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.

87 **2.4. Molecular barcoding of fish**

88 Total genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen) as per the manufacturer's
89 instructions. The 5' region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was
90 amplified with the primers FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-
91 TAGACTTCTGGGTGGCCAAAGAATCA-3') [19]. PCR reactions were performed in 20 µl, containing 1 ng
92 of DNA, 1× CoralLoad PCR buffer, 3 mM $MgCl_2$, 66 µM of each dNTP, 0.15 µM of each primer, and 0.5
93 units of Taq DNA polymerase (Qiagen). The amplification protocol was 4 min at 94°C, followed by 40
94 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.
95 PCR products were purified (Ampure XP Kit, Beckman Coulter) and sequenced in both directions on a
96 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems). We used CodonCode Aligner
97 version 3.7.1 software (CodonCode Corporation, Dedham, MA, USA) to edit sequences, which were
98 652 bp in length, compared them to the GenBank database content with BLAST, and deposited them
99 in GenBank under accession numbers KX926437 –KX926442. Species identification was confirmed
100 with the BOLD identification engine [20].
101
102 **2.5. COI sequences of monogeneans**
103 Total genomic DNA was isolated using QIAmp DNA Micro Kit (Qiagen). The specific primers JB3 (=COI-
104 ASmit1) (forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (=COI-ASmit2) (reverse 5'-
105 TAAAGAAAGAACATAATGAAAATG-3') were used to amplify a fragment of 424 bp of the COI gene [21,
106 22]. PCR reaction was performed in 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM
107 MgCl2, 0.25 mM dNTP, 0.15 µM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen).
108 Thermocycles consisted of an initial denaturation step at 94°C for 2 min, followed by 37 cycles of
109 denaturation at 94°C for 30 sec, annealing at 48°C for 40 sec, and extension at 72°C for 50 sec. The
110 final extension was conducted at 72°C for 5 min. Sequences were edited with CodonCode Aligner
111 software version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), compared to the GenBank

112 database content with BLAST, and deposited in GenBank under accession number KX926443 –
113 KX926447.
114
115 **2.6. Trees and distances**
116 A tree was constructed from our new sequences and several COI sequences of microcotylids already
117 in GenBank. In particular, we used a sequence of COI from *Microcotyle sebastis* from *Sebastes*
118 *schlegeli* collected in a fish farm in South Korea [23]. After estimating the best model with MEGA7
119 [24], the tree was inferred using Maximum Likelihood method based on the Hasegawa-Kishino-Yano
120 model [25] with invariant sites (HKY+I) in MEGA7 [24], with 100 bootstrap replications. Genetic
121 distances (Kimura-2 parameter distance) were estimated with MEGA7. All codon positions were
122 used.
123
124 **3. Results**
125
126 **3.1. Molecular identification of fish**
127 The provisional identification of fish species using morphological characteristics was
128 reconfirmed by DNA barcoding approach. BLAST analysis of the COI sequences of present study fish
129 species with NCBI and BOLD database showed sequence similarity values of 100% for *Scorpaena*
130 *notata* and 99-100% for *Helicolenus dactylopterus* specimens. For both fish species, the BOLD
131 database [20] includes many sequences with published information and thus we are confident that
132 the identifications are valid.
133
134 **3.2. Molecular characterization of monogeneans**
135 A tree built from available COI sequences of *Microcotyle* species, including our new
136 sequences, and other Microcotylidae, provided the following results (Figure 1). The analysis involved
137 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.

Materiel 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*

tristanensis in the Southeast Atlantic [28]. *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.

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289 **Figure Legends**

290

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

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298

299 **Figure 2.** *Microcotyle algeriensis* n. sp. from *Scorpaena notata*.
300 A, holotype. B, genital atrium. C, egg in utero. D-F, clamp: D, upper part, E, lower part, F, both parts
301 superposed.

302

303

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

306

Fish species	Fish Id	Fish COI Sequence	Monogenean Id	Monogenean COI sequence
Scorpaena notata	BrMO-01	KX926437	MO-01	KX926443
Scorpaena notata	BrMO-02	KX926438	MO-02	KX926444
Scorpaena notata	BrMO-03	KX926439	MO-03	KX926445
Helicolenus dactylopterus	BrMO-04	KX926440	MO-04	KX926446
Helicolenus dactylopterus	BrMO-05	KX926441	MO-05	-
Helicolenus dactylopterus	BrMO-06	KX926442	MO-06	KX926447

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308

309 **Table 2** Measurements of *Microcotyle algeriensis* from *S. notata* off Algeria, compared with *Microcotyle* specimens recorded from scorpaeniform hosts in

310 the Mediterranean. Bold, differences of interest for specific taxonomy.

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

311

312

313 **Table 3.** Measurements of *Microcotyle* spp. from scorpaeniform hosts (other than those in Table 1). All localities are in the Pacific Ocean.

	M. sebastis Goto, 1894	*M. sebastis* Goto, 1894	*M. sebastisci* Yamaguti, 1958	*M. victoriae* Woolcock, 1936	*M. caudata* Goto, 1894	*M. zealanicus* Dillon & Hargis,*1965*
Hosts	*Sebastes* spp. (2 species)	*Sebastodes maliger*, *Sebastodes caurinus*	*Sebasticus marmoratus*, *Sebastodes guntheri*, *Sebastichthys pachycephalus*, *Epinephelus akaara*	*Helicolenus percoides*	*Sebastes* spp. (2 species)	*Helicolenus percoides*
Locality	Off Hakodate, Hokkaido, Japan	Off Puget Sound, Washington State, USA	Inland Sea, Japan	Off Port Philipp, Victoria, Australia	Off Mitsugahama, Shikoku, Japan	Off Cape Campbell, South Island, New Zealand
Source	[26] Original description	[32]	[33]	[34]	[26]	[35]
Body length	5,500	3,100-5,200	1,700– 4,400	3,800–5,400	3,200	2,390 (1,460-2,920)
Haptor length	-	1,300 (950-1,700)	-	-	-	830 (560-1030)
Number of clamps	58	30 (23-31)	29–62	34-50 (often 42)	50	48-58
Clamp length	68-128	104 (88-117)	80	-	45-80	73 (68-78)
Clamp width				-	-	51 (47-54)
Buccal organ length		88 (60-100)	40–60	80-100	-	45 (37-51)
Pharynx length	-	70 (52-81)	40-78	-	-	50 (44-62)
Pharynx width	-		40-80	-	-	52 (47-61)
Genital atrium length			-	-	-	189 (172-210)
Genital atrium width			100–240		-	211 (177-256)
Total number of spines in genital atrium		150-200				>140 (counted on Figure 12)
Testes number	40	32 (21-48)	8–20	18–22	23	11-20
Egg length	-	240	200–220	-		231-238
Egg width	-	60	90–100	-		53-57

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316 **References**

317 [1] V.K. Machkewskyi, E.V. Dmitrieva, S. Al-Jufaili, N.A. Al-Mazrooei, *Microcotyle omanae* n. sp.
318 (Monogenea: Microcotylidae), a parasite of *Cheimerius nufar* (Valenciennes) (Sparidae) from the
319 Arabian Sea, Syst Parasitol 86(2) (2013) 153-63.

320 [2] S. Yamaguti, Systema Helminthum Volume IV Monogenea and Aspidocotylea, John Wiley &
321 Sons1963.

322 [3] R.V. Unnithan, On the functional morphology of a new fauna of Monogenoidea on fishes from
323 Trivandrum and environs. Part IV. Microcotylidae Sensu Stricto and its repartition into subsidiary
324 taxa, Am Midl Nat 85(2) (1971) 366-398.

325 [4] Y.L. Mamaev, [On one classification of monogeneans of the family Microcotylidae], Parazitologiya
326 11 (1977) 98-103.

327 [5] Y.L. Mamaev, The taxonomical composition of the family Microcotylidae Taschenberg, 1879
328 (Monogenea), Folia Parasitol 33 (1986) 199-206.

329 [6] Y.L. Mamaev, B.I. Lebedev, The system of Higher Monogeneans in the light of recent knowledge,
330 Zool Scr 8(1-4) (1979) 13-18.

331 [7] R.A. Bray, *Microcotyle* Van Beneden & Hesse, 1863. Accessed through: World Register of Marine
332 Species at *http://www.marinespecies.org/aphia.php?p=taxdetails&id=119381* on 2016-08-29,
333 (2004).

334 [8] L. Euzet, C. Combes, C. Caro, A check list of Monogenea of mediterranean fish, Second
335 International Symposium on Monogenea, Montpellier/Sète, 1993.

336 [9] B.M. Radujkovic, D. Sundic, Parasitic flatworms (Platyhelminthes: Monogenena, Digenea,
337 Cestoda) of fishes from the Adriatic Sea, Nat Monten 13(1) (2014) 7-280.

338 [10] B.M. Radujkovic, L. Euzet, Parasites des poissons marins du Monténégro: Monogènes. In:
339 Radujkovic, B. M & Raibaut, A. (Eds) Faune des parasites de poissons marins des côtes du
340 Monténégro (Adriatique Sud), Acta Adriat 30(1/2) (1989) 51-135.

341 [11] A. Pérez-del Olmo, M. Fernandez, D.I. Gibson, J.A. Raga, A. Kostadinova, Descriptions of fome
342 unusual digeneans from *Boops boops* L. (Sparidae) and a complete checklist of its metazoan
343 parasites, Syst Parasitol 66 (2007) 137-157.

344 [12] C. Schoelinck, C. Cruaud, J.-L. Justine, Are all species of *Pseudorhabdosynochus* strictly host
345 specific? – a molecular study, Parasitol Int 61 (2012) 356-359.

346 [13] A. Chaabane, L. Neifar, D. Gey, J.-L. Justine, Species of *Pseudorhabdosynochus* (Monogenea,
347 Diplectanidae) from groupers (*Mycteroperca* spp., Epinephelidae) in the Mediterranean and Eastern
348 Atlantic Ocean, with special reference to the "beverleyburtonae group" and description of two new
349 species, PLoS ONE 11(8) (2016) e0159886.

350 [14] A. Chaabane, L. Neifar, J.-L. Justine, *Pseudorhabdosynochus regius* n. sp. (Monogenea,
351 Diplectanidae) from the mottled grouper *Mycteroperca rubra* (Teleostei) in the Mediterranean Sea
352 and Eastern Atlantic, Parasite 22 (2015) 9.

[15] A. Chaabane, J.-L. Justine, D. Gey, M.D. Bakenhaster, L. Neifar, *Pseudorhabdosynochus sulamericanus* (Monogenea, Diplectanidae), a parasite of deep-sea groupers (Serranidae) occurs transatlantically on three congeneric hosts (*Hyporthodus* spp.), one from the Mediterranean Sea and two from the western Atlantic, PeerJ 4 (2016) e2233.

[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.

[17] W. Fischer, M.-L. Bauchot, M. Schneider, Fiches FAO d'identification des espèces pour les besoins de la pêche. (Révision 1). Méditerranée et mer Noire. Zone de pêche 37. Volume II. Vertébrés. Publication préparée par la FAO, résultat d'un accord entre la FAO et la Commission des Communautés Européennes (Projet GCP/INT/422/EEC) financée conjointement par ces deux organisations. Rome, FAO, Vo1.2: 761- 1530, 1987.

[18] J.-L. Justine, C. Rahmouni, D. Gey, C. Schoelinck, E.P. Hoberg, The monogenean which lost its clamps, PLoS ONE 8(11) (2013) e79155.

[19] R.D. Ward, T.S. Zemlak, B.H. Innes, P.R. Last, P.D. Hebert, DNA barcoding Australia's fish species, Philos Trans R Soc Lond B Biol Sci 360(1462) (2005) 1847-57.

[20] S. Ratnasingham, P.D.N. Hebert, BOLD: The Barcode of Life Data System (www. barcodinglife. org), Mol Ecol Notes 7(3) (2007) 355-364.

[21] J. Bowles, D. Blair, D.P. McManus, A molecular phylogeny of the human schistosomes, Mol Phylogenet Evol 4(2) (1995) 103-109.

[22] D.T.J. Littlewood, K. Rohde, K.A. Clough, Parasite speciation within or between host species? - Phylogenetic evidence from site-specific polystome monogeneans, Int J Parasitol 27 (1997) 1289-1297.

[23] J.K. Park, K.H. Kim, S. Kang, W. Kim, K.S. Eom, D.T.J. Littlewood, A common origin of complex life cycles in parasitic flatworms: evidence from the complete mitochondrial genome of *Microcotyle sebastis* (Monogenea: Platyhelminthes), BMC Evol Biol 7 (2007) 11.

[24] S. Kumar, G. Stecher, K. Tamura, MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets, Mol Biol Evol (2016) in press.

[25] M. Hasegawa, H. Kishino, T.-a. Yano, Dating of the human-ape splitting by a molecular clock of mitochondrial DNA, J Mol Evol 22(2) (1985) 160-174.

[26] S. Goto, Studies on the ectoparasitic Trematodes of Japan, Tokyo, 1894.

[27] J.-L. Justine, Species of *Pseudorhabdosynochus* Yamaguti, 1958 (Monogenea: Diplectanidae) from *Epinephelus fasciatus* and *E. merra* (Perciformes: Serranidae) off New Caledonia and other parts of the Indo-Pacific Ocean, with a comparison of measurements of specimens prepared using different methods, and a description of *P. caledonicus* n. sp, Syst Parasitol 62(1) (2005) 1-37.

[28] A.V. Gajevskaja, L.D. Aljoshkina, Fauna of monogenea of the south-east Atlantic, its ecological and geographical analysis, Zoologicheskij Zhurnal. Moscow 57(3) (1988) 325-330.

[29] A.M. Parukhin, Parasitic worms of benthic fishes of the southern seas, Naukova Dumka, Kiev (In Russian) (1989).

392 [30] L. Balboa, M. George-Nascimento, Variaciones ontogenéticas y entre años en las
393 infracomunidades de parásitos metazoos, Rev Chil Hist Nat 71 (1998) 27-37.

394 [31] M. Køie, Metazoan parasites of teleost fishes from atlantic waters off the Faroe Islands, Ophelia
395 52(1) (2000) 25-44.

396 [32] K. Bonham, J.E. Guberlet, Notes on *Microcotyle sebastis* Goto from Puget Sound, J Parasitol 23(3)
397 (1937) 281-290.

398 [33] S. Yamaguti, Studies on the helminth fauna of Japan. Part 53. Trematodes of fishes, XII, Publ Seto
399 Mar Biol Lab 7 (1958) 53-88.

400 [34] V. Woolcock, Monogenetic Trematodes from some Australian Fishes, Parasitology 28(1) (1936)
401 79-91.

402 [35] W.A. Dillon, W.J. Hargis, Monogenetic trematodes from the Southern Pacific Ocean. 2.
403 Polyopisthocotyleids from New Zealand fishes: the families Discocotylidae, Microcotylidae, Axinidae
404 and Gastrocotylidae, Biology of the Antarctic Seas II, Antarctic Research Series 5 (1965) 251-280.

405

406

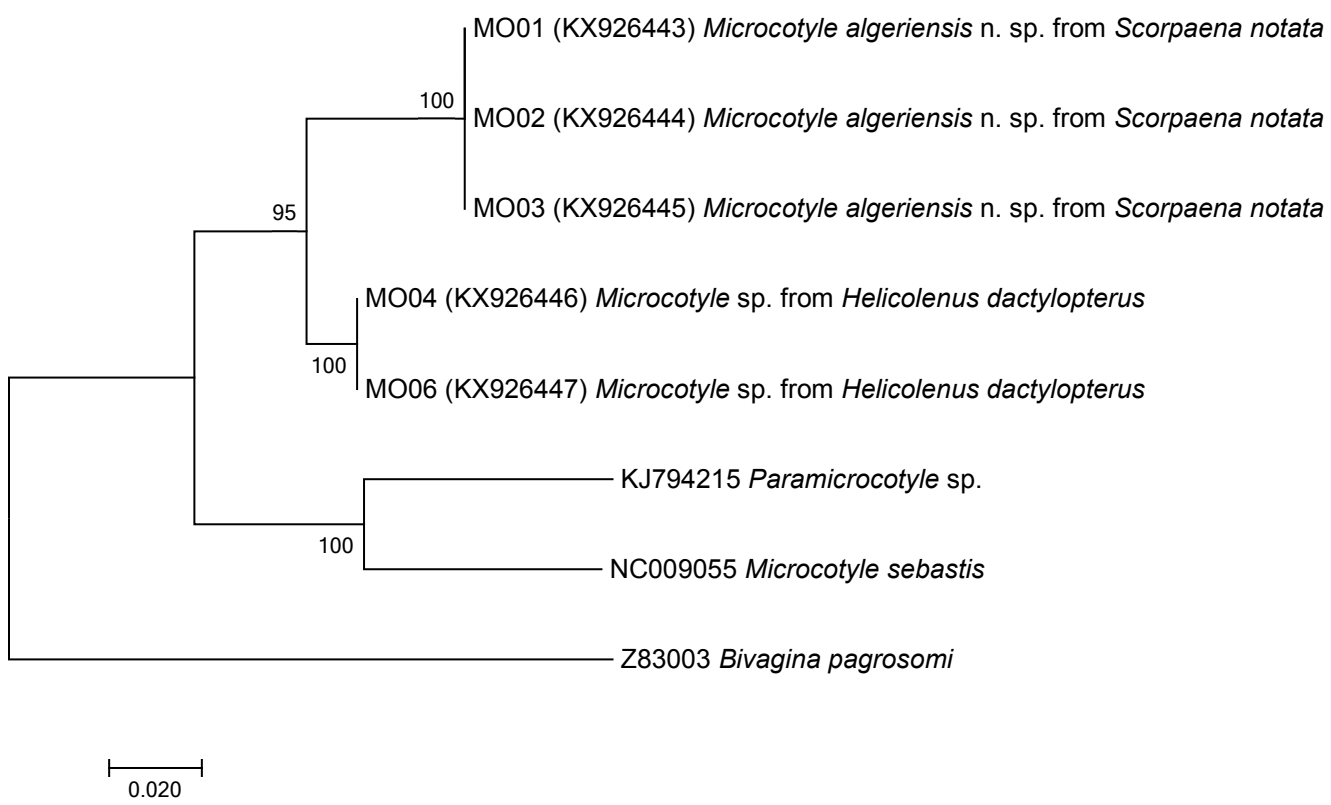
MO01 (KX926443) Microcotyle algeriensis n. sp. from Scorpaena notata
100
MO02 (KX926444) Microcotyle algeriensis n. sp. from Scorpaena notata
MO03 (KX926445) Microcotyle algeriensis n. sp. from Scorpaena notata
95
MO04 (KX926446) Microcotyle sp. from Helicolenus dactylopterus
100
MO06 (KX926447) Microcotyle sp. from Helicolenus dactylopterus
KJ794215 Paramicrocotyle sp.
100
NC009055 Microcotyle sebastis
Z83003 Bivagina pagrosomi
0.020

Fig. 1

Fig. 2