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Cystacanths of *Bolbosoma vasculosum* in the black scabbard fish *Aphanopus carbo*, oceanic horse mackerel *Trachurus picturatus* and common dolphin *Delphinus delphis* from Madeira, Portugal

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Abstract

Cystacanths of the acanthocephalan, *Bolbosoma vasculosum* Rudolphi 1819, were found to be encapsulated in the connective tissues of the viscera of the black scabbard fish, *Aphanopus carbo* and oceanic horse mackerel, *Trachurus picturatus* from Madeira, Atlantic Ocean. Juvenile worms were obtained from the intestine of a stranded common dolphin, *Delphinus delphis*, also from Madeira. Cystacanths were 11–15 mm long, with a proboscis of 18–19 longitudinal rows, eight hooks per row, and two sets of trunk spines. Overall, the morphology and dimensions of the proboscis, neck and trunk corresponded to previous descriptions. Scanning electron microscopy of the proboscis structures and trunk spines is provided for the first time. The prevalence of *B. vasculosum* in *A. carbo* increased with fish length. There were no statistical differences in the prevalence and intensity of infection between seasons. The intensity of infection was similar for male and female fishes, but there were significant differences in relation to length, longer fishes having heavier infections. *Aphanopus carbo* from Madeira represents a new host record and a new geographic location for *B. vasculosum.*

Introduction

The acanthocephalan genus *Bolbosoma* Porta 1908, established for Acanthocephala from whales, contains about 15 species (Amin, 1985). *Bolbosoma vasculosum* was described by Rudolphi (1819) as *Echinorhynchus vasculosus*. Meyer (1932) and Harada (1935, as *B. thunni* n.sp.) observed *B. vasculosum*, but due to the scarcity of materials from the definitive host, the species remains relatively unknown.

Acanthocephalans identified as *B. vasculosum* were found in black scabbard fish, *Aphanopus carbo* Lowe, oceanic horse mackerel, *Trachurus picturatus* Bowdich and a stranded common dolphin, *Delphinus delphis* L. from Madeira. The aim of this paper is to compare scanning electron micrographs of cystacanth and juvenile *B. vasculosum* and provide data on the occurrence of cystacanths in *A. carbo* and *T. picturatus*.

Materials and methods

One hundred and forty two *A. carbo* and 304 *T. picturatus* from the Atlantic Ocean, Madeira (33°7′30″–32°22′ 20″N and 16°16′30″–17°16′38″W) were examined. Most *A. carbo* were 105 to ⩾125 cm long, which is normal for commercial catches in Madeira, with seven juveniles 60–90 cm long, obtained from the Fisheries Department during October 1993 to June 1995. Adult *T. picturatus* 12–21 cm long, and juveniles 12–16 cm, were purchased from Funchal fish market during November 1995 to June 1996. The total length, weight and sex of each fish was

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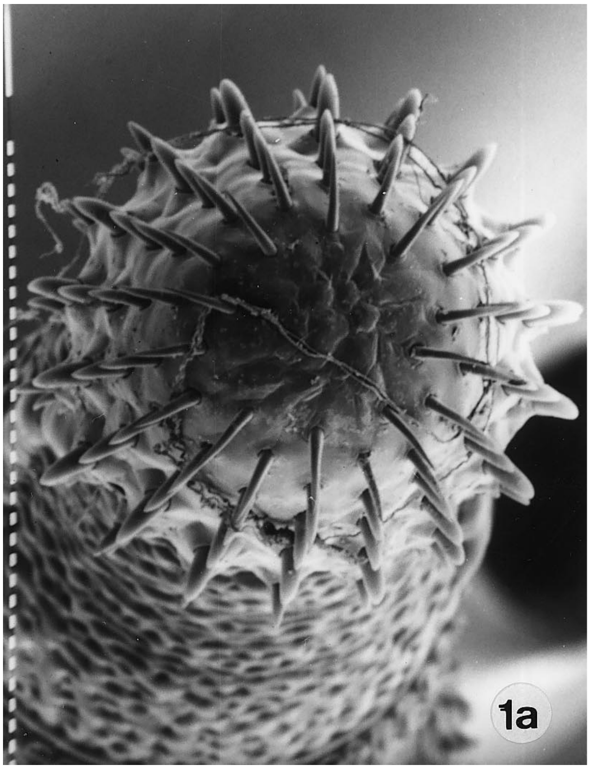
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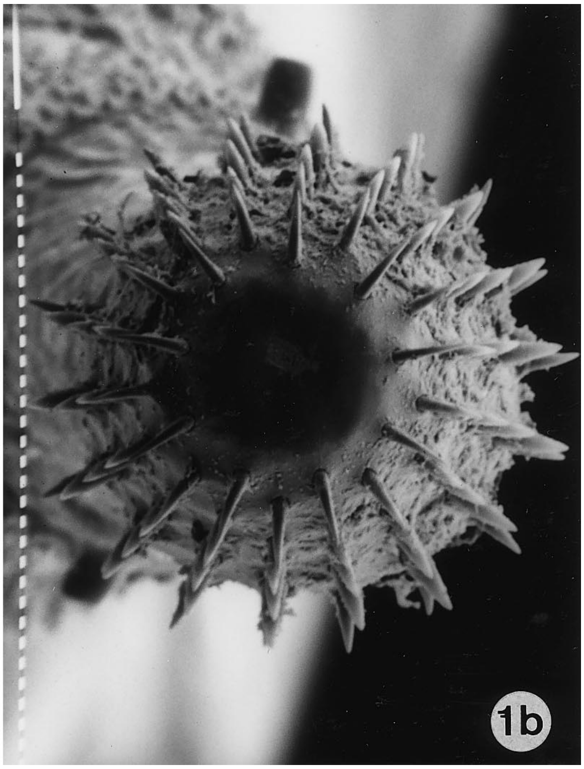
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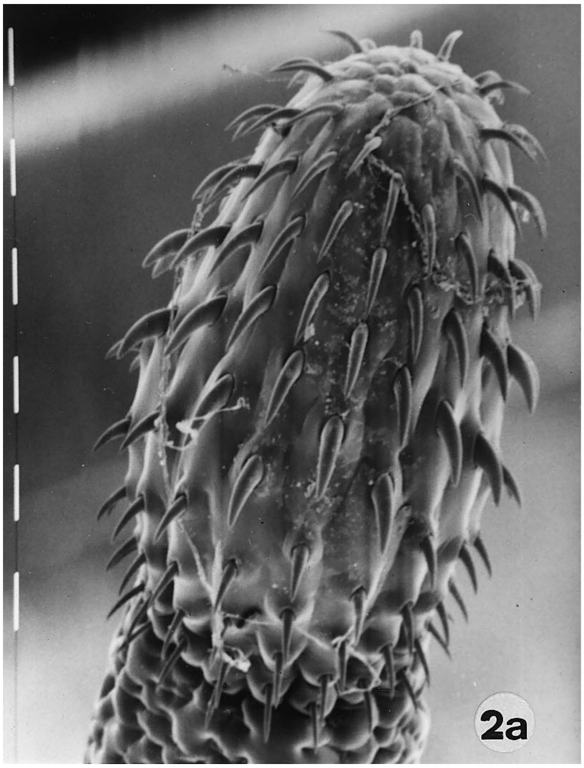
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Table 1. The total body length and width, proboscis and neck lengths and widths, of *Bolbosoma vasculosum*.

Host	Body length	Body width	Proboscis length	Proboscis width	Neck length	Neck width
Aphanopus carbo	12.25 ± 0.42 (11–15) n = 10	0.55 ± 0.03 (0.43–0.66)	0.89 ± 0.03 (0.72–1.00)	0.49 ± 0.03 (0.36–0.60)	0.90 ± 0.03 (0.72–1.00)	0.41 ± 0.02 (0.30–0.48)
Trachurus picturatus	13.70 ± 1.96 (11–15.3) n = 4	0.74 ± 0.15 (0.60–0.90)	0.77 ± 0.13 (0.60–0.90)	0.54 ± 0.06 (0.50–0.60)	no data	no data

Mean ± S.E. for *A. carbo*; mean ± S.D. for *T. picturatus*; range (min–max) in mm.

determined and juvenile acanthocephalans dissected from the visceral cavity, counted and recorded. The intestinal contents of one dead stranded *D. delphis* found January 1994 were examined for adult acanthocephalans. Acanthocephalans were relaxed in cold tap-water, fixed in 4% formaldehyde and stored in 70% ethanol. Optical microscopic observations and measurements were made after clearing worms in lactophenol. Selected acanthocephalans were dehydrated through an ethanol series, critical point dried (Polaron E 3000), coated with 60% gold-palladium (Polaron E5100 sputter coater) and viewed with a Philips 501B scanning electron microscope (SEM).

For statistical analysis of comparisons of factors such as host length, sex and prevalence and intensity of infection with acanthocephalans, a simple linear regression compared to a t-test and F-test, and one-way ANOVA were performed on the data. Results were considered statistically significant when $P \leqslant 0.05$ (Bailey, 1995). The terms prevalence, intensity, mean intensity and abundance follow the definitions of Margolis *et al.* (1982) and Bush *et al.* (1997).

Results

Orange coloured acanthocephalan cystacanths in host-response capsules were fastened to connective tissue on the outer walls of the intestine, stomach, liver and gonads of *A. carbo* and *T. picturatus*. No cystacanths were found in the muscles. After removal from the capsules, the trunk, neck and proboscis appear cylindrical, with the neck and proboscis whitish and the trunk orange in colour: measurements of specimens from *A. carbo* and *T. picturatus* are given in table 1. In *D. delphis*, juvenile acanthocephalans of similar size, with no evidence of genital development, are attached by their proboscis to the intestinal wall.

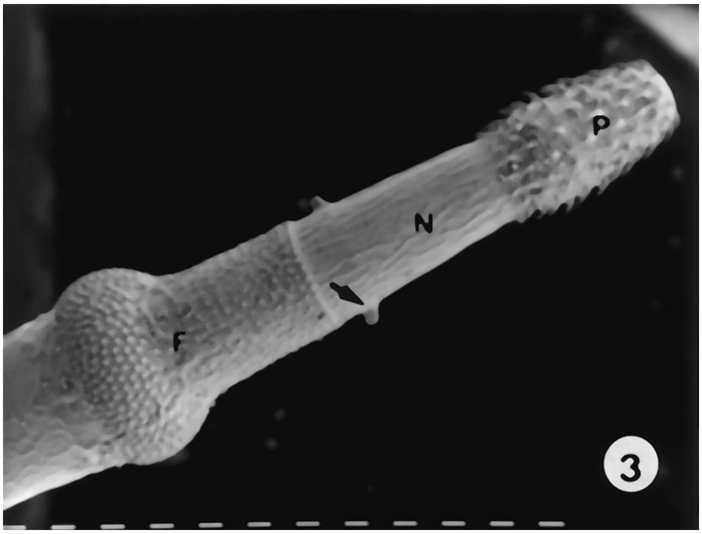
Acanthocephalans from *A. carbo* and *D. delphis* were compared using SEM. An apical view of the proboscis shows a central depression with 18 longitudinal rows with eight hooks in each row (fig. 1a,b). The proboscis is cylindrical, with an enlargment about two thirds along its length (fig. 2a,b) and hooks of three different sizes. Measurements of hooks (in μm, mean ± std. error in brackets) are as follows: H1 55.4–80.0 (69.2 ± 3.24), H2 61.5–86.1 (61.5 ± 3.06), H3 73.8–92.3 (83.8 ± 1.99), H4 73.8–92.3 (85.4 ± 2.16), H5 61.5–92.3 (76.9 ± 4.51), H6 61.5–80.0 (74.6 ± 2.16), H7 67.7–80.0 (73.1 ± 1.81), H8 61.5–80.0 (70.0 ± 3.07). The proboscis, neck and forebody are shown in fig. 3. The neck is about the same length as the proboscis, with prominent surface wrinkles in specimens from *A. carbo* (fig. 4a) but less so in worms from the intestine of *D. delphis* (fig. 4b). Two lateral, cylindrical protrusions of unknown function are present (figs 3 and 4). The anterior portion of the trunk had spines arranged in two collars (fig. 5), the anterior with about ten rings of spines around the body, the more posterior collar with seven to eight rings. The first collar of trunk spines (fig. 6) is on the cylindrical region of the trunk, whereas the second collar (fig. 7) is situated on a bulbous dilation.

Voucher specimens, reference number 4-1995, were deposited in the collection of the Marine Biology Laboratory, Department of Biology, University of Madeira.

The prevalence of cystacanths in *A. carbo* was 42.3% with seasonal variations (fig. 8) which were not statistically significant (ANOVA F = 0.945, d.f. = 141, $P = 0.427$). Prevalence increased with length (fig. 9) (F = 113.644, t = 10.66, $P = 0.0018$, r = 0.987, $r^2 = 0.974$, d.f. = 4, linear simple regression). Adult female *A. carbo* showed a higher prevalence of infection (51.30%) than males (31.59%). One of seven juvenile *A. carbo* (60–90 cm) was infected with one cystacanth. Overall *A. carbo* (60 to ⩾ 125 cm) had 1–21 cystacanths each (table 2). The intensity of infection showed no significant variations between seasons (d.f. = 3, F = 0.913, $P = 0.4369$), and there was no significant difference in the intensity of infection of females compared with males (d.f. = 1, F = 1.571, $P = 0.2123$). There was, however, a significant relationship between host length and mean intensity (r = 0.909, $r^2 = 0.826$, d.f. = 4, F = 14.242, $P = 0.0326$, t = 3.774).

In *T. picturatus* the overall prevalence was 1.3%, but fish less than 15 cm long were uninfected (n = 29), whereas fish ranging in length between 15 and 21 cm (n = 275) contained only a total of four cystacanths.

Fig. 1. Apical view of the proboscis of *Bolbosoma vasculosum* from *Aphanopus carbo* (a) and *Delphinus delphis* (b), showing rows of hooks. Scale bars = 10 μm. Fig. 2. Lateral view of the proboscis of *Bolbosoma vasculosum* from *Aphanopus carbo* (a) and *Delphinus delphis* (b). Scale bars = 62.5 μm (a) and 66.7 μm (b).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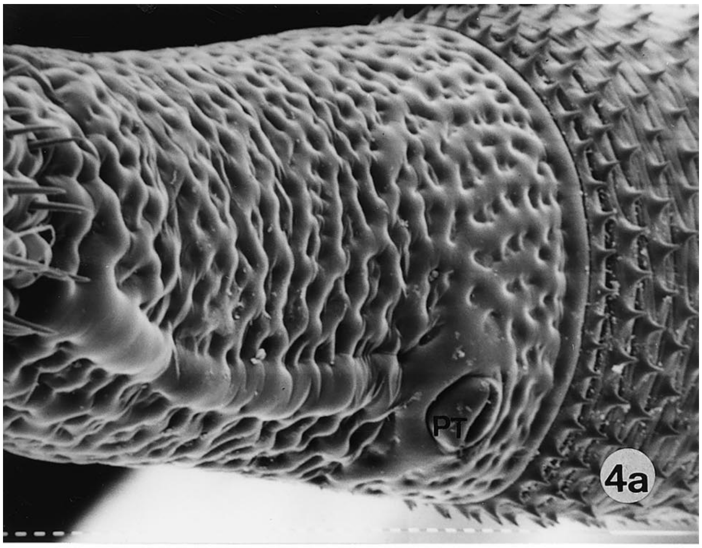
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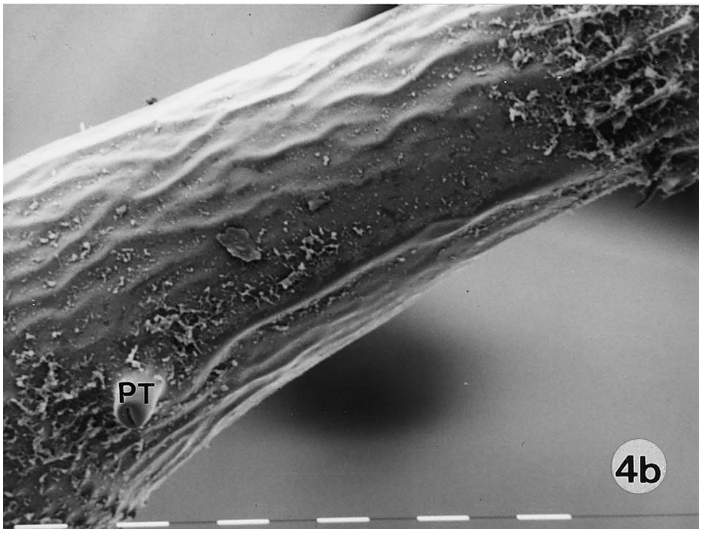
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Table 2. Intensity, abundance and prevalence of *Bolbosoma vasculosum* per length class, in *Aphanopus carbo* from October 93 to June 95.

Length class (cm)	Number of hosts	No. infected hosts	Prevalence (%)	No. parasites (range)	Mean intensity ± S.E.	Abundance ± S.E.
60–82	7	1	14.29	1 (0–1)	1	0.14 ± 0.14
105–110	12	3	25.00	4 (0–2)	1.33 ± 0.33	0.33 ± 0.19
110–115	45	14	31.11	19 (0–3)	1.36 ± 0.17	0.42 ± 0.11
115–120	35	17	48.57	33 (0–5)	1.94–0.29	0.94–0.22
120–125	29	16	55.17	69 (0–21)	4.31 ± 1.45	2.38 ± 0.89
⩾ 125	14	9	64.29	34 (0–10)	3.78 ± 1.02	2.43 ± 0.82

Total number of fish examined was 142.

Discussion

The acanthocephalan genus *Bolbosoma* Porta, 1908 includes 15 species (Amin, 1985). Adults of *B. turbinella* [two subspecies: *B. turbinella turbinella* (Diesing, 1851) Porta, 1908 and *B. turbinella australis* Skrjabin, 1972], *B. hamiltoni* Baylis, 1929, *B. capitatum* Linstow, 1880, *B. balaenae* Gmelin, 1790, *B. nipponicum* Yamaguti, 1939, *B. physeterii* Gubanow, 1952, *B. brevicolle* Malm, 1867 and *B. bobrovi* Krotov & Delamure, 1952 were collected from the intestine of marine mammals (Meyer, 1932; Petrochenko, 1958). *Bolbosoma caenoforme* and *B. heteracanthe* (Heitz, 1920) occur in salmonid fishes; *B. serpenticola* Fukui 1929 from the body cavity of *Elaphe quadrivirgata*, and *B. vasculosum* Rudolphi 1819 from dolphins and fishes. Meyer (1932) described cystacanths of *B. vasculosum* from several fish hosts and juveniles from *D. delphis*. Other sexually immature *B. vasculosum* were obtained from the Sowerby's beaked whale, *Mesoplodon bidens,* from North America (Van Cleave, 1953). *Bolbosoma thunni* Harada 1935 from the Sea of Japan, is a synonym of *B. vasculosum* according to Lincicome (1943) thus extending the distribution of this species from the Atlantic and Mediterranean to the Sea of Japan. Such a wide distribution is easily understandable, as the definitive hosts for this genus are marine mammals, mostly whales, which travel long distances (Van Cleave, 1953).

Our specimens were identified as *Bolbosoma vasculosum* Rudolphi, 1819 based on comparative measurements (table 1), proboscis hooks (number of rows of hooks and number per row) and a comparison of the morphology shown in figs 1–7. Unfortunately, some parameters were not detailed by earlier authors, so that comparisons are limited. All authors refer to the strong orange colour of the trunk of this acanthocephalan, although orange is a fairly frequent colour for acanthocephalan cystacanths. With respect to comparative measurements (table 1) worms usually were 11–15 mm long, except for those recovered from *T. thynnus* (Harada, 1935) and from *Kogia breviceps* (Pendergraph, 1971) which were a little smaller or larger respectively. Small differences in the dimensions of the body, neck and proboscis (table 1) occur due to incompletely relaxed specimens or variations in fixation procedures. In particular, the number of rows of hooks (18) and the number per longitudinal row (8–9) of our materials correspond to existing data (16–20 rows of hooks with up to 8–9 hooks per row as in Harada, 1935; Meyer, 1932; Van Cleave, 1953; Petrochenko, 1958). Hook measurements more closely resembled those described by Meyer (1932).

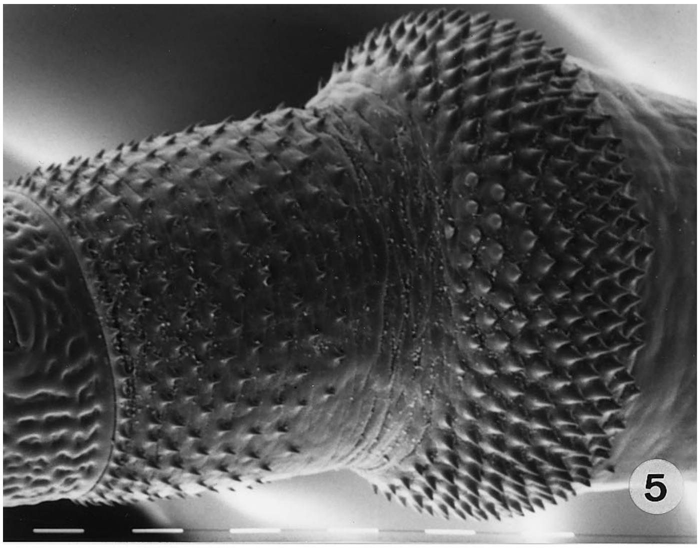
The small number and immature specimens of *B. vasculosum* seen in most investigations create a taxonomic conundrum: Van Cleave (1953) stated that 'The concept for this species has been very much in dispute. Founded on immature individuals more than a century ago, mature specimens have never yet been recorded'. Van Cleave was apparently unaware that Harada (1935, as *B. thunni* n.sp. = *B. vasculosum* according to Lincicome, 1943) examined three females with eggs, although no details other than egg measurements were provided.

The life cycle of *Bolbosoma* species involve pelagic marine zooplankton and fish as paratenic hosts (Hoberg *et al.*, 1993). Cystacanths of *B. vasculosum* have been recovered from a wide range of fish hosts (Meyer, 1932; Van Cleave, 1953) although the prevalence data from fish are not available in the literature. In our context, the considerable difference in the prevalence of cystacanths between *A. carbo* (42.3%) and *T. picturatus* (1.3%, four larvae) indicates that *A. carbo* is a more important paratenic host. Most *A. carbo* were adults (only one juvenile fish was infected with one cystacanth) while there were no significant seasonal variations in intensity of infection between male and female fishes. Prevalence increased with fish length suggesting that the infection is cumulative with each cystacanth likely to remain infective to a potential definitive host for an extended time and that the pathogenic effect of the parasite is negligible.

Larger *A. carbo* have a higher probability of acquiring new parasites, as they eat greater quantities of food, and feed on larger prey. Little is known about the diet of *A. carbo*, although Freitas (1998) found that *A. carbo* feeds on crustacean species, cephalopods and fish all from the meso- and bathypelagic zones. As *A. carbo* is a large predatory deep water fish, it is more likely to accumulate cystacanths than *T. picturatus* and serve as a reservoir of acanthocephalans for successful transmission to whales.

Fig. 3. Proboscis (P), neck (N) and forebody (F) of *Bolbosoma vasculosum*. Note two protrusions (arrow) in the neck. Scale bar = 100 μm.

Fig. 4. The neck of *Bolbosoma vasculosum* from *Aphanopus carbo* (a) and *Delphinus delphis* (b). Note neck protrusions (PT). Scale bars = 10 μm (a) and 66.7 μm (b).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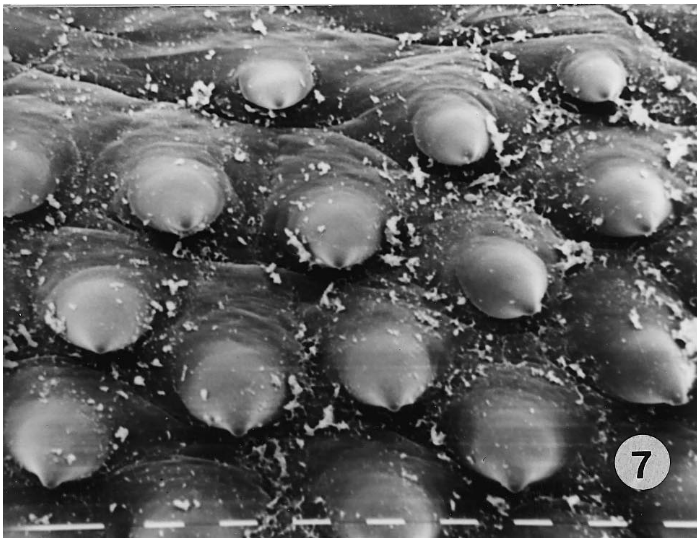
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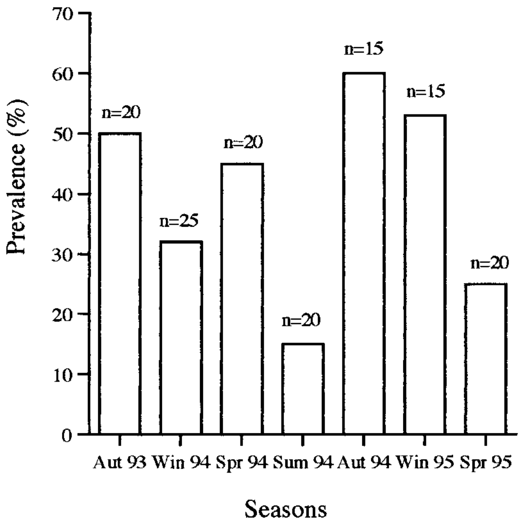

Fig. 8. Prevalence (%) of *Bolbosoma vasculosum* in *Aphanopus carbo* relative to season (n = number of fish examined).

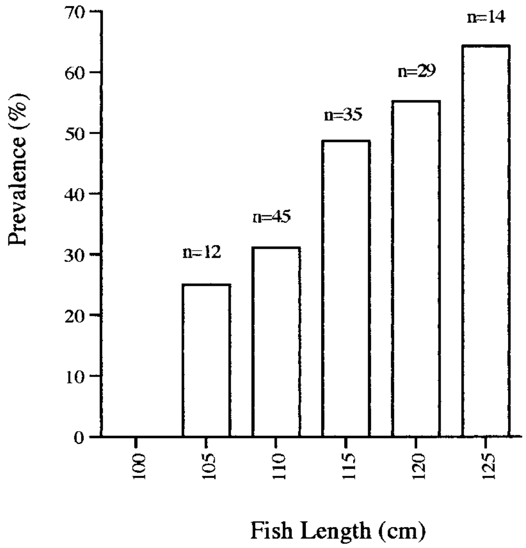

Fig. 9. Prevalence of *Bolbosoma vasculosum* in *Aphanopus carbo* relative to fish length (n = number of fish examined).

Hence a much higher prevalence of *B. vasculosum* occurs in *A. carbo* compared with *T. picturatus*. The reasons for the higher prevalence in female *A. carbo* is difficult to explain as available data on feeding ecology of this fish species is incomplete. However, differing feeding habits of male and female fish may affect rates of infection with endoparasites (Rohde, 1993).

The effect of intensity of infection on host condition in *A. carbo* was not tested, as *A. carbo* harboured concurrent infections of anisakid nematodes. As anisakids are also parasites of cetaceans (see, life cycle, Grabda, 1991) it is logical to find the two larval stages in the same paratenic host.

Juveniles of *B. vasculosum* have been most frequently reported in dolphins and whales (Meyer, 1932; Van Cleave,1953; Pendergraph, 1971; Costa *et al.*, 1996) from the Atlantic Ocean and Mediterranean. Van Cleave (1953) put forward the hypothesis that 'the fact that no sexually mature individuals have been encountered in any collection makes it seem possible that the normal definitive host has never yet been found'. If adult specimens of *B. thunni* with eggs from the Sea of Japan (Harada, 1935) are synonymous with *B. vasculosum* then they are the only adults ever reported. Van Cleave was categorical in his afirmation, that 'essential morphological features of this species seem to exclude the possibility that *B. vasculosum* might be the immature stage of one of the species which reach maturity in various whales'.

Gravid adults of several species of *Bolbosoma* are found in whales: *B. turbinella* in the blue whale *Balaenoptera musculus* (Measures, 1992); *B. capitatum* in the sperm whale *Physeter macrocephalus* (Hoberg *et al.*, 1993); *B. vasculosum* in pigmy sperm whale *Kogia breviceps* (Pendergraph, 1971) and *Mesoplodon bidens* (Van Cleave, 1953). *Kogia breviceps* and *M. bidens* occur around the coasts of Madeira: both whales could be definitive hosts, as they are capable of diving to depths of 1000 m (Mathias, 1988). Another potential deep-diving whale host, the sperm whale *Physeter catodon*, is also common around the Madeirean coasts (Mathias, 1988). *Mesoplodon bidens* and *P. catodon* feed on large benthic fish and squids (Lockyer, 1981).

The life cycle of *B. vasculosum* may have: (i) a pelagic pathway with crustacean intermediate hosts, and *T. picturatus* and dolphins as paratenic hosts, in which the parasite does not mature; and (ii) a deep-water pathway involving crustaceans, *A. carbo* as paratenic host and whales as definitive hosts where the parasites mature and produce eggs. Two alternative pathways are not unusual, for example as in *Anisakis simplex* (Oshima, 1972 quoted by Rohde, 1984). It is important, therefore, to examine freshly stranded whales and dolphins, although unfortunately strandings on the Madeirean coasts are quite rare.

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Fig. 5. Anterior portion of the trunk of *Bolbosoma vasculosum* showing spines arranged in two collars. Scale bar = 71.4 μm. Fig. 6. The trunk spines from the first collar in *Bolbosoma vasculosum*. Scale bar = 10 μm. Fig. 7. The trunk spines from the second collar in *Bolbosoma vasculosum*. Scale bar = 10 μm.

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