

Prey species of franciscana *Pontoporia blainvillei* as paratenic hosts of helminths

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ABSTRACT: The distribution of franciscana *Pontoporia blainvillei* Gervais & d'Orbigny, 1884, is restricted to the estuarine and coastal waters of the southwestern Atlantic. The diet of this dolphin is composed of fishes, squids, and shrimps, many of which harbor helminths that may infect franciscana. Larval forms of the trematode *Synthesium pontoporiae* and the acanthocephalan *Bolbosoma turbinella* have been recorded in franciscana; however, they have not yet been identified in any of the prey species of this cetacean. We evaluated 3 components of the diet of franciscana as possible transmission sources of parasitiasis. Specimens of São Paulo squid *Doryteuthis sanpaulensis* (n = 50), banded croaker *Paralonchurus brasiliensis* (n = 43), and rough scad *Trachurus lathami* (n = 50) were necropsied. Organs were washed and examined under a stereomicroscope. Helminths were collected and mounted on slides. None of the species analyzed showed infection by metacercariae of *S. pontoporiae*. Helminths found in São Paulo squid have not been recorded in franciscana. Cistacanth of *Corynosoma australe* were found in the coelomic cavity and mesentery of croaker (prevalence [P] = 53.49%; mean infection intensity [MII] = 6.74) and scad (P = 4%; MII = 1.50). Cistacanth of *B. turbinella* were also found in the same sites in scad (P = 14%; MII = 2.14). Banded croaker and rough scad are recorded in this study as new paratenic hosts for *C. australe*, while scad is a new paratenic host for *B. turbinella*.

KEY WORDS: Cetacean · Banded croaker · Rough scad · Squid · Parasite · Marine

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INTRODUCTION

Pontoporia blainvillei Gervais & d'Orbigny, 1884 (Cetacea: Pontoporiidae), commonly known as franciscana or La Plata dolphin, is a small cetacean whose distribution is restricted to the estuarine and coastal waters of the southwestern Atlantic, up to 50 km from the coast and around 30 m depth (Pinedo et al. 1989). The species is found from Espírito Santo State, Brazil (18° 25' S, 30° 42' W) (Siciliano 1994), to San Matias Gulf, Argentina (~42° S) (Crespo et al. 1998).

The diet of franciscana is mainly composed of fishes between 7 and 540 mm in length (Rodríguez et al. 2002). Among the main prey fish taxa are Sciaenidae (represented by *Cynoscion guatucupa*, *Paralonchurus brasiliensis*, *Macrodon ancylodon*, *Micropogonias furnieri*, and *Umbrina canosai*), Egraulidae (*Anchoa marmorata* and *Engraulis anchoita*), Trichiuridae (*Trichiurus lepturus*), and Carangidae (*Trachurus lathami*). Squids such as *Doryteuthis sanpaulensis* and *D. plei*, and shrimps, including *Pleoticus muelleri*, *Farfantepenaeus paulensis*, and *Artemisia longinaris*, are also an important part of

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the diet (Danilewicz et al. 2002, Rodríguez et al. 2002, B. Silva 2011).

Studies on gastrointestinal helminth communities of franciscana have recorded the presence of *Anisakis typica*, *A. simplex*, *Corynosoma australe*, *C. cetaceum*, *Procamallanus* sp., and *Contracaecum* sp. mainly in the stomach cavities (Brownell 1975, Kagei et al. 1976, Praderi 1984, Aznar et al. 1995, Andrade et al. 1997, Aznar et al. 2003, Silva & Cousin 2004, Pinto et al. 2011). Other helminths such as *Bolbosoma turbinella* and *Synthesium pontoporiae* infect the large and small intestine, respectively, while both can be found in the stomach (Raga et al. 1994, Andrade et al. 1997, Silva & Cousin 2004, 2006, Marigo et al. 2008). Franciscana acquire parasites by ingesting fishes, mollusks, and crustaceans. Many fishes that make up their diet have been diagnosed with different developmental stages of helminths that affect franciscana (Alves & Luque 2000, Luque et al. 2003, Timi et al. 2005). However, the intermediate host of the trematode *S. pontoporiae* and of the acanthocephalan *B. turbinella* are not known.

Little is known about the life cycle of most trematode parasites of cetaceans; however, it is estimated to involve 1 or more species of marine crustaceans and mollusks as intermediate hosts (Marigo et al. 2002, Rocha 2010). Therefore, the parasitological investigation of some of these organisms could reveal the presence of metacercariae of *S. pontoporiae*, indicating a source of infection to cetaceans. Infections by adult *Bolbosoma* spp. normally occur in mysticetes, and it is assumed that transmission occurs by the ingestion of infected euphausiids (Measures 1992). Although adult *B. turbinella* have been reported in franciscana (Andrade 1996, Rocha 2010), the presence of females with immature eggs has only been mentioned in a few studies (e.g. Andrade 1996). Infection by cystacanths of *B. turbinella* in fishes is known in *Trachurus picturatus* in the North Atlantic (Costa et al. 2000), in *T. japonicus* along the coast of Japan (Ichihara 1968), and in *T. murphyi* in the Pacific southwest (George-Nascimento & Arancibia 1992, George-Nascimento 2000, Muñoz & Olmos 2008). In the South Atlantic, *Bolbosoma* sp. was reported in *Scomber japonicus* from the coast of Rio de Janeiro (Rego & Santos 1983).

Due to lack of knowledge about the life cycle of some helminths that infect franciscana, we evaluated banded croaker *Paralanchurus brasiliensis*, rough scad *Trachurus lathami*, and São Paulo squid *D. sanpaulensis*, 3 components of the natural diet of franciscana, as possible transmitters of parasitic infections, to clarify some aspects of the biology of parasitism in franciscanas.

MATERIALS AND METHODS

Fifty specimens of the São Paulo squid *Doryteuthis sanpaulensis* (mean \pm SD) mantle length = 8.7 ± 1.4 cm, body weight = 32.4 ± 13.9 g) were necropsied. The squids were captured along the coast of Rio Grande do Sul State, Brazil, and frozen at -20°C until the necropsy. The identification of the species followed Pineda et al. (2002). The eyes, gills, gill hearts, main heart, buccal mass, esophagus, digestive gland, stomach, caecum, intestine, ink sac, liver, gonads, spermatophoric organ, spermatophoric sac, nidamental glands, and glandular oviduct as well as cuts of mantle, gills, arms, and tentacles were analyzed.

Forty-three specimens of banded croaker (total length, TL = 19.9 ± 1.2 cm, weight = 78.2 ± 15.6 g) were necropsied. The fish were captured along the Cassino Beach coast, Rio Grande, Rio Grande do Sul State ($32^{\circ} 14' 30''$ S, $52^{\circ} 12' 16''$ W); they were frozen at -20°C until the parasitological analysis. The species identification followed Menezes & Figueiredo (1980). The eyes, gills, heart, stomach, pyloric caeca, intestine, liver, spleen, kidneys, gonads, and swim bladder were examined. Coelomic cavity and nasal cavity lavages, as well as musculature cuts, were also performed and analyzed for parasites.

Fifty specimens of rough scad (TL = 16.5 ± 0.7 cm, weight = 40.3 ± 4.5 g) were necropsied. The fish were captured along the coast between Santa Catarina and Rio de Janeiro States, Brazil. They were frozen at -20°C until the necropsy. The identification of the species followed Menezes & Figueiredo (1980). During the necropsy, stomach, intestine, caeca, gonads, spleen, and heart were removed and analyzed. Coelomic cavity lavages and musculature cuts were also analyzed.

The material was distributed among Petri dishes, washed in a $150\ \mu\text{m}$ mesh sieve, and observed under a stereomicroscope. Helminths were collected, counted, and processed according to Amato et al. (1991), fixed in alcohol-formaldehyde-acid solution, stained with hematoxylin, cleared in Amman's lactophenol solution (beechwood creosote for acanthocephalans), and mounted on permanent slides with Enthelan[®]. Parasitological indices, viz. prevalence (P), mean infection intensity (MII), mean abundance (MA), and infection intensity variation (IIV), were calculated according to Bush et al. (1997). The measurements cited are given in millimeters (mm). The average values for morphometric variables are presented followed by the range of measures in parentheses, and by the number of measured specimens, if different from the above-mentioned sample sizes.

RESULTS

Specimens of squid and fishes were infected with larval and adult forms of helminths. However, in the present study, only those parasites recorded as belonging to the helminth assemblage of franciscana are presented.

Metacercariae of *Synthesium pontoporiae* were not found in any of the prey species studied. None of the parasites found in squid samples in this study have been recorded in franciscana; therefore, we do not report the squid findings here. Cystacanths of *Corynosoma australe* and *Bolbosoma turbinella*, as well as larval and adult forms of the nematode *Procamallanus* (formerly '*Spirocamlanus*') *pereirai* were found in both examined fish species. Host species and their respective parasites are presented below. Parasitological indices and sites of infection are shown in Table 1. All measurements are given in mm.

Banded croaker *Paralanchurus brasiliensis*

C. australe measured 2.68 (2.26–3.20) long by 0.76 (0.58–0.88) wide in the disencysted cystacanth stage (n = 6; measurements are based only on cystacanths with the proboscis and terminal end extroverted), and 1.41 (1.40–1.42) by 0.6 (n = 2) when encysted. The proboscis, total or partly extroverted in disencysted cystacanths, is armed with 20 hook rows containing 14 hooks in each row. Three smaller hooks at the baseline are present in each row. Males contain 2 testes and 6 cement glands, and the posterior end is surrounded by genital spines. Females contain a developed ovary and the posterior end is surrounded by genital spines that are shorter than those of males.

P. pereirai in the third larval stage (L3), fourth larval stage (L4), and adult forms were found. L3 measured 3.29 (2.38–4.24) long by 0.057 (0.03–0.08) wide. The buccal capsule presents 18–20 ridges that are obliquely organized, running to the left in the anterior region, and a posterior smooth portion not reached by the ridges. The muscular esophagus is followed by the glandular esophagus, and the tail has 3 or 4 spines. The L4 stage measured 6.25 (5.73–7.28) by 0.108 (0.084–0.125; n = 3). The buccal capsule does not present the subdivision that occurs in L3, showing 12 to 13 sinuous ridges, running to the right, in the anterior region. The muscular esophagus is also followed by the glandular esophagus, but the tail tip has only 2 spines. Adults measured 15.07 (13.23–16.93) long by 0.24 (0.21–0.28) wide (n = 5). Their buccal capsule contains 14 to 17 ridges running to the right and a distinct transverse basal ring. Adults also have a muscular and a glandular esophagus. The posterior end of the body has caudal alae, 3 pairs of pre-cloacal papillae, and 6 post-cloacal papillae. Uneven spicules were observed, the right larger than the left, and gubernaculum was absent. The tail has ventral bending, with 2 sharp terminal processes. The only female that was measured had a body size of 26.50 by 0.32, presenting a buccal capsule with basal ring and 14 ridges formed like those of the males. It also exhibited a muscular and a glandular esophagus and a conical tail with 2 sharp projections homologous with those of males.

Rough scad *Trachurus lathami*

B. turbinella in the cystacanth stage has an armed proboscis with 22 to 24 longitudinal rows, comprised of 8 to 9 hooks each; the most basal hook of each row is reduced, showing a rounded root. The lemniscs are

Table 1. Parasites of banded croaker *Paralanchurus brasiliensis* and rough scad *Trachurus lathami* previously reported in franciscana *Pontoporia blainvillei*, with their parasitological indices and infection sites. P: prevalence (%); MA: mean abundance; MII: mean infection intensity; IIV: infection intensity variation

Helminth species (stage[s])	<i>Paralanchurus brasiliensis</i> (n = 43)					<i>Trachurus lathami</i> (n = 50)				
	P	MA	MI	IIV	Site	P	MA	MI	IIV	Site
<i>Bolbosoma turbinella</i> (cystacanth)	-	0	-	-	-	14	0.30 (±0.90)	2.14 (±1.46)	1–5	Coelomic cavity and mesentery
<i>Corynosoma australe</i> (cystacanth)	53.49	3.60 (±6.72)	6.74 (±8.00)	1–33	Coelomic cavity and mesentery	4	0.04 (±0.31)	1.50 (±0.7)	1–2	Coelomic cavity
<i>Procamallanus pereirai</i> ^a (L3, L4, and adult)	32.56	0.63 (±1.36)	1.93 (±1.82)	1–8	Intestinal lumen	-	-	0	-	-

^aNematodes that do not infect franciscana; see the 'Discussion'

long, thin, and twisted, as is characteristic of the species. The cystacanth TL was 7.5 (2.5–10.5) (n = 10).

Two disencysted cystacanths of *C. australe* were 1.66 (1.40–1.91) long by 0.48 (0.43–0.53) wide, and the only encysted one measured was 1.38 by 0.53. The general morphology was similar to the acanthocephalans collected in banded croaker.

DISCUSSION

In this work, rough scad was found to be a paratenic host of *Bolbosoma turbinella*, which represents a new fish host species for this parasite. Also, this study is the first record of a food item of franciscana carrying cystacanths of *B. turbinella*. This finding highlights that rough scad could be an important infection source to other odontocete species, because the latter do not prey on Euphasiaceae or Copepoda. Also, in the present study, banded croaker and rough scad are recorded as new paratenic hosts for *Corynosoma australe*. Since these fishes were sampled from or near the area where franciscana are infected, we propose that these species are important transmitters of this acanthocephalan.

The *B. turbinella* cycle involves pelagic marine zooplankton (Euphasiaceae and Copepoda) as intermediate hosts, several fish species as paratenic hosts, and mysticetes as the preferred definitive hosts (Measures 1992, Bush et al. 2001, Gregori et al. 2012). This parasite has been recorded as part of the component community of franciscana in southern Brazil, parasitizing the main stomach, pyloric stomach, small intestine, and large intestine (Andrade et al. 1997, Silva & Cousin 2006, Rocha 2010, D. Silva 2011). Among odontocetes, northern bottlenose whale *Hyperoodon ampullatus* and striped dolphins *Stenella coeruleoalba* have also been parasitized by *B. turbinella* (Measures 1992, Andrade 1996, Luque et al. 2010); sperm whales *Physeter macrocephalus* and Guiana dolphins *Sotalia guianensis* have a record of parasitism by *Bolbosoma* sp. (Hoberg et al. 1993, Luque et al. 2010). However, to date, the origin of infection by *B. turbinella* in franciscana was unknown, as none of the prey consumed by franciscana in any of their geographic areas of occurrence had been reported with cystacanths of this parasite.

C. australe is more typically an intestinal parasite common to pinnipeds and piscivorous birds (Aznar et al. 2012). This acanthocephalan has been recorded in the gastrointestinal tract of franciscana from southern Brazil (Andrade et al. 1997, Marigo et al. 2002, Luque et al. 2010, Rocha 2010). Although some fishes

preyed upon by franciscana have previously been identified with cystacanths of *C. australe* (Pereira & Neves 1993, Alves & Luque 2000, Sabas & Luque 2003, Sardella et al. 2005, Paso-Viola et al. 2015), most geographical areas where the fishes are infected do not coincide with those where franciscana are infected by this parasite.

Procamallanus spp. are components of marine and freshwater fish parasite communities (Pinto et al. 1974, Luque et al. 2011). However, some studies indicate *Procamallanus* spp. as components of cetacean parasite communities. *Procamallanus* sp. has been found in the stomach of franciscana in Uruguayan and Brazilian waters (Kagei et al. 1976, Marigo et al. 2002); though some authors have commented that franciscana is not the definitive host for this nematode (e.g. Rocha 2010). *P. pereirai* has already been reported in Brazil as parasitizing franciscana (Pinto et al. 1984, Vicente et al. 1985), but the presence of adult forms in banded croaker indicates that this fish is a definitive host of this helminth life cycle. Furthermore, the occurrence of *P. pereirai* in spotbuck skate *Atlantoraja* (formerly *Raja castelnaui*, a cartilaginous fish, also along the coast of Rio Grande do Sul (Knoff et al. 2001), suggests that it is a species with low host specificity. We consider the presence of *Procamallanus* spp. in the stomach of franciscana to be an accidental infection.

The hypothesis that the trematode *Synthesium pontoporiae* could be transmitted via ingestion of *Doryteuthis sanpaulensis* was not supported by our study, since metacercariae of this helminth were not found in the squid samples. Juvenile franciscana usually have high prevalence (close to 100%) and high mean intensity of this trematode (Andrade 1996, Marigo et al. 2002, Rocha 2010), which should exclude the need for a large sample in order to find the parasites.

Two teleosts, viz. banded croaker and rough scad, are reported here as new paratenic hosts of polymorphid acanthocephalans from the southwest coast of the Atlantic Ocean. Banded croaker was more important as a transmitter of *C. australe* in areas near the coast, with up to 50 m depth, whereas rough scad was more important as a transmitter of *B. turbinella*; it is possible that other fish species are involved in the coastal cycle of both helminths. As experimental studies involving marine mammals are not practical, research on parasitology of marine organisms through food chains requires more attention, so that we can better understand the life cycles of the organisms that heavily infect franciscana, such as the trematode *S. pontoporiae*.

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