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First paleoparasitological record of acanthocephalan eggs from Northwestern Patagonia (Late Holocene, Argentina)

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ABSTRACT

Eggs representative of an acanthocephalan were found in an ancient fragment of raptor pellet, probably belonged to the barn owl, *Tyto alba*, from the archeological site named "Epullán Chica cave." This site is a cave located at the southern of Neuquén Province, Patagonia, Argentina. The fragment of pellet was found in a layer with charcoals dated at 1980 ± 80 years B.P. A total of 56 eggs were found. Eggs were brown colored and thick-shelled, and presented four membranes, the outer lightly sculpted. The embryos presented hooks in one extremity. Measurements ranged from 87.5 to 107.5 μm long and 50 to 57.5 μm wide. Eggs were very well-preserved, and were identified as belonged to Class Archiacanthocephala, Order Oligacanthorhynchida, Family Oligacanthorhynchidae, probably *Macracanthorhynchus* Travassos, 1917, or an unidentified species. This is the first report of small mammal acanthocephalans from ancient material worldwide.

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). A main characteristic of the phylum is a protrusible proboscis armed with recurved hooks, by which the worm attaches to the wall of the intestine of the definitive host.

The phylum Acanthocephala is divided into four classes: Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala and Polyacanthocephala (Amin, 2013). The class Archiacanthocephala is truly terrestrial: they use terrestrial insects and myriapods as intermediate hosts and predatory birds and mammals as definitive hosts. Small mammals are hosts to a great number of parasites. Nevertheless, the number of acanthocephalan species in small mammals is very low compared with other groups of parasites (Ribas and Casanova, 2006).

Owl regurgitate prey remains in the form of pellets are studied to understand their feeding habits and for inventorying small mammals at various actual and ancient sites from Patagonia, Argentina (e.g., Bellocq, 2000; Fernández et al., 2012). In the last times, owl pellets from Patagonia were also used for paleoparasitological studies (Beltrame et al., 2014; Fugassa et al., 2007). These authors have demonstrated that raptor pellets can be used as source of paleoparasitological information in archeological sites.

Eggs representative of an acanthocephalan species were found in a fragment of raptor pellet. This pellet is part of a series of 22 owl regurgitation pellets examined for paleoparasitological purposes from the Late Holocene archeological site named "Epullán Chica" (ECh). This cave site is located at the southern of Neuquén Province, Northwestern Patagonia, Argentina (40°23′10″S, 70°11′44″W, 680 m), about 5 km north of the Limay river valley. It opens to the north in an outcrop of volcanic tuffs of the Collón Curá Formation. This cave is 100 m east from "Epullán Grande," another archeological cave with rich small mammal materials (e.g., Crivelli Montero et al., 1996). 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.

The archeological sequence was dated between 2220 ± 50 years ^{14}C B.P. (charcoal sample recovered from the deeper layers) to 20th century (metal knife recovered from the upper layers). The pellets

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Fig. 1. Macroscopic aspect of the fragment of the regurgitation pellet examined.

Fig. 2. Acanthocephalan eggs found in a fragment of raptor pellet from the archeological site Epullán Chica Cave, Patagonia, Argentina, bar = 30 μm.

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, processed by spontaneous sedimentation and preserved in ethanol 70%. Up to 20 slides were prepared, along with the addition of one drop of glycerin, and examined at 10 and 40× using a light microscopy. Eggs of parasites were measured and photographed at 40× magnifications. Egg 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 to room temperature for diet analysis.

Only one fragment of regurgitation pellet (Fig. 1) was positive to acanthocephalan eggs. The fragment of pellet was found in a layer with charcoals dated at 1980 ± 80 years ^{14}C B.P. (Unit I). Measurements were 28.56 mm long by 13.31 mm wide, and the weight was 1.09 g. It seems to have been under the influence of desiccation due to the dry climate of the area. From a taphonomic point of view, all of the archeological units are characterized by the preservation of pellets, together with light digestive corrosion on <5% of bones and teeth of micromammals. These taphonomic features suggest that the main accumulator agent of micromammalian and pellet assemblages was an strigiform belonging to the Category I or Light modification (*sensu* Andrews, 1990) probably the barn owl, *Tyto alba*. This bird is the most common owl that inhabits in caves and open landscapes (Andrews, 1990), and displays an opportunistic behavior, feeding mostly on nocturnal small mammals, but can also prey on other classes of vertebrates and on insects (e.g., Bellocq, 2000; and references therein).

Macroscopic observations showed a mixture of vegetal inclusions. Bones and teeth were not observed. Prey identification was not possible. Microscopic examination revealed brown colored and thick-shelled eggs. Eggs presented four membranes, the outer lightly sculpted. The embryos presented hooks in one extremity (Fig. 2). Egg measurements (n = 56) ranged from 87.5 to 107.5 (96.6 ± 3.95) μm long and 50 to 57.5 (52.7 ± 2.04) μm wide. Eggs were very well-preserved and by the characteristics of the eggshell they were identified as an acanthocephalan, belonged to Class Archiacanthocephala, Order Oligacanthorhynchida, Family Oligacanthorhynchidae, probably or *Macracanthorhynchus* Travassos, 1917, or an unidentified species. Microscopic observations also revealed hairs, vegetal fibers, and pollen. The presence of microscopic hairs allowed the assumption of the small mammal ingestion.

Despite to the excellent egg preservation in the sample, it was not possible the assignment to species. This is due to scarce record of Oligacanthorhynchids 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 archeological site from Brazil (Ferreira et al., 1989). However, there are no records of small mammal acanthocephalans from ancient material at present (Beltrame et al., 2014).

Current knowledge of the Family Oligacanthorhynchidae from small mammals from South America is limited. *Oligacanthorhynchus* sp. was registered in the marsupial fat-tailed opossum, *Thylamys venustus*, from Northwestern Argentina (Jiménez et al., 2008). *Oligacanthorhynchus microcephalus* was found in two species of mouse opossum, *Marmosa demerarae* and *Marmosa murina*, from French Guiana (Byles et al., 2013). Richardson et al. (2014) reported *O. microcephalus* from different opossums from South America, and showed that *O. microcephalus* exhibits a broad distribution throughout much of the New World, using an array of hosts with substantially overlapping ranges. *Moniliformis moniliformis* and *Macracanthorhynchus hirudinaceus* are common acanthocephalans found in small mammals along the world (Ribas and Casanova, 2006). From South America, *M. moniliformis* were found in the black rat, *Rattus rattus*, in a coal mine from Río Grande do Sur, Brazil (Tietz Marques and Scroferneker, 2003) and from Perú (Tantaleán et al., 2005).

Genera *Macracanthorhynchus* and *Moniliformis* are also present in another mammals from South America. For example, *Macracanthorhynchus ingens* was reported from a kinkajou, *Potos flavos*, from Colombia (Richardson, 2014). This parasite appears to exhibit fairly low host specificity. *Oligacanthorhynchus major* was observed from a white-lipped peccary, *Tayassu pecari*, in Bolivia (Richardson and Barger, 2006) and in Perú (Gomez-Puerta, 2011) and from the collared-peccary, *Pecari tajacu*, from Brazil (Machado-Filho, 1963). *Oligacanthorhynchus carinii* was found in nine-banded armadillo, *Dasypus novemcinctus*, from Perú (Gomez-Puerta, 2011). *M. hirudinaceus* is observed frequently in wild and domestic pigs. From South America, it was observed in *T. pecari* from Brazil (Almeida Souza et al., 2005) and in *Sus scrofa* from Perú (Tantaleán et al., 2005; Gomez-Puerta, 2011). Some acanthocephalan species are characterized by a high degree of opportunism in relation to the host taxonomic affinity (Ribas and Casanova, 2006).

The earliest discovery of acanthocephalan infection was from the coprolite of a prehistoric human in Utah, which showed a significant number of *Moniliformis* eggs (Moore et al., 1969). These first findings made a sensation among acanthocephalan experts

(Schmidt, 1971). Acanthocephala findings also occurred in ancient mammal remains from United States and Brazil (e.g., Reinhard, 1990; Sianto et al., 2009). Currently, it is known that *M. 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). This suggests that humans, who inhabited in ECh cave during the last 2000 years, could have been exposed to this zoonotic parasite.

A search of the literature shows that most of the detailed parasitological studies on small mammals have been focused on other parasites, and that little knowledge exists on the acanthocephalan fauna of small mammals from South America. Schmidt (1972) noted that information about Oligacanthorhynchidae was confused and not clear. Richardson and Barger (2006) concluded, more than 30 years later, that this problem persists. The phylum Acanthocephala have simultaneously astonished and fascinated investigators attempting to understand their evolutionary and taxonomic relationships.

Futures studies are needed to clarify not only the specific filiation of the worms as more coprolites will be under study, but also the role of rodents and marsupials in the establishment of the acanthocephalan in the past, and the possibility of the small mammals to produce zoonotic illness on humans and animals in Patagonia.

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