Parasitological study of mountain viscacha fecal pellets from patagonia over the last 1200 years ('Cueva Peligro', Chubut province, Argentina)

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Abstract
The aim of the present study was to examine the parasite fauna present in mountain viscacha Lagidium viscacia (Caviomorpha, Chinchillidae) fecal pellets collected from 'Cueva Peligro', a cave located in Chubut province, Patagonia, Argentina, throughout the last 1200 years. A total of 84 samples were examined for parasites. Each pellet was whole processed: rehydrated, homogenized, sediment and examined using light microscopy. The samples and eggs of parasitites present were described, measured and photographed. Thirty-eight samples tested positive for the nematodes Heteroxyyna (Cavioxyyna) viscaciae Sutton & Hugot, 1989, Helminthoxys effilatus Schuurmans-Stekhoven, 1951 (Oxyurida: Oxyuridae), Trichuris sp. and one anoplocephalid species (Cestoda: Anoplocephalidae). This is the first time that H. effilatus is reported from ancient times. Significant differences of parasite occurrences through this temporal period were recorded. Parasitic life cycles and their presence along the studied period are so discussed.

Introduction
Mountain viscachas are large caviomorph rodents that inhabit arid 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. This rodent is found in rocky outcrops and is highly gregarious, living in colonies that may range widely in size. The presence of viscachas is readily detected by distinctive fecal pellets that can be found throughout the colony, on top of rocks or in sheltered crannies (Spotorno and Patton, 2015).

In the last years, several studies have been carried out in different areas of Patagonia involving micromammals fossil remains. The available data indicate that these communities had minor changes during the last 10 000 years. This holocene stability, despite the occurrence of climatic pulses, contrasts with the current structure of the assemblages, which shows a remarkable loss of diversity and even local or regional extinctions (e.g. Pardiñas et al., 2000, 2011, 2012; Udrizar Sauthier, 2009; Pardiñas and Teta, 2013; Teta et al., 2014). It has been suggested that these recent changes have been unleashed by anthropogenic activities, such as overgrazing, deforestation or the implantation of agroecosystems (Teta et al., 2014 and references cited therein).

Paleoparasitology is the study of parasites found in the archaeological or paleontological material. In a broad sense, paleoparasitologists are interested in the evolution of parasite-host–environment relationships, as well as in the origin and the evolution of infectious diseases within a paleoepidemiological perspective (Araújo et al., 2003). Beltrame et al. (2012, 2014, 2016) performed paleoparasitological studies on mountain viscacha coprolites from Patagonia, where nematode and cestode eggs were reported. The species found were also recorded in studies with modern mountain viscachas (Hugot and Sutton, 1989a; Denegri et al., 2003; Tantaleán et al., 2013). The environmental changes occurred in the last 100 years in Patagonia reflected in diversity changes of micromammals, could also be reflected in the evolution of parasite–mountain viscacha–environment relationships throughout time.

The aim of the present study was to examine the parasite remains from mountain viscacha fecal pellets from the site 'Cueva Peligro', Patagonia, through the last 1200 years and to discuss the paleoparasitological findings in temporal and paleoecological contexts. This is the first paleoparasitological examination of pellets of mountain viscachas throughout time, which include samples from modern, recent and ancient times. This study is part of an ongoing project in the area, where the impact of anthropic activities on micromammal community is under study.
Material and Methods

The paleontological site ‘Cueva Peligro’ (CP; 43°40′18″ S, 66°24′52″ W) is located near the southern margin of the Chubut River, about 6 km downstream of the Villa Dique Florentino Ameghino, Chubut Province, Argentina (Fig. 1). The site is a cave emplaced on a rocky front of approximately 35 m of height (Marrifil Formation, Jurassic), with a single entrance (4.57 m wide) and a single long gallery (ca. 30 m). The general dip of the floor of the cave is towards the outside, with some sectors that can reach 45° of inclination and others that approach 0°. From a phytogeographic point of view, CP is located in the Monte Phytogeographic Province (León et al., 1998). A 1 × 1 m² square grid was defined and the sequence was excavated by artificial layers of 3 cm thick until reaching the basement rock at a depth of 0.42 m. The extracted sedimentary material was sieved through a 5 mm mesh.

Feces samples from three layers were processed at the Laboratorio de Trítio y Radiocarbono (LATYR), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (LP), Argentina, to obtain radiocarbon dates. Calibration for the Southern Hemisphere was made through SHCal13 curve (Hogg et al., 2013) using OxCal 4.2 software (Bronk Ramsey, 2009) and weighted average was used as age estimator (Telford et al., 2004; Table 1). All the sequence was dated from the Late Holocene, including the historic period and actual times, approximately 1200 years throughout time. The radiocarbon dates of these layers range between 1220 ± 7114C years BP to modern years (Table 1).

The area has been under a progressive transformation of natural environments, mainly due to extensive sheep farming. The beginning of this activity corresponds to the 1880–1885 AD period. Based on the number of sheep feces found in each level, three time periods were defined: modern (1950–2015 AD, high sheep feces content), recent (1885–1950 AD, medium sheep feces content and ancient (previous to 1885 AD, low/null sheep feces content). Taking into account that: low/null: 0–15 sheep feces units 1.200 cm−3 of sediment, medium: 15–80 sheep feces units 1.200 cm−3 of sediment, high: >80 sheep feces units 1.200 cm−3 of sediment.

Six fecal pellets assigned to mountain viscachas from each layer were examined for parasites. A total of 84 samples were observed. Samples were inventoried and processed individually. Each sample was fully processed by rehydration in a 0.5% water solution of sodium phosphate in a glass tube for at least 3 days, followed by homogenization, processed by spontaneous sedimentation (Lutz, 1919) and preserved in 70% ethanol. Twenty slides were prepared from each sample, 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 400 × magnification.

Differences in parasite assemblages composition among modern, recent and ancient pellets were tested using permutation multivariate analyses of variance (PERMANOVA, Anderson, 2001). This is a non-parametric technique that uses label permutation to estimate the distribution of the test statistics under the hypothesis that within-group distances are not significantly different from between-group distances. The Sørensen similarity index was used as the distance measure for occurrence data. The Sørensen index of similarity was calculated on the basis of presence/absence between samples, according to the formula: S = 2C/(A + B), where S = index of similarity, A = number of species in one sample, B = number of species in the other sample and C = number of species common to both samples. A test of homogeneity of dispersions (PERMDISP, Anderson, 2006) was done in order to test differences in dispersions among groups because PERMANOVA is sensitive to differences in dispersions. A one-way PERMANOVA was performed using ‘Period’ as a factor (with three levels: modern, recent and ancient). Type III error and unrestricted permutation of raw data were used for unbalanced designs. Besides, a non-metric multidimensional scaling (NMDS, Clarke and Warwick, 2001) was used to ordinate samples in an ordination space. PERMDISP was used to avoid assumptions of linearity. Multivariate analyses were performed using the software PERMANOVA + and for PRIMER (Plymouth Routines In Marine Ecological Research) (v. 5.2) (Anderson et al., 2008).

Results

Fecal pellets were dark brown and cylindrical, with smooth surfaces and a flat end and the other point (Fig. 2). Average measurements of feces (N = 84) were 13.77 ± 2.38 mm long by 4.97 ± 0.49 mm wide. All layers examined contained parasite eggs (Table 1). Thirty-eight pellets were positive for parasites. Eggs of the parasite found were assigned to the nematodes *Heterostrongylum* (*Cavioxyura* viscaciae) Sutton and Hugot, 1989, *Helminthoxys effilatus* Schuurmans-Stekhoven, 1951 (Oxyurida: Oxyuridae), *Trichuris* sp. Roederer, 1761 (*Trichinellida: Trichuridae*) and one anoplocephalid (*Cestoda: Anoplocephalidae*).

Eggs of *H. viscaciae* (Fig. 3a), with single thick walls and with a rounded pole and the other sharp, without operculum, were found in five pellets from modern, recent and ancient times. Average egg measurements (N = 31) were 135.5–142.5 (135.2 ± 7.18) μm long by 57.5–70.0 (64.75 ± 3.59) μm wide.

Twelve pellets contained eggs of nematode attributed to *H. effilatus*. The samples belong to recent and ancient times. This nematode was not found from modern samples. The eggs were oblong, brown, with the striated and thick wall. One rounded pole and the other sharp, with a subterminal and notorious operculum (Fig. 3b), and some of them were embryonated. Average egg measurements (N = 29) were as follows: 110.0–122.5 (119.57 ± 4.77) μm long by 62.5–75.0 (67.74 ± 3.64) μm wide.

Eggs of *Trichuris* sp. (Fig. 3c) were observed in one pellet from modern times. These eggs were lemon-shaped, with a smooth surface and polar plugs. Measurements of eggs (N = 4) were 70.0–75.0 (71.63 ± 2.88) μm long by 37.5–42.5 (39.95 ± 2.5) μm wide.

Twenty-one samples were positive for anoplocephalids (Fig. 3d). Samples belong to modern, recent and ancient period. The eggs presented square or circular shape, with a piriform apparatus. The size ranges (means) of the eggs that were measured (N = 81) were 62.5–82.5 (69.53 ± 6.75) μm long by 62.5–80.5 (56.7 ± 1.5) μm wide.

Multivariate analysis performed on parasite occurrences in coprolites showed no significant differences in dispersions between groups (PERMDISP, P = 0.156), but significant differences between periods (PERMANOVA, P ≤ 0.01, Table 2). The Pair-wise result showed a significant difference between modern and ancient periods (P ≤ 0.01, Table 1). NMDS plot evidenced these differences with a stress value of 0.01 (Fig. 4). This difference between modern and ancient periods could be explained by the presence of *Trichuris* sp. and the absence of *H. effilatus* in actual coprolites in comparison with ancient samples.

Discussion

This is the first paleoparasitological study of mountain viscacha fecal pellets that includes a temporal period through the last 1200 years to modern times. Previously, Beltrame et al. (2012) studied mountain viscacha coprolites collected from Cueva Huenul 1, northern Neuquén (Patagonia, Argentina), an

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**Table 1.**

| Layer | Modern | Recent | Ancient |
|-------|--------|--------|---------|
| Fecal pellet content | 80.5 (70.6 ± 2.5) | 82.5 (69.53 ± 6.75) | 62.5–82.5 (69.53 ± 6.75) |
| Fecal pellet size | 57.5–70.0 (64.75 ± 3.59) | 62.5–75.0 (67.74 ± 3.64) | |
Anoplocephalids are parasites of zoonotic importance for animals and wild viscachas (*Lagostomus maximus*) from other areas from Argentina and Perú (Foster et al., 2002; Ferreira et al., 2007; Tantaleán et al., 2013).

*Helminthoxys* sp. (Oxyuridae: Syphacinae) is a parasite of neotropical caviomorph rodents. The genus *Helminthoxys* comprises seven species: *Helminthoxys caudatus* (syn. *Helminthoxys pujolii*), *Helminthoxys effilatus* (syn. *Helminthoxys velizi*), *Helminthoxys freitasi*, *Helminthoxys tifophila*, *Helminthoxys quentini*, *Helminthoxys urichi*, *Helminthoxys gigantea* and *Helminthoxys abrocoma* (Hugot and Sutton, 1989b). Several studies have demonstrated the high host-specificity among Oxyuridae (Hugot, 2003). In particular, these studies showed that the distribution of Syphacinae belonging to the same family of rodent hosts has a close parallel phylogeny with them. *Helminthoxys effilatus* is a parasite of the mountain viscacha (Hugot and Sutton, 1989b). Eggs of *Helminthoxys* spp. are similar. Nevertheless, due to the specificity of this genus, we can hypothesize that the eggs found in the studied samples belong to the species *H. effilatus*. This would be the first time that *H. effilatus* is reported from ancient times. This oxiurid was also registered in actual samples of mountain viscacha from Argentina and Perú (Hugot and Sutton, 1989b; Tantaleán et al., 2013). Nevertheless, in the present study, it was not found in samples from modern times. Due to the favourable conditions of humidity and temperature that these eggs need to continue the biological cycle, the environmental changes along the last period could be responsible for the absence/presence of this species in the samples under study.

*Trichuris* spp. include intestinal parasites of the caecum and colon of mammals, mainly humans, primates, pigs, ovines, goats, cervids, rodents and canids, with eggs that mature in the soil. 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 four molts before reaching the adult stage (Anderson, 2000). To date, a total of 24 species of *Trichuris* have been described from 10 families of American rodents (Robles and Navone, 2014). *Trichuris* spp. in South American rodents from ancient material were cited (Beltrame et al., 2014). Moreover, there is a previous record of two *Trichuris* species in coprolites of *L. viscacia* from fossil rodent middens of Cueva Huenul 1 archaeological site, northwest Patagonia, dated at 2540 ± 80, 5730 ± 70 and 8438 ± 37 years B.P. (Beltrame et al., 2016). Species of *Trichuris* have been infrequently recorded in high latitudes. This infrequent recording is probably related to the behavior of host species, characteristics of the environments where eggs are deposited, and/or lower degree of sampling effort in the area with respect to other areas of the country (Robles and Navone, 2014).

The anoplocephaline cestodes (Cyclophyllidea: Anoplocephalidae) represent a diverse group of parasites infecting both terrestrial mammals (placentals and marsupials) and birds. Based on the number of genera present in these hosts, the most important radiation of anoplocephalines has been in rodents and lagomorphs (Beveridge, 1994; Wickström et al., 2005). The intermediate hosts of these cestodes are oribatid mites, which are ingested by their herbivorous definitive hosts (Beveridge, 1994). Anoplocephalids are parasites of zoontic importance for animals and humans (Denegri et al., 1998). As previously mentioned, *V. quadrata* and *Monoecocestus* spp. were found in modern and ancient samples. In the present study, only one anoplocephalid morphotype egg was found with similar characteristics to those found from Los Altares and Cueva Huenul (Beltrame et al., 2013).

The difference between modern, recent and ancient periods could be explained by the presence of *Trichuris* sp. and the absence of *H. effilatus* in actual coprolites in comparison with recent and ancient samples. The fact that two of the recorded samples have been infrequently recorded in high latitudes. This infrequent recording is probably related to the behavior of host species, characteristics of the environments where eggs are deposited, and/or lower degree of sampling effort in the area with respect to other areas of the country (Robles and Navone, 2014).
Table 1. Depth, time period, radiocarbon dates, calibrated age, other fecal pellets present and parasite egg found from all studied layers from ‘Cueva Peligro’

| Temporal period | Layer | Depth (cm) | Other fecal pellets found | Dating \(14C\) BP | Calibrated age (cal AD; weighted average) | Pellet N° | Parasites found |
|-----------------|-------|------------|---------------------------|----------------|------------------------------------------|----------|----------------|
| Modern          | 1     | 0          | Micromammals and sheep    |                 |                                          | 1.1      | Negative       |
|                 |       |            |                           |                 |                                          | 1.2      | Anoplocephalid |
|                 |       |            |                           |                 |                                          | 1.3      | Anoplocephalid |
|                 |       |            |                           |                 |                                          | 1.4      | Negative       |
|                 |       |            |                           |                 |                                          | 1.5      | Anoplocephalid |
|                 |       |            |                           |                 |                                          | 1.6      | Negative       |
|                 | 2     | 0–3        | Micromammals and sheep    |                 |                                          | 2.1      | Anoplocephalid |
|                 |       |            |                           |                 |                                          | 2.2      | Anoplocephalid |
|                 |       |            |                           |                 |                                          | 2.3      | Negative       |
|                 |       |            |                           |                 |                                          | 2.4      | Negative       |
|                 |       |            |                           |                 |                                          | 2.5      | Anoplocephalid |
|                 |       |            |                           |                 |                                          | 2.6      | *Heteroxynema viscaciae* |
|                 | 3     | 3–6        | Micromammals and sheep    |                 |                                          | 3.1      | Negative       |
|                 |       |            |                           |                 |                                          | 3.2      | Negative       |
|                 |       |            |                           |                 |                                          | 3.3      | *Trichuris* sp. |
|                 |       |            |                           |                 |                                          | 3.4      | Negative       |
|                 |       |            |                           |                 |                                          | 3.5      | Anoplocephalid  |
|                 |       |            |                           |                 |                                          | 3.6      | Negative       |
| Recent          | 4     | 6–9        | Micromammals and sheep    |                 |                                          | 4.1      | Negative       |
|                 |       |            |                           |                 |                                          | 4.2      | Negative       |
|                 |       |            |                           |                 |                                          | 4.3      | *Heteroxynema viscaciae* |
|                 |       |            |                           |                 |                                          | 4.4      | Negative       |
|                 |       |            |                           |                 |                                          | 4.5      | Negative       |
|                 |       |            |                           |                 |                                          | 4.6      | Anoplocephalid  |
|                 | 5     | 9–12       | Micromammals              |                 |                                          | 5.1      | Negative       |
|                 |       |            |                           |                 |                                          | 5.2      | Negative       |
|                 |       |            |                           |                 |                                          | 5.3      | Anoplocephalid  |
|                 |       |            |                           |                 |                                          | 5.4      | Negative       |
|                 |       |            |                           |                 |                                          | 5.5      | Negative       |
|                 |       |            |                           |                 |                                          | 5.6      | Negative       |
|                 | 6     | 12–15      | Micromammals              |                 |                                          | 6.1      | *Heteroxynema viscaciae* |
|                 |       |            |                           |                 |                                          | 6.2      | Negative       |
|                 |       |            |                           |                 |                                          | 6.3      | Negative       |
|                 |       |            |                           |                 |                                          | 6.4      | Anoplocephalid  |
|                 |       |            |                           |                 |                                          | 6.5      | Anoplocephalid  |
|                 |       |            |                           |                 |                                          | 6.6      | Anoplocephalid  |
|                 | 7     | 15–18      | Micromammals              |                 |                                          | 7.1      | Anoplocephalid  |
|                 |       |            |                           |                 |                                          | 7.2      | Negative       |
|                 |       |            |                           |                 |                                          | 7.3      | Negative       |
|                 |       |            |                           |                 |                                          | 7.4      | *Helminthoxys effilatus* Anoplocephalid |
|                 |       |            |                           |                 |                                          | 7.5      | Negative       |
|                 |       |            |                           |                 |                                          | 7.6      | Negative       |
|                 | 8     | 18–21      | Micromammals Modern (LP-3076) |               |                                          | 8        | Without samples |
| Ancient         | 9     | 21–24      | Micromammals              |                 |                                          | 9.1      | Negative       |

(Continued)
species (i.e. *H. viscaciae* and an anoplocephaline species) along the span of time studied had remained constant, may be due to a different capacity of adapting to a changing environment or/and that the conditions were not different enough to affect them. The record of trichurid eggs in just one sample in the modern period strengthens the previous discussion that this species is infrequently recorded in high latitudes and very rare in the ancient material. Nonetheless, the pattern presented by *H. effilatus* where the high abundances recorded in the ancient period declined in the recent period, to finally be absent in modern

| Temporal period | Layer | Depth (cm) | Other fecal pellets found | Dating ($^{14}$C BP) | Calibrated age (cal AD; weighted average) | Pellet N° | Parasites found |
|-----------------|-------|------------|---------------------------|----------------------|------------------------------------------|----------|----------------|
| 10              | 24–27 | Micromammals | 880 ± 70 (LP-3153)        | 1183                 |                                          | 10.1     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 10.2     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 10.3     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 10.4     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 10.5     | Negative        |
|                 |       |            |                           |                      |                                          | 10.6     | *Heteroxynema viscaciae* |
| 11              | 27–30 | Micromammals | 11.1 Negative             |                      |                                          | 11.2     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 11.3     | Negative        |
|                 |       |            |                           |                      |                                          | 11.4     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 11.5     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 11.6     | Negative        |
| 12              | 30–33 | Micromammals | 12.1 Negative             |                      |                                          | 12.2     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 12.3     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 12.4     | Negative        |
|                 |       |            |                           |                      |                                          | 12.5     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 12.6     | Negative        |
| 13              | 33–36 | Micromammals | 13.1 Negative             |                      |                                          | 13.2     | Negative        |
|                 |       |            |                           |                      |                                          | 13.3     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 13.4     | *Helminthoxys effilatus* |
|                 |       |            |                           |                      |                                          | 13.5     | Negative        |
|                 |       |            |                           |                      |                                          | 13.6     | Negative        |
| 14              | 36–39 | Micromammals | 14.1 Negative             |                      |                                          | 14.2     | Negative        |
|                 |       |            |                           |                      |                                          | 14.3     | *Heteroxynema viscaciae* |
|                 |       |            |                           |                      |                                          | 14.4     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 14.5     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 14.6     | Negative        |
| 15              | 39–42 | Micromammals | 15.1 Negative             |                      |                                          | 15.2     | Negative        |
|                 |       |            |                           |                      |                                          | 15.3     | Anoplocephalid  |
|                 |       |            |                           |                      |                                          | 15.4     | Negative        |
|                 |       |            |                           |                      |                                          | 15.5     | Negative        |
|                 |       |            |                           |                      |                                          | 15.6     | *Helminthoxys effilatus* |
Fig. 2. Fecal pellets studied from the paleontological site 'Cueva Peligro'.

Fig. 3. Egg found from 'Cueva Peligro' (A) Heteroxynema viscaciae (Nematoda: Oxyuridae), (B) Helminthoxyx effilatus (Nematoda: Oxyuridae), (C) Trichuris sp. (Nematoda: Trichuridae), (D) Anoplocephalid (Cyclophyllidea: Anoplocephalidae). Bar: 20 µm.
times, is remarkable. This can be a consequence of a higher sensibility of this species to environmental changes. Indeed, oxyurid species are geohelminthes which undergo a period of development in the soil before being ingested. In a greater or lower degree, all these geohelminthes suffer the influence of the alterations of the soil characteristics and climate of the region (Chieffi, 2015). However, evidence showed that eggs of different species vary their resistance to extreme temperatures (Bunday and Cooper, 1989).

Concluding remarks

This is the first study of mountain viscacha parasites along the time. The presence of *H. viscaciae* and anoplocephalids in fecal pellets from mountain viscachas from CP have not changed significantly during the last 1200 years, although environmental conditions fluctuated throughout this period, indicating the stability of these associations over time. *Helminthoxys effilatus* was reported for the first time in ancient samples and was absent in modern fecal pellets studied. New studies are now needed and could contribute with the study of mountain viscacha–parasite–environment relationships throughout time.

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