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A new species of *Microcotyle* (Monogenea: Microcotylidae) from *Scorpaena notata* (Teleostei: Scorpaenidae) in the Mediterranean Sea

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Abstract

We collected specimens of Microcotyle spp. from two species of scorpaeniform fishes off Algeria, namely Scorpaena notata and Helicolenus dactylopterus. The identification of both fishes was confirmed by molecular barcoding of the COI gene. Sequences of COI gene were also obtained for both parasite species. The species from S. notata is described as Microcotyle algeriensis n. sp., on the basis of morphological differences from other species (number of clamps, number of spines in genital atrium, number of testes). Its COI sequence differs from M. sebastis Goto, 1894 (from Sebastes schlegeli from a fish farm in South Korea) by 14.6%. The species from H. dactylopterus is distinct from M. algeriensis on the basis of morphology (number of clamps, number of spines in genital atrium) and COI sequence (4.5% divergence) and is also distinct from M. sebastis in its COI sequence (12.3%). We refrained from describing it as new because M. sebastis, a species originally described from scorpaeniform fishes off Japan, has been recorded in various hosts in the North and South Pacific, Atlantic and Mediterranean (for the latter, in the same host, H. dactylopterus). We believe that correct specific assignment of species of Microcotyle from scorpaeniform fishes needs a detailed morphological and molecular study of representatives from various locations and hosts.

Keywords: Monogenea; Mediterranean Sea; COI; barcoding; Scorpaeniformes
1. Introduction

The genus *Microcotyle* Van Beneden & Hesse, 1863 includes many species, all parasites of marine fishes, mainly perciforms. This is "one of the oldest monogenean genera" [1] and it has been repeatedly revised [2-6]. Mamaev (1986) included 48 species but indicated that the validity of many species could not be estimated. He also added that identification was difficult because species of *Microcotyle* are not strictly specific. WoRMS listed 55 species [7].

In the Mediterranean, six valid species of *Microcotyle* have been reported [8, 9]: *M. canthari* Van Beneden & Hesse, 1863 from *Spondyliosoma cantharusa* (Linnaeus, 1758) (several references, see [9]); *M. donavini* Van Beneden & Hesse, 1863 from *Symphodus mediterraneus* (Linnaeus, 1758) [10]; *M. erythrini* Van Beneden & Hesse, 1863 from *Pagellus erythrinus* (Linnaeus, 1758) (several references, see [9]), *P. acarne* (Risso, 1827) [8], and *Boops boops* (Linnaeus, 1758) [11]; *M. lichiae* Ariola, 1899 from *Lichia amia* (Linnaeus, 1758) [8]; *M. pomatomi* Goto, 1899 from *Pomatomus saltatrix* (Linnaeus, 1766) [8]; and *M. sebastis* Goto, 1894 from *Helicolenus dactylopterus* (Delaroche, 1809) (several references; discussed below). Of these six species, only the last, *M. sebastis*, has been reported from a scorpaenid fish.

In this paper, we report the presence of two species of *Microcotyle* from scorpaenid fish off Algeria, and we describe one of the species, from *Scorpaena notata* Rafinesque, 1810, as new. We refrained from describing the second species (from *Helicolenus dactylopterus*) as new because problems of synonymies with *M. sebastis* were involved and will require examination of specimens from various localities and hosts. Since sequences of mitochondrial cytochrome c oxidase subunit I (COI) have proven reliable for distinguishing monogenean species [12] [13], we provide new sequences for both *Microcotyle* species. This paper is part of a project on the monogeneans of the south shores of the Mediterranean Sea [13-16].
2. Materials and Methods

2.1. Fish

Fishes of Scorpaenidae family were collected from Bouharoun, Algerian coast (36° 37′ 24.17″ N, 2° 39′ 17.38″ E) during February 2015 – March 2016. Fish specimens were identified using keys [17] and transferred to the laboratory shortly after capture. Gills were removed carefully from each fish and observed under microscope for the presence of monogeneans.

2.2. Monogeneans

Monogeneans were removed alive from gills using fine dissection needles, then fixed in 70% ethanol, stained with acetic carmine, dehydrated in ethanol series (70, 96 and 100%), cleared in clove oil, and finally mounted in Canada balsam. Some specimens were mounted in Berlese fluid to study the morphology of clamps and the genital atrium. All drawings were made with the help of an Olympus BH-2 microscope drawing tube. Drawings were scanned and redrawn on a computer with Adobe Illustrator. Measurements are in micrometres.

2.3. Traceability of fish, monogenean specimens and host-parasite relationships

For molecular study, special attention was given to ensure that hosts and monogeneans were labelled with respect of host-parasites relationships, i.e. complete traceability. For 3 individual S. notata and 3 H. dactylopterus, a tissue sample of the fish was taken and one monogenean was extracted; the monogenean was cut in two halves, the posterior half being processed for molecules and the anterior being kept for morphological assessment and preparation of a voucher slide [18]. This ensures that the molecular identification of the host fish and their monogenean parasites correspond perfectly, at the individual fish and parasite level. Slides were deposited in the Muséum National d’Histoire Naturelle, Paris, France (MNHN), under registration numbers MNHN HEL577-579.
2.4. Molecular barcoding of fish

Total genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen) as per the manufacturer’s instructions. The 5’ region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with the primers FishF1 (5’-TCAACCAACCACAAGACATTGCAC-3’) and FishR1 (5’-TAGACTTCTGGTGCCAAAGAATCA-3’) [19]. PCR reactions were performed in 20 μl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM MgCl2, 66 μM of each dNTP, 0.15 μM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen). The amplification protocol was 4 min at 94°C, followed by 40 cycles at 94°C for 30 sec, 48°C for 40 sec, and 72°C for 50 sec, with a final extension at 72°C for 7 min. PCR products were purified (Ampure XP Kit, Beckman Coulter) and sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems). We used CodonCode Aligner version 3.7.1 software (CodonCode Corporation, Dedham, MA, USA) to edit sequences, which were 652 bp in length, compared them to the GenBank database content with BLAST, and deposited them in GenBank under accession numbers KX926437 – KX926442. Species identification was confirmed with the BOLD identification engine [20].

2.5. COI sequences of monogeneans

Total genomic DNA was isolated using QIAamp DNA Micro Kit (Qiagen). The specific primers JB3 (=COI-ASmit1) (forward 5’-TTTTGTCATCGTGGTAT-3’) and JB4.5 (=COI-ASmit2) (reverse 5’-TAAAGAAAGAACATAATGAAAATG-3’) were used to amplify a fragment of 424 bp of the COI gene [21, 22]. PCR reaction was performed in 20 μl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM MgCl2, 0.25 mM dNTP, 0.15 μM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen). Thermocycles consisted of an initial denaturation step at 94°C for 2 min, followed by 37 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 40 sec, and extension at 72°C for 50 sec. The final extension was conducted at 72°C for 5 min. Sequences were edited with CodonCode Aligner software version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), compared to the GenBank
6

database content with BLAST, and deposited in GenBank under accession number KX926443 –
KX926447.

2.6. Trees and distances
A tree was constructed from our new sequences and several COI sequences of microcotylids already
in GenBank. In particular, we used a sequence of COI from *Microcotyle sebastis* from *Sebastes*
schlegeli collected in a fish farm in South Korea [23]. After estimating the best model with MEGA7
[24], the tree was inferred using Maximum Likelihood method based on the Hasegawa-Kishino-Yano
model [25] with invariant sites (HKY+I) in MEGA7 [24], with 100 bootstrap replications. Genetic
distances (Kimura-2 parameter distance) were estimated with MEGA7. All codon positions were
used.

3. Results

3.1. Molecular identification of fish
The provisional identification of fish species using morphological characteristics was
reconfirmed by DNA barcoding approach. BLAST analysis of the COI sequences of present study fish
species with NCBI and BOLD database showed sequence similarity values of 100% for *Scorpaena*
notata and 99-100% for *Helicolenus dactylopterus* specimens. For both fish species, the BOLD
database [20] includes many sequences with published information and thus we are confident that
the identifications are valid.

3.2. Molecular characterization of monogeneans
A tree built from available COI sequences of *Microcotyle* species, including our new
sequences, and other Microcotylidae, provided the following results (Figure 1). The analysis involved
8 nucleotide sequences, and there were a total of 391 positions in the final dataset. The three
sequences of *Microcotyle* sp. from *Scorpaena notata* were identical between them, and the two sequences of *Microcotyle* sp. from *Helicolenus dactylopterus* were identical between them (i.e. 0% intraspecific variation); however, they differed from each other (interspecific variation) by 4.5%. The sequences of the two *Microcotyle* sp. from *S. notata* and *H. dactylopterus* were different from the sequence of *Microcotyle sebastis* (from *Sebastes schlegeli*, South Korea; GenBank NC009055) by respectively, 14.6% and 12.3%.

These results strongly suggest that the two species of *Microcotyle* from scorpaenids from Algeria are distinct from *M. sebastis*. Since the species from *H. dactylopterus* has already been identified as *M. sebastis* in the literature [10], we decided to concentrate our morphological work on the specimens from *Scorpaena notata*. Possible relationships between the monogeneans from *S. notata* and *H. dactylopterus* are provided in the discussion section.

### 3.3. *Microcotyle algeriensis* n. sp.

3.3.1. Description (Fig. 2)

(Measurements based on 35 specimens in carmine, Table 1). Body symmetrical, elongate; total length of adult specimens 3,298 (1,900-4,300) (n=35); width at level of ovary 593 (1,900-4,300) (n=35). Posterior haptor subsymmetrical, continuous with body, 781 (450-1,040) (n=35) long. Haptor armed with a total of 31 (20-39) (n=35) clamps arranged in 2 subequal lateral rows. Buccal organs septate, oval, 59 (40-85) long, 60 (39-76) (n=28) wide. Pharynx globular, 74 (50-100) long, 69 (46-90) (n=28) wide. Oesophagus long and thin, without lateral diverticula. Intestine bifurcates at level of genital atrium; two lateral caeca, not united posteriorly, one ends at level of testes, one extends into haptor. Testes posterior to the ovary, intercaecal in posterior half of body proper, 13 (9–20) (n=9) in number, with irregular shape and size, 750 (600–900) long, 625 (500 –750) wide. Genital atrium located at 255 (110–400) (n= 20) from anterior end of body, 115 (77- 175) long and 106 (83–130) (n=11) wide; armed with numerous conical spines; spines arranged as one main anterior group and
two postero-lateral smaller groups (“pockets” of Mamaev, 1989). Number of spines in main group
102 (68-162) (n=12), in posterolateral groups 7 (4-9) on each side (n=12), total number of spines 116
(76-174) (n=12). Vaginal pore well visible in certain specimens, but not observed in several
specimens; located posterior to genital atrium. Ovary tubular. Uterus inconspicuous, extends
anteriorly and medially towards genital atrium. Genito-intestinal canal unites right caecum with
oviduct. Vitellarium, located around intestinal diverticula, extends from genital atrium to haptor. Two
vitello-vaginal ducts, conspicuous, unite posteriorly forming a common duct, with Y-shaped
structure. Eggs in utero fusiform, 236 (215-257) long, 68 (50- 85) wide (n=10), with long filaments at
both ends.

3.3.2. Taxonomic summary
Type host: *Scorpaena notata* (Scorpaeniformes; Scorpaenidae); identification of fish specimens
confirmed by molecular barcoding, see above.
Type locality: Off Bouharoun (36° 37′ 24.17″ N, 2° 39′ 17.38″ E), Algeria.
Microhabitat: gills.
Materiel examined: 108 specimens.
Prevalence: 21%
Type material: Holotype, MNHN HEL577, paratypes, MNHN HEL578.
Etymology: The species name refers to Algeria.

3.4. *Microcotyle* sp. from *Helicolenus dactylopterus*

3.4.1. Short description and selected measurements (see Table 1).
General morphology similar to *M. algeriensis*. Genital atrium located at 397 (270–520) (n=
18) from anterior end of body, 131 (95- 160) long and 133 (102–150) (n=5) wide; armed with
numerous conical spines; spines arranged as in *M. algeriensis* n. sp. (one main group and two
posterolateral “pockets”). Number of spines in main group 184 (104-307) (n=5), in posterolateral
groups 12 (6-19) on each side (n=10), total number of spines 210 (122-333) (n=5).

3.4.2. Taxonomic summary

Host: Helicolenus dactylopterus (Scorpaeniformes; Scorpaenidae); identification of fish specimens
confirmed by molecular barcoding, see above.

Microhabitat: gills.

Material examined: 20 specimens.

Material: vouchers deposited in MNHN HEL579.

3.5. A note on the variability of measurements of sclerotised parts under different conditions

It is well known that measurements of soft parts of monogeneans vary with flattening and
preparation (i.e. [26]). Further, the measurements of sclerotised parts also vary under various
conditions of flattening and preparation, but statistical analyses are rare in the literature. In
monopisthocotylean monogeneans, small sclerotised parts vary significantly between specimens
prepared in Carmine, which are moderately flattened, and specimens prepared in picrate, which are
more flattened [27]. We tested the differences in measurements for clamps of Microcotyle
algeriensis n. sp. Fifty-one clamps were measured in specimens prepared in Carmine and 51 were
measured in specimens prepared in Berlese. Measurements (indicated as mean ± SD and range, n=51
for all) were: Length, 70 ± 8.4 (48-85) in Carmine vs 86 ± 15.5 (48-120) in Berlese, and width 48 ± 7.1
(40-78) in Carmine vs 61 ± 8.9 (48-90) in Berlese. Measurements in Berlese were 23% greater for
length and 27% greater for width, and the differences were statistically significant. Clearly, methods
of preparation produce significantly different measurements in sclerotised parts.
4. Discussion

4.1. Differential diagnosis of *Microcotyle algeriensis* n. sp.

The use of measurements of soft body parts is not a reliable method for distinguishing microcotylid species; as soon as 1894, Goto (p. 186) wrote “I would point out also that the length of the body varies considerably according to the different state of contraction, and that therefore much weight should not be laid on it in the identification of species” [26]. Recently, Machkewskyi et al. (2013) reviewed fifteen morphometric characters and their significance for species differentiation of microcotylids. They noted “great intraspecific variability in practically all metrical characters used for the differentiation of representatives of *Microcotyle* spp”. They considered that the number of clamps and testes of adult worms were independent of body size and finally that seven measurements were independent of body length: pharynx length, genital atrium length, vitello-vaginal duct length, number of testes, number of clamps, length of clamps, width of clamps [1].

*Microcotyle algeriensis* is distinct from *Microcotyle* sp. from *Helicolenus dactylopterus* from off Algeria by the number of clamps (31 vs 54), which is clearly the easiest character to distinguish the species. In addition, the number of spines in the genital atrium is different (116 vs 210), especially in the pockets (7 vs 12).

Differentiation from the species reported from *Helicolenus dactylopterus* from off Montenegro by Radujkovic & Euzet as *Microcotyle sebastis* is less easy. First, this short description suffers from discrepancies, since the top of the paragraph mentions that one fish was found parasitized by one monogenean, but the rest of it includes measurements with ranges, i.e. based on several specimens [10]. The figure provided by Radujkovic & Euzet [10] lacks diagnostic features. The number of testes (15-17) and clamps (38-56) falls into the range of the two other species. The question remains why Radujkovic & Euzet (1989) decided to ascribe their specimens to this species. Apart from having scorpaeniform fish as host (but not the same species), broadly comparable body length (2,500-3,200 vs 5,500) and similar number of clamps (38-56 vs 58 in *M.*
sebastis), not many characters are shared. The number of testes (15-17 vs 40 in M. sebastis) is different. We assume that Radujkovic & Euzet considered that the number of clamps was the main character for distinguishing species of Microcotyle with similar morphologies.

4.2. Other microcotylids from scorpaeniform fish

We consider, on the basis of morphology and molecular data, that several conclusions on the systematic status of microcotylids from scorpaeniform fishes can be drawn. (1) Microcotyle algeriensis n. sp. is different from Microcotyle sp. from Helicolenus dactylopterus off Algeria, on the basis of different numbers of clamps and number of spines in the genital atrium. In addition, the monogeneans have different hosts (Scorpaena notata vs Helicolenus dactylopterus). COI divergence between the two species is 4.5%. This species is also clearly different from M. sebastis from the Pacific (COI divergence 14.6%, higher than the difference between Microcotyle from Helicolenus dactylopterus off Algeria and M. sebastis). (2) The species of Microcotyle collected from Helicolenus dactylopterus from off Montenegro assigned to M. sebastis by Radujkovic & Euzet [10] is probably not M. sebastis, on the basis of extremely geographically distant hosts (Pacific Ocean vs Mediterranean Sea) and absence of detailed morphological proofs of identity, not to mention absence of molecular information. (3) Microcotyle sp. from Helicolenus dactylopterus off Algeria might be the same or not as Microcotyle sp. from Helicolenus dactylopterus from off Montenegro, but it is impossible to conclude on the basis of available descriptions and material. For the same reason as the species above, this species is probably not Microcotyle sebastis; in addition, the COI sequence of the species has 12.3% difference with M. sebastis from the Pacific. (4) Species of Microcotyle found on scorpaeniform fishes in the Atlantic and the Mediterranean are probably distinct from M. sebastis. (5) Species of Microcotyle found on scorpaeniform fish on the Eastern side of the Pacific (USA coast) might be the same as M. sebastis (type-locality, off Japan), but to prove this would require a detailed morphological and molecular study of specimens from various localities and hosts. Many records have been published. Microcotyle sebastis was reported from Helicolenus
Microcotyle caudata was reported from Helicolenus dactylopterus in the Southeast Atlantic, from Helicolenus maculatus in the Southwest Indian Ocean and from Scorpaena scrofa in the Mediterranean, but without morphological descriptions [29]. Microcotyle spp. without specific identification were also reported from Helicolenus lengerichi off Chile, South-Eastern Pacific [30] and from Helicolenus dactylopterus in the Faroe Islands, North Atlantic [31]. Molecular data are only available for one record, the sequence attributed to M. sebastis from Sebastes schlegeli from a fish farm in South Korea, but the paper does not indicate any deposition of material in a collection [23].

For all these reasons, we consider that it was acceptable to describe our specimens from S. notata as a new species, because it is different on morphological and molecular characters from M. sebastis from the Pacific, but refrained from describing as new the Microcotyle sp. from Helicolenus dactylopterus off Algeria, pending a detailed comparison of Microcotyle spp. from the Mediterranean and other Seas, especially the Pacific.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Figure Legends

Figure 1. Molecular phylogenetic analysis by Maximum Likelihood method of COI sequences of monogeneans. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. There were a total of 391 positions in the final dataset.

Table 1. Fish, Monogeneans, and their COI sequences. To ensure full traceability and respect of host-parasite relationships, one monogenean was collected from one fish and each fish and monogenean individuals were sequenced.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Fish Id</th>
<th>Fish COI Sequence</th>
<th>Monogenean Id</th>
<th>Monogenean COI sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scorpaena notata</em></td>
<td>BrMO-01</td>
<td>KX926437</td>
<td>MO-01</td>
<td>KX926443</td>
</tr>
<tr>
<td><em>Scorpaena notata</em></td>
<td>BrMO-02</td>
<td>KX926438</td>
<td>MO-02</td>
<td>KX926444</td>
</tr>
<tr>
<td><em>Scorpaena notata</em></td>
<td>BrMO-03</td>
<td>KX926439</td>
<td>MO-03</td>
<td>KX926445</td>
</tr>
<tr>
<td><em>Helicolenus dactylopterus</em></td>
<td>BrMO-04</td>
<td>KX926440</td>
<td>MO-04</td>
<td>KX926446</td>
</tr>
<tr>
<td><em>Helicolenus dactylopterus</em></td>
<td>BrMO-05</td>
<td>KX926441</td>
<td>MO-05</td>
<td>-</td>
</tr>
<tr>
<td><em>Helicolenus dactylopterus</em></td>
<td>BrMO-06</td>
<td>KX926442</td>
<td>MO-06</td>
<td>KX926447</td>
</tr>
</tbody>
</table>
Table 2 Measurements of *Microcotyle algeriensis* from *S. notata* off Algeria, compared with *Microcotyle* specimens recorded from scorpaeniform hosts in the Mediterranean. Bold, differences of interest for specific taxonomy.

<table>
<thead>
<tr>
<th></th>
<th><em>Microcotyle algeriensis</em></th>
<th><em>Microcotyle sp.</em></th>
<th>“M. sebastis”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hosts</strong></td>
<td><em>Scorpaena notata</em></td>
<td><em>Helicolenus dactylopterus</em></td>
<td><em>Helicolenus dactylopterus</em></td>
</tr>
<tr>
<td><strong>Locality</strong></td>
<td>Off Algeria</td>
<td>Off Algeria</td>
<td>Off Montenegro</td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td>Present study</td>
<td>Present study</td>
<td>Radujkovic &amp; Euzet, 1989 [10]</td>
</tr>
<tr>
<td></td>
<td>(measurements in Carmine)</td>
<td>(measurements in Carmine)</td>
<td></td>
</tr>
<tr>
<td><strong>Body length</strong></td>
<td>3,298 ± 592 (1,900– 4,300, n = 35)</td>
<td>3,092 (410–3,800, n = 20)</td>
<td>2,500-3,200</td>
</tr>
<tr>
<td><strong>Haptor length</strong></td>
<td>781 ± 153 (450– 1040, n = 35)</td>
<td>962 (570–1,200, n = 20)</td>
<td></td>
</tr>
<tr>
<td><strong>Number of clamps</strong></td>
<td>31 ± 4 (20– 39, n = 32)</td>
<td>54 (49–58, n = 20)</td>
<td>38-56</td>
</tr>
<tr>
<td><strong>Clamp length</strong></td>
<td>70 ± 9 (48–85, n = 51)</td>
<td>64 ± 8 (42–74, n = 32)</td>
<td></td>
</tr>
<tr>
<td><strong>Clamp width</strong></td>
<td>48 ± 7 (40–78, n = 51)</td>
<td>44 ± 7 (40–69, n = 42)</td>
<td></td>
</tr>
<tr>
<td><strong>Buccal organ length</strong></td>
<td>59 ± 10 (40–85, n = 28)</td>
<td>61 (47–73, n = 20)</td>
<td></td>
</tr>
<tr>
<td><strong>Pharynx length</strong></td>
<td>74 ± 13 (50–100, n = 28)</td>
<td>61 (40–77, n = 20)</td>
<td></td>
</tr>
<tr>
<td><strong>Pharynx width</strong></td>
<td>69 ± 12 (46–90, n = 28)</td>
<td>58 (50–69, n = 20)</td>
<td></td>
</tr>
<tr>
<td><strong>Genital atrium length</strong></td>
<td>115 (77–175, n = 11)</td>
<td>131 (95–160, n = 5)</td>
<td>170</td>
</tr>
<tr>
<td><strong>Genital atrium width</strong></td>
<td>106 (83–130, n = 11)</td>
<td>133 (102–150, n = 5)</td>
<td>95</td>
</tr>
<tr>
<td><strong>Number of Genital atrium spines</strong></td>
<td>116 (76–174, n = 12)</td>
<td>210 (122–333, n = 5)</td>
<td></td>
</tr>
<tr>
<td><strong>Testes number</strong></td>
<td>13 (9–20, n = 9)</td>
<td>13 (10–17, n = 11)</td>
<td>15-17</td>
</tr>
<tr>
<td><strong>Egg length</strong></td>
<td>236 (215-257, n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Egg width</strong></td>
<td>68 (50- 85, n=10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Measurements of *Microcotyle* spp. from scorpaeniform hosts (other than those in Table 1). All localities are in the Pacific Ocean.

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Locality</th>
<th>Source</th>
<th>Body length</th>
<th>Haptor length</th>
<th>Number of clamps</th>
<th>Clamp length</th>
<th>Clamp width</th>
<th>Buccal organ length</th>
<th>Pharynx length</th>
<th>Pharynx width</th>
<th>Genital atrium length</th>
<th>Genital atrium width</th>
<th>Total number of spines in genital atrium</th>
<th>Testes number</th>
<th>Egg length</th>
<th>Egg width</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. sebastis</em> Goto, 1894</td>
<td>Off Hakodate, Hokkaido, Japan</td>
<td>[26] Original description</td>
<td>5,500</td>
<td>-</td>
<td>58</td>
<td>68-128</td>
<td>-</td>
<td>88 (60-100)</td>
<td>70 (52-81)</td>
<td>-</td>
<td>-</td>
<td>100-240</td>
<td>150-200</td>
<td>&gt;140 (counted on Figure 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. sebastisci</em> Yamaguti, 1958</td>
<td>Inland Sea, Japan</td>
<td></td>
<td>1,700-4,400</td>
<td>-</td>
<td>29-62</td>
<td>80</td>
<td>-</td>
<td>40-60</td>
<td>40-78</td>
<td>-</td>
<td>40-80</td>
<td>-</td>
<td>45 (37-51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. victoriae</em> Woolcock, 1936</td>
<td>Off Port Philipp, Victoria, Australia</td>
<td>[34]</td>
<td>3,800-5,400</td>
<td>-</td>
<td>34-50 (often 42)</td>
<td>-</td>
<td>-</td>
<td>80-100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 (44-62)</td>
<td>50 (47-61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. caudata</em> Goto, 1894</td>
<td>Off Mitsugahama, Shikoku, Japan</td>
<td>[26]</td>
<td>3,200</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>52</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>52</td>
<td>11 (7-12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. zealanicus</em> Dillon &amp; Hargis, 1965</td>
<td>Off Cape Campbell, South Island, New Zealand</td>
<td>[35]</td>
<td>2,390 (1,460-2,920)</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23</td>
<td>53-57</td>
<td></td>
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<td></td>
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</table>
References


[16] H. Kheddam, J.-L. Justine, F. Tazerouti, Hexabothriid monogeneans from the gills of deep-sea sharks off Algeria, with the description of *Squalonchocotyle euzeti* n. sp. (Hexabothriidae) from the kitefin shark *Dalatias licha* (Euselachii, Dalatiidae), *Helminthologia* (2016) in press.


[34] V. Woolcock, Monogenetic Trematodes from some Australian Fishes, Parasitology 28(1) (1936) 79-91.

MO01 (KX926443) *Microcotyle algeriensis* n. sp. from *Scorpaena notata*

MO02 (KX926444) *Microcotyle algeriensis* n. sp. from *Scorpaena notata*

MO03 (KX926445) *Microcotyle algeriensis* n. sp. from *Scorpaena notata*

MO04 (KX926446) *Microcotyle* sp. from *Helicolenus dactylopterus*

MO06 (KX926447) *Microcotyle* sp. from *Helicolenus dactylopterus*

KJ794215 *Paramicrocotyle* sp.

NC009055 *Microcotyle sebastis*

Z83003 *Bivagina pagrosomi*

Fig. 1
Fig. 2