Podium Presentation, Session 5, Friday 8:30 – 10:10

New functional evidence from the internal structure of the *Danuvius guggenmosi* lower limb

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Danuvius guggenmosi is an 11.62-million-year-old fossil great ape originally described as dentally most similar to dryopithecins, while the external morphology of the postcrania suggested adaptations to both suspensory locomotion and bipedalism [1]. The authors proposed a new positional behaviour — extended-limb clambering — that provides a model for the common ancestor of great apes and humans [1]. However, the bipedal component of the locomotor repertoire of *Danuvius* has been questioned [2].

The assessment of the structural variation in lower limb of the plastic ecophenotypic cortical and trabecular bone has the potential to provide functional information about the mechanical loading of the hip, knee, and ankle joints of *Danuvius* [3]. Here we investigate for the first time the internal bone structure of *Danuvius*, focusing on the femoral head, patella and distal tibia of male (GPIT/MA/10000) and female (GPIT/MA/10003) individuals. We compare its endostructure to Miocene apes *Rudapithecus* and *Oreopithecus* (patella only), australopiths (SKW 19, StW 311, StW 358, StW 389, StW 522, SWT1/B-2) and to a minimum (depending on the skeletal element) sample size of 9 humans, 10 *Pan*, and 5 each of *Gorilla*, *Pongo*, *Papio* and *Macaca*. We also include Sansuke, a bipedally-trained *Macaca fuscata* individual.

All specimens were microCT scanned with a spatial voxel size <90 microns. Bone tissues were segmented using medical image analysis clustering segmentation and the trainable Weka segmentation algorithm in ImageJ. A holistic morphometric analysis in medtool® was used to quantify trabecular bone volume fraction (BV/TV) throughout the entire preserved epiphyseal region (femoral head, distal tibia) or bone (patella). To statistically compare variation between groups, we applied canonical holistic morphometric analysis, that maps the trabecular values to a volumetric mesh of a canonical bone created by a statistical free-form deformation model [4]. We further compare variation in trabecular structure via principal component analyses (PCA).

Results of PCA reveal good separation among extant taxa for each skeletal element. Trabecular bone of the *Danuvius* femoral head shows a high BV/TV in the region of the fovea capitis that is also observed in the bipedally-trained macaque; both individuals found together in PC1-PC5 [5]. Such reinforcement might relate to the function of the ligamentum teres. The *Danuvius* patella shows high BV/TV and thick cortex mediosuperiorly; a pattern that is most similar to the terrestrial quadrupedal *Papio* and may reflect the action of the vastus medialis that plays an important role in the stabilisation of the patella during knee extension. *Danuvius* differs from the patterns observed in *Rudapithecus* and *Oreopithecus* patellae. The *Danuvius* distal tibia shows high BV/TV throughout the epiphysis. The BV/TV pattern suggests trabecular reinforcement at the anterior and posterior margins of the trochlear articulation, akin to non-human great apes, reflecting high loading in dorsiflexion and plantarflexion. However, unlike apes, these reinforcements are more centrally located on the articulation, possibly indicating lesser loading in dorsiflexion. The distal tibia also shows human-like reinforcement at the centre of the articulation, suggesting loadings of the tibiotalar joint related to weight bearing in a neutral axis.

The internal structure of the *Danuvius* femur, patella and tibia reveals novel information about the posture and loading of its lower limb. *Danuvius* shows a unique pattern that differs from the preserved elements of the suspensory apes *Rudapithecus* and *Oreopithecus*. Similarities to humans (distal tibia) and the bipedally-trained Sansuke (femoral head) are consistent with the use of more extended lower limb postures than in extant great apes.

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References: [1] Böhme, M., Spassov, N., Fuss, J., Tröscher, A., Deane, A.S., Prieto, J., Kirscher, U., Lechner, T., Begun, D.R., 2019. A new Miocene ape and locomotion in the ancestor of great apes and humans. Nature. 575, 489–493. [2] Williams, S.A., Prang, T.C., Meyer, M.R., Russo, G.A., Shapiro, L.J., 2020. Reevaluating bipedalism in Danuvius. Nature. 586, E1–E3. [3] Cazenave, M., Kivell, T.L., 2023. Challenges and perspectives on functional interpretations of australopith postcrania and the reconstruction of hominin locomotion. Journal of Human Evolution. 175, 103304. [4] Bachmann, S., Dunmore, C.J., Skinner, M.M., Pahr, D.H., Synek, A., 2022. A computational framework for canonical holistic morphometric analysis of trabecular bone. Scientific Reports. 12, 5187. [5] Cazenave, M., Nakatsukasa, M., Mazurier, A., M. Skinner, 2024. Identification of functionally related adaptations in the trabecular network of the proximal femur and tibia of a bipedally trained Japanese macaque. Anthropological Science. 132, 13–26.