UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Biociências
Câmpus do Litoral Paulista

PALEOECOLOGIA DA MEGAFAUNA TERRESTRE E MARINHA PLEISTOCÊNICA DO SUL DO BRASIL

THAYARA SILVEIRA CARRASCO

SÃO VICENTE, SP
2023

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THAYARA SILVEIRA CARRASCO
Orientador: Prof. Dr. Francisco Sekiguchi Buchmann

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APRESENTAÇÃO

A presente tese teve como foco principal o estudo da ecologia de mamíferos terrestres e marinhos que habitaram o sul do Brasil durante o Pleistoceno Médio-Superior, o que resultou em quatro capítulos. Todos os capítulos foram redigidos em língua inglesa e em formato de artigo de acordo com o periódico de escolha. Os capítulos 1 e 2 foram direcionados ao estudo da megafauna terrestre, enquanto os capítulos 3 e 4 abordaram a megafauna marinha.

O capítulo 1, intitulado "Paleodiet of Lamini camelids (Mammalia: Artiodactyla) from the Pleistocene of southern Brazil: insights from stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$)", foi publicado na revista *Paleobiology* e objetivou reconstituir a dieta de três espécies de camelídeos, com implicações sobre o paleoambiente do Rio Grande do Sul, bioma Pampa. Embora *Hemiauchenia paradoxa* esteja atualmente extinta, *Lama guanicoe* e *Vicugna vicugna* ainda habitam algumas regiões de clima frio e seco da América do Sul. O estudo da ecologia dessas espécies forneceu possíveis explicações para suas extinções locais, além de evidenciar as mudanças ambientais ocorridas no sul do Brasil durante os últimos milhares de anos.

O capítulo 2, intitulado "Paleobiology of Pleistocene large land mammals from the Brazilian Pampa", foi submetido para a revista *Quaternary International* e apresenta uma perspectiva mais ampla sobre a ecologia de grandes mamíferos terrestres do bioma Pampa. Assim como o capítulo 1, compreendeu a análise de isótopos estáveis de carbono ($\delta^{13}C$) e oxigênio ($\delta^{18}O$) em fósseis de diversos táxons e localidades, informações que foram complementadas com dados disponíveis na literatura.

O capítulo 3, intitulado "Pleistocene cetacean fossils from the coastal plain of Rio Grande do Sul in southern Brazil", foi publicado na revista *Alcheringa: An Australasian Journal of Palaeontology* e descreve estruturas ósseas do ouvido de cetáceos (bulas timpânicas e perióticos). Tais estruturas são diagnósticas a nível de família, gênero ou espécie, permitindo a identificação de táxons que ocorriam no Atlântico Sul-Ocidental durante o Pleistoceno, com implicações sobre suas paleodistribuições.

Por fim, o capítulo 4, intitulado "Ecological traits of the franciscana dolphin (*Pontoporia blainvillei*) from the Late Pleistocene to the present days based on stable isotope analysis", publicado na revista *Marine Mammal Science*, apresenta uma comparação ecológica, através de análise de isótopos estáveis, sobre a dieta e uso de habitat de toninhas fósseis (representando o Pleistoceno) e contemporâneas (representando o Holoceno), coletadas no sul do Brasil, ou FMA (i.e., *Franciscana Management Area*) III.

RESUMO

O Pleistoceno foi uma época geológica caracterizada pela sucessão de ciclos glaciais e interglaciais. Esses ciclos ocasionavam o recuo e avanço do nível do mar, respectivamente, ora expondo a plataforma continental e ora submergindo-a. Em consequência do avanço/retração do ambiente terrestre/marinho, a Planície Costeira do Rio Grande do Sul abriga depósitos fossilíferos submersos, que datam do Pleistoceno Médio-Superior, associados à ambos os ambientes. Atualmente, fósseis de mamíferos terrestres e marinhos, objetos de estudo da presente tese, são transportados pela ação das ondas e podem ser encontrados *ex situ* na zona de varrido das praias da região, principalmente da Praia dos Concheiros. Fósseis de mamíferos terrestres estão associados aos períodos glaciais e análises isotópicas de carbono ($\delta^{13}C$) e oxigênio ($\delta^{18}O$) de tais espécimes revelaram que estes habitavam campos, corroborando com reconstruções ambientais baseadas em palinologia. O clima, que provavelmente era frio e sazonal, favorecia o desenvolvimento de plantas C_3, especialmente gramíneas. Diante disso, a transição do Pleistoceno-Holoceno pode ter sido prejudicial à sobrevivência dos grandes herbívoros, devido ao estabelecimento de um clima mais quente e úmido que, associado a outros fatores, culminou na extinção da megafauna terrestre. A fauna de mamíferos marinhos do Pleistoceno, por outro lado, parece ter sido similar à atual. Com o desenvolvimento da presente tese, foram identificados fósseis de rorquais (*Balaenoptera* sp.), baleias-francas (*Eubalaena* sp.), delfinídeos (Delphinidae) e toninhas (*Pontoporia blainvillei*). Porém, a diversidade reportada para o Pleistoceno é subestimada e mais esforços devem ser realizados para a identificação da fauna passada. Entre os táxons de baleias e golfinhos presentes no registro fóssil da Planície Costeira do Rio Grande do Sul, a toninha é a mais abundante, fornecendo uma oportunidade única de estudar a ecologia dessa espécie ao longo do tempo geológico. A comparação entre espécimes atuais e fósseis evidenciou que o nicho isotópico da toninha ($\delta^{13}C$ *vs*. $\delta^{18}O$) era maior durante o Pleistoceno, devido aos valores de $\delta^{13}C$ mais amplos em fósseis. Os valores de $\delta^{18}O$, em contrapartida, foram mais amplos em toninhas atuais. Essas diferenças podem ser atribuídas a mudanças nas comunidades de peixes, com uma maior diversidade de presas pelágicas no passado, além de um maior fluxo de água doce e mudanças de salinidade na costa atual. Resultados que podem refletir também o impacto antrópico sobre os ecossistemas costeiros – empobrecimento das comunidades devido à sobrepesca.

Palavras-chave: Dieta, habitat, fóssil, Quaternário, Pleistoceno.

ABSTRACT

The Pleistocene epoch was characterized by the succession of glacial and interglacial cycles, leading to fluctuations in sea levels. As a result, the Rio Grande do Sul Coastal Plain provided favorable conditions for the fossilization of both terrestrial and marine mammals, although these deposits are now submerged. Fossils are typically found ex situ on the beach, after being transported by wave action. Land mammalian fossils are associated with glacial periods and isotopic analysis of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) of these specimens revealed that they inhabited grasslands, which aligns with environmental reconstructions based on palynology. The climate during this time was likely cool and seasonal, promoting the growth of C_3 plants, especially grasses. In view of this, the Pleistocene-Holocene transition, marked by a warmer and more humid climate, may have been detrimental to the survival of large herbivores, and combined with other factors, culminated in the extinction of terrestrial megafauna. In contrast, the marine mammalian fauna of the Pleistocene appears to be similar to that of the Holocene or present day. In the present study, fossil remains of rorquals (*Balaenoptera* sp.), right whales (*Eubalaena* sp.), dolphins (Delphinidae), and franciscanas (*Pontoporia blainvillei*) were identified. However, it is likely that the reported diversity is underestimated, and further efforts are required to identify past fauna accurately. Among the fossilized whale and dolphin taxa identified in Rio Grande do Sul Coastal Plain, the franciscana is the most abundant, providing a unique opportunity to study the species' ecology over geological time. A comparison between contemporary and fossil specimens revealed that franciscana's isotopic niche ($\delta^{13}C$ *vs.* $\delta^{18}O$) was wider during the Pleistocene, due to more disparate $\delta^{13}C$ values in fossils. In contrast, modern franciscanas showed more disparate $\delta^{18}O$ values. These differences can be attributed to changes in fish communities, with a higher diversity of pelagic prey in the past, as well as freshwater influx and associated salinity changes along the current coast. The results may also reflect the anthropogenic impact on coastal ecosystems, particularly the impoverishment of communities due to overfishing.

Keywords: Diet, habitat, fossil, Quaternary, Pleistocene.

INTRODUÇÃO GERAL

Pleistoceno

Antecedido pelo Plioceno e sucedido pelo Holoceno, o Pleistoceno é uma época geológica do Período Quaternário, Era Cenozóica, que compreende o intervalo temporal entre 2,58 milhões de anos (Ma) e 11,7 mil anos (ka) atrás (Cohen et al., 2013). Essa época foi marcada por uma alta variabilidade climática caracterizada pela sucessão de períodos frios e quentes, chamados de glaciais e interglaciais, respectivamente (Paillard, 1998).

A amplitude dos ciclos glaciais-interglaciais foi variável ao longo do Pleistoceno, mas estima-se que tenham ocorrido em uma frequência de cerca de 100 ka a partir do Pleistoceno Médio (Pisias & Moore, 1981; Elderfield et al., 2012), desencadeados por mudanças na insolação de verão no hemisfério norte devido a variações na órbita da Terra, ou ciclos de Milankovitch (Kawamura et al., 2007). No entanto, somente as variações na radiação solar são insuficientes para explicar tal periodicidade (Fig. 1). Possivelmente, mecanismos climáticos internos de retroalimentação, como o aumento e histerese do manto de gelo, também atuavam na manutenção dos ciclos glaciais-interglaciais (Bintanja & van de Wal, 2008; Abe-Ouchi et al., 2013; Hughes & Gibbard, 2018).

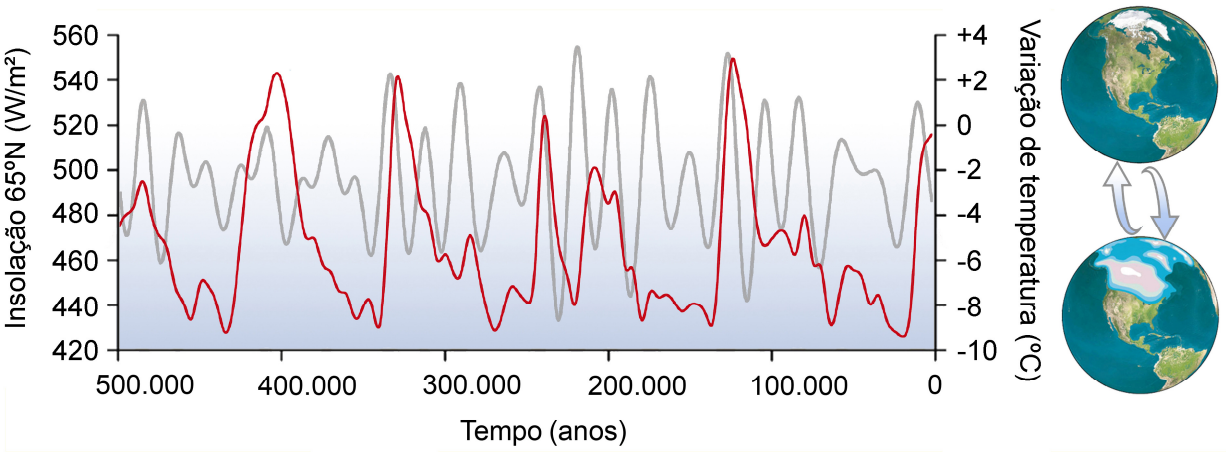


Fig. 1 Variações na insolação (linha cinza) e temperatura antártica (linha vermelha) durante os últimos 500 ka. Dados de Ellis & Palmer (2016).

As mudanças climáticas foram acompanhadas por mudanças nos ecossistemas terrestres da América do Sul. Condições mais frias resultavam em um predomínio de campos e ambientes áridos e, por outro lado, condições mais quentes favoreceriam a expansão de florestas remanescentes (Webb, 1991; Fig. 2). Por exemplo, no sul do Brasil, a floresta tropical, que

atualmente estende-se até 30ºS, era significativamente reduzida durante os períodos glaciais e substituída por campos dominados por gramíneas da família Poaceae (Behling, 2002; Behling et al., 2005; Roth et al., 2021). Dados do Pleistoceno Superior, com maior definição taxonômica, indicam que poáceas da subfamília Pooidae eram muito abundantes no bioma Pampa (Mourelle et al., 2020). Uma vegetação tipicamente campestre provavelmente favorecia populações de grandes herbívoros terrestres: gramíneas possuem baixo investimento em defesas contra herbivoria e são altamente nutritivas, especialmente aquelas de clima frio (Barbehenn et al., 2004; Mann et al., 2019).

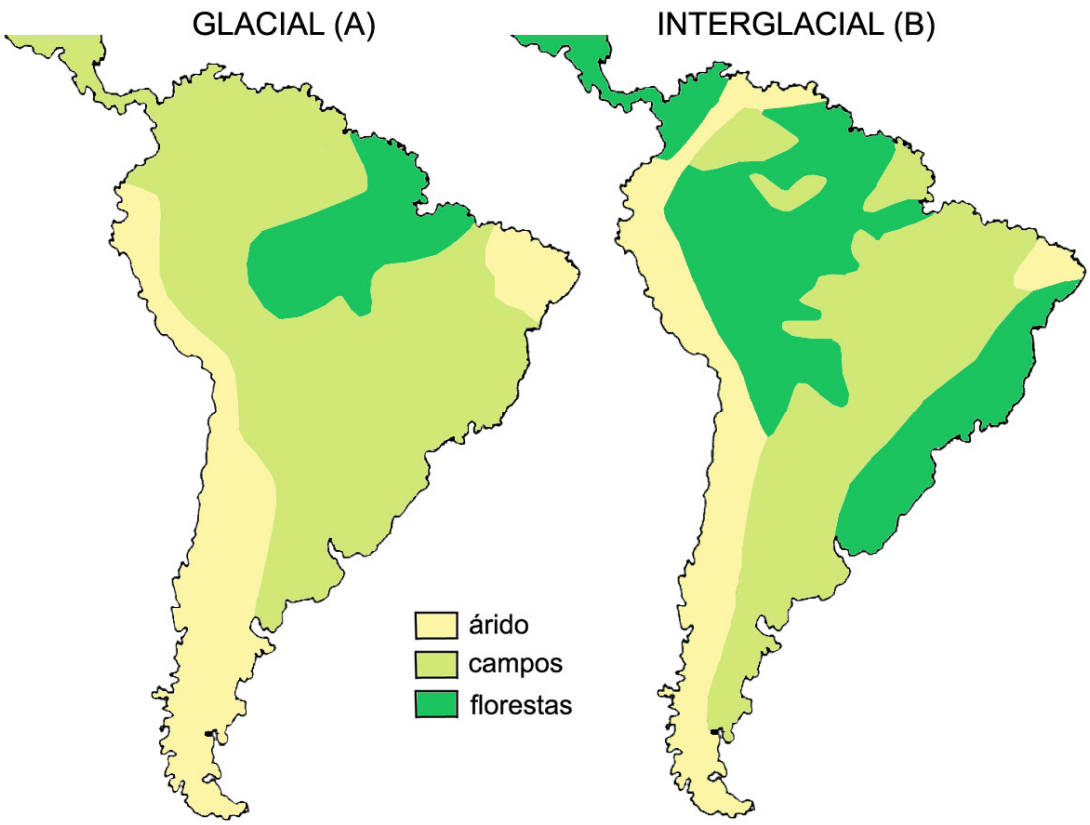


Fig. 2 Principais biomas encontrados na América do Sul durante o Pleistoceno: (A) predomínio de campos nos períodos glaciais; (B) períodos interglaciais caracterizados pelo avanço de florestas. Modificado de Webb (1991).

Além das evidentes mudanças supracitadas, a expansão do gelo marinho ocasionava o recuo do nível do mar e exposição da plataforma continental, aumentando a área habitável para a fauna terrestre (Borrero, 1996). Em contrapartida, nos períodos interglaciais, o derretimento do gelo era sucedido pelo aumento do nível do mar e a consequente inundação da plataforma continental (Denton et al., 2010). Na Planície Costeira do Rio Grande do Sul, estima-se que o nível do mar era cerca de 120 m mais baixo durante os períodos glaciais e, durante os

interglaciais, podia atingir cerca de 9,5 m acima do nível atual (Villwock & Tomazelli, 1995; Rosa et al., 2017).

Em decorrência dos deslocamentos da linha de costa (Fig. 3), o Rio Grande do Sul abriga diversos depósitos fossilíferos submersos que contém fósseis marinhos (associados aos períodos interglaciais) e terrestres (associados aos períodos glaciais), os quais são frequentemente transportados pela ação das ondas e encontrados *ex situ* na zona de varrido das praias da região (Paula Couto, 1961; Figueiredo, 1975; Buchmann, 1994; Lopes & Buchmann, 2011; Cruz et al., 2016).

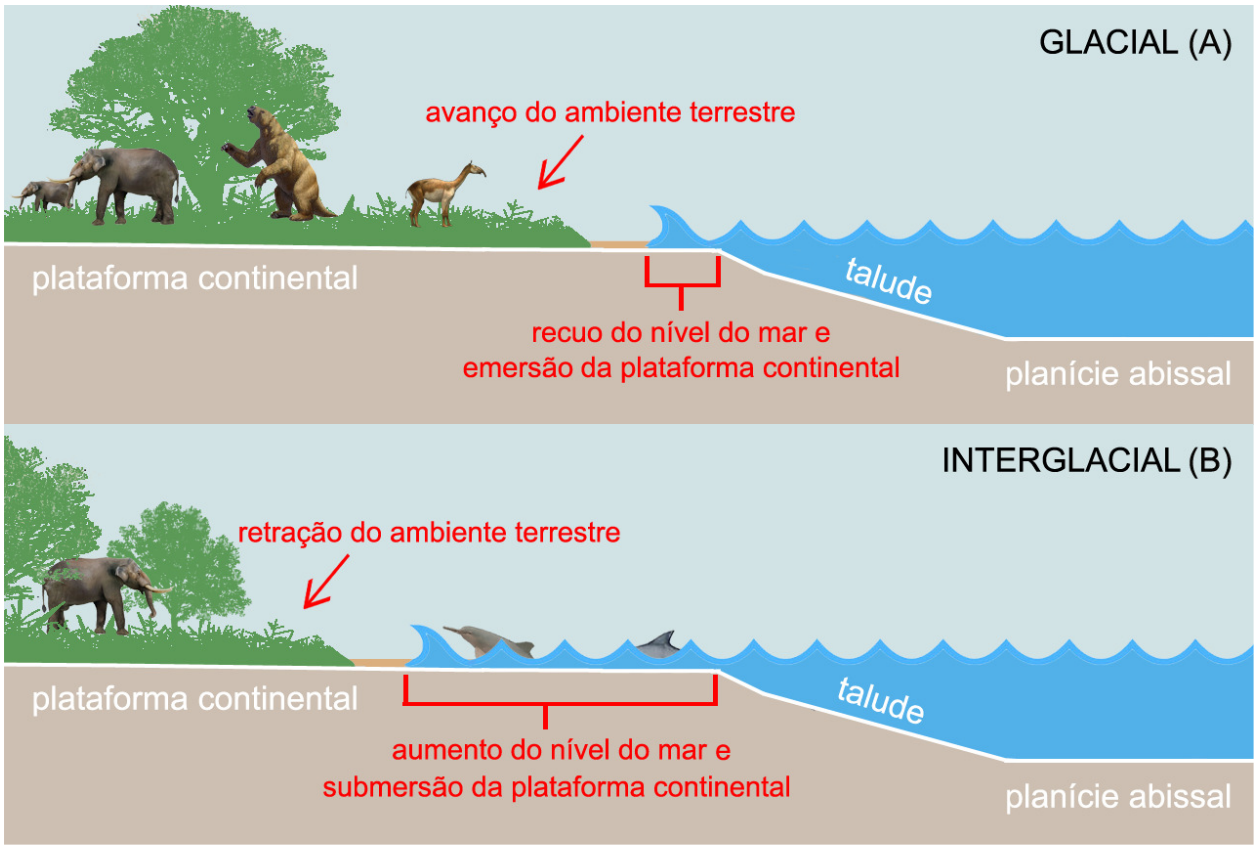


Fig. 3 Esquema representando as mudanças na plataforma continental do Rio Grande do Sul decorrentes das oscilações do nível do mar: (A) recuo do nível do mar e consequente avanço do ambiente terrestre durante os períodos glaciais; (B) aumento no nível do mar e retração do ambiente terrestre durante os períodos interglaciais. Fonte: autora.

Se por um lado, a área habitável para a fauna terrestre aumentava durante os períodos glaciais em decorrência do recuo do nível do mar, por outro, os habitats disponíveis para as espécies marinhas costeiras eram drasticamente reduzidos (Ludt & Rocha, 2015). Ademais, os ciclos de resfriamento e aquecimento da Terra alteravam a dinâmica marinha no hemisfério sul devido à expansão do gelo antártico (Otto-Bliesner et al., 2007; Ferrari et al., 2014). Mudanças na circulação oceânica, temperatura da água, estratificação, distribuição de nutrientes e

solubilidade de CO_2 na água do mar (e.g., Bischof & Darby, 1997; Stephens & Keeling, 2000; Mackensen et al., 2001; Ferrari et al., 2014; Lazar & Polyak, 2016; Martin-Garcia et al., 2018) impactaram a biota marinha desde o fitoplâncton até níveis tróficos mais elevados (e.g., Thatje et al., 2008). No entanto, o efeito das glaciações pode ter sido mais ameno em regiões subtropicais, por meio do armazenamento de águas com maior salinidade e mais quentes em comparação com latitudes mais altas (Santos et al., 2017).

Megafauna terrestre

A definição de megafauna é ampla e varia de acordo com a comunidade ou ecossistema estudado (Moleón et al., 2020). Por exemplo, Owen-Smith (1988) empregou o termo "mega" para designar animais herbívoros que excediam 1.000 kg, que incluiria elefantes, rinocerontes e hipopótamos, mas outros gigantes como as girafas estariam no limite dessa categoria. Trabalhos mais recentes, todavia, incluem uma margem consideravelmente maior – desde vertebrados com massa corporal de 10 kg até mais de duas toneladas (e.g., Wroe et al., 2013).

Na presente tese, enquadraram-se em "megafauna terrestre" os grandes mamíferos com peso entre 30 e 6.000 kg nas formas adultas, que habitavam o sul do Brasil durante o Pleistoceno, mas atualmente encontram-se extintos ou localmente extintos. Os grandes mamíferos pleistocênicos dessa região compreendiam principalmente herbívoros, como os xenartros, que incluem gliptodontes e preguiças-gigantes, uma ampla diversidade de artiodáctilos, como camelídeos e cervídeos, além de perissodáctilos, proboscídeos, litopternos e notoungulatos (Paula Couto, 1961; Soliani, 1973; Buchmann, 1994; Lopes et al., 2020a).

Parte dessa fauna, juntamente com mamíferos carnívoros, migrou da América do Norte para a América do Sul (e vice-versa) em um evento paleozoogeográfico que ficou conhecido como Grande Intercâmbio Americano (em inglês, *Great American Biotic Interchange* – GABI) (Webb, 1985; Fig. 4). Alguns pesquisadores consideram, inclusive, a migração do homem para a América do Sul como parte do GABI, o que teria culminado na extinção da megafauna (Cione et al., 2015). O GABI ocorreu em várias fases durante mais de 9 Ma, mas estima-se que a mais importante tenha ocorrido no Plioceno Superior, há aproximadamente 2,6 Ma, após o soerguimento do Istmo do Panamá e quando as condições climáticas estavam favoráveis (Webb, 1991; Woodburne, 2010; Cione et al., 2015).

O GABI foi um evento migratório assimétrico – mais táxons norte-americanos colonizaram a América do Sul do que o contrário, de modo que aproximadamente metade dos

gêneros encontrados atualmente no continente sul-americano são originários da América do Norte (Marshall, 1988; Webb, 2006). Essa assimetria pode ter sido resultado de uma maior suscetibilidade dos mamíferos sul-americanos à predação, pois a América do Sul possuía uma fauna de predadores menos desenvolvida (Faurby & Svenning, 2016). Além disso, o extenso e repetido intercâmbio de faunas entre a América do Norte e a Eurásia durante o Terciário pode ter resultado em uma maior resistência a patógenos na fauna norte-americana em comparação com a fauna sul-americana, que estava quase completamente isolada (Ferigolo, 1999).



Fig. 4 Exemplo de táxons norte- e sul-americanos envolvidos no Grande Intercâmbio Americano (GABI). Fonte: autora.

Os herbívoros norte-americanos possuíam uma dieta flexível e variável que incluía plantas C_3 e C_4, enquanto nativos como a macrauquênia (*Macrauchenia* sp.) apresentavam dieta mais restrita (Domingo et al., 2020). Apesar dessa aparente vantagem competitiva, o GABI não causou grandes extinções, mas sim comunidades extremamente diversas (Faurby & Svenning, 2016).

Embora a América do Sul tenha abrigado uma fauna diversa de grandes mamíferos durante o Pleistoceno – maiores até mesmo do que aqueles encontrados na África (Owen-Smith, 2013) – a maioria encontra-se extinta atualmente, fazendo com que o tamanho médio dos animais com mais de 10 kg diminuísse de 843 para 81 kg (Doughty et al., 2013). No sul do Brasil, a fauna que era dominada por espécies da Ordem Xenarthra no Pleistoceno, passou a ser dominada por roedores (Ordem Rodentia) no Holoceno (Lopes et al., 2020a).

Diversas explicações para a extinção do Pleistoceno Superior/Holoceno foram propostas. Por exemplo, Firestone e colaboradores (2007) sugeriram que a extinção da megafauna estaria relacionada com o impacto de um cometa sobre a América do Norte (há cerca de 12,9 ka) devido a geração de ondas de choque, pulsos termais e mudanças ambientais como a perda de biomassa vegetal. Além disso, esse impacto teria desestabilizado a camada de gelo, diminuindo a temperatura da Terra – período de resfriamento denominado *Younger Dryas* (Firestone et al., 2007; Sweatman, 2021), o que pode ter contribuído com a extinção da megafauna, como sugerido por outros autores (e.g., Stewart et al., 2021). No entanto, é mais provável que o impacto do cometa tenha tido um efeito local e esse evento por si só não seria capaz de explicar as extinções em massa do Pleistoceno Superior/Holoceno (Ruban, 2009). Ainda, Ferigolo (1999) sugeriu que essa extinção estaria relacionada à introdução de novos patógenos após o GABI, levantando mais uma hipótese. Entretanto, uma combinação de diferentes fatores, como mudanças ecossistêmicas relacionadas à última era glacial e a caça humana, é a explicação mais aceita atualmente (van Hoesel et al., 2014).

Após a última era glacial, o clima sofreu uma transição de frio e seco para quente e seco, no Holoceno Inferior, e quente e úmido, com algumas fases mais secas, a partir do Holoceno Médio (Prado & Alberdi, 1999; Behling et al., 2005; Roth et al., 2021). Consequentemente, houve mudanças na vegetação, como o aumento de gramíneas da família Cyperaceae, diminuição de poáceas da subfamília Pooidae e expansão das matas de galeria devido às condições mais úmidas (Behling et al., 2005; Mourelle et al., 2020; Roth et al., 2021). Registros polínicos obtidos de turfas do extremo sul do Brasil indicam uma melhoria climática no Holoceno marcada pela grande representatividade de polens arbóreos (Lima et al., 2020). Essas

mudanças ambientais foram provavelmente desvantajosas aos grandes mamíferos em decorrência de suas preferências ecológicas por campos (Bocherens et al., 2016).

O clima mais quente e úmido do Holoceno permitiu o desenvolvimento de plantas com maior capacidade competitiva, as quais tendem a apresentar um alto investimento em anatomia e defesas químicas contra a herbivoria, bem como baixo conteúdo proteico, fatores que teriam ocasionado na perda da diversidade de herbívoros (Mann et al., 2019). Porém, dados paleoclimáticos não indicam que a deglaciação mais recente foi mais rápida ou em maior magnitude do que as demais ocorridas nos últimos 700 ka (Barnosky et al., 2004), de modo que outros fatores devem ter atuado sinergicamente para a extinção da megafauna.

Mudanças na flora, desencadeadas por mudanças no clima, ocorreram diversas vezes ao longo do Pleistoceno sem causar grandes extinções e a chegada do homem à América do Sul foi a única novidade biológica que ocorreu no presente interglacial (Cione et al., 2009). O grande tamanho corporal dos mamíferos pleistocênicos conferia certas características adaptativas aos ecossistemas transitórios daquela época: eficiência metabólica e locomotora, resistência à inanição e longa expectativa de vida (Mann et al., 2019). Em contrapartida, também estava associado à uma baixa densidade populacional e taxa reprodutiva lenta, aumentando a vulnerabilidade da megafauna a ameaças previamente inexistentes, como a caça humana (Cione et al., 2009; Mann et al., 2019).

A cronologia do povoamento inicial da América do Sul ainda é um tema amplamente debatido. De acordo com a maioria dos arqueólogos, esse processo teria se iniciado entre 15,5 e 13 ka (Politis & Prates, 2018; Prates et al., 2020; Fiedel, 2022; Pérez-Balarezo et al., 2023). No entanto, registros mais antigos têm sido reportados na literatura, como é o caso de osteodermes de preguiças-gigantes da espécie *Glossotherium phoenesis*, encontrados na Serra das Araras (centro-oeste do Brasil) com idades de ~27 ka e modificados pelo homem (Pansani et al., 2023). A descontinuidade temporal e espacial de registros mais antigos do que 15 ka pode indicar que as populações humanas iniciais teriam sofrido extinções locais e regionais (Politis & Prates, 2018; Fiedel, 2022).

Os primeiros sinais de ocupação contínua no Cone Sul da América do Sul (i.e., Argentina, Chile, Uruguai e sul do Brasil) aparecem entre 14,5 e 14 ka (Politis & Prates, 2018; Fiedel, 2022) e especificamente a partir de 13 ka no sul do Brasil (Santos, 2023). Os últimos registros da megafauna no Cone Sul, por sua vez, datam de cerca de 7 ka (Politis et al., 2003; Politis et al., 2019), o que representaria uma coexistência de alguns milhares de anos (Fig. 5).

É possível que apenas a caça moderada e ocasional de fêmeas tenha sido suficiente para ocasionar a extinção da megafauna durante este período (Cione et al., 2009).

Artefatos líticos em associação estratigráfica com fósseis de megafauna são reportados para diversos sítios arqueológicos da América do Sul (Suárez & Santos, 2010). Alguns desses fósseis apresentam inclusive marcas de corte, a exemplo de ossos de *Megatherium americanum* (preguiça-gigante) encontrados em Campo Laborde na Argentina Pampeana (Politis et al., 2019). Entretanto, indícios diretos de que o homem foi a *causa mortis* de indivíduos da megafauna são raros.

Na busca por mais evidências sobre a influência humana nas extinções do Pleistoceno Superior/Holoceno, Prates & Perez (2021) analisaram a dinâmica temporal e distribuição espacial da megafauna sul-americana e pontas de projéteis do tipo *Fishtail*. Esses autores observaram uma forte relação entre o aumento de tais projéteis, bem como com as flutuações na demografia humana, e o desaparecimento de grandes mamíferos (Prates & Perez, 2021). No Brasil, um fóssil de mastodonte da espécie *Notiomastodon platensis*, recuperado da região de Lagoa Santa, Minas Gerais, foi descrito contendo um artefato lítico associado ao crânio, o que representaria a primeira evidência direta de abate de animais da megafauna pelo homem na América do Sul (Mothé et al., 2020).

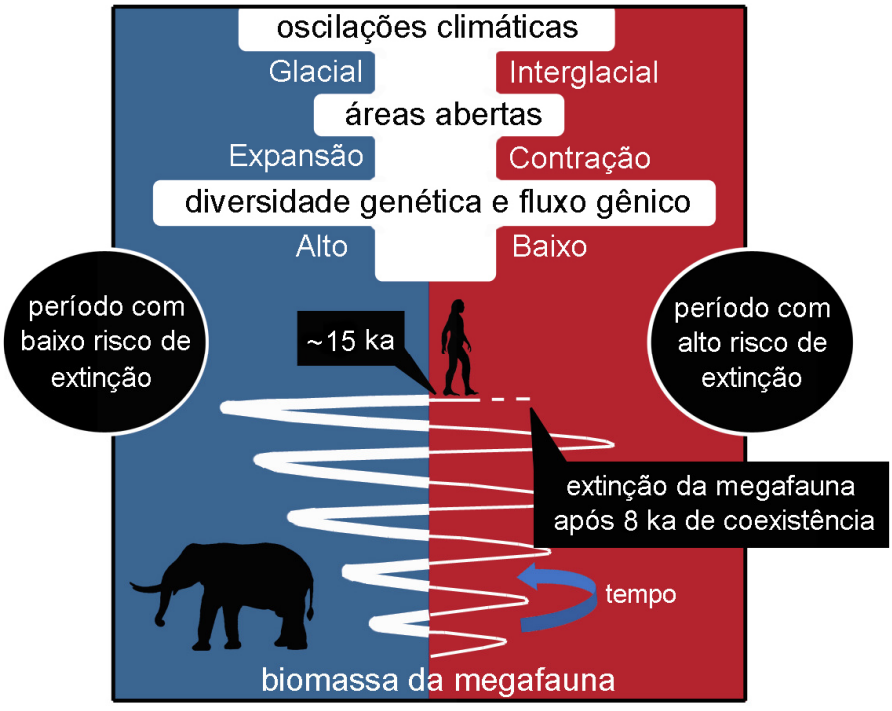


Fig. 5 Esquema sintetizando os principais fatores responsáveis pela extinção da megafauna. Adaptado de Soibelzon (2008) e Cione et al. (2015).

Considerando a escassez de evidências diretas de caça (em comparação com outras regiões com dados mais robustos, como é o caso da América do Norte), ainda é difícil avaliar a magnitude de cada variável – climática e antropogênica – sobre a extinção da megafauna na América do Sul (Barnosky et al., 2004). Com isso, diversos autores sugerem que sejam desenvolvidos estudos regionais para compreender a ecologia da fauna extinta, a dinâmica dessas espécies e, consequentemente, as reais causas da extinção (e.g, Barnosky et al., 2004; Hubbe et al., 2013). Tais estudos também permitiriam compreender os efeitos da mudança de fauna sobre as paisagens sul-americanas. Várias espécies da megafauna eram herbívoras pastadoras, como evidenciado por suas características morfológicas cranianas e dentárias (Janis, 2008; Madden, 2015) e, portanto, tinham papel importante na manutenção dos campos, impedindo a proliferação de árvores e arbustos, dispersando sementes, e auxiliando na ciclagem de nutrientes (Metera et al., 2010).

Megafauna marinha

Em um contexto pelágico marinho, classifica-se como megafauna as maiores espécies que ocorrem nesses ambientes (normalmente com massa corporal maior ou igual do que 45 kg, mas alguns autores consideram animais menores com respiração aérea), pertencentes a uma ampla variedade de grupos taxonômicos: peixes ósseos, elasmobrânquios, mamíferos, tartarugas, aves e até mesmo algumas espécies de moluscos, como lulas e polvos (Estes et al., 2016; Moleón et al., 2020).

No extremo sul do Brasil, registros pleistocênicos dessa fauna incluem fósseis de tubarão (e.g., *Carcharias taurus*, *Carcharodon carcharia*, *Galeocerdo cuvier*, *Isurus oxyrinchus*) e peixes ósseos como a miraguaia, *Pogonias cromis* (Richter, 1987; Buchmann, 1994; Buchmann & Rincon, 1997; Cruz et al., 2016), elementos cranianos e pós-cranianos de leão-marinho, *Otaria byronia* (Drehmer & Ribeiro, 1998; Rodrigues et al., 2004), vértebras de baleias (Balaenopteridae e Balaenidae) (Cunha & Nunan, 1980, Cunha, 1982, 1985; Cunha et al., 1992), fragmentos cranianos de toninha, *Pontoporia blainvillei* (Buchmann & Rincon, 1997; Ribeiro et al., 1998; Cruz et al., 2016; Fig. 6) e vértebras cervicais e segmento de tibiotarso de albatroz, *Thalassarche* sp. (Lopes et al., 2006). Porém, na presente tese será dado enfoque apenas aos mamíferos marinhos, em especial aos cetáceos (Ordem Cetacea – baleias e golfinhos).

Ao contrário da megafauna terrestre, a megafauna marinha sofreu uma redução na sua diversidade durante o Plioceno, como relatado em um estudo desenvolvido por Pimiento e colaboradores (2017), os quais calcularam a taxa de extinção de gêneros de mamíferos, aves aquáticas, tartarugas e tubarões do Paleoceno (66 Ma) ao Pleistoceno. Esses autores verificaram que o período com maior taxa de extinção foi entre 3,8 e 2,4 Ma, possivelmente uma consequência da alteração na circulação oceânica, mudanças na produtividade e redução nos habitats costeiros associadas ao início das glaciações. Com isso, cerca de 36% dos gêneros foram extintos e, quando considerados somente os mamíferos marinhos, a proporção é ainda maior: aproximadamente 55%. A megafauna moderna foi estabelecida após esse evento, durante o Pleistoceno (Pimiento et al., 2017), explicando, portanto, a aparição de táxons recentes nos sítios fossilíferos do Rio Grande do Sul. Enquanto os mamíferos terrestres atuais são significativamente menores do que aqueles do Pleistoceno, tal diferença não é observada na megafauna marinha (Estes et al., 2016).

Existem atualmente mais de 90 espécies de cetáceos (Society for Marine Mammalogy, 2023; Estes et al., 2016), sendo que aproximadamente metade ocorre no Atlântico Sul Ocidental (Bastida et al., 2018) e 33 já foram registradas em encalhes ao longo da área de estudo, a qual estende-se da Lagoa do Peixe até o Arroio Chuí (Prado et al., 2016). Entretanto, a diversidade fóssil registrada até o presente momento é bastante inferior. A grande maioria dos fósseis de mamíferos marinhos encontrados no extremo sul do Brasil compreende a restos de toninha, *P. blainvillei*, como reportado no trabalho de Ribeiro e colaboradores (1998).

Os fósseis de mais bem preservados compreendem a parte do rostro e do teto craniano, compostos principalmente por narinas ósseas, ossos nasais e frontais, vômer, maxilas e pré-maxilas (veja a Fig. 6). Além de serem atribuídos à mesma espécie de toninha vivente (Ribeiro et al., 1998), é possível que compreendam também à mesma população encontrada hoje em dia no sul do Brasil ou FMA (*Franciscana Management Area*) III, pois esta originou-se no Pleistoceno Inferior (Nara et al., 2022). Portanto, a presença desses fósseis possibilita estudar a ecologia da toninha em ambientes pristinos, sem influência humana. Atualmente, a toninha é classificada como vulnerável pela União Internacional para a Conservação da Natureza (IUCN) em consequência de sua alta captura acidental (e mortalidade) em redes de pesca, mas outras ameaças incluem ingestão de resíduos, perda de potenciais presas devido à pesca excessiva e degradação de habitat (Zerbini et al., 2017).

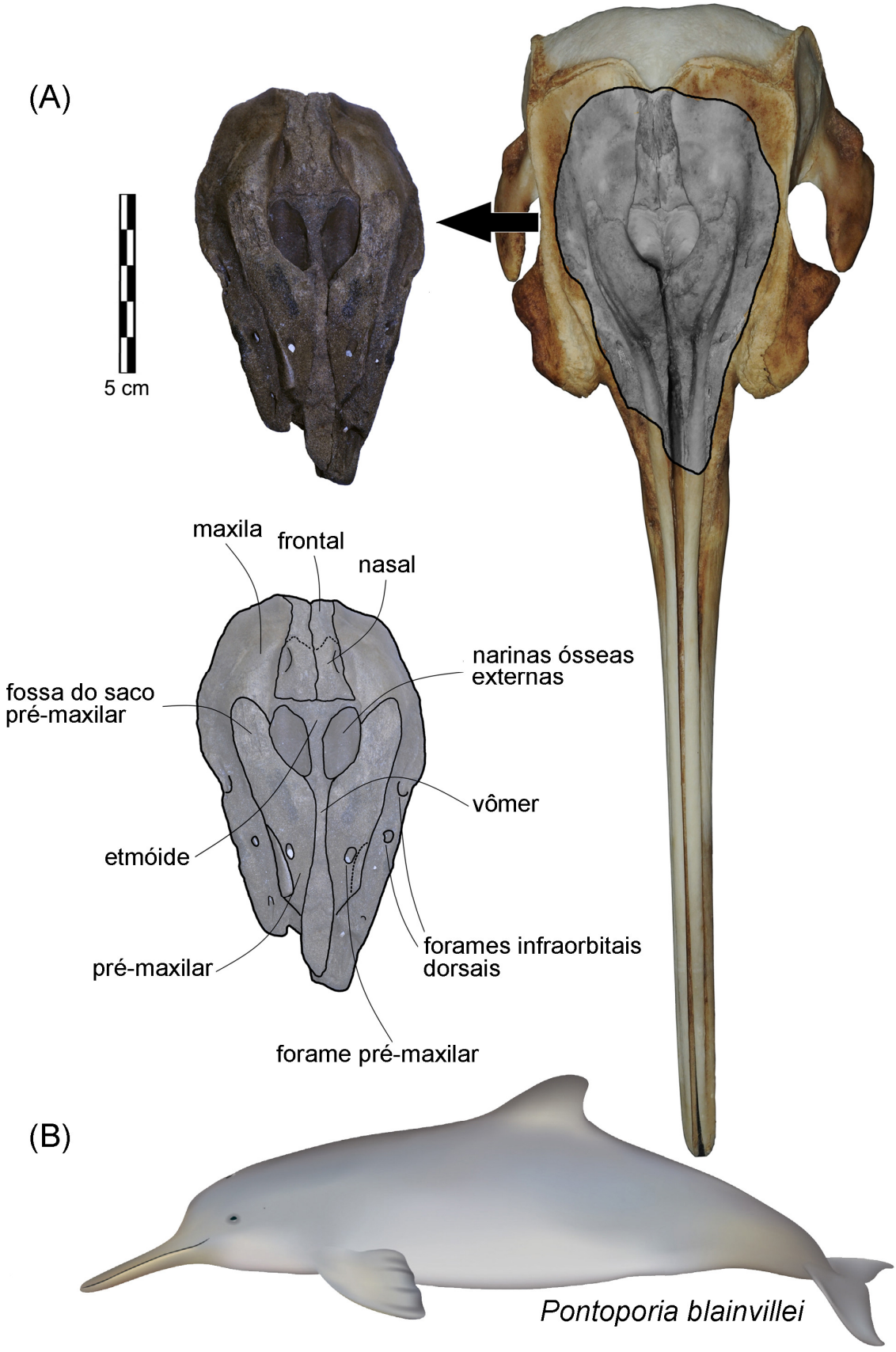


Fig. 6 Comparação entre um crânio fossilizado (MCN-PV 36963) e um crânio atual (A) e um desenho ilustrativo (Crespo, 2018) da toninha, *Pontoporia blainvillei* (B).

Outros registros quaternários para a costa brasileira são geologicamente mais recentes e incluem um fóssil de baleia-azul, *Balaenoptera musculus*, de idade holocênica, recuperado de fácies arenosas na costa de São Paulo (Buchmann et al., 2017) e balenídeos (Balaenidae), também de idade holocênica, recuperados da Lagoa Mirim, no Rio Grande do Sul (Lopes et al., 2020b). Infelizmente, os cetáceos têm um registro fóssil esparso, particularmente no Pleistoceno do hemisfério sul (Fordyce, 2018).

Dada a idade predominantemente pleistocênica dos sistemas de barreira da costa sul-brasileira, esses acabam sendo de suma importância para a reconstituição da história de baleias e golfinhos. O registro fóssil é fundamental para entender os efeitos das mudanças ambientais e climáticas sobre esses organismos ao longo do tempo e pode fornecer informações sobre a evolução, distribuição e extinção de espécies (Fordyce 2009). Novos fósseis são constantemente descobertos e existem ainda aqueles que estão depositados em coleções e museus, mas não foram devidamente descritos e, portanto, devem ser cientificamente explorados. Nesse sentido, ossos do ouvido, representados pela bula timpânica e periótico (Fig. 7), são especialmente importantes, pois exibem morfologias que são diagnósticas a nível de gênero e/ou espécie (Kasuya, 1973; Ekdale et al., 2011; Gol'din & Steeman, 2015).

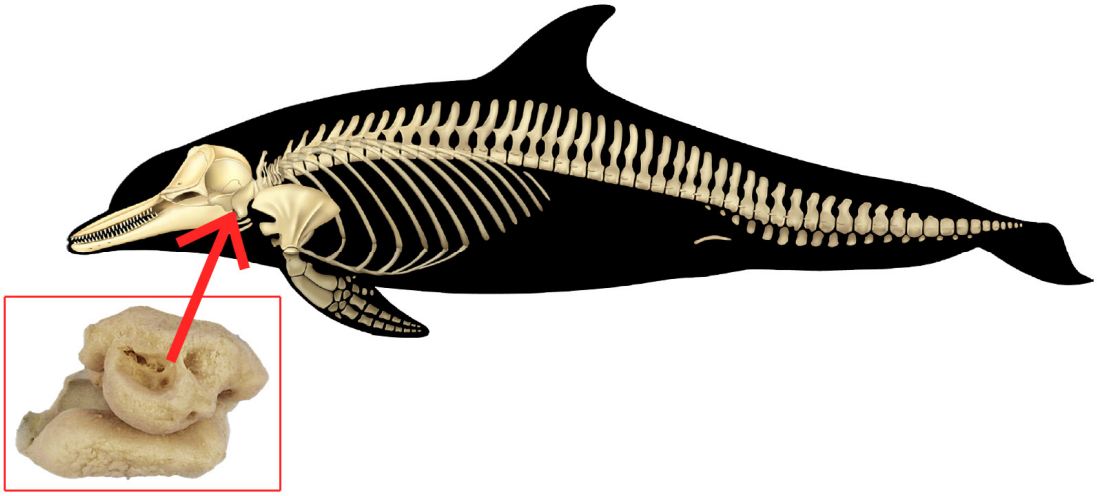


Fig. 7 Complexo timpanoperiótico de um golfinho-nariz-de-garrafa, *Tursiops truncatus*, e sua localização aproximada na face basal do crânio. Adaptado de Cozzi et al. (2017).

Geologia quaternária do Rio Grande do Sul

No Rio Grande do Sul afloram diversos depósitos de idade pleistocênica (Fig. 8) formados primariamente pela deposição de sedimentos em canais fluviais e planícies de

inundação (Da-Rosa, 2009). Os que deram origem ao material abordado neste estudo estão localizados ao longo das bacias do Arroio Touro Passo e Sanga da Cruz, no oeste do Rio Grande do Sul, próximos às fronteiras com o Uruguai e Argentina; do Arroio Pessegueiro, no centro do estado; e do Arroio Chuí, na planície costeira. Também se destacam os depósitos submersos marinhos, formados por sistemas deposicionais do tipo laguna-barreira em decorrência das oscilações eustáticas do nível do mar (Buchmann et al., 2009).

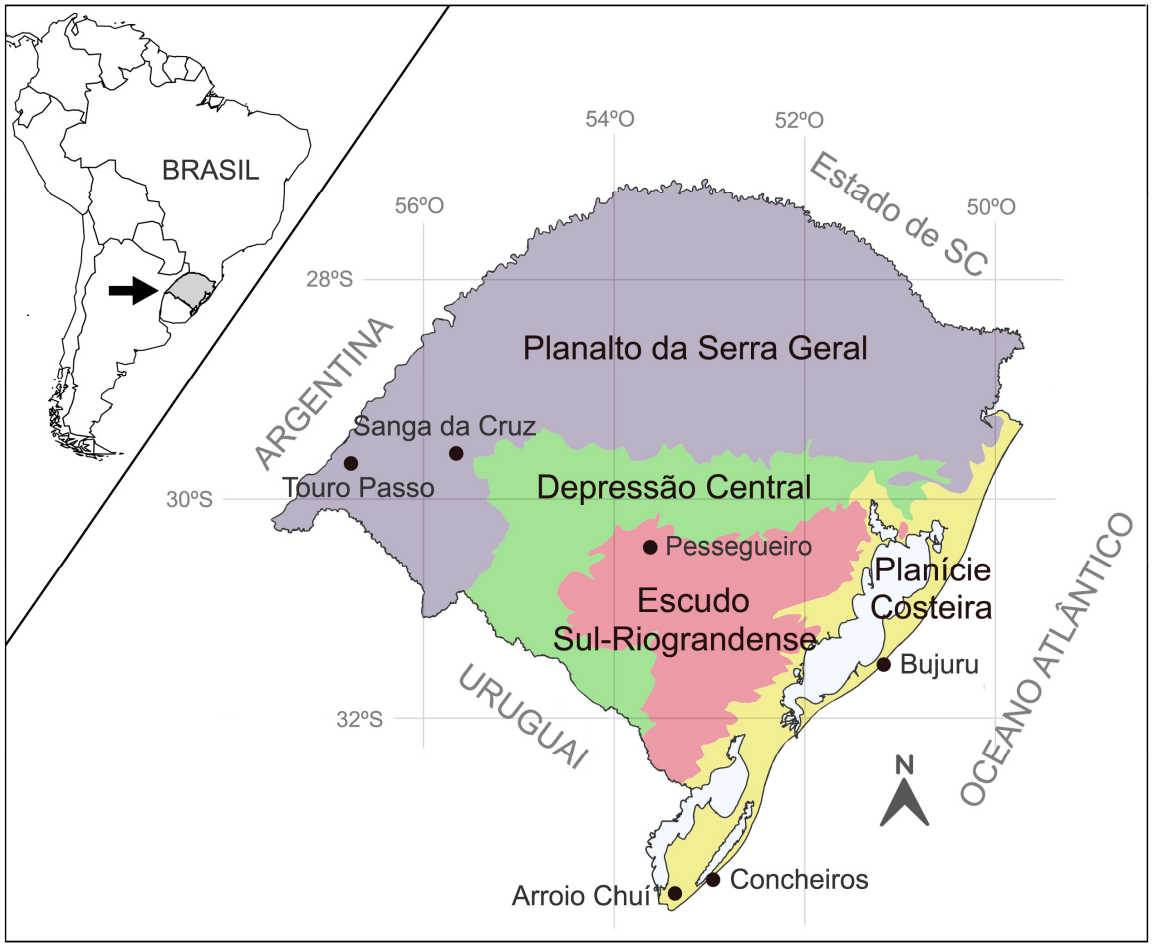


Fig. 8 Mapa geomorfológico do Rio Grande do Sul, com indicação dos sítios fossilíferos abordados no presente estudo. Adaptado de Da-Rosa (2009).

O Arroio Touro Passo é um afluente do Rio Uruguai e seus depósitos foram definidos por Bombim (1976) como pertencentes à Formação Touro Passo, a qual é composta por fácies conglomeráticas sobrepostas por níveis de areia e silte com concreções de carbonato de cálcio, $CaCO_3$ (Fig. 9). Essa formação remonta ao Pleistoceno Superior – seus sedimentos apresentam idades entre 42,9 e 6,4 ka de acordo com datações por termoluminescência de fácies conglomeráticas e lamíticas (Da-Rosa, 2009). A datação de mamíferos fósseis utilizando ressonância de *spin* eletrônico (provenientes da localidade Ponte Velha I) obteve idades similares: entre 42,2 e 10 ka (Kerber et al., 2011).

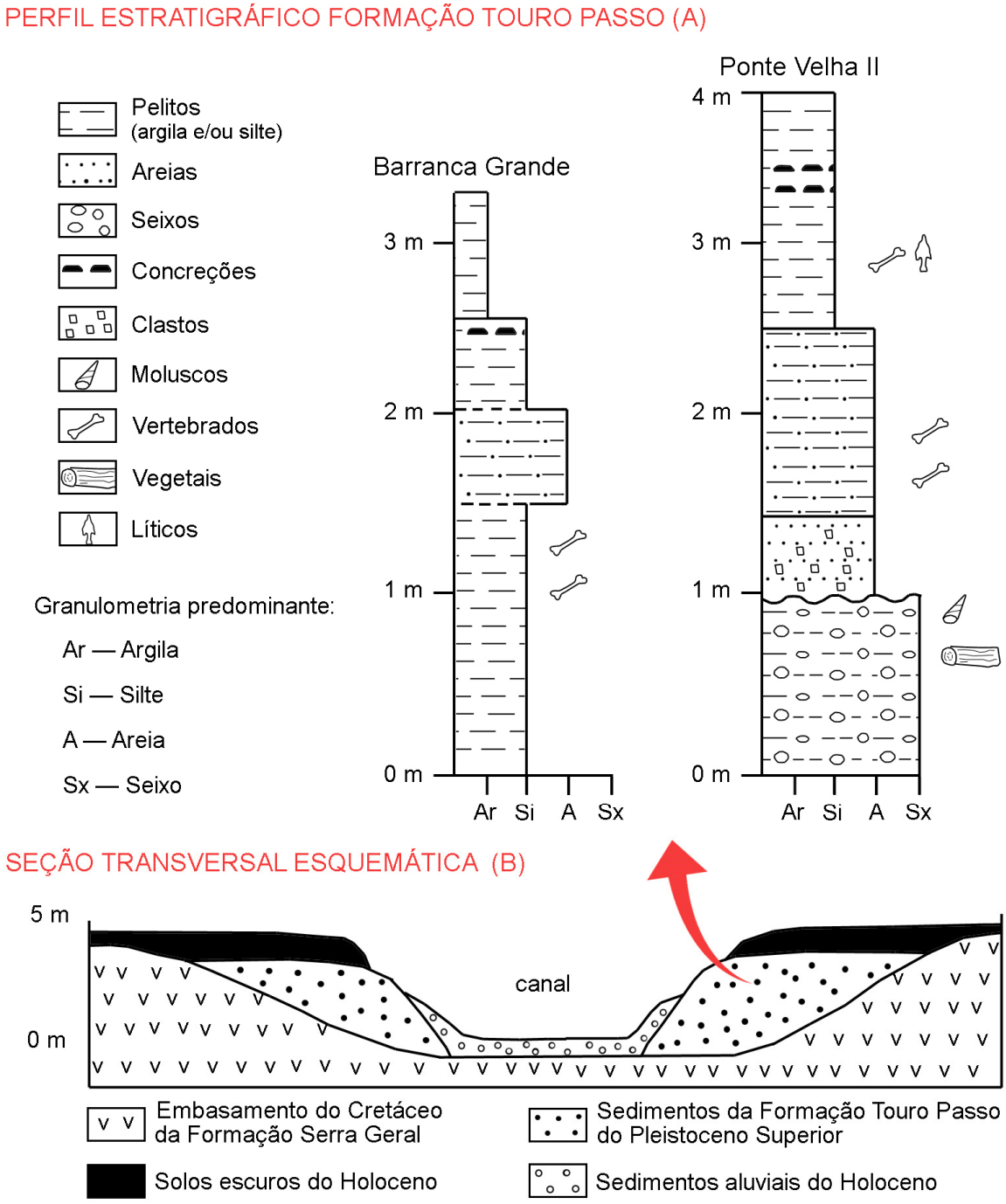


Fig. 9 Perfil estratigráfico das localidades Barranca Grande e Ponte Velha II, Formação Touro Passo (A), as quais expõem suas litologias ao longo das margens do Arroio Touro Passo (B). Imagens adaptadas de Oliveira & Kerber (2009) e Kerber et al. (2014).

Embora diversos afloramentos sejam descritos, como as localidades Ponte Velha I e II, Barranca Grande e Milton Almeida (Oliveira & Kerber, 2009), o material coletado carece, muitas vezes, de informações sobre a localidade e estrato proveniente (e.g., Scherer et al., 2007; Kerber & Oliveira, 2008). No entanto, estima-se que os fósseis depositados no Museu de

Ciências Naturais (SEMA, Porto Alegre–RS) sejam provenientes principalmente das localidades Barranca Grande e Milton Almeida (Oliveira & Kerber, 2009).

Restos de mamíferos da Formação Touro Passo apresentam como característica principal a intensa incrustação por carbonato (Fig. 10A), sendo normalmente recuperados de níveis de areia e silte, com elementos semi-articulados ou isolados, mas em proximidade (Kerber et al., 2014).

Sanga da Cruz, um afluente do Rio Ibicuí, contempla depósitos sedimentares consolidados sobre as rochas basálticas da Serra Geral da Bacia do Paraná (Da-Rosa, 2009). Não está associado a nenhuma formação geológica previamente descrita, porém a localidade Salatiel II apresenta litologia similar àquela encontrada na Formação Touro Passo, mas com estratos mais finos de lama e conglomerados (Scherer et al., 2009). Os fósseis ocorrem em diferentes níveis, principalmente nos conglomeráticos, normalmente incompletos e isolados (Fig. 10B), com sinais de retrabalhamento (Oliveira & Kerber, 2009; Scherer et al., 2009). Datações por diferentes métodos – ^{14}C em amostras biológicas (crânio de preguiça-gigante do gênero *Glossotherium* e troncos fossilizados) e termoluminescência em amostras de sedimentos – apontam para uma idade de deposição entre 17,8 e 11,7 ka (Miller, 1987; Milder, 2000).

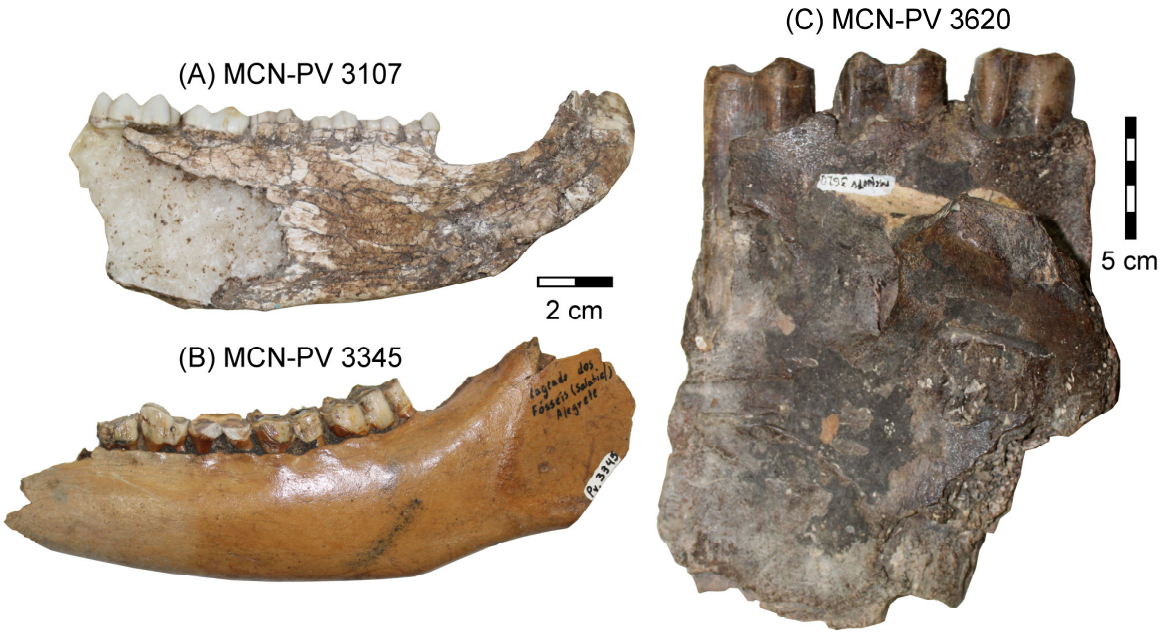


Fig. 10 Fragmento mandibular de queixada, *Tayassu pecari*, recuperado das barrancas do Arroio Touro Passo (A), de cervídeo, recuperado de Sanga da Cruz (B) e de preguiça-gigante, *Eremotherium laurillardi*, recuperado da Praia dos Concheiros (C). Fonte: autora.

Os depósitos sedimentares do Arroio Pessegueiro, consolidados sobre o Escudo Sul-Rio-Grandense (Da-Rosa, 2009), são formados por arenitos conglomeráticos, siltitos arenosos

e pelitos, com restos de mamíferos ocorrendo em fácies compostas por sedimentos pelíticos (Oliveira et al., 2002). Apesar de sua rara descrição geológica e ausência de datação absoluta, está associado a períodos de clima seco do Pleistoceno (Oliveira et al., 2002).

A Planície Costeira do Rio Grande do Sul, parte emersa da Bacia Sedimentar de Pelotas, abriga depósitos de diversas origens, associados ao Pleistoceno Médio-Superior, como os depósitos eólicos e fluviais do Arroio Chuí (Lopes et al., 2021; Fig. 11). Além disso, contempla diversos afloramentos submersos, localizados principalmente na costa sul, como o Parcel do Carpinteiro e o Banco Capela (Calliari et al., 1994; Buchmann, 1994; Buchmann et al., 2001; Buchmann & Tomazelli, 2003; Lopes & Buchmann, 2011).

A gênese da planície costeira está relacionada às oscilações de nível do mar do Quaternário, que resultaram em quatro sistemas deposicionais do tipo laguna-barreira, com idades correlacionadas com a cronologia de Imbrie et al. (1984): as Barreiras I, II e III com 325, 220 e 120 ka, respectivamente, e a Barreira IV, holocênica, com cerca de 6 ka (Villwock & Tomazelli, 1995; Tomazelli et al., 2000; Lopes et al., 2014). Cada idade representa, aproximadamente, um pico interglacial, no qual houve a deposição de sedimentos controlada pelo aumento do nível do mar. A subsequente diminuição do nível do mar, por sua vez, foi responsável pela progradação desses sistemas (Villwock & Tomazelli 1995, Tomazelli et al. 2000).

O Arroio Chuí está localizado entre as Barreiras II e III (veja a Fig. 11). Seus depósitos sedimentares foram inicialmente propostos por Soliani (1973) como parte da unidade litoestratigráfica Formação Santa Vitória. Posteriormente, Lopes e colaboradores (2021) propuseram a reclassificação dessa unidade como uma aloformação. Expõe fácies compostas por areia (com granulometria de fina a média) e argila, tendo como limite superior e inferior a Formação Cordão e a Barreira II, respectivamente (Lopes et al., 2013; 2021). Fósseis de mamíferos são recuperados principalmente das fácies de areia siltosa (Lopes et al., 2021), com idades que compreendem uma grande janela temporal, entre ~226 e 34 ka (datações por ressonância de *spin* eletrônico), provavelmente devido ao retrabalhamento dos depósitos através dos eventos transgressivos do nível do mar (Lopes et al., 2010).

Os fósseis provenientes da Formação Santa Vitória são relativamente frágeis, mas com alto grau de preservação (Lopes et al., 2005), a exemplo dos ossos de aves recuperados dessa formação (e.g., Lopes et al., 2019). Embora não esteja evidente na Fig. 12, fósseis do Arroio Chuí possuem coloração tipicamente mais clara do que aqueles encontrados *ex situ* na praia.

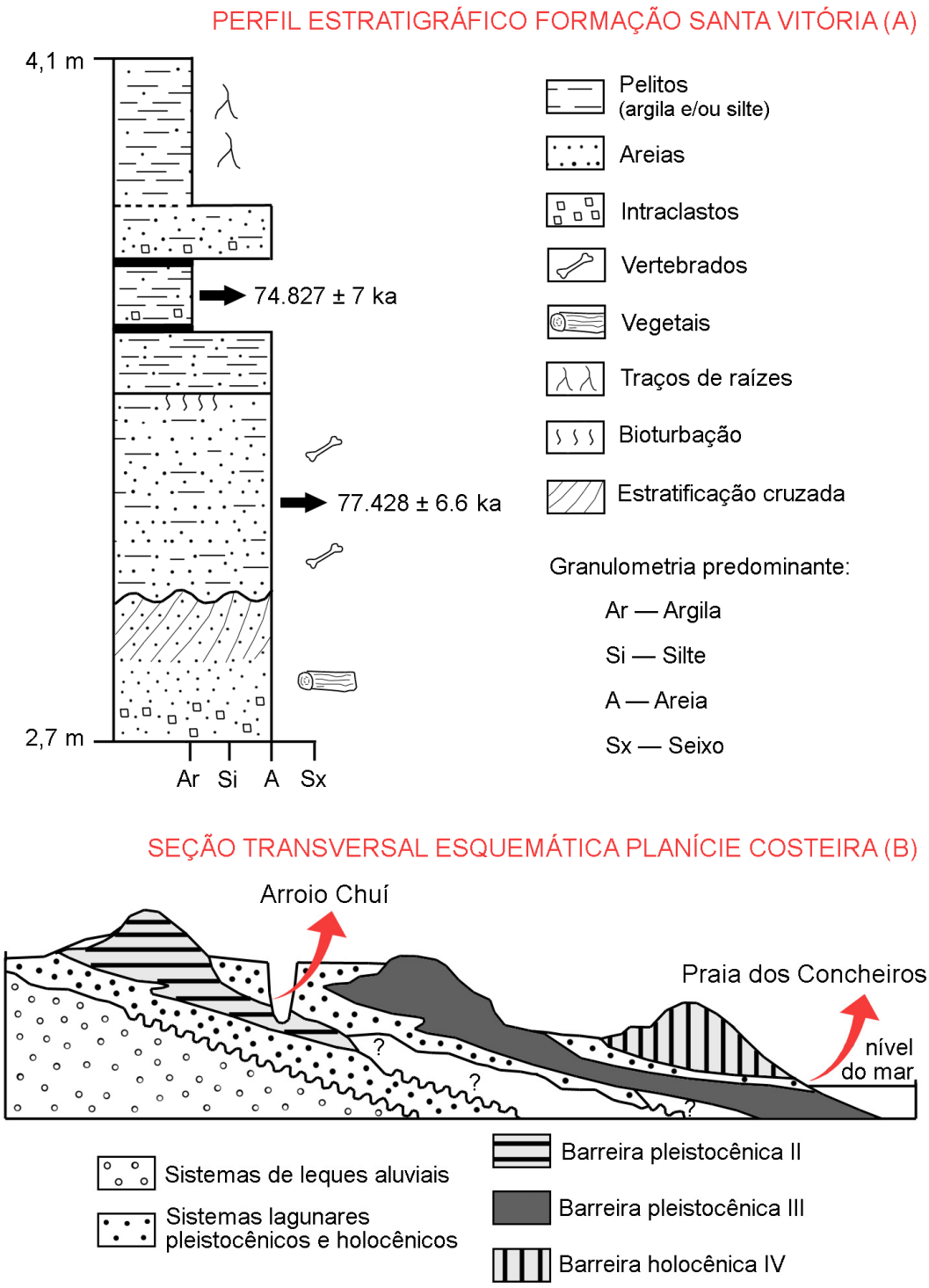


Fig. 11 Perfil estratigráfico da Formação Santa Vitória (A) e sua posição na Planície Costeira do Rio Grande do Sul (B). Representações esquemáticas modificadas de Lopes et al. (2021).

Os depósitos submersos apresentam uma assembleia terrestre fossilífera semelhante àquela encontrada no Arroio Chuí (Formação Santa Vitória) e, considerando suas proximidades geográficas, é possível que sejam de similar ou mesma procedência estratigráfica (Figueiredo, 1975; Buchmann, 1994). No entanto, os fósseis são encontrados *ex situ*, principalmente na Praia

dos Concheiros (Fig. 12), Praia do Hermenegildo e Praia do Albardão, dificultando tal afirmação. Ademais, a idade destes compreende uma janela temporal mais ampla – entre ~650 e 18 ka – de acordo com datações utilizando o método de ressonância de *spin* eletrônico (Lopes et al., 2010).

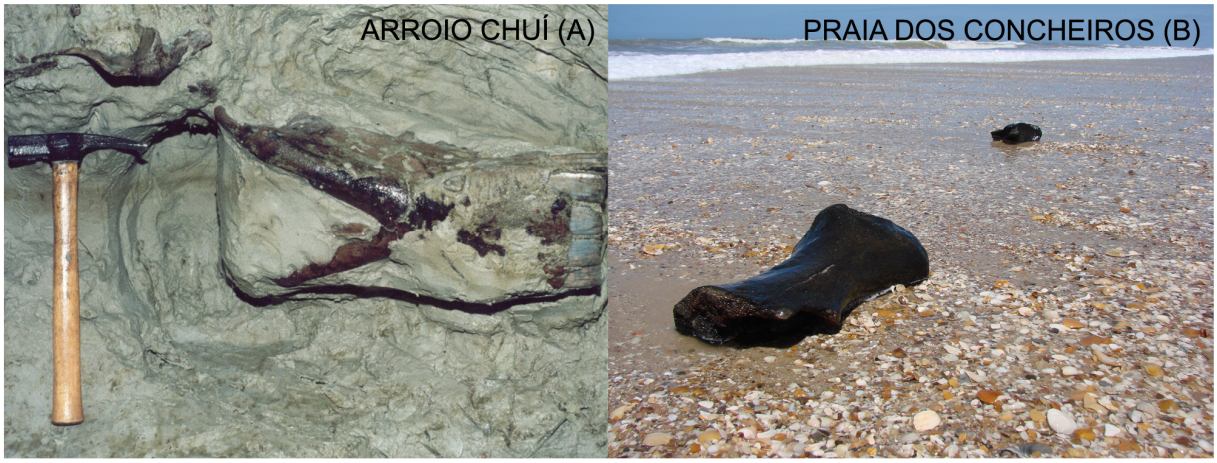


Fig. 12 Mandíbula de toxodonte, *Toxodon* sp., *in situ* nas barrancas do Arroio Chuí (Formação Santa Vitória) (A) e uma tíbia de preguiça-gigante, *Lestodon* sp., *ex situ* na zona de varrido da Praia dos Concheiros (B). Fotos: Buchmann.

As principais características dos fósseis provenientes dos afloramentos submersos são elevada densidade, dureza e coloração escura (Figueiredo, 1975; Buchmann, 1994; Fig. 10C) em consequência do alto conteúdo de metais incorporados durante a diagênese (i.e., conjunto de alterações químicas e físicas sofridas pelos fósseis desde a sua deposição), como ferro, bário e ítrio (Lopes & Ferigolo, 2015).

Além de fósseis de megafauna terrestre, os depósitos submersos preservaram fósseis de origem marinha (Fig. 6). Fósseis de cetáceos ainda não foram devidamente datados, porém a datação de corais da espécie *Oculina patagonica* por ^{14}C obteve uma idade superior a 40 ka, extrapolando o limite do método (Buchmann, 2002). Ademais, a datação por termoluminescência de um arenito (*beachrock*) localizado próximo ao Banco Capela obteve uma idade de aproximadamente 110 ka (Buchmann & Tomazelli, 2003). Considerando tais dados, juntamente com a atual configuração dos sistemas laguna-barreira da planície costeira, pressupõe-se que a principal fonte dos fósseis marinhos seja a Barreira III, pois é a mais bem preservada de idade pleistocênica e está sujeita à erosão direta pela ação das ondas (Buchmann, 2002). A Barreira III tem idade estimada entre 116 e 130 ka, período em que o nível do mar estava cerca de 8 m mais elevado do que atualmente (Villwock & Tomazelli, 1995; Rosa et al., 2017). Após esse evento transgressivo, o nível do mar baixou novamente e os fósseis provavelmente foram soterrados, sofrendo diagênese.

As acumulações fossilíferas submersas ocorrem em elevações topográficas em diversas profundidades, desde a borda do talude (~120 m de profundidade) até águas rasas, mas apenas aquelas localizadas em até ~20 m são retrabalhadas pela ação das ondas (Buchmann et al., 2001; Buchmann, 1994, 2002). Estão densamente distribuídas na costa sul, de modo que essa região possui maior ocorrência de fósseis encontrados na praia (Figueiredo, 1975; Correa, 1990; Buchmann, 2002). Esses depósitos são compostos de rochas carbonáticas e arenitos fortemente cimentados (Buchmann et al., 2001; Buchmann & Tomazelli, 2003), que são expostos, erodidos e transportados para a praia durante fortes tempestades, por ondas produzidas por ciclones extratropicais (Buchmann, 1994, 2002; Buchmann & Tomazelli, 2003; Buchmann et al., 2001; Cruz et al., 2016).

Métodos de estudo

Muitas vezes, paleontólogos se deparam com a ausência de representantes modernos ou análogos a formas fósseis, tornando difícil a reconstrução ecológica dessas espécies (Dantas et al., 2017). Nesse contexto, a análise de isótopos estáveis é um método amplamente difundido em estudos paleoecológicos (e.g., Lopes et al., 2013; Dantas et al., 2019; Oliveira et al., 2020; Gomes et al., 2023), pois permite reconstruções precisas da dieta real de mamíferos fósseis e, consequentemente, dos ambientes que esses costumavam forragear e habitar, independentemente de inferências morfológicas (Bocherens & Drucker, 2013).

Os isótopos estáveis são átomos energeticamente estáveis (ou seja, não sofrem decaimento radioativo ao longo do tempo) de um particular elemento químico que possuem o mesmo número de prótons e elétrons, mas diferem em relação ao número de nêutrons (Fig. 13) e, portanto, possuem massas atômicas (soma de prótons e nêutrons) distintas (Sulzman, 2007). Dos 118 elementos químicos conhecidos, 54 possuem ao menos dois isótopos estáveis, sendo que aqueles relacionados com a biosfera, hidrosfera e atmosfera são especialmente relevantes para pesquisas ecológicas (Ramos & González-Solís, 2012).

Os isótopos estáveis de um mesmo elemento são quimicamente equivalentes, entretanto seus comportamentos variam devido a diferenças em suas propriedades físicas relacionadas às suas massas (Ben-David & Flaherty, 2012). Essas características os permitem atuar como traçadores químicos, pois as composições isotópicas tendem a mudar de forma previsível ao longo dos compartimentos da biosfera (Peterson & Fry, 1987). Por exemplo, os isótopos de carbono presentes nos tecidos dos consumidores refletem o alimento digerido e assimilado,

porém com uma depleção dos isótopos leves devido à sua perda preferencial através da respiração (DeNiro & Epstein, 1978). Além disso, as razões isotópicas dos tecidos dependem dos constituintes dietéticos que participam de suas composições. Dessa forma, os consumidores são tipicamente mais enriquecidos do que a sua dieta e essa diferença é normalmente denominada de fator de discriminação trófica (e.g., Stephens et al., 2022) ou enriquecimento trófico (e.g., Tejada-Lara et al., 2018).

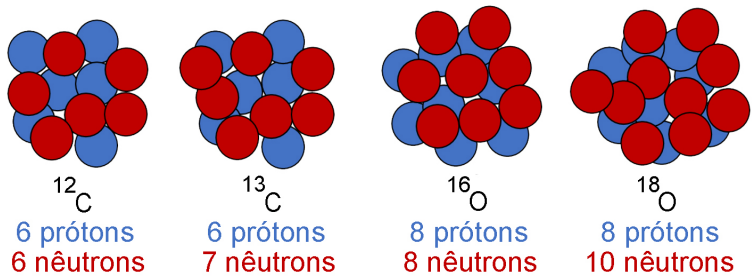


Fig. 13 Estrutura esquemática evidenciando prótons (em azul) e nêutrons (em vermelho) de isótopos de carbono e oxigênio comumente abordados em estudos paleoecológicos. Fonte: autora.

Usualmente, as composições isotópicas de materiais naturais são determinadas com grande precisão utilizando um espectrômetro de massas. São expressas pela notação delta (δ) em partes por mil (‰), de acordo com um padrão pré-definido para cada elemento, como segue: $\delta X = [(R_{AMOSTRA}/R_{PADRÃO}) - 1]$, onde X é o elemento na forma pesada e R é a razão entre isótopos pesados e leves na amostra (Peterson & Fry, 1987). Por convenção, os padrões são definidos internacionalmente de modo que os dados coletados por qualquer grupo de pesquisa ao redor do mundo sejam comparáveis (Ben-David & Flaherty, 2012).

Diversos materiais biológicos de mamíferos são passíveis de serem investigados isotopicamente, como sangue, pele, músculo e vibrissa, de modo que sua escolha depende da pergunta da pesquisa. Os elementos de um determinado tecido são assimilados durante intervalos de tempo específicos para a sua taxa metabólica, o que significa que diferentes tecidos refletem diferentes períodos (Dalerum & Angerbjörn, 2005). No entanto, normalmente apenas tecidos mineralizados como ossos e dentes estão disponíveis no caso dos fósseis. Essas estruturas são sintetizadas durante a vida do animal e compostas por duas frações: uma orgânica, formada majoritariamente por colágeno, e uma mineral, a "bioapatita", composta por fosfato de cálcio e carbonato (Bocherens & Drucker, 2013; Fig. 14).

A partir do colágeno presente nos ossos e dentina, é possível obter as razões isotópicas de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$), as quais refletem a dieta do animal durante o período de síntese do tecido (DeNiro & Epstein, 1978, 1981). Já a análise do carbonato fornece razões

isotópicas de oxigênio ($\delta^{18}O$), relacionadas principalmente com a água ambiental ingerida, e também de $\delta^{13}C$. Por fim, a análise do fosfato de cálcio permite a obtenção de $\delta^{18}O$ (Bocherens & Drucker, 2013), entretanto a análise do $\delta^{18}O$ do fosfato é mais custosa e complicada metodologicamente do que a análise do carbonato (Bryant et al., 1996).

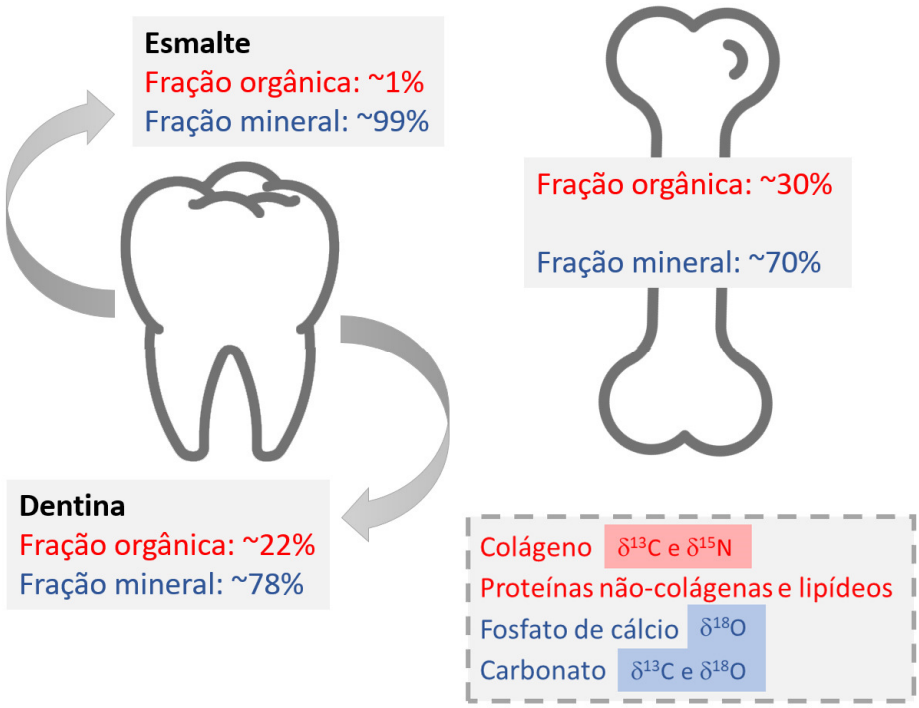


Fig. 14 Resumo da composição de ossos e dentes, evidenciando as principais composições isotópicas que podem ser recuperadas de cada componente. Modificado de Bocherens & Drucker (2013).

Por apresentar isótopos estáveis de dois elementos de origem dietética em sua composição ($\delta^{13}C$ e $\delta^{15}N$), o colágeno é o componente preferencialmente analisado em estudos que objetivam reconstruir a dieta dos animais e seus locais de forrageio. Quando essa proteína está disponível, é possível separá-la e analisar os isótopos estáveis específicos dos aminoácidos que a compõem, uma abordagem molecular que permite uma melhor compreensão das contribuições dietéticas (Whiteman et al., 2019). Um exemplo do emprego dessa ferramenta em amostras fósseis é o estudo desenvolvido por Tejada e colaboradores (2021). Esses autores analisaram as razões isotópicas de nitrogênio em glutamato e fenilalanina com o objetivo de reconstruir a posição trófica de uma espécie extinta de preguiça-gigante, *Mylodon darwinii*. A fenilalanina é caracterizada como um "aminoácido fonte", pois seus valores de $\delta^{15}N$ permanecem praticamente inalterados durante a assimilação pelos consumidores, já o ácido glutâmico (analisado como glutamato) é um "aminoácido trófico", pois sofre reações de desaminação e transaminação que resultam em um enriquecimento de ^{15}N a cada nível trófico

(Whitemann et al., 2019). Com isso, Tejada e colaboradores (2021) verificaram que as diferenças entre os valores de $\delta^{15}N$ desses dois aminoácidos ($\Delta\delta^{15}N_{Glu} - \delta^{15}N_{Fen}$) de *M. darwinii* se assemelhavam às de outros mamíferos onívoros.

Infelizmente, a matéria orgânica de fósseis muitas vezes encontra-se degradada e inadequada para análise isotópica, especialmente de regiões subtropicais úmidas, limitando os paleontólogos ao uso da bioapatita (Clementz, 2012; Zhao et al., 2021). Em tais contextos, a análise do carbonato é preferível, pois fornece informações de ao menos dois elementos de relevância paleoecológica, diferentemente do fosfato, que não apresenta carbono em sua composição (Fig. 14). Entretanto, apesar de ser quase sempre possível mensurar as razões isotópicas de carbono e oxigênio de ossos e dentes fósseis, é necessário determinar se os valores obtidos correspondem aos valores reais do organismo, de quando ele estava vivo (Bocherens & Drucker, 2013).

Normalmente, assume-se que os componentes minerais do esmalte permanecem bem preservados em amostras com até 100 Ma (Clementz, 2012). O esmalte é um tecido extremamente compacto, com baixo conteúdo orgânico, cristais grandes e de alta densidade, características que aumentam sua resistência à diagênese, enquanto o tecido ósseo é altamente poroso, e a dentina apresenta porosidade intermediária (Kohn & Cerling, 2002). Para avaliar o grau de preservação de amostras fósseis, diversos métodos podem ser aplicados, incluindo análise de cristalinidade e avaliação das semelhanças e diferenças nas composições de espécimes de uma mesma localidade (valores inesperados podem ser explicados por alteração diagenética) (Kohn & Cerling, 2002; Zhao et al., 2021).

Em amostras bem preservadas, o $\delta^{13}C$ do carbonato está relacionado com os processos que ocorrem na base da cadeia trófica e, portanto, reflete as razões isotópicas dos produtores primários (Kelly, 2000). As plantas fixam o carbono através de três vias fotossintéticas: do ciclo de Calvin, conhecido como via C_3, da via de Hatch-Slack ou de quatro carbonos (C_4) e do metabolismo ácido das crassuláceas (CAM).

Na fotossíntese C_3, a enzima ribulose 1,5-bifosfato (Rubisco) carboxilase/oxigenase catalisa a reação inicial do ciclo ao combinar-se com o dióxido de carbono (Marshall et al., 2007; Raven et al., 2007). Essa enzima possui alta afinidade pelo ^{12}C e discrimina contra o ^{13}C, fazendo com que as razões isotópicas das plantas C_3 sejam altamente empobrecidas, em torno de –28‰ (O'Leary, 1988), valores que podem variar ao longo do tempo geológico devido a mudanças no carbono atmosférico. Na via fotossintética do tipo C_4, o dióxido de carbono é inicialmente fixado ao fosfoenolpiruvato (PEP) em uma reação catalisada pela enzima PEP

carboxilase, onde o fracionamento é fracamente expresso (Marshall et al., 2007; Raven et al., 2007). Dessa forma, as plantas C_4 são menos empobrecidas no ^{13}C e apresentam valores de $\delta^{13}C$ ao redor de –14‰ (O'Leary, 1988). As reações simplificadas de fixação do carbono estão ilustradas na Figura 15.

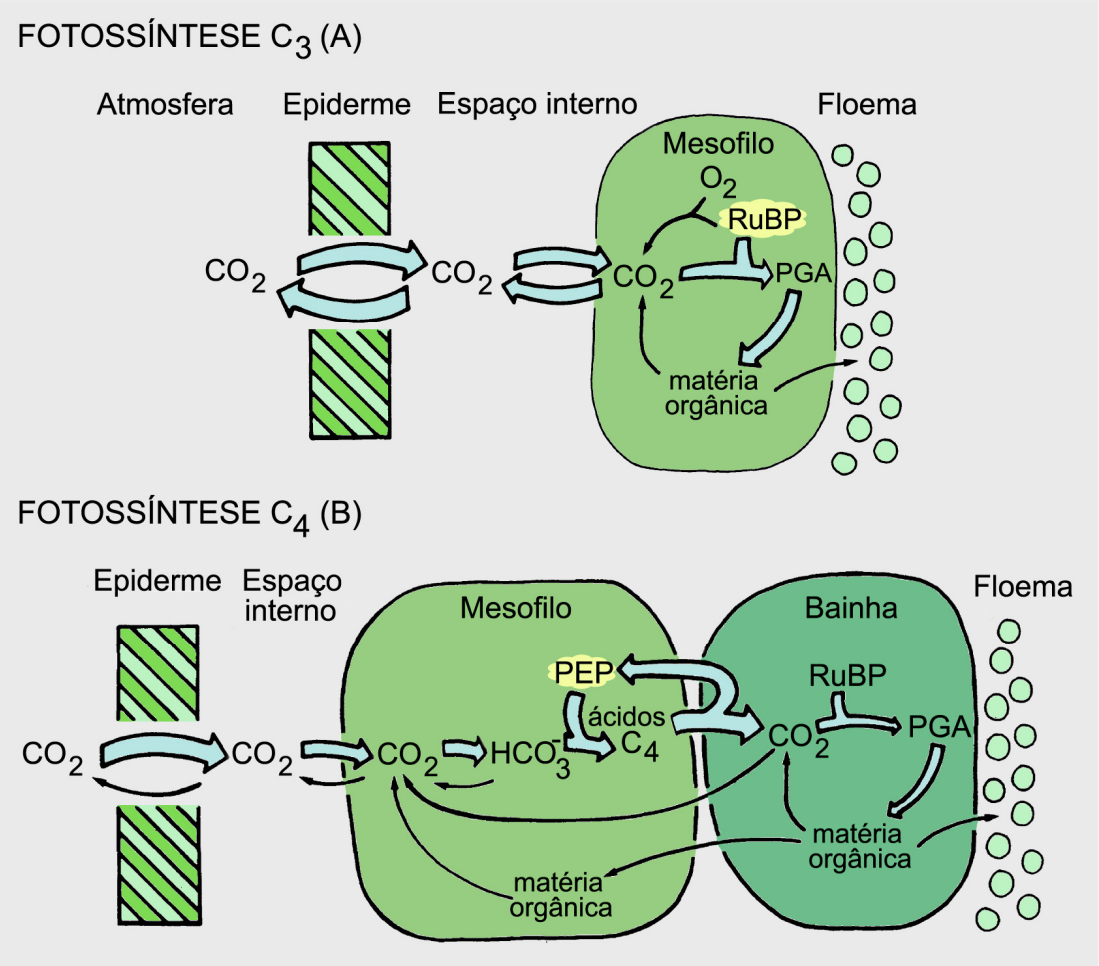


Fig. 15 Principais reações que levam à fixação de carbono nas vias fotossintéticas C_3 (A) e C_4 (B). Adaptado de O'Leary (1988).

Além da variação esperada de $\delta^{13}C$ devido à via metabólica, outros fatores podem afetar as razões isotópicas dos produtores primários terrestres. Por exemplo, as plantas C_3 de dossel fechado são tipicamente mais empobrecidas no ^{13}C do que aquelas que crescem em ambientes abertos (e.g., Cerling et al., 2004), como resultado da baixa irradiância e reciclagem de dióxido de carbono empobrecido em ^{13}C (Koch, 2007).

A via fotossintética CAM ocorre em plantas suculentas, as quais estão presentes principalmente em ambientes estressantes como desertos e, dessa forma, possuem baixa biomassa em outras regiões (Lüttge, 2004). Por esse motivo, essa via específica não será abordada na presente tese, já que as plantas CAM provavelmente não representavam

importantes fontes alimentares aos herbívoros pleistocênicos. No bioma Pampa, plantas C_3 e C_4 coexistem atualmente (Overbeck et al., 2007), sendo que as primeiras eram dominantes durante o Pleistoceno (Mourelle et al., 2020). Plantas com a via fotossintética C_3 compreendem árvores, arbustos e algumas gramíneas de estação fria, enquanto a via fotossintética C_4 geralmente ocorre em gramíneas de estação quente (Pearcy & Ehleringer, 1984).

Com exceção de alguns poucos exemplos, como o capim-marinho, *Spartina alterniflora*, uma planta encontrada em marismas, os produtores primários marinhos utilizam principalmente a via fotossintética C_3 (Fry & Sherr, 1989; Michener & Kaufman, 2007). Entretanto, apresentam ampla variação nos valores de $\delta^{13}C$ relacionada a uma série de fatores, como: barreias à difusão de carbono inorgânico dissolvido, HCO_3^- e CO_2, criadas por uma camada de água estagnada; temperatura e concentração de CO_2 dissolvido; densidade celular e taxas de crescimento do fitoplâncton (Fry & Sherr, 1989). Isso resulta em diferenças entre os ambientes aquáticos: algas planctônicas são tipicamente empobrecidas no ^{13}C em comparação com algas bentônicas (France, 1995) e tais diferenças persistem em consumidores de níveis tróficos superiores (Kelly, 2000).

Os valores de $\delta^{18}O$ de tecidos mineralizados dependem de várias reservas de oxigênio com composições isotópicas distintas, as quais interagem durante os processos biológicos (Luz & Kolodny, 1985), tornando a interpretação do $\delta^{18}O$ mais complexa (Ben-David & Flaherty, 2012). As principais fontes de entrada desse elemento no organismo de mamíferos incluem a ingestão (de água e alimentos) e a inalação (de vapor de água e gás oxigênio) durante a respiração, enquanto as principais perdas ocorrem através da expiração, excreção e transpiração (Luz & Kolodny, 1985; Koch, 2007). Os átomos de oxigênio provenientes das fontes de entrada compõem o fluido corporal do qual o biomineral se precipita, um processo dependente também da temperatura (Koch, 2007). Apesar da temperatura corporal de mamíferos e a composição isotópica do oxigênio atmosférico serem praticamente constantes, a composição da água ingerida é variável (revisado por Kohn et al., 1996). Além disso, o $\delta^{18}O$ pode variar entre indivíduos devido a diferenças fisiológicas relacionadas aos fluxos de entrada e saída de oxigênio (Kohn, 1996; Clementz & Koch, 2001).

A água ingerida fornece mais de 50% do oxigênio que compõe a água corporal de mamíferos terrestres (Koch, 2007) e, desse modo, corresponde à principal fonte de variação encontrada nos tecidos mineralizados desses animais (Podlesack et al., 2008). A água ingerida, por sua vez, tende a refletir a água ambiental, cujas razões isotópicas dependem do fracionamento isotópico durante o ciclo hidrológico (Fig. 16).

Diferentes espécies moleculares (isotopólogos) de H_2O têm pressões de vapor distintas que são proporcionais às suas massas (Ehleringer & Rundel, 1989). Consequentemente, as moléculas de água compostas por isótopos leves são mais propensas à evaporação e aquelas isotopicamente pesadas, por outro lado, são inicialmente eliminadas durante a precipitação (veja Pederzani & Britton, 2019). Tais processos resultam em uma variação preditiva na composição isotópica das águas ambientais (Fry, 2006) e, portanto, no $\delta^{18}O$ dos animais terrestres que as ingerem, variações essas intimamente relacionadas com o clima – principalmente temperatura e umidade.

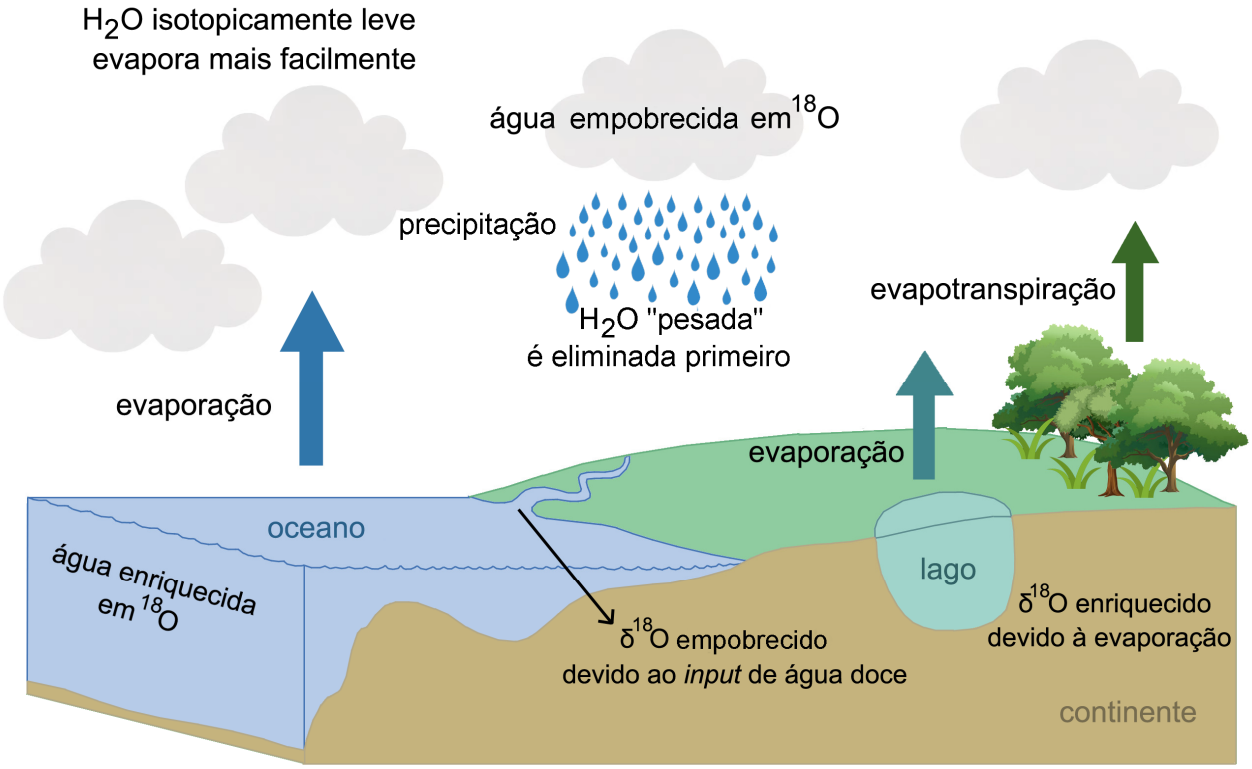


Fig. 16 Processos que influenciam a composição isotópica da água durante o ciclo hidrológico. Fonte: autora.

De modo geral, um clima mais frio/úmido resulta em valores de $\delta^{18}O$ mais negativos (ou empobrecidos), enquanto um clima mais quente/seco acarreta valores mais positivos (ou enriquecidos) em águas meteóricas (Koch, 2007; Bocherens & Drucker, 2013). Entretanto, o efeito da evaporação é mais pronunciado em plantas como resultado da transpiração foliar (Dawson et al., 2002), com um enriquecimento no ^{18}O inversamente proporcional à umidade relativa (Koch, 2007). Isso faz com que herbívoros com diferentes comportamentos de obtenção de água (através do ato de beber, ou indiretamente através da alimentação) reflitam aspectos distintos do ambiente: os que suprem suas necessidades de água por meio da dieta refletem a

aridez do ambiente, enquanto os bebedores obrigatórios normalmente rastreiam as razões isotópicas das águas meteóricas locais (Levin et al., 2006).

O $\delta^{18}O$ da bioapatita de mamíferos marinhos é semelhante ao da água ambiental (Newsome et al., 2010). As razões isotópicas de oxigênio da água do mar mostram uma relação estreita com a salinidade, pois ambas são fortemente influenciadas pela evaporação e entrada de água doce (Belem et al., 2019; Klein et al., 1996; LeGrande & Schmidt, 2006). Desse modo, espécies estuarinas costeiras são tipicamente empobrecidas no ^{18}O em comparação com espécies oceânicas e, consequentemente, o $\delta^{18}O$ de cetáceos evidencia seus habitats preferenciais (Drago et al., 2020). Portanto, embora guiados pelos mesmos processos, o $\delta^{18}O$ de mamíferos terrestres e mamíferos marinhos fornecem informações paleoambientais e paleoecológicas distintas.

Objetivos

Geral

A presente tese objetivou analisar aspectos paleoecológicos da megafauna marinha e terrestre do Pleistoceno do sul do Brasil. Para isso, utilizou-se principalmente da análise de isótopos estáveis de carbono e oxigênio ($\delta^{13}C$ e $\delta^{18}O$) a partir do carbonato da bioapatita de ossos e dentes. Espera-se que as informações obtidas sirvam como subsídios para melhor compreensão dos eventos que ocorreram no passado, como a extinção dos grandes herbívoros pleistocênicos, bem como para entender a ecologia de espécies marinhas viventes, como da toninha, *Pontoporia blainvillei*, em ambientes pristinos e sem influência humana.

Específicos

Seguem abaixo os objetivos específicos, cada um contemplado em um capítulo da presente tese:

CAPÍTULO 1. Paleodiet of Lamini camelids (Mammalia: Artiodactyla) from the Pleistocene of southern Brazil: insights from stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$)

Reconstruir a dieta de camelídeos sul-americanos (*Hemiauchenia paradoxa*, *Lama guanicoe* e *Vicugna vicugna*) do Pleistoceno do sul do Brasil e, com isso, obter informações sobre esse paleoambiente.

CAPÍTULO 2. Paleobiology of Pleistocene large land mammals from the Brazilian Pampa

Compreender a paleoecologia de grandes mamíferos do bioma Pampa brasileiro através de dados previamente publicados e novas análises isotópicas.

CAPÍTULO 3. Pleistocene cetacean fossils from the coastal plain of Rio Grande do Sul in southern Brazil

Descrever táxons de baleias e golfinhos que habitavam o Atlântico Sul Ocidental, utilizando uma coleção de ossos do ouvido (bulas timpânicas e perióticos), com implicações para a distribuição histórica desses cetáceos.

CAPÍTULO 4. Ecological traits of the franciscana dolphin (*Pontoporia blainvillei*) from the Late Pleistocene to the present days based on stable isotope analysis

Investigar aspectos ecológicos da toninha, *P. blainvillei*, através do tempo geológico – do Pleistoceno Tardio ao Holoceno (presente) – por meio da comparação das assinaturas isotópicas de espécimes fósseis e recentes.

Referências

Abe-Ouchi, A., Saito, F., Kawamura, K., Raymo, M.E., Okuno, J., Takahashi, K., Blatter, H., 2013. Insolation-driven 100,000-year glacial cycles and hysteresis of ice-sheet volume. Nature, 500: 190–193.

Barbehenn, R.V., Chen, Z., Karowe, D.N., Spickard, A., 2004. C_3 grasses have higher nutritional quality than C_4 grasses under ambient and elevated atmospheric CO_2. Global Change Biology, 10: 1565–1575.

Barnosky, A.D., Koch, P.L., Feranec, R.S., Wing, S.L., Shabel, A.B., 2004. Assessing the causes of Late Pleistocene extinctions on the continents. Science, 306: 70–75.

Bastida, R., Rodríguez, D., Secchi, E., Silva, V., 2018. Mamíferos Aquáticos da América do Sul e Antártica. Vázquez Mazzini Editores.

Behling, H., 2002. South and southeast Brazilian grasslands during Late Quaternary times: a synthesis. Palaeogeography, Palaeoclimatology, Palaeoecology, 177: 19–27.

Behling, H., Pillar, V.D., Bauermann, S.G., 2005. Late Quaternary grassland (campos), gallery forest, fire and climate dynamics, studied by pollen, charcoal and multivariate analysis of the São Francisco de Assis core in western Rio Grande do Sul (southern Brazil). Review of Palaeobotany and Palynology, 133: 235–248.

Belem, A.L., Caricchio, C., Albuquerque, A.L.S., Venancio, I.M., Zucchi, M.R., Santos, T.H.R., Alvarez, Y.G., 2019. Salinity and stable oxygen isotope relationship in the

southwestern Atlantic: constraints to paleoclimate reconstructions. Anais da Academia Brasileira de Ciências, 91: e20180226.

Ben-David, M., Flaherty, E.A., 2012. Stable isotopes in mammalian research: a beginner's guide. Journal of Mammalogy, 93: 312–328.

Bintanja, R., van de Wal, R.S.W., 2008. North American ice-sheet dynamics and the onset of 100,000-year glacial cycles. Nature, 454: 869–872.

Bischof, J.F., Darby, D.A., 1997. Mid- to Late Pleistocene ice drift in the western Arctic Ocean: evidence for a different circulation in the past. Science, 277: 74–78.

Bocherens, H., Drucker, D.G., 2013. Terrestrial teeth and bones. In: Elias, S.A., Mock, C.J. (Eds), Encyclopedia of Quaternary Science. Elsevier, pp. 304–314.

Bocherens, H., Cotte, M., Bonini, R., Scian, D., Straccia, P., Soibelzon, L., Prevosti, F.J., 2016. Paleobiology of sabretooth cat *Smilodon populator* in the Pampean Region (Buenos Aires Province, Argentina) around the Last Glacial Maximum: insights from carbon and nitrogen stable isotopes in bone collagen. Palaeogeography, Palaeoclimatology, Palaeoecology, 449: 463–474.

Bombim, M., 1976. Modelo paleoecológico evolutivo para o neoquaternário da região da Campanha – Oeste do Rio Grande do Sul (Brasil); a Formação Touro Passo, seu conteúdo fossilífero e a pedogênese pós-deposicional. Dissertação de Mestrado. Universidade Federal do Rio Grande do Sul.

Borrero, L.A., 1996. The Pleistocene-Holocene transition in southern South America. In: Straus, L.G., Eriksen, B.V., Erlandson, J.M., Yesner, D.R. (Eds), Humans at the End of the Ice Age: The Archaeology of the Pleistocene—Holocene Transition. Springer, pp. 339–354.

Bryant, D., Koch, P.L., Froelich, P.N., Showers, W.J., Genna, B.J., 1996. Oxygen isotope partitioning between phosphate and carbonate in mammalian apatite. Geochimica et Cosmochimica Acta, 60: 5145–5148.

Buchmann, F.S., 1994. Distribuição de fósseis pleistocênicos na zona costeira e plataforma continental interna no Rio Grande do Sul. Acta Geológica Leopoldensia, São Leopoldo, 39: 355–364.

Buchmann, F.S.C., 2002. Bioclastos de organismos terrestres e marinhos na praia e plataforma interna do Rio Grande do Sul: natureza, distribuição, origem e significado geológico. Tese de Doutorado, Universidade Federal do Rio Grande do Sul.

Buchmann, F.S., Rincon, G., 1997. Fósseis de vertebrados marinhos do Pleistoceno superior da porção Sul da Planície Costeira do Rio Grande do Sul, Brasil. Notas Técnicas, 8: 7–16.

Buchmann, F.S., Tomazelli, L.J., 2003. Relict nearshore shoals of Rio Grande do Sul, southern Brazil: origin and effects on nearby modern beaches. Journal of Coastal Research, 35: 318–322.

Buchmann, F.S., Seeliger, M., Zanella, L.R.C., Madureira, L.S.P., Tomazelli, L.J., Calliari, L.J., 2001. Análise batimétrica e sedimentológica no estudo do Parcel do Carpinteiro, uma paleolinha de praia pleistocênica na antepraia do Rio Grande do Sul, Brasil. Pesquisas em Geociências, 28: 109–115.

Buchmann, F.S., Caron, F., Lopes, R.P., Ugri, A., Lima, L.G., 2009. Panorama geológico da Planície Costeira do Rio Grande do Sul. In: Ribeiro, A.M., Bauermann, S.G., Scheres, C.S. (Eds), Quaternário do Rio Grande do Sul: Integrando Conhecimentos. Sociedade Brasileira de Paleontologia, pp. 34–56.

Buchmann, F.S., Zurlo, F.M., Vannucchi, F.S., Martins, C.C.A., 2017. First record of a fossil blue whale in São Paulo state, Brazil. Aquatic Mammals, 43: 649–654.

Calliari, L.J., Esteves, L.S., Oliveira, C.P.L., Tozzi, H.A.M., 1994. Padrões sonográficos de um afloramento de beachrock na plataforma continental interna do Rio Grande do Sul (COMEMIR/OSNLR). Notas Técnicas, 7: 27–32.

Cerling, T.E., Hart, J.A., Hart, T.B., 2004. Stable isotope ecology in the Ituri Forest. Oecologia, 138: 5–12.

Cione, A.L., Tonni, E.P., Soibelzon, L., 2009. Did humans cause the Late Pleistocene-Early Holocene mammalian extinctions in South America in a context of shrinking open areas? In: Haynes, G. (Ed), American Megafaunal Extinctions at the End of the Pleistocene. Springer, pp. 125–144.

Cione, A.L., Gasparini, G.M., Soibelzon, E., Soibelzon, L.H., Tonni, E.P., 2015. The Great American Biotic Interchange a South American Perspective. Springer.

Clementz, M.T., 2012. New insight from old bones: stable isotope analysis of fossil mammals. Journal of Mammalogy, 93: 368–380.

Clementz, M.T., Koch, P.L., 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia, 129: 461–472.

Cohen, K.M., Finney, S.C., Gibbard, P.L., Fan, J.-X., 2013. The ICS International chronostratigraphic Chart. Episodes, 36: 199–204.

Correa, I.C.S., 1990. Analyse morphostructurale et evolution paleogeographique de la plate-forme continentale atlantique sud-bresilienne (Rio Grande do Sul – Bresil). Tese de Doutorado, Universite de Bourdeaux.

Cozzi, B., Huggenberger, S., Oelschläger, H., 2017. Anatomy of Dolphins. Academic Press.

Crespo, E.A., 2018. Franciscana dolphin: *Pontoporia blainvillei*. In: Würsig, B., Thewissen, J.G.M., Kovacs, K. (Eds), Encyclopedia of Marine Mammals. Academic Press, pp. 388–392.

Cruz, E.A., Dillenburg, S.R., Buchmann, F.S., 2016. Description and controls on distribution of Pleistocene vertebrate fossils from the central and southern sectors of the coastal plain of Rio Grande do Sul, Brazil. Revista Brasileira de Paleontologia, 19: 425–438.

Cunha, F.L.S., 1982. Novos registros de baleias fósseis (Balaenopteridae e Balaenidae) no Pleistoceno de Santa Vitória do Palmar, RS, Brasil. Anais da Academia Brasileira de Ciências, 54: 264.

Cunha, F.L.S., 1985. Registros de cetáceos Balaenidae (*Eubalaena*) no Pleistoceno de Santa Vitória do Palmar, RS, Brasil. Anais da Academia Brasileira de Ciências, 57: 142.

Cunha, F.L.S., Nunan, G.W.A., 1980. Pleistocene marine vertebrates (Sciaenidae and Balaenopteridae) from litoral of Santa Vitória do Palmar, RS, Brasil. Anais do 31º Congresso Brasileiro de Geologia, 5: 3049–3055.

Cunha, F.L.S., Bergqvist, L.P., Capilla, R., 1992. Cetáceos fósseis e subfósseis da coleção de paleovertebrados do Museu Nacional. Anais da Academia Brasileira de Ciências, 64: 277–288.

Dalerum, F., Angerbjörn, A., 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia, 144: 647–658.

Dantas, M.A.T., Cherkinsky, A., Bocherens, H., Drefahl, M., Bernardes, C., França, L.M., 2017. Isotopic paleoecology of the Pleistocene megamammals from the Brazilian Intertropical Region: feeding ecology ($\delta^{13}C$), niche breadth and overlap. Quaternary Science Reviews, 170: 152–163.

Dantas, M.A.T., Araújo, A.V., Nogueira, E.E., Silva, L.A., Leoni, R.A., Fêlix, P.M., Cherkinsky, A., 2019. Isotopic paleoecology ($\delta^{13}C$) of mesoherbivores from Late Pleistocene of Gruta da Marota, Andaraí, Bahia, Brazil. Historical Biology, 33: 643–651.

Da-Rosa, A.A.S., 2009. Geologia do Quaternário continental do RS: estado da arte e perspectivas de trabalho. In: Ribeiro, A.M., Bauermann, S.G., Scherer, C.S. (Eds),

Quaternário do Rio Grande do Sul: Integrando Conhecimentos. Sociedade Brasileira de Paleontologia, pp. 17–34.

Dawson, T.E., Mandelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable isotopes in plant ecology. Annual Review of Ecology and Systematics, 33:507–559.

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta, 42: 495–506.

DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta, 45: 341–351.

Denton, G.H., Anderson, R.F., Toggweiler, J.R., Edwards, R.L., Schaefer, J.M., Putnam, A.E., 2010. The Last Glacial Termination. Science, 328: 1652–1656.

Domingo, L., Tomassini, R.L., Montalvo, C.I., Sanz-Pérez, D., Alberdi, M.T., 2020. The Great American Biotic Interchange revisited: a new perspective from the stable isotope record of Argentine Pampas fossil mammals. Scientific Reports, 10: 1608.

Doughty, C.E., Wolf, A., Malhi, Y., 2013. The legacy of the Pleistocene megafauna extinctions on nutrient availability in Amazonia. Nature Geoscience, 6: 761–764.

Drago, M., Valdivia, M., Bragg, D., González, E. M., Aguilar, A., Carbona, L., 2020. Stable oxygen isotopes reveal habitat use by marine mammals in the Río de la Plata estuary and adjoining Atlantic Ocean. Estuarine, Coastal and Shelf Science, 238: 106708.

Drehmer, C.J., Ribeiro, A.M., 1998. A temporal bone of an Otariidae (Mammalia, Pinnipedia) from the Late Pleistocene of Rio Grande do Sul state, Brazil. Revista da Universidade de Guarulhos Geociências, 6: 39–44.

Ehleringer, J.R., Rundel, P.W., 1989. Stable isotopes: history, units, and instrumentation. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds), Stable Isotopes in Ecological Research. Springer, pp. 1–15.

Ekdale, E.G., Berta, A., Deméré, T.A., 2011. The comparative osteology of the petrotympanic complex (ear region) of extant baleen whales (Cetacea: Mysticeti). PLoS ONE, 6: e21311.

Elderfield, H., Ferretti, P., Greaves, M., Crowhurst, S., McCave, I.N., Hodell, D., Piotrowski, A.M., 2012. Evolution of ocean temperature and ice volume through the mid-Pleistocene climate transition. Science, 337: 704–709.

Ellis, R., Palmer, M., 2016. Modulation of ice ages via precession and dust-albedo feedbacks. Geoscience Frontiers, 7: 891–909.

Estes, J.A., Heithaus, M., McCauley, D.J., Rasher, D.B., Worm, B., 2016. Megafaunal impacts on structure and function of ocean ecosystems. Annual Review of Environment and Resources, 41: 83–116.

Faurby, S., Svenning, J., 2016. The asymmetry in the Great American Biotic Interchange in mammals is consistent with differential susceptibility to mammalian predation. Global Ecology and Biogeography, 25: 1443–1453.

Ferigolo, J., 1999. Late Pleistocene South-American land-mammal extinctions: the infection hypothesis. In: Tonni, E.P., Cione, A.L. (Eds.), Quaternary Vertebrate Palaeontology in South America, pp. 279–310.

Ferrari, R., Jansen, M.F., Adkins, J.F., Burke, A., Stewart, A.L., Thompson, A.F., 2014. Antarctic sea ice control on ocean circulation in present and glacial climates. Proceedings of the National Academy of Sciences, 111: 8753–8758.

Fiedel, S.J., 2022. Initial human colonization of the Americas, redux. Radiocarbon, 64: 845–897.

Figueiredo, A.G., 1975. Geologia dos depósitos calcários biodetríticos da plataforma continental do Rio Grande do Sul. Dissertação de Mestrado. Universidade Federal do Rio Grande do Sul.

Firestone, R.B., West, A., Kennett, J.P., Becker, L., Bunch, T.E., Revay, Z.S., Schultz, P.H., Belgya, T., Kennett, D.J., Erlandson, J.M., Dickenson, O.J., Goodyear, A.C., Harris, R.S., Howard, G.A., Kloosterman, J.B., Lechler, P., Mayewski, P.A., Montgomery, J., Poreda, R., Darrah, T., Que Hee, S.S., Smith, A.R., Stich, A., Topping, W., Wittke, J.H., Wolbach, W.S., 2007. Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. Proceedings of the National Academy of Sciences, 104: 16016–16021.

Fordyce, R.E., 2009. Cetacean fossil record. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds), Encyclopedia of Marine Mammals 2nd Ed. Academic Press, pp. 207–215.

Fordyce, R.E., 2018. Cetacean evolution. In: Würsig, B., Thewissen, J.G.M., Kovacs, K., (Eds), Encyclopedia of Marine Mammals 3rd Ed. Academic Press, pp. 180–185.

France, R.L., 1995. Carbon-^{13} enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series, 124: 307–312.

Fry, B., 2006. Stable Isotope Ecology. Springer.

Fry, B., Sherr, E.B., 1989. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds), Stable Isotopes in Ecological Research. Springer, pp. 196–229.

Gol'din, P., Steeman, M.E., 2015. From problem taxa to problem solver: a new Miocene family, Tranatocetidae, brings perspective on baleen whale evolution. PLoS ONE, 10: e0135500.

Gomes, V.S., Lessa, C.M.B., Oliveira, G.R., Bantim, R.A.M., Sayão, J.M., Bocherens, H., Araújo-Júnior, H.I., Dantas, M.A.T., 2023. Seasonal variations in diet ($\delta^{13}C$) and climate ($\delta^{18}O$) inferred through toxodonts enamel teeth during the Late Pleistocene in the brazilian intertropical region. Journal of South American Earth Sciences, 121: 104148.

Hubbe, A., Hubbe, M., Neves, W.A., 2013. The Brazilian megamastofauna of the Pleistocene/Holocene transition and its relationship with the early human settlement of the continent. Earth-Science Reviews, 118: 1–10.

Hughes, P.D., Gibbard, P.L., 2018. Global glacier dynamics during 100 ka Pleistocene glacial cycles. Quaternary Research, 90: 222–243.

Imbrie, J., Hays, J.D., Martinson, D.G., McIntyre, A., Mix, A.C., Morley, J.J., Pisias, N.G., Prell, W.L., Shackleton, N.J., 1984. The orbital theory of Pleistocene climate: support from a revised chronology of the marine $\delta^{18}O$ record. In: Berger, A., Imbrie, J., Hays, J., Kukla, G., Saltzman, B. (Eds), Milankovitch and Climate, Part 1. D. Reidel Publishing Company, pp. 269–305.

Janis, C., 2008. An evolutionary history of browsing and grazing ungulates. In: Gordon, I.J., Prins, H.H.T. (Eds), The Ecology of Browsing and Grazing. Springer, pp. 21–45.

Kasuya, T., 1973. Systematic consideration of recent toothed whales based on the morphology of tympano-periotic bone. The Scientific Reports of the Whales Research Institute, 25: 1–103.

Kawamura, K., Parrenin, F., Lisiecki, L., Uemura, R., Vimeux, F., Severinghaus, J.P., Hutterli, M.A., Nakazawa, T., Aoki, S., Jouzel, J., Raymo, M.E., Matsumoto, K., Nakata, H., Motoyama, H., Fujita, S., Goto-Azuma, K., Fujii, Y., Watanabe, O., 2007. Northern Hemisphere forcing of climatic cycles in Antarctica over the past 360,000 years. Nature, 448: 912–916.

Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology, 78: 1–27.

Kerber, L., Oliveira, E.V., 2008. Fósseis de vertebrados da Formação Touro Passo (Pleistoceno Superior), Rio Grande do Sul, Brasil: atualização dos dados e novas contribuições. Gaea Journal of Geoscience, 4: 49–64.

Kerber, L., Kinoshita, A., José, F.A., Figueiredo, A.M.G., Oliveira, É.V., Baffa, O., 2011. Electron Spin Resonance dating of the southern Brazilian Pleistocene mammals from Touro Passo Formation, and remarks on the geochronology, fauna and palaeoenvironments. Quaternary International, 245: 201–208.

Kerber, L., Pitana, V.G., Ribeiro, A.M., Hsiou, A.S., Oliveira, E.V., 2014. Late Pleistocene vertebrates from Touro Passo Creek (Touro Passo Formation), southern Brazil: a review. Revista Mexicana de Ciencias Geológicas, 31: 248–259.

Klein, R.T., Lohmann, K.C., Thayer, C.W., 1996. Bivalve skeletons record sea-surface temperature and $\delta^{18}O$ via Mg/Ca and $^{18}O/^{16}O$ ratios. Geology, 24: 415–418.

Koch, P.L., 2007. Isotopic study of the biology of modern and fossil vertebrates. In: Michener, R., Lajtha, K. (Eds), Stable Isotopes in Ecology and Environmental Science. Blackwell Publishers, pp. 99–154.

Kohn, M.J., 1996. Predicting animal $\delta^{18}O$: accounting for diet and physiological adaptation. Geochimica et Cosmochimica Acta, 60: 4811–4829.

Kohn, M.J., Cerling, T.E., 2002. Stable isotope compositions of biological apatite. Reviews in mineralogy and geochemistry. In: Kohn, M.J., Rakovan, J., Hughes, J.M. (Eds), Phosphates, Geochemical, Geobiological, and Materials Importance. Reviews in Mineralogy and Geochemistry, Mineralogical Society of America, pp. 455–488.

Kohn, M.J., Schoeninger, M.J., Valley, J.W., 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. Geochimica et Cosmochimica Acta, 60: 3889–3896.

Lazar, K.B., Polyak, L., 2016. Pleistocene benthic foraminifers in the Arctic Ocean: implications for sea-ice and circulation history. Marine Micropaleontology, 126: 19–30.

LeGrande, A.N., Schmidt, G.A., 2006. Global gridded data set of the oxygen isotopic composition in seawater. Geophysical Research Letters, 33: L12604.

Levin, N.E., Cerling, T.E., Passey, B.H., Harris, J.M., Ehleringer, J.R., 2006. A stable isotope aridity index for terrestrial environments. Proceedings of the National Academy of Sciences, 103: 11201–11205.

Lima, L.G., Dillenburg, S., Buchmann, F., Parise, C., 2020. O significado paleogeográfico das turfeiras costeiras do Rio Grande do Sul. Revista Geociências, 39: 393–410.

Lopes, R.P., Buchmann, F.S., 2011. Pleistocene mammals from the southern Brazilian continental shelf. Journal of South American Earth Sciences, 31: 17–27.

Lopes, R.P., Ferigolo, J., 2015. Post mortem modifications (pseudopaleopathologies) in Middle-Late Pleistocene mammal fossils from southern Brazil. Revista Brasileira de Paleontologia, 18: 285–306.

Lopes, R.P., Buchmann, F.S.C., Caron, F., Itusarry, M.E.G.S., 2005. Barrancas fossilíferas do Arroio Chuí, RS – Importante megafauna pleistocênica no extremo sul do Brasil. In: Winge, M., Schobbenhaus, C., Berbert-Born, M., Queiroz, E.T., Campos, D.A., Souza, C.R.G., Fernandes, A.C.S. (Eds), Sítios Geológicos e Paleontológicos do Brasil. Brasília: Comissão Brasileira de Sítios Geológicos e Paleobiológicos, pp. 1–10.

Lopes, R.P., Buchmann, F.S.C., Caron, F., 2006. Primeiro registro de fósseis de aves marinhas na planície costeira do Rio Grande do Sul, Brasil. Revista Brasileira de Geociências, 36: 648–650.

Lopes, R.P., Oliveira, L.C., Figueiredo, A.M.G., Kinoshita, A., Baffa, O., Buchmann, F.S., 2010. ESR dating of Pleistocene mammal teeth and its implications for the biostratigraphy and geological evolution of the coastal plain, Rio Grande do Sul, southern Brazil. Quaternary International, 212: 213–222.

Lopes, R.P., Ribeiro, A.M., Dillenburg, S.R., Schultz, C.L., 2013. Late middle to late Pleistocene paleoecology and paleoenvironments in the coastal plain of Rio Grande do Sul State, southern Brazil, from stable isotopes in fossils of *Toxodon* and *Stegomastodon*. Palaeogeography, Palaeoclimatology, Palaeoecology, 369: 385–394.

Lopes, R.P., Kinoshita, A., Baffa, O., Figueiredo, A.M.G., Dillenburg, S.R., Schultz, C.L., Pereira, J.C., 2014. ESR dating of Pleistocene mammals and marine shells from the coastal plain of Rio Grande do Sul state, southern Brazil. Quaternary International, 352: 124–134.

Lopes, R.P., Pereira, J.C., Ferigolo, J., 2019. A late Pleistocene fossil stork (Ciconiiformes: Ciconiidae) from the Santa Vitoria Formation, southern Brazil and its paleoenviromental significance. Revista Brasileira de Paleontologia, 22: 199–216.

Lopes, R.P., Dillenburg, S.R., Savian, J.F., Pereira, J.C., 2021. The Santa Vitória Alloformation: an update on a Pleistocene fossil rich unit in Southern Brazil. Brazilian Journal of Geology, 51: e2020065.

Lopes, R.P., Pereira, J.C., Kerber, L., Dillenburg, S.R., 2020a. The extinction of the Pleistocene megafauna in the Pampa of southern Brazil. Quaternary Science Reviews, 242: 106428.

Lopes, R.P., Pereira, J.C., Dillenburg, S.R., Tatumi, S.H., Yee, M., Figueiredo, A.M.G., Kinoshita, A., Baffa, O., 2020b. Late Pleistocene-Holocene fossils from Mirim lake, southern Brazil, and their paleoenvironmental significance: I – vertebrates. Journal of South American Earth Sciences, 100: 102566.

Ludt, W.B., Rocha, L.A., 2015. Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. Journal of Biogeography, 42: 25–38.

Lüttge, U., 2004. Ecophysiology of crassulacean acid metabolism (CAM). Annals of Botany, 93: 629–652.

Luz, B., Kolodny, Y., 1985. Oxygen isotope variations in phosphate of biogenic apatites, IV. Mammal teeth and bones. Earth and Planetary Science Letters, 75: 29–36.

Mackensen, A., Rudolph, M., Kuhn, G., 2001. Late Pleistocene deepwater circulation in the subantarctic eastern Atlantic. Global and Planetary Change, 30: 197–229.

Madden, R.H., 2015. Hypsodonty in Mammals: Evolution, Geomorphology and the Role of Earth Surface Process. Cambridge University Press.

Mann, D.H., Groves, P., Gaglioti, B.V., Shapiro, B.A., 2019. Climate-driven ecological stability as a globally shared cause of Late Quaternary megafaunal extinctions: the Plaids and Stripes Hypothesis. Biological Reviews, 94: 328–352.

Marshall, L.G., 1988. Land mammals and the Great American Biotic Interchange. American Scientist, 76: 380–388.

Marshall, J.D., Brooks, J.R., Lajtha, K., 2007. Sources of variation in the stable isotopic composition of plants. In: Michener, R., Lajtha, K. (Eds), Stable Isotopes in Ecology and Environmental Science. Blackwell Publishers, pp. 22–60.

Martin-Garcia, G.M., Sierro, F.J., Flores, J.A., Abrantes, F., 2018. Change in the North Atlantic circulation associated with the Mid-Pleistocene transition. Climate of the Past, 14: 1639–1651.

Metera, E., Sakowski, T., Słoniewski, K., Romanowicz, B., 2010. Grazing as a tool to maintain biodiversity of grassland – a review. Animal Science Papers and Reports, 28: 315–334.

Michener, R.H., Kaufman, L., 2007. Stable isotope ratios as tracers in marine food webs: an update. In: Michener, R., Lajtha, K. (Eds), Stable Isotopes in Ecology and Environmental Science. Blackwell Publishers, pp. 238–282.

Milder, S.E.S., 2000. Arqueologia do sudoeste do Rio Grande do Sul: uma perspectiva geológica. Tese de Doutorado. Universidade de São Paulo.

Miller, E.T., 1987. Pesquisas arqueológicas paleoindígenas no Brasil Ocidental. Estudios Atameños, 8: 37–61.

Moleón, M., Sánchez-Zapata, J.A., Donázar, J.A., Revilla, E., Martín-López, B., Gutiérrez-Cánovas, C., Getz, W.M., Morales-Reyes, Z., Campos-Arceiz, A., Crowder, L.B., Galetti, M., González-Suárez, M., He, F., Jordano, P., Lewison, R., Naidoo, R., Owen-Smith, N., Selva, N., Svenning, J., Tella, J.L., Zarfl, C., Jähnig, S.C., Hayward, M.W., Faurby, S., García, N., Barnosky, A.D., Tockner, K., 2020. Rethinking megafauna. Proceedings of the Royal Society B, 287: 20192643.

Mothé, D., Avilla, L.S., Araújo-Júnior, H.I., Rotti, A., Prous, A., Azevedo, S.A.K., 2020. An artifact embedded in an extinct proboscidean sheds new light on human-megafaunal interactions in the Quaternary of South America. Quaternary Science Reviews, 229: 106125.

Mourelle, D., del Puerto, L., Perez, L., Bergamino, L., García-Rodríguez, F., 2020. Late Pleistocene and Late Holocene environment and climate in the campos region of southeastern South America inferred from phytolith, diatom and geochemical data. Palaeogeography, Palaeoclimatology, Palaeoecology, 543: 109586.

Nara, L., Cremer, M.J., Farro, A.P.C., Colosio, A.C., Barbosa, L.A., Bertozzi, C.P., Secchi, E.R., Pagliani, B., Costa-Urrutia, P., Gariboldi, M.C., Lazoski, C., Cunha, H.A., 2022. Phylogeography of the endangered franciscana dolphin: timing and geological setting of the evolution of populations. Journal of Mammalian Evolution, 29: 609–625.

Newsome, S.D., Clementz, M.T., Koch, P.L., 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science, 26: 509–572.

O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. BioScience, 38: 328–336.

Oliveira, E.V., Kerber, L., 2009. Paleontologia e aspectos geológicos das sucessões do final do Neógeno no sudoeste do Rio Grande do Sul, Brasil. Gaea – Journal of Geoscience, 5: 21–34.

Oliveira, E.V., Dutra, T.L., Zeltzer, F., 2002. Megaterídeos (Mammalia, Xenarthra) do Quaternário de Caçapava do Sul, Rio Grande do Sul, com considerações sobre a flora associada. Geologia Colombiana, 27: 77–86.

Oliveira, K., Araújo, T., Rotti, A., Mothé, D., Rivals, F., Avilla, L.S., 2020. Fantastic beasts and what they ate: revealing feeding habits and ecological niche of late Quaternary Macraucheniidae from South America. Quaternary Science Reviews, 231: 106178.

Otto-Bliesner, B.L., Hewitt, C.D., Marchitto, T.M., Brady, E., Abeouchi, A., Crucifix, M., Murakami, S., Weber, S.L., 2007. Last Glacial Maximum ocean thermohaline circulation: PMIP2 model intercomparisons and data constraints. Geophysical Research Letters, 34: L12706.

Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R., Forneck, E.D., 2007. Brazil's neglected biome: the South Brazilian Campos. Perspectives in Plant Ecology, Evolution and Systematics, 9: 101–116.

Owen-Smith, R.N., 1988. Megaherbivores. The influence of very large body size on ecology. Cambridge University Press.

Paillard, D., 1998. The timing of Pleistocene glaciations from a simple multiple-state climate model. Nature, 391: 378–381.

Pansani, T.R., Pobiner, B., Gueriau, P., Thoury, M., Tafforeau, P., Baranger, E., Vialou, A.V., Vialou, D., McSparron, C., Castro, M.C., Dantas, M.A.T., Bertrand, L., Pacheco, M.L.A.F., 2023. Evidence of artefacts made of giant sloth bones in central Brazil around the last glacial maximum. Proceedings of the Royal Society B, 290: 20230316.

Paula Couto, C., 1961. Considerações sobre o Pleistoceno Sul-Americano. Revista Brasileira de Geografia, 23: 122.

Pearcy, R.W., Ehleringer, J., 1984. Comparative ecophysiology of C_3 and C_4 plants. Plant, Cell & Environment, 7: 1–13.

Pederzani, S., Britton, K., 2019. Oxygen isotopes in bioarchaeology: principles and applications, challenges and opportunities. Earth-Science Reviews, 188: 77–107.

Pérez-Balarezo, A., González-Varas, M., van Havre, G., Zhou, Y., 2023. Early prehistory of South America and population dynamics: issues and hypotheses. L'anthropologie, 127: 103139.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18: 293–320.

Pimiento, C., Griffin, J.N., Clements, C.F., Silvestro, D., Varela, S., Uhen, M.D., Jaramillo, C., 2017. The Pliocene marine megafauna extinction and its impact on functional diversity. Nature Ecology & Evolution, 1: 1100-1106.

Pisias, N.G., Moore, T.C., 1981. The evolution of Pleistocene climate: a time series approach. Earth and Planetary Science Letters, 52: 450–458.

Podlesak, D.W., Torregrossa, A.M., Ehleringer, J.R., Dearing, M.D., Passey, B.H., Cerling, T.E., 2008. Turnover of oxygen and hydrogen isotopes in the body water, CO_2, hair, and enamel of a small mammal. Geochimica et Cosmochimica Acta, 72: 19–35.

Politis, G.G., Prates, L., 2018. Clocking the arrival of *Homo sapiens* in the Southern Cone of South America. In: Harvati, K., Jäger, G., Reyes-Centeno, H. (Eds), New Perspectives on the Peopling of the Americas. Kerns Verlag, pp. 79–106.

Politis, G.G., Johnson, E., Gutiérrez, M., Hartwell, W., 2003. Survival of the Pleistocene fauna: new radiocarbon dates on organic sediments from La Moderna (Pampean Region, Argentina). In: Miotti, L., Salemme, M., Flegenheimer, N. (Eds), Where the South Winds Blow: Ancient Evidence for Paleo South Americans. Texas University Press, pp. 45–50.

Politis, G.G., Messineo, P.G., Stafford, T.W., Lindsey, E.L., 2019. Campo Laborde: a Late Pleistocene giant ground sloth kill and butchering site in the Pampas. Science Advances, 5: eaau4546.

Prado, J.L., Alberdi, M.T., 1999. The mammalian record and climatic change over the last 30,000 years in the Pampean Region, Argentina. Quaternary International, 57/58: 165–174.

Prado, J.H.F., Mattos, P.H., Silva, K.G., Secchi, E.R., 2016. Longterm seasonal and interannual patterns of marine mammal strandings in Subtropical Western South Atlantic. PLoS ONE, 11: e0146339.

Prates, L., Perez, S.I., 2021. Late Pleistocene South American megafaunal extinctions associated with rise of Fishtail points and human population. Nature Communications, 12: 2175.

Prates, L., Politis, G.G., Perez, S.I., 2020. Rapid radiation of humans in South America after the last glacial maximum: a radiocarbon-based study. PLoS ONE, 15: e0236023.

Ramos, R., González-Solís, J., 2012. Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. Frontiers in Ecology and the Environment, 10: 258–266.

Raven, P.H., Evert, R.F., Eichhorn, S.E., 2007. Biologia vegetal. Guanabara Koogan.

Ribeiro, A.M., Drehmer, C.J., Buchmann, F.S.C., Simões-Lopes, P.C., 1998. Pleistocene skull remains of *Pontoporia blainvillei* (Cetacea, Pontoporiidae) from the coast plain of Rio Grande do Sul state, Brazil, and the relationship of Pontoporids. Revista Universidade de Guarulhos Geociências, 3: 71–77.

Richter, M., 1987. Osteichthyes e Elasmobranchii (Pisces) da Bacia de Pelotas, Quaternário do Rio Grande do Sul, Brasil. Paula-Coutiana, 1: 17–37.

Rodrigues, P.H., Prevosti, F.J., Ferigolo, J., Ribeiro, A.M., 2004. Novos materiais de carnívora para o Pleistoceno do estado do Rio Grande do Sul, Brasil. Revista Brasileira de Paleontologia, 7: 77–86.

Rosa, M.L.C.C., Barboza, E.G., Abreu, W.S., Tomazelli, L.J., Dillenburg, S.R., 2017. High-Frequency Sequences in the Quaternary of Pelotas Basin (coastal plain): a record of degradational stacking as a function of longer-term base-level fall. Brazilian Journal of Geology, 47: 183–207.

Roth, L., Lorscheitter, M.L., Masetto, E., 2021. Paleoenvironments of the last 24,000 years on the extreme northern Rio Grande do Sul coastal plain, southern Brazil. Quaternary International, 571:117–126.

Ruban, D.A., 2009. The survival of megafauna after the end-Pleistocene impact: a lesson from the Cretaceous/Tertiary boundary. Geologos, 15: 129–132.

Santos, M.C.P., 2023. Les premières occupations humaines dans le Sud du Brésil : une vision géoarchéologique intégrée. L'anthropogie 127, 103136.

Santos, T. P., Lessa, D. O., Venancio, I. M., Chiessi, C. M., Mulitza, S., Kuhnert, H., Govin, A., Machado, T., Costa, K. B., Toledo, F., Dias, B.B., Albuquerque, A.L.S., 2017. Prolonged warming of the Brazil Current precedes deglaciations. Earth and Planetary Science Letters, 463: 1–12.

Scherer, C.S., Ferigolo, J., Ribeiro, A.M., Guerra, C.C., 2007. Contribution to the knowledge of *Hemiauchenia paradoxa* (Artiodactyla, Camelidae) from the Pleistocene of southern Brazil. Revista Brasileira de Paleontologia, 10: 35–52.

Scherer, C.S., Pitana, V.G., Ribeiro, A.M., 2009. Proterotheriidae and Macraucheniidae (Litopterna, Mammalia) from the Pleistocene of Rio Grande do Sul State, Brazil. Revista Brasileira de Paleontologia, 12: 231–246.

Soibelzon, L.H., 2008. Broken zig-zag: una nueva hipótesis sobre las causas de la extinción de los megamamíferos em América del Sur. Museo, 3: 24–28.

Soliani, E., 1973. Geologia da região de Santa Vitória do Palmar, RS, e a posição estratigráfica dos fósseis de mamíferos pleistocênicos. Dissertação de Mestrado. Universidade Federal do Rio Grande.

Stephens, B.B., Keeling, R.F., 2000. The influence of Antarctic Sea ice on glacial-interglacial CO_2 variations. Nature, 404: 171–174.

Stephens, R.B., Ouimette, A.P., Hobbie, E.A., Rowe, R.J., 2022. Reevaluating trophic discrimination factors ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) for diet reconstruction. Ecological Monographs, 93: e1525.

Suárez, R., Santos, G.M., 2010. Cazadores-recolectores tempranos, supervivencia de fauna del Pleistoceno (*Equus* sp. y *Glyptodon* sp.) y tecnología lítica durante el Holoceno temprano en la frontera Uruguay-Brasil. Revista de Arqueología, 23: 20–39.

Sulzman, E.W., 2007. Stable isotope chemistry and measurement: a primer. In: Michener, R., Lajtha, K. (Eds), Stable Isotopes in Ecology and Environmental Science. Blackwell Publishers, pp. 1–21.

Sweatman, M.B., 2021. The Younger Dryas impact hypothesis: review of the impact evidence. Earth-Science Reviews, 218: 103677.

Tejada, J.V., Flynn, J.J., MacPhee, R., O'Connell, T.C., 2021. Isotope data from amino acids indicate Darwin's ground sloth was not an herbivore. Scientific Reports, 11: 18944.

Tejada-Lara, J.V., MacFadden, B.J., Bermudez, L., Rojas, G., Salas-Gismondi, R., Flynn, J.J., 2018. Body mass predicts isotope enrichment in herbivorous mammals. Proceedings of the Royal Society B, 285: 20181020.

Thatje, S., Hillenbrand, C., Mackensen, A., Larter, R., 2008. Life hung by a thread: endurance of Antarctic fauna in glacial periods. Ecology, 89: 682–692.

Tomazelli, L.J., Dillenburg, S.R., Villwock, J.A., 2000. Late Quaternary geological history of Rio Grande do Sul coastal plain, southern Brazil. Revista Brasileira de Geociências, 30: 474–476.

Van Hoesel, A., Hoek, W.Z., Pennock, G.M., Drury, M.R., 2014. The Younger Dryas impact hypothesis: a critical review. Quaternary Science Reviews, 83: 95–114.

Villwock, J.A., Tomazelli, L.J., 1995. Geologia costeira do Rio Grande do Sul. Notas Técnicas, 8: 1–45.

Webb, S.D., 1985. Late Cenozoic mammal dispersals between the Americas. In: Stehli, F.G., Webb, S.D. (Eds), The Great American Biotic Interchange. Topics in Geobiology. Boston: Springer, pp. 357–386.

Webb, S.D., 1991. Ecogeography and the Great American Interchange. Paleobiology, 17: 266–280.

Webb, S.D., 2006. The Great American Biotic Interchange: patterns and processes. Annals of the Missouri Botanical Garden, 93: 245–257.

Whiteman, J.P., Elliott Smith, E.A., Besser, A.C., Newsome, S.D., 2019. A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. Diversity, 11: 8.

Woodburne, M.O., 2010. The Great American Biotic Interchange: dispersals, tectonics, climate, sea level and holding pens. Journal of Mammalian Evolution, 17: 245–264.

Wroe, S., Field, J.H., Archer, M., Grayson, D.K., Price, G.J., Louys, J., Faith, J.T., Webb, G.E., Davidson, I., Mooney, S.D., 2013. Climate change frames debate over the extinction of megafauna in Sahul (Pleistocene Australia-New Guinea). Proceedings of the National Academy of Sciences, 28: 8777–8781.

Zerbini, A.N., Secchi, E., Crespo, E., Danilewicz, D., Reeves, R., 2017. *Pontoporia blainvillei* (errata version published in 2018). IUCN Red List of Threatened Species: e.T17978A123792204.

Zhao, Y., Yang, Y., Guo, Y., Ren, G., Zhang, F., 2022. Stable carbon isotope composition of bone hydroxylapatite: significance in paleodietary analysis. Paleoworld, 31: 169–184.

CAPÍTULO 1

Paleodiet of Lamini camelids (Mammalia: Artiodactyla) from the Pleistocene of southern Brazil: insights from stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$)

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Paleodiet of Lamini camelids (Mammalia, Artiodactyla) from the Pleistocene of southern Brazil: insights from stable isotopes analysis ($\delta^{13}C$, $\delta^{18}O$)

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Abstract

Camelids (Camelidae) were a diverse and widely distributed group in South America during the Pleistocene. According to the fossil record, three species inhabited southern Brazil in the recent past: *Hemiauchenia paradoxa*, *Lama guanicoe* and *Vicugna vicugna*. The analysis of carbon and oxygen stable isotope ratios in bioapatite provides insight into the paleobiology of non-living animals and the environment they used to inhabit. We applied this tool to investigate the diet of camelids from two geological localities in southern Brazil: Touro Passo and Santa Vitória Formations (*H. paradoxa*, n = 7; *L. guanicoe*, n = 6; *V. vicugna*, n = 4). Carbon stable isotopes from enamel, dentin, and bone indicated that *H. paradoxa* and *L. guanicoe* had diets comprising mostly C_3 grasses, but the latter showed a broader diet due to one individual with a mixed diet, whereas *V. vicugna* had a mixed C_3–C_4 diet. These different foraging behaviors may have minimized interspecific competition and favored niche partitioning and the coexistence of related species. Combined oxygen and carbon isotope data showed a consistent diet according to climate, probably due to the greater availability in glacial periods of cool-season grasses, which mainly use the C_3 photosynthetic pathway. Given their adaptations to grazing, the climate amelioration, followed by the loss of grasslands, likely had a great impact on camelid populations, leading to their extinction in southern Brazil. These results, therefore, contribute to the understanding of the dynamics of paleocommunities in this region.

Introduction

During the Pliocene, the rise of the Isthmus of Panama connected North and South American lands, triggering the major episode of trans-isthmian mammalian exchanges, an event

called the great American biotic interchange (Webb 2006; Woodburne 2010). At that time, the camelids (Mammalia: Camelidae) entered the southern continent and, by the Pleistocene, this group was widely distributed and diversified (Scherer 2013). South American camelids comprise the subfamily Camelinae, tribe Lamini, which diverged from the tribe Camelini during the Miocene, about 16 Ma, in North America (Lynch et al. 2020). South American camelid fossils have been recovered from fossiliferous deposits from Venezuela to southern Chile (see Rocha-dos-Santos et al. 2017). According to the fossil record of southern Brazil, three species of camelid Lamini inhabited this region in the recent past: *Hemiauchenia paradoxa* Gervais and Ameghino 1880, *Lama guanicoe* Müller 1776, and *Vicugna vicugna* Molina 1782 (Scherer 2009, 2013; Rocha-dos-Santos et al. 2017). While *H. paradoxa* is currently extinct, *L. guanicoe* and *V. vicugna* still live in some regions of South America – from the northern slope of the Andes to the island of Tierra del Fuego in the south (Baldi et al. 2016; Acebes et al. 2018; see also Fig. 1), and are popularly known as guanaco and vicuña, respectively. Given this scenario, Lopes et al. (2020) classified the two last taxa as pseudo-extinct. Following the same authors, the climatic changes of the Pleistocene/Holocene transition guided such extinctions in the southern Brazilian Pampa.

The Pleistocene climate was marked by several glaciation events that favored the development of grasslands in southern Brazil due to the colder and drier conditions (Behling, 2002). However, from ca. 18 ka onward, the last deglaciation process began (Cruz et al. 2006; Denton et al. 2010), increasing temperatures and humidity since mid-Holocene times (Behling et al. 2004, 2005; Medeanic and Corrêa 2010). Climate amelioration led to changes in vegetation composition: a progressively decrease of grassland pollen types, following an increase in forest taxa (Cordeiro and Lorscheitter 1994; Lorscheitter and Dillenburg 1998; Behling et al. 2004, 2005; Macedo et al. 2007; Masetto and Lorscheitter 2019; Roth et al. 2021). With the loss of grassland habitats, the extant Lamini became restricted to the Andean region, where cold and temperate weather is still predominant (González et al. 2006; Scherer 2013). Guanacos and vicuñas show ecophysiological adaptations to harsh environments that allowed them to survive and colonize the remaining open habitats of South America after the megafauna extinction of the Pleistocene/Holocene transition (González et al. 2006).

Pleistocene colder and drier conditions likely supported more heterogeneous vegetation, which allowed the co-occurrence of several herbivorous including closely related taxa (e.g., Yann 2014). Forage availability and quality are related to population dynamics, spatial distribution, and feeding habits of South American wild camelids (González et al. 2006; Shaw

et al. 2012; Rivals et al. 2013). Thus, assessing the paleoenvironment and the paleodiet of Pleistocene camelids can give us insights into the factors involved in the extinctions and pseudo-extinctions in southern Brazil. In addition, determining the diet of extinct animals contributes to the understanding of the function and structure of the south Brazilian paleocommunity, such as resource partitioning and competition between co-occurrent species (e.g., Feranec 2003).

The ecology of extinct species is typically analyzed through their dental morphologies and tooth wear (i.e., microwear and mesowear) (e.g., Hayek et al. 1992; Kaiser et al. 2000; Rivals and Semprebon 2011; Saarinen and Lister 2016). However, the specialized morphology observed in some herbivores does not always indicate a specialized diet (Bocherens and Drucker 2013). An alternative method for studying such species is stable isotope analysis (see Clementz 2012), because it allows for more accurate reconstructions of the actual diet of fossil mammals and, consequently, of the environments they inhabited (Bocherens and Drucker 2013). This analysis is based on the premise that the organism's isotopic composition changes in predictable ways, following the biogeochemical processes of the trophic web (Peterson and Fry 1987). In paleontological studies, the most common isotopes are carbon and oxygen, which can be measured in the inorganic fraction (biological apatite) of skeletal remains (e.g., Yann et al. 2016; Ma et al. 2019; Domingo et al. 2020; Asevedo et al. 2021). The isotopic ratios of oxygen ($\delta^{18}O$) in inert tissues are mainly related to water ingested by drinking and eating (Luz et al. 1984; Kohn 1996). Thus, the oxygen isotope composition of mammalian herbivores is strongly correlated with local meteoric water compositions and average humidity (Kohn 1996). The isotopic ratios of carbon ($\delta^{13}C$), in turn, are derived from the diet, which implies that the $\delta^{13}C$ values of herbivores reflect the isotopic ratios of the consumed plants (plus predictable changes – called isotopic enrichment), being considerably different between producers with physiological differences (e.g., plants with C_3 and C_4 photosynthetic pathways) (Kelly 2000).

Therefore, stable isotope analysis can provide proxies for habitat and diet information, regardless of morphological characters (Clementz 2012). Using this valuable tool, we assessed the diet of South American camelids from southern Brazil with insights into the paleoenvironment of this region.

Materials and methods

Samples

Teeth and bones of Lamini camelids were obtained from the paleovertebrate collection of the Museu de Ciências Naturais (Secretaria do Meio Ambiente e Infraestrutura – SEMA) (MCN–PV), located in Porto Alegre, RS, Brazil.

Because enamel is less prone to diagenetic alteration (Hedges et al. 2004; Clementz 2012; Bocherens and Drucker 2013), sampling focused on this tissue. However, when enamel was not available or to avoid damage to fossil specimens, bone or dentin was sampled (see Table 1).

Table 1 Sample information (collection site and sampled tissue) and stable isotope results of Lamini camelids: *Hemiauchenia paradoxa*, *Lama guanicoe*, and *Vicugna vicugna*. VPDB, Vienna Pee Dee Belemnite; VSMOW, Vienna Standard Mean Ocean Water.

Sample ID	Specie	Tissue	Site	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VSMOW}$
MCN-PV-6755	*H. paradoxa*	Enamel	Santa Vitória Formation	-7.9	29.5
MCN-PV-7276	*H. paradoxa*	Enamel	Santa Vitória Formation	-9.7	31.8
MCN-PV-36964	*H. paradoxa*	Enamel	Santa Vitória Formation	-10.6	31.1
MCN-PV-2159	*H. paradoxa*	Enamel	Touro Passo Formation	-7.7	30.3
MCN-PV-6947	*H. paradoxa*	Enamel	Touro Passo Formation	-7.4	31.4
MCN-PV-1474B	*H. paradoxa*	Dentin	Touro Passo Formation	-9.3	27.9
MCN-PV-1465	*H. paradoxa*	Bone	Touro Passo Formation	-8.5	25.0
MCN-PV-6741	*L. guanicoe*	Enamel	Santa Vitória Formation	-11.0	30.6
MCN-PV-6770	*L. guanicoe*	Enamel	Santa Vitória Formation	-10.1	29.1
MCN-PV-6794	*L. guanicoe*	Enamel	Santa Vitória Formation	-10.8	31.2
MCN-PV-36965	*L. guanicoe*	Enamel	Santa Vitória Formation	-11.5	29.6
MCN-PV-36966	*L. guanicoe*	Enamel	Santa Vitória Formation	-4.7	31.7
MCN-PV-6948	*L. guanicoe*	Bone	Touro Passo Formation	-8.4	27.5
MCN-PV-504	*V. vicugna*	Enamel	Santa Vitória Formation	-6.4	28.9
MCN-PV-6790	*V. vicugna*	Enamel	Santa Vitória Formation	-7.2	30.1
MCN-PV-7281	*V. vicugna*	Enamel	Santa Vitória Formation	-7.6	30.3
MCN-PV-2094	*V. vicugna*	Enamel	Touro Passo Formation	-6.2	31.4

Setting and geological context

The specimens analyzed in the present study are from two geological localities, both situated in southern Brazil: Touro Passo and Santa Vitória Formations (Table 1, Fig. 1).

Touro Passo Formation is located in the western region of Rio Grande do Sul, near the borders of Uruguay and Argentina (Fig. 1). It comprises sedimentary deposits generated on the Serra Geral Plateau of the Paraná Basin (Da-Rosa 2009). Fossils were recovered from the homonymous creek (i.e., Touro Passo Creek, 29º42’08’’S, 56º45’16’’W), a tributary of the

Uruguay River, which exposed late Pleistocene lithologies (conglomeratic facies overlapped by silt and sand levels) along its margins (Kerber et al. 2014). Thermoluminescence dating of sediments indicates a deposition age of 42 up to 6.4 ka (Da-Rosa 2009). Most vertebrate remains accumulated during the same period. According to electron spin resonance dating, fossils from Ponte Velha I locality, Touro Passo Creek, have ages between 42.6 and 10 ka and are therefore of the last glacial maximum. (Kerber et al. 2011).

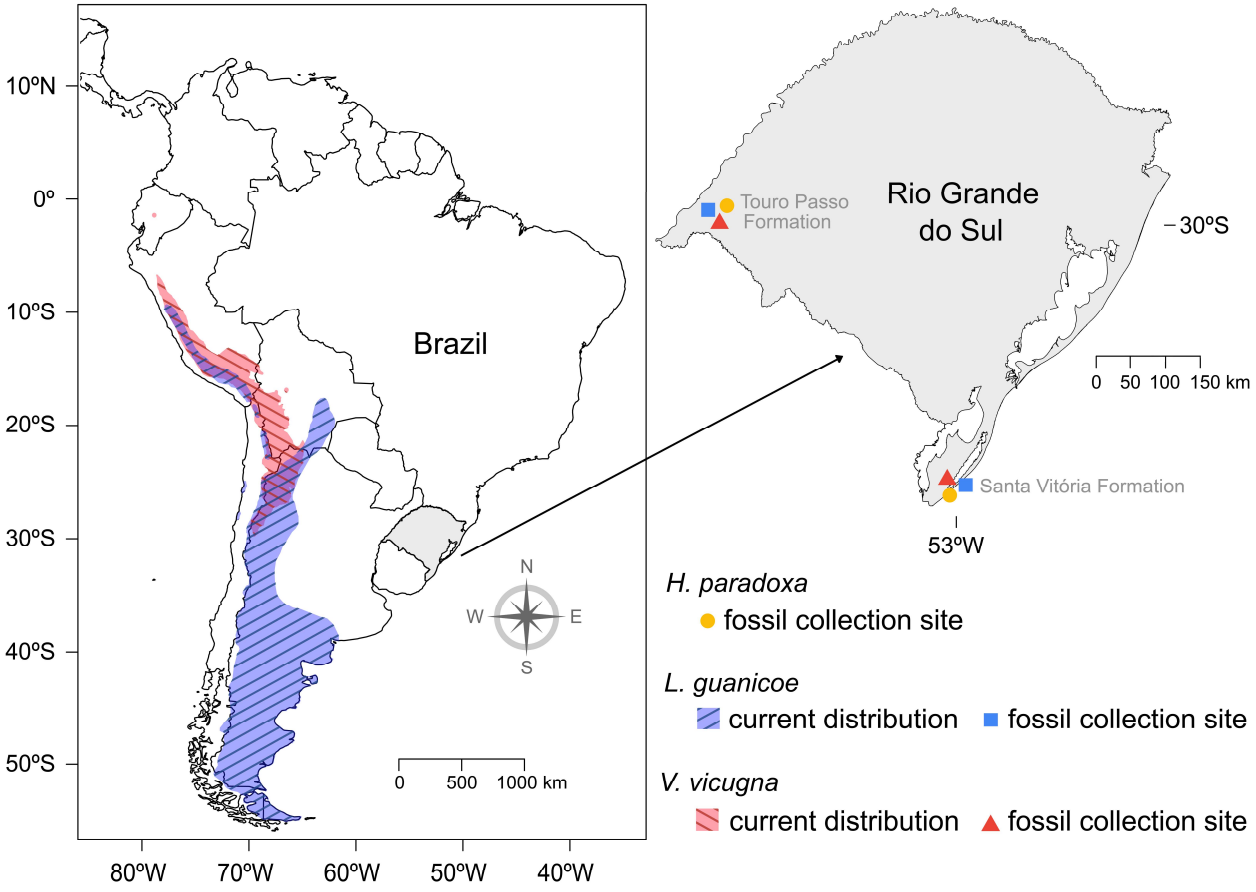


Fig. 1 Map showing the current distribution of the two endemic wild camelids of South America (*Lama guanicoe* and *Vicugna vicugna*; dashed area) (Baldi et al. 2016; Acebes et al. 2018) and the fossil collection sites (*L. guanicoe*, *V. vicugna*, and *Hemiauchenia paradoxa*) in the Rio Grande do Sul, southern Brazil.

Fossils were also recovered from Hermenegildo Beach (33º40'00''S, 53º15'35''W), typically found ex situ, after erosion of the submerged fossiliferous deposits by storm waves (Buchmann 2002). Thus, they lack any precise stratigraphic context. However, it is supposed that specimens recovered on the beach are from the same stratigraphic provenance as those found in situ in a near creek (Chuí Creek) – Santa Vitória Formation (Soliani 1973), as they have similar faunas. Santa Vitória Formation is located in the southern coastal plain, on the emerged portion of the Pelotas Sedimentary Basin (Fig. 1). Its formation is related to fluvial

and eolian deposits from the Middle-Upper Pleistocene (Lopes et al. 2021). Pleistocene sediments are mainly composed of quartz sediments as well as silty-clay sediments (Buchmann et al. 2009). As a result of rework and deposition guided by sea-level fluctuations, vertebrate fossils span a large temporal window. Submerged terrestrial remains are associated with glacial periods, when the continental shelf was exposed, and have ages between 700 and 18 ka, as revealed by electron spin resonance dating (Lopes et al. 2010).

The current climate of the study areas is warm temperate (Cfb) and subtropical (Cfa), according to Köppen climate zones, without a pronounced dry season. The Touro Passo Formation region's average summer and winter temperatures are 24ºC and 14ºC, respectively, while mean summer temperatures are below 24ºC and winter temperatures vary around 12ºC along the coast (Wrege et al. 2012). During the Pleistocene glacial periods, however, the climate was drier and temperatures were 5ºC–7ºC colder than today (Behling 2002), probably as a result of more frequent, intense, and persistent Antarctic cold fronts over the Southern Hemisphere (Behling 2002; Cruz et al. 2006).

Both Touro Passo and Santa Vitória Formations are currently located in the Pampa biome, with the landscape dominated by grasslands similar to those found in Uruguay and Argentina (Behling 2002; Overbeck et al. 2015). Despite the greater contribution of the grasslands, this region is quite heterogeneous concerning its plant physiognomies, with the presence of forest formations, although they do not dominate the landscape (Overbeck et al. 2015). Conforming to the climatic characteristics of Rio Grande do Sul (e.g., temperature and rainfall), models predict that the natural vegetation of this region should be composed of forests, rather than grasslands (Buriol et al. 2007). However, due to the Quaternary's history of changes, vegetation is likely still developing toward forest environments. Both forest and grassland environments of southern Brazil are strongly influenced by human activity for agriculture and livestock and, consequently, only small areas of natural vegetation remain (Behling 2002).

Several botanical families are found in the Pampa biome, such as Poaceae, Asteraceae, Fabaceae, Cyperaceae, and Verbenaceae, among others (Boldrini et al. 2015). The proportion of C_3 and C_4 grasses varies according to the climate. Toward the south, where the climate becomes temperate, there is an increase in the number of C_3 grasses, whereas from east to west, there is a greater proportion of C_4 grasses, due to higher temperatures (Overbeck et al. 2015). Grasslands were also the main biome in southern Brazil during the last glacial maximum, including in regions where different forest biomes exist today (Behling 2002).

Stable isotope analysis

Fossil bone, dentin and enamel were sampled using a small drill and then pretreated according to Bocherens et al. (1996), with some modifications:

First, to remove any residual organic matter, the powder was soaked in a 2.5% NaOCl solution for 24 h at room temperature and rinsed with distilled water. To remove the exogenous carbonate, a 0.1 M acetic acid solution was added to the samples. After 24 h at room temperature, the samples were rinsed again with distilled water and dried in an oven at 60ºC for 48 h. Approximately 20 mg of the resulting powder from each sample of bone, dentin, and enamel was packed into small tubes for further isotopic analysis at the University of Georgia, Center for Applied Isotope Studies (UGA–CAIS, Athens, GA, USA). Carbon and oxygen isotopic compositions were measured through a Thermo Gas Bench coupled to a Delta V Isotope Ratio Mass Spectrometer with a GC Pal autosampler (GasBench–IRMS). For this, the solid carbonate was converted to CO_2 gas by dissolution with phosphoric acid in a helium atmosphere.

The resulting isotopic ratio (R) of each element ($^{13}C/^{12}C$ and $^{18}O/^{16}O$) in the samples was reported in delta notation (δ) of the element in its heavy form (X) in parts per thousand (‰) relative to the international standard Vienna Pee Dee Belemnite (VPDB), as expressed by the following equation: $\delta X = [(R_{SAMPLE} / R_{STANDARD}) - 1]$. Internal laboratory control standards used for calibration were Fisher ($\delta^{13}C$ = -0.64‰; $\delta^{18}O$ = -14.90‰) and A1296 ($\delta^{13}C$ = 2.56‰; $\delta^{18}O$ = -0.60‰). The analytical uncertainty (σ) of the 12 repeated measurements of the standards was <0.02‰ for $\delta^{13}C$ and <0.10 for $\delta^{18}O$.

Data analysis and interpretation

The diet of the Lamini camelids that inhabited the southern Brazilian Pampa during the Pleistocene was reconstructed based on carbon isotope ratios. Such assessment is possible due to the carbon isotope fractionation during the photosynthetic process. For example, C_3 plants fix CO_2 through the action of the enzyme ribulose 1,5-biphosphate carboxylase, which discriminates against the heavy isotope, ^{13}C; whereas, in the C_4 photosynthetic pathway, the CO_2 is initially carboxylated in organic acids by the enzyme phosphoenolpyruvate carboxylase, with fractionation poorly expressed (O'Leary 1988). Because the biomass productivity of crassulacean acid metabolism plants is low at the community level and they are mostly present

in stressful environments like deserts (Lüttge 2004), it was assumed that they did not represent an important food source, and therefore this specific photosynthetic pathway was not addressed here.

Because carbonate bioapatite is derived from blood bicarbonate (Sullivan and Krueger 1981; Gannes et al. 1998), there is no significant fractionation between bone, dentin, and enamel (Tejada-Lara et al. 2018). Thus, correction factors were not applied to account for differences between tissue types.

The isotopic enrichment (ε*) between carbonate bioapatite of ungulate mammals and their diet is about 14‰ (Cerling and Harris 1999); however, enrichment is not fixed, because it depends on the digestive physiology of herbivorous species (Tejada-Lara et al. 2018). For a more accurate ε* value, the formula proposed by Tejada-Lara et al. (2018) for foregut fermenters was applied: ε* = 2.34 + 0.05 (BM), where BM is the natural logarithm of body mass in kilograms. For extant species, the body mass of living populations was adopted. Weight estimations for *Lama guanicoe* range from 80 to 120 kg (González et al. 2006 and references therein), therefore a conservative body mass value of 100 was considered here, whereas for a smaller camelid, *Vicugna vicugna* (weighing between 20 and 50 kg; Ccora et al. 2019), a value of 40 was considered. The estimated weight reported in the literature for the extinct *Hemiauchenia paradoxa* is 1000 kg (Prevosti and Vizcaíno 2006). Thus, after calculation the natural logarithm, ε* were estimated to be 13.1‰, 12.5‰ and 14.7‰ for *L. guanicoe*, *V. vicugna* and *H. paradoxa*, respectively.

As a consequence of differences in carbon isotope fractionation that occurs during photosynthesis, the mean $\delta^{13}C$ values of C_3 plants is -27.1 ± 2.0‰ and that of C_4 plants is -13.1 ± 1.2‰ (O'Leary 1988). However, these isotopic ratios were determined for modern plants, and therefore carbon isotope ratios must be corrected for the Suess effect (i.e., atmospheric ^{14}C decreases due to the burning of fossil fuels after the Industrial Revolution; Baxter and Walton 1970). For this, a correction value of 1.3‰ was applied to the values of modern plants, following the calculation proposed by Dombrosky (2020).

The resulting $\delta^{13}C$ values of C_3 and C_4 plants (-25.8 ± 2.0‰ and -11.8 ± 1.2‰, respectively), the estimated ε*, and $\delta^{13}C$ values of the consumers were then applied to *Stable Isotope Mixing Models in R* (simmr package; Parnell and Inger 2016) to estimate the contribution of C_3 and C_4 plants to the diet of the Lamini camelids. By definition, simmr is a Bayesian mixing model incorporating a Markov chain Monte Carlo method that uses the isotopic data to calculate the probability of contribution from potential sources to a mixture.

Bioapatite $\delta^{18}O$ values of herbivores with different behaviors and physiologies track distinct aspects of the environment. Herbivores that supply their water requirements by forage reflect changes in aridity, whereas those that are obligated drinkers trace local meteoric water (Levin et al. 2006).

Different molecular species (isotopologues) of H_2O have distinct vapor pressures that are proportional to their masses (Ehleringer and Rundel 1989). Consequently, water molecules composed of light isotopes are more prone to evaporation, so more positive $\delta^{18}O$ in meteoric waters indicate warmer or drier climates, whereas more negative $\delta^{18}O$ can represent colder or wetter climates (Bocherens and Drucker 2013). Conversely, when herbivores supply their water requirements by food, the interpretation of $\delta^{18}O$ values must take into account the fractionations that occur in plant organs and tissues. Although there is no fractionation during the passage of water through root membranes, leaf transpiration can result in enrichment in ^{18}O isotopes according to environmental conditions (e.g., atmospheric humidity and air temperature) (Dawson et al. 2002; Barbour 2007; Simonin et al. 2013). Hence, differences in $\delta^{18}O$ values indicate climate variability within a local population.

All oxygen isotopic ratios were converted from VPDB to Vienna Standard Mean Ocean Water (VSMOW) according to the equation: $\delta_{VSMOW} = 1.03086 \times \delta_{VPDB} + 30.86$ (Friedman and O'Neil 1977) before analysis.

Differences in carbon and oxygen isotope ratios between *H. paradoxa*, *L. guanicoe* and *V. vicugna* were assessed through parametric analysis of variance (ANOVA), as the data followed a normal distribution according to Shapiro–Wilk test. ANOVA was also applied to compare stable isotopic ratios of specimens from distinct collection sites. Unfortunately, this last analysis was only suitable for *H. paradoxa*, since it had a minimum of three observations per site, unlike *L. guanicoe* and *V. vicugna* (see Table 1). Finally, to verify whether the diet of South American camelids changes according to the climate, a linear regression was applied using $\delta^{13}C$ and $\delta^{18}O$ values.

All analyses were performed in the R statistical environment version 4.0.3 (R Core Team 2020). The significance level for two-sided tests was $\alpha = 0.05$.

Results

A total of 17 specimens were analyzed in the present study: 7 *Hemiauchenia paradoxa*, 6 *Lama guanicoe*, and 4 *Vicugna vicugna* (Table 1). $\delta^{13}C_{VPDB}$ values ranged from -11.5‰ to

-4.7‰, with mean and standard deviation (±SD) of -8.5 ± 1.9‰, while $\delta^{18}O_{VSMOW}$ values varied from 25.0‰ to 31.8‰ (mean 29.8 ± 1.8‰). Although the variation in $\delta^{18}O$ between individuals was high, the mean values were quite similar among the species analyzed (*H. paradoxa*, $\delta^{18}O$ = 29.6 ± 2.4‰; *L. guanicoe*, $\delta^{18}O$ = 29.9 ± 2.5‰; *V. vicugna*, $\delta^{18}O$ = 30.2 ± 1.0‰), whereas the mean carbon isotopic ratios varied broadly. *V. vicugna* showed higher mean values of $\delta^{13}C$ ($\delta^{13}C$ = -6.8 ± 0.6‰), compared with *H. paradoxa* ($\delta^{13}C$ = -8.7 ± 1.2‰) and *L. guanicoe* ($\delta^{13}C$ = -9.4 ± 2.5‰). Differences among species were nonsignificant in both $\delta^{13}C$ (p-value = 0.10) and $\delta^{18}O$ (p = 0.86) values. Isotopic ratios of *H. paradoxa* also indicated no differences between the collection sites (i.e., Touro Passo and Santa Vitória Formations) in $\delta^{13}C$ (p = 0.28) and $\delta^{18}O$ values (p = 0.28).

Considering the distinct isotopic enrichment (ε*) of camelids, the $\delta^{13}C$ values of each species instead of those of C_3 and C_4 plants were corrected for graphical representation and are shown in Figure 2. According to the scatter plot, specimens of *H. paradoxa* and *L. guanicoe* (except for one individual) are isotopically similar to C_3 plants. Specimens of *V. vicugna*, in contrast, are lying on the boundary between C_3 and C_4 plants, which points to a mixed diet. Mixing models indicated the same pattern. *H. paradoxa* from southern Brazil had a diet comprising mostly C_3 vegetation (>80% of the diet, credibility interval [CI]: 70%–90%), with a minimal contribution of C_4 vegetation (20%, CI: 8%–30%) (Fig. 3A). Similarly, the proportional contribution of C_3 plants to the diet of *L. guanicoe* was higher (~75%, CI: 55%–90%) than that of C_4 plants (24%, CI: 8%–45%) (Fig. 3B). Conversely, *V. vicugna* had a mixed diet; C_3 and C_4 vegetation comprise about 54 (CI: 40%–70%) and 46% (CI: 30%–60%) of its diet, respectively (Fig. 3C). No significant relationship was found between climate (represented by $\delta^{18}O$ values) and diet ($\delta^{13}C$ values) of the Lamini camelids (*H. paradoxa*, r^2 = 0.02, p = 0.78; *L. guanicoe*, r^2 = 0.05, p = 0.68; *V. vicugna*, r^2 = 0.004, p = 0.94) (see also Fig. 2).

Discussion

Isotopic analyzes of bone, dentin, and enamel bioapatite revealed the diet of camelid populations that inhabited southern Brazil during the Pleistocene. *Hemiauchenia paradoxa* and *Lama guanicoe* had diets predominantly composed of C_3 vegetation, whereas *Vicugna vicugna* had a mixed diet, as it consumed C_3 and C_4 plants in very similar proportions.

Studies with modern guanaco and vicuña have shown that the diet of these species comprises a wide range of plant resources, especially grass species, depending on the seasonal

and spatial availability of primary producers (Puig et al. 1996, 1997; Torres and Puig 2010; Puig et al. 2011; Rivals et al. 2013; Castellaro et al. 2020). Hence, based on their modern diet, it is possible to infer that the results found in the present study reflect the consumption of grasses rather than shrubs or trees. In addition, pollen analyses indicate a predominance of grassland environments in southern Brazil during glacial periods (Behling et al. 2005; Roth et al. 2021), which corroborates the high consumption of grasses by the Lamini camelids.

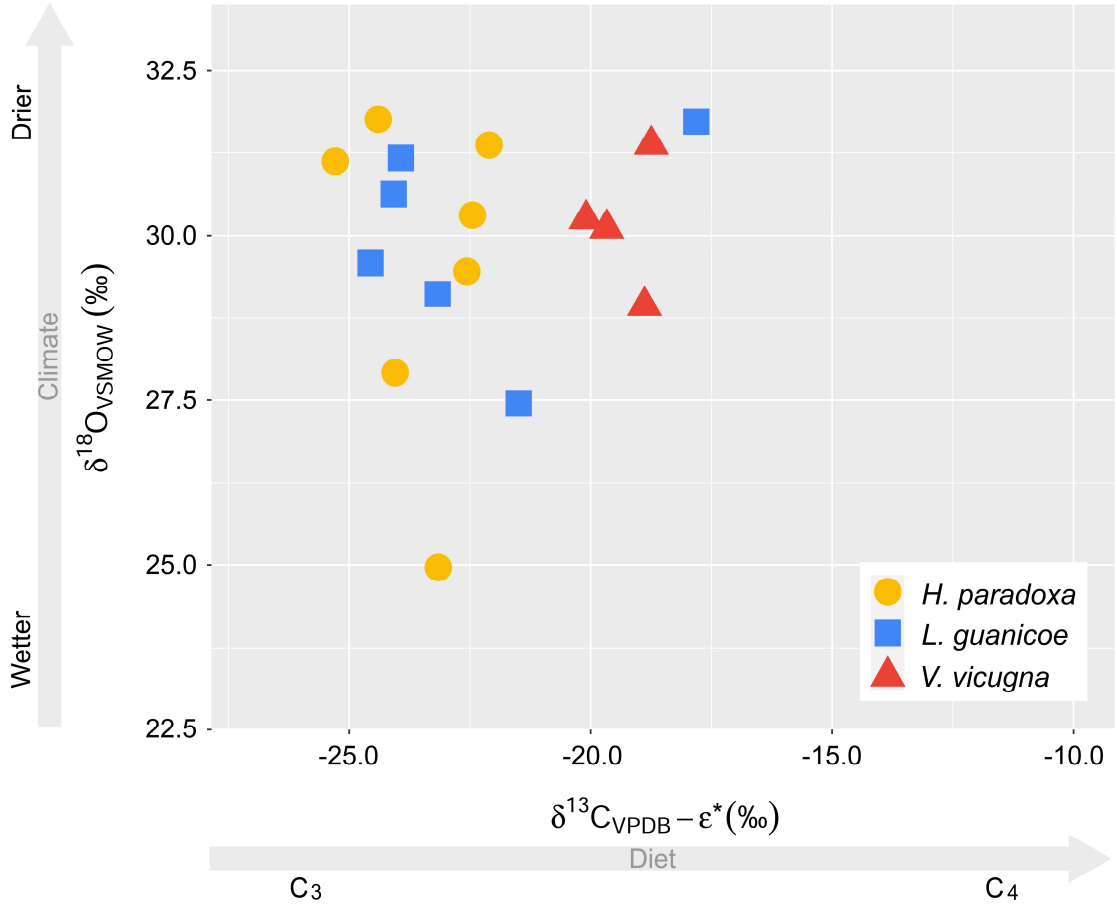


Fig. 2 Scatter plot of $\delta^{13}C$ and $\delta^{18}O$ values for the specimens of *Hemiauchenia paradoxa*, *Lama guanicoe*, and *Vicugna vicugna* from the Pleistocene of southern Brazil. Note that the carbon isotope data are corrected for trophic enrichment. VPDB, Vienna Pee Dee Belemnite; VSMOW, Vienna Standard Mean Ocean Water.

Similar to modern studies, paleontological and archaeological studies have reported high dietary plasticity, in which populations oscillate between C_3 to C_4 feeders (MacFadden and Schockey 1997; Feranec 2003; Gil et al. 2016; Yann et al. 2016; González-Guarda et al. 2017; Domingo et al. 2020; Gayo et al. 2020; Szpak and Valenzuela 2020). Given their opportunistic behavior, the feeding habits of southern Brazilian camelids may have been the result of forage availability, ecological opportunity, and intra- and interspecific competition, as discussed in the following paragraphs.

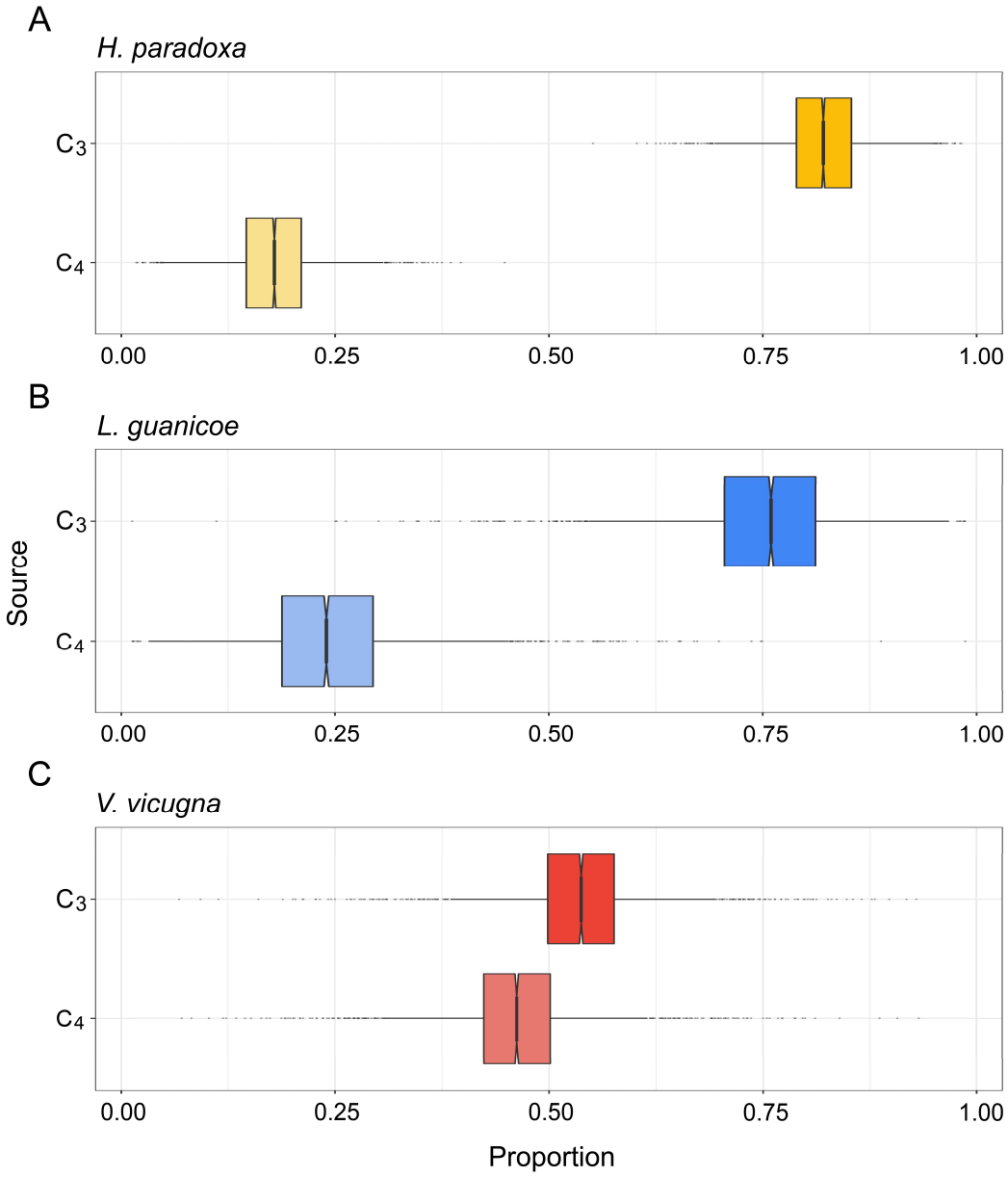


Fig. 3 Dietary contribution of C3 and C4 plants to the diet of *Hemiauchenia paradoxa* (A), *Lama guanicoe* (B), and *Vicugna vicugna* (C) according to simmr Bayesian models.

Variations in camelid diet are mainly linked to climate (Gil et al. 2016). Climatic conditions are responsible for the distribution of grass species and characterize their spatialization at a regional scale (Moreira et al. 2019; Preston and Fjellheim 2020). Due to the greater consumption of C_3 grasses compared with C_4 grasses, the former likely occurred in higher abundance in southern Brazil during the Pleistocene. Grasses that use the C_4 photosynthetic pathway require warm growing-season temperatures and, their growth is therefore limited by low temperatures and the occurrence of frost during winter; whereas C_3 photosynthesis occurs in cool-season grasses (Boldrini et al. 2015; Moreira et al. 2019). So, the

low temperatures of glacial periods may have favored the development of cool-season grasses over tropical grasses. Indeed, grasses that dominate temperate regions are from the subfamily Pooideae (family Poaceae) (Preston and Fjellheim 2020), all of which are C_3 photosynthesizers (Longhi-Wagner 2012). Pollen records from the coastal plain of northern Rio Grande do Sul state (municipality of Torres: sedimentary profiles dated from 24 ka onward) (Roth et al. 2021) and western Rio Grande do Sul (municipality of São Francisco de Assis: 22 ka to present) (Behling et al. 2005), corroborate the predominance of Poaceae grasses during the late Quaternary. Nonetheless, taxonomic identification of Poaceae grasses was not provided beyond the family level in both Roth et al. (2021) and Behling et al. (2005), due to pollen morphological similarities among members of this family. The comparable isotopic composition of specimens of Touro Passo (continental fluvial) and Santa Vitória (coastal plain) Formations also indicate similarities in the floristic composition of these two localities, with a predominance of grasslands during glacial periods, as stated by Bauermann et al. (2009).

Besides ecological differences, C_3 and C_4 grasses differ in their nutritional quality. Generally, C_4 grasses have lower nitrogen and higher cell wall concentrations than their C_3 counterparts (Ehleringer and Monson 1993). The South American camelids are foregut ferments with a great capacity to digest low-quality forages (López et al. 1998). They are even able to digest C_3 and C_4 plants more efficiently than hindgut fermenters (Sponheimer et al. 2003). Among them, the vicuña shows the highest degree of specialization for grazing, mainly evidenced by the adaptation of its lower incisors (Menegaz et al. 1989), which allowed this species to include large proportions of C_4 grasses in its diet, avoiding competition with other sympatric camelids. Contemporary vicuñas and guanacos also differ concerning food preferences, despite their generally similar diet (Cassini et al. 2009). For instance, in western Argentina, where populations of these species occur in sympatry, both guanacos and vicuñas consume several plant species in common; however, the guanaco's diet comprises a greater diversity of plant species and, consequently, more generalist feeding habits (Cajal 1989). Thus, different foraging behaviors may have favored niche partitioning and the coexistence of species with similar ecologies, as observed in paleocommunities worldwide (e.g., DeSantis et al. 2009; Ma et al. 2019).

A factor that contributes to niche partitioning between phylogenetically similar taxa is the different habitat use. Direct encounters between living populations of guanacos and vicuñas result in agonistic behavior which in turn leads to differences in habitat use (Cajal 1989). Unfortunately, such behaviors are almost impossible to assess in nonliving populations.

In summary, the results of this study likely reflect the opportunistic behavior of South American camelids. The higher nutritional quality of C_3 grasses, associated with their greater availability in the environment, may explain their high consumption by *H. paradoxa* and *L. guanicoe* during the Pleistocene of southern Brazil. Even so, among the guanaco specimens, one individual had an isotopic signature indicating a mixed-feed diet, probably as a result of individual variation in its foraging ecology to minimize intraspecific competition (Bolnick et al. 2003). Other studies also reported generalist feeding habits in guanaco populations, with some individuals differing from the general population (e.g., Gil et al. 2016) but further efforts are needed to fully understand past ecological dynamics.

The isotopic ratios of oxygen can provide information about paleoclimate, but such results should be interpreted with caution. Despite their strong phylogenetic proximity (Lynch et al. 2020), South American camelids are known to have some differences in their behavioral ecology (see Franklin 1983). For example, vicuñas are obligate water drinkers, whereas guanacos supply their water requirements by forage and are periodic water drinkers (Franklin 1983). The physiology and behavior of the extinct *H. paradoxa* are difficult to assess, nevertheless, by comparing the oxygen isotope ratios of proboscideans (obligated drinkers) and camelids of this genus, Yann et al. (2013) suggested that *Hemiauchenia* was more sensitive to $\delta^{18}O$ variations in plant leaves that they ate. These contrasting behaviors can lead to differences in the oxygen isotope ratios recorded in these species' tissues (Kohn 1996; Levin et al. 2006). In addition, C_4 feeders tend to have higher $\delta^{18}O$ values than C_3 feeders, following the isotopic composition of the consumed plants (Kohn 1996). Although South American camelids show different behaviors regarding water drinking and diet, significant differences in $\delta^{18}O$ values were not detected between guanacos, vicuñas, and the extinct *H. paradoxa*. One possible explanation is that both meteoric waters and the ingested plants had similar oxygen isotope compositions. The isotopic composition of plant leaf tissues is strongly influenced by leaf transpiration, which can vary with environmental conditions (Dawson et al. 2002; Barbour 2007; Simonin et al. 2013). So, glacial conditions likely provided slightly evaporative environments, leading to a weak fractionation in leaf water compared to source water.

According to oxygen and carbon isotope ratios from specimens analyzed in this study, climate does not influence the diet of the Lamini camelids. It is important to emphasize, however, that all samples were from glacial periods and, therefore, there may not have been substantial changes in climate and vegetation during this period to affect the feeding habits of these animals. On the other hand, during interglacial periods of global warming, cool-season

grasses likely became restricted to montane areas, such as the South American Andes (Preston and Fjellheim 2020). Likewise, the distribution of the South American camelids followed the distribution of these grasses, as they are specialized grazers (Menegaz et al. 1989).

Grazing is an important factor in maintaining the Pampa biome in southern Brazil, as stated by Overbeck et al. (2015). Intense grazing pressure favors the development of vegetation composed of grasses and increases the diversity of low-stratum species. In contrast, when the grazing pressure is low or absent, shrubs and tree species develop, reducing the diversity of smaller species. Therefore, the extinction of the Brazilian pampa megafauna may have led to strong changes in the landscape through reduced grazing pressure. Camelids also play other ecological roles in grasslands, as their excrement and urine increase soil fertility, which contributes to higher plant diversity and forage production (Franklin 1983).

Finally, it is important to recognize the possible limitations associated with stable isotope analysis in fossilized tissues. To date, there are no methods available to assess the preservation of carbon isotopic ratios in bioapatite (Domingo et al. 2020), and to test the preservation of oxygen isotopic ratios it is necessary to analyze both phosphate and carbonate compositions of bioapatite (Prado et al. 2011), which requires a greater quantity of samples. However, as noted by Domingo et al. (2020), a consistency in isotopic ratios among Pleistocene taxa suggests that they were derived from animal paleobiology rather than diagenesis. In addition, Demény et al. (2019) suggested that only $\delta^{18}O$ values of enamel should be included in past climate interpretations, due to large variations in OH related to $\delta^{18}O$ of bone. Despite this caveat, $\delta^{18}O$ values of bones were similar to those of enamel. Thus, we assume that bioapatite from bone samples is also reflecting natural changes in oxygen isotopic ratios. Furthermore, all bioapatite $\delta^{18}O$ values were obtained under the same analytical conditions, decreasing the chances of possible bias and ensuring that the values are comparable (Demény et al. 2019).

Conclusions

This was the first study to assess the paleobiology of extinct and pseudo-extinct camelids from southern Brazil. Based on stable isotope analysis, it was possible to reconstruct the diet of animals that inhabited the region during the Pleistocene. Carbon stable isotopes revealed that *Hemiauchenia paradoxa* (n = 7) fed mainly on C_3 grasses. Similar results were found for *Lama guanicoe* (n = 6), but it showed a broader diet due to one individual that

consumed C_3 and C_4 plants equally (i.e., mixed feeder). Conversely, *Vicugna vicugna* (n = 4) had a mixed diet. Distinct feeding habits may have contributed to niche partitioning and the coexistence of Lamini camelids. Combined oxygen and carbon isotope data indicated a consistent diet in glacial periods, probably marked by greater availability of C_3 grasses compared with C_4 grasses, as the former are adapted to cold weather. These results contribute to the understanding of the dynamics of paleocommunities of southern Brazil and the causes of Pleistocene/Holocene extinction. Given their adaptations to grazing, the climate amelioration followed by loss of grasslands likely had a major impact on camelid populations, leading to their extinction. For future studies, we recommend analyzing a larger number of samples to confirm the patterns reported here and also to account for individual variation.

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Literature cited

Acebes, P., Wheeler, J., Baldo, J., Tuppia, P., Lichtenstein, G., Hoces, D., and Franklin, W.L. 2018. *Vicugna vicugna* (errata version published in 2019). The IUCN Red List of Threatened Species 2018: e.T22956A145360542. Accessed 22 January, 2021. https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T22956A145360542.en

Asevedo, L., Ranzi, A., Kalliola, R., Pärssinen, M., Ruokolainen, K., Cozzuol, M.A., do Nascimento, E.R., Negri, F.R., Souza-Filho, J.P., Cherkinsky, A., and Dantas, M.A.T. 2021. Isotopic paleoecology ($\delta^{13}C$, $\delta^{18}O$) of late Quaternary herbivorous mammal assemblages from southwestern Amazon. *Quaternary Science Reviews* 251: 106700.

Baldi, R.B., Acebes, P., Cuéllar, E., Funes, M., Hoces, D., Puig, S., and Franklin, W.L. 2016. *Lama guanicoe*. The IUCN Red List of Threatened Species 2016: e.T11186A18540211.

Accessed 22 January, 2021. https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T11186A18540211.en

Barbour, M.M. 2007. Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology* 34: 83–94.

Bauermann, S.G., Behling, H., and Macedo, R.B. 2009. Biomas regionais e evolução da paisagem no Rio Grande do Sul com base em paleopalinologia. Pp. 81–94 *in* Ribeiro, A.M., Bauermann, S.G., and Scherer, C.S., eds. *Quaternário do Rio Grande do Sul: Integrando Conhecimentos*. Sociedade Brasileira de Paleontologia, Porto Alegre.

Baxter, M.S., and Walton, A. 1970. A theoretical approach to the Suess effect. *Proceedings of the Royal Society of London* 318: 213–230.

Behling, H. 2002. South and southeast Brazilian grasslands during Late Quaternary times: a synthesis. *Paleogeography, Palaeoclimatology, Palaeoecology* 177: 19–27.

Behling, H., Pillar, V. D., Orlóci, L., and Bauermann, S.G. 2004. Late Quaternary *Araucaria* forest, grassland (campos), fire and climate dynamics, studied by high-resolution pollen, charcoal and multivariate analysis of the Cambará do Sul core in southern Brazil. *Palaeogeography, Palaeoclimatology, Palaeoecology* 203: 277–297.

Behling, H., Pillar, V.D., and Bauermann, S.G. 2005. Late Quaternary grassland (campos), gallery forest, fire and climate dynamics, studied by pollen, charcoal and multivariate analysis of the São Francisco de Assis core in western Rio Grande do Sul (southern Brazil). *Review of Palaeobotany and Palynology* 133: 235–248.

Bocherens, H., and Drucker, D.G. 2013. Terrestrial teeth and bones. Pp. 304–314 *in* Elias, S.A., and Mock, C.J., eds. *Encyclopedia of Quaternary Science*, 2nd ed. Elsevier, Amsterdam.

Bocherens, H., Koch, P.L., Mariotti, A., Geraads, D., and Jaeger, J. 1996. Isotopic biogeochemistry (^{13}C, ^{18}O) of mammalian enamel from African Pleistocene hominid sites. *Palaios* 11: 306–318.

Boldrini, I.I., Overbeck, G.E., and Trevisan, R. 2015. Biodiversidade de plantas. Pp. 51–60 *in* Pillar, V.P., and Lange, O., eds. *Os Campos do Sul*. Rede Campos Sulinos – UFRGS, Porto Alegre.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., and Forister, M.L. 2003. The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist* 161: 1–28.

Buchmann, F.S.C. 2002. Bioclastos de organismos terrestres e marinhos na praia e plataforma interna do Rio Grande do Sul: natureza, distribuição, origem e significado geológico. PhD thesis, Universidade Federal do Rio Grande do Sul.

Buchmann, F.S.C., Caron, F., Lopes, R.P., Ugri, A., and de Lima, L.G. 2009. Panorama geológico da planície costeira do Rio Grande do Sul Pp. 34–56 *in* Ribeiro, A.M., Bauermann, S.G., and Scherer, C.S., eds. *Quaternário do Rio Grande do Sul: Integrando Conhecimentos*. Sociedade Brasileira de Paleontologia, Porto Alegre.

Buriol, G.A., Estefanel, V., de Chagas, A.C., and Eberhardt, D. 2007. Clima e vegetação natural do estado do Rio Grande do Sul segundo o diagrama climático de Walter e Lieth. *Ciência Florestal* 17: 91–100.

Cajal, J.L. 1989. Uso de hábitat por vicuñas y guanacos en la Reserva de Biosfera de San Guillermo. *Vida Silvestre Neotropical* 1–23.

Cassini, M., Borgnia, M., Arzamendia, Y., Benítez, V., and Vilá, B. 2009. Sociality, foraging and habitat use by vicuña. Pp. 35–48 *in* Gordon, I.J., ed. *The Vicuña*. Springer Science + Business Media.

Castellaro, G., Orellana, C., Escanilla, J., Bastías, C., Cerpa, P., and Raggi, L. 2020. Botanical composition and diet quality of the vicuñas (*Vicugna vicugna* Mol.) in highland range of Parinacota, Chile. *Animals* 10: 1205.

Ccora, E., Condori, A., Contreras, J.L., Curasma, J., Cordero, A.G., Valencia, N., Mayhua, P.H., and McGregor, B.A. 2019. Biometric characteristics in vicuñas (*Vicugna Vicugna mensalis*). *Small Ruminant Research* 175: 52–56.

Cerling, T.E., and Harris, J.M. 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia* 120: 347–363.

Clementz, M.T. 2012. New insight from old bones: stable isotope analysis of fossil mammals. *Journal of Mammalogy* 93: 368–380.

Cordeiro, S.H., and Lorscheitter, M.L. 1994. Palynology of Lagoa dos Patos sediments, Rio Grande do Sul, Brazil. *Journal of Paleolimnology* 10: 35–42.

Cruz, F.W., Jr., Burns, S.J., Karmann, I., Sharp, W.D., Vuille, M., and Ferrari, J.A. 2006. A stalagmite record of changes in atmospheric circulation and soil processes in the Brazilian subtropics during the Late Pleistocene. *Quaternary Science Reviews* 25: 2749–2761.

Da-Rosa, A.A.S. 2009. Geologia do Quaternário continental do RS: estado da arte e perspectivas de trabalho. Pp. 17–34 *in* Ribeiro, A.M., Bauermann, S.G., and Scherer,

C.S., eds. *Quaternário do Rio Grande do Sul: Integrando Conhecimentos*. Sociedade Brasileira de Paleontologia, Porto Alegre.

Dawson, T.E., Mandelli, S., Plamboeck, A.H., Templer, P.H., and Tu, K.P. 2002. Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* 33: 507–559.

Demény, A., Gugora, A.D., Kesjár, D., Lécuyer, C., and Fourel, F. 2019. Stable isotope analyses of the carbonate component of bones and teeth: the need for method standardization. *Journal of Archaeological Science* 109: 104979.

Denton, G.H., Anderson, R.F., Toggweiler, J.R., Edwards, R.L., Schaefer, J.M., and Putnam, A.E. 2010. The last glacial termination. *Science* 328: 1652–1656.

DeSantis, L.R.G., Feranec, R.S., and MacFadden, B.J. 2009. Effects of global warming on ancient mammalian communities and their environments. *PLoS ONE* 4: e5750.

Dombrosky, J. 2020. A ~1000-year ^{13}C Suess correction model for the study of past ecosystems. *The Holocene* 30: 474–478.

Domingo, L., Tomassini, R.L., Montalvo, C.I., Sanz-Pérez, D., and Alberdi, M.T. 2020. The Great American Biotic Interchange revisited: a new perspective from the stable isotope record of Argentine Pampas fossil mammals. *Scientific Reports* 10: 1608.

Ehleringer, J.R., and Rundel, P.W. 1989. Stable isotopes: history, units, and instrumentation. Pp. 1–15 *in* Rundel, P.W., Ehleringer, J.R., and Nagy, K.A., eds. *Stable Isotopes in Ecological Research*. Springer-Verlag, New York.

Ehleringer, J.R., and Monson, R.K. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* 24: 411–439.

Feranec, R.S. 2003. Stable isotopes, hypsodonty, and the paleodiet of *Hemiauchenia* (Mammalia: Camelidae): a morphological specialization creating ecological generalization. *Paleobiology* 29: 230–242.

Franklin, W.L. 1983. Contrasting socioecologies of South America's wild camelids: the vicuña and the guanaco. *Special Publications American Society of Mammalogists* 7: 573–629.

Friedman, I., and O'Neil, J.R. 1977. Compilation of stable isotope fractionation factors of geochemical interest. *Geological Survey Professional Paper* 440–KK: KK1–KK12.

Gannes, L.Z., de Rio, C.M., and Koch, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry & Physiology* 119A: 725–737.

Gayo, E.M., Martens, T., Stuart-Williams, H., Fenner, J., Santoro, C.M., Carter, C., and Cameron, J. 2020. Procurement of camelid fiber in the hyperarid Atacama Desert coast: insights from stable isotopes. *Quaternary International* 548: 71–83.

Gervais, H., and Ameghino, F. 1880. Les mammifères fossiles de l'Amérique du Sud. F. Savy, Paris, France.

Gil, A.F., Ugan, A., Otaola, C., Neme, G., Giardina, M., and Menéndez, L. 2016. Variation in camelid $\delta^{13}C$ and $\delta^{15}N$ values in relation to geography and climate: Holocene patterns and archaeological implications in central western Argentina. *Journal of Archaeological Science* 66: 7–20.

González, B.A., Palma, R.E., Zapata, B., and Marín, J.C. 2006. Taxonomic and biogeographical status of guanaco *Lama guanicoe* (Artiodactyla, Camelidae). *Mammal Review* 36: 157–178.

González-Guarda, E., Domingo, L., Tornero, C., Pino, M., Fernández, M.H., Sevilla, P., Villavicencio, N., and Agustí, J. 2017. Late Pleistocene ecological, environmental and climatic reconstruction based on megafauna stable isotopes from northwestern Chilean Patagonia. *Quaternary Science Reviews* 170: 188–202.

Hayek, L.C., Bernor, R.L., Solounias, N., and Steigerwald, P. 1992. Preliminary studies of hipparionine horse diet as measured by tooth microwear. *Annales Zoologici Fennici* 28: 187–200.

Hedges, R.E.M., Stevens, R.E., and Richards, M.P. 2004. Bone as a stable isotope archive for local climatic information. *Quaternary Science Reviews* 23: 959–965.

Kaiser, T.M., Solounias, N., Fortelius, M., Bernor, R.L., and Schrenk, F. 2000. Tooth mesowear analysis on *Hippotherium primigenium* from the Vallesian Dinotheriensande (Germany) – A blind test study. *Carolinea* 58: 103–114.

Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78: 1–27.

Kerber, L., Kinoshita, A., José, F.A., Figueiredo, A.M.G., Oliveira, É.V., and Baffa, O. 2011. Electron Spin Resonance dating of the southern Brazilian Pleistocene mammals from Touro Passo Formation, and remarks on the geochronology, fauna and palaeoenvironments. *Quaternary International* 245: 201–208.

Kerber, L., Pitana, V.G., Ribeiro, A.M., Hsiou, A.S., and Oliveira, E.V. 2014. Late Pleistocene vertebrates from Touro Passo Creek (Touro Passo Formation), southern Brazil: a review. *Revista Mexicana de Ciencias Geológicas* 31: 248–259.

Kohn, M.J. 1996. Predicting animal $\delta^{18}O$: Accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta* 60: 4811–4829.

Levin, N.E., Cerling, T.E., Passey, B.H., Harris, J.M., and Ehleringer, J.R. 2006. A stable isotope aridity index for terrestrial environments. *Proceedings of the National Academy of Sciences* 103: 11201–11205.

Longhi-Wagner, H.M. 2012. Poaceae: an overview with reference to Brazil. *Rodriguésia* 63: 89–100.

Lopes, R.P., Oliveira, L.C., Figueiredo, A.M.G., Kinoshita, A., Baffa, O., and Buchmann, F.S. 2010. ESR dating of Pleistocene mammal teeth and its implications for the biostratigraphy and geological evolution of the coastal plain, Rio Grande do Sul, southern Brazil. *Quaternary International* 212: 213–222.

Lopes, R.P., Pereira, J.C., Kerber, L., and Dillenburg, S.R. 2020. The extinction of the Pleistocene megafauna in the Pampa of southern Brazil. *Quaternary Science Reviews* 242: 106428.

Lopes, R.P., Dillenburg, S.R., Savian, J.F., and Pereira, J.C. 2021. The Santa Vitória Alloformation: an update on a Pleistocene fossil-rich unit Southern Brazil. *Brazilian Journal of Geology* 51: e2020065.

López, A., Maiztegui, J., and Cabrera, R. 1998. Voluntary intake and digestibility of forages with different nutritional quality in alpacas (*Lama pacos*). *Small Ruminant Research* 29: 295–301.

Lorscheitter, M.L., and Dillenburg, S.R. 1998. Holocene palaeoenvironments of the northern coastal plain of Rio Grande do Sul, Brazil, reconstructed from palynology of Tramandaí lagoon sediments. *Quaternary of South America and Antarctic Peninsula* 11: 73–97.

Lüttge, U. 2004. Ecophysiology of Crassulacean Acid Metabolism (CAM). *Annals of Botany* 93: 629–652.

Luz, B., Kolodny, Y., and Horowitz, M. 1984. Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental water. *Geochimica et Cosmochimica Acta* 48: 1689–1693.

Lynch, S., Sánchez-Villagra, R., and Balcarcel, A. 2020. Description of a fossil camelid from the Pleistocene of Argentina, and a cladistic analysis of the Camelinae. *Swiss Journal of Paleontology* 139: 8.

Ma, J., Wang, Y., Jin, C., Hu, Y., and Bocherens, H. 2019. Ecological flexibility and differential survival of Pleistocene *Stegodon orientalis* and *Elephas maximus* in mainland southeast

Asia revealed by stable isotopes (C, O) analysis. *Quaternary Science Reviews* 212: 33–44.

Macedo, R.B., Cancelli, R.R., Bauermann, S.G., Bordignon, S.A.L., and das Neves, P.C.P. 2007. Palinologia de níveis do Holoceno da planície costeira do Rio grande do Sul (localidade de Passinhos), Brasil. *Gaea* 3: 68–74.

MacFadden, B.J., and Shockey, B.J. 1997. Ancient feeding ecology and niche differentiation of Pleistocene mammalian herbivores from Tarija, Bolivia: morphological and isotopic evidence. *Paleobiology* 23: 77–100.

Masetto, E., and Lorscheitter, M.L. 2019. Vegetation dynamics during the last 7500 years on the extreme southern Brazilian coastal plain. *Quaternary International* 524: 48–56.

Medeanic, S., and Corrêa, I.C.S. 2010. Climatic changes in the coastal plain of Rio Grande do Sul state in the Holocene: palynomorph evidences. *Pan-American Journal of Aquatic Sciences* 5: 287–297.

Menegaz, A.N., Goin, F.J., and Jaureguizar, E.O. 1989. Analisis morfologico y morfométrico multivariado de los representantes fosiles y vivientes del genero *Lama* (Artiodactyla, Camelidae). Sus implicancias sistematicas, biogeográficas, ecologicas y biocronologicas. *Ameghiniana* 26: 153–172.

Molina, G.I. 1782. Sagio sulla Storia Naturale del Chili. Stamperia di S. Tommaso d'Aquino, Bologna, Italy.

Moreira, A., Fontana, D.C., Kuplich, T.M., and Guasselli, L.A. 2019. Phenological metrics of the grassland vegetation of Rio Grande do Sul, Brazil. *Revista Brasileira de Engenharia Agrícola e Ambiental* 12: 899–906.

Müller, P.L.S. 1776. Des Ritters C. von Linné vollständiges Natursystem nach der zwölften Lateinischen Ausgabe und nach Anleitung des holländischen houttuynischen Werks, mit einer ausfuhrlichen Erklarung ausgefertiget von P.L.S. Müller. G.N. Raspe, Nuremberg, Germany.

O'Leary, M.H. 1988. Carbon isotopes in photosynthesis. *BioScience* 38: 328–336.

Overbeck, G.E., Boldrini, I.I., do Carmo, M.R.B., Garcia, E.N., Moro, R.S., Pinto, C.E., Trevisan, R., and Zannin, A. 2015. Fisionomia dos campos. Pp. 31–42 *in* Pillar, V.P., and Lange, O., eds. *Os Campos do Sul*. Rede Campos Sulinos – UFRGS, Porto Alegre.

Parnell, A., and Inger, R. 2016. Stable isotope mixing models in R with simmr. Accessed 02 February, 2021. https://cran.r-project.org/web/packages/simmr/vignettes/simmr.html

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293–320.

Prado, J.L., Sánchez, B., and Alberdi, M.T. 2011. Ancient feeding ecology inferred from stable isotopic evidence from fossil horses in South America over the past 3 Ma. *BMC Ecology* 11: 15.

Preston, J.C., and Fjellheim, S. 2020. Understanding past, and predicting future, niche transitions based on grass flowering time variation. *Plant Physiology* 183: 822–839.

Prevosti, F.J., and Vizcaíno, S.F. 2006. Paleoecology of the large carnivore guild from the late Pleistocene of Argentina. *Acta Paleontologica Polonica* 51: 407–422.

Puig, S., Videla, F., Monge, S., and Roig, V. 1996. Seasonal variations in guanaco diet (*Lama guanicoe* Müller 1776) and food availability in northern Patagonia, Argentina. *Journal of Arid Environments* 34: 215–224.

Puig, S., Videla, F., and Cona, M.I. 1997. Diet and abundance of the guanaco (*Lama guanicoe* Müller 1776) in four habitats of northern Patagonia, Argentina. *Journal of Arid Environments* 36: 343–357.

Puig, S., Rosi, M.I., Videla, F., and Mendez, R. 2011. Summer and winter diet of the guanaco and food availability for a High Andean migratory population (Mendoza, Argentina). *Mammalian Biology* 76: 727–734.

R Core Team 2020. *R: A Language and Environment for Statistical Computing*. R Core Team, Geneva.

Rivals, F., and Semprebon, G.M. 2011. Dietary plasticity in ungulates: insight from tooth microwear analysis. *Quaternary International* 245: 279–284.

Rivals, F., Rindel, D., and Belardi, J.B. 2013. Dietary ecology of extant guanaco (*Lama guanicoe*) from southern Patagonia: seasonal leaf browsing and its archaeological implications. *Journal of Archaeological Science* 40: 2971–2980.

Rocha-dos-Santos, B.C.A., Avilla, L.S., and Scherer, C.S. 2017. The fossil Camelidae (Mammalia: Cetartiodactyla) from the Gruta do Urso cave, northern Brazil. *Quaternary International* 436: 181–191.

Roth, L., Lorscheitter, M.L., and Masetto, E. 2021. Paleoenvironments of the last 24,000 years on the extreme northern Rio Grande do Sul coastal plain, southern Brazil. *Quaternary International* 571: 117–126.

Saarinen, J., and Lister, A.M. 2016. Dental mesowear reflects local vegetation and niche separation in Pleistocene proboscideans from Britain. *Journal of Quaternary Science* 31: 799–808.

Scherer, C.S. 2009. Os Camelidae Lamini (Mammalia, Artiodactyla) do Pleistoceno da América do sul: aspectos taxonômicos e filogenéticos. PhD thesis, Universidade Federal do Rio Grande do Sul.

Scherer, C.S. 2013. The Camelidae (Mammalia, Artiodactyla) from the Quaternary of South America: cladistic and biogeographic hypotheses. *Journal of Mammalian Evolution* 20: 45–56.

Shaw, A.K., Galaz, J.L., and Marquet, P.A. 2012. Population dynamics of the vicuña (*Vicugna vicugna*): density-dependence, rainfall, and spatial distribution. *Journal of Mammalogy* 93: 658–666.

Simonin, K.A., Roddy, A.B., Link, P., Apodaca, R., Tu, K.P., Hu, J., Dawson, T.E., and Barbour, M.M. 2013. Isotopic composition of transpiration and rates of change in leaf water isotopologue storage in response to environmental variables. *Plant, Cell and Environment* 36: 2190–2206.

Society for Marine Mammalogy, 2023. List of marine mammal species and subspecies. Disponível em: https://marinemammalscience.org/science-and-publications/list-marine-mammal-species-subspecies/ (Acesso 27 de Setembro de 2023).

Soliani, E., Jr. 1973. Geologia da região de Santa Vitória do Palmar, RS, e a posição estratigráfica dos fósseis de mamíferos pleistocênicos. Dissertation, Programa de Pós-Graduação em Geociências, Universidade Federal do Rio Grande do Sul.

Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffer, L., Passey, B., Cerling, T., Dearing, D., and Ehleringer, J. 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits, and horses. *Small Ruminant Research* 48: 149–154.

Sullivan, C.H., and Krueger, H.W. 1981. Carbon isotope analysis of separate chemical phases in moderns and fossil bone. *Nature* 292: 333–335.

Szpak, P., and Valenzuela, D. 2020. Camelid husbandry in the Atacama Desert? A stable isotope study of camelid bone collagen and textiles from the Lluta and Camarones Valleys, northern Chile. *PLoS ONE* 15: e0228332.

Tejada-Lara, J.V., MacFadden, B.J., Bermudez, L., Rojas, G., Salas-Gismondi, R., and Flynn, J.J. 2018. Body mass predicts isotope enrichment in herbivorous mammals. *Proceedings of the Royal Society B* 285: 20181020.

Torres, M.E.M., and Puig, S. 2010. Seasonal diet of vicuñas in the Los Andes protected area (Salta, Argentina): are they optimal foragers? *Journal of Arid Environments* 74: 450–457.

Webb, S.D. 2006. The Great American Biotic Interchange: patterns and processes. *Annals of the Missouri Botanical Garden* 93: 245–257.

Woodburne, M.O. 2010. The Great American Biotic Interchange: dispersals, tectonics, climate, sea level and holding pens. *Journal of Mammalian Evolution* 17: 245–264.

Wrege, M.S., Steinmetz, S., Júnior, C.R., and Almeida, I.R. 2012. *Atlas Climático da Região Sul do Brasil: Estados do Paraná, Santa Catarina e Rio Grande do Sul (Segunda Edição)*. Embrapa, Brasília.

Yann, L.T. 2014. Diet and water source of Pleistocene Lamini camelids based on stable isotopes of tooth enamel: implications for North American vegetation and paleoclimate. PhD thesis, Vanderbilt University.

Yann, L.T., DeSantis, L.R.G., Haupt, R.J., Romer, J.L., Corapi, S.E., and Ettenson, D.J. 2013. The application of an oxygen isotope aridity index to terrestrial paleoenvironmental reconstructions in Pleistocene North America. *Paleobiology* 39: 576–590.

Yann, L.T., DeSantis, L.R.G., Koch, P.L., and Lundelius, E.L. 2016. Dietary ecology of Pleistocene camelids: influences of climate, environment, and sympatric taxa. *Palaeogeography, Palaeoclimatology, Palaeoecology* 461: 389–400.

CAPÍTULO 2

Paleobiology of Pleistocene large land mammals from the Brazilian Pampa

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Paleobiology of Pleistocene large land mammals from the Brazilian Pampa

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Abstract

The southern Brazilian Pleistocene fauna exhibited a rich diversity of large mammalian herbivores, which are now extinct or locally extinct. In this study, we employed stable isotope analysis to investigate the past ecology of these animals. Specifically, we examined the carbonate fraction of bones and teeth and utilized compiled carbon and oxygen isotopic ratios from previous research publications. The $\delta^{13}C$ values indicated that most specimens inhabited grassland environments, which aligns with environmental reconstructions based on pollen records of the "Campos" region. The dominant food resource for these herbivores consisted of C_3 photosynthesizers, mainly cool-season grasses. This preference can be attributed to the higher abundance and nutritional quality of cool-season grasses compared with warm-season grasses employing C_4 photosynthesis. The variability in $\delta^{18}O$ values within and between taxa may suggest a seasonal climate. Based on these findings, we conclude that the environmental changes during the Pleistocene-Holocene transition were detrimental to the survival of these large herbivores.

Introduction

Two main events guided the composition of mammalian fauna in South America during the Late Cenozoic: the great American biotic interchange (GABI; Webb, 1985), and the megafauna extinction (Fariña et al., 2013). The GABI spanned over nine million years, with its most crucial phase occurring approximately 2.5 Ma BP. This period coincided with the formation of the Panamá Isthmus and favorable climatic conditions, facilitating the faunal exchange between the Americas (Webb, 1991; Woodburne, 2010; Cione et al., 2015). The GABI primarily involved savanna-adapted animals and encompassed about 17 and 21 northern and southern families of land mammals, respectively (Webb, 2006). Prominent northern immigrants included gomphotherids, equids, camelids, and cervids, whereas toxodontids, glyptodonts, and an array of giant sloths were native to South America, collectively

representing the characteristic Pleistocene megafauna of the Pampa biome. However, this megafauna became extinct during the Pleistocene-Holocene transition due to factors that are not yet fully understood.

While some authors argue that hunting played a decisive role, others considered that climatic changes were primarily responsible for the extinction of Late Quaternary megafauna. For example, Prates and Perez (2021) proposed a connection between the spread of the South American Fishtail projectile points and the disappearance of large mammals. However, according to Hubbe et al. (2013), the available data for Brazil do not support the notion that specialized hunting was a major contributor to this extinction event. Additionally, Lopes et al. (2020) suggested that the megafauna disappeared from the Brazilian Pampa biome due to climate-induced environmental changes. A combination of factors is otherwise the most likely explanation for megafauna extinction. Given their ecological preferences for arid environments such as grasslands, the shift toward more humid and stable conditions in the Holocene could have been disadvantageous for large mammals (Bocherens et al., 2016). These climatic changes led to the establishment of a climax plant community with low protein content and high investment in defenses against herbivory (Mann et al., 2019). The large body size of mammalian herbivores, coupled with their low population densities and slow reproductive rates, became limiting factors in this new ecological context (Cione et al., 2009; Mann et al., 2019). Finally, the arrival of *Homo sapiens* in South America might have pushed populations beyond sustainable thresholds and disrupted the ecological balance of the continent, resulting in cascading extinctions (Cione et al., 2009).

To comprehend the patterns of this extinction and the dynamics of the species involved, regional paleoecological and paleoclimatic studies are crucial (Barnosky et al., 2004). Such studies not only help in assessing potential extinction causes but also shed light on the impacts of changing fauna on South American ecosystems. The extinction of large vertebrates triggers a cascade of ecological consequences, including changes in the plant community that alter the landscape, and disruptions in ecological interactions among animals that may ultimately result in co-extinctions (Johnson, 2009; Galetti et al., 2018). On a human timescale, extinctions are slow processes, and their actual ramifications are challenging to predict. Fossils, on the other hand, provide insights into longer timescales. Data derived from these records can aid in understanding the current effects of climate change and human-induced pressures on extant fauna.

Multiple sites in the southern Brazilian Pampa region have preserved Pleistocene mammalian fauna remains, providing an opportunity to investigate their past ecology and former habitats. Stable isotope analysis is a widely utilized method in paleoecological studies and has the advantage of providing dietary and environmental information independent of the animal's morphology (Bocherens and Drucker, 2013). This technique relies on analyzing the chemical composition of fossilized tissues like bone, dentin, or enamel (Clementz, 2012; Bocherens and Drucker, 2013). Carbon stable isotopes primarily reflect the isotopes acquired through ingested and metabolized food (DeNiro and Epstein, 1978). Considering the processes that occur at the base of the food web, the isotopic ratios of herbivores are not solely indicative of their diet but also reveal habitat preferences based on the characteristics of the dominant primary producers (Newsome et al., 2007), thus providing paleoenvironmental information. Oxygen isotopic ratios of mammalian tissues are controlled by the ingested water, either through food or drinking (Luz et al., 1984; Kohn, 1996). The isotopic signature of meteoric waters, in turn, reflects climatic conditions such as local precipitation and humidity, serving as a proxy for paleoclimatic conditions (e.g., Levin et al., 2006).

Here, we aimed to study the paleoecology of large mammals from the Pleistocene of southern Brazilian Pampa. To achieve this, we analyzed the carbonate fraction of bones and teeth from novel specimens and used compiled data from previous publications.

Materials and methods

Study area

The Pampa biome extends across southern Brazil, Uruguay, and northeastern Argentina (Fig. 1). This biome is characterized by grasslands, often referred to as "Campos", consisting of grasses, herbs, small shrubs, and sparse trees (Allen et al., 2011). The dominant plant families in southern Brazil are Asteraceae, Poaceae, Fabaceae, and Cyperaceae, respectively, with a mixture of C_3 and C_4 plants (Overbeck et al., 2007 and references therein). The predominant climate is subtropical according to the Köppen climate classification, with hot summers and cold winters, without a dry season (Wrege et al., 2012). In contrast to the current mosaic of grasslands and forest formations, the landscape during glacial periods was predominantly treeless, with riparian vegetation restricted to the margins of freshwater bodies, due to the colder and drier climatic conditions (Behling, 2002; Mourelle et al., 2020). Pollen records from such

periods indicate that Poaceae and Cyperaceae were the most common taxa (Behling et al., 2005), with C_3 grasses being dominant (Mourelle et al., 2020).

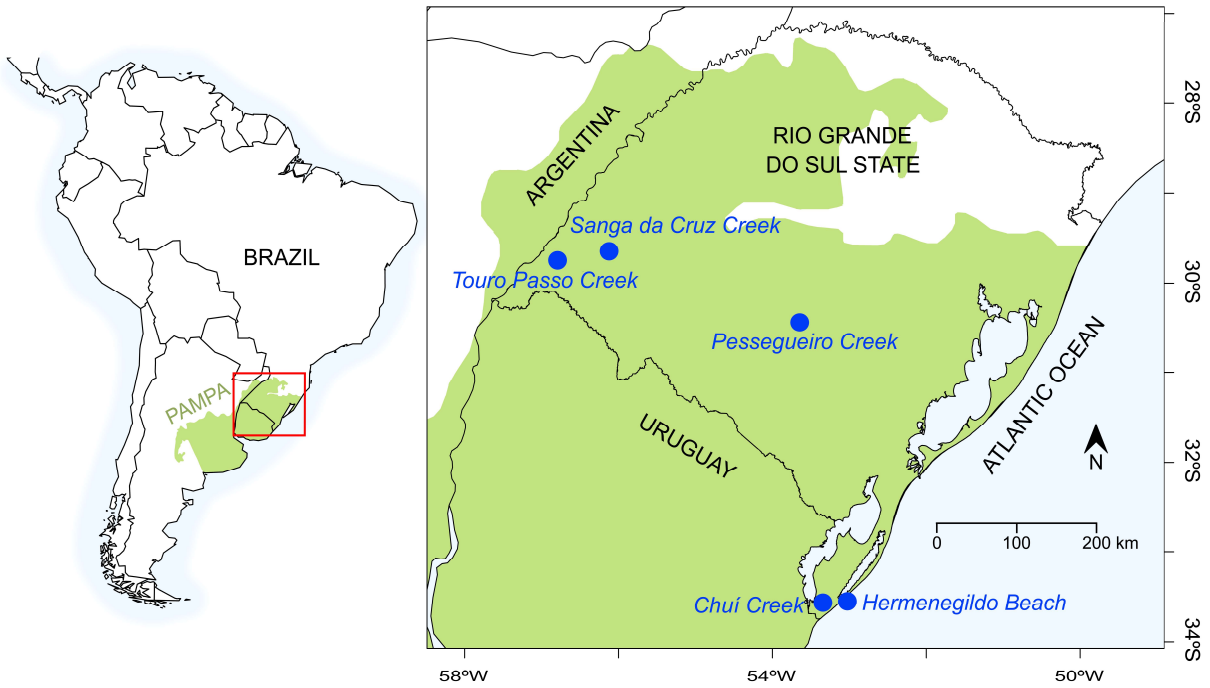


Fig. 1 Study area showing the Pampa biome and the fossiliferous sites in southern Brazil where Pleistocene megafauna remains have been discovered. It should be noted that the collection points Concheiros, Albardão, and Hermenegildo were considered as a single site named Hermenegildo due to their proximity.

To ensure a comprehensive representation of the Brazilian Pampa biome, we analyzed samples of teeth and bones from various locations: Touro Passo Creek, Sanga da Cruz Creek, and Hermenegildo Beach (refer to Table 2). These specimens are currently housed in the Paleontology section of the Museu de Ciências Naturais (MCN-PV), Secretaria do Meio Ambiente e Infraestrutura (SEMA), Porto Alegre, Rio Grande do Sul, Brazil. The specimens were classified at the lowest taxonomic level possible, based on the identification made by researchers from the MCN-PV. In order to expand our isotopic dataset, we also included data sourced from existing literature, belonging to the same ecological region and dating back to the Middle-Late Pleistocene. Because *Eremotherium laurillardi* is the only species from this genus recorded in Rio Grande do Sul fossil deposits, it was included in the analysis alongside *Eremotherium*. A similar situation applies to *Glossotherium robustum* (Lopes et al., 2020).

Touro Passo and Sanga da Cruz Creeks are located in western Rio Grande do Sul State, near the borders with Uruguay and Argentina. Both are fluvial deposits, generated on the basalts of the Serra Geral Plateau, Paraná Basin (Da-Rosa, 2009; Oliveira and Kerber, 2009). In Touro Passo, mammalian remains are typically found in sand and silt levels, with semi-articulated or

isolated elements, but in proximity to each other (Kerber et al., 2014). Fossils from Sanga da Cruz are usually isolated and recovered from conglomerate levels, which are overlaid by silt and sand levels (Oliveira and Kerber, 2009; Scherer et al., 2009). Based on sediment and fossil dating, the deposition age at Touro Passo Creek ranges from 42.6 and 10 ka (Da-Rosa, 2009; Kerber et al., 2011), and between 17.8 and 11.7 ka at Sanga da Cruz Creek (Miller, 1987; Milder, 2000).

Other sites of paleontological importance are those located in the Rio Grande do Sul Coastal Plain, on the Pelotas Sedimentary Basin. Whereas fossils from Chuí Creek are recovered *in situ*, those from Hermenegildo Beach are found *ex situ* on the foreshore, from submerged deposits (Buchmann, 1994). The formation of Rio Grande do Sul Coastal Plain is attributed to sea level fluctuations of the Late Quaternary, which resulted in the development of four barrier systems: Barriers I, II, and III with Pleistocene age of 325, 220, and 120 ka, respectively, and Barrier IV with a Holocene age of 6 ka (Villwock and Tomazelli, 1995; Lopes et al., 2014). Chuí Creek is located between Barriers II and III and exposes terrestrial facies composed of muddy sand (Lopes et al., 2013). The age of fossils from this site is estimated to be between 226 to 34 ka (Lopes et al., 2010). In contrast, the Hermenegildo Beach has a poor stratigraphical and chronological resolution – the dating of mammalian remains ranges from 650 to 18 ka (Lopes et al., 2010).

Pessegueiro Creek is situated in the central region of Rio Grande do Sul, on the Sul-Rio-Grandense Shield (Da-Rosa, 2009). This site is of fluvial origin and is composed by conglomerate sandstones, sandy siltstones, and pelites, with mammalian remains occurring within the deposits of pelitic sediments (Oliveira et al., 2002). Despite its rare geological description and lack of absolute dating, it is associated with periods of dry Pleistocene climate (Oliveira et al., 2002).

Stable isotope analysis

We sampled either individual or isolated teeth or bone fragments, with a random position, using a small drill to obtain a fine powder of approximately 50 mg. We then pretreated bulk samples of enamel, dentin, or bone following the protocol from Bocherens et al. (1996) with slight modifications. To remove organic residues, the samples were soaked in 2.5% NaOCl for 24 h at room temperature, followed by repeated rinsing (five times) with deionized water.

Subsequently, the samples were soaked in 0.1 M acetic acid solution during 24 h to remove exogenous carbonate, rinsed again, and dried in an oven at 60º C.

The carbon and oxygen isotope compositions were measured using a Thermo Gas Bench coupled to a Delta V IRMS with a GC Pal auto-sampler at the University of Georgia, Center for Applied Isotope Studies (UGA-CAIS) in Athens, GA, USA. During this process, solid carbonates were converted to CO_2 gas by dissolution in acid fosforic within a helium atmosphere. The CO_2 gas was then cryogenically preconcentrated and then released to IRMS.

The ratios of heavy to light isotopes in the samples (R) are expressed in parts per thousand (‰), using as scale the international standards Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW) for carbon ($^{13}C/^{12}C$, i.e., $\delta^{13}C$) and oxygen ($^{18}O/^{16}O$, i.e., $\delta^{18}O$), respectively: $\delta^{13}C$ or $\delta^{18}O = [(R_{SAMPLE} / R_{STANDARD}) - 1]$.

Internal laboratory controls (Fisher and A1296) were run every 12-sample set to ensure the analytical precision, which was better than 0.03‰ for carbon and 0.04‰ for oxygen.

Data analysis

We performed the statistical analysis using the R statistical environment version 4.0.3 (R Core Team, 2020). We assessed data normality using the Shapiro-Wilk test with a significance level of 0.05. To measure the association between carbon and oxygen stable isotopes, considering the entire dataset, we used the non-parametric Kendall correlation coefficient since the carbon isotopic data did not follow a normal distribution. To evaluate differences in $\delta^{13}C$ and $\delta^{18}O$ values among taxa with three or more observations we adopted a non-parametric comparison of multivariate samples, a statistics of Wilks' Lambda type, with 1000 permutations (npmv package; Ellis et al., 2017). Because the objective was to obtain information about the entire southern Brazilian Pampa, we do not consider possible differences in isotopic ratios between collection sites.

We applied Bayesian stable isotope mixing models in R (simmr package; Parnell and Inger, 2016) to estimate the contribution of C_3 and C_4 plants to the diet of herbivorous mammals. This analysis required three components: (1) the consumers' $\delta^{13}C$ values (Table 1), (2) the sources' $\delta^{13}C$ values, (3) and the expected offset between the isotopic composition of the herbivorous tissue and their diet, which resulted from the isotopic enrichment (ε*). We obtained the carbon isotopic signatures of C_3 and C_4 plants from the literature (O'Leary, 1988), and adjusted them for temporal differences in atmospheric $\delta^{13}C$ (Suess effect; Baxter and

Walton, 1970) following Dombrosky (2020), which resulted in mean values ± standard deviation (SD) of -25.8 ± 2.0‰ and -11.8 ± 1.2‰, respectively. We calculated the isotopic enrichment based on the formulae proposed by Tejada-Lara et al. (2018), considering on the digestive physiology and body mass of each taxon (Table 1). By on analogy with living armadillos, we considered glyptodonts hindgut fermenters (Estecondo et al., 1996). In the case of taxa belonging to extinct lineages with unknown digestive physiology (macraucheniids and toxodontids), we applied the general equation for all mammals. We used body mass estimations for extinct taxa and the standard weight in kilograms for extant species (see the references cited in Table 1).

Table 1 Body mass, expressed in kg, of the studied taxa and their corresponding references. We applied these data to a different formula for each digestive physiology type, which resulted in the isotopic enrichments reported below (‰). For more details, see Tejada-Lara et al. (2018).

Taxon	Body Mass	Reference	Formula	Enrichment
Cervidae	50	1; 2	Foregut fermenters	12.6
Hemiauchenia paradoxa	192	2	Foregut fermenters	13.5
Lama guanicoe	100	3	Foregut fermenters	13.1
Vicugna vicugna	40	4	Foregut fermenters	12.5
Tayassu pecari	32	5	Foregut fermenters	12.3
Equus	250	2	Hindgut fermenters	13.4
Hippidion	476	2	Hindgut fermenters	13.7
Macrauchenia	909	2	All taxa	13.9
Toxodon	1415	2	All taxa	14.1
Notiomastodon	5946	2	Hindgut fermenters	14.8
Megatheriidae	~3934	2; 6	Foregut fermenters	15.7
Eremotherium	3263	6	Foregut fermenters	15.6
Megatherium	4584	2	Foregut fermenters	15.8
Glossotherium	1713	1	Foregut fermenters	15.1
Lestodon	2590	2	Foregut fermenters	15.4
Glyptodon reticulatus	633	2	Hindgut fermenters	13.8
Panochthus tuberculatus	795	2	Hindgut fermenters	13.9

1 – Prevosti and Vizcaíno, 2006; 2 – Fariña et al., 2014; 3 – González et al., 2006; 4 – Ccora et al., 2019; 5 – Peres, 1996; 6 – Dantas, 2019.

We inferred habitat use based on $\delta^{13}C$ values corrected for isotopic enrichment. Plants with the C_3 photosynthetic pathway comprise trees and some cool-season grasses, whereas the C_4 photosynthetic pathway is typically found in warm-season grasses (Pearcy and Ehleringer 1984). In addition to the expected $\delta^{13}C$-variation due to metabolic pathway, C_3 plants growing in closed canopy environments are typically ^{13}C-depleted than those growing in open environments; on the other hand, plants in water stressed environments are ^{13}C-enriched (Cerling et al. 2004). Based on these differences, we applied the following ranges to distinguish

between habitat use: forest habitats from -34.6‰ to -28.6‰, mesic habitats between -28.6‰ and -23.6‰, and grassland habitats from -23.6‰ to -7.6‰ (Domingo et al., 2012).

Results

We analyzed a total of 43 specimens of large mammals from the Pleistocene of the southern Brazilian Pampa in this study, providing initial results for Cervidae, *Tayassu pecari*, *Equus*, *Hippidion*, *Macrauchenia*, *Glossotherium*, *Lestodon*, *Glyptodon reticulatus*, and *Panochthus tuberculatus*. To complement our dataset, we included $\delta^{13}C$ and $\delta^{18}O$ values of specimens from the same study area (n = 39), reported in the studies of Lopes et al. (2013; 2021) and Carrasco et al. (2022). Details regarding tissue type, paleontological site, and individual isotopic results are provided in Table 2.

Table 2 Tissue type analyzed, provenance, and $\delta^{13}C$ and $\delta^{18}O$ values of each specimen included in the present study.

Taxon	Tissue	Site	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VSMOW}$	Reference
Cervidae	Enamel	HB	-9.1	31.1	1
Cervidae	Enamel	HB	-5.4	30.3	1
Cervidae	Enamel	HB	-9.0	30.7	1
Cervidae	Enamel	HB	-9.3	30.3	1
Cervidae	Enamel	HB	-11.7	27.9	1
Hemiauchenia paradoxa	Enamel	HB	-7.9	29.5	2
Hemiauchenia paradoxa	Enamel	HB	-9.7	31.8	2
Hemiauchenia paradoxa	Enamel	HB	-10.6	31.1	2
Hemiauchenia paradoxa	Enamel	TPC	-7.7	30.3	2
Hemiauchenia paradoxa	Enamel	TPC	-7.4	31.4	2
Hemiauchenia paradoxa	Dentin	TPC	-9.3	27.9	2
Hemiauchenia paradoxa	Bone	TPC	-8.5	25.0	2
Lama guanicoe	Enamel	HB	-11.0	30.6	2
Lama guanicoe	Enamel	HB	-10.1	29.1	2
Lama guanicoe	Enamel	HB	-10.8	31.2	2
Lama guanicoe	Enamel	HB	-11.5	29.6	2
Lama guanicoe	Enamel	HB	-4.7	31.7	2
Lama guanicoe	Bone	TPC	-8.4	27.5	2
Vicugna vicugna	Enamel	HB	-6.4	28.9	2
Vicugna vicugna	Enamel	HB	-7.2	30.1	2
Vicugna vicugna	Enamel	HB	-7.6	30.3	2
Vicugna vicugna	Enamel	TPC	-6.2	31.4	2
Tayassu pecari	Enamel	TPC	-11.5	28.8	1
Equus	Enamel	HB	-6.2	29.6	1
Equus	Enamel	HB	-11.3	29.8	1

Equus	Enamel	HB	-9.9	31.1	1
Equus	Enamel	TPC	-4.2	32.4	1
Equus	Enamel	TPC	-5.0	29.4	1
Hippidion	Enamel	HB	-7.6	28.1	1
Hippidion	Enamel	HB	-9.5	30.3	1
Hippidion	Enamel	HB	-11.6	29.8	1
Hippidion	Enamel	TPC	-5.2	30.3	1
Macrauchenia	Enamel	HB	-9.6	29.7	1
Macrauchenia	Enamel	HB	-9.4	29.4	1
Macrauchenia	Enamel	HB	-6.8	29.2	1
Macrauchenia	Bone	TPC	-6.7	26.6	1
Macrauchenia	Bone	SCC	-7.1	27.2	1
Toxodon	Enamel	HB	-6.8	27.6	1
Toxodon	Enamel	HB	-6.6	29.5	1
Toxodon	Dentin	CC	-4.5	29.4	1
Toxodon	Enamel	CC	-4.5	31.0	3
Toxodon	Enamel	CC	-8.8	30.3	3
Toxodon	Enamel	CC	-4.9	30.3	3
Toxodon	Enamel	CC	-5.3	30.1	3
Toxodon	Enamel	CC	-4.5	30.2	3
Toxodon	Enamel	CC	-4.0	29.2	3
Toxodon	Enamel	CC	-4.4	29.7	3
Toxodon	Enamel	CC	-4.1	28.0	3
Toxodon	Enamel	CC	-5.5	30.1	3
Toxodon	Enamel	CC	-4.0	29.8	3
Toxodon	Dentin	TPC	-9.2	27.3	1
Notiomastodon	Enamel	HB	-9.9	29.3	1
Notiomastodon	Enamel	HB	-8.2	28.7	1
Notiomastodon	Enamel	HB	-7.8	29.9	1
Notiomastodon	Enamel	HB	-9.1	29.9	1
Notiomastodon	Enamel	CC	-8.2	27.9	3
Notiomastodon	Enamel	CC	-10.2	28.5	3
Notiomastodon	Enamel	CC	-7.3	30.1	3
Notiomastodon	Enamel	CC	-8.5	29.3	3
Notiomastodon	Enamel	CC	-8.2	29.1	3
Notiomastodon	Enamel	CC	-8.4	29.6	3
Megatheriidae	Dentin	CC	-6.9	30.4	4
Megatheriidae	Dentin	CC	-9.0	29.0	4
Eremotherium	Dentin	HB	-7.5	32.4	1
Eremotherium	Dentin	HB	-4.7	28.1	1
Eremotherium	Dentin	HB	-7.3	30.5	1
Eremotherium	Dentin	HB	-8.5	28.2	1
Eremotherium	Dentin	CC	-10.2	27.7	4
Eremotherium	Dentin	PC	-14.7	25.3	4
Eremotherium laurillardi	Dentin	HB	3.8	30.4	1
Megatherium	Dentin	CC	-6.9	28.7	4

Megatherium	Dentin	PC	-2.1	28.5	4
Glossotherium	Dentin	HB	-5.7	30.8	1
Glossotherium	Dentin	HB	-8.3	29.5	1
Glossotherium	Dentin	HB	-2.4	24.8	1
Glossotherium	Dentin	TPC	-4.9	27.1	1
Glossotherium robustum	Dentin	SCC	-9.8	26.9	1
Lestodon	Bone	HB	-8.0	27.9	1
Lestodon	Bone	HB	-8.4	29.4	1
Lestodon	Bone	HB	-8.6	29.4	1
Glyptodon reticulatus	Osteoderm	HB	-9.8	28.5	1
Panochthus tuberculatus	Osteoderm	HB	-4.1	29.2	1

HB – Hermenegildo Beach; TPC – Touro Passo Creek; SCC – Sanga da Cruz Creek; CC – Chuí Creek; PC – Pessegueiro Creek. 1 – This papper; 2 – Carrasco et al., 2022; 3 – Lopes et al., 2013; 4 – Lopes et al., 2021.

Carbon stable isotopes varied several parts per thousand among the analyzed taxa (mean ± SD = -7.5 ± 2.7‰). The lowest $\delta^{13}C$ value (-14.7‰) was observed in a *Eremotherium* fossil from Pessegueiro Creek, reported by Lopes et al. (2021), and the highest (3.8‰) was found in *E. laurillardi* analyzed in the presented study. Oxygen stable isotopes displayed less variation (mean ± SD = 29.3 ± 1.5‰) and ranged from 24.8‰, in a *Glossotherium* fossil, to 32.4‰, in an *Eremotherium* fossil, both analyzed in the present study, with literature data falling within this range. There was no significant correlation between $\delta^{13}C$ and $\delta^{18}O$ values ($R^2 = 0.05$, $p = 0.5$). Differences in stable isotope data among taxa were statistically significant ($p = 0.007$). As evidenced in Fig. 2, phylogenetically related taxa showed a tendency toward isotopic segregation, as observed in *Glyptodon reticulatus* and *P. tuberculatus* (Fig. 2).

Most of fossils' $\delta^{13}C$ values fall within the expected range of grassland habitats, with a few on the boundary between open and mesic environments, and a high ^{13}C-depleted individual from mesic/woodland habitats (Fig. 2). Plants with C_3 photosynthetic pathway were the most important dietary component for Cervidae, *Hemiauchenia paradoxa*, *Lama guanicoe*, *T. pecari*, *Equus*, *Hippidion*, *Macrauchenia*, *Notiomastodon*, Megatheriidae, *Eremotherium*, *Glossotherium*, *Lestodon*, and *Glyptodon reticulatus*, accounting for a median proportional contribution of over 60%. C_3 and C_4 plants contributed almost equally to the diet of *Vicugna vicugna*, *Toxodon*, *Megatherium*, and *P. tuberculatus* (Fig. 3).

Discussion

Stable isotope analysis of skeletal tissues from the Pleistocene of southern Brazil revealed a that most mammals predominantly consumed C_3 plants in open environments,

indicating a higher abundance of cool-season grasses. Similar findings have been reported for the Pampean regions of Uruguay and Argentina (Czerwonogora et al., 2011; Bocherens et al., 2016). Mammalian isotopic data align with phytolith and geochemical evidence derived from Late Pleistocene sediment cores, which indicated the dominance of grasslands consisting mainly of C_3 grasses, and riparian shrublands in areas with positive water balance (i.e., along margins of rivers and lakes) (Mourelle et al., 2020). Nevertheless, Sanz-Pérez et al. (2022) found a higher percentage of C_4 grasses in the diet of mammals from Playa del Barco and Santa Rosa localities, on the south and southwest boundaries of the Pampa biome, which could be attributed to more arid conditions in these regions during the last glacial maximum.

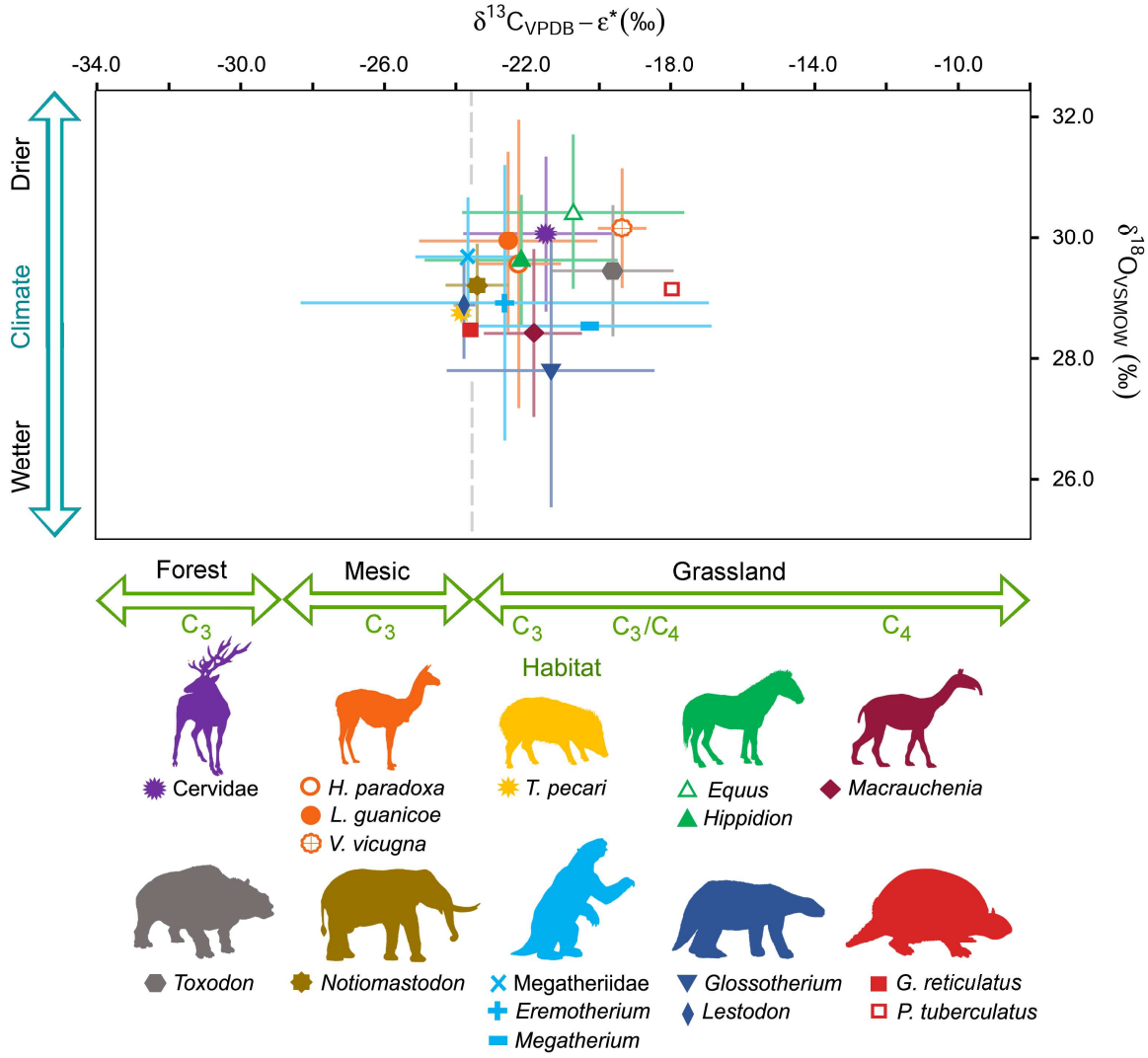


Fig. 2 Mean and standard deviation of $\delta^{13}C$, adjusted for isotopic enrichment, and $\delta^{18}O$ values from southern Brazilian land mammals. The carbon data provide insights into past ecologies (diet and habitat use), while oxygen isotopic data reflect environmental climatic conditions and drinking behavior.

According to Domingo et al. (2020), northern GABI immigrants such as proboscideans, perissodactyls, and camelids had flexible herbivore diets, conferring an ecological advantage during their immigration and establishment in the South America, whereas some native mammals like macraucheniids had a more specialized diet. In our study, we observed that Cervidae, *Hemiauchenia paradoxa*, *L. guanicoe*, *T. pecari*, *Equus*, *Hippidion*, and *Notiomastodon* primarily consumed C_3 plants, and only the camelid *V. vicugna* had a mixed diet that among the representatives of the northern taxa in the Pleistocene of Brazilian Pampa. The southern American natives *Macrauchenia*, Megatheriidae, *Eremotherium*, *Glossotherium*, *Lestodon*, and *Glyptodon reticulatus* fed mostly on C_3 plants, whereas *Toxodon*, *Megatherium*, and *P. tuberculatus* had a diet consisting of a combination of C_3 and C_4 plants.

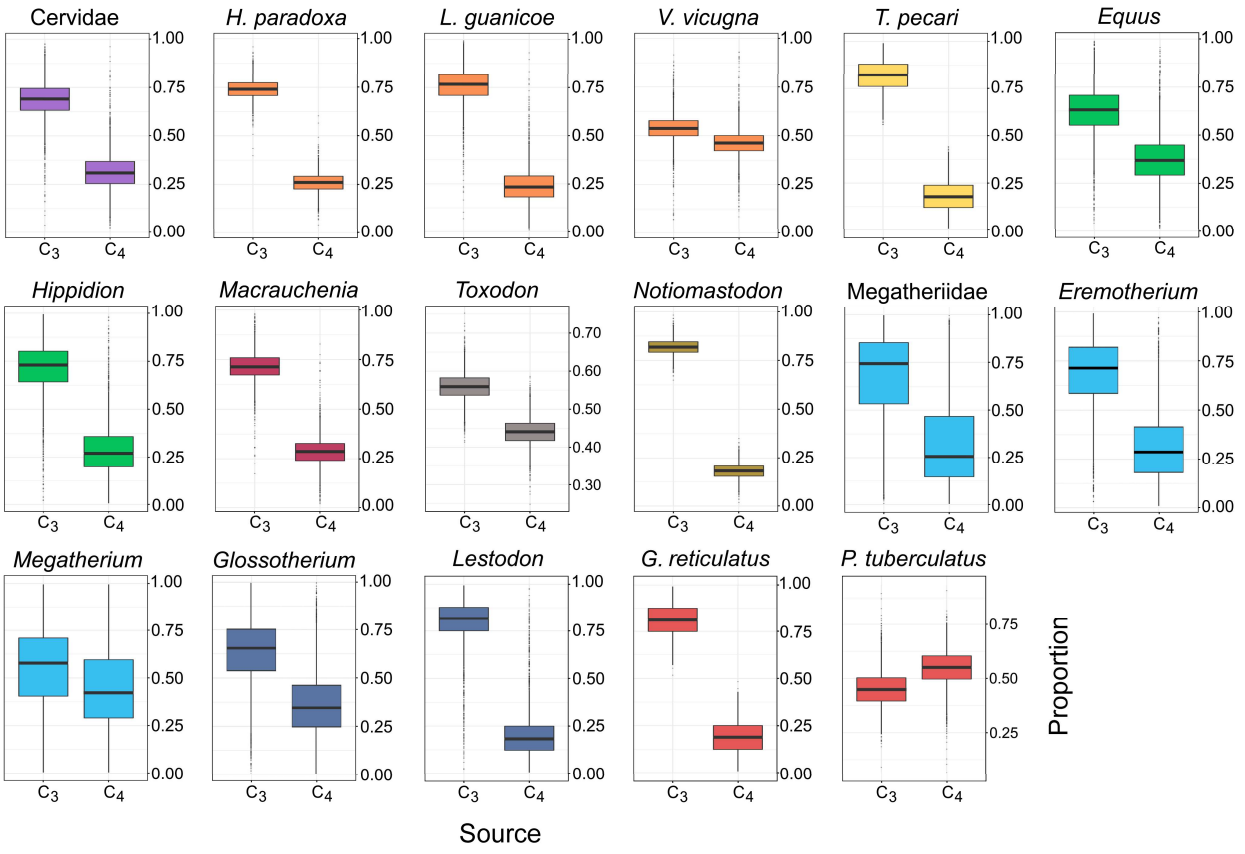


Fig. 3 Boxplot containing 95% credible interval of stable isotope mixing model solutions, representing the estimated contribution of C_3 and C_4 plants to the diet of land mammals in southern Brazil.

The predominance of C_3 diet in southern Brazil during the Pleistocene can be attributed to various factors. C_3 grasses, particularly those from the Pooideae lineage, were abundant in the "Campos" during that period (Mourelle et al., 2020). According to ecological modeling, these types of grasses are commonly associated with cold, dry, and open habitats, and have a

competitive advantage over C_4 taxa in such conditions (Pau et al., 2013). Moreover, C_3 grasses are more efficiently digested by herbivores (Sponheimer et al., 2003) and nutritionally superior to C_4 grasses due to higher levels of protein, nonstructural carbonates, and water (Barbehenn et al., 2004).

The abundance of nutritious C_3 plants likely favored the large herbivores in the Pleistocene of southern Brazil. Early successional plant communities consisting of grasses and shrubs can support dense populations of large mammals, whereas late successional stages are characterized by a reduction in forage availability, accompanied by an increase of plants with high investment in defenses (Mann et al., 2019). South America had extensive grasslands regions, especially during glacial periods, which were gradually replaced by forests with the onset of a more humid and warmer climate (Webb, 1991). This may be one of the reasons why Africa still maintains its megafauna and South America does not: the former preserves an arid area about four times larger than the latter (Mann et al., 2019).

A dietary overlap between grazing herbivores does not imply unlimited resources. Taking the African megafauna as an example, researchers had this initial impression, considering the large proportions of grass consumed by herbivores (e.g., de Iongh et al., 2011). However, the development of more detailed dietary studies revealed that herbivores discriminate their food based on certain pasture characteristics such as grass sward height, leading to resource partitioning (Voeten and Prins, 1999; Keynhans et al., 2011). Furthermore, plant DNA metabarcoding extracted from fecal samples clarified that herbivores consume different plant species (Kartzinel et al., 2015). Thus, it is possible that the southern Brazilian megafauna had a similar mechanism of resource partitioning. In addition, some specimens with highly ^{13}C-depleted bioapatite, for example *T. pecari*, *Glyptodon reticulatus* and some giant sloths, may reflect browsing habits in areas of riparian vegetation.

Despite the limitations in identifying specific plant types consumed using stable isotope analysis, the isotopic composition of Pleistocene herbivores indicates some level of resource partitioning. Resource partitioning is a strategy that reduces competition and facilitates the coexistence of related species, being evident by distinct isotopic composition between phylogenetically related mammals (e.g., Ma et al., 2019). Notable examples of this can be observed in glyptodontids (*Glyptodon reticulatus* and *P. tuberculatus*), camelids (*Hemiauchenia paradoxa*, *L. guanicoe*, and *Vicugna vicugna*), equids (*Equus* and *Hippidion*), and giant sloths (megatheriids and mylodontids). Besides the expected influence of interspecific competition, a variation in animals' feeding behavior can be a consequence of intraspecific

competition. According to Barabás et al. (2016), the impact of the competition is more pronounced on the species itself than on its competitors. Intraspecific variance in carbon isotope ratios may arise due to sexual and ontogenetic differences, as observed in *Notiomastodon* (e.g., Lopes et al., 2022), but also for reasons not attributable to these variables. In other words, individuals within a population have ecological differences, and mechanisms of between-individual variation to minimize resource use overlap (Bolnick et al., 2003; 2007). These mechanisms could have played a significant role to handle competition, especially among animals with social behavior, such as the giant sloths of the Mylodontidae family (Buchmann et al., 2016). Further exploration of intraspecific niche partitioning among megafauna herbivores through the analysis of serial samples would be interesting.

Giant sloths and other xenarthrans are the ones that raise most questions regarding their past feeding behavior. Bocherens et al. (2016) suggested that the variable $\delta^{13}C$ and $\delta^{15}N$ values obtained from *Megatherium* bone collagen could be attributed to the consumption of symbiotic invertebrates. In a subsequent study, the authors used the offset between the isotopic ratios of carbonate and collagen fractions as a proxy of trophic position. They found that $\Delta\delta^{13}C_{carb\text{-}coll}$ values of herbivores are typically higher than those of faunivorous (i.e., carnivores and omnivores) and the $\Delta\delta^{13}C_{carb\text{-}coll}$ values of giant sloths (*Megatherium* and *Glossotherium*) and glyptodontids (*Doedicurus* and *Glyptodon*) fell within the expected range of herbivores (Bocherens et al., 2017). Further, Tejada et al. (2021) analyzed nitrogen isotopic ratios of specific amino acids (glutamate and phenylalanine) and found that $\Delta\delta^{15}N_{Glx} - \delta^{15}N_{Phe}$ values for *Mylodon*, a taxon not included in the present study, were consistent with those of omnivorous taxa. Unfortunately, the absence of collagen in samples from southern Brazil prevents the application of such methodologies. However, $\delta^{13}C$ values, corrected for trophic enrichment, of bone carbonate indicate a high diversity of feeding habits.

Altogether, specimens of giant sloths and glyptodonts cover a broad isotopic range, exceeding that of all the other taxa included in the study. The more variable diet was observed in *Eremotherium*, in which $\delta^{13}C$ values varied by more than 10‰. Such results may indicate a consumption of woody plants as well as C_3 and C_4 grasses. This high dietary flexibility is consistent with morphological characteristics. According to Dantas et al. (2023), the relative muzzle width of *Eremotherium laurillardi* is indicative of a browsing mixed-feeder diet, allowing this species to consume different types of vegetation. Its high dietary flexibility, in turn, may ultimately be linked to its wide latitudinal distribution, which is greater than that of any other giant sloth (Dantas et al., 2023).

Several megafauna species exhibited adaptations for grazing, evidenced by their cranial morphologies, as an example of the premaxillary shape of *Macrauchenia patachonica* (Dantas et al., 2020). More pronounced adaptations can be observed in tooth morphology, such as high-crowned or hypsodont teeth, which provide greater resistance to abrasive diets (Janis, 2008). Additionally, elodont or ever-growing teeth are an effective adaptation to a grazing diet, capable of neutralizing the pressure of abrasives that would otherwise damage their teeth (Madden, 2015). Morphological tooth features for grazing emerged throughout the evolutionary history of several South American taxa, including camelids, equids, toxodontids, gomphotherids, and also xenarthrans (Janis, 2008; Madden, 2015).

Grazing animals perform important functions in natural ecosystems through defoliation, treading, and transport of seeds and nutrients by excreta (Metera et al., 2010), therefore maintaining grasslands by preventing the proliferation of weeds and shrubs. In the Brazilian Pampa, the current climatic conditions have favored the expansion of forests into grassland areas (Roesch et al., 2009) and, given this, some authors argue that cattle raising can help to preserve the remaining grasslands in the region (e.g., Overbeck et al., 2007). However, it is important to be mindful of the potential negative impacts of overgrazing since it can lead to a decrease in ecosystem diversity (Metera et al., 2010). It is also worth noting that grazing animals tend to have different food preferences, as discussed previously, and that a single species cannot replace the entire Pleistocene herbivore community.

While most of the species analyzed in this study are currently extinct, the guanaco, *L. guanicoe*, the vicuna, *V. vicugna*, and the white-lipped peccary, *T. pecari*, still inhabit certain regions of South America. The camelids are distributed along the Andean region, where cold weather conditions prevail (González et al., 2006; Scherer, 2013). The white-lipped peccary, on the other hand, is found in the Neotropical Region, preferably inhabiting tropical forests (Keuroghlian et al., 2013), but these three species coexisted during the Pleistocene of southern Brazil. Taking into account the ecological preferences of modern white-lipped peccary populations, Kerber et al. (2014) suggested that the presence of this species in the Touro Passo Formation would indicate forested areas in the Pleistocene of southern Brazil. In the present study, the white-lipped peccary showed values on the boundary between mesic/woodland and grassland habitats, suggesting that it may have inhabited areas of riparian vegetation of the Pampa biome. Conversely, the camelids would have inhabited open areas. This illustrates the importance of considering the entire fossil fauna for environmental inferences, as previously suggested by Gasparini et al. (2013).

The analysis of oxygen isotope ratios ($\delta^{18}O$) provides paleoenvironmental information that is complementary to that provided by $\delta^{13}C$ data. Herbivorous mammals can exhibit different responses to climate changes depending on their water intake behavior (Kohn, 1996). They can be categorized as either obligated drinkers or non-obligated drinkers (Levin et al., 2006). The effect of evaporation is more pronounced in leaves compared to continental water bodies, so herbivores that supply their water requirements primarily through diet (non-obligated drinkers) become progressively enriched in ^{18}O with increasing aridity. This means that the greater the aridity of a region, the larger the difference in $\delta^{18}O$ values between obligated and non-obligated drinkers (Levin et al., 2006; Lehmann et al., 2022).

The fossils analyzed in the present study include both obligated (e.g., equids) and non-obligated (e.g., cervids) drinkers (Sanz-Pérez et al., 2022), but a clear pattern according to behavior could not be traced. Furthermore, the lack of correlation between carbon and oxygen stable isotopes in the fossil dataset indicates that factors affecting plant consumption was independent of those affecting drinking water. These evidences may suggest that this region did not suffer with water deficit during the Pleistocene. However, it is important to note that the specimens have different ages of deposition, which may confound the results. In addition, sampling from different tissues is another source of bias, as it represents different periods of tissue synthesis. In this context, the variation between and within taxa may alternatively suggest a seasonal climate, as observed in paleoenvironmental studies (e.g., Mourelle et al., 2020).

The isotopic composition of bones and teeth ($\delta^{13}C$ and $\delta^{18}O$) of large herbivores from the Pleistocene of Brazilian Pampa indicates that they inhabited grassland environments characterized by the dominance of cool-season grasses and a seasonal climate. These herbivores played an important role in the maintenance of these ecosystems, but grazing alone cannot prevent changes in plant composition and ecological succession (Metera et al., 2010). The climate in the region transitioned from dry and cold in the Late Pleistocene to warm and dry in the early Holocene, which was followed by a decrease in Poaceae, particularly C_3 plants of the subfamily Pooideae, and an increase in Cyperaceae (Behling et al., 2005; Mourelle et al., 2020). Finally, after 5.1 ka BP, there was an expansion of gallery forests due to wetter conditions (Behling et al., 2005). These environmental changes, coupled with the arrival of humans in southern Brazil around 13 ka BP (Noelli, 2000; Santos, 2023), may have been disadvantageous to these animals. Consequently, the decline of large herbivores had an impact on apex predators such as the sabretooth cat, *Smilodon populator*, and the large canid *Protocyon* (Bocherens et al., 2016).

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References

Allen, V.G., Batello, C., Berretta, E.J., Hodgson, J., Kothmann, M., Li, X., McIvor, J., Milne, J., Morris, C., Peeters, A., Sanderson, M., 2011. An international terminology for grazing lands and grazing animals. Grass and Forage Science, 66: 2–28. https://doi.org/10.1111/j.1365-2494.2010.00780.x

Barabás, G., Michalska-Smith, M.J., Allesina, S., 2016. The effect of intra- and interspecific competition on coexistence in multispecies communities. The American Naturalist, 188: E1–E12. https://doi.org/10.1086/686901

Barbehenn, R.V., Chen, Z., Karowe, D.N., Spickard, A., 2004. C_3 grasses have higher nutritional quality than C_4 grasses under ambient and elevated atmospheric CO_2. Global Change Biology, 10: 1565–1575. https://doi.org/10.1111/j.1365-2486.2004.00833.x

Barnosky, A.D., Koch, P.L., Feranec, R.S., Wing, S.L., Shabel, A.B., 2004. Assessing the causes of Late Pleistocene extinctions on the continents. Science, 306: 70–75. https://doi.org/10.1126/science.1101476

Baxter, M.S., Walton, A., 1970. A theoretical approach to the Suess effect. Proceedings of the Royal Society of London A, 318: 213–230. https://doi.org/10.1098/rspa.1970.0141

Behling, H., 2002. South and southeast Brazilian grasslands during Late Quaternary times: a synthesis. Palaeogeography, Palaeoclimatology, Palaeoecology, 177: 19–27. https://doi.org/10.1016/S0031-0182(01)00349-2

Behling, H., Pillar, V.D., Bauermann, S.G., 2005. Late Quaternary grassland (campos), gallery forest, fire and climate dynamics, studied by pollen, charcoal and multivariate analysis of the São Francisco de Assis core in western Rio Grande do Sul (southern Brazil). Review

of Palaeobotany and Palynology, 133: 235–248. https://doi.org/10.1016/j.revpalbo.2004.10.004

Bocherens, H., Drucker, D.G., 2013. Terrestrial teeth and bones. In: Elias, S.A., Mock, C.J. (eds), Encyclopedia of Quaternary Science, 2nd ed. Elsevier, Amsterdam.

Bocherens, H., Koch, P.L., Mariotti, A., Geraads, D., Jaeger, J., 1996. Isotopic biogeochemistry (^{13}C, ^{18}O) of mammalian enamel from African Pleistocene hominid sites. Palaios, 11: 306–318. https://doi.org/10.2307/3515241

Bocherens, H., Cotte, M., Bonini, R., Scian, D., Straccia, P., Soibelzon, L., Prevosti, F.J., 2016. Paleobiology of sabretooth cat *Smilodon populator* in the Pampean Region (Buenos Aires Province, Argentina) around the Last Glacial Maximum: insights from carbon and nitrogen stable isotopes in bone collagen. Palaeogeography, Palaeoclimatology, Palaeoecology, 449: 463–474. https://doi.org/10.1016/j.palaeo.2016.02.017

Bocherens, H., Cotte, M., Bonini, R.A., Pablo Straccia, P., Scian, D., Soibelzon, L., Prevosti, F.J., 2017. Isotopic insight on paleodiet of extinct Pleistocene megafaunal Xenarthrans from Argentina. Gondwana Research, 48: 7–14. https://doi.org/10.1016/j.gr.2017.04.003

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L. 2003. The ecology of individuals: incidence and implications of individual specialization. The American Naturalist, 161: 1–28. https://doi.org/10.1086/343878

Bolnick, D.I., Svanbäck, R., Araújo, M.S., Persson, L., 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proceedings of the National Academy of Sciences, 104: 10075–10079. https://doi.org/10.1073/pnas.0703743104

Buchmann, F.S., 1994. Distribuição de fósseis pleistocênicos na zona costeira e plataforma continental interna no Rio Grande do Sul. Acta Geológica Leopoldensia, São Leopoldo, 39: 355–364.

Buchmann, F.S., Frank, H.T., Ferreira, V.M.S., Cruz, E.A., 2016. Evidência de vida gregária em paleotocas atribuídas a Mylodontidae (preguiças-gigantes). Revista Brasileira de Paleontologia, 19: 259–270. https://doi.org/10.4072/rbp.2016.2.09

Carrasco, T.S., Scherer, C.S., Ribeiro, A.M., Buchmann, F.S., 2022. Paleodiet of Lamini camelids (Mammalia: Artiodactyla) from the Pleistocene of southern Brazil: insights from stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$). Paleobiology, 48: 513–526. https://doi.org/10.1017/pab.2022.10

Ccora, E., Condori, A., Contreras, J.L., Curasma, J., Cordero, A.G., Valencia, N., Mayhua, P.H., McGregor, B.A., 2019. Biometric characteristics in vicuñas (*Vicugna Vicugna mensalis*). Small Ruminant Research, 175: 52–56. https://doi.org/10.1016/j.smallrumres.2019.04.002

Cerling, T.E., Hart, J.A., Hart, T.B., 2004. Stable isotope ecology in the Ituri Forest. Oecologia, 138: 5–12. https://doi.org/10.1007/s00442-003-1375-4

Cione, A.L., Tonni, E.P., Soibelzon, L., 2009. Did humans cause the Late Pleistocene-Early Holocene mammalian extinctions in South America in a context of shrinking open areas?. In: Haynes, G. (ed), American Megafaunal Extinctions at the End of the Pleistocene. Vertebrate Paleobiology and Paleoanthropology. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-8793-6_7

Cione, A.L., Gasparini, G.M., Soibelzon, E., Soibelzon, L.H., Tonni, E.P., 2015. The GABI in Southern South America. In: Rabassa, J., Lohmann, G., Notholt, J., Mysak, L.A., Unnithan, V. (eds), The Great American Biotic Interchange. SpringerBriefs in Earth System Sciences. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-9792-4_3

Clementz, M.T., 2012. New insight from old bones: stable isotope analysis of fossil mammals. Journal of Mammalogy, 93: 368–380. https://doi.org/10.1644/11-MAMM-S-179.1

Czerwonogora, A., Fariña, R.A., Tonni, E.P., 2011. Diet and isotopes of Late Pleistocene ground sloths: first results for *Lestodon* and *Glossotherium* (Xenarthra, Tardigrada). Neues Jahrbuch für Geologie und Paläontologie - Abhandlungen Band, 262: 257–266. https://doi.org/10.1127/0077-7749/2011/0197

Dantas, M.A.T., 2019. Atualizando a estimativa da massa corporal da megafauna do Pleistoceno Final da Região Intertropical Brasileira. In: XXVI Congresso Brasileira de Paleontologia, Uberlândia, MG. Paleontologia em Destaque.

Dantas, M.A.T., Lobo, L.S., Bernardes, C., 2020. Comments on "fantastic beasts and what they ate: Revealing feeding habits and ecological niche of late Quaternary Macraucheniidae from South America" by K. de Oliveira, T. Araújo, A. Rotti, D. Mothé, F. Rivals, L.S. Avilla, Quaternary Science Reviews 231 (2020) 106178. Quaternary Science Reviews, 250: 106662. https://doi.org/10.1016/j.quascirev.2020.106662

Dantas, M.A.T., Campbell, S.C., McDonald, H.G., 2023. Paleoecological inferences about the Late Quaternary giant sloths. Journal of Mammalian Evolution. https://doi.org/10.1007/s10914-023-09681-5

Da-Rosa, A.A.S., 2009. Geologia do Quaternário continental do RS: estado da arte e perspectivas de trabalho. In: Ribeiro, A.M., Bauermann, S.G., Scherer, C.S. (eds), Quaternário do Rio Grande do Sul: Integrando Conhecimentos. Sociedade Brasileira de Paleontologia, Porto Alegre.

de Iongh, H.H., de Jong, C.B., van Goethem, J., Klop, E., Brunsting, A.M.H., Loth, P.E., Prins, H.H.T., 2011. Resource partitioning among African savanna herbivores in North Cameroon: the importance of diet composition, food quality and body mass. Journal of Tropical Ecology, 27: 503–513. https://doi.org/10.1017/S0266467411000307

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta, 42: 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

Dombrosky, J., 2020. A ~1000-year ^{13}C Suess correction model for the study of past ecosystems. The Holocene, 30: 474–478. https://doi.org/10.1177/0959683619887416

Domingo, L., Prado, J.L., Alberdi, M.T., 2012. The effect of paleoecology and paleobiogeography on stable isotopes of Quaternary mammals from South America. Quaternary Science Reviews, 55: 103–113. https://doi.org/10.1016/j.quascirev.2012.08.017

Domingo, L., Tomassini, R.L., Montalvo, C.I., Sanz-Pérez, D., Alberdi, M.T., 2020. The Great American Biotic Interchange revisited: a new perspective from the stable isotope record of Argentine Pampas fossil mammals. Scientific Reports, 10: 1608. https://doi.org/10.1038/s41598-020-58575-6

Ellis, A.R., Burchett, W.W., Harrar, S.W., Bathke, A.C., 2017. Nonparametric inference for multivariate data: the R package npmv. Journal of Statistical Software, 76: 1–18. https://doi.org/10.18637/jss.v076.i04

Estecondo, S., Codón, S.M., Casanave, E.B., 1996. Histological study of the digestive tract of *Dasypus hybridus* (Desmarest, 1804) and *Zaedyus pichiy* (Desmarest, 1804) (Mammalia, Dasypodidae). Iheringia, Série Zoológica, 81: 7–12.

Fariña, R.A., Vizcaíno, S.F., De Iuliis, G., 2013. Megafauna: Giant Beasts of Pleistocene South America. Indiana University Press, Bloomington.

Fariña, R.A., Czerwonogora, A., Di Giacomo, M., 2014. Splendid oddness: revisiting the curious trophic relationships of South American Pleistocene mammals and their abundance. Anais da Academia Brasileira de Ciências, 86: 311–331. https://doi.org/10.1590/0001-3765201420120010

Galetti, M., Moleón, M., Jordano, P., Pires, M.M., Guimarães Jr., P.R., Pape, T., Nichols, E., Hansen, D., Olesen, J.M., Munk, M., de Mattos, J.S., Schweiger, A.H., Owen-Smith, N., Johnson, C.N., Marquis, R.J., Svenning, J., 2018. Ecological and evolutionary legacy of megafauna extinctions. Biological Reviews, 93: 845–862. https://doi.org/10.1111/brv.12374

Gasparini, G.M., Rodriguez, S.G., Soibelzon, L.H., Beilinson, E., Soibelzon, E., Missagia, R.V., 2014. *Tayassu pecari* (Link, 1795) (Mammalia, Cetartiodactyla): comments on its South American fossil record, taxonomy and paleobiogeography. Historical Biology, 26: 785–800. https://doi.org/10.1080/08912963.2013.858247

González, B.A., Palma, R.E., Zapata, B., Marín, J.C., 2006. Taxonomic and biogeographical status of guanaco *Lama guanicoe* (Artiodactyla, Camelidae). Mammal Review, 36: 157–178. https://doi.org/10.1111/j.1365-2907.2006.00084.x

Hubbe, A., Hubbe, M., Neves, W.A., 2013. The Brazilian megamastofauna of the Pleistocene/Holocene transition and its relationship with the early human settlement of the continent. Earth-Science Reviews, 118: 1–10. https://doi.org/10.1016/j.earscirev.2013.01.003

Janis, C., 2008. An evolutionary history of browsing and grazing ungulates. In: Gordon, I.J., Prins, H.H.T. (eds), The Ecology of Browsing and Grazing. Springer Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-72422-3

Johnson, C.N., 2009. Ecological consequences of Late Quaternary extinctions of megafauna. Proceedings of the Royal Society B, 276: 2509–2519. https://doi.org/10.1098/rspb.2008.1921

Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., Kress, W.J., Kuzmina, M.L., Rubenstein, D.I., Wang, W., Pringle, R.M., 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. Proceedings of the National Academy of Sciences, 112: 8019–8024. https://doi.org/10.1073/pnas.1503283112

Kerber, L., Kinoshita, A., José, F.A., Figueiredo, A.M.G., Oliveira, É.V., Baffa, O., 2011. Electron Spin Resonance dating of the southern Brazilian Pleistocene mammals from Touro Passo Formation, and remarks on the geochronology, fauna and palaeoenvironments. Quaternary International, 245: 201–208. https://doi.org/10.1016/j.quaint.2010.10.010

Kerber, L., Pitana, V.G., Ribeiro, A.M., Hsiou, A.S., Oliveira, E.V., 2014. Late Pleistocene vertebrates from Touro Passo Creek (Touro Passo Formation), southern Brazil: a review. Revista Mexicana de Ciencias Geológicas, 31: 248–259.

Keuroghlian, A., Desbiez, A., Reyna-Hurtado, R., Altrichter, M., Beck, H., Taber, A., Fragoso, J.M.V., 2013. *Tayassu pecari*. The IUCN Red List of Threatened Species 2013: e.T41778A44051115. Accessed on 11 April 2023. https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T41778A44051115.en

Kleynhans, E.J., Jolles, A.E., Bos, M.R.E., Olff, H., 2011. Resource partitioning along multiple niche dimensions in differently sized African savanna grazers. Oikos, 120: 591–600. https://doi.org/10.1111/j.1600-0706.2010.18712.x

Kohn, M.J., 1996. Predicting animal $\delta^{18}O$: accounting for diet and physiological adaptation. Geochimica et Cosmochimica Acta, 60: 4811–4829. https://doi.org/10.1016/S0016-7037(96)00240-2

Kohn, M.J., Schoeninger, M.J., Valley, J.W., 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. Geochimica et Cosmochimica Acta, 60: 3889–3896. https://doi.org/10.1016/0016-7037(96)00248-7

Lehmann, S.B., Levin, N.E., Passey, B.H., Huc, H., Cerling, T.E., Miller, J.H., Arppe, L., Beverly, E.J., Hoppe, K.A., Huth, T.E., Kelson, J.R., Luyt, J., Sealy, J., 2022. Triple oxygen isotope distribution in modern mammal teeth and potential geologic applications. Geochimica et Cosmochimica Acta, 331: 105–122. https://doi.org/10.1016/j.gca.2022.04.033

Levin, N.E., Cerling, T.E., Passey, B.H., Harris, J.M., Ehleringer, J.R., 2006. A stable isotope aridity index for terrestrial environments. Proceedings of the National Academy of Sciences, 103: 11201–11205. https://doi.org/10.1073/pnas.0604719103

Lopes, R.P., Oliveira, L.C., Figueiredo, A.M.G., Kinoshita, A., Baffa, O., Buchmann, F.S., 2010. ESR dating of Pleistocene mammal teeth and its implications for the biostratigraphy and geological evolution of the coastal plain, Rio Grande do Sul, southern Brazil. Quaternary International, 212: 213–222. https://doi.org/10.1016/j.quaint.2009.09.018

Lopes, R.P., Ribeiro, A.M., Dillenburg, S.R., Schultz, C.L., 2013. Late middle to late Pleistocene paleoecology and paleoenvironments in the coastal plain of Rio Grande do Sul State, southern Brazil, from stable isotopes in fossils of *Toxodon* and *Stegomastodon*.

Palaeogeography, Palaeoclimatology, Palaeoecology, 369: 385–394. https://doi.org/10.1016/j.palaeo.2012.10.042

Lopes, R.P., Kinoshita, A., Baffa, O., Figueiredo, A.M.G., Dillenburg, S.R., Schultz, C.L., Pereira, J.C., 2014. ESR dating of Pleistocene mammals and marine shells from the coastal plain of Rio Grande do Sul state, southern Brazil. Quaternary International, 352: 124–134. https://doi.org/10.1016/j.quaint.2013.07.020

Lopes, R.P., Pereira, J.C., Kerber, L., Dillenburg, S.R., 2020. The extinction of the Pleistocene megafauna in the Pampa of southern Brazil. Quaternary Science Reviews, 242: 106428. https://doi.org/10.1016/j.quascirev.2020.106428

Lopes, R.P., Dillenburg, S.R., Pereira, J.C., Sial, A.N., 2021. The paleoecology of Pleistocene giant megatheriid sloths: stable isotopes ($\delta^{13}C$, $\delta^{18}O$) of co-occurring *Megatherium* and *Eremotherium* from southern Brazil. Revista Brasileira de Paleontologia, 24: 245–264. https://doi.org/10.4072/rbp.2021.3.06

Lopes, R.P., Pereira, J.C., Sial, A.N., Dillenburg, S.R., 2022. Isotopic evidence for a diet shift in a Pleistocene sub-adult mastodon from the Brazilian Pampa. Historical Biology, 35: 388–402. https://doi.org/10.1080/08912963.2022.2043293

Luz, B., Kolodny, Y., Horowitz, M., 1984. Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. Geochimica et Cosmochimica Acta, 48: 1689–1693. https://doi.org/10.1016/0016-7037(84)90338-7

Ma, J., Wang, Y., Jin, C., Hu, Y., Bocherens, H. 2019. Ecological flexibility and differential survival of Pleistocene *Stegodon orientalis* and *Elephas maximus* in mainland southeast Asia revealed by stable isotopes (C, O) analysis. Quaternary Science Reviews, 212: 33–44. https://doi.org/10.1016/j.quascirev.2019.03.021

Madden, R.H., 2015. Hypsodonty in Mammals: Evolution, Geomorphology and the Role of Earth Surface Process. Cambridge University Press, Cambridge.

Mann, D.H., Groves, P., Gaglioti, B.V., Shapiro, B.A., 2019. Climate-driven ecological stability as a globally shared cause of Late Quaternary megafaunal extinctions: the Plaids and Stripes Hypothesis. Biological Reviews, 94: 328–352. https://doi.org/10.1111/brv.12456

Metera, E., Sakowski, T., Słoniewski, K., Romanowicz, B., 2010. Grazing as a tool to maintain biodiversity of grassland – a review. Animal Science Papers and Reports, 28: 315–334.

Milder, S.E.S., 2000. Arqueologia do Sudoeste do Rio Grande do Sul: uma perspectiva geológica. PhD Thesis, Universidade de São Paulo, Programa de Pós-graduação em Arqueologia.

Miller, E.T., 1987. Pesquisas arqueológicas paleoindígenas no Brasil Ocidental. Estudios Atameños, 8: 37–61.

Mourelle, D., del Puerto, L., Perez, L., Bergamino, L., García-Rodríguez, F., 2020. Late Pleistocene and Late Holocene environment and climate in the campos region of southeastern South America inferred from phytolith, diatom and geochemical data. Palaeogeography, Palaeoclimatology, Palaeoecology, 543: 109586. https://doi.org/10.1016/j.palaeo.2020.109586

Newsome, S.D., del Rio, C.M., Bearhop, S., Phillips, D.L., 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment, 5: 429–436. https://doi.org/10.1890/060150.1

Noelli, F.S., 2000. A ocupação humana na região sul do Brasil: arqueologia, debates e perspectivas 1872–2000. Revista USP, 44: 218–269.

O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. Bioscience, 38: 328–336. https://doi.org/10.2307/1310735

Oliveira, E.V., Kerber, L., 2009. Paleontologia e aspectos geológicos das sucessões do final do Neógeno no sudoeste do Rio Grande do Sul, Brasil. Gaea – Journal of Geoscience, 5: 21–34. https://doi.org/10.4013/gaea.2009.51.03

Oliveira, E.V., Dutra, T.L., Zeltzer, F., 2002. Megaterídeos (Mammalia, Xenarthra) do Quaternário de Caçapava do Sul, Rio Grande do Sul, com considerações sobre a flora associada. Geologia Colombiana, 27: 77–86.

Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R., Forneck, E.D., 2007. Brazil's neglected biome: the South Brazilian Campos. Perspectives in Plant Ecology, Evolution and Systematics, 9: 101–116. https://doi.org/10.1016/j.ppees.2007.07.005

Parnell, A., Inger, R., 2016. Stable isotope mixing models in R with simmr. Accessed on 05 January 2023. https://cran.r-project.org/web/packages/simmr/vignettes/simmr.html

Pau, S., Edwards, E.J., Still, C.J., 2013. Improving our understanding of environmental controls on the distribution of C_3 and C_4 grasses. Global Change Biology, 19: 184–196. https://doi.org/10.1111/gcb.12037

Pearcy, R.W., Ehleringer, J., 1984. Comparative ecophysiology of C_3 and C_4 plants. Plant, Cell & Environment, 7: 1–13. https://doi.org/10.1111/j.1365-3040.1984.tb01194.x

Peres, C.A., 1996. Population status of white-lipped *Tayassu pecari* and collared peccaries *T. tajacu* in hunted and unhunted Amazonian forests. Biological Conservation, 77: 115–123. https://doi.org/10.1016/0006-3207(96)00010-9

Prates, L., Perez, S.I., 2021. Late Pleistocene South American megafaunal extinctions associated with rise of Fishtail points and human population. Nature Communications 12: 2175. https://doi.org/10.1038/s41467-021-22506-4

Prevosti, F.J., Vizcaíno, S.F., 2006. Paleoecology of the large carnivore guild from the late Pleistocene of Argentina. Acta Palaeontologica Polonica, 51: 407–422.

R Core Team 2020. R: A Language and Environment for Statistical Computing. R Core Team, Geneva.

Roesch, L.F.W., Vieira, F.C.B., Pereira, V.A., Schünemann, A.L., Teixeira, I.F., Senna, A.J.T., Stefenon, V.M., 2009. The Brazilian Pampa: a fragile biome. Diversity, 1: 182–198. https://doi.org/10.3390/d1020182

Santos, M.C.P., 2023. Les premières occupations humaines dans le Sud du Brésil: une vision géoarchéologique intégrée. L'anthropogie, 127: 103136. https://doi.org/10.1016/j.anthro.2023.103136

Sanz-Pérez, D., Fernández, M.H., Tomassini, R.L., Montalvo, C.I., Beilinson, E., Gasparini, G.M., Domingo, L., 2022. The Pampean region (Argentina) underwent larger variation in aridity than in temperature during the Late Pleistocene: new evidence from the isotopic analysis of mammalian taxa. Quaternary Science Reviews, 286: 107555. https://doi.org/10.1016/j.quascirev.2022.107555

Scherer, C.S., 2013. The Camelidae (Mammalia, Artiodactyla) from the Quaternary of South America: cladistic and biogeographic hypotheses. Journal of Mammalian Evolution, 20: 45–56. https://doi.org/10.1007/s10914-012-9203-4

Scherer, C.S., Pitana, V.G., Ribeiro, A.M., 2009. Proterotheriidae and Macraucheniidae (Litopterna, Mammalia) from the Pleistocene of Rio Grande do Sul State, Brazil. Revista Brasileira de Paleontologia, 12: 231–246. https://doi.org/10.4072/rbp.2009.3.06

Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffer, L., Passey, B., Cerling, T., Dearing, D., Ehleringer, J., 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits, and horses. Small Ruminant Research, 48: 149–154. https://doi.org/10.1016/S0921-4488(03)00002-6

Tejada, J.V., Flynn, J.J., MacPhee, R., O'Connell, T.C., 2021. Isotope data from amino acids indicate Darwin's ground sloth was not an herbivore. Scientific Reports, 11: 18944. https://doi.org/10.1038/s41598-021-97996-9

Tejada-Lara, J.V., MacFadden, B.J., Bermudez, L., Rojas, G., Salas-Gismondi, R., Flynn, J.J., 2018. Body mass predicts isotope enrichment in herbivorous mammals. Proceedings of the Royal Society B, 285: 20181020. https://doi.org/10.1098/rspb.2018.1020

Villwock, J.A., Tomazelli, L.J., 1995. Geologia costeira do Rio Grande do Sul. Notas Técnicas, 8: 1–45.

Voeten, M., Prins, H., 1999. Resource partitioning between sympatric wild and domestic herbivores in the Tarangire region of Tanzania. Oecologia, 120: 287–294. https://doi.org/10.1007/s004420050860

Webb, S.D., 1985. Late Cenozoic mammal dispersals between the Americas. In: Stehli, F.G., Webb, S.D. (eds), The Great American Biotic Interchange. Topics in Geobiology, vol 4. Springer, Boston. https://doi.org/10.1007/978-1-4684-9181-4_14

Webb, S.D., 1991. Ecogeography and the Great American Interchange. Paleobiology, 17: 266–280. https://doi.org/10.1017/S0094837300010605

Webb, S.D., 2006. The Great American Biotic Interchange: patterns and processes. Annals of the Missouri Botanical Garden, 93: 245–257. https://doi.org/10.3417/0026-6493(2006)93[245:TGABIP]2.0.CO;2

Woodburne, M.O., 2010. The Great American Biotic Interchange: dispersals, tectonics, climate, sea level and holding pens. Journal of Mammalian Evolution, 17: 245–264. https://doi.org/10.1007/s10914-010-9144-8

Wrege, M.S., Steinmetz, S., Júnior, C.R., Almeida, I.R., 2012. Atlas Climático da Região Sul do Brasil: Estados do Paraná, Santa Catarina e Rio Grande do Sul (Segunda Edição). Embrapa, Brasília.

CAPÍTULO 3

Pleistocene cetacean fossils from the coastal plain of Rio Grande do Sul in southern Brazil

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Pleistocene cetacean fossils from the coastal plain of Rio Grande do Sul in southern Brazil

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Abstract

Cetacean ear bones are useful in paleontological studies because it is possible to identify most species based on their gross morphology, and thus glean information about past occurrences and distributions. This study describes cetacean tympanic bullae and periotics preserved in Middle-Late Pleistocene coastal barrier systems from Rio Grande do Sul in southern Brazil. The specimens were collected ex situ on the modern beach, and reveal the presence of the Franciscana dolphin, *Pontoporia blainvillei*, an indeterminate delphinid, the rorqual whale, *Balaenoptera*, and the right whale, *Eubalaena*. Given the current diversity of dolphins and whales in the Atlantic Ocean, these fossils suggest that the reported diversity of Pleistocene cetaceans is probably underestimated. The barrier systems of Rio Grande do Sul are the only Brazilian deposits that have preserved Pleistocene cetacean remains to date.

Introduction

The climate during the Pleistocene (2.58–0.01 Ma) was marked by cycles of cooling (glacial) followed by rapid warming (interglacial periods) triggered by variations in summer insolation (i.e., Milankovitch orbital influence; Kawamura et al. 2007) and hysteresis of the ice sheet volume in the Northern Hemisphere (Abe-Ouchi et al. 2013). During glacial periods, the expansion of the sea ice around Antarctica led to changes in Southern Hemisphere climate and geography (Otto-Bliesner et al. 2007, Ferrari et al. 2014). High ice volume corresponded to low sea-level, exposing the inner continental shelf and reducing the available habitat for coastal marine species (Ludt & Rocha 2015). Conversely, sea-level rise along with deglaciation events resulted in continental shelf flooding (Denton et al. 2010).

The glacial–interglacial cycles coincided with variations in the oceanic circulation, water temperature, stratification, nutrient distribution, and seawater solubility of CO_2 (e.g., Bischof & Darby 1997, Stephens & Keeling 2000, Mackensen et al. 2001, Ferrari et al. 2014, Lazar & Polyak 2016, Martin-Garcia et al. 2018). These changes impacted the marine biota from phytoplankton to higher trophic levels (e.g., Thatje et al. 2008). The fossil record is thus key to understanding the effects of climate change on such organisms over time, and can provide information on the evolution, distribution and extinction of species (Fordyce 2009). For example, glacial episodes affecting the past distribution of pygmy right whales (*Caperea* Gray, 1846) and right whales (*Eubalaena* Gray, 1864), as demonstrated by fossils, has shown that their regional ranges were once much broader than they are today (see Tsai et al. 2017, Tsai & Chang 2019).

Cetacean ear bones represented by the tympanic bullae and periotics or petrosals are especially useful for such studies because they exhibit morphologies that are diagnostic to genus and/or species level (Kasuya 1973, Ekdale et al. 2011, Gol'din & Steeman 2015). Unfortunately, cetaceans have a sparse fossil record (Fordyce 2018), particularly in the Pleistocene of the Southern Hemisphere. Here we therefore describe a collection of fossil cetacean ear bones recovered from the coastal plain of Rio Grande do Sul in southern Brazil, and discuss the implications for historical species distributions.

Geological and environmental setting

The coastal plain of Rio Grande do Sul (Fig. 1) corresponds to the onshore portion of the Pelotas Sedimentary Basin in southern Brazil. It originated with erosion of Precambrian and Palaeozoic basement rocks, which provided the main sediment source for barrier-lagoon depositional systems during the Quaternary (Closs 1970, Figueiredo Jr 1975, Villwock & Tomazelli 1995, Tomazelli et al. 2000). These were controlled by sea-level changes initiated by glacial–interglacial cycles. Four successive barrier systems have been recognized, and stratigraphically designated Barriers I, II, III and IV, with each developing during successive sea-level highstands. The intervening sea-level drops prograded these barrier systems seaward (Villwock & Tomazelli 1995, Tomazelli et al. 2000). The sea-level highstands reached up to 9 m, 8 m and 3 m above current levels as correlated from Barriers II, III during the Pleistocene, and Barrier IV during the Holocene; the maximum sea-level lowstand was otherwise more than 100 m below current levels (Rosa et al. 2017). The ages of the barrier systems correlate with

the oxygen isotope curves documented by Imbrie et al. (1984), with stages 9, 7e, 5, and 1 representing 325, 220, 120 and 6 ka (= thousand years ago), respectively (Villwock & Tomazelli 1995, Lopes et al. 2014). Almost 60% of the exposed deposits mapped on the coastal plain of Rio Grande do Sul are therefore of Pleistocene age (Rosa 2012). The barriers are composed mainly of sand and can be lithologically distinguished from lagoonal mud deposits by their undulating surfaces produced via aeolian activity. The eolian deposits in Barrier I reach up to 100 m, whereas Barriers II to IV are progressively lower (Rosa et al. 2017).

Geographically, the modern coastline of Rio Grande do Sul has no volcanic rocky promontories except in the extreme north. The wave-dominated beaches range from intermediate to dissipative (Dillenburg et al. 2009, Calliari & Toldo 2016), and are controlled by the Malvinas/Falkland Current, which transports Subantarctic shelf water northward, as well as by the Brazil Current, which transports tropical waters southward from the central South Atlantic. Discharges from the La Plata River and Patos Lagoon are also influential (Möller Jr et al. 2008), with the Patos Lagoon being established by the Late Pleistocene (~120 ka), as evidenced from Barrier III (Tomazelli et al. 2000). Diatom assemblages suggest a predominantly marine environment (Santos-Fischer et al. 2016) with inlets connecting to the sea. However, these palaeochannels filled with sediments during the Holocene when Barrier IV became more continuous (Weschenfelder et al. 2010, Santos-Fischer et al. 2016). Currently, the Patos Lagoon receives fluvial discharges from several tributary rivers and transports water to the ocean through a single large inlet (Vaz et al. 2006).

The fossil cetacean ear bones described here were collected by F.S.B. and Luis Rota between 1999 and 2015 on the beaches along the coastal plain of Rio Grande do Sul. They derived from submarine deposits formed during sea level oscillations in the Middle to Late Pleistocene (Lopes et al. 2014). The main source stratum was probably Barrier III, which is subject to direct erosion by wave action (Buchmann 2002). However, since the fossils were found ex situ, and therefore without stratigraphic context, this stratigraphic attribution remains speculative. Indeed, the assemblage is clearly time-averaged and incorporates both marine and terrestrial mammal remains (e.g., Ribeiro et al. 1998, Buchmann 2002, Lopes et al. 2006, Lopes & Buchmann 2011, Cruz et al. 2016). The terrestrial mammal fossils have been dated at between 700 ka and 18 ka using electron spin resonance (Lopes et al. 2010). Marine mammals thus presumably occupied the area when inter-glacial sea-levels were high, versus glacial intervals when the continental shelf was exposed and terrestrial mammal remains were able to accumulate (Lopes & Buchmann 2011).

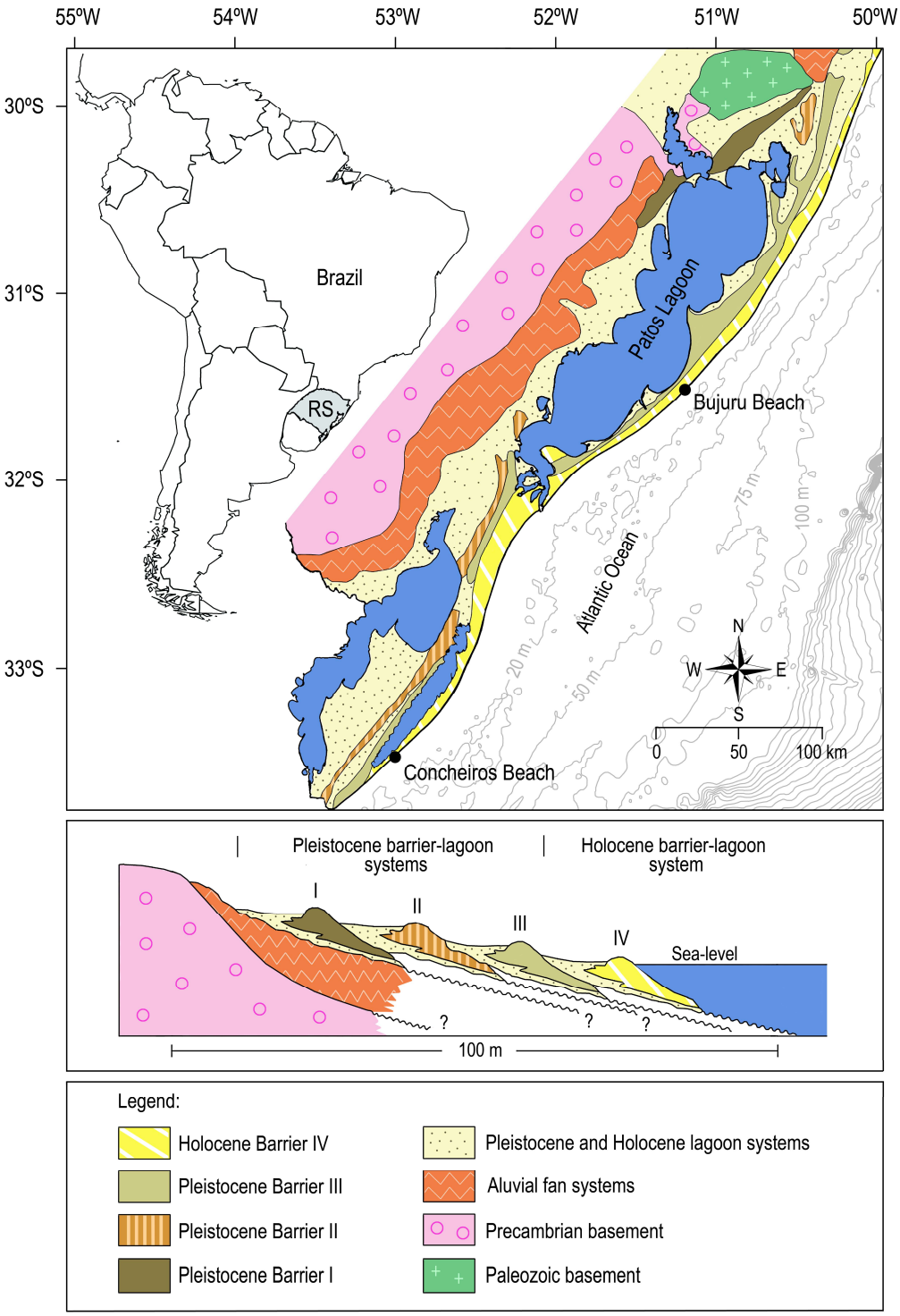


Fig. 1 Simplified geological map of the coastal plain of Rio Grande do Sul based on Dillenburg & Barboza (2014); schematic profile of the Pleistocene and Holocene barrier-lagoon systems follows Tomazelli et al. (2000). Isobaths are from the Geological Service of Brazil (CPRM) and follow Becker et al. (2009). Black circles indicate the main sites for cetacean fossils. Geographic datum WGS84.

Finally, the fossils at Rio Grande do Sul are known to accumulate on submarine topographic highs along the continental shelf (Buchmann et al. 2001, Buchmann, 2002). These

are most densely distributed along the southern coast, which also produces the most fossil occurrences (Correa 1990). Semi-diurnal microtides average about 0.5 m and waves are thus responsible for sediment transport and deposition via longshore drift (Dillenburg et al. 2000, 2004, Martinho et al. 2009). Erosion via wave action then transports material to the beach, and especially during storms (Buchmann 2002), when wave height can reach as much 7 m (Machado et al. 2010).

Materials and methods

Our anatomical terminology follows Mead & Fordyce (2009), except for mysticetes which follow Ekdale et al. (2011). Taxonomic identifications were based on Kasuya (1973), Oishi & Hasegawa (1994), Ekdale et al. (2011), and Gol'din & Steeman (2015). Tympanic bullae measurements were obtained using a digital calliper (error margin of 0.02mm) following the methodology of Kasuya (1973) for odontocetes: (1) length of the tympanic bulla, linear distance from the anterior tip to the end of the outer posterior prominence; (2) from the anterior tip to the end of the inner posterior prominence; (3) from the tip of the conical process to the end of the outer posterior prominence; (4) width of the tympanic bulla, distance from the margin of the outer lip to the extreme end of the involucrum; (5) width across outer and inner posterior prominences; and (6) width of the tympanic notch. For mysticetes, only the maximum length and width were obtained because their tympanic bullae tend to be poorly preserved at Rio Grande do Sul. Periotic measurements followed Bisconti (2005) as follows: (1) length of the posterior process; (2) width of the posterior process at mid-length, measured on the ventral side; (3) length of the anterior process; (4) width of the anterior process; and (5) diameter of the promontorium.

Institutional abbreviation

MCN-PV, Museu de Ciências Naturais (Secretaria do Meio Ambiente e Infraestrutura – SEMA), Porto Alegre, Brazil.

Systematic Palaeontology

Order CETACEA Brisson, 1762

Suborder ODONTOCETI Flower, 1867

Family PONTOPORIIDAE Kasuya, 1973

Genus **Pontoporia** Gray, 1846

Type species. *Pontoporia blainvillei* Gervais and d'Orbigny, 1844

Pontoporia blainvillei Gervais and d'Orbigny, 1844 (Fig. 2)

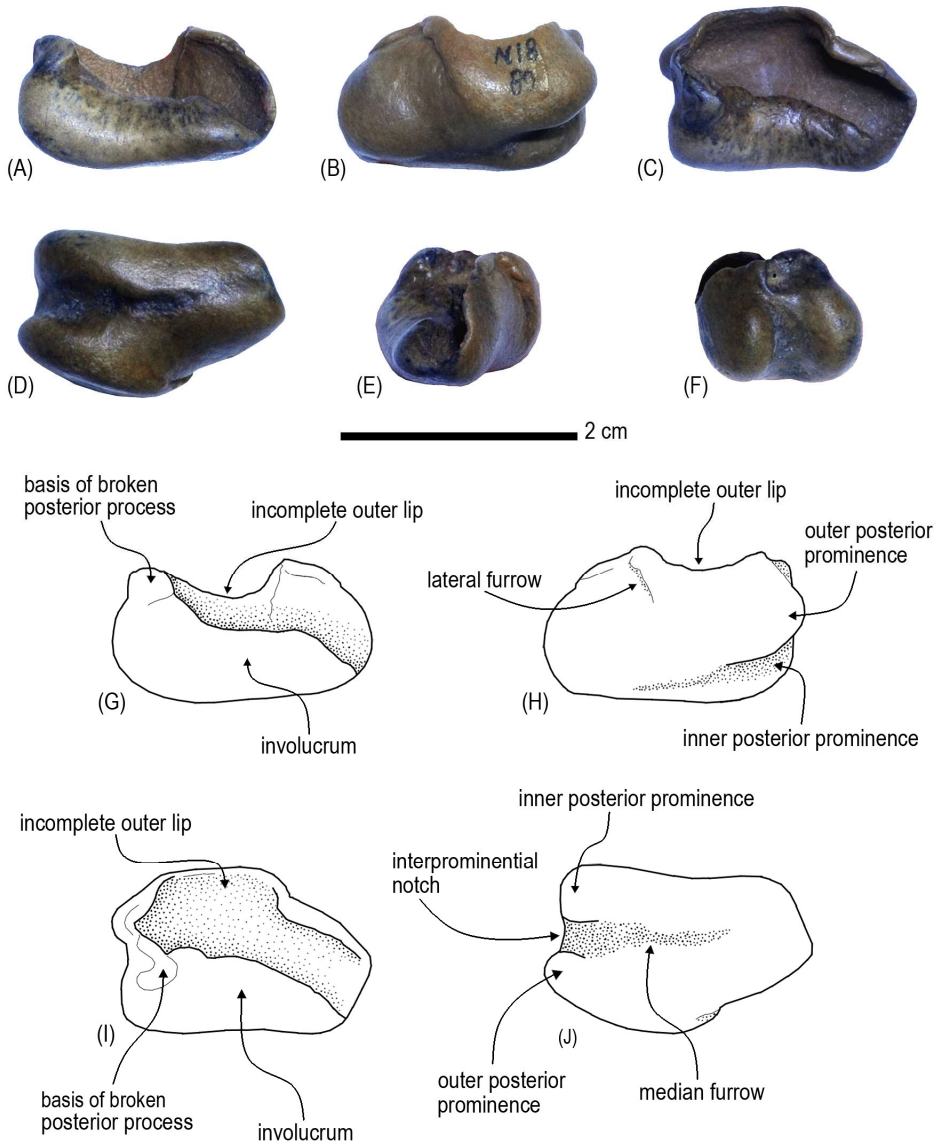


Fig. 2 Left tympanic bulla of *Pontoporia blainvillei* (MCN-PV-36954) in A, medial; B, lateral; C, dorsal; D, ventral; E, anterior; and F, posterior views; and the corresponding line drawings of the G, medial; H, lateral; I, dorsal; and J, ventral views.

Holotype. Skull collected by M. de Fréminville from the mouth of La Plata River, near Montevideo, Uruguay. Museum National d'Histoire Naturelle (MNHM), Paris, catalog number not specified (see Robineau 1989).

Referred material. MCN-PV-36954 left tympanic bulla.

Locality, unit and age. Collected on 3 May 1999 from undifferentiated Middle–Late Pleistocene deposits at Bujuru Beach (31º43'45.25''S, 51º28'055.04''W).

Description. MCN-PV-36954 is broken such that the posterior process, accessory ossicle, and part of the outer lip are missing. It measures 22.6 mm from the anterior tip to the end of the outer posterior prominence. Both posterior prominences are of similar size, with the inner being wider and shorter than the outer. As a result, the distance from the anterior tip to the end of the inner posterior prominence is about 22.1 mm. The width across the outer and inner posterior prominences is 11.6 mm, and the width of the entire tympanic bulla is 13.2 mm. The lateral furrow is visible in the outer lip (Fig. 2B, H). In ventral view, the tympanic bulla is laterally convex with the median furrow being parallel with the involucrum; the anterior tip is square. There is a conspicuous expansion along the outer lip between the lateral furrow and the outer posterior prominence (Fig. 2D, J).

Remarks. The tympanic bullae of platanistids, iniids and pontoporiids exhibit the ancestral condition of Odontoceti in possessing a lateral furrow, which differs from phocoenids and delphinids where the lateral furrow is almost absent. We identified MCN-PV-36954 as a Franciscana dolphin, *Pontoporia blainvillei*, based on expansion of the outer lip between the lateral furrow and the outer posterior prominence. The tympanic bullae of pontoporiids and kogiids are also the smallest among extant toothed whales, but posterior prominences are better developed in kogiids. Kasuya (1973) obtained a tympanic bulla length of 24.3 mm for *P. blainvillei*, which is similar to that of MCN-PV-36954. The convex lateral shape of the tympanic bulla and square anterior tip are further consistent with *P. blainvillei* as defined by Kasuya (1973).

Family DELPHINIDAE Gray, 1821

DELPHINIDAE gen. et sp. indet. (Fig. 3)

Referred material. MCN-PV-36955 left tympanic bulla

Locality, unit and age. Collected on 3 May 1999 from undifferentiated Middle-Late Pleistocene depositis at Bujuru Beach (31º40'37.84"S, 51º24'09.43"W).

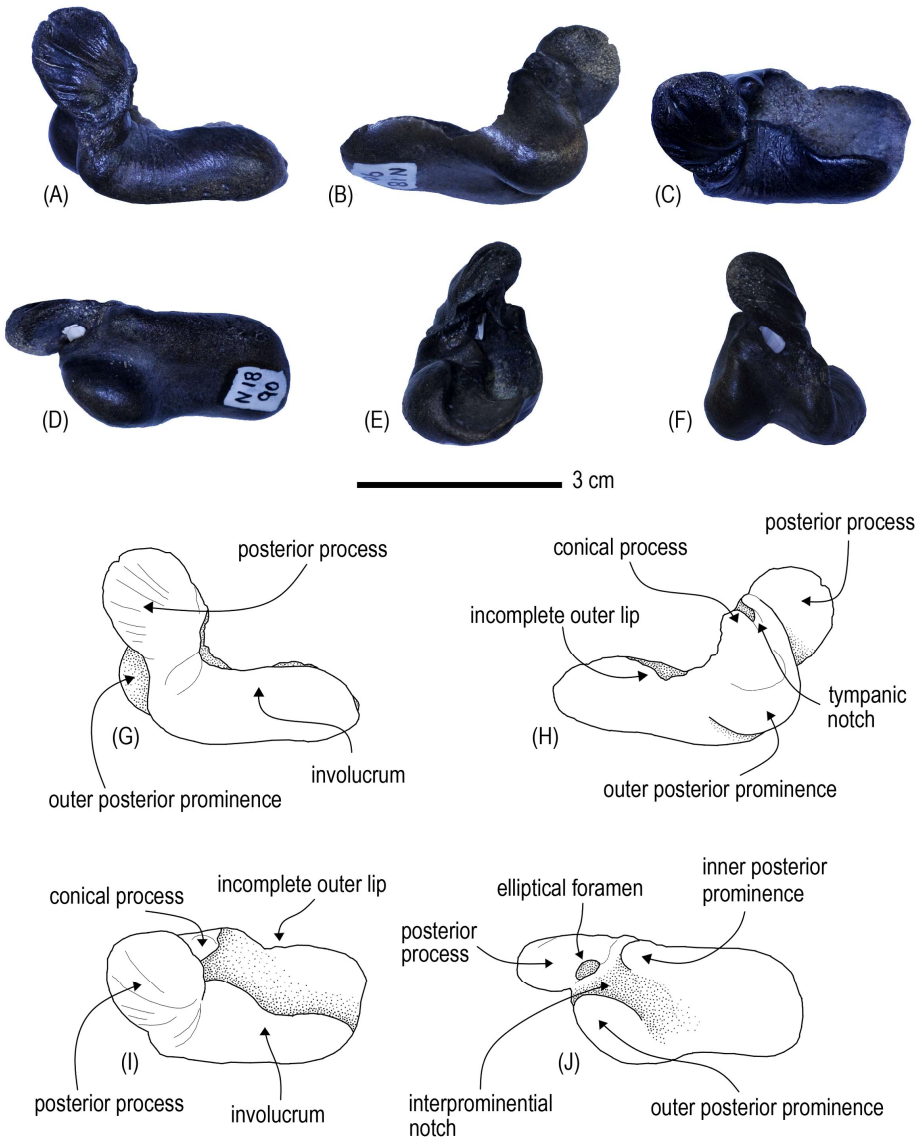


Fig. 3 Left tympanic bulla of Delphinidae gen. et sp. indet. (MCN-PV-36955) in A, medial; B, lateral; C, dorsal; D, ventral; E, anterior; and F, posterior views; and the corresponding line drawings of the G, medial; H, lateral; I, dorsal; and J, ventral views.

Description. MCN-PV-36955 is an incomplete tympanic bulla with a broken outer lip. The sigmoid process and accessory ossicle are therefore not observable. Conversely, the posterior process, conical process, involucrum and posterior prominences are well preserved. The

posterior process is laterally projecting (see Fig. 3D, F, J), and has longitudinal grooves along its facet (Fig. 3A, G). The inner posterior prominence is thinner and smaller than the outer posterior prominence. MCNPV-36955 also has a weak ventral keel developed from the anterior continuation of an eminence from the inner posterior prominence. Additional features include an elliptical foramen (Fig. 3J), and a deep notch between the anterior and posterior prominences (Fig. 3F). There is no evidence of bilateral compression. MCN-PV-36955 measures 35.8 mm long; 34.5 mm from the anterior tip to the end of the inner prominence; 18.2 mm from the tip of the conical process to the end of the outer prominence; 17.3 mm wide across the outer and inner prominences. The tympanic notch is 2.7 mm wide.

Remarks. MCN-PV-36955 is characteristic of delphinids. Key features include a laterally projecting posterior process with longitudinal grooves, and an outer posterior prominence that is larger than the inner posterior prominence (Kasuya 1973). MCN-PV-36955 is otherwise too incomplete for identification beyond family level.

Suborder MYSTICETI Flower, 1864
Family BALAENOPTERIDAE Gray, 1864

Genus **Balaenoptera** Lacépède, 1804

Type species. Balaenoptera physalus Linnaeus, 1758

Balaenoptera sp. (Figs 4, 5)

Referred material. MCN-PV-36959, left tympanic bulla; MCN-PV-36957, left tympanic bulla.

Locality, unit and age. MCN-PV-36959 collected in December 2010 at Concheiros Beach (33°32'23.57"S, 53°05'54.24"W); MCN-PV-36957 collected in May 2015 at Concheiros Beach. All deposits are undifferentiated Middle–Late Pleistocene in age.

Description. The tympanic bullae of MCN-PV-36959 and MCN-PV-36957 are both ovoid and slightly convex. They appear to have had shallow tympanic cavities and ovoid Eustachian openings. MCN-PV-36959 is the best preserved (Fig. 4), and shows a small depression on the

tympanic lip, which likely represents the lateral furrow (see Fig. 4A); the posterior lobe thus seems to have been longer than the anterior. The sigmoid and conical processes are not preserved. MCN-PV-36959 is 106.6mm long and 63.1mm wide. MCN-PV-36957 is similar to MCN-PV-36959, except for the posterior half, which is missing (Fig. 5). Based on its shape, it was probably broken around the lateral furrow prior to burial and eroded by wave action after fossilization. MCN-PV-36957 measures 53.7 mm long and 47.4 mm wide.

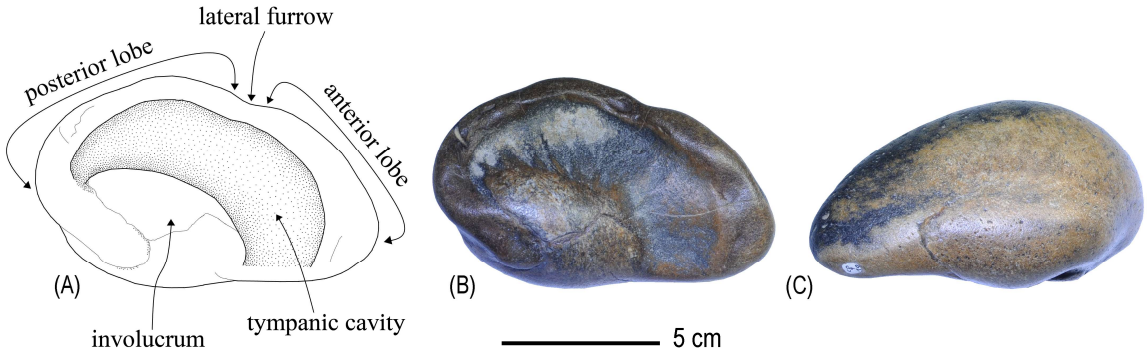


Fig. 4 Left tympanic bulla of *Balaenoptera* sp. (MCN-PV-36959) in A, B, dorsal; and C, ventral views.

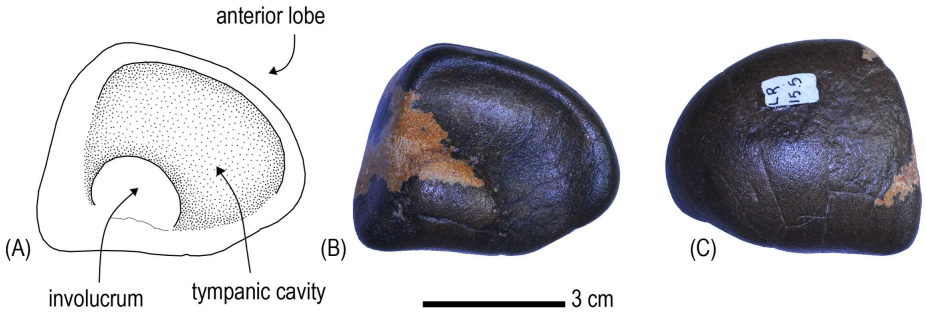


Fig. 5 Left tympanic bulla of *Balaenoptera* sp. (MCN-PV-36957) in A, B, dorsal; and C, ventral views.

Remarks. The tympanic bullae of mysticetes are diagnostic at family level (Gol'din & Steeman 2015). For instance, the tympanic bullae of balaenids are rhomboid, whereas those of balaenopterids and eschrichtiids are ovoid (Oishi & Hasegawa 1994, Ekdale et al. 2011, Gol'din & Steeman 2015). The tympanic bullae of MCN-PV-36959 and MCN-PV-36957 are likewise ovoid (Figs 4, 5), but differ from those of eschrichtiids in having a larger and longer posterior lobe and ovoid eustachian opening (Ekdale et al. 2011). This is consistent with balaenopterids. The extant balaenopterid genera *Balaenoptera* and *Megaptera* Gray, 1846 comprise at least seven species in the southwestern Atlantic Ocean: the Common minke whale, *Balaenoptera*

acutorostrata Lacépède, 1804, Antarctic minke whale, *Balaenoptera bonaerensis* Burmeister, 1867, Sei whale, *Balaenoptera borealis* Lesson, 1828, Bryde's whale, *Balaenoptera edeni* Anderson, 1878, Blue whale, *Balaenoptera musculus* Linnaeus, 1758, Fin whale, *Balaenoptera physalus*, and the Humpback whale, *Megaptera novaeangliae* Borowski, 1781 (Bastida et al. 2018); all of these taxa are known from stranding records on the Rio Grande do Sul (Prado et al. 2016). Based on their tympanic bullae, we infer that MCNPV-36959 and MCN-PV-36957 are not attributable to *M. novaeangliae*. This species is otherwise characterized by a deep and enlarged tympanic cavity and broadly convex ventral surface (Ekdale et al. 2011). Unfortunately, the broken sigmoid and conical processes prevent identification to species level (Oishi & Hasegawa 1994, Ekdale et al. 2011).

Family BALAENIDAE Gray, 1821

BALAENIDAE gen. et sp. indet. (Fig. 6)

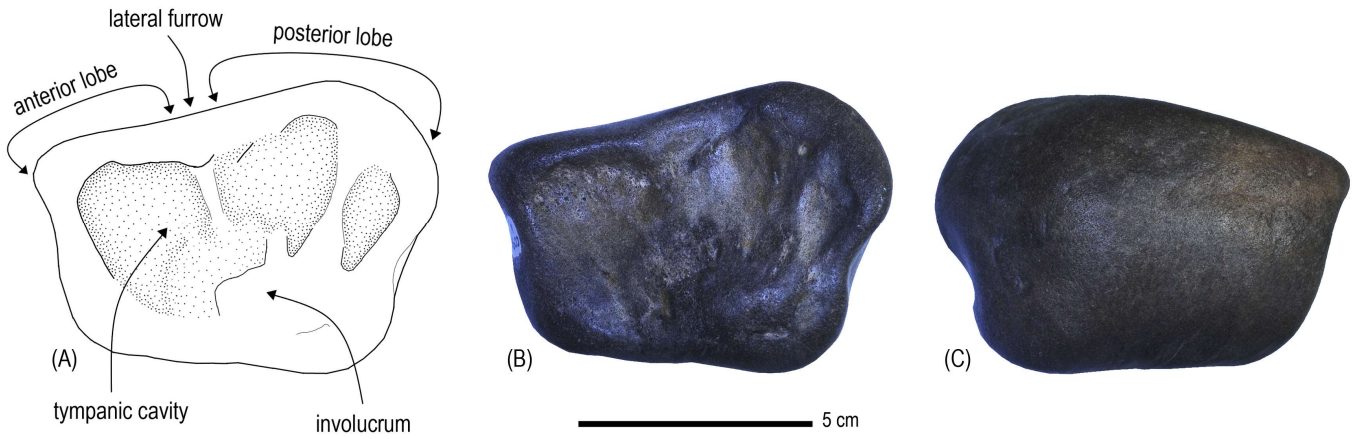


Fig. 6 Left tympanic bulla of Balaenidae gen. et sp. indet. (MCN-PV-36960) in A, B, dorsal; and C, ventral views.

Referred material. MCN-PV-36960 right tympanic bulla.

Locality, unit and age. Collected in 2014 from undifferentiated Middle-Late Pleistocene deposits at Concheiros Beach (33°32'23.57"S, 53°05'54.24"W).

Description. MCN-PV-36960 shows signs of abrasion by wave action and suspended sediments (Fig. 6). It is approximately 88.5 mm long and 59.3 mm wide. The tympanic bulla is rhomboid and apparently had a shallow tympanic cavity, squared Eustachian opening, and posterior lobe

that was larger than the anterior lobe (Fig. 6A, B). The development and orientation of the lateral furrow and sigmoid fissure, as well as size of the conical process are indeterminable.

Remarks. The rhomboid tympanic bulla of MCN-PV-36960 resembles those of balaenids (Oishi & Hasegawa 1994, Ekdale et al. 2011, Gol'din & Steeman 2015). Because periotics referrable to *Eubalaena* have been recovered from Concheiros Beach, we also suggest that MCN-PV-36960 may belong to this taxon.

Genus **Eubalaena** Gray, 1864

Type species. Eubalaena australis Desmoulins, 1822

Eubalaena sp. (Figs 7, 8)

Referred material. MCN-PV-36956 right periotic; MCN-PV-36958 right periotic.

Locality, unit and age. MCN-PV-36956 was collected in January 2015, and MCN-PV-36958 in December 2011 from undifferentiated Middle-Late Pleistocene deposits at Concheiros Beach (33°32'23.57"S, 53°05'54.24"W).

Description. MCN-PV-36956 and MCN-PV-36958 both exhibit posterior processes that form acute angles with the anterior processes (less evident in MCN-PV-36958 because of poor preservation). The anterior processes are also shorter and wider than the posterior processes, with the promontoria being rounded and convex on their ventral sides. Other features include a facial nerve sulcus formed by a deep groove with protuberant margins, and large, almost elliptical fenestra cochleae (Figs 7A, D, 8A, D). In dorsomedial view, the open hiatus Fallopii is visible at the junction of the promontorium and anterior process, as is the suprameatal region, endolymphatic and perilymphatic foramina, and the openings for the facial (CN VII) and vestibulocochlear (CN VIII) nerves, which are separated by the crista transversa (Figs 7C, E, 8C, E). The maximum length of the periotic in MCN-PV-36956 is 117.9 mm. The posterior process measures 73.5 mm long and 29.8 mm wide. The anterior process measures 29.8 mm long and 57.6 mm wide. The diameter of the promontorium is 37.4 mm. The periotic in MCN-

PV-36958 has suffered edge-rounding, thus its posterior process is 34.8 mm long and 27.7 mm wide; the anterior process is 39.2 mm long and 57.6 mm wide; the promontorium has a diameter of 37.4 mm.

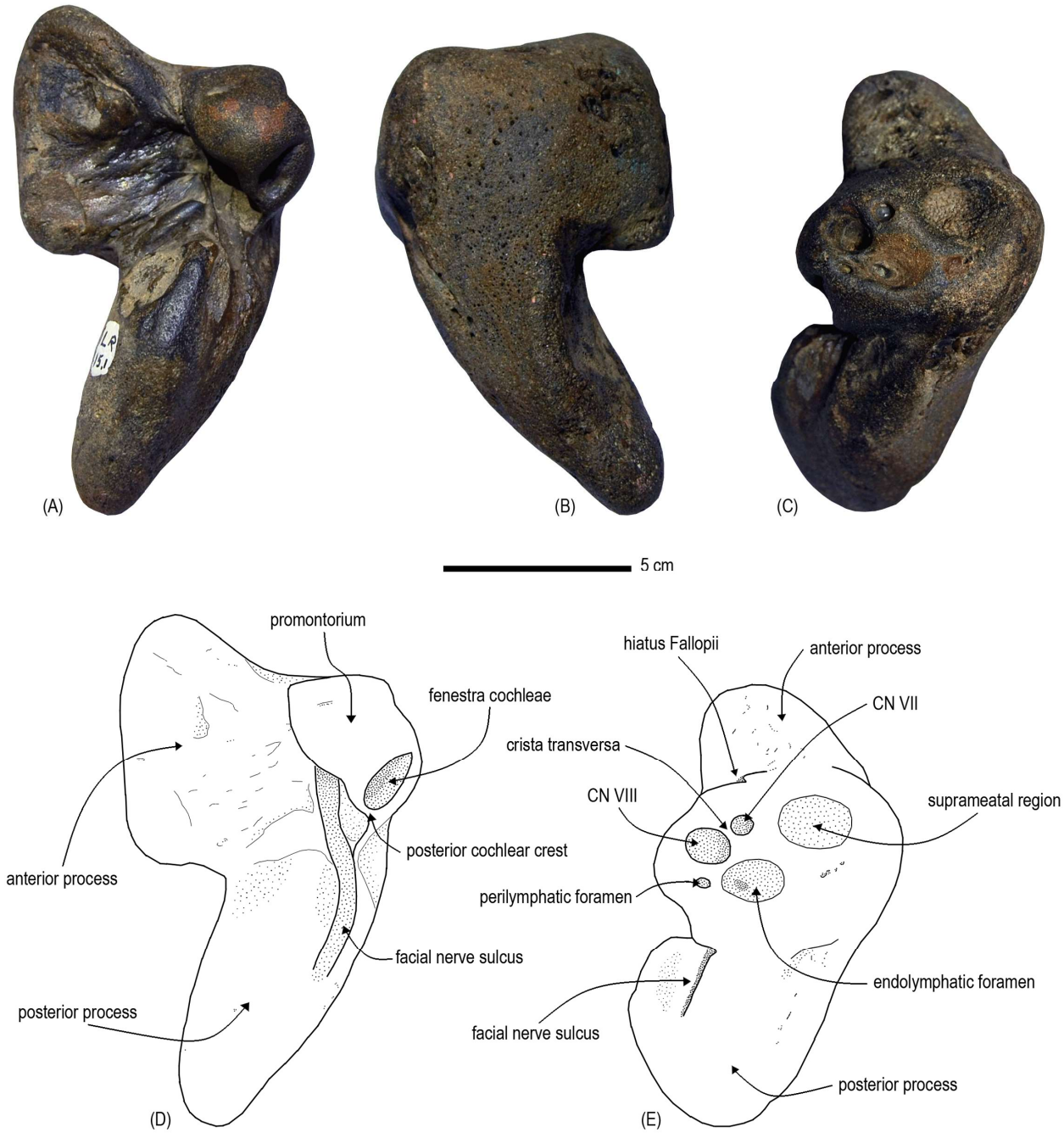


Fig. 7 Right periotic of *Eubalaena* sp. (MCN-PV-36956) in A, ventral; B, dorsal; and C, dorsomedial views; and the corresponding line drawings of the D, ventral; and E, dorsomedial views.

Remarks. MCN-PV-36956 and MCN-PV-36958 resemble the periotics of balaenids (see Ekdale et al. 2011), which also have posterior and anterior processes separated by an acute angle, and

rounded and convex promontoria in ventral view. Comparing between the balaenid genera *Eubalaena* and *Balaena* Linnaeus, 1758, the periotics of *Eubalaena* possess a longer posterior process and lack the planar area between the promontorium and anterior process typical of *Balaena*; they also exhibit a conspicuously concave suprameatal region (Ekdale et al. 2011).

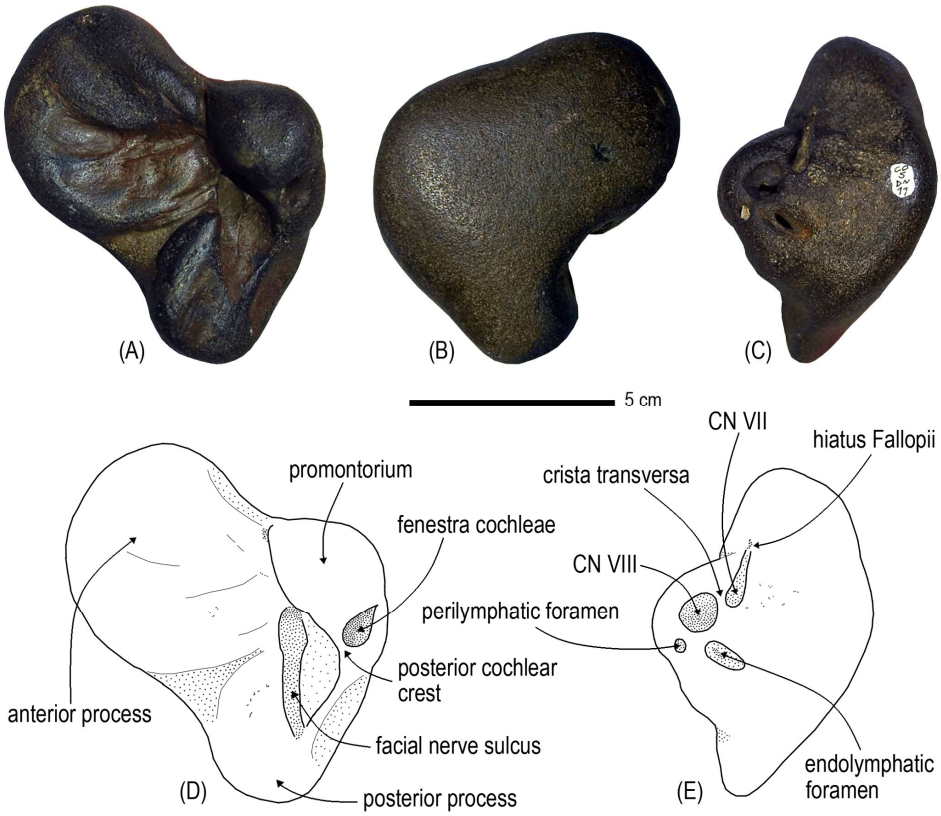


Fig. 8 Right periotic of *Eubalaena* sp. (MCN-PV-36958) in A, ventral; B, dorsal; and C dorsomedial views; and the corresponding line drawings of the D, ventral; and E, dorsomedial views.

Extant balaenids are antitropical (Davies 1963), but likely expanded their distributional ranges during past glacial periods (Rosenbaum et al. 2000). The southern right whale, *Eubalaena australis*, occurs in coastal waters of the southwestern Atlantic Ocean today (Seyboth et al. 2015). *E. australis* additionally has stranding records from the Rio Grande do Sul (Prado et al. 2016), and therefore might represent the species at Concheiros Beach, although an alternative species referral cannot be conclusively excluded.

Discussion

Taphonomic observations

All of the fossil tympanic bullae and periotics reported here are dark in colour, which is similar to terrestrial mammal fossils collected along the coastal plain of Rio Grande do Sul (e.g., Lopes et al. 2008). This distinctive coloration is produced by environmental agents (Fernández-Jalvo & Andrews 2016) involving diagenetic impregnation of iron, barium and yttrium (Lopes & Ferigolo 2015). MCN-PV-36957 also exhibits orange patches probably produced by preservation within a ferruginous lithic concretion (e.g., Haynes 2017). Recovered cetacean ear bones have suffered fragmentation (Fernández-Jalvo & Andrews 2016), and except for MCN-PV-36954 and MCN-PV-36955, extensive abrasion from wave action and transport (Lopes & Ferigolo 2015). Intense edge-rounding from coarse-grained sediments (e.g., Fernández-Jalvo & Andrews 2003, García-Morato et al. 2019) is visible on MCN-PV-36959 and MCN-PV-36957, which also exhibit extensive cracking possibly due to water and/or collagen loss (Fernández-Jalvo & Andrews 2016). On the other hand, MCN-PV-36954 and MCN-PV-36955 show minimal abrasion, but have fractures with jagged margins as would be typical of dried bones (Fernández-Jalvo & Andrews 2016).

Lastly, MCN-PV-36960 has small circular holes interconnected by shallow depressions on its ventral side that may represent bioerosion by sessile epibionts (e.g., Lopes 2012).

Biogeographical implications

Ambiguous stratigraphical differentiation and incomplete documentation has contributed to a scarce fossil record of Pleistocene cetaceans (do Amaral et al. 2018, Fordyce 2018). Our study therefore provides novel information on the past distribution of major clades in the southwestern Atlantic Ocean. About 40 species of toothed whales and nine baleen whales currently inhabit this region (Bastida et al. 2018). Of these, 33 species are occasionally found stranded along the coastal plain of Rio Grande do Sul (Prado et al. 2016). Our recognition of fossils representing the Franciscana dolphin, *Pontoporia blainvillei*, an indeterminate delphinid, the balaenopterid *Balaenoptera*, and balaenid *Eubalaena* thus constitutes a low diversity assemblage compared to the extant cetacean fauna.

Despite the Brazilian coast line stretching over 9,200 km, current records of Quaternary cetacean fossils are restricted to beach deposits in the south and southeast. These include Holocene subfossils recovered from sandy facies in São Paulo, which evidence the modern Blue whale, *Balaenoptera musculus* (Buchmann et al. 2017). Rio Grande do Sul has also previously produced Pleistocene cranial remains of *P. blainvillei* (Ribeiro et al. 1998), and adult and

juvenile balaenids that correlate with sea-level highstand during the Holocene (Lopes et al. 2020). Other marine mammal remains comprise cranial and postcranial elements of the South American sea lion, *Otaria byronia* de Blainville, 1820 (Drehmer & Ribeiro 1998, Rodrigues et al. 2004), which is a common species along the Atlantic coast today (Cappozzo & Perrin 2009). Given their predominantly Middle–Late Pleistocene age, the barrier systems of Rio Grande do Sul are thus important for reconstructing the past ecology and distribution of marine mammals in the Brazilian region.

The terrestrial mammal fossils from Rio Grande do Sul occupied the continental shelf when sea-levels were low (Bergqvist & Almeida 2004). This is comparable to many Quaternary coastal glacial–interglacial successions from around the world, such as the North Sea (van Kolfschoten & Laban 1995), the Pacific coast of Chile (López et al. 2016), and Taiwan (Tsai & Chang 2019). Unfortunately, in southern Brazil these glacio-eustatic sea-level oscillations reworked buried skeletal elements, thus confounding their precise stratigraphical context. Indeed, according to Cruz et al. (2016) about 80% of the bone material found at Rio Grande do Sul cannot be identified because of reworking. For cetaceans, the discovered specimens are often poorly preserved, and not taxonomically assignable beyond family or genus level.

Irrespectively, our recognition of the extant pontopoiid, *P. blainvillei*, is among the stratigraphically oldest occurrence of this species, and accords with previous studies (Ribeiro et al. 1998), as well as similar records from Buenos Aires in Argentina (Cozzuol 1996). Pontoporiids were historically distributed from the South Atlantic and Pacific to the North Atlantic during the Miocene–Pliocene (Post et al. 2017), but are currently restricted to the South Atlantic (Crespo 2018). Likewise, delphinids originated during the Miocene (e.g., Murakami et al. 2014), but radiated during the Plio-Pleistocene (e.g., Aguirre-Fernández et al. 2009, Bianucci 2013). In South America, previous delphinid records include specimens of *Tursiops* Gervais, 1855 and *Orcinus* Fitzinger, 1860, both from Pleistocene strata near Buenos Aires (Cozzuol 1996).

The earliest known balaenids diversified in the Early Miocene (Buono et al., 2017), with the extant genera *Eubalaena* and *Balaena* recognized from the Early Pliocene (e.g., Bisconti 2002, 2003, Field et al. 2017). Skeletal remains of the extant *Eubalaena australis* have been described from Holocene deposits (e.g., Cozzuol 1996, Fitzgerald 2005, Lopes et al. 2020), and likely represent the taxon at Rio Grande do Sul. Similarly, balaenopterids are today distributed across the Pacific and North Atlantic oceans, and have a fossil record that extends back to the Late Miocene (Deméré et al. 2005).

Conclusions

- We describe a collection of cetacean ear bones that were recovered ex situ on Bujuru and Concheiros beaches along the Rio Grande do Sul in southern Brazil.
- The remains were deposited during Pleistocene interglacial intervals when the sea levels were high. They also show traces of fragmentation and abrasion from wave action and transport within a coastal barrier system.
- Our identification of tympanic bullae and periotics representing the Franciscana dolphin, *Pontoporia blainvillei*, an indeterminate delphinid, the rorqual whale, *Balaenoptera*, and the right whale, *Eubalaena*, provides new information about cetacean diversity in the southwestern Atlantic during the Middle–Late Pleistocene.

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References

Abe-Ouchi, A., Saito, F., Kawamura, K., Raymo, M.E., Okuno, J., Takahashi, K. & Blatter, H., 2013. Insolation-driven 100,000-year glacial cycles and hysteresis of ice-sheet volume. *Nature 500*, 190–193.

Aguirre-Fernández, G., Barnes, L.G., Aranda-Manteca, F.J. & Fernández-Rivera, J.R., 2009. *Protoglobicephala mexicana*, a new genus and species of Pliocene fossil dolphin (Cetacea; Odontoceti; Delphinidae) from the Gulf of California, Mexico. *Boletín de la Sociedad Geológica Mexicana 61*, 245–265.

Anderson, J., 1878. *Anatomical and zoological research; comprising an account of zoological results of two expeditions to western Yunnan in 1868 and 1875*. London, 551–564, 44 pls.

Bastida, R., Rodríguez, D., Secchi, E. & Silva, V., 2018. *Mamíferos Aquáticos da América do Sul e Antártica*. Vázquez Mazzini Editores, Buenos Aires, 368 pp.

Becker, J.J. & 17 others, 2009. Global bathymetry and elevation data at 30 arc seconds resolution: SRTM30_PLUS. *Marine Geodesy 32*, 355–371.

Bergqvist, L.P. & Almeida, E.B., 2004. Biodiversidade de mamíferos fósseis brasileiros. *Revista Universidade de Guarulhos Geociências 9*, 54–68.

Bianucci, G., 2013. *Septidelphis morii*, n. gen. et sp., from the Pliocene of Italy: new evidence of the explosive radiation of true dolphins (Odontoceti, Delphinidae). *Journal of Vertebrate Paleontology 33*, 722–740.

Bischof, J.F. & Darby, D.A., 1997. Mid- to Late Pleistocene ice drift in the western Arctic Ocean: evidence for a different circulation in the past. *Science 277*, 74–78.

Bisconti, M., 2002. Na early Late Pliocene right whale (genus *Eubalaena*) from Tuscany (Central Italy). *Bollettino della Società Paleontologica Italiana 41*, 83–91.

Bisconti, M., 2003. Evolutionary history of Balaenidae. *Cranium 20*, 9–50.

Bisconti, M., 2005. Skull morphology and phylogenetic relationships of a new diminutive balaenid from the Lower Pliocene of Belgium. *Paleontology 48*, 793–816.

Borowski, G. H., 1781. *Gemeinnüzzige Naturgeschichte des Thierreichs*. Gottlieb August Lange, Berlin and Stralsund, 196 pp.

Brisson, A.D., 1762. *Regnum animale in classes IX Distributum, sive synopsis methodica*. Leiden, Lugdum Batarorum, apud. Theodorum Haak, 384 pp.

Buchmann, F.S.C., 2002. *Bioclastos de organismos terrestres e marinhos na praia e plataforma interna do Rio Grande do Sul: natureza, distribuição, origem e significado geológico*. PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 122 pp. (unpublished)

Buchmann, F.S.C., Seeliger, M., Zanella, L.R., Madureira, L.S.P., Tomazelli, L.J. & Calliari, L.J., 2001. Análise batimétrica e sedimentológica no estudo do Parcel do Carpinteiro, uma paleolinha de praia pleistocênica na antepraia do Rio Grande do Sul, Brasil. *Pesquisas 28*, 109–115.

Buchmann, F.S., Zurlo, F.M., Vannucchi, F.S. & Martins, C.C.A., 2017. First record of a fossil blue whale in São Paulo state, Brazil. *Aquatic Mammals 43*, 649–654.

Buono, M.R., Fernández, M.S., Cozzuol, M.A., Cuitiño, J.I. & Fitzgerald, E.M.G., 2017. The early Miocene balaenid *Morenocetus parvus* from Patagonia (Argentina) and the evolution of right whales. *PeerJ 5*, e4148.

Burmeister, H., 1867. Preliminary description of a new species of finner whale (*Balaenoptera bonaerensis*). *Proceedings of the Zoological Society 27*, 707–713.

Calliari, L.J. & Toldo, E.E., 2016. Ocean beaches of Rio Grande do Sul. In *Brazilian Beach Systems*. Short, A.D., & Klein, A.H.F., eds, Springer, 507–542.

Cappozzo, H.L. & Perrin, W.F., 2009. South American sea lion. In *Encyclopedia of Marine Mammals 2nd Ed*. Perrin, W.F., Würsig, B. & Thewissen, J.G.M., eds, Academic Press, Cambridge, 1076–1079.

Closs, D., 1970. Estratigrafia da Bacia de Pelotas, Rio Grande do Sul. *Iheringia (Série Geologia) 3*, 3–76.

Correa, I.C.S., 1990. *Analyse morphostructurale et evolution paleogeographique de la plate-forme continentale atlantique sud-bresilienne (Rio Grande do Sul – Bresil)*. PhD thesis, Universite de Bourdeaux, Bourdeaux, 314 pp. (unpublished)

Cozzuol, M.A., 1996. The record of the aquatic mammals in southern South America. *Münchner Geowissenschaftliche Abhandlungen 30*, 321–342.

Crespo, E.A., 2018. Franciscana dolphin: *Pontoporia blainvillei*. In *Encyclopedia of Marine Mammals 3rd Ed*. Würsig, B., Thewissen, J.G.M. & Kovacs, K., eds, Academic Press, Cambridge, 388–392.

Cruz, E.A., Dillenburg, S.R. & Buchmann, F.S., 2016. Description and controls on distribution of Pleistocene vertebrate fossils from the central and southern sectors of the coastal plain of Rio Grande do Sul, Brazil. *Revista Brasileira de Paleontologia 19*, 425–438.

Davies, J.T., 1963. The antitropical fator in cetacean speciation. *Evolution 17*, 107–116.

de Blainville, M.H.D., 1820. Sur quelques crânes des phoques. *Journal de Physique, de Chimie, d'Histoire Naturelle et des Arts 91*, 286–300.

Deméré, T.A., Berta, A. & Mcgowen, M.R., 2005. The taxonomic and evolutionary history of fossil and modern balaenopteroid mysticetes. *Journal of Mammalian Evolution 12*, 99–143.

Denton, G.H., Anderson, R.F., Toggweiler, J.R., Edwards, R.L., Schaefer, J.M. & Putnam, A.E., 2010, The Last Glacial Termination. *Science 328*, 1652–1656.

Desmoulins, A., 1822. Baleine. In *Dictionnaire Classique d'Histoire Naturelle 2*. Saint-Vicent, B., ed., Rey et Gravier, Paris, 155–165.

Dillenburg, S.R. & Barboza, E.G., 2014. The strike-fed sandy coast of southern Brazil. In *Sedimentary Coastal Zones from High to Low Latitudes: Similarities and Differences*.

Martini, I.P., & Wanless, H.R., eds, Geological Society, London, Special Publications 388, 333–352.

Dillenburg, S.R., Roy, P.S., Cowell, P.J. & Tomazelli, L.J., 2000. Influence of antecedent topography on coastal evolution as tested by the shoreface translation-barrier model (STM). *Journal of Coastal Research 16*, 71–81.

Dillenburg, S.R., Esteves, L.S. & Tomazelli, L.J., 2004. A critical evaluation of coastal erosion in Rio Grande do Sul, southern Brazil. *Anais da Academia Brasileira de Ciências 76*, 611–623.

Dillenburg, S.R., Barboza, E.G., Tomazelli, L.J., Hesp, P.A., Clerot, L.C.P. & Ayup-Zouain, R.N., 2009. The Holocene Costal Barriers of Rio Grande do Sul. In *Geology and Geomorphology of Holocene Coastal Barriers of Brazil*. Dillenburg, S.R. & Hesp, P.A., eds, Springer, 53–92.

Do Amaral, K.B., Amaral, A.R., Fordyce, R.E. & Moreno, I.B., 2018. Historical biogeography of Delphininae dolphins and related taxa (Artiodactyla: Delphinidae). *Journal of Mammalian Evolution 25*, 241–259.

Drehmer, C.J. & Ribeiro, A.M., 1998. A temporal bone of an Otariidae (Mammalia, Pinnipedia) from the Late Pleistocene of Rio Grande do Sul state, Brazil. *Revista da Universidade de Guarulhos Geociências 6*, 39–44.

Ekdale, E.G., Berta, A. & Deméré T.A., 2011. The comparative osteology of the petrotympanic complex (ear region) of extant baleen whales (Cetacea: Mysticeti). *PLoS ONE 6*, e21311.

Fernández-Jalvo, Y., & Andrews, P., 2003. Experimental effects of water abrasion on bone fragments. *Journal of taphonomy 1.3*, 147–163.

Fernández-Jalvo, Y. & Andrews, P., 2016. *Atlas of Taphonomic Identifications*. Springer, 359 pp.

Ferrari, R., Jansen, M.F., Adkins, J.F., Burke, A., Stewart, A.L. & Thompson, A.F., 2014. Antarctic sea ice control on ocean circulation in present and glacial climates. *PNAS 111*, 8753–8758.

Field, D.J., Boessenecker, R., Racicot, R.A., Áscjörnsdóttir, L., Jónasson, K., Hsiang, A.Y., Behleke, A.D. & Vinther, J., 2017. The oldest marine vertebrate fossil from the volcanic island of Iceland: a partial right whale skull from the high latitude Pliocene Tjörnes Formation. *Paleontology 60*, 141–148.

Figueiredo Jr., A.G., 1975. *Geologia dos depósitos calcários biodetríticos da plataforma continental do Rio Grande do Sul.* MSc dissertation. Universidade Federal do Rio Grande do Sul, Porto Alegre, 72 pp. (unpublished)

Fitzgerald, E.M.G., 2005. Pliocene marine mammals from the Whalers Bluff Formation of Portland, Victoria, Australia. *Memoirs of Museum Victoria 62*, 67–89.

Fitzinger, L.J., 1860. *Wissenschaftlich-populäre Naturgeschichte der Säugethiere in ihren sämmtlichen Hauptformen. Nebst einer Einleitung in die Naturgeschichte uberhaupt und in die Lehre von den Thieren insbesondere*. Aus der Kaiserlich-königlichen Hof- und Staatsdrucherei, Wien, 364 pp.

Flower, W.H., 1864. Notes on the skeletons of whales in the principal museums of Holland and Belgium, with descriptions of two species apparently new to science. *Proceedings of the Zoological Society of London 1864*, 384–420.

Flower, W.H., 1867. Description of the skeleton of *Inia geoffrensis* and of the skull of *Pontoporia blainvillei*, with remarks on the systematic position of these animals in the order Cetacea. *Transactions of the Zoological Society of London 6*, 87–116.

Fordyce, R.E., 2009. Cetacean fossil record. In *Encyclopedia of Marine Mammals 2nd Ed*. Perrin, W.F., Würsig, B. & Thewissen, J.G.M., eds, Academic Press, Cambridge, 207–215.

Fordyce, R.E., 2018. Cetacean evolution. In *Encyclopedia of Marine Mammals 3rd Ed*. Würsig, B., Thewissen, J.G.M. & Kovacs, K., eds, Academic Press, Cambridge, 180–185.

García-Morato, S., Marin-Monfort, M.D. & Fernández-Jalvo, Y., 2019. Rolling bones: a preliminary study of micromammal abrasion on different initial taphonomic stages. *Palaentologia Electronica 22*, 1–16.

Gervais, M.P., 1855. *Histoire naturelle des mammifères, avec l'indication de leurs moeurs, et de leurs rapports avec les arts, le commerce et l'agriculture* vol. 2. L. Curmer, Paris, 344 pp.

Gervais, P. & d'Orbigny, A., 1844. Mammalogie. *Bulletin de la Société Philomathique 1844*, 38–40.

Gol'din, P. & Steeman, M.E., 2015. From problem taxa to problem solver: a new Miocene family, Tranatocetidae, brings perspective on baleen whale evolution. *PLoS ONE 10*, e0135500.

Gray, J.E., 1821. On the natural arrangement of vertebrose animals. *The London Medical Repository 15*, 296–310.

Gray, J.E., 1846. On the cetaceous animals. In *The zoology of the voyage of H. M. S. Erebus and Terror under the command of Captain Sir James Clark Ross, R. N., F. R. S., during the years 1839 to 1843. Volume 1. Mammalia, birds*. RICHARDSON, J. & GRAY, J.E., eds, E. W. Janson, London, 13–53.

Gray, J.E., 1864. On the Cetacea which have been observed in the seas surrounding the British Islands. *Proceedings of the Zoological Society of London 1864*, 195–248.

Haynes, G., 2017. Taphonomy of the Inglewood mammoth (*Mammuthus columbi*) (Maryland, USA): green-bone fracturing of fossil bones. *Quaternary International 445*, 171–183.

Imbrie, J., Hays, J.D., Martinson, D.G., Mcintyre, A., Mix, A.C., Morley, J.J., Pisias, N.G., Prell, W.L. & Shackleton, N.J., 1984. The orbital theory of Pleistocene climate: support from a revised chronology of the marine $\delta^{18}O$ record. In *Milankovitch and Climate, Part 1*. Berger, A., Imbrie, J., Hays, J., Kukla, G. & Saltzman, B., eds, D. Reidel Publishing Company, Dordrecht, 269–305.

Kasuya, T., 1973. Systematic consideration of recent toothed whales based on the morphology of tympano-periotic bone. *The Scientific Reports of the Whales Research Institute 25*, 1–103.

Kawamura, K. & 17 others, 2007. Northern Hemisphere forcing of climatic cycles in Antarctica over the past 360,000 years. *Nature 448*, 912–916.

Lacépède, B.G.E., 1804. *Histoirie Naturelle des Cétacées*. Chez Plassan, Imprimeur-Libraire, Paris, 283 pp.

Lazar, K. B., & Polyak, L., 2016. Pleistocene benthic foraminifers in the Arctic Ocean: implications for sea-ice and circulation history. *Marine Micropaleontology 126*, 19–30.

Lesson, R.P., 1828. *Histoire naturelle générale et particulière des mammifères et des oiseaux décoverts depuis 1788 jusqu'a nos jours*. Chez Baudouin Frères, Paris, 442 pp.

Linnaeus, C., 1758. *Systema Naturae*. Salvii, Holmiae (Stockholm), 824 pp.

Lopes, R.P., 2012. Bioerosion and bioincrustation in body fossils from the coastal plain of Rio Grande do Sul state, southern Brazil. *Ichnology of Latin America–Selected Papers, Sociedade Brasileira de Paleontologia, Série Monografias 2*, 179–194.

Lopes, R.P. & Buchmann, F.S., 2011. Pleistocene mammals from the southern Brazilian continental shelf. *Journal of South American Earth Sciences 31*, 17–27.

Lopes, R.P. & Ferigolo, J., 2015. *Post mortem* modifications (pseudopaleopathologies) in middle-Late Pleistocene mammal fossils from southern Brazil. *Revista Brasileira de Paleontologia 18*, 285–306.

Lopes, R.P., Buchmann, F.S.C. & Caron, F., 2006. Primeiro registro de fósseis de aves marinhas na planície costeira do Rio Grande do Sul, Brasil. *Revista Brasileira de Geociências 36*, 648–650.

Lopes, R.P., Buchmann, F.S.C. & Caron, F., 2008. Taphonomic analysis on fossils of Pleistocene mammals from deposits submerged along southern Rio Grande do Sul coastal plain, Brazil. *Arquivos do Museu Nacional 66*, 213–229.

Lopes, R.P., Oliveira, L.C., Figueiredo, A.M.G., Kinoshita, A., Baffa, O. & Buchmann, F.S., 2010. ESR dating of Pleistocene mammal teeth and its implications for the biostratigraphy and geological evolution of the coastal plain, Rio Grande do Sul, southern Brazil. *Quaternary International 212*, 213–222.

Lopes, R.P., Dillenburg, S.R., Schultz, C.L., Ferigolo, J., Ribeiro, A.M., Pereira, J.C., Holanda, E.C., Pitana, V.G. & Kerber, L., 2014. The sea-level highstand correlated to marine isotope stage (MIS) 7 in the coastal plain of the state of Rio Grande do Sul, Brazil. *Anais da Academia Brasileira de Ciências 86*, 1573–1595.

Lopes, R.P., Pereira, J.C., Dillenburg, S.R., Tatumi, S.H., Yee, M., Figueiredo, A.M.G., Kinoshita, A. & Baffa, O., 2020. Late Pleistocene-Holocene fossils from Mirim lake, southern Brazil, and their paleoenvironmental significance: I – vertebrates. *Journal of South American Earth Sciences 100*, 102566.

López, P., Cartaneja, I., Carabias, D., Morales, C., Letelier, D. & Flores, V., 2016. Terrestrial and maritime taphonomy: differential effects on spatial distribution of a Late Pleistocene continental drowned faunal bone assemblage from the Pacific coast of Chile. *Archaeological and Anthropological Sciences 8*, 277–290.

Ludt, W.B. & Rocha, L.A., 2015. Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography 42*, 25–38.

Machado, A.A., Calliari, L.J., Melo, E. & Klein, A.H.F., 2010. Historical assessment of extreme coastal sea state conditions in southern Brazil and their relation to erosion episodes. *Pan-American Journal of Aquatic Sciences 5*, 105–114.

Mackensen, A., Rudolph, M. & Kuhn, G., 2001. Late Pleistocene deep-water circulation in the subantarctic eastern Atlantic. *Global and Planetary Change 30*, 197–229.

Martin-Garcia, G.M., Sierro, F.J., Flores, J.A. & Abrantes, F., 2018. Change in the North Atlantic circulation associated with the Mid-Pleistocene transition. *Climate of the Past 14*, 1639–1651.

Martinho, C.T., Dillenburg, S.R. & Hesp, P., 2009. Wave energy and longshore sediment transport gradients controlling barrier evolution in Rio Grande do Sul, Brazil. *Journal of Coastal Research 25*, 285–293.

Mead, J.G. & Fordyce, R.E., 2009. *The Therian Skull. A Lexicon with Emphasis on the Odontocetes*. Smithsonian Institution Scholarly Press, Washington, 261 pp.

Mezzalira, S., 1982. Ocorrências de ossadas semi-fossilizadas de baleias, no litoral paulista, município de Praia Grande – SP. *Revista do Instituto Geológico 3*, 37–38.

Möller Jr., O.O., Piola, A.R., Freitas, A.C. & Campos, E.J.D., 2008. The effects of river discharge and seasonal winds on the shelf off southeastern South America. *Continental Shelf Research 28*, 1607–1624.

Murakami, M., Shimada, C., Hikida, Y., Soeda, Y. & Hirano, H., 2014. *Eodelphis kabatensis*, a new name for the oldest true dolphin *Stenella kabatensis* Horikawa, 1977 (Cetacea, Odontoceti, Delphinidae), from the upper Miocene of Japan, and the phylogeny and paleobiogeography of Delphinoidea. *Journal of Vertebrate Paleontology 34*, 491–511.

Oishi, M. & Hasegawa, Y., 1994. Diversity of Pliocene mysticetes from eastern Japan. *The Island Arc 3*, 436–452.

Otto-Bliesner, B.L., Hewitt, C.D., Marchitto, T.M., Brady, E., Abe-Ouchi, A., Crucifix, M., Murakami, S. & Weber, S.L., 2007. Last Glacial Maximum ocean thermohaline circulation: PMIP2 model intercomparisons and data constraints. *Geophysical Research Letters 34*, L12706.

Post, K., Louwye, S. & Lambert, O., 2017. *Scaldiporia wandokkumi*, a new pontoporiid (Mammalia, Cetacea, Odontoceti) from the Late Miocene to earliest Pliocene of the Westerschelde estuary (The Netherlands). *PeerJ 5*, e3991.

Prado, J.H.F., Mattos, P.H., Silva, K.G. & Secchi, E.R., 2016. Long-term seasonal and interannual patterns of marine mammal strandings in Subtropical Western South Atlantic. *PLoS ONE 11*, e0146339.

Ribeiro, A.M., Drehmer, C.J., Buchmann, F.S.C. & Simões-Lopes, P.C., 1998. Pleistocene skull remains of *Pontoporia blainvillei* (Cetacea, Pontoporiidae) from the coast plain of Rio Grande do Sul state, Brazil, and the relationship of Pontoporids. *Revista Universidade de Guarulhos Geociências 3*, 71–77.

Robineau, D., 1989. Les types de cétacés actuels du Muséum national d'Histoire naturelle I. Balaenidae, Balaenopteridae, Kogiidae, Ziphiidae, Iniidae, Pontoporiidae. *Bulletin du*

Muséum national d'histoire naturelle. Section A, Zoologie, biologie et écologie animals 11, 271-289.

Rodrigues, P.H., Prevosti, F.J., Ferigolo, J. & Ribeiro, A.M., 2004. Novos materiais de carnívora para o Pleistoceno do estado do Rio Grande do Sul, Brasil. *Revista Brasileira de Paleontologia 7*, 77–86.

Rosa, M.L.C.C., 2012. *Geomorfologia, estratigrafia de sequências e potencial de preservação dos sistemas laguna-barreira do Quaternário costeiro do Rio Grande do Sul*. PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 251 pp. (unpublished)

Rosa, M.L.C.C., Barboza, E.G., Abreu, W.S., Tomazelli, L.J. & Dillenburg, S.R., 2017. High-Frequency Sequences in the Quaternary of Pelotas Basin (coastal plain): a record of degradational stacking as a function of longer-term base-level fall. *Brazilian Journal of Geology 47*, 183–207.

Rosenbaum, H.C. & 20 others, 2000. World-wide genetic differentiation of *Eubalaena*: questioning the number of right whale species. *Molecular Ecology 9*, 1793–1802.

Santos-Fischer, C.B., Corrêa, I.C.S., Weschenfelder, J., Torgan, L.C. & Stone, J.R., 2016. Paleoenvironmental insights into the Quaternary evolution of the southern Brazilian coast based on fossil and modern diatom assemblages. *Palaeogeography, Palaeoclimatology, Palaeoecology 446*, 108–124.

Seyboth, E., Groch, K.R., Secchi, E.R. & Rosa, L.D., 2015. Habitat use by southern right whales, *Eubalaena australis* (Desmoulins, 1822), in their main northernmost calving area in the western South Atlantic. *Marine Mammal Science 31*, 1521–1537.

Stephens, B.B. & Keeling, R.F., 2000. The influence of Antarctic sea ice on glacial-interglacial CO_2 variations. *Nature 404*, 171–174.

Thatje, S., Hillenbrand, C., Mackensen, A. & Larter, R., 2008. Life hung by a thread: endurance of Antarctic fauna in glacial periods. *Ecology 89*, 682–692.

Tomazelli, L.J., Dillenburg, S.R. & Villwock, J.A., 2000. Late Quaternary geological history of Rio Grande do Sul coastal plain, southern Brazil. *Revista Brasileira de Geociências 30*, 474–476.

Tsai, C. & 10 others, 2017. Northern pygmy right whales highlight Quaternary marine mammal interchange. *Current Biology 27*, R1037–R1059.

Tsai, C. & Chang, C., 2019. A right whale (Mysticeti, Balaenidae) from the Pleistocene of Taiwan. *Zoological Letters 5*, 37.

van Kolfschoten, T. & Laban, C., 1995. Pleistocene terrestrial mammal faunas from the North Sea. *Mededelingen Rijks Geologische Dienst 52*, 135–151.

Vaz, A.C., Möller Jr., O.O. & Almeida, T.L. 2006. Análise quantitativa da descarga dos rios afluentes da Lagoa dos Patos. *Atlântica 28*, 13–23.

Villwock, J.A. & Tomazelli, L.J., 1995. Geologia costeira do Rio Grande do Sul. *Notas Técnicas 8*, 1–45.

Weschenfelder, J., Corrêa, I.C.S., Aliotta, S. & Baitelli, R., 2010. Paleochannels related to Late Quaternary sea-level changes in southern Brazil. *Brazilian Journal of Oceanography 58*, 35–44.

CAPÍTULO 4

Ecological traits of the franciscana dolphin (*Pontoporia blainvillei*) from the Late Pleistocene to the present days based on stable isotope analysis

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Ecological traits of the franciscana dolphin (*Pontoporia blainvillei*) from the Late Pleistocene to the present days based on stable isotope analysis

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Abstract

Late Pleistocene fossils from southern Brazil provide a unique opportunity to study the diet and habitat use of the extant franciscana dolphin (*Pontoporia blainvillei*) prior to human influence. Here, we subject 19 fossil and 21 contemporary specimens to stable carbon and oxygen isotope analysis (after first ruling out diagenesis via Fourier transform infrared spectroscopy). The two groups differ in their isotopic composition (PERMANOVA, $p < .01$), with fossils yielding more disparate $\delta^{13}C$ values and recent samples more disparate $\delta^{18}O$ values. Their isotopic niches show an overlap of ~20%, with that of the fossils being slightly wider (SEAc = 2.25‰² versus 1.84‰²). We attribute these differences to impoverished modern fish communities and temporal changes in freshwater influx.

Introduction

Pontoporiids (Cetacea: Pontoporiidae) were widely distributed across the Atlantic and South Pacific Oceans during the Miocene and Early Pliocene (Post et al., 2017). However, most of them went extinct in the Plio-Pleistocene (Post et al., 2017), coincident with the Delphinidae radiation (Bianucci, 2013). Consequently, the group was reduced to a single species inhabiting the southwestern Atlantic Ocean (Crespo, 2018), the franciscana dolphin (*Pontoporia blainvillei* Gervais & d'Orbigny, 1844), representing a unique evolutionary lineage among cetaceans.

The franciscana's present distribution – from Espírito Santo State, Brazil (~18ºS), to Golfo Nuevo, Argentina (~42ºS) – is divided into four management units (i.e., Franciscana Management Areas, FMAs) due to genotypical, phenotypical, and populational differences.

FMA I encompasses the coastal waters of Espírito Santo and Rio de Janeiro States, Brazil. FMA II includes the area from São Paulo south to Santa Catarina, also in Brazilian coastal waters. FMA III includes the shallow waters of Rio Grande do Sul State in southern Brazil to Uruguay, and FMA IV is comprised of the coastal regions of Argentina (Secchi et al., 2003a). Further subdivision of these areas has been proposed recently by Nara et al. (2022). Differences in its distribution are mainly related to ecological divergence for niche occupation (Barbato et al., 2012) that lead to different degrees of reproductive isolation (e.g., Cunha et al., 2014; Nara et al., 2022). Temperature, salinity, bathymetry, and the availability of prey species are considered important environmental variables for their ecological niche (Amaral et al., 2018; Gomez & Cassini, 2015). Little is known about its paleobiology even though it is a well-studied species.

Franciscana fossils are known from Pleistocene deposits. The Pleistocene epoch was characterized by several glaciation events (Imbrie et al., 1984) and consequently, during most of this period, the South Atlantic Ocean was colder than it is today (Mulitza et al., 2007). Nevertheless, some interglacial periods of the Late Pleistocene showed higher temperatures than the present-day (Becquey & Gersonde, 2002). Concomitant with glacial-interglacial cycles, sea-levels fluctuated due to the increase and decrease of ice sheets, especially in the Arctic Ocean, leading to important changes in the hydrography, sedimentation, and biota (Darby et al., 2006).

In southern Brazil, several skull fragments (Ribeiro et al., 1998) and an isolated tympanic bulla (Carrasco & Buchmann, 2021) associated with Late Pleistocene deposits were described. Fossil skull fragments are typically composed of the roof of the skull and part of the rostrum, with bone nares, ethmoids, nasals, frontals, maxillae, and premaxillae partially preserved (for more details, see Ribeiro et al., 1998). Unlike currently stranded carcasses or bycatch samples, from which it is possible to extract valuable biological information, only bones are available as fossils. In this context, stable isotope analysis of skeletal components is a powerful tool for revealing the ecological responses of marine mammals to their environments (Newsome et al., 2010). The most common isotopes analyzed in paleontological studies are those of carbon, $^{13}C/^{12}C$ (i.e., $\delta^{13}C$), and oxygen, $^{18}/^{16}O$ (i.e., $\delta^{18}O$), which have been successfully extracted from inorganic components (carbonate) of cetacean fossils older than 20 Ma, from Oligocene, and even Eocene deposits (e.g., Clementz et al., 2006, 2014; Thewissen et al., 2011).

The $\delta^{13}C$ values of consumers depend mainly on the isotopic composition of food ingested and assimilated (DeNiro & Epstein, 1978). Prey items, in turn, tend to be $\delta^{13}C$-distinct

due to diet, taxonomic differences in isotopic fractionation, and ultimately changes that occur at the base of food webs during the process of photosynthesis (Clementz & Koch, 2001; Kelly, 2000; Newsome et al., 2010). $\delta^{18}O$ values depend mainly on ingested water, which is then incorporated into the animals' tissues (Luz et al., 1984; Newsome et al., 2010). Oxygen stable isotopes in seawater tend to show a close relationship with salinity, as both are controlled by evaporation and freshwater input (Belem et al., 2019; Klein et al., 1996; LeGrande & Schmidt, 2006). This means that $\delta^{18}O$ values obtained from cetaceans' carbonate bioapatite change along a salinity gradient (e.g., estuary versus coast versus ocean) (Clementz & Koch, 2001; Drago et al., 2020). Therefore, $\delta^{13}C$ and $\delta^{18}O$ values are proxies for the diet and habitat use of marine mammals, respectively (Clementz, 2012). They can be plotted in a bi-dimensional space, analogous to resources and environmental conditions required for population survival (i.e., the ecological niche of a population; Hutchinson, 1957); hereafter called isotopic niche (Newsome et al., 2007).

We investigated the ecological traits of the franciscana dolphin, *P. blainvillei*, through geological time – from the Late Pleistocene to the Holocene (i.e., present day) – by analyzing the bone carbonate isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) of fossil and contemporary specimens from southern Brazil (FMA III). Because the genetic split that originated in the FMA III occurred during the Early Pleistocene, as evidenced by mitochondrial DNA data (Nara et al., 2022), we considered fossil and contemporary franciscanas to belong to the same population.

Materials and methods

Study area and geological context

All specimens considered in this study were collected in the coastal plain of Rio Grande do Sul State (FMA III), southern Brazil (Fig. 1), in accordance with Brazilian legislation, by researchers from the Museu de Ciências Naturais (MCN-PV, Secretaria do Meio Ambiente e Infraestrutura – SEMA, Porto Alegre, RS) and the Laboratório de Ecologia e Conservação da Megafauna Marinha (ECOMEGA, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil). Fossil samples are comprised of skull fragments found ex situ on the beach from 1994 onward, and contemporary samples were obtained from stranded franciscanas during beach surveys from 1980 to 2004. The material is deposited in the aforementioned collections.

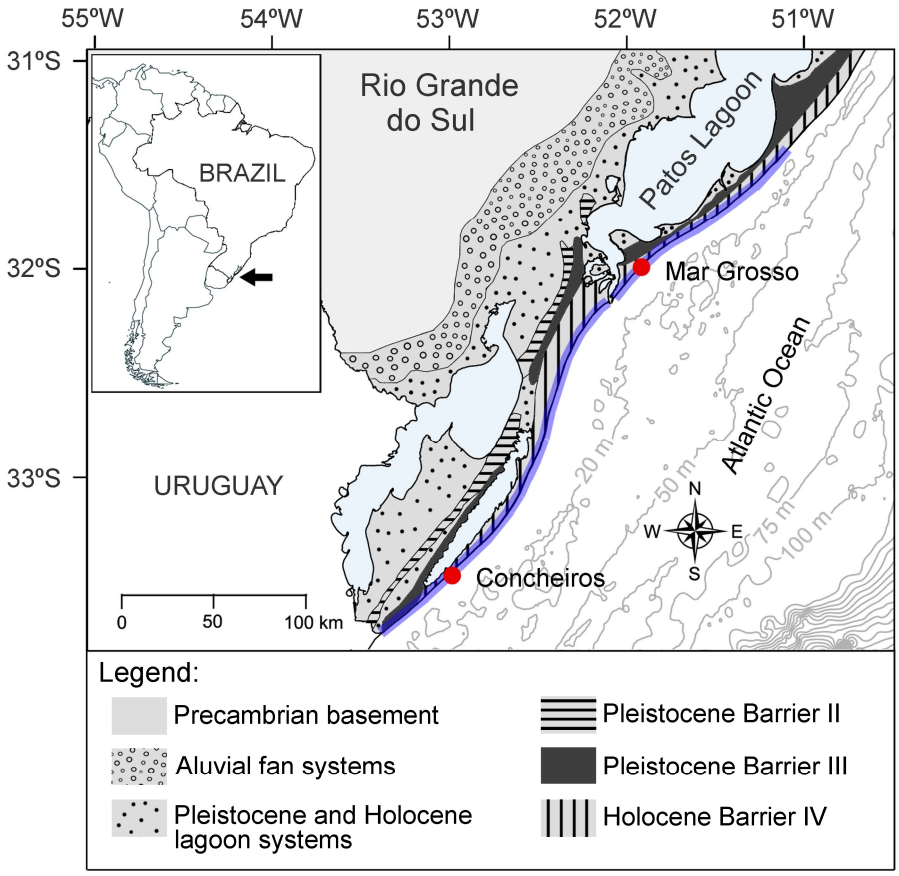


Fig. 1 Map showing the geomorphological features along the study area. Circles indicate the main fossil sites (Mar Grosso and Concheiros beaches), and the continuous line indicates the collection area of recent franciscanas, from Peixe Lagoon in the north to Chuí Creek in the south.

The study area is situated in a zone of the southwestern Atlantic Ocean that is under the influence of both the cold waters of the Falkland (Malvinas) Current and the warm waters of the Brazil Current (Piola & Matano, 2001). Oceanographic parameters such as temperature and salinity are variable in this region and, in the long term, related to the Earth's geological cycles of cooling and warming (Santos et al., 2017). The continental shelf in the region is also affected by freshwater discharges from the Río de la Plata and Patos Lagoon estuaries (Möller et al., 2008), with Río de la Plata having an older genesis (Early Pleistocene; Parker et al., 1994) than the Patos Lagoon (Late Pleistocene; Tomazelli et al., 2000).

The genesis of the Rio Grande do Sul coastal plain is primarily related to the erosion of Precambrian basement and Palaeozoic sedimentary rocks, which provided the sediments that compose four Quaternary barrier-lagoon depositional systems, forming the historic sequential architecture of the Pleistocene and Holocene: Barriers I, II, III, and IV (Tomazelli et al., 2000; Villwock & Tomazelli, 1995; Fig. 1). Each system was formed during sea-level highstands, and their respective ages are correlated with the marine isotope stages (MIS) of Imbrie et al. (1984):

9 (representing 325 ka, thousand years before present), 7e (220 ka), 5 (120 ka), and 1 (6 ka) (Lopes et al., 2010, 2014; Villwock & Tomazelli, 1995).

The source of the fossils are submarine deposits found at depths between 0 and 20 m of the shoreface, principally carbonate rocks and sandstones (Buchmann & Tomazelli, 2003; Buchmann et al., 2001) that are exposed and transported to the beach during strong storm waves produced by extratropical cyclones (Buchmann, 1994, 2002; Buchmann & Tomazelli, 2003; Buchmann et al., 2001; Cruz et al., 2016). Because the sea level was then more than 100 m below current levels (Rosa et al., 2017; Villwock & Tomazelli, 1995), and the continental shelf was occupied by terrestrial environments during glacial periods (Buchmann, 1994; Lopes & Buchmann, 2011), marine fossils are thus otherwise associated with interglacial periods. Among the strata of the Rio Grande do Sul coastal plain, it was suggested that the main source is the Barrier III, and the fossils' age would be estimated between 116 and 130 ka (MIS 5e; Buchmann, 2002) when the sea level reached up to 8 m above current levels (Rosa et al., 2017; Villwock & Tomazelli, 1995). After this event, the sea level decreased, and the fossils were likely buried, undergoing diagenesis (i.e., a set of physical and chemical alterations suffered by fossils after their deposition).

Chemical analysis

Preservation of bone biogenic hydroxyapatite should be assessed before stable isotope analysis, as diagenesis can change the animals' original isotopic composition (Clementz, 2012). For this purpose, we used Fourier transform infrared spectroscopy (FTIR) to compare the structure of fossil and contemporary bones (Zhao et al., 2022). The FTIR technique is a quali-quantitative tool to determine bioapatite diagenesis, since it provides the infrared spectrum absorbed and transmitted by the sample, creating both a molecular fingerprint (Dutta, 2017), and information on the concentration of the absorbing molecule (Sponheimer & Lee-Thorp, 1999).

We selected 19 fossil and 21 recent specimens for stable isotope analysis. FTIR requires a higher amount of material than stable isotope analysis, and therefore results in more destruction of material. Due to sampling restrictions, we designated only one representative of each group for FTIR spectroscopy. Tooth enamel and osteosclerotic bone are recommended tissues for analysis because of their high crystal density and increased resistance to diagenetic alteration (Clementz et al., 2014). However, no fossil had its teeth preserved and only one

isolated (broken) tympanic bulla was recovered from the Pleistocene deposits of southern Brazil, and further sampling would have damaged it. Therefore, we selected maxillary bones for sampling as this structure is preserved in both fossil and recent specimens (Fig. 2), ensuring the compatibility of the results (Smith et al., 2020).

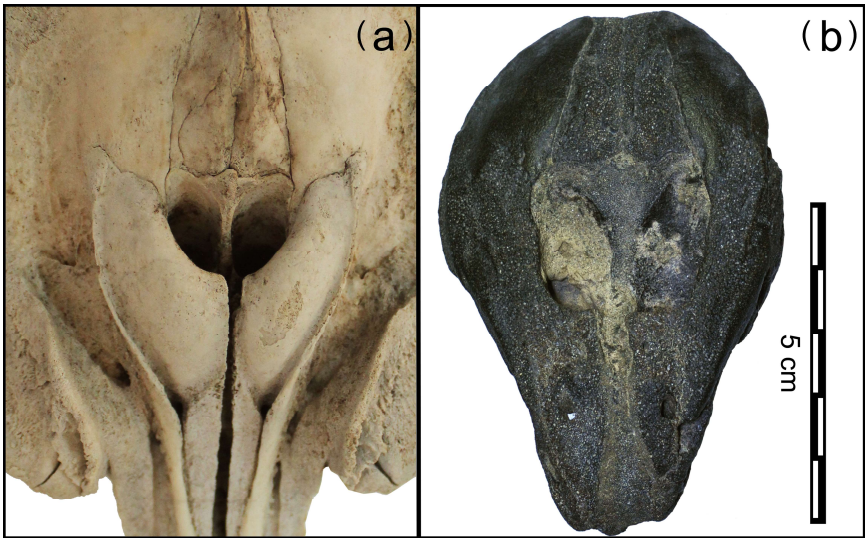


Fig. 2 Detail of a recent franciscana skull (specimen ID: ECOMEGA-SN136) (a) showing the structures typically preserved in fossils (specimen ID: MCN-PV 36962) (b).

We collected samples for both FTIR and isotopic analysis using a micro drill. We then pretreated the resulting powders according to the protocol of Bocherens et al. (1996): we soaked the samples in a 2.5% NaOCl solution, rinsed five times with distilled water through repeated centrifugations after 24 hr (for fossil samples) and 48 hr (in the case of the recent samples, due to the higher content of organic matter) in the solution at room temperature; we added an 0.1 M acetic acid solution for 24 hr to remove diagenetic carbonates, rinsed again, and dried in an oven at 60ºC.

We sent an aliquot of approximately 200 mg of both fossil and recent samples for FTIR analysis at the Laboratório de Materiais Poliméricos (LaPol – Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil). Each powdered sample was mixed with KBr powder to make a pellet and their infrared spectra was obtained through a PerkinElmer FT-IR Spectrometer Spectrum 1000, with 4 cm^{-1} resolution (32 scans).

We sent between 10 and 20 mg of each pretreated powder to the Center for Applied Isotope Studies (CAIS – University of Georgia, Athens, GA, USA) for stable isotope analysis. Carbon and oxygen isotopic compositions were measured using a Thermo Gas Bench coupled to a Delta V Isotope Ratio Mass Spectrometer with a GC Pal autosampler (GasBench–IRMS). For this, samples were acidified with phosphoric acid in a helium atmosphere to produce CO_2

gas. Isotopic compositions were expressed by δ-notation in parts per thousand (‰) following the equation: $\delta X^H = [(R_{Sample} / R_{Standard}) - 1]$, where X is the element, H is the mass of the heavy isotope, and R is $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratios of the sample and standard, respectively. The international standard is Vienna Pee Dee Belemnite (VPDB). The analytical precision was better than 0.03 for $\delta^{13}C$ and 0.08 for $\delta^{18}O$, based on repeated measurements of internal laboratory standards (Fisher and A1296).

Data analysis

Based on FTIR data, we calculated two indices to check the influence of digenesis on bioapatite: the crystallinity index (CI), which estimates changes in crystal size (Shemesh, 1990; Weiner & Bar-Yosef, 1990), and the carbonate/phosphate ratio (C/P), which indicates changes in the mineral bone fraction (Wright & Schwarcz, 1996) following the respective formulae: $CI = (A_{565} + A_{605}) / A_{595}$, and $C/P = A_{1415}/A_{1035}$, where A_x is the absorbance of a wavenumber x with baseline correction. Modern bone CI ranges between 2.5 and 3.6 (Beasley et al., 2014), and the C/P ratio of preserved samples is approximately 0.5, decreasing to 0.1 in degraded bones (Smith et al., 2007). Fossilized bones with CI and C/P outside these ranges had their bioapatite affected by diagenesis and are therefore unsuitable for isotopic analysis.

We corrected the carbon isotope ratios of franciscanas to account for the Suess effect (i.e., decrease in atmospheric ^{14}C due to the burning of fossil fuels since the Industrial Revolution; Baxter & Walton, 1970). For this, we assumed a $\delta^{13}C$ decrease of 0.005 per year since 1850 for the subtropical zone (Hilton et al., 2006). We then made corrections considering the year of collection of each recent specimen: $\delta^{13}C_{corrected} = \delta^{13}C + [0.005 \times (collection\ year - 1850)]$. No correction factors were applied to fossil samples. Before statistical analysis, we also converted the oxygen isotope ratios of both fossil and contemporary samples from VPDB to Vienna Standard Mean Ocean Water (VSMOW) following the equation: $\delta^{18}O_{VSMOW} = 1.03086 \times \delta^{18}O_{VPDB} + 30.86$ (Friedman & O'Neil, 1977).

We performed the statistical analyses using the statistical environment R version 4.2.2 (R Core Team, 2022). We used the permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001), using vegan: Community Ecology Package (Oksanen et al., 2022), to compare the isotopic composition ($\delta^{13}C$ and $\delta^{18}O$ values) of fossil and contemporary specimens, under the null hypothesis that the centroids and dispersion of the groups are equivalent. We chose the semimetric Bray–Curtis measures to calculate pairwise distances,

with 10,000 permutations, assuming a significance level of $\alpha = 0.05$. We used Stable Isotope Bayesian Ellipses in R (SIBER package; Jackson et al., 2011) to measure the isotopic niche width of fossil and contemporary franciscanas. Ellipses were generated with approximately 40% of the data (standard ellipse area, SEAc, expressed as per thousand squared, $‰^2$), which is recommended for small sample sizes. Finally, we calculated the overlap between the fossil and the contemporary group, expressed in percentage (%) ellipses, using the SEAc shared area.

Results

The fossil FTIR spectrum was similar to the contemporary, indicating that diagenesis did not affect carbonate bioapatite in the fossil sample. Both samples showed, respectively, the stretching band of OH^- at 3,421.93 cm^{-1} and 3,426.20 cm^{-1}, with peaks around 1,400 cm^{-1}, attributable to the group CO_3^{2-}, and peaks of 1,038.21, 1,033.87, 604.14, 604.91, 564, and 564.02 cm^{-1}, indicative of PO_4^{3-} groups (Fig. 3). CI and C/P indexes, calculated to estimate the degree of diagenetic alteration in hydroxyapatite, were within the expected range for preserved samples (fossil sample, CI = 2.80, C/P = 0.43; contemporary sample, CI = 2.84, C/P = 0.33).

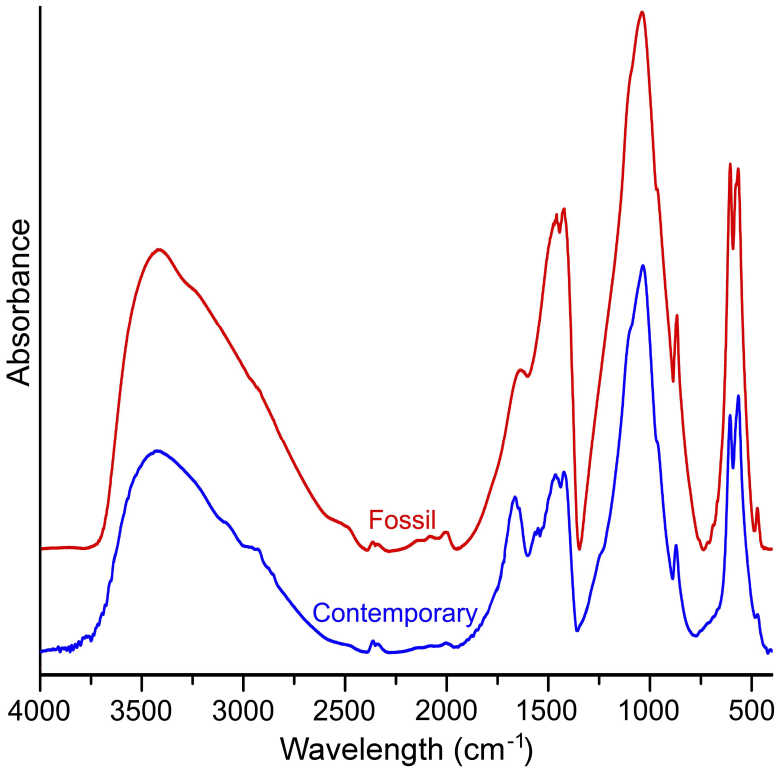


Fig. 3 Bone samples FTIR spectrum of fossil (top line, MCN-PV 36962) and recent (bottom line, ECOMEGA-SN136) franciscanas.

The two groups differ in their isotopic composition (PERMANOVA, p = .0005), with fossils yielding more disparate $\delta^{13}C$ values and recent samples more disparate $\delta^{18}O$ values. $\delta^{13}C$ values in fossil bones ranged from -9.62‰ to -6.34‰, with a mean (M) and standard deviation (± SD) of -8.01‰ ± 0.93‰ (Table 1), whereas in recent bones $\delta^{13}C$ values varied from -7.87‰ to -6.02‰ (M = -7.03‰ ± 0.49‰) after correction for the Suess effect (Table 2). $\delta^{18}O$ values ranged from 26.15‰ to 28.96‰ (M = 27.63‰ ± 0.76‰) and from 25.19‰ to 30.22‰ (M = 28.07‰ ± 1.13‰) in fossil and contemporary samples, respectively. The isotopic niche was slightly wider in fossil (SEAc = 2.25‰²) than contemporary (SEAc = 1.84‰²) franciscanas, with an overlap of ~20% (Fig 4).

Table 1 $\delta^{13}C$ and $\delta^{18}O$ values (‰) of franciscana fossil samples.

Sample ID	$\delta^{13}C_{VPDB}$	$\delta^{18}O_{VSMOW}$
MCN-PV 1526	-7.44	26.82
MCN-PV 2142	-9.24	28.22
MCN-PV 2144	-8.51	26.23
MCN-PV 2502	-8.14	26.15
MCN-PV 8343	-7.11	28.32
MCN-PV 30325	-7.64	27.69
MCN-PV 30326	-8.92	28.39
MCN-PV 36938	-7.02	27.48
MCN-PV 36939	-9.54	27.59
MCN-PV 36941	-7.76	27.55
MCN-PV 36945	-7.20	28.34
MCN-PV 36946	-8.67	27.20
MCN-PV 36947	-7.63	28.96
MCN-PV 36951	-6.34	28.34
MCN-PV 36952	-9.62	26.85
MCN-PV 36956	-8.08	27.72
MCN-PV 36957	-8.68	27.82
MCN-PV 36962	-7.64	27.13
MCN-PV 36963	-7.02	28.21

Table 2 $\delta^{13}C$ and $\delta^{18}O$ values (‰) of franciscana recent samples. The year of collection (used to calculate the predicted influence of the Suess effect on $\delta^{13}C$ values) and the resulting $\delta^{13}C_{corrected}$ values are also shown.

Sample ID	Year of collection	$\delta^{13}C_{VPDB}$	$\delta^{13}C_{VPDB\ CORRECTED}$	$\delta^{18}O_{VSMOW}$
ECOMEGA-SN136	1980	-6.67	-6.02	28.48
ECOMEGA-312	1980	-6.97	-6.32	27.68
ECOMEGA-321	1980	-8.52	-7.87	26.24
ECOMEGA-351	1980	-6.84	-6.19	27.42
ECOMEGA-667	1984	-7.59	-6.92	27.26

ECOMEGA-933	1986	-8.12	-7.44	28.02
ECOMEGA-967	1986	-7.31	-6.63	27.20
ECOMEGA-994	1986	-7.66	-6.98	27.78
ECOMEGA-1009	1986	-8.02	-7.34	27.81
ECOMEGA-1027	1986	-7.63	-6.95	25.19
ECOMEGA-1033	1986	-7.20	-6.52	27.96
ECOMEGA-1356	1993	-8.19	-7.47	28.18
ECOMEGA-2325	1999	-8.20	-7.45	30.22
ECOMEGA-2329	1999	-8.08	-7.34	29.77
ECOMEGA-2332	1999	-7.76	-7.02	29.29
ECOMEGA-2352	1999	-7.86	-7.12	29.01
ECOMEGA-2386	2000	-7.58	-6.83	29.02
ECOMEGA-2393	2000	-7.58	-6.83	28.75
ECOMEGA-2395	2000	-8.01	-7.26	28.59
ECOMEGA-26814	2004	-8.12	-7.35	27.60
ECOMEGA-26848	2004	-8.48	-7.71	27.99

Discussion

Diagenesis is a major concern when considering fossils, especially bone samples, for stable isotope analysis. Franciscana fossils share several characteristics, regardless of sampling location, such as high fragmentation due to transport and abrasion from wave action, and dark color. Here, we selected only one representative fossil to check for diagenetic alteration, and FTIR results indicated that bioapatite did not suffer chemical alterations and therefore maintained its original characteristics. Due to sampling constraints, we could not test the entire sample set (the ideal scenario) but other pieces of evidence suggested that fossils from the southern Brazilian coastal plain are suitable for stable isotope analysis. For instance, Lopes & Ferigolo (2015), using energy-dispersive X-ray spectroscopy, showed that the typically dark-coloration of fossils from this region results from the incorporation of trace metals, such as iron, barium, and yttrium, whereas biogenic skeletal components such as calcium and phosphorus show similar concentrations to modern bones. Thus, we assumed that isotopic ratios reflect those incorporated during the animals' life rather than post-burial alteration.

The extensive fossil record of the southern Brazilian coastal plain provided a unique opportunity to study the ecological traits of the franciscana dolphin through geological time. The isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) of maxillary bone bioapatite samples of fossil and contemporary specimens from FMA III revealed slight ecological differences over the last 130,000 years due to changes in trophic interactions and habitat features, as we discuss below.

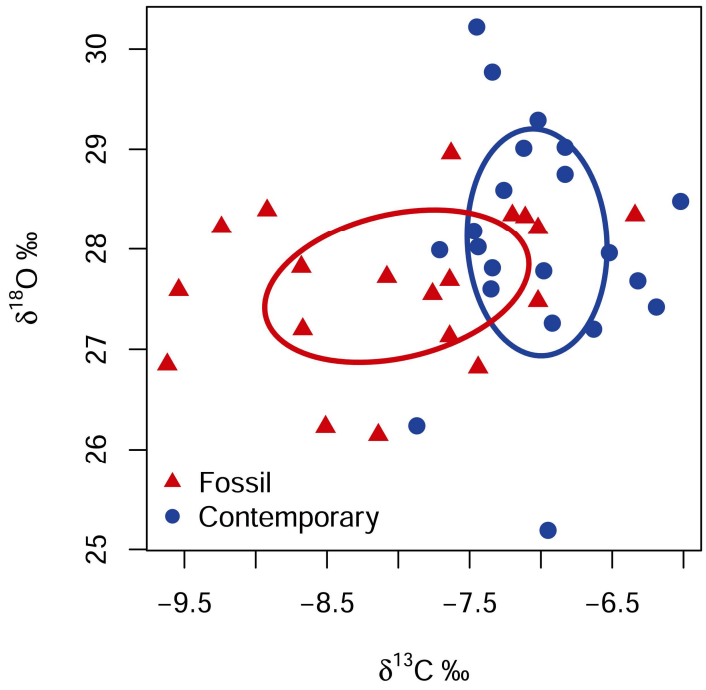


Fig. 4 Bone $\delta^{13}C$ and $\delta^{18}O$ values and the respective isotopic niche area for franciscana dolphin. Fossil and recent specimens are represented by triangles and circles, respectively. Note that contemporary $\delta^{13}C$ values are corrected for the Suess effect.

First, to interpret the results obtained here, it is essential to contextualize the environments inhabited by the species in both temporal scenarios: Late Pleistocene and present days (i.e., Holocene). The franciscana dolphin has small home ranges (Wells et al., 2021) and typically inhabits shallow coastal waters to a depth of 35 m (Danilewicz et al., 2009). The southern Brazilian coast is under the influence of freshwater discharges from Río de la Plata and Patos Lagoon estuaries (Möller Jr. et al., 2008), which affect salinity and nutrient concentration in addition to phytoplankton biomass and diversity (Burrage et al., 2008; Gonçalves-Araujo et al., 2018). The Río de la Plata is a component present in both geological scenarios, with dynamics related to changes in sea level (Beilinson et al., 2019), and an estimated age of 2.4 Ma (Parker et al., 1994). The Patos Lagoon, a productive area for some of the franciscanas' main prey (Secchi, 2010), has a genesis similar in age to that of fossils' deposition (~120 ka). The latter is predominantly a freshwater environment that discharges toward the coast through a large inlet, attaining a mean volumetric flow of 2,400 m^3/s (Vaz et al., 2006). The Patos Lagoon coastal plume is typically about 33 km wide and more than 100 km long, with salinity ranging between 12 to 27 psu (Burrage et al., 2008). Diatom assemblages, however, suggest that this estuary achieved characteristics similar to the current status only by

the Holocene, and Late Pleistocene sediments (older than 43,500 ka) show a typical assemblage of marine to marine-brackish environments (Santos-Fisher et al., 2016).

Another difference between the two scenarios includes human disturbances caused by fisheries activity. The Patos Lagoon estuary, and adjacent coastal waters, are marked by intense fishing by both industrial and artisanal boats, causing the impoverishment of local fish communities. This is particularly worrying in the case of fish species that spend part of their life cycle in the estuary, and those that use shallow coastal waters, which together represent more than 70% of the landings in this region (Haimovici & Cardoso, 2016). Given this situation, it has been suggested that changes in franciscana's diet over the years are caused by the depletion of fish stocks (Botta et al., 2022; Secchi et al., 2003b). Human disturbances may also be responsible for changes in franciscana's home ranges, with smaller ranges in heavily human-influenced regions compared to less-influenced areas (Wells et al., 2021). Fossil specimens, on the other hand, are of course from a period without anthropological influence.

Fossil franciscanas showed more depleted and variable $\delta^{13}C$ values than contemporary ones. Cetaceans' carbon stable isotopes are acquired through their diets, and mainly reflect the isotopic signature of primary producers and processes that occur along the food web, in conjunction with environmental conditions (Walker & Macko, 1999). Modern franciscana prey from the southern Brazilian coast are isotopically distinct (Troina et al., 2016): benthic and coastal prey are typically enriched in ^{13}C compared to pelagic or offshore prey (Clementz & Koch, 2001; France, 1995). In this context, considering the opportunistic behavior of the franciscana and its small spatial ranges (Bassoi et al., 2020; Wells et al., 2021), the diversity of prey was likely higher in the past, which would have resulted in the more diverse $\delta^{13}C$ values in the fossil group.

The franciscana feeds in marine and estuarine waters, preying upon fish, squid, and crustaceans, following the abundance of prey species (Bassoi et al., 2020, 2021; Rodríguez et al., 2002). The current diet of the species varies along its distribution range (Botta et al., 2022; Paso-Viola et al., 2014), as well as within aquatic (marine versus estuarine) habitats (Rodríguez et al., 2002). There appear to be no dietary differences between males and females (Bassoi et al., 2021; Troina et al., 2016). In the coastal waters of southern Brazil, under the influence of the Patos Lagoon estuary, the franciscana dolphin consumes about 23 species of fish, six cephalopods, and four crustaceans (Bassoi et al., 2021). The squid *Doryteuthis sanpaulensis*, the benthopelagic fish *Cynoscion guatucupa*, the shelf-pelagic fish *Anchoa marinii*, and the

costal-estuarine fish *Paralonchurus brasiliensis* are the most important prey items (Bassoi et al., 2021; Troina et al., 2016).

Depletion of fish stocks by fishery exploitation has resulted in changes in franciscana's diet over the years. Historical data showed evidence of a decrease in the consumption of *Macrodon ancylodon* and *Micropogonias furnieri*, while the occurrence of *Trichirus lepturus* and *Umbrina canosai* increased over a period of 20 years (Botta et al., 2022; Secchi et al., 2003b). A less varied trophic component in recent specimens can be linked to the extensive exploitation of fish stocks.

An alternative explanation is that the higher variation in $\delta^{13}C$ values of fossil specimens reflects changes at the base of the food web, but the little data that exists does not support this hypothesis. Samples from this group can differ in age by up to thousands of years, as they are associated with MIS 5e, which spans from 116 to 130 ka. A baseline variation of 0.70‰ was reported for the entire MIS 5e period, with a coarse temporal resolution, based on isotopic analysis of the benthic foraminifera *Cibicides wuellerstorfi* from the western South Atlantic (Santos et al., 2017). Mechanisms responsible for variations in carbon isotopic baseline include specific biological characteristics of the phytoplankton community and environmental conditions such as aqueous CO_2 concentrations (Graham et al., 2010). Oceanographic conditions are responsible for significant seasonal and annual variations in $\delta^{13}C$ values at the base of the food web of subtropical Brazilian waters, and can even exceed 2.00‰ (Troina et al., 2020). However, these data should be compared with caution considering the differences in temporal resolution. In addition, they were collected in shelf-break and offshore areas, whereas the franciscana dolphin inhabits shallow coastal waters, in which the influence of continental waters is expected to be higher.

Compared to carbon isotopic data, contemporary samples yield more variable $\delta^{18}O$ values. Cetaceans' oxygen stable isotopes are controlled by those of ingested seawater (Clementz, 2012; Luz et al., 1984; Newsome et al., 2010). Because seawater oxygen stable isotopes are driven by salinity, samples of estuarine-coastal species are typically ^{18}O-depleted compared to those inhabiting continental shelf waters, whereas oceanic species have ^{18}O-enriched tissues (i.e., higher $\delta^{18}O$ values; Drago et al., 2020). The franciscana dolphin is a coastal species living under the influence of the Patos Lagoon coastal plume, which appears to be a geologically recent feature of the southern Brazilian coastal habitat, as discussed above. The Patos Lagoon coastal plume is dynamic, and fundamentally influenced by the amount of freshwater load and wind direction (Marques et al., 2009). Therefore, the more variable $\delta^{18}O$

values of contemporary specimens are likely reflecting the fluctuations in seawater $\delta^{18}O$ values due to temporal changes in freshwater influx. Taking into account franciscana dolphin behavior, variability in $\delta^{18}O$ values is more likely to be related to habitat changes rather than animals' movement through distinct marine regions.

Fossils provided information about the species from a period without anthropological influence. This is especially important for the franciscana because it is classified as vulnerable by the International Union for Conservation of Nature (IUCN; Zerbini et al., 2017), and faces several human threats that include high incidental mortality in gillnet fisheries (Prado et al., 2016; Zerbini et al., 2017; Secchi et al., 2021, 2022). Although the isotopic niche of the contemporary group was slightly smaller than that of the fossil group, these data must be interpreted with caution. The isotopic niche width of recent franciscanas was strongly influenced by variation in oxygen isotopic values, which probably do not represent changes in habitat use but salinity variation throughout the study area, and thus habitat loss cannot be ruled out. The wider isotopic niche in fossils, on the other hand, may be related to higher dietary variability in pristine conditions. The ongoing exploitation of marine and estuarine resources negatively affected fish communities and abundance, resulting in an impact at the upper trophic levels.

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References

Amaral, K.B., Danilewicz, D., Zerbini, A., Di Beneditto, A.P., Adriolo, A., Alvares, D.J., Secchi, E., Ferreira, E., Sucunza, F., Borges-Martins, M., Santos, M.C.O., Cremer, M., Denuncio, P., Ott, P.H., & Moreno, I.B. (2018). Reassessment of the franciscana *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844) distribution and niche characteristics in Brazil. *Journal of Experimental Marine Biology and Ecology*, *508*, 1–12. https://doi.org/10.1016/j.jembe.2018.07.010

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Barbato, B. H. A., Secchi, E. R., Di Beneditto, A. P. M., Ramos, R. M. A., Bertozzi, C., Marigo, J., Bordino, P., & Kinas, P. G. (2019). Geographical variation in franciscana (*Pontoporia blainvillei*) external morphology. *Journal of the Marine Biological Association of the United Kingdom*, 92(8), 1645–1656. https://doi.org/10.1017/S0025315411000725

Bassoi, M., Secchi, E.R., Danilewicz, D., Moreno, I.B., Santos, R.A., & Shepherd, J.G. (2021). Intrapopulation variation in the diet of franciscana dolphin (*Pontoporia blainvillei*) off southern Brazil. *Journal of the Marine Biological Association of the United Kingdom*, *101*(3), 621–637. https://doi.org/10.1017/S0025315421000436

Bassoi, M., Shepherd, J.G., Secchi, E.R., Moreno, I.B., & Danilewicz, D. (2020). Oceanographic process driving the feeding ecology of franciscana dolphin off Southern Brazilian coast. *Continental Shelf Research*, *201*, 104124. https://doi.org/10.1016/j.csr.2020.104124

Baxter, M.S., & Walton, A. (1970). A theoretical approach to the Suess effect. *Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences*, *318*(1533), 213–230. https://doi.org/10.1098/rspa.1970.0141

Beasley, M.M., Bartelink, E.J., Taylor, L., & Miller, R.M. (2014). Comparison of transmission FTIR, ATR, and DRIFT spectra: implications for assessment of bone bioapatite diagenesis. *Journal of Archaeological Science*, *46*, 16–22. https://doi.org/10.1016/j.jas.2014.03.008

Becquey, S., & Gersonde, R. (2002). Past hydrographic and climatic changes in the Subantarctic Zone of the South Atlantic – The Pleistocene record from ODP Site 1090. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *182*(3–4), 221–239. https://doi.org/10.1016/S0031-0182(01)00497-7

Beilinson, E., Raigemborn, M.S., Rodriguez, S.G., Soibelzon, E., Gasparini, G.M., Calvo-Marcilese, L., Cusminsky, G.C., Mari, F., Iacona, F., & Soibelzon, L.H. (2019). A multi-proxy approach to paleoenvironmental changes in the southwestern Río de la Plata area (Argentina) during Late Pleistocene. *Quaternary International*, *512*, 6–17. https://doi.org/10.1016/j.quaint.2019.01.010

Belem, A.L., Caricchio, C., Albuquerque, A.L.S., Venancio, I.M., Zucchi, M.R., Santos, T.H.R., & Alvarez, Y.G. (2019). Salinity and stable oxygen isotope relationship in the southwestern Atlantic: constraints to paleoclimate reconstructions. *Anais da Academia Brasileira de Ciências*, *91*(03), e20180226. https://doi.org/10.1590/0001-3765201920180226

Bianucci, G. (2013). *Septidelphis morii*, n. gen. et sp., from the Pliocene of Italy: new evidence of the explosive radiation of true dolphins (Odontoceti, Delphinidae). *Journal of Vertebrate Paleontology*, *33*(3), 722–740. https://doi.org/10.1080/02724634.2013.744757

Bocherens, H., Koch, P.L., Mariotti, A., Geraads, D., & Jaeger, J. (1996). Isotopic biogeochemistry (^{13}C, ^{18}O) of mammalian enamel from African Pleistocene hominid sites. *PALAIOS*, *11*(4), 306–318. https://doi.org/10.2307/3515241

Botta, S., Bassoi, M., Troina, G.C. (2022). Overview of franciscana diet. In P.C. Simões-Lopes & M.J. Cremer (Eds.), *The franciscana dolphin on the edge of survival* (pp. 15–48). Elsevier.

Buchmann, F.S.C. (1994). Distribuição de fósseis pleistocênicos na zona costeira e plataforma continental interna no Rio Grande do Sul. *Acta Geologica Leopoldensia*, *17*(39/1), 355–364.

Buchmann, F.S.C. (2002). *Bioclastos de organismos terrestres e marinhos na praia e plataforma interna do Rio Grande do Sul: natureza, distribuição, origem e significado geológico* [Unpublished PhD's thesis]. Universidade Federal do Rio Grande do Sul.

Buchmann, F.S., & Tomazelli, L.J. (2003). Relict nearshore shoals of Rio Grande do Sul, southern Brazil: origin and effects on nearby modern beaches. *Journal of Coastal Research*, *35*, 318–322.

Buchmann, F.S., Seeliger, M., Zanella, L.R.C., Madureira, L.S.P., Tomazelli, L.J., & Calliari, L.J. (2001). Análise batimétrica e sedimentológica no estudo do Parcel do Carpinteiro, uma paleolinha de praia pleistocênica na antepraia do Rio Grande do Sul, Brasil. *Pesquisas em Geociências*, *28*(2), 109–115. https://doi.org/10.22456/1807-9806.20274

Burrage, D., Wesson, J., Martinez, C., Pérez, T., Möller Jr., O., & Piola, A. (2008). Patos Lagoon outflow within the Río de la Plata plume using an airborne salinity mapper: Observing an embedded plume. *Continental Shelf Reseach*, *28*(13), 1625–1638. https://doi.org/10.1016/j.csr.2007.02.014

Carrasco, T.S., & Buchmann, F.S. (2021). Pleistocene cetacean fossils from the coastal plain of Rio Grande do Sul in southern Brazil. *Alcheringa*, *45*(3), 369–381, https://doi.org/10.1080/03115518.2021.1966647

Clementz, M.T. (2012). New insight from old bones: stable isotope analysis of fossil mammals. *Journal of Mammalogy*, *93*(2), 368–380. https://doi.org/10.1644/11-MAMM-S-179.1

Clementz, M.T., & Koch, P.L. (2001). Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia*, *129*, 461–472. https://doi.org/10.1007/s004420100745

Clementz, M.T., Fordyce, R.E., Peek, S.L., & Fox, D.L. (2014). Ancient marine isoscapes and isotopic evidence of bulk-feeding by Oligocene cetaceans. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *400*(15), 28–40. https://doi.org/10.1016/j.palaeo.2012.09.009

Clementz, M.T., Goswani, A., Gingerich, P.D., & Koch, P.L. (2006). Isotopic records from early whales and sea cows: contrasting patterns of ecological transition. *Journal of Vertebrate Paleontology*, *26*(2), 355–370. https://doi.org/10.1671/0272-4634(2006)26[355:IRFEWA]2.0.CO;2

Crespo, E.A. (2018). Franciscana dolphin: *Pontoporia blainvillei*. In B. Würsig, J.G.M. Thewissen & K. Kovacs (Eds.), *Encyclopedia of marine mammals* (pp. 388–392). Academic Press.

Cruz, E.A., Dillenburg, S.R., & Buchmann, F.S. (2016). Description and controls on distribution of Pleistocene vertebrate fossil from the central and southern sectors of the coastal plain of Rio Grande do Sul, Brazil. *Revista Brasileira de Paleontologia*, *19*(3), 425–438. https://doi.org/10.4072/rbp.2016.3.08

Cunha, H.A., Medeiros, B.V., Barbosa, L.A., Cremer, M.J., Marigo, J., Lailson-Brito, J., Azevedo, A.F., & Solé-Cava, A.M. (2014). Population structure of the endangered

franciscana dolphin (*Pontoporia blainvillei*): Reassessing management units. *PloS One*, *9*(1), e85633. https://doi.org/10.1371/journal.pone.0085633

Danilewicz, D., Secchi, E.R., Ott, P.H., Moreno, I.B., Bassoi, M., & Borges-Martins, M. (2009). Habitat use patterns of franciscana dolphins (*Pontoporia blainvillei*) off southern Brazil in relation to water depth. *Journal of the Marine Biological Association of the United Kingdom*, *89*(5), 943–949. https://doi.org/10.1017/S002531540900054X

Darby, D.A., Polyak, L., & Bauch, H.A. (2006). Past glacial and interglacial conditions in the Artic Ocean and marginal seas – a review. *Progress in Oceanography*, *71*(2–4), 129–144. https://doi.org/10.1016/j.pocean.2006.09.009

DeNiro, M.J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, *42*(5), 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

Drago, M., Valdivia, M., Bragg, D., González, E.M., Aguilar, A., & Carbona, L. (2020). Stable oxygen isotopes reveal habitat use by marine mammals in the Río de la Plata estuary and adjoining Atlantic Ocean. *Estuarine, Coastal and Shelf Science*, *238*, 106708. https://doi.org/10.1016/j.ecss.2020.106708

Dutta, A. (2017). Fourier transform infrared spectroscopy. In S. Thomas, R. Thomas, A.K. Zachariah & R.K. Mishra (Eds.), *Spectroscopic methods for nanomaterials characterization* (pp. 73–94). Elsevier.

France, R.L. (1995). Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marine Ecology Progress Series*, *124*, 307–312. https://doi.org/10.3354/meps124307

Friedman, I. & O'Neil, J.R. (1977). Compilation of stable isotope fractionation factors of geochemical interest. *Geological Survey Professional Papers*, *440–KK*, KK1–KK12. https://doi.org/10.3133/pp1050

Gomez, J.J., & Cassini, M.H. (2015). Environmental predictors of habitat suitability and biogeographical range of Franciscana dolphins (*Pontoporia blainvillei*). *Global Ecology and Conservation*, *3*, 90–99. http://doi.org/10.1016/j.gecco.2014.11.007

Gonçalves-Araujo, R., de Souza, M.S., Tavano, V.M., Mendes, C.R., de Souza, R.B., Schultz, C., & Pollery, R.C. (2018). Phyto- and protozooplankton assemblages and hydrographic variability during an early winter survey in the Southern Brazilian Continental Shelf. *Journal of Marine Systems*, *184*, 36–49. https://doi.org/10.1016/j.jmarsys.2018.04.005

Graham, B.S., Koch, P.L., Newsome, S.D., McMahon, K.W., & Aurioles, D. (2010). Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In J.B. West, G.J. Bowen, T.E. Dawson & K.P. Tu (Eds.), *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping* (pp. 299–318). Springer Science + Business Media.

Haimovici, M., & Cardoso, L.G. (2016). Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. *Marine Biology Research*, *13*(1), 135–150. https://doi.org/10.1080/17451000.2016.1228978

Hilton, G.M., Thompson, D.R., Sagar, P.M., Cuthbert, R.J., Cherel, Y., & Bury, S.J. (2006). A stable isotopic investigation into the causes of decline in a sub-Antarctic predator, the rockhopper penguin *Eudyptes chrysocome*. *Global Change Biology*, *12*(4), 611–625. https://doi.org/10.1111/j.1365-2486.2006.01130.x

Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, 22, 415–427. https://doi.org/10.1101/SQB.1957.022.01.039

Imbrie, J., Hays, J.D., Martinson, D.G., McIntyre, A., Mix, A.C., Morley, J.J., Pisias, N.G., Prell, W.L., & Shackleton, N.J. (1984). The orbital theory of Pleistocene climate: support from a revised chronology of the marine $\delta^{18}O$ record. In A. Berger, J. Imbrie, J. Hays, G. Kukla & B. Saltzman (Eds.). *Milankovitch and climate, Part 1* (pp. 269–305). D. Reidel Publishing Company.

Jackson, A.L., Inger, R., Parnell, A.C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Klein, R.T., Lohmann, K.C., & Thayer, C.W. (1996). Bivalve skeletons record sea-surface temperature and $\delta^{18}O$ via Mg/Ca and $^{18}O/^{16}O$ ratios. *Geology*, *24*(5), 415–418. https://doi.org/10.1130/0091-7613(1996)024<0415:BSRSST>2.3.CO;2

LeGrande, A.N., & Schmidt, G.A. (2006). Global gridded data set of the oxygen isotopic composition in seawater. *Geophysical Research Letters*, *33*(12), L12604. https://doi.org/10.1029/2006GL026011

Lopes, R.P., & Buchmann, F.S. (2011). Pleistocene mammals from the southern Brazilian continental shelf. *Journal of South American Earth Sciences*, *31*(1), 17–27. https://doi.org/10.1016/j.jsames.2010.11.003

Lopes, R.P., & Ferigolo, J. (2015). Post mortem modifications (pseudopaleopathologies) in Middle-Late Pleistocene mammal fossils from southern Brazil. *Revista Brasileira de Paleontologia*, *18*(2), 285–306. https://doi.org/10.4072/rbp.2015.2.09

Lopes, R.P., Kinoshita, A., Baffa, O., Figueiredo, A.M.G., Dillenburg, S.R., Schultz, C.L., & Pereira, J.C. (2014). ESR dating of Pleistocene mammals and marine shells from the coastal plain of Rio Grande do Sul state, southern Brazil. *Quaternary International*, *352*, 124–134. https://doi.org/10.1016/j.quaint.2013.07.020

Lopes, R.P., Oliveira, L.C., Figueiredo, A.M.G., Kinoshita, A., Baffa, O., & Buchmann, F.S. (2010). ESR dating of pleistocene mammal teeth and its implications for the biostratigraphy and geological evolution of the coastal plain, Rio Grande do Sul, southern Brazil. *Quaternary International*, *212*(2), 213–222. https://doi.org/10.1016/j.quaint.2009.09.018

Luz, B., Kolodny, Y., & Horowitz, M. (1984). Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental water. *Geochimica et Cosmochimica Acta*, *48*(8), 1689–1693. https://doi.org/10.1016/0016-7037(84)90338-7

Marques, W.C., Fernandes, E.H., Monteiro, I.O, & Möller, O.O. (2009). Numerical modeling of the Patos Lagoon coastal plume, Brazil. *Continental Shelf Research*, *29*(3), 556–571. https://doi.org/10.1016/j.csr.2008.09.022

Möller, O.O., Piola, A.R., Freitas, A.C., & Campos, E.J.D. (2008). The effects of river discharge and seasonal winds on the shelf off southeastern South America. *Continental Shelf Research*, *28*(13), 1607–1624. https://doi.org/10.1016/j.csr.2008.03.012

Mulitza, S., Paul, A., & Wefer, G. (2007). Late Pleistocene South Atlantic. In S.A. Elias (Ed.), *Encyclopedia of Quaternary science* (pp. 1816–1831). Elsevier.

Nara, L., Cremer, M.J., Farro, A.P.C., Colosio, A.C., Barbosa, L.A., Bertozzi, C.P., Secchi, E.R., Pagliani, B., Costa-Urrutia, P., Gariboldi, M.C., Lazoski, C., & Cunha, H.A. (2022). Phylogeography of the endangered franciscana dolphin: timing and geological setting of the evolution of populations. *Journal of Mammalian Evolution*, *29*, 609–625. https://doi.org/10.1007/s10914-022-09607-7

Newsome, S.D., Clementz, M.T., & Koch, P.L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science*, *26*(3), 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Newsome, S.D., del Rio, C.M., Bearhop, S., & Phillips, D.L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, *5*(8), 429–436. https://doi.org/10.1890/060150.1

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., (…), & Weedon, J. (2022). *_vegan: Community Ecology Package_*. (R package version 2.6-4) [Computer software]. https://CRAN.R-project.org/package=vegan

Parker, G., Paterlini, C.M., & Violante, R.A. (1994). Edade y génesis del Río de la Plata. *Revista de la Asociación Geológica Argentina*, *49*(1–2), 11–18.

Paso-Viola, M.N., Denuncio, P., Negri, M.F., Rodríguez, D., Bastida, R., & Cappozzo, H.L. (2014). Diet composition of franciscana dolphin *Pontoporia blainvillei* from southern Buenos Aires, Argentina and its interaction with fisheries. *Revista de Biología Marina y Oceanografía*, *49*(2), 393–400.

Piola, A.R., & Matano, R.P. (2001). Brazil and Falklands (Malvinas) currents. In J.H. Steele (Ed.), *Encyclopedia of ocean sciences* (pp. 340–349). Elsevier. https://doi.org/10.1006/rwos.2001.0358

Post, K., Louwye, S., & Lambert, O. (2017). *Scaldiporia vandokkumi*, a new pontoporiid (Mammalia, Cetacea, Odontoceti) from the Late Miocene to earliest Pliocene of the Westerschelde estuary (The Netherlands). *PeerJ*, *5*, e3991. https://doi.org/10.7717/peerj.3991

Prado, J.H.F., Mattos, P.H., Silva, K.G., & Secchi, E.R. (2016). Long-term seasonal and interannual patterns of marine mammal strandings in Subtropical Western South Atlantic. *PLoS ONE*, *11*(1), e0146339. https://doi.org/10.1371/journal.pone.0146339

R Core Team. (2019). *R: a language and environment for statistical computing* [Computer software]. R Foundation for Statistical Computing.

Ribeiro, A.M., Drehmer, C.J., Buchmann, F.S.C., & Simões-Lopes, P.C. (1998). Pleistocene skull remains of *Pontoporia blainvillei* (Cetacea, Pontoporiidae) from the coast plain of Rio Grande do Sul state, Brazil, and the relationship of Pontoporids. *Revista Universidade Guarulhos Geociências*, *3*(6), 71–77.

Rodríguez, D., Rivero, L., & Bastida, R. (2002). Feeding ecology of the franciscana (*Pontoporia blainvillei*) in marine and estuarine waters of Argentina. *Latin American Journal of Aquatic Mammals*, *1*(1), 77–94. https://doi.org/10.5597/lajam00012

Rosa, M.L.C.C., Barboza, E.G., Abreu, V.S., Tomazelli, L.J., & Dillenburg, S.R. (2017). High-frequency sequences in the Quaternary of Pelotas Basin (coastal plain): a record of degradational stacking as a function of longer-term base-level fall. *Brazilian Journal of Geology*, *47*(02), 185–207. https://doi.org/10.1590/2317-4889201720160138

Santos, T.P., Lessa, D.O., Venancio, I.M., Chiessi, C.M., Mulitza, S., Kuhnert, H., Govin, A., Machado, T., Costa, K.B., Toledo, F., Dias, B.B., & Albuquerque, A.L.S. (2017). Prolonged warming of the Brazil Current precedes deglaciations. *Earth and Planetary Science Letters*, *463*, 1–12. http://doi.org/10.1016/j.epsl.2017.01.014

Santos-Fischer, C.B., Corrêa, I.C.S., Weschenfelder, J., Torgan, L.C., & Stone, J.R. (2016). Paleoenvironmental insights into the Quaternary evolution of the southern Brazilian coast based on fossil and modern diatom assemblages. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *446*, 108–124. https://doi.org/10.1016/j.palaeo.2016.01.018

Secchi, E.R. (2010). Life history and ecology of franciscana, *Pontoporia blainvillei* (Cetacea, Pontoporiidae). In M. Ruiz-García & J. Shostell (Eds), *Biology, Evolution and Conservation of River Dolphins within South America and Asia* (pp. 301–321). Nova Science Publishers.

Secchi, E.R., Cremer, M.J., Danilewicz, D., & Lailson-Brito, J. (2021). A synthesis of the ecology, human-related threats and conservation perspectives for the endangered franciscana dolphin. *Frontiers in Marine Science*, *8*, 617956. https://doi.org/10.3389/fmars.2021.617956

Secchi, E.R., Danilewicz, D., & Ott, P.H. (2003a). Applying the phylogeographic concept to identify franciscana dolphin stocks: implications to meet management objectives. *Journal of Cetacean Research and Management*, *5*(1), 61–68.

Secchi, E.R., Monteiro, D., & Claudino, R. (2022). Is the franciscana bycatch in gillnet fisheries sustainable?. In P.C. Simões-Lopes & M.J. Cremer (Eds), *The franciscana dolphin on the edge of survival* (pp. 201–234). Elsevier.

Secchi, E.R., Ott, P.H., & Danilewicz, D. (2003b). Effects of fishing bycatch and the conservation status of the franciscana dolphin, *Pontoporia blainvillei*. In N. Gales & M.K.R. Hindell (Eds.), *Marine mammals: fisheries, tourism, and management issues* (pp. 174–191). CSIRO Publishing Collingwood.

Shemesh, A. (1990). Crystallinity and diagenesis of sedimentary apatites. *Geochimica et Cosmochimica Acta*, *54*(9), 2433–2438. https://doi.org/10.1016/0016-7037(90)90230-I

Smith, C.I., Nielsen-Marsh, C.M., Jans, M.M.E., & Collins, M.J. (2007). Bone diagenesis in the European Holocene I: patterns and mechanisms. *Journal of Archaeological Science*, *34*(9), 1485–1493. https://doi.org/10.1016/j.jas.2006.11.006

Smith, K.J., Sparks, J.P., Timmons, Z.L., & Peterson, M.J. (2020). Cetacean skeletons demonstrate ecologically relevant variation in intraskeletal stable isotope values. *Frontiers in Marine Science*, *7*, 388. https://doi.org/10.3389/fmars.2020.00388

Sponheimer, M., & Lee-Thorp, J.A. (1999). Alteration of enamel carbonate environments during fossilization. *Journal of Archaeological Science*, *26*(2), 143–150. https://doi.org/10.1006/jasc.1998.0293

Thewissen, J.G.M., Sensor, J.D., Clementz, M.T., & Bajpai, S. (2011). Evolution of dental wear and diet during the origin of whales. *Paleobiology*, *37*(4), 655–669. https://doi.org/10.1666/10038.1

Tomazelli, L.J., Dillenburg, S.R., & Villwock, J.A. (2000). Late Quaternary geological history of Rio Grande do Sul coastal plain, southern Brazil. *Revista Brasileira de Geociências*, *30*(3), 474–476.

Troina, G., Botta, S., & Secchi, E.R. (2016). Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes. *Marine Mammal Science*, *32*(3), 1115–1137. https://doi.org/10.1111/mms.12316

Troina, G.C., Dehairs, F., Botta, S., Di Tullio, J.C., Elskens, M., & Secchi, E.R. (2020). Zooplankton-based $\delta^{13}C$ and $\delta^{15}N$ isoscapes from the outer continental shelf and slope in the subtropical western South Atlantic. *Deep-Sea Research Part I*, *159*, 103235. https://doi.org/10.1016/j.dsr.2020.103235

Vaz, A.C., Möller Jr., O.O., & Almeida, T.L. (2006). Análise quantitativa da descarga dos rios afluentes da Lagoa dos Patos. *Atlântica*, *28*(1), 13–23.

Villwock, J.A., & Tomazelli, L.J. (1995). Geologia costeira do Rio Grande do Sul. *Notas Técnicas*, *8*, 1–45.

Walker, J.L., & Macko, S.A. (1999). Dietary studies of marine mammals using stable carbon and nitrogen isotopic ratios of teeth. *Marine Mammal Science*, *15*(2), 314–334. https://doi.org/10.1111/j.1748-7692.1999.tb00804.x

Weiner, S., & Bar-Yosef, O. (1990). States of preservation of bones from prehistoric sites in the Near East: a survey. *Journal of Archaeological Science*, *17*(2), 187–196. https://doi.org/10.1016/0305-4403(90)90058-D

Wells, R.S., Cremer, M.J., Berninsone, L.G., Albareda, D., Wilkinson, K.A., Stamper, M.A., Paitach, R.L., & Bordino, P. (2021). Tagging, ranging patterns, and behavior of fransicana dolphins (*Pontoporia blainvillei*) off Argentina and Brazil: considerations for conservation. *Marine Mammal Science*, *38*(2), 571–605. https://doi.org/10.1111/mms.12879

Zerbini, A.N., Secchi, E., Crespo, E., Danilewicz, D., & Reeves, R. (2017). *Pontoporia blainvillei* (errata version published in 2018). *The IUCN Red List of Threatened Species*: e.T17978A123792204. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T17978A50371075.en

Zhao, Y., Yang, Y., Guo, Y., Ren, G., & Zhang, F. (2022). Stable carbon isotope composition of bone hydroxylapatite: significance in paleodietary analysis. *Paleoworld*, *31*(1), 169–184. https://doi.org/10.1016/j.palwor.2021.02.004

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

O estudo da fauna fóssil é, na grande maioria das vezes, desafiador em vista do escasso registro fóssil, degradação do material e ausência de contexto ambiental. Atrelado a isso, diversas espécies carecem de análogos atuais, pois pertencem a linhagens extintas. Nesse sentido, a análise de isótopos estáveis tem gerado resultados que superam boa parte das limitações inerentes ao registro fóssil. Na presente tese, o emprego dessa ferramenta permitiu a reconstituição das preferências alimentares de mamíferos terrestres do bioma Pampa brasileiro, além de ter fornecido uma perspectiva sobre as mudanças ecológicas sofridas por mamíferos marinhos na costa do Atlântico Sul-Ocidental. Cabe ressaltar aqui que tais inferências foram restringidas às razões isotópicas de carbono ($\delta^{13}C$) e oxigênio ($\delta^{18}O$), obtidas a partir da fração de bioapatita de ossos e dentes. Apesar de terem sido realizados esforços, não foi possível extrair com sucesso a fração de colágeno das amostras pleistocênicas coletadas no sul do Brasil.

A composição isotópica do material da megafauna terrestre sugere uma dieta baseada em plantas C_3, possivelmente gramíneas da família Poaceae, subfamília Pooidae, as quais eram abundantes durante períodos de clima frio do Pleistoceno. Ademais, essas gramíneas possuem alto valor nutricional e eram, portanto, capazes de sustentar uma alta diversidade de grandes herbívoros. Porém, uma alta sobreposição alimentar não significa que os recursos eram ilimitados naquela época. Possivelmente, os herbívoros pleistocênicos apresentavam algum nível de particionamento de recursos através do consumo de gramíneas com diferentes características ou espécies, a exemplo da megafauna africana atual.

As razões isotópicas de carbono não são apenas indicativas dos hábitos alimentares dos consumidores, mas também revelam suas preferências de habitat com base nas características dos produtores primários dominantes. Entre as espécies analisadas, três delas ainda ocorrem na América do Sul: o guanaco, *Lama guanicoe*, a vicunha, *Vicugna vicugna*, e a queixada, *Tayassu pecari*. Enquanto os camelídeos sul-americanos habitam regiões atualmente frias e secas, como os Andes, a queixada habita principalmente florestas. No entanto, essas espécies coexistiam no Pleistoceno do sul do Brasil, os camelídeos habitando campos, e a queixada habitando a vegetação ripária do bioma Pampa. Resultados esses que indicam a importância de considerar toda a fauna fóssil para inferências paleoambientais, como revelado no capítulo 2.

A interpretação das razões isotópicas de oxigênio provenientes de amostras de mamíferos terrestres é tipicamente mais complexa, pois depende de uma série de fatores, como comportamento de ingestão de água e fisiologia do animal, que podem gerar interpretações

errôneas sobre o paleoclima. Na presente tese, outros fatores que dificultaram tal interpretação foram a ausência de datação dos espécimes e o uso de diferentes tecidos (que compreendem janelas temporais distintas, pois apresentam diferentes períodos de síntese). A variação observada nos valores de $\delta^{18}O$ entre indivíduos e entre táxons distintos pode indicar que o sul do Brasil sofria com um clima sazonal durante períodos glaciais do Pleistoceno. No entanto, tais resultados devem ser interpretados com cautela. Para estudos posteriores, recomenda-se um maior rigor metodológico – em relação aos táxons analisados, idade dos espécimes e tecido, especialmente se o objetivo for reconstituir o paleoclima e/ou paleoambiente.

Em relação à megafauna marinha, as pesquisas paleontológicas ainda são incipientes. Comparado à megafauna terrestre, poucos táxons foram identificados e registrados para o Pleistoceno do sul do Brasil. Considerando essa carência de informações, um dos objetivos da presente tese foi identificar e descrever uma coleção de ossos do ouvido, os quais são diagnósticos a nível de família, gênero e/ou espécie. Com o auxílio de descrições previamente publicadas e disponíveis na literatura, registrou-se a presença de rorquais (*Balaenoptera* sp.), baleias-francas (*Eubalaena* sp.), delfinídeos (Delphinidae) e toninhas (*Pontoporia blainvillei*) – uma baixa (e provavelmente subestimada) diversidade comparada à atual. Em vista disso, recomenda-se que mais esforços sejam realizados para a identificação do material marinho proveniente dos sistemas laguna-barreira do Rio Grande do Sul, uma vez que são os únicos depósitos brasileiros conhecidos que preservaram fósseis de cetáceos pleistocênicos.

Entre os táxons supracitados, a toninha é a mais abundante no registro fóssil. Diversos fragmentos cranianos com idade estimada em aproximadamente 120.000 anos (período interglacial no qual o nível do mar estava mais alto) foram recuperados das praias do extremo sul do Brasil, alguns desses previamente descritos em detalhe no trabalho pioneiro de Ribeiro e colaboradores, publicado em 1998. A disponibilidade desse material permitiu o estudo da ecologia da espécie ao longo do tempo, através da análise isotópica ($\delta^{13}C$ e $\delta^{18}O$) de espécimes fósseis e recentes provenientes da FMA (i.e., *Franciscana Management Area*) III.

Similarmente à megafauna terrestre, os valores de $\delta^{13}C$ em mamíferos marinhos estão relacionados aos seus hábitos alimentares, porém os valores de $\delta^{18}O$ refletem o uso de habitat, pois oscilam de acordo com mudanças na salinidade. Quando ambos são plotados em um espaço bidimensional, configuram o que chamamos de nicho isotópico, fornecendo informações análogas ao nicho ecológico de uma população. Em decorrência dos valores de $\delta^{13}C$ mais amplos em fósseis, concluiu-se que o nicho ecológico da toninha era maior durante o Pleistoceno. Por outro lado, os valores de $\delta^{18}O$ foram mais variáveis na amostra recente.

Considerando as mudanças ambientais e geomorfológicas da planície costeira, tais resultados evidenciam um maior fluxo de água doce e mudanças de salinidade na costal atual.

Apesar de não ter sido o foco principal da presente tese, o estudo da megafauna terrestre e marinha evidenciou o impacto antrópico sofrido por essas populações. Continuando com o exemplo do capítulo 4, a menor amplitude trófica encontrada em toninhas atuais pode ser atribuída a uma menor diversidade de presas devido à sobrepesca. Além disso, sabe-se que a atividade pesqueira é responsável por uma alta taxa de mortalidade de toninhas no Rio Grande do Sul e em outras regiões ao longo de sua distribuição. Enquanto o impacto sobre os ecossistemas costeiros é um acontecimento mais recente, os efeitos sobre os ecossistemas terrestres começaram a milhares de anos atrás, culminando na extinção de grandes mamíferos terrestres, como discutido na introdução geral. Nesse contexto, o estudo da fauna fóssil pode ajudar a entender os processos de extinção e suas reais ramificações ecológicas.