

## Exceptional preservation of nonmineralized biomaterials in Cenozoic fossils of the Mammalia clade

Preservação excepcional de biomateriais não mineralizados em fósseis cenozoicos do clado

Mammalia

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### Abstract

The members of the Mammalia class experienced high ecological, morphological and taxonomic diversification during the Cenozoic Era. With technological advances in molecular paleontology in recent decades, many claims for the exceptional preservation of nonmineralized biomaterials have been made for this clade. However, these data are scattered in the literature and have associated such reports with unique and rare events. This study presents a review of the literature published in the last six decades, aiming to investigate the frequency with which vestigial of nonmineralized biomaterials are found in Mammalia fossils. The results identified 79 published studies describing endogenous biocomponents in Metatheria and Theria members. Of these, protein fragments were more frequent in sedimentary deposits (33.3%), and ancient DNA sequences were more frequent in depositional environments more favorable to exceptional preservation (53.1%), such as ice and cave deposits. The data on organic material for mammalian fossils are geographically and taxonomically comprehensive, with the superordinates Laurasiatheria and Afrotheria presenting the largest number of reports. With the emergence and improvement of new techniques for the recovery of organic material, it is believed that paleomolecular information for fossil mammals should increase, in addition to broadening the understanding of taphonomic factors related to exceptional preservation.

**Keywords:** Taphonomy; Ancient DNA; Paleogenomics; Ancient proteins; Paleoproteomics.

### Resumo

Os membros da classe Mammalia sofreram uma alta diversificação ecológica, morfológica e taxonômica durante a Era Cenozoica. Com os avanços tecnológicos em Paleontologia Molecular nas últimas décadas, muitas reivindicações de preservação excepcional de biomateriais não mineralizados foram realizadas para este clado. Entretanto, esses dados estão espalhados na literatura e têm associado tais relatos a eventos únicos e raros. Este trabalho apresenta uma revisão da literatura publicada nas últimas seis décadas, tendo por objetivo investigar a frequência com que vestígios de biomateriais não mineralizados são encontrados em fósseis de Mammalia. Os resultados identificaram 79 trabalhos publicados descrevendo biocomponentes endógenos em membros de Metatheria e Theria. Destes, os fragmentos de proteínas foram mais frequentes em depósitos sedimentares (33,3%) e os de sequências de DNA antigo mais frequentes em ambientes deposicionais mais favoráveis à preservação excepcional (53,1%), tais como depósitos de gelo e caverna. Os dados relativos a material orgânico para fósseis de mamíferos são geográfica e taxonomicamente abrangentes, com as superordens Laurasiatheria e Afrotheria apresentando a maior quantidade de relatos. Com o surgimento e aperfeiçoamento de novas técnicas de recuperação de material orgânico, acredita-se que informações paleomoleculares para mamíferos fósseis deverão aumentar, além de ampliar o entendimento dos fatores tafonômicos relacionados à preservação excepcional.

**Palavras-chave:** Tafonomia; DNA antigo; Paleogenômica; Proteína antiga; Paleoproteômica.

## Resumen

Los miembros de la clase Mammalia experimentaron una gran diversificación ecológica, morfológica y taxonómica durante la Era Cenozoica. Con los avances tecnológicos en Paleontología Molecular en las últimas décadas, se han hecho muchas afirmaciones de conservación excepcional de biomateriales no mineralizados para este clado. Sin embargo, estos datos están dispersos en la literatura y han asociado dichos informes con eventos únicos y raros. Este trabajo presenta una revisión de la literatura publicada en las últimas seis décadas, con el objetivo de investigar la frecuencia con la que se encuentran rastros de biomateriales no mineralizados en fósiles de mamíferos. Los resultados identificaron 79 trabajos publicados que describen biocomponentes endógenos en miembros de Metatheria y Theria. De estos, los fragmentos de proteínas fueron más frecuentes en depósitos sedimentarios (33,3 %) y los de secuencias de ADN antiguas fueron más frecuentes en ambientes de depósito más favorables a la conservación excepcional (53,1 %), como depósitos de hielo y cuevas. Los datos relacionados con el material orgánico de los fósiles de mamíferos son geográfica y taxonómicamente completos, y los superórdenes Laurasiatheria y Afrotheria tienen la mayoría de los informes. Con la aparición y mejora de nuevas técnicas de recuperación de materia orgánica, se cree que debe aumentar la información paleomolecular de los mamíferos fósiles, además de ampliar la comprensión de los factores tafonómicos relacionados con la conservación excepcional.

**Palabras clave:** Tafonomía; ADN antiguo; Paleogenómica; Proteína antigua; Paleoproteómica.

## 1. Introduction

Geological and paleontological evidence from the Cenozoic Era (65 Ma - present) indicates that this time interval showed, from its beginning, a high ecological, morphological and taxonomic diversity of mammals (Mammalia clade) (Benevento et al., 2019). This phenomenon is interpreted as the result of the ecological release of this group after the extinction of many other species, including non-avian dinosaurs, during the Cretaceous/Paleogene (K/Pg) transition, which marks the beginning of the Cenozoic Era (Slater, 2013).

Due to this rapid radiation experienced in a relatively short time, reconstructing the phylogenetic relationships of the clades of this taxonomic group proved to be particularly difficult (Romiguier et al., 2013; Morgan et al., 2013; Tarver et al., 2016; Benevento et al., 2019; Phillips & Zakaria, 2021). This occurs because, during this geological time, there are few accumulated phylogenetic signals and, when present, are almost always obscured by subsequent substitutions that probably occurred long after the speciation event (Benevento et al., 2019).

To provide more signals for the phylogenetic resolution of animals that experienced rapid radiation, as in this case, in recent years, cladistic reconstruction analyses have used genomic and proteomic scale data, as they were, for example, in tetrapods (Amemyia et al., 2013; Irisarri & Meyer, 2016), modern birds (Jarvis et al., 2014) and in the mammals themselves (Benevento et al., 2019; Phillips & Zakaria, 2021). However, the data obtained are uncertain and inconclusive for mammals (Benevento et al., 2019).

As the divergence between the main mammalian strains seems to have occurred in a period between 1 to 4 million years (Ma) only (Halström & Janke, 2010) and the phylogenetic analyses using modern samples, as mentioned above, are unsafe, the exceptional preservation of endogenous biomaterials in fossils can provide biomolecular information with high potential for phylogenetic use for the group (Alves & Machado, 2021a) and contribute to solving some common problems identified in all phylogenetic studies.

It is worth noting that even if the fossil record of mineralized bone elements of mammals is highly abundant, in turn, in the field of molecular paleontology, which is intended to recover, analyze and characterize nonmineralized biomaterials in fossils (Schweitzer, 2003), the report of findings of this type of material in the group, which has been done since the 1960s (Little et al., 1962; Wyckoff et al., 1964; Ho, 1965), is still dispersed in the literature and has associated this phenomenon with isolated and exceptional events (Gomes et al., 2021a, 2021b).

As the focus of this study is nonmineralized biomaterials, these can be represented in the fossil record by traces of soft tissues, cells, biomolecules and/or their degraded products, which are not originally biomineralized tissues (such as bones and

carapaces). For this reason, they were not replaced by the authigenic minerals present in the sediments during the fossil diagenesis (Schweitzer, 2003; 2004; Alves & Machado, 2020, 2021b; Alves, 2022).

Several types of vestigial biomaterials have already been found in fossils of various taxa (Thomas & Taylor, 2019; Alves & Machado, 2020, 2021a, 2021b, 2021c; Gomes et al., 2021a, 2021b) and are exemplified by epithelial and connective tissues and blood, structures of blood vessels, erythrocytes, osteocytes, biomolecules such as proteins, lipids, melanin pigments, peptide and amino acid sequences and even intracellular compounds chemically consistent with nuclear and mitochondrial DNA (Alves & Machado, 2020; Bailleul et al., 2020, 2021; Reest & Currie, 2020).

Given the degree of relevance of the findings of endogenous biological materials in the fossil record, this study aims to investigate the frequency of the claimed findings of nonmineralized biomaterials in fossils of the Mammalia clade from Cenozoic deposits.

## 2. Materials and Methods

We conducted a narrative review of the literature. This type of study allows to describe and analyze the state of the art on a given subject, without, however, requiring the establishment of a rigorous and replicable methodological path in terms of data reproduction and numerical answers to specific questions (Vosgerau & Romanowsk, 2014). On the other hand, it is a fundamental methodology for acquiring and updating knowledge on a specific subject, in order to highlight themes that have received little attention in the available literature (Rother, 2007).

The descriptors “mammals”, “Cenozoic fossils”, “Soft tissue”, “Exceptional preservation”, “Ancient protein” and “Ancient DNA” were used to search for articles, isolated or in combination, in Portuguese and English. The search was conducted in peer-reviewed journals available in the SciELO, CAPES and PubMed databases, with a search time interval of approximately six decades (from 1962 to 2021). When there was no specific literature on the subject in the aforementioned platforms, the search for Google Scholar was expanded.

The search resulted in the construction of two categorical tables, which describe the main information available in the publications about claims of traces of nonmineralized biomaterials in fossils of the Mammalia clade of the Cenozoic Era. Table 1 (consult supplementary material, hereinafter SM) consists of information from findings from sedimentary deposits (fluvial, marine, lacustrine, for example) and depositional environments considered to be unfavorable for the preservation of original biomaterials (Simões et al., 2014) since in these environments, biocomponents are exposed to environmental degradation factors, such as high temperatures, pressures that occur during the diagenesis of rocks, seasonality of temperature, humidity, solar radiation and biotic agents (Sansom, 2014; Alves, 2022). In contrast, Table 2 (SM) contains data on findings from different taphonomic contexts, such as tar wells, amber preservation, glacial deposits and caves, widely understood as deposition environments more favorable to the conservation of biomolecular structures, cells and tissues (Gobbo & Bertini, 2014).

The following exclusion criteria were used for the two tables: (a) articles that claimed the presence of totally or partially mineralized biomaterials, i.e., in which the biological material was replaced by the autogenous minerals of the sediments during fossil diagenesis; (b) articles that claimed the presence of endogenous biomaterials of hard parts (such as biominerals constituting skeletal parts or carapaces); (c) articles that claimed the presence of nonmineralized biomaterials in mammalian fossils from non-Cenozoic fossil layers; (d) articles that claim degradation products of organic molecules, however only showed organic chemical elements, such as organometallic and organosulfurized; (e) articles that claimed nonmineralized biomaterials, but that showed signs of microbial mimicry or exogenous geochemical precipitates; (f) articles that, although they described the presence of nonmineralized biomaterial in the research interest groups, did not present detailed information

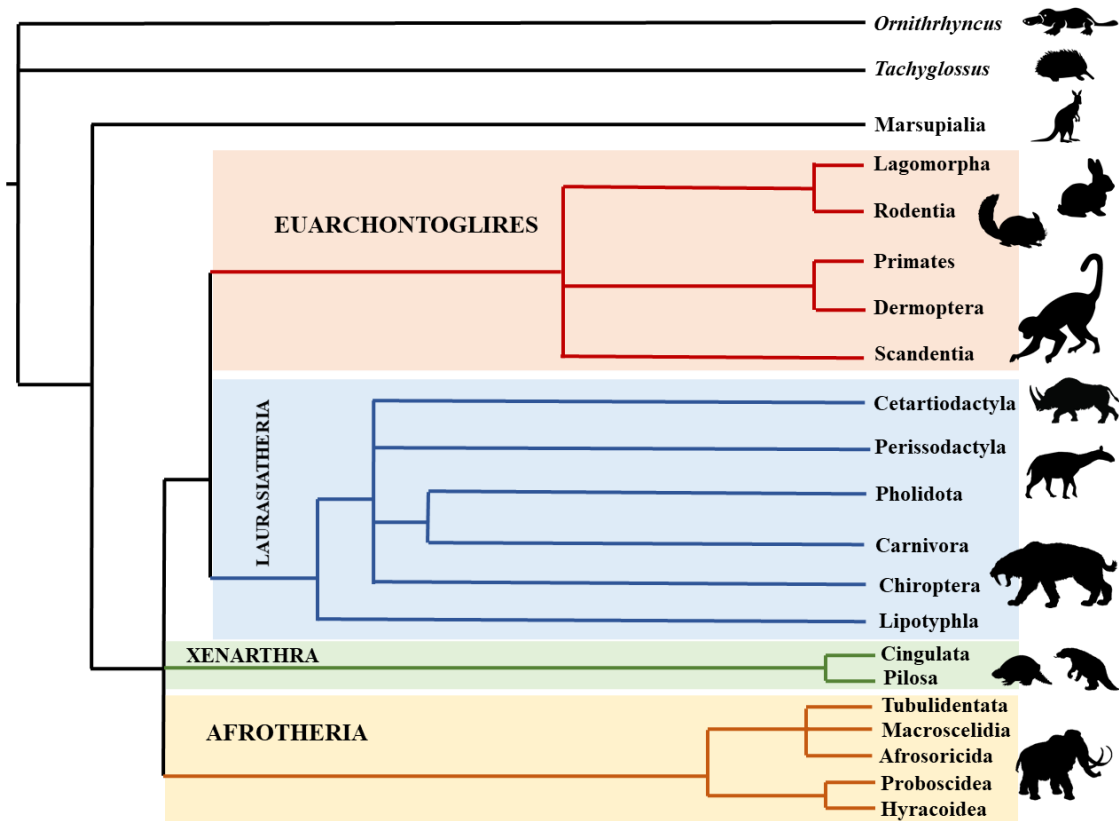
about the finding; and (g) articles that did not use analytical techniques for recovery, analysis and characterization of endogenous biological materials.

### 3. Theoretical Foundation

For the categorization of the findings of nonmineralized biomaterials by taxonomic groups of the Mammalia clade, the phylogenetic hypothesis developed by Phillips and Zakaria (2021) was used as a basis. When it was not possible to find information in this proposal about the phylogenetic grouping of the clade, the taxonomic base collected in the scientific articles that described the findings was used.

According to the phylogenetic hypothesis developed by Phillips and Zakaria, the Mammalia clade comprises all animals represented in Prototheria (the monotremes *Ornithorhynchus* and *Tachyglossus*) and in Theria, the most diversified clade. This, in turn, comprises the Metatheria (marsupial) and Eutheria (placental) groups. Eutheria, which is the clade with the largest number of representatives, includes four superorders: Euarchontoglires, Laurasiatheria, Xenarthra and Afrotheria (Figure 1). In the proposal developed by Phillips and Zakaria (2021), Euarchontoglires appears as a sister taxa to Laurasiatheria.

**Figure 1** - Phylogenetic proposal regarding the origin and diversification of the Mammalia clade. Evolutionary hypothesis constructed with the use of the mitogenomic phylogeny of ancient and currently living species.



Source: adapted from Phillips and Zakaria (2021).

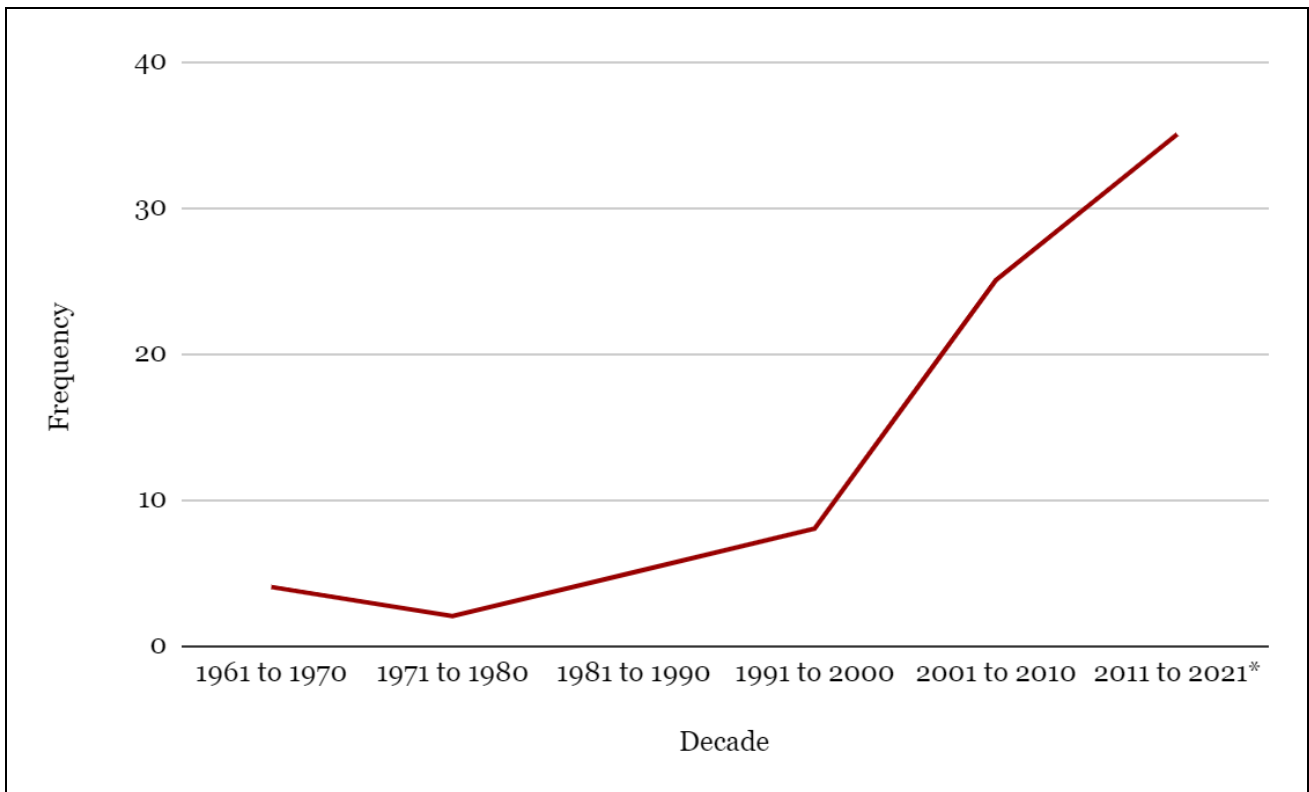
Therefore, based on the aforementioned phylogenetic proposal, we grouped the different findings of remaining nonmineralized biomaterials by taxonomic groups belonging to subclades within the main clades Metatheria and Eutheria.

## 4. Results and Discussion

### 4.1 General characterization of the findings

Regarding the numbers of publications per decade, the results can be seen in Figure 2. We observe that the works with claims of nonmineralized biomaterials began to be published in the early 60s, but remained scarce until the 2000s, with a set of 19 articles published in four decades. Since the beginning of the new century, the number of publications has increased, reaching 60 studies published in just two decades. This evolution is probably due to the official establishment of Molecular Paleontology as a research field in the early years of the 21st century and the introduction of new paleomolecular research techniques (Alves, 2020; Alves, 2022). With technological advances and new fossil discoveries, the trend will be that this frequency will continue to increase in the coming years.

**Figure 2** - Published studies claiming traces of nonmineralized biomaterials found in fossil mammals from different Cenozoic taphonomic contexts, organized by decade. Observation: it was decided to keep the year 2021 in the 2010s.

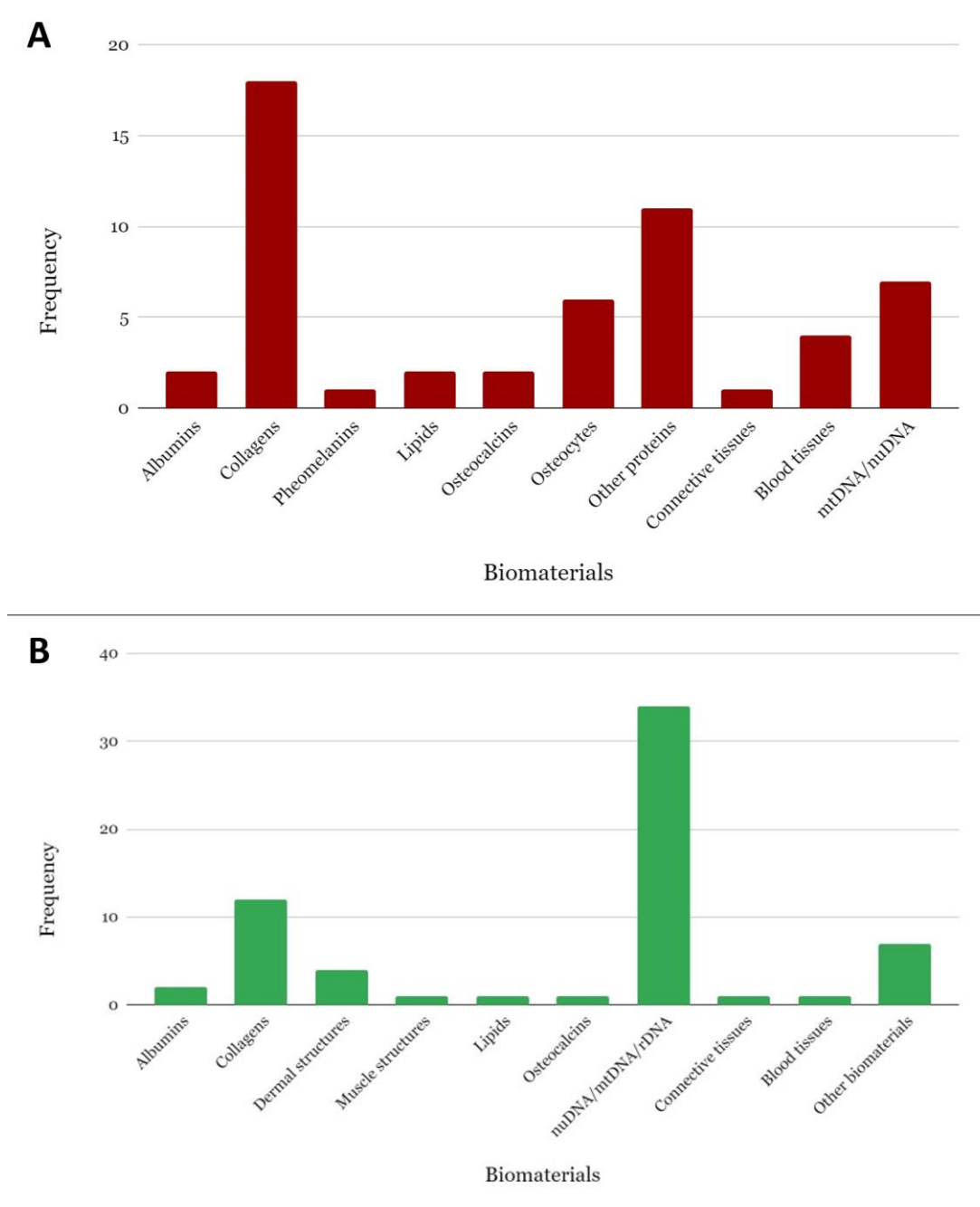


Source: Authors (2022).

In this study, 43 articles were identified that report findings of original biocomponents of the Mammalia clade in fossils recovered from sedimentary deposits little favorable to exceptional preservation (Table 1 of SM) and 36 articles with claims from other taphonomic contexts more favorable to this type of preservation. (Table 2 of the SM). Of these, there was a great diversity of types of nonmineralized biomaterials recovered from sedimentary deposits, with collagen traces being the most abundant (33.3%), followed by noncollagenous protein samples (20.4%) and distantly by genomic fragments of mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) (13.0%) (see Figure 3A). For findings of environments favorable to exceptional preservation, the genomic samples of mtDNA, nuDNA and ribosomal DNA (rDNA) led the claims (53.1%) in relation to the second most frequent biomaterial, collagen fragments (18.8%). (Figure 3B). It is worth noting that some articles

reported more than one type of endogenous biomaterial, as well as more than one type of analytical technique and more than one fossil analyzed, causing the data to overlap the number of published articles synthesized in both tables.

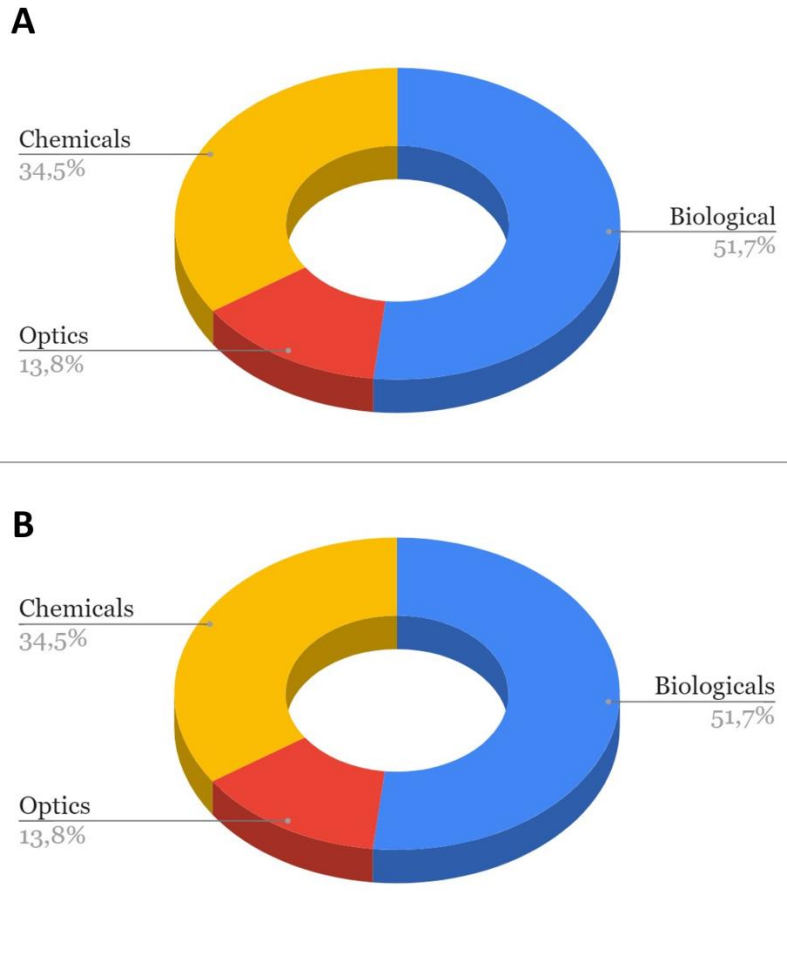
**Figure 3** - Frequency of the main types of nonmineralized vestigial biomaterials found in fossils of the Mammalia clade, recovered from the Cenozoic Era. (A) Biomaterials recovered from sedimentary deposits; (B) Biomaterials retrieved from other taphonomic contexts more favorable to exceptional preservation.



Source: Authors (2022).

There was also a wide range of analytical techniques used, with 44.7% being of chemical nature, 27.7% biological and 27.7% optical, for findings in sedimentary deposits (Figure 4A) and 51.7% of biological nature, 34.5% chemical and 13.8% optical, for findings of taphonomic contexts more favorable to exceptional preservation (Figure 4B).

**Figure 4** - Frequency of the main groups of analytical techniques used in the detection of traces of nonmineralized biomaterials found in fossils of the Mammalia clade recovered from the Cenozoic Era. (A) Techniques used in fossil samples of sedimentary deposits; (B) Techniques used in fossil samples from other taphonomic contexts more favorable to exceptional preservation.

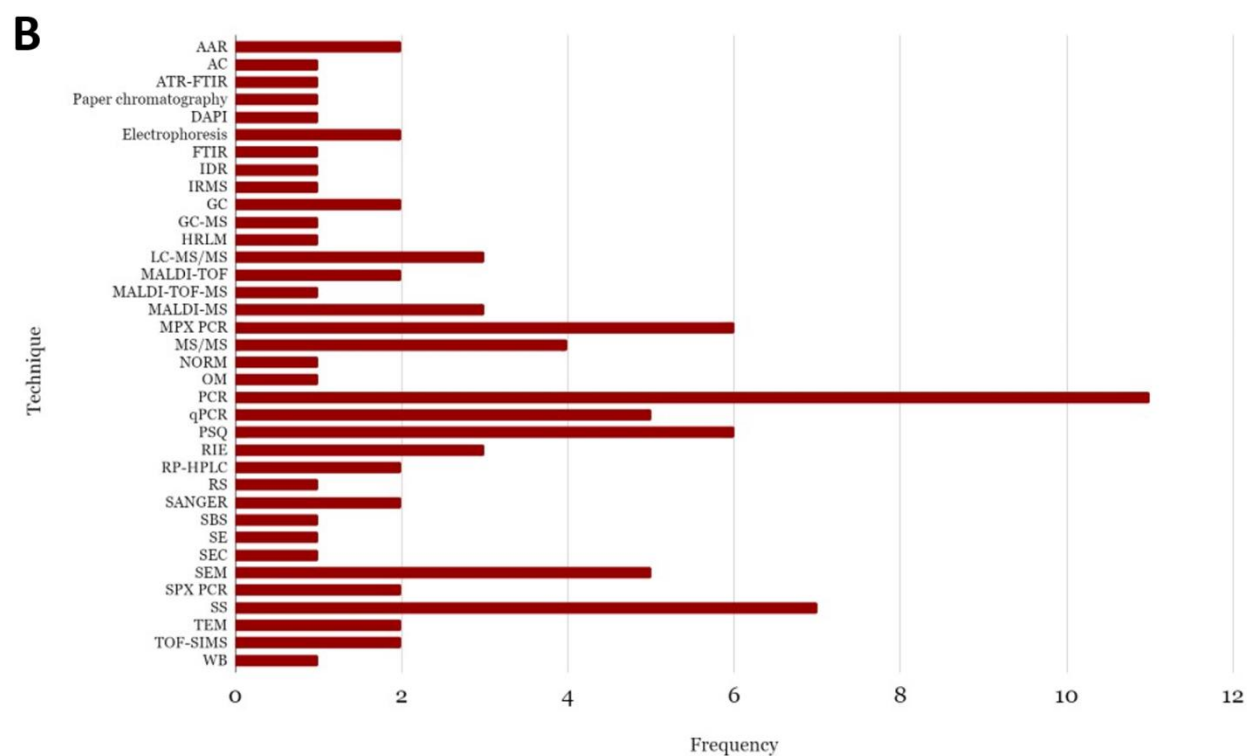
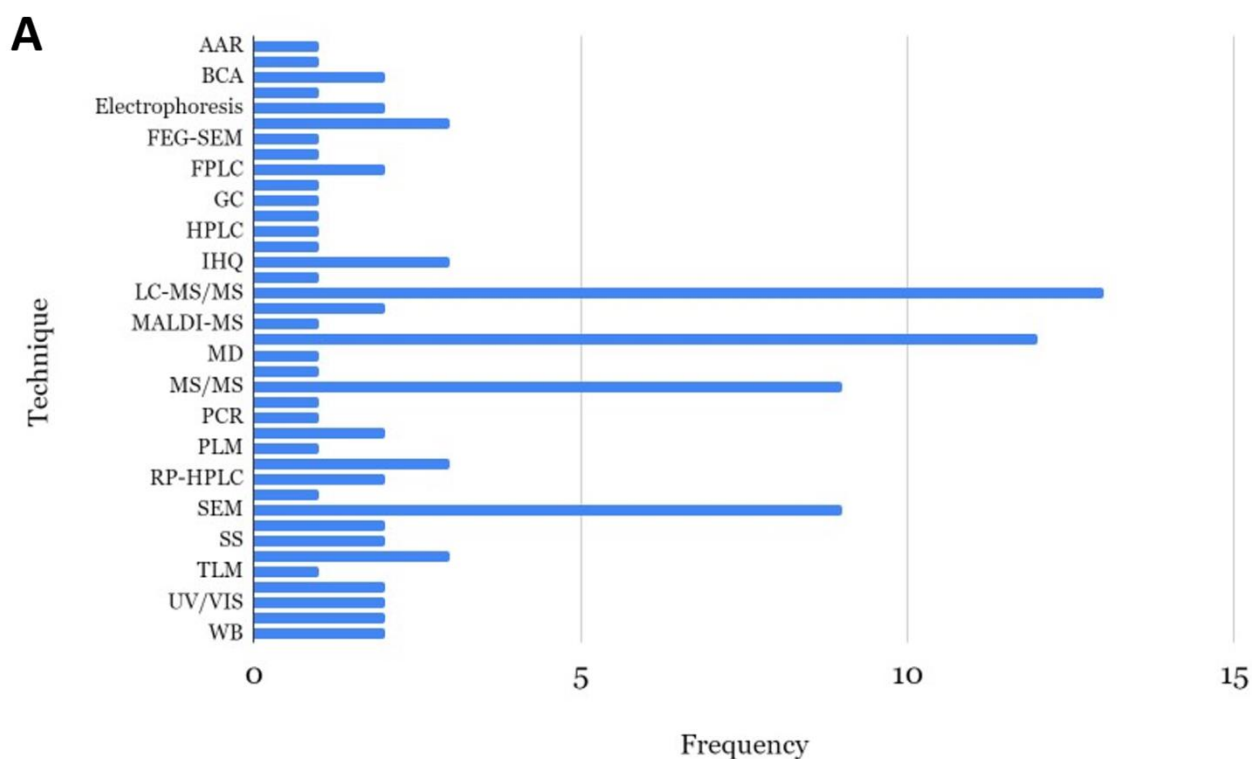


Source: Authors (2022).

In a deeper and more specific analysis, the analytical technique most frequently employed in the detection of endogenous biomaterials in fossils from sedimentary deposits was liquid chromatography coupled to mass spectrometry (LC–MS/MS), with 13.3% of the results, followed by mass spectrometry, flight time of matrix-assisted laser desorption/ionization (MALDI-TOF), with 12.2%, and by tandem mass spectrometry (MS/MS), with 9.2% (Figure 5A). For mammalian fossils from deposits more favorable to exceptional preservation, the polymerase chain reaction (PCR) technique stood out from the others, occupying 12.5% of the results, ahead of the Shotgun (SS) sequencing, with 8.0%, and multiplex PCR (MPX PCR) and pyrosequencing (PSQ), both with 6.8% (Figure 5B).



**Figure 5** - Frequency of analytical techniques used in the detection of traces of nonmineralized biomaterials in fossils of the Mammalia clade recovered from the Cenozoic Era. (A) Techniques applied to fossils from sedimentary deposits; (B) Techniques applied to fossils from taphonomic contexts more favorable to exceptional preservation.



Source: Authors (2022).

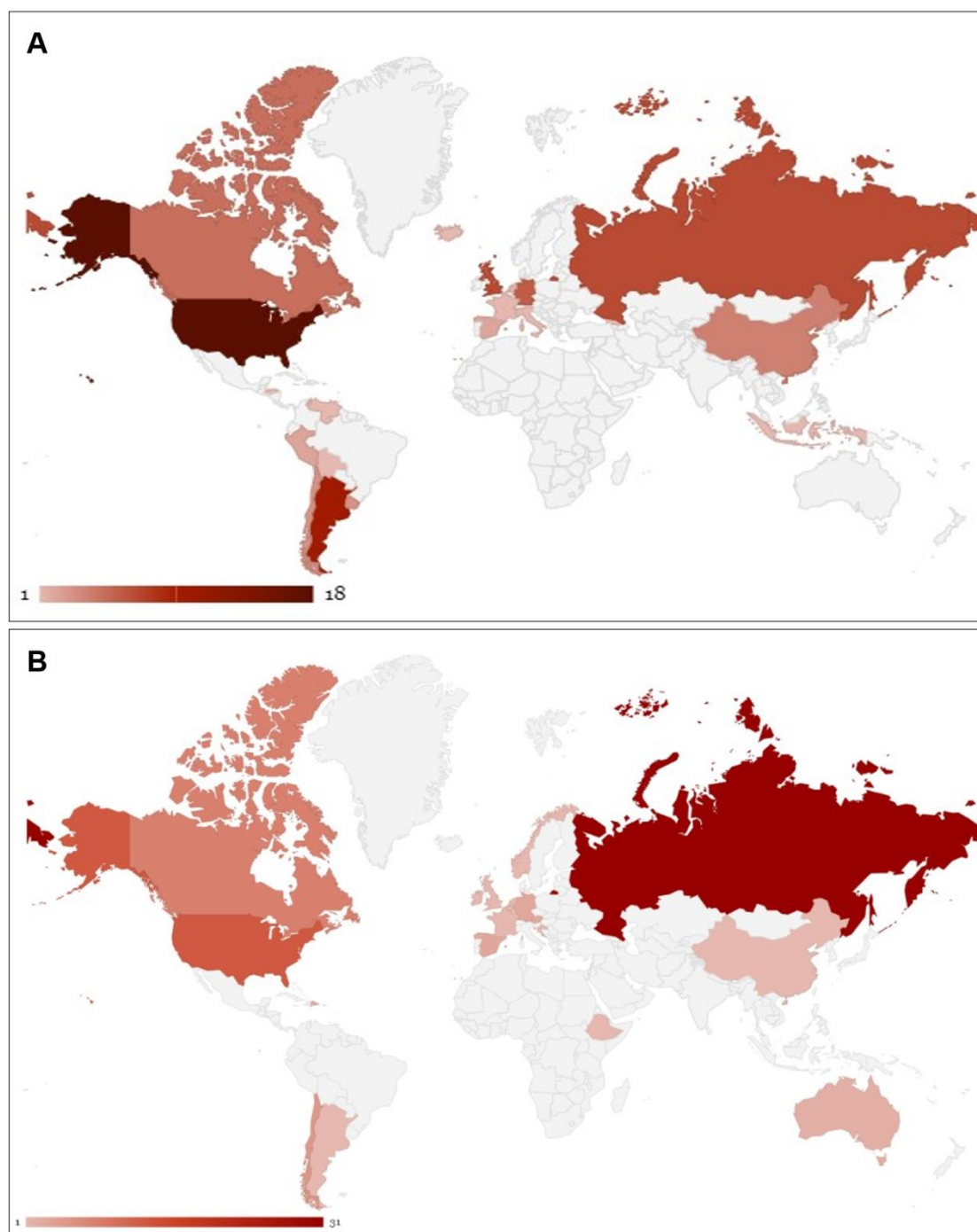


Regarding the Periods of the Cenozoic Era, the Pleistocene presented the highest number of original biomaterials, that is, 82.1% of the total for fossils of sedimentary deposits (Figure 1.2 of the SM) and 91.3% for fossils from other taphonomic contexts more favorable to preservation (Figure 2.2 of the SM). Regarding the deposition environments, there was a tendency to lack information in the articles regarding the specifications of the type of depositional environment and rock type, especially in the older ones (see Tables 1 and 2 of the SM). Of the articles that reported such information, for sedimentary environments, fluvial and alluvial deposits and gravitational flow were more frequent (8.0, 6.0 and 6.0%, respectively) (Figure 1.3 of the SM). For environments of other taphonomic contexts more favorable to preservation, the karst (caves) and periglacial (permafrosts) deposits prevailed, with 33.9 and 23.7% of cases, respectively (Figure 2.3 of the SM).

Of the articles in Table 1 of the SM that mention, in addition to the sedimentary environment, the type of rock in which the fossil was preserved, 24.1% of them reported sandstone, followed by claystone, siltite and gravel, all with the same proportion of 17.2% (Figure 1.4 of the SM). These results are consistent with what recent studies have shown, i.e., that the rapid burial of the organism or its carcass in a sandstone environment seems to favor exceptional preservation in relation to other sedimentary deposits, such as clay and schist, although the real reason is still unknown (Schweitzer et al., 2007b, 2009, 2019). To date, there is only one hypothesis proposed by Schweitzer et al. (2019), which refers to the porous nature of sand, which would facilitate the rapid dispersion of suppurating fluids (which are rich in microorganisms and degrading enzymes), thus facilitating the conservation of endogenous structures of living beings during the formation of the fossil.

In general, the findings are comprehensive and widely distributed. The data shown in Table 1 of the SM show that, with the exception of Africa, Oceania and Antarctica, all other continents have claims of endogenous biocomponents in fossil mammals (the United States is the country with the largest number of reports), as shown in Figure 6A. Similarly, the data in Table 2 of the SM reveal that nonmineralized biomaterials were found in representatives of the taxon studied in all continents of the world (except Antarctica), as shown in Figure 6B. Russia, more specifically the Siberia region, has the largest number of reported findings (31 in all), probably because this region represents one of the few areas of the globe in which layers of permafrosts older than the Holocene remain, together with the Yukon Valley in Canada and Antarctica (Liang et al., 2022).

**Figure 6** - Global distribution of different types of nonmineralized vestigial biomaterials found in fossil mammals recovered from Cenozoic deposits. (A) sedimentary deposits; (B) deposits of taphonomic contexts more favorable to exceptional preservation.



Source: Authors (2022).

Next, the results obtained are discussed and summarized in the tables for the Mammalia clades, in which nonmineralized biomaterials were identified.

## 4.2 Metatheria

The subclass Metatheria comprises marsupial mammals, which diverged from members of the subclass Eutheria in the second half of the Lower Cretaceous (130.7-120 Ma) or even earlier, according to some indications (Bi et al., 2018). Although marsupials represent animals that have undergone much less radiation than placentals, they fascinate researchers due to their highly specialized reproductive biology and the wide distribution of fossils on all continents (Eldridge, 2018). The metatherian fossils are distinguished from the euteriums by the shape of their dentition: they have four pairs of molar teeth in relation to the maximum of three pairs of euteriums (Benton, 1997; Bi et al., 2018). In the search, few records of nonmineralized biomaterials were found in this clade, all restricted to the superorder Australidelphia.

### 4.2.1 Australidelphia

The superorder Australidelphia comprises four orders, Notoryctemorphia, Peramelemorphia, Dasyuromorphia and Diprotodontia. The few endogenous organic materials recorded for this taxon are exclusive to taphonomic environments more favorable to exceptional preservation (in this case, swamps and caves). The first record was made by Rowley et al. (1986) of two kangaroos of indeterminate species of the family Macropodidae, order Diprotodontia, of the Upper Pleistocene (129-11.7 Ka). Collagen was identified in bone fragments not determined in the article using biological techniques based on monitoring antigen-antibody interaction, radioimmunoassay (RIE) and western blot (WB), which are based on well-documented specificity and sensitivity of the vertebrate immune response (Lowenstein & Scheuenstuhl, 1991). The specimens were recovered from a marshland and a cave in Australia.

Another published study used chemical and biological analytical techniques, such as real-time quantitative PCR (qPCR), SANGER sequencing and pyrosequencing, and identified the presence of mtDNA sequences of several bone fragments from fossil specimens of animals of the Diprotodontia (red kangaroo and kangaroo rat) and Dasyuridae (eastern quoll and tiger cat) families from the Upper Pleistocene (Haouchar et al., 2014).

The fact that there are few reported discoveries of nonmineralized biomaterials in metatherian mammals is probably due to the numerous gaps in the fossil record, and most taxa are known only from dental remains (Eldridge, 2018). Australia, for example, has few fossiliferous sites that preserve mammals since the emergence of the Paleogene, a period in which this group began to diverge (Duchêne et al., 2018). Even so, these discoveries, although few, demonstrate the potential of seeking to recover and analyze more metatherian fossils in caves, and Australia may be a location that can provide additional sources of information for paleomolecular investigations of vertebrates.

## 4.3 Eutheria

The infraclass Eutheria comprises placental mammals, which, unlike metatherians, have undergone high adaptive and morphophysiological diversification, reaching a community of more than 4,000 existing species (Mess, 2014). The analysis of molecular data sets led to a revolution in the phylogeny of this group, which was previously based only on morphological data (Archibald, 2003); now, the infraclass is divided into four superorders: Euarchontoglires (rodents, lagomorphs, primates, shrews, flying lemurs) that evolved independently across continents; Laurasiatheria (carnivores, cetartiodactyls, pangolins, bats) that evolved mainly on the continents of the Northern Hemisphere; Afrotheria (elephants, manatees, armadillos, elephant shrews, tenreks) that originated in Africa; and Xenarthra (sloths, anteaters, armadillos) whose main distribution area is South America (Mess, 2014). During the search, several reports of endogenous biomaterials were found in fossils of this clade.

#### 4.3.1 Euarchontoglires

This superorder currently comprises five orders of the Mammalia clade: Lagomorpha (rabbits and hares), Rodentia (rodents), Primates (primates), Dermoptera (colugos) and Scandentia (tupias) (Vander Linden et al., 2019). There are notable differences between the locomotor modes of the orders, including fossorial, ricocheted, arboreal, terrestrial and gliding locomotion (Geng et al., 2020), which can be found in more than one order, as in the case of the arboreal mode, which is present in Scandentia, Rodentia and Primates (Vander Linden et al., 2019). These forms of locomotion have long been studied as a field of investigation of morphophysiological convergence (Geng et al., 2020).

Of the findings of this clade, Kuch et al. (2002) identified, through the PCR technique, the presence of mtDNA fragments in a coprolite of the species *Phyllotis limatus* from the Upper Pleistocene of Chile in a sedimentary depositional environment of gravitational flux. By similarly studying a specimen recovered from the sedimentary environment, Cleland et al. (2016) analyzed, through hybrid Fourier transform mass spectrometry (hybrid FTMS), the nasal shells of a giant beaver *Castoroides ohioensis* from the Upper Pleistocene found in the United States and identified the presence of collagen from type 1, a biomaterial that apparently has a greater preservation potential than most proteins in sedimentary deposits, as our results showed (see Figure 3A). This is most likely because collagen is the main protein component of bones (Bray et al., 2020) and because it is a biomolecule with a triple-helix fibrous structure, whose long peptide chains intertwine to form fibrils, making it highly resistant to fossilization (Rana, 2016).

Regarding taphonomic environments more favorable to the preservation of nonmineralized biocomponents, nonnucleated red blood cells were found, supposedly of a Platyrrhini species, present inside the digestive tract of an amber-encapsulated tick dated to 20 Ma and recovered from a turbiditic sandstone deposit in the Dominican Republic (Poinar, 2017).

The results for Euarchontoglires reveal that, compared to other superordines of Theria, there are few reports of nonmineralized biomaterials in fossils for this clade. This trend may be a consequence of a shortage of fossil samples available for the taxonomic group (Beck & Baillie, 2018) or, perhaps, a lack of interest or resources to perform biochemical analyses in fossil samples of the clade. However, even so, the data obtained are in accordance with what was expected, that is, that most of the findings were from Rodentia, as it is the order of Euarchontoglires with the greatest amount of fossils ever found (Fostowicz-Frelik et al., 2021), and in this analysis they represented more than half of the findings of nonmineralized biomaterials (2 of the 3 reports).

#### 4.3.2 Laurasiatheria

This superorder represents a group of mammals that originated in the supercontinent Laurasia, currently comprising six orders, namely, Cetartiodactyla (cetaceans and artiodactyls), Perissodactyla (hippos, equidae, tapirs and rhinos), Pholidota (pangolins), Carnivora (ursids, felines) and mustelids), Chiroptera (bats) and Eulipotyphla (solenodonts, moles, shrews and hedgehogs).

This clade is seen as the top of the challenges for the establishment of phylogenetic relationships between mammalian taxa (Hu et al., 2013), since, except for Eulipotyphla, all other orders diversified in a short period from 1 to 4 million years (Hallström & Janke, 2010; Zhou et al., 2012). In the absence of a few genes with a strong phylogenetic signal related to the divergence of laurasiatherians, many phylogenomic hypotheses have been proposed (for example, only in the last decade: Hallström et al., 2011; Mccomack et al., 2012; Zhou et al., 2012; Nery et al., 2012; Romiguier et al., 2013; Benevento et al., 2019).

After several years of studies and discussions based on genomic data, there are only two positions with a certain degree of certainty: Eulipotyphla is a sister clade of all other laurasiatherians, and Pholidota is a sister group of Carnivora,

while the placements of the other four orders (Carnivora, Perissodactyla, Cetartiodactyla and Chiroptera) remain a subject of intense debate, even though thousands of genes are used for cladistic analysis in the comparison (Benevento et al., 2019).

However, there is a scientific consensus that long sequences of ancient DNA (aDNA) from fossils of Cenozoic mammals may help in this crucial endeavor to understand the evolution of mammals (Lv et al., 2021). Therefore, searching for fossilized bone elements with original biocomponents of the group, especially from paleogenomic data, is a valuable task.

The findings of nonmineralized biomaterials for the Laurasiatheria clade are numerous and of great taphonomic diversity, in addition to being represented in all the decades analyzed in this study. For example, since the beginning of paleomolecular research with mammalian fossils in the 1960s, the La Brea ranch, located in Los Angeles County, has stood out, revealing many biomolecular specimens. The tar infiltrations of this region have since been extensively studied to reconstruct the ecology and phylogeny of the last faunal communities of the Pleistocene, especially that of several extinct taxa of medium to large mammals, which are abundant at the site and are abundant sources of original biological material of the Mammalia clade during the Cenozoic Era (Coltrain et al., 2002).

These infiltrations are fuelled by oil originating from vertically inclined tar sands in the Los Angeles Basin, which is south of the Santa Monica Mountains (Coltrain et al., 2002). From these, different samples of amino acids and collagen fibers from laurasiatherians of the Upper Pleistocene were obtained from various biochemical analyses (Wyckoff et al., 1964; Ho et al., 1965; Wyckoff & Davidson, 1976).

Different endogenous biocomponents of this clade were also recovered from specimens from taphonomic environments with the presence of ice (glaciers, interglacial or periglacial). A considerable amount, for example, of skeletal remains and frozen cadavers of fossil mammals were preserved in the permafrosts of the Arctic zone of Yacutia during the late Pleistocene (Boeskorov et al., 2013). In this depositional environment, several representatives were found, including mammoths, woolly rhinos, horses and bison, and these were preserved with various organic information. The fossil deposits are widely distributed in the lowlands of the northern region of Yacutia, Siberia, with some permafrosts having reached more than 40 meters in thickness and with enormous ice complexes. These are very important because numerous sedimentological, paleontological, geochemical and isotopic data were stored in them (Schirrmeister et al., 2002).

The results of this study contribute to this trend, since several types of nonmineralized biomaterials were found in fossils of mammals of the Laurasiatheria clade from Yacutia. For example, in 1996, Bocherens et al. (1996) recovered collagen organic matter from different members of the orders Perissodactyla, Cetartiodactyla and Carnivora. Another potential of the area is the genetic data, that is, some studies with fossil samples of horses and bison from the region claimed the presence of original DNA sequences (Taylor et al., 1996; Nielsen-Marsh et al., 2002; Schubert et al., 2014).

Similarly, the territories of Canada and Russia are territories of Pleistocene glacial deposits with great possibilities for paleomolecular investigation, including mtDNA and nuDNA genomic sequences (Nielsen-Marsh et al., 2002; Gilbert et al., 2004; Orlando et al., 2013; Heintzman et al., 2015) and collagen peptide sequences (Orlando et al., 2013; Buckley et al., 2019). Other sites with preservation mechanisms involving ice were also found in Germany (Valdiosera et al., 2006) and Norway (Lindqvist et al., 2010), both with the presence of sequences of genetic material. These results show the effectiveness of exceptional preservation by cryopreservation, since temperature stability is a key factor in delaying necrolysis activity (Bettoni et al., 2019).

Undeniably, the recovery of nonmineralized biomaterials in fossils of laurasiatherians was higher in karst environments, probably because the caves seem to have more advantages, for example, in the temperature and pH of the rocks, thus favoring the survival of bone elements, including genomic material (Collins et al., 2002). Several studies have claimed the presence of mtDNA and nuDNA sequences in woolly rhinos, cave bears, giant camels, horses and macrauchenia recovered from Pleistocene caves in Europe, Chile, China, Canada and the United States (Orlando et al., 2003; Noonan et al., 2005; Lister

et al., 2005; Valdiosera et al., 2006; Orstrom et al., 2006; Krause et al., 2008; Dabney et al., 2013; Westbury et al., 2017). Data were also found regarding collagen samples in this type of environment (Buckley & Collins, 2011; Rao, 2020).

In addition to these, many examples of organic biomaterials recovered in various sedimentary deposits were claimed for this clade. Among these findings, the recovery of osteocytes and pheomelanins from fossils of bats and a pangolin dated with ages close to 48 Ma from the shale quarries of Messel Pit, Germany (Colleary et al., 2015; Cadena, 2016). This location has been recognized as having great potential for exceptional preservation of elements that would naturally mineralize, especially melanins (Vinther et al., 2010; O'Reilly et al., 2017). After these recoveries, recent experiments have shown that melanin seems to have a natural resistance to diagenetic processes (Rossi et al., 2019), surpassing other biomolecules. The hypothesis proposed is that during diagenesis, certain endogenous metals would provide an association with the biomolecule, making it more stable to survive in very old fossils, but new research still needs to be done.

In addition to the Messel Pit schist, samples of albumin, collagen and other proteins, such as melanins, were obtained from lacustrine deposits (Borja et al., 1997), fluvial-lacustrine deposits (Rybczynski et al., 2013), high terraces and fluvial (Buckley et al., 2019), marine and lagoon deposits (Boskovic, 2021). Other studies, although they did not specify the type of sedimentary depositional environment, reported the encounter of collagen fragments in bison, *Dremotherium*, camels, bovines and rhinos of the Pleistocene (Little et al., 1962; Ulrich, 1987; Welker et al., 2017; Buckley et al., 2019). In addition, a study claimed the presence of peptides, lipids and blood vessel-like structures of a Neogene whale fossil from the Pisco Formation, Peru (Vidal, 2006).

Other discoveries of nonmineralized biomaterials in fossils of laurasiatherians mammals were made by Peñalver and Grimaldi (2005). In the published article, they claimed, with the use of optical microscopy (OM), the presence of body hair of a possible solenodon in an amber, dating from the Miocene, found in the Dominican Republic. The resins of plants of the family Fabaceae, which are identified in several amber samples from the Dominican Republic, have a complex chemical composition and have many variables that simultaneously influence the processes of exceptional preservation, thus facilitating the recovery of larger samples, such as hairs (Lambert et al., 2015).

The results show that laurasiatherian fossils are abundant sources of paleobiochemistry and paleohistological information, as they represent an enormous number of reports of original biomaterials of extinct taxa. This propensity may be due to Laurasiatheria being higher in number of orders and having a dense and widely studied fossil record (Phillips & Zakarias, 2019).

### 4.3.3 Xenarthra

This superorder currently comprises two orders, namely, Pilosa (anteaters and sloths) and Cingulata (armadillos). The xenarths possibly appeared at approximately 59 Ma in the Paleocene (O'Leary et al., 2013) and diversified abundantly throughout America, with some representatives reaching great heights (giant sloths). Its fossil record is visibly abundant (Boscaini et al., 2019) and represents, together with Laurasiatheria, an important source of molecular paleontological research.

The research on Xenarthra Molecular Paleontology was conducted in fossils from different taphonomic contexts, but in greater quantity in giant sloths recovered from caves in South America, where the PCR and electrophoresis techniques allowed obtaining genomic data of these beings. For example, from the same individual of *Mylodon darwini* found in the Ultima Esperanza cave in Chile, dating from the end of the Upper Pleistocene, ribosomal DNA (rDNA), mtDNA and nuDNA sequences were identified in teeth, bones and coprolites (Höss et al., 1996; Taylor, 1996; Clack et al., 2012). Other mtDNA sequences were obtained from an indeterminate ground sloth, this time from a specimen from Argentina (Hofreiter et al., 2003). In addition, PCR and qPCR studies with museum fossil repository xenarths also yielded mitogenomic information (Greenwood et al., 2001; Delsuc et al., 2016).



Reports of findings of nonmineralized biomaterials for this clade in other depositional contexts have also occurred. For example, the fossil of a giant sloth of undetermined species, recovered from La Brea tar infiltrations, yielded the detection by gas chromatography (GC) of collagen amino acids (Wyckoff & Davidson, 1976). In turn, from a fluvial deposit in Uruguay, fossil elements of a *Lestodon armatus* showed conserved organic fragments of amino acids and peptides of type 1 collagen (Buckley et al., 2015). By means of digital microscopy (MD) and scanning electron microscopy (SEM), osteocytes, erythrocytes and pieces of blood vessels were identified in vertebrae and femurs of giant sloths of the Upper Pleistocene of the Gracías and Padre Miguel Formation in Honduras (Zúniga et al., 2019).

According to the results obtained here, the search for paleomolecular data of the Xenarthra clade consisted, over the years, of a greater weight of species recovered from karst environments, although some recent studies have claimed the presence of organic material from sedimentary deposits (Buckley et al., 2015; Zúniga et al., 2019). It is possible that with the recovery of new fossil samples and the improvement of analytical detection techniques, new information on nonmineralized biomaterials will be provided to the group.

#### 4.3.4 Afrotheria

Most groups of mammals included in this superorder share little or no morphological similarity (Stanhope, 1998) and currently comprise the orders Hyracoidea (hiraces), Proboscidea (elephants, including extinct mammoths and mastodons), Afrosoricida (golden moles and tenreques), Macroscelidia (elephant shrew) and Tubulidentata (ant-hog and armadillo). Among these, Afrotheria is a particularly interesting clade for the analyses of evolutionary uniqueness because, although it is relatively poor in living species (approximately 70), it contains an excessive amount of adaptive diversity (Kuntner, et al., 2011), and afrotherian fossils are widely known throughout the Cenozoic (Tabuce et al., 2008), especially by proboscidian representatives (Lister, 2013). The results presented in this study are in accordance with this trend, since the findings of endogenous biological materials are restricted to the order Proboscidea, intrinsically to the family Elephantidae and to the extinct family Mammuthidae.

The reports of nonmineralized biomaterials in the Afrotheria clade are numerous and taphonomically diverse, but the data from permafrosts in Siberia and Alaska outweigh the others. From this type of environment, several endogenous bioelements were recovered, such as collagen (Goodman et al., 1980; Lowenstein, 1981; Shoshani et al., 1985; Bocherens et al., 1996), albumin (Lowenstein, 1981; Shoshani et al., 1985), genetic material (Taylor, 1996; Krause et al., 2006; Rogaev et al., 2006; Gilbert et al., 2007; Barnes et al., 2007; Miller et al., 2008; Gilbert et al., 2008; Schwarz et al., 2009) and keratinocytes (Gilbert et al., 2007).

The importance of cryopreservation for the encapsulation of biomolecules, cells and tissues over deep time has already been discussed above (see section 4.3.2). Of these locations, the number of fossils of woolly mammoths (*Mammuthus primigenius*) with paleobiochemical information has been found. For example, a single study (Barnes et al., 2007) retrieved hundreds of mtDNA base pairs from teeth, ivories and short bones of forty-one woolly mammoths from the Upper Pleistocene from Yacutia, Siberia.

Deposits of taphonomic contexts less favorable to preservation were also abundant in afrotherian resources for Molecular Paleontology. From a marine deposit in northern Venezuela, a 13 Ka gomphothere fossil yielded a large sampling of albumin fragments (Tuross, 1989). From the beds of the Yellowstone River, in Montana, understood as a fluvial deposit, a fossil of mastodon (*Mammuth americanum*) and another of mammoth-colombian (*Mammuthus columbi*) were abundant sources of nonmineralized biomaterials, namely, collagen, osteocytes and blood vessels (Hill, 1998; Hill & Schweitzer, 1999; Schweitzer et al., 2002; Asara et al., 2007).



Another more recent study, with a species of gomphothere (*Anancus arvenensis*), recovered from the Clay Well of Lower Saxony, Germany, found the presence of extracellular bone matrix proteins in the cortex of the femoral neck dated to 3 Ma (Schmidt-Schultz et al., 2021). In addition, another study, with the application of various analytical techniques in mammoth specimens from a fine-grained flow channel and a sink deposit in the United States, found samples of amides, amino acids, lipids and collagen (Colleary et al., 2021). These environments, which are less favorable to exceptional preservation, as mentioned, have been shown to be interesting alternative sources of search for afrotherian fossils with paleomolecular data.

## 5. Conclusion

The fossil record of the Mammalia clade, from the Cenozoic Era, is extensive, abundant and, in this study, proved to be a remarkable source of various types of traces of nonmineralized biomaterials, regardless of the taphonomic context, favorable or not to exceptional preservation.

Regarding the taxonomic variability, the findings of fragments of endogenous biocomponents occurred in the infraclass Metatheria and Eutheria, with data on Eutheria being extensively higher, most likely because the number of species of this clade is much higher. Within Eutheria, the superordinates Laurasiatheria and Afrotheria were the ones with the highest number of claims, although a considerable number is also found in relation to Euarchontoglires and Xenarthra.

Regarding the frequency of types of trace original biomaterials, collagen was the most recovered molecule among studies with fossils from sedimentary deposits, namely, more than 30 reports, while for other taphonomic contexts, more favorable to exceptional preservation, the genetic elements were the most found, with more than 40 reports. Collagen has been shown to be a very resistant biomaterial to the adverse conditions of fossilization (Rana, 2016; Alves, 2022), although there is a common understanding, within paleontology, that more studies need to be developed for a more robust analysis of this trend. On the other hand, genetic material was also highlighted in this study, being prevalent in various taphonomic contexts, including deposits less favorable to exceptional preservation. This may represent a greater interest of researchers in seeking data regarding ancient DNA due to its great applicability (Brunson & Reich, 2019).

Regarding the geographic distribution, fossils of Cenozoic mammals with preserved biochemical information were found in virtually all continents, except Antarctica. North America, South America and Europe were the continents that presented the largest number of reports, which may mean that researchers from these sites have been more committed to paleomolecular research in recent decades or that they have had more resources for this type of detection, in addition to the possibility that the number of fossils with endogenous content is more common in these regions. Although the results presented here cannot predict this issue, further in-depth analyses may contribute.

From these findings, it is possible to preliminarily verify that many fossils of the Mammalia clade have the potential for exceptional preservation of traces of nonmineralized biomaterials and anticipate that, with the improvement of detection techniques and the emergence of new exploration methods of this field, as well as the increase in the number of studies being done with new fossils, these reports can be expanded. It is also worth noting the crucial need for further studies to broaden the understanding of the taphonomic processes responsible for the preservation of this type of material, specifically the environmental context and the geochemical conditions.

In summary, it is believed that the paleobiochemistry information produced from the field of study of molecular paleontology, as well as the frequency of occurrence of these findings, such as those presented here, can serve as a basis for new phylogenetic approaches, helping this field to solve old problems, especially regarding the mammal clade.

## References

- Alves, E. F., & Machado, M. F. (2020). Perspectivas atuais sobre tecidos moles não mineralizados em fósseis de dinossauros não avianos. *Terrae Didactica*, 16, e020028. <https://doi.org/10.20396/td.v16i0.8659539>
- Alves, E. F. (2020). 15 anos da Paleontologia molecular. *Khronos*, (9), 241-244. <https://doi.org/10.11606/khronos.v0i9.172317>
- Alves, E. F., & Machado, M. F. (2021a). Frequência de preservação de biomateriais não mineralizados no registro fóssil de répteis mesozoicos: uma abordagem sobre pterossauros e répteis marinhos. *Brazilian Journal of Development*, 7(5), 44797-44821. <https://doi.org/10.34117/bjdv7n5-076>
- Alves, E. F., & Machado, M. F. (2021b). Proposta de Plano de Aula sobre Paleontologia Molecular para inserção em disciplina de Paleontologia de cursos de graduação em Ciências Biológicas. *Pesquisa e Ensino em Ciências Exatas e da Natureza*, 5, e1695. <http://dx.doi.org/10.29215/pecen.v5i0.1695>
- Alves, E. F., & Machado, M. F. (2021c). Preservação excepcional de biomateriais não mineralizados em fósseis do clado Avialae. *Anuário do Instituto de Geociências*, 44, 37908. [https://doi.org/10.11137/1982-3908\\_2021\\_44\\_37908](https://doi.org/10.11137/1982-3908_2021_44_37908)
- Alves, E. F. (2022). *Introdução à Paleontologia Molecular: um guia básico para estudantes e professores universitários*. Maringá: Edição do Autor.
- Amemiya, C. T., Alföldi, J., Lee, A. P., Fan, S., Philippe, H., MacCallum, I., Braasch, I., Manousaki, T., Schneider, I., Rohner, N., Organ, C., Chalopin, D., Smith, J. J., Robinson, M., Dorrington, R. A., Gerdol, M., Aken, B., Biscotti, M. A., Barucca, M., Baurain, D., Berlin, A. M., Blatch, G. L., Buonocore, F., Burmester, T., Campbell, M. S., Canapa, A., Cannon, J. P., Christoffels, A., De Moro, G., Edkins, A. L., Fan, L., Fausto, A. M., Feiner, N., Forconi, M., Garmeldien, J., Gnerre, S., Gnerre, S., Gnerre, A., Goldstone, J. V., Haerty, W., Hahn, M. E., Hesse, U., Hoffmann, S., Johnson, J., Karchner, S. I., Kuraku, S., Lara, M., Levin, J. Z., Litman, G. W., Mauceli, E., Miyake, T., Mueller, M. G., Nelson, D. R., Nitsche, A., Olmo, E., Ota, T., Pallavicini, A., Panji, S., Picone, B., Ponting, C. P., Prohaska, S. J., Przybylski, D., Saha, N. R., Ravi, V., Ribeiro, F. J., Sauka-Spengler, P., Scapigliati, G., Searle, S. M. J., Sharpe, T., Simakov, O., Stadler, P. F., Stegeman, J. J., Sumiyama, K., Tabbaa, D., Tafer, H., Turner-Maier, J., Heusden, P., White, S., Williams, L., Yandell, M., Brinkmann, H., Volff, J., Tabin, C. J., Shubin, N., Scharl, M., Jaffe, D. B., Postlethwait, J. H., Ventakesh, B., Di Palma, F., Lander, E. S., Meyer, A., & Lindblad-Toh, K. (2013). The African coelacanth genome provides in-sights into tetrapod evolution. *Nature*, 496(7445), 311-316. <https://doi.org/10.1038/nature12027>
- Archibald, J. D. (2003). Timing and biogeography of the eutherian radiation: fossils and molecules compared. *Molecular Phylogenetics and Evolution*, 28(2), 350-359. [https://doi.org/10.1016/S1055-7903\(03\)00034-4](https://doi.org/10.1016/S1055-7903(03)00034-4)
- Asara, J. M., Schweitzer, M. H., Freimark, L. M., Phillips, M., & Cantley, L. C. (2007). Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science*, 316(5822), 280-285. <https://doi.org/10.1126/science.1137614>
- Bailleul, A. M., Zheng, W., Horner, J. R., Hall, B. K., Holliday, C. M., & Schweitzer, M. H. (2020). Evidence of proteins, chromosomes and chemical markers of DNA in exceptionally preserved dinosaur cartilage. *National Science Review*, 7(4), 815-822. <https://doi.org/10.1093/nsr/nwz206>
- Bailleul, A. M. (2021). Fossilized cell nuclei are not that rare: Review of the histological evidence in the Phanerozoic. *Earth-Science Reviews*, 216, 103599. <https://doi.org/10.1016/j.earscirev.2021.103599>
- Bailleul, A. M., & Li, Z. (2021). DNA staining in fossil cells beyond the Quaternary: Reassessment of the evidence and prospects for an improved understanding of DNA preservation in deep time. *Earth-Science Reviews*, 216, 103600. <https://doi.org/10.1016/j.earscirev.2021.103600>
- Barnes, I., Shapiro, B., Lister, A., Kuznetsova, T., Sher, A., Guthrie, D., & Thomas, M. G. (2007). Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. *Current Biology*, 17(12), 1072-1075. <https://doi.org/10.1016/j.cub.2007.05.035>
- Beck, R. M., & Baillie, C. (2018). Improvements in the fossil record may largely resolve current conflicts between morphological and molecular estimates of mammal phylogeny. *Proceedings of the Royal Society B*, 285(1893), 20181632. <https://doi.org/10.1098/rspb.2018.1632>
- Benevento, G. L., Benson, R. B., & Friedman, M. (2019). Patterns of mammalian jaw ecomorphological disparity during the Mesozoic/Cenozoic transition. *Proceedings of the Royal Society B*, 286(1902), 20190347. <https://doi.org/10.1098/rspb.2019.0347>
- Benton, M. J. (1997). *Vertebrate Paleontology*. London: Chapman & Hall, 306.
- Bettoni, J. C. (2019). Criopreservação: uma ferramenta para conservação de recursos genéticos de videira. *Agropecuária Catarinense*, 32(2), 92-97. <https://doi.org/10.22491/RAC.2019.v32n2.14>
- Bi, S., Zheng, X., Wang, X., Cignetti, N. E., Yang, S., & Wible, J. R. (2018). An Early Cretaceous eutherian and the placental–marsupial dichotomy. *Nature*, 558(7710), 390-395. <https://doi.org/10.1038/s41586-018-0210-3>
- Bocherens, H., Pécourt, G., Lazarev, P. A., & Mariotti, A. (1996). Stable isotope abundances ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) in collagen and soft tissues from Pleistocene mammals from Yakutia: implications for the palaeobiology of the Mammoth Steppe. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 126(1-2), 31-44. [https://doi.org/10.1016/S0031-0182\(96\)00068-5](https://doi.org/10.1016/S0031-0182(96)00068-5)
- Boeskorov, G. G., Protodopov, A. V., Mashchenko, E. N., Potapova, O. R., Kuznetsova, T. V., Plotnikov, V. V., Grigoryev, S. E., Belolyubskii, I. N., Tomshin, M. D., Shchelchkova, M. V., Kolesov, S. D., van der Plicht, I., & Tikhonov, A. N. (2013). New findings of unique preserved fossil mammals in the permafrost of Yakutia. *Doklady biological sciences: proceedings of the Academy of Sciences of the USSR, Biological sciences sections*, 452(1), 291–295. <https://doi.org/10.1134/S0012496613050116>
- Borja, C., García-Pacheco, M., Olivares, E. G., Scheuenstuhl, G., & Lowenstein, J. M. (1997). Immunospecificity of albumin detected in 1.6 million-year-old fossils from Venta Micena in Orce, Granada, Spain. *American Journal of Physical Anthropology*, 103(4), 433-441. [https://doi.org/10.1002/\(SICI\)1096-8644\(199708\)103:4<3C433::AID-AJPA1%3E3.0.CO;2-O](https://doi.org/10.1002/(SICI)1096-8644(199708)103:4<3C433::AID-AJPA1%3E3.0.CO;2-O)
- Boskovic, D. S., Vidal, U. L., Nick, K. E., Esperante, R., Brand, L. R., Wright, K. R., Sandberg, L. B., & Siviero, B. C. (2021). Structural and protein preservation in fossil whale bones from the Pisco Formation (Middle-Upper Miocene), Peru. *PALAIOS*, 36(4), 155-164. <https://doi.org/10.2110/palo.2020.032>

- Bray, F., Flament, S., Abrams, G., Bonjean, D., Di Modica, K., Rolando, C., Tokarski, C., & Auguste, P. (2020). Extinct species identification from Upper Pleistocene bone fragments not identifiable from their osteomorphological studies by proteomics analysis. *bioRxiv*. <https://doi.org/10.1101/2020.10.06.328021>
- Brunson, K., & Reich, D. (2019). The promise of paleogenomics beyond our own species. *Trends in Genetics*, 35(5), 319-329. <https://doi.org/10.1016/j.tig.2019.02.006>
- Buckley, M., & Collins, M. J. (2011). Collagen survival and its use for species identification in Holocene-lower Pleistocene bone fragments from British archaeological and paleontological sites. *Antiqua*, 1(1), e1-e1. <https://doi.org/10.4081/antiqua.2011.e1>
- Buckley, M., Larkin, N., & Collins, M. (2011). Mammoth and Mastodon collagen sequences; survival and utility. *Geochimica et Cosmochimica Acta*, 75(7), 2007-2016. <https://doi.org/10.1016/j.gca.2011.01.022>
- Buckley, M. (2015). Ancient collagen reveals evolutionary history of the endemic South American 'ungulates'. *Proceedings of the Royal Society B: Biological Sciences*, 282(1806), 20142671. <https://doi.org/10.1098/rspb.2014.2671>
- Buckley, M., Fariña, R. A., Lawless, C., Tambusso, P. S., Varela, L., Carlini, A. A., Powell, J. E., & Martinez, J. G. (2015). Collagen sequence analysis of the extinct giant ground sloths *Lestodon* and *Megatherium*. *PLoS ONE*, 10(11), e0139611. <https://doi.org/10.1371/journal.pone.0139611>
- Buckley, M., Lawless, C., & Rycyzynski, N. (2019). Collagen sequence analysis of fossil camels, *Camelops* and cf *Paracamelus*, from the Arctic and sub-Arctic of Plio-Pleistocene North America. *Journal of Proteomics*, 194, 218-225. <https://doi.org/10.1016/j.jprot.2018.11.014>
- Buckley, M., Recabarren, O. P., Lawless, C., García, N., & Pino, M. (2019). A molecular phylogeny of the extinct South American gomphothere through collagen sequence analysis. *Quaternary Science Reviews*, 224, 105882. <https://doi.org/10.1016/j.quascirev.2019.105882>
- Cadena, E. (2016). Microscopical and elemental FESEM and Phenom ProX-SEM-EDS analysis of osteocyte-and blood vessel-like microstructures obtained from fossil vertebrates of the Eocene Messel Pit, Germany. *PeerJ*, 4, e1618. <https://doi.org/10.7717/peerj.1618>
- Capellini, E., Welker, F., Pandolfi, L., Ramos-Madrigal, J., Samodova, D., Rütther, P. L., Fotakis, A. K., Lyon, D., Moreno-Mayar, J. V., Bukhsianidze, M., Jersie-Christensen, R. R., Mackie, M., Ginolhac, A., Ferring, R., Tappen, M., Palkopoulou, E., Dickinson, M. R., Stafford Jr, T. W., Chan, Y. L., Götherström, A., Nathan, S. K., Heintzman, P. D., Kapp, J. D., Kirillova, I., Moodley, Y., Agusti, J., Kahlke, R. D., Kiladze, G., Martínez-Navarro, B., Liu, S., Velasco, M. S., Sinding, M. S., Kelstrup, C. D., Allentoft, M. E., Orlando, L., Penkman, K., Shapiro, B., Rook, L., Dalén, L., Gilbert, M. P., Olsen, J. V., Lordkipanidze, D., & Willerslev, E. (2019). Early Pleistocene enamel proteome from Dmanisi resolves *Stephanorhinus* phylogeny. *Nature*, 574(7776), 103-107. <https://doi.org/10.1038/s41586-019-1555-y>
- Clack, A. A., MacPhee, R. D., & Poinar, H. N. (2012). Case study: ancient sloth DNA recovered from hairs preserved in paleofeces. *Methods Molecular Biology*, 840: 51–6. [https://doi.org/10.1007/978-1-61779-516-9\\_7](https://doi.org/10.1007/978-1-61779-516-9_7)
- Cleland, T. P., Schroeter, E. R., Feranec, R. S., & Vashishth, D. (2016). Peptide sequences from the first *Castoroides ohioensis* skull and the utility of old museum collections for palaeoproteomics. *Proceedings of the Royal Society B: Biological Sciences*, 283(1832), 20160593. <https://doi.org/10.1098/rspb.2016.0593>
- Colleary, C., Dolocan, A., Gardner, J., Singh, S., Wuttke, M., Rabenstein, R., Habersetzer, J., Schaal, S., Feseha, M., Clemens, M., Jacobs, B. F., Currano, E. D., Jacobs, L. L., Sylvestersen, R. L., Gabbott, S. E., & Vinther, J. (2015). Chemical, experimental, and morphological evidence for diagenetically altered melanin in exceptionally preserved fossils. *Proceedings of the National Academy of Sciences*, 112(41), 12592-12597. <https://doi.org/10.1073/pnas.1509831112>
- Colleary, C., Lamadrid, H. M., O'Reilly, S. S., Dolocan, A., & Nesbitt, S. J. (2021). Molecular preservation in mammoth bone and variation based on burial environment. *Scientific Reports*, 11(1), 1-9. <https://doi.org/10.1038/s41598-021-81849-6>
- Collins, M. J., Nielsen-Marsh, C. M., Hiller, J., Smith, C. I., Roberts, J. P., Prigodich, R. V., Prigodich, R. V., Wess, T. J., Csapo, J., Millard, A. R., & Turner-Walker, G. (2002). The survival of organic matter in bone: a review. *Archaeometry*, 44(3), 388. <https://doi.org/10.1111/1475-4754.t01-1-00071>
- Coltrain, J. B., Harris, J. M., Cerling, T. E., Ehleringer, J. R., Dearing, M. D., Ward, J., & Allen, J. (2004). Rancho La Brea stable isotope biogeochemistry and its implications for the palaeoecology of late Pleistocene, coastal southern California. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 205(3-4), 199-219. <https://doi.org/10.1016/j.palaeo.2003.12.008>
- Dabney, J., Knapp, M., Glocke, I., Gansauge, M., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J., & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences*, 110(39), 15758-15763. <https://doi.org/10.1073/pnas.1314445110>
- Delsuc, F., Gibb, G. C., Kuck, M., Billet, G., Hautier, L., Southon, J., Rouillard, J., Fericola, J. C., Vizcaino, S. F., MacPhee, R. D. E., & Poinar, H. N. (2016). The phylogenetic affinities of the extinct glyptodonts. *Current Biology*, 26(4), R155-R156. <https://doi.org/10.1016/j.cub.2016.01.039>
- Duchêne, D. A., Bragg, J. G., Duchêne, S., Neaves, L. E., Potter, S., Moritz, C., Johnson, R. N., Ho, S. Y. W., & Eldridge, M. D. (2018). Analysis of phylogenomic tree space resolves relationships among marsupial families. *Systematic Biology*, 67(3), 400-412. <https://doi.org/10.1093/sysbio/syx076>
- Eldridge, M. D., Beck, R. M., Croft, D. A., Travouillon, K. J., & Fox, B. J. (2019). An emerging consensus in the evolution, phylogeny, and systematics of marsupials and their fossil relatives (Metatheria). *Journal of Mammalogy*, 100(3), 802-837. <https://doi.org/10.1093/jmammal/gyz018>
- Fostowicz-Frelik, L., Ge, D., & Ruf, I. (2021). Recent advances in the evolution of Euarchontoglires. *Frontiers in Genetics*, 12, 773789. <https://doi.org/10.3389/fgene.2021.773789>
- Geng, W. H., Wang, X. P., Che, L. F., Wang, X., Liu, R., Zhou, T., Roos, C., Irwin, D. M., & Yu, L. (2020). Convergent evolution of locomotory modes in Euarchontoglires. *Frontiers in Ecology and Evolution*, 8, 476. <https://doi.org/10.3389/fevo.2020.615862>
- Gilbert, M. T. P., Wilson, A. S., Bunce, M., Hansen, A. J., Willerslev, E., Shapiro, B., Higham, T.F., Richards, M. P., O'Connell, T. C., Tobin, D. J., Cooper, A., & Janaway, R. C. (2004). Ancient mitochondrial DNA from hair. *Current Biology*, 14(12), R463-R464. <https://doi.org/10.1016/j.cub.2004.06.008>

- Gilbert, M. T. P., Tomsho, L. P., Rendulic, S., Packard, M., Drautz, D. I., Sher, A., Tikhonov, A., Dalen, L., Kuznetsova, T., Kosintsev, P., Higham, T., Collins, M. J., Wilson, A. S., Shidlovskiy, F., Buigues, B., Ericson, P. G., Germonpré, M., Götherström, A., Iacumin, P., Nikolaev, V., Nowak-Kemp, M., Willerslev, E., Knight, J. R., Irzyk, Gerard, Perbost, C. S., Fredrikson, K. M., Harkins, T. T., Sheridan, S., Miller, W., Schuster, S. C., & Campos, P. F. (2007). Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science*, 317(5846), 1927-1930. <https://doi.org/10.1126/science.1146971>
- Gilbert, M. T. P., Drautz, D. I., Lesk, A. M., Ho, S. Y., Qi, J., Ratan, A., Hsu, C., Sher, A., Dalén, L., Götherström, A., Tomsho, L. P., Rendulic, S., Packard, M., Campos, P. F., Kuznetsova, T. V., Shidlovskiy, F., Tikhonov, A., Willerslev, E., Iacumin, P., Buigues, B., Ericson, P. G. P., Germonpré, M., Kosintsev, P., Nikolaev, V., Nowak-Kemp, M., Knight, J. R., Irzyk, G. P., Perbost, C. S., Fredrikson, K. M., Harkins, T. T., Sheridan, S., Miller, W., & Schuster, S. C. (2008). Intraspecific phylogenetic analysis of Siberian woolly mammoths using complete mitochondrial genomes. *Proceedings of the National Academy of Sciences*, 105(24), 8327-8332. <https://doi.org/10.1073/pnas.0802315105>
- Gobbo, S. R., & Bertini, R. J. (2014). Tecidos moles (não resistentes): como se fossilizam? *Terrae Didactica*, 10(1), 2-13. <https://doi.org/10.20396/td.v10i1.8637374>
- Goin, F. J., Woodburne, M. O., Zimicz, A. N., Martin, G. M., & Chornogubsky, L. (2016). Dispersal of vertebrates from between the Americas, Antarctica, and Australia in the Late Cretaceous and Early Cenozoic. In *A brief history of South American metatherians*. Springer, Dordrecht, 77-124. [https://doi.org/10.1007/978-94-017-7420-8\\_3](https://doi.org/10.1007/978-94-017-7420-8_3)
- Gomes, W. A., Machado, M. F., Bélo, P., & Alves, E. F. (2021a). Biomoléculas em fósseis de mamíferos cenozoicos: reivindicações de tecidos moles não mineralizados em fósseis recuperados de diferentes contextos tafonômicos. *PaleoDest – Paleontologia em Destaque*, 36(74),45. <https://doi.org/10.13140/RG.2.2.11401.47206>
- Gomes, W. A., Machado, M. F., Bélo, P., & Alves, E. F. (2021b). Paleontologia molecular do Cenozoico: reivindicações de tecidos moles não mineralizados em mamíferos encontrados em depósitos sedimentares pouco favoráveis à preservação. *PaleoDest – Paleontologia em Destaque*, 36(74),45. <https://doi.org/10.13140/RG.2.2.18951.21928>
- Goodman, M., Birk, D. E., Romero-Herrera, A. E., Lande, M. A., Dene, H., & Barnhart, M. I. (1980). Collagen preservation in soft tissue from the Magadan mammoth. *FEBS Letters*, 114(1), 30-34. [https://doi.org/10.1016/0014-5793\(80\)80854-4](https://doi.org/10.1016/0014-5793(80)80854-4)
- Greenwood, A. D., Castresana, J., Feldmaier-Fuchs, G., & Pääbo, S. (2001). A molecular phylogeny of two extinct sloths. *Molecular Phylogenetics and Evolution*, 18(1), 94-103. <https://doi.org/10.1006/mpev.2000.0860>
- Hallström, B. M., & Janke, A. (2010). Mammalian evolution may not be strictly bifurcating. *Molecular Biology and Evolution*, 27(12), 2804-2816. <https://doi.org/10.1093/molbev/msq166>
- Hallström, B. M., Schneider, A., Zoller, S., & Janke, A. (2011). A genomic approach to examine the complex evolution of laurasiatherian mammals. *PLoS ONE*, 6(12), e28199. <https://doi.org/10.1371/journal.pone.0028199>
- Hauchar, D., Haile, J., McDowell, M. C., Murray, D. C., White, N. E., Allcock, R. J., Phillips, M. J., Prideaux, G. J., & Bunce, M. (2014). Thorough assessment of DNA preservation from fossil bone and sediments excavated from a late Pleistocene–Holocene cave deposit on Kangaroo Island, South Australia. *Quaternary Science Reviews*, 84, 56-64. <https://doi.org/10.1016/j.quascirev.2013.11.007>
- Heintzman, P. D., Zazula, G. D., Cahill, J. A., Reyes, A. V., MacPhee, R. D., & Shapiro, B. (2015). Genomic data from extinct North American *Camelops* revise camel evolutionary history. *Molecular Biology and Evolution*, 32(9), 2433-2440. <https://doi.org/10.1093/molbev/msv128>
- Hill, C. L. (1998). American mastodon (*Mammot americanum*) from the Doeden Gravels, No. 2 Terrace, Lower Yellowstone River. In *American Quaternary Association Program and Abstracts of the 15th Biennial Meeting*. Puerto Vallarta, Mexico, 180.
- Hill, C. L., & Schweitzer, M. H. (1999). Mammoth (*Mammuthus*) from the Doeden Gravels Eastern Montana: biometric and molecular analyses. *Current Research Pleistocene*, 16, 120-123.
- Hill, R. C., Wither, M. J., Nemkov, T., Barrett, A., D'Alessandro, A., Dzieciatkowska, M., & Hansen, K. C. (2015). Preserved proteins from extinct *Bison latifrons* identified by tandem mass spectrometry; hydroxylysine glycosides are a common feature of ancient collagen. *Molecular & Cellular Proteomics*, 14(7), 1946-1958. <https://doi.org/10.1074/mcp.M114.047787>
- Ho, T. Y. (1965). The amino acid composition of bone and tooth proteins in late Pleistocene mammals. *Proceedings of the National Academy of Sciences of the United States of America*, 54(1), 26-31. <https://doi.org/10.1073/pnas.54.1.26>
- Hofreiter, M., Poinar, H. N., Spaulding, W. G., Bauer, K., Martin, P. S., Possnert, G., & Pääbo, S. (2000). A molecular analysis of ground sloth diet through the last glaciation. *Molecular Ecology*, 9(12), 1975-1984. <https://doi.org/10.1046/j.1365-294x.2000.01106.x>
- Hofreiter, M., Betancourt, J. L., Sbriller, A. P., Markgraf, V., & McDonald, H. G. (2003). Phylogeny, diet, and habitat of an extinct ground sloth from Cuchillo Cura, Neuquen Province, southwest Argentina. *Quaternary Research*, 59(3), 364-378. [https://doi.org/10.1016/S0033-5894\(03\)00030-9](https://doi.org/10.1016/S0033-5894(03)00030-9)
- Höss, M., Dilling, A., Currant, A., & Pääbo, S. (1996). Molecular phylogeny of the extinct ground sloth *Myiodon darwini*. *Proceedings of the National Academy of Sciences*, 93(1), 181-185. <https://doi.org/10.1073/pnas.93.1.181>
- Hu, J., Zhang, Y., & Yu, L. (2012). Summary of Laurasiatheria (Mammalia) phylogeny. *Zoological Research*, 33(E5-6), E65-74. <https://doi.org/10.3724/SP.J.1141.2012.E05-06E65>
- Irisarri, I., & Meyer, A. (2016). The identification of the closest living relative(s) of tetrapods: phylogenomic lessons for resolving short ancient internodes. *Systematic Biology*, 65(6), 1057-1075. <https://doi.org/10.1093/sysbio/syw057>
- Jarvis, E. D., Mirarab, S., Aberer, A. J., Li, B., Houde, P., Li, C., Ho, S. Y. W., Faircloth, B. C., Nabholz, B., Howard, J. T., Suh, A., Weber, C. C., Fonseca, R. R., Li, J., Zhang, F., Li, H., Zhou, L., Narula, N., Liu, L., Ganapathy, G., Boussau, B., Bayzid, M. S., Zavidovych, V., Subramanian, S., Gabaldón, T.,



- Capella-Gutiérrez, S., Huerta-Cepas, J., Rekepalli, B., Munch, K., Schierup, M., Lindow, B., Warren, W. C., Ray, D., Green, R. G., Bruford, M. W., Zhan, X., Dixon, A., Li, S., Li, N., Huang, Y., Derryberry, E. P., Bertelsen, M. F., Sheldon, F. H., Brumfield, R. T., Mello, C. V., Lovell, P. V., Wirthlin, M., Schneider, M. P. C., Prosdocimi, F., Samaniego, J. A., Velazquez, A. M. V., Alfaro-Núñez, A., Campos, P. F., Petersen, B., Sichert-Ponten, T., Pas, A., Bailey, T., Scofield, P., Bunce, M., Lambert, D. M., Zhou, Q., Perelman, P., Driskell, A. C., Shapiro, B., Xiong, Z., Zeng, Y., Liu, S., Li, Z., Liu, B., Wu, K., Xiao, J., Yinqi, X., Zheng, Q., Zhang, Y., Yang, H., Wang, J., Smeds, L., Rheindt, F. E., Braun, M., Fjeldsa, J., Orlando, L., Barker, F. K., Jónsson, K. A., Johnson, W., Koepfli, K., O'Brien, S., Haussler, D., Ryder, O. A., Rahbek, C., Willerslev, E., Graves, G. R., Glenn, T. C., McCormack, J., Burt, D., Ellegren, H., Alström, P., Edwards, S. V., Stamatakis, A., Mindell, D. P., Cracraft, J., Braun, E. L., Warnow, T., Jun, W., Gilbert, M. T. P., & Zhang, G. (2014). Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science*, 346(6215), 1320-1331. <https://doi.org/10.1126/science.1253451>
- Krause, J., Dear, P. H., Pollack, J. L., Slatkin, M., Spriggs, H., Barnes, I., Lister, A. M., Ebersberger, I., Pääbo, S., & Hofreiter, M. (2006). Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. *Nature*, 439(7077), 724-727. <https://doi.org/10.1038/nature04432>
- Krause, J., Unger, T., Noçon, A., Malaspinas, A., Kolokotronis, S., Stiller, M., Soibelzon, L., Spriggs, H., Dear, P. H., Briggs, A. W., Bray, S. C. E., O'Brien, S. J., Rabeder, G., Matheus, P., Cooper, A., Slatkin, M., Pääbo, S., & Hofreiter, M. (2008). Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. *BMC Evolutionary Biology*, 8(1), 220. <https://doi.org/10.1186/1471-2148-8-220>
- Kuch, M., Rohland, N., Betancourt, J. L., Latorre, C., Stepan, S., & Poinar, H. N. (2002). Molecular analysis of a 11 700-year-old rodent midden from the Atacama Desert, Chile. *Molecular Ecology*, 11(5), 913-924. <https://doi.org/10.1046/j.1365-294X.2002.01492.x>
- Kuntner, M., May-Collado, L. J., & Agnarsson, I. (2011). Phylogeny and conservation priorities of afrotherian mammals (Afrotheria, Mammalia). *Zoologica Scripta*, 40(1), 1-15. <https://doi.org/10.1111/j.1463-6409.2010.00452.x>
- Lambert, J. B., Santiago-Blay, J. A., Wu, Y., & Levy, A. J. (2015). Examination of amber and related materials by NMR spectroscopy. *Magnetic Resonance in Chemistry*, 53(1), 2-8. <https://doi.org/10.1002/mrc.4121>
- Liang, R., Li, Z., LauVetter, M. C., Vishnivetskaya, T. A., Zanina, O. G., Lloyd, K. G., Pffnner, S. M., Riykina, E. M., Wang, W., Wiggins, J., Miller, J., Hettich, R. L., & Onstott, T. C. (2021). Genomic reconstruction of fossil and living microorganisms in ancient Siberian permafrost. *Microbiome*, 9(1), 1-20. <https://doi.org/10.1186/s40168-021-01057-2>
- Lindqvist, C., Schuster, S. C., Sun, Y., Talbot, S. L., Qi, J., Ratan, A., Tomsho, L. P., Kasson, L., Zeyl, E., Aars, J., Miller, W., Ingólfsson O., Bachmann, L., & Øystein, W. (2010). Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear. *Proceedings of the National Academy of Sciences*, 107(11), 5053-5057. <https://doi.org/10.1073/pnas.0914266107>
- Lister, A. M., Edwards, C. J., Nock, D. A. W., Bunce, M., van Pijlen, I. A., Bradley, D. G., Thomas, M. G., & Barnes, I. (2005). The phylogenetic position of the 'giant deer' *Megaloceros giganteus*. *Nature*, 438(7069), 850-853. <https://doi.org/10.1038/nature04134>
- Lister, A. M. (2013). The role of behaviour in adaptive morphological evolution of African proboscideans. *Nature*, 500(7462), 331-334. <https://doi.org/10.1038/nature12275>
- Little, K., Kelly, M., & Courts, A. (1962). Studies on bone matrix in normal and osteoporotic bone. *The Journal of Bone and Joint Surgery*, 44(3), 503-519. <https://doi.org/10.1302/0301-620X.44B3.503>
- Lowenstein, J. M. (1981). Immunological reactions from fossil material. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 292(1057), 143-149. <https://doi.org/10.1098/rstb.1981.0022>
- Lowenstein, J. M., & Scheuenstuhl, G. (1991). Immunological methods in molecular palaeontology. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 333(1268), 375-380. <https://doi.org/10.1098/rstb.1991.0087>
- Lv, X., Hu, J., Hu, Y., Li, Y., Xu, D., Ryder, O. A., Irwin, D. M., & Yu, L. (2021). Diverse phylogenomic datasets uncover a concordant scenario of laurasiatherian interordinal relationships. *Molecular Phylogenetics and Evolution*, 157, 107065. <https://doi.org/10.1016/j.ympev.2020.107065>
- McCormack, J. E., Faircloth, B. C., Crawford, N. G., Gowaty, P. A., Brumfield, R. T., & Glenn, T. C. (2012). Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Research*, 22(4), 746-754. <https://doi.org/10.1101/gr.125864.111>
- Mess, A. (2014). Placental evolution within the supraordinal clades of eutheria with the perspective of alternative animal models for human placentation. *Advances in Biology*, 2014, 639274. <https://doi.org/10.1155/2014/639274>
- Miller, W., Drautz, D. I., Ratan, A., Pusey, B., Qi, J., Lesk, A. M., Tomsho, L. P., Packard, M. D., Zhao, F., Sher, A., Tikhonov, A., Raney, B., Patterson, N., Lindblad-Toh, K., Lander, E. S., Knight, J. R., Irzyk, G. P., Fredrikson, K. M., Harkins, T. T., Sheridan, S., Pringle, T., & Schuster, S. C. (2008). Sequencing the nuclear genome of the extinct woolly mammoth. *Nature*, 456(7220), 387-390. <https://doi.org/10.1038/nature07446>
- Morgan, C. C., Foster, P. G., Webb, A. E., Pisani, D., McInerney, J. O., & O'Connell, M. J. (2013). Heterogeneous models place the root of the placental mammal phylogeny. *Molecular Biology and Evolution*, 30(9), 2145-2156. <https://doi.org/10.1093/molbev/mst117>
- Nery, M. F., González, D. J., Hoffmann, F. G., & Opazo, J. C. (2012). Resolution of the laurasiatherian phylogeny: evidence from genomic data. *Molecular Phylogenetics and Evolution*, 64(3), 685-689. <https://doi.org/10.1016/j.ympev.2012.04.012>
- Nielsen-Marsh, C. M., Ostrom, P. H., Gandhi, H., Shapiro, B., Cooper, A., Hauschka, P. V., & Collins, M. J. (2002). Sequence preservation of osteocalcin protein and mitochondrial DNA in bison bones older than 55 ka. *Geology*, 30(12), 1099-1102. [https://doi.org/10.1130/0091-7613\(2002\)030%3C1099:SPOOPA%3E2.0.CO;2](https://doi.org/10.1130/0091-7613(2002)030%3C1099:SPOOPA%3E2.0.CO;2)
- Noonan, J. P., Hofreiter, M., Smith, D., Priest, J. R., Rohland, N., Rabeder, G., Krause, J., Detter, J. C., Pääbo, S., & Rubin, E. M. (2005). Genomic sequencing of Pleistocene cave bears. *Science*, 309(5734), 597-599. <https://doi.org/10.1126/science.1113485>

- O'Leary, M. A., Bloch, J. I., Flynn, J. J., Gaudin, T. J., Giallombardo, A., Giannini, N. P., Goldberg, S. L., Kraatz, B. P., Luo, Z., Meng, J., Ni, X., Novacek, M. J., Perini, F. A., Randall, Z. S., Rougier, G. W., Sargis, E. J., Silcox, M. T., Simmons, N. B., Spaulding, M., Velazco, P. M., Weksler, M., Wible, J. R., & Cirranello, A. L. (2013). The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science*, 339(6120), 662-667. <https://doi.org/10.1126/science.1229237>
- Orlando, L., Leonard, J. A., Thenot, A., Laudet, V., Guerin, C., & Hänni, C. (2003). Ancient DNA analysis reveals woolly rhino evolutionary relationships. *Molecular Phylogenetics and Evolution*, 28(3), 485-499. [https://doi.org/10.1016/S1055-7903\(03\)00023-X](https://doi.org/10.1016/S1055-7903(03)00023-X)
- Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schuber, M., Cappellini, E., Petersen, B., Moltke, I., Johnson, P. L. F., Fumagalli, M., Vilstrup, J. T., Raghavan, M., Korneliusen, T., Malaspina, A., Vogt, J., Szklarczyk, D., Kelstrup, C. D., Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A. M. V., Cahill, J., Rasmussen, M., Wang, X., Min, J., Zazula, G. D., Seguin-Orlando, A., Mortensen, C., Magnussen, K., Thompson, J. F., Weinstock, J., Gregersen, K., Røed, K. H., Eisenmann, V., Rubin, C. J., Miller, D. C., Antczak, D. F., Bertelsen, M. F., Brunak, S., Al-Rasheid, K. A. S., Ryder, O., Anderson, L., Mundy, J., Krogh, A., Gilbert, M. T. P., Kjaer, K., Sicheritz-Ponten, T., Jensen, L. J., Olsen, J. V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J., & Willerslev, E. (2013). Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. *Nature*, 499(7456), 74-78. <https://doi.org/10.1038/nature12323>
- O'Reilly, S., Summons, R., Mayr, G., & Vinther, J. (2017). Preservation of uropygial gland lipids in a 48-million-year-old bird. *Proceedings of the Royal Society B: Biological Sciences*, 284(1865), 20171050. <https://doi.org/10.1098/rspb.2017.1050>
- Ostrom, P. H., Schall, M., Gandhi, H., Shen, T. L., Hauschka, P. V., Strahler, J. R., & Gage, D. A. (2000). New strategies for characterizing ancient proteins using matrix-assisted laser desorption ionization mass spectrometry. *Geochimica et Cosmochimica Acta*, 64(6), 1043-1050. [https://doi.org/10.1016/S0016-7037\(99\)00381-6](https://doi.org/10.1016/S0016-7037(99)00381-6)
- Ostrom, P. H., Gandhi, H., Strahler, J. R., Walker, A. K., Andrews, P. C., Leykam, J., Stafford, T. W., Kelly, R. L., Walker, D. N., Buckley, M., & Hampula, J. (2006). Unraveling the sequence and structure of the protein osteocalcin from a 42 ka fossil horse. *Geochimica et Cosmochimica Acta*, 70(8), 2034-2044. <https://doi.org/10.1016/j.gca.2006.01.004>
- Paijmans, J. L. A., Barnett, R., Gilbert, M. T. P., Zepeda-Mendoza, M. L., Reumer, J. W. F., Vos, J., Zazula, G., Nagel, D., Baryshnikov, G. F., Leonard, J. A., Rohland, N., Westbury, M. V., Barlow, A., & Hofreiter, M. (2017). Evolutionary history of saber-toothed cats based on ancient mitogenomics. *Current Biology*, 27(21), 3330-3336. <https://doi.org/10.1016/j.cub.2017.09.033>
- Peñalver, E., & Grimaldi, D. (2005). Assemblages of mammalian hair and blood-feeding midges (Insecta: Diptera: Psychodidae: Phlebotominae) in Miocene amber. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 96(2), 177-195. <https://doi.org/10.1017/S0263593300001292>
- Phillips, M. J., & Zakaria, S. S. (2021). Enhancing mitogenomic phylogeny and resolving the relationships of extinct megafaunal placental mammals. *Molecular Phylogenetics and Evolution*, 158, 107082. <https://doi.org/10.1016/j.ympev.2021.107082>
- Poinar Jr, G. (2017). Fossilized mammalian erythrocytes associated with a tick reveal ancient piroplasms. *Journal of Medical Entomology*, 54(4), 895-900. <https://doi.org/10.1093/jme/tjw247>
- Rana, F. (2016). *Dinosaur blood and the Age of the Earth*. RTB Press.
- Rao, H., Yang, Y., Liu, J., Westbury, M. V., Zhang, C., & Shao, Q. (2020). Palaeoproteomic analysis of Pleistocene cave hyenas from east Asia. *Scientific Reports*, 10(1), 1-12. <https://doi.org/10.1038/s41598-020-73542-x>
- Reest, A. J., & Currie, P. J. (2020). Preservation frequency of tissue-like structures in vertebrate remains from the upper Campanian of Alberta: Dinosaur Park Formation. *Cretaceous Research*, 109(104370). <https://doi.org/10.1016/j.cretres.2019.104370>
- Rogaev, E. I., Moliaka, Y. K., Malyarchuk, B. A., Kondrashov, F. A., Derenko, M. V., Chumakov, I., & Grigorenko, A. P. (2006). Complete mitochondrial genome and phylogeny of Pleistocene mammoth *Mammuthus primigenius*. *PLoS Biology*, 4(3), e73. <https://doi.org/10.1371/journal.pbio.0040073>
- Romiguier, J., Ranwez, V., Delsuc, F., Galtier, N., & Douzery, E. J. (2013). Less is more in mammalian phylogenomics: AT-rich genes minimize tree conflicts and unravel the root of placental mammals. *Molecular Biology and Evolution*, 30(9), 2134-2144. <https://doi.org/10.1093/molbev/mst116>
- Rossi, V., McNamara, M. E., Webb, S. M., Ito, S., & Wakamatsu, K. (2019). Tissue-specific geometry and chemistry of modern and fossilized melanosomes reveal internal anatomy of extinct vertebrates. *Proceedings of the National Academy of Sciences*, 116(36), 17880-17889. <https://doi.org/10.1073/pnas.1820285116>
- Rother, E. T. (2007). Revisão sistemática X revisão narrativa. *Acta Paulista de Enfermagem*, 20(2), v-vi. <https://doi.org/10.1590/S0103-21002007000200001>
- Rowley, M. J., Rich, P. V., Rich, T. H., & Mackay, I. R. (1986). Immunoreactive colla-gen in avian and mammalian fossils. *Naturwissenschaften*, 73(10), 620-623. <https://doi.org/10.1007/BF00368777>
- Rybczynski, N., Gosse, J. C., Harington, C. R., Wogelius, R. A., Hidy, A. J., & Buckley, M. (2013). Mid-Pliocene warm-period deposits in the High Arctic yield insight into camel evolution. *Nature Communications*, 4(1), 1-9. <https://doi.org/10.1038/ncomms2516>
- Sansom, R. S. (2014). Experimental decay of soft tissues. Reading and writing of the fossil record: preservational pathways to exceptional fossilization. *Paleontological Society Papers*, 20, 259-274. <https://doi.org/10.1017/S1089332600002886>
- Schubert, M., Jónsson, H., Chang, D., Der Sarkissian, C., Ermini, L., Ginolhac, A., Albrechtsen, A., Dupanloup, I., Foucal, A., Petersen, B., Fumagalli, M., Raghavan, M., Seguin-Orlando, A., Korneliusen, T. S., Velazquez, A. M. V., Stenderup, J., Hoover, C. R., Alfarhan, A. H., Alquraishi, S. A., Al-Rasheid, K. A. S., MacHugh, D. E., Kalbfleisch, T., MacLeod, J. N., Rubin, E. N., Sicheritz-Ponten, T., Andersson, L., Hofreiter, M., Marques-Bonet, T., Gilbert, M. T. P., Nielsen, R., Excoffier, L., Willerslev, E., Shapiro, B., & Orlando, L. (2014). Prehistoric genomes reveal the genetic foundation and cost of horse domestication. *Proceedings of the National Academy of Sciences*, 111(52), E5661-E5669. <https://doi.org/10.1073/pnas.1416991111>

- Schmidt-Schultz, T. H., Reich, M., & Schultz, M. (2021). Exceptionally preserved extracellular bone matrix proteins from the late Neogene proboscidean *Anancus* (Mammalia: Proboscidea). *PalZ*, 95, 757–765. <https://doi.org/10.1007/s12542-021-00566-7>
- Schwarz, C., Debruyne, R., Kuch, M., McNally, E., Schwarcz, H., Aubrey, A. D., Bada, J., & Poinar, H. (2009). New insights from old bones: DNA preservation and degradation in permafrost preserved mammoth remains. *Nucleic Acids Research*, 37(10), 3215–3229. <https://doi.org/10.1093/nar/gkp159>
- Schweitzer, M., Hill, C. L., Asara, J. M., Lane, W. S., & Pincus, S. H. (2002). Identification of immunoreactive material in mammoth fossils. *Journal of Molecular Evolution*, 55(6), 696–705. <https://doi.org/10.1007/s00239-002-2365-6>
- Schweitzer, M. H. (2003). Reviews and previews: the future of molecular biology. *Palaeontologia Electronica*, 5(2), 1–11.
- Schweitzer, M. H. (2004). Molecular paleontology: some current advances and problems. *Annales de Paléontologie*, 90(2), 81–102. <https://doi.org/10.1016/j.annpal.2004.02.001>
- Schweitzer, M. H., Wittmeyer, J. L., & Horner, J. R. (2007a). Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present. *Proceedings of the Royal Society B: Biological Sciences*, 274(1607), 183–197. <https://doi.org/10.1098/rspb.2006.3705>
- Schweitzer, M. H., Suo, Z., Avci, R., Asara, J. M., Allen, M. A., Arce, F. T., & Horner, J. R. (2007b). Analyses of soft tissue from *Tyrannosaurus rex* suggest the presence of protein. *Science*, 316(5822), 277–280. <https://doi.org/10.1126/science.1138709>
- Schweitzer, M. H., Zheng, W., Organ, C. L., Avci, R., Suo, Z., Freimark, L. M., Lebleu, V. S., Duncan, M. B., Heiden, M. G. V., Neveu, J. M., Lane, W. S., Cottrell, J. S., Horner, J. R., Cantley, L. C., Kalluri, R., & Asara, J. M. (2009). Biomolecular characterization and protein sequences of the Campanian hadrosaur *B. canadensis*. *Science*, 324(5927), 626–631. <https://doi.org/10.1126/science.1165069>
- Schweitzer, M. H., Schroeter, E. R., Cleland, T. P., & Zheng, W. (2019). Paleoproteomics of Mesozoic dinosaurs and other Mesozoic fossils. *Proteomics*, 19(16), 1800251. <https://doi.org/10.1002/pmic.201800251>
- Shoshani, J., Lowenstein, J. M., Walz, D. A., & Goodman, M. (1985). Proboscidean origins of mastodon and woolly mammoth demonstrated immunologically. *Paleobiology*, 11(4), 429–437. <https://doi.org/10.1017/S0094837300011714>
- Simões, M. G., Rodrigues, S. C., Bertoni-Machado, C., & Holz, M. (2014). Tafonomia: processos e ambientes de fossilização. In Carvalho, I. S. *Paleontologia: conceitos e métodos*. 3. ed. Editora Interciência, 1, 19–51.
- Slater, G. J. (2013). Phylogenetic evidence for a shift in the mode of mammalian body size evolution at the Cretaceous-Palaeogene boundary. *Methods in Ecology and Evolution*, 4(8), 734–744. <https://doi.org/10.1111/2041-210X.12084>
- Stach, P., Natkaniec-Nowak, L., Dumańska-Słowik, M., Kosakowski, P., Naglik, B., Drzewicz, P., Misiak, J., Pršek, J., George, C., & Gomez, R. E. R. (2021). The study of Dominican amber-bearing sediments from Siete Cañadas and La Cumbre with a discussion on their origin. *Scientific Reports*, 11, 18556. <https://doi.org/10.1038/s41598-021-96520-3>
- Stanhope, M. J., Waddell, V. G., Madsen, O., De Jong, W., Hedges, S. B., Cleven, G. C., Kao, D., & Springer, M. S. (1998). Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. *Proceedings of the National Academy of Sciences*, 95(17), 9967–9972. <https://doi.org/10.1073/pnas.95.17.9967>
- Tabuce, R., Asher, R. J., & Lehmann, T. (2008). Afrotherian mammals: a review of current data. *Mammalia*, 72(1), 2–14. <https://doi.org/10.1515/MAMM.2008.004>
- Tarver, J. E., Reis, M., Mirarab, S., Moran, R. J., Parker, S., O'Reilly, J. E., King, B. L., O'Connell, M. J., Asher, R. B., Warnow, T., Peterson, K. J., Donoghue, P. C. J., & Pisani, D. (2016). The interrelationships of placental mammals and the limits of phylogenetic inference. *Genome Biology and Evolution*, 8(2), 330–344. <https://doi.org/10.1093/gbe/evv261>
- Taylor, P. G. (1996). Reproducibility of ancient DNA sequences from extinct Pleistocene fauna. *Molecular Biology and Evolution*, 13(1), 283–285. <https://doi.org/10.1093/oxfordjournals.molbev.a025566>
- Thomas, B. D. (2018). *Collagen remnants in ancient bone*. PhD Thesis, University of Liverpool.
- Thomas, B., & Taylor, S. (2019). Proteomes of the past: the pursuit of proteins in Paleontology. *Expert Review of Proteomics*, 16(11–12), 881–895. <https://doi.org/10.1080/14789450.2019.1700114>
- Tuross, N. (1989). Albumin preservation in the Taima-taima mastodon skeleton. *Applied Geochemistry*, 4(3), 255–259. [https://doi.org/10.1016/0883-2927\(89\)90026-7](https://doi.org/10.1016/0883-2927(89)90026-7)
- Ulrich, M. M. W., Perizonius, W. R. K., Spoor, C. F., Sandberg, P., & Vermeer, C. (1987). Extraction of osteocalcin from fossil bones and teeth. *Biochemical and Biophysical Research Communications*, 149(2), 712–719. [https://doi.org/10.1016/0006-291X\(87\)90426-8](https://doi.org/10.1016/0006-291X(87)90426-8)
- Valdiosera, C., García, N., Dalén, L., Smith, C., Kahlke, R., Lidén, K., Angerbjörn, Arsuaga, J. L., & Götherström, A. (2006). Typing single polymorphic nucleotides in mitochondrial DNA as a way to access Middle Pleistocene DNA. *Biology Letters*, 2(4), 601–603. <https://doi.org/10.1098/rsbl.2006.0515>
- van der Valk, T., Pečnerová, P., Díez-del-Molino, D., Bergström, Oppenheimer, J., Hartmann, S., Xenikoudakis, G., Thomas, J. A., Dehasque, M., Saglican, E., Fidan, R. B., Barnes, I., Liu, S., Somel, M., Heintzman, P. D., Nikolskiy, P., Shapiro, B., Skoglund, P., Hofreiter, M., Lister, A. M., Götherström, A., & Dalén, L. (2021). Million-year-old DNA sheds light on the genomic history of mammoths. *Nature*, 591, 265–269. <https://doi.org/10.1038/s41586-021-03224-9>
- Vander Linden, A., Hedrick, B. P., Kamilar, J. M., & Dumont, E. R. (2019). Atlas morphology, scaling and locomotor behaviour in primates, rodents and relatives (Mammalia: Euarchontoglires). *Zoological Journal of the Linnean Society*, 185(1), 283–299. <https://doi.org/10.1093/zoolinnean/zly042>



- Vidal, U. L. (2010). Protein preservation in fossil whale bones of the Miocene/Pliocene Pisco Formation, Peru. Loma Linda University Electronic Theses, Dissertations & Projects, 12, 1-168.
- Vinther, J., Briggs, D. E., Clarke, J., Mayr, G., & Prum, R. O. (2010). Structural coloration in a fossil feather. *Biology Letters*, 6(1), 128-131. <https://doi.org/10.1098/rsbl.2009.0524>
- Vosgerau, D. S. A. R., & Romanowski, J. P. (2014) Estudos de revisão: implicações conceituais e metodológicas. *Revista de Diálogo Educacional*, (14)41, 165-189. <http://dx.doi.org/10.7213/dialogo.educ.14.041.DS08>
- Welker, F., Collins, M. J., Thomas, J. A., Wadsley, M., Brace, S., Cappellini, E., Turvey, S., Reguero, M., Gelfo, J., Kramarz, A., Burger, J., Thomas-Oates, J., Ashford, D., Ashton, P., Rowsell, K., Porter, D., Kessler, B., Fischer, R., Baessmann, C., Kaspar, S., Olsen, J., Kiley, P., Elliott, J., Kelstrup, K., Mullin, V., Hofreiter, M., Willerslev, E., Hublin, J., Orlando, L., Barnes, I., & MacPhee, R. (2015). Ancient proteins resolve the evolutionary history of Darwin's South American ungulates. *Nature*, 522(7554), 81-84. <https://doi.org/10.1038/nature14249>
- Welker, F., Smith, G. M., Hutson, J. M., Kindler, L., Garcia-Moreno, A., Villaluenga, A., Turner, E., Gaudzinski-Windheuser, S. (2017). Middle Pleistocene protein sequences from the rhinoceros genus *Stephanorhinus* and the phylogeny of extant and extinct Middle/Late Pleistocene Rhinocerotidae. *PeerJ*, 5, e3033. <https://doi.org/10.7717/peerj.3033>
- Westbury, M., Baleka, S., Barlow, A., Hartmann, S., Paijmans, J. L. A., Kramarz, A., Forasiepi, A. M., Bond, M., Gelfo, J. N., Reguero, M. A., López-Mendoza, P., Taglioretti, M., Scaglia, F., Rinderknecht, A., Jones, W., Mena, F., Billet, G., Muizon, C., Aguilar, J. L., MacPhee, R. D. E., & Hofreiter, M. (2017). A mitogenomic timetree for Darwin's enigmatic South American mammal *Macrauchenia patachonica*. *Nature Communications*, 8, 15951. <https://doi.org/10.1038/ncomms15951>
- Wyckoff, R. W., Mccaughey, W. F., & Doberenz, A. R. (1964). The amino acid composition of proteins from Pleistocene bones. *Biochimica et Biophysica Acta*, 93(2), 374-377. [https://doi.org/10.1016/0304-4165\(64\)90387-3](https://doi.org/10.1016/0304-4165(64)90387-3)
- Wyckoff, R. W., & Davidson, F. D. (1976). Pleistocene and dinosaur gelatins. *Comparative Biochemistry and Physiology Part B*, 55(1), 95-7. [https://doi.org/10.1016/0305-0491\(76\)90179-6](https://doi.org/10.1016/0305-0491(76)90179-6)
- Yuan, J., Sheng, G., Hou, X., Shuang, X., Yi, J., Yang, H., & Lai, X. (2014). Ancient DNA sequences from *Coelodonta antiquitatis* in China reveal its divergence and phylogeny. *Science China Earth Sciences*, 57(3), 388-396. <https://doi.org/10.1007/s11430-013-4702-6>
- Zhou, X., Xu, S., Xu, J., Chen, B., Zhou, K., & Yang, G. (2012). Phylogenomic analysis resolves the interordinal relationships and rapid diversification of the Laurasiatherian mammals. *Systematic Biology*, 61(1), 150. <https://doi.org/10.1093/sysbio/syr089>
- Zúniga, L. E., Enríquez, L., Vides, C., & Aguilar, D. (2019). Recuperación de tejidos blandos de perezosos fósiles gigantes (Mammalia, Xenarthra, Pilosa) de la zona central del Departamento de Yoro, Honduras. *Revista Ciencia y Tecnología*, (24), 32-4. <https://doi.org/10.5377/rct.v0i24.7875>