

# Helminths of South American fur seals (*Arctocephalus australis*) from the Subtropical Convergence Zone of the Southwestern Atlantic

Helmintos de lobos-marinhos-sul-americanos (*Arctocephalus australis*) da Zona de Convergência Subtropical do Atlântico Sudoeste

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## Abstract

Parasites are important components of ecosystems and may contribute to the ecological aspects of their hosts and indicate the integrity of their environment. To identify the gastrointestinal helminths of the South American fur seal, *Arctocephalus australis*, 52 animals found dead on the Rio Grande do Sul coast, Southern Brazil, were necropsied. All studied animals were parasitized, and 104,670 specimens of helminths from three phyla and 14 taxa were collected. Adult specimens represented five of the identified species: *Contracaecum ogmorhini*, *Adenocephalus pacificus*, *Stephanoprora uruguayense*, *Ascocotyle (Phagicola) longa*, and *Corynosoma australe*; and one of the identified genera: *Strongyloides* sp. Immature forms represented the other eight taxa: Anisakidae gen. sp., *Anisakis* sp., *Pseudoterranova* sp., *Contracaecum* sp., Tetrabothriidae gen. sp., Cestoda gen. sp., *Corynosoma cetaceum*, and *Bolbosoma turbinella*. The acanthocephalan *C. australe* was the most prevalent and abundant parasite, whereas *Strongyloides* sp. had the highest intensity. This is the first record of the nematode *Anisakis* sp., digenean *S. uruguayense*, and acanthocephalan *B. turbinella* in this host. Trophic generalist species such as *A. australis* can be good indicators of the composition of the helminth fauna of their ecosystems, indicating the presence of zoonotic parasites transmitted by the consumption of fish.

**Keywords:** Neotropical region, Pinnipedia, parasite ecology, zoonoses.

## Resumo

Os parasitas são componentes importantes dos ecossistemas e podem contribuir com os aspectos ecológicos de seus hospedeiros e indicar a integridade de seus ambientes. Com o objetivo de identificar os helmintos gastrointestinais de Lobos-marinhos-sul-americanos, *Arctocephalus australis*, 52 animais, encontrados mortos no litoral do Rio Grande do Sul, sul do Brasil, foram necropsiados. Todos os animais estudados estavam parasitados, e 104.670 espécimes de helmintos de três filos e 14 táxons foram coletados. Foram representadas por espécimes adultos cinco espécies: *Contracaecum ogmorhini*, *Adenocephalus pacificus*, *Stephanoprora uruguayense*, *Ascocotyle (Phagicola) longa*, e *Corynosoma australe*; e um gênero: *Strongyloides* sp. Oito táxons foram representados por formas imaturas: Anisakidae gen. sp., *Anisakis* sp., *Pseudoterranova* sp., *Contracaecum* sp., Tetrabothriidae gen. sp., Cestoda gen. sp., *Corynosoma cetaceum*, e *Bolbosoma turbinella*. O acantocéfalo *C. australe* foi o parasita mais prevalente e abundante, enquanto *Strongyloides* sp. foi o de maior intensidade. Este é o primeiro registro do nematódeo *Anisakis* sp., do digenético *S. uruguayense* e do acantocéfalo *B. turbinella* neste hospedeiro. Espécies de elevado nível trófico como *A. australis* podem ser bons indicadores da composição da helmintofauna de seus ecossistemas, alertando-se para a presença de parasitas zoonóticos transmitidos pelo consumo de peixes.

**Palavras-chave:** Região neotropical, Pinnipedia, ecologia de parasitas, zoonoses.

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## Introduction

South American fur seals *Arctocephalus australis* Zimmermann, 1783 (Carnivora: Otariidae) are distributed on the coasts and islands of the Atlantic and Pacific Oceans of South America (Vaz-Ferreira, 1982), are recorded from the southeastern Brazilian coast (Pinedo et al., 1992) to the northern region of Peru (Cárdenas-Alayza et al., 2016), occurring intermittently along the southern cone of South America. In Brazil, there are no reproductive colonies, and the Rio Grande do Sul State's coast is the most frequented place for this species, especially during the winter and spring months, between July and October (Silva et al., 2014a). The animals that reach the Brazilian coast are mainly young males dispersed after the reproductive period (Simões-Lopes et al., 1995; Silva et al., 2014a).

Research on the composition of *A. australis* helminth fauna is scarce (Morgades et al., 2006; Silva, 2012; Hernández-Orts et al., 2013; Jacobus et al., 2016) and the study of specific groups of parasites predominates (George-Nascimento et al., 1992; George-Nascimento & Marin, 1992; Timi et al., 2003; Aznar et al., 2004; Echenique et al., 2020). Regarding human health, pinnipeds are definitive hosts of some helminth species that can be transmitted to humans through the consumption of raw or undercooked fish, especially *Pseudoterranova* and *Adenocephalus* (Raga et al., 2018).

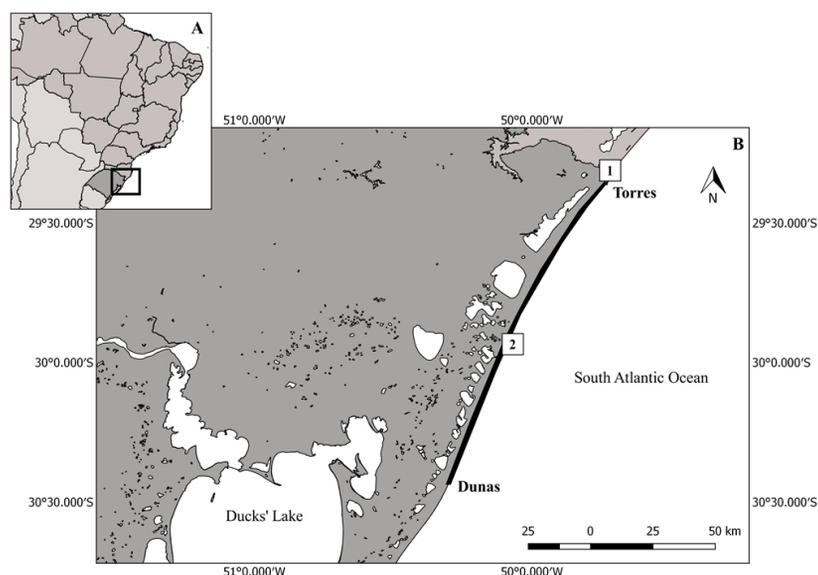
Parasites integrate biological communities and are fundamental for ecosystem balance, as they may influence the fitness of their hosts and lead to changes in the food chain, altering competitive interactions and availability of biomass (Hatcher et al., 2012). Macroparasites, an important tool for studies of phylogeny, migration, and social behavior of their host, can be used as biological markers in marine mammals (Balbuena & Raga, 1994; Aznar et al., 1995). They can also serve as bioindicators of marine ecosystem integrity (Mattiucci & Nascetti, 2008).

Little is known about the pelagic phase of South American fur seals in the South Atlantic Ocean, especially on the Brazilian coast, and knowledge of their parasites can contribute to elucidating aspects of their biology. Helminths collected from the gastrointestinal tract of stranded South American fur seals on the southern Brazilian coast were identified and related to host characteristics.

## Materials and Methods

### Study area

Beach monitoring was carried out in an area of the middle coast of Rio Grande do Sul, from Guarita beach, in Torres (29°21'32"S, 49°44'09"W) to Dunas Altas beach, Palmares do Sul (30°23'59"S, 50°17'16"W), representing 120 km of coastal line (Figure 1). This coastal area has sandy beaches with dunes, no recesses, and a low slope continental shelf. The climate is subtropical with temperature ranging from 16 to 20 °C and well-defined seasons (Seeliger et al., 1998).



**Figure 1.** Sampled area for the collection of *A. australis* beached carcasses at the Subtropical Convergence Zone, coast of Rio Grande do Sul, Brazil. (A) Location of the study area. (B) Sampling area (black stripe): from Torres beach to Dunas beach. Main drains of the study area: Mampituba River (1) and Tramandaí River (2).

## Animals

Fifty-two carcasses of *A. australis* found on the beaches were collected by the Monitoring Team of Center for Coastal, Limnological and Marine Studies (CECLIMAR) during the winters of 2012 (N = 47), 2016 (N = 3), and 2017 (N = 2) and forwarded to the CECLIMAR headquarters, where they were promptly stored in freezers at -20 °C. Carcass decomposition was evaluated based on the score proposed by Geraci & Lounsbury (1993). Only carcasses classified as code 2 (fresh) or code 3 (decomposed but intact) were included in the present study. Prior to necropsy, the carcasses were thawed at room temperature. Sex, age group, and biometric data (weight and total length expressed as mean  $\pm$  standard deviation), were obtained.

## Parasitological methods

The gastrointestinal tract was removed, separated into portions (stomach, small intestine, and large intestine), and opened in the longitudinal section. The other organs were cut open in plastic trays and washed thoroughly. The obtained contents were washed in running water over Tyler 100 metal mesh sieves, and the retained material was stored in Railliet and Henry's solution and inspected for helminths using stereomicroscopes. Parasites were sent to the Laboratory of Parasitic Diseases of the School of Agricultural and Veterinarian Sciences of the São Paulo State University (LabEPar, FCAV/Unesp) for identification.

The obtained helminths were clarified in 80% acetic acid solution and diaphanized in vegetable creosote if necessary. The cestodes and digenans were stained with hydrochloric acid. All measurements were expressed in mean ( $\mu\text{m}$ ) and were based on mature males and females for monoecious species and mature individuals for dioecious species. For morphological identification the keys of Delyamure (1969), Travassos et al. (1969), Khalil et al. (1994) and Anderson et al. (2009), and the papers of Sardella et al. (2005), and Fonseca et al. (2019), were used.

## Interpretation of data and parasite-host relationship

After the identification and counting of helminths, the ecological descriptors of infection prevalence, mean intensity, and mean abundance were calculated with confidence intervals according to Bush et al. (1997). The body condition index (BCI) of the host was determined by the ratio of body mass (kg) to total length (cm) (Arnould, 1995). Numeric variables were synthesized by mean, standard deviation and range in the form "mean  $\pm$  standard deviation (min-max)". Prior to statistical analyses, data distribution was evaluated using Kolmogorov-Smirnov and/or Shapiro-Wilk tests. The interaction of host sex and prevalence of infection was determined using Fisher's exact test, and the Mann-Whitney test was used to compare the total parasite intensity in relation to host sex. The relationship between the BCI and total parasite intensity was evaluated using Spearman's correlation coefficient. All tests were performed using GraphPadPrism 5.0 software, with  $P=0.05$ .

## Results

Sex, age group, total length, and body mass data were obtained for 42 of the 52 animals because of the poor conservation state of the animals. Thirty-four of them were males, with total length of (cm)  $98.27 \pm 18.03$  (80-159), and weight (kg)  $14.17 \pm 11.60$  (7.50-61.25). The eight females measured  $93.48 \pm 12.99$  (84.90-125.10) and weighed  $12.59 \pm 8.83$  (7.40-34.30). Only three of the studied animals were adults: two males and one female. The BCI of young females ranged from 0.080 to 0.126. Adult female had a BCI of 0.274. Among young males, the BCI ranged from 0.089 to 0.150, and adult males obtained a BCI of 0.361 and 0.385, respectively.

All the examined animals were parasitized by helminths. A total of 104,670 helminth specimens were collected, including 10,076 nematodes, 751 cestodes, 3,971 digeneans, and 89,872 acanthocephalans.

The nematodes *Contracaecum ogmorhini* (Johnston and Mawson, 1941) and *Strongyloides* sp., cestode *Adenocephalus pacificus* (Nybelin, 1931), digeneans *Stephanoprora uruguayense* (Holcman and Olagüe, 1989), *Ascocotyle (Phagicola) longa* (Ransom, 1920), and acanthocephalan *Corynosoma australe* (Johnston, 1937) were represented by mature specimens. Immature forms of Anisakidae gen. sp., *Anisakis* sp., *Pseudoterranova* sp., *Contracaecum* sp., Tetrabothriidae gen. sp., Cestoda gen. sp., *Corynosoma cetaceum* and *Bolbosoma turbinella* were found. *Corynosoma australe* had the highest prevalence (100%) and abundance (1716), whereas the highest intensity (8520) was exhibited by *Strongyloides* sp. (Table 1).

**Table 1.** Descriptors of infection observed on South American fur seals dispersed in the Subtropical Convergence Zone of the Southwestern Atlantic.

Species	Habitat	P (%) (95% CI)	MI (95% CI)	MA (95% CI)	Range
Nematoda					
Anisakidae					
Anisakidae gen. sp. (IF)	S & SI	25.00 (14.90-38.38)	23.0 (9.3-61.5)	5.8 (2.0-16.7)	1-153
<i>Anisakis</i> sp. (IF)	S & SI	9.60 (3.87-20.93)	8.8 (3.6-13.8)	0.8 (0.2-2.1)	1-16
<i>Pseudoterranova</i> sp. (IF)	S & SI	3.80 (0.69-13.16)	6.5 (3.0-6.5)	0.2 (0.0-0.9)	3-10
<i>Contracaecum</i> sp. (IF)	S & SI	38.46 (25.77-52.90)	47.6 (28.2-87.0)	18.3 (9.6-35.8)	4-248
<i>Contracaecum ogmorhini</i>	S & SI	9.60 (3.87-20.93)	49.8 (4.6-103.4)	4.8 (0.3-14.9)	3-138
Strongyloididae					
<i>Strongyloides</i> sp.	SI	1.90 (0.10-10.24)	8520*	163.8 (0.0-491.5)	-
Cestoda					
Cestoda gen. sp. (IF)	LI	5.76 (1.60-16.05)	1.30 (1-2)	0.1 (0.0-0.2)	1-2
Tetrabothriidae gen. sp. (IF)	SI	67.30 (52.91-79.06)	20.1 (12.5-32.5)	13.5 (8.1-22.7)	1-115
Diphyllobothriidae					
<i>Adenocephalus pacificus</i>	SI	19.2 (10.25-32.54)	4.4 (2.1-8.6)	0.8 (0.3-2.0)	1-17
Digenea					
Echinostomatidae					
<i>Stephanoprora uruguayense</i>	SI	30.80 (19.17- 45.14)	246.7 (73.3-605.8)	75.9 (20.6-208.2)	3-1590
Heterophyidae					
<i>Ascocotyle (Phagicola) longa</i>	SI	9.60 (3.87-20.93)	4.8 (2.0-7.8)	0.5 (0.1-1.2)	1-10
Acanthocephala					
Polymorphidae					
<i>Corynosoma australe</i>	S, SI & LI	100 (92.80-100)	1716.0 (1118.7-3232.8)	1716.0 (1116.8-3267.4)	2-22,380
<i>Corynosoma cetaceum</i> (IF)	S & SI	13.50 (6.46-2576)	4.43 (2-8.29)	0.6 (0.2 -1.5)	1-13
<i>Bolbosoma turbinella</i> (IF)	SI & LI	50.00 (36.45-67.79)	23.4 (11.9-50.69)	11.7 (5.5-26.6)	1-212

P: prevalence; MI: mean intensity; MA: mean abundance; CI: confidence interval; IF: immature forms; S: stomach; SI: small intestine; LI: large intestine.

\*Species found in a single host.

The total species richness was 14, ranging from one to seven. Larval forms and morphospecies were disregarded in the determination of this index. Four hosts (7.69%) were infected with only one species of helminth, another four (7.69%) with two species, 13 hosts (25.00%) with three, 16 (30.77%) with four, nine hosts (17.31%) with five, four hosts (7.69%) with six, and two (3.85%) with seven species of helminths. There was no difference in the prevalence of each parasite species according to the sex of the host. The comparison between the total parasite intensity and host sex was not significant ( $U=126.5$ ;  $P=0.7693$ ). The total parasite intensity and host sex in relation to IBC and host sex also did not show a statistically significant relationship ( $r=0.2602$ ;  $P=0.0502$ ). This is the first record of the nematode *Anisakis* sp., the digenetic *Stephanoprora uruguayense*, and the acanthocephalan *Bolbosoma turbinella* in South American fur seals.

## Discussion

The sample of animals in the present study was composed mainly of young males, representing 76.19% of the total ( $N=42$ ). This is because the sampled area is a post-reproductive dispersion zone used by animals from the colonies

of Cabo Polônio and Isla de Lobos, both in Uruguay (Oliveira, 2004). This was confirmed by the strong similarity of the helminth fauna observed in the parasitological findings described in that country (Morgades et al., 2006). The similar composition observed suggests that the foraging habits of the animals from the Brazilian shore are comparable to those of the animals in the Uruguayan colonies, or that the animals that reach the Brazilian coast are already infected by these parasites. Further studies focused on parasites of fish in Brazilian waters may help elucidate this.

Anisakid nematodes frequently parasitize the stomachs of mammals and seabirds (Mattiucci & Nascetti, 2008). Necropsy revealed three genera of this family: *Anisakis* Dujardin, 1845, *Pseudoterranova* Mozgovoï, 1951, and *Contracaecum* Railliet & Henry, 1912. *Anisakis* has already been described in odontocetes in Brazil (Carvalho et al., 2010) and *Anisakis typica* has been described in *Pontoporia blainvillei* (Gervais & D'Orbigny, 1844) in the same area of study (Silva & Cousin, 2006). *Contracaecum ogmorhini* is well documented in Otariidae (Mattiucci et al., 2003) including *Otaria flavescens* (Shaw, 1800) from the Rio Grande do Sul state coast (Machado-Pereira et al., 2017). However, adult parasites were found in only five animals, and most of the South American fur seals studied had immature forms of *Contracaecum* spp. The presence of immature forms may be associated with recent infections or could indicate that this species of helminth is not adapted to this host, since several species of *Contracaecum* have birds as their definitive hosts (Anderson, 2000).

The digeneans identified in this study have been previously described in *A. australis* in Uruguay (Morgades et al., 2006) and *O. flavescens* in Brazil (Pereira et al., 2013). The genus *Stephanoprora* had a higher prevalence than in the previously mentioned studies. Sea birds, such as *Larus dominicanus* (Laridae, Lichtenstein, 1823) and *Spheniscus magellanicus* (Spheniscidae, Forster, 1781), were also found to be naturally infected by *Stephanoprora uruguayense* and *Stephanoprora podicipes* (Núñez et al., 2004; Diaz et al., 2011; Brandão et al., 2013). Another digenean, *Ascocotyle (P.) longa*, is associated with coastal lagoons and estuaries and uses Mugilidae fish as the main intermediate host (Simões et al., 2010). Although species of Mugilidae are not described as feeding on *A. australis* (Naya et al., 2002), the general foraging characteristics of these pinnipeds may allow occasional predation of these fish. Another point to be considered is that this parasite can use other fish families as the second intermediate hosts in the evaluated region. Studies that allow the evaluation of the ecology of this digenean in the subtropical convergence zone of the Southwestern Atlantic can contribute to elucidating this issue.

*Corynosoma australe* is the most common acanthocephalan in otariids and it has the widest geographic distribution of the genus (García-Varela et al., 2021). These helminths can present high parasitic intensity without causing large inflammatory processes in *A. australis* (Silva et al., 2014b). Juvenile forms of *C. cetaceum* have been reported in otariids (Sardella et al., 2005); the prevalence was somewhat lower than that found in other studies on *A. australis*, but the intensity and abundance were similar (Hernández-Orts et al., 2013; Silva et al., 2014b). Following the contact/compatibility paradigm to explain host specificity patterns (Combes, 2001), Aznar et al. (2012) suggests that the compatibility filter prevents the establishment of *C. cetaceum* in *Otaria flavescens*, which can also occur in *A. australis*.

Tetrabothriidae gen. sp. and *Bolbosoma turbinella* (Diesing, 1851) were represented only by immature individuals but were the helminths that had higher prevalence after *C. australe*. *Bolbosoma turbinella* has cetaceans as the final host, and its cysticanths have been documented in Brazilian codling (*Urophycis brasiliensis*) (Pereira et al., 2014) and rough scad (*Trachurus lathami*) (Silveira et al., 2017) on the Brazilian coast. This species of fish is an important food item for *A. australis* throughout the eastern coast of South America (Naya et al., 2002; Oliveira et al., 2008), which may explain the high prevalence of *B. turbinella*, even though *A. australis* is an atypical host. The Tetrabothriidae gen. sp. did not present gravidic proglottids, but the scolex were similar to those found by Hernández-Orts et al. (2013) in *A. australis* in Argentina. *Adenocephalus pacificus* Nybelin, 1931, have broad distribution and low host specificity, occurring in several species of otariids (Hernández-Orts et al., 2015). Cestoda gen. sp. specimens were fragmented and did not have gravidic proglottids or scolex, which hindered their identification.

Helminth species of the Anisakidae and Diphyllbothriidae families, which have marine mammals as definitive hosts, have both economic and human health relevance (Oshima & Kliks, 1987). In the present study, we identified *Anisakis* sp., *Pseudoterranova* sp., *Contracaecum* sp., and *A. pacificus*, which belong to these families. In addition, the digenetic *A. (P.) longa* is responsible for heterophiosis in humans (Chieffi et al., 1990). Humans may act as accidental hosts for all these parasites and may be asymptomatic or present with nonspecific symptoms, such as abdominal pain, nausea, vomiting, diarrhea, fatigue, and anemia, resulting in a challenging diagnosis of the infection. Changes in human eating habits, such as increased consumption of ethnic dishes containing raw fish, may favor the emergence of foodborne diseases (Broglia & Kapel, 2011). Knowledge of the presence of these helminths on the Brazilian coast is essential for considering these parasites in the differential diagnosis of other diseases with similar signs and symptoms.

Although some species predominate in their diet, *A. australis* is a trophic generalist with the potential to prey on a wide range of species according to prey availability (Naya et al., 2002). Parasite diversity is not random within the food web and have cascading effects, being positively related to the upper levels of the trophic chain, so carnivores such as *A. australis* are a good model to study endoparasite richness (Chen et al., 2008; Rosalino et al., 2011). This is probably due to the fact that they frequently have expansive home ranges and distribution areas that cover a variety of landscape units, making them more likely to encounter opportunities for infestation; additionally, they typically have a wide prey range, which raises the likelihood that they will become infected by parasites; and finally, they are hosts to a wide variety of parasites with high interspecific variation (Rosalino et al., 2011).

## Conclusions

Most helminths reported in marine animals use the food chain as a form of transmission, favoring the encounter of a wide variety of underdeveloped parasites in unadapted hosts. Because South American fur seals have a wide distribution and diverse food habits, these pinnipeds are more likely to harbor nonspecific parasites. The present study identified 14 taxa, 8 in immature forms, which suggests that these animals may be good indicators of the composition of the local parasitic fauna, contributing to the recognition of important helminth species for human and animal health.

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## Ethics declaration

This study was approved by the Animal Ethics Committee of the FCAV/Unesp (protocol number 3063/2017).

## Conflict of interest

The authors declare no conflicts of interest.

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