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Parasites of cetaceans stranded on the Pacific coast of Costa Rica

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ABSTRACT

Information regarding parasitic fauna of cetaceans from Costa Rica is provided for the first time. A total of 25 stranded dolphins and whales were examined between 2001 and 2009, including striped dolphin (*Stenella coeruleoalba*) ($n = 19$), pantropical spotted dolphin (*S. attenuata*) ($n = 2$), spinner dolphin (*S. longirostris*) ($n = 1$), bottlenose dolphin (*Tursiops truncatus*) ($n = 1$), dwarf sperm whale (*Kogia sima*) ($n = 1$) and Cuvier's beaked whale (*Ziphius cavirostris*) ($n = 1$). Pathological findings associated with the parasites are also presented. In the most representative dolphin species, *S. coeruleoalba*, the prevalence of parasites was 89.5%; moreover, all examined specimens of *S. attenuata*, *S. longirostris*, *T. truncatus* and *Z. cavirostris* presented parasites. No parasites were recovered from *K. sima*. Fourteen helminth taxa were identified, including six species of cestodes (*Strobilocephalus triangularis*, *Tetrabothrius forsteri*, *Trigonocotyle* sp., *Phyllobothrium delphini*, *Monorygma grimaldi*, Tetraphyllidea gen. sp. plerocercoid), four digeneans (*Nasitrema globicephalae*, *Brachycladium palliatum*, *B. pacificum* and *Oschmarinella albamarina*) and four nematodes (*Anisakis* spp., *Halocercus lagenorhynchi*, *Halocercus* sp. and *Crassicauda anthonyi*). A commensal crustacean, *Xenobalanus globicipitis*, was also identified. All identified parasites representing new geographic records for the Pacific coast of Central America and new host records are presented. Parasitological information is valuable for conservation of cetaceans in Pacific coast of Costa Rica.

1. Introduction

The design and implementation of conservation plans for marine mammals is a matter of public concern. However, very little is known about the role of parasites in the dynamics of marine mammal populations (Raga et al., 1997). These animals commonly strand or are washed ashore along ocean beaches and the post mortem examination of them may provide information on causes of their death (Dailey and Walker, 1978; Raga et al., 1997; Gibson

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et al., 1998; Jaber et al., 2006). The parasitic diseases as a factor in cetacean stranding behavior is still an area of current speculation, although for some authors the parasites are considered as a cause of debilitation or death (Dailey and Stroud, 1978; Stroud and Roffe, 1979; Gibson et al., 1998; Dailey, 2001; Jaber et al., 2006). Damage 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).

Most Costa Rican marine mammals are cetaceans (5 families, 18 genera and 30 species), representing about 36% of the 83 species known worldwide, and 39% of all Latin American marine mammal species (Wehrtmann and Cortés, 2009). About 27 marine mammal species have been observed or are expected to occur in the Pacific (96.5% are confirmed) and 29 (only 28% are confirmed) in the Caribbean waters of Costa Rica (Wehrtmann and Cortés, 2009). Cetaceans are hosts of a great variety of parasites, which include nematodes, cestodes, trematodes, acanthocephalans and crustaceans (Aznar et al., 1994; Cerioni and Mariniello, 1996; Mignucci-Giannoni et al., 1998; Dailey, 2001; Fertl, 2002; Fernández et al., 2004; Colón-Llavina et al., 2009). Nevertheless, the parasite fauna of cetaceans from Costa Rica are unknown thereby the information gathered is valuable for conservation of these mammals.

In this work we provide the first records for parasite fauna of dolphins, dwarf sperm whale and Cuvier's beaked whale found stranded or dead along the Costa Rican Pacific coast. Additionally, pathological findings associated with them are also presented.

2. Materials and methods

Internal parasites were collected from 25 stranded and dead dolphins and whales on the Pacific coast from Costa Rica, during the period 2001–2009. In this period the following cetaceans were studied: 19 striped dolphin (*Stenella coeruleoalba*), 2 pantropical spotted dolphin (*S. attenuata*, previously known as *S. graffmani*), 1 spinner dolphin (*S. longirostris*), 1 bottlenose dolphin (*Tursiops truncatus*), 1 dwarf sperm whale (*Kogia sima*), and 1 Cuvier's beaked whale (*Ziphius cavirostris*). Biological data and geographic localities of these animals are presented in Table 1.

Either dead or moribund cetaceans were collected from the beach by the participants and volunteers of the Fundación Keto and transported to the Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica where the necropsies were carried on. During necropsy, major organ systems were analyzed, including skin, blubber, muscles, abdominal cavity, alimentary system (esophagus, stomach, small and large intestine, liver and pancreatic blood vessels, hepatic and pancreatic ducts), urinary system (kidneys, ureters and urinary bladder), reproductive system, thoracic cavity, respiratory system (air sinuses, trachea, bronchi and lungs), and central nervous system. Animals were necropsied fresh or refrigerated but never frozen. Samples of affected tissues were fixed in 10% buffered neutral formalin and processed for conventional histopathology examination.

Table 1
Biological data and geographic localities of stranded cetaceans on the Pacific coast of Costa Rica, 2001–2009.

Species/case number	Sex/age	Beach/province
Stenella coeruleoalba:		
ND14701	F/J	Hermosa, Guanacaste
N9303	M/J	Undetermined
N16503	F/U	Playa Grande, Guanacaste
ND12504	F/U	Tambor, Puntarenas
NP14004	F/U	Osa, Puntarenas
ND2105	M/A	Matapalo, Puntarenas
ND2505	M/J	Matapalo, Puntarenas
ND3005	F/A	Tambor, Puntarenas
ND3805	F/J	Tambor, Puntarenas
ND1406	F/A	Tambor, Puntarenas
ND2506	F/J	Bahia Ballena, Puntarenas
ND3106	M/J	Esterillos Oeste, Puntarenas
ND7506	F/A	Guacalillo, Puntarenas
ND2307	M/J	Matapalo, Puntarenas
ND2407	F/A	San Isidro, Puntarenas
ND8107	F/A	Quepos, Puntarenas
ND5808	M/J	Puntarenas, Puntarenas
ND5509	M/J	Barú, Puntarenas
ND7309	F/A	Malpaís, Punterenas
S. attenuata:		
ND204	M/J	Matapalo, Puntarenas
ND0109	M/S	Bajamar, Puntarenas
S. longirostris:		
ND7207	F/J	Tamarindo, Guanacaste
Tursiops truncatus:		
ND3909	F/A	Bahia Salinas, Guanacaste
Kogia sima:		
ND6106	F/C	Matapalo, Puntarenas
Ziphius cavirostris:		
ND4009	F/A	Matapalo, Puntarenas

A – adult; C – calf; F – female; J – juvenile; M – male; S – senile; U – undetermined.

Where the collection of all parasites specimens was not feasible, representative samples were taken. The parasites recovered during post mortem examination were transported to the laboratory, washed in physiological saline, and fixed and preserved in 70% ethanol or (93 parts 70% ethanol: 5 parts 10% formaldehyde: 2 parts acetic acid) AFA. Because the examination of all of the specimens under light microscope was not feasible, so the samples were scanned and sorted under stereomicroscope and representatives of the different forms were prepared for examination under light microscope. The staining and mounting of the helminths were carried out as described by Gibson et al. (1998). Platyhelminth specimens were stained with Mayer's paracarmine, dehydrated in an alcohol series, cleared in beechwood creosote and mounted in Canada balsam, whereas nematodes were examined as wet-mounts in clearing agents, such as glycerin (for small worms), lactophenol or beechwood creosote (for large worms). These compounds were removed from the specimens by the use of acid–alcohol prior to their being returned to the storage ethanol or AFA. On the other hand, the plerocercoids were processed as described by Agusti et al. (2005a). Most plerocercoids were collected alive and were stained with eosin, dehydrated in 70% (v/v) ethanol, and cleared with lactophenol.

Barnacles were observed under a stereomicroscope. The identification was based on Rajaguru and Shantha (1992).

Table 2
Parasites and commensals of cetaceans stranded on the Pacific coast of Costa Rica, 2001–2009.

Species/case number	Parasites	Infected organ
Stenella coeruleoalba:		
ND14701	*Tetrabothrius forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Stomach
	Halocercus lagenorhynchi	Lungs
N9303	*T. forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Stomach and intestine
	H. lagenorhynchi	Lungs
N16503	*T. forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Stomach and intestine
ND12504	*T. forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Esophagus and stomach
	H. lagenorhynchi	Lungs
ND2505	*T. forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Stomach
ND3005	*Anisakis* spp.	Stomach
	H. lagenorhynchi	Lungs
ND3805	*T. forsteri*	Intestine
	Anisakis spp.	Esophagus and stomach
ND1406	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Esophagus and stomach
	H. lagenorhynchi	Lungs
ND2506	*T. forsteri*	Intestine
	Anisakis spp.	Stomach
	H. lagenorhynchi	Lungs
ND3106	*T. forsteri*	Intestine
	Anisakis spp.	Stomach and intestine
	H. lagenorhynchi	Lungs
ND7506	*T. forsteri*	Intestine
	Strobilocephalus triangularis	Colon
	Phyllobothrium delphini	Subcutaneous blubber
	Monorygma grimaldi	Parallel to the rectum
	Anisakis spp.	Stomach and intestine
	H. lagenorhynchi	Lungs
ND2307	*S. triangularis*	Colon
	P. delphini	Subcutaneous blubber
	Anisakis spp.	Stomach
ND2407	*T. forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts, hepatic and pancreatic ducts
	P. delphini	Subcutaneous blubber
	M. grimaldi	Caudal area of the kidneys
	Brachycladium palliatum	Hepatic and pancreatic ducts
	Anisakis spp.	Esophagus, stomach, intestine
	H. lagenorhynchi	Lungs
ND8107	*T. forsteri*	Intestine
	Anisakis spp.	Stomach
	H. lagenorhynchi	Lungs
ND5808	*T. forsteri*	Intestine
	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Esophagus, stomach and intestine
	H. lagenorhynchi	Lungs
	Xenobalanus globicipitis	Skin (caudal fluke)
ND5509	*T. forsteri*	Intestine
	P. delphini	Subcutaneous blubber
	M. grimaldi	Testis
	Nasitrema globicephalae	Air sinuses
	Anisakis spp.	Stomach
	H. lagenorhynchi	Lungs
ND7309	*P. delphini*	Subcutaneous blubber
Stenella attenuata:		
ND204	Tetraphyllidean plerocercoids	Anal crypts
	Anisakis spp.	Stomach
	Halocercus sp.	Lungs

Table 2 (*Continued*)

Species/case number	Parasites	Infected organ
ND0109	*T. forsteri*	Intestine
	M. grimaldi	Testis
	N. globicephalae	Air sinuses
	Trematodes eggs	Lungs
	Anisakis spp.	Stomach
	X. globicipitis	Skin (caudal fluke)
Stenella longirostris:		
ND7207	*Trigonocotyle* sp.	Intestine
	B. pacificum	Hepatic and pancreatic ducts
	Anisakis spp.	Stomach
	Halocercus sp.	Lungs
Tursiops truncatus:		
ND3909	*M. grimaldi*	Ligament of the uterus
Ziphius cavirostris:		
ND4009	*Oschmarinella albamarina*	Hepatic and pancreatic ducts
	Anisakis spp.	Stomach
	Crassicauda anthonyi	Kidney

The numerical data collected are restricted to prevalence (Bush et al., 1997). Accurate estimates of intensity of infection were generally not possible.

3. Results

Overall, five of six cetacean species had parasites. In the most representative dolphin, *S. coeruleoalba*, prevalence of parasites was 89.5%; moreover, all examined specimens of *S. attenuata*, *S. longirostris*, *T. truncatus* and *Z. cavirostris* presented parasites. No parasites were recovered from the dwarf sperm whale calf *K. sima*.

Fourteen helminth taxa were found and morphologically identified, including six species of cestodes, four digeneans and four nematodes. The nematodes and cestodes were the most prevalent parasite groups (90.9%), followed by the digeneans (22.7%). The most prevalent species were *Anisakis* spp. (90.9%), followed by *Tetrabothrius forsteri* (63.6%), *Halocercus lagenorhynchi* (54.5%) and tetraphyllidean plerocercoids (40.9%).

Additionally, a commensal crustacean species was also found and identified.

Data of the hosts, parasites and infected organs, and prevalence of identified parasites are presented in Tables 2 and 3.

3.1. Eucestoda

Were collected many metacestodes (plerocercoids and merocercoids), scolices, strobilar fragments, and complete specimens of cestodes. Three cestodes species (Eucestoda, Tetrabothriidae) from the intestine of dolphins were identified: *T. forsteri* (63.6%) in *S. coeruleoalba* and *S. attenuata*; *Strobilocephalus triangularis* (9.1%) in *S. coeruleoalba*, and *Trigonocotyle* sp. (4.5%) in *S. longirostris*. Specimens of *T. forsteri* (Fig. 1) and *Trigonocotyle* sp. were recovered mainly from duodenum, whereas complete specimens of *S. triangularis* were recovered from the terminal colon. The enormous scolices of *S. triangularis* were deeply embedded in the sub-mucosa, no associated with lesions.

Two species of tetraphyllidean merocercoids were also collected: merocercoids of *Phyllobothrium delphini* (Eucestoda, Phylobothriidae) were encysted in the ventral abdominal subcutaneous blubber (Fig. 1) from *S. coeruleoalba*, whereas merocercoids of *Monorygma grimaldi* (Eucestoda, Phylobothriidae) were retroperitoneally encysted parallel to the rectum, in the caudal pole of the kidneys, in the broad ligament of the uterus and close to the testis (Fig. 1). Species infected with *M. grimaldi* were *S. coeruleoalba*, *S. attenuata* and *T. truncatus*. *P. delphini* and *M. grimaldi* presented the same prevalence (22.7%).

Tetraphyllidean plerocercoids (40.9%) were found inside the anal crypts (100%) and free in the hepatic and pancreatic ducts (11.1%) from *S. coeruleoalba* and *S. attenuata*. Two morphotypes were recognized: "small" plerocercoids (SP) and "large" plerocercoids (LP), according to the descriptions of Agusti et al. (2005a) and Aznar et al. (2007).

3.2. Digenea

Four digeneans of Brachycladiidae family (previously known as Campulidae and Nasitrematidae) were identified: *Nasitrema globicephalae* (9.1%), *Brachycladium palliatum* (4.5%), *B. pacificum* (4.5%) and *Oschmarinella alba-*

Table 3
Prevalence of parasites and commensals of cetaceans stranded on the Pacific coast of Costa Rica, 2001–2009.

Parasites	No. infected (prevalence %)
Anisakis spp.	20 (90.9)
Tetrabothrius forsteri	14 (63.6)
Halocercus lagenorhynchi	12 (54.5)
Tetraphyllidean plerocercoids	9 (40.9)
Phyllobothrium delphini	5 (22.7)
Monorygma grimaldi	5 (22.7)
Halocercus sp.	2 (9.1)
Strobilocephalus triangularis	2 (9.1)
Nasitrema globicephalae	2 (9.1)
X. globicipitis	2 (9.1)
Trigonocotyle sp.	1 (4.5)
Brachycladium palliata	1 (4.5)
B. pacificum	1 (4.5)
Oschmarinella albamarina	1 (4.5)
Crassicauda anthonyi	1 (4.5)
Total	22 (88.0)

marina (4.5%). The identification of digeneans was made according to Gibson and Bray (1997) and Gibson (2005).

Specimens of *N. globicephalae* were found in air sinuses (Fig. 1) of *S. coeruleoalba* and *S. attenuate*. The parasites (more than 40 trematodes per host) were associated with multiple erosions and mild sinusitis in infected dolphins. Moreover, the specimen of *S. attenuata* presented perineuristis in eighth cranial nerve (vestibulocochlear nerve). In a spotted dolphin *S. attenuata* (ND0109), which have adults of *N. globicephalae* in air sinuses, also were found trematode eggs in the mucous collected from the lungs. A slight granulomatous pneumonia was associated to the presence of eggs.

Liver flukes *B. palliatum* (Fig. 1), *B. pacificum* and *O. albamarina* (Fig. 1) were recovered from the hepatic and pancreatic ducts of *S. coeruleoalba*, *S. longirostris* and *Z. cavirostris* respectively. All infected cetaceans presented a slight nonsuppurative cholangitis associated with the presence of parasites.

3.3. Nematoda

Were identified nematodes of three genera: *Anisakis* (Nematoda, Anisakidae), *Halocercus* (Nematoda, Pseudaliidae) and *Crassicauda* (Nematoda, Tetrameridae).

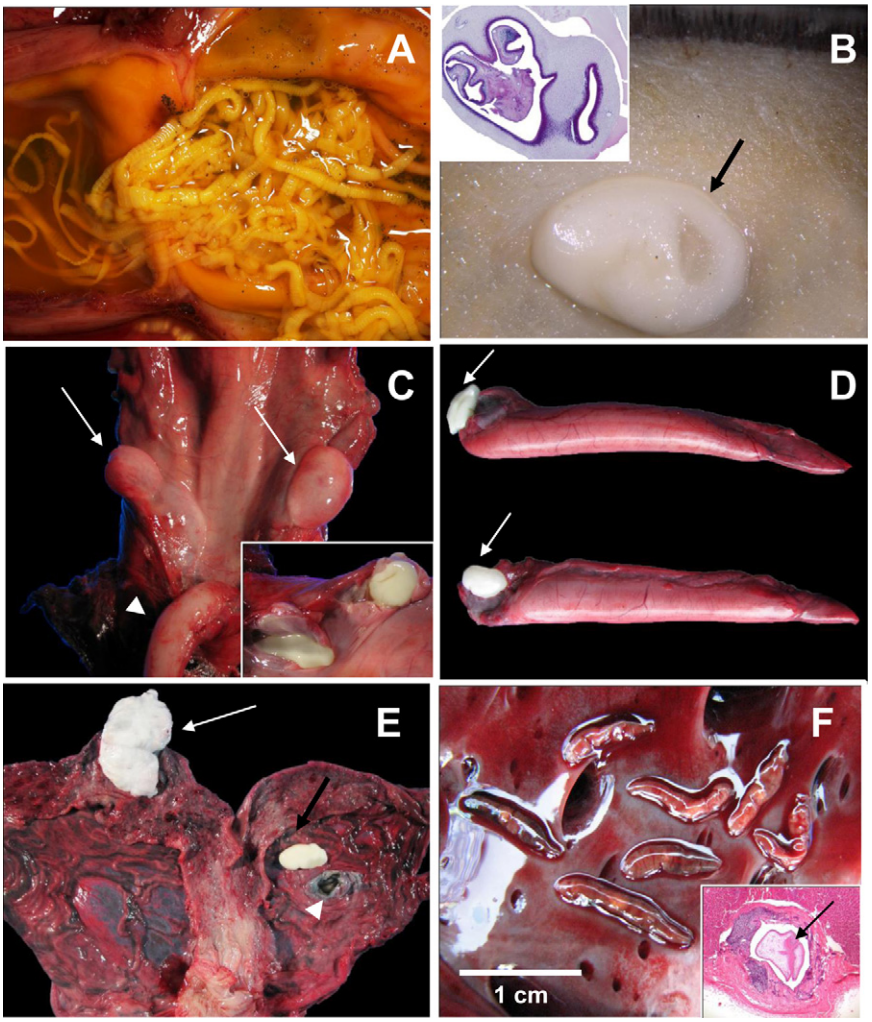

Fig. 1. Parasites of cetaceans stranded on the Pacific coast of Costa Rica, 2001–2009: (A) *Tetrabothrius forsteri* in the duodenum of striped dolphin *Stenella coeruleoalba*. (B) *Phyllobothrium delphini* cyst in the blubber of the abdominal area of *S. coeruleoalba* (black arrow); insert shows a histological section of this merocercoid (H&E X4). (C) Two cysts of *Monorygma grimaldi* (white arrows) close to the rectum (arrow head) from *S. coeruleoalba*; insert shows opened cyst with merocercoids exposed. (D) Two opened cysts of *M. grimaldi* (white arrows) from the testis of *S. coeruleoalba*. (E) Exposed merocercoid of *M. grimaldi* (black arrow) and empty cyst (arrow head) in the broad ligament of the uterus of *S. coeruleoalba*; note a non related tumor (with arrow). (F) *Brachycladium palliatum* from hepatic ducts of *S. coeruleoalba*; insert shows a histological section of a trematode (black arrow) inside a bile duct initiating a slight nonsupurative inflammation (H&E X4). (G) *Nasitrema globicephalae* in the air sinuses of *S. coeruleoalba* (black arrow) causing multiple erosions and mild sinusitis; magnification of a trematode in the paraotic sinuses is shown in the insert (white arrow). (H) *Oschmarinella albamarina* (white arrows) in the liver of Cuvier's beaked whale *Ziphius cavirostris*. (I) *Anisakis* spp. in the non glandular stomach of *S. coeruleoalba*. (J) *Halocercus lagenorhynchi* in the air passage of *S. coeruleoalba*; insert a granuloma (white arrow). (K) Kidney of *Z. cavirostris* showing atrophic ranulas (black arrows) caused by the presence of *Crassicauda anthonyi*. (L) *C. anthonyi* in the renal pelvis of *Z. cavirostris* opened kidney.

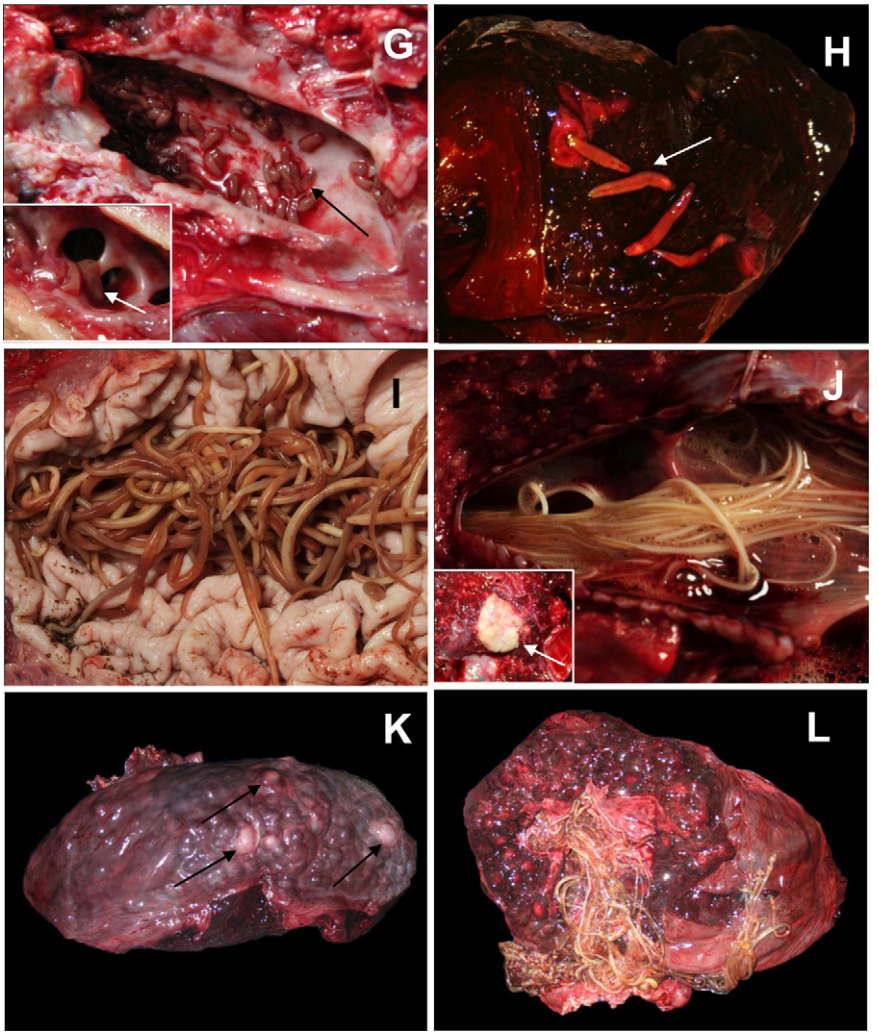

Fig. 1. (*Continued*).

Anisakis spp. was the most prevalent parasite (90.9%). The following hosts were infected by this nematode: *S. coeruleoalba*, *S. attenuata*, *S. longirostris* and *Z. cavirostris*. Larvae and adults of *Anisakis* spp. were collected in different digestive organs as stomach (100%), intestine (30%) and esophagus (25%) (Fig. 1). Four infected cetaceans presented a mild gastritis associated with a presence of parasites within the gastric mucosa.

H. lagenorhynchi (54.5%) was collected in the lungs of striped dolphins *S. coeruleoalba*, whereas *Halocercus* sp. (9.1%) was recovered from *S. attenuata* and *S. longirostris*. Adults of *H. lagenorhynchi* were collected in air passage of infected animals (Fig. 1), resulting in a moderate to severe granulomatous pneumonia associated with the presence of intralesional parasites.

The large nematode *C. anthonyi* was recovered from the kidneys of *Z. cavirostris* (4.5%). Compressive necrosis and fibrosis were observed, due to the huge number of parasites (Figs. 1 and 2) at the urinary pelvis and in some areas of the renal parenchyma, resulting in partial destruction of the organ.

Fig. 2. Free nematodes *C. anthonyi* from the kidney of *Ziphius cavirostris*.

3.4. Crustacea

A pedunculate barnacle, identified as *Xenobalanus globicipitis* (Crustacea, Coronulidae), was found in the caudal fluke of *S. coeruleoalba* and *S. attenuata*.

4. Discussion

Parasites in marine mammals are relatively common and some of them have been implicated in disease processes and as one of the prominent causes of strandings of cetaceans (Gibson et al., 1998; Mignucci-Giannoni et al., 1998; Colón-Llavina et al., 2009). This is the first systematic survey regarding the parasitic fauna of cetaceans from Pacific coast of Costa Rica. All identified parasites and commensals representing new geographic records for the Pacific coast of Central America and increased the occurrence of some parasites to new hosts.

4.1. Eucestoda

Cestodes were one of the most representative group of parasites. Among several cestodes species that may be found in the intestine of cetaceans the easiest to identify (because of their enormous scolex) is *S. triangularis*, a parasite of dolphin's intestine particularly *S. coeruleoalba* (Hoberg, 1994; Dhermain et al., 2002). This tapeworm had been reported solely in *S. coeruleoalba* from the Pacific coast of the United States and Mediterranean Sea (Dailey and Stroud, 1978; Raga and Carbonell, 1985; Cerioni and Mariniello, 1996; Dhermain et al., 2002), and *S. attenuata* from Pacific coast of the United States (Dailey and Perrin, 1973). *S. triangularis* is considered as the most pathogenic cestode of dolphins because penetrates the colon wall initiating an inflammatory response (Dailey, 2001; Dhermain et al., 2002), which was not observed in this search probably because of the low intensity of parasitism. Massive infections with this species have been reported as probable cause of death in certain age categories of *S. attenuata* in United States (Dailey and Perrin, 1973).

Besides of *S. triangularis*, tetrabothriids were also represented by *T. forsteri* and *Trigonocotyle* sp. *T. forsteri* was the most prevalent, particularly in *S. coeruleoalba* and *S. attenuata*. This parasite has been also reported in *S. coeruleoalba* and *S. attenuata* from the United States, Spain and Italy (Dailey and Perrin, 1973; Dailey and Stroud, 1978; Raga and Carbonell, 1985; Cerioni and Mariniello, 1996). On the other hand, *Trigonocotyle* sp. was found only in *S. longirostris*, similar to reported by Aguilar et al. (2001) in Mexico. Neither *T. forsteri* as *Trigonocotyle* sp. appear to have not obvious pathogenic effect (Gibson et al., 1998; Aguilar et al., 2001).

Four types of tetraphyllidean metacestodes infect cetaceans worldwide: two plerocercoids differing in size, "small" (SP) and "large" (LP), and two merocercoids referred as *P. delphini* and *M. grimaldii* (Aznar et al., 2007). In this search, tetraphyllidean plerocercoids (SP and LP) were collected from the anal crypts, hepatic and pancreatic conducts of *S. coeruleoalba* and *S. attenuata*. There are records of plerocercoids recovered from *S. coeruleoalba*, *Grampus griseus* and *Z. cavirostris* stranded on the Mediterranean coast of Spain (Fernández et al., 2004; Agusti et al., 2005a) as well as from *S. longirostris* on the Pacific and Atlantic coasts of Mexico and Brazil respectively (Aguilar et al., 2001; Carvalho et al., 2010). For the first time *S. attenuata* is presented as new host of tetraphyllidean plerocercoids.

Tetraphyllidean plerocercoids, initially referred as *Scolex pleuronectis* (Skrjabin, 1972; Fernández et al., 2004; Agusti et al., 2005a), showed high prevalence and abundance as well as a high degree of site specificity, particularly in the anal crypts. Moreover, the two morphotypes of tetraphyllidean plerocercoids coexists in the same individual hosts have been reported in some cetacean species (Fernández et al., 2004; Agusti et al., 2005a; Aznar et al., 2007). Some authors have speculated that SP and LP morphotypes are thought to be earlier stages of *P. delphini* and *M. grimaldi* that presumed to infect large pelagic sharks that feed on cetaceans (Agusti et al., 2005a; Aznar et al., 2007). Nevertheless, molecular analysis showed that LP of *P. delphini* and *M. grimaldi* metacestodes may represent separate species, whereas SP may be conspecific with *M. grimaldi* (Aznar et al., 2007). In the present work, solely one specimen of *S. coeruleoalba* (ND2407) parasitized by SP and LP plerocercoids presented co-infection by *P. delphini* and *M. grimaldi*. It seems to be clear that cetaceans act as natural intermediate hosts for *P. delphini* and *M. grimaldi*, as within these hosts they undergo development from the plerocercoid stage to the merocercoid stage (Agusti et al., 2005a,b; Aznar et al., 2007).

Merocercoids of *P. delphini* and *M. grimaldii* are well known from most cetaceans worldwide and the typical sites of infection of these encysted metacestodes are the subcutaneous blubber and the abdominal cavity, respectively (Norman, 1997; Mignucci-Giannoni et al., 1998; Agusti et al., 2005a,b; Colón-Llavina et al., 2009). In the present study, specimens of the *P. delphini* occurred in the blubber solely from *S. coeruleoalba*, which was also reported in England, Wales, United States, Spain and Italy (Dailey and Stroud, 1978; Dailey and Walker, 1978; Baker, 1992; Cerioni and Mariniello, 1996; Gibson et al., 1998; Agusti et al., 2005a,b). On the other hand, specimens of *M. grimaldi* were found retroperitoneally encysted in different locations of the abdominal cavity including rectum, testis, at the broad ligament of the uterus and close to the kidneys of *S. coeruleoalba*, *S. attenuata* and *T. truncatus*. Previously, several authors reported them from *S. coeruleoalba* and *S. attenuata* in England, Wales, United States Spain and Italy (Dailey and Perrin, 1973; Dailey and Stroud, 1978; Dailey and Walker, 1978; Baker, 1992; Cerioni and Mariniello, 1996; Gibson et al., 1998; Agusti et al., 2005a,b). In Italy, merocercoids of *M. grimaldi* were found solely in the diaphragm of *T. truncatus* (Macchioni and Barducci, 1989).

4.2. Digenea

Several species of flukes have been documented in cetaceous worldwide (Dailey and Perrin, 1973; Raga, 1986; O'Shea et al., 1991; Gibson and Bray, 1997; Gibson et al., 1998; Degollada et al., 2002). *N. globicephalae* normally resides in the air sinuses of small cetaceans (O'Shea et al., 1991), which was found in *S. coeruleoalba* (a juvenile dol-

phin) and *S. attenuata* (a senile dolphin) in this study. Degollada et al. (2002) observed a high prevalence of this parasite in the paraotic sinuses, ear complex, eighth cranial nerve and brain from *S. coeruleoalba* and *T. truncatus* stranded on the Canary Island. This is a new record of *N. globicephalae* in *S. attenuata*. Moderate ulcerative sinusitis and perineuritis of the eighth cranial nerve resulted of the presence of *N. globicephalae* in infected dolphins, but were not observed lesions in the brain. Differently of this investigation, brain lesions produced by *Nasitrema* have been implicated as a cause of death in single stranding of small cetaceans (Dailey and Walker, 1978; O'Shea et al., 1991; Degollada et al., 2002). However, it has not always been clear whether the trematode infection in the brain also debilitated the animal, leading to death from some secondary cause, or other events weakened the animals and allowed the parasites to invade the brain (O'Shea et al., 1991).

In a pantropical spotted dolphin (*S. attenuata*) infected by adults of *N. globicephalae* were also found trematode eggs in the lungs, which were morphologically indistinguishable those found in the air sinuses. O'Shea et al. (1991) noted a single *Nasitrema* sp. in the trachea of a striped dolphin stranded in the Gulf of Mexico; while Kumar et al. (1975) reported the potentialities of *N. attenuata* as an aetiological agent of pulmonary lesions in bottlenose dolphins (*T. truncatus*). According to Kumar et al. (1975), adults of *N. attenuata* occurring in the nasal sinuses and posterior nasal passage are considered as practically harmless for the host but their eggs, aspirated deep into the bronchial tree, may initiate a foreign-body of inflammatory reaction in the lungs and continuous aspiration of such eggs may provoke a chronic pneumonia condition. In the current survey, a slight granulomatous pneumonia was associated to the presence of trematode eggs. The eggs migration route of *Nasitrema* sp. is unclear.

Liver flukes *B. palliatum* (previously known as *Campula palliata*) and *B. pacificum* (previously known as *Zalophotrema pacificum*) were found in *S. coeruleoalba* and *S. longirostris* respectively, similar to reported in United States, Spain, Italy and Mexico (Dailey and Perrin, 1973; Lluch et al., 1987; Cerioni and Mariniello, 1996; Aguilar et al., 2001). Additionally, *B. palliatum* has been also reported in *S. attenuata* and *T. truncatus* from United States (Woodard et al., 1969; Dailey and Perrin, 1973). Parasitological information regarding to Cuvier's beaked whales *Z. cavirostris* is very limited (Demaree et al., 1997; Fernández et al., 2004; Berón-Vera et al., 2008) and the only record of digeneans in cetaceans of the family Ziphiidae was performed by Demaree et al. (1997), who identified a new species *O. macrorchis* from the liver sinuses of a Stejneger's beaked whale *Mesoplodon stejnegeri*. Thereby, *Z. cavirostris* is new host record for *O. albamarina*.

In this study, a slight nonsuppurative cholangitis was observed in all infected cetaceans. Likewise, there are numerous reports in the literature concerning the pathogenicity of liver flukes, which may produce severe damage to the bile ducts, eliciting focal suppurative cholangitis, severe necrotizing cholangitis or chronic granulomatous cholangitis (Gibson et al., 1998; Jaber et al., 2004, 2006). According to Woodard et al. (1969), pancreatic lesions occur with more regularity than the hepatic lesions in *T. truncatus* infected by *B. palliatum*.

4.3. Nematoda

Nematodes were one the most representative group of parasites.

Morphologically, all specimens recovered (adults and larvae) were identified as *Anisakis* genus. Nevertheless, species identification based on morphology is limited in this group of nematodes and the species identification is possible only through the use of genetic and molecular techniques (Mattiucci et al., 2002; Mattiucci and Nascetti, 2006). *Anisakis* genus include: *A. simplex* species complex (*A. simplex* s.s., *A. simplex* C and *A. pegreffii*), *A. typica*, *A. ziphidarum*, *A. physeteris* and *A. brevispiculata* (Mattiucci et al., 2002). All cetaceans studied in this survey are documented hosts for species of *Anisakis* and the major diversity (*Anisakis* sp., *A. ziphidarum*, *A. typica* and *A. physeteris*) have been reported from Cuvier's beaked whales in Puerto Rico, Virgin Islands and Spain (Mignucci-Giannoni et al., 1998; Fernández et al., 2004). *Anisakis* sp. and *A. typica* had been documented from *S. longirostris* in Puerto Rico, Virgin Islands and Mexico (Mignucci-Giannoni et al., 1998; Aguilar et al., 2001); while *A. simplex* is reported from *S. coeruleoalba* (Baker, 1992; Gibson et al., 1998) and *S. attenuata* (Dailey and Perrin, 1973). A molecular study should be performed for the identification of *Anisakis* species from Costa Rican cetaceans.

Larvae and adults of *Anisakis* spp. were found in stomach, intestine and esophagus. As observed in this work, the normal site for this worm is the stomach compartments; its occurrence outside this site is likely to be due to a post mortem migration (Gibson et al., 1998). *Anisakis* spp. is found within the lumen or attached to the mucosa of the stomach of marine mammals and can produce ulcers and cause hemorrhages (Abollo et al., 1998; Motta et al., 2008; Colón-Llavina et al., 2009). Four infected cetaceans presented a mild gastritis and no gastric ulcers were observed in any infected cetacean, similar to observed by Motta et al. (2008) in some cetaceans from Brazil. *Anisakis* spp. is medical and economic relevant because is a causative agent of human anisakiosis (Gibson et al., 1998).

In the current study *H. lagenorhynchi* was collected from the lungs of *S. coeruleoalba*, and *Halocercus* sp. from *S. attenuata* and *S. longirostris*. Previously, *H. lagenorhynchi* and *Halocercus* sp. has been recorded in *S. coeruleoalba* and *S. longirostris* from England, Wales and Brazil (Baker, 1992; Gibson et al., 1998; Carvalho et al., 2010); while *H. lagenorhynchi* and *H. delphini* are documented in *T. truncatus* and *S. attenuata* from Unites States and England (Dailey and Perrin, 1973; Dailey et al., 1991; Baker, 1992; Fauquier et al., 2009).

Parasitism of the respiratory system is a relatively common finding in stranded cetaceans, resulting in mild chronic lesions. Lungworms can be quite pathogenic, but the infections are not the primary cause of death (Gibson et al., 1998; Fauquier et al., 2009). The presence of parasites in the lungs of cetaceans stranded in Atlantic coast of Brazil was diagnosed as a highly debilitating condition, affecting respiratory capacity and diving (Carvalho et al., 2010).

In the present study a moderate to severe granulomatous pneumonia was associated with the presence of intralesional *Halocercus* parasites. Signs of active and chronic multifocal granuloma and granulomatous bronchopneumonia (consistent with secondary involvement following a primary lungworm infection) were observed in cetaceans stranded in the British Virgin Islands (Mignucci-Giannoni et al., 1998). Additionally, verminous pneumonia limited to the area immediately surrounding the parasite-laden air passage was reported by Woodard et al. (1969) in captive and wild Atlantic bottlenose dolphin *T. truncatus*.

Previous records of *Crassicauda* in cetaceans include *C. anthonyi*, *C. boopis* and *C. crassicauda* (Mignucci-Giannoni et al., 1998; Colón-Llavina et al., 2009). According to Gibson et al. (1998), the female worms are enormous, reaching up to 7 m in length and 1 cm in diameter. This is probably the second largest known species of nematode (Gibson et al., 1998). In this survey, the spirurid *C. anthonyi* was found solely in the kidneys of *Z. cavirostris*, resulting in necrosis, fibrosis and partial destruction of the organ due to huge number of parasites, similar to reported in Australia and Puerto Rico (Robson, 1984; Mignucci-Giannoni et al., 1998). Because the tissue damages the crassicaudosis is a parasitic disease threatening the health and population recovery of cetaceans (Lambertsen, 1992).

4.4. Crustacea

X. globicipitis, a nonpathogenic crustacean, have been documented in *S. coeruleoalba*, *S. attenuata* and *T. truncatus* worldwide (Rajaguru and Shantha, 1992; Aznar et al., 1994; Carvalho et al., 2010). This barnacle is referred to as commensals because it only use their hosts for fixing (Aznar et al., 1994; Dhermain et al., 2002; Fertl, 2002; Carvalho et al., 2010). *S. attenuata* is presented as a new host of *X. globicipitis*.

4.5. Final considerations

The parasite fauna of marine mammals has not yet received all the necessary attention although they could be used as biological tags of marine mammal populations and as an aid assessing mortality. As observed by Gibson et al. (1998), in this work were identified parasites that occur in a wide range of hosts (e.g. *Anisakis* spp.), parasites which might be associated with stranding and/or mortality (e.g. *N. globicephalae*) or have definite pathogenic effects and debilitate their host (e.g. *Anisakis* spp., *C. anthonyi*, *H. lagenorhynchi*, *Halocercus* sp., *B. palliatum*, *B. pacificum* and *O. albamarina*). The parasitological information presented in the current study is valuable for conservation of cetaceans in Costa Rica.

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