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Archaeobotanical study of Patagonian Holocene coprolites, indicators of diet, cultural practices and space use

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ABSTRACT

The analysis of coprolite inclusions is instrumental in exploring certain features of paleodiets and paleoenvironments, and in determining the time when the sites were occupied by those who deposited the feces. Plant remains found in palaeofeces can be recognized by means of optical microscopes and through microhistological techniques. The aim of this study is to perform a microhistological analysis of plant remains in probable human coprolites from the Cerro Casa de Piedra, Cueva 7 site, province of Santa Cruz, Argentina, with the purpose of identifying the plants included in the diet of the hunter - gatherers who lived in Patagonia during the Pleistocene - Holocene transition and the Holocene, of recognizing some of their cultural practices and of determining the seasonality of the shelters they used. Our microscope study of the samples revealed epidermal fragments of *Ephedra* sp. stem, leaf and fruit remains of *Empetrum rubrum* and *Gaultheria mucronata*, remains of vascular bundles of *Azorella monantha* leaves and of *Armeria maritima* epidermis. Only representatives of the Poaceae family were found among the monocots. Our microhistological study of coprolite provided evidence to endorse the consumption of fruits, namely of *E. rubrum* and G. mucronata. In addition, by determining the time of the year in which the mentioned species bear fruit, we were able to infer that humans used this cave in summer. Likewise, the identification of *Ephedra* sp., *Armeria maritima* and *Azorella monantha* also allowed us to infer that plants of these species were used as medicine and fuel.

1. Introduction

Archaeobotany is the discipline that aims at performing, recovering and conserving and taxonomic identification of plant remains found in archaeological contexts (Chevalier, 1996). Coprolites are fossil or subfossil feces with elements included in their matrixes. Plant remains such as seeds, fruits, pollen, phytoliths, diatoms, charcoal, small plant fragments, and animal remains, such as, parasites, bones and hairs are among the usual inclusions in coprolites of omnivorous animals. They result from elements consumed by the animals, or from elements attached to the feces surface subsequent to their deposition. Analysis of these inclusions reveals some aspects of paleodiets, paleopharmacology, paleoenvironment and the times in which the beings that deposited the feces occupied the sites (Callen and Cameron, 1960; Reinhard et al., 1985; Reinhard et al., 1991; Reinhard and Bryant, 1992; Carrión et al., 2004; Chaves and Reinhard, 2006; Reinhard et al., 2006; Rivera et al., 2014; Velázquez et al., 2010, 2015; Wang et al., 2015).

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Coprolites analysis also allows seasonality studies of various sites. Velázquez et al. (2014) performed palynological analyses in camelid coprolites found in Cerro Casa de Piedra (CCP), province of Santa Cruz, Argentina, by comparing them with the pollen spectrum of modern feces. They were able to identify the season when feces were deposited in the cave and, consequently, the seasonal occupation of the cave by these camelids.

Plant remains found in paleofeces are recognized by means the optical microscope and microhistological techniques. According to tissue features, mainly of epidermis, they can be identified up to species level (Yagueddú and Arriaga, 2010; Martínez Tosto and Yagueddú, 2012).

Through microhistological studies and comparison with reference material, Riley (2012) determined part of the diet of human coprolites from Texas's Hinds Cave. He identified epidermis of *Agave* sp., *Opuntia* sp., *Allium* sp., Poaceae, some *Opuntia* sp. seeds and *Allium* sp. buds. Through the availability of these species as feeding resources and their flowering and fructification periods, Riley determined coprolites seasonality, revealing that they had been deposited between winter - early spring and summer.

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There is not much background information from coprolites microhistological studies in Argentina. The work of Figuerero Torres (1986) stands among the few studies available. It mentions the existence of plant fragments in human coprolites of Cueva Las Buitreras archaeological site, Santa Cruz. Also, Hofreiter et al. (2003), Martínez Carretero et al. (2004, 2013) and García et al. (2008) were able to identify several taxa among the plant remains included in coprolites of extinct fauna, namely of *Hippidion* and *Megatherium* found in the province of San Juan.

Currently, they are carrying out microhistological, palynological and parasitological studies in coprolites of CCP archaeological sites with datings from *c*.10690 to 3480 ^{14}C yr BP (Yagueddú and Arriaga, 2010; Martínez Tosto and Yagueddú, 2012; Martínez Tosto et al. 2012; Burry et al., 2008, 2009; Velázquez et al., 2010, 2014, 2015; Fugassa et al., 2006, 2010; Taglioretti et al., 2015). These coprolites were found in excellent preservation conditions and have been assigned to different animals: Mylodontidae, camelids and probably humans. The exceptional preservation conditions of the plant material registered at CCP Cave 7 and the cave's extensive sequence of human occupation (Aschero, 1996) are ideal both to study the role of plant resources in Patagonian hunter-gatherers' economy and to complement the palaeoenvironmental studies that have already been done in the region (Mancini, 2007).

Regarding the use of plants by Patagonian indigenous groups, Ciampagna and Capparelli (2012) analyzed and recorded documentary information from 42 authors from the 16th to the 21st centuries. They later compared these records with evidences of archaeological macro and micro plant remains, thereby contributing to the archaeobotany interpretation of Argentine continental Patagonia.

The high diversity of taxa and the families identified in the archaeobotanical and documentary records predominantly Poaceae, Asteraceae and Fabaceae show the significant role of plants in the subsistence hunter - gatherer groups. These records indicate the use of plants to provide food, beverage, medicine and fuel. *Empetrum* sp., *Azorella* sp., *Oxalis* sp., *Prosopis* sp., *Bromus unioloides*, *Berberis buxifolia*, *Cyttaria darwini* and *Gaultheria mucronata* were among the plants with nutritional value.

The aim of this work is to perform a microhistological analysis of plant remains present in coprolites likely human origin from CCP Cave 7 archaeological site of the Pleistocene – Holocene transition and Holocene, with the purpose of identified the vegetable items in the diet of hunter - gatherers, of recognizing some of the latter's cultural practices and of determining the seasonality of shelter occupation.

2. Study area

Cerro Casa de Piedra (CCP) is a small elevation of volcanic origin, located in the south of Perito Moreno National Park (PMNP), province of Santa Cruz, Argentina (Fig. 1). It rises 900 m above sea level and lies about 500 m off the southern bank of Roble River, a forest-steppe ecotonal area close to Burmeister Lake.

Archaeological investigations recorded human presence there throughout the Holocene. In general, the earliest occupations were located on the southern bank of Roble river in the PMNP. Cerro Casa de Piedra has been dated as belonging to the early and middle Holocene, while dates of sites studied north of Roble River ranged from mid to late Holocene (Aschero et al., 1992; Goñi, 1988). The distribution of occupation sites has been linked to the presence of a paleolake (Goñi et al., 1994) that spread across much of the area of the park, connecting the basins of Belgrano and Burmeister Lakes (González, 1992).

The PMNP region is characterized by climatic and environmental extreme conditions. Annual rainfalls are 600 mm to the west, and 400 mm to the east of the park, and the average annual temperature is below to 4 °C (Paruelo et al., 1998). Vegetation in PMNP has been classified into two different units: (1) the forest, composed predominantly of *Nothofagus pumilio* and *N. Antarctica*. and *N. betuloides* as associated species, while shrubs and herbs as *Escallonia* sp. *Berberis* sp. *Fuchsia magellanica*, *Osmorrhiza* sp., *Acaena* sp. and *Perezia* sp. are found in the understory; (2) the shrubby - herbs steppe composed of *Chiliotrichium* sp., *Mulinum spinosum*, *Nardophyllum obtusifolium*. *Festuca pallescens*. *Stipa* sp., *Poa ligularis*, *Carex* sp., *Cerastium arvense*. *Adesmia lotoides*. *Nassauvia darwinii*. *Acaena pinnatifida*. *Rytidosperma picta*. *Colobanthus lycopodioides*. *Armeria maritima*. *Polygala darwiniana*. *Perezia recurvata*. *Mulinum microphyllum*. *Senecio filaginoides*. *Berberis heterophylla*. *Empetrum rubrum*. *Plantago barbata* and *Acaena magellanica* (Movia et al., 1987; Mermoz, 1998; Ferreyra et al., 2006).

There are a series of caves and rockshelters in the north of CCP, where caves 5 (CCP5) and 7 (CCP7) are considered the most successful in terms of archaeological evidence. They are the sites with the greatest definition of strata and occupation levels, as well as the ones with the largest number of rock art (Aschero et al., 2005).

The CCP7 site presents a stratigraphic sequence and human occupation dated between ca. 10,690 ± 72 and 3480 ± 70 ^{14}C yr BP (Civalero and Aschero, 2003; Aschero et al., 2007) (Fig. 2). A new dated radiocarbon, 1927 ± 41 ^{14}C yr BP, obtained from charcoal at the top of this site, would extend the occupation period of the cave (Civalero et al., 2007). As regards the vegetation around the site, from the base of the hill to Roble River, there is a strip with predominance of *Empetrum rubrum* and *Gaultheria mucronata*, both species with edible fruits. There is then a grassy steppe, followed by a dominance of *E. rubrum* "murtillar", with *Azorella monantha* and mature trees and seedlings of *Nothofagus pumilio* and shrubland predominance of *Nardophyllum obtusifolium* with *Mulinum spinosum*, *Senecio* sp. and *Adesmia boroniodes*. Moreover, on the riverbank there is a shrubby- grassy steppe dominated by *Nardophyllum obtusifolium* and *Acaena* sp.

The study of a sequence of pollen sediments in the CCP7 archaeological excavation (Mancini, 2007) led to a paleoenvironmental reconstruction. Prior to ca. 9000 ^{14}C yr BP the pollen spectrum showed high values of Poaceae (60–80%) indicating that the vegetation was represented by a grassy steppe, associated with wetter conditions than nowadays. After ca. 9000 ^{14}C yr BP, an increase of *Nothofagus* sp., shrubby taxa (*Empetrum* sp.) and cushion plants (*Azorella* sp.) was recorded, indicating similar environmental conditions to those of the current forest-steppe ecotone.

3. Materials and methods

Six coprolites found in CCP7 (M22, M30, M33, M34, M36 and M39) were studied. Because of their morphological characteristics, such as size, shape, color (Weir and Bonavia, 1985), and their inner contents: seeds, remains of chitin, hairs, bones, charcoal (Jouy Avantin et al., 2003) they were assigned to probable human origin. Although the new biotechnological techniques allow more accurate data, a diagnosis based on morphometric analyses allows the primary identification of the origin of taxonomic groups to support the best choice of subsequent analyses (Chame, 2003).

Moreover, in order to perform an analysis of all of human coprolites found at site CCP7, the results of two other human coprolites, namely M21 and M37, are here presented (Martínez Tosto and Yagueddú, 2012, Martínez Tosto et al., 2012). The coprolites derive from different archaeological levels of the Pleistocene-Holocene transition and the early and middle Holocene (Aschero, 1996; Civalero and Aschero, 2003; Aschero et al., 2007) (Table 1), and were stored under dehydration conditions. A 1-g sample was taken from each coprolite. They were hydrated with 0.5% trisodic phosphate at 4 °C for five days (Callen and Cameron, 1960). They were then filtered through a 500 μm mesh to separate coarse from fine fractions. The coarse fractions were dried at room temperature and observed under a stereomicroscope for determination of the gross macroscopic remains. Animal hairs were identified according to Chehébar and Martín (1989) and Reigadas (2007). These coarse fractions were rehydrated for three days in trisodium phosphate at 4 °C, and bleached with 50% sodium

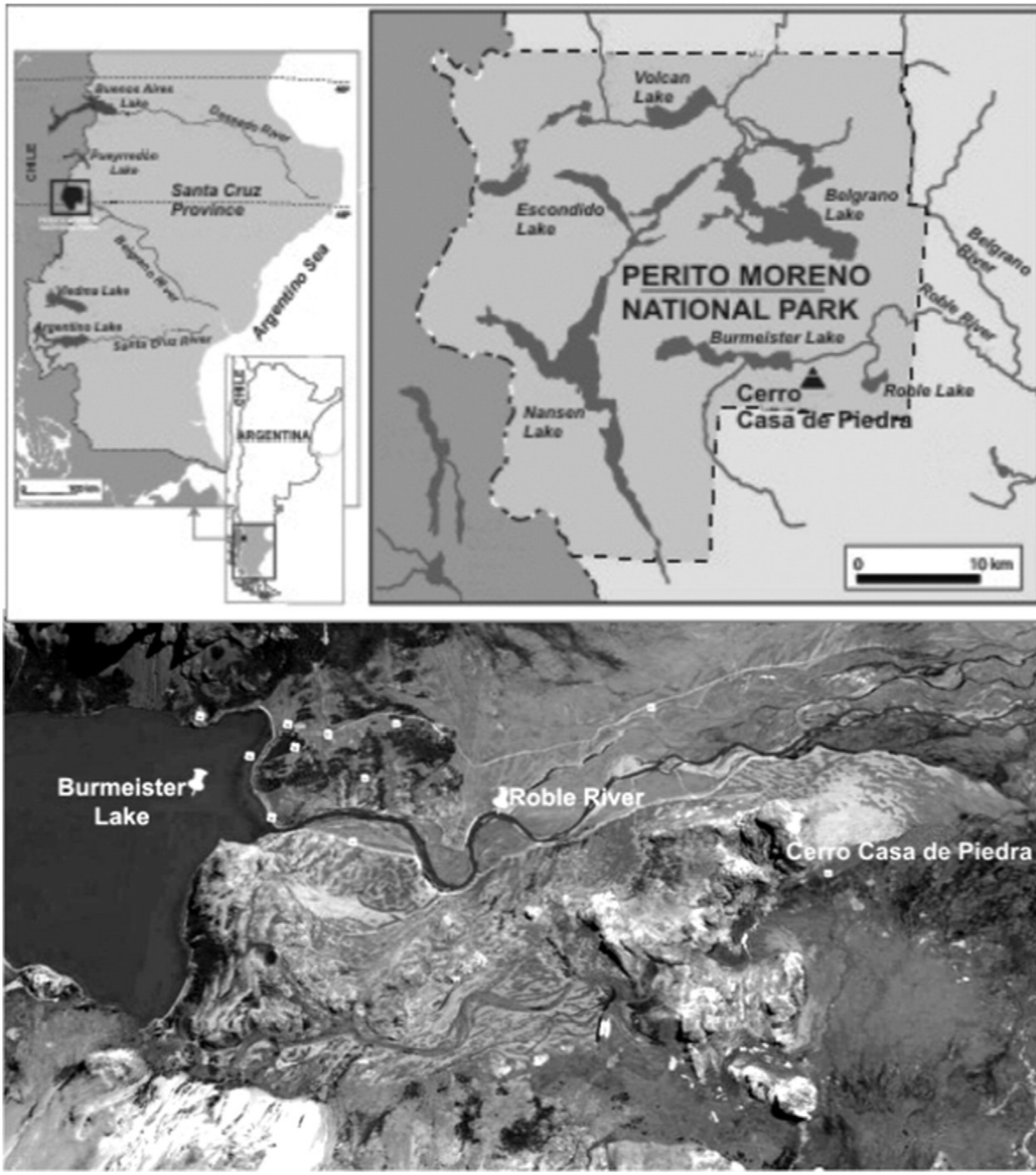

Fig. 1. Geographical location and satellite image of the study area.

hypochlorite for 2 min. They were washed with distilled water on a 200 μm mesh. The whole material of each sample was mounted on slides with glycerin – jelly for observation under the microscope.

Observations were made at 100× magnification. Plant remains from 100 microscopic fields randomly selected on each slide were identified and the relative percentage of each taxon was calculated. Besides, in the case of species with nutritional value (according Ciampagna and Capparelli (2012)), organic (leaves and fruits) percentages were calculated.

Determination of plant fragments was done by comparison with histological preparations of the species collected at the site, deposited at the Laboratory of Botany (Universidad Nacional de Mar del Plata). The results are plotted on a percentage diagram.

4. Results

Observations of the samples under the stereomicroscope allowed us to determine macro remains of different origins: charcoal, Coleoptera remains, bones, animal skin tissue, hairs of rodent, guanaco and huemul. Also observed were fruit fragments, whole leaves and seeds of *Empetrum rubrum*, vascular tissue of *Azorella monantha*, stem fragments, unidentified seeds and sclerenchyma cells (Table 2).

The microscopic study of the samples revealed fragments belonging to Gnetophytes (small group of vascular seed plants within the Gymnosperms, including only three genera: *Ephedra*, *Gnetum* and *Welwitschia*) and Angiosperms. Fragments of stem epidermis of *Ephedra* sp. (Ephedraceae) were found, whereas species of dicots and monocots

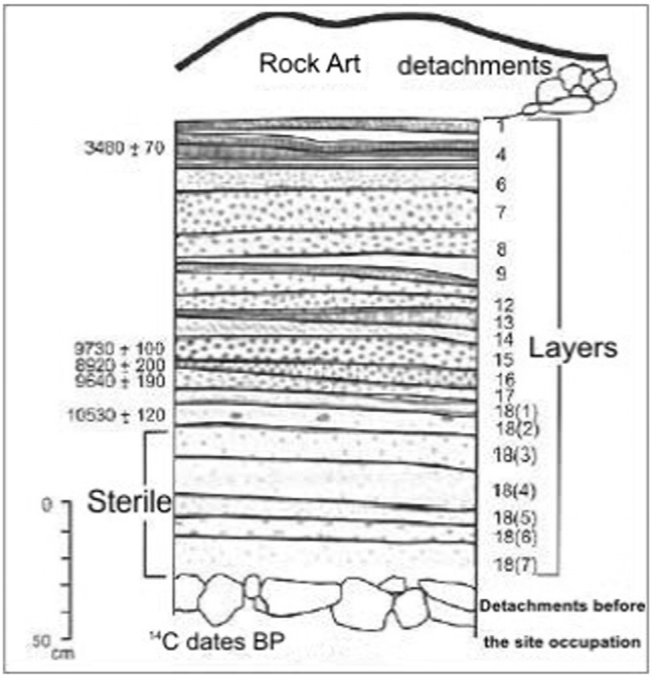

Fig. 2. Stratigraphy site and radiocarbon dating of Cerro Casa de Piedra, Cave 7. (Modified from Civalero and Aschero, 2003).

were identified among the Angiosperms. Leaves and fruits (epicarp) remains of *Empetrum rubrum* (Empetraceae) and *Gaultheria mucronata* (Ericaceae) were found among Dicotyledons. Leaf vascular bundles of *Azorella monantha* (Apiaceae) and epidermis of *Armeria marítima* (Plumbaginaceae) were also found. Only representatives of the Poaceae family were found among the Monocotyledons: leaf remains of *Stipa tenuis*. *S. chubutensis*, *Poa ligularis*. *Poa* sp. and *Festuca* sp. (Fig. 3).

Shown in the diagram of plant fragments percentage (Fig. 4) are the taxa rates found in each coprolite. In most samples, the dominant taxa were *Empetrum rubrum* and *Azorella monantha*. Between the coprolites associated with the oldest archaeological levels (M36, M39, M33) and the ones associated with intermediate levels (M22, M34, M37) there were differences both in the amount of the taxa determined, and in the percentages of the species found. The oldest samples (M36, M39 and M33) showed percentages of *A. monantha* ranging from 2 to 53%, lower than the intermediates (M22, M34 and M37) which ranged between 20 and 90%. Percentage of *E. rubrum* was 98% in M33, and a minimum of 10% in M22. With regard to Poaceae, higher percentages were found in samples of intermediate levels, and more species could be identified. The M30 sample (9950 cal BP) consists exclusively of large sclereids, which have not yet been determined.

Regarding plant fragments of species with nutritional value, leaves and fruits remains of *Empetrum rubrum* were found in all samples, in

Table 2
Macro remains observed under the stereomicroscope.

Sample	Macro-remains
M21	Charcoal, elytra of Coleoptera, bones, fruits, plant tissues.
M22	Charcoal, Coleoptera remains, bones, skin remains, hairs of huemul, abundant hairs of rodents, seeds of *Empetrum rubrum*, leaf vascular bundles of *Azorella monantha*.
M34	Charcoal, bones, rodent hairs, stems, leaves of *Empetrum rubrum*, fruit stalk, plant tissues.
M37	Charcoal, elytra of Coleoptera, bones, leaves of *Empetrum rubrum*, anther, stems, seed, vascular tissues.
M30	Charcoal, sclereids.
M33	Charcoal, stems, leaves of *Empetrum rubrum*.
M39	Charcoal, bones, hairs of guanaco, carbonized stems, leaves of *Empetrum rubrum*, leaf vascular bundles of *Azorella monantha*.
M36	Charcoal, elytra of Coleoptera, stems, seed, leaves of *Empetrum rubrum*, vascular tissues.

most cases with higher percentages of leaves. The highest percentages of leaf and fruit were found in M21 and M33, ranging between 23% and 57%. Likewise, *Gaultheria mucronata* fruit remains were identified in four samples, with 14.6% as the highest value found in M21 (Table 3).

5. Discussion

Our microhistological studies of the 8 coprolites of probably human origin from transition Pleistocene-Holocene, Early and Middle Holocene found in CCP7, provided evidence of the use of plants by hunter-gatherers in Patagonia, thus adding to the archaeobotanical information in the Ciampagna and Capparelli (2012) study, particularly concerning diet, seasonal occupation of the site and the use of plants for medicinal purposes and as fuel.

5.1. Diet and seasonality

The analysis of plant remains found in coprolites revealed the consumption of *Gaultheria mucronata* and *Empetrum rubrum* fruits. The presence of these fruits was recorded in these coprolites since the early Holocene. According to Rapoport et al. (2003), hunter – gatherers of Patagonia consumed fresh fruits of these species, and Martínez Crovetto (1982) assure that *G. mucronata* fruits were also used to prepare "chichas" (alcoholic drinks). *E. rubrum* fructifies in spring - early summer and *G. mucronata* fructifies in late summer to winter (Rapoport et al., 2003). Nowadays, vegetation just in front of CCP7 is dominated by *E. rubrum*, which forms dense myrtle fields ("murtillar") together with *G. mucronata*. The fructification period of these species led us to infer that man used the cave from spring to late summer during the Pleistocene-Holocene transition and the Early and Middle Holocene.

The fact that the leaves might also have been consumed together with the fruits may account for the high values of *E. rubrum* leaves found in all coprolites (Table 3). The fruits have a short peduncle and are very close to the leaves, which are small in size. In all likelihood,

Table 1
Coprolite samples analyzed and datings of the different levels in which they were found. ^{14}C ages were calibrated against the SHCal13 data set using the program Calib, version 7.0.2.

		Radiocarbon ages	Calibrated ages		
Lab. code	Level	^{14}C yr BP	Calibration median (yr BP) (2σ)	Material dated	Sample
UGA 7378	5	6150 ± 105	6989	Stem	M 21 (Martínez Tosto and Yagueddú, 2012)
UGA 7380	11	7880 ± 150	8689	Wood	M 22
UGA 7383	16	8920 ± 200	9950	Wood	M30
					M 34
					M 37 (Martínez Tosto et al., 2012)
UGA 7384	17	9640 ± 190	10,920	Wood	M 33
Beta 59,925	15	9730 ± 100	11,025	Stem	M39
UGA 873	19	10,690 ± 72	12,609	Osseous with cut marks	M36

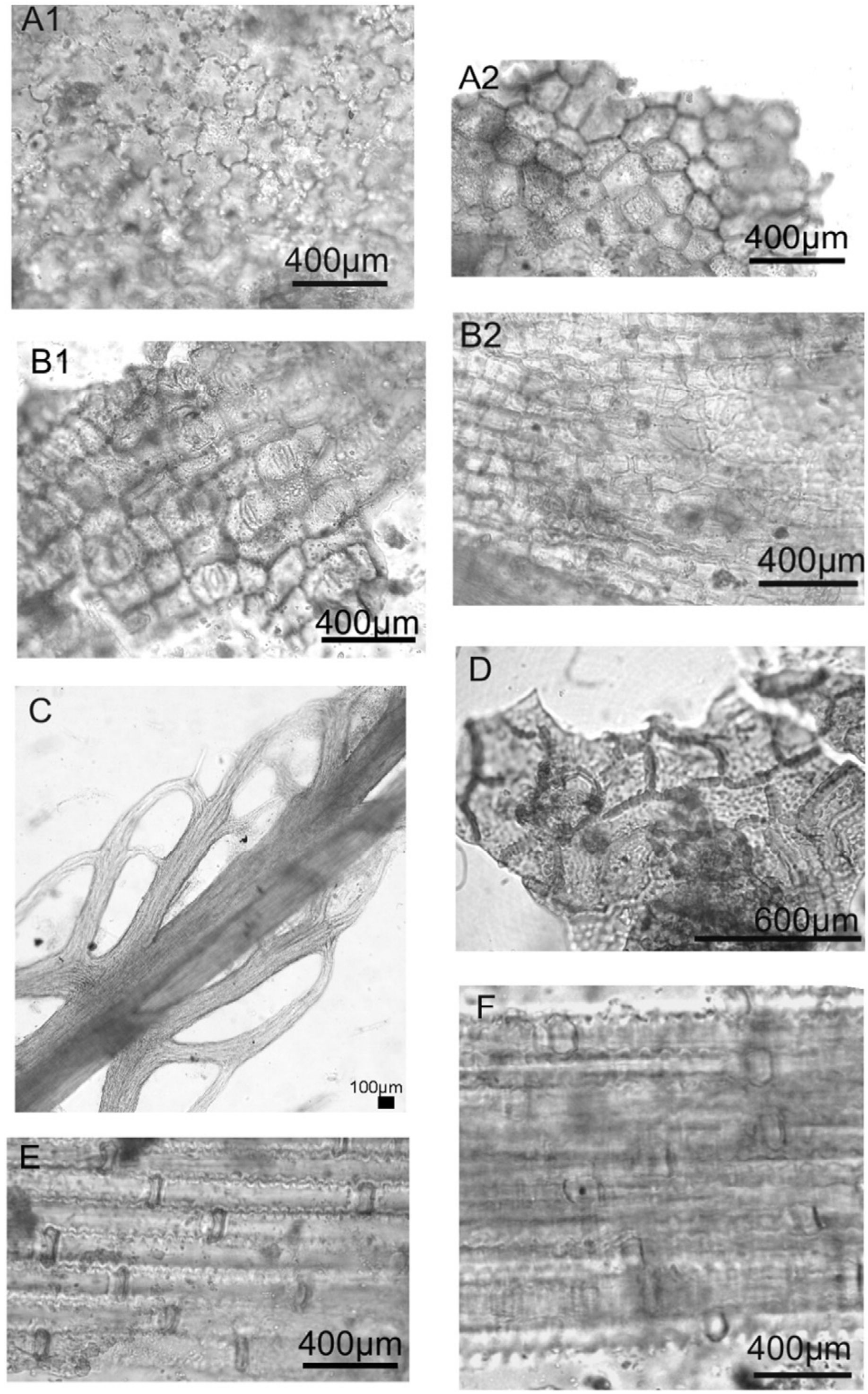

Fig. 3. Plant remains identified in the coprolites under optical microscope. A1 and A2. *Empetrum rubrum*, B1 and B2. *Gaultheria mucronata*. 1. leaf epidermis, 2. fruit epidermis; C. Remains of leaves vascular bundles of *Azorella monantha*; *D. armeria maritima* petal epidermis; E. *Stipa tenuis* leaf epidermis; F. *Poa ligularis* leaf epidermis.

hunter - gatherers took fruits off the plant along with the leaves and consumed them all together.

Poaceae epidermis has been observed to be present even in the oldest coprolites (12,609 cal BP) which could indicate the consumption of stomachs or intestines of herbivorous animals. These plants could have come from the intestinal contents of *Lama guanicoe* “guanaco”, hunter – gatherers' primary food source (Civalero and Franco, 2003), or from the intestinal contents of rodents. In line with the latter hypothesis, M22 (8689 cal BP) displays plenty of hairs of these small mammals (Table 2) as well as and skin remains, probably from the same rodents, which would indicate that they were consumed. To support this hypothesis, the chronicles of Pigafetta (2001) testified that during Magellan's voyage, the natives of San Julian, Santa Cruz, were seen to have caught rats on the ships, and to have eaten them unskinned. In addition, Reinhard et al. (2003) identified rodent hairs in human coprolites found in a cave near Pecos River, west to Texas. They mention that these mammals were consumed uncooked and mixed with other foodstuff.

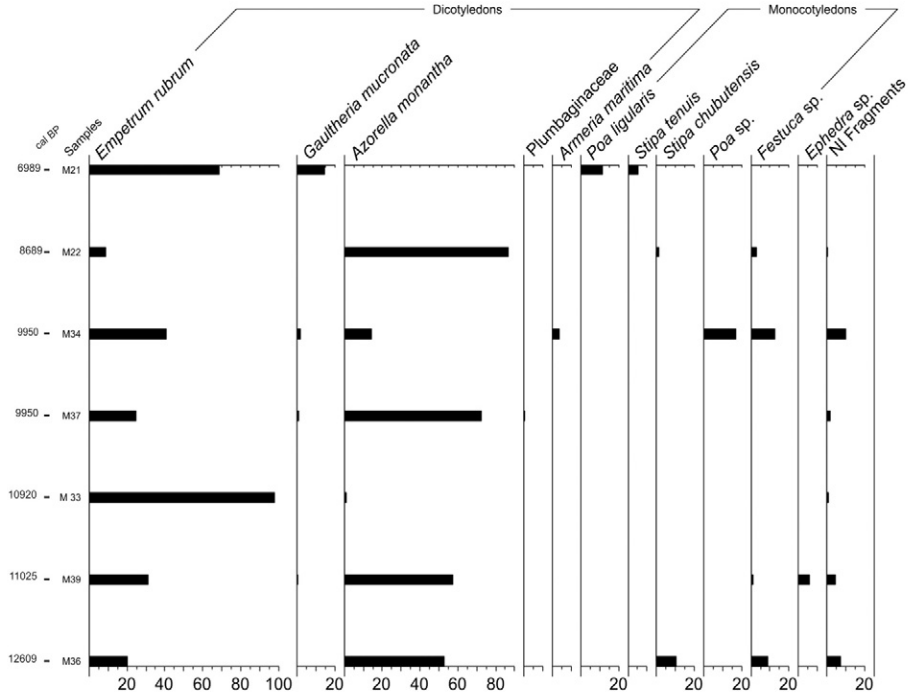

Fig. 4. Percentages of plant fragments found in coprolites. NI: Non identified. Sample M30 was not plotted because it only presents sclereids which have not yet been identified.

Furthermore, ethnohistorical sources indicate the consumption of native species of Poaceae. Ciampagna and Capparelli (2012) mention that the seeds of *Bromus unioloides* were consumed roasted. Also, varieties of *Bromus* genus were described to have been grown for consumption purposes in the central valleys of Chile by 1550 CE (Pérez et al., 2013). Also, the presence of charred cariopses of its genera was identified in hunter-gatherer contexts dated between 3340 and 3460 ^{14}C yr BP in Andean areas belonging to the Chile central region (Planella et al., 2011). Furthermore, ethnobotanical studies in Argentinian Patagonia indicate that edible festucoid species may have been valuable dietary resources during the slash and burn horticultural practice, which is postulated as intensive since 1100 CE (Pérez et al., 2015). However, there is to this date no data on consumption data of Poaceae leaves.

Concerning the use of *Azorella monantha* plants by hunter-gatherers, in this work t we found vascular tissue remains in leaves of this species in most coprolites analyzed. However, there are not ethnohistorical records on the use of leaves of this plant as foodstuff. Vignati (1941) notes only the consumption of ground roots of this species.

5.2. Medicinal uses

Plant fragments with medicinal properties, such as *Ephedra* sp., *Armeria maritima*, and *A. monantha* were found in coprolites. Occupiers of site CCP7 may have likely consumed these plants for medicinal purposes.

Ephedra sp. stems remains found in the coprolite dated to 11,025 cal BP could indicate the use of this taxon to cure a disease. Several species of this genus contain ephedrine, a phyto-composition characterized by its antitussive, cardiac stimulating, bronchodilating and vasopressing properties (Duke, 1985). Furthermore, it has also been cited for the Patagonian region as diuretic and as having properties against urinary tract disorders (Rapoport et al., 2003). Information by Harrington (1968), reports that boiled *Ephedra ochreata* root was used to treat diarrhoea. Moreover, Reinhard et al. (1991), suggest intentional consumption of *Ephedra* probably to treat diarrhoea, as inferred by pollen found in human coprolites in Bighorn Cave U.S.A.

Table 3
Percentages of nutritional value of taxa.

Species	M 21	M 22	M 34	M 37	M 33	M 39	M 36
Empetrum rubrum leaf	45,8	1,5	18,6	14	56,7	24,6	14,6
Empetrum rubrum fruit	23,1	7	22,1	11	41,3	6,3	5,7
Gaultheria mucronata fruit	14,6	–	1,7	0,9	–	0,5	–

A. maritime species, identified in the epidermal remains in one of the coprolites dated 8920 BP, could also have been used as a medicinal herb. According to ethnobotanical sources, this species is used to treat kidney diseases (Kutschker et al., 2002).

The high abundance *A. monantha* found in coprolites of the Pleistocene-Holocene transition and of the early Holocene, could also indicate its use as medicine, given that plants of this species exude a resin with stimulant, stomachic and vulnerary properties (Rapoport et al., 2003).

5.3. Combustion structures (hearths)

In addition to the use of *A. monantha* root for medicinal purposes, it was also used as firewood due to the large content of resin present in stems and roots (Boman, 1908; Ancibor and Perez de Micou, 2002). The high percentage of leaves fragments of this species present in samples M22, M37, M39 and M36 may indicate its use as fuel for hearths. Hunter - gatherers would use this species to light their fires. They would roast their preys directly upon the flames, whereby some leaves might have adhered to the meat and also been ingested.

This study provided archaeobotanical evidence from site CCP7. Its findings revealed a) the consumption of different plant organs as foodstuff; b) the seasonal occupation of the site; c) the utilization of plant material as fuel; and d) the use of plants for medicinal purposes, by hunter-gatherers from the Patagonian region throughout the Holocene.

On the other hand, the taxa found in this study are consistent with those reported by Mancini (2007) from sediments of the same archaeological site on his study of the paleoenvironmental reconstruction, for which the microhistological studies conducted in this work provide a higher level of taxonomic resolution than other proxies, mainly within the Poaceae family. In addition, they provide further evidence of plant resources available in the Pleistocene-Holocene transition, and in the Early and Middle Holocene.

6. Conclusions

The microhistological study of coprolites, likely of human origin, found in CCP7, provided evidence of the consumption of *Empetrum rubrum* and *Gaultheria mucronata* fruits. Also, the time of the year in which these species fructify, led us to infer the seasonal occupation of the cave, namely, in summer.

This work provided new evidence on the relevance of the use of plants during the Pleistocene - Holocene transition and the Holocene. Particularly, it determined that hunter - gatherers of that period consumed fruits, and Gramineae possibly by consuming animal intestines. It also determined that they ate plants with medicinal properties to cure diseases and used other plants as fuel for lighting fires.

The microhistological analysis of plant remains found in coprolites of different zoological origin also constitutes a valuable tool for paleoenvironmental reconstruction. It is an important technique because it provides a much higher level of taxonomic resolution than other proxies, and provides data on the availability of certain plant resources in the study area.

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