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An allometric study of *Macaca fascicularis* from the Late Pleistocene deposits at the Ille site (Philippines): a possible model for Southeast Asian Dwarf Hominins

Étude allométrique de *Macaca fascicularis* des dépôts du Pléistocène supérieur du site de Ille (Philippines) : un possible modèle pour les Homininés de petite taille du sud-est asiatique

T. Ingicco · N. Amano · J. Ochoa · F. Détroit

Abstract Recent discoveries of taxonomically challenging Southeast Asian dwarf hominins from Liang Bua in Indonesia and the Callao Cave in the Philippines have enabled us to investigate the general tendency towards dwarfism or gigantism already observed in endemic insular animals. One current hypothesis suggests that the pygmy human phenotype is the result of evolutionary selection in rainforest environments. In this paper we test the hypothesis that dwarfism is a response to forest habitats, using macaque (*Macaca fascicularis*) fossils from the well-stratified archaeological sequence at the Ille site in the Philippines. Our results show that changes in size may affect general conformations in forested environments, and therefore support the hypothesis put forward on the evolution of pygmy hominin populations in tropical rainforest habitats.

Keywords Non-human primates · Rainforest · Insular and Environmental dwarfism · Southeast Asian insularity · Hominin evolution · Elliptic Fourier

Résumé Les récentes découvertes d'homininés de petite taille en Asie du Sud-est insulaire à Liang Bua (Indonésie)

et Callao Cave (Philippines), dont la taxinomie est débattue, permettent de discuter de leur tendance au nanisme ou gigantisme par ailleurs déjà observé pour les faunes sujettes à l'endémisme insulaire. Une hypothèse actuelle propose que le phénotype pygmoïde soit le résultat d'une évolution en forêt tropicale. Nous cherchons ici à tester l'hypothèse du nanisme comme réponse à un habitat forestier, à partir de macaques (*Macaca fascicularis*) provenant des niveaux archéologiques stratifiés du site du site de Ille (Philippines). Nos résultats montrent que des changements de tailles peuvent avoir des conséquences sur la conformation générale dans le cadre d'un environnement forestier. Notre étude vient appuyer l'hypothèse de l'évolution du phénotype pygmoïde en forêt tropicale humide.

Mots clés Primates non-humains · Forêt tropicale humide · Nanisme insulaire et environnemental · Asie du Sud-Est insulaire · Évolution humaine · Fourier elliptique

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Introduction

The recent discovery of dwarf *Homo floresiensis* remains [1] in Liang Bua (Flores, Indonesia) points to the existence among hominins of a tendency towards reduced body size following Foster's rule [2], as previously observed in other vertebrates. Although the debate continues over the creation of a new species for these remains [3,4], it has been shown that these specimens fit into the allometric rule for island endemism [5-8]. As shown by the fossil record, the body size of animals tends to evolve rapidly in isolated island conditions: smaller animals tend to be larger and larger animals tend to decrease in size [9,10]. The size and the degree of isolation (*i.e.*, distance from a continental shelf) of the island also play a major role in the degree of endemism: small

islands tend to promote small species [11]. Therefore, the small size of Flores Island and the absence of any connection with the Sunda shelf during the Quaternary could explain the small size of *Homo floresiensis* [12].

The more recent discovery of a similar small-bodied hominin in Callao Cave on Luzon Island (Philippines) dated to 67,000 BP [13] appears to confirm the conclusions drawn from the Flores specimens, but a closer examination suggests that the reality is more complex. Firstly, Luzon is much larger than Flores, and the number and the heterogeneity of environment types is also greater. Secondly, although Luzon Island could have been the last island in the Philippines to be colonized during the Pleistocene [14,15], it probably never reached full island equilibrium, or at least maintained a significant rescue effect following the MacArthur-Wilson Equilibrium Model [16,17]. Therefore, Luzon cannot be directly compared to the smaller Flores Island. The reasons for the existence of a small-bodied hominin on Luzon Island during the Pleistocene would have to be accounted for otherwise than by Foster's rule. For the moment, the third metatarsal discovered in Callao Cave has been attributed to *Homo aff. sapiens* [13], despite the fossil's particular form (*i.e.* size and shape) and in contrast to the specimens from Flores. Taking into account the results of analyses of the Callao fossil, as well as its age of more than 60,000 years, the authors are "seriously considering the possibility that it could belong to a particular endemic type of *Homo* (mimicking *H. floresiensis*?)" [18]. To better understand this specimen, the authors of the original study compared the Callao fossil with present-day Negritos from the Philippines. Negrito pygmies may be the result of a different form of adaptation, the mechanism of which is still being debated today [19,20]. One of the hypotheses put forward is an evolutionary trend in response to the rainforest environment [21,22]. This hypothesis is difficult to test on human populations and apart from variation in size, the effects of endemism on the shape of individuals have not yet been described.

Our aim in this study is to contribute to the debate on the origins of small-bodied Pleistocene hominins by testing the importance of forest environments in allometric changes, defined as changes in the correlation of shape to size. We decided to quantify allometry in the forest-dependent *Macaca fascicularis*, which is a widespread species in insular Southeast Asia and therefore not endemic to any island [23]. However, *M. fascicularis* is dependent on forest environments [24], which may be considered as habitat endemism. The two macaque subspecies in the Philippines are identified by the colour of their fur: *M.f. philippinensis* (dark-coloured and the earliest colonizer of the archipelago) and *M.f. fascicularis* (light-coloured and a later disperser) [23]. They are morphometrically indistinguishable, includ-

ing from those in the fossil record at the Ille site (Palawan Island), where we found the oldest *M. fascicularis* ever discovered in the Philippines (Fig. 1) [25]. Ille (El Nido, Palawan Island, The Philippines) has yielded remains of *M. fascicularis* in each layer, from the silty clay Late Upper Pleistocene layers to activity areas 14 000-9400 cal BP and a c. 7000-5000 cal BP shell midden, through to the Late Holocene layers. The stratigraphic sequence at Ille has revealed changing patterns in human settlement and subsistence strategies, with a notable shift from adaptations to foraging and hunting in open grassland to closed rainforest environments [26,27]. Pleistocene *M. fascicularis* in the Ille site was observed to be larger in size than present-day macaques [25]. The purpose of this study was to investigate a possible change in shape in relation to size. We also tested the correlation between changes in allometry and environmental conditions over time along the entire stratigraphic profile of the Ille site. We expect the results to provide insights into insular and environnemental dwarfism as observed among hominin fossils and pygmy populations in general.
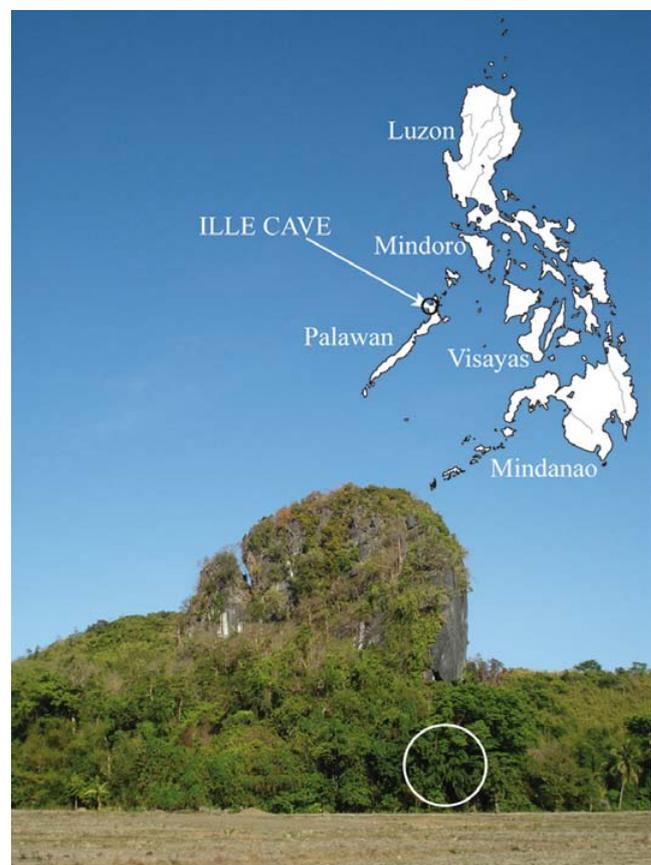
Fig. 1 Location of the Ille site in Palawan Island (Philippines) / *Situation géographique du site de Ille dans l'île de Palawan (Philippines)*

Materials and methods

The two clades of *Macaca fascicularis* were sampled without making any distinction. Our sample comprised 31 comparative specimens from the collections of the University of the Philippines Archaeological Studies Program (Manila) and the Museum national d'Histoire naturelle (Paris), and 42 fossil specimens from the Ille site: 6 from the Pleistocene layers, 10 from the 7000 cal. BP shell midden and 26 from the Late Holocene layers.

Teeth are well suited to the investigation of allometry since they are independent of any change in size during ontogeny. We chose the third left lower molar as its elongated shape made orientation easier.

Two-dimensional digital images were produced from photographs (Nikon D90 with an AF-S DX Nikkor 18-55mm lens) of the occlusal surface of each molar using a standardized protocol: the teeth were positioned horizontally along their cervix line, and parallel to the focal plane of the lens using two laser levels. Parallax was controlled with a grid paper on each photograph. Taking the buccal-talonid notch as the starting point, each outline was acquired with TPSdig [28] by recording the equidistant 2D coordinates of 120 successive points in the clockwise direction. We superimposed the outlines through a Procrustes analysis of the long axis of the tooth (Fig. 2). We applied the elliptic Fourier decomposition method to quantify the outlines on semi-landmarks [29,30] with 32 harmonics. This number was decided on after calculating the average deviation between the reconstructed outline with successive numbers of harmonics from the original outline [31] and the qualitative evaluation (Fig. 3). For statistical and multivariate analyses, we performed a principal component analysis (PCA) of the variance-covariance matrix of the Fourier coefficients. We described the differences between the mean-shapes of the different groups ("Pleistocene", "Shell-midden", "Holocene" and "reference material") through Thin-Plate Spline (TPS) visualizations and quantified the allometries through a Spearman's rank correlation test on the first principal component (PC1), which describes the shape and area of the first harmonic accounting for size. Finally, the
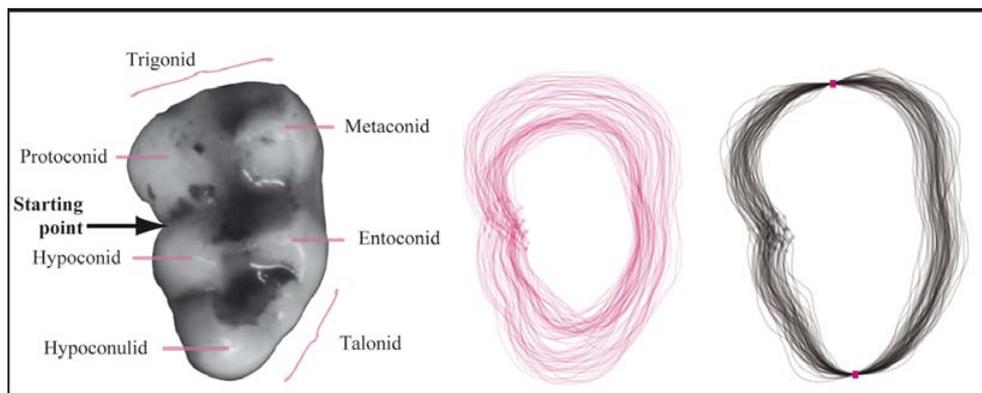
Fig. 2 Steps in the Procrustes superimposition of the outlines on the long axis of the third lower molars / *Étapes de la superposition Procuste des contours sur le grand axe des troisièmes molaires inférieures*
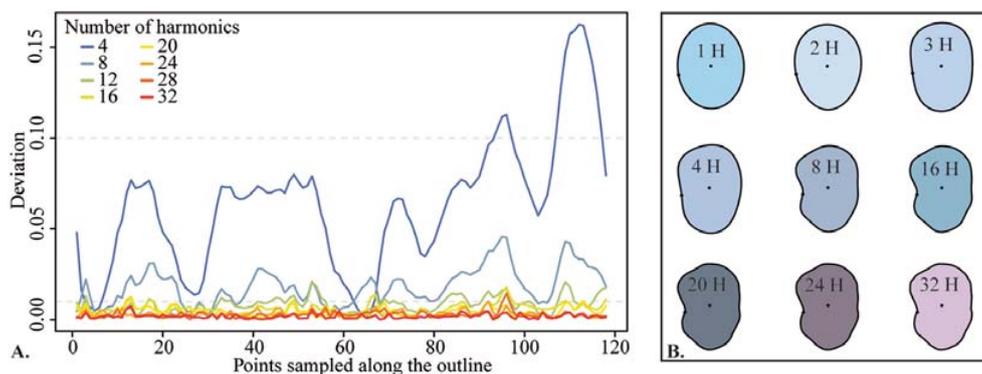
Fig. 3 Evaluation of the number of harmonics (n=1 to 32) to be chosen for the outline reconstructions, with quantification of the deviation from the original outline (A) and qualitative method (B) / *Estimation du nombre d'harmonique (n=1 à 32) à retenir pour la reconstruction des contours, par quantification de la déviation au contour d'origine (A) et par l'approche qualitative*

scores for the mean-shapes of the different groups obtained from the PCA were compared to environmental data for each layer through a Two-Block Partial Least Square analysis (2B-PLS) of the correlation matrix, to test whether the shape differences are correlated to the development of the forest [32]. The vegetation cover was estimated from the deer/pig ratio used as an index for the degree of opening/closure of the environment [27]. Analyses were performed using the Momocs package written by Bonhomme [33] and several functions written by Claude [34] for R [35].

Results

PC1 and PC2 account respectively for 53% and 14% of the total variance, with 99% of this variance represented by the 19 first PCs. None of the archaeological groups studied are isolated on the PCA (Fig. 4). Nevertheless, some patterns can be observed. The Pleistocene specimens are centred on PC1 and PC2, which correspond to an elongated molar of medium width. The Early Holocene specimens from the shell midden have a more pronounced development of the protoconid than the Pleistocene specimens, as they are clustered in the lower left-hand corner of the Fig. 4. The position of the protoconid shifts distally from the Early to the Late Holocene, as shown on the positive part of PC2. The present-day specimens cover the whole range of variability of our sample, displaying Pleistocene, Early Holocene and Late Holocene configurations and even a very narrow molar with a reduced metaconid and protoconid on the right-hand side of PC1.

There is a strong and highly significant negative correlation (Spearman's coefficient of correlation $Rs = -0.8876$, $P < 0.01$) between shape and size in the third lower molar of *M. fascicularis*. Although the morphological diversity of *M. fascicularis* is limited as a result of the PCA, a difference
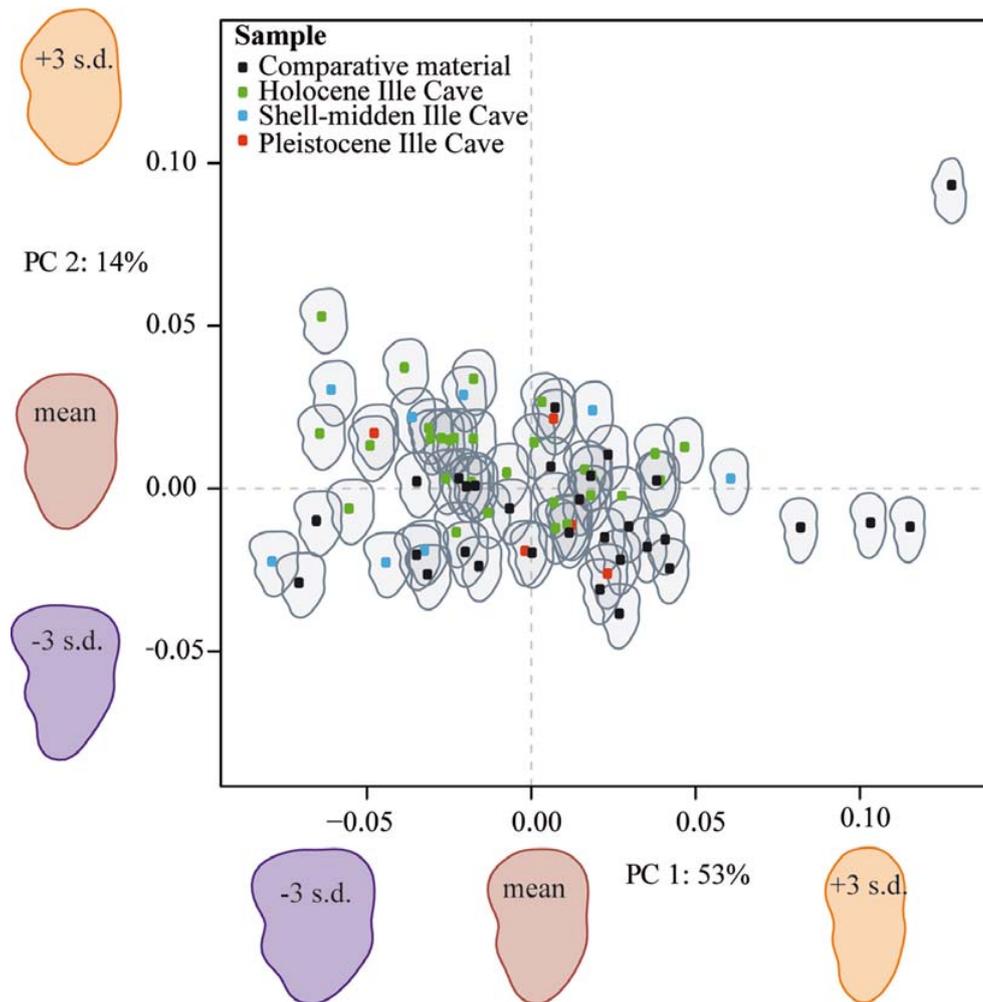
Fig. 4 Principal Component Analysis of the Fourier coefficients with the reconstructed outlines along each principal component for the mean, -3 and +3 standard deviations (s.d.) / *Analyse en Composante Principale sur les coefficients de Fourier avec reconstruction des contours le long de chaque composante principale pour la moyenne, -3 et +3 écart-types*

in shape organized through an allometry over time from the Pleistocene to the present day can be distinguished. This observation led us to a more detailed study of variations in shape over time from a TPS analysis (Fig. 5).

Observations on the PCA are confirmed here with the enlarged talonid and the more distal position of the protoconid in Early Holocene specimens compared to the robust Pleistocene *M. fascicularis* third lower molar (Fig. 5A). This sequence can be observed along the entire stratigraphic profile through to the shell midden (Fig. 5B) and the Late Holocene specimens (Fig. 5C).

The 2B-PLS between the mean-shapes of the different groups and the pig/deer ratio per archaeological layer was calculated to find patterns of correlation between shapes and the evolution of environments over time [36]. The highly significant Escoufier coefficient ($r=0.80$), which is a measure of the correlation between the two datasets analysed through the 2B-PLS, shows that the variations in shape of the third lower molar of *M. fascicularis* over time probably reflect the evolution of the environment in Palawan during the last 12,000 years, from closed forest during the Pleistocene to open landscape during the Holocene.

Discussion and concluding remarks

This study tested, for the first time, allometry in forest-dependent animals using macaques as an example. We have demonstrated here that forest environments may be one of the factors accounting for the evolution of pygmoid populations as suggested by Turnbull, and Perry and Dominy [21,22]. It is indeed possible to assess from the 2B-PLS result that the evolution of shape and size is closely related to the development of the forest, the allometry thus resulting from environmental dependency. We have also demonstrated that this change in size is highly correlated with changes in shape. The reduction in size correlated to the change in shape over time in *M. fascicularis* from the Ille site corresponds to the change in the environment with the development of the tropical rainforest after the Last Glacial Maximum [32]. Although further studies are needed to complete the picture of hominin history in Island Southeast Asia, we provide evidence here that could support the hypothesis on the evolution of pygmy hominin populations in forested environments.

Adaptation to a particular environment may not be the only factor accounting for shape and size. The isolation of populations in islands results in the evolution of endemic species [37]. In such insular conditions, there may be a lesser degree of predation, inter-species competition, genetic drift and food limitation, as well as a decrease in physiological efficiency in some cases (following Bergman's rule) [9,11,38]. Animals therefore tend to enhance their energy intake efficiency and ability to enter a state of torpor and store fats, increase their lifespan and reduce their reproduction rates [39,40]. On the other hand, their metabolic rates, energy expenditure during locomotion and the size of their brains and sense organs tend
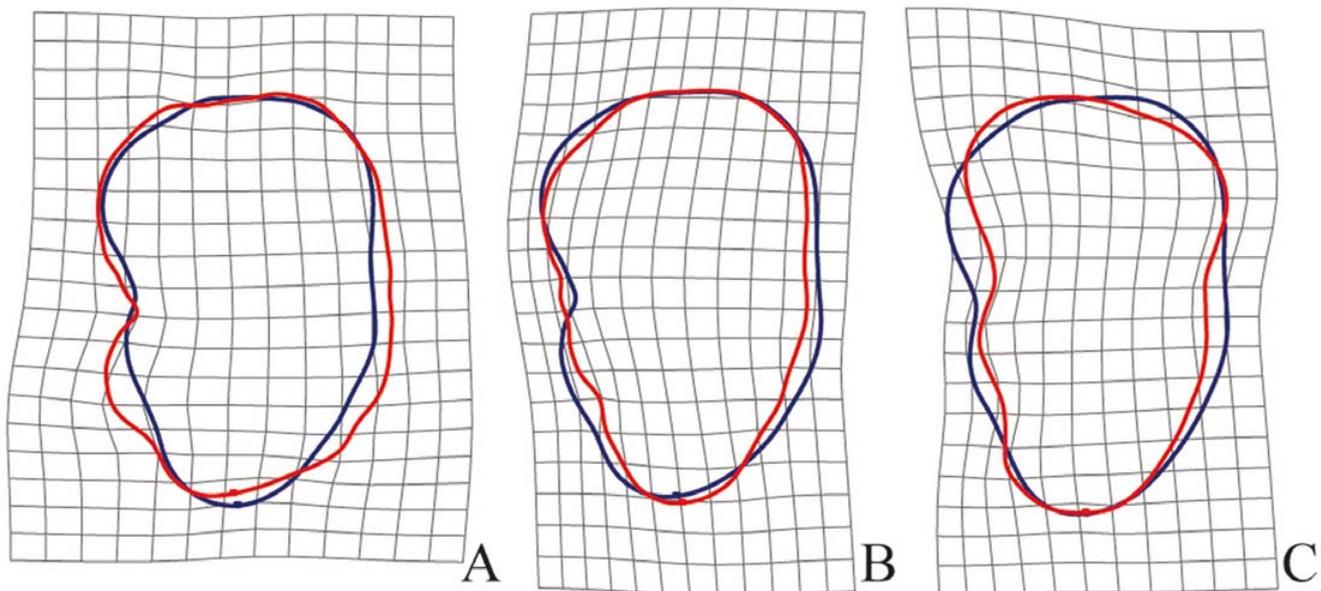
Fig. 5 Thin-Plate Spline grid deformation between the mean-shape of the Pleistocene (blue) and Shell Midden (red) (A), Shell Midden (blue) and Late Holocene (red) (B), Late Holocene (blue) and comparative (red) (C) samples / *Grilles de déformation de plaques minces entre les conformations moyennes au Pléistocène (bleu) et pour le niveau coquillier (rouge) (A), pour le niveau coquiller (bleu) et à l'Holocène récent (rouge) (B), à l'Holocène récent (bleu) et pour le matériel actuel de comparaison (rouge) (C)*

to decrease. The effects of endemic insularity include hypso-donty in herbivores, shifts in prey species in carnivores, fusion and shortening of limb bones and changes in body proportions that tend towards dwarfism in large species and gigantism in small ones [10]. This last point is crucial to understand the small-bodied Pleistocene hominins. Apart from size, a particular overall shape has also been described in *H. floresiensis* specimens, which has been used to describe similarities between this species and australopithecines (see hypothesis 3 in the conclusion of Argue et al. [41]). It should be noted here that the lesser degree of predation, which is an important consequence of island endemism, is known to result in shape homologies [38,42-44]. Therefore, any similarities between *H. floresiensis*, the Callao fossil and other Plio-Pleistocene hominins should be considered with extreme caution. Although australopithecines are known to have been preyed on by large carnivores, this is most unlikely to be the case for the dwarf Southeast Asian hominins [45, p.271]. The characteristics of these hominins have to be observed in the light of evolution within fragmented environments. This adaptation to forest habitats is clear from our study, but the processes governing selection of the pygmy phenotype are still unclear. Perry and Dominy [22] have proposed a convincing list of consequences of life in forest environments that would cause selection of the pygmy phenotype. In forest environments, high-calorie food resources are limited, whether from plants naturally present in forests as compared to starchier agricultural food, or from animals with little fat as compared to larger game animals living in open environments [46]. Furthermore, thermoregulation is inefficient because of the high humidity, while mobility is limited by dense undergrowth. In addition, Perry and Dominy [22] have suggested that pedomorphy is an efficient adaptation to the short lifespan of pygmy populations that may be due to the many parasites and infectious diseases in forests.

It must be acknowledged here that although we are considering the importance of forest habitats in the adaptation of the small-bodied Callao hominin, the palaeoenvironment of Callao Cave 67,000 years ago is not yet well known. Full understanding of the biological processes responsible for the small size and particular shape of the Callao hominin will only be possible once the palaeoenvironmental data become available. Island environments account for the presence or absence of species, but the reverse is also true. It has been observed that islands have few pioneer species promoting forest regeneration. No such species have been found in Callao Cave [13]. Nevertheless, the presence of the murid genera *Apomys* and *Batomys* in the 67,000 year-old fossil record of Callao Cave is evidence of the persistence of high-altitude forests (above 1350m) [47]. In addition, this study shows that forest adaptation may be a good model to account for the selection of small body size in the Callao hominin in the larger island of Luzon.

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