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Cerebral nasitremiasis in a Blainville's beaked whale (*Mesoplodon densirostris*) stranded in the Canary Islands

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A R T I C L E I N F O

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A B S T R A C T

Ten species within the genus *Nasitrema* (subfamily *Nasitrematinae,* family *Brachycladiidae*) have been reported infecting a wide variety of odontocetes worldwide, although there is still a lack of information about their presence in beaked whales (BWs). *Nasitrema* spp. are commonly described inhabiting the pterygoid sinus, the tympanic cavities, and the middle and inner ear; although aberrant migrations through the brain have been also reported. This trematode may cause different type of lesions, ranging from mild to severe saculitis, neuritis, otitis, and/or meningoencephalitis that may impede cetaceans to survive in the wild, resulting in incoordination, loss of equilibrium, and echolocation dysfunction ending in a stranding event. The presence of *Nasitrema* sp. was found in an adult female Blainville beaked whale stranded death in Fuerteventura, Canary Islands, on November 2016. The most relevant gross finding was a severe chronic-active multifocal pyogranulomatous and necrotizing encephalitis. Histologically, multiple areas of necrosis, pyogranulomatous and eosinophilic inflammation, haemorrhages and occasional cholesterol crystals were found associated with parasitic structures compatible with an adult trematode and its eggs. Molecular analysis, based on a quantitative polymerase chain reaction (qPCR) of the brain tissue sample detected 99% homology with a partial sequence of the NADH dehydrogenase subunit 3 (ND3) gene of *Nasitrema delphini*. In addition, liver, kidney, prescapular lymph node and brain samples were positive to herpesvirus (conventional nested PCR). Evidence of the presence of this parasite was not found in any of the 54 beaked whales ($n = 54$) stranded on the Canary Islands between 1999 and 2017, specifically 35 Cuvier's BWs and 19 specimens belonging to the *Mesoplodon* genus. To our knowledge, the current study represents the first description of a nasitremiasis in a member of the *Ziphiidae* family.

1. Introduction

Digeneans of the family *Brachycladiidae* (Phylum Platyhelminthes, class Trematoda) are restricted to marine mammals, from which ten species within the genus *Nasitrema*, (subfamily *Nasitrematinae*) (WoRMS Editorial Board, 2022) have been reported infecting a wide variety of toothed whales (odontocetes) worldwide (Table 1). *Nasitrema* spp. are commonly described inhabiting the pterygoid sinus, the tympanic cavities (Arbelo et al., 2013; Cowan et al., 1986; Dailey and Walker, 1978; Degollada et al., 2002; Díaz-Delgado et al., 2018; Lim et al., 2016; Neiland et al., 1970; Oliveira et al., 2011; Schwab, 1985; Shiozaki and Amano, 2017; Walker and Cowan, 1981) and the middle and inner ear (Dailey and Ridgway, 1976; Degollada et al., 2002; Díaz-Delgado et al., 2018), although ova and occasionally adults of these parasites have also been found damaging the vestibulocochlear nerve (VIII cranial nerve) (Degollada et al., 2002; Morimitsu et al., 1992; Morimitsu et al., 1987; Morimitsu et al., 1986; Oliveira et al., 2011) and the brain (Cowan et al., 1986; Dailey, 1985; Dailey and Walker, 1978; Degollada et al., 2002; Díaz-Delgado et al., 2018; Lewis and Berry, 1988; O'Shea et al., 1991; Phillips and Suepaul, 2017; Ridgway and Murray, 1972; Sierra et al., 2020). In those cases, *Nasitrema* may cause different type of lesions (Arbelo et al., 2013; Díaz-Delgado et al., 2018; Morimitsu et al., 1992; Morimitsu et al., 1987; Morimitsu et al., 1986; Ridgway and Murray, 1972), ranging from mild to severe saculitis, neuritis, otitis, and/or meningoencephalitis (St. Leger et al., 2018) that may impede cetaceans to survive in the wild, resulting in incoordination, loss of equilibrium,

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Table 1
Summary of reported worldwide cetacean species with nasitremiasis.

Cetacean species	References
Finless porpoise (*Neophocaena phocaenoides*)	(Ozaki, 1935; Yamaguti, 1951)
Dall's porpoise (*Phocoenoides dalli*)	(Cowan et al., 1986; Dailey and Walker, 1978; Yamaguti, 1951)
Short-finned pilot whale (*Globicephala macrorhynchus*)	(Degollada et al., 2002; Morimitsu et al., 1987; Neiland et al., 1970; Yamaguti, 1951)
False killer whales (*Pseudorca crassidens*)	(Morimitsu et al., 1987; Neiland et al., 1970)
Pacific white-side dolphin (*Lagenorhynchus obliquidens*)	(Dailey and Walker, 1978; Kikuchi et al., 1987; Lewis and Berry, 1988; Neiland et al., 1970; Ridgway and Murray, 1972)
Northern right whale dolphin (*Lissodelphis borealis*)	(Cowan et al., 1986; Dailey and Walker, 1978; Neiland et al., 1970)
Common dolphin (*Delphinus delphis*)	(Cowan et al., 1986; Dailey and Walker, 1978; Degollada et al., 2002; Díaz-Delgado et al., 2018; Fraija-Fernández et al., 2016; Neiland et al., 1970; Ridgway and Murray, 1972; Walker and Cowan, 1981)
Atlantic bottlenose dolphin (*Tursiops truncatus*)	(Arbelo et al., 2013; Dailey and Ridgway, 1976; Degollada et al., 2002; Díaz-Delgado et al., 2018; Kumar et al., 1975; Ridgway and Murray, 1972; Sierra et al., 2020)
Striped dolphin (*Stenella coeruleoalba*)	(Dailey and Walker, 1978; Degollada et al., 2002; O'Shea et al., 1991; Oliveira et al., 2011)
Pygmy killer whale (*Feresa attenuata*)	(Fraija-Fernández et al., 2015; Schwab, 1985)
Melon-headed whale (*Peponocephala electra*)	(Morimitsu et al., 1986; Phillips and Suepaul, 2017)
Pantropical spotted dolphin (*Stenella attenuata*)	(Forrester, 1991; Oliveira et al., 2011)
Rough-toothed dolphin (*Steno bredanensis*)	(Arbelo et al., 2013; Degollada et al., 2002; Ebert and Valente, 2013; Forrester, 1991)
Risso's dolphin (*Grampus griseus*)	(Morimitsu et al., 1992)
Pygmy sperm whale (*Kogia breviceps*)	(Degollada et al., 2002)
Atlantic spotted dolphin (*Stenella frontalis*)	(Degollada et al., 2002)
Spinner dolphin (*Stenella longirostris*)	(De La Fuente Marquez et al., 2016)
Guiana dolphin (*Sotalia guianensis*)	(Ebert and Valente, 2013)
Long-beaked Common Dolphin (*Delphius capensis*)	(Lim et al., 2016)
Long-finned Pilot Whale (*Globicephala melas*)	(Fraija-Fernández et al., 2016)
Narrow-ridged finless porpoise (*Neophocaena asiaeorientalis*)	(Shiozaki and Amano, 2017)

and echolocation dysfunction ending in a stranding event (Dailey and Walker, 1978; Lewis and Berry, 1988; Morimitsu et al., 1992; Morimitsu et al., 1987; Morimitsu et al., 1986; O'Shea et al., 1991).

Up to date, the life cycle of this trematode remains a subject of discussion, although it has been recently reported that *Brachycladiidae* are closely related to *Acanthocolpidae*, parasites of marine teleost fishes that typically possess three-host life cycles with gastropods acting as the first intermediate hosts and either fishes or bivalves acting as the second intermediate hosts (Kremnev et al., 2020). The presence of adults and eggs of *Nasitrema attenuata* in faeces reinforces the hypothesis that infection could be acquired through consumption of infected fish containing the larval stages of the parasite (Kleinertz et al., 2014; St. Leger et al., 2018).

The *Ziphiidade* family is widely distributed throughout the planet (MacLeod, 2018) and consists of 22 species and 6 genera, although a new beaked whale (BW) species, *Mesoplodon eueu*, has been recently described based on its genetic and morphological features (Carroll et al., 2021). The Canary Islands constitute an important geographical enclave located in the Atlantic Ocean, with an enormous diversity of marine mammals (Canarias Conservación, 2018a). Six different species of BWs, including hold species with a presence throughout the year and/or transient populations, have been recorded in this archipelago (Canarias Conservación, 2018b). Beaked whales are considered exceptional long and deep divers and because of their pelagic habits and by their scarcity, among other reasons, parasitological data from the *Ziphiidae* family are scarce (Di Azevedo et al., 2016; Díaz-Delgado et al., 2016; Fraija-Fernández et al., 2018; Jerdy et al., 2022; Paggi et al., 1998). Descriptions of the presence of trematodes of the family *Brachycladiidae* in these cetacean species are limited to liver parasitization, with no previous reports of Nasitremiasis (Fraija-Fernández et al., 2014; Nakagun et al., 2018; Nakagun and Kobayashi, 2020).

The present research represents the first description of cerebral nasitremiasis in a BW, which is associated with pyogranulomatous and necrotizing encephalitis severe enough to cause the stranding and/or death of the animal. Based on this finding, and due to the high number of beaked whale individuals analysed by our group, we reviewed in more detail all the specimens of the *Ziphiidae* family of our database for the occurrence of the presence of eggs, larvae and/or adults of *Nasitrema* spp.

2. Material and methods

A 454 cm-length, 814 kg-weight, adult female of Blainville's beaked whale (*Mesoplodon densirostris*) (CET 824), was found stranded dead at Playa Blanca, Fuerteventura, Canary Islands (N28° 28′ 33.665" N, 13° 52′ 0.033" W) on the afternoon of November 11th, 2016. The carcass was in a fresh conservation status (code 2) following the subsequent categories of decomposition stage: grade 1: very fresh; grade 2: fresh; grade 3: moderate autolysis; grade 4: advanced autolysis and grade 5: very advanced autolysis (Arbelo et al., 2013; Kuiken and García-Hartmann, 1991). The animal was transported, by the environmental agents of the Cabildo of Fuerterventura, to the Rescue Centre of La Oliva, Fuerteventura, and preserved at 4 °C until the next morning. The necropsy was performed by the veterinary staff of the Institute of Animal Health (IUSA) of the Universidad de Las Palmas de Gran Canaria following a complete standardized necropsy protocol for stranded cetaceans (Kuiken and García-Hartmann, 1991). The post-mortem study was possible thanks to the permission for the management of stranded cetaceans conceded by the Spanish Ministry of the Environment (SGPM/BDM/AUTSPP/70/2019). Physical development was estimated according to the total length of the animal (Perrin et al., 2009) and the histological study of the gonads (Geraci et al., 2005). Representative tissue samples from all the organs were taken and immersed in 10% neutral buffered formalin, carved, routinely processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (HE) for light microscopic examination (Olympus BX51, Tokyo, Japan) which use a Camara software for DP21 (Olympus DP21, Tokyo, Japan). Required additional stains included periodic acid–Schiff (PAS) and Luxol Fast Blue (LFB). The manipulation, fixation and brain sampling were made following the recently published methodology (Sacchini et al., 2022) applied by our group since 2009. In addition, a subset of samples (including skin, lung, kidney, liver, spleen, prescapular lymph node and brain) were stored at −80 °C for microbiological and virological analysis. For molecular viral screening, 0.5 g of each fresh frozen tissue sample was processed as previously described (Arbelo et al., 2012). Genomic DNA and RNA was simultaneously extracted by pressure filtration (QuickGene DNA tissue kit S, Kurabo, Japan), following the manufacturer's instructions with slight modifications (Sacristán et al., 2015). Molecular analyses for *cetacean morbillivirus* (CeMV) and herpesvirus detection were performed as previously reported (Felipe-Jiménez et al., 2022; Felipe-Jiménez et al., 2021).

In order to identify the genus and the species of the trematodes, a quantitative Polymerase Chain Reaction (q-PCR) based on SYBR® Green technique (Mini Opticon Real-Time PCR System. MJ Mini Personal Thermal Cycler of BIO RAD) was performed as previously published (Sierra et al., 2020). Two pairs of primers were designed, one of these amplifying a 230 bp nucleotide sequence of the NADH dehydrogenase, subunit 3 (ND3) gene of *Nasitrema delphini* (GenBank Accession no.

KT180216) (Fraija-Fernández et al., 2016): (Forward: 5′- CGG ATT GGT TTT CGT TGT CT −3′; Reverse: 5′- ACC CAA CCT AAG CAA GAG CA −3′), and the other set to amplify a 241 bp nucleotide sequence of the 18S rRNA gene, partial of *Nasitrema globicephalae* (GenBank Accession no. AJ004968) (Fernandez et al., 1998b): (Forward: 5′- CTG AAT CGG TGG GTT TGT CT −3′; Reverse: 5′- CCG TCT GTC CCT CTT AAC CA −3′). Two negative controls (for extraction and amplification) and an amplification-positive control were included (*Nasitrema* sp. from the brain tissue sample of a bottlenose dolphin previously obtained in our laboratory). The PCR product was purified using a Real Clean spin kit (REAL®, Durviz, s.l., Valencia, Spain) and sequenced (Sanger method). A BLAST search (BLAST: Basic Local Alignment Search Tool, 2021) was conducted to confirm the identity of the PCR amplicon, which was compared with other somewhat similar sequences published in GenBank. A set of closely related sequences were aligned using ClustalW algorithm through software MEGA X (Kumar et al., 2018). The phylogenetic tree was constructed using the Maximum Likelihood Method and the Hasegawa-Kishino-Yano model with a discrete Gamma distribution to model the evolutionary rate differences among sites (5 categories (+G, parameter = 0.3452)). To determine the reliability of the tree was used the Bootstrap method with 500 replicates.

The *Nasitrema* sp. obtained sequences from the NADH dehydrogenase, subunit 3 (ND3) gene, were identified from this study and were deposited in GenBank under accession no. OP480061.

Additionally, 54 beaked whales were reviewed in detail, representing the 60.7% of the total number ($n = 89$) of strandings involving BWs along the Canary coasts between January 1999 and December 2017, according to our database. The selection criteria were that all these specimens were subjected to a complete standardized necropsy and currently preserving recollected samples. Specifically, 35 animals were Cuvier's BWs (*Ziphius cavirostris*) and 19 specimens belonged to the *Mesoplodon* genus: 1 True's BW (*Mesoplodon mirus*), 2 Sowerby's BWs (*Mesoplodon bidens*), 6 Blainville's BWs (*Mesoplodon densirostris*) and 10 Gervais' BWs (*Mesoplodon europaeus*). All the information about the life history data (species, age category, sex, length weight, stranding date, location and stage), the decomposition stage, body condition (good, moderate, poor or emaciated) (Arbelo et al., 2013; Díaz-Delgado et al., 2018), and the reproductive status (mature or immature is registered and summarized in Supplementary Table 1. Postmortem examination included the careful gross and microscopic examination of the cephalic region (nasal air sacs system, pterygoid sinus, tympano-periotic complex and central nervous system (CNS)) as well as the respiratory tract (tracheobronchial tree and lungs) including evaluation of the presence of parasites.

3. Results

Gross examination of the CET 824 revealed a very poor body condition (814 kg weight) characterized by extremely concave dorsal profile with visible costal reliefs, and serous atrophy of fat. The thickness of blubber was measured in three positions, (dorsal, lateral, and ventral) in a skin sample from the cranial zone of the dorsal flipper: 3.4 centimetres (cm), 3 cm and 3.2 cm. In addition, no food remains were observed in the digestive tract. Moderate multifocal linear-bleeding and linear erosive skin lesions were mainly found in the flippers, rostrum, and ventral parts of the body, which were associated with a suspected active stranding event. Moderate and multifocal congestion and haemorrhages were also shown in the lung and mediastinal lymph nodes, acoustic fat and melon; although the most outstanding finding was brain lesions. More in detail, serial coronal sections of the brain revealed the presence of multifocal, tortuous, irregular, well-defined, yellow-green areas of soft texture, mainly located in the frontal lobe of the right cerebral hemisphere extending from the inner part of the white matter to the adjacent dorso-lateral cortex without affecting the meninges (Fig. 1 A). Similar lesions were also present in the diencephalon (Fig. 1 B).

Histologically, multiple areas of necrosis surrounded by pyogranulomatous inflammation (with pigment laden macrophages and scattered multinucleated giant cells), as well as multifocal haemorrhages associated with an intralesional adult trematode (Fig. 2 A, B and C) were observed. The trematode was 2 mm wide in a transversal cut (not evident grossly during the sampling of the brain) with a thin tegument where no spines were observed (probably lost post-mortem) surrounded by spongy parenchyma where no other structures were recognizable (because of necrosis) except the vitellaria (Fig. 2 C). Refringent yellow triangular to oval-shape eggs of about 50 to 75 μm in diameter were found in the cortex and diencephalon. The eggs were found in the same lesion area but not near the adult parasite (Fig. 2 D). Other histopathological changes observed in the CNS were diffuse gliosis, multifocal

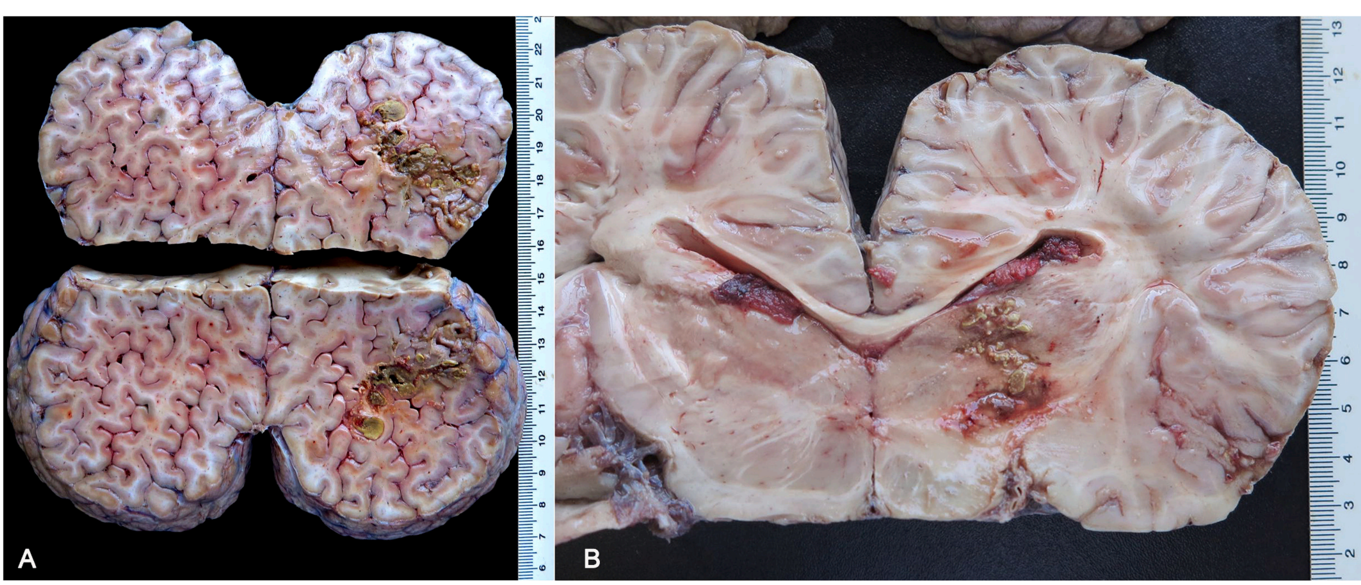

Fig. 1. Gross findings in the brain from a Blainville beaked whale (CET 824): coronal sections (post fixation in formalin). A) Sections from the frontal lobe of the right cerebral hemisphere showed a severe chronic-active multifocal pyogranulomatous and necrotizing encephalitis. B) The diencephalon section also presented multifocal, irregular, well-demarcated and elevated yellow-green lesions, surrounded by a hemorrhagic halo, which are compatible with the aberrant parasitic migratory route. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

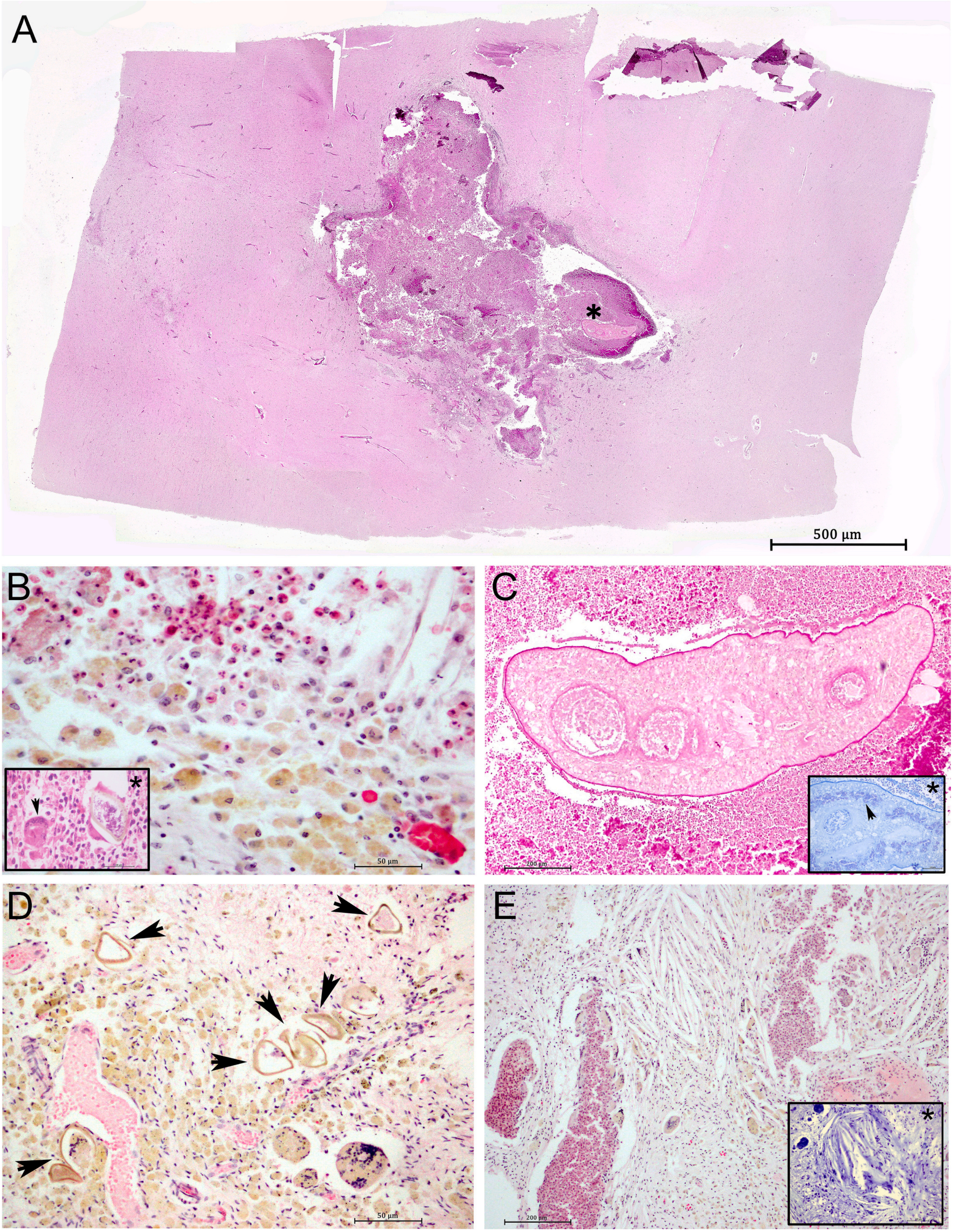

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Fig. 2. Histopathology: brain findings from a Blainville beaked whale (CET 824). A) Histological overview showing an area of necrosis and pyogranulomatous inflammatory reaction associated with an intralesional adult trematode (asterisk) at the diencephalon. PAS stain, low-power magnification. B) Detail of the inflammatory infiltrate mainly composed of neutrophils and pigment laden macrophages cells. HE stain, high-power magnification Inset (*): Detail of a multinucleated giant cell (arrow). HE stain, high-power magnification. C) A closer view showing the only one adult *Nasitrema* sp. found at the histopathological examination. The trematode was surrounded by necrosis, macrophages and polymorphonuclear cells. HE stain, medium-power magnification. Inset (*): Detail of the trematode's internal structures compatible with vitellaria (arrow). LFB stain, medium-power magnification. D) Triangular to oval-shape refringent yellow of trematode eggs (arrows) surrounded by macrophages with intracytoplasmic yellowish pigment. HE stain, high-power magnification. E) Multifocal cholesterol clefts associated with the presence of *Nasitrema* sp. HE stain, medium-power magnification. Inset (*): cholesterol clefts stained with LFB stain, medium-power magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

lymphoplasmacytic perivascular cuffs, perivascular edema and multifocal presence of cholesterol clefts (Fig. 2 E). The liver presented lymphoplasmacytic multifocal pericholangitis and periductal fibrosis associated with the presence of adult flukes and eggs, compatible with trematodes species belonging to the *Brachycladiidae* family. Mild multifocal granulomatous fasciitis and panniculitis with intralesional degenerating metazoan cuticle (compatible with *Crassicauda* sp.), multiorgan moderate haemorrhages (lymph nodes, acoustic fat and melon), mild multifocal lymphoplasmacytic myocarditis, as well as mild-moderate multifocal acute degenerative changes in the skeletal (hypercontracted myofibers and segmental necrosis) and cardiac muscle (juxtanuclear vacuolization and increased acidophilic cytoplasm of the myocardiocytes), and mild multifocal lymphoplasmacytic gastroenteritis and adrenalitis were observed.

A partial sequence of the ND3 gene (152 base pairs in length excluding primers) was obtained from brain sample of CET 824, which presented the highest percentage of similarity, (99.34% with a 99% Query cover (QC)) with a *N. delphini* sequence (GenBank Accession no. KT180216) detected in a common dolphin stranded in Spain (Canary Islands) (Fraija-Fernández et al., 2016). The sequence also showed 91.33% of homology (99% QC) with a *N. attenuatum* sequence (GenBank Accession no. MN264280) obtained from a Guiana dolphin stranded in Brazil, and 90.07% of homology (99% QC) with a *N. globicephalae* sequence (GenBank Accession no. AF034557) from a long-finned pilot whale stranded in Chile (Fernandez et al., 1998a). The phylogenetic tree analysis involved ten nucleotide sequences (Fig. 3). The tree was divided into two branches, one of them contained five *Nasitrema* genus sequences and the other is formed by five sequences from several trematodes belonging to the *Brachycladiidae* family which also constitute the root of the tree. The phylogenetic analysis showed that the sequence obtained from the Blainville's BW of our study is clustered, with a bootstrap value of 79, with the only available sequence of *N. delphini* (KT180216) detected in the common dolphin stranded in the same geographic area. The branches were condensed with a bootstrap value over 50%. All the tested samples from CET 824 were CeMV negative by RT-qPCR (Felipe-Jiménez et al., 2022). However, Herpesvirus DNA was detected by conventional PCR in the kidney, liver, prescapular lymph node and brain tissue samples with no associated lesions. (Felipe-Jiménez et al., 2021).

Evidence of the presence of these parasites was not found in any of the other BWs from our study for which the following anatomical regions were grossly inspected: the respiratory track (100%), the pterygoid sinus (40.7%), the nasal air sacs (12.9%), the tympano-periotic complex (11.1%), and the CNS (55.5%). Some of these regions were also microscopically analysed; the brain (55.5%), the respiratory track (100%), and the tympano-periotic complex (7.4%). This information is compiled in Table 2. Notwithstanding, some of the microscopic findings displayed by brain sample of CET 824 were also observed in animal identified as CET 770; specifically, a localized mild granulomatous meningitis with pigment-laden macrophages was noticed. However, this case was negative for *Nasitrema* spp. by the two q-PCRs.

4. Discussion

We report the presence of a platyhelminth parasite, identified as *N. delphini*, in a BW stranded in the Canary Islands. Evidence of the presence of this parasite was not found in any of the revised 54 beaked whales. Thus, the presence of *Nasitrema* sp. detected in one BW stranded in the Canary Islands represents the 0.2% of the animals in which at least three regions (lungs, pterygoid sinuses and CNS) were consistently examined (1/20). The fluke was associated with brain lesions that could have been responsible for the stranding and/or death of the animal. In the recent years, an increased number of neuropathological findings has been reported to be related to cetaceans stranding (Arbelo et al., 2013; Díaz-Delgado et al., 2018; Pintore et al., 2018; St. Leger et al., 2018), from which infectious and inflammatory conditions of the CNS are among the most common leading natural causes of stranding and death. A wide spectrum of pathogens (viruses, bacteria, fungi and parasites) has been reported affecting the CNS of these marine mammals, which can result in fatal acute, subacute, or chronic meningitis and/or encephalitis (Pintore et al., 2018; Sierra et al., 2022; Sierra et al., 2020). Parasitic meningoencephalitis has been more frequently related to protozoans (*Toxoplasma gondii*) (Gulland et al., 2018; St. Leger et al., 2018), while helminthic brain lesions are less frequently described and usually involve necrotizing encephalitis along the migratory path in which intralesional nematodes and trematodes (adults, eggs, and/or

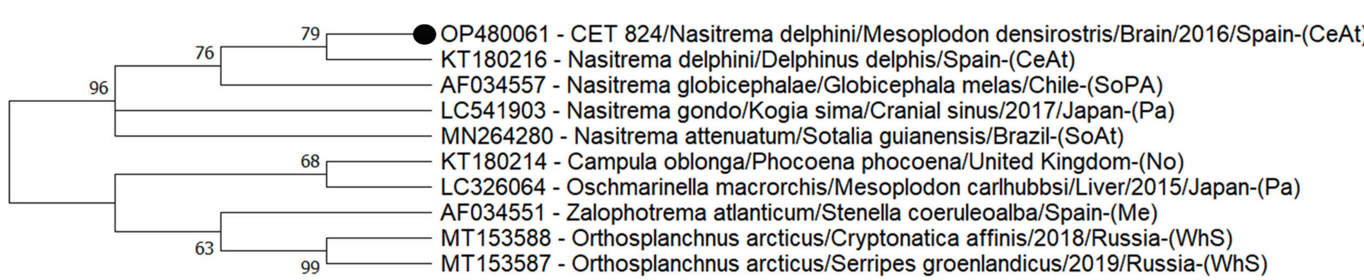

Fig. 3. Maximum likelihood phylogenetic tree for the nucleotide partial sequences of the ND3 gene. The phylogenetic tree consists of 10 sequences from reported cases of *Nasitrema* spp. and trematodes belonging to the *Brachycladiidae* family. The Neighbour-Join and BioNJ algorithms together with the Hasegawa-Kishino-Yano model and Gamma distribution to model the evolutionary rate differences among sites [5 categories (+G, parameter = 0.3452)], were chosen to construct the tree. The Bootstrap method was performed to resample 500 replicates and evaluate the reliability of the tree. The branches were condensed with a bootstrap value over 50%. The accession number from GenBank, the trematode species from the *Brachycladiidae* family, the host, the date of collection, and the geographic area of stranding were used to identify the nucleotide sequences. Abbreviations: CeAt (Central Atlantic Ocean); Me (Mediterranean Sea); No (North Sea); Pa (Pacific Ocean); SoAt (South Atlantic Ocean); SoPA (South Pacific Ocean); WhS (White Sea).

Table 2
Summary of the study's anatomical regions examined for detecting *Nasitrema* spp. in the beaked whales of our study. Notes. G: gross examination; M: microscopic examination; (–): not examined; A: absence of parasites; P: presence of parasites.

		Respiratory tract		Pterygoid sinus	Nasal air sac system	Tympano-periotic complex		CNS	
ID CODE	SPECIES	G	M	G	G	G	M	G	M
CET 86	*Ziphius cavirostris*	A	A	–	–	–	–	A	A
CET 103	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 108	*Ziphius cavirostris*	A	A	A	–	–	–	–	–
CET 113	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 134	*Mesoplodon europaeus*	A	A	–	–	–	–	–	–
CET 180	*Mesoplodon densirostris*	A	A	A	–	–	–	A	A
CET 181	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 182	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 183	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 184	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 185	*Mesoplodon europaeus*	A	A	A	–	–	–	A	A
CET 189	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 213	*Mesoplodon densirostris*	A	A	–	–	–	–	A	A
CET 236	*Ziphius cavirostris*	A	A	–	–	A	A	A	A
CET 243	*Mesoplodon densirostris*	A	A	–	–	–	–	A	A
CET 259	*Mesoplodon europaeus*	A	A	–	–	–	–	A	A
CET 264	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 265	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 294	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 304	*Ziphius cavirostris*	A	A	–	–	–	–	A	A
CET 322	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 333	*Mesoplodon europaeus*	A	A	–	A	–	–	A	A
CET 334	*Mesoplodon europaeus*	A	A	–	–	–	–	A	A
CET 338	*Mesoplodon europaeus*	A	A	A	–	A	A	A	A
CET 352	*Ziphius cavirostris*	A	A	–	–	–	–	A	A
CET 354	*Mesoplodon europaeus*	A	A	–	–	A	–	–	–
CET 379	*Mesoplodon bidens*	A	A	A	–	–	–	A	A
CET 471	*Ziphius cavirostris*	A	A	–	–	–	–	A	A
CET 503	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 510	*Mesoplodon europaeus*	A	A	A	–	–	–	A	A
CET 547	*Mesoplodon europaeus*	A	A	A	–	–	–	A	A
CET 576	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 579	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 591	*Ziphius cavirostris*	A	A	–	A	–	–	–	–
CET 593	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 620	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 624	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 631	*Mesoplodon europaeus*	A	A	A	–	–	–	A	A
CET 636	*Mesoplodon mirus*	A	A	A	–	–	–	A	A
CET 645	*Ziphius cavirostris*	A	A	–	A	–	–	–	–
CET 680	*Ziphius cavirostris*	A	A	–	A	–	–	–	–
CET 688	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 695	*Mesoplodon densirostris*	A	A	A	–	–	–	A	A
CET 711	*Mesoplodon densirostris*	A	A	–	–	–	–		–
CET 712	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 719	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 720	*Ziphius cavirostris*	A	A	A	A	–	–	–	–
CET 770	*Ziphius cavirostris*	A	A	A	A	A	A	A	A
CET 771	*Ziphius cavirostris*	A	A	A	–	–	–	A	A
CET 818	*Ziphius cavirostris*	A	A	–	–	–	–	–	–
CET 824	*Mesoplodon densirostris*	A	A	A	A	A	A	A	**P**
CET 827	*Mesoplodon bidens*	A	A	A	–	A	A	A	A
CET 833	*Ziphius cavirostris*	A	A	A	–	–	–	–	–
CET 852	*Mesoplodon densirostris*	A	A	A	A	A	–	A	A
CET 855	*Ziphius cavirostris*	A	A	–	–	–	–	A	A

larvae) can be found (Summers et al., 1995; Vandevelde et al., 2012).

The presence of adults and/or eggs of *Nasitrema* spp. have been previously identified in odontocete cetacean brain lesions, which typically are described as grossly multiple, irregularly, brownish areas with cavitation and necrosis, and thickened meninges (Cowan et al., 1986; Dailey, 1985; Dailey and Walker, 1978; Degollada et al., 2002; Forrester, 1991; Lewis and Berry, 1988; O'Shea et al., 1991; Phillips and Suepaul, 2017; Ridgway and Murray, 1972; Sierra et al., 2020). Similar lesions were grossly observed in the animal from our study. Histologically, liquefactive necrotic abscessed areas bordered by diffuse infiltrates of macrophages with occasional hemosiderophages, giant cells, gliosis and infiltrative lymphoplasmacytic or fibrinopurulent meningoencephalitis have been observed associated to the presence of eggs and/or adult worms of these flukes (Degollada et al., 2002; Díaz-Delgado et al., 2018; Lewis and Berry, 1988; O'Shea et al., 1991; Phillips and Suepaul, 2017; Ridgway and Murray, 1972; Sierra et al., 2020); although the participation of *Nasitrema* spp. in chronic nervous lesions, such as non-suppurative leptomeningitis, cannot be ruled out (Degollada et al., 2002). Similar lesions were found in the animal from our study in addition to the presence of cholesterol clefts within the necrotic tissue, which have been less frequently described in brain nasitremiasis (Ridgway and Murray, 1972). We only noticed brain lesions during the histopathological examination in two out of the 30 brain microscopically examined (6.7%), although these lesions could only be associated to the presence of *Nasitrema* sp. in one of these brains. Despite the life cycle of *Nasitrema* spp. remains unknown (Howard, 1983; Lewis and

Berry, 1988), brain lesions are thought to be the result of an aberrant migration of the parasite through the VIII cranial nerve (Cowan et al., 1986; Howard, 1983; Lewis and Berry, 1988; Morimitsu et al., 1992; Ridgway and Murray, 1972). In odontocetes, this nerve is externalized, extending from the brain stem to the retro-bullar space of the tympanoperiotic complex without an osseous cover (Cozzi et al., 2017; Ketten, 1992; Morimitsu et al., 1992; Morimitsu et al., 1987; Morimitsu et al., 1986; Reysenbach de Haan, 1957). In spite of that the head and air sacs are the most common anatomic locations where these parasites inhabit (Arbelo et al., 2013; Cowan et al., 1986; Dailey and Ridgway, 1976; Dailey and Walker, 1978; Degollada et al., 2002; Díaz-Delgado et al., 2018; Ebert and Valente, 2013; Forrester, 1991; Kikuchi et al., 1987; Kumar et al., 1975; Lewis and Berry, 1988; Morimitsu et al., 1986, 1987, 1992; Neiland et al., 1970; O'Shea et al., 1991; Oliveira et al., 2011; Phillips and Suepaul, 2017; Ridgway and Murray, 1972; Schwab, 1985; Shiozaki and Amano, 2017; Sierra et al., 2020; Walker and Cowan, 1981), any trematode was found neither in the inner ear, the middle ear, the VIII cranial nerve, the pterygoids' sinus or the cranial sacs neither in animal CET 824 nor in other BW including in this study. Thus, in accordance, it remains unsolved, how the parasite reached the brain in the animal of our study.

Incoordination, loss of equilibrium, and echolocation dysfunction are some of the clinical signs exhibited by the affected animals by brain nasitremiasis, highlighting the possible role of this trematode parasite in the stranding events due to alterations in echolocation and balance orientation (Dailey and Walker, 1978; Lewis and Berry, 1988; Morimitsu et al., 1992; Morimitsu et al., 1987; Morimitsu et al., 1986; O'Shea et al., 1991). In that sense, despite being found death, a live stranding event was suspected in the animal from our study according to the extremely freshness of the carcass and several necropsy findings, such as skin erosive lesions, congestion of acoustic submandibular fat and melon and mild-moderate acute myodegenerative changes in cardiac muscle, as previously described (Herráez et al., 2013; Herráez et al., 2007). The severe lesions of the brain, produced by the widely distributed nasitremiasis, might could affect the foraging and diving pattern of the animal and potentially cause its stranding.

The animal of our study displayed an extremely poor body condition and herpesvirus co-infection, which could indicate a debilitated and immunocompromised state. *Nasitrema* spp. co-infections with other pathogens have been previously reported in cetaceans: in a captive Atlantic bottlenose dolphin with a bacterial and mycotic saculitis (Wright et al., 1979), in a striped dolphin with pneumonia produced by *Vibrio damsela* (O'Shea et al., 1991), in a Pacific white-side dolphin with *Edwardsiella tarda* septicaemia (Lewis and Berry, 1988), and in a melon-headed whale from which *Edwardsiella* sp. was recovered from purulent material of brain lesions (Lewis and Berry, 1988; Phillips and Suepaul, 2017).

Different species within the genus *Nasitrema* have been detected in a wide range of odontocete cetacean (Table 3). In our study, the parasitic identification mostly relied on the molecular and phylogenetic analyses, as most of the internal parasitic structures were indistinguishable. The remaining morphohistological features displayed by the parasite from our study (specifically the size of the adult fluke and eggs) are in concordance with those of different species of the genus *Nasitrema;* specifically, *dalli*, *delphini*, *globicephalae* (Ebert and Valente, 2013; Kikuchi et al., 1987; Neiland et al., 1970) and *attenuata* (Kumar et al., 1975; Lim et al., 2016). However, the sequence obtained from this study presented the highest nucleotide similarity with a sequence of *N. delphini* detected in a common dolphin stranded in the same geographic area as the animal from our study (Canary Islands) (Fraija-Fernández et al., 2016). The presence of the *N. delphini* had been additionally reported in two common bottlenose dolphins also stranded in the Canary Islands (morphologically or molecularly identified) (Díaz-Delgado et al., 2018; NIH: National Library of Medicine; National Center for Biotechnology Information, 2021). Apart from this geographical area, this species within the genus *Nasitrema* had been only morphologically identified in the common dolphin species from southern California (Dailey and Walker, 1978; Neiland et al., 1970).

Table 3
Summary of the detected *Nasitrema* species in cetaceans.

Nasitrema spp.	Cetacean host	References
N. delphini	Bottlenose dolphin	(Díaz-Delgado et al., 2018; NIH: National Library of Medicine; National Center for Biotechnology Information, 2021)
	Common dolphin	(Dailey and Walker, 1978; Fraija-Fernández et al., 2016; Neiland et al., 1970)
N. globicephalae	Common dolphin	(Dailey and Walker, 1978)
	Long-finned pilot whale	(Fernandez et al., 1998a; Fraija-Fernández et al., 2016)
	Rough-toothed dolphin	(Ebert and Valente, 2013)
	Striped dolphin	(Oliveira et al., 2011)
	Pantropical spotted dolphin	(Oliveira et al., 2011)
	Short-finned pilot whale	(Neiland et al., 1970)
	False killer whale	(Neiland et al., 1970)
	Pacific white-side dolphin	(Dailey and Walker, 1978)
	Northern right whale dolphin	(Dailey and Walker, 1978; Neiland et al., 1970)
N. attenuata	Long-beaked common dolphin	(Lim et al., 2016)
	Indo-Pacific Bottlenose Dolphin (*Tursiops aduncus*)	(Kleinertz et al., 2014)
	Guiana dolphin	(Ebert and Valente, 2013)
	Rough-toothed dolphin	(Ebert and Valente, 2013)
	False killer whale	(Neiland et al., 1970)
	Bottlenose dolphin	(Kumar et al., 1975)
N. gondo	Melon-headed whale	(Morimitsu et al., 1986)
	Long-finned pilot whale	(Morimitsu et al., 1987; Yamaguti, 1951)
	False killer whale	(Morimitsu et al., 1987)
	Risso's Dolphin	(Morimitsu et al., 1992)
N. spathulatum	Narrow-ridged finless porpoise	(Shiozaki and Amano, 2017)
	Finless porpoise	(Ozaki, 1935)
N. sunameri	Narrow-ridged finless porpoise	(Shiozaki and Amano, 2017)
	Finless porpoise	(Yamaguti, 1951)
N. lanceolata	Long-finned pilot whale	(Neiland et al., 1970)
N. lagenorhynchus	Pacific white-side dolphin	(Kikuchi et al., 1987)
N. dalli	Dall's porpoise	(Dailey and Walker, 1978; Yamaguti, 1951)
N. stenosoma	Common dolphin	(Dailey and Walker, 1978)

5. Conclusion

Nasitremiasis have been reported in several cetacean species, with the presence of intralesional trematodes in lungs, pterygoids sinus, middle and inner ear, VIII cranial nerve and brain. Although this is, to the authors' best knowledge, the first description of *Nasitrema* sp. (molecularly identified as *N. delphini*) in a member of the *Ziphiidae* family (namely, a Blainville 's BW).

Due to the severe clinical signs (incoordination, loss of equilibrium, and echolocation dysfunction) that may be derived from tissues damage caused by the presence of adult flukes or their eggs in the cephalic region, a careful postmortem diagnostic (including a deep inspection of the cranial structures) is needed to increase the potential diagnostic of the disease.

Additionally, further investigation is required to a better understanding of the biology, epidemiology, and pathogenesis of *Nasitrema* spp. in BWs and other cetacean species.

Institutional review board statement

The necropsy of the animal from this study was possible thanks to the permission for the management of stranded cetaceans conceded by the Spanish Ministry of the Environment (SGPM/BDM/AUTSPP/70/2019). Furthermore, neither animal was sacrificed and not experiments were performed with live animals, so ethical review and approval were waived.

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CRediT authorship contribution statement

Idaira Felipe-Jiménez: Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Antonio Fernández:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Funding acquisition. **Yara Bernaldo de Quirós:** Methodology, Formal analysis. **Marina Arregui-Gil:** Methodology, Formal analysis. **Raquel Puig-Lozano:** Methodology, Formal analysis. **Eva Sierra:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The *Nasitrema delphini* obtained sequence from a Blainville beaked whale (*Mesoplodon densirostris*) stranded in the Canary Islands has been deposited in GenBank (under accession number: OP480061).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2023.03.009.

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