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Additional records of metazoan parasites from Caribbean marine mammals, including genetically identified anisakid nematodes

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Abstract Studies of marine mammal parasites in the Caribbean are scarce. An assessment for marine mammal endo- and ectoparasites from Puerto Rico and the Virgin Islands, but extending to other areas of the Caribbean, was conducted between 1989 and 1994. The present study complements the latter and enhances identification of anisakid nematodes using molecular markers. Parasites were collected from 59 carcasses of stranded cetaceans and manatees from 1994 to 2006, including *Globicephala macrorhynchus*, *Kogia breviceps*, *Kogia sima*, *Lagenodelphis hosei*, *Mesoplodon densirostris*, *Peponocephala electra*, *Stenella longirostris*, *Steno bredanensis*, *Trichechus manatus*. *Tursiops truncatus*, and *Ziphius cavirostris*. Sixteen species of endoparasitic helminthes were morphologically identified, including two species of acanthocephalans (*Bolbosoma capitatum*, *Bolbosoma vasculosum*), nine species of nematodes (*Anisakis* sp., *Anisakis brevispiculata*, *Anisakis paggiae*, *Anisakis simplex*, *Anisakis typica*, *Anisakis ziphidarium*, *Crassicauda anthonyi*, *Heterocheilus tunicatus*, *Pseudoterranova ceticola*), two species of cestodes (*Monorygma grimaldi*, *Phyllobothrium delphini*), and three species of trematodes (*Chiorchis groschafti*, *Pulmonicola cochleotrema*, *Monoligerum blairi*). The nematodes belonging to the genus *Anisakis* recovered in some stranded animals were genetically identified to species level based on their sequence analysis of mitochondrial DNA (629 bp of mtDNA *cox 2*). A total of five new host records and six new geographic records are presented.

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Introduction

Marine mammals are parasitized by a variety of helminthes, including acanthocephalans, cestodes, nematodes, and digeneans (Dierauf 1990; Aznar et al. 2001). Some of them cause diseases but rarely death of the host (Raga et al. 1997; Dailey 2001). In the Caribbean, studies concerning marine mammal parasites are scarce. Prior to the mid-1990s, only Arnold and Gaskin (1975), Morales-Vela and Olivera-Gómez (1993), and Debrot and Barros (1994) reported finding endoparasites in cetaceans from the area. Mignucci-Giannoni et al. (1998) conducted the most comprehensive study of the metazoan parasitic fauna of cetaceans in the Caribbean followed by an assessment of the endoparasites of the West Indian manatee (*Trichechus manatus*) in Puerto Rico (Mignucci-Giannoni et al. 1999a) and the Dominican Republic (Mignucci-Giannoni et al. 1999b). Mora-Pinto (2000) studied the morphological differences between trematodes found in the manatee's intestine. Cintrón-de Jesús et al. (Cintrón-de Jesús 2001; Cintrón-de Jesús et al. 1999, 2005) identified the barnacles

associated with Caribbean marine mammals, while Ortíz et al. (1992), Bonde et al. (2005a, b), and Morales-Vela et al. (2008) described crustacean epibionts on manatees from Cuba, Belize, and Mexico, respectively. Valentini et al. (2006) included anisakid nematodes from Caribbean cetaceans in establishing the genetic relationship among the genetically recognized *Anisakis* species, inferred from the mitochondrial cytochrome oxidase 2 gene (mtDNA *cox2*) sequences as well as allozyme data. However, genetic–molecular tools have not previously been used to corroborate taxonomic identification of Caribbean parasites.

Here, we update the taxonomic composition of helminthes in Caribbean marine mammals with additional collections and use genetic and molecular techniques to identify anisakid nematodes found in cetaceans from these tropical waters.

Materials and methods

Parasites were collected from 59 carcasses of stranded cetaceans and manatees salvaged in Puerto Rico and the Virgin Islands between 1994 and 2006. Marine mammals examined included one roughtooth dolphin (*Steno bredanensis*), one spinner dolphin (*Stenella longirostris*), one melonhead whale (*Peponocephala electra*), one pygmy sperm whale (*Kogia breviceps*), two Fraser's dolphins (*Lagenodelphis hosei*), two Blainville's beaked whales (*Mesoplodon densirostris*), three dwarf sperm whales (*Kogia sima*), four bottlenose dolphins (*Tursiops truncatus*), four shortfin pilot whales (*Globicephala macrorhynchus*), 10 Cuvier's beaked whales (*Ziphius cavirostris*), and 30 West Indian manatees.

During necropsy, we searched for endoparasites in the entire gastrointestinal tract, major organ systems, blubber, inner ear canals, and nares. Subsamples of parasites were fixed in 10% formalin and stored in glass vials in 70% ethanol between 1994 and 2000. Beginning in 2001, and thereafter, all parasites collected were preserved only in 70% ethanol to provide for both morphologic and genetic–molecular identification. Each vial was labeled with information from the stranding or mortality event, the date of collection, the host, and the location in the host.

Helminthes were identified morphologically with a phase-contrast or dissection stereomicroscope for external features and with a compound-light microscope for morphologic landmarks. For nematodes, we examined their cephalic and caudal end (i.e., spicules, postanal papillae, preanal papillae), postanal tail with typical mucron (for larvae identification), esophagus, lips, and body at level of intestinal ventricular junction by mounting the helminth in lactophenol and comparing them to descriptions given in keys of Yamaguti (1959, 1961, 1963a, b, c), Davey (1971), Anderson et al. (1974–1982), and Mattiucci et al. (2005). For cestodes, we used keys in Schmidt (1986) and Khalil et al. (1994) and for trematodes, we used keys in Yamaguti (1971), Schell (1985), Gibson et al. (2002), Jones et al. (2005), and Bray et al. (2008).

Fourteen nematode specimens belonging to the genera *Anisakis* and *Pseudoterranova* were identified genetically based on the sequence analysis of the mtDNA *cox2* gene. These specimens were collected in this study from a roughtooth dolphin, bottlenose dolphin, Blainville's beaked whale, Cuvier's beaked whales, and dwarf sperm whales. Total DNA was extracted from 2 mg of individual nematode tissue, using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). Total DNAs from some specimens fixed in formalin were extracted using cetyltriethylammonium bromide as described by Yang et al. (1997). The *cox2* gene from each species of *Anisakis* was amplified using the primers 210 5′CACCAACTCTTAAAATTATC and 211 5′ TTTTCTAGTTATATAGATTGRTTYAT from Nadler and Hudspeth (2000) spanning mtDNA nucleotide position 10639–11248 as defined in *Ascaris suum* (GenBank Accession #X54253). PCR amplification was carried out in a volume of 50 μl containing 30 pmol of each primer, $MgCl_2$ 2.5 mM (Amersham Pharmacia Biotech, Piscataway, NJ), PCR buffer 1× (Amersham Pharmacia Biotech), DMSO 0.08 mM, dNTPs 0.4 mM (Sigma–Aldrich, St. Louis, MO) 5 U of *Taq* Polymerase (Amersham Pharmacia Biotech), and 10 ng of total DNA. The mixture was denatured at 94°C for 3 min, followed by 34 cycles at 94°C for 30 s, 46°C for 1 min, 72°C for 90 s, followed by post amplification at 72°C for 10 min. The PCR product was purified using PEG precipitation and automated DNA sequencing was performed by Macrogen (Seoul, Korea; Valentini et al. 2006). The mtDNA *cox2* (629 bp) sequences obtained in the specimens of *Anisakis* sequenced were compared to those corresponding to the species of *Anisakis* so far genetically characterized by us using allozyme markers and the mtDNA *cox2* locus and deposited in GenBank with the following accession number: *Anisakis* sp. (DQ116431), *Anisakis brevispiculata* (DQ116433), *Anisakis paggiae* (DQ116434), *Anisakis pegreffii* (DQ116428), *Anisakis physeteris* (DQ116432), *Anisakis simplex* C (DQ116429), *A. simplex* (*sensu stricto*; DQ116426), *Anisakis typica* (DQ116427), *Anisakis ziphidarum* (DQ116430), and *Pseudoterranova ceticola* (DQ116435). The *cox2* sequences were aligned using ClustalW (Thompson et al. 1994) as implemented in BioEdit 7.0.1 (Hall 1999), using default parameters. Phylogenetic analysis was performed using "Maximum Parsimony" (MP) by MEGA 3.1 (Kumar et al. 2001). The reliability of phylogenetic relationships was evaluated using nonparametric bootstrap analysis (Felsenstein 1985) for MP tree. Bootstrap values ≥70 were considered well supported (Hillis and Bull 1993). *P. ceticola* was considered as outgroup to root the MP tree, as in previous phylogenetic analysis of *Anisakis* spp. (Valentini et al. 2006).

Following identification, representative voucher specimens were deposited in the US National Parasite Collection, Biosystematics and National Parasite Collection Unit, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705.

Results

In the 59 carcasses of marine mammals examined, we found and morphologically identified 16 species of helminthes, including two species of acanthocephalans, nine species of nematodes, two species of cestodes, and three species of trematodes (Table 1). Larval nematodes and some other specimens in poor condition were only morphologically identifiable to genus. Genetic identification performed on *Anisakis* and *Pseudoterranova* nematode specimens allowed us to document the presence of *Anisakis* sp. (see Valentini et al. 2006), *A. brevispiculata* (see Mattiucci et al. 2001), *A. paggiae* Mattiucci et al. 2005, *A. typica* (see Mattiucci et al. 2002), *A. ziphidarum* Paggi et al. 1998, and *P. ceticola* (Table 1). A total of six new geographic records and five new host records was found (Table 2).

Discussion

Acanthocephala

Species of *Bolbosoma* are characteristic parasites in the intestine of odontocete and mysticete cetaceans (Delyamure 1955). Mignucci-Giannoni et al. (1998) reported two species for the Caribbean: *Bolbosoma capitatum* from the intestine of a shortfin pilot whale and *Bolbosoma vasculosum* from the intestine of a pigmy killer whale (*Feresa attenuata*) and an Atlantic spotted dolphin (*Stenella frontalis*). *B. capitatum* has been reported among large odontocetes (reviewed by Hoberg et al. 1993) and has been found in the longfin pilot whale (*Globicephala melas*) in Canadian waters (Cowan 1967). *B. vasculosum* is known from the shortsnout common dolphin (*Delphinus delphis*), pygmy sperm whale and Sowerby's beaked whale (*Mesoplodon bidens*; see Pendergraph 1971; McAlpine et al. 1997), Blainville's beaked whale (Bannister et al. 1996, Ross 2006), and Atlantic spotted dolphin and the pygmy killer whale (Mignucci-Giannoni et al. 1998). Specimens of *B. capitatum* from a shortfin pilot whale and a melonhead whale are reported, the latter being a new host record.

Nematoda

Species of *Anisakis* were the most prevalent nematodes in Caribbean cetaceans. Species identification based on morphology is limited in this group of nematodes and often is possible at morphospecies level only using adult male worms. As larvae L4, L3 (i.e., undeveloped individuals of anisakid adults), and adult females were abundant in our collection, species identification was possible only through the use of genetic and molecular techniques. Genetic species-level identification was successful in our collected specimens, except in the case of nematodes recovered from a shortfin pilot whale, and, thus, their identification remained as *A. simplex* (*sensu lato*).

Although *A. simplex* (*sensu lato*) was particularly prevalent in Caribbean pilot whales studied, Kagei et al. (1967), Dailey and Brownell (1972) and Mignucci-Giannoni et al. (1998) only reported *Anisakis* sp. in this odontocete. Genetic–molecular studies on the taxonomy of *A. simplex* (*sensu lato*) revealed the existence of three biological species in this complex: *A. simplex* (*sensu stricto*), widespread between 30° N and the Arctic polar circle; *A. pegreffii*, occurring between 35° S and 55° S as well as in the Mediterranean Sea; and *A. simplex* C, having a discontinuous range including Pacific Canada and the region south of 35° S (Mattiucci et al. 1997; Mattiucci and Nascetti 2006, 2008). *A. simplex (sensu lato*) was found consistently in the stomach of the shortfin pilot whale. This represents a new geographical record. However, because the identification to species level of these specimens was not possible (due to the worm's storage media), we did not consider the shortfin pilot whale a new host record. The alignment of the mtDNA *cox2* sequences (629 bp) of the specimens of *Anisakis* in the present study and those from our previous study (Valentini et al. 2006) are reported in Fig. 1.

All cetaceans studied (Table 2) are known hosts for species of *Anisakis* so far genetically characterized (Mattiucci and Nascetti 2006). *A. brevispiculata* and *A. paggiae* are known from the pygmy and dwarf sperm whales considered in this study. These nematode species have been demonstrated to be genetically distinct at both nuclear and mitochondrial levels (Mattiucci et al. 2001, 2005; Valentini et al. 2006), although they often occur sympatrically in the same definitive host (Mattiucci et al. 2005). However, some diagnostic morphological features distinguish adult worms of *A. brevispiculata* from *A. paggiae* and *A. physeteris* (see Mattiucci et al. 2005). We genetically confirmed the presence of *A. brevispiculata* and *A. paggiae* from the dwarf sperm whale collected in the Caribbean, constituting new geographic records. The sequences of the specimens collected from this host matched the sequences reported in GenBank for *A. brevispiculata* and *A. paggiae* (Valentini et al. 2006; Fig. 1). Moreover, MP analysis (Fig. 2) confirms that the specimens sequenced clustered with those of *A. brevispiculata* or *A. paggiae* genetically sequenced in our previous

Table 1 Endoparasites identified from marine mammals examined in Puerto Rico and the US and British Virgin Islands

Host Field number	Collection date	Sex	Length (cm)	Locality	Parasite	Location in host
Roughtooth dolphin (*Steno bredanensis*)						
NEPST859	30 Mar 2003	F	236	Arroyo, PRI	*Anisakis typica*[a]	Stomach, intestine
					Phyllobothrium delphini	Skin
Bottlenose dolphin (*Tursiops truncatus*)						
NEPST381	29 Jul 1998	U	251	Manatí, PRI	*Phyllobothrium delphini*	Blubber
NEPST549	10 Jun 1999	M	192	San Juan, PRI	*Anisakis typica*	Stomach
NEPST550	5 Jul 1999	F	273	San Juan, PRI	*Anisakis typica*	Stomach
					Phyllobothrium delphini	Skin
NEPST558	23 Aug 1999	M	193	Vega Baja, PRI	*Anisakis typica*[a]	Stomach
Spinner dolphin (*Stenella longirostris*)						
NEPST850	23 Sep 2002	F	186	Ponce, PRI	*Anisakis* sp. (L4)	Liver, stomach
					Monorygma grimaldi	Gonads
Fraser's dolphin (*Lagenodelphis hosei*)						
NEPST319	22 May 1994	M	227	Guánica, PRI	*Anisakis* sp. (L4)	Stomach
NEPST842	9 Jun 2002	M	236	Humacao, PRI	*Anisakis* sp. (L4)	Stomach
					Monorygma grimaldi	Urinary bladder
					Phyllobothrium delphini	Blubber
Melonhead whale (*Peponocephala electra*)						
NEPST848	21 Jul 2002	M	214	San Juan, PRI	*Bolbosoma capitatum*	–
					Monorygma grimaldi	Urinary bladder
Shortfin pilot whale (*Globicephala macrorhynchus*)						
NEPST481	1 Sep 1998	M	236	Cabo Rojo, PRI	*Bolbosoma capitatum*	Intestine
					Monorygma grimaldi	Blubber
NEPST560	28 Aug 1999	F	250	Anegada, VGB	*Anisakis simplex (sensu lato)*	Stomach
NEPST562	28 Aug 1999	M	340	Anegada, VGB	*Anisakis simplex (sensu lato)*	Stomach
NEPST563	30 Aug 1999	F	300	Anegada, VGB	*Anisakis simplex (sensu lato)*	Stomach
Cuvier's beaked whale (*Ziphius cavirostris*)						
NEPST382	29 Jul 1998	M	452	Aguadilla, PRI	*Crassicauda anthonyi*	Kidney
					Anisakis ziphidarum	Stomach
					Phyllobothrium delphini	Blubber
NEPST385	29 Jul 1998	M	530	Aguadilla, PRI	*Anisakis* Type II larvae	Intestine
					Crassicauda anthonyi	Kidney
					Monorygma grimaldi	Abdominal cavity
NEPST392	30 Jul 1998	M	505	Aguadilla, PRI	*Anisakis ziphidarum*[a]	Stomach
					Crassicauda anthonyi	Kidney
					Phyllobothrium delphini	Blubber
NEPST401	30 Jul 1998	M	474	Aguada, PRI	*Anisakis ziphidarum*[a]	Stomach
					Crassicauda anthonyi	Kidney
NEPST505	25 Nov 1998	M	528	Aguada, PRI	*Crassicauda anthonyi*	Kidney
					Anisakis ziphidarum	Stomach
					Phyllobothrium delphini	Blubber
NEPST506	25 Nov 1998	F	498	Hatillo, PRI	*Crassicauda anthonyi*	Kidney
					Phyllobothrium delphini	Skin
NEPST421	12 Apr 1999	U	452	Aguadilla, PRI	*Crassicauda anthonyi*	Kidney
					Phyllobothrium delphini	Blubber
NEPST575	4 Oct 1999	F	494	St. Thomas, VIR	*Crassicauda anthonyi*	Kidney
					Anisakis ziphidarum	Stomach
					Phyllbothrium delphini	Blubber
NEPST576	3 Oct 1999	F	520	St. John, VIR	*Anisakis ziphidarum*[a]	Stomach

Table 1 (continued)

Host Field number	Collection date	Sex	Length (cm)	Locality	Parasite	Location in host
NEPST601	3 May 2000	M	453	Vieques Island, PRI	*Anisakis ziphidarum*[a]	Stomach
					Crassicauda anthonyi	Kidney
					Phyllobothrium delphini	Blubber
Blainville's beaked whale (*Mesoplodon densirostris*)						
NEPST838	9 April 2002	M	335	Dorado, PRI	*Anisakis* sp. (L4)[a]	Stomach
NEPST881	11 Feb 2004	M	410	Ceiba, PRI	*Bolbosoma vasculosum*	Stomach
Pygmy sperm whale (*Kogia breviceps*)						
NEPST617	10 Apr 2001	M	295	Culebra, PRI	*Phyllobothrium delphini*	Blubber
Dwarf sperm whale (*Kogia sima*)						
NEPST393	17 Jul 1998	M	223	Mayagüez, PRI	*Anisakis brevispiculata*[a]	Stomach
					Phyllobothrium delphini	Blubber
NEPST846	2 Jul 2002	F	214	Luquillo, PRI	*Phyllobothrium delphini*	Blubber
					Pseudoterranova ceticola	Stomach
NEPST845	4 Jul 2002	F	135	Rio Grande, PRI	*Anisakis paggiae*[a]	Stomach
					Phyllobothrium delphini	Blubber
West Indian manatee (*Trichechus manatus*)						
NEPST534	9 Jun 1998	F	230	Guayanilla, PRI	*Chiorchis groschafti*	Intestine
NEPST371	5 Jul 1998	F	168	Toa Baja, PRI	*Pulmonicola cochleotrema*	Nares
NEPST488	27 Sep 1998	M	277	Toa Baja, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST535	2 Jan 1999	M	271	Arroyo, PRI	*Heterocheilus tunicatus*	Stomach
					Pulmonicola cochleotrema	Nares
NEPST553	18 Jul 1999	F	327	Juana Díaz, PRI	*Chiorchis groschafti*	Intestine
					Pulmonicola cochleotrema	Nares
NEPST559	26 Aug 1999	F	330	Guayanilla, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
NEPST599	14 Apr 2000	F	217	Yabucoa, PRI	*Chiorchis groschafti*	Intestine
					Pulmonicola cochleotrema	Nares
NEPST612	26 Oct 2000	F	260	Guánica, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST619	16 May 2001	M	305	Salinas, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST620	19 May 2001	F	259	Loíza, PRI	*Pulmonicola cochleotrema*	Nares
NEPST639	20 Oct 2001	F	300	Salinas, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
NEPST640	28 Nov 2001	M	206	Peñuelas, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST851	20 Oct 2002	F	310	Ponce, PRI	*Chiorchis groschafti*	Intestine
					Pulmonicola cochleotrema	Nares
NEPST852	11 Nov 2002	F	238	Ponce, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST853	23 Nov 2002	M	220	Ceiba, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares

Table 1 (continued)

Host Field number	Collection date	Sex	Length (cm)	Locality	Parasite	Location in host
NEPST854	12 Dec 2002	M	284	Guayama, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares, trachea, lungs
NEPST860	9 Apr 2003	M	221	Patillas, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Moniligerum blairi	Intestine
					Pulmonicola cochleotrema	Nares, trachea
NEPST861	18 May 2003	F	308	Arroyo, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST863	5 Jun 2003	F	317	Cabo Rojo, PRI	*Heterocheilus tunicatus*	Stomach
NEPST865	19 Jul 2003	F	279	Guayanilla, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
NEPST866	21 Jul 2003	F	245	Guayanilla, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares, lungs
NEPST873	9 Nov 2003	F	290	Guayanilla, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
					Pulmonicola cochleotrema	Nares
NEPST875	23 Nov 2003	F	273	Naguabo, PRI	*Chiorchis groschafti*	Intestine
					Heterocheilus tunicatus	Stomach
NEPST886	18 Apr 2004	F	101	Salinas, PRI	*Heterocheilus tunicatus*	Stomach
NEPST890	13 Jul 2004	F	–	Fajardo, PRI	*Heterocheilus tunicatus*	Stomach
NEPST905	18 May 2005	F	243	San Juan, PRI	*Chiorchis groschafti*	Intestine
					Pulmonicola cochleotrema	Nares
NEPST916	13 Jun 2006	F	280	Salinas, PRI	*Heterocheilus tunicatus*	Stomach
					Pulmonicola cochleotrema	Nares, lungs
NEPST918	16 Aug 2006	M	310	Cataño, PRI	*Chiorchis groschafti*	Intestine
					Pulmonicola cochleotrema	Nares
NEPST919	16 Aug 2006	M	309	Cataño, PRI	*Chiorchis groschafti*	Intestine
					Pulmonicola cochleotrema	Nares
NEPST929	30 Sep 2006	M	274	Cabo Rojo, PRI	*Heterocheilus tunicatus*	Stomach

M male, *F* female, *U* undetermined, *PRI* Puerto Rico, *VGB* British Virgin Islands, *VIR* US Virgin Islands

[a] Identified by molecular marker (mtDNA *cox2*)

study (Valentini et al. 2006). Our report of *A. brevispiculata* in the dwarf sperm whale is a new host record, as well as a new geographic record for this species in Atlantic waters. Records of Mignucci-Giannoni et al. (1998) of *A. physeteris* in a pygmy sperm whale and *Anisakis* sp. in a dwarf sperm whale are probably *A. brevispiculata* and/or *A. paggiae*. The last species was previously genetically detected, based on 19 allozyme (nuclear) markers, and morphologically described in pygmy and dwarf sperm whales from Florida (Mattiucci et al. 2005). Later, its genetic relationship with respect to the other *Anisakis* spp. was inferred also by mtDNA *cox2* sequence analysis (Valentini et al. 2006) and reviewed in Mattiucci and Nascetti (2008).

Mignucci-Giannoni et al. (1998) reported *A. typica* from the Atlantic spotted dolphin and shortfin pilot whale, providing the first records of *A. typica* from pelagic odontocetes in the Caribbean. Mattiucci et al. (2002) genetically identified and characterized *A. typica* from a marine tucuxi (*Sotalia guianensis*) from the Brazilian coast. Specimens of *A. typica* were genetically identified based on mtDNA *cox2* sequences from the stomach and intestine of a roughtooth dolphin and stomach of a bottlenose dolphin. The sequences matched those reported for *A. typica* and deposited in GenBank (Fig. 1) and in the MP analysis (Fig. 2), those specimens clustered together in the same clade as formed by *A. typica* previously genetically

Table 2 Revised annotated list of endoparasites from marine mammals from the Caribbean

Taxonomic group Helminth Host	Collection locality	Location in host	References
Phylum Acanthocephala[a]			
Family Polymorphidae[a]			
Bolbosoma capitatum[b]			
Melonhead whale (*Peponocephala electra*)[c]	PRI	Intestine	This paper
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	PRI	Intestine	4, 5, this paper
Bolbosoma vasculosum[b]			
Atlantic spotted dolphin (*Stenella frontalis*)[c]	VIR	Intestine	5
Blainville's beaked whale (*Mesoplodon densirostris*)[c]	PRI	Intestine	This paper
Pygmy killer whale (*Feresa attenuata*)[c]	VGB	Intestine	5
Bolbosoma sp.[b]			
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI	Intestine	5
Phylum Nemathelminthes[a]			
Family Anisakidae[a]			
Anisakis brevispiculata[b]			
Dwarf sperm whale (*Kogia sima*)[c]	PRI	Stomach	This paper
Anisakis paggiae[b]			
Dwarf sperm whale (*Kogia sima*)[c]	PRI	Stomach	This paper
Anisakis ziphidarum[b]			
Cuvier's beaked whale (*Ziphius cavirostris*)[c]	PRI, VIR	Stomach	This paper
Anisakis typica[b]			
Atlantic spotted dolphin (*Stenella frontalis*)[c]	PRI, VIR	Stomach	5
Bottlenose dolphin (*Tursiops truncatus*)[c]	PRI	Stomach	This paper
Roughtooth dolphin (*Steno bredanensis*)[c]	PRI	Stomach	This paper
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	PRI, JAM	Stomach	5
Anisakis physeteris[b]			
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI, VIR	Stomach	5
Anisakis simplex[b]			
Longsnout common dolphin (*Delphinus capensis*)[c]	VEN	Stomach	5
Anisakis simplex (*sensu lato*)[b]			
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	VGB	Stomach	This paper
Anisakis sp.[b]			
Atlantic spotted dolphin (*Stenella frontalis*)[c]	PRI, VIR	Stomach	5
Blainville's beaked whale (*Mesoplodon densirostris*)[c]	PRI	Stomach	This paper
Cuvier's beaked whale (*Ziphius cavirostris*)[c]	PRI, VIR	GI tract	5
Dwarf sperm whale (*Kogia sima*)[c]	PRI	Stomach	5
Fraser's dolphin (*Lagenodelphis hosei*)[c]	PRI	Stomach	This paper
Pygmy killer whale (*Feresa attenuata*)[c]	PRI, VGB	Stomach	5
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI, VIR	Stomach	5
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	PRI, JAM	Stomach	5
Spinner dolphin (*Stenella longirostris*)[c]	PRI	Liver, stomach	This paper
Pseudoterranova ceticola[b]			
Dwarf sperm whale (*Kogia sima*)[c]	PRI	Stomach	This paper
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI, VIR	Stomach	5
Pseudoterranova sp.[b]			
Dwarf sperm whale (*Kogia sima*)[c]	PRI	Stomach	5
Pygmy killer whale (*Feresa attenuata*)[c]	PRI, VGB	Stomach	5
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI, VIR	Stomach	5

Table 2 (continued)

Taxonomic group Helminth Host	Collection locality	Location in host	References
Family Heterocheilidae[a]			
Heterocheilus tunicatus[b]			
West Indian manatee (*Trichechus manatus*)[c]	PRI, DOM	Stomach	6, 7, this paper
Family Tetrameridae[a]			
Crassicauda anthonyi[b]			
Cuvier's beaked whale (*Ziphius cavirostris*)[c]	PRI	Kidney	5, this paper
Crassicauda duguyi[b]			
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI, VIR	Neck muscle	5
Crassicauda sp.[b]			
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	MEX	Pelvic girdle	3, 5
Family Pseudaliidae[a]			
Stenurus globicephalae[b]			
Melonhead whale (*Peponocephala electra*)[c]	PRI	Ear	5
Pygmy killer whale (*Feresa attenuata*)[c]	VGB	Stomach	5
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	LCA, PRI JAM, MEX	Cranial sinus	1, 3, 5
Stenurus minor[b]			
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	MEX	Cranial sinus	3
Halocercus sp./*Pharurus* sp.[b]			
Pygmy killer whale (*Feresa attenuata*)[c]	VGB	Bronchi	5
Phylum Platyhelminthes[a]			
Family Brachycladiidae[a]			
Synthesium tursionis[b]			
Bottlenose dolphin (*Tursiops truncatus*)[c]	PRI	Intestine	5
Nasitrema globicephalae[b]			
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	MEX	–	3
Family Diphyllobotrhiidae[a]			
Diphyllobothrium sp.[b]			
Melonhead whale (*Peponocephala electra*)[c]	PRI	Intestine	5
Family Tetrabothriidae[a]			
Tetrabothrius forsteri[b]			
Fraser's dolphin (Lagenodelphis hosei)[c]	PRI	Intestine	5
Trigonocotyle sexitesticulae[b]			
Pygmy killer whale (*Feresa attenuata*)[c]	PRI, VGB	Intestine	5
Trigonocotyle sp.[b]			
Pygmy killer whale (*Feresa attenuata*)[c]	PRI, VGB	Intestine	5
Family Phyllobotrhiidae[a]			
Monorygma grimaldi[b]			
Cuvier's beaked whale (*Ziphius cavirostris*)[c]	PRI	Abdomen	This paper
Fraser's dolphin (*Lagenodelphis hosei*)[c]	PRI	Abdomen	5
Melonhead whale (*Peponocephala electra*)[c]	PRI	Urinary bladder	This paper
Pygmy killer whale (*Feresa attenuata*)[c]	PRI, VGB	Blubber	5
Shortfin pilot whale (*Globicephala macrorhynchus*)[c]	PRI	Abdomen	5
Spinner dolphin (*Stenella longirostris*)[c]	PRI	Gonads	This paper
Phyllobothrium delphini[b]			
Atlantic spotted dolphin (*Stenella frontalis*)[c]	VIR	Blubber	5
Bottlenose dolphin (*Tursiops truncatus*)[c]	PRI	Blubber	This paper

Table 2 (continued)

Taxonomic group Helminth Host	Collection locality	Location in host	References
Cuvier's beaked whale (*Ziphius cavirostris*)[c]	VIR, PRI	Blubber	5, this paper
Dwarf sperm whale (*Kogia sima*)[c]	PRI	Blubber	This paper
Fraser's dolphin (*Lagenodelphis hosei*)[c]	PRI	Blubber	5, this paper
Pygmy sperm whale (*Kogia breviceps*)[c]	PRI	Blubber	5, this paper
Risso's dolphin (*Grampus griseus*)[c]	PRI	Blubber	5
Roughtooth dolphin (*Steno bredanensis*)[c]	PRI	Blubber	This paper
Sperm whale (*Physeter macrocephalus*)[c]	PRI	Blubber	5
Family Paramphistomatidae[a]			
Chiorchis groschafti[b]			
West Indian manatee (*Trichechus manatus*)[c]	PRI, MEX CUB, DOM	Intestine	2, 6, 7, 8, this paper
Family Opisthotrematidae[a]			
Pulmonicola cochleotrema[b]			
West Indian manatee (*Trichechus manatus*)[c]	PRI, DOM	Nares	6, 7, this paper
Moniligerum blairi[b]			
West Indian manatee (*Trichechus manatus*)[c]	PRI	Intestine	This paper

CUB Cuba, *DOM* Dominican Republic, *JAM* Jamaica, *LCA* Saint Lucia, *MEX* Mexico, *PRI* Puerto Rico, *VEN* Venezuela, *VGB* British Virgin Islands, *VIR* US Virgin Islands, *1* Arnold and Gaskin 1975, *2* Coy-Otero 1989, *3* Morales-Vela and Olivera-Gómez 1993, *4* Williams and Bunkley-Williams 1996, *5* Mignucci-Giannoni et al. 1998, *6* Mignucci-Giannoni et al. 1999a, *7* Mignucci-Giannoni et al. 1999b, *8* Mora-Pinto 2000

[a] Taxonomic group

[b] Helminth

[c] Host

characterized with the same molecular markers (Valentini et al. 2006). *A. typica* in our roughtooth dolphin is a new host record.

A. ziphidarum was differentiated genetically and morphologically from other species of *Anisakis* (Paggi et al. 1998) and found in a Cuvier's beaked whale from the Mediterranean and South Africa and a Layard's beaked whale (*Mesoplodon layardi*) from South Africa. We have sequenced specimens collected from the Cuvier's beaked whales from Puerto Rico and the US Virgin Islands, and they matched the sequences of *A. ziphidarum* deposited in GenBank (Fig. 1). Those specimens clustered in the same clade (Fig. 2) with *A. ziphidarum* previously genetically characterized by us and deposited in GenBank (Valentini et al. 2006). This constitutes a new geographical record for *A. ziphidarum*.

The L4 specimens of *Anisakis* collected from the Blainville's beaked whale and sequenced at the mtDNA *cox2* perfectly corresponded to the species deposited in GenBank as *Anisakis* sp. (Fig. 1) and genetically reported for the first time by Valentini et al. (2006) in the True's beaked whale (*Mesoplodon mirus*) and Gray's beaked whale (*M. grayi*), respectively, from the Southeast Atlantic Ocean (South African coast) and western South Pacific Ocean (New Zealand coast) and reviewed in Mattiucci and Nascetti (2008). In addition, MP analysis (Fig. 2) revealed that the specimens sequenced corresponded to the same clade formed by *Anisakis* sp. in Valentini et al. (2006). Gibson et al. (1998) reported *A. simplex* from a Blainville's beaked whale stranded on the British coast. Recently, Iglesias et al. (2008) demonstrated by using sequences analysis of the mtDNA *cox2* that the taxon *Anisakis* sp. detected as preadults in beaked whales in Valentini et al. (2006) corresponds to that documented in fish at larval stages from Madeira and reported as *Anisakis* sp. A by Pontes et al. (2005). Iglesias et al. (2008) detected the presence of some adult worms from Blainville's beaked whales stranded along the Atlantic coasts of Spain; however, the nominal designation of the species was not possible because of the lack of male specimens in their collection that were in suitable condition to be analyzed morphologically. Our Caribbean data further confirms that this taxon belonging to the genus *Anisakis* is a species genetically closely related to *A. ziphidarum,* one with host preference for beaked whales (Ziphiidae; Mattiucci and Nascetti 2008).

We found specimens of *Anisakis* type II (*sensu*; Berland 1961) larvae in one of the Cuvier's beaked whales studied. This had been previously reported in two Cuvier's beaked whales from the Western Mediterranean (Fernández et al. 2004).

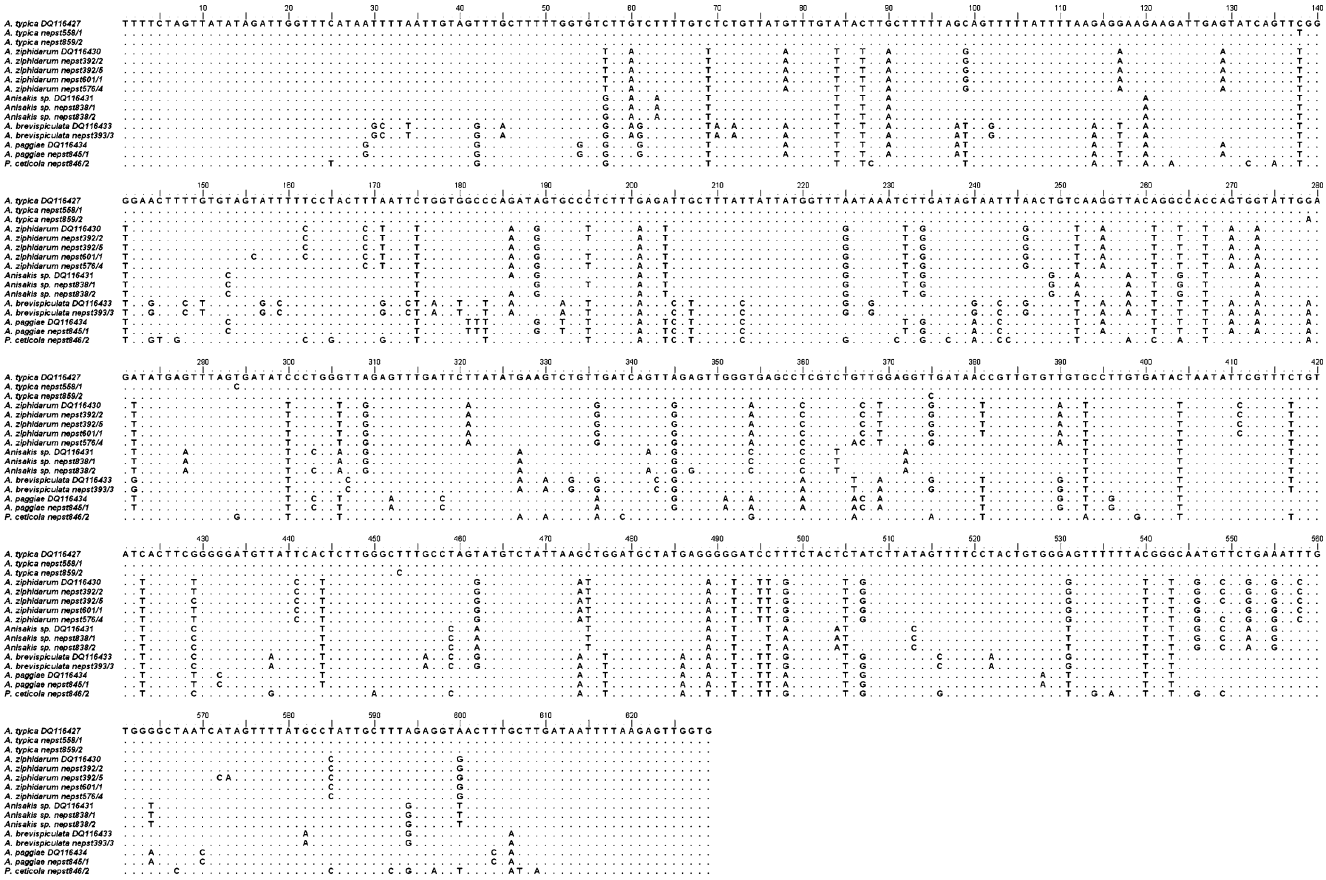

Fig. 1 Alignment of mtDNA *cox2* (629 bp) sequences of *Anisakis* specimens, using BioEdit (Hall 1999), in comparison with the deposited sequences in GenBank of all *Anisakis* spp., thus far, genetically characterized (specimen code is that reported in Table 1)

P. ceticola was identified genetically using mtDNA *cox2* sequence analysis, from the stomach of a dwarf sperm whale (Fig. 1). Gunter and Overstreet (1974) and Deardorff and Overstreet (1981) reported *P. ceticola* from a dwarf sperm whale from the Mississippi Sound. Our finding is a new geographical record for the Caribbean.

The nematode *Heterocheilus tunicatus* is a characteristic parasite in the stomach and rarely in the duodenum and intestine of sirenians (Dailey et al. 1988; Upton et al. 1989; Beck and Forrester 1988; Mignucci-Giannoni et al. 1999a, b). They were commonly found in most manatees examined in Puerto Rico. Morphological comparisons of manatee trematodes found in different geographical locations and habitats (riverine vs. marine) yielded differentiation of helminth species thought to be the same (i.e., *Chiorchis* spp., Mora-Pinto 2000). We suspect that a morphological as well as genetic comparative study of manatee's *Heterocheilus* nematode would probably yield similar results.

Spirurids were represented by *Crassicauda anthonyi* in all of our Cuvier's beaked whale specimens. Previous records of *Crassicauda* spp. from Cuvier's beaked whales include *C. anthonyi*, *Crassicauda boopis*, and *Crassicauda crassicauda* (Baylis 1932; Delyamure 1955; Heyning 1989; Raga 1994). However, Dollfus (1966) and Raga and Balbuena (1990) questioned some of these records. *Crassicauda* sp. was reported from a shortfin pilot whale by Morales-Vela and Olivera-Gómez (1993). Mignucci-Giannoni et al. (1998) reported *C. anthonyi* for the Cuvier's beaked whale and *C. duguyi* for the pygmy sperm whale.

Digenea

Five species of trematodes have been documented in the West Indian manatee: *Chiorchis fabaceus*, *Chiorchis groschafti*, *Moniligerum blairi*, and *Nudacotyle undicola* from the intestine and *Pulmonicola cochleotrema* (previously recognized as *Cochleotrema cochleotrema*; see Blair 2005) from the nares (Beck and Forrester 1988; Dailey et al. 1988; Coy-Otero 1989; Upton et al. 1989; Mignucci-Giannoni et al. 1999a, b; Mora-Pinto 2000). We found *P. cochleotrema* and *C. groschafti* in the manatees studied. Following the distinction by Mora-Pinto (2000) between *C. groschafti* (from manatees in the Caribbean and south Florida) and *C. fabaceus* (from manatees throughout Florida), the identification of all *C. fabaceus* reported in

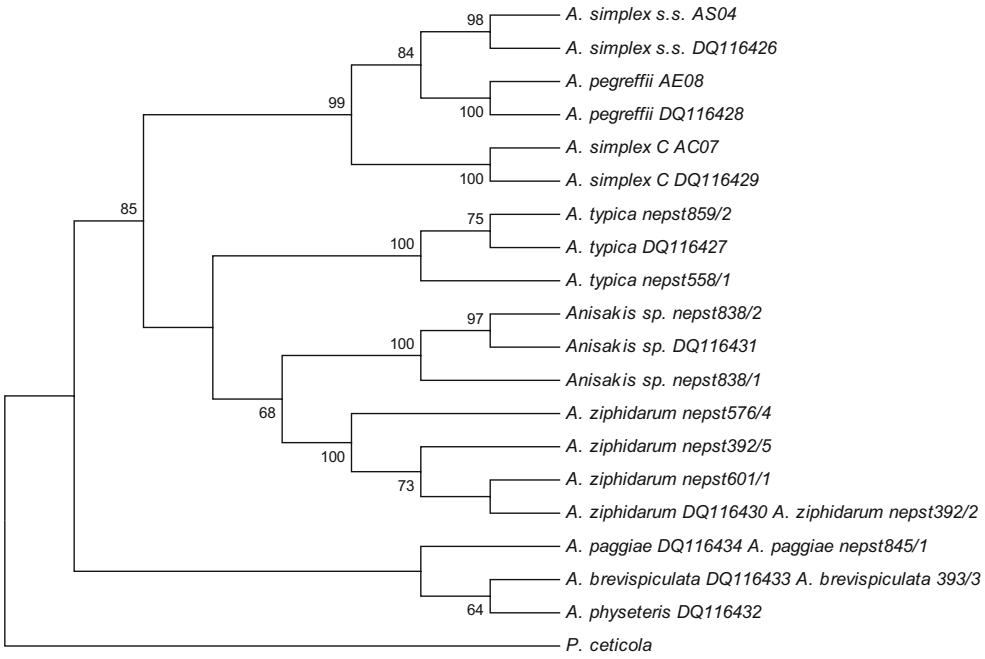

Fig. 2 *Cox2*-derived MP tree using MEGA for the *Anisakis* specimens sequenced (specimen code is that reported in Table 1). The sequences deposited in GenBank are also included. Bootstrap values ≥60 are shown at the *internal nodes*. *P. ceticola* is included as an outgroup

Mignucci-Giannoni et al. (1999a, b) are corrected as *C. groschafti*. We collected specimens of *M. blairi* from the intestine of a manatee for the first time from Puerto Rico. This trematode commonly parasitized manatees in Florida (Beck and Forrester 1988; Dailey et al. 1988; Upton et al. 1989); thus, our finding in a Caribbean manatee constitutes a new geographic record.

Eucestoda

Specimens of the tetraphyllidean metacestode *Phyllobothrium delphini* occurred in the blubber of a pygmy sperm whale, roughtooth dolphin, Fraser's dolphin, two bottlenose dolphins, three dwarf sperm whales, and in seven Cuvier's beaked whales. Morphological types of *Phyllobothrium* larvae have been described from cetaceans throughout the world's oceans and in some cases may represent discrete species (Delyamure 1955; Skrjabin 1972; Testa and Dailey 1976). These larvae have previously been reported as *P. delphini* in the Cuvier's beaked whale (Tomilin 1957), sperm whale (Sokolov 1955; Testa and Dailey 1976; Rice 1989; McAlpine et al. 1997), dwarf sperm whale (Zam et al. 1971; Ross 1978), and Risso's dolphin and Fraser's dolphin (McColl and Obendorf 1982). In the Caribbean, Mignucci-Giannoni et al. (1998) reported them from the Risso's dolphin, pygmy sperm whale, Fraser's dolphin, Cuvier's beaked whale, and Atlantic spotted dolphin. *P. delphini* in the roughtooth dolphin in this study is a new host record.

Specimens of *Monorygma grimaldi* were found in the blubber of a shortfin pilot whale, in the urinary bladder of a Fraser's dolphin and a melonhead whale, in the abdominal cavity of a Cuvier's beaked whale, and in the gonads of a spinner dolphin. The typical site of infection of these cestodes is the abdominal cavity. Forrester (1992) reported them from the striped dolphin (*Stenella coeruleoalba*) in the Atlantic Ocean, Dailey and Brownell (1972) from the shortfin pilot whale, McColl and Obendorf (1982) from the Fraser's dolphin, and Dailey and Brownell (1972) and Bryden et al. (1976) from the melonhead whale. Previously, Mignucci-Giannoni et al. (1998) reported them from the pygmy killer whale, shortfin pilot whale, and Fraser's dolphin in the Caribbean Sea. *M. grimaldi* in the Cuvier's beaked whale constitute a new host record.

Conclusions

Morphological species identification was successful in the main four endoparasitic groups. Genetic identification of anisakid nematodes allowed geographic ranges to be confirmed and/or extended and expand the list of hosts for genetically recognized *Anisakis* spp. Our results agree with the previous *Anisakis* cetacean host preferences found by Mattiucci and Nascetti (2006, 2008). *A. typica* parasitized oceanic dolphins of warmer temperate and tropical waters, and *A. ziphidarum* and adult *Anisakis* sp. parasitized beaked whales. The dwarf and pygmy sperm whales were suitable hosts for *A. brevispiculata* and *A. paggiae* (Valentini et al. 2006; Mattiucci and Nascetti 2006, 2008). The phylogenetic relationships proposed for species of the genus *Anisakis* seem to align with that proposed for their cetacean hosts (Milinkovitch 1995; Nikaido et al. 2001), suggesting that some level of parallelism or co-evolutionary

events, including co-divergence and host-switching, could have accompanied the speciation of these endoparasitic nematodes (Mattiucci and Nascetti 2008). Additional sampling and genetic identification of both the hosts and their parasite fauna is needed to obtain a more complete analysis.

Mignucci-Giannoni et al. (1998) indicated that the utility of helminthes in Caribbean marine mammals as biogeographical indicators or tags needed to be evaluated in future parasitic studies. *A. typica* only occurs in offshore cetaceans. It is the only anisakid infecting bottlenose dolphins in Puerto Rico, suggesting that this host has a more pelagic distribution in Puerto Rico. Results in a recent phylogeographic analysis of Caribbean bottlenose dolphins are in agreement, indicating that bottlenose dolphins found in Puerto Rico share more affinities with genetically identified pelagic type *Tursiops*, similar to those found in the Mediterranean Sea and the Azores (Mignucci-Giannoni, Caballero and Islas, unpubl. data). The use of *A. typica* as a biological tag, as seen in the latter relationship in Puerto Rico, should be examined on a broader geographic scale in the tropics to determine its distribution and possible significance. Similarly, the tetraphyllidean cestodes *P. delphini* and *M. grimaldi*, for which sharks seem to be the definitive host and cetaceans an intermediate host (Testa and Dailey 1976), appear to serve as biological tags for the preferred habitat of dolphins and whales in Puerto Rico's archipelago, as they are acquired mostly offshore (Aznar et al. 2007). All species of cetaceans in the present study were infested with either *P. delphini* or *M. grimaldi*, indicating their common pelagic distribution. In manatees, the distinction of the two species of trematodes (*C. groschafti* in South Florida, Mexico, Dominican Republic and Puerto Rico, and *C. fabaceus* throughout Florida) serves as well as a biogeographic indicator.

The established phylogeography of the West Indian manatee (García-Rodríguez et al. 1998; Vianna et al. 2006) should be compared with a genetic differentiation of geographic populations of *H. tunicatus*, *C. fabaceus*, *C. groschafti*, *P. cochleotrema*, and *M. blairi* within Puerto Rico and throughout the range of the West Indian manatee as a future study.

This second report of the taxonomic composition of helminthes in Caribbean marine mammals adds new information about the diversity of its parasite fauna. A detailed knowledge of both the parasite fauna and their interactions with their marine mammal hosts is necessary to obtain basic life history information on the latter and, thus, assist management and conservation of these legally protected and sometimes endangered species.

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