



# Reptile and rodent parasites in raptor pellets in an archaeological context: the case of Epullán Chica (northwestern Patagonia, Argentina)



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## ABSTRACT

Paleoparasitology is the study of parasite remains from archaeological and paleontological sites. Raptor pellets can be used as source for paleoparasitological information in archaeological sites. However, this zooarchaeological material has been scarcely studied. Epullán Chica (ECh) is an archaeological site in northwestern Patagonia. This cave yielded remains from more than 2000 years before present. The aim of this paper was to study the parasite remains found in owl pellets from the archaeological site ECh, and to discuss the paleoparasitological findings in an archaeological context. Twenty two raptor pellets were examined for parasites. The pellets were whole processed by rehydration in a 0.5% water solution of trisodium phosphate, followed by homogenization, filtered and processed by spontaneous sedimentation. Eight out of 22 bird pellets examined were positive for parasites from reptiles and rodents. Representatives of 12 parasite taxa were recorded; nine of this parasitic species were reported for the first time from ancient samples from Patagonia. This is the first time that pellets give evidences of ancient reptile parasites from archaeological contexts. It is noteworthy that Late Holocene hunter-gatherers of the upper Limay River basin, could have been exposed to some of these zoonotic parasites. Future paleoparasitological studies on owl pellets may reflect even more the parasitological diversity of all micromammal and reptile species presents in ancient times.

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## 1. Introduction

Paleoparasitology is the study of parasite remains from archaeological and paleontological sites (Ferreira et al., 1979; Ferreira, 2014). It is focused on the knowledge of parasite-induced illness of humans in the past and on the paleoecological knowledge of the environment, ecology, settlement, diet, hygiene, and health in the antiquity (Reinhard, 1992). Parasites are useful tools for understanding host ecology (Poulin, 2007).

Owl regurgitation pellets collected from Patagonia were recently used for paleoparasitological studies (Fugassa et al., 2007; Beltrame

et al., 2011). These authors demonstrated that raptor pellets can be used as source for paleoparasitological information in archaeological sites. However, this zooarchaeological material has been scarcely studied.

Epullán Chica is an archaeological site in northwestern Patagonia. Excavations were conducted during the early 1990's (Crivelli Montero et al., 1996). This cave yielded remains from more than 2000 years before present. During this temporal segment, the cave was used by groups of hunter-gatherers, as well as armadillos and owls. Several archaeological and biological materials, including owl pellets, were incorporated in sediments. The micromammal assemblages formed by these accumulations of pellets were studied and compared over a broad range of recent owl pellet samples (Fernández et al., 2015).

The barn owl, *Tyto alba*, is the strigiform with the most worldwide distribution, that inhabits mostly open areas. The feeding habits of this raptor bird are well known for the southern regions of

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South America. Although small mammals are the main food source, they also prey on other vertebrates, such as reptiles, amphibians, and insects (Bellocq, 2000, and references therein). The great diversity on diet that owls can display could be reflected on parasite diversity, often present in pellets. The aim of this paper was to study the parasite remains found in owl pellets from the archaeological site Epullán Chica, and to discuss the paleoparasitological findings in an archaeological context.

## 2. Materials and methods

The archaeological site named Epullán Chica (ECh) is a cave located at the southern of Neuquén Province, Argentina, 5 km north of the Limay river valley (40°23'10"S, 70°11'44"W, 680 m) (Fig. 1). It opens to the north in an outcrop of volcanic tuffs of the Collón Curá Formation. This cave is closed (100 m east) to Epullán Grande, another archaeological cave with rich small mammals records and dated around  $9970 \pm 100$  years  $^{14}\text{C}$  B.P. (Crivelli Montero et al., 1996; Pardiñas and Teta, 2013). It is 5 m wide at the mouth, 3.5 m long and covers 11 m<sup>2</sup>. Maximum depth of the fill was 1.40 m. The archaeological sequence was dated around  $2220 \pm 50$  years  $^{14}\text{C}$  B.P. (from charcoal sample recovered from the deeper layers) to 20th century (from metal knife collected from the upper layers). Four temporal units were defined: unit I ( $2220 \pm 50$  to  $1980 \pm 80$  yrs  $^{14}\text{C}$  B.P.), unit II ( $1740 \pm 60$  yrs  $^{14}\text{C}$  B.P.), unit III ( $1680 \pm 80$  to  $1510 \pm 80$  yrs  $^{14}\text{C}$  B.P.) and unit IV (20th century). ECh is located in the Monte-Patagonia ecotone of the three major vegetation units that occur in Patagonia: forest, Patagonian steppe, and Monte desert. The annual precipitation is around 300 mm; this arid environment is mainly composed by Monte shrubs, Patagonian shrubs and grasses that grow in low organic sandy soils and pyroclastic rocks, or in isolated wetlands. Archaeological materials were recovered, including lithic artifacts, plant remains, owl pellets, fresh-water mollusc shells (*Diplodon chilensis*), bones of *Cyanoliseus patagonus* (burrowing parakeet), bones and egg shell fragments of

Rheidae birds (*Rhea americana* [greater rhea] and *R. pennata* [lesser rhea]), and bones and teeth from large, medium and small-sized mammals, such as *Lama guanicoe* (guanaco), *ChaetophRACTUS villosus* (large hairy armadillo), *Leopardus geoffroyi* (Geoffroy's cat), *Conepatus chinga* (Molina's hog-nosed skunk), and *Microcavia australis* (southern mountain cavy) (Fernández et al., 2015). In addition, from a taphonomic point of view, all of the archaeological units are characterized by light digestive corrosion on <5% of bone and teeth of micromammals, suggesting that the main accumulator agent of micromammalian and pellet assemblages was a strigiform belonging to the Category I or Light modification (*sensu* Andrews, 1990) probably *T. alba* (Fernández et al., 2015).

Twenty two raptor pellets collected from units I to IV from ECh were examined for parasites. The pellets were described, measured and weighed. The samples were whole processed by rehydration in a 0.5% water solution of trisodium phosphate (TSP) in a glass tube for at least 72 h, followed by homogenization, filtered and processed by spontaneous sedimentation (Lutz, 1919). Samples were preserved in ethanol 70%. At least 10 slides of each sample were made with the aid of a drop of sediment mixed with one drop of glycerin, and examined at 10× and 40× using a light microscopy. The measurements are based on those taken from well-preserved eggs. Eggs dimensions and morphologies were compared with data from the literature in order to identify the parasites at the lowest taxonomic level.

The macroscopic remains were separated and dried at room temperature for diet analysis. Taxonomic identifications of the small mammals recovered from pellets were made exclusively based on cranial and dental remains by comparison with reference materials from the Colección de Mamíferos del Museo de Ciencias Naturales de La Plata (La Plata, Buenos Aires) as well as with specific bibliographic (e.g., Pearson, 1995; Fernández et al., 2011). Since fragmentary remains of *Ctenomys* (tuco tuco) and *Eligmodontia* (gerbil mouse) are very difficult to identify to species, they were characterized only to the generic level.



Fig. 1. Map of the archaeological site Epullán Chica cave, northwestern Patagonia, Argentina.

### 3. Results

Table 1 presents measurements of pellets, microscopic and macroscopic remains, identified preys, and parasitological records for each pellet. Macroscopic observations of examined pellets showed a mixture of inclusions such as hairs, numerous bones (specially cranial and postcranial bones) and teeth of micro-mammals and reptiles, chitin of arthropods (e.g., ants, scorpions). Microscopic observations also revealed hairs, pollen grains, vegetal fibers and fungi remains.

In several samples it was possible to identify the micromammal remains. All of them were identified as belonged to order Rodentia. Four individuals were identified as belonged to order Rodentia. Four individuals were identified as *Reithrodon auritus* (bunny rat) (Cricetidae, Sigmodontinae), two as *Eligmodontia* sp. (Cricetidae, Sigmodontinae), two as *M. australis* (Hystricomorpha, Caviidae), and five as *Ctenomys* sp. (Hystricomorpha, Ctenomyidae). Two individuals were recorded as unidentified Caviidae and Sigmodontidae, respectively. Two samples also contained scales, teeth and bones assigned to Iguania (Squamata), commonly known as small lizards.

Eight out of 22 bird pellets examined contained eggs; representatives of 12 parasite taxa were recorded. Pellets belonged to units I, III and IV. Samples examined from Unit II were negative for parasites. Helminth eggs of nematodes, one cestode, and one acanthocephalan, pentastomid eggs, and coccidian oocysts were found.

Microscopic examination revealed in the pellet number one, eggs with smooth walls with plugs at each poles, compatible with a trichurid (Nematoda:Trichuridae) (Fig. 2). The egg measurements were 55.0–60.0  $\mu$  ( $57.61 \pm 2.06$ ; N = 23; without plugs) and 60.0 to 67.5  $\mu$  ( $63.93 \pm 2.44$ ; N = 7; with plugs) in length and 30.0 to 37.5  $\mu$



Fig. 2. Egg found from Epullán Chica cave compatible with a trichurid (nematode). Bar = 20  $\mu$ m.

( $31.82 \pm 1.76$ ; N = 23) in width. Eggs were not well-preserved. The pellet had prey remains belonged to the rodent *Ctenomys* sp.

Eggs compatible with the capillariid *Echinocoleus* sp. (Nematoda: Capillariidae) (Fig. 3), were found in pellet number 5. In this case, the surface of the wall of this egg exhibited grooves. The egg measurements were 50.0–55.0  $\mu$  ( $52.92 \pm 1.88$ ; N = 6; without plugs) and 60.0 to 65.0  $\mu$  ( $62.5 \pm 1.77$ ; N = 6; with plugs) in length and 27.5

Table 1

Measurements of pellets, microscopic and macroscopic remains, identified preys, and parasitological records for each pellet studied from Epullán Chica cave.

Temporal units	Pellet	Measurements (mm)	Macroscopic and microscopic remains	Identified preys	Parasites found
IV 20th century (metal knife)	1	43.67 × 18.06	Hairs, small mammal bones	<i>Ctenomys</i> sp.	Trichurid
	2	27.01 × 16.55	Mite egg, pollen and vegetal tissues, ants		Negative
	3	46.29 × 18.85	Pollen, vegetal tissues, hairs, small mammal bones	<i>Ctenomys</i> sp.	Negative
	4	29.29 × 14.10	Hairs, ants	Unidentified Mammalia	Negative
	5	31.59 × 12.37	One mite, hairs, small mammal bones	Unidentified Mammalia	<i>Echinocoleus</i> sp., one oxyurid
	6	31.00 × 2.13	Hairs, small mammal bones	Unidentified Mammalia	<i>Heteroxynema</i> sp.
	7	25.25 × 10.78	One mite egg, vegetal hairs and tissues, ants, small vertebrate bones, insect remains	Small Iguania, scorpion	Eimeriid, <i>Paraspidodera uncinata</i> , <i>Parapharingodon riojensis</i> , Pentastomid, unidentified nematode
III 1510 ± 80 <sup>14</sup> C BP 1680 ± 80 <sup>14</sup> C BP	8	14.63	Mite egg, hairs, small mammal bones	<i>Ctenomys</i> sp.	<i>Paraspidodera uncinata</i>
	9	33.22 × 20.36	One mite, hairs, small mammal bones and teeth	<i>Microcavia australis</i>	Negative
	10	25.76 × 15.38	Pollen, hairs, small mammal bones, insect remains	Rodentia	Negative
II 1740 ± 60 <sup>14</sup> C BP	11	16.81	Hairs, small mammal bones	<i>Ctenomys</i> sp.	negative
	12	7.35	Hairs, small mammal bones	<i>Reithrodon auritus</i> and <i>Eligmodontia</i> sp.	Negative
	13	24.68 × 19.91	Vegetal tissues, small mammal bones, insect remains	Scorpions	Negative
	14	41.32 × 28.53	Hairs, small mammal bones and teeth, insect remains	<i>Reithrodon auritus</i>	Negative
	15	46.30 × 30.45	Hairs, small mammal bones	<i>Ctenomys</i> sp. and <i>Eligmodontia</i> sp.	Negative
	16	41.62 × 28.62	Vegetal tissues, insect remains, seeds, vegetals remains, small mammal bones	Small Iguania	Negative
	17	39.66 × 30.72	Hairs, small mammal bones and teeth	<i>Reithrodon auritus</i>	Negative
	18	10.01 × 10.08	hairs, small mammal bones	Unidentified Sigmodontidae	negative
	19	50.07 × 23.73	Hairs, small mammal bones, insect remains	Unidentified Caviidae, insects	Negative
I 1980 ± 80 <sup>14</sup> C BP 2200 ± 60 <sup>14</sup> C BP 2220 ± 50 <sup>14</sup> C BP	20	33.29 × 16.21	Vegetal tissues, hairs, small mammal bones and teeth	<i>Reithrodon auritus</i>	Ascaridid, <i>Trichuris</i> sp.
	21	28.56 × 13.31	Mites, vegetal, hairs and tissues, insect remains	Insects	<b><i>Oligacanthorynchid</i></b>
	22	25.78	Hairs, small mammal bones	<i>Microcavia australis</i>	Anoplocephalid





**Fig. 3.** Eggs found from Epullán Chica cave compatible with the capillariid *Echinocoleus* sp. Bar = 20  $\mu\text{m}$ .



**Fig. 5.** Egg found from Epullán Chica cave identified as an oxyurid, probably *Heteroxynema* (*Cavioxyura*) sp. (Nematoda: Oxyuridae). Bar = 40  $\mu\text{m}$ .

to 30.0  $\mu\text{m}$  ( $28.33 \pm 1.29$ ;  $N = 6$ ) in width. Moreover, one egg with a subterminal operculum at one side, compatible with an oxyurid was found. Their measurements were 30  $\mu\text{m}$  in length and 17.5  $\mu\text{m}$  in width. In this pellet, unidentified micromammal bones were found (Fig. 4).

Eggs of a nematode (Fig. 5) were collected from the pellet 6. These eggs had single thick walls, with a differentiation in the wall near 1 pole, and were identified as an oxyurid (Nematoda: Oxyuridae). Egg measurements ranged from 110.0 to 125.0 ( $117.5 \pm 7.5$ ;  $N = 3$ )  $\mu\text{m}$  long by 52.5–62.5 ( $57.5 \pm 5.0$ ;  $N = 3$ )  $\mu\text{m}$  wide. Their measurements and morphology were similar to that of species of *Heteroxynema* Hall, 1916, subgenus *Cavioxyura* Quentin 1975 (Oxyurida, Heteroxynematidae). Eggs were not well preserved.

Two different nematodes were found in pellet number 7. In one case, eggs were embryonated and thick-walled. Their measurements were 47.5–50.0  $\mu\text{m}$  ( $48.75 \pm 1.32$ ;  $N = 13$ ) in length and 30.0 to 37.5  $\mu\text{m}$  ( $33.25 \pm 2.06$ ;  $N = 13$ ) in width. Based on eggshell morphology and measurements, the identity of eggs was tentatively attributed to *Paraspidodera uncinata* Travassos, 1914 (Heterakoidea, Aspidoderidae) (Fig. 6). The other egg was oblong, thick-shelled, with punctuated shell and with a subterminal operculum. Egg

measurements were 92.5–105.0  $\mu\text{m}$  ( $99.17 \pm 6.29$ ;  $N = 3$ ) in length and 55.0 to 60.0  $\mu\text{m}$  ( $57.50 \pm 2.50$ ;  $N = 3$ ) in width. This eggs were attributed to *Parapharyngodon* Chatterji, 1933, probably *Parapharyngodon riojensis* (Oxyuroidea: Pharyngodonidae) (Fig. 7).

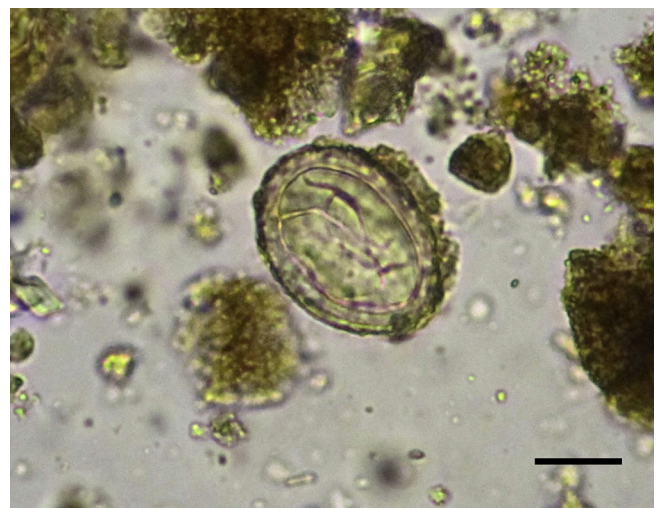
In pellet 7, eggs ( $N = 2$ ) ovoid and embryonated were found, surrounded by a thin external membrane, compatible with a pentastomid (Arthropoda, Crustacea, Maxillipoda) (Fig. 8). Egg measurements were 42.5  $\mu\text{m}$  in length and 32.5  $\mu\text{m}$  in width. An unidentified helminth egg (probably nematode) was observed. Egg measurements were 70.0  $\mu\text{m}$  in length and 42.5  $\mu\text{m}$  in width ( $N = 1$ ) (Fig. 9).

Eimeriid coccidia (Apicomplexa) oocysts were also found. Oocyst measurements were 37.5–50.0  $\mu\text{m}$  ( $43.66 \pm 2.41$ ;  $N = 54$ ) in length and 30.0 to 37.5  $\mu\text{m}$  ( $33.29 \pm 2.65$ ;  $N = 54$ ) in width (Fig. 10). In this sample, bones and scales belonged to a small iguana were present.

Eggs identified as *P. uncinata* were found in sample number 8. Egg measurements were 40.0–50.0  $\mu\text{m}$  ( $46.25 \pm 4.33$ ;  $N = 4$ ) in length and 32.5 to 35.0  $\mu\text{m}$  ( $33.75 \pm 1.44$ ;  $N = 4$ ) in width. Bones belonged to *Ctenomys* sp. were found in this sample.



**Fig. 4.** Egg found from Epullán Chica cave compatible with an oxyurid (Nematoda: Oxyuridae). Bar = 20  $\mu\text{m}$ .



**Fig. 6.** Eggs found from Epullán Chica cave tentatively attributed to *Paraspidodera uncinata* (Heterakoidea, Aspidoderidae). Bar = 20  $\mu\text{m}$ .





**Fig. 7.** Eggs found from Epullán Chica cave attributed to *Parapharyngodon*, probably *Parapharyngodon riojensis* (Oxyuroidea: Pharyngodontidae). Bar = 40  $\mu\text{m}$ .

Eggs of *Trichuris* sp. were also identified in the pellet number 20 (Fig. 11). Eggs were barrel shaped, with thick and smooth walls, and plugs at each pole. Only two eggs were found. Their measurements were 35.0  $\mu\text{m}$  in width by 65  $\mu\text{m}$  in length, and 40  $\mu\text{m}$  in width by 77.5  $\mu\text{m}$  in length. In the same pellet, 11 embryonated eggs, with irregular mammilated surfaces and thick-walled were observed. Egg measurements were 62.5–72.5  $\mu\text{m}$  ( $66.39 \pm 3.33$ ;  $N = 9$ ) in length and 47.5 to 55.0  $\mu\text{m}$  ( $50.83 \pm 2.80$ ;  $N = 9$ ) in width. Based on eggshell morphology, the identity of the eggs was tentatively attributed to an ascaridid, belonged to the family Aspidoderidae (Ascaridida: Heterakoidea). Skeletal remains belonged to *R. auritus* were identified in this pellet (Fig. 12).

Brown colored and thick-shelled eggs were found in pellet 21. Eggs present 4 membranes, the outer lightly sculpted. The embryos present hooks in one extremity (Fig. 13). Egg measurements ( $N = 56$ ) ranged from 87.5 to 107.5 ( $96.6 \pm 3.95$ )  $\mu\text{m}$  long and 50 to 57.5 ( $52.7 \pm 2.04$ )  $\mu\text{m}$  wide. Eggs were very well-preserved and were identified as an acanthocephalan, belonged to Class Archiacanthocephala, Order Oligacanthorhynchida, Family Oligacanthorhynchidae, probably or *Macracanthorhynchus* Travassos, 1917 or an



**Fig. 8.** Eggs found from Epullán Chica cave compatible with a pentastomid (Arthropoda, Crustacea, Maxillipoda). Bar = 20  $\mu\text{m}$ .



**Fig. 9.** Unidentified helminth egg (nematode) found from Epullán Chica cave. Bar = 20  $\mu\text{m}$ .

unidentified species. The presence of microscopic hairs allowed the assumption of the small mammal ingestion.

Finally, the pellet number 22 contained eggs of anoplocephalids (Cestoda: Anoplocephalidae). Eggs were rounded and yellowish (Fig. 14). The embryophore was in one side and exhibits a form of a pyriform apparatus, without horns. The average measurements of eggs were  $75.83 \pm 1.44$   $\mu\text{m}$  by  $73.33 \pm 1.44$   $\mu\text{m}$  ( $N = 3$ ). Eggs were attributed to genus *Monoecocestus* Beddard 1914 or to *Andrya* Railliet 1893. Remains of *M. australis* were found in this pellet.



**Fig. 10.** Eimeriid coccidia (Apicomplexa) oocysts found from Epullán Chica cave. Bar = 20  $\mu\text{m}$ .





Fig. 11. Eggs found from Epullán Chica cave attributed to *Trichuris* sp. (Nematoda: Trichuridae). Bar = 20  $\mu$ m.

#### 4. Discussion

Beltrame et al. (2011) stated that raptor pellets are an important source of archaeological evidences in paleoparasitological studies. In the present survey, 12 parasite taxa were reported in 8 regurgitation pellets, and showed the diversity of parasites that this zooarchaeological material can display. Nine of this parasitic species were reported for the first time from ancient samples from Patagonia (*Parapharyngodon riojensis*, the acanthocephalan, one ascarid, one unidentified oxyurid, *Heteroxynema* sp., *Eimeria* sp., one unidentified nematode, one anoplocephalid, and one pentastomid).

The abundances of the small mammals recovered from the archaeological site ECh were detailed by Fernández et al. (2015). Taxa found include one didelphid marsupial *Thylamys pallidior* (mouse opossum), nine sigmodontine rodents *Akodon iniscatus* (Patagonian grass mouse), *Abrothrix olivacea* (olive grass mouse), *Abrothrix hirta* (long-haired grass mouse), *Chelemys macronyx* (Andean long-clawed mouse), *Reithrodon auritus*, *Phyllotis xanthopygus*, *Loxodontomys micropus* (southern pericote), *Eligmodontia* sp.



Fig. 13. Eggs found from Epullán Chica cave identified as *Macracanthorhynchus* or an unidentified acanthocephala (Archiacanthocephala: Oligacanthorhynchidae). Bar = 40  $\mu$ m.

and *Euneomys chinchilloides* (chinchilla rat), and three caviomorph rodents *M. australis*, *Galea leucoblephara* (common yellow-toothed cavy), and *Ctenomys* spp. In this sense, the small mammal diversity on *T. alba* diet is reflected by the parasites found.

The presence of reptile parasites (*Parapharyngodon* sp. and one pentastomid) and lizard remains (scales, bones and teeth) in the same pellet, is an interesting finding, adding new information for the archaeological context of ECh. This is the first time that

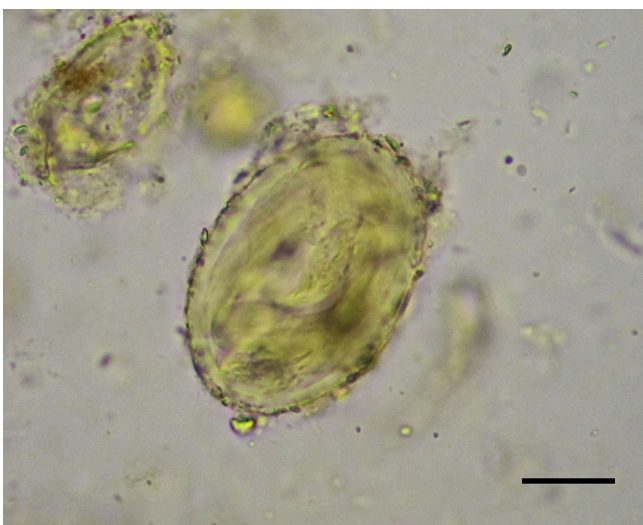


Fig. 12. Eggs found from Epullán Chica cave tentatively attributed to an ascaridid belonged to the family Aspidoderidae (Ascaridida: Heterakoidea). Bar = 20  $\mu$ m.

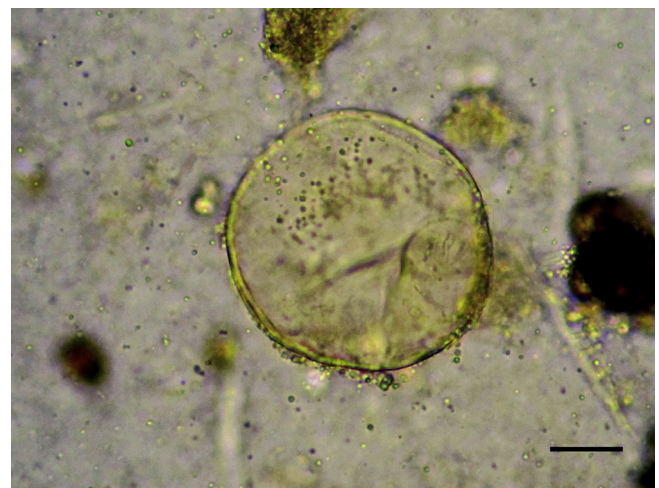


Fig. 14. Eggs found from Epullán Chica cave attributed to genus *Monoecocestus* or to *Andrya* (Cestoda: Anoplocephalidae). Bar = 20  $\mu$ m.

pellets give evidences of ancient reptile parasites from archaeological contexts.

The subclass Pentastomida (Arthropoda, Crustacea, Maxillipoda) comprises 2 orders: Cephalobaenida, with 2 families, and Porocephalida, with 7 families (Riley, 1986). Pentastomids (tongue worms) are long-lived endoparasites of the respiratory system of vertebrates, and are the oldest metazoan parasites known to science. They comprise ~130 living species, and the 90% are parasites of reptiles. They also infect toads, birds (seabirds and vultures), and mammals (canids, felids, reindeer, sugar gliders, and humans) (Almeida and Christoffersen, 1999; Paré, 2008). Pentastomids generally have an indirect life cycle, using at least one intermediate host. Suitable intermediate hosts for pentastomids span diverse taxa (e.g., mammals, reptiles, insects, fish, and amphibians), but for most species the intermediate host is unknown (Riley, 1986; Paré, 2008). Lizards may serve as definitive or as intermediate hosts for pentastomids. In this case, lizard remains, were found in the same sample. This is the first paleoparasitological record of a pentastomid from an archaeological site from South America. The pentastomid fauna of lizards from South America are not very known. In Argentina, Goldberg et al. (2004) found nymphs of *Kiricephalus* sp., from the lizard *Liolaemus lemniscatus* from Neuquén Province.

Pentastomosis is a zoonotic parasitic disease caused by pentastomes. The adult pentastome resides in the lungs or in other tissue of the reptile host. Humans may get infected with pentastomids by ingestion of raw or insufficiently cooked reptile meat contaminated with the larval stage of the parasite, or by ingestion of infectious eggs contaminating the carcasses of infected reptiles, by water or food (Magnino et al., 2009). *Armillifer armillatus* and *Linguatula serrata* are the most common species. In general, humans are tolerant to pentastome infections. Usually is asymptomatic and its clinical features are non-specific, this uncommon disease is usually misdiagnosed. However, a large number of infective parasites invading, or larvae migrating to some key positions (such as the eyes or brain) may cause serious symptoms (Koebsler et al., 2011; Wang et al., 2013; Tappe et al., 2014).

Species of oxyurid nematodes are monoxenic parasites that live in the posterior third of the digestive tract of various vertebrates and arthropods (Anderson, 2000). The family Pharyngodonidae is known to commonly occur in several reptiles and amphibians of America. *Parapharyngodon* spp. are distinguished on the basis of the pattern of caudal papillae, morphology of the anterior cloacal lip, the location of the ovary, and their geographical distribution (Burse and Goldberg, 1999). The egg is also a distinctive characteristic. Nine species have been described from Neotropics. All of them are parasites of lizards (Ramallo et al., 2002). *Parapharyngodon riojensis* was found in the large intestines of the lizard *Phymaturus punae* from La Rioja Province, Argentina (Ramallo et al., 2002). It was also found in the lizards *Liolaemus buergeri* from Mendoza Province and *Phymaturus palluna* from Neuquén Province (Goldberg et al., 2004). Argentina has a diverse and taxonomic complex of lizard fauna. Around 261 species of lizards were listed from Argentina (Avila et al., 2013).

Paleoparasitological studies of 9000-year-old coprolites of lizards (probably *Tropidurus* sp.) from central Brazil were carried out by Araújo et al. (1982). In this case, eggs of *Parapharyngodon sceleratus* were found. Sianto et al. (2012) found eggs of Pharyngodonidae and lizard remains in human coprolites from remote regions of Northeast Brazil, and provided evidence that humans consumed reptiles at least 10,000 years ago.

Reptiles are an important source of protein for human populations in many parts of the world. Several cases of human disease associated to the consumption of food from reptiles were reported. Biological risks associated with the consumption of products from

both farmed and wild reptile meat and eggs include infections caused by bacteria (*Salmonella* spp., *Vibrio* spp.), parasites (*Spirometra*, *Trichinella*, *Gnathostoma*, pentastomids), as well as intoxications by biotoxins. Traditionally, iguanas have been used as food for more than 10,000 years in part of Central and South America (Magnino et al., 2009; Sianto et al., 2012). The consumption of raw or undercooked meat from lizards and other reptiles may have played a role in the transmission of a wide range of zoonotic agents to humans in the past.

Another oxyurid nematode egg was also found in this study, tentatively *Heteroxynema* sp. In the same sample, hairs and bones remains belonged to a small mammal were recovered. The most ancient record (240 million years old) of representatives of the family Heteroxynematidae came from a cynodont reptile coprolite shedding light on the early origin of pinworms in vertebrates (Hugot et al., 2014). In the family Heteroxynematidae, four genera make up an independent line which evolved in Sciuromorpha, Caviomorpha and Myomorpha, namely *Heteroxynema*, a primitive genus divided into three subgenera, *Heteroxynema* from nearctic Sciuridae, *Proxyuronema* from palaeartic Sciuridae and *Cavioxyura* from neotropical Caviomorpha (Petter and Quentin, 2009). Based on the known parasitic specificity of *Heteroxynema* spp. by caviomorphs, the feces were tentatively assigned to a small, or medium-sized rodent, possibly *M. australis*, *G. leucoblephara*, or *Ctenomys* sp., found in this studied site.

Nematodes of the family Capillariidae include numerous species parasitizing a wide range of domestic and wild animals worldwide. The family is composed of 22 genera and 300 species. Many species of this family are zoonotic, mostly with low specificity at host level but high specificity regarding their location in the host (Anderson, 2000; Moravec, 2001). In America, 16 species of Capillariidae are known as parasites of 10 families of mammals, 4 of them are parasites of rodents of the families Cricetidae, Muridae, Caviidae and Sciuridae (Robles, 2008). Capillariidae exhibit both direct and indirect cycles, with a vertebrate being the definitive host. In the case of the heteroxenic cycles, the intermediate host ingests the eggs and the infective larvae develop in it (Anderson, 2000; Morand et al., 2006). The structure and ornamentation of the outer layer of the eggshell of capillariids is very often typical of each species and can be used as a taxonomic key. From archaeological and paleontological sites of Argentina, numerous eggs of capillariids have been found in coprolites from a wide range of hosts (Taglioretti et al., 2014). They were also found in raptor pellets from the archaeological site Cerro Casa de Piedra, Santa Cruz province, Patagonia (Beltrame et al., 2011). The genus *Echinocoleus* is currently distributed in America, Asia and Oceania and comprises 6 species parasitizing the intestine of birds and mammals (Moravec, 2001). The first communication of this genus was from one cricetid rodent from Buenos Aires province (Robles, 2008). The first paleoparasitological record of *Echinocoleus* was in rodent coprolites collected from the archaeological site Alero Destacamento Guardaparque, located in the Perito Moreno National Park, Santa Cruz Province, dated at 3440 ± 70 years B.P. (Sardella et al., 2010).

The Aspidoderidae (Ascaridida: Heterakoidea) currently includes 17 species divided among 4 genera. The members of this family are parasites with direct life cycles, and occur in the caecum and large intestine of mammals, with southern Nearctic and general Neotropical distributions. The known host range for species in the family includes xenarthrans, didelphimorphs, and hystricognath and sigmodontine rodents (Jiménez-Ruiz et al., 2012). *Paraspidodera* spp. parasitize hystricognath rodents, including species of *Cavia*, *Cuniculus*, *Kerodon*, and *Ctenomys*, and exhibit a high specificity for octodontid rodents of the genus *Ctenomys*, in which they are usually present in high densities (Gardner, 1991). *Paraspidodera uncinata* is notorious for their wide geographic and



host spectrum, occurring from Argentina to México. In Argentina, it was found in *Cavia aperea* (Caviidae) (Sutton, 1976), from *Ctenomys talarum* (Ctenomyidae) in Buenos Aires province (Rossin et al., 2010) and from *Euryzgomatomys spinosus* (Rodentia, Echimyidae) in Misiones province (Robles et al., 2012). Paleoparasitological records includes patagonian samples from the archaeological site Cerro Casa de Piedra, located in Perito Moreno National Park, Santa Cruz Province, with an antiquity considered as  $7920 \pm 130$  yr B.P. The rodent host was tentatively identified as *Ctenomys* spp. (Sardella and Fugassa, 2009b). In this study, *P. uncinata* was found in a pellet with a prey identified as *Ctenomys* sp. This species was also found in another pellet, where reptile and arthropod remains were present.

Another ascarid egg was identified as belonged to the family Aspidoderidae. Egg morphology was similar to that of the *P. uncinata*, although measurements were higher. Moreover, in the same sample, sigmodontine rodent remains were present. As mentioned above, *Paraspidodera* spp. parasitizes hystricognath rodents. This species was not cited in the literature at present, and could be a new species not yet reported.

Species of *Trichuris* Roederer, 1761 (Nematoda: Trichuridae) have a cosmopolitan distribution and include intestinal parasites of the caecum and colon of mammals, mainly humans, primates, pigs, ovines, goats, cervids, rodents, lagomorphs, African antelopes, marsupials, felids, and canids. They hatch in the small intestine of the definitive host and larvae migrate to the large intestine, where they penetrate the intestinal mucosa and develop through 4 molts before reaching the adult stage (Anderson, 2000). Species of *Trichuris* are included among the geohelminths, where their eggs are deposited from host feces to the soil where infective larvae develop within the egg. A total of 24 species of *Trichuris* have been described from 10 families of rodents in America (Robles and Navone, 2014). Eight of these species have been reported from Argentina, including *Trichuris dolichotis*, *Trichuris myocastoris*, *Trichuris laevitesticis*, *Trichuris bursacaudata*, *Trichuris pampeana*, *Trichuris pardinasi*, *Trichuris navonae*, *Trichuris thrichomyssi*, and 4 additional species, not identified to specific level (Robles and Navone, 2014). A total of 9 species of *Trichuris* have been recorded from 14 American sigmodontines. In the present study, the sigmodontine rodent *R. auritus* was registered in the pellet where *Trichuris* eggs were found. From Patagonia, it was recently reported for the first time *T. pardinasi* and *Trichuris* sp. (Robles and Navone, 2014). There are previous paleoparasitological findings of *Trichuris* from rodent coprolites and raptor pellets with rodent remains from archaeological sites of Patagonia (Sardella and Fugassa, 2009a; Sardella et al., 2010; Beltrame et al., 2011; Sardella and Fugassa, 2011).

The phylum Acanthocephala is exclusively parasitic. It includes approximately 1150 described species with indirect life cycles always involving arthropods as intermediate hosts and vertebrates as definitive hosts (Kennedy, 2006). The number of acanthocephalan species in small mammals is very low compared with other groups of parasites (Ribas and Casanova, 2006). The presence of microscopic hairs allowed the assumption of the small mammal ingestion. Eggs were identified as or *Macracanthorhynchus* or as *Oligacanthorhynchus*. A search of the literature shows that there is little knowledge on the acanthocephalan fauna of small mammals from South America. The first report of acanthocephalan eggs in ancient material from South America was *Gigantorhynchus echinodiscus* (Archiacanthocephala, Gigantorhynchidae) in anteaters coprolites of southern tamandua, *Tamandua tetradactyla*, from an archaeological site from Brazil (Ferreira et al., 1989). However, there are no records of small mammal acanthocephalans from ancient material at present (Beltrame et al., 2014b). *Moniliformis moniliformis* and *Macracanthorhynchus* spp. are species that rarely causes intestinal disease in humans, but there are clinical reports of

human infection with these parasites around the world (e.g. Schmidt, 1971; Andres et al., 2014). Acanthocephalan human infection was a focus of research early in the history of archaeoparasitology (Moore et al., 1969). More recently, prehistoric acanthocephalan human infection was recorded from Antelope Cave in extreme northwest Arizona (Fugassa et al., 2011).

Cestodes are ubiquitous parasites of intestinal and tistular location of quite all vertebrates, and are currently found in small mammals (Morand et al., 2006). Based on the number of genera present in these hosts, the important radiation of the anoplocephalines took place in rodents and lagomorphs (Beveridge, 1994; Wickström et al., 2005). Intermediate hosts are oribatid mites ingested by their herbivorous definitive hosts (Beveridge, 1994). Anoplocephalids have been reported from mammals from all major zoogeographic regions; however are not commonly found from Central and South America (except for species of *Monoecocestus*). Anoplocephalids (Cyclophyllidea) are very well represented in small mammals with 25 genera described at present (Wickström et al., 2005). Fewer than 30 species of anoplocephaline cestodes (mostly *Monoecocestus* spp.) have been described at present from mammals in the Neotropics, and all of them were found in hystricognath and sigmodontine rodents (Haverkost and Gardner, 2010).

Coccidia (Apicomplexa: Eimeriidae) are ubiquitous protists, obligate intracellular parasites of vertebrates and invertebrates; they represent some of the most prevalent and abundant parasites known. One important feature of most species of coccidia is their high degree of host specificity (Joyner, 1982). The genus *Eimeria* is considered the most specific. In the life-cycle of a coccidium, the oocyst is a resistant stage that leaves the host, usually with the faeces, and is the stage most easily collected and studied. Because features of sporulated oocysts can vary considerably from host to host, oocyst structure has been used historically to distinguish among species. However, in this study it was not possible to diagnose the species and to determine if it belonged to the reptile or to the rodent prey of the owl.

In this study, skeletal remains of the hystricognath rodent *M. australis* were found in the same sample. The only known valid anoplocephalid genera parasites of South American rodents are *Monoecocestus*, *Andrya* and *Viscachataenia* (Global Cestode Database). The present species is similar to some species belonging to genus *Monoecocestus* or *Andrya* from South America, but their measurements are higher. For this reason, this species probably belongs to one of these genera. Anoplocephalid species from rodent coprolites were previously reported from archaeological sites from Patagonia (Sardella and Fugassa, 2009a; Sardella et al., 2010; Beltrame et al., 2012, 2013, 2014a, b). Oribatid mites are intermediate hosts for anoplocephalids, and are commonly ingested by herbivorous where infection occurs. Anoplocephalids are parasites of zoonotic importance for animals and humans. They can cause human disease if humans eat mites present in the soil (Denegri et al., 1998). It is highlighted that some bones remains of *M. australis* from the same unit (cf. Unit I; the earlier occupations of the cave), showed evidences of human consumption (Fernández et al., 2015).

## 5. Conclusions

Nocturnal owls such as *T. alba* produce bone assemblages with low degree of destruction (Andrews, 1990). This was reflected on the excellent preservation of the most parasite remains recorded. Results reinforce the study of barn owl pellets for paleoparasitological purposes. They represent a valuable and excellent source of small mammals and reptile parasites from archaeological sites. In this study, this zooarchaeological material allowed to report 12



parasites species from reptiles and rodents from the archaeological site ECh. Nine of this species were found for the first time from ancient material from Patagonia. It is noteworthy that Late Holocene hunter-gatherers of the upper Limay River basin, could have been exposed to some of these zoonotic parasites. Future paleoparasitological studies on owl pellets may reflect even more the parasitological diversity of all micromammal and reptile species presents in ancient times.

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