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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.

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 [5] 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

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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′-TCAACCAACCACAAAGACATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) [19]. PCR reactions were performed in 20 μl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM $MgCl_2$, 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 s, 48 °C for 40 s, and 72 °C for 50 s, 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 QIAmp DNA Micro Kit (Qiagen). The specific primers JB3 (=COI-ASmit1) (forward 5′-TTTTTTGGGCATCCTGAGGTTTAT-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 s, annealing at 48 °C for 40 s, and extension at 72 °C for 50 s. 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 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 (Fig. 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 2). Body symmetrical, elongate; total length of adult specimens 3298 (1900–4300) (n = 35); width at level of ovary 593 (300–860) (n = 35). Posterior haptor subsymmetrical, continuous with body, 781 (450–1040) (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