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Research Article

New records of nematodes from three aquatic mammals in Mexico with notes about the nematode fauna recorded in Mexican waters

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ABSTRACT. Nematodes are among the most common parasites in aquatic mammals. Because of their cuticular covering, these worms are frequently collected from the examination of feces or stranded hosts. This work provides new records of three nematode taxa infecting three different aquatic mammal species (*Lontra longicaudis annectens*, *Trichechus manatus*, and *Zalophus californianus*) in Mexico. Additionally, it brings together all available published records about this country's nematode fauna parasitizing aquatic mammals. The aim is to describe the current state of the art, which sets the basis for the strategic design of future research. Given the diversity of printed sources recording nematode fauna, we gathered literature, including theses and articles in journals profiled in the Journal Citation Reports or similar academic databases. The compiled information shows that 24 nominal nematode taxa have been reported from 11 species of aquatic mammals in Mexico. Our study exhibits irregular patterns of geographic distribution and host spectrum, existing entire marine ecoregions, and numerous host species lacking records.

Keywords: *Lontra longicaudis annectens*; *Trichechus manatus*; *Zalophus californianus*; *Anisakis simplex*; *Heterocheilus tunicatus*; *Terranova*; Nematoda; Mexican marine ecoregions

INTRODUCTION

Nematodes are one of the main metazoan groups parasitizing aquatic mammals (Geraci & Aubin 1987). However, the diversity and distribution of nematodes in Mexico are poorly known. Currently, 50 species of aquatic mammals have been reported inhabiting the Mexican Economic Exclusive Zone (MEEZ) (Muzquiz-Villalobos & Pompa-Mansilla 2018, Ladrón de Guevara & Elorriaga 2019, Medrano & Urbán 2019). However, most lists exclude non-marine species, such as the Neotropical otter *Lontra longicaudis annectens*.

Based on the distribution of marine mammals, Muzquiz-Villalobos & Pompa-Mansilla (2018) suggested areas with high species richness, such as the northeast Pacific and the southwestern portion of the Gulf of California, and areas with relatively lower richness (around 25 species) located at the Tropical Pacific and the Gulf of Mexico. The knowledge of parasites infecting marine mammals in Mexico is currently poor. Since the collection of aquatic mammals in Mexico is highly restricted, parasitological studies are limited to freshly stranded specimens (Aguilar-Aguilar & Delgado-Estrella 2017) or the examination of fecal samples (e.g. García-Varela et al. 2021). Consequently, most of the parasites found are damaged or deteriorated, and the only specimens that can be collected are those protected by a cuticle, such as the nematodes. This work reports new records of three nematode species collected from three species of aquatic mammals in Mexico. Additionally, we performed an analysis summarizing the current knowledge

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about the diversity of parasitic nematodes in Mexican aquatic mammals to contribute to future management and conservation strategies.

MATERIALS AND METHODS

One subadult male of the California sea lion (*Zalophus californianus*), stranded in the Gulf of Santa Clara (31°40'23"N, 114°28'50.5"W), Sonora in November 1993, and one subadult male of the Antillean manatee (*Trichechus manatus manatus*) stranded in laguna de Pom (18°36'24.6"N, 92°09'49.7"W), Campeche, Mexico on April 28, 2019, were examined for nematodes. Additionally, some nematode specimens were collected from fresh feces of Neotropical otter (*Lontra longicaudis annectens*) in Palizada-Laguna del Este (18°26'56.3"-18°27'32.3"N; 91°44'48"-91°45'49.8"W), Campeche, Mexico.

Nematodes were fixed and preserved in 4% formalin and later cleared in a glycerine-water mixture (3:1) for morphological examination. Nematodes were identified following Delyamure (1955), Anderson et al. (2009), and Gibbons (2010). Temporary mounts of whole specimens were examined using a Leica DM500® microscope. Photomicrographs were captured using the Leica ICC50 HD® capture imaging system (Leica, Germany), except for specimens from the California sea lion, which were captured with a conventional (non-digital) reflex camera attached to a microscope. Voucher specimens were deposited at the Parasite-Host Reference Collection (CRPH, by its Spanish acronym) of the Laboratory of Aquatic Zoology, Faculty of Sciences, Universidad Nacional Autónoma de México, Mexico City, Mexico, and at the National Collection of Helminths (CNHE, by its Spanish acronym), Institute of Biology, Universidad Nacional Autónoma de México, Mexico City, Mexico.

To analyze the current knowledge on the diversity of parasitic nematodes of aquatic mammals in Mexico, we extensively searched records published as of December 2021. This information was obtained from databases such as Zoological Record, Biological Abstracts, ISI Web of Knowledge, Google Scholar, Aquatic Sciences and Fisheries Abstracts, Biological and Agricultural Index Plus, Scopus, Latindex, and SERIUNAM. Given the relatively few published works on parasites of aquatic mammals in Mexico, we also included information from non-published theses on nematode taxa parasitizing these hosts; these works were classified into three categories: A) studies published in academic journals profiled in the Journal Citation Reports; B) works published in peer-reviewed local journals; and C) data published in undergraduate or postgraduate theses. Records are presented in a table organized by alphabetically listed nematode family/taxon, followed by hostname(s), site(s), marine ecoregion, locality, and reference. Valid names of parasites and host species were updated according to WoRMS (2021).

The classification of the MEEZ into marine ecoregions was based on Spalding et al. (2017). Presence and distribution records of aquatic mammals in the MEEZ were based on Gallo-Reynoso (1997), Aurioles-Gamboa et al. (2004), Meraz & Sánchez-Díaz (2008), Vázquez-Castán et al. (2009), Niño-Torres et al. (2015), Heckel et al. (2018), Villegas-Zurita et al. (2018), and Meraz et al. (2019).

RESULTS

Three nematodes were identified in this study based on morphological traits: 1) *Anisakis simplex* (Rudolphi, 1809) (Anisakidae, adults, and larvae 3) collected from the stomach of *Zalophus californianus* (Fig. 1); 2) *Terranova* sp. (Anisakidae, larvae 3) from feces of *Lontra longicaudis annectens* (Fig. 2); and 3) *Heterocheilus tunicatus* Diesing, 1839 (Heterocheilidae, immature adults) from the stomach of *Trichechus manatus manatus* (Fig. 3). These records increase the number of nematode taxa parasitizing aquatic mammals in Mexico reported to date and the collection localities (Table 1).

There are 24 nominal taxa of nematode parasites reported from 11 host species of aquatic mammals in Mexico. Most of them (58.33%) have been reported from marine environments. In comparison, nine taxa (37.5%) were found in *L. longicaudis annectens* from riverside freshwater or brackish lagoon systems, and a single species (4.16%) was collected from manatees living in lagoon environments. There are 19 published studies reporting nematode parasites; 6 (31.57%) used indirect analyses to find and identify eggs in feces, while the remaining studies have examined nematode parasite specimens collected directly from dead hosts or fresh feces.

The 19 published works (including the present one) have been produced irregularly over 86 years, from 1935 to 2021. Most works have been conducted in three marine ecoregions, with large information gaps for other entire ecoregions. However, the current knowledge on nematodes parasitizing aquatic mammals derives from these studies. This knowledge is summarized in Table 1 and Figure 4, which show the number of published studies by marine ecoregion, the

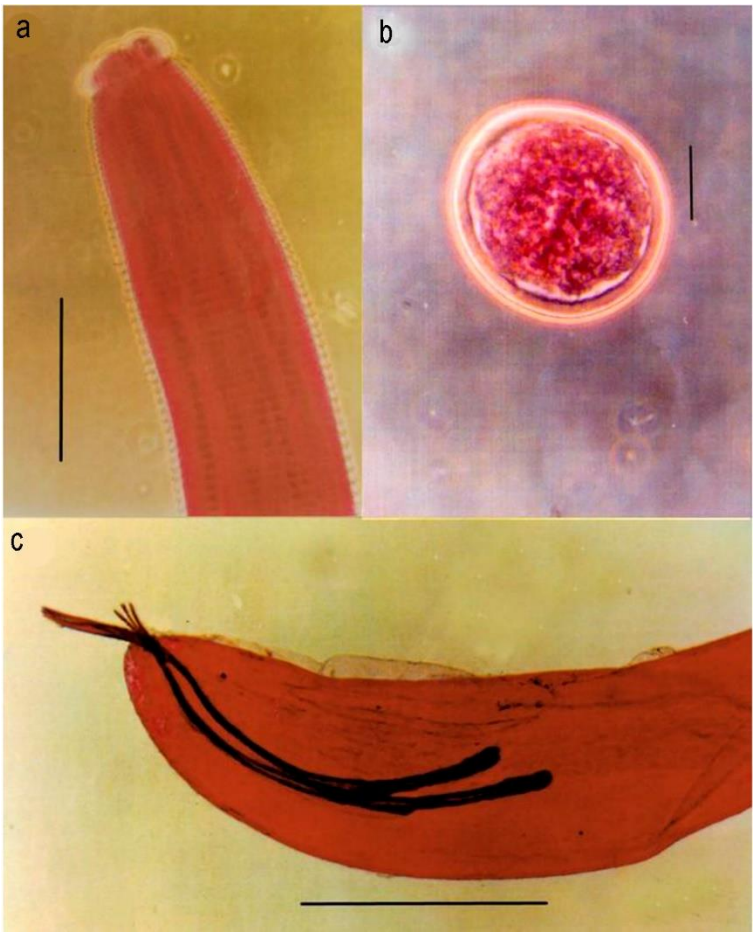

Figure 1. *Anisakis simplex* from *Zalophus californianus*. a) Anterior view, scale bar = 1.5 mm, b) egg, scale bar = 20 μm, c) posterior end, scale bar = 1.0 mm.

number of host species and taxonomic orders, and the number of nematode taxa recorded. Figure 4 also shows the number of records of nematode parasite taxa by type of aquatic mammal host and the percentage of studies about host species concerning the total number of host species distributed in Mexico.

DISCUSSION

The relatively low number of available studies illustrates the scarce information about the diversity of nematode parasites of aquatic mammals in Mexico. Only a few host species have been analyzed from a parasitological point of view, and there are marked differences in the sampling effort across host groups. Most studies (including this one) have focused on the California sea lion, *Zalophus californianus*. This species is an abundant and accessible aquatic mammal, widely distributed along the Pacific coasts and islands of Mexico (Melin et al. 2018). It is worth mentioning that there are very few records of nematode parasites for the other species of pinnipeds in Mexico. In fact, besides those California sea lions, the only available reports date back more than 80 years ago for the northern elephant seal, *Mirounga angustirostris*, by Schroeder & Wegeforth (1935) and Caballero y Caballero & Peregrina (1938). There are no records for the other pinniped species well-distributed in Mexican waters, such as the harbor seal (*Phoca vitulina*) and the Guadalupe fur seal (*Arctocephalus townsendi*).

Species of the order Cetartiodactyla have also been scarcely studied. About 80% of the species of odontocetes and 75% of mysticetes distributed in Mexico lack records of nematodes parasitizing them. Records for Odontoceti are available for four species of delphinids (*Feresa attenuata*, *Globicephala macrorhynchus*, *Stenella clymene*, and *S. longirostris*) and a single species in the family Kogiidae (*Kogia bre-*

Figure 2. *Terranova* sp. from *Lontra longicaudis annectens*, anterior view (scale bar = 200 μm).

Figure 3. *Heterocheilus tunicatus* from *Trichechus manatus manatus*, anterior view (scale bar = 500 μm).

viceps). Reports for Mysticeti are limited to two species of the genus *Balaenoptera* (*B. musculus* and *B. physalus*), with data still missing for most species, including some well-known and broadly distributed species (extensively studied from other perspectives), such as the bottlenose dolphin (*Tursiops truncates*) or the gray whale (*Eschrichtius robustus*).

The Neotropical otter (*Lontra longicaudis annectens*) harbors the most diverse nematode parasitic fauna among aquatic mammals in Mexico, with nine recorded taxa from seven different families. This high diversity is likely due to the broad feeding spectrum of this host, a top predator in aquatic environments, with an opportunistic and generalist diet (Brito-Ríos et al. 2022), including crustaceans, fishes, mollusks, and even reptiles, birds, and other mammals (Corona-Figueroa et al. 2002, Vázquez-Maldonado et al. 2021, Brito-Ríos et al. 2022). Moreover, collecting nematode parasites from this host species seems easy because of its semi-terrestrial habits (García-Prieto et al. 2012, Serrano et al. 2017, Badillo-Flores et al. 2020, present study), thus increasing the possibility of recovering well-preserved parasite eggs or worms. The relatively low number of parasitological studies for this species - widely distributed in Mexican rivers and coastal lagoons suggests a more extensive diversity of nematodes. On the other hand, the Antillean manatee (*Trichechus manatus manatus*) is an entirely aquatic species. Whose coastal habits have allowed conducting two studies (Hernández-Olascoaga et al. 2017, present study), from which the nematode species *Heterocheilus tunicatus* has been consistently recorded.

The diversity of nematodes parasitizing aquatic mammals in Mexico is represented by 24 nominal taxa belonging to 10 families. Most of the host-parasite relationships reported here involve anisakid nematodes parasitizing in pinnipeds, cetaceans, and the Neotropical otter. Anisakidae species have been recorded as adults or third larval stage, reaching their definitive host when feeding on fish or squid infected with the larvae (Nagasawa 1990, Ángeles-Hernández et al. 2020). Cetacean species also have adult parasite species of Pseudaliidae, which probably also infect their final hosts by feeding on intermediate hosts in the food chain (Balbuena et al. 1994, Faulkner 1995). However, transplacental infections should not be ruled out for lungworms such as *Halocercus* spp. (Balbuena et al. 1994), and tetramerid worms of the genus *Crassicauda*. Besides anisakids, pinniped hosts harbor parasitic worms of the family Ancylostomatidae, which possess

Table 1. Records of nematode parasites in aquatic mammals from Mexico. The alphabetical character following the references indicates the type of published work as described in the Materials and Methods section. A: studies published in academic journals profiled in the Journal Citation Reports or similar databases, B: works published in local peer-reviewed journals, C: data published in undergraduate or postgraduate theses.

Nematode taxon	Host species (Order)	Site	Marine ecoregion/locality	Reference
Ancylostomatidae: *Uncinaria* sp.				
	Zalophus californianus (Lesson, 1828) (Carnivora)	Feces: eggs and individual specimens	Cortezian/Isla Granito, Baja California	Abundes-Gallegos (1995) [C]
Ancylostomatidae: *Uncinaria lucasi* Stiles, 1901				
	Zalophus californianus (Carnivora)	Intestine	Magdalena Transition/Isla Asunción, Baja California Sur	García-Prieto et al. (2012) [A]
Anisakidae: *Anisakis* sp.				
	Balaenoptera musculus (Linnaeus, 1758) (Cetartiodactyla)	Feces: eggs	Cortezian/Loreto to La Paz, Baja California Sur	Flores (2012) [C]
	Feresa attennuata Gray, 1874 (Cetartiodactyla)	Stomach	Southern Gulf of Mexico/Punta Villa Rica, Veracruz	Aguilar-Aguilar et al. (2002) [B]
	Stenella clymene (Gray, 1850) (Cetartiodactyla)	Stomach	Western Caribbean/Playa Kantenah, Quintana Roo	Aguilar-Aguilar et al. (2010) [A]
	Zalophus californianus (Carnivora)	Feces: eggs	Cortezian/Gulf of Californa	Barberi (1992) [C]
		Feces: eggs and individual specimens	Cortezian/Los Islotes, Baja California Sur	Méndez (2002) [C]
Anisakidae: *Anisakis brevispiculata* Dollfus, 1966				
	Kogia breviceps (de Blainville, 1838) (Cetartiodactyla)	Digestive tract	Southern Gulf of Mexico/ Progreso and El Cuyo, Yucatán	González-Solís et al. (2006) [A]
			Western Caribbean/Cozumel, Quintana Roo	González-Solís et al. (2006) [A]
Anisakidae: *Anisakis pegreffi* Campana-Rouget and Biocca, 1955				
	Mirounga angustirostris Gill, 1866 (Carnivora)	Intestine	Southern California/Isla Guadalupe, Baja California	Schroeder & Wegeforth (1935) [A]; Caballero y Caballero & Peregrina (1938) [B]
Note: These records were originally made as *Anisakis similis* and later synonymized with *A. pegreffi* by Rocka (2006)				
Anisakidae: *Anisakis simplex* (Rudolphi, 1809).				
	Kogia breviceps (de Blainville, 1838) (Cetartiodactyla)	Digestive tract	Southern Gulf of Mexico/ Progreso, Yucatán	González-Solís et al. (2006) [A]
			Western Caribbean/Cozumel, Quintana Roo	González-Solís et al. (2006) [A]
	Zalophus californianus (Carnivora)	Stomach	Cortezian/Gulf of Santa Clara, Sonora	Present work [A]
Anisakidae: *Anisakis typica* (Diesing, 1860)				
	Stenella longirostris (Gray, 1828) (Cetartiodactyla)	Stomach	Cortezian/Bahía de La Paz, Baja California Sur	Aguilar-Aguilar et al. (2001) [A]
Anisakidae: *Contracaecum ogmorhini* Johnston & Mawson, 1941				
	Zalophus californianus (Carnivora)	Brain, intestine	Magdalena Transition/Isla Asunción, Baja California Sur	Flores-Barroeta & Hidalgo-Escalante (1961) [A]
		Intestine	Southern California/Isla San Benito	Schroeder & Wegeforth (1935) [A]
			Southern California/Isla Coronado	Fagerholm & Gibson (1987) [A]
Anisakidae: *Contracaecum osculatum* (Rudolphi, 1802)				
	Mirounga angustirostris (Carnivora)	Intestine	Southern California/Isla Guadalupe, Baja California	Caballero y Caballero & Peregrina (1938) [B]
Anisakidae: *Pseudoterranova ceticola* (Deardorff & Overstreet, 1981)				
	Kogia breviceps (de Blainville, 1838) (Cetartiodactyla)	Digestive tract	Southern Gulf of Mexico/ Progreso and El Cuyo, Yucatán	González-Solís et al. (2006) [A]
Anisakidae: *Terranova* sp.				
	Lontra longicaudis annectens (Major, 1897) (Carnivora)	Feces: individual specimens	Southern Gulf of Mexico/ Palizada-Laguna del Este system, Campeche	Present work [A]
Ascarididae: *Ascaris* sp.				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Inland freshwater systems/Necaxa River	Badillo-Flores et al. (2020) [A]
Ascarididae: *Toxocara* sp.				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Southern Gulf of Mexico/Alvarado Lagoon System, Veracruz	Serrano et al. (2017) [A]

Continuation

Nematode taxon	Host species (Order)	Site	Marine ecoregion/locality	Reference
Dioctophymidae: *Eustrongylides* sp.				
	Lontra longicaudis annectens (Carnivora)	Feces: individual specimens	Inland freshwater systems/ Unknown	García-Prieto et al. (2012) [A]
Heterocheilidae: *Heterocheilus tunicatus* Diesing, 1839				
	Trichechus manatus (Sirenia)	Feces: eggs	Southern Gulf of Mexico/Usumacinta and Grijalva basins, Tabasco	Hernández-Olascoaga & González-Solís (2006) [D]; Hernández-Olascoaga et al. (2017) [A]
		Feces: eggs	Southern Gulf of Mexico/ Ascención and Chetumal Bays	Hernández-Olascoaga et al. (2017) [A]
		Feces: individual specimens	Southern Gulf of Mexico/Pom lagoon, Campeche	Present work [A]
Oxyuridae: *Enterobius* sp.				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Southern Gulf of Mexico/Alvarado Lagoon System, Veracruz	Serrano et al. (2017) [A]
Oxyuridae: *Oxyuris equi* (Schrank, 1788)				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Inland freshwater systems/Necaxa River	Badillo-Flores et al. (2020) [A]
Pseudaliidae: *Halocercus delphini* Baylis & Daubney, 1925				
	Stenella clymene (Gray, 1850) (Cetartiodactyla)	Bronchi	Western Caribbean/Playa Kantenah, Quintana Roo	Aguilar-Aguilar et al. (2010) [A]
Note: These records were originally made as *Skrjabinalius guevarai*, and later synonymized with *H. delphini* by Pool et al. (2020)				
Pseudaliidae: *Stenurus globicephalae* Baylis & Daubney, 1925.				
	Globicephala macrorhynchus Gray, 1846 (Cetartiodactyla)	Cranial sinuses	Western Caribbean/Cozumel, Quintana Roo	Morales-Vela & Olivera-Gómez (1993) [B]
Pseudaliidae: *Stenurus minor* (Kuhn, 1829)				
	Globicephala macrorhynchus (Cetartiodactyla)	Cranial sinuses	Western Caribbean/Cozumel, Quintana Roo	Morales-Vela & Olivera-Gómez (1993) [B]
Strongylidae: *Strongylus equinus* Mueller, 1780				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Inland freshwater systems/Necaxa River	Badillo-Flores et al. (2020) [A]
Strongyloididae: *Strongyloides* sp.				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Southern Gulf of Mexico/Alvarado Lagoon System, Veracruz	Serrano et al. (2017) [A]
Tetrameridae: *Crassicauda* sp.				
	Globicephala macrorhynchus (Cetartiodactyla)	Cranial sinuses	Western Caribbean/Cozumel, Quintana Roo	Morales-Vela & Olivera-Gómez (1993) [B]
	Balaenoptera musculus (Linnaeus, 1758) (Cetartiodactyla)	Feces: eggs	Cortezian/Loreto to La Paz, Baja California Sur	Flores-Cascante (2012) [C]
	Balaenoptera physalus (Linnaeus, 1758) (Cetartiodactyla)	Feces: eggs	Cortezian/Loreto to La Paz, Baja California Sur	Flores-Cascante et al. (2019) [A]
Trichuridae: *Trichuris* sp.				
	Lontra longicaudis annectens (Carnivora)	Feces: eggs	Southern Gulf of Mexico/Alvarado Lagoon System, Veracruz	Serrano et al. (2017) [A]

a direct life cycle, whereby pinnipeds are infected orally and percutaneously with eggs and larvae deposited on the soil (Olsen & Lyons 1965, Lyons et al. 2001). Anisakids are absent in manatees, which seem to harbor a single species of Heterocheilidae, acquiring the infection by consuming the intermediate hosts while feeding on vegetation (Hernández-Olascoaga et al. 2017). As mentioned above, the Neotropical otter harbors the highest diversity of nematode parasites belonging to seven different families. These nematode species occur as eggs or larvae, which probably enter the hosts with food.

The present study evidence that scarce studies are recording the diversity of nematode parasites of aquatic mammals in Mexico. Most records have been conducted in the southern Gulf of Mexico, the Mexican Caribbean area, and the coasts of the Baja California Peninsula (both the Pacific and the Gulf of California). Besides, these studies have been conducted only on mammals inhabiting some areas. Besides, there are significant information gaps. The ecoregions of Revillagigedos, the Mexican Tropical Pacific, Chiapas-Nicaragua, and the Mexican part of the northern Gulf of Mexico are large marine areas with no records of nematode parasites of the aquatic mammal species. Fu-

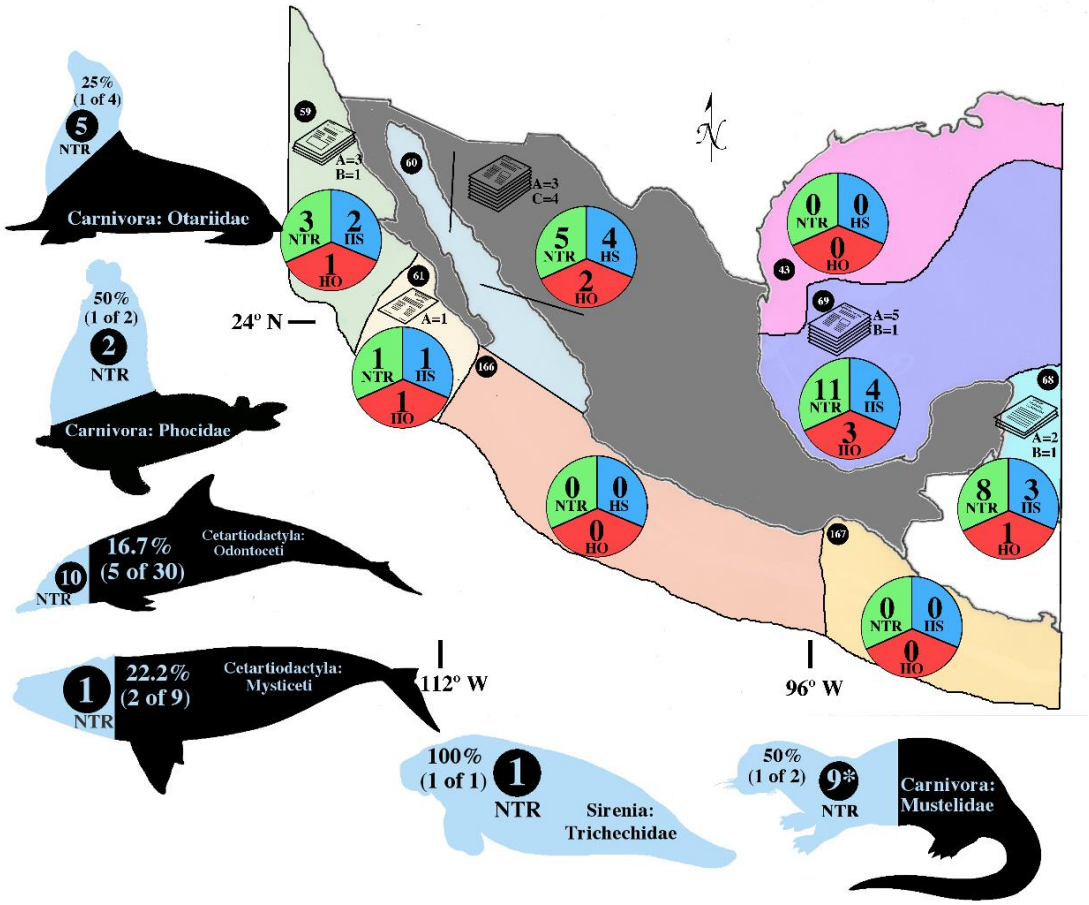

Figure 4. Map of Mexico showing data about the diversity of nematode parasites of aquatic mammals by marine ecoregion. Nomenclature of marine ecoregions is based on Spalding et al. (2007), 43: northern Gulf of Mexico; 59: southern California Bight; 60: Cortezian; 61: Magdalena Transition; 68: Western Caribbean; 69: southern Gulf of Mexico; 166: Mexican Tropical Pacific; 167: Chiapas-Nicaragua. NTR: Nematode taxa recorded; HS: Host species; HO: Host orders; A: publication type A; B: publication type B; and C: publication type C. Values inside silhouettes indicate the number of nematode taxa recorded and the percentage of host species examined for nematodes for each mammal group distributed in Mexico. *Indicates that three nematode taxa were collected from inland fluvial environments.

ture studies aimed at improving the inventory of nematode biodiversity should be conducted following a strategic approach, i.e. combining the need to target missing species within the host spectrum while selecting areas that remain to be investigated. In this sense, increasing the current knowledge on the parasite diversity in aquatic mammals in Mexico largely depends on the collaboration between wildlife parasitologists and the numerous networks attending mammal strandings in the country. Thus, a substantial advancement of the information by surveying poorly studied host taxa inhabiting different geographic areas is desirable to achieve a more accurate evaluation of the nematode parasite biodiversity and to set the basis for further analyses from different contemporary approaches, such as molecular and morphometrical comparisons.

To date, there are no studies on nematode parasites of aquatic mammals in Mexico using molecular procedures. Molecular data are currently used as an affordable taxonomical tool supplementing the morphologically-based taxonomic approach to establish the delimitation between species (e.g. Umehara et al. 2006, Irigoitia et al. 2021); or analyze the genetic structure (Mattiucci et al. 2008, Bello et al. 2021), phylogenetic relationships (Matiucci & Nascetti 2006, 2008),

hybridization processes (Bello et al. 2021); and exploring the existence of sibling species -a relatively common phenomenon among marine nematodes, where an apparently well-known cosmopolitan or widespread parasite taxa represent complexes of species (Mattiucci et al. 2003, 2014, Raga et al. 2018, Irigoitia et al. 2021).

Parasites, including nematodes, are essential components of biodiversity, and their interactions with their hosts should be considered as a part of conservation research on their aquatic mammal hosts (Aznar et al. 2010, Lehnert et al. 2019). Further research on the parasitic species affecting aquatic mammal populations and the biodiversity and ecology of parasites of aquatic mammals is essential to inform species, habitat, and population assessments and to guide some conservation strategies.

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