# GASTROINTESTINAL HELMINTHS OF THE FRANCISCANA Pontoporia blainvillei (MAMMALIA: CETARTIODACTYLA) IN THE NORTHERN COAST OF RIO GRANDE DO SUL STATE, SOUTHERN BRAZIL

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## ABSTRACT

The franciscana, *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844), is an endemic dolphin species from the southwestern Atlantic, occurring exclusively in coastal waters of Brazil, Uruguay and Argentina. This species is considered threatened with extinction at both global and regional levels and is listed as õCritically Endangeredö (CR) according to the IUCN criteria in the state of Rio Grande do Sul (RS), southern Brazil. The franciscanas from RS and Uruguay are considered to be part of the same management stock, defined as Franciscana Management Area III (FMA III). This study evaluated the helminths of the stomach of 34 specimens of franciscana, incidentally caught in fishing nets or stranded in the northern coast of RS, between 1993 and 2010. Additionally, the helminths found in the small and large intestines of four of those specimens were also analyzed. Although six parasite taxa have been identified, based on infection levels and reproductive status, three of them were considered as the most important helminths of franciscanas: *Anisakis typica, Corynosoma cetaceum* and *Synthesium pontoporiae*. The parasite assemblages and the parasitological indices were different from those reported in the literature for southern RS (*i.e.*, Rio Grande). The most striking differences between the two areas were the lower prevalence of *C. cetaceum* as well as the absence of *C. australe* in the dolphins from northern RS. Based on these findings, we hypothesized the existence of an ecological differentiation on the helminth fauna of franciscanas inside the FMA III.

Keywords: cetacean; dolphin; helminth fauna; parasitism.

### **INTRODUCTION**

The franciscana dolphin, *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844), is the only living species of the family Pontoporiidae (Rice 1998). This small cetacean is restricted to the coastal waters of Argentina, Uruguay, and Brazil (Crespo 2009). The greatest threat to *P. blainvillei* is its incidental capture in coastal gillnets, mainly along the Brazilian coast (Ott *et al.* 2002). This species is the most endangered dolphin in the southwestern Atlantic (Secchi *et al.* 2002) and is currently classified as "Vulnerable" (VU) in the IUCN Red List of Threatened Species (Reeves *et al.* 2012), and as "Critically Endangered" (CR) by the Brazilian Ministry of Environment (MMA 2014).

In order to implement management actions to

conserve this species, understanding how its populations are structured is crucial. In this way, Secchi *et al.* (2003) compiled all available data on the species and identified four population stocks, called Franciscana Management Areas (FMAs I to IV). However, even within each stock, it is still possible to have ecological variations not yet detected that can be important to the conservation of the species (*e.g.*, Mendez *et al.* 2010, Cunha *et al.* 2014). Referring to franciscanasø stock inhabiting the coastal waters of Rio Grande do Sul (RS), southernmost Brazil, and Uruguay (FMA III), genetic studies indicate the need for a better definition of stock boundaries (Ott 2002, Cunha *et al.* 2014).

In this context, the characterization of helminths infections may be useful to define the ecological niche differentiation of aquatic animals, especially in cetacean populations (Dailey & Vogelbein 1991, Aznar *et al.* 1995, Mackenzie 2002). Comparisons of parasitological indices of one or more species of helminths among host groups within a given geographic area can help identify populations that are partially segregated, potentially comprising different ecological stocks (*e.g.*, MacKenzie 1987, Dailey & Vogelbein 1991, Aznar *et al.* 1995). However, helminthological studies with this particular group of mammals involve methodological limitations (Praderi *et al.* 1989, Torres *et al.* 1992, Aznar *et al.* 1995, Andrade *et al.* 1997).

Research on the helminths of P. blainvillei has been conducted since the 1970s (Kagei et al. 1976). Since 1990, the efforts and improvements to the methodology used in data collection have intensified, especially in relation to gastrointestinal parasites. Some studies have described the helminth fauna, as well as the infection levels of the helminths, especially in southern FMA III and FMA IV (Aznar et al. 1994, Andrade et al. 1997, Marigo et al. 2002, Rocha 2010). In those studies, few species of helminths infected P. blainvillei, and the stomach was the organ that harbored the largest number of species, due to its complexity and tissue compartmentalization (Andrade et al. 1997). No studies focusing on parasite data have been performed in franciscanas from the northern boundary of the FMA III, including the northern coast of the state of Rio Grande do Sul, Brazil.

The investigation on possible segregation of franciscana ecological stocks within FMA III is a priority, considering its broad shoreline distribution of about 1,168 km and the high levels of incidental mortality of the species in this region (*e.g.*, Ott *et al.* 2002). Given the lack of parasitological studies for the northern coast of RS, this study aims to identify the helminths species and estimate their infrapopulation parameters in franciscanas. Moreover, this study compares this data to the parasitological information available in literature of the FMA III in order to find possible ecological and parasitological differentiations.

### MATERIAL AND METHODS

The esophagus and stomach of 34 franciscanas were collected from by-caught or stranded dolphins in the northern coast of RS, Brazil between 1993 and 2010 by a marine mammal research team (Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul - GEMARS). The number of samples of each year were: 1993 (n=1), 1994 (n=1), 1995 (n=2) 1996 (n=1), 1998 (n=1), 2000 (n=3), 2002 (n=2), 2004 (n=4), 2005 (n=5), 2006 (n=5), 2007(n=3), 2008 (n=1), 2009 (n=2), 2010 (n=3). Additionally, the entire small and large intestines of four of those specimens were also analyzed for helminths recovery. The surveyed area covered about 250 km of exposed sandy beaches, from Torres (29°19'S, 49°43'W) to the Lagoa do Peixe National Park (31°15'S, 50°54'W).

Franciscanas were necropsied on the beach and the organs were kept frozen at -20°C to be analyzed in the laboratory. Total body length (TL) was measured for 17 franciscanas according to the methodology used by the American Society of Mammalogists (1961). The stage of decomposition of the specimens was evaluated based on the scale (from 1 to 5) proposed by Geraci & Lounsbury (2005), where stage one represents an alive animal, stage two represents a fresh sample and stage 5 indicates advanced decomposition. All the 34 franciscanas analyzed were in stage two or three. The gender of the specimens was identified, whenever possible, and the age group was estimated from the TL according to the methodology used by Danilewicz (2003) and Danilewicz et al. (2004). In this sense, dolphins up to 129.0 cm were considered immature, and greater than 130.0 cm were classified as mature.

Each stomach chamber (i.e., main stomach and pyloric stomach, Yamazaki et al. 1974) was analyzed separately. Parasites recovered from the esophagus of some franciscanas were considered from the main stomach. The small intestine was divided in five equal parts and each segment was analyzed separately. The large intestine was not segmented for the analysis. Each compartment was washed on a sieve with 150 µm mesh and all contents were inspected using a stereomicroscope (5 to 40 x) to collect helminths. The stomach and intestine walls were examined, and the attached worms, collected. All parasites were fixed and stained according to Amato et al. (1991). Species identifications were based on taxonomic keys and specific descriptions (e.g., Yamaguti 1958, 1961, 1963, Foreyt 2005, Marigo et al. 2008, Aznar et al. 2012).

Prevalence and mean intensity of infection

were calculated according to Bush et al. (1997). Prevalence was defined as the percentage of hosts in the sample infected by a helminth species; mean intensity of infection as the average number of a helminth species among the infected hosts, and mean abundance as the total number of helminths of each species divided by the total number of hosts. A Mann-Whitney U test was applied to compare mean intensity of infection and mean abundance between males and females. A Chi-square contingency test was applied to compare prevalence between males and females and between previously reported in southern RS (Andrade et al. 1997, Rocha 2010). The geographical localities from southern RS extended from Lagoa do Peixe National Park to Arroio Chuí (33°45'S; 53°22'W). We conducted the statistical analyses using the values of gastric helminths (i.e., main stomach+pyloric stomach). Significance level used was 0.05 in all the analyses.

# RESULTS

Twenty franciscanas' stomachs collected were from males and ten from females, and in four, the gender could not be determined. Total length varied between 70.0 cm and 159.6 cm (mean=107.8, sd=18.6), corresponding to 23 immature and six mature animals. For the other five, this information was not accessed. Twenty franciscanas were infected with at least one helminth species (overall prevalence of 58.8%). The total length of the infected individuals ranged from 85.7 cm to 155.5 cm.

Seven hundred and one helminth specimens were collected overall during the study period (Table 1). Six parasite taxa were found: Anisakis typica (Diesing, 1860), Raphidascaris sp. Railliet & Henry, 1915 (Nematoda: Anisakidae); Bolbosoma turbinella (Diesing, 1851), Corynosoma cetaceum Johnston & Best, 1942 (Acanthocephala: Polymorphidae); Synthesium pontoporiae (Raga, Aznar, Balbuena & Dailey, 1994) (Trematoda: Brachycladiidae), and one unidentified species of trematode (Hemiuridae) (Table 1). Anisakis typica, C. cetaceum and S. pontoporiae presented the highest prevalence and mean intensity of infection in both stomach and intestine of the analyzed specimens (Table 1). In relation to the stage of development of the helminths, it should be noted that only 6% of A. typica found were adults (i.e., with egg production or spicules clearly visible and developed). The two specimens of B. turbinella found in the pyloric stomach of a single franciscana were also sexually immature, the same occurring with Raphidascaris sp.. The sexual development of the Hemiuridae was not accessed.

		Site	of In	nfecti	on		Stomach (n:	=34)		Intestine (n=	<del>(</del>
Helminths	Ν	SM	$\mathbf{PS}$	SI	LI	P%	(ps) IIIM	(ps) WA	P%	(ps) IIIM	MA (sd)
Anisakis typica	150	х	х	Х		20.59	$21.43(\pm 20.40)$	4.41 (± 12.68)	25	1	$0.25(\pm 0.43)$
Bolbosoma turbinella	0		х			2.94	5	$0.06 (\pm 0.34)$	0	0	0
Corynosoma cetaceum	48	х	x		х	35.29	4 (± 5.87)	1.41 (± 3.98)	25	1	$0.25(\pm 0.43)$
Hemiuridae	61	Х	Х			17.65	$10.17(\pm 8.01)$	1.79 (± 5.13)	0	0	0
Raphidascaris sp.	1	х				2.94	1	$0.03 (\pm 0.17)$	0	0	0
Synthesium pontoporiae	439	х	х	x	x	38.24	33.77(± 87.02)	12.91 (± 56.26)	75	$125.33(\pm 87.40)$	94(± 93.13)
Total	701										

**Table 1.** Prevalence (P%), mean intensity of infection (MII) and mean abundance (MA) of helminth species recovered from the stomach (n=34)

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Regarding the host gender, we found no significant difference in prevalence, mean abundance or mean intensity for the helminth species (Tables 2 and 3). However, only males were infected with *B. turbinella* and *Raphidascaris* sp. (Table 2). Comparison between age classes was not performed due to small and unequal samples of juveniles and adults.

With respect to the comparison between the prevalence of northern and southern RS, five out of ten helminth taxa found were shared between these regions (Table 4). Among them, significant differences were observed between the present study and by Andrade *et al.* (1997) for *A. typica* and *C. cetaceum*, with larger prevalence of these species in Andrade's and col. study (Tables 4 and 5). We also observed significant differences between our results and the study of Rocha (2010) for *C. cetaceum*, with larger prevalence for that study in southern RS (Tables 4 and 5).

**Table 2.** Prevalence (P%), mean intensity of infection (MII) and mean abundance (MA) of gastrointestinal helminths of male and female franciscanas, *Pontoporia blainvillei*, on the northern coast of Rio Grande do Sul, southern Brazil (1993-2010) n=sample size: sd=standard deviation Parasites are listed in alphabetical order

		Males (n=20			Females (n	=10)
Helminths	P%	(ps) III	MA (sd)	P%	(ps) IIIW	MA (sd)
Anisakis typica	20	20.25(± 25.43)	$4.1(\pm 13.96)$	20	$31.5(\pm 5.5)$	$6.3(\pm 12.84)$
Bolbosoma turbinella	5	2	$0.1(\pm 0.44)$	0	0	0
Corynosoma cetaceum	30	$2.33(\pm 1.7)$	$0.7(\pm 1.42)$	50	$6.6(\pm 8.21)$	$3.3(\pm 6.68)$
Hemiuridae	5	1	$0.05(\pm 0.22)$	0	0	0
Raphidascaris sp.	5	1	$0.05(\pm 0.22)$	0	0	0
Synthesium pontoporiae	40	$51.25(\pm 107.26)$	$21(\pm 72.34)$	40	$2.69(\pm 2.6)$	$2.6(\pm 3.61)$

**Table 3** - Chi-squared test results (df=1) for prevalence (P%) and Mann-Whitney *U* test results (two-tailed) for mean intensity of infection (MII) and mean abundance (MA) of gastrointestinal helminths between males (n=20) and females (n=10) franciscanas, *Pontoporia blainvillei*, on the northern coast of Rio Grande do Sul, southern Brazil(1993-2010). Parasites are listed in alphabetical order.

-	P%		Ν	<b>1</b> II	Μ	[A
Helminths	$X^2$	р	<b>U-value</b>	р	U-value	р
Anisakis typica	0.000	>0.9999	2	0.4667	98	0.9442
Bolbosoma turbinella	0.517	>0.9999	-	-	95	0.8418
Corynosoma cetaceum	1.148	0.4253	8	0.2446	70.5	0.2713
Hemiuridade	0.517	>0.9999	-	-	87.5	0.5961
Raphidascaris sp.	0.517	>0.9999	-	-	95	0.8414
Synthesium pontoporiae	0.000	0.7170	14.5	0.8364	98.5	0.9681

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Table 4 - Prevalence (P%) and mean intensity of infection (MII) of helminths found in the stomach of Pontoporia
blainvillei in the northern (this study) and southern (Andrade et al. 1997, Rocha 2010) Rio Grande do Sul, southern Brazil
Gastric Helminths (GH); main Stomach (MS); pyloric Stomach (PS). Parasites are listed in alphabetical order.

		Northern RS			Southern ]	RS		
		Presen (n=	t study 34)	Andrade e (n=	<i>t al.</i> (1997) 53)	Rocha (n=	(2010) =44)	
Helminths	Infection site	P%	MII	P%	MII	P%	MII	
Anisakis simplex	GH	0	0	0	0	4.5	1	
	MS	0	0	0	0	3.1	1	
	PS	0	0	0	0	0	0	
Anisakis typica	GH	<b>21.9<sup>a</sup></b>	18.8	72.7 <sup>b</sup>	9	27.2 <sup>a</sup>	1.6	
	MS	17.7	23.5	56	4.8	18.7	1.6	
	PS	5.9	5.5	56	6.6	0	0	
Bolbosoma turbinella	GH	3.1	2	7	1.9	6.2	3.3	
	MS	0	0	7	1.3	6.2	1	
	PS	3.1	2	7	1.6	6.2	1	
Contracaecum sp.	GH	0	0	0	0	4.5	16	
	MS	0	0	0	0	3.1	16	
	PS	0	0	0	0	0	0	
Corynosoma australe	GH	0	0	40.9	5.6	27.2	3	
	MS	0	0	12	1	6.2	2	
	PS	0	0	29	7	6.2	4	
Corynosoma cetaceum	GH	<b>37.5</b> <sup>a</sup>	4	90.7 <sup>b</sup>	30.5	86.3 <sup>b</sup>	74.7	
	MS	29.5	1.6	29	3.3	68.7	3.7	
	PS	20.6	4.7	80	22.3	78.1	43.1	
Hemiuridae	GH	17.65	10.17	0	0	0	0	
	MS	11.76	10.75	0	0	0	0	
	PS	8.82	6	0	0	0	0	
Lecithochirium sp.	GH	0	0	0	0	4.5	16	
	MS	0	0	0	0	3.1	16	
	PS	0	0	0	0	0	0	
Raphidascaris sp.	GH	3.1	1	0	0	4.5	1	
	MS	3.3	1	0	0	3.1	1	
	PS	0	0	0	0	0	0	
Synthesium pontoporiae	GH	40.6	36.6	60.38	201.7	43.3	217.5	
	MS	20.6	5.8	27	11.2	43.7	10.8	
	PS	32.4	36.5	59	12.7	50	16.5	

Gastric values (GH) identified with different bold letters are statistically different from each other (p < 0.05).

**Table 5** - Chi-squared ( $X^2$ ) test results (df=1) for prevalence of helminths prevalence found in the stomach of *Pontoporia* blainvillei between the present study (northern RS) and the previous studies in southern RS (Andrade *et al.* 1997, Rocha 2010), in Rio Grande do Sul, southern Brazil. Parasites are listed in alphabetical order.

		Preval	ence	
	Andrade	et al. (1997)	Rocha	a (2010)
Helminths	$X^2$	р	$X^2$	р
Anisakis typica	23.347	0.0001*	0.465	0.4952
Bolbosoma turbinella	0.811	0.3678	2.318	0.1437
Corynosoma cetaceum	27.070	0.0001*	19.629	0.0001*
Raphidascaris sp.	1.577	0.2092	0.133	0.7149
Synthesium pontoporiae	3.065	0.0800	0.032	0.8589

\* Significant values (p<0.05).



# DISCUSSION

All the parasite taxa (except the Hemiuridae) found in the franciscanas in the present study have already been reported for this dolphin along its distributional range (*e.g.*, Aznar *et al.* 1994, Andrade *et al.* 1997, Marigo *et al.* 2002) (Table 6).

In the present study, dolphins with total length between 91.0 and 100.0 cm already presented most of helminth species harboured by franciscanas in the region. Weaning size in franciscanas from Uruguay and Argentina is around 100.0-105.0 cm (Rodríguez et al. 2002, Denuncio et al. 2013). In this early predation period, a variety of different preys are consumed by the species, including fishes, squids and crustaceans (Rodríguez et al. 2002, Denuncio et al. 2013). Therefore, the intermediate hosts of these parasites should reflect some of the earliest species of prey consumed by the franciscanas, as the sciaenid fishes [e.g., Cynoscion guatucupa (Cuvier, 1830) and Macrodon ancylodon (Bloch & Schneider, 1801)] (Perciformes: Sciaenidae), the long-Doryteuthis finned squid, sanpaulensis (Brakoniecki, 1984) (Myopsida: Loliginidae), and shrimps [e.g., Artemesia longinaris Spence Bate, 1888 (Decapoda: Penaeidae) and Pleoticus muelleri (Spence Bate, 1888) (Decapoda: Solenoceridae]. These species are also important preys of franciscanas in the northern coast of Rio Grande do Sul (Ott 1994, Bassoi 2005).

Regarding the gender of the host, we found no significant differences for prevalence, mean intensity of infection and mean abundance of the helminth fauna. In fact, the first analysis on the feeding ecology of P. blainvillei conducted in southern Brazil concluded that the prey species are basically the same, regardless of the sex or the age class of franciscanas (Pinedo 1982). Although more recent studies have found some tendencies in the prey composition of males and females in southern Brazil, these groups seem to share many prey species (Ott 1994, Bassoi 1997, 2005). Therefore, this similarity on the feeding ecology of males and females suggests that the parasitological indices should not present differences between gender, once franciscanas are infected by parasites through prey consumption.

**Table 6-** Comparison of identified gastrointestinal helminth species in *Pontoporia blainvillei* along its distribution, from Argentina to Brazil (RS=Rio Grande do Sul, PR=Paraná, SP=São Paulo and RJ=Rio de Janeiro. FMA=Franciscana Management Areas (*sensu* Secchi *et al.* 2003); S=stomach; I=intestine. Adapted from Marigo *et al.* (2002). Parasites are listed in alphabetical order.

II a base for the se	<b>C!</b> 4.	FMA IV		FMA III		J	FMA II	FMA I
Heiminuns	Sile	Argentina	Uruguay	South RS	North RS	PR	SP	$\mathbf{RJ}^{\mathrm{a}}$
Andracantha sp.		1						
Anisakis simplex	S	2		3				
Anisakis typica	S		4	3, 5	6			
Bolbosoma turbinella	S			3, 5	6			
Contracaecum sp.	S	2	7, 8	3				
Corynosoma australe	S	1		3, 5				
Corynosoma cetaceum <sup>b</sup>	S, I	1, 2	4, 7, 8	3, 5	6	9 <sup>c</sup>	Reviewed <sup>d</sup>	
Hemiuridae	S				6			
Lecithochrium sp.	S			3				
Pholeter gastrophilus	S	2						
Procamallanus sp.	S, I		4	3				
Raphidascaris sp.	S			3	6			
Synthesium pontoporiae <sup>e</sup>	S, I	2	3	3, 5	6	9,10	9,10	

<sup>a</sup> Santos et al. (1996) did not find any parasite in 42 franciscanas from Rio de Janeiro State, southeastern Brazil.

<sup>b</sup> Previously called *Polymorphus cetaceum*.

<sup>c</sup> Although referred as *Polymorphus* sp. by Marigo *et al.* (2002) and Marigo (2003), these specimens were later identified as *C. cetaceum* by ALS Valente (unpublished data).

<sup>d</sup> The presence of *Polymorphus* sp. (*i.e.*, *C. cetaceum*) in franciscanas from São Paulo was mistakenly reported by Marigo *et al.* (2002). The correct locality for this taxon is Paraná, as reported in Marigo (2003). J Marigo (personal communication).

<sup>e</sup> Previously called *Hadwenius pontoporiae*. References:
1-Aznar *et al.* 2012 (n=43)
2-Aznar *et al.* 1994 (n=46)

- 3-Rocha 2010 (n=44)
- 4-Kagei *et al.* 1976 (n=1)
- 5-Andrade et al. 1997 (n=53)
- 6-Present study (n=34)
- 7-Dailey & Brownell 1972 (n=unknown)
- 8-Brownell 1975 (n=191)
- 9-Marigo (2003) (n=56)

10-Marigo et al. 2002 (n=41)

Specimens of *A. typica*, *C. cetaceum* and *S. pontoporiae* recovered from the esophagus of some of the franciscanas were considered in an atypical site, because in most of the dolphins analyzed the former two species were found mainly in the stomach and the latter primarily in the intestine (*e.g.*, Aznar *et al.* 1995, Marigo *et al.* 2002, 2008). This abnormal finding suggests a post-mortem migration or parasite replacement due to reflux from the main stomach towards the mouth, and it has also been reported for

another small cetacean species (e.g., Berón-Vera et al. 2001).

The digenean *S. pontoporiae* was the dominant species among *P. blainvillei* helminths. This species only infects franciscanas (Raga *et al.* 1994) and occurs in the helminth assembly mainly on the small and large intestines of this dolphin along its entire distribution (see Marigo *et al.* 2002, 2008, Rocha 2010) (Table 6). *Synthesium pontoporiae* was not previously considered part of the helminth assembly of franciscanas along the Uruguayan coast (also within the FMA III) (Aznar *et al.* 1995), which was not supported by Rocha (2010), who found this parasite occurring with similar prevalence (P%>90) in both southern RS and Uruguayan dolphins.

The presence of Anisakidae predominantly in the stomach of franciscanas corroborates the results obtained in other studies of several species of cetaceans (Deardorff & Overstreet 1981, Dans et al. 1999, Berón-Vera et al. 2008). Similar to the findings of Andrade et al. (1997) in franciscanas from southern RS, most of the A. typica specimens collected in the present study were immature, which suggests, in both studies, a late recruitment, once all dolphins were sampled during the spring-summer seasons. It is also noteworthy that A. typica was recorded only in dolphins from RS and Uruguay and there are no findings of this anisakid in franciscanas from Paraná and São Paulo (ca. from 25°00'S to 25°30'S), Brazil (Table 6), further north of the study area (see Marigo et al. 2002). This species does not occur in franciscanas from Argentina, where the occurrence of its congeneric A. simplex has been reported (Aznar et al. 1995). This last species was also reported in a single franciscana from southern RS (Rocha 2010), but it was not recorded in the northern RS or in localities further north (Table 6).

The acanthocephalan C. cetaceum is known to infect mainly the pyloric stomach of P. blainvillei (Aznar et al. 1995, 2001, Andrade et al. 1997). In the present study, C. cetaceum had lower prevalence and mean intensity of infection levels than those reported for the southern coast of RS (Andrade et al. 1997) (Tables 4 and 5), Uruguay and Argentina (Aznar et al. 1995, 2001). Possibly, these observed differences are related to a low availability of intermediate hosts for this parasite in the northern RS. Previous records of the prevalence of C. cetaceum in franciscanas suggest that this parasite tends to increase from north to south, being higher in those studies from southern RS, Uruguay and Argentina (Aznar et al. 1995, Andrade et al. 1997). Moreover, along the Argentine coast (Aznar et al. 1995), infection levels of C. cetaceum were higher than those found by Andrade et al. (1997) in southern RS. Therefore, it seems that there is a latitudinal variation in the distribution of this species in the southwestern Atlantic, with a clinal distribution and higher parasitological indexes at higher latitudes. This hypothesis is also supported by the low levels of infection of *C. cetaceum* in franciscanas from the coast of Paraná (Marigo 2003), and the absence of this parasite species in franciscanas collected along the coasts of and São Paulo (Marigo 2003) and Rio de Janeiro (Santos *et al.* 1996), in southeastern Brazil (Table 6).

Hemiurid species are not historically considered as part of the component community of franciscanas (see Table 1 of Marigo *et al.* 2002), moreover, this family is known to infect fishes (Almeida *et al.* 2009). It is possible that our findings are related with recent fish digestion, once the helminth's cuticles were very fragile. This hypothesis can be confirmed only after the species identification.

The immature form, low prevalence and mean intensity of *Raphidascaris* sp. in both this study and Rocha (2010), along with previous records of this nematode in Brazilian waters exclusively from teleost and elasmobranch fishes (*e.g.*, Knoff *et al.* 2001, Fujimoto *et al.* 2012, Fonseca *et al.* 2016), indicate that its occurrence in franciscanas is accidental.

Based on our results *B. turbinella* seems to be a rare parasite for franciscanas, once only immature worms were found with low prevalence and mean intensity. However, this acanthocephalan has been observed in *P. blainvillei* from southern RS with higher prevalence, which could be explained by the use of a larger sample of intestines in the southern studies (Andrade *et al.* 1997, Rocha 2010), the preferred site of infection of this parasite in *P. blainvillei* (Andrade *et al.* 1997).

Despite the five helminth taxa shared between the present study and southern RS' one (Andrade *et al.* 1997, Rocha 2010), other five species were exclusively found in the southern region (Tables 4 and 6). The most striking differences were the presence of *C. australe* only in franciscanas from southern RS, and the higher prevalence of *C. cetaceum* in both studies from southern RS.

Although *C. australe* was recorded with prevalence around 30% in the southern area (Andrade *et al.* 1997, Rocha 2010), this acanthocephalan does not mature in franciscanas and its presence could be accidental or erratic due to the absence of adult worms.

In fact, Aznar et al. (2012), based on the presence of pregnant females, suggested that C. australe is specific to pinnipeds, whereas C. cetaceum is specific to cetaceans. The absence of C. australe in franciscanas of the northern coast of RS can be linked to differences in the diet of P. blainvillei, such as the variations in the consumption rate of the whitemouth croaker, Micropogonias furnieri (Desmarest, 1823) (Perciformes: Sciaenidae), which is an important intermediate host of C. australe (Pereira & Neves 1993). This fish species is not an important prey for franciscana in northern RS (Ott 1994) but it was an important component of the diet of this mammal in southern RS in the late 1970's (Pinedo 1982), although its occurrence in the diet composition of P. blainvillei decreased drastically since early 1990's (Bassoi 1997, 2005). This shift in diet could also be responsible for temporal differences observed in the infection levels of C. australe in southern RS, where Rocha (2010) found C. australe with lower prevalence than that found by Andrade et al. (1997) (Table 4).

Marigo *et al.* (2002) suggest that *S. pontoporiae* could be a useful biological tag for identifying franciscana populations along the species distribution. The present study suggests that *C. cetaceum* has also a great potentiality as an alternative biological tag for franciscanas within the FMA III as well between the FMAs. This species shows clear different levels of infection between the areas and seems to be more resistant and easier to collect, in comparison to *S. pontoporiae*, - a small and fragile trematode - distributed in approximately 40 m of intestine of *P. blainvillei*. The potential use of *C. cetaceum* as a biological tag for discrimination of franciscana's populations was previously mentioned by Aznar *et al.* (1995).

Nevertheless, the use of several different parasites as tags has been considered as a more reliable tool than using a single species (Catalano *et al.* 2014). In this sense, the lower prevalence of *C. cetaceum* in franciscanas observed in the northern RS in comparison to southern RS, as well as the absence of *C. australe* in northern RS, suggest the existence of ecological differences between franciscanas from these areas within the FMA III. This fact can be related to latitudinal changes of the temperature or other physical factors, which may influence on the intensity of infection of the parasite fauna of aquatic organisms (*e.g.*, Mosquera *et al.* 2003).

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### REFERENCES

- Almeida, F. M., Barquete, V. & Pereira, J. 2009. Progenetic metacercariae of *Parahemiurus merus* (Platyhelminthes, Digenea, Hemiuridae) infecting *Parasagitta friderici* (Chaetognatha) from southern coast Brazil. Atlântica, 31(1), 35-38. DOI: 10.5088/atl. 2009.31.1.35
- Amato, J. F. R., Boeger, W. A. & Amato, S. B. (Eds). 1991. Protocolo para laboratório, - coleta e processamento de parasitos de pescado. Rio de Janeiro: Universidade Rural do Rio de Janeiro: p. 78.
- American Society of Mammalogists. 1961. Standardized methods for measuring and recording data on the smaller cetaceans. Journal of Mammalogy, 42(4), 471-476. DOI: 10.2307/ 1377364
- Andrade, A. L. V., Pinedo, M. C. & Pereira, J. 1997. The gastrointestinal helminths of franciscana, *Pontoporia blainvillei*, in Southern Brazil. Reports of the International Whaling Commission, 47, 669-673.
- Aznar, F. J., Balbuena, J. A. & Raga, J. A. 1994. Helminths communities of *Pontoporia blainvillei* (Cetacea: Pontoporiidae) in Argentinian waters. Canadian Journal of Zoology, 72(4), 702-706. DOI: 10.1139/z94-094
- Azna,r F. J., Raga, J. A., Corcuera, J. & Monzón, F. 1995. Helminths as biological tags for franciscana (*Pontoporia blainvillei*) (Cetacea: Pontoporidae) in Argentinian and Uruguayan waters. Mammalia, 59(3), 427-435.
- Aznar, F. J., Bush, A. O., Balbuena, J. A. & Raga, J. A. 2001. *Corynosoma cetaceum* in the stomach of franciscanas, *Pontoporia blainvillei* (Cetacea): an exceptional case of habitat selection by an acanthocephalan. Journal of Parasitology, 87(3), 536-541. DOI: 10.1645/0022-3395(2001)087[0536:CCITSO] 2.0.CO;2
- Aznar, F. J., Hernández-Orts, J. S., Suárez, A. A., García-Varela, M., Raga, J. A. & Cappozzo, H. L. 2012. Assessing host-

parasite specificity through coprological analysis: a case study with species of *Corynosoma* (Acanthocephala: Polymorphidae) from marine mammals. Journal of Helminthology, 86, 156-164. DOI: 10.1017/S0022149X11000149

- Bassoi, M. 1997. Avaliação da dieta alimentar de toninhas, *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844), capturadas acidentalmente na pesca costeira de emalhe, no sul do Rio Grande do Sul. Instituto de Oceanografia da Universidade Federal do Rio Grande. p. 68.
- Bassoi, M. 2005. Feeding ecology of franciscana dolphin, *Pontoporia blainvillei* (Cetacea: Pontoporiidae), and oceanographic processes on the Southern Brazilian coast. National Oceanographic Centre, University of Southampton. p. 190.
- Berón-Vera, B., Pedraza, S. N., Raga, J. A., Pertierra, A. G., Crespo, E. A., Alonso, M. K. & Goodall, R. N. P. 2001. Gastrointestinal helminths of Commerson's dolphins *Cephalorhynchus commersonii* from central Patagonia and Tierra del Fuego. Diseases of Aquatic Organisms, 47(3): 201-208.DOI: 10.3354/ dao047201
- Berón-Vera, B, Crespo, E. A., Raga, J. A. 2008. Parasites in stranded cetaceans of Patagonia. Journal of Parasitology, 94(4), 946-948. DOI: 10.1645/GE-1296.1.
- Brownell, Jr., R. L. 1975. Progress report on the biology of the franciscana dolphin *Pontoporia blainvillei*, in Uruguayan waters. Journal of the Fisheries Research Board of Canada, 32(7), 1073-1078. DOI: 10.1139/f75-127
- Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. 1997. Parasitology meets ecology on terms: Margolis *et al.* revisited. The Journal of Parasitology, 83(4), 575-583. DOI: 10.2307/ 3284227
- Catalano, S. R., Whittington, I. D., Donnellan, S. C. & Gillanders, B. M. 2014. Parasites as biological tags to assess host population structure: guidelines, recent genetic advances and comments on a holistic approach. International Journal for Parasitology: Parasites and Wildlife, 3(2), 220-226. DOI: 10.1016/j.ijppaw.2013.11.001
- Crespo, E. A. 2009. Franciscana *Pontoporia blainvillei*. In: W. Perrin, B. Wursig & J. G. M. Thewissen (Eds.), Encyclopedia of marine mammals, 2nd ed. pp. 466-469. Amsterdam: Academic Press.
- Cunha, H. A., Medeiros, B. V., Barbosa, L. A., Cremer, M. J., Marigo, J., Lailson-Brito, J., Azevedo, A. F. & Solé-Cava, A. M. 2014. Population structure of the endangered franciscana dolphin (*Pontoporia blainvillei*): reassessing management units. PLoS ONE, 9(1), e85633. DOI: 10.1371/ journal.pone.0085633
- Dailey, M. D. & Brownell Jr. R. I. 1972. A checklist of marine mammal's parasites. In: S.R. Rigdway (Ed.), Mammals of the sea, biology and medicine. pp. 528-589. Springfield, IL: Charles C. Thomas.
- Dailey, M. D. & Vogelbein, W. K. 1991. Parasite fauna of three species of Antarctic whales with reference to their use as potential stock indicators. Fishery Bulletin, 89(3), 355-365.
- Danilewicz, D. 2003. Reproduction of female franciscana (*Pontoporia blainvillei*) in Rio Grande do Sul, southern Brazil. Latin American Journal of Aquatic Mammals, 2(2), 67-78. DOI: 10.5597/lajam00034

- Danilewicz, D., Claver, J. A., Secchi, E. R., Fontoura, N. F. 2004. Reproductive biology of male franciscanas (*Pontoporia blainvillei*) (Mammalia: Cetacea) from Rio Grande do Sul, southern Brazil. Fishery Bulletin, 102(4), 581-592.
- Dans, S. L., Reyes, L. M., Pedraza, S. N., Raga, J. A. & Crespo, E. A. 1999. Gastrointestinal helminths of the dusky dolphin, *Lagenorhynchus obscurus* (Gray, 1828), off Patagonia, in the southwestern Atlantic. Marine Mammal Science, 15(3), 649-660. DOI: 10.1111/j.1748-7692.1999.tb00834.x
- Deardorff, T. L. & Overstreet, R. M. 1981. *Terranova ceticola* n. sp. (Nematoda: Anisakidae) from the dwarf sperm whale, *Kogia simus* (Owen), in the Gulf of Mexico. Systematic Parasitology, 3(1), 25-28. DOI: 10.1007/BF00012236
- Denuncio, P. E., Bastida, R. O., Danilewicz, D., Morón, S., Rodríguez-Heredia, S. & Rodríguez, D. H. 2013. Calf chronology of the franciscana dolphin (*Pontoporia blainvillei*): birth, onset of feeding, and duration of lactation in coastal waters of Argentina. Aquatic Mammals, 39(1), 73-80. DOI: 10.1578/AM.39.1.2013.73
- Foreyt, W. J. 2005. Parasitologia veterinária: manual de referência. 5.ed. São Paulo: Roca: p. 240.
- Fonseca, M. C. G, Knoff, M., Felizardo, N. N., Di Azevedo, M. I. N., Torres, E. J. L., Gomes, D. C., Iñiguez, A. M. & São Clemente, S. C. 2016. Integrative taxonomy of Anisakidae and Raphidascarididae (Nematoda) in *Paralichthys patagonicus* and *Xystreurys rasile* (Pisces: Teleostei) from Brazil, International Journal of Food Microbiology, 235, 113-124. DOI: 10.1016/j.ijfoodmicro.2016.07.026.
- Fujimoto, R. Y., Sarmento, A. De M. B., Diniz, D. G. & Eiras, J.
  C. 2012. Nematode parasites of pescada Gó, *Macrodon* ancylodon Bloch and Schneider, 1801 (Osteichthyes, Sciaenidae), from Vila dos Pescadores, Bragança-PA, Brazil.
  Brazilian Archives of Biology and Technology, 55(6), 865-870. DOI: 10.1590/s1516-89132012000600009
- Geraci, J. R. & Lounsbury, V. J. 2005. Marine mammals ashore. A field guide for strandings, 2nd ed. Baltimore: National Aquarium: p. 371.
- Kagei, N., Tobayama, T. & Nagasaki, Y. 1976. On the helminthum of franciscana, *Pontoporia blainvillei*. Scientific Report of the Whales Research Institute, 28, 161-166.
- Knoff, M., De São Clemente, S. C., Pinto, R. M. & Gomes, D. C. 2001. Nematodes of elasmobranch fishes from the southern coast of Brazil. Memórias do Instituto Oswaldo Cruz, 96(1), 81-87. DOI: 10.1590/S0074-02762001000100009.
- Mackenzie, K. 1987. Parasites as indicators of host populations. International Journal for Parasitology, 17(2), 345-352. DOI: 10.1016/0020-7519(87)90109-3
- Mackenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology, 124 (7), 153-163. DOI: 10.1016/j.ijppaw.2013.11.001
- Marigo, J. 2003. Patologia comparada das principais enfermidades parasitárias de mamíferos marinhos encontrados na costa sudeste e sul do Brasil. Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo. p. 131. (Available at: http://www.biopesca.org.br/pdfs/2003/Marigo\_MSc\_ PatCompParasitariasMM.pdf)
- Marigo, J., Rosas, F. C. W., Andrade, A. L. V., Oliveira, M. R.,

Dias, R. A. & Catão-Dias, J. L. 2002. Parasites of franciscana (*Pontoporia blainvillei*) from São Paulo and Paraná states, Brazil. Latin American Journal of Aquatic Mammals, 1(1), 115-122. DOI: 10.5597/lajam00015

- Marigo, J., Vicente, A. C. P., Valente, A. L. S., Measures, L. & Santos C. P. 2008. Redescription of *Synthesium pontoporiae* n. comb. with notes on *S. tursionis* and *S. seymouri* n. comb. (Digenea: Brachycladiidae Odhner, 1905). Journal of Parasitology, 94, 505-514. DOI:10.1645/GE-1306.1
- Mendez, M., Rosenbaum, H., Subramaniam, A., Yackulic, C. & Bordino, P. 2010. Isolation by environmental distance in mobile marine species: molecular ecology of franciscana dolphins at their southern range. Molecular Ecology, 19(11), 2212-2228. DOI:10.1111/j.1365-294X.2010.04647.x
- MMA. Ministério do Meio Ambiente. 2014. Lista Nacional das Espécies da Fauna Brasileira Ameaçadas de Extinção. Portaria nº 444 de 17 de dezembro de 2014. Diário Oficial da União. <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/ index.jsp?jornal=1&pagina=121&data=18/12/2014>. (Accessed in 15/02/2017).
- Mosquera, J., de Castro, M. & Gomez-Gesteira, M. 2003. Parasites as biological tags of fish populations: advantages and limitations. Comments on Theoretical Biology, 8, 69-91. DOI: 10.1080/08948550302442
- Ott, P. H. 1994. Estudo da ecologia alimentar de *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844) (Cetacea, Pontoporiidae) no litoral norte do Rio Grande do Sul, sul do Brasil. Departamento de Zoologia da Universidade Federal do Rio Grande do Sul. p. 113.
- Ott, P. H. 2002. Diversidade genética e estrutura populacional de duas espécies de cetáceos do Atlântico Sul Ocidental: *Pontoporia blainvillei* e *Eubalaena australis*. Departamento de Genética da Universidade Federal do Rio Grande do Sul. p. 150
- Ott, P. H., Secchi, E. R., Moreno, I. B., Danilewicz, D., Crespo, E. A., Bordino, P., Ramos, R., Di Beneditto, A. P., Bertozzi, C., Bastida, R., Zanelatto, R., Perez, J. & Kinas, P. G. 2002. Report of the working group on fishery interactions. Latin American Journal of Aquatic Mammals, 1(1), 55-64. DOI: 10.5597/lajam00008
- Pereira, J. & Neves, L. F. M. 1993. Corynosoma australe Johnston, 1937 (Acanthocephala, Polymorphia) em Micropogonias furnieri (Desmarest, 1823) (Perciformes, Sciaenidae) do litoral do Rio Grande do Sul. Comunicações do Museu de Ciências da PUCRS. Série Zoologia, 6, 51-61.
- Pinedo, M. C. 1982. Análise dos conteúdos estomacais de Pontoporia blanvillei (Gervais & d'Orbigny, 1844) e Tursiops gephyreus (Lahille, 1908) (Cetacea, Platanistidae e Delphinidae) na zona estuarial e costeira de Rio Grande, RS, Brasil. Instituto de Oceanografia da Universidade Federal do Rio Grande. p. 95.
- Praderi, R., Pinedo, M. C. &, Crespo, E. A. 1989. Conservation and management of *Pontoporia blainvillei* in Uruguay, Brazil and Argentina. In: R. L. Brownell, J. Liu, W. F. Perrin & K. Zhou (Eds.), Biology and conservation of the river dolphins.

Occasional Papers of the IUCN Species Survival Commission 3. pp. 52-56. Lawrence: Allen Press.

- Raga, J. A., Aznar, J., Balbuena, J. A. & Dailey, M. D. 1994. *Hadwenius pontoporiae* sp. n. (Digenea: Campulidae) from the intestine of franciscana (Cetacea: Pontoporiidae) in Argentinian waters. Journal of the Helminthological Society of Washington, 61(1), 45-49.
- Reeves, R., Dalebout, M., Jefferson, T.A., Karkzmarski, L., Laidre, K., O'Corry-Crowe, G., Rojas-Bracho, L., Secchi, E., Slooten, E., Smith, B. D., Wang, J. Y., Zerbini, A. N., Zhou, K. 2012. *Pontoporia blainvillei*. The IUCN red list of threatened species 2012. e.T17978A17623386. Retrieved December 15, 2016, from http://dx.doi.org/10.2305/IUCN.UK.2012. RLTS.T17978A17623386.en.
- Rice, D. W. 1998. Marine mammals of the world: Systematics and distribution. Special publication (4). Lawrence: Society for Marine Mammalogy: p. 231.
- Rocha, A. 2010. Comunidade componente de helmintos gastrointestinais de *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844) do litoral sul do Rio Grande do Sul: relação com a dieta e determinação de estoques ecológicos. Instituto de Oceanografia da Universidade Federal do Rio Grande. p. 133.
- Rodríguez, D., Rivero, L. & Bastida, R. 2002. Feeding ecology of the Franciscana (*Pontoporia blainvillei*) in marine and estuarine waters of Argentina. Latin American Journal of Aquatic Mammals, 1(1), 77-94. DOI: 10.5597/lajam00012
- Santos, C. P., Rohde, K., Ramos, R. & Di Beneditto, A. P. 1996. Helminths of cetaceans on the southeastern coast of Brazil. Journal of the Helminthological Society of Washington, 63(1), 149-152.
- Secchi, E. R., Ott, P. H. & Danilewicz, D. 2002. Report of the IV Workshop for the coordinated research and conservation of the franciscana dolphin (*Pontoporia blainvillei*) in the western south Atlantic. Latin American Journal of Aquatic Mammals, 1(1), 11-20. DOI: 10.5597/lajam00003
- Secchi, E. R., Danilewicz, D. & Ott, P. H. 2003. Applying the phylogeographic concept to identify franciscana dolphin stocks: implications to meet management objectives. Journal of Cetacean Research and Management, 5(1), 61-68.
- Torres, P., Oporto, J. A., Brieva, L. M. & Escare, L. 1992. Gastrointestinal helminths of the cetaceans *Phocoena spinipinnis* (Burmeister, 1865) and *Cephalorhynchus eutropia* (Gray, 1846) from the southern coast of Chile. Journal of Wildlife Diseases, 28(2), 313-315. DOI: 10.7589/0090-3558-28.2.313
- Yamaguti, S. 1958. Systema helminthum, Trematoda. New York: Interscience: p. 1575.
- Yamaguti, S. 1961. Systema Helminthum, Nematoda. New York: Interscience: p. 679.
- Yamaguti, S. 1963. Systema Helminthum, Acanthocephala. New York: Interscience: p. 423.
- Yamazak, F., Takahashi, K. & Kamiya, T. 1974. Digestive tract of la Plata dolphin, *Pontoporia blainvillei* I. Oesophagus and Stomach. Okajimas Folia Anatomica Japonica, 51(1), 29-25. DOI: 10.2535/ofaj1936.51.1\_29

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