



Corynosoma cetaceum Johnston & Best, 1942 (Acanthocephala, Polymorphidae) in *Arctocephalus australis* Zimmermann, 1783 (Mammalia: Pinnipedia): Histopathology, parasitological indices, seasonality and host gender influences

Corynosoma cetaceum (Acanthocephala: Polymorphidae) em
Arctocephalus australis (Mammalia: Pinnipedia): histopatologia, índices
parasitológicos, sazonalidade e influência do gênero sexual do hospedeiro

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Abstract

The parasitological indices [Prevalence (P%), Mean Intensity (MI), Mean Abundance (MA) and the Range of Variation (Ax)] of *Corynosoma cetaceum* in *Arctocephalus australis* and the histopathology caused by this parasite are presented based on the necropsy of 32 specimens found stranded dead on the shore of Rio Grande do Sul, Brazil, from 2008 to 2010. Differences between the genders of the hosts as well as for their reproductive status [males (pups, prepubertals) and females (pups, reproductive-breeding)] were analyzed for P% (Chi-square test, χ^2) and MI (Bootstrap Confidence Interval, BCI_p , $p < 0,05$) and between genders of the hosts for season groups (Spring-Summer: S-S; Autumn-Winter: A-W). Abnormal tissue samplings of the stomachs (histopathology caused by the parasite) and the gonads (for analysis of the sexual maturity) of the hosts were submitted to the histological procedure of paraffin embedding and mounting of permanent slides in balsam. The infection occurred along the sampling period with low P%, MI and MA (respectively) for the genders of the hosts (males: 37.5, 2.83 and 1.5; females: 31.3, 3 and 1), for the reproductive status of the hosts (males pups: 25, 1.33 and 1; prepubertal males: 75, 4.33 and 5; female pups: 28.6, 2.5 and 1; reproductive-breeding females: 50, 5 and 5) and for season groups (S-S: 16.7, 4 and 0.67; A-W: 45, 2.78 and 1.25). Concerning to the hosts, there were no parasitological indices-related differences between genders and sexual maturation status. There were no season-related differences for P% and MI. The results suggest an accidental infection of *C. cetaceum* in *A. australis* despite the absence of severe inflammatory processes added with the parasite's inadequate development.

Keywords: Accidental infection. Arctocephalinae. Corynosomiasis. Parasite attachment. Stomach abnormalities.

Resumo

Índices parasitológicos [Prevalência (P%), Intensidade Média de Infecção (MI), Abundância Média (MA) e Amplitude de Variação (Ax)] de *Corynosoma cetaceum* em *Arctocephalus australis* e a histopatologia causada por este parasito são apresentados com base na necropsia de 32 espécimes encontrados mortos e encalhados no litoral do Rio Grande do Sul, Brasil, entre 2008 e 2010. Os gêneros sexuais e condições reprodutivas dos hospedeiros [machos (filhotes, pré-púberes) e fêmeas (filhotes, reprodutivas-reprodutoras)] foram analisados para P% (Qui-quadrado, χ^2) e MI (Intervalo de Confiança via "Bootstrap", BCI_p , $p < 0,05$) e entre os gêneros sexuais dos hospedeiros para os grupos sazonais (Primavera-Verão: P-V; Outono-Inverno: O-I). Amostras de tecido estomacal anormal (histopatologia causada pelo parasito) e as gônadas dos hospedeiros (análise da maturidade sexual) foram submetidas ao procedimento histológico de inclusão em parafina e montagem de lâminas permanentes em bálsamo. A infecção ocorreu durante todo o período amostral com baixas P%, MI e MA (respectivamente) para os gêneros sexuais dos hospedeiros (machos: 37,5; 2,83 e 1,5; fêmeas: 31,3; 3 e 1), para as condições reprodutivas de cada gênero sexual dos hospedeiros (machos filhotes: 25; 1,33 e 1; machos pré-púberes: 75; 4,33 e 5; fêmeas filhotes: 28,6; 2,5 e 1; fêmeas reprodutivas-reprodutoras: 50; 5 e 5) e para os grupos sazonais (P-V: 16,7; 4 e 0,67; O-I: 45; 2,78 e 1,25). Não houveram diferenças parasitológicas de acordo com os gêneros sexuais ou nas categorias de maturidade sexual dos hospedeiros. Grupos sazonais não diferiram em P% ou MI. Os resultados sugerem tratar de uma infecção acidental de *C. cetaceum* em *A. australis*, apesar da ausência de processos inflamatórios severos em adição com o desenvolvimento inadequado dos parasitos.

Palavras-chave: Infecção acidental. Arctocephalinae. Corynosimíase. Ancoragem parasitária. Anormalidade estomacal.

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Introduction

Helminthiasis commonly occurs in marine mammals populations (Geraci & Aubin, 1979) and there is no doubt that the gastrointestinal helminths are highly successful groups to adapt to their micro-environment (Hayunga, 1991). Definitive hosts, as aquatic mammals, have tissue abnormalities caused by helminths (Dailey & Stroud, 1978; Silva & Cousin, 2004, 2006a, b; Motta et al., 2008) due to several parasite mechanisms, such as oral apparatus actions, body holdfasts and/or enzymes (Thomson, 1983; Hayunga, 1991). In aquatic mammals, pathologies *sensu lato* are generally and simply recorded as macroscopic necropsy findings of tissue alterations (Abollo et al. 1998; Liskins, 2002; Mazzariol et al. 2007) but there is a paucity of microanatomical analysis (Parsons & Jefferson, 2000; Spraker et al., 2003; Jaber et al., 2004, 2006; Silva & Cousin, 2004, 2006a, b; Motta et al., 2008; Colegrove et al., 2009).

The reports for diseases in marine mammals are technologically biased showing peaks according to temporal die-offs of host species (i.e., helminths, biotoxins, viruses, bacteria, human interactions) and temporal technologic advances (Gulland & Hall, 2007). Concerning to the marine mammals, the helminthological studies were focused to understand the mass strandings of species with strong social behavior to formation of herds, mainly in the 1970s and 1980s (Ridgway & Dailey, 1972; Dailey & Stroud, 1978; Geraci, 1978, Geraci & Aubin, 1979; Colongue et al., 1985). Large scale die-offs receive considerable attention and concern by scientists, politicians, managers and public thinking about the deteriorating ocean "health" because "charismatic" marine mammal species have been affected by several sources of human impact (Jennings et al., 2001; Reddy et al., 2001a, b; Reddy & Ridgway, 2003; Bossart, 2006, 2007; Gulland & Hall, 2007). After intense investigations for parasite-related mass die-offs of marine mammals, parasites were broadly used to investigate the social structure and stock identity of their hosts from middle of the 1990s (Balbuena et al., 1995; Valente et al., 1997).

Several Pinnipedia are definitive hosts for *Corynosoma* spp. (Acanthocephala, Polymorphidae) (Van Cleave, 1952; Zdzitowiecki, 1984, 1986; Raga et al., 2002; Valtonen et al., 2004), including the South American fur seal *Arctocephalus australis*, the most common stranded species in Southern Brazilian shore

(Pinedo, 1990; Silva, 2004). Although Ridgway (1972), Bonner (1990) and Vlasman and Campbell (2003) consider Acanthocephala as a group of little pathological parasites for Pinnipedia, there are evidences of Acanthocephala-related mortality for mammals (Ponce de León, 2000; Kreuder et al., 2003; McKenzie et al., 2005; Taman, 2009). There is lack of information, whether anthropogenic-related or parasitosis-related, concerning to the true incidence of diseases in marine mammals. This is probably a consequence of the historical focus on domestic animal health and its economic implications rather than wildlife diseases and conservation of the wild animal populations (Gulland & Hall, 2007). Several ecological studies were carried out for *Corynosoma* spp. (George-Nascimento & Marín, 1992; Aznar et al., 2001, 2004; Sinisalo et al., 2004; Valtonen et al., 2004; Sardella et al., 2005) but histopathological aspects of corynosomiasis on mammalian hosts are scarcely known (Silva & Cousin, 2006b) as well as parasitological indices for *A. australis*. This corroborate the broad paucity concerning to the microanatomical analysis of lesions caused by helminths that are found in aquatic mammals (Parsons & Jefferson, 2000; Ruoppolo, 2003; Spraker et al., 2003; Jaber et al., 2004, 2006; Silva & Cousin, 2004, 2006a, b; Motta et al., 2008, Silva et al., 2013). Moreover, several intrinsic features of the hosts such as age, sex and immunity (Combes, 1997; Wilson et al., 2002; Klein, 2004; Khokhlova et al., 2010), as well as, the intra- inter-specific parasite relationships (Mettrick & Podesta, 1974; Wilson et al., 2002) tend to drive the parasitological indices (infection levels) or parasite distribution patterns (pattern of parasite spread within the host population) for a male-biased higher levels on several parasitosis, such as, arthropodiasis, nematodiasis and acanthocephalosis (Barriga & Al-Khalidi, 1991; Cowan et al., 2007; Ferrari et al., 2007; Khokhlova et al., 2010). On the other hand, female-biased helminthiasis such as cestodiasis (Barriga and Al-Khalidi, 1991; Escobedo et al., 2004, Morales-Montor et al., 2004) and nematodiasis (Rossin et al., 2010), are recorded. Klein (2004) postulated that according to the Prevalence and Mean Intensity there is a male-biased susceptibility for parasitic infections in mammals than in females. On the other hand, Morales-Montor et al. (2004) refuse this conception and discuss that there is no a female host supremacy paradigm against the parasitic infection. Moreover, Wirsing et al. (2007) indicate that the gender-biased parasitic levels depend of the analysis context.

The gastric histopathological pattern and parasitological indices of *C. cetaceum* in *A. australis* are presented according to the gender of the host and seasonality.

Materials and methods

Hosts sampling: The specimens of *A. australis* (16males: 16 females) were collected from Cassino Beach (ca. 32°11'14,23"S, 52°09'21,70"W) to Chui (ca. 33°44'35,96"S; 53°22'12,70"W), Rio Grande do Sul State, Brazil. The total lengths (TL) of the hosts were measured linearly in meters (m) (following Dierauf 1994) and their genders were determined by external examination. Normal and abnormal (attached *C. cetaceum*) tissue samplings were taken from the stomachs (fundic and pyloric regions). Samplings for hosts were performed within two major seasons grouping, i.e., Autumn-Winter (A-W) and Spring-Summer (S-S) from 2008 A-W to 2010 A-W.

The conservation status of carcasses utilized in this study followed Dierauf (1994). Concerning to this author, only carcasses code 2 and 3 were collected for necropsy procedures. Additionally other post-mortem alterations were observed, such as *rigor-mortis*, *livor-mortis*, abdominal expansion by decomposition gases and absence of decomposers (as larval forms of insects) (Thomson, 1983).

The opening of the thoracic and abdominal cavities for the removal of the digestive system, reproductive organs and gross analyses of the organs were performed according to Dierauf (1994). Thus, the digestive tube was divided in its anatomical parts (esophagus and stomach, small and large intestines). These parts were tied, labeled, bagged and frozen for the parasite screening. The small intestine was separated from the mesentery and from the large intestine, linearly measured and divided in a cranial portion and other caudal portion for analyze the parasite's distribution.

Sexual maturity of the hosts: The entire reproductive system of the females was extracted for analysis of the sexual maturity as well as the male gonads (both left and right ones) with epididymis. The stereomicroscopy in the female gonads was performed to observe ovarian follicles, luteogenesis and luteolysis *sensu* Bukovsky et al. (2005), Katz et al. (2009), Lucacin and Pinto-Neto (2009) and Palma-Cerda et al. (2011). Ovaries (both left and right ones) were separate from the uterus and ovarian sections

(1-2mm) were performed and observed for folliculogenesis. The histomorphology of the spermatogenesis followed Wistuba et al. (2007).

Histological protocols: Histological samplings of the testicles were made by transversal sections at the center of the gonad in order to analyze the epididymis. Midsagittal sections from both ovaries were submitted to the histological routine processing. Tissue samplings from stomachs and gonads were fixed (Bouin's fluid) for histological routine by using paraffin-embedding method; sectioned (7µm thickness); stained (Hematoxylin-Eosin) and mounted on permanent slides with balsam (Silva & Cousin, 2004, 2006 a, b). According to the spermatogenesis, the male hosts can be categorized as: pups (spermatogonia and spermatocytes), prepubertal (spermatids); pubertal (spermatozoa within seminiferous tubules = testicular spermatozoa) and adult reproductive (spermatozoa within epididymis) (Wistuba et al., 2007). Female hosts can be categorized for the sexual maturity according to the follicle-type population within ovarian stroma as: pups (mainly presence of primary follicles); prepubertal (presence of Antral and Graafian follicles) and adult reproductive-breeding females (presence of corpus luteum-corpora albicans) (Bukovsky et al. 2005, Katz et al., 2009, Lucacin & Pinto-Neto, 2009, Palma-Cerda et al., 2011).

Parasite protocols: The stomachs of the hosts were opened and washed into a plastic bowl. The small and large intestines were separated. The small intestines were separated from the mesentery to be linearly measured. They were divided in two equal portions (first and last halves) of equal lengths. Each small intestine half was divided in subsets to be opened and washed into a plastic bowl. The parasites were detached (when no free within the intestine lumen) from the intestine wall according to each subset from each small intestine half. The content from the plastic bowl for the stomach as well as for each subset from each intestine half was sieved and observed under stereomicroscope for parasite's screening. Parasites were collected and counted according to stomach and each half of the small intestine as well as for the large intestine. They were prepared to be mounted on permanent slides with balsam and stained (Semichon's Carmim and Eosin) following a protocol adapted from Amato et al. (1991). Parasites were identified *sensu* Petrochenko (1971) and Sardella et al. (2005) according to the criteria of the holdfast: proboscis oncotaxia (structure and distribution pattern of the hooks), body shape,

trunk (presoma and metasoma) spine (structure and distribution pattern of the somatic spines) and organology. Infrapopulations were analyzed for cystacanth and adult forms (*sensu* Nickol 1985). Females were analyzed for fertilization and maturity status (mated; non-mated) following the criteria of the eggs (sheltered acanthors) within body cavity *sensu* Sinisalo et al. (2004) and Valtonen et al. (2004). Fertilized females represent those containing developing/developed acanthors and mature females as those containing gonads, uterine and vaginal structures completely formed.

Parasitological indices such as Prevalence (P%), Mean Intensity (MI) and Mean Abundance (MA) *sensu* Bush et al. (1997, 2001) were obtained and analyzed *sensu* Rozsa et al. (2000) using the software Quantitative Parasitology 3.0. The P% (Chi-square test, χ^2) and MI (Bootstrap confidence interval, BC_{cr} , $p < 0,05$) were compared between genders of the host (total males and females), gender maturity of the host (pup and prepubertal males; male and female pups; female pups and reproductive-breeding females; and prepubertal males and reproductive-breeding females).

Seasonal variations for P% and MI were comparatively analyzed by grouped seasons, i.e., Autumn-Winter (A-W) and Spring-Summer (S-S).

The infection terminology was utilized considering parasites as antigens infecting their hosts *sensu* Frank (2002), Wilson et al. (2002) and Paul (2008).

Results

Lengths of infected hosts and sexual maturity of the hosts: Samples comprised male pups (n = 12) to prepubertal males (n = 4) as well as female pups (n = 14) and reproductive/breeding females (n = 2). Corynosomiasis occurred in all categories for males and females hosts.

The ranges of the total lengths (TL) for the genders of the hosts were: $0.84m \geq TL \leq 1.76m$ to males and $0.8m \geq TL \leq 1.34m$ to females. Male and female hosts infected by *C. cetaceum* comprised $0.97m \geq TL \leq 1.76m$ and $0.87m \geq TL \leq 1.33m$, respectively. From 32 hosts, six out of 16 males and five out of 16 females presented *C. cetaceum*-related (n = 11) corynosomiasis (Table 1).

Table 1 - Annual general distribution of *Corynosoma cetaceum* - related cases through the grouped season in *Arctocephalus australis* found stranded died in the of Rio Grande do Sul - Brazil. A-W: Autumn-Winter; S-S: Spring-Summer.

Gender of the Host	Year and Seasonal Grouping					
	2008		2009		2010	
	A-W	S-S	A-W	S-S	A-W	S-S
Male (n = 6/16)		5			1	
Female (n = 5/16)	1					4

Legend: Black bars represent the distribution of the parasite-related cases; White numbers (in black bars) represent the total of reported cases.

Source: Research data.

Infection sites and reproductive status of the para-site: *Corynosoma cetaceum* was found within the stomachal lumen and/or the duodenal lumen of the small intestine of *A. australis*. Corynosomiasis was observed exclusively in the stomach in five out of 11 cases (45.45%) and in the initial duodenum in four out of 11 cases (36.36%). Corynosomiasis within both gastrointestinal regions (stomach and duodenum) were two out of 11 (18.18%). There were no fertilized female parasites.

The stomachal corynosomiasis revealed the pyloric region as the main site of the cases of infection

(P% = 80), and the fundic region (closer to fundus-pylorus transitional region) as the site with fewer cases of infection. Few parasites were found superficially attached to the stomachal mucosa; in this case a concavity formed by the body of the parasite could be noted, after parasite's removal. This concavity comprised the presoma (foretrunk) and metasoma (hindtrunk) of the parasite. The duodenal corynosomiasis comprised only free specimens in the intestinal lumen.

Seasonality: *Corynosoma cetaceum. cetaceum* infected *A. australis* throughout the years and seasons but with low intensities for both genders of the

host (Table 1) as well as low parasitological indices (Table 2) were found for this host-parasite relationship. Males of *A. australis* were mainly infected between 2008 and 2009. *Corynosoma cetaceum* occurred in male hosts throughout the year 2008 (A-W and S-S) and only during A-W of 2009, which represent 83.33% of the cases. Only 16.66% of the cases occurred isolated in A-W of 2010. Concerning

to the year 2010, there was an inversion of the infective pattern, when females of *A. australis* were the principal gender infected by *C. cetaceum*. In this later case, only 20% occurred during the A-W of 2008 and 80% of the infection occurred throughout the 2010 (A-W and S-S). There were no season-related differences for P% and MI of *C. cetaceum* in *A. australis* (Table 2).

Table 2 - Parasitological indices (Prevalence - P%; Mean Intensity - MI; Mean Abundance - MA), Range of Variation (Ax) between grouped seasons and statistical results for P% (X^2) and MI for *Corynosoma cetaceum* collected from *Arctocephalus australis* found stranded died in the coast of Rio Grande do Sul, Brazil between 2008-2010. A-W: Autumn-Winter; S-S: Spring-Summer

Parasite	Grouped Seasons							
	A-W				S-S			
	P%	MI	MA	Ax	P%	MI	MA	Ax
<i>Corynosoma cetaceum</i>	45 (9/20)	2.78	1.25	1-7	16.7 (2/12)	4	0.67	1-7

Legend: P% values: numbers in parenthesis represent proportion of *Corynosoma cetaceum* - related cases for *Arctocephalus australis*.
Source: Research data.

Infection by gender and reproductive status of the host: The parasitological indices of *C. cetaceum* infestation in sampled *A. australis* were: P% = 34.4, MI = 2.91 and MA = 1.0.

There were no significant differences for P% and MI of *C. cetaceum* between genders or sexual maturation status of the host (Table 3).

Table 3 - Parasitological indices (Prevalence - P%; Mean Intensity - MI; Mean Abundance - MA) and Range of Variation (Ax) for *Corynosoma cetaceum* - related cases (n = 11) in *Arctocephalus australis* (n = 32) found stranded died in the coast of Rio Grande do Sul, Brazil

Host Sexual Parameters	Host Sex							
	Male*				Female**			
	P%	MI	MA	Ax	P%	MI	MA	Ax
Exclusively	37.5 (6/16)	2.83	1.5	1-7	31.3 (5/16)	3	1	1-7
Pups	25 (3/12)	1.33	1	1-2	28.6 (4/14)	2.5	1	1-7
Prepubertal*/Reproductive-Breeding**	75 (3/4)	4.33	5	1-7	50 (1/2)	5	5	5

Legend: P% values: numbers in parenthesis represent proportion of *Corynosoma cetaceum* - related cases for *Arctocephalus australis*.
Source: Research data.

Histopathology: The parasites attach superficially (middle-outward) in the fundic mucosa and form a concavity with their body as result of the holdfast embedding: proboscis hooks, foretrunk

(presoma) spines and hindtrunk (metasoma) spines. Inner layers of the stomach wall, i.e., submucosa, muscular layer and serosa, were not mechanically affected by the parasite.

In the fundic stomachal mucosa, *C. cetaceum* caused necrosis, erosion and reduction in length of the simple or branched tubular gastric glands. The glandular erosion occurs in the middle-apical region, degenerating the gastric crypts and glandular neck, reaching the middle of the glandular body and releasing cell remains from glandular epithelial cells

and from the papillary- or finger-like lamina propria. The affected glands by the holdfast of the parasite showed mainly karyorrhexis and karyolysis of the muciparous neck cells, chief (zymogenic) cells and parietal (oxyntic) cells. The retraction of the proboscis caused deformations and bending of the gastric glands (Figure 1).

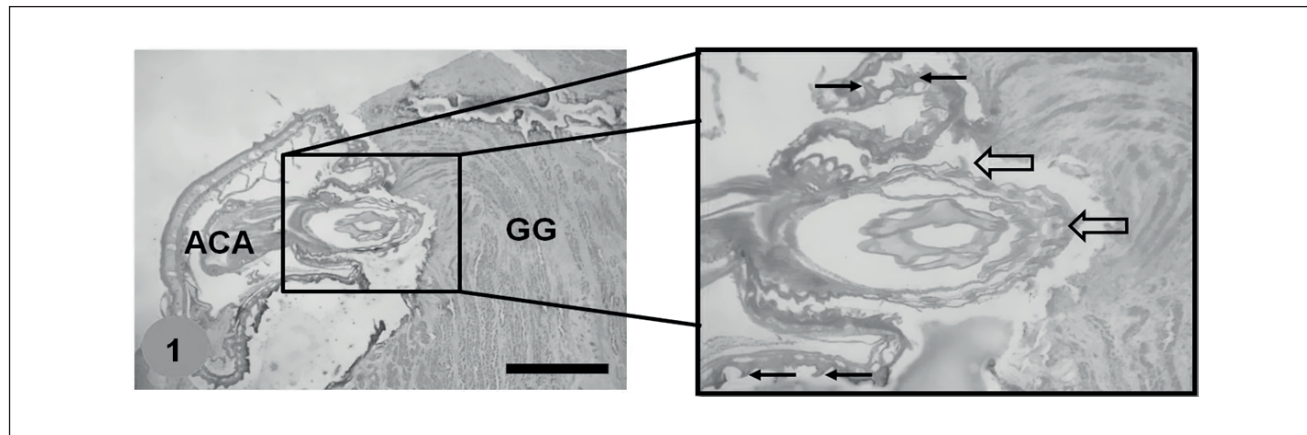


Figure 1 - Microphotograph of the attachment of *Corynosoma cetaceum* in the stomach mucosa of *Arctocephalus australis*. Observe the concavity harboring the parasite (ACA) and the erosion and deformities of the gastric glands (GG) from the mucosa. The detail on right shows the parasite's proboscis and cute proboscis hooks (open arrows) and trunk spines (solid arrows). Stain: H-E. Scale bar: 560µm

Source: Research data.

All abnormal features of the pyloric mucosa were similar to those of the fundic mucosa. However, it is important to elucidate that the fundic mucosa is composed exclusively by simple or branched tubular muciparous glands (pyloric glands) and the necrotic features are related exclusively with muciparous cells.

Discussion

Helminthiasis in *A. australis* is poorly recorded and generally focused to investigate the biological parameters of a parasite-target species (Aznar et al., 2004; Sardella et al., 2005). In Uruguayan waters, the parasite assemblage is recorded and compared by using similarity index for the sympatric *A. australis* and *Otaria flavescens* (Shaw, 1800) but parasitological indices as prevalence (P%), mean intensity (MI), mean abundance (MA) and range of variation (Ax) are not available (Morgades et al., 2006) as in the present study. The influence of the same species of the hosts on the morphology (ecotype) and/or

fecundity of intestinal *Corynosoma* sp. (George-Nascimento & Marin, 1992) and nematode parasite (George-Nascimento et al., 1992) were also analyzed. Morgades et al. (2006) reported *Corynosoma* sp. for pups and *Corynosoma* spp. for juveniles and adults *A. australis*. Morgades et al. (2006) provided a clue to think that *Corynosoma* spp. most likely are both *C. cetaceum* and *C. australe* each in its proper gastrointestinal niche space, i.e., within the stomach and intestines respectively, as record Aznar et al. (2004) and Sardella et al. (2005). *Corynosoma* sp. analyzed by George-Nascimento and Marín (1992) from intestine of *A. australis* is most likely *C. australe*. The morphological characteristics analyzed, in this study, corroborate Aznar et al. (1999a, b) for *C. cetaceum*. The infection of the pups of the host by this parasite, in the present work, supports the first intake of solid food as a way to allow the parasite settlement (Vaz-Ferreira & Ponce de León, 1987) but little is known about the food habits of the host that are stranded in Brazilian shores (Santos & Haimovici, 2001).

Normally adult Acanthocephala settles and recruits within the intestinal lumen of endothermic vertebrates (Crompton, 1973; Nickol, 1985; Bush et al., 2001; Aznar et al., 2004; Sinisalo et al., 2004), but some species represent exceptions (Aznar et al., 2001; Morgades et al., 2006; Muñoz & Olmos, 2008). *Corynosoma cetaceum* was recorded with an atypical settlement site within the gastrointestinal tract of *Pontoporia blainvillei* (Cetacea, Pontoporiidae) and this pattern of occurrence is confirmed for *A. australis* by this study. In polygastric *P. blainvillei* (= polycompartmentalized) (main stomachal chamber, connecting channel and pyloric stomachal chamber) (Yamasaki et al., 1974) host, this parasite species showed its higher P% and MA in the pyloric stomach lumen followed by the duodenal ampulla (small intestine) (Valente et al., 1997; Aznar et al., 2001). Also, the fecundity proportional ratio for the females of *C. cetaceum* in the pyloric stomachal compartment of *P. blainvillei* showed that it is the appropriated niche to the parasite reaches its sexual maturation, thus this parasite constitute part of the helminth community for this host (Valente et al., 1997; Aznar et al., 1994, 2001). The largest stomachal compartment (main stomach) of *P. blainvillei* had the lower values of *C. cetaceum* and this could be related with the lower nutritional concentrations, because the digestion is in its initial process (if compared with higher free nutrient values toward pyloric compartment of the stomach) and also due to the anti-mechanical protection during the breakdown of the food items in the main stomach (Aznar et al., 2001). Other Cetacea with polycompartmentalized stomachs, such as *Cephalorhynchus eutropia* (Delphinidae) and *Phocaena spinipinnis* (Phocoenidae), are recorded typically harboring *C. cetaceum* within their pyloric stomachal chamber (Muñoz & Olmos, 2008) reinforcing the fitness of this parasite species to these mammals, but suggesting to be different (not typical) for *A. australis*.

On the other hand, the histochemical characteristics of the mucosa of the compartments and portions of the stomach surface should be considered. The anti-corrosive (anti-acid and anti-enzymatic) properties of the mucous in the pyloric region should be added with the reflections of Aznar et al. (2001). This could help to explain why *C. cetaceum* occurs in the pyloric region of monogastric (monocompartmentalized) species such as *A. australis* (Ridgway, 1972) and closer to the duodenum portion. The pyloric stomachal chamber of Cetacea shares the same

muciparous glandular characteristic (Harrison et al., 1970; Smith, 1972; Chaves & Silva, 1988; Silva & Cousin, 2006b). In the present study, gastric corynosomiasis in the monocompartmentalized stomach of *A. australis*, showed higher P% in the pyloric portion composed only by mucosal muciparous glands. The host-parasite relationship, *sensu* Zander (2001), is adjusted by biochemical coevolutionary factor to taxis of the parasite, its settlement and its recruitment in/within an adequate host or body site of the host to adequately finish the life-cycle of the parasite (Combes, 1997; Dybdahl & Storfer, 2003) and the muciparous characteristics of the pyloric stomachal portion suggest to support the occurrence of *C. cetaceum* there, even at very low parasitological loads.

In evolutionary studies, parasitological indices, such as P%, are commonly used to predict and measure the "parasite load" or "parasite pressure" upon a host species (Gregory & Blackburn, 1991), as a selective pathogen (= parasite) force that helps the process of natural selection (Anderson & Gordon, 1982; Anderson & May, 1982). The weak occurrence of parasites across sampled years, the low values of the P%, MI and MA as well as no-gravid females of *C. cetaceum* suggest to show that, at least, *A. australis* is an inadequate or accidental host for this parasite. According to the structure of the population and recruitment patterns, the congeneric *C. australe* is considered as a typical parasite of the intestinal infrapopulation of *A. australis* (Vaz-Ferreira & Ponce de León, 1987; Aznar et al., 2001; Morgades et al., 2006). On the other hand, *C. cetaceum* is considered a typical parasite of Cetacea due to its parasitological indices and dynamics of the population (Andrade et al., 1997; Béron-Vera et al., 2007).

Corynosoma cetaceum fitness to Cetacea perhaps is due to the gastric anatomy (polycompartmentalized) that allows the anatomical isolation by well-defined compartments forming adequate microhabitats for this parasite species (Berón-Vera et al. 2007). These well-defined microhabitats help to avoid or decrease interspecific competitions with other gastrointestinal parasite infrapopulations and/or infracommunities (Von Zuben, 1997; Poulin, 2001). The monocompartmentalized stomach condition, typical of the Pinnipedia and other Carnivora, perhaps could contribute for the interspecific parasite competition. *Arctocephalus australis* harbors well-defined helminths species according to its gastrointestinal niche space, *i.e.*, anatomic division of the digestive tube (Morgades et al., 2006). Hosts wide distributed,

as *A. australis*, tend to demonstrate higher species richness, concerning to the parasites (Bush et al., 2001) due to the higher variety of preys (Naya et al., 2002; Casper et al., 2006). The interactions between concomitant parasite infrapopulations in individual hosts will determine how many parasites species could coexist in single hosts, and ultimately in the parasite component community (Bush et al., 1997, 2001) under a maximum load of the parasites (Bush & Lotz, 2000; Read, 2000; Roberts, 2000; Cattadori et al., 2008).

Concerning to the genders and/or sexual maturity status of the hosts, the absence of differences for parasitological indices, observed in this work, could be related with the common high food plasticity within Arctocephalinae (Gales et al., 1993; Georges et al., 2000; Harcourt et al., 2002; Casper et al., 2006; Ciaputa & Sicinski, 2006; Boren, 2010) and Otariinae (Drago et al., 2009). The typical sexual dimorphism of the mammals (Fairbairn, 1997; Isaac, 2005; Ferguson & Larivière, 2008) implies in possible differences in the parasitic loads due to gender-related heterogeneities concerning to the behaviour, endocrinology, morphology (length and body mass = hyperallometry), energetic requirements and genetic factors of the hosts (Cassini, 2000; Klein, 2004; Morales-Montor et al., 2004; Cattadori et al., 2008) as well as environmental selective pressure (Ferguson & Larivière, 2008). The absence of heterogeneity in exposure (or no differences in susceptibility) could rapidly results an aggregated distribution of parasites, but until the present moment is unclear what is the relative significance of these different mechanisms, and the importance of their interactions for accentuating individual differences in parasite loads (Wilson et al., 2002).

Puberty is not fixed in Pinnipedia. It occurs more similarly over a range of age among females of Phocidae and Otariidae and it is more variable among males of Pinnipedia (Atkinson, 1997). The higher variation in males of Pinnipedia is partially due to the need to gain behavioural experience and body mass to sustain successfully the energetic costs for the competition by the breeding rookeries (Atkinson, 1997). This results in a sexual selection, according to high degree of sexual dimorphism in polygynous Otariidae, generally marked by the larger body size of the male several times heavier than females, as an advantage in intrasexual dominance in male-male combat for breeding territory (Clinton & Le Bouef, 1993). Moreover, the higher male body mass

is correlated with the increasing of the home range (Ferguson & Larivière, 2008). Within the sampled specimens, the male hosts were very closer to female hosts according to the TL. This corroborate the absence of hyperallometric growth of organs and structures [or higher body mass according to Bergmann's rule influence *sensu* Ferguson and Larivière, (2008)] associated with combat or display in males (Fairbairn, 1997). This could explain the absence of differences in P% and MI concerning to the sampled genders of the host, at the least for *C. cetaceum*, probably due to the similar energetic requirements and/or superposition of foraging areas between the specimens without hyperallometric sexual dimorphism, reflecting in food uniformity between genders of the host within the sampled length classes.

On the other hand, the season-related equalities observed for P% and MI and the low values of MA and Ax should be considered as reinforce for the diet plasticity and uniformity for the hosts, i.e., Otariidae are very flexible foragers (Casper et al., 2006; Georges et al., 2000; Harcourt et al., 2002; Ciaputa & Sicinski, 2006; Drago et al., 2009; Boren, 2010). Moreover, these results suggest the hypothesis of accidental occurrence of *C. cetaceum* in *A. australis* as previously discussed. Food plasticity could result in the crowded (in patches) pattern of occurrence of gastrointestinal parasite groups within a host trophic level, generally secondary and definitive hosts (this latter such as marine mammals) (Bush et al., 2001; Rószka et al., 2000; Reiczigel et al., 2005). The crowded parasite pattern to definitive hosts is related with stochastic environmental changes that can influence the dynamic of the population of the intermediate host, such as Russel cycle and primary production (Lalli & Parson, 2004) (for the oceanographic environment), the susceptibility of the host (influenced by the immunology, genetic and behaviour), intra- and/or inter-specific competition by the parasites in a host and differences between trophic levels occupied by the host (ontogeny-associated) associated with its food necessities (Von Zuben, 1997; Bush & Lotz, 2000; Read, 2000; Roberts, 2000; Cattadori et al., 2008). The host immunological efficiency aspects (Von Zuben, 1997; Klein, 2004) could explain the observed low parasitological indices, the immaturity of *C. cetaceum* in the gastric lumen and the low inflammatory effect of this parasite for *A. australis*, as in the present work.

Histopathologically, the stomachal attachment of *C. cetaceum* in *A. australis* was similar to that of *Pontoporia blainvillei* (Cetacea, Pontoporiidae)

(Silva & Cousin, 2006b). However, other polymorphidiasis result in different host tissue reaction, such as *Bolbosoma*-associated intestinal colonic granuloma (Silva & Cousin, 2006b) or intestinal erosion without severe inflammatory consequences (Silva et al., 2013). The histopathological pattern of attachment of *C. cetaceum* in the stomach of *A. australis* was very closer from that caused by *C. australe* in the intestine of the same host species (Silva et al., 2013). Nevertheless, acanthocephalosis are recorded in mammalian hosts contributing to the mortalities via inflammatory processes as septic peritonitis associated with the migration of *Proflicolis* spp. (Proflicolidae) in *Enhydra lutris* (Kreuder et al., 2003) and *Macracanthorhynchus*-associated (Macracanthorhynchidae) neoplasia, chronic enteritis, intestinal perforation and intestinal eosinophilic granuloma in *Hemiechinus auritus* (Taman, 2009). Some authors (Ridgway, 1972; Vlasman & Campbell, 2003), from a strict point of view, believe that the acanthocephalosis is not significant for the mortalities in Pinnipedia, but it is known that parasites influence the health of their hosts in several ways (p.e., physiological and behavioural) (Thomson, 1983; Bush et al., 2001).

Conclusion

Corynosoma cetaceum was found mainly in the pyloric region of the stomach and early duodenum of *A. australis*. The parasite occurred throughout the year for both genders of *A. australis* at very low prevalence (P%), mean intensity (MI) and mean abundance (MA). There were no genders-related differences for P% and MI as well as for the analyzed sexual maturation categories (male pups, prepubertal males, female pups and breeding females) of the *A. australis* parasite by *C. cetaceum*. There were no season-related differences for P% and MI of *C. cetaceum* in *A. australis*, suggesting that the occurrence of this parasite is accidental for this host. There were no severe inflammatory processes caused by *C. cetaceum* in the stomach of *A. australis*.

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