

Epidemiological and Pathological Analysis of Cranial Crassicaudasis in Stranded Cetaceans in the Canary Islands

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Abstract

This retrospective study focuses on 55 out of 852 (6.45%) cetaceans stranded in the Canary Islands over a 25-year period, from 1999 to 2023, which presented parasitic infestation of the pterygoid sacs, which could be an important morbidity factor due to the intimate and close connection between the air sacs, the tympanic bullae, and the acoustic nerves. From these, nematodes of the genus *Crassicauda* were observed in 39 animals (71%), with different levels of severity (classified into mild, moderate and severe) and with our without co-infection with other nematodes (*Stenurus* spp.) and/or trematodes (*Nasitrema* spp.). The epidemiological study allowed us to identify that the incidence was higher in *Stenella frontalis* (26%) and *Grampus griseus* (21%) species in addition with more severe level of parasitization. The prevalence was higher in the juvenile/subadults age group (55%), while regarding the sex, we found the same proportion in males and females.

In addition, due to the lack of gross descriptions in most cases of parasitic sinusitis, we aimed to histologically characterize the effect of parasitic infestation on the tissues of the pterygoid sacs. For this purpose, histological samples from apparently non parasitized pterygoid sacs from 2 cetaceans were analysed; describing the normal histological features of the sacs, which were compared with the histological samples from 3 cetaceans that had *Crassicauda* sp. in the pterygoid sacs at the time of necropsy. As a result of the parasitization, a mild inflammatory reaction, hyperkeratosis, and desquamation of the pseudostratified columnar epithelium was observed.

Since migration of nematode parasites to the central nervous system (CNS) can occur under certain circumstances, causing damage or serving as a vector of pathogenic microorganisms (e.g. *Brucella* spp.), we looked for brain lesions and associatedpathogens described in their anatomopathological reports to relate them with the presence and level of parasitization by *Crassicauda* sp. in the pterygoid sacs. No relationship was found between the presence of *Crassicauda* sp. and any of the pathological agents (morbillivirus, herpesvirus, *Brucella* spp. and *Toxoplasma gondii*) detected in the CNS when the cetaceans were tested for.

Introduction

Theoretical fundament

The Canary Islands constitutes the place with the highest diversity of marine mammals throughout Europe. Currently, and according to the Biodiversity Database of the Canary Islands (BIOTA), there is a record of 31 species of cetaceans in the Canarian oceans.

Cetaceans can be affected by different human activities such as maritime traffic for the transportation of goods and people, the fishing industry, chemical pollution, tourist activities and noise pollution, among others. Additionally, they face adverse environmental conditions, including exposure to chemical contaminants, algal biotoxins, and pathogenic agents (viral, bacterial, fungal, parasitic), all of which can contribute to instances of stranding (Arbelo et al., 2013; Diaz-Delgado et al., 2018; Puig-Lozano et al., 2020a,2020b). Parasitic infections are also common among wild cetaceans, which are often found as incidental findings during necropsies. Thanks to various authors, it is known that parasites impact population dynamics (Lambertsen,1992), suggesting that parasite infestation may lead to an increase in mortality. Parasitization can also indicates alterations in the health of cetacean populations and marine ecosystem (Aznar et al., 2005; Van Bressem et al., 2009) and have ecological implications, acting as biomarkers to unveil habitat utilization and social structure (Aznar et al., 1995; Balbuena and Raga, 1994).

In this study, we aimed to determine whether the severity of the lesions caused by *Crassicauda* sp. in the pterygoid sacs of cetaceans is related to the level of infestation and if the CNS lesions reported in those animals could be associated to the parasitic sinusitis or to the infection by pathogenic agents. Moreover, the role of cranial crassicaudasis in the stranding events is considered.

Background and current status

Crassicauda

Nematodes infesting tissues and organs of cetaceans are most commonly members of the family *Tetrameridae*, a family consisting of two genera of large nematodes, *Crassicauda* and *Placentonema*. The genus *Crassicauda* is included in the order Spirurida (Tetrameridae). This parasite has been documented in a wide variety of tissues of their hosts and has been reported as an endemic disease in some species of cetaceans (Lambertsen 1992). Furthermore, previous studies have established a directly linked

between the infection and the morbidity and mortality in parasitized mysticetes and odontocetes cetaceans (Lambertsen 1985).

Fourteen different species have been described within the genus *Crassicauda* Nemys eds. (2024). Theses parasites have been documented to cause a deep and significant damage to surrounding tissues (Van Bressem et al., 2006). Although they can be found in a variety of organs and body tissues, such as the vasculature, urogenital organs, placenta, mammary glands, cranial sinuses, lungs, musculature, and beneath the subcutaneous fat layer (Gibson et al., 1998), there is an apparaently specific tissular localization for the different crassicaudid species. So, accordingly, *C. fuelleborni, C. duguyi* (probably a synonym of *C. magna*), and *C. magna* can be find in the musculature, *C. grampicola* is usually present in the pterygoid bone, mammary glands and subcutaneous adipose tissue, and *C. boopis* (synonym of *C. pacifica*), *C. crassicauda*, *C. anthonyi*, *C. delamureana*, *C. costata*, *C. bennetti*, *C. giliakiana*, and *C. carbonelli* have been described in the urogenital system (Caneiro, 2019).

The biological cycle of *Crassicauda* spp. is not well understood because it is largely unknown, although it has been proposed a transmission via intermediate hosts (e. g., crustaceans, cephalopods, fishes) involved in the diet of cetaceans (Díaz-Delgado et al., 2016). In this sense, the prevalence of *Crassicauda* in cetaceans could be associated with certain species of fish and cephalopods (Lehnert *et al.*, 2014; Kumagai et al., 2023). Other proposed transmission pathways for crassicaudids include transplacental and through milk or urine (Geraci et al., 1978; Lambertsen, 1986; Suárez-Santana et al., 2018) due in part to the detection of eggs or developing larvae in milk, urine or tissue (Dailey, 2007).

Little information is currently available about the prevalence of *Crassicauda* spp. across species, within species across sex, and within sex across length and life history categories. However, contrary to expectations, Keenan-Bateman and colleagues (2016) recently observed a positive correlation between *Kogia* spp. infection and female total length, with a higher prevalence among females than males. This could be due to the lowered immune system of females during the reproductive stage (Domit et al., 2018). In a similar way, Van Bressem et al., (2020) found a significant difference in lesion prevalence by age, with a higher incidence in immature versus mature animals within the *Tursiops aduncus* and *Sousa plumbea* species.

Crassicauda spp. usually infest the cranial sinuses of members of the Delphinidae (Dailey & Perrin 1973), causing round, lytic bone lesions with a basket-like appearance typically associated with this parasite (Van Bressem et al 2006). Most of this took place as eroded areas in the frontalorbitosphenoid and pterygoid regions, with nasal and orbitary perforations extending into the brain cavity. These irreversible lesions occur because of the migration of the nematodes, since they release excretory and secretory enzymes that play a fundamental role in invading host tissues, resulting in degradation of glucosaminoglycans and chondroitin sulfates (Pascual et al., .2000). It is known that *Crassicauda* spp. can lead to the inflammation of the mucosa, purulent sinusitis, and osteitis (Raga et al., 1982) which can additionally affect echolocation (Dhermain et al. 2002) or facilitate bacterial and viral infections.

It has been also recognized that nematodes, including different crassicaudid species, can cause lesions affecting the CNS, with necrotizing encephalitis, multiple parasitic tracts, and intralesional nematodes as the main described lesions (Martin et al., 1970; Perrin and Powers, 1980; Cowan et al., 1986; Zucca et al., 2004). In addition, it has been previously described that helminth nematodes are able to transmit *Brucella* spp. between marine mammalian hosts (Dawson et al., 2008). This bacterium has been detected, through immunohistochemical techniques, in the uterus and the intestinal lumen of lungworms; which was confirmed by electron microscopy, demonstrating the presence of intrauterine Brucella sp. in adult lungworms, predominantly located in the membranes that separate and surround the developing larvae (Garner et al., 1997). Brucella spp. are Gram-negative bacteria that can cause a wide range of pathogenic lesions in mammals, such as abortions, male infertility, cardiopathies, and bone and skin lesions. However, there have even been cases where Brucella ceti has led to specific the most representative lesions CNS infections. with being meningitis, meningoencephalitis, or meningoencephalomyelitis (Davison et al., 2009). Differential etiological diagnosis for these neuropathological lesions in marine mammals include: morbillivirus, herpesvirus and Toxoplasma gondii, among others (Sierra et al., 2020).

The only identified species of *Crassicauda* in the pterygoid sacs is *C. grampicola*, which has been reported infecting the nasal cavity, the tympanic bullae and the pterygoid and air sinuses of the Risso's dolpin species (*Grampus griseus*) (Zucca et al., 2004). If we focus on morphological features, *C. grampicola* has anterior end narrow, somewhat compressed laterally, mouth opening a dorsoventral slit, with two lateral lips,

each of them has a papilla-like projection, four submedian cephalic papillae and two lateral amphids. There are some differences between both of them: in female vulva opening ventrally, anus subterminal, has pair of phasmids near tail end, and the eggs have a thick-shelled, larvated; and in male caudal end coiled spirally, moreover males had 11-15 pairs of caudal papillae; 3-4 pairs preanal and 8-11 pairs postanal. Near the end of the tail has a pair of phasmids (Araki et al, 1994; Raga, 1987).

Pterygoid sacs

Cetaceans have three types of distinct air sinuses or sacs: nasal sinuses, pterygoid sinuses, and laryngeal sinuses. These sacs could play a role in gas exchange, thermoregulation, resonance, and skeletal pneumatization (Reidenberg y Laitman, 2008). In this study, we will focus on the pterygoid sacs. These sacs represent a continuation of the Eustachian tube, surrounding the middle and inner ear, allowing for directional hearing. Some researchers contend that fluctuations in sinus volume enable the adjustment of pressure on the ear bones, thereby offsetting volume loss of air sacs during immersion. This aids in preserving hydrostatic equilibrium and ensuring stability in sound transmission to the ear, consequently enhancing the efficacy of the auditory system (Fraser & Purves, 1960).

Figure. 1. Odontocete head PT, pterygoid sac; B, blowhole; L, laryngeal sacs; N, nasofrontal sac; PM, premaxillary sac; V, vestibular sac. Reidenberg et al., 2008.

Both of pterygoid sacs located ventral to the skull connect the pharynx and middle ear cavities (Reidenberg y Laitman, 2008). It is important to note that they are not contained by bony walls (Costidis & Rommel 2012) and are located beneath the pterygoid bone. They extend superiorly under the palatine, maxillary, and frontal bones,

and caudally under the occipital and temporal bones, surround the ear region. Caudally, they help in forming of the peribullar space surrounding the ear. Medially, they are bordered by the pterygoid plates of the sphenoid bone and connected with Pterygoid, Palatine, Basisphenoids an Alisphenoid bones. In numerous species, there is absence of bone surrounding sections of the ventral and lateral aspects of the sacs; while in other cases they can appear ossified at the level of the sphenoid bone. The pterygoid sacs have various diverticula or lobes: the pre- and postorbital lobes, located rostrally and caudally respectively in the orbital area of the skull, and the hamular lobe, which is situated between the lateral and medial laminae of the pterygoid hamulus of the pterygoid bone (Fraser y Purves, 1960). These sacs and are coated with an intricate network of vessels or blood sinuses (Reidenberg y Laitman, 2008).

Although the available information is limited, Cowan (1996) describes the histology of the sacs as a respiratory epithelium of simple columnar nature over lax connective tissue containing blood vessels of considerable size.

The prevalence of *Crassicauda* spp. in 5 cetacean species from SouthAmerica range from 26.5 to 0% (VanBressem et al., 2007) (Figure 2).

AREA AND SPECIES	HABITAT	SAMPLING	BONE			LYTIC LESIONS		
		PERIOD	REMAINS	N	CRASSICAUDA SPP.	OSTEOMYELITIS	OSTEOLYSIS	PLD
Peru	-			-	-			
Delphinus capensis	Offshore/neritic	1985-2000	Skulls	103	26.5%ª	1.9%	4.9%	1%
Lagenorhynchus obscurus	Offshore/neritic	1984-2001	Skulls	46	4.4%	0%	2.1%	8.7%
Phocoena spinipinnis	Inshore/neritic	1985-1999	Skulls	37	0%	2.7%	5.4%	2.7%
Tursiops truncatus	Inshore/neritic	1984-2000	Skulls	26	7.7%	7.7%	7.7%	15.4%
Tursiops truncatus	Offshore/pelagic	1984-2000	Skulls	42	26.2%	4.8%	9.5%	16.7% ^b
Brazil								
Sotalia guianensis	Inshore/estuarine	1987-1998	Skulls	53	1.9%¢	1.9%	0%	15.1%
Sotalia guianensis	Inshore/estuarine	1987-1998	Axia1 skeleton	53	-	7.6%	0%	-
Sotalia guianensis	Inshore/estuarine	2001-2006	Axial skeletons	31	-	3.2%	0%	-
Tursiops truncatus	Inshore & offshore	2001-2005	Skulls	8	25%	0%	0%	indet.
Tursiops truncatus	Inshore & offshore	2001-2005	Skeletons	8	-	12.5%	12.5%	-
Venezuela								
Sotalia guianensis	Inshore/estuarine	1991-2003	Skulls	46	0%	2.2%	6.5%	10.9%

Table 7. Prevalence of lytic lesions in small odontocetes from Peru, Brazil and Venezuela.

N = total number of specimens, PLD= periodontal lytic disease; indet.=indeterminate, *N =98; b this percentage may be an underestimate; ^c possible *Crassicauda* spp. infestation in one *Sotalia guianensis*.

Figure 2. Prevalence of *Crassicauda* sp. in 5 different cetacean species from Peru, Brazil and Venezuela. VanBressem et al., 2007.

Approach and Objectives

This retrospective study aims to investigate the prevalence and the pathological consequences of *Crassicauda* spp. infestation in stranded cetaceans in the Canary Islands, focusing specifically on the lesions produced in the skull and pterygoid sacs (cranial crassicaudasis). In addition, we will also intent to establish a possible correlation between the pterygoid infestation with *Crassicauda* sp. and the neuropathological findings observed in the parasitized animals.

The specific objectives are:

- To determine the prevalence of cranial crassicaudasis (in total, by age group and by sex) in stranded cetaceans in the Canary Islands.
- To correlate the level of infestation of *Crassicauda* sp. in pterygoid sacs with the histopathological lesions in the same anatomical location.
- To correlate the pterygoid infestation with *Crassicauda* sp. with the histopathological lesions observed in the central nervous system.
- To determine the relevance of cranial crassicaudasis in the causes of death and strandings in cetaceans from the Canary Islands.

Materials and methods

Post-mortem examinations, following standardized protocols (Kuiken & García-Hartmann 1991), were performed on 852 stranded cetaceans in the Canary Islands during the period of study (1999 – 2023). During necropsy, all the major organs and lesions were analysed and samples were obtained for subsequent anatomopathological study. Parasites found in a good state of conservation, whenever possible, were kept in 70% ethanol for identification. For morphological identification and better observation of the internal structures of nematodes and acanthocephalans, they were clarified for 24-48 hours with lactophenol. For observation, a magnifying glass and mostly an optical microscope equipped with a monitor that converts parasite length in µm with Motic® Images Plus 2.0 ML software and Moticam digital camera were used. Parasites were identified according to (Dailey & Gilmartin, 1980; Delyamure, 1955; Gibson, 2005).

Molecular identification was performed in one nematode. Genomic DNA was extracted from an adult nematode recovered from the pterygoid sac of a juvenile Atlantic spotted

dolphin (Stenella frontalis) (CET1287) by means of the kit Quick-DNA/RNATM MagBead according to manufacturer's instructions (Zymo Research). A conventional PCR amplifying the ITS2 region of ribosomal DNA was performed (crassicauda-5.8S-F 5' - TACTCTTAGCGGTGGATCAC - 3', crassicauda-28S-R; 5' - AATCAC-GACTGAGCTGAGGT -3), as previously described with some modifications to adapt the protocol to the conditions of the molecular laboratory at University Institute of Animal Health and Food Safet (IUSA) (Kumagai et al., 2023). Horizontal gel electrophoresis was performed in 2% agarose containing GelRed® (Biotium, Inc., CA, United States) for 5 µL of the obtained amplicons. Purification of PCR products was carried out using a Real Clean Spin kit (REAL®, Durviz, S.L., Valencia, Spain) to perform bidirectional sequencing (the Sanger method) (Secugen S.L., Madrid, Spain). The Crassicauda sp. sequences obtained (excluding primers) were aligned through the ClustalW algorithm using MEGA11 software (Pennsylvania, PA, United States) (Kumar et al., 2018; Tamura et al., 2011) to attain a consensus sequence. 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.

Cetaceans with presence of parasites in the pterygoid sacs according to the anatomopathological reports (APRs) of stranded cetaceans in the Canary Islands performed by the "Red Canaria de Varamientos de Cetáceos (RCV)," established by the IUSA at the University of Las Palmas de Gran Canaria (ULPGC) were retrospectively selected for this study. Once these animals were identified, we tried to discriminate between trematodes (*Nasitrema* spp.) and nematodes (*Stenurus* spp. and *Crassicauda* spp.) according to the APRs and the images taken during necropsies. Severity of *Crassicauda* spp. infestation was established according to the number of parasites described/observed in the pterygoid sacs: mild (1-2), moderate (3-4) and severe (>4).

An epidemiological investigation was conducted aiming to identify predisposing or risk factors, e. g. some species for which there is some concern that the pterygoid sac infestation may be important in the conservation of this species. The prevalence of cranial crassicaudasis (in total, by species, by age group and by sex, etc.) in stranded cetaceans in the Canary Islands was determined according to the total number of animal in the period of the study and the epidemiology of the stranding and the life history data (systematically recorded during *post-mortem* examination): species, sex, age class,

nutritional condition, decomposition code, stranding condition (active/passive stranding), stranding date and stranding location (island). Age class was determined based on total body length and sexual maturity, assessed through morphological and histological examination of the gonads (Geraci & Lounsbury, 2005; Perrin et al., 2009). This resulted in the following categories: calf, juvenile - subadult, and adult. Nutritional condition was estimated based on the external body shape (the degree of epaxial muscle concavity or convexity, nuchal depression, visualization of ribs and vertebral transverse processes, as well as the presence or absence of nuchal and epicardial fat) in very poor, poor, fair and good body condition (Joblon et al., 2014). For decomposition code, five codes were applied: code 1 (very fresh), code 2 (fresh), code 3 (moderate autolysis), code 4 (advanced autolysis), or code 5 (very advanced autolysis) (IJsseldijk et al., 2019).

Gross lesions from pterygoid sacs were compiled from the APRs; while the histopathological analysis of the pterygoid sacs was performed on 5 parasitized individuals and 2 individuals without evidence of cranial crassicaudasis, by means of two histochemical techniques: Hematoxylin and Eosin (H&E), and phosphotungstic acid hematoxylin (PTAH).

Neuropathological findings were systematically recorded from each APR of each animal. In some parasitized animals with brain inflammatory lesions, results for the molecular identification of the involved pathogen (previously performed at the Molecular Pathology Laboratory at the IUSA) were compiled.

For the epidemiological study, a chi-square (χ^2) test has been used to determine whether there are significant differences in the presence of parasites based on species, age and sex. P-values were estimated by means of confidence intervals at 95%. Statistical significance was set at p < .05. IBM SPSS Statistics version 23.0 software was used for statistical analysis.

Results

1. Cetaceans with parasites in the pterygoid sacs

Eight hundred and fifty-two cetaceans stranded in the Canary Islands during the period of the study and were submitted to a standardized necropsy, with an annual average of strandings of 35 cetaceans per year (Figure 3).

Figure 3. Number of stranded cetaceans per year during the period of study (1999-2023).

The cetacean species that most frequently stranded during the period of study was Atlantic spotted dolphin, representing 21%, followed by striped dolphin (*Stenella coeruleoalba*) with 20%, common dolphin (*Delphinus delphis*) with 11%, bottlenose dolphin (*Tursiops truncates*) and short-finned pilot whale (*Globicephala macrorhynchus*) with 7%; respectively, and Cuvier's beaked whale (*Ziphius cavirostris*), pygmy sperm whale (*Kogia breviceps*) and sperm whale (*Physeter microcephalus*) with 5%. The remaining stranded species each accounted for less than 5% of the strandings (Figure 4).

Figure 4. Number of stranded cetaceans by species between 1999-2023.

During necropsies, parasites (nematodes and/or trematodes) were found in the pterygoid sacs of 55 out of the 852 analysed cetaceans, representing 6.45%, according to the IUSA database (Supplementary Table 1, Supplementary Table 2 and Supplementary Table 3). From these, a varied distribution among several species (n=9) was observed: Atlantic spotted dolphin 17, Risso's Dolphin (*Grampus griseus*) 10, bottlenose dolphin 8, short-finned pilot whale 8, roughtothed dolphin (*Steno bredanensis*) 4, common dolphin 3, striped dolphin 3, Fraser's dolphin (*Lagenodelphis hosei*) 1, and pygmy sperm whale 1 (Figure 5).

Figure 5. Number of stranded cetaceans by species with parasites in the pterygoid sacs.

Most of the cetaceans with parasitic infestation in their pterygoid sacs were *Stenella frontalis* and *Grampus griseus*, representing 31% and 18%, respectively.

Thirty animals were males and 24 females (only one was pregnant) and the sex could not be identified in one animal. Most of the animals belonged to the juvenile/subadult age group (32/55; 58.2%), followed by the adults (14/55; 25.4%) and the calves (7/55; 12.7%). In two animals, the age class could not be determined.

2. *Post-mortem* examination: type of stranding, decomposition code and nutritional score

Of the cetaceans stranded in the Canary Islands during the period of study, 83% (n=707) stranded passively and 17% (n=145) stranded actively. The majority of strandings of the 55 cetaceans with parasitic infestation of the pterygoid sacs

were passive, representing 87.27%, compared to active strandings with 12.75% (Figure 6). The decomposition code of these 55 cetaceans at the time of necropsy were: fresh at 38.18%, followed by moderate autolysis at 27.27%, very fresh 12.72%, advanced autolysis 12.18% and very advanced autolysis 3.63% (Figure 7). Regarding the nutritional score of cetaceans with parasites in their pterygoid sacs: 36.36% (n=20) were found in a fair state, 27.27% (n=15) were considered poor, 12.77% (n=9) were in good nutritional status, and 7.25% (n=4) in very poor. The nutritional status could not be estimated (ND) in 12.77% (n=7) of these 55 stranded cetaceans (Figure 8).

Figure 6. Stranding condition. Stranding dead (passive) or alive (active).

Figure 7. Decomposition code: very fresh (1), fresh (2), moderate autolysis (3), advance autolysis (4) and very advance autolysis (5).

Figure 8 Nutritional score. ND = non determined.

3. Cetaceans with Crassicauda sp. in the pterygoid sacs

From the 55 cetaceans initially identified with parasites in the pterygoid sacs, we subsequently proceeded to exclude those individuals in which *Crassicauda* sp. was no described within the APRs neither identified during the visualization of the images but taking into account the possibility of coinfection with other nematodes (*Stenurus* spp.) and/or trematodes (*Nasitrema* spp.). As a result, a total of 39 (23 with only *Crassicauda* sp., 8 co-infested with *Stenurus* sp. and 8 co-infested with *Nasitrema* sp.) out of 55 (71%) cetaceans were selected,

representing the 4.77% of the total stranded cetaceans in the period of study (Table 1). In 14 cetaceans, the parasites present in the pterygoid sacs could not be included in any taxonomic level due to the lack of available information.

Genus	Nº	Percentage
Crassicauda sp.	23	42%
Crassicauda sp. and Stenurus sp.	8	14,5%
Crassicauda sp. and Nasitrema sp.	8	14,5%
Nasitrema sp.	1	2%
Anisakis	1	2%
ND	14	25%

Table 1. Type of parasites found in the pterygoid sacs of the stranded cetaceans during the period of study. ND= non determined.

4. Severity of cranial crassicaudasis

According to the level of parasitic infestation, 15/39 (38.5%) had mild infestation, 13/39 (33.3%) moderate, 6/39 (15.38%) severe, and in 5/39 (12.8%) animals the level of infestation could not be determined (Figure 9).

Figure 9. Severity of cranial crassicaudasis. Photos taken by IUSA during the necropsies (1999-2023). A) Mild infestation (CET 456 Grampus griseus); B) Mild co-infestation by Crassicauda sp. and Stenurus sp. (CET 533 Grampus griseus); C) and D) Moderate co-infestation by Crassicauda sp. and Nasitrema sp. (CET 305 Tursiops truncatus); E) Moderate infestation (CET 1287 Stenella frontalis); F) Skull eroded by parasitic migration tracks caused by Crassicauda sp. (CET 1287 Stenella frontalis); G) Severe infestation with congestive

pterygoid sac (CET 1213 *Grampus griseus*); **H**) Severe co-infestation by *Crassicauda* sp. and *Stenurus* sp. (CET 534 *Grampus griseus*).

In addition, the severity of cranial crassicaudasis was classified as: mild, moderate, and severe according to the cetacean species and the presence of *Crassicauda* sp. alone or in combination with other nematodes or trematodes (Supplementary Table 4) (Figure 10).

Figure 10. Panel of graphs by species illustrating the incidence of *Crassicauda* sp. based on the level of infestation and the presence of coinfection.

These data suggest that *Grampus griseus* is more susceptible to severe infestations and coinfestation compared to *Stenella frontalis*, which tends to experience milder

infestations without the simultaneous presence of different types of parasites. Regarding the species *Lagenodelphis hosei*, *Stenella coeruleoalba*, and *Delphinus delphis*; infestations were mild and there was no presence of co-infestation with other type of parasites. On the other hand, in the species *Tursiops truncatus*, co-infestation can occur but parasitization is usually not severe. In *Steno bredanensis*, co-infestation can also occur and infestation use to be moderate or mild. In *Globicephala macrorhynchus*, the infestation ranges from moderate to severe if only the species *Crassicauda* is present, while milder infestations occur in case of co-infestation.

In 10 out of the 40 cases, *Crassicauda grampicola* was identified as the causative agent of the parasitic infestation, accounting for 25% of the cases. The identification was carried out through the morphological observation of the parasite, following the previously described characteristics. It was present in the cetaceans: CET 418, CET 446, CET 450, CET 456, CET 594, CET 751, CET 1057, CET 1057, CET 1059, CET 1213, and CET 1287 (also identified by molecular methods) (Supplementary Table 3).

5. Epidemiological study Species -Sex – Age

As a result of the epidemiological study regarding the species, sex and age of the animals that presented cranial crassicaudasis, we found a higher number of cetaceans within the *Stenella frontalis* and *Grampus griseus* species, and the juvenile/subadults age group of animals (Figure 11). However, a similar proportion was found for males and females (Figure 12). Statistical difference was only observed concerning the species (P<0.005.) (Supplementary Table 6).

Figure 11 Cetaceans classified by age: calf, juvenile/subadult, adult, and undetermined.

Figure 12. Cetaceans classified by sex: Male, Female and Not determined.

Year

The higher number of animals with cranial crassicaudasis was recorded in the years 2008 and 2020, with a total of 5 cases each year (Figure 13).

Figure 13. Cetaceans stranded in the Canary Islands with *Crassicauda* sp. in the pterygoid sacs between 1999 and 2023.

Stranding location

The cetaceans in this study were primarily found stranded on the main islands of Gran Canaria and Tenerife, with Fuerteventura following closely (Figure 14).

Figure 14. Location of stranding for the cetaceans with *Crassicauda* sp. in the pterygoid sacs between 1999-2023.

6. Gross pathology in the pterygoid sacs

There are no gross descriptions for 33 out of the 39 cetaceans in our study with cranial crassicaudasis (Supplementary Table 5). Regarding the remaining 6 cetaceans, bone lesions (eroded bone and bone proliferation) (Figure 15) were described in 3 animals (CET 94, CET 479 and CET 1287), mucosal lesions (in the form of patches of white epithelium) in 2 animals (CET 94 and CET 1056) and congestion and haemorrhages in 3 animals (CET 395, CET 549 and CET 1287) (Figure 16).

Figure 15. Bone lesions during gross examination. A) CET 94. Eroded bone B) CET 479. Bone proliferation.

Figure 16. Macroscopic lesions in pterygoid sacs in cetaceans with Crassicauda sp.

7. Microscopic lesions in the pterygoid sacs

- Histology of the pterygoid sacs

In two animals with no macroscopic evidence of cranial crassicaudasis, the sacs were lined by a pseudostratified epithelium that regularly invaginate into the mesenchyme. A muscular layer was observed beneath the mucosa (Figures 17 and 18).

Figure 17. Panel Cetacean 1037/21. A) x40 (H&E) and B) x 10 (H&E) The epithelium is shown at different magnifications, with the respiratory-type epithelium present in the sacs being a pseudostratified columnar epithelium. C) (PTAH) x 4. The natural conformation is shown, with the invagination indicated by an arrow, the muscular layer marked with a dot, and the venous and arterial network that forms the sacs visible. D) x 20 (PTAH). A higher magnification of the invaginated epithelium within the submucosa.

Figure 18. Cetacean 132/10. Image A) x20 (PTAH) and B) x40 (PTAH) of the pseudostratified columnar epithelium.

- Histopathology of the pterygoid sacs.

The main histopathological lesions, when described (CET 94, CET 751, CET 963, CET 1218 and CET 1287) consisted on erosion and ulceration of the epithelium, hyperplasia and squamous metaplasia of the epithelium (with presence of keratin spicules), oedema and haemorrhages in the submucosa, intravascular leucocytosis and lymphoplasmacytic and histiocytic inflammatory reaction in the submucosa (Figures 19-21).

Figure 19. **CET 963 A)** The epithelium is eroded in some points. H&E, 20x. **B)** Lymphoplasmacytic inflammatory reaction within the submucosa associated to necrotic foci. H&E, 20x. **C)** Edema in the relatively loose connective tissue stroma (submucosa). PTAH, x10. **D)** Extensive hemorrhages within the submucosa. PTAH, x10.

Figure 20. CET 94: **A**) and **B**) Histologically, at 4x magnification (H&E and PTAH), a parasitic structure compatible with *Crassicauda sp.* is shown, indicated by an arrow (*), associated to erosion and ulceration of the epithelium; **C**) Lymphoplasmacytic and histiocytic inflammatory reaction within the submucosa in association with keratin spicules inside invaginated epithelium. H&E, 20x. **D**) and **E**) In both stains (H&E and PTAH at 20x) hyperkeratosis and squamous metaplasia of the epithelium is evident. **F**) Intravascular leukocytosis. PTAH, 40x.

Figure 21. CET 1218: A) At 4x magnification (H&E), the normal anatomical structure of the pterygoid sacs is displayed, with invaginations indicated by an asterisk, circulatory tract structures such as arteries

and veins marked with arrows, as well as trabecular spaces forming the venous plexus. Additionally, in the bottom right corner, muscle tissue is observed; **B**) A nerve can be observed at 20x (H&E) magnification, representing one of the possible routes utilized by nematodes during migration inside their hosts. **C**) Erythrocyte extravasation can be observed. H&E, 20x and **D**) Lymphoplasmacytic and histiocytic inflammatory reaction in the submucosa. PTHA, 40x.

8. Nervous System lesions

During the study, morphological diagnoses of brain lesions from the 39 cetaceans with cranial crassicaudasis were compiled. Approximately 1/3 of the cetaceans with cranial crassicaudasis displayed inflammation in the brain. The diagnoses were grouped as: meningitis (15%), meningoencephalitis (13%), encephalitis (5%), apparently normal (49%) and non-inflammatory injuries (18%) (Figure 22). Congestion and/or micro/haemorrhages were detected in 6 animals. Moreover, 1 cetacean present leptomeningeal thickness (CET 751). According to the type of inflammatory infiltrate, meningoencephalitis was predominantly non suppurative (4/5), while meningitis and encephalitis were represented in similar proportion by non-suppurative infiltrates and other types of inflammatory cells (Figure 23).

Figure 22. Main brain pathologies described in the central nervous system of the 39 cetaceans with cranial crassicaudasis. NIL: Non-inflammatory lesions. AN: Apparently normal

Meningitis	N	Meningoencephalitis	N1	Encephalitis	N2
Non-suppurative	2	non-suppurative	4	Necrosuppurative	1
pyogranulomatous	2	Prurulent	1	Lymphoplasmacytic	1
	·				
No determinated	1				
Lymphotic	1				

Figure 23. Type of inflammation present in the Central Nervous system.

Regarding the level of infestation and the presence of brain lesions, we found that 60% of cetaceans with mild infestation, the 57.14% with moderate infestation and the 33% with severe infestation did not present brain lesions (Figure 24). A single cetacean could present both inflammatory and non-inflammatory lesions, so the figure 24 does not represent the total number of cetaceans per infestation level.

Five cetaceans whose infestation level was unknown were not considered, although two of them presented significant lesions.

Figure 24. Number of cetaceans with inflammatory and Non-inflammatory brain lesions according to the level of parasitization. AN: Apparently normal.

9. Etiological diagnosis

As a part of the routine laboratory diagnosis of infectious diseases, *Brucella* spp., Herpesvirus, Morbillivirus, and *Toxoplasma gondii* were test in the brain of some of the cetaceans with cranial crassicaudasis (Table 2). As a result, 3 animals were positives for *Brucella* sp. (CET 305, CET 618 and CET 1287) and 2 for Morbillivirus (CET 305 and CET 594).

Etiological	Positive	Negative	Non tested
<i>Brucella</i> s p.	3	4	32
HV	0	12	27
CeMV	2	12	25
T. gondii	0	0	39

Table 2. Test results for: *Brucella* sp., Herpesvirus (HV), Cetacean Morbillivirus (CeMV), and *Toxoplasma gondii* (*T. gondii*).

Discussion

The main objectives of the study was to characterize the gross and microscopic lesions found in the pterygoid sacs of cetaceans with different levels of parasitic sinusitis and to give approximative data about the prevalence among species, sex and age of the stranded cetaceans in the Canary Islands. As a result, we found that, during this 25-year period, the 6.5% of the cetaceans stranded in the Canary Islands presented parasitic sinusitis, and specifically, 4.6% presented cranial crassicaudasis. We have found Crassicauda sp. in the cranial region of 9 out 26 cetaceans species recorded stranded in the Canarian archipelago during the period of study. In relation to the species susceptibility to this parasitization, most cetaceans with Crassicauda sp. in their cranial region were Atlantic spotted dolphins and Risso's dolphins; results that were supported by the statistical analysis (there is a higher predisposition for at least two species). Stenella frontalis is the cetacean species with the highest number of strandings in the Canary Islands, during the period of study and presented a prevalence of Crassicauda sp. of 7.1%, similar to that for the Tursiops truncatus species in Venezuela for the period 1984-200 (7.7%). However, although Stenella coeruleoalba ranks second in terms of most strandings, our study only recorded 1 case of cranial crassicaudasis (CET 1054). In a similar way, only 3 out of the 33 strandings of Steno bradensis had Crassicauda sp. in the pterygoid sacs (CET 269, CET 430 and CET 446). Grampus griseus accounted for less than 5% of the total strandings; however, it was the second species with most animals presenting Crassicauda sp. in the cranial region, with a prevalence for the species of 38.5% (CET 431, CET 456, CET 533, CET 534, CET 549, CET 634, CET 751, CET 984, CET 1059 and CET 1213). Similar results had been reported by Zucca et al., (2004) and Raga et al., (1997) regarding the role of Crassicauda grampicola in the regulation of cetacean populations, especially in Grampus griseus. We have not detected Crassicauda sp. in the skulls and/or pterygoid sacs of any individuals from the remaining 17 species, despite the prevalence of strandings in the Canary Islands for some species such as Ziphius cavirostris and Physeter macrocephalus in the period of study is about the 5%.

The prevalence of cranial crassicaudiasis in cetaceans varies significantly by species and geographical areas. Studies conducted on Indo-Pacific bottlenose dolphins and Indian Ocean humpback dolphins off the coast of South Africa described a prevalence of 31.9% and 13%., respectively (Van Bressem et al., 2020). For other species and

regions, specific prevalence data are less available, but *Crassicauda* sp. infestations are generally noted in various odontocetes across different areas, including the North Atlantic and Mediterranean Sea. Further research is needed to map out the prevalence in a broader range of cetacean species and locations.

Regarding severity of parasitic infestation, our results showed that *Grampus griseus* species seems to be more susceptible to severe infestations compared to *Stenella frontalis* and the other host species from our study, as it has been previously described by Zucca et al. (2004).

Young animals also look more susceptible to parasitization, according to our results: a higher prevalence was observed in juveniles/subadults, accounting for 55.3%. It has been also reported that nematodes of the family *Tetrameridae* have been associated with severe and irreversible chronic skull lesions in various dolphin species, apparently more prevalent in young tropical spotted dolphins (*Stenella attenuate*) than in older animals (Dailey and Perrin, 1973). Van Bressem et al., (2020) also found that immature *Tursiops aduncus* were 4.6 times more likely to be affected by cranial crassicaudasis than adults, while for *Sousa plumbea*, the difference was 6.5-fold. These findings emphasize the importance of age as a critical factor in the prevalence of cranial crassicaudiasis, with immature dolphins being more affected due to their developing immune systems and skeletal structures.

Sexual dimorphism in immune responses can lead to differences in susceptibility and severity of infections across species. For instance, male cetaceans might experience a higher prevalence of parasitic infections due to testosterone's immunosuppressive effects, whereas females might exhibit different infection rates or severities due to hormonal influences such as estrogen and progesterone, which can modulate immune responses differently (Zuk et al., 2010). However, susceptibility to *Crassicauda* sp. doesn't seem to be influenced by sex, based on our findings and previous publications (Van Bressem et al., 2020). The cetaceans in this study were composed of 51% male and 46% female, with no significant difference between both sexes. Within the females, only one of the cetaceans with *Crassicauda* sp. was pregnant (CET 418), so it was not demonstrated that there was a higher prevalence in females than in males due to the decreased immune system during the reproductive stage, as previously suggested (Keenan-Bateman et al., 2016). However, more animals are needed to corroborate or not this hypothesis.

In most cases, *Crassicauda* sp. was identified within the pterygoid sacs of cetaceans from our study among the animals with parasitic sinusitis (70.9%), although coinfection with *Stenurus* sp. (8/39; 20.5%) and *Nasitrema* sp. (8/39; 20.5%) can occur. Only in 4% of the cases with parasites in the pterygoid sacs, the parasite was not identified as *Crassicauda* sp; although in 25% of the animals, the parasites identification could not be made. When the co-infection was by *Stenurus* sp., the parasitization was more present in *Grampus griseus* (n=4/8; 50%). When the coinfection was by *Nasitrema* sp., all the cases occurred in *Steno bredanensis* and *Tursiops truncatus*, without severe infection observed. We didn't find any case of triple co-infestation by *Crassicauda* sp., *Stenurus* sp. and *Nasitrema* sp.

It is well known that cranial crassicaudasis play a fundamental role in regulating cetacean populations, potentially causing natural mortality rate of 11-14% in some species (Perrin and Powers, 1980), often being identified as *Crassicauda grampicola* (Zucca et al., 2004, Raga et al., 1982, Kikuchi & Nakajima, 1996). Morphological and molecular identification of the nematodes causing cranial crassicaudasis in stranded cetaceans in the Canary Islands was possible in 1.1% of the cases from our study, being identified as *Crassicauda grampicola*.

Typical lesions in cranial crassicaudasis consist of purulent inflammation of the pterygoid mucosa with variable extension to the underlying bony structures causing osteomyelitis (Raga et al., 1982) and potential neuritis and/or inflammation of the adjacent neuroparenchyma. In our study, we only found gross pathological description in 6/39 (15.4%) cetaceans. Specifically, 3 animals (CET 94, CET 479, CET 1287) showed bone lesions, characterized by bone destruction or dissolution (osteolysis) and new bone formation, following the typical trabecular pattern, probably due to the parasitic damage mechanisms (Dailey & Perrin, 1973). Consequently, this may lead to significant inflammatory reactions, affecting echolocation (Dhermain et al., 2002), and facilitating infections caused by other opportunistic agents (bacteria and/or viruses) (Pascual et al., 2000). Two cetaceans (CET 94 and CET 1056) showed non detailed lesions in the mucosa of the sacs, although CET 94 showed a co-infection with Nasitrema sp. These trematodes have lateral spicules, which can cause damage by the mechanical action of the parasite migrating in the pterygoid sacs (Neiland K. A et al., 1970). Two cetaceans from our study (CET 395 and CET 549) showed haemorrhages and congestion, respectively. Prolonged parasitic infestation of the sacs has been

previously described as commonly associated with production of a bloody, brown to black mucoid secretion as well as ulcers and patches of white epithelium (Cowan, 1996).

Histologically, erosion of the epithelium with hyperkeratosis and squamous metaplasia and ulceration, associated with an inflammatory reaction in the submucosa and extravasation of erythrocytes were observed in the sacs of 3 cetaceans with parasitic sinusitis in our study. Although the literature relating to the normal histological appearance of the pterygoid sinuses is scarce, a study by Cowan (1996) describes ulcerative lesions and haemorrhages in the pterygoid sinuses because of trematode infestation, as well as the presence of squamous metaplasia due to mucosal irritation. Due to anatomical difficulties in visualizing and sampling the pterygoid sacs, routine sampling was not conducted in stranded cetaceans, resulting in a lack of knowledge regarding the natural conformation of these pterygoid sacs. This posed a challenge in identifying pathological findings. Additionally, in many cases, reports did not record either the number or type of parasites found, making it difficult to correlate the genera *Crassicauda, Nasitrema*, or *Stenurus* with lesions observed at both macroscopic and microscopic levels. More samples from animals with and without lesions should be analysed to better characterized lesions associated to this type or parasitization.

Perrin and Powers proposed in 1980 that future studies of *Crassicauda* sp. in dolphins should include a search for brain histopathology associated with bone lesions. In our study, we looked for CNS lesions in animals with cranial crassicaudasis regardless the presence or not of bone lesions (considering that gross pathology was only available for the 15.4% of cetaceans with parasitic sinusitis). As a result, the 15% of the cetacean with *Crassicauda* sp. in the cranial region presented meningitis (CET 168, CET 450, CET 534, CET 816, CET 1059 and CET 1145), 13% meningoencephalitis (CET 305, CET 456, CET 594, CET 618, and CET 1287) and o 5% presented encephalitis (CET 1103) with two type of lesions). These brain inflammatory lesions are non-specific and can be caused by a wide range of pathogens (viruses, bacteria, parasites, fungi), although characteristics patterns can be associated to each one (Sierra et al., 2020). From these 12 cetaceans with inflammatory brain lesions, one or two associated pathogens could be determined in 4 animals (33.3%). *Brucella* sp. was detected in 3 out of 7 (42.9%) tested samples from the 39 cetaceans with cranial crassicaudasis in our study. Meningoencephalitis is a common lesions of *Brucella ceti* in the CNS of

cetaceans (Davison et al., 2009), although parasitic lesions in the CNS can also lead to meningoencephalitis, especially related to *T. gondii*, and are frequently non-suppurative, but not exclusively, since helminths have also been widely recognized as affecting the CNS. We would highlight necrotizing encephalitis in the frontal brain with multiple parasitic tracts and intralesional nematodes (Martin et al., 1970). This nematode was presumably associated with the genus *Contracoecum*, although other later authors refuted this classification, considering the genus *Crassicauda* more likely (Cowan et al., 1986b), which has been associated with CNS lesions with relative frequency (Perrin et al., 1980; Zucca et al., 2004). However, this type of lesions can be caused by *Nasitrema* sp. resulting in encephalitis due to the damage caused by the parasitic pathway. If the cause is a virus, non-suppurative meningoencephalitis is frequently associated with morbillivirus and herpesvirus (Sierra et al., 2020). For the other 8 cetaceans, despite some pathogens have been excluded, more molecular analysis (including those to test the presence of DNA from *Crassicauda* sp. in brain samples) should be performed to try to identify the responsible agent of such lesions.

It has been proposed that lungworms carrying *Brucella* spp. can infect marine mammals (Dawson et al., 2008). *Brucella* sp. infection in the CNS of cetaceans is most diagnosed in *Stenella coeruleoalba* (Hernández-Mora et al., 2008) and *Delphinus delphis* (Davison et al., 2013). In this study, the only two *Delphinus delphis* and one *Stenella coeruleoalba* with cranial crassicaudasis that stranded in the Canary Island during the period of study (CET848, CET 1186, and CET 1054) were not tested for *Brucella* sp. because they didn't display inflammatory brain lesions. The three *Brucella* sp.-positive animals from our study belonged to the species *Stenella frontalis* (CET 618 and CET 1287) and *Tursiops truncatus* (CET 305), with CET 1287 also presenting erosive bone lesions caused by *Crassicauda grampicola*. This hight incidence (3/12; 25%) of neurobrucelosis in stranded cetaceans in the Canary Islands with cranial crassicaudasis and brain lesions should be considered, and more animals should be included in the molecular analysis for *Brucella* sp. detection.

Tursiops truncatus (CET 305) was also tested positive for Morbillivirus. Additionally, fourteen more cetaceans with cranial crassicaudasis were tested for morbillivirus and CET 594 were positive. Furthemore, twelve of these cetaceans were tested for herpesvirus, but all of them were negative. None of the 39 cetaceans that presented *Crassicauda* sp. were tested for *Toxoplasma gondii*, even though the brain lesions

displayed for some of them are compatible with the lesions associated to the presence of these protozoa, as previously described (Di Guardo et al., 2010; Díaz-Delgado et al., 2018).

Regarding the CNS, six animals showed congestion and/or haemorrhage (CET 305, CET 430, CET 479, CET 984, CET 1020, CET 1287), that could be related to parasitic migrations (Sprent, 1955), among other causes.

Given that this parasite is responsible for strandings and is known for its high pathogenic capacity (Lambertsen, 1992), the incidence in our study on the Canary coasts, affecting 4.57% of the pterygoid sacs, underscores the need for more thorough monitoring and study of *Crassicauda sp*. This will help us better understand its impact on the health of cetacean populations.

Conclusions

- 1. There is a host susceptibility to cranial crassicaudasis for two species from our study, *Stenella frontalis* and *Grampus griseus*.
- 2. The prevalence seems to be higher in younger animals compared to adults for the species from our study for the 25-year period (1999-2023).
- 3. There is no sex-related difference in the occurrence of cranial crassicaudiasis in the species from our study for the 25-year period (1999-2023).
- 4. There is not enough available information from our study to correlate the level of *Crassicauda* sp. infestation to the severity of lesions in the pterygoid sacs.
- 5. Brain lesions is a frequent finding (46.1%) in stranded cetaceans with cranial crassicaudasis in the Canary Islands in a 25-year period (1999-2023).
- 6. *Brucella* is the pathogen most frequently implicated in inflammatory lesions of the CNS in cetaceans with Crassicaudasis (25%), compared to other tested pathogens such as Morbillivirus and Herpesvirus in a 25-year period (1999-2023).
- 7. Stranded cetaceans should be routinely tested for other pathogens such as *Brucella* sp. and pterygoid sacs must be systematically examined.

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ANEXO

Nº IUSA	CET	Specie	Age	Sex	Gestation	Located	Stranding date	Necropsy date
1 44/00		93 Tursiops truncatus	Juv/Sub	Male		Tenerife	2000	20-01-00
2 48/00		94 Tursiops truncatus	Juv/Sub	Male		Tenerife	2000	24-01-00
3 266/00		106 Stenella frontalis	Juv/Sub	Male		Tenerife	2000	28-04-00
4 i82/01		123 Stenella frontalis	Adult	Male		Gran canaria	2001	26-03-01
5 i083/02		168 Tursiops truncatus	Juv/Sub	Male		Tenerife	2002	11-05-02
6 i145/04		269 Steno bredanensis	Juv/Sub	Male		Gran canaria	2004	06-08-04
7 i225/05		305 Tursiops truncatus	Juv/Sub	Female	No	Lanzarote	2005	18-07-05
8 i283/07		395 Stenella frontalis	Adult	Male		Fuerteventura	2007	24-06-07
9 i127/08		418 Stenella frontalis	Adult	Female	Yes	Gran Canaria	2008	26-03-08
10 i141/08		430 Steno bredanensis	Juv/Sub	Female	No	Gran Canaria	2008	14-04-08
11 i149/08		431 Grampus griseus	Juv/Sub	Male		Tenerife	2008	21-04-08
12 i199/08		446 Steno bredanensis	Juv/Sub	Male		Gran Canaria	2008	05-05-08
13 1208/08		450 Tursiops truncatus	Juv/Sub	Female	No	Tenerife	2008	13-05-08
14 i237/08		456 Grampus griseus	Adult	Female	No	Gran Canaria	2008	17-06-08
15 i039/09		479 Globicephala macrohynchus	ND	NS		Gran canaria	2009	18-02-09
16 I-132/10		533 Grampus griseus	Juv/Sub	Male		Fuerteventura	2010	20-04-10
17 1-136/10		534 Grampus griseus	Juv/Sub	Male		Tenerife	2010	22-04-10
18 i284/10		549 Grampus griseus	Adult	Female	No	Tenerife	2010	14-09-10
19 i102/11		571 Globicephala macrohynchus	Adult	Female	No	Gran Canaria	2011	01-04-11
20 1-379/11		594 Globicephala macrohynchus	Juv/Sub	Male		Tenerife	2011	02-11-11
21 1-42/12		607 Delphinus delphis	Juv/Sub	Male		Lanzarote	2012	12-03-12
22 1-071/12		618 Stenella frontalis	Juv/Sub	Male		Tenerife	2012	12-05-12
23 1-39/13		634 Grampus griseus	Adult	Male		Fuerteventura	2012	03-11-12
24 1-33/13		641 Stenella coeruleoalba	Juv/Sub	Male		La gomera	2013	28-01-13
25 193/15		746 Globicephala macrohynchus	Adult	Female	No	Lanzarote	2015	29-02-15
26 i249/15		751 Grampus ariseus	Juv/Sub	Female	No	Tenerife	2015	16-03-15
27 i249/15		761 Stenella frontalis	Juv/Sub	Female	No	El hierro	2015	24-05-15
28 i343/15		762 Stenella frontalis	Juv/Sub	Male		La gomera	2015	01-06-15
29 19581/16		816 Tursiops truncatus	Juv/Sub	Female	No	Tenerife	2016	01-08-16
30 11014/16		825 Kogia breviceps	Adult	Male		Fuerteventura	2016	15-11-16
31 10104/17		829 Stenella frontalis	Juv/Sub	Female	No	Tenerife	2016	22-12-16
32 1301/17		848 Delphinus delphis	Calf	Male		Fuerteventura	2017	14-04-17
33 1374/17		872 Stenella frontalis	Juv/Sub	Female	No	Fuerteventura	2017	22-10-17
34 1348/17		876 Steno bredanensis	Adult	Female	No	Lanzarote	2017	08-11-17
35 SA38/18		884 Stenella frontalis	Calf	Female	No	Tenerife	2018	16-01-18
36 Sa 221/19		963 Lagenodelphis hosei	Calf	Male		Gran Canaria	2019	03-03-19
37 Sa249/19		984 Grampus griseus	Juv/Sub	Male		Gran Canaria	2019	26-04-19
38 Sa266/19		995 Globicephala macrohynchus	Juv/Sub	Male		Gran Canaria	2019	20-05-19
39 SA277/19		996 Globicephala macrohynchus	Juv/Sub	Male		Fuerteventura	2019	20-05-19
40 SA280/19		1000 Globicephala macrohynchus	Adult	Female	ND	Lanzarote	2019	31-05-19
41 SA373/19		1020 Tursiops truncatus	Juv/Sub	Female	No	Tenerife	2019	09-08-19
42 SA370/20		1054 Stenella coeruleoalba	Adult	Female	No	Lanzarote	2020	18-01-20
43 SA031/20		1056 Stenella frontalis	Calf	Male		Tenerife	2020	24-01-20
44 SA289/20		1057 Stenella frontalis	Adult	Female	No	Gran canaria	2020	26-01-20
45 SA054/20		1059 Grampus ariseus	Juv/Sub	Male		Fuerteventura	2020	28-01-20
46 SA181/20		1103 Tursiops truncatus	Juv/Sub	Male		Gran canaria	2020	13-06-20
47 SA218/21		1145 Stenella frontalis	Calf	Male		Fuerteventura	2021	18-01-21
48 SA297/21		1152 Stenella frontalis	Calf	Male		Gran Canaria	2021	26-02-21
49 SA345/21		1162 Stenella frontalis	Juv/Sub	Female	No	Gran Canaria	2021	21-03-21
50 SA672/21		1186 Delphinus delphis	Adult	Female	No	Fuerteventura	2021	08-07-21
51 SA199/22		1213 Grampus ariseus	ND	Female	No	Fuerteventura	2022	15-02-22
52 SA308/22		1218 Stenella frontalis	Calf	Male		Tenerife	2022	07-03-22
53 SA1404/22		1257 Globicephala macrohypchus	Juy/Sub	Male		Tenerife	2022	15-09-22
54 SA1890/22		1259 Stenella coeruleoalba	Juv/Sub	Female	No	Fuerteventura	2022	07-10-22
55 54380/23		1287 Stenella frontalis	luv/Sub	Female	No	Gran Canaria	2022	23-05-23

Supplementary Table 1. Biological and stranding conditions of the 55 cetaceans stranded in the Canary Islands with parasites in their pterygoid sacs during the period of study.

N٥	IUSA	CET	Especie	DC	NC	Columna2	Columna3
	1 44/00		93 Tursiops truncatus	3	poor	Р	1
	2 48/00		94 Tursiops truncatus	2	good	Р	1
	3 266/00		106 Stenella frontalis	2	good	Р	1
	4 i82/01	1	123 Stenella frontalis	3	good	Р	1
	5 i083/02		168 Tursiops truncatus	2	good	Р	1
	6 i145/04		269 Steno bredanensis	2	very poor	А	1
	7 i225/05		305 Tursiops truncatus	1	fair	A	1
	8 i283/07		395 Stenella frontalis	2	good	Р	1
	9 i127/08		418 Stenella frontalis	2	good	Р	1
	10 i141/08		430 Steno bredanensis	4	ND	Р	1
	11 i149/08		431 Grampus griseus	2	poor	A	1
	12 i199/08		446 Steno bredanensis	5	good	Р	1
	13 1208/08		450 Tursiops truncatus	1	fair	Р	1
	14 i237/08		456 Grampus griseus	2	poor	A	1
	15 i039/09		479 Globicephala macrohynchus	1	ND	Р	1
	16 I-132/10		533 Grampus griseus	2	poor	Р	1
	17 -136/10		534 Grampus griseus	1	poor	Р	1
	18 i284/10		549 Grampus griseus	2	poor	Р	1
	19 i102/11		571 Globicephala macrohynchus	4	fair	Р	1
	20 -379/11		594 Globicephala macrohynchus	2	poor	Р	1
	21 -42/12		607 Delphinus delphis	4	poor	Р	1
	22 1-071/12		618 Stenella frontalis	4	fair	Р	1
	23 -39/13		634 Grampus griseus	4	poor	P	1
	24 -33/13		641 Stenella coeruleoalba	4	poor	P	1
	25 193/15		746 Globicephala macrohynchus	3	fair	Р	1
	26 i249/15		751 Grampus griseus	3	good	P	1
	27 i249/15		761 Stenella frontalis	3	good	P	1
	28 i343/15		762 Stenella frontalis	4	fair	P	1
	28 19581/16		816 Tursiops truncatus	3	fair	P	1
	30 11014/16		825 Kogia braviceps	2	fair	Р	1
	31 0104/17		829 Stenella frontalis	2	fair	P	1
	32 301/17		848 Delphinus delphis	3	poor	Р	1
	33 374/17		872 Stenella frontalis	3	poor	P	1
	34 348/17		876 Steno bredanensis	3	fair	Р	1
	35 SA38/18		884 Stenella frontalis	2	fair	Р	1
	36 Sa 221/19		963 Logonedelphis hosei	3	fair	Р	1
	37 Sa249/19		984 Grampus griseus	2	Very poor	A	1
	38 Sa 266/19		995 Globicephala macrohynchus	3	fair	P	1
	39 SA277/19		996 Globicephala macrohynchus	3	poor	Р	1
	40 SA280/19	1	1000 Globicephala macrohynchus	4	ND	Р	1
	41 SA373/19	1	1020 Tursiops truncatus	3	fair	Р	1
	42 SA370/20	1	1054 Stenella coeruleoalba	3	very poor	Р	1
	43 SA031/20		1056 Stenella frontalis	2	fair	Р	1
	44 SA289/20		1057 Stenella frontalis	3	fair	Р	1
	45 SA054/20	1	1059 Grampus griseus	4	ND	P	1
	46 SA181/20	1 1	1103 Tursiops truncatus	1	poor	A	1
	47 SA218/21		1145 Stenella frontalis	5	ND	Р	1
	48 SA297/21		1152 Stenella frontalis	1	fair	A	1
	49 SA345/21		1162 Stenella frontalis	2	fair	Р	1
	50 SA672/21	-	1186 Delphinus delphis	2	fair	Р	1
	51 SA199/22	1	1213 Grampus griseus	4	ND	Р	1
	52 SA308/22	-	1218 Stenella frontalis	2	very poor	Р	1
	53 SA1404/22	-	1257 Globicephala macrohynchus	2	fair	P	1
	54 SA1890/22	-	1259 Stenella coeruleoalba	1	ND	Р	1
	55 SA380/23		1287 Stenella frontalis	2	poor	Р	1
					1		

Supplementary Table 2. Decomposition Score (DC), Nutrition Score (NC), and active or passive stranding of the 55 cetaceans stranded in the Canary Islands with parasites in their pterygoid sacs during the period of study.

Number		CET	Cetacean	Phylumm	Genus	Species	Degree	Coinfection	SNC
	1	Cet 93	Tursiops truncatus	Nematodes	Crassicaud a	ND	Moderate	NO	AN
				Nematodes	Grassicaud				
			Tursiops	y	аY				
	2	Cet 94	Stenella	Trematodes	Crassicaud	ND	MILO	51	AN
	3	Cet 106	frontalis Tursiops	Nematodes	a Crassicaud	ND	Moderate	NO	AN Lymphocytic
	5	Cet 168	truncatus	Nematodes	а	ND	Severe	NO	leptomeningitis
			Steno	Nematodes	Crassicaud				
	6	Cet 269	S	y Trematodes	Nasitrema	ND	Moderate	SI	AN
									purulent
			Tursiops	Nematodes y	Crassicaud a Y				meningoencephalit is.
	7	Cet 305	truncatus	Trematodes	Nasitrema	ND	Moderate*	SI	Microhemorrhage PNET primitive
	8	Cet 395	Stenella frontalis	Nematodes	Crassicaud a	ND	ND	NO	neuroectoderm tumor
			Stenella		Crassicaud	Crassicauda			
	9	Cet 418	frontalis	Nematodes	a	grampicola	Mild	NO	AN
			Steno	Nematodes	Crassicaud				
	10	Cet 430	S	y Trematodes	Nasitrema	ND	Moderate	SI	Congestion
			Steno	Nematodes	Crassicaud				
	12	Cet 446	bredanensi s	y Trematodes	a Y Nasitrema	Crassicauda grampicola	Mild*	si	AN
				Nematodes	Crassicaud				
	13	Cet 450	Tursiops truncatus	y Trematodes	a Y Nasitrema	Crassicauda grampicola	ND	SI	Non-suppurative meningitis
			Grampus		Crassicaud	Crassicauda			Purulent meningoencephalit
	14	Cet 456	griseus	Nematodes	a	grampicola	Mild*	NO	is
			la magneti		Crassicaud				
	15	Cet 479	nacrohync hus	Nematodes	a r Stenurus	Stenerus minor	Mild	SI	Congestion
			Grampus		Crassicaud a Y				
	16	Cet 533	griseus	Nematodes	Stenurus Crassicaud	ND	Mild*	SI	AN
	17	Out 524	Grampus	Nomatodor	a Y Stopurur	ND	Sminro*	CI.	Pyogranulomatous
	1/	061304	griseus	Weinatodes	Crassicaud	ND	Jevere	31	meningius
	18	cet 549	griseus	Nematodes	a r Stenurus	Stenerus minor	Moderate	si	AN
			Globicepha la						
	19	Cet 571	macrohync hus	Nematodes	Crassicaud a	ND	Severe	NO	AN
									Non-suppurative
			Globicepha		Crassicand				meningoencephalit
			macrohync		a Y	Crassicauda			suppurative
	20	cet 594	hus	Nematodes	Stenurus	grampicola	Mild	si	Non-suppurative
	22	cet 618	Stenella frontalis	Nematodes	Crassicaud a	ND	Mild*	NO	leptomeningoence phalitis
			Grampus		Crassicaud a Y	Crassicauda grampicola y			Leptomeningeal
	26	cet 751	griseus	Nematodes	Stenurus Crassicaud	Stenerus minor	Severe*	SI	thickening
	27	cet 761	Stenella frontalis	Nematodes	a Y Stenurus	ND	Moderate*	9	AN
	20	cot 767	Stenella	Nomatodor	Crassicaud	ND	Mildt	ND	AN
	20	Cel 762	ITUITIdus	Nemaloues	d	ND	milu.	ND	AN
			Tursiops	Nematodes y	Crassicaud a Y				Mild subacute multifocal
	29	cet 816	truncatus Delphinus	Trematodes	Nasitrema Crassicaud	ND	ND	SI	meningitis
	32	cet 848	delphis Lagenodelp	Nematodes	a Crassicaud	ND	Mild*	NO	AN
	36	Cet 963	his hosei Gramnus	Nematodes	a Crassicaud	ND	Moderate*	ND	AN Thrombosis and
	37	cet 984	griseus	Nematodes	a	ND	Moderate*	NO	hemorrhage
			la						
	40	cet1000	macrohync hus	Nematodes	Crassicaud a	ND	Moderate	NO	AN
			Tursiops		Crassicaud a Y				
	41	cet 1020	truncatus Stenella	Nematodes	Stenurus	ND	Moderate*	SI	Hemorrhages
	42	CET 1054	coeruleoal ba	Nematodec	Crassicaud a	ND	Mild	NO	AN
	+2	CET 1050	Stenella	Nometral	Crassicaud	ND	Mild	NO	41
	43	0011030	Stenella	Nen	Crassicaud	Crassicuada	Miles	NO	AN
	44	UEI 1057	rrontalis	Nematodes	a	grampicola	Mild*	NO	Focal
	45	CET 1059	Grampus griseus	Nematodes	Crassicaud a	Crassicauda grampicola	Severe*	NO	pyogranulomatous meningitis
									Necrosuppurative encephalitis.
			Tursions	Nematodes v	Crassicuda				Lymphoplasmacyti c encephalitis and
	46	CET 1103	truncatus	Trematodes	y Nasitrema	ND	Mild*	SI	meningitis
1		057.11.45	Stenella	Namet	Crassicaud	ND	Milat	ND	multifocal
	4/	uer 1145	Stenella	ivernatodes	d Crassicaud	UND	MIII.0.^	UND	Edema- astrocytic
	48	UEI 1152	rrontalis	Nematodes	a	UN	ND	NO	pattooning Moderate
									multifocal satellitelitosis,
1	50	CET 1186	Delphinus delphis	Nematodes	Crassicaud a	ND	ND	NO	neuronal lipofuscinosis
	51	OFT 1213	Grampus	Nematodos	Crassicaud a	Crassicauda	Severe*	NO	AN
	-01	CET 1210	Stenella	Nomet	Crassicaud	ND	Moderatio	NO	AN
	52	GET 1218	n ontalis	ivernatodes	d	UND	rnoderate*	UNU	Lymphoplasmacyti
									c meningoencephalit
									is, Lymphoplasmacyti
									c choroiditis, Lymphoplasmacvti
									c polydariculitis.
						0			and edema.
			stenella		crassicaud	Grassicauda			Endotnelial

Supplementary Table 3. Parasitic infestations. The cetaceans marked with an asterisk indicating the level of infestation were confirmed through images.

				Crassicauda	Crassicauda								
	Crassicauda			and Nasitrema			Crassicauda and Stenurus			ND	Total		
	Mild	Moderate	Severe	Mild	Moderate	Severe	Mid	Moderate	Severe				
Tursiops													
truncatus	C) 1	1 1	1	. 1	1 () () :	2	0	2 8		
Stenella frontalis	6	6 3	3 0	0	(0 0) () :	1	0	2 12		
Steno													
bredanensis	C) (0 C	1	. 2	2 () () (D	0	0 3		
Grampus griseus	1	1 1	1 2	0	() () 1	1 :	1	2	0 8		
Globicephala													
macrohynchus	C) 1	1 1	0) () 2	2 (D	0	0 4		
Stenella													
coeruleoalba	1		0 0	0	0	0 0) () (D	0	0 1		
Delphinus delphis	1	. (0 C	0) () () (0	0	1 2		
Lagenodelphis													
hosei	C		1 0	0	(0 0) () (0	0	0 1		

Supplementary Table 4. Parasitic infestation severity according to the host species and the type of parasitization (only *Crassicauda* sp. or in combination with *Nasitrema* sp. or *Stenurus* sp.).

Number CET	Cetacean	Phylium	Genus	Lesions	MBV	HV	Brucella	Тохо
1 Cet 93	Tursiops	Nematodec	Crassicaud a	N	Negative	Negative	Non tested	Non tested
1 001 35	. Snoutus	Man	0	tata -				
	Tursiops	Nematodes y	a Y	Injury to mucosa				
2 Cet 94	truncatus	Trematodes	Nasitrema	and bone	Negative	Negative	Non tested	Non tested
3 Cet 106	frontalis	Nematodes	a	N	Non tested	Non tested	Non tested	Non tested
5 Cet 168	Tursiops truncatus	Nematodes	Crassicaud a	N	Negative	Negative	Non tested	Non tested
	01		Quere la cond		U	U.		
	steno bredanensi	y y	a Y					
6 Cet 269	S	Trematodes	Nasitrema	N	Non tested	Non tested	Non tested	Non tested
		Nematodes	Crassicaud					
7 Cet 305	Tursiops truncatus	y Trematodes	a Y Nasitrema	N	Positive	Negative	Positive	Non tested
8 Oat 205	Stenella	Nomatodoo	Crassicaud	Hemorrahg	Non toctod	Non toctod	Non toctod	Non tostod
8 Cet 395	Stenella	Nemaloues	a Crassicaud	e	Non tested	Non tested	Non tested	Non testeu
9 Cet 418	frontalis	Nematodes	а	N	Non tested	Non tested	Non tested	Non tested
	Steno	Nematodes	Crassicaud					
10 Cet 430	s	y Trematodes	a r Nasitrema	N	Non tested	Non tested	Non tested	Non tested
	Steno	Nematodes	Crassicaud					
	bredanensi	y	a Y					
12 Cet 446	S	Trematodes	Nasitrema	N	Non tested	Non tested	Non tested	Non tested
	Tursions	Nematodes v	Crassicaud a Y					
13 Cet 450	truncatus	, Trematodes	Nasitrema	Ν	Negative	Negative	Non tested	Non tested
14 Cet 456	Grampus griseus	Nematodes	Crassicaud a	N	Negative	Negative	Negative	Non tested
	Globicepha		Craceleaud					
	ia macrohync		a Y	Injury in				
15 Cet 479	hus	Nematodes	Stenurus Crassicaud	bone	Non tested	Non tested	Non tested	Non tested
	Grampus		аY					
16 Cet 533	griseus	Nematodes	Stenurus Crassicaud	N	Negative	Negative	Non tested	Non tested
17 Oct 524	Grampus	Nomatodoo	a Y Stopurus	N	Norativo	Norativo	Norativo	Non tostod
17 061 334	griseus	Nematodes	Crassicaud	IN .	wegauve	Negauve	Negauve	Nontesteu
18 cet 549	Grampus griseus	Nematodes	a Y Stenurus	Congestion	Negative	Negative	Non tested	Non tested
	Globicepha			Ū	U	U.		
	ia macrohync		Crassicaud					
19 Cet 571	hus Globicenha	Nematodes	а	N	Negative	Negative	Non tested	Non tested
	la .		Crassicaud					
20 cet 594	macrohync hus	Nematodes	a Y Stenurus	N	Positive	Non tested	Non tested	Non tested
22 cet 618	Stenella frontalis	Nematodes	Crassicaud a	N	Negative	Non tested	Positive	Non tested
			Crassicaud		nogauro			
26 cet 751	griseus	Nematodes	a Y Stenurus	N	Negative	Negative	Negative	Non tested
	Stenella		Crassicaud					
27 cet 761	frontalis	Nematodes	Stenurus	N	Non tested	Non tested	Non tested	Non tested
28 cet 762	Stenella frontalis	Nematodes	Crassicaud a	N	Non tested	Non tested	Non tested	Non tested
		Nomatodoo	Crassicaud					
	Tursiops	y y	a Y					
29 cet 816	truncatus Delphinus	Trematodes	Nasitrema Crassicaud	N	Non tested	Non tested	Non tested	Non tested
32 cet 848	delphis	Nematodes	a	N	Non tested	Non tested	Non tested	Non tested
36 Cet 963	his hosei	Nematodes	a a	N	Non tested	Non tested	Non tested	Non tested
37 cet 984	Grampus griseus	Nematodes	Crassicaud a	N	Non tested	Non tested	Non tested	Non tested
	Globicepha							
	ta macrohync		Crassicaud					
40 cet1000	hus	Nematodes	a Crassicaud	N	Non tested	Non tested	Non tested	Non tested
	Tursiops	Man-+	a Y	N	Nontro	Nontro	Nontro	Nontrol
41 cet 1020	truncatus Stenella	ivernatodes	stenurus	Ń	NON TESTED	NON TESTED	NON TESTED	Non tested
42 CET 1054	coeruleoal ba	Nematodes	Crassicaud a	N	Non tested	Non tested	Non tested	Non tested
42 0011004	Stenella		Crassicaud	Injury to				
43 CET1056	frontalis Stenella	Nematodes	a Crassicaud	mucosa	Non tested	Non tested	Non tested	Non tested
44 CET 1057	frontalis	Nematodes	a Crassicaud	N	Non tested	Non tested	Non tested	Non tested
45 CET1059	griseus	Nematodes	a	N	Non tested	Non tested	Non tested	Non tested
		Nematodes						
	Tursiops	<i>y</i>	Crassicuda		Neger	Manad	Manad	
46 CET1103	truncatus Stenella	rematodes	y Nasitrema Crassicaud	N	Negative	Negative	Negative	Non tested
47 CET1145	frontalis	Nematodes	a	Ν	Non tested	Non tested	Non tested	Non tested
48 CET1152	frontalis	Nematodes	a a	N	Non tested	Non tested	Non tested	Non tested
50 CFT1186	Delphinus delphis	Nematodes	Crassicaud a	N	Non tested	Non tested	Non tested	Non tested
	Grampus	Manut	Crassicaud	N	Nentra	Nontro	Non	Nontro
51 CEI 1213	griseus Stenella	wernatodes	a Crassicaud	N	won tested	Non tested	won tested	NON TESTED
52 CET 1218	frontalis	Nematodes	a Craceleaud	N	Non tested	Non tested	Non tested	Non tested
55 CET 1287	frontalis	Nematodes	a	injuries	Non tested	Non tested	Positivo	Non tostod

Supplementary Table 5. Gross lesions in the pterygoid sacs and etiological pathogens tested in samples from the CNS of the 55 cetaceans with parasitic sinusitis. N= non reported.

Supplementary Table 6 (Statistics)

Species

						Ca	SOS				
		Va	alido			Per	dido			l otal	
		N	N Porce		N		Porc	entaje		N	Porcentaj
pecies * Cr	assicauda	852	1	00,0%		0		0,0%		852	100,05
				Cr	assic	auda					
			No		Ye	s	Tota				
Species	Balaenop acutorost	Balaenoptera acutorostrata					0		7		
	Lagenode	elphis hose			5		1		6		
	Megaptera novaeangliae				2		0		2		
	Mesoplodon bidens				2		0		2		
	Mesoplodon densirostris				10		0	1	0		
	Mesoploc	lon europa	eus		16		0	1	6		
	Mesoploc		1		0		1				
	Orcinus o	orca			1		0		1		
	Phocoena	a phocoena			1		0		1		
	Physeter macrocephalus				46		0	4	6		
	Pseudorca crassidens				2		0		2		
	Balaenoptera borealis				3		0		3		
	Stenella o	oeruleoalb	a	1	79		1	18	0		
	Stenella f	rontalis		1	57		13	17	0		
	Stenella l	ongirostris			3		0		3		
	Steno bre	danensis			30		3	3	3		
	Tursiops	truncatus			61		8	6	9		
	Ziphius c	avirostris			51		0	5	1		
	Peponoce	ephala elec	tra		1		0		1		
	Balaenop	tera edeni			3		0		3		
	Balaenop	tera physa	lus		9		0		9		
	Delphinu	s delphis			94		2	9	6		
	Globicep macrorhy	hala /nchus			55		4	5	9		
	Grampus	griseus			19		7	2	6		
	Kogia bre	eviceps			45		0	4	5		
	Kogia sin	Kogia sima			10		0	1	0		
Total				8	13		39	85	2		

Pruebas de chi-cuadrado

	Valor	df	Significación asintótica (bilateral)
Chi-cuadrado de Pearson	63,620 ^a	25	,000
Razón de verosimilitud	58,532	25	,000
N de casos válidos	852		

P<0.005. Statistical difference is observed.

Sex

Resumen de procesamiento de casos

	Casos					
	Válido		Perdido		Total	
	Ν	Porcentaje	Ν	Porcentaje	Ν	Porcentaje
Gender * Crassicauda	852	100,0%	0	0,0%	852	100,0%

Tabla cruzada Gender*Crassicauda

Recuento

		Crassi		
		No	Yes	Total
Gender	ND	19	1	20
	Female	377	18	395
	Male	417	20	437
Total		813	39	852

Pruebas de chi-cuadrado

	Valor	df	Significación asintótica (bilateral)
Chi-cuadrado de Pearson	,009 ^a	2	,996
Razón de verosimilitud	,008	2	,996
N de casos válidos	852		

a. 1 casillas (16,7%) han esperado un recuento menor que
 5. El recuento mínimo esperado es ,92.

No statistical differences are observed Age

Resumen de procesamiento de casos

		Casos				
	Válido		Perdido		Total	
N Porcentaje		N	Porcentaje	N	Porcentaje	
Age * Crassicauda	852	100,0%	0	0,0%	852	100,0%

Tabla cruzada Age*Crassicauda

Recuento

		Crassie		
		No	Yes	Total
Age	0	16	0	16
	Neonate-calve	207	13	220
	Juvenile-subadult	213	15	228
	Adult	377	11	388
Total		813	39	852

Pruebas de chi-cuadrado

	Valor	df	Significación asintótica (bilateral)
Chi-cuadrado de Pearson	6,449 ^a	3	,092
Razón de verosimilitud	7,276	3	,064
N de casos válidos	852		

a. 1 casillas (12,5%) han esperado un recuento menor que
 5. El recuento mínimo esperado es ,73.

No statistical differences are observed