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MORPHOMETRICAL CATEGORIZATION OF *PHYLLOBOTHRIUM DELPHINI* (CESTOIDEA, TETRAPHYLLIDEA) CYSTS FROM FRASER'S DOLPHIN, *LAGENODELPHIS HOSEI* (CETACEA, DELPHINIDAE)

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Abstract – The larvae of phyllobothriid cestode worms found in marine mammals have been classified as either "*delphinii group*" or "*grimaldii group*". The first group has been divided into 11 morphotypes by previous studies. However, these categories are vague and often overlap, as they were described from different host species within a wide geographical range. The goal of this study is to simplify the categorization of larval shape and size based on samples obtained from a single host species. Eleven Fraser's dolphin *Lagenodelphis hosei* Fraser, 1956 stranded on the coast of Uruguay in November 2001 and subsequently died. From these animals, 190 cysts of *Phyllobothrium delphini* (Bosc, 1802) were obtained from the perigenital blubber of one female and one male. The prevalence of *P. delphini* was 18.18%, density was 159.38 larvae/kg of perigenital blubber, mean intensity was 95 and mean abundance was 17.27. The following measurements were taken: total length (BL), width and thickness of cysts, neck length (NL), scolex length, scolex width, external diameter of the suckers, and the ratio BL/NL. In order to facilitate further identification of these phyllobothriid cysts, they were grouped into three categories according to shape and size: category "A" (22.35%, neck always straight); category "B" (62.35%, all with curved neck); category "C" (15.30%, U-shaped or with projections from the wall of the cyst, also curved neck). These morphological differences could correlate to time spent in the host. One other larval form (*Tetrabothrius* sp.) was also found in the blubber of these dolphins.

Resumen – Las larvas de filobotridios en mamíferos marinos fueron clasificadas como "grupo *delphinii*" y "grupo *grimaldii*". En estudios previos el primer grupo fue dividido en 11 morfotipos. Sin embargo, estas categorías son vagas y a menudo se solapan, ya que fueron descritas a partir de diferentes especies de hospedadores provenientes de una amplia distribución geográfica. El objetivo de este estudio es simplificar la categorización de tamaños y formas larvarias a partir de una muestra obtenida de un único hospedador. Se estudiaron once ejemplares muertos de *Lagenodelphis hosei* Fraser 1956 varados en la costa de Uruguay en noviembre de 2001. Ciento noventa quistes de *Phyllobothrium delphini* (Bosc, 1802) fueron obtenidas de la grasa perigenital de una hembra y un macho. La prevalencia de *P. delphini* fue del 18.18%, la densidad 159.38 larvas/kg de grasa perigenital, la intensidad media 95 y la abundancia media 17.27. Se tomaron las siguientes medidas: largo total (BL), ancho y grosor del quiste, largo del cuello (NL), largo del scolex, ancho del scolex, diámetro externo de las ventosas y el cociente BL/NL. Para facilitar futuras identificaciones, estas larvas filobotrídeas fueron agrupadas en tres categorías de acuerdo a su tamaño y forma: categoría "A" (22.35%, cuello siempre recto); categoría "B" (62.35%, todas con cuello curvado) y categoría "C" (15.30%, cuello curvado, quistes en forma de U o con proyecciones de la pared). Estas diferencias morfológicas podrían correlacionarse con el tiempo de permanencia en el hospedador. Sólo una forma larvaria fue determinada como *Tetrabothrius* sp., también encontrada en el panículo graso de estos delfines.

Key words: *Lagenodelphis hosei*, Phyllobothriidae, *Phyllobothrium delphini,* stranding, Uruguay.

Introduction

Two types of phyllobothriid cysts are recognized in marine mammals: *Phyllobothrium delphini* (Bosc, 1802), parasitizing the blubber coat, and *Monorygma grimaldii* (Monier, 1809), usually present in coelomic mesenteries. Baer (1932) named them the "*delphinii* group" and "*grimaldii* group" respectively.

The presence of the larval cestode *Phyllobothrium delphini*, has been reported for many cetacean species (Delyamure, 1955; Testa and Dailey, 1977; Dailey and Walker, 1978; Dailey, 1985; Abollo *et al*., 1998; Gibson *et al*., 1998). This parasite is commonly found within the blubber, typically concentrated in the perigenital region. Adult worms are found in the spiral valves of elasmobranch and holocephalan fishes, which ingest infected flesh either by predation or by scavenging (Euzet, 1994; Walker, 2001; Raga *et al*., 2002). The life cycle of this parasite is not clear and little is known about the biology of these larval forms.

Dailey and Walker (1978) found larval *P. delphini* to be the most common parasite in the blubber of 56 cetaceans (representing 4 species) stranded along the coast of South California, USA. Geraci *et al*. (1976) reported up to 570 larvae *per* host in the Atlantic white-sided dolphin, *Lagenorhynchus acutus,* from the western north Atlantic. Larvae of *P. delphini* have also been cited for Fraser's dolphin, *Lagenodelphis hosei* Fraser, 1956 (Tobayama *et al*., 1973; Testa and Dailey, 1977; McColl and Obendorf, 1982; van Bree *et al*., 1986; Abollo *et al*., 1998; Mignucci-Giannoni *et al*., 1999; Walker, 2001; Moreno *et al*., 2003).

Larval forms of the "*delphinii* group" exhibit great diversity in shape: Guiart (1935) described four cyst morphotypes, Delyamure (1955) documented two more and Testa and Dailey (1977) added another five to give the current total of 11. Unfortunately, previous descriptions lack clear differences between similar forms making the classification of morphotypes difficult. In addition, the definition of individual morphotypes is vague and does not contribute to the identification of phyllobothriid larvae. The size and

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shape of cestode larvae, including those of the Tetraphillidea, are extremely variable and depend on many factors, especially the age of larvae. This raises the question of the usefulness of previously described morphotypes and supports the need for a simpler categorization until a conclusive systematic determination can be made.

The present study was conducted to describe and characterize the *P. delphini* larvae recovered from Fraser's dolphins, based on their morphological variation within a single host. Consequently, a simple classification into three groups is presented. This is the first record of *P. delphini* parasitizing *L. hosei* for Uruguayan waters. In addition, an "atypical" morphology of one cyst is discussed.

Material and Methods

In November 2001, approximately 60 Fraser's dolphins were observed alive at the mouth of Santa Lucía river (34º47'S; 56º21'W, Uruguay). Approximately 35-40 dolphins were conducted to open waters and saved, but at least 21 individuals perished. Eleven dead specimens were recorded as ZVCM2133 to ZVCM2143 and were sampled for parasites within a day of death. From each individual, body measurements, sex and color pattern were recorded. A field necropsy was carried out for sampled animals to determine cause of death. Skulls were deposited in the Mammalian Collection of the Vertebrate Zoology Section and parasitological samples entered the Helminthological Collection of Invertebrate Zoology Section as BP/9004, Faculty of Sciences, Montevideo, Uruguay.

Whole skin surrounding the genital slit and anus was removed up to a distance of 30cm for each animal, weighed *in situ* and fixed in 10% isotonic formalin solution. Two days later, samples were serially sectioned for cyst identification. Cysts of *P. delphini* were obtained from the perigenital blubber of one female (ZVCM2139) and one male (ZVCM2142). All larvae were removed, dehydrated and cleared in beechwood creosote for morphometrical study and a small sample (N=8) was used to perform SEM studies. They were critically point dried and coated with a Coating Denton Vacuum apparatus for 60s at 40mA. Images were obtained from a JEOL JSM-5900LV electron microscope. Measurements were made by ocular micrometer using a Nikon SMZ-10 Axioskope microscope equipped with a drawing tube as follows:- BL: bladder length; BW: bladder width and BT: bladder thickness. Length and width of neck (NL, NW) and scolex (SL, SW) and outer diameter of accessory bothridial suckers (BS) (Fig.1). In order to characterize the shape of cysts, BL/NL ratio was used. Several scolices were stained with acetic carmin and mounted in Canada Balsam.

The total number of recovered cysts was 190. The criteria adopted to consider an entire cyst was the presence of a complete scolex (although the bladder cyst could be partially missing). One hundred and fourteen cysts were used for external and bothridial sucker measurements, twenty of which were used to measure the fifth apical sucker on the myzorhynchus. Eighty five cysts were used to estimate the three categories proposed. Images were obtained by an Olympus BX40 microscope with an adapted digital camera (50x). Standard parasitological parameters were used according to Bush *et al*. (1997). All parasitological measurements in *P. delphini* are expressed in mm. Normality of errors of the transformed data set was determined by the Kolmogorov-Smirnov test at $p \geq 0.05$ in order to perform descriptive statistics (Sokal and Rohlf, 1995). Statistical analysis were performed using Statistica 6.0 software.

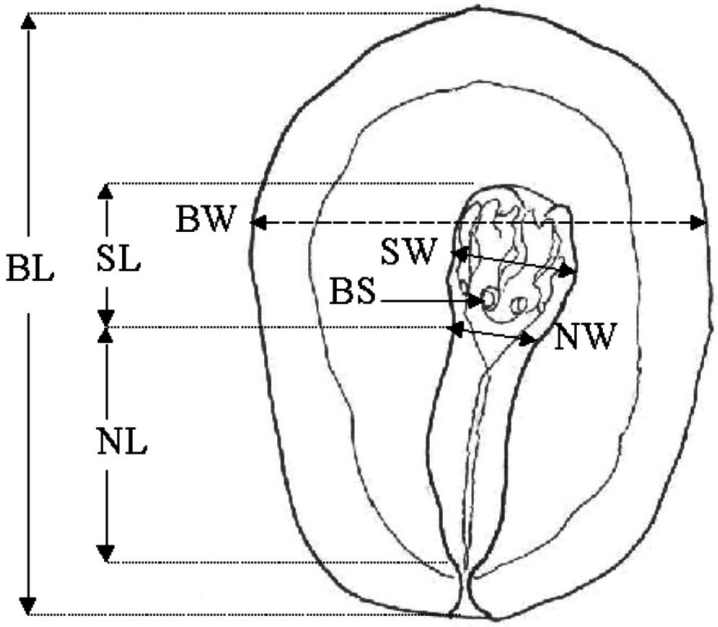

Figure 1. Schematic *Phyllobothrium delphini* cyst and its measurements: (BL) bladder length, (BW) bladder width, (NL) length of neck, (NW) width of neck, (SL) scolex length, (SW) scolex width, (BS) outer diameter of bothridial suckers.

Results

One hundred and ninety cysts were recovered from two individuals ZVCM2142 ♂ (N=117) and ZVCM2139 ♀ (N=73). Ten to 15 hours after host death, larvae were alive and easily separated themselves from host capsules. Microscopic examination of the scolex revealed 4 sessile auricular bothridia with smooth and folded edges, often loculated with adjacent edges touching (Fig.2c). One accessory sucker in each bothridium was observed (Fig.2a,c,e) and the myzorhynchus possessed an apical 5th sucker (Fig.2b,d,e). Overall cyst prevalence was 18.18% (N=190), density was 159.38 larvae *per* kg of blubber, mean intensity (total number of cysts/total number of infected hosts) was 95 and mean abundance (total number of cysts/ total number of sampled hosts) was 17.27.

Larvae were measured as follows:- neck length: NL, neck width: NW, scolex length: SL, scolex width: SW, bothridial suckers: BS, 5th apical sucker length: ASL, and width: ASW. As the data fit the normal distribution (Kolmogorov-Smirnov d=0.10449, p>0.05), basic descriptive statistics were calculated: mean values on the 114 cysts measured for size and the 20 measured for 5th apical sucker are shown in Table 1.

According to shape and size, bladder worms were grouped into 3 categories (N=85, Table 2). For category "A" (22.35%), neck always straight, BL ranging from 2.10 to 4.10mm (Fig.3b,c). In category "B" (62.35%), all with curved neck, BL ranging from 4.10 to 7.14mm (Fig.3a,d,e). Category "C" (15.30%), U-shaped (Fig.4a,b) or with projections from the wall of the cyst (Fig.4c,d), also curved neck, usually BL>7.14mm. There was a tendency for the ratio BL/NL to be >1 and <1 in categories A and C respectively, but in category B it could give either of both results.

We also found one cyst with an external morphology similar to all others except the scolex morphology consisted of 4 muscular round and smooth bothridia, without an accessory apical sucker. This specimens'

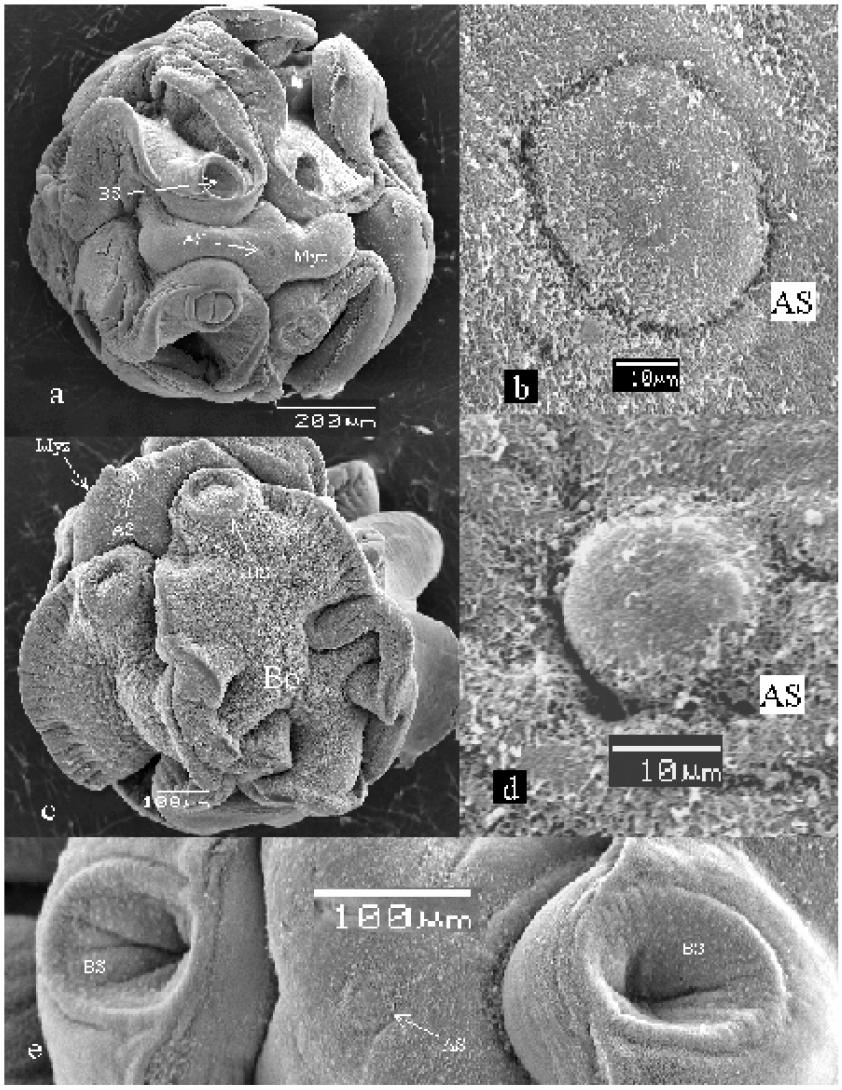

Figure 2. Scanning electron micrographs of the scolex of *Phyllobothrium delphini* (cyst removed) taken from five different scolices: (a) scolex, front view showing 4 bothridial suckers and one 5th apical sucker; (b) 5th apical sucker; (c) scolex, lateral view; (d) 5th apical sucker; (e) apical and bothridial suckers; (AS) apical fifth sucker, (Bo) bothridium, (BS) bothridial sucker, (Myz) myzorhynchus.

measurements were BL:3.57; WL:2.66; BL/NL:1.88; NL:1.89; NW:0.63 and SL:0.49, and was determined as *Tetrabothrius* sp.

The stranded dolphins were identified as *L. hosei* based on their color pattern and external morphology. No apparent pathologies were found during the inspection of the carcasses, therefore the causes of this stranding event remain unknown.

Discussion

The great scattering of our data suggests the existence of high morphological and size variability in larval development. Baer's (1932) groups "*delphinii*" and "*grimaldii*" are morphologically and spatially separated so no overlapping occurs, although they may be found in the same or different hosts. All larvae considered for this study were of the "*delphinii* group".

Table 1. Mean values (mm) of the 5th apical sucker and cysts of *Phyllobothrium delphini*.

	BL	BT	BW	BS	ASL	ASW
Mean	5.25	3.68	3.07	0.150	0.073	0.067
Standard Error	0.12	0.09	0.07	0.002	0.001	0.002
Median	5.50	4.00	3.15	0.150	0.075	0.075
Mode	6.00	4.00	3.50	0.150	0.075	0.075
Standard deviation	1.25	0.98	0.81	0.019	0.004	0.010
Variance	15.50	9.57	6.50	0.015	0.000	0.004
Range	5.80	4.30	4.10	0.075	0.012	0.025
Minimum	2.50	1.20	1.20	0.100	0.062	0.050
Maximum	8.30	5.50	5.30	0.175	0.075	0.075
N	114	114	114	114	20	20

(ASL) apical sucker length, (ASW) apical sucker width, (BL) bladder length, (BS) outer diameter of bothridial suckers, (BT) bladder thickness, (BW) bladder width.

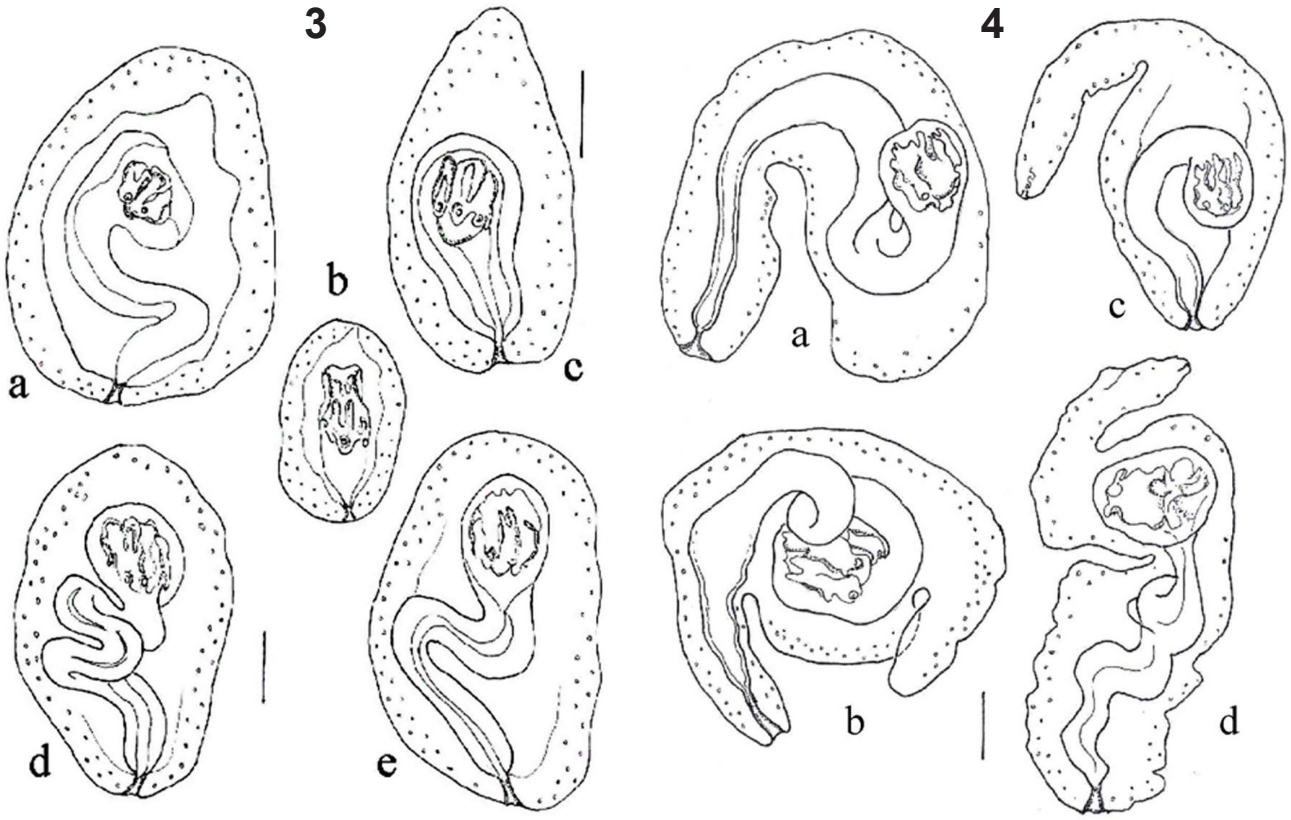

Figure 3. *Phyllobothrium delphini* morphotypes. According to the proposed classification, a,d,e = category "B"; b,c = category "A". Bar=1mm (a,b,d,e); 0.5mm (c). Figure 4. Category "C" cysts of *Phyllobothrium delphini*: (a,b) U-shaped cysts; (c,d) cysts with projections from the wall of the cyst. Bar=1mm.

Table 2. Mean values (mm) of the morphometric measurements for the 3 proposed categories in *Phyllobothrium delphini* cysts.

	BL	NL	NW	SL	SW
		Category A			
Mean	3.11	1.12	0.59	1.20	1.07
Standard Error	0.12	0.28	0.06	0.06	0.06
Median	3.01	0.77	0.63	1.26	1.05
Mode	4.06	0.35	0.70	0.84	1.05
Standard deviation	0.50	1.23	0.28	0.24	0.27
Variance	0.25	1.52	0.08	0.06	0.07
Range	1.61	5.24	1.04	0.84	0.98
Minimun	2.45	0.01	0.01	0.84	0.70
Maximun	4.06	5.25	1.05	1.68	1.68
N	19	19	19	19	19
		Category B			
Mean	5.74	5.08	0.86	1.66	1.51
Standard Error	0.10	0.23	0.02	0.04	0.04
Median	5.81	5.25	0.84	1.61	1.54
Mode	5.95	5.74	0.84	1.54	1.75
Standard deviation	0.72	1.64	0.18	0.30	0.28
Variance	0.52	2.70	0.03	0.09	0.08
Range	2.94	7.63	0.91	1.33	1.40
Minimun	4.20	1.12	0.49	0.91	0.70
Maximun	7.14	8.75	1.40	2.24	2.10
N	53	53	53	53	53
		Category C			
Mean	9.89	7.90	0.83	1.56	1.52
Standard Error	0.47	0.51	0.05	0.08	0.06
Median	10.15	8.12	0.77	1.61	1.47
Mode	12.25	8.75	0.77	1.61	1.54
Standard deviation	1.68	1.84	0.17	0.30	0.21
Variance	2.83	3.37	0.03	0.09	0.04
Range	4.55	6.30	0.63	1.05	0.77
Minimun	7.70	4.55	0.63	1.05	1.26
Maximun	12.25	10.85	1.26	2.10	2.03
N	13	13	13	13	13

(BL) bladder length, (NL) neck length, (NW) neck width, (SL) scolex length, (SW) scolex width.

Our larvae categories are difficult to compare with those previously described, due to overlapping measurements. It is not possible to compare our categories with those of Testa and Dailey (1977), which are the result of a pool of larvae (N=81) extracted from 12 different cetacean hosts (including only 7 bladder worms from *L. hosei*) within a wide geographical range. Therefore, following the assumption that our larvae correspond to *P. delphini*, we attribute the differences in size and shape to adaptation to hosts tissues and size, geographical variation, and the larval stage of development in the host. Geographical differences have been observed previously for *P. delphini* in Dall's porpoise (Walker, 2001).

The fifth apical sucker on the myzorhynchus was difficult to observe because in some specimens it was extremely reduced. This apical sucker could not be seen in the stained scolices under the optical microscope and the structure observed under the electron microscope cast doubt on its use as a fixation organ, at least at this level of development.

Larval permanence within the host could explain size and shape diversity. A possible consequence of this process could be the enlargement or folding of some cysts (category "C" cysts: 15.30%). On the other hand, cysts could be affected by density of parasites, host blubber characteristics and geographical location of both intermediate(s) and definitive hosts. Geographical location is not a factor in our samples, which come from the same species, social group and locality.

To assess the meaning of presented categories a temporal hypothesis can be suggested: category "C" cysts could be those inhabiting host tissues for longer periods, while category "A" (22.35%) could represent more recent infections. Category "B" (62.35%) cysts may be taken as intermediate, involving the main stock of oval-like, curved neck cysts. Consequently,

the morphological differences in the 3 categories of cysts could correlate to time spent in the host. The ratio BL/NL was not useful for the three categorizations proposed.

One larval form had similar external characteristics to the other bladder worms but differed internally. According to Hoberg (1994) it was determined to be *Tetrabothrius* sp. Rudolphi, 1819. This parasite has been found in *L. hosei* by Tobayama *et al*. (1973) in the stomach, and Mignucci-Giannoni *et al*. (1999) in the blubber. Due to the fact that they could share the same site of infection and external similarities these larvae could be easily confused with *P. delphini*.

We emphasize the monospecific character of this study, relating to the cetacean host *L. hosei*. Further studies on other hosts will be needed to extrapolate the categories proposed.

As previously mentioned, Fraser's dolphins could act as intermediate or accidental hosts, and definitive host infection could occur by predation on cetaceans. According to Walker (2001) the concentration of *P. delphini* cysts in the posterior-abdominal blubber layer of the hosts could be advantageous for the successful transmission of this larval cestode. The presence of bite marks probably caused by the cookie-cutter shark *Isistius brasiliensis* on stranded specimens of *L. hosei* supports this hypothesis (J. Lailson-Brito Jr, UFRJ, Rio de Janeiro, Brazil, pers. comm.; Perrin *et al*., 1993).

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