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## Diet and environment of *Mylodon darwini* based on pollen of a Late-Glacial coprolite from the Mylodon Cave in southern Chile

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### ABSTRACT

We studied the pollen content of a well-preserved coprolite of a Late-Glacial giant ground sloth (*Mylodon darwini*) from the Mylodon Cave, province Última Esperanza, southern Chile. The specimen was obtained in 1909 and has been stored in a museum in the Netherlands since. It was radiocarbon dated to  $13,140 \pm 55$  BP ( $15,927$ – $15,522$  cal BP), which fits with other radiocarbon dates showing the early Late-Glacial presence of *M. darwini* in the province Última Esperanza. Contemporaneous oxygen isotope data from Antarctic EPICA Dome C indicates that our *Mylodon* specimen lived during a warming phase of the Late-Glacial, ca. 1000 years before the start of the Antarctic Cold Reversal. We compared our pollen data with pollen records showing contemporaneous regional vegetation and discuss the uncertainties in the interpretation of pollen spectra from faeces. To expand on the pollen data, we tested ancient DNA preservation in the sample; we sequenced ~9.4 million DNA reads and found that the concentration of ancient plant DNA is below detectable levels. Pollen analysis confirms earlier findings that the *Mylodon* was a grazer, but the discovery of large amounts of *Fragaria* and *Azorella* pollen in the faeces may indicate that *Mylodon* was also able to select and consume specific plants, and therefore could also be regarded as a selective feeder.

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## 1. Introduction

### 1.1. History of research

The Naturalis Biodiversity Center in Leiden, the Netherlands, hosts an extraordinary collection of Pleistocene mammal bones and other remains from southern South America. The Dutch biologist Jan Herman Kruimel (1885–1916) obtained this collection in 1909, including bones, skin fragments, and coprolites (fossilised faeces), in Punta Arenas in South Chile. The material originates from the Mylodon Cave (Cueva del Milodón), also known as Cave Última Esperanza, Eberhardt Cave, and Last Hope Cave. The cave is in the southern part of Chile (province Última Esperanza; coordinates  $51^{\circ}33'49''\text{S}$ ,  $72^{\circ}37'07''\text{W}$ ) 160 m above sea level, and 24 km from Puerto Natales (Fig. 1). It is situated in the Benítez mountain, north of the Strait of Magellan that separates the island Tierra del Fuego from mainland South America. The entrance of the cave is 170 m wide, 30 m high, and the cave is 270 m deep.

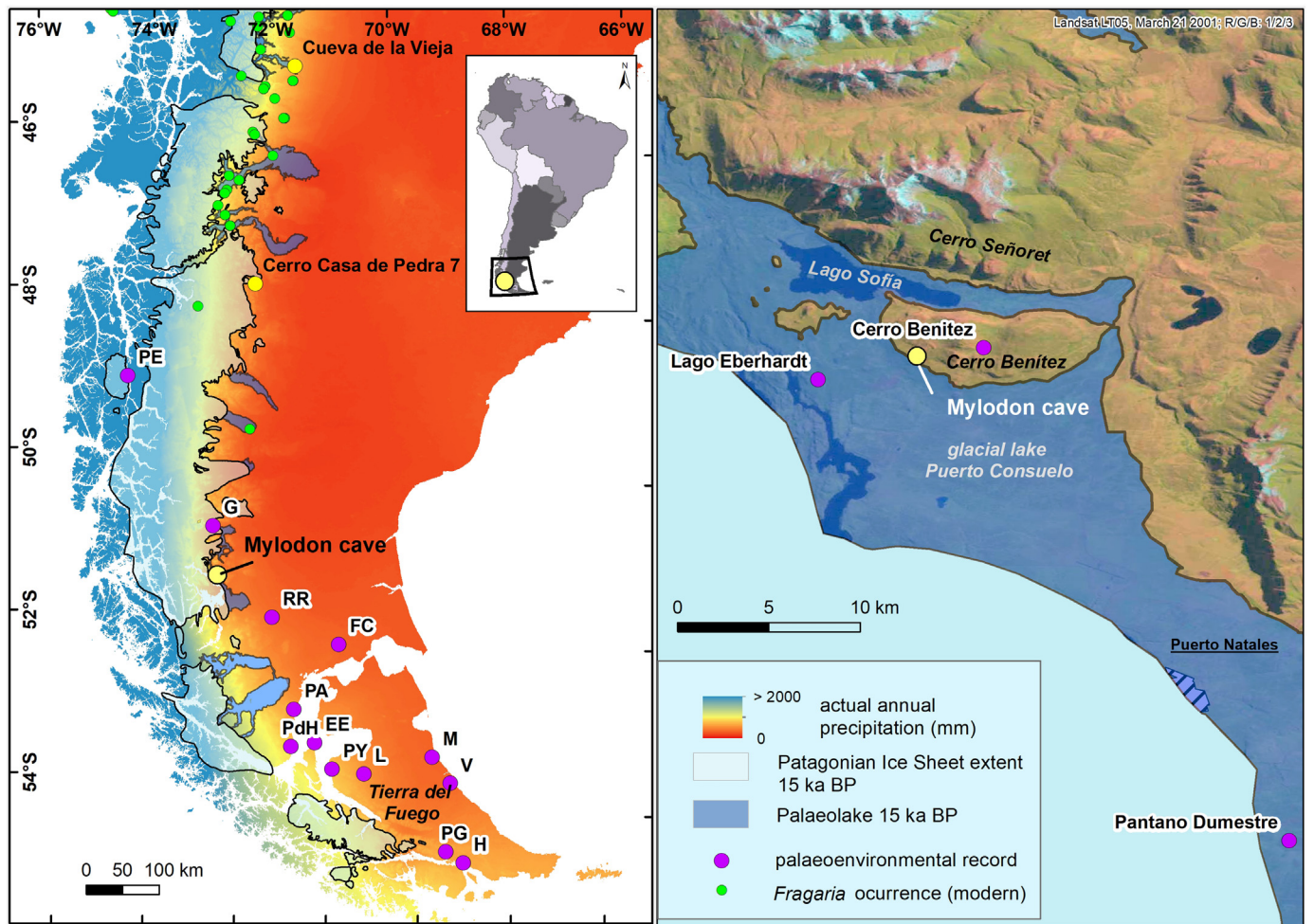
Landowner Hermann Eberhardt (1852–1908) made several discoveries in the cave and unearthed large bones and skin. He showed the specimens to the Swedish explorer A.E. Nordenskjöld in 1896. Nordenskjöld subsequently visited the cave and dug up more bones, and also some skin fragments, similar to the remains unearthed by Eberhardt (Nordenskjöld, 1898, 1899a, 1899b, 1899c, 1900). He also found a keratin nail sheath of a ground sloth. The discoveries of Nordenskjöld were described by Lönnberg (1899, 1900).

Shortly after the departure of Nordenskjöld in 1899, the geologist R. Hauthal carried out an excavation and found some more pieces of skin, as well as faeces from the giant ground sloth *Mylodon darwini* (Hauthal et al., 1899; Hauthal, 1900, 1904). Hauthal also dug in a smaller cave, three kilometres east of the main cave. Both Hauthal and Nordenskjöld mentioned up to one-meter thick layers of faeces, which they attributed to *M. darwini*, and they concluded that the animals used the cave over a long period.

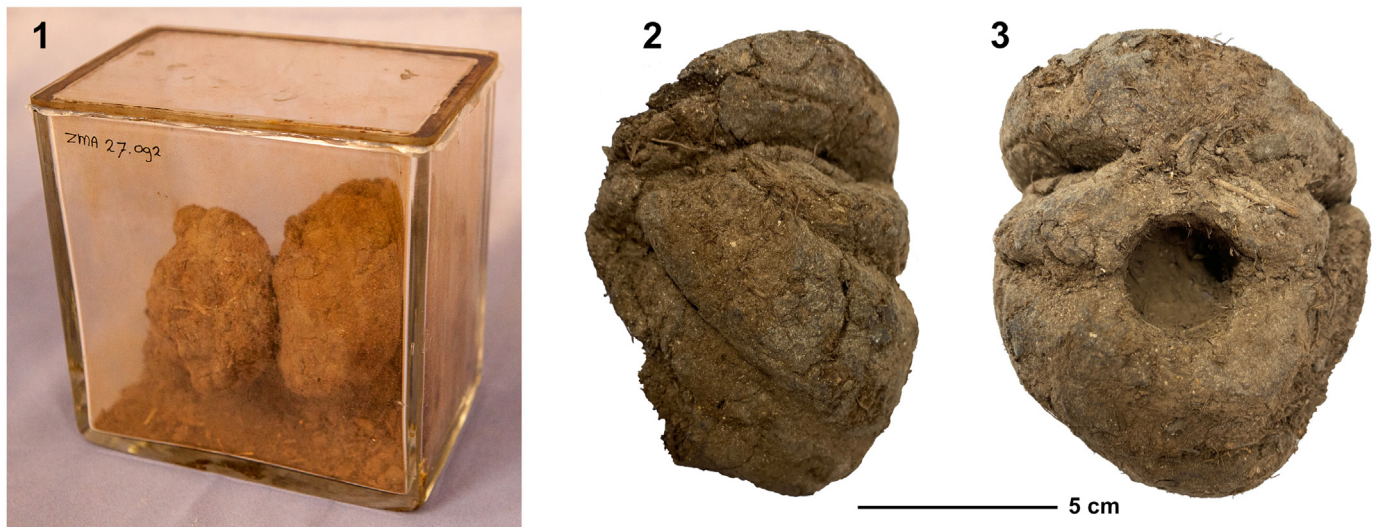
J.H. Kruimel visited the Mylodon Cave in 1909 but did not excavate any remains himself. Rather, he obtained a collection from Mr. Charles Milward, the British Consul in Punta Arenas, who also sold a collection of bones and other remains from the cave to the Natural History

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**Fig. 1.** Location of Mylodon Cave in southern Chile, and palaeoenvironmental sites discussed in the text (Markgraf, 1993; Kilian and Lamy, 2012, and references therein). Indicated are the estimated extent of the Patagonian Ice Sheet and palaeolakes around 15 ka BP (after Davies et al., 2020), annual precipitation (1950–2000) calculated with WorldClim version 1.4 (Hijmans et al., 2005), and modern occurrence of *Fragaria* species (GBIF, 2021). Abbreviations: V = Viamonte, PE = Puerto Eden, H = Haberton, PG = Paso Garibaldi, M = La Misión, FC = Fells Cave, L = Lynch, PY = Punta Yartou, PdH = Puerto del Hambre, EE = Estancia Esmeralda, PA = Punta Arenas, RR = Rio Rubens, G = Guanaco.



**Plate I. 1:** Two *Mylodon darwini* coprolite fragments from the Mylodon Cave, province Última Esperanza, southern Chile. The material is stored in a closed container (15 × 10.5 × 15 cm) in the Kruiemel Collection at the Naturalis Biodiversity Center in Leiden, Netherlands (collection number ZMA.MAM.27092); 2 and 3: coprolite specimen 1, showing the borehole where the radiocarbon sample and pollen sample 1 were taken.



Museum in Berlin, Germany. Kruimel's collection was donated to the Zoological Museum, University of Amsterdam (ZMA), by the Amsterdamse Universiteits-Vereniging, and was integrated into the Naturalis Biodiversity Center, Leiden, in 2011. The Kruimel collection includes 202 numbered items and a large selection of unnumbered vertebrae from mainly *Mylodon darwini*. In addition, there are still a few boxes with unidentified fragments.

The identification of specimens in the Kruimel collection was carried out mainly by Dr. A. Smith Woodward of the British Museum of Natural History in London, who was visited by J.H. Kruimel (mentioned in a letter from Kruimel to professor M.W.C. Weber dated 7/29/1910). The collection is dominated by the remains of ground sloths and felines (Felidae). The items were catalogued in the ZMA as *M. darwini* (n = 94), *Neomylodon listai* = *M. darwini* (n = 1), Felidae (n = 40), *Felis concolor* (n = 5), *Smilodon ensedanensis* (n = 16), *Smilodon neogens* (skull, plaster = plaster cast of skull), *Equus argentinus* (n = 1), *Equus curvidens* (n = 1), *Onohippidon saldiassi* (n = 2), *Lama huanachus* (n = 35), *Lama guanicoe* (n = 5), and *Propalaeohop* (= panther scale; n = 1). Some coprolites are still intact, among which are two large *M. darwini* coprolites (one is object of our study), which are housed in a closed glass container numbered ZMA.MAM.27092 (Plate 1).

In recent decades, skin fragments, bones, and coprolites of *M. darwini* from the Mylodon Cave have attracted the attention of archaeologists, palaeoecologists, and molecular biologists, including Salmi (1955), Moore (1978), Saxon (1979), Bird (1988), Heusser et al. (1994), Höss et al. (1996), Martinic (1996), Mol et al. (2003), Tonni et al. (2003), Steadman et al. (2005), Brandoni et al. (2010), Borrero and Martin (2012), Clack et al. (2012), Martin et al. (2013), Varela and Fariña (2016), Villavicencio et al. (2016), Delsuc et al. (2018), McDonald and Werdelin (2018), and Hunt and Lucas (2018, 2020).

## 1.2. Aspects of Late-Glacial climate and vegetation in the province Última Esperanza

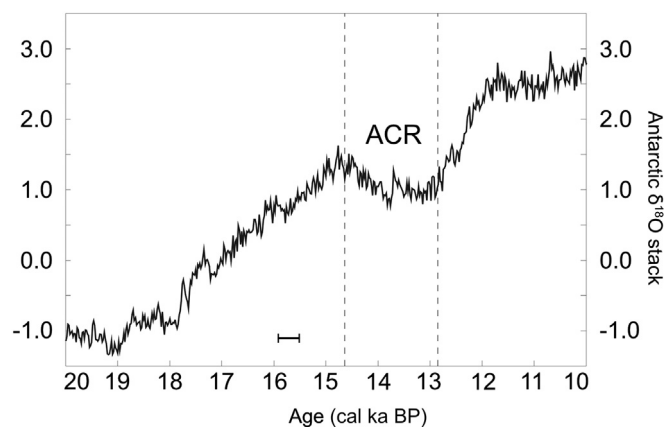
The general distribution of the major vegetation types in southern Patagonia is determined by topography and climate. The prevailing westerly winds arriving from the Pacific Ocean are laden with moisture, which is mostly deposited as the wind rises to cross the southern Andes and their outlying systems. The west–east rainfall gradient ranges from 5000 mm per year to about 200 mm per year (Moore, 1978; Schneider et al., 2003; Garreaud et al., 2013).

The province Última Esperanza is located at the transition between the humid western side of the Andes and the drier, more continental Argentine steppe (Fig. 1). The region of the Mylodon Cave has high precipitation in autumn and winter and lower precipitation in spring and summer.

The present vegetation in the surroundings of the Mylodon Cave is open, with scattered stands of *Nothofagus* woodland. Moore (1978) and Heusser et al. (1994) described general features of the modern vegetation zones of southern Patagonia and the principal plant associations of Última Esperanza. The location of Mylodon Cave is in an area where the natural vegetation would be deciduous forest dominated by *Nothofagus pumilio* and *N. antarctica*. However, during the past 150 years human influence has considerably altered the natural vegetation (Moore, 1978).

Estimates based on EPICA DOME C, east Antarctica (Jouzel et al., 2007; Villavicencio et al., 2016; Pedro et al., 2015), indicate ca. 10.3 °C lower 100-year mean surface temperatures during the Last Glacial Maximum (20–18 ka BP) than during the last millennium (Fig. 2). An increase of ca. 6.5 °C between 18 and 14.5 ka BP was followed by the Antarctic Cold Reversal (ACR) between 14.7 and 13 ka BP, when the 100-yr mean dropped by about 2 °C. After 13 ka BP, warming commenced again with an increase of 4 °C by 11.9 ka BP.

Markgraf (1991, 1993), Lumley and Switsur (1993), Markgraf and Kenny (1997), Kilian and Lamy (2012), and Moreno et al. (2012) summarised major climate changes in southern Chile. Relevant for our



**Fig. 2.** Oxygen isotope stack of EPICA DOME C, east Antarctica and the calibrated age range (horizontal bar: 15,927–15,522 cal BP) of our radiocarbon dated coprolite. According to Pedro et al. (2015) the oxygen isotope fluctuations indicate an increase of about 6.5 °C between 18 and 14.5 ka BP, followed by the Antarctic Cold Reversal (ACR) between 14.7 and 13 ka cal BP, when the 100-yr mean dropped by about 2 °C. After 13 ka BP, warming commenced again with an increase of 4 °C by 11.9 ka BP.

study is the observation that shortly after 15 ka cal BP, glaciers retreated rapidly (McCulloch and Bentley, 1998; McCulloch et al., 2019, 2020; Kilian et al., 2007), and plant invasion started (Heusser, 1995; Moreno et al., 2012). The early Late-Glacial warming phase allowed immigration of various plant species from the north, which caused an increase of the available botanical biomass. The carrying capacity of the landscape increased, allowing mammals to explore new areas and to move into southern South America.

In this paper, we present and discuss the botanical analysis of a *M. darwini* coprolite from the Kruimel collection (collection number ZMA.MAM.27092, Plate 1). The specimen was radiocarbon dated and investigated using pollen, to elucidate the diet and contemporary environment of the individual. We further investigated the specimen with ancient DNA shotgun sequencing analysis. The ancient DNA study was intended to complement inferences based on pollen analysis and may detect the presence of small low-density populations that cannot be identified by looking at the fossil pollen record (Parducci et al., 2015).

## 2. Material and methods

### 2.1. Sampling and preparation

The two coprolites attributed to *M. darwini*, the only ground sloth that occurs in the Última Esperanza fauna (McDonald and Werdelin, 2018; Hunt and Lucas, 2018), are housed in the Kruimel collection of the Naturalis Biodiversity Center in Leiden, Netherlands (Plate 1). The coprolite specimens in the collection are recognisable as *M. darwini* faeces because of their large size and distinctive morphology (Hunt and Lucas, 2018). Specimen 1 consist of five compressed segments which are similar to those described and figured by Hauthal et al. in 1899 (Hunt and Lucas, 2018). The measurements of specimen 1 are: maximum height 69 mm, maximum width 75 mm. Specimen 2 consist of three relatively flat compressed segments. The measurements of specimen 2 are: maximum height 39 mm, the maximum width 77 mm. Both specimen 1 and 2 are not complete coprolites. We chose to minimise damage to the museum collection, and therefore we only sampled specimen 1. We took a sub-sample for radiocarbon dating from inside that coprolite. For pollen analysis we took three sub-samples (1, 2 and 3). Pollen sample 1 was taken from inside the coprolite, so its content was not contaminated with 'secondary' microfossils landing on the coprolite surface after its dropping by the ground sloth. To be able to distinguish possible contamination, pollen samples 2 and 3 were taken from the surface of coprolite specimen 1. Pollen samples 4 and 5 comprised

dusty, non-compacted faecal material from the bottom of the glass container (Plate I). The preparation of the material for radiocarbon dating was according to Dee et al. (2019).

### 2.1.1. Pollen analysis

The five sub-samples used for pollen analysis were prepared according to Faegri and Iversen (1989). The microfossils were embedded in glycerol gelatine and sealed in with paraffin wax on microscope slides. Pollen grains were identified using the pollen reference collection at the Institute for Biodiversity and Ecosystem Dynamics (University of Amsterdam) and the pollen floras by Markgraf and d'Antoni (1978) and Heusser (1971).

### 2.1.2. Ancient DNA

For the ancient DNA (aDNA) analysis, we sub-sampled the inside of coprolite specimen 1. The outer layer of the specimen was removed before sampling to minimise 'secondary' contaminants, as explained above. Sub-sampling was carried out inside a flowhood at the Centre for Geogenetics - Ancient DNA lab (University of Copenhagen) with proper decontamination and using sterile material for the sampling. All the pre-PCR steps (including DNA extraction, library build, index PCR set up) were also carried out in appropriate facilities for ancient DNA work. Extraction blanks with no coprolite added were used to monitor possible contamination.

To incorporate within-sample variability, three sub-samples of ~200 mg were taken from different areas within coprolite specimen 1. For aDNA extraction we used ~200 mg of starting material. This was achieved by combining up to 70 mg from each sub-sample in an Eppendorf tube.

DNA extraction was carried out following method B (Modified MinElute Protocol) from Hagan et al. (2019) with some modifications. Post-incubation, the supernatant was added to a 30 KDa Amicon® Ultra-4. The sample was spun down at 4000 rpm, until the supernatant was concentrated down to 70 µl. The concentrate was combined with 13× Qiagen PB buffer and purified using Monarch columns (NEB). We performed two washes with Qiagen PE buffer as recommended by Hagan et al. (2019). DNA elution was performed in two steps to reach 60 µl of eluate. We added 30 µl of Qiagen EB buffer to the Monarch column, incubated for 5 min at room temperature, and centrifuged at 13,000 rpm (max speed) for 1 min.

The aDNA sequencing library was prepared as in Kapp et al. (2021) and double-indexed using KAPA HiFi uracil+ premix (KAPA Biosystems). The number of cycles for the index PCR was determined from qPCR analysis. The resulting indexed library was quantified on an Agilent 2100 Bioanalyser, combined with other indexed libraries, and shotgun sequenced on an Illumina HiSeq 4000 SR 80 basepairs (bp) at Geogenetics Sequencing Core, University of Copenhagen.

After sequencing, the raw fastq file was filtered computationally by removing remnant adapter sequences with AdapterRemoval (v 2.3.0), which, in addition, was set to discard reads shorter than 30 bp and to trim stretches of N's from the 5 and 3' ends. To reduce false positives from low-complexity regions, low-complexity reads were filtered out using sga preprocess (v.0.10.15), with a dust threshold of 1.0. The resulting pre-processed fastq files were mapped against databases of all Reference Sequences (RefSeqs) for mitochondria and chloroplasts, respectively, using bowtie2 (v2.3.2) set to report up to 500 alignments per read. Lastly, the lowest common ancestor of the best hit(s) to the database was evaluated using the getLCA script from <https://github.com/frederikseersholm/getLCA> (Seersholm et al., 2016).

## 3. Results

### 3.1. Radiocarbon dating

Our *M. darwini* coprolite was dated to 13,140 ± 55 BP (GrM-21,338) at the Groningen Centre for Isotope Research. The Late-Glacial climate

history as based on Greenland ice cores differs from Antarctic data, and we therefore compare the age interval of our South American coprolite with the chronological and climatological records of the Antarctic EPICA Dome C (Jouzel et al., 2007; Pedro et al., 2015). After calibration using the SHCal20 calibration curve published by Hogg et al. (2020) for samples originating from the Southern Hemisphere, the calendar age was estimated between 15,927 and 15,522 cal BP (95.4% probability).

### 3.2. Pollen analysis

Fig. 3 shows the results of the pollen analysis. Percentages are expressed on a sum of pollen and the spores of vascular plants. Sample 1 was taken from inside the coprolite, so its pollen content is likely not contaminated with 'secondary' pollen grains landing on the surface of the coprolite after its dropping by the ground sloth. Samples 2 and 3 were taken from the surface of the coprolite, and samples 4 and 5 comprise coprolite crumbs from the bottom of the glass container (Plate I) and may contain other pollen from the surroundings of the cave. The sample 'Σ' at the top of the diagram shows the combined microfossil record for the five investigated samples. Plates II, III and IV show a selection of the fossil material that we encountered.

### 3.3. Ancient DNA analysis

We sequenced a total of 9,383,710 DNA reads, which after filtering and pre-processing resulted in 5,374,323 DNA sequences. Consistent with the ancient nature of the sample, the DNA appears highly fragmented, with an average read length of 55.2 bp and a high fraction of DNA reads discarded because of the 30 bp threshold used. We investigated the content of plant and animal remains in the sample by mapping reads against reference mitochondrial and chloroplast genomes. Unfortunately, we did not find evidence of endogenous aDNA for vertebrates or plants. For vertebrates, very few reads had a match in the database, and no taxon was detected more than three times. No DNA sequences mapped to *Myiodon*, or to the order Pilosa. For plants, 1028 reads could be assigned to a taxon. However, these identifications all represent exotic species that are commonly detected in blank and negative controls. In addition, upon further investigation of the mapping characteristics of the most commonly detected plant taxa, we found that they all had an uneven distribution of mapped reads across the reference genome. This finding suggests that the plant taxa identified are false positives from low-complexity DNA.

## 4. Discussion

Villavicencio et al. (2016), Hunt and Lucas (2018), and Perez et al. (2021) published a series of radiocarbon dates of *M. darwini* material from the Myiodon Cave, which encompassed the period from ca 13,560 to 10,200 BP. The coprolite studied by us is one of the oldest found in the cave, and corresponds with many dates on *Myiodon* bone, hair, and skin recovered from the Myiodon Cave (Pérez et al., 2021). Based on the oxygen isotope curves and our radiocarbon date, we conclude (Fig. 2) that the *M. darwini* faeces specimen was deposited during an early Late-Glacial warming phase, about 1000 years before the start of the Antarctic Cold Reversal.

Faeces are an important source of information for the reconstruction of diets, vegetation, and other environmental aspects (Birks et al., 2018; Carrión et al., 2007; Gil-Romera et al., 2014; Gravendeel et al., 2014; Hunt and Lucas, 2018, 2020; Scott, 1987; Scott et al., 2004; van Geel et al., 2008, 2011a, 2011b), but the pollen content in faecal samples will be biased. Faeces mainly represent a few days at most, and the pollen taxa composition depends on the food choice and (flowering) season. Inflorescences may have been consumed preferentially (van Geel et al., 2019). Consumed plant species may have contained pollen of other species sticking on leaf surfaces, representing the pollen rain for





a shorter or longer period. Therefore, some pollen taxa may be present that were not a direct part of the animal's diet, and some rare taxa may even have arrived via long-distance transport. On the other hand, vegetative parts of some plant species may have been consumed when the flowering period was over, and in such cases no pollen of the consumed plant species was ingested. Regrettably, our ancient DNA analysis of the sample did not provide any useable DNA that could shed further light on this.

The five dominant pollen and spore types show comparable percentages in all five sub-samples, without major differences between them (Fig. 3). Five pollen types and the fern spores that were not recorded in the centre of the coprolite (sample 1; *Alnus*, *Podocarpus*, Malvaceae, *Solanum*-type, Trilete fern spore) have mostly a single occurrence in the other samples 2–5.

The high abundance of *Azorella* and *Fragaria chilensis* in our data, and probably also of Poaceae and *Empetrum rubrum*, likely reflect the pollen-bearing plant consumption of the *M. darwini* individual. Recorded clumps of unripe pollen of *Azorella*-type, *Empetrum*, Poaceae, and Cyperaceae in our samples also point to consumption of these plants. The recorded *Azorella* may well be *A. monantha*, a cushion plant of alpine Patagonian plant communities (Arroyo et al., 2003).

Our results primarily reflect the flora, be it selectively, and not so much the vegetation in terms of dominant plant taxa and structure. The pollen types in the coprolite may largely be derived from the vegetation near the cave, but their relative abundance in the coprolite will heavily depend on the season and the animal's ingestion of pollen-bearing plants. Heusser et al. (1994) summarised this as follows: "pollen (...) assemblages of (...) dung are not considered entirely reliable to characterise (...) vegetation (...), due to the mixture of ingested and wind-blown pollen." Nevertheless, faeces are an important source of information for the reconstruction of diets, vegetation, and other environmental aspects.

Markgraf (1985) and Heusser et al. (1994) also studied fossil pollen assemblages of *M. darwini* coprolites sampled in the Mylodon Cave. The deposits sampled by Heusser et al. were more than 3 m thick. Faeces were dated between  $13,470 \pm 180$  and  $10,575 \pm 400$  BP. The pollen assemblages were dominated by Poaceae (grass steppe) and *Empetrum* (tundra or dwarf scrub heath). Grazing habits and habitats of *M. darwini* may have been selective, forming a source of distortion of the vegetation composition as derived from pollen in lake sediments. In our pollen record, secondary (post-depositional) contamination did not take place, as samples 2–5 show similar pollen taxa in comparable percentages relative to sample 1, which was taken from inside the compact *M. darwini* coprolite (Fig. 3).

A direct comparison of the pollen assemblage in the *M. darwini* coprolite with pollen records from nearby Lake Eberhard and Pantano Dumestre (Fig. 1; Moreno et al., 2012) is not possible. Around 15 ka BP, a large palaeolake was present at these sites (after Davies et al., 2020), and thick glaciolacustrine clay and silts were deposited that lack sufficient pollen for palaeoenvironmental reconstructions (Moreno et al., 2012).

McCulloch et al. (2021) published a Late-Glacial–Holocene pollen sequence from the closed basin mire Cerro Benítez ( $51^{\circ}33'40.45''S$ ,  $72^{\circ}35'10.24''W$ ). The mire is at an altitude of 211 m asl, at only 4 km distance from the Mylodon Cave (Fig. 1). The pollen record at Cerro Benítez provides insights into the changing nature of landscape and resources encountered by the fauna and early humans. The sediment sequence begins ca. 16.3 ka cal BP, after glacier retreat from the area (deglaciation between 18 and 15 ka BP, McCulloch et al., 2021) and the pollen records indicates a treeless tundra/steppe, favoured by megaherbivores. At ca. 14.9 ka cal BP, *Nothofagus* began to migrate into the area, but the landscape remained open with sufficient open ground for grazers. At ca. 12.0 ka cal BP there was a dramatic expansion of woodland, but the decline of large mammals appears to have started about 700 years earlier and is coincident with the arrival of hunter-gatherers in the area ca. 12.7 ka cal BP. For the discussion about man-magafauna interactions we refer to McCulloch et al. (2021).

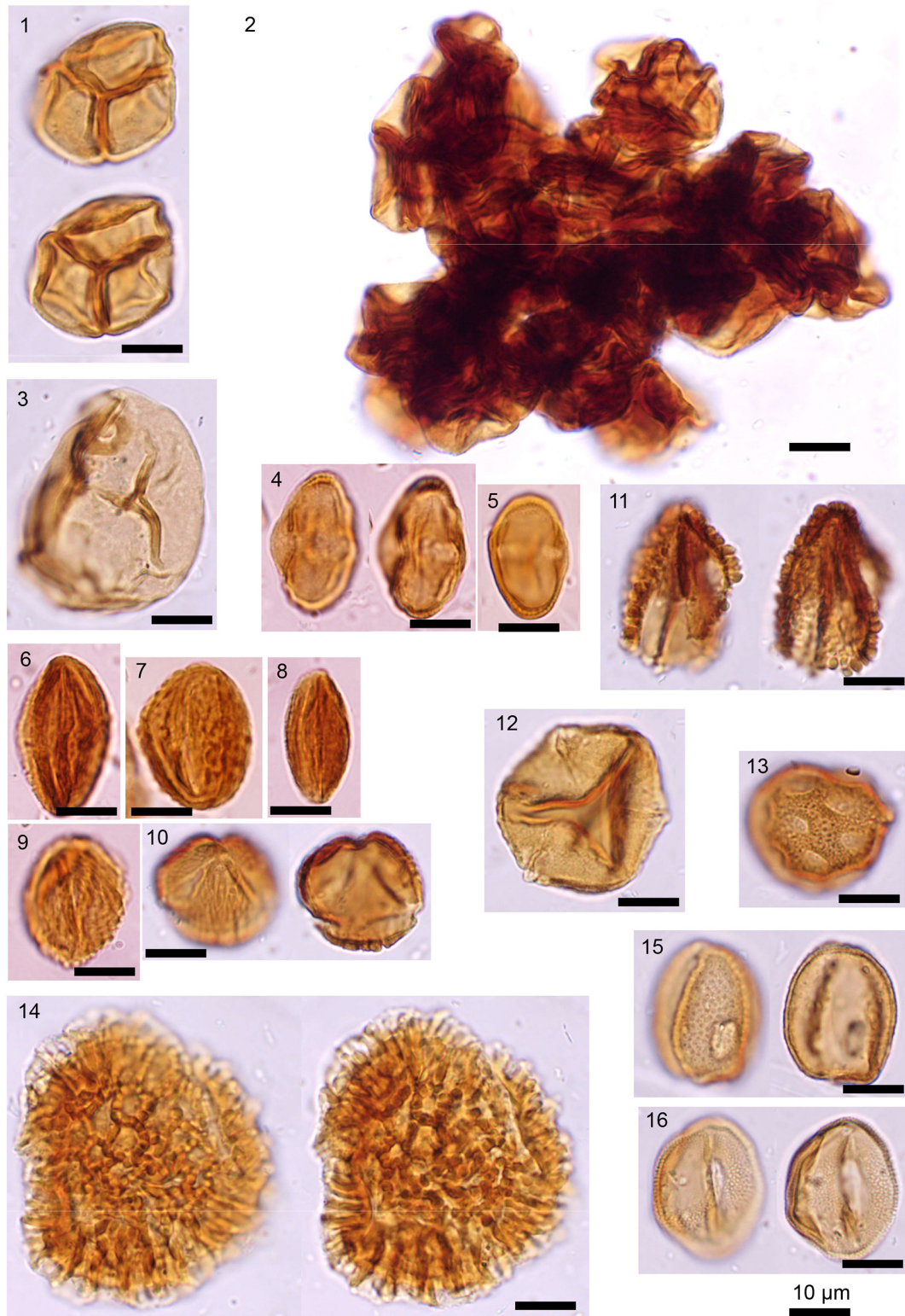
Comparison of the Cerro Benítez pollen diagram with our *Mylodon* pollen spectra shows high percentages of Poaceae, *Empetrum* and Asteraceae in both records, but there are also major differences in the percentages: Apiaceae (*Azorella* in the *Mylodon* samples) and *Fragaria* respectively show low percentages of are absent in the Cerro Benítez spectra, while high in the *Mylodon* record. This we consider as an indication for consumption of *Azorella* and *Fragaria*.

The pollen record of *Mylodon* coprolites published by Markgraf (1985) and Heusser et al. (1994) also show a high abundance of Poaceae (= Gramineae) and *Empetrum rubrum*. The sum of Asteraceae (named Compositae by Markgraf, 1985 and Tubuliflorae by Heusser et al., 1994) reaches comparable values in both studies. Major differences are shown by *Azorella*-type (= Umbelliferae in Heusser et al., 1994). This pollen type is most abundant in our samples, but has low values in the samples studied by Markgraf and Heusser.

Our fourth-most abundant type, *Fragaria chilensis*, is absent from Heusser's and Markgraf's pollen record, and also Velázquez et al. (2015) did not find *Fragaria* pollen in Mylodontid coprolites from Cerro Casa de Piedra 7 (Fig. 1). *Fragaria* macrofossils were not present in other Mylodontid coprolites from Mylodon Cave (Moore, 1978). However, *Fragaria* seeds were encountered in early Holocene cave deposits at Cueva de la Vieja (Fig. 1; Méndez et al., 2018), associated with early human activity. *Fragaria* species grow in the Andean mountains and foothills, but are nowadays not often described or reported south of 50°S (Fig. 1; GBIF.org). Markgraf (1993) discussed pollen results of numerous 'pollen profile sites' and mentioned a dominance of Poaceae and *Empetrum rubrum* (dry, cold tundra) for Late-Glacial vegetation up to 12.5 ka BP, which is in agreement with our results. Furthermore, Markgraf (1993) mentioned the presence of characteristic herbs that also show up in our results, including *Acena*, *Azorella*, and *Gunnera*. However, a major difference is the high abundance in our material of *Azorella* and *Fragaria chilensis*.

Based on microfossils and macrofossils from coprolites from the Mylodon Cave, Moore (1978) showed that *M. darwini* fed on a diet of Cyperaceae, Poaceae, and species including *Marsippospermum grandiflorum*, *Plagiobotrys albiflorus*, and *Oreobolus obtusangulus*. These species nowadays occur in the open, cool, wet sedge-grasslands of western Patagonia. *Empetrum rubrum* occupies a wide range of open habitats, from dry steppe to Magellanic moorlands.

In the pollen record of Musotto et al. (2012), Poaceae pollen dominates the three modern steppe samples, and *Empetrum*/Ericaceae (equivalent to *Empetrum rubrum* in our data) dominates the two modern ecotone samples. The closest similarity with our data is one modern ecotone sample that has abundant Poaceae pollen next to dominant *Empetrum*/Ericaceae. Cyperaceae pollen is abundant in two modern steppe samples but has relatively low values in our data. *Azorella*, the most abundant pollen type in our data (>25%), has higher values in one of the modern steppe samples (ca. 12%). The past vegetation reflected by our *Mylodon* pollen record may have looked like the patches in the modern steppe and modern ecotone that have abundant *Empetrum rubrum* and Poaceae. Musotto et al. (2012) related modern pollen assemblages to modern vegetation in Tierra del Fuego: three samples from steppe, nine from forest, and two from the ecotone between steppe and forest. Pollen of the major forest tree *Nothofagus* and its parasite *Misodendron* was found to reach everywhere, even into the steppe. Pollen transport of *Nothofagus* over long distances was also shown by Van der Knaap and Van Leeuwen (1993). In material younger than 300 years old from the South Shetland Islands, which are situated ca. 1000 km south of Tierra del Fuego, *Nothofagus* comprised ca. 79% of all long-distance transported pollen. In our study, *Nothofagus* pollen is extremely scarce and *Misodendron* is even absent from our *M. darwini* coprolite, which shows that forest was absent in the surroundings and even far away from the Mylodon Cave (compare the Cerro Benítez pollen diagram by McCulloch et al. (2021). Markgraf (1985) concluded on the basis of pollen and cuticulae in *Mylodon* dung that the animals were grazers, not browsers. Our data however,



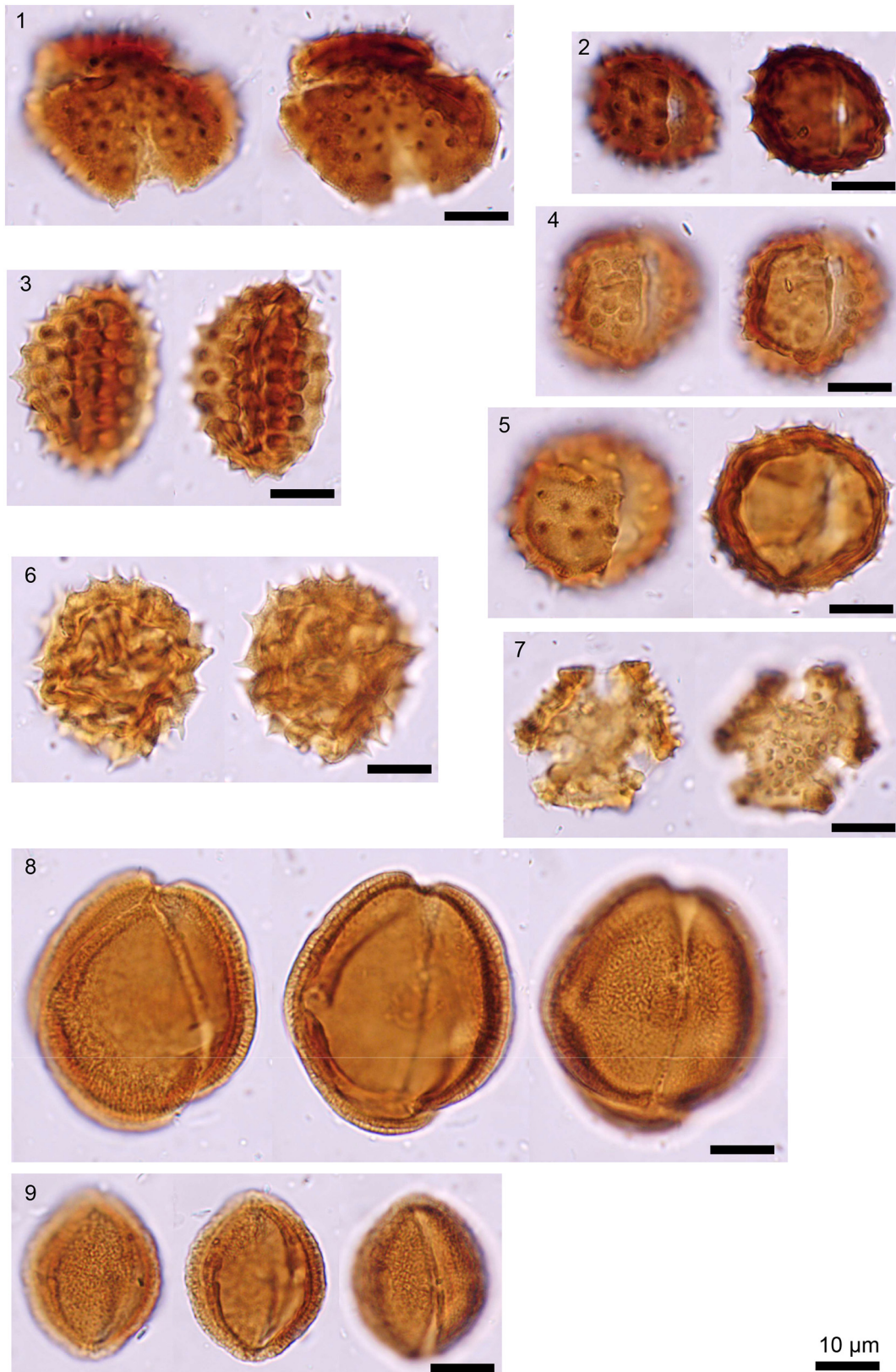
**Plate II.** Selection of pollen from the *Mylodon darwini* coprolite studied. 1: Ericaceae, *Empetrum rubrum*; 2: clump of *Empetrum rubrum*; 3: Poaceae, *Hordeum* type; 4 and 5: Apiaceae, *Azorella* type; 6–10: Rosaceae, *Fragaria chiloensis*; 11: Aquifoliaceae, *Ilex*; 12: Rosaceae, *Acaena*; 13: Caryophyllaceae, *Cerastium* type; 14: Plumbaginaceae, *Armeria*; 15: Ranunculaceae, *Ranunculus* type; 16: Fabaceae, *Adesmia* type.

suggest that *Mylodon* may have been a selective feeder during part of the year, explaining the high abundance of *Fragaria* and *Azorella* pollen in the faeces studied.

A way to improve the DNA results could be to perform deeper shotgun sequencing of the DNA library. This would increase the amount of

sequencing data available for analysis; however, it would not guarantee an increase in the sequencing of target ancient DNA. In samples with poor DNA preservation, this may rather lead to the further sequencing of contaminants and artefacts, but not real ancient DNA. Hybridization by capture prior to sequencing is an effective way to enrich for DNA of

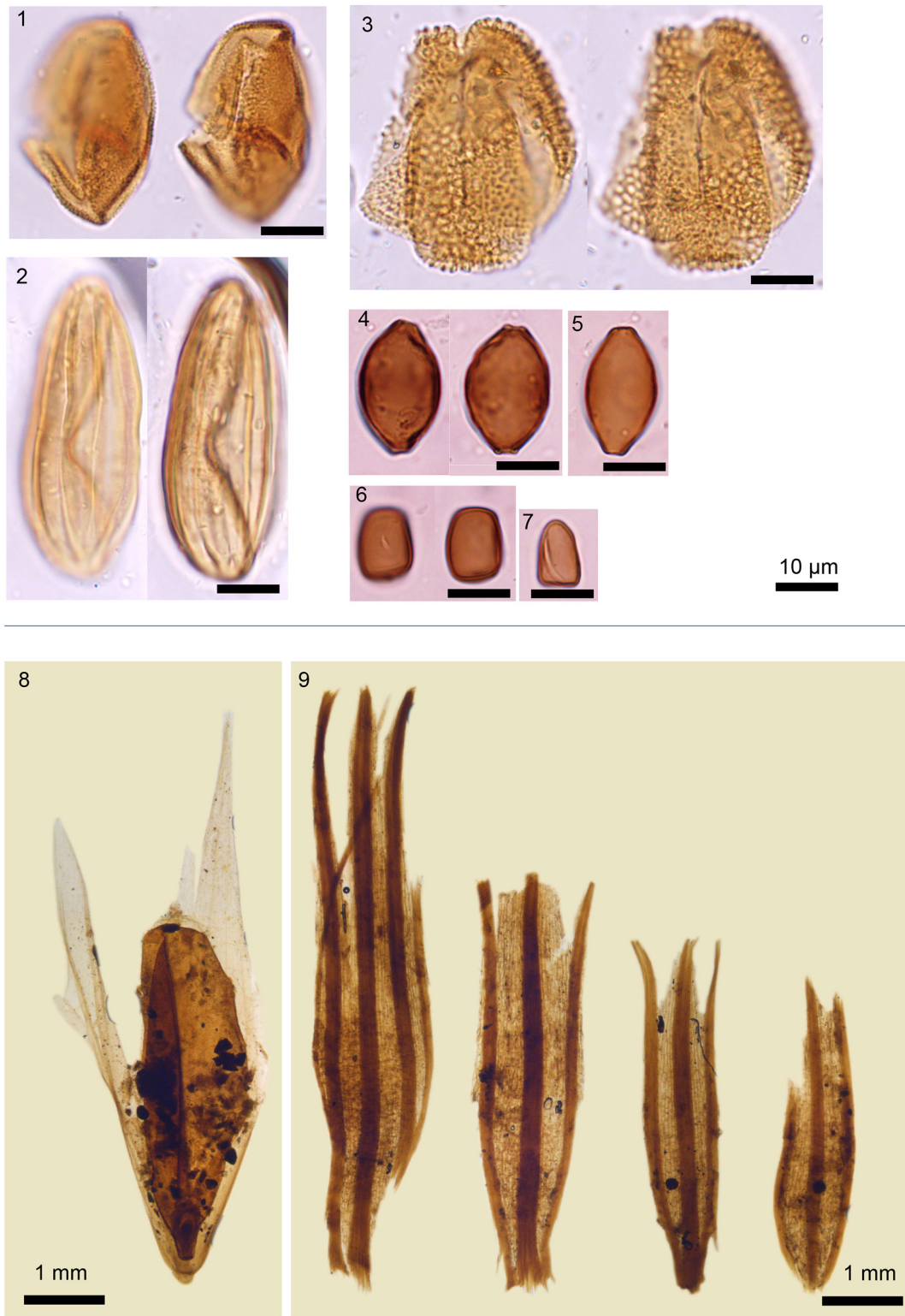




**Plate III.** Selection of pollen from the *Mylodon darwinii* coprolite studied. 1: Asteraceae, Asteroideae; 2: *Baccharis* type; 3–5: *Senecio* type; 6 and 7: Cichorioidae; 8: *Mutisia* type; 9: *Perezia/Nassauvia*.

interest, and this approach has successfully been applied to complex samples (e.g., Slon et al., 2017; Schulte et al., 2021). However, it requires designing capture baits, either using a long-range PCR approach (Maricic et al., 2010) or having a company synthesising them. The first

approach is challenging for complex samples (such as faeces or sediments), where the target DNA is not a single species, as in our case. This approach would require using long-range PCR for every taxon of potential interest, which is both time and resource consuming.



**Plate IV.** Selection of fossil material from the *Mylodon darwini* coprolite studied. 1: Orobanchaceae, *Euphrasia* type; 2: Ephedraceae, *Ephedra*; 3: non-identified pollen; 4 and 5: diporate ascospores; 6 and 7: *Sporormiella*; 8: Poaceae part of inflorescence; 9: unidentified plant remains (objects 8 and 9 were found in the dusty material at the bottom of the glass container).

Commercial baits would be a better solution for a multi-species approach; however, this is also prohibitively expensive.

## 5. Conclusions

A well-preserved Late-Glacial coprolite of the giant ground sloth *Mylodon darwini*, which was obtained more than 110 years ago from the

*Mylodon* Cave in southern Chile, was successfully studied for pollen. The specimen was radiocarbon dated to  $13,140 \pm 55$  BP (15,927–15,522 cal BP) and contemporaneous oxygen isotope data from Antarctic EPICA Dome C indicates that our *Mylodon* specimen lived during a warming phase of the early Late-Glacial, ca. 1000 years before the start of the Antarctic Cold Reversal. We provide a discussion of the contemporaneous vegetation and food choice of the giant ground sloth that produced



the dropping. The absence of tree pollen and the predominance of pollen of low-growing plants in the *Mylodon faeces* (*Azorella*, Cyperaceae, *Empetrum*, *Fragaria*, Poaceae) show that our animal was a grazer, but based on the high representation of *Fragaria* and *Azorella* pollen it was a selective feeder as well.

### Declaration of Competing Interest

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

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