are associated with ancestry contributions from MSEA that were probably already part of the NTT genomic landscape when Austronesians arrived but were absent from the North Moluccas until recently.

Papuan ancestry in Wallacea. All newly presented ancient Wallaceans are genetically closer to present-day Papuans than to the pre-Neolithic Leang Panninge individual from Sulawesi⁴², suggesting little direct continuity between pre-Neolithic and post-Austronesian Wallaceans. Additionally, the ancestry of the newly presented Wallaceans is closer to the ancestry of Papuans than indigenous Australians. This suggests that either the group that gave rise to Australians split first or there was contact between Wallacea and New Guinea after their initial settlement. The second scenario is supported by an mtDNA study that inferred major influxes of Papuan ancestry into Wallacea: after the Last Glacial Maximum (around 15 ka); and Austronesian contact (around 3 ka)⁴⁵.

Previous studies also reported elevated amounts of Denisovan ancestry in present-day Wallaceans, which correlate with the amount of Papuan-related ancestry⁴⁶. We confirmed that the same relationship holds for ancient Wallaceans (Extended Data Fig. 5); therefore, their Denisovan-related ancestry was probably contributed via Papuan-related admixture.

Early SEA ancestry in NTT. Wallacea is generally assumed to have been mostly isolated and shaped by two main streams of ancestry: one related to the first settlement of Sahul and another associated with the Austronesian expansion^{28–30}. The results presented in this study show that the genetic variation of ancient individuals from NTT also requires ancestry contributions from MSEA. The inferred order of events makes it unlikely that the SEA and Austronesian-related ancestries were introduced together from Western Indonesia, where both ancestries are found. Instead, it seems that human groups from MSEA crossed into southern Wallacea before the Austronesian-related groups spread into the region. Further support for this scenario comes from genetic analyses of the commensal black rat—often a good indicator of human migration—which suggests that this species was also first introduced in NTT from MSEA⁴⁷.

The broad geographical distribution of groups best matching the MSEA ancestry of southern Wallaceans raises questions about the actual origin(s) of peoples who reached those islands.

The best present-day proxies, the Mlabri from Thailand/Laos and the Nicobarese from the Nicobar islands, speak Austroasiatic languages and have been relatively isolated compared to other MSEA groups that recently experienced extensive admixture^{48–51}. Their isolation might explain why they appear as best proxies without being necessarily connected to the inferred migration event. Moreover, there is no clear link between any specific ancient group from MSEA and the actual source that contributed ancestry to NTT since we identified several equivalent ancient proxies for this ancestry.

Other evidence for this migration is equivocal. NTT languages are either Austronesian or Papuan-related and no influences from MSEA language families have been reported. Similarly, there is no archaeological evidence for pre-Austronesian contact between MSEA and southern Wallacea; the earliest evidence is the appearance of the Đông Sơn bronze drums, which spread to southern but not northern Wallacea around the early centuries AD, following maritime trade routes⁵². These drums probably originated in northern Vietnam or adjacent provinces of southern China⁵². Although we cannot rule out some MSEA ancestry contributions from the Đông Sơn period (or even later) for the younger NTT individuals, the high amount of MSEA ancestry in the Liang Bua individual (2,600 BP) and our AHG inferences support an earlier presence in southern Wallacea. This has important implications for our understanding of the Neolithic expansion into ISEA since it brings to light a previously undescribed human dispersal from MSEA. Future archaeological studies in SEA might help link this human dispersal to any contemporaneous material culture. Additionally, ancient DNA from older periods will help clarify the time of arrival of this ancestry.

Austronesian expansion into the North Moluccas and Pacific.

The fine-scale structure observed among Austronesian-related groups from ISEA and the Pacific, and the higher genetic proximity of the ancient North Moluccans to the latter, are pertinent for previous considerations of the role of the North Moluccas in dispersals to the Pacific²⁰. When analysed through seafaring and climatic models, the North Moluccas is one of the most likely starting points for settlers that ventured into the Palau or Mariana Islands (western Micronesia)^{53,54}. Their geographical setting also led archaeologists to search the region for pottery that might be ancestral to the Lapita cultural complex (distributed from the Bismarck Archipelago to Samoa), as well as the Marianas Redware culture^{11,18}. However, current evidence does not connect the North Moluccas red-slipped pottery to either of these material cultures but instead to pottery from the Talaud Islands, northern and western Sulawesi, North Luzon, Batanes and south-eastern Taiwan¹¹.

The genetic affinity between the ancient individuals from the North Moluccas and the Mariana Islands suggested by our results has some parallels in mtDNA studies based on present-day groups⁵⁵. However, ancient DNA from Guam supports an origin for the settlement of the Mariana Islands from the Philippines³⁹. Under a simple expansion scenario, without back migration, the increasing amounts of Austronesian ancestry characteristic of the Pacific (and decrease of ancestry characteristic of Taiwan/Philippines) from the ancient North Moluccas to Guam (2,200 BP), Vanuatu (2,900 BP) and Tonga (2,500 BP) could reflect their relative position along the peopling wave that eventually reached the eastern parts of the Pacific (Extended Data Fig. 3). Yet, the position in this study refers to the split order of groups without any necessary attachment to their geographical location. Therefore, it is possible that the higher proximity between the North Moluccas and groups from the Pacific, compared to NTT or Sulawesi, simply reflects their more recent ancestry tracing back to a common Austronesian source, regardless of its location. This scenario also implies that the Austronesian-related ancestry found in NTT or Sulawesi is somewhat differentiated from that found in the North Moluccas. Nonetheless, we cannot exclude the possibility of more complex migration scenarios (for example, involving back migrations).

It is also important to consider that the dates of the oldest individuals from the North Moluccas (2,150 BP in Morotai and 1,900 BP in Kayoa Island) overlap with the start of the Early Metal Age 2,300–2,000 ya in this region¹¹. This period is characterized by the appearance of copper, bronze and iron artefacts and glass beads in the region, as well as the spread of pottery into Morotai. Thus, these individuals might not be good representatives of the first Austronesians, thought to have reached Kayoa island 3,500 ya^{11,18}, but instead might reflect additional genetic influences brought by later contacts.

However, linguistic evidence parallels the genetic evidence for a closer relationship of North Moluccans with Oceanians, compared to peoples from NTT. The Austronesian (Malayo-Polynesian major subgroup) languages of the Northern Moluccas are part of the South Halmahera–West New Guinea (SHWNG) regional subgroup, which are closer to Oceanic languages than to any other Western Malayo-Polynesian major subgroup^{13,56,57}, whereas the languages spoken in NTT are an outgroup to both SHWNG and Oceanic languages¹³.

The timing of admixture. Besides providing direct evidence for Austronesian–Papuan contact before 2,150 BP in the North Moluccas and 2,600 BP in NTT, the oldest individuals gave admixture date estimates close to 3,000 BP. This period is slightly younger than the earliest archaeological traces of the Neolithic (Austronesian) arrival in the North Moluccas (approximately 3,500 BP for Kayoa Island^{11,18}) but predates the adoption of pottery on Morotai Island (2,300–2,000 BP), where the oldest North Moluccan individuals in this study were found^{11,58}. However, it is similar to some of the earliest secure dates from NTT (3,000 BP for eastern Flores)¹⁷. Previous studies conducted on present-day eastern Indonesian populations suggested that this admixture lagged about a millennium behind the arrival of Austronesian populations³⁰. Our admixture analysis for ancient individuals, and the comparison with present-day data, provides an alternative explanation and helps to clarify previous debates concerning admixture times^{28–30,32,59}. The decreasing trend in admixture time estimates from the oldest individuals until present-day populations is a strong indicator of multiple pulses or continuous admixture. Therefore, even our oldest estimates might not correspond to the actual start of admixture but to a more recent time due to additional gene flow.

Gene flow events might have been facilitated by emergent maritime networks and spice trade interactions in the Metal Age¹¹. In the North Moluccas, this period not only corresponds to a more

rapid spread of material culture between regions^{11,21,22,58} but also to the period of language levelling or radiation described for both the Austronesian (SHWNG) and Papuan (West Papuan phylum, Northern Halmahera stock) languages¹¹. The historical socio-economic systems of the North Moluccas and western Papua also brought together Papuan-speaking resident populations and a Malay-speaking elite¹¹, thus mixing could have occurred until very recently. In contrast to the North Moluccas, NTT and Sulawesi individuals do not show genetic traces of very recent contact. Still, their demographic history was nonetheless characterized by a long-term process of admixture involving at least two Asian-related ancestries.

The evidence for ongoing contact in Wallacea has important implications for efforts that use present-day genomic data to discern the direction and number of human migrations to Sahul (for example, Brucato et al.⁶⁰). Failure to consider such contact may result in wrongly considering the genetic affinity between Papuans and northern versus southern Wallaceans to reflect ancestral relationships of these groups rather than differences in the degree of contact. Overall, our findings suggest different histories for northern versus southern Wallaceans that reflect differences in contact with MSEA, in the duration of contact with Papuans and perhaps even with different Austronesian-related groups. Future ancient DNA studies involving individuals from earlier periods will help to improve our understanding of the demographic changes occurring before and after the arrival of Austronesians in Wallacea.

Methods

Sampling. All samples were processed in dedicated ancient DNA laboratories at the Max Planck Institute for the Science of Human History and the Max Planck Institute for Evolutionary Anthropology. At the Max Planck Institute for the Science of Human History, the petrous bone of samples AMA001, AMA004 and AMA009 was first drilled from the outside, identifying the position of the densest part by orienting on the internal acoustic metre and drilling parallel to it into the target area to avoid damaging the semicircular ducts⁶¹ (protocol: <https://doi.org/10.17504/protocols.io.bqd8ms9w>). After that, the petrous bone was cut along the margo superior partis petrosae (crista pyramidis) and 50–150 mg of bone powder were drilled from the densest part around the cochlea⁶². All other elements processed at the Max Planck Institute for the Science of Human History (AMA003, AMA005, AMA008, JAB001, KMO001, LIA001, LIA002, LIT001, TOP002, TOP004) were sampled by cutting and drilling the densest part. At the Max Planck Institute for Evolutionary Anthropology, the Tanjung Pinang and Uattamdi specimens were sampled by targeting the cochlea from the outside. For this, a thin layer of surface (approximately 1 mm) was removed with a sterile dentistry drill. Small holes were then drilled into the cleaned areas, yielding between 42 and 63 mg of bone powder. Detailed information on the analysed samples, radiocarbon dating and archaeological context are provided in the supplementary information and in Supplementary Tables 2 and 12.

DNA extraction. DNA extraction in both laboratories was carried out using a silica-based method optimized for the recovery of highly degraded DNA^{63,64}. To release DNA from 50–100 mg of bone powder, a solution of 900 µl EDTA, 75 µl H₂O and 25 µl proteinase K was added. In a rotator, samples were digested for at least 16 h at 37°C, followed by an additional hour at 56°C. The suspension was then centrifuged and transferred into a binding buffer. To bind DNA, large-volume silica spin columns (High Pure Viral Nucleic Acid Large Volume Kit; Roche Molecular Systems) were used. After two washing steps using the manufacturer's wash buffer, DNA was eluted in TET (10 mM Tris, 1 mM EDTA and 0.05% Tween 20). At the Max Planck Institute for the Science of Human History, the second elution of DNA from the spin column was carried out using a fresh aliquot of elution buffer for a total of 100 µl DNA extract, whereas at the Max Planck Institute for Evolutionary Anthropology the same aliquot of elution buffer was loaded twice for a total of 50 µl DNA extract (protocol: <https://doi.org/10.17504/protocols.io.baksicwe>).

Library preparation. At the Max Planck Institute for the Science of Human History, double-stranded DNA libraries were built from 25 µl of DNA extract in the presence of uracil DNA glycosylase (UDG) (half libraries) according to a protocol that uses the UDG enzyme to reduce, but not eliminate, the amount of deamination-induced damage towards the ends of ancient DNA fragments⁶⁵. Negative and positive controls were carried alongside each experiment (extraction and library preparation) (protocol: <https://doi.org/10.17504/protocols.io.bmh6k39e>). Libraries were quantified with the IS7 and IS8 primers⁶⁶ in a quantification assay using a DyNAmo SYBR Green qPCR Kit (Thermo Fisher Scientific) on the LightCycler 480 (Roche). Each ancient DNA library was double-indexed⁶⁷ in parallel 100 µl reactions using PfuTurbo DNA Polymerase

(Agilent Technologies) (protocol: <https://doi.org/10.17504/protocols.io.bakticwn>). The indexed products for each library were pooled, purified over MinElute columns (QIAGEN), eluted in 50 µl TET and again quantified with the IS5 and IS6 primers⁶⁶ using the quantification method described above; 4 µl of the purified product were amplified in multiple 100 µl reactions using Herculase II Fusion DNA Polymerase (Agilent Technologies) according to the manufacturer's specifications with 0.3 µM of the IS5/IS6 primers. After another MinElute purification, the product was quantified with the Agilent 2100 Bioanalyzer DNA 1000 chip. An equimolar pool of all libraries was then prepared for shotgun sequencing on the Illumina HiSeq 4000 platform using an SR75 sequencing kit. Libraries were further amplified with IS5/IS6 primers to reach a concentration of 200–400 ng µl⁻¹ as measured on a NanoDrop spectrophotometer (Thermo Fisher Scientific). At the Max Planck Institute for Evolutionary Anthropology, single-stranded DNA libraries were prepared without UDG treatment using the Bravo NGS Workstation B (Agilent Technologies), exactly as described in Gansauge et al.⁶⁸. Briefly, after an initial denaturation step, adaptor oligonucleotides were ligated to the 3' ends of the single-stranded ancient DNA fragments using T4 DNA ligase. Using streptavidin-covered magnetic beads, the ligation products and excess adaptors were immobilized, a primer hybridized to the adaptor and a copy of the ancient DNA molecule generated using the Klenow fragment of *Escherichia coli* DNA polymerase I. Excess primer was then removed in a washing step at increased temperature, which prevented the formation of adaptor dimers. Blunt-end ligation with T4 DNA ligase was used to ligate a second, double-stranded adaptor. Finally, the library strand was released from the beads by heat denaturation. Libraries were quantified through two probe-based quantitative PCR assays and amplified and indexed via PCR⁶⁸.

Targeted enrichment and high-throughput sequencing. MtDNA capture⁶⁹ was performed on screened libraries which, after shotgun sequencing, showed the presence of ancient DNA, highlighted by the typical C to T and G to A substitution pattern towards the 5' and 3' molecule ends, respectively. Furthermore, samples with a percentage of human DNA in shotgun data around 0.1% or greater were enriched for a set of 1,237,207 targeted SNPs across the human genome (1,240 K capture)³³. The enriched DNA product was sequenced on an Illumina HiSeq 4000 instrument with 75 cycles single reads or 50 cycles paired-end reads according to the manufacturer's protocol (at the Max Planck Institute for the Science of Human History) or on a HiSeq 2500 with 75 paired-end reads (at the Max Planck Institute for Evolutionary Anthropology). The output was demultiplexed using in-house scripts requiring either a perfect match of the expected and observed index sequences (Max Planck Institute for Evolutionary Anthropology samples) or allowing a single mismatch between the expected and observed index sequences (Max Planck Institute for the Science of Human History samples).

Genomic data processing. Preprocessing of the sequenced reads was performed using EAGER v.1.92.55 (ref. ⁷⁰). The resulting reads were clipped to remove residual adaptor sequences using Clip&Merge v.1.7.6⁷¹ and AdapterRemoval v.2 (ref. ⁷²). Clipped sequences were then mapped against the human reference genome hg19 using the Burrows–Wheeler Aligner v.0.7.12 (ref. ⁷³), disabling seeding (-l 16,500) and allowing for 2 mismatches (--n 0.01). Duplicates were removed with DeDup v.0.12.2 (ref. ⁷⁰). Additionally, a mapping quality filter of 30 was applied using SAMtools v.1.3 (ref. ⁷⁴). Different sequencing runs and libraries from the same individuals were merged and duplicates were removed and sorted again using SAMtools v.1.3 (ref. ⁷⁴). Genotype calling was performed separately for trimmed and untrimmed reads using pileupCaller v.8.6.5 (<https://github.com/stschiff/sequenceTools>), a tool that randomly draws one allele at each of the targeted SNPs covered at least once. For the UDG-treated libraries produced at the Max Planck Institute for the Science of Human History, two bases were trimmed on both ends of the reads. For libraries produced at the Max Planck Institute for Evolutionary Anthropology (without UDG treatment), the damage plots were inspected to determine the number of bases to trim from each read. For all libraries, the residual damage extended 8 base pairs into the read, after which it was below 0.05%, and trimmed accordingly. We combined the genotypes keeping all transversions from the untrimmed genotypes and transitions only from the trimmed genotypes to eliminate problematic, damage-related transitions overrepresented at the ends of reads. The generated pseudo-haploid calls were merged with previously published ancient data^{38,39,42,48,75–79}, present-day genomes from the Simons Genome Diversity Project⁸⁰, and worldwide populations genotyped on the Affymetrix Human Origins array^{38,41,50,75,76,81–86}. For the PCA and DyStruct analyses, we additionally merged the data with populations from ISEA genotyped on the Affymetrix 6.0 (refs. ^{46,87}) (dataset 1) or Affymetrix Axiom Genome-Wide Human Array³⁰ (dataset 2), filtering out SNPs with a missing rate higher than 10%. Related individuals were excluded if they exhibited a proportion of identity by descent (IBD) higher than 0.3, computed in PLINK v.1.9 (ref. ⁸⁸) as $P(\text{IBD}=2) + 0.5 \times P(\text{IBD}=1)$. We additionally pruned $r^2 > 0.4$ in 200 kilobase windows, shifted at 25-SNP intervals. After pruning, a total of 89,597 and 65,880 SNPs remained in datasets 1 and 2, respectively.

Y-chromosome haplogroups were identified by calling the SNPs covered on the Y chromosome of all male individuals using the pileup from the Rsamtools v.1.3.⁸⁹ package and by recording the number and form of derived and ancestral SNPs

overlapping with the International Society of Genetic Genealogy SNP index v.14.07 (<https://github.com/Integrative-Transcriptomics/DamageProfiler>)⁹⁰.

Authentication of ancient DNA. The typical features of ancient DNA were inspected with DamageProfiler v.0.3.1 (<http://bintray.com/apeltzer/EAGER/DamageProfiler>)⁷⁰. Sex determination was performed by comparing the coverage on the targeted X-chromosome SNPs to the coverage on the Y-chromosome SNPs, both normalized by the coverage on the autosomal SNPs⁷¹ (Supplementary Table 2). For male individuals, ANGSD v.0.919 was run to measure the rate of heterozygosity of polymorphic sites on the X chromosome after accounting for sequencing errors in the flanking regions⁹¹. This provides an estimate of nuclear DNA contamination in males since they are expected to have only one allele at each site. For both male and female individuals, mtDNA-captured data were used to jointly reconstruct the mtDNA consensus sequence and estimate contamination levels with contamMix v.1.0–10⁹⁹ (Supplementary Table 2) using an in-house pipeline (<https://github.com/alexhbnr-mitoBench-ancientMT>)⁹⁹.

Statistical analyses. PCAs were carried out using smartpca v.10210 (ref. ⁹²) based on present-day Asian and Oceanian populations from datasets 1 and 2. Ancient individuals were projected onto the calculated components using the options lsqproject: YES and numoutlieriter: 0. We used DyStruct v.1.1.0 (ref. ⁴⁰) to infer shared genetic ancestry taking into account archaeological age. The uncalibrated radiocarbon dates of each ancient sample were converted to generations, assuming a generation time of 29 years⁹³. For each dataset (1 and 2), we performed 25 independent runs, using 2–15 ancestral populations (K). To compare runs for different values of K , a subset of loci (5%) was held out during training and the conditional log-likelihood was subsequently evaluated (Supplementary Fig. 1a,b). Within the best K , the run with the highest objective function was selected (Supplementary Fig. 1c,f).

To formally test population relationships we used the f_4 -statistics implemented in the ADMIXTOOLS software v.4.1⁴¹. This analysis was carried out using the admixr v.0.9.1 R package⁹⁴. To evaluate differences in f_4 -statistics for individuals from NTT and the North Moluccas, we built a Bayesian linear regression model:

$$B_{\text{OBS},i} \sim \text{Normal}(B_{\text{TRUE},i}, B_{\text{SE},i})$$

$$B_{\text{TRUE},i} \sim \text{Normal}(\mu_i, \sigma)$$

$$\mu_i = \alpha_{\text{REGION}[i]} + \beta_{\text{REGION}[i]} A_{\text{TRUE},i}$$

$$A_{\text{OBS},i} \sim \text{Normal}(A_{\text{TRUE},i}, A_{\text{SE},i})$$

$$A_{\text{TRUE},i} \sim \text{Normal}(0, 1)$$

$$\alpha_{\text{REGION}[i]} \sim \text{Normal}(0, 1)$$

$$\beta_{\text{REGION}[i]} \sim \text{Normal}(0, 10)$$

$$\sigma \sim \text{Exponential}(1)$$

The model was stratified by region (NTT versus North Moluccas) and takes into account measurement error in both A_{OBS} and B_{OBS} variables (corresponding to the f_4 -statistics displayed on the x and y axes of the biplots, respectively)⁹⁵. The parameters μ and σ represent the mean and s.d. A_{OBS} and B_{TRUE} correspond to the unobserved true values of A and B . Both variables were standardized. The posterior distribution was obtained via Hamiltonian Monte Carlo approximation as implemented in the R package rethinking v.2.21 (<https://github.com/rmcelreath/rethinking>), using 6 chains of 4,000 samples. We used a non-centred parameterization of the error model to aid in posterior exploration. Convergence of the chains was assessed by inspection of the trace plots, Rhat and the effective number of samples. All of these criteria indicate reliable sampling. All Rhat values were equal to 1.00 and all effective number of samples values were above 500. This procedure was applied to each pair of f_4 -statistics separately. The code is available at https://github.com/sroliveira/ancient-Wallacea_f4_differences.

We used qpWave v.410 (ref. ⁹⁶) and qpAdm v.650 (ref. ⁴¹) to test two- and three-wave admixture models, using a 'rotating' strategy⁹⁷. A reference set of populations was chosen to represent diverse human groups and include potential source populations for the ancient Wallacean individuals: Mbuti, English, Brahui, Onge, Yakut, Oroqen, Lahu, Miao, Dai, Khomu, Denisova, Papuan, Kankanaey and Mlabri. We rejected models if their P values were lower than 0.01, if there were negative admixture proportions or if the s.e. was larger than the corresponding admixture proportion. When more than one model was accepted (Supplementary Table 7), the estimated admixture proportions under the model with the highest P value was preferred and used in subsequent analyses (Supplementary Fig. 10 and Extended Data Fig. 5) because the results better matched the DyStruct

ancestry proportions and the ability to reject models might be affected by several factors (for example, the ancestry proportion, the quality of the target sample, the combination of ancient and present-day samples in the same analysis). The correlation between ancestry proportions inferred with qpAdm and DyStruct was assessed with a Mantel test with 10,000 permutations of the distance matrix to determine significance.

The relative order of the mixing of different ancestries was inferred using the AHG approach³⁹. Assuming that an admixed population with two ancestry components (A and B) later receives a third component (C) via admixture, then ancestry components A and B from the first admixture event will covary with the component that comes later (C) but the ratio of A and B throughout the population will be independent from C. Thus, the covariance of the recent ancestry C with the ratio of the two older ancestries A and B should be zero. The AHG approach thus involves estimating the covariance of the frequencies of A/B with C, A/C with B and B/C with A across all individuals in the population; the covariance closest to 0 then indicates the order of admixture events. We used the DyStruct ancestry proportions for each ancient and present-day Wallacean individual included in dataset 1 and 2 (Supplementary Tables 8 and 9) to calculate the covariances between ancestry components as indicated in Supplementary Table 10. The sequence of admixture events was then determined by the configuration that produced the smallest absolute value of the covariance estimate. The time since admixture was estimated based on the decay of ancestry covariance using the software DATES v.753 (ref. ⁴⁴) with the following parameters: binsize = 0.001; maxdis = 1.0; jackknife, YES; qbin = 10; runfit, YES; affit, YES; lovalfit = 0.45; mincount = 1. In our main analysis, to maximize the number of SNPs included in the analysis and have equal sample sizes, we used as sources 16 Papuan individuals and 16 Asian-related individuals (2 Amis, 1 Atayal, 2 Kankanaey, 5 Dai, 2 Dusun, 2 She, 2 Kinh) with data covering the approximate 1,240,000 SNPs captured in the ancient samples. Two additional admixture tests were conducted using the same Papuan source and either Austronesians (2 Amis, 1 Atayal, 2 Kankanaey) or MSEA (2 Cambodian, 2 Kinh, 2 Thai) as the Asian-related source.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All newly reported ancient DNA data, including nuclear DNA and mtDNA alignment sequences, are archived in the European Nucleotide Archive (accession no. [PRJEB48109](#)).

Received: 26 October 2021; Accepted: 13 April 2022;

Published online: 9 June 2022

References

- Dickerson, R. E. Distribution of life in the Philippines. *Monogr. Bur. Sci. (Manila)* **21**, 1–322 (1928).
- O'Connell, J. F. et al. When did *Homo sapiens* first reach Southeast Asia and Sahul? *Proc. Natl Acad. Sci. USA* **115**, 8482–8490 (2018).
- Allen, J. & O'Connell, J. Both half right: updating the evidence for dating first human arrivals in Sahul. *Aust. Archaeol.* **79**, 86–108 (2014).
- Veth, P. Breaking through the radiocarbon barrier: Madjedbebe and the new chronology for Aboriginal occupation of Australia. *Aust. Archaeol.* **83**, 165–167 (2017).
- Allen, J. & O'Connell, J. F. A different paradigm for the initial colonisation of Sahul. *Archaeol. Ocean.* **55**, 1–14 (2020).
- Clarkson, C. et al. Human occupation of northern Australia by 65,000 years ago. *Nature* **547**, 306–310 (2017).
- Hawkins, S. et al. Oldest human occupation of Wallacea at Laili Cave, Timor-Leste, shows broad-spectrum foraging responses to late Pleistocene environments. *Quat. Sci. Rev.* **171**, 58–72 (2017).
- Shipton, C., O'Connell, S., Reepmeyer, C., Kealy, S. & Jankowski, N. Shell Adzes, exotic obsidian, and inter-island voyaging in the early and middle Holocene of Wallacea. *J. Isl. Coast. Archaeol.* **15**, 525–546 (2020).
- Sutikna, T. et al. The spatio-temporal distribution of archaeological and faunal finds at Liang Bua (Flores, Indonesia) in light of the revised chronology for *Homo floresiensis*. *J. Hum. Evol.* **124**, 52–74 (2018).
- Brumm, A. et al. Oldest cave art found in Sulawesi. *Sci. Adv.* **7**, eabd4648 (2021).
- Bellwood, P. *The Spice Islands in Prehistory: Archaeology in the Northern Moluccas, Indonesia* (ANU Press, 2019).
- Bellwood, P. & Dizon, E. The Batanes archaeological project and the “Out of Taiwan” hypothesis for Austronesian dispersal. *J. Austronesian Stud.* **1**, 1–31 (2005).
- Gray, R. D., Drummond, A. J. & Greenhill, S. J. Language phylogenies reveal expansion pulses and pauses in Pacific settlement. *Science* **323**, 479–483 (2009).
- Ko, A. M.-S. et al. Early Austronesians: into and out of Taiwan. *Am. J. Hum. Genet.* **94**, 426–436 (2014).
- Anggraeni, S. T., Bellwood, P. & Piper, P. Neolithic foundations in the Karama valley, West Sulawesi, Indonesia. *Antiquity* **88**, 740–756 (2014).
- O'Connor, S. Rethinking the Neolithic in island Southeast Asia, with particular reference to the archaeology of Timor-Leste and Sulawesi. *Archipel* **90**, 15–47 (2015).
- Galipaud, J.-C. et al. The Pain Haka burial ground on Flores: Indonesian evidence for a shared Neolithic belief system in Southeast Asia. *Antiquity* **90**, 1505–1521 (2016).
- Ono, R., Oktaviana, A. A. & Sriwigati, A. N. in *The Archaeology of Island Colonization: Global Approaches to Initial Human Settlement* (eds Napolitano, M. F. et al.) 293–326 (Univ. Press of Florida, 2021).
- Bellwood, P., Waluyo, A., Nitihaminoto, G. & Irwin, G. Archaeological research in the Northern Moluccas; interim results, 1991 field season. *Bull. Indo-Pacific Prehistory Assoc.* **13**, 20–33 (1993).
- Bellwood, P. S. *First Islanders: Prehistory and Human Migration in Island Southeast Asia* (Wiley Blackwell, 2017).
- Ono, R. et al. Development of regional maritime networks during the Early Metal Age in Northern Maluku Islands: a view from excavated glass ornaments and pottery variation. *J. Isl. Coast. Archaeol.* **13**, 90–108 (2018).
- Ono, R. et al. The development of pottery making traditions and maritime networks during the Early Metal Age in Northern Maluku Islands. *AMERTA* **35**, 109–122 (2017).
- Lape, P. V. Political dynamics and religious change in the late pre-colonial Banda Islands, Eastern Indonesia. *World Archaeol.* **32**, 138–155 (2000).
- Solheim, W. G. The University of Hawai'i archaeological programme in Eastern Indonesia. *Southeast Asian Archaeol.* **1996**, 61–73 (1998).
- Blust, R. The Austronesian homeland and dispersal. *Annu. Rev. Linguist.* **5**, 417–434 (2019).
- Holton, G. & Klamer, M. in *The Languages and Linguistics of the New Guinea Area* (ed. Palmer, B.) 569–640 (De Gruyter Mouton, 2017).
- Klamer, M. The dispersal of Austronesian languages in Island South East Asia: current findings and debates. *Lang. Linguist. Compass* **13**, e12325 (2019).
- Xu, S. et al. Genetic dating indicates that the Asian–Papuan admixture through Eastern Indonesia corresponds to the Austronesian expansion. *Proc. Natl Acad. Sci. USA* **109**, 4574–4579 (2012).
- Lipson, M. et al. Reconstructing Austronesian population history in Island Southeast Asia. *Nat. Commun.* **5**, 4689 (2014).
- Hudjashov, G. et al. Complex patterns of admixture across the Indonesian archipelago. *Mol. Biol. Evol.* **34**, 2439–2452 (2017).
- Pugach, I. et al. The gateway from near into remote Oceania: new insights from genome-wide data. *Mol. Biol. Evol.* **35**, 871–886 (2018).
- Denham, T. & Donohue, M. Lack of correspondence between Asian–Papuan genetic admixture and Austronesian language dispersal in eastern Indonesia. *Proc. Natl Acad. Sci. USA* **109**, E2577 (2012).
- Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
- Hudjashov, G. et al. Revealing the prehistoric settlement of Australia by Y chromosome and mtDNA analysis. *Proc. Natl Acad. Sci. USA* **104**, 8726–8730 (2007).
- Soares, P. et al. Ancient voyaging and Polynesian origins. *Am. J. Hum. Genet.* **88**, 239–247 (2011).
- Mona, S. et al. Genetic admixture history of Eastern Indonesia as revealed by Y-chromosome and mitochondrial DNA analysis. *Mol. Biol. Evol.* **26**, 1865–1877 (2009).
- Tumonggor, M. K. et al. The Indonesian archipelago: an ancient genetic highway linking Asia and the Pacific. *J. Hum. Genet.* **58**, 165–173 (2013).
- Skoglund, P. et al. Genomic insights into the peopling of the Southwest Pacific. *Nature* **538**, 510–513 (2016).
- Pugach, I. Ancient DNA from Guam and the peopling of the Pacific. *Proc. Natl Acad. Sci. USA* **118**, e2022112118 (2021).
- Joseph, T. A. & Peër, I. Inference of population structure from time-series genotype data. *Am. J. Hum. Genet.* **105**, 317–333 (2019).
- Patterson, N. et al. Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
- Carlhoff, S. et al. Genome of a middle Holocene hunter-gatherer from Wallacea. *Nature* **596**, 543–547 (2021).
- Pugach, I. et al. The complex admixture history and recent southern origins of Siberian populations. *Mol. Biol. Evol.* **33**, 1777–1795 (2016).
- Narasimhan, V. M. et al. The formation of human populations in South and Central Asia. *Science* **365**, eaat7487 (2019).
- Purnomo, G. A. et al. Mitogenomes reveal two major influxes of Papuan ancestry across Wallacea following the Last Glacial Maximum and Austronesian contact. *Genes* **12**, 965 (2021).
- Reich, D. et al. Denisova admixture and the first modern human dispersals into Southeast Asia and Oceania. *Am. J. Hum. Genet.* **89**, 516–528 (2011).

47. Louys, J. et al. Expanding population edge craniometrics and genetics provide insights into dispersal of commensal rats through Nusa Tenggara, Indonesia. *Rec. Aust. Mus.* **72**, 287–303 (2020).
48. Lipson, M. et al. Ancient genomes document multiple waves of migration in Southeast Asian prehistory. *Science* **361**, 92–95 (2018).
49. Oota, H. et al. Recent origin and cultural reversion of a hunter–gatherer group. *PLoS Biol.* **3**, e71 (2005).
50. Liu, D. et al. Extensive ethnolinguistic diversity in Vietnam reflects multiple sources of genetic diversity. *Mol. Biol. Evol.* **37**, 2503–2519 (2020).
51. Kutanan, W. et al. Reconstructing the human genetic history of mainland Southeast Asia: insights from genome-wide data from Thailand and Laos. *Mol. Biol. Evol.* **38**, 3459–3477 (2021).
52. Calò, A. *Trails of Bronze Drums Across Early Southeast Asia: Exchange Routes and Connected Cultural Spheres* (Institute of Southeast Asian Studies, 2013).
53. Montenegro, A., Callaghan, R. T. & Fitzpatrick, S. M. Using seafaring simulations and shortest-hop trajectories to model the prehistoric colonization of Remote Oceania. *Proc. Natl Acad. Sci. USA* **113**, 12685–12690 (2016).
54. Fitzpatrick, S. M. & Callaghan, R. T. Estimating trajectories of colonisation to the Mariana Islands, western Pacific. *Antiquity* **87**, 840–853 (2013).
55. Vilar, M. G. et al. The origins and genetic distinctiveness of the Chamorros of the Marianas Islands: an mtDNA perspective. *Am. J. Hum. Biol.* **25**, 116–122 (2013).
56. Blust, R. Eastern Malayo-Polynesian: a subgrouping argument. In *Proc. Second International Conference on Austronesian Linguistics 1* (eds Wurm, S. A. & Carrington, L.) 181–234 (Department of Linguistics, Research School of Pacific Studies, Australian National University, 1978).
57. Kamholz, D. C. *Austronesians in Papua: Diversification and Change in South Halmahera–West New Guinea*. PhD thesis, Univ. of California, Berkeley (2014).
58. Ono, R. et al. Early Metal Age interactions in Island Southeast Asia and Oceania: jar burials from Aru Manara, northern Moluccas. *Antiquity* **92**, 1023–1039 (2018).
59. Xu, S. & Stoneking, M. Reply to Denham and Donohue: Asian-Papuan genetic admixture is in excellent agreement with Austronesian dispersal in eastern Indonesia. *Proc. Natl Acad. Sci. USA* **109**, E2578 (2012).
60. Brucato, N. et al. Papua New Guinean genomes reveal the complex settlement of north Sahul. *Mol. Biol. Evol.* **38**, 5107–5121 (2021).
61. Himmel, M. *Etablierung und Evaluierung einer CT-Scan-informierten minimalinvasiven Methode zur Maximierung der DNA-Gewinnung aus prähistorischem Skelettmateriale* (Establishment and Evaluation of a CT Scan-informed Minimally Invasive Method to Maximize DNA Recovery from Prehistoric Skeletal Material). BSc thesis, Ernst-Abbe-Hochschule Jena (2017).
62. Pinhasi, R. et al. Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS ONE* **10**, e0129102 (2015).
63. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* **110**, 15758–15763 (2013).
64. Rohland, N., Glocke, I., Aximu-Petri, A. & Meyer, M. Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat. Protoc.* **13**, 2447–2461 (2018).
65. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
66. Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, pdb.prot5448 (2010).
67. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
68. Gansauge, M.-T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**, 2279–2300 (2020).
69. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
70. Peltzer, A. et al. EAGER: efficient ancient genome reconstruction. *Genome Biol.* **17**, 60 (2016).
71. Fu, Q. et al. The genetic history of Ice Age Europe. *Nature* **534**, 200–205 (2016).
72. Schubert, M., Lindgreen, S. & Orlando, L. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Res. Notes* **9**, 88 (2016).
73. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
74. Li, H. et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
75. Lipson, M. et al. Population turnover in Remote Oceania shortly after initial settlement. *Curr. Biol.* **28**, 1157–1165.e7 (2018).
76. McColl, H. et al. The prehistoric peopling of Southeast Asia. *Science* **361**, 88–92 (2018).
77. Posth, C. et al. Language continuity despite population replacement in Remote Oceania. *Nat. Ecol. Evol.* **2**, 731–740 (2018).
78. Yang, M. A. et al. Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* **369**, 282–288 (2020).
79. Yang, M. A. et al. 40,000-year-old individual from Asia provides insight into early population structure in Eurasia. *Curr. Biol.* **27**, 3202–3208.e9 (2017).
80. Mallick, S. et al. The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**, 201–206 (2016).
81. Lazaridis, I. et al. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
82. Skoglund, P. et al. Genetic evidence for two founding populations of the Americas. *Nature* **525**, 104–108 (2015).
83. Meyer, M. et al. A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222–226 (2012).
84. Nakatsuka, N. et al. The promise of discovering population-specific disease-associated genes in South Asia. *Nat. Genet.* **49**, 1403–1407 (2017).
85. Prüfer, K. et al. The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**, 43–49 (2014).
86. Qin, P. & Stoneking, M. Denisovan ancestry in East Eurasian and Native American populations. *Mol. Biol. Evol.* **32**, 2665–2674 (2015).
87. Jinam, T. A. et al. Discerning the origins of the Negritos, First Sundaland People: deep divergence and archaic admixture. *Genome Biol. Evol.* **9**, 2013–2022 (2017).
88. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
89. Morgan, M., Pagès, H., Obenchain, V. & Hayden, N. Rsamtools: Binary alignment (BAM), FASTA, variant call (BCF), and tabix file import. R package version 1.34.1 <https://bioconductor.org/packages/release/bioc/html/Rsamtools.html> (2019).
90. Rohrlach, A. B. et al. Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-Path Neolithic expansion to Western Europe. *Sci. Rep.* **11**, 15005 (2021).
91. Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics* **15**, 356 (2014).
92. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
93. Fenner, J. N. Cross-cultural estimation of the human generation interval for use in genetics-based population divergence studies. *Am. J. Phys. Anthropol.* **128**, 415–423 (2005).
94. Petr, M., Vernot, B. & Kelso, J. admixr—R package for reproducible analyses using ADMIXTOOLS. *Bioinformatics* **35**, 3194–3195 (2019).
95. McElreath, R. *Statistical Rethinking: A Bayesian Course with Examples in R and Stan* 495–497 (CRC Press, Francis & Taylor, 2020).
96. Reich, D. et al. Reconstructing Native American population history. *Nature* **488**, 370–374 (2012).
97. Harney, E., Patterson, N., Reich, D. & Wakeley, J. Assessing the performance of qpAdm: a statistical tool for studying population admixture. *Genetics* **217**, iyaa045 (2021).

Acknowledgements

We thank R. Radzeviciute, A. Wissgott, the Max Planck Institute for Evolutionary Anthropology lab technicians and the Max Planck Institute for Evolutionary Anthropology Sequencing and Bioinformatics Groups for their excellent support, C. Jeong for valuable comments, L. Iasi for helpful discussions on admixture dating and R. McElreath for help with statistics. This research was supported by the Max Planck Society. K.N., S.C. and A.P. were supported by the European Research Council Starting Grant ‘Waves’ (no. ERC758967). The research conducted on the samples from Liang Toge, Liang Bua and Komodo was part of a New Zealand Fast-Start Marsden Grant (no. 18-UOO-135). The research conducted on the Jareng Bori site was part of a joint project between the Australian National University Universitas Gadjah Maja funded by an ARC Laureate Project no. FL120100156.

Author contributions

R.O., P.B., R.K., T.K. and S.H. contributed archaeological material, collected with the critical support of A.A.O., M.T., C.K., D.B.M., R.S.P., M., A.P. and S.O.C. T.H., C.F.W.H., R.K., F.P. and P.B. contributed the radiocarbon data. K.N., S.C. and M.M. conducted the ancient DNA laboratory work. S.O., K.N., S.C., I.P. and A.H. performed the genetic analysis. S.O. and K.N. wrote the manuscript with input from all authors. M.S., C.P. and J.K. conceived and coordinated the study.

Funding

Open access funding provided by Max Planck Society

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41559-022-01775-2>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41559-022-01775-2>.

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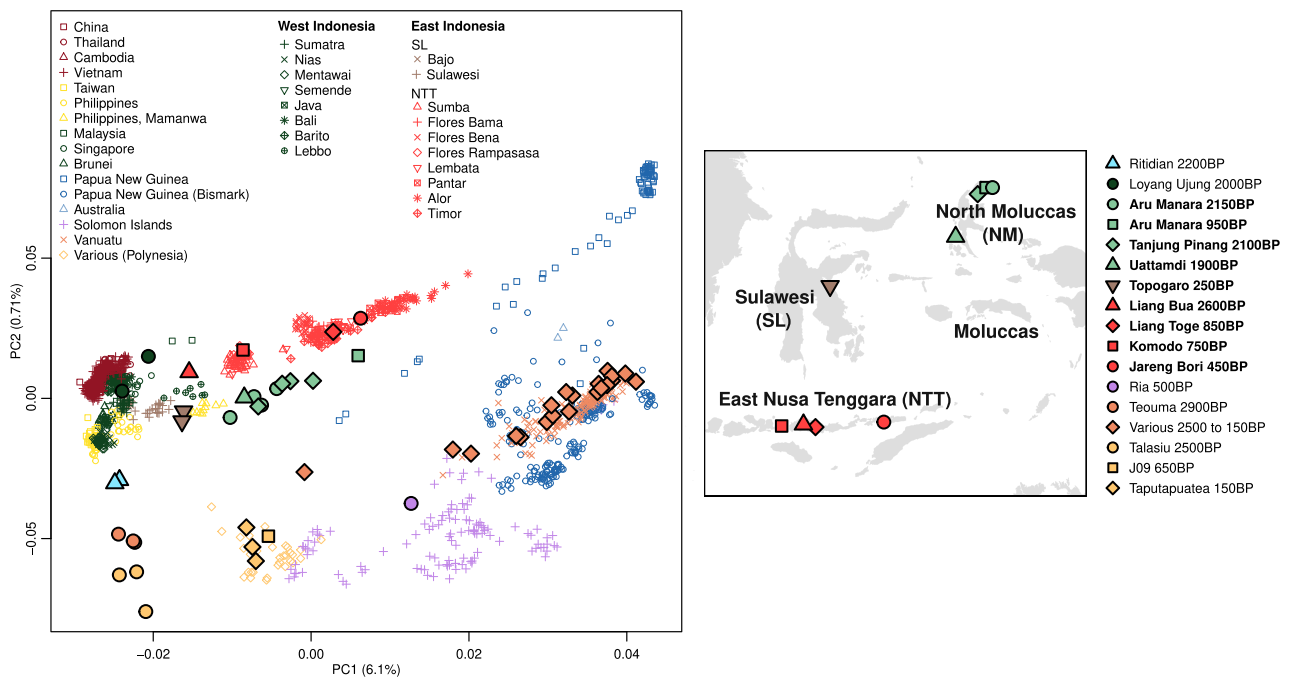
Peer review information *Nature Ecology & Evolution* thanks Lluís Quintana-Murci, Guy Jacobs and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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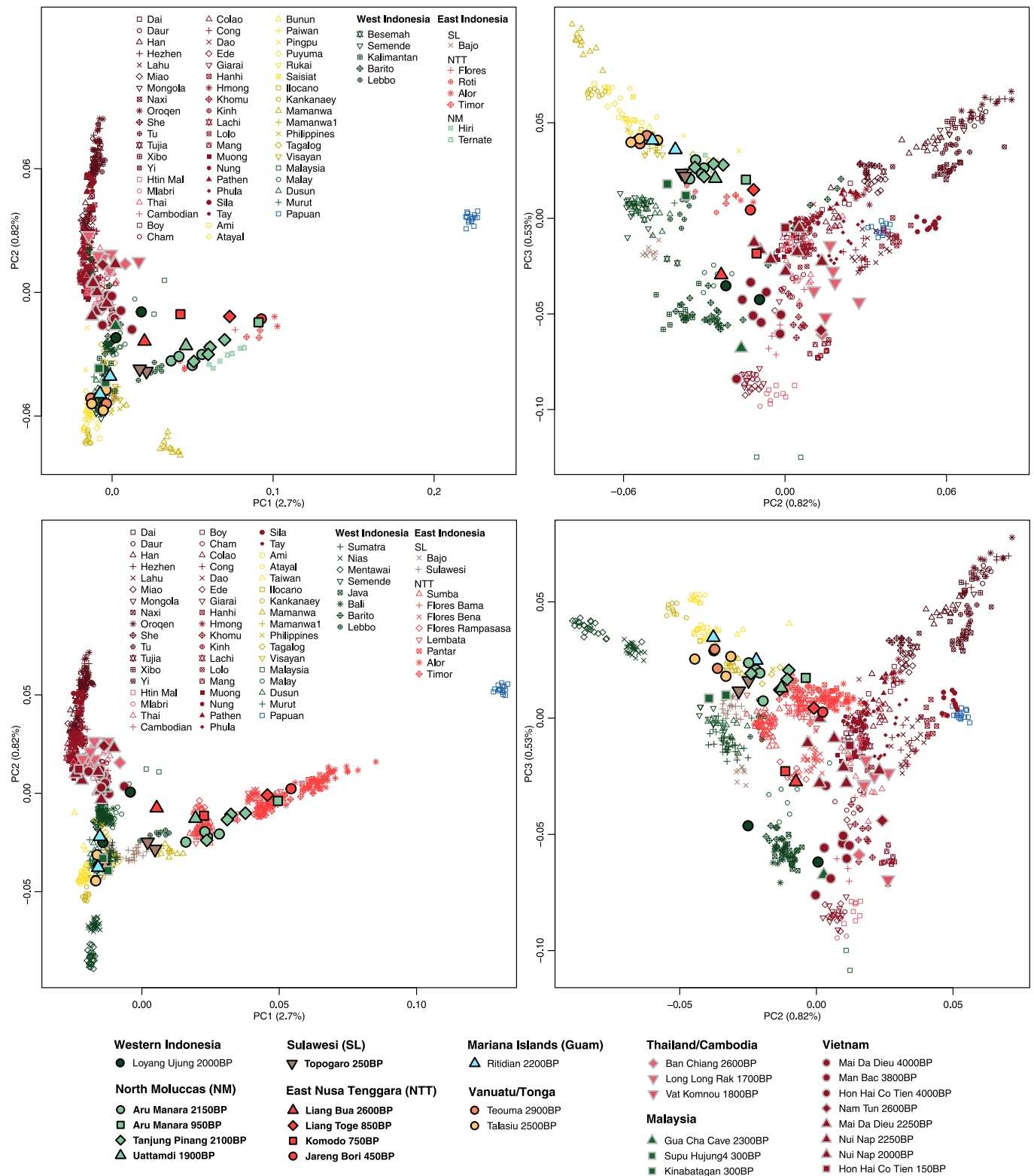
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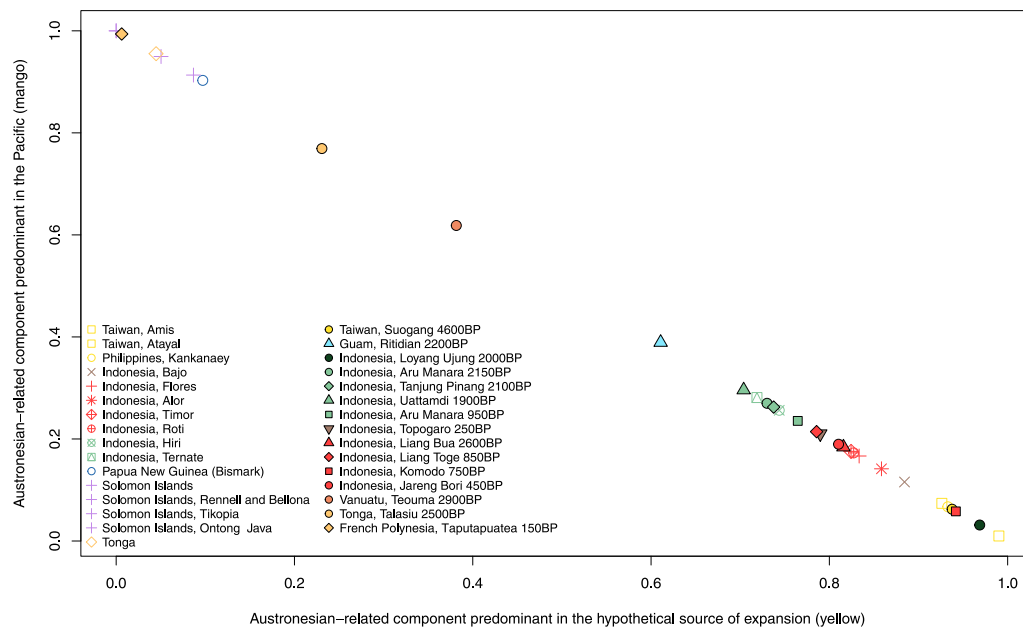
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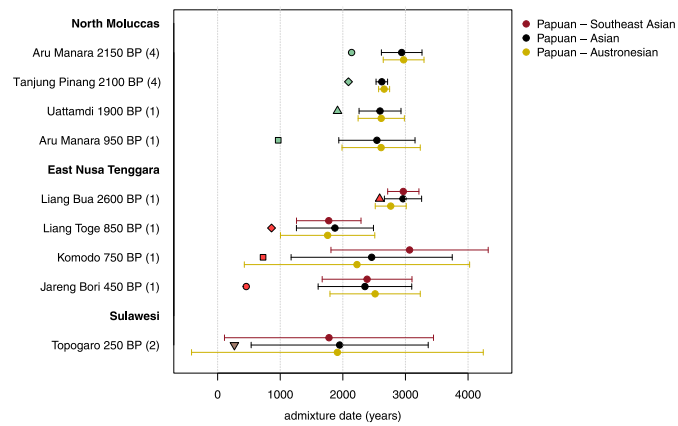
Extended Data Fig. 1 | PCA of publicly available whole genome data merged with Human Origins genotype data and Affymetrix Axiom Genome-Wide Human genotype data (dataset 2). Ancient individuals (shown with a black contour) are projected and their fill color matches the color of present-day samples from the same geographic area. The location of the ancient individuals newly presented in this study is shown in the map on the right panel. For our purposes East Nusa Tenggara is abbreviated to NTT.



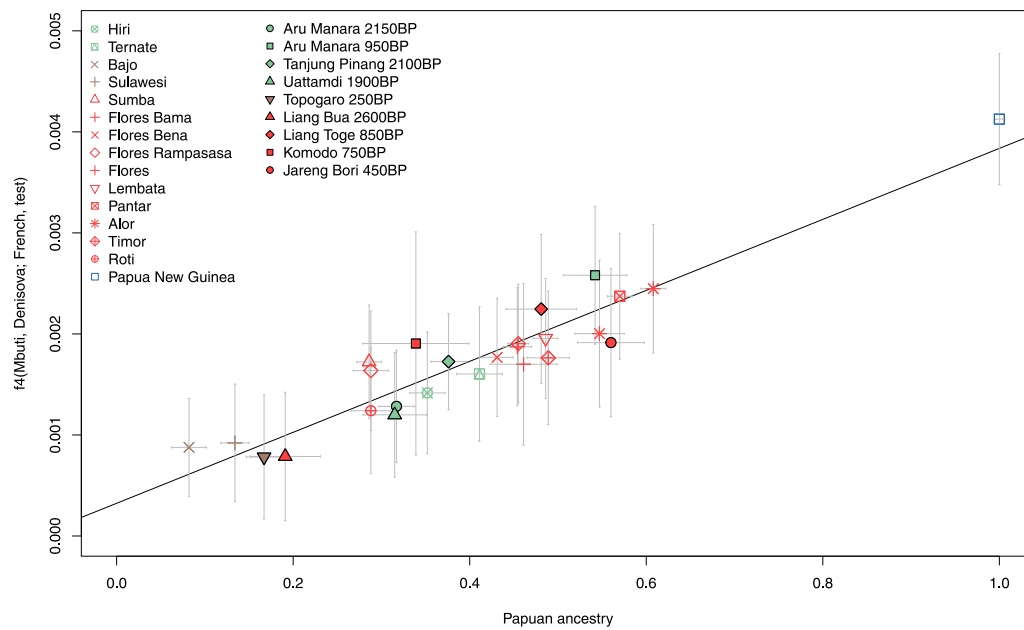
Extended Data Fig. 2 | PCA generated with a subset of present-day populations from dataset 1 (A) and dataset 2 (B) that emphasize differences between closely related Asian ancestries. Ancient individuals (shown with a black/grey contour) are projected and their fill color matches the color of present-day samples from the same geographic area. For our purposes East Nusa Tenggara is abbreviated to NTT.



Extended Data Fig. 3 | Representation of two Austronesian-related components. The frequencies of the “yellow” and “mango” components identified in Supplementary Figure 1E were normalized to sum to 1.



Extended Data Fig. 4 | Admixture dates estimated with different source groups. The admixture dates estimated with a pool of Asian groups are shown in black, while the admixture dates estimated with a more specific set of Asian-related groups (SEA or Austronesians) are shown in dark red and yellow. Data are presented as point estimates ± 2 SE. The individuals age is shown by filled symbols with a black contour. The number of individuals included in each group is shown in parenthesis, next to the group label.



Extended Data Fig. 5 | Papuan vs Denisova ancestry. The x-axis presents the Papuan-related ancestry proportion ± 2 SE calculated with block jackknife in the qpAdm software. The Denisova ancestry is represented in the y-axis by an f_4 -statistic of the form $f_4(Mbuti, Denisova; French, test) \pm 2$ SE.

3.3 Manuscript C: Genomic portrait and relatedness patterns of the Iron Age Log Coffin culture in northwestern Thailand

Selina Carlhoff, Wibhu Kutanan, Adam B Rohrlach, Cosimo Posth, Mark Stoneking, Kathrin Nägele, Rasmi Shoocongdej, Johannes Krause (submitted)

Previous archaeogenetic studies of Neolithic individuals from Southeast Asia have described wide-spread admixture between the local ‘Hòabinhian’-associated hunter-gatherers, who are related to present-day Andamanese Islanders, and incoming southern East Asian-related agriculturalists. Archaeological excavations trace this expansion of inhuman rituals, material culture, and domestic animals along different river valleys, including the Salween river running through the highlands of northwestern Thailand. Dating to the later Iron Age (2.3-1 ka), wooden coffins mounted on stilts in caves and shelters mark the Log Coffin culture of the region. The vast number of archaeological sites and analyses of the distinctive carvings of the coffins point towards a large, interconnected community. In this manuscript, we present genome-wide data for 33 individuals from four Log Coffin-associated sites to assess the genetic profile and community connectivity. Our analyses show that the Log Coffin-associated individuals were genetically homogeneous and harboured ancestry related to ‘Hòabinhian’-associated individuals, as well as Yangtze- and Yellow River-related farmers. The Yellow River genetic component is absent from Bronze and Iron Age individuals from northeastern Thailand, demonstrating significant genetic structure in northern Thailand, corresponding to differences in material culture and mortuary practices. We also compared the newly-sequenced ancient individuals to present-day inhabitants of the region, who speak a variety of languages, and find no distinct show evidence of additional gene pool changes since the Iron Age, corresponding to oral traditions of mobility during historical times. Focussing on the Log Coffin-associated community, we find that close genetic relatives were more likely to be buried in the same cave, suggesting a genetic or kin aspect to the culture’s mortuary behaviour. The high number and genomic coverage of the Log Coffin-associated individuals also allowed for investigations of more distant genetic relations through analysis of identity-by-descent blocks for the first time in Southeast Asia. The many detected third to sixth degree relationships demonstrate interconnectedness and movement between different sites and river valleys and illustrate the value of small-scale studies integrating archaeological and archaeogenetic findings.

Genomic portrait and relatedness patterns of the Iron Age Log Coffin culture in northwestern Thailand

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Abstract

The Iron Age of highland Pang Mapha, northwestern Thailand, is characterised by a unique mortuary practice known as Log Coffin culture. Over 40 natural caves and rock shelters, dating between 2,300 and 1,000 years ago, have been identified where large coffins carved from individual teak trees were mounted on stilts. While numerous multidisciplinary studies have been carried out to investigate the cultural development of the Log Coffin-associated sites, the origins of the practice, connections with other wooden coffin-using groups in Southeast Asia, and social structure within the region remains understudied. Here, we present genome-wide data from 33 individuals associated with five Log Coffin culture sites to study genetic ancestry profiles and genetic interconnectedness within and between archaeological sites. The Log Coffin-associated genomes can be modelled as an admixture between Hòabìnghian hunter-gatherer-, Yangtze River farmer-, and Yellow River farmer-related ancestry. Bronze and Iron Age individuals from northeastern Thailand show no evidence of the Yellow River farmer-related component, indicating different influence spheres also reflected by cultural practices. Our analyses also identify close genetic relationships between individuals from the same site and more distant connections between sites in the same river valley. In combination with high mitochondrial haplogroup diversity and genome-wide homogeneity, the Log Coffin-associated groups from northwestern Thailand seem to have been a large, well-connected community, where relatedness played a significant role in the mortuary ritual.

Introduction

A vast number of caves intersperse the limestone karst formations of the southern Shan Hills in the northwestern part of Thailand. These diverse habitats, dominated by deciduous forests and controlled by tropical monsoon climate, provided a favourable setting for Palaeolithic hunter-gatherers and subsequent human settlements^{1–3}. While the first lithic tools in northern Thailand were dated to 550 thousand years (ka) ago⁴, excavations of hunter-gatherer sites suggest modern humans occupied the region from at least 35 ka ago⁵, where high environmental heterogeneity allowed this subsistence strategy and so-called ‘Hòabìnghian’ stone artefact technology to persist for over 20 ka⁶. As cultural and genetic variation within the hunter-gatherers is only beginning to be uncovered^{1,7,8}, archaeological and archaeogenetic studies have chronicled large-scale genetic and cultural shifts in local hunter gatherers groups with the expansion of early sedentary food producers from East Asia^{9–11}. Many habitational

sites spreading along south-flowing river valleys appear in the archaeological record around 4.5 – 4 ka ago. They share inhumation rituals, pottery styles and domestic animals such as dogs, pigs, and cattle^{11–13}. Genetic evidence indicates that their inhabitants harboured both Neolithic southern East Asian and local hunter-gatherer ancestry^{7,8,10,14}.

Based on the current evidence found across Thailand, it appears that the expansion of Neolithic material culture and technologies involved different migration routes and groups of people. One route may have followed the Salween river in the northwest, another along the Red and Mekong rivers on the northeastern and eastern coast of Thailand¹⁴. However, little is known about the genetic diversity in the northwestern corridor. Nowadays, highland Pang Mapha, a region in the Mae Hong Son province in northwestern Thailand, located near the western Salween river, is inhabited by many ethnic groups, including the Shan, Karen, Red and Black Lahu, Chinese, Lawa and Thai², speaking Sino-Tibetan, Tai-Kadai, Hmong-Mien and Austroasiatic languages¹⁵. Early speakers of these language families might have interacted, or even been part of the same ancient communities in southern China 3-1 ka ago¹⁶. Previous studies have suggested that the different ethnic groups, called ‘Baiyue tribes’ by the Han, might be connected to the Hanging Coffin and Log Coffin archaeological cultures of southern China and northwestern Thailand, respectively¹⁷. In highland Pang Mapha, over 40 caves and shelters with large wooden coffins, dating between 2.3 and 1 ka ago, have been discovered in caves and rock shelters of five river valleys. The Log Coffin culture is a unique mortuary practice that reflects the regional variations of highland culture in the Late prehistory of mainland Southeast Asia¹⁸. Coffins are cut from a single tree and feature distinct carvings at the head and foot ends, which may reflect societal beliefs, the status of the deceased, the skill of the coffin’s maker, or indicate family or clan cemeteries^{19–22}. Other artefacts recovered from these caves include pottery sherds and faunal remains, and iron tools and bronze objects place them in the Iron Age of the region^{2,3}. Dendrochronological analysis and radiocarbon dating suggest use as cemeteries for several hundred years^{23,24}. The conspicuousness of the wooden coffins has generated a lot of scientific and public interest but due to many instances of looting and the lack of habitation sites associated with the cemeteries, detailed insights into the life of the associated people and how they are connected to previous and present-day inhabitants of the region remain challenging. Since DNA preservation is poor in Southeast Asia, to date, only a few mostly low-coverage genomes associated with log coffins excavated at Long Long Rak⁸ are available (Fig. 1). However, higher coverage data from a larger number of sampled individuals would be desirable, as this

would enable more fine-scale analyses of genetic relatedness, such as shared identity-by-descent (IBD) blocks²⁵, community connectivity, and cemetery structure.

Here, we present the genetic and archaeological analyses of 33 newly-sequenced individuals from five Iron Age Log Coffin culture sites in northwestern Thailand. We evaluate genetic ancestry profiles of ancient individuals from Thailand and the surrounding regions by focussing on the differences in hunter-gatherer- and Neolithic farmer-related ancestries. Additionally, we compare the ancient genetic signatures with those of the present-day inhabitants of the region and investigate genetic relatedness and connectivity within and between different burial caves.

Results

For this study, we assessed 64 pieces of skeletal material from eight archaeological sites in northwestern Thailand, including 42 petrous parts of the temporal bone and 22 teeth, of which 60 were sampled for ancient DNA (Fig. S1, Table S2). We were able to recover genetic material suitable for down-stream analyses from the sites Ban Rai (n=1), Lahu Pot (n=1), Tham Lod (n=3), Yappa Nhae 1 (n=2) and Yappa Nhae 2 (n=26; see Supplementary Text for descriptions of all sites). A subsample of petrous bones was directly ¹⁴C dated to 1.6-1.8 ka calBP (Fig. 1, Table S1).

After double-stranded library preparation and shotgun screening, we evaluated the authenticity and preservation level of ancient DNA fragments by considering short DNA fragment length, the percentage of endogenous human DNA, and the elevation of C→T mis-incorporations at the 5' end of DNA strands²⁶ (Fig. S2, Table S2). Based on these criteria, we selected 33 DNA libraries to be enriched for ~1.2 million (1240K capture) single nucleotide polymorphisms (SNPs) using in-solution DNA hybridization capture^{27,28}. Final coverage was variable, with a median number of 489,787 SNPs on the 1240K panel (range: 52,907 - 935,000 SNPs) and minimal contamination levels (Table S2). The main mitochondrial haplogroups were F1a1a, F2f, M7b1a1, and N8*, while the Y-haplogroups were mostly O1b and O2a, in addition to N1b and C2b (Table S2).

We assessed genetic relatedness with KIN²⁹ and determined that two genomes were likely from the same individual (YPN032-YPN033) within Yappa Nhae 1, while there was one parent-child pair (YPN014-YPN021) within Yappa Nhae 2 (Table S3). We also determined four second-degree relationships, including one grandparent-grandchild (YPN010-YPN011)

and three avuncular or half-sibling relationships within and between Yappa Nhae 1 and 2. We also analyzed the published genomes from Long Long Rak and identified two as the same individual (Th519-Th703) and a potential sibling pair (Th530-Th531). Based on runs of homozygosity³⁰, we also assigned four individuals from Yappa Nhae 2 as offspring of closely related parents (YPN001, YPN002, YPN006, YPN022; Fig. S3).

To study more distant genetic relationships, we detected shared IBD blocks >20 cM after imputation between high-coverage individuals from Lahu Pot, Tham Lod, and Yappa Nhae 2 (Suppl. Mat.). We found the highest amount of IBD sharing within Yappa Nhae 2, with the highest total IBD block length shared between YPN010 and YPN011 (Fig. S15). A cluster of individuals from Yappa Nhae 2 (YPN001, YPN006, YPN012, YPN027, YPN030) were all closely related to each other, while other individuals from the site were only distantly related to one or two individuals of this group. YPN003 and YPN007, who shared 825 cM of IBD blocks and MT- and Y-haplogroups, did not share long IBD blocks or haplogroups with any other individuals. Some individuals from Yappa Nhae 2 also shared distant relationships with those from Tham Lod and Lahu Pot (Fig. 2c). Still, the frequency and length of shared blocks between pairs of sites differed, with a maximum of 12 blocks of 659.4 cM total Tham Lod-Yappa Nhae 2 (THL002 – YPN030) compared to a maximum of two blocks of 54.4 cM total shared by Lahu Pot-Yappa Nhae 2 (LHP002 – YPN022) and no shared IBD blocks between Lahu Pot and Tham Lod (Fig. S15).

We performed a principal component analysis to assess the genetic similarities between the newly sequenced individuals and ancient and present-day individuals from the region^{7–10,31–38} (Fig. 2a). The Log Coffin-associated individuals formed a cluster which also contained individuals from the previously studied Log Coffin site Long Long Rak⁸ and fell on top of present-day individuals from Thailand³¹ (Sino-Tibetan speaking Karen Padaung and Austroasiatic speaking Lawa Eastern, Lawa Western, and Mon). This position was distinct from other ancient individuals, including Bronze Age and Iron Age individuals from Ban Chiang in northeastern Thailand¹⁰.

To formally investigate the genetic processes underlying the observed positions of the individuals in PCA space, we calculated a series of f -statistics (Suppl. Mat.). When testing the relationship between the newly sequenced individuals and Bronze Age individuals from northeastern Thailand compared to ancient genomes from East Asia (f_4 (Mbuti, ancient East Asia; Bronze Age Ban Chiang, Log Coffin)), we observed a significant affinity of the Log

Coffin-associated individuals to ancient groups from northern East Asia, particularly from the Yellow River valley³⁸, Mongolia³², and Nepal³⁷ (Fig. S8). Based on these results, we were able to model the newly-generated genomes, as well as the previously published ones from Long Long Rak⁸, as a two-way admixture of Bronze Age individuals from Ban Chiang, and Late Neolithic/Iron Age Yellow River or West Liao River groups using qpAdm³⁵ ($p=0.4789$, Fig. S11). In contrast, these models did not fit for individuals from the Iron Age in Ban Chiang (nested $p=0.4665$). When dividing the ancestry of Bronze Age individuals into hunter-gatherer and early farmer components^{7–10}, the Hòabìnhian-associated individual from Laos was determined to be the best fitting hunter-gatherer source for the Log Coffin-associated individuals, while both Hòabìnhian- and Longlin-related individuals resulted in similarly well-fitting models for the individuals from Ban Chiang (Fig. S12). We successfully modelled the newly-generated Log Coffin-associated genomes as a three-way admixture between $13.0 \pm 1.5\%$ Hòabìnhian-, $43.0 \pm 2.4\%$ southern East Asian- and $44.0 \pm 2.1\%$ Upper Yellow River-related ancestry ($p=0.8799$), similar to Laos 2–10 ka and Late Neolithic and historic Vietnam (Fig. 2b, 3a, Fig. S13, Table S4). Conversely, no Upper Yellow River-related ancestry was required for modelling the Bronze Age and Iron Age individuals from Ban Chiang, as well as the Neolithic individuals from Man Bac in Vietnam (Fig. 2b, 3a, Table S4). We also observed slight variation in the relative amounts of these ancestries between the Log Coffin-associated individuals from northwestern Thailand (Fig. S14).

We then tested for genetic continuity from the ancient individuals to present-day groups from Thailand³¹. While some present-day groups clustered with the ancient individuals in PCA space (Fig. 2a, S4), ADMIXTURE³⁹ analysis showed that most present-day groups contained more and other combinations of ancestry components compared to the ancient genomes (Fig. 3b, S6). Further testing with $f_4(\text{Mbuti, present-day Thailand; Bronze Age Ban Chiang, Log Coffin})$ confirmed a generalized affinity of present-day groups to the newly reported ancient genomes, when compared to Bronze Age Ban Chiang, but with no attraction to any specific group or language family (Fig. S9, S10).

Discussion

Here, we present genome-wide data of 33 individuals associated with the Iron Age Log Coffin culture of northwestern Thailand. The low level of long runs of homozygosity and the high mitochondrial haplogroup diversity indicate that a large community lived in the highlands, which is supported by the high number of log coffin sites found in the area^{18,21}.

From a population genetics perspective, the Log Coffin-associated individuals appear to be genetically homogeneous with little variation in PCA position and admixture components (Fig. 2a, Suppl. Mat.). This pattern indicates older genetic contact with genetically-distinct communities or a large amount of genetic exchange within the recently admixed community. The Log Coffin-associated communities in northwestern Thailand retained ancestry from Hòabìnhiàn-associated groups, whereas 8 ka-old groups in southern China carry Longlin-related ancestry and younger groups from Guangxi and Bronze Age Vietnam do not carry either hunter-gatherer-related component (Table S4). This indicates multiple interactions of incoming groups with local hunter-gatherers, as well as movement with little local admixture, resulting in a diverse (post-) Neolithic genetic landscape in Southeast Asia. This is further corroborated by the occupational history of some Log Coffin-associated caves reaching back into the Late Pleistocene and Early Holocene^{3,5} and underlines the importance of understanding exactly when and how these interactions and transitions took place. However, poor DNA preservation in the region makes DNA retrieval from older periods particularly challenging.

At the regional level, we identify at least two distinct ancestry profiles in ancient individuals from Thailand. The Log Coffin-associated individuals from the highlands show a large additional genetic component related to groups from the Yellow River valley, which is not present in Bronze Age (3.1 ka) and Iron Age (2.5 ka) individuals from Ban Chiang in northeastern Thailand¹⁰. The genetic results are indicative of different spheres of influence for these groups during the Iron Age which is paralleled by the similarity of wooden coffin burials in northwestern Thailand, the central highlands of Vietnam, and southern China²¹, compared to the burials underneath residence structures in northeastern Thailand⁴⁰. Additionally, palaeoproteomic studies indicate a strongly C₃-based diet for Log Coffin-associated individuals from Long Long Rak, likely consisting of rice and freshwater fish among other food items, while the diets of Neolithic and Iron Age northeastern Thailand groups were more diverse, but depleted in C₃ food sources⁴¹. Although an analysis of mtDNA data concluded that the Log Coffin culture arrived via cultural transmission from Yunnan Province, China¹⁷, the newly generated autosomal data shows an external genetic influence before or with the introduction of log coffins to northwestern Thailand. This differentiation could also reflect different migration routes of different Neolithic groups, with highland Pang Mapha being part of the northwestern route along the Salween river and mountain ranges.

The substantial gene flow into the studied Log Coffin communities from groups carrying Yellow River-related ancestry has parallels in other parts of East and Southeast Asia (Fig. 3a). Detected in ancient individuals from Fujian Province, China, after the Late Neolithic⁹, this component arrived in Guangxi Province, China, between 6.4 and 1.5 ka ago⁷ and is later present in Neolithic and Bronze Age individuals (4.2 – 2.1 ka) from Malaysia, Myanmar, Laos, and Vietnam^{7,8}. Recently published ancient human genomic data also showed that Upper Yellow River-related ancestry contributed >80% to some early and present-day Tibetan Plateau groups, indicating genetic links before the introduction of barley agriculture onto the Plateau⁴². Since present-day groups of the Chinese Central Plain form a genetic cline between Upper Yellow River- and southern East Asian-related ancestry, the former has been associated with the spread of Sino-Tibetan languages from their homeland along the Upper Yellow River during the late Cishan/early Yangshao period (7.4 ka) to mainland Southeast Asia^{32,43}. Bronze Age and Iron Age Ban Chiang from northeastern Thailand and Neolithic Man Bac from northern Vietnam stand out in their lack of this genetic component, potentially pointing towards a temporal pattern, where this component only arrived in Thailand and Vietnam later, or perhaps cultural boundaries limited further propagation. Additional ancient genomes, in combination with novel admixture modelling and dating techniques, will help to date the introduction of this northern East Asian component, and understand the distribution and spread of this ancestry throughout East and Southeast Asia in the future.

Although a study of the mitochondrial haplogroup frequency spectra identified present-day Khon Mueang and Thai Yuan as most similar to ancient Log Coffin-associated individuals from northwestern Thailand¹⁷, on a genome-wide level, present-day groups³¹ show a generalized affinity to the ancient individuals from northwestern Thailand, but also harbour additional ancestry components (Fig. 3b, Suppl. Mat.). This indicates that significant changes in the gene pool took place after 1700 BP, including migrations from Laos, Myanmar, and southern China in historical times^{44,45}. Still, groups in northwestern Thailand retain a level of genetic differentiation from other regions in Thailand^{31,46,47}.

Generating genomic data from many individuals associated with the same archaeological complex and recovered in close proximity also allowed the first investigations into the social structure of an ancient Southeast Asian community. At the Log Coffin-associated sites, we identified several close genetic relationships between sampled individuals from Yappa Nhae 1 and 2, situated in the Mae Lana valley, and within the previously-published genomes from

Long Long Rak. Furthermore, individuals from Lahu Pot and Tham Lod, located in the Lang river valley, and Yappa Nhae 2 shared long IBD blocks, suggesting close connections and exchange between the communities from different river valleys. Individuals from Yappa Nhae 2 and Tham Lod shared 3rd to 6th degrees of relatedness, while Yappa Nhae 2 and Lahu Pot were more distantly related through 5 to 6th degrees of relatedness. Present-day groups in the region practise both matri- and patrilocality traceable through uniparental markers⁴⁵, but analysis of the uniparental markers suggests connections of the ancient related individuals through the maternal (e.g., YPN014-YPN021) and paternal lines (e.g., YPN014-YPN027-YPN030) within Yappa Nhae 2. Several 1st and 2nd degree relationships within Yappa Nhae and Long Long Rak, in addition to the six individuals from Yappa Nhae 2 and one from Tham Lod being more closely related to each other compared to other individuals from the site, could indicate a tendency for genetic relatives to be buried in the same cave. This corresponds with a social network analysis based on the coffin handles that suggested that the cemetery sites in each river valley were closely related and the respective communities interacted across the river valleys^{48,49}. Since the two first degree-related individuals at Long Long Rak were also buried in the same coffin⁸, the variety of the coffin handles may also reflect the diversity of families living in highland Pang Mapha from different periods. Incorporating our archaeogenetic results into archaeological findings demonstrates the value of the in-depth study of single sites and archaeological complexes to reveal more about the lives and beliefs of past communities. Radiocarbon dating and genomic analyses of other sites with log coffin burials will help to further resolve the social structure of these communities, and to study the trans-regional connections and the spread of this mortuary practice.

Methods

Sampling, extraction, library preparation, and indexing were performed in a dedicated clean room for ancient DNA at the Max Planck Institute for the Science of Human History (now MPI of Geoanthropology) in Jena, Germany. We obtained bone powder from 40 petrous parts of the temporal bone by cutting along the margo superior and drilling near the cochlea⁵⁰ and from 22 teeth by cutting along the enamel-dentin junction and drilling from the pulp (<https://dx.doi.org/10.17504/protocols.io.bqebmtan>). We extracted DNA (<https://dx.doi.org/10.17504/protocols.io.baksicwe>) and built double-stranded UDG-half libraries after partial uracil-DNA glycosylase treatment⁵¹. After double-indexing with unique

index combinations⁵², the libraries were shotgun-sequenced on an Illumina HiSeq 4000 using a 75 bp single-read configuration.

After initial quality assessment, 33 libraries were hybridized in-solution to enrich for a targeted set of 1,237,207 single nucleotide polymorphisms^{27,28}. The capture products were sequenced on an Illumina HiSeq 4000 using a 75 bp single-read configuration. The resulting reads were aligned to the human reference genome (hg19) using mapping quality filter 30 and duplicates removed in *nf-core/eager* v.2.4.0⁵³.

We estimated nuclear contamination using *ANGSD* v.0.935⁵⁴ and mitochondrial contamination with *ContamMix* v.1.0-10⁵⁵. We trimmed 2 bp off both ends of each read of the 1240K-captured sequences and genotyped these for the 1240K panel with *pileupCaller* v.1.4.0.5 (<https://github.com/stschiff/sequenceTools>). We determined genetic relatedness with *KIN* v.3.1.3²⁹ and parental relatedness using runs of homozygosity in *hapROH* v.0.3a4³⁰. For IBD calling, the ancient individuals were imputed to the 1000 Genomes positions⁵⁶ using *ATLAS* v.0.9⁵⁷ and *GLIMPSE* v1.0.0⁵⁸ and IBD blocks called with *ancIBD* v.0.2a2 (<https://pypi.org/project/ancIBD/>).

Initial population genetic analyses were conducted with *smartpca* v.1.6000⁵⁹, where all ancient individuals were projected, and *ADMIXTURE* v.1.3.0³⁹. All f_3 - and f_4 -statistics were calculated with *qp3pop* v.651 and *qpDstat* v.980, respectively, while admixture proportions were estimated with *qpAdm* v.1520³⁵. The employed methods are described in detail in the supplementary materials.

Data availability

The raw nuclear sequences are available at the European Nucleotide Archive under the accession number PRJEB59488.

Acknowledgements

The research was funded by the Thailand Research Fund (RDG55H0006 and RTA6080001), the ERC Starting Grant ‘Waves’ (ERC758967), and the Max Planck Society. W.K. was also funded by Institute of Suvanabhumi Studies, TASSHA (Thailand Academy of Social Sciences, Humanities and Arts), Ministry of Higher Education, Science, Research and Innovation, and Naresuan University under global and frontier research university fund (R2566C051). Permits for export and archaeogenetic analyses were issued by the Fine Arts

Department of Thailand under permit numbers 497/2561 and WTh-0417-236. We thank Benchawan Phonprasoe and Siriluck Kanthasri for coordinating sample collection. We thank the laboratory and bioinformatics team at the Max Planck Institute for Evolutionary Anthropology, in particular Rita Radzeviciute and Antje Wissgott, for their excellent support, as well as Valentina Zaro for help during sampling.

Author contributions

M.S., W.K., J.K., and R.S. conceived the study. R.S. led the archaeological excavations and surveys. W.K. organised sample collection. S.C. and K.N. sampled and extracted DNA and analysed the data with input from A.B.R., C.P., and M.S. S.C. and K.N. interpreted the data with critical input and contextualisation from R.S. and W.K. S.C. wrote the manuscript with critical input from K.N. and the remaining authors.

Figures

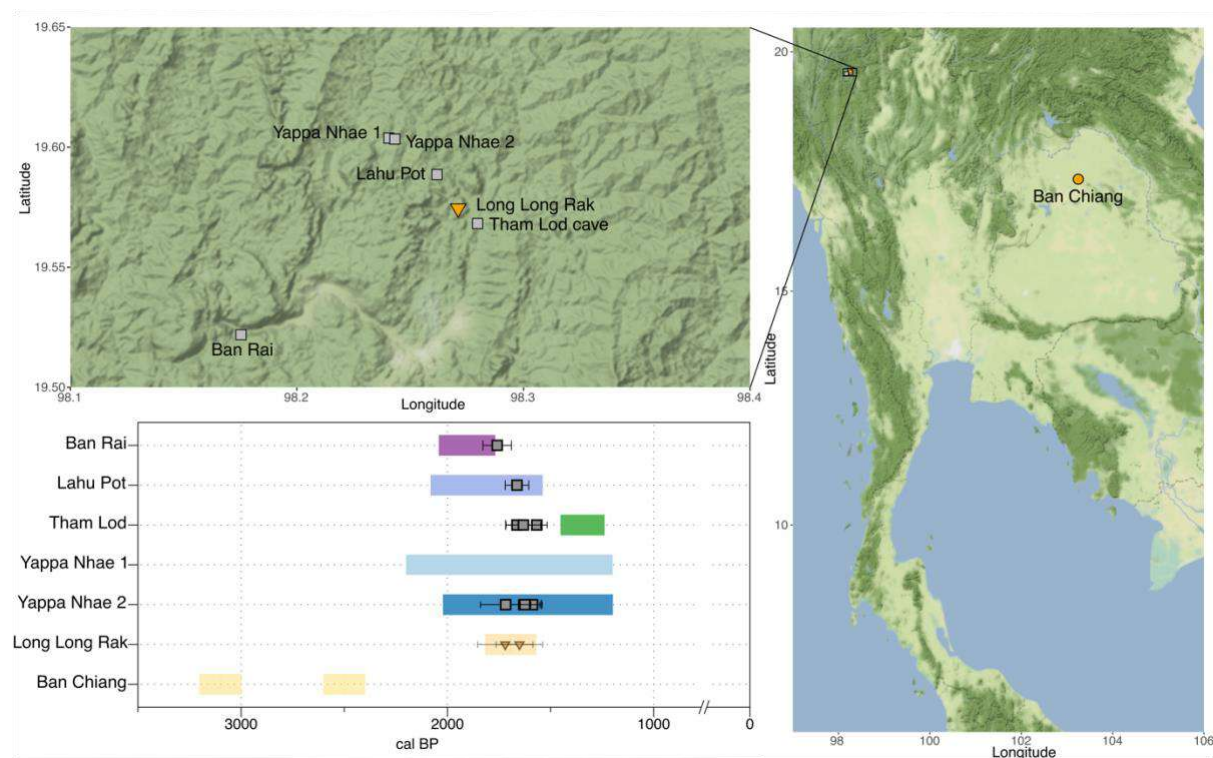


Figure 1. Map showing the location and age of ancient individuals from Thailand; previously published sites marked in orange^{8,10}, bars denote archaeological age and symbols denote direct calibrated ¹⁴C dates ± 1 standard error, plotted with ggmap⁶⁰, map tiles by Stamen Design (CC BY 3.0), data by OpenStreetMap (ODbL).

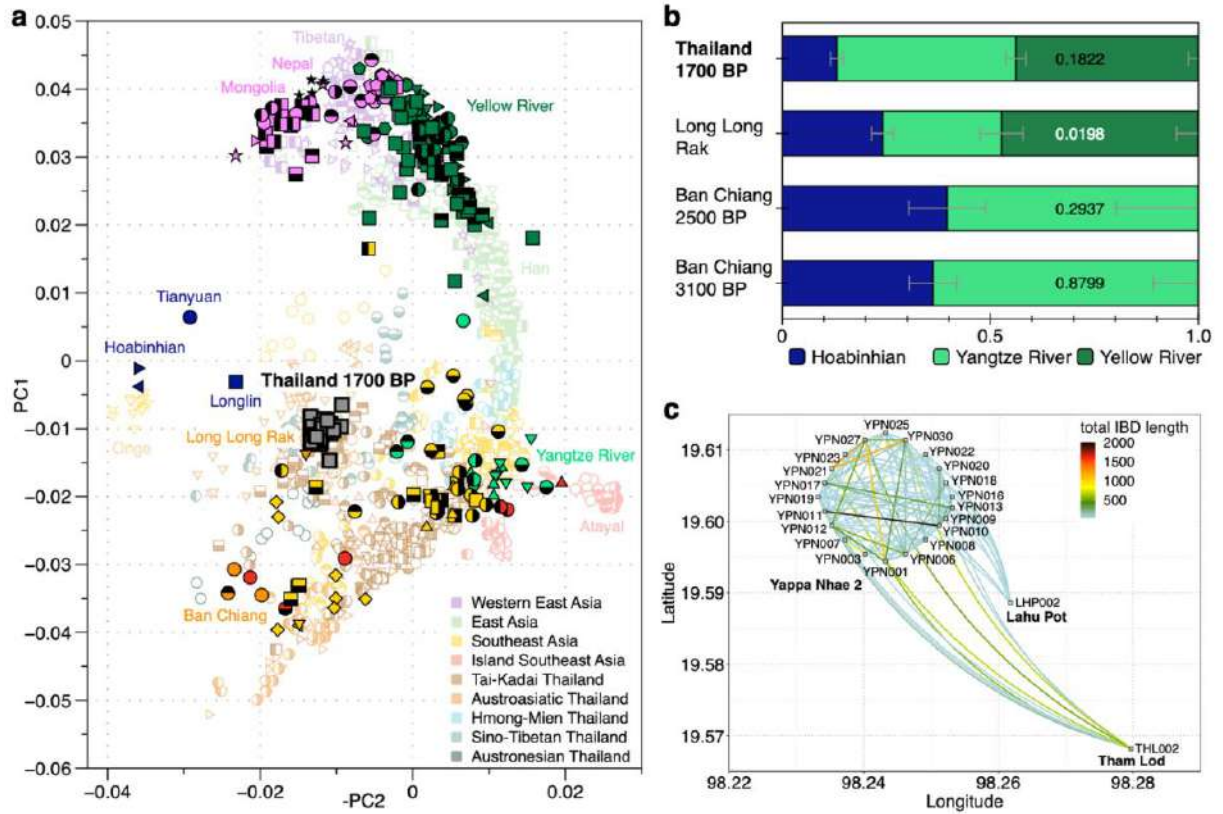


Figure 2. **a**, PCA calculated on present-day individuals from East and Southeast Asia^{8,31–35}, ancient individuals are projected^{7–10,32,36–38}, full legend is available in Fig. S5. **b**, Admixture proportions modelling Log Coffin culture-associated individuals from northwestern Thailand, including Long Long Rak⁸, and Bronze and Iron Age individuals from Ban Chiang, northeastern Thailand¹⁰ as a combination of ancient Hòabìnhian-⁸, Yellow River-³⁸ and Yangtze River-related⁹ ancient individuals, error bars show ± 1 standard error, calculated with block jackknife in qpAdm³⁵, p-values are printed within the bars, p-values in white indicate an unsatisfactory fit ($p < 0.05$). **c**, Shared IBD block lengths between newly generated ancient genomes plotted in the geographical location of the respective sites; individuals from Yappa Nhae 2 have been artificially spread out to show within-site connections.

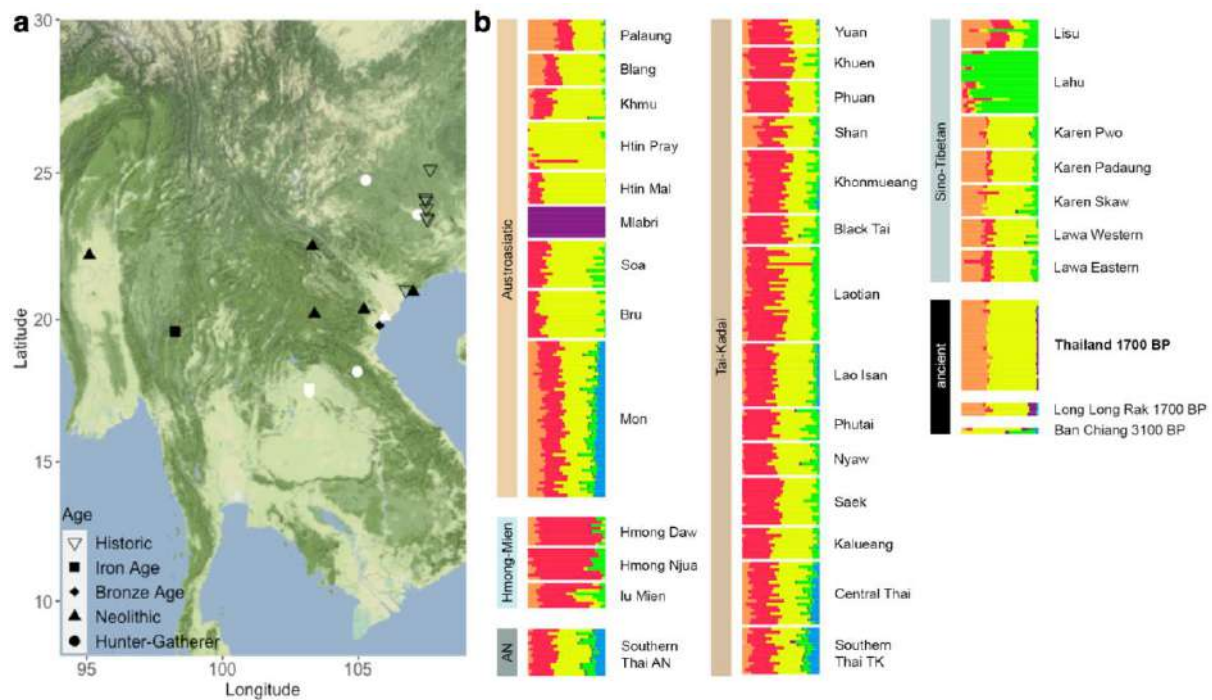


Figure 3. a, Map of mainland Southeast Asia showing the presence (black) and absence (white) of northern East Asian ancestry in ancient individuals^{7,8,10} (Suppl. Mat.), plotted with ggmap⁶⁰, map tiles by Stamen Design (CC BY 3.0), data by OpenStreetMap (ODbL); **b**, ADMIXTURE components of ancient and present-day groups from Thailand^{8,10,31}, represented at K = 8, separated by language family (AN = Austronesian, TK = Tai-Kadai; Suppl. Mat.).

References

1. Zeitoun, V. *et al.* Discovery of an outstanding Hoabinhian site from the Late Pleistocene at Doi Pha Kan (Lampang province, northern Thailand). *Archaeological Research in Asia* **18**, 1–16 (2019).
2. Shoocongdej, R. Late pleistocene activities at the Tham Lod rockshelter in highland Pang Mapha, Mae hong Son province, northwestern Thailand. in *Uncovering Southeast Asia's Past: Selected Papers from the 10th International Conference of the European Association of Southeast Asian Archaeologists* 22–37 (NUS press Singapore, 2006).
3. Treerayapiwat, C. Patterns of Habitation and Burial Activity in the Ban Rai Rock Shelter, Northwestern Thailand. *Asian Perspectives* **44**, 231–245 (2005).
4. Forestier, H. *et al.* The first lithic industry of mainland Southeast Asia: Evidence of the earliest hominin in a tropical context. *Anthropologie* **126**, 102996 (2022).

5. Chitkament, T., Gaillard, C. & Shoocongdej, R. Tham Lod rockshelter (Pang Mapha district, north-western Thailand): Evolution of the lithic assemblages during the late Pleistocene. *Quat. Int.* **416**, 151–161 (2016).
6. Suraprasit, K., Shoocongdej, R., Chintakanon, K. & Bocherens, H. Late Pleistocene human paleoecology in the highland savanna ecosystem of mainland Southeast Asia. *Sci. Rep.* **11**, 16756 (2021).
7. Wang, T. *et al.* Human population history at the crossroads of East and Southeast Asia since 11,000 years ago. *Cell* (2021) doi:10.1016/j.cell.2021.05.018.
8. McColl, H. *et al.* The prehistoric peopling of Southeast Asia. *Science* **361**, 88–92 (2018).
9. Yang, M. A. *et al.* Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* **369**, 282–288 (2020).
10. Lipson, M. *et al.* Ancient genomes document multiple waves of migration in Southeast Asian prehistory. *Science* **361**, 92–95 (2018).
11. Oxenham, M. & Tayles, N. *Bioarchaeology of Southeast Asia*. (Cambridge University Press, 2006).
12. Bellwood, P. *First Farmers: the Origins of Agricultural Societies*. (Backwell, 2005).
13. Diamond, J. & Bellwood, P. Farmers and their languages: the first expansions. *Science* **300**, 597–603 (2003).
14. Higham, C. F. First farmers in mainland Southeast Asia. *JIPA* **41**, 13–21 (2017).
15. Enfield, N. J. & Comrie, B. Mainland Southeast Asian languages. in *Languages of Southeast Asia* (eds. Enfield, N. J. & Comrie, B.) 1–27 (Walter de Gruyter Inc., 2015).
16. Lee, H. Y.-H. & Clontz, J. Reviewing the prehistoric linguistic relationships of the Tai–Kadai language family and its putative linguistic affiliations: a meta–analysis study and abbreviation edition. *JLA*. **4**, 48–48 (2012).
17. Zhang, X. *et al.* A Matrilineal Genetic Perspective of Hanging Coffin Custom in Southern China and Northern Thailand. *iScience* **23**, 101032 (2020).
18. Shoocongdej, R. Craft-specialization in Iron age log coffin culture on Highland Pang Mapha District, Mae Hong Son Province. *Journal of Damrong Vichakarn* **13**, 75–106 (2014).
19. Shoocongdej, R. *et al.* *Final Report of Prehistoric Population and Cultural Dynamic in Highland Pang Mapha, Mae Hong Son Province. Volume II: Archaeology*. (2021).
20. Shoocongdej, R. *Log Coffin in Thailand in Southeast Asian Context*. (Charnsanitwong Press, 2016).

21. Pumijumnong, N. & Wannasri, S. Teak Log Coffins in Northwest Thailand: Dated by Dendrochronology and ^{14}C wiggle Matching. *Applied Environmental Research* **37**, 1–16 (2015).
22. Wannasri, S., Pumijumnong, N. & Shoocongdej, R. Teak log coffin head styles in northern Thailand: Time sequencing with dendrochronology. *Sci. Asia*. **33**, 047 (2007).
23. Buajan, S. *et al.* The stable oxygen isotope ($\delta^{18}\text{O}$) composition of ancient teak log coffins captures the Asian monsoon 2000 years ago in northwestern Thailand. *Theor. Appl. Climatol.* (2021) doi:10.1007/s00704-021-03662-5.
24. Preechamart, S. *et al.* Tree-ring oxygen isotope chronology of teak log coffins in northwestern Thailand and its relationship with Pacific Decadal Oscillation and El Niño-Southern Oscillation. *Quat. Int.* (2021) doi:10.1016/j.quaint.2021.04.007.
25. Fernandes, D. M. *et al.* A genetic history of the pre-contact Caribbean. *Nature* **590**, 103–110 (2021).
26. Sawyer, S., Krause, J., Guschanski, K., Savolainen, V. & Pääbo, S. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLoS One* **7**, e34131 (2012).
27. Fu, Q. *et al.* An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
28. Mathieson, I. *et al.* Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503 (2015).
29. Popli, D., Peyrégne, S. & Peter, B. M. KIN: a method to infer relatedness from low-coverage ancient DNA. *Genome Biol.* **24**, 10 (2023).
30. Ringbauer, H., Novembre, J. & Steinrücken, M. Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* **12**, 5425 (2021).
31. Kutanan, W. *et al.* Reconstructing the Human Genetic History of Mainland Southeast Asia: Insights from Genome-Wide Data from Thailand and Laos. *Mol. Biol. Evol.* **38**, 3459–3477 (2021).
32. Wang, C.-C. *et al.* Genomic insights into the formation of human populations in East Asia. *Nature* **591**, 413–419 (2021).
33. Skoglund, P. *et al.* Genomic insights into the peopling of the Southwest Pacific. *Nature* **538**, 510–513 (2016).
34. Lazaridis, I. *et al.* Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).

35. Patterson, N. *et al.* Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
36. Yang, M. A. *et al.* 40,000-Year-Old Individual from Asia Provides Insight into Early Population Structure in Eurasia. *Curr. Biol.* **27**, 3202–3208.e9 (2017).
37. Jeong, C. *et al.* Long-term genetic stability and a high-altitude East Asian origin for the peoples of the high valleys of the Himalayan arc. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 7485–7490 (2016).
38. Ning, C. *et al.* Ancient genomes from northern China suggest links between subsistence changes and human migration. *Nat. Commun.* **11**, 2700 (2020).
39. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).
40. Higham, C. F. W. From site formation to social structure in prehistoric Thailand. *J. Field Archaeol.* **40**, 383–396 (2015).
41. Wasinger, V. C. *et al.* Analysis of the Preserved Amino Acid Bias in Peptide Profiles of Iron Age Teeth from a Tropical Environment Enable Sexing of Individuals Using Amelogenin MRM. *Proteomics* **19**, e1800341 (2019).
42. Liu, C.-C. *et al.* Ancient genomes from the Himalayas illuminate the genetic history of Tibetans and their Tibeto-Burman speaking neighbors. *Nat. Commun.* **13**, 1203 (2022).
43. Sagart, L. *et al.* Dated language phylogenies shed light on the ancestry of Sino-Tibetan. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 10317–10322 (2019).
44. Schliesinger, J. *The non Tai speaking people*. (White Lotus, 2000).
45. Kutanan, W. *et al.* Cultural variation impacts paternal and maternal genetic lineages of the Hmong-Mien and Sino-Tibetan groups from Thailand. *Eur. J. Hum. Genet.* **28**, 1563–1579 (2020).
46. Changmai, P. *et al.* Indian genetic heritage in Southeast Asian populations. *bioRxiv* 2021.01.21.427591 (2021) doi:10.1101/2021.01.21.427591.
47. Wangkumhang, P. *et al.* Insight into the peopling of Mainland Southeast Asia from Thai population genetic structure. *PLoS One* **8**, e79522 (2013).
48. Samrit, C. Social Network of Log Coffin Culture in Highland Pang Mapha, Mae Hong Son. in *Archaeology of Pre Thai in Highland Pang Mapha, Mae Hong Son Province* 95–112 (Ruen Khew Printing, 2020).
49. Samrit, C. & Shoocongdej, R. Social networks in Log Coffin culture, in highland Pang Mapha district, Mae Hong Son province (2,120–1,250 B.P.). *Damrong Journal*.

50. Pinhasi, R. *et al.* Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS One* **10**, e0129102 (2015).
51. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
52. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
53. Fellows Yates, J. A. *et al.* Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager. *PeerJ* **9**, e10947 (2021).
54. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics* **15**, 356 (2014).
55. Fu, Q. *et al.* A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
56. 1000 Genomes Project Consortium *et al.* A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).
57. Link, V. *et al.* ATLAS: Analysis Tools for Low-depth and Ancient Samples. *bioRxiv* 105346 (2017) doi:10.1101/105346.
58. Rubinacci, S., Ribeiro, D. M., Hofmeister, R. J. & Delaneau, O. Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nat. Genet.* **53**, 120–126 (2021).
59. Patterson, N., Richter, D. J., Gnerre, S., Lander, E. S. & Reich, D. Genetic evidence for complex speciation of humans and chimpanzees. *Nature* **441**, 1103–1108 (2006).
60. Kahle, D. & Wickham, H. ggmap: Spatial visualization with ggplot2. *The R Journal* **5**, 144–161 (2013).

3.4 Manuscript D: Investigating the occupation history, social hierarchy, and life histories at an early colonial site in Taiwan through archaeology, archaeoethanatology, and archaeogenetics

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Beginning in the 16th century, the majority of Southeast Asia was colonised by European countries, the consequences of which are still felt today. From their base in Manila on the Philippines, Spain commissioned the colonisation of Heping Dao in northern Taiwan in 1626 to increase trade with China. Dominican priests were closely involved in the establishment of the settlement of San Salvador and had a large stone church built. Excavations on Heping Dao revealed the foundations of this convent, an associated cemetery, and a total of 19 burials between 2011 and 2019. In this project, we apply archaeological, archaeoethanological, and archaeogenetic methods to an early colonial graveyard for the first time in the Asia-Pacific region. Five individuals were interred within the church, the most desirable location in Christian communities. In Heping Dao, all individuals most closely related to medieval and present-day groups from Europe are buried inside the church, suggesting that social hierarchy was at least in part driven by genetic ancestry. Four of these burials were oriented south-north, following the church orientation, and were genetically most similar to groups from medieval Spain, while one burial was oriented west-east and harboured more northern European-related ancestry. Since San Salvador was taken over by Dutch forces in 1642, this might indicate the continued use of the originally Catholic church and hallowed grounds by the Protestants. Following this argument, the burials outside the church belong to both occupation phases. Corresponding to the vast abundance of Chinese material culture and historical descriptions of a Chinese residential quarters, most individuals in the cemetery were Christian converts of costal Han Chinese-related ancestry. Buried among these are also five individuals most closely related to present-day Southeast Asian groups. While two individuals buried in supine position harbour Island Southeast Asian-related ancestry, three individuals – two adults in flexed position and one child jar burial – are genetically most similar to ancient and Indigenous groups from Taiwan. The practice of these Indigenous, Austronesian-related rites within the Christian cemetery, potentially during the Dutch occupation, raises new questions about the interactions of local groups and colonisers at the colonial periphery.

Investigating the occupation history, social hierarchy, and life histories at an early colonial site in Taiwan through archaeology, archaeoethanatology, and archaeogenetics

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Abstract

Excavations at Heping Dao, an island on the northern coast of Taiwan, have revealed archaeological evidence of a colonial settlement successively occupied by Taiwanese in prehistory, and Spanish, Dutch, Chinese, Japanese, and the Kuomintang from the early historical period to this day. During the early colonial period, in the 17th and 18th centuries, a church and an adjacent cemetery were built, where nineteen individuals were recovered between 2011 and 2019. The burials displayed various body treatments and orientations and were located inside and outside the church. We used archaeological, archaeoethanatomical, and archaeogenetic methods to study this distinctive spatial pattern and to investigate colonial community structure. Our results showed that the distribution of burials likely reflected the different occupation periods, social structure, and life histories of the Heping Dao colony, which in turn is connected to the individual biological identity of the dead. As the only early colonial-period cemetery exhaustively excavated and analysed so far in the Asia-Pacific, the

combination of different scientific approaches allowed for the rendition of a more detailed picture of early colonial settlements in the Asia-Pacific region.

Introduction

The colonization of the majority of the Asia-Pacific region by European countries had a vast effect on past and present-day inhabitants of the region. With its beginnings in the 16th century, a large number of historical documents about the colonial occupation are available and have been studied to understand the relationships between colonizers and Indigenous peoples and also the biases present in these primary sources¹, mainly focusing on Chinese, Malay, and Spanish perspectives on pre-colonial Philippine society². However, archaeological excavations of colonial sites in the region, which allow the direct study of initial contact, development of the site and impact on the local communities and environment³, are rare⁴⁻⁷.

The Spanish colonization of Taiwan has been well-documented in Spanish letters, administration and missionary reports, as well as records of the Dutch East India Company^{8,9}. Driven by the desire to counterbalance increasing Dutch influence in the region, re-establish trading with China and accelerate evangelization¹⁰, the Spanish governor of Manila Fernando de Silva commissioned the founding of San Salvador de Quelang on Heping Dao, an island situated off the coast of present-day Keelung in northern Taiwan in 1626 and Santo Domingo in present-day Tamsui in 1629^{9,10} (Fig. 1). Since the Dutch had already founded a first settlement in the bay of Tainan in 1624, the Spanish arrived in San Salvador with three infantry companies and established a large fortress comparable to those in the Caribbean, three auxiliary forts, a stone church, a convent, and residential quarters^{3,11,12}. According to Spanish sources, they encountered Indigenous Taiwanese who were living in a village called Quimaurri, comprised of 300-1,500 houses and made their living as fishers, hunters, crafters, traders, and farm workers for other Indigenous groups, as well as a small number of Chinese fishermen and traders^{9,12,13}. As intermediaries between Chinese boats and other Taiwanese groups, the inhabitants of Quimaurri exchanged deerskins and Sulphur for rice, silk, pottery, iron or cereals using a trade language understandable by groups speaking various Austronesian languages¹³. However, the initial contact between the colonists and Indigenous people was limited, as the village was abandoned upon the arrival of the Spanish and trade was only established slowly¹³. To support the new colony and establish trade connections, Manila regularly sent galleons to San Salvador, which employed Spaniards, Filipinos, Chinese, mestizos, people from Malabar, and possibly also Africa¹⁴. Historical records also

indicate a substantial number of Chinese labourers in San Salvador working as stonemasons, carpenters, and sawyers¹⁴. Combined with trade agreements with China and the large number of Europeans stationed there^{9,12,13}, San Salvador was a contact point between people from all over the world¹².

Archaeological excavations in Heping Dao started in 2011^{11,12} and revealed archaeological stratigraphy reaching from Middle Neolithic Yuanshan- (from ca. 3,500 years ago) over Iron Age Shisanhang-associated material (from ca. 2000 years ago) into contemporary times¹¹. During the early historical period, Heping Dao was under Spanish rule from 1626 to 1642, Dutch rule (from 1642 to 1662) and conquered by Qing forces in 1662¹¹. Most recovered artefacts are associated with the later Japanese occupation between 1895 and 1945¹¹.

Since the Dominican order was a fundamental axis of the Spanish conquest of Taiwan and the foundation of San Salvador, the Catholic priests established a very visible presence in the new colony with the construction of the Convent of All Saints (Convento de Todos los Santos) of big carved sandstone blocks, which stood out among the surrounding thatched wooden houses^{11,12}. The excavations revealed the convent's foundations and an associated cemetery in 2012. Likely established soon after the church's construction in the 1630s¹¹, the cemetery offers the unique opportunity to study the people interacting and being buried during the early colonial period.

Previously, we have reported the discovery of five burials, which already indicated a variety in burial practices in Heping Dao^{11,12}. Oriented perpendicular to the church (West to East), four adult individuals in supine position and a child in flexed position within a jar were buried near the foundations of the church¹². Traces of a coffin and the interment within the church interior for burial Area1UE107 suggest the burial of a prominent figure or priest, while the jar burial reflects an Austronesian practice^{12,15}. PCR-based analyses of mitochondrial DNA extracted from burial T3P2UE26, interred outside the church, indicated a European haplogroup, but attempts to extract ancient DNA from another individual (T2P9UE40) were unsuccessful¹². However, this was the first direct bioarchaeological evidence of the European presence at the site, which is otherwise only represented by two buckles and a Caravaca cross^{11,16} and mostly indirectly inferred by the abundance of Chinese material culture³. However, Chinese stoneware from Heping Dao dated to the 15th-17th century and evidence of contact since the late 9th century in other regions of Taiwan indicated that Han material culture was well-established before European contact¹¹. Material culture studies showed the

reliance of the European colonists on the existing Taiwanese trading networks and ways of life, which is also corroborated by the usage of trees native to the Taiwanese cloud forests for the coffin of burial Area1UE107¹⁶.

In this study, we summarize the results of further archaeological excavations at the colonial graveyard of San Salvador on Heping Dao, Taiwan (site name: Heping Dao-B, Fig. 1). We use archaeological and archaeothanatological methods to study the burial practices of 19 individuals excavated until 2019. The burials include 18 adults, of whom 16 are buried in supine and two in flexed position, and one child jar burial. We evaluate these in conjunction with genetic ancestry inferred through ancient DNA to investigate social structure at a colonial-era site in the Asia-Pacific region.

Results

A total of nineteen burials were excavated at Heping Dao-B between 2011 and 2019 by an international team under a Spanish and Taiwanese consortium, led by M.C.B., C.-h.T., and E.H. (since 2019; Fig. 1b, 2c). Four of these burials (Area1ue107-HPD001/6, Area1ue132-HPD002/5, T2P2ue26-HPD003/7, T2P9ue72-HPD004) have been described in detail previously^{11,12}. North of HPD001 within the church foundations, four adult burials in supine position (HPD008-11) and one pit (Area2ue38) were excavated. As opposed to HPD001, these were oriented south-north. We recovered six adult burials in supine position oriented west-east just south of HPD002/5 and HPD003/7 outside the church. South of these were four adult burials in supine position oriented south-north and one adult burial in supine position oriented north-south. To the left and right of these, we discovered two adult flexed burials oriented west-east placed on the right side of the body and a child buried within a jar (HPD004). Analyses of burials recovered since 2019 are still forthcoming.

Samples HPD001-7 were retrieved by F.V., while S.C. and the archaeological team recovered petrous bones and teeth of individuals HPD008-21 in situ in 2019. We extracted ancient DNA from the petrous portion of the temporal bone and teeth of 18 individuals from Heping Dao at the Max Planck Institute for the Science of Human History, Jena, Germany (now MPI of Geoanthropology). After library preparation and shotgun screening, we confirmed the authenticity of the retrieved DNA fragments by short median fragment size and elevated rates of cytosine to thymine transversions on the 5' end of reads (Fig. S1). We then used in-solution hybridization capture to enrich for ca. 1,240,000 single nucleotide polymorphisms (SNPs)

across the human genome (1240K capture^{17,18}), which resulted in a median of 503,148 SNPs for each individual (SI Table 1). We identified 16 individuals as XY and two individuals as XX and attained Y-haplogroups O2a2b2a, O1a1a and R1b1a1b1a for the XY individuals, while the mitochondrial haplogroups recovered after mitochondrial capture¹⁹ were much more diverse (B, D, F, H, J, K, M, U) (SI Table 1, Suppl. Mat.). We could confirm the association of sampled teeth and petrous bones as genetically identical individuals but did not identify any other genetic relationships between the sampled individuals (Suppl. Mat.). Three individuals showed elevated runs of homozygosity which indicated small population size (HPD004) and close genetic relationships between parents (HPD001, HPD013) (Fig. S2).

To initiate the population genetic analyses, we calculated a principal component analysis (PCA) on present-day individuals from Eurasia^{20–24} (Fig. S3). Five individuals from Heping Dao clustered with groups from Southeast and eight with groups from East Asia, while another five individuals clustered with groups from western Eurasia. Two regional PCAs with exclusively groups from eastern and western Eurasia, respectively, showed further separation among the ancient individuals (Fig. 2a, b, Fig. S4). We could also confirm these visual affiliations with genetic drift calculations of the form $f_3(\text{Mbuti, present-day group, test})$ and ADMIXTURE proportions (Fig. S5, S7).

Within eastern Eurasia, HPD004, HPD017, and HPD020 clustered with present-day Indigenous and ancient individuals from Taiwan and were genetically consistent with 1st-8th century CE Hanben from Taiwan²⁴ in qpWave²⁰ (SI Table 2). The comparison with present-day genomes from multiple Indigenous groups from Taiwan showed closest similarity of the ancient individuals to Rukai, Atayal, and Tao²⁵, and did not show evidence for additional ancestry coefficients in ADMIXTURE analysis (Fig. S7). Individuals HPD012 and HPD015 clustered with individuals from Southeast Asia in the PCA. While HPD015 was consistent with present-day Igorot from the Philippines²⁶, HPD012 showed different Southeast-related ancestry in qpWave and ADMIXTURE (Fig. S7, SI Table 2). HPD002-3, HPD013-14, HPD016, HPD018-19, and HPD021 clustered with present-day Han Chinese²⁴ and were genetically consistent with the 300-year-old Chuanyun individual from Fujian Province, coastal China²⁷ in qpWave²⁰ (SI Table 2).

On the western Eurasian PCA, HPD008 and HPD010 clustered on top of present-day Spanish and near 8th-12th century individuals from Girona in northern Spain²⁸ (Fig. 2b), who they were genetically consistent with in qpWave²⁰ (SI Table 4). HPD011 clustered and shared most drift

with present-day northern Spanish and Basque individuals (Fig. S5). HPD009 clustered within a group from 10th-16th century Granada, southern Spain²⁸ and can similarly be modelled as a genetic admixture between 8th-12th century Gironan, northern African and Middle Eastern individuals²⁸ (Fig. S9, SI Table 5). HPD001 did not fall with previously published contemporary Iberian individuals on the PCA but clustered with present-day French, a 10th-12th century individual from northern Germany and near 7th-11th century individuals from the Netherlands²⁹. Using qpWave²⁰, we identified this individual as most similar to 7th-11th century individuals from the Netherlands with previously identified Dutch and Spanish genetic components²⁹ and could model their ancestry as ~80% 7th-11th century Netherlands and ~20% 8th-12th century Girona (Fig. S8, SI Table 3).

Discussion

One of the most striking spatial patterns at Heping Dao-B is the selective interment of five individuals within the church foundations, while the remaining individuals were buried in the church adjacent cemetery. Based on contemporary Christian burial practices in Europe³⁰, a church's interior, cloistral area or crypt were the preferred places of interment for more prominent congregation members, of which the clergy were buried closer towards the altar¹². San Salvador reportedly hosted more than 48 primarily Dominican priests who were likely all Europeans, for example, the priests Fr. Antonio de Viana (Portugal) or Fr. Mateo de Cobissa (Spain)⁹. The burial of prominent Spanish colonists in the church interior was also attested in the Philippines, where it was only possible for Filipinos to be buried inside the church once a wealthy upper class had been established who could afford the large sums required³¹. This stratification of burials, therefore, likely reflected the social hierarchy during everyday life within the colonial community at San Salvador. The spatial separation between individuals genetically similar to present-day groups from East and Southeast Asia buried outside the church and those similar to groups from Europe inside the church also suggests a genetic ancestry aspect to the burial placements and social structure.

Inside the church, four burials and one empty pit were oriented south-north, while one was interred with the body facing west-east. Similar to the orientation of medieval Christian churches, according to the Christian rite, burials were preferably placed along a west-east axis with the head in the west facing towards the altar¹². Strict observation of this rite has been documented in Spanish^{32,33} and post-reformation Dutch territories in the 16th-19th century^{34,35}. However, exact orientation towards geographic east during church construction started losing

importance from the beginning of the 16th century in favour of adaptation to the local geographic situation³⁶, as was likely the case in San Salvador, where the church was oriented north-south. Therefore, it seems likely that the graveyard was used during two phases with differences in orientation priorities, where the south-north oriented burials follow the orientation of the church and the later burials are oriented traditionally west-east, also within the church. This would fit well within the context of the initial Spanish (Catholic) colony conquered by the (Protestant) Dutch in 1642 and might indicate the continued use of the cemetery and church despite the contemporary wars between Christian denominations in Europe. The Dutch reportedly gathered all inhabitants of San Salvador in the convent during their conquest¹², so it must have still been standing at the time, and archaeological intrusions into burials date to the consequent Qing period¹¹. A strong argument for the continued use of the Catholic sacred grounds by the Protestants in San Salvador is the exact correspondence between burial orientation and genetic similarity of the burials within the church, as south-north orientation corresponded to genetic similarity with medieval Spanish and west-east to medieval Dutch individuals.

In the cemetery outside the church eleven individuals were interred in a supine position with varying arm placements, of which three burials were oriented south-north, one north-south, and nine west-east. While these individuals were all most closely related to individuals from eastern Eurasia, the burial orientation did not correspond to closest genetic ancestries, indicating presence of Han Chinese- and Southeast Asian-related individuals during the Spanish and the Dutch occupation. The majority of individuals were genetically most similar to present-day Han from coastal and central China, correspondent to the abundant Chinese material culture recovered from the site^{3,11} and a large number of Fujianese emigrants to other Spanish colonies, such as Manila³⁷. Their large presence on the colonial graveyard is noteworthy in the light of the xenophobic attitude and massacres of Spanish authorities towards the Chinese community in Manila despite its reliance on Chinese-offered products and services^{10,37,38}, as differences in size, reliance on trading with, and pre-colonial presence of Chinese settlers between San Salvador and Manila might have resulted in differences in racial politics³. A Chinese residential and market quarter (parian) was recorded to have developed in San Salvador just before 1630 and was claimed by Chinese authorities as in Manila and other parts of Southeast Asia¹⁰. The supine burial position, the praying attitude of the hands, and the use of shrouds instead of the traditional concrete and stone coffins with coin grave goods^{12,39,40} suggest that the excavated individuals were Christian converts, of

which there were reportedly ‘not few’ in San Salvador and who had a small chapel in the parian¹⁰. Two of these individuals were genetically most similar to present-day Southeast Asian groups, as indicated by Filipino pottery sherds¹¹. Indeed, many Filipino draftees and labourers are mentioned in historical documents and would have been the main demographic component of the colony^{12,14}. Interestingly, one of these burials was oriented west-east, belonging to the later Dutch period.

Similarly oriented west-east were also two flexed adult burials positioned on the side and a jar burial of a child. These mortuary practices were common among contemporary Taiwanese and Austronesian groups^{12,15} and stratigraphic analyses have indicated that these Indigenous burials were contemporary to the cemetery. Some rite modifications (e.g. the Chinese stoneware vessel used as a burial jar) were consistent with contact outside the community¹¹. These individuals were also genetically most similar to present-day Indigenous groups from Taiwan and shared the same mitochondrial haplogroup (M7c1c), which nowadays occurs at a very high frequency in the Puyuma and Tao communities from southeast Taiwan⁴¹. The elevated levels of homozygosity within the child’s genome indicated a small Indigenous community at the site before or during the colonial occupation. The west-east orientation of the adult Indigenous burials might indicate interment in the European colonial cemetery during the Dutch occupation. This raises new questions about the position of the Indigenous community and their potential conversion to Christianity under the European rule. Both Spanish and Dutch systematically married Indigenous Taiwanese women as a way to consolidate their presence in Taiwan^{9,12,42}. The use of the Indigenous rite within the Christian cemetery opens up questions on the application of religious orthodoxy in these remote colonies.

Our results show that the burial location, orientation, and rite near the Convento de Todos los Santos reflect the social hierarchy, occupation history, and impact on the Indigenous communities during the colonial occupation. We demonstrate that archaeological and archaeothanatological analyses, supported by archaeogenetics, have great potential to better understand the community structure and development of early colonial sites in the Asia-Pacific region, a place and period which remain understudied.

Methods

Sampling, extraction, library preparation and indexing were performed in a dedicated clean room for ancient DNA at the Max Planck Institute for the Science of Human History in Jena,

Germany (now MPI of Geoanthropology). We obtained bone powder by cutting pars petrosa along the margo superior and drilling near the cochlear⁴³ and by cutting teeth along the cemento-enamel junction and drilling bone powder from the tooth pulp. For HPD001-007, we extracted DNA using a modified version of the standard ancient DNA protocol⁴⁴ and built double-stranded libraries after partial uracil-DNA glycosylase treatment⁴⁵. We used a bead-based extraction method for the remaining samples⁴⁶ and built single-stranded libraries on an automated liquid handling system⁴⁷. After double indexing with unique index combinations⁴⁸, all libraries were shotgun-sequenced for a depth of 3-7 million reads on an Illumina HiSeq 4000 using a 75 bp single-read configuration. After further amplification, the libraries were hybridized in-solution to enrich for a targeted set of 1,237,207 SNPs across the human genome ('1240K' capture^{17,18}).

After sequencing, we removed adapters and duplicates, mapped to *hs37d5*, calculated karyotype and nuclear contamination, and genotyped the captured sequences with *nf-core/eager* v2.4.4⁴⁹. We built mitochondrial consensus sequences with *Geneious* v2019.2.3⁵⁰ and used these to determine mitochondrial haplogroups with *Haplogrep* v2.4.0⁵¹ and to estimate mitochondrial contamination with *ContamMix* v1.0-10¹⁹. We remapped the 1240K-captured sequences to include neighbouring SNPs and compared all ancestral and derived SNP positions on the Y-chromosome to determine Y-haplogroups⁵². We used *READ* vf541d55⁵³ to assess genetic relatedness within the dataset and detected runs of homozygosity in all individuals with *hapROH* v0.3a4⁵⁴. Principal component analyses (PCAs) were performed using *smartpca* v1600⁵⁵ with shrinkmode and lsqmode enabled, calculating the PCs from present-day Eurasian individuals²⁰⁻²⁴ and projecting all ancient individuals^{24,28}. All f_3 -statistics were calculated using *qp3pop* v651²⁰ setting 'inbred: YES'. We used *qpWave* v1520 and *qpAdm* v1520 with 'inbred: NO', 'allsnps: YES', and 'details: YES'²⁰.

Data availability

The raw and aligned sequences will be available at the European Nucleotide Archive upon submission.

Acknowledgements

M.C.B. was funded by STAR2-Santander Universidades and Ministry of Education, Culture and Sports (Program Campus de Excelencia Internacional, call CEI 2015 of the project Cantabria Campus Internacional), Chiang Ching-kuo Foundation for International Scholarly

Exchange, Taiwan (RG011-U-12, RG003-U-20), Ministry of Culture and Sport, Spain (T002020N0000044971), Ministry of Culture, Spain (32-T002018N0000042763), Fundación Palarq (54.S079.64699), Zukunftskolleg of the University of Konstanz (EU FP7 Marie Curie Zukunftskolleg Incoming Fellowship Program, University of Konstanz, grant no. 291784), Ministry of Economy and Competitiveness, Spain (HAR2011-16017-E), the FORMOSA 2010 Program CSIC/National Science Council, Taiwan (2010TW0023), and the Ministry of Science and Innovation, Spain (PID2020-116196GB-I00). C.-h.T., E.H., and M.C.B. were also funded by the Keelung Cultural Affairs Bureau, Taiwan (1080001631). The genetic research was funded by the ERC Starting Grant ‘Waves’ (ERC758967) and the Max Planck Society. We thank the laboratory and bioinformatics team at the Max Planck Institute for Evolutionary Anthropology, in particular Rita Radzeviciute and Ana Gomes, for their excellent support.

Author contributions

M.C.B., C.-h.T., F.V., C.P., and J.K. conceived the study. M.C.B. and C.-h.T. led the archaeological excavations. F.V. conducted the anthropological analyses. S.C. analysed the genomic data with critical input from K.N., C.P., M.C.B., and F.V. S.C. wrote the manuscript with critical input from K.N., M.C.B., F.V., and the remaining authors.

Figures

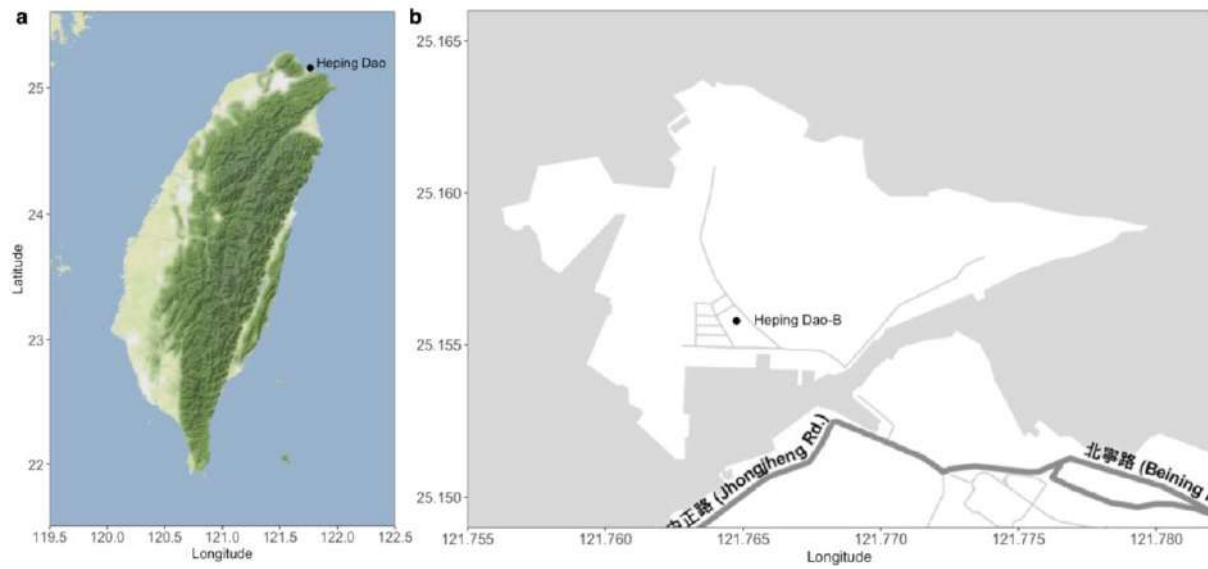


Figure 1. **a**, Position of Heping Dao off the northern coast of Taiwan; **b**, location of the excavation site Heping Dao-B on the island; plotted with ggmap⁵⁶, map tiles by Stamen Design and data by OpenStreetMap.

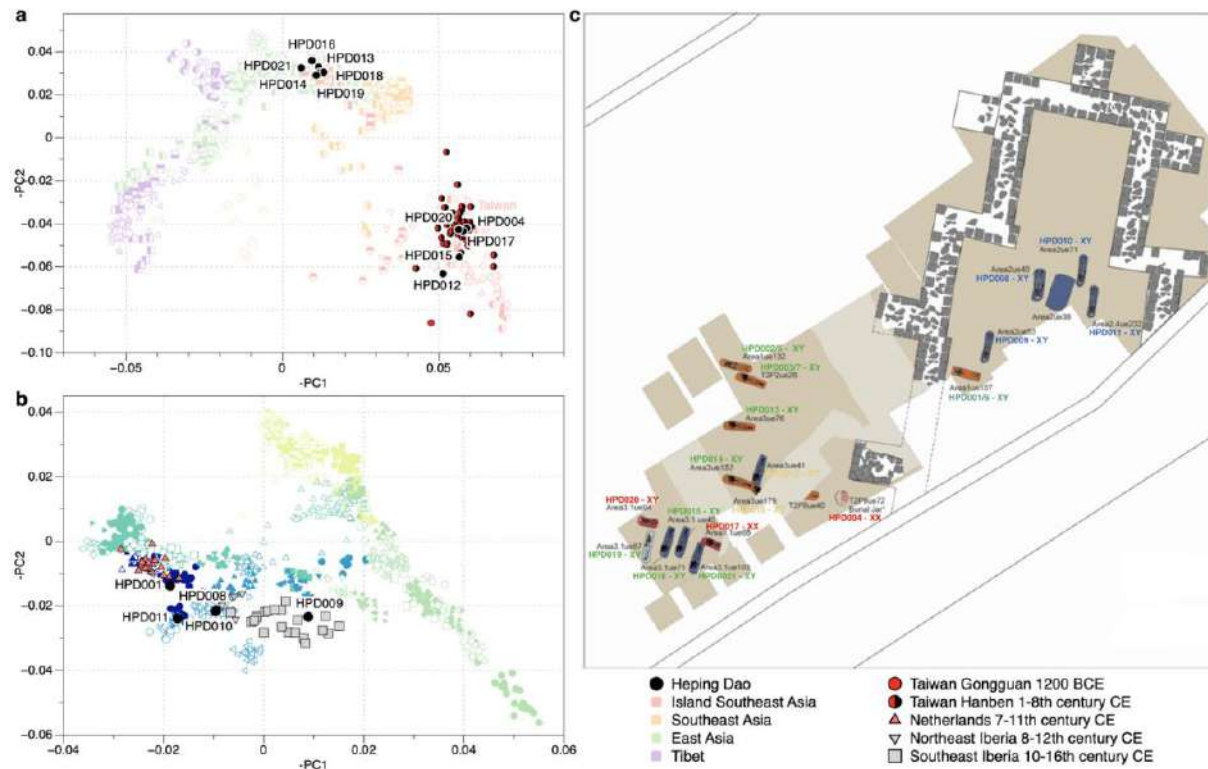


Figure 2. Ancient individuals^{24,28,29} projected onto PCAs calculated on present-day individuals from eastern (a) and western Eurasia (b)^{20–25}; excavation plan of the church foundations and associated burials and pits at Heping Dao, labels denote archaeological and laboratory ID and karyotype, colour indicates genetic ancestry, adapted from Elena Serrano Herrero.

References

1. Lape, P. Archaeological approaches to the study of Islam in Island Southeast Asia. *Antiquity* **79**, (2005).
2. Junker, L. L. Integrating History and Archaeology in the Study of Contact Period Philippine Chiefdoms. *Int. J. Hist. Archaeol.* **2**, 291–320 (1998).
3. Cruz Berrocal, M. Ilha Formosa, Seventeenth Century: Archaeology in Small Islands, History of Global Processes. in *Archaeologies of Early Modern Spanish Colonialism* (eds. Montón-Subías, S., Cruz Berrocal, M. & Ruiz Martínez, A.) 281–302 (Springer International Publishing, 2016).
4. Peterson, J. A. *et al.* Visayan Settlement by the River: Archaeological Investigations at the late 16th and 17th century Site of Salug in Carcar, Cebu. *Philipp. Q. Cult. Soc.* **33**, 155–217 (2005).

5. Cuevas, N. T., Calugay, C. G. & Bersamira, E. D. A preliminary archaeological investigation of the c. 13th century pre-Spanish Cebuano community in the municipalities of Carcar and Sibonga, Southern Cebu. *Philipp. Q. Cult. Soc.* (2010).
6. Bersales, J. E. R. & De Leon, A. The Archaeology of an Augustinian Frontier Mission: A Report on the Fifth Phase of Excavations at the Boljoon Parish Church, Cebu. *Philipp. Q. Cult. Soc.* **39**, 185–213 (2011).
7. Montón-Subías, S., Moragas Segura, N. & Bayman, J. M. The First Missions in Oceania: Excavations at the colonial church and cemetery of San Dionisio at Humåtak (Guam, Mariana Islands). *Journal of Pacific Archaeology* **10**, (2020).
8. Blussé, L. & Everts, N. The Formosan encounter. Notes on Formosa's aboriginal society: A selection of documents from Dutch archival sources. Vol. II: 1636--1645. Preprint at (2000).
9. Borao, J. E., Heyns, P., Gomez, C. & Zanduetta Nisce, A. M. *Spaniards in Taiwan Vol. I & Vol. II*. (SMC Publishing, Taipei, 2002).
10. Borao, J. E. An overview of the Spaniards in Taiwan (1626-1642). in *Proceedings of the Conference on China and Spain during the Ming and Qing Dynasties* (2007).
11. Cruz Berrocal, M. *et al.* A Comprised Archaeological History of Taiwan through the Long-Term Record of Heping Dao, Keelung. *Int. J. Hist. Archaeol.* **22**, 905–940 (2018).
12. Cruz Berrocal, M. *et al.* The Study of European Migration in Asia-Pacific During the Early Modern Period: San Salvador de Isla Hermosa (Keelung, Taiwan). *Int. J. Hist. Archaeol.* **24**, 233–283 (2020).
13. Ollé. Castilians in the North of Taiwan: Frontier Interactions at Isla Hermosa. *Taiwan—A Bridge between the East and South China* (2011).
14. Fang, C. Naufragio, colonización y comercio: relaciones entre Filipinas y Taiwán en los siglos XVI y XVII. *Boletín del Instituto de Estudios Latinoamericanos de Kyoto* **12**, (2014).
15. Valentin, F., Choi, J., Lin, H., Bedford, S. & Spriggs, M. Three-thousand-year-old jar-burials at the Teouma cemetery (Vanuatu): A Southeast Asian-Lapita connection. in *The Lapita cultural complex in time and space: Expansion routes, chronologies and typologies* 81–101 (2015).
16. Martín Seijo, M., Cruz Berrocal, M., Serrano Herrero, E. & Tsang, C. Wooden material culture and long-term historical processes in Heping Dao (Keelung, Taiwan). *J. Archaeol. Sci.* **133**, 105443 (2021).

17. Fu, Q. *et al.* An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
18. Mathieson, I. *et al.* Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503 (2015).
19. Fu, Q. *et al.* A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
20. Patterson, N. *et al.* Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
21. Lazaridis, I. *et al.* Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
22. Skoglund, P. *et al.* Genomic insights into the peopling of the Southwest Pacific. *Nature* **538**, 510–513 (2016).
23. Jeong, C. *et al.* The genetic history of admixture across inner Eurasia. *Nat Ecol Evol* **3**, 966–976 (2019).
24. Wang, C.-C. *et al.* Genomic insights into the formation of human populations in East Asia. *Nature* **591**, 413–419 (2021).
25. Liu, D., Ko, A. M.-S. & Stoneking, M. The genomic diversity of Taiwanese Austronesian groups: implications for the ‘Into and Out of Taiwan’ models. *bioRxiv* 2023.01.09.523210 (2023) doi:10.1101/2023.01.09.523210.
26. Mallick, S. *et al.* The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**, 201–206 (2016).
27. Yang, M. A. *et al.* Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* **369**, 282–288 (2020).
28. Olalde, I. *et al.* The genomic history of the Iberian Peninsula over the past 8000 years. *Science* **363**, 1230–1234 (2019).
29. Gretzinger, J. *et al.* The Anglo-Saxon migration and the formation of the early English gene pool. *Nature* **610**, 112–119 (2022).
30. Dexeus, A. The Bones of our ancestors. The end of burials in churches in the late 18th century. *Contributions to science* **11**, 85–94 (2016).
31. Dakudao, M. E. The Development of Cemeteries in Manila before 1941. *Philipp. Q. Cult. Soc.* **20**, 133–150 (1992).

32. Lawrence, J. W. Archaeology and Ethnohistory on the Spanish Colonial Periphery: Excavations at the Templo Colonial in Nicoya, Guanacaste, Costa Rica. *Hist. Archaeol.* **43**, 65–80 (2009).
33. Santana, J. *et al.* The early colonial atlantic world: New insights on the African Diaspora from isotopic and ancient DNA analyses of a multiethnic 15th-17th century burial population from the Canary Islands, Spain. *Am. J. Phys. Anthropol.* **159**, 300–312 (2016).
34. Abrahams-Willis, G. & Fouche, K. Burial from the seventeenth century Dutch Fort De Goede Hoop at the Cape. *Southern African Field Archaeology* **4**, 95–102 (1995).
35. Hagemeyer, A. Buried in the chapel of the St. Josbgasthuis in Utrecht. (Saxion University of Applied Sciences, 2021).
36. Arneitz, P., Draxler, A., Rauch, R. & Leonhardt, R. Orientation of churches by magnetic compasses? *Geophys. J. Int.* **198**, 1–7 (2014).
37. Chia, L. The Butcher, the Baker, and the Carpenter: Chinese Sojourners in the Spanish Philippines and their impact on Southern Fujian (Sixteenth-Eighteenth Centuries). *J. Econ. Soc. Hist. Orient* **49**, 509–534 (2006).
38. Borao, J. E. The Massacre of 1603 Chinese Perception of the Spanish in the Philippines. *Itinerario* **22**, 22–40 (1998).
39. Salmon, C. Ancient Chinese Cemeteries of Indonesia as Vanishing Landmarks of the Past (17th-20th c.). *Arch.* 23–61 (2016).
40. Sebillaud, P., Berger, E., Xiaoxi, L. & Kan, H. A Glimpse into Burial Practices of Medieval (10th to 13th c. AD) Elites in the Horqin Region, Northeast China. *Bulletin de l'École française d'Extrême-Orient* **105**, 255–320 (2019).
41. Trejaut, J. A. *et al.* Traces of archaic mitochondrial lineages persist in Austronesian-speaking Formosan populations. *PLoS Biol.* **3**, e247 (2005).
42. Fetzer, J. & Soper, J. C. Church and state in Spanish Formosa. *Rev. Relig. Chin. Soc.* **1**, 236–248 (2014).
43. Pinhasi, R. *et al.* Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS One* **10**, e0129102 (2015).
44. Dabney, J. *et al.* Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 15758–15763 (2013).

45. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
46. Dabney, J. & Meyer, M. Extraction of Highly Degraded DNA from Ancient Bones and Teeth. *Methods Mol. Biol.* **1963**, 25–29 (2019).
47. Gansauge, M.-T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**, 2279–2300 (2020).
48. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
49. Fellows Yates, J. A. *et al.* Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager. *PeerJ* **9**, e10947 (2021).
50. Kears, M. *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
51. Weissensteiner, H. *et al.* HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* **44**, 58–63 (2016).
52. Rohrlach, A. B. *et al.* Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-path Neolithic expansion to Western Europe. *Sci. Rep.* **11**, 15005 (2021).
53. Monroy Kuhn, J. M., Jakobsson, M. & Günther, T. Estimating genetic kin relationships in prehistoric populations. *PLoS One* **13**, e0195491 (2018).
54. Ringbauer, H., Novembre, J. & Steinrücken, M. Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* **12**, 5425 (2021).
55. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
56. Kahle, D. & Wickham, H. ggmap: Spatial visualization with ggplot2. *The R Journal* **5**, 144–161 (2013).

4. The Potential and Challenges of Archaeogenetics in Southeast Asia

4.1 Overcoming challenging DNA preservation conditions

For the archaeogenetic studies presented in this thesis, we sampled a total of 136 pieces of skeletal material for ancient DNA in the clean room facilities of the Max Planck Institute for the Science of Human History in Jena, Germany. We produced bone powder from 92 petrous parts of the temporal bone, 38 teeth, and two unspecified bones, from which DNA was extracted and converted into genetic libraries.

DNA preservation after shot-gun screening was highly variable, but the amount of human endogenous DNA tended towards the lower end of the scale with only several thousand human reads per library (Fig. 2a). In line with previous findings, petrous bones and teeth yielded the highest amounts of human endogenous DNA, while there was not sufficient evidence for preserved DNA in other elements of the skeleton [69,74]. A maximum endogenous DNA yield of 57.92% for petrous bones and 70.77% for teeth shows that outstanding preservation in tropical regions is possible. However, the medians of 1.93% and 0.15% for petrous bones and teeth, respectively, illustrate that those are rather exceptions of the region's norm. We also observe that endogenous DNA varies wildly between and within sites (Fig. 2b). DNA preservation seems particularly low in Island Southeast Asia, even in the Metal Age individuals from Wallacea, where DNA is not better preserved than in the 7,200-year-old individual from Leang Panninge. The Log Coffin-associated individuals also show high variability, despite originating from a very confined geographic region and similar cave environments. The highest median endogenous DNA preservation was achieved in the youngest site, Heping Dao, with a median of 4.27%. However, considering these individuals are only around 400 years old, our results may suggest that DNA decays particularly rapidly in Southeast Asia and then stagnates near the boundary of detection for a long time, similar to other tropical environments [337]. This pattern is comparable to collagen, the main structural protein of bones, which degrades faster in the tropics - sometimes even wholly - compared to staying at a low level in temperate climates [338].

Fifty-two individuals yielded an extremely low amount of endogenous DNA, and the few recovered human sequences were likely not ancient, suggesting bacterial and human contamination affecting endogenous DNA and damage rate, respectively (Fig. 2c). These compound the observed challenging DNA preservation and, in combination, represent the

main difficulty of ancient DNA retrieval from Southeast Asia. Nevertheless, many samples with low amounts of human DNA still contained a large portion of ancient molecules, as indicated by high levels of DNA damage. The presence of endogenous DNA and DNA damage in 84 individuals resulted in these being selected for enrichment with in-solution capture for the ‘1240K’ SNP panel ascertained for differences in population history [99,100]. Analogous to the endogenous DNA results, sequences from petrous bones were also genotyped for higher numbers of SNP positions than those resulting from teeth, but there were no direct relationships between those two measurements for individual samples (Fig. 2d).

The possibility of producing well-covered genomes from low-preservation samples underlines the importance of enrichment approaches, such as in-solution capture, in tropical regions, where whole-genome sequencing is uneconomical. However, the currently used in-solution capture arrays, particularly the common ‘1240K’ array targeting around 1.24 million ancestry-informative SNPs [99,100], show a variability of effectiveness in capturing different sites and biases towards certain alleles from the same site [339]. Additionally, the restriction to a specified SNP set discards information retrieved outside of the targeted positions, adds ascertainment bias to the analyses, when comparing with data generated through other less-restrictive methods, and reduces the resolution of loci, such as the Y-chromosome [97]. Due to the prohibition of oligonucleotide probe re-distribution by the producing company and high probe synthesis costs per sample for small-scale laboratories, access to the ‘1240K’ probe set has been limited to well-funded, high-throughput laboratories [339]. The recent publication of promising fully commercially available extended ‘1240K’ capture arrays may help contribute to more equitable ancient DNA processing from Southeast Asia until methodological innovations allow for better DNA recovery.

The climate in Southeast Asia is likely one of the contributors to the rapid decay of DNA recovered from skeletal remains. While the assessment of biological impacts on DNA may be highly variable per site, the chemical vulnerability of DNA has been shown to increase with temperature. Particularly the rate of depurination, where adenine and guanine are released from the DNA strand, is positively affected by temperature [43]. Similarly, higher site mean temperature supplies the activation energy for hydrolysis more often, resulting in higher cytosine deamination rates [42]. Constructing a thermal age model for DNA decay even demonstrated that from 10^{12} copies of a DNA fragment at 10°C, the last remains after 359

years, while at 35°C, this is estimated for 11.5 years [340]. This has considerable implications for Southeast Asia, where the annual mean temperature accounts for 27°C, compared to Europe with 10°C [341]. Additionally, both the more seasonal monsoon-influenced mainland and the uniformly humid insular Southeast Asia [342] provide large amounts of moisture for the hydrolytic reactions. However, we clearly also observe very large inter- and intra-site variation in DNA preservation, even in the same climatic zones. Therefore, it seems that micro-scale environmental and geological factors, such as mineral and moisture content, have a strong influence on DNA preservation, as previously suggested for the tropics of Oceania and the Caribbean [337]. Future systematic studies of soil properties and environmental conditions in relation to ancient DNA yield will help predicting DNA preservation at a specific site and aid project planning.

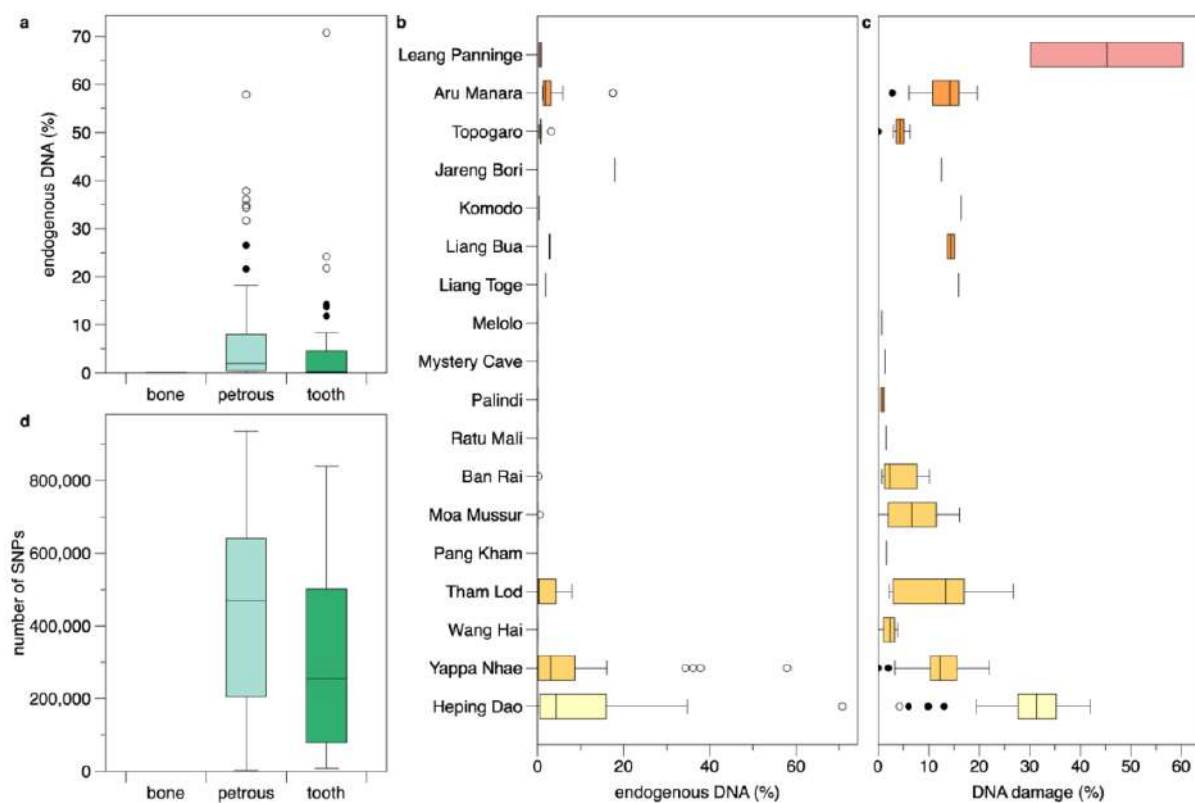


Figure 2. Ancient DNA preservation in Southeast Asia assessed through endogenous human DNA recovered from different sampled skeletal elements (a) and archaeological sites (b), rate of cytosine to thymine misincorporation or ‘DNA damage’ from different archaeological sites (c) and number of single-nucleotide polymorphisms (SNPs) genotyped for different sampled skeletal elements after 1240K SNP capture (d).

4.2 Tracking the settlement of Southeast Asia and hunter-gatherer diversity

In Manuscript A, we describe the first burial associated with the ‘Toalean’ technocomplex from Sulawesi and analyse the first ancient DNA from Wallacea. While the associated material culture was first described in 1902, exactly which groups were living at the many associated sites in Sulawesi Selatan and persisted until after the arrival of Austronesian groups remained a challenge to assess from artefacts only. Radiocarbon dated to 7.2 ka ago on an associated burnt *Canarium* sp. seed, our analyses at Leang Panninge finally connected a burial with the distinctive ‘Maros points’ and sawlettes of the ‘Toalean’. The flexed burial covered with large cobbles and the use of ochre in the direct vicinity of the cranium has parallels across Southeast Asia during the middle Holocene, ranging from Laos and Thailand, over the Malay Peninsular and Java to Borneo and the Philippines [343,344]. Perhaps originating in Borneo 10 ka ago before spreading across Island Southeast Asia [345], flexed burials as a shared mortuary practice might indicate connectivity between Holocene groups, such as shown for obsidian trade [346], or a common baseline from which distinctive assemblages, such as the ‘Toalean’, developed.

From a morphological perspective, pre-Neolithic individuals from Mainland and Island Southeast Asia share an affinity to present-day Near Oceanians, suggesting common ancestry of groups in Sunda and Sahul [245]. Fitting with the craniometric similarities with Sahul-Pacific groups of the burial from Leang Panninge, our population genetic analysis demonstrated this individual’s genetic affinity to present-day Near Oceanians. On an admixture graph, the newly-published individual branched off after the Andamanese Islanders, and consequently the ‘Hòabinhian’-associated genome from Laos, but before Papuans and Australians split. Distinct present-day groups from Island Southeast Asia supposedly represent the ‘first Sunda people’ and fall into the same phylogenetic position [241,347]. Our reported dentomorphological similarities between the ‘Toalean’-associated individual and the Semang, a hunter-gatherer group from Malaysia, certainly require further investigations into the genetic relationships between these potential descendants of early Wallaceans. While any cultural relationship to early groups in Wallacea or the people producing the 46-ka-old cave art on Sulawesi remains unknown [157], the lineage that the Leang Panninge individual was part of dates to before 37 ka ago, when the ancestors of Australian and Papuan groups diverged [185]. The split time of 53 ka ago between groups from the Philippines and New Guinea [347] further suggests a deep structure within early

modern humans in Southeast Asia shortly after the separation of Oceanian and Eurasian groups around 58 ka ago [185].

So far, the only other pre-Neolithic individuals for whom genetic data is available are from Mainland Southeast Asia. The two currently available ‘Hòabìnhian’-associated genomes from Pha Faen in Laos (7.8 ka) and Gua Cha in Malaysia (4.4 ka) genetically mirror the spatio-temporal consistency assigned to this technocomplex, but the criticism leveraged against the archaeological homogeneity could also be reflected in so far undetected genetic diversity. While these individuals are closely related to present-day Andamanese Islanders, the Longlin hunter-gatherer from Guangxi in China, dated to 10.5 ka ago and not connected to any artefacts, falls on the East Asian lineage, basal to later northern and southern East Asian individuals [249]. Younger individuals from Guangxi, such as the 8.7-ka-old Dushan and the 6.4-ka-old Baojianshan individual, demonstrate genetic contact between different lineages already during the early Holocene. Dushan harbours both south East Asian and Longlin ancestry, while Baojianshan is additionally admixed with a ‘Hòabìnhian’-related group [249]. This illustrates that hunter-gatherer diversity is likely higher than previously assumed and that interactions between hunter-gatherer groups already started the formation of the complex genetic landscape we observe today, thousands of years prior to the Neolithic expansion.

The genome from Leang Panninge establishes that ancient DNA recovery from Middle Holocene Wallacea is possible and highlights the feasibility of studying the relationships between mainland and island hunter-gatherer groups. Recent excavations of a 31-ka-old individual at Liang Tebo on Borneo, whose left lower leg had been surgically removed [348], a human maxilla from Leang Bulu Bettue on Sulawesi, dated 25-16 ka ago [349], and the cranial remains from Tron Bon Lei, dated to 13 ka ago [343,350], show the immense potential of archaeological and archaeogenetic studies in Wallacea. Despite challenging preservation conditions, genomic data from these and other archaeologically well-studied sites are key to understanding the settlement history and distribution of lineages from Sunda into Wallacea and Sahul. Previously published lineage divergence time estimates are consistent with modern human presence in Southeast Asia around 50 ka ago. However, the split locations are undeterminable through genetic data alone and radiocarbon dating becomes unreliable near 50 ka ago due to exponential decay [351]. Ancient DNA from such human fossils could anchor these lineages in time and space and genetically date the emerging diversification.

4.3 Illuminating the Denisovan introgression events

Initially identified through the genetic analysis of hominin remains in Siberia, the Denisovans and related ancestry are ubiquitous questions in any genetic study in Eastern Eurasia. In the present day, Denisovan ancestry has mainly been identified in groups living past the Wallace line. While Papuan and Australian groups harbour ca. 3% Denisovan-related ancestry [197], groups from the Philippines show more Denisovan-related ancestry than expected based on the shared Oceanian-related ancestry, indicating an additional introgression event after their ancestors split from Near Oceanians [347]. The high amount of Denisovan-related ancestry in the Philippines, particularly the Ayta, starkly contrasts with the very little amount thereof in Andamanese Islanders and Austronesian-related groups, such as Ami and Kankanaey [198]. There is also no evidence of Denisovan introgression into the ancestors of the ‘Hòabìnhian’-associated individuals, despite the recent discoveries of potential Denisovan remains in Laos and Tibet [206,207], where distributions might have overlapped.

As the oldest and most western individual with significant Denisovan-related ancestry to date, the Denisovan ancestry tracks identified in the Leang Panninge genome represent a crucial data point in understanding the timing and location of Denisovan contact in the region. Despite the phylogenetic position prior to the split between Papuan and Australian groups, the Denisovan-related DNA segments in Leang Panninge and Papuan groups are correlated, indicating a shared introgression event. On the other hand, the Ayta from the Philippines contain additional Denisovan input from a separate admixture event, corroborating previous evidence of divergent Denisovan lineages admixing at different time points [200,201,352]. The equal genetic distance of Leang Panninge to all Near Oceanians also places the time of Denisovan admixture before 37 ka ago, when Papuan and Australian groups split [185]. As the Ayta and Papuans split around 53 ka ago [347], determining the split order and date between Leang Panninge, the Ayta, and related lineages and will be crucial in narrowing down the timing of the different Denisovan introgression events.

So far, it seems possible that at least one of the Denisovan contact periods occurred in peninsular or Island Southeast Asia. The presence of other archaic hominins, such as *Homo erectus*, *H. floresiensis*, and *H. luzonensis*, indicate the ability of early hominins to cross to and persist in Wallacea, which could also apply to the Denisovans. However, the split date between Ayta and Papuan and the associated additional Denisovan introgression, as well as the lower amount of Denisovan ancestry in the ancestors of Tibetans, all date the interactions

back to the initial human settlement of the region [347,353]. Another Denisovan lineage is also present in the 40-ka-old Tianyuan and 34-ka-old Salkhit individuals from China and Mongolia, respectively [188,354]. Such old genetic contributions could indicate that population structure between modern human lineages arose early in Sunda, and divergent groups interacted with different Denisovan groups before crossing the Wallace line and in Wallacea independently. A hitherto lack of Denisovan fossils from Wallacea and archaic fossils from Sahul support this hypothesis [355].

Recent re-evaluations have suggested that several previously unspecified archaic hominin fossils from Eastern Eurasia also represent Denisovans, including the Penghu 1 mandible recovered from a submarine channel in the Taiwan Strait [356,357]. Since most archaic hominin fossils are represented by fragmentary mandibles or teeth, ancient DNA could assist in determining such material as Denisovan and identify the different lineages and their geographical extent. Collagen fingerprinting and sediment DNA have been also used successfully to identify additional Denisovan bones and presence [358–362]. More genetic data from Denisovans would also further our understanding of the general interaction dynamics between Denisovans and early modern humans. At Denisova cave, ancient DNA from sediments suggests that while Neanderthals and Denisovans both frequented the site during the early and middle Middle Palaeolithic, modern humans only occupied the site from the Initial Upper Paleolithic [361]. The comparatively fast arrival and establishment of modern humans in Eastern compared to Western Eurasia point towards different interaction dynamics between modern humans and Denisovans or Neanderthals, respectively [363]. Positions in the genome where Denisovan ancestry is found in groups from Southeast Asia and Near Oceania could be screened for any environmentally-influenced variants, such as marine or seafaring-related adaptations, similar to how high-altitude adapted variants in Tibetan groups are supposed to have introgressed from Denisovans, but were not under selection until the early Holocene [353,364].

4.4 Characterising regional connectivity and exchange

The manuscripts presented in this thesis illustrate how archaeogenetic studies can track genetic exchange between local and distant groups. While contact between different groups and individual mobility can also be traced through the presence of distinctive artefacts (e.g. the Dong Son drums) and *Sr* isotopes can identify if a person grew up or died in a different place by comparing values from tooth enamel and bones [365], genomic evidence can

determine if the material or technology transfer took place via trans-cultural or demic diffusion. Additionally, ancient DNA can suggest if incoming people had offspring with the local community and on what magnitude this contribution to the gene pool happened.

On Heping Dao in Taiwan (Manuscript D), our genomic analyses revealed the presence of at least five different ancestries in a colonial-era graveyard. While some historical records suggest Han Chinese settlements on Heping Dao from the 9th century CE, the Spanish only established the first European colony in 1626 [366]. Short-lived until only 1642, the numerous burials near the church foundations demonstrate that Indigenous Taiwanese-, Southeast Asian-, Han Chinese-, southern, and northern European-related individuals were all part of the life in the colonial settlement and took part in the associated Christian congregation to some extent. However, the current state of knowledge suggests no evidence of genetic interactions between groups, despite historical records describing numerous occasions of intermarriage between European soldiers and Indigenous women, who supposedly even accompanied the Europeans on the abandonment of the colony [366–368].

Our conclusions in Heping Dao are certainly limited by the scarcity of child burials, which the transience of the colony would determine as the most direct evidence of genetic exchange on a short time scale. We only identify one individual aged approximately four years old, who is most closely related to present-day Indigenous Taiwanese groups. Despite the burial in a ceramic jar representing the traditional Austronesian rite, also practised in the pre-colonial Philippines [369,370], the find of a jade bracelet of Chinese make and body placement half outside the jar illustrate that cultural contact was influencing and modifying even long-lasting cultural practices, such as mortuary customs [366]. Modifications of burials rites in the presence of other cultural groups are also common at early Atlantic slave plantations, illustrated by a 15th-17th century cemetery on the Canary Islands, where Eurasian, Sub-Saharan African, and Canarian enslaved individuals were interred according to a syncretistic funerary ritual incorporating Catholic, Islamic, and African aspects [371]. The lack of other child burials could alternatively indicate that they were buried elsewhere, as common for unbaptised infants in Medieval Europe [372,373]. The jar burial and another individual of Indigenous Taiwanese ancestry also represent the only genetically female individuals recovered from the site, which further suggests that the cemetery was only used by a selected fraction of the community, e.g. soldiers or clergy members. The remains of the rest of the

community and any potential evidence of admixture remain hidden from archaeological and genomic analyses for now.

Differences in genetic ancestry profile and admixture dating are central to our study of Metal Age genomes from northern, western, and southern Wallacea (Manuscript B). Despite the geographical proximity, these individuals show no genetic connection to the Leang Panninge individual. Instead, they demonstrate the far-reaching genetic impact of the Austronesian expansion, admixed with a different Papuan-like ancestry. On Sulawesi and the southern Indonesian islands, we also identify a third admixture component most closely related to present-day Austroasiatic speakers from Mainland Southeast Asia. Both associated with the spread of agriculture, Austroasiatic- and Austronesian-related ancestries co-occurring in Wallacea might point towards people from the two different Neolithic traditions interacting in Wallacea. The genetic dates indeed suggest that the Austroasiatic- and Austronesian-related pulses occurred close to the first archaeological occurrence of Neolithic artefacts in the region, corroborating that we observe a pattern originating closer to the Neolithic than the later Metal Ages, where archaeological finds evidence increasing trade connections.

Additionally, admixture dating tentatively suggests that the admixture of the Austroasiatic- and Papuan-related components might have occurred prior to the introduction of the Austronesian-related ancestry. Two previously published individuals from Sumatra, dated between 2.3 and 1.8 ka ago, can even be modelled directly as an admixture between Austroasiatic- and Austronesian-related ancestry, without a Papuan-related component [291]. Thus, while Austroasiatic-associated presence has been proposed for western Indonesia, such as Sumatra and Sarawak, our ancient genomes push the extent of this ancestry across the Wallace line. If we assume the Papuan-related ancestry in the Wallacean ancient genomes to originate from the first modern human settlement of the region, its lack across the Wallace line might also have some implications for the preferential presence of Papuan-related hunter-gatherer ancestry in Wallacea or different interactions between hunter-gatherers and early farmers in the different regions. Dating the admixture times of the ancestries present in these individuals and older Neolithic ancient genomes from Island Southeast Asia will help to understand the interactions between these three ancestry components and potentially associated cultural phenomena.

Present-day individuals from Sumatra and Java also harbour both Austronesian- and Austroasiatic-related ancestry [291]. Due to the lack of Austroasiatic languages in western

Indonesia nowadays and the ubiquitous presence of both ancestries in the whole region, these components have been suggested to have admixed when Austronesian-related people arrived in Vietnam or the Malay Peninsula and then distributed to the islands [370]. However, linguistic evidence of similarities between branches of Austroasiatic in Mainland Southeast Asia and the Malay Peninsula and Austronesian languages on Borneo support a potential Austroasiatic substrate in Borneo, originating from Vietnam before the Austronesian arrival, when they admixed and spread into Sumatra and the Malay Peninsula [374].

Archaeologically, this Austroasiatic presence might be connected to the paddle-impressed pottery found in western Indonesia, while the classic Austronesian red-slipped pottery is mainly found in eastern Indonesia [297,371]. Interesting are sites, such as Liang Abu in Kalimantan and Loyang Mendale in Aceh, where both potteries co-occur, but a lack of comparative sites and a resolved chronology make interpretations challenging [297,375]. The identification of Austroasiatic-related ancestry in Metal Age Wallacea also underlines the importance of discussing the history of Mainland and Island Southeast Asia together rather than as two separate zones.

In contrast, the studied Log Coffin-associated individuals from Pang Mapha in northwestern Thailand, show similar relative shares of ancestry related to ‘Hòabinhian’-associated hunter-gatherers and Yangtze and Yellow River farmers (Manuscript C). Such a homogenous gene pool may result from the constant slow assimilation of ancestry components over a long period or high rates of genetic exchange after initial genetic contact. Since the two farming-associated groups are connected to independent developments of rice and millet agriculture, the presence of both components in Iron Age northwestern Thailand might indicate the sequential interaction of local hunter-gatherers with different incoming farmer groups introducing rice and millet or farming groups already carrying both ancestries and crops. The introduction of Yellow River-related ancestry into the Yangtze River gene pool from the Late Neolithic and the detection of both ancestries in the majority of ancient genomes from Southeast Asia make an admixed introduction likely. However, the admixture proportions between different regions vary. Ancient individuals from Guangxi and Vietnam carry higher Yangtze River-related ancestry [249], while the Log Coffin-associated individuals carry relatively equal proportions of both. We also find exclusive admixtures of hunter-gatherer- and Yangtze River farmer-related groups at Neolithic Mán Bạc on the Red River delta in Vietnam and Bronze and Iron Age of Ban Chiang in northeastern Thailand [257,372], corresponding with similarities in material culture and dentometrics of Mán Bạc, An Son near

the Mekong Delta in southern Vietnam, and Khok Phanom Di in central Thailand with Neolithic Yangtze River sites [257,376]. Conversely, Late Neolithic Myanmar only shows Yellow River-related ancestry admixed with hunter-gatherers and the recovery of millet at Non Pa Wai in the Lopburi region of Thailand might represent direct archaeological evidence for the influence of groups from the Yellow River valley [265]. Since ancient and present-day Tibetans also carry Upper Yellow River ancestry [257], it seems that this ancestry is present to a higher degree in the western part of Southeast Asia. This could indicate different and broader dispersal routes of Yellow River ancestry, while Yangtze River ancestry was more directly introduced in the western parts of Southeast Asia, both likely spread along different rivers [257].

However, the occurrence of both ancestries already during the Neolithic of Southeast Asian sites indicates that interactions between groups occurred often and soon after initial arrival. Additionally, the Yangtze and Yellow River-related groups are the most distal sources of the observed ancestries. They likely do not represent the gene pools of the admixing groups accurately, who may or may not have been admixed already. While we were not able to date the admixture between the three components for the Log Coffin-associated individuals, more geographically dispersed sites will facilitate the step from observing said ancestries to understanding the dispersal and contact between different Neolithic groups. Genetic time transects and detailed archaeological analysis of other wooden coffin sites in Southeast Asia will also help inform about the connections of these groups and if the dispersal followed previous introduction paths from southern China, where the oldest wooden coffin sites are found [377].

The admixture graph analyses showing that the ‘Toalean’-associated individual also harbours ‘Deep Asian’- in addition to the Oceanian-related ancestry, was certainly an unexpected result (Manuscript A). Although only the uppermost layer of the site’s stratigraphy, dated between 3.5 and 1.5 ka ago, yielded both Austronesian pottery sherds and ‘Toalean’ stone tools, consistent with reports of Austronesian settlements in the Karama valley in Central Sulawesi from 3.5 ka ago [374,375], our results indicate long-distance genetic exchange thousands of years before the Austronesian expansion. However, if this represents an isolated case or the general ‘Toalean’-associated ancestry profile remains unknown. Recent excavations at the Cappa Lombo rock shelter have revealed seven additional burials associated with ‘Toalean’ artefacts [378,379]. Dated 7.4 to 6.5 ka ago, genetic information from these burials could help

understand the ‘Toalean’-associated gene pool. Furthermore, relatedness tests could illuminate the social constellations and mortuary rituals. Supposedly displaying distinctive craniometric morphologies mixed between Asian and Australian, these individuals could represent admixture between the different groups ‘in real time’ [379]. Despite no detailed insight into the location or archaeological association of the ‘Deep Asian’-related component through any closely related ancient groups or direct material evidence, the occurrence of unifacially retouched stone points, hollow-based ‘Maros points’, backed microliths, and bone points has previously drawn connections between the ‘Toalean’-associated people and contemporary groups from Java, Japan, South Asia, and northern Australia, respectively [380]. In addition to the necessity of seafaring for the initial modern human settlement of Wallacea and Sahul around 50 ka ago, archaeological finds from Mindoro and Palawan in the Philippines, dating from 35 ka ago, indicate open seafaring and long-distance movements of people and material [381] underlining the importance of pre-Neolithic mobility and connections in Southeast Asia.

4.5 Understanding social structure at an archaeological site level

Manuscripts C and D provide an exciting precedent for studying more than ten individuals from the same locality. While a singular genome contains the genetic heritage of many ancestors and can therefore be used to investigate questions of demographic change and population history, multiple individuals from the same site or cemetery allow for the investigation of small-scale social structure and genetic relatedness. Understanding the community through their connections between different sites and the treatment of the deceased permits insight into a limited but central part of past societies [382].

One of the notable features of the excavations at Heping Dao is the structuring of burials inside and outside the 17th-century colonial church foundations (Manuscript D). While the burials are either oriented south-north or west-east, the body position varies only minimally for most interred individuals. Near the foundations of the ‘Convento de Todos los Santos’, individuals are buried in a supine position, facing skywards, with evidence of shrouding, and arms extended alongside or flexed on the torso, exemplifying Christian mortuary rites [373,383]. These customs travelled with the Spanish colonisers to their South American colonies, such as 17th-19th century Spanish Venezuela [384] and Costa Rica [385]. At Heping Dao, eight individuals buried outside the church foundations are most closely related to present-day Han Chinese groups and another two harbour Island Southeast Asian-related

ancestry. Since the funerary rites deviate from contemporary burial practices in other Chinese communities, where the deceased were placed in supine positions in brick coffins and given coins as grave goods [386], these burials likely represent Christian converts.

In contrast, the most desirable burial placement inside the church near the altar [373] was exclusively occupied by individuals with European ancestry. This is paralleled in the earliest churches of Manila, where Spanish governors and colonists were buried within San Augustin Church and Manila Cathedral, while it was only from the late 18th century that wealthy Filipino Christians could be buried inside the churches in exchange for large sums of money [370]. Generally, it was typical for cemeteries to have at least separate sections for baptised pagans and unbaptised infants [373]. In Manila, the Chinese Christian community had separate cemeteries on Binondo and burial stones with both Spanish and Chinese inscriptions indicate Chinese Christian burials in other churches and churchyards [387]. As one's earthly rank had direct implications for the placement of one's burial [370], the church and adjoining graveyard likely illustrate the social hierarchy at colonial Heping Dao, with people of European ancestry occupying the highest positions. With over 48 named Dominican priests from Portugal, Spain, and Italy mentioned in historical records, the burials inside the church most closely related to southern Europeans likely represent such [368,383].

Only one individual inside the church is oriented west-east and has northern European-related ancestry. Considering the takeover of Heping Dao by the Protestant Dutch in 1642 and the Spanish custom of burying with the feet directed towards the church altar [373], which faces north-south here, it seems likely that this individual belongs to the second colonial occupation. However, descriptions of separate Catholic and Protestant cemeteries from Portuguese Macau and British Hongkong [388] and the strong emphasis on only burying baptised Catholics on consecrated grounds, even to the exclusion of unbaptised infants [372,373], illustrate the desire of different Christian denominations to separate one another and would make the use of the same burial ground unlikely. However, these examples are from later and well-established colonies, where both faiths were practised simultaneously. At Heping Dao, the transience and sequentiality of the colonial settlements already located on the colonial periphery might have influenced the local interpretation of religious and moral codes [383].

This is potentially also reflected in two adult burials in a flexed position and a child jar burial among the churchyard burials. Placed on the side, they represent the local Indigenous burial

rite, as recovered at the large Iron Age site of Shihsanhang I in northern Taiwan [389]. The genomic analyses show that these three individuals also harbour exclusively Indigenous Taiwanese-related ancestry, likely representing members of the local village Quimaurri or other Indigenous groups interacting with the colonisers. While there is a possibility these burials date prior to the European colonisation, stratigraphic investigations and a lack of intrusions from later Christian burials or the extensive terrain modifications necessary for the construction of the church suggest that they belong to the colonial cemetery [366].

Unfortunately, detailed radiocarbon dating is not possible, as the initial Spanish colonisation falls within a plateau of the radiocarbon calibration curve lasting from 1480 to 1630 CE, when the ^{14}C to ^{12}C ratio in the atmosphere decreases at the same rate as the radiocarbon decay in the sample [390]. Archaeogenetic studies of other Spanish colonial cemeteries or colonies with changing foreign powers could contribute to better understand these social dynamics and, with the help of historical sources and anthropological analyses, illuminate the relationships between different colonial settlements and Indigenous groups.

Another aspect crucial for understanding the studied community better is if and how they expressed genetic relatedness in death. In genomics, two individuals are determined to be related if they share alleles due to descending from the same ancestors (identical-by-descent) and are more closely related, the more alleles they share [391]. Initially utilising forensic and microsatellite techniques, ancient DNA allows for the direct assessment of genetic relatedness [392,393]. High-coverage autosomal data in combination with advanced statistical methods now produce reliable results up to third-degree relationships [394–396]. Despite the high-quality data available, we did not identify any close genetic relatives at Heping Dao, potentially another consequence of intentional separation between different groups, as fostered by colonial powers in later Southeast Asian contexts [397].

Interestingly, we determined several Log Coffin-associated individuals as parent-offspring and siblings, or second-degree related, i.e. grandparent-grandchild or aunt/uncle-niece/nephew. The two first- and four second-degree relationships all occur within Yappa Nhae and Long Long Rak [394], where the siblings from Long Long Rak were even buried within the same coffin. Although the burials at the newly studied Log Coffin sites were too disturbed to associate burials with coffins, our results indicate that genetic relatedness likely played a role in the choice of burial site and structure. Similarly, six secondary burials in a single jar dating to early Medieval Korea were recently shown to represent a couple, their two

children, and both paternal and maternal relatives [398]. While this burial differs from the surrounding stone-cist and stone-chamber burials, aDNA offers insights into the potential intentions of unique burials. Moreover, three burials in the immediate vicinity of a two-room house associated with the Longshan culture of the Yellow River valley demonstrate the co-habitation of extended families through their second-degree relationships [399]. At the transition from extended to nuclear family units, these individuals also attest to the presence of levirate/sororate mating, which describes the inheritance of a deceased sibling's mate and was recorded in historical documents from the Late Bronze Age Eastern Zhou dynasty. While analyses of large cemeteries, such as Middle Neolithic Gurgy 'les Noisats' in France, where a pedigree including 62 individuals over seven generations could be reconstructed [400], are not feasible in Southeast Asia presently, newly available software and laboratory methods will facilitate the study of relatedness on a local and regional level.

Because of the large number of high-coverage genome-wide data from several Log Coffin-associated sites, we were also able to determine and compare blocks of the genome shared through identity-by-descent (IBD) for the first time in the region. This analysis leverages the relatively long persistence of a few long IBD segments through several meioses to detect more distant relatedness, when exponential decay has decreased the overall IBD alleles to very low levels [401]. Focusing on IBD segments longer than 20 cM, we could show that most of the studied individuals from the same site shared third- to sixth-degree relationships. We also identified shared IBD blocks between individuals from different sites, although no close relationships were found. These findings suggest genetic contact between people buried at different sites, but a preference for burying close genetic relatives at the same location.

Our results integrate well into archaeological analyses of the log coffins themselves. Dendrochronological dating of the coffins showed that the cemeteries were each used continuously over several centuries, with simultaneous use of different carving styles at the head and foot end of the wooden coffins [402,403]. The carvings ranging from simple geometric to animal or human shapes have previously been divided into several styles and trace relatedness within and connectivity across river valleys [404]. Carved from an individual log with an average diameter of 0.5 m and a length of 4 m, the felling, sculpturing, and transport of the coffins required the collaboration of several individuals, which suggests the presence of large communities with shared mortuary practices [403].

While we focused here on first- to sixth-degree relatives, IBD analyses also allow the detection of even more distant genetic relatives who only share singular or very few short IBD segments. This has previously been employed to study the Ceramic Age in the Caribbean, where shared IBD blocks generally decreased with geographical distance, but also identified multiple distantly related individuals across islands [405]. Previous studies of mitochondrial DNA of Log Coffin-associated individuals have suggested that this custom was introduced to northwestern Thailand through trans-cultural diffusion since the mitochondrial DNA haplogroups of Hanging Coffin-associated individuals from southern China were differentially distributed [406]. As similar mortuary rituals were practised from Myanmar in Mainland to the Philippines in Island Southeast Asia, ancient DNA has enormous potential for studying local patterns in Log Coffin-associated groups and potential long-distance connections between these throughout Southeast Asia. Recent surveys also report Log Coffin-like findings from Nagaland in India and Laos [403], raising further questions of the connectivity of these phenomena and, if determined as of a singular origin, how the custom was propagated through the region.

While we were able to produce genome-wide data for 33 individuals from Log Coffin-associated sites overall, our IBD block analyses are limited by the numerical bias towards individuals from the site Yappa Nhae 2. Due to preservation issues, only one individual from Ban Rai and Lahu Pot was suitable for imputation and IBD identification, while none from Tham Lod cave had sufficient coverage. This demonstrates the value of many high-coverage genomes from the same site or archaeological complex and the additional questions that can be investigated with such an extended dataset.

4.6 Developing equitable archaeogenetic research in Southeast Asia

Technological advances, such as Next-generation sequencing, in-solution capture, and ancient DNA-specific protocols, have led to a large increase in archaeogenetic projects and studies utilising ancient DNA methods. Records of the PubMed database show rapid increases in published papers mentioning ‘ancient DNA’ around 1994 and 2013 [407]. While we also observe this steep surge in studies investigating the genomic history of Europe and East Asia, albeit to a higher degree in the former, the number of studies focussing on ancient Southeast Asia stagnates at a lower level (Fig. 3a).

This geographical imbalance is compounded by papers tackling human history in Europe now often analysing 100-500 individuals at once (e.g. Viking Age Scandinavia, Anglo-Saxon Great Britain or Neolithic-Bronze Age Anatolia [408–410]). For previously published papers studying human history in Southeast Asia through ancient DNA, most sites only yielded singular or few genomes and are clustered in northern Southeast Asia (Fig. 3b). Likely resulting from the challenging preservation conditions in the region, only five sites from southern China, northern Vietnam, and northwestern Thailand allowed for the generation of more than two genomes. Particularly Island Southeast Asia is understudied, with only four sites in the northern part but no ancient genomes from further south than Kuala Lumpur, Malaysia (<3.5°N).

With the manuscripts presented in this thesis, we increase the number of archaeological sites with genome-wide data for more than two individuals in northern Southeast Asia and show that ancient DNA is preserved throughout Wallacea. However, there are still large geographical and temporal gaps. Especially for hunter-gatherers, only minimal genetic data is available and, similar to the many singular genomes associated with a particular period or archaeological complex, are currently being assumed to be representative for the genetic ancestry of whole regions or groups. A similar phenomenon is observed in archaeological excavations, which have historically focussed on in-depth analyses of prominent sites [411]. Archaeogenetic data from more dense geographical sampling and time transects would be beneficial to trace hypothesised migrations on a smaller scale and compare with the many observed technological and cultural differences. However, the region's poor bone and DNA preservation make all ancient human remains more precious, both archaeologically and spiritually. Implementing destructive methods, such as ancient DNA, should be evaluated carefully under the consultation of all stakeholders, from archaeogeneticists and archaeologists to local governments and communities [107,412].

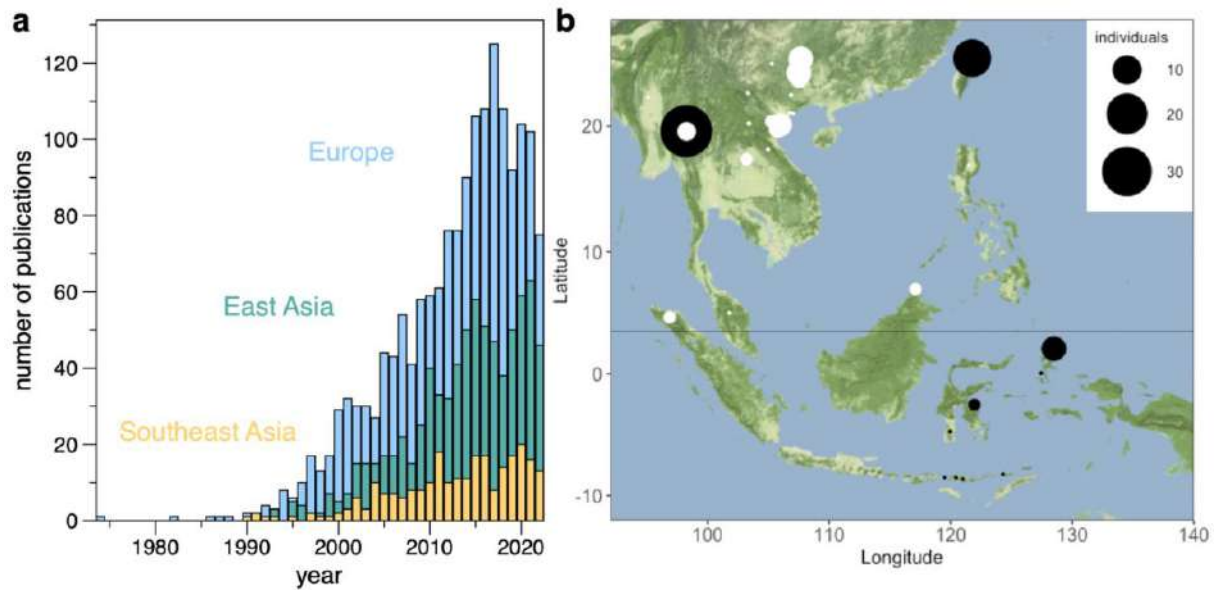


Figure 3. (a) Number of publications on the PubMed database queried for ‘ancient DNA’ in combination with ‘Europe’, ‘East Asia’ or ‘Southeast Asia’ plotted by year of publication; (b) Map of Southeast Asia showing the number of ancient genomes available from archaeological sites ranging from the Holocene to historical times, previously published sites are indicated in white [243,249,268], sites studied in manuscripts of this thesis are marked in black, plotted with ggmap [409], map tiles by Stamen Design (CC BY 3.0), data by OpenStreetMap (ODbL).

Archaeogenetic data and archaeological sites generally are limited in Southeast Asia. Due to the many sea level changes over human history in the region, large parts of what used to be Sunda nowadays lie underwater, limiting access to potentially key hunter-gatherer sites and human remains. With the exceptional emphasis on seafaring throughout human history in the region, changes in the coastal morphology would also affect Neolithic, Metal Age, and historical sites. These also include shipwrecks, many of which are still inaccessible to maritime archaeology and vulnerable to destruction [413]. Furthermore, even accessible sites are not always easy to study and interpret. Particularly open sites, such as the wooden coffins erected in rock shelters in northwestern Thailand, and ongoing excavations are vulnerable to looting, which results in the loss of artefacts and remains and the disturbance of the stratigraphic integrity of the site [412,414]. Reconstructing the original placement and temporal sequence of artefacts and burials is challenging but crucial for interpreting any derived data, such as ancient DNA results.

Due to the paucity of comparable ancient data, the analysis of ancient genomes from Southeast Asia also relies heavily on present-day reference groups to reconstruct past from present-day diversity. For many regions, such comprehensive genetic information is lacking. Alternatively, when available, the methodology employed to produce present-day genomic data is very variable and the resulting genome-wide data are not always comparable between different sequencing efforts or to ancient individuals. For example, investigating the Denisovan ancestry in present-day groups from Island Southeast Asia and their relationships with each other and ancient individuals, such as Leang Panninge, is currently limited by a low number of high-coverage present-day genomes. Most present-day data is produced using SNP chips with different SNP sets, so the overlap in genetic sites between previously generated present-day data and ancient genomes is currently not sufficient for robust analyses [98,198,241,347,415,416]. There is an impetus to study historical questions through present-day genomic data [417–422], such as the recent study of over 400 genomes from 35 ethnolinguistic groups in Thailand and Laos, which revealed differences in connectivity within and between linguistic groups throughout recent and historical periods [422,423]. Although ascertainment biases and differences in the chemical properties of generated present-day and ancient DNA limits their comparability, the results and genomic data from such initiatives will help provide more context for ancient genomes. However, this obviously requires rigorous ethical examination when working with Indigenous peoples [424,425].

Despite political independence, archaeological research in Southeast Asia also continues to be affected by colonial legacies. First established during the rule of western colonial powers, Southeast Asian archaeology was long dominated by colonial institutions and western researchers and focussed on artefacts and monuments from historical periods [412,426]. Prehistoric archaeology only developed post-World War II, and while local archaeologists have conducted the majority of archaeological work since the 1970s, many are trained in western countries, continuing to implement western research traditions and frameworks [426]. Additionally, the majority of researchers are located in national government departments, which focus on rescue excavations and the development of heritage tourism rather than research excavations [426,427]. Due to mainly operating within one country, archaeological reports and papers are often only available in the local language, which, combined with the designation of English publications for a ‘global’ audience, leads to a citation and narration bias towards western researchers and institutions [426,428,429]. Another consequence of colonial archaeology is the storage of many human remains and artefacts in western

institutions, but recent repatriation efforts have successfully returned human remains to local communities [412,429].

As a fast-developing field reliant on expensive technologies and extensive training currently mainly found in Europe and North America, archaeogenetics has the potential to exacerbate the already existing power asymmetries [412,430–432]. The dependency on overseas collaboration for laboratory and computational infrastructure, as well as funding, also facilitates ‘parachute’ or ‘parasitic science’. This describes a neo-colonial practice, whereby researchers from high-income, ‘core’ countries extract data or samples from the ‘periphery’ and benefit from the generated knowledge and resulting academic merit without acknowledging local expertise or establishing long-term collaborations [107,430,431,433].

Additionally, the interest of local scholars and communities in their historical roots and ancestry and the western focus on humanity’s development on a global scale can lead to disparities in research goals [426]. This is also visible in the continental-scale narratives presented in many archaeogenetic papers, which preclude exploring small-scale questions and the full complexity of the studied samples [430]. Western concepts commonly employed in archaeogenetic studies, such as the ‘Three Age system’ describing the Neolithic, Bronze, and Iron Age, are also not directly applicable to Southeast Asian contexts [434,435]. The transition from hunter-gatherers to farmers occurred on different time scales in different regions and involved various combinations of pottery, agriculture, husbandry, weaving, and sedentism commonly associated with the ‘Neolithic’ [435]. The ‘early Neolithic’ in Vietnam, when pottery and ‘Hòa Bìnhian’ stone tools co-occur [432], and the long process of Neolithisation in Japan [436] illustrate the presence of several ‘Neolithicities’ in Southeast Asia [434]. In Island Southeast Asia, the Neolithic is also not immediately accompanied by rice and millet agriculture, and bronze and iron metallurgy appear simultaneously [426]. Such details demonstrate the value and necessity of close collaboration with local archaeologists, who have thorough knowledge of the studied sites and regional processes [430].

Therefore, developing reciprocal relationships throughout project planning, analysis, interpretation, and publication is key to establishing equitable collaborations within and across disciplines. Bridging the gap between ‘core’ and ‘periphery’ also requires capacity-building through trainee exchange programs and financial support for infrastructure development and upkeep [412,431]. Southeast Asian archaeologists are already applying decolonial approaches, such as community archaeology, which could be extended with

archaeogenetic aspects [430]. Community archaeology aims to bridge the gap between local communities and scholars to create more meaningful research outcomes [437]. Local groups, local administration, government institutions, and private landowners are integrated into the excavation, which includes prior consultation, spiritual ceremonies before entering sites associated with ancestors or spirits, and dissemination of knowledge throughout and after the excavation [412,427,438,439]. In multi-ethnic countries of communities with different connections to the history of the studied region, Indigenous and local engagement require long-term commitment but can also inform about the past through local traditions and oral histories [438]. Communication about the potential and interpretation of genomic results is critical to avoid bioessentialist views [430,439].

While close collaborations with involved archaeologists formed the basis of all projects presented in this thesis, there is always more potential to foster capacity building and community engagement in future projects. With archaeogenetics still in its infancy in Southeast Asia, we, as the scientific community in collaboration with other disciplines and communities, have the opportunity and obligation to develop sustainable and equitable practices for exploring the many exciting open questions in Southeast Asia.

5. Conclusions and Outlook

With genome-wide data generated from 68 ancient human individuals, the manuscripts included in this thesis provide a substantial contribution to the establishment of archaeogenetics in Southeast Asia. Manuscripts A and B offer the first insights into the genetic history of Wallacea from the Middle Holocene through the Austronesian expansion and the Metal Age, while manuscripts C and D break new ground in tackling more localised questions on the social organisation during pre- and historical times.

One of the big challenges working in Southeast Asia is low DNA preservation and we indeed observed great inter- and intra-site variability for the skeletal materials studied in manuscripts A-D. The decay of DNA in the Southeast Asian tropics is likely expedited by high annual mean temperature, but the recovery of a suitable and even high proportion of human endogenous DNA, also facilitated by in-solution capture enrichment, is possible. Future studies of the influence of micro-environmental properties on DNA preservation and technological improvements targeting the recovery and analysis of small DNA fragments will help to improve the success rate of ancient DNA studies in Southeast Asia.

With such innovations, it will also be possible to understand the rapid human settlement of Sunda, Wallacea, and Sahul better and investigate the involved genetic lineages. While most early Southeast Asian individuals cluster together in morphological studies, genetic studies of the Longlin hunter-gatherer from southern China, two ‘Hòabìnhian’-associated individuals from Laos and Malaysia, and the newly described Leang Panninge (Manuscript A) indicate the co-occurrence of different genetic lineages. Understanding how these lineages are related to other pre-Neolithic individuals and present-day groups from Island Southeast Asia and Oceania will be an important aim of future archaeological and archaeogenetic studies.

In this context, the different amounts of Denisovan-related ancestry are crucial to discerning the structure of hunter-gatherer groups and the location and distribution of the Denisovans. Manuscript A provides the first precedent of Denisovan ancestry recovered from an ancient individual. The Denisovan-related segments found in the genetic data from Leang Panninge on Sulawesi dating to the middle Holocene likely stem from the same admixture event as the ancestors of present-day Oceanians. High-quality genomic data from other Holocene individuals from Sunda and Wallacea will be helpful to explore the interactions between

modern humans and Denisovans further and illuminate the number, locations, and impact of these introgression events.

All four presented manuscripts also clearly demonstrate that modern humans in Southeast Asia of all periods have been moving and interacting over long distances and their demographic histories have been shaped by genetic admixture. The studies describe different stages of admixture from initial contact and parallel habitation, as found at the early colonial site of Heping Dao in Taiwan (Manuscript D), through variations in the relative amounts of ancestries, exemplified by the differences in Austroasiatic ancestry in Metal Age Wallacea (Manuscript B), to a well-admixed, homogenous group ancestry profile, such as for the Log Coffin-associated individuals from the Iron Age of Thailand (Manuscript C). Evidence of admixture with a ‘Deep Asian’-related lineage in Leang Panning illustrates that movement and interactions have a long history in the region. Future archaeological and archaeogenetic studies will help to study such early interactions, but also the introduction routes of agriculture and metallurgy to Southeast Asia and the continental trade networks in later periods.

The generation of genome-wide data for more than 15 individuals in manuscripts C and D allowed for the study of local genetic connections for the first time in the region. The arrangement of burials at Heping Dao by genetic ancestry representing the social hierarchy (Manuscript D), as well as the identification of a relatedness-driven burial pattern at the Log Coffin-associated sites (Manuscript C), demonstrate the value of ancient DNA in the context of small-scale studies of a singular or several geographically close cemeteries. Where archaeoethnologists study the treatment of the body and the skeleton itself, archaeogenetics can contribute another puzzle piece to reconstruct the attitudes of past communities towards death and the deceased and what mortuary practices can tell about the life of the community.

As demonstrated, there is vast potential for archaeogenetic studies in Southeast Asia, but due to challenging preservation conditions, the field lags behind the rapid development in other parts of the world. In front of the colonial histories of many parts of the region and the highly-specialised knowledge and financial means necessary for archaeogenetic studies, the development of future archaeogenetic studies is closely connected to the establishment of equitable relationships and engagement with local researchers, administration, and communities.

Acknowledgements

PhDs are often compared to marathons, and now close to the finish line looking back, I would like to thank the many people that kept me going, showed me shortcuts, and cheered on the sidelines.

First of all, I would like to thank my supervisors, Johannes and Cosimo, for giving a Marine Biologist a chance and allowing me to work on such fascinating projects. Already early on, you gave me a lot of freedom to explore and develop the projects into directions I found interesting.

A very special ‘thank you’ has to go to Kathrin for showing me how to make the change from marine biology to archaeogenetics. I could not have undertaken this journey without your guidance and support during my first steps in the wet and dry lab, learning how to lead a study, and evolving into an independent researcher with my own ideas and principles.

I am also very grateful to all my collaborators who introduced me to Southeast Asian archaeology and history and welcomed me into their projects with open arms. I hope we can continue to work together in the future – there are still many exciting questions to investigate.

I also would like to commend the whole DAG community. So many brilliant people being welcoming, asking questions, and sharing their knowledge is one of the big things that make this a dream job. The support from the lab team has also been invaluable and I would like to thank Rita in particular for training me in the lab and giving me an appreciation for where our data come from.

I’m also extremely grateful to my friends, old and new, who were there for me during the ups and downs of the past years and provided much needed distraction.

And finally, to my family: words cannot express my gratitude for your unwavering love and support.

References

1. Wyckoff, R.W.G. *The Biochemistry of Animal Fossils*; Sciencetechnica, Bristol, 1972;
2. Weiner, S.; Lowenstam, H.A.; Hood, L. Characterization of 80-Million-Year-Old Mollusk Shell Proteins. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73*, 2541–2545.
3. Poinar, G.O., Jr; Hess, R. Ultrastructure of 40-Million-Year-Old Insect Tissue. *Science* **1982**, *215*, 1241–1242.
4. Pääbo Über Den Nachweis von DNA in Altägyptischen Mumien. *Altertum* **1984**.
5. Higuchi, R.; Bowman, B.; Freiberger, M.; Ryder, O.A.; Wilson, A.C. DNA Sequences from the Quagga, an Extinct Member of the Horse Family. *Nature* **1984**, *312*, 282–284.
6. Pääbo, S. Molecular Cloning of Ancient Egyptian Mummy DNA. *Nature* **1985**, *314*, 644–645.
7. Saiki, R.K.; Scharf, S.; Faloona, F.; Mullis, K.B.; Horn, G.T.; Erlich, H.A.; Arnheim, N. Enzymatic Amplification of Beta-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia. *Science* **1985**, *230*, 1350–1354.
8. Svante Pääbo, Russell G. Higuchi, Allan C. Wilson Ancient DNA and the Polymerase Chain Reaction. *THE JOURNAL OF BIOLOGICAL CHEMISTRY* **1989**.
9. Pääbo, S.; Gifford, J.A.; Wilson, A.C. Mitochondrial DNA Sequences from a 7000-Year Old Brain. *Nucleic Acids Res.* **1988**, *16*, 9775–9787.
10. Pääbo, S. Ancient DNA: Extraction, Characterization, Molecular Cloning, and Enzymatic Amplification. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 1939–1943.
11. Hagelberg, E.; Hofreiter, M.; Keyser, C. Ancient DNA: The First Three Decades. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2015**, *370*, 20130371.
12. Hagelberg, E.; Sykes, B.; Hedges, R. Ancient Bone DNA Amplified. *Nature* **1989**, *342*, 485.
13. Horai, S.; Hayasaka, K.; Murayama, K.; Wate, N.; Koike, H.; Nakai, N. DNA Amplification from Ancient Human Skeletal Remains and Their Sequence Analysis. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **1989**, *65*, 229–233.
14. Hagelberg, E.; Gray, I.C.; Jeffreys, A.J. Identification of the Skeletal Remains of a Murder Victim by DNA Analysis. *Nature* **1991**, *352*, 427–429.
15. Hagelberg, E.; Clegg, J.B. Genetic Polymorphisms in Prehistoric Pacific Islanders Determined by Analysis of Ancient Bone DNA. *Proc. Biol. Sci.* **1993**, *252*, 163–170.
16. Stone, A.C.; Stoneking, M. Ancient DNA from a Pre-Columbian Amerindian Population. *Am. J. Phys. Anthropol.* **1993**, *92*, 463–471.
17. Handt, O.; Höss, M.; Krings, M.; Pääbo, S. Ancient DNA: Methodological Challenges. *Experientia* **1994**, *50*, 524–529.
18. Woodward, S.R.; Weyand, N.J.; Bunnell, M. DNA Sequence from Cretaceous Period Bone Fragments. *Science* **1994**, *266*, 1229–1232.
19. Golenberg, E.M.; Giannasi, D.E.; Clegg, M.T.; Smiley, C.J.; Durbin, M.; Henderson, D.; Zurawski, G. Chloroplast DNA Sequence from a Miocene Magnolia Species. *Nature* **1990**, *344*, 656–658.
20. Stoneking, M. Ancient DNA: How Do You Know When You Have It and What Can You Do with It? *Am. J. Hum. Genet.* **1995**, *57*, 1259–1262.

21. Cooper, A. Reply to Stoneking: Ancient DNA--How Do You Really Know When You Have It? *Am. J. Hum. Genet.* 1997, 60, 1001–1003.
22. Béraud-Colomb, E.; Roubin, R.; Martin, J.; Maroc, N.; Gardeisen, A.; Trabuchet, G.; Goossens, M. Reply to Cooper. *Am. J. Hum. Genet.* **1997**, 60, 1002.
23. Krings, M.; Stone, A.; Schmitz, R.W.; Krainitzki, H.; Stoneking, M.; Pääbo, S. Neandertal DNA Sequences and the Origin of Modern Humans. *Cell* **1997**, 90, 19–30.
24. Cooper, A.; Poinar, H.N. Ancient DNA: Do It Right or Not at All. *Science* 2000, 289, 1139.
25. Richards, M.B.; Sykes, B.C.; Hedges, R.E.M. Authenticating DNA Extracted From Ancient Skeletal Remains. *J. Archaeol. Sci.* **1995**, 22, 291–299.
26. Der Sarkissian, C.; Allentoft, M.E.; Ávila-Arcos, M.C.; Barnett, R.; Campos, P.F.; Cappellini, E.; Ermini, L.; Fernández, R.; da Fonseca, R.; Ginolhac, A.; et al. Ancient Genomics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2015**, 370, 20130387.
27. Noonan, J.P.; Hofreiter, M.; Smith, D.; Priest, J.R.; Rohland, N.; Rabeder, G.; Krause, J.; Detter, J.C.; Pääbo, S.; Rubin, E.M. Genomic Sequencing of Pleistocene Cave Bears. *Science* **2005**, 309, 597–599.
28. Margulies, M.; Egholm, M.; Altman, W.E.; Attiya, S.; Bader, J.S.; Bemben, L.A.; Berka, J.; Braverman, M.S.; Chen, Y.-J.; Chen, Z.; et al. Genome Sequencing in Microfabricated High-Density Picolitre Reactors. *Nature* **2005**, 437, 376–380.
29. Poinar, H.N.; Schwarz, C.; Qi, J.; Shapiro, B.; Macphee, R.D.E.; Buigues, B.; Tikhonov, A.; Huson, D.H.; Tomsho, L.P.; Auch, A.; et al. Metagenomics to Paleogenomics: Large-Scale Sequencing of Mammoth DNA. *Science* **2006**, 311, 392–394.
30. Gilbert, M.T.P.; Tomsho, L.P.; Rendulic, S.; Packard, M.; Drautz, D.I.; Sher, A.; Tikhonov, A.; Dalén, L.; Kuznetsova, T.; Kosintsev, P.; et al. Whole-Genome Shotgun Sequencing of Mitochondria from Ancient Hair Shafts. *Science* **2007**, 317, 1927–1930.
31. Bentley, D.R.; Balasubramanian, S.; Swerdlow, H.P.; Smith, G.P.; Milton, J.; Brown, C.G.; Hall, K.P.; Evers, D.J.; Barnes, C.L.; Bignell, H.R.; et al. Accurate Whole Human Genome Sequencing Using Reversible Terminator Chemistry. *Nature* **2008**, 456, 53–59.
32. Rasmussen, M.; Li, Y.; Lindgreen, S.; Pedersen, J.S.; Albrechtsen, A.; Moltke, I.; Metspalu, M.; Metspalu, E.; Kivisild, T.; Gupta, R.; et al. Ancient Human Genome Sequence of an Extinct Palaeo-Eskimo. *Nature* **2010**, 463, 757–762.
33. Orlando, L.; Allaby, R.; Skoglund, P.; Der Sarkissian, C.; Stockhammer, P.W.; Ávila-Arcos, M.C.; Fu, Q.; Krause, J.; Willerslev, E.; Stone, A.C.; et al. Ancient DNA Analysis. *Nature Reviews Methods Primers* **2021**, 1, 1–26.
34. Orlando, L.; Ginolhac, A.; Zhang, G.; Froese, D.; Albrechtsen, A.; Stiller, M.; Schubert, M.; Cappellini, E.; Petersen, B.; Moltke, I.; et al. Recalibrating Equus Evolution Using the Genome Sequence of an Early Middle Pleistocene Horse. *Nature* **2013**, 499, 74–78.
35. Krause, J.; Fu, Q.; Good, J.M.; Viola, B.; Shunkov, M.V.; Derevianko, A.P.; Pääbo, S. The Complete Mitochondrial DNA Genome of an Unknown Hominin from Southern Siberia. *Nature* **2010**, 464, 894–897.
36. Reich, D.; Green, R.E.; Kircher, M.; Krause, J.; Patterson, N.; Durand, E.Y.; Viola, B.; Briggs, A.W.; Stenzel, U.; Johnson, P.L.F.; et al. Genetic History of an Archaic Hominin Group from Denisova Cave in Siberia. *Nature* **2010**, 468, 1053–1060.

37. Bos, K.I.; Schuenemann, V.J.; Golding, G.B.; Burbano, H.A.; Waglechner, N.; Coombes, B.K.; McPhee, J.B.; DeWitte, S.N.; Meyer, M.; Schmedes, S.; et al. A Draft Genome of *Yersinia Pestis* from Victims of the Black Death. *Nature* **2011**, *478*, 506–510.
38. Warinner, C.; Rodrigues, J.F.M.; Vyas, R.; Trachsel, C.; Shved, N.; Grossmann, J.; Radini, A.; Hancock, Y.; Tito, R.Y.; Fiddyment, S.; et al. Pathogens and Host Immunity in the Ancient Human Oral Cavity. *Nat. Genet.* **2014**, *46*, 336–344.
39. Pedersen, J.S.; Valen, E.; Velazquez, A.M.V.; Parker, B.J.; Rasmussen, M.; Lindgreen, S.; Lilje, B.; Tobin, D.J.; Kelly, T.K.; Vang, S.; et al. Genome-Wide Nucleosome Map and Cytosine Methylation Levels of an Ancient Human Genome. *Genome Res.* **2014**, *24*, 454–466.
40. Lindahl, T.; Wood, R.D. Quality Control by DNA Repair. *Science* **1999**, *286*, 1897–1905.
41. Hofreiter, M.; Serre, D.; Poinar, H.N.; Kuch, M.; Pääbo, S. Ancient DNA. *Nat. Rev. Genet.* **2001**, *2*, 353–359.
42. Kistler, L.; Ware, R.; Smith, O.; Collins, M.; Allaby, R.G. A New Model for Ancient DNA Decay Based on Paleogenomic Meta-Analysis. *Nucleic Acids Res.* **2017**, *45*, 6310–6320.
43. Lindahl, T. Instability and Decay of the Primary Structure of DNA. *Nature* **1993**, *362*.
44. Allentoft, M.E.; Collins, M.; Harker, D.; Haile, J.; Oskam, C.L.; Hale, M.L.; Campos, P.F.; Samaniego, J.A.; Gilbert, M.T.P.; Willerslev, E.; et al. The Half-Life of DNA in Bone: Measuring Decay Kinetics in 158 Dated Fossils. *Proc. Biol. Sci.* **2012**, *279*, 4724–4733.
45. Sawyer, S.; Krause, J.; Guschanski, K.; Savolainen, V.; Pääbo, S. Temporal Patterns of Nucleotide Misincorporations and DNA Fragmentation in Ancient DNA. *PLoS One* **2012**, *7*, e34131.
46. Lindahl, T.; Andersson, A. Rate of Chain Breakage at Apurinic Sites in Double-Stranded Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3618–3623.
47. Briggs, A.W.; Stenzel, U.; Johnson, P.L.F.; Green, R.E.; Kelso, J.; Prüfer, K.; Meyer, M.; Krause, J.; Ronan, M.T.; Lachmann, M.; et al. Patterns of Damage in Genomic DNA Sequences from a Neandertal. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 14616–14621.
48. Hofreiter, M.; Jaenicke, V.; Serre, D.; von Haeseler, A.; Pääbo, S. DNA Sequences from Multiple Amplifications Reveal Artifacts Induced by Cytosine Deamination in Ancient DNA. *Nucleic Acids Res.* **2001**, *29*, 4793–4799.
49. Krause, J.; Briggs, A.W.; Kircher, M.; Maricic, T.; Zwyns, N.; Derevianko, A.; Pääbo, S. A Complete MtDNA Genome of an Early Modern Human from Kostenki, Russia. *Curr. Biol.* **2010**, *20*, 231–236.
50. Der Sarkissian, C.; Ermini, L.; Jónsson, H.; Alekseev, A.N.; Crubezy, E.; Shapiro, B.; Orlando, L. Shotgun Microbial Profiling of Fossil Remains. *Mol. Ecol.* **2014**, *23*, 1780–1798.
51. Key, F.M.; Posth, C.; Krause, J.; Herbig, A.; Bos, K.I. Mining Metagenomic Data Sets for Ancient DNA: Recommended Protocols for Authentication. *Trends Genet.* **2017**, *33*, 508–520.
52. Leonard, J.A.; Shanks, O.; Hofreiter, M.; Kreuz, E.; Hodges, L.; Ream, W.; Wayne, R.K.; Fleischer, R.C. Animal DNA in PCR Reagents Plagues Ancient DNA Research. *J. Archaeol. Sci.* **2007**, *34*, 1361–1366.

53. Llamas, B.; Valverde, G.; Fehren-Schmitz, L.; Weyrich, L.S.; Cooper, A.; Haak, W. From the Field to the Laboratory: Controlling DNA Contamination in Human Ancient DNA Research in the High-Throughput Sequencing Era. *STAR: Science & Technology of Archaeological Research* **2017**, *3*, 1–14.
54. Ballantyne, K.N.; Salemi, R.; Guarino, F.; Pearson, J.R.; Garlepp, D.; Fowler, S.; van Oorschot, R.A.H. DNA Contamination Minimisation – Finding an Effective Cleaning Method. *Aust. J. Forensic Sci.* **2015**, *47*, 428–439.
55. Schubert, M.; Ginolhac, A.; Lindgreen, S.; Thompson, J.F.; Al-Rasheid, K.A.S.; Willerslev, E.; Krogh, A.; Orlando, L. Improving Ancient DNA Read Mapping against Modern Reference Genomes. *BMC Genomics* **2012**, *13*, 178.
56. Peyrégne, S.; Prüfer, K. Present-Day DNA Contamination in Ancient DNA Datasets. *Bioessays* **2020**, *42*, e2000081.
57. Renaud, G.; Slon, V.; Duggan, A.T.; Kelso, J. Schmutzi: Estimation of Contamination and Endogenous Mitochondrial Consensus Calling for Ancient DNA. *Genome Biol.* **2015**, *16*, 224.
58. Fu, Q.; Mitnik, A.; Johnson, P.L.F.; Bos, K.; Lari, M.; Bollongino, R.; Sun, C.; Giemsch, L.; Schmitz, R.; Burger, J.; et al. A Revised Timescale for Human Evolution Based on Ancient Mitochondrial Genomes. *Curr. Biol.* **2013**, *23*, 553–559.
59. Furtwängler, A.; Reiter, E.; Neumann, G.U.; Siebke, I.; Steuri, N.; Hafner, A.; Lösch, S.; Anthes, N.; Schuenemann, V.J.; Krause, J. Ratio of Mitochondrial to Nuclear DNA Affects Contamination Estimates in Ancient DNA Analysis. *Sci. Rep.* **2018**, *8*, 14075.
60. Rasmussen, M.; Guo, X.; Wang, Y.; Lohmueller, K.E.; Rasmussen, S.; Albrechtsen, A.; Skotte, L.; Lindgreen, S.; Metspalu, M.; Jombart, T.; et al. An Aboriginal Australian Genome Reveals Separate Human Dispersals into Asia. *Science* **2011**, *334*, 94–98.
61. Moreno-Mayar, J.V.; Korneliussen, T.S.; Dalal, J.; Renaud, G.; Albrechtsen, A.; Nielsen, R.; Malaspina, A.-S. A Likelihood Method for Estimating Present-Day Human Contamination in Ancient Male Samples Using Low-Depth X-Chromosome Data. *Bioinformatics* **2020**, *36*, 828–841.
62. Huang, Y.; Ringbauer, H. HapCon: Estimating Contamination of Ancient Genomes by Copying from Reference Haplotypes. *Bioinformatics* **2022**, *38*, 3768–3777.
63. Peyrégne, S.; Peter, B.M. AuthenticCT: A Model of Ancient DNA Damage to Estimate the Proportion of Present-Day DNA Contamination. *Genome Biol.* **2020**, *21*, 246.
64. Skoglund, P.; Northoff, B.H.; Shunkov, M.V.; Derevianko, A.P.; Pääbo, S.; Krause, J.; Jakobsson, M. Separating Endogenous Ancient DNA from Modern Day Contamination in a Siberian Neandertal. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 2229–2234.
65. Pedersen, M.W.; De Sanctis, B.; Saremi, N.F.; Sikora, M.; Puckett, E.E.; Gu, Z.; Moon, K.L.; Kapp, J.D.; Vinner, L.; Vardanyan, Z.; et al. Environmental Genomics of Late Pleistocene Black Bears and Giant Short-Faced Bears. *Curr. Biol.* **2021**, *31*, 2728–2736.e8.
66. O’Sullivan, N.J.; Teasdale, M.D.; Mattiangeli, V.; Maixner, F.; Pinhasi, R.; Bradley, D.G.; Zink, A. A Whole Mitochondria Analysis of the Tyrolean Iceman’s Leather Provides Insights into the Animal Sources of Copper Age Clothing. *Sci. Rep.* **2016**, *6*, 31279.
67. Green, E.J.; Speller, C.F. Novel Substrates as Sources of Ancient DNA: Prospects and Hurdles. *Genes (Basel)* **2017**, *8*, 180.

68. Gamba, C.; Jones, E.R.; Teasdale, M.D.; McLaughlin, R.L.; Gonzalez-Fortes, G.; Mattiangeli, V.; Domboróczy, L.; Kővári, I.; Pap, I.; Anders, A.; et al. Genome Flux and Stasis in a Five Millennium Transect of European Prehistory. *Nat. Commun.* **2014**, *5*, 5257.
69. Pinhasi, R.; Fernandes, D.; Sirak, K.; Novak, M.; Connell, S.; Alpaslan-Roodenberg, S.; Gerritsen, F.; Moiseyev, V.; Gromov, A.; Raczky, P.; et al. Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone. *PLoS One* **2015**, *10*, e0129102.
70. de León, M.S.P.; Koesbardiati, T.; Weissmann, J.D.; Milella, M.; Reyna-Blanco, C.S.; Suwa, G.; Kondo, O.; Malaspinas, A.-S.; White, T.D.; Zollikofer, C.P.E. Human Bony Labyrinth Is an Indicator of Population History and Dispersal from Africa. *Proceedings of the National Academy of Sciences* **2018**, *115*, 4128–4133.
71. Charlton, S.; Booth, T.; Barnes, I. The Problem with Petrous? A Consideration of the Potential Biases in the Utilization of Pars Petrosa for Ancient DNA Analysis. *World Archaeol.* **2019**, *51*, 574–585.
72. Sirak, K.A.; Fernandes, D.M.; Cheronet, O.; Novak, M.; Gamarra, B.; Balassa, T.; Bernert, Z.; Cséki, A.; Dani, J.; Gallina, J.Z.; et al. A Minimally-Invasive Method for Sampling Human Petrous Bones from the Cranial Base for Ancient DNA Analysis. *Biotechniques* **2017**, *62*, 283–289.
73. Sirak, K.; Fernandes, D.; Cheronet, O.; Harney, E.; Mah, M.; Mallick, S.; Rohland, N.; Adamski, N.; Broomandkhoshbacht, N.; Callan, K.; et al. Human Auditory Ossicles as an Alternative Optimal Source of Ancient DNA. *Genome Res.* **2020**, *30*, 427–436.
74. Parker, C.; Rohrlach, A.B.; Friederich, S.; Nagel, S.; Meyer, M.; Krause, J.; Bos, K.I.; Haak, W. A Systematic Investigation of Human DNA Preservation in Medieval Skeletons. *Sci. Rep.* **2020**, *10*, 18225.
75. Alberti, F.; Gonzalez, J.; Paijmans, J.L.A.; Basler, N.; Preick, M.; Henneberger, K.; Trinks, A.; Rabeder, G.; Conard, N.J.; Münzel, S.C.; et al. Optimized DNA Sampling of Ancient Bones Using Computed Tomography Scans. *Mol. Ecol. Resour.* **2018**, *18*, 1196–1208.
76. Sponheimer, M.; Ryder, C.M.; Fewlass, H.; Smith, E.K.; Pestle, W.J.; Talamo, S. Saving Old Bones: A Non-Destructive Method for Bone Collagen Prescreening. *Sci. Rep.* **2019**, *9*, 13928.
77. Harney, É.; Cheronet, O.; Fernandes, D.M.; Sirak, K.; Mah, M.; Bernardos, R.; Adamski, N.; Broomandkhoshbacht, N.; Callan, K.; Lawson, A.M.; et al. A Minimally Destructive Protocol for DNA Extraction from Ancient Teeth. *Genome Res.* **2021**, *31*, 472–483.
78. Margaryan, A.; Hansen, H.B.; Rasmussen, S.; Sikora, M.; Moiseyev, V.; Khoklov, A.; Epimakhov, A.; Yepiskoposyan, L.; Kriiska, A.; Varul, L.; et al. Ancient Pathogen DNA in Human Teeth and Petrous Bones. *Ecol. Evol.* **2018**, *8*, 3534–3542.
79. Fagernäs, Z.; García-Collado, M.I.; Hendy, J.; Hofman, C.A.; Speller, C.; Velsko, I.; Warinner, C. A Unified Protocol for Simultaneous Extraction of DNA and Proteins from Archaeological Dental Calculus. *J. Archaeol. Sci.* **2020**, *118*, 105135.
80. Mann, A.E.; Sabin, S.; Ziesemer, K.; Vågane, Å.J.; Schroeder, H.; Ozga, A.T.; Sankaranarayanan, K.; Hofman, C.A.; Fellows Yates, J.A.; Salazar-García, D.C.; et al.

Differential Preservation of Endogenous Human and Microbial DNA in Dental Calculus and Dentin. *Sci. Rep.* **2018**, *8*, 9822.

81. Velsko, I.M.; Overmyer, K.A.; Speller, C.; Klaus, L.; Collins, M.J.; Loe, L.; Frantz, L.A.F.; Sankaranarayanan, K.; Lewis, C.M., Jr; Martinez, J.B.R.; et al. The Dental Calculus Metabolome in Modern and Historic Samples. *Metabolomics* **2017**, *13*, 134.
82. Wright, S.L.; Dobney, K.; Weyrich, L.S. Advancing and Refining Archaeological Dental Calculus Research Using Multiomic Frameworks. *STAR: Science & Technology of Archaeological Research* **2021**, *7*, 13–30.
83. Höss, M.; Pääbo, S. DNA Extraction from Pleistocene Bones by a Silica-Based Purification Method. *Nucleic Acids Res.* **1993**, *21*, 3913–3914.
84. Rohland, N.; Hofreiter, M. Ancient DNA Extraction from Bones and Teeth. *Nat. Protoc.* **2007**, *2*, 1756–1762.
85. Dabney, J.; Knapp, M.; Glocke, I.; Gansauge, M.-T.; Weihmann, A.; Nickel, B.; Valdiosera, C.; García, N.; Pääbo, S.; Arsuaga, J.-L.; et al. Complete Mitochondrial Genome Sequence of a Middle Pleistocene Cave Bear Reconstructed from Ultrashort DNA Fragments. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 15758–15763.
86. Rohland, N.; Glocke, I.; Aximu-Petri, A.; Meyer, M. Extraction of Highly Degraded DNA from Ancient Bones, Teeth and Sediments for High-Throughput Sequencing. *Nat. Protoc.* **2018**, *13*, 2447–2461.
87. Meyer, M.; Kircher, M. Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing. *Cold Spring Harb. Protoc.* **2010**, *2010*, db.prot5448.
88. Meyer, M.; Kircher, M.; Gansauge, M.-T.; Li, H.; Racimo, F.; Mallick, S.; Schraiber, J.G.; Jay, F.; Prüfer, K.; de Filippo, C.; et al. A High-Coverage Genome Sequence from an Archaic Denisovan Individual. *Science* **2012**, *338*, 222–226.
89. Gansauge, M.-T.; Meyer, M. Single-Stranded DNA Library Preparation for the Sequencing of Ancient or Damaged DNA. *Nat. Protoc.* **2013**, *8*, 737–748.
90. Gansauge, M.-T.; Aximu-Petri, A.; Nagel, S.; Meyer, M. Manual and Automated Preparation of Single-Stranded DNA Libraries for the Sequencing of DNA from Ancient Biological Remains and Other Sources of Highly Degraded DNA. *Nat. Protoc.* **2020**, *15*, 2279–2300.
91. Korlević, P.; Gerber, T.; Gansauge, M.-T.; Hajdinjak, M.; Nagel, S.; Aximu-Petri, A.; Meyer, M. Reducing Microbial and Human Contamination in DNA Extractions from Ancient Bones and Teeth. *Biotechniques* **2015**, *59*, 87–93.
92. Gansauge, M.-T.; Meyer, M. Selective Enrichment of Damaged DNA Molecules for Ancient Genome Sequencing. *Genome Res.* **2014**, *24*, 1543–1549.
93. Briggs, A.W.; Stenzel, U.; Meyer, M.; Krause, J.; Kircher, M.; Pääbo, S. Removal of Deaminated Cytosines and Detection of in Vivo Methylation in Ancient DNA. *Nucleic Acids Res.* **2010**, *38*, e87.
94. Rohland, N.; Harney, E.; Mallick, S.; Nordenfelt, S.; Reich, D. Partial Uracil–DNA–Glycosylase Treatment for Screening of Ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2015**, *370*, 20130624.

95. Maricic, T.; Whitten, M.; Pääbo, S. Multiplexed DNA Sequence Capture of Mitochondrial Genomes Using PCR Products. *PLoS One* **2010**, *5*, e14004.
96. Avila-Arcos, M.C.; Cappellini, E.; Romero-Navarro, J.A.; Wales, N.; Moreno-Mayar, J.V.; Rasmussen, M.; Fordyce, S.L.; Montiel, R.; Vielle-Calzada, J.-P.; Willerslev, E.; et al. Application and Comparison of Large-Scale Solution-Based DNA Capture-Enrichment Methods on Ancient DNA. *Sci. Rep.* **2011**, *1*, 74.
97. Rohrlach, A.B.; Papac, L.; Childebayeva, A.; Rivollat, M.; Villalba-Mouco, V.; Neumann, G.U.; Penske, S.; Skourtanioti, E.; van de Loosdrecht, M.; Akar, M.; et al. Using Y-Chromosome Capture Enrichment to Resolve Haplogroup H2 Shows New Evidence for a Two-Path Neolithic Expansion to Western Europe. *Sci. Rep.* **2021**, *11*, 15005.
98. Patterson, N.; Moorjani, P.; Luo, Y.; Mallick, S.; Rohland, N.; Zhan, Y.; Genschoreck, T.; Webster, T.; Reich, D. Ancient Admixture in Human History. *Genetics* **2012**, *192*, 1065–1093.
99. Fu, Q.; Hajdinjak, M.; Moldovan, O.T.; Constantin, S.; Mallick, S.; Skoglund, P.; Patterson, N.; Rohland, N.; Lazaridis, I.; Nickel, B.; et al. An Early Modern Human from Romania with a Recent Neanderthal Ancestor. *Nature* **2015**, *524*, 216–219.
100. Mathieson, I.; Lazaridis, I.; Rohland, N.; Mallick, S.; Patterson, N.; Roodenberg, S.A.; Harney, E.; Stewardson, K.; Fernandes, D.; Novak, M.; et al. Genome-Wide Patterns of Selection in 230 Ancient Eurasians. *Nature* **2015**, *528*, 499–503.
101. Immel, A.; Key, F.M.; Szolek, A.; Barquera, R.; Robinson, M.K.; Harrison, G.F.; Palmer, W.H.; Spyrou, M.A.; Susat, J.; Krause-Kyora, B.; et al. Analysis of Genomic DNA from Medieval Plague Victims Suggests Long-Term Effect of *Yersinia Pestis* on Human Immunity Genes. *Mol. Biol. Evol.* **2021**, *38*, 4059–4076.
102. Andrades Valtueña, A.; Mittnik, A.; Key, F.M.; Haak, W.; Allmäe, R.; Belinskij, A.; Daubaras, M.; Feldman, M.; Jankauskas, R.; Janković, I.; et al. The Stone Age Plague and Its Persistence in Eurasia. *Curr. Biol.* **2017**, *27*, 3683–3691.e8.
103. Kocher, A.; Papac, L.; Barquera, R.; Key, F.M.; Spyrou, M.A.; Hübner, R.; Rohrlach, A.B.; Aron, F.; Stahl, R.; Wissgott, A.; et al. Ten Millennia of Hepatitis B Virus Evolution. *Science* **2021**, *374*, 182–188.
104. Pearson, M.P.; Schadla-Hall, T.; Moshenska, G. Resolving the Human Remains Crisis in British Archaeology. *Papers from the Institute of Archaeology* 2011, *21*, 5.
105. Fox, K.; Hawks, J. Use Ancient Remains More Wisely. *Nature* **2019**, *572*, 581–583.
106. Makarewicz, C.; Marom, N.; Bar-Oz, G. Ensure Equal Access to Ancient DNA Available online: <http://dx.doi.org/10.1038/548158a> (accessed on 27 September 2022).
107. Prendergast, M.E.; Sawchuk, E. Boots on the Ground in Africa’s Ancient DNA ‘Revolution’: Archaeological Perspectives on Ethics and Best Practices. *Antiquity* **2018**, *92*, 803–815.
108. Kahle, D.; Wickham, H. Ggmap: Spatial Visualization with Ggplot2. *The R Journal* **2013**, *5*, 144–161.
109. Kaifu, Y. Archaic Hominin Populations in Asia before the Arrival of Modern Humans: Their Phylogeny and Implications for the “Southern Denisovans”. *Curr. Anthropol.* **2017**, *58*, S418–S433.

110. Larick, R.; Ciochon, R.L.; Zaim, Y.; Sudijono; Suminto; Rizal, Y.; Aziz, F.; Reagan, M.; Heizler, M. Early Pleistocene $^{40}\text{Ar}/^{39}\text{Ar}$ Ages for Bapang Formation Hominins, Central Jawa, Indonesia. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 4866–4871.
111. Zeitoun, V.; Déroit, F.; Grimaud-Hervé, D.; Widiyanto, H. Solo Man in Question: Convergent Views to Split Indonesian Homo Erectus in Two Categories. *Quat. Int.* **2010**, *223–224*, 281–292.
112. Baab, K.L.; Zaim, Y. Global and Local Perspectives on Cranial Shape Variation in Indonesian *Homo Erectus*. *Anthropol. Sci.* **2017**, *125*, 67–83.
113. Swisher, C.C., 3rd; Rink, W.J.; Antón, S.C.; Schwarcz, H.P.; Curtis, G.H.; Suprijo, A.; Widiasmoro Latest Homo Erectus of Java: Potential Contemporaneity with Homo Sapiens in Southeast Asia. *Science* **1996**, *274*, 1870–1874.
114. Yokoyama, Y.; Falguères, C.; Sémah, F.; Jacob, T.; Grün, R. Gamma-Ray Spectrometric Dating of Late Homo Erectus Skulls from Ngandong and Sambungmacan, Central Java, Indonesia. *J. Hum. Evol.* **2008**, *55*, 274–277.
115. Rizal, Y.; Westaway, K.E.; Zaim, Y.; van den Bergh, G.D.; Bettis, E.A., 3rd; Morwood, M.J.; Huffman, O.F.; Grün, R.; Joannes-Boyau, R.; Bailey, R.M.; et al. Last Appearance of Homo Erectus at Ngandong, Java, 117,000–108,000 Years Ago. *Nature* **2020**, *577*, 381–385.
116. Brown, P.; Sutikna, T.; Morwood, M.J.; Soejono, R.P.; Jatmiko; Saptomo, E.W.; Due, R.A. A New Small-Bodied Hominin from the Late Pleistocene of Flores, Indonesia. *Nature* **2004**, *431*, 1055–1061.
117. Morwood, M.J.; Brown, P.; Jatmiko; Sutikna, T.; Saptomo, E.W.; Westaway, K.E.; Due, R.A.; Roberts, R.G.; Maeda, T.; Wasisto, S.; et al. Further Evidence for Small-Bodied Hominins from the Late Pleistocene of Flores, Indonesia. *Nature* **2005**, *437*, 1012–1017.
118. Weber, J.; Czarnetzki, A.; Pusch, C.M. Comment on ‘The Brain of LB1, *Homo Floresiensis*’. *Science* **2005**, *310*, 236–236.
119. Martin, R.D.; Maclarnon, A.M.; Phillips, J.L.; Dussubieux, L.; Williams, P.R.; Dobyns, W.B. Comment on ‘The Brain of LB1, *Homo Floresiensis*’. *Science* **2006**, *312*, 999; author reply 999.
120. Jacob, T.; Indriati, E.; Soejono, R.P.; Hsü, K.; Frayer, D.W.; Eckhardt, R.B.; Kuperavage, A.J.; Thorne, A.; Henneberg, M. Pygmoid Australomelanesian Homo Sapiens Skeletal Remains from Liang Bua, Flores: Population Affinities and Pathological Abnormalities. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 13421–13426.
121. Martin, R.D.; Maclarnon, A.M.; Phillips, J.L.; Dobyns, W.B. Flores Hominid: New Species or Microcephalic Dwarf? *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **2006**, *288*, 1123–1145.
122. Falk, D.; Hildebolt, C.; Smith, K.; Morwood, M.J.; Sutikna, T.; Jatmiko; Saptomo, E.W.; Imhof, H.; Seidler, H.; Prior, F. Brain Shape in Human Microcephalics and Homo Floresiensis. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 2513–2518.
123. Argue, D.; Donlon, D.; Groves, C.; Wright, R. Homo Floresiensis: Microcephalic, Pygmoid, Australopithecus, or Homo? *J. Hum. Evol.* **2006**, *51*, 360–374.

124. Sutikna, T.; Tocheri, M.W.; Morwood, M.J.; Saptomo, E.W.; Jatmiko; Awe, R.D.; Wasisto, S.; Westaway, K.E.; Aubert, M.; Li, B.; et al. Revised Stratigraphy and Chronology for Homo Floresiensis at Liang Bua in Indonesia. *Nature* **2016**, *532*, 366–369.
125. Brumm, A.; Jensen, G.M.; van den Bergh, G.D.; Morwood, M.J.; Kurniawan, I.; Aziz, F.; Storey, M. Hominins on Flores, Indonesia, by One Million Years Ago. *Nature* **2010**, *464*, 748–752.
126. van den Bergh, G.D.; Kaifu, Y.; Kurniawan, I.; Kono, R.T.; Brumm, A.; Setiyabudi, E.; Aziz, F.; Morwood, M.J. Homo Floresiensis-like Fossils from the Early Middle Pleistocene of Flores. *Nature* **2016**, *534*, 245–248.
127. Détroit, F.; Mijares, A.S.; Corny, J.; Daver, G.; Zanolli, C.; Dizon, E.; Robles, E.; Grün, R.; Piper, P.J. A New Species of Homo from the Late Pleistocene of the Philippines. *Nature* **2019**, *568*, 181–186.
128. Zanolli, C.; Kaifu, Y.; Pan, L.; Xing, S.; Mijares, A.S.; Kullmer, O.; Schrenk, F.; Corny, J.; Dizon, E.; Robles, E.; et al. Further Analyses of the Structural Organization of Homo Luzonensis Teeth: Evolutionary Implications. *J. Hum. Evol.* **2022**, *163*, 103124.
129. Harvati, K.; Röding, C.; Bosman, A.M.; Karakostis, F.A.; Grün, R.; Stringer, C.; Karkanas, P.; Thompson, N.C.; Koutoulidis, V.; Mouloupoulos, L.A.; et al. Apidima Cave Fossils Provide Earliest Evidence of Homo Sapiens in Eurasia. *Nature* **2019**, *571*, 500–504.
130. Liu, W.; Jin, C.-Z.; Zhang, Y.-Q.; Cai, Y.-J.; Xing, S.; Wu, X.-J.; Cheng, H.; Edwards, R.L.; Pan, W.-S.; Qin, D.-G.; et al. Human Remains from Zhirendong, South China, and Modern Human Emergence in East Asia. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 19201–19206.
131. Bae, C.J.; Wang, W.; Zhao, J.; Huang, S.; Tian, F.; Shen, G. Modern Human Teeth from Late Pleistocene Luna Cave (Guangxi, China). *Quat. Int.* **2014**, *354*, 169–183.
132. Liu, W.; Martín-Torres, M.; Cai, Y.-J.; Xing, S.; Tong, H.-W.; Pei, S.-W.; Sier, M.J.; Wu, X.-H.; Edwards, R.L.; Cheng, H.; et al. The Earliest Unequivocally Modern Humans in Southern China. *Nature* **2015**, *526*, 696–699.
133. Shen, G.; Wu, X.; Wang, Q.; Tu, H.; Feng, Y.-X.; Zhao, J.-X. Mass Spectrometric U-Series Dating of Huanglong Cave in Hubei Province, Central China: Evidence for Early Presence of Modern Humans in Eastern Asia. *J. Hum. Evol.* **2013**, *65*, 162–167.
134. Curnoe, D.; Ji, X.; Shaojin, H.; Taçon, P.S.C.; Li, Y. Dental Remains from Longtanshan Cave 1 (Yunnan, China), and the Initial Presence of Anatomically Modern Humans in East Asia. *Quat. Int.* **2016**, *400*, 180–186.
135. Sun, X.-F.; Wen, S.-Q.; Lu, C.-Q.; Zhou, B.-Y.; Curnoe, D.; Lu, H.-Y.; Li, H.-C.; Wang, W.; Cheng, H.; Yi, S.-W.; et al. Ancient DNA and Multimethod Dating Confirm the Late Arrival of Anatomically Modern Humans in Southern China. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, doi:10.1073/pnas.2019158118.
136. Higham, T.; Douka, K. The Impact of Reliable Radiocarbon Dating on Understanding Early Homo Sapiens in Asia. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
137. Storm, P.; Aziz, F.; de Vos, J.; Kosasih, D.; Baskoro, S.; Ngaliman; van den Hoek Ostende, L.W. Late Pleistocene Homo Sapiens in a Tropical Rainforest Fauna in East Java. *J. Hum. Evol.* **2005**, *49*, 536–545.

138. Westaway, K.E.; Morwood, M.J.; Roberts, R.G.; Rokus, A.D.; Zhao, J.-X.; Storm, P.; Aziz, F.; van den Bergh, G.; Hadi, P.; Jatmiko; et al. Age and Biostratigraphic Significance of the Punung Rainforest Fauna, East Java, Indonesia, and Implications for Pongo and Homo. *J. Hum. Evol.* **2007**, *53*, 709–717.
139. Barker; Barton; Bird; Daly; Datan The 'human Revolution' in Lowland Tropical Southeast Asia: The Antiquity and Behavior of Anatomically Modern Humans at Niah Cave (Sarawak, Borneo). *of human evolution* **2007**.
140. Bacon, A.-M.; Demeter, F.; Düringer, P.; Helm, C.; Bano, M.; Long, V.T.; Thuy, N.T.K.; Antoine, P.-O.; Mai, B.T.; Huong, N.T.M.; et al. The Late Pleistocene Duoi U'Oi Cave in Northern Vietnam: Palaeontology, Sedimentology, Taphonomy and Palaeoenvironments. *Quat. Sci. Rev.* **2008**, *27*, 1627–1654.
141. Westaway, K.E. An Early Modern Human Presence in Sumatra 73,000- 63, 000 Years Ago. *Nature* **2017**, *548*, 322–325.
142. Mijares, A.S.; Détroit, F.; Piper, P.; Grün, R.; Bellwood, P.; Aubert, M.; Champion, G.; Cuevas, N.; De Leon, A.; Dizon, E. New Evidence for a 67,000-Year-Old Human Presence at Callao Cave, Luzon, Philippines. *J. Hum. Evol.* **2010**, *59*, 123–132.
143. Grün, R.; Eggers, S.; Kinsley, L.; Moseley, H.; Sambridge, M. Laser Ablation U-Series Analysis of Fossil Bones and Teeth. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2014**, *416*, 150–167.
144. Clarkson, C.; Jacobs, Z.; Marwick, B.; Fullagar, R.; Wallis, L.; Smith, M.; Roberts, R.G.; Hayes, E.; Lowe, K.; Carah, X.; et al. Human Occupation of Northern Australia by 65,000 Years Ago. *Nature* **2017**, *547*, 306–310.
145. O'Connell, J.F.; Allen, J.; Williams, M.A.J.; Williams, A.N.; Turney, C.S.M.; Spooner, N.A.; Kamminga, J.; Brown, G.; Cooper, A. When Did Homo Sapiens First Reach Southeast Asia and Sahul? *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 8482–8490.
146. Llamas, B.; Rada, X.R.; Collen, E. Ancient DNA Helps Trace the Peopling of the World. *Biochem. (Lond.)* **2020**, *42*, 18–22.
147. Demeter, F.; Shackelford, L.L.; Bacon, A.-M.; Düringer, P.; Westaway, K.; Sayavongkhamdy, T.; Braga, J.; Sichanthongtip, P.; Khamdalavong, P.; Ponche, J.-L.; et al. Anatomically Modern Human in Southeast Asia (Laos) by 46 Ka. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 14375–14380.
148. Demeter, F.; Shackelford, L.; Westaway, K.; Düringer, P.; Bacon, A.-M.; Ponche, J.-L.; Wu, X.; Sayavongkhamdy, T.; Zhao, J.-X.; Barnes, L.; et al. Early Modern Humans and Morphological Variation in Southeast Asia: Fossil Evidence from Tam Pa Ling, Laos. *PLoS One* **2015**, *10*, e0121193.
149. Demeter, F.; Shackelford, L.; Westaway, K.; Barnes, L.; Düringer, P.; Ponche, J.-L.; Dumoncel, J.; Sénégas, F.; Sayavongkhamdy, T.; Zhao, J.-X.; et al. Early Modern Humans from Tam Pà Ling, Laos. *Curr. Anthropol.* **2017**, *58*, S527–S538.
150. Shackelford, L.; Demeter, F.; Westaway, K.; Düringer, P.; Ponche, J.-L.; Sayavongkhamdy, T.; Zhao, J.-X.; Barnes, L.; Boyon, M.; Sichanthongtip, P.; et al. Additional Evidence for Early Modern Human Morphological Diversity in Southeast Asia at Tam Pa Ling, Laos. *Quat. Int.* **2018**, *466*, 93–106.

151. Shackelford, L.L.; Bacon, A.M.; Demeter, F. Tam Pà Ling and the Eastern Asian Hominin Fossil Record Available online: <https://hal.archives-ouvertes.fr/hal-03377703/document> (accessed on 30 September 2022).
152. Corny, J.; Garong, A.M.; Sémah, F.; Dizon, E.Z.; Bolunia, M.J.L.A.; Bautista, R.; Déroit, F. Paleoanthropological Significance and Morphological Variability of the Human Bones and Teeth from Tabon Cave. *Quat. Int.* **2016**, *416*, 210–218.
153. Barnes, L.M. Establishing Robust Chronologies for Models of Modern Human Dispersal in Southeast Asia: Implications for Arrival and Occupation in Sunda and Sahul, Macquarie University, Sydney, 2014.
154. Shen, G.; Wang, W.; Cheng, H.; Edwards, R.L. Mass Spectrometric U-Series Dating of Laibin Hominid Site in Guangxi, Southern China. *J. Archaeol. Sci.* **2007**, *34*, 2109–2114.
155. Bowler, J.M.; Johnston, H.; Olley, J.M.; Prescott, J.R.; Roberts, R.G.; Shawcross, W.; Spooner, N.A. New Ages for Human Occupation and Climatic Change at Lake Mungo, Australia. *Nature* **2003**, *421*, 837–840.
156. Thorne, A.; Grün, R.; Mortimer, G.; Spooner, N.A.; Simpson, J.J.; McCulloch, M.; Taylor, L.; Curnoe, D. Australia's Oldest Human Remains: Age of the Lake Mungo 3 Skeleton. *J. Hum. Evol.* **1999**, *36*, 591–612.
157. Aubert, M.; Lebe, R.; Oktaviana, A.A.; Tang, M.; Burhan, B.; Hamrullah; Jusdi, A.; Abdullah; Hakim, B.; Zhao, J.-X.; et al. Earliest Hunting Scene in Prehistoric Art. *Nature* **2019**, *576*, 442–445.
158. Hawkins, S.; O'Connor, S.; Maloney, T.R.; Litster, M.; Kealy, S.; Fenner, J.N.; Aplin, K.; Boulanger, C.; Brockwell, S.; Willan, R.; et al. Oldest Human Occupation of Wallacea at Laili Cave, Timor-Leste, Shows Broad-Spectrum Foraging Responses to Late Pleistocene Environments. *Quat. Sci. Rev.* **2017**, *171*, 58–72.
159. Morley, M.W.; Goldberg, P.; Sutikna, T.; Tocheri, M.W.; Prinsloo, L.C.; Jatmiko; Saptomo, E.W.; Wasisto, S.; Roberts, R.G. Initial Micromorphological Results from Liang Bua, Flores (Indonesia): Site Formation Processes and Hominin Activities at the Type Locality of Homo Floresiensis. *J. Archaeol. Sci.* **2017**, *77*, 125–142.
160. O'Connell, J.F.; Allen, J.; Hawkes, K. Pleistocene Sahul and the Origins of Seafaring. In *The global origins and development of seafaring*; Anderson, A., , Barrett, J H, Boyle, K V, Eds.; McDonald Institute for Archaeological Research, Cambridge University: Cambridge, 2010.
161. Davidson, I.; Noble, W. Why the First Colonisation of the Australian Region Is the Earliest Evidence of Modern Human Behaviour. *Archaeology in Oceania* **1992**, *27*, 135–142.
162. Smith Did Early Hominids Cross Sea Gaps on Natural Rafts. *Faunal and Floral Migrations and Evolution in* **2001**.
163. Bradshaw, C.J.A.; Ulm, S.; Williams, A.N.; Bird, M.I.; Roberts, R.G.; Jacobs, Z.; Laviano, F.; Weyrich, L.S.; Friedrich, T.; Norman, K.; et al. Minimum Founding Populations for the First Peopling of Sahul. *Nat Ecol Evol* **2019**, *3*, 1057–1063.
164. Bird, M.I.; Condie, S.A.; O'Connor, S.; O'Grady, D.; Reepmeyer, C.; Ulm, S.; Zega, M.; Saltré, F.; Bradshaw, C.J.A. Early Human Settlement of Sahul Was Not an Accident. *Scientific Reports* **2019**, *9*.

165. Balme, J.; Davidson, I.; McDonald, J.; Stern, N.; Veth, P. Symbolic Behaviour and the Peopling of the Southern Arc Route to Australia. *Quat. Int.* **2009**, *202*, 59–68.
166. Bulbeck, D. Where River Meets Sea: A Parsimonious Model for Homo Sapiens Colonization of the Indian Ocean Rim and Sahul. *Curr. Anthropol.* **2007**, *48*, 315–321.
167. Anderson, A. Ecological Contingency Accounts for Earliest Seagoing in the Western Pacific Ocean. *The Journal of Island and Coastal Archaeology* **2018**, *13*, 224–234.
168. O’Connell, J.F.; Allen, J. The Process, Biotic Impact, and Global Implications of the Human Colonization of Sahul about 47,000 Years Ago. *J. Archaeol. Sci.* **2015**, *56*, 73–84.
169. Kuijper, E.; Haigh, I.; Marsh, R.; Farr, H. Changing Tidal Dynamics and the Role of the Marine Environment in the Maritime Migration to Sahul. *PaleoAnthropology* **2022**.
170. Birdsell, J.B. The Recalibration of a Paradigm for the First Peopling of Greater Australia. *Sunda and Sahul: Prehistoric Studies in Southeast Asia, Melanesia, and Australia*. **1977**, 113–167.
171. Kealy, S.; Louys, J.; O’Connor, S. Reconstructing Palaeogeography and Inter-island Visibility in the Wallacean Archipelago during the Likely Period of Sahul Colonization, 65–45 000 Years Ago. *Archaeol. Prospect.* **2017**, *24*, 259–272.
172. Kealy, S.; Louys, J.; O’Connor, S. Least-Cost Pathway Models Indicate Northern Human Dispersal from Sunda to Sahul. *J. Hum. Evol.* **2018**, *125*, 59–70.
173. Bird, M.I.; Beaman, R.J.; Condie, S.A.; Cooper, A.; Ulm, S.; Veth, P. Palaeogeography and Voyage Modeling Indicates Early Human Colonization of Australia Was Likely from Timor-Roti. *Quat. Sci. Rev.* **2018**, *191*, 431–439.
174. Norman, K.; Inglis, J.; Clarkson, C.; Faith, J.T.; Shulmeister, J.; Harris, D. An Early Colonisation Pathway into Northwest Australia 70-60,000 Years Ago. *Quat. Sci. Rev.* **2018**, *180*, 229–239.
175. Bradshaw, C.J.A.; Norman, K.; Ulm, S.; Williams, A.N.; Clarkson, C.; Chadœuf, J.; Lin, S.C.; Jacobs, Z.; Roberts, R.G.; Bird, M.I.; et al. Stochastic Models Support Rapid Peopling of Late Pleistocene Sahul. *Nat. Commun.* **2021**, *12*, 2440.
176. Macaulay, V.; Hill, C.; Achilli, A.; Rengo, C.; Clarke, D.; Meehan, W.; Blackburn, J.; Semino, O.; Scozzari, R.; Cruciani, F.; et al. Single, Rapid Coastal Settlement of Asia Revealed by Analysis of Complete Mitochondrial Genomes. *Science* **2005**, *308*, 1034–1036.
177. Hudjashov, G.; Kivisild, T.; Underhill, P.A.; Endicott, P.; Sanchez, J.J.; Lin, A.A.; Shen, P.; Oefner, P.; Renfrew, C.; Villems, R.; et al. Revealing the Prehistoric Settlement of Australia by Y Chromosome and MtDNA Analysis. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 8726–8730.
178. Bergström, A.; Nagle, N.; Chen, Y.; McCarthy, S.; Pollard, M.O.; Ayub, Q.; Wilcox, S.; Wilcox, L.; van Oorschot, R.A.H.; McAllister, P.; et al. Deep Roots for Aboriginal Australian Y Chromosomes. *Curr. Biol.* **2016**, *26*, 809–813.
179. Tobler, R.; Rohrlach, A.; Soubrier, J.; Bover, P.; Llamas, B.; Tuke, J.; Bean, N.; Abdullah-Highfold, A.; Agius, S.; O’Donoghue, A.; et al. Aboriginal Mitogenomes Reveal 50,000 Years of Regionalism in Australia. *Nature* **2017**, *544*, 180–184.
180. Nagle, N.; van Oven, M.; Wilcox, S.; van Holst Pellekaan, S.; Tyler-Smith, C.; Xue, Y.; Ballantyne, K.N.; Wilcox, L.; Papac, L.; Cooke, K.; et al. Aboriginal Australian

- Mitochondrial Genome Variation - an Increased Understanding of Population Antiquity and Diversity. *Sci. Rep.* **2017**, *7*, 43041.
181. Pedro, N.; Brucato, N.; Fernandes, V.; André, M.; Saag, L.; Pomat, W.; Besse, C.; Boland, A.; Deleuze, J.-F.; Clarkson, C.; et al. Papuan Mitochondrial Genomes and the Settlement of Sahul. *J. Hum. Genet.* **2020**, *65*, 875–887.
 182. Huoponen, K.; Schurr, T.G.; Chen, Y.; Wallace, D.C. Mitochondrial DNA Variation in an Aboriginal Australian Population: Evidence for Genetic Isolation and Regional Differentiation. *Hum. Immunol.* **2001**, *62*, 954–969.
 183. Nagle, N.; Ballantyne, K.N.; van Oven, M.; Tyler-Smith, C.; Xue, Y.; Wilcox, S.; Wilcox, L.; Turkalov, R.; van Oorschot, R.A.H.; van Holst Pellekaan, S.; et al. Mitochondrial DNA Diversity of Present-Day Aboriginal Australians and Implications for Human Evolution in Oceania. *J. Hum. Genet.* **2017**, *62*, 343–353.
 184. Nagle, N.; Ballantyne, K.N.; van Oven, M.; Tyler-Smith, C.; Xue, Y.; Taylor, D.; Wilcox, S.; Wilcox, L.; Turkalov, R.; van Oorschot, R.A.H.; et al. Antiquity and Diversity of Aboriginal Australian Y-Chromosomes. *Am. J. Phys. Anthropol.* **2016**, *159*, 367–381.
 185. Malaspinas, A.-S.; Westaway, M.C.; Muller, C.; Sousa, V.C.; Lao, O.; Alves, I.; Bergström, A.; Athanasiadis, G.; Cheng, J.Y.; Crawford, J.E.; et al. A Genomic History of Aboriginal Australia. *Nature* **2016**, *538*, 207–214.
 186. Brucato, N.; André, M.; Tsang, R.; Saag, L.; Kariwiga, J.; Sesuki, K.; Beni, T.; Pomat, W.; Muke, J.; Meyer, V.; et al. Papua New Guinean Genomes Reveal the Complex Settlement of North Sahul. *Mol. Biol. Evol.* **2021**, *38*, 5107–5121.
 187. Purnomo, G.A.; Mitchell, K.J.; O'Connor, S.; Kealy, S.; Taufik, L.; Schiller, S.; Rohrlach, A.; Cooper, A.; Llamas, B.; Sudoyo, H.; et al. Mitogenomes Reveal Two Major Influxes of Papuan Ancestry across Wallacea Following the Last Glacial Maximum and Austronesian Contact. *Genes* **2021**, *12*, doi:10.3390/genes12070965.
 188. Yang, M.A.; Gao, X.; Theunert, C.; Tong, H.; Aximu-Petri, A.; Nickel, B.; Slatkin, M.; Meyer, M.; Pääbo, S.; Kelso, J.; et al. 40,000-Year-Old Individual from Asia Provides Insight into Early Population Structure in Eurasia. *Curr. Biol.* **2017**, *27*, 3202–3208.e9.
 189. Fu, Q.; Meyer, M.; Gao, X.; Stenzel, U.; Burbano, H.A.; Kelso, J.; Pääbo, S. DNA Analysis of an Early Modern Human from Tianyuan Cave, China. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 2223–2227.
 190. Hajdinjak, M.; Mafessoni, F.; Skov, L.; Vernot, B.; Hübner, A.; Fu, Q.; Essel, E.; Nagel, S.; Nickel, B.; Richter, J.; et al. Initial Upper Palaeolithic Humans in Europe Had Recent Neanderthal Ancestry. *Nature* **2021**, *592*, 253–257.
 191. Fu, Q.; Li, H.; Moorjani, P.; Jay, F.; Slepchenko, S.M.; Bondarev, A.A.; Johnson, P.L.F.; Aximu-Petri, A.; Prüfer, K.; de Filippo, C.; et al. Genome Sequence of a 45,000-Year-Old Modern Human from Western Siberia. *Nature* **2014**, *514*, 445–449.
 192. Green, R.E.; Krause, J.; Briggs, A.W.; Maricic, T.; Stenzel, U.; Kircher, M.; Patterson, N.; Li, H.; Zhai, W.; Fritz, M.H.-Y.; et al. A Draft Sequence of the Neandertal Genome. *Science* **2010**, *328*, 710–722.
 193. Prüfer, K.; Racimo, F.; Patterson, N.; Jay, F.; Sankararaman, S.; Sawyer, S.; Heinze, A.; Renaud, G.; Sudmant, P.H.; de Filippo, C.; et al. The Complete Genome Sequence of a Neanderthal from the Altai Mountains. *Nature* **2014**, *505*, 43–49.

194. Sawyer, S.; Renaud, G.; Viola, B.; Hublin, J.-J.; Gansauge, M.-T.; Shunkov, M.V.; Derevianko, A.P.; Prüfer, K.; Kelso, J.; Pääbo, S. Nuclear and Mitochondrial DNA Sequences from Two Denisovan Individuals. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 15696–15700.
195. Slon, V.; Viola, B.; Renaud, G.; Gansauge, M.-T.; Benazzi, S.; Sawyer, S.; Hublin, J.-J.; Shunkov, M.V.; Derevianko, A.P.; Kelso, J.; et al. A Fourth Denisovan Individual. *Sci. Adv.* **2017**, *3*, e1700186.
196. Prüfer, K.; de Filippo, C.; Grote, S.; Mafessoni, F.; Korlević, P.; Hajdinjak, M.; Vernot, B.; Skov, L.; Hsieh, P.; Peyrégne, S.; et al. A High-Coverage Neandertal Genome from Vindija Cave in Croatia. *Science* **2017**, *358*, 655–658.
197. Qin, P.; Stoneking, M. Denisovan Ancestry in East Eurasian and Native American Populations. *Mol. Biol. Evol.* **2015**, *32*, 2665–2674.
198. Reich, D.; Patterson, N.; Kircher, M.; Delfin, F.; Nandineni, M.R.; Pugach, I.; Ko, A.M.-S.; Ko, Y.-C.; Jinam, T.A.; Phipps, M.E.; et al. Denisova Admixture and the First Modern Human Dispersals into Southeast Asia and Oceania. *Am. J. Hum. Genet.* **2011**, *89*, 516–528.
199. Larena, M.; Sanchez-Quinto, F.; Sjödin, P.; McKenna, J.; Ebeo, C.; Reyes, R.; Casel, O.; Huang, J.-Y.; Hagada, K.P.; Guilay, D.; et al. Multiple Migrations to the Philippines during the Last 50,000 Years. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, doi:10.1073/pnas.2026132118.
200. Browning, S.R.; Browning, B.L.; Zhou, Y.; Tucci, S.; Akey, J.M. Analysis of Human Sequence Data Reveals Two Pulses of Archaic Denisovan Admixture. *Cell* **2018**, *173*, 53–61.e9.
201. Jacobs, G.S.; Hudjashov, G.; Saag, L.; Kusuma, P.; Darusallam, C.C.; Lawson, D.J.; Mondal, M.; Pagani, L.; Ricaut, F.-X.; Stoneking, M.; et al. Multiple Deeply Divergent Denisovan Ancestries in Papuans. *Cell* **2019**, *177*, 1010–1021.e32.
202. Choin, J.; Mendoza-Revilla, J.; Arauna, L.R.; Cuadros-Espinoza, S.; Cassar, O.; Larena, M.; Ko, A.M.-S.; Harmant, C.; Laurent, R.; Verdu, P.; et al. Genomic Insights into Population History and Biological Adaptation in Oceania. *Nature* **2021**, doi:10.1038/s41586-021-03236-5.
203. Mondal, M.; Bertranpetit, J.; Lao, O. Approximate Bayesian Computation with Deep Learning Supports a Third Archaic Introgression in Asia and Oceania. *Nat. Commun.* **2019**, *10*, 246.
204. Teixeira, J.C.; Jacobs, G.S.; Stringer, C.; Tuke, J.; Hudjashov, G.; Purnomo, G.A.; Sudoyo, H.; Cox, M.P.; Tobler, R.; Turney, C.S.M.; et al. Widespread Denisovan Ancestry in Island Southeast Asia but No Evidence of Substantial Super-Archaic Hominin Admixture. *Nat Ecol Evol* **2021**, doi:10.1038/s41559-021-01408-0.
205. Tucci, S.; Vohr, S.H.; McCoy, R.C.; Vernot, B.; Robinson, M.R.; Barbieri, C.; Nelson, B.J.; Fu, W.; Purnomo, G.A.; Sudoyo, H.; et al. Evolutionary History and Adaptation of a Human Pygmy Population of Flores Island, Indonesia. *Science* **2018**, *361*, 511–516.
206. Chen, F.; Welker, F.; Shen, C.-C.; Bailey, S.E.; Bergmann, I.; Davis, S.; Xia, H.; Wang, H.; Fischer, R.; Freidline, S.E.; et al. A Late Middle Pleistocene Denisovan Mandible from the Tibetan Plateau. *Nature* **2019**, *569*, 409–412.

207. Demeter, F.; Zanolli, C.; Westaway, K.E.; Joannes-Boyau, R.; Durringer, P.; Morley, M.W.; Welker, F.; R  ther, P.L.; Skinner, M.M.; McColl, H.; et al. A Middle Pleistocene Denisovan Molar from the Annamite Chain of Northern Laos. *Nat. Commun.* **2022**, *13*, 2557.
208. Zhang, D.; Xia, H.; Chen, F.; Li, B.; Slon, V.; Cheng, T.; Yang, R.; Jacobs, Z.; Dai, Q.; Massilani, D.; et al. Denisovan DNA in Late Pleistocene Sediments from Baishiya Karst Cave on the Tibetan Plateau. *Science* **2020**, *370*, 584–587.
209. Bulbeck, D.; Marwick, B. Stone Industries of Mainland and Island Southeast Asia. **2022**, doi:10.1093/oxfordhb/9780199355358.013.15.
210. Ji, X.; Kuman, K.; Clarke, R.J.; Forestier, H.; Li, Y.; Ma, J.; Qiu, K.; Li, H.; Wu, Y. The Oldest Hoabinhian Technocomplex in Asia (43.5 Ka) at Xiaodong Rockshelter, Yunnan Province, Southwest China. *Quat. Int.* **2016**, *400*, 166–174.
211. Shoocongdej, R. Late Pleistocene Activities at the Tham Lod Rockshelter in Highland Pang Mapha, Mae Hong Son Province, Northwestern Thailand. In Proceedings of the Uncovering Southeast Asia’s Past: Selected Papers from the 10th International Conference of the European Association of Southeast Asian Archaeologists; NUS press Singapore, 2006; pp. 22–37.
212. Marwick, B. Multiple Optima in Hoabinhian Flaked Stone Artefact Palaeoeconomics and Palaeoecology at Two Archaeological Sites in Northwest Thailand. *Journal of Anthropological Archaeology* **2013**, *32*, 553–564.
213. Shoocongdej, R.; Wattanapitaksakul, A. Faunal Assemblages and Demography during the Late Pleistocene (MIS 2-1) to Early Holocene in Highland Pang Mapha, Northwest Thailand. *Quat. Int.* **2020**, *563*, 51–63.
214. Shoocongdej, R. *Highland Archaeology in Pang Mapha*; Silpakorn University Press, 2004;
215. Chitkament, T.; Gaillard, C.; Shoocongdej, R. Tham Lod Rockshelter (Pang Mapha District, North-Western Thailand): Evolution of the Lithic Assemblages during the Late Pleistocene. *Quat. Int.* **2016**, *416*, 151–161.
216. Shoocongdej, R. Forager Mobility Organization in Seasonal Tropical Environments of Western Thailand. *World Archaeol.* **2000**, *32*, 14–40.
217. Marwick, B. Approaches to Flaked Stone Artefact Archaeology in Thailand: A Historical Review. *Silpakorn University International Journal* **2007**, *7*.
218. Patole-Edoumba, E.; Durringer, P.; Richardin, P.; Shackelford, L.; Bacon, A.-M.; Sayavongkhamdy, T.; Ponche, J.-L.; Demeter, F. Evolution of the Hoabinhian Techno-Complex of Tam Hang Rock Shelter in Northeastern Laos. *Archaeol. discov.* **2015**, *03*, 140–157.
219. Forestier, H.; Sophady, H.; Puaud, S.; Celiberti, V. The Hoabinhian from Laang Spean Cave in Its Stratigraphic, Chronological, Typo-Technological and Environmental Context (Cambodia, Battambang Province). *Journal of* **2015**.
220. Forestier, H.; Zeitoun, V.; Winayalai, C.; M  tais, C. The Open-Air Site of Huai Hin (Northwestern Thailand): Chronological Perspectives for the Hoabinhian. *C. R. Palevol* **2013**, *12*, 45–55.

221. Marwick, B. Human Behavioural Ecology and Stone Artefacts in Northwest Thailand during the Terminal Pleistocene and Holocene. *Southeast Asian Prehistory: The 11th EurASEAA Conference* **2008**, 73–80.
222. Suraprasit, K.; Shoocongdej, R.; Chintakanon, K.; Bocherens, H. Late Pleistocene Human Paleoecology in the Highland Savanna Ecosystem of Mainland Southeast Asia. *Sci. Rep.* **2021**, *11*, 16756.
223. Gorman, C. The Hoabinhian and after: Subsistence Patterns in Southeast Asia during the Late Pleistocene and Early Recent Periods. *World Archaeol.* **1971**, *2*, 300–320.
224. Shoocongdej, R. The Hoabinhian: The Late and Post-Pleistocene Cultural Systems of Southeast Asia. **2022**, doi:10.1093/oxfordhb/9780199355358.013.16.
225. Treerayapiwat, C. Patterns of Habitation and Burial Activity in the Ban Rai Rock Shelter, Northwestern Thailand. *Asian Perspectives* **2005**, *44*, 231–245.
226. Zeitoun, V.; Auetrakulvit, P.; Forestier, H.; Zazzo, A.; Davtian, G.; Nakbunlung, S.; Tiamtinkrit, C. Discovery of a Mesolithic Burial near the Painted Rock-Shelter of Ban Tha Si (Lampang Province, Northern Thailand): Implications for Regional Mortuary Practices. *Comptes rendus - Palevol* **2013**, *2*, 127–136.
227. Matsumura, H.; Yoneda, M.; Dodo, Y.; Oxenham, M.F.; Cuong, N.L.; Thuy, N.K.; Dung, L.M.Y.; Long, V.U.T.; Yamagata, M.; Sawada, J.; et al. Terminal Pleistocene Human Skeleton from Hang Cho Cave, Northern Vietnam: Implications for the Biological Affinities of Hoabinhian People. *Anthropol. Sci.* **2008**, *advpub*, 0805190039–0805190039.
228. Simanjuntak, T.; Asikin, I.N. Early Holocene Human Settlement in Eastern Java. *Bulletin of the Indo-Pacific Prehistory Association* **2004**, *24*, 13–19.
229. Lewis, H.; Paz, V.; Lara, M.; Barton, H.; Piper, P.; Ochoa, J.; Vitales, T.; Carlos, A.J.; Higham, T.; Neri, L.; et al. Terminal Pleistocene to Mid-Holocene Occupation and an Early Cremation Burial at Ille Cave, Palawan, Philippines. *Antiquity* **2008**, *82*, 318–335.
230. Bulbeck, D. The Last Glacial Maximum Human Burial from Liang Lemdubu in Northern Sahulland. In *The Archaeology of the Aru Islands, Eastern Indonesia*; O'Connor, S., Spriggs, M., Veth, P., Eds.; ANU Press: Canberra, Australia, 2005; Vol. 22, pp. 255–294.
231. Lloyd-Smith, L. EARLY HOLOCENE BURIAL PRACTICE AT NIAH CAVE, SARAWAK. *BIPPA* **2014**, *32*, doi:10.7152/jipa.v32i0.12844.
232. Perston, Y.L.; Moore, M.; Suryatman; Langley, M.; Hakim, B.; Oktaviana, A.A.; Brumm, A. A Standardised Classification Scheme for the Mid-Holocene Toalean Artefacts of South Sulawesi, Indonesia. *PLoS One* **2021**, *16*, e0251138.
233. Bulbeck, D.; Pasqua, M.; Di Lello, A. Culture History of the Toalean of South Sulawesi, Indonesia. *Asian Perspectives* **2000**, *39*, 71–108.
234. Bulbeck, D. Divided in Space, United in Time: The Holocene Prehistory of South Sulawesi. *Quaternary research in Indonesia* **2004**, 129–166.
235. Larson, G.; Cucchi, T.; Fujita, M.; Matisoo-Smith, E.; Robins, J.; Anderson, A.; Rolett, B.; Spriggs, M.; Dolman, G.; Kim, T.-H.; et al. Phylogeny and Ancient DNA of *Sus* Provides Insights into Neolithic Expansion in Island Southeast Asia and Oceania. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 4834–4839.

236. Fillios, M.A.; Taçon, P.S.C. Who Let the Dogs in? A Review of the Recent Genetic Evidence for the Introduction of the Dingo to Australia and Implications for the Movement of People. *Journal of Archaeological Science: Reports* **2016**, *7*, 782–792.
237. Hooijer, D.A. *Man and Other Mammals from Toalian Sites in Southwestern Celebes*; North Holland Pub. Co., 1950;
238. Matsumura, H.; Zuraina, M. Metric Analyses of an Early Holocene Human Skeleton From. *Am. J. Phys. Anthropol.* **1999**, *109*, 327–340.
239. Matsumura, H.; Hung, H.-C.; Cuong, N.L.; Zhao, Y.-F.; He, G.; Chi, Z. Mid-Holocene Hunter-Gatherers ‘Gaomiao’ in Hunan, China: The First of the Two-Layer Model in the Population History of East/Southeast Asia. *terra australis* **45** **2017**, 61.
240. Matsumura, H.; Hudson, M.J. Dental Perspectives on the Population History of Southeast Asia. *Am. J. Phys. Anthropol.* **2005**, *127*, 182–209.
241. Jinam, T.A.; Phipps, M.E.; Aghakhanian, F.; Majumder, P.P.; Datar, F.; Stoneking, M.; Sawai, H.; Nishida, N.; Tokunaga, K.; Kawamura, S.; et al. Discerning the Origins of the Negritos, First Sundaland People: Deep Divergence and Archaic Admixture. *Genome Biol. Evol.* **2017**, *9*, 2013–2022.
242. Hung, H.-C.; Matsumura, H.; Nguyen, L.C.; Hanihara, T.; Huang, S.-C.; Carson, M.T. Negritos in Taiwan and the Wider Prehistory of Southeast Asia: New Discovery from the Xiaoma Caves. *World Archaeol.* **2022**, 1–22.
243. McColl, H.; Racimo, F.; Vinner, L.; Demeter, F.; Gakuhari, T.; Moreno-Mayar, J.V.; van Driem, G.; Gram Wilken, U.; Seguin-Orlando, A.; de la Fuente Castro, C.; et al. The Prehistoric Peopling of Southeast Asia. *Science* **2018**, *361*, 88–92.
244. Saitou, N.; Jinam, T.A.; Kanzawa-Kiriyama, H.; Tokunaga, K. Initial Movements of Modern Humans in East Eurasia. *terra australis* **45** **2017**, 43.
245. Matsumura, H.; Hung, H.-C.; Higham, C.; Zhang, C.; Yamagata, M.; Nguyen, L.C.; Li, Z.; Fan, X.-C.; Simanjuntak, T.; Oktaviana, A.A.; et al. Craniometrics Reveal ‘Two Layers’ of Prehistoric Human Dispersal in Eastern Eurasia. *Sci. Rep.* **2019**, *9*, 1451.
246. Kanzawa-Kiriyama, H.; Kryukov, K.; Jinam, T.A.; Hosomichi, K.; Saso, A.; Suwa, G.; Ueda, S.; Yoneda, M.; Tajima, A.; Shinoda, K.-I.; et al. A Partial Nuclear Genome of the Jomons Who Lived 3000 Years Ago in Fukushima, Japan. *J. Hum. Genet.* **2017**, *62*, 213–221.
247. Gakuhari, T.; Nakagome, S.; Rasmussen, S.; Allentoft, M.E.; Sato, T.; Korneliussen, T.; Chuiñeagáin, B.N.; Matsumae, H.; Koganebuchi, K.; Schmidt, R.; et al. Ancient Jomon Genome Sequence Analysis Sheds Light on Migration Patterns of Early East Asian Populations. *Commun Biol* **2020**, *3*, 437.
248. Kanzawa-Kiriyama, H.; Jinam, T.A.; Kawai, Y.; Sato, T.; Hosomichi, K.; Tajima, A.; Adachi, N.; Matsumura, H.; Kryukov, K.; Saitou, N.; et al. Late Jomon Male and Female Genome Sequences from the Funadomari Site in Hokkaido, Japan. *Anthropol. Sci.* **2019**, *advpub*, doi:10.1537/ase.190415.
249. Wang, T.; Wang, W.; Xie, G.; Li, Z.; Fan, X.; Yang, Q.; Wu, X.; Cao, P.; Liu, Y.; Yang, R.; et al. Human Population History at the Crossroads of East and Southeast Asia since 11,000 Years Ago. *Cell* **2021**, doi:10.1016/j.cell.2021.05.018.

250. Yang, M.A.; Fan, X.; Sun, B.; Chen, C.; Lang, J.; Ko, Y.-C.; Tsang, C.-H.; Chiu, H.; Wang, T.; Bao, Q.; et al. Ancient DNA Indicates Human Population Shifts and Admixture in Northern and Southern China. *Science* **2020**, *369*, 282–288.
251. Liu, L.; Lee, G.-A.; Jiang, L.; Zhang, J. Evidence for the Early Beginning (c. 9000 Cal. BP) of Rice Domestication in China: A Response. *Holocene* **2007**, *17*, 1059–1068.
252. Yang, X.; Wan, Z.; Perry, L.; Lu, H.; Wang, Q.; Zhao, C.; Li, J.; Xie, F.; Yu, J.; Cui, T.; et al. Early Millet Use in Northern China. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 3726–3730.
253. Gao, Y.; Dong, G.; Yang, X.; Chen, F. A Review on the Spread of Prehistoric Agriculture from Southern China to Mainland Southeast Asia. *Sci. China Earth Sci.* **2020**, *63*, doi:10.1007/s11430-019-9552-5.
254. Higham, C.F. First Farmers in Mainland Southeast Asia. *JIPA* **2017**, *41*, 13–21.
255. Matsumura, H.; Oxenham, M.F. Demographic Transitions and Migration in Prehistoric East/Southeast Asia through the Lens of Nonmetric Dental Traits. *Am. J. Phys. Anthropol.* **2014**, *155*, 45–65.
256. Higham, C.; Thosarat, R. *Prehistoric Thailand: From Early Settlement to Sukhothai*; Da Capo Press, Incorporated, 1998;
257. Higham, C.F.W. The Neolithic of Mainland Southeast Asia. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, 2022.
258. Sawada, J.; Thuy, N.K.; Tuan, N.A. Faunal Remains at Man Bac. In *Man Bac: The Excavation of a Neolithic Site in Northern Vietnam*; Oxenham, M., Matsumura, H., Nguyen, D.K., Eds.; ANU Press: Canberra, Australia, 2010; pp. 105–116.
259. Hanihara, T.; Matsumura, H.; Kawakubo, Y.; Coung, N.L.; Thuy, N.K.; Oxenham, M.F.; Dodo, Y. Population History of Northern Vietnamese Inferred from Nonmetric Cranial Trait Variation. *Anthropol. Sci.* **2012**, *120*, 157–165.
260. Weber, S.; Lehman, H.; Barela, T.; Hawks, S.; Harriman, D. Rice or Millets: Early Farming Strategies in Prehistoric Central Thailand. *Archaeol. Anthropol. Sci.* **2010**, *2*, 79–88.
261. King, C.L.; Alexander Bentley, R.; Higham, C.; Tayles, N.; Viðarsdóttir, U.S.; Layton, R.; Macpherson, C.G.; Nowell, G. Economic Change after the Agricultural Revolution in Southeast Asia? *Antiquity* **2014**, *88*, 112–125.
262. Ning, C.; Li, T.; Wang, K.; Zhang, F.; Li, T.; Wu, X.; Gao, S.; Zhang, Q.; Zhang, H.; Hudson, M.J.; et al. Ancient Genomes from Northern China Suggest Links between Subsistence Changes and Human Migration. *Nat. Commun.* **2020**, *11*, 2700.
263. Wang, C.-C.; Yeh, H.-Y.; Popov, A.N.; Zhang, H.-Q.; Matsumura, H.; Sirak, K.; Cheronet, O.; Kovalev, A.; Rohland, N.; Kim, A.M.; et al. Genomic Insights into the Formation of Human Populations in East Asia. *Nature* **2021**, *591*, 413–419.
264. Sagart, L.; Jacques, G.; Lai, Y.; Ryder, R.J.; Thouzeau, V.; Greenhill, S.J.; List, J.-M. Dated Language Phylogenies Shed Light on the Ancestry of Sino-Tibetan. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 10317–10322.
265. Liu, C.-C.; Witonsky, D.; Gosling, A.; Lee, J.H.; Ringbauer, H.; Hagan, R.; Patel, N.; Stahl, R.; Novembre, J.; Aldenderfer, M.; et al. Ancient Genomes from the Himalayas

- Illuminate the Genetic History of Tibetans and Their Tibeto-Burman Speaking Neighbors. *Nat. Commun.* **2022**, *13*, 1203.
266. Blust, R. Beyond the Austronesian Homeland: The Austric Hypothesis and Its Implications for Archaeology. *Transactions of the American Philosophical Society* **1996**, *86*, 117–158.
267. Diamond, J.; Bellwood, P. Farmers and Their Languages: The First Expansions. *Science* **2003**, *300*, 597–603.
268. Lipson, M.; Cheronet, O.; Mallick, S.; Rohland, N.; Oxenham, M.; Pietrusewsky, M.; Pryce, T.O.; Willis, A.; Matsumura, H.; Buckley, H.; et al. Ancient Genomes Document Multiple Waves of Migration in Southeast Asian Prehistory. *Science* **2018**, *361*, 92–95.
269. Kuo, S.-C. *New Frontiers in the Neolithic Archaeology of Taiwan (5600–1800 BP)*; Springer Nature Singapore, 2019;
270. Tsang, C.-H.; Li, K.-T.; Hsu, T.-F.; Tsai, Y.-C.; Fang, P.-H.; Hsing, Y.-I.C. Broomcorn and Foxtail Millet Were Cultivated in Taiwan about 5000 Years Ago. *Bot. Stud.* **2017**, *58*, 3.
271. Piper, P.J.; Campos, F.Z.; Hung, H.-C. A Study of the Animal Bones Recovered from Pits 9 and 10 at the Site of Nagsabaran in Northern Luzon, Philippines. *Hukay* **2009**, *14*, 47–90.
272. Hung, H.-C.; Tsang, C.-H.; Deng, Z.; Bolunia, M.J.L.; Santiago, R.A.; Carson, M.T.; Bellwood, P. Preceramic Riverside Hunter-Gatherers and the Arrival of Neolithic Farmers in Northern Luzon. *Antiquity* **2022**, *96*, 848–867.
273. Anggraeni; Simanjuntak, T.; Bellwood, P.; Piper, P. Neolithic Foundations in the Karama Valley, West Sulawesi, Indonesia. *Antiquity* **2014**, *88*, 740–756.
274. Hung, H.-C.; Carson, M.T.; Bellwood, P.; Campos, F.Z.; Piper, P.J.; Dizon, E.; Bolunia, M.J.L.; Oxenham, M.; Chi, Z. The First Settlement of Remote Oceania: The Philippines to the Marianas. *Antiquity* **2011**, *85*, 909–926.
275. Bellwood, P. Archaeological Investigations at Bukit Tengkorak and Segarong, Southeastern Sabah. *Bull. Indo-Pac. Prehistory Assoc.* **1989**, *9*, doi:10.7152/bippa.v9i0.11286.
276. Galipaud, J.-C.; Kinaston, R.; Halcrow, S.; Foster, A.; Harris, N.; Simanjuntak, T.; Javelle, J.; Buckley, H. The Pain Haka Burial Ground on Flores: Indonesian Evidence for a Shared Neolithic Belief System in Southeast Asia. *Antiquity* **2016**, *90*, 1505–1521.
277. Carson, M.T.; Hung, H.-C.; Summerhayes, G.; Bellwood, P. The Pottery Trail From Southeast Asia to Remote Oceania. *The Journal of Island and Coastal Archaeology* **2013**, *8*, 17–36.
278. Summerhayes, G.R.; Matisoo-Smith, E.; Mandui, H.; Allen, J.; McPherson, S. Tamuarawai (EQS): An Early Lapita Site on Emirau, New Ireland, PNG. *Journal of Pacific Archaeology* **2010**, *1*, 62–75.
279. Valentin, F.; Bedford, S.; Buckley, H.R.; Spriggs, M. Lapita Burial Practices: Evidence for Complex Body and Bone Treatment at the Teouma Cemetery, Vanuatu, Southwest Pacific. *The Journal of Island and Coastal Archaeology* **2010**, *5*, 212–235.
280. Gray, R.D.; Drummond, A.J.; Greenhill, S.J. Language Phylogenies Reveal Expansion Pulses and Pauses in Pacific Settlement. *Science* **2009**, *323*, 479–483.

281. Blust, R. The Austronesian Homeland and Dispersal. *Annu. Rev. Linguist.* **2019**, *5*, 417–434.
282. Donohue, M.; Denham, T. Farming and Language in Island Southeast Asia: Reframing Austronesian History. *Curr. Anthropol.* **2010**, *51*, 223–256.
283. Tabbada, K.A.; Trejaut, J.; Loo, J.-H.; Chen, Y.-M.; Lin, M.; Mirazón-Lahr, M.; Kivisild, T.; De Ungria, M.C.A. Philippine Mitochondrial DNA Diversity: A Populated Viaduct between Taiwan and Indonesia? *Mol. Biol. Evol.* **2010**, *27*, 21–31.
284. Melton, T.; Peterson, R.; Redd, A.J.; Saha, N.; Sofro, A.S.; Martinson, J.; Stoneking, M. Polynesian Genetic Affinities with Southeast Asian Populations as Identified by MtDNA Analysis. *Am. J. Hum. Genet.* **1995**, *57*, 403–414.
285. Tätte, K.; Metspalu, E.; Post, H.; Palencia-Madrid, L.; Luis, J.R.; Reidla, M.; Rea, A.; Tamm, E.; Moding, E.J.; de Pancorbo, M.M.; et al. The Ami and Yami Aborigines of Taiwan and Their Genetic Relationship to East Asian and Pacific Populations. *Eur. J. Hum. Genet.* **2021**, *29*, 1092–1102.
286. Trejaut, J.A.; Kivisild, T.; Loo, J.H.; Lee, C.L.; He, C.L.; Hsu, C.J.; Lee, Z.Y.; Lin, M. Traces of Archaic Mitochondrial Lineages Persist in Austronesian-Speaking Formosan Populations. *PLoS Biol.* **2005**, *3*, e247.
287. Melton, T.; Clifford, S.; Martinson, J.; Batzer, M.; Stoneking, M. Genetic Evidence for the Proto-Austronesian Homeland in Asia: MtDNA and Nuclear DNA Variation in Taiwanese Aboriginal Tribes. *Am. J. Hum. Genet.* **1998**, *63*, 1807–1823.
288. Mirabal, S.; Cadenas, A.M.; Garcia-Bertrand, R.; Herrera, R.J. Ascertaining the Role of Taiwan as a Source for the Austronesian Expansion. *Am. J. Phys. Anthropol.* **2013**, *150*, 551–564.
289. Richards, M.; Oppenheimer, S.; Sykes, B. MtDNA Suggests Polynesian Origins in Eastern Indonesia. *Am. J. Hum. Genet.* **1998**, *63*, 1234–1236.
290. Su, B.; Jin, L.; Underhill, P.; Martinson, J.; Saha, N.; McGarvey, S.T.; Shriver, M.D.; Chu, J.; Oefner, P.; Chakraborty, R.; et al. Polynesian Origins: Insights from the Y Chromosome. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 8225–8228.
291. Lipson, M.; Loh, P.-R.; Patterson, N.; Moorjani, P.; Ko, Y.-C.; Stoneking, M.; Berger, B.; Reich, D. Reconstructing Austronesian Population History in Island Southeast Asia. *Nat. Commun.* **2014**, *5*, 4689.
292. Skoglund, P.; Posth, C.; Sirak, K.; Spriggs, M.; Valentin, F.; Bedford, S.; Clark, G.R.; Reepmeyer, C.; Petchey, F.; Fernandes, D.; et al. Genomic Insights into the Peopling of the Southwest Pacific. *Nature* **2016**, *538*, 510–513.
293. Posth, C.; Nägele, K.; Colleran, H.; Valentin, F.; Bedford, S.; Kami, K.W.; Shing, R.; Buckley, H.; Kinaston, R.; Walworth, M.; et al. Language Continuity despite Population Replacement in Remote Oceania. *Nat Ecol Evol* **2018**, *2*, 731–740.
294. Ko, A.M.-S.; Chen, C.-Y.; Fu, Q.; Delfin, F.; Li, M.; Chiu, H.-L.; Stoneking, M.; Ko, Y.-C. Early Austronesians: Into and out of Taiwan. *Am. J. Hum. Genet.* **2014**, *94*, 426–436.
295. Mörseburg, A.; Pagani, L.; Ricaut, F.-X.; Yngvadottir, B.; Harney, E.; Castillo, C.; Hoogervorst, T.; Antao, T.; Kusuma, P.; Brucato, N.; et al. Multi-Layered Population Structure in Island Southeast Asians. *Eur. J. Hum. Genet.* **2016**, *24*, 1605–1611.

296. Hudjashov, G.; Karafet, T.M.; Lawson, D.J.; Downey, S.; Savina, O.; Sudoyo, H.; Lansing, J.S.; Hammer, M.F.; Cox, M.P. Complex Patterns of Admixture across the Indonesian Archipelago. *Mol. Biol. Evol.* **2017**, *34*, 2439–2452.
297. Simanjuntak, T. The Western Route Migration: A Second Probable Neolithic Diffusion to Indonesia. *Terra Australis* **2017**, *45*, 201–212.
298. Higham, C.F.W.; Cawte, H. Bronze Metallurgy in Southeast Asia with Particular Reference to Northeast Thailand. *Journal of World Prehistory* **2021**.
299. White, J.C.; Hamilton, E.G. The Transmission of Early Bronze Technology to Thailand: New Perspectives. In *Archaeometallurgy in Global Perspective: Methods and Syntheses*; Roberts, B.W., Thornton, C.P., Eds.; Springer New York: New York, NY, 2014; pp. 805–852 ISBN 9781461490173.
300. Higham, C.; Higham, T.; Ciarla, R.; Douka, K.; Kijngam, A.; Rispoli, F. The Origins of the Bronze Age of Southeast Asia. *J. World Prehist.* **2011**, *24*, 227–274.
301. Higham, C.F.W.; Douka, K.; Higham, T.F.G. A New Chronology for the Bronze Age of Northeastern Thailand and Its Implications for Southeast Asian Prehistory. *PLoS One* **2015**, *10*, e0137542.
302. Ciarla, R. The Origins of the Bronze Age in Mainland Southeast Asia. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, 2022.
303. Tucci, A.; Sayavongkhamdy, T.; Chang, N.; Souksavatdy, V. Ancient Copper Mining in Laos: Heterarchies, Incipient States or Post-State Anarchists. *Journal of Anthropology and Archaeology* **2014**, *2*, 1–15.
304. Pigott, V.C.; Pryce, T.O. Prehistoric Copper Production and Exchange in Southeast Asia. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.
305. Higham, T.F.G.; Weiss, A.D.; Higham, C.F.W.; Ramsey, C.B.; Hanson, S.; Weber, S.A.; Rispoli, F.; Ciarla, R.; Pryce, T.O.; Pigott, V.C. A Prehistoric Copper-Production Centre in Central Thailand: Its Dating and Wider Implications. *Antiquity* **2020**, *94*, 948–965.
306. White, J.C. Incorporating Heterarchy into Theory on Socio-Political Development: The Case from Southeast Asia. *Archeol. pap. Am. Anthropol. Assoc.* **1995**, *6*, 101–123.
307. Fochesato, M.; Higham, C.; Bogaard, A.; Castillo, C.C. Changing Social Inequality from First Farmers to Early States in Southeast Asia. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, doi:10.1073/pnas.2113598118.
308. Higham, C.F.W. From Site Formation to Social Structure in Prehistoric Thailand. *J. Field Archaeol.* **2015**, *40*, 383–396.
309. Masanari, N. Settlement Patterns on the Red River Plain from the Late Prehistoric Period to the 10th Century AD. *Bull. Indo-Pac. Prehistory Assoc.* **2007**, *25*, doi:10.7152/bippa.v25i0.11920.
310. Kim, N.C. The Dong Son Culture of Vietnam. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, UK, 2022.
311. Oliveira, N.V.; Bellwood, P. Dong Son Drums from Timor-Leste: Prehistoric Bronze Artefacts in Island Southeast Asia. *Antiquity* **2019**, *93*, 163–180.

312. Reinecke, A. The Sa Huynh Culture and Related Cultures in Southern Vietnam and Cambodia. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, UK, 2022.
313. Yamagata, M.; Matsumura, H. Austronesian Migration to Central Vietnam: Crossing over the Iron Age Southeast Asian Sea. *terra australis* **45** **2017**, 333.
314. Higham, C.F.W. Social Change in Southeast Asia during the Iron Age. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, UK, 2022.
315. Shoocongdej, R. *Log Coffin Culture of Thailand in the Southeast Asian Context*; Charansanitwong Printing Company Limited: Bangkok, 2018;
316. Pyae, S.K. New Archaeological Discoveries of Coffin Caves in Kayah State, Myanmar. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
317. Jamir, T. Practice of Wood Coffin Burial Among Naga Communities of Highland Northeast India. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
318. Roberts, N. Wooden Coffins and the ‘Coffin Culture’ in Lao PDR: Current Research and Future Directions. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
319. Beavan, N.; Halcrow, S.; McFadgen, B.; Hamilton, D.; Buckley, B.; Sokha, T.; Shewan, L.; Sokha, O.; Fallon, S.; Miksic, J.; et al. Radiocarbon Dates from Jar and Coffin Burials of the Cardamom Mountains Reveal a Unique Mortuary Ritual in Cambodia’s Late-to Post-Angkor Period (15th-17th Centuries AD). *Radiocarbon* **2012**, *54*, 1–22.
320. Chia, S. *Wood Coffin Burial of Kinabatangan, Sabah (Penerbit USM)*; Penerbit USM, 2014; ISBN 9789838617260.
321. Chua, J. Log Coffin Burials in the Archaeological Record of the Philippines. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
322. Murillo-Barroso, M.; Pryce, T.O.; Bellina, B.; Martínón-Torres, M. Khao Sam Kaeo – an Archaeometallurgical Crossroads for Trans-Asiatic Technological Traditions. *J. Archaeol. Sci.* **2010**, *37*, 1761–1772.
323. Glover, I.C.; Bellina, B. Ban Don Tha Phet and Khao Sam Kaeo: The Earliest Indian Contacts Re-Assessed. In *Early Interactions Between South and Southeast Asia: Reflections on Cross-cultural Exchange*; Manguin, P.-Y., Mani, A., Wade, G., Eds.; Institute of Southeast Asian Studies, 2011 ISBN 9789814345101.
324. Biggs, L.; Bellina, B.; Martínón-Torres, M.; Pryce, T.O. Prehistoric Iron Production Technologies in the Upper Thai-Malay Peninsula: Metallography and Slag Inclusion Analyses of Iron Artefacts from Khao Sam Kaeo and Phu Khao Thong. *Archaeol. Anthropol. Sci.* **2013**, *5*, 311–329.
325. Ono, R.; Aziz, F.; Oktaviana, A.A.; Prastiningtyas, D.; Ririmasse, M.; Iriyanto, N.; Zesse, I.; Hisa, Y.; Yoneda, M. Development of Regional Maritime Networks during the Early Metal Age in Northern Maluku Islands: A View from Excavated Glass Ornaments and Pottery Variation. *The Journal of Island and Coastal Archaeology* **2018**, *13*, 90–108.
326. Bellina, B. Southeast Asian Evidence for Early Maritime Silk Road Exchange and Trade-Related Polities. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.

327. Bellwood, P. The Northern Spice Islands in Prehistory, from 40,000 Years Ago to the Recent Past. In *The Spice Islands in Prehistory: Archaeology in the Northern Moluccas, Indonesia*; Bellwood, P., Ed.; ANU Press: Acton, Australia, 2019.
328. Hawkins, S.; Arumdhati, F.S.; Litster, M.; Lim, T.S.; Basile, G.; Leclerc, M.; Reepmeyer, C.; Maloney, T.R.; Boulanger, C.; Louys, J.; et al. Metal-Age Maritime Culture at Jareng Bori Rockshelter, Pantar Island, Eastern Indonesia. *Rec. Aust. Mus.* **2020**, *72*, 26.
329. Dizon, E. The Prehistory of the Philippines. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.
330. Manguin, P.-Y.; Stark, M.T. Mainland Southeast Asia's Earliest Kingdoms and the Case of 'Funan'. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.
331. Clarke, W.; Gallon, M. Early States in Thailand: Dvāravatī. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.
332. Manguin, P.-Y. Early States of Insular Southeast Asia. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.
333. Miksic, J.N. The Civilizations of Central and East Java and Bali. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, England, 2022.
334. Berrocal, M.C.; Tsang, C.-H. Introduction - Understanding Early Modern Colonialism in Asia and the Pacific. In *Historical Archaeology of Early Modern Colonialism in Asia-Pacific: The Asia-Pacific Region*; Berrocal, M.C., Tsang, C.-H., Eds.; University Press of Florida: Gainesville, FL, 2017.
335. Luque-Talaván, M. Impact of First Conquest on Indigenous Populations of the Philippines. In *Historical Archaeology of Early Modern Colonialism in Asia-Pacific: The Asia-Pacific Region*; Berrocal, M.C., Tsang, C.-H., Eds.; University Press of Florida: Gainesville, FL, 2017.
336. Skowronek, R.K. European Ceramics in the Spanish Philippines. In *Historical Archaeology of Early Modern Colonialism in Asia-Pacific: The Asia-Pacific Region*; Berrocal, M.C., Tsang, C.-H., Eds.; University Press of Florida: Gainesville, FL, 2017.
337. Nägele, K. An Ancient Genomic Perspective on the Human Dispersals to Tropical Islands – Implications for the Settlement History of the Ancient Caribbean and the Pacific. PhD, Eberhard Karls Universität: Tübingen, Germany, 2020.
338. Pestle, W.J.; Colvard, M. Bone Collagen Preservation in the Tropics: A Case Study from Ancient Puerto Rico. *J. Archaeol. Sci.* **2012**, *39*, 2079–2090.
339. Rohland, N.; Mallick, S.; Mah, M.; Maier, R.; Patterson, N.; Reich, D. Three Assays for In-Solution Enrichment of Ancient Human DNA at More than a Million SNPs. *Genome Res.* **2022**, doi:10.1101/gr.276728.122.
340. Smith, C.I.; Chamberlain, A.T.; Riley, M.S.; Stringer, C.; Collins, M.J. The Thermal History of Human Fossils and the Likelihood of Successful DNA Amplification. *J. Hum. Evol.* **2003**, *45*, 203–217.

341. Mourshed, M. Climatic Parameters for Building Energy Applications: A Temporal-Geospatial Assessment of Temperature Indicators. *Renewable Energy* **2016**, *94*, 55–71.
342. Chuan, G.K. The Climate of Southeast Asia. In *The Physical Geography of Southeast Asia*; Gupta, A., Ed.; 2005.
343. Samper-Carro, S.C.; O'Connor, S.; Mahirta; Kealy, S.; Shipton, C. Talking Dead. New Burials from Tron Bon Lei (Alor Island, Indonesia) Inform on the Evolution of Mortuary Practices from the Terminal Pleistocene to the Holocene in Southeast Asia. *PLoS One* **2022**, *17*, e0267635.
344. Samper Carro, S.C.; Stewart, T.J.; Mahirta; Wood, R.; O'Connor, S. Burial Practices in the Early Mid-Holocene of the Wallacean Islands: A Sub-Adult Burial from Gua Makpan, Alor Island, Indonesia. *Quat. Int.* **2021**, *603*, 125–138.
345. Piper, P.J. Human Cultural, Technological and Adaptive Changes from the End of the Pleistocene to the Mid-Holocene in Southeast Asia. *The Routledge Handbook of Bioarchaeology in* **2015**, doi:10.4324/9781315725444-13/human-cultural-technological-adaptive-changes-end-pleistocene-mid-holocene-southeast-asia-philip-piper.
346. Reepmeyer, C.; O'Connor, S.; Mahirta; Kealy, S.; Maloney, T. Kisar, a Small Island Participant in an Extensive Maritime Obsidian Network in the Wallacean Archipelago. *Archaeological Research in Asia* **2019**, *19*, 100139.
347. Larena, M.; McKenna, J.; Sanchez-Quinto, F.; Bernhardsson, C.; Ebeo, C.; Reyes, R.; Casel, O.; Huang, J.-Y.; Hagada, K.P.; Guilay, D.; et al. Philippine Ayta Possess the Highest Level of Denisovan Ancestry in the World. *Curr. Biol.* **2021**, *31*, 4219–4230.e10.
348. Maloney, T.R.; Dilkes-Hall, I.E.; Vlok, M.; Oktaviana, A.A.; Setiawan, P.; Priyatno, A.A.D.; Ririmasse, M.; Geria, I.M.; Effendy, M.A.R.; Istiawan, B.; et al. Surgical Amputation of a Limb 31,000 Years Ago in Borneo. *Nature* **2022**, 1–5.
349. Brumm, A.; Bulbeck, D.; Hakim, B.; Burhan, B.; Oktaviana, A.A.; Sumantri, I.; Zhao, J.-X.; Aubert, M.; Sardi, R.; McGahan, D.; et al. Skeletal Remains of a Pleistocene Modern Human (*Homo Sapiens*) from Sulawesi. *PLoS One* **2021**, *16*, e0257273.
350. Samper Carro, S.C.; Gilbert, F.; Bulbeck, D.; O'Connor, S.; Louys, J.; Spooner, N.; Questiaux, D.; Arnold, L.; Price, G.J.; Wood, R.; et al. Somewhere beyond the Sea: Human Cranial Remains from the Lesser Sunda Islands (Alor Island, Indonesia) Provide Insights on Late Pleistocene Peopling of Island Southeast Asia. *J. Hum. Evol.* **2019**, *134*, 102638.
351. Higham, T.; Brock, F.; Peresani, M.; Broglio, A.; Wood, R.; Douka, K. Problems with Radiocarbon Dating the Middle to Upper Palaeolithic Transition in Italy. *Quat. Sci. Rev.* **2009**, *28*, 1257–1267.
352. Schaefer, N.K.; Shapiro, B.; Green, R.E. An Ancestral Recombination Graph of Human, Neanderthal, and Denisovan Genomes. *Sci Adv* **2021**, *7*, doi:10.1126/sciadv.abc0776.
353. Zhang, X.; Witt, K.E.; Bañuelos, M.M.; Ko, A.; Yuan, K.; Xu, S.; Nielsen, R.; Huerta-Sanchez, E. The History and Evolution of the Denisovan-*EPAS1* Haplotype in Tibetans. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, doi:10.1073/pnas.2020803118.
354. Massilani, D.; Skov, L.; Hajdinjak, M.; Gunchinsuren, B.; Tseveendorj, D.; Yi, S.; Lee, J.; Nagel, S.; Nickel, B.; Devière, T.; et al. Denisovan Ancestry and Population History of Early East Asians. *Science* **2020**, *370*, 579–583.

355. Teixeira, J.C.; Cooper, A. Using Hominin Introgression to Trace Modern Human Dispersals. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 15327–15332.
356. Chang, C.-H.; Kaifu, Y.; Takai, M.; Kono, R.T.; Grün, R.; Matsu'ura, S.; Kinsley, L.; Lin, L.-K. The First Archaic Homo from Taiwan. *Nat. Commun.* **2015**, *6*, 6037.
357. Zanolli, C. Denisovans: How to Recognize Them in the Fossil Record. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
358. Brown, S.; Higham, T.; Slon, V.; Pääbo, S.; Meyer, M.; Douka, K.; Brock, F.; Comeskey, D.; Procopio, N.; Shunkov, M.; et al. Identification of a New Hominin Bone from Denisova Cave, Siberia Using Collagen Fingerprinting and Mitochondrial DNA Analysis. *Sci. Rep.* **2016**, *6*, 23559.
359. Slon, V.; Mafessoni, F.; Vernot, B.; de Filippo, C.; Grote, S.; Viola, B.; Hajdinjak, M.; Peyrégne, S.; Nagel, S.; Brown, S.; et al. The Genome of the Offspring of a Neanderthal Mother and a Denisovan Father. *Nature* **2018**, *561*, 113–116.
360. Vernot, B.; Zavala, E.I.; Gómez-Olivencia, A.; Jacobs, Z.; Slon, V.; Mafessoni, F.; Romagné, F.; Pearson, A.; Petr, M.; Sala, N.; et al. Unearthing Neanderthal Population History Using Nuclear and Mitochondrial DNA from Cave Sediments. *Science* **2021**, *372*, doi:10.1126/science.abf1667.
361. Zavala, E.I.; Jacobs, Z.; Vernot, B.; Shunkov, M.V.; Kozlikin, M.B.; Derevianko, A.P.; Essel, E.; de Filippo, C.; Nagel, S.; Richter, J.; et al. Pleistocene Sediment DNA Reveals Hominin and Faunal Turnovers at Denisova Cave. *Nature* **2021**, *595*, 399–403.
362. Brown, S.; Massilani, D.; Kozlikin, M.B.; Shunkov, M.V.; Derevianko, A.P.; Stoessel, A.; Jope-Street, B.; Meyer, M.; Kelso, J.; Pääbo, S.; et al. The Earliest Denisovans and Their Cultural Adaptation. *Nat Ecol Evol* **2022**, *6*, 28–35.
363. R. Vahdati, A.; Weissmann, J.D.; Timmermann, A.; Ponce de León, M.; Zollikofer, C.P.E. Exploring Late Pleistocene Hominin Dispersals, Coexistence and Extinction with Agent-Based Multi-Factor Models. *Quat. Sci. Rev.* **2022**, *279*, 107391.
364. Huerta-Sánchez, E.; Jin, X.; Asan; Bianba, Z.; Peter, B.M.; Vinckenbosch, N.; Liang, Y.; Yi, X.; He, M.; Somel, M.; et al. Altitude Adaptation in Tibetans Caused by Introgression of Denisovan-like DNA. *Nature* **2014**, *512*, 194–197.
365. Price, T.D.; Burton, J.H.; Bentley, R.A. The Characterization of Biologically Available Strontium Isotope Ratios for the Study of Prehistoric Migration. *Archaeometry* **2002**, *44*, 117–135.
366. Cruz Berrocal, M.; Serrano Herrero, E.; Gener Moret, M.; Uriarte González, A.; Torra Pérez, M.; Consuegra Rodríguez, S.; Chevalier, A.; Valentin, F.; Tsang, C.-H. A Comprised Archaeological History of Taiwan through the Long-Term Record of Heping Dao, Keelung. *Int. J. Hist. Archaeol.* **2018**, *22*, 905–940.
367. Cruz Berrocal, M. Ilha Formosa, Seventeenth Century: Archaeology in Small Islands, History of Global Processes. In *Archaeologies of Early Modern Spanish Colonialism*; Montón-Subías, S., Cruz Berrocal, M., Ruiz Martínez, A., Eds.; Springer International Publishing: Cham, 2016; pp. 281–302 ISBN 9783319218854.
368. Borao, J.E.; Heyns, P.; Gomez, C.; Zanduetta Nisce, A.M. *Spaniards in Taiwan Vol. I & Vol. II*; SMC Publishing, Taipei, 2002;

369. Valentin, F.; Choi, J.; Lin, H.; Bedford, S.; Spriggs, M. Three-Thousand-Year-Old Jar-Burials at the Teouma Cemetery (Vanuatu): A Southeast Asian-Lapita Connection. In *The Lapita cultural complex in time and space: Expansion routes, chronologies and typologies*; 2015; pp. 81–101.
370. Dakudao, M.E. The Development of Cemeteries in Manila before 1941. *Philipp. Q. Cult. Soc.* **1992**, *20*, 133–150.
371. Santana, J.; Fregel, R.; Lightfoot, E.; Morales, J.; Alamón, M.; Guillén, J.; Moreno, M.; Rodríguez, A. The Early Colonial Atlantic World: New Insights on the African Diaspora from Isotopic and Ancient DNA Analyses of a Multiethnic 15th-17th Century Burial Population from the Canary Islands, Spain. *Am. J. Phys. Anthropol.* **2016**, *159*, 300–312.
372. Hausmair, B. Topographies of the Afterlife: Reconsidering Infant Burials in Medieval Mortuary Space. *Journal of Social Archaeology* **2017**, *17*, 210–236.
373. Jacobi, K.P. *Last Rites for the Tipu Maya: Genetic Structuring in a Colonial Cemetery*; University of Alabama Press, 2000; ISBN 9780817310257.
374. Blench, R. Was There an Austroasiatic Presence in Island Southeast Asia Prior to the Austronesian Expansion? *BIPPA* **2010**, *30*, 133–144.
375. Plutniak, S.; Araujo, A.; Puaud, S.; Ferrié, J.-G.; Oktaviana, A.A.; Sugiyanto, B.; Chazine, J.-M.; Ricaut, F.-X. Borneo as a Half Empty Pot: Pottery Assemblage from Liang Abu, East Kalimantan. *Quat. Int.* **2016**, *416*, 228–242.
376. Bellwood, P.; Oxenham, M.; Hoang, B.C.; Dzung, N.K.; Willis, A.; Sarjeant, C.; Piper, P.; Matsumura, H.; Tanaka, K.; Beavan-Athfield, N.; et al. An Son and the Neolithic of Southern Vietnam. *Asian Perspectives* **2011**, *50*, 144–175.
377. Preechamart, S.; Pumijumnong, N.; Bräuning, A.; Muangsong, C.; Cai, B.; Payomrat, P.; Buajan, S.; Wang, F.; Li, M. Tree-Ring Oxygen Isotope Chronology of Teak Log Coffins in Northwestern Thailand and Its Relationship with Pacific Decadal Oscillation and El Niño-Southern Oscillation. *Quat. Int.* **2021**, doi:10.1016/j.quaint.2021.04.007.
378. Hakim, B.; Perston, Y.; Sardi, R.; Newman, K.; Nur, M.; Muda, K.; Others Incised Stone Artefact in the Context of Middle Holocene Burials at Cappalombo 1, South Sulawesi, Indonesia| Artefak Batu Bergores Dalam Konteks Penguburan Holosen Tengah Di Situs Cappalombo 1, Sulawesi Selatan, Indonesia. *SPAFA Journal* **2021**, *5*.
379. Fakhri Flexed Burials From Sulawesi: New Evidence of Mid Holocene Mortuary Practices in South East Asia. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.
380. Bulbeck, D. An Integrated Perspective On The Austronesian Diaspora: The Switch from Cereal Agriculture to Maritime Foraging in the Colonisation of Island Southeast Asia. *Australian Archaeology* **2008**, *67*, 31–51.
381. Pawlik, A.F. Technology, Adaptation, and Mobility in Maritime Environments in the Philippines from the Late Pleistocene to Early/Mid-Holocene. *Quat. Int.* **2021**, *596*, 109–123.
382. Bocquentin, F.; Knüsel, C.J. Beyond the Formal Analysis of Funerary Practices?: Archaeothanatology as a Reflexive Tool for Considering the Role of the Dead amongst the Living. A Natufian Case Study. In *The Routledge Handbook of Archaeothanatology*; Knüsel, C.J., Schotsmans, E.M.J., Eds.; Routledge: London, 2022.
383. Cruz Berrocal, M.; Serrano, E.; Valentin, F.; Tsang, C.-H.; Gorostiza, A.; Campoy, E.; Pereira, R.; Martín, A.G.; Bracker, K. The Study of European Migration in Asia-Pacific

- During the Early Modern Period: San Salvador de Isla Hermosa (Keelung, Taiwan). *Int. J. Hist. Archaeol.* **2020**, *24*, 233–283.
384. Zucchi, A. Tombs and Testaments: Mortuary Practices during the Seventeenth to Nineteenth Centuries in the Spanish-Venezuelan Catholic Tradition. *Hist. Archaeol.* **1997**, *31*, 31–41.
385. Lawrence, J.W. Archaeology and Ethnohistory on the Spanish Colonial Periphery: Excavations at the Templo Colonial in Nicoya, Guanacaste, Costa Rica. *Hist. Archaeol.* **2009**, *43*, 65–80.
386. Lu, T.-K.; Li, K.-T. *The Discovery of the Group of Burials of the Early Qing Dynasty at Shuijiaoshe, Tainan*; Tainan National University of the Arts: Tainan, Taiwan, 2009;
387. Chu, R.T.; Ang See, T. Toward a History of Chinese Burial Grounds in Manila during the Spanish Colonial Period. *Archipel. Études interdisciplinaires sur le monde insulindien* **2016**, 63–90.
388. Müller, G. *Challenging Dead: A Look into Foreigners' Cemeteries in Macau, Hong Kong, and Taiwan*; CrossAsia-Repository, 2018; ISBN 9783946742524.
389. Pietrusewsky, M.; Lauer, A.; Douglas, M.T.; Tsang, C.-H.; Li, K.-T. Patterns of Health in Early Neolithic and Iron Age Taiwan. *Anthropological Science* **2016**, *124*, 117–133.
390. Taylor, R.E.; Stuiver, M.; Reimer, P.J. Development and Extension of the Calibration of the Radiocarbon Time Scale: Archaeological Applications. *Quat. Sci. Rev.* **1996**, *15*, 655–668.
391. Weir, B.S.; Anderson, A.D.; Hepler, A.B. Genetic Relatedness Analysis: Modern Data and New Challenges. *Nat. Rev. Genet.* **2006**, *7*, 771–780.
392. Gamba, C.; Fernández, E.; Tirado, M.; Pastor, F.; Arroyo-Pardo, E. Brief Communication: Ancient Nuclear DNA and Kinship Analysis: The Case of a Medieval Burial in San Esteban Church in Cuellar (Segovia, Central Spain). *Am. J. Phys. Anthropol.* **2011**, *144*, 485–491.
393. Baca, M.; Doan, K.; Sobczyk, M.; Stankovic, A.; Węgleński, P. Ancient DNA Reveals Kinship Burial Patterns of a Pre-Columbian Andean Community. *BMC Genet.* **2012**, *13*, 30.
394. Monroy Kuhn, J.M.; Jakobsson, M.; Günther, T. Estimating Genetic Kin Relationships in Prehistoric Populations. *PLoS One* **2018**, *13*, e0195491.
395. Lipatov, M.; Sanjeev, K.; Patro, R.; Veeramah, K. Maximum Likelihood Estimation of Biological Relatedness from Low Coverage Sequencing Data. *bioRxiv* 2015.
396. Popli, D.; Peyrégne, S.; Peter, B.M. KIN: A Method to Infer Relatedness from Low-Coverage Ancient DNA. *Genome Biol.* **2023**, *24*, 10.
397. Jonsson, H. Ethnology and the Issue of Human Diversity in Mainland Southeast Asia. In *Dynamics of human diversity: The case of mainland Southeast Asia*; Enfield, N.J., Ed.; Canberra: Pacific Linguistics, 2011; pp. 109–122.
398. Lee, D.-N.; Jeon, C.L.; Kang, J.; Burri, M.; Krause, J.; Woo, E.J.; Jeong, C. Genomic Detection of a Secondary Family Burial in a Single Jar Coffin in Early Medieval Korea. *Am. J. Biol. Anthropol.* **2022**, *179*, 585–597.
399. Ning, C.; Zhang, F.; Cao, Y.; Qin, L.; Hudson, M.J.; Gao, S.; Ma, P.; Li, W.; Zhu, S.; Li, C.; et al. Ancient Genome Analyses Shed Light on Kinship Organization and Mating Practice of Late Neolithic Society in China. *iScience* **2021**, *24*, 103352.

400. Rivollat, M. Investigating Neolithic Social Structures on the Basis of Unprecedentedly Large Family Trees from the Site Gurgy “Les Noisats” in France. Presented at the EAA 2021, Kiel, Germany, 2021.
401. Browning, S.R.; Browning, B.L. Identity by Descent between Distant Relatives: Detection and Applications. *Annu. Rev. Genet.* **2012**, *46*, 617–633.
402. Wannasri, S.; Pumijumnong, N.; Shoocongdej, R. Teak Log Coffin Head Styles in Northern Thailand: Time Sequencing with Dendrochronology. *Sci. Asia.* **2007**, *33*, 047.
403. Pumijumnong, N.; Wannasri, S. Teak Log Coffins in Northwest Thailand: Dated by Dendrochronology and 14Cwiggle Matching. *Applied Environmental Research* **2015**, *37*, 1–16.
404. Samrit, C. Social Network of Log Coffin Culture in Highland Pang Mapha, Mae Hong Son. In *Archaeology of Pre Thai in Highland Pang Mapha, Mae Hong Son Province*; Ruen Khew Printing: Bangkok, 2020; pp. 95–112.
405. Fernandes, D.M.; Sirak, K.A.; Ringbauer, H.; Sedig, J.; Rohland, N.; Cheronet, O.; Mah, M.; Mallick, S.; Olalde, I.; Cullen, B.J.; et al. A Genetic History of the Pre-Contact Caribbean. *Nature* **2021**, *590*, 103–110.
406. Zhang, X.; Li, C.; Zhou, Y.; Huang, J.; Yu, T.; Liu, X.; Shi, H.; Liu, H.; Chia, S.; Huang, S.; et al. A Matrilineal Genetic Perspective of Hanging Coffin Custom in Southern China and Northern Thailand. *iScience* **2020**, *23*, 101032.
407. Sayers, E.W.; Bolton, E.E.; Brister, J.R.; Canese, K.; Chan, J.; Comeau, D.C.; Connor, R.; Funk, K.; Kelly, C.; Kim, S.; et al. Database Resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **2022**, *50*, D20–D26.
408. Gretzinger, J.; Sayer, D.; Justeau, P.; Altena, E.; Pala, M.; Dulias, K.; Edwards, C.J.; Jodoin, S.; Lacher, L.; Sabin, S.; et al. The Anglo-Saxon Migration and the Formation of the Early English Gene Pool. *Nature* **2022**, *610*, 112–119.
409. Margaryan, A.; Lawson, D.J.; Sikora, M.; Racimo, F.; Rasmussen, S.; Moltke, I.; Cassidy, L.M.; Jørsboe, E.; Ingason, A.; Pedersen, M.W.; et al. Population Genomics of the Viking World. *Nature* **2020**, *585*, 390–396.
410. Skourtanioti, E.; Erdal, Y.S.; Frangipane, M.; Balossi Restelli, F.; Yener, K.A.; Pinnock, F.; Matthiae, P.; Özbal, R.; Schoop, U.-D.; Guliyev, F.; et al. Genomic History of Neolithic to Bronze Age Anatolia, Northern Levant, and Southern Caucasus. *Cell* **2020**, *181*, 1158–1175.e28.
411. Shoocongdej, R. Working toward an Anthropological Perspective on Thai Prehistory: Current Research on the Post-Pleistocene. *the Indo-Pacific Prehistory Association* **1996**.
412. Halcrow, S.E.; Crozier, R.; Domett, K.M.; Lertcharnrit, T.; Newton, J.S.; Shewan, L.G.; Ward, S.M. Ethical Issues of Bioarchaeology in Southeast Asia. In *Ethical Approaches to Human Remains: A Global Challenge in Bioarchaeology and Forensic Anthropology*; Squires, K., Errickson, D., Márquez-Grant, N., Eds.; Springer International Publishing: Cham, 2019; pp. 465–484 ISBN 9783030329266.
413. Flecker, M. The Ethics, Politics, and Realities of Maritime Archaeology in Southeast Asia. *Int. J. Naut. Archaeol.* **2002**, *31*, 12–24.
414. Byrne, D. The Problem with Looting: An Alternative Perspective on Antiquities Trafficking in Southeast Asia. *J. Field Archaeol.* **2016**, *41*, 344–354.

415. Basu, A.; Sarkar-Roy, N.; Majumder, P.P. Genomic Reconstruction of the History of Extant Populations of India Reveals Five Distinct Ancestral Components and a Complex Structure. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 1594–1599.
416. Aghakhanian, F.; Yunus, Y.; Naidu, R.; Jinam, T.; Manica, A.; Hoh, B.P.; Phipps, M.E. Unravelling the Genetic History of Negritos and Indigenous Populations of Southeast Asia. *Genome Biol. Evol.* **2015**, *7*, 1206–1215.
417. Changmai, P.; Koci, J.; Flegontov, P. Reconstructing the Genetic History of Kra-Dai Speakers from Thailand. *bioRxiv* 2022, 2022.06.30.498332.
418. Srithawong, S.; Muisuk, K.; Srikumool, M.; Kampuansai, J.; Pittayaporn, P.; Ruangchai, S.; Liu, D.; Kutanan, W. Close Genetic Relationship between Central Thai and Mon People in Thailand Revealed by Autosomal Microsatellites. *Int. J. Legal Med.* **2021**, *135*, 445–448.
419. Mawan, A.; Prakhun, N.; Muisuk, K.; Srithawong, S.; Srikumool, M.; Kampuansai, J.; Shoocongdej, R.; Inta, A.; Ruangchai, S.; Kutanan, W. Autosomal Microsatellite Investigation Reveals Multiple Genetic Components of the Highlanders from Thailand. *Genes* **2021**, *12*, doi:10.3390/genes12030383.
420. Kutanan, W.; Shoocongdej, R.; Srikumool, M.; Hübner, A.; Suttipai, T.; Srithawong, S.; Kampuansai, J.; Stoneking, M. Cultural Variation Impacts Paternal and Maternal Genetic Lineages of the Hmong-Mien and Sino-Tibetan Groups from Thailand. *Eur. J. Hum. Genet.* **2020**, *28*, 1563–1579.
421. Kutanan, W.; Kampuansai, J.; Srikumool, M.; Brunelli, A.; Ghirotto, S.; Arias, L.; Macholdt, E.; Hübner, A.; Schröder, R.; Stoneking, M. Contrasting Paternal and Maternal Genetic Histories of Thai and Lao Populations. *Mol. Biol. Evol.* **2019**, *36*, 1490–1506.
422. Kutanan, W.; Liu, D.; Kampuansai, J.; Srikumool, M.; Srithawong, S.; Shoocongdej, R.; Sangkhano, S.; Ruangchai, S.; Pittayaporn, P.; Arias, L.; et al. Reconstructing the Human Genetic History of Mainland Southeast Asia: Insights from Genome-Wide Data from Thailand and Laos. *Mol. Biol. Evol.* **2021**, *38*, 3459–3477.
423. GenomeAsia100K Consortium The GenomeAsia 100K Project Enables Genetic Discoveries across Asia. *Nature* **2019**, *576*, 106–111.
424. Rodriguez, J.J.R.B.; Cuares, J.M.D.; Herrera, M.J.B.; Zubiri, L.A.M.; Muallil, R.N.; Ishmael, A.I.; Jimenez, E.B.; Stoneking, M.; De Ungria, M.C.A. Ethical Challenges in Genetic Research among Philippine Indigenous Peoples: Insights from Fieldwork in Zamboanga and the Sulu Archipelago. *Front. Genet.* **2022**, *13*, 901515.
425. Claw, K.G.; Anderson, M.Z.; Begay, R.L.; Tsosie, K.S.; Fox, K.; Garrison, N.A.; Summer internship for INdigenous peoples in Genomics (SING) Consortium A Framework for Enhancing Ethical Genomic Research with Indigenous Communities. *Nat. Commun.* **2018**, *9*, 2957.
426. Shoocongdej, R. Contemporary Archaeology as a Global Dialogue: Reflections from Southeast Asia. In *Comparative Archaeologies: A Sociological View of the Science of the Past*; Lozny, L.R., Ed.; Springer New York: New York, NY, 2011; pp. 707–729 ISBN 9781441982254.
427. Marwick, B.; Shoocongdej, R.; Thongcharoenchaikit, C.; Chaisuwan, B.; Khowkhiew, C.; Kwak, S.; Others Hierarchies of Engagement and Understanding: Community

Engagement during Archaeological Excavations at Khao Toh Chong Rockshelter, Krabi, Thailand. *Transcending the Culture-Nature Divide in Cultural Heritage: Views from the Asia-Pacific Region* **2013**.

428. Yuki, N. Legacies of Colonialism in Southeast Asian Archaeology and How to Move Forward. Presented at the IPPA 22, Chiang Mai, Thailand, 2022.

429. Kaharudin, H.A.F.; Asyraf, M. Archaeology in the Making of Nations: The Juxtaposition of Postcolonial Archaeological Study. *AMERTA* **2019**, *37*, 55.

430. Yáñez, B.; Fuentes, A.; Silva, C.P.; Figueiro, G.; Menéndez, L.P.; García-Deister, V.; de la Fuente-Castro, C.; González-Duarte, C.; Tamburrini, C.; Argüelles, J.M. Pace and Space in the Practice of ADNA Research: Concerns from the Periphery. *Am. J. Biol. Anthropol.* **2022**, doi:10.1002/ajpa.24683.

431. Ávila-Arcos, M.C.; de la Fuente Castro, C.; Nieves-Colón, M.A.; Raghavan, M. Recommendations for Sustainable Ancient DNA Research in the Global South: Voices From a New Generation of Paleogenomicists. *Front. Genet.* **2022**, *13*, doi:10.3389/fgene.2022.880170.

432. Argüelles, J.M.; Fuentes, A.; Yáñez, B. Analyzing Asymmetries and Praxis in ADNA Research: A Bioanthropological Critique. *Am. Anthropol.* **2022**, *124*, 130–140.

433. Odeny, B.; Bosurgi, R. Time to End Parachute Science. *PLoS Med.* **2022**, *19*, e1004099.

434. Rispoli, F. The Expansion of Rice and Millet Farmers into Southeast Asia. In *The Oxford Handbook of Early Southeast Asia*; Higham, C.F.W., Kim, N.C., Eds.; Oxford University Press: Oxford, 2022.

435. Rispoli, F. The Incised & Impressed Pottery Style of Mainland Southeast Asia: Following the Paths of Neolithization. *East and West* **2007**, *57*, 235–304.

436. Hudson, M.J. Slouching toward the Neolithic: Complexity, Simplification, and Resilience in the Japanese Archipelago. *The Routledge Handbook of the Bioarchaeology of* **2020**.

437. Acabado, S. Current Archaeological Practice in Southeast Asia: Collaboration, Engagement, and Community Involvement in Field Research in Southeast Asia. *Journal of Community Archaeology & Heritage* **2020**, *7*, 158–160.

438. Shoocongdej, R. The Challenge of Archaeological Interpretation and Practice That Integrates between the Science of the Past and Local Knowledge. *SPAFA Journal* **2020**, *4*.

439. Wagner, J.K.; Colwell, C.; Claw, K.G.; Stone, A.C.; Bolnick, D.A.; Hawks, J.; Brothers, K.B.; Garrison, N.A. Fostering Responsible Research on Ancient DNA. *Am. J. Hum. Genet.* **2020**, *107*, 183–195.

Supplementary Information for Manuscripts A-D

Supplementary information

Genome of a middle Holocene hunter-gatherer from Wallacea

In the format provided by the
authors and unedited

SUPPLEMENTARY INFORMATION FOR:

Genome of a middle Holocene hunter-gatherer from Wallacea

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The Toalean technocomplex of South Sulawesi

For more than a century, prehistorians have used the label Toalean¹⁻⁸ (also spelt Toalian) to denote a unique hunter-gatherer technocomplex that existed in the southern portion of the southwestern peninsula of Sulawesi between approximately 8–1.5 thousand years ago (kya)⁷⁻⁹. Stratified Toalean archaeological assemblages are typically found in the upper layers of cave and shelter deposits¹⁻⁸. The technocomplex is characterised by small bone and tooth points, backed microliths, and Maros points³⁻⁴. The latter are distinctive stone projectiles with pressure-flaked denticulations along the lateral margins⁷, and, oftentimes, indented or hollow bases⁸⁻⁹. Current archaeological evidence⁷⁻⁸ suggests the Toalean technocomplex was highly localised. Thus far, characteristic assemblages have only ever been identified at Holocene sites located within a roughly 10,000 km² area of coastal lowlands and interior uplands in the southwestern arm of Sulawesi⁷⁻⁸ (Fig. 1b).

Toalean assemblages were first identified in the early 1900s, when Swiss naturalists Paul and Fritz Sarasin journeyed into the highlands of South Sulawesi in search of ethnological data^{1,10}. In the hills of south Bone the Sarasins encountered the To Ala' (Forest People), a modern group of cave-dwellers whom they considered to be descendants of Sulawesi's earliest human inhabitants¹. The Sarasins excavated several local rock-shelters in search of the prehistory of the To Ala', unearthing Maros points and other distinctive tools which they attributed to the direct lineal ancestors of the To Ala'¹. The To Ala', whose ethnographic material culture suggests continuity of a pre-Bugis heritage¹¹, had disappeared from the historical record prior to the outbreak of World War II (WWII)¹¹. However, later archaeological excavations involving Indonesian, Dutch², and Australian researchers³⁻⁶ uncovered similar prehistoric assemblages attributed to what had become widely known by the 1970s as the Toalean culture^{7-8,10}. It has recently been speculated¹²⁻¹⁴ that a group of middle Holocene Toalean foragers may have journeyed by watercraft from South Sulawesi to northern Australia, introducing dogs – that is, the domesticated ancestors of Australia's wild native canid, the dingo (*Canis dingo*)¹²⁻¹⁴ – an exotic language family (Pama-Nyungan)¹³⁻¹⁴, and a novel approach to stone tool-making (microlithic technology)¹³⁻¹⁴.

Although considerable numbers of Toalean artefacts have been amassed over the last century of fieldwork in South Sulawesi, the nature of the Toalean has long remained ambiguous⁷. Most Toalean sites on record were last investigated in the pre-WWII Dutch colonial period before the advent of radiocarbon dating. These early excavations were generally low quality and scantily reported², while seminal work conducted by Dutch archaeologists in the 1950s² improved but little on these pioneering efforts⁷. Until recently, the best-documented Toalean localities were investigated by Australian-Indonesian archaeologists over four decades ago³⁻⁶. However, the resulting Toalean sequences were typically ill-defined and at best coarsely dated⁷⁻⁸. A 1972 review of the Toalean archaeological record² listed only 20 published sites, while the most recent reviews (2000/2008) include only two more^{7,15}. According to a 2018 study¹⁶ there are only about 15 published, acceptable ¹⁴C determinations related to the entire Toalean period, and almost all of these dates came from the two Toalean master sequence sites, Leang Burung 1³⁻⁵ and Ulu Leang 1⁶. Recent Indonesian-led excavations at Leang Jarie in Maros and Bonto Cani in interior South Sulawesi^{8,9,17} have considerably improved chronological documentation of the Toalean technocomplex. It is now clear, for example, that prior models in which the development of Toalean backed microlithic technology preceded

that of Maros point technology, and, in fact, the emergence of the latter was linked to the arrival of Austronesian farmers, are questionable⁹. Despite recent advances in our knowledge of the history of the Toalean technocomplex, however, published human skeletal remains from Toalean contexts are still meagre⁷⁻⁸. The extant evidence consists mostly of fragmentary materials of uncertain antiquity. Hence, up until now the identity of the people responsible for producing the pre-Neolithic Toalean technocomplex has remained unresolved⁷⁻⁸.

Archaeological context of the Toalean human burial from Leang Panninge

Leang Panninge (Bat Cave in the Bugis language; alternative names and transcriptions include: Leang Panningnge, Leang Panning'nge, Leang Panning, Liang Panning, and Gua Panninge) is a limestone cave located 169.8 m above sea level in the Mallawa district of the Maros Regency, South Sulawesi. The cave is formed in a relict river-cut passage that extends for 120 m through a low karst hill. Situated in the upper Walanae valley, and separated from the lowland karsts of Maros-Pangkep by the Western Dividing Range, Mallawa is strategically positioned on the main pass through the rugged cordillera that loops behind the southern and western coastal lowlands of the peninsula. The area has received limited archaeological exploration.

Stratigraphic sequence at Leang Panninge

Leang Panninge has been the focus of several archaeological excavations undertaken by various teams, resulting in a total of 13 excavation squares in different areas of the site¹⁸⁻²⁵ (see Extended Data Fig. 2). All excavation squares have measured 1 m², and consistently encountered thick, well-stratified aceramic Toalean deposits (layers 2-4) located below a layer of mixed modern and Neolithic/Austronesian-associated sediments (layer 1). The sedimentary sequence for layers 1-5 is generally quite similar, suggesting a broadly similar depositional environment during the accumulation of these strata. Stratigraphic boundaries were for the most part difficult to identify consistently during excavation of layers 1-5, although layer changes tended to become clearer with depth. There is evidence for some minor post-depositional mixing affecting layer 4. However, the consistent angle of incline of artefacts and faunal remains throughout the profile section suggests that the stratigraphy is largely undisturbed, and that post-depositional mixing has been limited.

Layer 1 is a moist silty clay with ashy lenses. The relatively low-density cultural assemblages excavated from this level include prehistoric Austronesian pottery sherds and minor traces of

Toalean stone artefacts (i.e., backed microliths). Layer 2 comprises a silty clay that is very similar to layer 1 in terms of sedimentary composition and colour, although it is noticeably drier. This stratum contains a rich aceramic Toalean stone artefact assemblage. The most diagnostic Toalean tool type consists of geometric backed microliths. Maros points were not recovered from this archaeological horizon. Layer 3 (silty clay) is a low-intensity occupational horizon that contains relatively few cultural remains compared with the immediately overlying and underlying strata (layers 3 and 4, respectively). Layer 4 is a thick silty clay unit containing a dense concentration of cultural and faunal remains, with the rich Toalean lithic assemblage including Maros points but no backed microliths (characteristic Toalean osseous points, often as not made on lower incisor roots of *Suidae*, were found throughout layers 2 and 4). Layer 4 yielded the deepest clear evidence for Toalean occupation in stratified deposits at the site. Layer 5 is a silty clay with a similar sedimentary composition to layer 4, but it contains fewer stone artefacts and faunal remains generally and no Maros points or other diagnostic Toalean tool types. Layers 6 and 7 consist of silty clay and sand layers, respectively, with abundant gravel and few cultural findings. Layer 8 comprises clay with fossils and lithic artefacts. Exposed clearly for the first time in 2019, layers 6-8 represent a transition to larger artefacts including limestone and volcanic materials. No macroscopically observable plant carbon remains or other materials amenable to ^{14}C dating were recovered from these lower strata, which are probably Late Pleistocene but are as-yet undated.

Recovered skeletal remains

The Toalean human skeleton from Leang Panninge was uncovered in 2015 within squares S16T6 and S17T6, and removed in a recovery excavation in 2018²³⁻²⁴. The 2015 excavations were conducted in arbitrary 10-cm-thick spits and wet-sieved through a 3 mm mesh, to a depth of ~190 cm, at which point the human skeletal remains were encountered. The 2019 excavations took place between 20 July and 9 September. The backfilled squares, S16T6 and S17T6, were reopened and the deposit excavated in 5 cm spits to a depth of 200 cm (spit 19). Square S16T6 was extended vertically an additional 130 cm to a maximum depth of 330 cm and S17T6 extended an additional 60 cm to a maximum depth of 260 cm. These trenches continued to be excavated in 5 cm spits and all spit labels were continued from 2015. Squares S16T7 and S17T7 were opened to the east contiguous with S16T6 and S17T6. Both squares were excavated to a maximum depth of 235 cm (spit 45). Artefact locations were recorded using a Leica total station. All excavated deposits were wet sieved through a 3 mm screen.

Descriptions of the human skeletal remains are based on original field documentation by the 2015 UNHAS/USM/Balar Sulse team²³, observations by BB who removed the sediment blocks with AMS in 2018, and a laboratory excavation of these blocks by DM. During the 2015 excavation of squares S16T6 and S17T6 a clear burial cut could not be identified in the largely homogenous sediment profile. However, the semi-articulated condition of the remains indicates a primary inhumation. It was also observed that the skeletal remains were partially covered over by five large water-worn volcanic cobbles and numerous smaller stones (Extended Data Figs. 2f, 4). Much of the skull was represented, but in a fragmentary condition. Only the mandible, the four podials, and to a lesser degree the pelvis were semi-complete. The left and right pelvis were articulated with the fragmented sacrum and included well preserved left and right greater sciatic notches²³. The skull and pelvic region were both extremely fragile; hence, it was necessary to remove these parts of the skeleton from the deposit *en bloc* for later processing in a laboratory. The remaining post-crania were poorly represented. The upper limb elements were represented by a fragmented right clavicular shaft, left and right scapulae, distal right radius, and relatively complete left and right hands. The recovered lower limb components included fragments of both distal right tibia and distal left fibula, and relatively complete left and right feet. The post-cranial axial skeleton was represented only by fragments of twelve ribs (2nd to 7th, left and right) and associated thoracic vertebrae, and fragments of lumbar vertebrae²³. In 2018, cervical vertebral fragments were recovered from the skull sediment block in association with the occipital region of the skull. (For additional details on skeletal parts represented, see **Morphological description of the Leang Panninge human skeleton**).

As observed during the 2015 and 2018 fieldwork²³, the Leang Panninge skeleton was orientated in a north-south direction and lying in a tightly flexed or crouched position, with the skull facing left (towards the east) in the southern end of S16T6 (Extended Data Figs. 2f, 4). Most of the post-cranial remains were located in S17T6. The cranium was crushed post-mortem, although the position and orientation of the cranial elements recovered suggest it was intact when interred. The associated cobbles appear to have been deliberately arranged over the human remains (Extended Data Figs. 2f, 4). Two of the largest cobbles, with a combined mass of 12 kg, flanked the skull²³. A hand comprised of five fractured metapodials and a single phalanx was located below the largest of these (#1). A smaller cobble (#2) was placed immediately to the right (to the east) of the skull. Another of the largest cobbles (#4)

was positioned over the left foot. The southernmost cobble (#4) was placed over the pelvic region. The three cobbles in the pelvic region (#3, #4, and #5) had a combined mass of 13 kg.

A number of skeletal remains from non-human fauna were also excavated from spits 19 and 20 of squares S16T6 and S17T6 in the same context as the human skeleton. These included several phalanges, long bones, vertebrae, carpals and metacarpals of unidentified animal taxa as well as Bovidae teeth, Suidae astragalus, Siluriformes barbs, one *Macaca* sp. molar, various Chiroptera teeth, and 64 mollusc shells, including the freshwater gastropod *Tylomelania* (= *Brotia*) *perfecta* and several bivalves²³. Tools made on bone or tooth, and over 2,000 stone artefacts – including around 13 modified flakes displaying clear attributes of Maros point technology (Extended Data Fig. 5i-k) – were also recovered²³.

Toalean context of the human skeletal remains

The Leang Panninge individual was interred in a secure Toalean context, with distinctively Toalean artefacts recovered from the grave fill and surrounding and overlying deposits. As already noted, Toalean assemblages are commonly marked by the presence of distinctive artefact types, namely small points made on osseous materials, small stone Maros points, and/or backed microliths, occurring alongside more generic flakes and cores of generally small dimensions¹⁻⁹. Maros points are distinctive and apparently unique in the world, consisting of pressure-flaked stone points made on a small flake blank with an indented base and/or regular denticulations around the remaining margins. None of these classic Toalean artefact types have ever been recorded in pre-Toalean sites, and hence they are used as a diagnostic cultural marker for this South Sulawesi technocomplex of ~8–1.5 kya⁷. A specialist analysis of the large lithic assemblage excavated from Leang Panninge is ongoing; however, tens of thousands of largely chert artefacts have been recovered from S16T6 and S17T6, including dozens of Maros points (e.g., Extended Data Fig. 5d-p), backed microliths, and bone/tooth points^{23,25}. Among 4079 artefacts (total weight, 16.3 kg) recovered during excavation from spits 19 and 20 of S16T6 and S16T7, a total of 11 display diagnostic Maros point features (Extended Data Fig. 5i-k). While only two of these artefacts are classic Maros points, with an indented base and edge denticulations, the remaining specimens also have varying degrees of denticulations and represent incomplete or damaged Maros points.

Within the area of sediment removed *en bloc* around the skull of the individual in 2018, a total of 118 stone artefacts was recovered in association with the human remains, and these

were subjected to more detailed analysis. The majority of these stone artefacts (N = 71, 60%) were made from non-descript honey- to grey-coloured cherts common at the site. However, six were formed on a dark, fine-grained volcanic material with fine quartz veining that is exotic to the site. The distinctive appearance of the raw material of these particular artefacts strongly suggests that they were all struck from the same core during a single reduction event and likely deposited simultaneously, with minimal post-depositional disturbance.

In addition to this, two Maros points were recovered in direct association with the human skeleton during the laboratory excavation of the sediment blocks (Extended Data Figs. 2h-i, 5a-c). A chert Maros point (spit 19-20, square S17T6) was found directly associated with the pelvic area (~40 mm from the greater sciatic notch) of the Leang Panninge individual (Extended Data Figs. 2i, 5a-c). This artefact is a classic example of the tool type⁸, with a deep, well-defined basal notch and neat denticulations around the remaining margin. The point is 20 mm long, 17 mm wide and 3.5 mm thick. It should be noted, however, that the point has been broken by excessive heat and hence the first measurement probably only represents three-quarters of the original length. The stone contains embedded heat-induced potlids and a small number of potlid scars, further demonstrating that this Maros point was exposed to extreme heat. Differential gloss only occurs on the heat-damaged surfaces, indicating it was not deliberately heat-treated during manufacture and that the burning occurred after initial production and breakage. The artefact is pinkish red in colour, possibly the result of chemical change from the burning event. The point was produced using the classic Maros point reduction sequence, with pressure-flaked denticulations on the lateral margins converging to form the tip, and a basal notch at the proximal end of the flake blank. The point was manufactured on a small, almost flat flake with a single parallel dorsal ridge. A second chert Maros point (32.2 [incomplete] x 15.3 x 5.2 mm; Extended Data Figs. 2h, 5c) with minimal edge denticulations, also broken, was recovered from the skull block.

Faunal remains

The archaeological deposits at Leang Panninge are rich in non-human faunal remains, some of which may be attributed to human activities. Across the 11 excavation units, faunal assemblages are largely dominated by Chiroptera and Rodentia bones, sometimes articulated. The Toalean deposits (layers 2-4), include the two endemic Suidae species *Sus celebensis* (Sulawesi warty pig) and *Babyrousa* sp. (babirusa), as well as *Bubalus* sp. (anoa), *Ailurops ursinus*, *Strigocuscus* sp., *Varanus* sp., *Macaca* sp., *Tarsius* sp., one possible *Canis* sp. bone

(S8T6, layer 2)²³ and members of the Anura, Aves, Osteichthyes, Serpentes, Viverridae, and Cervidae taxa¹⁸⁻²⁴. The 2019 excavations revealed a near-complete Suidae skeleton in S16T7 spit 19 and 20, and extending into S17T7. Both species of Suidae, *S. celebensis* and babirusa, show signs of being butchered on site during the Toalean phase of occupation, in the form of processing marks on the bones and an assemblage dominated by sub-adult individuals²⁴. High linear enamel hypoplasia levels were identified on Suidae teeth^{24,26}. Modified faunal remains at Leang Panninge include osseous points, sometimes manufactured using Suidae incisors and canines²³, a drilled shark tooth, and a small fragment of shell with a possible partial perforation²². Mollusc findings are dominated by specimens of the locally common freshwater gastropod *T. perfecta* and a small number of shells from the Bivalvia class²³.

Dating evidence

In total, 13 AMS ¹⁴C dates have been obtained on charcoal (N = 8), seeds (N = 3), and shells (N = 2) excavated from Leang Panninge, including three samples associated directly with the human burial context. These are reported in SI Table 1 (for additional contextual information, see Extended Data Figs. 2e, 3). All of the dated samples were hand-picked from the sediments under excavation – no samples were collected from sieve residues. The two dated shells were hand-picked from the layer 4 Toalean human burial feature under excavation in 2015. While exposing the skull area, several stone flakes and various other artefacts, including shells and non-human bones, were uncovered. An unidentified freshwater gastropod shell exposed near the human cranium (S17T6; spit 19, 181 cm depth) was collected for radiocarbon dating (Sample Wk-42781) using clean metal tweezers and aluminium foil to avoid any possible contamination. Another unidentified freshwater gastropod shell was collected using the same methods (Sample Wk-42782) during excavation of the burial area in S17T6 at spit 18 (170-180 cm). These two shells yielded AMS ¹⁴C dates of 7423 ± 22 BP (8325–8176 cal BP) and 7008 ± 22 BP (7926–7748 cal BP), respectively (SI Table 1). Environmental offsets are unavailable to correct for the freshwater reservoir effect³⁰, the magnitude of which is unknown in this region. Notably, the ¹⁴C-dates on gastropod shells associated with the Toalean human skull are earlier than the ¹⁴C age of a charred *Canarium* sp. seed (7264–7165 cal BP [Wk-48639]) recovered from the excavated sediment block containing the cranial bones, mandible, and dental remains (SI Table 1). It is therefore likely that the ¹⁴C dates on freshwater shell are erroneously old – in the order of at least several centuries in this particular instance – owing to absorption of “dead” carbon from the ancient river or lake environment³⁰. In this study, we inferred the age of the human burial

using the more reliable chronology obtained from ^{14}C -dating of charcoal and charred seeds (SI Table 1). We attempted to directly date the Leang Panninge burial by conducting AMS ^{14}C dating on the human petrous bone that yielded the ancient DNA. This effort was unsuccessful, however, owing to the lack of a sufficient amount of collagen in the petrous bone.

SI Table 1: Radiocarbon dates from Leang Panninge.

Year	Lab Code	Sample context			Material	Conventional ^{14}C age BP ^c	Calibrated ^{14}C age BP ^c
		L ^a	Squ ^b	Spit (depth [cm])			
2019	D-AMS 035761	1	S17T7	7 (90-95 cm)	Charcoal ^c	1594 ± 23	1526–1404
2019	D-AMS 035753	2	S16T7	17 (90-95 cm)	Charcoal ^c	3519 ± 26	3848–3692
2019	D-AMS 035754	2	S17T7	22 (115-120 cm)	Charcoal ^c	3656 ± 32	4083–3849
2019	D-AMS 035755	2	S16T7	29 (150-153 cm)	Charcoal ^c	3577 ± 30	3968–3723
2019	D-AMS 035762	3	S16T7	32 (165-170 cm)	Charcoal ^c	3989 ± 28	4524–4300
2019	D-AMS 035756	4	S16T7	33 (170-175 cm)	Charcoal ^c	4555 ± 28	5315–5051

2019	D-AMS 035757	4	S16T7	35 (180- 185 cm)	Charcoal ^e	6978 ± 36	7920–7690
2019	D-AMS 035758	4	S17T7	38 (195- 200 cm)	Burnt seed ^{f,g}	6200 ± 31	7166–6977
2019	D-AMS 035759	5	S16T7	40 (205-210 cm)	Charcoal ^e	7993 ± 37	8994–8650
2019	D-AMS 035760	5	S16T7	42 (215- 220 cm)	Burnt seed ^{f,g}	8261 ± 36	9403–9031
2018	Wk- 48639	Sample recovered from the excavated sediment block containing the human cranium (layer 4 burial) that yielded ancient DNA			Burnt seed ^{f,h}	6317 ± 19	7264–7165
2015	Wk- 42781	Sample uncovered while exposing the skull area of the human burial (S17T6; spit 19, 181 cm depth)			Shell ^{i,j}	7423 ± 22	8325–8176
2015	Wk- 42782	Sample uncovered during excavation of the burial area (S17T6; spit 18, 170-180 cm)			Shell ^{i,j}	7008 ± 22	7926–7748

^aL = Stratigraphic layer.

^bSqu = Excavation square.

^cUncertainties reported at 95% confidence interval.

^dConventional ¹⁴C ages were calibrated using the OxCal 4.4 program and a mix_curve fixed at 50%:50% IntCal20:SHCal20²⁷.

^ePretreatment processes and dating methods used by the DirectAMS laboratory (lab code identifier = D-AMS) for AMS ¹⁴C-dating of charcoal were as follows: upon arrival, samples that passed initial inspection for quality and integrity were subsampled to obtain a clean, representative portion for chemical pretreatment. During subsampling, gross contaminants such as rootlets, fungal fibres, sediment, or mineral deposits were removed

manually. The selected portion was then broken into smaller pieces, as needed, to maximise surface area. Charcoal and plant materials were subjected to an acid-base-acid (ABA) treatment based on established methods²⁸⁻²⁹ of sequential HCl and KOH immersions. Initial acid immersions remove carbonates, iron and sulfur; base immersions remove adsorbed organic compounds, collectively known as humic acids; and the final acid immersion removes any carbon fixed during the preceding base immersions. Pretreated material was then lyophilised prior to graphitisation for measurement by AMS.

^f*Canarium* sp. (kenari nut).

^gPretreatment processes and dating methods used by the DirectAMS laboratory for AMS ¹⁴C-dating of seeds followed the same methods used for charcoal, as outlined above.

^hPretreatment processes and dating methods used by the Radiocarbon Dating Laboratory at the University of Waikato (lab code identifier = Wk) for AMS ¹⁴C-dating of seeds were as follows: following initial sample inspection, extraneous material such as rootlets or other visible contaminants were removed; the sample was then washed in distilled water and milled and pretreated using a dilute acid/dilute alkali/dilute acid treatment prior to AMS measurement (<https://radiocarbon dating.com/operating-procedures/pretreatment>).

ⁱThe dated shells derived from an unidentified freshwater gastropod species, probably *Tylomelania* (= *Brotia*) *perfecta*.

^jShells dated by the Radiocarbon Dating Laboratory at the University of Waikato (lab code identifier = Wk) were processed as follows: physical pretreatment involved cleaning surfaces and washing the samples in an ultrasonic bath; the samples were tested for secondary recrystallisation using the Feigl method; staining showed that both shells were comprised of aragonite. Chemical pretreatment involved acid washing with 0.1N HCl, after which the samples were rinsed and dried.

Morphological description of the Leang Panninge human skeleton

The Leang Panninge human remains (SI Table 2) are stored at the Archaeology Laboratory of the Archaeology Department (Departemen Arkeologi Fakultas Ilmu Budaya) at UNHAS, Makassar, South Sulawesi.

SI Table 2: Skeletal elements represented in the Leang Panninge human burial

Part of skeleton	Representation	Weight (grams)	Percent of total
Cranium	Loose teeth, face, frontal, left and right temporals, right parietal, occipital	78	30
Mandible	Semi-complete	44	17
Vertebral column	Mainly cervical, some thoracic and lumbar	9	3
Ribs	2 nd to 7 th rib fragments, left and right	10	4
Clavicle	Right shaft fragment	2	1

Scapula	Left and right fragments	6	2
Radius	Distal right shaft/epiphysis	3	1
Hands	Left and right complete elements and fragments	6	2
Pelvic girdle	Left and right pelves, fragmentary sacrum	80	30
Tibia	Distal right shaft (tentative identification)	4	2
Fibula	Left(?) distal shaft	3	1
Feet	Left and right complete elements and fragments	18	7
Total		263	100

Surface red colouration was observed on the following elements: anterior mandible, axis vertebra, upper thoracic ribs, medial thoracic right ribs, right distal radius, suspected right tibia, distal fibula, some left foot bones. However, this possible evidence of red painting of some of the bones is not limited to the human remains, because a large rib fragment (probably *Suidae*) is also stained red. Evidence for some sort of ritual use of ochre for the burial is suggested by the discovery of a nodule of red ochre beneath the cranium.

Age and sex

The Leang Panninge individual's age can be placed at approximately 17–18 years old. This determination is based on the status of the third molars (cf. ref. 31) and the postcranial epiphyses (cf. Figure 3.4 in ref. 32) as detailed in SI Table 3.

SI Table 3: Anatomical observations relevant to determining the age of the Leang Panninge individual

Element(s)	Status	Age evidence
Four third molars	Roots not quite completely formed, teeth unerupted	Root formation starts at 15; fully occluded at 21 years old
Scapula inferior angle	Unfused suture	Fusion at 17–22 years old
Right distal radius	Unfused distal epiphysis	Fusion at 13–19 years old

Hands	Recently fused epiphyses: right 2 nd –3 rd metacarpals, left and right 1 st distal phalanges, right 5 th distal phalanx. Unfused epiphyses: left 3 rd –5 th metacarpals, right 4 th metacarpal, right 3 rd –5 th proximal phalanges, right 3 rd medial phalanx.	Fusion at 14–21 years old
Right ischium	Fused to main body of pelvis	Fusion at 17–25 years old
Feet	Recently fused epiphyses: left 2 nd metatarsal, left 1 st proximal phalanx, left 1 st distal phalanx. Unfused epiphyses: left 1 st , 3 rd and 5 th metatarsals, right 2 nd –4 th metatarsals, left 2 nd –5 th proximal phalanges, right 3 rd proximal phalanx.	Fusion at 12–22 years old

The individual is mature enough to be treated as an adult and on that basis its sex may be determined as female, from the shape of the greater sciatic of the pelvis, the gracile status of the cranium, and the small and gracile status of the mandible. The right sciatic notch angle is ~80°, which is high within the female range (Figure 3.2 in ref. 32). Its visually assessed shape is “2” of the system published in ref. 33, which is also feminine. Its measured width of ~50.2 mm compares better with the Australian Aboriginal female average of 50.9 (range 42–60) mm than the male average of 45.2 (range 37–55) mm. In addition, the acetabulum vertical diameter, measured as 44.8 mm left and right, albeit exaggerated from horizontal compression of the pelvis, is central within the Australian Aboriginal female range of 41–50 mm (average 45.9 mm) but falls barely within the male range of 45–58 mm for this group³⁴.

With regards to the Leang Panninge cranium, four of the seven sexually dimorphic characters highlighted by ref. 35 for sexing Aboriginal Australians are represented. These are: slight (right) zygomatic trigone, absent (left) malar tuberosity, small (right) mastoid process with an estimated module of 17.6, and small development of the occipital markings. Leang Panninge scores 4 out of a possible range of 4–12, where a low score represents a female status.

Concerning the mandible, the weight of observations is consistent with a female status. These include bilaterally inverted gonias, with a narrow estimated bigonial breadth <91 mm, a small symphysis height <30 mm, a bilaterally slight anterior marginal tubercle, and a *sulcus intertoralis* that is slight on the right side and medium on the left side. One masculine feature, however, is a broad left ramus with a minimum ramus breadth >33 mm, producing an overall score of 8.5/18, which, if extrapolated to all 11 sexually dimorphic features in ref. 36, would equate to a total score of 15.6, central within the female Australian Aboriginal range and below the male range of 17–30. Another masculine feature is flexure of the left ramus below the occlusal line³⁷.

In sum, the Leang Panninge skeletal remains are generally consistent with a small and female individual, as will become clear in the description that follows.

Dentition

The tooth sizes of the Leang Panninge individual can be compared with those recorded by DB for the Lake Towuti region of central Sulawesi, including Gua Mo'o hono (mid-Holocene) and the 134 teeth from second millennium CE burials from Gua Lampetia, Gua Andomo and Gua Sambagoala (SI Tables 4 and 5). The Leang Panninge teeth either show no occlusal wear (Smith's stage 1—see ref. 31), enamel polishing or at most slight exposure of the dentine (Smith's stages 2–3), and so their diameters are not affected by interstitial wear. Nonetheless, for the purposes of full documentation, the Leang Panninge tooth diameters were also measured at the cemento-enamel junction, as presented in **Additional observations on the teeth** (see below).

The Leang Panninge teeth are generally small. Their diameters fall below the Lake Towuti range for the second upper incisor and lower canine, and for the length of the third upper molar and breadths of the first upper incisor and first lower incisor. On other comparisons, except the second lower incisor length, Leang Panninge falls within the Lake Towuti range.

SI Table 4: Maximum mesio-distal diameters (mm) of the Leang Panninge teeth, with prehistoric Sulawesi comparisons

	Leang Panninge	Gua Mo'o hono	Gua Lampetia/Gua Andomo/ Gua Sambagoala

Tooth	Left	Right	Available data	Average (range)
First upper incisor	–	8.6	–	8.2 (7.3–9.3)
Second upper incisor	–	6.0	–	7.1 (6.1–8.6)
Upper canine	7.2	–	–	7.8 (7.1–8.3)
First upper premolar	–	7.8	–	7.3 (6.7–8.2)
Second upper premolar	7.35	–	8.5	7.0 (6.5–7.6)
First upper molar	10.6	–	–	10.4 (9.5–11.4)
Second upper molar	9.5	9.65	–	9.7 (9.0–10.7)
Third upper molar	8.5	9.1	–	9.8 (9.5–10.0)
First lower incisor	5.5	5.5	–	5.8 (4.9–6.3)
Second lower incisor	6.1	6.2	–	5.4 (5.1–6.0)
Lower canine	6.7	6.2	–	7.8 (7.1–8.3)
First lower premolar	7.2	–	–	7.2 (6.8–7.9)
Second lower premolar	7.0	7.6	–	7.2 (6.2–8.5)
First lower molar	10.9	11.5	–	11.5 (10.9–12.1)
Second lower molar	11.1	11.6	11.0	11.2 (10.0–11.7)
Third lower molar	10.7	10.8	–	10.9 (10.7, 11.1)

SI Table 5: Maximum bucco-lingual diameters (mm) of the Leang Panninge teeth, with prehistoric Sulawesi comparisons

	Leang Panninge		Gua Mo'o hono	Gua Lampetia/Gua Andomo/Sambagoala
Tooth	Left	Right	Available data	Average (range)
First upper incisor	–	6.9	–	7.4 (7.0–7.6)
Second upper incisor	–	5.9	–	6.7 (6.1–7.0)
Upper canine	8.2	–	–	8.2 (6.6–9.5)
First upper premolar	–	9.8	–	9.2 (7.3–10.4)
Second upper premolar	9.1	–	9.3	9.5 (9.1–10.5)
First upper molar	11.4	–	–	11.7 (10.6–12.6)
Second upper molar	11.5	11.5	–	11.6 (10.9–13.4)
Third upper molar	11.3	11.3	–	11.5 (11.0–12.4)
First lower incisor	5.6	5.5	–	6.4 (5.9–7.6)

Second lower incisor	5.7	5.8	–	6.0 (5.5–6.5)
Lower canine	7.3	6.7	–	8.2 (7.6–8.5)
First lower premolar	8.5	–	–	8.3 (7.6–9.2)
Second lower premolar	8.1	8.4	–	8.0 (6.8–9.0)
First lower molar	10.8	10.9	–	10.5 (10.0–10.9)
Second lower molar	10.0	10.3	9.3, 11.0	10.2 (9.7–10.6)
Third lower molar	9.9	10.4	–	10.4 (10.2, 10.5)

Overall tooth size can be summarised by multiplying the average lengths and breadths of each of the 32 teeth and summing the products to calculate overall tooth area³⁸, applying this method to Asia-Pacific populations of determined sex (SI Table 6). Unsurprisingly, in any given population, males consistently have larger tooth area than females. Also, as expected, Aboriginal Australians have the largest teeth, after taking sex into account. Melanesians however have widely variable tooth area, from populations equivalent to Aboriginal Australians (New Guinea Highlanders) to populations with a tooth area that resembles that of the smallest-toothed Indo-Malaysian populations (coastal Papuan Motupore). Motupore males in fact have a tooth area very similar to that of Leang Panninge, along with Semang “Negrito” and Surabaya males. The closest female populations to Leang Panninge in summed tooth area are the Temiar Senoi and Semang, both small-bodied populations of the Malay Peninsula hinterland⁴⁴. The most informative comparison would probably be the Semang because, unlike the other populations but as inferred for Leang Panninge, they traditionally had a foraging economy.

SI Table 6: Summed tooth area (mm²) of the Leang Panninge teeth, with comparisons

Region	Sex	Population	Tooth area	Source
Australia	Male	Western Australia	1449	ref. 39
Australia	Male	Euston. Murray Valley	1443	ref. 40
Melanesia	Male	New Guinea Highlands	1443	ref. 41
Australia	Male	Walbiri	1436	ref. 42
Melanesia	Male	Nasioi, Bougainville	1362	ref. 42
Australia	Female	Euston. Murray Valley	1361	ref. 40

Melanesia	Male	New Britain	1359	ref. 43
Australia	Female	Walbiri	1358	ref. 42
Australia	Female	Western Australia	1291	ref. 39
Indo-Malaysia	Male	Temiar Senoi	1290	ref. 44
Indo-Malaysia	Male	Temuan Aboriginal Malays	1288	ref. 44
Indo-Malaysia	Male	Semelai Aboriginal Malays	1279	ref. 44
Indo-Malaysia	Male	Batawi (Java)	1272	ref. 42
Melanesia	Female	New Britain	1267	ref. 43
Melanesia	Female	Nasioi, Bougainville	1253	ref. 42
Indo-Malaysia	Male	Malays	1238	ref. 44
Indo-Malaysia	Female	Temiar Senoi	1219	ref. 44
Melanesia	Male	Motupore, coastal Papua	1218	ref. 40
Indo-Malaysia	Male	Semang Negritos	1209	ref. 44
<i>Toalean</i>	<i>Female</i>	<i>Leang Panninge</i>	<i>1206</i>	<i>This paper</i>
Indo-Malaysia	Male	Surabaya, Java	1193	ref. 43
Indo-Malaysia	Female	Semang Negritos	1189	ref. 44
Indo-Malaysia	Female	Temuan Aboriginal Malays	1170	ref. 44
Indo-Malaysia	Female	Batawi (Java)	1158	ref. 42
Indo-Malaysia	Female	Semelai Aboriginal Malays	1136	ref. 44
Melanesia	Female	Motupore, coastal Papua	1132	ref. 40

Macroscopic enamel hypoplasia was recorded for a considerable proportion of the Leang Panninge teeth. Ref. 31 recommends a system of assigning the observed defects to broad developmental categories, without making assumptions about the exact age at which the teeth developed. Consistency of hypoplastic events across the different tooth categories supports the inference that the individual experienced a generalised interruption to growth, whereas inconsistency would be better assigned to localised effects at the specific tooth sites observed. Based on this approach (SI Tables 7 to 11), the strongest evidence is for a “D” event. Although the evidence is not entirely consistent (SI Table 9), the right lower canine could also be included if we allowed for the defect recorded at its occlusal part of the crown, as could the right upper second molar and left lower second molar if we allowed for the defects recorded at their intermediate part of the crown.

SI Table 7: Evidence for A–B events as reflected in macroscopic enamel hypoplasia, following the system of ref. 31 (Table 6.3).

Event type	Right M ¹	Left M ₁	Right M ₁	Right I ¹	Left I ₁	Right I ₁	Left I ₂	Right I ₂
A	O X	O X	O X	O √	O X	O X		
B	I X	I √	I √	I √	I X	I X	O X	O X

Key: M¹, upper first molar; M₁, lower first molar; I¹, upper first incisor; I₁, lower first incisor; I₂, lower second incisor; O, occlusal part of crown side; I, intermediate part of crown side; √, present; X, absent.

SI Table 8: Evidence for a C event as reflected in macroscopic enamel hypoplasia, following the system of ref. 31 (Table 6.3).

Right M ¹	Left M ₁	Right M ₁	Right I ¹	Left I ₁	Right I ₁	Left I ₂	Right I ₂	Left <u>C</u>	Left $\hat{C}$	Right $\hat{C}$
C √	C X	C X	I √	I X	I X	I X	I X	O √	O X	O √

Key: C, upper canine; $\hat{C}$, lower canine; C, cervical part of crown side; other symbols as per SI Table 7.

SI Table 9: Evidence for a D event as reflected in macroscopic enamel hypoplasia, following the system of ref. 31 (Table 6.3).

R I ¹	L I ₁	R I ₁	L I ₂	R I ₂	L <u>C</u>	L $\hat{C}$	R $\hat{C}$	R I ²	R P ¹	L P ²	L P ₁	L P ₂	R P ₂	L M ²	R M ²	L M ₂	R M ₂
C √	C √	C √	C √	C √	I √	I √	I X	O √	O √	O X	O X	O X	O X	O X	O X	O X	O X

Key: R, right; L, left; I², upper second incisor; P¹, upper first premolar; P², upper second premolar; P₁, lower first premolar; P₂, lower second premolar; M², upper second molar; M₂, lower second molar; other symbols as per SI Tables 7 and 8.

SI Table 10: Evidence for E–F events as reflected in macroscopic enamel hypoplasia, following the system of ref. 31 (Table 6.3).

Event type	L <u>C</u>	L $\hat{C}$	R $\hat{C}$	R I ²	R P ¹	L P ²	L P ₁	L P ₂	R P ₂	L M ²	R M ²	L M ₂	R M ₂
E	I √	I √	I X	C X	I √	I X	I X	I X	I X	I X	I √	I √	I X
F	C X	C X	C X		C X	C X	C X	C X	C X	C X	C X	C X	C X

Key: symbols as per SI Table 9.

SI Table 11: Evidence for a G event as reflected in macroscopic enamel hypoplasia, following the system of ref. 31 (Table 6.3).

Left upper third molar	Right upper third molar	Left lower third molar	Right lower third molar
O X, I √, C X	O √, I X, C X	OIC X	OIC X

Key: symbols as per SI Table 9.

A “D” event corresponds to a developmental age of three to four years old, and may have been caused by weaning at that age when the Leang Panninge individual was no longer protected by antibodies from breast milk.

Numerous observations on the dental morphology of the Leang Panninge individual were possible, and these are presented in **Additional observations on the teeth** (see below). Leang Panninge’s expressions for focus tooth traits⁴⁵ are presented in SI Table 12, along with how Leang Panninge compares with Sahul-Pacific (Indigenous Australians, New Guineans/Papuans, and Melanesians), Sunda-Pacific (prehistoric and recent Southeast Asians, Polynesians, and Micronesians) and Sino-American populations. Some traits, such as 2-rooted lower canines and premolar odontomes, are rare for any population. However, if we focus on the traits for which there is an appreciable difference between the three population complexes, we see the most agreement overall with Sahul-Pacific populations (most similar for 12/18 comparisons) rather than Sunda-Pacific or Sino-American populations (both most similar for 3/18 comparisons).

SI Table 12: Focus dental morphology traits⁴⁵ and proportions of circum-Pacific populations that have the same expression as Leang Panninge.

Trait	Leang Panninge		Sahul-Pacific populations	Sunda-Pacific populations	Sino-American populations*
	Left	Right			
Upper central incisor shovelling	—	< grade 3	80–100%	65–78%	5–62%

Upper central incisor double-shovelling	–	< grade 2	95–100%	83–95%	30–80%
Upper second incisor interruption grooves	–	Absent	82–84%	61–75%	34–57%
Trait	Leang Panninge		Sahul-Pacific populations	Sunda-Pacific populations	Sino-American populations*
	Left	Right			
Upper canine mesial ridge	Absent	–	97–98%	94–97%	92–100%
Lower canine 2-rooted	Not observable	1-rooted	100%	98–99%	97–100%
Upper first premolar 2-rooted	–	Present	30–46%	34–56%	5–32%
Lower first premolar 2-rooted	Absent	–	73–93%	78–84%	80–96%
Premolar odontomes	Absent	Absent	97–100%	97–99%	93–99%
Upper first molar Carabelli's trait	< grade 5	–	77–82%	76–82%	83–96%
Upper first molar cusp 5	Present	–	44–63%	29–43%	12–28%
Upper first molar enamel extension	Absent	–	89–95%	64–92%	45–72%
Upper second molar hypocone	Absent grade 1	Present grade 3	Present 93–96%	Present 85–92%	Present 68–88%
Upper second molar 3-rooted	Present	Present	56–80%	50–77%	40–70%
Lower first molar hypoconulid	Present	Present	96–99%	98–100%	97–100%
Lower first molar cusp 6	Absent	Absent	38–83%	48–67%	43–80%

Lower first molar cusp 7	Absent	Absent	88–95%	93–94%	90–95%
Lower first molar deflecting wrinkle	Absent	Absent	82–96%	77–84%	<i>60–85%</i>
Lower first molar distal trigonid crest	Absent	Absent	96–100%	90–96%	<i>82–96%</i>
Lower first molar 3-rooted	Not observable	Absent	95–100%	86–97%	<i>70–97%</i>
Lower second molar hypoconulid	Present	Absent	Present 10–60%	Present 20–35%	Present 5–55%
Lower second molar Y-groove pattern	Present	Present	23–40%	19–23%	<i>10–23%</i>
Lower second lower molar 1-rooted	Not observable	Absent	84–94%	68–84%	<i>37–68%</i>

*Excluding the prehistoric Jōmon of Japan, whose dental morphological profile resembles that of Sunda-Pacific populations.

Note: Most similar shown bold, least similar shown italicised, except where the Leang Panninge expression is asymmetrical.

Similarities to the Leang Panninge individual can be shown with a simple concordance statistic, which is the average of the percentage similarities for the traits in SI Table 12 where Leang Panninge does not have an asymmetric expression. In descending order to whole numbers, the results are: New Guinea 84%; Australia 81%; Melanesia 80%; Micronesia 78%; Jōmon 77%; prehistoric Southeast Asia, Polynesia 75%; recent Southeast Asia 74%; South Siberia 72%; recent Japan, China-Mongolia 67%; Northeast Siberia 65%; and American populations the least similar (e.g., “North and South America” 60%). These results confirm the inference that the Leang Panninge dental morphology is most similar to that of Sahul-Pacific populations, with the further indication of a greater similarity to Sunda-Pacific/Jōmon than to Sino-American populations.

Cranium

Reconstruction of the cranium proceeded as far as the right/median frontal, left zygomatic region, right and left temporal regions, right parietal, median occipital and left occipital condyle. Further joins could not be found and the general size and shape of the cranium is unknown. The recorded total weight of 78 grams of cranial bone and maxillary teeth (SI Table 2) excludes the right petrous portion of the tympanic, which had been collected for ancient DNA analysis. Italicised character states in the description below are those recommended⁴⁶ for distinguishing Aboriginal Australians from other populations.

In addition to the *slight* (right) zygomatic trigone, noted above, the frontal bone has a slight frontal crest on the right side, strong frontal bosses and *absence* of the median frontal ridge. As reconstructed, the frontal bone is broad anteriorly, with fronto-malar breadth of ref. 47 estimated at 107 mm, and so the maximum supraorbital breadth would be *large* (greater than 108 mm). Cranial bone thickness is 4.7 mm at the left frontal boss and 5.2 mm at the right. In addition to the absence of a malar tuberosity, noted above, the left malar bone has an orbital margin that is trending towards being *distinctly rounded*, while the size of the marginal process of the malar frontal process measures 4.4 mm or *large*. The maximum cheek height (as extant) of ref. 47 is 13 mm. The external auditory meatus on the right temporal bone slants strongly postero-inferiorly, with a height of 17 mm and a width of 8 mm. The development of the supramastoid crest is *slight*. There are no useful additional observations for the left temporal region. The right parietal fragment shows only *slight* development of the parietal boss, where the thickness of the cranial bone is 5.2 mm. In addition to the gracile occipital markings, noted above, there is merely *trace* development of the transverse occipital torus, while the external occipital protuberance is *absent*. There are no useful additional observations for the occipital condyle.

The methodology of ref. 46 assigns scores of 0, 1, 2 and 3 to their characters (italicised above) depending on their state. To see how the available comparative data on Aboriginal Australian, Melanesian and Indo-Malaysian crania would assist the classification of Leang Panninge, the following procedure was followed:

1. Find all of the female crania scored for all of the characters scored for Leang Panninge
2. Focus on the female crania with a similar aggregate score to Leang Panninge (in practice, with a total difference of either 3 or 4 from Leang Panninge)

3. Focus on the characters for which the focus female crania either score identically with Leang Panninge (zygomatic trigone, orbital border rounding) or differ by at most one grade (median frontal ridge, parietal bossing, external occipital protuberance, supramastoid crest)
4. Accept a match with Leang Panninge for all female crania scored for these six characters whose expressions fall within the bounds described in (3) above
5. To extend the comparisons to include male crania, drop the requirement on zygomatic trigone, which is one of the characters used for sexing between males and females³⁵.

SI Table 13: Proportions of Australian Aboriginal, Melanesian and Indo-Malaysian crania whose morphology matches Leang Panninge

Formula for match with Leang Panninge	Sex	Australia ^(a)	Melanesia ^(b)	Indo-Malaysia ^(c)
Zygomatic trigone = 1 AND orbital border rounding = 3 AND median frontal ridge $\leq$ 1 AND parietal bossing $\geq$ 2 AND external occipital protuberance $\geq$ 1 AND supramastoid crest $\geq$ 2	Females	31/92 (34%)	55/186 (30%)	6/43 (14%)
Orbital border rounding = 3 AND median frontal ridge $\leq$ 1 AND parietal bossing $\geq$ 2 AND external occipital protuberance $\geq$ 1 AND supramastoid crest $\geq$ 2	Both	51/92 (F), 27/130 (M), 78/222 (35%)	69/187 (F), 177/524 (M), 246/711 (35%)	6/43 (F), 12/119 (M), 18/162 (11%)

(a) Coastal New South Wales and Queensland, recorded by Stanley Larnach

(b) Papua New Guinea⁴⁸, Solomon Islands⁴², New Britain, Vanuatu and New Caledonia (DB laboratory notes)

(c) Java, Nicobar Islands (recorded by J. Kamminga), Singapore Malays (recorded by D. Rayner), Borneo, Sulawesi, southeastern Indonesia including Melolo (DB laboratory notes)

As shown in SI Table 13, between 30–35% of Aboriginal Australian and Melanesian crania match Leang Panninge in their morphology, whereas the proportion for Indo-Malaysian crania is much lower (11–14%). Thus, although Leang Panninge does not lie outside the recent Indo-Malaysian range of variation in its cranial morphology, it more closely matches southwest Pacific populations in this regard.

Mandible

The observations recorded on the Leang Panninge mandible are detailed in **Detailed observations on the mandible** (see below). Detailed biometric analysis lies beyond the scope of this report. The mandible is U-shaped rather than V-shaped from above and below. The mandible's small size can be shown by comparing its measurements with the means published⁴⁹ for two English cemetery series, Punjabi Indians (males only) and Aboriginal Australians. For three of the seven comparative measurements, Leang Panninge is smaller than the female range of means, and for another two measurements, it falls within the female (as well as the male) range of means (SI Table 14).

SI Table 14: Comparison of Leang Panninge measurements (mm) with the means published in ref. 49.

Measurement*	Leang Panninge	Male range of means	Female range of means
	Leang Panninge smaller than female range of means		
gogo	~71.4	92.8–97.7	85.7–87.7
h1	26.5	30.9–33.3	28.9–30.8
m2h	22.2 left, 22.9 right	24.9–26.4	23.4–24.5
	Leang Panninge in overlap between male and female ranges of means		
cpl	74	74.0–83.2	67.7–75.9
zz	~45	43.9–47.4	42.9–45.8
	Leang Panninge within male range of means		
m2pl	31.3 left, 30.1 right	28.0–30.4	26.3–29.2
	Leang Panninge larger than male range of means		
rb'	36 left	30.9–34.3	28.3–31.6

*For explanation of acronyms, refer to **Detailed observations on the mandible** (see below).

Ref. 36 recommends a list of 12 anatomical characters for distinguishing between Australian Aboriginal and mainland East Asian mandibles. Ten of these can be observed for Leang Panninge, with their scores as detailed in **Detailed observations on the mandible** (see below). Use of these characters to assist classification of Leang Panninge followed a similar method as that used for crania:

1. Find all of the mandibles scored for all of the characters scored for Leang Panninge
2. Focus on the mandibles with a similar aggregate score to Leang Panninge (in practice, with a total difference of either 2 or 3 from Leang Panninge)
3. Focus on the characters for which the focus mandibles differ by at most one grade (*sulcus extramolaris*, lateral prominence, anterior mandibular incurvature, chin projection, *planum alveolare* decline, lingula development)
4. Accept a match with Leang Panninge for all mandibles scored for these six characters whose expressions fall within the bounds described in (3) above (SI Table 15).

SI Table 15: Proportions of Australian Aboriginal, Melanesian and Indo-Malaysian mandibles whose morphology matches Leang Panninge

Formula for match with Leang Panninge	Sex	Australia ^(a)	Melanesia ^(b)	Indo-Malaysia ^(c)
<i>Sulcus extramolaris</i> ≤ 2 AND lateral prominence ≥ 2 AND anterior mandibular incurvature ≥ 2 AND chin projection ≥ 2 AND <i>planum alveolare</i> decline ≥ 2 AND lingula > 2	Both	25/78 (32%)	19/91 (21%)	3/36 (8%)

(a) Coastal New South Wales and Queensland males, recorded by S. Larnach (individual records for female mandibles could not be found in his unpublished papers)

(b) Papua New Guinea, Solomon Islands, New Britain, Vanuatu and New Caledonia (DB laboratory notes)

(c) Talaud Islands⁴², Singapore Malays (recorded by D. Rayner), Kuala Selinsing, Sulawesi, southeastern Indonesia including Golo and Melolo (DB laboratory notes)

Around one-third of specimens classified as Aboriginal Australians and one-fifth of Melanesians have a mandibular morphology that matches Leang Panninge. This, however, is the case for less than one-tenth of Indo-Malaysian mandibles, again indicating that Leang Panninge does not lie outside of the recent Indo-Malaysian range of variation, but is more compatible with a southwest Pacific affinity.

Vertebral column

The vertebral column (above the pelvic girdle) is represented by a fragmentary axis vertebra and seven other fragments from the third to seventh cervical vertebrae, a seventh thoracic vertebra fragment and a process fragment to another thoracic vertebra, and a lumbar vertebra

fragment. None of these fragments are complete enough to provide information on the size or shape of the original vertebrae. In summary, the entire vertebral column (above the pelvic girdle) is sparsely represented, better so for the neck than the back.

Ribs

The ribcage is represented by fragments of the left second to fourth ribs and sixth to seventh ribs, and the right fourth to seventh ribs. None of these fragments are complete enough to provide information on the size or morphology of the original ribs. In summary, much of the ribcage is represented but in a very fragmentary manner.

Upper appendicular skeleton

The right clavicle is represented by two joining fragments that extend medially from the conoid tubercle to the mid-shaft. The extant measurements defined by ref. 50, compared with the same ranges measured for Australian Aboriginal right female clavicles, are a minimum width of 9.2 mm (Aboriginal Australian range 8–13 mm), a height at the midpoint of 7.1 mm (Aboriginal Australian range of 7–17mm), a width at the conoid of 10.7 mm (Aboriginal Australian range of 10–20 mm), and a height at the conoid of 10.8 mm (Aboriginal Australian range of 8–14 mm). The Leang Panninge measurements all fall within the Australian Aboriginal female range but towards the lower end. The right scapula is represented by two fragments joining to make the inferior angle, whose suture is unfused with the epiphysis. The left scapula is represented by the junction between the acromion and the blade. None of these fragments are complete enough to provide information on the size or morphology of the original elements. The right radius is represented by three joining fragment to the distal shaft and the epiphysis, which can be joined to the shaft along the unfused line of epiphyseal union. No measurements are available.

The right hand is represented by a cluster of bones that included the trapezium, the second metacarpal with the head recently fused to the distal shaft, the third metacarpal with a recently fused head, the fourth metacarpal with an unfused head, a shaft fragment to the fifth metacarpal, the third proximal manual phalanx with an unfused base, the fourth proximal manual phalanx distal shaft and head, the fifth proximal manual phalanx base and head, a shaft fragment to the second medial manual phalanx, a complete fifth medial manual phalanx with a fused base, and a complete distal manual phalanx with a fused base. A fragmentary capitate was also found with this cluster of bones, and although its morphology strongly

suggests it was a left capitate, it is more likely to be the right capitate based on its association. The length of the fifth medial manual phalanx is 19.7 mm, which is shorter than the average of 21.1 mm recorded for Australian Aboriginal males⁵¹. The length of the first distal manual phalanx is 20.5 mm, which is shorter than the average of 21.5 mm recorded for Australian Aboriginal males⁵¹.

The left hand is represented by the first distal manual phalanx, complete with a fused base, and fragmentary third to fifth metacarpals with unfused heads. Measuring 21.7 mm, the length of the first distal manual phalanx approximates the average for Australian Aboriginal males⁵¹.

Pelvic girdle

Both the right and left pelves are represented by their acetabulum and the surrounding bone. In both cases, lateral compression has exaggerated the measurable, vertical diameter of the acetabulum, and so the recorded measurements (44.8 mm on both sides) are a maximum estimate. These measurements strongly suggest a female status, by the standards of Aboriginal Australians, because their female average is 45.9 mm and their male range is 45–58 mm³⁴. Other measurements taken on the right pelvis are 27.5 for the acetabulum horizontal diameter, 20.9 mm for the greatest depth of the greater sciatic notch, and 50.2 mm for the greatest width of the greater sciatic notch. Lateral compression means that the measurement for the acetabulum horizontal diameter is a gross underestimate, as can be seen by comparing it with the Australian Aboriginal female (40–50 mm) and male (45–57 mm) ranges. The greatest depth of the greater sciatic notch falls within the range of overlap between Australian Aboriginal female (21–34 mm) and male (18–31 mm) ranges. On the other hand, the greatest width of the greater sciatic notch is very close to the average for female Aboriginal Australians (50.86 mm) but barely within the range for males (37–55 mm).

The pelvic arcuate line is moderately developed bilaterally. The extant ischium is moderately developed on the right side but gracile on the left side. The fragmentary sacrum is represented by its right upper lateral area, part of its median sacral crest joined to the intermediate sacral crest, the disc to the fourth sacral vertebra joined to the right sacral corna, and the first coccygeal vertebra joined to the cornea. The available fragments suggest a small sacrum even though no measurements are available.

Lower appendicular skeleton

The right leg is represented by two joining fragments tentatively assigned to the distal tibia, and the left leg by a compressed fragment of the distal fibula. The left foot is represented by the cuboid and an articulating navicular fragment, all of the metatarsals and proximal pedal phalanges, and the first distal phalanx. Where measurable, the lengths of the Leang Panninge metatarsals and pedal phalanges generally approximate the Australian Aboriginal male averages but in some cases are about 3 mm shorter (SI Table 16). The right foot is represented by the right second metatarsal missing its unfused head, the right fourth metatarsal which is complete after being joined to its unfused head, the right third proximal phalanx missing its unfused base, and three fragments to the shaft of another proximal pedal phalanx. The length of the right fourth metatarsal (60.3 mm) is short compared to the average length of 65.8 mm recorded for Australian Aboriginal males⁵¹.

SI Table 16: Details of the left metatarsals and pedal phalanges

Element	Extant element	Length (mm)	Australian Aboriginal male average (mm)
First metatarsal	Unfused distal epiphysis	–	N/A
Second metatarsal	Semi-complete, recently fused head	72.2	72.0
Third metatarsal	Semi-complete, unfused head	–	N/A
Fourth metatarsal	Shaft fragment	–	N/A
Fifth metatarsal	Semi-complete, unfused head	–	N/A
First proximal phalanx	Complete, recently fused base	27.5	30.5
Second proximal phalanx	Complete, unfused base	25.3	28.1
Third proximal phalanx	Semi-complete, unfused base	25.2	25.5
Fourth proximal phalanx	Complete except for unfused base	–	N/A
Fifth proximal phalanx	Complete, unfused base	21.7	21.6
First distal phalanx	Complete, recently fused base	21.8	22.3

Note: Australian male Aboriginal averages from ref. 51.

Summary

The Leang Panninge skeleton represents an adult female of about 17–18 years old at the time of death. Her growth as a child appears to have been interrupted at around 3–4 years old, potentially corresponding to her age of weaning. There are no osteological markers of her cause of death. Cranial, mandibular and dental morphology do not lie outside of the range of recent Indo-Malaysian variation but are considerably more compatible with the morphology of southwest Pacific populations in Indigenous Australia and Melanesia. Tooth size is small, as also found for coastal Melanesians and the Semang Negritos of Peninsular Malaysia.

Additional observations on the teeth

Diameters at the cemento-enamel junction

Mesio-distal diameters (mm) at the cemento-enamel junction of the Leang Panninge teeth, with prehistoric Sulawesi comparisons

	Leang Panninge		Gua Mo'o hono	Gua Lampetia/Gua Andomo
Tooth	Left	Right	Available data	Average (range)
First upper incisor	—	5.8	—	6.4 (5.2–7.3)
Second upper incisor	—	5.3	—	6.8 (6.3–7.0)
Upper canine	5.1	—	—	6.2 (5.3–7.2)
First upper premolar	—	5.0	—	5.9 (4.7–6.9)
Second upper premolar	4.2	—	—	5.4 (4.9–6.5)
First upper molar	7.7	—	9.6	8.6 (8.0–9.5)
Second upper molar	6.9	7.3	9.5	8.3 (7.0–9.6)
Third upper molar	7.6	7.1	—	9.0 (8.9–9.1)
First lower incisor	3.4	3.4	5.4	4.3 (3.8–4.8)
Second lower incisor	4.4	4.1	5.1	4.4 (3.5–5.1)
Lower canine	4.9	4.4	—	6.4 (5.5–7.9)
First lower premolar	5.1	—	—	5.9 (5.2–6.3)
Second lower premolar	6.8	6.4	—	5.7 (5.1–7.6)
First lower molar	8.8	9.1	9.5	10.4 (9.5–10.9)
Second lower molar	9.7	9.4	9.7–10.0	9.9 (9.2–10.7)
Third lower molar	8.9	8.5	9.4	9.5 (8.9, 10.1)

Bucco-lingual diameters (mm) at the cemento-enamel junction of the Leang Panninge teeth, with prehistoric Sulawesi comparisons

	Leang Panninge		Gua Mo'o hono	Gua Lampetia/Gua Andomo
Tooth	Left	Right	Available data	Average (range)
First upper incisor	—	6.0	—	7.0 (6.5–7.5)
Second upper incisor	—	5.7	—	6.5 (5.8–6.8)
Upper canine	7.8	—	—	7.9 (7.0–8.7)
First upper premolar	—	8.4	—	8.6 (7.3–9.7)
Second upper premolar	8.1	—	—	9.1 (8.2–9.9)
First upper molar	10.4	—	10.4	11.3 (10.3–12.4)
Second upper molar	10.35	10.4	9.7	11.0 (10.0–13.0)
Third upper molar	10.4	9.9	—	11.3 (10.8–12.2)
First lower incisor	5.6	5.5	7.8	6.3 (5.6–7.6)
Second lower incisor	5.6	5.6	7.4	5.9 (5.5–6.6)
Lower canine	7.1	5.8	—	8.2 (7.5–8.4)
First lower premolar	7.5	—	—	7.8 (7.0–8.4)
Second lower premolar	6.6	7.7	—	7.7 (7.3–8.5)
First lower molar	9.8	9.7	9.1	9.8 (9.1–10.6)
Second lower molar	8.7	9.4	8.5–9.6	9.6 (8.5–10.5)
Third lower molar	8.9	9.5	—	9.2 (8.4, 9.9)

Detailed dental morphology (following ref. 45)

Right upper central incisor: shovelling ASU 1, double-shovelling ASU 0, slight tuberculum dentale, no interruption grooves

Right upper lateral incisor: shovelling ASU 0, double-shovelling ASU 0, slight tuberculum dentale, no interruption grooves, normal variant

Left upper canine: buccal shovelling ASU 1, slight tuberculum dentale, no interruption grooves, no mesial canine ridge, distal accessory ridge ASU 2, 1-rooted

Right upper first premolar: no distal accessory ridges, no accessory marginal tubercle, no odontome, distal accessory ridge present (faint), 2-rooted

Left upper second premolar: no distal accessory ridges, no accessory marginal tubercle, no odontome, distal accessory ridge absent, 1-rooted

Left upper first molar: hypocone ASU 4, Carabelli's trait ASU 0, cusp 5 ASU 1, no mesial marginal accessory tubercle, no parastyle, no enamel extension, 3-rooted

Left upper second molar: hypocone ASU 1, Carabelli's trait ASU 0, cusp 5 ASU 0, no mesial marginal accessory tubercle, no parastyle, no enamel extension, 3-rooted

Right upper second molar: hypocone ASU 3, Carabelli's trait ASU 0, cusp 5 ASU 3, no mesial marginal accessory tubercle, no parastyle, no enamel extension, 3-rooted

Left upper third molar: hypocone ASU 2, Carabelli's trait ASU 0, cusp 5 ASU 3, no mesial marginal accessory tubercle, no parastyle, slight enamel extension buccally and lingually, 3-rooted

Right upper third molar: hypocone ASU 3, Carabelli's trait ASU 0, cusp 5 ASU 0, mesial marginal accessory tubercle present, no parastyle, no enamel extension, 3-rooted

Left lower central incisor: absent shovelling, absent winging

Right lower central incisor: absent shovelling, absent winging

Left lower lateral incisor: absent shovelling

Right lower central incisor: absent shovelling

Left lower canine: shovelling ASU 0, distal accessory ridge ASU 0

Right lower canine: shovelling ASU 1, distal accessory ridge ASU 0, 1-rooted (recorded during reconstruction)

L lower first premolar: absent accessory ridge, absent odontome, multiple lingual cusps ASU 1, 1-rooted (recorded during reconstruction)

L lower second premolar: absent accessory ridge, absent odontome, multiple lingual cusps ASU 2, 1-rooted (recorded during reconstruction)

R lower second premolar: absent accessory ridge, multiple lingual cusps ASU 0, absent odontome

Left lower first molar: cusp 5 present, Y-pattern, cusps 6 and 7 absent, protostylid grade 1, absent deflecting wrinkle, present anterior fova, absent distal trigonal crest, medium lingual and slight buccal enamel extension

Right lower first molar: cusp 5 present, Y-pattern, cusps 6 and 7 absent, protostylid grade 1, absent deflecting wrinkle, present anterior fova, absent distal trigonal crest, slight lingual enamel extension, 2-rooted (recorded during reconstruction)

Left lower second molar: cusp 5 present (small), Y-pattern, cusps 6 and 7 absent, protostylid grade 2, present deflecting wrinkle, present anterior fova, absent distal trigonal crest, no enamel extension

Right lower second molar: cusp 5 absent, Y-pattern, cusps 6 and 7 absent, protostylid absent, absent deflecting wrinkle, absent anterior fova, absent distal trigonal crest, slight enamel extension buccally and lingually, 2-rooted (recorded during reconstruction)

Left lower third molar: 4-cusped molar even though wrinkled, cusp 5 absent, Y-pattern, cusps 6 and 7 absent, protostylid absent, present deflecting wrinkle, absent anterior fova, absent distal trigonal crest, slight lingual enamel extension, 2-rooted (recorded during reconstruction)

Right lower third molar: 4-cusped molar even though wrinkled, cusp 5 absent, Y-pattern, cusp 6 absent, cusp 7 present, protostylid absent, absent deflecting wrinkle, present anterior fova, present distal trigonal crest, slight lingual enamel extension (buccally cannot be observed), 2-rooted (recorded during reconstruction)

Detailed observations on the mandible

Measurements in mm

Defined in ref. 36

Bigonial width (w2 in the Biometric system): ~71.4

Symphysis height: 27.5

Minimum ramus breadth (rb' in the Biometric system): 36 (left)

Defined in the Biometric system⁴⁹

Bigonial width, respectively inner and outer, gogo, w2: ~71.4

Corpus length, cpl: 74

Gnathion–gonion length, gngo: 78.75 (right)

Chin height, h1: 26.5

Breadth between the mental foramina, zz: ~45

Corpus height at the second molar, m2h: 22.2 (left), 22.9 (right)

Corpus thickness at the second molar, m2t: 15.3 (left)

Chord length between the crown midpoints of the first premolar and second molar, m2p1: 31.3 (left), 30.1 (right)

Minimum ramus breadth, rb': 36 (left)

Defined in ref. 52

Symphysis thickness: 12

Corpus height at the junction between the first and second molars: 22.6 (left), 23.3 (right)

Corpus thickness at the junction between the first and second molars: 13.6 (left)

Chord length between the anterior point of the first premolar and posterior point of the second molar, M2P1 (crown interproximal points): 37.2 (left), 36.0 (right)

Other measurements

Corpus height at mental forâmen, M69(1) of ref. 53: 26.5 (right)

Corpus thickness at mental forâmen, M69(3) of ref. 53: 13.0 (right)

Interoblique breadth of ref. 54: 75.3

Anatomical observations following ref. 36

Traits for sexing

Gonial eversion: Inverted left, right (1)

Anterior marginal tubercle: Slight left, right (1)

Intertoral sulcus: Medium left, Slight right (1.5)

Traits for distinguishing Aboriginal Australians from East Asians

Sulcus extramolaris: Absent left (1)

Lateral prominence: Slight left, right (3)

Mental trigone development: Slight (3)

Anterior mandibular incurvature: Medium (2)

Chin projection: Negative (3)

Planum alveolare decline: Slight (2)

Precoronoid fossa: Shallow left (1)

Mylo-hyoid groove development: Medium left (2)

Lingula development: Slight left (3)

Mylo-hyoid ridge development: Medium right (2)

Other traits

Superior lateral torus: Absent left, right

Marginal torus: Medium left

Posterior marginal tubercle: Slight left, right
Mental foramen position: Between first and second lower premolars left, right
Mental foramen size: small R (1.6 mm)
Submental notch: Medium
Fossa mentalis: Absent left, right
Superior transverse torus: Medium left, right
Digastric fossa: Medium left, right
Trigonum basale: Medium
Sulcus praedigastricus: Faint
Fossa masseterica: Depressed left, right
Lateral ramus eminence: Slight left
Crista endocondyloidea: Medium right
Crista pharyngea: Absent left
Torus triangularis: Slight left
Sulcus colli: Medium left
Sublingual fossa: Slight left
Submaxillary fossa: Marked left
Mandibular torus: Absent left, right

Anatomical observations based on other sources

Non-rocker jaw⁵⁵
Genial pit⁵⁶: Present
Genial spines type⁵⁶: Small inferior tubercle
Genial grooves⁵⁶: Absent
Mental foramen number⁵⁶: single right
Mental foramen direction⁵⁶: lateral right
Mylo-hyoid bridge⁵⁷: Absent left, right

Ancient DNA processing

Sampling, extraction, library preparation and indexing were performed in a dedicated clean room for ancient DNA at the Max Planck Institute for the Science of Human History (MPI-SHH) in Jena, Germany.

Sampling and extraction

The petrous bone of the Leang Panninge individual was sampled by sawing along the margo superior partis petrosae (crista pyramidis) and drilling 56.5 mg of bone powder from the densest part around the cochlea⁵⁸. From the bone powder, DNA was extracted following a modified version of a previously published protocol⁵⁹. The sample was digested in a mixture of EDTA, UV-H₂O and Proteinase K for approximately 24 hrs while rotating in an incubator at 37°C. After centrifugation to remove the remaining bone material from solution, the supernatant was transferred into a binding buffer (GuHCl, UV-H₂O, Isopropanol) and then into a silica column (High Pure Viral Nucleic Acid Kit; Roche). This was washed twice using the High Pure Viral Nucleic Acid Kit wash buffer (Roche) and eluted in 100 µl TE-buffer containing 0.05% Tween. An extraction blank to check for cross- and background-contamination and a positive control were added to this step and processed in parallel with the sample.

Library preparation

From the resulting extract we built two libraries. Twenty-five µl were used to build a double-stranded library after partial Uracil DNA Glycosylase treatment⁶⁰. Illumina Adapters were ligated to the fragments using the Quick Ligation Kit (NBE) and the solution purified with a MinElute kit (QIAGEN). The DNA copy number of a 2 µl aliquot of the library was quantified by DyNAmo SYBR Green qPCR (Thermo Fisher Scientific) using IS7/IS8 primers on the LightCycler 480 (Roche⁶¹). Based on those values, the library was double-indexed with unique index combinations⁶² and amplified using Pfu Turbo DNA Polymerase (Agilent). It was then purified over MinElute columns, eluted in 50 µl TE-buffer containing 0.05% Tween and an aliquot quantified with IS5/IS6 primers using DyNAmo SYBR Green qPCR (Thermo Fisher Scientific) on the LightCycler 480 (Roche⁶¹). The remaining solution was further amplified with Herculase II Fusion DNA Polymerase (Agilent), purified again, quantified on the TapeStation (TapeStation Nucleic Acid System, Agilent 4200), and diluted to 10 nM for shotgun sequencing. Throughout library preparation a new blank as well as the extraction blank were processed in an identical manner to the sample. In addition to the

above-noted procedures, another 30 µl of extract was used to build a single-stranded library using the automated liquid handling system Bravo NGS Workstation (Agilent Technologies)⁶³.

Shotgun screening and in-solution enrichment

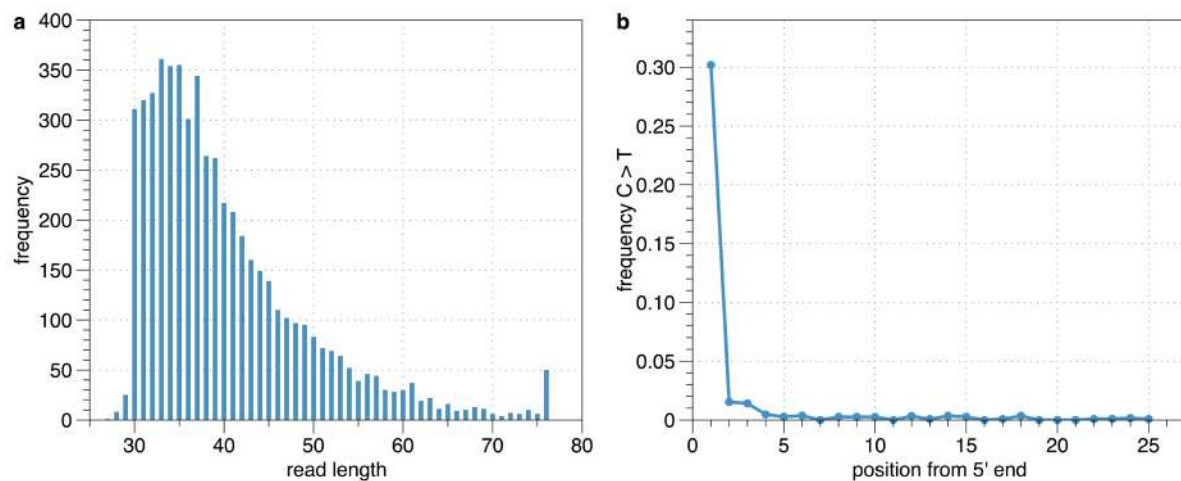
The prepared libraries were shotgun-sequenced for a depth of 3-5 million reads on an Illumina HiSeq 4000 using a 75 bp single-read configuration. The resulting reads were demultiplexed according to expected index combinations and subsequently filtered for lengths of more than 30 bp. They were then aligned to the human reference genome *hg19* with quality filter 30 and analysed with *EAGER* v.1.92.55 (ref. 64). The sample showed the damage pattern characteristic for ancient DNA (SI Fig. 1b) and an endogenous DNA content suitable for in-solution capture (0.305% for double-stranded, 0.925% for single-stranded). After further amplification with the IS5/IS6 primers, the libraries were hybridised in-solution to oligonucleotide probe sets (Agilent) in order to enrich for the complete mitogenome from the double-stranded library (mtDNA capture⁶⁵), and twice for a targeted set of 1,237,207 single nucleotide polymorphisms (SNPs) across the human genome (two rounds of “1240K” capture⁶⁶). The single-stranded library was additionally enriched for two rounds for a targeted set of 1,749,385 SNPs forming the “archaic ancestry” panel (Panel 4 in ref. 66). After sequencing the 1240K and archaic ancestry capture products to 23 and 22 million reads, respectively, and the mtDNA capture product to ~1 million reads on the Illumina HiSeq 4000 using a 75 bp single-read configuration, the reads were demultiplexed again and aligned to the human genome *hg19* (quality filter 30, seedlength 16500, -n 0.01) with *BWA*⁶⁷. Adapters and duplicates were removed with *AdapterRM/Merging* and *DeDup*, respectively, and damage calculated with *MapDamage* as implemented in *EAGER* v.1.92.56 (ref. 64). Overall the enrichment was successful, with a 95-fold for the double- and 552-fold increase for the single-stranded library in on-target endogenous DNA for the 1240K captured sequences compared to the shotgun results. To increase the SNP coverage for subsequent analyses, the 1240K-enriched double-stranded library was deeper-sequenced for a total of 120 million reads. The mtDNA-enriched library was also deeper-sequenced for a total of 8 million reads and aligned to the mitochondrial reference genome sequences (rCRS) with the *Circular Mapper* (seedlength 16500, -n 0.01, extended reference NC_012920.1, quality filter 30). Adapters and duplicates were removed with *AdapterRM/Merging* and *DeDup*, respectively, and damage calculated with *MapDamage* as implemented in *EAGER* v.1.92.56 (ref. 64). All sequences for each enriched library were then merged with *samtools* v.1.3

(<https://github.com/samtools/samtools>), duplicates removed using *DeDup* v.0.12.2 (<https://github.com/apeltzer/DeDup>), and the resulting bam files used for all following analyses.

Quality control

Ancient DNA authentication

To assess the authenticity of the ancient genome from Leang Panninge, we determined the read length distribution and frequency of C to T transitions at the 5' end ("damage") from the unique mapped reads of the double-stranded library after shotgun sequencing using *mapDamage* v.2.0.6 as implemented in *EAGER* v.1.92.55 (ref. 64). The reads were mostly between 30 and 40 bp long, with a median fragment length of 38 bp (SI Fig. 1a). The reads also show a strongly elevated rate of 30.19% C to T transitions at the first position from the 5' end (SI Fig. 1b). This drops to 1.53% at the second position due to the UDG-half treatment. These characteristics are consistent with other examples of DNA from ancient compared to present-day samples (e.g., ref. 68). Additionally, present-day DNA contamination was estimated as $0.1 \pm 0.436\%$ in the single-stranded library using *AuthentiCT* v.1.0⁶⁹.



SI Figure 1: Quality control plots of the Leang Panninge genome; **a**, low median fragment length; **b**, high frequency of C-T transitions at the 5' end of the DNA fragments; plotted with DataGraph v.4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

Mitochondrial analysis

All unique mitochondrial reads (16.72x coverage) were used to reconstruct the mtDNA consensus sequence and estimate mitochondrial estimation using *schmutzi*⁷⁰ with quality

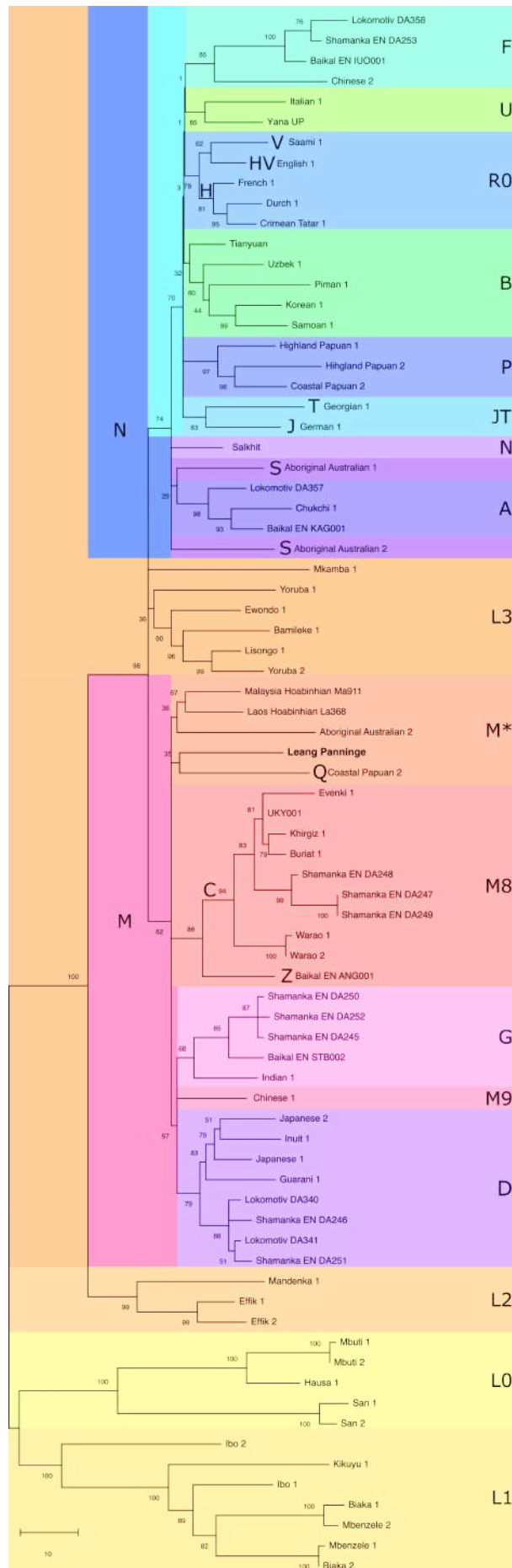
filters 0, 10, 20 and 30. Mitochondrial contamination was estimated to be $2 \pm 1\%$. Since the mitochondrial/nuclear ratio of the shotgun sequence was low (61.78), the mitochondrial contamination can serve as a proxy for nuclear DNA contamination⁷¹. The mitochondrial haplogroup was ascertained as M1 using *Haplofind*⁷² but with a low confidence score (SI Table 17).

SI Table 17: Mitochondrial haplogroup assignment of the Leang Panninge mitochondrial sequence based on consensus sequences assigned to be “endogenous” with *schmutzi*⁷⁰ (using quality filtering levels 0 to 30).

Quality filter	Assigned haplogroup	Score	# of missing positions
q0	M1	0.3	25
q10	M1	0.3	63
q20	M1	0.3	124
q30	M1	0.3	227

The q30 consensus sequence was aligned against the consensus sequences of 53 present-day human and several ancient mitochondrial genomes from China⁷³, Siberia⁷⁴⁻⁷⁶, Mongolia⁷⁷ and Southeast Asia⁷⁸ using MAFFT⁷⁹. We then constructed a maximum parsimony tree, eliminating all positions with less than 97% coverage (~2 bp missing/position) and calculating 500 bootstrap replicates in MEGA v.10.1.5 (ref. 80). The Leang Panninge mitochondrial genome falls basal of a haplogroup M clade, but on a different branch than the Hòabìnhians⁷⁸ (SI Fig. 2).

Detailed assessment of the mutations differentiating the Leang Panninge mitochondrial sequence (quality filter 30) from the Reconstructed Sapiens Reference Sequence (RSRS) as identified using *Haplofind*⁷² and *phylotree*⁸¹ showed 12 mutations exclusively determining haplogroup M (T489C, C10400T, T14783C, G15043A) and various subgroups of M (T629C, G5252A, T6374C, T6680C, G7859A, G8584A, G10373A, G16438A), as well as six private mutations (314insC, 4131G, 6218G, 10653A, 13958C, 15777A). These polymorphisms corroborate the phylogenetic placement of the mtDNA sequence branching from the base of the haplogroup M clade.



SI Figure 2: Maximum parsimony tree estimated from a dataset of the Leang Panninge mitochondrial genome aligned against 53 present-day human and several ancient mitochondrial genomes from China⁷³, Siberia⁷⁴⁻⁷⁶, Mongolia⁷⁷ and Southeast Asia⁷⁸ using MAFFT⁷⁹, eliminating all positions with less than 97% coverage (~2 bp missing/position) and calculating 500 bootstrap replicates in MEGA v.10.1.5 (ref. 80).

PMD filtering

To confirm that quality control results were not affected by present-day DNA contamination, the merged 1240K-captured sequences were filtered for degraded DNA sequences with *PMDtools* v.0.6 restricting to a PMD score of 3 (<https://github.com/pontussk/PMDtools>⁸²). Using the --UDGhalf parameter for the double-stranded library, this reduced the number of mapped reads from 735,985 to 81,525. For the single-stranded data the number reduced from 536,455 to 159,286 mapped reads.

Genetic sex determination

Based on the misincorporation pattern (SI Fig. 1b) of the double-stranded library, two base pairs were trimmed off the double-stranded 1240K-captured sequence on both ends of all reads to reduce damage-induced bias. From the resulting reads, the relative coverage of X- and Y-chromosomes was compared to that of the autosome, calculating the X- and Y-rate respectively. Due to the high X-rate (0.789) and low Y-rate (0.042), we concluded that the sample most likely possessed two X-chromosomes. Results after PMD-filtering were comparable with an X-rate of 0.740 and Y-rate of 0.024.

Genotyping

The double-stranded 1240K-captured sequence was genotyped for the aforementioned 1,237,207 SNP panel by genotyping the untrimmed and 2 bp-trimmed bam files separately using the *samtools* v.1.3 mpileup command and then *pileupCaller* from SequenceTools v.1.4.0.2 (<https://github.com/stschiff/sequenceTools>), which calls one SNP per position considering the human genome as a pseudo-haploid. The untrimmed and trimmed genotypes were then combined, retaining only transversion from the untrimmed genotype and transitions from the trimmed genotype to maximise information from the trimmed ends. The resulting coverage was suitable for population genetics analyses, with 163,099 SNPs on the 1240K and

84,514 SNPs on the Human Origins panel (HO⁸³⁻⁸⁶). The same procedure was also applied to the PMD-filtered sequence and resulted in a genotype covering 20,961 SNPs on the 1240K and 10,906 SNPs on the HO panel.

The single-stranded 1240K-captured and the respective PMD-filtered sequence were also genotyped using the *samtools* v.1.3 *mpileup* command and *pileupCaller*, but utilising the --single-stranded mode which removes damage by ignoring reads aligning to the forward strand at C/T polymorphisms and at the reverse strand for G/A polymorphisms. The resulting genotypes covered 127,048 and 41,577 SNPs on the 1240K panel for full and PMD-filtered genotype, respectively.

To maximise SNP coverage, the double- and single-stranded genotypes were merged using a custom Python script, which resulted in 263,207 SNPs on the 1240K and 135,432 SNPs on the HO panel.

Population genetic analyses

Principal component analyses

Principle component analyses (PCA) were performed using *smartpca* v.16000 from the EIGENSOFT package v.7.2.1 (<https://github.com/DReichLab/EIG>) with shrinkmode and lsqmode enabled⁸⁷. Genetic data of present-day individuals from East and Southeast Asia and Near Oceania included in the HO dataset⁸³⁻⁸⁶ were used to calculate the principal components, whereas ancient samples were projected (Leang Panninge; PMD-filtered Leang Panninge; Vanuatu/Tonga^{86,88}; Jōmon, Japan⁸⁹; Tianyuan, China⁹⁰; Neolithic/Hòabìnhian-associated Indonesia/Laos/Malaysia⁷⁸; Devils Cave, Russia⁷⁶ and; Qihe/Bianbian, China⁹¹).

In both PCAs calculated on only East and Southeast Asian groups (Extended Data Fig. 6a), and including Near Oceanian groups (Extended Data Fig. 6b), the Leang Panninge genome falls into PCA space not occupied by any present-day populations. However, they are broadly located between Indigenous Australians and Onge from the Andaman Islands, which overlap with the Hòabìnhian-associated individuals⁷⁸. The PMD-filtered genomes behave very similarly. The observed slight shift could also be due to the low coverage on the HO panel used for these calculations.

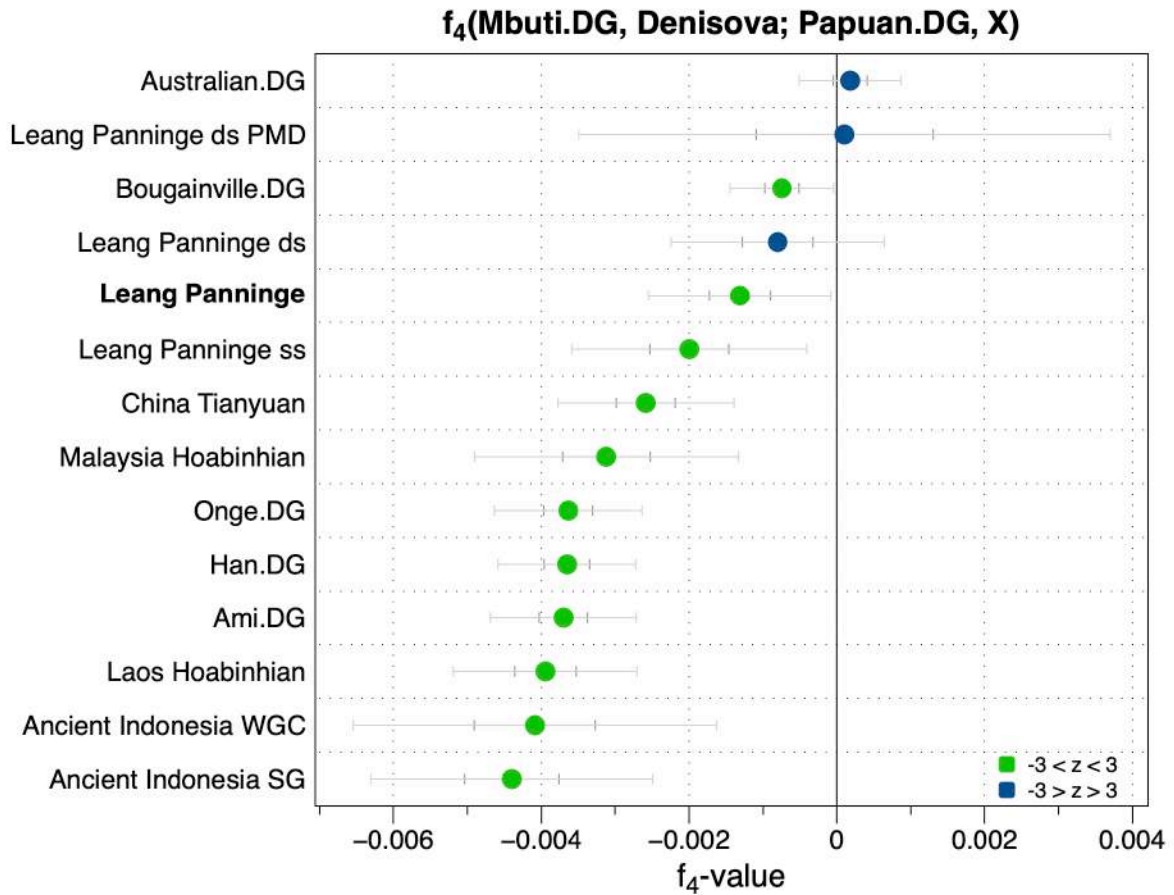
f -statistics

All f_3 - and f_4 -statistics were calculated with *AdmixTools* package v.5.0 (<https://github.com/DReichLab/AdmixTools>) using *qp3pop* v.420 and *qpDstat* v.721, respectively, with the inbreed option enabled for *qp3pop*⁸⁴. For f_3 -statistics we used East and Southeast Asian and Oceanian groups from the HO panel to cover a broader geographic area. However, for f_4 -statistics the use of a more restricted dataset genotyped on the 1240K panel⁹²⁻⁹³ was necessary to maximise the number of overlapping SNPs with the Leang Panninge genome, particularly when comparing to the PMD-filtered data or to other ancient genomes.

To further investigate the observed shared drift between Leang Panninge and Oceanians we calculated $f_3(\text{Mbuti.DG, Leang Panninge, X})$ and $f_4(\text{Mbuti.DG, X; Papuan.DG, Leang Panninge})$. The resulting values indicated that Papuans are genetically closer to the Leang Panninge genome than any other Eastern Eurasian group (Fig. 2b, Extended Data Fig. 7a). For Near Oceanians, however, the values are significantly negative (z-score > -3), with individuals from both Indigenous Australia and Bougainville forming a clade with Papuans in comparison to Leang Panninge.

Focusing on the position of Leang Panninge within groups from Near Oceania, f_4 -statistics of the form $f_4(\text{Mbuti.DG, X; Y, Z})$ were calculated, varying the position of Leang Panninge, Papuan.DG, Australian.DG und Bougainville.DG between X, Y and Z (Extended Data Fig. 7b). The results show that while the Bougainville group forms a clade with Papuan individuals to the exclusion of Indigenous Australia, these three groups are mostly closer to each other when compared to Leang Panninge, thus placing this ancient individual outside of the Oceanian clade.

The gene pool of present-day populations from Oceania contains the highest proportion of genetic ancestry from Denisovans^{85,94-97}. We therefore investigated if Leang Panninge also carried a genetic contribution from this hominin group. Using Mbuti as outgroup, Leang Panninge showed stronger affinity to the Denisovan genome than Onge and other East Asian groups, but less than Papuan and Indigenous Australian individuals (Extended Data Fig. 7c). We confirmed these observations when Papuans were kept in the third position in the f_4 -statistics (Mbuti, Denisova; Papuan, X) where X are ancient and present-day individuals from the Asia-Pacific region (SI Fig. 3).

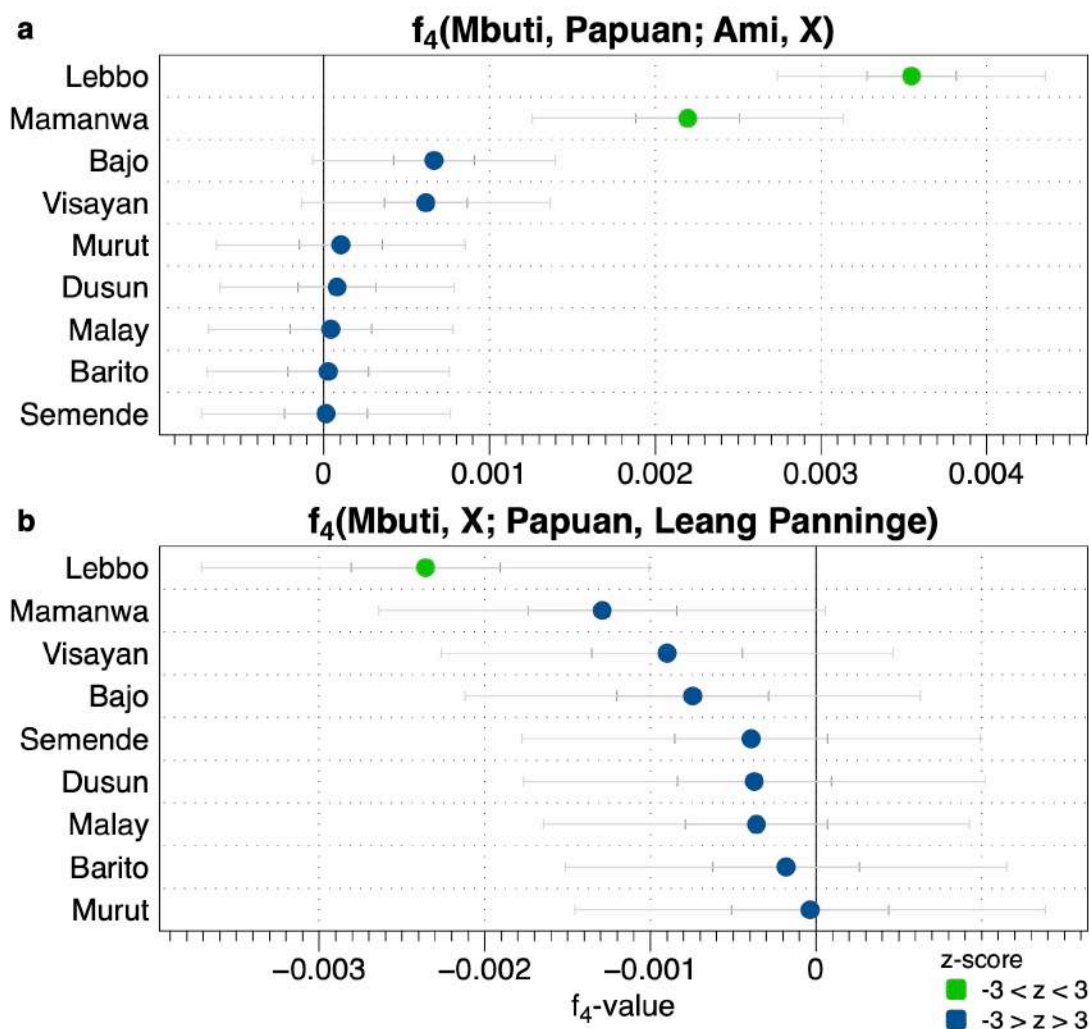


SI Figure 3: The affinity of Papuan individuals to the Denisova genome in comparison to Asia-Pacific present-day and ancient groups and individuals, including the combined Leang Panninge data, the data from double- and single-stranded libraries (ds/ss) separately and from the PMD-filtered double-stranded library sequences. Data are presented as exact f_4 -values $\pm$ one and three standard errors indicated as dark and light grey lines, respectively; green symbols denote z-scores larger than $|3|$.

While present-day groups show no affinity to Leang Panninge stronger than Papuans, ancient individuals from Southeast Asia^{78,91} do show some positive values (Extended Data Fig. 7d). A note of caution is warranted, however, since these statistics might in part be affected by ancient versus modern samples attraction.

Present-day groups in Wallacea, and particularly Sulawesi, speak Austronesian languages. However, as published previously and indicated by the PCAs (Extended Data Fig. 6), there might also be Papuan ancestry present in these groups, similar to what has been observed in Vanuatu⁸⁸. Therefore, we first tested if Papuan-like ancestry was present in modern-day groups from around the region in comparison to Ami individuals from Taiwan (HO dataset)

as representatives of Austronesian ancestry (SI Fig. 4a). These statistics were significantly positive only for the Lebbo and Mamanwa (traditionally, hunter-gatherer populations in Sarawak and the Philippines, respectively), confirming the dominance of Austronesian-like ancestry in the region. Targeting the minor Papuan-like component, a second statistic was performed to test potential differential affinities of present-day groups in the region towards Leang Panninge or Papuans. This showed that any detectable Papuan-like ancestry seemed to be more directly Papuan- than Leang Panninge-related (SI Fig. 4b). This could be a result of exclusive admixture with Papuan ancestry, as shown for the Bajo⁹⁸, or any signals of other Papuan-like ancestry (such as Leang Panninge-related) being too limited to be picked up at the current resolution or diminished over time by other demographic processes.



SI Figure 4: The genetic ancestry of present-day groups in Wallacea and the surrounding region⁸³⁻⁸⁶; results of f_4 -statistics showing the genetic affinities comparing **a**, Papuan and Ami and **b**, Papuan and Leang Panninge. Data are presented as exact f_4 -values $\pm$ one and three

standard errors indicated as dark and light grey lines, respectively; green symbols denote z-scores larger than $|3|$.

f_4 -ratio

To investigate the proportion of Denisovan-related ancestry present in the Leang Panninge genome we calculate several f_4 -ratio statistics using *f4ratio* from the *AdmixTools* package v.5.0 (<https://github.com/DReichLab/AdmixTools>).

We calculated the percentage of Denisova-related ancestry directly as in ref. 85:

$$\alpha = 1 - \frac{f_4(\text{Yoruba}, \text{Neanderthal}, \text{Han}, X)}{f_4(\text{Yoruba}, \text{Neanderthal}, \text{Han}, \text{Denisova})}$$

and indirectly as a proportion of the Denisovan ancestry found in Papuans as in ref. 96:

$$\alpha = 1 - \frac{f_4(\text{YRI.SG}, \text{Denisova}, \text{CHB.SG}, X)}{f_4(\text{YRI.SG}, \text{Denisova}, \text{CHB.SG}, \text{Papuan.DG})}$$

where we used Yoruba individuals, Han Chinese and Papuans from the 1000 Genomes, Simon Genomes Diversity Project (SGDP) or HO panel^{83-86,92-93,99} as baselines, where Denisova indicates the ancient genome from Denisova cave⁹⁴ and X is replaced by the test groups.

Estimating the Denisovan proportion directly using HO groups with the statistics presented in ref. 85, results for Papuans and Indigenous Australians including both the Altai¹⁰⁰ or Vindija Neanderthals¹⁰¹ in the calculations show values very similar to the ones in ref. 84 (~3.4%, SI Table 18). The Nasioi from Bougainville achieve ~3.1% Denisova-related ancestry and Leang Panninge and Tianyuan⁹⁰ achieve ~2.2%, while values drop to below 1% for all other tested groups, including the Hòabìnhian-associated individuals⁷⁸.

SI Table 18: Results of f_4 -ratio statistics calculating the Denisova-related proportion (α) in present-day and ancient Asia-Pacific groups (HO^{78,90,91}) as in ref. 85.

Baselines	Yoruba, Han	Yoruba, Han
Neanderthal	Altai published.DG	Vindija.DG

	α	Z	% Denisova	% SE	α	z	% Denisova	% SE
Ami	0.0017	1.255	0.167	0.1331	0.0021	1.557	0.2098	0.1347
Indigenous Australian	0.0346	8.498	3.4603	0.4072	0.03472	8.179	3.4717	0.4245
Tianyuan	0.0220	4.855	2.201	0.4533	0.02285	4.987	2.2848	0.4581
Leang Panninge	0.0222	4.041	2.2187	0.549	0.02329	4.148	2.3286	0.5613
Laos Hoabinhian	-0.004	-0.95	-0.4141	0.4358	-0.0045	-1.009	-0.4531	0.4492
Malaysia Hoabinhian	-	-0.063	-0.0464	0.7391	-0.0019	-0.238	-0.1872	0.7857
Nasioi	0.0308	8.854	3.0768	0.3475	0.03145	8.713	3.1451	0.361
Onge	0.0031	1.218	0.3089	0.2537	0.00288	1.095	0.2878	0.2629
Papuan	0.0338	9.198	3.3844	0.3679	0.03330	8.787	3.3302	0.379
Qihe	0.0074	1.67	0.7426	0.4446	0.00885	1.952	0.885	0.4534

To increase the SNP overlap of the test groups and the Leang Panninge genome, we also calculated the same statistics using the Vindija Neanderthal, Yoruba and Han from the 1000 Genomes project and present-day groups from the SGDP panel. Using YRI.SG and CHB.SG with other groups from SGDP achieved slightly higher values for all groups than the statistics using HO groups, while using all groups from SGDP panel resulted in lower values, notably 2.5% and 2.1% for Leang Panninge, respectively (SI Table 19).

SI Table 19: Results of f_4 -ratio statistics calculating the Denisova-related proportion (α) in present-day and ancient Asia-Pacific groups⁹⁹, and SGDP^{78,90,91} as in ref. 85.

Baselines	YRI.SG, CHB.SG				Yoruba.DG, Han.DG			
Neanderthal	Vindija.DG				Vindija.DG			
	α	Z	% Denisova	% SE	α	z	% Denisova	% SE
Ami.DG	0.0060	2.944	0.6018	0.2044	-0.0013	-0.497	-0.1262	0.2542
Australian.DG	0.0356	8.997	3.5562	0.3953	0.02933	6.82	2.9333	0.4301
Bougainville. DG	0.0353	9.201	3.5274	0.3834	0.02896	6.769	2.8958	0.4278

Tianyuan	0.0269	6.091	2.6962	0.4426	0.01741	3.538	1.7413	0.4921
Leang Panninge	0.0254	5.078	2.5401	0.5002	0.02168	3.971	2.1678	0.5459
Laos Hoabinhian	-0.004	-0.94	-0.3698	0.3934	-0.0106	-2.408	-1.0613	0.4407
Malaysia Hoabinhian	-0.003	-0.42	-0.2598	0.6189	-0.0094	-1.316	-0.9367	0.7119
Onge.DG	0.0070	2.333	0.7002	0.3001	-0.0021	-0.599	-0.2112	0.3525
Papuan.DG	0.0367	10.047	3.6669	0.365	0.02921	7.399	2.9205	0.3947
Qihе	0.0088	2.197	0.8796	0.4004	0.00060	0.131	0.0603	0.4589

When using the statistic presented in ref. 96, where Denisova-related ancestry is calculated as the proportion of Denisovan-related ancestry present in Papuan groups, we again observe that Indigenous Australian and Papuan groups carry nearly identical amounts of Denisovan introgression, while Bougainville and Leang Panninge individuals carry less (SI Table 20a-b). There is no detectable Denisovan ancestry in the oldest Hòabìnhian-associated individual from Laos⁷⁸, but this increases slightly with the younger Hòabìnhian-associated individual from Malaysia⁷⁸, with values slightly higher than present-day Onge also. Assuming 3% Denisovan introgression into Papuans^{85,97,102}, the calculated percentage of Denisovan-related ancestry in Leang Panninge is $1.9 \pm 0.3\%$ for the HO, $2.2 \pm 0.3\%$ for SGDP/DG and $2.4 \pm 0.3\%$ for DG panel.

SI Table 20a: Results of f_4 -ratio statistics calculating the Denisova-related proportion (α) as a fraction of the 3% Denisovan proportion present in Papuans in present-day and ancient Asia-Pacific groups (HO⁹⁹, SGDP^{78,90,91}) as in ref. 96.

Test groups	Yoruba, Han, Papuan					YRI.SG, CHB.SG, Papuan.DG			
	α	z	% Denisova	% SE		α	z	% Denisova	% SE
Ami	0.0255	1.12	0.076371	0.0682	Ami.DG	0.09318	2.766	0.279549	0.10106
Indigenous Australian	1.0026	18.586	3.007893	0.1618	Australian. DG	1.00835	19.701	3.02505	0.15355
Nasioi	0.8502	18.965	2.550561	0.1345	Bougainvill e.DG	0.85570	16.98	2.567106	0.15118

Tianyuan	0.4291	5.252	1.287291	0.2451	Tianyuan	0.48027	6.754	1.440798	0.21333
Leang Panninge	0.6629	6.452	1.988613	0.3082	Leang Panninge	0.74728	8.539	2.24184	0.26253
Laos Hoabinhian	0.0269	0.324	0.080853	0.2499	Laos Hoabinhian	0.03402	0.498	0.102063	0.20505
Malaysia Hoabinhian	0.1697	1.235	0.509046	0.4122	Malaysia Hoabinhian	0.18104	1.656	0.543129	0.32805
Onge	0.0392	0.847	0.117609	0.1388	Onge.DG	0.13625	2.866	0.408747	0.14262
Qihe	0.1629	1.967	0.488778	0.2485	Qihe	0.14816	2.076	0.444486	0.21412

SI Table 20b: Results of f_4 -ratio statistics calculating the Denisova-related proportion (α) as a fraction of the 3% Denisovan proportion present in Papuans in present-day and ancient Asia-Pacific groups (HO⁹⁹, SGDP^{78,90,91}) as in ref. 96.

Test groups	Yoruba.DG, Han.DG, Papuan.DG			
	A	z	% Denisova	% SE
Ami.DG	-0.03461	-0.711	-0.10383	0.145989
Australian.DG	1.036123	16.381	3.108369	0.189759
Bianbian	0.07679	1.082	0.23037	0.212904
Bougainville.DG	0.858306	14.164	2.574918	0.181794
Tianyuan	0.348121	4.099	1.044363	0.254763
Leang Panninge	0.803749	7.146	2.411247	0.337407
Laos Hoabinhian	-0.086022	-0.711	-0.258066	0.268494
Malaysia Hoabinhian	0.081115	16.381	0.243345	0.405702
Onge.DG	-0.024938	1.082	-0.074814	0.201423
Qihe	0.02768	14.164	0.08304	0.280392

D-statistics

Based on the archaic ancestry array (panel 4)⁶⁷, we calculated D -statistics to gain insight into archaic ancestry in the Leang Panninge individual. Since the archaic ancestry array capture probes were applied to a single-stranded sequencing library, the orientation of deamination induced substitutions as C to T exchanges in forward oriented sequence alignments and G to A exchanges in reverse alignments was preserved. To reduce the effect of this deamination, we did not consider forward aligning sequences when the human reference genome, the two archaic human genomes or the inferred ancestor from the four ape genomes carried a C allele,

and did not consider reverse aligning sequences when any of these genomes carried a G allele. An allele from the Leang Panninge genome was randomly chosen from the remaining sequences at each position. A random allele from the genotype calls of a Yoruban, San, Mbuti, French, Han Chinese and Papuan individual (from panel B in ref. 100) and the genotype calls of the Altai Neanderthal (Denisova 5), Denisovan (Denisova 3), and Ust’Ishim high-coverage genomes were added. We then annotated the archaic ancestry sites⁶⁷ with an ancestral state inferred from the alignments of four ape genomes (chimpanzee (panTro4), bonobo (panpan1.1), gorilla (gorGor3), and orangutan (ponabe2)) by requiring identical bases in all called genomes.

We calculated *D*-statistics for all pairwise comparisons of modern human genomes to the Denisovan and the Altai Neanderthal genomes using the inferred ape ancestor as an outgroup and estimated significance using a block jackknife procedure over 5Mb windows^{84,103}. The archaic ancestry SNPs of the Leang Panninge genome showed more allele sharing with Denisovans than all genomes, albeit not always significant, with the exception of the Papuan individual who shared significantly more alleles with Denisovans than does Leang Panninge (SI Table 21). No significant differences were found in the sharing of derived alleles with the Altai Neanderthal between the Han Chinese and Leang Panninge. Furthermore, the differences in allele sharing with the Altai Neanderthal were comparatively small between all tested genomes, except the French.

SI Table 21: Allele sharing of the Leang Panninge genome and present-day individuals with the Denisova, Altai and Vindija genome, using the inferred great ape ancestor as outgroup.

Test	<i>D</i> -value	z-score
D(Yoruba, Leang Panninge , Denisova, ancestor)	-0.532	-10.466
D(Mbuti, Leang Panninge , Denisova, ancestor)	-0.354	-6.98
D(French, Leang Panninge , Denisova, ancestor)	-0.296	-5.341
D(Han, Leang Panninge , Denisova, ancestor)	-0.261	-4.923
D(Ust’Ishim, Leang Panninge , Denisova, ancestor)	-0.128	-2.566
D(San, Leang Panninge , Denisova, ancestor)	-0.111	-2.358
D(Papuan, Leang Panninge , Denisova, ancestor)	0.158	3.44
D(Yoruba, Leang Panninge , Altai, ancestor)	-0.605	-13.851
D(Mbuti, Leang Panninge , Altai, ancestor)	-0.474	-10.715

D(San, Leang Panninge , Altai, ancestor)	-0.251	-5.691
D(French, Leang Panninge , Altai, ancestor)	-0.048	-1.05
D(Han, Leang Panninge , Altai, ancestor)	0.055	1.318
D(Papuan, Leang Panninge , Altai, ancestor)	0.102	2.386
D(Ust'Ishim, Leang Panninge , Altai, ancestor)	0.203	4.155
D(Yoruba, Leang Panninge , Vindija, ancestor)	-0.651	-15.362
D(Mbuti, Leang Panninge , Vindija, ancestor)	-0.512	-11.829
D(San, Leang Panninge , Vindija, ancestor)	-0.284	-6.515
D(French, Leang Panninge , Vindija, ancestor)	-0.074	-1.57
D(Han, Leang Panninge , Vindija, ancestor)	0.048	1.153
D(Papuan, Leang Panninge , Vindija, ancestor)	0.092	2.153
D(Ust'Ishim, Leang Panninge , Vindija, ancestor)	0.155	3.091

We also calculated D -statistics using data from the Tianyuan individual⁹⁰. For this, we randomly sampled a single read at captured archaic ancestry sites from the Tianyuan data. Similar to the Leang Panninge data, higher sharing with Denisovans is observed (SI Table 22).

SI Table 22: Allele sharing of the Tianyuan genome and present-day individuals with the Denisova, Altai and Vindija Neanderthal genome, using the inferred great ape ancestor as an outgroup.

Test	D-value	z-score
D(Yoruba, Tianyuan, Denisova, ancestor)	-0.405	-15.048
D(Mbuti, Tianyuan, Denisova, ancestor)	-0.188	-6.848
D(French, Tianyuan, Denisova, ancestor)	-0.179	-6.292
D(Han, Tianyuan, Denisova, ancestor)	-0.096	-3.413
D(San, Tianyuan, Denisova, ancestor)	-0.042	-1.583
D(Ust'Ishim, Tianyuan, Denisova, ancestor)	-0.019	-0.743
D(Papuan, Tianyuan, Denisova, ancestor)	0.283	9.603
D(Yoruba, Tianyuan, Altai, ancestor)	-0.688	-28.546
D(Mbuti, Tianyuan, Altai, ancestor)	-0.521	-20.079
D(San, Tianyuan, Altai, ancestor)	-0.37	-13.328
D(French, Tianyuan, Altai, ancestor)	-0.176	-5.648

D(Han, Tianyuan, Altai, ancestor)	-0.059	-2.013
D(Papuan, Tianyuan, Altai, ancestor)	0.012	0.368
D(Ust'Ishim, Tianyuan, Altai, ancestor)	0.076	2.112
D(Yoruba, Tianyuan, Vindija, ancestor)	-0.709	-29.547
D(Mbuti, Tianyuan, Vindija, ancestor)	-0.544	-20.821
D(San, Tianyuan, Vindija, ancestor)	-0.395	-14.235
D(French, Tianyuan, Vindija, ancestor)	-0.171	-5.344
D(Han, Tianyuan, Vindija, ancestor)	-0.053	-1.781
D(Papuan, Tianyuan, Vindija, ancestor)	0.015	0.479
D(Ust'Ishim, Tianyuan, Vindija, ancestor)	0.064	1.704

In a last step, we joined the Leang Panninge and Tianyuan tables, retaining only those sites where both Leang Panninge and Tianyuan had coverage. In this direct comparison of Leang Panninge and Tianyuan, we observe significantly higher sharing of Leang Panninge with Denisovans ($|Z| > 3$) in many comparisons and a trend towards more Neanderthal sharing for Tianyuan as opposed to Leang Panninge. D -statistics of the form $D(\text{Leang Panninge}, \text{Tianyuan}, \text{Denisovan}, \text{Neanderthal})$ combine these two trends and are significant in all comparisons. Comparisons between Ust'Ishim and Tianyuan show no significant differences ($|Z| < 2.5$) in sharing with the Neanderthal genomes or the Denisovan genome. Comparisons of Ust'Ishim and Leang Panninge show higher Neanderthal sharing for Ust'Ishim and trends towards higher Denisovan ancestry in Leang Panninge. All combined statistics of the form $D(\text{Ust'Ishim}, \text{Leang Panninge}, \text{Denisovan}, \text{Neanderthal})$ are significant (SI Table 23).

SI Table 23: Allele sharing of the Leang Panninge, Tianyuan and Ust'Ishim genomes with the Denisova, Altai and Vindija Neanderthal genomes, using the inferred great ape ancestor as an outgroup.

test	<i>D</i> -value	z-score
D(Leang Panninge , Tianyuan, Denisova, ancestor)	0.161	3.122
D(Leang Panninge , Tianyuan, Altai, ancestor)	-0.087	-1.843
D(Leang Panninge , Tianyuan, Vindija, ancestor)	-0.073	-1.545
D(Leang Panninge , Tianyuan, Denisova, Yoruba)	0.153	3.17
D(Leang Panninge , Tianyuan, Altai, Yoruba)	-0.064	-1.431
D(Leang Panninge , Tianyuan, Vindija, Yoruba)	-0.056	-1.244
D(Leang Panninge , Tianyuan, Denisova, Altai)	0.202	4.295

D(Leang Panninge , Tianyuan ,Denisova, Vindija)	0.188	3.993
D(Ust'Ishim, Tianyuan, Altai, ancestor)	0.112	2.259
D(Ust'Ishim, Tianyuan, Altai, Yoruba)	0.121	2.427
D(Ust'Ishim, Tianyuan, Denisova, Altai)	-0.091	-1.896
D(Ust'Ishim, Tianyuan, Denisova, ancestor)	0.053	1.063
D(Ust'Ishim, Tianyuan, Denisova, Vindija)	-0.053	-1.058
D(Ust'Ishim, Tianyuan, Denisova, Yoruba)	0.095	1.75
D(Ust'Ishim, Tianyuan, Vindija, ancestor)	0.078	1.534
D(Ust'Ishim, Tianyuan, Vindija, Yoruba)	0.089	1.755
D(Ust'Ishim, Leang Panninge , Altai, ancestor)	0.202	3.899
D(Ust'Ishim, Leang Panninge , Altai, Yoruba)	0.19	3.791
D(Ust'Ishim, Leang Panninge , Denisova, Altai)	-0.284	-5.964
D(Ust'Ishim, Leang Panninge , Denisova, ancestor)	-0.095	-1.777
D(Ust'Ishim, Leang Panninge , Denisova, Vindija)	-0.237	-4.863
D(Ust'Ishim, Leang Panninge , Denisova, Yoruba)	-0.05	-0.951
D(Ust'Ishim, Leang Panninge , Vindija, ancestor)	0.153	2.911
D(Ust'Ishim, Leang Panninge , Vindija, Yoruba)	0.148	2.929

Admixfrog

We used *admixfrog*¹⁰⁴ for inference of genomic regions of Denisovan and Neanderthal ancestry, restricting analyses to the sites of the archaic admixture array (Panel 4 in ref. 67). For comparison we used the SGDP Sub-Saharan Africans⁹², three high-coverage Neanderthals and the high-coverage Denisovan as putative reference populations, and the chimpanzee reference genome (panTro4) as the putative ancestral allele. As no fine-scale recombination maps for ancient populations from the region under investigation were available, we ran the program using bins of size 10kb without incorporating a recombination map. Furthermore, as contamination has been estimated to be very low, we did not estimate contamination and error rates, but rather assumed a constant error rate of 0.01 for all reads. The exact command run was:

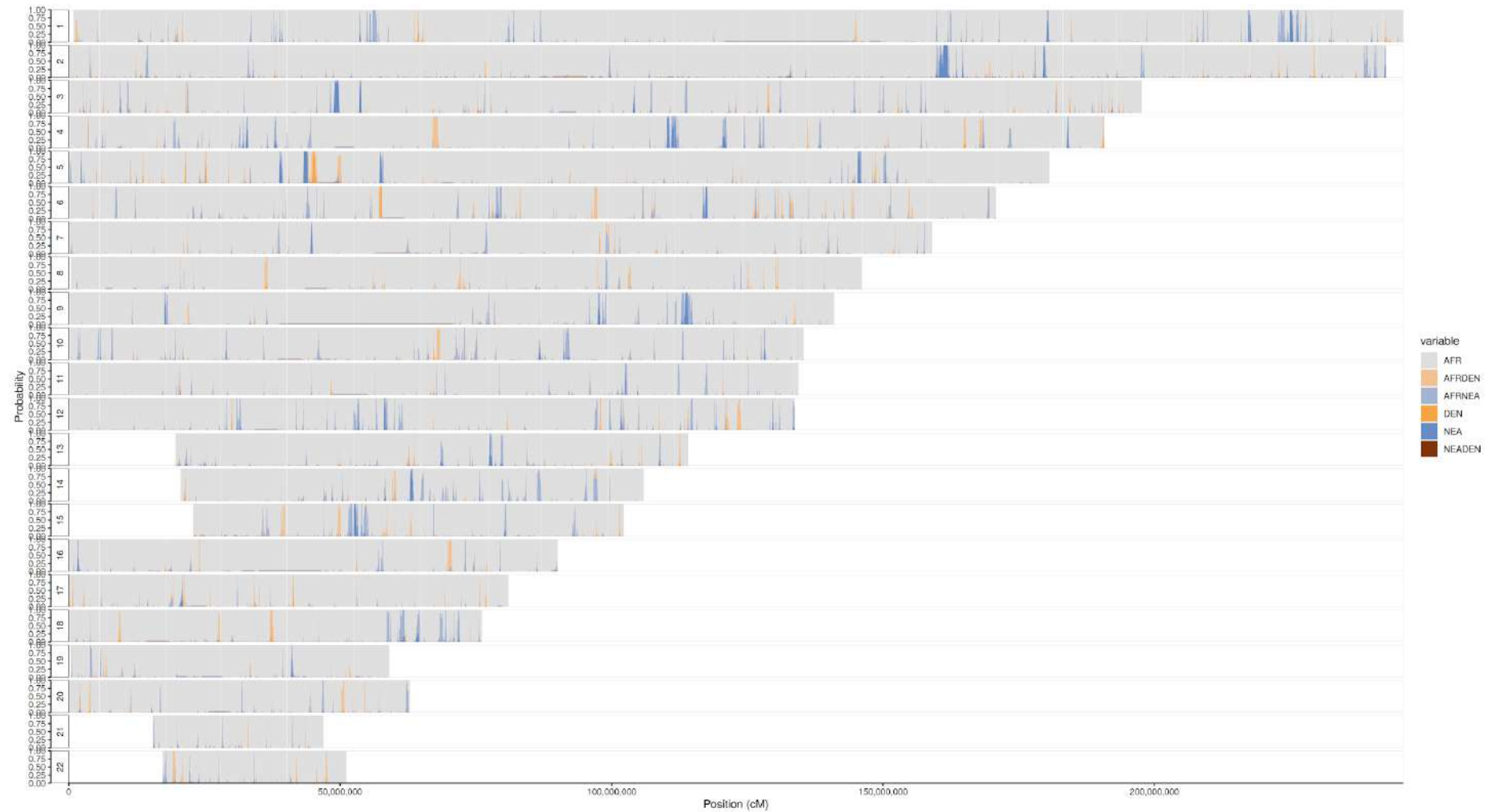
```
admixfrog --infile sulawesi_archaicadmixture.in.xz --ref
ref/ref_archaicadmixture.csv.xz -o
admixfrog/posmode2/50000/AFR_NEA_DEN/sulawesi_archaicadmixture --
states AFR NEA DEN --ll-tol 0.01 --bin-size 10000 --est-F --est-tau
```

```
--freq-F 3 --dont-est-contamination --e0 0.01 --c0 0 --pos-mode --  
ancestral PAN --run-penalty 0.4 --max-iter 250 --n-post-replicates 200
```

In order to evaluate the robustness of some key parameters, we also used all East Asians from the SGDP data set as an alternative reference and varied the bin size to 20kb and 50kb. As results are consistent, we will focus the discussion on the primary parameter combination. For comparison, we also used *admixture* on all SGDP individuals using similar parameters, but calling the ancestry from the available genotypes by using the `--gt-mode` flag.

Admixture, as run above, results in a posterior decoding of the archaic ancestry; that is, for each window we obtained the posterior probability of whether it is homozygous modern human, Neanderthal or Denisovan ancestry, or heterozygous for any combination of those ancestry states. In this analysis a total of 299,047 SNPs overlapped the archaic admixture position, corresponding to a genome-wide coverage of 0.206 on target sites. We found 49.2 (± 2.5) Mb of Neanderthal ancestry and 22.4 (± 1.9) Mb of Denisovan ancestry in tracts of at least 200kb length, for which *admixture* has high detection power. Therefore, the amount of Denisovan ancestry in the Leang Panninge individual is around half of the amount found in Papuans (50.7 Mb), but more than what is found in East Asians and South Asians and in the Upper Paleolithic Tianyuan individual, which was also captured for the “archaic admixture” SNP panel (Fig. 2c). The calculated amount of Neanderthal ancestry is similar to what previous methods have detected in present-day human populations (comparison in ref. 105).

The Denisovan ancestry found in the Leang Panninge genome is located in a total of 31 called fragments, the longest of which is just over 1 Mb (1.04 Mb) long, and is located on chr5:44,660,000-45,700,000 (SI Fig. 5)



SI Figure 5: Archaic ancestry fragments in Leang Panninge after posterior decoding using *admixfrog* of the autosomes. Heterozygous Neanderthal and Denisovan fragments are given in light blue and orange, respectively, homozygous fragments in darker colors. Bar height is proportional to the posterior probability of ancestry. Grey bars correspond to African ancestry.

We then investigated whether the 31 Denisovan fragments overlapped with known Denisova fragments from 109 modern human populations from Simons Genome Diversity Project (SGDP⁹⁰) using the same approach described in a previous study¹⁰⁶. We summarised the overlap in terms of overlapping sequence and calculated p-values and 95% confidence intervals using 500 reshufflings of the archaic fragments in the Leang Panninge individual. We found that there is a significant correlation between the Denisova fragments in Leang Panninge and those in Papuans (n=15 individuals), Indigenous Australians (n=2 individuals) and people from Bougainville (n=2 individuals), suggesting their Denisovan ancestry is shared (p-value < 1/500, Extended Data Fig. 8).

Multidimensional scaling plot

We calculated the values of $1-f_3(\text{Mbuti, Leang Panninge, X})$ to visualise the pair-wise genetic distance between Leang Panninge and present-day East Eurasian and Near Oceanian groups in a multidimensional scaling plot created in *RStudio* v.1.2.1335 (Extended Data Fig. 9). Overall the plot shows great similarities to the PCAs (Extended Data Fig. 6), with Leang Panninge falling in unoccupied space removed from Oceanian towards Onge and East Asian groups.

qpWave and qpAdm

Admixture proportions were estimated with *qpWave* v.410 and *qpAdm* v.810 from the AdmixTools package v.5.0 (<https://github.com/DReichLab/AdmixTools>) with the allsnps and details option enabled as implemented in the *qpWrapper* script (<https://github.com/TCLamnidis/qpWrapper>).

To investigate the apparent shift from Near Oceanians in PCA space and in the MDS plot despite their cladal behaviour compared to other present-day East Eurasians (Extended Data Fig. 9), we used *qpWave* to test if Leang Panninge and Papuan⁹³ are equally related to a set of “reference” populations. We used the Mbuti⁹², Kostenki-14¹⁰⁷ and Onge⁹² as a basic reference set and added Denisova⁹³ and ancient Asians^{77,89,90} in turn to this set (SI Table 24). While the basic reference set was not able to differentiate between Leang Panninge and Papuan, these two groups are always consistent as deriving from at least two distinct waves of ancestry when using Denisova and/or ancient Asian individuals as additional reference groups.

SI Table 24: Results of *qpWave* analyses showing the p-value testing for “2 waves” detected for Papuan and Leang Panninge with varying reference groups; p-values below 0.05 are indicated in bold.

test groups (left)	p-value	reference groups (right)
Papuan.DG, Leang Panninge	0.000010	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Xitoucun
Papuan.DG, Leang Panninge	0.000054	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Laos_Hoabinhian.SG
Papuan.DG, Leang Panninge	0.000066	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Liangdao1
Papuan.DG, Leang Panninge	0.000124	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Tanshishan
Papuan.DG, Leang Panninge	0.000277	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Qihe
Papuan.DG, Leang Panninge	0.000435	Mbuti.DG, Russia_Kostenki14, Onge.DG, Xitoucun
Papuan.DG, Leang Panninge	0.000483	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, China_Tianyuan
Papuan.DG, Leang Panninge	0.000520	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Xiaogao
Papuan.DG, Leang Panninge	0.001148	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Suogang
Papuan.DG, Leang Panninge	0.001483	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Liangdao2
Papuan.DG, Leang Panninge	0.001560	Mbuti.DG, Denisova_published.DG
Papuan.DG, Leang Panninge	0.001715	Mbuti.DG, Russia_Kostenki14, Onge.DG, Laos_Hoabinhian.SG
Papuan.DG, Leang Panninge	0.001947	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14
Papuan.DG, Leang Panninge	0.002277	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG
Papuan.DG, Leang Panninge	0.002618	Mbuti.DG, Russia_Kostenki14, Onge.DG, Liangdao1
Papuan.DG, Leang Panninge	0.004338	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Xiaojingshan
Papuan.DG, Leang Panninge	0.004880	Mbuti.DG, Denisova_published.DG, Onge.DG
Papuan.DG, Leang Panninge	0.005355	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Boshan

Papuan.DG, Leang Panninge	0.005518	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Malaysia_Hoabinhian.WGC
Papuan.DG, Leang Panninge	0.005589	Mbuti.DG, Russia_Kostenki14, Onge.DG, Tanshishan
Papuan.DG, Leang Panninge	0.005630	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, Yumin
Papuan.DG, Leang Panninge	0.005787	Mbuti.DG, Denisova_published.DG, Russia_Kostenki14, Onge.DG, DevilsCave_N
Papuan.DG, Leang Panninge	0.010384	Mbuti.DG, Russia_Kostenki14, Onge.DG, Qihe
Papuan.DG, Leang Panninge	0.013869	Mbuti.DG, Russia_Kostenki14, Onge.DG, Xiaogao
Papuan.DG, Leang Panninge	0.019466	Mbuti.DG, Russia_Kostenki14, Onge.DG, China_Tianyuan
Papuan.DG, Leang Panninge	0.043432	Mbuti.DG, Russia_Kostenki14, Onge.DG, Liangdao2
Papuan.DG, Leang Panninge	0.044791	Mbuti.DG, Russia_Kostenki14, Onge.DG, Suogang
Papuan.DG, Leang Panninge	0.083579	Mbuti.DG, Russia_Kostenki14, Onge.DG
Papuan.DG, Leang Panninge	0.083579	Mbuti.DG, Russia_Kostenki14, Onge.DG
Papuan.DG, Leang Panninge	0.116857	Mbuti.DG, Russia_Kostenki14
Papuan.DG, Leang Panninge	0.132633	Mbuti.DG, Russia_Kostenki14, Onge.DG, Xiaojingshan
Papuan.DG, Leang Panninge	0.165804	Mbuti.DG, Russia_Kostenki14, Onge.DG, Malaysia_Hoabinhian.WGC
Papuan.DG, Leang Panninge	0.167992	Mbuti.DG, Russia_Kostenki14, Onge.DG, Boshan
Papuan.DG, Leang Panninge	0.171997	Mbuti.DG, Russia_Kostenki14, Onge.DG, Yumin
Papuan.DG, Leang Panninge	0.349595	Mbuti.DG, Onge.DG

Based on these results, we used *qpAdm* to model Leang Panninge as a mixture between Papuan and ancient Asian groups using the basic reference set (Mbuti, Kostenki14, Onge, Denisova) plus adding one ancient Asian group in turn (SI Table 25). We observe that using Tianyuan as a source or as a reference group provides the highest number of fitting models (i.e., the admixture models achieved a significant p-value (>0.05) and a low p-value for the nested model (<0.05)). The worst fits were observed for Laos Hòabinhian as a source, likely due to its close affinity to Onge among the reference populations.

SI Table 25: Results of *qpAdm* analyses showing the p-value and nested p-value when modelling Leang Panninge as an admixture between Papuan and ancient individuals from East Asia using Mbuti, Kostenki14, Onge, the Denisova and an additional ancient Asian individual as references; p-values above 0.05 are indicated in bold.

sources (left)	p-value	nested p	source1 %	source2 %	std. err. %	additional reference
Papuan.DG, Tianyuan	0.048365	0.210081	0.299	0.701	0.246	Laos Hoabinhian
Papuan.DG, Tianyuan	0.925551	0.025674	0.368	0.632	0.138	Liangdao1
Papuan.DG, Tianyuan	0.651633	0.000208	0.544	0.456	0.115	Liangdao2
Papuan.DG, Tianyuan	0.920368	0.034874	0.375	0.625	0.148	Qihe
Papuan.DG, Tianyuan	0.952534	0.031107	0.394	0.606	0.154	Suogang
Papuan.DG, Tianyuan	0.980153	0.012916	0.405	0.595	0.13	Tanshishan
Papuan.DG, Tianyuan	0.821696	0.000289	0.514	0.486	0.112	Xiaogao
Papuan.DG, Tianyuan	0.985877	0.002719	0.424	0.576	0.112	Xitoucun
Papuan.DG, Laos Hoabinhian	0.000271	0.345217	0.815	0.185	0.25	Tianyuan
Papuan.DG, Laos Hoabinhian	0.000069		0.686	0.314	0.261	Liangdao1
Papuan.DG, Laos Hoabinhian	0.001501		0.745	0.255	0.2	Liangdao2
Papuan.DG, Laos Hoabinhian	0.000372		0.687	0.313	0.214	Qihe
Papuan.DG, Laos Hoabinhian	0.004771		0.681	0.319	0.139	Suogang
Papuan.DG, Laos Hoabinhian	0.000284		0.663	0.337	0.19	Tanshishan
Papuan.DG, Laos Hoabinhian	0.002306		0.651	0.349	0.153	Xiaogao
Papuan.DG, Laos Hoabinhian	0.000182		0.564	0.436	0.158	Xitoucun
Papuan.DG, Liangdao1	0.084229	0.002523	0.426	0.574	0.141	Tianyuan
Papuan.DG, Liangdao1	0.019140		0.248	0.752	0.212	Laos Hoabinhian
Papuan.DG, Liangdao1	0.005859		0.868	0.132	0.058	Liangdao2
Papuan.DG, Liangdao1	0.013679		0.755	0.245	0.075	Qihe

Papuan.DG, Liangdao1	0.014740		0.73	0.27	0.105	Suogang
Papuan.DG, Liangdao1	0.009572		0.808	0.192	0.054	Tanshishan
Papuan.DG, Liangdao1	0.011913		0.773	0.227	0.074	Xiaogao
Papuan.DG, Liangdao1	0.010854		0.796	0.204	0.047	Xitoucun
Papuan.DG, Liangdao2	0.048679	0.000502	0.704	0.296	0.087	Tianyuan
Papuan.DG, Liangdao2	0.000370		0.737	0.263	0.124	Laos Hoabinhian
Papuan.DG, Liangdao2	0.039308		0.821	0.179	0.043	Liangdao1
Papuan.DG, Liangdao2	0.045997		0.799	0.201	0.053	Qihe
Papuan.DG, Liangdao2	0.055109		0.766	0.234	0.068	Suogang
Papuan.DG, Liangdao2	0.033535		0.835	0.165	0.043	Tanshishan
Papuan.DG, Liangdao2	0.031693		0.837	0.163	0.048	Xiaogao
Papuan.DG, Liangdao2	0.033404		0.839	0.161	0.036	Xitoucun
Papuan.DG, Qihe	0.436190	0.000088	0.469	0.531	0.108	Tianyuan
Papuan.DG, Qihe	0.022257		0.342	0.658	0.162	Laos Hoabinhian
Papuan.DG, Qihe	0.073110		0.709	0.291	0.066	Liangdao1
Papuan.DG, Qihe	0.018344		0.817	0.183	0.062	Liangdao2
Papuan.DG, Qihe	0.045800		0.726	0.274	0.093	Suogang
Papuan.DG, Qihe	0.024852		0.804	0.196	0.051	Tanshishan
Papuan.DG, Qihe	0.045813		0.744	0.256	0.068	Xiaogao
Papuan.DG, Qihe	0.032164		0.789	0.211	0.044	Xitoucun
Papuan.DG, Suogang	0.764642	0.001596	0.255	0.745	0.086	Tianyuan
Papuan.DG, Suogang	0.490427		0.197	0.803	0.083	Laos Hoabinhian

Papuan.DG, Suogang	0.037382		0.6	0.4	0.084	Liangdao1
Papuan.DG, Suogang	0.025269		0.623	0.377	0.1	Liangdao2
Papuan.DG, Suogang	0.037940		0.582	0.418	0.108	Qihe
Papuan.DG, Suogang	0.027064		0.634	0.366	0.083	Tanshishan
Papuan.DG, Suogang	0.072029		0.516	0.484	0.098	Xiaogao
Papuan.DG, Suogang	0.025077		0.656	0.344	0.062	Xitoucun
Papuan.DG, Tanshishan	0.112507	0.000184	0.518	0.482	0.119	Tianyuan
Papuan.DG, Tanshishan	0.006714		0.477	0.523	0.157	Laos Hoabinhian
Papuan.DG, Tanshishan	0.040368		0.761	0.239	0.056	Liangdao1
Papuan.DG, Tanshishan	0.014040		0.846	0.154	0.057	Liangdao2
Papuan.DG, Tanshishan	0.027621		0.782	0.218	0.072	Suogang
Papuan.DG, Tanshishan	0.021931		0.813	0.187	0.055	Xiaogao
Papuan.DG, Tanshishan	0.018115		0.84	0.16	0.035	Xitoucun
Papuan.DG, Xiaogao	0.187825	0.000097	0.639	0.361	0.096	Tianyuan
Papuan.DG, Xiaogao	0.021444	0.000126	0.569	0.431	0.133	Laos Hoabinhian
Papuan.DG, Xiaogao	0.134800	0.000015	0.74	0.26	0.058	Liangdao1
Papuan.DG, Xiaogao	0.033701	0.002991	0.845	0.155	0.051	Liangdao2
Papuan.DG, Xiaogao	0.114497	0.000094	0.754	0.246	0.06	Qihe
Papuan.DG, Xiaogao	0.162277	0.000317	0.705	0.295	0.08	Suogang
Papuan.DG, Xiaogao	0.065057	0.000073	0.808	0.192	0.047	Tanshishan
Papuan.DG, Xiaogao	0.061302	0.000005	0.818	0.182	0.039	Xitoucun
Papuan.DG, Xitoucun	0.296973	0.000054	0.573	0.427	0.098	Tianyuan
Papuan.DG, Xitoucun	0.023580		0.495	0.505	0.135	Laos Hoabinhian
Papuan.DG, Xitoucun	0.057260		0.796	0.204	0.048	Liangdao1
Papuan.DG, Xitoucun	0.016774		0.873	0.127	0.046	Liangdao2
Papuan.DG, Xitoucun	0.049152		0.803	0.197	0.052	Qihe
Papuan.DG, Xitoucun	0.054845		0.785	0.215	0.064	Suogang

Papuan.DG, Xitoucun	0.022820		0.862	0.138	0.036	Tanshishan
Papuan.DG, Xitoucun	0.030045		0.838	0.162	0.048	Xiaogao

Using the *qpWave* results, we also attempted to find the best fitting source using the “rotating” approach described in ref. 108, keeping Mbuti as the calculation baseline and cycling in turn Kostenki14, Onge, Denisova, and the Asians groups listed above from the references to the source alongside Papuan. In this instance, Onge and Tianyuan are the only two sources that provide a working model, resulting in around 50% Asian- and 50% Papuan-related contributions (Fig. 3a and SI Table 26).

SI Table 26: Results of *qpAdm* analyses showing the p-value and nested p-value when modelling Leang Panninge as an admixture between Papuan and ancient individuals using the “rotating” approach¹⁰⁸; p-values above 0.05 are indicated in bold.

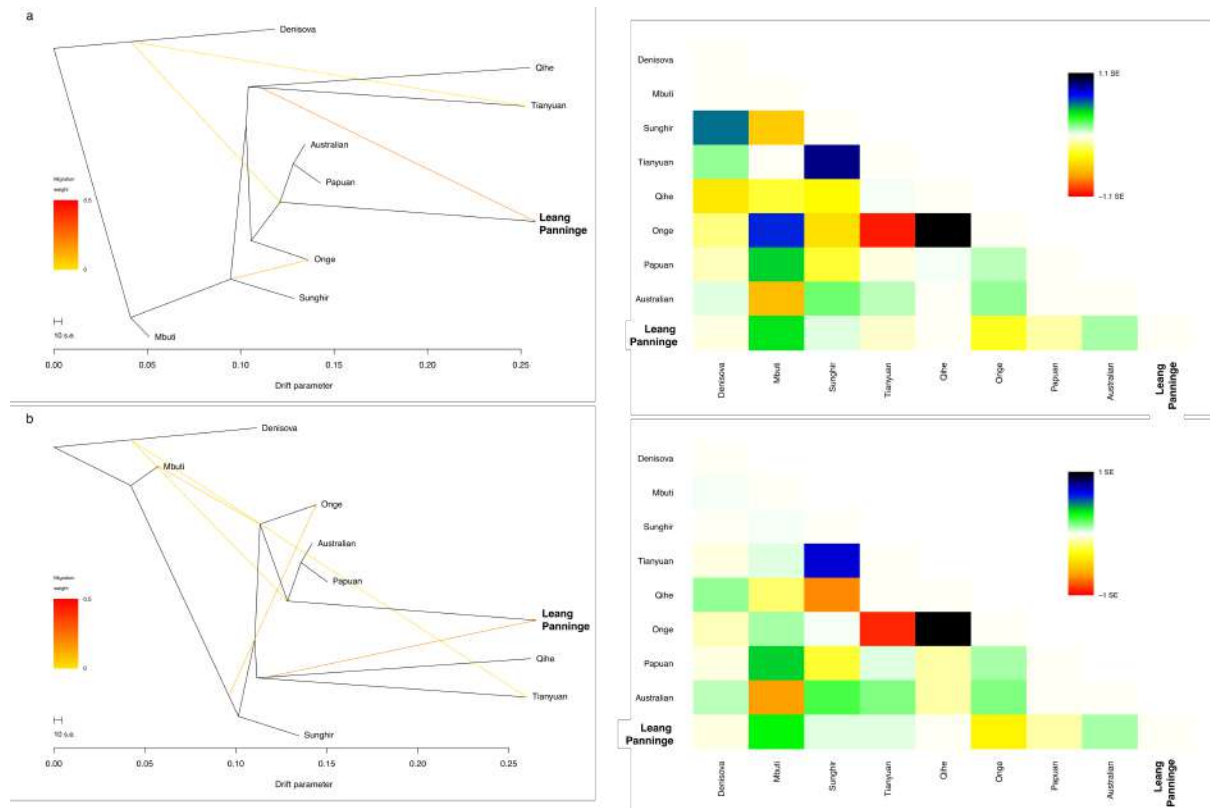
sources (left)	p-value	nested p	source1 %	source2 %	std. err. %
Papuan.DG, Onge.DG	0.500733	0.000003	57.2	42.8	8.9
Papuan.DG, Tianyuan	0.12648	0.000118	48.7	51.3	10.7
Papuan.DG, Xiaogao	0.023194	0.000002	83.3	16.7	3.4
Papuan.DG, Suogang	0.017916	0.000003	68.5	31.5	5.5
Papuan.DG, Liangdao2	0.011545	0.000005	86	14	3
Papuan.DG, Xitoucun	0.007175	0.000059	88	12	2.9
Papuan.DG, Qihe	0.007	0.000014	83	17	3.7
Papuan.DG, Tanshishan	0.006288	0.000022	85.8	14.2	3.2
Papuan.DG, Liangdao1	0.003605	0.000078	84.3	15.7	3.8
Papuan.DG, Denisova	0.001328	0.059912	101.5	-1.5	0.8
Papuan.DG, Laos Hoabinhian	0.000398	0.024648	64.7	35.3	19.9
Papuan.DG, Kostenki14	0.000005	0.723388	95.3	4.7	9.5

TreeMix

The Eigenstrat genotypes of a subset of eight groups were first converted into PED format using *convertf* from the EIGENSOFT package v.7.2.1 (<https://github.com/DReichLab/EIG>)⁸⁷, and then to PLINK format, and stratified allele frequencies were then calculated using *PLINK* v.1.9 (www.cog-genomics.org/plink/1.9/)¹⁰⁹. These files were converted into *TreeMix* format with a python script (*plink2treemix.py*, <https://bitbucket.org/nygcresearch/treemix/downloads/>). Finally, all (0,0) alleles were

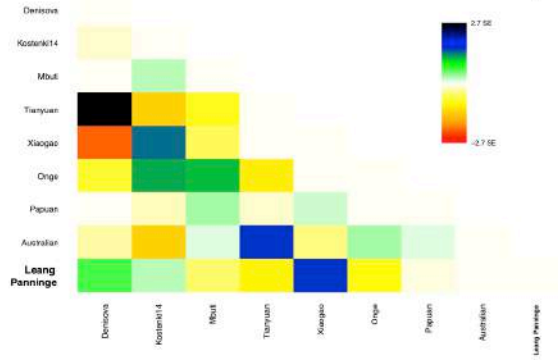
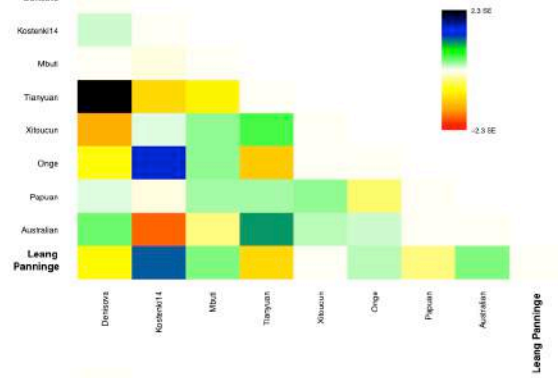
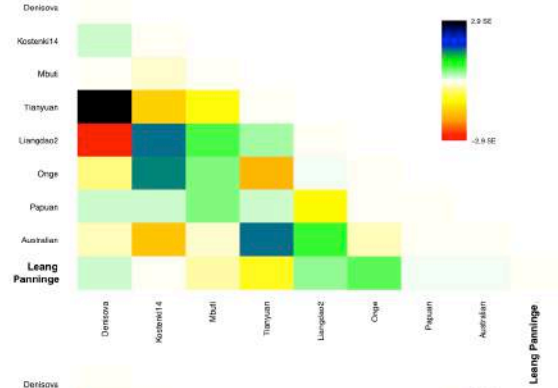
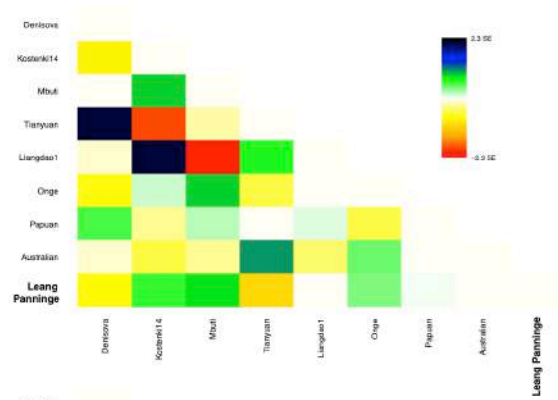
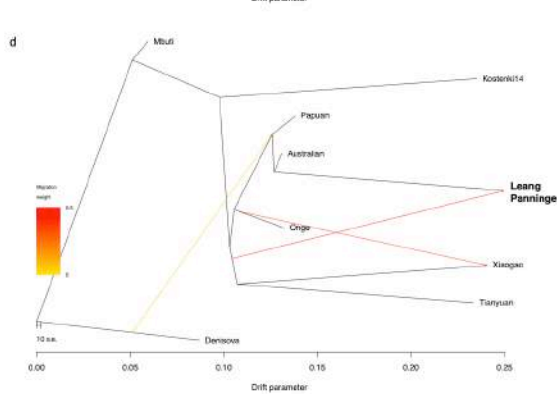
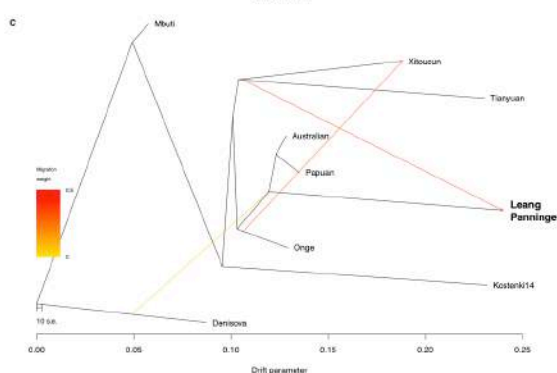
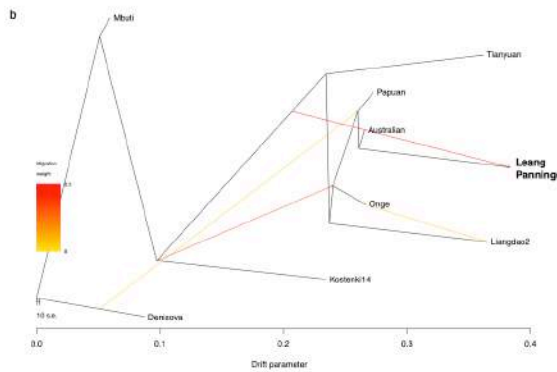
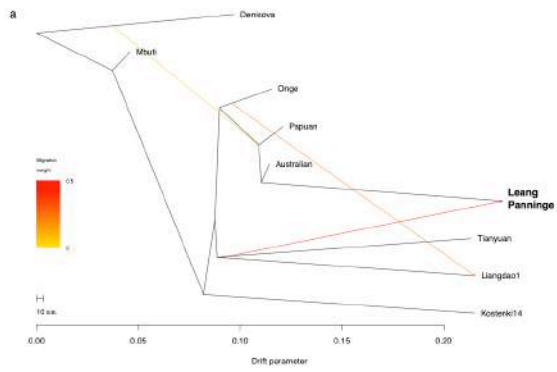
removed. *TreeMix* v.1.12¹¹⁰ was run using the Denisova genome⁹⁴ as a root. The resulting phylogenies were plotted using the R functions provided with *TreeMix* in *RStudio* v.1.2.1335 utilising the package *RColorBrewer* v.1.1.2.

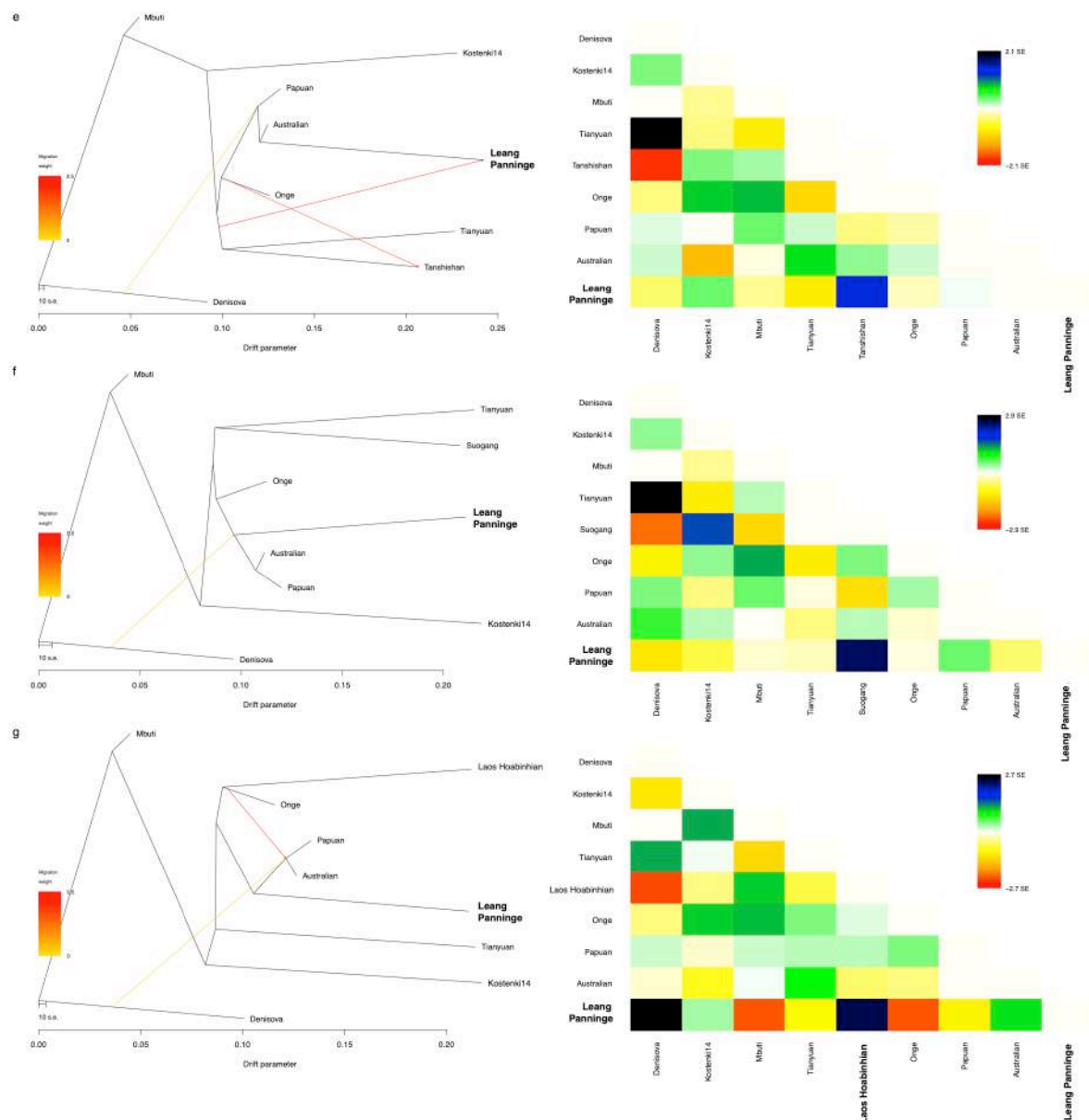
We then used *TreeMix* to model the relationships between the Denisova⁹⁴, Mbuti⁹², Onge⁹², Indigenous Australian⁹², Papuan⁹³, Sunghir¹¹¹, Tianyuan⁹⁰, Qihe⁹¹ and the Leang Panninge genome. The initial maximum likelihood tree showed that Leang Panninge fell outside the Oceanian clade as indicated by f -statistics (Extended Data Fig. 7b), but with relatively large residuals (Extended Data Fig. 10a). The first migration edge added by the algorithm introduced Denisovan-related ancestry into the clade containing Leang Panninge, Papuan, and Indigenous Australian individuals (Extended Data Fig. 10b). However, large standard errors remained when comparing the relationship of Leang Panninge to Qihe and the Denisovan. Allowing for another migration event, which branched off basally on the Qihe-related lineage and led into Leang Panninge, reduced the standard errors of all Leang Panninge-related statistics to around 0 (Fig. 3b, Extended Data Fig. 10c). A third migration event introduced Denisovan introgression into Tianyuan as it is also observed in the f_4 -ratio analyses (SI Tables 18-20, Extended Data Fig. 10d). Results of additional migration events are included here for completeness (SI Fig. 6).



SI Figure 6a: Maximum likelihood trees estimating the genetic relationship of Leang Panninge with other Asia-Pacific groups adding additional migration events than the one reported in Extended Data Fig. 10; **a**, adding four migration events; **b**, adding five migration events; presented with corresponding residuals on the right.

In *TreeMix* we also tested the relationship of ancient Asian groups other than Qihhe showing an affinity to Leang Panninge in *qpWave* analyses. All tested groups showed a gene flow event as the third or fourth inferred admixture edge from a deep Asian-related lineage into Leang Panninge (SI Fig. 6b). Suogang and the Hòabìnhian individual from Laos represent the only exceptions, reaching residuals lower than $Z=|3|$ with fewer admixture edges, possibly due to their low SNP coverage.



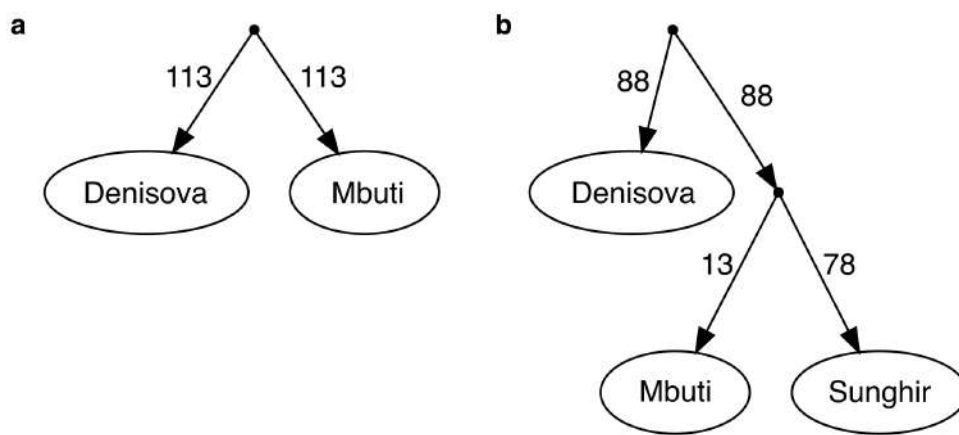


SI Figure 6b: Maximum likelihood trees estimating the genetic relationship of Leang Panninge with other Asia-Pacific groups^{78,91}, presenting the lowest number of migration events resulting in worst z-scores below $|3|$.

qpGraph

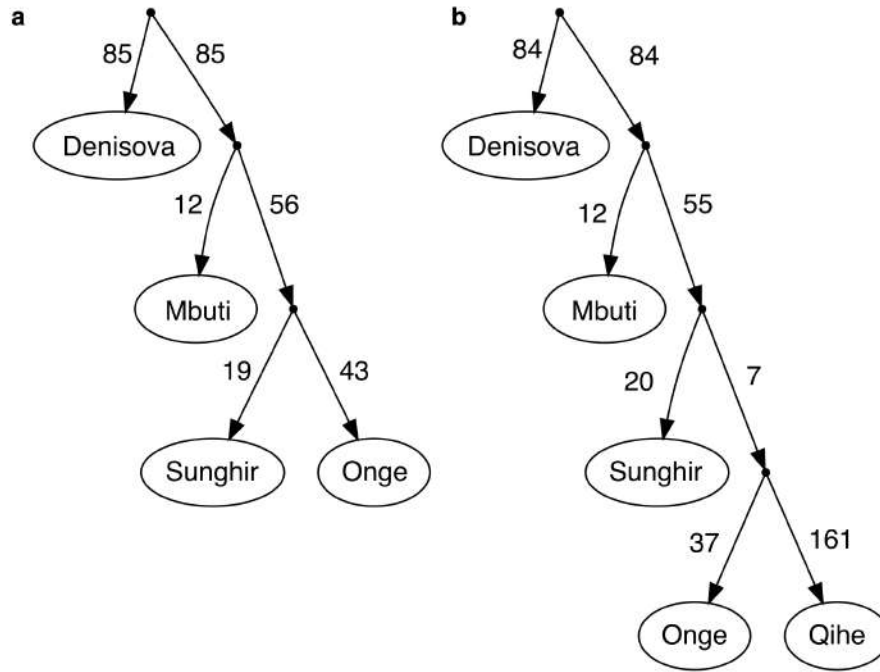
We constructed *qpGraph* scaffolds using these parameters under AdmixTools v.5.0⁸⁴: outpop: NULL, useallsnps: YES, blgsize: 0.05, forcezmode: YES, lsqmode: YES, diag: .0001, bigiter: 15, hires: YES, lambdascale: 1, initmix: 1000, inbreed: YES. We added one group after the other, moving from archaic humans to modern groups and ancient samples according to previously established branching patterns, testing all possible one- and two-way mixtures using a custom-made script.

The first scaffold was composed of five Mbuti individuals⁹² and the Denisovan genome⁹⁴. As expected, this two-group model did not result in any “outlier” – that is, f_4 -statistics where the calculated value using the proposed tree compared to the observed values differ with a z-score larger than 3 or smaller than -3 - and achieved a worst z-score of 0.013 (SI Fig. 7a). Then, we added the genomes of four Upper Paleolithic individuals from Sunghir (Russia)¹¹¹ as representatives of ancient non-African European-related lineages. The lowest worst z-score was achieved by fitting Sunghir as a sister group to Mbuti (worst z-score 0.024, SI Fig. 7b).



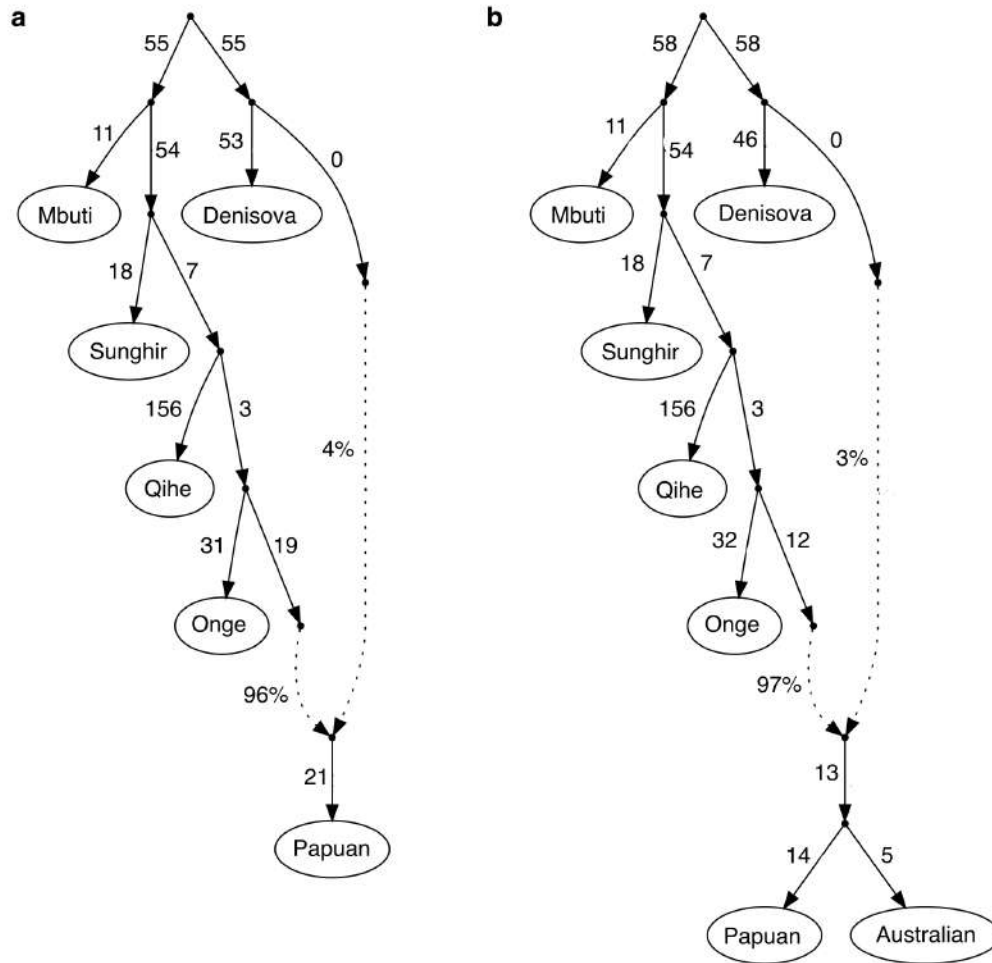
SI Figure 7: The *qpGraph* scaffolds with the lowest worst z-scores modelling the relationships between: **a**, the Denisova and Mbuti (worst z-score 0.013), plus; **b**, ancient individuals from Sunghir, Russia (worst z-score 0.024).

Next, we added the genomes of two Onge individuals from the Andaman Islands⁹² to introduce eastern Eurasian ancestry. The best fitting tree models Onge as a sister group to ancient Sunghir individuals (worst z-score 1.873, SI Fig. 8a). To represent more specifically mainland East Asian ancestry, the Neolithic Qihe individual⁹¹ from present-day China was then added, forming a sister group to Onge (worst z-score -2.447, SI Fig. 8b).



SI Figure 8: The *qpGraph* scaffolds with the lowest worst z-scores modelling the relationships between: **a**, African, western and eastern Eurasian (worst z-score 1.873); and **b**, ancient East Asian lineages (worst z-score -2.447).

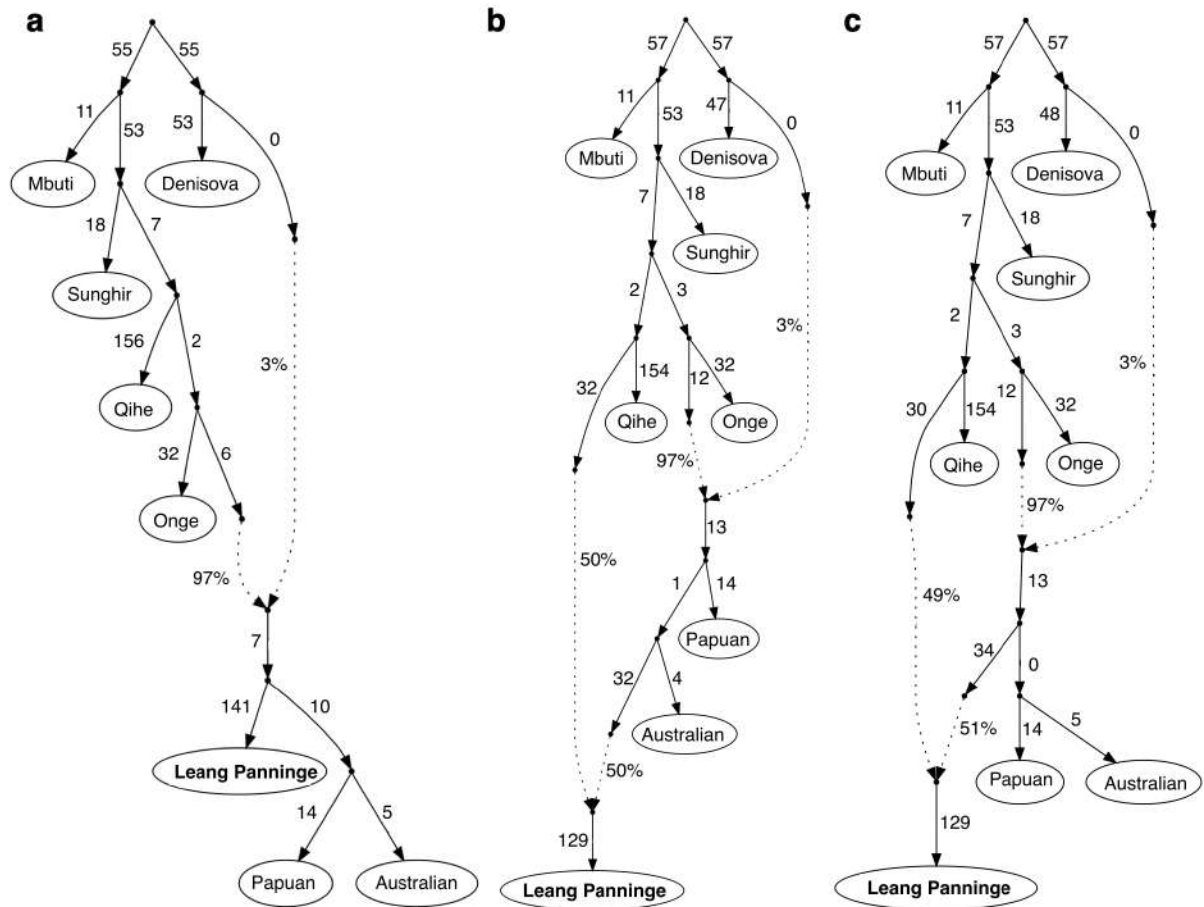
The next added group is formed by 16 individuals from Papua New Guinea⁹³. The lowest worst z-score was calculated for a tree modeling Papuan as a mixture of Onge- and Denisova-related ancestry (worst z-score 1.556, SI Fig. 9a). As expected, two individuals from Aboriginal Australia⁹² were placed on the tree as a sister group to the Papuan individuals (worst z-score 2.085, SI Fig. 9b), with 3% Denisova-related introgression into the common ancestor as reported in previous publications^{85,97,102}.



SI Figure 9: The *qpGraph* scaffolds with the lowest worst z-scores modelling the relationships between: **a**, deep African and Asia-Pacific lineages (worst z-score 1.556); plus **b**, Indigenous Australian individuals showing the Denisovan-related introgression into Papuan and Indigenous Australian groups (worst z-score 2.085).

Finally, we added the Leang Panninge genome to the scaffold at all 91 possible positions resulting from one- and two-way admixtures. The simplest tree, where the new genome simply branches off before Papuan and Indigenous Australian groups split, does not fit, achieving a worst z-score of -4.818 (SI Fig. 10a). The lowest worst z-scores are achieved by modelling Leang Panninge as a two-way admixture between Near Oceanian and Qihe-related ancestries (SI Fig. 10b and c), which is congruent with previous analyses (Fig. 3a, SI Table 26). Any other branching position from the Asian-related portion of the admixture graph results in branch lengths of zero between Qihe and the edge leading to Leang Panninge. The branching point on the Near Oceanian portion of the admixture graph is instead more uncertain. The overall lowest worst z-score of -2.190 is observed when Leang Panninge is

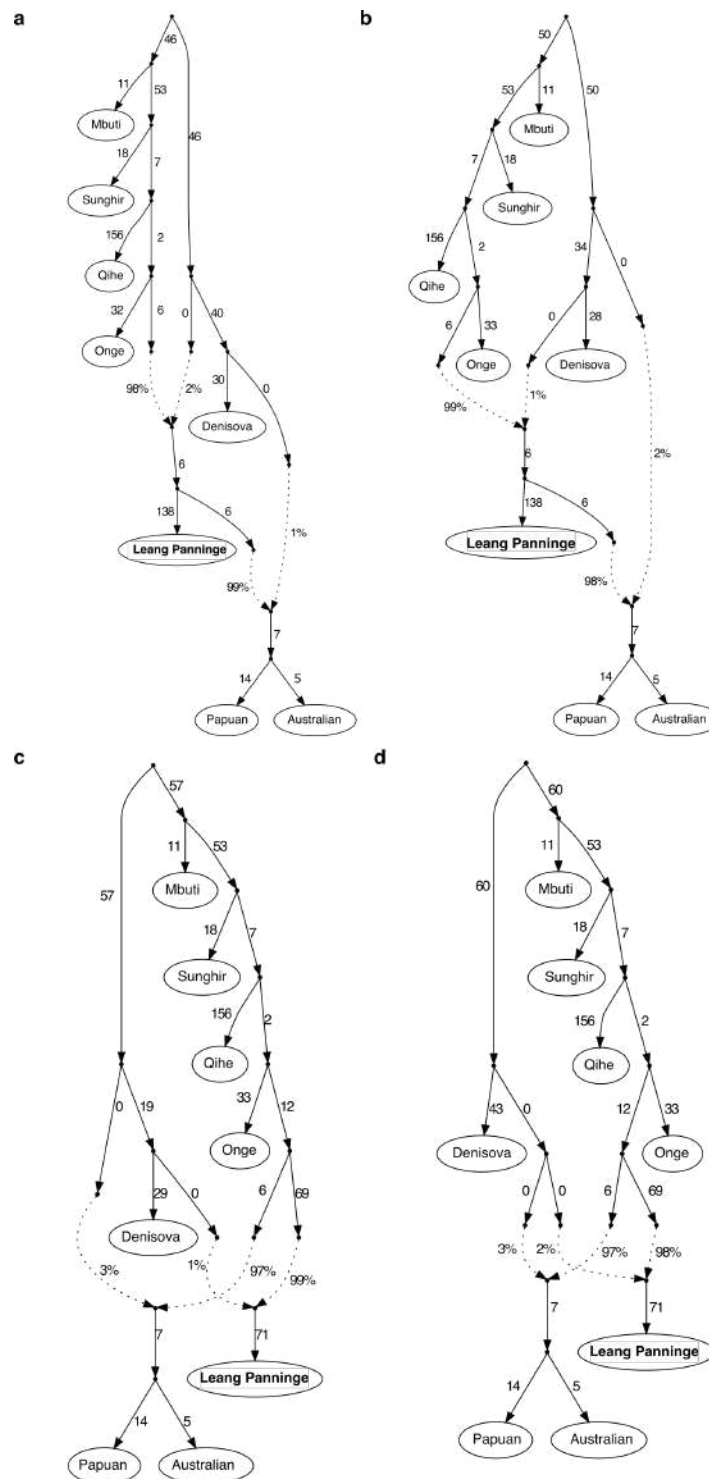
closer to Indigenous Australian than Papuan groups. However, this is only marginally different from the graph where Leang Panninge branches before the Near Oceanian populations split from each other (worst z-score -2.194), consistent with the f_4 -statistic results (Extended Data Fig. 7b). Both scaffolds contain branch length of 1 or 0, respectively, within the Leang Panninge-Oceanian clade; therefore, we present these groups as deriving from a trifurcation (Fig. 3c).



SI Figure 10: Fitting Leang Panninge on the *qpGraph* scaffold: **a**, as a one-way admixture (worst z-score -4.818); **b**, as a two-way admixture branching closer to Indigenous Australian than Papuan (worst z-score -2.190); and **c**, as a two-way admixture branching before the Australo-Papuan population split (worst z-score -2.194).

To rule out the possibility that the observed reduced amount of Denisovan ancestry in Leang Panninge compared with present-day Near Oceanians is due to repeated interactions with different Denisovan groups, we also modelled this scenario in four additional admixture graphs (SI Fig. 11). While these fit better than the one-way Denisovan admixture model

presented in SI Fig 10a – likely due to the different amounts of Denisovan-related ancestry present in Leang Panninge and Near Oceanians – the worst z-scores remain close to $|4|$. This is opposed to the graphs with one common Denisovan-related admixture into Oceanians Leang Panninge followed by an Asian-related admixed into Leang Panninge which provide worst z-scores below $|3|$ (SI Fig. 10b-c).



SI Figure 11: Fitting Leang Panninge on the *qpGraph* scaffold as a sister group to Near Oceanians: **a**, with sequential Denisovan admixtures (worst z-score -3.814); **b**, with sequential Denisova admixtures where the admixture into Near Oceanians is more basal on the Denisovan lineage (worst z-score 4.068); **c**, with separate Denisovan admixtures where the lineage into Leang Panninge is more closely related to the sequenced Denisovan individual (worst z-score -4.044); and **d**, with separate Denisovan admixtures where the lineage into Leang Panninge is equally distant to the sequenced Denisovan individual (worst z-score -3.913).

DATES

Having established different lines of evidence for the presence of Asian-related admixture into Leang Panninge, we attempted to date the admixture using *DATES* v.753¹¹². However, the values calculated for ancient as well as a grouping of present-day individuals (Ami, Atayal, Han, Igorot, Kinh, Dai, Lahu, She; 1240K panel) did not yield reliable results (SI Table 27).

SI Table 27: Inferred admixture date estimates simulating Leang Panninge as an admixture between Papuan and Asian-related ancestry with DATES.

target	source 1	source 2	mean admixture time	std. err.
Leang Panninge	Papuan.DG	Liangdao1	48.418	55.827
Leang Panninge	Papuan.DG	Qihe	-52,220.398	26,877.414
Leang Panninge	Papuan.DG	Suogang	-10,009.262	4,068.558
Leang Panninge	Papuan.DG	Tianyuan	187.816	200.514
Leang Panninge	Papuan.DG	East Asians	422.033	728.927

References

1. Sarasin, P. & Sarasin, F. *Reisen in Celebes* (C.W. Kreidel's Verlag, Wiesbaden, 1905).
2. van Heekeren, H. R. *The Stone Age of Indonesia* (Martinus Nijhoff, The Hague, 1972).
3. Mulvaney, D. J. & Soejono, R. P. Archaeology in Sulawesi, Indonesia. *Antiquity* **45**, 26–33 (1970).
4. Mulvaney, D. J. & Soejono, R. P. The Australian-Indonesian Archaeological Expedition to Sulawesi. *Asian Perspect.* **13**, 163–177 (1970).
5. Mulvaney, D. J. *The Prehistory of Australia* (Thames & Hudson, London, 1975).
6. Glover, I. C. Ulu Leang Cave, Maros: A preliminary sequence of post-Pleistocene cultural development in South Sulawesi. *Archipel* **11**, 113–154 (1976).
7. Bulbeck, D., Pasqua, M., & Di Lello, A. Culture history of the Toalean of South Sulawesi, Indonesia. *Asian Perspect.* **39**(1), 71–108 (2000).
8. Perston, Y.L. et al. A standardised classification scheme for the Mid-Holocene Toalean artefacts of South Sulawesi, Indonesia. *PLoS ONE* **16**(5), e0251138 (2021).
9. Suryatman, et al. Artefak batu preneolitik situs Leang Jarie: bukti teknologi Maros Point tertua di Kawasan budaya Toalean, Sulawesi Selatan. *AMERTA* **37**(1), 1–17 (2019).
10. Macknight, C. C. The joint Australian–Indonesian archaeological expedition to South Sulawesi. In *The Archaeology of Sulawesi: Current Research on the Pleistocene to the Historic Period* (eds. O'Connor, S., Bulbeck, D. & Meyer, J.) 43–59 (Terra Australis 48, ANU Press, Canberra, 2018).

11. Bulbeck, D. Economic and technological change during the middle and late Holocene in the Lamongcong highlands, South Sulawesi, Indonesia. In *Uncovering Southeast Asia's Past* (eds. Bacus, E. A., Glover, I. C. & Pigott, V. C.) 393–410 (National University of Singapore, Press Singapore, 2006).
12. Fillios, M. A. & Taçon, P. S. C. Who let the dogs in? A review of the recent genetic evidence for the introduction of the dingo to Australia and implications for the movement of people. *J. Arch. Sci.: Rep.* **7**, 782–792 (2016).
13. Bellwood, P. *First Migrants: Ancient Migration in Global Perspective* (Wiley, Chichester, 2013).
14. Bellwood, P. *First Islanders: Prehistory and Human Migration in Island Southeast Asia* (Wiley, Hoboken, 2017).
15. Bulbeck, D. An archaeological perspective on the diversification of the languages of the South Sulawesi stock. In *Austronesian in Sulawesi* (ed. Simanjuntak, T.) 185–212 (Center for Prehistoric and Austronesian Studies, Jakarta, 2008).
16. Bulbeck, D. Holocene site occupancy in Sulawesi. In *The Archaeology of Sulawesi: Current Research on the Pleistocene to the Historic Period* (eds. O'Connor, S., Bulbeck, D. & Meyer, J.) 93–116 (Terra Australis 48, ANU Press, Canberra, 2018).
17. Hakim, B. Interpretasi awal temuan gigi manusia di situs Bala Metti, Bone dan situs Leang Jarie, Maros, Sulawesi Selatan. *Walennae* **15**(10), 19–30 (2017).
18. Balar Sulsel Research Team. Laporan penelitian arkeologi di Mallawa, Kabupaten Maros, Sulawesi Selatan (Unpublished report, Balai Arkeologi Sulawesi Selatan, Makassar, 2014).
19. Balai Pelestarian Cagar Budaya Research Team. Laporan ekskavasi penyelamatan situs Leang Panning di desa Batu Putih kecamatan Mallawa kabupaten Maros provinsi Sulawesi Selatan (Unpublished report, Balai Pelestarian Cagar Budaya, Sulawesi Selatan, Makassar, 2015).

20. Hasanuddin. Gua Panningnge di Mallawa, Maros: kajian tentang gua hunian berdasarkan artefak batu dan sisa fauna. *Naditira Widya* **11**, 81–96 (2017).
21. Balar Sulsel, Universitas Hasanuddin & Universitas Sains Malaysia Research Team. Laporan Ekskavasi Liang Panningnge (Unpublished report, Balai Arkeologi Sulawesi Selatan, Makassar, 2016).
22. Balar Sulsel Research Team. Laporan penelitian arkeologi ekskavasi Gua Panningge Mallawa, Maros (Unpublished report, Balai Arkeologi Sulawesi Selatan, Makassar, 2016).
23. Duli, A., Akhmar, A. M. & Nur, M. Awal peradaban di Sulawesi, kajian arkeologi pada situs gua Panningnge di kecamatan Mallawa, kabupaten Maros (Unpublished report, Universitas Hasanuddin, Makassar, 2018).
24. Saiful, M. A. & Anggraeni. Eksploitasi Suidae pada kala Holosen di Liang Panningge, Maros, Sulawesi Selatan. *Purbawidya* **8**(2), 81–100 (2019).
25. Al Anshari, K. Teknologi lancipan Maros di situs Leang Panningge kecamatan Mallawa kabupaten Maros (Unpublished undergraduate thesis, Universitas Hasanuddin, Makassar, 2018).
26. Saiful, M. A. Suidae dalam strategi subsistensi penghuni Liang Panningnge, Maros, Sulawesi Selatan (Unpublished MA thesis, Universitas Gadjah Mada, Jogjakarta, 2019).
- 27 Hogg, A. et al. SHCal20 Southern Hemisphere Calibration, 0–55,000 Years cal BP. *Radiocarbon* **62**(4), 759–778 (2020).
28. Taylor, R. E. *Radiocarbon Dating: An Archaeological Perspective* (Academic Press, Orlando, 1987).
29. Brock, F., Higham, T., Ditchfield, P. & Ramsey, C. Current pretreatment methods for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (Orau). *Radiocarbon* **52**(1), 103–112 (2010).

30. Philippsen, B. The freshwater reservoir effect in radiocarbon dating. *Herit. Sci.* **1**, 24 (2013).
31. Hillson, S. *Dental Anthropology* (Cambridge University Press, Cambridge, 1996).
32. Brothwell, D. R. *Digging up Bones* (British Museum, London, 1981).
33. White, T. & Folkens, P. A. *Human Bone Manual* (Elsevier Academic Press, Amsterdam, 2005).
34. Davivongs, V. The pelvic girdle of the Australian Aborigine: Sex differences and sex determination. *Am. J. Phys. Anthropol.* **21**, 443–455 (1963).
35. Larnach, S. L. & Freedman, L. Sex determination of Aboriginal crania from coastal New South Wales. *Rec. Aust. Mus.* **26**, 295–308 (1964).
36. Larnach, S. L. & Macintosh, N.W.G. *The Mandible in Eastern Australian Aborigines* (Oceania Monographs 17, University of Sydney, Sydney, 1971).
37. Loth, S. R. & Henneberg, M. Mandibular ramus flexure: a new morphologic indicator of sexual dimorphism in the human skeleton. *Am. J. Phys. Anthropol.* **99**, 473–485 (1996).
38. Brace, C. L. Australian tooth-size clines and the death of a stereotype. *Curr. Anthropol.* **21**, 141–164 (1980).
39. Freedman, L. & Lofgren, M. Odontometrics of Western Australian Aborigines. *Archaeol. Oceania* **16**, 87–93 (1981).
40. Brown, P. J. The ultrastructure of dental abrasion: its relationship to diet (Unpublished BA Honours thesis, The Australian National University, Canberra, 1978).
41. Doran, G. A. & Freedman, L. Metrical features of the dentition and arches of populations for Goroka and Lufa, Papua New Guinea. *Hum. Biol.* **3**, 583–594 (1974).

42. Bulbeck, F. D. Continuities in Southeast Asian evolution since the Late Pleistocene (Unpublished MA thesis, The Australian National University, Canberra, 1981).
43. Snell, C. A. R. D. *Menschelijke Skeletresten uit de Duin Formatie van Java's Zuidkust nabij Poeger (Z. Banjoewangi)* (G. Kolff, Surabaya, 1938).
44. Bulbeck, D., Kadir, R. A., Lauer, A., Radzi, Z. & Rayner, D. Tooth sizes in the Malay Peninsula past and present: insights into the time depth of the Indigenous inhabitants' adaptations. *Int. J. Indig. Res.* **1**, 41–50 (2005).
45. Scott, G. R. & Turner II, C. G. *The Anthropology of Modern Human Teeth: Dental Morphology and its Variation in Recent Human Populations* (Cambridge University Press, Cambridge, 1997).
46. Larnach, S. L. & Macintosh, N. W. G. *The Craniology of the Aborigines of Queensland* (Oceania Monographs 15, University of Sydney, Sydney, 1970).
47. Howells, W. W. *Skull Shapes and the Map* (Papers of the Peabody Museum of Archaeology and Ethnology 79, Harvard University, Cambridge, MS, 1989).
48. Green, M. K. Prehistoric cranial variation in Papua New Guinea (Unpublished PhD thesis, The Australian National University, Canberra, 1990).
49. Cleaver, F.H. A contribution to the biometric study of the human mandible. *Biometrika* **29**, 80–112 (1937).
50. Ray, L. J. Metrical and non-metrical features of the clavicle of the Australian Aboriginal. *Am. J. Phys. Anthropol.* **17**, 217–226 (1959).
51. Rao, P. D. P. The anatomy of the distal limb segments of the Aboriginal skeleton (Unpublished PhD thesis, University of Adelaide, Adelaide, 1966).
52. Brown, P. J. *Coobool Creek* (Terra Australis 13, The Australian National University, Canberra, 1989).

53. Bräuer, G. 2. Osteometrie. a. Kraniometrie. In *Lehrbuch der Anthropologie. Band I. Wesen und Methoden der Anthropologie. 1. Teil. Wissenschaftstheorie, Geschichte, Morphologischen Methoden* (ed. Knußman, R.) 160–192 (Gustav Fischer Verlag, Stuttgart, 1988).
54. Jacob, T. *Some Problems Pertaining to the Racial History of the Indonesian Region* (Netherlands Bureau for Technical Assistance, Utrecht, 1967).
55. Houghton, P. Rocker jaws. *Am. J. Phys. Anthropol.* **47**, 365–370 (1977).
56. Villiers, H. de. *The Skull of the South African Negro: A Biometric and Morphological Study* (Witwatersrand University Press, Johannesburg, 1968).
57. Sawyer, D. F., Allison, M. J., Elzay, R. P. & Pezzia, A. The mylohyoid bridge of pre-Columbian Peruvians. *Am. J. Phys. Anthropol.* **48**, 9–16 (1978).
58. Pinhasi, R. et al. Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PloS One* **10**(6), e0129102 (2015).
59. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U.S.A.* **110**(39), 15758–15763 (2013).
60. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **370**(1660), 20130624 (2015).
61. Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **5**(6) pdb.prot5448 (2010).
62. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**(1), e3 (2012).

63. Gansauge, M.-T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**(8), 2279–2300 (2020).
64. Peltzer, A. et al. EAGER: efficient ancient genome reconstruction. *Genome Biol.* **17**, 60 (2016).
65. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**(7), 553–559 (2013).
66. Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**(7564), 216–219 (2015).
67. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**(14), 1754–1760 (2009).
68. Sawyer, S., Krause, J., Guschanski, K., Savolainen, V. & Pääbo, S. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PloS One* **7**(3), e34131 (2012).
69. Peyrégne, S., & Peter, B. M. AuthentiCT: a model of ancient DNA damage to estimate the proportion of present-day DNA contamination. *Genome Biol.* **21**(1), 1–16 (2020).
70. Renaud, G., Slon, V., Duggan, A. T. & Kelso, J. Schmutzi: estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA. *Genome Biol.* **16**(1), 224 (2015).
71. Furtwängler, A. et al. Ratio of mitochondrial to nuclear DNA affects contamination estimates in ancient DNA analysis. *Sci. Rep.* **8**(1), 14075 (2018).
72. Vianello, D. et al. HAPLOFIND: a new method for high-throughput mtDNA haplogroup assignment. *Hum. Mutat.* **34**(9), 1189–1194 (2013).

73. Fu, Q. et al. DNA analysis of an early modern human from Tianyuan Cave, China. *Proc. Natl. Acad. Sci. U.S.A.* **110**(6), 2223–2227 (2013).
74. de Barros Damgaard, P. et al. The first horse herders and the impact of early Bronze Age steppe expansions into Asia. *Science* **360**(6396), eaar7711 (2018).
75. Yu, H. et al. Paleolithic to Bronze Age Siberians reveal connections with first Americans and across Eurasia. *Cell* **181**, 1232–1245 (2020).
76. Sikora, M. et al. The population history of northeastern Siberia since the Pleistocene. *Nature* **570**(7760), 182–188 (2019).
77. Devière, T. et al. Compound-specific radiocarbon dating and mitochondrial DNA analysis of the Pleistocene hominin from Salkhit Mongolia. *Nat. Commun.* **10**, 274 (2019).
78. McColl, H. et al. The prehistoric peopling of Southeast Asia. *Science* **361**(6397), 88–92 (2018).
79. Katoh, K., Misawa, K., Kuma, K.-i. & Miyata, T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**(14), 3059–3066 (2002).
80. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **35**(6), 1547–1549 (2018).
81. van Oven, M. & Kayser, M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum. Mutat.* **30**(2), E386–394 (2009).
82. Skoglund, P. et al. Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. *Proc. Natl. Acad. Sci. U.S.A.* **111**(6), 2229–2234 (2014).
83. Lazaridis, I. et al. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**(7518), 409–413 (2014).

84. Patterson, N. et al. Ancient admixture in human history. *Genetics* **192**(3), 1065–1093 (2012).
85. Qin, P. & Stoneking, M. Denisovan ancestry in east Eurasian and Native American populations. *Mol. Biol. Evol.* **32**(10), 2665–2674 (2015).
86. Skoglund, P. et al. Genomic insights into the peopling of the Southwest Pacific. *Nature* **538**(7626), 510–513 (2016).
87. Patterson, N., Richter, D. J., Gnerre, S., Lander, E. S. & Reich, D. Genetic evidence for complex speciation of humans and chimpanzees. *Nature* **441**(7097), 1103–1108 (2006).
88. Posth, C. et al. Language continuity despite population replacement in Remote Oceania. *Nat. Ecol. Evol.* **2**(4), 731–740 (2018).
89. Kanzawa-Kiriyama, H. et al. A partial nuclear genome of the Jomons who lived 3000 years ago in Fukushima, Japan. *J. Hum. Genet.* **62**(2), 213–221 (2017).
90. Yang, M. A. et al. 40,000-year-old individual from Asia provides insight into early population structure in Eurasia. *Curr. Biol.* **27**(20), 3202–3208.e9. (2017).
91. Yang, M. A. et al. Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* **369**(6501), 282–288 (2020).
92. Mallick, S. et al. The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**(7624), 201–206 (2016).
93. Skoglund, P. et al. Genetic evidence for two founding populations of the Americas. *Nature* **525**(7567), 104–108 (2015).
94. Meyer, M. et al. A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**(6104), 222–226 (2012).

95. Reich, D. et al. Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* **468**(7327), 1053–1060 (2010).
96. Reich, D. et al. Denisova admixture and the first modern human dispersals into Southeast Asia and Oceania. *Am. J. Hum. Genet.* **89**(4), 516–528 (2011).
97. Vernot, B. et al. Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals. *Science* **352**(6282), 235–239 (2016).
98. Kusuma, P. et al. The last sea nomads of the Indonesian archipelago: genomic origins and dispersal. *Eur. J. Hum. Genet.* **25**(8), 1004–1010 (2017).
99. 1000 Genomes Project Consortium, et al. A global reference for human genetic variation. *Nature* **526**(7571), 68–74 (2015).
100. Prüfer, K. et al. The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**(7481), 43–49 (2014).
101. Prüfer, K. et al. A high-coverage Neandertal genome from Vindija Cave in Croatia. *Science* **358**(6363), 655–658 (2017).
102. Bergström, A. et al. Insights into human genetic variation and population history from 929 diverse genomes. *Science* **367**(6484), eaay5012 (2020).
103. Busing, F. M. T. A. et al. Delete-m jackknife for unequal m. *Stat. Comput.* **9**, 3 (1999).
104. Peter, B. M. 100,000 years of gene flow between Neandertals and Denisovans in the Altai mountains. *bioRxiv* (2020).
105. Skov, L. et al. Detecting archaic introgression using an unadmixed outgroup. *PLoS Genet.* **14**(9), e1007641 (2018).
106. Massilani, D. et al. Denisovan ancestry and population history of early East Asians. *Science* **370**(6516), 579–583 (2020).

107. Seguin-Orlando, A. et al. Genomic structure in Europeans dating back at least 36,200 years. *Science* **346**(6213), 1113–1118 (2014).
108. Harney, É. et al. Assessing the performance of qpAdm: a statistical tool for studying population admixture. *bioRxiv* (2020).
109. Chang, C. C. et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* **4**(1), 7 (2015).
110. Pickrell, J. K. & Pritchard, J. K. Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet.* **8**(11), e1002967 (2012).
111. Sikora, M. et al. Ancient genomes show social and reproductive behavior of early Upper Paleolithic foragers. *Science* **358**(6363), 659–662 (2017).
112. Narasimhan, V.M. et al. The formation of human populations in South and Central Asia. *Science*, **365**(6457), eaat7487 (2019).

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- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

EAGER v.1.92.56
BWA v.0.7.12
circularmapper v.1.93.5
AdapterRemoval v.2.3.1
dedup v.0.12.2
mapDamage v.2.0.9
AuthentiCT v1.0
samtools v.1.3
pileupCaller v.1.4.0.2
PMDtools v.0.6
EIGENSOFT v.7.2.1 (convertf)
PLINK v.1.9

Data analysis

schmutzi (NB: schmutzi does not have a version number)
Haplofind (NB: Haplofind does not have a version number)
mafft v.7.305
MEGA v.10.1.5
EIGENSOFT v.7.2.1 (smartpca)
AdmixTools 5.1 (qp3Pop, qpDstat, qpF4Ratio, qpDstat, qpWave, qpAdm, qpGraph)
admixture v.0.6.1
RStudio v.1.2.1335
Treemix v.1.12
RColorBrewer v.1.1.2

ggplot2 v.3.3.3
DATES v.753

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Alignment files of the nuclear and mitochondrial DNA sequences for the newly sequenced Leang Panninge individual are available at the ENA database under the accession number PRJEB43715. These were made public on June 19, 2021.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All genomic analyses were based on genetic material extracted from the petrous portion of the temporal bone of the Leang Panninge individual, a partial skeleton of a 17-18 year old adult human female excavated from middle Holocene archaeological deposits at the limestone cave of Leang Panninge in the Mallawa district of South Sulawesi, Indonesia. Therefore, no statistical methods were used to determine ancient DNA sample size a priori.
Data exclusions	No data were excluded.
Replication	Replication is achieved by comparing the results obtained with the shotgun and the nuclear capture datasets, as well as the single- and double-stranded sequencing data, which was successful.
Randomization	Since the data derive from a single individuals, no randomization was performed.
Blinding	No blinding was performed, as experimental group assignment is not relevant for this type of population genetic analyses. The genotypes of the single- and double-stranded libraries were combined after confirmation of similar statistical behaviour.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	The human skeletal remains that yielded the ancient genome were initially uncovered during University of Hasanuddin-led excavations at the limestone cave of Leang Panninge in 2015. They were found at a depth of 2 m below the surface in stratified archaeological deposits of middle Holocene age (~7.3-7.2 kya). The individual was buried in a shallow pit and partially covered with rocks. Artefacts characteristic of the middle to late Holocene Toalean technocomplex were recovered in association with the skeleton

and in adjacent and overlying deposits. Owing to the fragmentary condition and fragility of the skeletal remains, both the skull and pelvic areas were removed (during Indonesian-led fieldwork) in intact sediment blocks for excavation under laboratory conditions. The petrous bone was recovered during this process and later sent for DNA analysis at the Max Planck Institute for the Science of Human History (MPI-SHH) in Jena, Germany. Joint Indonesian-Australian excavations were conducted at Leang Panninge in 2019 in order to clarify the stratigraphic context of the burial and to collect additional samples for radiocarbon dating. This field research and related work was conducted in collaboration with Prof. Adam Brumm's primary institutional counterpart, the Pusat Penelitian Arkeologi Nasional (ARKENAS) in Jakarta, under the terms of a foreign research permit issued to Brumm by Indonesia's State Ministry of Research and Technology (Permit No: 154/SIP/FRP/E5/Dit.KI/VII/2017).

Specimen deposition

Skeletal remains attributed to the Leang Panninge individual are curated in the archaeology department (Departemen Arkeologi Fakultas Ilmu Budaya) at the University of Hasanuddin in Makassar, Sulawesi (Indonesia). The remaining portion of the petrous bone sampled for DNA analysis is stored at the MPI-SHH under the curation of Prof. Johannes Krause. Requests to access the skeletal material for research should be directed to Prof. Akin Duli (akinduli@yahoo.co.id) at the University of Hasanuddin.

Dating methods

For the study we obtained 13 new Accelerator Mass Spectrometer (AMS) radiocarbon dates on in situ plant charcoal (N = 8), seeds (N = 3), and freshwater gastropod shells (N = 2) excavated from Leang Panninge, including three samples associated directly with the human burial context. Conventional radiocarbon ages were calibrated using the OxCal 4.4 program and a mix_curve IntCal20/SHCal20 calibration curve, with uncertainties reported at 95% confidence interval. Two radiocarbon dating labs were used: DirectAMS (USA), and the Radiocarbon Dating Laboratory at the University of Waikato (New Zealand). Pretreatment processes, quality control protocols, and dating methods used by both labs are provided in Supplementary Information (see especially SI Table 1).

☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

No ethical approval or guidance was required by Griffith University as the research did not involve human participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Supplementary information

Ancient genomes from the last three millennia support multiple human dispersals into Wallacea

In the format provided by the
authors and unedited

Supplementary Information

1. Ethics statement

The human remains analyzed in this study are considered the cultural heritage of the originating countries. Appropriate research permits and export approvals were obtained from Indonesia to conduct the current research. For the skeletal material from Tanjung Pinang and Uattamdi, the research was undertaken as part of a collaborative project between Pusat Penelitian Arkeologi Nasional (Jakarta) and the Australian National University, under Lembaga Ilmu Pengetahuan Indonesia Research Permits 6939/S.K./1990, 307/I/KS/1994, and 10290/V3/KS/1995. For the skeletal material from Aru Manara, the research was undertaken as part of a collaborative project between Pusat Penelitian Arkeologi Nasional (Jakarta) and the Tokai University, under Kementerian Riset dan Teknologi Research Permits 0291/SIP/FRP/VIII/2011 and Pusat Penelitian Arkeologi Nasional export permit UM.001/2595/PAN/KPK/IX/2012, 2634/H5/TU/2017. For the skeletal material from Topogaro, the research was undertaken as part of a collaborative project between Pusat Penelitian Arkeologi Nasional (Jakarta) and the Tokai University, under Kementerian Riset dan Teknologi Research Permits 40/EXT/SIP/FRP/SM/VII/2015 and 194/SIP/FRP/E5/Dit.KI/VII/2017 and Pusat Penelitian Arkeologi Nasional export permit 2634/H5/TU/2017. For the skeletal material from Liang Bua, Liang Toge and Komodo, the research was undertaken as part of a collaborative project between Universitas Airlangga (Surabaya, Indonesia) and the Max Planck Institute for the Science of Human History (Jena, Germany) under the Kementerian Ristekdikti Research Permit 303/SIP/FRP/E5/Dit.KI/IX/2017 and Pusat Penelitian Arkeologi Nasional export permit 11404H5/TU/2017. Research of the human remains from Jareng Bori were carried out as part of a collaboration between Australian National University and Universitas Gadjadjar under the Kementerian Ristekdikti Research Permit 1209/FRP/E5/Dit.KI/VI/2016.

2. Radiocarbon dating

Nine new radiocarbon dates for this study (Supplementary Table 12) were produced at the Curt-Engelhorn-Zentrum Archäometrie gGmbH in Mannheim, Germany. There, collagen from bone and dentin was extracted using a modified Longin method¹ and long molecules removed with ultrafiltration before freeze-drying the product². After pretreatment, organic samples were combusted in an elemental analyser (Microcube, Elementar, Hanau, Germany). Following catalytic reduction to graphite, the ¹⁴C content was measured with an AMS-System type MICADAS³. The Liang Bua individual was dated at the Radiocarbon Dating Laboratory, University of Waikato. The bone was decalcified and then gelatinized before ultrafiltration. Pressed graphite was analysed at the Keck Radiocarbon Dating Laboratory, the University of California on a NEC 0.5MV 1.5SDH-1 AMS system coupled with an in-house modified ion source⁴. Reported $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ values for dietary calibration correction were measured by Isotope Ratio Mass Spectrometry (IRMS) at Iso-trace Research Department of Chemistry, the University of Otago on a Carlo Erba NA 1500 elemental analyser (EA), coupled with either a Europa Scientific '20/20 Hydra' or a Thermo Finnigan Delta Plus Advantage. The Uattamdi individual was dated in the University of Oxford's Radiocarbon Accelerator Unit (ORAU). The bone powder was given a solvent wash due to possible glue present. This comprised a sequence of extraction in acetone, methanol and chloroform. This was then followed by routine ABA pretreatment and ultrafiltration⁵. The ultrafiltered collagen was combusted using an automated carbon and nitrogen elemental analyzer (Carlo Erba EA1108) coupled with a continuous-flow isotope monitoring mass spectrometer (Europa Geo 20/20). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, % carbon values, as well as C:N atomic ratios were obtained. We used VPDB as the standard for determining $\delta^{13}\text{C}$ values and AIR for the $\delta^{15}\text{N}$ equivalent. AMS radiocarbon measurement was undertaken using the MiCaDaS accelerator^{6,7,8}. The Tanjung Pinang individual was dated in the Australian National University. The sample was crushed and treated with Acetic Acid then rinsed to separate the bone apatite.

The bone protein samples were corrected for marine reservoir effect using the Mixed_Curves approach in OxCal 4.4 with Marine20 and INTCAL20 curves^{9,10} assuming a regional ΔR average for the Indonesian islands of -173 ± 75 ¹⁴C yrs obtained from the Marine Reservoir Corrections Database (<http://calib.org/marine/>). The percent contribution of

the marine curve was determined based on a linear interpolation of the measured $\delta^{13}\text{C}$ value between assumed endpoints of -21 and -12‰ (terrestrial and marine). Bone carbonate $\delta^{13}\text{C}$ (e.g., ANU-8439) is a function of the bulk diet and is offset by around +12‰ to +14‰¹¹; consequently, the reported value represents a terrestrial diet and has been calibrated accordingly.

The radiocarbon dates and quality collagen indicators (collagen yields, C/N ratios, %C and %N) are reported in Supplementary Table 12.

3. Archaeological information

Aru Manara. Aru Manara is a limestone cave located about 275 m inland from the southeastern coast of Morotai island, North Maluku Islands, Eastern Indonesia, excavated in 2011. The stratigraphy of the site comprises four deep sediment layers 2 m thick that terminated in the bedrock. The individuals studied here were excavated from Layer 3. The excavations yielded a large number of fragmented human bone (NISP=57,273) and teeth (n=1550), as well as early Metal Age sherds dated to between 2300 and 1800 cal BP, (mainly from Layer 3)¹²⁻¹⁴. The other sherds found at the site (n=504) included 32 red-slipped and 79 decorated specimens. One type of pottery decoration, with linear and curvilinear motifs, are similar to those in contemporary Moluccan assemblages at Tanjung Pinang on Morotai, also analyzed in this study¹⁵. However, the occurrence of anthropo- and zoomorphic decorations are unique to this site within the Northern Moluccas. The existence of pottery with applied-relief decorations, including a human face and lizard, suggest the development of maritime networks with East Indonesia and Near Oceania from 2700 to 2000 years BP, but is also linked with ceramics from Lapita associated burials in Remote Oceania¹⁶. The use of large burial jars and box typed jars may indicate possible human interaction between the Northern Moluccas and the Philippines¹⁷. The skeletal remains from Aru Manara are curated by the Pusat Penelitian Arkeologi Nasional (Jakarta, Indonesia).

Topogaro 1. Topogaro 1 is a cave that is part of a larger system of three caves (Topogaro 1-3) and rockshelters (Topogaro 4-7), located about 3.5 km inland from the eastern coast of Central Sulawesi in Morowali District. Topogaro 1 contains more than 30 broken coffins with human skeletal remains and surface finds, including fragments of prehistoric pottery, Chinese and European ceramics, achert flakes (including finely retouched tools), and shell. The presence of a number of flakes and shellfish clearly indicate that the cave had been used as a prehistoric habitation or tool production site, while wooden coffins and the variety of Chinese and European ceramics show the site had been used as a burial site more recently, between 300 and 100 years BP, based on AMS dates acquired from the coffin itself and some associated human teeth. The skeletal remains from Topogaro are curated by the Pusat Penelitian Arkeologi Nasional (Jakarta, Indonesia).

Gua Uattamdi. Gua Uattamdi is a limestone rock shelter situated on Kayoa Island in the northern Moluccas (0° 127°20'E). This site has yielded skull fragments associated with jar burial, incised pottery, glass beads and metal fragments, with one individual analysed in this report directly dated to 1820 - 1530 cal BP. The oldest material culture from Uattamdi contained red-slipped Neolithic pottery, dated to c.3300–2500 BP, with connections with the Philippines and eastern Taiwan, as well as other Neolithic sites in Wallacea. This Neolithic layer contained no human remains. The younger incised, impressed and appliqué pottery associated with the analysed skull is paralleled in sites on Morotai, including the two sites of Aru Manara and Tanjung Pinang that are also analysed in this study (ref¹⁵, ch.7). This style of pottery dates to c.2500 BP and later. The skeletal remains from Gua Uattamdi are curated by the Pusat Penelitian Arkeologi Nasional (Jakarta, Indonesia).

Tanjung Pinang. Tanjung Pinang is a rock shelter situated on Morotai Island (2°05'N, 128°40'E). There is a direct date for one of the four individuals included in this study (TanjungPinang1) of 2690 - 1600 cal BP. The date was produced from bone apatite in 1993, therefore most likely showing a minimum date (Rachel Wood, ANU Radiocarbon Laboratory, pers. Comm.), and it exhibits a large calibration range. However, the incised decoration of the pottery found at this site is similar to the Metal Age pottery in Gua Uattamdi (ref¹⁵, ch7), supporting a date younger than the Neolithic red-slipped pottery in Uattamdi. Nevertheless, the associated pottery and the apatite

analysis allow a possibility that the Tanjung Pinang sample could be as old as 2500 BP. A morphological analysis of the Tanjung Pinang skulls showed Papuan features¹⁸. The skeletal remains from Tanjung Pinang are curated by the Pusat Penelitian Arkeologi Nasional (Jakarta, Indonesia).

Liang Bua. Liang Bua is best known as the cave where *Homo floresiensis* was discovered^{19,20}. However, Liang Bua also contains archaeological deposits dating to the Neolithic and Proto-Metallic periods. These later deposits were first excavated in 1965 by T.H. Verhoeven who discovered six skeletons^{21,22}. Five skulls from that excavation are curated at Universitas Airlangga and loose petrous bones from Burials 1 and 2 were sampled for the current study. It was later found that both petrous fragments belonged to the crania of Burial 2. The individual included²³ in this study was directly dated to 2588 ± 23 BP (Wk-51763). In both the earlier and later excavations, the burials were found associated with Neolithic and Proto-Metallic material culture and domestic fauna, such as plain and decorated pottery (*periuk* [jars], *kendi* [pitcher/ewer], *buli buli* [little jars], and *tutup* [lid]), flaked adzes, bone tools, pig tusks and a bronze axe^{19,22,24}. Bioarchaeological analysis confirmed that 3/3 (100%) of the Liang Bua individuals curated at Universitas Airlangga who had preserved anterior maxillary teeth (including burial 2) had undergone ritual tooth ablation, a practice thought to have been introduced by the Neolithic Austronesian settlers to East Nusa Tenggara^{23, 25-27}.

Liang Toge. One individual was analysed in this study from the cave site of Liang Toge on Flores Island, located near Warukia, 1 km south of Lepa, in the Manggarai district of Flores²⁸. Excavated by T.H. Verhoeven in the mid 20th century, there is little published information about the human remains from Liang Toge, which are currently curated at Universitas Airlangga, Surabaya. Visual inspection of the Liang Toge skeletal assemblage suggests that these remains may have been (or become after excavation) commingled because there were mixed cranial and post-cranial elements from multiple individuals in the collection and no apparent burial numbers. The petrous bone analyzed for this study was dated to 800 – 720 cal BP (MAMS 40606), in addition to two ribs which provided dates of 1066 – 973 cal BP (MAMS 35084) and 911 – 786 cal BP (MAMS 35085).

Komodo. The individual (Komodo I) analysed from Komodo Island, West Manggarai Regency, East Nusa Tenggara (S 08° 32' 35.9988" E 119° 29' 21.9876") was one of two burials found during excavations carried out by Walter Auffenberg (University of Florida, USA) and Putra Sastrawan (Universitas Udayana, Indonesia) in 1969-1970. During their survey for Komodo dragons, Auffenberg and Putra Sastrawan identified 18 archaeological sites across Komodo and the two burials were found at the hill site of Ntodo Leseh. The crania were removed from the primary flexed (or squatting) burials (both with their heads facing west) and they were designated Komodo I and Komodo II^{29,30}. Komodo I was buried 16 cm below the surface and had a circle of flat stones marking the grave. Komodo II was buried 19 cm below the surface and had a triangle of flat stones marking the grave. From comparisons of burial type with other prehistoric interments on Flores and East Java, in addition to material culture (stone flakes and blades and Neolithic hand axes) found at the other sites on Komodo, ref.²³ proposed that the Komodo burials were between 3000-5000 years old. However, recent direct dating of the petrous bone from Komodo I provided a much more modern date of 570 - 240 cal BP (MAMS 40602) indicating that this individual lived during the period when East Nusa Tenggara was under the rule of the Majapahit Empire³¹. The skeletal remains from Jareng Bori are curated at Universitas Airlangga (Surabaya, Indonesia).

Jareng Bori. The site of Jareng Bori, Pantar island, East Nusa Tenggara (S 08° 15'51.7 E 124°17'55.4) was excavated in 2016 as part of a joint research project between researchers from the Australian National University (Australia) and Universitas Gaja Madja (Indonesia). The small rock shelter (40 m² living floor) was located beside a large boulder at the base of a cliff on a coastal beach flat approximately 120 m from the current shoreline. A 1 x 1 m test pit (test pit A) was excavated and seven stratigraphic layers were identified at the site. The period of site use was dated between the Metal Age, ca 1800 BP, and the late Historic Period. A site report detailing the full excavation and results of the dating, faunal and material culture analyses is available³². At Jareng Bori, a poorly preserved human burial (analyzed for the current study) was found cut into the upper layers with a skeleton in a flexed position in the south area of the test pit. Only the upper half (thorax, upper limbs and skull) of the skeleton was excavated and the remainder was left

in situ because it extended into the southern baulk of the test pit. The skeletal remains were analysed at Australian National University and a full bioarchaeology report is forthcoming. Of the 21 charcoal dates analysed from the site, four were associated with the burial (ANU 53127, ANU 53130, ANU 53129, and ANU136) and ranged between 0–429 cal BP³². The direct date of the petrous bone for this burial provided results of 530 – 490 cal BP (MAMS 40607), indicating that this individual lived during the rule of the Majahapit Empire in the region, directly before the first Portuguese contact with the area at the beginning of the 16th century AD³¹. Tooth modifications in the form of labial filing of the maxillary incisors was found on the dentition of the Jareng Bori individual. This type of tooth modification has been observed on skeletons dating to a similar time period in Java, Bali, Sumba and Flores. Tooth filing has been suggested as a cultural tradition that is a unique characteristic of later inhabitants of the region^{25, 27, 33}, whereas tooth ablation (in the form of the removal of the maxillary lateral incisors and canines) has been interpreted as an earlier Neolithic/Austronesian ritual practiced across East Nusa Tenggara *ca.* 3000 – 2000 BP^{25–27}. The skeletal remains from Jareng Bori are curated at Universitas Airlangga (Surabaya, Indonesia).

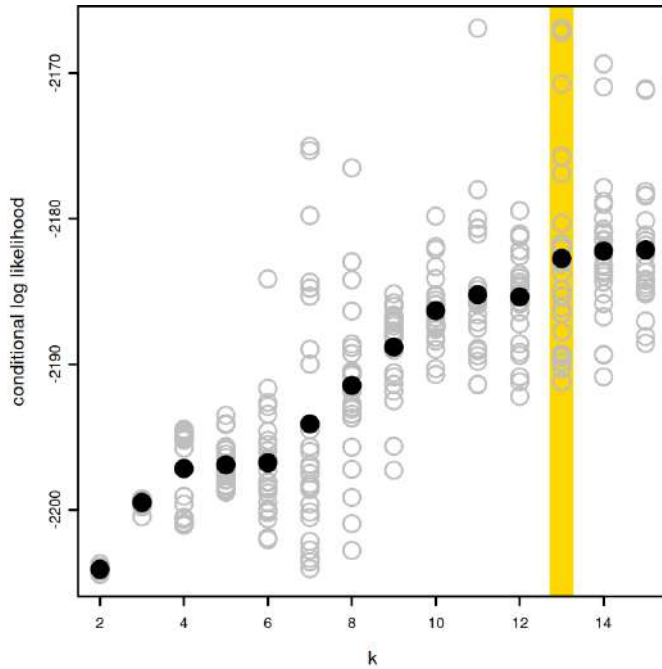
References

- 1 Longin, R. New method of collagen extraction for radiocarbon dating. *Nature* **230**, 241–242 (1971).
- 2 Higham, T. F., Jacobi, R. M. & Ramsey, C. B. AMS radiocarbon dating of ancient bone using ultrafiltration. *Radiocarbon* **48**, 179–195 (2006).
- 3 Kromer, B., Lindauer, S., Synal, H.-A., Wacker, L. MAMS - a new AMS facility at the Curt-Engelhorn-Centre for archaeometry, Mannheim, Germany. *Nucl Instrum Methods Phys Res, Sect. B* **294**, 11–13 (2013).
- 4 Beverly, R.K. *et al.* The Keck Carbon Cycle AMS Laboratory, University of California, Irvine: Status Report. *Radiocarbon*, **52** (2–3), 301–309 (2010)
- 5 Brock, F., Higham, T., Ditchfield, P. & Ramsey, C. B. Current pretreatment methods for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (ORAU). *Radiocarbon* **52**, 103–112 (2010).
- 6 Stuiver, M. & Polach, H. A. Discussion reporting of 14 C data. *Radiocarbon* **19**, 355–363 (1977).
- 7 Dee, M. & Ramsey, C. B. Refinement of graphite target production at ORAU. *Nucl Instrum Methods Phys Res B* **172**, 449–453 (2000).
- 8 Ramsey, C. B., Higham, T. & Leach, P. Towards high-precision AMS: progress and limitations. *Radiocarbon* **46**, 17–24 (2004).
- 9 Reimer, P. J. *et al.* The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* **62**, 725–757 (2020).
- 10 Heaton, T. J. *et al.* Marine20—the marine radiocarbon age calibration curve (0–55,000 cal BP). *Radiocarbon* **62**, 779–820 (2020).
- 11 Petchey, F., Spriggs, M., Bedford, S., & Valentin, F. The chronology of occupation at Teouma, Vanuatu: Use of a modified chronometric hygiene protocol and Bayesian modelling to evaluate midden remains. *Journal of Archaeological Science: Reports* **4**, 95–105 (2015).
- 12 Ono, R. *et al.* Development of regional maritime networks during the Early Metal Age in northern Maluku Islands: A view from excavated glass ornaments and pottery variation. *The J Island Coast Archaeol* **13**, 90–108 (2018).
- 13 Ono, R. *et al.* Development of pottery making tradition and maritime networks during the Early Metal Ages in Northern Maluku Islands. *AMERTA* 35 **2**, 109–122 (2017).
- 14 Ono, R. *et al.* Early Metal Age interactions in Island Southeast Asia and Oceania: jar burials from Aru Manara, northern Moluccas. *Antiquity* **92**, 1023–1039 (2018).
- 15 Bellwood, P. *The Spice Islands in Prehistory: Archaeology in the Northern Moluccas, Indonesia*. (ANU Press, 2019).
- 16 Spriggs, M. Archaeological and Ethnoarchaeological Research (1990).
- 17 Ono, R, Oktaviana AA, Sriwigati, Aziz N. In *The Archaeology of Island Colonization* (ed Matthew F. Napolitano, Robert J. DiNapoli), 293–326 (University Press of Florida, 2021).

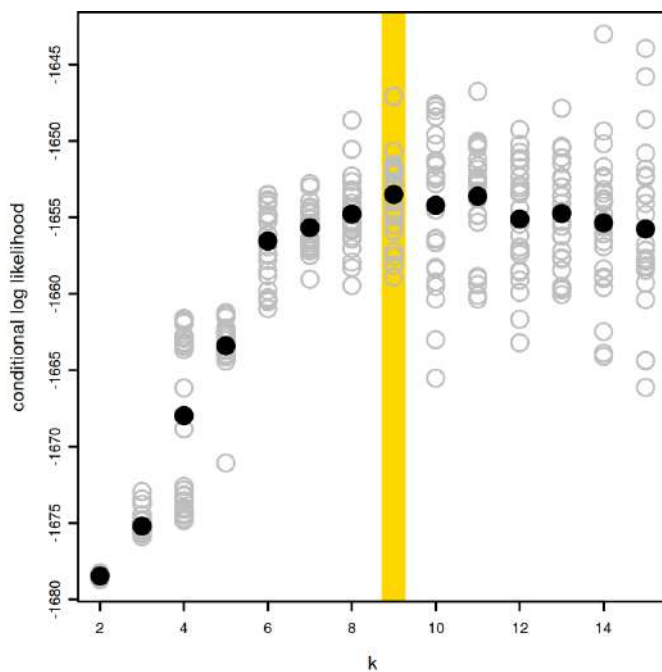
- 18 Bulbeck, D. in *The Spice Islands in Prehistory Archaeology in the Northern Moluccas, Indonesia* (JSTOR, 2019).
- 19 Morwood, M. J. *et al.* Archaeology and age of a new hominin from Flores in eastern Indonesia. *Nature* **431**, 1087-1091 (2004).
- 20 Sutikna, T. *et al.* The spatio-temporal distribution of archaeological and faunal finds at Liang Bua (Flores, Indonesia) in light of the revised chronology for *Homo floresiensis*. *J Hum Evol* **124**, 52-74 (2018).
- 21 Koesbardiati, T., Murti, D. B., Herina, D. A. & Sari, A. A. The occurrence of enamel hypoplasia, porotic hyperostosis and cribra orbitalia in three prehistoric skeletal assemblages from Indonesia. *Bull Int Assoc Paleodont* **12**, 33-40 (2018).
- 22 Soejono, R. Prehistoric development in Indonesia. In *Research Conference on Early Southeast Asia*, 8-13 (1985)
- 23 Koesbardiati, T. & Suriyanto, R. Dental modification in Flores: a biocultural perspective. *Recent advances on Southeast Asian paleoanthropology and archaeology*. Yogyakarta: Yogyakarta: Laboratory of Bioanthropology and Paleoanthropology Faculty of Medicine Gadjah Mada University, 259-268 (2007).
- 24 Morwood, M. J. Time, space and prehistoric art: a principal components analysis. *Archaeology & Physical Anthropology in Oceania* **15**, 98-109 (1980).
- 25 Kinaston, R. L. *et al.* Ritual tooth ablation and the Austronesian expansion: Evidence from eastern Indonesia and the Pacific Islands. *The J Island Coast Archaeol*, 1-32 (2020).
- 26 Koesbardiati, T., Murti, D. B. & Suriyanto, R. A. Cultural dental modification in prehistoric population in Indonesia. *Bull Int Assoc Paleodont* **9**, 52-60 (2015).
- 27 Suriyanto, R., Koesbardiati, T., Murti, D. & Indriati, E. In *Proceeding Book the 2nd International Joint Symposium on Oral and Dental Sciences*, 213-224 (2012).
- 28 Hooijer, D. Three new giant prehistoric rats from Flores, Lesser Sunda Islands. *Zoologische Mededelingen* **35**, 299-314 (1957).
- 29 Sukadana, A. Tengkorak-tengkorak purba dari P. Komodo. *Kumpulan Naskah Pertemuan Nasional Ahli Anatomi Indonesia III* (1973).
- 30 Sukadana, A. Studi Politiisme dan Polimorfisme populasi pada beberapa peninggalan di Nusa Tenggara Timur. *Disertasi*. Surabaya: Universitas Airlangga. Tidak dipublikasikan (1984).
- 31 Ricklefs, M. C. *A History of Modern Indonesia since c. 1200* (Macmillan International Higher Education, 2008).
- 32 Hawkins, S. *et al.* Metal-Age maritime culture at Jareng Bori rockshelter, Pantar Island, Eastern Indonesia. *Rec Aust Mus* **72**, 237-262 (2020).
- 33 Kasnowihardjo, G., Suriyanto, R. A., Koesbardiati, T. & Murti, D. B. Human Teeth Modification in Binangun and Leran: New findings in the Northern Coast of Rembang District, Central Java. *Berkala Arkeologi* **33** (2013).

Supplementary Figure 1 - DyStruct results. A-B Conditional log likelihood (CLL) values across $K = 2$ to $K = 15$ obtained for 25 independent runs (grey circles) using dataset 1 (A) and dataset 2 (B). Black dots show the average CLL per K . The yellow bar indicates the value of K at which the CLL starts to plateau. C-D ADMIXTURE-like barplots for $K = 2$ to $K = 15$ for dataset 1 (C) and dataset 2 (D). E-F Expanded barplots for the highlighted K value of each respective dataset: $K = 13$ for dataset 1 (E) and $K = 9$ for dataset 2 (F).

A)



B)



C)

$$K = 2$$
$$K = 3$$
$$K = 4$$
$$K = 5$$
$$K = 6$$
$$K = 7$$
$$K = 8$$

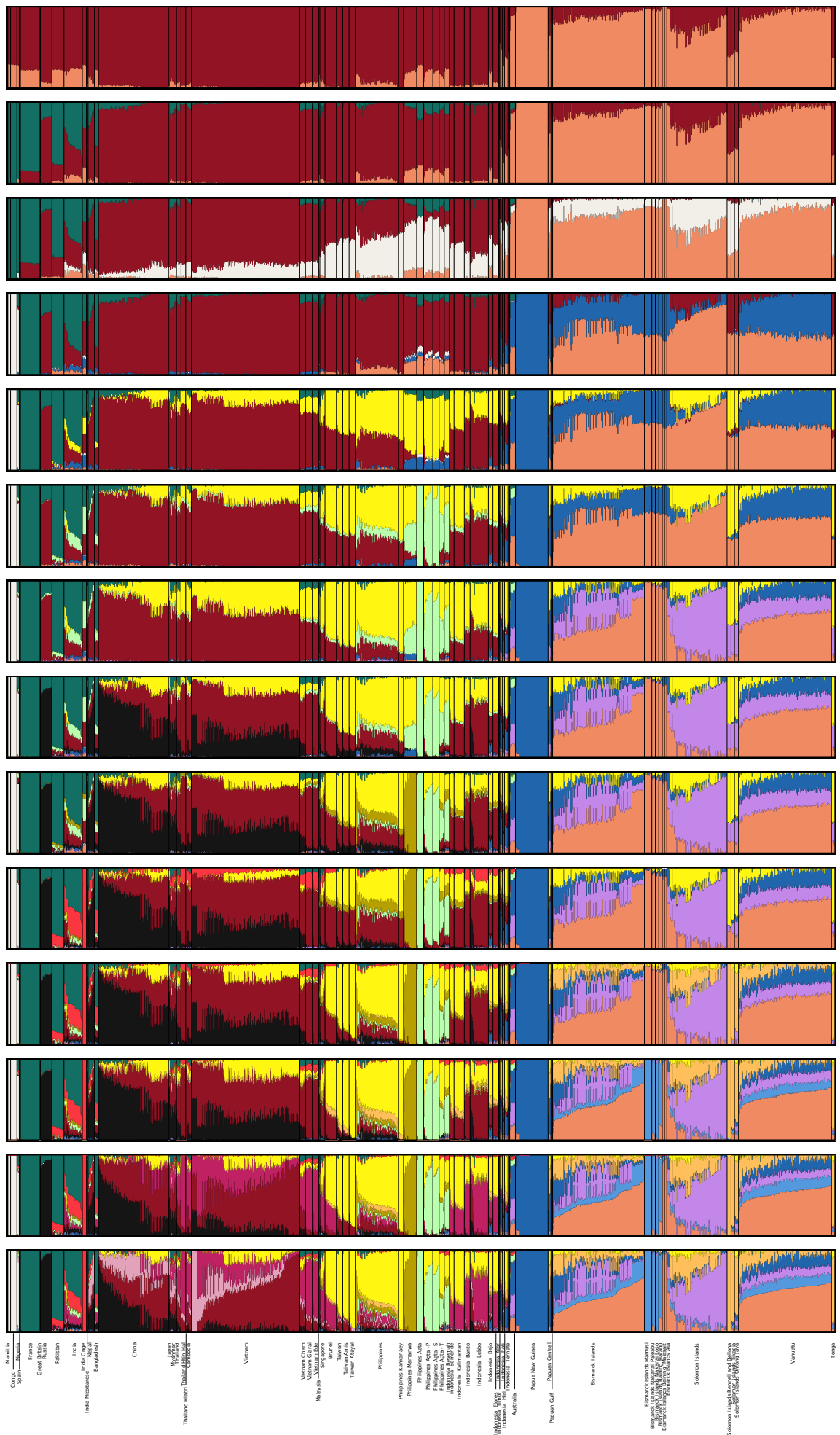
$K = 9$

$$K = 10$$
$$K = 11$$
$$K = 12$$

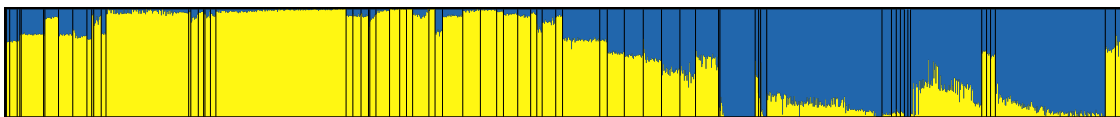
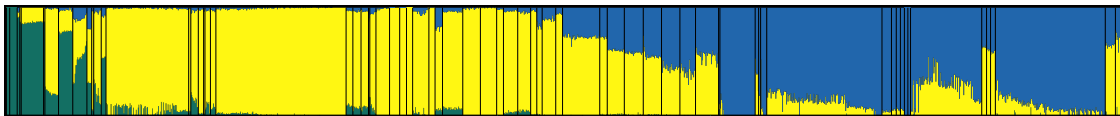
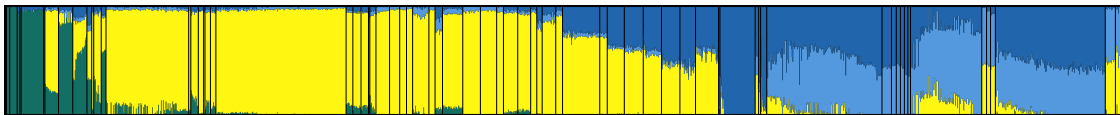
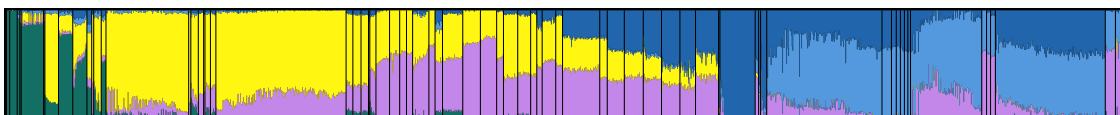
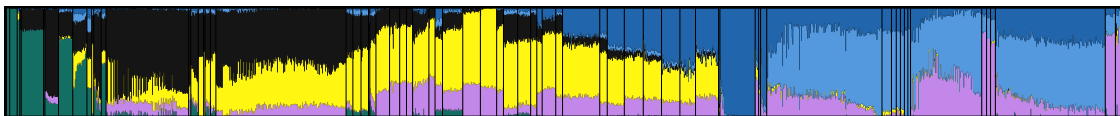
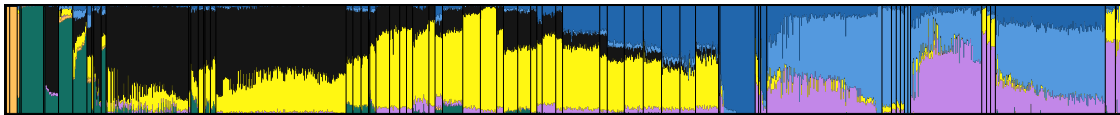
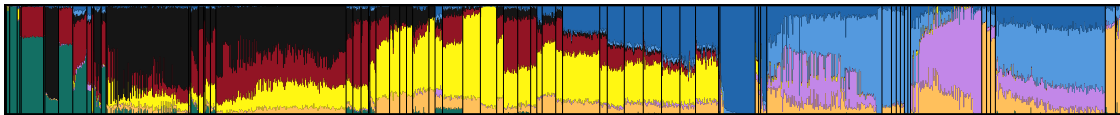
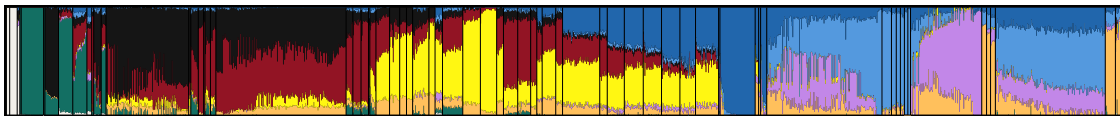
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$$K = 14$$

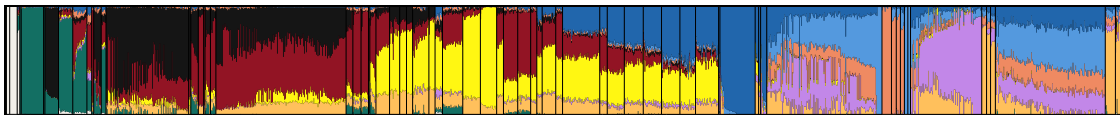
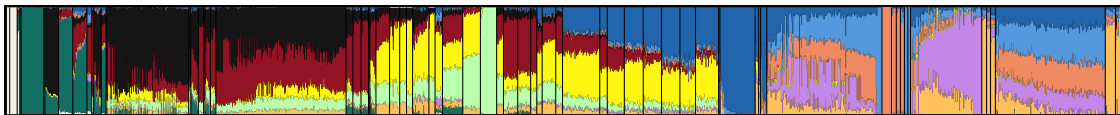
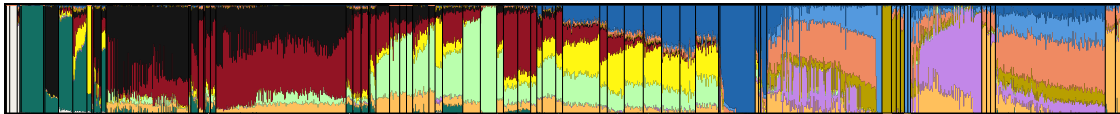
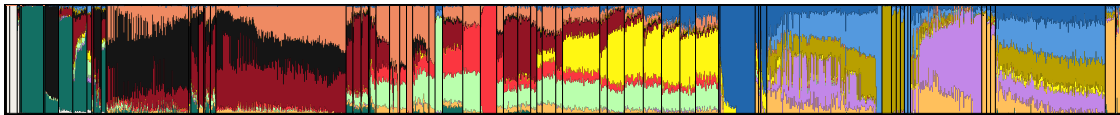
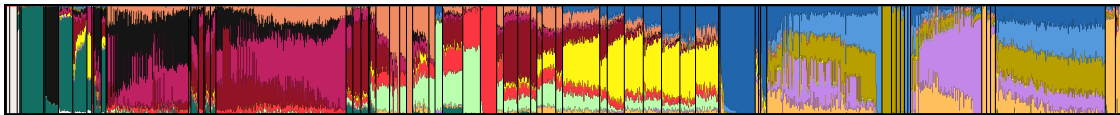
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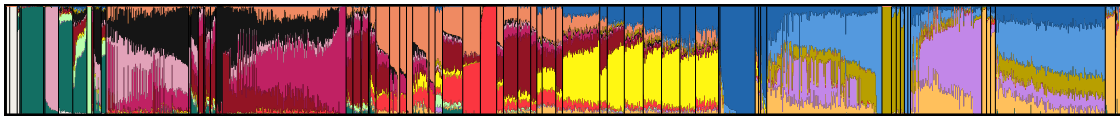
D)

$$K = 2$$
 $K = 3$ 
$$K = 4$$

$$K = 5$$

$$K = 6$$

$$K = 7$$

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$$K = 9$$


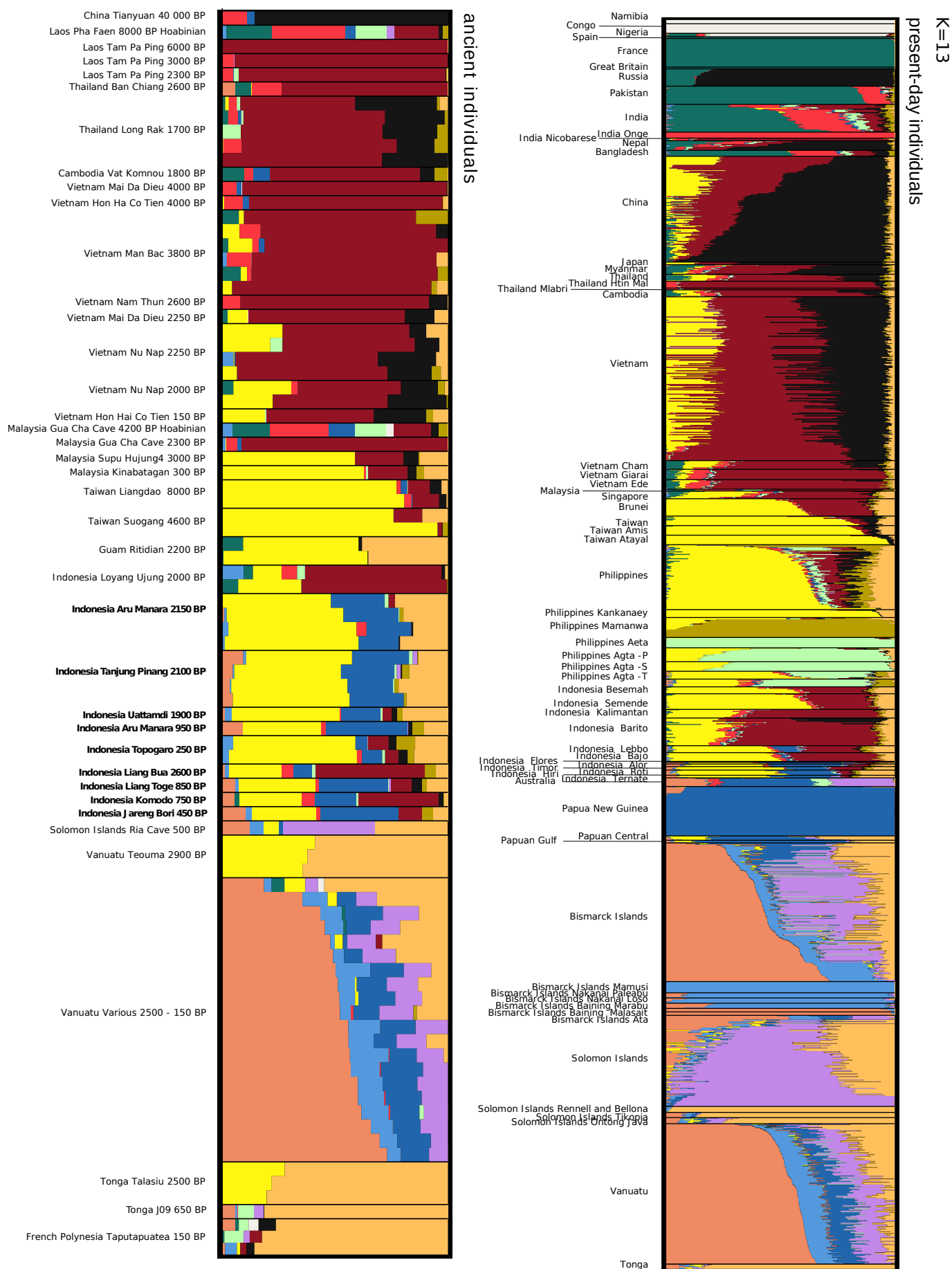
$K = 10$


$$K = 11$$

$$K = 12$$

$$K = 13$$

$$K = 14$$


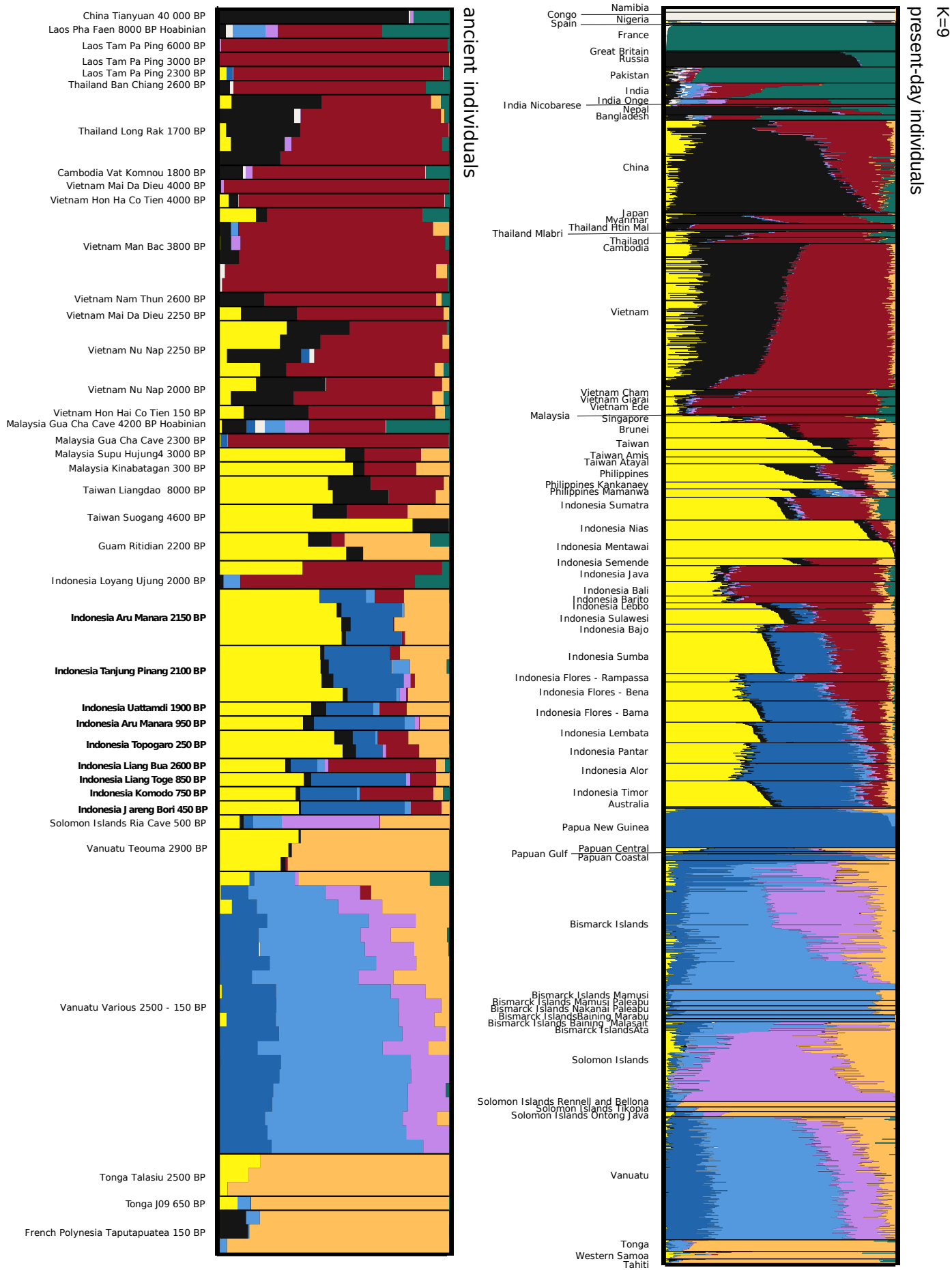
$K = 15$

[illegible]

E)

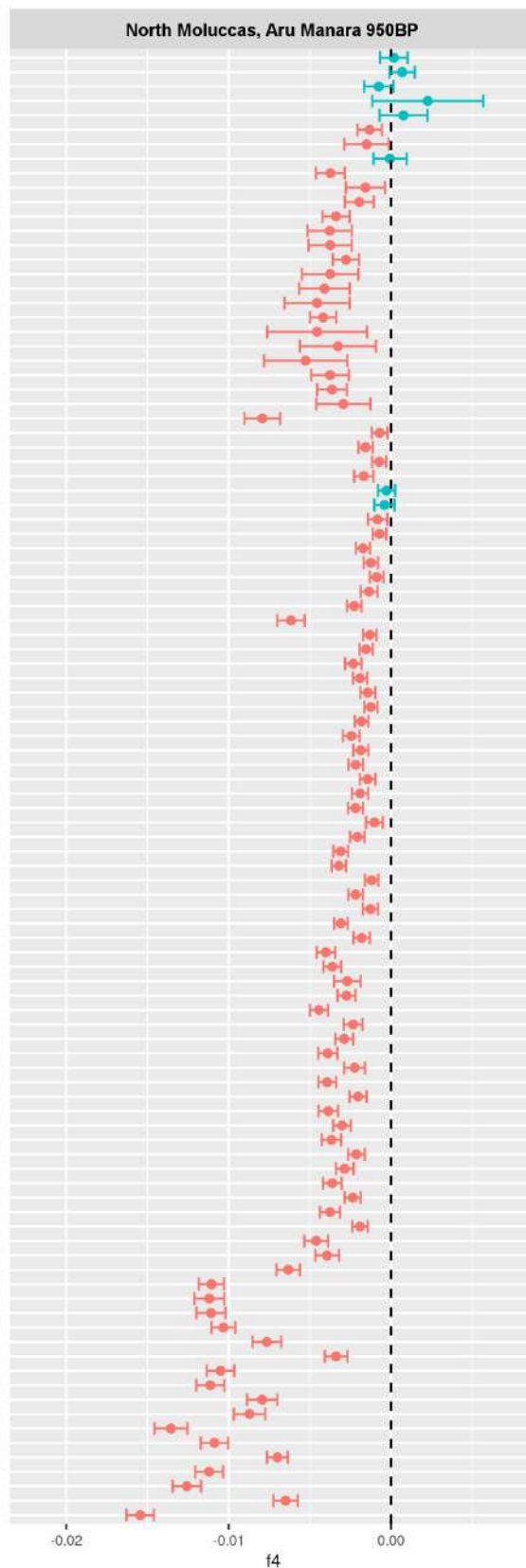
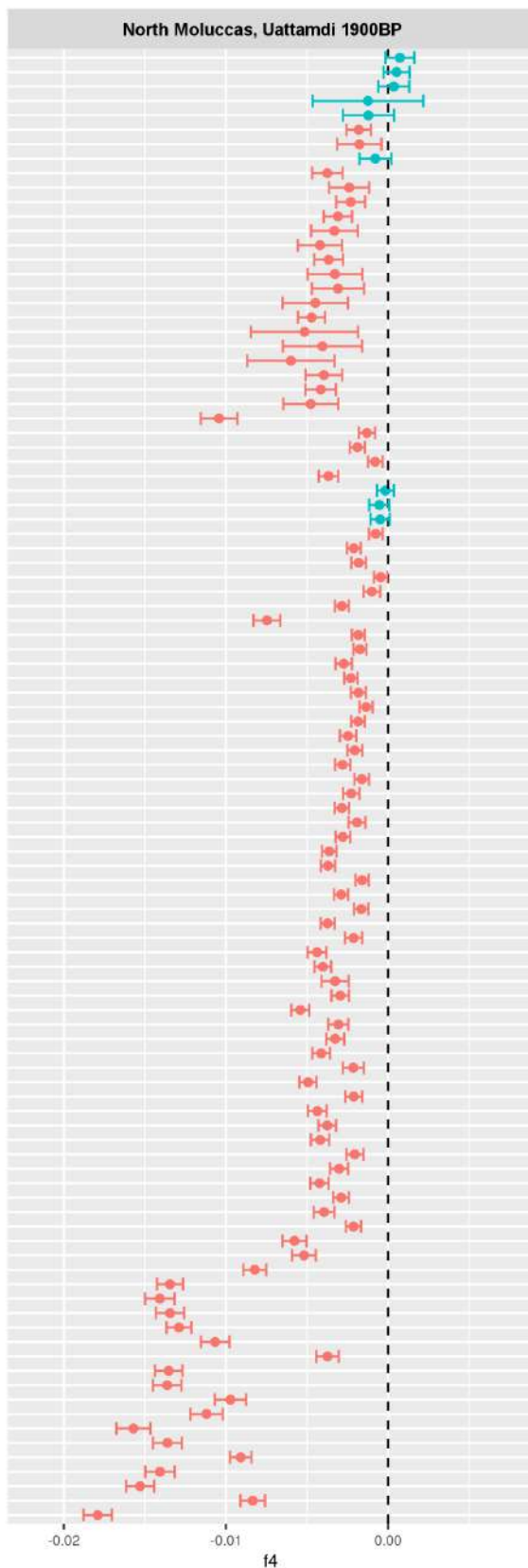


F)

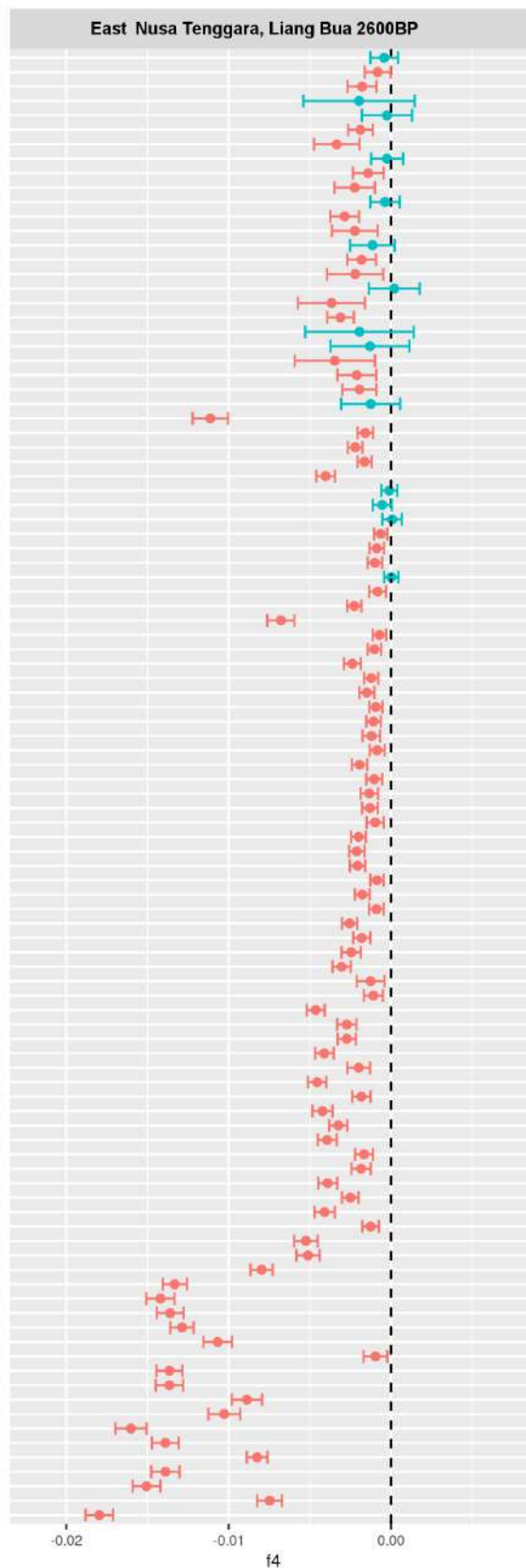
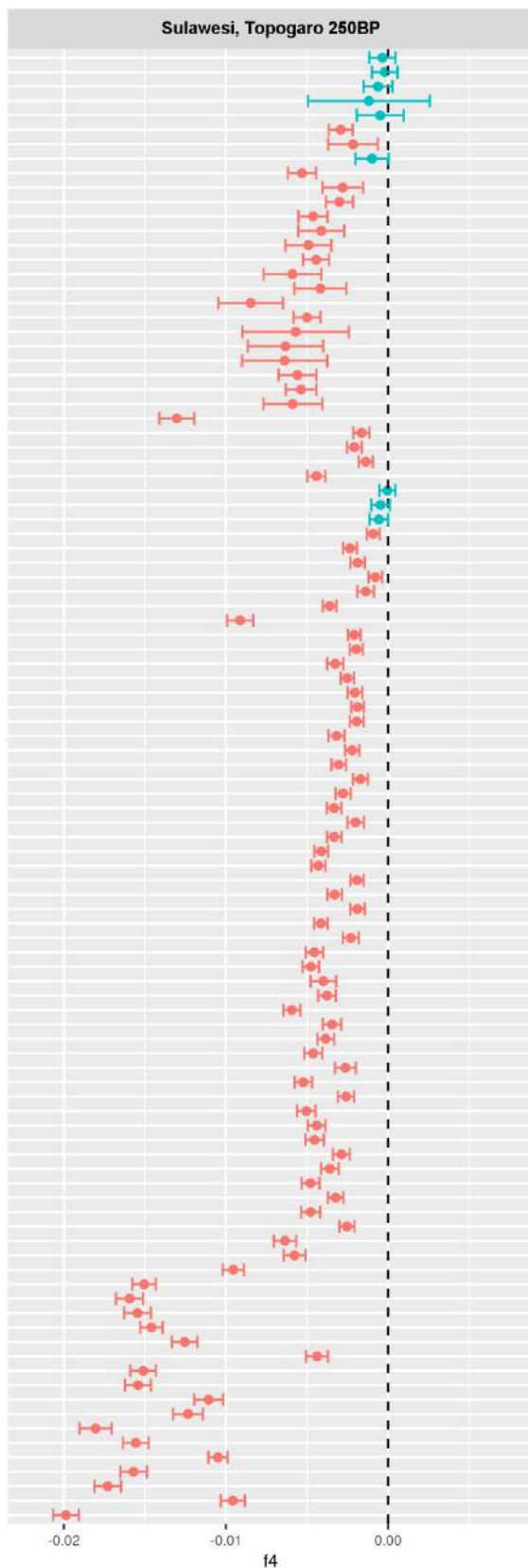


Supplementary Figure 2 - f_4 -statistic of the form $f_4(\text{Mbuti, ancient Wallacean; Amis, test})$ computed for each ancient group from Wallacea separately. The test groups are shown in the y-axis and include ancient and present-day groups from mainland Asia, Island southeast Asia, and the Pacific that have no discernible Papuan-related ancestry. Data are presented as exact f_4 -values ± 2 SE. Values in green are not significantly different from zero ($|Z| < 2$), whereas values in red are significantly different from zero ($|Z| > 2$).



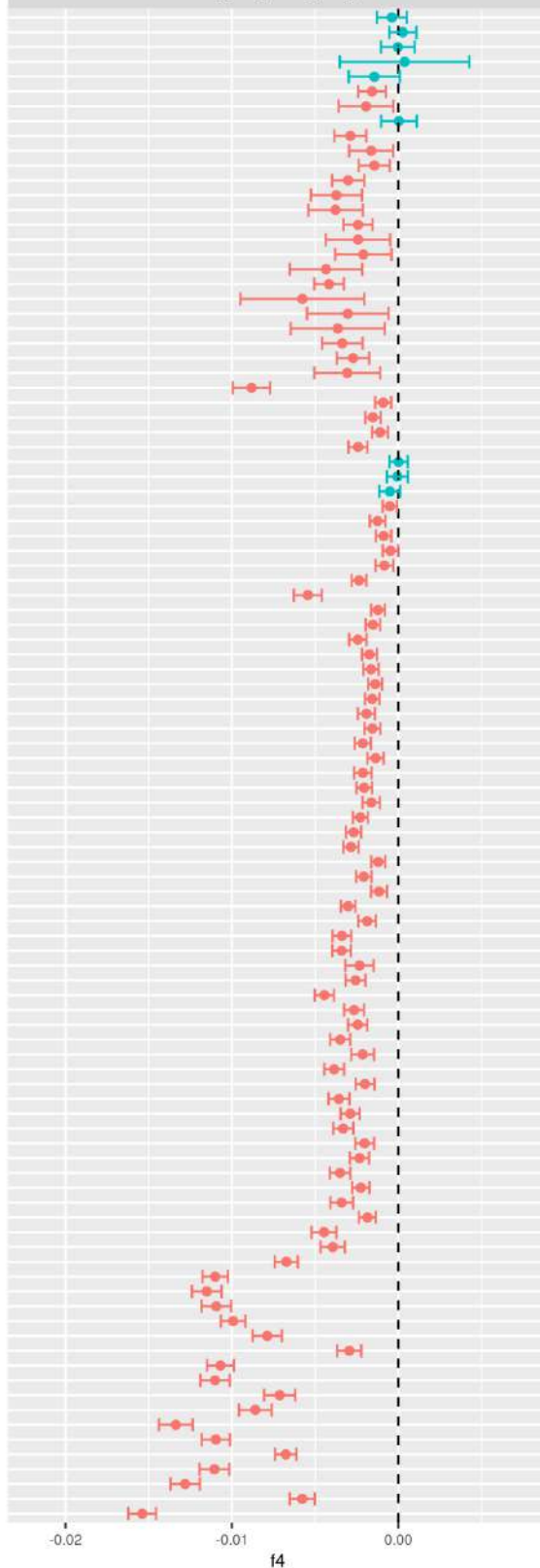


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- Vanuatu, Tecuma 2900BP
- US (Guam), Ritidian 2200BP
- Philippines, Nagsabaran 1800BP
- Taiwan, Suogang 4600BP
- Taiwan, Liangdao 8000BP
- Malaysia, Supu Hujung4 300BP
- Malaysia, Kinabatangan 300BP
- Malaysia, Gua Cha Cave 2300BP
- Vietnam, Hon Hai Co Tien 150BP
- Vietnam, Nui Nap 2000BP
- Vietnam, Nui Nap 2250BP
- Vietnam, Mai Da Dieu 2250BP
- Vietnam, Nam Tun 2600BP
- Vietnam, Man Bac 3800BP
- Vietnam, Hon Hai Co Tien 4000BP
- Vietnam, Mai Da Dieu 4000BP
- Cambodia, Vat Komnou 1800BP
- Thailand, Long Long Rak 1700BP
- Thailand, Ban Chiang 2500BP
- Thailand, Ban Chiang 2600BP
- Thailand, Ban Chiang 3100BP
- Laos, Tam Pa Ping 2300BP
- Laos, Tam Pa Ping 3000BP
- Laos, Tam Pa Ping 6000BP
- China, Tianyuan 40000BP
- Philippines, Visayan
- Philippines, Tagalog
- Philippines, Mamanwa1
- Philippines, Mamanwa
- Philippines, Kankanaey
- Philippines, Ilocano
- Taiwan, Atayal
- Indonesia, Semende
- Indonesia, Barito
- Indonesia, Bajo
- Brunei, Murut
- Brunei, Dusun
- Singapore, Malay
- Malaysia, undefined
- Vietnam, Thai
- Vietnam, Tay
- Vietnam, Sila
- Vietnam, Phula
- Vietnam, Pathen
- Vietnam, Nung
- Vietnam, Muong
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- Thailand, Htin Mal
- Myanmar, Burmese
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- China, Tu
- China, She
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- China, Han
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- China, Dai
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- Nepal, Sherpa
- Nepal, Kusunda
- Bangladesh, Bengali
- India, Yadava
- India, Relli
- India, Paliyar
- India, Onge
- India, Nicobarese
- India, Mala
- India, Madiga
- India, Khonda Dora
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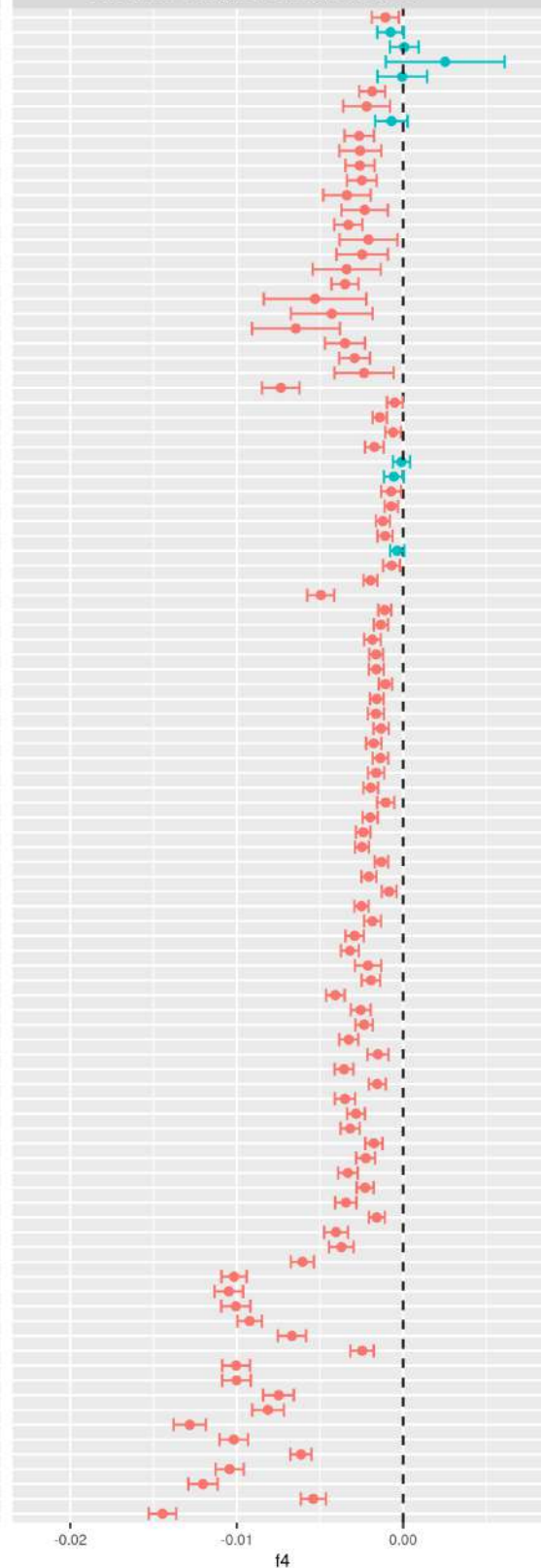


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- Vanuatu, Tecuma 2900BP
- US (Guam), Ritidian 2200BP
- Philippines, Nagsabaran 1800BP
- Taiwan, Suogang 4600BP
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- Malaysia, Supu Hujung4 300BP
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- Vietnam, Nam Tun 2600BP
- Vietnam, Man Bac 3800BP
- Vietnam, Mai Da Dieu 4000BP
- Vietnam, Hon Hai Co Tien 4000BP
- Cambodia, Vat Komnou 1800BP
- Thailand, Long Long Rak 1700BP
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- Laos, Tam Pa Ping 6000BP
- China, Tianyuan 40000BP
- Philippines, Visayan
- Philippines, Tagalog
- Philippines, Mamanwa1
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- Nepal, Sherpa
- Nepal, Kusunda
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- India, Yadava
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- India, Padiyar
- India, Onge
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- India, Mala
- India, Madiga
- India, Khonda Dora
- India, Kharia
- India, Kashmiri Pandit
- India, Kapu
- India, Juang
- India, Irula
- India, Brahmin
- India, Bondo
- Pakistan, Brahui

East Nusa Tenggara, Liang Toge 850BP

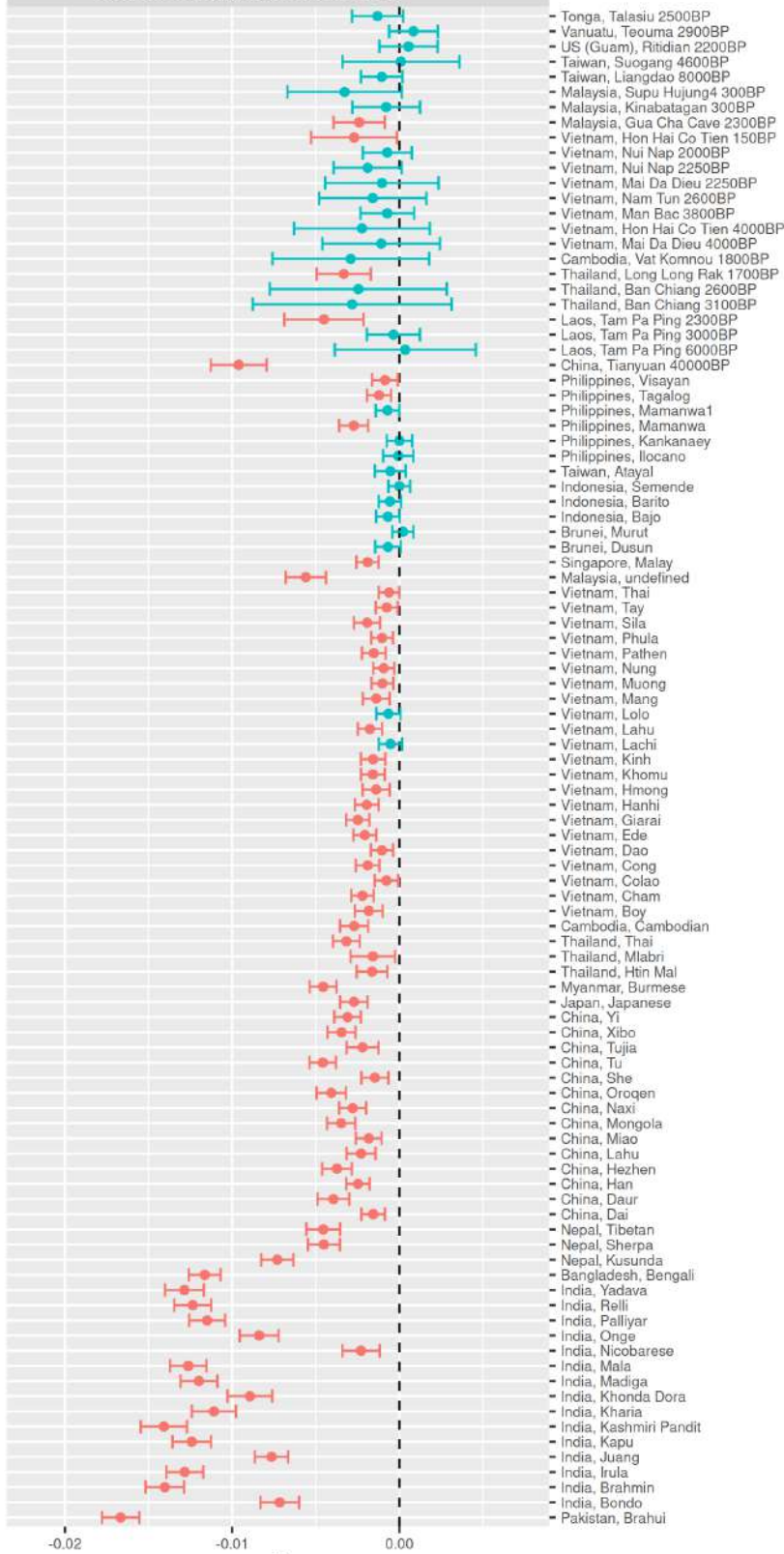


East Nusa Tenggara, Jareng Bori 450BP

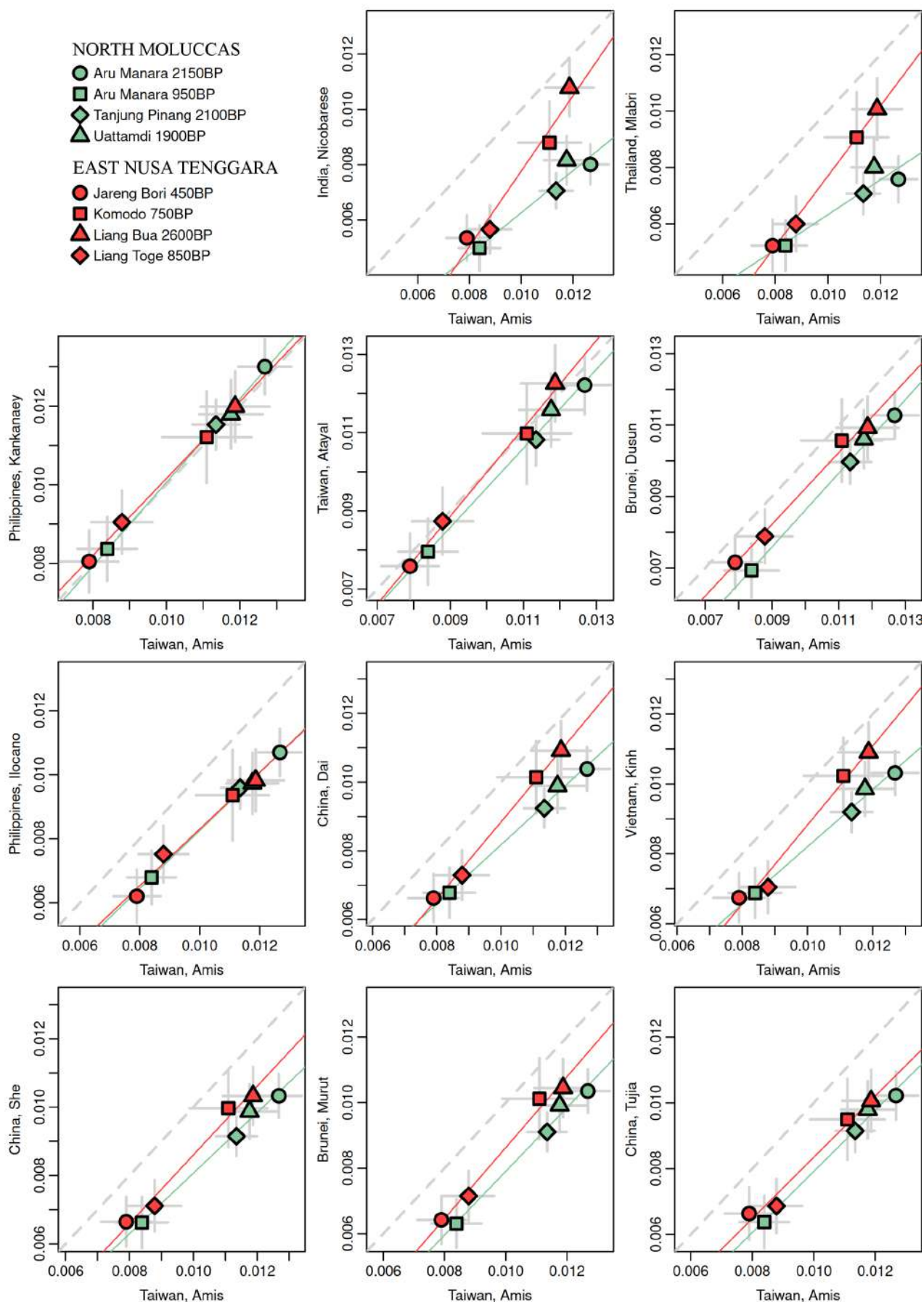


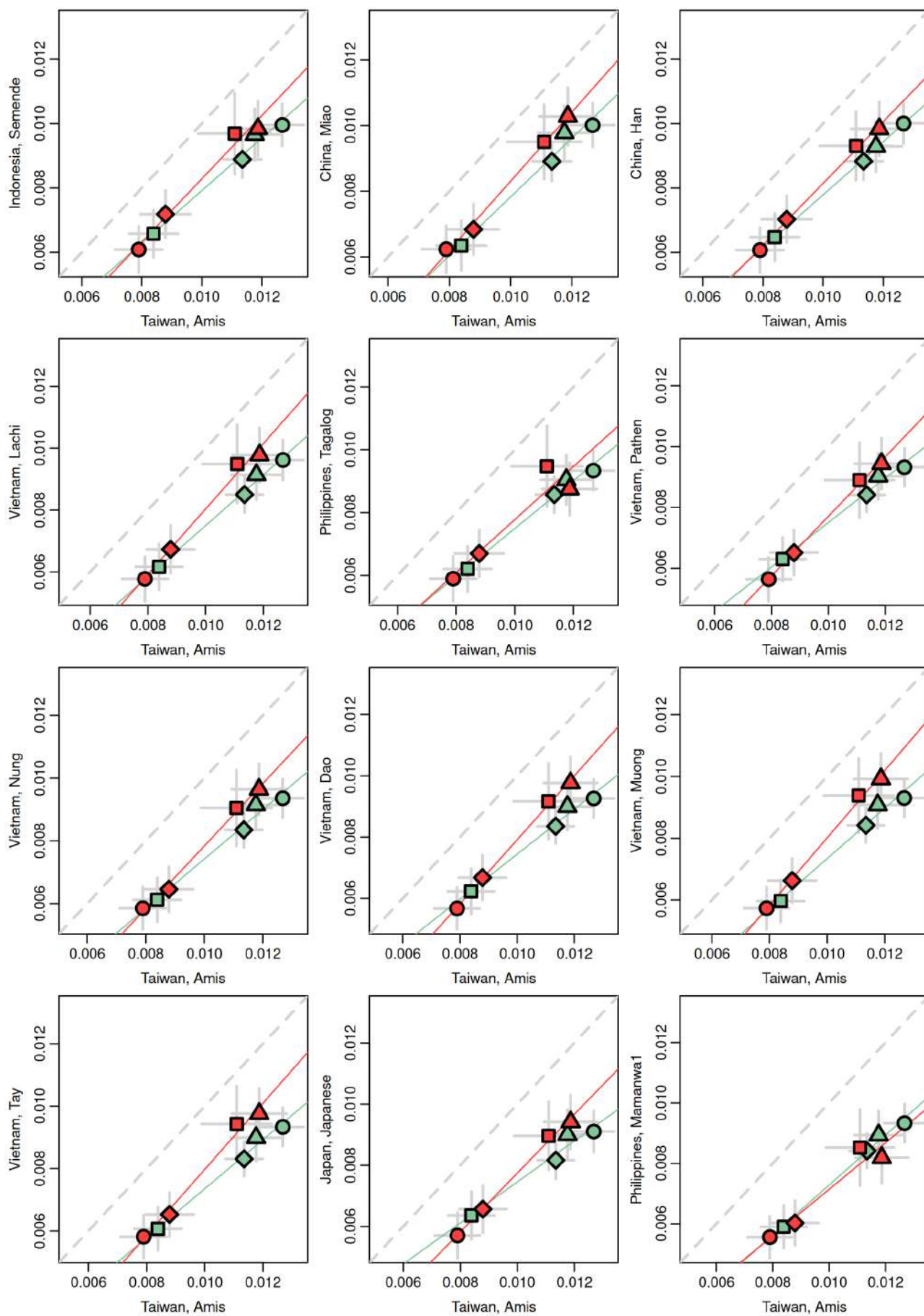
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- Malaysia, Kinabatangan 300BP
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- Thailand, Long Long Rak 1700BP
- Thailand, Ban Chiang 2500BP
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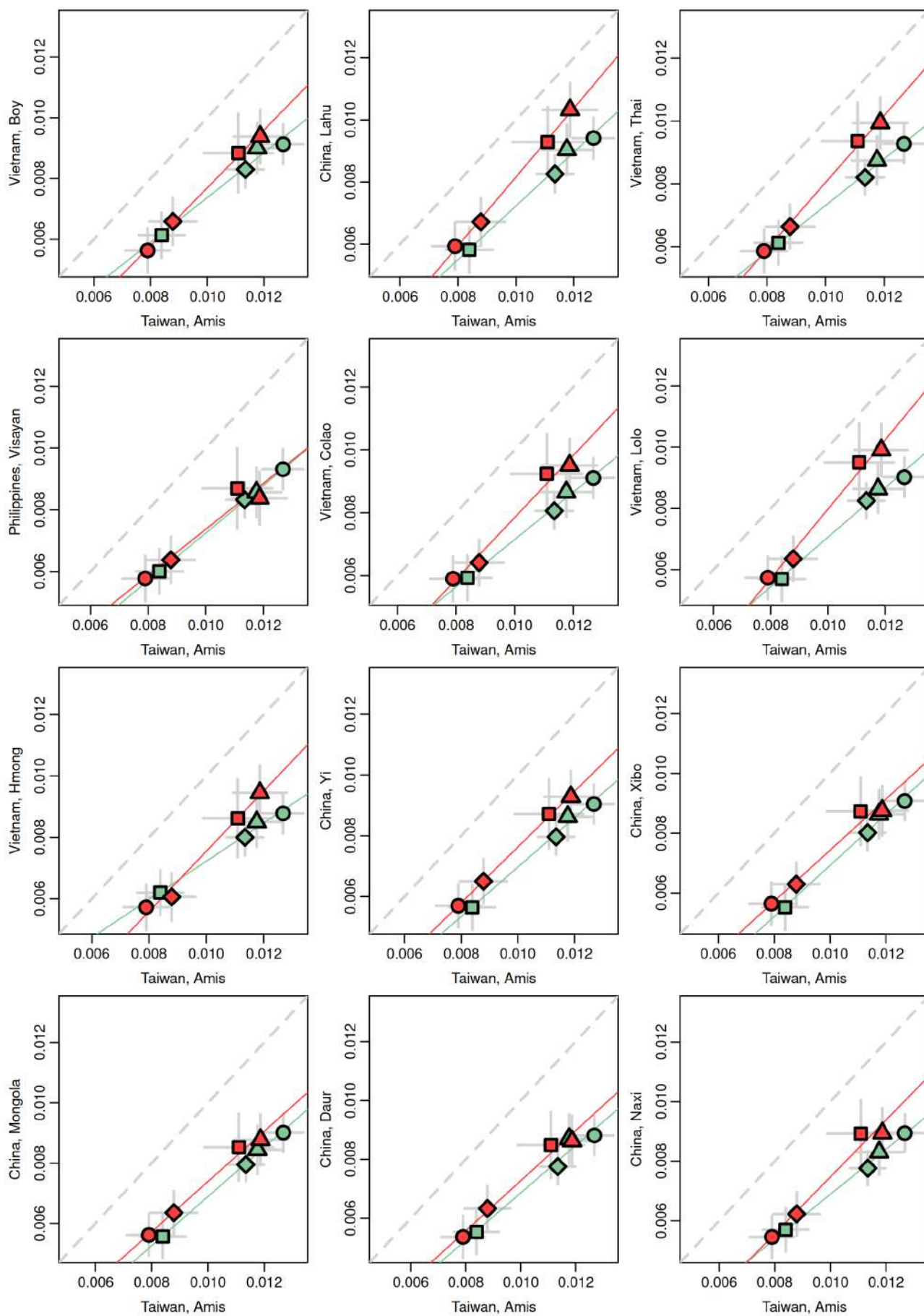
East Nusa Tenggara, Komodo 750BP

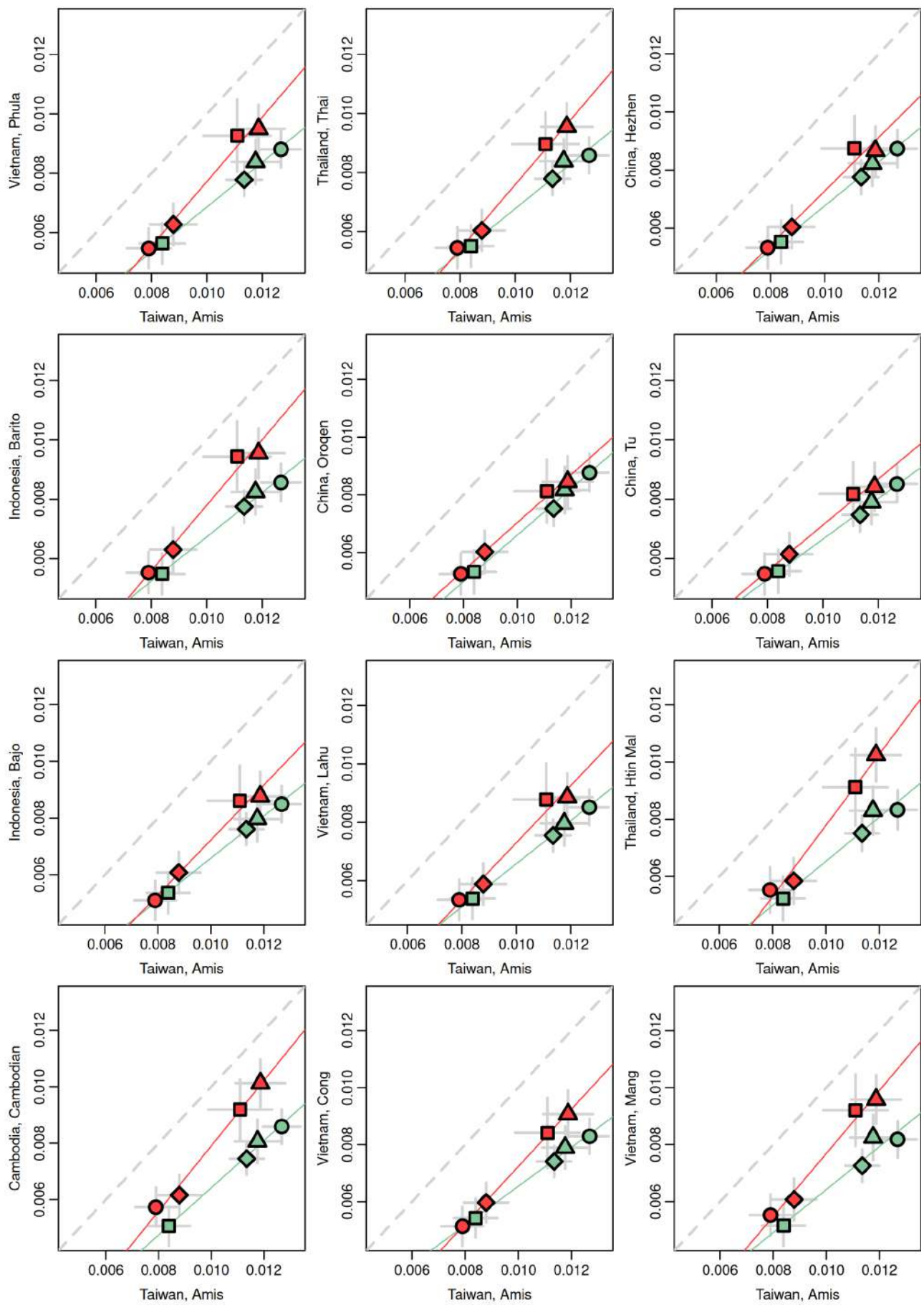


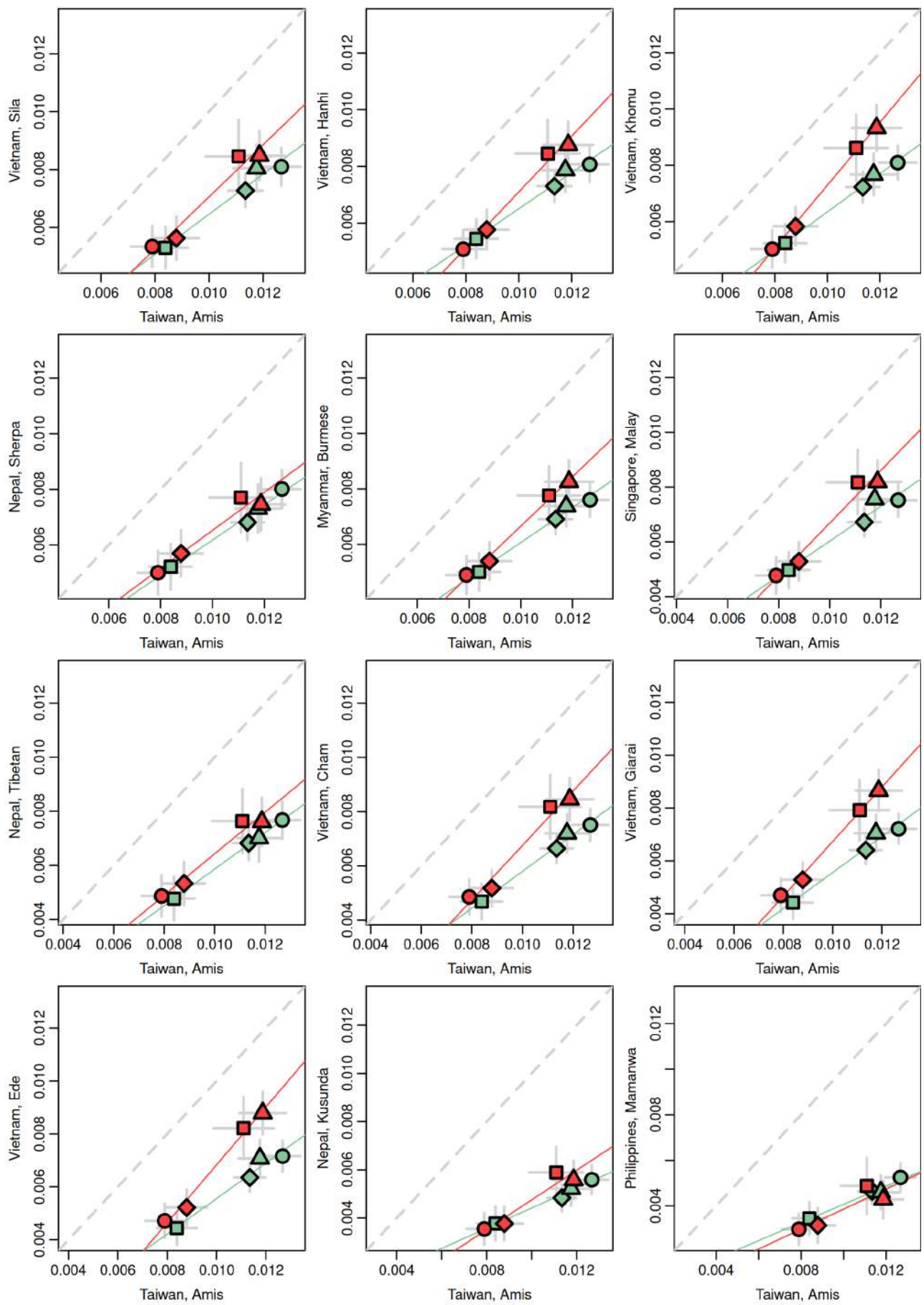
Supplementary Figure 3 - Biplots showing the results of pairs of f_4 -statistics of the form: $f_4(\text{Mbuti, test; New Guinea Highlanders, ancient Wallacea})$. The test groups, shown on the x-y axis label, include present-day groups from mainland Asia, Island southeast Asia, and Oceania that have no discernible Papuan-related ancestry based on the DyStruct analysis. Data are presented as exact f_4 -values $\pm$ 2 SE indicated by grey lines. Linear regression lines for the North Moluccas and East Nusa Tenggara individuals are shown in green and red, respectively.

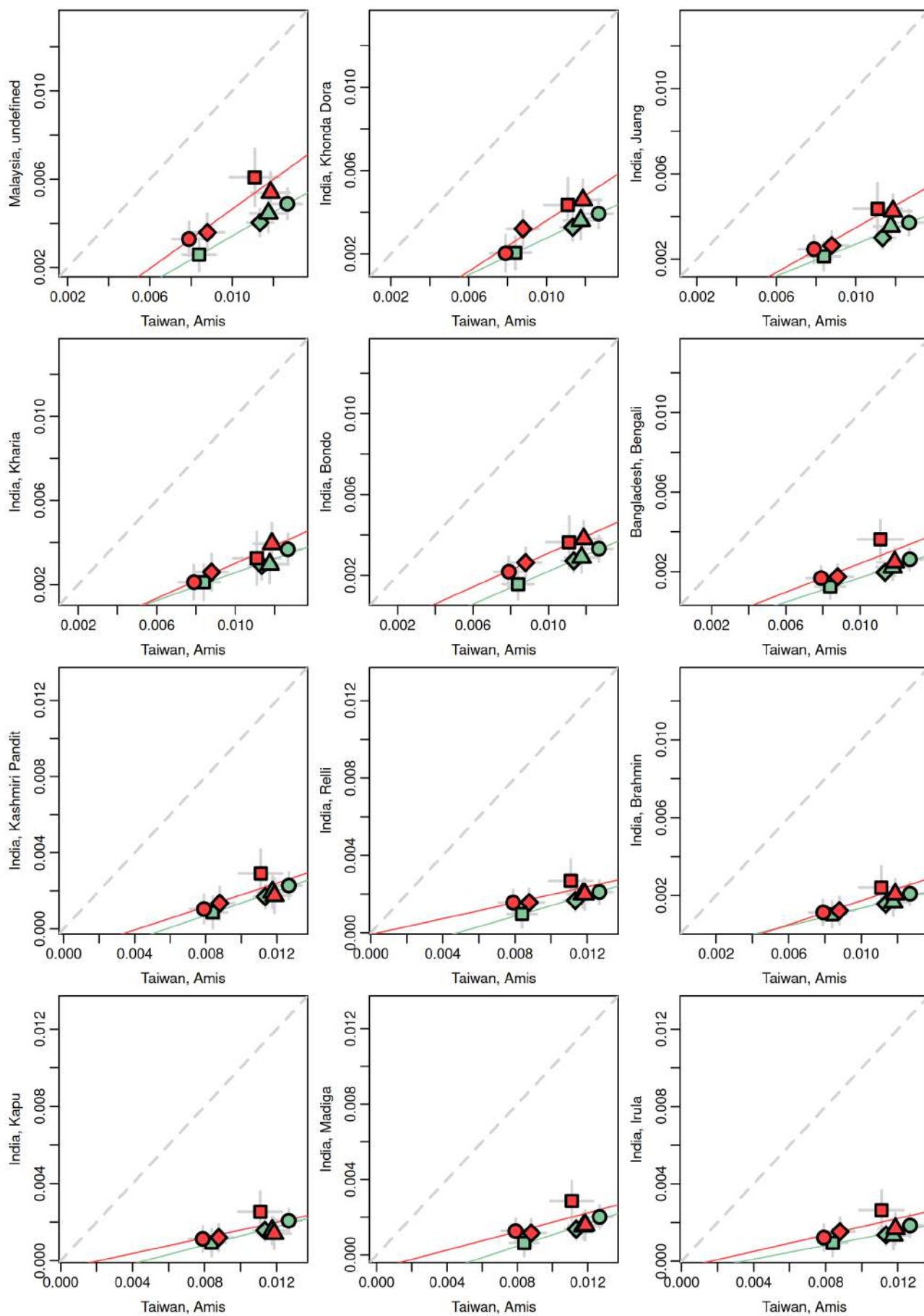


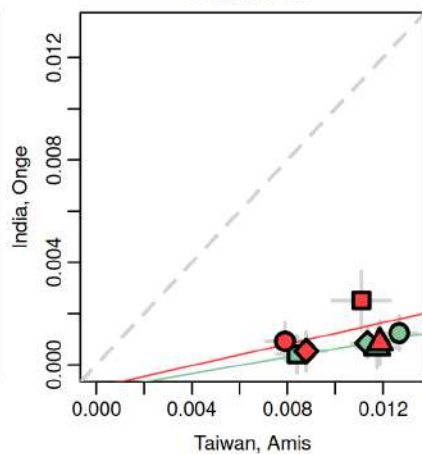
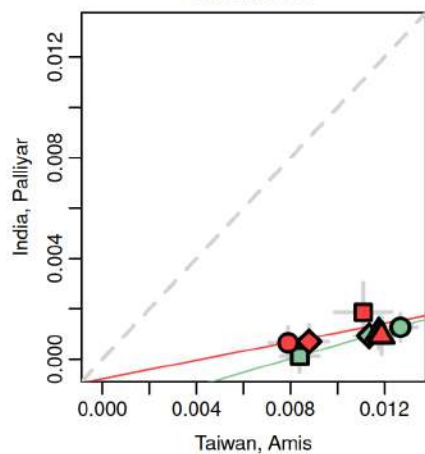
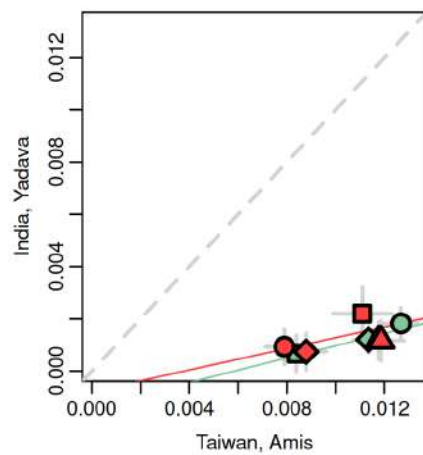
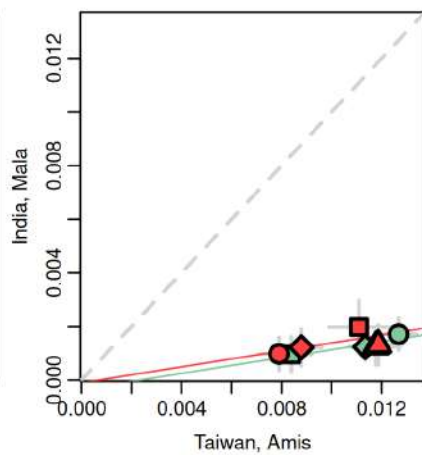
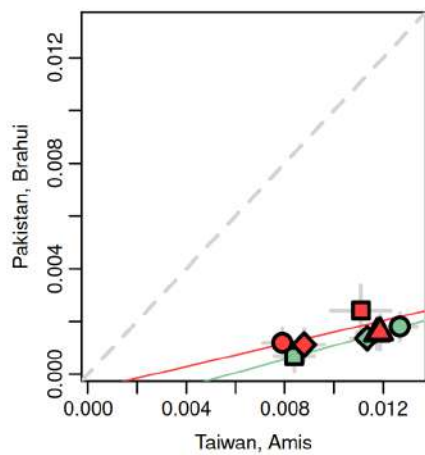




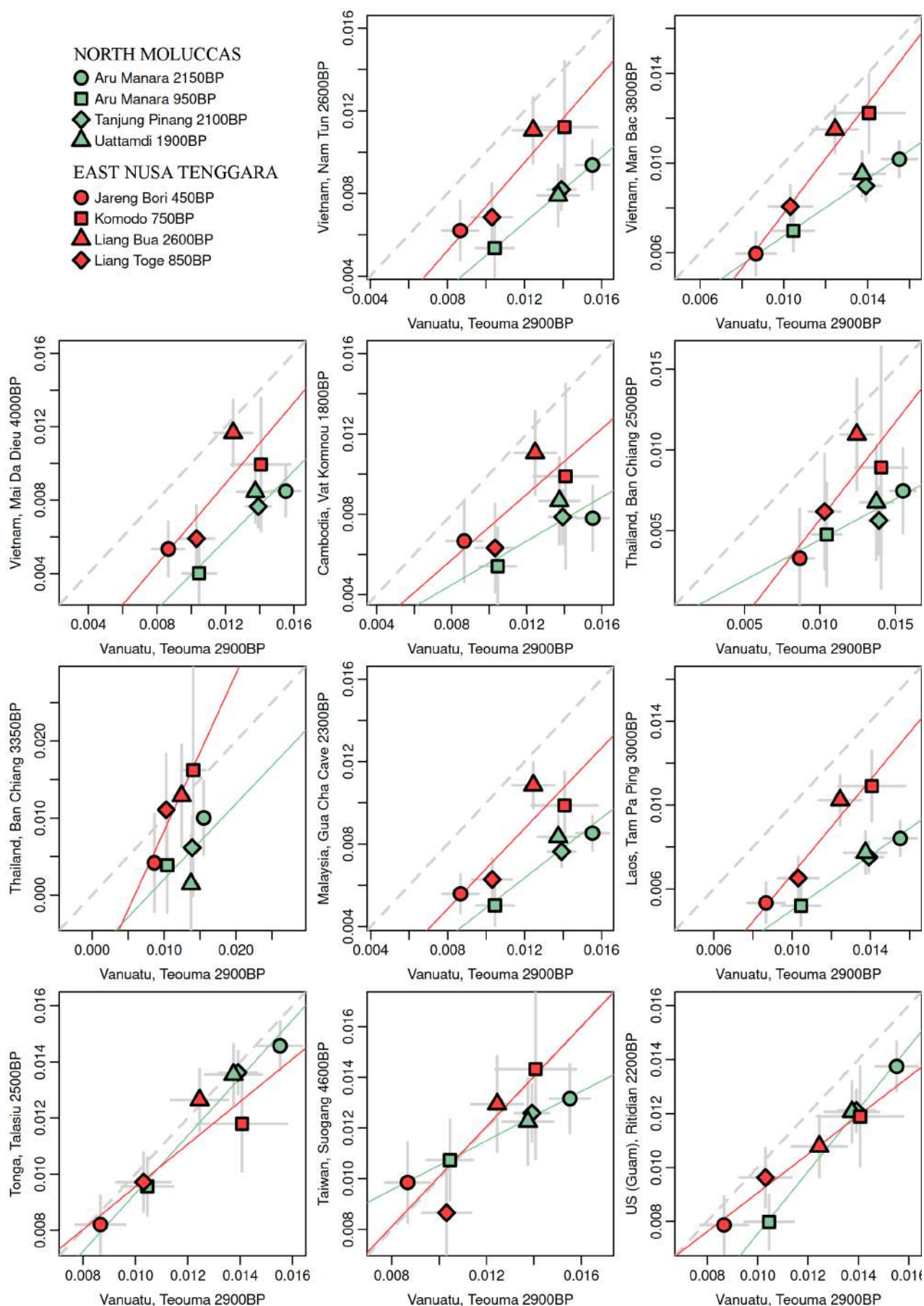


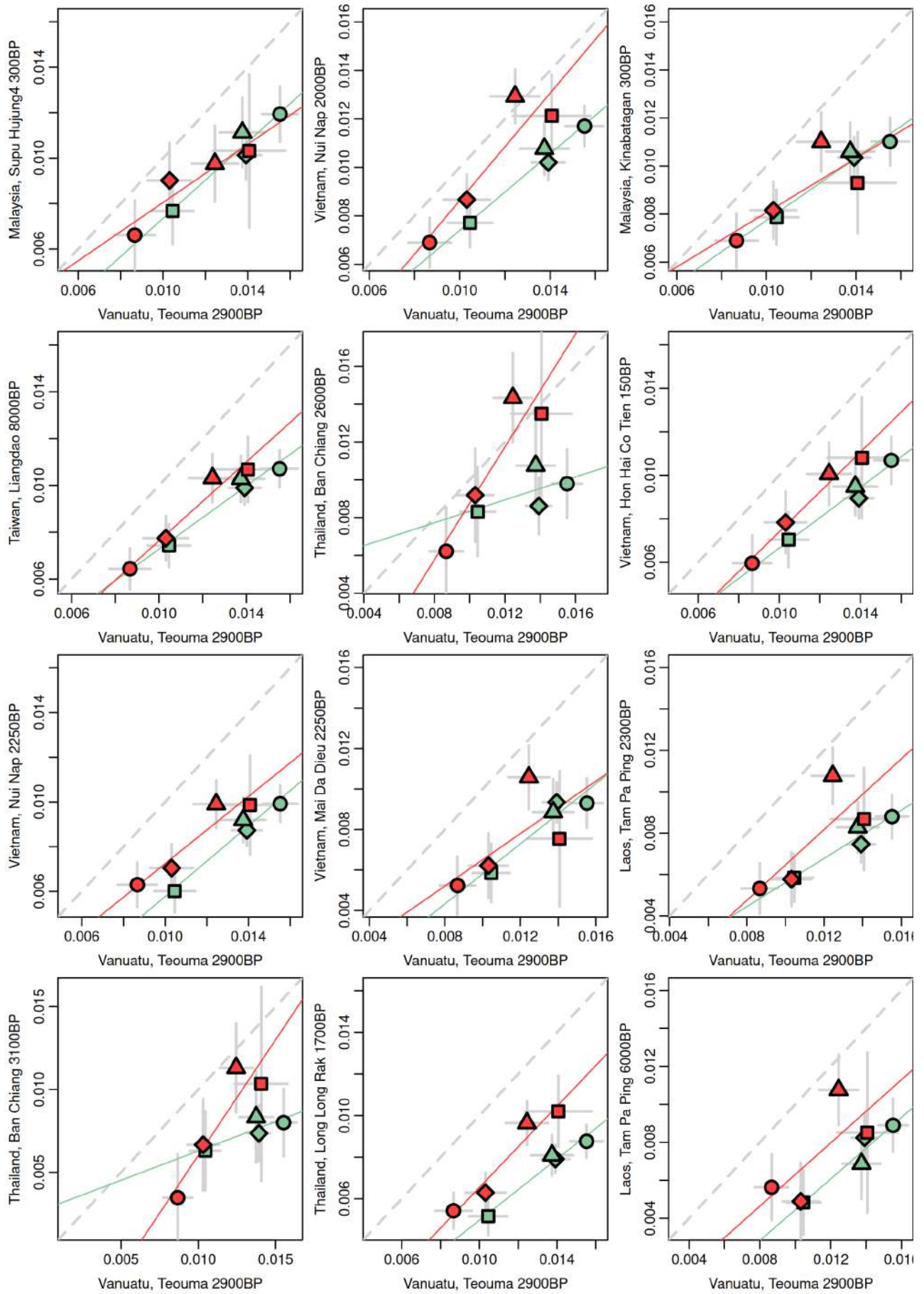


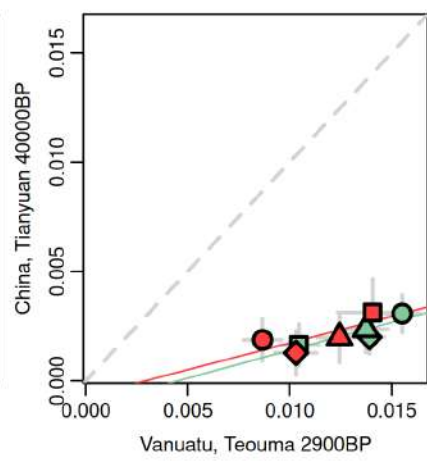
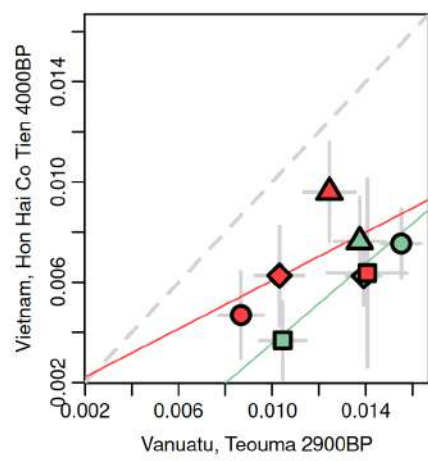




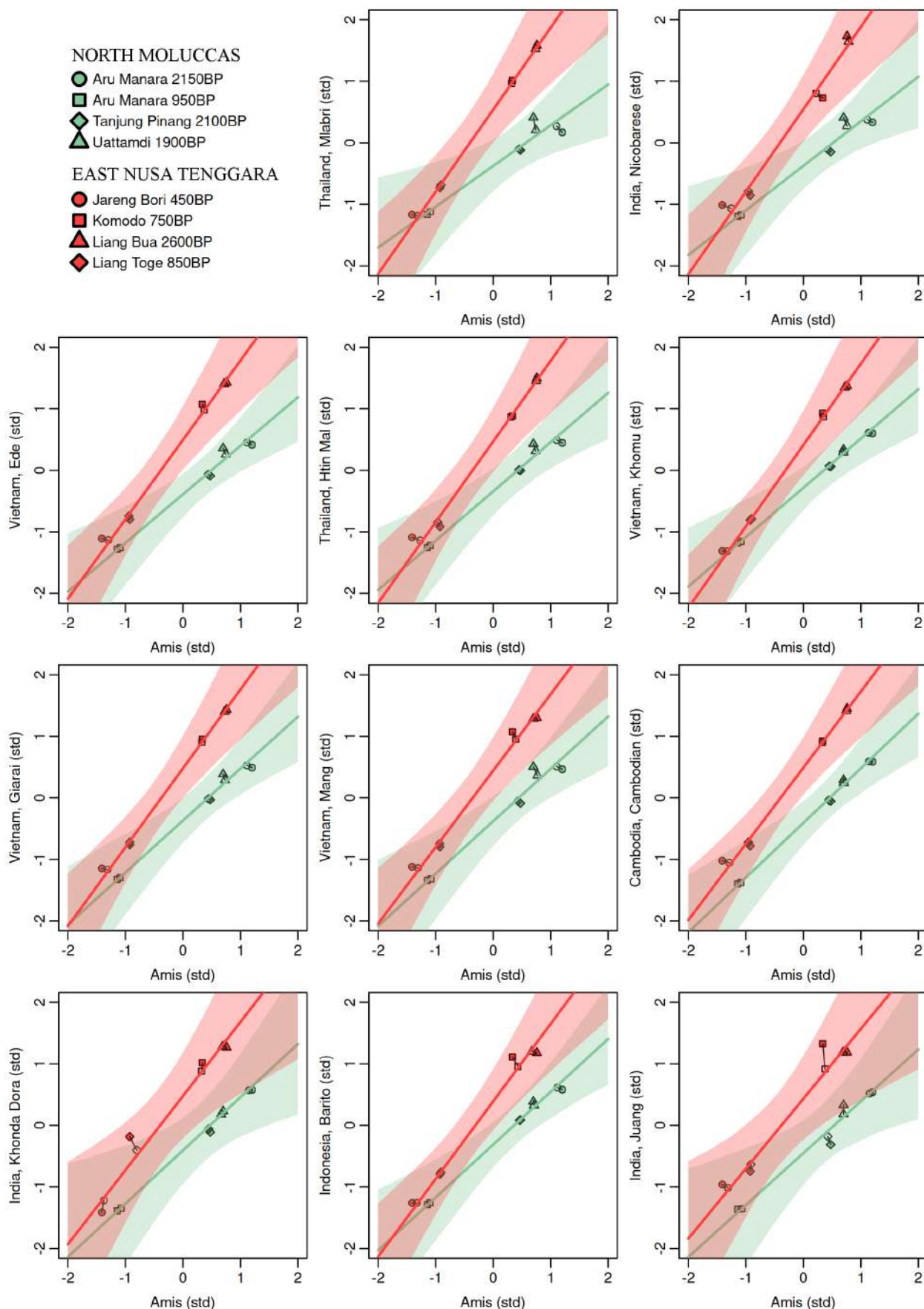
Supplementary Figure 4 - Biplots showing the results of pairs of f_4 -statistics of the form: $f_4(\text{Mbuti}, \text{test}; \text{New Guinea Highlanders}, \text{ancient Wallacea})$. The test groups, shown on the x-y axis label, include ancient groups from mainland Asia, Island southeast Asia, and Oceania that have no discernible Papuan-related ancestry based on the DyStruct analysis. Data are presented as exact f_4 -values $\pm$ 2 SE indicated by grey lines. Linear regression lines for the North Moluccas and East Nusa Tenggara individuals are shown in green and red, respectively.

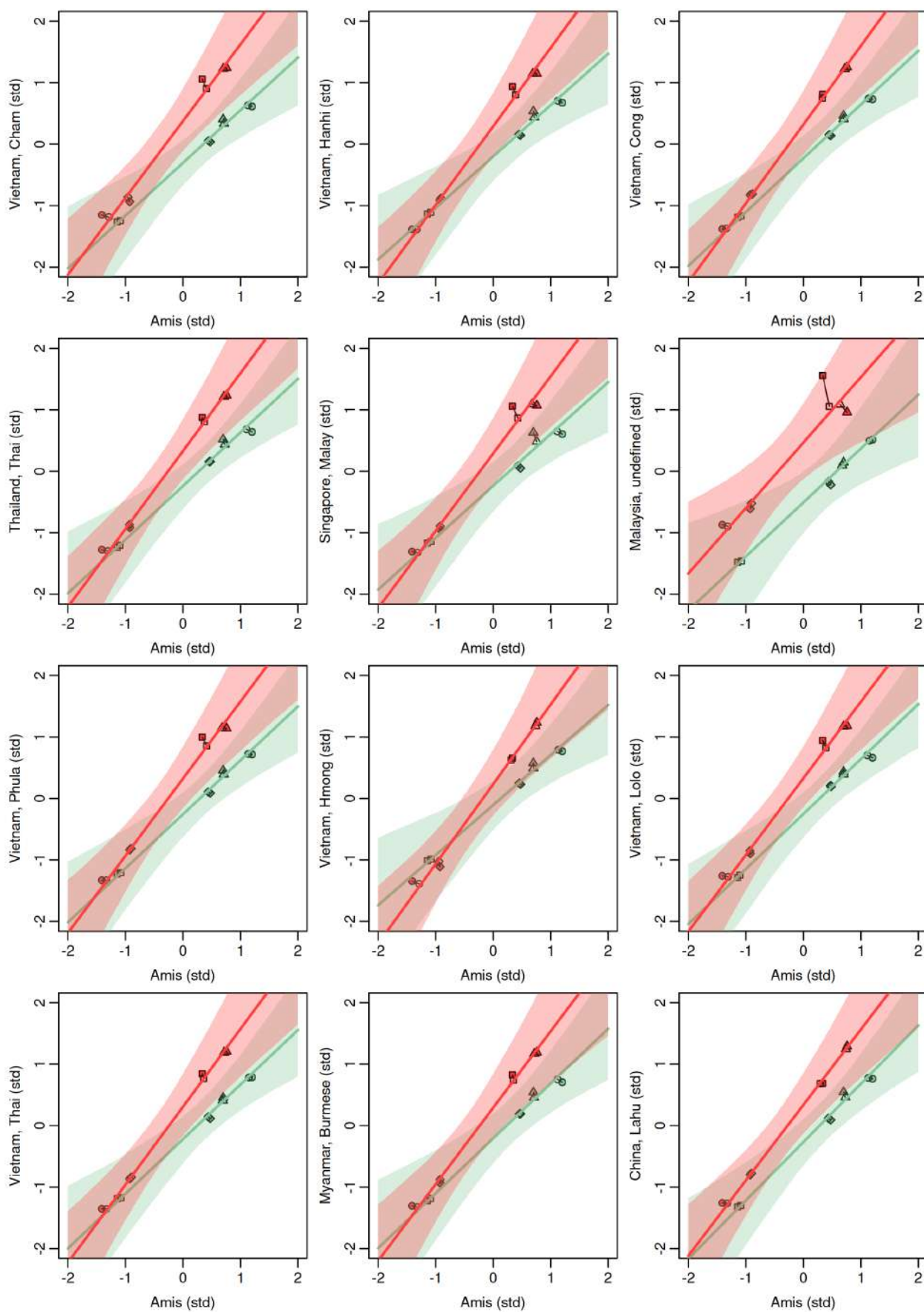


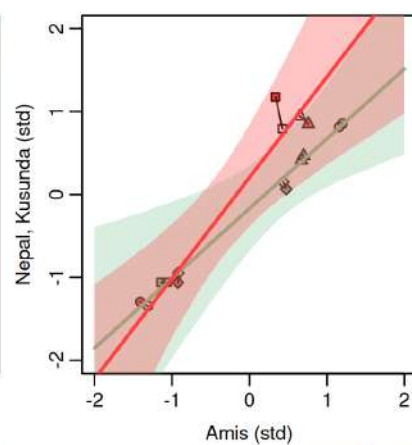
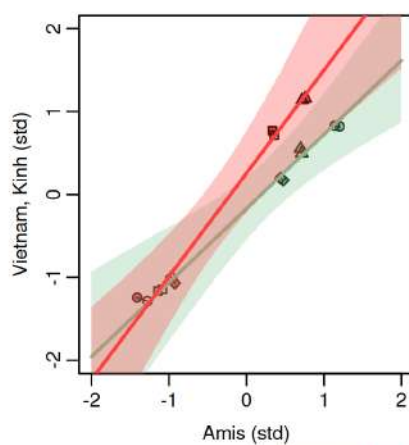
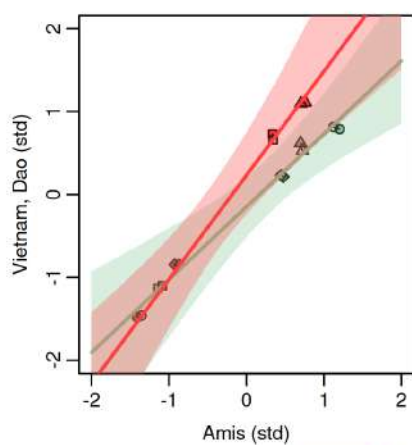


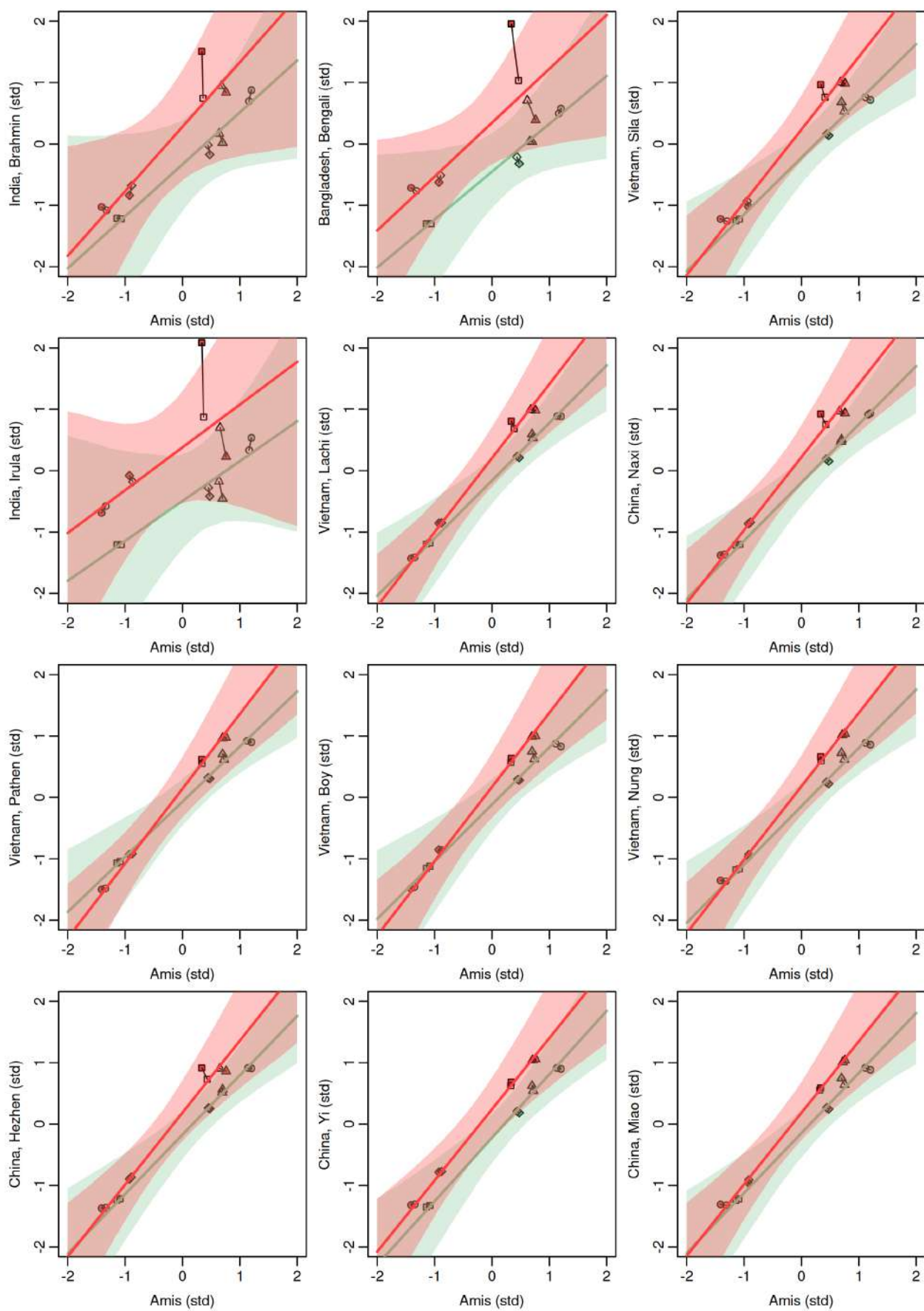


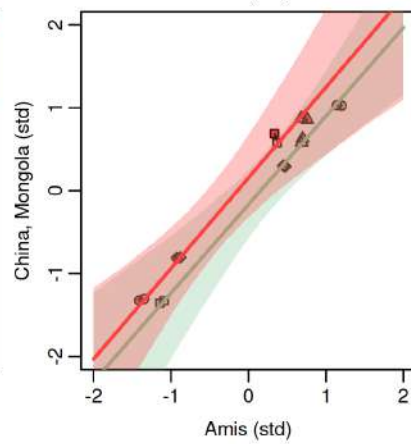
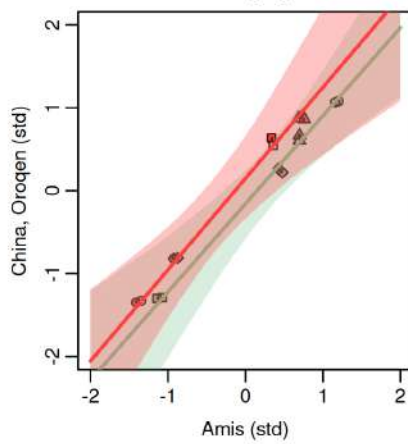
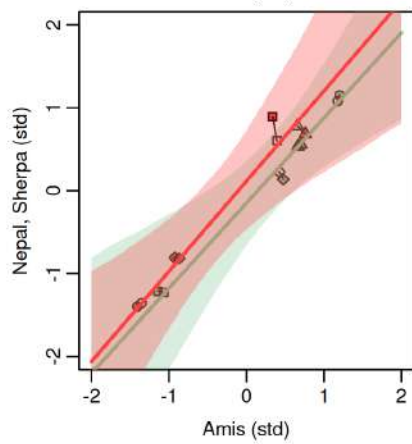
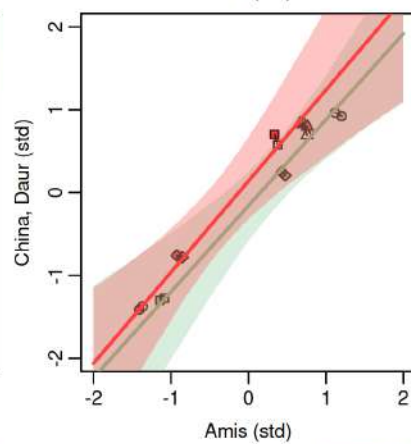
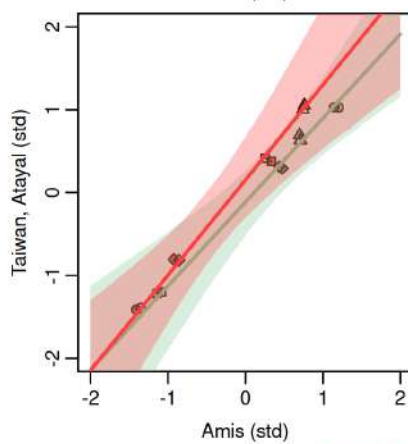
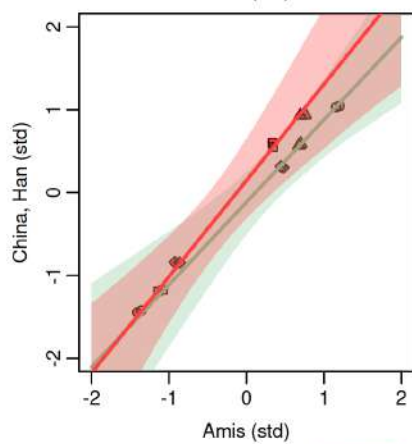
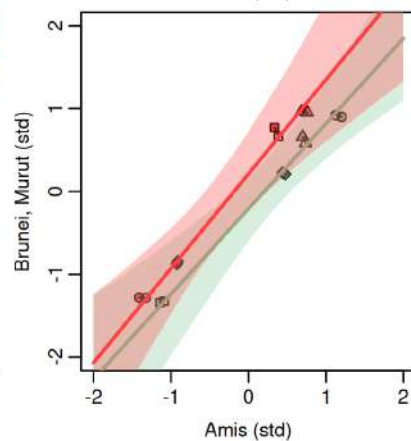
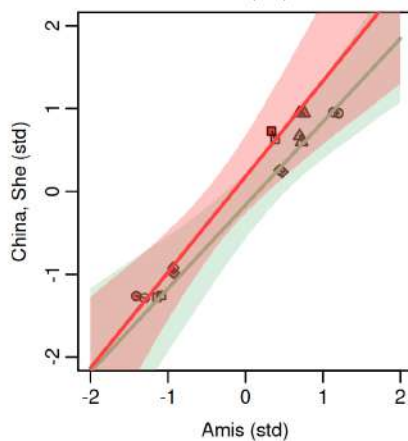
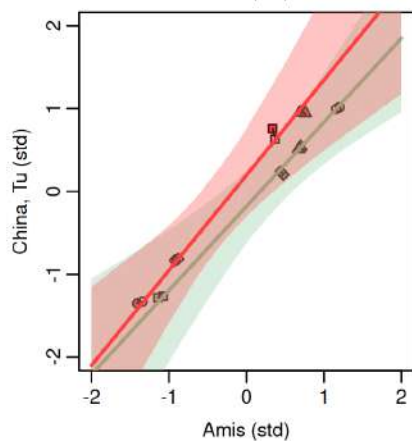
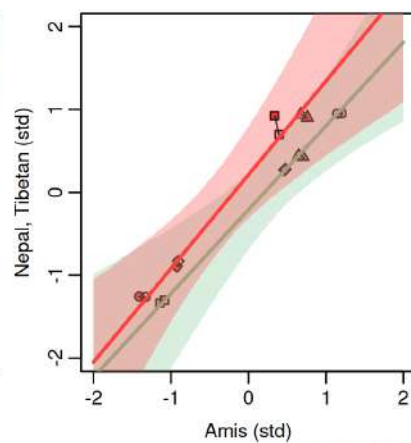
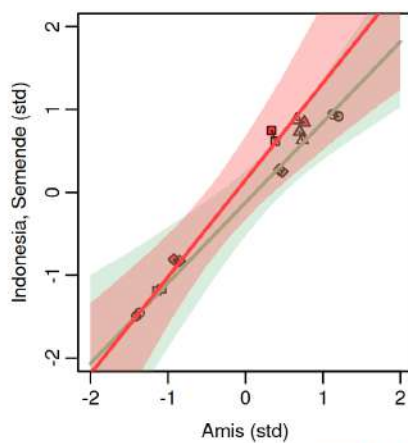
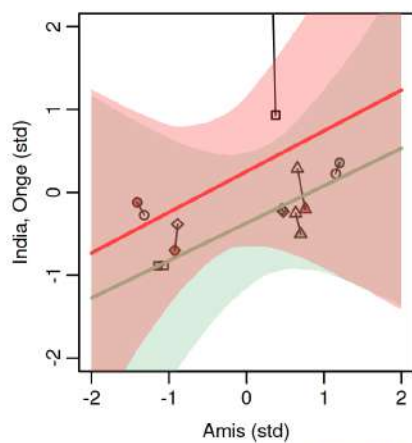
Supplementary Figure 5 - Bayesian linear regression for pairs of f_4 -statistics of the form: $f_4(\text{Mbuti}, \text{test}; \text{New Guinea Highlanders, ancient Wallacea})$, where *test* corresponds to the present-day groups shown in the x-y axis labels. The lines show the posterior means for the North Moluccas (green) and East Nusa Tenggara (red) groups, while the shades show the 95% credible interval. Filled symbols show the observed values (standardized) and non-filled symbols show their posterior means, with the lines connecting pairs of points from the same ancient sample.

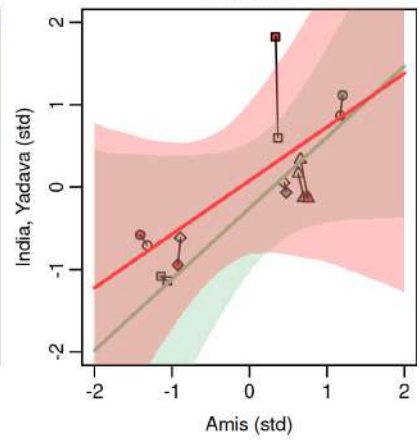
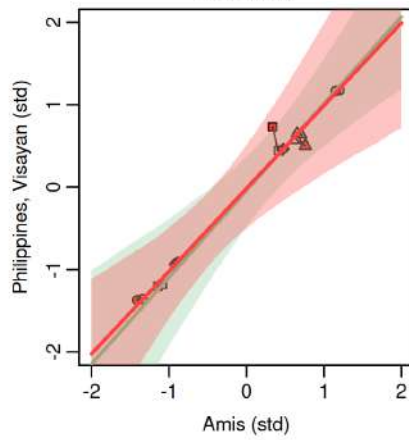
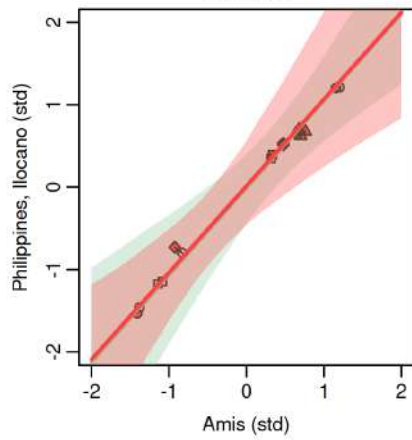
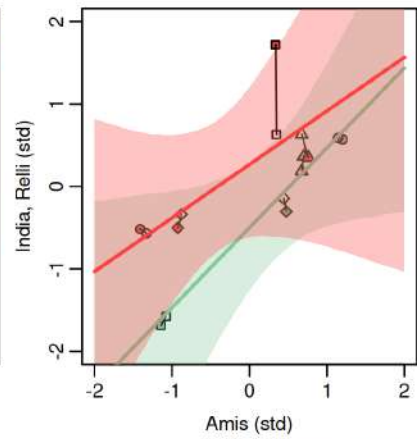
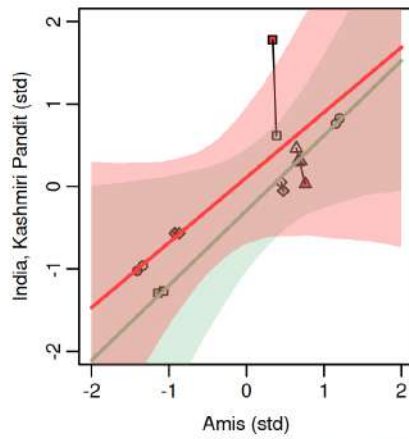
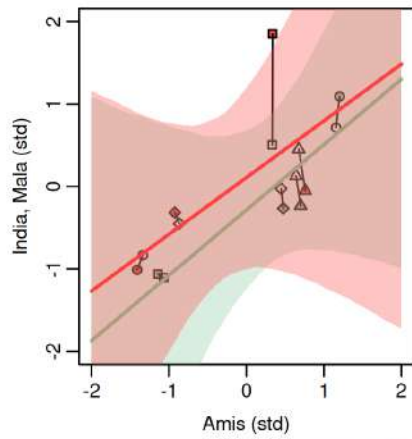
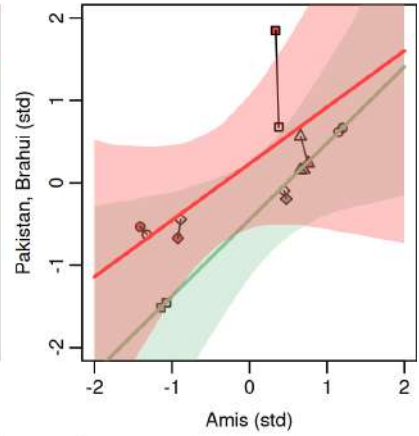
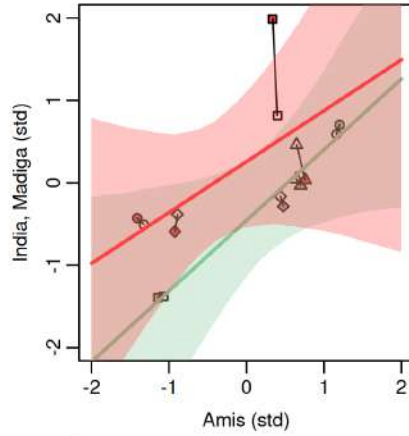
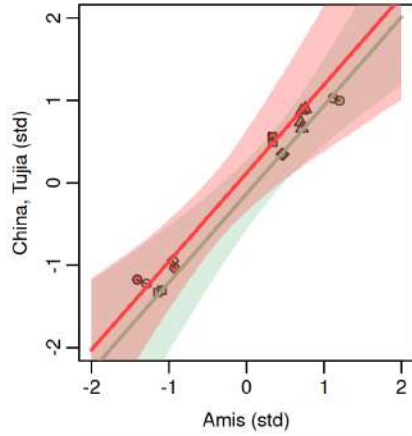
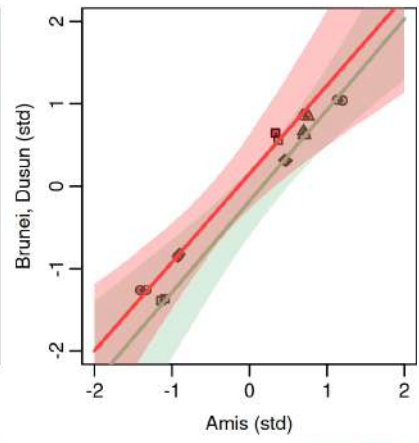
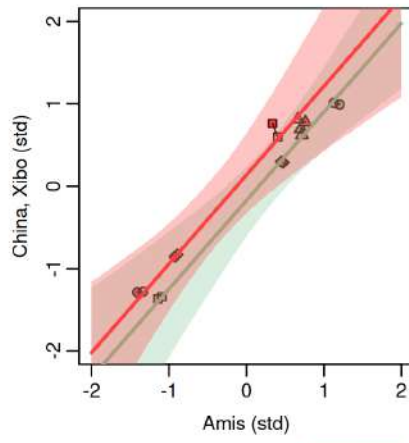
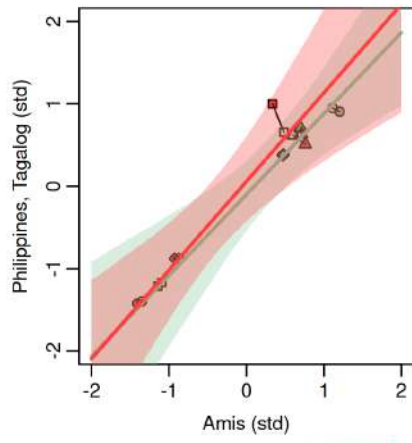


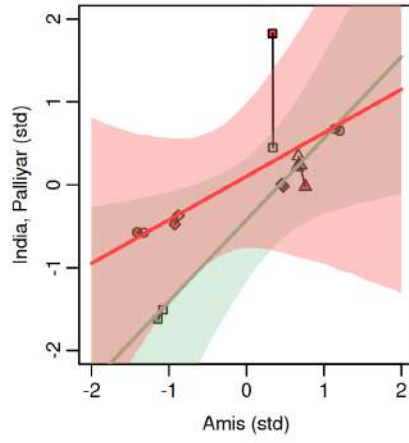
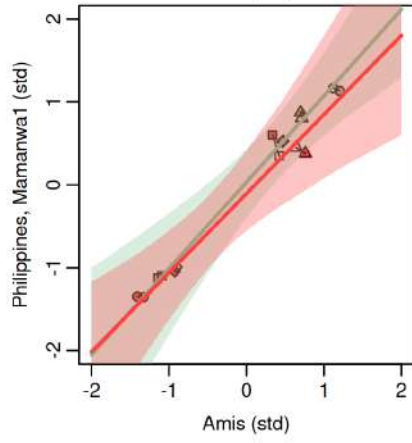
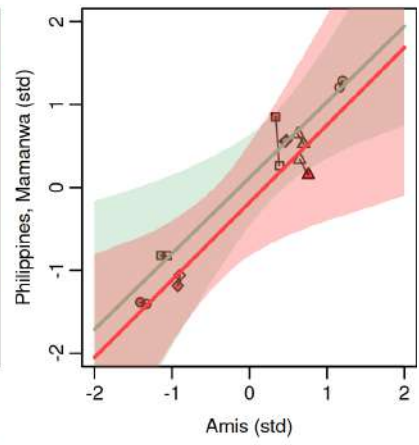
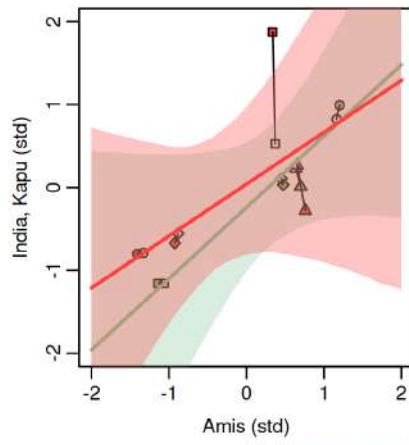
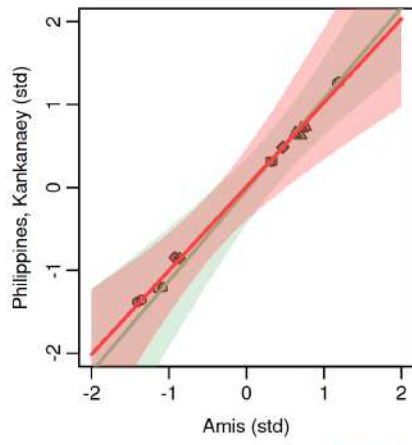




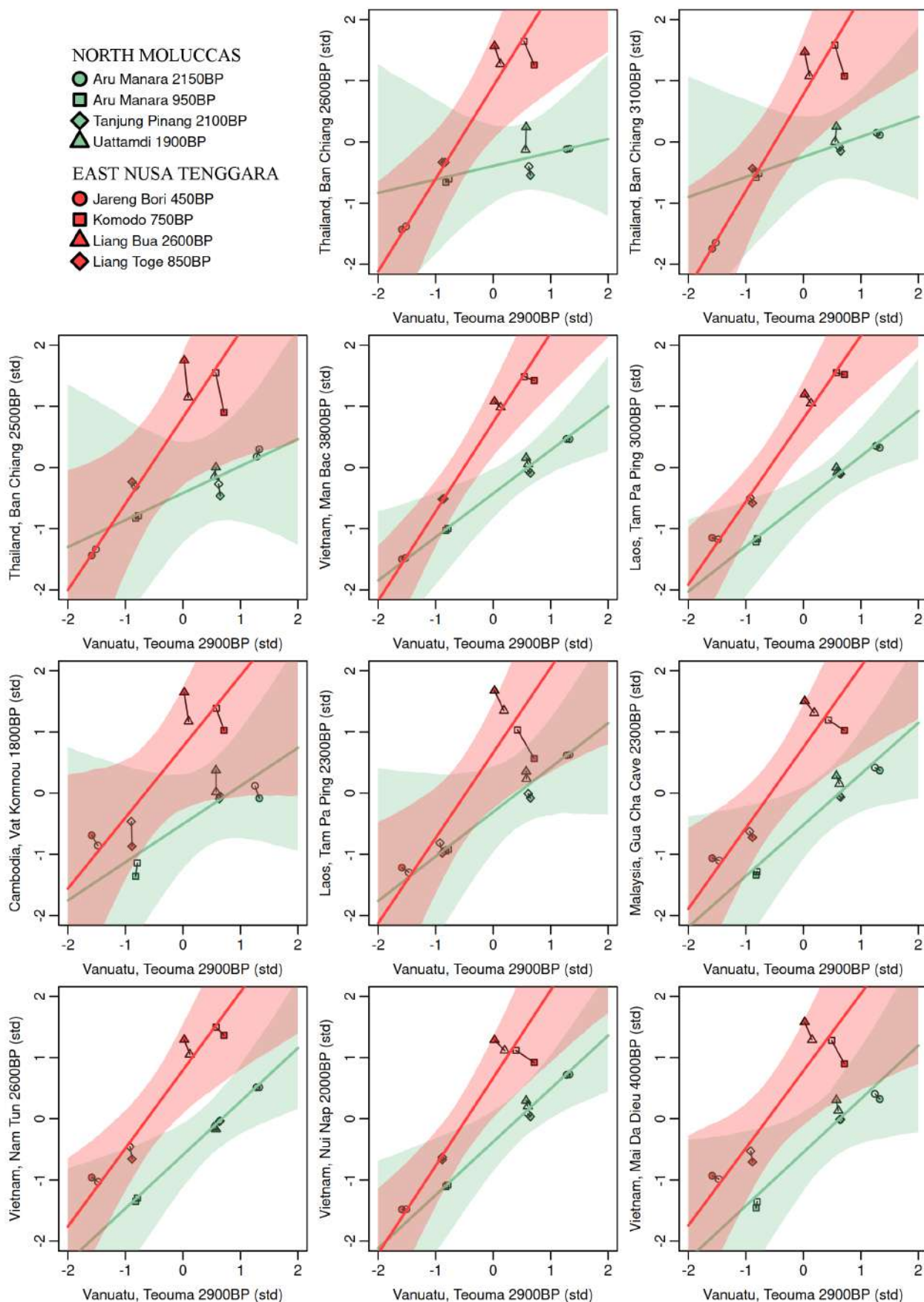


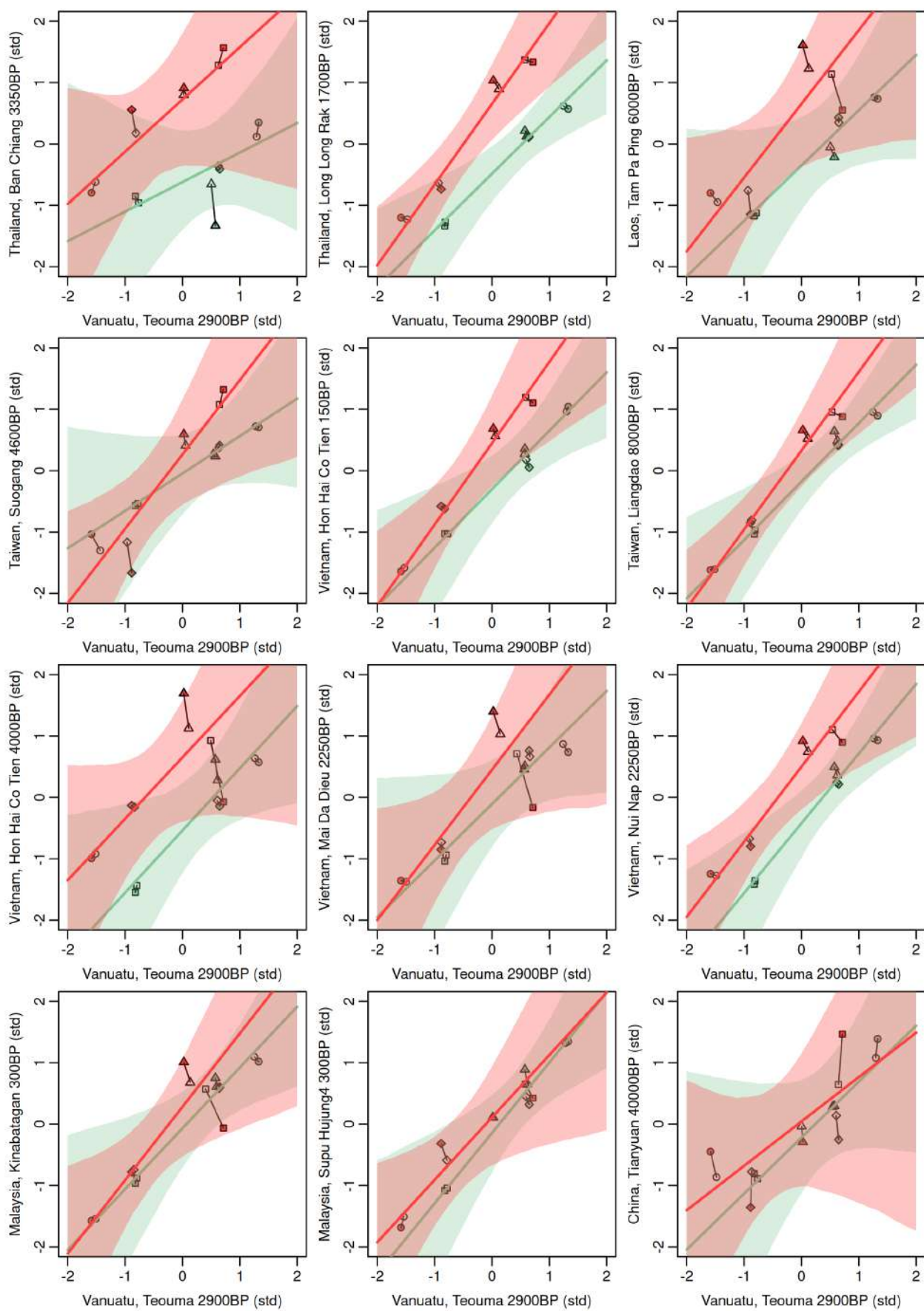


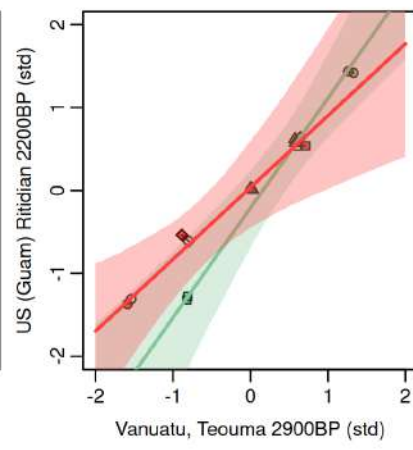
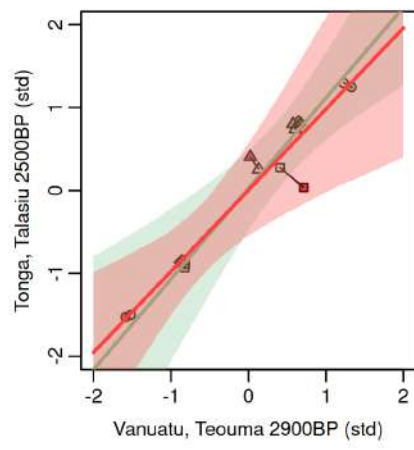




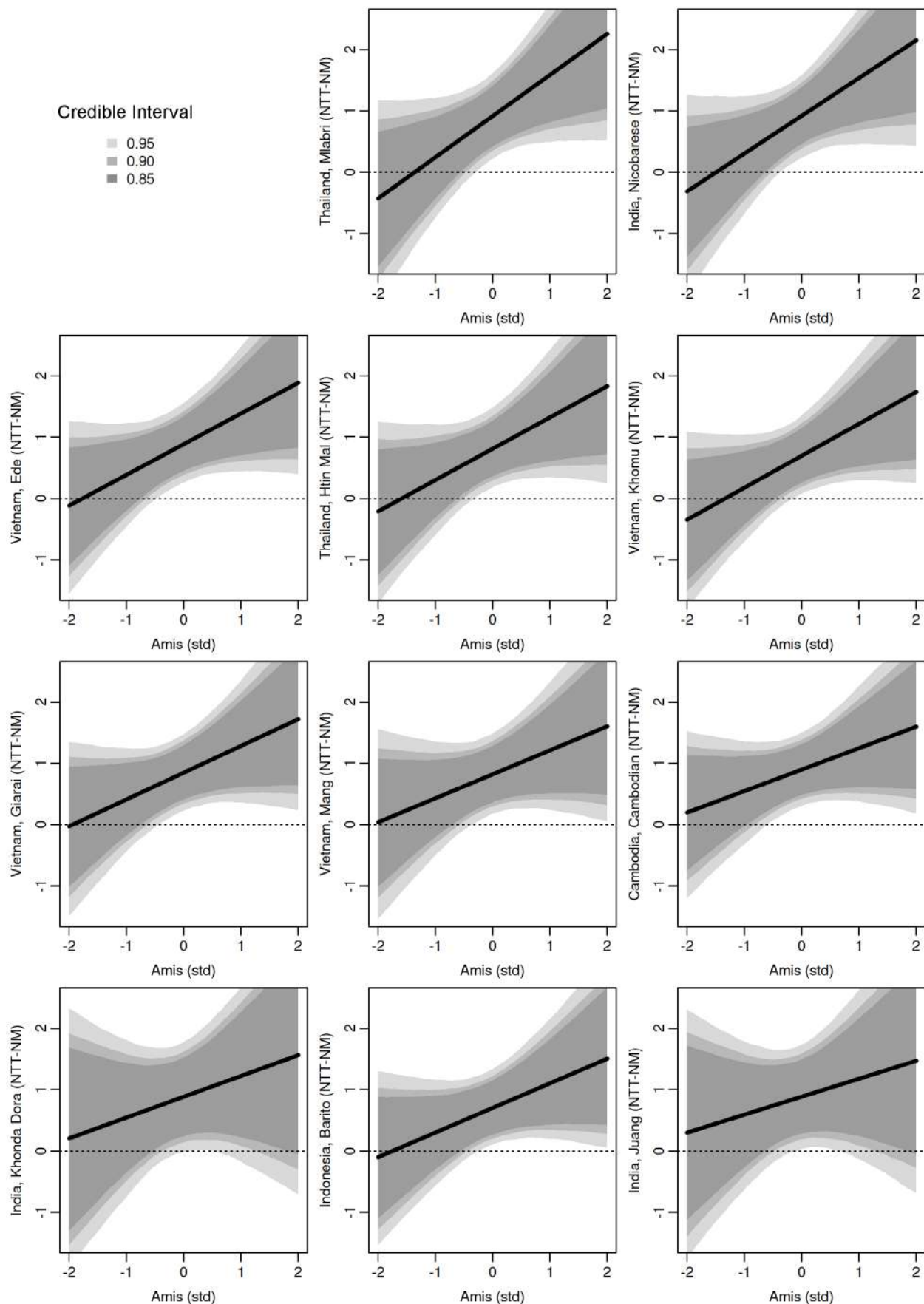
Supplementary Figure 6 - Bayesian linear regression for pairs of f_4 -statistics of the form: $f_4(\text{Mbuti}, \text{test}; \text{New Guinea Highlanders}, \text{ancient Wallacea})$, where *test* corresponds to the ancient groups shown in the x-y axis labels. The lines show the posterior means for the North Moluccas (green) and East Nusa Tenggara (red) groups, while the shades show the 95% credible interval. Filled symbols show the observed values (standardized) and non-filled symbols show their posterior means, with the lines connecting pairs of points from the same ancient sample.

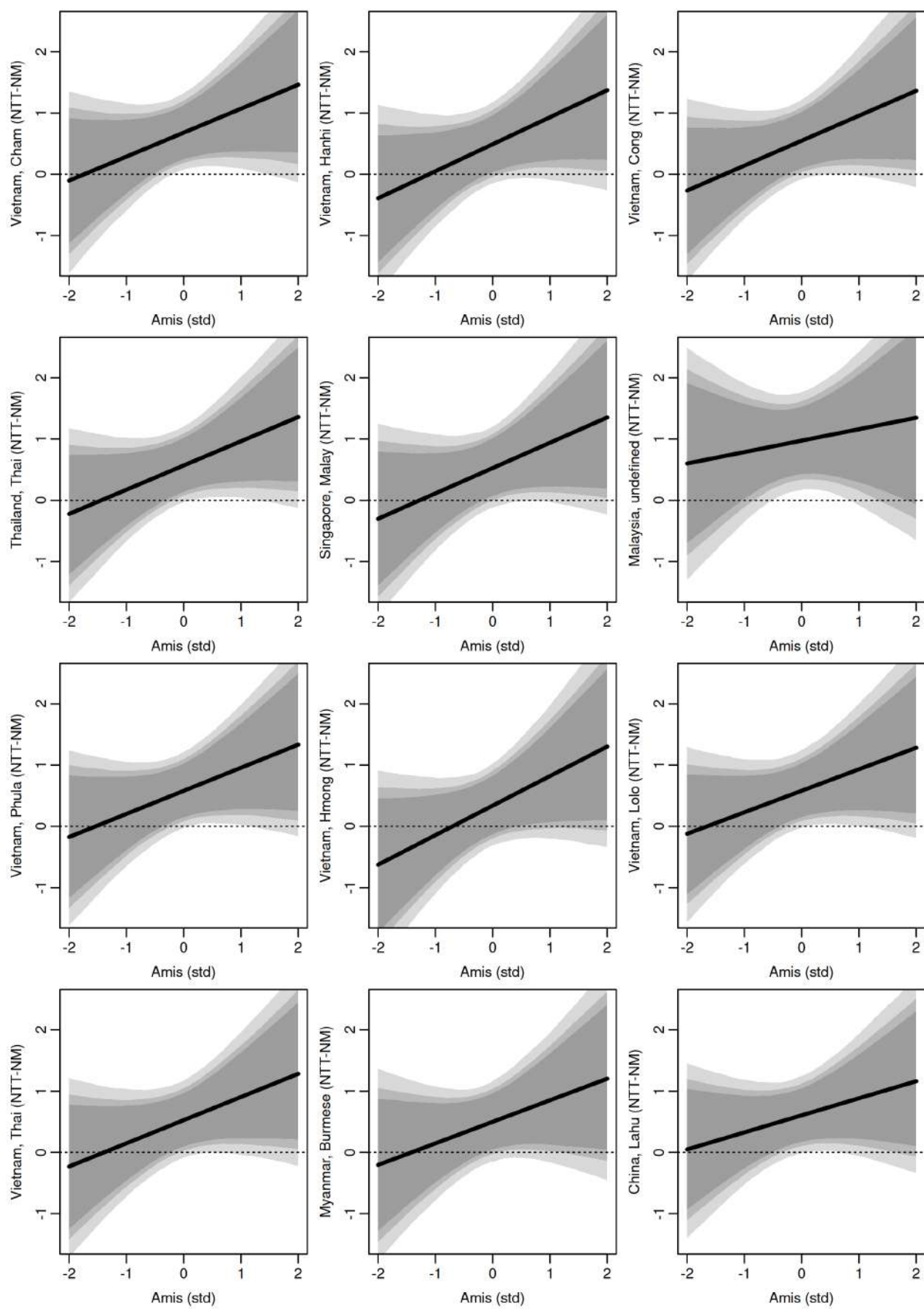


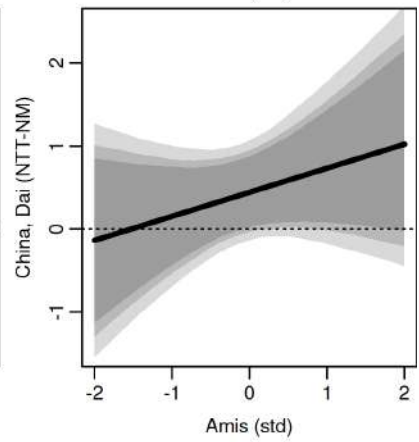
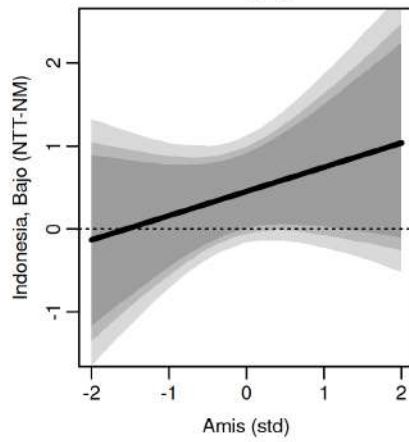
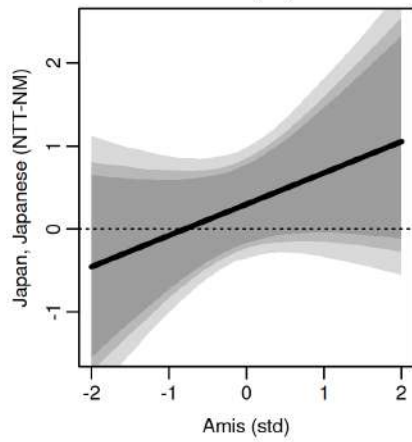
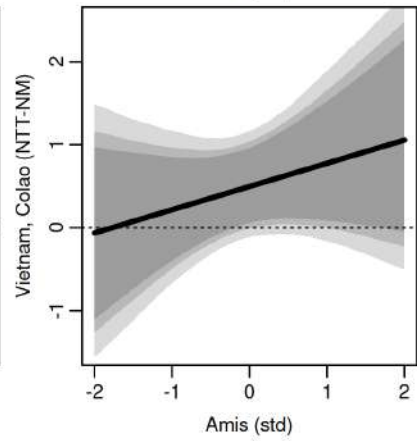
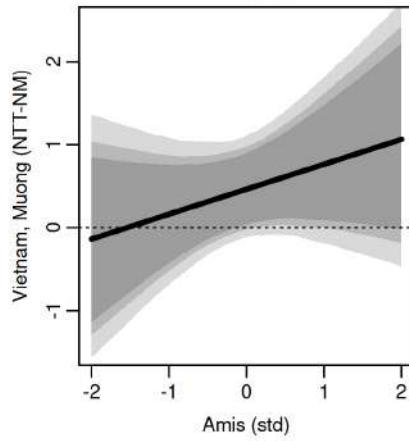
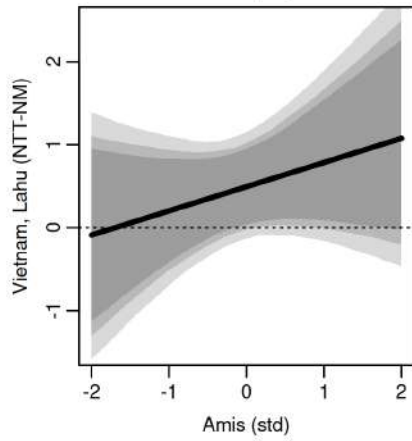
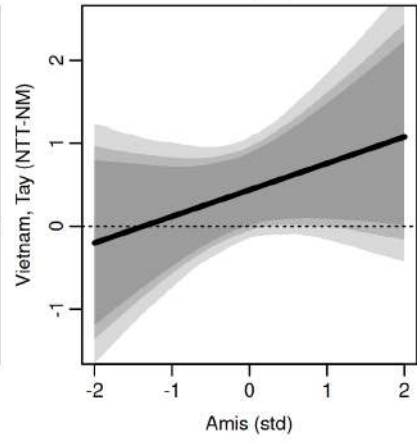
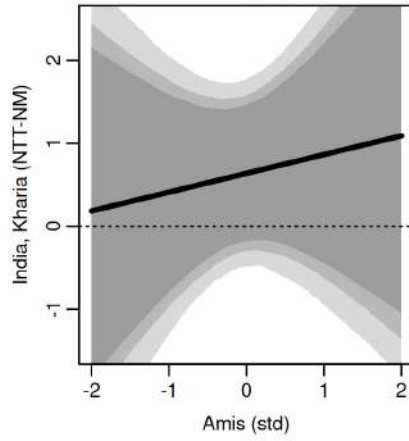
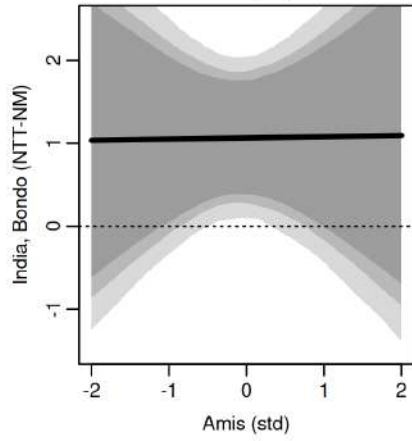
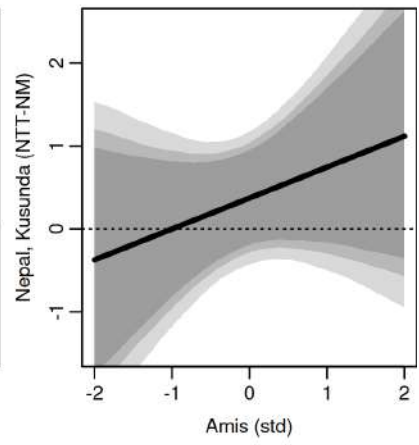
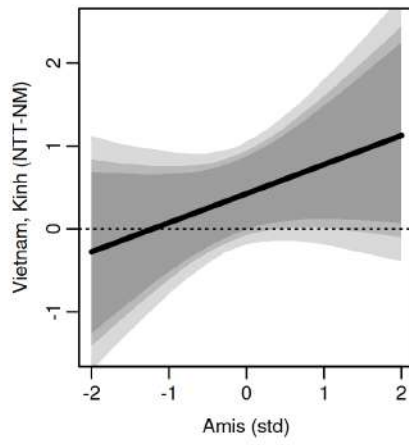
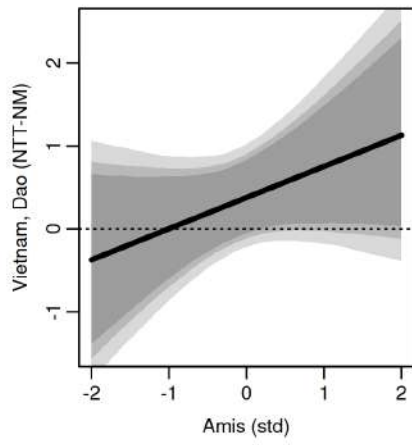


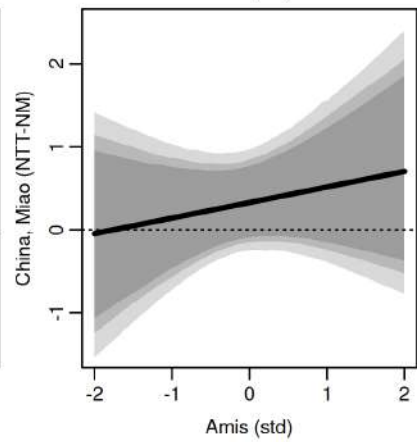
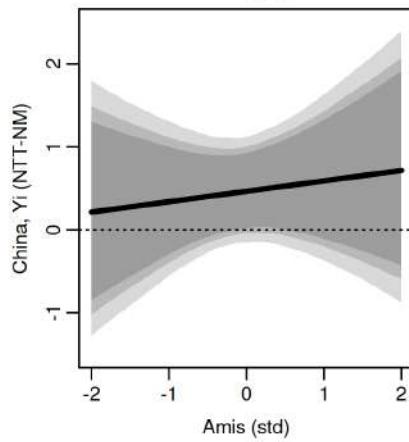
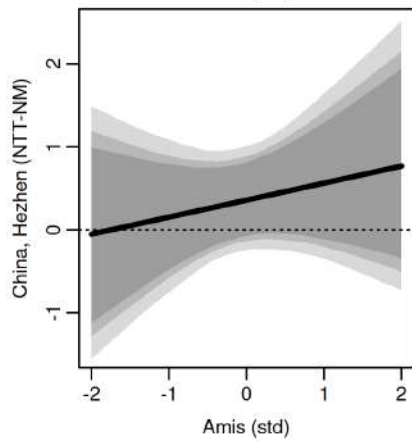
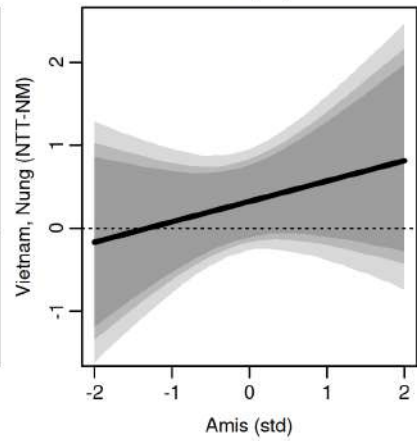
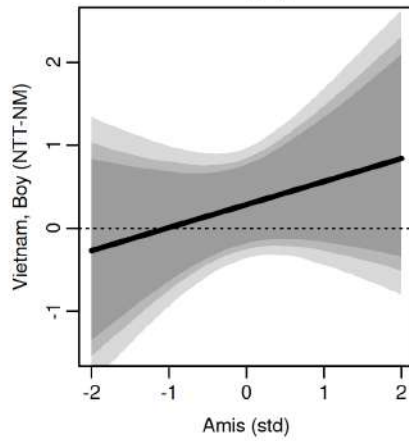
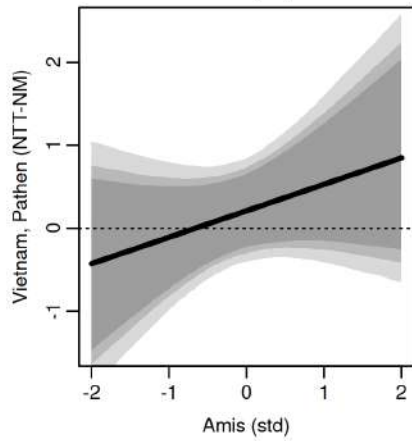
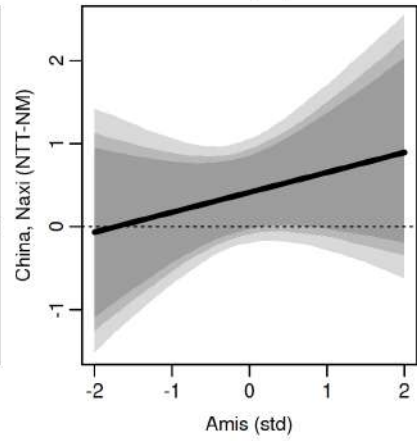
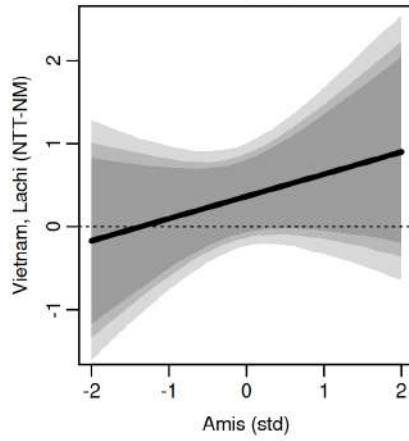
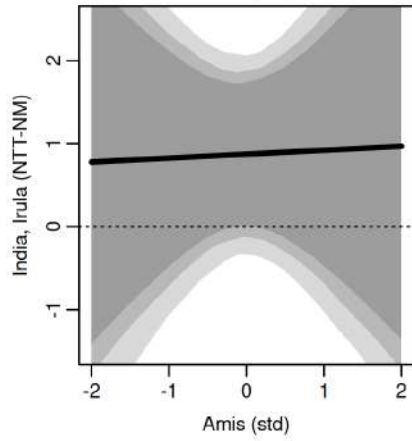
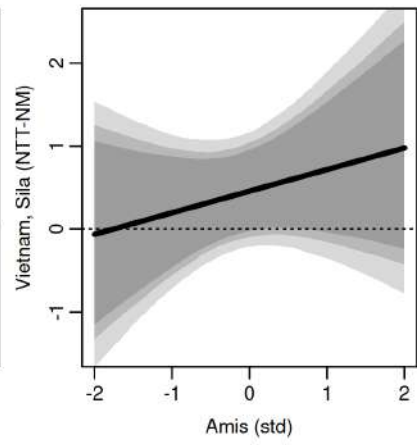
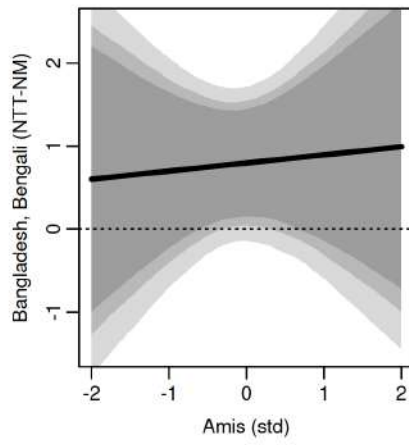
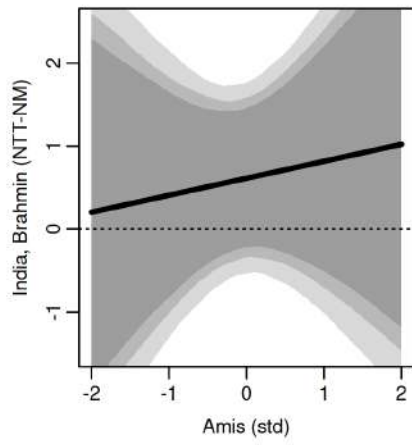


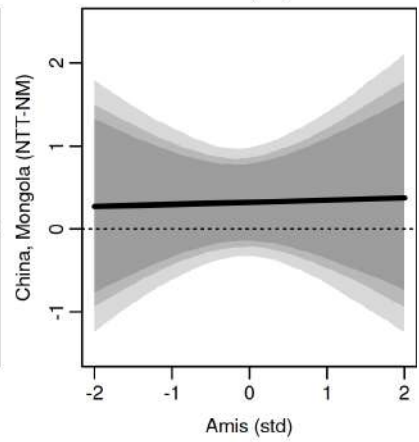
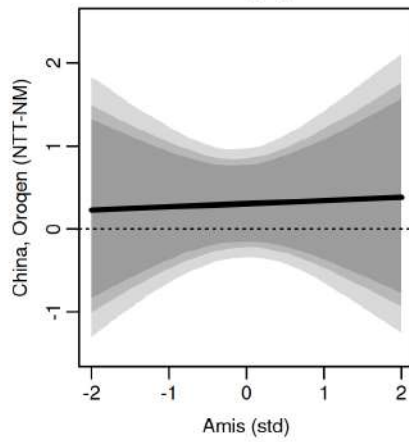
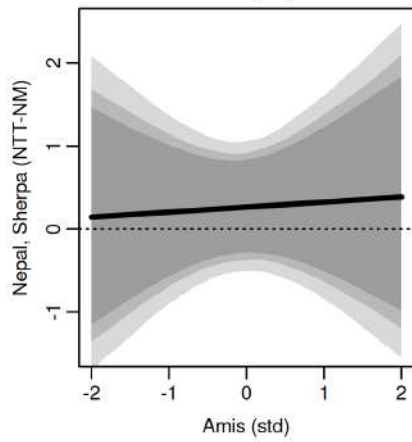
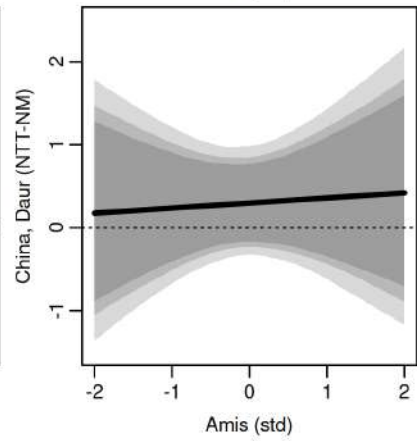
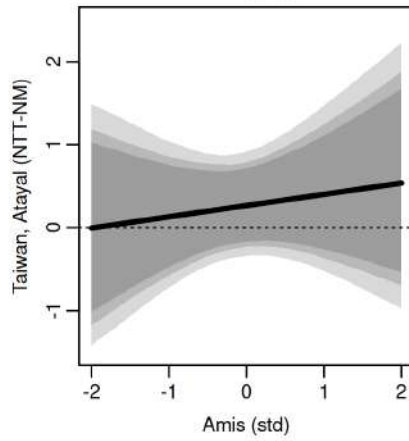
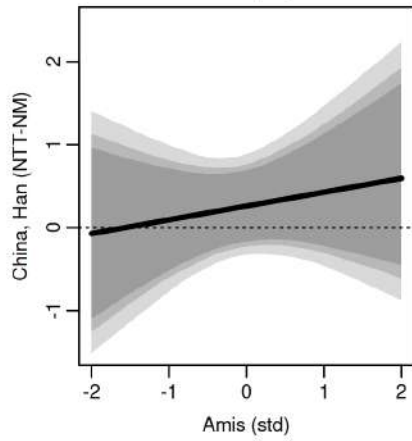
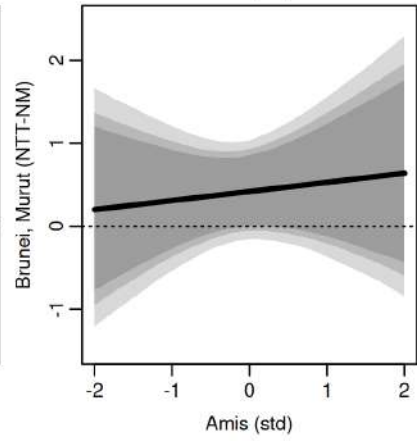
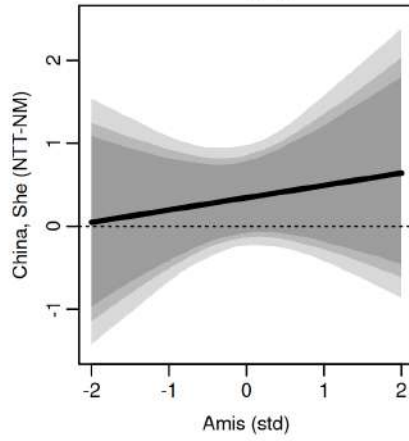
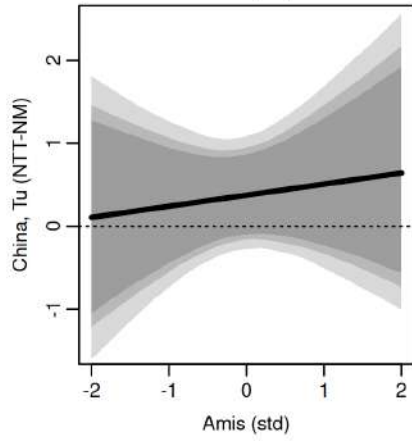
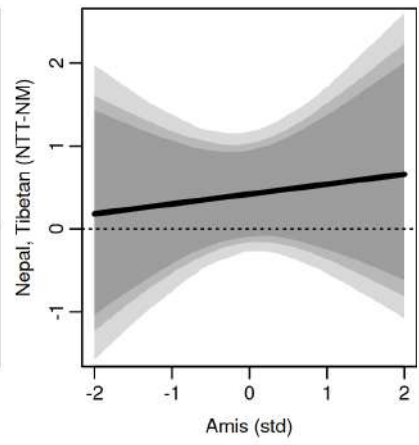
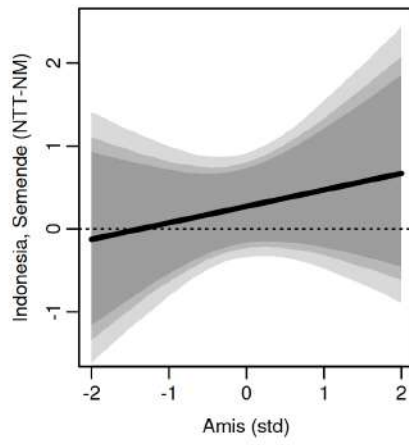
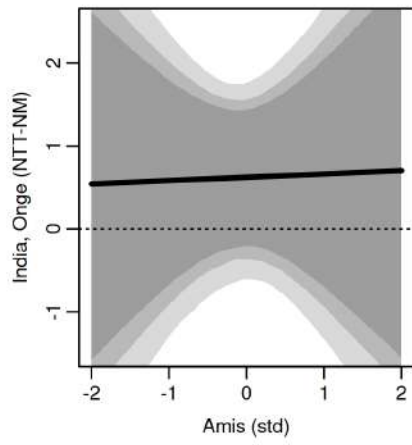
Supplementary Figure 7 - Differences between the posterior distribution of the regression models for the East Nusa Tenggara and North Moluccas (contrasts). Each plot shows the contrasts for the pair of present-day groups shown in the x-y axis labels. The black line represents differences in the posterior means and the shades show the differences in the credible intervals.

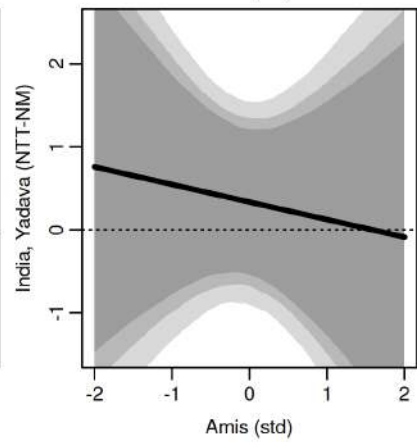
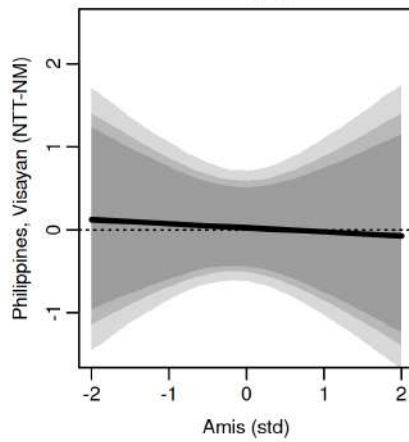
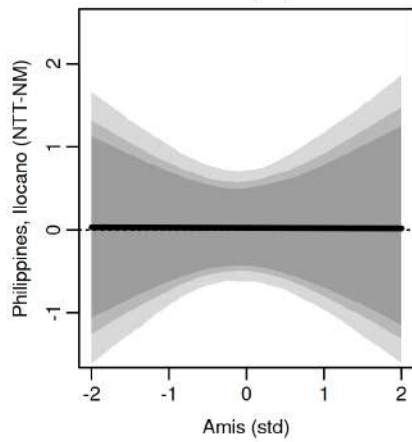
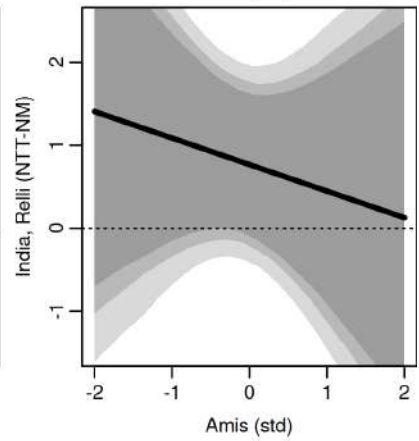
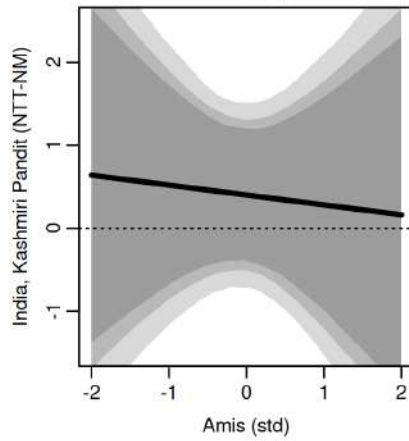
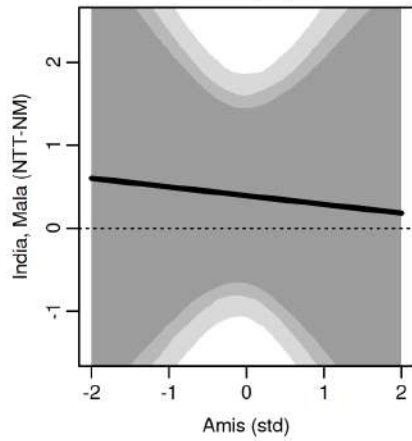
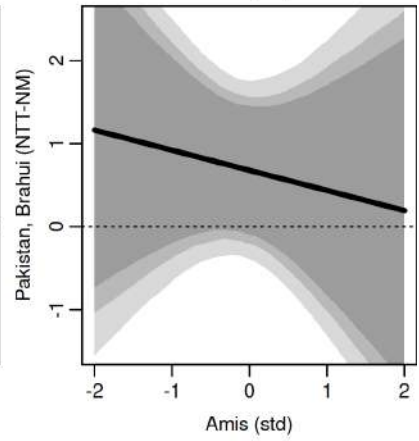
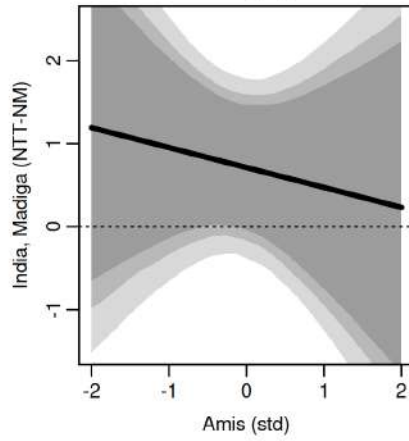
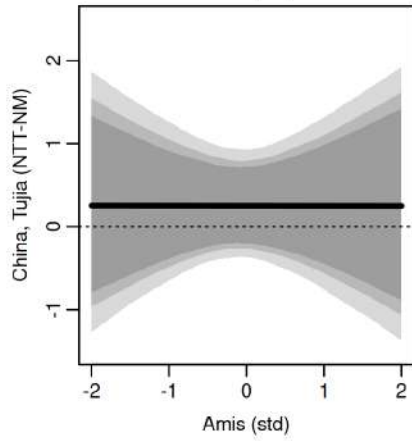
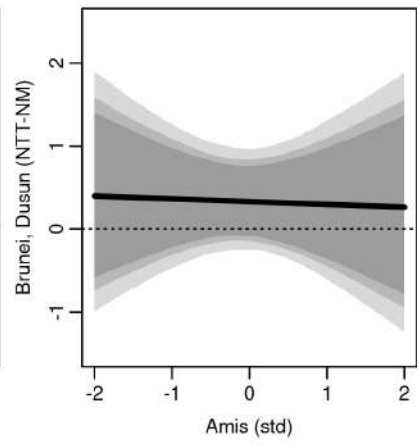
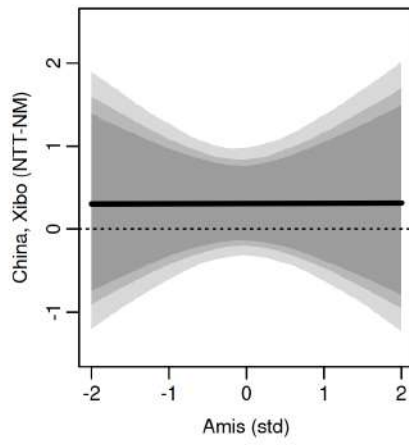
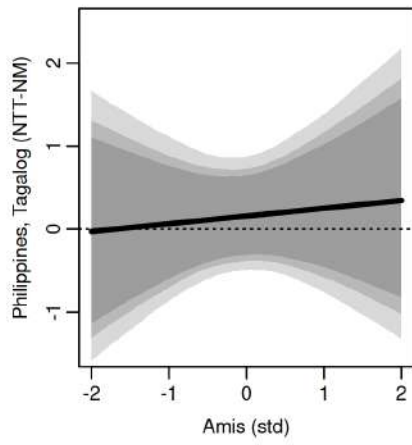


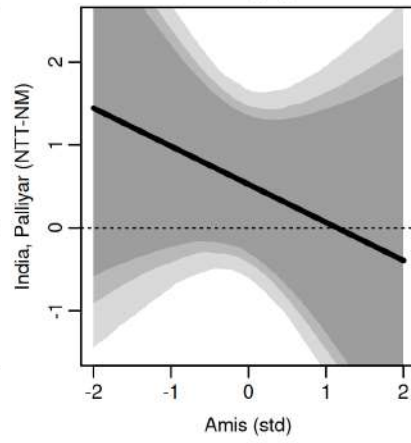
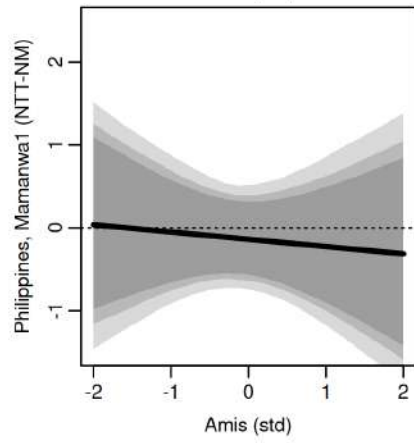
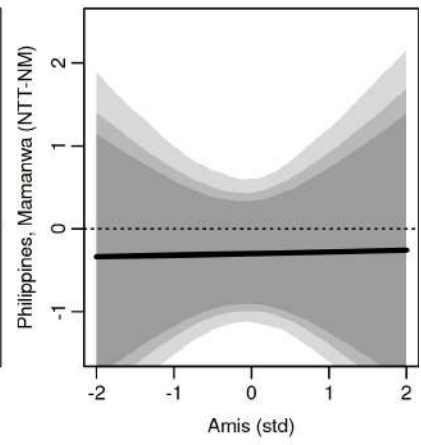
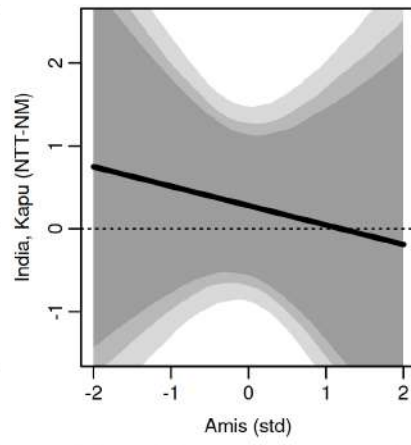
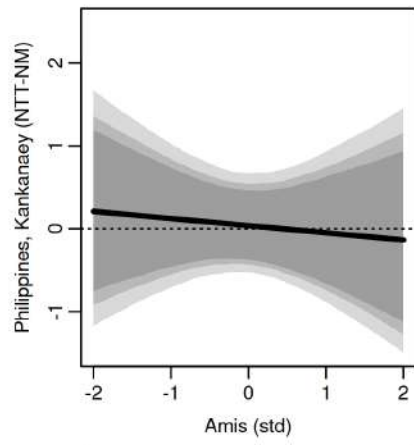




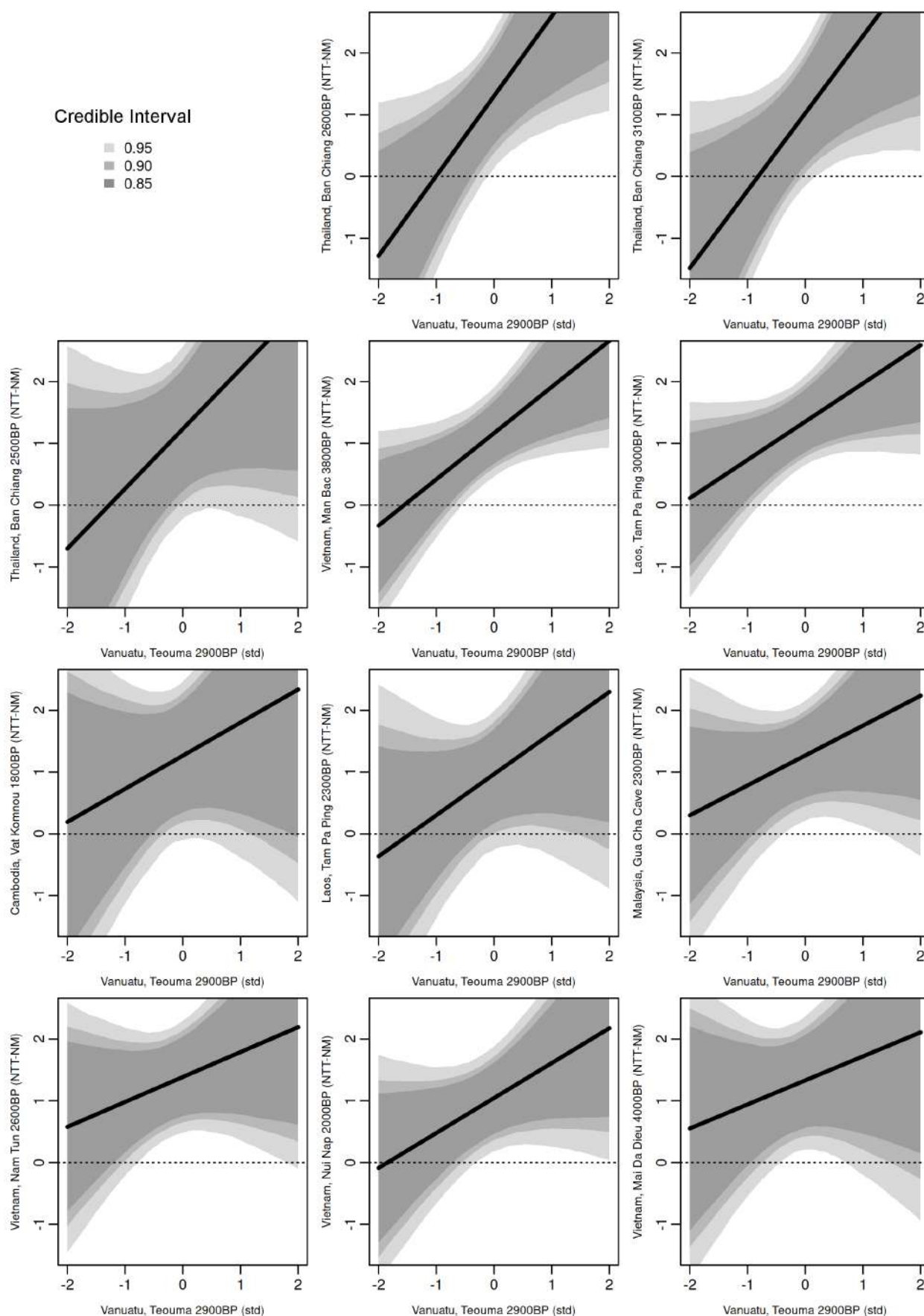


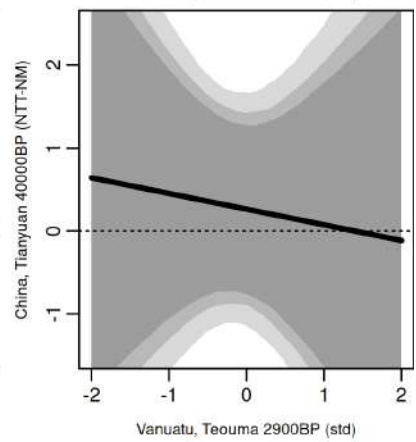
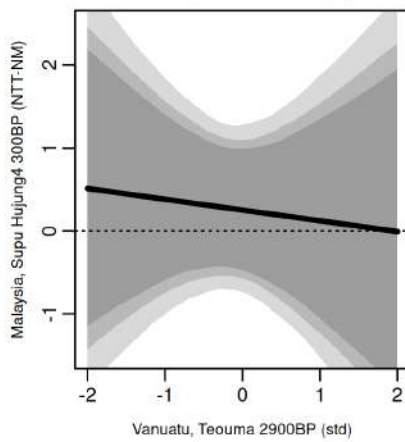
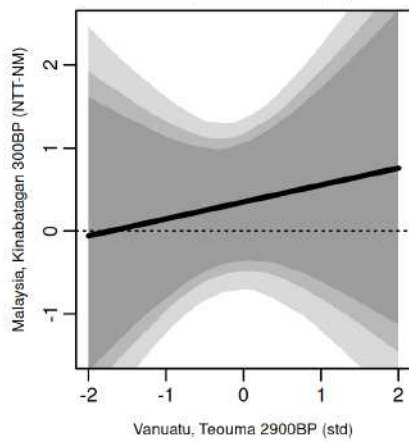
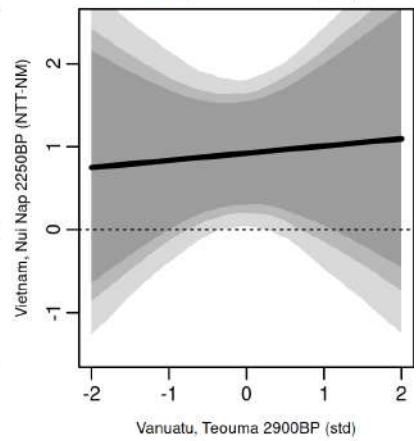
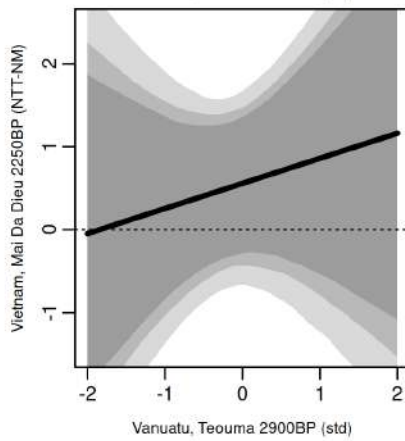
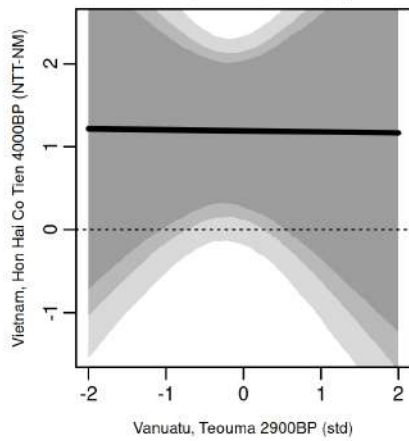
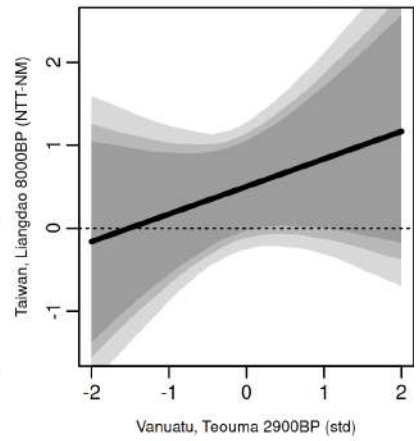
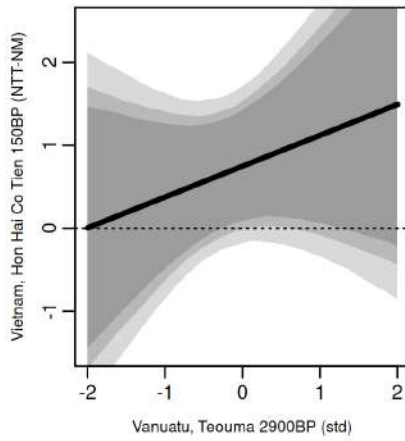
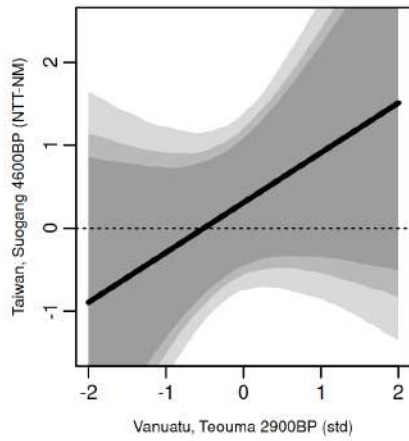
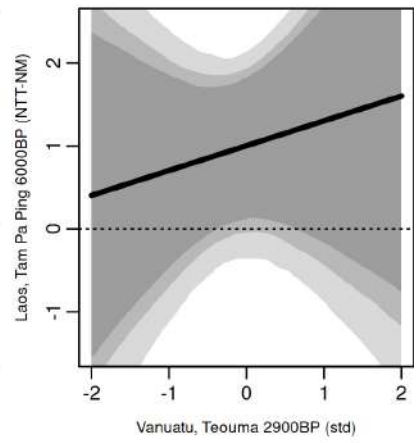
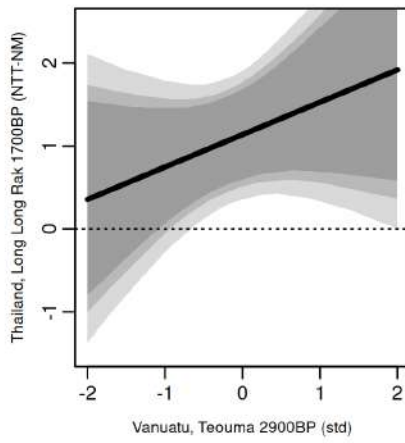
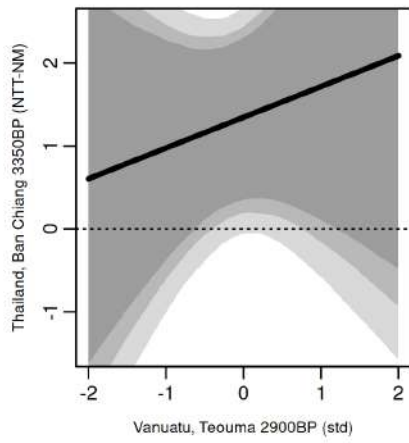


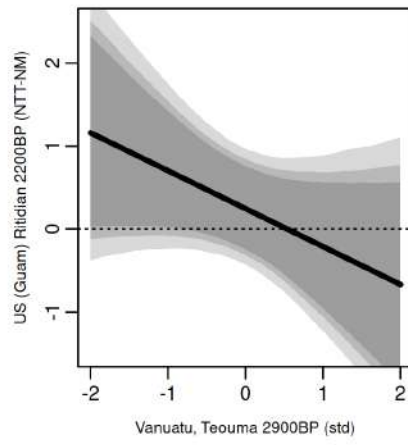
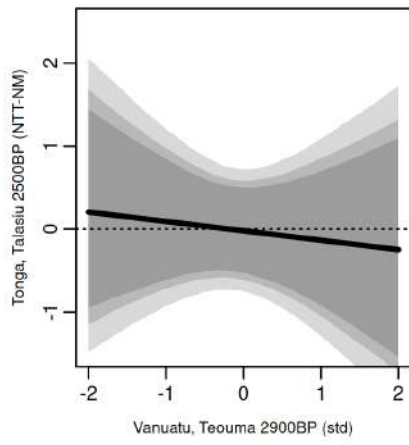




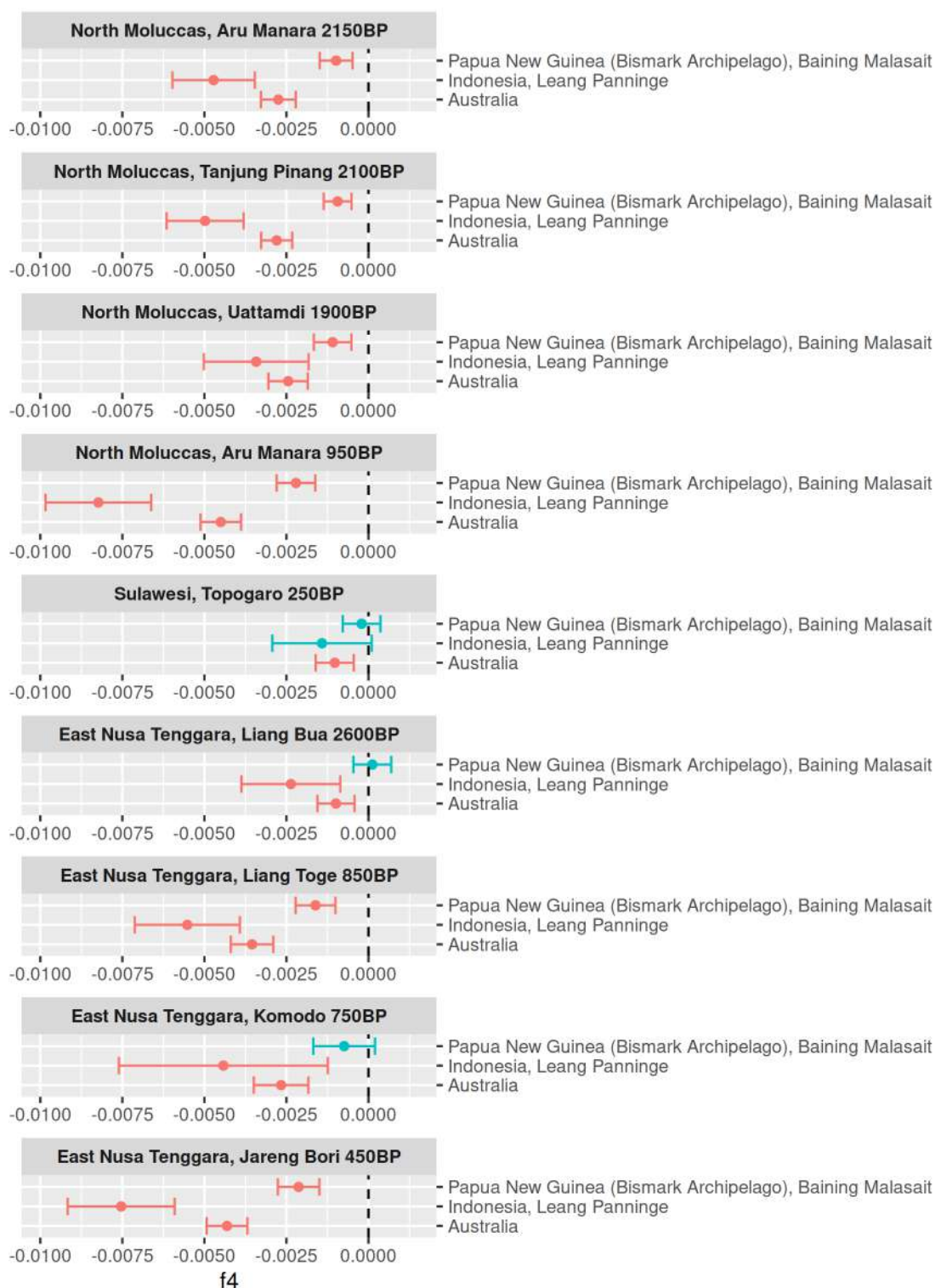
Supplementary Figure 8 - Differences between the posterior distribution of the regression models for the East Nusa Tenggara and North Moluccas (contrasts). Each plot shows the contrasts for the pair of ancient groups shown in the x-y axis labels. The black line represents differences in the posterior means and the shades show the differences in the credible intervals.



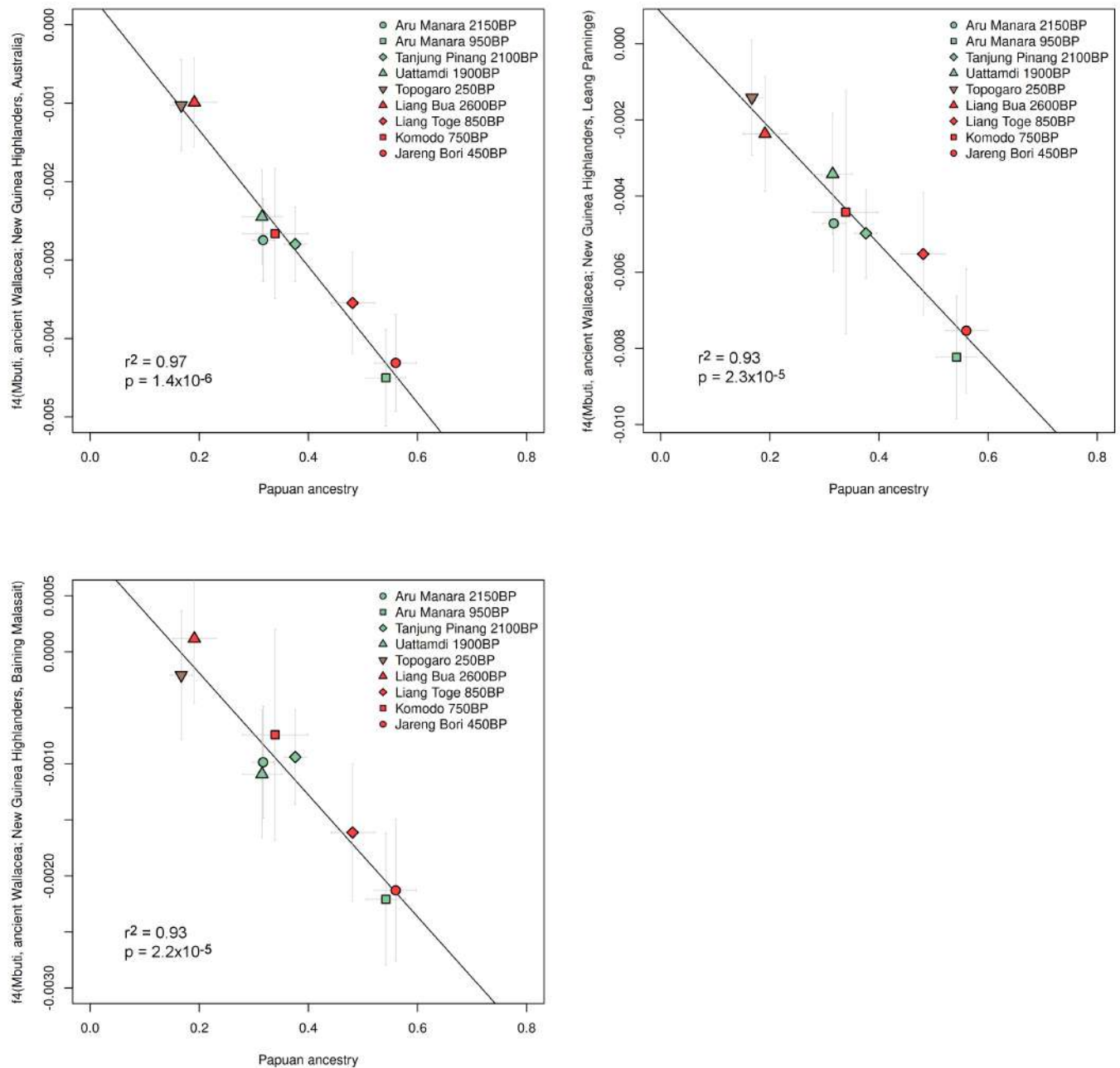




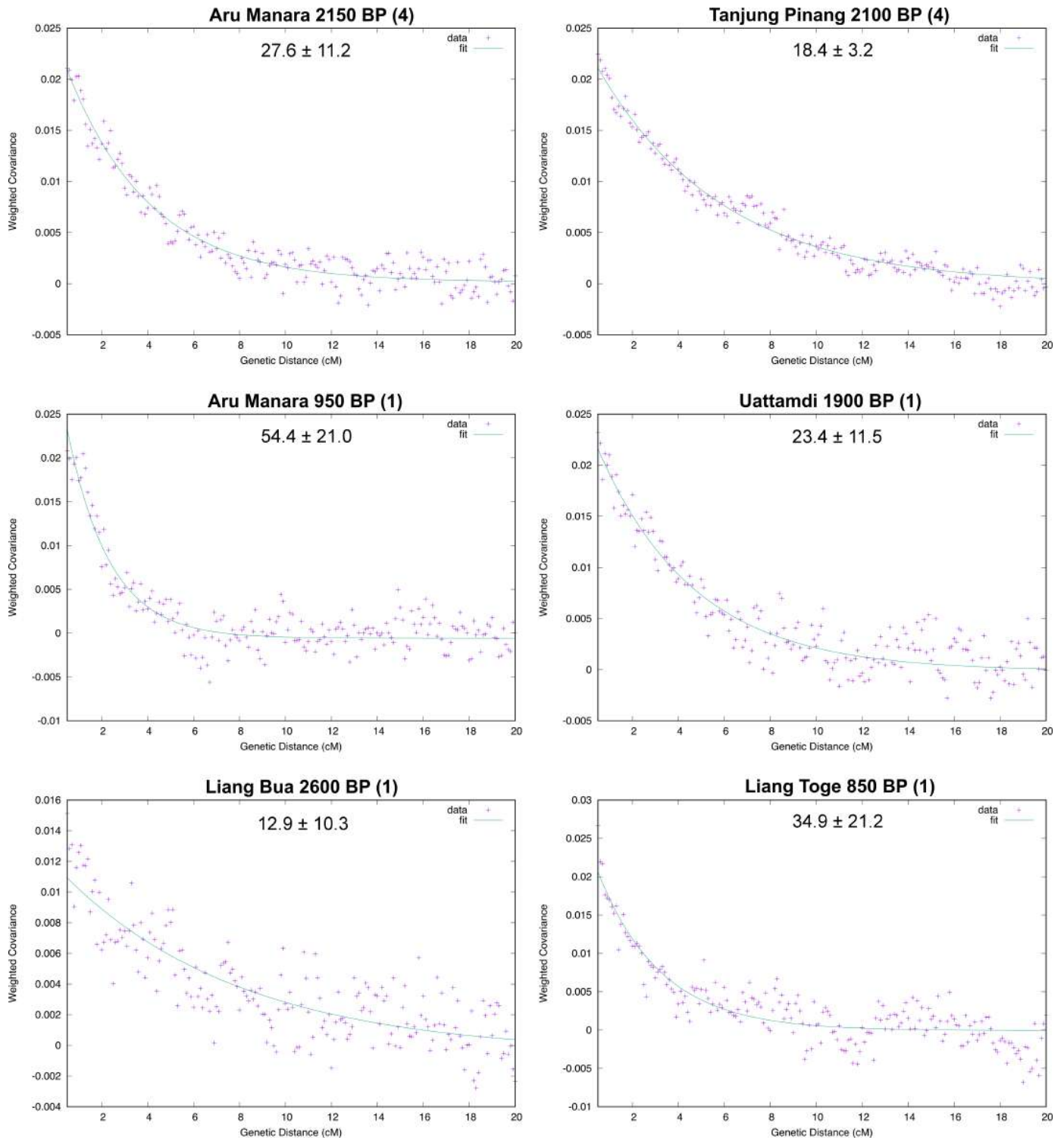
Supplementary Figure 9 - f_4 -statistic of the form $f_4(\text{Mbuti, new ancient Wallacean; New Guinea Highlanders, test})$ computed for each ancient group from Wallacea separately. The test groups (shown to the right of each value) consist of Australo-Papuans with no discernable Asian ancestry and a recently published pre-Neolithic individual from Sulawesi (Leang Panninge). Data are presented as exact f_4 -values ± 2 SE. Values in green are not significantly different from zero ($|Z| < 2$), whereas values in red are significantly different from zero ($|Z| > 2$). Significant negative results indicate that the new ancient Wallacean shares additional drift with the New Guinea Highlands, while positive results indicate that the new ancient Wallacean shares additional drift with the test population.

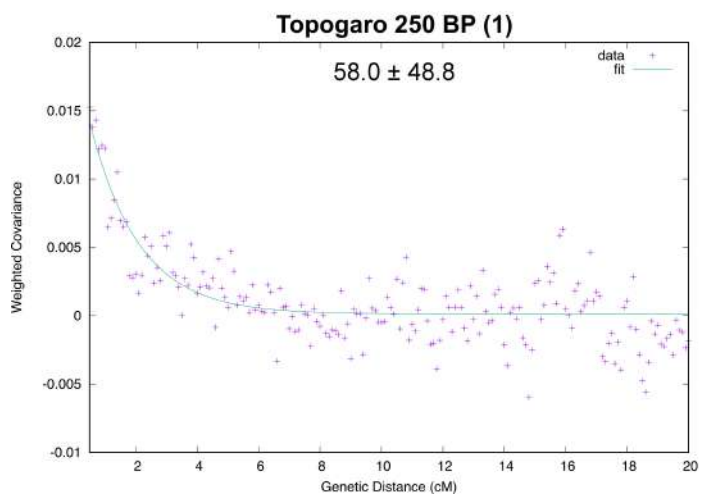
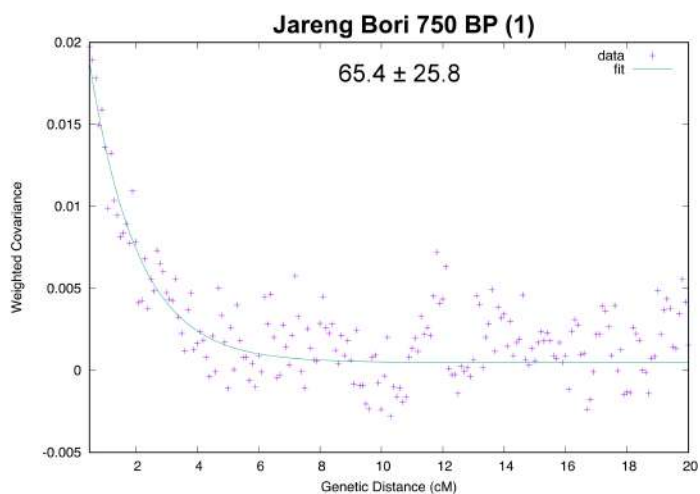
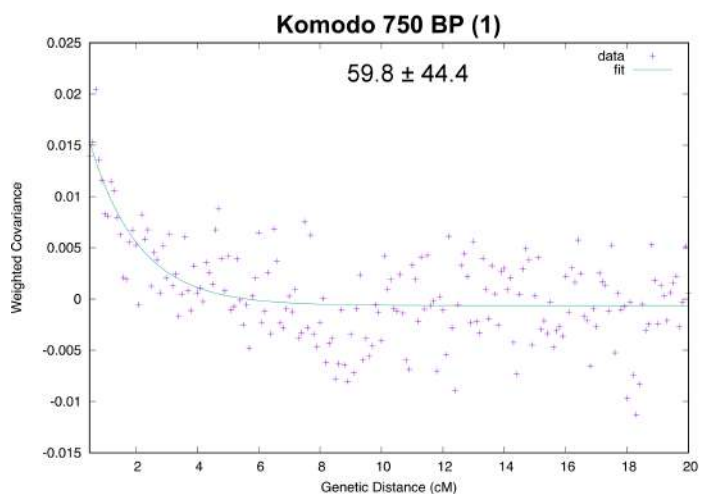


Supplementary Figure 10 – Correlation between the amount of Papuan-related ancestry in ancient Wallacean groups and their shared drift with different Australo-Papuan groups, as well as a pre-Neolithic individual from Sulawesi (Leang Panninge). The x-axis presents the Papuan-related ancestry proportion ± 1 SE calculated with block jackknife in the qpAdm software. The shared drift is presented as exact f_4 -values ± 2 SE (y-axis).



Supplementary Figure 11 – Ancestry covariance in ancient Wallaceans. The number of individuals included in each group is shown in parenthesis, next to the group label. The inferred admixture date (before adding the sample age) ± 2 SE are shown in generations.





Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☐ ☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

EAGER v.1.92.55
 ClipAndMerge
 AdapterRemoval v.2
 BWA v.0.7.12
 DeDup v.0.12.2
 SAMtools v1.3
 pileupCaller v.8.6.5
 EIGENSOFT v.7.2.1 (mergeit, convertf)
 DamageProfiler v.0.3.1
 ANGSD v.0.919
 contamMix
 PLINK v.1.9
 smartpca v.10210
 DyStruct v.1.1.0
 ADMIXTOOLS v.4.1
 admixr R package
 rethinking R package
 DATES v.753
 RStudio v.1.1.38
 OxCal v.4.4

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- Accession codes, unique identifiers, or web links for publicly available datasets
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Alignment files of the nuclear and mitochondrial DNA sequences for the newly sequenced individuals are available at the ENA database under the accession number PRJEB48109.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was not predetermined and was based on the availability of skeletal material.
Data exclusions	No data from the newly generated ancient samples were excluded. Related individuals, identified among the previously published datasets used to contextualize genetic variation, were excluded according to a pre-defined criteria outlined in the methods.
Replication	Principal component, DyStruct, qpAdm, Admixture History Graph (AHG), and admixture dating analyses were carried out based on two different datasets that included present-day populations from Island Southeast Asia genotyped on the Affymetrix 6.0 (dataset 1) and the Affymetrix Axiom Genome-Wide Human Array (dataset 2). The findings were consistent for all analyses.
Randomization	We grouped ancient samples from the same archaeological site based on radiocarbon dates or archaeological context when relevant to improve the power of estimations, and grouped present-day samples according to the population of origin. We confirmed that samples from the same site and age have a similar genetic profile in unsupervised analysis (PCA and DyStruct) before grouping them.
Blinding	Blinding was not relevant for this study. The data analysis was performed for all individuals separately or into subgroups defined by external information (archaeological context and dates).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	The specimens reported in this study come from eight archaeological sites in Indonesia: Aru Manara, Tanjung Pinang, Gua Uattamdi, Topogaro, Liang Bua, Liang Toge, Komodo, and Jareng Bori. The archaeological context and detailed information on the specimens collection are described in the Supplementary information.
Specimen deposition	The remaining portion of the bones sampled for DNA analysis from the Aru Manara, Topogaro, Tanjung Pinang, and Uattamdi sites will be deposited at the Pusat Penelitian Arkeologi Nasional (Jakarta, Indonesia), and those from Jareng Bori, Komodo, Liang Bua, and Liang Toge will be returned to Prof. Koesbardiati at Universitas Airlangga (Surabaya, Indonesia).
Dating methods	For this study we obtained 13 direct radiocarbon dates. Five radiocarbon dating laboratories were used: Curt-Engelhorn-Zentrum Archäometrie gGmbH in Mannheim (Germany), Radiocarbon Dating Laboratory at the University of Waikato (New Zealand), the University of Oxford's Radiocarbon Accelerator Unit (UK), the Australian National University in Canberra (Australia), and the Iso-trace Research Department of Chemistry, in the University of Otago (New Zealand). Pretreatment processes, quality control protocols, and dating methods used by each laboratory are provided in Supplementary Information and Supplementary Table 12. Conventional radiocarbon ages were calibrated using the OxCal 4.4 program and the INTCAL20 calibration curve, with uncertainties reported at 68% and 95% confidence interval.
☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	For the skeletal material from Tanjung Pinang and Uattamdi, the research was undertaken as part of a collaborative project between Pusat Penelitian Arkeologi Nasional (Jakarta) and the Australian National University, under Lembaga Ilmu Pengetahuan Indonesia Research Permits 6939/S.K./1990, 307/I/KS/1994, and 10290/V3/KS/1995. For the skeletal material from Aru Manara, the research was undertaken as part of a collaborative project between Pusat Penelitian Arkeologi Nasional (Jakarta) and the Tokai University, under Kementerian Riset dan Teknologi Research Permits 0291/SIP/FRP/VIII/2011 and Pusat Penelitian Arkeologi Nasional export permit UM.001/2595/PAN/KPK/IX/2012, 2634/H5/TU/2017. For the skeletal material from Topogaro, the research was undertaken as part of a collaborative project between Pusat Penelitian Arkeologi Nasional (Jakarta) and the Tokai University, under Kementerian Riset dan Teknologi Research Permits 40/EXT/SIP/FRP/SM/VII/2015 and 194/SIP/FRP/E5/Dit.KI/VII/2017 and Pusat Penelitian Arkeologi Nasional export permit 2634/H5/TU/2017. For the skeletal material from Liang Bua, Liang Toge and Komodo, the research was undertaken as part of a collaborative project between Universitas Airlangga (Surabaya, Indonesia) and the Max Planck Institute for the Science of Human History (Jena, Germany) under the Kementerian Ristekdikti Research Permit 303/SIP/FRP/E5/Dit.KI/IX/2017 and Pusat Penelitian Arkeologi Nasional export permit 11404H5/TU/2017. Research of the human remains from Jareng Bori were carried out as part of a collaboration between Australian National University and Universitas Gadjadara under the Kementerian Ristekdikti Research Permit 1209/FRP/E5/Dit.KI/VI/2016.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Supplementary Information for:

Genomic portrait and relatedness patterns of the Iron Age Log Coffin culture in northwestern Thailand

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Ethics statement

The human remains were exported and sampled with permission of the Fine Arts Department of Thailand (permit numbers 497/2561 and WTh-0417-236).

Site descriptions

Ban Rai (BRB)

Ban Rai (19° 31'18" N 98° 10'31" E) is a rock shelter on top of the southern edge of the Lang River valley near Ban Huai Rai (Ban Hai) village in the Pang Mapha District of Mae Hong Son Province. Excavations under the Highland Archaeology Project led by Rasmi Shoocongdej yielded a rich, well-dated stratigraphic sequence indicating two occupation layers and prehistoric rock paintings of human figures and animals¹⁻⁹. The pre-Log Coffin culture layers have been dated to 12.5 to 8 k BP and were characterized by a wide range of lithics^{10,11}, an abundance of faunal remains¹², and a primary flexed burial¹.

In its second phase, the rock shelter was used exclusively as a burial site assigned to the Log Coffin culture (2040±30-1770±30 BP)¹³. This layer yielded human remains, pottery sherds, iron tools (chisel, hoop), and 15 log coffins and supporting posts dug into older layers^{1,9}. Dendrochronological and ¹⁴C studies of log coffin heads showed the simultaneous use of different carving styles^{14,15}, while oxygen chronology indicated that precipitation was higher during the Log Coffin period¹⁶.

Lahu Pot (LHP)/Moa Mussur (MMS)

Lahu Pot (19° 35' N, 98° 15' E) is a stalagmite cave located in the Lang River basin near Wa-Na Luang village in the Pang Mapha District. The lower cave yielded six log coffins, human bones, and teeth, while four log coffins and human bones were found in the upper cave. Dates from Lahu Pot Cave range from 1,540 ± 120 to 2,080 ± 60 BP¹⁷.

Pang Kham 1 (PKL)

Pang Kham 1 Cave (19° 40' N, 98° 12' E) is located 1,034 m above sea level in the Huay Pong Saen Pig basin in the Pang Mapha District. It can be divided into three areas of which only two housed log coffins. The second area yielded several log coffins, iron tools, bronze decorations, earthenware, and human bones. The third area housed many coffins,

earthenware, and a limestone-embedded skull. A total of 10 log coffins were recovered and dated to $1,343 \pm 59$ BP and $1,698 \pm 59$ BP¹⁸.

Tham Lod rock shelter (THL)

The Tham Lod rock shelter ($19^{\circ} 34' N$ $98^{\circ} 16' E$) is located at the base of a limestone cliff overhang around 250 m from a Nam Lang River tributary in the vicinity of the Tham Lod Natural and Wildlife Education Center. It was excavated from 2002 to 2006 under the direction of Rasmi Shoocondej (Highland Archeological Project in Pang Mapha) supported by Thailand Research Funds (TRF) and Silpakorn University²⁻⁹.

Tham Lod yielded the longest stratigraphic sequence of the region (10 layers over 4.5 m). More than 100,000 archaeological remains date from the late Pleistocene into the Holocene, including lithic artefacts, faunal and floral remains, pottery sherds, and metal objects from upper layers^{7,12}. Dates produced from various materials indicate use as a temporary settlement for lithic production²⁰, including Hòabìnhian-typical stone artefacts (sumatraliths) in layers 4 to 6²¹, food preparation, and as a cemetery^{8,9,21} from $35,782 \pm 266$ BP (layer 10) over $27,620 \pm 170$ BP (layer 8) to ~3 kaa ago (layer 2), albeit the absence of the Middle Holocene¹⁷. Recent isotopic studies of human and faunal teeth enamel tracked the expansion of forest-grassland ecosystems in the region. They suggested a northern expansion of the savanna corridor during the terminal Pleistocene providing favourable conditions for hunter-gatherers across mountainous areas²².

Two burials and two fragmentary skeletons were discovered in the youngest Pleistocene layers associated with faunal remains, shells, flakes, and used cobble tools^{8,23,24}.

Tham Lod cave

Tham Lod cave ($19^{\circ} 34' N$, $98^{\circ} 17' E$) is situated near the Tham Lod Natural and Wildlife Education Center, about 300 m from the Tham Lod rockshelter. The cave is a multi-chamber cave complex with the Nam Lang river passing through the cave. The burial site is located in the upper chambers, where three chambers contain fragments of ten log coffins, earthenware sherds, iron rings, and a decorated tooth, but the site has been badly disturbed by human and animals. Pieces of log coffin from the site date to $1,450 \pm 110$ - $1,240 \pm 90$ BP²⁶.

Ban Wang Hai (WAH)

Ban Wang Hai (18° 32' N, 99° 00' E) is situated in Kuang river valley, Mueang Lamphun district, Lamphun province. The site was first excavated by the 4th Regional Office of Fine Arts Department in 1987. Seven skeletal remains were discovered along with grave goods including bronze and glass bracelets, iron tools, stone tools, spindle whorls and earthenware pottery as well as stone and glass beads (the 4th Regional Office of Fine Arts Department 1987). The excavation was continued as part of the Thai-French Prehistoric Project between 1996-1998 and dated to 429-657 CE²⁵.

Yappa Nhae 1 (YPN)

Yappa Nhae 1 Cave (19° 36' N, 98° 14' E) is located 900 m above sea level in the Mae La Na Basin near Ya-Pa Nae village in the Pang Mapha District. Indirectly dated between 2,200 and 1,200 BP, four log coffins were found in Chamber 3 and 4 in addition to human remains of at least six individuals, a decorated tooth, iron tools, bronze coins, and earthenware.

Yappa Nhae 2 (YPN)

Yappa Nhae 2 Cave (19° 36' N, 98° 14' E) is located 964 m above sea level in the Mae La Na basin near Ya-Pa Nae village in the Pang Mapha District. Four log coffins were discovered in the middle, upper and lower chamber indirectly dated to 2,020 to 1,200 BP from the log coffin heads²⁶. These were associated with human remains of at least 23 individuals, earthenware, chisels, animal bones, and iron tools.

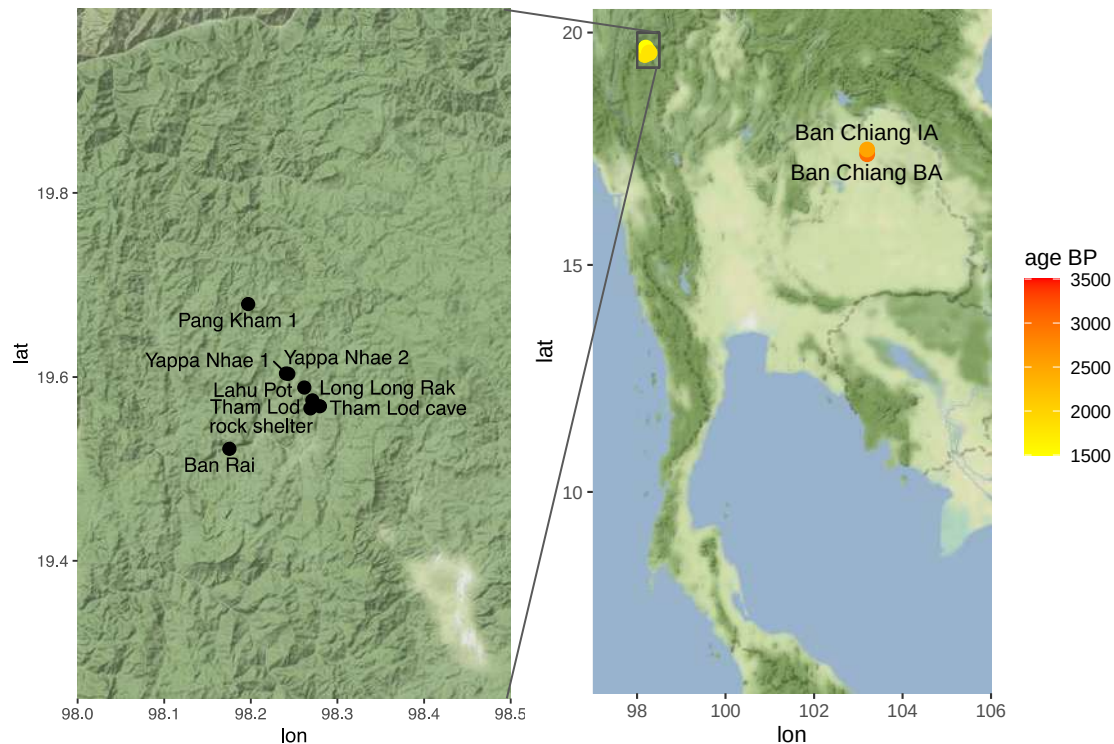


Figure S1. Maps of archaeological sites used in this study; (A) the location of the archaeological sites from which data was produced for this study and Long Long Rak²⁷; (B) the location of archaeological sites in Thailand for which ancient DNA data has been published²⁸; map created with ggmap v3.0.0²⁹, map tiles by Stamen Design (CC BY 3.0), data by OpenStreetMap (ODbL).

Radiocarbon dating

A selection of petrous bones from sampled individuals was directly dated at the Klaus-Tschira-Archäometrie-Zentrum, Mannheim, Germany. All samples are dated to a similar period between 1,698 and 1,811 uncal. years BP (Table S1).

Ancient DNA processing

Sampling, extraction, library preparation, and indexing were performed in a dedicated clean room for ancient DNA at the Max Planck Institute for the Science of Human History in Jena, Germany (now Max Planck Institute of Geoanthropology).

Sampling and extraction

We assessed the preservation of 64 pieces of skeletal material from seven archaeological sites and selected 42 petrous bones and 22 teeth for ancient DNA analysis (Table S2).

The petrous bones were sampled by cutting them in half along the margo superior partis petrosae (crista pyramidis) and drilling bone powder from the densest part around the cochlea³⁰. The hardest part of the petrous bone was missing for two samples, and another two elements were too soft to sample successfully. Therefore, these samples were not further processed.

The teeth were sampled by cutting at the cemento-enamel junction and drilling bone powder from the pulp of the tooth (<https://dx.doi.org/10.17504/protocols.io.bqebmtan>).

From the resulting bone powder, DNA was extracted following a modified version of the Dabney, et al. (2013)³¹ protocol (<https://dx.doi.org/10.17504/protocols.io.baksicwe>). The sample was digested in a mixture of EDTA, UV-irradiated High Performance Liquid Chromatography-grade H₂O (UV-H₂O), and Proteinase K for approximately 24 hrs while rotating in an incubator at 37°C. After centrifugation to remove the remaining bone material from solution, the supernatant was transferred into a binding buffer (GuHCl, UV-H₂O, Isopropanol) and then into a silica column (High Pure Viral Nucleic Acid Kit; Roche). The column was washed twice using the High Pure Viral Nucleic Acid Kit wash buffer (Roche) and eluted in a 100 µl TE-buffer containing 0.05% Tween. An extraction blank to check for cross- and background contamination and a positive control were added to this step and processed in parallel with the sample.

Library preparation

From the resulting extracts, 25 µl were used to build double-stranded libraries after partial Uracil DNA Glycosylase treatment³². Illumina adapters were ligated to the fragments using the Quick Ligation Kit (NBE) and the solution purified with a MinElute kit (QIAGEN). The DNA copy number of a 2 µl aliquot of the library was quantified by DyNAmo SYBR Green qPCR (Thermo Fisher Scientific) using IS7/IS8 primers on the LightCycler 480 (Roche)³³. Based on those values, the library was double-indexed with unique index combinations³⁴ and amplified using Pfu Turbo DNA Polymerase (Agilent; <https://dx.doi.org/10.17504/protocols.io.bvt8n6rw>). Then it was purified over MinElute columns, eluted in 50 µl TE-buffer containing 0.05% Tween, and an aliquot quantified with IS5/IS6 primers using DyNAmo SYBR Green qPCR (Thermo Fisher Scientific) on the LightCycler 480 (Roche)³³. The remaining solution was further amplified with Herculanse II Fusion DNA Polymerase (Agilent; <https://dx.doi.org/10.17504/protocols.io.beqkjduw>), purified again, quantified on the TapeStation (TapeStation Nucleic Acid System, Agilent

4200), and diluted to 10 nM for shotgun sequencing. Throughout library preparation, a new blank, as well as the extraction blank, were taken along.

Shotgun screening

The prepared libraries were shotgun-sequenced for a depth of 2-10 million reads on an Illumina HiSeq 4000 using a 75 bp single-read configuration (Table S2). The resulting reads were demultiplexed according to expected index combinations.

Further processing was done in nf-core/eager v2.4.0 (<https://nf-co.re/eager>)³⁵ using Nextflow v21.04.0³⁶. General sequencing quality control was conducted with FastQC v0.11.9³⁷ and fastP v0.20.1³⁸. Forward and reverse adapters were removed using AdapterRemoval v2.3.2³⁹ with one overlapping position trimming reads based on 30 bp read length and 20 base quality and skipping base quality trimming on the 5' end. Using BWA v0.7.17-r1188^{40,41}, the reads were aligned to the human reference genome *hg19* allowing for 1 mismatch per read, maximum edit distance of 2, disabling the seeding and two gaps. The resulting bam files were then filtered with SAMtools v1.12⁴² for a minimum mapping quality and minimum read length of 30, and unmapped reads were discarded. Mapping statistics were generated with Qualimap v2.2.2-dev⁴³ and bedtools v2.30.0⁴⁴. Duplicates were removed using Picard MarkDuplicates v2.26.0 (<http://broadinstitute.github.io/picard/>) and C->T misincorporation frequency assessed with DamageProfiler v0.4.9⁴⁵ of all reads up to 100 bp, considering 15 bp of each read and setting the maximum misincorporation frequency to be displayed. Endogenous DNA was estimated using endorS.py v0.4 (<https://github.com/aidaanva/endorS.py>), and the final report generated with MultiQC v1.11⁴⁶.

```
nextflow run nf-core/eager
```

```
-profile eva,archgen,big_data
-r 2.4.0
--input Thailand.tsv
--min_adap_overlap 1
--clip_readlength 30
--clip_min_read_quality 20
--preserve5p
--mapper bwaaln
--bwaalnnn 0.01
--bwaalno 2
```

```
--run_bam_filtering true
--bam_mapping_quality_threshold 30
--bam_filter_minreadlength 30
--bam_unmapped_type discard
--dedupper markduplicates
--damageprofiler_length 100
--damageprofiler_threshold 15
--damageprofiler_yaxis 0.3
```

Ancient DNA authentication

After shotgun sequencing, the level of endogenous human DNA content ranged from <0.1% to 57.92%, with a median of 0.285% (Table S2). Overall, samples from Yappa Nhae were better preserved compared to the other sites, and petrous bones yielded much more endogenous DNA compared to teeth.

We assessed if the sequenced reads were ancient rather than present-day human DNA by the rate of cytosine to thymine mis-incorporations at the 5' end of reads ('ancient DNA damage', Figure S2A, Table S2). Most samples showed an elevated damage rate (7-26%) at the first base, which drops off further into the read due to UDG-half treatment.

Since ancient DNA reads are known to be fragmented, we also evaluated the median read length, which ranged between 30 and 55 bp (Figure S2B, Table S2).

Based on previous comparisons of ancient and present-day DNA⁴⁷, 33 sequences showed the damage pattern and read length characteristic of ancient DNA and an endogenous DNA content suitable for in-solution capture (>0.1%).

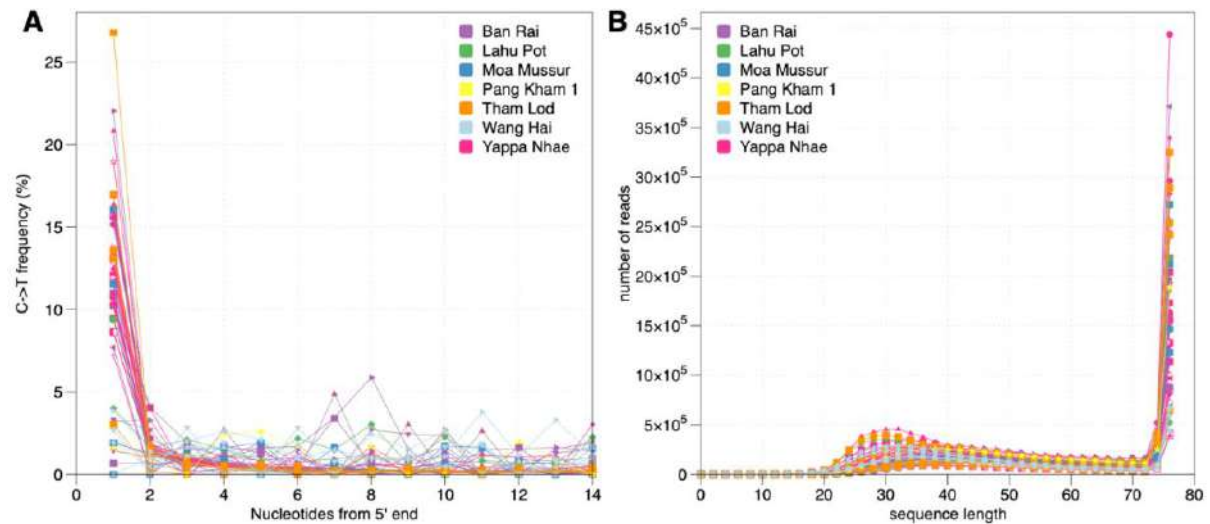


Figure S2. Quality control plots of ancient genomes from seven archaeological sites in northwestern Thailand; A, frequency of C-T transitions at the 5' end of the DNA fragments; B, read length distribution; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

In-solution enrichment

The selected libraries were further amplified with the IS5/IS6 primers and hybridized in-solution to oligonucleotide probe sets (Agilent) to enrich for a targeted set of 1,237,207 single nucleotide polymorphisms (SNPs) across the human genome (1240K capture)^{48,49}. Due to low preservation, BRB002, LHP002, THL003, and YPN036 went through two rounds of 1240K-capture before sequencing.

After sequencing the 1240K capture products to 13-73 million reads on the Illumina HiSeq 4000 using a 75 bp single-read configuration, the reads were demultiplexed again.

The 1240K-enriched sequences were processed with nf-core/eager v2.4.0 as described above with the addition of providing the 1240K positions in a bedfile, mitochondrial to nuclear ratio calculation using MTNucRatioCalculator v0.7, genetic sex determination with sexdetermine v1.1.2⁵⁰ and nuclear contamination estimation with ANGSD v0.935⁵¹. After trimming off 2 bp at both ends of the reads with bamUtil v1.0.15⁵², the resulting bam files were genotyped with pileupCaller from sequenceTools v1.4.0.5 (<https://github.com/stschiff/sequenceTools>) on the 1240K SNP panel calling random haploids and utilizing all sites.

nextflow run nf-core/eager

-profile eva,archgen,big_data

```
-r 2.4.0
--input Thailand.tsv
--min_adap_overlap 1
--clip_readlength 0
--clip_min_read_quality 20
--preserve5p
--mapper bwaaln
--bwaalnnn 0.01
--bwaalno 2
--run_bam_filtering true
--bam_mapping_quality_threshold 30
--bam_filter_minreadlength 30
--bam_unmapped_type discard
--dedupper markduplicates
--damageprofiler_length 100
--damageprofiler_threshold 15
--damageprofiler_yaxis 0.3
--snpcature_bed
--run_mtnucratio true
--mtnucratio_header MT
--run_sextederrmine true
--run_nuclear_contamination true
--contamination_chrom_name chrX
--run_trim_bam true
--bamutils_clip_half_udg_left 2
--bamutils_clip_half_udg_right 2
--run_genotyping true
--genotyping_tool pileupcaller
--genotyping_source trimmed
--pileupcaller_method randomHaploid
--pileupcaller_transition AllSites
```

Karyotype determination

We calculated the relative coverage of X- and Y-chromosomes in comparison to that of the autosome (X- and Y-rate) using sexdeterrmine v1.1.2⁵⁰ as implemented in nf-core/eager v2.4.0³⁵. Seventeen individuals with high X-rates most likely possessed the karyotype XX and YPN likely XXX, while 15 individuals had elevated Y-rates indicating XY (Table S2).

Contamination estimation

We estimated nuclear contamination in individuals with karyotype XY based on heterozygosity levels of X-chromosome SNPs using the xContamination tool of ANGSD v0.935⁵¹ as implemented in nf-core/eager v2.4.0³⁵. No contamination could be detected for individuals with elevated Y-rates with the exception of YPN036, which was excluded from population genetic analyses (Table S2).

We further estimated the mtDNA contamination with ContamMix v1.0-10⁵³, and found that all individuals had estimated contamination levels <3%, except for YPN036 (6%, Table S2).

Genetic relatedness

We determined genetic relatedness between individuals using the software KIN v3.1.3⁵⁴. The results indicated several first- and second-degree relationships between individuals from Yappa Nhae (Table S3). YPN032 and YPN033 likely originated from the same individual or identical twins and shared a second-degree relationship with YPN002. YPN014 and YPN021 are first-degree related, likely parent-child, while YPN014 and YPN027 (avuncular related or half-siblings) and YPN010 and YPN011 (grandparent-grandchild) are second-degree related. To avoid biasing population genetic analyses, we selected the lower coverage genotypes to be excluded from the data set (YPN002, YPN014, YPN033). Within the previously published individuals from Long Long Rak²⁷, we identified Th519 and Th703 as to be the same individual/identical twins and Th530 and Th531 as first-degree related.

Runs of homozygosity

To evaluate parental relatedness, we detected runs of homozygosity (ROH) in all individuals using hapROH v0.6⁵⁵ in Jupyter notebooks v6.4.10. We ran the habsb_ind command with the provided down-sampled 1000 Genomes data as a reference panel and evaluated ROH on 22 chromosomes (chs=range(1,23)) with parameters matching our data (e_model="haploid", p_model="Eigenstrat", n_ref=2504, random_allele=True, readcounts=False, delete=False, logfile=True, combine=True).

When compared with simulated data part of the python package, we could show that four individuals from Yappa Nhae and one from Long Long Rak had close genetic relations between their parents, resulting in many long ROH (>20 cM, Fig. S3A). Particularly YPN001 was likely offspring of parent's siblings' children or even more closely related parents (Figure S3B+C).

We also used hapROH to estimate effective population size (N_e), i.e., the size of an idealized population that would give rise to the same variance of allele frequencies, with a maximum likelihood approach (MLE_ROH_ N_e) to 977 (845-1,130) after removing the four individuals with ROH >20 cM. For the individual sites, we calculated the following N_e values: 50,000 (2,610-50,000) for Ban Rai, 1,047 (483-2,936) for Lahu Pot, 1,316 (784-2,453) for Tham Lod, and 1,047 (881-1,254) for Yappa Nhae and 518 (382-716) for Long Long Rak.

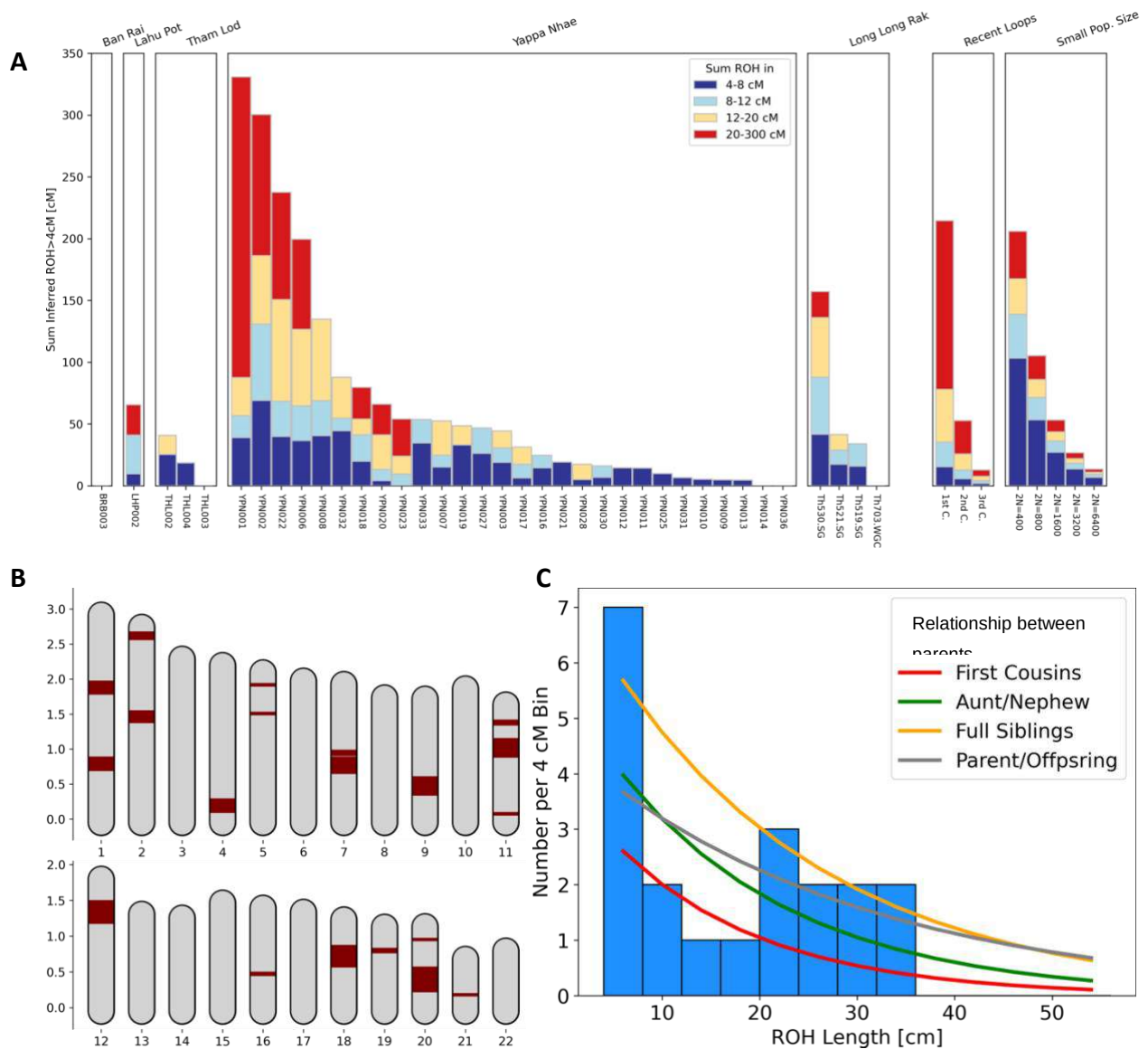


Figure S3. Runs of homozygosity (ROH) of ancient individuals from northwestern Thailand: (A) lengths of ROH for each ancient individual, previously simulated data expected for different parental relationships and population sizes displayed on the right⁵⁵, (B) location of ROH within the karyotype of YPN001 and (C) ROH length distribution of YPN001 (blue bars) with lines depicting the “expected” ROH trajectory of relationships between parents superimposed.

Uniparental analyses

Mitochondrial haplogroups

We built consensus sequences using the export function in Geneious v2019.2.3⁵⁶ setting "If no coverage call" and "Call {} if coverage < {}" to N/X with a coverage threshold of 5, "highest quality" and >50% Sanger heterozygotes. Mitochondrial haplogroups were determined with HaploGrep v2.4.0⁵⁷. The main haplogroups we identified were F1f, F1a1a, M7b1a1, and N8* (Table S2) and correspond with previously published mtDNA data from the region⁵⁸.

Y-chromosome haplogroups

For Y-chromosome assessment, we mapped the 1240K-enriched data to the human reference genome as described for the shotgun screening above. We then called all SNP positions on the Y-chromosome and compared the amount of ancestral and derived calls from the ISOGG database (<https://isogg.org/tree/>) to determine the Y-haplogroups of XY individuals⁵⁹. We mainly identified O1b and O2a in addition to N1b and C2b (Table S2).

Population genetic analyses

Principal component analysis

We merged the newly generated genotype calls with published data from present-day and ancient individuals from northern to southern East Asia^{27,28,60–69} and used smartpca v16000 (lsqmode: YES, shrinkmode: YES) from EIGENSOFT v7.2.1⁷⁰ to calculate several sets of principal components.

The initial PCA was calculated on present-day East and Southeast Asian individuals^{28,60,61,63,67,69} and with all ancient individuals projected onto the scaffold from the present-day individuals (Fig. S4A). Along PC1 we can observe a north-south cline, while PC2

stretches between the Onge from the Andaman Islands and the Taiwanese Atayal. The newly genotyped ancient Thai individuals cluster together with present-day Thai individuals (Karen Padaung, Lawa Eastern, Lawa Western, Mon) as well as the Log Coffin-associated individuals from Long Long Rak²⁷. This position is markedly different from Bronze and Iron Age individuals from Ban Chiang, northeastern Thailand²⁸ and other contemporaneous individuals from Guangxi, China⁶⁸.

The second PCA was calculated on the present-day Thai individuals only⁶⁷ which separated according to the genetic drift of specific groups (Fig. S4B). The ancient individuals clustered with present-day groups affiliated with all language families except Hmong-Mien (Blang, Karen Padaung, Laotian, Lawa Eastern, Lawa Western). However, the Ban Chiang individuals were only associated with Austroasiatic-speaking groups, such as the Bru, Khmu, and Soa, and the younger individuals from Guangxi clustered with Hmong-Mien speakers.

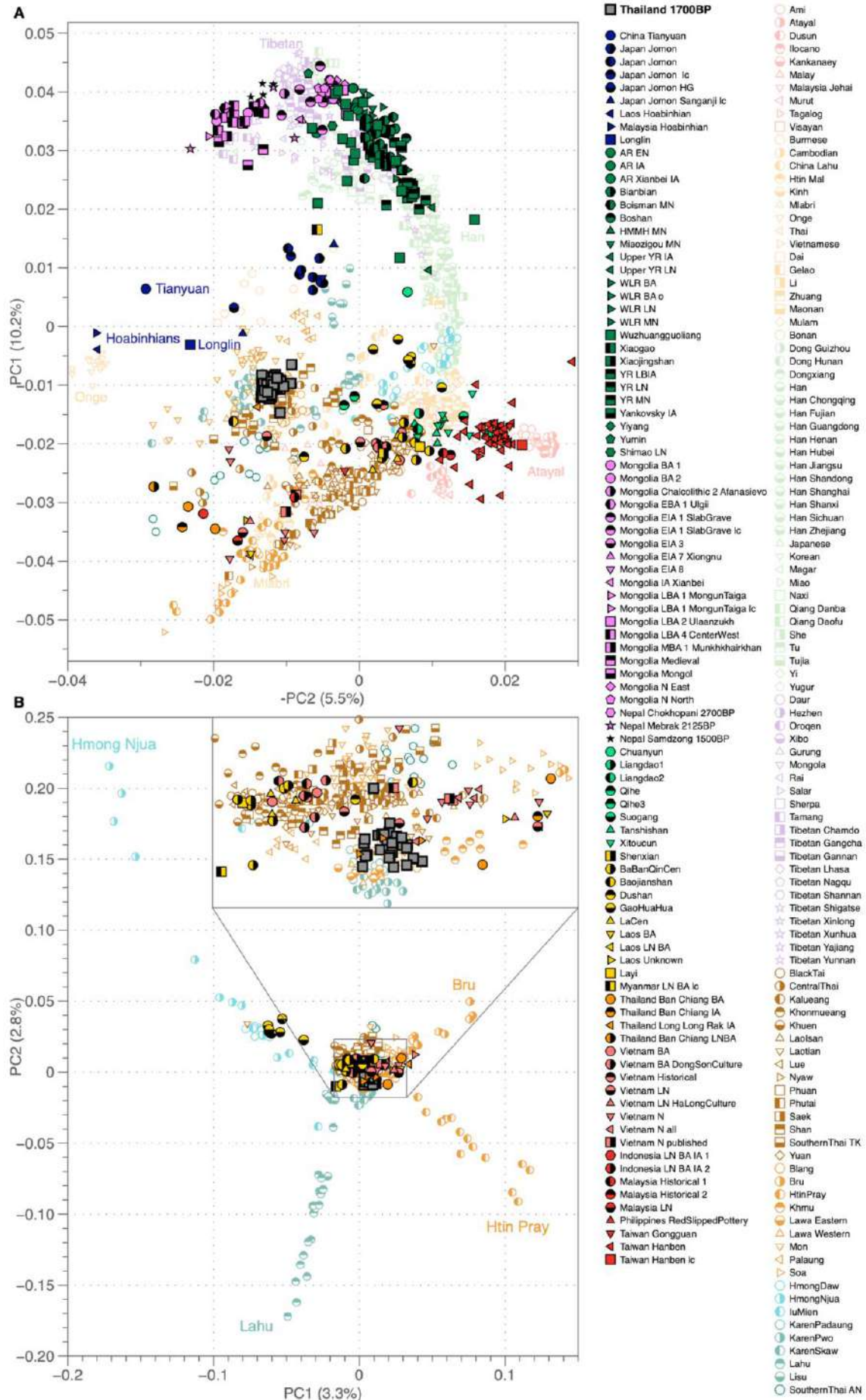


Figure S4. Principal component analyses calculated on (A) present-day East Asian individuals^{28,60,61,63,67,69} and (B) present-day individuals from Thailand⁶⁷; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

ADMIXTURE

We estimated proportions of ancestry using ADMIXTURE v1.3.0⁷¹ on a dataset containing present-day and ancient individuals from East and Southeast Asia^{27,28,60,61,64,66–68,72}. The analyses were conducted unsupervised on 4 parallel threads (-j 4), a random seed was selected (-s \${RANDOM}) and cross-validation errors calculated (--cv). We estimated K=2 through K=15 and selected the run with the highest likelihood out of five replicates per K. The lowest cross-validation errors were at K=8 (Fig. S5).

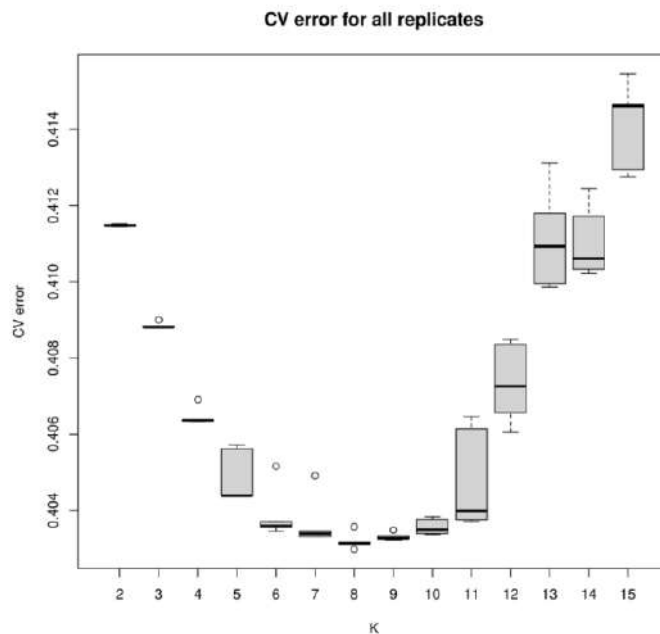


Figure S5. Cross-validation errors of ADMIXTURE analysis of present-day and ancient individuals from East and Southeast Asia^{27,28,60,61,64,66–68,72}, plotted using CLErrorBoxplotPlotter.R (<https://github.com/TCLamnidis/AdmixturePlotter>).

For K=8 the corresponding ancestry components are maximized in the following groups: Mbuti (pink), French (light blue), Onge (dark green), Mlabri (dark purple), Atayal (red), Lahu (light green), Htin Pray (yellow), Boisman_MN (orange; Fig. S6). The newly generated genomes from northwestern Thailand are split into ~65% Boisman and ~35% Htin Pray ancestry component with most individuals additionally containing a few per cent Mlabri, and some individuals very little French or Atayal ancestry. This is similar to the individuals from

Long Long Rak (Thailand_IA.SG/WGC)²⁷, but differs from Ban Chiang (Thailand_BA)²⁸, which lack the Boisman and contain more Lahu component.

f_3 -statistics

To formally investigate the observed patterns in the PCA and ADMIXTURE analyses, we used qp3Pop v651 and qpDstat v980 from AdmixTools v7.0.2⁶⁰.

The statistic $f_3(\text{Mbuti}; \text{present-day groups, Thailand 1700BP})$ indicated that the ancient Thai individuals shared most drift with the Austroasiatic-speaking Mlabri and Htin from Thailand²⁸ followed by the Kra-Dai-speaking Maonan and Gelao from southern China⁶⁹ (Fig. S7). The tested groups also included present-day groups from Thailand⁶⁷, Malaysia²⁷ and other South and East Asian groups^{60,61}.

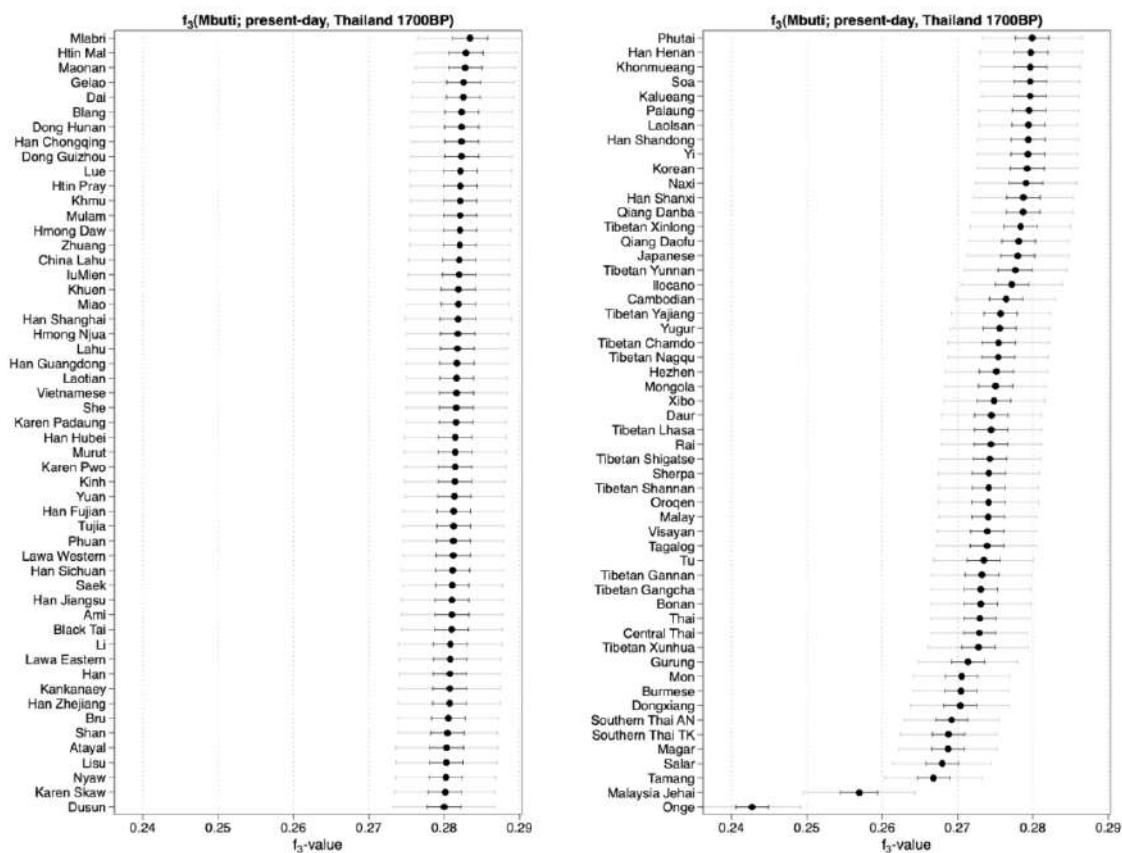


Figure S7. Observed f_3 -statistics showing shared genetic drift between ancient genomes from northwestern Thailand and present-day groups from the region^{27,28,60,61,67,69}; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

We then tested the different affinities of the Ban Chiang Bronze Age individuals²⁸ and the ancient Thai individuals to all published ancient groups from East Asia^{27,28,62,64–66,68,69} with $f_4(\text{Mbuti.DG, ancient East Asia; Thailand\_BA, Thailand\_1700BP})$. While there were no

ancient groups that showed closer affinity to the Bronze Age individuals, ancient individuals from northern East Asia (e.g., Miaoziyou_MN, Upper_YR_IA, WLR_LN, Mongolia_EIA_7_Xiogou, Upper_YR_LN, YR_LBIA, Mongolia_EIA_3, Mongolia_LBA_2_Ulaanzukh) and Nepal (Mebrak_2125BP.SG, Samdzong_1500BP.SG) resulted in significantly positive values indicating extra genetic affinity present in the ancient northwestern Thai individuals (Fig. S8A).

We also observed this pattern when comparing with the Iron Age individual from Ban Chiang²⁸, albeit with a lower number of overlapping SNPs.

Tests with Long Long Rak in the third position showed significant attraction of the ancient Thai individuals to Neolithic individuals from Vietnam, Tanshishan, Mongolia_EIA_8, Bianbian, and Boisman_MN, while Vietnam_BA_DongSonCulture.SG was significantly closer to Long Long Rak (Fig. S8B).

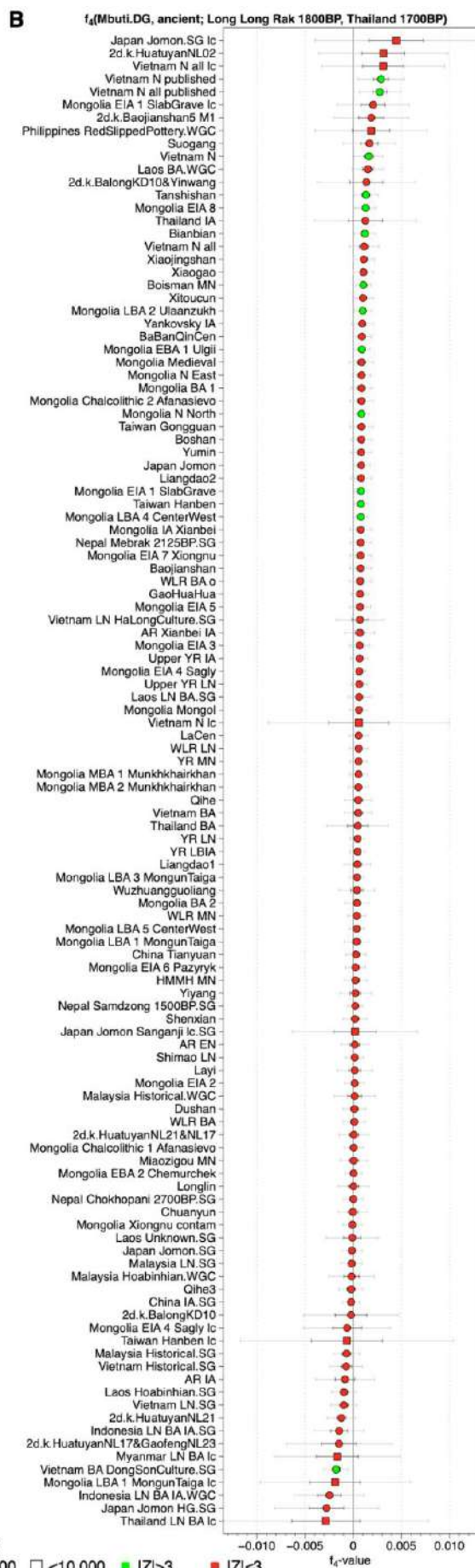
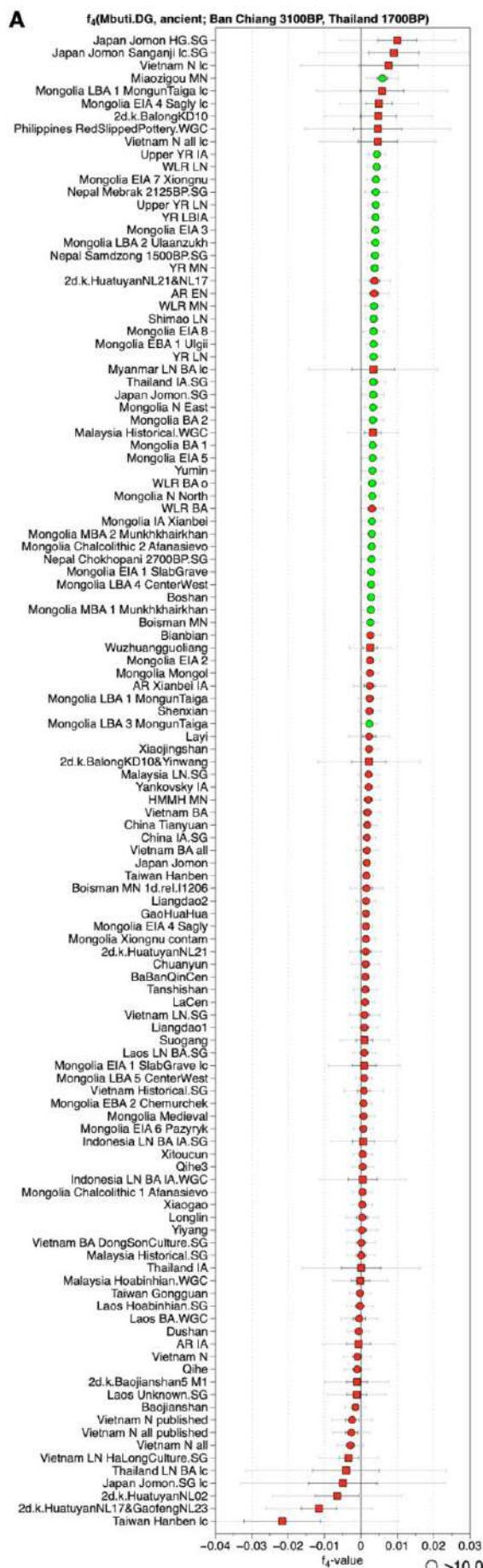


Figure S8. Observed f_4 -statistics comparing the genetic affinities of the newly genotyped individuals from northwestern Thailand with (A) Bronze Age northeastern Thailand²⁸ and (B) Iron Age northwestern Thailand²⁷; red and green indicate z-scores such that $|Z| \leq 3$ and $|Z| > 3$ respectively, circles and squares denote f_4 -statistics where there were more and less than 10,000 overlapping SNPs respectively; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

The results of f_4 (Mbuti, present-day Thai; Bronze Age Ban Chiang, Thailand 1700BP) showed a closer affinity of all present-day groups from Thailand⁶⁷ to the newly genotyped ancient individuals compared to the Bronze Age individuals from northeastern Thailand²⁸. The values were particularly high for the Palaung, Karen Skaw, Iu Mien, and Shan, whereas tests including the Khmu, Kalueang, Soa, Southern Thai Austronesian speakers, Htin Pray and Bru were not significant (Fig. S9).

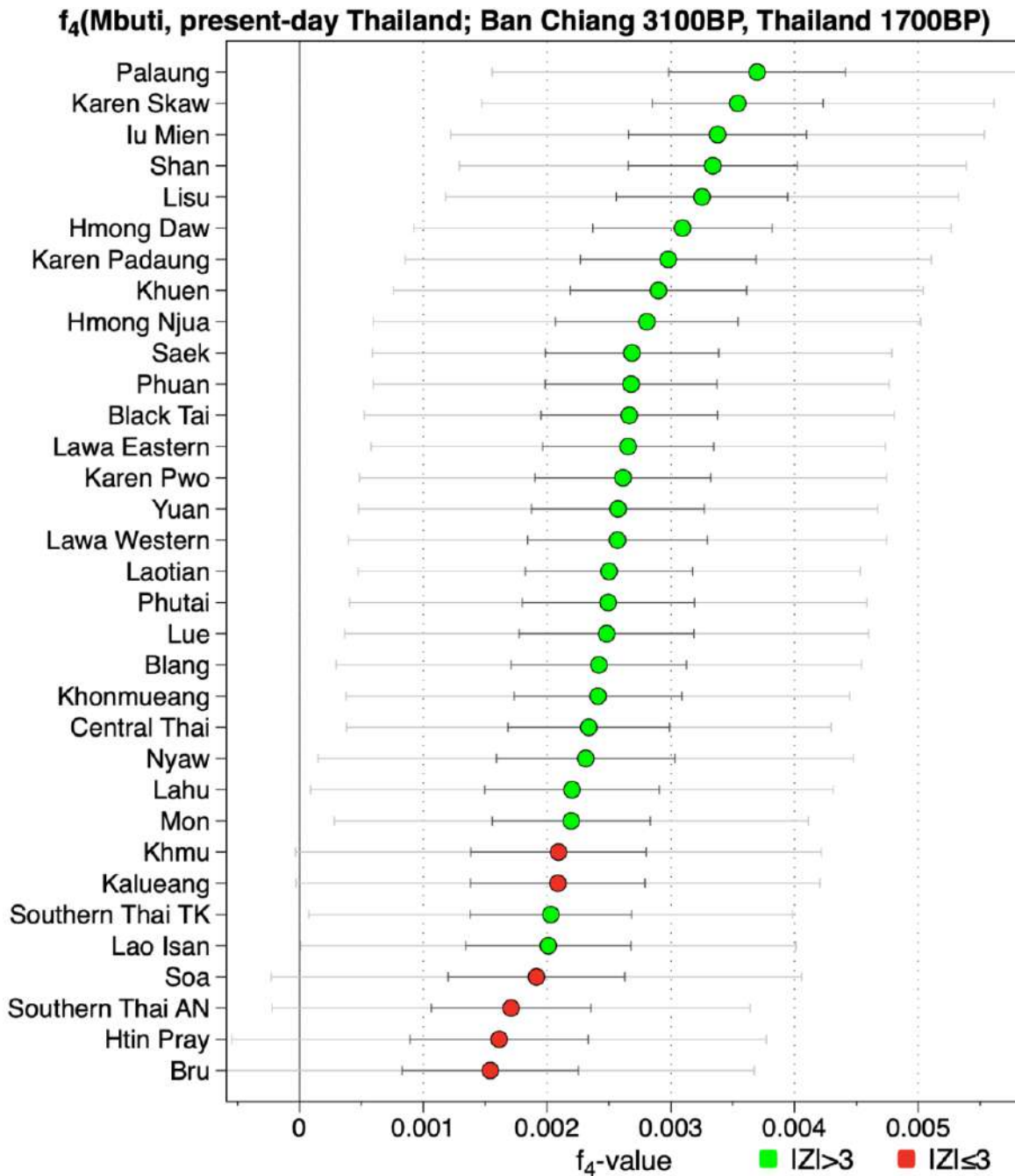


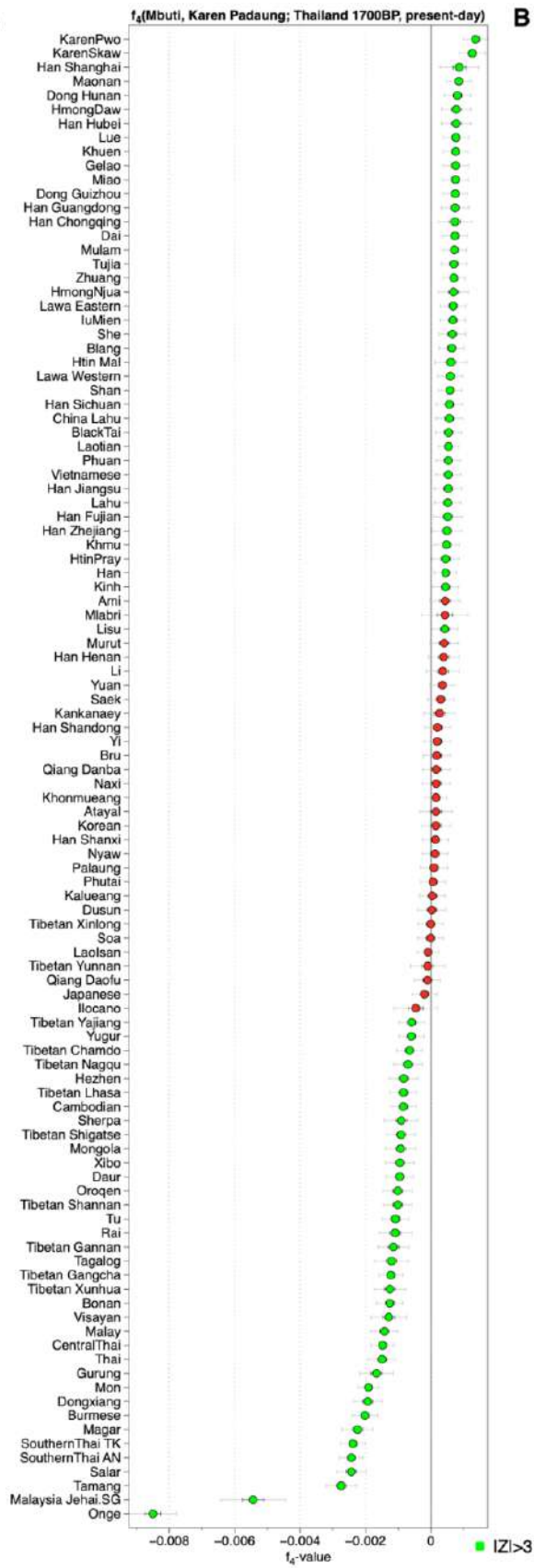
Figure S9. Observed f_4 -statistics comparing the affinity of the newly generated genomes from ancient northwestern Thailand and Bronze Age northeastern Thailand²⁸ to present-day groups from Thailand⁶⁷; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

We also tested if any present-day groups from Thailand showed closer affinity to the Log Coffin-associated genomes compared to other present-day groups ($f_4(\text{Mbuti, present-day$

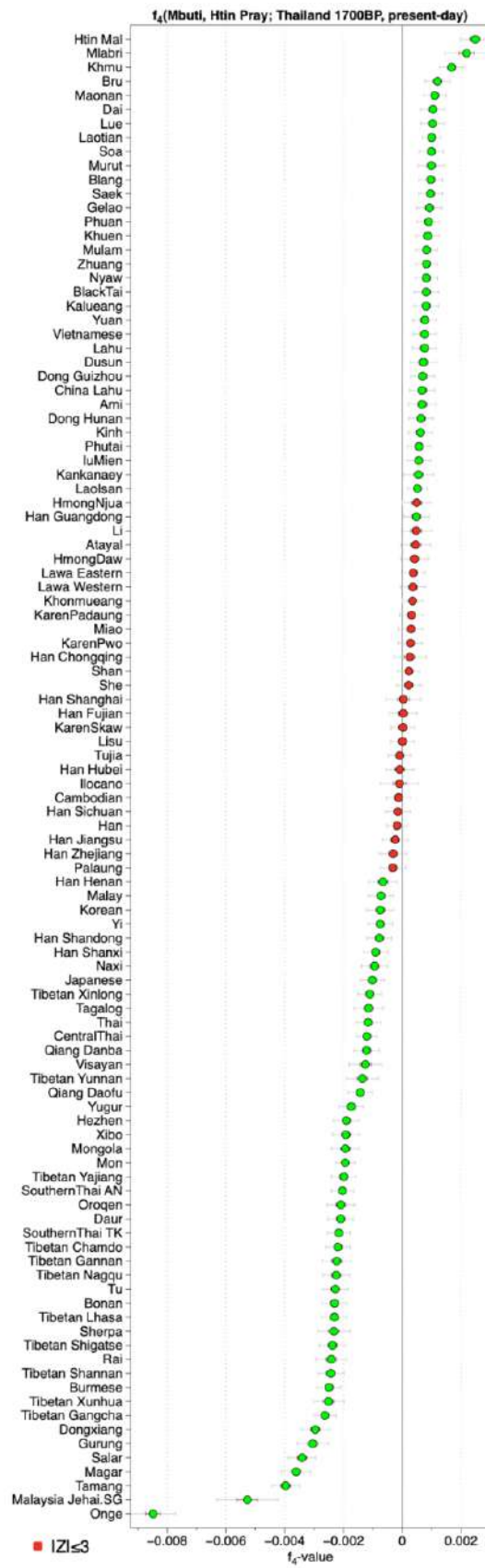
Thailand; Thailand1700BP, present-day groups). Here, we were particularly interested in the hill tribes living in the highlands (Karen, Lahu, Lisu, Akha, Lawa, Htin, Khmu, Hmong, and Iu Mien), who are culturally and genetically isolated from the lowlanders⁶⁷, and chose the Sino-Tibetan-speaking Karen Padaung, the Austroasiatic-speaking Htin Pray and the Hmong Mien-speaking Hmong Njua for statistical testing based on close geographical or position in PCA space. Since Tai-Kadai languages have been hypothesized to have spread from southern China in the Iron Age⁷³, we repeated the same test with the Tai-Kadai speaking Shan in the second position, who live near the excavation site.

The results indicate that the Log Coffin-associated individuals are closer to the tested groups when compared with the Onge, Jehai, and Salar, while the tested groups are all genetically closer to other groups of the same language family than to the ancient genomes (Fig. S10). Since there is no present-day group or groups from one language family, which show a closer genetic affinity to the ancient genomes, we conclude that the present-day genetic diversity in Thailand is the result of different demographic processes than captured by the ancient genomes from northwestern Thailand.

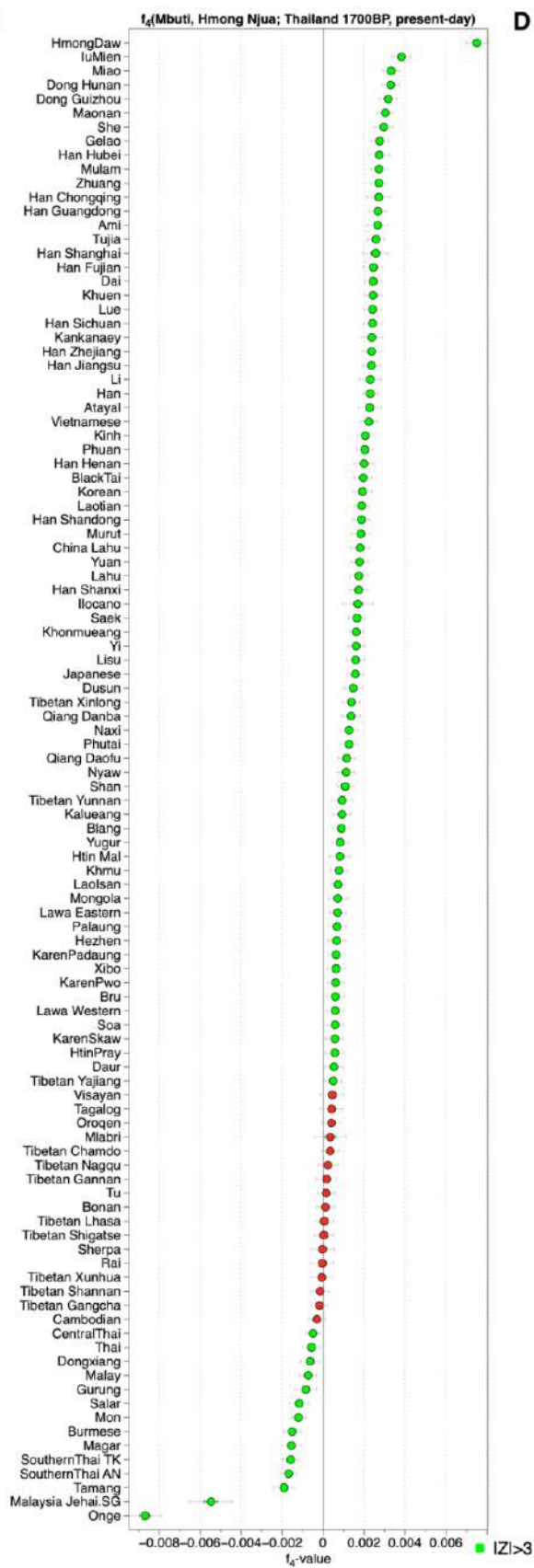
A



B



C



D

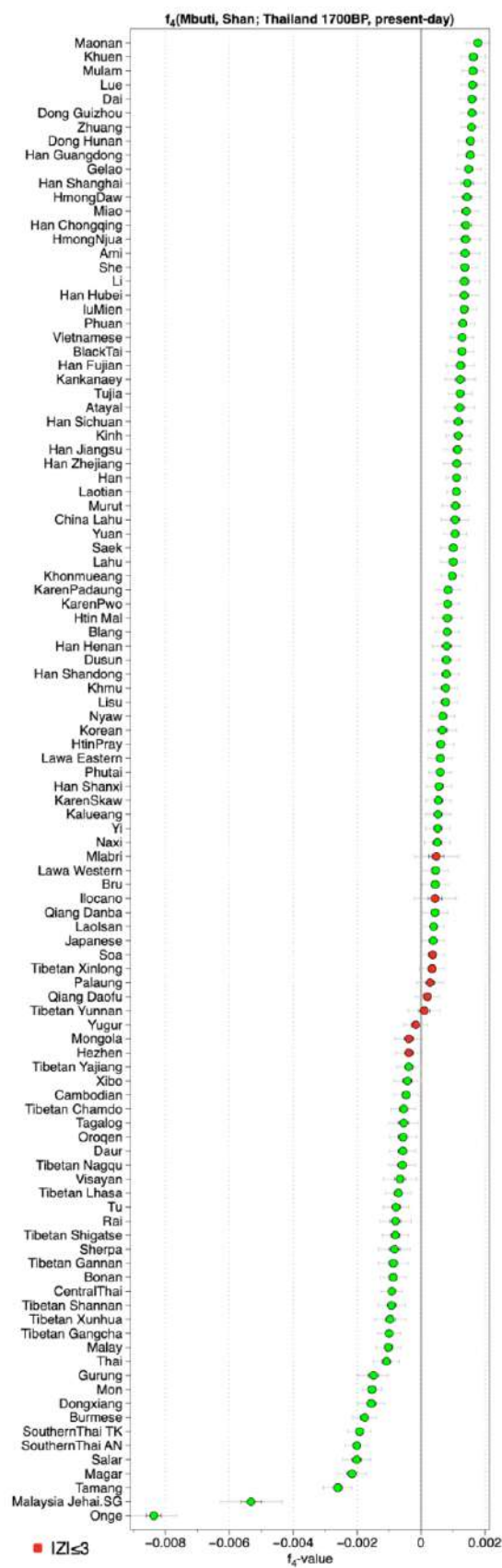


Figure S10. Observed f_4 -statistics comparing the affinity of the newly generated genomes from ancient northwestern Thailand to present-day groups^{27,28,60,61,67,69} and hill tribes from Thailand⁶⁷; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

qpAdm

We then used the f_4 results to inform ancestry modelling with qpAdm v1520 from AdmixTools v7.0.2⁶⁰ in a rotating model selection approach⁷⁴. We set inbreed: NO to be able to use single individuals in the model and used Mbuti.DG as a SNP calculation basis in the references. The input files for qpAdm were generated with qpWrapper v1.0.0, and results inspected with qpParser v0.1.1 (<https://github.com/TCLamnidis/qpWrapper>).

We first tried to model the ancient individuals from Thailand as an admixture between Bronze Age Ban Chiang and ancient individuals from northern East Asia, which showed the strongest affinity to the Log Coffin-associated individuals (Miaozigou_MN, Upper_YR_IA, WLR_LN, Mongolia_EIA_7_Xiognu, Upper_YR_LN, YR_LBIA, Mongolia_EIA_3, Mongolia_LBA_2_Ulaanzukh, Mebrak_2125BP.SG, Samdzong_1500BP.SG, Fig. S8). The published ancient individuals were rotated from left (sources) to right (references). The tested groups from the Yellow River region resulted in well-fit models estimating around 50% northern East Asian ancestry in the ancient Thai individuals (Fig. S11).

A parallel setup modelling the ancestry of the Long Long Rak individuals resulted in very similar results. However, the amount of Bronze Age Ban Chiang ancestry was slightly higher in all models.

Setting the Iron Age individuals from Ban Chiang as the target, however, showed that no genetic input from for northern East Asian groups was required (nested $p > 0.05$).

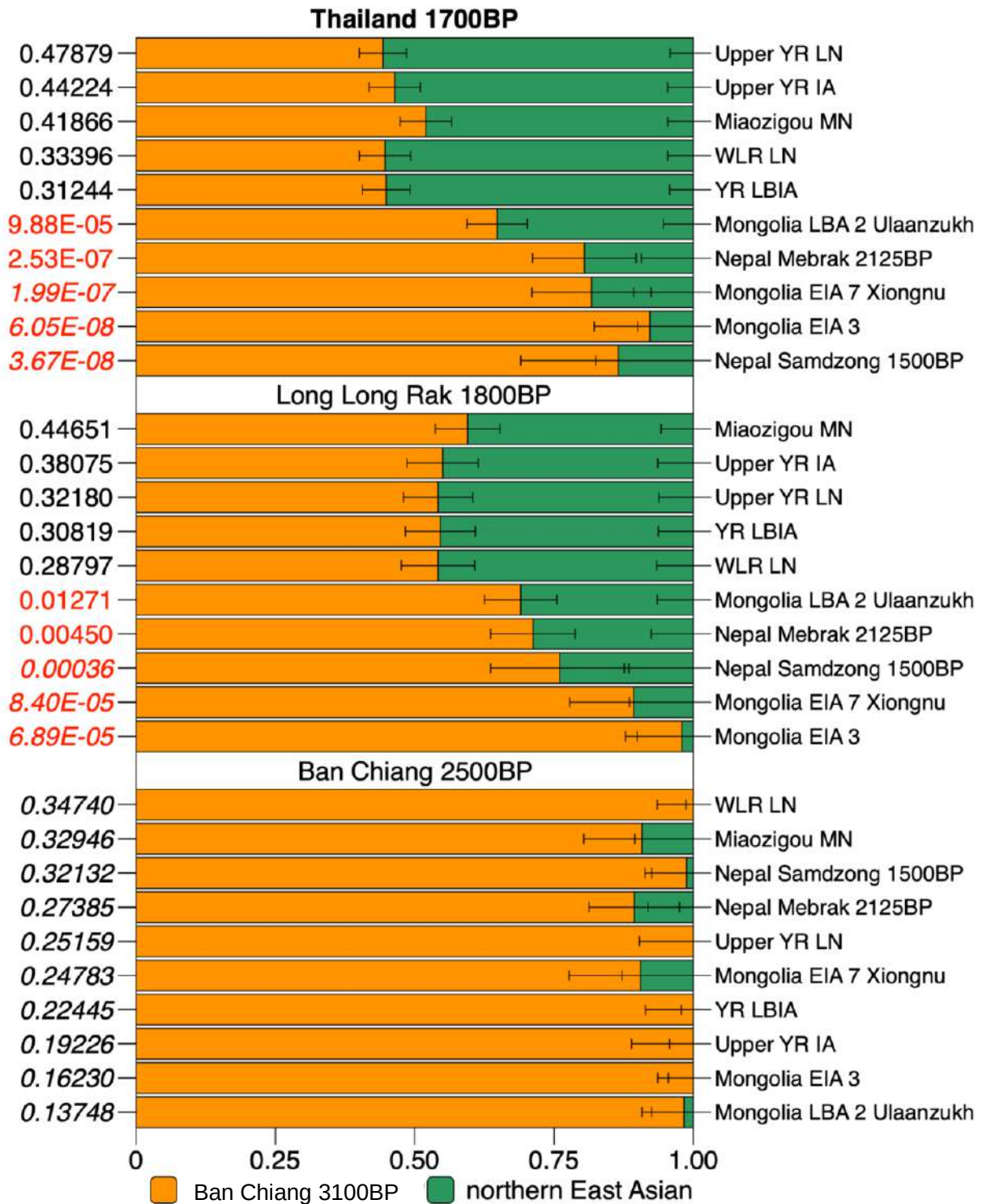


Figure S11. Results of qpAdm analyses modelling ancient individuals from Iron Age northeastern and -western Thailand^{27,28} as an admixture between Bronze Age northeastern Thailand²⁸ and ancient groups from northern East Asia⁶⁴; p-values are given on the left where red indicates $p < 0.05$ and italicized values indicate nested $p > 0.05$, northern East Asian groups

are displayed on the left; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

Initially it was suggested that all Neolithic Southeast Asian individuals, including the individuals from Ban Chiang, represent an admixture between deep Onge- (hunter-gatherer) and Ami-related (early farmer) groups²⁸, where Hòabìnhian-associated individuals from Laos and Malaysia were the best proxies for the hunter-gatherer ancestry²⁷. However, later studies showed that another hunter-gatherer source, Longlin from Guangxi Province⁶⁸, produced better fitting models for Neolithic Man Bac in Vietnam and some other ancient Southeast Asians, while others retained Hòabìnhian-associated influence⁶⁸. To account for this diverse ancestry set present in the region and assess the predominant genetic influence for all ancient groups from Thailand, we tested models using both the Hòabìnhian-associated individual from Laos²⁷ and the Longlin individual from Guangxi⁶⁸ as the hunter-gatherer source or in the reference groups. As representatives for early farmer ancestry we rotated the Neolithic individuals from southern China⁶⁶ from sources to references.

For the newly genotyped individuals using the Hòabìnhian-associated individual as the hunter-gatherer source resulted in better fitting models, i.e., higher p-values, although most models with the Longlin individual did fit ($p > 0.05$). The best-fitting model estimated $36.0 \pm 3.1\%$ Hòabìnhian-related and $64.0 \pm 3.1\%$ Suogang-related ancestry (Fig. S12). The Hòabìnhian-associated individual was also the preferred source for Long Long Rak with the best-fitting model favouring Liangdao1 as the southern East Asian source. The models for the Iron Age individuals from Ban Chiang resulted in larger hunter-gatherer ancestry components compared to the Iron Age groups from northwestern Thailand and the differentiation between models including the Hòabìnhian and Longlin as sources was less pronounced. Bronze Age Ban Chiang was best modelled as an admixture between Longlin and Qihe. Overall, the best-performing southern East Asian source varied, but most models involving Liangdao1 showed sufficient model-fit, and hence was selected for further modelling.

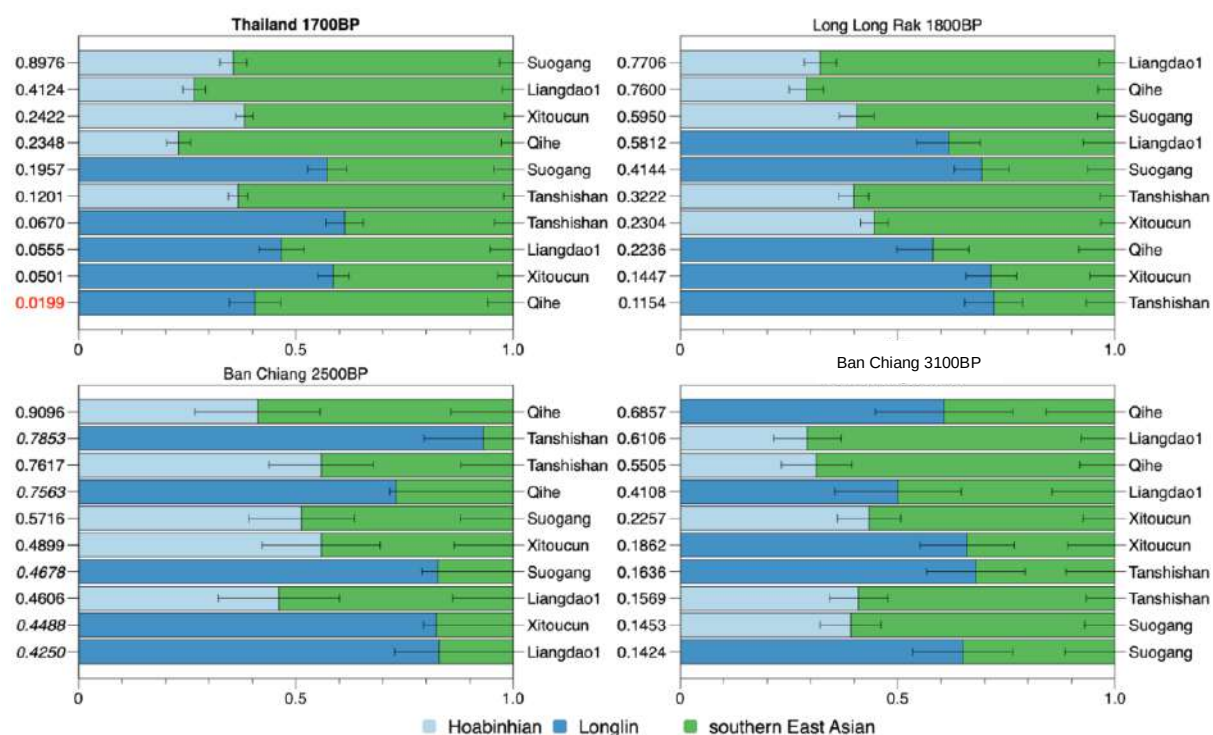


Figure S12. Results of qpAdm analyses modelling the ancestry of ancient genomes from Thailand as an admixture between the Hòabìnhian-associated individual from Laos²⁷, Longlin⁶⁸ and southern East Asian individuals⁶⁶; p-values are displayed on the left, where red indicates $p < 0.05$ and italicized values indicate nested $p > 0.05$, the southern East Asian sources are displayed on the right; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

Combining the information from the previous two analyses, we simultaneously estimated ancestry proportions related to hunter-gatherers, early farmers, and northern East Asians. We set the Hòabìnhian-associated individual from Laos and Liangdao1 as sources, while the remaining southern East Asian individuals were added to the references, and all northern East Asians rotated from references to sources.

The model could not assign any ancestry related to northern East Asia in the Bronze and Iron Age individuals from Ban Chiang, whereas the new individuals from northwestern Thailand showed a large component related to Upper_YR_LN, Miaoziyou_MN, and Upper_YR_IA (Fig. S13). However, using this setup, we could not find a sufficiently well-fit model for Long Long Rak ($p < 0.05$).

To investigate the geographical spread of the northern East Asian-related ancestry, we attempted to fit most previously published individuals as an admixture between a Longlin/Hòabìnhian-associated individual, Liangdao1 and northern East Asian^{27,28,66,68} (Table S4). While we could model Laos_Unknown.SG, Thailand 1700BP, Vietnam_Historical.SG and Vietnam_LN.SG as Longlin, Liangdao1 and Upper Yellow River, the models with the Hòabìnhian-associated individual achieved higher p-values and fit for more northern East Asian groups. Our analyses indicate that hunter-gatherer-related ancestry is not necessary for the 1.5-0.5-ka-old individuals from Guangxi (BaBanQinCen, GaoHuaHua, LaCen, Shenxian and Yiyang), Myanmar_LN_BA_lc from Oakaie and Vietnam_BA from Nui Nap (nested model 100 p>0.05 or negative admixture proportion). Conversely, northern East Asian ancestry is not required for modelling Baojianshan, Dushan, Thailand_BA, Thailand_IA (Ban Chiang) and Vietnam_N (Man Bac) (nested model p>0.05).

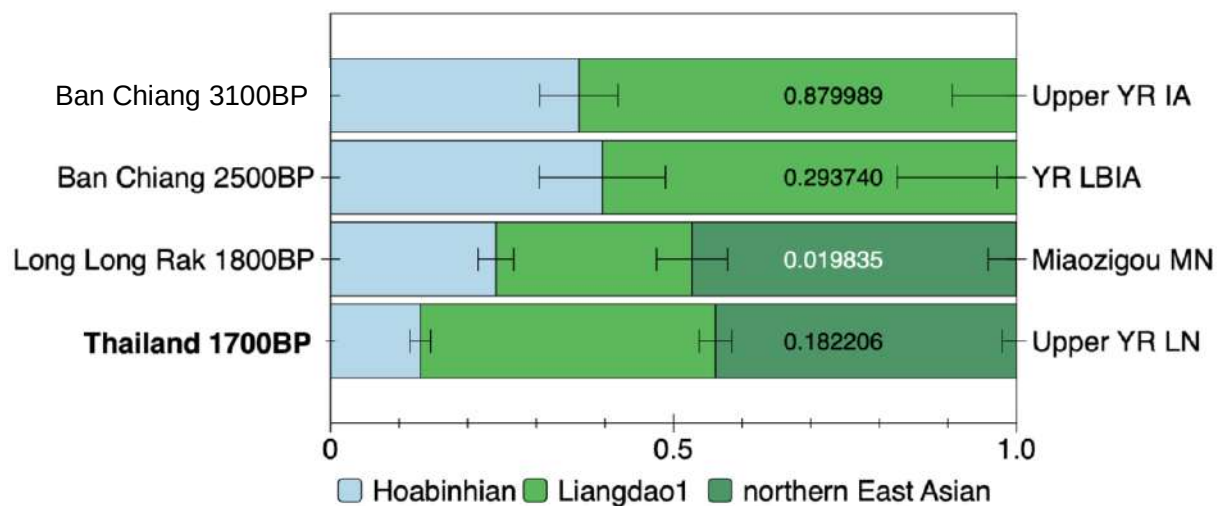


Figure S13. Results of qpAdm analyses modelling ancient individuals from Thailand as a three-way admixture between hunter-gatherers (light blue), ancient southern (light green), and ancient northern East Asian groups (dark green); p-values are displayed within the bars, northern East Asian sources on the left, white p-values indicate a poor fit, bold indicates the newly generated genomes; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

We repeated the analysis described above for the newly sequenced genomes individually using the Hòabìnhian-associated individual, Liangdao1, and Upper YR LN as respective proxies with the remaining ancient genomes as references (Mbuti.DG, Longlin, Qihe,

Xitoucun, Tanshishan, Suogang, Miaozigou_MN, Mongolia_EIA_3, Mongolia_EIA_7_Xiongnu, Mongolia_LBA_2_Ulaanzukh, Nepal_Mebrak_2125BP.SG, Nepal_Samdzung_1500BP.SG, Upper_YR_IA, WLR_LN, YR_LBIA). We could show some variation in hunter-gatherer, early farmer, and northern East Asian ancestry, but overall, the proportions were similar, around 15%, 45%, and 40%, respectively (Fig. S14). The lowest northern East Asian component was estimated for THL003, while THL004 could also be modelled without any hunter-gatherer contribution (nested $p > 0.05$). This ancestry model did not fit for YPN010, YPN017, and YPN030 ($p < 0.05$).

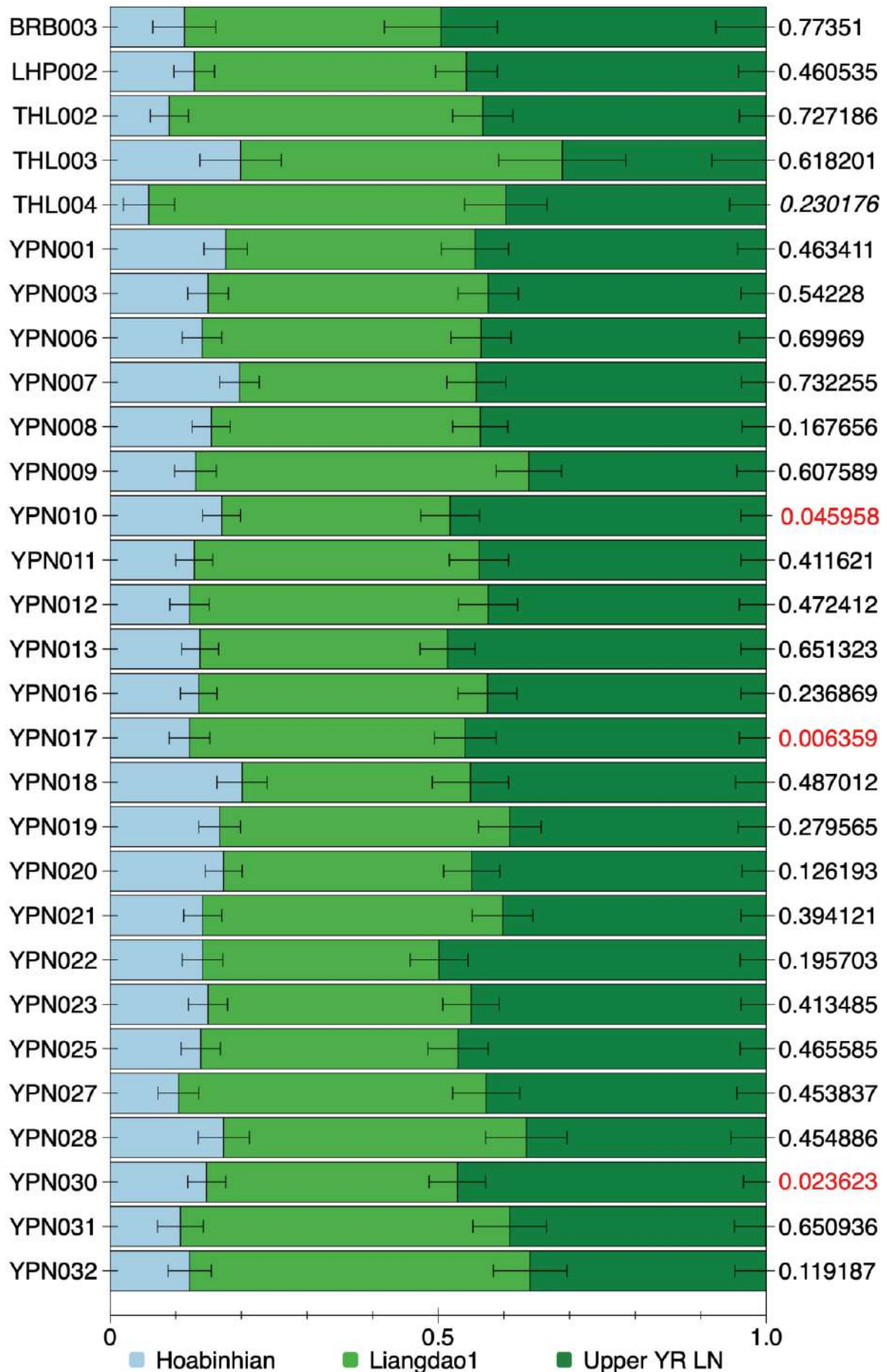


Figure S14. Results of qpAdm analyses modelling the newly generated ancient genomes from northwestern Thailand as an admixture of Hòabìnhiàn-associated individual from Laos²⁷, Liangdao1⁶⁶ and Upper Yellow River Late Neolithic (Upper YR LN)⁶⁴; p-values are displayed on the right, where red numbers indicate $p < 0.05$ and italic nested $p > 0.05$; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

Sex-biased admixture

Due to the lack of X-chromosome coverage in individuals from northern East Asia⁶⁴, we were not able to conduct the qpAdm analyses exclusively on X-chromosome data to assess differences in admixture proportions on the autosome and X-chromosome potentially related to sex-biased admixture.

An ADMIXTURE analysis including present-day Thai individuals was similarly inconclusive.

Admixture time dating

We used DATES v753⁷⁵ to estimate the time of admixture between the hunter-gatherer/Yangtze River, hunter-gatherer/Yellow River and Yangtze River/Yellow River components. However, the linkage disequilibrium decay curves were flat and the resulting standard errors very large, when using Laos_Hoabinhian, Liangdao1 and Upper_YR_LN as representatives for these ancestry components.

IBD calculation

To further investigate the genetic relationships between the studied individuals, we analysed long shared segments of the genome, also called identity-by-descent (IBD) blocks.

In preparation for imputation, we used ATLAS v.0.9⁷⁶ to call genotype likelihoods (GL) for the 33 newly-generated individuals. We called GL (method=MLE) for all positions from the 1000 Genomes Phase 3 release⁷⁷ after recalibrating the base quality scores (regions="88_mammals.epo_low_coverage.10M_GRCh37.masked.bed") according to post-mortem damage (length=50). These GL were then used for imputation with GLIMPSE v1.0.0⁷⁸, followed by extraction of the 1240K SNPs. We assessed imputation quality by counting the number of SNPs with genotype probabilities above 0.99 and excluded individuals with less than 600,000 high-quality SNPs (BRB003, THL003, THL004, YPN002,

YPN014, YPN018, YPN028, YPN031, YPN032, YPN033, YPN036). Due to sequencing differences and low coverage, we were not able to include the published individuals from Long Long Rak in our analysis²⁷.

To assess imputation accuracy, we also plotted genotypes before and after imputation onto a PCA calculated on present-day East and Southeast Asian groups, as described previously. In PCA space, the imputed individuals are more spread out and show a slight drift towards groups from East Asia, but generally overlap with the positions of the non-imputed individuals (Fig. S15A).

We then used ancIBD v.0.2a2 (<https://pypi.org/project/ancIBD/>) to calculate IBD blocks per individual from the 1240K-extracted data and compared these against each other, focusing on chunks with more than 220 SNPs and shared segments longer than 20 cM to identify relationships up to sixth-degree.

The results show complex relationship patterns within and between sites (Fig. S15B). While the pairs with the most and largest IBD blocks longer than 20 cM were excavated at Yappa Nhae 2, some individuals from Yappa Nhae 2 also share a third-/fourth-degree relationship with THL002. We also identified shared IBD blocks between Yappa Nhae 2 individuals and LHP002, but these are much less frequent and shorter, with a maximum of two blocks of 54.4 cM total (LHP002 – YPN022) compared to a maximum of 12 blocks of 659.4 cM total (THL002 – YPN030).

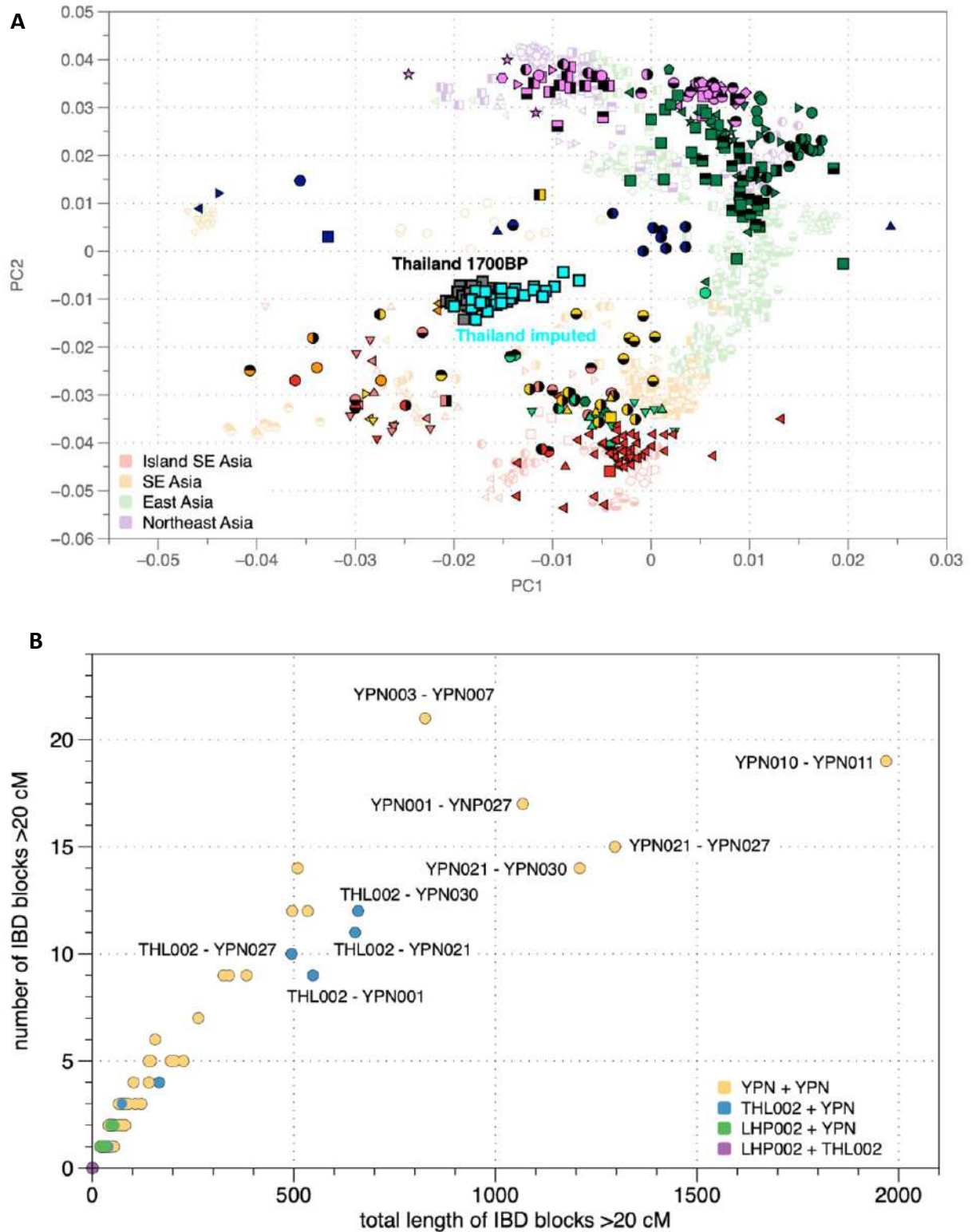


Figure S15. (A) Principal component analyses calculated on present-day East and Southeast Asian individuals^{28,60,61,63,69} showing the position of the Log Coffin-associated genomes before (grey squares) and after imputation (turquoise squares), full legend as in Fig. S5; (B)

Shared IBD blocks between Iron Age individuals from Thailand coloured by genetic relationships between individuals from different excavation sites; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

References

1. Treerayapiwat, C. Patterns of Habitation and Burial Activity in the Ban Rai Rock Shelter, Northwestern Thailand. *Asian Perspectives* 44, 231–245 (2005).
2. Shoocongdej, R. *The Final Report of Survey and Database of Caves in Pang Mapha district, Mae Hong Son Province*. (2000).
3. Shoocongdej, R. *Progress Report 1. Highland Archaeology Project in Pang Mapha Phase I*. (2001).
4. Shoocongdej, R. *Progress Report 2. Highland Archaeology Project in Pang Mapha Phase I*. (2002).
5. Shoocongdej, R. *Progress Report 3. Highland Archaeology Project in Pang Mapha Phase I*. (2002).
6. Shoocongdej, R. *Progress Report 4. Highland Archaeology Project in Pang Mapha Phase I*. (2002).
7. Shoocongdej, R. *Final Report 1. Highland Archaeology Project in Pang Mapha Phase I*. (2003).
8. Shoocongdej, R. *Highland Archaeology in Pang Mapha*. (Silpakorn University Press, 2004).
9. Shoocongdej, R. Late pleistocene activities at the Tham Lod rockshelter in highland Pang Mapha, Mae hong Son province, northwestern Thailand. in *Uncovering Southeast Asia's Past: Selected Papers from the 10th International Conference of the European Association of Southeast Asian Archaeologists* 22–37 (NUS press Singapore, 2006).
10. Marwick, B. Human behavioural ecology and stone artefacts in northwest Thailand during the terminal Pleistocene and Holocene. *Southeast Asian Prehistory: The 11th EurASEAA Conference* 73–80 (2008).
11. Marwick, B. Multiple Optima in Hoabinhian flaked stone artefact palaeoeconomics and palaeoecology at two archaeological sites in Northwest Thailand. *Journal of Anthropological Archaeology* 32, 553–564 (2013).

12. Shoocongdej, R. & Wattanapituksakul, A. Faunal assemblages and demography during the Late Pleistocene (MIS 2-1) to Early Holocene in Highland Pang Mapha, Northwest Thailand. *Quat. Int.* 563, 51–63 (2020).
13. Shoocongdej, R. *et al.* *Final Report of Prehistoric Population and Cultural Dynamic in Highland Pang Mapha, Mae Hong Son Province. Volume II: Archaeology.* (2021).
14. Wannasri, S., Pumijumnong, N. & Shoocongdej, R. Teak log coffin head styles in northern Thailand: Time sequencing with dendrochronology. *Sci. Asia.* 33, 047 (2007).
15. Pumijumnong, N. & Wannasri, S. Teak Log Coffins in Northwest Thailand: Dated by Dendrochronology and 14Cwiggle Matching. *Applied Environmental Research* 37, 1–16 (2015).
16. Preechamart, S. *et al.* Tree-ring oxygen isotope chronology of teak log coffins in northwestern Thailand and its relationship with Pacific Decadal Oscillation and El Niño-Southern Oscillation. *Quat. Int.* (2021) doi:10.1016/j.quaint.2021.04.007.
17. Grave, P. Dating of Log Coffins in Northwestern Thailand. (1994).
18. Dilokwanich, S. *An Exploration and Database System of the Caves, Mae Hong Son Province.* (2000).
19. Marwick, B. What attributes are important for the measurement of assemblage reduction intensity? Results from an experimental stone artefact assemblage with relevance to the Hoabinhian of mainland Southeast Asia. *J. Archaeol. Sci.* 35, 1189–1200 (2008).
20. Chitkament, T., Gaillard, C. & Shoocongdej, R. Tham Lod rockshelter (Pang Mapha district, north-western Thailand): Evolution of the lithic assemblages during the late Pleistocene. *Quat. Int.* 416, 151–161 (2016).
21. Shoocongdej, R. *Final Report 2. Highland Archaeological Project in Pang Mapha Phase II.* (2007).
22. Suraprasit, K., Shoocongdej, R., Chintakanon, K. & Bocherens, H. Late Pleistocene human paleoecology in the highland savanna ecosystem of mainland Southeast Asia. *Sci. Rep.* 11, 16756 (2021).
23. Khaewkamput, V. Archaeology of Tham Lod: Late Pleistocene-Late Holocene life ways. in *A Conference Proceeding on Human, Culture, and Paleoenvironment on Highland Pang Pang Mapha, Mae Hong Song Province* (Silpakorn University, Bangkok, 2003).

24. Wattanapituksakul, S. Late Pleistocene Mammal Teeth from the Tham Lod Rockshelter, Amphoe Pang Mapha, Changwat Mae Hong Son. (Chulalongkorn University, Bangkok, 2006).
25. Pautreau, J.-P., Mornais, P. & Doy-Asa, T. *Ban Wang Hai: excavations of an Iron-Age cemetery in northern Thailand*. (Silkworm Books, 2003).
26. Wannasri, S. *A Dendroarchaeological Study of Log Coffins: Bo Krai Cave and Ban Rai Rockshelter in Pang Mapha District, Mae Hong Son Province*. (Mahidol University, 2004).
27. McColl, H. *et al.* The prehistoric peopling of Southeast Asia. *Science* 361, 88–92 (2018).
28. Lipson, M. *et al.* Ancient genomes document multiple waves of migration in Southeast Asian prehistory. *Science* 361, 92–95 (2018).
29. Kahle, D. & Wickham, H. Ggmap: Spatial visualization with ggplot2. *The R Journal* 5, 144–161 (2013).
30. Pinhasi, R. *et al.* Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS One* 10, e0129102 (2015).
31. Dabney, J. *et al.* Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U. S. A.* 110, 15758–15763 (2013).
32. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20130624 (2015).
33. Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* 2010, db.prot5448 (2010).
34. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* 40, e3 (2012).
35. Fellows Yates, J. A. *et al.* Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager. *PeerJ* 9, e10947 (2021).
36. Di Tommaso, P. *et al.* Nextflow enables reproducible computational workflows. *Nat. Biotechnol.* 35, 316–319 (2017).

37. Andrews, S. FastQC A Quality Control tool for High Throughput Sequence Data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
38. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884–i890 (2018).
39. Schubert, M., Lindgreen, S. & Orlando, L. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Res. Notes* 9, 88 (2016).
40. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760 (2009).
41. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv [q-bio.GN]* (2013).
42. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079 (2009).
43. Okonechnikov, K., Conesa, A. & García-Alcalde, F. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics* 32, 292–294 (2016).
44. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842 (2010).
45. Neukamm, J., Peltzer, A. & Nieselt, K. DamageProfiler: Fast damage pattern calculation for ancient DNA. *Bioinformatics* (2021) doi:10.1093/bioinformatics/btab190.
46. Ewels, P., Magnusson, M., Lundin, S. & Käller, M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32, 3047–3048 (2016).
47. Sawyer, S., Krause, J., Guschanski, K., Savolainen, V. & Pääbo, S. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLoS One* 7, e34131 (2012).
48. Fu, Q. *et al.* An early modern human from Romania with a recent Neanderthal ancestor. *Nature* 524, 216–219 (2015).
49. Mathieson, I. *et al.* Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* 528, 499–503 (2015).
50. Lamnidis, T. C. *et al.* Ancient Fennoscandian genomes reveal origin and spread of Siberian ancestry in Europe. *Nat. Commun.* 9, 5018 (2018).

51. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics* 15, 356 (2014).
52. Jun, G., Wing, M. K., Abecasis, G. R. & Kang, H. M. An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Res.* 25, 918–925 (2015).
53. Fu, Q. *et al.* A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* 23, 553–559 (2013).
54. Popli, D., Peyrégne, S. & Peter, B. M. KIN: a method to infer relatedness from low-coverage ancient DNA. *Genome Biol.* 24, 10 (2023).
55. Ringbauer, H., Novembre, J. & Steinrücken, M. Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* 12, 5425 (2021).
56. Kearse, M. *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649 (2012).
57. Weissensteiner, H. *et al.* HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* 44, 58–63 (2016).
58. Zhang, X. *et al.* A Matrilineal Genetic Perspective of Hanging Coffin Custom in Southern China and Northern Thailand. *iScience* 23, 101032 (2020).
59. Rohrlach, A. B. *et al.* Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-path Neolithic expansion to Western Europe. *Sci. Rep.* 11, 15005 (2021).
60. Patterson, N. *et al.* Ancient admixture in human history. *Genetics* 192, 1065–1093 (2012).
61. Lazaridis, I. *et al.* Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 513, 409–413 (2014).
62. Jeong, C. *et al.* Long-term genetic stability and a high-altitude East Asian origin for the peoples of the high valleys of the Himalayan arc. *Proc. Natl. Acad. Sci. U. S. A.* 113, 7485–7490 (2016).
63. Skoglund, P. *et al.* Genomic insights into the peopling of the Southwest Pacific. *Nature* 538, 510–513 (2016).
64. Ning, C. *et al.* Ancient genomes from northern China suggest links between subsistence changes and human migration. *Nat. Commun.* 11, 2700 (2020).

65. Yang, M. A. *et al.* 40,000-Year-Old Individual from Asia Provides Insight into Early Population Structure in Eurasia. *Curr. Biol.* 27, 3202-3208.e9 (2017).
66. Yang, M. A. *et al.* Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* 369, 282–288 (2020).
67. Kutanan, W. *et al.* Reconstructing the Human Genetic History of Mainland Southeast Asia: Insights from Genome-Wide Data from Thailand and Laos. *Mol. Biol. Evol.* 38, 3459–3477 (2021).
68. Wang, T. *et al.* Human population history at the crossroads of East and Southeast Asia since 11,000 years ago. *Cell* (2021) doi:10.1016/j.cell.2021.05.018.
69. Wang, C.-C. *et al.* Genomic insights into the formation of human populations in East Asia. *Nature* 591, 413–419 (2021).
70. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* 2, e190 (2006).
71. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19, 1655–1664 (2009).
72. Mallick, S. *et al.* The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* 538, 201–206 (2016).
73. Pittayaporn, P. Layers of Chinese Loanwords in Protosouthwestern Tai as Evidence for the Dating of the Spread of Southwestern Tai. *Manusya: Journal of Humanities* 17, 47–68 (2014).
74. Harney, É., Patterson, N., Reich, D. & Wakeley, J. Assessing the Performance of qpAdm: A Statistical Tool for Studying Population Admixture. *Genetics* 217, (2021).
75. Narasimhan, V. M. *et al.* The formation of human populations in South and Central Asia. *Science* 365, (2019).
76. Link, V. *et al.* ATLAS: Analysis Tools for Low-depth and Ancient Samples. *bioRxiv* 105346 (2017) doi:10.1101/105346.
77. 1000 Genomes Project Consortium *et al.* A global reference for human genetic variation. *Nature* 526, 68–74 (2015).
78. Rubinacci, S., Ribeiro, D. M., Hofmeister, R. J. & Delaneau, O. Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nat. Genet.* 53, 120–126 (2021).

Table S1. Direct dating results of a selection of petrous bones from Log Coffin sites in Pang Mapha, Thailand as provided by the Klaus-Tschira-Archäometrie-Zentrum, Germany.

Sample Name	Archaeological ID	Site	14C Lab Nr.	14C age [yr BP]	[+/-]	$\delta^{13}\text{C}$ AMS [‰]	Cal 1-sigma	Cal 2-sigma	C:N	C [%]	Collagen [%]
BRB003	BN 554 #44	Ban Rai	MAMS-40600	1811	21	-18,4	cal AD 142-240	cal AD 132-310	2,9	40,8	8,8
LHP002	LHP#17	Lahu Pot/Moa Mussur	MAMS-40601	1754	23	-26,6	cal AD 246-327	cal AD 229-377	3,3	17,7	0,4
THL002	TL#24/10	Tham Lod Cave	MAMS-40597	1754	21	-18,8	cal AD 248-326	cal AD 232-375	2,9	29,1	5,6
THL003	TL 880 S2 #14	Tham Lod Cave	MAMS-40598	1653	21	-20,9	cal AD 384-418	cal AD 343-424	3,2	20,2	24,0
THL004	TL 878 S2 #14	Tham Lod Cave	MAMS-40599	1724	21	-23,8	cal AD 258-377	cal AD 253-384	2,9	41,2	10,8
YPN008	YN2#Mdcave02	Yappa Nhae 2	MAMS-40608	1717	20	-20,2	cal AD 260-380	cal AD 254-388	2,8	24,1	2,3
YPN011	YP2#mdcave04	Yappa Nhae 2	MAMS-40609	1698	20	-19,3	cal AD 336-387	cal AD 258-399	2,9	32,7	4,8
YPN016	YN2#RT-04	Yappa Nhae 2	MAMS-40610	1706	21	-25,1	cal AD 265-384	cal AD 257-395	2,2	17,7	2,4
YPN025	YN2#LT-08	Yappa Nhae 2	MAMS-40611	1793	26	-16,7	cal AD 145-318	cal AD 135-325	2,5	19,4	1,0

Table S2. Overview of archaeological and genomic information of Iron Age Log Coffin culture-associated individuals from northwestern Thailand.

Sample Name	Archaeological ID	Site	Provi	Material	SG reads	Endogenous DNA (%)	5' C→T frequency (%)	Median read length	# of 1240K captures	1240K reads	1240K SNPs	X-rate	Y-rate	Karyotype	nuclear contam (%)	MT contam (%)	MT Haplogroup	Y-haplogroup	terminal SNPs
BRB001	BR - B#1 Burial 1	Ban Rai	Mac Hong Son	tooth	5,436,036	0.03	0.7	35.0											
BRB002	BN 574 #45	Ban Rai	Mac Hong Son	tooth	7,086,237	0.01	3.2	31.0											
BRB003	BN 554 #44	Ban Rai	Mac Hong Son	tooth	5,423,492	0.21	10.1	40.0		2	18,853,846	74,647	0.7	0.1 XX			1 F1f		
BRB004	BN 553 #24	Ban Rai	Mac Hong Son	tooth	4,826,271	0.03	1.4	35.0											
BRB005	BN 551 #45	Ban Rai	Mac Hong Son	tooth	6,798,699	0.01	7.7	44.0											
LHP001	LHP#28.5	Lahu Pot/Moa Mussur	Mac Hong Son	petrous	4,986,261	0.02	4.0	30.0											
LHP002	LHP#17	Lahu Pot/Moa Mussur	Mac Hong Son	petrous	5,586,504	0.48	9.4	40.0		2	19,214,185	469,997	0.4	0.6 XY	0	0	M2375	O1b1a1a1b1	FGC29900/Y9322/Z23667
MMS001	MS 706 #15	Moa Mussur	Mac Hong Son	tooth	5,853,704	0.04	11.5	41.0											
MMS002	MS 368 #34	Moa Mussur	Mac Hong Son	tooth	4,512,518	0.01	0.0	31.0											
MMS003	MS 385 #15	Moa Mussur	Mac Hong Son	tooth	3,997,455	0.04	16.1	40.0											
MMS004	MS 384 #15	Moa Mussur	Mac Hong Son	tooth	5,782,476	0.02	1.9	46.5											
PKL001	PK1#11	Pang Kham 1	Mac Hong Son	petrous	6,487,050	0.01	1.6	31.0											
THL001	Tham Lod (TL) Burial#2 TL-SK2/B#2	Tham Lod rock shelter	Mac Hong Son	petrous	4,496,578	0.27	2.2	51.0											
THL002	TL#2410 #2410	Tham Lod cave	Mac Hong Son	petrous	5,088,921	7.96	26.8			1	54,190,994	698,906	0.9	0 XX				1 F1f	
THL003	TL 880 S2 #14	Tham Lod cave	Mac Hong Son	tooth	4,829,588	0.3	13.1	39.0		2	21,191,683	52,907	0.7	0.1 XX				2 F1f	
THL004	TL 878 S2 #14	Tham Lod cave	Mac Hong Son	tooth	5,749,714	4.25	17.0	37.0		1	14,843,196	192,331	0.8	0 XX				1 F1a1a1	
THL005	TL 351 S1 #25	Tham Lod cave	Mac Hong Son	tooth	6,195,535	0.06	13.6	40.0											
THL006	TL 350 #24 S1	Tham Lod cave	Mac Hong Son	tooth	5,218,611	0.07	3.0	55.0											
WAH001.A	#001-003 Wang Hai Skeleton#1	Ban Wang Hai	Lamphun	petrous	3,012,757	0.02	0.0	32.0											
WAH001.B	#001-003 Wang Hai Skeleton#1	Ban Wang Hai	Lamphun	petrous	3,400,841	0.02	2.7	31.0											
WAH001.D	#001-003 Wang Hai Skeleton#1	Ban Wang Hai	Lamphun	tooth	4,844,173	0.04	2.0	33.0											
WAH002.A	#004-005 Wang Hai WH#7 TP1 Burial 1 Skeleton #2	Ban Wang Hai	Lamphun	tooth	4,381,992	0.02	3.8	30.0											
WAH002.B	#004-005 Wang Hai WH#7 TP1 Burial 1 Skeleton #2	Ban Wang Hai	Lamphun	cranial															
YPN001	YN2#LM01	Yappa Nhae 2	Mac Hong Son	petrous	5,703,478	2.07	13.0	46.0		1	24,240,439	403,681	0.7	0 XX			1 M55		
YPN002	YN2#LP01	Yappa Nhae 2	Mac Hong Son	petrous	5,631,311	1.09	16.8	40.0		1	22,435,077	203,446	0.7	0 XX			1 F1a1a1		
YPN003	YN2#RM-01	Yappa Nhae 2	Mac Hong Son	petrous	4,357,628	5.68	16.1	42.0		1	21,298,377	499,444	0.4	0.5 XY	0	0	1 M24b	N1b	M1918/Z5034
YPN004	YN2#RN-04	Yappa Nhae 2	Mac Hong Son	petrous	7,615,604	0.01	10.5	34.0											
YPN005	YN2#RM-03	Yappa Nhae 2	Mac Hong Son	cranial															
YPN006	YN2#RM-05	Yappa Nhae 2	Mac Hong Son	petrous	7,594,864	3.41	10.2	49.0		1	20,736,306	489,787	0.4	0.5 XY	0	0	1 F1f	O1b1a1	CTS8589/M1425
YPN007	YN2#RM-02	Yappa Nhae 2	Mac Hong Son	petrous	5,009,245	3.28	9.5	51.0		1	22,379,333	537,949	0.4	0.5 XY	0	0	1 M24b	N1b1	Y6604/Z19701
YPN008	YN2#Mdcav02	Yappa Nhae 2	Mac Hong Son	petrous	5,477,996	36.07	10.8	48.0		1	22,281,187	755,278	0.4	0.5 XY	0	0	1 D5a2a1-+@16172	N1b1	F4201
YPN009	YN2#Mdcav03	Yappa Nhae 2	Mac Hong Son	petrous	5,928,759	3.88	11.6	44.0		1	25,168,096	415,482	0.4	0.5 XY	0	0	2 N8*	O2a2b1a1a1a4a	CTS4658, CTS9332
YPN010	YN2#Mdcav01	Yappa Nhae 2	Mac Hong Son	petrous	5,998,803	16.1	8.7	55.0		1	19,685,704	699,987	0.7	0 XX	0	0	1 F1a1a1		
YPN011	YP2#Mdcav04	Yappa Nhae 2	Mac Hong Son	petrous	5,862,164	34.32	13.3	48.0		1	22,590,279	709,086	0.4	0.5 XY	0	0	1 F1f	O1b1a1a1b	F4181/M1357, F2522/M1410, F2028/M1367
YPN012	YN2#RT-02	Yappa Nhae 2	Mac Hong Son	petrous	5,131,698	5.33	12.2	47.0		1	19,068,936	469,818	0.7	0 XX			1 M3256		
YPN013	YN2#RT-01	Yappa Nhae 2	Mac Hong Son	petrous	5,191,086	7.95	9.4	51.0		1	23,218,901	651,991	0.8	0 XX	0	0	1 F1a1a1		
YPN014	YN2#RT-06	Yappa Nhae 2	Mac Hong Son	petrous	4,665,553	0.65	13.2	44.0		1	22,215,941	150,455	0.4	0.5 XY	0	0	0 M7b1a1	O2a2b1a1a1	F42
YPN015	YN2#RT-03	Yappa Nhae 2	Mac Hong Son	petrous															
YPN016	YN2#RT-04	Yappa Nhae 2	Mac Hong Son	petrous	3,759,755	57.92	10.6	53.0		1	13,656,511	647,252	0.8	0 XX			2 N8*		
YPN017	YN2#RT-05	Yappa Nhae 2	Mac Hong Son	petrous	6,334,027	8.47	12.3	44.0		1	19,564,682	509,713	0.4	0.5 XY	0	0	1 N8*	O1b1a1a1b	F789/M1283
YPN018	YN2#LT-07	Yappa Nhae 2	Mac Hong Son	petrous	7,150,078	1.01	13.8	42.0		1	21,845,249	209,963	0.4	0.5 XY	0	0	0 G2b1a	O2a2b1a1a1a4a2	Z25929
YPN019	YN2#LT-03	Yappa Nhae 2	Mac Hong Son	petrous	1,956,047	3.47	13.1	42.0		1	21,586,212	413,444	0.4	0.5 XY	0	0	1 M69	O1b1a1a1b	F789/M1283
YPN020	YN2#LT-04	Yappa Nhae 2	Mac Hong Son	petrous	2,563,537	14.8	8.6	51.0		1	22,849,997	710,459	1.1	0 XXXX			1 M74		
YPN021	YN2#LT-05	Yappa Nhae 2	Mac Hong Son	petrous	5,081,717	13.29	15.6	41.0		1	19,443,033	529,293	0.7	0 XX			1 M7b1a1		
YPN022	YN2#LT-02	Yappa Nhae 2	Mac Hong Son	petrous	5,311,234	13.86	12.1	48.0		1	16,120,416	559,709	0.7	0 XX			1 F1a1a1		
YPN023	YP2#LT01	Yappa Nhae 2	Mac Hong Son	petrous	3,818,974	8.6	12.8	49.0		1	21,539,845	624,088	0.7	0 XX			2 G2b1a		
YPN024	YN2#LT - 06	Yappa Nhae 2	Mac Hong Son	petrous															
YPN025	YN2#LT-08	Yappa Nhae 2	Mac Hong Son	petrous	4,813,579	37.83	19.0	41.0		1	20,389,261	633,197	0.4	0.5 XY	0	0	2 D5a2a1-+@16172	C2b1	Z4132
YPN026	YN2#ZoneA2-01	Yappa Nhae 2	Mac Hong Son	petrous	3,628,925	0.02	7.2	36.0											
YPN027	YN2#ZoneA2-02	Yappa Nhae 2	Mac Hong Son	petrous	4,828,481	13.75	10.6	50.0		1	13,853,274	517,358	0.4	0.5 XY	0	0	0 F1f	O2a2b1a1a1a4a	CTS4658
YPN028	YN2#29/41-1	Yappa Nhae 2	Mac Hong Son	petrous	9,286,948	0.38	15.6	41.0		1	73,305,948	193,760	0.9	0 XX			3 n/a		
YPN029	YN2#29/41-2	Yappa Nhae 2	Mac Hong Son	petrous	5,932,497	0.08	20.8	35.0											
YPN030	YN2#29/41-3	Yappa Nhae 2	Mac Hong Son	petrous	6,223,614	8.65	10.6	43.0		1	62,878,538	935,220	0.5	0.6 XY	0	0	1 F1a1a1	O2a2b1a1a1a4a2	Z25928
YPN031	YN2#29/41-4	Yappa Nhae 2	Mac Hong Son	petrous	4,632,050	0.66	15.6	39.0		1	51,328,182	261,374	0.9	0.1 XX			3 M7b1a1		
YPN032	YN1-MH0505	Yappa Nhae 1	Mac Hong Son	petrous	4,500,075	29.0	16.3	39.0		1	18,298,564	266,799	0.7	0 XX			2 F1f		
YPN033	YN1#1918	Yappa Nhae 1	Mac Hong Son	petrous	5,291,354	0.21	16.0	41.0		2	20,562,941	224,205	0.6	0 XX			1 n/a		
YPN034	YN2 481 #35	Yappa Nhae 2	Mac Hong Son	tooth	5,295,550	0.1	12.5	40.0											
YPN035	YN2 482 #45 Y	Yappa Nhae 2	Mac Hong Son	tooth	6,229,621	0	10.3	36.0											
YPN036	YN2 478 #35	Yappa Nhae 2	Mac Hong Son	tooth	6,754,045	0.1	15.2	42.0		2	21,849,911	101,945	0.4	0.4 n/a	20	20	6 n/a	O?	P191
YPN037	YN2 477 #45 U	Yappa Nhae 2	Mac Hong Son	tooth	5,925,888	0	0.0	31.0											
YPN038	YN1 858 #35	Yappa Nhae 1	Mac Hong Son	tooth	5,129,747	0.01	3.3	40.0											
YPN039	YN1 846 #15	Yappa Nhae 1	Mac Hong Son	tooth	5,457,002	0.01	2.0	36.0											
YPN040	YN1 844 #24	Yappa Nhae 1	Mac Hong Son	tooth	4,131,554	0.08	22.0	41.0											
YPN041	YN1 837 #25	Yappa Nhae 1	Mac Hong Son	tooth	5,624,442	0.02	11.0	36.0											

Table S3. Results of genetic relatedness inference with KIN, k0 shows the proportion of the genome without IBD sharing, k1 the proportion with one chromosome in IBD, k2 the proportion with both chromosomes in IBD.

Pair	Relatedness	Second most likely relatedness	Log Likelihood Ratio	Within Degree 2nd most likely	Within Degree Log Likelihood Ratio	k0	k1	k2	IBD Length	IBD Number
YPN032 - YPN033	Identical	Siblings	72.963			0	0	1	0	1
Th519 - Th703	Identical	Siblings	60.959			0	0	1	0	1
Th530 - Th531	Siblings	Grandparent-Grandchild	0.965	Parent-Child	1.485	0.272	0.559	0.169	223	11
YPN014 - YPN021	Parent-Child	Grandparent-Grandchild	20.003	Siblings	52.497	0	1	0	290	1
YPN014 - YPN027	Half-siblings	Unrelated	8.201	Avuncular	0.530	0.477	0.523	0	151	21
YPN002 - YPN032	Avuncular	Unrelated	17.484	Half-siblings	0.850	0.373	0.627	0	190	24
YPN002 - YPN033	Avuncular	Unrelated	17.735	Half-siblings	1.186	0.392	0.608	0	170	26
YPN010 - YPN011	Grandparent-Grandchild	Unrelated	13.991	Half-siblings	8.576	0.372	0.628	0	178	12
THL002 - YPN014	Unrelated	Unrelated	1.347			0.802	0.198	0	46	11
THL002 - YPN021	Unrelated	Unrelated	1.782			0.779	0.221	0	64	14
THL002 - YPN027	Unrelated	Unrelated	0.178			0.802	0.198	0	51	12
THL002 - YPN030	Unrelated	Unrelated	1.393			0.756	0.244	0	70	16
THL004 - Th521	Unrelated	Unrelated	0.258			0.771	0.229	0	57	9
YPN001 - YPN014	Unrelated	Half-siblings	1.290			0.664	0.336	0	87	9
YPN001 - YPN027	Unrelated	Grandparent-Grandchild	2.431			0.631	0.369	0	102	10
YPN003 - YPN007	Unrelated	Unrelated	1.381			0.749	0.251	0	70	16
YPN012 - YPN027	Unrelated	Unrelated	0.995			0.77	0.23	0	46	13
YPN014 - YPN030	Unrelated	Avuncular	1.229			0.723	0.277	0	69	13
YPN021 - YPN027	Unrelated	Grandparent-Grandchild	0.831			0.626	0.374	0	105	15
YPN021 - YPN030	Unrelated	Grandparent-Grandchild	0.787			0.631	0.369	0	109	11
Th519 - Th521	Unrelated	Avuncular	1.468			0.7	0.3	0	80	13
Th521 - Th703	Unrelated	Unrelated	2.541			0.724	0.276	0	56	8
BRB003 - THL003	Unrelated	Unrelated	0.965			0.875	0.125	0	0	1
BRB003 - YPN014	Unrelated	Unrelated	2.571			0.877	0.123	0	15	1
THL002 - THL003	Unrelated	Unrelated	3.554			0.894	0.106	0	26	4
THL002 - YPN001	Unrelated	Unrelated	2.374			0.857	0.143	0	41	7
THL003 - YPN014	Unrelated	Unrelated	1.487			0.871	0.129	0	0	1
THL003 - YPN030	Unrelated	Unrelated	2.643			0.875	0.125	0	32	5
THL003 - Th519	Unrelated	Unrelated	1.088			0.876	0.124	0	0	1
YPN001 - YPN012	Unrelated	Unrelated	3.451			0.879	0.121	0	30	8
YPN006 - YPN012	Unrelated	Unrelated	1.689			0.839	0.161	0	38	8
YPN006 - Th521	Unrelated	Unrelated	2.530			0.867	0.133	0	33	9
YPN012 - YPN014	Unrelated	Unrelated	1.892			0.843	0.157	0	40	9
YPN012 - Th519	Unrelated	Unrelated	2.939			0.866	0.134	0	36	10
YPN013 - YPN019	Unrelated	Unrelated	3.822			0.87	0.13	0	35	11
YPN027 - YPN030	Unrelated	Unrelated	2.149			0.847	0.153	0	39	13
YPN033 - Th703	Unrelated	Unrelated	2.815			0.885	0.115	0	16	1
BRB003 - THL002	Unrelated	Unrelated	4.994			0.909	0.091	0	21	3
BRB003 - THL004	Unrelated	Unrelated	3.667			0.931	0.069	0	8	1
BRB003 - YPN001	Unrelated	Unrelated	5.058			0.916	0.084	0	18	2
BRB003 - YPN006	Unrelated	Unrelated	7.398			0.946	0.054	0	5	1
BRB003 - YPN012	Unrelated	Unrelated	6.542			0.935	0.065	0	5	2
BRB003 - YPN017	Unrelated	Unrelated	8.336			0.948	0.052	0	9	1
BRB003 - YPN025	Unrelated	Unrelated	9.199			0.953	0.047	0	5	2
BRB003 - YPN027	Unrelated	Unrelated	5.837			0.934	0.066	0	5	1
BRB003 - YPN030	Unrelated	Unrelated	6.165			0.923	0.077	0	16	5
BRB003 - YPN032	Unrelated	Unrelated	4.830			0.926	0.074	0	3	1
BRB003 - Th519	Unrelated	Unrelated	2.823			0.935	0.065	0	0	1
BRB003 - Th521	Unrelated	Unrelated	3.495			0.925	0.075	0	3	1
BRB003 - Th531	Unrelated	Unrelated	1.378			0.937	0.063	0	0	1

BRB003 - Th703	Unrelated	Unrelated	2.645	0.939	0.061	0	0	1
LHP002 - YPN002	Unrelated	Unrelated	10.689	0.962	0.038	0	8	4
LHP002 - YPN008	Unrelated	Unrelated	12.778	0.978	0.022	0	5	4
LHP002 - YPN009	Unrelated	Unrelated	12.288	0.97	0.03	0	6	3
LHP002 - YPN012	Unrelated	Unrelated	12.746	0.977	0.023	0	5	3
LHP002 - YPN013	Unrelated	Unrelated	7.996	0.937	0.063	0	15	6
LHP002 - YPN016	Unrelated	Unrelated	11.886	0.972	0.028	0	4	1
LHP002 - YPN017	Unrelated	Unrelated	10.346	0.959	0.041	0	7	3
LHP002 - YPN019	Unrelated	Unrelated	10.231	0.963	0.037	0	9	4
LHP002 - YPN020	Unrelated	Unrelated	11.542	0.973	0.027	0	3	3
LHP002 - YPN023	Unrelated	Unrelated	11.790	0.962	0.038	0	11	7
LHP002 - YPN027	Unrelated	Unrelated	11.698	0.964	0.036	0	8	4
LHP002 - YPN031	Unrelated	Unrelated	11.957	0.98	0.02	0	3	3
LHP002 - YPN032	Unrelated	Unrelated	11.604	0.971	0.029	0	7	4
LHP002 - Th519	Unrelated	Unrelated	8.652	0.943	0.057	0	13	5
LHP002 - Th521	Unrelated	Unrelated	8.076	0.936	0.064	0	12	4
LHP002 - Th703	Unrelated	Unrelated	7.182	0.948	0.052	0	2	1
THL002 - YPN006	Unrelated	Unrelated	12.063	0.975	0.025	0	6	4
THL002 - YPN008	Unrelated	Unrelated	12.372	0.983	0.017	0	3	2
THL002 - YPN010	Unrelated	Unrelated	12.462	0.98	0.02	0	4	3
THL002 - YPN011	Unrelated	Unrelated	12.219	0.971	0.029	0	5	3
THL002 - YPN012	Unrelated	Unrelated	9.572	0.95	0.05	0	11	3
THL002 - YPN017	Unrelated	Unrelated	10.785	0.973	0.027	0	6	3
THL002 - YPN019	Unrelated	Unrelated	10.990	0.963	0.037	0	6	4
THL002 - YPN022	Unrelated	Unrelated	11.194	0.972	0.028	0	7	4
THL002 - Th519	Unrelated	Unrelated	11.597	0.971	0.029	0	7	3
THL002 - Th521	Unrelated	Unrelated	12.441	0.98	0.02	0	2	2
THL002 - Th703	Unrelated	Unrelated	10.088	0.96	0.04	0	6	1
THL003 - YPN001	Unrelated	Unrelated	7.834	0.95	0.05	0	2	1
THL003 - YPN010	Unrelated	Unrelated	9.788	0.96	0.04	0	7	2
THL003 - YPN012	Unrelated	Unrelated	7.598	0.946	0.054	0	3	1
THL003 - YPN017	Unrelated	Unrelated	9.166	0.955	0.045	0	5	2
THL003 - YPN021	Unrelated	Unrelated	4.377	0.918	0.082	0	22	2
THL003 - YPN027	Unrelated	Unrelated	5.548	0.928	0.072	0	7	1
THL003 - YPN031	Unrelated	Unrelated	7.841	0.946	0.054	0	0	1
THL003 - Th703	Unrelated	Unrelated	1.174	0.936	0.064	0	0	1
THL004 - YPN001	Unrelated	Unrelated	11.355	0.976	0.024	0	2	2
THL004 - YPN002	Unrelated	Unrelated	6.251	0.935	0.065	0	9	4
THL004 - YPN003	Unrelated	Unrelated	10.558	0.97	0.03	0	2	1
THL004 - YPN006	Unrelated	Unrelated	10.514	0.955	0.045	0	12	6
THL004 - YPN009	Unrelated	Unrelated	11.490	0.97	0.03	0	2	1
THL004 - YPN010	Unrelated	Unrelated	9.863	0.949	0.051	0	12	4
THL004 - YPN011	Unrelated	Unrelated	10.723	0.957	0.043	0	10	5
THL004 - YPN012	Unrelated	Unrelated	9.721	0.955	0.045	0	13	2
THL004 - YPN014	Unrelated	Unrelated	9.392	0.964	0.036	0	2	1
THL004 - YPN019	Unrelated	Unrelated	8.273	0.942	0.058	0	12	4
THL004 - YPN023	Unrelated	Unrelated	8.283	0.946	0.054	0	5	4
THL004 - YPN027	Unrelated	Unrelated	10.412	0.964	0.036	0	4	2
THL004 - YPN030	Unrelated	Unrelated	9.082	0.954	0.046	0	9	5
THL004 - YPN031	Unrelated	Unrelated	9.927	0.957	0.043	0	8	2
THL004 - YPN032	Unrelated	Unrelated	6.375	0.932	0.068	0	14	3
THL004 - YPN033	Unrelated	Unrelated	4.856	0.921	0.079	0	12	3
THL004 - Th519	Unrelated	Unrelated	7.136	0.942	0.058	0	3	1

THL004 - Th530	Unrelated	Unrelated	9.469	0.961	0.039	0	3	2
THL004 - Th703	Unrelated	Unrelated	4.044	0.944	0.056	0	0	1
YPN001 - YPN003	Unrelated	Unrelated	12.545	0.985	0.015	0	1	1
YPN001 - YPN006	Unrelated	Unrelated	7.073	0.918	0.082	0	22	7
YPN001 - YPN007	Unrelated	Unrelated	12.804	0.982	0.018	0	3	2
YPN001 - YPN009	Unrelated	Unrelated	11.867	0.975	0.025	0	5	3
YPN001 - YPN011	Unrelated	Unrelated	12.295	0.979	0.021	0	4	2
YPN001 - YPN013	Unrelated	Unrelated	11.567	0.971	0.029	0	4	3
YPN001 - YPN016	Unrelated	Unrelated	10.518	0.956	0.044	0	12	3
YPN001 - YPN017	Unrelated	Unrelated	12.848	0.979	0.021	0	4	2
YPN001 - YPN018	Unrelated	Unrelated	11.473	0.973	0.027	0	2	1
YPN001 - YPN019	Unrelated	Unrelated	12.092	0.978	0.022	0	3	1
YPN001 - YPN023	Unrelated	Unrelated	12.168	0.975	0.025	0	1	1
YPN001 - YPN030	Unrelated	Unrelated	5.347	0.923	0.077	0	22	8
YPN001 - Th521	Unrelated	Unrelated	8.988	0.95	0.05	0	11	5
YPN001 - Th530	Unrelated	Unrelated	11.691	0.972	0.028	0	3	3
YPN002 - YPN006	Unrelated	Unrelated	11.015	0.959	0.041	0	9	3
YPN002 - YPN009	Unrelated	Unrelated	9.711	0.952	0.048	0	6	3
YPN002 - YPN012	Unrelated	Unrelated	10.234	0.955	0.045	0	7	4
YPN002 - YPN013	Unrelated	Unrelated	11.565	0.965	0.035	0	8	5
YPN002 - YPN017	Unrelated	Unrelated	8.735	0.945	0.055	0	10	3
YPN002 - YPN019	Unrelated	Unrelated	8.954	0.94	0.06	0	12	4
YPN002 - YPN025	Unrelated	Unrelated	10.329	0.949	0.051	0	12	5
YPN002 - YPN030	Unrelated	Unrelated	11.641	0.967	0.033	0	8	3
YPN002 - Th521	Unrelated	Unrelated	10.396	0.958	0.042	0	8	3
YPN002 - Th530	Unrelated	Unrelated	9.240	0.955	0.045	0	8	2
YPN003 - YPN006	Unrelated	Unrelated	11.783	0.971	0.029	0	6	2
YPN003 - YPN008	Unrelated	Unrelated	11.682	0.97	0.03	0	6	2
YPN003 - YPN012	Unrelated	Unrelated	11.576	0.962	0.038	0	9	4
YPN003 - YPN016	Unrelated	Unrelated	12.313	0.975	0.025	0	6	3
YPN003 - YPN018	Unrelated	Unrelated	10.327	0.961	0.039	0	5	4
YPN003 - YPN019	Unrelated	Unrelated	12.399	0.976	0.024	0	3	2
YPN003 - YPN022	Unrelated	Unrelated	11.823	0.978	0.022	0	3	1
YPN003 - Th530	Unrelated	Unrelated	10.039	0.956	0.044	0	9	5
YPN003 - Th703	Unrelated	Unrelated	7.748	0.947	0.053	0	0	1
YPN006 - YPN008	Unrelated	Unrelated	8.589	0.925	0.075	0	20	8
YPN006 - YPN010	Unrelated	Unrelated	11.025	0.96	0.04	0	9	5
YPN006 - YPN014	Unrelated	Unrelated	4.288	0.891	0.109	0	31	8
YPN006 - YPN016	Unrelated	Unrelated	12.330	0.984	0.016	0	1	1
YPN006 - YPN017	Unrelated	Unrelated	11.509	0.969	0.031	0	7	4
YPN006 - YPN018	Unrelated	Unrelated	7.266	0.913	0.087	0	22	6
YPN006 - YPN019	Unrelated	Unrelated	11.859	0.968	0.032	0	8	3
YPN006 - YPN021	Unrelated	Unrelated	12.657	0.976	0.024	0	6	4
YPN006 - YPN023	Unrelated	Unrelated	6.145	0.908	0.092	0	20	9
YPN006 - YPN025	Unrelated	Unrelated	11.521	0.963	0.037	0	7	3
YPN006 - YPN027	Unrelated	Unrelated	6.851	0.902	0.098	0	24	13
YPN006 - YPN030	Unrelated	Unrelated	6.132	0.909	0.091	0	24	10
YPN006 - YPN032	Unrelated	Unrelated	8.416	0.941	0.059	0	13	5
YPN006 - YPN033	Unrelated	Unrelated	11.241	0.969	0.031	0	7	2
YPN006 - Th519	Unrelated	Unrelated	7.915	0.941	0.059	0	3	1
YPN006 - Th703	Unrelated	Unrelated	4.501	0.929	0.071	0	11	3
YPN007 - YPN013	Unrelated	Unrelated	13.126	0.979	0.021	0	6	5
YPN007 - YPN014	Unrelated	Unrelated	12.432	0.977	0.023	0	2	1

YPN007 - YPN018	Unrelated	Unrelated	10.365	0.961	0.039	0	4	3
YPN007 - YPN027	Unrelated	Unrelated	11.268	0.967	0.033	0	6	5
YPN007 - Th521	Unrelated	Unrelated	12.127	0.97	0.03	0	5	2
YPN008 - YPN010	Unrelated	Unrelated	10.737	0.968	0.032	0	4	3
YPN008 - YPN011	Unrelated	Unrelated	12.217	0.982	0.018	0	2	1
YPN008 - YPN012	Unrelated	Unrelated	10.569	0.954	0.046	0	9	4
YPN008 - YPN013	Unrelated	Unrelated	11.667	0.968	0.032	0	8	4
YPN008 - YPN016	Unrelated	Unrelated	12.258	0.976	0.024	0	5	3
YPN008 - YPN017	Unrelated	Unrelated	11.497	0.965	0.035	0	6	3
YPN008 - YPN018	Unrelated	Unrelated	10.534	0.968	0.032	0	3	2
YPN008 - YPN020	Unrelated	Unrelated	11.725	0.969	0.031	0	5	5
YPN008 - YPN023	Unrelated	Unrelated	11.883	0.969	0.031	0	5	4
YPN008 - YPN025	Unrelated	Unrelated	7.051	0.927	0.073	0	15	8
YPN008 - YPN027	Unrelated	Unrelated	12.477	0.975	0.025	0	4	4
YPN008 - YPN030	Unrelated	Unrelated	11.298	0.957	0.043	0	11	6
YPN008 - YPN033	Unrelated	Unrelated	10.852	0.97	0.03	0	3	2
YPN008 - Th519	Unrelated	Unrelated	10.276	0.958	0.042	0	6	2
YPN008 - Th530	Unrelated	Unrelated	12.238	0.974	0.026	0	6	3
YPN008 - Th703	Unrelated	Unrelated	8.941	0.951	0.049	0	8	2
YPN009 - YPN013	Unrelated	Unrelated	9.554	0.936	0.064	0	14	5
YPN009 - YPN016	Unrelated	Unrelated	11.213	0.978	0.022	0	4	4
YPN009 - YPN017	Unrelated	Unrelated	12.227	0.976	0.024	0	4	2
YPN009 - YPN019	Unrelated	Unrelated	9.677	0.945	0.055	0	10	4
YPN009 - YPN020	Unrelated	Unrelated	12.257	0.972	0.028	0	7	3
YPN009 - YPN023	Unrelated	Unrelated	11.485	0.973	0.027	0	3	3
YPN009 - YPN027	Unrelated	Unrelated	11.478	0.967	0.033	0	6	4
YPN009 - YPN030	Unrelated	Unrelated	10.482	0.967	0.033	0	3	2
YPN009 - YPN031	Unrelated	Unrelated	11.399	0.964	0.036	0	6	3
YPN009 - YPN032	Unrelated	Unrelated	9.479	0.955	0.045	0	7	2
YPN009 - Th521	Unrelated	Unrelated	8.140	0.947	0.053	0	8	5
YPN009 - Th530	Unrelated	Unrelated	9.702	0.962	0.038	0	4	3
YPN010 - YPN012	Unrelated	Unrelated	12.009	0.973	0.027	0	5	3
YPN010 - YPN013	Unrelated	Unrelated	7.734	0.923	0.077	0	21	9
YPN010 - YPN016	Unrelated	Unrelated	8.354	0.939	0.061	0	12	4
YPN010 - YPN019	Unrelated	Unrelated	11.385	0.973	0.027	0	3	2
YPN010 - YPN020	Unrelated	Unrelated	6.009	0.902	0.098	0	28	9
YPN010 - YPN022	Unrelated	Unrelated	12.673	0.98	0.02	0	5	2
YPN010 - YPN023	Unrelated	Unrelated	11.773	0.971	0.029	0	8	6
YPN010 - YPN030	Unrelated	Unrelated	12.381	0.973	0.027	0	5	2
YPN010 - YPN032	Unrelated	Unrelated	11.388	0.975	0.025	0	5	4
YPN010 - Th519	Unrelated	Unrelated	9.379	0.953	0.047	0	11	4
YPN010 - Th521	Unrelated	Unrelated	10.992	0.961	0.039	0	9	5
YPN011 - YPN012	Unrelated	Unrelated	11.429	0.967	0.033	0	8	3
YPN011 - YPN013	Unrelated	Unrelated	12.368	0.979	0.021	0	4	2
YPN011 - YPN014	Unrelated	Unrelated	11.716	0.978	0.022	0	1	1
YPN011 - YPN016	Unrelated	Unrelated	7.709	0.938	0.062	0	12	8
YPN011 - YPN020	Unrelated	Unrelated	11.176	0.976	0.024	0	3	2
YPN011 - YPN022	Unrelated	Unrelated	12.575	0.981	0.019	0	2	1
YPN011 - YPN023	Unrelated	Unrelated	10.892	0.972	0.028	0	2	1
YPN011 - YPN027	Unrelated	Unrelated	11.247	0.961	0.039	0	7	5
YPN011 - YPN030	Unrelated	Unrelated	13.517	0.985	0.015	0	3	2
YPN011 - YPN031	Unrelated	Unrelated	11.245	0.978	0.022	0	4	2
YPN011 - YPN032	Unrelated	Unrelated	9.831	0.968	0.032	0	6	2

YPN011 - YPN033	Unrelated	Unrelated	10.750	0.971	0.029	0	5	1
YPN011 - Th530	Unrelated	Unrelated	11.671	0.97	0.03	0	6	5
YPN012 - YPN013	Unrelated	Unrelated	10.265	0.954	0.046	0	7	4
YPN012 - YPN016	Unrelated	Unrelated	12.162	0.975	0.025	0	6	2
YPN012 - YPN017	Unrelated	Unrelated	11.090	0.963	0.037	0	8	6
YPN012 - YPN018	Unrelated	Unrelated	10.993	0.972	0.028	0	4	2
YPN012 - YPN019	Unrelated	Unrelated	11.349	0.967	0.033	0	5	2
YPN012 - YPN020	Unrelated	Unrelated	11.908	0.972	0.028	0	5	2
YPN012 - YPN021	Unrelated	Unrelated	12.265	0.97	0.03	0	7	4
YPN012 - YPN023	Unrelated	Unrelated	9.291	0.941	0.059	0	12	6
YPN012 - YPN025	Unrelated	Unrelated	9.203	0.939	0.061	0	13	5
YPN012 - YPN030	Unrelated	Unrelated	8.033	0.923	0.077	0	16	6
YPN012 - YPN032	Unrelated	Unrelated	10.443	0.961	0.039	0	8	4
YPN012 - YPN036	Unrelated	Unrelated	8.851	0.948	0.052	0	10	3
YPN012 - Th521	Unrelated	Unrelated	4.394	0.895	0.105	0	27	10
YPN012 - Th530	Unrelated	Unrelated	11.449	0.97	0.03	0	5	3
YPN012 - Th703	Unrelated	Unrelated	3.144	0.911	0.089	0	13	3
YPN013 - YPN016	Unrelated	Unrelated	11.685	0.971	0.029	0	6	4
YPN013 - YPN017	Unrelated	Unrelated	10.948	0.964	0.036	0	9	4
YPN013 - YPN018	Unrelated	Unrelated	10.315	0.961	0.039	0	9	3
YPN013 - YPN022	Unrelated	Unrelated	12.642	0.984	0.016	0	5	4
YPN013 - YPN023	Unrelated	Unrelated	11.502	0.967	0.033	0	6	5
YPN013 - YPN025	Unrelated	Unrelated	12.519	0.984	0.016	0	1	1
YPN013 - YPN027	Unrelated	Unrelated	9.518	0.955	0.045	0	8	6
YPN013 - Th519	Unrelated	Unrelated	9.756	0.956	0.044	0	8	3
YPN013 - Th521	Unrelated	Unrelated	10.411	0.953	0.047	0	12	7
YPN013 - Th530	Unrelated	Unrelated	10.803	0.965	0.035	0	4	2
YPN014 - YPN017	Unrelated	Unrelated	10.236	0.962	0.038	0	3	2
YPN014 - YPN025	Unrelated	Unrelated	11.982	0.971	0.029	0	7	3
YPN014 - Th519	Unrelated	Unrelated	6.814	0.943	0.057	0	0	1
YPN014 - Th521	Unrelated	Unrelated	6.112	0.937	0.063	0	9	2
YPN016 - YPN018	Unrelated	Unrelated	12.133	0.975	0.025	0	5	1
YPN016 - YPN020	Unrelated	Unrelated	8.993	0.949	0.051	0	13	3
YPN016 - YPN022	Unrelated	Unrelated	9.206	0.947	0.053	0	8	5
YPN016 - YPN023	Unrelated	Unrelated	10.146	0.963	0.037	0	8	3
YPN016 - Th521	Unrelated	Unrelated	11.309	0.97	0.03	0	2	2
YPN016 - Th703	Unrelated	Unrelated	9.014	0.955	0.045	0	3	1
YPN017 - YPN019	Unrelated	Unrelated	11.653	0.968	0.032	0	5	4
YPN017 - YPN020	Unrelated	Unrelated	12.640	0.979	0.021	0	5	2
YPN017 - YPN023	Unrelated	Unrelated	11.534	0.969	0.031	0	6	2
YPN017 - YPN025	Unrelated	Unrelated	11.183	0.966	0.034	0	6	2
YPN017 - YPN027	Unrelated	Unrelated	11.590	0.97	0.03	0	4	3
YPN017 - YPN030	Unrelated	Unrelated	11.803	0.971	0.029	0	6	3
YPN017 - YPN032	Unrelated	Unrelated	9.918	0.953	0.047	0	10	2
YPN017 - YPN033	Unrelated	Unrelated	8.226	0.935	0.065	0	15	3
YPN017 - Th519	Unrelated	Unrelated	8.963	0.951	0.049	0	8	4
YPN017 - Th521	Unrelated	Unrelated	9.495	0.946	0.054	0	12	5
YPN017 - Th530	Unrelated	Unrelated	10.555	0.968	0.032	0	5	3
YPN017 - Th703	Unrelated	Unrelated	7.456	0.954	0.046	0	2	1
YPN018 - YPN030	Unrelated	Unrelated	11.152	0.967	0.033	0	9	4
YPN018 - Th521	Unrelated	Unrelated	9.979	0.965	0.035	0	4	2
YPN018 - Th531	Unrelated	Unrelated	4.831	0.944	0.056	0	0	1
YPN018 - Th703	Unrelated	Unrelated	5.559	0.949	0.051	0	0	1

YPN019 - YPN020	Unrelated	Unrelated	11.645	0.969	0.031	0	6	4
YPN019 - YPN023	Unrelated	Unrelated	10.560	0.963	0.037	0	9	6
YPN019 - YPN025	Unrelated	Unrelated	12.504	0.973	0.027	0	6	2
YPN019 - YPN027	Unrelated	Unrelated	9.651	0.956	0.044	0	6	4
YPN019 - YPN030	Unrelated	Unrelated	10.171	0.959	0.041	0	7	4
YPN019 - YPN031	Unrelated	Unrelated	7.329	0.931	0.069	0	19	6
YPN019 - YPN032	Unrelated	Unrelated	6.041	0.919	0.081	0	19	7
YPN019 - YPN033	Unrelated	Unrelated	6.501	0.917	0.083	0	20	6
YPN019 - YPN036	Unrelated	Unrelated	8.830	0.952	0.048	0	4	2
YPN019 - Th519	Unrelated	Unrelated	8.773	0.951	0.049	0	4	2
YPN019 - Th521	Unrelated	Unrelated	9.484	0.947	0.053	0	10	4
YPN020 - YPN023	Unrelated	Unrelated	11.204	0.957	0.043	0	9	6
YPN020 - YPN025	Unrelated	Unrelated	12.932	0.987	0.013	0	1	1
YPN020 - Th521	Unrelated	Unrelated	11.409	0.969	0.031	0	5	2
YPN021 - YPN028	Unrelated	Unrelated	10.557	0.963	0.037	0	6	2
YPN021 - Th519	Unrelated	Unrelated	9.977	0.959	0.041	0	6	3
YPN022 - YPN023	Unrelated	Unrelated	11.778	0.962	0.038	0	9	5
YPN022 - YPN025	Unrelated	Unrelated	12.749	0.984	0.016	0	1	1
YPN022 - Th519	Unrelated	Unrelated	9.929	0.956	0.044	0	3	1
YPN022 - Th521	Unrelated	Unrelated	11.121	0.973	0.027	0	3	2
YPN022 - Th703	Unrelated	Unrelated	7.227	0.944	0.056	0	4	2
YPN023 - YPN025	Unrelated	Unrelated	11.641	0.967	0.033	0	5	5
YPN023 - YPN027	Unrelated	Unrelated	9.700	0.943	0.057	0	14	6
YPN023 - YPN030	Unrelated	Unrelated	12.097	0.97	0.03	0	6	4
YPN023 - YPN031	Unrelated	Unrelated	11.760	0.97	0.03	0	8	4
YPN023 - YPN033	Unrelated	Unrelated	9.483	0.956	0.044	0	10	4
YPN023 - Th521	Unrelated	Unrelated	9.700	0.954	0.046	0	12	3
YPN023 - Th530	Unrelated	Unrelated	12.080	0.978	0.022	0	3	2
YPN025 - YPN027	Unrelated	Unrelated	11.307	0.959	0.041	0	9	5
YPN025 - YPN030	Unrelated	Unrelated	11.755	0.973	0.027	0	8	4
YPN025 - YPN032	Unrelated	Unrelated	9.783	0.953	0.047	0	11	4
YPN025 - YPN033	Unrelated	Unrelated	11.303	0.963	0.037	0	10	4
YPN025 - Th519	Unrelated	Unrelated	8.370	0.955	0.045	0	4	1
YPN025 - Th521	Unrelated	Unrelated	9.830	0.96	0.04	0	10	5
YPN025 - Th530	Unrelated	Unrelated	11.207	0.958	0.042	0	10	5
YPN025 - Th703	Unrelated	Unrelated	6.273	0.939	0.061	0	10	4
YPN027 - YPN028	Unrelated	Unrelated	11.108	0.962	0.038	0	7	4
YPN027 - YPN031	Unrelated	Unrelated	11.248	0.975	0.025	0	3	1
YPN027 - YPN032	Unrelated	Unrelated	9.891	0.95	0.05	0	10	3
YPN027 - YPN033	Unrelated	Unrelated	9.711	0.944	0.056	0	13	4
YPN027 - Th519	Unrelated	Unrelated	9.812	0.959	0.041	0	4	2
YPN027 - Th521	Unrelated	Unrelated	9.479	0.95	0.05	0	11	5
YPN027 - Th530	Unrelated	Unrelated	11.609	0.964	0.036	0	8	4
YPN028 - Th703	Unrelated	Unrelated	6.184	0.949	0.051	0	3	2
YPN030 - YPN031	Unrelated	Unrelated	11.379	0.968	0.032	0	9	4
YPN030 - YPN032	Unrelated	Unrelated	10.830	0.957	0.043	0	6	2
YPN030 - YPN033	Unrelated	Unrelated	10.568	0.956	0.044	0	9	3
YPN030 - Th519	Unrelated	Unrelated	11.625	0.973	0.027	0	5	2
YPN030 - Th521	Unrelated	Unrelated	8.926	0.95	0.05	0	10	5
YPN030 - Th703	Unrelated	Unrelated	9.829	0.96	0.04	0	7	2
YPN031 - YPN032	Unrelated	Unrelated	11.736	0.974	0.026	0	4	2
YPN031 - Th519	Unrelated	Unrelated	10.025	0.961	0.039	0	6	3
YPN031 - Th521	Unrelated	Unrelated	10.832	0.964	0.036	0	8	2

YPN032 - Th519	Unrelated	Unrelated	7.223	0.945	0.055	0	7	2
YPN032 - Th521	Unrelated	Unrelated	10.630	0.967	0.033	0	7	3
YPN032 - Th703	Unrelated	Unrelated	5.458	0.946	0.054	0	0	1
YPN033 - YPN036	Unrelated	Unrelated	8.844	0.954	0.046	0	5	1
YPN036 - Th530	Unrelated	Unrelated	5.420	0.936	0.064	0	0	1
YPN036 - Th703	Unrelated	Unrelated	1.968	0.933	0.067	0	0	1
Th519 - Th530	Unrelated	Unrelated	7.118	0.948	0.052	0	1	1
Th519 - Th531	Unrelated	Unrelated	2.837	0.934	0.066	0	0	1
Th521 - Th530	Unrelated	Unrelated	10.530	0.966	0.034	0	4	3
Th521 - Th531	Unrelated	Unrelated	7.308	0.949	0.051	0	4	3
Th530 - Th703	Unrelated	Unrelated	4.026	0.93	0.07	0	3	1
BRB003 - LHP002	Unrelated	Unrelated	12.147	1	0	0	0	1
BRB003 - YPN002	Unrelated	Unrelated	7.714	1	0	0	0	1
BRB003 - YPN003	Unrelated	Unrelated	13.972	1	0	0	0	1
BRB003 - YPN007	Unrelated	Unrelated	14.496	1	0	0	0	1
BRB003 - YPN008	Unrelated	Unrelated	9.422	1	0	0	0	1
BRB003 - YPN009	Unrelated	Unrelated	12.006	1	0	0	0	1
BRB003 - YPN010	Unrelated	Unrelated	14.855	1	0	0	0	1
BRB003 - YPN011	Unrelated	Unrelated	14.730	1	0	0	0	1
BRB003 - YPN013	Unrelated	Unrelated	10.371	1	0	0	0	1
BRB003 - YPN016	Unrelated	Unrelated	11.439	1	0	0	0	1
BRB003 - YPN018	Unrelated	Unrelated	9.797	1	0	0	0	1
BRB003 - YPN019	Unrelated	Unrelated	14.776	1	0	0	0	1
BRB003 - YPN020	Unrelated	Unrelated	14.786	1	0	0	0	1
BRB003 - YPN021	Unrelated	Unrelated	11.245	1	0	0	0	1
BRB003 - YPN022	Unrelated	Unrelated	15.954	1	0	0	0	1
BRB003 - YPN023	Unrelated	Unrelated	14.647	1	0	0	0	1
BRB003 - YPN028	Unrelated	Unrelated	11.984	1	0	0	0	1
BRB003 - YPN031	Unrelated	Unrelated	9.755	1	0	0	0	1
BRB003 - YPN033	Unrelated	Unrelated	7.618	1	0	0	0	1
BRB003 - YPN036	Unrelated	Unrelated	5.507	1	0	0	0	1
BRB003 - Th530	Unrelated	Unrelated	8.008	1	0	0	0	1
LHP002 - THL002	Unrelated	Unrelated	17.154	1	0	0	0	1
LHP002 - THL003	Unrelated	Unrelated	13.949	1	0	0	0	1
LHP002 - THL004	Unrelated	Unrelated	13.041	1	0	0	0	1
LHP002 - YPN001	Unrelated	Unrelated	16.962	1	0	0	0	1
LHP002 - YPN003	Unrelated	Unrelated	16.383	1	0	0	0	1
LHP002 - YPN006	Unrelated	Unrelated	15.311	1	0	0	0	1
LHP002 - YPN007	Unrelated	Unrelated	15.770	1	0	0	0	1
LHP002 - YPN010	Unrelated	Unrelated	14.776	1	0	0	0	1
LHP002 - YPN011	Unrelated	Unrelated	14.610	1	0	0	0	1
LHP002 - YPN014	Unrelated	Unrelated	12.941	1	0	0	0	1
LHP002 - YPN018	Unrelated	Unrelated	12.678	1	0	0	0	1
LHP002 - YPN021	Unrelated	Unrelated	14.892	1	0	0	0	1
LHP002 - YPN022	Unrelated	Unrelated	15.640	1	0	0	0	1
LHP002 - YPN025	Unrelated	Unrelated	13.684	1	0	0	0	1
LHP002 - YPN028	Unrelated	Unrelated	18.993	1	0	0	0	1
LHP002 - YPN030	Unrelated	Unrelated	17.723	1	0	0	0	1
LHP002 - YPN033	Unrelated	Unrelated	12.280	1	0	0	0	1
LHP002 - YPN036	Unrelated	Unrelated	14.837	1	0	0	0	1
LHP002 - Th530	Unrelated	Unrelated	16.629	1	0	0	0	1
LHP002 - Th531	Unrelated	Unrelated	9.431	1	0	0	0	1
THL002 - THL004	Unrelated	Unrelated	15.327	1	0	0	0	1

THL002 - YPN002	Unrelated	Unrelated	17.074	1	0	0	0	1
THL002 - YPN003	Unrelated	Unrelated	18.183	1	0	0	0	1
THL002 - YPN007	Unrelated	Unrelated	18.783	1	0	0	0	1
THL002 - YPN009	Unrelated	Unrelated	16.507	1	0	0	0	1
THL002 - YPN013	Unrelated	Unrelated	16.096	1	0	0	0	1
THL002 - YPN016	Unrelated	Unrelated	13.089	1	0	0	0	1
THL002 - YPN018	Unrelated	Unrelated	14.638	1	0	0	0	1
THL002 - YPN020	Unrelated	Unrelated	16.631	1	0	0	0	1
THL002 - YPN023	Unrelated	Unrelated	14.535	1	0	0	0	1
THL002 - YPN025	Unrelated	Unrelated	16.728	1	0	0	0	1
THL002 - YPN028	Unrelated	Unrelated	13.328	1	0	0	0	1
THL002 - YPN031	Unrelated	Unrelated	20.207	1	0	0	0	1
THL002 - YPN032	Unrelated	Unrelated	17.056	1	0	0	0	1
THL002 - YPN033	Unrelated	Unrelated	17.095	1	0	0	0	1
THL002 - YPN036	Unrelated	Unrelated	13.788	1	0	0	0	1
THL002 - Th530	Unrelated	Unrelated	19.139	1	0	0	0	1
THL002 - Th531	Unrelated	Unrelated	15.051	1	0	0	0	1
THL003 - THL004	Unrelated	Unrelated	6.640	1	0	0	0	1
THL003 - YPN002	Unrelated	Unrelated	7.134	1	0	0	0	1
THL003 - YPN003	Unrelated	Unrelated	11.363	1	0	0	0	1
THL003 - YPN006	Unrelated	Unrelated	12.077	1	0	0	0	1
THL003 - YPN007	Unrelated	Unrelated	13.509	1	0	0	0	1
THL003 - YPN008	Unrelated	Unrelated	13.803	1	0	0	0	1
THL003 - YPN009	Unrelated	Unrelated	14.906	1	0	0	0	1
THL003 - YPN011	Unrelated	Unrelated	13.098	1	0	0	0	1
THL003 - YPN013	Unrelated	Unrelated	14.999	1	0	0	0	1
THL003 - YPN016	Unrelated	Unrelated	13.245	1	0	0	0	1
THL003 - YPN018	Unrelated	Unrelated	6.381	1	0	0	0	1
THL003 - YPN019	Unrelated	Unrelated	15.288	1	0	0	0	1
THL003 - YPN020	Unrelated	Unrelated	14.581	1	0	0	0	1
THL003 - YPN022	Unrelated	Unrelated	12.273	1	0	0	0	1
THL003 - YPN023	Unrelated	Unrelated	11.015	1	0	0	0	1
THL003 - YPN025	Unrelated	Unrelated	9.902	1	0	0	0	1
THL003 - YPN028	Unrelated	Unrelated	9.308	1	0	0	0	1
THL003 - YPN032	Unrelated	Unrelated	8.854	1	0	0	0	1
THL003 - YPN033	Unrelated	Unrelated	11.468	1	0	0	0	1
THL003 - YPN036	Unrelated	Unrelated	5.670	1	0	0	0	1
THL003 - Th521	Unrelated	Unrelated	10.588	1	0	0	0	1
THL003 - Th530	Unrelated	Unrelated	8.330	1	0	0	0	1
THL003 - Th531	Unrelated	Unrelated	5.185	1	0	0	0	1
THL004 - YPN007	Unrelated	Unrelated	14.905	1	0	0	0	1
THL004 - YPN008	Unrelated	Unrelated	11.667	1	0	0	0	1
THL004 - YPN013	Unrelated	Unrelated	12.987	1	0	0	0	1
THL004 - YPN016	Unrelated	Unrelated	15.005	1	0	0	0	1
THL004 - YPN017	Unrelated	Unrelated	13.910	1	0	0	0	1
THL004 - YPN018	Unrelated	Unrelated	15.416	1	0	0	0	1
THL004 - YPN020	Unrelated	Unrelated	16.874	1	0	0	0	1
THL004 - YPN021	Unrelated	Unrelated	17.463	1	0	0	0	1
THL004 - YPN022	Unrelated	Unrelated	13.945	1	0	0	0	1
THL004 - YPN025	Unrelated	Unrelated	13.254	1	0	0	0	1
THL004 - YPN028	Unrelated	Unrelated	10.985	1	0	0	0	1
THL004 - YPN036	Unrelated	Unrelated	9.541	1	0	0	0	1
THL004 - Th531	Unrelated	Unrelated	6.732	1	0	0	0	1

YPN001 - YPN002	Unrelated	Unrelated	12.493	1	0	0	0	1
YPN001 - YPN008	Unrelated	Unrelated	17.637	1	0	0	0	1
YPN001 - YPN010	Unrelated	Unrelated	18.660	1	0	0	0	1
YPN001 - YPN020	Unrelated	Unrelated	19.122	1	0	0	0	1
YPN001 - YPN021	Unrelated	Unrelated	18.646	1	0	0	0	1
YPN001 - YPN022	Unrelated	Unrelated	19.296	1	0	0	0	1
YPN001 - YPN025	Unrelated	Unrelated	17.511	1	0	0	0	1
YPN001 - YPN028	Unrelated	Unrelated	19.034	1	0	0	0	1
YPN001 - YPN031	Unrelated	Unrelated	17.967	1	0	0	0	1
YPN001 - YPN032	Unrelated	Unrelated	17.838	1	0	0	0	1
YPN001 - YPN033	Unrelated	Unrelated	15.967	1	0	0	0	1
YPN001 - YPN036	Unrelated	Unrelated	10.725	1	0	0	0	1
YPN001 - Th519	Unrelated	Unrelated	14.196	1	0	0	0	1
YPN001 - Th531	Unrelated	Unrelated	13.510	1	0	0	0	1
YPN001 - Th703	Unrelated	Unrelated	12.563	1	0	0	0	1
YPN002 - YPN003	Unrelated	Unrelated	13.373	1	0	0	0	1
YPN002 - YPN007	Unrelated	Unrelated	17.525	1	0	0	0	1
YPN002 - YPN008	Unrelated	Unrelated	15.788	1	0	0	0	1
YPN002 - YPN010	Unrelated	Unrelated	15.418	1	0	0	0	1
YPN002 - YPN011	Unrelated	Unrelated	17.484	1	0	0	0	1
YPN002 - YPN014	Unrelated	Unrelated	14.061	1	0	0	0	1
YPN002 - YPN016	Unrelated	Unrelated	18.079	1	0	0	0	1
YPN002 - YPN018	Unrelated	Unrelated	14.127	1	0	0	0	1
YPN002 - YPN020	Unrelated	Unrelated	18.700	1	0	0	0	1
YPN002 - YPN021	Unrelated	Unrelated	17.190	1	0	0	0	1
YPN002 - YPN022	Unrelated	Unrelated	18.888	1	0	0	0	1
YPN002 - YPN023	Unrelated	Unrelated	16.930	1	0	0	0	1
YPN002 - YPN027	Unrelated	Unrelated	12.252	1	0	0	0	1
YPN002 - YPN028	Unrelated	Unrelated	15.381	1	0	0	0	1
YPN002 - YPN031	Unrelated	Unrelated	17.242	1	0	0	0	1
YPN002 - YPN036	Unrelated	Unrelated	10.495	1	0	0	0	1
YPN002 - Th519	Unrelated	Unrelated	9.161	1	0	0	0	1
YPN002 - Th531	Unrelated	Unrelated	10.110	1	0	0	0	1
YPN002 - Th703	Unrelated	Unrelated	7.897	1	0	0	0	1
YPN003 - YPN009	Unrelated	Unrelated	13.015	1	0	0	0	1
YPN003 - YPN010	Unrelated	Unrelated	19.238	1	0	0	0	1
YPN003 - YPN011	Unrelated	Unrelated	17.465	1	0	0	0	1
YPN003 - YPN013	Unrelated	Unrelated	14.497	1	0	0	0	1
YPN003 - YPN014	Unrelated	Unrelated	15.258	1	0	0	0	1
YPN003 - YPN017	Unrelated	Unrelated	16.053	1	0	0	0	1
YPN003 - YPN020	Unrelated	Unrelated	16.045	1	0	0	0	1
YPN003 - YPN021	Unrelated	Unrelated	15.064	1	0	0	0	1
YPN003 - YPN023	Unrelated	Unrelated	15.511	1	0	0	0	1
YPN003 - YPN025	Unrelated	Unrelated	17.440	1	0	0	0	1
YPN003 - YPN027	Unrelated	Unrelated	13.239	1	0	0	0	1
YPN003 - YPN028	Unrelated	Unrelated	18.997	1	0	0	0	1
YPN003 - YPN030	Unrelated	Unrelated	17.000	1	0	0	0	1
YPN003 - YPN031	Unrelated	Unrelated	14.534	1	0	0	0	1
YPN003 - YPN032	Unrelated	Unrelated	18.103	1	0	0	0	1
YPN003 - YPN033	Unrelated	Unrelated	17.649	1	0	0	0	1
YPN003 - YPN036	Unrelated	Unrelated	12.824	1	0	0	0	1
YPN003 - Th519	Unrelated	Unrelated	13.067	1	0	0	0	1
YPN003 - Th521	Unrelated	Unrelated	14.695	1	0	0	0	1

YPN003 - Th531	Unrelated	Unrelated	8.784	1	0	0	0	1
YPN006 - YPN007	Unrelated	Unrelated	14.006	1	0	0	0	1
YPN006 - YPN009	Unrelated	Unrelated	15.901	1	0	0	0	1
YPN006 - YPN011	Unrelated	Unrelated	13.596	1	0	0	0	1
YPN006 - YPN013	Unrelated	Unrelated	15.037	1	0	0	0	1
YPN006 - YPN020	Unrelated	Unrelated	14.342	1	0	0	0	1
YPN006 - YPN022	Unrelated	Unrelated	14.461	1	0	0	0	1
YPN006 - YPN028	Unrelated	Unrelated	18.530	1	0	0	0	1
YPN006 - YPN031	Unrelated	Unrelated	14.343	1	0	0	0	1
YPN006 - YPN036	Unrelated	Unrelated	10.570	1	0	0	0	1
YPN006 - Th530	Unrelated	Unrelated	16.547	1	0	0	0	1
YPN006 - Th531	Unrelated	Unrelated	11.264	1	0	0	0	1
YPN007 - YPN008	Unrelated	Unrelated	13.137	1	0	0	0	1
YPN007 - YPN009	Unrelated	Unrelated	16.817	1	0	0	0	1
YPN007 - YPN010	Unrelated	Unrelated	19.351	1	0	0	0	1
YPN007 - YPN011	Unrelated	Unrelated	15.833	1	0	0	0	1
YPN007 - YPN012	Unrelated	Unrelated	16.567	1	0	0	0	1
YPN007 - YPN016	Unrelated	Unrelated	16.210	1	0	0	0	1
YPN007 - YPN017	Unrelated	Unrelated	16.945	1	0	0	0	1
YPN007 - YPN019	Unrelated	Unrelated	17.642	1	0	0	0	1
YPN007 - YPN020	Unrelated	Unrelated	16.537	1	0	0	0	1
YPN007 - YPN021	Unrelated	Unrelated	18.874	1	0	0	0	1
YPN007 - YPN022	Unrelated	Unrelated	15.627	1	0	0	0	1
YPN007 - YPN023	Unrelated	Unrelated	14.072	1	0	0	0	1
YPN007 - YPN025	Unrelated	Unrelated	18.747	1	0	0	0	1
YPN007 - YPN028	Unrelated	Unrelated	18.683	1	0	0	0	1
YPN007 - YPN030	Unrelated	Unrelated	15.117	1	0	0	0	1
YPN007 - YPN031	Unrelated	Unrelated	18.714	1	0	0	0	1
YPN007 - YPN032	Unrelated	Unrelated	18.925	1	0	0	0	1
YPN007 - YPN033	Unrelated	Unrelated	16.216	1	0	0	0	1
YPN007 - YPN036	Unrelated	Unrelated	13.327	1	0	0	0	1
YPN007 - Th519	Unrelated	Unrelated	15.048	1	0	0	0	1
YPN007 - Th530	Unrelated	Unrelated	11.655	1	0	0	0	1
YPN007 - Th531	Unrelated	Unrelated	8.546	1	0	0	0	1
YPN007 - Th703	Unrelated	Unrelated	11.153	1	0	0	0	1
YPN008 - YPN009	Unrelated	Unrelated	14.231	1	0	0	0	1
YPN008 - YPN014	Unrelated	Unrelated	15.052	1	0	0	0	1
YPN008 - YPN019	Unrelated	Unrelated	13.810	1	0	0	0	1
YPN008 - YPN021	Unrelated	Unrelated	16.282	1	0	0	0	1
YPN008 - YPN022	Unrelated	Unrelated	15.707	1	0	0	0	1
YPN008 - YPN028	Unrelated	Unrelated	14.667	1	0	0	0	1
YPN008 - YPN031	Unrelated	Unrelated	19.486	1	0	0	0	1
YPN008 - YPN032	Unrelated	Unrelated	11.332	1	0	0	0	1
YPN008 - YPN036	Unrelated	Unrelated	16.961	1	0	0	0	1
YPN008 - Th521	Unrelated	Unrelated	16.461	1	0	0	0	1
YPN008 - Th531	Unrelated	Unrelated	7.715	1	0	0	0	1
YPN009 - YPN010	Unrelated	Unrelated	14.251	1	0	0	0	1
YPN009 - YPN011	Unrelated	Unrelated	8.973	1	0	0	0	1
YPN009 - YPN012	Unrelated	Unrelated	13.956	1	0	0	0	1
YPN009 - YPN014	Unrelated	Unrelated	16.988	1	0	0	0	1
YPN009 - YPN018	Unrelated	Unrelated	16.043	1	0	0	0	1
YPN009 - YPN021	Unrelated	Unrelated	19.404	1	0	0	0	1
YPN009 - YPN022	Unrelated	Unrelated	15.708	1	0	0	0	1

YPN009 - YPN025	Unrelated	Unrelated	15.743	1	0	0	0	1
YPN009 - YPN028	Unrelated	Unrelated	15.375	1	0	0	0	1
YPN009 - YPN033	Unrelated	Unrelated	13.799	1	0	0	0	1
YPN009 - YPN036	Unrelated	Unrelated	13.731	1	0	0	0	1
YPN009 - Th519	Unrelated	Unrelated	11.798	1	0	0	0	1
YPN009 - Th531	Unrelated	Unrelated	7.779	1	0	0	0	1
YPN009 - Th703	Unrelated	Unrelated	8.228	1	0	0	0	1
YPN010 - YPN014	Unrelated	Unrelated	13.744	1	0	0	0	1
YPN010 - YPN017	Unrelated	Unrelated	15.429	1	0	0	0	1
YPN010 - YPN018	Unrelated	Unrelated	15.222	1	0	0	0	1
YPN010 - YPN021	Unrelated	Unrelated	18.563	1	0	0	0	1
YPN010 - YPN025	Unrelated	Unrelated	16.949	1	0	0	0	1
YPN010 - YPN027	Unrelated	Unrelated	14.046	1	0	0	0	1
YPN010 - YPN028	Unrelated	Unrelated	15.466	1	0	0	0	1
YPN010 - YPN031	Unrelated	Unrelated	15.343	1	0	0	0	1
YPN010 - YPN033	Unrelated	Unrelated	16.758	1	0	0	0	1
YPN010 - YPN036	Unrelated	Unrelated	10.868	1	0	0	0	1
YPN010 - Th530	Unrelated	Unrelated	17.314	1	0	0	0	1
YPN010 - Th531	Unrelated	Unrelated	14.591	1	0	0	0	1
YPN010 - Th703	Unrelated	Unrelated	12.984	1	0	0	0	1
YPN011 - YPN017	Unrelated	Unrelated	16.968	1	0	0	0	1
YPN011 - YPN018	Unrelated	Unrelated	15.530	1	0	0	0	1
YPN011 - YPN019	Unrelated	Unrelated	15.255	1	0	0	0	1
YPN011 - YPN021	Unrelated	Unrelated	19.571	1	0	0	0	1
YPN011 - YPN025	Unrelated	Unrelated	16.691	1	0	0	0	1
YPN011 - YPN028	Unrelated	Unrelated	17.364	1	0	0	0	1
YPN011 - YPN036	Unrelated	Unrelated	12.887	1	0	0	0	1
YPN011 - Th519	Unrelated	Unrelated	14.037	1	0	0	0	1
YPN011 - Th521	Unrelated	Unrelated	13.238	1	0	0	0	1
YPN011 - Th531	Unrelated	Unrelated	15.693	1	0	0	0	1
YPN011 - Th703	Unrelated	Unrelated	9.652	1	0	0	0	1
YPN012 - YPN022	Unrelated	Unrelated	15.149	1	0	0	0	1
YPN012 - YPN028	Unrelated	Unrelated	17.108	1	0	0	0	1
YPN012 - YPN031	Unrelated	Unrelated	12.011	1	0	0	0	1
YPN012 - YPN033	Unrelated	Unrelated	12.979	1	0	0	0	1
YPN012 - Th531	Unrelated	Unrelated	8.300	1	0	0	0	1
YPN013 - YPN014	Unrelated	Unrelated	16.965	1	0	0	0	1
YPN013 - YPN020	Unrelated	Unrelated	13.677	1	0	0	0	1
YPN013 - YPN021	Unrelated	Unrelated	15.631	1	0	0	0	1
YPN013 - YPN028	Unrelated	Unrelated	18.015	1	0	0	0	1
YPN013 - YPN030	Unrelated	Unrelated	17.100	1	0	0	0	1
YPN013 - YPN031	Unrelated	Unrelated	18.731	1	0	0	0	1
YPN013 - YPN032	Unrelated	Unrelated	13.333	1	0	0	0	1
YPN013 - YPN033	Unrelated	Unrelated	14.957	1	0	0	0	1
YPN013 - YPN036	Unrelated	Unrelated	15.847	1	0	0	0	1
YPN013 - Th531	Unrelated	Unrelated	15.024	1	0	0	0	1
YPN013 - Th703	Unrelated	Unrelated	9.860	1	0	0	0	1
YPN014 - YPN016	Unrelated	Unrelated	16.370	1	0	0	0	1
YPN014 - YPN018	Unrelated	Unrelated	12.925	1	0	0	0	1
YPN014 - YPN019	Unrelated	Unrelated	14.883	1	0	0	0	1
YPN014 - YPN020	Unrelated	Unrelated	16.727	1	0	0	0	1
YPN014 - YPN022	Unrelated	Unrelated	18.290	1	0	0	0	1
YPN014 - YPN023	Unrelated	Unrelated	10.928	1	0	0	0	1

YPN014 - YPN028	Unrelated	Unrelated	11.217	1	0	0	0	1
YPN014 - YPN031	Unrelated	Unrelated	14.539	1	0	0	0	1
YPN014 - YPN032	Unrelated	Unrelated	15.424	1	0	0	0	1
YPN014 - YPN033	Unrelated	Unrelated	14.987	1	0	0	0	1
YPN014 - YPN036	Unrelated	Unrelated	12.594	1	0	0	0	1
YPN014 - Th530	Unrelated	Unrelated	10.238	1	0	0	0	1
YPN014 - Th531	Unrelated	Unrelated	5.755	1	0	0	0	1
YPN014 - Th703	Unrelated	Unrelated	6.358	1	0	0	0	1
YPN016 - YPN017	Unrelated	Unrelated	16.413	1	0	0	0	1
YPN016 - YPN019	Unrelated	Unrelated	17.142	1	0	0	0	1
YPN016 - YPN021	Unrelated	Unrelated	18.883	1	0	0	0	1
YPN016 - YPN025	Unrelated	Unrelated	17.522	1	0	0	0	1
YPN016 - YPN027	Unrelated	Unrelated	17.445	1	0	0	0	1
YPN016 - YPN028	Unrelated	Unrelated	15.741	1	0	0	0	1
YPN016 - YPN030	Unrelated	Unrelated	19.201	1	0	0	0	1
YPN016 - YPN031	Unrelated	Unrelated	15.515	1	0	0	0	1
YPN016 - YPN032	Unrelated	Unrelated	18.738	1	0	0	0	1
YPN016 - YPN033	Unrelated	Unrelated	14.447	1	0	0	0	1
YPN016 - YPN036	Unrelated	Unrelated	14.613	1	0	0	0	1
YPN016 - Th519	Unrelated	Unrelated	11.022	1	0	0	0	1
YPN016 - Th530	Unrelated	Unrelated	12.699	1	0	0	0	1
YPN016 - Th531	Unrelated	Unrelated	15.645	1	0	0	0	1
YPN017 - YPN018	Unrelated	Unrelated	13.991	1	0	0	0	1
YPN017 - YPN021	Unrelated	Unrelated	16.895	1	0	0	0	1
YPN017 - YPN022	Unrelated	Unrelated	15.231	1	0	0	0	1
YPN017 - YPN028	Unrelated	Unrelated	17.883	1	0	0	0	1
YPN017 - YPN031	Unrelated	Unrelated	15.239	1	0	0	0	1
YPN017 - YPN036	Unrelated	Unrelated	13.475	1	0	0	0	1
YPN017 - Th531	Unrelated	Unrelated	13.496	1	0	0	0	1
YPN018 - YPN019	Unrelated	Unrelated	16.358	1	0	0	0	1
YPN018 - YPN020	Unrelated	Unrelated	17.378	1	0	0	0	1
YPN018 - YPN021	Unrelated	Unrelated	17.515	1	0	0	0	1
YPN018 - YPN022	Unrelated	Unrelated	15.223	1	0	0	0	1
YPN018 - YPN023	Unrelated	Unrelated	11.648	1	0	0	0	1
YPN018 - YPN025	Unrelated	Unrelated	15.591	1	0	0	0	1
YPN018 - YPN027	Unrelated	Unrelated	12.182	1	0	0	0	1
YPN018 - YPN028	Unrelated	Unrelated	15.921	1	0	0	0	1
YPN018 - YPN031	Unrelated	Unrelated	17.200	1	0	0	0	1
YPN018 - YPN032	Unrelated	Unrelated	17.399	1	0	0	0	1
YPN018 - YPN033	Unrelated	Unrelated	15.899	1	0	0	0	1
YPN018 - YPN036	Unrelated	Unrelated	11.714	1	0	0	0	1
YPN018 - Th519	Unrelated	Unrelated	8.727	1	0	0	0	1
YPN018 - Th530	Unrelated	Unrelated	12.753	1	0	0	0	1
YPN019 - YPN021	Unrelated	Unrelated	16.446	1	0	0	0	1
YPN019 - YPN022	Unrelated	Unrelated	15.628	1	0	0	0	1
YPN019 - YPN028	Unrelated	Unrelated	16.623	1	0	0	0	1
YPN019 - Th530	Unrelated	Unrelated	14.589	1	0	0	0	1
YPN019 - Th531	Unrelated	Unrelated	13.009	1	0	0	0	1
YPN019 - Th703	Unrelated	Unrelated	8.988	1	0	0	0	1
YPN020 - YPN021	Unrelated	Unrelated	16.533	1	0	0	0	1
YPN020 - YPN022	Unrelated	Unrelated	16.484	1	0	0	0	1
YPN020 - YPN027	Unrelated	Unrelated	14.043	1	0	0	0	1
YPN020 - YPN028	Unrelated	Unrelated	18.665	1	0	0	0	1

YPN020 - YPN030	Unrelated	Unrelated	20.290	1	0	0	0	1
YPN020 - YPN031	Unrelated	Unrelated	19.735	1	0	0	0	1
YPN020 - YPN032	Unrelated	Unrelated	17.044	1	0	0	0	1
YPN020 - YPN033	Unrelated	Unrelated	15.332	1	0	0	0	1
YPN020 - YPN036	Unrelated	Unrelated	15.181	1	0	0	0	1
YPN020 - Th519	Unrelated	Unrelated	14.373	1	0	0	0	1
YPN020 - Th530	Unrelated	Unrelated	18.643	1	0	0	0	1
YPN020 - Th531	Unrelated	Unrelated	15.458	1	0	0	0	1
YPN020 - Th703	Unrelated	Unrelated	10.138	1	0	0	0	1
YPN021 - YPN022	Unrelated	Unrelated	15.341	1	0	0	0	1
YPN021 - YPN023	Unrelated	Unrelated	16.327	1	0	0	0	1
YPN021 - YPN025	Unrelated	Unrelated	14.824	1	0	0	0	1
YPN021 - YPN031	Unrelated	Unrelated	13.243	1	0	0	0	1
YPN021 - YPN032	Unrelated	Unrelated	18.468	1	0	0	0	1
YPN021 - YPN033	Unrelated	Unrelated	16.536	1	0	0	0	1
YPN021 - YPN036	Unrelated	Unrelated	17.191	1	0	0	0	1
YPN021 - Th521	Unrelated	Unrelated	15.265	1	0	0	0	1
YPN021 - Th530	Unrelated	Unrelated	14.573	1	0	0	0	1
YPN021 - Th531	Unrelated	Unrelated	14.909	1	0	0	0	1
YPN021 - Th703	Unrelated	Unrelated	7.748	1	0	0	0	1
YPN022 - YPN027	Unrelated	Unrelated	12.955	1	0	0	0	1
YPN022 - YPN028	Unrelated	Unrelated	17.076	1	0	0	0	1
YPN022 - YPN030	Unrelated	Unrelated	19.264	1	0	0	0	1
YPN022 - YPN031	Unrelated	Unrelated	19.230	1	0	0	0	1
YPN022 - YPN032	Unrelated	Unrelated	12.227	1	0	0	0	1
YPN022 - YPN033	Unrelated	Unrelated	12.753	1	0	0	0	1
YPN022 - YPN036	Unrelated	Unrelated	18.302	1	0	0	0	1
YPN022 - Th530	Unrelated	Unrelated	13.315	1	0	0	0	1
YPN022 - Th531	Unrelated	Unrelated	10.148	1	0	0	0	1
YPN023 - YPN028	Unrelated	Unrelated	17.151	1	0	0	0	1
YPN023 - YPN032	Unrelated	Unrelated	14.251	1	0	0	0	1
YPN023 - YPN036	Unrelated	Unrelated	15.267	1	0	0	0	1
YPN023 - Th519	Unrelated	Unrelated	11.277	1	0	0	0	1
YPN023 - Th531	Unrelated	Unrelated	13.035	1	0	0	0	1
YPN023 - Th703	Unrelated	Unrelated	11.282	1	0	0	0	1
YPN025 - YPN028	Unrelated	Unrelated	18.015	1	0	0	0	1
YPN025 - YPN031	Unrelated	Unrelated	19.644	1	0	0	0	1
YPN025 - YPN036	Unrelated	Unrelated	15.075	1	0	0	0	1
YPN025 - Th531	Unrelated	Unrelated	16.505	1	0	0	0	1
YPN027 - YPN036	Unrelated	Unrelated	12.629	1	0	0	0	1
YPN027 - Th531	Unrelated	Unrelated	12.106	1	0	0	0	1
YPN027 - Th703	Unrelated	Unrelated	11.720	1	0	0	0	1
YPN028 - YPN030	Unrelated	Unrelated	17.036	1	0	0	0	1
YPN028 - YPN031	Unrelated	Unrelated	18.436	1	0	0	0	1
YPN028 - YPN032	Unrelated	Unrelated	16.660	1	0	0	0	1
YPN028 - YPN033	Unrelated	Unrelated	15.252	1	0	0	0	1
YPN028 - YPN036	Unrelated	Unrelated	12.747	1	0	0	0	1
YPN028 - Th519	Unrelated	Unrelated	10.715	1	0	0	0	1
YPN028 - Th521	Unrelated	Unrelated	17.017	1	0	0	0	1
YPN028 - Th530	Unrelated	Unrelated	13.228	1	0	0	0	1
YPN028 - Th531	Unrelated	Unrelated	8.588	1	0	0	0	1
YPN030 - YPN036	Unrelated	Unrelated	14.474	1	0	0	0	1
YPN030 - Th530	Unrelated	Unrelated	16.161	1	0	0	0	1

YPN030 - Th531	Unrelated	Unrelated	15.624	1	0	0	0	1
YPN031 - YPN033	Unrelated	Unrelated	15.447	1	0	0	0	1
YPN031 - YPN036	Unrelated	Unrelated	9.516	1	0	0	0	1
YPN031 - Th530	Unrelated	Unrelated	18.384	1	0	0	0	1
YPN031 - Th531	Unrelated	Unrelated	10.194	1	0	0	0	1
YPN031 - Th703	Unrelated	Unrelated	11.791	1	0	0	0	1
YPN032 - YPN036	Unrelated	Unrelated	14.792	1	0	0	0	1
YPN032 - Th530	Unrelated	Unrelated	10.696	1	0	0	0	1
YPN032 - Th531	Unrelated	Unrelated	9.568	1	0	0	0	1
YPN033 - Th519	Unrelated	Unrelated	9.551	1	0	0	0	1
YPN033 - Th521	Unrelated	Unrelated	10.395	1	0	0	0	1
YPN033 - Th530	Unrelated	Unrelated	12.095	1	0	0	0	1
YPN033 - Th531	Unrelated	Unrelated	9.425	1	0	0	0	1
YPN036 - Th519	Unrelated	Unrelated	8.990	1	0	0	0	1
YPN036 - Th521	Unrelated	Unrelated	11.476	1	0	0	0	1
YPN036 - Th531	Unrelated	Unrelated	7.439	1	0	0	0	1
Th531 - Th703	Unrelated	Unrelated	4.762	1	0	0	0	1

Table S4. Admixture proportion estimations modelling ancient genomes from mainland Southeast Asia as a three-way admixture of hunter-gatherer, Yangtze River- and Yellow River-related ancestry with qpAdm.

[illegible]

N2 SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.N	0.00028	0.394	0.338	0.268	0.107
Vietnam_N1S3 SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_WLR_I.N	0.34779	0.443	0.313	0.245	0.086
Vietnam_N1S3 SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.11260	0.437	0.35	0.213	0.09
Vietnam_N1S3 SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.A	0.09066	0.425	0.348	0.227	0.091
Vietnam_N1S3 SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.N	0.19688	0.379	0.386	0.236	0.086
Vietnam_N1S3 SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Longlin.Langdaol_YR_LBIA	0.28397	0.447	0.305	0.248	0.083
Vietnam_N1 HalongCulture SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN	Longlin.Langdaol_Maoizougou MN	0.01723	0.453	0.339	0.183	0.107
Vietnam_N1 HalongCulture SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.01920	0.546	0.399	0.055	0.119
Vietnam_N1 HalongCulture SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.A	0.03091	0.551	0.403	0.046	0.116
Vietnam_N1 HalongCulture SG	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.N	0.01866	0.537	0.432	0.051	0.117
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_WLR_I.N	0.06029	0.554	0.392	0.034	0.113
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Longlin.Langdaol_YR_LBIA	0.4947	0.504	0.361	0.204	0.107
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.01723	0.453	0.339	0.183	0.107
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.A	0.02097	0.428	0.436	0.137	0.101
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN	Longlin.Langdaol_Upper_YR_I.A	0.02030	0.439	0.335	0.226	0.101
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_WLR_I.N	0.02199	0.432	0.323	0.243	0.103
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.N	0.10224	0.272	0.817	-0.09	0.113
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Longlin.Langdaol_YR_LBIA	0.25316	0.282	0.816	-0.08	0.113
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.10192	0.263	0.817	-0.081	0.113
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.N	0.30306	0.279	0.753	-0.032	0.106
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Longlin.Langdaol_YR_LBIA	0.10600	0.261	0.828	-0.09	0.116
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_WLR_I.N	0.70189	0.206	0.975	-0.181	0.073
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.A	0.73725	0.202	0.963	-0.072	0.072
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Longlin.Langdaol_YR_LBIA	0.06448	0.233	0.969	-0.203	0.073
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.39401	0.297	0.912	-0.122	0.073
Vietnam_N2	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.62670	0.183	0.965	-0.148	0.073
Yiyang	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Longlin.Langdaol_YR_LBIA	0.92007	-0.189	0.881	0.308	0.073
Yiyang	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.A	0.73200	-0.216	0.924	0.291	0.076
Yiyang	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Longlin.Langdaol_Upper_YR_I.N	0.58217	-0.212	0.909	0.247	0.082
Yiyang	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_WLR_I.N	0.74637	-0.255	1.008	0.305	0.086
Yiyang	Mbuti DG.Laos_Hoabinhan SG.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Longlin.Langdaol_Maoizougou MN	0.69602	-0.277	0.929	0.267	0.087
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Laos_Hoabinhan SG.Giangdaol_YR_LBIA	0.84212	-0.052	0.844	0.207	0.028
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.N	0.42626	-0.076	0.886	0.029	0.028
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.50687	-0.064	0.865	0.199	0.028
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_WLR_I.N	0.66318	-0.061	0.86	0.201	0.028
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Maoizougou MN	0.50687	-0.064	0.865	0.199	0.028
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.Upper_YR.I.N.WLR LN	Laos_Hoabinhan SG.Giangdaol_YR_LBIA	0.19874	0.394	0.653	-0.047	0.032
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.18858	0.394	0.649	-0.043	0.032
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_IA.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.N	0.19149	0.394	0.636	-0.04	0.032
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_WLR_I.N	0.23775	0.393	0.646	-0.04	0.032
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Maoizougou MN	0.33982	0.393	0.642	-0.037	0.032
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.42088	0.183	0.866	-0.05	0.037
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.N	0.42088	0.183	0.866	-0.05	0.037
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_YR_LBIA	0.42715	0.181	0.882	-0.063	0.037
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.43579	0.182	0.881	-0.063	0.037
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.N	0.71702	0.184	0.869	-0.053	0.036
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Maoizougou MN	0.06117	-0.023	0.618	0.044	0.024
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.06117	-0.023	0.618	0.044	0.024
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_WLR_I.N	0.10321	-0.009	0.648	0.361	0.024
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.09540	-0.023	0.59	0.423	0.023
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_YR_LBIA	0.85520	-0.008	0.588	0.42	0.022
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.39482	0.012	0.749	0.239	0.029
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Maoizougou MN	0.09471	-0.002	0.766	0.233	0.03
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.05802	-0.007	0.753	0.243	0.029
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_WLR_I.N	0.05802	-0.007	0.753	0.243	0.029
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.05533	-0.005	0.766	0.239	0.03
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.N	0.07772	0.279	0.584	0.136	0.038
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_YR_LBIA	0.01401	0.284	0.589	0.127	0.038
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Maoizougou MN	0.01048	0.279	0.602	0.12	0.038
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.00400	0.276	0.588	0.136	0.038
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_WLR_I.N	0.29272	0.256	0.434	0.31	0.03
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.03722	0.241	0.443	0.316	0.031
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_WLR_I.N	0.01529	0.229	0.508	0.263	0.032
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Maoizougou MN	0.10574	0.252	0.465	0.283	0.031
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.09735	0.251	0.464	0.285	0.031
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Upper_YR_IA.Upper_YR.I.N.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_YR_LBIA	0.83836	0.214	0.466	0.32	0.034
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA_2_Ulaanzkh.Nepal_Mekrab_2125BP SG.Nepal_Samdzong_1500BP SG.Maizougou MN.Upper_YR_I.N.WLR LN.YR.LBIA	Laos_Hoabinhan SG.Giangdaol_Upper_YR_I.A	0.59470	0.214	0.439	0.347	0.054
BaBarQcEn	Mbuti DG.Longlin.Qhc.Xitocoun.Tanshinan.Suagong.Mongolia_EIA_3.Mongolia_EIA_7_Xiongnu.Mongolia_LBA						

Thailand_1A	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.08486	0.367	0.684	-0.051	
Thailand_1A	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.13004	0.371	0.669	-0.004	0.0991
Thailand_1A	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.09166	0.362	0.711	-0.073	0.0993
Thailand_1A_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.08086	0.222	0.218	0.56	0.0025
Thailand_1A_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.00316	0.194	0.337	0.469	0.0024
Thailand_1A_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.08086	0.222	0.218	0.56	0.0025
Thailand_1A_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.01917	0.22	0.248	0.532	0.0024
Thailand_1A_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.01983	0.241	0.286	0.472	0.0026
Vietnam_BA_DongSonCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.00010	0.142	0.638	0.22	0.0031
Vietnam_BA_DongSonCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.00067	0.15	0.624	0.226	0.003
Vietnam_BA_DongSonCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.00067	0.15	0.624	0.226	0.003
Vietnam_BA_DongSonCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.00008	0.133	0.679	0.118	0.0031
Vietnam_BA_DongSonCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.00017	0.151	0.646	0.203	0.0031
Vietnam_BA	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.66621	-0.04	0.878	0.163	-0.0036
Vietnam_BA	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.49497	-0.057	0.918	0.139	-0.0037
Vietnam_BA	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.59718	-0.046	0.889	0.057	-0.0036
Vietnam_BA	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.59718	-0.046	0.889	0.057	-0.0036
Vietnam_BA	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.56625	-0.046	0.897	0.149	-0.0036
Vietnam_Historical SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.00337	0.027	0.446	0.527	0.0039
Vietnam_Historical SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.66670	0.081	0.285	0.633	0.0037
Vietnam_Historical SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.66987	0.057	0.313	0.613	0.0037
Vietnam_Historical SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.66987	0.057	0.313	0.613	0.0037
Vietnam_Historical SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.08845	0.054	0.296	0.065	0.0038
Vietnam_LN2 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.07794	0.213	0.394	0.393	0.0044
Vietnam_LN2 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.02322	0.215	0.401	0.384	0.0044
Vietnam_LN2 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.04971	0.23	0.416	0.354	0.0044
Vietnam_LN2 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.00759	0.196	0.475	0.329	0.0041
Vietnam_LN2 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.00759	0.196	0.475	0.329	0.0041
Vietnam_LN3 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.02505	0.208	0.498	0.294	0.0041
Vietnam_LN3 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.15119	0.214	0.527	0.259	0.0041
Vietnam_LN3 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.29391	0.211	0.483	0.306	0.0042
Vietnam_LN3 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.04180	0.188	0.525	0.287	0.0051
Vietnam_LN3 SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.01600	0.188	0.525	0.287	0.0051
Vietnam_LN_HalOnCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.14040	0.233	0.601	0.163	0.0052
Vietnam_LN_HalOnCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.09692	0.231	0.608	0.161	0.0053
Vietnam_LN_HalOnCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.07562	0.215	0.649	0.136	0.0052
Vietnam_LN_HalOnCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.32468	0.245	0.584	0.071	0.0052
Vietnam_LN_HalOnCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.19870	0.231	0.593	0.176	0.0051
Vietnam_LN_HalOnCulture SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.04180	0.188	0.525	0.287	0.0051
Vietnam_LN_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.14834	0.2	0.502	0.298	0.0047
Vietnam_LN_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.43274	0.219	0.484	0.297	0.0047
Vietnam_LN_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.09818	0.161	0.617	0.197	0.0048
Vietnam_LN_SG	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.14319	0.196	0.495	0.309	0.0047
Vietnam_LN2	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.37978	0.22	0.895	-0.115	0.0057
Vietnam_LN2	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.09284	0.194	0.629	0.124	0.0052
Vietnam_LN2	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.68759	0.23	0.836	-0.066	0.0052
Vietnam_LN2	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.43436	0.223	0.916	-0.139	0.0054
Vietnam_LN2	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.41294	0.228	0.909	-0.137	0.0054
Vietnam_LN	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.78220	0.115	1.049	-0.164	0.0041
Vietnam_LN	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.71231	0.115	1.049	-0.164	0.0041
Vietnam_LN	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.79456	0.115	1.037	-0.151	0.0041
Vietnam_LN	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.94260	0.118	0.987	-0.105	0.0041
Vietnam_LN	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.94591	0.125	0.958	-0.183	0.0041
Yiyang	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.16313	-0.007	0.822	-0.027	0.0038
Yiyang	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_WLR_LN	0.77714	-0.07	0.806	-0.282	0.0037
Yiyang	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.02642	-0.11	0.817	-0.212	0.0037
Yiyang	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Maoziougou MN_Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Upper_YR_1A	0.55302	-0.098	0.833	-0.265	0.0038
Yiyang	Mbuti DG_Longlin Qhe_Xitocun Tanshishan Suoguang Mongolia_EIA_3_Mongolia_EIA_7_Xiongmu Mongolia_LBA_2_Ulanzhukh Nepal_Mekrah 2125BP SG_Nepal_Samdzung 1500BP SG_Upper_YR_1A/Upper_YR_1A_WLR_LN_YR_LB1A	Laos_Hoabinhian SG_Liangdao1_Maoziougou MN	0.54336	-0.094	0.857	-0.237	0.0038

Supplementary Information for:

Investigating the occupation history, social hierarchy, and life histories at an early colonial site in Taiwan through archaeology, archaeoethnatology, and archaeogenetics

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Ancient DNA processing

Sampling, extraction, library preparation, and indexing were performed in a dedicated clean room for ancient DNA at the Max Planck Institute for the Science of Human History in Jena, Germany (now MPI for Geoanthropology).

Sampling

We received skeletal remains of 18 individuals from Heping Dao in Taiwan, including 18 petrous parts of the temporal bone, 15 teeth, two proximal phalanges, and one parietal bone (SI Table 1). The phalanges and the parietal bone were not sampled.

The petrous bones were sampled by cutting them in half along the margo superior partis petrosae (crista pyramidis) and drilling bone powder from the densest part around the cochlea¹. The teeth were sampled by cutting at the cemento-enamel junction and drilling bone powder from the pulp of the tooth (<https://dx.doi.org/10.17504/protocols.io.bqebmtan>).

DNA extraction and library preparation

An extraction blank to check for cross- and background contamination and a positive control were added to this step and processed in parallel with the samples. Throughout library preparation an additional blank as well as the extraction blank were processed in an identical manner to the samples.

For samples HPD001 - HPD007, DNA was extracted following a modified version of a previously published protocol² (<https://dx.doi.org/10.17504/protocols.io.baksicwe>). The bone powder was digested in a mixture of EDTA, UV-H₂O, and Proteinase K for approximately 24 hrs while rotating in an incubator at 37°C. After centrifugation, to remove the remaining bone material from solution, the supernatant was transferred into a binding buffer (GuHCl, UV-H₂O, Isopropanol) and then into a silica column (High Pure Viral Nucleic Acid Kit; Roche). The columns were washed twice using the High Pure Viral Nucleic Acid Kit wash buffer (Roche) and eluted in a 100 µl TE-buffer containing 0.05% Tween. From the resulting extracts, 25 µl were used to build double-stranded libraries after partial Uracil DNA Glycosylase treatment³. Illumina adapters were ligated to the fragments using the Quick Ligation Kit (NBE) and the solution purified with a MinElute kit (QIAGEN). The DNA copy number of a 2 µl aliquot of the library was quantified by DyNAmo SYBR Green qPCR (Thermo Fisher Scientific) using IS7/IS8 primers on the LightCycler 480 (Roche)⁴.

A bead-based extraction method was used for the remaining samples⁵ and 30 µl of resulting extract used to build a single-stranded library using the automated liquid handling system Bravo NGS Workstation (Agilent Technologies)⁶.

All libraries were double-indexed with unique index combinations⁷ and amplified using Pfu Turbo DNA Polymerase (Agilent; <https://dx.doi.org/10.17504/protocols.io.bvt8n6rw>). They were purified over MinElute columns, eluted in 50 µl TE-buffer containing 0.05% Tween, and an aliquot quantified with IS5/IS6 primers using DyNAmo SYBR Green qPCR (Thermo

Fisher Scientific) on the LightCycler 480 (Roche)⁴. The remaining solution was further amplified with Herculanase II Fusion DNA Polymerase (Agilent; <https://dx.doi.org/10.17504/protocols.io.beqkjduw>), purified again, quantified on a TapeStation (TapeStation Nucleic Acid System, Agilent 4200), and diluted to 10 nM for shotgun sequencing.

Shotgun screening

The prepared libraries were shotgun-sequenced for a depth of 3-7 million reads on an Illumina HiSeq 4000 using a 75 bp single-read configuration (SI Table 1). The resulting reads were demultiplexed according to expected index combinations.

Further processing was done in nf-core/eager v2.4.4 (<https://nf-co.re/eager>)⁸ using Nextflow v20.10.0⁹. General sequencing quality control was conducted with FastQC v0.11.9¹⁰ and fastP v0.20.1¹¹. Forward and reverse adapters were removed using AdapterRemoval v2.3.2¹² with one overlapping position trimming reads based on read length 30 and base quality 20 and skipping base quality trimming on the 5' end. Using BWA v0.7.17-r1188^{13,14}, the reads were aligned to the human reference genome *hs37d5* allowing for 0.01 mismatches, maximum edit distance of 2, disabling the seeding and two gaps. The resulting bam files were then filtered with SAMtools v1.12¹⁵ for a minimum mapping quality and minimum read length of 30, and unmapped reads were discarded. Mapping statistics were generated with Qualimap v2.2.2-dev¹⁶ and bedtools v2.30.0¹⁷. Duplicates were removed using Picard MarkDuplicates v2.26.0 (<http://broadinstitute.github.io/picard/>) and C to T misincorporation frequency assessed with DamageProfiler v0.4.9¹⁸ of all reads up to 100 bp, considering 15 bp of each read and setting the maximum misincorporation frequency to be displayed. Endogenous DNA was estimated using endorS.py v0.4 (<https://github.com/aidaanva/endorS.py>), and the final report generated with MultiQC v1.12¹⁹.

nextflow run nf-core/eager

```
-profile eva,archgen  
-r 2.4.4  
--input HPD_SG.tsv  
--min_adap_overlap 1  
--clip_readlength 30
```

```

--clip_min_read_quality 20
--preserve5p
--mapper bwaaln
--bwaalnnn 0.01
--bwaalno 2
--run_bam_filtering true
--bam_mapping_quality_threshold 30
--bam_filter_minreadlength 30
--bam_unmapped_type discard
--dedupper markduplicates
--damageprofiler_length 100
--damageprofiler_threshold 15
--damageprofiler_yaxis 0.3

```

Ancient DNA authentication

The endogenous human DNA content after shot-gun screening showed very heterogenous DNA preservation ranging from 0.03 to 70.77% with a median of 4.27% (SI Table 1). We concluded that the sequenced reads were ancient due to elevated rates of cytosine to thymine mis-incorporations at the 5' end of reads ('ancient DNA damage', SI Table 1, Fig. S1). As expected, damage was higher at the end of the reads and dropped off quickly for the UDG-half treated libraries. Additionally, the median read length was low (35-59 bp, SI Table 1) which is characteristic for ancient DNA compared to present-day DNA²⁰.

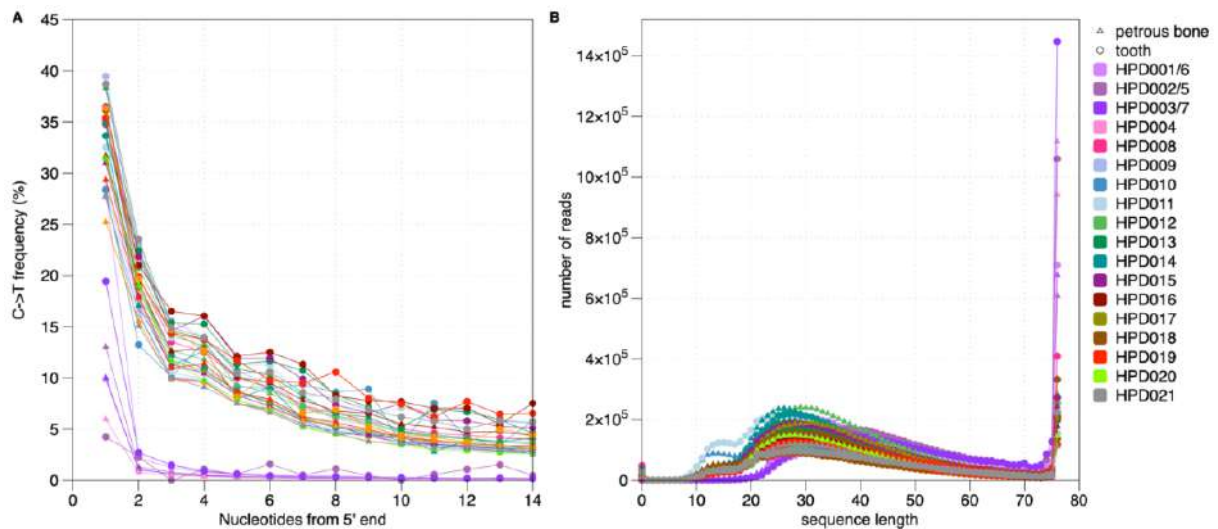


Figure S1. Quality control plots of ancient genomes from Heping Dao, Taiwan; **A**, frequency of C to T transitions at the 5' end of the DNA fragments; **B**, read length distribution; plotted with DataGraph v4.4 (Visual Data Tools, Inc., <http://www.visualdatatools.com/DataGraph/>).

In-solution enrichment

After further amplification with the IS5/IS6 primers, the libraries were hybridized in-solution to oligonucleotide probe sets (Agilent) to enrich for a targeted set of 1,237,207 single nucleotide polymorphisms (SNPs) across the human genome ('1240K' capture)^{21,22}. Since endogenous DNA and DNA damage were low, HPD006 was not captured.

After sequencing the 1240K capture products to 12-29 million reads on the Illumina HiSeq 4000 using a 75 bp single-read configuration (SI Table 1), the reads were demultiplexed again according to expected index combinations.

Further processing was again done in nf-core/eager v2.4.4 (<https://nf-co.re/eager>)⁸ using Nextflow v21.04.3⁹ following the same parameters as the shot-gun data. In addition to those, the 1240K positions were provided in a bedfile, mitochondrial to nuclear ratio were calculated using MTNucRatioCalculator v0.7, genetic sex determined with sexdetermine v1.1.2²³ and nuclear contamination estimated with ANGSD v0.935²⁴. After trimming 2 bp at both ends of the reads with bamUtil v1.0.15²⁵ for the UDG-half-treated libraries, the resulting bam files were genotyped (--run_genotyping true) with pileupCaller from sequenceTools v1.4.0.5 (<https://github.com/stschiff/sequenceTools>) on the 1240K SNP panel calling random haploids and utilizing all sites.

```
nextflow run nf-core/eager
    -profile eva,archgen
    -r 2.4.4
    --input HPD_TF.tsv
    --min_adap_overlap 1
    --clip_readlength 30
    --clip_min_read_quality 20
```

```
--preserve5p
--mapper bwaaln
--bwaalnnn 0.01
--bwaalno 2
--run_bam_filtering true
--bam_mapping_quality_threshold 30
--bam_filter_minreadlength 30
--bam_unmapped_type discard
--dedupper markduplicates
--damageprofiler_length 100
--damageprofiler_threshold 15
--damageprofiler_yaxis 0.3
--snpcapture_bed
--run_mtnucratio true
--mtnucratio_header MT
--run_sexterrmine true
--run_nuclear_contamination true
--contamination_chrom_name X
--run_trim_bam true
--bamutils_clip_half_udg_left 2
--bamutils_clip_half_udg_right 2
--run_genotyping true
--genotyping_tool pileupcaller
--genotyping_source trimmed
--pileupcaller_method randomHaploid
--pileupcaller_transition AllSites
```

The libraries were also captured for the full mitochondrial genome ('MT capture')²⁶ and sequenced to 300,000 to 1.6 million reads on an Illumina HiSeq 4000 using a 75 bp single-read configuration. The sequencing data were processed in nf-core/eager v2.4.4 (<https://nf-co.re/eager>)⁸ using Nextflow v22.04.0⁹. General sequencing quality control was conducted with FastQC v0.11.9¹⁰ and fastP v0.20.1¹¹. Forward and reverse adapters were removed using AdapterRemoval v2.3.2¹² with one overlapping position trimming reads based on read length

30 and base quality 20 and skipping base quality trimming on the 5' end. The reads were aligned to the human mitochondrial genome of reference *hs37d5* using circularmapper²⁷ and extending by 500 bp and specifying the reference header to NC_012920.1. The resulting bam files were then filtered with SAMtools v1.12¹⁵ for a minimum mapping quality and minimum read length of 30, and unmapped reads were discarded. Mapping statistics were generated with Qualimap v2.2.2-dev¹⁶ and duplicates were removed using Picard MarkDuplicates v2.26.0 (<http://broadinstitute.github.io/picard/>). Endogenous DNA was estimated using endorS.py v0.4 (<https://github.com/aidaanva/endorS.py>), and the final report generated with MultiQC v1.12¹⁹.

nextflow run nf-core/eager

```
-profile eva,archgen
-r 2.4.4
--input HPD_MT.tsv
--min_adap_overlap 1
--clip_readlength 30
--clip_min_read_quality 20
-- skip_damage_calculation true
--preserve5p
--mapper circularmapper
--circulartarget NC_012920.1
--circularextension 500
--run_bam_filtering true
--bam_mapping_quality_threshold 30
--bam_filter_minreadlength 30
--bam_unmapped_type discard
--dedupper markduplicates
```

Uniparental marker analysis

Karyotype determination

We calculated the relative coverage of X- and Y-chromosomes in comparison to that of the autosome (X- and Y-rate) using sexdetermine v1.1.2²³ as implemented in nf-core/eager

v2.4.4⁸. Sixteen individuals with elevated Y-rates most likely possessed the karyotype XY, while HPD004 and HPD017 had high X-rates indicating XX (SI Table 1).

Contamination estimation

We estimated nuclear contamination based on heterozygosity levels of X-chromosome SNPs using the xContamination tool of ANGSD v0.935²⁴ as implemented in nf-core/eager v2.4.4⁸. No contamination could be detected for individuals with elevated Y-rates (SI Table 1).

We also used the MT-capture data to estimate mitochondrial contamination with ContamMix v1.0-10²⁶. Overall, mitochondrial contamination was higher than nuclear contamination with a median of 3.13% and highest in HPD009 with 6.5% (SI Table 1).

Mitochondrial haplogroups

We built consensus sequences from the MT-captured bam files using the export function in Geneious v2019.2.3²⁸ setting ‘If no coverage call’ and ‘Call {} if coverage < {}’ to N/X with a coverage threshold of 5, ‘highest quality’ and >50% Sanger heterozygotes. Mitochondrial haplogroups were determined with HaploGrep v2.4.0²⁹.

We mainly identified subgroups of haplogroups B and D in addition to one individual each of haplogroups F, H, J, K, M and U (SI Table 1).

Y-haplogroups

To be able to include neighbouring SNPs from the 1240K capture, we remapped the 1240K-captured sequences with nf-core/eager v2.4.4 (<https://nf-co.re/eager>)⁸ using Nextflow v21.04.0⁹ following the same parameters as for the 1240K capture data but without providing the 1240K positions bed file. We then called all SNP positions on the Y-chromosome and compared the amount of ancestral and derived positions from the ISOGG database (<https://isogg.org/tree/>) to determine the Y-haplogroups of XY individuals.

We identified mainly O2a2b2a, O1a1a and R1b1a1b1a (SI Table 1).

Population genomic analyses

Genetic relatedness

We used READ vf541d55³⁰ to determine genetic relatedness within the dataset and could confirm that the sampled teeth and petrous bones archaeologically and genetically belong to the same individuals, but did not identify any other genetic relationships (SI Table 1).

Runs of homozygosity

To evaluate parental relatedness, we detected runs of homozygosity (ROH) in all individuals using hapROH v0.3a4³¹ in Jupyter notebooks v6.4.4. We ran the habsb_ind command with the provided down-sampled 1000 Genomes data as a reference panel and evaluated ROH on 22 chromosomes (chs=range(1,23)) with parameters matching our data (e_model='haploid', p_model='Eigenstrat', n_ref=2504, random_allele=True, readcounts=False, delete=False, logfile=True, combine=True).

We observed that most individuals do not have any or only very short ROH (Fig. S2).

However, HPD004 had many short and long ROH which indicated that this individual was from a rather small population, while HPD001 and HPD013 showed elevated rates of long ROH suggestive of close genetic relationships between the parents.

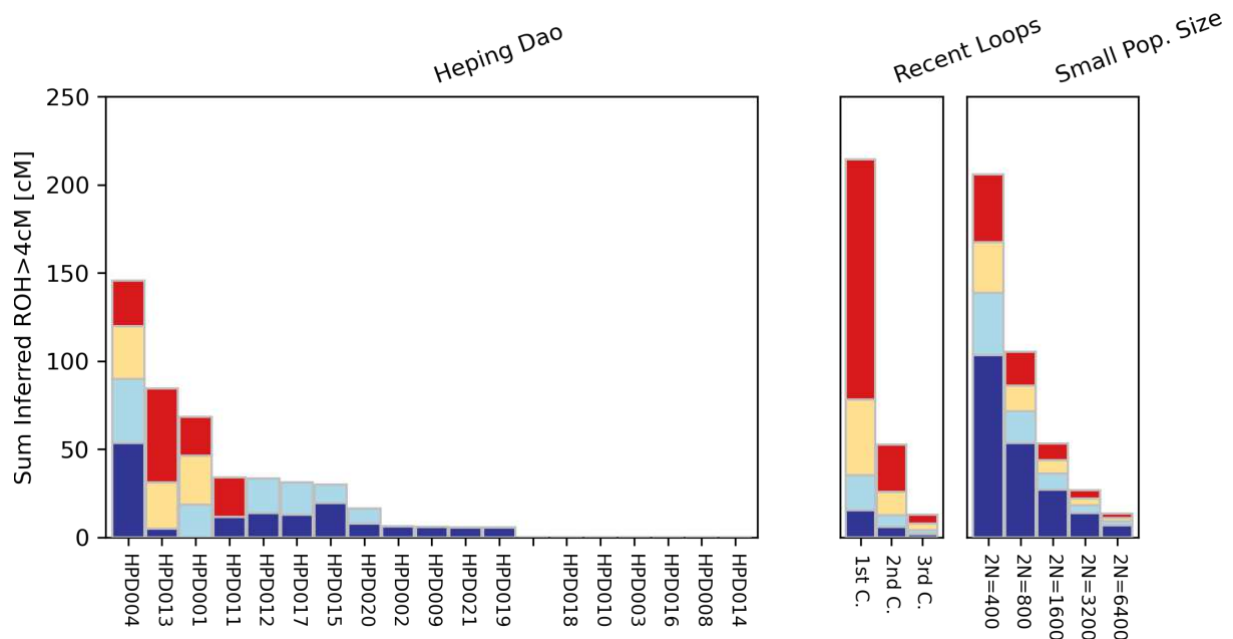


Figure S2. Lengths of runs of homozygosity of ancient individuals from Heping Dao, Taiwan, previously simulated data expected for different parental relationships and population sizes displayed on the right³¹.

Principal component analysis

We merged the ancient genotypes with published individuals from present-day and ancient Eurasia^{32–37} and used smartpca v1600 (lsqmode: YES, shrinkmode: YES) from EIGENSOFT v7.2.1³⁸ to calculate several sets of principal components (PCs).

The initial PCA was calculated on a selection of present-day Eurasian individuals^{32,33,35} with all ancient individuals projected (Fig. S3). Along PC1 we observed a cline between western and eastern Eurasia, whereas PC2 represented a north-south cline in Eastern Eurasia. The ancient individuals split into three distinct clusters with HPD001 and HPD008-11 plotting on top of western Eurasian, HPD004/12/15/17/20 on Southeast and the remaining individuals on East Asian individuals.

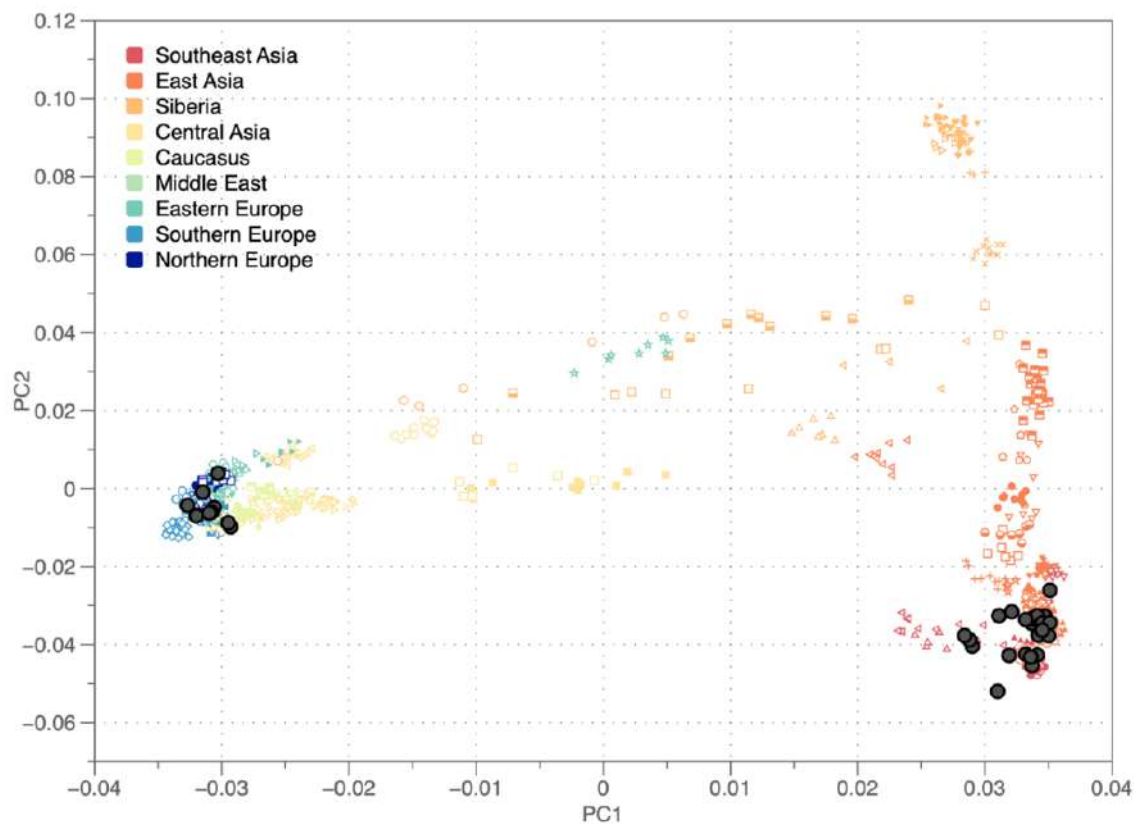


Figure S3. Principal component analyses calculated on present-day individuals from Eurasia^{32,33,35}, ancient individuals from Heping Dao are projected and marked by grey circles.

To focus on these clusters individually, we calculated two regional PCAs (Fig. S4). The first PCA included individuals from East and Southeast Asia (Fig. S4A). HPD004, HPD017 and HPD020 clearly clustered with present-day Indigenous groups from Taiwan, while HPD012

and HPD015 clustered with individuals from the Philippines and Malaysia. HPD002-3, HPD013-14, HPD016, HPD018-19 and HPD021 plotted on top of Han Chinese.

On the west Eurasian PCA (Fig. S4B), HPD001 clustered with present-day individuals from France and Visigoth individuals from Girona, northern Spain, while HPD008 and HPD010 fell very close to each other on present-day Spanish individuals. HPD011 clustered with present-day individuals from northern Spain and Basque, whereas HPD009 fell near Visigoth and Muslim Period individuals from Granada, southern Spain.

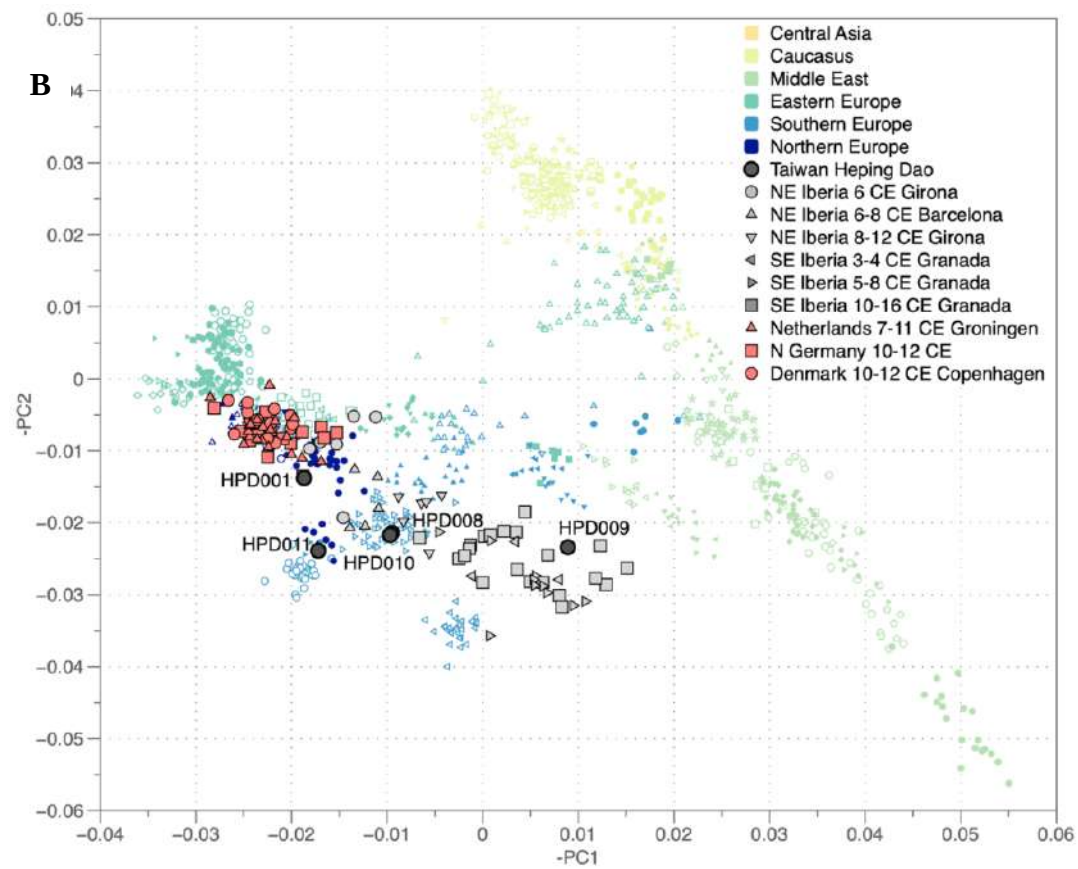
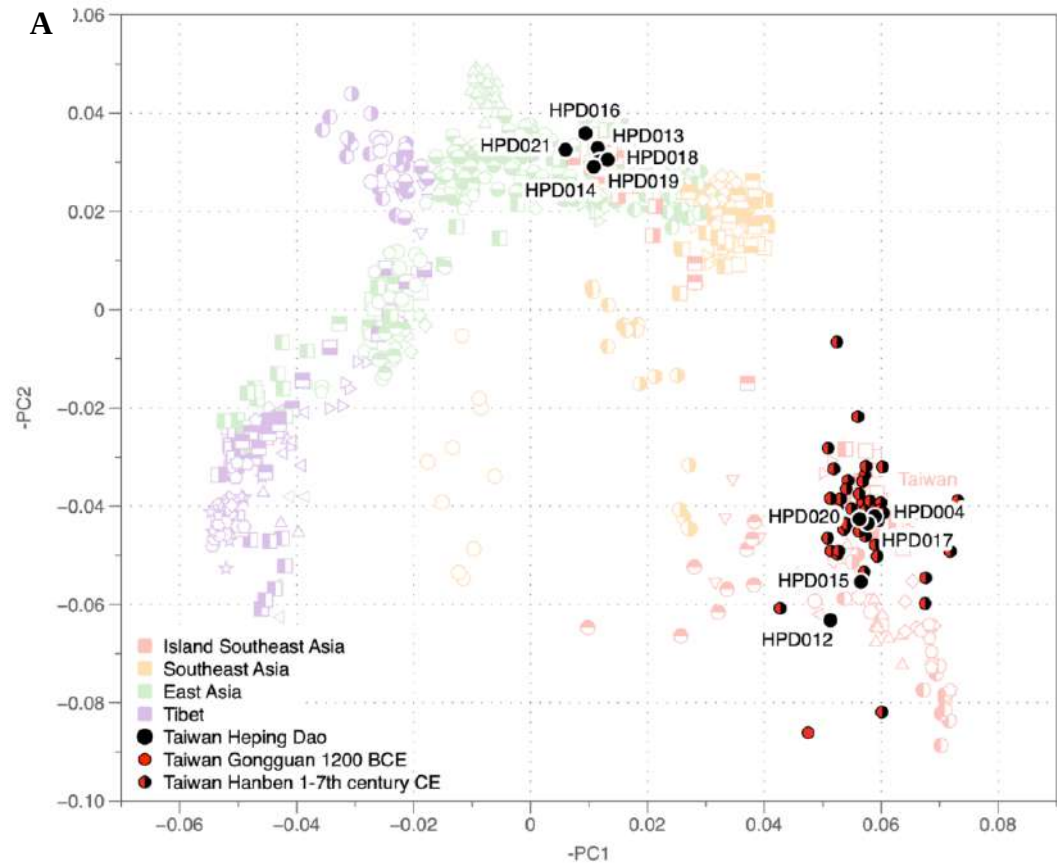


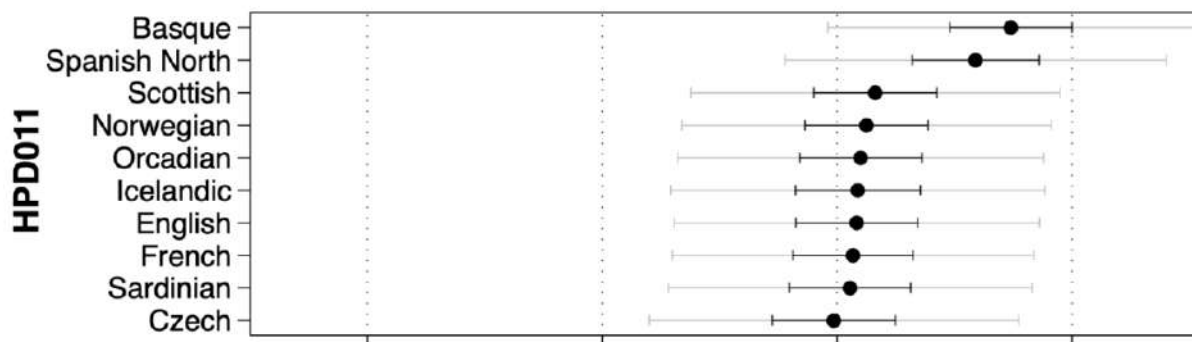
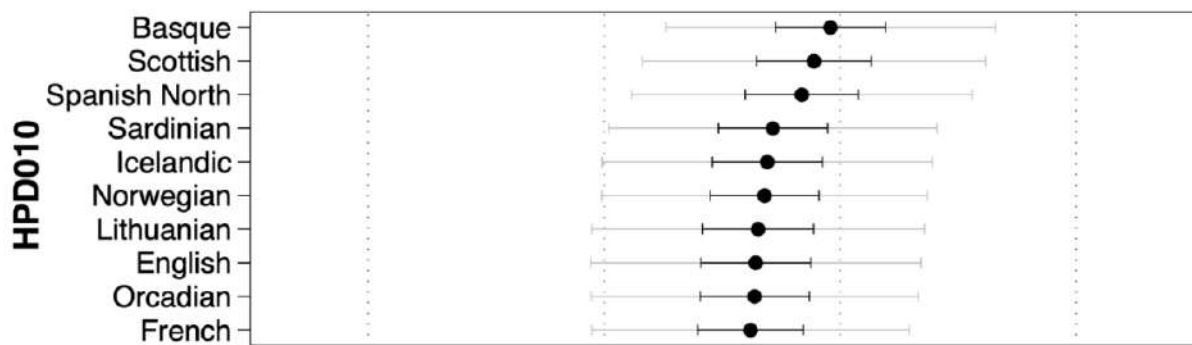
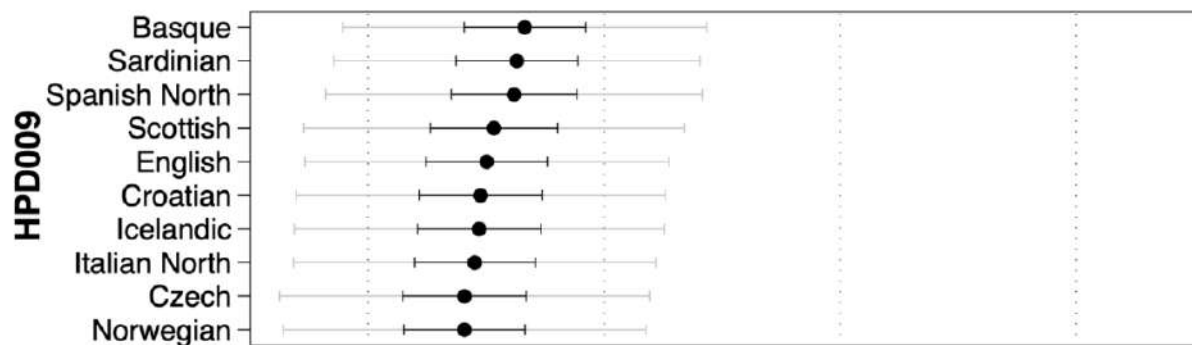
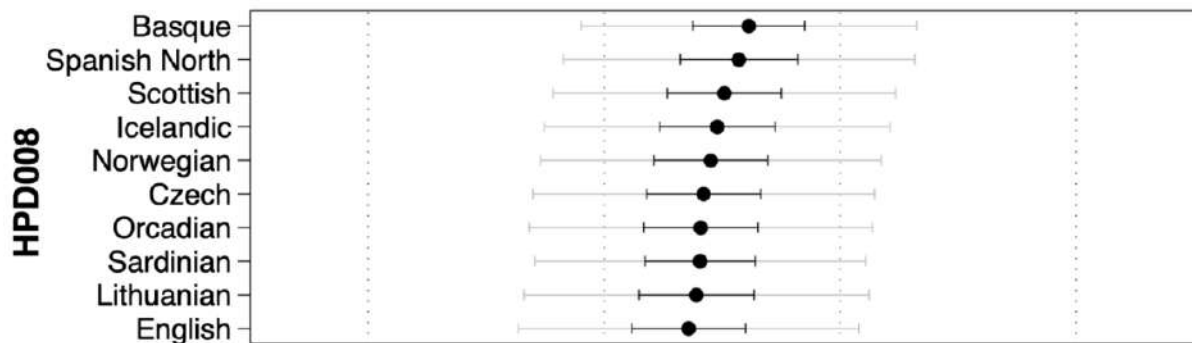
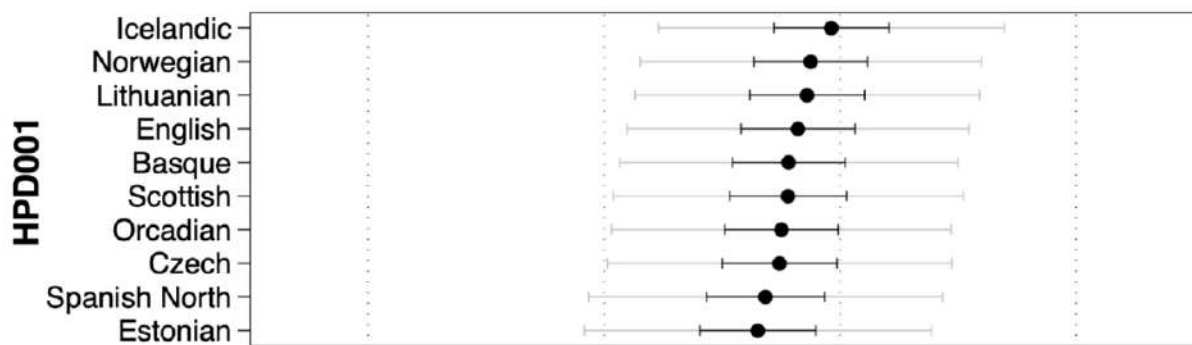
Figure S4. Principal component analyses calculated on present-day western (A) and eastern (B) Eurasian groups, ancient individuals from Heping Dao and previous publications are projected^{36,37,39}.

f_3 -statistics

We calculated f_3 -statistics with *qp3Pop* v651 from ADMIXTOOLS v7.0.2³² using ‘inbreed: YES’.

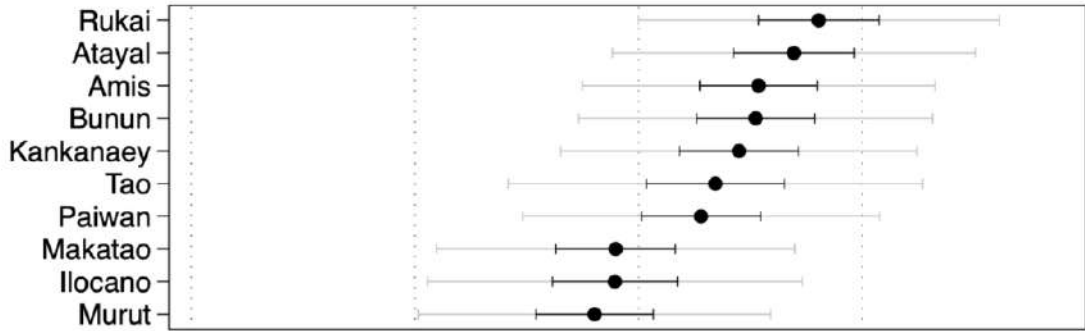
Using the same present-day groups from west Eurasia as for the PCA as *test*^{32,33,35}, we calculated $f_3(\text{Mbuti}, \text{test}, \text{Heping Dao})$ for HPD001 and HPD008-11 (Fig. S5). HPD001 shared the most genetic drift with northern European groups such as Icelandic, Norwegian and Lithuanian, while the remaining individuals shared most drift with Basque, Spanish_North and Sardinian. However, the overall low f_3 -values suggest that HP009 didn’t share as much drift with any present-day group which corresponds to falling outside the present-day variation in PCA (Fig. S4B). Additionally, HPD011 was the only individual who shows clear attraction to two groups (Basque and Spanish_North) compared to other *test* groups.

Similarly, we used the present-day eastern Eurasian groups^{32–34,37,40} from PCA as *test* to calculate $f_3(\text{Mbuti}, \text{test}, \text{Heping Dao})$ for HPD002-4 and HPD012-21 (Fig. S5). We could show that HPD004, HPD017, and HPD020 shared most drift with Indigenous groups from Taiwan, with the highest values achieved for Rukai, Atayal, and Tao, respectively. HPD012 and HPD015 shared most drift with Kankanaey from the Philippines. The remaining individuals shared most drift with Han Chinese groups from Hubei, Shanghai, Fujian, Zhejiang, Chongqing (Fig. S5).

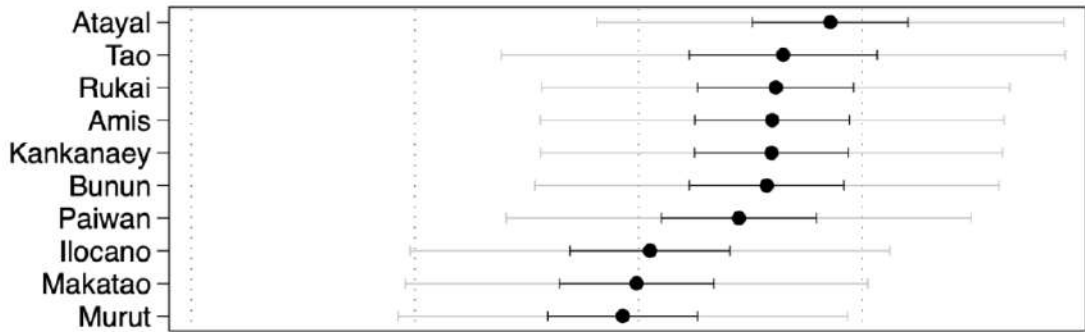


0.24 0.25 0.26 0.27 f_3 -value

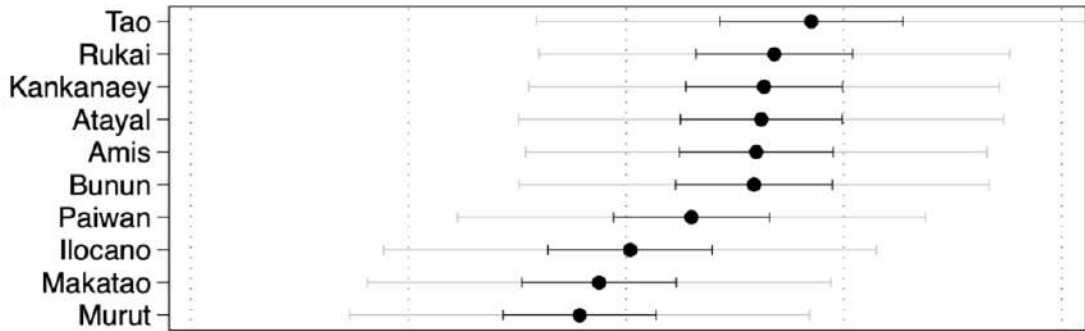
HPD004



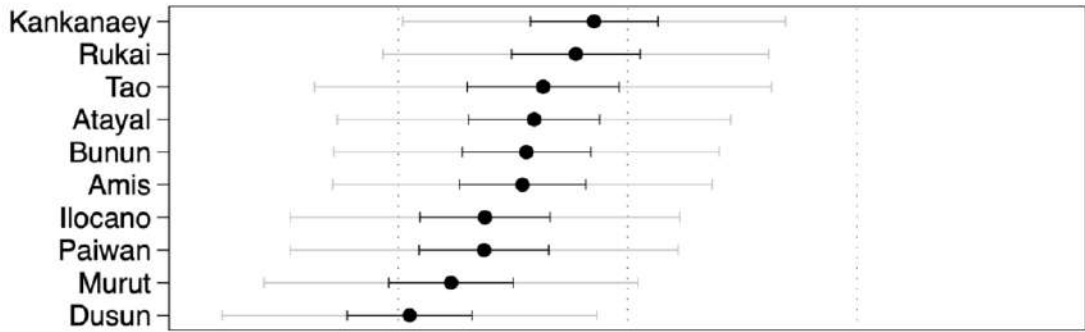
HPD017



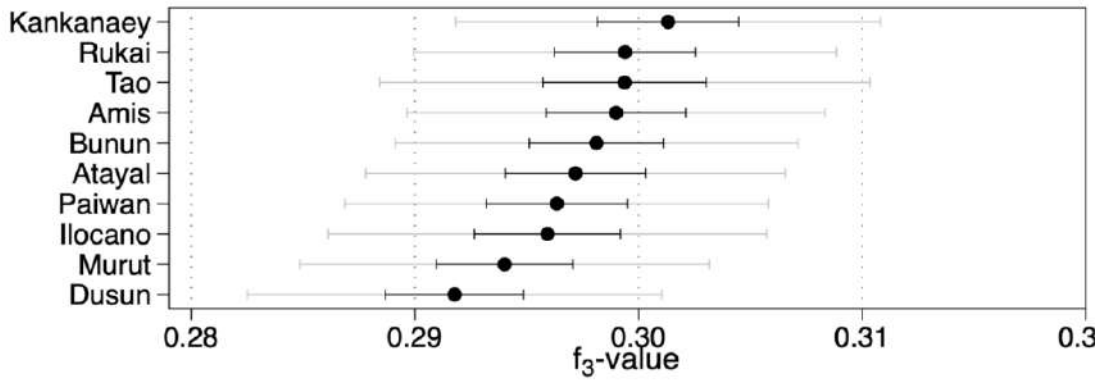
HPD020



HPD012



HPD015



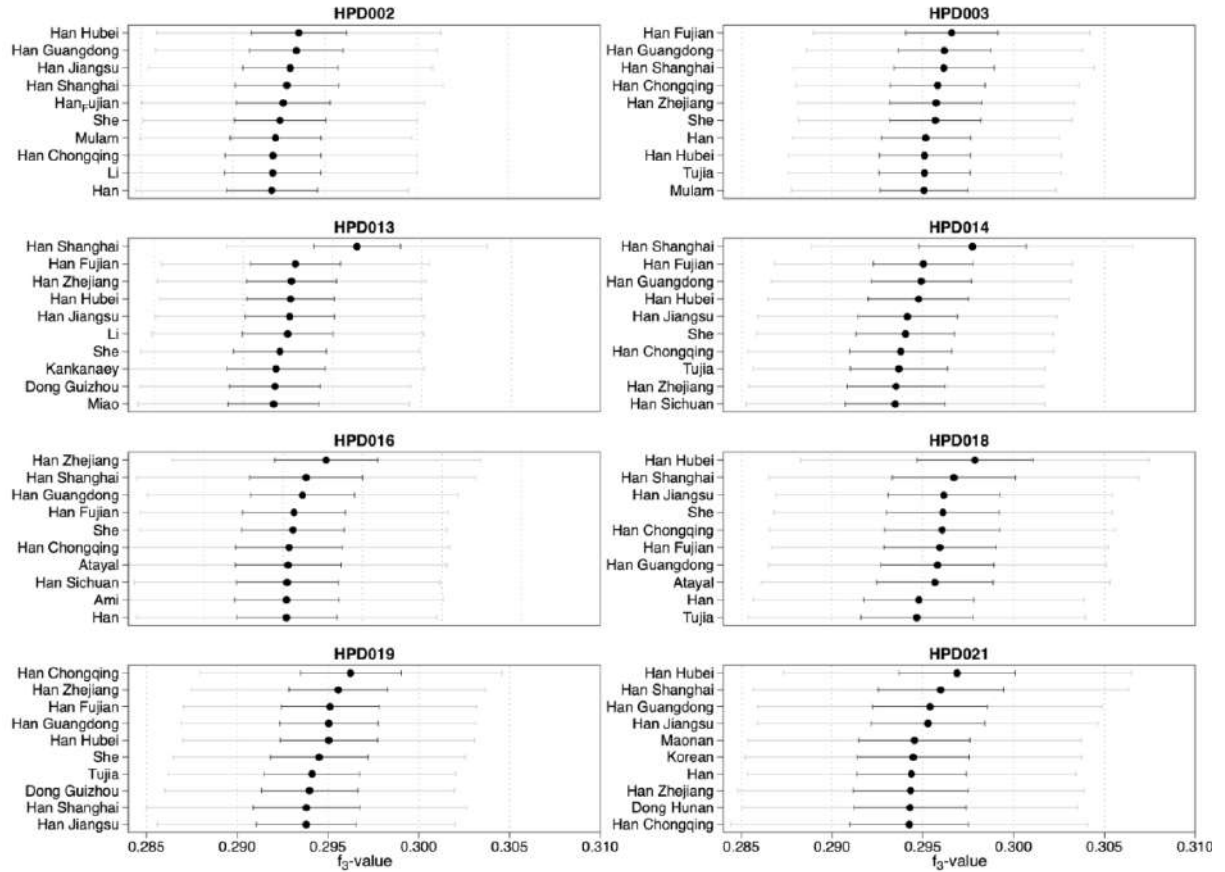


Figure S5. Shared drift of ancient individuals from Heping Dao with present-day western and eastern Eurasian groups as calculated with f_3 -statistics.

ADMIXTURE

We estimated ancestry coefficients using ADMIXTURE v1.3.0⁴¹ on a dataset containing present-day and ancient individuals from East and Southeast Asia^{32–34,40,42}. The analyses were conducted on 4 parallel threads (`-j 4`), a random seed was selected (`-s ${RANDOM}`) and cross-validation errors calculated (`--cv`). We estimated $K=2$ through $K=12$ and selected the run with the highest likelihood out of five replicates per K . The lowest cross-validation errors were achieved by $K=7$ (Fig. S6).

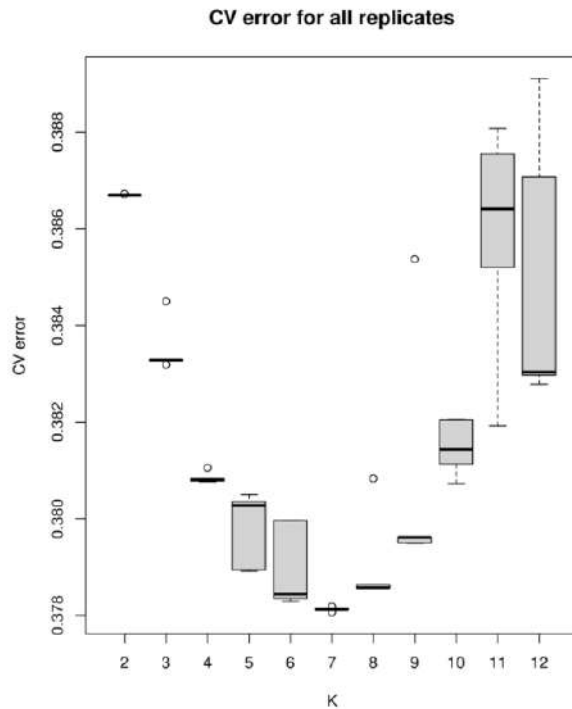


Figure S6. Cross-validation errors of ADMIXTURE analysis of present-day and ancient individuals from East and Southeast Asia^{32,33,35,42}, plotted using CLErrorBoxplotPlotter.R (<https://github.com/TCLamnidis/AdmixturePlotter>).

At K=7 the corresponding ancestry components were maximized in the following groups: Mbuti (pink), French (light blue), Papuan (dark green), Atayal (orange), Dai (purple), Tibetan (yellow) and Japanese (red; Fig. S7). The ancient genomes from Heping Dao showed very diverse ancestry components. As expected, HPD001/HPD008-11 showed exclusively the French components and HPD004/HPD017/HPD020 exclusively the Atayal component. HPD012 was split into ~75% Atayal, 1% Tibetan, 15% Dai, 3% Papuan and 6% French; HPD015 was decomposed into three components (90% Atayal, 6%Tibetan, 4% Papuan). While these admixture compositions are not present in the exact proportions in any of the comparative individuals, the combination of components is generally found for individuals from Southeast Asia. The remaining individuals HPD002-3/HPD013-16/HPD018-19/HPD021 were modelled as 60-70% Japanese, 20-30% Dai and 2-10% Atayal similar to Han and Han_Guangdong, with only HPD002 showing 2% Mbuti instead of Atayal.

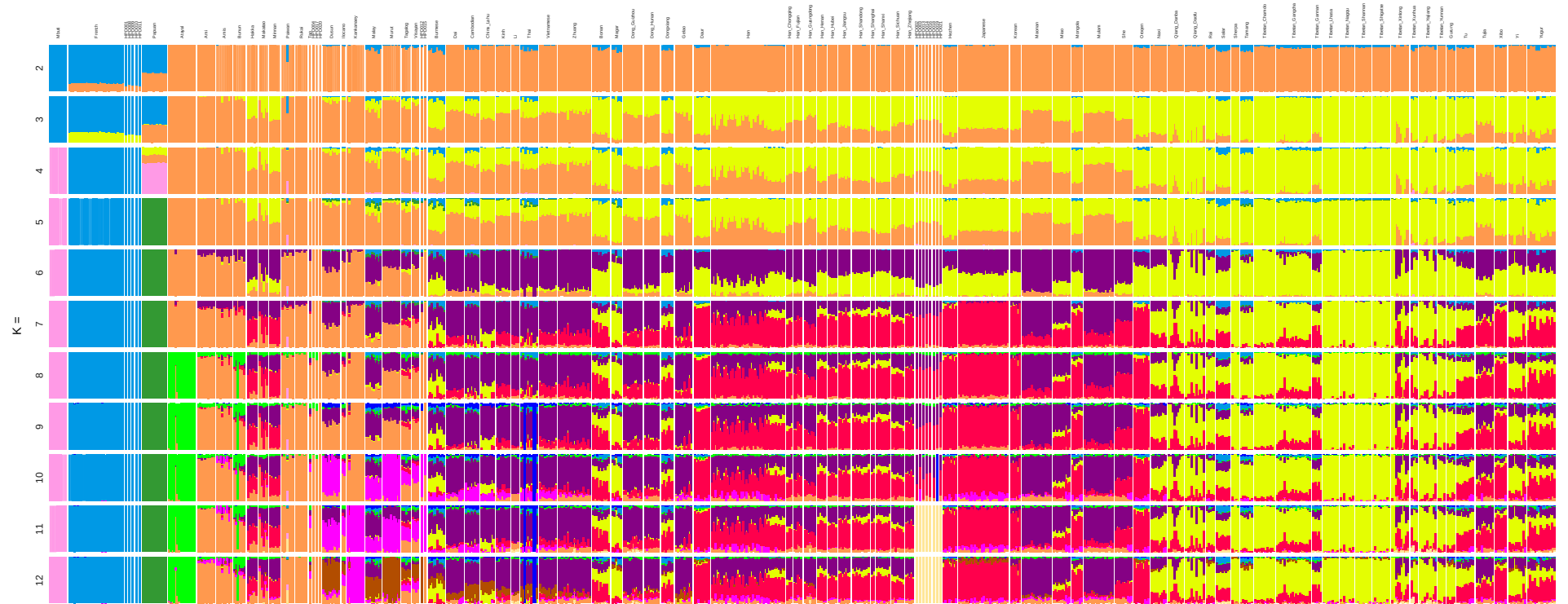


Figure S7. ADMIXTURE results of present-day and ancient individuals from East and Southeast Asia^{32,33,40,42,43}; plotted with AdmixturePlotter.R (<https://github.com/TCLamnidis/AdmixturePlotter>).

qpWave and qpAdm

We used *qpWave* v1520 and *qpAdm* v1520 from ADMIXTOOLS v7.0.2³² setting inbreed: NO', 'allsnps: YES' and 'details: YES' to investigate the genetic admixture flows present in the ancient individuals.

To confirm the previously established genetic affinity to several East and Southeast Asian groups, we tested if HPD002-4/HPD012-21 were indistinguishably from 1st-8th century Hanben from Taiwan³⁷, 300-year-old Chuanyun from southern China⁴⁴, present-day Igorot from the Philippines⁴³ or present-day Dusun from Indonesia⁴³ in *qpWave*. The remaining 'test' groups were added to a set of fixed 'right'/reference groups consisting of Mbuti⁴³, Tianyuan⁴⁵, Liangdao1 from the Taiwanese Strait⁴⁴, Gongguan from Taiwan³⁷, Philippines Red Slipped Pottery⁴⁶, Papuan⁴³, Han⁴³ (SI Table 1).

We could show that HPD004, HPD017 and HPD020 formed 'one wave' with 1st-8th century Hanben from Taiwan, while HPD002-3/HPD013-14/HPD016/HPD018-19/HPD021 were genetically consistent with Chuanyun from Fujian, China ($p > 0.05$). The remaining individuals with Southeast Asian-related ancestry were more difficult to model but clearly showed differences in affinity, since HPD015 formed 'one wave' with present-day Igorot from the Philippines, while HPD012 was not consistent with any tested group, but borderline consistent with Dusun from Indonesia ($p = 0.046$).

SI Table 2. Results of *qpWave* analyses showing the p-value testing for ‘two waves’ detected for Heping Dao and various ancient and present-day groups from eastern Eurasia; p-values above 0.05 are indicated in bold.

Left groups	p-value
Taiwan Hanben,HPD020	0.97924654
Taiwan Hanben,HPD017	0.76605374
Taiwan Hanben,HPD004	0.34646248
Taiwan Hanben,HPD015	0.00818898
Taiwan Hanben,HPD012	2.22E-06
Taiwan Hanben,HPD021	2.39E-27
Taiwan Hanben,HPD016	3.38E-31
Taiwan Hanben,HPD013	9.20E-36
Taiwan Hanben,HPD018	4.04E-37
Taiwan Hanben,HPD019	3.87E-37
Taiwan Hanben,HPD014	6.75E-40
Taiwan Hanben,HPD002	2.43E-50
Taiwan Hanben,HPD003	2.13E-50
Chuanyun,HPD019	0.23954808
Chuanyun,HPD016	0.5750046
Chuanyun,HPD013	0.44312037
Chuanyun,HPD018	0.38697842
Chuanyun,HPD014	0.38000561
Chuanyun,HPD021	0.20329477
Chuanyun,HPD002	0.18875193
Chuanyun,HPD003	0.05223892
Chuanyun,HPD012	5.48E-16
Chuanyun,HPD015	4.14E-18
Chuanyun,HPD020	4.01E-22
Chuanyun,HPD017	5.05E-26
Chuanyun,HPD004	6.42E-27
Igorot,HPD015	0.09712855
Igorot,HPD020	0.00174485
Igorot,HPD004	0.00112357

Igorot,HPD017	2.55E-05
Igorot,HPD012	3.73E-06
Igorot,HPD016	5.13E-28
Igorot,HPD021	2.23E-31
Igorot,HPD018	4.12E-35
Igorot,HPD013	3.13E-40
Igorot,HPD002	9.65E-46
Igorot,HPD003	1.74E-47
Igorot,HPD014	6.31E-48
Igorot,HPD019	4.74E-48
Dusun,HPD012	0.04601591
Dusun,HPD015	0.0007833
Dusun,HPD021	2.13E-14
Dusun,HPD017	5.32E-16
Dusun,HPD020	2.01E-16
Dusun,HPD004	2.27E-19
Dusun,HPD016	2.50E-20
Dusun,HPD013	1.05E-20
Dusun,HPD018	3.71E-22
Dusun,HPD003	4.98E-25
Dusun,HPD019	1.22E-26
Dusun,HPD014	3.34E-27
Dusun,HPD002	7.16E-28

Based on the western Eurasian PCA, we first tested if HPD001 was genetically indistinguishable from the most temporally proximal ancient groups including northeast Iberia 8th-12th century from Girona³⁶, Netherlands 7th-11th century from Groningen, northern Germany 10th-12th century from Schleswig, and Denmark 10th-12th century from Copenhagen³⁹. We also separated individuals GRO006, GRO012 and GRO024 from the main Netherlands 7th-11th century group since they were shown to harbor southern European ancestry which corresponds to their PCA position³⁹. The remaining ‘test’ groups were added to a set of fixed ‘right’/reference groups consisting of Mbuti.DG⁴³, Russia_Kostenki14⁴⁷,

Iberia_ElMiron⁴⁷, Germany_EN_LBK_published⁴⁸, Iberia_IA³⁶ and Netherlands 4-7th century from Midlum³⁹.

The *qpWave* results showed that HPD001 only formed ‘one wave’ with the main and admixed group from Netherlands 7-11th century ($p > 0.05$, SI Table 2). We then tested if NE Iberia, Netherlands 7-11th and Netherlands 7-11th admixed formed two waves, indicating that the Spanish ancestry reported for Netherlands 7-11th century admixed could be represented by NE Iberia 8-12th century. Using HPD001, we found that a comparison of these groups also only formed 2 waves ($p > 0.05$).

SI Table 3. Results of *qpWave* analyses showing the p-value testing for ‘2 waves’ detected for HPD001 and ancient groups from western Eurasia; p-values above 0.05 are indicated in bold.

Left groups	p-value
NE Iberia 8-12 th c., HPD001	1.23x10 ⁻¹¹
Denmark 10-12 th c., HPD001	0.008423
Germany 10-12 th c., HPD001	0.011609
Netherlands 7-11 th c., HPD001	0.120794
Netherlands 7-11 th c. admixed, HPD001	0.183693
NE Iberia 8-12 th c., Netherlands 7-11 th c., Netherlands 7-11 th c. admixed	0.877916
NE Iberia 8-12 th c., Netherlands 7-11 th c., HPD001	0.386208

Subsequently, we ran *qpAdm* to estimate admixture proportions for Netherlands 7-11th century admixed and HPD001 using the same reference groups as for *qpWave*. We could successfully model both ancient groups as and admixture between ~80% NE Iberia 8-12th century and ~20% Netherlands 7-11th century (Fig. S8).

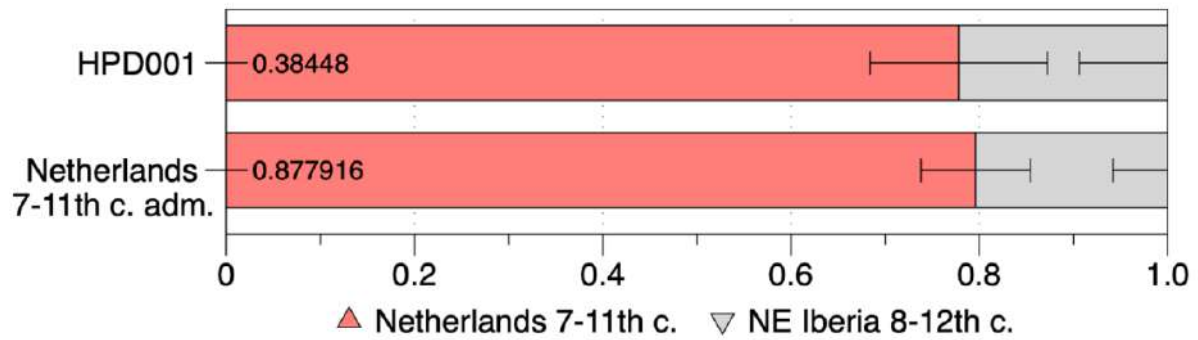


Figure S8. Admixture proportions modelling HPD001 and admixed individuals from Netherlands 7-11th century as a combination of Netherlands 7-11th century³⁹ and northeast Iberia 8-12th century³⁶ as estimated by *qpAdm*, error bars denote standard errors as calculated with block jackknife, p-values are shown within the bars.

We then tested if HPD008 and HPD010 were consistent with ‘one wave’ of genetic ancestry with contemporary ancient groups (NE Iberia 8-12th century, SE Iberia 10-16th century, Netherlands 7-11th century and Netherlands 7-11th century admixed) and found that they were both genetically indistinguishable from NE Iberia 8-12th century and from each other (SI Table 3). We used Mbuti.DG, Russia_Kostenki14, Iberia_ElMiron, Germany_EN_LBK_published, Iberia_IA, NorthSea_NL, Morocco_LN.SG⁴⁹, NorthSea_NL_Groningen_admix, SE_Iberia_c.10-16CE, NorthSea_NL_Groningen and NE_Iberia_c.8-12CE as references.

SI Table 4. Results of *qpWave* analyses showing the p-value testing for ‘two waves’ detected for HPD008 and HPD010 with ancient groups from Iberia; p-values above 0.05 are indicated in bold.

Left groups	p-value
NE Iberia 8-12 th c., HPD010	0.56106887
NE Iberia 8-12 th c., HPD008	0.42765117
HPD008, HPD010	0.2790247
SE Iberia 10-16 th c., HPD008	1.24E-06
SE Iberia 10-16 th c, HPD010	1.58E-11
NE Iberia 8-12 th c., HPD008, HPD010	0.48567134

Since HPD009 fell within their variation on PCA, we included previously reported individuals from 10-16th century SE Iberia and their reported genetic components in the analysis³⁶ (outgroups: Mbuti.DG, Russia_Kostenki14, Iberia_ElMiron, Germany_EN_LBK_published, Iberia_IA, NorthSea_NL, Morocco_LN.SG, NorthSea_NL_Groningen, Israel_Natufian, SE_Iberia_c.10-16CE, Jordan_EBA⁵⁰, Canary Islands Guanche⁵¹). We could show that HPD009 doesn't form one wave with one particular group, but is genetically consistent with 3 waves when using Guanches, Jordan EBA and NE Iberia 8-12th century (SI Table 4).

SI Table 5. Results of *qpWave* analyses showing the p-value testing for 'two waves' detected for HPD009 and ancient groups from western Eurasia; p-values above 0.05 are indicated in bold.

Left groups	p-value
SE Iberia 10-16 th c., HPD009	0.01861141
Jordan EBA, HPD009	5.93E-12
NE Iberia 8-12 th c., HPD009	3.60E-16
Canary Islands Guanche, HPD009	7.80E-51
Canary Islands Guanche, Jordan EBA, NE Iberia 8-12 th c., HPD009	0.21745788

Accordingly, we could model HPD009 as an admixture between ~50% NE Iberia 8-12th century, ~26% Guanche from the Canary Islands and ~23% Jordan EBA which are also present in SE Iberia 10-16th century in vastly variable proportions³⁶ (Fig. S9).

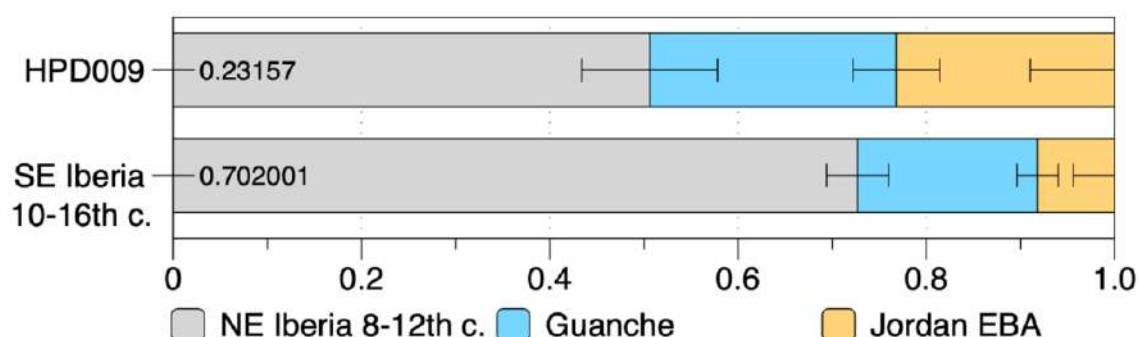


Figure S9. Admixture proportions modelling HPD009 and ancient individuals from southeastern Iberia 10-16th century as a combination of northeast Iberia 8-12th century³⁶,

Guanche from the Canary Islands⁵¹, and Early Bronze Age from Jordan⁵⁰ as estimated by *qpAdm*, error bars denote standard errors as calculated with block jackknife, p-values are shown within the bars.

References

1. Pinhasi, R. *et al.* Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS One* **10**, e0129102 (2015).
2. Dabney, J. *et al.* Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 15758–15763 (2013).
3. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
4. Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, db.prot5448 (2010).
5. Dabney, J. & Meyer, M. Extraction of Highly Degraded DNA from Ancient Bones and Teeth. *Methods Mol. Biol.* **1963**, 25–29 (2019).
6. Gansauge, M.-T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**, 2279–2300 (2020).
7. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
8. Fellows Yates, J. A. *et al.* Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager. *PeerJ* **9**, e10947 (2021).
9. Di Tommaso, P. *et al.* Nextflow enables reproducible computational workflows. *Nat. Biotechnol.* **35**, 316–319 (2017).
10. Andrews, S. FastQC A Quality Control tool for High Throughput Sequence Data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
11. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
12. Schubert, M., Lindgreen, S. & Orlando, L. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Res. Notes* **9**, 88 (2016).

13. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
14. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv [q-bio.GN]* (2013).
15. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
16. Okonechnikov, K., Conesa, A. & García-Alcalde, F. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics* **32**, 292–294 (2016).
17. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).
18. Neukamm, J., Peltzer, A. & Nieselt, K. DamageProfiler: Fast damage pattern calculation for ancient DNA. *Bioinformatics* (2021) doi:10.1093/bioinformatics/btab190.
19. Ewels, P., Magnusson, M., Lundin, S. & Käller, M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* **32**, 3047–3048 (2016).
20. Sawyer, S., Krause, J., Guschanski, K., Savolainen, V. & Pääbo, S. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLoS One* **7**, e34131 (2012).
21. Fu, Q. *et al.* An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
22. Mathieson, I. *et al.* Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503 (2015).
23. Lamnidis, T. C. *et al.* Ancient Fennoscandian genomes reveal origin and spread of Siberian ancestry in Europe. *Nat. Commun.* **9**, 5018 (2018).
24. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics* **15**, 356 (2014).
25. Jun, G., Wing, M. K., Abecasis, G. R. & Kang, H. M. An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Res.* **25**, 918–925 (2015).
26. Fu, Q. *et al.* A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
27. Peltzer, A. *et al.* EAGER: efficient ancient genome reconstruction. *Genome Biol.* **17**, 60 (2016).

28. Kearse, M. *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
29. Weissensteiner, H. *et al.* HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* **44**, 58–63 (2016).
30. Monroy Kuhn, J. M., Jakobsson, M. & Günther, T. Estimating genetic kin relationships in prehistoric populations. *PLoS One* **13**, e0195491 (2018).
31. Ringbauer, H., Novembre, J. & Steinrücken, M. Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* **12**, 5425 (2021).
32. Patterson, N. *et al.* Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
33. Lazaridis, I. *et al.* Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409–413 (2014).
34. Skoglund, P. *et al.* Genomic insights into the peopling of the Southwest Pacific. *Nature* **538**, 510–513 (2016).
35. Jeong, C. *et al.* The genetic history of admixture across inner Eurasia. *Nat Ecol Evol* **3**, 966–976 (2019).
36. Olalde, I. *et al.* The genomic history of the Iberian Peninsula over the past 8000 years. *Science* **363**, 1230–1234 (2019).
37. Wang, C.-C. *et al.* Genomic insights into the formation of human populations in East Asia. *Nature* **591**, 413–419 (2021).
38. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
39. Gretzinger, J. *et al.* The Anglo-Saxon migration and the formation of the early English gene pool. *Nature* **610**, 112–119 (2022).
40. Liu, D., Ko, A. M.-S. & Stoneking, M. The genomic diversity of Taiwanese Austronesian groups: implications for the ‘Into and Out of Taiwan’ models. *bioRxiv* 2023.01.09.523210 (2023) doi:10.1101/2023.01.09.523210.
41. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).
42. Wang, T. *et al.* Human population history at the crossroads of East and Southeast Asia since 11,000 years ago. *Cell* (2021) doi:10.1016/j.cell.2021.05.018.

43. Mallick, S. *et al.* The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**, 201–206 (2016).
44. Yang, M. A. *et al.* Ancient DNA indicates human population shifts and admixture in northern and southern China. *Science* **369**, 282–288 (2020).
45. Yang, M. A. *et al.* 40,000-Year-Old Individual from Asia Provides Insight into Early Population Structure in Eurasia. *Curr. Biol.* **27**, 3202–3208.e9 (2017).
46. McColl, H. *et al.* The prehistoric peopling of Southeast Asia. *Science* **361**, 88–92 (2018).
47. Fu, Q. *et al.* The genetic history of Ice Age Europe. *Nature* **534**, 200–205 (2016).
48. Lipson, M. *et al.* Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* **551**, 368–372 (2017).
49. Fregel, R. *et al.* Ancient genomes from North Africa evidence prehistoric migrations to the Maghreb from both the Levant and Europe. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 6774–6779 (2018).
50. Lazaridis, I. *et al.* Genomic insights into the origin of farming in the ancient Near East. *Nature* **536**, 419–424 (2016).
51. Rodríguez-Varela, R. *et al.* Genomic Analyses of Pre-European Conquest Human Remains from the Canary Islands Reveal Close Affinity to Modern North Africans. *Curr. Biol.* **28**, 1677–1679 (2018).

Table S1. Overview of archaeological and genomic information of individuals from Heping Dao-B, an early colonial site in Taiwan.

Lab ID	Archaeological ID	Site	Country	Latitude	Longitude	Material	Age	anthr. Sex	Burial location	Burial position	Burial orientation	SG reads	Endogenous 5' C>T s DNA (%)	X-rate (%)	Median read length (bp)	1240K reads	Merged 1240K		X-rate	Y-rate	Karotype	nuclear mapped contam	MT contam (%)	MT haplogroup	Y-haplogroup	terminal SNPs	
																	1240K SNPs	SNPs									
HPD001	HPD-B-2016 / Area1ue16	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	adult M		Inside	Supine	W-E	5,155,170	6.43	9.80	59	22,053,705	647,048	647,048	0.5	0.6	XY	0	16.744	2.87	H	R1b1a1b1a2a	P312/PP6547/S116	
HPD002	HPD-B-2016 / Area1ue132	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	adult M		Outside	Supine	W-E	4,247,923	15.92	13.00	47	20,512,271	753,094	996,138	0.5	0.6	XY	0	56.024	0.67	D5a2	N1a2a	Z19785	
HPD003	HPD-B-2014 / T3P2ue26	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	adult M		Outside	Supine	W-E	5,489,716	21.78	10.00	50	22,389,106	812,867	934,566	0.5	0.6	XY	0	55.444	0.76	B5a1c*	O1a1a	F54	
HPD004	HPD-B / T2P6 Jb1/T2P9ue7	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	4 f		Outside	Jar Burial	W-E	5,584,143	26.53	6.00	59	29,193,689	905,806	905,806	0.9	0	XX	0.2	28.070	2.38	M7c1c3	N/A	N/A	
HPD004B	HPD-B / T2P6 Jb1/T2P9ue7	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Parietal bone	4 f		Outside	Jar Burial	W-E																	
HPD005	HPD-B-2016 / Area1ue132	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left upper II	adult M		Outside	Supine	W-E	5,442,832	70.77	28.40	39	23,961,728	839,756		0.5	0.6	XY	0	-	-	-	N1a2a	Y3054	
HPD006	HPD-B-2016 / Area1ue107	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Lower I1	adult M		Inside	Supine	W-E	4,683,160	0.03	4.20	41										-	-	-	
HPD007	HPD-B-2014 / T3P2ue26	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower I2	adult M		Outside	Supine	W-E	5,680,742	8.24	19.40	43	27,802,596	607,567		0.4	0.5	XY	0	-	-	-	O1a1a	F54	
HPD008A	HPD-B-2019 / Area2au60	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower P2	30-35 M		Inside	Supine	S-N	3,887,754	18.19	34.70	43	19,999,423		685,252	34.70	0.4	0.5	XY	0	55.877	3.75	U2e1a1	O1a1a2b-	Y6862
HPD008B	HPD-B-2019 / Area2au60	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	30-35 M		Inside	Supine	S-N	5,967,672	24.17	27.60	45	14,815,307	407,055		0.4	0.5	XY	0	-	-	-	-	-	
HPD009A	HPD-B-2019 / Area2au53	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower P2	25-30 M		Inside	Supine	S-N	4,032,074	9.14	39.50	38	17,844,074	299,802	471,118	0.4	0.5	XY	0	34.078	6.46	K1a+195	R1b1a1b1a2a	P312/PP6547/S116	
HPD009B	HPD-B-2019 / Area2au53	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	25-30 M		Inside	Supine	S-N	6,193,501	6.64	28.50	44	18,814,002	268,763		0.4	0.5	XY	0	-	-	-	-	-	
HPD010A	HPD-B-2019 / Area2au71	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower P2	25-30 M		Inside	Supine	S-N	3,670,182	0.11	28.40	44	15,623,482	3,128	535,178	0.4	0.5	XY	N/A	28.400	2.40	H1e	R1b1a1b1a2a	P312/PP6547/S116	
HPD010B	HPD-B-2019 / Area2au71	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	25-30 M		Inside	Supine	S-N	5,622,072	14.24	27.70	45	25,285,208	533,992		0.4	0.5	XY	0	-	-	-	-	-	
HPD011A	HPD-B-2019 / Area2.4su232	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower P2	17-20 M		Inside	Supine	S-N	7,002,386	0.44	32.50	40	17,872,417	27,981	391,265	0.4	0.5	XY	0	34.052	2.81	J2a1a1	I2a1a	PF3966/Z2612	
HPD011B	HPD-B-2019 / Area2.4su232	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	17-20 M		Inside	Supine	S-N	6,222,020	13.72	27.80	45	13,677,026	376,051		0.4	0.5	XY	0	-	-	-	-	-	
HPD012A	HPD-B-2019 / Area3su41	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower P1	20-25 M		Outside	Supine	S-N	4,407,926	21.62	35.20	41	15,195,493	391,057	660,971	0.4	0.5	XY	0	48.361	3.69	B4b1a2b*	O2a2b2a2b2b	AM00465	
HPD012B	HPD-B-2019 / Area3su41	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	20-25 M		Outside	Supine	S-N	7,341,507	11.86	31.30	45	21,919,852	469,038		0.4	0.5	XY	0	-	-	-	-	-	
HPD013A	HPD-B-2019 / Area3su76	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right lower P2	25-30 M		Outside	Supine	W-E	5,863,846	0.3	33.70	34	16,708,289	15,678	269,813	0.4	0.6	XY	N/A	30.827	4.43	D5b3	O?	F84M1741	
HPD013B	HPD-B-2019 / Area3su76	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	25-30 M		Outside	Supine	W-E	5,309,393	3.13	38.30	42	25,331,525	259,414		0.4	0.6	XY	0	-	-	-	-	-	
HPD014A	HPD-B-2019 / Area3su153	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left lower P1	30-35 M		Outside	Supine	S-N	6,175,401	31.61	34.90	40	26,835,758	625,041	641,426	0.4	0.5	XY	0	26.815	1.26	D4b2b1	O2a1b1a1a1a	CTS858	
HPD014B	HPD-B-2019 / Area3su153	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	30-35 M		Outside	Supine	S-N	6,018,412	33.60	48.80	40	26,835,758	625,041		0.4	0.5	XY	0	-	-	-	-	-	
HPD015A	HPD-B-2019 / Area3su179	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right upper P2	~35 M		Outside	Supine	W-E	6,120,560	0.2	36.50	39	12,619,984	10,811	259,110	0.4	0.5	XY	N/A	26.561	4.74	B5b1c	O2a2b2a2b	AM01750	
HPD015B	HPD-B-2019 / Area3su179	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	~35 M		Outside	Supine	W-E	4,988,325	4.61	31.00	46	22,318,614	251,853		0.4	0.5	XY	0	-	-	-	-	-	
HPD016A	HPD-B-2019 / Area3.1su45	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right upper II	35-40 M		Outside	Supine	S-N	4,450,443	0.51	36.20	34	21,671,260	31,900	223,825	0.4	0.5	XY	0	44.609	4.86	F2a	O2a2a1a	F2221	
HPD016B	HPD-B-2019 / Area3.1su45	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left petrous bone	35-40 M		Outside	Supine	S-N	4,593,088	3.93	31.70	46	15,989,126	199,995		0.4	0.5	XY	0	-	-	-	-	-	
HPD017A	HPD-B-2019 / Area3.1su68	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right proximal phalanx	40-45 F		Outside	Flexed	W-E														-	-	-	
HPD017B	HPD-B-2019 / Area3.1su68	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	40-45 F		Outside	Flexed	W-E	6,040,750	2.91	31.50	44	19,068,614	190,540	190,540	0.8	0	XX	0.2	9.213	3.21	M7c1c3	N/A	N/A	
HPD018A	HPD-B-2019 / Area3.1su71	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left upper P1	25-30 M		Outside	Supine	S-N	3,165,038	0.48	35.40	43	17,196,236	44,900	258,664	0.4	0.5	XY	0	33.051	5.43	D5a3a	O2a2b2a1a	Z44739	
HPD018B	HPD-B-2019 / Area3.1su71	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	25-30 M		Outside	Supine	S-N	5,531,384	3.58	29.30	45	17,861,310	226,833		0.4	0.5	XY	0	-	-	-	-	-	
HPD019A	HPD-B-2019 / Area3.1su87	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Left lower P1	35-45 M		Outside	Supine	S-N	3,740,750	0.25	36.30	35	13,169,905	8,075	555,498	0.3	0.5	XY	N/A	37.687	3.59	B4a4	O2a2b1a1a	F204	
HPD019B	HPD-B-2019 / Area3.1su87	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	35-45 M		Outside	Supine	S-N	4,732,726	34.82	25.10	51	16,649,256	552,730		0.4	0.5	XY	0	-	-	-	-	-	
HPD020A	HPD-B-2019 / Area3.1su94	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right proximal phalanx	~45 F		Outside	Flexed	W-E														-	-	-	
HPD020B	HPD-B-2019 / Area3.1su94	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	~45 F		Outside	Flexed	W-E	4,510,748	3.43	31.40	43	13,389,233	167,346	167,346	0.4	0.5	XY	0	6.658	2.94	M7c1c1	O1a1a1a	FGC15382/Z23466	
HPD021A	HPD-B-2019 / Area3.1su103	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right upper P2	25-35 M		Outside	Supine	S-N	3,690,990	1.63	38.70	39	14,262,573	84,461	216,601	0.4	0.5	XY	0	35.234	3.06	B4c1b2c2	O2a	CTS8399	
HPD021B	HPD-B-2019 / Area3.1su103	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	Right petrous bone	25-35 M		Outside	Supine	S-N	3,734,142	2.87	27.90	45	13,821,579	148,872		0.4	0.5	XY	0	-	-	-	-	-	
-	HPD-B-2016 / T2P9ue40	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	-	adult	-	Outside	Supine	W-E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
-	HPD-B / Area2ue38	Heping Dao B Taiwan	25°09'20.86 N	121°45'53.15 E	-	-	-	Inside	-	N-S/S-N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	