The Fossils of *Speothos pacivorus* (Carnivora: Canidae) at the Peter Lund/Quaternary Collection of the Natural History Museum of Denmark

Juan V. Ruiz[1,2,3,4*], Christina Kyriakouli[3,4], Kasper Hansen[5], Carsten Gundlach[6], Gabriel S. Ferreira[3,4], Fabio A. Machado[7], Pedro L. Godoy[8,9], Mariela C. Castro[10], Felipe C. Montefeltro[1]

[1] *Laboratório de Paleontologia e Evolução de Ilha Solteira, UNESP, Ilha Solteira, Brazil.*
[2] *Programa de Pós-Graduação em Biodiversidade, UNESP, São José do Rio Preto, Brazil.*
[3] *Senckenberg Centre for Human Evolution and Palaeoenvironment at the University of Tübingen, Tübingen, Germany.*
[4] *Department of Geosciences, Eberhard Karls University of Tübingen, Tübingen, Germany.*
[5] *Archaeoscience, Globe Institute, Copenhagen University, DenmarkDepartment of Physics, Technical University of Denmark, Kgs. Lyngby, Denmark.*
[6] *3D Imaging Centre, Technical University of Denmark, Kgs. Lyngby, Denmark.*
[7] *Department of Integrative Biology, Oklahoma State University, USA.*
[8] *Department of Zoology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil.*
[9] *Department of Anatomical Sciences, Stony Brook University, Stony Brook, USA.*
[10] *Laboratório de Biologia Integrativa e Conservação, Universidade Federal de Catalão, Catalão, Brazil.*
*__Corresponding author__: juanvitorruiz@gmail.com

Abstract

Speothos pacivorus is an extinct South American canid (Canidae: Cerdocyonina) from the Pleistocene of Lagoa Santa Karst, Central Brazil. This taxon is one of the hypercarnivore canids that vanished from the continent at the end of Pleistocene. Although all remains of *Speothos pacivorus* were collected in the 19th century by the Danish naturalist Peter W. Lund, few studies have committed to an in-depth analysis of the taxon and the known specimens. Here, we analyzed all biological remains of *S. pacivorus* hosted in the Peter Lund/Quaternary Collection at the Natural History Museum of Denmark, Copenhagen, by listing and illustrating all its specimens known to date. We also conducted a reconstruction of the holotype, an almost complete cranium, based on a µCT scan, producing an undeformed and crack-free three-dimensional model. With this data available we aim to foster new research on this elusive species.

Keywords: 3D reconstruction, Canidae, Lagoa Santa Karst, Pleistocene, *Speothos*

Submitted:18/04/2024, published online:14/05/2024. https://doi.org/10.18563/journal.m3.229

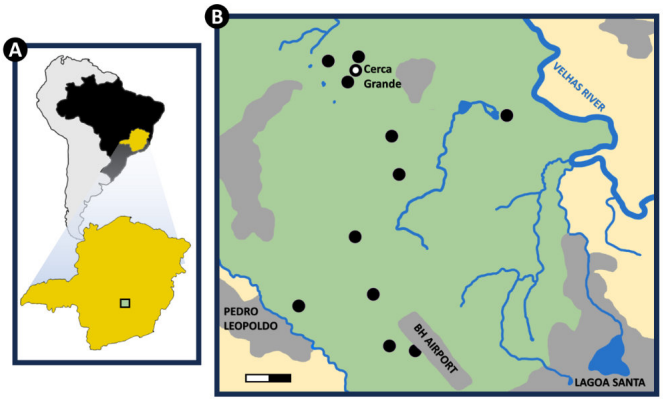

Figure 1. Lagoa Santa Karst location. South America map indicating Brazil (black) and, within, Minas Gerais State (yellow), with Lagoa Santa region highlighted in the rectangle (a). Detail of the Carste de Lagoa Santa Environmental Protection Area (green area), indicating the original location of caves with palaeontological remains (black dots); Lapa da Cerca Grande cave indicated with a black-and-white dot (b). Scale bar = 2 km. Lagoa Santa map adapted from Kohler *et al.* (1978).

INTRODUCTION

The Lagoa Santa Karst in Minas Gerais, Central Brazil, is one of the most important Pleistocene fossil sites in South America, yielding a vast array of mammal and bird remains, many of these unique to the region (Auler, 2020; Cartelle, 2020). This cave system was first scientifically explored by Peter W. Lund (1801-1880), who conducted significant palaeontological and geological works in these sites during most of his life, which granted him the title of "father of Brazilian palaeontology and speleology" (Holten & Sterll, 2000, 2011). One of the most remarkable species discovered by him is the cave jackal *Speothos pacivorus* (Lund, 1839). *Speothos pacivorus* is a member of the South American lineage of canids (Canidae: Cerdocyonina), and its closest living relative is the bush dog *S. venaticus* (Okrinova, 2013; Zrzavý *et al.* 2017). All materials assigned to *Speothos pacivorus* come from a single cave, the Lapa da Cerca Grande cave (Figure 1), collected by Lund and sent to Copenhagen, where they are housed in the Peter Lund/Quaternary Collection of the Natural History Museum of Denmark (NHMD). This species received little attention compared to other fossil Cerdo-

cyonina, usually only being referred to in phylogenetic analyses (Perini *et al*. 2010; Okrinova, 2013; Zrzavý *et al.* 2017) and in summarized faunal accounts (Prevosti & Forasiepi, 2018). The only modern study completely dedicated to it was published four decades ago (Berta, 1984). Our goal here was to reanalyse all the remains associated to *S. pacivorus* hosted at the Peter Lund/Quaternary Collection of the NHMD, listing and illustrating all elements, and creating a three-dimensional model of the holotype, based on µCT scan of its almost complete cranium, hoping to promote more studies including this poorly-known canid.

METHODS

The specimens of *Speothos pacivorus*

All known remains of *S. pacivorus* are deposited in the Peter Lund/Quaternary Collection of the Natural History Museum of Denmark, Copenhagen (Table 1). They comprise two cranial elements (Figure 2), three incomplete mandibles (Figure 3), one vertebra (Figure 4), 18 elements of the appendicular skeleton (Figure 5), and 54 isolated teeth (Figure 6). The materials were photographed using a Nikon P530 and mostly included in the plates in at least two different views.

The holotype of *S. pacivorus* (NHMD:211341), a cranium, is the best preserved and most significant material. It preserves most of the bones; an unfused suture between the sphenoid and occipital indicates that this individual is a subadult. Its dentition is well preserved, missing left I1-2, right I2, left PM1, right PM2-3, and M2 on both sides, and lacks significant wear. Berta (1984) argued that this cranium and the main postcranial remains are from the same individual based on the relative size and stage of development.

3D acquisition and segmentation

Two specimens were scanned for the purpose of this reconstruction. The complete cranium of the holotype of *S. pacivorus* was µCT-scanned at the 3D Imaging Centre, Technical University of Denmark, using a Nikon XT H225 ST system. The scan was conducted using an aluminium filter with thickness of .002 mm, using a voltage of 120 kV and current of 417 µA, resulting in 3142 projections (voxel size of .1 mm). For comparison, a skull of the extant *S. venaticus*, SNHMS:19136, was also scanned at the 3D Imaging Lab of the University of Tubingen, Germany, using a Nikon XT H 320 system and an aluminium filter (thickness of .25 mm), with a voltage of 180 kV and current of 83 µA, resulting in 4476 projections (voxel size of .053 mm). The separation of organic from inorganic materials (e.g. sand and other sediments) and the segmentation of the bones were performed using manual and semi-automatic tools (e.g. brushes, wand) with the software Amira 2021.1 (Thermo Fisher Scientific) and the online tool Biomedisa (Lösel *et al.* 2020). The crania and teeth were segmented into separate materials in order to generate triangulated surface mesh models which were then exported in .PLY format.

Visual reconstruction of the cranium

The mesh models of both crania were imported into the open-source 3D-modeling software Blender 3.4.1 (available at https://www.blender.org). Specimen NHMD:211341 is mostly well preserved but separated into posterior and anterior parts along the unfused coronal suture. The alisphenoid is partially preserved on the right side while the orbitosphenoids, presphenoid, and vomer bones are mostly missing. The anterior and posterior parts were manually repositioned to achieve the best alignment possible both in all orthographic planes. Since the left side is the best preserved and the least deformed side of the cranium, the reconstruction was focused there. The cranium was positioned in an orthographic view and split into halves along an anteroposterior plane (antimeres) that would ensure that the preferred left side, when doubled and mirrored, would maintain the original volume of the cranium. Next, internal cranial structures such as teeth roots and vascular canals that remained from the surface extraction in Amira were isolated and removed manually. A few elements were present only on the right side and thus were mirrored and added to the left. Namely, the I1 and PM1 and the partially preserved alisphenoid bone on the right side, which included the round foramen and part of the orbital fissure were mirrored to complete the left half. The cranial roof had undergone a slight deformation along the midline, mainly at the nasals, which bend inwards. For that, the slightly better preserved right nasal bone was mirrored and repositioned by rotating it clockwise along the anteroposterior axis to correct the angle of the deformed cranial surface. The parietal bone, also deformed along the suture with the temporal, bending inwards, was also retro deformed, so that the suture is closed. The rest of the missing parts were reconstructed based on the extant species of *Speothos*, *S. venaticus*. The half missing alisphenoid and orbitosphenoid bones were isolated from the reference and fused into our reconstructing model. The inconsistencies left from this fusion, as well as all unfused sutures and fractures, were digitally infilled and smoothed to achieve a sturdy cranial volume. Missing parts at the zygomatic process of the temporal bone, the parietal and the palatine-presphenoid contact were sculpted to match the extant reference, as well as the crest of the left side to match the better-preserved side. Finally, the model was tested for mesh errors (bad geometries). For this, the Blender tool "3DPrint" was used to pinpoint the non-manifold edges and intersecting faces. Non-manifold faces were then cleaned up with the same tool while the intersecting faces were removed manually by merging each intersection together. The reconstructed cranium was then exported in .PLY format, retopologised and remeshed into an isotropic triangular mesh using the open-source software InstantMeshes (Wenzel et al., 2015), and exported in .STL format. The final reconstruction can be seen in Figure 7.

DISCUSSION

Our reanalysis of all specimens assigned to *S. pacivorus* makes more easily available the information about this taxon by presenting **a)** a list of all the specimens of *S. pacivorus*, including

ISSN: 2274-0422

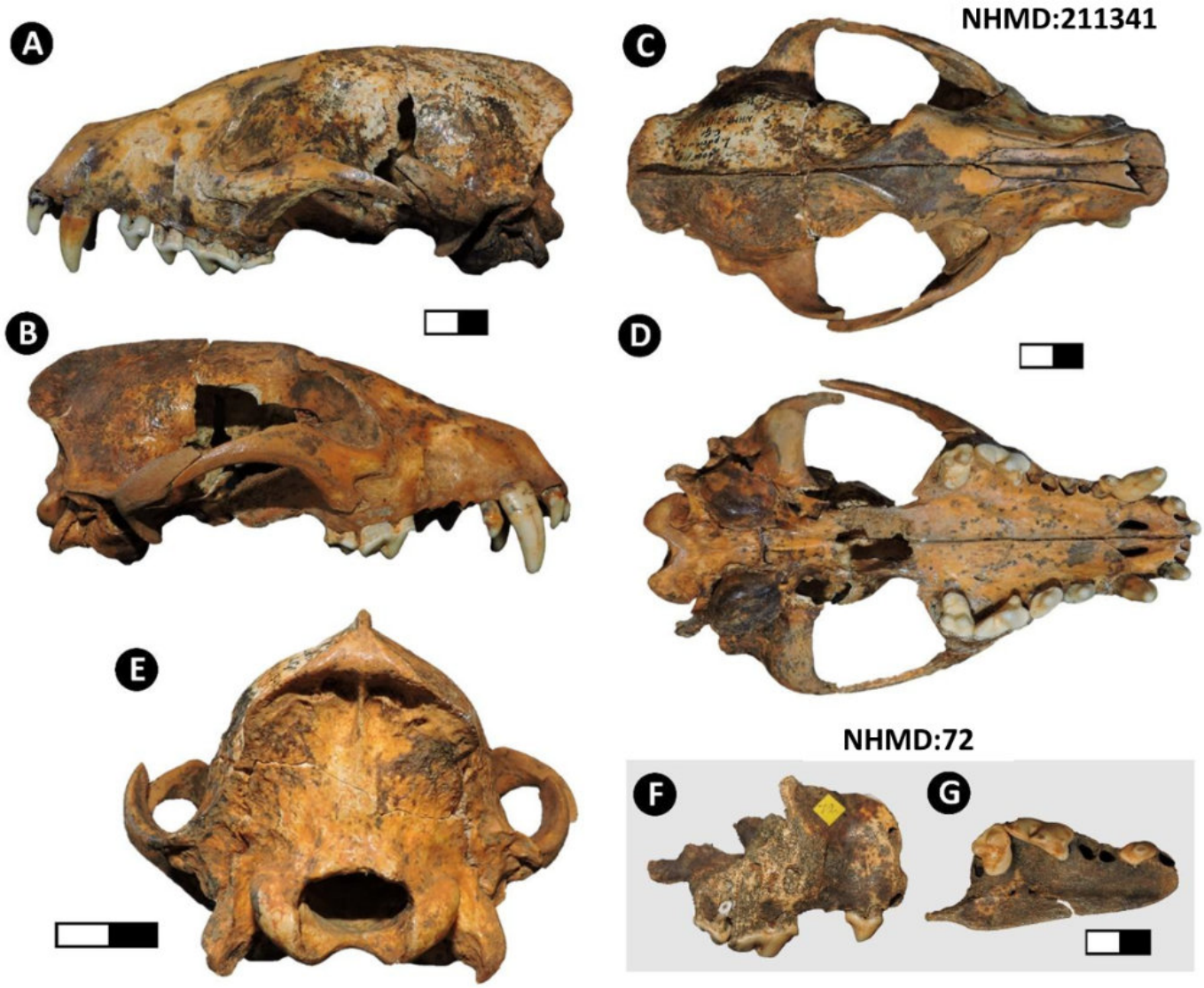

Figure 2. Cranial specimens of *Speothos pacivorus*. Holotype NHMD:211341 in left lateral (a), right lateral (b), dorsal (c), ventral (d), and posterior (e) views. Maxillary fragment NHMD:72 in right lateral (f) and ventral (g) views. Scale bars = 2 cm.

ISSN: 2274-0422

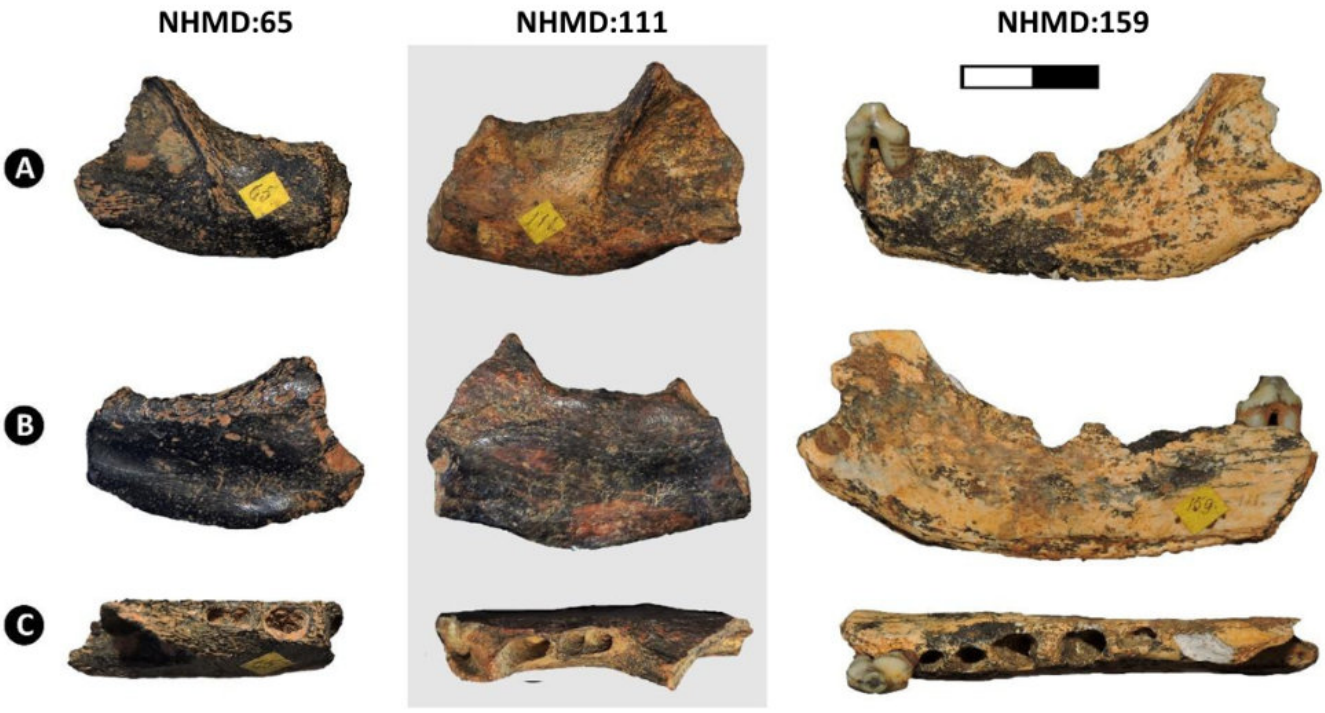

Figure 3. Mandibular fragments of *Speothos pacivorus* in lateral (a), medial (b), and dorsal (c) views. Scale bar = 2 cm.

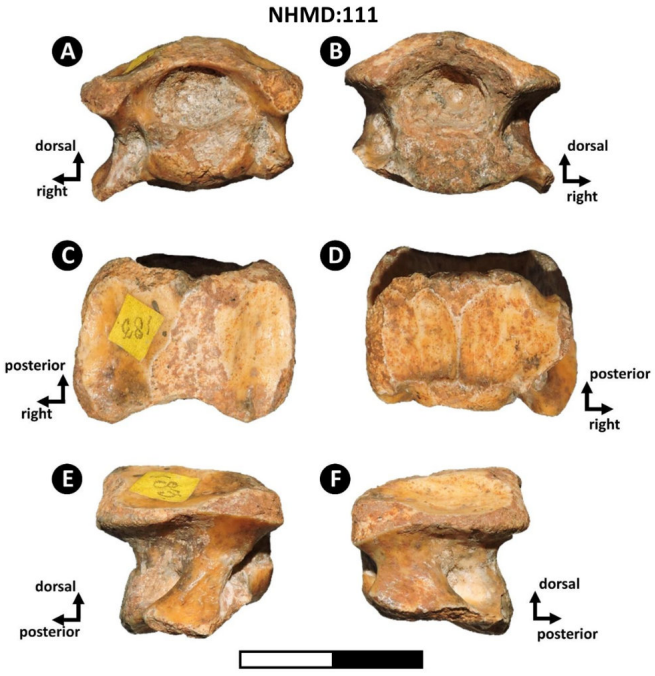

Figure 4. Cervical vertebra VI NHMD:111 of *Speothos pacivorus* in anterior (a), posterior (b), dorsal (c), ventral (d), right lateral (e), and left lateral (f) views. Scale bar = 2 cm.

illustrations; and **b)** a restored three-dimensional model of its holotype. The list of specimens can facilitate works on the taxonomy, faunal accounts of absolute and relative abundance, and taphonomy by presenting every known fossil of the species, as well as giving a detailed view of what is present in the Peter Lund/Quaternary Collection of the NHMD. The illustrations can be useful in comparative descriptions, morphological reconstructions, and bi-dimensional morphometrics analyses. The three-dimensional model made available here can allow peers to check the anatomy of the holotype in a reconstructed condition, without taphonomic deformation. In addition to the already mentioned potentials, this model can be used in studies based on modelled organic entities, as finite element analyses and geometric morphometrics analyses. It can also be 3D printed in its natural size and function as an educational tool, in exhibitions, etc. We hope that the information available here will allow researchers and interested peers all over the world to access the biological information of this poorly-studied canid.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 (PROEX 88887.486529/2020-00 and CAPES-DAAD 88881.650314/2021-01 to JVR; PROBRAL 88881.628 047/2021-01 to FCM, JVR, and MCC); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2020/12786-2 to FCM, JVR, GSF, and MCC); Deutscher Akademischer Austauschdienst (DAAD) 57598274 to GSF); Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG 202110267000072 to MCC, FCM, and JVR); and the National Science Foundation

ISSN: 2274-0422

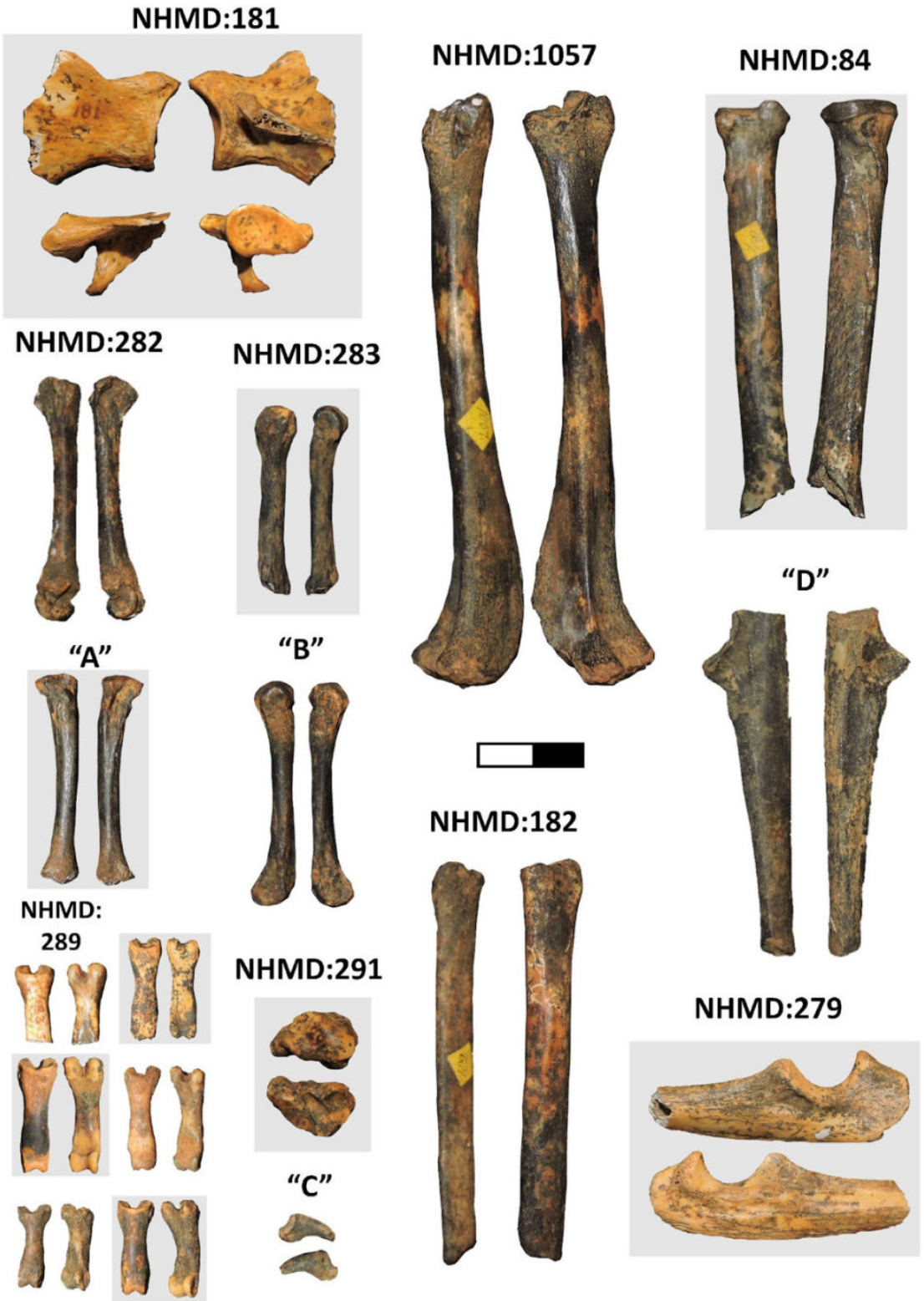

Figure 5. Appendicular elements of *Speothos pacivorus*. Specimen numbers are assigned next to each material, when present. "A", "B", "C", and "D" are unnumbered materials provisionally listed for easy crosscheck with the data in Table 1. Scale bar = 2 cm.

ISSN: 2274-0422

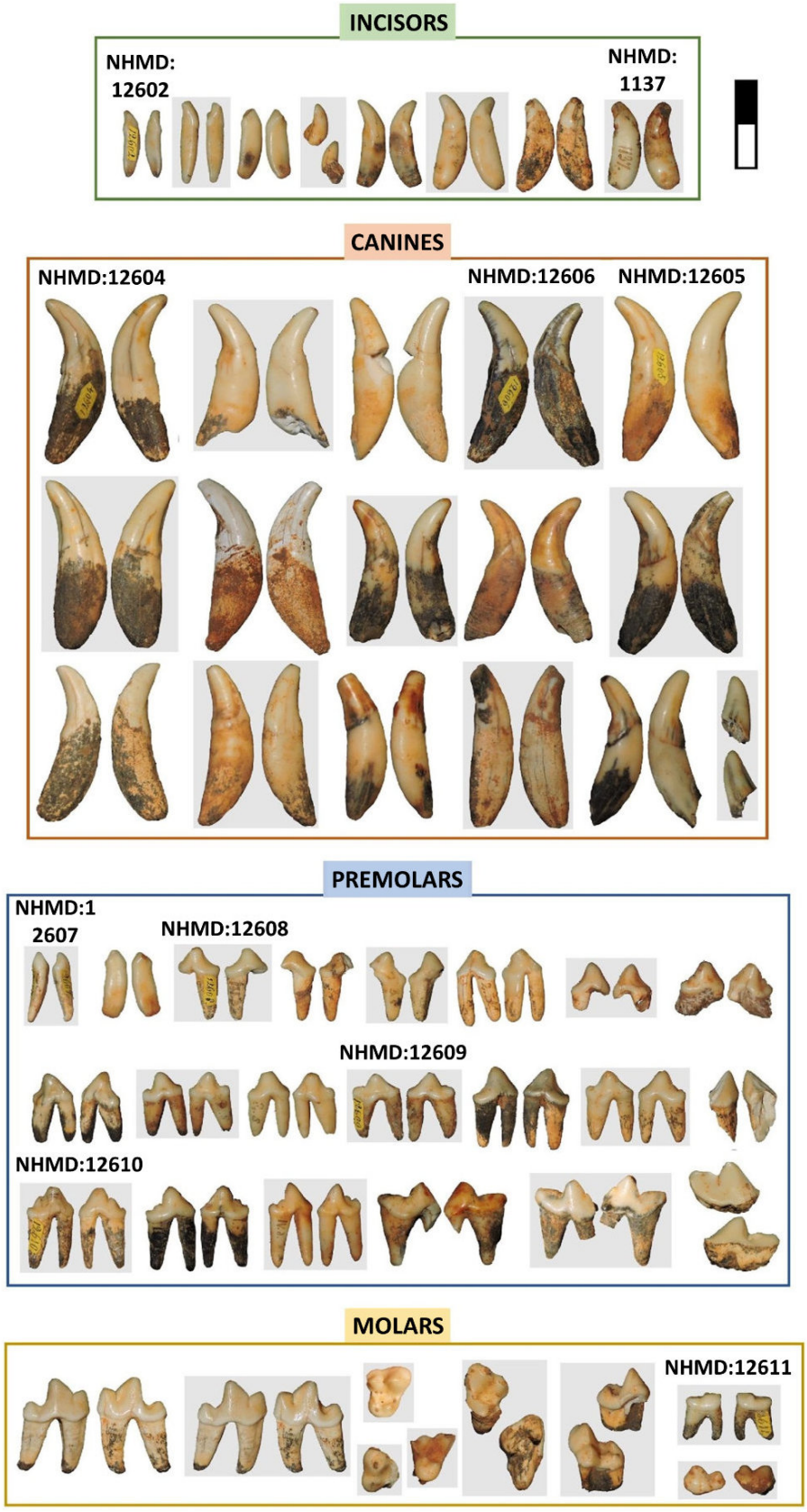

Figure 6. Isolated teeth of *Speothos pacivorus*. Specimen numbers are assigned next to each material, when present. Scale bar = 2 cm.

ISSN: 2274-0422

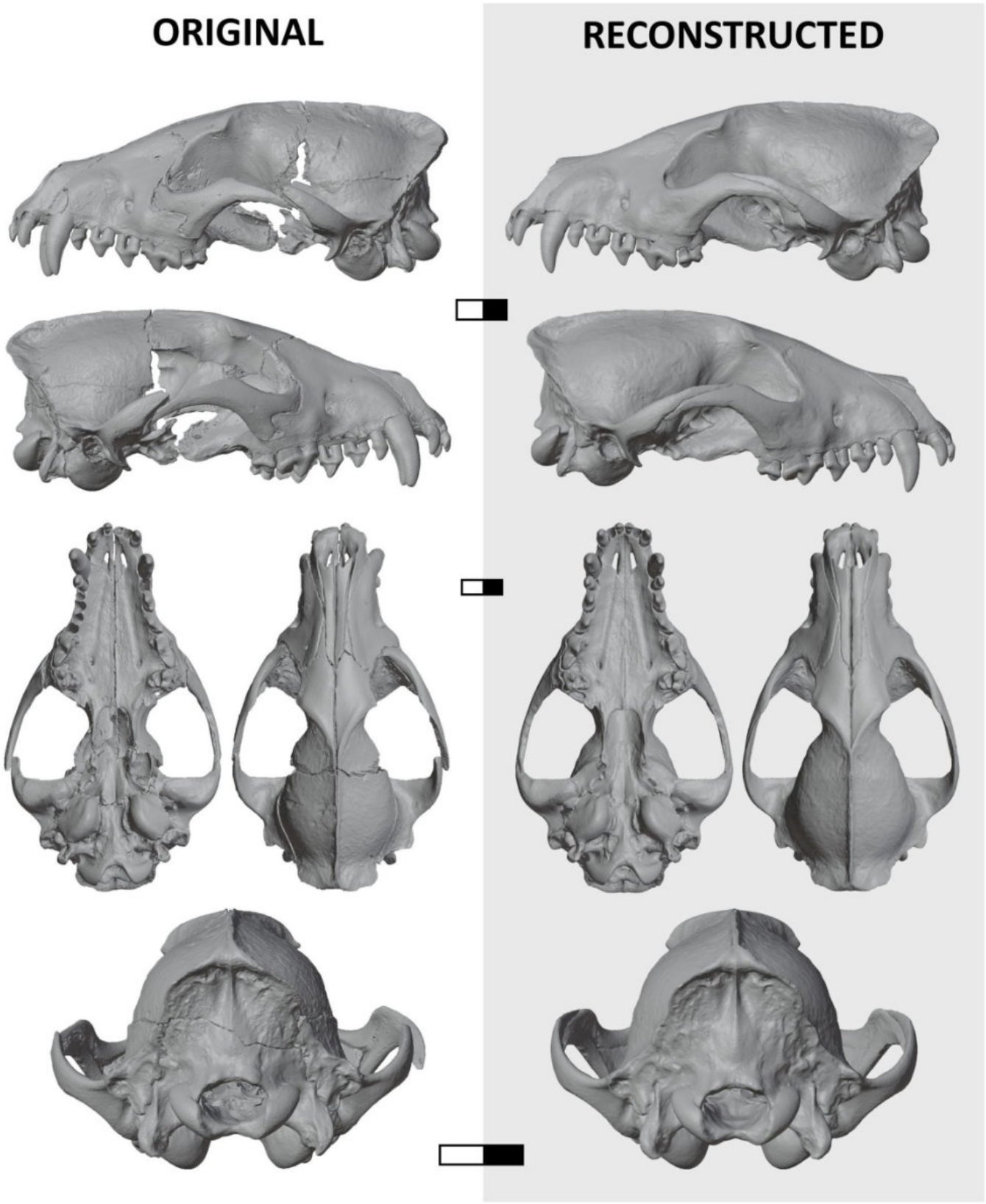

Figure 7. Comparison between the original (left) and reconstructed (right) cranium of *Speothos pacivorus* NHMD:211341. Scale bars = 2 cm.

ISSN: 2274-0422

(NSF DEB 1754596 to PLG). The instrumentation in the 3D Imaging Centre at DTU is partly funded by an infrastructure grant from the Danish agency for higher education and science (grant no. 5072-00030B).

BIBLIOGRAPHY

Auler, A. S., 2020. History of research in the Lagoa Santa Karst. In: *Lagoa Santa Karst: Brazil's Iconic Karst Region (Auler, A. S., and Pessoa, P., eds.).* Springer Nature, 1-11. https://doi.org/10.1007/978-3-030-35940-9_1

Berta, A., 1984. The pleistocene bush dog *Speothos pacivorus* (Canidae) from the Lagoa Santa caves, Brazil. *Journal of Mammalogy*, *65*(4), 549-559. https://doi.org/10.2307/1380837

Cartelle, C., 2020. Cave Paleontology in the Lagoa Santa Karst. In: *Lagoa Santa Karst: Brazil's Iconic Karst Region (Auler, A. S., and Pessoa, P., eds.).* Springer Nature, 209-225. https://doi.org/10.1007/978-3-030-35940-9_11

Holten, B., and Sterll, M., 2000. The Danish Naturalist Peter Wilhelm Lund (1801-80): Research on early man in Minas Gerais. *Luso-Brazilian Review*, 33-45.

Holten, B., and Sterll, M., 2011. *Peter Wilhelm Lund e as grutas com ossos em Lagoa Santa*. Editora UFMG, Belo Horizonte.

Kohler, H. C., Cançado, A. M., Gomes, D., Macieira, F. L., & Nascimento, N., 1978. Carte du Karst. *Laboratoire d'Analyse et Cartographie des Formations Superficielles de l'Université de Caen et le Centre de Geomorphologie, CNRS, Caen.*

Lösel, P. D., van de Kamp, T., Jayme, A., Ershov, A., Faragó, T., Pichler, O., ... & Heuveline, V. (2020). Introducing Biomedisa as an open-source online platform for biomedical image segmentation. *Nature communications*, 11(1), 5577. https://doi.org/10.1038/s41467-020-19303-w

Lund P. W. (1839).Coup d'oeil sur les espèces éteintes de Mammifères du Brésil, extrait de quelques mémoires présentés à l'Académie Royale des Sciences de Copenhague. In: *Annales des Sciences Naturelles*, 11, 214-234.

Okřinová, I., 2013. *Paleoecology of fossil species of canids (Carnivora, Mammalia)*. Master thesis. Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic, 53 p.

Perini, F. A., Russo, C. A. M., and Schrago, C. G., 2010. The evolution of South American endemic canids: a history of rapid diversification and morphological parallelism. *Journal of evolutionary biology*, *23*(2), 311-322. https://doi.org/10.1111/j.1420-9101.2009.01901.x

Prevosti, F. J., Forasiepi, A. M., 2018. South American fossil carnivorans (order Carnivora). In: Prevosti, F. J., and Forasiepi, A. M. (ed) *Evolution of South American Mammalian Predators During the Cenozoic: Paleobiogeographic and Paleoenvironmental Contingencies.* Springer, 85-136. https://doi.org/10.1007/978-3-319-03701-1_4

Wenzel J., Tarini M, Panozzo D. and Sorkine-Hornung O., 2015. Instant Field-Aligned Meshes. *ACM Transactions on Graphics (Proceedings of SIGGRAPH Asia 2015).*

Zrzavý, J., Duda, P., Robovský, J., Okřinová, I., and Pavelková Řičánková, V., 2018. Phylogeny of the Caninae (Carnivora): combining morphology, behaviour, genes and fossils. *Zoologica Scripta*, *47*(4), 373-389. https://doi.org/10.1111/zsc.12293

In situ	In Berta (1984)	Notes
NHMD:211341	UZM L “406”?180	Holotype; nearly complete cranium missing left I1-2, P1, M2, and right I1, P2-3, M2
NHMD:72	UZM L72	Right fragmentary maxilla with P2, P4, M1
NHMD:65	UZM L65	Right hemimandible with m1-2 alveoli
NHMD:111	UZM L111	Left hemimandible with m1-2 alveoli
NHMD:159	-	Left hemimandible with pm3
NHMD:183	UZM L183	Cervical vertebra VI
NHMD:1057	UZM L1057	Right tibia missing distal end
NHMD:84	UZM L84	Right radius missing proximal end
NHMD:181	UZM L181	Head portion of left scapula
NHMD:182	-	Radius or fibula missing end
NHMD:279	UZM L279	Right proximal ulna
NHMD:282	UZM L282	Right metacarpal IV
NHMD:283	UZM L283	Left metatarsal IV missing distal end
NHMD:289	UZM L289	Proximal phalanx
NHMD:291	UZM L291	Right scapholunar
No number (A)	?	Metapodial
No number (B)	-	Metapodial
No number (C)	?	Ungual phalanx
No number (D)	-	Ulna missing head
No number	-	Phalanx
No number	-	Phalanx
No number	-	Phalanx
No number	-	Phalanx
No number	-	Phalanx
NHMD:1137	-	Incisive
?	UZM L12603	Left lower incisive 3
NHMD:12602	-	Incisor
NHMD:12604	-	Canine
NHMD:12605	-	Canine
NHMD:12606	UZM L12606	Right lower canine
NHMD:12607	UZM L12607	Right lower premolar 1
NHMD:12608	UZM L12608	Right lower premolar 2
NHMD:12609	UZM L12609	Left lower premolar 3
NHMD:12610	UZM L12610	Left lower premolar 4
NHMD:12611	UZM L12611	Right lower molar 2
?	UZM L12613	Left upper premolar 4
?	UZM L?	Right lower molar 1
No number	-	Incisor
No number	-	Incisor
No number	-	Incisor
No number	-	Incisor
No number	-	Incisor
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine
No number	-	Canine

ISSN: 2274-0422

No number	-	Canine
No number	-	Canine
No number	-	Right upper premolar 4
No number	-	Right upper premolar 4
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Premolar
No number	-	Left lower molar 1
No number	-	Molar
No number	-	Molar
No number	-	Molar
No number	-	Molar
No number	-	Molar
No number	-	Molar

Table 1. Specimens of *Speothos pacivorus* hosted at the Peter Lund/Quaternary Collection of the Natural History Museum of Denmark. "In situ" refers to as specimens photographed by the authors in the collection; the specimens (and synonyms) listed by Berta (1984) are also presented.

ISSN: 2274-0422