Parasites in rodent coprolites from the historical archaeological site Alero Mazquiarán, Chubut Province, Argentina

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The aim of this study was to examine the parasitic remains that were found in rodent coprolites collected from the archaeological site Alero Mazquiarán (Chubut Province, 45º44'15"S, 70°25'9"W), which is assigned to the interface of the Araucanian and Tehuelche cultures, dated at 212 ± 35 years B.P. The faecal material from two unidentified rodent species (X-10 and X-11) was collected from one human pelvic cavity found in a multiple burial. The faecal samples were processed and examined using paleoparasitological procedures. The X-10 coprolites were positive for eggs of Monoecocestus *sp. (Cestoda: Anoplocephalidae) and the X-11 faeces were positive for* Pterygodermatites *sp. (Nematoda: Rictulariidae),* Trichosomoides *sp. (Nematoda: Trichosomoididae) and* Monoecocestus *sp. In this study, we discuss parasitic life cycles, the zoonotic importance of parasites and the behaviour of the aboriginal people.*

Key words: paleoparasitology - helminths - rodents - Patagonia - nematodes - anoplocephaliids

Parasites are useful as biological markers for the interpretation of the ecology of their hosts; they also play important roles in regulating the populations and communities of the hosts that they infect (Williams et al. 1992, Poulin 2007). In addition, parasites can be used as tags to determine the history of their hosts (Manter 1967, Brooks 1979, Morand et al. 2006). Helminth eggs, found in faeces and in sediments or other materials, vary widely in their ability to resist desiccation and destruction. Eggs are one of the most resilient stages in the life cycle of parasitic worms. Therefore, inferences can be made regarding evolutionary time using the recognition of eggs collected from old infected materials, i.e., from coprolites (Pike 1968).

In the archaeological site named Alero Mazquiarán (Chubut Province, Argentina), a multiple human burial was discovered. Parasites attributed to rodents, including several eggs of *Trichuris* sp. (Nematoda), one egg probably of taeniid, and two eggs of Anoplocephalidae (Cestoda) were recovered from sediment in one human pelvic cavity. Along with the sediment, rodent coprolites of two distinct morphological aspects (X-10 and X-11) were discovered. These samples were processed and examined microscopically. The presence of oribatid mites, *Moniezia* sp., *Capillaria* sp., *Trichuris* sp., ascaridid eggs, and differences in the parasite burden of both rodent faeces were available from previously published parasitological data (Fugassa 2006a, b).

The aim of this paper was to broaden the current paleoparasitological information using a new set of observations of the preserved coprolites, X-10 and X-11. The purpose of this study was to review the parasite fauna and to examine the existence of potentially zoonotic parasites and the role of rodents in ancient human populations in Patagonia.

MATERIALS AND METHODS

Alero Mazquiarán is an archaeological site located in the South of Chubut Province, Argentina (45º44'15"S, 70°25'9"W) (Fig. 1), assigned to the interface of the Araucanian and Tehuelche cultures. This site is a rock shelter oriented eastwards antecedent to Rio Guenguel, and protected from the prevailing winds in the region. A multiple burial composed of five individuals was discovered at this site. Samples from the traces of clothes were dated to 212 ± 35 years B.P., corresponding to historical times (Pérez de Micou 2006).

Coprolites were inventoried and processed individually as described in Fugassa (2006a). The examination consisted of the external observation of faeces (colour, texture, inclusions and measures) according to Chame (2003) and Jouy-Avantin (2003). Two types of faeces with similar dimensions were identified and processed, X-10 and X-11 (Fig. 2). Three 1,5 mL microcentrifuge tubes with a mixture of X-10 coprolites (7,1 mm length x 3 mm width) (soft, crumbled, fibrous, light textured, gray-green ochre coloured) and three tubes with a mixture of X-11 coprolites (7 mm length x 2,4 mm width) (hard and heavier textured, compact, gray-brown coloured, dark content) were sampled (Fugassa 2006a, b). Faeces were fully processed by rehydration in an aqueous solution of tris-sodium phosphate 0.5% in a glass tube for one week, followed by incubation at 4°C to prevent overgrowth of fungi and spontaneous sedimentation (Lutz 1919). The material that was sedimented into the tube was recovered with a pipette and 10 slides of each sample were observed, with the addition of one drop of glycerine. Eggs of the parasites were measured and photographed at 40X magnification.

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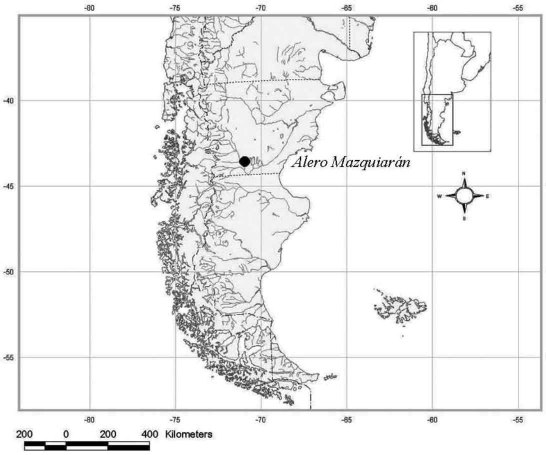

Fig. 1: geographic location of Alero Mazquiarán, Chubut Province, Argentina.

Fig. 2: macroscopic aspect of X-10 and X-11 coprolites examined. Bar = 20 mm.

RESULTS

Paleoparasitological results indicated that X-10 and X-11 coprolites belonged to two different individuals and likely to two distinct species. The parasite contents of both faeces were as follows.

Sample X-10 - A total of 47 Cestoda eggs belonging to the order Cyclophyllidea, family Anoplocephalidae, with characteristics attributable to genus *Monoecocestus* Beddard, 1914, were found (Fig. 3A). The measurements of the eggs were 50-62.5 µm in length (58.87 ± 3.07; n = 30) and 50-62.5 µm in width (58.42 ± 3.02; n = 30).

Sample X-11 **-** Thick walled smoothed eggs (n = 8) embryonated with coiled larvae, 51.72 µm ± 2.4 long (48.75-51.25) by 37.97 µm ± 1.88 wide (35-37.5) were found (Fig. 3B). Through examination of the egg wall characteristics, the eggs were identified as being from nematodes of the genus *Pterygodermatites* Wedl, 1861 (class Adenophorea, order Spirurida, family Rictulariidae). Microscopic remains of unidentified insect cuticles were also discovered in the coprolites. Eggs from an additional nematode (n = 13) with smooth walls and somewhat prominent polar plugs were also recovered. Most of these eggs contained larvae. These eggs measured 68.52 µm ± 3.66 in length (65-75) and 49.66 µm ± 2.31 in width (45-52.5). During the rehydration process, a larva was extruded from one of the eggs (Fig. 3C, D). This parasite was identified as belonging to order Enoplida, superfamily Trichinelloidea, family Trichosomoididae Hall, 1916, *Trichosomoides* Raillet, 1895, likely *Trichosomoides crassicauda* (Bellingham, 1840) Raillet, 1895. Only five eggs of Cestoda Anoplocephalidae were found in sample X-11 and one of these eggs could be measured. This egg measured at 62.5 µm long and 62.5 µm wide and was identified as *Monoecocestus* sp., similar to those of sample X-10.

DISCUSSION

It was not currently possible to identify the specific biological origin of the rodent coprolites. Nevertheless, there is information available with respect to both the current and fossil rodent communities living in relative proximity of the area covered in this study (45°S, 70°W). These known rodents have the ability to produce faeces with aspects and sizes similar to those that we discovered at the Alero Mazquiarán site. Monjeau et al. (1997), Pardiñas et al. (2003) and Teta et al. (2005) recognized the ubiquitous presence of sigmodontine rodents of small to medium size of *Abrothrix olivaceus*, *Abrothrix longipilis*, *Eligmodontia morgani*, *Reithrodon auritus* and others, in Chubut province. Pardiñas et al. (2003) communicated that *Rattus* sp. was absent in samples from Miocene to Holocene, in Patagonia. Two of the species mentioned below in addition to another two unidentified species could be hosts of *Trichosomoides*.

Spirurina include a diverse group of nematodes that use intermediate hosts such as arthropods, insects and crustaceans. Rictulariodea are a homogeneous group comprising approximately 50 species. They are found freely in the lumen, or firmly attached to the intestinal mucosa of their hosts. Representative eggs of the genus *Pterygodermatites* are oval, smooth, thick walled and contain a single larva in their interior. In the digestive tract of the intermediate hosts, including orthopterans, cockroaches, locusts, beetles e. g., depending on temperature, develop potentially infective larvae 3. There are parathenesis in carnivores, with evidence of disease in humans (Anderson 2000). Definitive hosts are carnivores (canids, felids, mustelids), rodents (Anderson 2000), dasipodids and didelphids (Navone & Lombardero 1980) and bats (Quentin 1969).

The genus *Pterygodermatites* Wedl, 1861 includes five sub-genera: *Pterygodermatites*, *Paucipectines*, *Neopaucipectines*, *Mesopectines* and *Multipectines*. These sub-types are differentiated by their anatomy and parasitic specificity (Navone & Lombardero 1980). In Argentina, *P. (Multipectines) cahirensis* was found in several felids

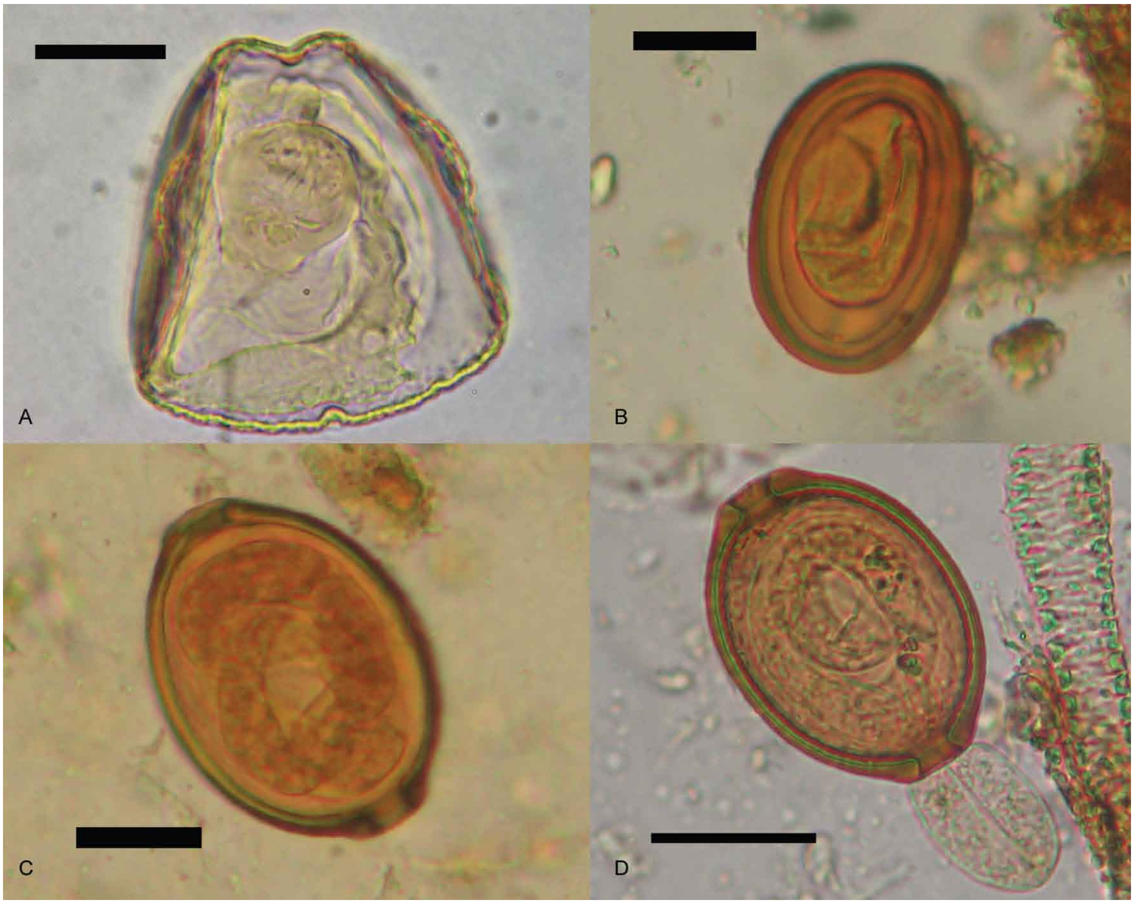

Fig. 3: A: egg of *Monoecocestus* sp. at 100X (onchospheral hooks can be observed); B: embryonated egg of *Pterygodermatites* sp.; C: embryonated egg of *Trichosomoides*, probably *T. crassicauda:* D: embryonated egg of *Trichosomoides*, probably *T. crassicauda*, with the larvae partially outside. Bar = 20 µm.

(Beldoménico et al. 2005). The subgenus *Paucipectines* is represented by the following species that infect xenarthrans, marsupials and cricetid rodents: *Pterygodermatites* (*P.*) *massoiai* (Sutton 1979) in *Graomys griseoflavus griseoflavus* from Mendoza (Sutton 1979); *Pterygodermatites* (*P.*) *chaetophracti* (Navone & Lombardero 1980) in *Chaetophractus villosus* and *Dasypus hybridus* from Santa Fé, Cordoba and Buenos Aires (Navone & Lombardero 1980) and in *Tolypeutes matacus* from Santiago del Estero (Navone 1987); *Pterygodermatites* (*P.*) *azarai* (Sutton 1984) in *Akodon azarae* from Buenos Aires (Sutton 1984); *Pterygodermatites* (*P.*) *kozeki* (Chabaud & Bain 1981) in *Didelphys albiventris* from Santiago del Estero, in *Thylamys pusilla* (= *pusillus*) from Mendoza and *Lestodelphis halli* from Rio Negro (Navone 1989) and in *Thylamys pallidior* and *Trimeresurus venustus* from Salta and Jujuy (Ramallo & Claps 2007). Navone (1989) stated that dasipodids and didelphids (marsupials) established over 60 million years ago in South America and that parasitism by rictulariids must have been established before the arrival of the cricetids during the Pliocene-Pleistocene time period. *Pterygodermatites* (*P.*) *spinicaudatis* Navone and Suriano, 1992 was found in *Microbiotheria* (= *australis*) from Rio Negro (Navone & Suriano 1992); *Pterygodermatites* (*P.*) *zygodontomys* from *Bolomys* (= *Zygodontomys*) *lasiurus*, *Graomys* (= *Phyllotys*) *grisoflavus* and *A. azarae* and *Pterygodermatites* (*P.*) *jagerskjoldi* in *Caluromys phillander*, whose distribution extends from Southern Mexico to Northern Argentina (Lent & Texeira de Freitas 1935).

O'Brien and Etges (1981) registered seasonal fluctuations in the parasitic load of *Pterygodermatites coloradensis* in the rodent *Promyscus leucopus*. These researchers argue that seasonality may be due to the short life of the adult parasite in the gut of the host, and/or to the availability of infected hosts. In addition, they report that the intensive feeding on grasshoppers outside the caves in the spring and summer months expose rodents to higher loads of parasites. The constant conditions inside the caves, with sheltered and warmer environmental conditions, could facilitate the persistence of viable eggs throughout the winter. In this regard, the presence of *Pterygodermatites* sp. in rodent faecal samples from Alero Mazquiarán would indicate that they could have been deposited in the most active months (spring-summer). Eggs found in this study are larger than those reported from other rodent and didelphid species (Table) and may belong to a new species and a possible new host in Argentina, TABLE

Pterygodermatites species records in Argentina and egg measurements in micrometers

	Egg measurements (µ)		
Pterygodermatites sp.	Length range mean	Width range mean	References
P. (*P.*) *massoiai*	32-36	24	Sutton 1979
P. (*P.*) *chaetophracti*	36-40 (38)	24-26 (28)	Navone & Lombardero 1980, Navone 1987
P. (*P.*) *azarai*	32-40	24-28	Sutton 1984, Navone 1989, Ramallo & Claps 2007
P. (*P.*) *kozeki*	21-39 (31)	18-27 (24)	Navone 1989
P. (*P.*) *spinicaudatis*	30-50 (40)	20-40 (30)	Navone & Suriano 1992
P. (*P.*) *jagerskjoldi*	38-46	24-30	Lent & Texeira de Freitas 1935
P. (*P.*) *zygodontomys*	without measurements	without measurements	Lent & Texeira de Freitas 1935
Pterygodermatites sp.	48.75-51.25 (51.72 ± 2,4)	35-37.5 (37.97 ± 1.88)	Present finding

current or extinct. This represents the first paleoparasitological report of this nematode genus worldwide.

The family Trichosomoididae consists of only four genera of nematode parasites: *Anatrichosoma* (in the oral cavity of marsupials), *Huffmanela* (in the gills and other organs of sharks), *Trichuroides* (in the bladder of bats) and *Trichosomoides* (in the urinary tract of rodents). Eggs of trichosomoidids have characteristics resembling those of trichinellids, being completely embryonated when deposited, thick-walled and having dark shells (Moravec 2000, 2001). Thomas (1924) described the cycle of *T. crassicauda*, a parasite of the urinary bladder of *Rattus norvegicus*, exhibiting the peculiar characteristic that the male is parasitic to the female uterus. This parasite produces papillomas, which are highly pathogenic to the host. The author of this study experimentally reported the zoonotic potential of this nematode in dogs. Eggs are eliminated with the urine of rats in groups, with the production of adhesive substances. Parasites found in X-11 coprolites were identified as *Trichosomoides* and likely *T. crassicauda* (Bellingham 1840, Raillet 1895). While it is a parasite of the rodent urinary system, the presence in faeces would likely be due to contamination of faeces with urine. The present study constitutes the first time that this nematode genus is cited from old or new material in Argentina.

Cestodes are common parasites in small mammals (insectivorous, lagomorphs, bats and rodents). They appeared long before the origin of mammals and their hosts accompanied them wherever they were. Whether as intermediate or as a definitive host, rodents provided excellent opportunities for cestodes to diverge in both geographic area and time, playing a major role in the evolutionary ecology of their hosts. The passage of cestodes from one host to another occurs through food webs (Georgiev et al. 2006). All cestode species listed as adults in rodents belong to six families and 47 genera within the order Cyclophyllidea: Catenotaeniidae, Hymenolepididae, Anoplocephalidae, Paruterinidae, Davaineidae and Dilepydidae. Hymenolepididae and Anoplocephalidae (45 genera and 25, respectively) are very well represented in small mammals (Wickström et al. 2005). Intermediate hosts for cestodes in small mammals include the terrestrial arthropods (insects, mites, and also myriapods and arachnids). Anoplocephalids recorded at the current time in rodents are representatives of the following genera: *Andrya*, *Anoplocephaloides* (= *Paranoplocephaloides*), *Bertiella* (= *Indotaenia*), *Ctenotaenia*, *Diandrya*, *Gallegoides*, *Hokkaidocephala*, *Moniezia*, *Monoecocestus*, *Parandrya*, *Paranoplocephala*, *Pseudocittotaenia*, *Sdarikovina*, *Viscachataenia*, *Mathevotaenia* (= *Schizorchodes*), *Sinaiotaenia*, *Witenbergitaenia*, *Inermicapsifer* and *Metacapsifer* (Beveridge 1994, Georgiev et al. 2006). Beveridge (1994) stated that *Moniezia* consists of a diverse collection of cestodes. In addition to the well-documented records in ruminants and suids, their normal hosts, there is only one species in rodents (*Monoecocestus bequarti*). Beveridge (1994) states that the genus structure needs serious revision, as many of the records of cestodes attributed to *Moniezia* were found in rodents, do not correspond to the genus, and are mainly *Monoecocestus*. Tenora (1976) examined the taxonomy of anoplocephalids both globally and in South America and Rego (1961) conducted a review of the genus *Monoecocestus* Beddard, 1914, recording several parasitic species from rodents living in Brazil, Paraguay, Perú and Chile. These species included *Monoecocestus hagmanni* from *Hydrochoerus hydrochoeri*, eggs: 50 x 63 µm (Brazil); *Monoecocestus hydrochoeri* from *H. hydrochoeri*, eggs: 50 x 67 µm (Paraguay and Brazil); *Monoecocestus parcitesticulatus* from *Cavia porcellus*; eggs: 50 µm (Brazil); *Monoecocestus minor* from Brazilian guinea pig, eggs: 50 µm (Brazil) and *Monoecocestus macrobursatum* from *H. hydrochoeri*, eggs: 50 x 58 µm (Brazil). Magalhães Pinto et al. (2002) reported *M. parcitesticulatus* in *C. porcellus* with eggs 40-51 (average: 47) x 25-28 (average: 26) µm.

In Argentina, Sutton (1973) described a new species, *Monoecocestus myopotami*, in the otter *Myocastor coypus*, with eggs 53.3 µm long and 53.6 µm wide. These eggs harboured a pyriform apparatus excluding any terminal filaments. Navone (1988) cited the presence of anoplocephalids in three species of dasypodids, belonging to the genus *Mathevotaenia*: *Mathevotaenia surinamensis* in *Dasypus novemcinctus* of Corrientes, and described two new species: *Mathevotaenia diminuta* in *Chaetophractus vellerosus* of Formosa and *Mathevotaenia matacus* in *T. matacus* from Chaco. Denegri et al. (2003) created a new genus and species to incorporate *Viscachataenia quadrata*, the parasite of *Lagidium viscacia*, a rodent of the family Chinchillidae endemic in Argentina, whose eggs measured 15-19 µm (average: 17) in diameter, and with a pyriform apparatus elongated, and four lobes of different sizes. Recently, Wickström et al. (2005), in a phylogenetic analysis of morphological and molecular genitalia of nine genera and 35 species of anoplocephalids, established that the majority of the radiation of the group was among rodents and lagomorphs. They distinguished a large monophyletic group that includes all species of arvicoline rodents (voles and lemmings), primarily represented by the genera *Anoplocephaloides* and *Paranoplocephala*. Intermediate hosts for these cestodes are Oribatid mites and are ingested by herbivorous where infection occurs. In this regard, Denegri et al. (2002) confirmed the role of *Zygoribatula striassima* and *Oribatella* sp. in the transmission of *Thysanosoma actinioides*. Anoplocephalids can cause human disease if humans eat mites present in the soil (Denegri et al. 1998).

Fugassa (2006a, b) found coprolites eggs attributed to *Moniezia* sp. in sample X-10, although we stated that rodents have not yet been reported as hosts for this anoplocephalid genus. Re-examination of the material carried out in the present study identified these eggs as *Monoecocestus* sp. Freeman (2004) reported that full development of cysticercoids of *Monoecocestus americanus* and *Monoecocestus variabilis* take place between 15-25°C. As related with this report, together with the finding of *Pterygodermatites*, it is possible that faeces examined in this study would have been deposited in the sediment of the pelvic cavity in the archaeological site during the warmer months.

From the pelvic sediment, one egg of *Trichuris*, two eggs of anoplocephalids and many remnants of mites (deutonymph hyponus) were reported by Fugassa (2006a, b). In X-11 coprolites, we discovered an unidentified ascaridid, one egg of *Capillaria* sp., and one egg of *Trichuris*. Some of the parasites cited by Fugassa (2006a, b) were not found in this study (i.e., ascaridid and *Capillaria*) and *vice versa* (*Pterygodermatites*). With respect to the finding of *Trichuris* and *Capillaria* in rodent coprolites (sample X-11) by Fugassa (2006a, b), it probably was erroneously identified, corresponding to *Trichosomoides* sp.

The behaviour of the aboriginals living in Patagonia during the XIX century can partially explain the parasite findings of rodent parasites in the burial examined. Domeyko (Pérez de Micou 2006) cited that it was common in the past to offer food to the dead, who spent days above ground before burial. This type of situation is conducive to rodents coming in search of food and depositing their faeces, thereby contaminating the area. In this case, the faeces may have been deposited in the pelvic cavity, with the parasites contained in the faecal material.

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