First study of fossil rodent middens as source of paleoparasitological evidences (northwestern Patagonia, Argentina)

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ABSTRACT

The present paper reports the first paleoparasitological results obtained from coprolites of fossil rodent middens and demonstrates the potential of rodent middens as a source of paleoparasitological evidences in South America. Ten fossil rodent middens from northwestern Patagonia, Argentina, were studied. Five coprolites of each midden were fully processed, rehydrated, homogenized, subjected to spontaneous sedimentation, and examined through light microscopy. Eight of the 10 examined rodent middens contained parasite eggs. The eggs of parasites were assigned to *Heteroxynema* (*Cavioxyura*) *viscaciae* Sutton & Hugot, 1989 and *Helminthoxys* sp. (Nematoda: Oxyuridae), *Trichuris* sp. (Nematoda: Trichuridae) and one unidentified nematode. Fossil rodent middens were assigned to *Lagidium viscacia* (Caviomorph: Chinchillidae). The excellent preservation of parasite remains in coprolites from fossil rodent middens provided an opportunity to performe paleoparasitological inferences. The results of this papers demonstrates that fossil rodent middens offer an excellent opportunity for the recovery of parasite remains for future paleoparasitological studies in the southwest of South America.

KEYWORDS: fossil rodent middens – coprolites – paleoparasitology - Patagonia

INTRODUCTION

Rodent middens are accumulations of organic debris (plant macrofossils, coprolites, pollen) and sediment, encased in hardened urine preserved in rockshelters, caves and crevices [1]. When water from rodent urine is evaporated, these accumulations hardened, allowing the preservation of the deposited materials which can remain intact for several thousand years [2].

Studies of South American fossil rodent middens have been mainly focused on the analysis of pollen and plant macrofossil records. Such studies provided fundamental paleoecological and paleoclimatological evidence from arid and semi-arid regions of South America for the late Quaternary (last 50ka) [2- 8]. Midden agents in South America includes rodent genera such as *Lagidium* spp. (Chinchillidae), *Phyllotis* spp. (Cricetidae), *Abrocoma* spp. (Abrocomidae), *Microcavia* spp. (Caviidae), *Octomys* sp. and *Octodontomys* sp. (Octodontidae) [2]. Rodent middens have become a valuable archive in arid and semi-arid regions of South America due to their multiproxy evidence which allows to address questions in diverse areas of knowledge such as botany, zoology, ecology, biogeography, archaeology, climatology, molecular biology, among others.

Paleoparasitoloical studies have been traditionally applied to coprolites, mummified remains and latrines which are the most common sources of evidences [9]. During the past few years, several samples of rodent coprolites collected from archaeological and paleontological sites from Patagonia were examined for parasites [10- 16] providing important insights into the biodiversity of parasites in ancient times. In that sense, the presence of rodent parasites in fossil middens would provide useful and valuable information, as rodent paleoparasitological data can be used from several point of views [17]. Despite

their unique conditions for preservation in arid and semi-arid settings from South America, fossil rodent middens have not been used for paleoparasitological purposes.

The present paper reports, therefore, the first paleoparasitological results obtained from coprolites of fossil rodent middens from northwestern Patagonia, Argentina and demonstrates the potential of rodent middens as a source of paleoparasitological evidences in South America.

MATERIAL AND METHODS

Northwestern Patagonia is located on the lee side of the Andes and the adjacent volcanic fields and plateaus between 35°-39°S. The region is characterized by a west-east climatic gradient from humid conditions along the Andes and dry conditions at the easternmost tip. Vegetation distribution follows the west-east climatic and environmental gradient ranging from the forest and the grass steppes at the lee side of the Andes to the shrub steppes developing on volcanic fields and plateaus [18, 19].

The Huenul locality, where rodent middens were collected, is located near the grass steppe- shrub steppe (locally known as Monte) ecotone, at the easternmost tip of the climatic gradient. It is close to Cueva Huenul 1 archaeological site (CH1) (36°56′45"S, 69°47′32"W) which presents excellent preservation conditions of archaeological and paleontological deposits [20, 21].

Ten fossil rodent middens were collected at Huenul site and specifically together to CH1 and within a 200m-far gorge from CH1 (Fig. 1; Table 1). Eight

of the ten middens were ^{14}C dated at LATYR (Laboratorio de Tritio y Radiocarbono, Argentina) and calibrated with the Southern Hemisphere curve (SHCal13; Hogg et al., 2013) from CALIB 7.0.2 program (Table 1). Five coprolites from each rodent midden were examined for parasites. Coprolites were inventoried and processed individually. The examination of the external shape of feces was conducted according to Chame [22] and Jouy-Avantin [23]. Each coprolite was fully processed by rehydration in a 0.5% water solution of tris-sodium phosphate (TSP) in a glass tube for a week, followed by homogenization, processed by spontaneous sedimentation [24] and preserved in 70% ethanol. Ten slides were prepared from each coprolite, along with the addition of one drop of glycerin to each slide, and were examined using light microscopy. Eggs of parasites were measured and photographed at 400X magnification. Broken eggs were discarded.

RESULTS

Coprolites were dark brown and cylindrical, with smooth surfaces (Fig. 2). Average measurements of feces (N=50) were 10.3 ± 1.1 mm long by 3.7 ± 0.7 mm wide.

Eight of the ten rodent middens examined contained parasite eggs (Table 1). Sixteen coprolites contained eggs of nematodes. Eggs of parasite were assigned to *Heteroxynema* (Cavioxyura) *viscaciae* Sutton & Hugot, 1989 and *Helminthoxys* sp. (Nematoda: Oxyuridae), *Trichuris* sp. (Nematoda: Trichuridae) and one unidentified nematode.

Eggs of *H. viscaciae* (Fig. 3), with single thick wall and with a rounded pole and the other sharp, without operculum, were found in 5 coprolites from 4 middens. Average egg measurements (N=5) were 137.5 to 152.5 (143.5 ± 5.47) μm long by 62.5 to 67.5 (65.63 ± 2.39) μm wide.

Asymmetrical and yellowish eggs, with only one side convex and thin and smooth wall, with a dark-yellow embryonic mass, were assigned to *Helminthoxys* sp. (Fig. 4) and were found in 6 coprolites from five middens. Average egg measurements (N=20) were as follows: 100.0 to 112.5 (106.9 ± 4.12) μm long by 42.5 to 55.0 (48.63 ± 3.19) μm wide.

Eggs of *Trichuris* sp. (Fig. 5) were observed in 4 coprolites from 4 different rodent middens. These eggs were lemon shaped, with a smooth surface and polar plugs. Measurements of eggs were different between middens, and were attributed to two different *Trichuris* species. Average egg measurements (N=6) from one of the middens were as follows: 70.0 to 77.5 (74.17 ± 2.58) μm without plugs and 77.5 to 80.0 (79.00 ± 1.37) μm with plugs long by 37.5 to 40.0 (38.5 ± 1.37) μm wide. From 2 middens only one egg in each one were found. Measurements were 55 μm without plugs and 65 μm with plugs long by 42,5 μm wide and 60 μm without plugs long by 32,5 μm wide. Two *Trichuris* eggs were found in another midden, their measurements were 52,5 and 57,5 μm without plugs and 60 and 62,5 μm with plugs by 30 μm wide.

Two oval and embryonated egg belong to an unidentified nematode were found in one coprolite, the measurements were 45 μm by 40 μm (Figure 6).

DISCUSSION

Species of oxyurid nematodes are monoxenic parasites that live in the posterior third of the digestive tract of various vertebrates and arthropods [25]. Oxyuroidea from vertebrates can be grouped into 3 families: Pharyngodonidae, Oxyuridae and Heteroxynematidae [26]. Heteroxynematidae includes nematodes that evolved in sciuromorph, caviomorph and miomorph mammals. *Heteroxynema viscaciae* (Heteroxynematidae) is a parasite found in the caecum and large intestine from *Lagidium viscacia* from Chubut Province, Argentina, first described by Hugot and Sutton [27]. It was also observed by Foster et al. [28] and by Ferreira et al. [29] from the wild viscachas *Lagostomus maximus* from La Pampa and Chaco Provinces (Argentina), respectively. *Heteroxynema viscaciae* was also found in ancient coprolites assigned to *L. viscacia* from Cueva Huenul 1 [14] and from the paleontological site Los Altares [16].

Several studies have demonstrated the high host-specificity among Oxyuridae (e.g. 30, 31]. In particular, these studies showed that the distribution of Syphaciinae belonging to the same family of rodent hosts have a closely parallel phylogeny with them. *Helminthoxys* sp. (Oxyuridae: Syphaciinae) is a parasite of neotropical caviomorph rodents. The genus *Helminthoxys* comprises seven species: *H. caudatus* (syn. *H. pujoli*), *H. effilatus* (syn. *H. velizi*), *H. freitasi, H. tiflophila, H. quentini, H. urichi, H. gigantea* and *H. abrocomae* [32]. The measurements and morphology of eggs found in this study are similar to those of *H. caudatus,* described parasitizing *Microcavia australis* from Los Altares, Chubut Province, Argentina [33].

Trichuris spp. include intestinal parasites of the caecum and colon of mammals, mainly humans, primates, pigs, ovines, goats, cervids, rodent and

canids, with eggs that mature in soil. Eggs of *Trichuris* are characteristics in the shape of a barrel, with thick and smooth walls, and plugs at each pole. They hatch in the small intestine of the definitive hosts and the larvae migrate to the large intestine, where they penetrate the intestinal mucosa and develop through 4 molts before reaching the adult stage [25]. To date, a total of 24 species of *Trichuris* have been described from 10 families of American rodents [34]. *Trichuris* spp. in South American rodents from ancient material were reported [10, 12, 35, 36, 37, 38]. Based on egg measurements, there were differences in the archaeofaunistic composition among the rodent middens, indicative of the existence of different *Trichuris* spp.

Rodents represent one of the most important sources of zoonoses for mammals and their increasing densities forced their dispersion to occur and brought them into closer contact with humans [39]. Species of *Trichuris* are included among the zoonotic geohelminths [40] since infection involves ingestion of contaminated soils. It seems probable that *Trichuris* spp., living in rodents from northwestern Patagonia, could be infectious for humans given that, during the Holocene, both humans and animals occupied caves and shelters.

A previous paleoparasitological study was done in CH1 [12], in this case coprolites were recolected from different levels of the cave, an archaeological site. In this case, micromammal coprolites were studied and were positive for *Viscachataenia quadrata* and *Monoecocestus* sp. (Cestoda: Anoplocephalidae) and for *Heteroxynema* (*Cavioxyura*) *viscaciae* (Nematoda: Oxyuridae). The coprolites examined were tentatively attributed to *Lagidium viscacia*.

Midden fecal-pellet sizes and shapes are tools for distinguishing the midden-forming agents. In this study, based on the rodent species present in the study area [41], the aspect of fossil rodent middens and the coprolites examined, the eggs of the parasites found in this study, and the knowledge of the parasitic fauna of viscachas, the feces were attributed to *Lagidium viscacia* (Caviomorph: Chinchillidae) named "chinchillón or vizcacha serrana". This family comprises the genus *Chinchilla* (chinchillas), *Lagidium* (mountain viscachas), and *Lagostomus* (plains viscachas), mostly limited to the driest regions of western and southern South America, from the highlands of Ecuador through the Andes of Peru and Bolivia to the coastal mountains of Chile and Patagonian steppe of Argentina. Three species of *Lagidium* are recognized, one with an extensive distribution down the spine of the Andes, from southern Peru through Bolivia and northern Chile to central Argentina (*L. viscacia*,), a second from a small area in the mountains of west- central Argentina and adjacent to Chile (*L. wolffsohni*), and a third, recently described from southern Ecuador (*L. ahuacaense*) [42].

Rodent middens in South America are discontinuous in nature but a fundamental multiproxy archive that provide the chance to peer in the past environmental and climatic dynamic of arid and semiarid areas [1,3]. From a paleoparasitological point of view, and based on the results of this paper, fossil rodent middens have proven to be an excellent source of parasites from the past. Thus, the good preservation of parasite remains in coprolites from fossil rodent middens provided an opportunity for paleoparasitological inferences. Fossil rodent middens offer an excellent opportunity for the recovery of parasite remains for future paleoparasitological studies in the southwest of South

America. In order to improve the scale of the inferences, a larger number of middens will be needed to be robust regarding the parasite information. This paper, therefore, state the basis for future research employing rodent middens, an unexplored archive up to now for parasitological and paleoparasitological studies.

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Figure captions:

Figure 1: Photographs showing a. Cueva Huenul 1 (CH1); b. gorge close to CH1; c. and d. rodent middens collected at Huenul site.

Figure 2: Macroscopic aspect of the examined coprolites.

Figure 3: Egg of *Heteroxynema viscaciae* (Nematoda: Oxyuridae) observed from fossil rodent middens. Bar= 20 µm.

Figure 4: Egg of *Helminthoxys* sp*.* (Nematoda: Oxyuridae) observed from fossil rodent middens. Bar= 20 µm.

Figure 5: Eggs of *Trichuris* sp. (Nematoda: Trichuridae) observed from fossil rodent middens. Bar= 20 μm.

Figure 6: Egg of unidentified nematode observed from fossil rodent middens. Bar= 20 μm.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Table 1: Locality, height, age and parasites found from all fossil rodent middens studied from Huenul site, northwestern Patagonia, Argentina.

Midden	locality	Height (m)	Age (yrs BP)	Age (cal yrs BP)	Parasites found
HU584B	36°57'34.7''S 69°49'40.3''W	1,167			negative
HU558D	36°57'32.9''S 69°49'21.7''W	1,145	5,730 ± 70	6,480	*Trichuris* sp.
HU558E1	36°57'32.9''S 69°49'21.7''W	1,145			*Heteroxynema viscaciae*, *Trichuris* sp.
HU582-1	36°57'31.0''S 69°49'34.0''W	1,171	8,280 ± 120	9,213	*Helminthoxys* sp.
HU559A	36°57'31.0''S 69°49'34.0''W	1,171	2,540 ± 80	2,569	*H. viscaciae*, *Trichuris* sp.
HU584A	36°57'34.7''S 69°49'40.3''W	1,167	3,320 ± 90	3,512	*H. viscaciae*, *Helminthoxys* sp.
HU558A2	36°57'32.9''S 69°49'21.7''W	1,145	8,920 ± 180	9,947	negative
HU558A1	36°57'32.9''S 69°49'21.7''W	1,145	9,240 ± 130	10,389	*H. viscaciae*, *Helminthoxys* sp.
HU558C	36°57'32.9''S 69°49'21.7''W	1,145	8,680 ± 120	9,963	*Helminthoxys* sp., unidentified nematode
HU558E2	36°57'32.9''S	1,145	8,830 ±	9,834	*Trichuris* sp., *Helminthoxys* sp.

69°49'21.7''W	90
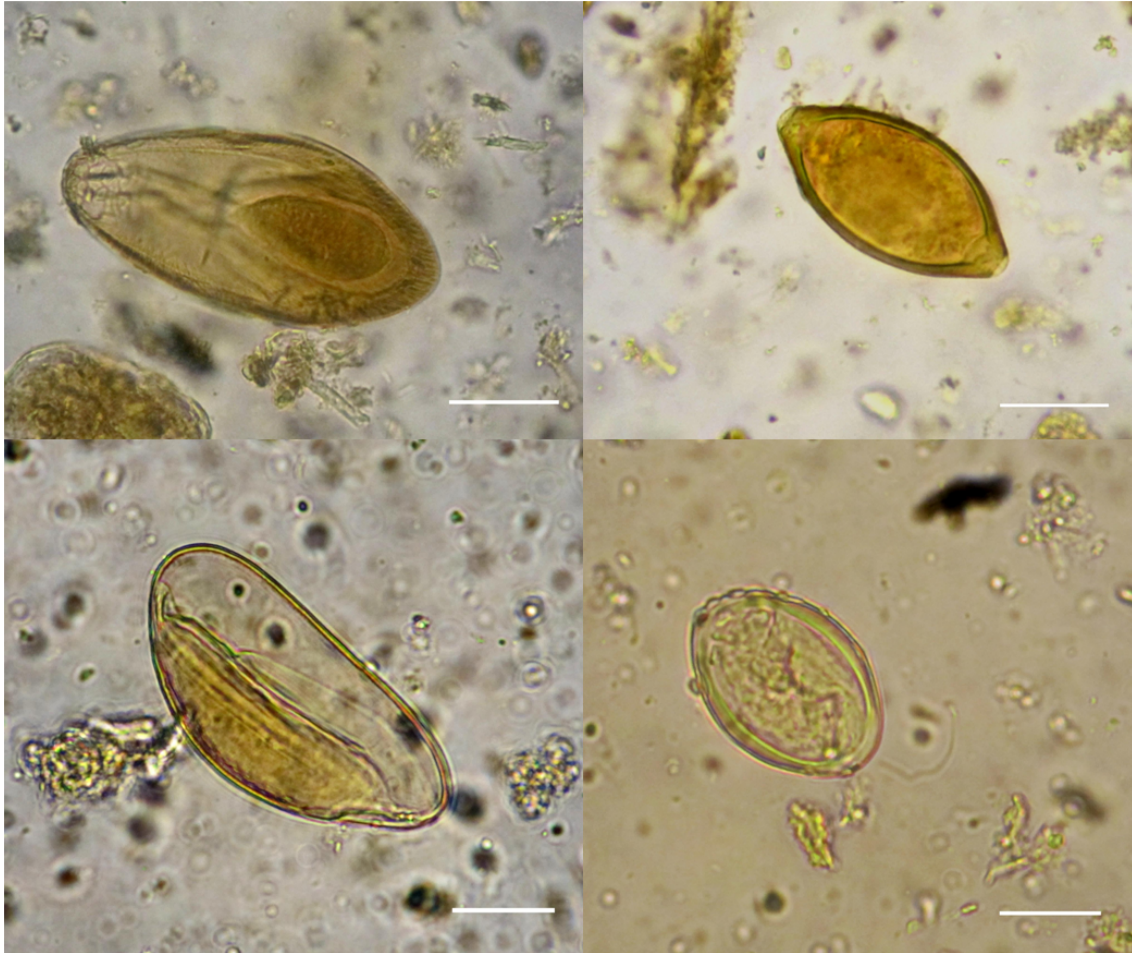
Graphical abstract

Highlights

- Fist paleoparasitological results from coprolites of fossil rodent middens

- Coprolites from 10 fossil middens (northwestern Patagonia, Argentina) were studied

- Parasite remains in excellent preservation were found in coprolites

- Fossil rodent middens were assigned to *Lagidium viscacia* (Caviomorph)

- Middens offer an excellent opportunity for the recovery of ancient parasite remains