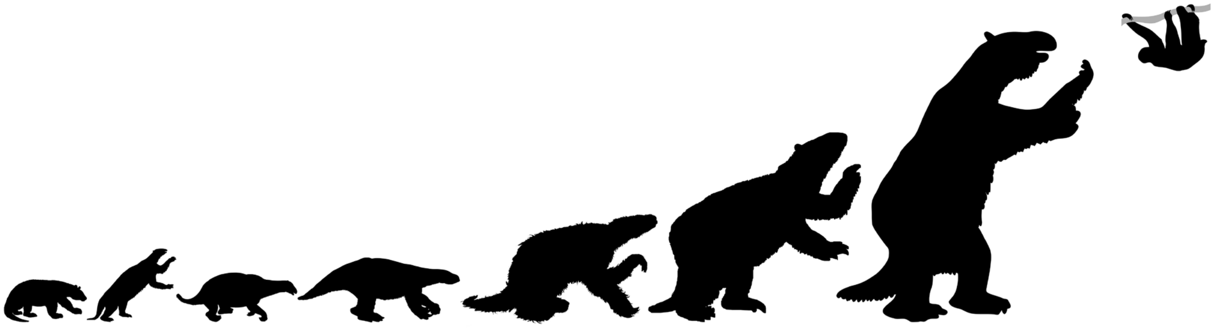
Paleobiología de xenartros fósiles gigantes: enfoques macroevolutivos y paleoecológicos



Tesis de Doctorado

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Resumen

La presente tesis aborda el estudio de los miembros extintos del superorden Xenarthra, en particular aquellos que alcanzaron grandes tamaños. La tesis surge de la combinación de diferentes enfoques centrados en el estudio de aspectos paleobiológicos de estos animales gigantes con el fin de mejorar el conocimiento actual sobre sus preferencias dietarias y climáticas, la relación entre la forma y la función en el complejo cráneo-mandíbula, así como también aspectos relacionados al desarrollo de la dentición y la capacidad aeróbica de las formas gigantes. En esa línea, se presentan cinco trabajos, de los cuales cuatro se encuentran publicados y uno se encuentra en revisión. Los principales resultados de la tesis permiten mejorar el entendimiento sobre el rol de estos megamamíferos en los ecosistemas pasados. Por medio de modelos de elementos finitos y morfología geométrica se obtuvo evidencia sobre la existencia de adaptaciones a diferentes dietas en perezosos. El estudio de la dentición en perezosos permitió reconocer un patrón macroevolutivo presente en otros clados conocido como cascada inhibitoria. Los resultados mostraron la existencia de procesos de activación y inhibición durante el desarrollo de la dentición similares a los existentes en otros grupos pese a las particularidades existentes en la dentición de los perezosos. Por otro lado, el estudio de fósiles provenientes del sitio Arroyo del Vizcaíno en el sur de Uruguay permitió reconocer un taxón previamente no registrado en Uruguay y explorar aspectos paleoecológicos de los xenartros gigantes en el contexto de una comunidad de megamamíferos típica del Pleistoceno final de la región. Por último, el estudio de los forámenes nutritivos en fémures permitió obtener evidencia en favor de una alta capacidad aeróbica en las formas gigantes, comparable a otros mamíferos placentarios de gran tamaño y no a los xenartros actuales.

Abstract

The present thesis addresses the study of extinct members of the superorder Xenarthra, particularly those that reached large sizes. The thesis arises from the combination of different approaches focused on the study of paleobiological aspects of these giant animals in order to enhance current knowledge about their dietary and climatic preferences, the relationship between form and function in the skull-jaw system, as well as aspects related to the development of dentition and the aerobic capacity of giant forms. In this line, five papers are presented, four of which are published, and one is under review. The main results of the thesis improve the understanding of the role of these megamammals in past ecosystems. Through finite element models and geometric morphology, evidence was obtained for adaptations to different diets in sloths. The study of dentition in sloths allowed the recognition of a macroevolutionary pattern present in other clades known as inhibitory cascade. The results showed the existence of activation and inhibition processes during dentition development, similar to those in other groups, despite the peculiarities in sloth dentition. On the other hand, the study of fossils from the Arroyo del Vizcaíno site in southern Uruguay allowed the recognition of a taxon previously unrecorded in Uruguay and the exploration of paleoecological aspects of giant xenarthrans in the context of a typical megamammal community from the late Pleistocene of the region. Finally, the study of nutrient foramina in femurs provided evidence in favor of a high aerobic capacity in giant forms, comparable to other large placental mammals and not to current xenarthrans.

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Introducción

Los xenartros son un superorden dentro de la clase Mammalia que representa uno de los cuatro grandes clados de mamíferos placentarios. Su ubicación en el árbol de los mamíferos ha sido motivo de debate a lo largo de muchos años. Inicialmente fueron agrupados con otros mamíferos placentarios como los pangolines (orden Pholidota) y los cerdos hormigueros (orden Tubulidentata), dentro de un grupo conocido como Edentata, principalmente por las peculiares denticiones que estos taxones presentan (Cuvier 1798). Más recientemente se los ha reconocido como un clado, aunque su grado de parentesco con otros mamíferos placentarios ha sido difícil de determinar. En este aspecto, existen básicamente tres posibles configuraciones respecto a la ubicación de la divergencia que dio origen a los xenartros, otorgando a dicha divergencia posiciones más o menos cercanas a la raíz del árbol filogenético de los mamíferos placentarios (Fig. 1). Entre estas posibilidades, las evidencias más recientes tienden a ubicar a los xenartros como uno de los grandes grupos de mamíferos placentarios, siendo éstos el grupo hermano de los Afrotheria, formando así el magnorden

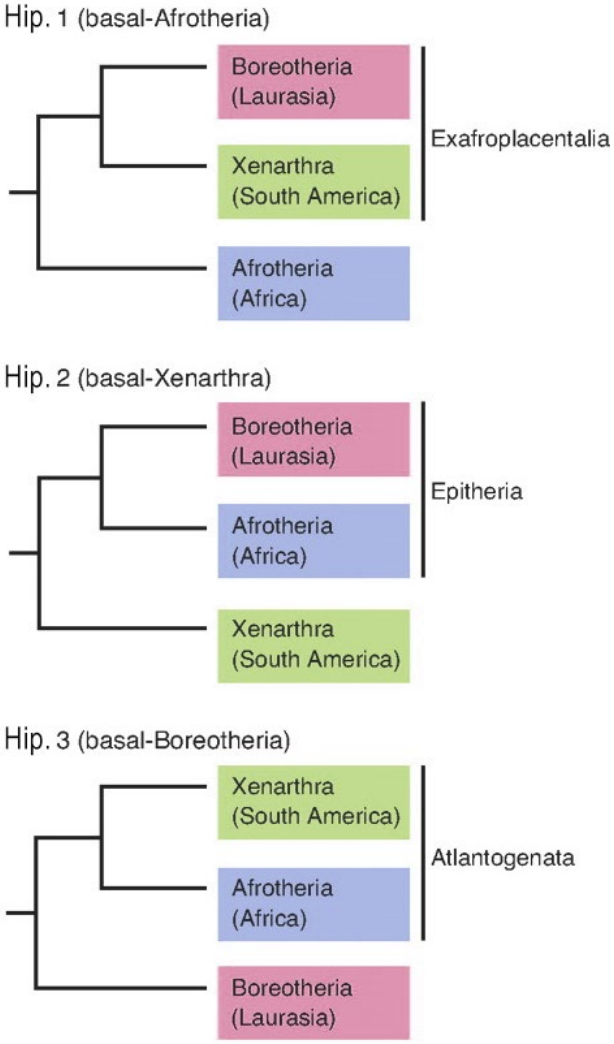

Figura 1. Hipótesis alternativas sobre las relaciones filogenéticas entre los tres grandes clados de mamíferos placentarios. Modificado de Nishihara et al. (2009).

Atlantogenata (Morgan et al. 2013). Independientemente de su posición exacta en el árbol de los mamíferos, los xenartros indudablemente tienen su origen en una de las divergencias más antiguas dentro de los mamíferos placentarios, haciéndolos sumamente importantes para la comprensión de la evolución de los mamíferos (Svartman et al. 2006).

Los xenartros se dividen en dos grupos, el orden Cingulata (armadillos) y el orden Pilosa, el cual se divide en Vermilingua (osos hormigueros) y Folivora (perezosos). La diversidad actual de los xenartros es considerablemente baja si se compara con la de otros grupos de mamíferos, reconociéndose aproximadamente 31 especies (Superina y Loughry 2015). Entre estas especies, los armadillos representan la mayor diversidad taxonómica, mientras que los osos hormigueros y los perezosos se reúnen en solo tres y dos géneros, respectivamente. Si bien todos los xenartros comparten ciertas características sinapomórficas como articulaciones extra en sus vértebras o la ausencia de esmalte en sus dientes, entre los integrantes del grupo también existen grandes diferencias morfológicas. Por ejemplo, los perezosos son conocidos por su movimiento extremadamente lento y sus hábitos arbóreos, los cuales fueron adquiridos de forma independiente por los dos géneros vivientes y representan un caso de convergencia evolutiva excepcional (Nyakatura 2012; Delsuc et al. 2019). Por otro lado, los osos hormigueros y los armadillos muestran adaptaciones a hábitos excavadores y dietas basadas en insectos y pequeños animales, con muchos taxones considerados como fosoriales (Superina y Abba 2020).

A diferencia de su reducida diversidad actual, los xenartros representan uno de los grupos de mamíferos sudamericanos más importantes en el registro fósil, con representantes en todo el continente e incluso en América del Norte y el Caribe (Croft 2016). Esta diversidad en el registro fósil no sólo se evidencia en la gran cantidad de especies reconocidas, sino también en la gran disparidad morfológica alcanzada por estos animales. La gran diversidad existente en el registro fósil en comparación con el número de especies actuales, sumado a las importantes diferencias morfológicas entre muchos taxones extintos y sus parientes actuales, ha resultado en diversas hipótesis sobre las relaciones filogenéticas entre los xenartros actuales y fósiles, en particular dentro de los diferentes órdenes. A su vez, los avances recientes en la obtención de datos moleculares en taxones extintos han generado nueva evidencia que en muchos casos contrasta con las hipótesis más sustentadas en base a datos morfológicos.

En el caso de los cingulados, clásicamente los gliptodontes y pampatérios han sido considerados dentro de la superfamilia Glyptodontoidea mientras que los armadillos actuales y extintos se han agrupado dentro de la superfamilia Dasypodoidea (McKenna y Bell 1997). Por otro lado, más recientemente algunos estudios han sustentado su identidad como grupos monofiléticos en base a información morfológica, pero los han ubicado cercanamente emparentados con la subfamilia de armadillos Euphractinae (Gaudin y Wible 2006; Billet et al. 2011). En los últimos años, en base a datos moleculares, se ha propuesto que los gliptodontes representan un clado dentro de la familia Chlamyphoridae, en gran parte compatibles con las hipótesis morfológicas más recientes (Delsuc et al. 2016), contrastando claramente con las hipótesis clásicas en base a datos morfológicos. En relación a sus características morfológicas, los gliptodontes muestran una marcada divergencia respecto al morfotipo general de los armadillos, con una clara tendencia al gigantismo durante su historia evolutiva y portando una coraza rígida sin bandas móviles y con un alto grado de fusión a nivel del esqueleto axial (Fariña et al. 2013).

Por otro lado, los perezosos extintos muestran un gran rango de formas y tamaños, en algunos casos mostrando cierta semejanza con los taxones vivientes, como en el caso de los perezosos de las Antillas (White y MacPhee 2001), pero también existiendo representantes gigantes de distintas familias como Mylodontidae y Megatheriidae, que probablemente alcanzaban más de 3000 kg (Fariña et al. 2013; Varela et al. 2019), e incluso con especies adaptadas a la vida acuática (Amson et al. 2014). Las grandes diferencias morfológicas entre las pocas especies actuales y muchas de las especies fósiles, así como la ausencia total de restos fósiles con afinidades claras con las especies actuales, han resultado en una gran dificultad para establecer relaciones filogenéticas confiables en el grupo. En la actualidad, la mayor cantidad de estudios basados en caracteres morfológicos coinciden en la existencia de dos grandes clados, Megatherioidea (incluyendo las familias Megatheriidae, Nothrotheriidae y Megalonychidae) y Mylodontoidea (incluyendo las familias Orophodontidae, Scelidotheriidae y Mylodontidae), los cuales comprenden prácticamente la totalidad de la diversidad fósil del grupo y a uno de los dos géneros actuales (*Choloepus*, dentro de la familia Megalonychidae), mientras que el otro género viviente (*Bradypus*, único representante de la familia Bradypodidae) representaría un grupo hermano a todos los perezosos (Gaudin 2004; Varela et al. 2019; Casali et al. 2022). Por otro lado, dos estudios recientes basados en información molecular obtenida de taxones fósiles mostraron un panorama considerablemente diferente al obtenido a partir de información morfológica, con una configuración en la cual *Choloepus* se encuentra dentro de Mylodontoidea y *Bradypus* dentro de Megatherioidea, mientras que los perezosos de las Antillas formarían un grupo hermano a todos los perezosos denominado Megalocnoidea (elevando el número de familias a al menos ocho; Delsuc et al. 2019; Presslee et al. 2019).

Si bien existen xenartros fósiles de gran tamaño en distintos momentos del registro geológico, la mayor diversidad taxonómica y los mayores tamaños corporales se observan durante el

Pleistoceno, con más de 10 géneros con tamaños cercanos o por arriba de la tonelada ocurriendo en regiones América del Sur como las Pampas (Fariña et al. 2013; Toledo et al. 2015). Es indudable que estos animales eran componentes importantes en los ecosistemas del Pleistoceno, donde cumplían funciones relevantes relacionadas, entre otras, con la conectividad entre regiones (Berti y Svenning 2020), la dispersión de semillas (Pires et al. 2018) y el movimiento de nutrientes en el suelo (Doughty et al. 2013). Más allá de su gran éxito evolutivo si se considera el registro fósil del Cenozoico, todos los xenartros gigantes se extinguieron al final del Pleistoceno (hace unos 11700 años), en un evento de extinción masivo en el cual prácticamente todas las especies de gran tamaño desaparecieron y que fue determinante para la composición de la fauna actual del continente (Fariña et al. 2013).

Por estos motivos, resulta de suma importancia no sólo comprender los motivos detrás de la extinción de estos animales (muchas veces relacionados a los cambios climáticos al final del Pleistoceno y/o a la llegada de los primeros humanos modernos al continente, Barnosky et al. 2004), sino también diversos aspectos paleobiológicos y paleoecológicos que permitan comprender mejor los procesos por los cuales evolucionaron estas formas y los roles que cumplían en los ecosistemas. Estos aspectos son aún de mayor importancia si se considera el contexto de cambio global y que las especies de gran tamaño se encuentran desproporcionadamente en mayor peligro de extinción en la actualidad (Smith et al. 2018). En última instancia, el estudio de los xenartros extintos es crucial para comprender y valorar los pocos representantes actuales del clado, así como también generar conciencia sobre su importancia y conservación (Vizcaíno y Bargo 2014; Superina y Loughry 2015).

Los xenartros fósiles han sido motivo de gran diversidad de estudios durante al menos los últimos 150 años. Los primeros hallazgos de xenartros extintos y su estudio sistemático se remontan a el final del Siglo XVIII, con la descripción de un esqueleto de *Megatherium americanum* por parte de Cuvier en 1796, el cual había sido excavado en Luján, Argentina años antes y enviado a España (Pasquali y Tonni 2008). Los xenartros fósiles también fueron motivo de estudio a partir de hallazgos durante las expediciones de Charles Darwin en la región del Río de la Plata, donde además tuvieron un rol importante en el desarrollo de las ideas del naturalista sobre los procesos evolutivos (Vizcaíno et al. 2009). Si bien los estudios más abundantes tienen como objetivo la mejor comprensión de la sistemática del grupo, durante los últimos 50 años se han incrementado los enfoques paleobiológicos sobre estos animales. En este contexto, es importante destacar los esfuerzos enfocados en la relación entre la forma y la función, los cuales han permitido obtener valiosa información sobre diversos aspectos de estos animales. A modo de ejemplo, la relación entre forma y función observada en el complejo cráneo-mandíbula ha permitido la generación de ciertas hipótesis en relación con las dietas de muchas especies fósiles de gliptodontes y perezosos, mostrando evidencia en favor de dietas diferentes a las observadas en los taxones actuales para muchas especies fósiles (Bargo 2003). Este tipo de estudios han sido fundamentales para avanzar en una mejor comprensión de los xenartros extintos, evidenciando que las comparaciones directas con sus parientes actuales no necesariamente aportan información fiable de estos animales extintos (Vizcaíno et al. 2018).

Objetivos y Estructura de la Tesis

En la presente tesis se pretende profundizar el conocimiento en diversos aspectos paleobiológicos de los xenartros fósiles a partir de la combinación de diferentes enfoques que contemplen la forma y la función, así como también la estimación de importantes aspectos del desarrollo, paleoecológicos y fisiológicos desde una perspectiva macroevolutiva. Como objetivo principal se plantea determinar la dieta como un aspecto clave de la paleobiología de los xenartros fósiles gigantes. En particular, se pretende abordar la temática por medio del desarrollo de distintos aspectos ligados al desarrollo y sus cambios evolutivos, a la relación entre forma y función desde un punto de vista biomecánico y a las preferencias dietarias de diferentes xenartros fósiles en el contexto de una comunidad de megamamíferos del Pleistoceno tardío por medio de análisis de isótopos estables. De forma adicional, se describe el hallazgo en el sur de Uruguay de un fósil asignable a un género de perezosos comúnmente hallado en América del Norte y se discuten sus implicancias biogeográficas y paleoecológicas. Por último, se realiza un análisis centrado en la estimación de la capacidad aeróbica de los xenartros fósiles gigantes a partir del estudio de los forámenes nutritivos en fémures con el fin de profundizar en los requerimientos metabólicos de estos animales.

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UNEXPECTED INHIBITORY CASCADE IN THE MOLARIFORMS OF SLOTHS (FOLIVORA, XENARTHRA): A CASE STUDY IN XENARTHRANS HONOURING GERHARD STORCH'S OPEN-MINDEDNESS

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Abstract: The inhibitory cascade (IC) represents a developmental model that explains the evolution of molar relative sizes, originally described in rodents but later validated in several mammalian groups. The IC comprises signalling molecules produced by the first molar buds that inhibit the development of subsequent molars and molecules from surrounding tissues that have opposite effects. Sloths, as xenarthrans, present many peculiarities in their dentition, like tooth and enamel loss, homodonty, and changes in the typically mammalian dental formula. Here, we test the existence of an IC and explore the evolution of the lower dentition in sloths. We studied the variability of molariform proportions in 20 specimens of the Late Pleistocene ground sloth *Lestodon armatus*. We also analysed molariforms proportions in 53 sloth genera to explore evolutionary trends. Our results show that the lower dentition of most sloths complies with the IC model, despite the difficulties of assessing dental homologies with other mammals. Furthermore, we tested the existence of different patterns among families, obtaining support for models taking mylodontids and orophodontids separately from the rest of sloths. Also, members of Mylodontidae show a unique IC pattern, with a slope considerably higher than 2 and an $mf1 \leq mf2 << mf3$ configuration. This pattern could be related to the morphological adaptations to grazing showed by mylodontids during most of their evolutionary history.

Key words: sloths, inhibitory cascade, macroevolution, tooth evolution, molariforms

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Introduction

One of us (RAF) had the pleasure to meet Gerhard Storch in connection with the preparation of the proceedings of the Xenarthra meeting held in Jena in 2001, published as a special issue of Senckenbergiana biologica (Fariña et al. 2003). Here we intend to honour his wide legacy that dealt with taxonomy, palaeobiology, biochronology, and palaeobiogeography of mainly small mammals in which he made relevant contributions (Franzen et al. 2018). Among the several Palaeogene taxa Storch described, an astonishing finding of a complete skeleton of a clearly ant-eating mammal in Eocene sediments from a pit not far from the city of Frankfurt am Main, led Storch (1981) to create the genus *Eurotamandua*. Originally, as the name implies, considered as the first myrmecophagid (Xenarthra) outside of South America, it was later classified as a basal member of Afrotheria (see Hunter and Janis 2006), and finally as a pholidotan without scales (Gaudin et al. 2009).

Xenarthrans are a group of mammals that include small to medium size armadillos, anteaters, and tree sloths as living species. However, their diversity in the fossil record is astonishing, including many glyptodonts and ground sloths (some of gigantic size; see Fariña et al. 1998, Christiansen and Fariña 2003) as completely extinct forms. Xenarthrans originated in South America during the early Cenozoic and, following a long-term diversification in that subcontinent, they spread into Central and North America following the Great American Biotic Interchange (Fariña et al. 2013). Prior to the extinctions near the Pleistocene-Holocene limit, the group reached great taxonomic and morphological diversity (Varela et al. 2018). Folivora (see Fariña and Vizcaíno 2003), the group that includes both living and extinct sloths, is currently represented by only two genera, the obligatory

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arboreal sloths *Bradypus* and *Choloepus*. However, as mentioned before, the fossil history of this clade was much more diverse along the Cenozoic, including about 90 genera (McKenna and Bell 1997), a number continuously growing with new findings in less-explored parts of the Americas (e.g., Pujos et al. 2012).

Xenarthrans differ from the rest of the mammalian clades by the individual morphology and number of teeth (McDonald 2003). When present, teeth in most adult xenarthrans lack enamel and are usually homodont, hypselodont, tubular, and primarily composed of orthodentine and vasodentine, which makes it difficult to identify homologies with the teeth and cusps of other mammals (Vizcaíno 2009, Hautier et al. 2016). In sloths, the dentition is reduced to a maximum of five upper and four lower teeth, with caniniforms (cf) present in megalonychids and some nothrotheriids, megatheriids, and mylodontids (Vizcaíno 2009). Hautier et al. (2016) studied the prenatal dental ontogeny of extant sloths, showing that the upper caniniforms are not homologous in both genera and their lower caniniforms are not homologous to the lower canines of other mammals. Furthermore, based on the timing of mineralizations of each tooth, they showed that the lower molariforms (mf) of both sloths are probably homologous to the dp3, dp4, and m1 of the rest of mammals. These results depict the complex evolution of the dentition in these two distantly related sloths (Gaudin 2004, Varela et al. 2018, Delsuc et al. 2019, Presslee et al. 2019) as well as in Folivora in general, which highlights the difficulties of defining dental homologies in extant and extinct sloths.

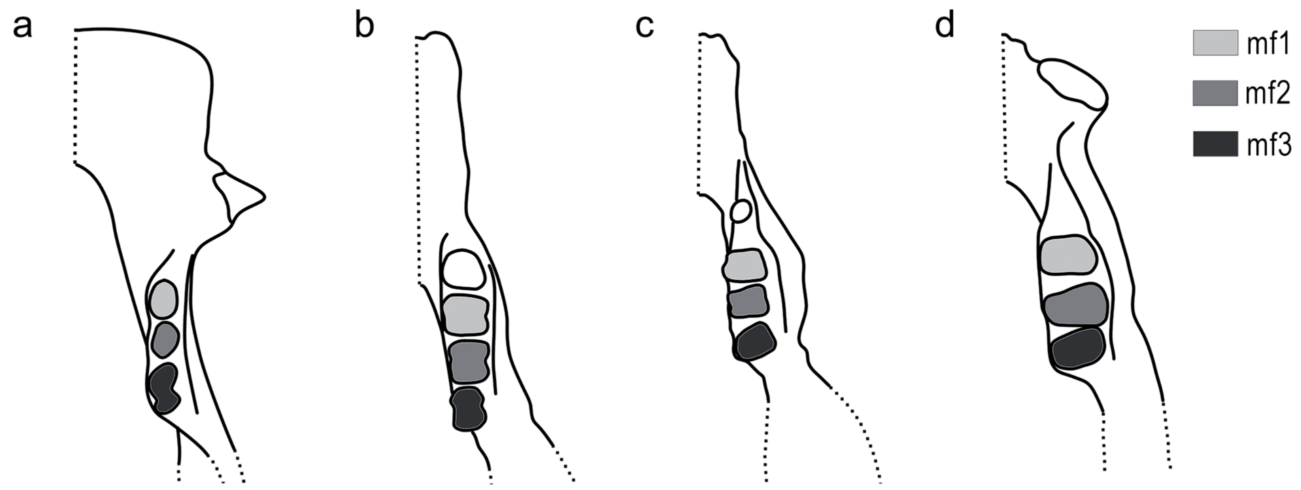
The evolutionary developmental (evo-devo) field consists of the integration of evolutionary, adaptational, and developmental approaches to explore the mechanistic relationships between the processes of individual development and phenotypic change during evolution (Müller 2007). The mammalian dentition represents a great system for the study of macroevolutionary patterns and the link between phenotypic variation, development, and evolutionary processes in deep time, allowing the inclusion of fossil taxa many times largely known by their tooth remains. Some years ago, Kavanagh et al. (2007) established a developmental model that can explain the evolution of the relative sizes of lower molars in murine rodents. The inhibitory cascade model (IC model) showed that signalling molecules produced by the developing first molar buds inhibited the development of subsequent molars, while molecules from the surrounding tissues had the opposite effect. Consequently, this balance between inhibition and activation during molar development results in a predictable relationship between molar sizes, with phenotypes (and their change across taxa and time) constrained to fall within expected patterns, namely m1 > m2 > m3, m1 ≈ m2 ≈ m3, or m1 < m2 < m3. The IC model has been studied in several extant (Kavanagh et al. 2007, Polly 2007, Labonne et al. 2012, Asahara 2013, Bernal et al. 2013) and extinct (Wilson et al. 2012, Schroer and Wood 2015, Asahara et al. 2016, Evans et al. 2016, Gomes Rodrigues et al. 2017) mammals, with evidence supporting its existence deep in mammalian evolution (Halliday and Goswami 2013). Although it applies to many clades, it cannot be generalized across all mammals, and some examples of mammalian groups that fall outside the model prediction also exist (Polly 2007). Moreover, different patterns in different mammalian groups were found, supporting the existence of different intensities in inhibition and activation across the cascade, and allowing the evolution of different morphotypes not strictly predicted by the model (Asahara 2013). Interestingly, in many reported cases, this variability associated with the inhibitory cascade is related to different adaptations to certain diets. For example, in murine rodents, faunivorous species show larger m1 (and loss of m3), whereas herbivorous species have approximately equal-sized molars (Kavanagh et al. 2007). In canids, carnivorous species exhibited the pattern m1 >> m2 >> m3, omnivorous species exhibited m1 > m2 > m3 and insectivorous species have equal-sized molars (Asahara 2013). Finally, Evans et al. (2016) reported that an inhibitory cascade pattern was present along the dp3, dp4, and m1 in hominins, integrating to the already proposed cascade through m1, m2, and m3 and supporting the existence of a developmental control across the five teeth (the idea of a consistent pattern across the primary dentition was originally proposed by Butler 1939, but the exact mechanism was unknown at the time).

In this study, considering the dental homologies of sloths (Hautier et al. 2016) and the existence of an inhibitory cascade in dp3, dp4, and m1 in hominins (Evans et al. 2016), we explore the evolution of the lower dentition in sloths aiming at testing the existence of the inhibitory cascade in their molariforms. We also discuss the significance of the observed patterns across sloth families and their potential relationship with diet adaptations.

Materials and methods

Taxon sampling and data acquisition

In order to study the existence of the inhibitory cascade in the lower dentition of sloths, we focused on the dental dimensions of the last three molariforms. These last teeth are commonly named mf1, mf2, and mf3 and are morphologically different from the first lower tooth, which has a caniniform morphology in most sloths and is referred as cf1. However, some sloths, like the Megatheriinae, present a first tooth with molariform morphology and thus have a lower dental formula composed of mf1, mf2, mf3, and mf4. In those cases, we considered the last three molariforms as homologous with the last three molariforms of the rest of sloths (as tentatively proposed by Hautier et al. 2016) and the first molariform as homologous with the caniniform, with mf2, mf3, and mf4 considered as mf1, mf2, and mf3 in those taxa (Text-fig. 1). We collected data from juvenile and adult specimens of *Lestodon armatus*, a fossil ground sloth belonging to the family Mylodontidae, deposited in the collections of Museo Nacional de Historia Natural, Montevideo, Uruguay (MNHN); Museo Municipal “Bautista Rebuffo”, Colonia, Uruguay (MMBR); Museo Paleontológico “Armando Calcaterra”, Colonia, Uruguay (MPAC); Colección Arroyo del Vizcaíno, Sauce, Uruguay (CAV); Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina (MACN); Museo de La Plata, La Plata, Argentina (MLP), in order to explore the molariforms variability among a single species (Tab. 1). The



Text-fig. 1. Examples of lower dentition and molariforms homologies in the four families of extinct sloths. a: Mylodontidae, b: Megatheriidae, c: Nothrotheriidae, d: Megalonychidae.

largest mylodontid *Lestodon armatus* was selected because it is probably the most abundant ground sloth found in Pleistocene deposits of Uruguay (Fariña et al. 2014, Varela and Fariña 2016), along with the large number of remains found in Argentina and Brazil, and because, despite this, it remains relatively little studied. We also collected data from sloth genera covering all sloth families from a revision of the literature, as well as published images and measurements of museum specimens (Tab. 2) to evaluate the IC model in this clade. In this case, a single adult individual was measured, which (although not ideal, is common in macroevolutionary analyses covering fossil species due to the limited sample or fragmentary nature of many specimens) could produce some artefacts in the analysis and should be considered when discussing the results. A total of 20 specimens of *L. armatus* were included in the first analysis, while a total of 53 sloth genera were included in the second approach.

Table 1. Molariforms area measurements and proportions in studied specimens of *Lestodon armatus*. Measurements in mm^2. %mf2: percentage of area occupied by mf2 in relation to the total area occupied by the last three teeth.

Specimen	mf1	mf2	mf3	mf2/ mf1	mf3/ mf1	%mf2
CAV 125	543.0	563.2	720.0	1.04	1.33	30.8
CAV 391	364.9	419.3	639.5	1.15	1.75	29.5
CAV 595	404.2	458.5	650.0	1.13	1.61	30.3
CAV 648	501.4	536.8	685.4	1.07	1.37	31.1
CAV 847	405.1	436.0	543.2	1.08	1.34	31.5
CAV 897	486.8	488.4	687.5	1.00	1.41	29.4
CAV 898	122.6	155.7	212.0	1.27	1.73	31.8
CAV 1261	381.2	401.1	499.9	1.05	1.31	31.3
CAV 1571	406.4	405.0	484.2	1.00	1.19	31.3
CAV 1572	327.7	344.5	438.5	1.05	1.34	31.0
MACN 9470	467.8	496.8	722.9	1.06	1.55	29.4
MACN 10830	471.6	582.7	867.7	1.24	1.84	30.3
MLP 3-29	529.4	619.1	806.8	1.17	1.52	31.7
MLP 3-30	342.4	385.8	607.5	1.13	1.77	28.9
MMBR 1110	68.5	68.5	92.9	1.00	1.36	29.8
MMBR sn-5	295.5	339.6	503.1	1.15	1.70	29.8
MNHN 2776	388.7	396.8	515.0	1.02	1.32	30.5
MNHN 2784	327.7	360.0	549.0	1.10	1.68	29.1
MNHN 2785	429.1	447.3	614.1	1.04	1.43	30.0
MNHN 2786	472.2	488.6	690.8	1.03	1.46	29.6

Tooth area is commonly measured by the product of the tooth length and width in most mammalian groups. However, in the case of sloths, especially mylodontids, the teeth are circular, elliptical, and/or lobated in section (Text-fig. 1). Therefore, we measured occlusal tooth area using ImageJ (Rueden and Eliceiri 2019) in images of the lower molariform row from published literature and collection specimens (Tabs 1, 2). A similar approach was previously used by Vizcaíno et al. (2006) in sloths for the study of the dental occlusal surface area and its relation with body mass and food habits.

The inhibitory cascade model

The IC model, originally proposed by Kavanagh et al. (2007), assumes a linear effect of the activator and inhibitor ratio on tooth proportions. Relative molar size and position follows the equation $y = 1 + [(a - i)/i](x - 1)$ where y is the relative molar size estimated from occlusal area, x is the position of the molar in the tooth row, a is the strength of activation, and i is the strength of inhibition. This equation predicts that M1 = 1, M2 = a/i and M3 = $2a/i - 1$.

The majority of the published research on the IC model used the RMA model in order to fit regressions. However, there is a current discussion regarding the use of OLS and RMA regressions and their impact on parameter estimations (Smith 2009). Considering this, and in order to compare our results with published analyses, we used both OLS and RMA to fit regressions to the *Lestodon* data. Furthermore, the lack of independency in biological data due to the existence of phylogenetic relationships between species has been

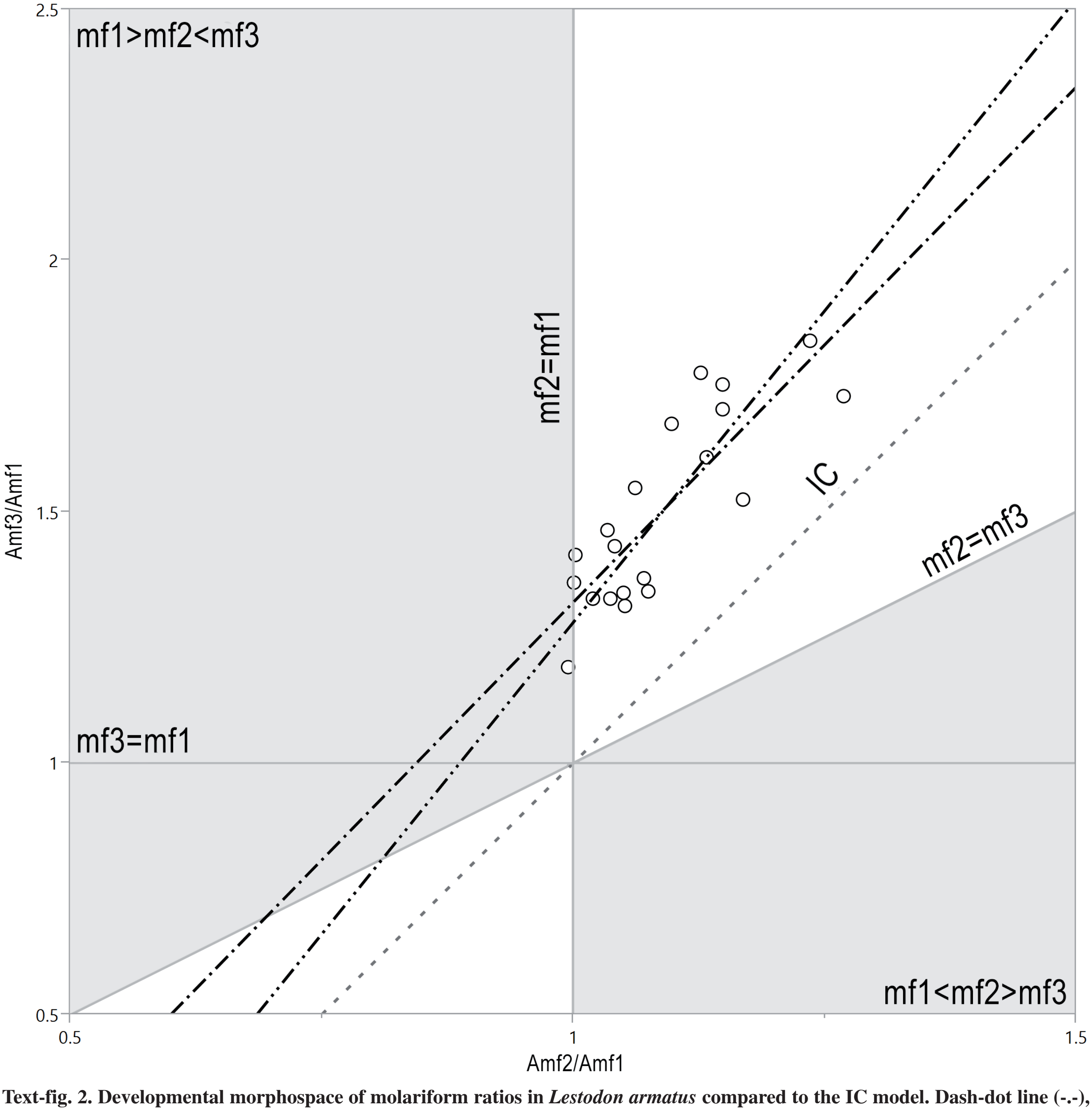
Table 2. Molariforms area measurements and proportions in studied sloth genera. Measurements in mm^2. cf: caniniform, mf: molariform, %mf2: percentage of area occupied by mf2 in relation to the total area occupied by the last three teeth.

Genus	cf1	mf1	mf2	mf3	mf2/mf1	mf3/mf1	%mf2	Reference
Octodontotherium	106.9	141.8	308.1	257.5	2.17	1.82	43.6	Hoffstetter 1954
Octomylodon	–	665.2	745.4	761.0	1.12	1.14	34.3	Scillato-Yané 1977
Pseudoprepotherium	215.8	191.4	210.8	349.2	1.10	1.82	28.1	Hirschfeld 1985
Thinobadistes	333.7	150.5	227.2	489.7	1.51	3.25	26.2	Webb 1989
Lestodon	505.5	394.6	427.1	587.4	1.09	1.50	30.3	This work
Pleurolestodon	453.0	328.0	340.4	660.5	1.04	2.01	25.6	Rovereto 1914, Saint-André et al. 2010
Glossotherium	374.6	424.2	567.7	1097.7	1.34	2.59	27.2	Mcafee 2009
Paramylodon	242.8	380.5	411.6	792.9	1.08	2.08	26.0	Mcafee 2009
Nematherium	35.5	44.8	51.6	79.0	1.15	1.76	29.4	Scott 1904
Catonyx	337.0	233.1	231.9	342.4	0.99	1.47	28.7	Cartelle et al. 2009
Scelidotherium	196.9	182.8	186.9	304.6	1.02	1.67	27.7	Bargo 2001a
Mylodon	222.7	316.2	354.3	462.8	1.12	1.46	31.3	Bargo 2001a
Acratocnus	61.2	50.0	56.3	59.7	1.13	1.20	33.9	Matthew and Paula Couto 1959
Neocnus	10.3	20.4	21.4	25.2	1.05	1.23	32.0	Matthew and Paula Couto 1959
Parocnus	24.8	57.5	69.3	89.3	1.21	1.55	32.1	Matthew and Paula Couto 1959
Megalocnus	150.3	291.2	342.9	404.0	1.18	1.39	33.0	Matthew and Paula Couto 1959
Pliometanastes	102.4	245.5	261.0	271.2	1.06	1.10	33.6	Hirschfeld 1981
Megalonyx	350.1	259.1	273.8	278.3	1.06	1.07	33.8	Savage 1946
Pronothrotherium	22.1	80.0	93.0	91.7	1.16	1.15	35.1	Rovereto 1914
Mionothropus	37.9	115.5	97.6	102.7	0.85	0.89	30.9	De Iuliis et al. 2011
Nothrotherium	–	84.8	95.2	98.0	1.12	1.16	34.2	Quiñones et al. 2017
Nothrotheriops	–	186.2	216.6	221.8	1.16	1.19	34.7	Quiñones et al. 2017
Eucholoeops	62.1	75.7	79.8	86.9	1.05	1.15	32.9	De Iuliis et al. 2014
Hapalops	33.7	48.8	54.6	60.5	1.12	1.24	33.3	Scott 1903
Eremotherium	2013.5	2686.3	2275.5	1770.8	0.85	0.66	33.8	McDonald and Lundelius 2009
Megatherium	1902.7	2396.8	2155.5	1442.2	0.90	0.60	36.0	Bargo 2001a
Schismotherium	20.8	36.4	46.4	54.3	1.27	1.49	33.8	Scott 1904
Pelecyodon	8.5	32.8	42.2	44.1	1.29	1.34	35.5	Scott 1904
Ahytherium	88.0	321.9	329.1	360.4	1.02	1.12	32.5	Cartelle et al. 2008
Proscelidodon	103.3	98.0	108.1	138.5	1.10	1.41	31.4	Taglioretti et al. 2014
Prepoplanops	47.2	119.9	123.9	146.0	1.03	1.22	31.8	Carlini et al. 2013
Anisodontherium	684.1	695.7	560.9	418.6	0.81	0.60	33.5	Brandoni et al. 2011
Urumacotherium	186.9	140.8	125.3	117.1	0.89	0.83	32.7	Negri and Ferigolo 2004
Simomylodon	138.6	99.0	106.0	193.5	1.07	1.95	26.6	Saint-André et al. 2010
Octodontobradys	223.1	271.6	349.3	330.9	1.29	1.22	36.7	Dos Santos et al. 1993
Brievabradys	42.8	19.7	21.2	28.7	1.07	1.46	30.4	Villarroel 2000
Lestobradys	310.0	231.3	268.5	448.4	1.16	1.94	28.3	Rinderknetcht et al. 2010
Valgipes	139.5	124.2	122.0	136.7	0.98	1.10	31.9	Cartelle et al. 2009
Bolivartherium	305.3	243.9	326.0	507.0	1.34	2.08	30.3	Carlini et al. 2006
Thalassocnus	–	146.9	161.7	183.7	1.10	1.25	32.9	Mcdonald and Muizon 2002, Muizon et al. 2003
Pyramiodontherium	1325.5	1678.4	1470.4	903.4	0.88	0.54	36.3	Carlini et al. 2002
Pseudortotherium	24.0	72.2	67.7	65.7	0.94	0.91	32.9	Scillato-Yané 1981
Xyophorus	15.1	56.5	63.9	62.1	1.13	1.10	35.0	Brandoni 2014
Megathericulus	276.1	333.2	346.5	351.1	1.04	1.05	33.6	Pujos et al. 2013
Diabolotherium	131.4	143.8	163.6	154.7	1.14	1.08	35.4	Pujos et al. 2007
Pseudoglyptodon	50.3	31.9	41.7	47.9	1.31	1.50	34.3	Engelmann 1987
Baraguatherium	–	252.7	285.8	222.9	1.13	0.88	37.5	Rincón et al. 2017
Aymaratherium	33.8	172.1	185.9	159.3	1.08	0.93	35.9	Pujos et al. 2016
Australonyx	152.1	283.8	302.4	305.8	1.07	1.08	33.9	De Iuliis et al. 2009
Lakukullus	34.7	129.8	153.8	172.8	1.19	1.33	33.7	Pujos et al. 2014
Prepotherium	25.6	65.5	66.1	69.7	1.01	1.06	32.8	Scott 1904
Bradypus	7.8	12.0	13.2	16.8	1.10	1.40	31.4	This work*
Choloepus	31.6	16.8	17.8	21.7	1.05	1.29	31.6	This work*

* Measurements taken from computerised tomography in digimorph.org.

recognized for a long time (Felsenstein 1985). In fact, some studies have used phylogenetic comparative methods to study the IC model in some groups (Bernal et al. 2013, Carter and Worthington 2016). In the present study, we employed PGLS to address the existence of the IC model in sloths. Specifically, we used the function corPagel from the package ape (Paradis and Schliep 2018) to define a correlation structure derived from Brownian motion allowing lambda to be estimated by the function. Also, we accounted for unequal tip variances due to a non-ultrametric tree. The OLS and RMA models were fitted using the software Past (Hammer et al. 2001), while the PGLS models were fitted using the package nlme (Pinheiro et al. 2012) for the software R (R core team 2019). In order to contemplate the phylogenetic relationships among the studied sloths, we used the phylogenetic framework previously used by Delsuc et al. (2019) for the study of the ancestral reconstruction of the dental configuration. For this, we used a backbone constraint based on Delsuc et al. (2019) molecular results on the morphological phylogeny proposed by Varela et al. (2018).

First, we evaluated the existence of the IC in all the data. However, we further tested the model after the exclusion of *Octodontotherium*, as this taxon was clearly an outlier when the data were plotted (see Text-fig. 4). The placement of this taxon could be explained by the unclear relationship of orophodontids with the rest of sloths and, therefore, the potential inexistence of homologies among their teeth. Alternatively, we tested the existence of an IC in the first three teeth of sloths, i.e., the first caniniform/molariform and the subsequent mf1 and mf2, to account for the existence of a different pattern as well as the potential influence of the first tooth on the last three teeth.



Text-fig. 2. Developmental morphospace of molariform ratios in *Lestodon armatus* compared to the IC model. Dash-dot line (-.-), show OLS line; dash double-dot line (-..-) shows RMA line.

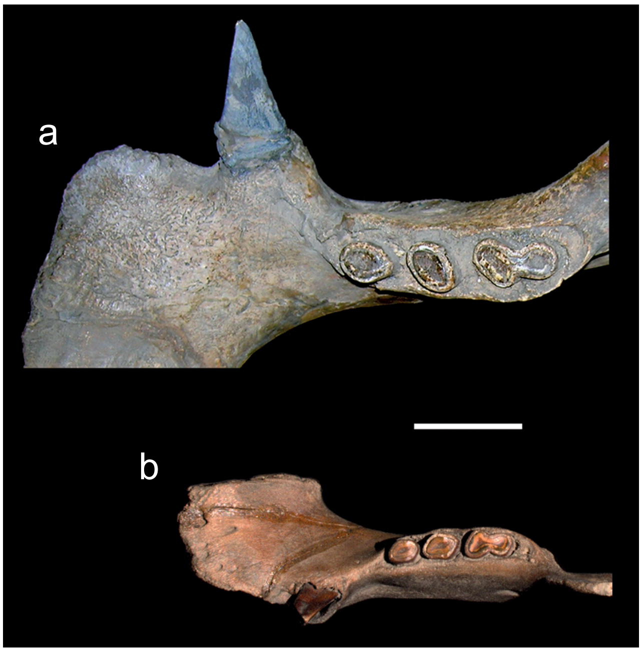
Furthermore, considering that the inhibitory cascade model predicts that the tooth placed in the middle should occupy 1/3 of the sum of the occlusal area, we calculated this value for each family and tested for significant differences with ANOVA and Tukey's multiple comparisons test ($p < 0.05$).

Finally, based on Tukey's test, we evaluated the possibility of different families showing different patterns regarding the existence and intensity of the IC model. For this, we included families as a categorical variable and compare different models using AIC.

Results

Lestodon armatus

A significant positive correlation ($r^2 = 0.66$, $p < 0.01$) was found between the ratios of mf2 and mf1, and mf3 and mf1 in *L. armatus*. Regarding the IC model predictions, the regression analysis showed a slope of 2.05 (CI: 1.13–2.54) for OLS and 2.48 (CI: 1.38–3.05) for RMA, and an intercept of −0.73 (CI: −1.26–0.22) for OLS and −1.20 (CI: −1.82–0.02) for RMA, consistent with the IC model (Text-fig. 2). However, the results indicate that, even if *L. armatus* comply with the IC model, some specimens almost fall outside the strict IC model prediction, with mf1 and mf3 larger than mf2. When observing the different specimens of *L. armatus* in detail, it is evident that the mf3 is always the largest tooth (even in juvenile specimens), while mf1 and mf2 show more variability and overlap in their size (Text-fig. 3). On the other hand, the mf2 occupied roughly 30 % on average among the studied individuals, well below the prediction of the IC model. Alternatively, considering the great variability in the caniniform size in this taxon, a preliminary test (data not shown) exploring the existence of an IC among the first three teeth with a lower sample showed a significant correlation and a slope of 1, clearly inconsistent with the IC model.



Text-fig. 3. Mandibles of (a) adult (MACN 10830) and (b) juvenile (CAV 898) specimens of *Lestodon armatus*. Scale bar 5 cm.

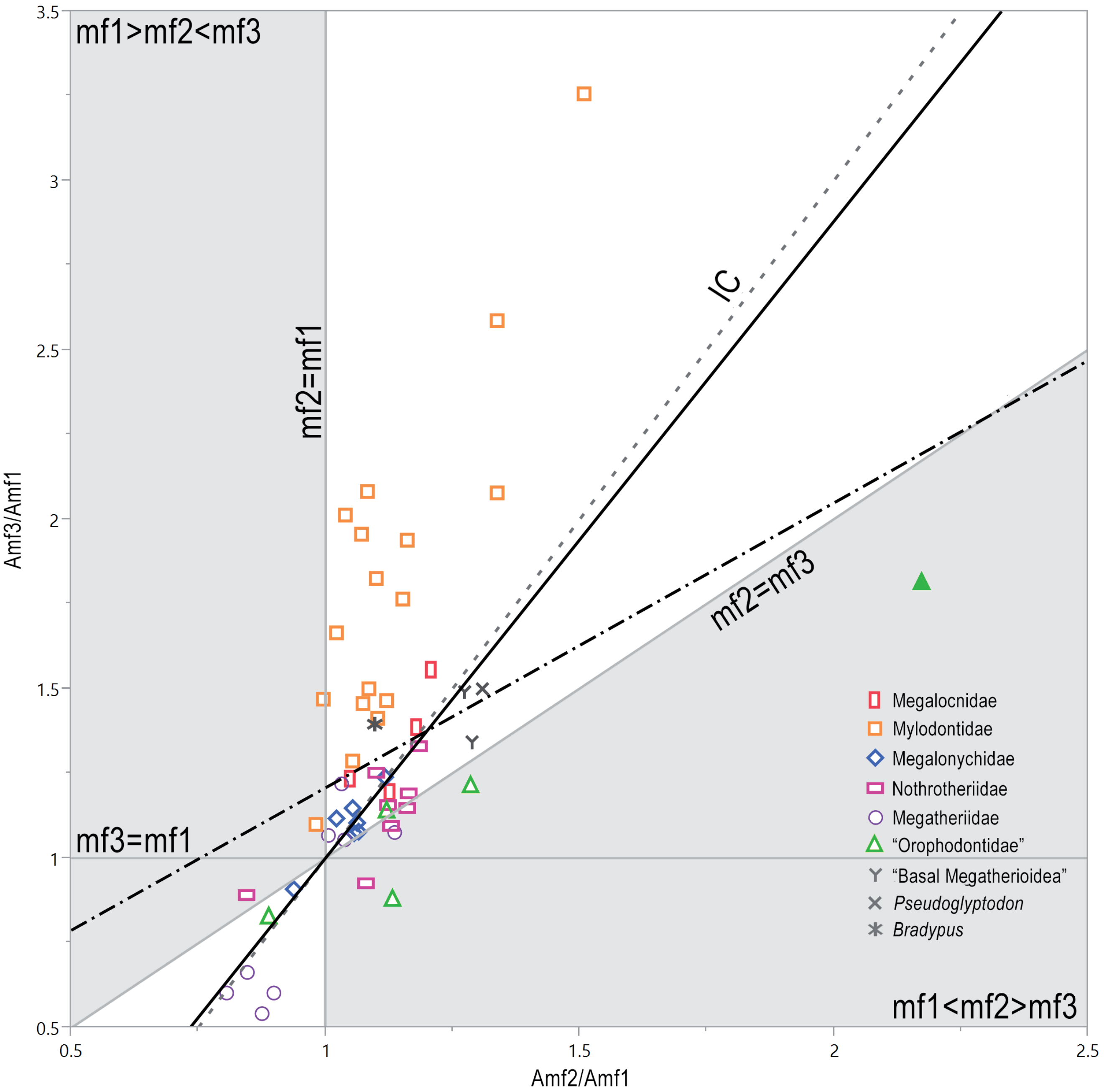
Sloths

Regarding the data set that includes taxa from all the families of sloths, a significant correlation was found between the ratios (Slope: 0.84 [0.53–1.15]; Intercept: 0.37 [−0.15–0.88], $r^2 = 0.63$, $p < 0.01$). However, although roughly 85 % of the studied sloth genera fall inside the area predicted by de IC model, the regression results are not consistent with the IC model. However, considering the plotted data (Text-fig. 4), it is evident that only one taxon falls well outside the IC model predicted area. In fact, the exclusion of this single taxon (*Octodontotherium*) significantly changes the obtained regression (Slope: 1.88 [1.37–2.39]; Intercept: −0.87 [−1.55–(−0.20)], $r^2 = 0.75$, $p < 0.01$), producing results largely consistent with the IC model.

Furthermore, when testing the existence of a similar pattern between the first three teeth (Text-fig. 5), the results show much less spread of the data (although, again, *Octodontotherium* falls considerably away from the rest of the taxa) as well as the lack of a pattern expected by the existence of IC (Slope: 1.11 [1.00–1.21]; Intercept: 0.05 [−0.16–0.27], $r^2 = 0.90$, $p < 0.01$).

Moreover, the mf2 roughly occupied 1/3 of the tooth row in all sloth families, but values in Mylodontidae were significantly lower ($p < 0.05$) than in the rest of the families while the values of the orophodontids were considerably higher than most other taxa (Tab. 3, Text-fig. 6).

Considering the spread of the data, as well as the results of the Tukey's post-hoc test, we tested the existence of different patterns among families. The results (Tab. 4) showed support for models taking mylodontids and orophodontids separately. The most supported model was the one with different regressions for mylodontids, orophodontids, and the rest of the sloths. In that case, the mylodontids showed a pattern consistent with the IC model, with a slope moderately higher than 2 (Slope: 2.88 [1.92–3.84]; Intercept: −1.52 [–2.05–(−0.99)], $r^2 = 0.70$, $p < 0.01$) which is consistent with the $mf1 \leq mf2 << mf3$ pattern. In particular, the Scelidotheriinae *Catonyx* and *Valgipes* were placed outside the area predicted by the IC model, showing an $mf1 > mf2 < mf3$ configuration. On the other hand, the orophodontids showed a pattern inconsistent with the IC, with a slope slightly below 1 (Slope: 0.78 [0.63–0.93]; Intercept: 0.12 [−0.15–0.39], $r^2 = 0.69$, $p < 0.01$). Specifically, it is worth noting that the orophodontids represented the taxa that fell most distant from the area expected by the IC, with *Octodontotherium*, *Octodontobradys*, and *Baraguatherium* showing an $mf1 < mf2 > mf3$ configuration. The rest of the sloth taxa from the other families showed values almost consistent with the IC model and similar to values previously reported in other taxa where the IC model is present (see Tab. 5), with a slope below 2 but well above 1 (Slope: 1.57 [1.56–1.58]; Intercept: −0.54 [−0.71–(−0.37)], $r^2 = 0.80$, $p < 0.01$). Also, it is worth mentioning the placement of two Nothrotheriidae, *Aymaratherium* and *Mionothropus*, which fell well outside the predicted area with $mf1 < mf2 > mf3$ and $mf1 > mf2 < mf3$ configurations, respectively. Table 5 shows the comparisons of the obtained results in the regression analyses with the strict IC model prediction and previously obtained results in other mammalian groups.



Text-fig. 4. Macroevolutionary trends related to the IC model in the last three teeth of the six families of extinct sloths, as well as specimens of the "basal Megatherioidea", *Pseudoglyptodon*, and *Bradypus*. Dash-dot line (-.-) shows the regression including all data; solid line shows the regression after the exclusion of *Octodontotherium* (shown in the plot as a filled triangle).

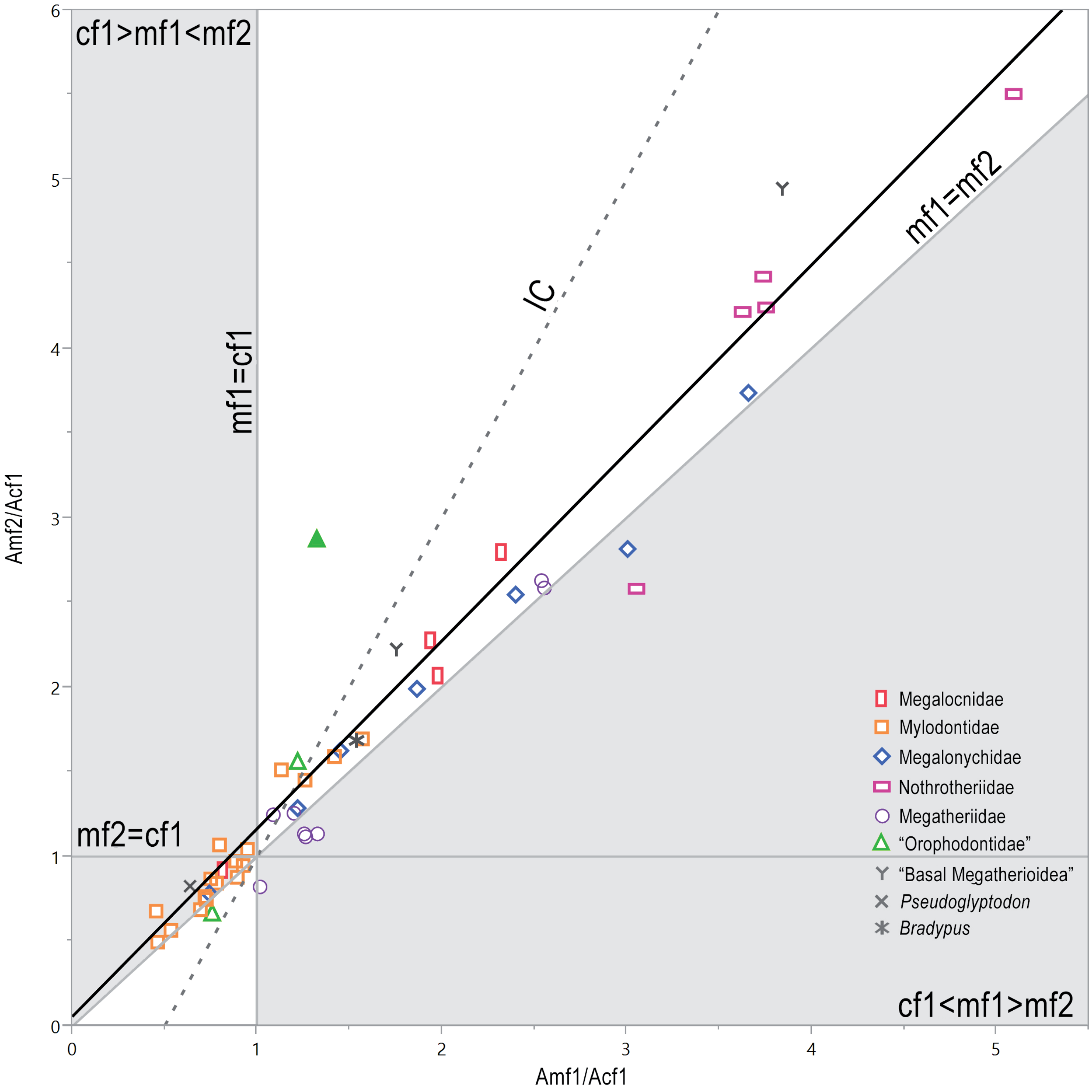
Discussion

The inhibitory cascade in sloths

The inhibitory cascade has been proven to be present in many groups of extant mammals, such as rodents, carnivorans, and primates, as well as extinct ungulates and Mesozoic basal mammals (Kavanagh et al. 2007, Polly 2007, Labonne et al. 2012, Wilson et al. 2012, Asahara 2013, Bernal et al. 2013, Halliday and Goswami 2013, Schroer and Wood 2015, Asahara et al. 2016, Evans et al. 2016, Gomes Rodrigues et al. 2017). In this work, we demonstrate that it is also present in at least one group of Xenarthrans, the Folivora, even with the many peculiarities in the dentition of the group: tooth loss, enamel loss, homodonty, and changes in the typical mammalian dental formula, among others.

In *Lestodon armatus*, our results show that the observed variability largely complies with the IC predictions. In this sloth, the last molariform is always the largest tooth in the series, but the mf1 and mf2 are similar in size, with some specimens with mf1 approximately equal to mf2. This variation could be related to ontogeny, with juvenile specimens having larger mf1, but mf2 and mf3 increasing in relative size during ontogeny. However, this pattern is not clear since many adult individuals present similarly sized mf1 and mf2 (Text-fig. 3) and the juvenile individuals were not clustered in any zone of the morphospace.

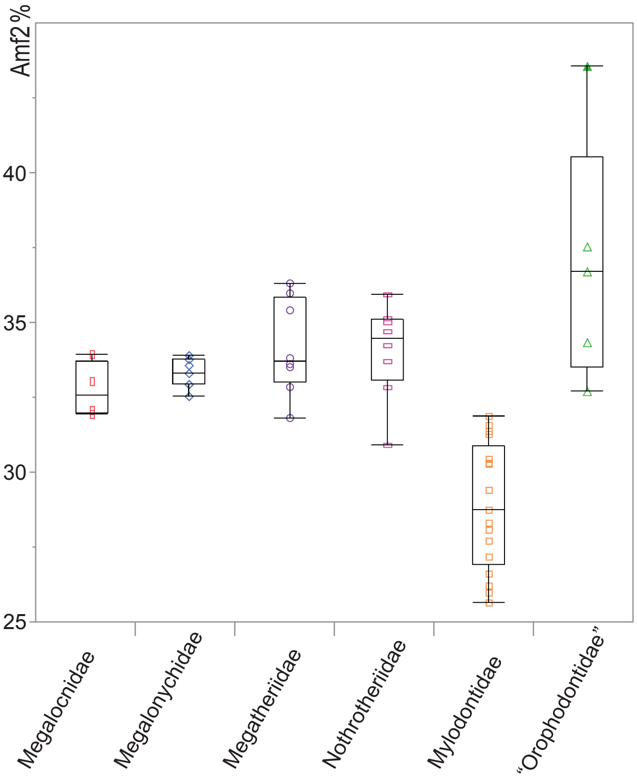
For all sloths, the existence of the IC in the last three teeth is largely supported in the clade. However, as mentioned



Text-fig. 5. Macroevolutionary trends related to the IC model in the first three teeth of the six families of extinct sloths, as well as specimens of the "basal Megatherioidea", *Pseudoglyptodon*, and *Bradypus*. Dashed line (- -) shows the regression including all data; solid line shows the regression after the exclusion of *Octodontotherium* (shown in the plot as a filled triangle).

before, the placement of one genus, *Octodontotherium*, as a clear outlier, required its exclusion to obtain significant results. Contrarily, a pattern consistent with the prediction of the IC model was not recovered for the first three teeth regardless of the first tooth morphology (caniniform or molariform).

Furthermore, significant differences were obtained between families when considering the area of mf2, with mylodontids and orophodontids respectively having an mf2 considerably smaller or larger than expected under the IC model. These differences were further explored after the evaluation of different slopes in these families. A slight departure from the strict prediction of the IC model was detected in the case of mylodontids. In this family, especially in mylodontines, a slope considerably larger than 2 was obtained between the molariform ratios, showing a pattern in which mf3 tends to be remarkably larger while mf1 and mf2 remain smaller and sometimes similar in size. In fact, some mylodontids, namely *Catonyx* and *Valgipes*, showed an mf1 > mf2 < mf3 configuration, which was proposed by Kavanagh et al. (2007) as the developmentally least likely phenotype to occur. For orophodontids, the slope was slightly lower than 1, showing a significant departure from thee IC model. However, as observed in Text-fig. 5, a pattern consistent with the IC model could be present in the first three teeth in orophodontids, but this cannot be confirmed with the current data due to the poor preservation or absence of cf1 in the specimens. These trends in orophodontids could be the result of different dental homologies in this clade compared with the rest of sloths. Nonetheless, a similar



Text-fig. 6. Boxplots showing the proportion of mf2 in relation to the sum of the occlusal area in the five families of extinct sloths.

pattern was previously reported in ursids, with evidence supporting the existence of a unique IC pattern in this group related to evolutionary changes in the expression or function of low diffusible inhibitory molecules (or their antagonists) affecting m2/m1, but not m3/m1 (Asahara et al. 2016). On the other hand, the observed slope after the exclusion of mylodontids and orophodontids showed that most sloths comply with the IC model predictions. Although the observed slope was slightly less steep than the strict prediction of the IC model, it was comparable to those observed in many mammalian groups, including the murine rodents originally studied by Kavanagh et al. (2007).

Even though the molariform loci of sloths seem not to be homologous with those of molars of other mammalian groups, the existence of the IC allows observing that the mechanisms described for other clades are probably also working during the development of the molariforms of sloths and constraining the evolution of relative tooth sizes. In this regard, it must be pointed out that only the mf3 of sloths would be homologous to a molar, particularly the m1 (Hautier et al. 2016), but the observed pattern across relative tooth sizes clearly indicates the influence of the front teeth on the hind teeth (i.e., the IC). On the other hand, premolars in mammals originate from back to front (unlike molars, which develop from front to back; Luckett 1993, van Nievelt and Smith 2005), and their influence over other teeth is not extensively studied.

Table 3. Proportion of the mf2 relative to the three last molariforms in sloths. Levels not connected by the same letters are significantly different ($p < 0.05$).

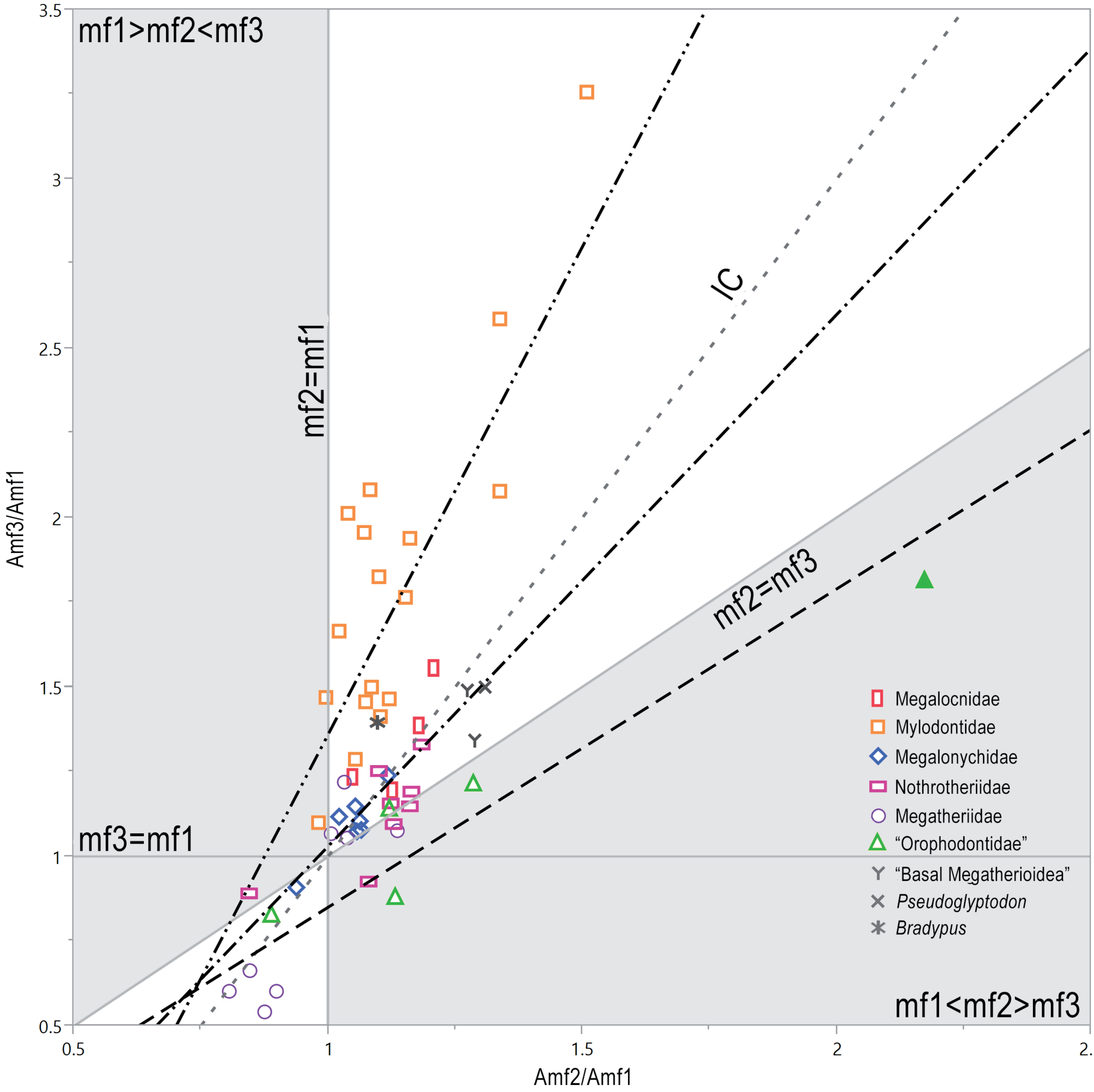
Taxon	Mean	SD	
All sloths	0.32	0.03	–
Megalocnidae	0.33	0.01	B
Megalonychidae	0.33	0.01	B
Megatheriidae	0.34	0.02	AB
Nothrotheriidae	0.34	0.02	AB
Mylodontidae	0.29	0.02	C
"Orophodontidae"	0.37	0.04	A

However, Labonne et al. (2012) found that the p4, when present, significantly influences the IC in rodents, constraining the development of m1 and affecting molars relative sizes. Furthermore, Evans et al. (2016) results indicate the existence of an expanded IC in hominins affecting all primary post-canine dentition, namely the deciduous premolars and molars. In most mammals, including hominins, these deciduous premolars are replaced by permanent premolars, so many studies are commonly based on this permanent dentition (Dahlberg 1945, Townsend and Brown 1981). However, sloths (and most xenarthrans) do not show tooth replacement and their mf1 and mf2 are probably homologous to the dp3 and dp4 of most mammals (see Hautier et al. 2016). Thus, the observed pattern in the molariforms ratios in sloths could be related to the existence of a similar IC pattern to that in hominins, in which the development of all present postcanine teeth is governed by the equilibrium between activation and inhibition between teeth, with the peculiarity that sloths would have lost their last two molars. Also, the clearly different IC pattern in mylodontids could be related to changes in inhibition and activation intensities along the cascade, in particular, an extreme activation of the mf3 development. However, considering the limited information regarding tooth development in fossil sloths, differences in tooth homologies in the case of mylodontids should not be ruled out. Moreover, given the fact that the first lower tooth (the caniniform in most sloths) seems to be a dp2, its potential effects over the IC should be taken into account, especially in cases where it shows a molariform morphology.

Finally, it must be mentioned that in most sloths the upper dentition has one more tooth, although according to the previously mentioned developmental studies the extra tooth is the first one and the last three molariforms still would be homologous to the dp3, dp4, and m1 of other mammals. However, in many sloths, including many in which the last lower tooth is the largest in the series, the last upper tooth is the smallest in the series. This apparent difference between upper and lower dentition regarding the trends in relative tooth sizes will be further studied in future research in order to explore differences between inhibition and activation intensities in both toothrows, a topic scarcely explored in previous works (Gomes Rodrigues et al. 2017).

IC and dietary adaptations in fossil sloths

Several authors reported variations in the IC model among taxa that related to different dietary adaptations. In rodents, an increase in the relative sizes of m2 and m3 led to an m1 = m2 = m3 configuration that was related to



Text-fig. 7. PGLS regressions for Mylodontidae, "Orophodontidae", and the rest of the sloths considered separately, Mylodontidae: dash double-dot line (-..-), "Orophodontidae": dash-dot line (-.-), rest of sloths: dash line (--).

more herbivorous diets (Kavanagh et al. 2007). Similarly, in canids, insectivorous and omnivorous taxa showed relatively smaller m1 and larger m2 and m3 than taxa with carnivorous habits (Asahara 2013). Moreover, a similar pattern was recovered by Halliday and Goswami (2013) for 135 genera from several extinct mammalian taxa, with more faunivorous taxa arranged in the bottom left of the morphospace and more herbivorous taxa in the top right.

In the case of sloths, the only proposed major group with dietary adaptations more related to bulk feeding or

Table 4. Model support of the regression models fitted to the sloth molariform ratios.

Model	AICc	ΔAICc	AICcw
All families considered separately	3.96	10.41	0.005
Mylodontidae and "Orophodontidae" considered separately	**−6.45**	**0**	**0.994**
Mylodontidae considered separately	32.15	38.6	< 0.001
"Orophodontidae" considered separately	7.88	14.33	0.001
All data	29.38	35.83	< 0.001

Table 5. PGLS regression analysis of sloth molariform ratios compared to previously published results in other mammalian groups.

	Slope	C.I. min	C.I. max	Intercept	C.I. min	C.I. max	r
IC model	2	–	–	0	–	–	1
Murinae (Kavanagh et al. 2007)	2.15	1.77	2.69	−1.22	−1.65	−0.93	0.74
Arvicolinae (Renvoisé et al. 2009)	1.39	1.21	1.56	−0.31	−0.41	−0.21	0.77
Carnivora (Asahara 2013)	0.59	0.49	0.74	−0.17	−0.22	−0.13	0.62
Astrapotheria (Wilson et al. 2012)	1.27	1.06	1.48	−0.29	−0.55	−0.04	0.91
Notoungulata (Wilson et al. 2012)	1.76	1.58	1.99	−0.66	−0.88	−0.46	0.81
Creodonta (Asahara et al. 2016)	2.57	1.30	7.25	−2.31	−8.59	−0.72	0.39
Dasyuromorphia (Asahara et al. 2016)	2.01	1.88	3.20	−1.31	−2.90	0.27	0.70
Folivora	0.84	0.53	1.15	0.37	−0.15	0.88	0.63
Folivora excluding *Octodontotherium*	1.88	1.37	2.39	−0.87	−1.55	−0.20	0.75
Mylodontidae	2.88	1.92	3.84	−1.52	−2.05	−0.99	0.70
“Orophodontidae”	0.78	0.63	0.93	0.12	−0.15	0.39	0.69
Folivora excluding Mylodontidae and “Orophodontidae”	1.57	1.56	1.58	−0.54	−0.71	−0.37	0.80

grazing are mylodontids, with some members of the family exhibiting these adaptations as early in the fossil record as the Oligocene (Bargo and Vizcaíno 2008, Shockey and Anaya 2011, Pujos et al. 2012). Considering our results, these adaptations could be related to the remarkable departure from the strict expected relative teeth sizes under the IC model, with mylodontids, especially mylodontines, showing an mf1 ≤ mf2 << mf3 configuration. In this case, the mf2 occupies ~29 % on average, while the mf3 occupies ~45 % of the molariform area.

On the other hand, all other sloths have been commonly interpreted as browsers, and they are mainly placed in the centre of the morphospace. However, it is particularly interesting that some megatheriids, specifically the largest megatheriines, were placed in the other extreme of the morphospace in the left bottom part of the graph, with almost all members having an mf1 > mf2 > mf3 configuration. As mentioned above, this part of the morphospace is associated with less herbivorous diets, with many omnivorous, insectivorous, and carnivorous taxa showing similar configurations. Interestingly, the giant megatheriine sloth *Megatherium americanum* from southern South America has so far been the only species with a proposed not strictly herbivorous diet (Fariña 1996, Fariña and Blanco 1996, Fariña and Varela 2018; see also Bargo 2001b). However, other evidence also suggests an herbivorous diet for this sloth (Green and Kalthoff 2015, Bocherens et al. 2017) and other developmental or morphofunctional reasons should not be discarded. In particular, the dental formula of megatheriines, with the first tooth having a molariform morphology in contrast to the caniniform present in most other sloths, could have affected the IC and should be further studied.

Conclusions

Our results show that, despite having extremely derived dentitions among mammals, sloths dental development is largely constrained by the same mechanisms acting in many other mammalian clades. At the same time, these mechanisms were important during the evolution of the group and, in part, produced the diversity of morphologies found in extinct and extant sloths. Moreover, important departures of the strictly expected morphology under the IC model seem to have allowed some sloths, specifically some mylodontids, a dentition arrangement better suited to grazing.

The previous findings regarding the development of teeth in extant sloths (Hautier et al. 2016), coupled to the analysis of the abundant extinct diversity of a group with only two extant genera, allowed us to test an important developmental model in a macroevolutionary context in a group of mammals in which the presence of the IC affecting tooth proportions would be considered improbable at first glance.

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RESEARCH

Occurrence of the ground sloth *Nothrotheriops* (Xenarthra, Folivora) in the Late Pleistocene of Uruguay: new information on its dietary and habitat preferences based on stable isotope analysis

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Abstract

Sloths represent one of the most successful South American clades that reached North America during the Great American Biotic Interchange (GABI). At least four families are known from the Pleistocene of North America: Mylodontidae, Megalonychidae, Megatheriidae, and Nothrotheriidae. Members of the Nothrotheriidae are known from the Middle Miocene to the Pleistocene/Holocene transition in the Americas, but *Nothrotheriops* is the only genus in the family recorded in North America. Recently, femora with characteristic nothrothere morphology were recovered from Santa Fe, Argentina, and assigned to *Nothrotheriops*, thus greatly expanding the geographic distribution of this taxon. In Uruguay, records of Quaternary Nothrotheriidae are scarce, and previous reports are limited to the genus *Nothrotherium*. In this work, we describe a humerus from Arroyo del Vizcaíno (AdV), a ~30 ka site located in southern Uruguay, and assign it to the nothrotheriid *Nothrotheriops* sp. We also discuss several specimens from Uruguay originally assigned to *Nothrotherium*, which upon re-examination can be tentatively reassigned to *Nothrotheriops* sp. Furthermore, we employ stable isotope analysis to explore the climatic and dietary preferences of this taxon in South America. The new records are consistent with the materials reported from Argentina, thus providing new knowledge of the geographic distribution of the taxon, as well as its climatic and dietary preferences. Stable isotope results showed $\delta^{13}C$ values consistent with diets based mainly on C_3 plants, similar to those published for *N. shastensis* in North America.

Keywords Megatherioidea · Nothrotheriops · Paleoecology · Quaternary · South America

Introduction

Within the Xenarthra Cope, 1889 (armadillos, anteaters, and sloths), the Folivora Delsuc et al., 2001 (sloths) represents a small part of the diversity seen today for the clade (Gibb et al. 2016). However, in marked contrast, the fossil record of sloths is significantly more diverse than the two extant genera, *Bradypus* and *Choloepus* (McKenna and Bell 1997). Several families within Folivora have been traditionally recognized, with the Bradypodidae, Mylodontidae, Megalonychidae, Nothrotheriidae, and Megatheriidae being the most accepted based on morphological studies (Gaudin 2004; Varela et al. 2019). Recently, molecular studies based on ancient DNA recovered from several extinct sloth taxa have proposed a different arrangement within the clade, with *Bradypus* nesting within the Megatherioidea, *Choloepus* grouping with the Mylodontidae, and the Great Antillean sloths representing a family level sister group to

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all other sloths (Delsuc et al. 2019; Presslee et al. 2019). In this context, members of the Nothrotheriidae (Xenarthra, Folivora) are recognized as a family within the superfamily Megatherioidea (Gaudin 2004) based on both morphological and molecular data. The group is known from the Middle Miocene to the Pleistocene/Holocene transition in the Americas, when its last members became extinct along with many other members of the Pleistocene megafauna in both South, Central, and North America (Fariña et al. 2013; Brandoni and Vezzosi 2019). The family is not as well-represented in the fossil record of South America as other sloths and has the smallest diversity of genera of all the sloth families, although several recent discoveries from South America during the last decades have considerably increased the records of pre-Pleistocene members of the family, thus providing more information on their diversity, evolution, and different ecological adaptations (De Iuliis et al. 2011; Pujos et al. 2014, 2016; Gaudin et al. 2022). In fact, within this family is the only sloth considered semi-aquatic, the South American genus *Thalassocnus*, which shows clear and progressive adaptations to aquatic life over several million years (Muizon et al. 2003, 2004a, b; Amson et al. 2015a, b, c; Quiñones et al. 2022; but see Amson et al. 2017 for a different classification of this monogeneric subfamily). The oldest undoubted record of the clade is represented by the genus *Mcdonaldocnus* from the Middle Miocene of Bolivia and Argentina (Gaudin et al. 2022), while records of other taxa show that at least by the Late Miocene representatives of the family were widely distributed across South America (Pujos et al. 2017). For the Neogene deposits of Uruguay, *Pronothrotherium* is the only nothrothere currently recognized, with records from the Late Miocene to the Pliocene of San José (Perea 2007). During the Quaternary, the Nothrotheriidae are represented only by three genera, the South American *Nothropus* (represented by only scarce and fragmentary materials), *Nothrotherium*, which is also endemic to South America, and *Nothrotheriops*, which has recently been recorded for South America (Brandoni and Vezzosi 2019), but is better known in North America from the Irvingtonian and Rancholabrean North American Land Mammal Ages (McDonald and Jefferson 2008).

In addition to the type species, *Nothrotherium maquinense* along with *N. escrivanense* from Argentina and Brazil (Reinhardt 1878; Pujos 2001), other species referred to this genus include *N. torresi* and *N. roverei* from Argentina (Kraglievich 1926, 1931; Vezzosi et al. 2019), and *N. normaroselli* from Uruguay (Roselli 1976). *Nothrotherium torresi* is based on a left femur as is *N. normaroselli*, while *N. roverei* is based on a left humerus. Regarding *Nothropus*, three species from the Late Pleistocene have been described from Argentina and Bolivia (Burmeister 1882; Bordas 1942) based primarily on isolated and fragmentary dentaries (see Quiñones et al. 2017). The recovery of ancient DNA from sloth dung from Cuchillo Curá, Neuquén Province in Argentina, did not provide an exact determination of the taxon that produced the dung, but the closest match to known samples of fossil sloths suggests it was produced by a nothrothere (Hofreiter et al. 2003). Unfortunately, the lack of any skeletal remains associated with the dung prevents any possible assignment to the currently known Late Pleistocene nothrotheres in South America.

Nothrotheriops represents one of the successful Folivora evolutionary lineages that colonized North America after the closure of the Isthmus of Panama, during the Great American Biotic Interchange (GABI). The first records of *Nothrotheriops* in North America are dated to the Irvingtonian (1.9–0.25 Ma; McDonald 1995), and several occurrences of the taxon showed that it was well established in the western United States and south into Belize during the Rancholabrean (0.25–0.011 Ma; McDonald and Jefferson 2008; De Iuliis et al. 2015). Recently, the presence of *Nothrotheriops* has been documented in Argentina and Brazil based on postcranial remains (i.e, femora) from the Late Pleistocene (Brandoni and McDonald 2015; Oliveira et al. 2017; Brandoni and Vezzosi 2019). Based on these occurrences and the assigned chronological ages of northern and southern specimens, Brandoni and Vezzosi (2019) proposed that the occurrence of *Nothrotheriops* in South America is indicative of a re-entrance to South America by a northern taxon (Brandoni and Vezzosi 2019). This proposition is not unheard-of, as other similar cases have been documented in other clades. For example, Scillato-Yané et al. (2005) showed the probable dispersal of the tropical pampathere *Holmesina* (Xenarthra, Cingulata) into South America following its diversification in North America, indicating the occurrence of a re-entrance of the clade into the continent after its original dispersal to the north. Similarly, Carlini et al. (2006, 2008a, 2018) proposed that an ancestor of the ground sloth *Eremotherium* migrated to North America and, after a cladogenetic process, gave rise to *E. laurillardi* which in turn migrated back to colonize the northern and central lowlands of South America. Carlini et al. (2008b) proposed a similar pattern in the glyptodont *Glyptotherium*, which occurs typically in North America but is also found in northern South America. Recently, the first unequivocal records of Teratornithidae birds from the Pleistocene of South America were described, potentially representing a late recolonization event of a northern member of this highly diversified guild of large carnivorous flying birds in the Americas, although other hypotheses cannot be completely discarded (Cenizo et al. 2021).

In this work, we describe a new record of the nothrotheriid *Nothrotheriops* sp. for the Late Pleistocene of South America. The specimen comes from the Arroyo del Vizcaíno site (AdV), a fossiliferous site located in southern Uruguay and dated to ~30 ka (Fariña et al. 2014). Furthermore, we discuss the affinities of other nothrotheriid specimens from Uruguay. Finally, we provide the first isotope data for the taxon in the Southern Hemisphere and discuss its implications regarding its dietary preferences and environment.

Geographical, geological, and paleontological settings

The specimen, a humerus, was collected at the AdV in Sauce, Canelones, Uruguay (34° 37′ 3″ S, 56° 2′ 33″ W; 35 m above sea level; Fig. 1). The site represents a Late Pleistocene deposit found at a natural pond within a streambed that lies on Cretaceous silicified sandstones of the Mercedes Formation (Fariña et al. 2014). Fossil remains of several taxa typical of the Late Pleistocene megafauna are located in a bed that transitions from a muddy sandy gravel facies, to a muddy sand facies, with polymictic clasts. The site has been interpreted as a fluvial system deposit that probably formed during a relatively short period of time (see Fariña et al. 2014). Among the fauna recorded for this site other sloths present include, three mylodontids (*Lestodon armatus*, *Glossotherium robustum*, and *Mylodon darwinii*) and one scelidotheriid (*Valgipes bucklandi*). Amongst these taxa, *Lestodon* represents the vast majority of fossils found. The co-occurrence of multiple mylodontids at AdV was discussed by Varela and Fariña (2016) and the presence of *V. bucklandi* was reported recently by Lobato et al. (2021), further increasing the sloth species diversity for the site and providing valuable information on the potential interactions among these taxa. Other taxa recorded for the site include: three glyptodonts, *Glyptodon reticulatus*, *Doedicurus clavicaudatus*, and *Panochthus tuberculatus*; the notoungulate *Toxodon platensis*; the proboscidean *Notiomastodon platensis*; the equid *Hippidion* sp.; as well as indeterminate Cervidae and Camelidae remains; and a single predator, the felid,

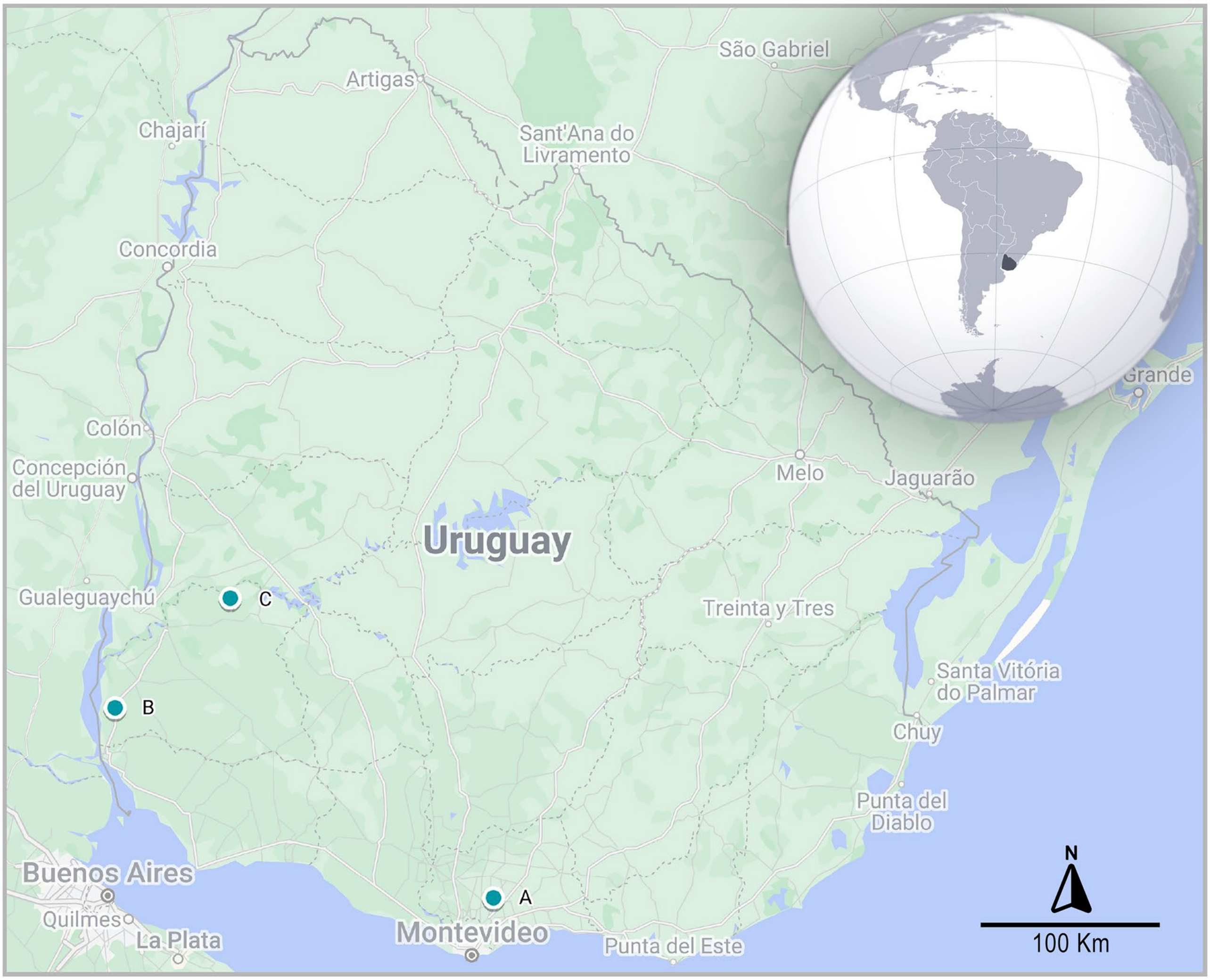

Fig. 1 Map of Uruguay showing the localities mentioned in the text. **a**. Arroyo del Vizcaíno; **b**. Arenal Chico River; and **c**. Perico Flaco Creek

Smilodon populator (Fariña et al. 2014). The nothrothere specimen is housed at the SAUCE-P Laboratory and Collection, Sauce, Uruguay.

Materials and methods

Description and analysis

The studied specimens were measured using a steel caliper with millimetric accuracy. Furthermore, a 3D digital reconstruction of the AdV specimen was made through photogrammetry using the software Agisoft Photoscan and post-processed in MeshLab (Cignoni et al. 2008). The specimens were compared with other nothrotheriid specimens from North and South America, as well as published descriptions of *Nothrotheriops* (Stock 1925; McDonald 1985), *Nothrotherium* (Reinhardt 1878; Cartelle and Fonseca 1982), and other nothrotheriids, namely *Pronothrotherium*, *Mionothropus*, and *Thalassocnus* (De Iuliis et al. 2011; Amson et al. 2015a; Pujos et al. 2016). Measurements of the studied specimens are provided in Table 1. A principal components analysis (PCA) was carried out to explore the relationships among the studied specimens. The PCA was performed in the software PAST (Hammer et al. 2001) using the nine measurements provided in Table 1. Data were transformed using $\log_{10}$ prior to the analysis to normalize it and missing values in specimens were completed using the iterative method provided in PAST (Jackson 1991; Hammer et al. 2001).

Stable isotopes analysis

Samples of bone from the specimens were obtained and analyzed to determine the isotopic composition of the preserved collagen and carbonate from the bioapatite. The extraction of collagen and carbonate, according to established standards (Longin 1971; DeNiro and Epstein 1981; Gupta and Polach 1985; Koch et al. 1997; Jørkov et al. 2007), as well as the stable isotope analysis, was conducted at the Alaska Stable Isotope Facility (University of Alaska Fairbanks). Since no previous isotopic results exist for *Nothrotheriops* in South America, the results were compared with data from North American specimens (Bonde 2013).

The results were also evaluated in comparison to data obtained from other taxa in the AdV site (Czerwonogora et al. 2011; Lobato et al. 2021) and the Pampean region of Argentina (Domingo et al. 2020). The results are reported using the delta notation, $\delta = [(R_{sample}/R_{standard} - 1) * 1000]$ (Coplen 1994), while the reference for carbon ($R = {}^{13}C/{}^{12}C$) and oxygen ($R = {}^{18}O/{}^{16}O$) isotopic values is V-PDB and for nitrogen isotopic values ($R = {}^{15}N/{}^{14}N$) is atmospheric nitrogen (AIR). The fossils from AdV are considerably well-preserved, with almost no chemical alterations visually identifiable, so contamination of the bioapatite by exogenous carbonates is unlikely. Despite this, these results should be considered cautiously as different processes can alter the isotopic composition of bioapatite (Koch et al. 2007). Therefore, we evaluated the validity of the carbonate results considering the quality of the obtained collagen, the expected difference between $\delta^{13}C$ values from collagen and carbonate for an herbivorous mammal, and the results for two other taxa from the AdV for which data for the region is known (*Lestodon* and *Equus*; Bocherens et al. 2017). The preservation of organic components at the site is noteworthy, with organic preservation allowing the analysis of proteomic and DNA sequences as well (see Buckley et al. 2015 and Baleka et al. 2022). Previous studies have shown good preservation of collagen in bones from the site (Czerwonogora et al. 2011; Fariña et al. 2014; Lobato et al. 2021), with $C{:}N_{atomic}$ in the traditionally accepted range (Clementz 2012).

Isotopic data can provide important information on the diet of both extant and extinct organisms. For example, in herbivores, the carbon isotopic composition of collagen can be correlated with the plants on which the animals fed, as different types of plants (C_3, C_4, and CAM) have carbon isotopic signatures reflecting the differential discrimination of $^{13}CO_2$ (Bocherens et al. 1996). As an enrichment of $\delta^{13}C$ from +3‰ to +5‰ occurs at each trophic level, herbivores feeding exclusively on C_3 plants (average $\delta^{13}C$ value of -26 ± 2‰) will show an average $\delta^{13}C$ value of -22‰ while those feeding exclusively on C_4 plants (average $\delta^{13}C$ value of -12 ± 1‰) will show an average $\delta^{13}C$ value of -8‰. Similarly, the nitrogen isotopic composition shows an enrichment of 2–5‰ on average at each trophic step, providing important information on the trophic level of organisms (DeNiro and Epstein 1981). Regarding the mineral bioapatite, the $\delta^{18}O$ values are indicative of the $\delta^{18}O$ value of the water consumed by the animals, providing information on the humidity and temperature of the environment where the animal lived (Larmon et al. 2019). Nevertheless, $\delta^{18}O$ also varies in relation to animals' diets, with grazers having higher $\delta^{18}O$ values than browsers (Bocherens et al. 1996). On the other hand, herbivorous mammals record the $\delta^{13}C$ values of the ingested vegetation with an enrichment of $\delta^{13}C$ values on the bioapatite that differs from that of the collagen, with the difference being dependent on the body mass (Fariña and Varela 2018; Tejada-Lara et al. 2018).

Institutional abbreviations CAV, Colección Arroyo del Vizcaíno, Sauce, Canelones, Uruguay; FC, Fauna Cedazo, Aguascalientes, Mexico; FMNH, The Field Museum, Chicago, USA; GRCA, Grand Canyon National Park (US National Park Service), Arizona, USA; LACM, Los Angeles County Museum, California, USA; MACN, Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina; MLP, Museo de La Plata, La Plata, Argentina; MMPLR,

Table 1 Comparison of measurements of the nothrothere humerus (CAV 1466) from AdV with other nothrothere taxa

			Measurement								
Taxon	Site	Cat No.	TL	PW	PD	SW	DW	DAW	DDL	DDM	PLS
Nothrotheriops sp.	Arroyo del Vizcaino, Canelones, Uru.	CAV 1466	433	104.1	70.5*	57	142.7	84.9	48.8	39.6	126.6
Nothrotheriops texanum	Leisey, FL, USA	UF 65821	360.5	91.1	65.4	46.9	142.3	79.1	40	33.9	109.8
Nothrotheriops texanum	Leisey, FL, USA	UF 86185		101.7	69.3	49.2					
Nothrotheriops texanum	Leisey, FL, USA	UF 86987	382.1	91.9	68	53	141.9	76.2	42.1	35.7	120.1
Nothrotheriops texanum	Leisey, FL, USA	UF 86989	361.2	92.6	69.4	46	143	79.3	46.2	34.7	116.6
Nothrotheriops texanum	Leisey, FL, USA	UF 86990				48.4	145.2	80.1	38.5	34.7	139.6
Nothrotheriops texanum	Leisey, FL, USA	UF 86991		84.4	71.7	55.2					
Nothrotheriops texanum	Pool Branch, FL, USA	UF 11452	376			62.5		89			
Nothrotheriops shastensis	San Josecito Cave, Nuevo León, Mex.	LACM 21604	413	105.2	79	66.6	157.1	91.7	49	44.6	150.5
Nothrotheriops shastensis	San Josecito Cave, Nuevo León, Mex.	LACM 21605	430	102	75	67.5	160	101.4	48.8	40.7	155.6
Nothrotheriops shastensis	San Josecito Cave, Nuevo León, Mex.	LACM 21606	428.4	102.9	80.6	64.6	162*				
Nothrotheriops shastensis	San Josecito Cave, Nuevo León, Mex.	LACM 21607	406.7	103.2	79.2	56.2	152.9	93.2	50.7	43.1	152.4
Nothrotheriops shastensis	San Josecito Cave, Nuevo León, Mex.	LACM 21613	395.4	97.3	75.4	55.8	143.9	87.3	44.1	37.7	127.1
Nothrotheriops shastensis	San Josecito Cave, Nuevo León, Mex.	LACM 21614	433.6	113	84.1	63.9	172	103.5	52.5	45.1	158
Nothrotheriops shastensis	Conkling Cavern, NM, USA	LACM 21746	385.1	99.9	73.2			88.8	47.7	40.2	
Nothrotheriops shastensis	Rancho La Brea, CA, USA	LACM HC 1874	431.9	106.3	80.6	53.6	168.7	97	51.2	40.2	143.4
Nothrotheriops shastensis	Devil Peak, NV, USA	NSMLV	377.8	99.5	70.2	59.6	151.8	88.9	47.1	37	106
Nothrotheriops shastensis	Rampart Cave, AZ, USA	GRCA 21657		81.7	64.5	37.8	133.3				106
Nothrotheriops shastensis	Rampart Cave, AZ, USA	GRCA 21651		84.5	65	37.6	134				106
Nothrotheriops shastensis	Payeatt Cave, AZ, USA	UALP 2563					165	96	51.6	40.7	
Nothrotheriops shastensis	Payeatt Cave, AZ, USA	OMNH 59264			83.7						
Nothrotheriops shastensis	Cedazo, Aguascalientes, Mex.	FC 640	398	95.9	74.3	51.6	153.6				
Nothrotherium roverei	Laguna Setubai, Santa Fe, Arg.	MACN 11070	592.5	137.4	108		189.3	128.7	66.8	61.3	158.5
Nothrotherium maquinense	Gruta dos Brejoes, Bahia, Bra.	1020/092 U.C.M.G	256	57		29	85				
Pronothrotherium typicum	Corral Quemado, Catamarca, Arg.	FMNH P14515		78.8	53.5	44.8	108.9	63.1	30.9	29.8	104

TL Length head to distal lateral condyle, *PW* Mediolateral width of proximal end, *PD* Anteroposterior depth of proximal end, *SW* Mediolateral width of shaft across deltoid crest, *DW* Distal width across epicondyles, *DAW* Mediolateral width across distal articular surface, *DDL* Anteroposterior depth of distal lateral condyle, *DDM* Anteroposterior depth of distal

*denotes measurements taken on incomplete specimens

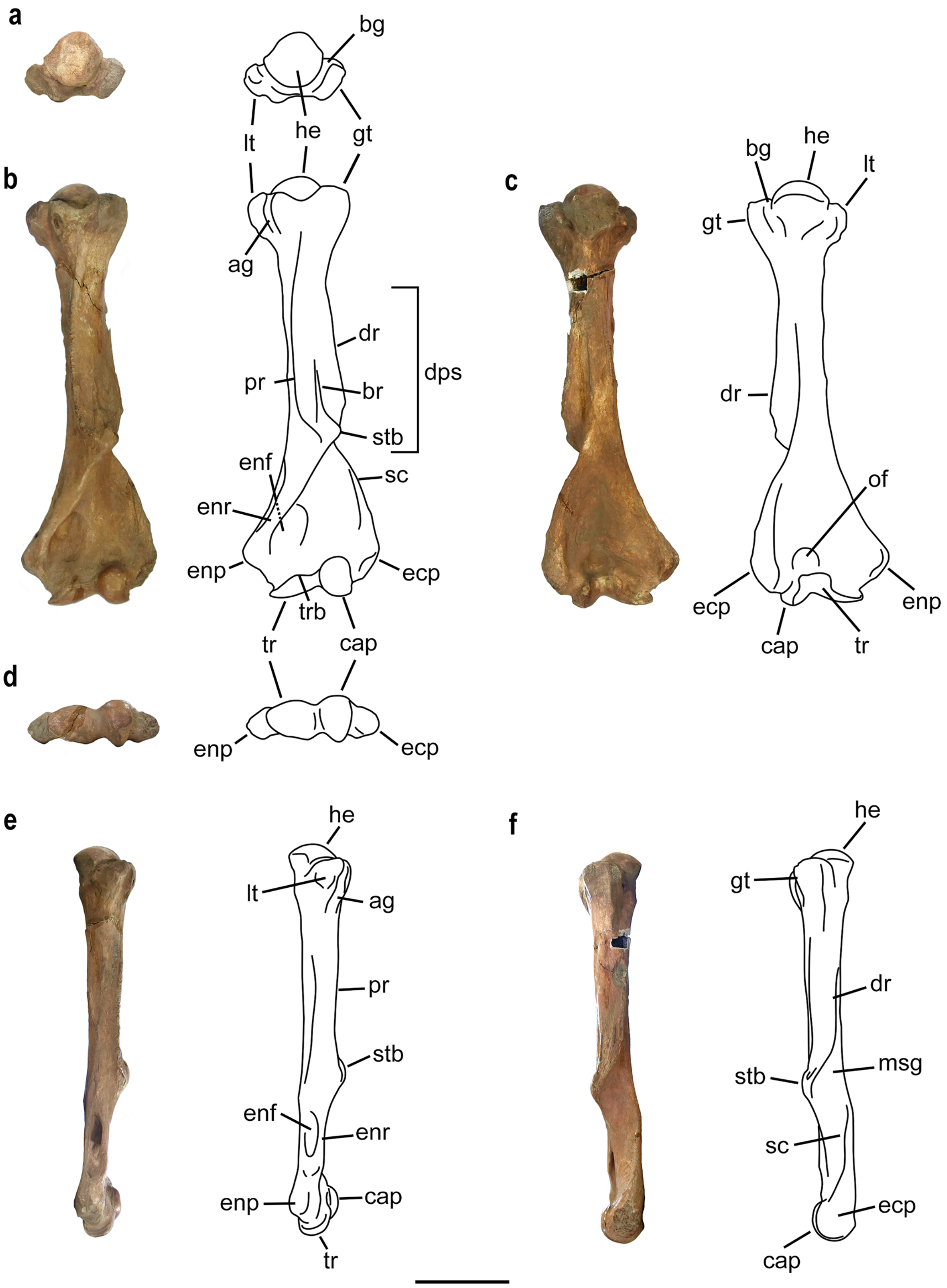

Fig. 2 Left humerus CAV 1466. **a**. proximal view, posterior towards top and medial towards left; **b**. anterior view; **c**. posterior view; **d**. distal view, anterior towards top and medial towards left; **e**. medial view; **f**. lateral view. Abbreviations: **ag**, anterior groove; **br**, brachiocephalic ridge; **bg**, bicipital groove; **cap**, capitulum; **dps**, deltopectoral shelf; **dr**, deltoid ridge; **ecp**, ectepicondyle; **enf**, entepicondylar foramen; **enp**, entepicondyle; **enr**, entepicondylar ridge; **gt**, greater tubercle; **he**, head; **lt**, lesser tubercle; **msg**, musculospiral groove; **of**, olecranon fossa; **pr**, pectoral ridge; **sc**, supinator crest; **stb**, shelf tubercle; **tr**, trochlea; **trb**, anterior border of the trochlea. Scale bar equals 100 mm

Museo Municipal Profesor Lucas Roselli, Soriano, Uruguay; MPAB, Museo Paleontológico Alejandro Berro, Mercedes, Soriano; NSMLV, Nevada State Museum, Las Vegas, USA; OMNH, Sam Noble Oklahoma Museum of Natural History, Oklahoma, USA; UALP, University of Arizona Laboratory of Paleontology, Arizona, USA; UCMG, Universidade Católica de Minas Gerais, Minas Gerais, Brazil; UF, University of Florida, Florida, USA.

Results

Systematic paleontology

XENARTHRA Cope, 1889.
FOLIVORA Delsuc et al., 2001.
MEGATHERIOIDEA Gray, 1821.
NOTHROTHERIIDAE (Ameghino, 1920) Muizon et al. 2004a, b.
NOTHROTHERIINAE Ameghino, 1920.
Nothrotheriops Hoffstetter, 1954.

Type species: *Nothrotheriops shastensis* (Sinclair, 1905).

Referred Specimen: CAV 1466, complete left humerus (Fig. 2).

Geographic and stratigraphic provenance: Arroyo del Vizcaíno site, Canelones, Uruguay. Late Pleistocene. The specimen was collected at the AdV site and comes from the same level where several Late Pleistocene taxa have been found. Radiocarbon dating of the bone collagen of several taxa, as well as from wood remains, gives the site an age of ~30 ka (Fariña et al. 2014).

Description and comparison: CAV 1466 is a slender left humerus with well-developed greater and lesser tubercles (Fig. 2a-c), as in the Late Miocene *Pronothrotherium typicum* (Pujos et al. 2016), being more laterally extended than in *Nothrotheriops shastensis*, *Nothrotheriops texanus*, *Nothrotherium maquinense*, and *Nothrotherium roverei* (Table 1). Both tubercles are almost symmetrical and connected by a broad shelf as in *N. shastensis* and *N. texanus* leaving the head slightly exposed in anterior view (Fig. 2b). In *N. maquinense* the tubercles are asymmetric, with the greater tubercle more proximally extended than the lesser tubercle.

The bicipital groove is poorly developed (Fig. 2a) as in *Nothrotheriops shastensis*, *Nothrotherium maquinense*, and *Nothrotherium roverei*. A deep groove is present on the anterior surface of the lesser tubercle (Fig. 2b), as in *N. shastensis*, *N. maquinense, N. roverei*, and the Late Miocene *Mionothropus cartellei* (De Iuliis et al. 2011). The head is positioned at the middle of both tubercles and in line with the proximodistal axis. In proximal view, the head is slightly oval, with the major axis oriented mediolaterally as in *N. shastensis*, rather than anteroposteriorly as in *N. maquinense* and *N. roverei*.

The deltopectoral shelf is well developed and raised, running along the anterolateral part of the diaphysis (Fig. 2b). The deltoid ridge is prominent and more developed than the pectoral ridge, and projects laterally as in *Nothrotheriops texanus*, *Nothrotherium roverei* and *Pronothrotherium typicum*. In *Nothrotheriops shastensis* and *Nothrotherium maquinense* the deltopectoral shelf is less prominent, with a weaker deltoid ridge (De Iuliis et al. 2011; Pujos et al. 2016). Between the deltoid and pectoral ridges is a small ridge that could correspond to the brachiocephalic ridge (De Iuliis 2003; Amson et al. 2015a; Fig. 2b); these three crests converge distally to form a prominent tuberosity. As in *N. texanus*, the deltoid ridge forms a large and rounded notch on the lateral surface of the humerus defined as the musculospiral groove (McDonald 1985; Fig. 2f). This notch is less developed in *N. roverei* and not present in *N. shastensis* and *N. maquinense.*

The distal epiphysis expands transversally, with a well-developed and medially extended entepicondyle (Fig. 2b-d) as in *Nothrotheriops shastensis*, *Nothrotheriops texanus*, *Nothrotherium maquinense,* and *Nothrotherium roverei*. The entepicondyle is connected to the deltopectoral shelf by a well-developed entepicondylar ridge, delimiting a large and oval entepicondylar foramen (Fig. 2b, e). The ectepicondyle is smaller than the entepicondyle, but more robust. The supinator crest gently slopes proximomedially as in *N. shastensis*, *N. texanus* and *N. maquinense*, so the supinator plate appears small and triangular (Fig. 2b). In contrast, in *N. roverei* the supinator crest is almost vertical, giving to the supinator plate a larger and squared appearance, as in *Mionothropus cartellei* (De Iuliis et al. 2011). The trochlea is convex both transversely and anteroposteriorly, and it is mediolaterally wider than the capitulum (Fig. 2b, d) as in *M. cartellei*, unlike *N. shastensis*, *N. texanus, N. maquinense* and *N. roverei*. The capitulum is slightly elongated, with the proximodistal axis being larger than the mediolateral one (Fig. 2b, d). This could involve having a smaller range of rotation (supination) of the arm, compared to other nothrotherids. In *N. shastensis*, *N. texanus*, *N. maquinense* and *N. roverei*, the capitulum is well-rounded, with equal proximodistal and mediolateral axes, as observed in most nothrotheres (De Iuliis et al. 2011; Amson et al. 2015a; Pujos et al. 2016). The anterior border of the trochlea recurves sharply on the surface of the bone which produces a notch between the trochlea and the capitulum (Fig. 2b) as in *N. shastensis* and *N. texanus*, rather than a straight border as in *N. maquinense* and *N. roverei.*

The Fig. 3 quantitative analyses based on the linear measurements showed that CAV 1466 falls within the size range recorded for specimens of *Nothrotheriops*. In particular, the size of CAV 1466 is similar to that of

Nothrotheriops shastensis based on the maximum length and the proximal width. On the other hand, the distal width of CAV 1466 is in the range of the values observed in *Nothrotheriops texanus*, which shows that the distal epiphysis of CAV 1466 is considerably narrower for its total length. Regarding the PCA, the first two principal components explained 84.37% and 12.69% of the variance, respectively, together accounting for more than 95% of the observed variance. When plotted, the two principal components showed considerable separation among the studied taxa, with the *N. shastensis* and *N. texanus* specimens clustering in the center of the morphospace while *Nothrotherium maquinense*, *Nothrotherium roverei*, and *Pronothrotherium typicum* plotted in the margins of the morphospace. The AdV specimen was plotted well within the variability observed in *Nothrotheriops*, specifically within the convex hull depicted by the *N. shastensis* sample.

Other referred material from Uruguay assignable to *Nothrotheriops*

For the Quaternary deposits of Uruguay, several lithostratigraphical units with fossils of sloths are recognized (Ubilla and Martínez 2016). However, nothrothere records are very scarce, with only *Nothrotherium* cf. *N. maquinense* previously being confidently recorded in the Sopas Formation (Late Pleistocene; Perea 2007). Here we describe and discuss three records from the Late Pleistocene of Uruguay which were originally assigned to *Nothrotherium*, but upon reexamination, we have tentatively reassigned them to *Nothrotheriops* sp.

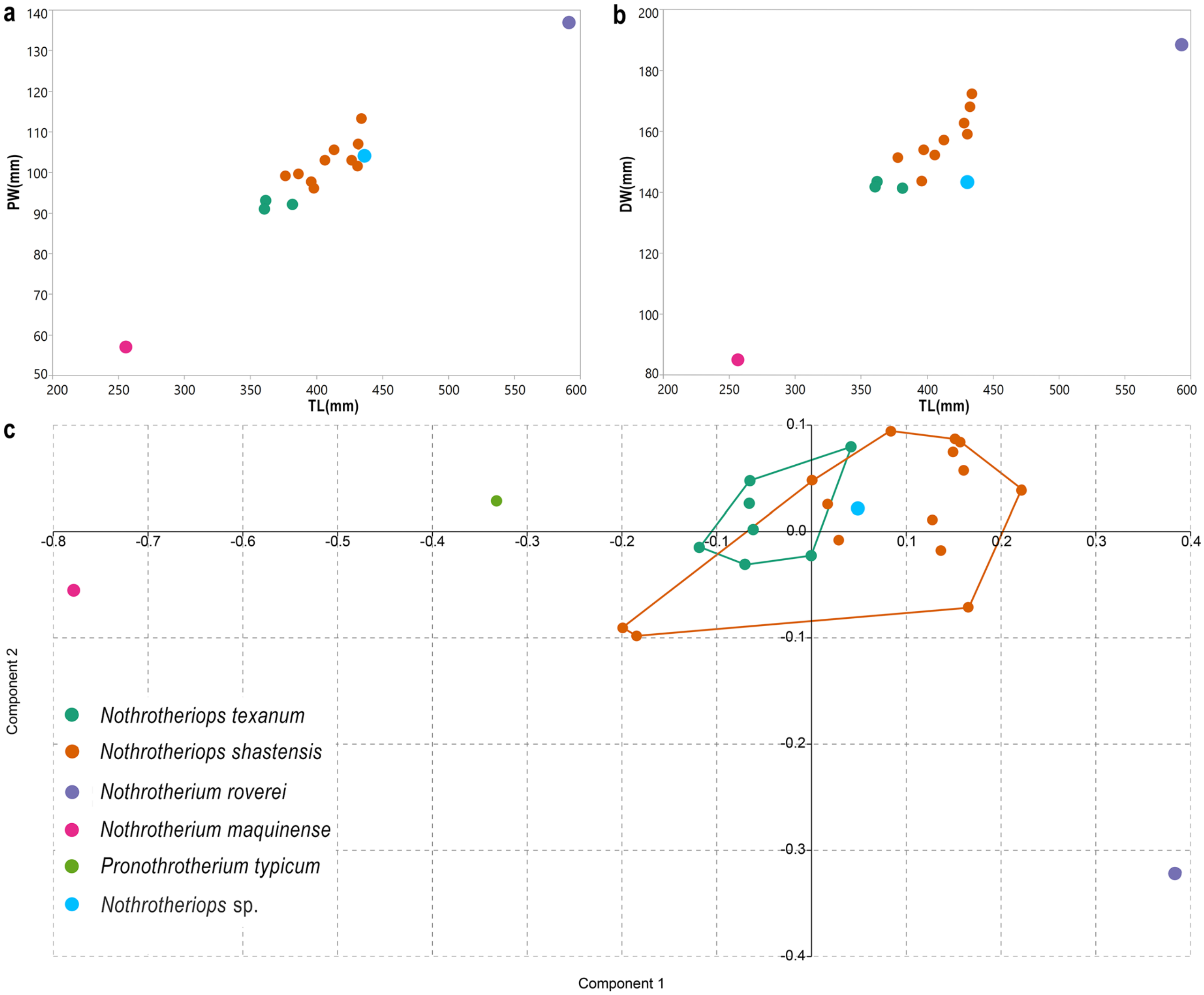

Fig. 3 Plots showing the results of the bivariate analyses (**a** and **b**) and principal components analysis (**c**) based on the linear measurements of the humerus of the studied specimens. Abbreviations: **DW**, distal width; **PW**, proximal width; **TL**, total length

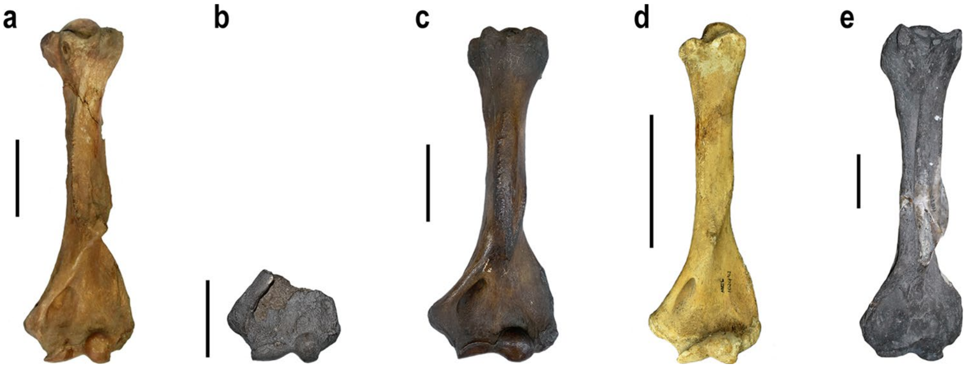

Fig. 4 Nothrothere humeri. **a**. *Nothrotheriops* sp., CAV 1466; **b**. MACN–Pv 10848; **c**. *Nothrotheriops shastensis* (reversed), LACM HC 1874-R-1; **d**. *Nothrotherium maquinense*, MCL1020-42-1; **e**. *Nothrotherium roverei*, MACN 11070. Scale bars equal 100 mm

Referred Specimen: MACN–Pv 10848, distal epiphysis of left humerus, recovered from the stream bed of the Río Negro River, Uruguay (Pampeano; Pleistocene sensu lato; Cohen et al. 2013; ICC 2022).

Description: Distal portion of humerus preserving the trochlea, capitulum, both epicondyles, and part of the entepicondylar ridge (Fig. 4b). It was assigned to *Nothrotherium* sp. by Perea (2007). The morphology and size of MACN–Pv 10848 are almost equal to that of CAV 1466 (Fig. 4), therefore the previous description and comparison apply to this specimen. It should be noted that MACN–Pv 10848 presents the notch between the trochlea and the capitulum as in *Nothrotheriops*. The trochlea is mediolaterally wider than the capitulum as in CAV 1466 and *Mionothropus*. Furthermore, the capitulum has the same shape and proportions as in CAV 1466.

Referred Specimen: MMPLR 482, left femur, recovered from the Arenal Chico River, Soriano, Uruguay (Libertad Formation, Upper Pleistocene; Cohen et al. 2013; ICC 2022).

Description: Almost complete femur, missing only some parts of the area of the lesser and third trochanters (Fig. 5a). This femur was described and assigned to *Nothrotherium normarosellii* by Roselli (1976). However, the author already noted several characteristics similar to *Nothrotheriops*. The femur resembles that of *Nothrotheriops* in being mediolaterally broader distally than proximally (Roselli 1976; Brandoni and McDonald 2015; Brandoni and Vezzosi 2019). The greater trochanter is positioned distally to the head, and in anterior view, it is connected to it by a nearly straight crest as in *Nothrotheriops* (Brandoni and Vezzosi 2019). Most of the lesser trochanter is missing. Although the third trochanter is missing a portion, it is possible to observe and recognize that its surface is connected to the ectepicondyle as in *Nothrotheriops*, rather than being separated from the ectepicondyle as in *Nothrotherium* (Roselli 1976; Brandoni and McDonald 2015; Brandoni and Vezzosi 2019). As in *Nothrotheriops* and *Nothrotherium*, the patellar trochlea is separated from the medial and lateral condyles. The medial condyle is pyriform in outline, and the lateral condyle is pentagonal as is clearly noted in *Nothrotheriops* (Brandoni and McDonald 2015; Brandoni and Vezzosi 2019).

Referred Specimen: MPAB 959, distal epiphysis of left tibia, recovered from the Perico Flaco Creek, Soriano, Uruguay (Pampeano superior, Dolores Formation, Upper Pleistocene; Cohen et al. 2013; ICC 2022).

Description: Distal portion of tibia preserving the articular surfaces for the astragalus and part of the fibular surface (Fig. 6). The specimen was collected by Berro in 1928 along with some rib fragments and was cataloged as *Nothrotherium maquinense*. The medial malleolus is prominent and bears two tendinous grooves (Fig. 6a, b) as observed in *Nothrotheriops* and *Nothrotherium* (Reinhardt 1878; Stock 1925). The medial articular surface, for the odontoid facet of the astragalus, is roughly circular and markedly concave; it separates from the lateral surface, for the discoid facet of the astragalus, by a well-marked ridge (Fig. 6c) as in *Nothrotheriops* (Stock 1925; Amson et al. 2015b). This differs with *Nothrotherium*, in which the surfaces are less separated and the medial one is less circular and less concave (Amson et al. 2015b). The greatest mediolateral width (128 mm) and the greatest

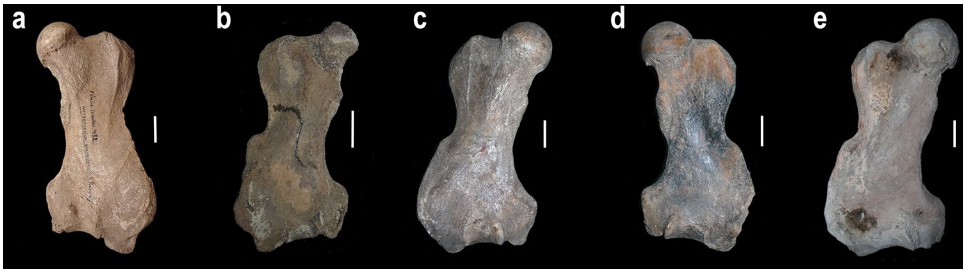

Fig. 5 *Nothrotheriops* sp. femora of from Uruguay (**a**) and Argentina (**b-e**): **a**. MMPLR 482; **b**. MCRS 199; **c**. MACN-Pv 14148; **d**. MACN-Pv 14149; **e**. MACN-Pv 14172. Scale bars equal 100 mm

anteroposterior depth (63 mm) measurements are similar to that of *Nothrotheriops* (Stock 1925) and larger than those observed in *Nothrotherium* (Cartelle and Fonseca 1982).

Isotopic analysis

The analyzed sample from the AdV specimen provided a collagen $C:N_{atomic}$ of 3.67, and the percentages of C and N were 6.6% and 2.1%, respectively. Traditionally, an upper limit of 3.6 has been considered to address the quality of extracted collagen (DeNiro 1985). However, recent research centered on the preservation of ancient specimens and the consequences of common contamination, has provided a more specific framework for the analysis of potentially contaminated samples (Harbeck and Grupe 2009; Guiry and Szpak 2021). Samples with values $C:N_{atomic} > 3.25$ are commonly contaminated with humic acids, but this contamination rarely has significant effects on the $\delta^{13}C$ and $\delta^{15}N$ isotopic values. In fact, $\delta^{13}C$ values tend to be more affected than $\delta^{15}N$, especially in animals with C_4 diets in C_3 environments, with values becoming more negative as contamination increases. Based on their results, Guiry and Szpak (2021) proposed "conservative" and "liberal" $C:N_{atomic}$ limits based on the expected $\delta^{13}C$ value of the studied taxa, with the "liberal" upper limits for $\delta^{13}C$ values of 20‰ and 19‰ (those expected for a herbivorous C_3/mixed consumer) being 3.90 and 3.70, respectively. Therefore, the obtained values of the AdV *Nothrotheriops* can be regarded as valid for paleobiological reconstruction, especially considering that the potential error for the $\delta^{13}C$ value is probably less than 1‰ (producing a more negative value and, ultimately, not changing the predicted herbivorous feeding guild). Moreover, previous results obtained from the AdV site in other megamammals (Czerwonogora et al. 2011; Fariña et al. 2014; Buckley et al. 2015; Lobato et al. 2021; Baleka et al. 2022) have shown the expected $C:N_{atomic}$, and %C and %N values were above the lower threshold proposed by Ambrose (1993) for the isotopic analysis of paleodiets (3% and 1% for C and N, respectively) in all cases, showing that collagen preservation is commonly good in the site. Regarding the potential contamination of the carbonate fraction, we employed two approaches (Bocherens et al. 2017). First, the obtained difference between $\delta^{13}C$ values from collagen and carbonate ($\Delta^{13}C_{carb\text{-}coll} = 8.23$) was well within the expected range for a Pleistocene ground sloth from the region (8.65 ± 1; Bocherens et al. 2017) and an herbivorour mammal (Codron et al. 2018). Second, the obtained $\delta^{13}C$ values from collagen and carbonate for two taxa from the AdV site showed the expected values based on previous data generated in other studies for the same time and region, showing that the carbonate fraction produced the expected values for these taxa (Online Resource 1). Therefore, the isotopic data obtained from the carbonate fraction of the bone can be considered for paleodietary inference. The collagen sample showed $\delta^{13}C$ and $\delta^{15}N$ values of -21.34‰ and 9.51‰, respectively. On the other hand, the carbonate fraction showed $\delta^{13}C$ and $\delta^{18}O$ values of -13.11‰ and 0.24‰, respectively. Finally, the sample obtained from MPAB 959 did not yield well-preserved collagen and, therefore, the carbonate fraction was not considered as reliable either.

Discussion

Considerations about the morphology and size of the Arroyo del Vizcaíno specimen and its taxonomic allocation to *Nothrotheriops*

Until recently, the Nothrotheriinae from the Late Pleistocene of South America were represented by *Nothrotherium* and *Nothropus*. However, the records of several femora from Santa Fe Province, Argentina (Brandoni and McDonald 2015; Brandoni and Vezzosi 2019) have indicated the presence of *Nothrotheriops* in South America. The species of *Nothrotherium* and *Nothrotheriops* are known by multiple postcranial remains, whereas the species of *Nothropus* are based only on isolated dentaries (i.e., *Nothropus carcaranensis*, *Nothropus priscus* and *Northropus tarijensis*). Additionally, there is no agreement regarding the validity of some species of *Nothropus*, as well as other South American nothrotheres. The lack of associated postcranial elements to the mandibles of *Nothropus* precludes direct

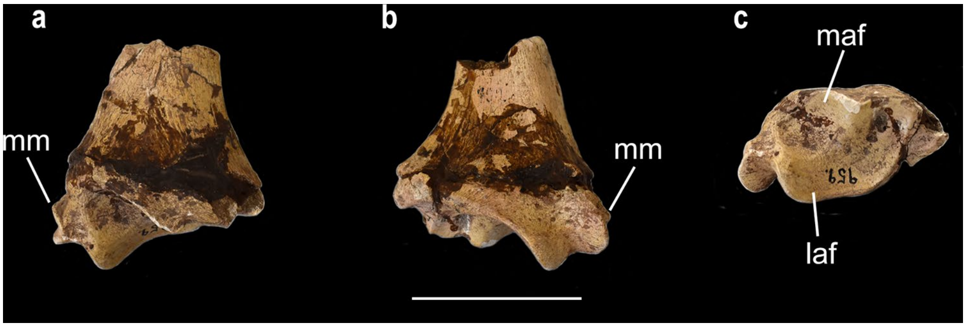

Fig. 6 Left tibia, MPAB 959: **a**. anterior view; **b**. posterior view; **c**. distal view. Abbreviations: **lar**, lateral articular facet; **maf**, medial articular facet; **mm**, medial malleolus. Scale bar equals 100 mm

comparison with other nothrothere specimens. This situation, and its systematic consequences, have recently been discussed (see Brandoni and McDonald 2015; Quiñones et al. 2017; Brambilla et al. 2019), even considering some Late Pleistocene species as junior synonyms of *Nothrotheriops* (Brandoni and Vezzosi 2019). A systematic review of Pleistocene Nothrotheriinae would shed light on the diversity of this group for the Quaternary of South America. However, that kind of study is beyond the goal of this work and more specimens of South American nothrotheres would be needed for its proper accomplishment.

The humerus of *Nothrotherium* has asymmetric tubercles and an oval head with its major axis anteroposteriorly oriented, whereas in *Nothrotheriops* both tubercles are almost symmetrical and the major axis of the head is mediolaterally oriented. Furthermore, at the distal epiphysis, *Nothrotheriops* have a notch between the trochlea and the capitulum, whereas *Nothrotherium* has a straight border. The size of *Nothrotherium* is smaller than *Nothrotheriops* (Brandoni and McDonald 2015; Brandoni and Vezzosi 2019), with the exception of the humerus of *Nothrotherium roverei*, and the femur of *Nothrotherium torresi*, as both of them are larger than any *Nothrotheriops* (it would be possible that both specimens belong to the same taxon, but at the moment it is not possible to know since they do not represent homologous elements). In *Nothrotheriops*, *N. shastensis* is generally larger than *N. texanus*, as corroborated by humerus dimensions (McDonald 1995). The size and proportions of the humerus CAV 1466 fall in the range of *Nothrotheriops shastensis*. However, as noted above, it presents some morphological differences with that species.

According to the description given above, the specimen CAV 1466 is assigned to *Nothrotheriops* sp. on the basis of the following morphological characteristics: (1) size; (2) greater and lesser tubercles symmetric and well developed; (3) head larger mediolaterally, (4) deltopectoral shelf well developed; and (5) notch between the trochlea and the capitulum in anterior view.

Implications of the new record in southern Uruguay and other specimens from Uruguay

The records of Quaternary Nothrotheriidae from Uruguay are scarce, with previous reports being limited to the genus *Nothrotherium*. Ubilla et al. (1994), with doubts, assigned a fragment of a tooth from the Sopas Formation to *Nothrotherium* and, later, Perea (2007) described a hemimandible from the same Formation and assigned it to *N. maquinense*. A Nothrotheriinae gen. et sp. indet. was mentioned by Ubilla et al. (2004, table 1), though it was not specified which bone was recorded; it is possibly the same molariform mentioned by Ubilla et al. (1994). Furthermore, there are two other specimens mentioned for Uruguay, but their assignments to *Nothrotherium* are doubtful and have been reviewed in the present work. Roselli (1976) described a femur, probably from Pleistocene deposits, and defined a new species, *N. normaroselli*. However, as described above, the femur shows the distinctive characteristics of the genus *Nothrotheriops*, namely, medio-laterally broader distally than proximally, greater trochanter connected to the head by a nearly straight crest, and connection between the third trochanter and the ectepicondyle. The distal fragment of a humerus assigned to *Nothrotherium* sp. mentioned but not figured by Perea (2007) is at the Museo Argentino de Ciencias Naturales but was collected in the Río Negro in Uruguay. As mentioned before, that specimen is almost identical in morphology and size to the material from AdV (Fig 4), both being larger than *Nothrotherium maquinense*, but smaller than *Nothrotherium roverei*. Furthermore, the morphology of the entepicondyle, ectepicondyle, trochlea and capitulum are congruent with that described for *Nothrotheriops*. Lastly, the distal fragment of a tibia, housed at the Museo Paleontológico Alejandro Berro (Soriano, Uruguay) and assigned to *Nothrotherium maquinense*, is larger than expected for this species and shows a morphology of the articular surfaces congruent with that of *Nothrotheriops*. In this context, the new record from Arroyo del Vizcaíno reported here, as well as the other specimens previously assigned to *Nothrotherium* (femur, distal humerus, and distal tibia) but whose anatomical characters do not coincide with the characteristics of this genus, further supports the presence of *Nothrotheriops* in the Late Pleistocene fauna of Uruguay.

Ecological and biogeographical implications

Considering the records from northern Uruguay, it is important to mention that there are several taxa in the Sopas Formation that show an important connection with Brazilian taxa, indicating a strong biogeographical connection between northern and southern regions of South America (Ubilla et al. 2004). This pattern has also been recorded in several Late Pleistocene sites by different authors based on the occurrence of typical Pampean taxa with taxa of tropical or intertropical environments, relating the Rio Grande do Sul and São Paulo areas with the Argentinian Mesopotamia and western Uruguay (e.g., Oliveira 1996, 1999; Scillato-Yané et al. 1998; Ferrero et al. 2017). Furthermore, this pattern is often interpreted as evidence of warmer and more humid climates in these southern regions during some periods (Carlini et al. 2004, 2008a, b). The existence of these non-analog faunas in the region has been discussed extensively, with hypotheses centered around the process involved in the formation of these sites and whether these faunas represent the actual coexistence of the recorded taxa or just an artifact of the time-averaging nature of the outcrops (Ghilardi et al. 2011; Gasparini et al. 2016). Lobato et al. (2021) reported the occurrence of the scelidothere *Valgipes bucklandi*, a sloth typical from the Brazilian Intertropical Region, in the AdV site, supporting the hypothesis that these non-analog faunas actually coexisted based on the short time span represented in the site. The occurrence of *Nothrotheriops* sp. in Santa Fe, Uruguay, and, potentially, Mato Grosso do Sul (see Oliveira et al. 2017; Brandoni and Vezzosi 2019) further shows the biogeographic connections in the region during the Late Pleistocene. These records show that the environments in the region during the Late Pleistocene probably included patches of mixed vegetation, allowing for the survival of taxa with different ecological requirements. In the case of *Nothrotheriops*, the North American species have been interpreted to have inhabited dry open environments like deserts, based on the analysis of plants in dung preserved in caves (Thompson et al. 1980; McDonald and Jefferson 2008; Ghilardi et al. 2011; McDonald 2021, 2022). Previous results for Mexico and the United States showed similar high $\delta^{18}O$ values for *N. shastensis* (Bonde 2013; Pérez-Crespo et al. 2018). The $\delta^{18}O$ values from southern Nevada were -6.9‰ (Devil Peak) and -6.1‰ (Tule Springs), while the values for the northern California sites were -5.7‰ and -5.2‰ (Potter Creek Cave), -4.7‰ and -4.3‰ (Samwell Cave), and -4.2‰ (Hawver Cave), and the value reported for central Mexico was -3.8‰ (Cedral). The specimen from the AdV site also showed high $\delta^{18}O$ values (0.24‰) typical of dry or warm climates. Brandoni and Vezzosi (2019), in contrast, predicted somewhat more humid conditions for Santa Fe Province, Argentina during the Late Pleistocene based on the known taxa as well as the geological and geomorphological context. Finally, Oliveira et al. (2017) suggested a dry environment for the Mato Grosso do Sul specimens possibly assignable to *Nothrotheriops* sp. Overall, the results are compatible with the predicted environments for the northern species of *Nothrotheriops*, *N. shastensis*, and are consistent with previous results supporting the existence of complex open mosaic habitats with patches of mixed vegetation in the Río de la Plata region (Varela and Fariña 2016; Varela et al. 2018; Lobato et al. 2021).

Another relevant aspect of the occurrence of *Nothrotheriops* sp. in the AdV site is related to the presence of other sloths recorded at the site and the potential niche partitioning between these taxa, similar to that seen in North America (McDonald 2021). As mentioned above, four sloths have been previously recognized for the site, *Lestodon armatus*, *Glossotherium robustum*, *Mylodon darwinii*, and *Valgipes bucklandi* (Fariña et al. 2014; Varela and Fariña 2016; Lobato et al. 2021). Considering the nature of the site, which is interpreted to have formed during a relatively short time span (Fariña et al. 2014), populations of these five sloths should have coexisted in southern Uruguay during the Late Pleistocene. This is not common in the fossil record of America, where only a few sites record the co-occurrence of ground sloths (McDonald 1996; Varela and Fariña 2016; Varela et al. 2018). Varela and Fariña (2016) discussed the co-occurrence of the three mylodontids, *L. armatus*, *G. robustum*, and *M. darwinii*, in the AdV, showing considerably different climatic preferences for the three sloths and the overlap of potentially suitable areas mainly in the Río de la Plata region. Moreover, Lobato et al. (2021) expanded the discussion to include the scelidotheriid *V. bucklandi*, further discussing the potential niche partitioning between these taxa. Their results showed that $\delta^{13}C$ values for *V. bucklandi* are consistent with a browsing diet based on C_3 plants, in contrast to the grass-dominated diets commonly predicted for the three mylodontids (Bargo and Vizcaíno 2008; Czerwonogora et al. 2011). In this regard, the North American species of *Nothrotheriops*, *N. texanus* and *N. shastensis*, have been predicted to be browsers feeding on xerophytic vegetation in open semi-desert habitats based on different approaches including morphological, biomechanical, coprolites, and isotopic analyses (McDonald 2021, 2022). In particular, previous results based on isotopic analyses have shown $\delta^{13}C$ values consistent with a diet highly based on C_3 plants for Mexico (Pérez-Crespo et al. 2018) or a mix between C_3 and C_4 plants for the United States (Bonde 2013). The data from two sites in southern Nevada (Tule Springs and Devil Peak Cave) by Bonde (2013) were consistent with data from the dung, showing that the animal's diet was predominately xeric vegetation. However, analysis of specimens from northern California cave sites (Potter Creek Cave, Hawver Cave, and Samwel Cave) indicated that this species had a much more isotopically diverse diet than previously thought based on the dung samples, showing an enrichment in ^{13}C through life, with a tendency to consume C_3 or mixed vegetation in earlier years and increasing percentages of C_4 vegetation as it aged. The $\delta^{13}C$ for the Devil Peak and Tule Springs individuals were -4.1‰ and -4.7‰, respectively. In contrast, the $\delta^{13}C$ values for Potter Creek Cave, Samwell Cave, and Hawver Cave were -8.6‰ and -8.4‰, -8.9‰ and -8.0‰, and -11.4‰, respectively. The $\delta^{13}C$ results obtained for *Nothrotheriops* sp. from the AdV site indicate a diet based mainly on C_3 plants, consistent with those published for *N. shastensis* in North America and similar to that obtained for *V. bucklandi* in the AdV site. In this context, a relative dietary differentiation among the sloths at the AdV site can be assumed, with mylodontids having more grazing habits (probably with different levels of specialization), as also observed for other mylodontids in the Pampean region (Domingo et al. 2020), and the scelidotheriid *Valgipes* and the nothrotheriid *Nothrotheriops* being more selective browsers. Nevertheless, it is important to mention that recent results obtained by Tejada et al. (2021) show evidence of a more diverse diet for the mylodontid *Mylodon darwinii*, probably including some amount of animal sources. Therefore, a more complex scenario regarding the diets of these five sloths cannot be entirely ruled out and will require further studies.

Conclusions

Several characteristics of the humerus CAV 1466 (i.e., size, development of greater and lesser tubercles, shape of the head, deltopectoral shelf, and the notch between the trochlea and the capitulum) are similar to those of *Nothrotheriops*, allowing us to assign CAV 1466, as well as MACN–Pv 10848, to *Nothrotheriops* sp. After reexamination of other materials (femur, tibia) originally assigned to *Nothrotherium*, they must be reassigned to *Nothrotheriops* sp., providing additional strong evidence for the presence of this taxon in several parts of Uruguay. These records are consistent with the recently reported materials from Argentina, providing new knowledge on the geographic distribution of this taxon. The analysis of stable isotopes shows contrasting results between the $\delta^{18}O$ values from the AdV *Nothrotheriops* and those obtained from other ground sloths recorded at the site. This is consistent with the existence of complex open mosaic habitats with patches of mixed vegetation in the region and the climatic variations that occurred during the Late Pleistocene. Lastly, the $\delta^{13}C$ results for *Nothrotheriops* sp. from AdV are consistent with diets based mainly on C_3 plants. In this way, and taking into account previous results, it is possible to infer a relative dietary differentiation among the sloths at the AdV site, with mylodontids being grazers at different levels of specialization, and the scelidotheriid and nothrotheriid being more selective browsers.

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Data availability All the data required to reproduce the analyses reported in this work is available in the main text or the available supporting information. The 3D model of the specimen is available at MorphoMuseuM (Varela 2023; https://doi.org/10.18563/journal.m3.191).

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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3D finite element analysis and geometric morphometrics of sloths (Xenarthra, Folivora) mandibles show insights on the dietary specializations of fossil taxa

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ABSTRACT

Sloths are represented today only by two distantly-related small, arboreal, and folivorous genera. However, the fossil record of the clade is composed of many more taxa, with much more diverse morphologies, including giant terrestrial forms with no clear modern analogs. In this context, several approaches have been implemented in order to explore the ecological adaptations of fossil taxa and, in particular, their dietary preferences. In this work we used 3D Finite Elements Analysis (FEA) coupled with Geometric Morphometrics (GMM) to explore the ecomorphology of sloths and possible differences among taxa related to dietary adaptations. Digital models of the mandibles of 14 taxa were obtained (two extant and twelve extinct), representing members of all the major clades within the group. We modeled the actions of the three major muscles involved in mastication (masseter, temporalis, and pterygoideus) and simulated unilateral mastication in four different conditions, one for each tooth along the toothrow. The results were analyzed qualitatively regarding the distribution of von Mises stress (vMs) and quantitatively using the mesh-weighted arithmetic mean (MWAM) vMs, strain energy (SE), and mechanical efficiency (ME). Differences in the distribution of high-vMs areas and lower SE values were found among taxa predicted to be grazers in comparison to those predicted to be browsers. Furthermore, when simulating biting with the most anterior tooth, we observed considerably higher vMs and SE values in taxa with a caniniform, which could indicate its involvement in sexual display rather than in food processing, acquisition, or other strenuous activities.

1. Introduction

The superorder Xenarthra has been known in South America since the Paleocene (Scillato-Yané, 1976; Pascual and Ortiz Jaureguizar, 2007), with some taxa being present in the Caribbean Islands as early as the Oligocene (MacPhee, 1995), and several genera reaching Central and North America during the Great American Biotic Interchange (Webb, 1976, 2006; Woodburne, 2010; Campbell Jr. et al., 2010). It is made up of the Orders Cingulata (armadillos, pampatheres, and glyptodonts) and Pilosa, with the latter composed of the suborders Vermilingua (anteaters) and Folivora (arboreal and ground sloths; Gaudin and McDonald, 2008). Although the Folivora are represented today by just two genera, the arboreal and relatively distantly related sloths *Bradypus* and *Choloepus*, their fossil history is much more diverse, with more than 90 recorded genera (McKenna and Bell, 1997). Traditionally, based on morphological characters, the Folivora are divided into five monophyletic families: Bradypodidae, Megatheriidae, Nothrotheriidae, Megalonychidae, and Mylodontidae (Gaudin, 2004; Varela et al., 2019). However, recent studies based on molecular data have shown a different picture regarding the systematics of the group. In this regard, the two most recent studies using protein (Presslee et al., 2019) and DNA (Delsuc et al., 2019) data, respectively, showed the existence of at least eight families, separating the Greater Antilles sloths in their own family (Megalocnidae) and considering the Scelidotheriidae and

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Choloepodidae as families within Mylodontoidea, as well as placing Bradypodidae within the Megatherioidea.

Several cranial and mandibular morphological characteristics of sloths have been used in paleobiological analyses to infer the dietary preferences of extinct taxa (Bargo, 2001; McDonald, 2005; Bargo et al., 2006a; Bargo and Vizcaíno, 2008; Pujos et al., 2012). Sloths have a characteristic dentition, lacking enamel in all teeth and showing morphologies that do not resemble any of the common characteristics observed in the rest of the mammals (Vizcaíno, 2009). In particular, sloths show lack of deciduous teeth and hypselodont ever-growing teeth, as well as a reduction of their dental formula, lacking incisors and true canines, and having no more than five upper and four lower teeth per side (with several taxa showing also the loss of the first tooth). Furthermore, sloths lack a clear differentiation between canines, premolars, and molars, making assumptions of homology between their teeth loci and that of other mammals difficult. In this regard, several sloths possess a first tooth with a caniniform morphology. The presence of caniniform is common in the Megalocnidae, Mylodontidae, Megalonychidae, and some Nothrotheriidae. On the other hand, changes of this tooth to a molariform morphology are recorded for example in the Scelidotheriidae and Megatheriidae, while its complete loss is well registered in derived members of Nothrotheriidae (Pujos et al., 2016). Furthermore, the exact homology of each tooth loci in sloths, both in relation to other mammal groups as well as within the clade, and the evolution and ancestral form of the sloth dental formula remains considerably unknown. In fact, recently, Hautier et al. (2016) showed that the first upper and lower teeth in *Bradypus* and *Choloepus* are not homologous, while Delsuc et al. (2019) predicted the presence of caniniform as an ancestral character for the clade when using both molecular and morphological phylogenies. Moreover, Varela et al. (2020) showed the existence of a pattern consistent with the Inhibitory Cascade Model, indicating that for most of the sloths, the last three molariforms seem to have evolved following constraints imposed by the balance between inhibition and activation during molar development, as is seen in other mammalian clades (Kavanagh et al., 2007). However, this pattern was not apparent when including the caniniform, showing that the highly-variable first tooth probably evolved under different evolutionary pressures. Among extant sloths, *Bradypus* does not present a caniniform, while *Choloepus* has a caniniform with vertical projection, similar to some extinct megalonychid genera (Naples, 1982). The use of the caniniform in *Choloepus* is mainly related to the acquisition of food and the cutting function (Naples, 1985), although there are also records of its use in active defense against threats (Naples, 1982). Furthermore, the wear of the caniniforms and the formation of the occlusal surface in *Choloepus* is produced by the interaction between both caniniforms in exclusive and independent movements, not related to its use in feeding (Naples, 1985). While the use of the caniniform in fossil taxa has not been extensively studied, a recent study by Varela et al. (2022) proposed that the extensive enlargement of the caniniform in the mylodontid *Lestodon armatus* represents a dimorphic structure and could be related to sexual selection, showing that caniniforms could have been used for different purposes besides feeding. Furthermore, other studies have also shown dimorphism in the caniniforms of fossil taxa (McDonald, 2006; Cartelle et al., 2019), and preliminary results of one of us indicate the existence of sexual dimorphism in the caniniforms of extant taxa (RKM; unpublished data).

Most fossil ground sloths are interpreted to have been herbivorous animals. Some taxa have been previously interpreted as selective browsers, while others have been suggested to be grazers (see for example Bargo and Vizcaíno, 2008; Shockey and Anaya, 2011). Furthermore, some fossil sloths have been proposed to be opportunistic omnivores, potentially feeding on animal matter, perhaps as carrion-eaters (Fariña, 1996; see also Tejada et al., 2021). In this context, several approaches have been implemented to explore the ecological adaptations of fossil taxa and, in particular, their dietary preferences. For example, several authors studied the jaw biomechanics of extinct species in order to explore potential dietary implications related to form and function (e.g., Naples, 1985; Bargo and Vizcaíno, 2008; McAfee, 2011), showing a wide range of variation and possibly correlations with dietary habits. Likewise, studies based on the form of the skull, specifically the muzzle, were conducted by Bargo et al. (2006b) and Shockey and Anaya (2011), indicating some degree of grazing and bulk feeding in some extinct taxa (specifically some mylodontids). Furthermore, other studies have focused on the signals provided by the microwear (e.g., Resar et al., 2013; Kalthoff and Green, 2017) and mesowear (Saarinen and Karme, 2017) observed in teeth, showing more evidence of specializations to different plant materials among taxa. On the other hand, some studies have looked at the contents of coprolites found mostly in caves in South and North America and the Great Antilles, finding evidence of varied diets (see Hunt and Lucas, 2018 for a review). Finally, stable isotopes analyses have further increased the knowledge on the dietary preferences of extinct taxa, with examples showing differences among taxa, but also some intraspecific differences probably related to age or geographic precedence (e.g., Czerwonogora et al., 2011; Larmon et al., 2019; Lobato et al., 2021). Interestingly, a recent study by Tejada et al. (2021) based on isotope data from specific amino acids showed evidence in favor of an omnivorous diet for the mylodontid *Mylodon darwinii*. This finding would be compatible with the previous propositions regarding *Megatherium americanum*, for which isotope analyses were inconclusive (see Bocherens et al., 2017; Fariña and Varela, 2018).

In this context, the absence of living close relatives of fossil ground sloths that can be considered as analogs for comparative analyses pose certain limitations for these types of studies (Vizcaíno and Bargo, 2014). Therefore, it is important to explore other potential living analogs despite their phylogenetic closeness, as well as incorporate new techniques and methods for the evaluation of potential dietary adaptations in fossil taxa without clear analogs. The use of three-dimensional reconstructions, simulations, and Finite Element Analysis (FEA) (Hughes, 2012) for the study of various biological structures has recently increased. These techniques allow the evaluation of stresses in complex structures submitted to different loading conditions. The geometry of biological structures is idealized and modeled using meshes formed by finite elements such as tetrahedra, and boundary conditions such as supports or forces are imposed to the surface or nodes of the mesh. For this reason, they are widely used in studies of biological structures, since they allow us to appreciate their reactions to different possible scenarios (Richmond et al., 2005; Ross, 2005). In paleontology, cranial (Whitenack et al., 2011; Young et al., 2012) and postcranial (Arbour and Snively, 2009; Milne and O'Higgins, 2012) structures have been analyzed. In particular, several authors have used these approximations for the study of the dietary habits of fossil species. For example, Marcé-Nogué et al. (2020) analyzed fossil hominins to evaluate adaptations to hard-food processing, Fletcher et al. (2010) studied the mandibles of ungulates to predict the mode of digestive physiology, McHenry et al. (2007) explored the hunting strategy of the American sabercat *Smilodon fatalis* through FEAs, while Tseng et al. (2016) tested a unique prey-capture-mastication sequence in the extinct marine arctoid *Kolponomos*.

Regarding the Xenarthra, Serrano-Fochs et al. (2015) studied the jaws of several extant and extinct Cingulata in 2D to evaluate differences and predict the diets of extinct taxa. Their results showed a high correlation between the stress patterns of the jaw with potential diet preferences, differentiating between insectivorous, omnivorous, and herbivorous taxa. In another work, Patiño et al. (2021) analyzed xenarthran's ungual phalanxes using 2D FEA to explore the locomotor behavior of extinct taxa, showing that mylodontid ground sloths were probably better adapted to strenuous activities such as digging. Recently, Bomfim Melki et al. (2022) performed FEAs in 2D sloth's mandibles to explore dietary adaptations. Their results did not display a clear correlation with the dietary habits previously predicted for several extinct taxa, but the authors argued that the obtained grouping could be related to ecological specialization. However, the results of 2D analyses when studying primate mandibles have recently been questioned by Smith et al. (2023), who argued that this type of analysis should not be used to draw functional, ecological, or evolutionary conclusions. Nevertheless, it is important to note that in certain conditions, plane models can display the same differences as 3D models and therefore be useful for comparative purposes (Lautenschlager et al., 2020). In addition, FEA has been extended to the study of various sexual traits to understand their operation and importance during mating. Finite element analyses have been applied to the study of horns in dynastic beetles (McCullough et al., 2016) and jaws in lucanid beetles (Goyens et al., 2015) in intraspecific combat. In another example, Farke (2008) used FEA to study horns in goats during intraspecific fighting. For this reason, McCullough et al. (2016) suggests FEAs as an ideal methodology for the study of exaggerated structures, especially weapons, that may be the product of sexual selection.

Geometric morphometrics (GMM) is a growing field based on the study of form through the use of Cartesian landmarks representing the key features describing the form of organisms (Zelditch et al., 2004). Geometric morphometrics expanded and improved the power of traditional morphometrics which rely mostly on length measurements, allowing researchers to better account for the variations in shape. Furthermore, geometric morphometric approaches have been used in conjunction with FEA to further explore the relationship between form and function. In fact, Polly et al. (2016) recently argued that combining FEA and GMM represents a unique approach capable of addressing new questions regarding the evolution of the form and function of extant and extinct taxa.

In this contribution, we use 3D FEA in combination with GMM to explore the ecomorphology of sloths and the potential differences among taxa that could indicate different dietary and ecological adaptations in fossil members of the clade.

2. Methods

Institutional abbreviations - CAV: Colección Arroyo del Vizcaíno, Sauce, Canelones, Uruguay; MHND: Statens Naturhistoriske Museum, Copenhagen, Denmark; MLP: Museo de La Plata, La Plata, Argentina; MNHN.F: Muséum National d'Histoire Naturelle, Paris, France; MNHN-M: Museo Nacional de Historia Natural, Montevideo, Uruguay; MNHNSD: Museo Nacional de Historia Natural, Santo Domingo, República Dominicana; MPAC: Museo Paleontológico Profesor Armando Calcaterra, Colonia, Uruguay; VCU 3D: Virtual Curation Lab, Virginia Commonwealth University, Virginia, USA.

2.1. Taxon sampling and data acquisition

In order to collect 3D data, we used different methods that have proven to provide comparable data (Giacomini et al., 2019). In particular, we obtained surface data using a combination of a DAVID-SLS2 structured light scanner and photogrammetry. Three-dimensional digitizations of the jaw surface of the following fossil species were made: the megalocnids *Acratocnus ye* (MNHNSD FOS 25.1217) and *Neocnus* cf. *dousman* (MNHNSD FOS 25.1214); the mylodontids *Lestodon armatus* (MPAC 899), *Glossotherium robustum* (MNHN-M 914), *Paramylodon harlani* (VCU 3D 3213), and *Mylodon darwinii* (CAV 379); the scelidotheriids *Scelidotherium leptocephalum* (MNHN-M 137,722) and *Valgipes bucklandi* (NHMD.Z.M.K. 1/1845:3540); the nothrotheriids *Nothrotherium maquinense* (NHMD.Z.M.K. 1/1845:13937) and *Thalassocnus natans* (MNHN.F.SAS734); the megalonychid *Megalonyx jeffersonii* (VCU 3D 3943); and the megatheriid *Megatherium americanum* (MLP 2–56). In addition, 3D models of the extant sloths *Choloepus hoffmanni* and *Bradypus variegatus* were obtained from the Digimorph online repository (Rowe, 2002). The 3D models were processed with Meshlab v2016.12 (Cignoni et al., 2008) in order to clean the scanned data. Missing parts in the scanned specimens were filled using their symmetrical counterparts and, in a minority of cases, holes and missing teeth were filled so that the surface of the scan would follow the expected continuity of the bone. Some of the 3D models used for the study are available in a data paper by Varela and Tambusso (2023).

The sampled taxa cover all the main groups of sloths commonly recognized in the literature, with representatives of all the currently identified families, namely Megalocnidae, Megalonychidae, Megatheriidae, Mylodontidae, Nothrotheriidae, and Scelidotheriidae. Furthermore, the taxa cover a wide range of the dental morphologies seen in fossil Folivora, including small and enlarged caniniforms, as well as the change of the caniniform to a molariform morphology or its complete loss (Naples, 1985; Pujos et al., 2016; Varela et al., 2022). Moreover, different dietary preferences have been predicted for the included taxa, namely semi-arboreal browsers, open-habitat browsers, and grazers (Bargo and Vizcaíno, 2008; Pujos et al., 2012). Also, several of the studied taxa inhabited similar environments and some of them are recorded as co-occurring at some sites, showing that these taxa probably competed for resources or specialized in different food types (McDonald et al., 2013; Varela and Fariña, 2016; Varela et al., 2018; McDonald, 2021).

Furthermore, in order to account for the phylogenetic relationships of the studied taxa and its influence on the form and function of the evaluated mandibles, we used a phylogenetic framework based on the morphological phylogeny proposed by Varela et al. (2019) with a backbone constraint derived from the molecular results of Delsuc et al. (2019). For our study, all the taxa that were not sampled in our analysis were pruned from the final tree.

2.2. Finite element analysis

The FEA models were meshed using solid tetrahedral elements in the NetGen v6.0 software (Schöberl, 1997). The resulting meshes were composed of ~300,000 elements, as was shown to be the optimum in a study by Tseng et al. (2011). The meshes were then imported in the FEBio Studio software tool (Maas et al., 2012) to execute the finite element analyses. FEBio is an open-source software tool developed for FEA in biomechanics and biophysics.

The jaws were treated as homogeneous solids with a linear isotropic constitutive behavior. Although more complex models with differentiation of structures and material behaviors could provide more realistic results, the main objective of this work focuses on large structural differences in the mandible during mastication based on comparisons between different taxa. Such approximations have been previously used for the study of other taxa with success (Marcé-Nogué, 2020). Furthermore, more complex models result in computational-heavy analyses and, more importantly, can result in problems when modeling certain structures that lack reliable information in some extinct taxa, ultimately reducing the comparability between models (Marcé-Nogué, 2020). Therefore, this simplification is not considered to represent a drawback for the present study (Gil Espert et al., 2015). As sloths present a completely fused symphysis, this part of the mandible was given the same properties as the rest of the bone. The properties of the material were obtained from the literature. Since there are no estimates of Young's modulus or specific Poisson's ratio for xenarthrans, lamellar bone values previously estimated in carnivores were used, with a Young's modulus of 20 GPa and a Poisson's ratio of 0.3 (Tseng, 2008; Tseng et al., 2011).

The areas of insertion of the masseter, temporalis, and pterygoideus muscles and their respective lines of action were delimited based on observations on fossils and existing descriptions in the literature (Fig. 1; Naples, 1982, 1985; Bargo and Vizcaíno, 2008; McAfee, 2011). In all cases, a homogeneous pressure equal to 0.3 N/mm^2 was used (maximum tension produced by mammalian muscle fibers; Wroe et al., 2005), which allowed the different simulations to be carried out without the use of volume or force scaling (Dumont et al., 2009). Therefore, the total force input of each model was calculated by multiplying the muscles

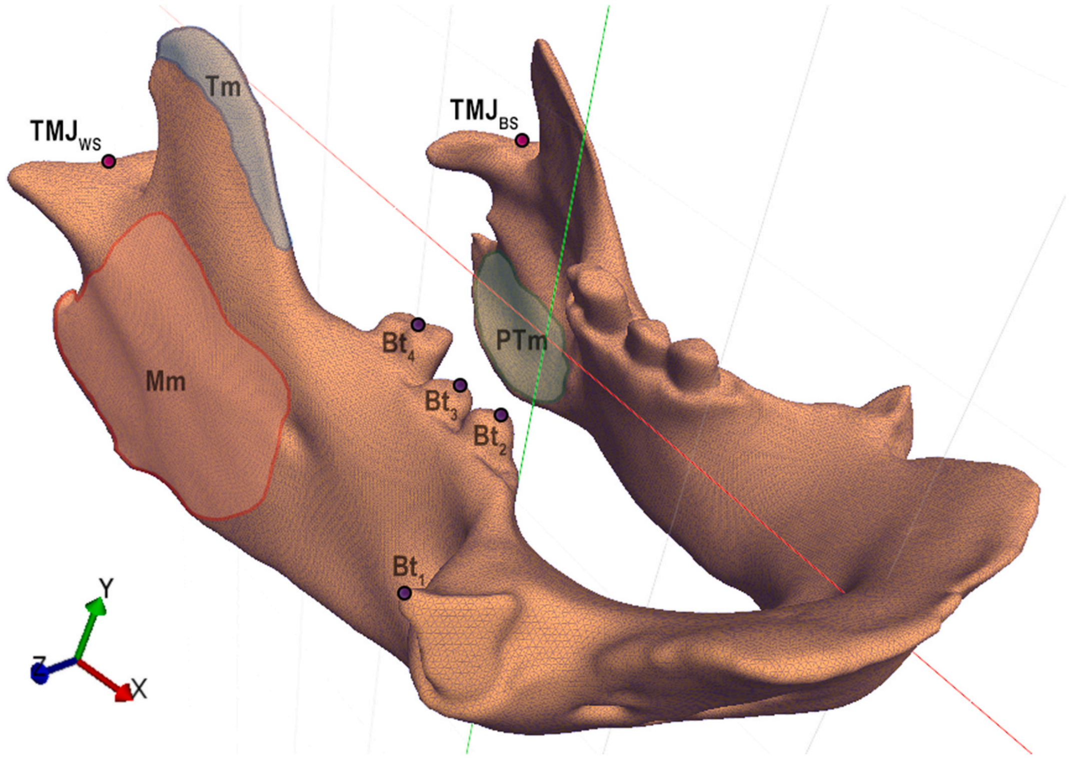

Fig. 1. Diagram of the finite element mesh of a mandible of *Lestodon armatus* with reference points used for support conditions or loads application. Bt: biting point, TMJ: temporomandibular joint, Mm: Masseter muscle insertion area, Tm: Temporalis muscle insertion area, PTm: Pterygoideus muscle insertion area.

insertion areas and the maximum tension.

The boundary conditions were established to simulate different situations during mastication. We simulated unilateral mastication (the balancing-side muscle forces were adjusted to 60%; Dessem, 1989), setting the following boundary conditions using three nodal constraints: left and right temporomandibular joints (TMJ; preventing all translational movement except in the mediolateral "z" axis of the joint on the balancing side) and bite point (preventing translational movement in the vertical "y" axis). We modeled four different scenarios, one for each tooth along the toothrow (Fig. 1; Bt1, Bt2, Bt3, and Bt4). Considering the different dental formulas in different taxa, we considered Bt1 as the first tooth in all taxa except the Nothrotheriidae, where the loss of the caniniform is well documented.

The results were analyzed in terms of von Mises stress (vMs), which represents a good estimate to detect failure in isotropic materials under a ductile deformation regime and have been used previously in bone (Nalla et al., 2003), and total strain energy, which represents a robust metric for comparing the mechanical efficiency of structures modeled with finite elements (Dumont et al., 2009). Stresses were qualitatively evaluated in relation to their distribution in the jaws. For quantitative comparisons, the average vMs and the vMs for each element in each simulation were recorded. Considering that Marcé-Nogué et al. (2016) showed that the common central tendency statistics used to compare among models are problematic due to the nature of FEA data when the used meshes are not uniform regarding element size (something probable in meshes representing biological complex structures), we used the mesh-weighted arithmetic mean (MWAM) of the vMs to compare among models. Furthermore, we recorded the total strain energy and obtained the Adjusted Strain Energy (SE; Dumont et al., 2009), using *Glossotherium robustum* as standard, as: $SE_{x,adjusted} = (Volume_x/Volume_{G.\ robustum})^{1/3}(InputLoad_{G.\ robustum}/InputLoad_x)^2*SE_x$. This adjustment is necessary considering that strain energy requires equal loads and volumes to make comparisons among models, but our models have equal force:surface area ratios (which allow the direct comparison of stress values; Dumont et al., 2009). Finally, we calculated the phylogenetic signal for the MWAM vMs values in the Bt4 scenario using the phytools R package function "phylosig" and plotted the ancestral reconstruction for these values using the function "contMap" (Revell, 2012).

2.3. Geometric morphometrics analysis

For the GMM analysis we employed an automatic landmark generation approach implemented in the Auto3dgm (Boyer et al., 2015) module in the software 3D Slicer (Fedorov et al., 2012). In contrast to other GMM methods available, which are based in the manual placement of homologous landmarks, Auto3dgm employs a fully automated method to place equidistant landmarks on the 3D model in order to fully describe its surface. This approach is ideal in cases where homology is difficult to address, and has the potential to accurately describe shape and allow comparisons in significantly different forms, reducing the subjectivity related to the placement of landmarks. Furthermore, the possibility to place several hundreds or thousands of landmarks per model with minimal user intervention and in a reduced time, greatly expands the capacity to study complex morphologies and large samples while accounting for the whole shape. Nonetheless, it is important to address that the absence of homology between landmarks can result in different artifacts when comparing taxa, particularly if one is interested in specific structures and not the general form. In our case, we considered the use of Auto3dgm as appropriate considering that we are interested in the changes in the general form of the mandible in sloths. For this analysis we used only hemimandibles and set 1024 landmarks homogeneously distributed on the surface of the models. These landmarks were saved in their original orientation and scale and were later further processed and analyzed in the R environment (R Core Team, 2022).

Landmark data were analyzed using the R package geomorph (Adams and Otárola-Castillo, 2013). Specifically, three-dimensional landmark coordinates were submitted to a Generalized Procrustes Analysis (GPA; Gower, 1975) using the geomorph's function "gpagen" in order to translate all specimens to the origin, scale them to unit-centroid size, and optimally rotate them. Then, we calculated the phylogenetic signal from Procrustes shape variables using the geomorph's function "physignal". In particular, the function employs a multivariate version of the K-statistic (Adams, 2014), evaluating the strength of the phylogenetic signal in relation to what would be expected under a Brownian motion model of evolution. Also, the resulting aligned Procrustes coordinates were used to perform a phylogenetically-aligned components (PaCA; Collyer and Adams, 2021) using the geomorph's function "gm.prcomp" in order to generate a depiction of the morphospace based on the ordination of shape data. Finally, we used the "btShapes" function of the StereoMorph package to generate theoretical back-transformed mandible shapes to depict shape variability across the morphospace defined by PC1 and PC2 (see Olsen, 2017).

3. Results

3.1. Finite element analysis

In general terms, all the studied specimens showed relatively high-stress areas in the condylar process, as well as the anterior margin of the coronoid process and the mandibular symphysis (Fig. 2). As expected, the highest vMs values were located at, or near, the locations where constraints were placed. There was no clear pattern among all taxa showing higher vMs areas in any specific scenario, with some taxa like *Lestodon armatus*, *Glossotherium robustum*, *Neocnus* sp., and

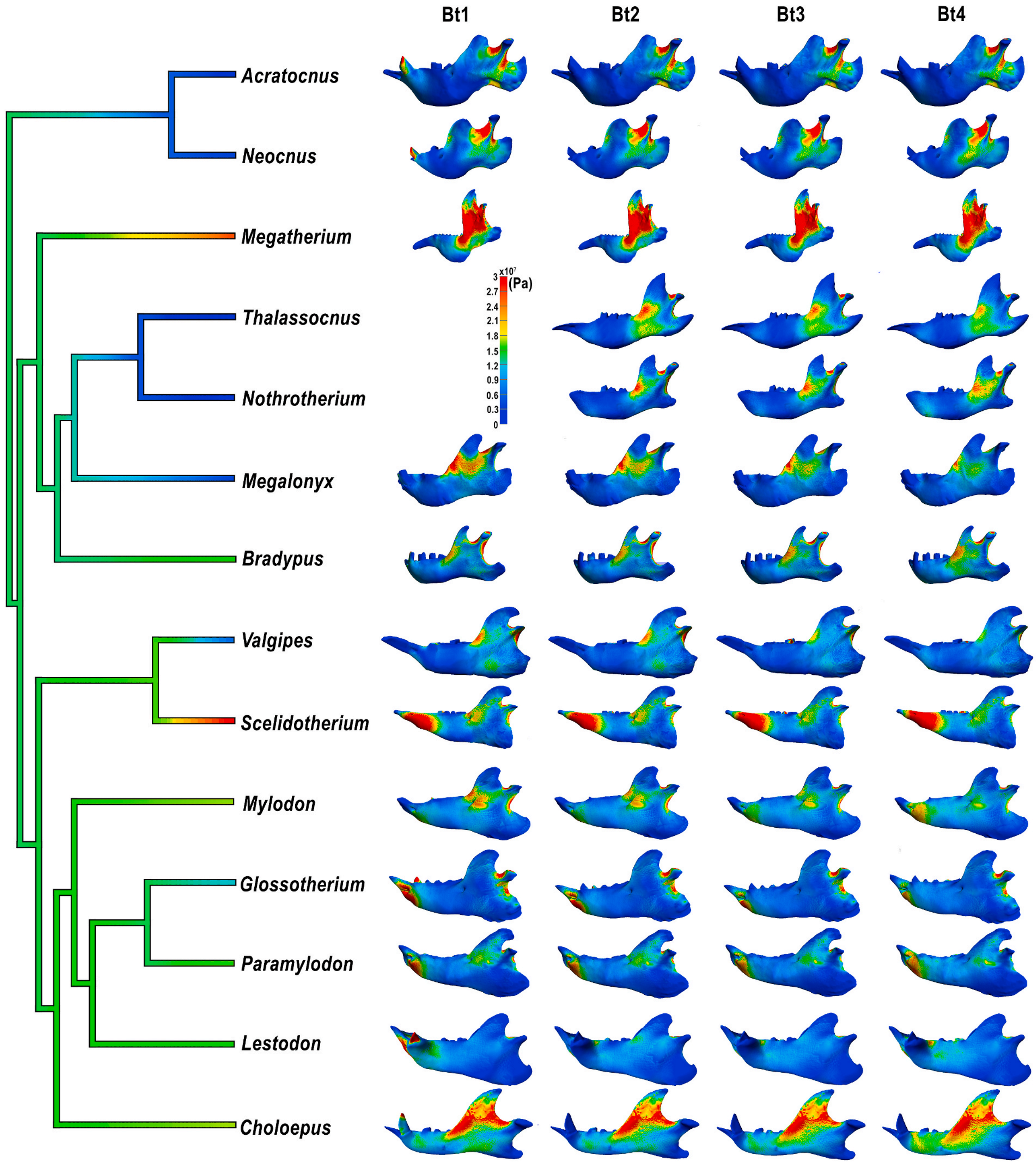

Fig. 2. Phylogeny showing the ancestral reconstruction of the MWAM vMs values for the Bt_4 simulation, as well as the vMs distribution on the mandibles for each biting point along the toothrow for each taxa.

Acratocnus ye showing relatively higher vMs when biting with the most anterior tooth and other taxa like *Mylodon darwinii*, *Paramylodon harlani*, and *Scelidotherium leptocephalum* showing higher vMs when biting with the posterior teeth. This pattern seemed to be related to the presence, or not, of a caniniform in the first tooth loci rather than to the phylogenetic closeness of taxa. In other words, taxa with a caniniform tended to show higher vMs values when biting with these teeth, while other taxa without the caniniforms showed lower vMs values in this scenario but vMs became higher in the posterior teeth simulations. Other taxa, like the Antillean sloths, *Neocnus* sp., and *Acratocnus ye*, and *Megalonyx jeffersonii* and *Thalassocnus natans* showed a flatter pattern, with all biting simulations showing considerable similar vMs values (Fig. 3). This result is interesting, considering that different morphologies are present among these taxa, with *M. jeffersonii* having upper and lower caniniforms that “crush” when occluding while Antillean sloths such as *A. ye* show a “shearing” or “cutting” occlusion pattern, and *T. natans* lack caniniforms. On the other hand, when comparing the absolute values among the studied taxa, considerable differences in the distribution of high-vMs areas and SE values were found. With some taxa showing higher vMs and SE values in all scenarios. In particular, *Megatherium americanum*, *Scelidotherium leptocephalum*, and the extant sloths *Choloepus hoffmanni* and *Bradypus variegatus* showed the highest vMs, while *Valgipes bucklandi*, *Megalonyx jeffersonii*, *Neocnus* sp., and *Acratocnus ye* showed the lowest vMs values (Figs. 3–4). Furthermore, the performance of the mandible, evaluated as vMs, showed no significant phylogenetic signal (Bt_4 scenario: K = 0.45; $p = 0.66$).

3.2. Geometric morphometrics analysis

The obtained phylomorphospace showed considerable spread of the studied taxa, with a low level of overlap, especially between non-closely related species (Fig. 5). This pattern clearly shows a general lack of convergent evolution, and a strong tendency of closely related taxa to show similar mandibular shapes. In fact, the phylogenetic signal was significant for the shape (K = 0.925; $p < 0.001$) and centroid size (K = 1.221; $p = 0.003$), confirming the observed tendency. Furthermore, the results showed a clear segregation along the PC1 of the two main clades of sloths, with Mylodontoidea placed on the negative size and Megatherioidea on the positive size. When considering the two more informative components depicting the phylomorphospace, the variation in PC1 seemed to depict the horizontal length of the mandible (with taxa on the positive size showing shorter mandibles but more prominent spouts), while the variation in PC2 showed the vertical length, as well as the robustness of the horizontal ramus (with taxa on the negative size showing the prominent bulge commonly observed on Megatherioidea).

4. Discussion

We found considerable differences in the distribution of high-vMs areas and MWAM vMs and adjusted SE values among the studied taxa. Moreover, differences were also found when considering the different biting scenarios, with some taxa showing higher efficiencies when biting with anterior teeth and others being more efficient when biting with posterior teeth, which could be indicative of the use of front teeth in food procurement in more selective species and the use of back teeth for tougher food processing in bulk feeders. On the other hand, the phylogenetic signal for the vMs values was non-significant, indicating that more closely related species do not particularly tend to show similarly MWAM vMs values. Considering the diversity of dietary habits predicted for different fossil sloths’ taxa, the obtained results could be related to different dietary adaptations that would pose constraints on the performance of the mandible. Our results show differences with those of Bomfim Melki et al. (2022) regarding the observed stress patterns and similarities among the studied taxa. However, it is important to note that Bomfin Melki et al. (2022) chose the anterior tip of the mandible as the fixed position, and therefore the “biting” point, instead of the approximate position of each tooth. This decision could have a great influence on the outcome of FEAs and the biological interpretation of the results, since the simulation would not clearly represent mastication in sloths (which lack incisors and many times present a diastema between the first tooth and the cheek teeth), but the potential use of the spout in the anterior part of the mandible. In fact, previous studies have related the shape of the muzzle, and consequently the mandible’s spout, to the level of food selectivity (Bargo et al., 2006b; Shockey and Anaya, 2011; Boscaini et al., 2019). Considering this, it is reasonable that, as the autors point out, Bomfim Melki et al. (2022) results could potentially relate to ecological specialization and not specifically dietary habits related to mastication.

Our results for the two extant sloths were consistent with their known diets. *Bradypus* showed relatively high vMs and SE values, consistent with a diet based almost completely on leaves (Saarinen and Karme, 2017). On the other hand, *Choloepus* showed similar vMs values, but a comparably stiffer (lower SE) mandible, which could correspond with the more variable diet known for this sloth (Adam, 1999). Nevertheless, both extant sloths showed relatively high vMs and SE values, as well as extended areas of high vMs in their mandibles, which clearly show the lack of adaptations to hard food processing. Mylodontid taxa generally considered to be grazers, like *Glossotherium robustum* and *Lestodon armatus*, showed smaller high-vMs areas and lower MWAM vMs and SE values when biting with posterior teeth, consistent with the requirements of grass processing and an enlargement of the masseter muscle (Bargo and Vizcaíno, 2008; Varela and Fariña, 2015). Other mylodontids, such as *Mylodon darwinii*, *Paramylodon harlani*, and,

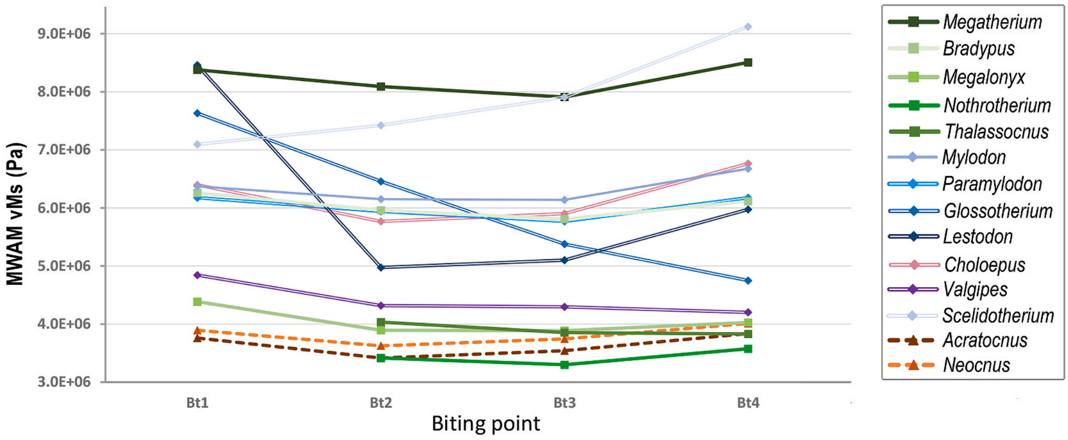

Fig. 3. Plots showing the MWAM vMs for each biting point along the toothrow for each taxa.

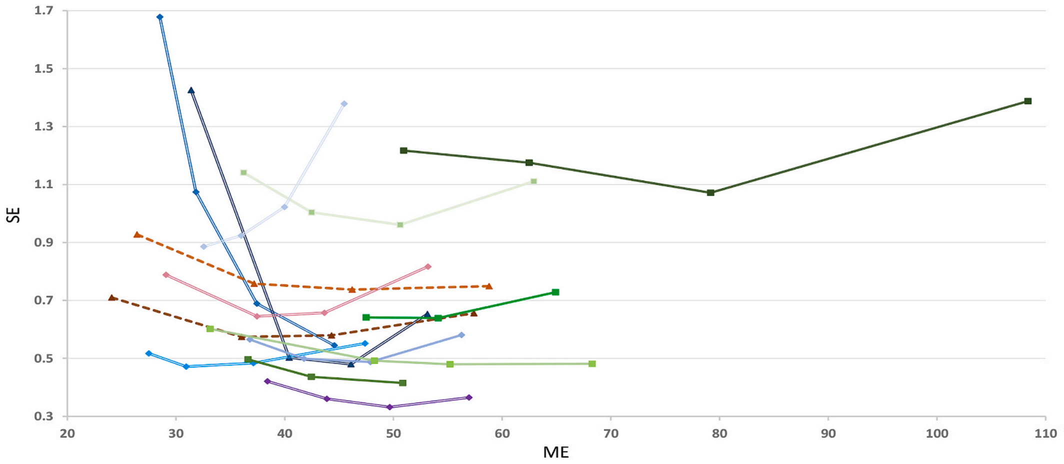

Fig. 4. Bivariate plot showing the Adjusted Strain Energy (SE) and Mechanical Efficiency (ME) for each biting point along the toothrow for each taxa. Color references as in Fig. 3.

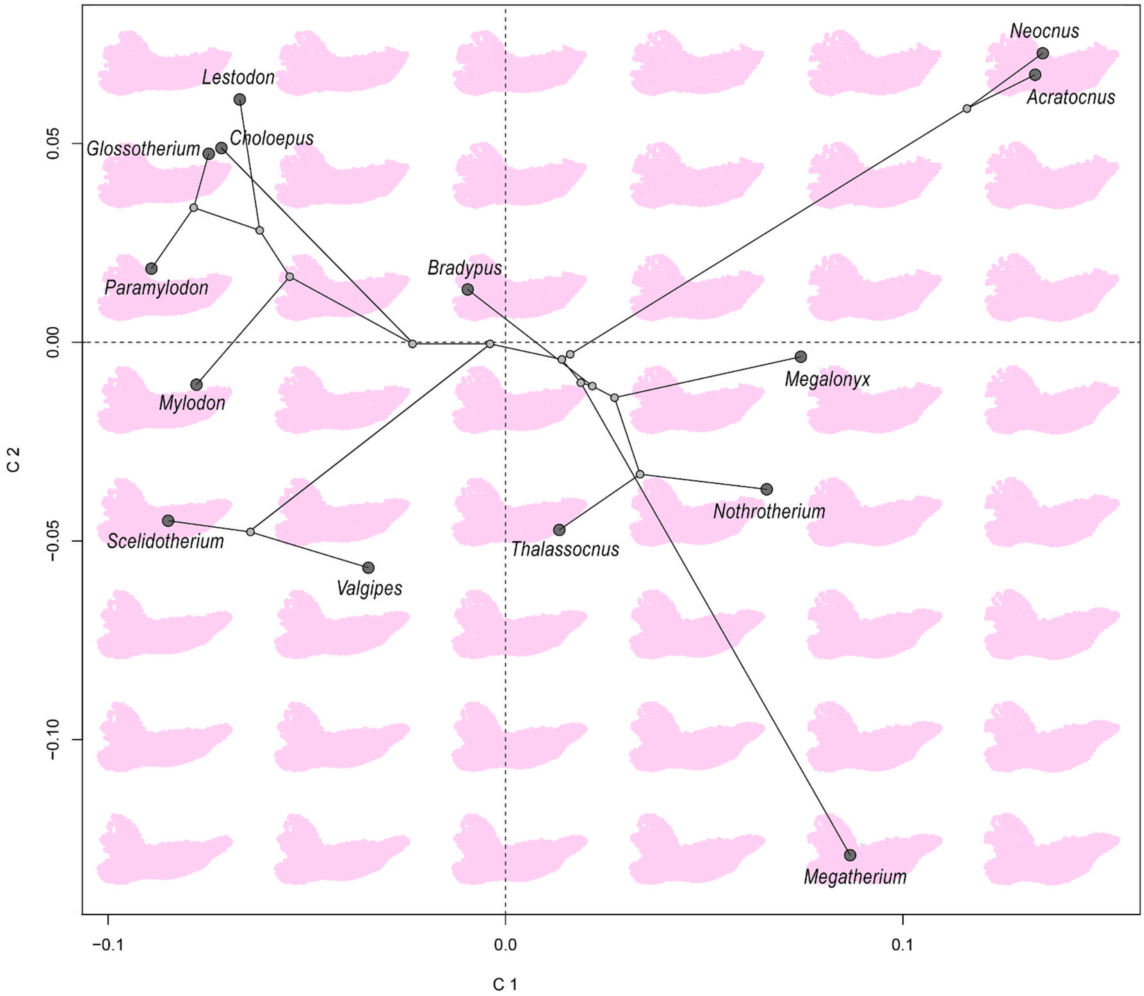

Fig. 5. Phylomorphospace depicted by the first two components of the GMM results. The shades represent theoretical backtransformed shapes obtained from the principal coordinates analysis.

especially, the scelidotheriid *Scelidotherium leptocephalum*, showed higher vMs and SE values, consistent with the increasing consumption of softer foods and more selective/browser habits (Bargo and Vizcaíno, 2008). Contrarily, the scelidotheriid *Valgipes bucklandi* showed considerably lower vMs and SE values, not expected based on its predicted browsing habits (Dantas et al., 2017; Lobato et al., 2021). However, it is important to note that *V. bucklandi* shows a clear morphological convergence with megatherioid sloths, something reflected by its complicated taxonomic history and its original classification as a megalonychid (see Cartelle et al., 2009). In fact, our GMM results showed a clear proximity between *V. bucklandi* and *Thalassocnus natans* regarding the general morphology of their mandibles. This particularity could indicate different feeding habits for this taxon compared to those predicted for the other studied scelidotheriid, *S. leptocephalum*, diverging from the specialist, selective/browser habits predicted for most members of the family (Miño-Boilini, 2012) and including more varied foods. Nevertheless, the obtained differentiation among mylodontoids is interesting, since these taxa have been found co-occurring at some sites and their potential distributions widely overlap at the Río de la Plata region, indicating that a certain niche differentiation could exist among these taxa (Varela and Fariña, 2016; Varela et al., 2018). For example, at least five sloth genera have been recorded at the Arroyo del Vizcaíno site in southern Uruguay, namely *Lestodon armatus*, *Glossotherium robustum*, *Mylodon darwinii*, *Valgipes bucklandi*, and *Nothrotheriops* sp., and different evidence points to the existence of different dietary habits among these taxa (Fariña et al., 2014; Varela and Fariña, 2016; Lobato et al., 2021; Varela et al., 2023). On the other hand, *Megatherium americanum* showed the highest vMs and SE values among the studied taxa, indicating a mandible less efficient for the processing of hard food objects. These results would be consistent with diets composed of fleshy food, like succulent plants and fruits, but also with previous predictions of meat consumption (Fariña and Blanco, 1996; Bargo, 2001). Moreover, *M. americanum* showed considerably higher ME values compared to all the other taxa analyzed, showing a highly efficient jaw for transferring a large part of the muscle input force to the biting force. In fact, we obtained ME values slightly higher than 1 for the Bt_4, indicating more output force than input force and the potential functioning of the mandible as a second-class lever (Davis, 1955; Turnbull, 1970). Cox (2017) discussed the potential functioning of the mandible as a second-class lever in the hystricomorph rodent *Pedetes capensis*, showing the highly efficient jaw and the consequent high-force bites without the increase of the overall adductor muscle mass. In *M. americanum*, this could be the result of constraints on muscular development due to its extremely large size and requirements to maintain high output forces for the processing of food objects. Greater Antilles taxa, *Acratocnus ye* and *Neocnus* sp., and the North American *Megalonyx jeffersonii* showed results consistent with the processing of hard food objects (lower vMs and SE values), which does not correspond with previous findings indicating mostly browsing habits (McAfee, 2011; McDonald et al., 2019). However, our results could indicate the processing of harder food objects like roots and tubers, something that was previously suggested by Antúnez and Suárez (2013) for Greater Antillean sloths based on coprolites analyses. The semi-aquatic nothrotheriid *Thalassocnus natans* showed a similar pattern, although this sloth has been commonly thought as a grazer feeding on seagrass (De Muizon et al., 2004). Although the results are partially compatible with grass diets, an adaptation to processing harder food elements common in marine environments like mollusks and crustaceans can not be entirely ruled out. In fact, De Muizon et al. (2004) mentioned the striking similarity between the dentition of some species of *Thalassocnus* and *Megatherium americanum*, arguing that, according to the analysis of Bargo (2001) in *M. americanum*, this morphology would be indicative of powerful puncturing and cutting. Powerful puncturing would be needed for the processing of the hard exoskeleton of crustaceans and mollusks. For example, Tseng et al. (2016) found low vMs and SE values for the durophagous marine bear *Kolponomos*, showing that it probably crushed prey with emphasis on stiffness rather than increasing force and ME. Finally, the nothrotheriid *Nothrotherium maquinense* showed extremely low MWAM vMs and SE values, which could indicate an adaptation to hard-food processing. However, this sloth has been predicted as a tree climber that fed on leaves and fruits of xeric vegetation in hot and dry climates (Duarte and Souza, 1991; Cartelle, 1999). In this case, it is important to note that the scanned mandible probably belongs to a sub-adult individual, and this fact could pose a limitation to the analysis due to a limited development of the skull and masticatory muscles in the specimen.

A common pattern observed between the studied taxa is related to the presence and relative development of a caniniform morphology in the first tooth. Taxa with a caniniform showed a pattern of stress and stiffness where significant higher values were observed when biting with this tooth. This pattern was present in the megalocnids, but was really evident in the mylodontids with enlarged caniniform like *Lestodon armatus* and *Glossotherium robustum*. Overall, taxa with caniniforms showed much higher SE values when biting with the caniniform than when biting with molariforms. Moreover, in some cases, like *Lestodon armatus* and *Glossotherium robustum*, the obtained SE were considerably higher than the maximum values observed in all the studied taxa. These results show that, when biting with the large caniniform present in some taxa, the mandible showed considerably low ME and high vMs and SE values, which would render it less efficient for biting hard objects. These results show that, besides diet, the presence of such attributes as potentially sexually selected large caniniforms (Varela et al., 2022) in some taxa, might also be an influence on the mechanical response of the mandible when biting. In this regard, Varela et al. (2022) argued that in *Lestodon armatus*, the caniniforms could be considered as analogous to the “defenses’’ or “tusks” (incisors or highly modified canines, with continuous growth and projecting outside the oral cavity) present in other groups of mammals such as Hippopotamidae, Moschidae, Odontoceti, Pinnipedia, Proboscidea, Rhinocerotidae, Sirenia, and Suidae (Mansfield, 1958; Peyer, 1968; Schmidt and Neil, 1971; Marsh, 1980; Best, 1981; Haynes, 1991; Hillson, 2005; Mihlbachler, 2005), where these large teeth are used as weapons and/or sexual display (Silverman and Dunbar, 1980; Haynes, 1991; Lincoln, 1994; Anderson, 2002). On the other hand, Boscaini et al. (2019) recently showed considerable differences in the feeding apparatus of the Late Miocene–Late Pliocene mylodontid *Simomylodon uccasamamensis* related to sexual dimorphism. The authors hypothesized that these differences would be related to differences in feeding preferences between sexes. Therefore, these considerations should not be completely discarded and further studies should be conducted in order to explore the potential differences among sexes in certain taxa.

Finally, the results of the GMM analysis did not show a clear correspondence with the FEA results. Nevertheless, some common patterns were observed within superfamilies. The GMM results showed a strong correlation with the phylogeny, evident in the morphospace and confirmed by a high phylogenetic signal. In this aspect, different tendencies were observed at the superfamily and family levels. The Mylodontoidea were characterized by relatively longer mandibles, shorter vertically, and with less marked spouts, while the Megatherioidea tended to show horizontally shorter and taller mandibles and a more developed spout. The Megalocnidae showed both horizontally and vertically shorter mandibles with small spouts. These differences did not seem to relate to the differences observed in the distribution of vMs, since both high and low vMs were observed in all groups. Nevertheless, within Mylodontoidea, the increase in mandible height seemed to be related with higher vMs. *Valgipes bucklandi* represented an exception, showing lower vMs values than other mylodontoids. However, as mentioned before, *V. bucklandi* shows a clear morphological convergence with some Megatherioidea, evidenced by its proximity to *Thalassocnus natans* in the morphospace, and its mandible is considerably more robust than that of the Scelidotheriidae analyzed, *Scelidotherium leptocephalum*. Within Megatherioidea, a similar pattern can be observed, with the extreme vertical elongation of the mandible of *Megatherium americanum* being related to the high vMs obtained when compared to the relatively vertically shorter mandibles of *Megalonyx jeffersonii*, *Nothrotherium maquinense*, and *Thalassocnus natans*. Finally, the two studied Megalocnidae, *Neocnus* sp.and *Acratocnus ye*, showed a similar morphology between them and a relative proximity to *M. jeffersonii* in the morphospace, which is probably related to the similar vMs and SE patterns obtained for the different biting scenarios. These results show that, although a general pattern of stress is not observed when considering the variation in form among all the studied sloths, a tendency within clades shows lower vMs and SE values in vertically shorter mandibles, indicating considerable adaptations to hard-food processing in these taxa. This pattern could be related to specific specializations, like the grazing habits predicted for most mylodontoids (Casali et al., 2023) or potential habits not commonly predicted for sloths like the possible processing of roots, tubers, fruits, or seeds in Greater Antillean taxa.

5. Conclusions

The results of the FEA and GMM showed significant differences among the studied taxa, providing valuable insights on the potential masticatory adaptations of fossil sloths, as well as the relationship between form and function. The observed differences among taxa are relatively in accordance with the known dietary preferences of extant sloths, as well as previous results and predicted dietary preferences of some extinct taxa, specifically the browser/selective and grazing/bulk-feeding habits in mylodontoidea taxa. Furthermore, some results could show adaptations not previously considered for some taxa, specifically the processing of hard-food objects like roots, tubers, or even certain fruits and seeds in Greater Antillean taxa, or the inclusion of hard elements like mollusks and crustaceans in the diet of *Thalassocnus*. Finally, the results in taxa with extremely enlarged caniniforms do not seem to indicate their use in highly strenuous feeding or digging activities, while their involvement in sex-related activities like sexual display could explain their exaggerated size and should be further explored. The present study shows that the coupling of FEAs with GMM provides a useful approach to explore different aspects of the ecological adaptation of fossil xenarthrans when no clear extant analogs exist. Further efforts could improve the modeling including soft parts and differentiating hard tissues, including more taxa, and considering intraspecific variability, specially in cases where sexual dimorphism has been proposed.

CRediT authorship contribution statement

Luciano Varela: Writing – original draft, Visualization, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **P. Sebastián Tambusso:** Writing – review & editing, Resources. **Jorge M. Pérez Zerpa:** Writing – review & editing, Methodology. **Robert K. McAfee:** Writing – review & editing, Resources. **Richard A. Fariña:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Main data produced and analyzed in the current study is provided as a supplementary file. Other data will be available on request.

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Appendix A. Supplementary data

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A window into a late Pleistocene megafauna community: Stable isotopes show niche partitioning among herbivorous taxa at the Arroyo del Vizcaíno site (Uruguay)

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ABSTRACT

The analysis of stable isotopes on fossil mammals has become a widely used tool for understanding the paleoecology and paleodiet of these organisms. In this work, we study the stable isotope composition of collagen ($\delta^{13}C$ and $\delta^{15}N$) and bioapatite ($\delta^{13}C$ and $\delta^{18}O$) of fossil bones from Arroyo del Vizcaíno, a fossiliferous site in southern Uruguay dated to ~32 ka cal BP. Fourteen taxa were analyzed: the ground sloths *Lestodon armatus*, *Glossotherium robustum*, *Mylodon darwinii*, *Valgipes bucklandi* and *Nothrotheriops* sp.; the glyptodonts *Glyptodon reticulatus*, *Panochthus tuberculatus*, and *Doedicurus clavicaudatus*; the equids *Hippidion principale* and *Equus neogeus*; the proboscidean *Notiomastodon platensis*; the notoungulate *Toxodon platensis*; the saber-tooth felid *Smilodon populator*; and an indeterminate cervid. In general, the percentage of nitrogen in collagen and the C:N ratio were within the limits expected for collagen preservation in fossil bone. The $\delta^{13}C$ and $\delta^{15}N$ results of the herbivores indicated that most taxa consumed high proportions of C_3 plants in open, relatively dry environments, but significant variations were observed. Furthermore, relatively high values of $\delta^{18}O$ in bone bioapatite, which approximately tracks local drinking water, indicated low precipitation and/or high evaporation. Our results show that the herbivorous taxa present at the site covered a considerable range of the grazer-browser spectrum and support the existence of niche partitioning among closely related taxa. Overall, these kinds of approaches are indispensable to better understand how these communities thrived during the Pleistocene in the region, supporting an outstanding number of giant species, before their extinction at the Pleistocene-Holocene transition.

1. Introduction

The study of the biology of fossil organisms usually presents numerous challenges since in many occasions only fragmentary materials are found and these fossil taxa do not have extant analogs. Likewise, a similar situation occurs when studying the paleocommunities to which fossils belong. In this context, the analysis of stable isotopes on fossil mammals has become a widely used tool for understanding the paleoecology and paleodiet of fossil organisms (Domingo et al., 2012; Bocherens et al., 2016, 2017; Dantas et al., 2017, 2019).

An organism's diet can be reflected, for example, in the carbon and nitrogen isotopic composition of the preserved bone collagen. There are different types of plants that herbivores can feed on (C_3, C_4, and CAM). Each of these types has a different carbon isotopic signature since they discriminate $^{13}CO_2$ differently. That is why C_3 plants have an average $\delta^{13}C$ value of −26 ± 2‰, while C_4 plants have an average $\delta^{13}C$ value of −12 ± 1‰ (Bocherens et al., 1996; Gröcke, 1997; Koch, 1998; Zazzo et al., 2000). At each trophic level, the enrichment of $\delta^{13}C$ ranges from +3‰ to +5‰. Therefore, a mammal that consumes C_3 vegetation will show an average $\delta^{13}C$ collagen value of −22‰, while those that feed on C_4 plants will exhibit a value close to −8‰. In carnivores, a predator that feeds on a primary consumer who feeds on C_3 plants will record an average collagen $\delta^{13}C$ value of −18‰, while those that consume C_4 plants will show a value close to −4‰ (DeNiro and Epstein, 1978; Van der Merwe, 1982; Klepinger and Mintel, 1986; Gröcke, 1997; Koch, 1998).

On the other hand, the mineral portion of the bone (bioapatite) also provides information about the food animals consume, but also provides

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information on the environment in which these animals live. In the case of ^{13}C, the information is similar to that obtained from collagen, although the expected enrichment at each trophic level differs, with values ranging from +8‰ to +14‰ and an important influence of body mass (Cerling and Harris, 1999; Froehle et al., 2010; Tejada-Lara et al., 2018).

The $\delta^{18}O$ values are indicators of humidity and temperature. In general, higher $\delta^{18}O$ values both in local water and, subsequently, in bone, are related to high evaporation and/or low precipitation, often caused by warm and/or dry conditions (Larmon et al., 2019). In certain contexts, especially in taxa that obtain water from food (non-obligate drinkers), $\delta^{18}O$ values can indicate to which guild a vertebrate belongs, as, based on its diet, grazers have $\delta^{18}O$ higher values than browsers since C_4 plants are enriched in ^{18}O in relation to C_3 plants (Bocherens et al., 1996; Kingston and Harrison, 2007; Cerling et al., 2008). Furthermore, an enrichment of ^{18}O in certain tissues of C_3 plants can be deduced from the results found for forest vertebrates. For example, Nelson (2013) showed that fruits and roots are more enriched than leaves (~2‰ and ~4‰, respectively), allowing for further distinction between species' dietary preferences.

The late Pleistocene fauna of southern South America has been the focus of several studies since the beginning of the 19th Century, when Charles Darwin collected several species of strange giant mammals during his travels aboard the Beagle (Vizcaíno et al. 2009). The composition of these mammalian faunas was the product of different evolutionary and biogeographical processes that occurred in South America during the Cenozoic (Fariña et al., 2013). In particular, the fact that the continent was mainly isolated from other continental masses during most of the Cenozoic allowed the evolution and diversification of many endemic clades, while the formation of the Panama's Isthmus ended this isolation and produced extensive migration of taxa between North and South America in an event known as the Great American Biotic Interchange (Croft, 2016).

In this context, the spectacularity of the late Pleistocene South American megafauna lies mainly in the abundance of giant taxa, with probably more than 10 species reaching body masses of more than 1000 kg (Fariña et al., 2013). Moreover, many of these taxa do not have modern close relatives or clear analogs, posing challenges for studying them (Vizcaíno et al., 2018). In fact, these peculiarities have been the center of many studies focusing on the ecology of these communities, exploring their trophic relationships, energetics, and specific abundances (Fariña, 1996; Fariña et al., 2014b; Di Giacomo and Fariña, 2017).

In this line, Pires et al. (2015) showed how megafauna interaction networks became more vulnerable after the arrival of modern humans to the continent, while Segura et al. (2016) found that the structure of the Pleistocene food webs was similar to modern faunas despite the exceptional body sizes of many taxa. Also, Varela and Fariña (2016) and Varela et al. (2018) studied the potential distributions of many giant xenarthrans and explored the potential co-occurrence of these taxa in South America, showing that many taxa probably exploited different resources when inhabiting the same regions. Several other studies have focused on the dietary habits of different species in order to better understand their probable roles in their ecosystems.

For example, Bargo et al. (2006) studied the muzzle of several taxa to explore different ecological adaptations for the consumption of different plant materials among some taxa. Similarly, Dantas and Santos (2022) included several more taxa and used a similar approach to study the ground sloths of the Brazilian Intertropical Region, showing differences among taxa in relation to dietary habits. Moreover, Bocherens et al. (2017) and Dantas et al. (2017) used stable isotopes to address the dietary preferences of several late Pleistocene megamammals from Argentina and Brazil, respectively, further showing the existence of different specializations as well as potential overlaps among some taxa.

However, when considering the isotopic studies focusing on the South American late Pleistocene Megafauna, the vast majority of the cases are focused on the study of specimens coming from different localities or from sites formed during large spans of time, limiting the capacity to make accurate observations related to the possible interactions among taxa, like competition for specific resources, or the existence of niche differentiation, like resource partitioning.

The South American late Pleistocene megafauna is well represented in Uruguay, with several records comprising typical Pampean species as well as some taxa with more northern affinities. In particular, the Arroyo del Vizcaíno (AdV) is a paleontological site dated ~32 ka cal BP in Southern Uruguay where many taxa have been recorded. Although ~95% of the bones found in the AdV correspond to the giant ground sloth *Lestodon armatus*, other xenarthra as well as Equidae, Cervidae, Notoungulata, Proboscidea, and Carnivora are also recorded (Fariña et al., 2014a) .

Considering this, the AdV represents an excellent opportunity, and almost unique for its age and region, for the study of a late Pleistocene megafauna community where variations due to geographic provenance or time-averaging can be ruled out. Furthermore, the high-quality preservation observed on the fossils from the site has allowed the successful extraction of well-preserved collagen in several taxa and DNA in a *Notiomastodon* specimen (Czerwonogora et al., 2011; Fariña et al., 2014a, 2022; Buckley et al., 2015; Lobato et al., 2021; Baleka et al., 2022; Varela et al., 2023a), ultimately allowing for the accurate dating of the fossil site, as well as providing insights on the evolution and ecology of some taxa.

In this study, we explore the dietary and climatic preferences of the megamammals found at the Arroyo del Vizcaíno site using stable isotopes analysis. Furthermore, we conduct mixing models to address the consumption of different plant resources by herbivorous taxa and discuss the existence of niche partitioning among closely related species.

2. Geographical, geological, and paleontological settings

The AdV site is located in Sauce, Canelones, Uruguay (34°37′3″ S, 56°2′33″ W; Fig. 1). The site is found at the bottom of a stream where a natural pond is formed due to the erosion of Cretaceous silicified sandstones (Mercedes Formation). The fossiliferous layer is composed of a bed that transitions from a muddy sandy gravel facies to a muddy sand facies with polymictic clasts and has been described as a fluvial system deposit that likely originated over a brief period of time (Fariña et al., 2014a; Domínguez-Rodrigo et al., 2021). In fact, several AMS dates have provided overlapping ranges (Fariña et al., 2022).

During the last decade, several excavations have allowed the extraction of more than 2000 fossil specimens, including several typical late Pleistocene Pampean taxa, namely *Lestodon armatus, Glossotherium robustum, Mylodon darwinii, Nothrotheriops* sp., *Glyptodon reticulatus, Doedicurus clavicaudatus, Panochthus tuberculatus, Toxodon platensis, Notiomastodon platensis, Equus neogeus, Hippidion principale, Smilodon populator*, and indeterminate Cervidae and Camelidae remains (Fariña et al., 2014a; Lobato et al., 2021; Varela et al., 2023a). Interestingly, the site represents one of the relatively scarce records of several sloth taxa co-occurring, with at least five genera having been recorded (Varela and Fariña, 2016), as well as the presence of three glyptodont genera and two equids, making it extremely interesting for the study of potential differences among closely related fossil taxa.

3. Materials and methods

All the samples were collected from well-preserved specimens that allowed accurate taxonomic assignments. The obtained samples correspond to the ground sloths *Lestodon armatus*, *Glossotherium robustum*, *Mylodon darwinii*, *Valgipes bucklandi* and *Nothrotheriops* sp.; the glyptodonts *Glyptodon reticulatus*, *Panochthus tuberculatus*, and *Doedicurus clavicaudatus*; the equids *Hippidion principale* and *Equus neogeus*; the proboscidean *Notiomastodon platensis* (for which only collagen extracts were analyzed); the notoungulate *Toxodon platensis*; the saber-tooth

Fig. 1. Map of Uruguay showing the location of the Arroyo del Vizcaíno site.

felid *Smilodon populator*; and an indeterminate cervid.

Collagen extraction was performed by acid hydrolysis with 1 M hydrochloric acid (HCl) in a ratio of 80 ml/4 g of sample. Constantly stirring on a magnetic stirrer at 4 °C for 1 h (Jørkov et al., 2007). In this digestion step, the inorganic components of the sample were eliminated, as well as other organic contaminants that it could contain (Longin, 1971; Gupta and Polach, 1985). After the digestion with HCl, all the digestion liquid in the sample was transferred to 50 ml Falcon tubes. Distilled water was added up to 40 ml capacity per tube. It was placed in the centrifuge for 15–20 min at 2500 rpm, thus obtaining the visibly precipitated and concentrated collagen. The washes were repeated in the centrifuge, with distilled water, until the supernatant cleared and reached a neutral pH. Subsequently, each sample was left to stand in 30 ml of 0.25 M sodium hydroxide (NaOH) (between 16 and 20 h), this step removes humic acids (DeNiro and Epstein, 1981). After resting, the precipitated collagen was centrifuged with distilled water until reaching neutral pH (approximately 6–8 washes). The supernatant for each wash was discarded. Finally, the sample was transferred to 30 ml bohemian glasses where it was allowed to crystallize in an oven at 65–70 °C.

For bioapatite extraction the pulverized bone was soaked for 3 days in 2% NaOCl (sodium hypochlorite), using 0.04 ml for mg of sample, rinsed five times with an excess of distilled water. Finally, the samples were transferred to 30 ml bohemian glasses where it was allowed to crystallize in an oven at 65–70 °C (Koch et al., 1997). The products of these extractions were weighed and stored in Eppendorf tubes.

Isotopic measurements were carried out either at the Yale University Stable Isotope Laboratory (YASIC) or the Alaska Stable Isotope Facility (ASIF, University of Alaska Fairbanks). The reference for carbon isotope values ($\delta^{13}C$; $R = {}^{13}C/{}^{12}C$) and oxygen isotope values ($\delta^{18}O$; $R = {}^{18}O/{}^{16}O$) is Vienna Pee Dee Belemnite (V-PDB), except for the analysis regarding drinking water (where VSMOW was used), and the reference for nitrogen isotope values ($\delta^{15}N$; $R = {}^{15}N/{}^{14}N$) is atmospheric nitrogen (AIR).

In order to address the quality of the obtained extracts, and subsequently, the reliability of the data, we conducted a series of analyses. Regarding the collagen extracts, we followed the standard practice of considering minimum values of C% = 3 and N% = 1 (Ambrose, 1993), as well as the expected $C{:}N_{atomic}$ ratio, which is traditionally expected to be less than 3.6 (DeNiro, 1985), although recent research has shown that more variable limits could be considered depending on different factors (Harbeck and Grupe, 2009; Guiry and Szpak, 2021).

Considering this, we checked for significant correlations between $C{:}N_{atomic}$ and the obtained isotopic values to address the impact of potential contamination. Regarding the carbonate extracts, to address the possible contamination of the samples and the potential modification of the original isotopic signals we checked for the expected difference between $\delta^{13}C$ values obtained from collagen and carbonate according to trophic guilds. Furthermore, we checked our results for taxa with known diets like *Equus*, as well as taxa for which previous studies have obtained data.

Furthermore, in order to better explore the possible resource partitioning among taxa, we applied Stable Isotope Mixing Models using the R package simmr (Parnell, 2019). These models are considered standard to quantify source contributions to a mixture in trophic web studies (Phillips et al., 2014). In particular, simmr uses a Bayesian framework to solve mixing equations for stable isotopic data, allowing the determination of the proportional contribution of sources to a mixture (in this case, the proportion of plant types in the diet of fossil herbivorous taxa).

For these analyses, we used only the $\delta^{13}C$ values, as information regarding the Oxygen and Nitrogen isotopic values of the potential sources (C_3 and C_4 vegetation) for the late Pleistocene of the region is not available. Therefore, we used the obtained $\delta^{13}C$ values, both from collagen and apatite, for each taxon as consumers and the previously determined $\delta^{13}C$ ranges of C_3 and C_4 vegetation as sources (Froehle et al., 2010). Also, we used the Trophic Discrimination Factors (TDFs) commonly used in the literature in order to account for the differential enrichments occurring at each trophic level and in different tissues (Koch et al., 2007; Froehle et al., 2010). Specifically, we set the TDFs as 3.9 ± 0.3 and 3.7 ± 0.3 in collagen and 10.9 ± 1.4 and 10.7 ± 1.4 in apatite for C_3 and C_4 vegetation, respectively.

Finally, in order to obtain approximate $\delta^{18}O$ values of the animal's drinking water ($\delta^{18}O_{dw}$), we used the equation proposed by Dotsika (2020), which allows for the direct comparison of values obtained from apatite carbonates. For this purpose, we converted the obtained values from VPDB to VSMOW following Coplen (1988), therefore all $\delta^{18}O_{dw}$ are expressed in VSMOW. The obtained $\delta^{18}O_{dw}$ were compared to the available isoscapes for South America showing the $\delta^{18}O$ values for rainfall and river water, as well as the expected differences for the late Pleistocene (Terzer et al., 2013; Jasechko, 2019; Nan et al., 2019).

Nevertheless, it should be noted that several different equations have been proposed and results generally show considerable spread, so correlations should be taken with caution (Ueda and Bell, 2021). Furthermore, this approximation is based on the assumption that all these taxa were obligate drinkers (i.e., they obtained most of their water drinking from water bodies and not from their food; Bryant and Froelich, 1995). This assumption can be considered sound based on the large body size of most of the studied taxa (Fariña et al., 2013), but particular cases like the indeterminate cervid could instead be non-obligate drinkers considering some of their extant relatives (Miller et al., 2019).

4. Results

4.1. Fossil preservation and reliability of data

In general, the collagen extracts showed C% and N% values higher than 3 and 1, respectively. Only the sample corresponding to *Valgipes bucklandi* showed values slightly below the mentioned limits, and therefore was evaluated with caution. Furthermore, most of the samples showed $C:N_{atomic}$ values lower than 3.6, indicating good preservation. Some specimens showed elevated $C:N_{atomic}$ values higher than 3.6, namely *Panochthus tuberculatus*, *Nothrotheriops* sp., and *Valgipes bucklandi*, with values of 3.66, 3.67, and 3.76, respectively. However, despite the probable contamination with humic acids in these specific samples, these values fall well within those reported to be reliable for ancient samples by Guiry and Szpak (2021), who proposed a 3.7–3.9 "liberal" upper limit when studying the impact of contamination on the reliability of isotopic signals.

Moreover, the regressions of the obtained $\delta^{13}C$ and $\delta^{15}N$ values with the $C:N_{atomic}$ values showed no significant correlations ($p = 0.17$ and 0.42, respectively, Fig. S1), indicating that the slightly elevated $C:N_{atomic}$ values in a minority of the samples did not have an important impact over the reliability of the obtained data. Finally, the obtained results for the $^{13}C_{carb\text{-}coll}$ were consistent with the expected values for herbivore and carnivore taxa, as well as with previous results for the late Pleistocene South American megafauna (Bocherens et al., 2017; Codron et al., 2018), and did not show any significant correlation with $C:N_{atomic}$ values ($p = 0.39$).

4.2. Stable isotopes results

The obtained results showed considerable variation in all the studied isotopes, covering a relatively wide range of environmental and dietary preferences for the included taxa (Fig. 2; Table S1). In particular, the $\delta^{18}O$ results showed a wide range, with *Valgipes bucklandi* and *Nothrotheriops* sp. Representing the lowest (−8.1‰) and highest (0.2‰) values, respectively (Table S2). The rest of the studied taxa showed intermediate values between these extremes, with considerable spread and the lack of clear clustering.

Regarding the $\delta^{13}C$ values, both the collagen and carbonate results were skewed towards the expected C_3 plant values, but differences among taxa were visible. For the carbonate extracts, the obtained range was −13.1‰ to −4.4‰, represented by *Nothrotheriops* sp. and one of the two sampled specimens of *Lestodon armatus*, respectively. On the other hand, the collagen extracts showed less spread, although a similar pattern to those of carbonate was clear. In this case, the obtained range was −21.3‰ to −17.8‰, represented by *Nothrotheriops* sp. and *Smilodon populator*, respectively. The fact that *Smilodon populator* represented the most positive $\delta^{13}C$ value is expected considering that isotopic enrichment in relation to prey occurs in carnivores and *S. populator* was probably consuming some of the studied herbivorous taxa.

Finally, the $\delta^{15}N$ results showed considerable clustering in high values, with many taxa having values close to 10‰. The range of all the samples was 7.2‰–13.6‰, represented by *Toxodon platensis* and *Smilodon populator*. Again, as expected, the highest value observed in *S. populator*, the only carnivore analyzed in our sample. The $\Delta^{13}C_{coll\text{-}carb}$ values among the herbivore taxa showed considerable variation

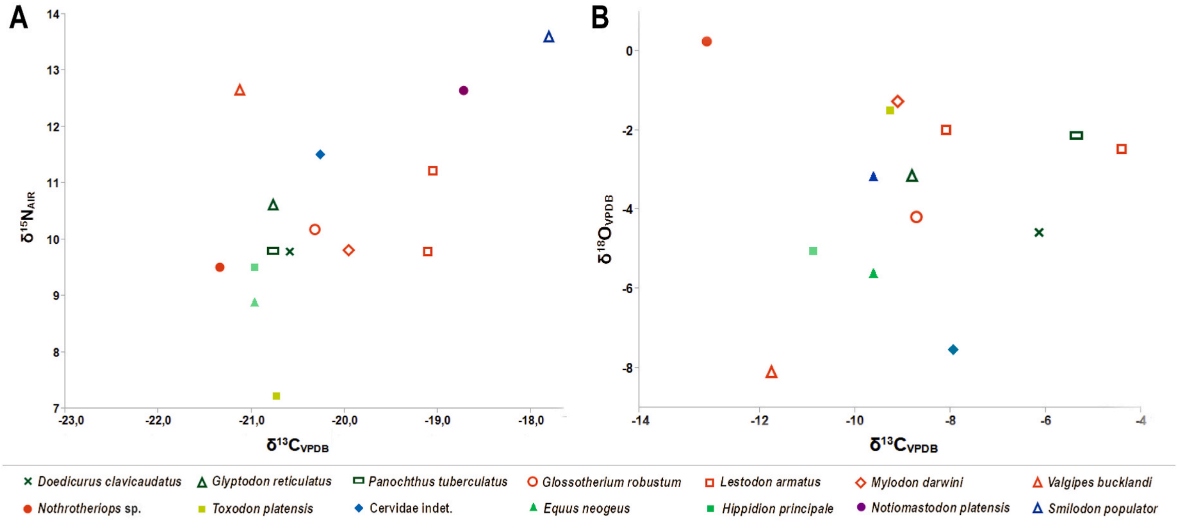

Fig. 2. Scatter-plot of A: collagen $\delta^{15}N$ versus $\delta^{13}C$ values and B: carbonate $\delta^{18}O$ versus $\delta^{13}C$.

(8.2–15.4). In particular, *Lestodon armatus*, *Doedicurus clavicaudatus*, *Panochthus tuberculatus*, and the indeterminate cervid showed the largest spacings. However, the collagen values showed less variation among taxa, indicating that the obtained $\Delta^{13}C_{coll\text{-}carb}$ was largely influenced by the carbonate values.

The Stable Isotope Mixing Models showed different results for apatite and collagen values (Fig. 3). For collagen, the results showed less differentiation among taxa regarding the consumption of C_4 vegetation, with almost all taxa overlapping in their posterior distributions and clustering below a 0.25 proportion of C_4 plants in their diet. On the other hand, the results based on the apatite $\delta^{13}C$ values showed much more differentiation among taxa, with several taxa showing non-overlapping posterior distributions and species like *Lestodon armatus*, the glyptodonts *Doedicurus clavicaudatus* and *Panochthus tuberculatus*, and the indeterminate cervid showing mean proportions of C_4 plants consumption above 0.5.

However, it is important to note that the same general pattern regarding differences in C_4 plants consumption can be observed among taxa in the carbonate and collagen $\delta^{13}C$ results, with the taxa showing higher consumption of C_4 plants being the same in both analyses. In other words, both the results of carbonate and collagen are consistent when showing higher consumption of C_4 plants in some taxa in relation to other taxa despite the greater overlaps observed in the collagen results.

5. Discussion

5.1. Paleoenvironmental information

The $\delta^{18}O_b$ values among the studied taxa showed considerable variation, with the minimum and maximum being −8.1‰ (*Valgipes bucklandi*) and 0.2‰ (*Nothrotheriops* sp.), respectively. Considering the diversity of taxa recorded at the AdV and their potential habitat preferences, the results represent interesting information on the environments present at Southern Uruguay during the late Pleistocene.

For example, several taxa recorded at the site, like *Glossotherium robustum*, *Mylodon darwinii*, *Glyptodon reticulatus*, and *Doedicurus clavicaudatus*, are commonly found in southern regions of the continent, while others like *Notiomastodon platensis* and *Valgipes bucklandi* have geographical distributions including northern regions of the continent (Mothé et al., 2017; Varela et al., 2018). This mixture of taxa with southern and northern affinities, and therefore preferences for colder and hotter habitats, respectively, would imply the existence of an environment where these taxa could survive. Likewise, taxa like *Nothrotheriops* probably had preference for more arid environments (McDonald, 2022), while others like *Hippidion* were probably more adapted to more humid environments (Alberdi and Prado, 1992).

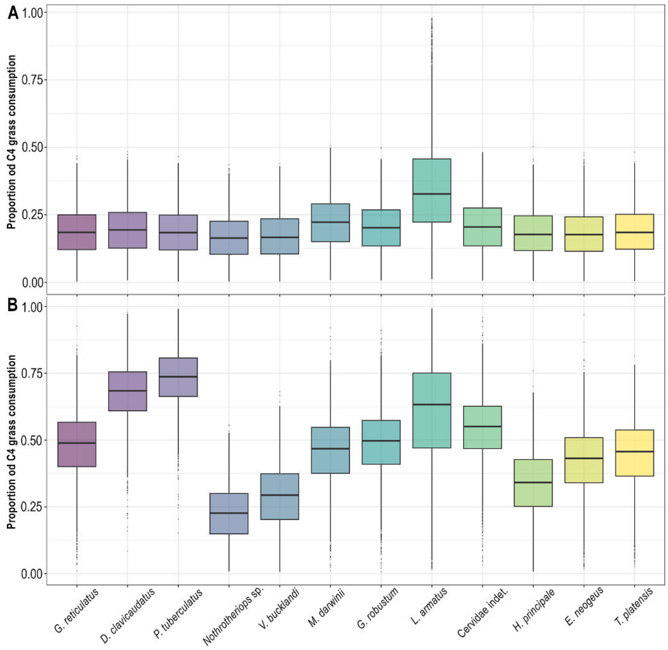

Fig. 3. Proportion of C_4 plants in the diet of studied taxa based on the results of a mixing model using A: $\delta^{13}C$ collagen and B: $\delta^{13}C$ bioapatite values.

Interestingly, these differences seem to be in accordance with the obtained results, with most taxa showing the expected $\delta^{18}O_{dw}$ values based on their predicted environmental preferences. For example, *Nothrotheriops* sp. showed a $\delta^{18}O_{dw}$ value lower than 2‰, which are commonly related to hot and arid environments, like the Dry Chaco and Caatinga ecoregions (Nan et al., 2019). Other taxa, more commonly associated with the Pampean ecoregion, like *Glossotherium robustum*, *Mylodon darwinii*, *Lestodon armatus*, *Doedicurus clavicaudatus*, and *Glyptodon reticulatus* showed slightly more negative values (~3–6‰) in accordance with those observed in river water in the Pampean region (Nan et al., 2019).

Remarkably, while *Mylodon darwinii* is also recorded in more southern regions (Varela and Fariña, 2016), it is important to mention that the river water $\delta^{18}O$ values predicted for the Argentine Monte and Patagonian steppe are considerably positive, despite the cold environment and probably due to the dry conditions (Nan et al., 2019). Further, taxa like *Hippidion principale* and the indeterminate cervid showed more negative values (~6–9‰), probably indicating affinities to more humid and closed environments. Interestingly, similar values are predicted in river water from higher lands in southeastern and northeastern Uruguay (Nan et al., 2019).

Finally, *Valgipes bucklandi* showed significantly more negative values than the other studied taxa, probably indicating colder and more humid conditions. However, *V. bucklandi* is commonly found in the Brazilian Intertropical Region (BIR), which is characterized by drier and hotter environments (Pereira et al., 2013). This result could be related to the extreme distance from the AdV specimen to the populations from Brazil, indicating different habits, although contamination altering the original isotopic value should not be completely ruled out (Lobato et al., 2021).

Nevertheless, it is important to point out that Uruguay is located in a zone where river water shows considerable variations due to water provenance from extensive river systems, altitude, and coastal influence (Nan et al., 2019). This situation is relevant when considering the variations observed among taxa, and further supports the existence of an open mosaic habitat with patches of mixed vegetation in the region (Varela and Fariña, 2016; Lobato et al., 2021).

In fact, Varela et al. (2018) proposed that the Río de la Plata region could represent an ecotone between southern and northern ecoregions, where taxa from both regions could coexist, as well as the occurrence of endemic taxa with more bounded distribution limits. Furthermore, the considerable spread of the obtained $\delta^{18}O$ values could also indicate considerable seasonality in the region during the Last Pleistocene, specifically on the onset of the Last Glacial Maximum. In fact, previous research has proposed higher seasonality than in the present for the pampas during the late Pleistocene (Iriondo and García, 1993).

However, the signal recovered from bone bioapatite represents an extended period of time, as isotopes are incorporated to this tissue during the life of the animal, and therefore averaged across a considerable timespan (Lee-Thorp, 2008). Considering this, future research should focus on sequential sampling approaches in incremental tissues like tooth dentine or enamel to better explore these variations (Sharp and Cerling, 1998).

5.2. Paleodietary reconstruction

The $\delta^{15}N$ values were high in general, with all taxa showing values higher than 7‰. These high values are commonly found on animals that consume animal food sources (i.e., omnivores and carnivores), due to the enrichment occurring at each trophic level (~4‰; Bocherens and Drucker, 2003) and the fact that plant's $\delta^{15}N$ values are rarely >4‰ (Bowen and West, 2008). However, previous results in fossil taxa, particularly for the Pampean region, have shown the existence of considerably high $\delta^{15}N$ values in herbivore taxa. For example, Bocherens et al. (2016) reported values higher than 10‰ for some xenarthrans, as well as *Hippidion*, *Toxodon* and *Macrauchenia*. Interestingly, the giant ground sloth *Megatherium americanum* was among the taxa with highest $\delta^{15}N$ values, although the authors discarded the possibility of a non-herbivore diet for this sloth based on the also high values obtained for the other species. Indeed, high $\delta^{15}N$ are commonly found in animals living in hot and arid environments, a pattern probably arising from their diet based on plants adapted to these environments (Hartman, 2011).

Considering the $\delta^{15}N$ isoscape for plant tissue as well as the potentially more arid environments occurring in the region during the late Pleistocene (Tonni et al., 1999; Bowen and West, 2008), the observed high $\delta^{15}N$ values seem to be reasonable for taxa living in these environments. Nevertheless, the fact that some sloths show considerably high values should be pointed out, especially considering the debate over the potential consumption of animal food sources by these taxa (Fariña, 1996; Fariña and Blanco, 1996; Bargo, 2001; Bocherens et al., 2017; Fariña and Varela, 2018). In fact, a recent study by Tejada-Lara et al. (2021) showed the probable consumption of animal sources by the ground sloth *Mylodon*, showing that more specific studies are probably needed in order to evaluate this group of mammals.

On the other hand, the $\delta^{15}N$ values obtained for *Smilodon populator* are in accordance with the expected values for a large carnivore and also to those obtained by Bocherens et al. (2016) for specimens from the Argentinean Pampas. In fact, following the simple approach used by Bocherens et al. (2016) to recognize potential preys (subtracting 0.8‰ and 1.3‰ to $\delta^{13}C$ and 3‰ and 5‰ to $\delta^{15}N$ values), we obtain the following ranges for potential preys of the AdV *S. populator*: −18.6‰ to −19.1‰ and 8.6‰–10.6‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Fig. S2). Similarly to the results of Bocherens et al. (2016), the expected values showed a match with *Lestodon armatus*, especially with one of the two studied specimens, which produced both values within the expected ranges. Nevertheless, it is important to point out that *Notiomastodon platensis* also falls within the range expected for $\delta^{13}C$ values, and several taxa fall within the range expected for $\delta^{15}N$ values, indicating that the AdV *S. populator* was probably consuming a combination of taxa.

Regarding the $\delta^{13}C$ values obtained for the herbivore taxa, the results of collagen and apatite seemed to show a similar pattern, with differences among taxa that potentially indicate differences in the exploited resources. However, these differences were more pronounced when in the apatite data, where many taxa showed non-overlapping posterior probabilities regarding C_4 plants consumption. Interestingly, the largest $\Delta^{13}C_{\text{coll-carb}}$ spacing was seen in taxa with high C_4 consumption (i.e., grazers), with some taxa showing values similar to the highest values reported by Codron et al. (2018). In fact, the obtained results are in accordance with those obtained by Codron et al. (2018), who showed that $\Delta^{13}C_{\text{coll-carb}}$ spacing increases with increased levels of C_4 grass intake. In their work, the authors argue that this pattern is the result of a tendency toward ^{13}C-depleted C_3 foods in $\delta^{13}C_{\text{collagen}}$ due to higher protein contents in C_3 plants compared to C_4 plants (Jim et al., 2004), and the increase in $\delta^{13}C_{\text{carbonate}}$ due to higher levels of ^{13}C-depleted methane production associated with grass-rich diets (Cerling and Harris, 1999).

Furthermore, the differences observed in $\Delta^{13}C_{\text{coll-carb}}$ could be largely influenced by the proteins consumed by taxa (which are mainly reflected in the $\delta^{13}C_{\text{collagen}}$ values) and also be responsible for the less evident differences observed in $\delta^{13}C_{\text{collagen}}$ values among taxa. In fact, Froehle et al. (2010) showed how collagen values can be less informative to estimate animal diets in some situations and the inclusion of apatite should be necessary to correctly predict diets in fossil specimens.

It should be pointed out that, in the present, for Southern Uruguay and the Pampas in general, the proportion of C_4 grasses is considerably low, with estimates assigning a composition of ~50% C_4 for the open habitats present in the region (Powell and Still, 2009). This pattern in the distribution of C_3 and C_4 grasses implies that any taxa consuming grasses almost exclusively would hardly show a $\delta^{13}C$ profile characteristic of C_4 grazers, i.e., a strict grazer would probably show a $\delta^{13}C$ value compatible with a consumption of ~50% C_4 plants unless it would actively select C_4 grasses over C_3 grasses.

Based on this, the resolution obtained through $\delta^{13}C$ to infer the dietary habits of herbivorous taxa (e.g., browser vs. grazer) becomes reduced when compared to other parts of the planet, probably resulting in more overlap between species in their $\delta^{13}C$ values despite their potential differences in grass consumption. Despite this, the differentiation observed in the $\delta^{13}C_{\text{carbonate}}$ values gives important insights regarding the partitioning of resources in the taxa found in the AdV site. Considering the clustered datings obtained for the site and the evidence indicating a potential single depositional event (Fariña et al., 2014a; Fariña et al., 2022), these results provide a unique picture of the ecology of a late Pleistocene community of megamammals, showing some patterns among taxa that allow the comparison with previous results from different geographic zones as well as with predictions based on other methods, like morphological and biomechanical approximations.

Ground sloths represent the most diverse group found at the AdV site, with five genera recorded, belonging to three different clades (Delsuc et al., 2019; Varela et al., 2019). The occurrence of five sloth genera at a single site is extremely uncommon in the late Pleistocene of America (McDonald et al., 2013; Varela and Fariña, 2016). Nevertheless, the co-occurrence of the mylodontids *Lestodon armatus*, *Mylodon darwinii*, and *Glossotherium robustum* has been registered in some sites, as well as the co-occurrence of two of them (Varela and Fariña, 2016). Although these three taxa have been proposed as predominantly grazers, some differences in their morphology and the fact that they co-occur in some sites are indicators that they probably had some degree of niche differentiation that allowed them to partition resources and limit competition (Bargo and Vizcaíno, 2008; McAfee, 2009; Brandoni et al., 2010; Varela et al., 2020; Casali et al., 2023). Moreover, recent research exploring the biomechanical efficiency of sloths' mandibles using Finite Element Analysis showed differences among these mylodontids, indicating different grades of specialization on hard-food processing (Varela et al., 2023b). The results obtained from the AdV specimens further supports their preference for grasses, although some differences are seen in the predicted proportion of C_4, with *L. armatus* being the one showing the largest C_4 grass consumption and *M. darwinii* showing the least C_4 grass consumption. In fact, the predicted consumption of C_4 grasses for *L. armatus* (~50–75%) indicates that it was probably a strict grazer considering the expected proportion of C_3 and C_4 for southern Uruguay.

Furthermore, the obtained results are in accordance with the expected resource partitioning observed among closely related herbivores from the same herbivorous guild (Pansu et al., 2022). On the other hand, the other two sloths recorded in the site, *Valgipes bucklandi* and *Nothrotheriops* sp., showed values compatible with the predominant consumption of C_3 plants, with scarce amounts of C_4 grass consumption. These results are largely in accordance with previous results for these taxa, and would indicate that these taxa probably dwelled on more closed habitats and showed more browser habits (Bonde, 2013; Pereira et al., 2013; Pérez-Crespo et al., 2018; Lobato et al., 2021; Varela et al., 2023a).

Overall, the results obtained for the ground sloths found at the AdV site show the existence of resource partitioning among the taxa and the existence of differences that show that members of this clade probably occupied a considerable range of the browser-grazer continuum in the region. However, future research should explore the potential differences among individuals, especially considering the proposals of sexual dimorphism in many ground sloths (McDonald, 2006; Cartelle et al., 2019; McAfee and Beery, 2021), including *L. armatus* (Varela et al., 2022), and the potential niche differentiation that sexually dimorphic forms could have had (Boscaini et al., 2019).

The glyptodonts found at the AdV site are represented by the most commonly recorded taxa in the region, *Glyptodon reticulatus*, *Panochthus tuberculatus*, and *Doedicurus clavicaudatus*, which represent the largest cingulates for the South American late Pleistocene (Fariña et al., 2013). Previous studies for these taxa have shown some differences in their potential feeding and habitat preferences based on their morphology (Vizcaíno et al., 2011). In particular, the authors analyzed the hypsodonty index and relative muzzle-width of several fossil taxa and showed that some of them were probably better adapted to bulk-feeding in open habitats, like *Panochthus tuberculatus* and *Doedicurus clavicaudatus*, while *Glyptodon reticulatus* was predicted to have had a more selective feeding habit in more closed environments. Moreover, a study based on stable isotopes in South American late Pleistocene mammals proposed a flexible dietary behavior for *Glyptodon reticulatus* (Domingo et al., 2012). Furthermore, Varela et al. (2018) showed considerable overlap of potential geographic distributions among these taxa using Ecological Niche Models, which would also support the idea of niche partitioning among these taxa.

The new isotopic results for the AdV site show that all these taxa were consuming some degree of C_4 grass, but are in accordance with previous studies showing *Glyptodon reticulatus* as the species less specialized in grass consumption and probably that with a more flexible diet. These results are important to further support the fact that these related species with generally similar morphologies probably adapted to relatively different resources in order to co-exist in the same region.

Regarding the ungulates recorded at the AdV site, the indeterminate cervid showed the highest $\delta^{13}C$, and therefore, the highest predicted proportion of C_4 grasses in its diet. This result is interesting considering the diversity of cervids recognized for the late Pleistocene of the region and the suggested dietary preferences that have been proposed for them. In fact, most extant cervids are commonly considered as mixed feeders, with several taxa occupying different niches along the grazer-browser spectrum, and grass consumption was probably present early in the evolutionary history of the clade (Pérez-Barbería et al., 2001; DeMiguel et al., 2008).

Furthermore, the diversity of taxa occurring in the region during the late Pleistocene encompassed a wide range of body masses, with some taxa reaching body masses of more than 100 Kg. Among these taxa, *Morenelaphus*, a medium-sized deer (Fariña et al., 2013), has been interpreted as a mixed feeder to grazer based on microwear analysis (Rotti et al., 2018). On the other hand, one of the extant cervids of the region, the Pampas deer's (*Ozotoceros bezoarticus*) is commonly considered a grazer associated with open habitats (Jackson and Giulietti, 1988; Rodrigues and Monteiro-Filho, 1999). In this regard, the obtained results for the AdV cervid are consistent with these reports, although more research is needed to gain knowledge from other cervid taxa, as well as to obtain a taxonomic determination for the specimen.

The two equids recorded at the AdV site showed relatively low differences in their isotopic values, with both species showing a considerable amount of C_4 consumption. Nevertheless, *Equus neogeus* showed a higher $\delta^{13}C$ value than *Hippidion principale*, indicating more grass consumption in its diet. This result is in line with previous studies on these taxa, which showed a preference for open habitats and grazing diets for *E. neogeus* and more diverse diets and environments for *H. principale* (Prado and Alberdi, 2017). Furthermore, Villavicencio et al. (2019) obtained similar results for these species using Ecological Niche Models, indicating some differences in their climatic preferences but considerable overlap in their potential distributions in the Pampas. Our results for the AdV specimens show that these two equid species probably preferred slightly different resources and thus, avoided direct competition when inhabiting the same regions.

Finally, the notoungulate *Toxodon platensis* have been frequently associated with a grazer diet based on different data. For example, previous results based on isotopic analyses have shown $\delta^{13}C$ values compatible with a predominant consumption C_3 and C_4 grasses in Argentina, Bolivia, and Brazil (MacFadden and Shockey, 1997; MacFadden, 2005; Domingo et al., 2012). Furthermore, the high-crowned teeth of *T. platensis*, as well as its masticatory muscle organization, have been interpreted as an adaptation to grass consumption and/or preference for dust-rich open habitats (MacFadden and Shockey, 1997; Varela and Fariña, 2015). However, MacFadden (2005) also showed the existence of considerable variability in the diet of *T. platensis* in different regions, which indicates that this toxodontid was capable of consuming different plant resources based on availability. In the case of the AdV specimen, the obtained values seem to be in accordance with the previous results for this taxon, especially with those of the Province of Buenos Aires, Argentina and Bahia, Brazil, showing values associated to a mixed C_3 and C_4 diet.

Based on all the obtained data, the existence of niche partitioning among the herbivorous taxa recorded at the AdV site can be proposed. Accordingly, the two herbivore guilds can be recognized based on the grazer-browser spectrum. This pattern is expected based on previous results showing considerable differences in grass consumption among herbivores co-occurring at specific geographical regions, with taxa often covering all the grazer-browser spectrum (Codron et al., 2007; Pansu et al., 2022). Interestingly, Pansu et al. (2022) also found differences within guilds, showing that species tend to differentiate even when being strict browsers or grazers. In the megamammal community recorded at the AdV site, results show that taxa are distributed across the grazer-browser spectrum based on the $\delta^{13}C$ values, with both guilds being represented by more than one taxon. Furthermore, the observed within-guild differentiation could be related to small differences in the consumption of specific plant taxa, but also be related to the preference for different habitats, as was shown above for the $\delta^{18}O$ results.

For example, the glyptodonts, *Panochthus tuberculatus* and *Doedicurus clavicaudatus* and ground sloths *Lestodon armatus*, *Glossotherium robustum*, and *Mylodon darwinii* showed slight differences in percentage of C_4 consumption but showed some differences in $\delta^{18}O$ and $\delta^{15}N$ values. Moreover, the indeterminate cervid, which showed $\delta^{13}C$ values compatible with a grazer diet, also showed considerably different $\delta^{18}O$ values compared to the other grazers. On the other hand, the two sloths that showed $\delta^{13}C$ values compatible with a browser diet showed opposite values for both $\delta^{18}O$ and $\delta^{15}N$, indicating that these taxa probably preferred different habitats and consumed different kinds of C_3 plants. This pattern among taxa would be further promoted considering an open mosaic habitat with patches of mixed vegetation that would allow the coexistence of several taxa.

6. Conclusions

Several studies have shown considerable variations in the diet of fossil taxa when analyzing different geographic regions or time periods (MacFadden, 2005; Prado et al., 2011; França et al., 2015). Considering this, it is important to consider the influence of these factors when studying fossil faunas at the community level in order to address questions regarding potential competition or niche differentiation among its members.

In this study, we analyzed samples obtained from a South American late Pleistocene fossil site where evidence supports site formation to have occurred during a short timespan, providing an accurate picture of the megafauna community isotopic ecology. Our results show that most of the herbivorous taxa present at the site were inhabiting open landscapes and had preference for C_3 vegetation but covered a considerable range of the grazer-browser spectrum, supporting the existence of niche partitioning among closely related taxa.

Overall, these kinds of approaches are essential to better understand how these communities thrived during the Pleistocene in the region, supporting an outstanding number of giant species, before their extinction at the Pleistocene-Holocene transition.

Authors contribution

Luciano Varela: Conceptualization, Investigation, Methodology, Formal analysis, Resources, Visualization, Funding acquisition, Writing – Original Draft. Lucía Clavijo: Investigation, Methodology, Formal analysis, Resources, Visualization, Writing – Original Draft. P. Sebastián Tambusso: Resources, Writing – Review & Editing. Richard A. Fariña: Conceptualization, Resources, Funding acquisition, Writing – Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data is shared as a supplementary material attached during the submission

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2023.108286.

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Title: Femora Nutrient Foramina and Aerobic Capacity in Giant Extinct Xenarthrans

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Abstract

Nutrient foramina are small openings in the periosteal surface of long bones that traverse the cortical layer and reach the medullary cavity. They are important for the delivery of nutrients and oxygen to bone tissue, and are crucial for the repair and remodeling of bones over time. The nutrient foramina in the femur's diaphysis are related to the energetic needs of the femur, and have been shown to be related to the maximum metabolic rate (MMR) of taxa. Here, we investigate the relationship between nutrient foramen size and body mass as a proxy to the aerobic capacity of taxa in living and extinct xenarthrans, including living sloths, anteaters, and armadillos, as well as extinct xenarthrans such as glyptodonts, pampatheres, and ground sloths. Sixtynine femora were sampled, including 19 from extant taxa and 50 from extinct taxa. We obtained the blood flow index (Q_i) based on foramina area and performed PGLS and phylogenetic ANCOVA in order to explore differences among mammalian groups. Our results show that among mammals, taxa commonly associated with lower metabolism like marsupials and living xenarthrans showed relatively smaller foramina, while the foramina of giant extinct xenarthrans like ground sloths and glyptodonts overlapped with non-xenarthran placentals. Consequently, Q_i estimations indicated aerobic capacities comparable to other placental giant taxa like elephants or some ungulates. Furthermore, the estimation of the MMR for fossil giant taxa showed similar results, with almost all taxa showing high values except for those for which strong semi-arboreal or fossorial habits have been described. Moreover, the results are compatible with the diets predicted for extinct taxa, which indicate a strong consumption of grass similar to ungulates and in contrast to the folivorous or insectivorous diets of extant xenarthrans. The ancestral reconstruction of the MMR values indicated a lack of a common pattern for all xenarthrans, strongly supporting the occurrence of low metabolic rates in extant forms due to their particular dietary preferences and arboreal or fossorial habits. Our results highlight the importance of considering different evidence beyond the phylogenetic position of extinct taxa, especially when extinct forms are exceptionally different from their extant relatives. Future studies evaluating the energetic needs of giant extinct xenarthrans should not assume lower metabolic rates for these extinct animals based solely on their phylogenetic position and the observations on their extant relatives.

Keywords: South America, Folivora, Cingulata, Quaternary, Macroevolution, Metabolism

Introduction

Nutrient foramina are small openings in bones that allow blood vessels to enter and exit the inner parts of the bone. These foramina are clearly seen in the periosteal surface of long bones and traverse the cortical layer and ultimately reach the medullary cavity. These openings are important for the delivery of nutrients and oxygen to bone tissue, and are also involved in the repair and remodeling of bones over time (Lieberman et al. 2003; Robling et al. 2006; Eriksen 2010). Nutrient foramina can be found in almost all long bones of tetrapods, with fossil groups like dinosaurs or stem-mammals showing these openings (Seymour et al. 2012; Newham et al. 2020). The size and shape of nutrient foramina can vary widely between different species of animals, and can be influenced by a variety of factors including type and zone of long bone, body size, age, among others. In the case of the femur, previous research has shown that most bones show a reduced number of nutrient foramina (1–2) in the diaphysis of the bone, which account for 50–70% of the blood flow of the femur (Trueta 1963). The mechanical loading on the femur has a direct influence over bone formation, while both the body mass and exercise level determine the loadings suffered by the bone (Foote 1911). Furthermore, the nutrient foramina in the femur's diaphysis are related to the energetic needs of the femur, which are largely related to the generation of microfractures due to mechanical loading stress and the consequent bone remodeling (Burr et al. 2002).

Seymour et al. (2012) showed that the area of the femur's nutrient foramina and the femora total length can be used to calculate a relative quotient of blood flow (Q_i) that can be compared among taxa from different taxonomic groups, allowing for a direct comparison of the energetic needs of the femur across a wide spectrum of species. Furthermore, the authors showed that the obtained estimates of blood flow, when controlled for body mass, are directly related to the aerobic capacity of taxa and, ultimately, its maximum metabolic rate (MMR). In fact, endotherms like extant mammals and birds significantly differ from ectotherms like nonvaranid reptiles when comparing their nutrient foramina according to their body mass, with the former showing significantly higher intercepts in their regressions lines (Seymour et al. 2012). Considering this, several studies have used the nutrient foramina to compare the aerobic capacity of fossil taxa, as well as a proxy for the estimation of MMR in these species, including dinosaurs, birds, non-mammalian synapsids, and stem-mammals (Seymour et al. 2012; Allan et al. 2014; Newham et al. 2020; Knaus et al. 2021).

Xenarthrans are a peculiar group of mammals that are found exclusively in the Americas, with approximately 31 living species (Superina and Loughry 2015). Within the Xenarthra, sloths (Folivora) are known for their slow-moving and arboreal lifestyles, with the two extant genera (*Choloepus* and *Bradypus*) representing an exceptional case of convergent evolution (Nyakatura 2012; Delsuc et al. 2019). On the other hand, anteaters (Vermilingua) and armadillos (Cingulata) are adapted for digging and feeding on insects and other small animals, with many taxa showing considerable fossorial habits (Superina and Abba 2020). Despite their distinct morphologies and ecological roles, studies have confidently shown that all xenarthrans form a clade, and their last common ancestor was probably a myrmecophagous animal with adaptations to digging and climbing (Gaudin and Croft 2015). Furthermore, xenarthrans represent one of the four major clades within placental mammals, and their phylogenetic position has been the center of debate (Kriegs et al. 2006; Murphy et al. 2007; Morgan et al. 2013). In fact, xenarthrans could represent one of the most basal divergences within the placental mammals, making them particularly important for understanding the evolution of mammals (Svartman et al. 2006). Moreover, they show a unique set of characteristics that often represent less derived forms, but also particular adaptations product of their peculiar evolutionary history (Vizcaíno and Bargo 2014). One of these characteristics is related to their body temperature and basal metabolic rate, with studies showing members of the clade with the lowest values among mammals, oftentimes in ranges similar to those of non-placental mammals with similar habits like monotremes and marsupials (McNab 1984, 1986). In particular, sloths have received special attention due to their slow-moving and seemingly sluggish behavior. Living sloths are known for their extremely low metabolic rates and reduced muscle mass, which are thought to be adaptations to their folivorous diet and arboreal lifestyle (McNab 1985). Nevertheless, other members of the clade also present lower than expected body temperatures or basal metabolic rates, probably related to their specialized diets or fossorial habits (McNab 1985).

The fossil record of the Xenarthra is significantly more varied than the living members of the clade, both in terms of species diversity as well as morphological and ecological adaptations (Vizcaíno and Loughry 2008). In particular, within Cingulata, the glyptodonts represent a clade within the armadillo family Chlamyphoridae (Delsuc et al. 2016) that shows considerable differences with their close living relatives, being mostly giant terrestrial animals with more rigid carapaces (Fariña et al. 2013). Moreover, within sloths, several fossil forms also show tendencies towards gigantism, with several families like Megatheriidae and Mylodontidae having members with estimated body masses of more than 3,000 kg (Toledo et al. 2015). These giant ground sloths were certainly not arboreal like their extant relatives, and some of them probably had the capacity to move using only their hindlimbs (Casinos 1996; Fariña et al. 2013), form groups of individuals with a certain level of social behavior (Tomassini et al. 2020; Varela et al. 2022), or dig extensive tunnel systems (Vizcaíno et al. 2001; Frank et al. 2015). Considering the extreme differences between the fossil xenarthrans and their few extant relatives, recent research has shown that direct comparisons with the latter could not be appropriate to perform paleobiological reconstructions (Vizcaíno et al. 2018). In fact, one of the key characteristics of living xenarthrans, their low body temperature and metabolic rate, has been elusive to study in their fossil relatives since they are impossible to directly measure in commonly fossilized body parts, like bones or teeth. However, it is commonly assumed that the extinct xenarthrans, including the giant terrestrial forms like glyptodonts and ground sloths, had low metabolic rates based on their phylogenetic position (McNab 1985; Vizcaíno et al.

2023). Nevertheless, some research has suggested that some giant members of the clade could have had higher agility levels and, potentially, higher metabolic rates (Billet et al. 2013; Boscaini et al. 2018; Tambusso et al. 2021; Dantas and Santos 2022). These results could have important consequences on our understanding of the evolution of xenarthrans since they would indicate that the metabolic rates observed in most of the extant members of the clade would be the result of convergent evolution due to their particular lifestyles and not necessarily the product of an evolutionary constraint due to common ancestry.

Here, we aim to investigate the relationship between nutrient foramen size and body mass as a proxy to the aerobic capacity of taxa in living and extinct xenarthrans. Specifically, we analyzed the nutrient foramina in the femora of several species, including living sloths, anteaters, and armadillos, as well as extinct xenarthrans such as glyptodonts, pampatheres, and ground sloths. Our objectives were to determine whether the size of nutrient foramina in extant and extinct xenarthrans shows differences that could be associated to the aerobic capacity and maximum metabolic rates in these species, with particular interest in the implications on our understanding of the behavior and ecology of fossil giant xenarthrans.

Methods

Taxon Sampling

We extracted all the available information of mammalian taxa in the data published by Seymour (2012). Furthermore, we expanded the dataset with new data of extant mammals from specimens housed in the Statens Naturhistoriske Museum in Denmark (MHND) in Copenhagen. These new data comprised several extant xenarthrans, as well as members of other clades like marsupials and non-xenarthran placentals. Regarding the extant xenarthrans, we obtained data for the following species: the folivorans *Bradypus torquatus* and *Bradypus tridactylus*; the vermilinguans *Cyclopes didactylus*, *Myrmecophaga tridactyla* and *Tamandua tetradactyla*; and the cingulates *Cabassous unicinctus*, *Chaetophractus vellerosus*, *Dasypus hybridus*, *Dasypus novemcinctus*, *Euphractus sexcinctus*, and *Priodontes maximus*. Also, we obtained data for several fossil xenarthrans housed in the Arroyo del Vizcaíno collection (AdV), the Museo Paleontológico Profesor Armando Calcaterra (MPAC), and the Museum of Natural History (MNHN-M) in Uruguay; the Muséum National d'Histoire Naturelle (MNHN-F) in France; and the Statens Naturhistoriske Museum (MHND) in Denmark. In particular, the sampled fossil xenarthrans are represented by the following taxa: the folivorans *Catonyx cuvieri*, *Glossotherium robustum*, *Lestodon armatus*, *Megatherium americanum*, *Mylodon darwinii*, *Nothrotherium maquinense*, *Scelidotherium leptocephalum*, and *Valgipes bucklandi* and the cingulates *Dasypus punctatus*, *Glyptodon reticulatus*, *Holmesina majus*, *Neoglyptatelus* sp., *Neosclerocalyptus paskoensis*, *Neosclerocalyptus ornatus*, *Panochthus tuberculatus*, and *Propraopus sulcatus*. Sixtynine femora were sampled, including 19 from extant taxa and 50 from extinct taxa. All the newly generated data is available as supplementary material (Table S1).

Figure 1. Images of the femur of a *Lestodon armatus* specimen (CAV 977). A. Entire femur in anterior view. Nutrient foramen is visible on the medial part of the diaphysis (black arrow). Scale bar equals 10 cm. B. Close view of the nutrient foramen. Scale bar equals 1 mm.

Foramen area and Q_i calculation

For the analysis, we measured the nutrient foramina present in the femoral diaphysis of the studied specimens (Fig. 1). In cases where more than one foramen was present on the specimen, we measured the largest one. In the case of fossil specimens, when sediment was adhered to the bone surface and potentially interfered with the measurement of the nutrient foramen, we removed the sediment in order to completely expose its complete size. In all cases, the specimens were checked for cracks and breakages that could modify the original size of the nutrient foramen, and in those cases the foramen was not sampled. Measurements were made digitally using the software Fiji (Schindelin et al. 2012) on high-resolution digital images following Seymour (2012) and Hu et al. (2020). We measured the minor diameter of the nutrient foramen for the Q_i calculation. For each specimen we also measured the femur's total length using a digital caliper or a measuring tape for very large fossil specimens. The index of blood flow into the femur through the nutrient foramen was calculated as $Q_i = r^4/L$, where r is the nutrient foramen radius and L is the femur total length. Also, we obtained the average body mass (*Bm*) for each extant and extinct taxa from a literature review (Table S1).

Phylogeny

In order to account for the phylogenetic relatedness of the studied taxa in all the statistical analyses, we obtained an updated phylogeny of extant mammals from TimeTree (Kumar et al. 2022) and pruned all the taxa that were not present in our dataset. Also, we added the fossil taxa to this phylogeny following the most recent publications on fossil xenarthran systematics, namely Wible (2006); Delsuc et al. (2016), Tambusso et al. (2021) for Cingulata, Casali et al. (2020) for Vermilingua, and Varela et al. (2019), Delsuc et al. (2019), and Presslee et al. (2019) for Folivora. The final tree was constructed using Mesquite (Maddison and

Maddison 2007). Since the original phylogeny was time-calibrated, the inclusion of fossil taxa resulted in a non-ultrametric tree in which the tips corresponding to older fossil taxa are consequently placed with shorter branch lengths that do not reach 0 Ma. This approach produced a phylogeny including 88 tips, with 69 of those representing extant taxa and 19 representing extinct taxa.

Q_i evolution, PGLS, and ANCOVA

The phylogenetic tree was used to address the relevance of the phylogenetic signal on the evolution of Q_i and *Bm*. In particular, we fitted three evolutionary models to the data using the "fitContinuous" function of the R package Geiger (Harmon et al. 2008), namely Brownian Motion (BM), Ornstein-Uhlenbeck (OU), and White Noise (WN; i.e., absence of phylogenetic signal). We addressed model significance using Akaike weights and selected the model showing the best fit for the data for further analyses.

To establish the relationship between the Q_i and *Bm* we used Phylogenetic Generalized Least Squares (PGLS) regressions, which allow to account for the non-independence of biological data due to phylogenetic relatedness in a flexible way. The flexibility of PGLS not only allows for the incorporation of several variables, but also allows for the implementation of different correlation structures like BM or OU (Harmon 2019). Considering this, we set the correlation structure according to the results of the analysis described in the previous step. These analyses were conducted using the R package "nlme" (Pinheiro et al. 2017) with the function "gls", implementing phylogenetic weights and the correlation structure "corMartins" of the package "ape" (Paradis et al. 2004). We fitted different models accounting for the existence of differences in slopes and/or intercepts and compared them in relation to the significance of their respective coefficients, as well as using the AIC and BIC metrics. In particular, we tested the significance of a model including a categorical variable depicting a grouping based on previous assumptions about mammalian metabolism, i.e., the fact that non-placental mammals and xenarthran placentals tend to show lower metabolisms than the rest of the placentals (McNab 1984, 1986). Furthermore, in order to incorporate our hypotheses concerning fossil giant xenarthrans, we grouped both glyptodonts and ground sloths as a separate group. The normality of the residual error of the fitted models was addressed following the approach of Butler et al. (2000), by transforming the residuals by the Cholesky decomposition of the inverse of the phylogenetic covariance matrix and testing for normality of the transformed residuals using the “lille.test” function of the R package “nortest” (Gross and Ligges 2015).

In order to explore differences among the major mammalian clades, we conducted a *post hoc* test using an ANCOVA approach. Specifically, we tested for the significance of a categorical variable representing three extant mammalian groups, namely non-placentals (Prototheria and Metatheria), Xenarthra, and Epitheria (i.e., non-xenarthran placentals), and the fossil giant xenarthrans groups that are recognised as widely diverging from extant forms (i.e., Folivora, excluding both extant genera, and Glyptodontiinae). In this regard, based on the lower metabolic rates often reported for non-placentals and xenarthrans, we hypothesized that there were significant differences between their regression lines and that of the rest of the placentals. We used the “glht” function of the R package “multcomp” (Hothorn et al. 2016) and adjusted P-values according to the Bonferroni method in order to account for the effect of multiple comparisons.

Estimation of MMR

To estimate the Maximum Metabolic Rate of the studied fossil xenarthrans, we conducted an analysis using the R package "mvMorph" (Clavel et al. 2015), which allowed us to estimate missing values for fossil tips as well as ancestral states for the mammalian phylogeny. For this analysis we fitted a multivariate OU model to the phylogeny accounting for the covariance of MMR, *Qi*, and *Bm* of taxa using the function "mvOU". Then, we used the "estimate" function to estimate ancestral and missing tip values. In order to explore the predicting capacity of our model, we compared the estimated and observed values for taxa with MMR data and performed a linear regression to obtain a R^2 value. This approach allowed us to obtain average and 95% confidence values for each of the studied taxa and check the reliability of the estimates against the observed values in taxa with available MMR data. We performed ancestral state reconstruction using the best model and the functions "estim" of the R package "mvMorph" and "contMap" of the package "phytools" (Revell 2012). Finally, we report Mass-independent MMR values as $mLO_2h^{-1}g^{-0.67}$ for better comparison with previous literature (Hemmingsen 1960; Knaus et al. 2021).

Results

Nutrient Foramina Size and Q_i in Extant and Extinct Xenarthrans

The results showed considerable variation in the nutrient foramina size across the extant and extinct xenarthrans analyzed, and, as expected, a strong correlation with body mass. Most of the studied specimens showed only one nutrient foramen in the femur diaphysis, but a minority of the specimens showed two, which were almost always located in close proximity from one another. Some fossil specimens apparently lacked any nutrient foramina in their diaphysis; however, we could not confirm if this condition was the product of sediment deposition or taphonomic alterations. Nevertheless, the absence of nutrient foramina in long bones is reported as relatively common in humans (Mysorekar 1967). The obtained Q_i values for the extant xenarthrans spanned from 2.73*10^{-7} mm^3 for the species (*Cyclopes didactylus*) to 2.09*10^{-4} mm^3 for the largest species (*Priodontes maximus*). The Q_i values for the sampled extinct xenarthrans ranged from 6.06*10^{-5} mm^3 for the smallest armadillo (*Neoglyptatelus* sp.) to 1.72*10^{-2} mm^3 for the largest sloth (*Megatherium americanum*). When plotting the studied extinct giant xenarthrans' Q_i against *Bm*, many taxa fell considerably above the OLS regression line of mammals, well within the expected values for placental mammals of similar size (Fig. 2).

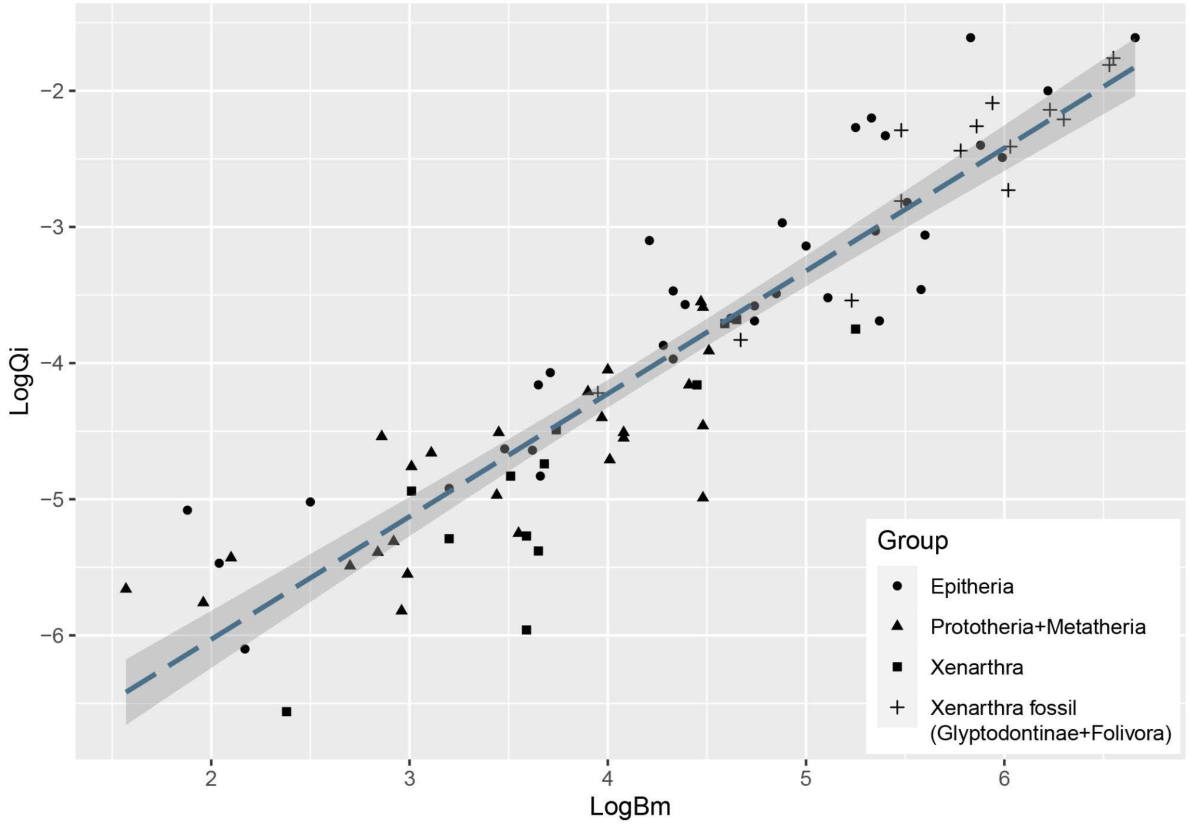

Figure 2. Bivariate plot of log Q_i vs log *Bm* showing the placement of the different mammalian groups mentioned in the text. OLS regression is plotted for comparison with previous studies.

Phylogenetic Generalized Least Squares and ANCOVA

The best fitting models for *Bm* and Q_i were different. For *Bm*, the best fitting model was BM (AIC.w = 0.744), while an OU model was the best fit for Q_i data (AIC.w = 0.687). In both cases, the white noise model showed extremely low fit compared to the alternatives (AIC.w < 0.001), indicating the existence of phylogenetic signals in both variables. Considering this, we used an OU model for the PGSL. As expected, the PGLS analysis showed a strong correlation between *Bm* and Q_i. Furthermore, the inclusion of the categorical grouping variable in the analysis supported the distinction between some of the groups. In particular, the results showed support for the existence of different intercepts between groups ($p = 0.0001$), but no distinction between slopes (the interaction coefficient was not significant, $p = 0.084$; while the BIC showed a better fit for the model without interaction, $\Delta BIC > 6$, and the AIC showed no preference between both models, $\Delta AIC < 2$). The Lillefors test showed normality of the transformed residuals ($p = 0.315$). The post-hoc pairwise ANCOVA showed a significant difference between the Epitheria and both extant xenarthrans and non-placentals (Bonferroni-adjusted $p < 0.001$ and $p = 0.014$, respectively). On the other hand, the giant fossil xenarthrans (ground sloths and glyptodonts) showed a significant difference in intercept with the extant xenarthra ($p = 0.002$), but no significant difference with the Epitheria ($p = 1$).

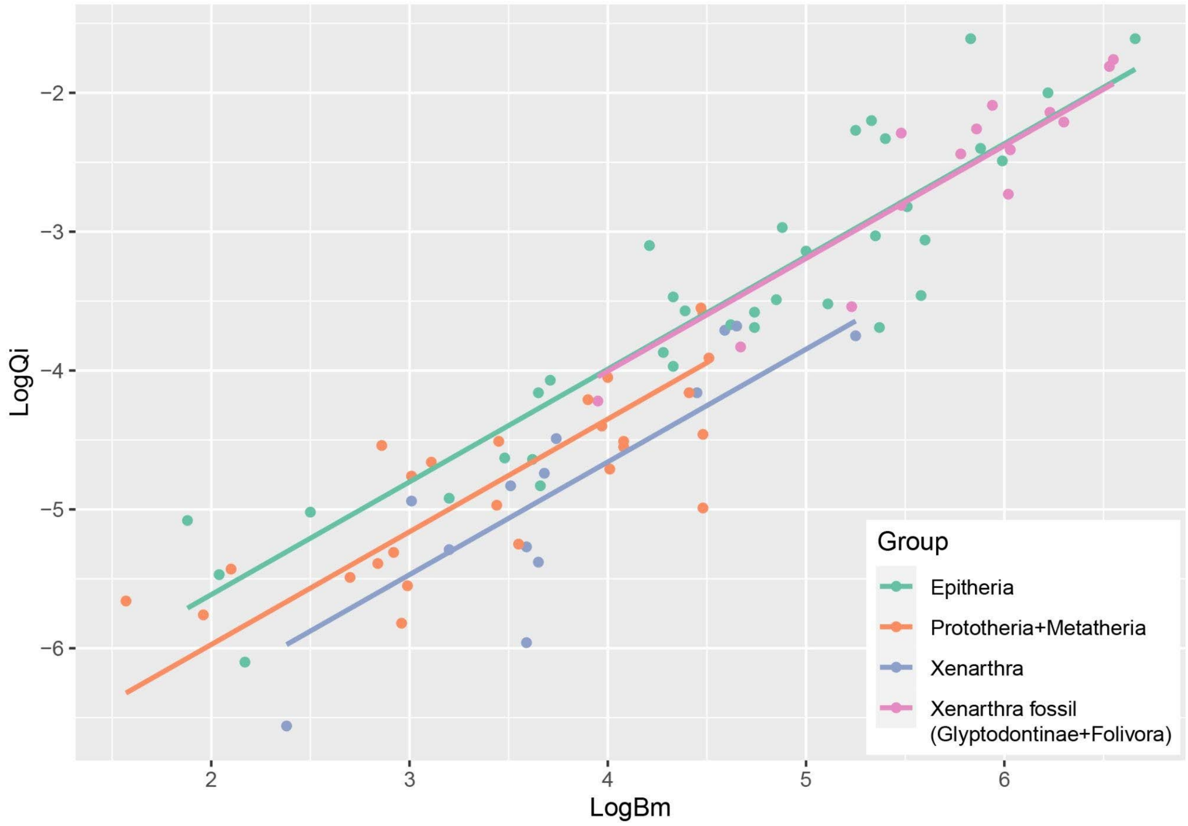

Figure 3. PGLS results showing the difference in intercepts recovered for the mammalian groups considered in the study.

Estimation of MMR and Ancestral Character Reconstruction

The OU model was the best fitting model when compared to a BM model in the multivariate analysis (ΔAIC > 10). The observed vs predicted regression showed a $R^2 = 0.92$, indicating a good prediction capacity of the model. The predicted MMR values when considering *Bm* and Q_i as predictors ranged from 6.09 mL $O_2h^{-1}g^{-0.67}$ for the sloth *Bradypus torquatus* to 589.72 mL $O_2h^{-1}g^{-0.67}$ for the equid *Equus caballus*. The lowest predicted values corresponded to three extant xenarthrans, the sloth *Bradypus torquatus*, the anteater *Cyclopes didactylus*, and the armadillo *Dasypus hybridus*, all with values lower than 50 mL $O_2h^{-1}g^{-0.67}$ and lower than the values registered or estimated for all the analyzed Epitheria. Epitherians showed the highest estimated values, with *Canis familiaris* and *Equus caballus* showing values higher than 300 and 500 mL $O_2h^{-1}g^{-0.67}$, respectively. A distinction between extant xenarthrans an fossil giant xenarthrans was seen in the predicted values, with the former showing estimated MMR values lower than 100 mL $O_2h^{-1}g^{-0.67}$ in all cases and the latter showing values higher than 100 mL $O_2h^{-1}g^{-0.67}$ except for two sloths, *Nothrotherium maquinense* and *Scelidotherium leptocephalum*.

The ancestral reconstruction showed a pattern consistent with multiple independent cases of high or low MMRs, with almost all ancestral nodes showing intermediate MMR values. This pattern is consistent with the presence of taxa with low and high MMRs in all groups and the fact that our sample is relatively small and covers a wide spectrum of mammal species with a deep phylogenetic history. In the case of the Xenarthra, the results show an ancestor with an intermediate MMR and the independent acquisition of low MMR in the three extant clades.

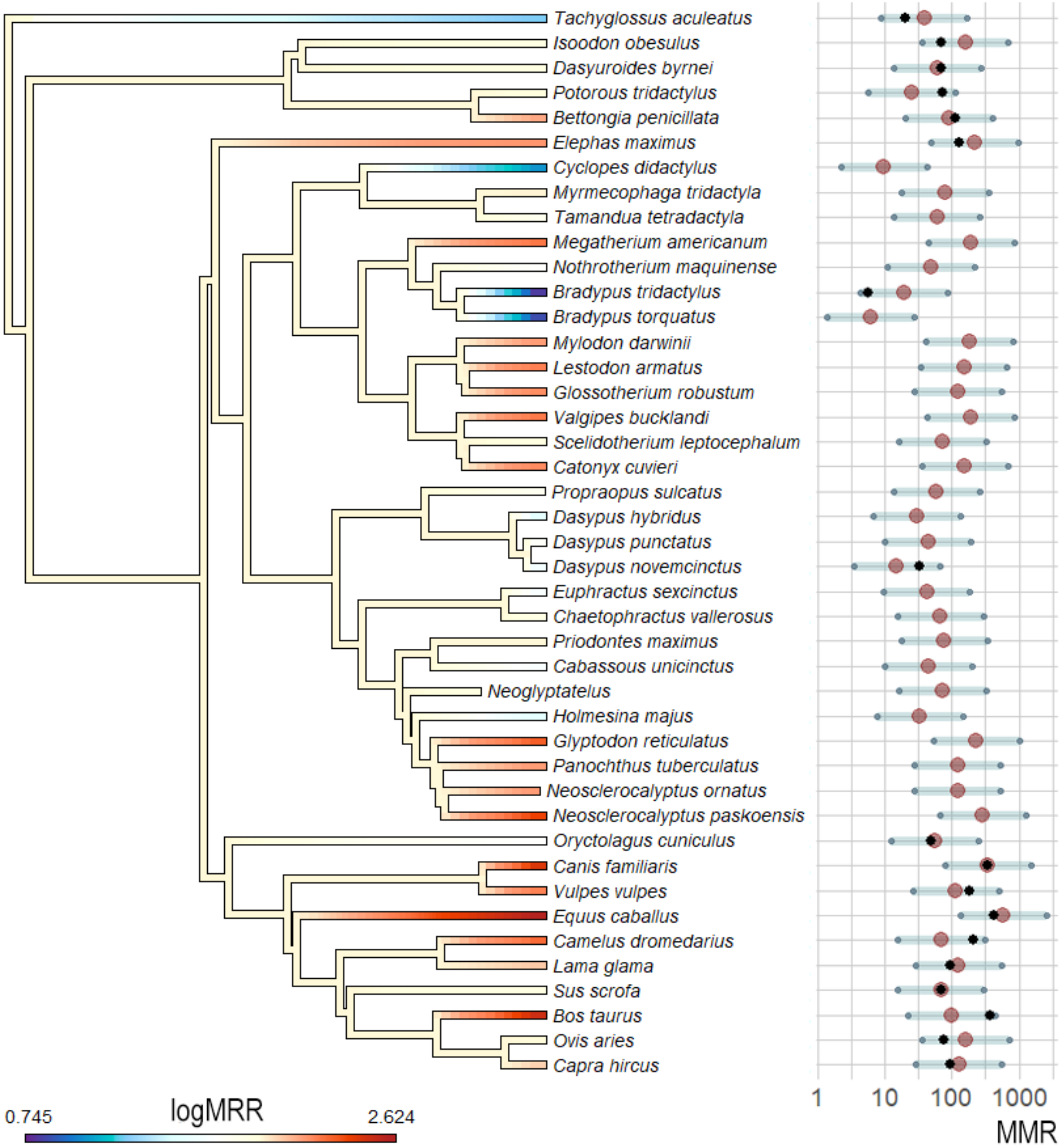

Figure 4. MMR phylogenetic estimations and ancestral state reconstruction using *Bm* and Q_i as predictor variables and the OU model. MMR values in the phylogeny are log-transformed for better visualization. MMR average estimations and 95% confidence intervals are shown as red dots and gray bars, respectively. Black dots represent MMR values for taxa with empirical data available.

Discussion

Size of nutrient foramina and femoral blood flow in xenarthrans

Previous research has shown consistent differences between some tetrapods regarding the size of the femur nutrient foramina, and the consequent blood flow into the femur diaphysis, and its relationship to the aerobic capacity of different groups. In particular, among extant tetrapods, birds and mammals have shown significantly larger nutrient foramina when compared to other tetrapods, which has been associated with their higher metabolic needs and more active lifestyles (Seymour et al. 2012). Furthermore, these differences have allowed researchers to study the aerobic capacity and maximum metabolic rates of fossil taxa, gaining insights regarding the metabolism of groups like dinosaurs, basal mammaliforms, among others (Seymour et al. 2012; Allan et al. 2014; Newham et al. 2020). This approach not only provides information on specific extinct taxa, but also has allowed the improvement of the understanding of the evolution of aspects such as endothermy in the fossil record (Grigg et al. 2022). In fact, it is noteworthy that some early synapsids showed evidence in favor of elevated aerobic capacity based on Q_i values similar to those observed in mammals, pointing to an early evolution of the high aerobic capacity commonly seen in mammals (Knaus et al. 2021). However, no previous research has focused on the potential differences in nutrient foramina size within mammals, especially considering the large variations that exist in the clade regarding aerobic capacity and metabolic rate.

In this regard, our results show significant differences in the nutrient foramina size and the consequent inferred femora blood flow between different mammalian groups. The PGLS regressions showed considerable differences between the Epitheria and the rest of the sampled mammals, indicating that extant xenarthrans and non-placentals have proportionally smaller nutrient foramina and less blood flow in their femora compared with the Epitheria. In particular, the xenarthrans most adapted to a slow-moving lifestyle, represented by the extant sloth *Bradypus* and the vermilinguan *Cyclopes didactylus*, showed the smallest foramina sizes reported for mammals, which would be in accordance with the extremely low metabolism reported for these taxa (McNab 1978; Nagya and Montgomery 2012). On the contrary, the results showed that the extinct giant xenarthrans like glyptodonts and ground sloths had proportionately larger nutrient foramina in their femora, indicating higher aerobic capacity and agility levels when compared to their extant relatives. In fact, some of the largest xenarthrans like the sloths *Megatherium americanum* and *Lestodon armatus*, or the glyptodont *Glyptodon reticulatus* showed Q_i values comparable to those observed in other giant placental mammals like elephants in our dataset.

Aerobic capacity and maximum metabolic rate in extinct giant xenarthrans

Our results place most giant fossil xenarthra as animals with aerobic capacities just like other similarly giant placentals, such as elephants or some ungulates. In fact, two non-xenarthra taxa from the late Pleistocene megafauna, the Meridiungulata (relatively closely related to extant ungulates; Buckley 2015) *Macrauchenia patachonica* and *Toxodon platensis* also showed values similar to those of the extinct giant xenarthrans. The relative size of nutrient foramina in giant xenarthrans, as well as the estimations of MMR point to a relatively high aerobic lifestyle for these animals, which would greatly differ from that of their extant relatives. In fact, the results for *Megatherium americanum*, the largest sloth analyzed, are compatible with the previous studies mentioned before, and depict an animal probably capable of a considerable level of aerobic activity non different to what is observed in a similarly sized placental mammal like the elephant. Furthermore, most of the sampled mylodontids (the other xenarthran clade with members reaching more than 1000 kg) showed Q_i and MMR values similar to those observed in placentals of similar size, which would indicate similarly aerobic capacity to *Megatherium americanum* and elephants. Interestingly, the Scelidotheriinae

Scelidotherium leptocephalum, which is largely associated with fossorial habits based on its morphology and its association to fossil burrows (Vizcaíno et al. 2001, Patiño et al. 2021) showed lower Q_i and MMR values when compared to other scelidotheriines of similar size like *Catonyx cuvieri* and *Valgipes bucklandi*. Moreover, the relatively small nothrotheriid *Nothrotherium maquinense* also showed reduced Q_i and MMR values in comparison to giant terrestrial taxa. Interestingly, *N. maquinense* was a relatively small sloth interpreted as climbers that probably fed on leaves, which would be compatible with our results based on what is known from extant sloths (Dantas and Santos 2022; Santos et al. 2023). Regarding cingulates, it is interesting to note that fossil armadillos closely related to extant taxa, like *Propraopus sulcatus* and *Dasypus punctatus*, and morphologically similar pampatheres, like *Holmesina majus*, showed overlapping MMR values with extant taxa, even in cases where extinct forms are considerable larger that extant ones. These fossil taxa are often depicted as capable burrowers and probably had similar diets and habits to those observed in extant armadillos, which are characteristics related to the low metabolic rates observed in extant xenarthrans. On the other hand, giant glyptodonts showed elevated Q_i and MMR values compatible with higher aerobic capacities and metabolic needs. These findings are interesting considering glyptodonts are often depicted as slow-moving, heavy-weighted, and armored taxa that probably were not capable of agile movements (Gillette and Ray 1981). However, the results would be more in line with predictions based on certain morphological traits seen in glyptodonts that proposed the existence of intraspecific fights using their club-like tails (Alexander et al. 1999). In fact, one of the glyptodonts that showed high MMR values was *Glyptodon reticulatus*, which has been proposed as capable of acquiring a bipedal stance in agonistic contexts (Fariña 1995).

Most giant fossil xenarthrans probably presented extreme departures from the common habits and morphology observed in their closely-related extant forms. In particular, some fossil taxa show extreme gigantism, which impedes the consideration of extant forms as potential analogs for their study (Vizcaíno et al. 2018). Furthermore, their large body size would also have had a significant impact over other aspects of these taxa, including their behavior, ecology, and evolution. Moreover, aspects like the extreme modification of the carapace in giant glyptodonts or the inability to climb trees of giant ground sloths further increases their unlikeness to extant forms. On the other hand, these fossil xenarthrans have been associated with what is known of their extant relatives, often depicting them as slow and not agile (Gillette and Ray 1981; Toledo 1996). Likewise, low body temperatures and metabolic rates have been assumed for extinct giant xenarthrans mostly based on their phylogenetic position (McNab 1985).

However, some research has shown evidence in favor of a more active lifestyle for some extinct xenarthra in comparison to their extant relatives. For example, several studies concerning the intracranial morphology of extinct xenarthrans, in particular the auditory region, have shown evidence in favor of a more active lifestyle in comparison with extant members of the clade. Billet et al. (2013) studied the inner ear of several xenarthrans and showed that the megatheriid *Megatherium americanum* was more agile than extant sloths based on the scaling of its semicircular canals. Boscaini et al. (2018) recovered similar results for the mylodontid *Glossotherium robustum*, arguing that this ground sloth had an inner ear morphology more similar to terrestrial taxa like *Tamandua* than to other xenarthran with fossorial habits. In fact, Boscaini et al. (2018) results placed *G. robustum* among mammals of similar size categorized as having a “medium” level of agility according to Spoor et al. (2007). Furthermore, another study by Tambusso et al. (2021) noted that extinct glyptodonts like *Glyptodon reticulatus*, *Panochthus tuberculatus*, and *Doedicurus clavicaudatus* would fall within the agility range observed in most extant Cingulata and Vermilingua, but considerable overlap among agility categories was present based on semicircular canals’ morphology. These studies would indicate that giant fossil xenarthrans could have had aerobic capacities comparable to other giant mammals in opposition to most of the extant members of the clade like sloths or fossorial armadillos. Other studies also point in a similar direction, indicating the possibility of considerable aerobic capacity. For example, the crural index of the fossil sloth *Pyramiodontherium scillatoyanei* was considerable high when compared to other xenarthra, with a value similar to those found in ungulates like llamas and horses, potentially indicating a relatively high agility (De Iuliis et al. 2004). Furthermore, the estimation of *Megatherium americanum* walking speed based on fossil tracks by Casinos (1996) and Blanco and Czerwonogora (2003) showed a range of 0.8 to 2.2 m/s, which would be similar to the normal walking speed reported for Asian elephants of similar size (1.37 ± 0.28 ms^{-1}; Ren et al. 2008). For glyptodonts, some studies have proposed the existence of interspecific fights, as well as a certain capacity to adopt bipedal locomotion (Fariña et al.1995; Alexander et al. 1999; Vizcaíno et al. 2011).

Implications for the understanding of fossil giant xenarthrans metabolism

Regarding the metabolic rate of fossil xenarthrans, most studies have focused on exploring the basal metabolic rate of members of the group. However, minimum and maximum metabolic rates are correlated in vertebrates and, therefore, our results provide important insights that can be compared to previous research (Auer et al. 2017). A study by Vizcaíno et al. (2006) showed some differences in the occlusal surface areas of fossil giant sloths, with *Megatherium americanum* having higher than expected oral processing capacity, pointing to high energetic needs in this taxon, while mylodontids showed lower processing capacities. A recent study by Dantas and Santos (2022) expanded this approach and showed results consistent with high metabolic rates for most of the Brazilian Intertropical Region fossil ground sloths. In particular, the results showed probable high metabolic rates for members of the Mylodontidae, Megatheriidae and Megalonychidae, while the Nothrotheriidae showed lower values comparable to those of extant xenarthrans. Other studies have also pointed to probable high metabolic rates in fossil sloths based on different methods. Recently, Tejada-Lara et al. (2021) proposed that the interpretation of low metabolic needs in fossil mylodontids could be instead explained by the consumption of higher-quality food like meat based on the finding that *Mylodon darwinii* probably consumed animal protein according to Isotope data from amino acids, in line with other proposals of animal items in the diet of Pleistocene sloths (Fariña 1996; Fariña and Blanco 1996; Fariña and Varela 2018). Furthermore, this interpretation would also explain the capacity of some sloths like *M. darwinii* to have lived in fairly extreme cold weathers like southern Patagonia (Varela and Fariña 2016; Varela et al. 2018). Similarly, the megalonychid *Megalonyx jeffersonii* has been recovered in northern North America, with a latitude record at 68ºN in Canada, indicating that this species could also endure considerable cold climates (Harington 1978). Other indirect evidence that would point to higher metabolic rates in fossil sloths in comparison to extant forms could be related to the evolution of a complete marine lifestyle in the nothrotheriid genera *Thalassocnus* (Amson et al. 2014). In this aspect, many extant marine mammals like odontocetes, otariids, and sea otters show elevated metabolic rates for their body mass when compared with terrestrial forms (with the clear exception of sirenians, which show lower metabolic rates for their body mass; Costa 2009). Furthermore, besides their arboreal and fossorial habits, the low metabolism seen in extant xenarthrans has also been related to their particular diets. McNab (1985, 1986) showed that the constraints imposed by the diets of extant xenarthrans (myrmecophagy and folivory) were important determinants for their low body temperatures and metabolic rates. Concerning this, it is important to note that many, if not all, fossil giant xenarthrans were probably adapted to other food sources. For example, many studies have shown that most of the late Pleistocene mylodontids were adapted to the consumption of grass, with many taxa probably consuming high percentage of grass similar to ungulate grazers (Bargo and Vizcaíno 2008; Varela et al. 2023a; Varela et al. 2023b). In fact, these adaptations would be favored by a developmental mechanism in mylodontids, where the posterior teeth would be enlarged due to an unique inhibitory cascade pattern within sloths (Varela et al. 2020). In this scenario, the mere assumption of low metabolic rates for extinct giant xenarthrans based solely on their phylogenetic relatedness to extant forms is not supported, and studies should contemplate metabolic rates equal to those of other Eutheria when studying giant fossil xenarthrans.

Finally, based on the ancestral reconstruction of MMR, it would seem that the low metabolism often reported for extant xenarthrans would not represent a trait acquired early in the evolution of the clade, but, more probably, a later and independent acquisition. Interestingly, these low metabolic rates could be the product of the ecological preferences of the extant xenarthrans, representing an exemplary case of convergent evolution due to peculiar habits shared by almost all extant members of the clade, like insectivory (and myrmecophagy in some cases) and folivory, or by contrasting habits like the fossoriality of armadillos or the suspensory habits of sloths, which are both associated to lower metabolic rates (McNab 1985). Considering this, extant xenarthrans would not represent good analogues for studying the metabolism of extinct taxa. Likewise, the fact that extant xenarthrans show relatively low agility levels and metabolic rates should not be considered as evidence for the presence of these characteristics in extinct taxa, especially in giant forms like glyptodonts and ground sloths. This has important implications when addressing different ecological questions like the roles these giant animals had in past ecosystems (Vizcaíno et al. 2023). For example, Fariña (1996) made his calculations about the thermodynamics of the trophic relationships in Lujanian mammals using values of metabolic rates according only to body size, i.e., without phylogenetic correction or any other variable, which would be reasonable for the giant xenarthrans of this fauna considering our results. Furthermore, more recent and refined calculation did use those corrections without major changes in the results (Fariña et al. 2013).

Conclusions

The study of physiological traits like metabolism is often difficult in fossil taxa due to the common lack of preservation of soft body parts in the fossil record. Considering this, it is indispensable to explore morphological features that can be associated to species' metabolic rates but also commonly present in the fossil record. In this regard, the study of femora nutrient foramina represents an excellent opportunity to explore the aerobic capacity and maximum metabolic rate in extinct species without clear extant analogues like the giant xenarthrans. Despite this, the results of these kinds of analyses have some limitations and the limited data on extant taxa's MMR reduce models capacity to estimate extinct taxa's values. Future research on extinct giant xenarthrans should focus on expanding the available MMR data for extant taxa, but also on incorporating other variables associated with metabolic rate in fossil taxa to further improve our understanding of these strange animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

All the data used in this paper is available either in the main text or the supplementary materials. The code used in the study is provided in: https://github.com/lvar/Varela_et_al._2023

Authors contribution

Luciano Varela: Conceptualization, Investigation, Methodology, Formal analysis, Resources, Visualization, Writing – Original Draft. **P. Sebastián Tambusso**: Resources, Writing – Review & Editing. **Richard A. Fariña**: Resources, Funding acquisition, Writing – Review & Editing.

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Conclusiones y Perspectivas

Los trabajos publicados como resultado de la presente tesis permitieron expandir los conocimientos respecto a los xenartros fósiles, en particular a aquellos taxones que presentaron una marcada tendencia al gigantismo. En conjunto, los trabajos presentados ofrecen una combinación de diferentes metodologías para abordar algunos aspectos claves para un mejor entendimiento de la paleobiología de estos animales. En este sentido, los trabajos aportan conocimientos vinculados con la biología evolutiva del desarrollo, la biomecánica, la morfología, la ecología isotópica y la evolución del metabolismo, en muchos casos empleando metodologías novedosas e implementando análisis filogenéticos como herramientas indispensables para el estudio de la evolución de diferentes tipos de rasgos en el registro fósil.

Se mostró la existencia en los perezosos de un patrón macroevolutivo previamente descrito en varios grupos de mamíferos por el cual se rigen los tamaños de los tres molares (molariformes en el caso de los perezosos) en base a una cascada de activación-inactivación durante el desarrollo. Este resultado es interesante, ya que los perezosos no presentan la dentición típica de los mamíferos y no existe certeza sobre la homología de sus dientes respecto a la diferenciación de las piezas dentarias en el resto del grupo. Asimismo, los resultados mostraron que los milodóntidos presentan un patrón diferente dentro de los perezosos, el cual parecería haber sido determinante para las adaptaciones a dietas pastadoras observadas en el grupo. En base a estos resultados, en el futuro se espera expandir esta aproximación a la dentición superior de los perezosos y contrastar recientes propuestas en relación con patrones alométricos relacionados al desarrollo de los molares en los mamíferos.

Por medio de una aproximación biomecánica basada en análisis de elementos finitos en mandíbulas de perezosos, se pudo explorar adaptaciones a distintos tipos de dietas en varios taxones. Los resultados permitieron observar adaptaciones compatibles con dietas basadas en pastos en los milodóntidos (corroborando estudios anteriores), así como también señales de posibles dietas basadas en objetos duros como tubérculos o frutos tipo nuez en algunas especies para las cuales no existen predicciones anteriores. En este contexto, la incorporación de este tipo de análisis podrá resultar de gran importancia para estudiar distintos aspectos biomecánicos de la megafauna del Pleistoceno de América.

Se corroboraron estos resultados en una comunidad de megamamíferos en el Pleistoceno final a partir de un análisis de isótopos estables en especímenes provenientes del sitio Arroyo del Vizcaíno en el sur de Uruguay. Estos resultados permitieron cuantificar la proporción de pastos C_4 en la dieta de los diferentes taxones presentes en el sitio, incluidos perezosos y gliptodontes, y explorar la existencia de partición de recursos entre taxones emparentados. De forma adicional, estos análisis permitieron obtener información relevante sobre las condiciones climáticas en la región justo antes del comienzo del último máximo glaciar. En este contexto, el hallazgo de un nuevo taxón descrito para el Arroyo del Vizcaíno aportó datos novedosos y permitió expandir el registro del género *Nothrotheriops* en América del Sur, así como también describir sus preferencias climáticas y dietarias en la región. En el futuro se pretende abordar aspectos relacionados a la variabilidad en y entre individuos en algunas de las especies presentes en el sitio Arroyo del Vizcaíno por medio del análisis de muestras en dientes de crecimiento continuo y la inclusión de individuos de diferentes sitios de la región del Río de la Plata. De forma adicional, los datos generados podrán ser utilizados junto con modelos de nicho ecológico para estudiar la evolución de las preferencias dietarias y climáticas en estos animales por medio de la utilización de métodos filogenéticos comparativos.

Por último, las estimaciones de capacidad aeróbica y metabolismo máximo en xenartros fósiles gigantes supone un aporte novedoso para el estudio del grupo, ya que representa una de las pocas investigaciones relacionadas a los requerimientos metabólicos de estos animales. Los resultados de este estudio permitieron no sólo evaluar a los taxones gigantes extintos, los cuales mostraron metabolismos máximos comparables a los observados en otros placentarios no xenartros y no en los xenartros actuales, si no que también permitieron obtener una visión macroevolutiva de esta característica, evidenciando la ausencia de un patrón común a todos los xenartros. Esto resulta importante ya que supone que los bajos metabolismos observados en los xenartros actuales no serían producto de una característica ancestral si no que habrían sido adquiridos de forma independiente en los distintos grupos probablemente como producto de sus adaptaciones para dietas basadas en insectos u hojas y sus hábitos arbóreos o fosoriales.

Es importante destacar que los trabajos desarrollados también permitieron generar tres publicaciones de datos abiertos a partir de los modelos tridimensionales utilizados, los cuales representan un aporte más de esta tesis (Anexo; Varela et al. 2021; Varela 2023; Varela y Tambusso 2023). De igual forma, las investigaciones desarrolladas formaron parte de una serie de libros digitales y abiertos (arroyodelvizcaino.org/publicaciones) y de un libro impreso (megafauna3d.org/un-libro-de-huesos) de divulgación científica, generando así una contribución en un aspecto importante de la generación de conocimiento científico como es la comunicación científica.

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Anexo. Publicaciones de datos abiertos a partir de los modelos tridimensionales utilizados:

- **Varela, L.**, Lobato, C., Tambusso, P. S. (2021). 3D model related to the publication: Presence of the ground sloth *Valgipes bucklandi* (Xenarthra, Folivora, Scelidotheriinae) in southern Uruguay during the Late Pleistocene: Ecological and biogeographical implications. MorphoMuseuM, 7(147), 1-2.
- **Varela, L.** (2023). 3D model related to the publication: Occurrence of the ground sloth *Nothrotheriops* (Xenarthra, Folivora) in the Late Pleistocene of Uruguay: New information on its dietary and habitat preferences based on stable isotope. MorphoMuseuM, 9(2), 1-2.
- **Varela, L.**, Tambusso, P. S. (2023). 3D Models Related to the Publication: 3D Finite Element Analysis and Geometric Morphometrics of Sloths (Xenarthra, Folivora) Mandibles Shows Insights on the Dietary Specializations of Fossil Taxa. MorphoMuseuM, 9(2), 1-2.

3D model related to the publication: Presence of the ground sloth *Valgipes bucklandi* (Xenarthra, Folivora, Scelidotheriinae) in southern Uruguay during the Late Pleistocene: Ecological and biogeographical implications

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Abstract
The present 3D Dataset contains the 3D model analyzed in Presence of the ground sloth *Valgipes bucklandi* (Xenarthra, Folivora, Scelidotheriinae) in southern Uruguay during the Late Pleistocene: Ecological and biogeographical implications. Quaternary International. https://doi.org/10.1016/j.quaint.2021.06.011.

Keywords: Ground sloth, Mylodontidae, Quaternary, Scelidotheriinae, South America

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Inv nr.	Taxon	Description
CAV1573	*Valgipes bucklandi*	Left tibia-fibula

Table 1. Involved specimen. Collection: Servicio Académico Universitario y Centro de Estudio Paleontológicos (SAUCE-P), Universidad de la República.

INTRODUCTION

We present the surface model (Fig. 1 and Table 1) of a specimen of *Valgipes bucklandi* (Mammalia, Folivora) from the Arroyo del Vizcaíno site (AdV; Canelones, Uruguay; Table 1). The AdV is a fossiliferous site dated to 30 ka, where numerous taxa typical of the Late Pleistocene South American megafauna have been found (Fariña *et al.*, 2014). The specimen represents the southernmost occurrence record of this sloth and provides evidence showing a greater climatic tolerance. Furthermore, the presence of *V. bucklandi* increases to four the number of sloth taxa found in the site, where the mylodontids *Lestodon armatus*, *Glossotherium robustum*, and *Mylodon darwinii* were previously reported (Varela and Fariña, 2016).

METHODS

The fossil specimen was scanned using a DAVID SLS-2 Scanner and processed with the DAVID 3D software. The 3D surfaces scans were semi-automatically aligned with the DAVID 3D software. Due to the preservation state of the specimen, two parts were scanned independently and later aligned in an anatomically correct position in order to be fused into a single 3D model. The 3D surface model is provided in .obj format, which can be opened by an extensive list of free and open-source software.

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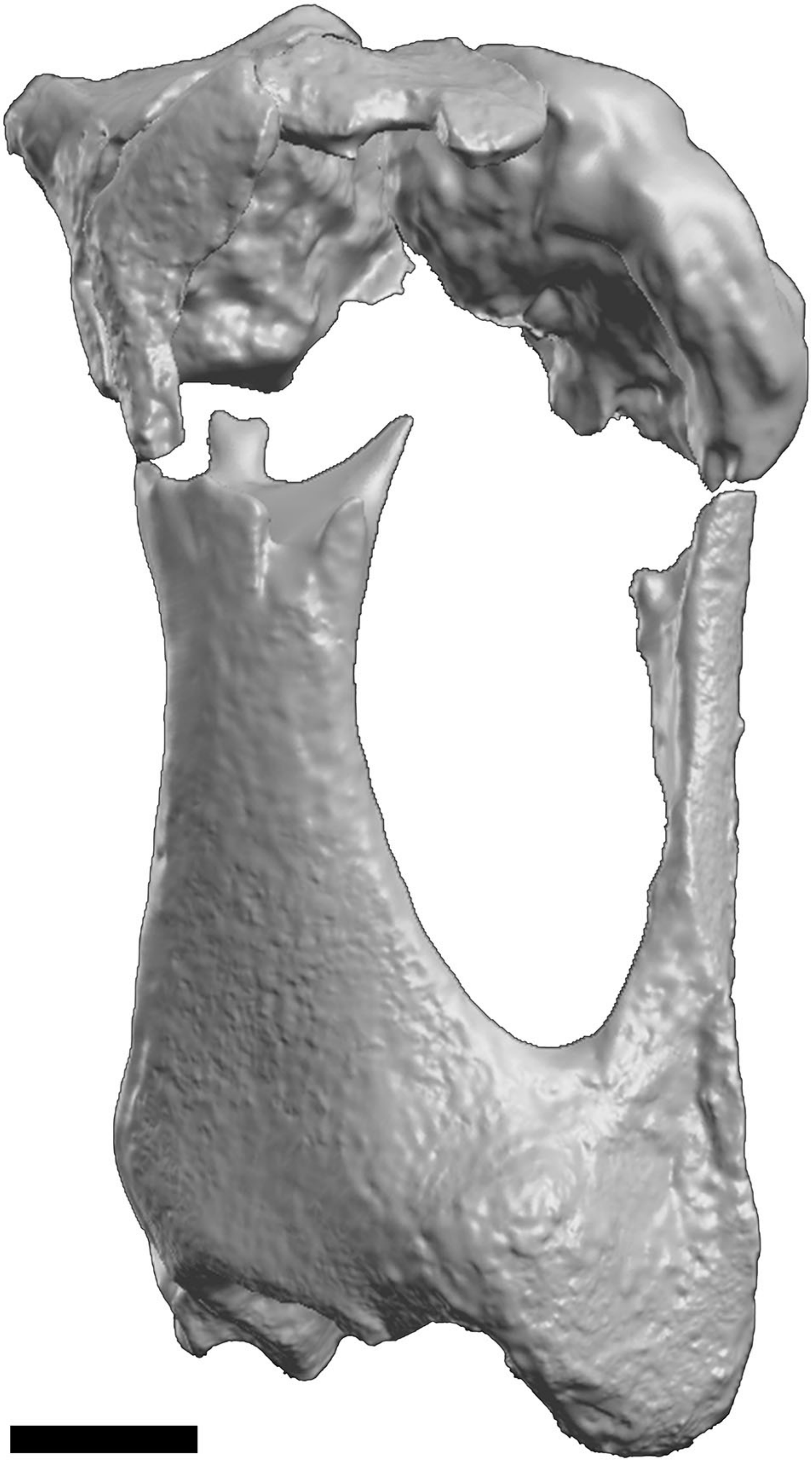

Figure 1. 3D surface model of CAV 1573 (*Valgipes bucklandi*) in anterior view. Scale bar: 5 cm.

MorphoMuseuM

3D model related to the publication: Occurrence of the ground sloth *Nothrotheriops* (Xenarthra, Folivora) in the Late Pleistocene of Uruguay: New information on its dietary and habitat preferences based on stable isotope analysis

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Abstract

The present 3D Dataset contains the 3D model analyzed in the following publication: Occurrence of the ground sloth Nothrotheriops (Xenarthra, Folivora) in the Late Pleistocene of Uruguay: New information on its dietary and habitat preferences based on stable isotope analysis. Journal of Mammalian Evolution. https://doi.org/10.1007/s10914-023-09660-w

Keywords: Ground sloth, Nothrotheriidae, Nothrotheriinae, Quaternary, South America

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Inv nr.	Taxon	Description
CAV1466	*Nothrotheriops sp.*	Left humerus

Table 1. Involved specimen. Collection: Servicio Académico Universitario y Centro de Estudio Paleontológicos (SAUCE-P), Universidad de la República.

INTRODUCTION

We present the surface model (Fig. 1) of a specimen of *Nothrotheriops* sp. (Mammalia, Folivora) from the Arroyo del Vizcaíno site (AdV; Canelones, Uruguay; Table 1). The AdV was previously dated to 30 ka through 14C, and the recovered fauna is composed of several typical South American Late Pleistocene taxa (Fariña *et al.*, 2014). The specimen represents one of the scarce records of this taxon for South America and contributes to the knowledge of its distribution in the continent. Furthermore, the new record of *Nothrotheriops* increases to five the number of sloth taxa found at the site, where the mylodontids *Lestodon armatus*, *Glossotherium robustum*, and *Mylodon darwinii* and the scelidotheriid *Valgipes bucklandi* were previously reported (Varela and Fariña, 2016; Lobato et al. 2021). The data regarding the specimen is published in Varela et al. (2023).

METHODS

The fossil specimen was scanned using photogrammetry with the software Agisoft Photoscan. The 3D surface model is provided in .obj format, which can be opened by an extensive list of free and open-source software.

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Figure 1. 3D surface model of CAV 1466 (*Nothrotheriops* sp.) in anterior view. Scale bar: 5 cm.

morphomuseum

Dataset

3D models related to the publication: 3D Finite Element Analysis and Geometric Morphometrics of Sloths (Xenarthra, Folivora) Mandibles Show Insights on the Dietary Specializations of Fossil Taxa

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Abstract

The present 3D Dataset contains the 3D models analyzed in 3D Finite Element Analysis and Geometric Morphometrics of Sloths (Xenarthra, Folivora) Mandibles Show Insights on the Dietary Specializations of Fossil Taxa. Journal of South American Earth Sciences. https://doi.org/10.1016/j.jsames.2023.104445.

Keywords: Ground Sloths, Mandibles, Photogrammetry, Quaternary, South America

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INTRODUCTION

We present the surface models (Fig. 1; Table 1) of several specimens of Folivora (Mammalia, Xenarthra) used in Varela et al. (2023). The scanned specimens represent mandibles of adult individuals of representatives of one of the mayor clades of sloths, the Mylodontoidea (Delsuc et al. 2019; Varela et al. 2019), namely *Lestodon armatus*, *Glossotherium robustum*, *Mylodon darwinii*, *Scelidotherium leptocephalum*, and *Valgipes bucklandi*. The specimens also correspond the some of the most representative taxa of the Río de la Plata region during the Late Pleistocene (Varela and Fariña 2016; Varela et a. 2018). The models were used in Varela et al. (2023) to perform Finite Elements Analysis and Geometric Morphometrics to explore potential differences among taxa regarding their dietary specializations. The results supported the existence of adaptations to hard-food processing in some taxa and consequently the probable resource partitioning among members of the Mylodontoidea coexisting in the same region during the Late Pleistocene.

METHODS

The fossil specimens were scanned using photogrammetry with the software Agisoft Photoscan. The 3D surface models are provided in .stl format, which can be opened by an extensive list of free and open-source software.

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Inv nr.	Taxon	Description	Collection
CAV 379	*Mylodon darwinii*	Right hemimandible	SAUCE-P, Uruguay
MNHN-M 137,722	*Scelidotheriun leptocephalum*	Mandible	MNHN, Montevideo, Uruguay
MNHN-M 914	*Glossotherium robustum*	Mandible	MNHN, Montevideo, Uruguay
MPAC 899	*Lestodon armatus*	Mandible	MPAC, Colonia, Uruguay
NHMD.Z.M.K 1/1845:3540	*Valgipes bucklandi*	Mandible	NHMD, Copenhagen, Denmark

Table 1. Involved specimens. Collections: Statens Naturhistoriske Museum, Copenhagen, Denmark (NHMD); Museo Nacional de Historia Natural, Montevideo, Uruguay (MNHN-M); Museo Paleontológico Profesor Armando Calcaterra, Colonia, Uruguay (MPAC); Servicio Académico Universitario y Centro de Estudio Paleontológicos, Universidad de la República, Canelones, Uruguay (SAUCE-P).

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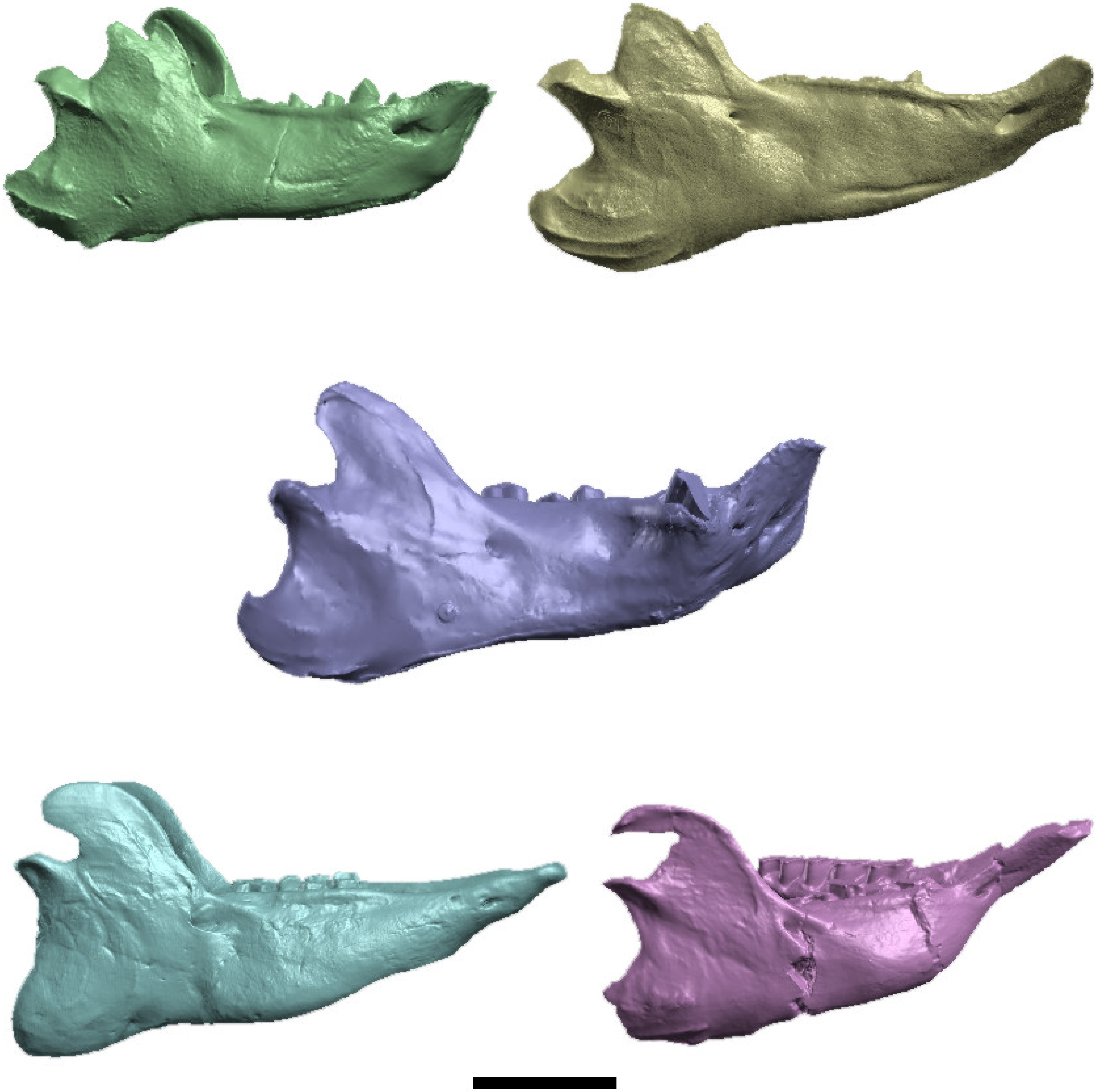

Figure 1. 3D surface models of: CAV 379 (*Mylodon darwinii*), MPAC 899 (*Lestodon* armatus), MNHN-M 914 (*Glossotherium* robustum), MNHN-M 137,722 (*Scelidotherium leptocephalum*), and NHMD.Z.M.K. 1/1845:3540 (*Valgipes* bucklandi) in lateral view. Scale bar: 10 cm.

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