Endohelminth parasites of the blacktail comber *Serranus atricauda* (Pisces: Serranidae), from Madeira Archipelago (Atlantic Ocean)

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ABSTRACT: Four different endohelminth parasite taxa were found in the viscera of the blacktail comber *Serranus atricauda* Günther, 1874 caught in the Madeira Archipelago. Nematodes were the dominant group, represented by 2 different taxa, *Hysterothylacium* spp. Ward & Magath, 1917 and *Procamallanus* (*Spirocamallanus*) *halitrophus* Fusco & Overstreet, 1978 comb. n. Plerocerci of the trypanorhynch *Pseudogrillotia epinepheli* (synonym: *Grillotia epinepheli*) Scholz, Garippa & Scala, 1993, and cystacanths of the acanthocephalan *Bolbosoma vasculosum* Rudolphi, 1819 were found in the visceral cavity. New host records for *P.* (*S.*) *halitrophus* and *P. epinepheli* and the extension of the geographic distribution of these 2 parasite species provide evidence of parasite transference between the Madeira Archipelago, the Mediterranean and the Gulf of Mexico. The paucity of the parasite fauna of blacktail comber reflect a combination of fish host selective feeding on particular dietary items and its territorial behaviour.

KEY WORDS: Serranidae · *Serranus atricauda* · Trypanorhyncha · Anisakid · *Hysterothylacium* · *Bolbosoma vasculosum* · Acanthocephala · Madeira Island

INTRODUCTION

Serranids are important components of coastal marine ecosystems, being active predators of a diverse array of invertebrates and fishes (Heemstra & Randall 1993, Tuset et al. 1996). Several authors have described the helminth parasites infecting fishes from the family Serranidae (Moravec et al. 1995a,b, 1996, 1997, 2004, 2006, Rigby & Adamson 1997), with a focus on groupers (genus *Epinephelus*). Few studies have focused on the genus *Serranus*, despite their commercial importance and abundance (Tuya et al. 2006). Parasite surveys have been conducted on only 4 of the 30 valid species of this genus, revealing

infections with ectoparasitic copepods, isopods and hirudeans as well as endohelminth taxa, including myxozoans, nematodes, cestodes and digeneans (Arculeo et al. 1997, Cuyás et al. 2004, Figus et al. 2005, Braicovich & Timi 2009). The blacktail comber *Serranus atricauda* Günther, 1874 (Pisces: Serranidae), is a benthic and highly territorial fish species (Morato et al. 2000), with a broad geographic distribution, encompassing the rocky shores of the Northeast Atlantic from the Bay of Biscay to Mauritania, including the archipelagos of Madeira, the Azores and the Canary Islands, as well as the Mediterranean (Tortonese 1986). As the blacktail comber is a site-attached fish species, with such a broad geographic

range, its parasites may prove to be valuable as environmental and population structure indicators (MacKenzie 1999, Cuyás et al. 2004), as well as providing an understanding of parasite zoogeographic relationships. The present study: (1) established a baseline for the parasite fauna of *S. atricauda* caught off Madeira and the Selvagens Islands (Macaronesian Biogeographic Region, Atlantic Ocean), (2) examined changes in parasite biodiversity between 2 sampling events, (3) described the geographic links of parasites found in the Madeira Archipelago with the Mediterranean and Gulf of Mexico.

MATERIALS AND METHODS

Fish hosts, collection and morphological examination of parasites

From February to June 1998, 31 blacktail comber *Serranus atricauda* were caught off the Selvagens Islands and examined for endohelminth parasites. Fish ranged in length from 13.8 to 31.5 cm (24.3 ± 0.8; mean ± standard error). The viscera of 34 fish obtained at the Funchal fish market (Madeira Island) were also examined. Both sampling regions, Madeira and the Selvagens Islands, are part of the Madeira Archipelago, Macaronesian Biogeographic Region, 30° 01′ 35″ to 30° 09′ 10″ N and 15° 52′ 15″ to 16° 03′ 15″ W). The viscera of the 34 market blacktail comber were examined fresh, and the 31 fish from the Selvagens Islands were frozen and later defrosted in the laboratory. A second sampling event, in October 2010, consisted of 23 blacktail comber ranging in length from 17 to 36.0 cm (26.5 ± 0.9) caught off the Selvagens Islands. These fish were examined fresh, immediately after being caught. The liver, stomach, pyloric caeca and intestines of all fish were examined with the aid of a binocular microscope (Zeiss Stemi 2000). Fresh squashes were also examined with a high power microscope (Zeiss Axioplan Photomicroscope). Recovered parasites were preserved in either 70 or 95% ethanol and identified according to zoological group following Berland (1961), Anderson (1992), Campbell & Beveridge (1994), Amin (1998), Palm (2004) and Gibbons (2010). Nematodes, cestodes and acanthocephalans used for morphological study were cleared in lactophenol and mounted in glycerol jelly. Measurements of morphological characters of the parasites are expressed as means (± standard error), with the range in brackets. Five larval nematodes and 4 larval cestodes fixed in 70% ethanol

were rehydrated in a descending ethanol series, post-fixed in 1% osmium tetroxide in cacodylate buffer, dehydrated in an ascending ethanol series, critical point dried (Baltec CPD 030), coated with gold/palladium and viewed with a FEI-Quanta 400F scanning electron microscope (SEM). Nomenclatural terms for description of trypanorhynchs follow Campbell & Beveridge (1994) and Palm (2004). Ratios between character measurements in trypanorhynchs were calculated by dividing each measurement by the smallest value (Palm 2004). Prevalence, mean intensity and mean abundance of the parasites were calculated according to Bush et al. (1997).

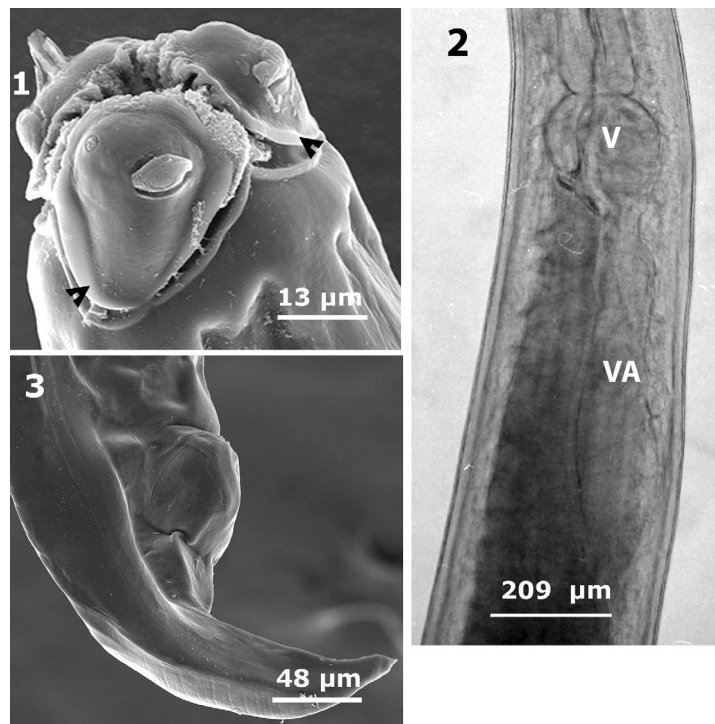### Molecular analysis of nematodes

Nineteen larval nematodes, recovered from the viscera of 2 blacktail comber caught in July 2010, were used for molecular identification. Each specimen was first inserted into a sterile 1.5 ml Eppendorf tube, frozen in liquid nitrogen, and homogenized in 100 µl of lysis solution (0.1M Tris-HCl, 0.01M EDTA, 0.1M NaCl including 1% SDS). Then 10 µl of proteinase K was added, and the tube was vortexed, followed by incubation in a water bath at 40°C for 6 h. After that period 50 µl of 5 M NaCl was added to each homogenate, and the mixture was centrifuged for 5 min at 12 000 rpm (11 269 × g). The supernatants were transferred to new tubes with addition of 30 µl of 3 M NaOAC, kept at −20°C for 20 min, and thereafter centrifuged at 12 000 rpm for 5 min. Supernatants were transferred to new microtubes, adding 60 µl of 0.2 M NaCl and 540 µl of cold absolute ethanol, and then centrifuged at 12 000 rpm for 20 min. Pellets were washed with 70% ethanol. To amplify the ribosomal DNA region (spanning the internal transcribed spacer regions ITS-1 and ITS-2) 2 primers were used, namely the forward primer NC5 and the reverse primer NC2 (Zhu et al. 1998). All PCR reactions were carried out in a final volume of 25 µl containing 1 µl of purified DNA, 0.5 µl of each primer, 2.5 µl of buffer solution 10×, 1.25 µl of MgCl$_2$, 0.2 µl of Taq DNA polymerase, 2.0 µl of each dNTP and 17 µl of ultra-pure H$_2$O in a thermocycler (Perkin Elmer Gene AMP system 2400, Applied Biosystems) under the following conditions: 94°C for 4 min, followed by 30 cycles at 95°C for 30 s, 52°C for 40 s, 72°C for 1 min and 12 s, and a final elongation at 72°C for 7 min. Amplified fragments were subjected to RFLP analysis using 2 restriction enzymes, *Hha*I and *Hae*III. Restriction was performed using 6 µl of

amplified products, 0.5 µl of enzyme, 1 µl of specific buffer (according to Fermentas Molecular Biology) and 3 µl of ultra-pure H$_2$O. Digested amplicons were separated on 1% agarose gels at 100 V at room temperature for 30 min. PCR products were purified with Qiaquick columns (Qiagen) prior to sequencing. Sequencing was performed on an ABI Prism 3700 sequencer (Applied Biosystems) by Macrogen Inc. Sequences were assembled using the SeqMan program of the DNASTAR Lasergene software package, and then aligned with previous sequences of Raphidascaridinae using Clustal W (1.8) multiple sequence alignments (Thompson et al. 1994). To construct a phylogenetic tree sequences were edited with MacClade (Version 4.0) (Maddison & Maddison 2000) and compared with available sequences of anisakid nematodes in the GenBank database. The phylogenetic tree was constructed with the neighbour joining (NJ) method, using molecular evolutionary genetics analysis (MEGA 4) software (Tamura et al. 2007). Gaps and missing data were excluded from the analysis. The new sequence was deposited in the EMBL database with the Accession No. HE610414.

Figs. 1 to 3. *Hysterothylacium* spp. from the viscera of *Serranus atricauda*. Fig. 1. Ventral view of the cephalic end, showing the lips (black arrowheads). Fig. 2. Ventricular region, showing the nearly spherical shaped ventriculus (V) and the ventricular appendage (VA). Fig. 3. Tail with pointed tip

RESULTS

Hysterothylacium spp.

A total of 599 larval nematodes of the genus *Hysterothylacium* Ward & Magath, 1917 (Nematoda: Ascarididae: Raphidascaridinae) were recovered from 65 blacktail comber *Serranus atricauda* examined in 1998, whereas in the 23 fish examined in October 2010, 1089 larval *Hysterothylacium* spp. were found. Larvae were dissected out of the brown nodules attached to the liver, stomach, intestine, intestinal caeca and gonads. Most larvae had weakly developed lips and a conical tail with a small terminal cuticular spike, suggesting they were all in L3 developmental stage. Some larval worms had a cephalic end with 3 lips of equal size, with inner margins indented and with no interlabia (Figs. 1 & 2). A short intestinal caecum, not easily seen in most individuals, varied in length from 55 to 120 µm. The ventrally curved tail showed a pointed distal tip (Fig. 3). Measurements of the oesophagus, ventriculus, ventricular appendage and tail of a sub-sample of these larvae are presented in Table 1. An 832 base

pair (bp) portion of rDNA spanning both the ITS-1 and ITS-2 regions was amplified from 12 individual nematode larvae. The digestion of this fragment with the restriction enzyme *Hha*I gave 3 fragments of 349, 150 to 154 and 77 bp, while with *Hae*III 2 fragments of 250 and 334 bp were obtained. These patterns were not previously known for anisakid nematodes. The ITS sequences (832 bp) of this species were identical to the newly described species *Hysterothylacium deardorffoverstreetorum* (Knoff et al. 2012) and showed 99% similarity with *Ra-*

Table 1. Morphometric data for *Hysterothylacium* spp. (n = 50) from the viscera of the blacktail comber *Serranus atricauda*. All measurements are in µm, except body length

Character	Range	Mean ± SE
Body length (mm)	10–25	15.5 ± 0.5
Oesophagus	750–1570	1094.5 ± 23.1
Ventriculus	95–350	192.8 ± 9.1
Ventricular appendage	450–1700	869.1 ± 16.3
Tail	125–500	289.2 ± 16.3

phidascaris acus Bloch, 1779 and *Hysterothylacium muraenesoxin* (Luo, 1999) Li, Xu & Zhang, 2006 (synonym: *Contracaecum muraenesoxin* Luo, 1999). Fig. 4 compares the sequence obtained in the present study with the available sequences of *Hysterothylacium* spp. and of 2 species of *Raphidascaris*. Prevalence, mean intensity and mean abundance of this and other endohelminths are presented in Table 2.

Table 2. Prevalence, mean intensity and mean abundance of endohelminth parasites found infecting *Serranus atricauda* from the Madeira Archipelago, in 2 sampling events (1998 and 2010)

Parasite species	Sampling year	Prevalence (%)	Mean intensity	Mean abundance
Hysterothylacium spp.	1998	83.1	11.1	9.2
(Nematoda)	2010	100.0	47.3	47.3
Pseudogrillotia epinepheli	1998	32.3	6.2	2.0
(Cestoda)	2010	0.0	0.0	0.0
Procamallanus (*Spirocamallanus*)	1998	3.1	5.5	0.2
halitrophus (Nematoda)	2010	65.2	2.7	1.8
Bolbosoma vasculosum	1998	3.1	1.5	0.05
(Acantocephala)	2010	0.0	0.0	0.0

Procamallanus (*Spirocamallanus*) *halitrophus* comb. n.

From the intestines of 2 out of 31 fish caught in 1998, 11 gravid females of *Procamallanus* (*Spirocamallanus*) *halitrophus* Fusco & Overstreet, 1978 comb. n. (Nematoda: Camallanidae) were recovered. Eighteen males and 23 gravid females were recovered from the intestines of 15 out of 23 fish caught in 2010. When alive, these large nematodes were reddish in colour with a brown buccal capsule (Fig. 5). Females were longer than males, with total body lengths from 25 to 35 mm, whereas males were 12 to 17 mm long. Gravid females contained hundreds of embryos and larvae, with each larva about 275 µm long. The tip of the female's tail was armed with 2 minute spines. In contrast, the posterior region of males was ventrally bent with wide caudal alae and

2 spike-like structures on the tip. Around the cloacal opening of the male, papillae were organized into 3 pairs of precloacal, 6 pairs of postcloacal and 2 pairs of adanal papillae. Spicules were dissimilar, with one partially protruding through the cloacal opening. Comparative morphometry of specimens of *P.* (*S.*) *halitrophus* from the Gulf of Mexico and Selvagens Islands is given in Table 3. Prevalence, mean inten-

Fig. 5. *Procamallanus* (*Spirocamallanus*) *halitrophus* comb. n. Cephalic end (from the intestine of *Serranus atricauda*), showing the buccal capsule (B) with spiral thickenings and basal ring (arrow)
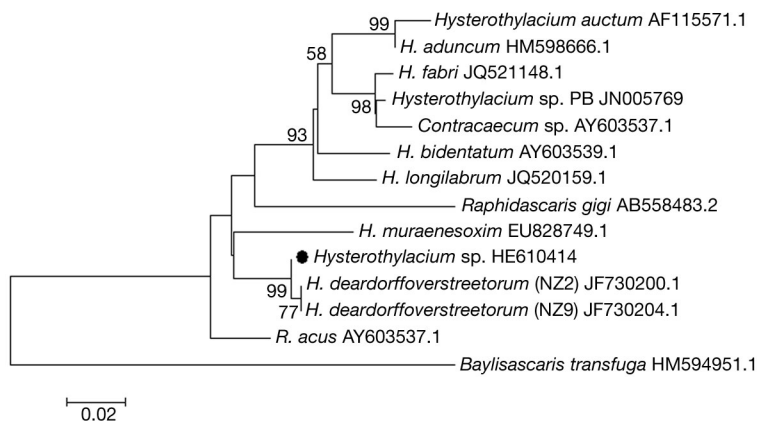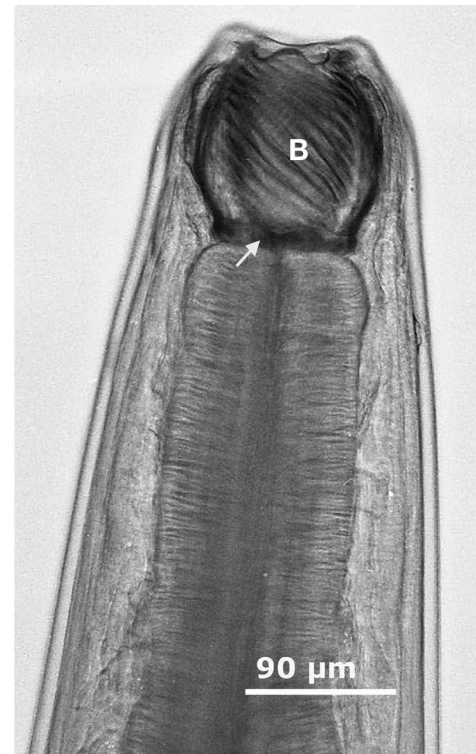

Fig. 4. Neighbour-joining phylogenetic tree inferred from internal transcribed spacer region sequence data from *Hysterothylacium* spp. in this study (●), and sequences of other Raphidascaridinae species, available from GenBank. Gaps and missing data were excluded from analysis. Bootstrap values >50% are indicated by numerals

Table 3. *Procamallanus* (*Spirocamallanus*) *halitrophus* comb. n. Comparison of measurements of specimens from the Gulf of Mexico (Fusco & Overstreet 1978) and Madeira Archipelago (present study). All measurements are in µm, except body length (mm). Values are means (±SE) with range in brackets for specimens collected from *Serranus atricauda*

Morphological character	Gulf of Mexico samples		Madeira Archipelago samples	
	Males (n = 16)	Females (n = 15)	Males (n = 6)	Females (n = 5)
Body length (mm)	13.3–26.9	22.5–44.5	14.0±0.8 (12–17)	31.0±1.9 (25–35)
Buccal capsule (length × width)	86–98 × 63–81	96–121 × 84–96	82.9±2.4 (75–85) × 68.3±4.4 (60–75)	101.3±5.9 (85–110) × 110.0±13.5 (90–150)
Spiral thickenings	10–14 (12)	10–14 (12)	12	12
Length of muscular oesophagus	390–465	459–609	448.3±12.2 (410–500)	626.7±43.3 (520–700)
Length of glandular oesophagus	517–928	696–963	833.3±40.1 (700–950)	1000±100.0 (800–1100)
Ratio length of muscular oeso-phagus:glandular oesophagus	–	1:1.5	1:1.5–1.8	1:1.5–1.8
Anus	160–189	191–255	150	160–275
Caudal alae	425–610	–	400–450	–
Caudal papillae	3, 6, 2	–	3, 6, 2	–
Right spicule	257–459	–	386.0±30.4 (315–450)	–
Left spicule	183–324	–	225	–
Spicule ratio	1:1.3–1.8	–	1:1.4	–
Tail digit	–	36–73	–	55.0±5.0 (37.5–60)
Host	*Scyacium papillosum*	*Scyacium papillosum*	*Serranus atricauda*	*Serranus atricauda*

sity and mean abundance values of *P.* (*S.*) *halitrophus* are presented in Table 2.

Pseudogrillotia epinepheli

A total of 130 plerocerci of *Pseudogrillotia epinepheli* Scholz, Garippa & Scala, 1993 (Platyhelminths: Lacistorhynchidae: Grillotiinae) were recovered from 21 of the 65 fish examined in 1998 only. They were found inside whitish blastocysts, which were in yellowish nodules, adherent to the external walls of the stomach, intestine, pyloric caeca, liver and gonads (Fig. 6). The dimensions of morphological features (scolex length [sl], length and width of pars bothridialis [pbo], length and width of pars vaginalis [pv], length and width of bulbs [bl and bw, respectively]) in specimens from the Madeira Archipelago were compared with previous results for specimens collected from the Mediterranean by Scholz et al. (1993) (Table 4). The ratios pbo:pv:bl of 1:4.2:1.5 and bl:bw of 1.9:1 were consistent with those previously obtained for this species (see Table 4). Moreover, the type and patterns of the tentacular armature (both basal and metabasal armatures), and the presence of multidigitated palmate microtriches in the bothrial surface (Figs. 7 to 9) were in agreement with previous morphological descriptions for the species. In addition, the bulbs were longer than wider, as expect for *P. epinepheli* (Fig. 10). Prevalence, mean intensity
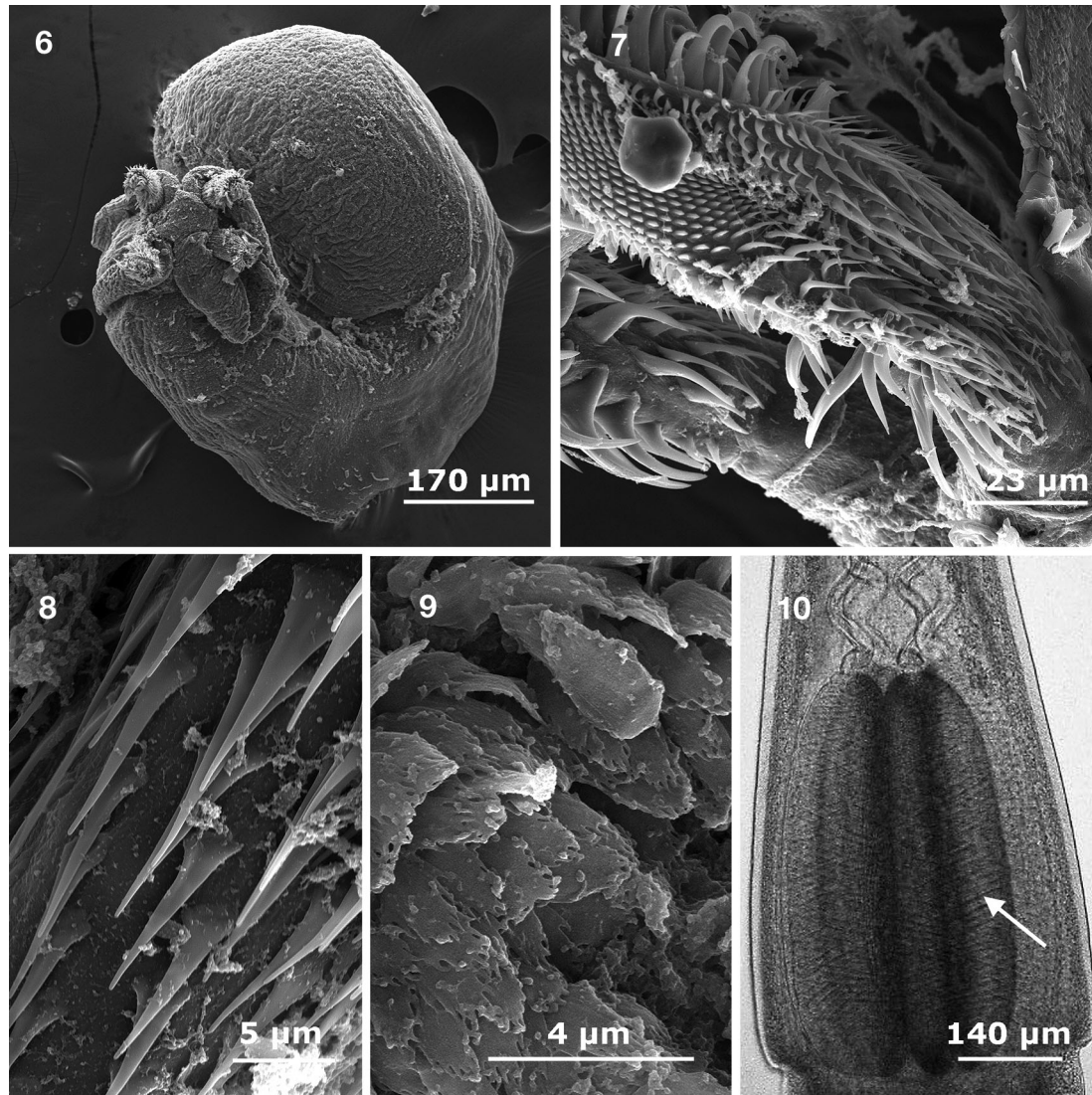
and abundance values of this parasite can be seen in Table 2.

Bolbosoma vasculosum

Three orange coloured cystacanths of *Bolbosoma vasculosum* Rudolphi, 1819 (Acanthocephala: Polymorphidae) were found in the visceral cavity of 2 fish in 1998. The cylindrical-shaped proboscis, measuring 730 µm in length and 500 µm in width, was armed with 18 longitudinal rows of 8 hooks each, followed by a neck without ornamentations, 880 µm long and 490 µm wide. The trunk showed an anterior portion armed with 2 collars of spines, the most anterior with 12 transversal rows of spines, followed by a second collar with 9 transversal rows of spines. Total body length was 11 to 13 mm. The morphological features of these specimens were in agreement with previous descriptions given for this species. Prevalence mean intensity and mean abundance values of this acanthocephalan are presented in Table 2.

DISCUSSION

The present study represents the first survey of the endohelminth parasites of the blacktail comber *Serranus atricauda* from the Macaronesian Islands (Selvagens and Madeira), adding new host and geo-

Figs. 6 to 10. *Pseudogrillotia epinepheli*. Fig. 6. Plerocercus (from the viscera of *Serranus atricauda*). Fig. 7. Detail of basal armature and basal part of metabasal armature of external face of tentacles. Fig. 8. Detail of the spatulated hooks of basal armature. Fig. 9. Multidigitate microtriches, from the bothrial surface. Fig. 10. Detail of the tentacular bulbs of *P. epinepheli* (arrow) occurring in *S. atricauda*

graphic records for the parasites found. In detail, the endohelminth fauna of this fish was characterized by parasite groups usually infecting serranids, such as camallanid and anisakid nematodes, larval trypanorhynchs and cystacanths of a generalist species, *Bolbosoma vasculosum* (Moravec et al. 1995b, 1997, Rigby & Adamson 1997, Figus et al. 2005).

Identification, hosts and geographic range of the endohelminths

The morphology and ITS sequence of the larval *Hysterothylacium* spp. found in the present study matched the description of a recently found new species, *Hysterothylacium deardorffoverstreetorum*, from the flounder *Paralichthys isosceles* caught off Rio de Janeiro, Brazil (Knoff et al. 2012). As in our study, only larvae were found in the visceral cavity and associated organs. This fact suggests that the flounder, like the blacktail comber, is a paratenic host, not a definitive host. The validity of this new species, however, is questionable on the grounds that classification was entirely based on the morphological description of larvae because no adults were found. In our opinion, the genus *Hysterothylacium* is still genetically poorly known, with only 6 species sequenced, out of the 61 species morphologically

Table 4. *Pseudogrillotia epinepheli*. Comparison of the dimensions of the scolex characters (in µm) of the plerocerci of *P. epinepheli* between Mediterranean, Italy, Bermuda samples (Scholz et al. 1993, Palm 2004) and samples from the Madeira Archipelago (present study; values are means ± SE, with range in brackets). sl: scolex length; pbo: pars bothrialis; pv: pars vaginalis; bl: bulbs length; bw: bulbs width

Body parameter	Mediterranean, Italy, Bermuda (n = 11)	Madeira Archipelago (n = 23)
sl	2440–3350	2610 ± 117.9 (1632–3536)
pbo length	300–440	400.9 ± 12.6 (280–528)
pbo width	290–380	428.4 ± 16.5 (248–552)
pv length	1100–2230	1683.9 ± 71.1 (912–2250)
pv width		455.7 ± 86.0 (256–2250)
bl	500–640	584.6 ± 11.8 (480–700)
bw	130–180	308.2 ± 46.0 (150–376)
Ratio bl:bw	2.7–5.0:1	1.9:1
Ratio pbo:pv:bl	1.0:4.5:1.5	1.0:4.2:1.5
Tentacle length	Elongated	823.7 ± 44.1 (424–1200)
Tentacle width		51.0 ± 1.3 (40–64)
Hosts	*Epinephalus guaza, Carcharhinus leucas*	*Serranus atricauda*

described (Li et al. 2007). In addition, the morphology of our larvae was similar to that of the larvae of another species, *Hysterothylacium eurycheilum* Olsen, 1952, a parasite of the serranid *Epinephelus itajara* collected off Tortugas, Florida (Deardorff & Overstreet 1981). Our observations were similar to their description of the morphometry of this nematode, which has a long ventricular appendage, a nearly spherical ventriculus, a very short intestinal caecum (sometimes not easily seen) and a conically shaped tail without ornamentation (Deardorff & Overstreet 1981, Moravec et al. 1995b). The type host of *H. eurycheilum*, the grouper *E. itajara*, has a geographical distribution encompassing the Gulf of Mexico and the Atlantic coast of North Africa, including the Canary Islands (Froese & Pauly 2011), which are close to our sampling location of the Selvagens and Madeira Islands. Considering that groupers can feed on smaller serranids (Lopez & Orvay 2005), it is possible that groupers may be the definitive hosts of our larval nematodes and that the blacktail comber is a paratenic host. Further surveys will target the endohelminths of groupers, with the aim of collecting adult nematodes for morphological and molecular characterization.

The nematode *Procamallanus* (*Spirocamallanus*) *halitrophus* comb. n. was first reported from the Gulf of Mexico, infecting the benthic dusky flounder *Syacium papillosum* Linnaeus, 1758 (Fusco & Overstreet 1978). Later, Cárdenas & Lanfredi (2005) found this parasite species in the intestines of 2 pleuronectiform fish species, the type host and the spotted

whiff *Citharichthys macrops* Dressel, 1889, both occurring in shallow-water habitats; thus, the geographical range of this parasite was extended to South Atlantic waters. The presence of *P.* (*S.*) *halitrophus* in the Madeira Archipelago may suggest that the geographic range of this camallanid encompasses the Gulf of Mexico (Florida, Mississippi, coast of Alabama), the south-western coast of the Atlantic (Rio de Janeiro) and Central North Atlantic waters (Madeira and Selvagens Islands). The 3 fish hosts infected with *P.* (*S.*) *halitrophus*, the dusky flounder, the spotted whiff and the blacktail comber, have a common habitat in benthic shallow waters and feed on invertebrates and small benthic fishes (Tortonese 1986, Morato et al. 2000, Froese & Pauly 2011). Furthermore, pelagic fishes may be paratenic hosts for camallanid nematodes, which may enhance the dispersal capacities of these nematodes (Rigby et al. 1997) and lead to the wide geographic distribution of the camallanid *P.* (*S.*) *istiblenni* (Rigby & Font 1997).

Plerocerci of *Pseudogrillotia epinepheli* have previously been found to infect the body cavity and peritoneum of the serranid *Epinephelus guaza* in the Mediterranean (Scholz et al. 1993). The morphometric values given for *P. epinepheli* in the original description by Scholz et al. (1993) are very similar to our observations (see Table 4). In addition, the SEM details of the tentacular hooks are in agreement with our observations. The presence of plerocerci of this trypanorhynch in the blacktail comber may be due to a decline of groupers in our study area. Alternatively, blacktail comber is another intermediate host for this parasite, as many trypanorhynchs have low host specificity at the intermediate host level (Palm 2004). Adults of this trypanorhynch have been described from the spiral valve of the bull shark *Carcharhinus leucas* Müller & Henle, 1839 from the Bermudas (Rees 1969). This shark has a wide geographic distribution in warm waters (Palm 2004). It appears that plerocerci of *P. epinepheli* are specific to serranids, as they occurred only in fish of that family.

On the other hand, cystacanths of *Bolbosoma vasculosum* have previously been found in a variety of hosts, including dolphins and several pelagic and demersal fish species in the Atlantic, including waters off Madeira and in the Mediterranean (Meyer 1932, Costa et al. 2000).

Transmission of the endohelminths to the blacktail comber

Investigations of the feeding habits of *Serranus atricauda* from the Azores Archipelago (also belonging to the Macaronesian Biogeographic Region) identified planktonic and benthic crustaceans and small littoral and benthic fishes as their main prey items (Gomes 1995, Morato et al. 2000). In detail, the frequency of occurrence of mysids was 26%, of fishes of the genus *Parablennius* was 10.3% and of amphipods was 3.1%. Mysids and amphipods are known to be intermediate hosts of *Hysterothylacium* spp. (Køie 1993). Therefore, the importance of feeding on these invertebrates explains the high prevalence of *Hysterothylacium* spp. found in the present study. Ingestion of small benthic fish such as species of rockpool fishes of the genus *Parablennius* could transmit *Procamallanus* (*Spirocamallanus*) *halitrophus*. In fact, a female of *Spirocamallanus* sp. was found infecting the rockpool fish *Mauligobius maderensis* off Madeira Island (Gibson & Costa 1997). This suggests that the main food items of blacktail comber examined in the present study were demersal and benthic invertebrates, as well as small fishes, which explains the higher prevalence of the nematodes *Hysterothylacium* spp. and *P.* (*S.*) *halitrophus* and the trypanorhynch *Pseudogrillotia epinepheli* transmitted by planktonic, benthic invertebrates and small fishes (Køie 1993, Marcogliese 1995). The very low prevalence of *Bolbosoma vasculosum* and the absence of digeneans could possibly be attributable to a lower or sporadic consumption of the typical intermediate hosts in benthic habitats, namely isopods (1.9%) and amphipods (3.1%) for the transmission of acanthocephalans and molluscs for the transmission of digeneans (Køie 1992, Russell-Pinto et al. 2006).

Zoogeographical relationships

Some genetic studies, using mitochondrial DNA, and parasitological surveys of fish and sea turtles collected in the North Atlantic suggested the existence of a link between the Gulf of Mexico and Madeira Archipelago, due to the influence of the Gulf Stream (Sedberry et al. 1996, Bolten et al. 1998, Valente et al. 2009), and another link with the Mediterranean (Gibson & Costa 1997). Furthermore, genetic studies using cytochrome *b* have demonstrated that serranids present in the eastern Atlantic are closely related to the New World species (Maggio et al. 2005). Briggs (1995) considered that some of the

Madeiran shore fishes could be considered transatlantic, because they are also present on the western side of this ocean, probably coming from the Caribbean region with the Gulf Stream. In our study the presence of *Procamallanus* (*Spirocamallanus*) *halitrophus* and *Hysterothylacium* spp. (genetically identical to *H. deardorffoverstreetorum*) in blacktail comber could indicate a relationship with the Gulf of Mexico (see Cárdenas & Lanfredi 2005, Knoff et al. 2012). There is also some evidence of links to the Mediterranean, due to the infection with plerocerci of *Pseudogrillotia epinepheli* (see Scholz et al. 1993). Many parasites in the marine environment are generalists at intermediate and definitive host levels, in order to ensure their survival in a dilute environment (Marcogliese 1995). They pass through different intermediate hosts and switch to new intermediate hosts, in order to spread to new hosts and new geographic regions (Marcogliese 1995, 2002). Future parasite surveys of blacktail comber from the Azores and Canary Islands, as well as from the Mediterranean, would help to better understand parasite dispersal.

In conclusion, the endohelminths infecting the blacktail comber suggested a link to the northwestern Atlantic region and the Mediterranean. These links can be explained by the oceanographic currents arriving at Madeira, namely the North Atlantic drift derived from the Gulf Stream, as well as outflow currents from the Mediterranean into the Atlantic (Briggs 1995).

LITERATURE CITED

Amin OM (1998) Marine flora and fauna of the eastern United States, Acanthocephala. NOAA Tecnhical Report NMFS 135, Washington, DC

Anderson RC (1992) Nematode parasites of vertebrates, their development and transmission. CAB International, Wallingford

Arculeo M, Hristoski N, Riggio S (1997) Helminth infestation of three fishes (*Serranus scriba*, *Mullus surmuletus*, *Scorpaena porcus*) from a coastal seaground in the Gulf of Palermo (Tyrrhenian Sea). Ital J Zool 64:283–286

Berland B (1961) Nematodes from some Norwegian marine fishes. Sarsia 2:1−50

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada SE, Bowen BW (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. Ecol Appl 8:1−7

Braicovich PE, Timi JT (2009) *Acanthochondria serrani* sp. n. (Copepoda: Chondracanthidae) parasitic on *Serranus auriga* (Perciformes: Serranidae) from Argentinean waters. Folia Parasitol (Praha) 56:313−316

Briggs JC (1995) Global biogeography. Developments in palaeontology and stratigraphy, Vol 14. Elsevier Science, Amsterdam

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83: 575-583.

Campbell RA, Beveridge I (1994) Order Trypanorhyncha Diesing 1863. In: Khalil LF, Jones A, Bray RA (eds) Keys to the cestode parasites of vertebrates. CAB International, Wallingford, Oxon, p 51−148

Cárdenas MQ, Lanfredi RM (2005) Further description of *Procamallanus* (*Spirocamallanus*) *halitrophus* comb. n. (Nematoda: Camallanidae) from flounder off the Brazilian coast by light and scanning electron microscopy. J Parasitol 91:606−613

Costa G, Chubb JC, Veltkamp CJ (2000) Cystacanths of *Bolbosoma vasculosum* in the black scabbard fish *Aphanopus carbo*, oceanic horse mackerel *Trachurus picturatus* and common dolphin *Delphinus delphis* from Madeira, Portugal. J Helminthol 74:113−120

Cuyás C, Castro JJ, Santana-Ortega AT, Carbonell E (2004) Insular stock identification of *Serranus atricauda* (Pisces: Serranidae) through the presence of *Ceratothoa steindachneri* (Isopoda: Cymothoidae) and *Pentacapsula cutanea* (Myxozoa: Pentacapsulidae) in the Canary Islands. Sci Mar 68:159−163

Deardorff TL, Overstreet RM (1981) *Raphidascaris camura* sp.n., *Hysterothylacium eurycheilum* (Olsen) comb. n., and comments on *Heterotyphlum* Spaul (Nematoda: Ascaridoidea) in marine fishes. J Parasitol 67:426−432

Figus V, D'Amico V, Loddo SL, Siddu NL, Trotti GC (2005) Elminti parassiti di *Serranus cabrilla* (L.) (Osteichthyes, Serranidae) del Golfo di Cagliari, Mediterraneo sud-occidentale. Ittiopatologia 2:207−215

Froese R, Pauly D (2011) FishBase. Available at: www.fishbase.org, Version 08/2011 (accessed on 31 October 2011)

Fusco A, Overstreet RM (1978) *Spirocamallanus cricotus* sp. n. and *S. halitrophus* sp. n. (Nematoda: Camallanidae) from fishes in the northern Gulf of Mexico. J Parasitol 64: 239−244

Gibbons LM (2010) Keys to the nematode parasites of vertebrates (Suppl Vol) CAB International, Wallingford, Oxon

Gibson D, Costa G (1997) Helminth parasites of Madeiran rockpool fishes, with a redescription of *Schikhobalotrema longivesiculatum* Orecchia and Paggi, 1975 (Digenea: Haplosplanchnidae) and some comments on their zoogeographical relationships. Syst Parasitol 38:73−79

Gomes T (1995) Ecologia alimentar de *Serranus atricauda* (Günther, 1874) dos Açores. Diplom dissertation, University of Algarve, Faro

Heemstra PC, Randall JE (1993) Groupers of the world (family Serranidae, subfamily Epinephelinae): an annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. Species Catalogue 16, FAO, Rome

Knoff M, Felizardo NN, Iñiguez AM, Maldonado A Jr, Torres EJL, Pinto RM, Gomes DC (2012) Genetic and morphological characterisation of a new species of the genus *Hysterothylacium* (Nematoda) from *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) of the Neotropical Region, state of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 107:186−193

Køie M (1992) Life cycle and structure of the fish digenean *Brachyphallus crenatus* (Hemiuridae). J Parasitol 78: 338−343

Køie M (1993) Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can J Zool 71:1289−1296

Li L, An R, Zhang L (2007) A new species of *Hysterothylacium* (Nematoda: Anisakidae) from marine fishes from Yellow Sea, China, with a key to the species of the genus *Hysterothylacium.* Zootaxa 1614:43−52

Lopez VG, Orvay FC (2005) Food habits of groupers *Epinephelus marginatus* (Lowe, 1834) and *Epinephelus costae* (Steindachner, 1878) in the Mediterranean coast of Spain. Hidrobiológica 15:27−34

MacKenzie K (1999) Parasites as pollution indicators in marine ecosystems: a proposed early warning system. Mar Pollut Bull 38:955−959

Maddison DR, Maddison WP (2000) MacClade 4: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, MA

Maggio T, Andaloro F, Hemida F, Arculeo M (2005) A molecular analysis of some eastern Atlantic grouper from the *Epinephelus* and *Mycteroperca* genus. J Exp Mar Biol Ecol 321:83−92

Marcogliese DJ (1995) The role of zooplankton in the transmission of helminth parasites of fish. Rev Fish Biol Fish 5: 336−371

Marcogliese DJ (2002) Food webs and parasite transmission. Parasitology 124:83−99

Meyer A (1932) Acanthocephala. In: Bronns HG (ed) Klassen und Ordnungen des Tier-Reichs. Akademische Verlagsgesellschaft, Leipzig, p 1−332

Morato T, Santos RS, Andrade JP (2000) Feeding habits, seasonal and ontogenetic diet shift of black-tail comber, *Serranus atricauda* (Pisces: Serranidae) from the Azores, north-eastern Atlantic. Fish Res 49:51−59

Moravec F, Salgado-Maldonado G, Vivas-Rodriguez C (1995a) *Ascarophis mexicana* n. sp. (Nematoda: Cystidicolidae) from two species of *Epinephelus* (Pisces) from the Gulf of Mexico in southeastern Mexico. J Parasitol 81:952−955

Moravec F, Vidal-Martinez VM, Aguirre-Macedo ML (1995b) Some helminth parasites of *Epinephelus morio* (Pisces: Serranidae) from the peninsula of Yucatan, Mexico. Studies Nat Hist Carib Reg 72:55−68

Moravec F, Mendoza-Franco E, Vargas-Vásquez J (1996) *Paracapillaria epinepheli* n. sp. (Nematoda: Capillariidae) from the red grouper *Epinephelus morio* (Pisces) from Mexico. Syst Parasitol 33:149−153

Moravec F, Vidal-Martinez VM, Vargas-Vásquez J, Vivas-Rodriguez C and others (1997) Helminth parasites of *Epinephelus morio* (Pisces: Serranidae) of Yucatan Peninsula, southeastern Mexico. Folia Parasitol (Praha) 44: 255−266

Moravec F, Cruz-Lacierda ER, Nagasawa K (2004) Two *Procamallanus* spp. (Nematoda, Camallidae) from fishes in the Philippines. Acta Parasitol 49:309−318

Moravec F, Justine JL, Rigby MC (2006) Some camallanid

nematodes from marine perciform fishes off New Caledonia. Folia Parasitol (Praha) 53:223–239

Palm HW (2004) The Trypanorhyncha Diesing, 1863. PKSPL-IPB Press, Bogor

Rees G (1969) Cestodes from Bermuda fishes and an account of *Acompsocephalum tortum* (Linton, 1905) gen. nov. from the lizard fish *Synodus intermedius* (Agassiz). Parasitology 59:519–548

Rigby MC, Adamson ML (1997) *Spirocamallanus* species of French Polynesian coral reef fishes. Can J Zool 75: 1270–1279

Rigby MC, Font WF (1997) Redescription and range extension of *Spirocamallanus istiblenni* Noble, 1966 (Nematoda: Cammallanidae) from coral reef fishes in the Pacific. J Helminthol Soc Wash 64:227–233

Rigby MC, Holmes JC, Cribb TH, Morand S (1997) Patterns of species diversity in the gastrointestinal helminthes of a coral reef fish, *Epinephelus merra* (Serranidae), from French Polynesia and the South Pacific Ocean. Can J Zool 75:1818–1827

Russell-Pinto F, Gonçalves JF, Bowers E (2006) Digenean larvae parasitizing *Cerastoderma edule* (Bivalvia) and *Nassarius reticulates* (Gastropoda) from the Ria de Aveiro, Portugal. J Parasitol 92:319–332

Scholz T, Garippa G, Scala A (1993) *Grillotia epinepheli* sp. n. (Cestoda: Trypanorhyncha) plerocerci from the teleost, *Epinephelus guaza*, in Sardinia, Italy. Folia Parasitol (Praha) 40:23–28

Sedberry GR, Carlin LL, Chapman RW, Eleby B (1996) Population structure in the pan-oceanic wreck fish *Polyprion*

americanus (Teleostei: Polyprionidae) as indicated by mtDNA variation. J Fish Biol 49(Issue Suppl):318–329

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: molecular evolutionary genetics analysis (MEGA) software Version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Higgins DG, Gibson TJ, Clustal W (1994) Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tortonese E (1986) Serranidae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E (eds) Fishes of the north-eastern Atlantic and Mediterranean. UNESCO, Paris, p 780–792

Tuset VM, González JA, Garcia-Díaz MM, Santana JI (1996) Feeding habits of *Serranus cabrilla* (Serranidae) in the Canary Islands. Cybium 20:161–167

Tuya F, Sanchez-Jerez P, Haroun R (2006) Populations of inshore serranids across the Canarian Archipelago: relationships with human pressure and implications for conservation. Biol Conserv 128:13–24

Valente AL, Delgado C, Moreira C, Ferreira S, Dellinger T, Pinheiro de Carvalho MA, Costa G (2009) Helminth component community of the loggerhead sea turtle *Caretta caretta* from Madeira Archipelago, Portugal. J Parasitol 95:249–252

Zhu X, Gasser RB, Podolska M, Chilton NB (1998) Characterization of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. Int J Parasitol 28: 1911–1921