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Juan Vitor Ruiz

A new look on *Speothos* Lund, 1839: investigating the evolutionary history of bush dogs and cave jackals through morphofunctional and quantitative approaches

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Back in my academic world, this meant that you were truly in limbo

(SAPOLSKY, 2001, p. 219)

RESUMO

Cerdocyonina, os canídeos (Canidae, Carnivora) sul-americanos, evoluíram em diferentes linhagens nos últimos 3 milhões de anos. Entre eles, o gênero *Speothos* compreende duas espécies com morfologia e história evolutiva únicas: a extinta espécie-tipo *Speothos pacivorus*, o chacal-das-cavernas, e *S. venaticus*, o cachorro-vinagre. Poucos estudos foram conduzidos sobre o chacal-das-cavernas, cujos registros fósseis são restritos ao Pleistoceno; em relação ao cachorro-vinagre, dada a dificuldade de estudo em seu ambiente natural, muito do que se sabe sobre sua biologia é oriundo de estudos realizados em cativeiro e informações anedóticas. Nos últimos anos, avanços em técnicas computacionais permitiram a zoólogos conduzirem análises *in silico* (i.e., simulações computacionais) para reconstruir sistemas orgânicos numa perspectiva quantitativa, facilitando estudos de táxons elusivos e extintos. Assim, essa tese objetivou investigar o comportamento predatório e a taxonomia de *Speothos* por meio de diferentes aplicações quantitativas. As análises do Capítulo 1 comparam a biomecânica do aparato craniomandibular do cachorro-vinagre com o de outros canídeos viventes com diferentes dietas e tamanhos, baseando-se em simulações oriundas de análises de elementos finitos (FEA). O segundo capítulo se baseia em duas abordagens distintas. A primeira delas é de cunho taxonômico, no qual a variação morfológica do crânio de *Speothos* foi avaliada através de análise de morfometria geométrica (GMM), esperando-se investigar o *status* taxonômico do cachorro-vinagre como espécie distinta. A segunda abordagem é similar à do Capítulo 1, comparando as performances biomecânicas do crânio do cachorro-vinagre e chacal-das-cavernas, permitindo inferências paleoecológicas. As análises do Capítulo 1 resultaram em novas perspectivas sobre o comportamento predatório do cachorro-vinagre, incluindo a sugestão de um aparato craniomandibular robusto e força de mordida considerável para um canídeo de porte pequeno, corroborando o pressuposto de se tratar de um caçador grupal de grandes roedores e xenártros (e.g., pacas, cutias, tatus). Os resultados também sugerem a ocasional predação de animais maiores (e.g., capivaras, antas), usualmente baseadas em registros não confirmados, como sendo atos plausíveis. As análises GMM no Capítulo 2 indicaram que a morfologia do crânio do chacal-das-cavernas não se encontra na variação esperada para o crânio do cachorro-vinagre, reforçando a hipótese dessas duas formas como espécies separadas. Por outro lado, os resultados da FEA se mostraram muito semelhantes para ambas as espécies, sugerindo modos similares de captura de presa e indicando uma possível especialização para presas de diferentes tamanhos, evitando a competição direta por recursos, bem como sugerindo uma pressão seletiva para o menor tamanho no cachorro-vinagre. Por fim, no Capítulo 3, todos os materiais fósseis do chacal-das-cavernas, atualmente depositados na Dinamarca, são listados e ilustrados, assim como a reconstrução do holótipo, um crânio, em modelo 3D. Ao valer-se de perspectivas comuns tanto à paleontologia quanto à neontologia, usando abordagens quantitativas *in silico*, os resultados combinados desses três capítulos contribuem para nosso conhecimento da biologia e história evolutiva de *Speothos*. As metodologias e dados originais empregados também podem ser úteis em novos estudos sobre esse clado peculiar de canídeos sul-americanos e suas formas associadas.

Palavras–chave: Canidae. Biomecânica. Método dos elementos finitos. Paleoecologia. Zoologia classificação.

ABSTRACT

Cerdocyonina, the South American canids (Canidae, Carnivora) evolved in various forms in the last 3 million years. Among them, the genus *Speothos* comprises two species unique in morphology and taxonomical history: the extinct and first described cavel jackal *S. pacivorus* and the extant bush dog *S. venaticus*. Few studies were conducted with the cave jackal, whose remains are restricted to the Pleistocene; regarding the bush dog, due to the difficulty of studying it in its natural habitats, most of our knowledge of its biology comes from observations of captive individuals and anecdotal records. In the past years, advances in computer techniques have allowed zoologists to employ in silico analyses (i.e., computer simulation) aiming to reconstruct organic materials through quantitative perspectives, enabling studies on elusive and extinct taxa. This thesis aimed to investigate the predatory behaviour and taxonomy of the *Speothos* genus by using different quantitative approaches. The analyses of the first chapter compare the biomechanics of the skull of the bush dog with that of other living canids with different sizes and diets based on finite element analysis (FEA). The second chapter presented two distinct approaches. The first of them is taxonomical, in which the cranial morphology variation of *Speothos* was tested through geometric morphometrics analysis (GMM), aiming to investigate the taxonomical status of the bush dog as a distinct species. The second approach is similar to that seen in Chapter 1, comparing the biomechanical performances of the cranium of both bush dog and cave jackal, allowing inferences on their palaeoecology. The analyses of Chapter 1 provided new insights into the predatory behaviour of the bush dog, including the suggestion of a strong skull and considerable bite force for a small-sized canid, corroborating the assumption of it being a pack hunter of large rodents and xenarthrans (e.g., pacas, agoutis, armadillos) and indicating that occasional predation on larger prey (e.g., capybaras, tapirs), usually based on non-confirmed records, as plausible events. The GMM analysis in the second chapter showed that the morphology of the cave jackal’s cranium is not within the expected variation for the bush dog, reinforcing the hypothesis of these two entities as separate species. The results of the FEA analysis in the same chapter, however, are very similar for both species, indicating similar ways to capture prey and suggesting a possible specialization of different prey sizes to avoid competition and a selective pressure to a smaller size in the bush dog. Finally, in the third chapter, all the fossil remains of the cavel jackal, currently housed in Denmark, are listed and illustrated, and the holotype, a cranium, was reconstructed in a 3D model. By using both palaeontological and neontological perspectives associated with quantitative, in silico approaches, the combined results of these three chapters helped to improve our knowledge of the biology and evolutionary history of *Speothos*. The original data and methodology also can be useful to new studies on this singular clade of South American canids and their associated forms.

Keywords: Canidae. Biomechanics. Finite element analysis. Palaeoecology. Zoology classification.

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LIST OF ABBREVIATIONS AND ACRONYMS

b.p.	(years) before present
m.y.	million of years (ago)
GABI	Great American Biotic Interchange
sp./spp.	species (singular and plural, respectively)
3D	tridimensional
FEA	Finite Element Analysis
CanU	unilateral canine bite
CanB	bilateral canine bite
CarU	unilateral carnassial bite
CarB	bilateral carnassial bite
BIR	Brazilian Intertropical Region
µCT	micro-computer tomography
GPA	generalized procrustes analysis
PC/PCA	principal component/principal component analysis
CS	centroid size
npMANOVA	non-parametric multivariate analysis
M1/m2	upper molar 1/lower molar 2
PM3/pm4	upper premolar 4/lower premolar 3
Df	degree of freedom
SS	sum of squares
MS	mean squares
R^2	percentage of the variation explained by a factor
F	Fisher' F
Z	Effect size
Pr(>F)	P value

RegScore	regression coefficients
HS	head-shake
HT	head-twist
PB	pull-back
I1/i2	upper incisor 1/lower incisor 2
.PLY	polygon file format
.STL	stereolithography file format

LIST OF SYMBOLS

kg	Kilogram
mm	Milimetre
kV	Kilovolt
µA	Microampère
E	Young's modulus
GPa	Gigapascal
υ	Poisson’s ratio
MPa	Megapascal
cm	centimeter
n	x number (of specimens)
km	Kilometre

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Figure 1.1 A raining morning at Lagoa Santa, 10.000 b.p.: a *Palaeolama* is attacked by a pack of cave jackals *Speothos pacivorus*.

Source: Julia D’Oliveira

1. DRAMATIS PERSONAE

1.1 The arrival of canids in South America

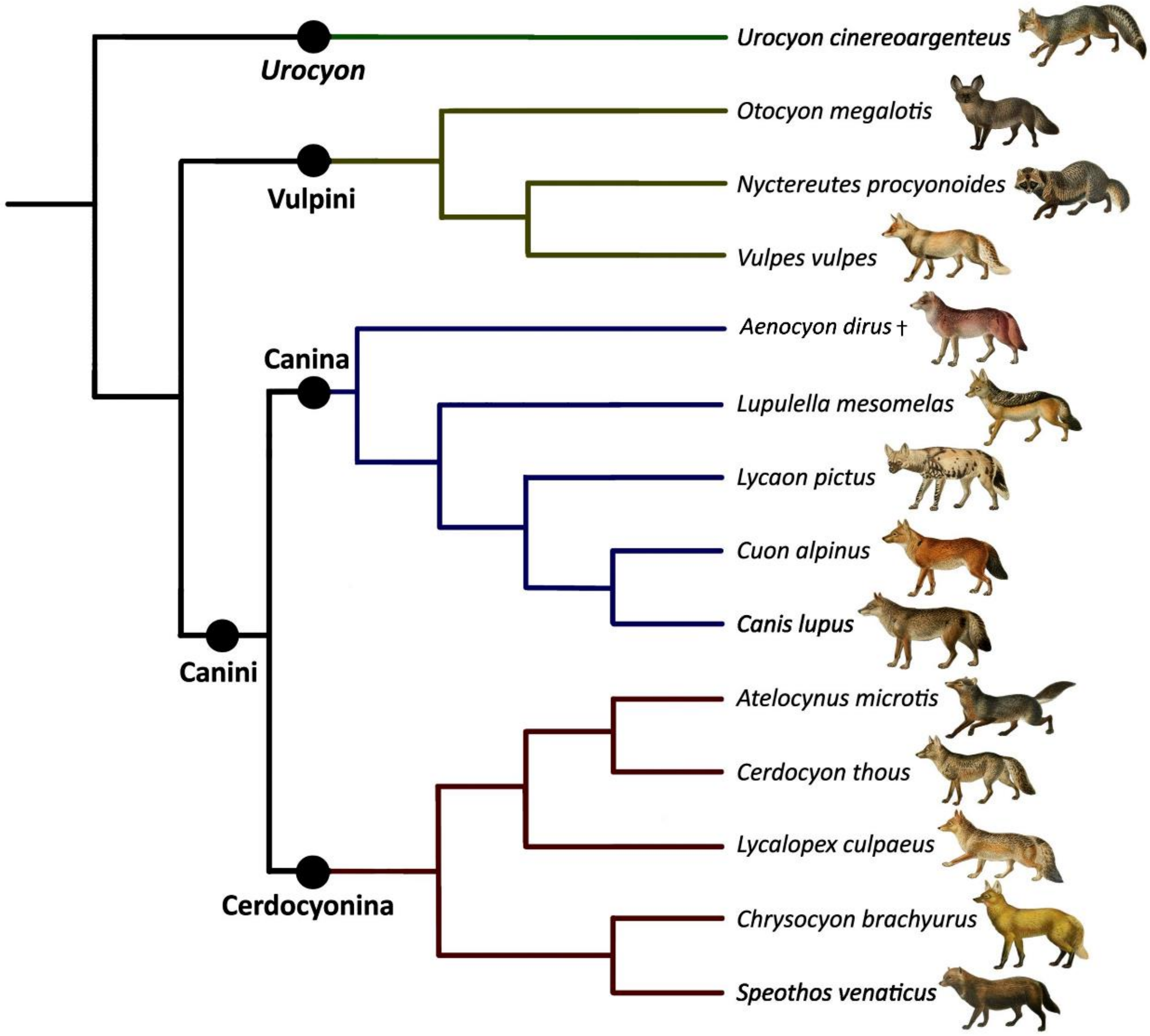
For most of the Cenozoic (66 m.y. to present), South American was an island continent, with sporadic and brief contact with other landmasses, especially Antarctica (YANBIN, 1998; GOIN *et al*. 2016; HOFFMEISTER, 2020). Therefore, the terrestrial fauna evolved into a unique assembly found nowhere else in the world, with representatives including terrestrial raptorial birds (Phorusrhacidae, LABARGE; GARDNER; ORGAN, 2024), large, hypercarnivorous notosuchians (Sebecidae, KELLNER; PINHEIRO; CAMPOS, 2014), giant semiaquatic crocodilians (Caimaninae, Gryposuchinae, CIDADE; FORTIER; HSIOU, 2019), and massive turtles (Podocnemididae, FERREIRA *et al*. 2024). Among them, the mammalian community stood apart. Typical South American clades of mammals that diversified during much of the Cenozoic include sloths and armadillos (Xenarthra), marsupials and sparossodonts (Metatheria), a diverse group of “ungulates” (Meridioungulata), as well as endemic rodents (Caviomorpha), and primates (Platyrrhini), among other (DEFLER, 2019a). The uniqueness of the South American endemic mammals and their peculiar evolutionary history was famously defined by the American palaeontologist George Gaylord Simpson as the resulting of a “splendid isolation” (SIMPSON, 1980).

This isolation was significantly reduced from the Pliocene (2.7 m.y.), with the connection of the South and North Americas through the Panama Isthmus (O’DEA *et al*. 2016), leading to major faunal changes. With this land bridge, terrestrial animals, especially mammals, fluxed from south to north and, primarily, from north to south, an event so important in shaping the faunal composition of both landmasses that was aptly named Great American Biotic Interchange, GABI for short (DEFLER, 2019b). Most of the Laurasiatherian mammals that currently are present in South America arrived in this continent during the GABI, including cervids, peccarids, tapirids, camelids, and carnivorans (DEFLER, 2019b). Although some carnivorans dispersed to South America before the GABI, as indicated by the presence of widespread Miocene procyonids (7.3 m.y., WOODBURN, 2010), most lineages only established in the continent after this event (HOFFMEISTER, 2020). One of the first carnivorans to do that was members of the Canidae lineage.

The clade Canidae Fischer von Waldheim, 1817 (hereafter canids) originated in North America, and for most of its evolutionary history, it was confined to this landmass. Fossil remains of the extinct Hesperocyoninae and Borophaginae were restricted to North America, while the third main lineage of canids, Caninae, firstly spread to Eurasia by the middle to late Miocene (16-7 m.y., WANG; TEDFORD, 2008; PERINI, 2009; PERINI; RUSSO; SCHRAGO, 2010); currently, all the extant, cosmopolitan canids belong to this clade (TEDFORD; WANG; TAYLOR, 2009). Regarding South America, non-anthropogenic dispersion of canids occurred in at least three waves. The first and major event took place during the Pliocene (3.9-3.5 m.y.) from a unique canid species that originated the endemic South American living canids, the Cerdocyonina (CHAVEZ *et al*. 2022). During the Late Pleistocene (12.700-11.500 b.p.) is detected the dispersal of *Aenocyon dirus* and *Canis nehringi* (a possible synonym of the former, PREVOSTI, 2006) from the North American, an influx that encompass large canids of the grey wolf lineage (PREVOSTI, 2006, 2010; PREVOSTI; RINCÓN, 2007), which was followed by the dispersal of the small grey fox *Urocyon cinereoargenteum* (PREVOSTI; RINCÓN, 2007). A phylogeny including all the major clades of Caninae is depicted in **Figure 1.2**.

Prior to the GABI, the terrestrial guild of South American carnivores was mostly limited to metatherians such as sparassodonts, some didelphids, phorurhascids birds, and at least one genus of armadillos, *Macroeuphractus* (PREVOSTI; FORASIEPI; ZIMICZ, 2013; ERCOLI; PREVOSTI; FORASIEPI, 2014; PREVOSTI; FORASIEPI, 2018). The sparassodonts were the main mammalian predators; although its extinction was traditionally viewed as the result of the putative competition with the Laurasiatherian carnivorans after the GABI, we now know that sparassodonts and carnivore marsupials were already in decline when the first canids appeared in the continent (PINO *et al*. 2022). With a depauperate assembly of mammalian carnivores and being some of the first arrivals in the GABI, the ancestral Cerdocyonina rapidly irradiated into several lineages, some of them bearing highly specialized morphological and, presumably, behavioural traits (DEFLER, 2019b; CHAVEZ *et al*. 2022). Today, the South American Cerdocyonina comprises the most diverse assembly of canids of any continent (BERTA 1987), including fox-like forms such as the crab-eating dog *Cerdocyon thous* and the South American foxes, *Lycalopex* spp., the fish-eating short-eared dog *Atelocynus microtis*, the large, long legged maned wolf *Chrysocyon brachyurus*, and the small, mustelid-like *Speothos venaticus* (CASTELLÓ, 2018).

Figure 1.2 Simplified phylogenetic relationship of Caninae, including species referred to in the *Dramatis personae* section. Phylogeny adapted from Lindblad-Toh *et al.* (2005) and Perri *et al.* (2021).



Source: Adapted from RUIZ *et al*. (2022).

1.2. Cave jackals and bush dogs: the genus *Speothos* Lund, 1839

The genus *Speothos* groups two species of small to medium size Cerdocyonina, the cave jackal *S*. *pacivorus* and the bush dog *S. venaticus*. The cave jackal is known for few fossils discovered on Lagoa Santa, Minas Gerais, Brazil, by Peter Lund (1839). The bush dog is the sole extant member of the genus *Speothos* and one of the most peculiar and understudied species of extant canids (BEISIEGEL; ADES, 2002). It is notable among living canids by presenting a long torso, shortened legs and tail, partial interdigital membranes, small, rounded ears, and a characteristic short snout, giving it a mustelid/bear-like appearance (BEISIEGEL; ZUERCHER, 2005). The remains of cave jackal indicate a similar body proportion when compared to the bush dog, but it was substantially larger and had longer, slender limbs (BERTA, 1984). The dentition of *Speothos* is also specialized, presenting 40-41 teeth instead of the usual 42 for the family, and exhibiting highly developed canines and carnassials (BERTA, 1984; SILLERO-ZUBIRI, 2009). As in the painted dog *Lycaon pictus* and the dhole *Cuon alpinus*, the lower carnassial of *Speothos* is trenchantly heeled, presenting a single cuspid in the talonid, contrary to the normal condition of two cuspids seen in other canids (VAN VALKENBURGH, 1991). This unique character was historically used to group these three, non-related genus in Simocyoninae (SIMPSON, 1957).

Some of these morphological characteristics can be directly linked to another particularity of *Speothos*, its hypercarnivore diet. An organism is considered hypercarnivorous when its diet is composed of, at least, 70% of vertebrate “meat”, including muscles, bones, viscera, and connective tissues (VAN VALKENBURGH, 2007; WANG; TEDFORD, 2008; PERINI; RUSSO; SCHRAGO, 2010). Among the common hypercanivory adaptations to capture and process vertebrate prey are the reduction of the dentition, the shortening of the snout, the development of specialized teeth (e.g., canines and carnassials in carnivorans, including the trenchant heel in canids), a reinforcement of the robustness of the skull elements, and an increase of the bite force, all of these presented by both the bush dog as well as the cave jackal (TSENG; WANG, 2010; RUIZ *et al*. 2022). Among living canids, the bush dog is one of the only four species considered to be hypercarnivores, and the only Cerdocyonina with this dietary specialization (SILLERO-ZUBIRI, 2009). In the Pleistocene South America, however, this guild was occupied by other specialized Cerdocyoninae, the larger *Theriodictis* and *Protocyon* (ZRZAVÝ *et al*. 2018), the latter being sympatric to both cave jackals and bush dogs in the Brazilian Intertropical Region (MOUCHARD, 2019).

The bush dog also presents distinct behavioural traits. It is one of the most gregarious canid species, living in packs of up to 12 individuals usually based on a reproductive pair and its offspring of different ages (BEISIEGEL; ZUERCHER, 2005; LIMA *et al.* 2014). Although individuals were seen foraging alone (DEUTSCH, 1983), they usually hunt in groups, allowing them to attack and capture considerably large prey, as other hypercarnivore canids such as grey wolves *Canis lupus* (WALLACE; PAINTER; SALDANIA, 2002; RUIZ *et al*. 2022).

1.3. Goals

Morphofunction (i.e., the relation between shape and function in a system) is gaining attraction in palaeontological and neontological studies through a quantitative perspective. The advances in 3D modelling and statistical analyses in this field lead to insights unable to be obtained as recently as two decades ago. The use of finite element analysis and geometric morphometric analysis, the two main methodologies applied in the following chapters, in studies involving biomechanics, dietary behaviour, evolutionary trends, and taxonomy and systematics, *inter alia*, is already well established (RAYFIELD, 2007; CURRAN, 2018), being used in analyses involving both extinct (FIGUEIRIDO *et al.* 2018; MONTEFELTRO *et al.* 2020; CHATAR *et al.* 2021; SILVA-JUNIOR *et al.* 2022) as extant organisms (SLATER; DUMONT; VAN VALKENBURGH, 2009; SLATER *et al.* 2010; GRUBICH *et al.* 2012; FABRE *et al.* 2014; MACHADO; TETA, 2020).

The genus *Speothos* is an excellent "cryptic" clade to be investigated through quantitative, *in silico* approaches. The extant living bush dog is an elusive species difficult to study in the field, whose much basic information came from the observation of captive individuals and even anecdotal records; the extinct *S. pacivorus*, although very similar, is considerably larger than the bush dog, is represented by excellent fossil remains but was historically understudied. Considering the substantial gaps in our knowledge of *Speothos* biology and evolutionary history, this thesis aimed to investigate in detail some of its peculiar characteristics, focusing especially on the skull morphofunction and unique, hypercarnivore specialization. To do so, the first two chapters investigated the following hypothesis:

Chapter 1 – The bush dog's skull is biomechanically more similar to other hypercarnivorous canids than to mesocarnivorous species.

Chapter 2 – The Pleistocene cave jackal, *Speothos pacivorus*, is distinct from the bush dog in both taxonomical and palaeoecological traits.

To test these hypotheses, I used an array of *in silico* and statistical methods, including finite element analysis and geometric morphometric analysis, to investigate morphofunctional features difficult or even impossible to obtain using living specimens, such as bite forces and bone strength. Additionally, **Chapter 3** focused on an account of all the known materials of *S. pacivorus*, as well as a detailed description of the reconstruction of the holotype's cranium into a complete, undeformed 3D model. The main goal of this descriptive chapter was to list and illustrate, for the first time, all its fossil remains. The results of this thesis contributed by presenting new data on the predatory and feeding behaviours of this unique but otherwise understudied clade, as well as, hopefully, by popularizing these two species to the scientific community.

Note: this thesis is comprised by three chapters that are already published (Chapter 1 and Chapter 3) or is currently under review (Chapter 2).

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2. CHAPTER 1: Different, but the same: Inferring the hunting behaviour of the hypercarnivorous bush dog (*Speothos venaticus*) through finite element analysis

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2.1. CHAPTER ABSTRACT

Cerdocyonina is a clade composed by the South American canids in which the bush dog (*Speothos venaticus*) is one of the most elusive species. Known for its unique morphology within the group, this small, bear-like faced canid is the only member of the clade adapted to hypercarnivory, an almost exclusively meat-based diet currently present only in usually large, pack-hunting canids such as the grey wolf (*Canis lupus*). However, much of the biology of the bush dog is poorly understood, and inferences about its ecology, hunting strategies and diet are usually based on observation of captive individuals and anecdotal records, with reduced quantitative data to offer support. Here, the craniomandibular functional morphology of the bush dog was investigated through finite element analysis (FEA). FEA was employed to model the biting behaviour and to create extrinsic and intrinsic functional scenarios with different loads, corresponding to different bites used to subdue and process the prey. For comparison, the same modelling was applied to the skull of a grey wolf and a grey fox (*Urocyon cinereoargenteus*). The analyses showed that the bush dog's responses to loading are more similar to the wolf's than to the fox's in most scenarios, suggesting a convergent craniomandibular functional morphology between these two hypercarnivorous species, despite their distinct phylogenetic positions and body sizes. Differences between the three taxa are noteworthy and suggested to be related to the size of the usual prey. The modelled bite force for the bush dog is relatively strong, about half of that estimated for the wolf and about 40% stronger than the fox's bite. The results strengthen with quantitative data the inferences of the bush dog as a pack-

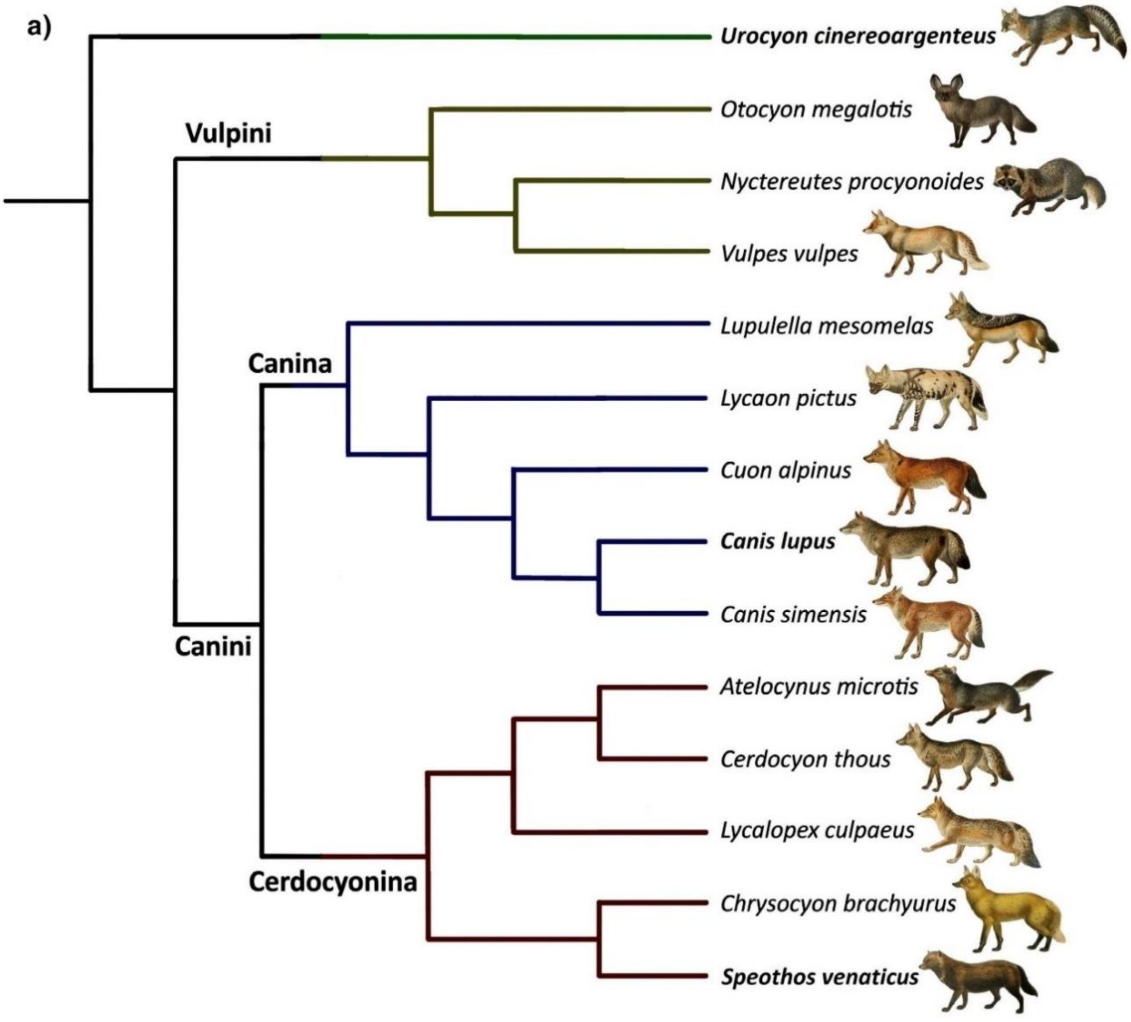
hunting predator with prey size similar to its own, such as large rodents and armadillos, being specialised in subduing and killing its prey using multiple bites. Its similarity to the wolf also confirms anecdotal accounts of predation on mammals that are much larger than itself, such as peccaries and tapirs. These data highlight the ecological specialisation of this small canid in a continent where large, pack-hunting canids are absent**.**

2.2. Introduction

Cerdocyonina is a lineage of South American endemic canids that include some of the least-known extant canid species (**Figure 2.1**, CHAVEZ *et al*. 2022; DEMATTEO; LOISELLE, 2008; PITMAN; BEISIEGEL, 2013; TENSEN, 2018). This group comprises generalist fox-like morphotypes (*Cerdocyon*, *Atelocynus* and *Lycalopex*), as well as highly specialised species such as the maned wolf (Chrysocyon brachyurus) and the bush dog (*Speothos venaticus*, CHAVEZ *et al*. 2022; LINDBLAD-TOH *et al*. 2005; SEGURA; CASSINI; PREVOSTI, 2021; WOZENCRAFT, 2005). Most Cerdocyonina are mesocarnivores (i.e. with a diet comprised of 50%–70% of vertebrate tissues, balanced with non-vertebrate food items such as insects, fruits and seeds; SLATER; DUMONT; VAN VALKENBURGH, 2009; VAN VALKENBURGH, 2007), which is the most common condition in living canids. The bush dog, however, is the only living member of Cerdocyonina adapted to hypercarnivory (i.e. diet comprising >70% of vertebrate tissues, PERINI; RUSSO; SCHRAGO, 2010; VAN VALKENBURGH, 2007; WANG; TEDFORD, 2008). The hypercarnivorous diet is only observed in three other species of living canids, all included in the Canina clade, the sister group of Cerdocyonina (**Figure 2.1**; LINDBLAD-TOH *et al*. 2005; ZRZAVÝ *et al*. 2018): the painted wolf (*Lycaon pictus*) from Africa, the dhole (*Cuon alpinus*) from Asia and the grey wolf (*Canis lupus*) with a Holarctic distribution (VAN VALKENBURGH, 1991).

The bush dog is a small, short-legged canid (weighing 4–7 kg, BEISIEGEL; ZUERCHER, 2005), and its general biology is poorly studied in its natural habitat, with most of its biological information obtained from observation of captive animals (BEISIEGEL; ADES, 2002). Anecdotal accounts comprise most of the information about individuals in the wild, including social and hunting behaviours and habitat use patterns (BEISIEGEL; ZUERCHER, 2005; HILDEBRAND, 1954). Most of the known aspects of the functional morphology of the bush dog were made through generic inferences based on its unique morphology. Its massive and deep skull, with short rostrum, reduced dentition and enlarged canines and carnassials points to a specialised diet overwhelmingly based on meat (DAMASCENO; HINGST-ZAHER; ASTÚA, 2013; VAN VALKENBURGH, 1991). Indeed, the bush dog was historically placed in an exclusively hypercarnivorous subfamily of Canidae called Simocyonina, together with the painted wolf and the dhole, based on the unique traits of its carnassial teeth (SIMPSON, 1957). The hypercarnivorous diet of the bush dog is verified by observations of captive animals (MACDONALD, 1996) and faeces analyses of wild individuals (LIMA; PINTO; DALPONTE, 2009; ZUERCHER; GIPSON; CARRILLO, 2005). However, there are few available quantitative data on the bush dog's predatory behaviours from a functional morphology perspective (CHRISTIANSEN; ADOLFSSEN, 2005; CHRISTIANSEN; WROE, 2007; PENROSE, 2019; PENROSE *et al*. 2020).

Figure 2.1. Simplified phylogenetic relationship of Canidae showing its main lineages, after Lindblad-Toh *et al*. (2005). Illustration of the taxa from Mivart (1890).

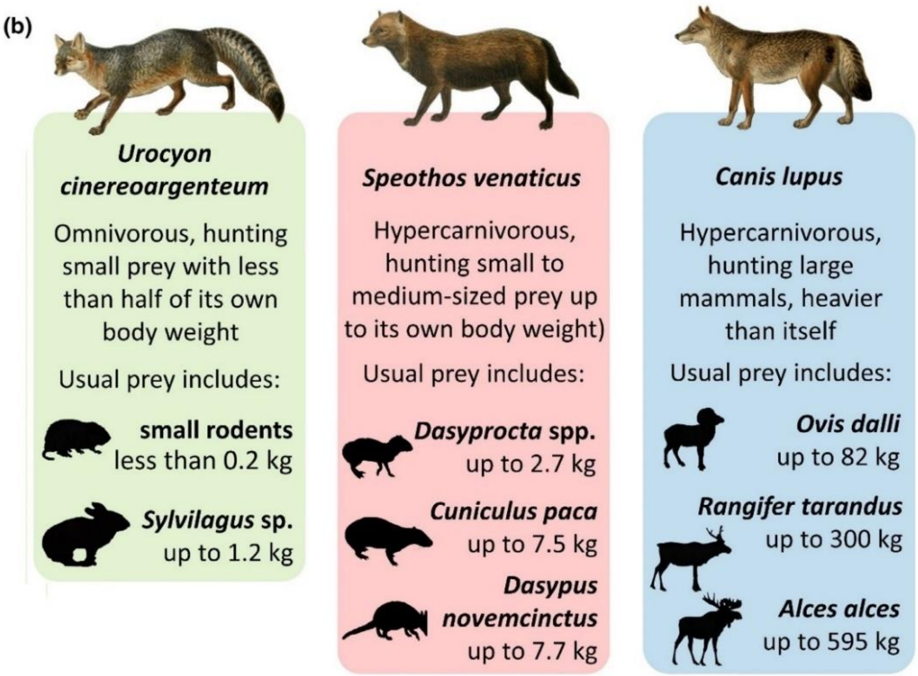


Source: adapted from Ruiz *et al*. (2022)

Here, it is used a functional morphology approach to analyse the skull of the bush dog using finite element analysis (FEA) and compare it to the models of the hypercarnivorous grey wolf (**Figure 2.2**), a large, pack-hunting canid (weighing up to

50 kg, MECH, 1974), and the grey fox (*Urocyon cinereoargenteus*), a similar sized species (weighing 3–7 kg, FRITZELL; HAROLDSON, 1982) that has a mesocarnivorous diet (**Figure 2.2**, ALLEN *et al*. 2021). Bite forces and simulated biomechanical scenarios were tested to understand the functional morphology of the bush dog and, thus, its possible hunting strategies. By testing previously formulated hypotheses of its hunting and feeding behaviour, the main goal of this study is to fill gaps in the limited data from field studies. The results shed light on biomechanical aspects that may bridge the lack of quantitative data regarding the predation strategies of the bush dog and contribute to a better understanding of this elusive species.

Figure 2.2. Size of the usual prey of the grey fox (*Urocyon cinereoargenteus*, FRITZELL; HAROLDSON, 1982), bush dog (*Speothos venaticus*, LIMA; PINTO; DALPONTE, 2009; ZUERCHER; GIPSON; CARRILLO, 2005) and (*Canis lupus*, PETERSON; CIUCCI, 2003). For a detailed account of the usual prey of each species, see **Appendix 2.1**. Illustration of the taxa from Mivart (1890).



Source: adapted from Ruiz *et al*. (2022)

2.3. Methods

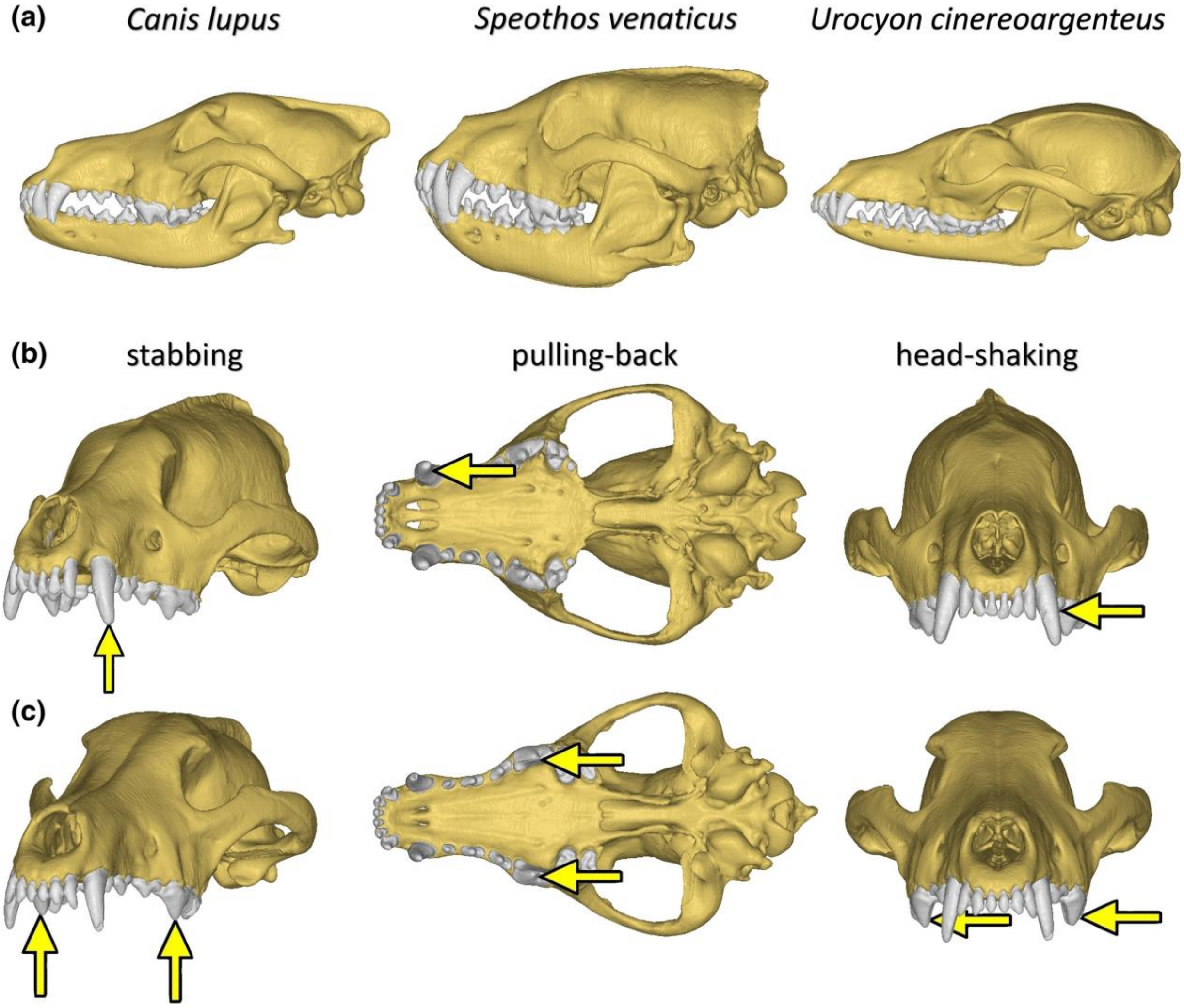
2.3.1. Specimens and Segmentation

The specimen of bush dog modelled for this study (MVZ 184054—Museum of Vertebrate Zoology, Berkeley, USA) was scanned in the University of Texas High-

Resolution X-ray CT Facility, with 0.31 mm slice thickness, voltage of 150 kV and current of 160 µA, and made available on the online platform digimorph.org (**Figure 2.3**). For comparison, a specimen of grey wolf (*C. lupus pambasileus*, LACM(M)23,010—Department of Mammalogy, Natural History Museum of Los Angeles County, Los Angeles, USA) was obtained through published data (TSENG; GROHÉ; FLYNN, 2016) deposited in the online platform morphosource.org (media ID 000009038), and modelled herein (**Figure 2.3**). This specimen was scanned at UCLA Medical Center, with 0.6 mm slice thickness, voltage of 150 kV and current of 198 µA. The grey wolf was chosen for comparison since it is the most studied hypercarnivorous canid (TENSEN, 2018), with a solid history of publications about its hunting and dietary habits, which can serve as a model for inferences on the bush dog. Additionally, a specimen of grey fox (*U. cinereoargenteus*, UCLA 6928—University of California, Los Angeles, USA) was also tested, scanned in the University of Texas High-Resolution X-ray CT Facility, with 0.24 slice thickness, voltage of 120 kV and current of 160 µA, also made available on the online platform digimorph.org (**Figure 2.3**). The grey fox was tested because of it omnivorous, non-specialised diet (FRITZELL; HAROLDSON, 1982), a condition that could be used as a proxy for the general dietary habits of Canidae. Further information about the computed tomography scans is provided on their respective repositories.

To model the extrinsic (i.e. ‘external’ forces, generated by a struggling prey) and intrinsic (i.e. ‘internal’ forces, generated by the canids' own musculature) scenarios, the skull elements were segmented into osteological (cranium and mandible) and dental (upper and lower dentition) components using the software Amira 5.3.3 (Thermo Fisher Scientific). The segmentation was conducted using automatic and manual tools. The three-dimensional models were exported to the software Hypermesh 13. 0 (Altair Engineering) and were treated as isotropic and homogeneous (solid model consisting of approximately 900,000 tetrahedral elements per model). The material properties of bones and teeth (bone: $E = 13.7$ GPa, $\upsilon = 0.3$ N; tooth: $E = 38,6$ GPa, $\upsilon = 0.4$ N) were assigned based on previous works on carnivore mammals, including canids (ATTARD *et al*. 2011; CHAMOLI; WROE, 2011; FIGUEIRIDO *et al*. 2018; SLATER; DUMONT; VAN VALKENBURGH, 2009; SLATER; VAN VALKENBURGH, 2009). In the absence of direct material testing for each modelled species, material properties obtained for other Carnivora species and applied to all models allowed the functional study of the different skull morphologies in a maximally comparative context. Each modelled scenario was solved in the software Abaqus 6.14-1 (Simulia). The performance of each model was assessed via contour plots of von Mises stress distribution and mean von Mises stress and displacement value of the elements, considering 99% of the values to avoid individual stress singularities on nodes (FIGUEIRIDO *et al*. 2018; MONTEFELTRO *et al*. 2020).

Figure 2.3. (a) Digital reconstructions of the analysed specimens of grey wolf (*Canis lupus*), bush dog (*Speothos venaticus*) and grey fox (*Urocyon cinereoargenteus*). Unilateral and bilateral extrinsic scenarios applied to the cranium models: (b) unilateral bite with the canine in the bush dog; (c) bilateral bite with the carnassials in the grey wolf.



Source: Ruiz *et al*. (2022)

2.3.2. Extrinsic Scenarios

Twelve extrinsic scenarios with different load assignments were tested to understand the craniomandibular functional properties in different conditions, based on the application of an extrinsic load of 500 N on each tested tooth (**Figure 2.3**), a magnitude which concerns the bite force recovered for both species (see **Section 2.4.2).** The scenarios follow Figueirido *et al*. (2018) and consist of: (i) stabbing, in which a dorsally direct load was placed at the tip of the tooth; (ii) pulling back, in which an anteriorly direct extrinsic load was placed at the posterior surface of the tooth; and (iii) head shaking, in which a laterally direct load was placed at the mid-left surface of the tooth (**Figure 2.3**). Both canines and carnassials were tested in unilateral and bilateral extrinsic scenarios (**Figure 2.3**). The tests aim to investigate cranial properties in situations that simulate possible behaviours during prey capture and consumption, including bite (stabbing) and postcranial guided head movements (pulling back and head shaking, MONTEFELTRO *et al*. 2020). For comparison, the same scenarios were applied to the corresponding teeth on the lower jaw, that is, the lower canines and carnassials, of the three species in mandible-driven bilateral bites. The wolf and the grey fox models were scaled to the same surface area of the bush dog model to avoid incongruities due to the difference size between the specimens (DUMONT; GROSSE; SLATER, 2009). In the cranium models, constraints were applied on the occipital condyle (10 in total) and the temporomandibular joint (five on each side); in the mandible models, they were applied at the condyloid process (five on each side), with each node constrained in all directions (*x*, *y* and *z*).

2.3.3. Intrinsic Scenarios and Bite Force

To calculate bite force, intrinsic scenarios were simulated for the mandible models, using a simplified jaw adductor muscle-driven biting (following FIGUEIRIDO *et al*. 2018; MONTEFELTRO *et al*. 2020). The *m. temporalis*, *m. masseter*, and *m. pterygoideus* (including the medialis and lateralis) were reconstructed based on the dry-skull method and without considering their subdivisions, following previous studies (PENROSE *et al*. 2020; TSENG; GROHÉ; FLYNN, 2016; TSENG *et al*. 2017). The adductor force of each muscle (**Table 2.1**) was estimated by considering its attachment area, as in previous works on canids and other Carnivora (PENROSE *et al*. 2020; TSENG *et al*. 2017). The attachment areas were used as a proxy for physiological cross-sectional area, which was then multiplied by an isometric muscle stress value of 37.0 N cm^{-2} (CHRISTIANSEN; ADOLFSSEN, 2005; CHRISTIANSEN; WROE, 2007; Koolstra *et al*. 1988; PENROSE *et al*. 2020; WEIJS; HILLEN, 1985).

Table 2.1. Total force inferred from the mandible attachments for each modelled muscle.

	Temporalis (N)	Pterygoideus (N)	Masseter (N)
Speothos venaticus	833.61	422.54	152.81
Canis lupus	2205.2	959.52	468.79
Urocyon	608.65	216.93	114.7

Source: Ruiz *et al*. (2022)

Four intrinsic scenarios were simulated in the mandibles: (i) unilateral bite with the canine; (ii) bilateral bite with both canines; (iii) unilateral bite with the carnassial; and (iv) bilateral bite with both carnassials. For each test, constraints were placed on the condyloid process (five on each side) and on the tip of each tested tooth (for the unilateral scenarios, on the left tooth; for bilateral scenarios, on the teeth of both sides); each node was constrained in all directions (*x*, *y* and *z*).

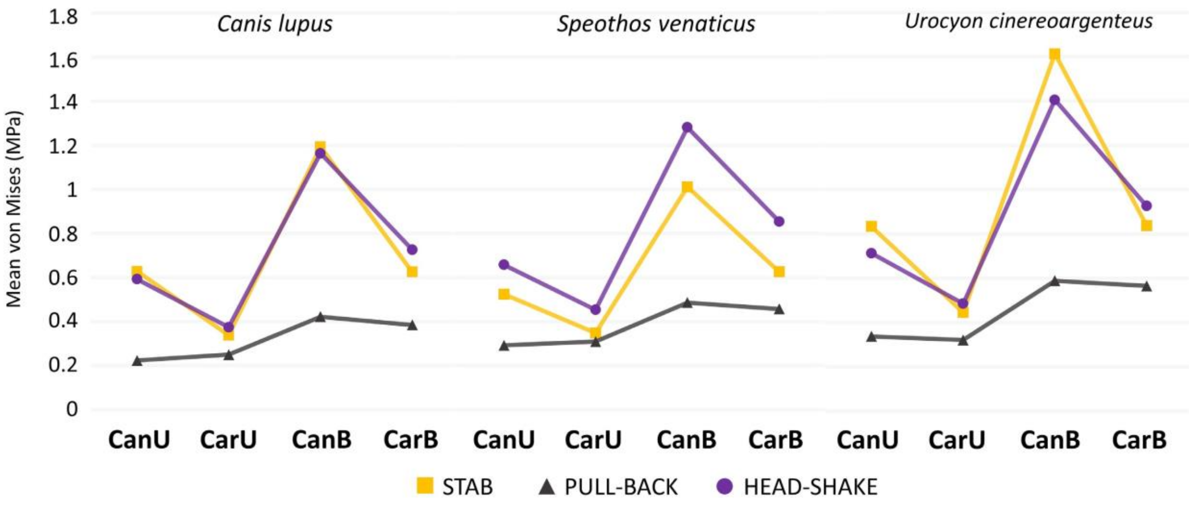
2.4. Results

2.4.1. Extrinsic Scenarios

In general, the extrinsic scenarios in the cranium do not show great differences in mean von Mises stress per element between the three species, being similar in all tested taxa (**Figure 2.4**). In most tests, the bilateral scenarios recorded higher stresses than the unilateral ones, and the carnassial bites proved to be less prone to stress than the canine bites in all but the pullback unilateral scenarios of the hypercarnivorous taxa (even in these, the differences were minimal). Regarding how the crania reacted to the different scenarios, the stabbing and head shaking behaved relatively similar to each other, while the pulling back stood out from the others with the lowest mean von Mises stress per element. The contour plots and mean von Mises stress per element of each cranial scenario is shown in **Figures 2.5-2.7**.

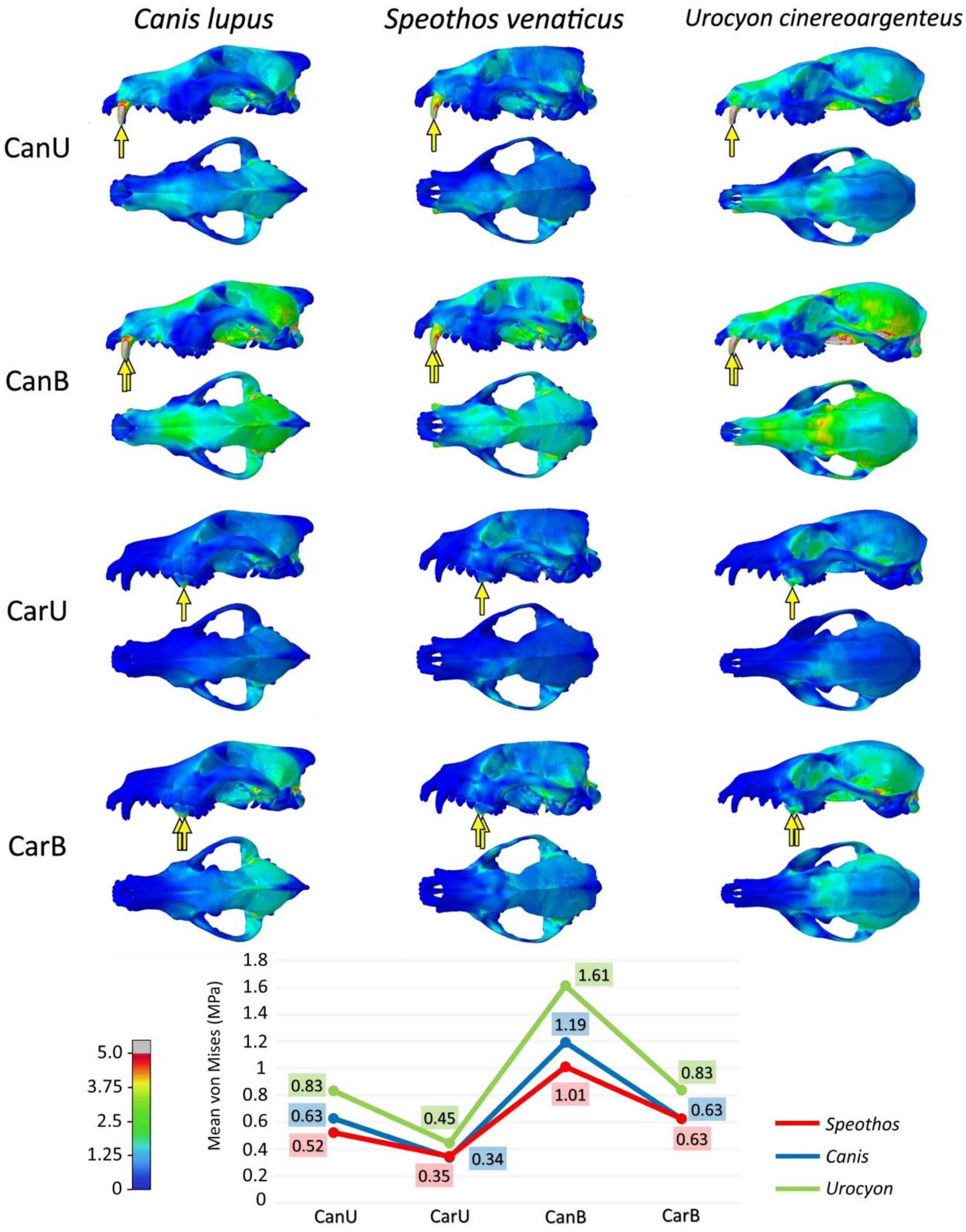
The stabbing and head-shaking scenarios not only showed more stress per element than the pulling back scenario, but also had more widespread stress across the crania (**Figures 2.5** and **2.6**), while in the pulling back scenario most of the stress was limited to the ventral region of the cranium (**Figure 2.7**). Between the three taxa, the stress varied more in terms of magnitude (i.e. the mean von Mises stress per element) than spatial distribution (i.e. the regions more or less stressed in the cranium). This suggests the same pathway, as stress is distributed in similar areas of the cranium of these canids, as represented in the contour plots of von Mises stress (**Figures 2.5-2.7**). The most affected areas in the stabbing and head-shaking scenarios are the attachment region of the temporalis muscle, including most of the temporal, parietal and the orbital region of the frontal, the zygomatic arch (especially in the head-shaking scenarios), and the maxilla and nasal (**Figures 2.5** and **2.6**). In the grey fox, the canine scenarios also presented higher stress levels in the anterior portion of the rostrum when compared to the hypercarnivorous taxa, especially in the stabbing and pulling back scenarios (**Figures 2.5** and **2.7**). In the pulling- back scenarios, most of the stress is restricted to the palatines and pterygoids in all species (**Figure 2.7**).

Figure 2.4. Relation among the mean von Mises stress in the three extrinsic cranial scenarios tested. CanU: Unilateral canine-driven bite; CarU: Unilateral carnassial-driven bite; CanB: Bilateral canine-driven bite; CarB: Bilateral carnassial-driven bite.



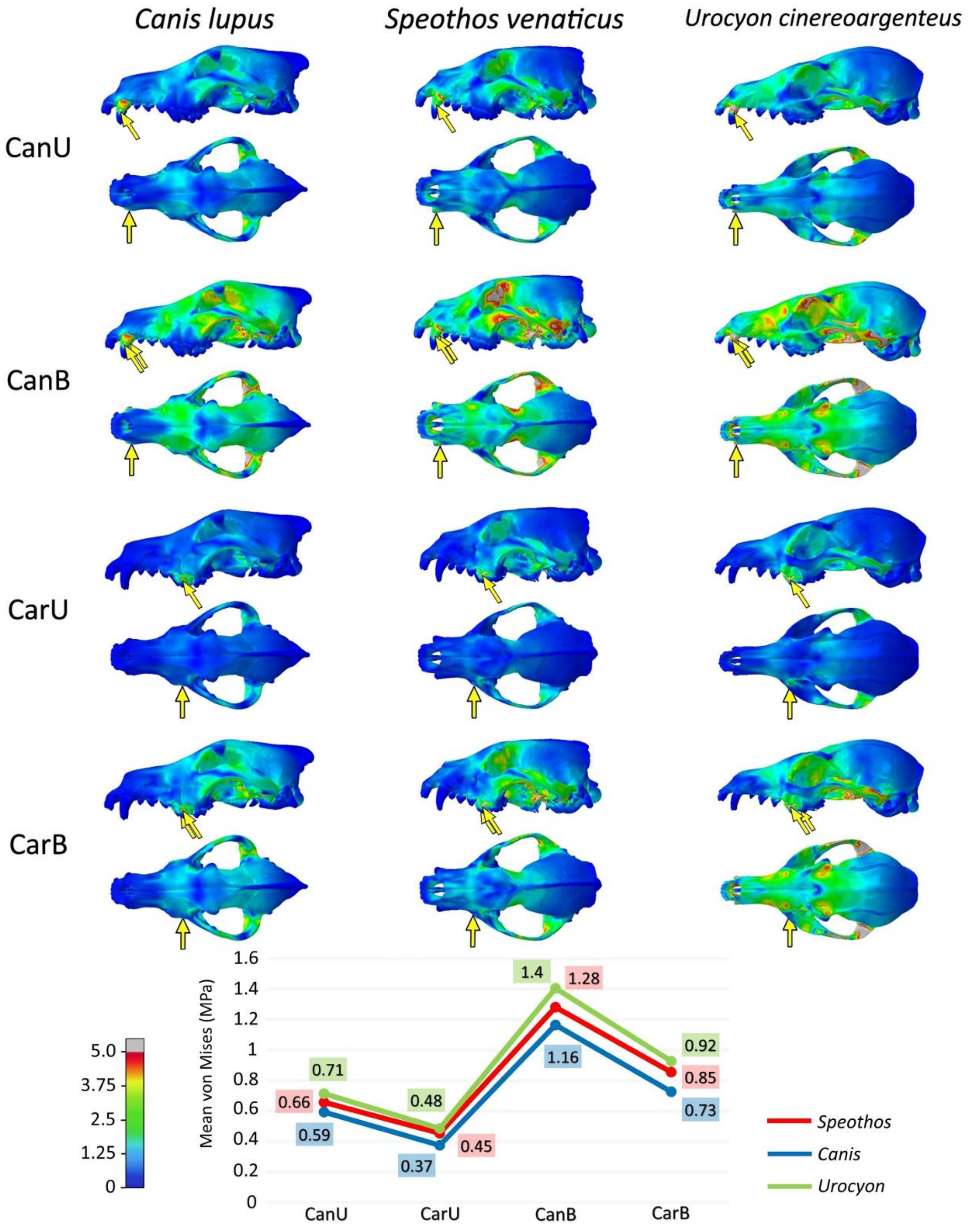
Source: Ruiz *et al*. (2022)

Figure 2.5. Von Mises stress contour plots from finite element analysis of the extrinsic stabbing scenarios modelled to the crania of the bush dog (*Speothos venaticus*), grey wolf (*Canis lupus*) and grey fox (*Urocyon cinereoargenteus*). Arrows indicate the direction of the applied forces in each scenario. The mean von Mises stress of each scenario is shown in the bottom right.



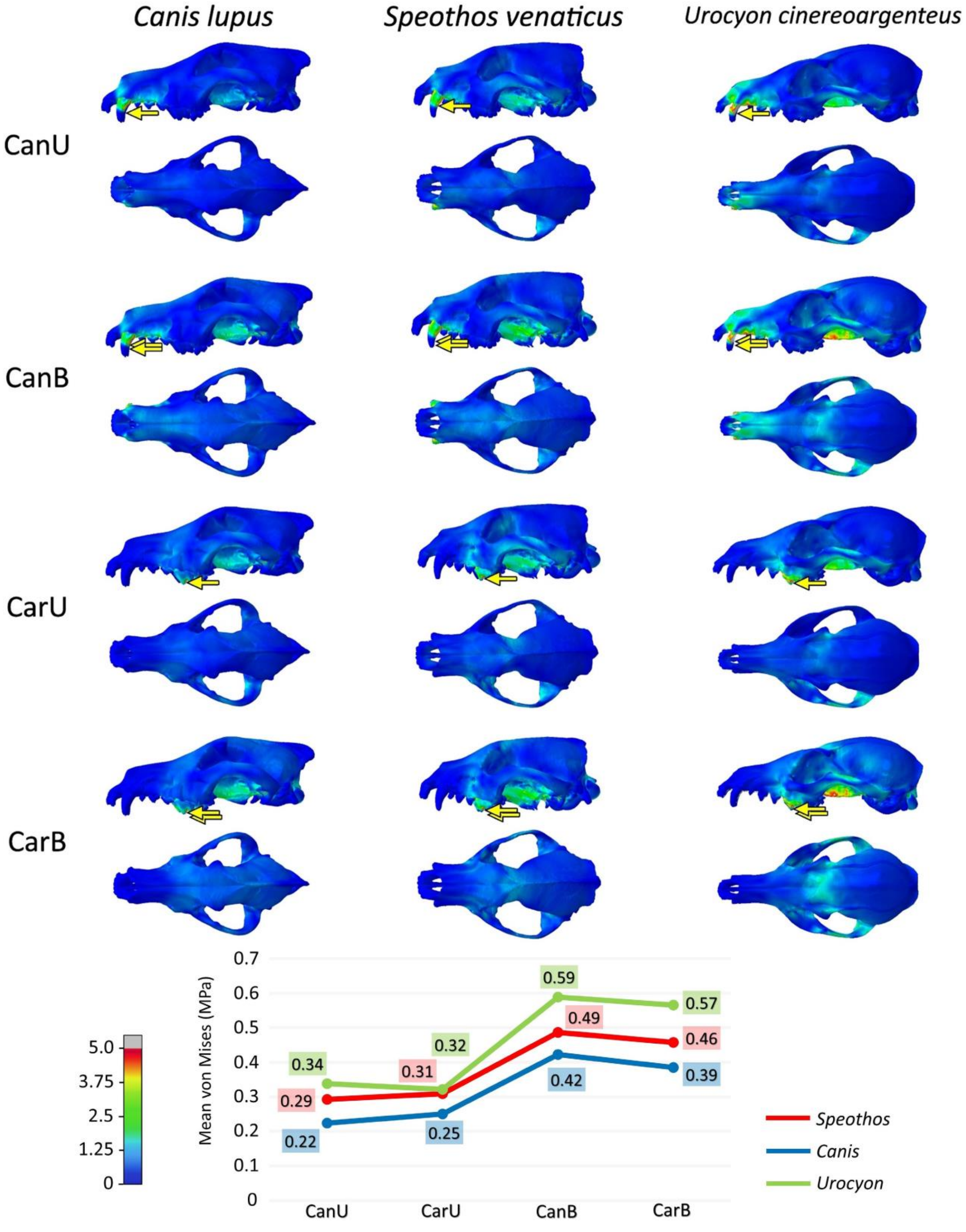
Source: Ruiz *et al*. (2022)

Figure 2.6. Von Mises stress contour plots from finite element analysis of the extrinsic head shaking scenarios modelled to the crania of the bush dog (*Speothos venaticus*), grey wolf (*Canis lupus*) and grey fox (*Urocyon cinereoargenteus*). Arrows indicate the direction of the applied forces in each scenario. The mean von Mises stress of each scenario is shown in the bottom right.



Source: Ruiz *et al*. (2022)

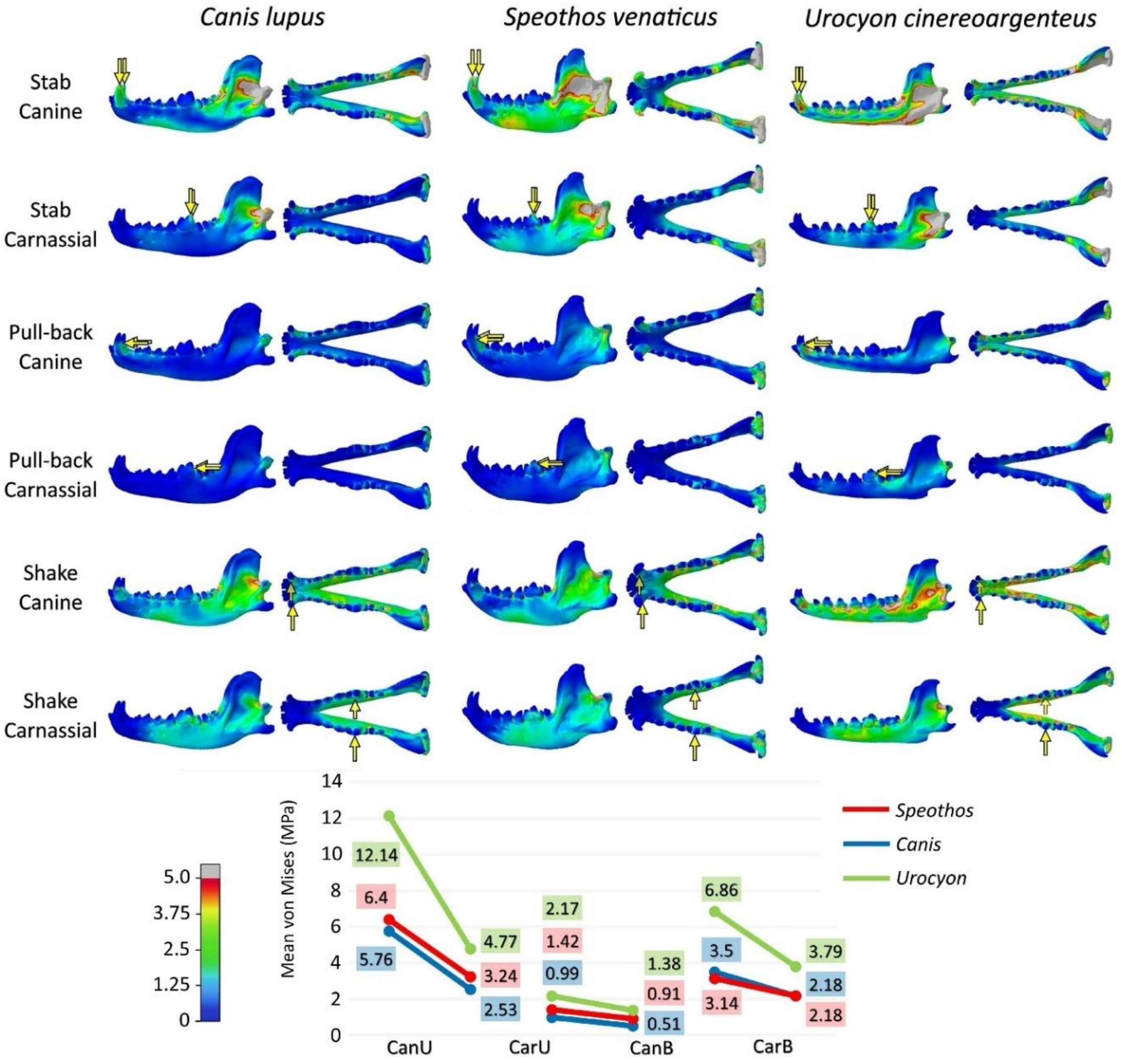
Figure 2.7. Von Mises stress contour plots from finite element analysis of the extrinsic pulling back scenarios modelled to the crania of the bush dog (*Speothos venaticus*), grey wolf (*Canis lupus*) and grey fox (*Urocyon cinereoargenteus*). Arrows indicate the direction of the applied forces in each scenario. The mean von Mises stress of each scenario is shown in the bottom right.



Source: Ruiz *et al*. (2022)

The bilateral extrinsic scenarios of the mandibles in the hypercarnivorous species showed similar results to the cranial scenarios in both mean stress per element and stress spatial distribution, while in the grey fox tests the mandible was significantly more stressed in the stabbing and head shaking scenarios (**Figure 2.8**). The main difference noted in the tests performed with the mandibles is that the stabbing scenario with the canines presented a much higher mean stress than any other scenario, both in the mandibles and in the cranium in the modelled species.

Figure 2.8. Von Mises stress contour plots from finite element analysis of the extrinsic bilateral scenarios modelled to the mandibles of the bush dog (*Speothos venaticus*), grey wolf (*Canis lupus*) and grey fox (*Urocyon cinereoargenteus*). Arrows indicate the direction of the applied forces in each scenario. The mean von Mises stress of each scenario is shown in the bottom right. HS, head shake; PB, pull-back.



Source: Ruiz *et al*. (2022)

The bush dog cranium proved to be more stressed in most of the scenarios than the grey wolf. When comparing these two hypercarnivorous species, the head shaking and the pulling back tests presented similar differences between the mean stress per element. Although the bush dog cranium showed greater stress in most tests (**Figures 2.6** and **2.7**), the stabbing scenarios deviate from the pattern (**Figure 2.5**). In the latter, the bush dog cranium was less stressed than the wolf during the canine bites (in both unilateral and bilateral scenarios) and similarly during the carnassial bite (in the bilateral scenario). The only other scenarios where a bush dog bite was less or as stressed as the wolf bite is during the head shaking bilateral bites with the mandibles (**Figure 2.8**). In these scenarios, the canine bite showed less stress in the bush dog, while the carnassial bite had the same mean stress as the wolf's bite.

The grey fox is more stressed in all tested scenarios when compared to the hypercarnivorous species, especially in the stabbing of both cranium (**Figure 2.5**) and mandible (**Figure 2.8**). Both bush dog and grey fox showed very similar results in the carnassial unilateral bites in the head shaking and pulling back scenarios (**Figures 2.6** and **2.7**) and, in general, the head shaking results as a whole are more similar between these two small species than either is to the wolf. The stress in the mandible, however, is far greater in the grey fox than in the other two species in every scenario, but the enormous discrepancy of the results for this species in the head shaking and, above all, the stabbing scenario, is noteworthy, showing virtually double the stress when compared to the hypercarnivorous species.

Comparing the different scenarios within the same species, the stabbing scenarios of the cranium have a lower mean stress than head shaking in the bush dog (**Figure 2.4**), suggesting a cranium less suited to lateral shaking movements. This condition is dissimilar to the wolf, which presents mean stress values that are similar in these different scenarios (**Figure 2.4**). The mean stress of the carnassial bites in the stabbing and head shaking scenarios of the grey fox is very similar to each other, resembling the condition seen in the wolf (**Figure 2.4**), but when taking into account the canine bites in these same scenarios, the stabbing bites evidently generate more stress, unlike in the other two species.

2.4.2. Intrinsic Scenarios and Bite Force

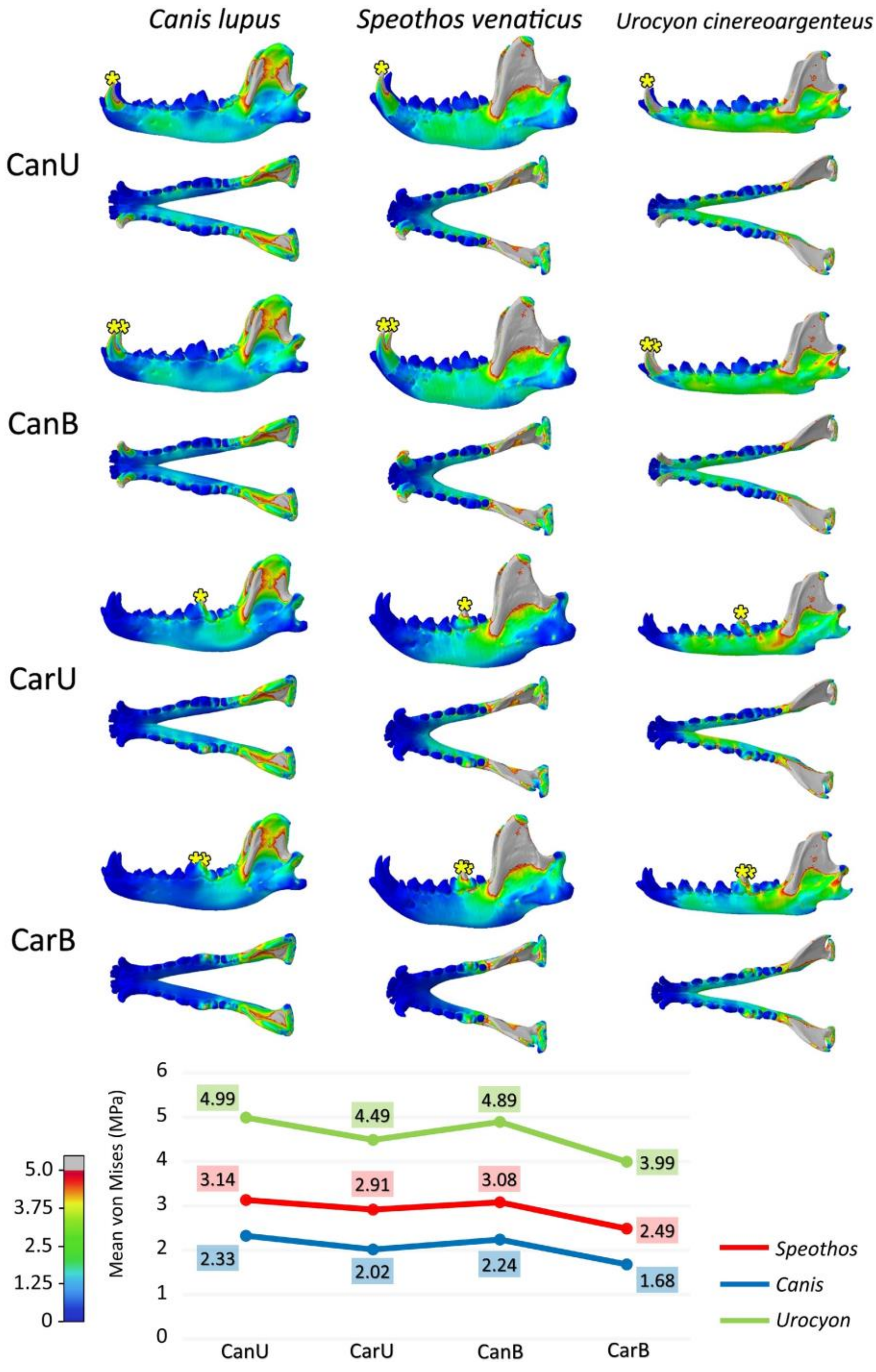
The magnitude of von Mises stress per element varies between the species, with higher mean stress in the mandible of the grey fox and lower mean stress in the mandible of the grey wolf, with the bush dog presenting results more similar to the wolf than to the fox in all tested intrinsic scenarios (**Figure 2.9**). The differences between stress mean in each scenario remain relatively constant, with the bush dog's mandible exhibiting approximately 30% more stress than the wolf's mandible and about 60% less stress than the fox's mandible. In the three species, the bilateral bite presents less stress per element than the unilateral ones, a subtle difference that is more evident in the fox results.

The mandibles reacted similarly (**Figure 2.9**) in terms of von Mises stress distribution, with the same stress pathways, as observed in the extrinsic scenarios (**Figures 2.5-2.9**). As expected, in all models, the greatest von Mises stress was observed in the region of attachment of the adductor muscles, especially in the coronoid process, corresponding to the area of attachment of the *m. temporalis*, the largest adductor muscle in Canidae, but also in the masseteric fossa, where the *m. masseter* is attached. The mandibular rami also show more stress posteriorly according to the tested tooth.

The bite forces modelled in all intrinsic scenarios are expressed in **Table 2.2**. The relationship between the forces exerted by the canines and the carnassials proved to be roughly the same with each other in both the unilateral and bilateral scenarios. When comparing the hypercarnivorous species, the raw bush dog's bite force was about half of the wolf's bite force in all tested scenarios (48% weaker in unilateral bites scenarios and 45%–50% in the bilateral bites scenarios). Compared to the grey fox, the raw bush dog's bite was significantly stronger (36%–43% stronger in the unilateral bites and about 50% in the bilateral bites scenarios).

In general, the canine bites resulted in lower bite forces when compared to the carnassial bites (56% weaker in the unilateral scenarios of both hypercanivorous species and 33% weaker in the fox's bites). In all species, the total force of the carnassial bite was similar in the unilateral and bilateral scenarios, but the canine bilateral bite proved to be slightly stronger than the unilateral bites, with the estimated force being about 106%, 115% and 119% higher in the bush dog, wolf and fox respectively.

Figure 2.9. Von Mises stress contour plots from finite element analysis of the intrinsic scenarios modelled to the mandibles of the bush dog (*Speothos venaticus*), grey wolf (*Canis lupus*), and grey fox (*Urocyon cinereoargenteus*). Asterisks indicate the placement of the tooth constraint in each scenario. The mean von Mises stress of each scenario is shown in the bottom right. CanU: Unilateral canine-driven bite; CarU: Unilateral carnassial-driven bite; CanB: Bilateral canine-driven bite; CarB: Bilateral carnassial-driven bite.



Source: Ruiz *et al.* (2022)

Table 2.2. Total force inferred for the bites in the intrinsic scenarios.

	Speothos venaticus **(N)**	***Canis lupus*** **(N)**	***U. cinereoargenteus*** **(N)**
Canine unilateral	591.74	1210.14	337.74
Canine bilateral (left + right)	342.41 + 286.85	712.87 + 686.39	188.35 + 214.29
Carnassial unilateral	1051.94	2153.54	502.31
Carnassial bilateral (left + right)	570.49 + 497.16	1112.51 + 1010.86	292.09 + 279.98

Source: Ruiz *et al*. (2022)

2.5. Discussion

Regarding the von Mises stress, the differences between all species are restricted to specific regions of the skull and variation is seen more in terms of magnitude of stress than in the distribution of affected areas. Slater, Dumont, and Van Valkenburgh (2009) performed similar extrinsic tests (stabbing, pulling back and head shaking scenarios) to the ones presented here, with different Canina species, including hypercarnivorous (painted wolf *Lycaon pictus*) and non-hypercarnivorous (Ethiopian wolf *Canis simensis*, black-backed jackal *Lupulella mesomelas*). Their substantially similar results indicate that Canina present a similar stress distribution in the cranium. The results of the present chapter reinforce that of Slater, Dumont, and Van Valkenburgh (2009) in showing that the specialisation on diet and prey size will be represented more as a variation in the stress gradient than in different stress areas in the von Mises stress plot (**Figures 2.5-2.9**).

The extrinsic and intrinsic tests modelled lead to a complex panorama since the bush dog's results do not entirely mirror the results of the grey wolf, a much larger species with an equivalent, hypercarnivorous diet, nor the results of the grey fox, a similar-sized species but with a distinct, more generalist diet. Still, the mean element von Mises stress of the bush dog is similar to that of the wolf in most scenarios (with the exception of cranial head shaking), except in few particular cases in which they are closer to the fox (**Figures 2.5-2.9**). Next, the similarities and differences found in each species considering (i) the selection and capture of prey, and (ii) the bite force are discussed.

2.5.1. Prey Selection and Capture

In general, differences in functional morphology in the tested species can be attributed to the relative size of the usual prey. A skull better suited to withstand high amounts of stress can be crucial to deal with larger prey since, unlike the grey fox, bush dogs can occasionally hunt animals much larger than themselves, even in a prey–predator proportion similar to the wolf and its usual large prey (see **Appendix 2.1**). The mechanical efficiency of the skull in the stabbing and pulling back scenarios and the powerful bite force (see **Section 2.4.2**) makes the inferences and anecdotal records of bush dogs hunting large mammals such as *Mazama* deer, peccaries and capybaras (BEISIEGEL, 1999; BEISIEGEL; ZUERCHER, 2005; see **Appendix 2.1**) quite plausible.

Perhaps the most notable report of large-prey hunting by bush dogs was made by Wallace, Painter, and Saldania (2002) of a pack of six individuals chasing and badly injuring an adult tapir (*Tapirus terrestris*) in Bolivia. The chase was watched for 3 hours and after the dogs fled as the witnesses approached, the tapir was found lying down, exhausted and with numerous bite wounds on the lower parts of all four legs. The record of a group of bush dogs chasing and exhausting an adult tapir, weighing between 150 and 250 kg (PADILLA; DOWLER, 1994), is impressive. This implies a predator–prey size relation of at least 1:21, which is possible unique among Canidae and even Carnivora as a whole. Even populations of lions (*Panthera leo*) specialised in hunting bush elephants (*Loxodonta africana*) in Botswana and Zimbabwe do not deal with a body size difference of this magnitude, with a predator–prey size relation of 1:10–15 (JOUBERT, 2006; LOVERIDGE *et al*. 2006; POWER; COMPION, 2009).

A trade-off of hunting big, dangerous prey is that it poses a greater risk to the predators' lives. Reports of wolves severely and even fatally injured during hunting are common (LAZAR *et al*. 2008; MECH; PETERSON, 2003 and references therein; SEVERTSOV *et al*. 2016). To date, the only occurrence of injuries resulting from hunting in the bush dog is the record of a broken canine when a solitary individual attempted to subdue a paca (DEUTSCH, 1983). The scarcity of records like this may be related to the lower volume of published data on the bush dog when compared to the wolf. The results presented here, however, also suggest that these types of injuries may be less common in the bush dog, given the preference for smaller, less demanding prey. As expected, traumas and injuries derived from struggling prey are uncommon in the grey fox; in an analysis with 569 specimens, Evenhuis *et al*. (2018) found only terminal or postmortem fractures in 56 (9.8%) of the specimens and concluded that skull traumas are most likely to be caused by interspecific aggression or predation and anthropogenic causes.

Besides prey size, another factor that may allow the bush dog to deal with less stress than the wolf during prey subjugation is related to habitat use. As the short legs of the bush dog reduce its speed (HILDEBRAND, 1954), it is unlike that it hunts in long chases as other hypercarnivorous canids do, relying instead on environmental factors to subdue its prey. This species is usually associated with rivers and watercourses (BARNETT; SHAPLEY; ENGSTROM, 2001; DEUTSCH, 1983; LINARES, 1967; PERES, 1991; WALLACE; PAINTER; SALDANIA, 2002); indeed, it presents interdigital membranes (BEISIEGEL; ZUERCHER, 2005) and is considered semi-aquatic by some authors (LANGGUTH, 1975; NOWAK, 1999). Contrary to the wolf, whose prey tends to enter the water as a defence mechanism (MECH; SMITH; MACNULTY, 2015), some records and anecdotes point out that the bush dog tends to take its prey into water bodies (STRAHL *et al*. 1992; TATE, 1931). Another hypercarnivorous canid, the dhole, has a similar behaviour (FOX, 1984).

The short legs of the bush dog also allow it to hunt in narrow spaces, where the movements of its prey are also limited (LIMA *et al.* 2012). It is known by the Matse people of north-eastern Peru that the bush dog actively hunts the long-nosed armadillo (*Dasypus kappleri*) by entering the armadillo's burrow, capturing it in the tunnels and dragging it to the entrance of the burrow, where it is finally consumed (FLECK; VOSS, 2016). Some authors suggest that its interdigital membranes are also helpful while digging (CHAVEZ *et al*. 2022), and its robust forelimbs are distinct to the slender bones of cursorial Carnivora, being more similar—although not as robust—to those of the Canadian river otter (*Lontra canadensis*) and European badger (*Meles meles*), mustelids of aquatic and burrowing habits respectively (MARTÍN-SERRA; FIGUEIRIDO; PALMQVIST, 2014). These behaviour and anatomic adaptations could help the bush dogs since, presumably, prey movements become reduced in the water and in narrow spaces, and also may be related to the bush dog's cranium being less suited to deal with lateral movements, as seen in the head shaking scenarios (**Figure 2.6**). Once in the water or inside a burrow, the bush dog is much more apt to apply its powerful bite (see **Section 2.4.2**) in conditions where the prey would be slower and struggling less than if it were on land surface.

In the three tested species, the pulling back scenarios are pointed out as the less stressful of all scenarios. Smaller mean stress in pulling back scenarios was also detected in previous analyses of canids (SLATER; DUMONT; VAN VALKENBURGH, 2009). Unlike felids, which rely on the combination of bite and forelimbs action to capture their prey (FIGUEIRIDO *et al*. 2018; MONTUELLE; KANE, 2019), canids are usually cursorial predators that do not use their legs in prey capturing, but only their heads (SLATER; DUMONT; VAN VALKENBURGH, 2009). Hypercarnivorous canids usually hunt in groups using successive bites, subduing and tearing the prey in the process (CREEL, 2001; CREEL; CREEL, 2002; GRASSMAN *et al*. 2005; MECH; SMITH; MACNULTY, 2015). The pulling back scenario is also supported by the common ‘tug-of-war’ behaviour when a hypercarnivorous canid constrains large prey through a bite (usually in the face) while other members of the pack simultaneously pull in opposite directions (BUSKIRK; CREEL, 2001; GIPSON, 1978; GRASSMAN et al. 2005; MECH; SMITH; MACNULTY, 2015). The act of taking down an animal through the combination of bite and movement of the postcranium during the capture could explain why the pulling back scenarios are less stressful in all tested taxa, but especially in the bush dog and the wolf, which need to apply several bites to subdue its medium to large-sized prey.

Pull-back movements in hunting are extensively documented in the wolf (MECH; SMITH; MACNULTY, 2015). In the bush dog, there are observations in captivity and in the wild. Macdonald (1996) describes six captive bush dogs disembowelling a duck by biting and pulling their head back. In central Brazil, Deutsch (1983) recorded photos and described a single bush dog struggling with a paca after dragging it about 200 metres across a pasture; the photo record on this reference clearly shows the pull-back movement.

2.5.2. Bite Force

Due to the many ways in which it can be measured, estimates of bite from biomechanical models serve more as a comparative tool than as absolute values (ELLIS *et al*. 2008). One of the most used ways to calculate bite force is the dry-skull method developed by Thomason (1991), which reconstructs the adductor musculature to estimate the muscle forces solely using the skull morphology, modelling the jaws as simple levers. It has been applied in analyses of muscular and bite force in many organisms, including canids (CHRISTIANSEN; WROE, 2007; ELLIS *et al*. 2008; NANOVA *et al*. 2017; PENROSE *et al*. 2020; SLATER; DUMONT; VAN VALKENBURGH, 2009; WROE; MCHENRY; THOMASON, 2005).

The analysis conducted herein show a strong bite for the bush dog when compared to the other tested species (**Table 2.2**). In all tested scenarios, the bush dog exerted a bite force of about half the strength of that of the wolf, a canid about seven times heavier (see **Section 2.4.2**). This is likely directly related to the skull morphology of the bush dog, as its short rostrum and deep cranium is linked to a strong bite (PENROSE *et al*. 2020; SLATER; DUMONT; VAN VALKENBURGH, 2009). In other analyses using the dry-skull methods to calculate bite force in both bush dog and wolf, the results were not so extreme (**Table 2.3**). A more similar result was obtained by Penrose *et al*. (2020), who tested the bite force in canids using reduced physiological cross-sectional area (a methodology that considers the muscle mass and angle of pinnation of the fibres to estimate muscular force; ANAPOL; SHAHNOOR; ROSS, 2008) instead of the dry-skull method and, in near occlusion scenarios, obtained bite force estimates for the bush dog about half of those of the wolf.

The increase in the bite forces during the evolution of Carnivora occurs as an adaptation to two specialised diets: as a specialisation to herbivory (e.g. giant panda *Ailuropoda melanoleuca*, red panda *Ailurus fulgens*) and in carnivores that feed on large preys (CHRISTIANSEN; WROE, 2007). Hypercarnivorous canids tend to have stronger bites than mesocarnivorous taxa (SLATER; DUMONT; VAN VALKENBURGH, 2009), as a functional adaptation both for subduing and killing larger prey, as for dismembering and processing it (CHRISTIANSEN; WROE, 2007). The results reinforce this hypothesis since the grey fox not only presented weaker bites

(**Table 2.2**) as its mandible stressed notably more than the other two species in all tested bites (**Figure 2.9**).

In canids, the canines and incisors teeth are used to grab and cut the prey's tissues (BUSKIRK; GIPSON, 1978; PETERSON; CIUCCI, 2003; SEVERTSOV *et al*. 2016). In the hypercarnivorous taxa, the canine's robust shape and size allow it to slash the skin and muscles during the bite, promoting laceration and extensive bleeding (PETERSON; CIUCCI, 2003). The records of paca (DEUTSCH, 1983) and tapir (WALLACE; PAINTER; SALDANIA, 2002) predation by the bush dog attest to the importance of these teeth to subdue prey in this species, including, presumably, in long-lasting battles, as in wolves. Bilateral bites with canines proved to be slightly stronger than unilateral ones in all tested species, a condition which can guarantee more effective, stable bites during the submission of prey and, allied with high bite forces, are of extreme importance in the capture process, especially for the hypercarnivorous species.

Table 2.3 Comparison between bite forces of the bush dog (*Speothos venaticus*, in *S.*) and the grey wolf (*Canis lupus*, in *C.*) in different studies. The grey fox (*Urocyon cinereoargenteus*) is not represented here since it was not tested in the referred studies.

	Canine			Carnassial		
	***S.* (N)**	***C.* (N)**	**Proportion (%)**	***S.* (N)**	***C.* (N)**	**Proportion (%)**
Present study	591.74	1210.14	48.89	1051.94	2153.54	48.84
CHRISTIANSEN AND ADOLFSSEN (2005)	170.1	743	22.89	272	1262.3	21.54
CHRISTIANSEN AND WROE (2007)	150	493.5	30.39	233.5	773.9	30.17
PENROSE *et al*. (2020)	222	508	43.7	319	715	44.61

Source: Ruiz *et al*. (2022)

For all tested species, the greatest forces occurred during carnassial bites. In the unilateral scenarios, the canine bite corresponded to about 56% of the force of the carnassial bite in both bush dog and wolf, suggesting a similar importance of these teeth in both species; this relation could be directly linked to the role of the carnassials in the disintegration of the food, including processing skin, muscles and hard tissues as tendons and bones of large prey. In the grey fox, the differences between the bite forces are less pronounced, with the canine bites being around 70% of the carnassial bites. Given the importance of insects and fruits for this species, a less powerful mastication was expected.

The typical traits of the skull and teeth of a hypercarnivorous canid, such as short rostrum, reduced dentition, robustness of the skull elements and teeth and, of course, higher bite forces, are even more marked in the extinct bone-crushing forms (TSENG; WANG, 2010; WANG; TEDFORD, 2008). Bone elements do not comprise the majority of the biomass found in faeces (LIMA; PINTO; DALPONTE, 2009; ZUERCHER; GIPSON; CARRILLO, 2005) to consider the bush dog a bone-crushing species. However, the high predominance of dermal plates of nine- banded armadillos in the diet of northern Pantanal bush dogs described by Lima, Pinto, and Dalponte (2009) suggests an ability to process hard materials. The carapace of this armadillo species has a complex architecture that can handle forces up to 500 MPa in the banded part of the shell and 1500 MPa in the forward and rear shells (RHEE; HORSTEMEYER; RAMSAY, 2011); the bush dog is not only able to deal with the banded carapace, but seems to prefer armadillos over medium-sized rodents in some locations (LIMA; PINTO; DALPONTE, 2009).

2.6. Chapter Conclusions

The *in silico* tests align with previous assumptions and anecdotes about the bush dog and allow inferring hunting behaviours by comparing it with the better-known grey wolf and grey fox. The results indicate that the unusual morphology of the bush dog is also reflected in its biomechanical performance. The bush dog's functional morphology holds many similarities, but also important differences to both the grey wolf—a much larger species with a similar diet and hunting strategies—and the grey fox—a similar-sized species, but with a very distinct, generalist diet. The bush dog's cranium performs more similarly to the grey fox's cranium in lateral head movements, presumably related to the habit of subdue prey constrained in limited spaces. On the other hand, it has a robust skull and teeth that deal much better with stress overall, and generates a relatively strong bite force, being more similar to the grey wolf in these aspects, which supports the conjectures of the bush dog being, as the wolf, a pack hunter. These results can be explained by the preferred prey size of the different canid species analysed. While wolf prey on larger and potentially dangerous mammals, and grey fox hunt animals usually half its own body weight, the bush dog generally prefers rodents and armadillos that are similar or slightly larger than its own size. This may explain the bush dog stress distribution and magnitude fitting in between the other two species, albeit slightly more similar to the wolf. The results of this study, when combined with morphological and behavioural characteristics, for example, robustness of forelimbs, webbed digits and preference to live near waterbodies, suggest that bush dogs typically pursue prey in conditions where its movements are limited, like in the water and inside bur- rows, unlike most of other hypercarnivorous, pack-hunting canids, as the grey and painted wolves, which chase their prey for long distances. These results also stress the possibility of using FEA as a tool to infer hunting behaviour in species that would otherwise be difficult or even impossible to study *in vivo*, and provide new insights about the biology of the bush dog. One of the main threats to this species is habitat transformation (DEMATTEO; LOISELLE, 2008); thus, a better understanding of its hunting strategies (e.g. preferred type of prey, habitat selection) can be useful to propose conservation actions.

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2.8. Appendix 2.1 – Usual prey of the bush dog, grey wolf, and grey fox

Although both bush dogs (*Speothos venaticus*) and grey wolves (*Canis lupus*) are considered hypercarnivorous, they hunt different prey in terms of body mass and taxa. Christiansen and Wroe (2007) classified the bush dog's diet as based on small to medium-sized mammals, up to its own body mass, while the wolf's diet is based on medium to large-sized prey, frequently exceeding its own body mass. The grey fox (*Urocyon cinereoa*rgenteus) is set apart from the other species since it hunts prey much smaller than itself and relies on a more generalist diet. Here, a short literature review is presented, describing the usual prey hunted by these three species.

Pacas (*Cuniculus paca*) and agoutis (*Dasyprocta azarae*) are reported to comprise about 95% of the total biomass in the analysed faeces of bush dogs in a fragment of the Atlantic Forest in Paraguay (ZUERCHER; GIPSON; CARRILLO, 2005). In the Brazilian northern Pantanal, faeces analysis by Lima, Pinto, and Dalponte (2009) found an overwhelming contribution of nine-banded armadillos (*Dasypus novemcinctus*) in its diet (about 95% of the relative biomass ingested). The bush dog weight 4-7 kg (BEISIEGEL; ZUERCHER, 2005), while pacas and agoutis weigh, respectively, 7.5 kg and 2.7 kg (EISENBERG; REDFORD, 1989) and nine-banded armadillos up to 7.7 kg (MCBEE; BAKER, 1982). This shows that the bush dog selects prey of similar body size to its own, but there are records that indicates exceptions. Animals larger than pacas reported as preyed on by bush dogs include, according to Beisiegel (1999), the deer *Mazama* (weighting up to 34.9 kg in *Maz. Americana*; SILVA-CABALLERO; ORTEGA, 2022) and collared peccaries (*Dicotyles tajacu*, weighting up to 30 kg, CEBALLOS, 2014), and, according to Beisiegel and Zuercher (2005), capybaras (*Hydrochoerus hydrochaeris*, capable to attains 91 kg, MONES; OJASTI, 1986). A record of a pack of six bush dogs chasing an adult tapir (*Tapirus terrestris*, WALLACE; PAINTER; SALDANIA, 2002) is noteworthy since it can weight up to 250 kg (PADILLA; DOWLER, 1994).

The diet and hunting strategies of the grey wolf are far more studied (ANDERSONE; OZOLIŅŠ, 2004; LAFFERTY *et al*. 2014; LAKE *et al*. 2013). Although wolves will eat a wide variety of prey, in North America they usually prefer ungulates (PETERSON; CIUCCI, 2003), and, in the case of the subspecies *C. lupus pambasileus*, Butler *et al*. (2006) recorded a prevalence of moose (*Alces alces*), caribous (*Rangifer tarandus*) and, less frequently, Dall's sheep (*Ovis dalli*). Since some of the largest wolves in the world are found in Western Canada and Alaska (HAYES, 2010), it is reasonable to assign a weight of more than 50 kg to the specimen tested here (BUTLER; BALLARD; WHITLAW., 2006; MECH, 1974;). Thus, wolves' prey are far more massive animals (moose weigh up to 595 kg, FRANZMANN, 1981; caribous up to 300 kg, NAUGHTON, 2012; and Dall's sheep up to 82 kg, BOWYER; LESLIE, 1992).

Unlike the bush dog and grey wolf, the grey fox is an omnivorous species, with arthropods and vegetal matter being an important part of its diet (JASLOW, 1987; NEALE; SACKS, 2001), a condition shared by other taxa in all main lineages of Canidae (CHRISTIANSEN; WROE, 2007). Due to its wide distribution, the food composition on the grey fox's diet varies both spatially as temporally, highly influenced by the seasons. An overview of studies carried across the United States made by Fritzell and Haroldson (1982) returned insects as a major food item during the summer, fruits in autumn, and rodents and lagomorphs in winter, although the latter are usually consumed, in minor proportions, all over the year. According to the same study, cottontail rabbits (*Sylvilagus* sp.), which weights in average 1.2 kg (*Syl. floridanus*, CHAPMAN; HOCKMAN; OJEDA, 1980), are the main prey in the Central and Eastern United States, being significantly smaller than the foxes (which usually weights 3-7 kg, FRITZELL; HAROLDSON, 1982). Evidence of larger prey are scarce. The predation of a striped skunk (*Mephitis mephitis*), a species which weights up to 5.3 kg, is known but potentially represents exceptional situations of near-starvation, as mammals usually do not hunt skunks (WADE-SMITH; VERTS, 1982). Records of consumption of deer *Odocoileus* are unusual acts of scavenging (FRITZELL; HAROLDSON, 1982).

2.8.1. Appendix References

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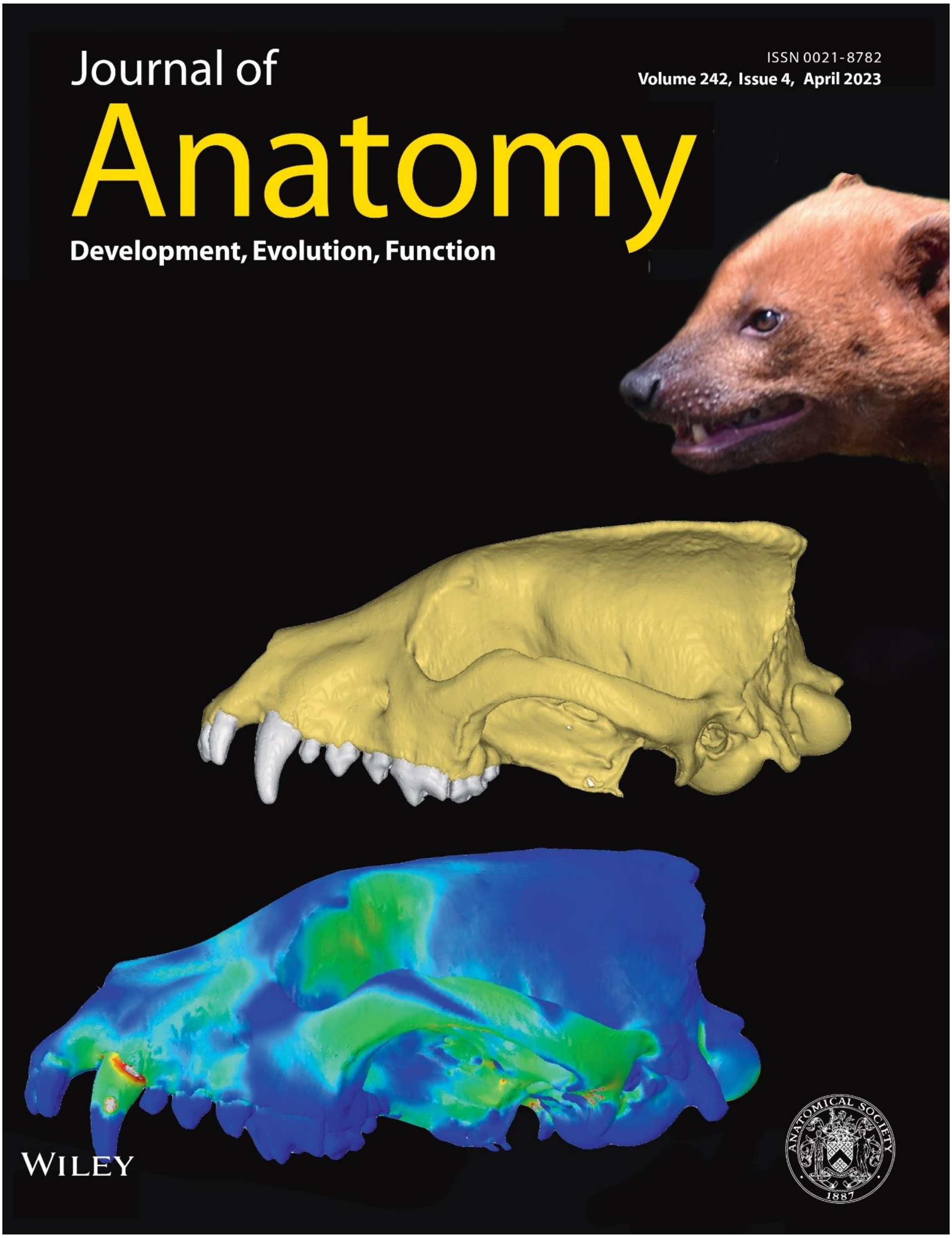
2.9. Appendix 2.2 – Journal of Anatomy Issue Cover

Figure A.2.1. Journal of Anatomy Volume 242, Issue 4 cover depicting the article derived from Chapter 2.



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3. CHAPTER 2: The lost jackals from the Brazilian caves: insights on the taxonomy and paleoecology of Pleistocene bush dog *Speothos pacivorus* (Carnivora, Canidae)

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**Chapter submitted at* Journal of Vertebrate Paleontology *(currently under review)*

3.1. Chapter Abstract

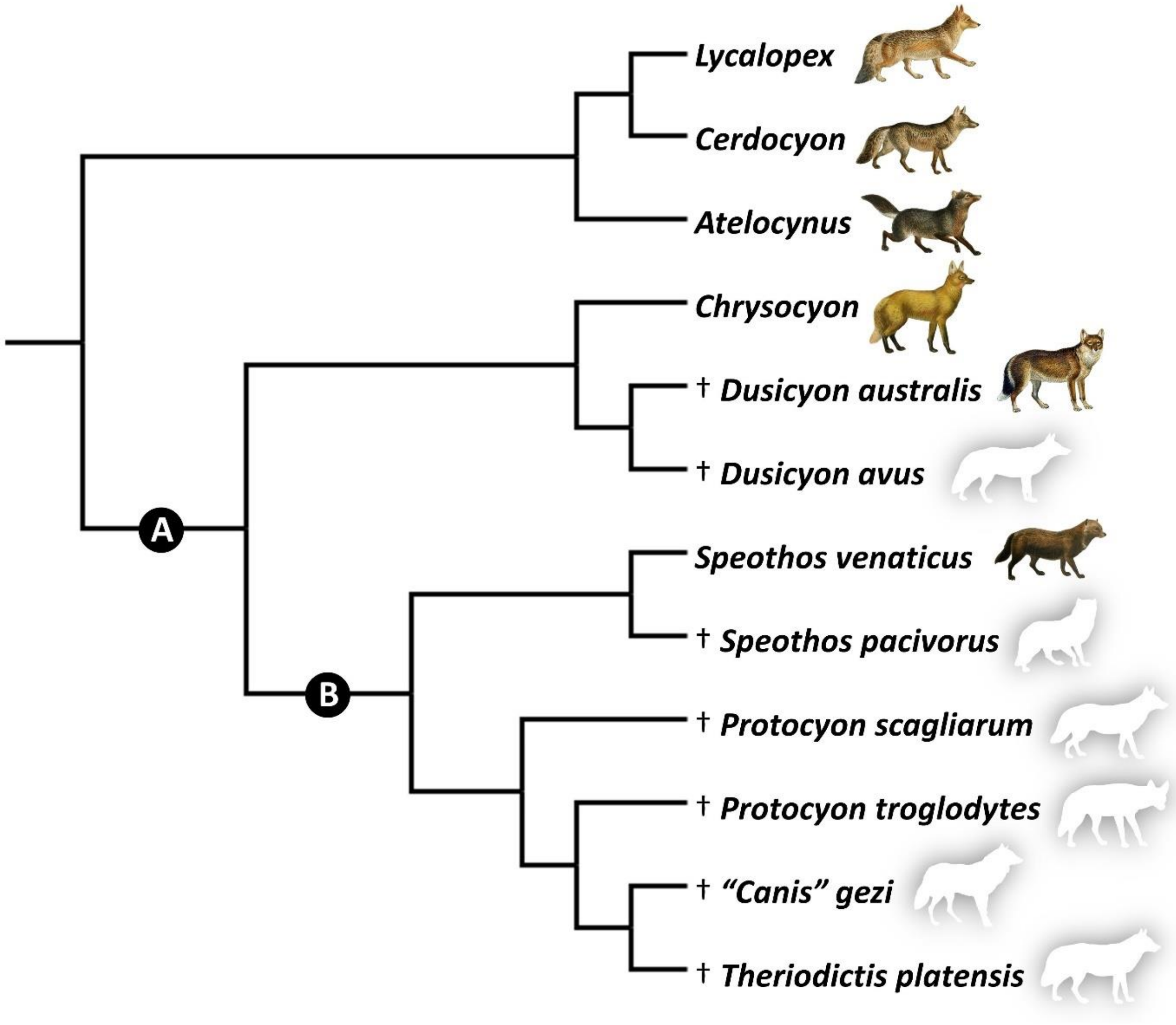
Speothos pacivorus from the Pleistocene of Brazilian Intertropical Region (BIR) is distinguished from the extant bush dog *S. venaticus* by its larger size and key characters of the skeleton and teeth. Nonetheless, its taxonomic status is volatile, with some authors still considering both species as synonymous. Here, the goal is to summarize morphofunctional differences between *S. pacivorus* and *S. venaticus* through comparative description of their skull and dentition, geometric morphometric analysis (GMM), and finite element analysis (FEA). The GMM data revealed *S. pacivorus* significantly separated from the sampled specimens of *S. venaticus*, and a regression analysis showed that the morphological differences of its cranium are not size-related, reinforcing its status as a separate species. Both exhibited very akin von Mises' stress average and distribution on FEA's results, suggesting similar prey-capturing strategies. The results bolster the hypothesis that the coexistence of these hypercarnivorous canids in the BIR could be explained by a megaherbivore community more diverse, allowing resource partitioning between different canid species. Following the demise of these megaherbivores in the Pleistocene-Holocene boundary, other large hypercarnivorous canids also became extinct, leaving the small *S. venaticus*, able to subsist on smaller prey, as the sole surviving species of this specialized lineage.

3.2. Introduction

The Lagoa Santa Karst region in Minas Gerais, Brazil, is of great importance for Brazilian Paleontology. It was the place in which the Danish naturalist Peter Lund (1801-1880) conducted his famous works on the natural history of the Brazilian central plateau (HOLTEN; STERLL, 2011). By the mid-19th century, Lund collected numerous fossils and described 22 extinct and 8 living species of mammals from the caves of the region, from rodents to saber-toothed cats and even human remains (AULER, 2020; CARTELLE, 2020), a pioneering work that gave him the title of father of Brazilian Paleontology (HOLTEN; STERLL, 2000). Among the specimens, Lund identified the remains of five species of Cerdocyonina, the South American canids, including the only known fossils of the genus *Speothos* (RUIZ *et al*. 2024)

Cerdocyonina encompasses an interesting diversity of living and extinct species (CHAVEZ *et al*. 2022; ZRZAVÝ *et al*. 2018). This clade has two major lineages: a foxlike clade, with small, generalist species, and another clade composed by only two distinctive living species, the bush dog (*Speothos venaticus*) and the maned wolf (*Chrysocyon brachyurus,* CHAVEZ *et al*. 2022; ZRZAVÝ *et al*. 2018). The *Speothos-Chrysocyon* lineage also contains many extinct taxa, including the genus *Dusicyon*, jackal-like forms closely related to the maned wolf, and the medium to large-sized hypercarnivores *Protocyon*, *Theriodictis*, and associated forms (PREVOSTI, 2010; ZRZAVÝ *et al*. 2018). The bush dog is the only living species of this hypercarnivorous lineage (**Figure 3.1**, clade B), being also the only living Cerdocyonina which presents this specialized diet and other unique traits, such as pack-hunting behavior and polyestrous reproduction (BEISIEGEL; ZUERCHER, 2005; PERINI; RUSSO; SCHRAGO, 2010; PORTON; KLEIMAN; RODDEN, 1987; VAN VALKENBURGH, 2007).

Figure 3.1. Simplified phylogenetic relationships within Cerdocyonina after Zrzavý *et al.* (2018); **A**, *Speothos*-*Chrysocyon* lineage; **B**, the clade of hypercarnivorous cerdocyonines. Illustrations from Mivart (1890), with silhouettes of extinct Pleistocene taxa (not to scale).



Source: Ruiz *et al*. (under review)

The taxonomy of *Speothos* is historically confusing. The genus was first erected by Lund (1839, 1840), after the discovery of the remains in the Lapa da Cerca Grande cave (Lagoa Santa Karst region, Brazil), which encompass the materials referred here as *S. pacivorus*. After that, Lund also described fossil and extant specimens of the bush dog, first as a mustelid, *Cynogale venatica* (LUND, 1842) and then as a canid, *Icticyon venaticus* (LUND, 1842/43). Winge (1895) noticed the similarities between *Speothos* and *Icticyon venaticus*, but erroneously argued that *Icticyon* has priority as the genus name and considered *I. pacivorus* the ancestor of *I. venaticus* (BERTA, 1984). The priority of *Speothos* was only recognized in the middle of the next century, by Kraglievich (1930). Although Berta (1984) concluded that there are enough morphological traits to differentiate *S. pacivorus* from *S. venaticus*, some studies considered the observed differences as intraspecific variation (PREVOSTI; FORASIEPI, 2018), with *S. venaticus* as a potential synonym of *S. pacivorus*. Finally, there are few phylogenetic analyses that include *S. pacivorus*. The most consensual view (i.e. *Speothos* as a clade containing two species, *S. pacivorus* and *S. venaticus*) is recovered by Okřinová (2013) and Zrzavý *et al*. (2018), in their analysis based on morphological, developmental, ecological, behavioral, and cytogenetic characters. In contrast, Perini, Russo, and Schrago (2010), in a combined analysis with molecular and morphological data, did not recover *Speothos* as a monophyletic genus, with *S. venaticus* as the sister group of the clade *S. pacivorus*+(*Protocyon*+*Theriodictis*).Even with the rich assembly of extinct Cerdocyonina, most of the works with this clade are oriented to new geographical records (OLIVEIRA; PREVOSTI; PEREIRA, 2005; PREVOSTI; RINCÓN, 2007; PREVOSTI *et al*. 2009, 2011; RUIZ-RAMONI; WANG; RINCÓN, 2022), phylogenetic relationships (PERINI; RUSSO; SCHRAGO, 2010; PREVOSTI, 2010), or ecological analyses, mostly based on the diet, but *Speothos* is mostly absent in such works (DANTAS *et al*. 2022; JACOB *et al*. 2024; PREVOSTI; ZURITA; CARLINI, 2005; PREVOSTI; SCHUBERT 2012). In this study, quantitative analyses were applied to study the biology of the Pleistocene *Speothos*, focusing on *S. pacivorus*, aiming to **(a)** elucidate the taxonomy of the genus based on a geometric morphometrics analysis performed with key characters of the cranium, and **(b)** investigate its hunting strategies using finite element analysis. With these two combined techniques, the most comprehensive examination on the evolution and functional morphology of the genus in forty years was conduct.

3.3. Material And Methods

3.3.1. The Materials of *Speothos pacivorus*

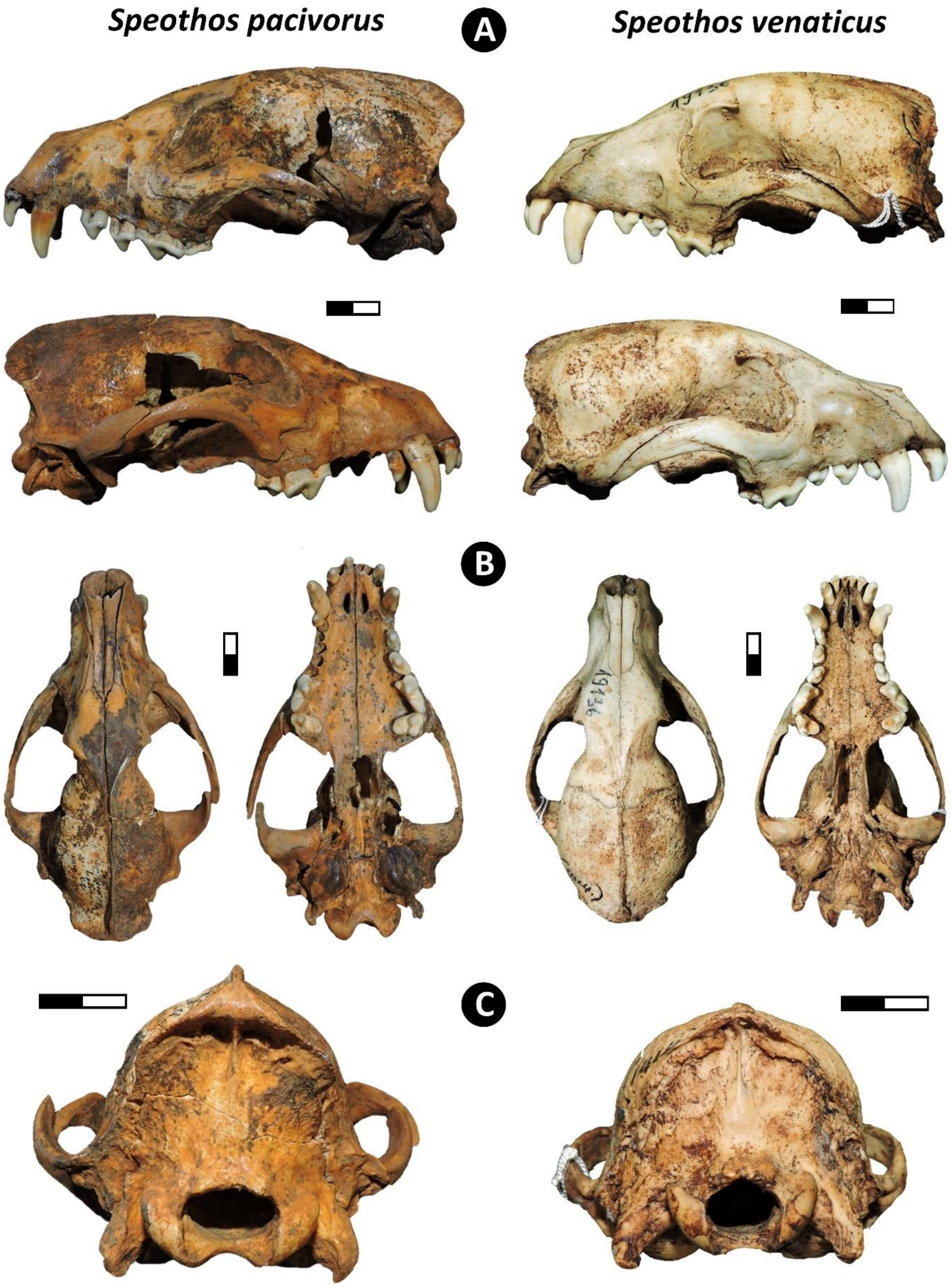
The known specimens of *Speothos pacivorus* are deposited in the Peter Lund/Quaternary Collection of the Natural History Museum of Denmark, Copenhagen, including a nearly complete cranium (the holotype, NHMD:211341, **Figure 3.2**), as well as four other cranial and mandibular fragments (**Figure 3.3**), at least 54 isolated teeth, and fragments of postcranial skeleton, including appendicular bones and one vertebra

(RUIZ *et al*. 2024). Berta (1984) assumed that the holotype and the postcranial remains belong to the same individual based on the similar stage of development; considering the incomplete epiphyseal closure of ulna and tibia, she also concluded it was a subadult of about 7-8 months of age, though no more details are discussed. The holotype's cranium also presents unfused bones (see **Section 3.4.1**), and, although all the preserved teeth are permanent, it does not present extensive wear, which fits with the "adult 1" category proposed by Segura and Prevosti (2012), an ontogenetic classification considered for the analyses. Linear measurements discussed in the comparative description (e.g. cranial length, palate width, etc.) were taken from pictures using the software ImageJ (ABRÀMOFF; MAGALHÃES; RAM, 2004). The osteological and dental nomenclatures follow Evans and De Lahunta (2012) and Berta (1984), respectively.

3.3.2. CT-Scanning

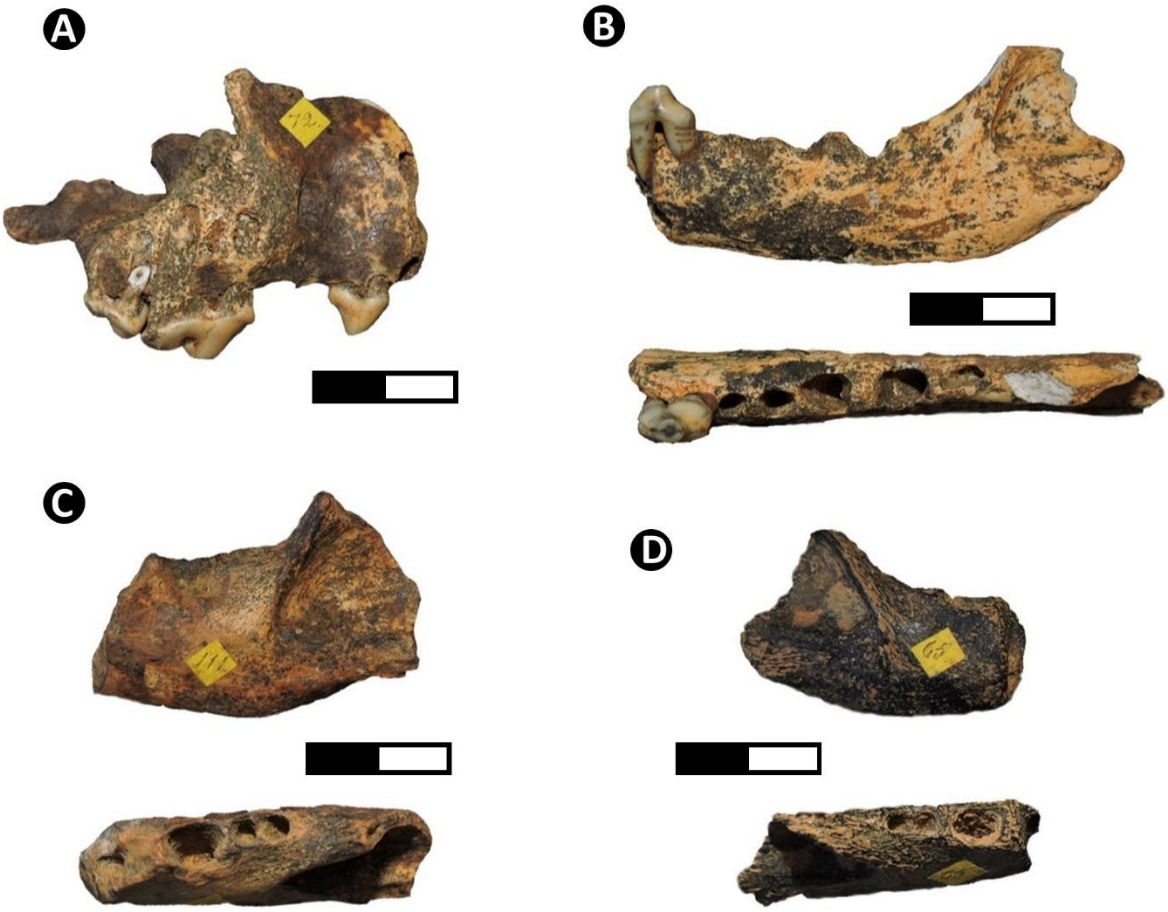
For this study, the specimen NHMD:211341 (complete cranium) was µCT-scanned at the 3D Imaging Centre, Technical University of Denmark, using a XT H225 ST system from Nikon. The scan was conducted using an aluminum filter (thickness of .002 mm), with a voltage of 120 kV and current of 417 µA, resulting in 3142 projections (voxel size of .1 mm). For comparison, a skull of *S. venaticus* (hereafter bush dog), SNHMS:19136, was also scanned, which represents a large wild adult from Suriname, at the 3D Imaging Lab of the University of Tübingen, Germany, using a XT H 320 system from Nikon, scanned with an aluminum 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 segmentation of the slices into a single material was performed with Amira 2021.1 (Thermo Fisher Scientific) and Biomedisa (LÖSEL *et al*. 2020), with small cracks and holes in NHMD:211341 being reconstructed posteriorly with Blender version 3.4 (DEVRIES *et al*. 2022).

Figure 3.2. The cranium of *Speothos pacivorus* (NHMD:211341) and *S. venaticus* (SNHMS:19136). **A**, Left and right lateral views; **B**, Dorsal and ventral views; **C**, Occipital views. Scale bars equals 2 cm.



Source: Ruiz *et al*. (under review)

Figure 3.3. The fragmentary skull elements of *Speothos pacivorus*. **A**, right maxilla NHMD:72 in lateral view; **B**, Partial left hemimandible NHMD:159 in labial and dorsal view; **C**, Partial left hemimandible NHMD:711 inlabial and dorsal view; **D**, Partial right hemimandible NHMD:65 in labial and dorsal view. Scale bars = 2 cm.



Source: Ruiz *et al*. (under review)

3.3.3. Geometric Morphometrics

For geometric morphometric analyses, the ventral view of 77 individuals of Cerdocyonina was photographed with a scale for. The sample consisted of the type specimen of *Speothos pacivorus* and 59 individuals of bush dog (**Table A.3.1**), including only adult specimens from A1 to A3 categories proposed by Segura and Prevosti (2012) considering dental eruption and wear. Of the 56 specimens with confirmed categorization, 24 were labeled as A1, 16 as A2, and 16 as A3 (see **Table A.3.2**). For comparison, other species of the *Speothos-Chrysocyon* lineage were added, including the extant *Chrysocyon brachyurus* (n=6) and the extinct *Dusicyon avus* (n=2), *D. australis* (n=4), *Protocyon scagliorum* (n=1), *P. troglodytes* (n=2), *‘Canis’ gezi* (n=1), and *Theriodictis platensis* (n=1). See **Appendix 3.1** for a complete list of specimens (**Table A.3.1**) and institutional abbreviations.

The x and y coordinates of 35 landmarks were registered on both sides of the cranium in ventral view (**Table A.3.3**, **Figure A.3.1**), as well as the scale, with the software TPSDig (ROHLF, 2015). Because the resulting configurations are arbitrarily rotated, translated, and scaled, a Generalized Procrustes Analysis (GPA) was conducted to standardize for those effects (ROHLF; SLICE, 1990). This analysis was performed with the R (version 4.2.2 R Core Team) package Morpho, which allows for the removal of bilateral asymmetry (SCHLAGER, 2017). The resulting superimposed configurations were subjected to a principal component analysis (PCA) for dimensionality reduction and data visualization.

To evaluate possible differences between *S. pacivorus* and the bush dog, a non-parametric multivariate analysis of covariance (npMANCOVA, ANDERSON, 2001) was conducted using size as a covariate, and species as factor. This analysis was performed only for the *Speothos* specimens to assess if differences among species can be explained only by allometric changes. If a significant effect of size was found, but not of species, that would be consistent with the existence of a single taxon with allometric differences among individuals. If a significant effect of species despite the presence of allometry was found, that would be consistent with non-allometric shape differences between both taxa. Size was measured as the log-transformed centroid size (logCS). The CS is calculated as the square-root of the sum of the distances among each landmark and the centroid of the configuration, which is the center of mass of the configuration.

Significance was tested using 9,999 permutations of the species identities and calculating effect sizes using the R package geomorph (ADAMS *et al*. 2019). Because there is a single specimen of *S. pacivorus*, this would be equivalent to calculating the within-group variation only from bush dog and the between-group variation from the average of bush dog and the specimen of *S. pacivorus*. Permutations would then construct the null distribution of effect sizes by considering *S. pacivorus* as part of bush dog and randomly drawing a specimen of bush dog to be associated to *S. pacivorus*. Significance was calculated as the number of effect sizes greater than the ones observed through permutations. To visualize the allometric shape differences within the *Speothos* sample, the regression scores between shape and size was calculated

(DRAKE; KLINGEBERG, 2008). This method calculates the projection score of individual shapes onto the coefficient of regression between size and shape (obtained from the npMANOVA), and thus describes the shape variation more associated with size (DRAKE; KLINGEBERG, 2008).

3.3.4. Finite Element Analysis

To understand the cranial functional properties in different hunting movements for both species of *Speothos*, different extrinsic (i.e. 'external' forces, generated by a struggling prey) scenarios on the cranium of *S. pacivorus* and the bush dog (SMNH:19136) were tested. The three-dimensional surfaces were composed of a single material, with no distinction between bone and teeth, as in Slater, Dumont, and Van Valkenburgh (2009). These surfaces were then imported in the software Hypermesh 13.0 (Altair Engineering) and treated as isotropic and homogeneous (solid model consisting of approximately 800,000 tetrahedral elements per model). The material properties of cortical bones (E = 13.7 GPa, υ = 0.3) were assigned based on previous works on carnivore mammals, including canids (CHAMOLI; WROE, 2011; FIGUEIRIDO *et al*. 2018; SLATER; VAN VALKENBURGH, 2009; SLATER; DUMONT; VAN VALKENBURGH, 2009). To avoid incongruities related to size difference, the cranium of *S. pacivorus* was scaled to the same volume of that of the bush dog (SHARP, 2015). Constraints were applied on the occipital condyle (10 nodes in total) and the temporomandibular joint (five on each side), with each node constrained in all directions (x, y, and z). Each modelled scenario was solved in the software Abaqus 6.14-1 (Simulia Engineering). The performance of each model was assessed via contour plots of von Mises stress distribution and mean von Mises stress per element and displacement value of the elements; to avoid individual stress singularities on nodes, 99% of the values were considered (FIGUEIRIDO *et al*. 2018; MONTEFELTRO *et al*. 2020).

Eight extrinsic scenarios with different load assignments were tested to understand the craniomandibular functional properties in different conditions, based on the application of an extrinsic load of 315 N on each canine and 530 N on each carnassial, according to the bite force estimated for the bush dog by Ruiz *et al*. (2022). Each scenario aims to investigate possible movements of prey capture, including bite and postcranial-guided head movements (MONTEFELTRO *et al*. 2020). Six bilateral scenarios follow Figueirido *et al*. (2018) and Ruiz *et al*. (2022), and consist of: stabbing, in which a dorsally directed load was placed at the tip of the tooth; pulling back, in which an anteriorly directed load was placed at the posterior surface of the tooth; and head shaking, in which a laterally directed load was placed at the mid-left surface of the tooth.

Additionally, two unilateral, postcranially guided scenarios were tested following Montefeltro *et al*. (2020), the head twisting, in which a dorsally directed load was placed in the left tested tooth and a ventrally directed force was applied on the opposite, right tooth. This movement of grabbing a prey and twisting the skull laterally is characteristic for semiaquatic and aquatic vertebrates, being better known for crocodiles as the 'death roll' (DRUMHELLER; DARLINGTON; VLIET, 2019). It was not tested in canids through FEA in previous studies (e.g., Ruiz *et al*. 2020; SLATER; DUMONT; VAN VALKENBURGH, 2009) and was included here due to the semiaquatic habits of the bush dog (BEISIEGEL; ZUERCHER, 2005).

3.4. Results

3.4.1. Comparative Description of the Skull

In this section the anatomy of the preserved cranial and mandibular elements of *Speothos pacivorus* and its respective dentition (**Figure 3.3-3.4**) is described, comparing it with the bush dog (*S. venaticus*).

Cranium—overall, the cranium of *S. pacivorus* is a bit larger than those of the bush dog (**Table A.3.2**), being longer and wider (at the zygomatic arches) in dorsal and ventral views (**Figure 3.2**). In general, its shape is akin to that of the dhole (*Cuon alpinus*, **Figure A.3.2**), appearing to be less compact than the stumpy cranium of the bush dog.

The rostrum is superficially shorter in relation to the whole cranium than in the bush dog (BERTA, 1984). In dorsal view, the contact of the nasals with the frontal has an acute shape, as in most bush dogs, although some specimens can present a blunt to square-shaped suture. The frontal of *S. pacivorus* has an anterior process situated between the nasal and maxilla, almost contacting the incisive and limiting the nasal-maxilla suture to a small segment. In the bush dog, the condition is usually similar but, in many specimens, the frontal and incisive contact each other, preventing a nasal-maxilla contact. The close proximity between the incisive and the anterior process of the frontal is a typical condition in short-rostrum species of living canids, being especially prominent in the hypercarnivorous painted dog (*Lycaon pictus*, **Figure A.3.2**). In dorsal and lateral views, the nasal does not reach the most posterior limit of the maxilla, a condition shared by *Speothos* with the short-eared dog (*Atelocynus microtis*), as noted by Berta (1984), and the crab-eating dog (*Cerdocyon thous*, **Figure A.3.2**).

Apart from the dentition (see below), the palatal region of both species of *Speothos* is very similar. Compared with any other living canid, it is proportionally shorter and wider. At the widest point (the contact of M1-M2), the width of the palate of *S. pacivorus* comprises about 30% of the condylobasal length of the cranium; in the studied bush dog specimens, the proportional width is similar, ranging from 26% to 33% (**Table A.3.4**). Most of the pterygoid-palatine fossa and the nasopharyngeal region is missing in NHMD:211341.

The basicranium of NHMD:211341 is noteworthy by presenting unfused contact between the sphenoid (basisphenoid part) and the occipital (basioccipital part); the sphenoid (alisphenoid part) and temporal (squamous part), anteriorly to the tympanic bullae; and the occipital (basioccipital part) and temporal (tympanic part), around the medial and posterior margins of the tympanic bullae, as seen in ventral view (**Figure 3.2B**). In posterior view (**Figure 3.2C**), the temporal (tympanic and petrosal parts) is also unfused to the occipital (especially with the exoccipital but also with a small part of the supraoccipital). Apart from this, the basicranium of this specimen does not present any considerable deformation or displacement and looks very similar to the basicranium of the bush dog. In this species, the overall shape of the paracondylar process and the position of the jugular and hypoglossal foramina, as well as the overall shape of the occipital condyles in ventral view, differ among the analyzed specimens.

Although Berta (1984) attested that the tympanic bullae of *S. pacivorus* are larger than that of the bush dog, due to the great variety of size of this structure in the latter species, it is difficult to assume that an enlarged bulla is characteristic for *S. pacivorus* or if it is only a condition of this specimen. As cubs and juveniles of South American canids present larger bullae compared to the adults (SEGURA; CASSINI;

PREVOSTI, 2021), the large, globulous bullae of the holotype of *S. pacivorus* reinforces the possibility of it representing a subadult specimen.

The clearest difference between both species is the proportion of the braincase. In *S. pacivorus* it is considerably smaller in relation to the cranium as a whole (being approximately 42% of the cranium total length and with a width, at its widest point, of 57% compared to the cranium's widest point at the zygomatic arches), while the bush dog possess a much more developed braincase (being approximately 50% of the cranium total length and with a width at widest point of 68.4% compared to the cranium's widest point at the zygomatic arches in SNHMS:19136). This enlarged braincase in the bush dog is even enhanced by a postorbital constriction more accentuated in *S. pacivorus*, which reduces the area of the anterior portion of the braincase in dorsal and ventral views (**Figure 3.2B**). Although some specimens of bush dog also possess a considerable compression of the postorbital region, the degree of this compression is not so extreme as seen in *S. pacivorus*.

The sagittal crest of *S. pacivorus* is more developed than that of most bush dog specimens, which show crests of different sizes. This variation across bush dogs may be related to the ontogenetic stage of the specimens, since the sagittal crest tends to increase in size with age, allowing the attachment of the powerful adductor muscles (GARCIA-PEREA, 1996; SEGURA, 2013; VAN VALKENBURGH, 2007). Considering that the sampling of bush dogs in this study represents adults of different ages, however, it may indicate that the development of the sagittal crest in the bush dog can be related to other factors, such as sexual dimorphism or demographic variations.

The nuchal crest is mildly developed compared to other canids and is anteroventrally directed in *S. pacivorus*, with a concave margin that follows the lateral limits of the occipital region of the braincase, in lateral view. As with the sagittal crest, in the bush dog this trait is variable but, overall, it is less concave and more dorsoventrally directed, being almost straight in some specimens. In both species, the ventral limit of the nuchal crest ends on a small eminence almost contacting the external acoustic meatus. The more developed nuchal crest of *S. pacivorus* prevents the visualization of the occipital region of the cranium in lateral view, a condition present in some bush dog specimens.

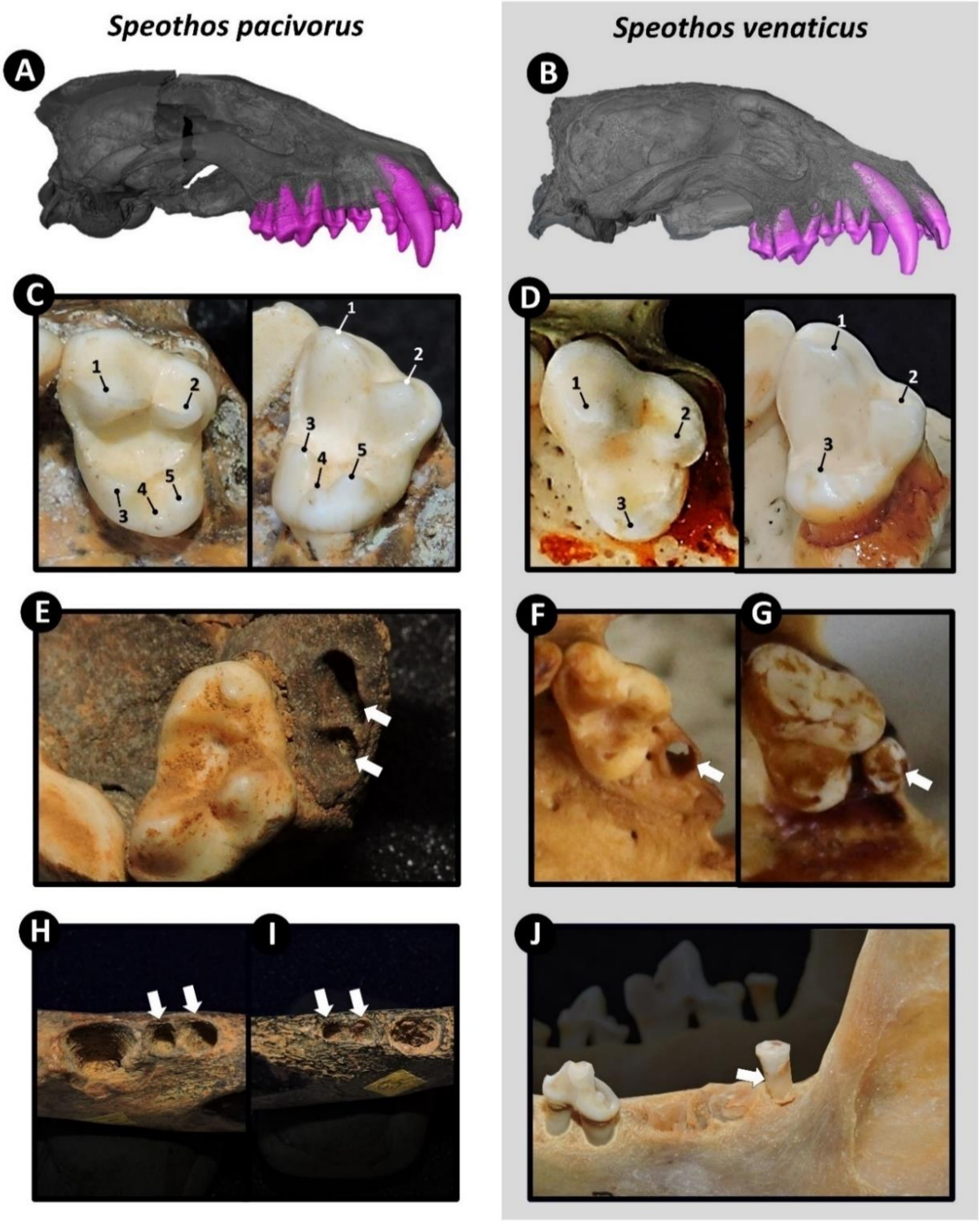
The occipital region of *S. pacivorus* has a squared shape on its dorsal portion, with its lower limits (outlined by the nuchal crest) ventrolaterally directed, while in the bush dog it appears to be more rounded (**Figure 3.2C**). The scars of the cervical muscles are marked by deeper depressions when compared to the blunter surface of the bush dog, a condition exaggerated by the larger nuchal crests of *S. pacivorus.* The foramen magnum of *S. pacivorus* is oval and dorsoventrally compressed in posterior view, being larger in relation to the cranium than that of the bush dog (**Figure 3.2C**). The dorsal margin of the foramen magnum bears a crest that is present in both *Speothos* species, but it is far more developed in the bush dog, notably seen in lateral view, just above the occipital condyles. The foramen magnum region, including the occipital condyles, is more posteriorly displaced in the bush dog than in *S. pacivorus*, a condition noted by Langguth (1980) and Berta (1984).

Mandible—there are three fragmentary mandibular specimens preserved for *S. pacivorus* (**Figure 3.3**): NHMD:159 (a partial left hemimandible preserved from pm2 to the anterior half of the masseteric fossa), NHMD:65 (a small fragment of the right hemimandible preserved from the posterior wall of the anterior alveoli of m1 to the anterior portion of the masseteric fossa), and NHMD:711 (same as NHMD:65, but on the left hemimandible). The morphology of the preserved portions is very similar to that seen in the mandibles of the bush dog, being short and deep and possessing a slightly developed subangular lobe (Berta, 1984). The masseteric fossa extends to the level of m2, a condition that is also present in most bush dog specimens included in this study.

By estimating the length of the molar section of the mandible, is suggested that the mandible of the bush dog is proportionally deeper than that of *S. pacivorus*. While in the latter the height of the mandibular ramus at the level of m1 is about 65-80% of the length from the anterior part of the m1 to the posterior part of the m2, in the five largest bush dog specimens, it represents about 98-108% (**Table A.3.5**). A shorter and deeper mandible can be directly related to the stumpy condition of the cranium of the bush dog, with its shorter rostrum and reduced dentition.

Dentition—the dentition in the holotype of *S. pacivorus* is relatively complete, and without extensive wear on the crowns (**Figure 3.3**). Teeth are also preserved in the maxillary fragment (NHMD:72) and in the right hemimandible (NHMD:159); there are also 54 isolated teeth assigned to the species. For a detailed description of the dentition, see Berta (1984).

Figure 3.4. Diagnostic dental characters in *Speothos pacivorus* (left column) and *S. venaticus* (right column). **A**, and **B**, digital reconstruction of the upper dentition. **C**, and **D**, left M1 in ventral (left) and lingual (right) views, in which **1**: paracone, **2**: metacone, **3**: protocone, **4**: hypocone, and **5**: metaconule (following Berta 1984), **E**, alveoli of the right M2 in *S. pacivorus*, showing the double rooted anatomy of the tooth (indicated by the arrows). Left M2 in *S. venaticus*, showing: **F**, the single alveolus for the tooth (USNM270370), and **G,** the preserved M2 (MPEG8794), both indicated by the arrows. Double alveoli for m2 (indicated by the arrows) in: **H**, left hemimandible (NHMD:711), and **I**, right hemimandible of *S. pacivorus* (NHMD:65). **J**, detached m2 of *S. venaticus*, showing its single root, indicated by the arrow (MZUSP35732).



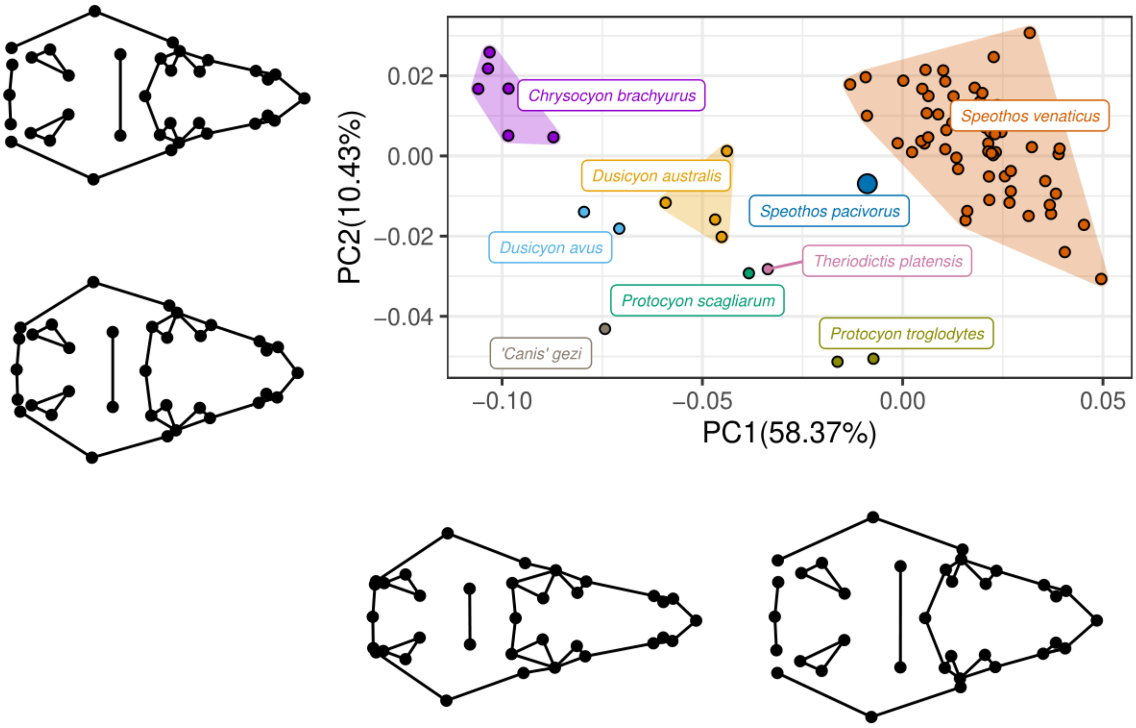
Source: Ruiz *et al*. (under review)

Overall, the dentition of *S. pacivorus* is very similar to that of the bush dog (**Figures 3.2A-B, 3.4**) with specific differences. Of the four proposed autapomorphies of *S. pacivorus*, three are related to its molars (BERTA, 1984). First, the M1 of *S. pacivorus* presents well-developed metaconule and hypocone, thus appearing larger and more developed, square-shaped in ventral view (**Figure 3.4C**). In the bush dog, the metaconule and hypocone are absent (BERTA, 1984), and the tooth has a more delicate, triangular appearance in ventral view (**Figure 3.4D**). Second, unlike most bush dogs, *S. pacivorus* presents a M2, as there is an alveolus posterior to M1. In the holotype, it is single-rooted in the left side, but double-rooted in the right side (**Figure A.3.3**); in the maxillary fragment preserved it is also double-rooted (**Figure 3.4E**). Usually, bush dogs do not possess M2: of the five specimens analyzed by Gudinho and Weksler (2021), only one had it (20% of the sampling). In the dataset of 59 specimens, the M2 is present in 11 specimens (about 18% of the sampling); of these, only three bear the additional molar in both sides of the toothrow. In general, this extra tooth is small and peg-like, circular in cross-section, unlike the larger and wider alveolus present in *S. pacivorus* (**Figure 3.4F-G**). Finally, the m2 in *S. pacivorus* is double rooted, as seen in the alveoli of NHMD:65 and NHMD:711 (**Figure 3.4H-I**), but difficult to interpret in NHMD:159 due to its preservation. In bush dogs, this tooth is very reduced, presenting a single root (Winge, 1895; **Figure 3.4J**). However, it is important to note that root number in molars is not a reliable character, especially in carnivorans with a specialized diet (DEMERS, 2023).

3.4.2. Cranial shape

The two first Principal Components (PCs) explain 68.8% of the total variation in the sample (**Figure 3.5**; for higher PCs, see **Figure A.3.4**). The PC1 describes an overall contrast between a greater contribution of molar teeth (M1-2) in the overall length of the toothrow and a narrower braincase and zygomatic arches with negative values and a greater contribution of the carnassial (PM4) in relation to the overall length of the toothrow and a wider braincase and zygomatic arches with positive values. The PC2 mostly describes changes in the length of the rostrum, with specimens with short, wider rostra at more negative values and those with longer, narrower rostra at more positive values. For the bush dog, specimens are concentrated on the positive values of PC1 highlighting the overall shortening and robustness of the rostrum, and reduction of the number of teeth. Within the non-*Speothos* sample, there is a contrast between *Chrysocyon brachyurus*, with a gracile cranium and elongated rostrum (PC1-, PC2+), and *P. troglodyctes*, with a robust cranium and short rostrum (PC1+, PC2-). ‘*Canis’ gezi* is the only species positioned closer to the negative quadrant of both PCs (PC1-, PC2-), which refers to a gracile cranium with a relatively short rostrum. On the PC1 vs. PC2 morphospace, *S. pacivorus* is positioned between the bush dog and the remaining species and is outside of a minimum convex hull representing the variation of bush dog on the first two PCs.

Figure 3.5. Morphospace occupation of nine species of the *Speothos*-*Chrysocyon* clade. PC1 vs PC2 plot of cranial shape variation in ventral view, with different colours representing different species and polygons (minimum convex hulls) used for species with multiple specimens included in the sample.



Source: Ruiz *et al*. (under review)

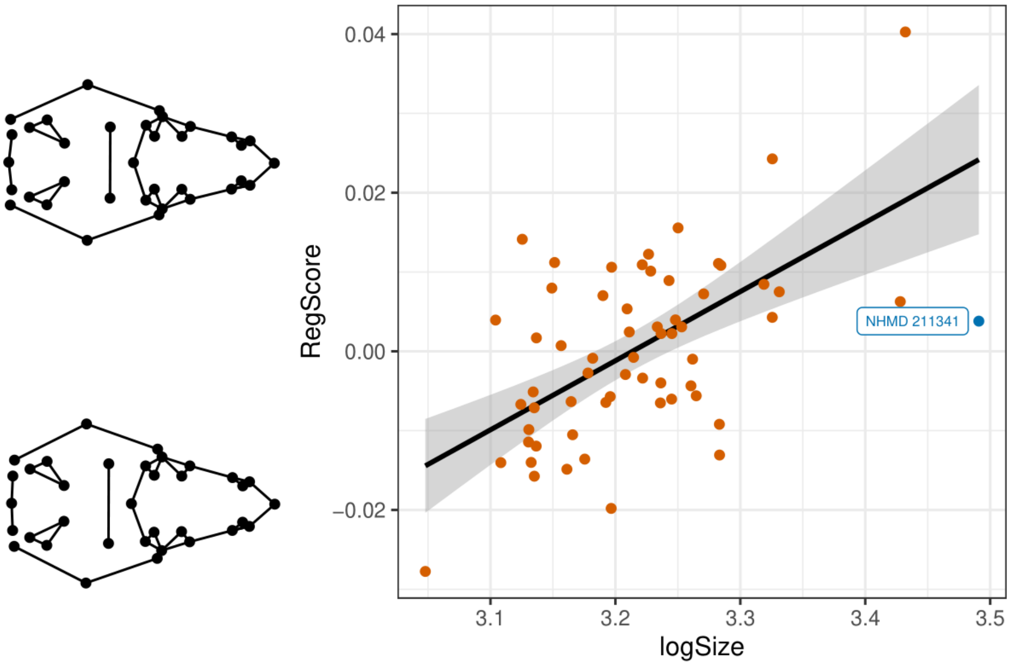
The npMANOVA showed a significant effect of both size and species on shape variation, suggesting that the differences observed between *S. pacivorus* and bush dog cannot be explained by allometric differences alone (**Table 3.1**). The visualization of shape regression scores shows a weak association between size and shape (**Table 3.1**, **Figure 3.6**). Shape differences between small and large individuals are subtle, with smaller individuals having proportionally shorter rostra, and larger individuals having longer rostra. Nevertheless, the regression scores illustrate the result of the npMANOVA, showing that, despite being larger in size, the type specimen of *S. pacivorus* does not follow the allometric expectation of bush dog.

Table 3.1. Non-parametric MANCOVA table of species differences with log(size) as a covariate. **Df:** Degrees of freedom; **SS:** Sum of Squares; **MS:** Mean Squares; **R^2:** Percentage of the variation explained by a factor; **F:** Fisher' F; **Z**: Effect size; **Pr(>F):** P-value: proportion of the non-parametric null-distribution that is as extreme or more than the observed value.

	Df	SS	MS	R^2	F	Z	Pr(>F)
log(size)	1	2.46E+00	2.46E+00	0.038	2.321	2.081	0.019
Species	1	2.46E+00	2.46E+00	0.037	2.318	1.995	0.022
Residuals	57	6.05E+01	1.06E+00	0.922			
Total	59	6.56E+01					

Source: Ruiz *et al*. (under review)

Figure 3.6. Relationship between the shape regression coefficients (RegScore) and size (logSize) in *Speothos* (*S. venaticus* represented by orange dots and the type specimen of *S. pacivorus* represented by the blue dot). Configurations represent shape changes associated with size differences on the ventral view of the crania.



Source: Ruiz *et al*. (under review)

3.4.3. Finite Element Analysis

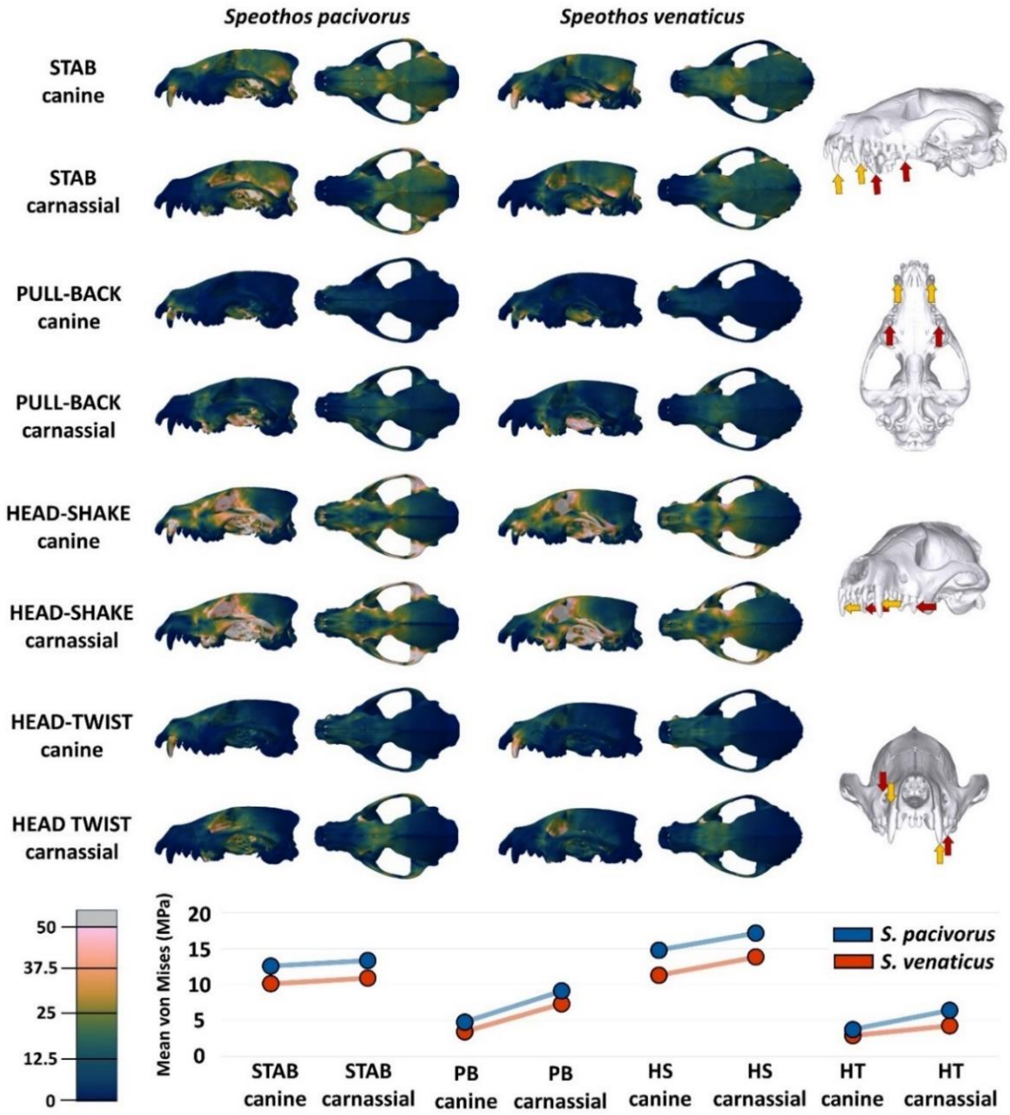
In relation to the distribution of von Mises stress, the differences between the species are more related to the degree of stress (i.e. how the magnitude varies in the same affected area) than to difference in the foci of stress through the cranium (i.e. distinct points of stress between the species). In all extrinsic scenarios tested, both species presented minor differences regarding the average von Mises stress per element, with the bush dog stressing slightly less than *S. pacivorus*, especially in the stabbing and head-shake scenarios (**Figure 3.7**); these differences, albeit small, might be explained by the more stocky and higher cranium of the bush dog. Loads on the carnassials yielded more stress than on the canines. It is noteworthy, however, that the differences in mean values between both teeth in all scenarios are not considerable, even considering that the loads on the carnassial are about 170% that of the canines.

The head-shake scenarios were the most stressful of all tests, a condition also recovered for bush dogs in Ruiz *et al*. (2022), a study conducted with different models with distinct volumes, material properties, and loads. In these scenarios, the carnassial bites were slightly more stressful. Conversely, the difference of stress average between canines and carnassials in the stabbing scenarios are minimal. In the headshake, the stress is more anteriorly distributed, especially at the orbital, lateral palatine surface, and zygomatic arch, with the rostrum being also affected but to a lesser degree. In the stabbing scenarios, the stress is distributed mainly on the braincase, orbital region, and lateral surface of the palatines, with minor focus of stress on the dorsal surface of the rostrum (in the canine scenarios).

The pull-back scenario stands out as the less stressed movement, similar to previous studies with canids (RUIZ *et al*. 2022; SLATER; DUMONT; VAN VALKENBURGH, 2009). Compared to the other scenarios, the differences between the mean values of the canines and carnassials are higher, albeit only slightly. The stress distribution in the pull-back scenarios is limited mainly to the ventral portion of the cranium, with minor areas of stress at the anterior portion of the rostrum (in the canine tests), and in the posterior portion of the rostrum and at the frontal (in the carnassial tests).

The head-twist scenarios are also characterized by lower stress, responding very similarly, on average, to the pull-back scenario. These lower values can be attributed to it being a unilateral scenario. Although the bush dog shows similar mean values between the canine and carnassial tests, in *S. pacivorus* the canine-driven model is markedly less stressed than the carnassial one. This comprises the only remarkable, albeit subtle, difference between both species in all scenarios tested. The stress distribution is quite similar to that of the pull-back scenario, with less stress on the lateral surface of the palatines and more stress on the zygomatic arch.

Figure 3.7. von Mises stress contour plots from finite element analysis of the extrinsic scenarios modelled to the crania of the *Speothos pacivorus* and *S. venaticus*, with reconstruction of the loads of each scenario illustrated on the right, and relation among the mean von Mises values per element in the tested scenarios. **HS**: head-shake; **HT**: head-twist; **PB**: pull-back.



Source: Ruiz *et al*. (under review)

3.5. Discussion

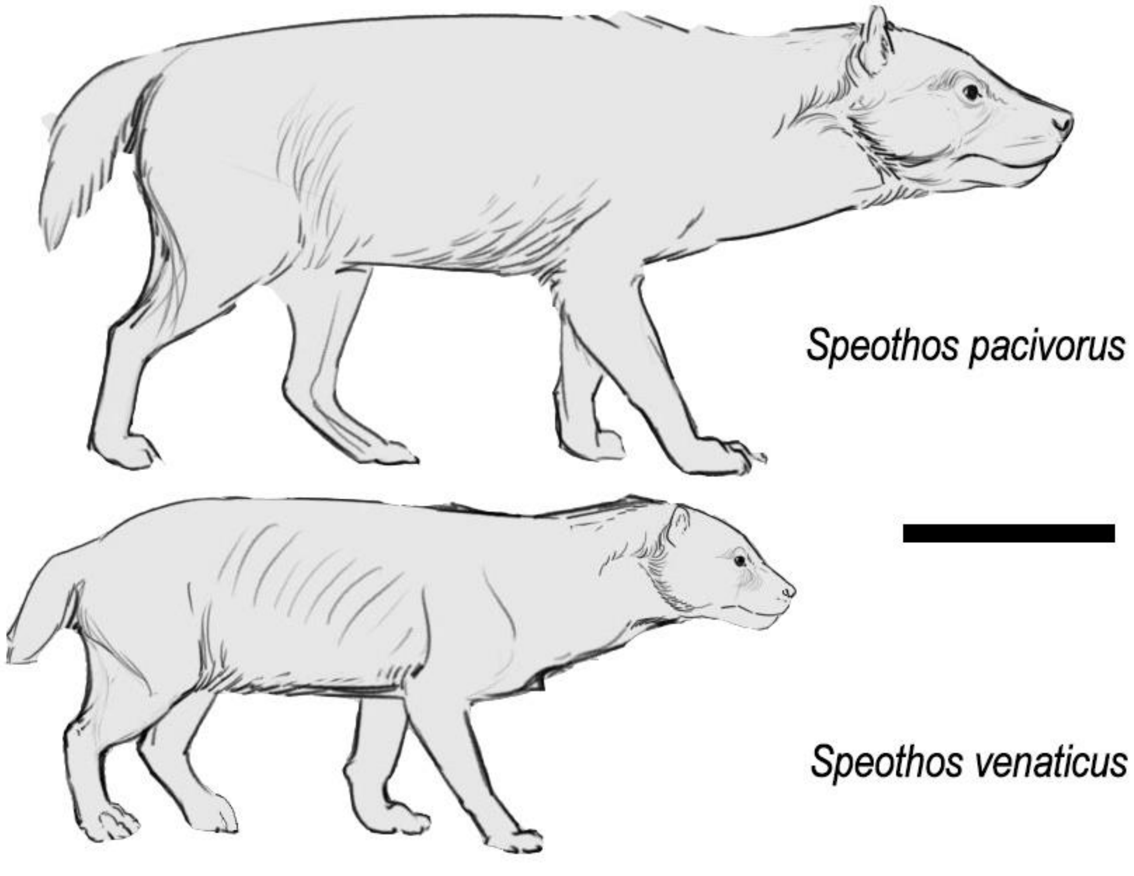
3.5.1. *Speothos venaticus* as a Distinct Species

The identification of canid species by osteological and external features is difficult, especially when considering recent species (MACHADO; TETA, 2020). Morphological similarities may not be determinant of conspecifics, but subtle morphological discontinuities are a common criterion to distinguish species (DIERSING; WILSON, 2017; GAUBERT; ANTUNES, 2005; MACHADO; TETA, 2020). Although, overall, the crania and dentition of *S. pacivorus* and the bush dog are very akin, close inspection shows that **(a)** the proportions, especially regarding the rostrum and braincase, are different; and **(b)** the series of distinct cranial characters used to distinguish both species — such as the number of teeth, shape of tooth crown, and number of roots — are not consistent with the intraspecific variation found within the tested sample of bush dog. Finally, postcranial elements also show distinct characters, as listed by Berta (1984).

Among living canids, the bush dog has arguably the most peculiar cranium in terms of proportion, resembling those of mustelids (VIEIRA, 1948). The geometric morphometrics analysis showed that the shortening of the rostrum (the most extreme within the sampled species) occurs together with hypercarnivory related dental adaptations (i.e., reduced number of molars and the development of a carnassial tooth PM4), with shorter rostra in individuals with a more hypercarnivorous dentition (**Figure 3.5**). Larger, hypercarnivorous Cerdocyonina also present a shorter rostrum and, in the case of *Protocyon troglodytes*, also a noticeably wide cranium, while *S. pacivorus* appears closer to the bush dog as an intermediate condition between *P. troglodytes* and *S. venaticus*. Because only a single specimen of *S. pacivorus* with a complete cranium is known, it is difficult to confirm its distribution in relation to the other included hypercarnivorous taxa; however, its placement near but not within the variability of the bush dog supports the hypothesis of it being a distinct, but closely related species.

Additionally, one of the proposed diagnoses of *S. pacivorus* is its larger size (**Figure 3.8**). Allometric scaling led to significant morphological differences in canids, with large individuals of a species being sometimes misleadingly classified as different taxa (MACHADO; TETA, 2020; WAYNE, 1986). The npMANCOVA results clearly showed that the specimen of *S. pacivorus* is not within the expected shape of an oversized bush dog. Most bush dogs analyzed have a similar age to *Speothos pacivorus* (A1; **Table A.3.2**), so its position in the morphospace cannot be interpreted as due ontogenetic differences. But even if *S. pacivorus* deviation from *S. venaticus'* allometric change is due to its ontogenetic stage, considering it a not fully grown "subadult", as assumed by Berta (1984), that would mean that the final adult morphology of *S. pacivorus* would extrapolate the variation of the extant *Speothos*. In light of these results and considering that *Speothos pacivorus* and the bush dog are distinguished from each other by discrete morphological characters (BERTA, 1984), the most consensual hypothesis of *Speothos* taxonomy is endorsed, rejecting *S. venaticus* as a junior synonym of *S. pacivorus*.

Figure 3.8. Diagram comparing the putative size of adult *Speothos pacivorus* (above) and *S. venaticus* (bellow), based on estimations made by Berta (1984). Scale bars equals 20 cm.



Source: Julia D'Oliveira, adapted

3.5.2. *Speothos* as Pleistocene Predators

Although morphologically distinct, the cranium of both *Speothos pacivorus* and the bush dog present very similar biomechanical performances. The FEA results do not suggest different hunting movements for capturing prey for each taxon. With both *Speothos* species being slightly different in size and form, and assuming a sympatric distribution for it in the Pleistocene of Central Brazil, two main questions arose: **(a)** how such similar taxa could coexist temporally and spatially; and **(b)** why *S. pacivorus* became extinct but the bush dog did not. Different ecological, dietary, and reproductive strategies of modern canids can provide some insights into it.

Coexistence of canid species in modern ecosystems occurs with similar sized but generalist species (DI BITETTI *et al*. 2009; MACDONALD; LOVERIDGE; ATKINSON, 2004; NOVARO; FUNES; JIMÉNEZ, 2004), species with different sizes, or species with different ecological and/or dietary niches (FOWLER *et al*. 2022; KOZLOWSKI; GESE; ARJO, 2008; PETROELJE *et al*. 2020). In the case of the Pleistocene *Speothos*, the results suggest that both species were highly specialized hypercarnivorous, indicating a similar diet and, consequently, ecological niche. In modern ecosystems, such examples do not usually overlap in their distribution (CASTELLÓ, 2018) and, when they do, such as in the case of the grey wolf (*Canis lupus*) and the dhole (*Cuon alpinus*), they are antagonists and usually avoid each other spatially at the microhabitat scale (HEPTNER; NAUMOV, 1998, but see GHASKADBI *et al*. 2021). Although some canid communities show evidence of species displacement, black-backed jackals (*Lupulella mesomelas*), side-striped jackals (*Lu. adusta*), and African golden wolves (*Canis lupaster*), three species of generalist feeders of similar sizes, coexist in Kenya (SILLERO-ZUBIRI, 2009). Could this be the case of *Speothos* in the Brazilian Intertropical Region (BIR)? Comparing modern and Pleistocene ecosystems, however, is not simple since the faunal compositions are substantially different.

A factor that could allow the spatial coexistence of *S. pacivorus* and bush dogs is a richer community of herbivorous mammals in the Pleistocene. Modern bush dogs are pack-hunters that eat small to medium-sized prey, especially caviomorphs and armadillos (LIMA; PINTO; DALPONTE, 2009; ZUERCHER; GIPSON; CARRILLO, 2005), also appearing to be specialized in some prey in certain regions (e.g. armadillos in Pantanal, LIMA; PINTO; DALPONTE, 2009). Their robust skulls also allow the bush dogs to occasionally hunt bigger prey (RUIZ *et al*. 2022), such as brocket deer, peccaries, capybaras, and even tapirs (BEISIEGEL, 1999; BEISIEGEL; ZUERCHER, 2005; WALLACE; PAINTER; SALDANIA, 2002). In the Pleistocene, the Lagoa Santa region supported a rich community of herbivorous mammals, with at least 21 species of rodents, xenarthrans, and eungulates registered to date (CARTELLE, 2020). Many of these species are larger relatives of known prey of modern bush dogs, such as the armadillos *Propraopus* spp. and the rodents *Brasiliochoerus stenocephalus*, *Neochoerus sulcidens*, and, noteworthy, the large paca *Cuniculus rucigeps* (CARTELLE, 2020).

Since the modern, slightly smaller *Speothos* hunts extensively on modern pacas — also smaller than their extinct relatives — it is reasonable to think that part of the diet of the larger *S. pacivorus* was based on the larger, contemporaneous pacas. The specific epithet of *S. pacivorus* means ‘paca eater’, in allusion to the large bed of bones of those rodents in the same caves where the remains of *S. pacivorus* were found (PAULA-COUTO, 1979). The collector and describer of the species, Peter Lund, attributed both *S. pacivorus* and *Protocyon troglodytes* as being the main responsible for the accumulation of paca carcasses in these caves (MOUCHARD, 2019). The skull morphology of *S. pacivorus*, in addition, suggests it could have preyed on even larger prey when hunting in packs, occasionally pursuing species that could not be targeted by the smaller bush dogs, avoiding an extensive overlap in food resources between both species. Although there is no direct evidence of pack hunting in *S. pacivorus*, it is a behavior also postulate for *Theriodictis* (PREVOSTI; PALMQVIST, 2001), and known for both extant bush dogs and extinct *Protocyon* (BEISIEGEL; ZUERCHER, 2005; DANTAS *et al*. 2022), as well as non-Cerdocyonina extant hypercarnivores canids such as gray wolves, painted dogs, and dholes (SILLERO-ZUBIRI, 2009).

By also presenting longer leg bones (BERTA, 1984), *S. pacivorus* could have had different hunting strategies that are not suggested by its skull biomechanics. The short legs of the bush dog are one of its most striking characters, and it was assumed to be an adaptation to facilitate moving in narrow spaces (BEISIEGEL; ZUERCHER, 2005; KLEIMAN, 1972), presumably allowing it to pursue prey through thick vegetation. It could also be related to digging behaviour, since it is known to chase prey in their burrows (FLECK; VOSS, 2016), presenting also wider forepaws with strong claws, characters that enable a better digging performance (LIMA; PINTO; DALPONTE, 2009). Shorter legs, in contrast, did not allow bush dogs to maintain high speed for long periods when compared to longer legged pack hunters as grey wolves and painted dogs (RUIZ *et al*. 2022). The short legs of bush dogs, thus, can indicate a specialization to prey capture in conditions better suited for smaller bodies (i.e., hunting through undercover and riparian thick vegetation or inside burrows) when compared to the sympatric *S. pacivorus* and *P. troglodytes*.

The Pleistocene of the BIR had a community of land mammals with comparatively more herbivores than modern assemblages, which could handle a more complex carnivore assembly as well. Similarly rich faunal compositions in other parts of the world today accommodate more than one species of canids specialized in hypercarnivory. For example, the current overlapping distribution of the dhole and the grey wolf in certain parts of India, Nepal, Bhutan, and China (BOITANI; PHILLIPS, M; JHALA, 2023; CASTELLÓ, 2018; KAMLER; SONGSASEN; JENKS, 2015). In the Pleistocene, however, dholes were far more widespread, having a distribution very similar to that of the modern Eurasian wolf, with records in Europe (PETRUCCI *et al*. 2012; RIPOLL *et al*. 2009). Paleoclimatic range reconstructions indicates also its presence in the Levant, where the dhole has coexisted and admixed with the very similar painted dog (ROCHA *et al*. 2022). Finally, its presence in North America is attested by fossils found in Mexico, a region with Pleistocene records not only for grey wolves and red wolves (*Canis rufus*) but also for the larger *Aenocyon dirus* (BRAVO-CUEVAS *et al*. 2018; CEBALLOS; ARROYO-CABRALES; PONCE, 2010; HODNETT; MEAD; BAEZ, 2009) and the similar-sized *P. troglodytes* (although the temporal and spatial coexistence with this species is unclear, see SCHUBERT *et al*. 2019).

Assuming that the extinction of different-sized hypercarnivore predators can be directly related to the extinction of the megaherbivores after the Pleistocene (Berta 1987, 1988), the same reason could also explain why the smaller bush dog was the only hypercarnivorous canid to cross the Pleistocene-Holocene boundary. There is no data to confirm how exactly the process of extinction of megafauna has occurred in the Lagoa Santa region, but a study conducted in the Brazilian Pampas region showed that, after a major extinction event of mega xenarthrans, smaller rodents and carnivorans became the most abundant mammals in Holocene deposits (LOPES *et al*. 2020). Modern large canids are usually associated with habitats in which prey are at least abundant (e.g., Ethiopian wolves, *Canis simensis*) and, more generally, abundant and large (e.g., painted dogs, grey wolves, SILLERO-ZUBIRI, 2009). Considering the size and hunting habits of Pleistocene Cerdocyonina, Berta (1987) noted that larger, packing-hunters 'wolflike' forms (i.e. *Protocyon*, *Theriodictis*) became extinct along with the megaherbivores, while smaller, 'foxlike' solitary hunters (i.e. *Cerdocyon*, *Lycalopex*), increased in diversity in the Pleistocene-Holocene transition. The bush dog has a mixture of these traits: it is a hypercarnivorous pack-hunter but with fox size, and can deal with smaller-sized prey, as observed today.

Hypercarnivory in canids is correlated to an increase of body size and the specialization on large prey with, at least, half their body weight (VAN VALKENBURGH, 2007), a trend observed in both living and extinct lineages (VAN VALKENBURGH, 1991; Van VAN VALKENBURGH; WANG; DAMUTH, 2004). The bush dog is the only extant small-sized hypercarnivorous canid, and it could be precisely this trait that led to its survival through the Pleistocene-Holocene boundary. Three of the four species of living hypercarnivorous canids are not only larger than their sister species, but also the larger canids in their ecosystems (CASTELLÓ, 2018; SILLERO-ZUBIRI, 2009). The only exception is the small-bodied bush dog, smaller than most sympatric, non-hypercarnivore species (CASTELLÓ, 2018; CHAVÉZ *et al*. 2022; SILLERO-ZUBIRI, 2009).

3.6. Chapter Conclusions

Although superficially similar, the skull of *Speothos pacivorus* bears considerably differences when compared to that of the bush dog, especially regarding the proportions of the rostrum and braincase, the width of the cranium, the morphology of the molars M1 and m2, and the presence of a well-developed M2, which sums with differences in postcranial elements reported before. It is important to mention that the best-preserved materials of *S. pacivorus* almost certainly represent a young adult individual, which reinforces the difference of size between the two species. The quantitative data from the GMM gives further support to the descriptive information, and therefore could be argued that the consensual view of *S. pacivorus* and *S. venaticus* as different species.

Both species performed very similarly in all tested extrinsic FEA scenarios, suggesting similar biting movements to capture prey. The coexistence of these similarly specialized hypercarnivorous canids in both space and time could be explained by a richer community of prey, already described to the Lagoa Santa Karst. A better aptitude of the bush dog in subduing smaller prey than *S. pacivorus* and other larger hypercarnivores from the BIR might explain its survival through the Pleistocene-Holocene boundary. This hypothesis could also explain one of the most remarkable characters of the bush dog: its small size, unique among living hypercarnivorous canids.

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3.8. Appendix 3.1 – Supplementary tables

Table A.3.1. List of specimens analysed in the geometrical morphometric analysis.

SPECIMENS ANALYSED IN GMM	
Species	**Specimen**
***Speothos pacivorus* (n=1)**	NHMD:211341
***Speothos venaticus* (n=59)**	AMNH136285
	AMNH167846
	AMNH175306
	AMNH184688
	AMNH37472
	AMNH76035
	AMNH76805
	AMNH76806
	AMNH98558
	AMNH98559
	AMNH98560
	AMNH98640
	FMNH121544
	FMNH125402
	FMNH60290
	FMNH87861
	MACN16510
	MACN33154
	MACN5067
	MCZ28056
	MCZ41096
	MCZ42086
	MNRJ25668
	MNRJ25670
	MNRJ3033

(continue)

(continuation)

***Speothos venaticus* (n=59)**	MNRJ3035
	MPEG1042
	MPEG111
	MPEG1394
	MPEG1778
	MPEG1780
	MPEG5614
	MPEG5615
	MPEG6535
	MPEG6536
	MPEG8793
	MPEG8794
	MVZ126276
	MVZ184054
	MZUSP19743
	MZUSP19744
	MZUSP2684
	MZUSP2685
	MZUSP35732
	SNHMS19136
	SNHMS478
	USNM253504
	USNM270165
	USNM270368
	USNM270370
	USNM307650
	USNM314048
	USNM395841
	USNM398030

(continue)

(conclusion)

***Speothos venaticus* (n=59)**	USNM521045 USNM538307 USNM544414 USNM582465 ZMK-1-1845-2033
***Chrysocyon brachyurus* (n=6)**	MZUSP3025 MZUSP3338 MZUSP19732 MZUSP32039 MZUSP2479 MNRJ17502
***Dusicyon avus* (n=2)**	CEHA 5131 Pr1 090
***Dusicyon australis* (n=4)**	BMNH 55.12.24.431 BMNH 1642 BMNH 1837.3.15.48 BMNH 1869.2.24.3
***Potrocyon scagliorum* (n=1)**	MMP S 164
***Protocyon troglodytes* (n=2)**	FUMDHAM 145517 DP 5867
***Theriodictis platensis* (n=1)**	MLP 96-IX-1-1
***“Canis” gezi* (n=1)**	MACN-Pv 5120

Source: Ruiz *et al*. (under review)

Table A.3.2. Linear measurements of the condylobasal length of the cranium of *Speothos pacivorus* and *S. venaticus*. The proposed ages follow the categorization by Segura and Prevosti (2012). Specimens listed in descending order of size. Specimens marked with an asterisk were not included in the geometric morphometric analyses.

CONDYLOBASAL LENGHT		
Specimen	**Condylobasal length (in cm)**	**Age**
Speothos pacivorus NHMD:211341	17.26	A1
SNHMS19136	16.56	A2
MNRJ3033	16.09	A1
ZMK-1-1845-2033	15.75	A3
MNRJ3035	15.03	A3
SNHMS478	15 (approximate)	A2
MZUSP2684	14.89	A3
MZUSP2685	14.55	A2
MACN5067	14.45	A3
MNRJ25668	14.38	A2
MZUSP19744	14.29 (specimen with osteopatology)	?
MPEG1778	13.93	A1
MVZ184054	13.85	A1
MPEG6536	13.82	A2
AMNH167846	13.81	A2
AMNH175306	13.76	A3
MACN33154	13.74	A1
MPEG8793	13.67	A1
AMNH98640	13.65	A2
MZUSP19743	13.65	A1
MPEG111	13.63	A1
MPEG1780	13.56	A2
MZUSP35732	13.53	A2
MVZ126276	13.49	A3
MPEG8794	13.48	A1
AMNH76806	13.43	A3

(continue)

(continuation)

MPEG5614	13.4	A1
MCZ42086	13.36	A1
MPEG26180*	13.3	A2
AMNH76035	13.29	A2
MPEG1042	13.29	A3
MNRJ25670	13.19	A1
FMNH125402	13.1	A2
MACN16510	13.06	A1
USNM270370	13.03	A3
MPEG5615	12.91	A1
AMNH98558	12.8	A1
USNM521045	12.77	A3
MCZ28056	12.75	A3
AMNH98560	12.65	A2
USNM582465	12.65	A2
USNM307650	12.56	A3
MCZ41096	12.54	A3
USNM538307	12.53	A1
USNM270368	12.51	A3
FMNH87861	12.38	A1
AMNH98559	12.24	A1
USNM395841	12.21	A3
AMNH76805	12.19	A1
USNM398030	12.11 (specimen with osteopatology)	A2?
USNM253504	12.08	A1
AMNH184688	12.02 (incisors absent)	A1?
MPEG1394	11.99	A1
AMNH37472	11.96	A2
USNM314048	11.91	A2
USNM270165	11.9	A1

(continue)

(conclusion)

FMNH121544	11.76	A1
MPEG6535	11.56	A1
FMNH60290	11.5	A1
USNM179047*	11.31 (a juvenile specimen)	J3
AMNH136285	? (occipital condyle absent)	A3
USNM544414	? (occipital condyle absent)	A2
MNRJ68179*	? (right lateral rostrum absent)	A1

Source: Ruiz *et al*. (under review)

Table A.3.3. Landmark and semilandmarks definitions. In parenthesis, corresponding landmarks in Machado and Teta (2020). Landmark topology in **Figure A.3.1**.

LANDMARKS AND SEMILANDMARKS DEFINITIONS	
Landmark	**Definition**
1	anteriormost point of the incisive (IS*)
2	lateral tip of the occipital condyle, left
3	lateral of the occipital condyle, right
4	incisive-maxilla contact medial to the canine, left (PM*)
5	incisive-maxilla contact medial to the canine, right (PM*)
6	nasal spine, left (PNS*)
7	ventralmost point of the zygomatic bone, left (ZI*)
8	ventralmost point of the zygomatic bone, right (ZI*)
9	ventral contact between the zygomatic and the temporal. left (ZYGI*)
10	ventral contact between the zygomatic and the temporal, right (ZYGI*)
11	ventral margin of the external acoustic meatus, left (EAM*)
12	ventral margin of the external acoustic meatus, right (EAM*)
13	anteriormost point of the auditory bulla, left
14	anteriormost point of the auditory bulla, right
15	posteriormost point of the auditory bulla, left
16	posteriormost point of the auditory bulla, right
17	ventral tip of the paracondylar process, left
18	ventral tip of the paracondylar process, right

(continue)

(conclusion)

19	medial point of the ventral margin of the foramen magnum (BA*)
20	canine, anterior left (CA*)
21	canine, anterior right (CA*)
22	canine, posterior left (CP*)
23	canine, posterior right (CP*)
24	carnassial, anterior left (CAA*)
25	carnassial, anterior right (CAA*)
26	carnassial, medial left
27	carnassial, medial right
28	carnassial, posterior left (CAP*)
29	carnassial, posterior right (CAP*)
30	M1, medial left
31	M1, medial right
32	posterior limit of the left toothrow
33	posterior limit of the right toothrow
34	lateralmost point of contact between frontal and temporalis, left
35	lateralmost point of contact between frontal and temporalis, right
curve a	11->13 (13 points)
curve b	13->15 (16 points)
curve c	15->11 (8 points)
curve d	12->14 (13 points)
curve e	14->16 (16 points)
curve f	16->12 (8 points)
curve g	2->19 (16 points)
curve h	19->3 (16 points)

Source: Ruiz *et al*. (under review)

Table A.3.4. Maxillary and palatal measurements of *Speothos pacivorus* and *S. venaticus*.

MAXILLARY AND PALATAL MEASUREMENTS (in cm)				
Species	**Specimen**	**Palate to PM4-M1 contact**	**PM3-M1 distance**	**PM4 total length**
S. pacivorus	**NHMD:211341**	2.76	4.99	1.8
	NHMD:72	1.81	3.73	1.42
S. venaticus	**ZMK-1-1845-2033**	2.62	4.41	1.69
	SNHMS19136	2.42	4.54	1.74
	MNRJ3033	2.34	3.76	1.52
	MNRJ3035	1.97	4.06	1.5
	MZUSP2684	1.93	3.88	1.53

Source: Ruiz *et al*. (under review)

Table A.3.5. Mandibular lateral measurements of *Speothos pacivorus* and *S. venaticus*.

MANDIBULAR MEASUREMENTS (in cm; e = estimated)				
Species	**Specimen**	**pm4-m2**	**m1-m2**	**height under m1**
S. pacivorus	**NHMD:159**	4.66	2.59	1.86
	NHMD:65	-	2.45 (e)	1.6
	NHMD:711	-	2.6 (e)	2.08
S. venaticus	**ZMK-2041**	2.69	1.85	2.09
	ZMK-1-1845-2033	2.46	1.64	1.64
	SNHMS19136	2.19	1.4	1.37
	MNRJ3033	2.57	1.77	1.74
	MNRJ3035	2.68	1.76	1.9
	MZUSP2684	2.78	1.87	1.87

Source: Ruiz *et al*. (under review)

3.8.1. Appendix References

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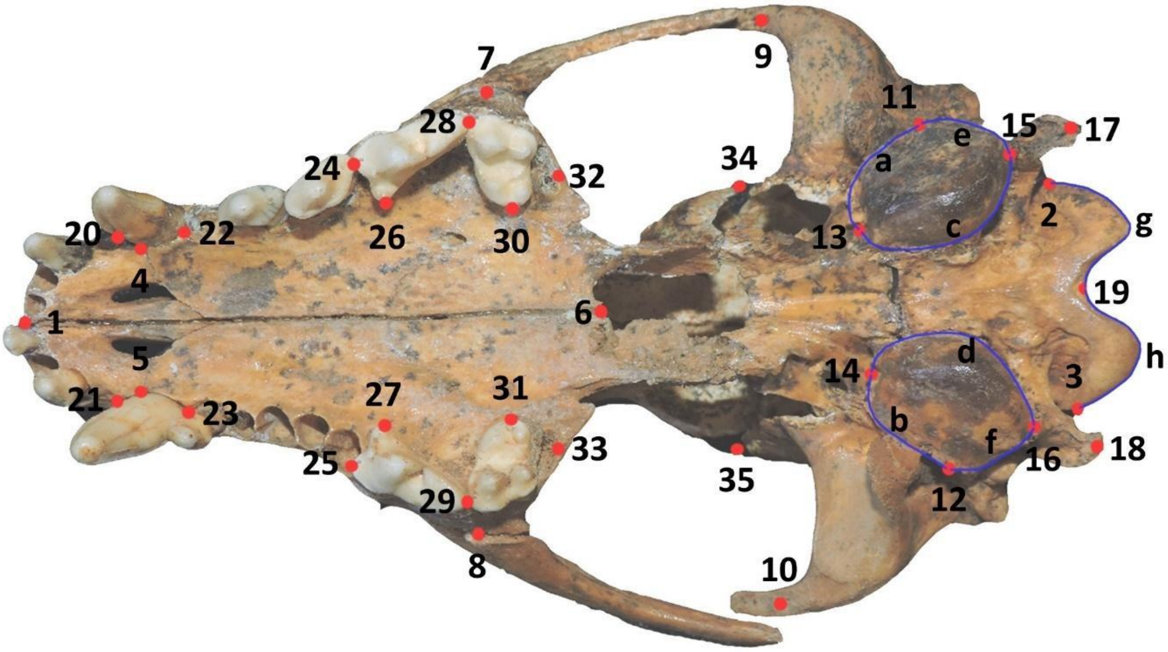
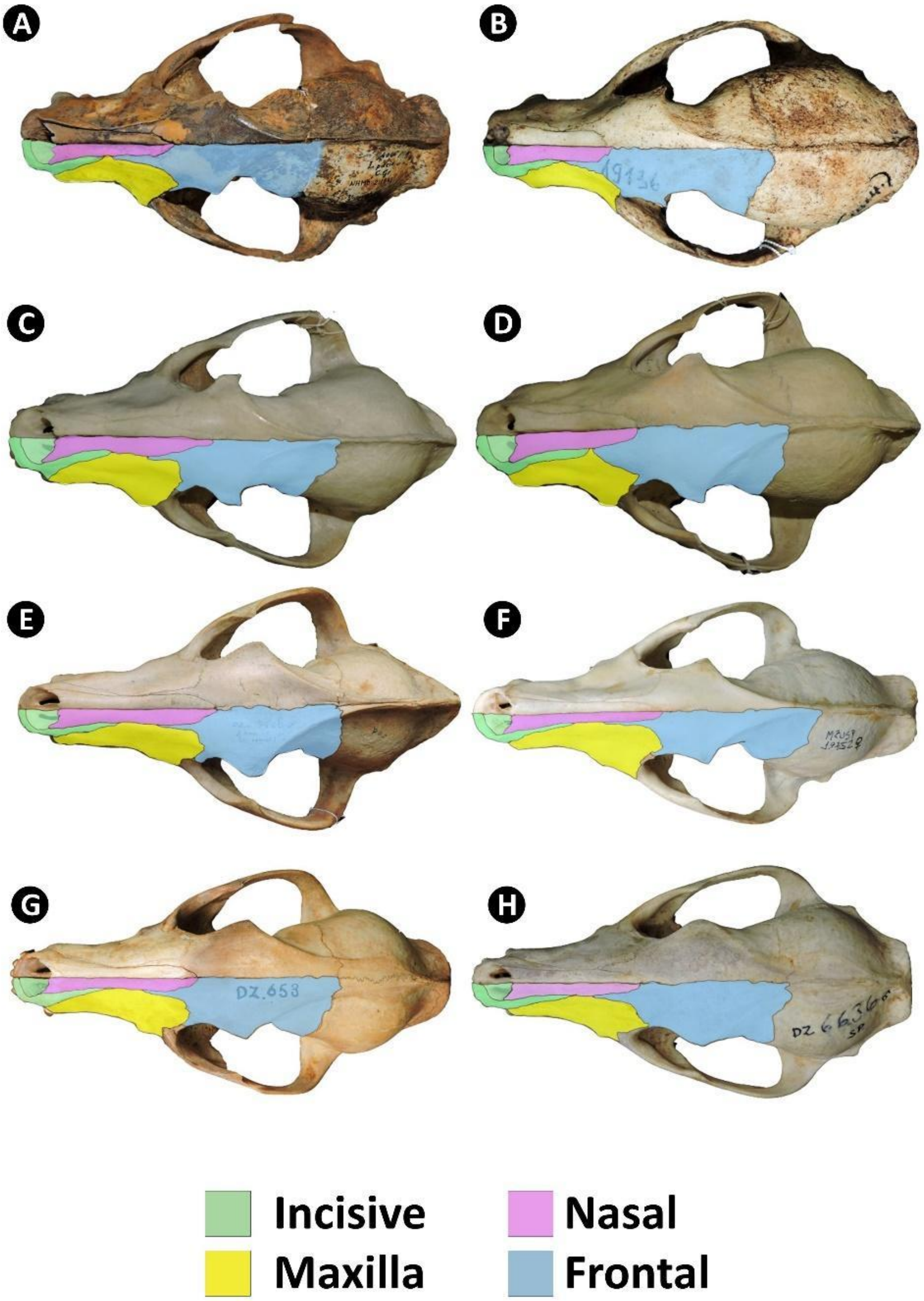
3.9. Appendix 2 – Supplementary figures

Figure A.3.1. Landmarks and semilandmarks used in the geometric morphometry, represented in the cranium of *Speothos pacivorus* (NHMD:211341). Landmark definitions on **Table A.3.3**.



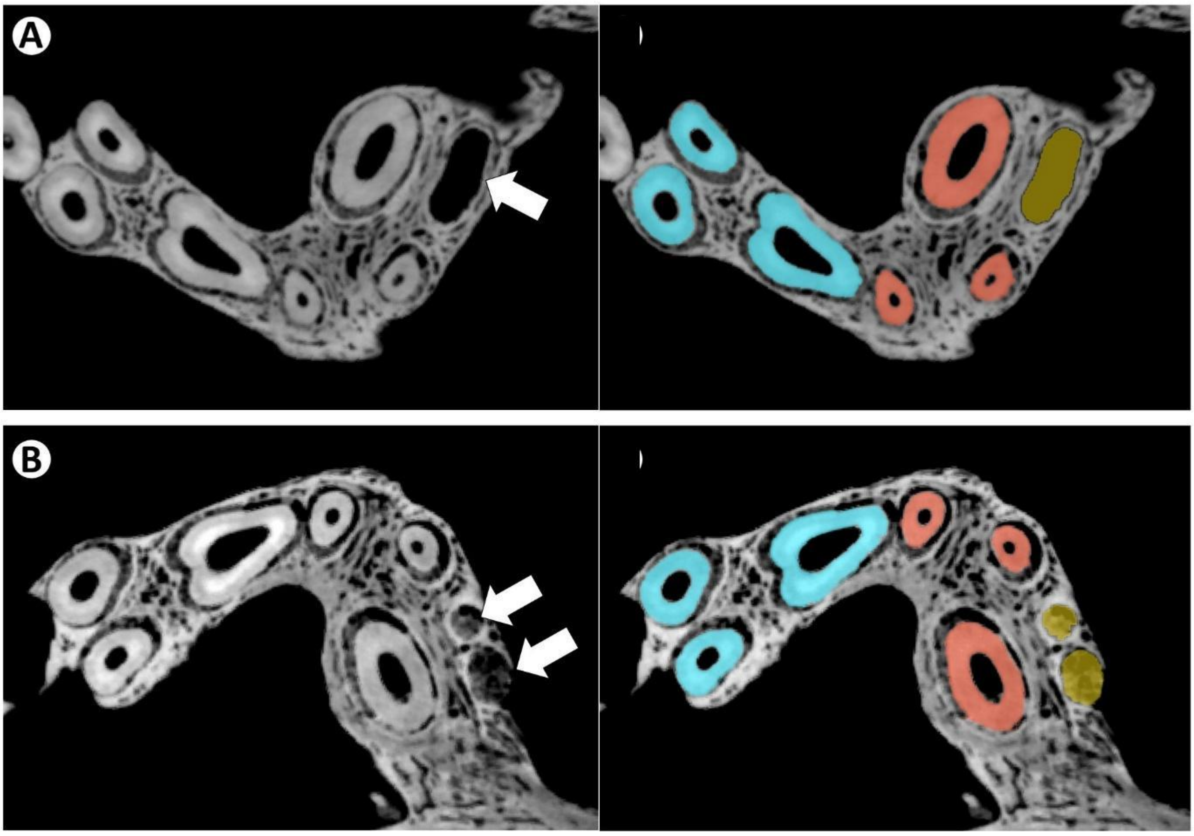
Source: Ruiz *et al*. (under review)

Figure A.3.2. Dorsal view of the crania of different canids, with the bones discussed in the comparative description highlighted. **(a)** *Speothos pacivorus*; **(b)** *S. venaticus*; **(c)** *Cuon alpinus*; **(d)** *Lycaon pictus*; **(e)** *Chrysocyon brachyurus*; **(f)** *Atelocynus microtis*; **(g)** *Lycalopex gymnocercus*; **(h)** *Cerdocyon thous*. Not to scale.



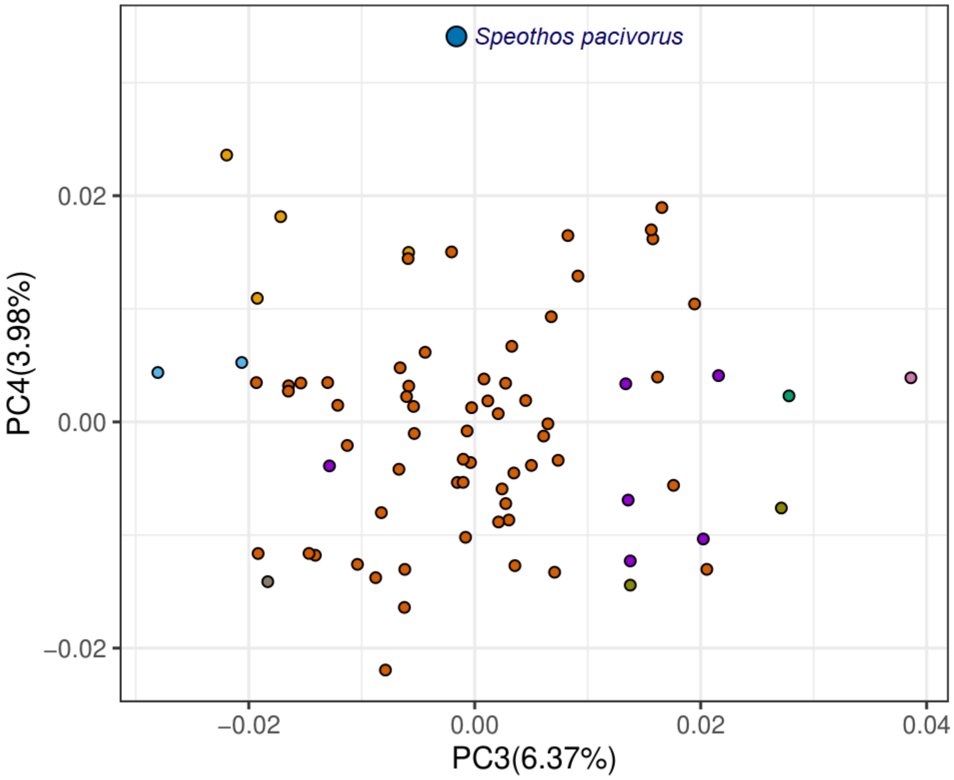
Source: Ruiz *et al*. (under review)

Figure A.3.3. Cross section of the cranium of *Speothos pacivorus* (NHMD:211341) in ventral view, showing the tooth root and alveoli of the last three teeth in (a) right; and (b) left side. From left to right, PM4 (blue), M1 (red), and M2 (yellow). Arrows indicate the root alveoli of M2.



Source: Ruiz *et al*. (under review)

Figure A.3.4. PC3 vs PC4 occupation of nine species of the *Speothos-Chrysocyon* clade.



Source: Ruiz *et al*. (under review)

4. CHAPTER 3 – The Fossils of *Speothos pacivorus* (Carnivora: Canidae) at the Peter Lund/Quaternary Collection of the Natural History Museum of Denmark

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4.1. Chapter 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, all biological remains of *S. pacivorus* hosted in the Peter Lund/Quaternary Collection at the Natural History Museum of Denmark, Copenhagen, were analised by listing and illustrating all its specimens known to date. A reconstruction of the holotype, an almost complete cranium, based on a μCT scan, was also conducted, producing an undeformed and crack-free three-dimensional model. This data available it is expected to foster new research on this elusive species.

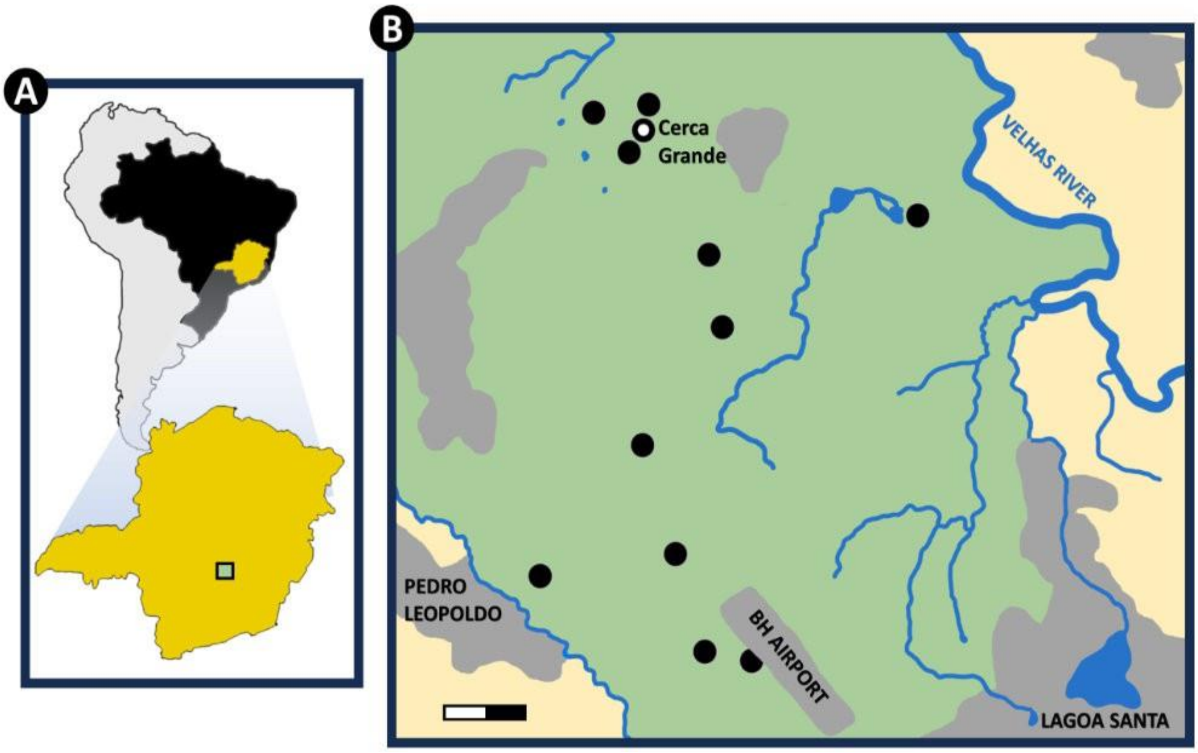
4.2. INTRODUCTION

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* (OKŘINOVÁ, 2013; ZRZAVÝ *et al.* 2017). All materials assigned to *Speothos pacivorus* come from a single cave, the Lapa da Cerca Grande cave (**Figure 4.1**), 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 Cerdocyonina, usually only being referred to in phylogenetic analyses (OKŘINOVÁ, 2013; PERINI; RUSSO; SCHRAGO, 2010; 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). The goals of the present study 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.

Figure 4.1. 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).



Source: Ruiz *et al*. (2024)

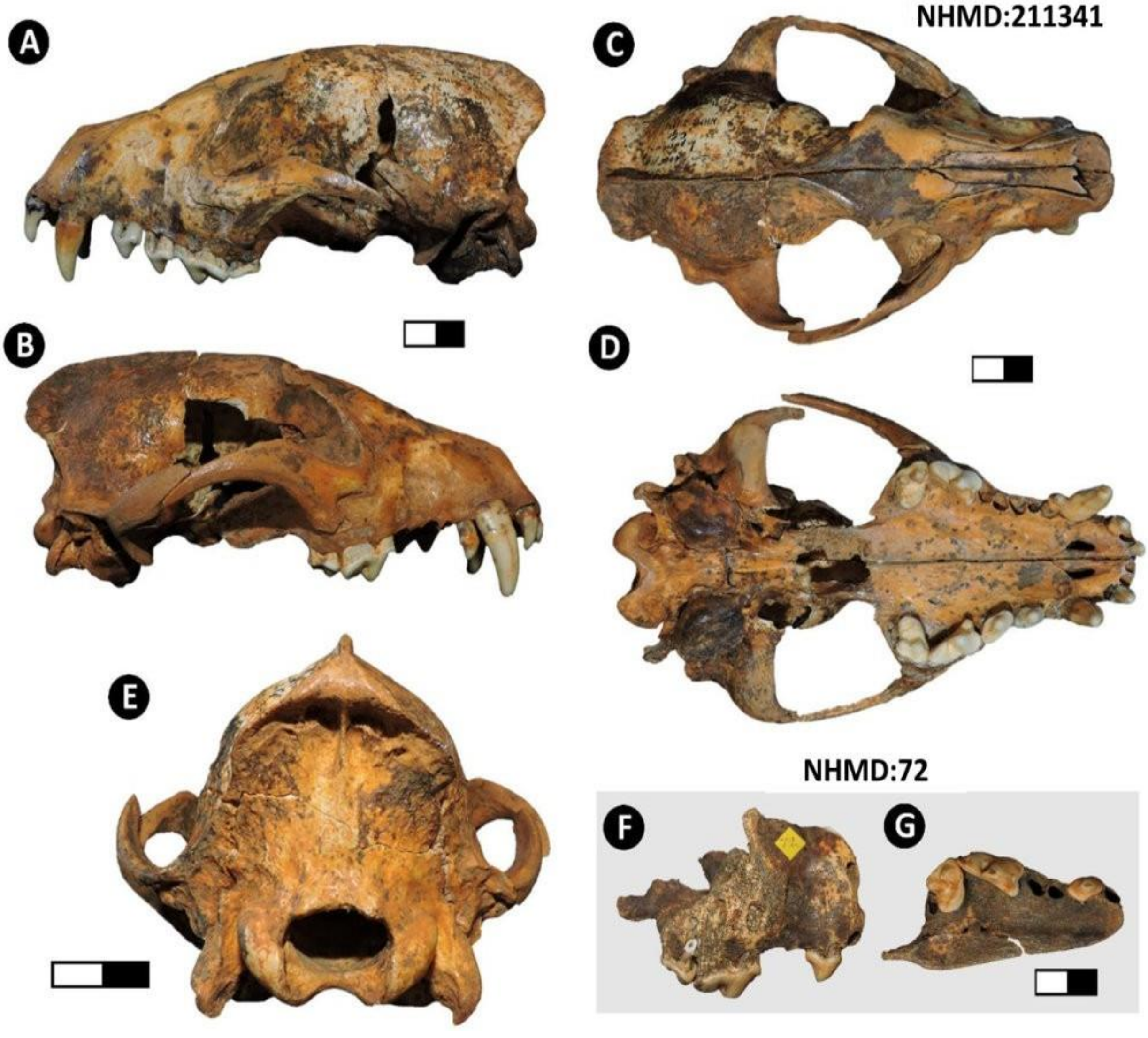
4.3. Methods

4.3.1. 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 A.4.1**). They comprise two cranial elements (**Figure 4.2**), three incomplete mandibles (**Figure 4.3**), one vertebra (**Figure 4.4**), 18 elements of the appendicular skeleton (**Figure 4.5**), and 54 isolated teeth (**Figure 4.6**). 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.

Figure 4.2. 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 equals 2 cm.



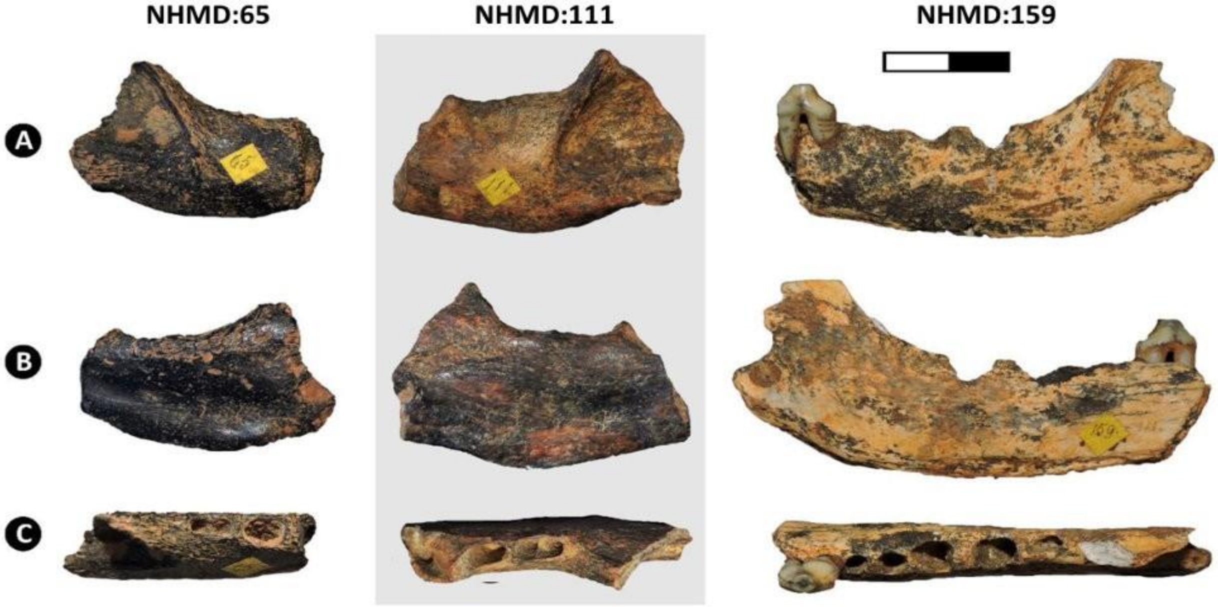
Source: Ruiz *et al*. (2024)

4.3.2. 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

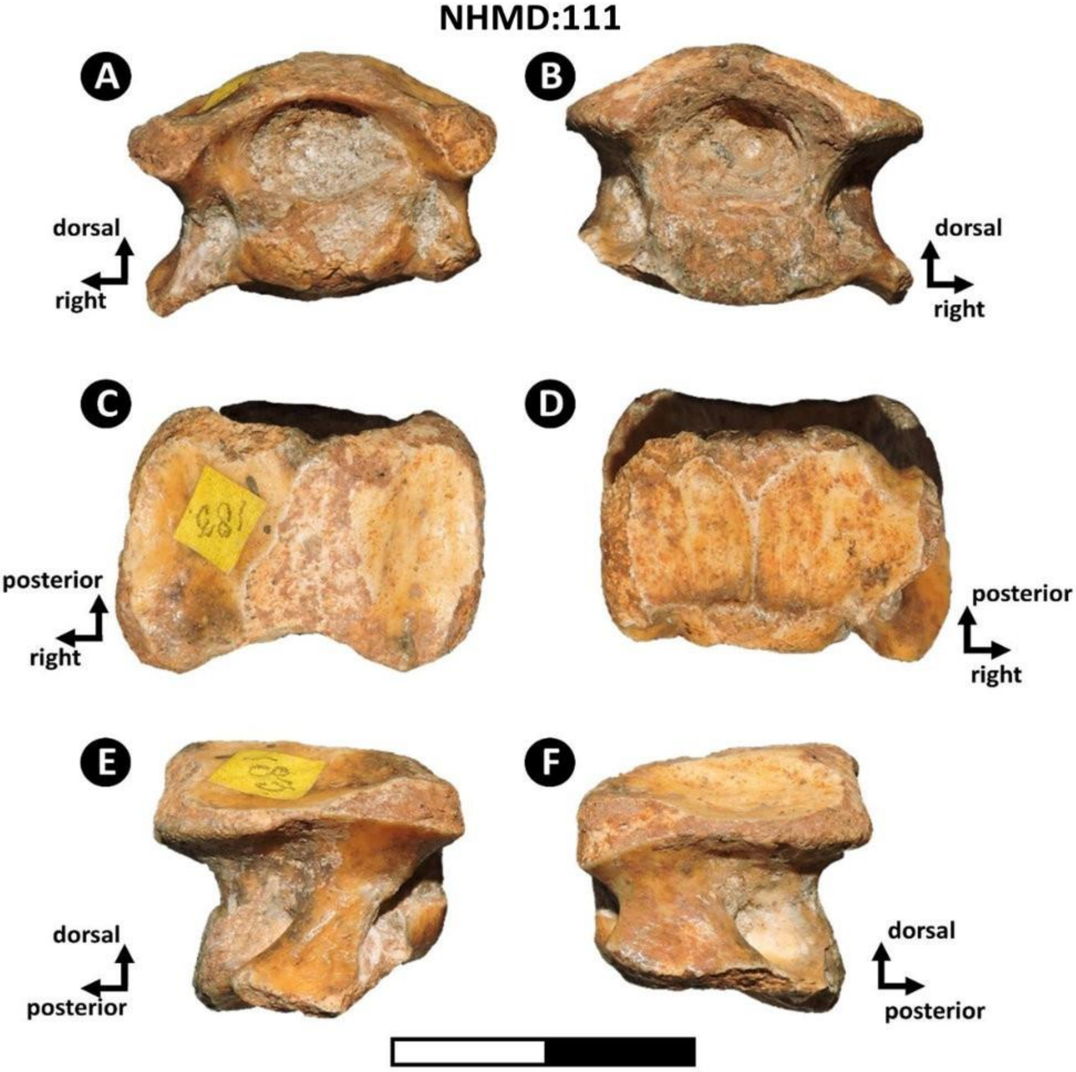
Centre, Technical University of Denmark, using a Nikon XT H225 ST system. The scan was conducted using an aluminium filter with thickness of .002 mm, using a voltage of 120 kV and current of 417 µA, resulting in 3142 projections (voxel size of .1 mm). For comparison, a skull of the extant *S. venaticus*, 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 (thick- ness 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.

Figure 4.3. Mandibular fragments of *Speothos pacivorus* in lateral (a), medial (b), and dorsal (c) views. Scale bar equals 2 cm.



Source: Ruiz *et al*. (2024)

Figure 4.4. 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 equals 2 cm.



Source: Ruiz *et al*. (2024)

4.3.3. Visual reconstruction of the cranium

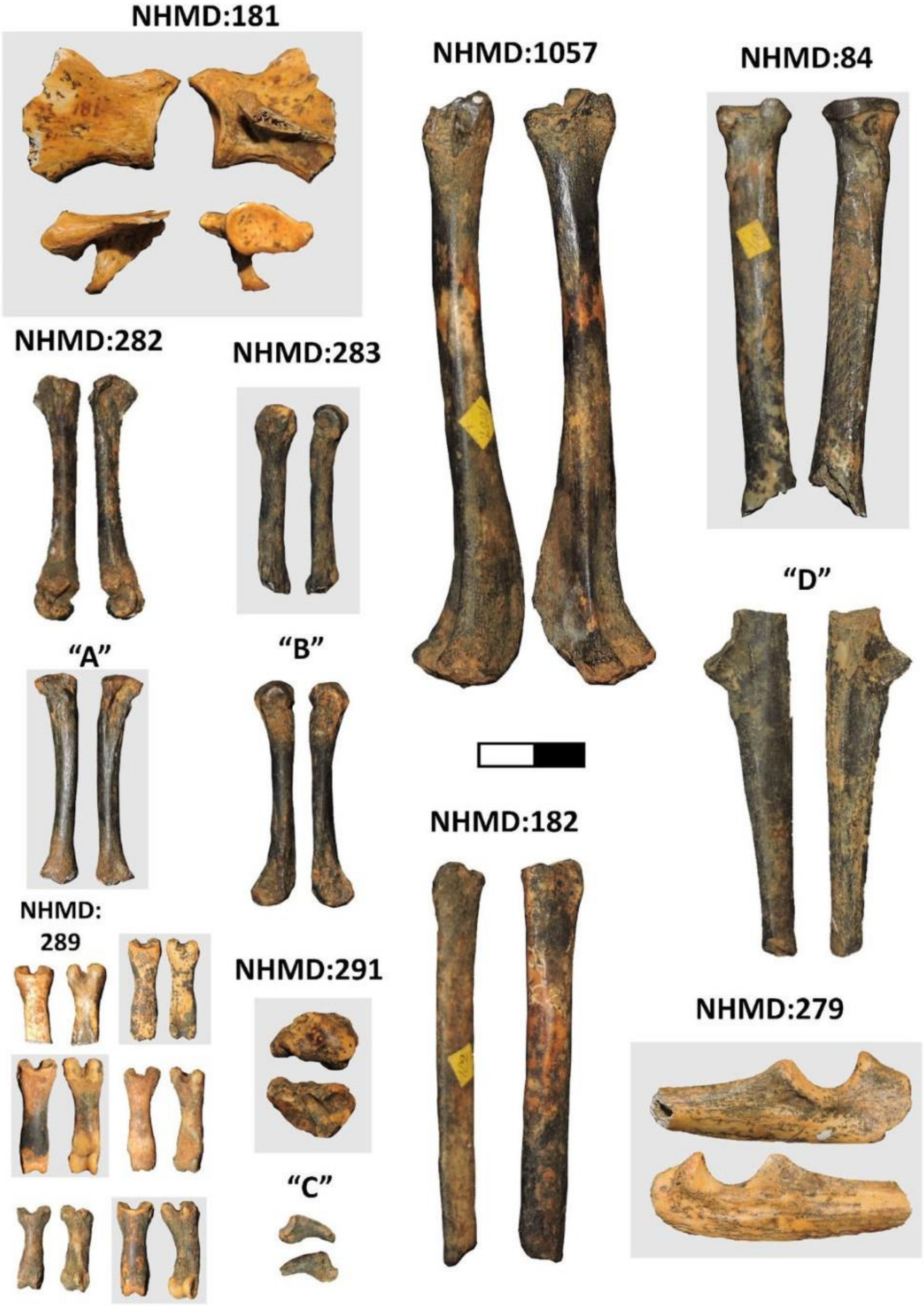
The mesh models of both crania were imported into the open- source 3D-modeling software Blender 3.4.1 (available at blender.org). Specimen NHMD:211341 is mostly well preserved but separated into posterior and anterior parts along the unfused coronal suture. The alisphenoid is partially preserved on the 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 the 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.

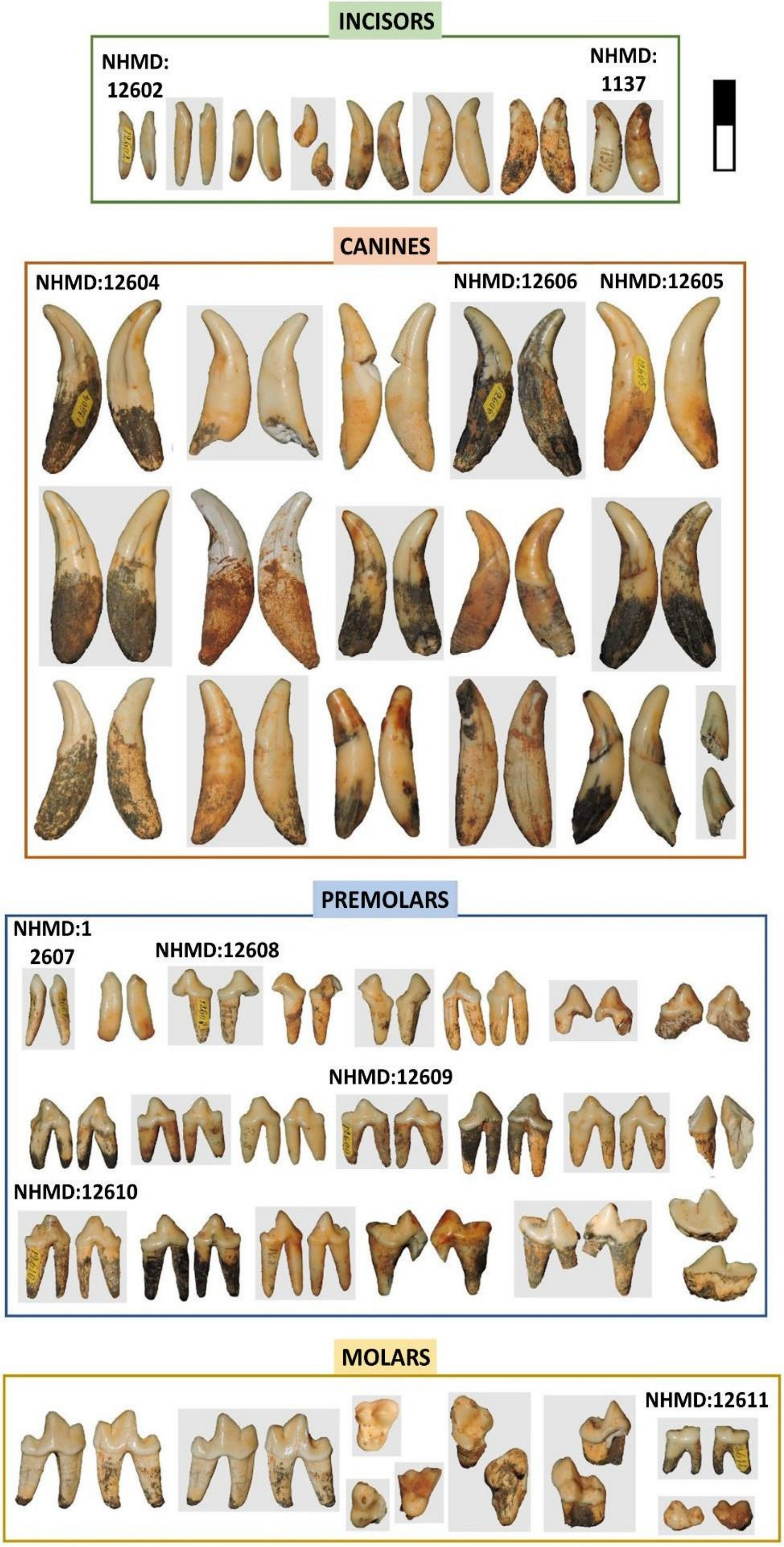
Finally, the model was tested for mesh errors (bad geometries). For this, the Blender tool “3DPrint” was used to pinpoint the non-manifold edges and intersecting faces. Non-manifold faces were then cleaned up with the same tool while the intersecting faces were removed manually by merging each intersection 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 4.7**.

Figure 4.5. 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 A.4.1**. Scale bar equals 2 cm.



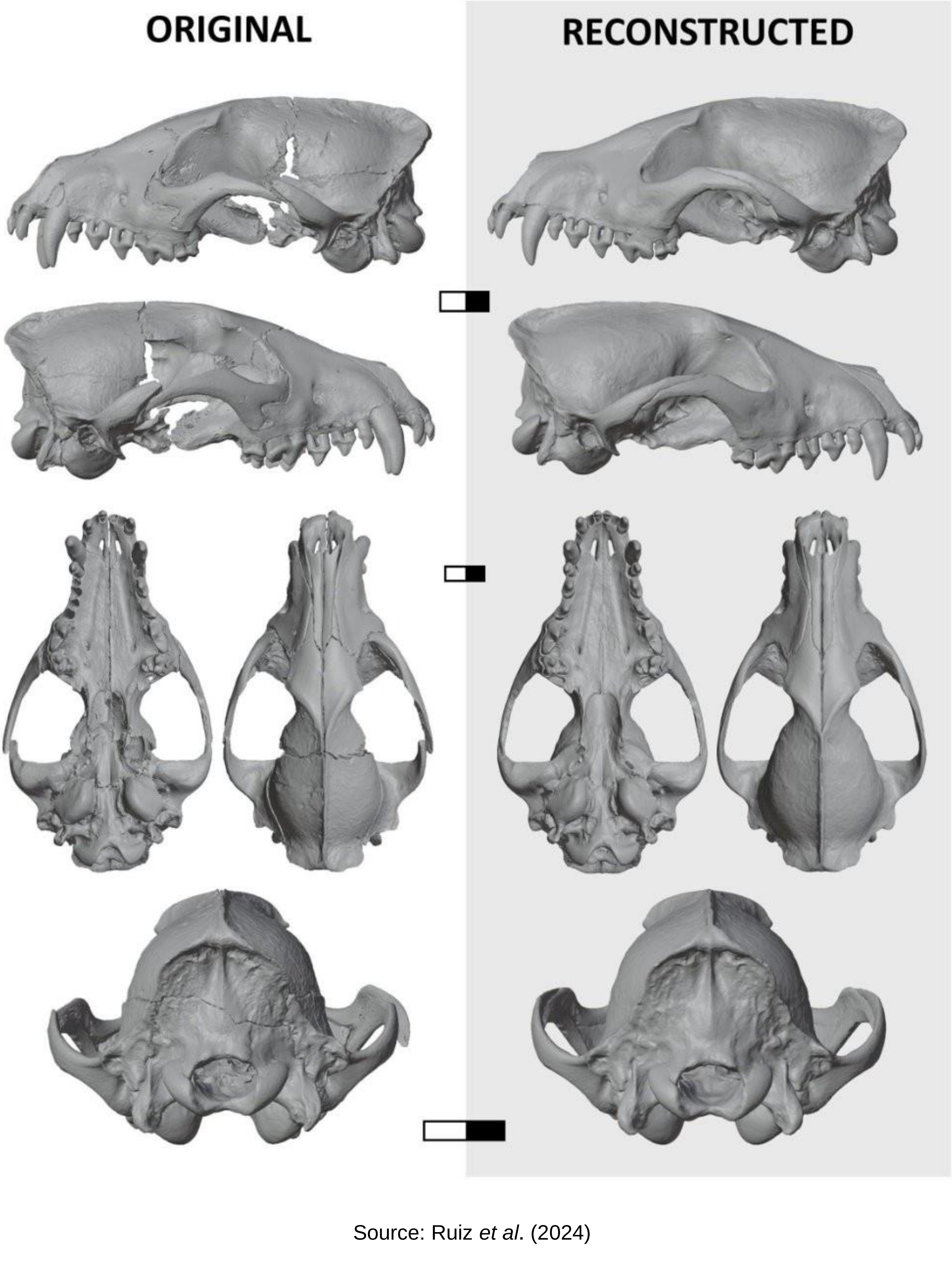
Source: Ruiz *et al*. (2024)

Figure 4.6. Isolated teeth of *Speothos pacivorus*. Specimen numbers are assigned next to each material, when present. Scale bar equals 2 cm.



Source: Ruiz *et al*. (2024)

Figure 4.7. Comparison between the original (left) and reconstructed (right) cranium of *Speothos pacivorus* NHMD:211341. Scale bars equal 2 cm.



Source: Ruiz *et al*. (2024)

4.4. Discussion

The presented 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 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 bidimensional morphometrics analyses. The three-dimensional model made available 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. Is to be expected 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.

4.5. References

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4.6. Appendix 4.1 – Supplementary table

Table A.4.1. 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.

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

(continue)

(continuation)

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

(continue)

(conclusion)

No number	-	Canine
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

Source: Ruiz *et al*. 2024

5. FINAL CONSIDERATIONS

The taxonomical history of *Speothos* is a remarkable case of which a new taxon (in this case, a genus) is erected after an extinct species, *S. pacivorus*, and a living species, *S. venaticus*, is described only later, a variation of the “Lazarus effect” (FLESSA; JABLONSKI, 1983). This is only the tip of the uniqueness of these peculiar canids. As discussed in the previous chapters, both species present a high degree of specialization to a hypercarnivorous diet compared to any other living canid, reflecting on peculiar morphological and, for the bush dog, known behaviours. They are, however, understudied, the bush dog by the difficulty of conduct research on the field and the cave jackal, for its only remains being housed in a collection outside South America.

Considering these difficulties, this thesis was heavily based on *in silico* approaches to test hypotheses related to the biology of both bush dog and cave jackals that are, otherwise, difficult or impossible to test. The comparative investigation of bush dog, grey wolf, and grey fox in **Chapter 1** showed that bush dogs have a very robust skull, consistent with its hunting strategies and usual prey captured. Also, it presents a strong bite force for a canid of its size, allowing it to a better grip on its prey when hunting in packs, facilitating the it disembowelling and the consumption of hard tissues, such as armadillo’s armour. These results also indicates that extraordinary sights of bush dogs preying larger prey than habitual, as capybaras, peccaries, and even tapirs, usually based on anecdotal accounts instead of well-based observations, are plausible. Finally, the results of this chapter highlighted the potentials of the application of finite element analysis (FEA) to infer the predatory behaviour of taxa which are difficult to study or extinct.

Bearing this in mind, similar tests were conducted with the cave jackal, as seen in **Chapter 2**. In this study, by using geometric morphometrics analysis based on cranial landmarks, it was possible to reinforce that both *Speothos* species are distinct taxonomical entities, contrary to what some researchers inferred (PREVOSTI; FORASIEPI, 2018). The FEA results, by the other side, recovered a strong similarity between the crania of cave jackals and bush dogs in all tested scenarios. Thus, considering the sympatry of three hypercarnivorous canids (i.e., both *Speothos* species and *Protocyon troglodytes*) in the Late Pleistocene of the Brazilian Intertropical

Region, a possible explanation to the co-occurrence of these forms can be related to specialization of different prey sizes, avoiding an extended overlapping on resources usage. This trend leaded to the small size of the bush dog; after the demise of the megaherbivores in the Pleistocene-Holocene boundary and the extinction of the large mammal hunters, *Protocyon* and the cave jackal, the rodent-specialized bush dog was the only species able to survive to this day, explaining also why it is the only species of extant hypercarnivorous canid with a small size. This hypothesis is also, strengthened by the results of chapter 1, which reinforced the bush dog as a predator of medium to large rodents and occasional hunter of larger mammals.

The Brazilian zoology and palaeontology are historically important for the study of *Speothos* since both species were formally described in Brazil, where the only fossils of bush dogs and cave jackals were found. Although all their fossil remains were found in the Lagoa Santa karst, in central Minas Gerais, Brazil, they are currently housed in the Peter Lund/Quaternary Collection at the Natural History Museum of Denmark, which can potentially difficult its study by researchers focused on the evolution of Cerdocyonina, usually based in South America. To facilitate the virtual access of the remains of the cave jackal, **Chapter 3** presents all its known fossils, listed and illustrated, as well as a complete reconstruction of the holotype, a cranium.

Combined, it is expected that the information provided in the three chapters of this thesis could contribute to our understanding of the evolutionary history of one of the most unusual clades of Carnivora, fostering other studies based on the original data presented and the methodologies developed. One of the most important disciplines that could benefit from the data generated here is conservation biology. The bush dog populations are decreasing (DE MATTEO; MICHALSKI; LEITE-PITIMAN, 2011); there is so little data on its distribution patterns and other ecological aspects that some authors suggest a high priority for its conservation assessment (HURTADO *et al.* 2016). Additionally, due to their ecological interactions as top predators, the protection of bush dogs could be useful in broader, ecosystemic scales (SERGIO *et al.* 2008). Finally, being the last member of a once highly diverse and specialized lineage of Cerdocyonia enhances the urge to protect this species.

In conclusion, the robustness of the data derived from this thesis, considering its multidisciplinary approach, distinct methodologies applied, and insights on ecological topics from both palaeontological and neontological perspectives, can assist conservation considerations for the bush dog. This thesis presents unprecedented quantitative data on its predatory behaviour and also reinforces the evolutionary history of *Speothos* as one of the most unusual among the predators of the tropical Americas.

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