

Combining geometric morphometrics and paleoproteomics to enlighten the paleodiversity of *Pongo*

Jülide Kubat^{1,2}, Ryan Paterson³, Graeme Barker⁴, Fabrice Demeter^{5,6}, Arnaud Filoux⁷, Ottmar Kullmer², Meaghan Mackie³, Tomas Marques-Bonet⁸, Nguyen Thi Mai Huong⁹, Nguyen Anh Tuan⁹, Sytha Pheng¹⁰, Jessica Rippenga¹⁴, Friedemann Schrenk², Viengkeo Souksavatdy¹¹, Lim Tze Tshen¹², Athiwat Wattanapituksakul⁷, Wei Wang¹³, Clément Zanolli¹⁴, Anne-Marie Bacon¹, Enrico Cappellini³

1 - University of Paris, CNRS UMR 8045 Babel, Montrouge, France · 2 - Department of Palaeoanthropology, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt am Main, Germany · 3 - Section for Evolutionary Genomics, Globe Institute, University of Copenhagen, Copenhagen, Denmark · 4 - McDonald Institute for Archaeological Research, University of Cambridge, Cambridge, United Kingdom · 5 - Lundbeck Foundation GeoGenetics Centre, GLOBE Institute, Copenhagen, Denmark · 6 - UMR 7206 Eco Anthropologie, Muséum National d'Histoire Naturelle, CNRS, Paris, France · 7 - Palaeontological Research and Education Centre, Mahasarakham University, Mahasarakham, Thailand · 8 - Institute of Evolutionary Biology (UPF-CSIC), University Pompeu Fabra, Barcelona, Spain · 9 - Anthropological and palaeoenvironmental Department, Institute of Archaeology, Ha Noi, Vietnam · 10 - Royal University of Fine Arts, Phnom Penh, Cambodia · 11 - Department of Heritage, Ministry of Information, Culture and Tourism, Lao People's Democratic Republic · 12 - Department of Geology, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia · 13 - Institute of Cultural Heritage, Shandong University, Qingdao, China · 14 - University of Bordeaux, CNRS, MCC, PACEA, UMR 5199, 33600 Pessac, France.

The spatio-temporal distribution of orangutans (*Pongo*) is evidenced by the numerous Pleistocene fossil sites in Southeast Asia ranging from southern China to Insular Indonesia (Vietnam, Laos, Cambodia, Thailand, Malaysia; [1]). Modern and fossil *Pongo* show a high degree of variation in tooth size and morphology, which obscures taxonomic identifications at species level [2]. The fossil record of *Pongo* largely consists of isolated dental remains, hence several attempts to distinguish taxa at (sub-)species level have been proposed exclusively based on odontometric measurements and non-metric features recorded on isolated teeth [3]. However, there are still debates regarding the recognition of fossil species of *Pongo*. In order to attempt characterizing and distinguishing extinct orangutan species, we investigated the internal structure of 70 lower molars of Pleistocene *Pongo* from various sites and origins (Punung and Sangiran from Indonesia; Lang Trang, Tham Om, Hang Hum, Mai Da Dieu, and Duoi U'Oi from Vietnam; Tam Hang and Marklot from Laos; Ganxian and Chinese Apothecary Collection from China). For comparison, we have analyzed 25 modern orangutan lower molars, representing the species *Pongo pygmaeus* and *Pongo abelii*. We used geometric morphometrics to analyze the shape of the enamel-dentine-junction (EDJ) by placing five landmarks on the tip of the main dentine horns, and 142 semilandmarks along the marginal ridges. We also applied paleoproteomic analyses on 14 dental specimens of Late Pleistocene *Pongo* from Vietnam, Laos, Malaysia and Thailand by utilizing high-resolution tandem mass spectrometry to sequence ancient proteins [4]. Our results show that fossil molars have a lower EDJ relief height with lower dentine horns than modern molars. Extant *Pongo* molars are morphologically quite distinct with relatively higher dentine horns and a more elongated and narrow molar shape than the fossil counterparts. Moreover, *P. pygmaeus* and *P. abelii* show a large morphometric overlap in EDJ shape. Specimens from Vietnam and Laos are dated to the final Middle Pleistocene to Late Pleistocene and share the same morphospace, which is consistent with their allocation to the same chrono-geographical variant. This also applies to fossils from the Middle Pleistocene of China as well, which tend to group in a separate morphospace with some overlap with specimens from Vietnam and Laos. The Late Pleistocene site of Punung, Indonesia shows the highest variability, with a mix of specimens exhibiting a modern morphotype and others grouping with Pleistocene *Pongo*. As published recently [5], it suggests that the Punung assemblage might be a mix of Pleistocene and Holocene remains. Preliminary

paleoproteomic data does not allow a consistent discrimination of fossil specimens as either *P. pygmaeus* or *P. abelii*. This is partially confirmed also by the morphometric results indicating that a clear attribution of the fossil specimens to modern species is not possible, except for one sub-sample from Punung. The morphological differences between fossil *Pongo* molar crowns and living orangutans reveal a higher diversity in the genus *Pongo* during the Pleistocene than today. Combining EDJ morphology and paleoproteomics provide an exciting basis for further studying the evolutionary biodiversity in the only nonhuman great ape genus living today outside of Africa.

This project has received funding from the European Union's Horizon 2020 research, Innovative Training Networks PUSHH *Palaeoproteomics to Unleash Studies on Human History* (Pr. Enrico Cappellini) under the Marie Skłodowska-Curie grant agreement (No.861389).

References:

- [1] Tshen, L.T., 2016. Biogeographic distribution and metric dental variation of fossil and living orangutans (*Pongo* spp.). *Primates* 57, 39-50.
- [2] Harrison, T., Jin, C., Zhang, Y., Wang, Y., Zhu, M., 2014. Fossil *Pongo* from the Early Pleistocene *Gigantopithecus* fauna of Chongzuo, Guangxi, southern China. *Quat. Int.* 354, 59-67.
- [3] Harrison, T., Zhang, Y., Yang, L., Yuan, Z., 2021. Evolutionary trend in dental size in fossil orangutans from the Pleistocene of Chongzuo, Guangxi, southern China. *J. Hum. Evol.* 161, 103090.
- [4] Welker, F., Ramos-Madrigal, J., Gutenbrunner, P., Mackie, M., Tiwary, S., Rakownikow Jersie-Christensen, R., Chiva, C., Dickinson, M.R., Kuhlwilm, M., de Manuel, M., Gelabert, P., Martín-Torres, M., Margvelashvili, A., Arsuaga, J. L., Carbonell, E., Marques-Bonet, T., Penkman, K., Sabidó, E., Cox, J., Olsen, J.V., Lordkipanidze, D., Racimo, F., Lalueza-Fox, C., Bermúdez de Castro, J.M., Willerslev E., Cappellini, E., 2020. The dental proteome of *Homo antecessor*. *Nature* 580, 235-238.
- [5] Kaifu, Y., Kurniawan, I., Yurnaldi, D., Setiawan, R., Setiyabudi, E., Insani, H., Takai, M., Nishioka, Y., Takahashi, A., Aziz, F., Yoneda, M., 2022. Modern human teeth unearthed from below the ~128,000-year-old level at Punung, Java: A case highlighting the problem of recent intrusion in cave sediments. *J. Hum. Evol.* 163, 103122.