LARVAL TRYPANORHYNCHS (PLATYHELMINTHES: EUCESTODA: TRYPANORHYNCHA) FROM BLACK-SCABBARD FISH, *APHANOPUS CARBO* AND OCEANIC HORSE MACKEREL, *TRACHURUS PICTURATUS* IN MADEIRA (PORTUGAL)

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Summary:

Four different types of trypanorhynch postlarvae were found attached to the stomach mucosa, external stomach wall or free in the body cavity of two marine fishes from Madeira, namely black-scabbard fish, *Aphanopus carbo* and oceanic horse mackerel, *Trachurus picturatus*. Morphological features shown by SEM indicated that the postlarvae belonged to the species *Tentacularia coryphaenae*, *Sphyriocephalus tergestinus*, *Nybelinia lingualis* and possibly *N. yamagutii*. Prevalence [mean intensity (range)] of *T. coryphaenae*, *S. tergestinus* and *Nybelinia* spp. in *A. carbo* (n = 135) was 12.6 % [1.65 ± 1.27 (1-6)], 5.9 % [1.57 ± 0.79 (1-3)] and 2.2 % [1.33 ± 0.58 (1-2)] respectively. The prevalence of *T. coryphaenae* and *S. tergestinus* showed some seasonality, with a rise in prevalence of *T. coryphaenae* corresponding to a decrease in prevalence of *S. tergestinus*. However these differences were not significant. In *T. picturatus* (n = 304) only *N. lingualis* was found at a prevalence of 9.6 %. Both *S. tergestinus* and *N. lingualis* were recovered only from the stomach mucosa or external stomach wall, while *T. coryphaenae* was observed either attached to the stomach mucosa or free in the visceral cavity of the fish. The paper presents the first scanning electron micrographs (SEM) of *Sphyriocephalus tergestinus* and a new geographical record of *N. lingualis* in *T. picturatus*.

KEY WORDS : Cestodes, Trypanorhyncha, postlarvae, morphology, prevalence, marine fishes.

Résumé : LARVES DE TRYPANORHYNQUES (PLATYHELMINTHES: EUCESTODA: TRYPANORHYNCHA) CHEZ LE SABRE NOIR, *APHANOPUS CARBO* ET LE CHINCHARD DU LARGE, *TRACHURUS PICTURATUS* DE MADÈRE (PORTUGAL)

Quatre sortes de trypanorhynques postlarvaires ont été trouvées fixées à la paroi externe de l'estomac où à l'état libre dans la cavité générale de deux espèces de poissons marins provenant de Madère : le sabre noir, Aphanopus carbo *et le chinchard du large,* Trachurus picturatus. *Les caractéristiques morphologiques, étudiées au microscope à balayage indiquent que les postlarvae appartiennent aux espèces suivantes :* Tentacularia coryphaenae, Sphyriocephalus tergestinus, Nybelinia lingualis *et enfin probablement* N. yamagutii. *Le taux d'infestation [intensité moyenne (range)] de* T. coryphaenae, S. tergestinus *et* Nybelinia *spp. chez* A. carbo *(n = 135) est respectivement de 12.6 % [1.65 ± 1.27 (1-6)], 5.9 % [1.57 ± 0.79 (1-3)] et 2.2 % [1.33 ± 0.58 (1-2)]. La prévalence de* T. coryphaenae *et* S. tergestinus *semble fluctuer selon les températures saisonnières, avec une hausse de la prévalence de* T. coryphaenae *correspondant à la baisse de la prévalence de* S. tergestinus. *Néanmoins, ces différences ne sont pas significatives. Chez* T. picturatus, N. lingualis *a une prévalence de seulement 9.6 % (n = 304).* S. tergestinus *et* N. lingualis *ont été retrouvés seulement dans l'estomac ou dans sa paroi externe alors que l'on constate que le T. coryphaenae se trouve soit fixé à l'estomac soit à l'état libre dans la cavité viscérale du poisson. Ce travail présente les premières images réalisées en microscopie à balayage de* S. tergestinus *et une nouvelle mention géographique sur la présence de* N. lingualis *chez* T. picturatus.

MOTS CLÉS : *Cestodes, Trypanorhyncha, post-larvae, morphologie, taux d'infestation, poissons marins.*

INTRODUCTION

Postlarvae of the trypanorhynchs, *Tentacularia coryphaenae* Bosc, 1802, *Nybelinia lingualis* Cuvier, 1817 and *Sphyriocephalus tergestinus* Pintner, 1913 occur in a wide variety of fish species, attaining maturity in elasmobranchs (Dollfus, 1942; Bates, 1990; Campbell & Beveridge, 1994). The cestode species *T. coryphaenae* and *S. tergestinus* are widely distributed in all oceans, whereas *N. lingualis* is known from the Atlantic and Mediterranean (Dollfus, 1942; Gaevskaya & Kovaleva, 1985; Bates, 1990). The occurrence of these cestodes in Madeiran waters was previously reported by Costa *et al.* (1996), from the black-scabbard fish, *Aphanopus carbo*. Moreover *Nybelinia* sp. larvae were found in the rockpool fish, *Mauligobius maderensis* in Madeira (Gibson & Costa, 1997). Precise identification of these cestodes is possible, at the larval stage, by examining the hook arrangements on the surface of tentacles, studying the morphology of tentacle hooks, counting number of longitudinal rows of hooks and number of hooks per row (Dollfus, 1942; Campbell & Beveridge, 1994). For these morphological

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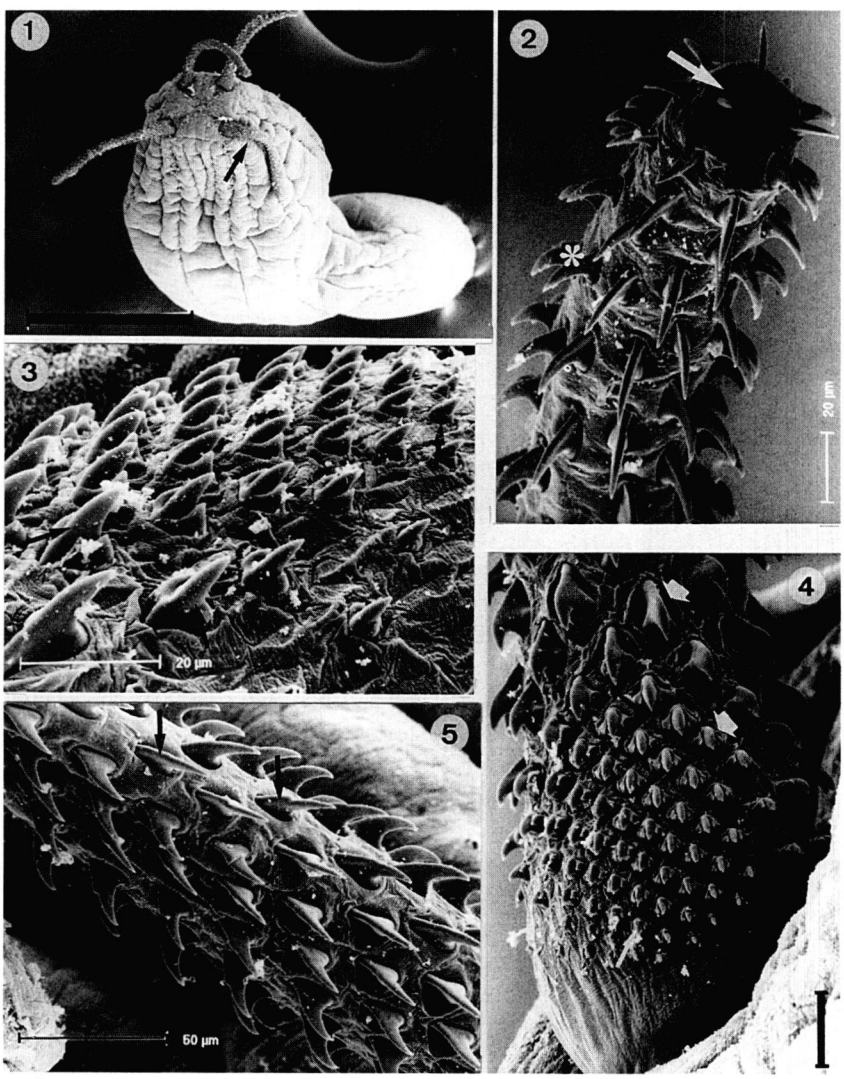
studies, scanning electron microscopy (SEM) is a powerful tool, allowing a clear observation of tentacle and hook morphology (Scholz *et al.*, 1993; Palm, 1995). In the present study, hook arrangement, hook morphology at the base and tip of tentacles, general basal and metabasal armature, number of hooks per row and number of rows of hooks were examined, by means of SEM, in order to confirm species identification. Morphological details were compared with previous reports for these species of cestodes (Dollfus, 1942; Campbell & Beveridge, 1994; Palm, 1995; Beveridge & Campbell, 1996; Palm, 1999; Palm & Walter, 2000). Furthermore prevalence and intensity values for both *A. carbo* and *Trachurus picturatus* are given.

MATERIALS AND METHODS

One hundred and thirty five *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 for cestode infections. Adult *A. carbo* ranging in length from 105 to 125 cm and both juvenile and adult *T. picturatus* ranging in lengths from 12-16 cm and 12-21 cm were obtained either from the Fisheries Department or the Fish Market at Funchal during October 1993 to June 1995 (for *A. carbo*) and November 1995 to June 1996 (for *T. picturatus*). Live postlarvae were removed from infected stomachs, by cutting a small piece of the stomach around the parasite, and placing it in tap water so that worms would release themselves naturally, without deleterious effect on parasite morphology. Those found in *T. picturatus* encapsulated in the external wall of the stomach, were released by dissection of the capsules. Relaxed worms were fixed in 70 % ethanol, dehydrated through an ethanol series, critical point dried (Polaron E3000), coated with 60 % goldpalladium (Polaron E5100 sputter coater) and viewed with Philips XL30 scanning electron microscope. Measurements are given in millimeters (mm) or micrometers (µm), with means and standard errors in brackets. Scolex and



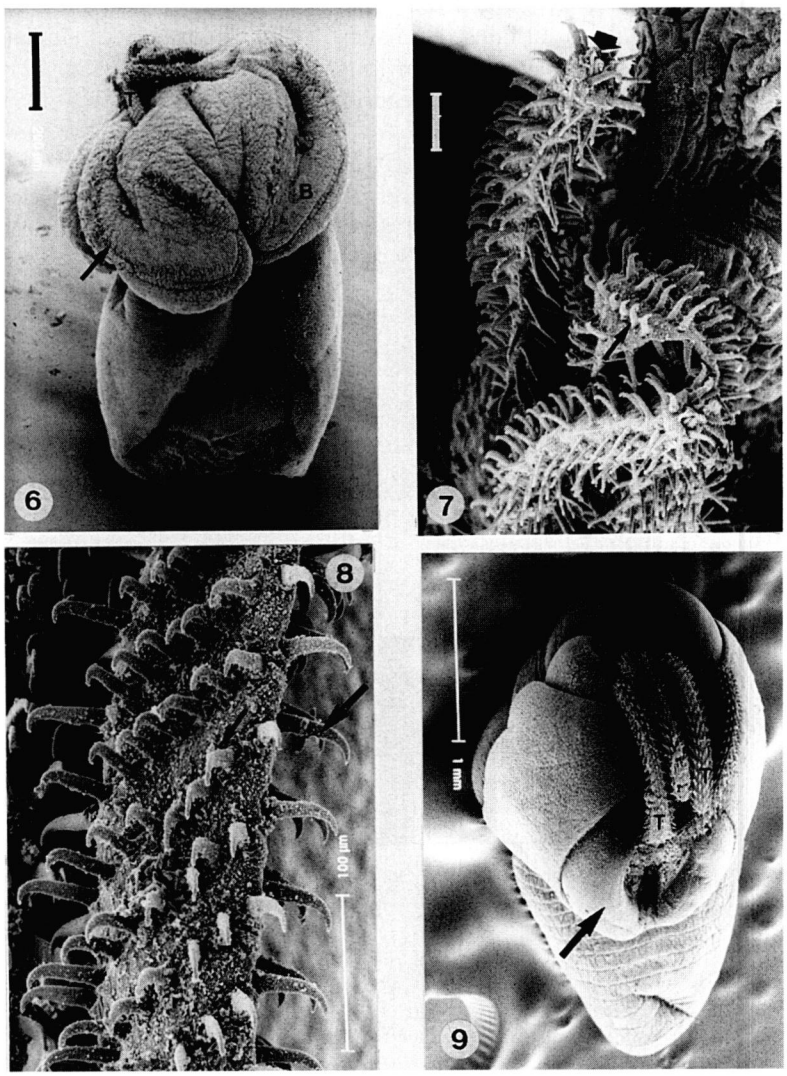
Figs 1-5. – 1: SEM view of the postlarva of *Tentacularia coryphaenae*, with four everted tentacles (arrows) (bar = 1 mm). 2: anterior extremity of tentacle of *T. coryphaenae* showing apex of tentacle (arrow) and homeomorphous hooks (star) (bar = 20 µm). 3: view of basal armature of *T. coryphaenae* showing uncinate shaped hooks (arrows) of decreasing size (bar = 20 µm). 4: detail of basal armature of *T. coryphaenae* with hooks of different sizes (arrows) (bar = 20 µm). 5: detail of metabasal armature of *T. coryphaenae* showing curved uncinate hooks (arrows) (bar = 50 µm).

hook measurements follow Palm (1999) and Palm & Walter (2000). The terminology of larval cestodes follows Chervy (2002). Mann-Whitney tests were used to compare prevalences of cestodes. Results were considered significant for $p < 0.05$ in the same host. Seasons of the year were abbreviated to Aut (autumn), Spr (spring), Sum (summer) and Win (winter). The terms prevalence and intensity follow the definitions of Bush *et al.* (1997).

RESULTS

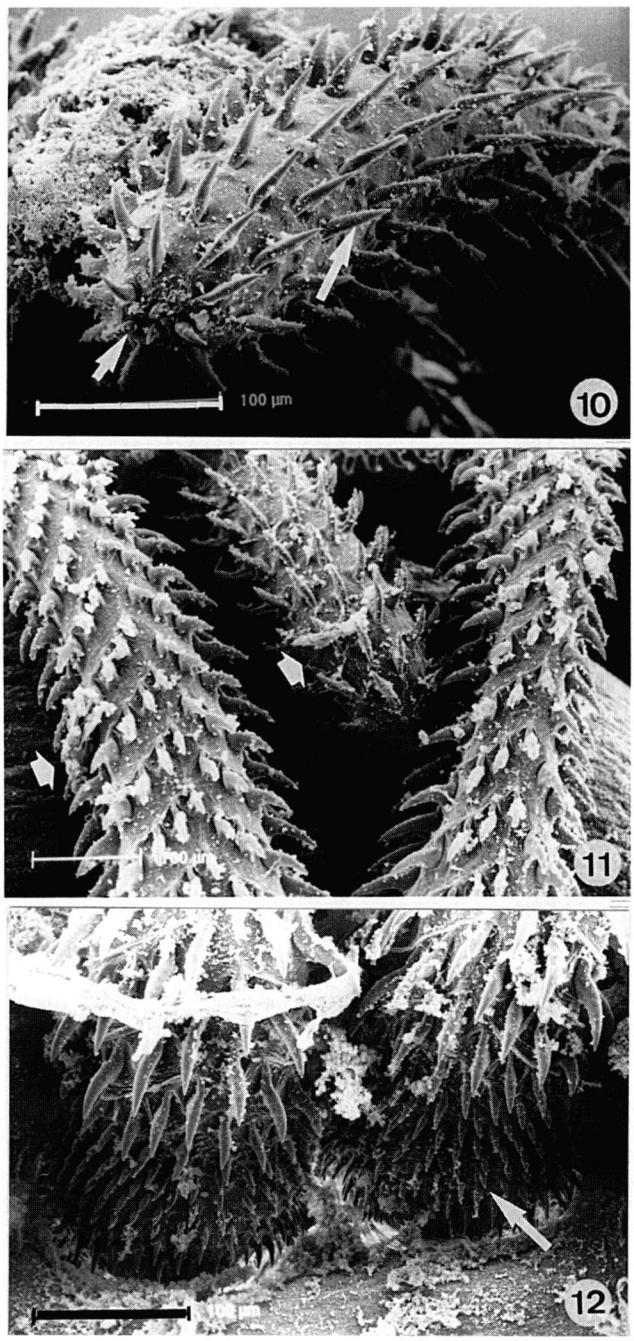
Postlarvae of *Tentacularia coryphaenae* and *Sphyriocephalus tergestinus* were found attached to the stomach mucosa of *A. carbo* only. Some postlarvae of *T. coryphaenae* were found encapsulated in the stomach lumen (smaller specimens) or free in the visceral cavity. The total scolex length (SL) of *T. coryphaenae* postlarvae ranged from 4 to 9 mm (7.83 ± 0.46, n = 12). *T. coryphaenae* had four equal-length cylindrical tentacles of about 800 µm long when fully extended (772.88 ± 22.02, n = 17) (Fig. 1). The tip of the tentacles was slightly concave with a diameter of 33 µm (Fig. 2). Basal armature presented 15-20 longitudinal rows of hooks. The hooks were uncinate shaped, measuring from 3.5 µm up to 17.6 µm in length (L = 3.5, 5.9, 7.1, 8.2, 11.8, 17.6) (Figs 3 & 4), the smallest ones at the base of the tentacle. Metabasal armature showed strong almost equally sized hooks, slightly curved and pointed, falciform shaped (24.3 to 28.6 µm, n = 7). At the apex of the tentacle, hooks were slightly smaller increasing in length towards the middle of the metabasal armature (17.5 to 25 µm). The metabasal armature carried about 12 longitudinal rows of hooks (Fig. 5), each with about 25-28 hooks.

Postlarvae of *Nybelinia lingualis*, recovered from *T. picturatus*, were found adhering to the external stomach wall, enclosed in an ovoid white capsule. Upon careful dissection of the capsule, small transparent white larvae were released. Scolex length (SL) of larvae was 1.7 ± 0.31 mm (1.3-2.8) (n = 34) (Fig. 6). The scolex had



Figs 6-9. – 6: SEM view of the postlarva of *Nybelinia lingualis* with four partially everted tentacles (T) and characteristic folliar bothridia (B and arrow) (bar = 200 µm). 7: metabasal armature of *Nybelinia yamagutii* from *Aphanopus carbo* (arrows) (bar = 74.1 mm). 8: detail of metabasal armature of *Nybelinia yamagutii* from *A. carbo* showing hooks of different sizes (arrows) (bar = 100 µm). 9: SEM view of the postlarva of *Sphyriocephalus tergestinus* showing circular bothridia (arrow) and everted tentacles (T) (bar = 1 mm).

four foliar bothridia (pbo) of 573.3 to 769.3 µm (636.92 ± 34.48, n = 7) (Fig. 6), with four tentacles emerging at the top, up to 1,600 µm in length in completely relaxed specimens. Each tentacle carried about 16 longitudinal rows of hooks. At the tip of the tentacles there was a small group of upwarded oriented hooks. Hooks at basal armature were smaller (L = 10.0-13.0 µm) than at the metabasal armature (L = 13.0-20.0 µm). Bulb length (BL) was 350 µm and bulb width (BW) was 130 µm giving a ratio of 2.7:1 typical of *N. lingualis*. Pars vaginalis (pv) slightly longer than pars bothridialis (pbo). In *A. carbo* two postlarvae of *Nybelinia* sp. and two of *Nybelinia lingualis* were attached to the stomach mucosa. Tentacular armature of the two specimens of *Nybelinia* sp. (Fig. 7), with scolex lengths (SL) 3.5 and 4.0 mm, and bothridia measuring 1,120 µm in length (pbo), was composed of strong hooks, slender and curved at the tip, falciform shaped, ranging in size from 25 to 43 µm (L = 25.7, 28.6, 31.4, 42.9) (Fig. 8). *Sphyriocephalus tergestinus* Pintner, 1913 ranging in length (SL) from 4 to 9 mm (6.2 ± 0.61, n = 10), showed two circular sucker-like bothridia (pbo) measuring 1,180 µm in diameter, with four armed tentacules of up to 1,100 µm in length (880 ± 140, n = 7) (Fig. 9). The tip of tentacles all had four hooks at the centre measuring 25 µm each (Fig. 10). The metabasal armature comprised about 16-18 longitudinal rows of curved and pointed falciform shaped hooks. These hooks were all the same size, about 41.7 µm long (Figs 10 & 11). The hooks arrangement in the metabasal armature was in spiral rows. The basal armature consisted of about 32 lines of smaller hooks, apparently of similar size, 16.7 to 20 µm long, falciform shaped (Fig. 12). Prevalences and mean intensities in *A. carbo* (n = 135) were: *T. coryphaenae*, 12.6 %, 1.65 ± 1.27; *S. tergestinus* 5.9 %, 1.57 ± 0.79 and *Nybelinia* spp. 2.2 %, 1.33 ± 0.58 respectively. Intensity of postlarvae was low with a maximum of six specimens per host for *T. coryphaenae*, three for *S. tergestinus* and two for *Nybelinia* spp. Prevalence of *T. coryphaenae* and *S. tergestinus*



Figs 10-12. – 10: metabasal armature of the tentacle of *Sphyriocephalus tergestinus* showing arrangement of hooks (arrows) (bar = 100 µm). 11: view of metabasal armature of two of the tentacles in *S. tergestinus* and apex of a third tentacle (arrows) (bar = 100 µm). 12: view of basal armature of *S. tergestinus* (arrow) (bar = 100 µm).

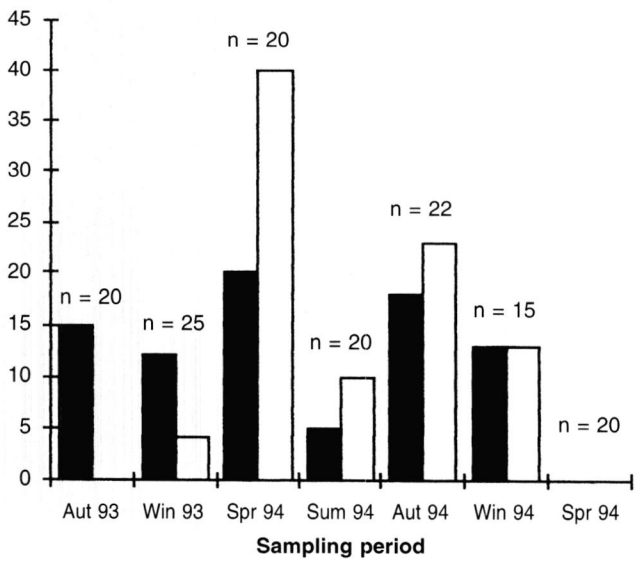


Fig. 13. – Prevalence of *Tentacularia coryphaenae* (black columns) and *Sphyriocephalus tergestinus* (white columns) in *Aphanopus carbo* related to season of the year. Abbreviations: Aut (autumn); Spr (spring); Sum (summer); Win (winter); n = number of hosts examined.

showed fluctuations with different seasons of the year (Fig. 13) with a slightly higher prevalence of *T. coryphaenae* that corresponded to a lower prevalence of *S. tergestinus*. In the autumn 1993 (Aut 93) a prevalence of 15 % was obtained for *T. coryphaenae* corresponding to a 0 % prevalence for *S. tergestinus* whilst both species had a 0 % prevalence in spring 1995 (Spr 95). The prevalences of the two species were not significantly different (Mann-Witney U: u = 21.5, z = – 0.383, p = 0.702). In *T. picturatus* only *N. lingualis* was found with a prevalence of 9.6 % (n = 304), 0 % in the winter (n = 60), 24 % in spring (n = 95) and 4.6 % in summer (n = 149).

DISCUSSION

General body morphology, total body length, the number of rows and number of hooks per row found in the present investigation for *Tentacularia coryphaenae* were identical to those reported by Dollfus (1942). Differences were found for tentacle lengths, which according to Dollfus (1942) were 100 µm long (not completely extruded) and in our study were 800 µm (completely extruded). The arrangement of the hooks in the basal (Dollfus, 1942) and metabasal armatures were similar to those seen in Dollfus (1942) and Palm (1995, 2000). Beveridge & Campbell (1996) remarked that tentacular basal armature of *T. coryphaenae* showed bilateral symmetry, with identical internal and external surfaces of the tentacle and hooks arranged in ascending rows. They pointed out that this type of arrangement is typical of heteroacanths and thus *T. coryphaenae* presents a mixed-pattern of tentacular armature, typical of homeoacanths (at the metabasal armature) and of hetereoacanths (basal armature). The arrangement of hooks at basal armature is well ilustrated in Figure 4 of the present work. Likewise scolex lengths and morphology for our *Nybelinia lingualis* found in *T. picturatus*, corresponded to the descriptions of Dollfus (1942), Palm (1995, 1999), Palm & Walter (2000) for that species, especially in details of the form and arrangement of hooks at the tip and middle of tentacles, the shape of bothridia, body size and overall morphology. Two of the specimens infecting *A. carbo* showed a different hook morphology and size (see Figs 7 & 8). The arrangement of hooks, shape and size of the metabasal armature of these individuals were closer to those found in *Nybelinia yamagutii* (Dollfus, 1960), a species described based on postlarvae from *Liosaccus cutaneus* from the west coast of North Africa. This author commented on the remarkable similarity of the species with *N. syngenes* (Pintner, 1929). Palm (1999) erected a new genus *Heteronybelinia* for those species with tentacular armature with heteromorphous hooks on different tentacle surfaces, and placed *Nybelinia yamagutii* into this new genus, renaming it as *Heteronybelinia yamagutii*. This species was redescribed by Palm (1999) and Palm *et al.* (1997) based on postlarvae from six teleosts and one cephalopod, *Todarodes angolensis* (ommastrephid squid) from the Moçambique coast, and one adult from *Sphyrna lewini* (South Africa). Our specimens from *A. carbo* are tentatively placed as *Heteronybelinia yamagutii*. Postlarvae of *Sphyriocephalus tergestinus* corresponded to previous morphological descriptions (Dollfus, 1942; Campbell & Beveridge, 1994) except that the length of tentacles in our specimens was slightly smaller, perhaps owing to incomplete eversion. The number of rows of hooks in the metabasal armature corresponded to values reported by previous workers (Dollfus, 1942; Beveridge & Campbell, 1996). Because we were unable to get clear SEM close-ups of basal armature we cannot give a detailed description of the morphology of these hooks. Nevertheless based on the size of the bulbs, twice as long as wide (see Dollfus, 1942), the fact that tentacular metabasal armature appears to have glide reflection symmetry (Fig. 11) as reported for *S. tergestinus* in Beveridge & Campbell (1996) and the arrangement and size of hooks of basal armature (Fig. 12) these postlarvae were assigned to *S. tergestinus*.

Postlarvae of *T. coryphaenae* have been recovered from a wide variety of teleosts, and the adults from sharks (Dollfus, 1942). This indicates that this cestode has a low host specificity both at the final larval and adult stage. Adults of *N. lingualis* have been found in the pyloric stomach and spiral valve of sharks and rays, namely *Mustelus* species, *Galeus* species and *Isurus oxyrhynchus* (Dollfus, 1942; Gómez Cabrera, 1990) which are found in Madeira. Postlarvae of *N. lingualis* were found in cephalopods (Dollfus, 1929) as well as in several teleosts (Dollfus, 1942). Here too low host specificity applies. Adults of *S. tergestinus* were reported from some shark hosts, from the Mediterranean and Pacific coast of Japan (Dollfus, 1942). Postlarvae of the genus *Sphyriocephalus* were recovered from the shark *Centroscymnus coelolepsis* (Dollfus, 1942; Bates, 1990). This shark species was commonly caught in Madeiran waters, at the time of sampling, and preys on *A. carbo*. Mature stages of *S. tergestinus* may occur in the spiral intestine of this shark. Adults were also recovered from *Isurus glaucus* and *Alopias vulpinus* (Dollfus, 1942). Cephalopods are important elements of the diet of *A. carbo* and *T. picturatus* in Madeira, including the families Ommastrephidae, Sepiolidae and Loliginidae (Jesus, 1992; Freitas, 1998). These families, and in particular the genera *Loligo*, *Sepia* and *Ommatostrephes* were implicated in the transmission of *Nybelinia lingualis* (Dollfus, 1942). The fact that those cephalopod genera did not represent a significant role in the diet of *A.carbo* (Freitas, 1998) might explain the low pre-

valence of *Nybelinia* in this fish host. As for *T. picturatus*, its position in the food chain as mainly a zooplankton feeder, where low numbers of larval cestodes, are expected to be present, could explain the absence of *S. tergestinus* and *T. coryphaenae* and the relative low prevalence of *N. lingualis*, probably transmitted by the ingestion of some cephalopod prey. Postlarvae of *T. coryphaenae* are known to be widespread in several zooplankton species (Dollfus, 1942), and their transmission to *A. carbo* could be through an initial infection of its cephalopod prey. It appears that *T. coryphaenae* and *N. lingualis* are characteristic cestodes of the epi- and mesopelagic regions. This might explain the low prevalences and intensities in *A. carbo* (a deep-water fish) compared to other literature results in which prevalences were much higher. As an example we refer to Amato *et al.* (1990), who reported infestations of the muscles of *Katsuwonus pelamis* (skipjack tuna) by postlarvae of *T. coryphaenae*, with intensities from one up to 110 larvae, which led to commercial rejection of infected tuna. Prevalence was very high (92.9 %, n = 28), which indicates that possibly the life cycle of this cestode is concentrated in the epipelagic environment. Although in the black-scabbard fish, *A. carbo*, prevalence of this cestode was comparatively low (12.6 %, n = 135), the fact that some postlarvae were seen free in the visceral cavity, could provide a potential cause for rejection by consumers. Likewise, prevalence with *S. tergestinus* in *A. carbo* was low, not more than 5.9 %. Such low prevalence may be related to a decrease in the stock of the shark definitive host, *Centroscymnus coelolepis* in Madeiran waters (unpublished data from the Fisheries Department of Funchal, Madeira). On the other hand, *A. carbo* is a deep-water fish caught mainly between 800 to 1,600 m depth (Nakamura & Parin, 1993); most fish are found to have everted stomachs owing to the abrupt change in pressure on lifting to the surface. Consequently, a percentage of larval cestodes may have been lost due to eversion of stomachs in this fish. This could be particularly true for *Nybelinia* specimens which were the smaller of the larval trypanorhynchs found in the present work.

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