Veterinary Parasitology 173 (2010) 116–122

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Metazoan parasites of cetaceans off the northeastern coast of Brazil

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A R T I C L E I N F O

Article history:
Received 20 October 2009
Received in revised form 20 June 2010
Accepted 21 June 2010

Keywords:
Cetaceans
Endoparasites
Ectoparasites
Commensals

A B S T R A C T

This study represents the first survey of the parasitic fauna of cetaceans off the northeastern coast of Brazil. Parasites were collected from 82 animals rescued from the states of Ceará to Bahia, including the archipelago of Fernando de Noronha. A total of 14 species of cetaceans were evaluated: *Sotalia guianensis*, *Stenella* sp., *Stenella clymene*, *Stenella longirostris*, *Stenella coeruleoalba*, *Stenella frontalis*, *Megaptera novaeangliae*, *Peponocephala electra*, *Steno bredanensis*, *Kogia breviceps*, *Kogia sima*, *Globicephala macrorhynchus*, *Tursiops truncatus*, *Physeter macrocephalus* and *Lagenodelphis hosei*. The parasites were fixed and preserved in 70% ethanol or alcohol–formalin–acetic acid solution (AFA), clarified in phenol and mounted on slides for morphological identification. In total, 11 species and 8 genera of endo- and ectoparasites were identified: *Halocercus brasiliensis*, *Halocercus kleinenbergi*, *Stenurus globicephalae*, *Halocercus* sp., *Anisakis* sp., *Crassicauda* sp. (Nematoda), *Phyllobothrium delphini*, *Monorygma grimaldii*, *Scolex pleuronectis*, *Strobicephalus triangularis*, *Tetrabothrius forsteri*, *Tetrabothrius* sp., *Trigonocotyle* sp., *Diphyllobothrium* sp. (Cestoda), *Campula* sp. (Trematoda), *Bolbosoma* sp. (Acanthocephala), *Cyamus boopis*, *Syncyamus pseudorcae* and *Xenobalanus globicipitis* (Crustacea). The identification of some species represented novel records for the country and increased the occurrence of some parasites to new hosts. The use of standardized methodologies for collecting and evaluating a larger number of animals is essential for a better understanding of host-parasite relationships in cetaceans and their use as biological indicators in the region.

1. Introduction

Cetaceans are parasitized by a wide diversity of endo- and ectoparasites in various tissues, organs and cavities (Dailey, 2001). In general, the presence of parasites is of little relevance to the health of hosts (Geraci and St Aubin,

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doi:10.1016/j.vetpar.2010.06.023

1987). Damage to and mortality of individuals and populations caused by parasitic infections are dependent upon several factors, including the parasite species, its abundance, the health status of the host and competition with other pathogens (Raga et al., 2002). In addition to knowledge about the pathological effects of these organisms, their importance in ecological and evolutionary studies is highlighted, as parasites of cetaceans can influence the behavior of their hosts, population size, the dynamics of the food chain and community structure (Raga et al., 2002).

The parasitic fauna of cetaceans in Brazil is still little known. Although there has been a greater interest in the study and collection of parasites in recent years, only few studies have been conducted in a systematic way using standardized methods. However, such methods are mostly limited to records in strandings, occurring mainly in the south and southeast regions. Some 24 species of parasites and commensals have been identified morphologically in the country. The nematodes *Anisakis* sp., *Halocercus* sp. and the digenean *Synthesium* sp. are the most commonly described (Marigo, 2003). Among these, *Anisakis typica* and *Synthesium* spp. have also been characterized by molecular techniques (Motta et al., 2008; Iñiguez et al., 2009; Marigo, 2009).

Parasites have been reported in more than 20 species of cetaceans in Brazil, including dolphins and whales (Marigo, 2003). The parasitic fauna of the Guiana dolphin (*Sotalia guianensis*) and Franciscana (*Pontoporia blainvillei*) have been further studied, mainly because of the high accidental capture in fishing gear that these species suffer along the Brazilian coast, allowing the evaluation of a larger number of animals (Santos et al., 1996; Melo et al., 2006; Marigo et al., 2002, 2008; Iñiguez et al., 2009).

Considering the lack of information available for the region, the present study aims to identify the metazoan parasites that occur in cetaceans in the northeast of Brazil.

2. Materials and methods

Parasites were collected from 82 carcasses of cetaceans rescued off the northeastern coast of Brazil between 1994 and 2009, comprising 2.600 km of coastline from the states of Ceará to Bahia, in addition to the archipelago of Fernando de Noronha (Fig. 1). The animals were rescued by institutions of the Aquatic Mammals Stranding Network of the Northeast (Remane), these being: 21 Guiana dolphins (*S. guianensis*), 2 *Stenella* sp., 16 Clymene dolphins (*Stenella clymene*), 9 spinner dolphins (*Stenella longirostris*), 2 striped dolphins (*Stenella coeruleoalba*), 1 Atlantic spotted dolphin (*Stenella frontalis*), 6 humpback whales (*Megaptera novaeangliae*), 5 melon-headed whales (*Peponocephala

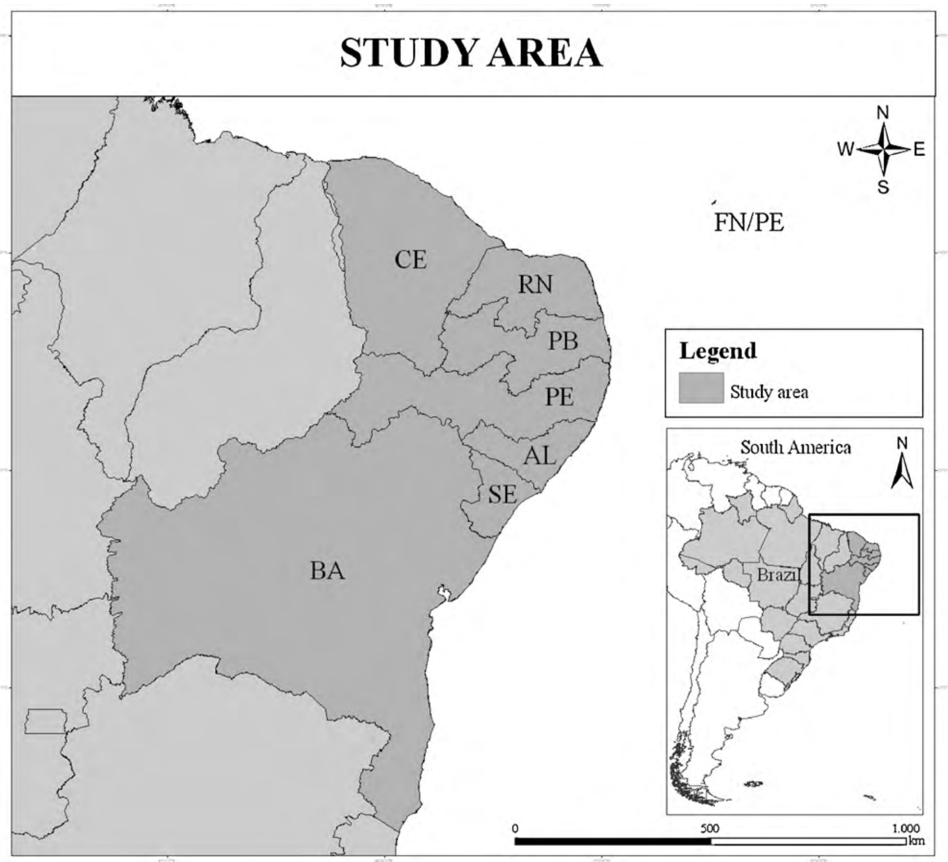

Fig. 1. Study area including Ceará (CE), Rio Grande do Norte (RN), Paraíba (PB), Pernambuco (PE), Alagoas (AL), Sergipe (SE) and Bahia (BA) states and the archipelago of Fernando de Noronha (FN/PE), on northeast region of Brazil. The strandings of cetaceans were most frequent in CE and BA.

electra), 4 rough-toothed dolphins (*Steno bredanensis*), 4 pygmy sperm whales (*Kogia breviceps*), 3 dwarf sperm whales (*Kogia sima*) 3 short-finned pilot whales (*Globicephala macrorhynchus*), 2 bottlenose dolphins (*Tursiops truncatus*), 2 sperm whales (*Physeter macrocephalus*) and 2 Fraser's dolphins (*Lagenodelphis hosei*).

Parasites were collected during the necropsy of animals. After collection, the helminths were fixed and preserved in

Table 1
Parasites and commensals identified in cetaceans off northeastern coast of Brazil.

Host	Parasite	Localization
Humpback whale (*Megaptera novaeangliae*)	*Cyamus boopis*	Skin
Short-finned pilot whale (*Globicephala macrohynchus*)	*Stenurus globicephalae*	Tympanic bullae
	Anisakis typica	Stomach
	Halocercus kleinebergi	Lung
	Pyllobothrium delphini	Blubber
	Diphyllobothrium sp.	Intestine
Guianna dolphin (*Sotalia guianensis*)	*Anisakis* sp.	Stomach
	Halocercus sp.	Lung
	Halocercus brasiliensis	Lung
Sperm whale (*Physeter macrocephalus*)	Crustacea: Cirripedia	Skin
	P. delphini	Blubber
Dwarf sperm whale (*Kogia sima*)	*Anisakis* sp.	Stomach
	P. delphini	Blubber
Pygmy sperm whale (*Kogia breviceps*)	*Anisakis* sp.	Stomachs
	Halocercus sp.	Lung
	Crassicauda sp.	Muscle, pleura, penis
	P. delphini	Blubber
	Monorygma grimaldii	Abdominal cavity
	Diphyllobothrium sp.	Intestine
Melon-headed whale (*Peponocephala electra*)	*S. globicephalae*	Tympanic bullae
	Anisakis sp.	Stomach
	P. delphini	Blubber
	M. grimaldii	Abdominal cavity
	Strobicephalus triangularis	Rectum
Clymene dolphin (*Stenella clymene*)	*Synciamus pseudorcae*	Skin, mouth, blowhole
	Anisakis sp.	Stomach, faeces
	Halocercus sp.	Lung
	H. brasiliensis	Lung
	Bolbosoma sp.	Stomach, intestine
	P. delphini	Blubber
	M. grimaldii	Abdominal cavity
	Scolex pleuronectis	Liver
	Trigonocotyle sp.	Intestine
	Tetrabothrius forsteri	Intestine
	S. triangularis	Rectum
Rough-toothed dolphin (*Steno bredanensis*)	*Anisakis* sp.	Stomach
Fraser's dolphin (*Lagenodelphis hosei*)	*Anisakis* sp.	Faeces
	P. delphini	Blubber
	M. grimaldii	Abdominal cavity
Striped dolphin (*Stenella coeruleoalba*)	*Xenobalanus globicipitis*	Caudal fluke
	Anisakis sp.	Anterior and piloric stomachs
	Halocercus sp.	Lung
	P. delphini	Blubber
	M. grimaldii	Abdominal cavity
Bottlenose dolphin (*Tursiops truncatus*)	*Anisakis* sp.	Intestine
	Bolbosoma sp.	Intestine
Atlantic spotted dolphin (*Stenella frontalis*)	*Anisakis* sp.	Stomach
	P. delphini	Blubber
	S. triangularis	Intestine
Spinner dolphin (*Stenella longirostris*)	*Anisakis* sp.	Stomach, regurgitated, faeces
	Halocercus sp.	Lung
	H. brasiliensis	Lung
	Bolbosoma sp.	Intestine
	Campula sp.	Hepatopancreatic duct
	P. delphini	Blubber
	M. grimaldii	Abdominal cavity
	S. pleuronectis	Hepatopancretic duct
	Tetrabothrius sp.	Intestine
	T. forsteri	Intestine
	Trigonocotyle sp.	Intestine
	S. triangularis	Intestine
Stenella sp.	*Halocercus* sp.	Lung
	M. grimaldii	Abdominal cavity

Table 2
Prevalence and intensity of parasitism in cetaceans of the northeastern coast of Brazil.

Parasites	*n*	Hosts	*P* (%)	Intensity
Nematoda				
H. brasiliensis	8	*S. guianensis*, *S. clymene*, *S. longirostris*	9.8	10–122
H. kleinebergi	1	*Globicephala macrorhynchus*	1.2	100–150
S. globicephalae	3	*P. electra*, *G. macrorhynchus*	3.7	300–1348
Anisakis sp.	49	*S. guianensis*, *S. clymene*, *S. longirostris*, *S. frontalis*, *S. coeruleoalba*, *S. bredanensis*, *K. sima*, *Kogia breviceps*, *L. hosei*, *P. electra*, *T. truncatus*, *G. macrorhynchus*	59.8	1–1100
Halocercus sp.	14	*S. guianensis*, *S. clymene*, *S. longirostris*, *S. coeruleoalba*, *Stenella* sp., *Kogia breviceps*	17.1	2–64
Crassicauda sp.	1	*Kogia breviceps*	1.2	3
Cestoda				
P. delphini	18	*K. breviceps*, *L. hosei*, *S. clymene*, *S. longirostris*, *P. electra*, *S. frontalis*, *S. coeruleoalba*, *G. macrorhynchus*, *P. macrocephalus*	22.0	1–100
M. grimaldii	16	*S. coeruleoalba*, *S. clymene*, *S. longirostris*, *K. breviceps*, *K. sima*, *P. electra*, *L. hosei*, *Stenella* sp.	19.5	1–10
S. triangularis	5	*P. electra*, *S. clymene*, *S. longirostris*, *S. frontalis*	6.1	1–5
T. forsteri	3	*S. clymene*, *Stenella logirostris*	3.7	7–8
S. pleuronectis	2	*S. longirostris*, *S. clymene*	2.4	20–327
Tetrabothrius sp.	1	*S. longirostris*	1.2	6
Trigonocotyle sp.	4	*S. clymene*, *S. longirostris*	4.9	49–59
Diphyllobothrium sp.	2	*K. breviceps*, *G. macrorhynchus*	2.4	–
Trematoda				
Campula sp.	1	*S. longirostris*	1.2	50
Acantocephala				
Bolbosoma sp.	4	*T. truncatus*, *S. longirostris*	4.9	8–13
Crustacea				
C. boopis	6	*M. novaeangliae*	7.3	7–600
S. pseudorcae	6	*S. clymene*	7.3	4–10
X. globicipitis	1	*Stenella coeruleolba*	1.2	1

70% ethanol or (93 parts 70% ethanol: 5 parts 10% formaldehyde: 2 parts acetic acid) AFA, while the ectoparasites were fixed in 70% ethanol.

Nematodes were cleared in phenol from 5 to 60 min, depending on the size and thickness of the parasite. Platyhelminths and Acanthocephala were stained with hematoxylin and cleared in phenol for similar times, according to a methodology adapted from Amato (1985) and Hoffmann (1987). After clarification, the parasites were mounted on slides with the permanent synthetic resin Entellan® (Amato, 1985; Hoffmann, 1987). The internal structures were visualized under an optical microscope, and morphological identification was based on identification keys available in the literature (Almeida, 1933; Delyamure, 1955; Yamaguti, 1958; Davey, 1971; Wardle et al., 1974; Lambertsen, 1985). Amphipods and barnacles were placed in Petri dishes and observed under a stereomicroscope for evaluation of external characteristics. The identification was based on Leung (1967) and Rajaguru and Shantha (1992).

The prevalence and intensity of infection were assessed for each parasite species according to Bush et al. (1997). The statistical test Mann–Whitney (U) was used with significance level $\alpha = 0.05$, to determine if there was a significant difference between the parasite richness of coastal (*S. guianensis*, *S. bredanensis* and *M. novaeangliae*) and pelagic cetaceans species (remainder).

3. Results

In the evaluated animals, 11 species and 8 genera of metazoan endo and ectoparasites were identified: *Halocercus brasiliensis*, *Halocercus kleinenbergi*, *Stenurus globicephalae*, *Halocercus* sp., *Anisakis* sp., *Crassicauda* sp. (Nematoda), *Phyllobothrium delphini*, *Monorygma grimaldii*, *Scolex pleuronectis*, *Strobicephalus triangularis*, *Tetrabothrius forsteri*, *Tetrabothrius* sp., *Trigonocotyle* sp., *Diphyllobothrium* sp. (Cestoda), *Campula* sp. (Trematoda), *Bolbosoma* sp. (Acanthocephala), *Cyamus boopis*, *Syncyamus pseudorcae* and *Xenobalanus globicipitis* (Crustacea) (Table 1).

The nematodes were the most prevalent parasite group (79.3%), followed by cestodes (36.6%), crustaceans (15.9%), acanthocephalans (4.9%) and digeneans (1.2%). Among the most prevalent species, the most common were the nematodes *Anisakis* sp. (59.8%) and *Halocercus* spp. (28.1%) and the merocercoids *P. delphini* (22.0%) and *M. grimaldii* (19.5%). Most of the parasites were generalists, being observed in several host species, while cyamids *C. boopis* and *S. pseudorcae* were considered specialists, found exclusively in humpback whales and Clymene dolphins, respectively, in all areas analyzed (Table 2).

Between 1 and 6 species of parasites were found in the individuals analyzed, with an average of 1.74 species of parasites per host. The comparison between parasite richness of coastal and pelagic species, revealed that pelagic cetaceans are significantly ($U = 356.5$; $p < 0.05$) more parasitized than costal (Table 1).

4. Discussion

This study is the first survey of the parasitic fauna of cetaceans rescued in northeast Brazil. The results of this work are new records for the country and increased the occurrence of some parasites to new hosts.

There has been little work done using different host species of marine mammals in the country. In the southeastern and southern coasts, Santos et al. (1996) identified four species of helminths in *S. guianensis*, *T. truncatus* and *S. bredanensis*. Most records involved individual or single host species, mainly *P. blainvillei* (Andrade, 1996; Marigo et al., 2002, 2008), *S. guianensis* (Melo et al., 2006; Iñiguez et al., 2009), and species that have suffered mass strandings (Andrade et al., 2001).

4.1. Nematodes

The genus *Anisakis* was the most prevalent in animals evaluated. Larvae and adults were found mainly in the stomach chambers. The parasites were not identified at the species level, as morphological analysis is limited and is only possible at the level of morphospecies using adult male specimens (Colón-Llavina et al., 2009). *Anisakis* species have been reported previously for all hosts evaluated in this work. The presence of these parasites in the stomach can lead to ulcers and cause bleeding (Dailey, 2001). Motta et al. (2008) observed the formation of ulcers, gastritis and granulomatous inflammation caused by *Anisakis typica* in cetaceans stranded on the coast of Ceará, northeast Brazil.

Pulmonary nematodes belonging to the genus *Halocercus* were one of the most prevalent. *H. kleinenbergi* was first identified apparently in the Atlantic Ocean, parasitizing *G. macrorhynchus*. *S. guianensis* and *G. macrorhynchus* newborns had lung parasites, indicating transmission through the placenta, previously reported in *T. truncatus* infected by *H. lagenorhynchi* (Dailey et al., 1991). The presence of parasites in the lungs of cetaceans stranded in the state of Ceará included in this study was diagnosed as a highly debilitating condition, affecting respiratory capacity and diving (Motta, 2006).

Three animals showed severe infection by *S. globicephalae* in the inner ear. Infection by these parasites can interfere with echolocation and orientation (Geraci and St Aubin, 1987). A live, stranded individual of *P. electra* showed signs of disorientation, poor buoyancy and swimming in circles related with the presence of parasites, mainly adult females full of eggs (Costa and Monteiro-Neto, 1998).

Specimens of *Crassicauda* sp. were found encysted in the diaphragm, suprascapular muscles, and penis of *K. breviceps*, despite the high pathogenicity of this parasite, there were no associated lesions with implications for the health of the current specimen (Altieri et al., 2008).

4.2. Cestodes

Four types of tetraphyllidean larvae have been recognized in marine mammals: two plerocercoids that differ in size, identified as *S. pleuronectis*, and two merocercoids, *P. delphini* and *M. grimaldii* (Aznar et al., 2007). *P. delphini* it was found in the blubber from nine host species, while. *M. grimaldii* was found in the abdominal cavity, generally located near the perigenital region. The merocercoids were among the most prevalent parasites in cetaceans stranded in the northeast, mostly found in pelagic animals. *S. pleuronectis* was found in two animals, one *S. longirostris*, where large plerocercoids were observed, and one S. clymene, where small plerocercoids were detected. Adult stages are unknown for all types of worms, but there is molecular, anatomical and ecological evidence that the large plerocercoids, *P. delphini* and *M. grimaldii* are distinct species, and small plerocercoids are early stages of *M. grimaldii* (Agusti et al., 2005; Aznar et al., 2007). Most of the evaluated animals were infected by two species of merocercoids. In the two dolphins parasitized by *S. pleuronectis*, co-infection by *M. grimaldii* was observed, but it was not possible to indicate whether they were distinct stages of the same species. Molecular studies are needed to establish a relationship between the different types of larvae.

S. triangularis was found with its scolex embedded in the intestine and rectal mucosa at a low intensity of infection. *T. forsteri* and *Trigonocotyle* sp. were found in small intestine of *S. clymene* and *S. longirostris*.

Diphyllobothrium sp. was observed in the intestine of *K. breviceps* and *G. macrorhynchus*. This species is usually harmless, but may be encysted in the colon wall, forming masses that block the lumen of the organ (Geraci and St Aubin, 1987).

4.3. Digeneans

Only the genus *Campula* was found in the hepatopancreatic duct of a specimen of *S. longirostris*. The animal had a low intensity of infection, and no pathology was described related to the presence of the parasite. At high infections, *Campula* sp. can cause fibrosis, chronic inflammation and hyperplasia of the ducts, affecting the digestive and endocrine functions (Dailey, 2001). The prevalence of these parasites and other digeneans may be underestimated in the region, mainly due to inadequate data collection protocols. Few digeneans were identified in Brazil. Parasites of the genus *Synthesium* are commonly observed in the guts of *P. blainvillei* and *S. guianensis* that inhabit the southeast and south regions (Marigo et al., 1999, 2008).

4.4. Acanthocephalans

Bolbosoma sp. was the only acanthocephalan found in the animals studied and was present in the stomach and intestine of *T. truncatus* and *S. longirostris*, *Bolbosoma* sp. occurs mainly in small pelagic cetaceans. They are generally associated with some diseases, causing local irritation and occasional ulcers (Geraci and St Aubin, 1987; Raga et al., 2002).

4.5. Crustaceans

Three species of crustaceans were identified in the studied cetaceans. *C. boopis* was found in the skin, callosities and fins of *M. novaeangliae*. *S. pseudorcae* was observed in the skin around the mouth and blowhole of *S. clymene*. Both cyamid species were found in various localities in the same host species, demonstrating the specificity of these parasites. They are transmitted by direct contact between infected individuals (Pfeiffer, 2002). This is the first record of *S. pseudorcae* for Brazil and for the Clymene dolphin.

A pedunculate barnacle, identified as *X. globicipitis*, was found in the caudal fluke of *S. coeruleoalba* (Ribeiro et al., 2010). They are better referred to as commensals because they only use their hosts for fixing (Fertl, 2002).

The presence of ectoparasites and commensals in small cetaceans is common in slower animals with impaired skin immune function (Aznar et al., 1994). Most of the dolphins that had ectoparasites were stranded alive, showing signs of previous illness and various pathological findings in necropsies. The presence of these organisms may indicate the existence of poor prognosis in clinical evaluation of small living cetaceans.

The collections of parasites were conducted by different research groups in the northeast of the country over 15 years. Samples were collected opportunistically from stranded cetaceans. The lack of a standard methodology for the collection and storage of samples hinders detailed systematic studies. Moreover, the incidence and prevalence of many species of parasites may be underestimated.

It was not possible to evaluate the parasitic fauna of different host species at the level of parasite communities, as there were differences in the methodology of collection and research efforts.

Most cetacean species in this study inhabit pelagic environments, According to Raga et al. (2002), pelagic animals have distinct and poorer parasitic fauna compared to coastal animals, since there is a lower abundance of intermediate hosts used by digeneans, mainly gastropods and bivalves, and the infective stages are much more diluted, reducing transmission rates in this habitat (Raga et al., 2002). This finding may explain the low prevalence of digeneans among all groups of parasites found. However, the pelagic cetacean species, especially *S. clymene* and *S. longirostris*, exhibited the richest parasitic fauna among the cetaceans studied. On the other hand, *S. guianensis*, which inhabits the coastal zone and had the largest number of individuals analyzed, showed poor parasite fauna. It is important to emphasize that parasites, such as *P. delphini*, *M. grimaldii*, *S. pleuronectis*, *T. forsteri* and *Trigonocotyle* sp., especially observed in dolphins of the genus *Stenella* in this study, occur mainly in pelagic cetaceans (Raga et al., 2002). In general, specimens of *S. guianensis* strand in an advanced state of decomposition throughout the northeast coast of Brazil, making it difficult to collect samples and diagnose parasitic infections in these animals.

Most species of parasites identified proved to be generalists, such as *Anisakis* sp., *Halocercus* sp., *P. delphini* and *M. grimaldii*, suggesting that different species of cetaceans can share food items and/or that the parasites use various intermediate hosts to complete their life cycles. However, studies of the composition of the diet of cetaceans are needed to assist the elucidation of life cycles.

The identification of the parasitic fauna of cetaceans not only contributes to a better understanding of the causes of stranding and mortality, but also provides valuable information on the biology and ecology of cetaceans. The use of standardized methodologies and evaluation of a larger number of animals are essential for studies of host–parasite relationships and their use as biological indicators in the region.

Acknowledgements

This work received the financial support of CAPES. Dr. Bevilaqua has a grant from CNPq. Authors are thankful for the assistance of Mr. Iran Normande from Aquatic Mammals Stranding Network of Northeast (REMANE); AQUASIS, IMA, CMA, FMA, CGR and PPC teams for their work recovering and performing cetaceans necropsies.

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