

1RH: SHORT COMMUNICATIONS

2Apex Predators, Rockshelters, and Zoonoses in the Patagonian Holocene

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7ABSTRACT: Carnivorous mammals are a trophic group (guild?) with an important role in
8parasite infective stages (larvae, eggs, cysts, and oocysts). In the present study, new
9samples of coprolites attributed to carnivorous mammals, obtained from two archaeological
10caves, were analyzed for the presence of parasites, with the aim to increase the knowledge
11about parasites in rockshelters that could have spread to humans and other mammals. To
12this purpose, fragments of 3 coprolites from Cerro Casa de Piedra, cave 5 (CCP5) and cave
137 (CCP7) were examined. Coprolites were rehydrated in aqueous trisodium phosphate and
14processed by spontaneous sedimentation. A high parasite richness was observed and new
15parasite species for archaeological contexts were found. The parasitological findings in
16*Puma concolor* coprolites associated with caves suggest the importance of these
17carnivorous in the dissemination of parasites in areas with high redundancy in space use
18and steady conditions of temperature, humidity, and radiation.

19 In the last 12 yr, some evidence about the occurrence of parasites in areas inhabited
20by hunter-gatherers during the Patagonian Holocene and by European settlers in more
21recent times have been performed. Some studies have been carried out on samples from
22open-air burial (Fugassa et al., 2006; Fugassa and Dubois, 2009), burial caves and middens
23(Fugassa and Barberena, 2006; Sardella and Fugassa, 2009b). However, most of the
24knowledge about Holocene parasitology from Patagonia has been produced by the analysis
25of coprolites found in 2 archaeological sites: Cerro Casa de Piedra, cave 5 (CCP5) and cave

267 (CCP7) (e.g., Fugassa et al., 2006, 2009, 2013; Sardella and Fugassa, 2009a; Taglioretti
27et al., 2015). Since the frequency of zoonotic parasites in these 2 sites has been found to be
28high, these sites are considered as a transmission focus where parasites were spread within
29mammal populations and communities (Fugassa et al., 2009).

30 In the presente study, new samples of coprolites attributed to carnivorous mammals
31were analyzed to increase the knowledge about parasites that could have spread in these
32rockshelters. Considering an ecological approach, carnivorous mammals are a trophic guild
33with an important role in the fecal dissemination of infective parasitic stages because of the
34consumption of definitive hosts parasitized by gastrointestinal parasites or by release of
35own parasites.

36 Coprolites were selected according to the similar aspect to carnivorous or
37omnivorous mammals stool. Fragments of 3 coprolites from archaeological sites CCP5 and
38CCP7 were examined. Cerro Casa de Piedra is a volcanic hill located in the Perito Moreno
39National Park in the west of the province of Santa Cruz, Argentina (Civalero and Aschero,
402003). Coprolites N° 521 and 246 came from site CCP7, from layer IV (dated in $5,120 \pm 90$
41– $3,970 \pm 80$ yr before present (BP)) and layer XVII/XVIII (dated in $9,100 \pm 150 - 10,530$
42± 620 yr BP), respectively. Coprolite N°521 was identified as *Puma concolor* by using
43molecular tools, whereassample N° 246 could not be identified as feline or human (Petri
44and Fugassa, 2017). Site CCP7 is located to $47^{\circ}57'S$, $72^{\circ}05'W$ at an altitude of 900 masl.
45Inside the site, area 1 displays a stratigraphic sequence of 19 levels, which was occupied by
46humans between *ca.* $9,700 \pm 100$ and $3,600 \pm 70$ yr BP (Aschero, 1996; Civalero and
47Aschero, 2003). Coprolite N° 398 was extracted from layer II of CCP5 site, dated in $2,740 \pm$
48 100 yr BP. Site CCP5 is a deep cave containing 7 levels. The radiocarbon dating showed
49human occupation in 3 periods *ca.* $6,780-6,540$ yr BP, *ca.* $5,170-4,330$ B.P. and *ca.* $2,740-$
50 $2,550$ BP (Aschero, 1996). Molecular identification indicated that the sample corresponds

51to *Puma concolor* (Petrigh and Fugassa, 2017). Coprolites were processed by spontaneous
52sedimentation (Lutz, 1919) and rehydrated in 30 ml of aqueous trisodium phosphate 0.5%
53for 48 hr (Callen and Cameron, 1960). Thirty transient slides were observed with x40 in a
54light microscope.

55 Results showed a high diversity of gastrointestinal parasites, but in low frequency.
56*Calodium* sp. was the only species shared by 2 coprolites.

57 *Eimeria macusaniensis*: one pyriform oocyst was found in *Puma concolor* (Sample
58N° 398). It showed a prominent micropyle and was composed of 3 membranes: an external
59thin and translucent membrane, a medium-thickness brown membrane, and an internal
60light-brown thinner wall (Fig. 1). Dimensions: 77.5 x 45.0 µm. *Eimeria macusaniensis* is a
61specific parasite of camelids, its presence suggests the consumption of camelid viscera.
62This parasite has been reported in archaeological remains of Peru (Leguía and Casas, 1995)
63and carnivorous coprolites (e.g., Fugassa et al., 2008; 2009), camelid coprolites (Fugassa,
642007, Taglioretti et al., 2015) and in archaeological middens (Fugassa and Barberena,
652006) from Patagonia.

66 *Isospora* sp.: one oocyst was recovered from *Puma concolor* (sample N° 521). The
67oocyst had hemispherical body, smooth and refracting thin wall without apparent
68micropyle, and an internal mass occupying most of the inside (Fig. 2). Dimension: 30x30
69µm. Ancient remains containing *Isospora* sp. were found by Jones (1987) and *Isospora*
70*belli* was identified by Allison et al. (1999).

71 *Lamanema* sp. or *Nematodirus* sp.: 6 ellipsoid light-brown eggs with rounded ends,
72thick-walled, and ornaments on the poles (Fig. 3) were found in *Puma concolor* (sample
73N°398). Dimensions: 169.6(155-175 ± 7.3; n = 6) x 81.7(77.5-85 ± 2.6; n = 6) µm. These
74parasites are more frequent in camelid coprolites from Patagonia (Fugassa, 2007;
75Taglioretti et al., 2015). *Lamanema* sp. has been reported in alpacas and llamas mummified

76remains (Leguia and Casas, 1995) and in modern Patagonian guanacos only from Chile
77(Ruiz-Aravena and Correa, 2010).

78 Spirurid nematode: one small translucent egg with rough and light-brown outer wall
79with apparent ornaments was recorded in *Puma concolor* (sample N° 521). This egg had a
80thick, translucent and smooth inner layer and contained larvae (Fig. 4). Dimensions: 40 x
8128.75µm. It was identified as probably *Protospirura* sp. No parasitological records of
82spirurids have been reported in Holocene coprolites, but it parasite has been found in
83current foxes, skunks and rodents from Patagonia (Fugassa, 2015).

84 Capillariid type 1: 2 elliptical eggs with asymmetric shape, bioperculated, with
85plugs in both poles were found in *Puma concolor* (sample N°398). The eggshell was
86unornamented, with 3 layers: a medium-thickness wall, light-colored thin inner layer, and a
87dark external layer (Fig. 5). Dimensions: 85.0x45.0µm. Both shape and ornamentation of
88eggs remind to *Eucoleus* genus. Similar eggs have been found in camelid coprolites from
89Patagonia (Taglioretti et al., 2015). *Eucoleus* sp. parasites have not yet been found in
90modern wild mammals of Patagonia (Fugassa, 2015).

91 Capillariid type 2: 2 ellipsoidal eggs with asymmetric sizes were recorded in *Puma*
92*concolor* (sample N° 398). The ends of the eggs had a polar operculum,with non-extruded
93and flat plugs (Fig. 6). The wall was thick and composed of a thin dark-brown inner layer, a
94thick medium layer, and hyaline external layer. The surface was ornamented and
95reticulated,and the surface cross-section showed a radially arranged canaliculus .
96Dimensions: 63.75-70.0 x 35.0-41.25µm. In addition,, 4 eggs were recovered from *Puma*
97*concolor* (sample N° 521). Dimensions: (60.0-70.0 ± 4.37; n = 4) x 38.1(37.5-40.0 ± 1.25;
98n = 4) µm. These eggs were similar to those of *Calodium hepaticum*. This parasite has been
99recorded in the most ancient samples of Patagonia (Taglioretti et al. 2014), although it has
100not been recorded in current samples of this region.

101 Capillariid sp. type 3: 2 poorly ellipsoidal eggs with an operculum and plugs in the
 102poles were found in an unidentified concretion (sample N°246). The walls were damaged,
 103with a few visible features. They presented longitudinal ornamental lines. Dimensions: 72.5
 104x 27.5; n = 1. The eggs could not be assigned to any known morphotype because of the
 105small number of eggs and poor conservation state.

106 Trichosomoidid nematode: 1 ellipsoidal brown egg with asymmetric sides was
 107recovered from *Puma concolor* (sample N° 398). It has bipolar operculated, without plugs.
 108The wall was irregular, with apparent nipples and 2 layers: a long, smooth and internal
 109layer and an external, thick, rough and dark layer. The egg contained embryo (Fig. 7).
 110Dimensions: 80.0 x 46.25 µm. *Anatrichosoma* eggs have been found in *Abrothrix olivaceus*
 111from Patagonian Chile and Argentina (Robles et al., 2012; Fugassa et al., 2014).
 112Trichosomoidid eggs have also been found in rodent coprolites from an archaeological site
 113in Chubut Province, Argentina (Sardella and Fugassa, 2009b), although they were smaller
 114than the one found here.

115 *Taenia sp. or Echinococcus sp.*: 3 greenish brown eggs with striated embryophore
 116were found in *Puma concolor* (sample N° 398). The eggshell had numerous small spots and
 117a thin translucent inner layer. The oncosphere contained at least 4 visible hooks (Fig. 8).
 118Dimensions: 36.5(36-37 ± 0.7; n = 2) x 32.75(31.5-34 ± 1.77; n = 2) µm. Several records
 119have been reported in Patagonia, but only the findings in site CCP5 (specially layer II) have
 120achieved all specific features (Beltrame et al., 2010, 2011). In current samples, the genera
 121*Echinococcus* and *Taenia* have been reported in various mammals of Patagonia, as
 122intermediate and definitive hosts (Fugassa, 2015). Specifically, *E. oligarthrus*, *T. omissa*, *T.*
 123*taeniaeformis*, *T. ovis krabbei*, *T. multiceps* y *T. hydatigena* have been reported in *P.*
 124*concolor* and others wild felids as definitive hosts (Fugassa, 2015).

125 The 246 concretions studied showed only 3 parasitic remains. The low density of
126intestinal parasites and the lack of identification of defecators as carnivores by using
127molecular tools (Petrigh and Fugassa, 2017) suggest that these concretions are not fecal.
128High parasite richness was found in *Puma concolor*. This suggests a combination between
129patent parasitism and parasites in transit by consumption of viscera preys. This hypothesis
130is supported by the finding of *E. macusaniensis* oocysts which are camelid-specific
131parasites. In the present study, the abundance of parasites found was low. Besides, some of
132the parasitological findings could not be validated due to the absence or alteration of
133features with taxonomic value in ancient eggs, cysts, oocysts or larvae. Often, the small
134number of parasitic remains prevented us from finding individuals which fulfilled all
135characteristics.

136 These parasitological findings allow to strengthening patterns previously established
137for sites CCP5 and CCP7: 1) capillariids were present in all samples. *Calodium* sp. type
138eggs was the most common; 2) high frequency of *E. macusaniensis* and *Nematodirus* sp. or
139*Lamanema* sp. nematode in coprolites evidenced consumption of camelid viscera by *Puma*
140*concolor* (Sample 398) (the association between *E. macusaniensis* and *Nematodirus* sp. or
141*Lamanema* sp. is common in predator coprolites that feed on camelids); and 3) predator's
142coprolites can exhibit a high parasitic diversity due to a combination of spurious and patent
143parasitism.

144 In addition, new and uncommon information was obtained for Patagonian record: 1)
145taeniid eggs (*Taenia* sp. or *Echinococcus* sp.) were present in sample N° 398; 2)
146*Protospirura* sp. is the first report for to Patagonian Holocene and even for ancient
147samples; 3) trichosomoidid nematode, another rare parasite, was recorded, which suggests a
148high preservation level; 4) spirurids and trichosomoidids found in predator coprolites

149increased the spectrum of parasites that could have spread mammals in caves, thus
150evidencing potential zoonotic implications.

151 Although parasitological studies in wild mammals of Patagonia are scarce (Fugassa,
1522015), a stark contrast between current and ancient reports in this region was observed.
153*Eimeria macusaniensis* was only reported in a study of modern guanaco feces from
154Patagonia (Beldomenico et al., 2003). Nevertheless, *E. macusaniensis* oocysts have been
155widely found in Patagonian archaeological samples. Besides, in Patagonia, eggs that are
156compatible with *Lamanema* sp. have been recorded only in wild rodents and Chilean
157guanacos (Fugassa, 2015). Only 6 records of capillariids have been identified in modern
158wild mammals of Patagonia (Fugassa, 2015), mainly in samples from Santa Cruz.

159 In Meridional Patagonia, the presence of strong snowfalls for at least nine months
160could emphasize the importance of rockshelters as refuge of many mammals. The
161parasitological findings in carnivores associated with caves suggest the importance of these
162mammals for the dissemination of their own parasites and those to their prey in areas with
163high redundancy in space use. This generates higher density of parasitic remains, and more
164stable temperature, humidity and radiation conditions would stimulate the viability and
165maturation of eggs and oocysts until its infective stage.

166 Archaeologists Maria Teresa Civalero (INAPL, CONICET) and Carlos Aschero
167(CONICET) conducted fieldwork and laboratory that provided the important collection of
168coprolites CCP5 and CCP7. We also thank to Muriel Fugassa for english revision. We
169would also like to thank two anonymous reviewers for their useful comments on an earlier
170version of the manuscript. This study was funded by the National University of Mar del
171Plata (EXA 680), CONICET (PIP 090) and FONCyT (PICT 2316 and PICT 3126).

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244Figure 1. *Eimeria macusaniensis* oocyst.

245Figure 2. Small oocyst assigned to *Isospora* sp.

246Figure 3. *Nematodirus* sp. or *Lamanema* sp. egg.

247Figure 4. Spirurid egg assigned to *Protospirura* sp.

248Figure 5. Capillariid egg.

249Figure 6. Capillariid egg similar to *Calodium hepaticum*.

250Figure 7. Trichosomoidid egg containing larva.

251Figure 8. Cestode (taenid) egg.

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