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Paleoparasitological and Archaeobotanical Studies of Fecal Remains from the Argentine Puna (Pueblo Viejo de Tucute archaeological site, province of Jujuy, 11th to 15th centuries)

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ABSTRACT

Paleoparasitological and archaeobotanical studies provide useful information to reconstruct past scenarios where humans and animals inhabited. However, multi-proxy studies including these research lines are scarce. Here we applied paleoparasitological and archaeobotanical techniques to study a carnivore coprolite and a dung ball from Pueblo Viejo de Tucute archaeological site, in the Argentine Puna. Among parasites, *Physaloptera* sp., acantocephalan, and ascaridid-like eggs were identified, which shed light on potential parasitic infections and their possible implications for human health. Regarding the botanical evidence, morphotypes related to wild – *Prosopis* sp. and *Geoffroea decorticans* – and cultivated plants -*Solanum tuberosum* and *Zea mays*- were identified. In addition, pollen grains corresponding to the Amaranthaceae-Chenopodiaceae complex, the families Asteraceae, Myrtaceae and Poaceae, and the genera *Taraxacum*, *Atriplex* and *Nototriche* were found. Also, six diatom genera: *Denticula*, *Pinnularia*, *Encyonema*, *Cocconeis*, *Surirella* and *Rhoicosphenia* were identified. These botanical results suggest that the animals were living with humans or wandering around the settlements, which reinforce the hypothesis of possible zoonotic infections. This is the first ancient dung ball analyzed for this kind of study, proving to be a valuable source of information. Altogether, the results provide novel data to reconstruct paleoecological conditions of the region, which complement the available archaeological information.

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coprolite; dung ball; parasitism; ancient health; paleoecology; carnivore; microremains

Introduction

In recent years, the study of coprolites, i.e. desiccated or mineralised feces, has contributed to the knowledge of different aspects of ancient populations worldwide (Fuks and Dunseth 2021). Using multiple lines of evidence, paleoparasitological studies of human and animal coprolites have provided insight into the presence of parasites in the past, which have contributed to paleoecological knowledge of the environment, settlement, diet, hygiene, and health in the antiquity (Ferreira, Reinhard, and Araújo 2014; Araújo et al. 2013). In addition, archaeobotanical studies combine different proxies, such as the recovery of seeds, pollen, plant tissues and phytoliths, which have allowed a better understanding the dietary habits of ancient populations, gaining information on wild and domestic resources, and suggesting environmental aspects of the sites studied (Ball et al. 2016; Martínez Tosto, Burry, and Civalero 2012; Martínez and Yagueddú 2012; Velázquez et al. 2019; 2010). The multidisciplinary study of coprolites recovered from archaeological sites can provide valuable insights to reconstruct past scenarios, especially concerning ecological relationships, herding practices, and handling and selection of natural resources. Despite the great potential displayed by these studies, few paleoparasitological works have been conducted in fecal remains from archaeological sites from the Puna of Argentina (Petrigh et al. 2019; 2021; Tietze, Urquiza, and Beltrame 2021), and no multidisciplinary studies on ancient feces have been applied in the region so far.

The Puna is a large ecological region, distributed along the Andean mountain range in northwestern Argentina, western Bolivia, northern Chile, and

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southern Peru. Despite its harsh climatic conditions, the Puna has been long inhabited by human populations. The Argentine Puna is characterised by a high-altitude desert biome located at more than 3,500 metres above sea level (masl), with an arid and cold climate, intense solar radiation by altitude, high thermal day/night amplitude, marked seasonality with scarce summer rains, and low atmospheric pressure (Basso et al. 2010; Bruniard 1999). Its vegetation is scarce and mainly xerophytic with high-altitude wetlands on the banks of small permanent rivers, where different environments are found, such as meadows, wetlands, salt flats, and lagoons, which are mainly composed of *Deyeuxia* sp. and *Mulembergia* sp. grasslands. Particularly in the meadows, rhizomatous pygmy species predominate, mainly junciform or graminiform monocotyledons (Cabrera 1957; Fernández and Panarello 2001; Killian Galván 2015). Between 3,500 and 3,900 masl, the shrubby steppe (tolar) is found, made up mostly of species from the Puna, such as *Parastrephia lepidophylla* (Asteraceae) and *Fabiana densa* (Solanaceae), an endemic species from Argentina (Killian Galván 2015). In streams close to 4,000 masl, groves of queñoa (*Polylepis australis*) are present, and between 4,100 and 4,700 masl, the herbaceous steppe (pajonal) includes grass of the genus *Festuca* sp. and, to a lesser extent, *Poa* sp. and *Stipa* sp. (Cabrera 1976; Killian Galván 2015). Concerning the current fauna, different species inhabit semi-arid and dry areas of the Puna (Morello et al. 2012). Thus, carnivores such as the Andean and gray foxes (*Lycalopex culpaeus* and *L. griseus*, respectively), the lesser grison (*Galictis cuja*), the Andean and pajonal cats (*Oreailurus jacobita* and *Oncifelis colocolo*, respectively), and the puma (*Puma concolor*) have been described for the region (Perovic 1998). Besides, large herbivores such as South American camelids, and different rodents like *Thylamys pusilla*, *Galea musteloides*, *Calomys lepidus*, and *Phyllotis darwini* (Bonaventura et al. 1998). Wetlands and lagoons in the region provide the habitat for large populations of flamingoes (*Phoenicoparrus jamesi* and *P. andinus*), giant and great-crowned coots (*Fulica gigantea* and *F. cornuta*, respectively), in addition to the choique (*Rhea pennata*) and the plover (*Phegornis mitchellii*) (Birdlife International 2011; Collar and Andrew 1988; Morello et al. 2012).

Pueblo Viejo de Tucute (PVT) is a large pre-Hispanic settlement located in the Argentine Puna, at 3,700 masl southwest of Casabindo, in Cochinoca, province of Jujuy, Northwest Argentina (NWA). The site, distributed over two elevations (Loma Baja and Loma Alta) separated by a stream, shows unique architectural characteristics for this region, with Altiplanic roots and a period of occupation from the 11th and 15th centuries (Basso et al. 2010; Albeck 2007; 2010; Albeck and Zaburlín 2007; Albeck et al. 2020). Around 600 circular enclosures have been counted, most corresponding to dwellings, distributed mainly over the two hills (Albeck 2010). Different investigations have shown domestic activity areas inside the residential structures related to the lifestyles and subsistence strategies of the ancient settlers (Basso et al. 2016). The aim of this work is to carry out a multiproxy study (parasites, pollen, silica phytoliths, starch grains, and diatoms) of two fecal remains, by paleoparasitological and archaeobotanical techniques, to shed light on the parasitic and plant diversity of Pueblo Viejo de Tucute in ancient times, and discuss results from a paleoecological perspective.

Materials and Methods

Samples Studied

Two samples (ID01 and ID02) were obtained from the residential area of the PVT archaeological site (Figure 1a). Samples were recovered from the west of the Loma Baja area (Figure 1b), in an open space free of archaeological structures, a surface depression defined as a pathway within the site (Tolaba 2011). The samples were found at a depth of about 25 cm and in different sectors of the excavated 1 × 1 metre unit, where highly eroded ceramic fragments were also found. An associated radiocarbon dating of a charcoal obtained from the excavation revealed an age of 569 ± 38 years BP (1324-1449 calCE) (Albeck et al. 2020).

The sample ID01 was brown and cylindrical, with a length of 12.3 cm by a width of 2.4 cm (Figure 1d). According to this morphology, the sample was attributed to a carnivore, possibly a felid or canid coprolite (Chame 2003). The sample ID02 consisted of a circular ball, brown, with smooth surface and a diameter of 3.5 by 2.5 cm (Figure 1c), identified as a dung ball formed by a dung beetle (Coleoptera: Scarabaeidae), composed by feces of an unknown origin.

Paleoparasitological Studies

Two fragments of each sample were processed and examined independently in two different laboratories (Paleogenetic Laboratory of the IDACOR, CONICET-UNC, Córdoba, Argentina, and Paleoparasitology Laboratory of the IIPROSAM, CONICET, Mar del Plata, Argentina). Subsamples were rehydrated in a 0.5% Tris-sodium phosphate solution (Callen and Cameron 1960) for 72 h at 4°C and then filtered through a double-folded gauze. Next, spontaneous sedimentation (Lutz 1919) was applied at 4°C for 24 h to concentrate the solid remains. Finally, aliquots of 400 µl were taken from the bottom of the sedimentation vessels and observed by light microscopy (Labklass XSZ 107 CCD and Zeiss Primo Star microscopes) at 100x (total magnification). Photographs were taken at 400x and

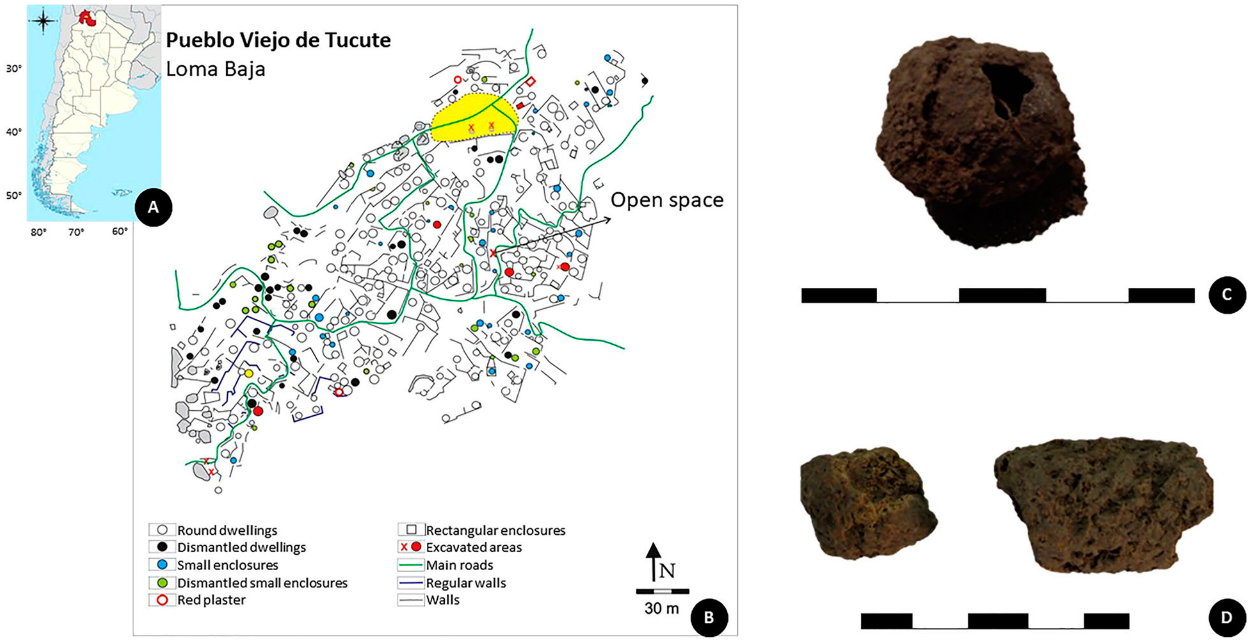

Figure 1. A. Location of Pueblo Viejo de Tucute in the province of Jujuy, Northwest Argentina. B. Schematic representation of the Low hill of the archaeological site, indicating the open space from which the samples were taken. C. Dung ball. D. Carnivore coprolite.

parasite remains were identified based on their morphological and morphometric characteristics.

Phytolith and Starch Archaeobotanical Studies

A subsample of 100 mg of each fecal material was crushed in a porcelain mortar and mounted directly on a slide with two drops of cedar oil to allow observation. To describe and identify the qualitative and quantitative attributes of the siliceous microremains and starchy elements, we complied with the classifications proposed by the International Code for Phytolith Nomenclature (Neumann et al. 2019) and International Code for Starch Nomenclature (ICSN 2011). For the recognition and quantification of the elements, a Nikon Eclipse E200 petrographic microscope with a built-in AmScope x14pp digital camera was used. Each sample was observed at 400x.

Palynological and Diatomological Studies

A subsample from each of the fecal remains was taken, rehydrated with distilled water, and settled for 48 h. The liquid excess was then removed and a portion of the residue was stained with safranin. For the microscopic analysis, semipermanent preparations were made with both stained and unstained residues, and cedar oil was used as a mounting medium. The identification and counting of pollen and diatom grains were carried out under a Nikon Eclipse E200 petrographic microscope (400x and 1000x), and the photomicrographs were taken with an AmScope x14pp camera. For identification of pollen record groups (I, II, III and IV), references proposed by Collao-Alvarado et al. (2015) were followed. This includes the analysis of conglomerates, floristic criteria, composition, and coverage percentages of each taxon, as well as height above sea level.

Results

Paleoparasitological Studies

Helminth eggs were found in both samples. In the carnivore coprolite (ID01), 36 nematode eggs attributed to *Physaloptera* sp. (Spirurida: Physalopteridae) were observed. The eggs were 47.98-56.32 µm long by 28.40-32.50 µm wide (mean = 51.25 × 30.10 µm, n = 17). They were ovoid-shaped, thick-shelled, and presented translucent to light brown colour (Figure 2A). Moreover, four ascaridid-like eggs (Ascaridida: Ascarididae), partially or completely decorticated, round-shaped, thick-shelled, and brown-coloured were found (Figure 2B), measuring 62.50-65 µm long by 57.50-65 µm wide (mean = 63.33 × 61.67 µm). Finally, one acanthocephalan egg (Archiacanthocephala: Oligacanthorhynchidae) was recovered, exhibiting four layers, the outer one lightly sculpted, brown colour and internal content, with no distinguishable embryo, measuring 55 by 42.50 µm (Figure 2C). In the dung ball (ID02), one ascaridid-like egg was observed, with similar characteristics to those found in the carnivore sample, measuring 81 by 73 µm.

Phytolith and Starch Archaeobotanical Studies

Six slide preparations were observed, three from each sample. In sample ID01, 106 silicophytoliths and four

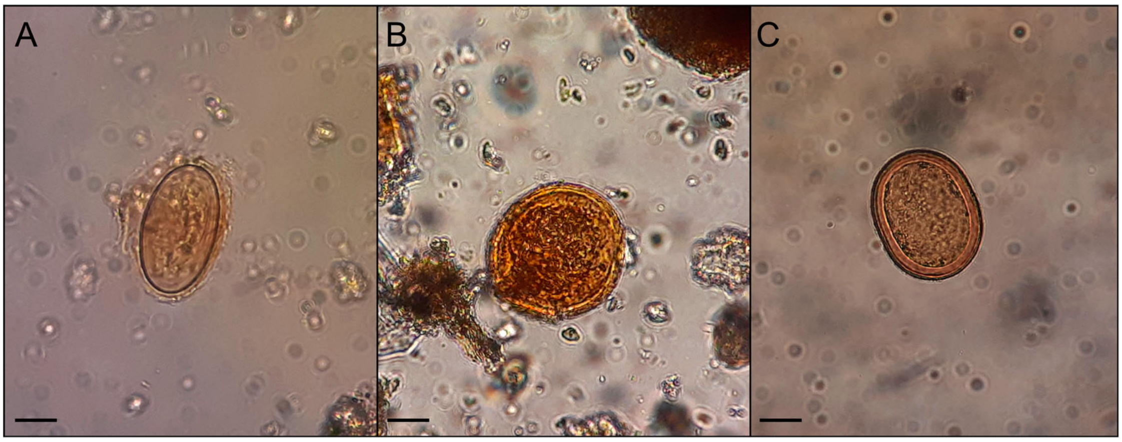

Figure 2. Parasite remains registered at Pueblo Viejo de Tucute archaeological site, Puna of Argentina. A. *Physaloptera* sp. (Spirurida: Physalopteridae) egg. B. Ascaridid-like egg (Ascaridida, Ascarididae). C. Acantocephalan (Archiacanthocephala: Oligacanthorhynchidae) egg. Bar: 20 µm.

starch grains were observed, while in sample ID02, 69 siliceous structures and two starch grains were found. Different siliceous morphotypes related to wild and domestic plants were found. Main morphologies included graminoid affinity (ID01 = 100), predominantly elongate and bilobate (ID01 = 65, ID02 = 38), and trapeziform (ID01 = 16, ID02 = 11), while in lower frequency, saddle (ID01 = 9, ID02 = 7), acute (ID01 = 10, ID02 = 5), and oblong (ID01 = 0, ID02 = 6) morphologies were found.

Regarding botanical affinities, polyhedral elements with flat and colourless texture were observed (ID01 = 3, ID02 = 1), resembling the morphotypes registered in *Geoffroea decorticans* (Fabaceae) (chañar) (Korstanje and Babot 2007; Medina, López, and Berberián 2009). The circular morphotypes (ID01 = 2) were recorded isolated, in pink or yellowish colours. Babot (2005) links this morphology to edible fruits of the genus *Prosopis* (Fabaceae), or algarrobo. In addition, a spiky globular phytolith of blunt apexes, similar to Arecaceae (Benvenuto et al. 2015) was found in sample ID01. A cross-shaped morphotype related to panicoid elements was observed in sample ID02, which is associated with the presence of corn (*Zea mays*) (Piperno 2006) (Figure 3).

Fewer starch grains were recorded in relation to silicophytoliths. Four starchy elements were found in sample ID01, two were circular and two polygonal. The circular grains could not be associated with any known plant group, while the two remaining polygonal grains matched those described for *Zea mays* varieties (Korstanje and Babot 2007). In these cases, the hilum was spherical, with a central and symmetric Maltese cross with four visible arms and sizes ranging between 23 and 27 µm (Korstanje and Babot 2007). In sample ID02, a circular grain and an oval grain were observed. The circular grain was related to *Prosopis* sp. In this case, a high birefringence was observed, and the size coincides with that proposed by the authors for this genus (size = 13 µm) (Giovannetti et al. 2008). The oval grain was possibly associated to *Solanum tuberosum* (potato) (Korstanje and Babot 2007). It was 37 µm long and 23 µm wide, with four visible arms, two long and two short, with evidence of damage in its eccentric hilum (Figure 3).

Palynological Studies

The palynological content in ID01 showed a general good preservation. Its colour varied from yellow to light yellow, almost transparent. Within the pollen remains, six families and three species were represented, corresponding to vascular plants. Shrub (Amaranthaceae), herbaceous (Asteraceae, Malvaceae, Poaceae), and arboreal/shrub (Anacardiaceae, Myrtaceae) taxa were recognised (Figure 4). Within the non-pollen palynomorphs, mostly non-structured organic matter, vascular and epidermal tissues, and some fungal remains were observed.

The most represented family was Asteraceae (22 specimens). Among them, seven specimens of the genus *Taraxacum* could be identified, showing a spheноidal, fenestrated, and trizonocolpate pollen grain. Its walls were made up of thorns of up to 4 µm, and a size of 30–35 µm. Chenopodiaceae (16 specimens) was a well-represented taxa, with 8 specimens corresponding to the genus *Atriplex*, presenting spheroidal pollen grains, pantoporate, with circular and opercular pores, scabbed ornamentation, and a size of 20–25 µm. Malvaceae (16 specimens) was also found; four of which corresponded to the genus *Nototriche*, having spheroidal pollen grains, periporate, equine with spines of about 5 µm, and a size of 40–45 µm.

To a lesser extent, Poaceae (10 specimens) was identified, showing spheroidal to ovoidal pollen grains, monoporate, and with a ring or annulus surrounding the aperture. They were psilate or with very small granules, and their size was between 30-

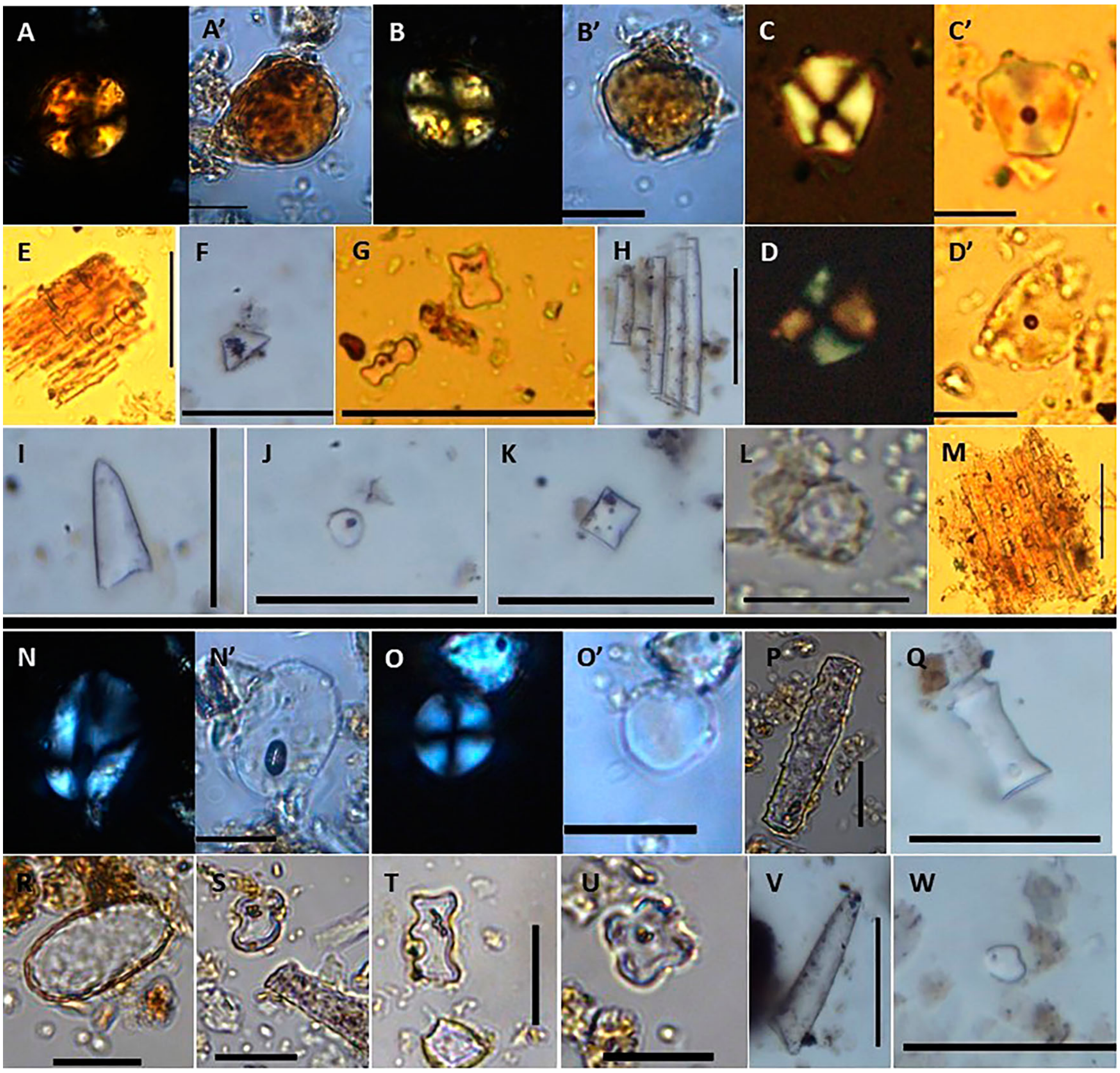

Figure 3. Vegetable microremains found in samples from Pueblo Viejo de Tucute archaeological site, Puna of Argentina. Sample ID01: A-A', B-B'. circular starch grains. C-C', D-D'. polygonal starch grain. E. silica skeleton. F. trapeziform. G. bilobate and polilobate elements. H. silica skeleton. I. acute. J. spheric subcircular scrobiculate. K. geometrical flat. L. spheroid echinate. M. silica skeleton. Sample ID02: N-N'. oval starch grain. O-O'. circular starch grain. P. elongate. Q. trapeziform. R. oblong. S. bilobate. T. polilobate. U. cross-shaped type. V. acute. W. probably saddle. Scale bars. 20 µm.

60 µm. Myrtaceae was also found, with only two representatives and seven indeterminate taxa between pollen and spores.

The palynological content of ID02 showed moderate to poor preservation. The pollen grains were brown to dark brown and some were translucent, making it difficult to observe diagnostic characters for classification. Only 14 taxa corresponding to pollen grains of the Amaranthaceae-Chenopodiaceae complex were identified, with spheroidal and pantoporate shape and circular pores, ranging in size from 19 to 22 µm.

Diatomological Studies

The diatoms present in ID01 were scarce, with relatively good preservation. Ten taxa corresponding to six genera were identified: *Denticula*, *Pinnularia*, *Encyonema*, *Cocconeis*, *Surirella*, and *Rhoicosphenia*. The diatoms in sample ID02 were scarce and poorly preserved, most having only a few fragments. Two taxa belonging to the genus *Denticula* were identified (Figure 5).

Discussion

The paleoparasitological study allowed the discovery of parasitic remains in both samples. A total of four different taxa were observed. *Physaloptera* sp. eggs were found in the coprolite of carnivore origin. This worldwide distributed genus of parasites is commonly found in a broad range of definitive animal hosts and requires mainly arthropods as intermediate hosts (Pereira et al. 2012; Taylor, Wall, and Coop 2007). In canids and felids, the infection can be asymptomatic or cause catarrhal gastritis, including chronic vomiting, weight loss, and stomach ulcers leading to blood loss (Naem, Farshid, and Marand 2006; Taylor,

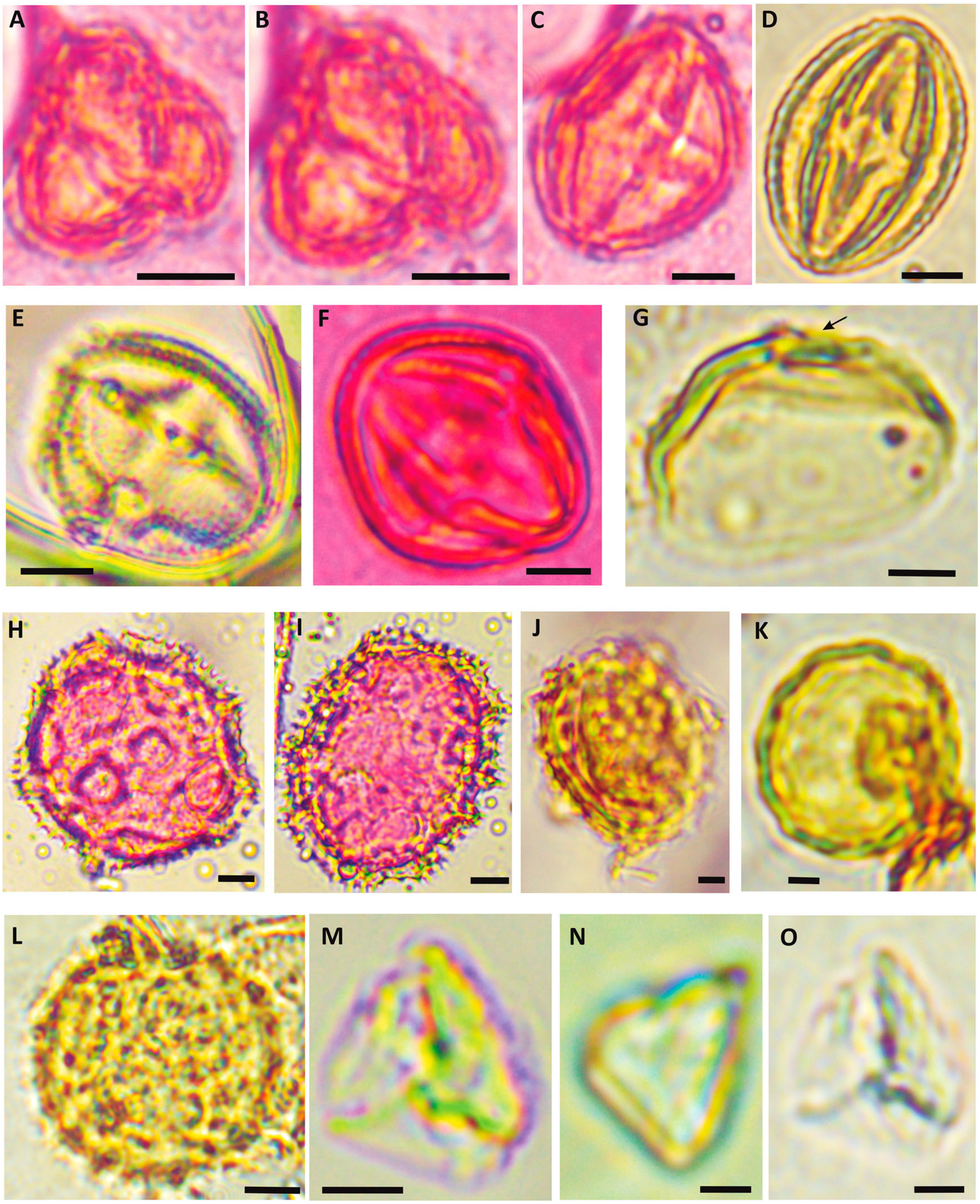

Figure 4. Pollen types identified at Pueblo Viejo de Tucute archaeological site, Puna of Argentina. A-F. Anacardiaceae. A-B Polar view, C-F Equatorial view. G. Poaceae. Arrow indicating the pore. H-I. Asteraceae, *Taraxacum* Sp. J. Asteraceae. K. Chenopodiaceae, *Atriplex* sp. L. Malvaceae, *Nototriche* sp. M. Myrtaceae. N. cf. *Carex*. O. Indeterminate spore. Scale A-F, H-K, M-O bar = 5 µm; G, L bar = 10 µm.

Wall, and Coop 2007). Paleoparasitological studies have reported infection with *Physaloptera* sp. in humans, canids, and felids from archaeological sites in Argentina, Mexico, and Iran (Cleeland et al. 2013; Beltrame et al. 2018; Makki et al. 2017; Fugassa et al. 2006). Nowadays, infection of humans with *Physaloptera* is not common and reports are scarce (Naem, Farshid, and Marand 2006).

Ascaridid-like eggs were found in the two samples studied. Their morphometric characteristics are similar to those of the genera *Toxocara*/*Toxascaris*, which include species having canids and felids as definitive hosts (Okulewicz et al. 2012). Evidence of the presence of this nematode has been reported in ancient samples from both animal and human origin, in a time span from millions of years to the last centuries (Petrigh et al. 2019; Bouchet et al. 2003; Perri et al. 2017; Sianto et al. 2014; 2017; Tietze, Barberena, and Beltrame 2019). These are some of the most common parasites in current canids and felids, and some species are recognised for their zoonotic importance (Acha and Szyfres 2003). The potential occurrence of this

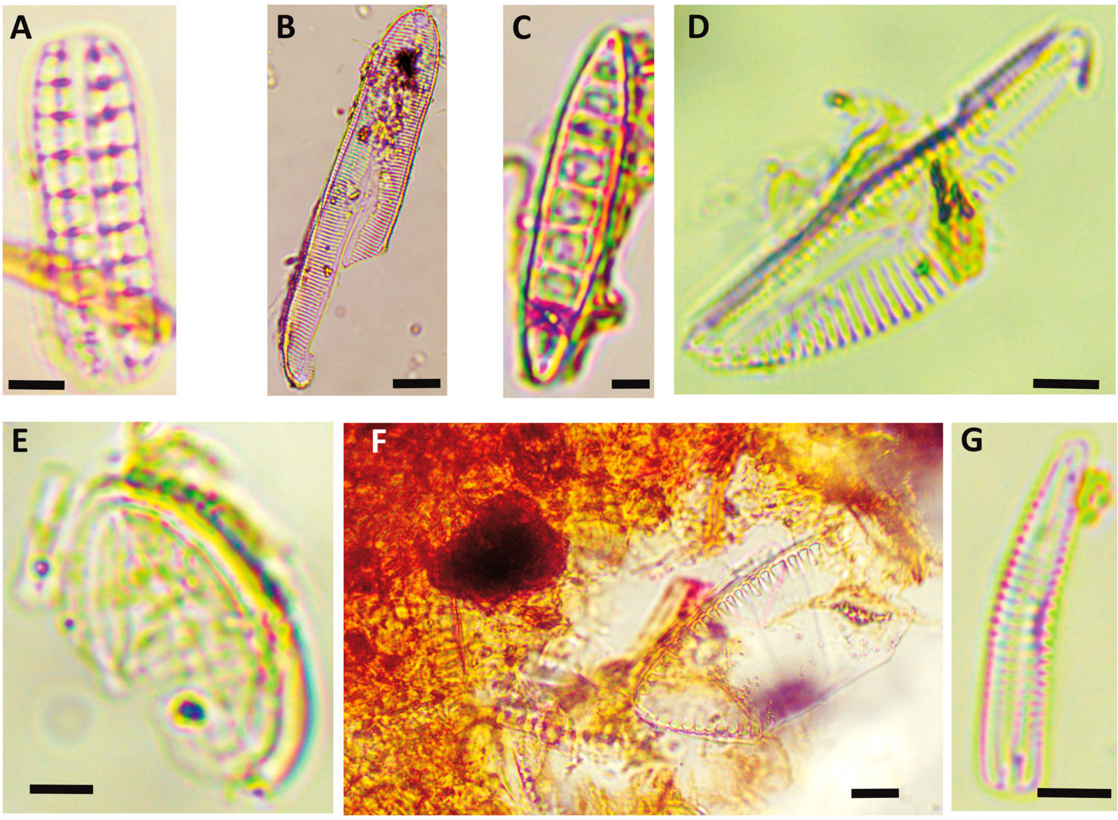

Figure 5. Diatoms. A. *Denticula* sp. B. *Pinnularia* aff. major. C. *Denticula sp.* D. Cf. *Encyonema* sp. E. *Cocconeis* sp. F. *Surirella* sp. frustules, which are surrounded by amber-colored organic matter. G. *Rhoicosphenia* sp. Scale A, C, F, G bar = 5 µ. B bar = 20 µm. D bar = 10 µm.

nematode in PVT, considered a semi-clustered settlement where the presence and processing of animals within residential units were registered (Albeck and Zaburlín 1996), may indicate a zoonotic risk for past populations. While these parasitosis can cause severe damage in the respiratory and gastrointestinal tracts of animals, especially in cubs with high parasite burdens (Eslahi et al. 2020), in humans they are usually found as *larva migrans,* where the larvae travel into the body of a non-definitive host causing minor inflammatory response. Sometimes, the infection can cause severe damage in humans, especially in the brain and eyes (Acha and Szyfres 2003).

The phylum Acanthocephala presents more than one thousand species of obligate parasites, which involve the presence of an arthropod as an intermediate host, and can infect different animals as definitive hosts, including canids and felids (Goater, Goater, and Esch 2014). Thorny-headed worms are usually reported in the paleoparasitological analysis of rodents, canids, felids, and humans (Beltrame, Fernández, and Sardella 2015; Beltrame et al. 2018; Ferreira et al. 1989; Fugassa et al. 2011; Moore, Fry, and Englert 1969; Mowlavi et al. 2015; Noronha et al. 1994; Sianto et al. 2014). Although it is expected to observe the embryo's rostellar hooks in most acanthocephalan eggs, it is not always possible to visualise them (Vieira de Souza et al. 2020). The egg found here resembles that reported by Noronha and collaborators (1994) in a feline coprolite dated 9000 years BP, although the rostellar hooks are not visible. However, since only one egg was found, no further identification was possible.

These findings are the first paleoparasitological results in the province of Jujuy, NWA, and allowed obtaining a first glance at the parasitic diversity of the region in the past. Future analyses of new samples will provide a broader picture of the paleoparasitological scenario in the region.

Phytolith studies allowed the identification of wild and cultivated plants in both samples. Among wild ones, we can include grasses (Poaceae), *Geoffroea decorticans* (chañar), *Prosopis* sp. (algarrobo), and palms (Arecaceae). Among those cultivated, microremains related to corn (*Zea mays*) and possibly potato (*Solanum tuberosum*) were found.

Previous palynological studies conducted in PVT reported the presence of pollen grains belonging to the families Asteraceae, Chenopodiaceae, Malvaceae, Myrtaceae, and Poaceae (Lupo et al. 2011), in agreement with the present findings. The family Anacardiaceae is cited for the first time in the Puna, although this family was recorded in melissopalynologycal studies carried out in the western sector of the Yungas, province of Jujuy (Mendez et al. 2016; Sánchez and Lupo 2011). Studies on pollen associations during the Late Quaternary in the Argentine Puna have linked the Asteraceae family with shrub steppes, Anacardiaceae and Myrtaceae with forests, and Poaceae with high Andean grasslands and herbaceous steppes. The Malvaceae family and the Chenopodiaceae-Amaranthaceae complex are considered indicators of disturbance by human activities, e.g. overgrazing and plant cultivation (Lupo et al. 2018). This complex is predominant in sample ID02, while the Asteraceae family is highly represented in the ID01 coprolite, in agreement with that reported by Lupo et al. (2011) in enclosure 7. Within this family, the genus *Taraxacum* was identified for the first time in the site, which corresponds to a cosmopolitan herbaceous plant. Its fruits contain only one seed, they bloom in spring and are very tolerant to frost and wind. It has yellow flowers and a high dispersal rate. In addition, the genus *Atriplex* was identified, corresponding to the Chenopodiaceae family, which is represented by herbs and shrubs and widely distributed in high altitude areas. They are known as 'salt plants' because they tolerate high levels of salinity. In the III Region of Atacama, Chile, located at 2,000 masl, several species belonging to the genus *Atriplex* have been recorded (*A. atacamensis*, *A. imbricata*, *A. microphylla*, and *A. oreophylla*), which are characteristic of different types of scrub (Orellana et al. 2013). The species *Atriplex imbricata* has also been recorded in northern Chile, in the Pozo Almonte-Salar de Huasco area, where it is predominant in Group I located between 3,050 and 3,400 masl, as described by Collao-Alvarado and collaborators (2015). The genus *Nototriche* (family Malvaceae) corresponds to a perennial herb that grows on rocky substrates and areas exposed to cold winds, shaped as a cushion. It is characteristic (but not endemic) of the Southern Cone, and some of its species can be found from 1,900–4,500 masl (Galán de Mera, Cáceres, and González 2003; Zuloaga, Morrone, and Belgrano 2008). Currently, the species *Nototriche compacta* has been recorded in the northern Chile at 4,150 masl (Group IV) (Collao-Alvarado et al. 2015). According to the results found in this work, the relative coverage percentages show dominance of the Asteraceae family in Group IV (Collao-Alvarado et al. 2015).

The archaeobotanical remains studied in this work displayed the presence of wild and domestic plants in the fecal remains studied. The presence of cultivated plants, such as corn and potatoes, suggests that these animals were possibly associated with human groups or were roaming amongst the crops. In addition, as the identified trees (chañar and algarrobo) bloom between September and November, and respectively bear fruit from December to February and April (M. Medina 2015), feces deposition should have been around summer time.

Pollen grains cannot move on their own, but depend on several factors for their dispersal (water, wind, animals, insects, among others), which are closely correlated with their morphology and productivity rate (Velázquez et al. 2010). Identifying each pollen grain allows us to infer the type of dispersal of the plant, as well as the surrounding paleoenvironment at the time of deposition of feces. In some cases, it is even possible to deduce whether the organism that generated the fecal matter travelled considerable distances (e.g. if pollen remains not consistent with the surrounding vegetation are found). Based on the palynological remains found at PVT site, it was observed that the Asteraceae family, mostly present in sample ID01, is represented by pollen grains with a morphology similar to a type of dispersal by animals (zoophily). They present slightly aerodynamic shapes, with complex and particularly ornamented walls that allow them to adhere to the pollinator. According to Pearsall (2000), the presence of this pollen type could indicate consumption of flowers, seeds, and fruits with attached pollen grains, including consumption of insects that pollinate the plant (e.g. beetles, butterflies or flies). This may be related to the high percentage of organic matter found in the samples, which may indicate consumption of pollinating animals or ingestion of grass to purge their digestive system, as observed in modern animals. The information obtained from the palynological studies carried out in sample ID01 agrees with that gathered from phytolith and starch studies, concluding that the coprolite possibly came from an omnivorous animal. Regarding sample ID02, a predominance of pollen grains belonging to the Chenopodiaceae-Amaranthaceae complex was observed, closely linked to anthropic activities (overgrazing and cultivation). This information is consistent with what was previously stated about the possibility that both animals cohabited with or near human groups.

From the diatomological study, the presence of *Pinnularia* aff. *major* was identified. This species was previously documented in high altitude wetlands located between 2,420 and 4,240 masl, in the Prepuna, Puna, and Altoandina ecoregions of the province of Catamarca, NWA (Maidana and Seeligmann 2006; 2015). In turn, the genus *Pinnularia* sp. has been recorded in saline lagoons of the Puna region in the province of Jujuy, together with the genera *Cocconeis*, *Denticula*, *Surirella*, *Rhoicosphenia*, and *Encyonema* (Seeligmann and Maidana 2019; Maidana, Seeligmann, and Morales 2009; Apumaita, Maidana, and Rodríguez 2019; Seeligmann, Maidana, and Morales 2008). Finally, the genera *Denticula* and *Surirella* have been identified in the Bolivian altiplano salar, located at more than 4,100 masl (Álvarez-Blanco et al. 2011). The findings of the present work agree with those expected in saline environments. The Puna exhibits a latitudinal gradient that determines two subregions: the Dry or Northern Puna and the Salt or Southern Puna. While both have similar vegetation compositions, the Salt Puna is dominated by salt-lakes and saline soils (Cabrera 1976).

The genera and species of diatoms identified in both samples come from saline water bodies similar to those recorded near the archaeological site. Several studies show that this water is suitable for animal consumption, since their tolerance to salt (approximately 10 g/l) far exceeds the degree of mineralisation allowed for human consumption (1.5–1.7 g/l) (Puig 1992; Vilá 2007). In summer, when the feces would have been deposited, there is a greater evaporation that leads to the drying of water sources (Bavera 2006), so the animals that made the depositions may have drunk water located near the place where the coprolites studied were finally found. We hope that future studies on the water bodies surrounding the site will allow us to further expand and refine the information obtained in these analyses.

Sample ID01 was attributed by morphology to a carnivore coprolite, possibly to a canid or a felid species. A large diversity of Neotropical carnivores inhabits in the southern cone of South America (Bárquez, Díaz, and Ojeda 2006), and many species are registered in the Puna, as mentioned above. Evidence of the presence of these species has been found for several years in the archaeological record. The zooarchaeological studies of faunal remains found in PVT revealed that they mostly belong to members of the taxonomic groups *Lama* sp. (Camelidae), Rodentia, and *Lagidium viscacia* (Chinchillidae), and a single pelvic fragment of Canidae was identified (Albeck and Zaburlín 1996). A similar scenario was observed through the examination of faunal assemblages from several sites near PVT dated between the XIII and XVII centuries, revealing the presence of camelids in all of them (Torres Vega, Angiorama, and Mercolli 2021), while canid bones were also recovered from the nearby Cochinoca 1 archaeological site (Mercolli, Mamaní, and Basso 2018; Mercolli and Basso 2020). On other archaeological sites in the NWA region, the presence of canid remains was registered, mainly in association with human burials (Belotti López de Medina 2012), suggesting a ritual importance for these animals. In this sense, skeletonised remains of the Andean fox (*L. culpaeus*) were identified in a human burial (Boman 2007), and different findings of canid remains were registered by Ambrosetti (1908) near Casabindo, both in the Argentine Puna. In addition, the identification of a domestic dog (*Canis lupus familiaris)* in a pre-Hispanic cemetery of the Quebrada de Humahuaca, province of Jujuy (Belotti López de Medina 2012), and the presence of dog remains in burials from the Agua Caliente site (Gallardo 1964), close to PVT, were reported. Based on current and ancient records of carnivores in the study area, the coprolite studied here was likely deposited by either a domestic or a wild canid cohabiting with humans at PVT.

Sample ID02 was identified as a dung ball created by a dung beetle. These belong to a highly diversified group of insects associated with pasture lands, using dung for the feeding and development of their larvae (Nichols et al. 2008). These insects are important organisms involved in many ecosystem processes, such as nutrient recycling, ecosystem productivity, bioturbation, seed dispersal, and control of pest flies and parasites of vertebrates (Bang et al. 2005; Horgan 2005; Maldonado et al. 2019; Noriega et al. 2018). Dung beetles bury the dung as a food source and a nesting substrate (nest balls). Our findings suggest that the feces from which the dung ball was formed were also produced by a carnivore, but do not provide enough information for further identification.

Conclusions

This is the first time that a carnivore coprolite and a dung ball, two types of zooarchaeological remains, were studied using paleoparasitological and archaeobotanical methods. The diverse proxies used in this work: parasites, pollen, silica phytoliths, starch grains, and diatoms, demonstrate that coprolites and dung balls are potential sources of evidence in the past. The results suggest the importance of these types of multidisciplinary studies in the reconstruction of paleoecological scenarios. This is also the first study of its kind at the PVT archaeological site. The proxies studied in this work shed insights into the paleoecological context in which past societies lived and suggest potential interactions in everyday life between humans and domestic or wild canids. The latter is to be expected due to the natural behaviour of foxes, the only wild canids in the region, which are characterised by prowling in peri-domestic spaces. Our paleoparasitological and archaeobotanical studies on fecal remains proved to be an important source of knowledge of the diversity of parasites and potential zoonosis between the 11th-15th centuries, documenting paleoenvironmental conditions, anthropic activities, and plant species present. Note that this study includes the first results of the presence of pollen grains and diatoms in this kind of samples in the Puna region. Finally, we believe it is relevant to continue conducting this type of multiproxy analyses, which provide complementary information to ongoing archaeological analyses.

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No potential conflict of interest was reported by the author(s).

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