Specificity of Stenurus (Metastrongyloidea: Pseudaliidae) infections in odontocetes stranded along the north-west Spanish coast

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ABSTRACT

Parasites extracted from the lungs and the pterygoid sinus complex of 6 species of odontocetes stranded along the north-west Spanish coast (Northeast Atlantic) between 2009 and 2019 were morphologically identified. The samples belonged to 14 specimens, including 3 harbour porpoises, Phocoena phocoena, 6 short-finned pilot whales, Globicephala macrorhynchus, 1 long-finned pilot whale, Globicephala melas, 1 Risso’s dolphin, Grampus griseus, 1 striped dolphin, Stenella coeruleoalba and 2 bottlenose dolphins, Tursiops truncatus. All animals (14/14) were infected by nematodes of the genus Stenurus spp.; moreover, two of them presented a mixed lung nematode infection by Stenurus spp. and Halocercus spp., and another two a mixed infection by Stenurus spp. and the trematode Nasitrema spp. in the pterygoid sinuses. The morphological characterization of the Stenurus specimens revealed the existence of three different species: Stenurus minor, present in the pterygoid sinuses of harbour porpoises with a mean intensity of 43.0 ± 9.0; Stenurus globicephalae, in the pterygoid sinuses of pilot whales and the Risso’s dolphin (370.3 ± 579.4); and Stenurus ovatus infecting bottlenosed and striped dolphins’ lungs (47.7 ± 76.5). This is the first citation of St. minor and St. ovatus in odontoceti from the Galician coast. Nematodes of the genus Stenurus are frequent in odontocetes stranded along the north-west Spanish coast. A clear host-parasite association was observed between St. minor and the Phocoenidae family, between S. globicephalae and the subfamily Globicephalinae and between S. ovatus and subfamily Delphininae. Different trophic position and niche segregation may lead to different patterns of specificity.

1. Introduction

The Galician coast in north-western (NW) Spain is considered an important area for strandings due to its geographical characteristics, high cetacean biodiversity and abundance, and high fishery by-catch levels (López et al., 2002, 2003). Strandings provide valuable information not only on the presence and relative abundance of cetacean species but also on their anatomy, behaviour, and health status (Soares-Castro et al., 2019, García de los Ríos et al. 2021). However, only a very few studies have dealt on parasitic diversity and prevalence in marine mammals stranded in the area (Abolilo et al., 1998; Reboredo-Fernández et al., 2014; Pons-Bordas et al., 2020).

Pseudaliids Railliet & Henry, 1909 (Nematoda: Metastrongyloidea) are found in the lungs, cranial sinuses, middle ear, and/or circulatory system of their odontocete hosts (Delyamure 1955). Little is known about their life cycle or mode of transmission (Faulkner et al., 1998); moreover, data about the specificity of these nematodes are also scarce, mainly because host sampling is very difficult and experimental work virtually impossible (Pool et al., 2021). Pseudaliids have evolved and adapted to their host over thousands of years, and probably light infections pose no serious problems for otherwise healthy animals (Measures 2018).

Nine species of Stenurus have been reported in odontocetes throughout the world (Zylber et al., 2002). Most species are found in the middle ear, eustachian tube and cranial sinuses, while a few of them are found in bronchi and bronchioles (Measures 2001). The presence of Stenurus, often packed together in huge numbers, has been associated with obstruction of the auditive ducts and with osseous lesions, leading to the hypothesis that this infection could represent a potential factor in the stranding of Odontoceti (Delyamure 1955; Dailey and Stroud 1978;
In this study we identified the species of *Stenurus* collected from the lungs and the pterygoid sinus complex of 6 species of odontocetes found stranded along the Galician coastline (NW Spain). The intensity of infection in relation to the host’s sex, size and species was also analyzed.

2. Materials and methods

2.1. Animals and samples

The parasitic samples collected from the pterygoid sinus complex and lungs of 14 odontocetes stranded along the Galicia coast (41° 54’ 9” N, 8° 52’ 25” W - 43° 32’14”N 7° 02’35”W) in the northeast Atlantic between February 2009 and August 2019 were analyzed. A total of eleven parasite samples were collected from the pterygoid sinuses of three harbour porpoises (*Phocoena phocoena* Linnaeus, 1758), six short-finned pilot whales (*Globicephala macrorhynchus* Gray, 1846), one long-finned pilot whale (*Globicephala melas* Traill, 1809), one Risso’s dolphin (*Grampus griseus* Cuvier, 1812) and, three from the lungs of two bottlenose dolphins (*Tursiops truncatus* Montagu, 1821) and one striped dolphin (*Stenella coeruleoalba* Mayen, 1833) (Table 1).

Parasites were collected during the on-site necropsies by members of the Coordinadora para o Estudo dos mamíferos Mariñas (CEMMA). Necropsies were carried out following standardized protocols (Kuiken and Garcia 1991; Geraci and Lounsbury 1993). The abdominal and thoracic cavities were opened and lungs and other organs were inspected. The cranium was disarticulated from the trunk and the removal of soft parts allowed air sinuses to be examined (Fig. 1). All available parasites in the cranium was disarticulated from the trunk and the removal of soft parts allowed air sinuses to be examined (Fig. 1). All available parasites in the table were collected from the pterygoid sinus complex.

2.2. Morphological identification of parasites

All the worms extracted from the lungs and pterygoid sinuses were counted, measured, and classified by stage and sex using an Olympus SZX7 binocular stereomicroscope at 30x. Those that were preliminary identified as *Stenurus* spp. were classified as mature or immature according to the sclerotization of the spicules (males) and gravidity (females).

For specific identification all the specimens were cleared with Amann’s lactophenol with 0.01% blue cotton and mounted for morphological identification using an Olympus CX43 microscope, following the keys of Baylis and Daubney (1925), Baylis (1928) and Delamare (1955). Measurements were taken using a digital camera (Olympus EP50) attached to a compound microscope with Olympus software.

2.3. Statistical analysis

All the statistical analysis were performed with R statistical package (R v.4.1.2; R Core Team, 2020). The significance was set at *P* < 0.05. The nonparametric Wilcoxon rank sum exact test was used to compare the intensity of infection in relation to the host’s species and sex. The one-tailed Spearman correlation test was used to relate worm abundance with host body length.

Bipartite multigraph was created with bmgraph () function from multigraph package (Ostoic, 2021) and then modified with Inkscape v.1.2 to add the image of the females of the three species of *Stenurus*.

3. Results

All individuals (14/14) examined were infected with nematodes of the family Pseudaliidae. All the specimens found in the pterygoid sinuses belonged to the genus *Stenurus*, whereas two out of the three samples of nematodes collected from the lungs presented mixed infections by *Stenurus* and *Halocercus* (Table 2). Moreover, two of the short-finned pilot whales harboured in its pterygoid sinuses a mixed infection by *Stenurus* and a trematode Digenea of the genus *Nastrema* (Family Brachycladiidae).

When comparing the number of *Stenurus* collected in the different species, the Wilcoxon test showed that short-finned pilot whales were more intensely infected than the other odontocete species (W = 41; *P* = 0.0293). Moreover, the Spearman correlation test showed a positive and significant correlation between the parasite burden and the host length (S = 128; *R* = 0.719; *P* = 0.005). On the contrary, no differences in the intensity of infections were found taking into consideration the sex of the animals (W = 21; *P* = 0.9451).

All the specimens of the *Stenurus* genus were classified as mature with a clear predominance of females (85.2%) over males (14.8%), both in the pterygoid sinuses (ratio 4.6:1) and in the lungs (ratio 3.2:1), with
anus and the vagina (Fig. 2F). The mean intensity of infection by a spherical cuticular swelling on the anterior and posterior lips of the vulva (Fig. 2D). Mean intensity of infection by Stenurus spp. was 1:3.25.

The male of this species has the bursa divided into three lobes (Fig. 2C) and the ratio of the mixed infection of *S. ovatus* was 1:3.25. Mixed infection with *Nasitrema* sp.

**Table 2**

| Host          | Stenurus spp. | n | Location | Females n | Females Length | Males n | Males Length | F:M |
|---------------|---------------|---|----------|------------|---------------|---------|-------------|-----|
| PPH*          | *S. minor*    | 52 | PS       | 37         | 17.7 ± 3.1    | 15      | 16.7 ± 3    | 2.5:1 |
| PPH*          | *S. minor*    | 34 | PS       | 17         | 19.9 ± 2.9    | 17      | 16.3 ± 1.9  | 1:1  |
| PPH*          | *S. minor*    | 43 | PS       | 36         | 22.9 ± 2.1    | 7       | 19.3 ± 2.4  | 5.1:1 |
| GMA*          | *S. globiceps* | 496 | PS       | 437        | 38.3 ± 3.7    | 59      | 19.2 ± 4.5  | 6.8:1 |
| GMA*          | *S. globiceps* | 189 | PS       | 153        | 38.1 ± 4.2    | 36      | 28.4 ± 4.5  | 3.6:1 |
| GMA*          | *S. globiceps* | 18  | PS       | 15         | 38.3 ± 8.6    | 3       | 22.6 ± 6.6  | 4.3:1 |
| GMA*          | *S. globiceps* | 1761 | PS      | 1585       | 29 ± 7.6      | 176     | 20.3 ± 4.4  | 7.2:1 |
| GMA*          | *S. globiceps* | 121 | PS       | 100        | 36.1 ± 6.8    | 21      | 26.1 ± 2.7  | 4.8:1 |
| GMA*          | *S. globiceps* | 110 | PS       | 99         | 26.6 ± 7      | 11      | 19.5 ± 5    | 9.0:1 |
| GME*          | *S. globiceps* | 107 | PS       | 84         | 44.2 ± 2.6    | 23      | 25.2 ± 3.9  | 3.7:1 |
| GME*          | *S. globiceps* | 161 | PS       | 121        | 39.7 ± 3.4    | 40      | 20.7 ± 2.6  | 3.0:1 |
| GGB*          | *S. ovatus*    | 1  | L        | 1          | 23 ± 0        | 0       | –           | 1:0  |
| SCO*          | *S. ovatus*    | 6  | L        | 4          | 14.5 ± 6.4    | 2       | 10 ± 0      | 2:1  |
| TTR*          | *S. ovatus*    | 136| L        | 118        | 17.8 ± 6      | 18      | 9.4 ± 1.9   | 6.6:1 |
| Total         |               | 3235|          | 2807       | 29.0 ± 4.6    | 428     | 19.5 ± 3.3  | 4.3:1 |

* Phocoena phocea.
* Globicephala macrocephalus.
* Globicephala melas.
* Grampus griseus.
* Stenella coeruleoalba.
* Tursiops truncatus.

The host-parasite associations observed in the northeastern Atlantic Ocean, including those involving *Halocercus inermis*, were congruent with the molecular analysis of the ITS2 barcoding region of ribosomal DNA, so it can be assumed that the morphological identification for those species is reliable.

### 4. Discussion

Pseudaliid nematodes are common parasites of the respiratory tract and the pterygoid sinuses of delphinids and porpoises in both hemispheres (Lehner et al., 2005; Tomo et al., 2010). All the odontoceti included in this study were infected by pseudaliid nematodes of the genus *Stenurus*. In addition, mixed infections with other pseudaliid, *Halocercus delphini* Baylis and Daubney (1925), were found in the lungs of one striped and bottlenose dolphins; mixed infection with the trematode *Digenea Nasitrema* was detected in the pterygoid sinuses of two short-finned pilot whales.

Despite the small sample size, there is strong evidence that *Stenurus* is the most prevalent parasite in the pterygoid sinuses and lungs of odontoceti in the northeastern Spanish Atlantic coast. Two out of the three lung parasitic samples contained *Halocercus delphini*, so a high prevalence of this lungworm could also be expected. In a previous study carried out by Abollo et al. (1998) in the same area *Stenurus globiceps* was identified with a prevalence of 50% in the air sinuses of long-finned whales, whereas the genus *Halocercus* was found in the lungs of harbour porpoises (25%), bottlenose (10%), striped (25%) and common (18%) dolphins.

Three out of the nine species included in the genus *Stenurus* were morphologically identified from the six odontoceti species stranded in the Galician coast: *S. minor*, *S. ovatus* and *S. globiceps*, making this the first description of *S. minor* and *S. ovatus* in Galician waters. A recent study (Pool et al., 2021) has shown that the species identification following the morphological descriptions by Delamare (1955) for *Stenurus* spp. was congruent with the molecular analysis of the ITS2 barcoding region of ribosomal DNA so, it can be assumed that the morphological identification for those species is reliable.

A clear pattern of host specificity was observed for the species *Stenurus*, with *S. minor* showing a specificity for Phocoenidae (harbour porpoises), *S. globiceps* for Globicephalinae (pilot whales and Bottlenose dolphin) and *S. ovatus* for Delphininae (bottlenose and striped dolphins) (Fig. 3).

The host-parasite associations observed in the northeastern Atlantic Ocean are important because they provide insights into the ecological interactions and the health of the marine mammals involved.
waters match with those found by Pool et al. (2021) in western Mediterranean, who observed that both S. ovatus and Halocercus delphini were restricted to the clade of the Delphininae, and S. globicephalae to the clade of the Globicephalinae. In addition, our findings also support a clear association between S. minor and Phocoenidae.

Marine mammal parasites constitute valuable indicators of host habitat use, diet, migration, and population dynamics (Balbuena and Raga, 1994). Pool et al. (2021) found a weak but significant association between prey overlap and lungworm species similarity. In our study, predominantly teuthophagous species like pilot whales and Risso’s dolphins were infected by S. globicephalae, whereas piscivorous species such as the bottlenose dolphin and species with a mixed diet like the striped dolphin, harboured S. ovatus. However, harbour porpoises which have a mixed diet harboured S. minor. Méndez-Fernández et al. (2013) confirmed the existence of a niche segregation of these odontocete species in our study area, with the harbour porpoise in the highest trophic level. Pierce et al. (2010) affirmed that even though the three main cetaceans in Galician waters (porpoises, bottle nose and common dolphins) feed primarily on fish, there are differences in the species of prey eaten and also differences in habitat use to avoid food competition and, in the case of porpoises, it is possible that they do not use areas frequented by bottlenose dolphins in order to avoid aggressive interactions (Ross and Wilson 1996; Santos and Pierce 2003). Therefore, trophic specialization and differences in the niche occupied for each cetacean

Fig. 2. (A–B) Stenurus minor: posterior end of a male (A) with an undivided bursa and broad spicules and of a female (B) with a finger-shaped cuticular process on the anterior lip of the vulva (•) and first stage larvae (L1) in the uterus (⇨). (C–D) Stenurus globicephalae: posterior end of a male (C) and a female (D) with multiple cuticular swellings (•) and L1 in the uterus (⇨). (E–F) Stenurus ovatus: posterior end of a male (E) with a divided bursa showing three rays and a female (F) showing a conical terminal process (•) and a cuticular swelling (⇨).
Fig. 3. Bipartite multigraph relating the three *Stenurus* species with their hosts. The width of the links (gray arrows) represents the mean parasitic load on each host.
species may lead to different patterns of parasite specificity. In relation to the anatomical localization, most Stenurus species occur in the middle ear, eustachian tube and cranial sinuses, whereas a few species have been reported to occur in bronchi and bronchioles (Measures, 2001). However, Zylber et al. (2002) detected the presence of abundant lungworms, identified as causing discomfort to the host.

Pseudaliids located in the cranial sinuses and middle ear provoke minor Stroud, 1978; Measures, 2001). Moreover, no gross lesions associated cause a subacute purulent focal pneumonia (Cowan, 1967; Dailey and Stroud, 1978). In the same host species, that had the highest parasite burden. On the contrary, Faulkner et al. (1998) found an inverse relationship between the intensity of S. minor infection and mean worm length, which was suggestive to a crowding effect. The one-tailed Spearman correlation test was used to relate worm abundance with host body length, since larger hosts could have more infection opportunities due to higher food consumption (Measures, 2001).

Some adult pulmonary pseudaliids stimulate none or little inflammatory response in bronchi or bronchioles, but larvae in alveoli can cause a subacute purulent focal pneumonia (Cowan, 1967; Dailey and Stroud 1978; Measures, 2001). Moreover, no gross lesions associated with the presence of numerous S. minor in the cranial sinuses were observed by Faulkner et al. (1998). According to different authors, Pseudaliids located in the cranial sinuses and middle ear provoke minor hemorrhage, mild to moderate nonsuppurative chronic inflammation and thickening of the sinus mucosal lining, rarely purulent sinuses (Delyamure, 1955; Measures, 2001). However, Zylber et al. (2002) detected the presence of abundant lungworms, identified as G. globicephalae in the cranial air sinuses of a false killer whale (Pseudorca crassidens Owen, 1846) which revealed loss of osseous mass with the disappearance of the left zygomatic arch, and the left jaw had three osseous fenestrations in the region related to the organ of acoustic reception. These lesions supported the hypothesis of these authors that this infection was related to the stranding. In our study, it has not been possible to establish a relationship between the presence of Stenurus in the host and the strandings. However, its presence in the pterygoid sinuses and in the lungs could induce lesions given a stressful situation, causing discomfort to the host.

In conclusion, nematodes of the genus Stenurus are frequent in odontocetes stranded along the north-west Spanish coast, showing a clear host-parasite association propitiated by niche segregation and trophic position.

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Declaration of competing interest
None.

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