1 GEOMETRIC MORPHOMETRIC ANALYSIS OF THE INTERMEDIATE PHALANX OF *OURANOPITHECUS MACEDONIENSIS*—A PILOT STUDY

Jana Kunze[1,*], Fotios Alexandros Karakostis[2], Melania Ioannidou[1], George D. Koufos[3], Katerina Harvati[1,2]

[1]*Paleoanthropology, Institute for Archaeological Sciences and Senckenberg Centre for Human Evolution and Palaeoenvironment, Department of Geosciences, Eberhard Karls University of Tübingen, Tübingen, Germany*
[2]*DFG Centre of Advanced Studies 'Words, Bones, Genes, Tools', Eberhard Karls University of Tübingen, Tübingen, Germany*
[3]*Laboratory of Geology and Palaeontology, School of Geology, Aristotle University of Thessaloniki, Thessaloniki, Greece*
**jana.kunze@outlook.com*

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1.1 INTRODUCTION

Ravin de la Pluie (RPl) in the Axios valley, Greece, is one of the three localities where the material of the Late Miocene ape *Ouranopithecus macedoniensis* has been found to date (de Bonis et al., 1974; de Bonis and Melentis, 1978; Koufos and de Bonis, 2006; Koufos et al., 2016). The material from RPl is rich; it includes maxillary and mandibular remains, multiple isolated teeth, and just a few postcranial specimens, which to date represent the only postcranial remains associated with this species: two phalanges (proximal: RPl-86, intermediate: RPl-87).

A first analysis of the phalanges was conducted in 2014 (de Bonis and Koufos, 2014). In that study, de Bonis and Koufos followed a comparative approach, using multivariate analyses of linear measurements to assess morphological similarities to extant and fossil primates. De Bonis and Koufos concluded that both phalanges likely belong to the hand, but that an allocation to the foot cannot be excluded due to the lack of comparative material from the same species. However, since the phalanges are the only postcranial remains associated with this species so far, identifying them as either manual or pedal phalanges, as well as allocating them to a ray, is crucial. Having this information would allow for further, more detailed examinations, such as more accurate comparative analyses and reconstruction of locomotor behavior. Therefore, the goal of this study is to contribute to the identification of the intermediate phalanx RPl-87 through the application of three-dimensional (3D) geometric morphometrics (GM). Geometric morphometrics is a statistical analysis of form (shape

J. Kunze: https://orcid.org/0000-0002-4408-1362
F. A. Karakostis: https://orcid.org/0000-0003-3913-4332
M. Ioannidou: https://orcid.org/0000-0002-8199-7126
G. D. Koufos: https://orcid.org/0000-0001-9669-4884
K. Harvati: https://orcid.org/0000-0001-5998-4794

and size) in which, in contrast to traditional morphometrics, geometric information of the investigated structures can be retained throughout the analysis to provide a more detailed evaluation of shape differences (Slice, 2007). By comparing the shape of the better-preserved phalanx RPl-87 with manual and pedal intermediate phalanges of extant primates, we aim to address the following research questions:

1. Does RPl-87 belong to the foot or the hand?
2. What ray can RPl-87 be assigned to?
3. Does the specimen show morphological affinities to extant primates?

1.2 MATERIALS AND METHODS

Our comparative sample consists of scans of intermediate phalanges of *Gorilla gorilla* (n=23, 4 individuals), *Pan troglodytes* (n=24, 4 individuals), *Pongo pygmaeus* (n=24, 5 individuals), *Papio hamadryas* (n=15, 2 individuals), and *Macaca arctoides* (n=7, 1 individual) from the American Museum of Natural History Mammal Collections downloaded from MorphoSource.org. Manual and pedal phalanges from the second to fifth ray were selected for the shape analysis. Generally, it was attempted to keep the number of individuals low by including hand and foot bones from the same individual. However, this was not possible in some cases due to issues with preservation or scan quality. Moreover, a second intermediate phalanx from *G. gorilla* had to be removed as it presented as an outlier throughout the statistical analyses. Since the anatomical side of RPl-87 could not be determined so far, we analyzed bones from left and right anatomical sides together.

1.2.1. SHAPE ANALYSIS

The shape analysis was conducted in R-Studio (R version 4.1.2 for Windows; R Core Team, 2021) using the geomorph package (Adams and Otárola-Castillo, 2013). Eleven fixed landmarks were placed in geometrically corresponding positions (Table 1) together with 70 surface semilandmarks.

No	Orientation*	Descritpion*
1	Medial	Most distal point of the medial head condyle
2	Lateral	Most distal point of the lateral head condyle
3	Medial	Most palmar point of the medial head condyle
4	Lateral	Most palmar point of the lateral head condyle
5	Distal, dorsal up	Most dorsal point of the medial half of the head
6		Most dorsal point of the lateral half of the head
7	Proximal, palmar up	App. midpoint of palmar border of proximal articular surface, often most palmarly projecting point
8		App. midpoint of left border of proximal articular surface, often most medially projecting point
9		App. midpoint of right border of proximal articular surface, often most laterally projecting point
10		App. midpoint of dorsal border of proximal articular surface, often most proximally projecting point
11	Medial	Most distal point of base curvature

*Anatomical sides were pooled in the analysis, and bones were not mirrored. Therefore, to simplify landmark description, the indication of medial and lateral in this table refer to the bone as if it were right (i.e., for a bone from the left side, medial should be replaced by lateral and vice versa).

Table 1: Landmark description of the eleven fixed landmarks.

For RPl-87, the landmark placing procedure was repeated three additional times. The precision of landmark placement was calculated based on the total of four repetitions using the centroid radius approach (von Cramon-Taubadel et al., 2007).

The bones were divided into different subgroups for the analyses to address our research questions. Procrustes superimposition was performed for each group separately by rotating, centering, and scaling. The resulting Procrustes coordinates were analyzed in a principal component analysis (PCA) based on a covariance matrix. RPl-87 was not included in the original PCAs; instead, its principal component scores were calculated, and it subsequently was projected onto the PC plots. The principal components depicted in the PC plot were chosen based on what best separated the sample. To display the associated shape changes, the 'warpRefMesh' function from the package geomorph was used to create thin-plate spline grids (Bookstein, 1989). In each analysis, the specimen most similar in shape to the average sample was warped to depict the shapes at the extremes of the principal components. Additionally, a matrix including the pairwise Procrustes distances was exported from R-Studio and imported into PAST (Hammer et al., 2001), to create a neighbor joining tree.

1.3 RESULTS

1.3.1. HAND OR FOOT

The precision of landmark placement was high with a maximum error rate of 2.77%.

As our first goal was to assess whether RPl-87 belonged to the hand or the foot, the sample was divided by ray, resulting in four subgroups including both hand and foot bones.

The PC plot depicting the analysis of second intermediate phalanges (Fig. 1, top left) shows the clearest separation of hand and foot bones when PC6 (2.76% of variance) is plotted against PC1 (65.88% of variance). RPl-87 plots inside the convex hull of foot bones extending mainly towards PC1 negative, whereas the hand bones are concentrated more towards PC1 positive and PC6 positive. It should be noted that the variation on PC6 appears to be mainly driven by one *Gorilla* specimen. In the neighbor joining tree (Fig. 1, top right), RPl-87 is closest to some *Gorilla* and *Pan* foot bones, as well as *Papio* foot bones. This is also reflected in the Procrustes distance values (not shown), as the *Ouranopithecus* phalanx shows the least mean distance to the *Papio* species.

In the analysis of the third ray (not shown), in which the best separation was achieved through a combination of PC1 (63.24% of variance) and PC4 (4.56% of variance), the *Ouranopithecus* specimen shows a distinctively higher negative loading on PC1 than the rest of the sample, placing it outside the convex hulls of either hand or foot bones. Nonetheless, it is closer to the majority of foot bones, in particular those of *Gorilla* and *Pan*, than to hand bones, which plot more towards PC1 positive and PC4 negative. The neighbor joining tree shows RPl-87 positioned at its bottom with two *Pan* foot bones in the closest proximity. As in the PC plot, the majority of hand bones are positioned further away from the *Ouranopithecus* bone, indicating that the bone is more similar to the foot bones in the sample. The species means of Procrustes distances place RPl-87 closest to *Gorilla* and *Papio*, whereas the similarity to *Pan*, as indicated by the neighbor joining tree, is limited to the two foot bones.

The shape analysis of the fourth ray (not shown) consistently showed poor separation of hand and foot bones, irrespective of the combination of principal components. Therefore, it was decided to focus on the PC axes reflecting the highest amount of variance: PC1 (62.07%) and PC2 (10.33%). As

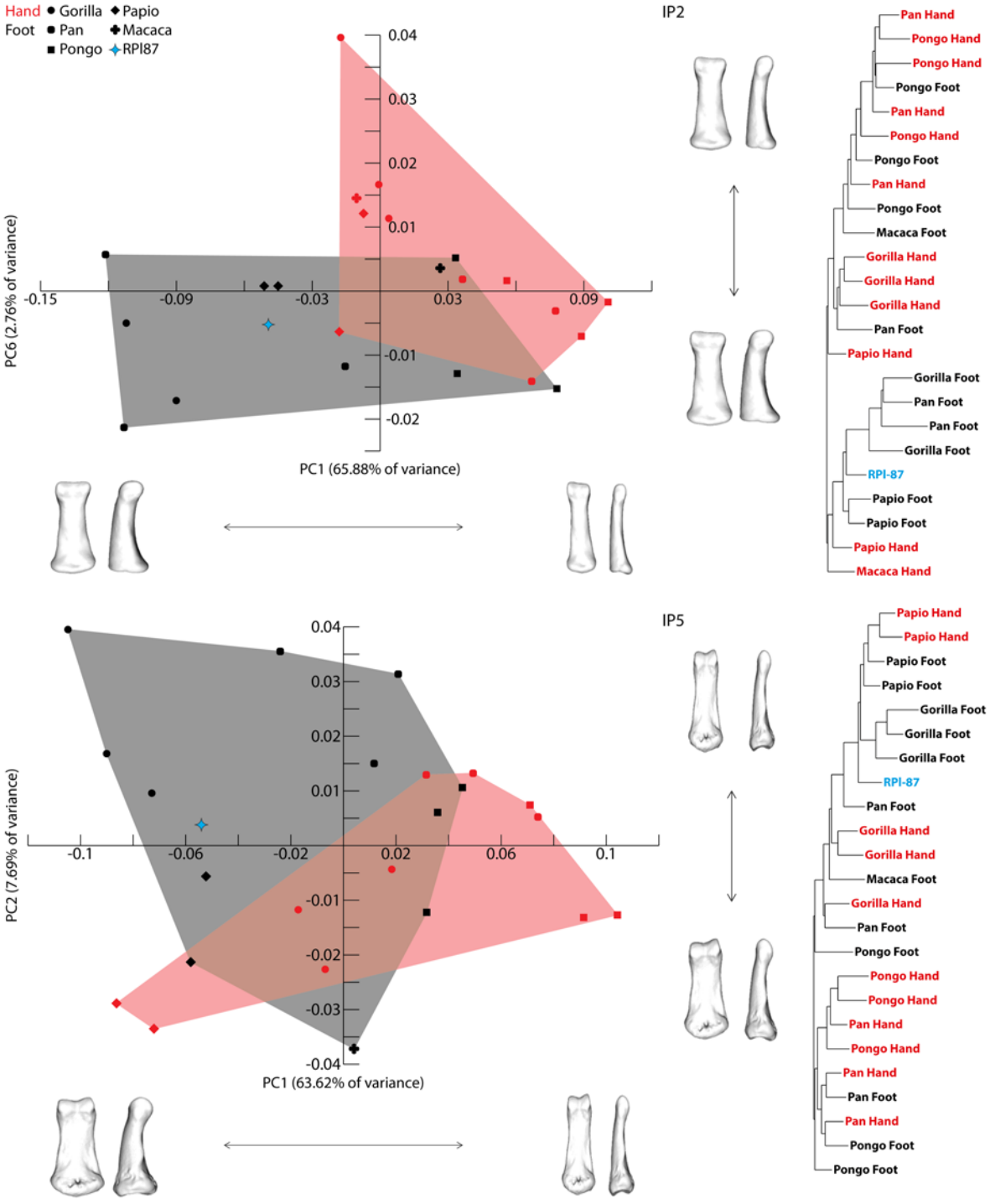

Figure 1: Top: PCA of Procrustes superimposed landmarks of the second intermediate phalanx without a priori group association. PC1 compared to PC6. Shape changes along PC1 and PC6 are illustrated below and to the right of the plot respectively. The neighbor joining tree based on the Procrustes distances is depicted on the right.
Bottom: PCA of Procrustes superimposed landmarks of the fifth intermediate phalanx without a priori group association. PC1 compared to PC2. Shape changes along PC1 and 2 are illustrated below and to the right of the plot respectively. The neighbor joining tree based on the Procrustes distances is depicted on the right. Coloration and symbology follow the legend in the top image.

in the analysis of the third intermediate phalanx, RPl-87 plots outside the range of either hand or foot bones. On PC1, it is most similar to a *Papio* hand bone that differs from the remaining hand bones in the sample by its high negative loading on that axis. When considering both PC1 and PC2, two *Gorilla* foot bones on the margin of the foot bone convex hull are slightly closer to the *Ouranopithecus* bone. On the neighbor joining tree, the *Papio* hand bone is situated on a sister branch to RPl-87 and three *Gorilla* foot bones are located on branches in the proximity. This is also reflected in the Procrustes distance values, as RPl-87 is closest to the *Papio* and *Gorilla* species. As in the PC plot, the separation of hand and foot bones is poor in the tree due to the high amount of overlap. However, there is a higher concentration of foot bones towards the bottom of the tree and in the proximity of RPl-87, while most hand bones, particularly those of *Pan* and *Pongo*, are located further away from the fossil.

Finally, when analyzing the intermediate phalanges of the fifth ray (Fig. 1, bottom left), the best separation was achieved by plotting PC1 (63.62% of variance) and PC2 (7.69% of variance). RPl-87 plots inside the shape variation of foot bones, closest to a *Papio* and a *Gorilla* specimen. The neighbor joining tree (Fig. 1, bottom right) shows the *Ouranopithecus* specimen situated close to a *Pan* and three *Gorilla* foot bones, but also in the proximity of *Papio* bones of both hand and foot. The latter is also reflected in the Procrustes distance values, as the mean distance is smaller from RPl-87 to *Papio* than to *Gorilla*.

Correlation analyses of PC1 and centroid size show that there is a moderate correlation (Pearson's r=0.55-0.66, assumptions are met) between these variables. However, this correlation does not appear to be related to size differences among species, but rather to differences in size among hand and foot bones, the latter being typically larger than the former.

1.3.2. RAY ALLOCATION AND AFFINITIES TO EXTANT PRIMATES

As the previous analyses have generally placed RPl-87 closer to foot bones than to hand bones, the latter were removed from the sample. In order to determine the ray RPl-87 belongs to, the foot bones of all rays were analyzed together in the subsequent analyses.

The plot of PC1 (60.9% of variance) and PC2 (9.08% of variance) shows substantial overlap between the different rays (Fig. 2, top left). The shape changes along PC1 appear to be mostly related to length-to-width proportions, with longer and more slender bones on PC1 positive and shorter and broader bones on PC1 negative. Shape changes on PC2 can be attributed to bone robusticity, particularly at the head and base, asymmetry in distal extension of the head condyles and the degree of proximal extension of the palmar area of the base. While the overlap among rays is particularly visible around the center of PC1, some degree of separation of the different rays is apparent at the extremes of this axis. The convex hulls of the second and fifth intermediate phalanx extend towards PC1 negative, while PC1 positive shows a higher concentration of bones from the third and fourth ray. With its negative PC1 loading, RPl-87 plots inside the range of variation of both second and fifth intermediate phalanges. The neighbor joining tree also reflects the overlap among rays (Fig. 2, top right), although the branches in the proximity of RPl-87 are mainly associated with bones from the second and fifth ray, with the only exception of a *Gorilla* fourth intermediate phalanx. In terms of Procrustes distances, this *Gorilla* bone appears to be closest to the fossil specimen. Nonetheless, when calculating the mean distance of each ray, RPl-87 shows the least distance to fifth intermediate phalanges, followed by bones of the second ray.

An improved separation is achieved when the phalanges are grouped by species instead of ray

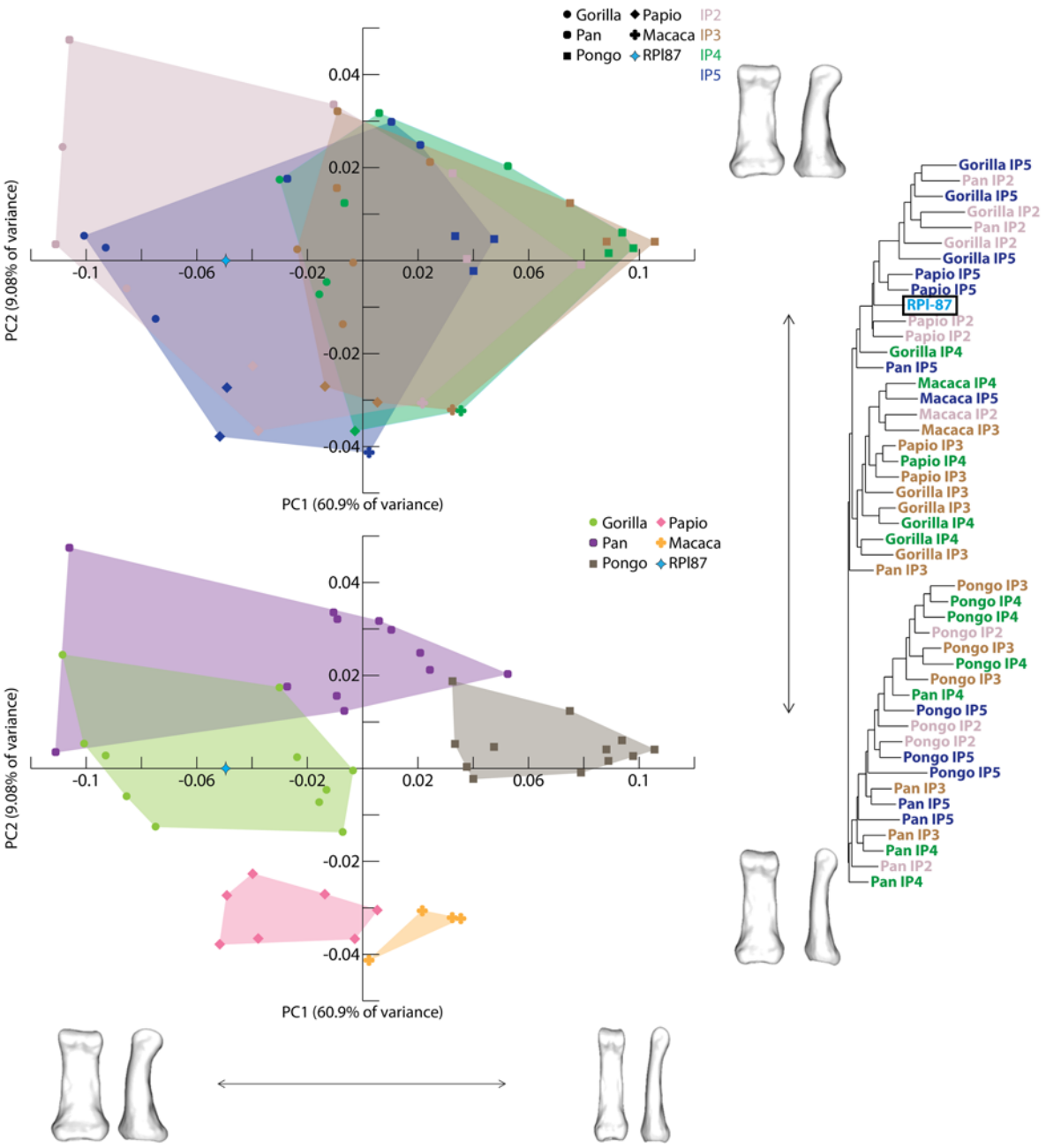

Figure 2: PCA of Procrustes superimposed landmarks of the pedal phalanges without a priori group association. PC1 compared to PC2. The upper plot depicts grouping by ray while the lower plot depicts grouping by species. Since the two plots represent the same analysis, the shape changes along PC1 and 2, illustrated below and to the right of the plots, respectively, apply for both plots. The neighbor joining tree based on the Procrustes distances is depicted on the right with coloration based on the different rays.

(Fig. 2, bottom left). This suggests that the variation driving the principal component analysis is not related to the differences in shape among rays, but to the differences among species. With its longer and more slender foot bones, *Pongo* is located at the extreme of PC1 positive. In contrast, *Gorilla* plots towards PC1 negative, together with two *Pan* foot bones. The majority of *Pan* bones, however, are clustered around the center of PC1 and towards PC2 positive. *Papio* and *Macaca* have

a similar loading on PC2 as they both fall on the negative extreme of this component, with *Papio* on PC1 negative and *Macaca* on PC1 positive. RPl-87 plots in the center of the *Gorilla* convex hull, indicating a possible similarity in shape to that species. Interestingly, in the neighbor joining tree, as well as in Procrustes distance values, the *Ouranopithecus* bone is more similar to the *Papio* foot bones. These contradictory results can be explained by that the PC plot focuses only on two axes of shape variance, whereas the Procrustes distances cover the entire shape variation in the sample.

PC1 of this analysis is again moderately correlated with centroid size (Pearson's r=0.52, assumptions are met). In this case, however, this correlation appears to be driven by size differences among the rays (ray 2 and 5 compared to ray 3 and 4) instead of species-related size differences, as the two species with the overall largest phalanges (*Gorilla* and *Pongo*) are represented on both extremes of PC1.

To further localize the ray RPl-87 belongs to, an analysis was conducted including only pedal intermediate phalanges of the second and fifth ray (not shown). However, the overlap between bones of the two rays is too extensive both in the PC plot and the neighbor joining tree to draw meaningful conclusions. Procrustes distance values indicate that RPl-87 is slightly more similar to fifth intermediate phalanges than to those of the second ray, but only by a small margin (0.085 as compared to 0.087).

1.4 DISCUSSION

This pilot study was an attempt at identifying the intermediate phalanx RPl-87, one of the only two postcranial elements so far associated with *Ouranopithecus macedoniensis,* through the application of 3D geometric morphometrics. According to the results of the principal component analyses and the neighbor joining trees, the shape of RPl-87 more closely resembles the shape of foot bones in our comparative sample. It differs from most hand bones analyzed here. This would suggest that, in contrast to a previous assessment of this specimen (de Bonis and Koufos, 2014), RPl-87 can likely be attributed to the foot. Additionally, the fact that the fossil plots outside the shape variation of both hand and foot bones of the third and fourth ray suggests that its shape diverges notably from the bones of these rays. This is supported by our analysis including foot bones only, which focused on ray identification. Despite the overlap among rays, the PC plot indicates a similarity in shape of RPl-87 and intermediate phalanges of the second and fifth ray. These results are in accordance with those by de Bonis and Koufos (2014), who suggested that the specimen represents a paramedian digit (two and five). Unfortunately, it was not possible to further specify the ray in this pilot study, as the overlap in shape of these two rays is too great. This could possibly be due to the fact that our sample includes bones from both the right and left side. The two paramedian digits are asymmetric in their morphology, especially of the head condyles, but in the opposite direction (i.e., a more distally extending head condyle can usually be observed on the lateral side of the second and the medial side of the fifth intermediate phalanx). Therefore, for a clearer identification, it would be necessary to identify the anatomical side of RPl-87. If this is not possible without first identifying the ray, the second option is to analyze a larger sample with balanced proportions of left and right phalanges to better observe and interpret potential patterns. While our analyses have identified a moderate correlation between the respective PC1s and size, this does not appear to be associated with species-related size differences, but rather with differences in size among hands, feet, and rays. Therefore, we propose that the variance on the first PCs is mainly

driven by variation in shape, whereas their correlation with size is likely an ancillary effect.

Our results additionally suggest RPl-87 to be most similar in shape to *Gorilla* and *Papio* foot bones. While reconstructing individual behavior from overall bone shape can be misleading (e.g., see Wallace et al., 2020), the fact that RPl-87 is more similar in shape to the terrestrial species than it is to *Pongo*—the most arboreal species in our sample—could suggest that *Ouranopithecus macedoniensis* was a terrestrial primate as well. A comparable conclusion has been drawn by de Bonis and Koufos (2014), as they have found similar morphological affinities in their analysis. Further support can be found in reconstructions of the paleoenvironment at Ravin de la Pluie, indicating an open and dry environment in this area (Koufos, 2006), and in the results of the microwear analysis on teeth of other fossils attributed to *O. macedoniensis* (Ungar, 1996; Merceron et al., 2005).

1.5 CONCLUSION AND OUTLOOK

The analysis of the overall bone shape of the intermediate phalanx RPl-87 has shown that the specimen can likely be attributed to the second or fifth ray of the foot. Additionally, its morphology appears to be most similar to intermediate phalanges of *Gorilla gorilla* and *Papio hamadryas*. While this study succeeded in showing the great potential of using 3D geometric morphometric analysis to identify the intermediate phalanx RPl-87, it also presented some issues that impede drawing secure conclusions. To corroborate and specify the results of this pilot study, future analyses of this bone should increase the size of the comparative sample. Not only should the number of individuals of the species analyzed here be increased, but future studies ideally should also include additional species of extant and—more crucially—fossil primates. Another important step is to apply a more detailed and specialized landmark configuration. RPl-87 shows some distinctive morphological traits such as the subtle curvature of its bone shaft and the faint development of its flexor sheath ridges. These traits could be captured with more specific landmark placement or with curve semilandmarks.

Finally, the shape analysis should be extended to the proximal phalanx RPl-86. As the two specimens represent a proximal and an intermediate phalanx, respectively, thorough analyses of not only the overall shapes of the bones, but also of their articular surfaces specifically, could shed light on whether these bones are from the same individual, the same foot or even from the same ray.

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