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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

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

São José do Rio Preto
2024

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Financiadora: CAPES
FAPESP – Processos
2020/12786-2 e 2020/07997-4

Orientador: Prof. Dr. Felipe Chinaglia Montefeltro
Coorientadora: Prof.ª Dr.ª Mariela C. de Castro

São José do Rio Preto
2024

R934n Ruiz, Juan Vitor
A new look on *Speothos* Lund, 1839: investigating the evolutionary history of bush dogs and cave jackals through morphofunctional and quantitative approaches / Juan Vitor Ruiz. -- São José do Rio Preto, 2024
129 f. : il., tabs., fotos, mapas

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto
Orientador: Felipe Chinaglia Montefeltro
Coorientadora: Mariela Cordeiro Castro

1. Canidae. 2. biomecânica. 3. método dos elementos finitos. 4. paleoecologia. 5. zoologia classificação. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Universidade Estadual Paulista (UNESP), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto. Dados fornecidos pelo autor(a).

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2020/12786-2 e 2020/07997-4

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AGRADECIMENTOS

Como todo texto científico, as páginas a seguir são apáticas e insensíveis; logo, a única oportunidade de gerar uma mínima demonstração de emoção (um sorrisinho, talvez até mesmo um olho molhado) seria aqui, nos agradecimentos. Vamos lá.

O que seria de um aluno sem seus orientadores? Felipe Montefeltro, sempre presente, não apenas cumpriu sua função de orientador *de facto*, oferecendo todos os meios que me permitiram crescer como pesquisador, como também se mostrou um colega e um amigo em vários momentos nos últimos quase 10 anos. Ainda que essa seja a etapa final de nossa parceria como orientador-orientando, espero continuar sendo seu aprendiz no futuro.

Regarding supervisors, a special thanks to Madelaine Böhme, who received me at her study group at the Senckenberg/University of Tübingen during my nine months of German life.

Coorientadores dão aquela ajuda necessária quando o seu orientador está ocupado demais ~~provavelmente limpando aquários~~. E eu que tive a sorte de não ter um, mas três coorientadores? Um agradecimento à Mariela Castro, a “coorientadora oficial”, a quem compartilho quase que exclusivamente um interesse sobre mamíferos em meio a uma panela arrogante e centrada em répteis, credo. Ao Fabio “Haeckeliano” Machado, o cara dos canídeos, cujo conhecimento geral e experiência em morfometria ajudou a elevar o nível do que é apresentado no Capítulo 2, abrindo caminho, eu espero, para projetinhos futuros. E, finalmente, um agradecimento sem tamanho ao Gabriel Ferreira, que não apenas me recebeu em Tübingen como, ao compartilhar suas ideias, me ajudou a entender o que significa ser um cientista. Além disso, se fosse comentar todas as vezes em que me tirou de apuros, essa seção ia ter umas 30 páginas.

Agradeço também às inúmeras pessoas que me receberam e compartilharam espécimes depositados em museus e instituições de pesquisa, incluindo Joyce Prado, Fabio Nascimento, Juliana Gualda e Ismael Jesus (Coleção de Mamíferos, MZUSP), Erich Weber e Jürgen Rösinger (Zoological Colection, University of Tübingen), Stefan Merker (Section Mammalogy, State Museum of Natural History Stuttgart); Irina Ruf (Section Mammalogy, Senckenberg Frankfurt); Roberto Portela Miguez (Mammal Collection, Natural History Museum London); e Kasper Hansen (Quaternary Collection, Natural History Museum of Denmark). To the researchers who made their data available online, something crucial to a young Brazilian researcher. Special thanks to Narimane Chatar and Anne-Claire Fabre, who shared models (*a lot* of models) and knowledge.

Aos meus amigos e parceiros “da faculdade pra vida”, Marcos “Pelanza”, Aymar, Paulo “The Enguia”, Jaurés “Fifi” e Ivo, por quem, não importa a distância, o carinho e apreço se mantiveram ao longo de nossas vidas. Aos colegas do LAPEISA, “Menino” Kawan, Alice, aos Gabriéis Gonzalez e David, aos Paulos “Tavinho” e Roberto, ao Arthur e ao Giovane Cidade, por fazerem me sentir num laboratório de paleontologia *de verdade*. To the study group in Tübingen, to Tobias Massonne, who helped me with my carnivoran samplings, and especially to Christina Kyriakouli, whose wizardly skills in everything computer-related resulted in the model that was crucial to the analyses of Chapters 2 and 3. À Simone Neves, cujos conselhos e dicas valiosíssimos me auxiliaram a chegar à Dinamarca. Ao Zé, que me ajudou a superar minhas dificuldades básicas com o inglês, e ao Tiago Pezzuti, que chegou quase que no finzinho do doutorado, mas já se mostrou um parceiro e amigo (e por ter me introduzido ao VERDADEIRO pão de queijo). À Julia d’Oliveira, que fez o retrato definitivo do chacal-das-cavernas (finalmente temos um!). E ao pessoal do Paleolab de Ribeirão & agregados, sobretudo ao Gustavo Darlim, Mario Bronzati e Pedro “Tomate”, por ajudarem a me desenvolver academicamente ao longo desses anos e servirem de exemplos profissionais. Um agradecimento especial ao Donatinho, pela camaradagem em terras europeias e por mostrar que não sou (o único) doido por gostar de bonequinhos colecionáveis de bicho.

Essa tese se desenrolou num dos períodos mais difíceis e quase surreais dos últimos tempos: o Brasil pós-2020. Devo agradecer às UNESPs de São José do Rio Preto e de Ilha Solteira e, sobretudo, ao PPG Biodiversidade, por jamais deixar de apoiar seus alunos, não importa se o mundo estivesse desabando ao nosso redor. Agradeço também aos professores Lilian Casatti e Francisco Langeani, membros da banca de qualificação e que sempre se mostraram prestativos a qualquer dúvida ou necessidade que possam ter surgidos. Agradeço também os membros da banca de defesa, Rafaela Missagia, Julio Dalponte, Simone Neves e Fabio Nascimento, por seu tempo e atenção.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001 (processos PROEX 88887.486529/2020-00, PROEX

88887.817849/2022-00, CAPES-DAAD 88881.650314/2021-01). Essa tese também foi financiada em parte pela FAPESP (processos 2020/12786-2 e 2020/07997-4).

Gostaria de agradecer aos meus primos queridos, Denise e Rafael, por terem me recebido tão bem em Londres. O mesmo agradecimento vale à Marina, Leandro, Margotzinha e Cookie. Foi uma ocasião especial ao qual sempre vou lembrar com carinho e conforto. Obrigado.

Aos meus “familiares postiços”, Maria Luzia, Alaércio e Heitour, por me acolherem como filho/irmão, o que de fato acabei me tornando. E aos meus “familiares reais”, meus irmãos Diego e Sofia, por sempre me alegrarem durante minhas visitas ao meu lar de criação, que nunca deixará de ser um dos meus cantinhos no mundo. Ao Lineu Aparecido, infame Lineuzinho, o pequeno quadrúpede que nos ajudou a passar boa parte da pandemia com mais leveza e otimismo, dentro do possível. E aos meus pais, Nira e Ricardo, por terem sempre me incentivado e acreditado, seja estando próximos, seja estando distantes.

Por fim, à Helo, minha amiga, namorada, companheira, parceira e agora esposa, por ter me acompanhado por todo esse período e além, sempre dando suporte, conselhos sábios, palpites especulativos quase mágicos e que sempre se concretizaram, petelecos necessários e todas as risadas que me permitiram manter a sanidade em vários momentos. E pela quase infinita paciência. E por ser meu lar. Obrigado por tudo.

As the surest sign that I was growing up a bit, I now had my PhD (…)
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

SUMMARY

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

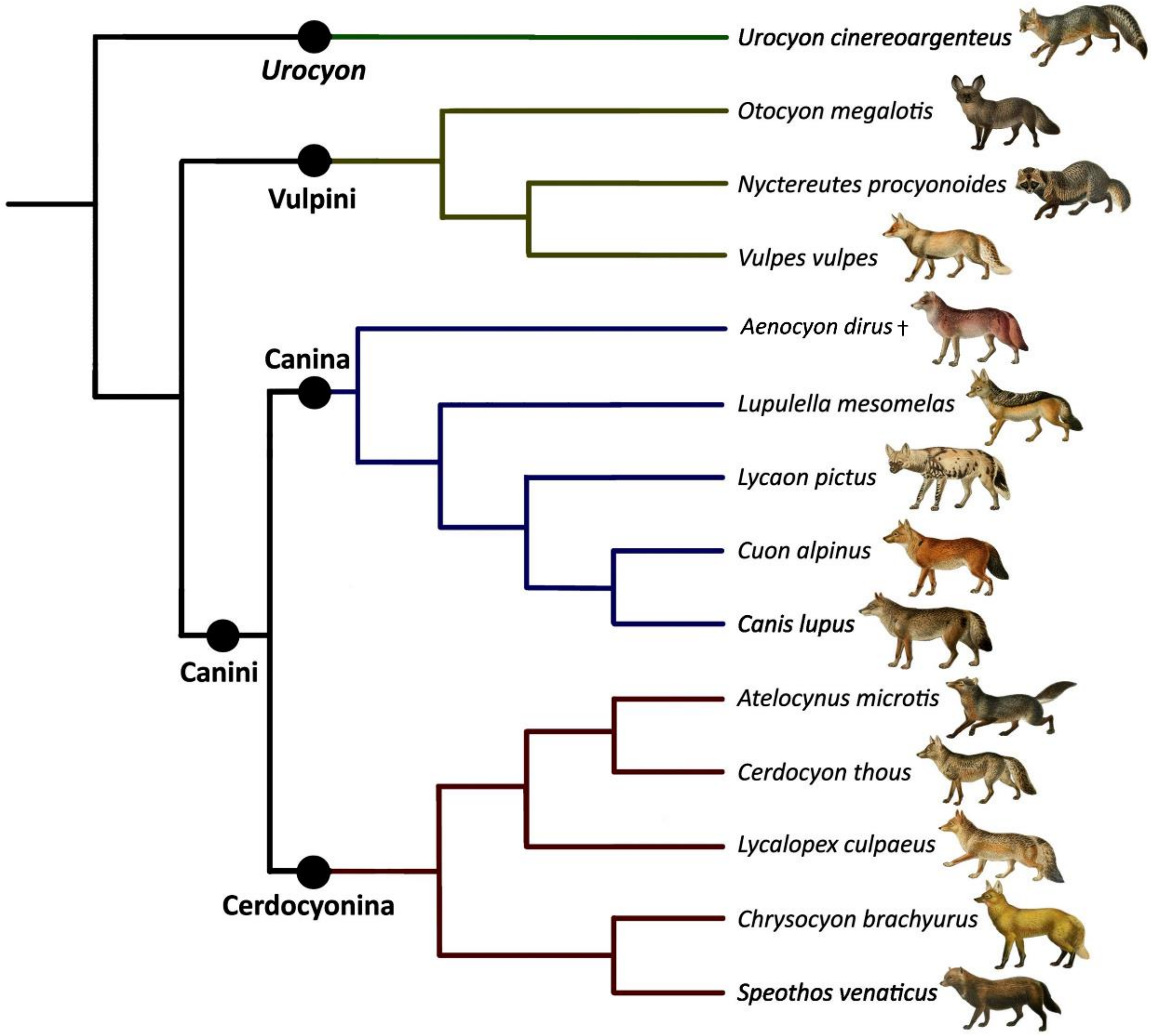
For most of the Cenozoic (66 m.y. to present), South American was an island continent, with sporadic and brief contact with other landmasses, especially Antarctica (YANBIN, 1998; GOIN *et al*. 2016; HOFFMEISTER, 2020). Therefore, the terrestrial fauna evolved into a unique assembly found nowhere else 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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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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