Reassessment of the phylogenetic relationships of the late Miocene apes *Hispanopithecus* and *Rudapithecus* based on vestibular morphology

Alessandro Urucioli1,a,2, Clément Zanolib, Sergio Almécija3,c,e, Amélie Beaudet4,e,f,g, Jean Dumoncelb, Naoki Morimoto1,a, Masato Nakatsukasa1,a, Salvador Moyà-Solà1,j,k, David R. Begun4, and David M. Alba5,1

1Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain; 2Univ. Bordeaux, CNRS, MCC, PACEA, UMR 5199, F-33600 Pessac, France; 3Division of Anthropology, American Museum of Natural History, New York, NY 10024; 4New York Consortium in Evolutionary Primatology, New York, NY 10016; 5Department of Anthropology, University of Cambridge, Cambridge CB2 1QH, United Kingdom; 6School of Geography, Archaeology, and Environmental Studies, University of the Witwatersrand, Johannesburg, WITS 2050, South Africa; 7Department of Anatomy, University of Pretoria, Pretoria 0001, South Africa; 8Laboratoire Anthropologie and Image Synthesis, UMR 5288 CNRS, Université de Toulouse, 31073 Toulouse, France; 9Laboratory of Physical Anthropology, Graduate School of Science, Kyoto University, 606 8502 Kyoto, Japan; 10Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain; 11Unitat d’Antropologia, Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain; and 12Department of Anthropology, University of Toronto, Toronto, ON M5S 2Z5, Canada

Edited by Justin S. Sipilä, University of Iowa, Iowa City, IA, and accepted by Editorial Board Member C. O. Lovejoy December 3, 2020 (received for review July 19, 2020)

Late Miocene great apes are key to reconstructing the ancestral morphotype from which earliest hominins evolved. Despite consensus that the late Miocene dryopithecoid great apes *Hispanopithecus laietanus* (Spain) and *Rudapithecus hungaricus* (Hungary) are closely related (Hominidae), ongoing debate on their phylogenetic relationships with extant apes (stem hominoids, hominines, or pongines) complicates our understanding of great ape and human relationships with extant apes (stem hominids, hominines, or pongines). While most Asian extinct great apes, such as *Abocador de Can Mata* (6, 17–20), were included within the genus *Rudapithecus* (2, 3, 5, 7, 18, 21–26), *Hispanopithecus* (Spain) and *Rudapithecus* (Hungary) were classified in a subfamily (Dryopithecinae) (6, 20, 26) or tribe (Dryopithecini) (3, 7, 21) of their own, distinct from pongines. Both taxa possess a hominid-like cranial morphology (6, 11–13, 21, 25, 28, 29), as shown by the high zygomatic root, reduced midfacial prognathism, lack of subarcuate fossa, deep glenoidridge, and hominoid (apes and humans) origins. Given the relevance of the phylogenetic signal of the bony labyrinth, here we assess the phylogenetic affinities of the late Miocene great apes *Hispanopithecus* and *Rudapithecus* by studying their inner ear morphology. Our results are consistent with the distinction of *Rudapithecus* and *Hispanopithecus* at the genus rank and further support their allocation to the Hominidae based on their derived semicircular canal volumetric proportions. Compared with extant hominoids, the vestibular morphology of *Hispanopithecus* and *Rudapithecus* most closely resembles that of African apes, and differs from the derived condition of orangutans. However, the vestibular morphologies reconstructed for the last common ancestors of dryopithecids, crown hominines, and crown hominids are very similar, indicating that hominines are plesiomorphic in this regard. Therefore, our results do not conclusively favor a hominine or stem hominid status for the investigated dryopiths.

Significance

Reconstructing the phylogenetic relationships of extinct apes is challenging due to their fragmentary fossil record and the recurrent independent evolution of morphological features. Given the relevance of the phylogenetic signal of the bony labyrinth, here we assess the phylogenetic affinities of the late Miocene great apes *Hispanopithecus* and *Rudapithecus* by studying their inner ear morphology. Our results are consistent with the distinct generic status of these dryopithecids, which further differ from the derived condition of orangutans and most closely resemble African apes. However, the latter appear largely primitive (similar to the last common ancestor of most apes and humans). Hence, our results do not conclusively favor a closer relationship with African apes as opposed to great apes as a whole.


The authors declare no competing interest.

This article is a PNAS Direct Submission. J.S.S. is a guest editor invited by the Editorial Board. Published under the PNAS license.

Published under the PNAS license.

1To whom correspondence may be addressed. Email: alessandro.urucioli@icp.cat or david.alba@icp.cat.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2015215118/-/DCSupplemental.

Published January 25, 2021.

PNAS 2021 Vol. 118 No. 5 e2015215118

https://doi.org/10.1073/pnas.2015215118 | 1 of 12
fossa, and prominent entoglenoid process. However, there is no consensus regarding the phylogenetic position of this group—being either considered stem hominids (6, 19, 30), stem hominines (2, 3, 14, 16, 25), or even pongines (10, 28, 29)—which may be informally referred to as “dryopiths.” Resolving the phylogenetic position of dryopiths has important implications for the evolution of the great ape and human clade, since their purported hominine status has led to paleobiogeographic scenarios favoring a European origin and subsequent back-to-Africa dispersal for the African and human clade (2, 3, 15, 24, 25). Disagreements and uncertainties about the phylogenetic position of extinct apes are persistent, and stem from a combination of factors, including the incomplete and fragmentary hominoid fossil record, the decimated current diversity of the group, and pervasive homoplasy coupled with mosaic evolution (6, 23, 31–35).

Fig. 1. The vestibular apparatus morphology of *R. hungaricus* (A–C), *H. laietanus* (D), fossil hominoids (E and F), and individuals from extant hominoid genera (G–M) as depicted by renderings of the 3D models. From left to right, in posterolateral, superior, and posterior medial views: (A) *R. hungaricus* (RUD 77L); (B) *R. hungaricus* (RUD 77R); (C) *R. hungaricus* (RUD 200); (D) *H. laietanus* (IPS18000); (E) *Oreopithecus* (BAC 208); (F) *Nacholapithecus* (BG 42744); (G) *Hoolock hoolock* (AMNH.M 83425); (H) *Symphalangus syndactylus* (AMNH.M 106583); (I) *Hylobates lar* (MCZ 41424); (J) *Pongo* (IPS10647); (K) *Gorilla gorilla* (AMNH.M 167338); (L) *Pan troglodytes* (AMNH.M 51204); (M) *Homo sapiens* (F 04). (Scale bars, 5 mm.)
The morphology of the semicircular canals (SCs), which partly constitute the inner ear’s bony labyrinth, has been classically related to locomotion (36–42). However, several studies have highlighted the possibility of inferring phylogenetic relatedness based on this portion of the inner ear morphology (43–47). Recently, it has been shown that this anatomical structure also embeds a strong phylogenetic signal among catarrhine primates by means of three-dimensional geometric morphometric (3DGM) analyses (5, 47–49), thus being potentially useful to test phylogenetic hypotheses for extinct hominoids. Previous studies relied on the SC radius of *Nacholapithecus* and *Hispanopithecus* to infer slow and deliberate arboreal locomotion for these species (41). However, recent analyses raised doubts about the reliability of locomotor behavior predictions based on the SC radius only (50, 51). In contrast, here we rely on microcomputed tomography (μCT) scans of the same specimens and a deformation-based (landmark-free) 3DGM approach to assess their closest affinities in SC morphology with extant hominoids and interpret them from an evolutionary viewpoint. First, we describe the fossil remains and qualitatively compare them with extant hominoids. Second, we assess if the volumetric proportions of their SCs more closely resemble those of hominids than those of other anthropoids. Third, we quantitatively evaluate changes in SC and vestibule morphology by means of a between-group principal component analysis (bgPCA) applied to a sample of extant and extinct hominoids. The affinities of the investigated fossil taxa are further assessed by means of cluster analyses and group membership probabilities based on reconstructed ancestral morphotypes under various phylogenetic assumptions for dryopithecids.

**Results**

**Descriptions and Comparisons.** Three-dimensional renderings of the vestibular apparatus of fossil and extant hominoids investigated here are illustrated in the Fig. 1. The vestibular apparatus of *Rudapithecus* is well preserved in the three available specimens (Fig. 1A–C). As in extant hominids, the SCs are stout—although less so than in orangutans (Fig. 1J), most humans (Fig. 1M), and gorillas (Fig. 1K)—and the vestibule is large relative to theSC volume occupied by SCs. The anterior and posterior canals are large and similar in size (Fig. 1A and B). The anterior canal is slightly vertically compressed, as in extant hominoids and the fossil apes *Nyanzapithecus alesi* (4) and *Nacholapithecus kerioi* (Fig. 1F), and somewhat larger in RUD 77 than in RUD 200. The anterior canal is somewhat anterosuperiorly projecting, albeit much less so than in *Pongo* (Fig. 1J) and *Oreopithecus* (Fig. 1E). The lateral and posterior canals are slightly different between the two individuals. In RUD 77, the lateral canal is noticeably smaller than the other SCs (Fig. 1A and B), slightly compressed horizontally, and slenderer than in RUD 200. The lateral canal of RUD 200 is stout and large, almost reaching the size of the vertical SCs (similar to the condition in African apes, yet smaller than in gorillas) (Fig. 1C), and its slender portion connects with the vestibule somewhat more inferiorly than in RUD 77. The junction of the slender portion of the lateral canal and the ampulla further differs between the two individuals, as it protrudes anteriorly in RUD 200, while it is posterolaterally oriented in RUD 77. In both individuals, the ampullary portion bends superiorly and the slender segment between the connection with the vestibule and the posterolateral tip of the lateral canal is straight, as in *Rudapithecus* (Fig. 1A–C), *Hoolock* (Fig. 1G), and most hominids (Fig. 1J–M), and laterally oriented, as in *Pongo* (Fig. 1J), some humans (Fig. 1M), and RUD 77 (Fig. 1A and B). The ampullary portion of the lateral canal is bent superiorly, as in *Rudapithecus* (Fig. 1A–C) and extant hominoids (Fig. 1G–M). However, unlike extant great apes (Fig. 1J–L) and *Rudapithecus* (Fig. 1A–C), the portion between the ampulla and the tip of the lateral canal is inflated. The posterior canal is small and rounded, with a large ampulla. The CC is longer than in *Rudapithecus* (Fig. 1A–C) and in most extant great apes (with *Pongo* showing the shortest), yet more inflated (even if much less so that in orangutans) (Fig. 1J), and the CC apex forms an obtuse angle. As in *Rudapithecus* and extant hominoids, the planes identified by the lateral and posterior canals form a right angle and their trajectories do not intersect.

**Volumetric Proportions.** Allometric regressions of SC volume vs. length were performed separately for hominoids and the rest of anthropoids included in the sample (Fig. 24; measurements for the dryopiths are given in SI Appendix, Table S1), because it has been previously shown that the former display an allometric grade shift toward relatively higher volumes at a comparable length once size-scaling effects have been taken into account (5), with only minimal overlap. *Hispanopithecus* falls above the hominid regression line, while *Rudapithecus* is situated more (RUD 77) or less (RUD 200) below the line, close to *Nacholapithecus*, but in all cases within the range of extant hominids and well above the regression line of other anthropoids (Fig. 24). Gorillas are variable in this regard, while humans and orangutans display stouter proportions than chimpanzees and bonobos (Fig. 2B). The SCs of *Hispanopithecus* appear intermediate between these aforementioned taxa (closer to humans and orangutans), while those of *Rudapithecus, Oreopithecus*, and *Nacholapithecus* are slenderer and more comparable to those of chimpanzees and bonobos. Overall, given their range of variation, all the extinct apes analyzed here display extant hominid-like volumetric proportions of the vestibular apparatus.

**Shape Analysis.** The bgPCA (Fig. 3), based on the deformation fields computed for the hominoid sample, allows us to discriminate extant hominoid species, as shown by classification results...
Fig. 2. (A) Allometric regressions of cube root of SC volume (ln VolSC, in millimeters) vs. SC length (ln L, in millimeters) in anthropoids. Lines represent ordinary least-squares best-fit lines for extant hominids (red) and other extant anthropoids (blue). Both hominoids and other anthropoids show a negatively allometric relationship between VolSC and L, but with a marked allometric grade shift, such that hominoids possess stouter canals than other anthropoid taxa at comparable lengths (see SI Appendix, Table S1 for comparative sample measurements). (B) Boxplots of allometric residuals computed using the best-fit line of the nonhominid anthropoid regression as baseline. Horizontal line is the median, boxes represent the interquartile range, whiskers represent maximum (mostly positive values) from hylobatids (negative values), with others overlapping with orangutans and humans, while the Rudapithecus specimens fall within the African great ape range. Both Rudap 77 and Rudap 200 closely approach the origin, with the latter showing slightly more positive values. Oreopithecus and Nacholapithecus are found on moderate negative values, within the lower range of Pan and Gorilla, due to their slighter slender SCs (albeit clearly stouter than in hylobatids).

The patterns of shape variation captured by bgPC2 (33.4% of total variance) (Fig. 3A) reflect changes in the shape of three canals as well as their relative proportions. In particular, bgPC2 clearly discriminates Homo (with most negative values) from the rest of the sample, due to the presence in the former of a large and rounded (sometimes even slightly superiorly elongated) anterior canal, a posterolaterally displaced inferior portion of the posterior canal, and a small, fairly anterolaterally elongated lateral canal, whose slender portion connects to the vestibule more superiority and anterolaterally than in apes. The latter fall on intermediate and positive values, with hylobatids considerably overlapping with Pan spp. (Fig. 3A). To a large extent, this is due to their anterior canal shape, which appears intermediate (99% of correctly classified individuals before and after cross-validation). These results closely resemble those computed using a cross-validated bgPCA (SI Appendix, Fig. S1). We also recover very significant group mean differences (P < 0.001) for the raw shape data (SI Appendix, Table S2), confirming that group structure does not artifactualy result from the bgPCA (52). Indeed, group differences account for a substantial amount of structure does not artifactually result from the bgPCA (52). In-
Fig. 3. Results of the bgPCA based on vestibular shape (as reflected by deformation data) in hominoids using genera as grouping factor (variance explained by each bgPC is included within parentheses): (A) bgPC2 vs. bgPC1; (B) bgPC3 vs. bgPC1. Extreme conformations for each bgPC are displayed: (C) bgPC1; (D) bgPC2; (E) bgPC3. Convex hulls are drawn for each hominoid genus and colored as follows: blue, *Pongo*; black, *Gorilla*; green, *Pan*; lilac, *Homo*; red, *Hylobates*; orange, *Symphalangus*; cyan, *Hoolock*.
between the rounded morphology of humans and the marked vertical compression of *Pongo* and *Gorilla* (the latter taxa occupying more positive values with only very slight overlap with *Pan* and hylobatids). Both *Rudapithecus* and *Hispanopithecus*, like *Oreopithecus* and *Nacholapithecus*, show intermediate values along this axis, overlapping with hylobatids and *Pan* spp. (as well as the *Australopithecus* specimen StW 573), but not with *Pongo* and *Gorilla*. Conversely, the other australopith (StW 578) more closely approaches humans due to its larger vertical SCs.

bgPC3 (11.4% of variance) (Fig. 3B) is driven by the shape of the anterior canal, its relative size compared with that of the lateral one, the length of the CC, and the amount of torsion of the lateral canal. Thus, negative values reflect a large and anterosuperiorly projecting anterior canal, coupled with a small lateral one, and a short CC. This axis discriminates *Pongo* (most negative values) from the rest of the sample, only minimally overlapping with some *Hylobates*. One individual of *Rudapithecus* (RUD 77) and *Oreopithecus* overlap with the range of orangutans due to their anterosuperiorly projecting anterior canal (albeit less so in RUD 77), short CC, and markedly small lateral canal. A similar morphology of the anterior canal is also found in *Australopithecus* (most RUD 200) fall at the negative end of the gorilla and human variation, due to their intermediate anterior canal morphology, longer CC (yet less so than in most *Pan* and *Gorilla* individuals), and a larger lateral canal. *Nacholapithecus* and the other australopith specimen (StW 578) fall among moderate positive values, overlapping with gorillas, chimpanzees, bonobos, humans, and hylobatids, due to their long CC and more vertically aligned (i.e., superiorly directed) connection of the anterior canal with the CC.

When the inspected bgPCs are considered simultaneously to compute posterior probabilities of group membership (Table 1), the *Rudapithecus* RUD 77 individual occupies a position in the morphospace that does not fit well with most extant hominoid genera (*P* < 0.05), rather approaching the position of *Nacholapithecus* and *Oreopithecus* in the morphospace (Table 2). Conversely, RUD 200 shows considerable similarities with *Pan* (*P* = 0.549) and *Nacholapithecus*. The three *Rudapithecus* specimens fall closer to one another than either approaches the single specimen of *Hispanopithecus* (Table 2), which is also more distant than *Nacholapithecus* from all the considered specimens (Table 2). *Hispanopithecus* mostly differs along bgPC1, sharing similarities in the volumetric proportions of the SCs and in the vertically compressed anterior canal morphology with *Australopithecus* individual StW 573 (Table 2). IPS18000 marginally differs from *Pan* (*P* = 0.053) and is clearly an outlier compared to the remaining extant genera.

The cluster analyses based on the significant bgPCs (Fig. 4A) and raw shape data (Fig. 4B) further support the aforementioned results, since *Rudapithecus* and *Hispanopithecus* do not cluster with one another and show affinities with different taxa. In particular, the cluster based on the bgPCA results (Fig. 4A) indicates that *Rudapithecus* is most similar to both *Pan* and *Nacholapithecus*, while *Hispanopithecus* approaches hominins. This is further supported by the raw shape data cluster (Fig. 4B), which mainly differs by recovering a great ape cluster.

**Phylomorphospace and Reconstruction of Ancestral Morphologies.** The shape data, as captured by the bgPCA performed on the extant hominoid sample, approaches the Brownian motion model of evolution, as supported by the phylogenetic signal computed for the bgPCs (*K*nl = 0.864, *P* = 0.019) and for the raw data (i.e., the deformation fields; *K*nl = 0.863, *P* = 0.017). We used phylogenetically informed techniques on the shape data to visualize the direction and magnitude of vestibular shape change during hominoid evolution as well as to depict the internal nodes of the phylogeny—that is, the inferred vestibular morphology of the last common ancestors (LCAs) of major groups—as reconstructed by maximum likelihood. The results are very similar irrespective of the precise phylogenetic placement of dryopithecids as stem hominines (Fig. 5 and SI Appendix, Fig. S2B), stem hominins (*SI Appendix*, Figs. S2A and S3 A and C), or stem pongines (*SI Appendix*, Figs. S2C and S3 B and D). The crown hominoid LCA (Figs. 5 and 6A) is reconstructed as possessing evenly sized and moderately inflated SCs, a moderately long and not inflated CC, a fairly vertically compressed, yet not anterosuperiorly projecting anterior canal, an almost rounded posterior canal, and an obtuse angle among the planes identified by the anterior and posterior canals (close to the right angle), and a right angle among the SCs merging at the CC apex

| Table 1. Mahalanobis squared distances (D²) between fossil scores and extant hominoid group centroids and associated posterior probabilities (P) of group membership for all fossil individuals |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|  | Hoook | Symphalangus | Hylobates | Pongo | Gorilla | Pan | Homo |
| **D²** | | | | | | | | | | | | |
| IPS18000 (Hispanopithecus laietanus) | 6.086 | 6.861 | 10.532 | 9.229 | 5.329 | 1.407 | 5.076 |
| RUD 77R (Rudapithecus hungaricus) | 2.044 | 2.776 | 4.233 | 8.821 | 9.809 | 2.736 | 4.063 |
| RUD 77L (Rudapithecus hungaricus) | 2.252 | 3.325 | 4.104 | 6.735 | 10.091 | 3.132 | 5.621 |
| RUD 200 (Rudapithecus hungaricus) | 2.478 | 3.312 | 5.338 | 7.475 | 5.174 | 0.745 | 6.566 |
| BAC 208 (Oreopithecus bambolii) | 4.359 | 6.261 | 4.809 | 4.087 | 15.042 | 7.218 | 11.210 |
| BG 42744 (Nacholapithecus kerioi) | 1.200 | 1.662 | 3.546 | 10.016 | 4.942 | 0.683 | 7.673 |
| STW 573 (Australopithecus sp.) | 5.323 | 5.367 | 9.865 | 14.341 | 5.192 | 1.246 | 4.182 |
| **P** | | | | | | | | | | | | |
| IPS18000 (Hispanopithecus laietanus) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.053 | <0.001 |
| RUD 77R (Rudapithecus hungaricus) | 0.006 | 0.003 | <0.001 | <0.001 | <0.001 | 0.011 | <0.001 |
| RUD 77L (Rudapithecus hungaricus) | 0.015 | 0.005 | <0.001 | <0.001 | <0.001 | 0.012 | <0.001 |
| RUD 200 (Rudapithecus hungaricus) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.549 | <0.001 |
| BAC 208 (Oreopithecus bambolii) | 0.016 | 0.001 | <0.001 | <0.001 | <0.001 | 0.026 | <0.001 |
| BG 42744 (Nacholapithecus kerioi) | 0.035 | 0.012 | <0.001 | <0.001 | <0.001 | 0.184 | <0.001 |
| STW 573 (Australopithecus sp.) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.026 | <0.001 |
| STW 578 (Australopithecus sp.) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.001 | 0.013 |

Note that these are probability estimates of having a particular score given membership in a particular group, not the likelihood of group membership in each of a priori defined groups given a particular score. The lowest D² and the highest probability for each specimen are in bold.
Irrespective of the phylogenetic assumptions for dryopiths, the reconstructed LCA for crown hominoids is closer to hominids (especially Nacholapithecus, Rudapithecus and, among extant taxa, Pan) than to hylobatids (Fig. 5), in terms of their intermediate volumetric proportions, contrasting with the markedly slenderer SCs of gibbons and siamangs. In turn, the LCAs of crown hominines and dryopiths (Rudapithecus + Hispanopithecus) closely resemble one another irrespective of the underlying phylogenetic assumptions for the fossil species (Fig. 5 and SI Appendix, Fig. S3), being extant hominid-like in volumetric proportions but otherwise showing a more plesiomorphic morphology in the evenly sized and fairly rounded SCs.

The inferred LCA of crown hominids, in particular, closely resembles that of crown hominoids, except for the stouter volumetric proportions, more derived toward the extant hominid condition (Figs. 5 and 6B). It displays equally sized SCs, an obtuse to right angle in the apex of a moderately long CC, and a slightly laterally elongated posterior canal (Fig. 6B). Orangutans appear derived from the LCA by displaying more inflated SCs (especially the anterior one) (Figs. 1L and 6B), further diverging in the opposite direction from African great apes and humans because its short and extremely stout CC, as well as its anterosuperiorly projecting anterior canal, differing from the hominid LCA by the somewhat less vertically compressed anterior canal (Fig. 6B and D).

Hispanopithecus and Rudapithecus appear to have diverged in opposite directions from their LCA (Fig. 5). The former seems derived in the volumetric proportions (similarly to Pongo, Australopithecus, and Homo), whereas the Rudapithecus condition in this regard is very similar to that of Pan as well as the reconstructed hominid LCA, and (to a lesser extent) to those of Nacholapithecus and Oreopithecus (Figs. 5 and 6B). Similarly, the fairly short CC and a somewhat anterosuperiorly projecting anterior canal found in Rudapithecus (less so than in orangutans and Oreopithecus) contrast with the longer CC and the rectangular-shaped anterior canal found in Hispanopithecus (Fig. 1D). In these regards, Hispanopithecus more closely resembles the members of the African ape and human clade (Fig. 1A–C).

In summary, each extant hominid genus is derived in a particular direction from the ancestral morphology, with Pan remaining close to the hominid and hominine LCAs;

Table 2. Mahalanobis distances ($D^2$) between dryopiths and other fossils

<table>
<thead>
<tr>
<th>$D^2$</th>
<th>IPS18000</th>
<th>RUD 77R</th>
<th>RUD 77L</th>
<th>RUD 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPS18000 (Hispanopithecus laietanus)</td>
<td>—</td>
<td>2.037</td>
<td>2.504</td>
<td>1.012</td>
</tr>
<tr>
<td>RUD 77R (Rudapithecus hungaricus)</td>
<td>2.037</td>
<td>—</td>
<td>0.179</td>
<td>0.772</td>
</tr>
<tr>
<td>RUD 77L (Rudapithecus hungaricus)</td>
<td>2.504</td>
<td>0.179</td>
<td>—</td>
<td>0.848</td>
</tr>
<tr>
<td>RUD 200 (Rudapithecus hungaricus)</td>
<td>1.012</td>
<td>0.772</td>
<td>0.848</td>
<td>—</td>
</tr>
<tr>
<td>BAC 208 (Oreopithecus bambolii)</td>
<td>6.678</td>
<td>2.495</td>
<td>1.385</td>
<td>3.571</td>
</tr>
<tr>
<td>BG 47244 (Nacholapithecus keriol)</td>
<td>2.286</td>
<td>1.270</td>
<td>1.505</td>
<td>0.416</td>
</tr>
<tr>
<td>StW 573 (Australopithecus sp.)</td>
<td>0.703</td>
<td>2.587</td>
<td>3.657</td>
<td>1.637</td>
</tr>
<tr>
<td>StW 578 (Australopithecus sp.)</td>
<td>3.080</td>
<td>2.745</td>
<td>3.087</td>
<td>4.479</td>
</tr>
</tbody>
</table>

These distances are based on the scores of the significant bgPCs (bgPC1–bgPC3).

(Fig. 6A). Irrespective of the phylogenetic assumptions for dryopiths, the reconstructed LCA for crown hominoids is closer to hominids (especially Nacholapithecus, Rudapithecus and, among extant taxa, Pan) than to hylobatids (Fig. 5), in terms of their intermediate volumetric proportions, contrasting with the markedly slenderer SCs of gibbons and siamangs. In turn, the LCAs of crown hominines and dryopiths (Rudapithecus + Hispanopithecus) closely resemble one another irrespective of the underlying phylogenetic assumptions for the fossil species (Fig. 5 and SI Appendix, Fig. S3), being extant hominid-like in volumetric proportions but otherwise showing a more plesiomorphic morphology in the evenly sized and fairly rounded SCs.

The inferred LCA of crown hominids, in particular, closely resembles that of crown hominoids, except for the stouter volumetric proportions, more derived toward the extant hominid condition (Figs. 5 and 6B). It displays equally sized SCs, an obtuse to right angle in the apex of a moderately long CC, and a slightly laterally elongated posterior canal (Fig. 6B). Orangutans appear derived from the LCA by displaying more inflated SCs (especially the anterior one) (Figs. 1L and 6B), further diverging in the opposite direction from African great apes and humans because its short and extremely stout CC, as well as its anterosuperiorly projecting anterior canal, differing from the hominid LCA by the somewhat less vertically compressed anterior canal (Fig. 6B and D).

Hispanopithecus and Rudapithecus appear to have diverged in opposite directions from their LCA (Fig. 5). The former seems derived in the volumetric proportions (similarly to Pongo, Australopithecus, and Homo), whereas the Rudapithecus condition in this regard is very similar to that of Pan as well as the reconstructed hominid LCA, and (to a lesser extent) to those of Nacholapithecus and Oreopithecus (Figs. 5 and 6B). Similarly, the fairly short CC and a somewhat anterosuperiorly projecting anterior canal found in Rudapithecus (less so than in orangutans and Oreopithecus) contrast with the longer CC and the rectangular-shaped anterior canal found in Hispanopithecus (Fig. 1D). In these regards, Hispanopithecus more closely resembles the members of the African ape and human clade (Fig. 1A–C).

In summary, each extant hominid genus is derived in a particular direction from the ancestral morphology, with Pan remaining close to the hominid and hominine LCAs;
Nacholapithecus appears as the least derived among both extant and fossil hominid taxa, together with projecting anterior canal (Fig. 1 taxon also shows similarities with 8o f1 2

Phylomorphospaces of the vestibular apparatus in hominoids, Fig. 5. clade of stem hominines (obtained by projecting the phylogenetic tree that considers dryopithecines a bgPC1; (arrowheads. See SI Appendix maximum likelihood for the LCAs of various clades are depicted by means of phylogenetic hypotheses (bgPCs. The tips correspond to genus bgPCA score centroids: (not shown) are virtually identical. key ancestral morphologies reconstructed using deformation-interest as inferred using maximum-likelihood methods for deformation-

Fig. 6. Reconstructed vestibular shape for the LCA of the main clades of interest as inferred using maximum-likelihood methods for deformation-based 3DGM analyses applied to the hominoid sample under the stem-hominine phylogenetic hypothesis for dryopithecids (SI Appendix, Fig. 51 B), in postero-lateral (left), superior (center), and posteromedial (right) views. The reconstructed LCAs depicted are the following: (A) Crown hominoids; (B) crown hominines; (C) crown hominines; (D) dryopithecines (Hispanopithecus + Rudapithecus). The results for the other phylogenetic hypotheses (not shown) are virtually identical. LCA and, to a lesser extent, Nacholapithecus), with hominines (particularly gorillas) and especially orangutans having subsequently derived in opposite directions.

Discussion

Our results show that the vestibular morphology of both Hispanopithecus and Rudapithecus more closely resembles that of extant great apes and humans than than of hyllobatids, in agreement with the current consensus that they belong to the great-ape-and-human clade (2, 3, 6, 26). These similarities particularly concern the volumetric proportions of the SCs as well as the size of the latter relative to the vestibular recesses. Volumetric proportions, as reflected by the ratio between the volume and the length of the SCs, appear particularly relevant given that an allometric grade shift has been previously identified to characterize all extant hominids, so that they display relatively more voluminous SCs than other anthropoids (including hyllobatids) at comparable


https://doi.org/10.1073/pnas.2015215118 Reassessment of the phylogenetic relationships of the late Miocene apes Hispanopithecus and Rudapithecus based on vestibular morphology

Urciuoli et al.

8 of 12 | PNAS
https://doi.org/10.1073/pnas.2015215118
lengths (5). The derived condition of hominoids has been linked with locomotion (5), but is noteworthy that chimpanzees, bonobos, and gorillas are slightly more variable in SC volumetric proportions than orangutans and humans. Given the relationship between SC shape variation and locomotion noted by some authors (53, 54), our results might reflect stronger locomotion-related selection pressures in orangutans and humans.

The classification results based on the bgPCA as well as the cluster analyses indicate that the two investigated dryopiths are distinguishable from one another, with the three specimens (two individuals) of Rudapithecus being more similar to one another than to the single specimen of Hispanopithecus. This result, together with other cranial differences (e.g., morphology of the frontal squama, premaxilla, and zygomatic), supports the distinction of these taxa at the genus rank (2, 3, 7, 21, 25, 35). Rudapithecus generally displays a somewhat more primitive morphology, closer to the one inferred for the crown hominid LCA. It shows some similarities with the fossil hominoids Oreopithecus and Nacholapithecus. The latter taxon appears more primitive than other hominoids, in agreement with a previous study based on the entire inner ear morphology (49).

However, both the hominid-like volumetric proportions of Nacholapithecus and the lack of a subarcuate fossa (55) support its item hominid status, closely resembling the morphotype reconstructed for the crown hominin LCA. The morphology of Rudapithecus also resembles that of crown hominoids, such as Pan (volumetric proportions and the relative size of the SCs) and, to a lesser extent, orangutans (the somewhat anterosuperiorly projecting anterior canal and the short CC). As previously noted (5), chimpanzees and bonobos appear least derived than other extant hominoids. This is shown by the possession of similarly sized SCs (shared with the reconstructed crown hominin and crown hominid LCA, while the dryopith LCA displays a slightly smaller lateral canal) and, especially, by the fairly slender volumetric proportions (intermediate between the hominine and hominid LCA, yet closer to the latter). This is also supported by the similarities between Pan species and Miocene apes, especially Nacholapithecus. Nonetheless, chimpanzees and bonobos appear derived in some features (the small and rounded posterior canal as well as the obtuse angle of the CC apex), just like gorillas and humans are derived in other directions (largest lateral canal radius, the other SCs and markedly enlarged vertical canals, respectively).

Among hominoids, orangutans and humans show the most extreme condition in the volumetric proportions of the SCs. Orangutans further diverge from the hominid LCA by the anterosuperiorly projecting anterior canal (even more so than in hylobatids). Hispanopithecus appears more derived than the other Miocene taxa, especially by the stouter SCs, while it does not fit well within the variation of any extant genus. More clearly than Rudapithecus, Hispanopithecus displays a mosaic of features that is unknown among extant hominoids, including similarities with chimpanzees and bonobos (in the long CC), humans (the obtuse angle of the CC apex and the right angle between the planes of the posterior and lateral canals), and orangutans (the stout CC and the voluminous vestibular recesses, the latter also shared with humans) coupled with some unique features (the swollen area between the ampulla and the tip of the lateral canal, and the markedly inflated ampullae).

Interpreting the similarities of the investigated dryopiths in evolutionary terms is not straightforward. The results of the phylomorphospace approach and the reconstructed ancestral vestibular morphologies suggest that modern hominid-like volumetric proportions of the SCs would have been present in the LCA of crown hominoids, while that of crown hominoids as a whole would have displayed somewhat intermediate proportions between hylobatids and hominoids (yet closer to the latter). Differences in volumetric proportions of the SCs have been related to locomotor adaptations, because they directly affect the sensitivity and steadiness of the SCs in response to angular accelerations (5, 56). Hence, the moderately stout SCs of the LCA of crown hominoids indicate that it showed a slow type of locomotion, which was present, to a large extent, also in the LCA of crown hominoids, as previously inferred based on the size of the SC radius alone (41). Both Rudapithecus and Hispanopithecus show a wide gap between the lateral and posterior canals (the planes defined by them are well separated and do not intersect), caused by the anterolateral location of the lateral canal. This trait has been linked to orthograde behaviors (42), in agreement with the fossil evidence available for these taxa (6, 11, 23, 25, 35, 57–61). However, from a phylogenetic viewpoint, the presence of the aforementioned feature in the investigated dryopiths is less informative than their hominid-like volumetric proportions, since the former have been identified as a synapomorphy of crown hominoids as a whole (5).

We conclude that, with differences that are consistent with their distinction at the genus rank, both Hispanopithecus and Rudapithecus display a unique hominid-like vestibular morphology that differs from that of any extant hominid genera but that appears quite close to that ancestral for crown hominids and crown hominines, mainly diverging from that of hylobatids by the stouter volumetric proportions of the SCs that are uniquely characteristic of great apes and humans among anthropoids. Orangutans appear most derived from such an ancestral vestibular morphology, whereas the investigated dryopiths lack most orangutan-like derived features, except for the slightly anterosuperiorly projecting anterior canal in Rudapithecus (not found in Hylobates) and some torsion in the shape of the lateral canal (a character that appears to be quite variable within hominoids). The lack of orangutan-derived features in dryopithecines does not completely rule out a stem pongine status, as previously supported by some authors (10, 28, 29), as it represents a more primitive morphology that probably precedes the subsequent evolution of the orangutan-like features in the pongine lineage. However, our results are more consistent with a stem hominid (6, 30) or a stem hominine (2, 3, 14, 16, 25) status for the investigated dryopiths. Our results suggest that African apes and hominin genera evolved in different directions from an ancestral morphology that more closely resembles that of Pan among extant hominines, and which is largely plesiomorphic for hominoids, as further supported by similarities with the stem hominid Nacholapithecus (except for the slenderer volumetric proportions of the latter). Therefore, similarities between the SC morphology of the studied dryopiths and that of African apes do not necessarily imply a hominine status, but overall support the previous claim (5), based on extant taxa alone, that extant hominines evolved from an ancestral condition quite similar to that of the crown hominid LCA, and that the latter was characterized by derived volumetric proportions of the SCs. Pending the analysis of other Miocene apes, Pan among the extant taxa and Rudapithecus among extinct apes constitute the best available proxies for such ancestral morphologies, being already somewhat more derived from the crown hominoid condition that is best approximated by Nacholapithecus. In the future, the inclusion in the analyses of additional extinct hominoids will hopefully clarify further the evolutionary history of these hominoids during the Miocene.

**Materials and Methods**

**Sample Composition and Acquisition.** We inspected three petrosals from two individuals of *H. hungaricus* from Rudabánya, Hungary (RUD 77, left [RUD 77L] and right [RUD 77R]; and RUD 200, right) (12, 13) and the single available petrosal of *H. laetanius* from Cal Ussabites 2, Spain (IPS18000, right) (10, 28, 29). The specimens of Rudapithecus are housed at the Geological Museum of the Mining and Geological Survey of Hungary and were scanned with a SkyScan 1172 (obtaining a resolution of 0.0136 mm) at the
Max Plank Institute for Evolutionary Anthropology (Leipzig, Germany), with the following parameters: 100-kV voltage and 100 mA. In turn, IPS18000 is housed at the Institut Catalá de Paleontologia Miquel Crusafont in Sabadell (Spain) and was scanned with a GE Phoenix VTomeX X 240 (obtaining a resolution of 0.0295 mm) at the Centro Nacional de Investigación sobre la Evolución Humana (Burgos, Spain), with the following parameters: 125-kV voltage and 120 mA. The three-dimensional (3D) virtual models of IPS18000, RUD 200 and RUD 77R were mirrored to enable the comparison with extant species. The segmented surfaces of the SCs of these fossils are available from MorphoSource (https://www.morphosource.org) (SI Appendix, Table S3).

The comparative sample for the volumetric proportion evaluation has been taken from a previous analysis that evaluated the phylogenetic signal embedded in the vestibule morphology (5), and integrated with recently published material of extant hominoids (4) and humans (62), together with the stem hominid N. kerioi (49). Overall, it consists of μCT scans of 169 dried crania and petrosals belonging to 27 extant anthropoid species, including all hominid genera and a selection of hylobatids, cercopithecoids, and platyrrhines, together with fossil taxa (SI Appendix, Table S3). The 3D meshes of the inner ear bony labyrinth of STW 573 and STW 578 were downloaded from the Sterkfontein project of the digital repository http://MorphoSource.org. The juvenile status of a few specimens should not affect their vestibular morphology since the bony labyrinth ossifies in early prenatal stages and does not change subsequently (63). The analysis of the patterns of shape variation was performed using the procedure described using 77 individuals representing all extant hominoid genera (SI Appendix, Table S4). Part of the scans used in the study originally appeared in refs. 64 and 65.

The μCT scans (voxel size for the extant and fossil specimens added in the present analysis to those originally published in ref. 5 can be found in SI Appendix, Table S4) were segmented using Avizo 9.0.1 (FEI Visualization Sciences Group) to digitally extract the left bony labyrinth, when available, or that from the right side (mirrored before the surface alignment). The vestibular apparatus was separated from the cochlea by cutting the generated 3D surfaces right under the saccule and the oval window and filling the resulting holes withGeomagic Studio 2014 (3D Systems) using a flat surface (5).

The anatomical axes used for describing SC morphology corresponds to those employed in the vast majority of inner ear analysis focusing on primates (36, 42, 43) and phylogenetically for great apes and humans using SPSS Statistics v. 17.0 for Windows (see figure 7b in ref. 5). The regression for the nonhominid sample was used as a baseline for computing the allometric residuals (SI Appendix, Table S1) (see table S5 in ref. 5) for the extant and extinct species. Comparisons between the latter and extinct groups are depicted by means of box-and-whisker plots.

Shape Analysis. Shape was analyzed using a deformation-based 3DGM technique that does not rely on a priori defined landmarks and examines the geometrical correspondences between continuous surfaces (5, 48, 66–68). This method quantifies the deformation from the analyzed surfaces from a constructed sample-average surface (template) (66, 68), mathematically models them as a diffeomorphism, and computes a set of vectors (momenta) that describe the direction and magnitude of deformation from the average template. The unscaled 3D models were aligned with Avizo 9.0.1 using the “Align Surface” module before running the analyses. The diffeomorphisms and the momenta were computed in the Barcelona Supercomputing Center (Barcelona, Spain) with Deformetica 4 software. The 3D models of the fossil specimens (except for IPS:18000) were constructed from the surface generated by means of bgPCA ran on the set of momenta for the hominoid-only sample using genera as grouping factor. The bgPCA was computed in R Studio v1.1.453 for R v3.5.0 using the ade4 package (69), while the cross-validated bgPCA were derived using the “groupPCA” function of the Morpho v2.6.70 library. Group mean differences were tested by computing a permutational ANOVA obtained when the inspected mode of evolution can adequately be described using a stochastic Brownian motion model. For Kmult < 1 is obtained when the inspected mode of evolution can adequately be described using a stochastic Brownian motion model. For Kvaric > 1 indicates that variance–covariance pattern found in the shape data. It is informative about the accumulation of the variance in the phylogeny. Thus, Kvaric < 1 indicates that variance is mostly found among different clades, being obtained close taxa are less diverse that expected under Brownian motion (suggesting that phenomena of stabilizing selection might have occurred). Additionally, the correlation between the log-transformed cube root of SC volume (in VolsC, in millimeters) and log-transformed SC length (in L, in millimeters) was assessed using the method described by the relationship between these variables has previously been shown to display an allometric grade shift between hominids and other anthropoids (5). Two separate regressions were computed for the nonhominid anthropoids and for great apes and humans using SPSS Statistics v. 17.0 for Windows (see figure 7b in ref. 5). The regression for the nonhominid sample was used as a baseline for computing the allometric residuals (SI Appendix, Table S1) (see table S5 in ref. 5) for the extant and extinct species. Comparisons between the latter and extinct groups are depicted by means of box-and-whisker plots.

Phylomorphospace and Phylogenetic Signal. Major patterns of vestibular shape variation were quantified using a phylomorphospace approach (74), obtained by projecting a phylogeny on to the tangent space derived from the bgPCA of a 3DGM shape analysis. In this method, the tips of the phylogeny correspond to the genus bgPC centroid, while the internal nodes (i.e., the ancestral states) of the tree are estimated using a maximum-likelihood method for continuous characters, assuming that the reconstructed nodes approximate the true morphology of the ancestors. Thus, when a time-calibrated phylogeny is used, its two-dimensional representation enables the intuitive interpretation of the magnitude and direction of differences based on branching patterns and orientations. Phylogenetic signal for extant hominoids used in this analysis was downloaded from the 10kTrees Website (v2; https://10ktrees.fas.harvard.edu), while Hispanopithecus and Rudapithecus were added based on the assumption that they are closely related and constitute a clade, with the tips corresponding to 9.6 Ma and 10.1 Ma, respectively (20), and diverging at a time considered as recent phylogenetic event. The divergence between pongines and hominines (crown hominoids), thus always preceding the divergence of dryopiths in all the phylogenetic hypotheses, and its tip corresponds to 14.77 Ma (55, 75). The divergence between hominoids and Rudapithecus has been placed 1 Myr older than the divergence between hylobatids and hominoids and its tip corresponds to its last occurrence in the fossil record (7.0 to 6.5 Ma) (76). For the South African Australopithecus sp., we used the published first appearance datum for Australopithecus africanus (4.02 Ma) that includes the Jacovec specimens into the species (77).

The position in the morphospace of the internal nodes of the phylogeny (ancestral morphologies) was estimated via a maximum-likelihood method for continuous characters (78) using the “fastAnc” function of phytools v0.6-60 R package (79). Subsequently, the bgPCs scores of the ancestral states were recalculated by translated point shape data between the taxa as discussed in the literature during the last two decades (see above): stem hominids, stem hominines, and stem pongines (SI Appendix, Fig. S2).

Analyses were repeated based on the resulting three different cladograms and their results compared to evaluate the effect of phylogenetic uncertainties surrounding these taxa. Rudapithecus is here considered as a stem hominoid as indicated by most recent clade analyses (4). Nacholapithecus is placed in a stem hominoid position, 2 Myr older than the divergence between pongines and hominines (crown hominoids), thus always preceding the divergence of dryopiths in all the phylogenetic hypotheses, and its tip corresponds to 14.77 Ma (55, 75). The divergence between crown hominoids and Rudapithecus has been placed 1 Myr older than the divergence between hylobatids and hominoids and its tip corresponds to its last occurrence in the fossil record (7.0 to 6.5 Ma) (76). For the South African Australopithecus sp., we used the published first appearance datum for Australopithecus africanus (4.02 Ma) that includes the Jacovec specimens into the species (77).

The 3D mesh data have been deposited in MorphoSource, https://morphosource.org/ (Rudapithecus hungaricus: RUD:77 R: https://doi.org/10.17602/M2/M126214; RUD:77 L: https://doi.org/10.17602/M2/M126215; RUD:200: https://doi.org/10.17602/M2/M126216; Hispanopithecus laietanus: IPS:18000: https://doi.org/10.17602/M2/M126217; Nacholapithecus keriobi:...
ACKNOWLEDGMENTS. We thank Jose Braga for allowing us to use the CT scans of the human specimens; the Max Plank Institute for providing access to the microCT scans of RUD 77 and RUD 200; the European Synchrotron Radiation Facility heritage database for paleoanthropology, evolutionary biology, and archaeology; providing access to a part of the hominoid scans used in the present analysis; and Lynn Copes, Lynn Lucas, and the Museum of Comparative Zoology (Cambridge, MA) for providing access to a part of the scans used in the study, and funded by NSF DDIG #0925793, and a Wenner-Gren Foundation Dissertation Grant #8102 (both to Lynn Copes). These scans were downloaded from MorphoSource.org, a web-accessible archival and threedimensional storage house at Duke University. This research has been funded by the Agencia Estatal de Investigación CGL2016-76431-P and CGL2017-82654-P, AEI/FEDER, UE; and BES-2015-071318 (to A.U.); the Generalitat de Catalunya (CERCA Programme); the consolidated research groups 2017 SGR 86 and 2017 SGR 116 GRC; and the French Centre National de la Recherche Scientifique. Part of the analyses were performed using Barcelona Supercomputing Center resources (BCV-2020-1-0008). A.B. was funded by the University of the Witwatersrand.


