

RESEARCH NOTE

Larval cestodes infecting the deep-water fish, *Cataetyx laticeps* (Pisces: Bythitidae) from Madeira Archipelago, Atlantic Ocean

Graça Costa^{1*}, Mahnaz Khadem², Thomas Dellinger¹, Manuel Bischoito¹ and Egberto Melo-Moreira¹

¹Estação de Biologia Marinha do Funchal, Promenade Orla Marítima do Funchal, 9000-107 Funchal, Portugal;

²ISOPlexis, Universidade da Madeira, Campus da Penteadá, 9000-390 Funchal, Portugal

Abstract

Parasites of deep-water fishes are less known in comparison to the parasites of fishes living in the demersal and epipelagic ocean zones. In the present research note we report the occurrence of larval trypanorhynch cestodes in a rare deep-water fish, the deep-water brotula, *Cataetyx laticeps*. Based on the 28S rDNA (region D1-D3) sequence homology and the phylogenetic analysis, the larval cestodes are putatively assigned to the genus *Grillotia*. It is suggested that the definitive host of this trypanorhynch is a batoid.

Keywords

Trypanorhyncha, *Grillotia*, deep-water fish, *Cataetyx laticeps*

Parasites of deep-water fishes are less known in comparison to parasites of demersal and epipelagic fishes (Klimpel *et al.* 2009). The reason behind this lack of knowledge, relates to the limited exploitation of deep-water fish species, in contrast with that of epipelagic and demersal fish resources. Haedrich and Merrett (1988) listed 338 fish species, living in deep-water in the North Atlantic, and recently, Bergstad *et al.* (2012) and Bergstad (2013) increased this number to 593 species. This increase relates to more studies conducted in the deep-waters of oceanic islands and seamounts in North Atlantic. Nevertheless the studies focused on the distribution of deep-water demersal fishes, without consideration of their parasite faunas. A few surveys exploiting the deep-water realm have been conducted in the North Atlantic, concentrating on the description of their parasites (see Klimpel *et al.* 2009). Two earlier studies by Noble and Orias (1975) and Orias *et al.* (1978) studied 84 bathypelagic fishes recording low prevalence of helminth parasites. On the other hand a survey of 52 benthic deep-water fishes, from Northwest Atlantic, registered high prevalence of parasites (Campbell *et al.* 1980). Based on these

results, the authors suggested that, benthic deep-water fishes harboured rich diversity of adult and larval forms of parasites, whereas pelagic deep-water fishes had more impoverished parasite faunas, mainly composed of larval cestodes and nematodes. Little is known about the parasites of the deep water brotula, *Cataetyx laticeps* Koefoed, 1927 (Pisces: Bythitidae), a bathydemersal fish species, with a geographic distribution encompassing temperate and subtropical North Atlantic, Mediterranean and eastern South Atlantic (500–2400 m depth) (Froese and Pauly 2011). A literature search for records of parasites of this fish species returned only two records of the occurrence of digeneans of the genera *Merlucciotrema* and *Steringophorus* (Bray 1996; Bray *et al.* 1999), and two further records of blood haematozoans (Davies and Merrett 2000; Davies *et al.* 2012).

The liver, stomach, intestine and visceral cavity of a single deep-water brotula, *C. laticeps*, found dead on the south coast of Madeira Island, were examined for the presence of parasites. Recovered parasites were examined alive, under binocular and high power microscopes, thereafter fixed in 70 and 100%

*Corresponding author: g.costa@uma.pt

ethanol. Specimens fixed in 70% ethanol were stained with haematoxylin, dehydrated in ascending ethanol series, cleared in eugenol, mounted in Entellan, and studied with the aid of a Zeiss Axioplan Photomicroscope. Because larval cestodes can be difficult to identify based only on morphological examination, we used the molecular approach of Jensen and Bullard (2010), to amplify a region of the 28S rDNA gene (region D1-D3), as this region is adequate to identify larval cestodes (see Brickle *et al.* 2001; Agusti *et al.* 2005; Jensen and Bullard 2010). For molecular identification, total genomic DNA was extracted from larvae previously preserved in 100% ethanol, as follows: each specimen was inserted in sterile 1.5 ml Eppendorf tube and tissue was digested in 100 µl of cell lysis buffer (0.1M Tris-HCl, 0.01M EDTA, 0.1M NaCl and 0.1% sodium dodecyl sulphate) and 10 µl of proteinase K at 40°C for 6hr. After digestion, protein was precipitated adding 50 µl of 5M NaCl followed by centrifugation for 5 min at 12000 rpm (12,269 g). The protein was discarded and the supernatants (DNA) transferred to new eppendorfs with addition of 30 µl of 3M NaOAc, kept at -20°C for 20 min, centrifuged again for 5 min (12000 rpm). Supernatants were transferred to new tubes, for precipitation of DNA, addition of 60 µl of 0.2 NaCl and 540 µl of cold 100% ethanol and again centrifuged for 20 min at 12,000 rpm. DNA pellet was washed with 70% ethanol. To amplify the region D1-D3 of the 28S rDNA gene we used the primers recommended by Jensen and Bullard (2010), namely the forward primer LSU5 and the reverse primer 1200R. PCR was performed in 25 µl reactions composed of 0.5 µl Primer 1 (forward), 0.5 µl Primer 2 (Reverse), 2.5 µl 10x(buffer), 1.25 µl MgCl₂, 2 µl dNTPs, 17 µl H₂O, 0.2 µl *Taq*, 1 µl sample DNA. Primers used were the forward primer LSU5 (5'-TAGGTC-GACCCGCTGAAYTTAAGCA-3') and reverse primer 1200R (5'-GCATAGTTCACCATCTTTCGG-3'). Cycling conditions were as follows: initial denaturation for 5 min at 94°C, 35 cycles of 30s at 94°C, 45s at 52°C, 2 min at 72°C and a final elongation of 5 min at 72°C. PCR products were purified with NucleoFast® 96 PCR (Macherey- Nagel) plates and used as template for sequencing with the same amplification primers. Both strands were cycle-sequenced using BigDye™ Terminators 3.1 Kit (Applied Biosystems) and run on an ABI Prism 3700 sequencer by STABVIDA Inc.

Sequences of 646 bp were assembled using the SeqMan program of the DNASTAR Lasergene software package, and then aligned with previous sequences of larval and adult cestodes 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 and Maddison 2000) and compared with available sequences of larval and adult cestodes in the GenBank database. The phylogenetic tree was constructed using maximum likelihood method in MEGA version 6 (Tamura *et al.* 2013). The best fit model for the data was chosen from model test implemented in MEGA 6. General Time Reversible mode with gamma distribution with 1000 bootstrap replicates (GTR+I+G) was used to construct the phylogenetic tree (Tavaré 1986; Rodrigues

et al. 1990; Yang *et al.* 1994). One sequence was deposited in GenBank with accession number KT 183342.

Several whitish cysts were found attached to the outer wall of the stomach of the deep-water brotula. These cysts contained larvae of an unknown cestode with total body length of 6 mm, and width of 2 mm. No parasites were found inside the stomach. A total of 57 plerocercoid cestode larvae were recovered from the fish host. The plerocercoids were cylindrical in shape, and no particular features could be detected on their morphology, to allow us to assign them to a particular order or family. Voucher specimens, reference number MMF 44487, were deposited in the collection of the Marine Biological Station of Funchal (Funchal, Madeira). Molecular analysis based on the aligned 28S rDNA gene sequence of 4 larvae allowed the detection of only one haplotype. Maximum likelihood phylogenetic analysis clustered our sample well within the genus *Grillotia*, close to the species *Grillotia rowei* Campbell, 1977, with 95% similarity (Fig. 1). The same topology was observed using neighbour joining and Bayesian analysis (data not shown).

It is estimated that 671 different parasite species or taxa occur in deep-water fishes (Klimpel *et al.* 2009). Almost half of that number refers to digeneans, and the other half to monogeneans, cestodes, nematodes, crustaceans and acanthocephalans all together. In respect to cestodes, Noble and Orias (1975) highlighted the lack of knowledge on the cestode fauna of deep-water fishes, in particular of bathypelagic fish species. From that date onwards some new results on the occurrence of parasites as a whole and cestodes in particular have been published (Bray and Gibson 1980, 1986; Beveridge and Justine 2006; Costa *et al.* 1996; Costa *et al.* 2003; Klimpel *et al.* 2006, 2008; Kuchta and Scholz 2004, 2006; Palm and Klimpel 2008; Scholz and Bray 2001). Based on the comprehensive check list of metazoan parasites from deep-water fishes published by Klimpel *et al.* in 2001 and 2009 (see Klimpel *et al.* 2009), it was found that, the great majority of cestodes of deep-water fishes, belong to the order Trypanorhyncha (48 species recorded), followed by the order Tetracanthocephala (32 species, including *Scolex pleuronectis*) and Bothriocephalida (24 species) (Campbell and Gartner 1982; Campbell *et al.* 1982; Klimpel *et al.* 2009; Kuchta and Scholz 2004; 2006). Some cestode parasites belonging to Gyrocotylidae (7 species), 2 species to the Diphyllidea and one to the Spathebothriidea were also found infecting deep-water fishes. Nevertheless parasite surveys of fishes from the family Bythitidae (deep-sea brotulas), and in particular from *C. laticeps* are largely unknown, apart from the records of the occurrence of digeneans (Bray 1996; Bray *et al.* 1999) and haematozoans (Davies and Merrett 2000; Davies *et al.* 2012). Plerocerci of trypanorhynch cestodes of the genus *Grillotia* have been described from several fish species, including many inhabiting deep waters (Palm 2004; Palm *et al.* 1997). *Grillotia rowei* plerocerci are typical parasites of macrourid fishes (Campbell 1977; Palm 2004) being firstly described from deep-water *Coryphaenoides* spp. (bathypelagic fish species). Adults are parasites of the deep-water skate, *Bathyraja richardsoni* (see Palm 2004). The try-

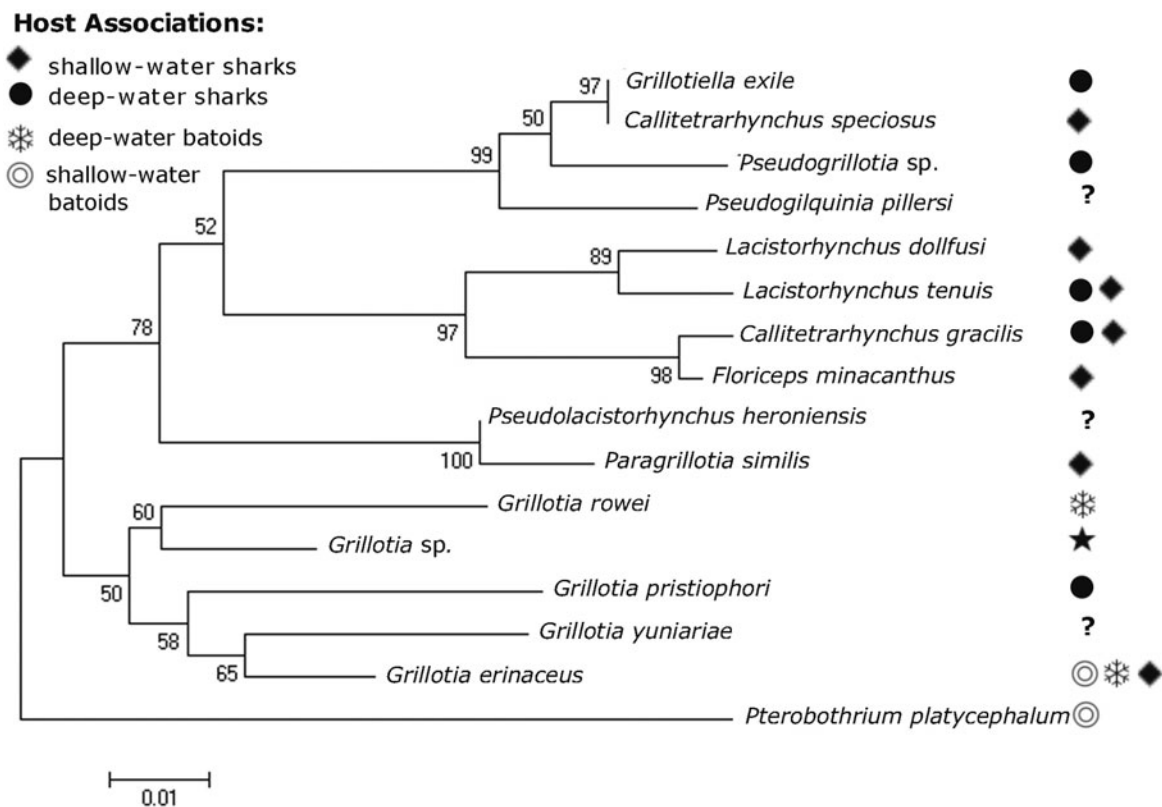


Fig. 1. Maximum-likelihood tree constructed with the GTR+I+G model, showing the phylogenetic relationship of the trypanorhyncha found in the present study (marked with a start symbol), with other trypanorhyncha species. The bootstraps values above 50% are indicated on branches. Host associations are indicated with symbols. Question marks (?) indicate species for which the definitive host is not known

panorhynch larvae of the genus *Grillotia* found in the present work in the deep-water brotula, may represent a new species, or yet belong to one of the 8 already described *Grillotia* species, excluding *G. rowei*, *G. erinaceus*, *G. yuniariae* and *G. pristiophori* whose sequences are included in Figure 1. Although we obtained rather low bootstrap values for the *Grillotia* group, probably caused by using only partial sequence of the 28S rDNA, compared to complete sequence of that gene used by Olson *et al.* (2010), we were able to putatively identify our larvae as members of the genus *Grillotia* and phylogenetically closer to *G. rowei*, which happens to be a trypanorhynch maturing in deep-water rays. Considering that the deep-water brotula is a bathydemersal fish species, we suggest that the definitive host of our *Grillotia* could be a batoid.

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