

# Pan-American marine coastal distribution of the acanthocephalan *Profilicollis altmani* based on morphometric and phylogenetic analyses of cystacanths from the mole crab *Emerita brasiliensis*

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## Abstract

Thorny-headed acanthocephalan worms of the genus *Profilicollis* are widely distributed in the oceans of the world and present complex life cycles with intermediate and definitive hosts. The genus is still poorly known, with an unstable taxonomy and, for most species, incompletely characterized geographical distributions. In this study, based on molecular and morphological evidence, we report that the species *Profilicollis altmani* is also distributed along the South American Atlantic coast, using the mole crab *Emerita brasiliensis* as an intermediate host. As such, our record shows that *P. altmani* has a Pan-American distribution where five species of *Emerita* are utilized as intermediate hosts.

## Introduction

*Profilicollis* Meyer, 1931 encompasses polymorphid acanthocephalans with long necks, females and males with a fully ovoid proboscis, eggs with concentric membranes and decapods as intermediate hosts (Nickol *et al.*, 1999). The species richness of the genus is still unclear due to the taxonomic status of some forms being unstable as well as the lack of revisionary studies. As currently understood (e.g. Brouckerhoff & Smales, 2002; Tantaleán & Cárdenas, 2004; Amin, 2013; Goulding & Cohen, 2014), the genus encompasses nine species: *P. altmani* (Perry, 1942), which includes *P. bullocki* (Mateo, 1982), *P. kenti* (Van Cleave, 1947) and *P. texensis* (Webster, 1948) in its synonymy; *P. antarcticus* Zdzitowiecki, 1985; *P. arcticus* (Van Cleave, 1920); *P. botulus* (Van Cleave, 1916) (type species of the genus); *P. chasmagnathi*

(Holcman-Spector, Mane-Garzon & Dei-Cas, 1978); *P. formosus* (Schmidt & Kuntz, 1967); *P. major* (Lundström, 1942); *P. novaezelandensis* Brouckerhoff & Smales, 2002; and *P. sphaerocephalus* (Bremser in Rudolphi, 1819).

Three species, *P. altmani*, *P. antarcticus* and *P. bullocki*, were traditionally recognized as inhabiting the coasts of the South American Pacific Ocean. *Profilicollis antarcticus* uses the estuarine crab *Hemigrapsus crenulatus* as intermediate host. Meanwhile, *P. altmani* and *P. bullocki* have the mole crab *Emerita analoga* as intermediate host and different species of sea birds as definitive hosts (Torres *et al.*, 1993; Riquelme *et al.*, 2006). A recent phylogeographic study (Goulding & Cohen, 2014) corroborates the morphology-based suggestion made by Tantaleán & Cárdenas (2004) that *P. bullocki* is a subjective junior synonym of *P. altmani*. This latter species has a large bi-oceanic distribution and has been recorded along the Pacific coast of North America (California) and South America (Chile) and along the Atlantic coast of North America (Rhode Island, North Carolina) and the Gulf of Mexico (Florida

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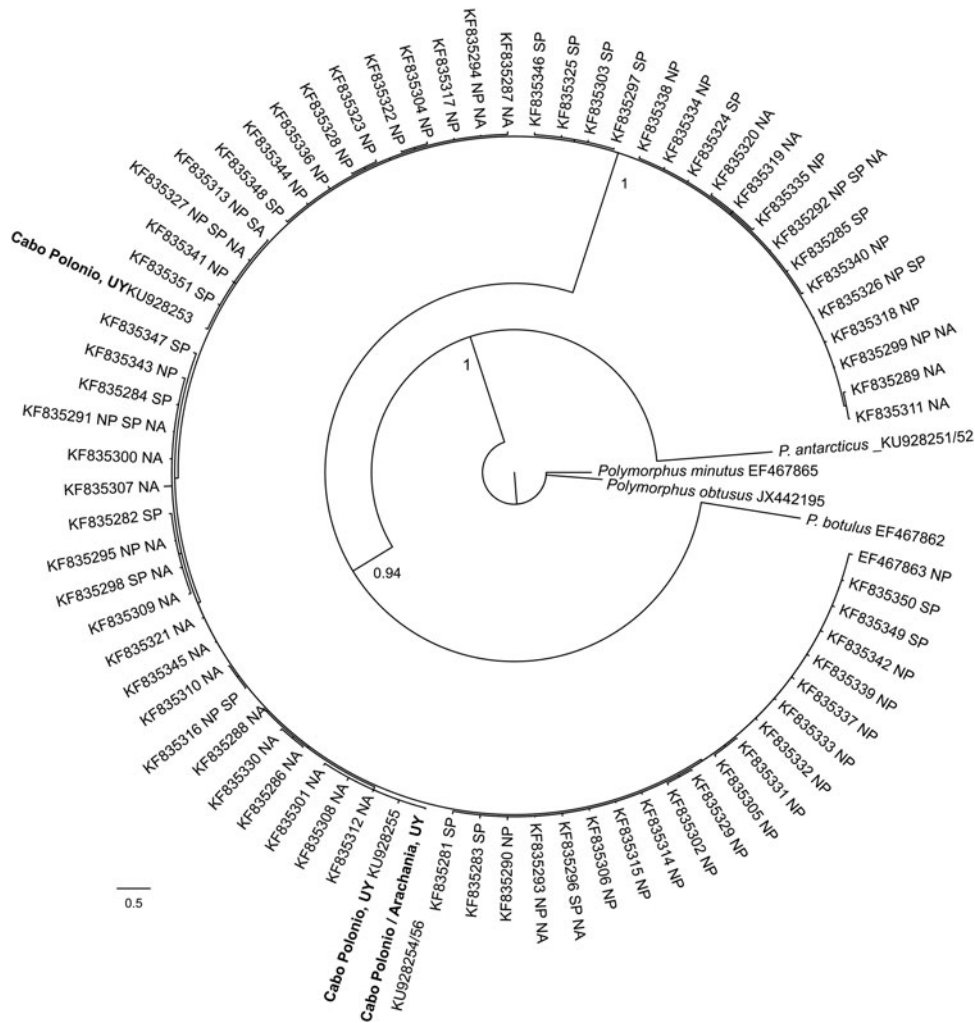


Fig. 1. Genealogical relationships of haplotypes of the COI gene of the genus *Profilicollis* using Bayesian analysis; support values correspond to posterior probability (only those for species and relationships among species are shown) with GenBank accession numbers given at the terminal labels. NP, northern Pacific Ocean; SP, southern Pacific Ocean; NA, northern Atlantic Ocean; UY, Uruguayan Atlantic Ocean.

and Mississippi). Meanwhile, for the South American Atlantic coast the thorny-headed worm *P. chasmagnathi* has been cited with the crab species *Neohelice granulata* and *Cyrtograpsus angulatus* as intermediate hosts (Holcman-Spector *et al.*, 1977; Alda *et al.*, 2011; La Sala *et al.*, 2012) and *Larus atlanticus* and *L. dominicanus* as definitive hosts (Diaz *et al.*, 2011; La Sala *et al.*, 2013). There is a mention of cystacanths of *P. altmani* infecting *Emerita brasiliensis* and *Calidris canutus* in Rio, southern Brazil (Buehler *et al.*, 2010). However, this record is ambiguous because the parasite species was mentioned in the publication as either *Profilicollis* sp. or *P. altmani*, and no indication of the evidence used to identify the specimens was given.

Here, by means of morphological and molecular evidence, we analyse a sample of *Profilicollis* retrieved from specimens of the mole crab *E. brasiliensis* collected on the southern Atlantic coast of Uruguay. We assign these specimens to

*P. altmani*, thereby extending the known geographical range of the acanthocephalan, previously shown to have a Pan-American distribution (Goulding & Cohen, 2014).

### Materials and methods

Thirty-seven specimens of *E. brasiliensis* were collected by hand on two sandy beaches of Uruguay: Cabo Polonio, Rocha (34°24.200'S, 53°47.700'W; December 2014) and Arachania, Rocha (34°37.179'S, 54°8.800'W; January 2016). Mole crabs were stored in 95% ethanol, transported to the laboratory and dissected for the presence of cystacanths. These were placed on distilled water to force osmotic eversion of the proboscis, examined under a stereomicroscope and identified using Nickol *et al.* (2002).

Table 1. Morphometrics (measurements in  $\mu\text{m}$  and mean values given in brackets) of cystacanths of *Profilicollis altmani* from the USA, Chile and Uruguay.

Study site	USA	Chile	Uruguay
Host	<i>E. talpoida</i>	<i>E. analoga</i>	<i>E. brasiliensis</i>
Source	Nickol <i>et al.</i> , 2002	Balboa <i>et al.</i> , 2009	This study
Total body length	3986–6017 (4949)	5000–6500 (5700)	4500–6200 (5533)
Maximum body width	624–845 (663)	470–1490 (1143)	660–915 (755)
Proboscis			
shape	Ovoid	Ovoid	Ovoid
length	518–648 (571)	500–900 (696)	505–730 (608)
width	230–364 (269)	329–600 (456)	383–610 (485)
Proboscis receptacle			
length	1718–2026 (1868)	1400–2970 (2562)	1600–2010 (1783)
width	–	240–470 (338)	318–450 (369)
Neck			
length	1000–1740 (1512)	850–1690 (1232)	805–1100 (939)
width	336–470 (406)	350–580 (473)	379–595 (461)
Rows of hooks	28	26–30	26–29
Hooks/row	12	14–16	14–15
Length of apical hooks	34–43 (37)	56	41–48 (44)
Length of medial hooks	41–53 (45)	73	48–61 (54)
Length of end hooks	43–57 (52)	90	52–79 (64)

Genetic comparisons and phylogenetic analyses were based on DNA sequences of the mitochondrial cytochrome oxidase I gene (hereafter *COI*; a fragment of 578 base pairs (bp)) gathered from four specimens. These sequences were integrated to a matrix composed of all *COI* sequences of *Profilicollis* available in GenBank (see fig. 1), with most sequences being recorded by Goulding & Cohen (2014). To enlarge the taxonomic coverage of *Profilicollis*, we also sequenced two specimens of *P. antarcticus* retrieved from specimens of the estuarine crab *H. crenulatus* collected at Niebla, Los Ríos, Chile (39°52.446'S, 73°24.041'W). As such, 206 sequences of *Profilicollis* were analysed. The outgroup was formed with sequences of two species of *Polymorphus*, the sister genus of *Profilicollis* (García-Varela *et al.*, 2013). All sequences were obtained using universal primers presented by Folmer *et al.* (1994) and the protocol outlined by García-Varela & Nadler (2006). Amplicons were sequenced using an external sequencing service (Macrogen, Inc. Seoul, South Korea). New DNA sequences were edited using CodonCode (Codon-Code, Dedham, Massachusetts, USA) and deposited in GenBank (KU928251–KU928256).

Sequences were aligned in MEGA 6 (Tamura *et al.*, 2013) using default parameter values. Observed genetic distances ( $p$ ) were calculated in MEGA 6. Phylogenetic relationships were inferred via Bayesian analyses (BA) using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). To reduce computational time we analysed a matrix consisting of only one sequence per haplotypic class (e.g. both specimens of *P. antarcticus* analysed share the same haplotype), as such the matrix included 77 sequences of *Profilicollis*. Analysis of each matrix consisted of two independent runs, each with five heated and one cold Markov chain. The substitution model HKY+G was selected using jModelTest (Darriba *et al.*, 2012). All model parameters were estimated in MrBayes. Uniform-interval priors were assumed for all parameters except base composition and substitution model parameters, which assumed a

Dirichlet prior. Runs were allowed to proceed for 20 million generations, with trees sampled every 1000 generations per chain. To check for convergence on a stable log-likelihood value, we plotted the log-likelihood values against generation time for each. The first 25% of the trees were discarded as burn-in and the remaining trees were used to compute a 50% majority rule consensus tree and to obtain posterior probability (PP) estimates for each clade.

## Results and discussion

Of the 37 specimens of *E. brasiliensis* examined, three were infected with *Profilicollis* (prevalence = 8.1%), with one, one and two parasites each (total = 4; mean intensity = 1.3). These four *Profilicollis* were sequenced and three distinct haplotypes were found. Observed average divergence of the Uruguayan sequences is 1.0%. In the genealogical analysis the haplotypes do not form a monophyletic group (fig. 1) and fall within the large clade of *P. altmani*, which is strongly supported (PP = 1). As such, the Bayesian analysis of DNA sequences suggests that cystacanths recovered from *E. brasiliensis* on the southern Atlantic coast belong to the same species, *P. altmani*, that has already been recorded on the Californian and Chilean Pacific coasts, as well as on the Atlantic coast of North America. Observed  $p$  distances among species of *Profilicollis* ranged from 22.2 to 25.1%.

A morphological assessment of the cystacanths collected in Uruguay points in the same direction, as it clearly differentiates them from *P. chasmagnathi* and *P. bullocki*, the other southern South American thorny-headed worms, in the number of hooks per head row as well as in the shape of the proboscis (table 1; Holman-Spector *et al.*, 1977; Balboa *et al.*, 2009). Cystacanths collected in Uruguay have an elongated body and an ovoid proboscis, with 26–29 longitudinal rows of 14–15 hooks each. Similarly, cystacanths from the Pacific coast of Chile have between 14 and 16 hooks per row (Balboa *et al.*,

2009; reported as *P. bullocki*). Interestingly, specimens of *P. altmani* from the Atlantic and Pacific coasts of the USA have 12 hooks in each longitudinal row (Nickol *et al.*, 2002).

Taken as a whole, the evidence suggests that cystacanths recovered from *E. brasiliensis* along the southern Atlantic coast belong to the same species, *P. altmani*, already recorded on the Californian and Chilean Pacific coast as well as on the Atlantic coast of North America. These results confirm that the acanthocephalan *P. altmani* has a Pan-American distribution with much variation (table 1), although the biological significance of this at the intermediate host stage remains unclear.

We also enlarge the list of intermediate hosts of *P. altmani*. Goulding & Cohen (2014) reported cystacanths of *P. altmani* parasitizing the mole crab species *E. analoga*, *E. talpoidea*, *E. benedicti* and *E. rathbunae*. Here we show that *P. altmani* also parasitizes *E. brasiliensis* (see also Buehler *et al.*, 2010).

As on the southern Pacific coast of South America, on the South American Atlantic coast two species of *Profilicollis* are also found. *Profilicollis chasmagnathi* uses crabs from estuarine environments as intermediate hosts (Holcman-Spector *et al.*, 1977; Martorelli, 1989; La Sala *et al.*, 2012) and the gulls *L. atlanticus* and *L. dominicanus* as definitive hosts (e.g. Díaz *et al.*, 2011; La Sala *et al.*, 2013). *Profilicollis altmani* has the mole crab *E. brasiliensis*, which inhabits sandy beaches, as intermediate host. Future studies should clarify the definitive host of *P. altmani* in this environment (but see Buehler *et al.*, 2010, who reported a mortality event of the sandpiper *Calidris canutus* that may be linked to the presence of *P. altmani*). Finally, the recording of *P. altmani* on the South American Atlantic coast is of relevance, given that infection with this parasite has caused the mortality of marine mammals in North America (Kreuder *et al.*, 2003; Mayer *et al.*, 2003). Similarly, there are suggestions that *P. altmani* can infect humans and domestic animals that accidentally ingest cystacanths when eating mole crabs (Tantaleán *et al.*, 2002; Rojas & Sebastián, 2011).

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### Conflict of interest

None.

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