



# *Microcotyle visa* n. sp. (Monogenea: Microcotylidae), a gill parasite of *Pagrus caeruleostictus* (Valenciennes) (Teleostei: Sparidae) off the Algerian coast, Western Mediterranean

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Received: 30 November 2018 / Accepted: 8 January 2019 / Published online: 30 January 2019  
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**Abstract** Parasite biodiversity of fish of the southern part of the Mediterranean sea is still incompletely explored. We describe here *Microcotyle visa* n. sp. from the gill filaments of the bluespotted seabream *Pagrus caeruleostictus* (Valenciennes) (Sparidae) collected off the Algerian coast. The identity of fish hosts was confirmed by barcoding. *Microcotyle visa* n. sp. is herein described and illustrated. Analysis of the *cox1* gene of the monogeneans revealed minor intraspecific variation (1.4%), an order of magnitude

lower than the distance between this species and other *Microcotyle* species (10–15 %). *Microcotyle visa* n. sp. is distinguished from *Microcotyle erythrini* van Beneden & Hesse, 1863, a congener infesting sparids, on the basis of morphological (size of clamps, number of testes) and molecular (*cox1*) differences. This is the fourth member of the genus known to parasitise a sparid host. A species of *Paramicrocotyle* sp. included in the molecular analysis was nested within a robust *Microcotyle* + *Paramicrocotyle* clade; in the absence of demonstrated molecular and morphological differences, we consider that *Paramicrocotyle* Caballero & Bravo-Hollis, 1972 is a junior synonym of *Microcotyle* van Beneden & Hesse, 1863 and transfer two species of *Paramicrocotyle* as *Microcotyle danielcarrioni* (Martinez & Barrantes, 1977) n. comb. and *Microcotyle moyanoi* (Villalba & Fernandes, 1986) n. comb.

This article was registered in the *Official Register of Zoological Nomenclature* (ZooBank) as 28EDA724-010F-454A-AD99-B384C1CB9F04. This article was published as an Online First article on the online publication date shown on this page. The article should be cited by using the doi number. This is the Version of Record.

This article is part of the Topical Collection Monogenea.

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## Introduction

The Microcotylidae Taschenberg, 1879 is one of the largest families of polyopisthocotylean monogeneans. It has received much attention by taxonomists (Dillon & Hargis, 1965; Mamaev, 1986; Tripathi, 1956; Unnithan, 1971). Still, the specific composition and status of some genera remain unclear (Mamaev, 1986). Tripathi (1956), in an early effort, arranged the species of the genus *Microcotyle* van Beneden & Hesse, 1863 into four subgenera: *Microcotyle* Tripathi, 1956, *Bispina* Tripathi, 1956, *Vaginaespina* Tripathi, 1956 and *Aspina* Tripathi, 1956, based on the characters of the cirrus and vagina (Tripathi, 1956). Dillon & Hargis (1965), in light of the insufficiency of the older literature and of Tripathi's systematic work as he omitted certain species unquestionably belonging to *Microcotyle*, found only little merit to his arrangement (Dillon & Hargis, 1965). Unnithan (1971) as well, considered Tripathi's subdivision of *Microcotyle* to be "too arbitrary" to be adopted without considerable modification (Unnithan, 1971), and erected several subfamilies. Mamaev (1977) considered that, regardless of some positive results of Unnithan's work, his attempt "to eliminate ambiguities as far as possible" (Unnithan, 1971) "failed and caused only additional confusion" (Mamaev, 1977); he rejected all Unnithan's subfamilies, and recognised only five of his 13 new genera, with a need of critical review of specific composition of four of them (Mamaev, 1977). Mamaev (1986) revised again the family and proposed eight subfamilies, 39 genera and 150 species (Mamaev, 1986).

During the 21st century, new genera (*Omanicotyle* Yoon, Al-Jufaili, Freeman, Bron, Paladini & Shinn, 2013, *Paranaella* Kohn, Baptista-Farias & Cohen, 2000) (see Kohn et al., 2000; Yoon et al., 2013) and species [*Microcotyle algeriensis* Ayadi, Gey, Justine & Tazerouti, 2017, *M. argenticus* Hadi & Bilqees, 2011, *M. rubrum* Hadi & Bilqees, *M. jonii* Hadi, Bilqees & Khatoun, 2011, *M. omanae* Machkewskyi, Dmitrieva, Al-Jufaili & Al-Mazrooei, 2013 and *Polylabris bengalensis* Sailaja & Madhavi, 2011] have been added to this family (Hadi & Bilqees 2010, 2011; Hadi et al., 2011; Sailaja & Madhavi, 2011; Machkewskyi et al., 2013; Ayadi et al., 2017).

Within the Microcotylidae, *Microcotyle* is the largest genus and has been considered one of the most difficult genera of Monogenea (see Sproston, 1946).

Several attempts have been made to subdivide this genus (Unnithan, 1971 [1967]; Mamaev, 1977, 1986). Currently, WoRMS list about 60 valid species (WoRMS, 2018). As for other monogeneans, it is likely that studies integrating both morphology and molecules are necessary to elucidate phylogenetic relationships and limits between species.

Recent studies have shown that the biodiversity of monogeneans of marine fish in the southern part of the Mediterranean is far from being completely known (Chaabane et al., 2015, 2016a, b, 2017; Chaari et al., 2016; Kheddami et al., 2016; Boudaya & Neifar, 2016; Ayadi et al., 2017). In the course of a parasitological study of monogeneans of sparid fishes off the Algerian coast, we collected representatives of an undescribed species of *Microcotyle* on the gills of *Pagrus caeruleostictus*. The species is described here.

## Materials and methods

### Fishes

During 2016 and 2017, 73 *Pagrus caeruleostictus* were collected at Zemmouri El Bahri (36°48'4.58"N, 3°34'7.01"E) off the Algerian coast. Fish specimens were transferred to the laboratory shortly after capture and identified using keys (Fischer et al., 1987). Gills were removed from each fish and observed under microscope for the presence of monogeneans.

### Morphological methods

Monogeneans were removed alive from gills using fine dissection needles, then fixed in 70% ethanol, stained with acetic carmine, dehydrated in graded ethanol series (70, 96 and 100%), cleared in clove oil, and mounted in Canada balsam. Some specimens were mounted in Berlese's fluid to study the morphology of clamps and the genital atrium. Drawings were made with the help of an Olympus BH-2 microscope equipped with a drawing tube. Drawings were scanned and redrawn on a computer with Adobe Illustrator. Measurements are in micrometres, and indicated as the range followed by the mean  $\pm$  standard deviation (for  $n > 30$ ) and the number of measurements in parentheses; measurements of the holotype are also indicated.

### Molecular methods

To ensure that hosts and monogenean were labelled with respect of host-parasites relationships we

followed Justine et al. (2013) and Ayadi et al. (2017). Specimens of *Microcotyle* sp. were extracted from the same host fish, and a tissue sample from the gills of the fish was taken. The extracted monogeneans were cut in three parts using a scalpel blade. Their anterior parts (which include the genital atrium) and posterior parts (which include the haptor) were mounted (both parts on a single slide) for drawing and deposition in a museum; their median parts were fixed in absolute ethanol then subjected to molecular analyses. Three specimens were analysed.

#### *Molecular barcoding of fish*

Total genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France) as per the manufacturer's instructions. The 5' region of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene was amplified with the primers FishF1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FishR1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') (Ward et al., 2005). PCR reactions were performed in a total volume of 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM MgCl<sub>2</sub>, 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 step at 72°C for 7 min. PCR products were purified (Ampure XP Kit, Beckman Coulter, Brea, USA) and sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems, Foster City, USA). We used CodonCode Aligner version 3.7.1 software (CodonCode Corporation, Dedham, MA, USA) to edit the sequence, which was 652 bp in length, compared it to the GenBank database content with BLAST, and deposited it in GenBank under the accession number MK275650. Species identification was confirmed with the BOLD identification engine (Ratnasingham & Hebert, 2007).

#### *cox1 sequences of monogeneans*

Total genomic DNA was isolated using QIAamp DNA Micro Kit (Qiagen). The specific primers JB3 (=COIASmit1) (forward 5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and JB4.5 (=COI-ASmit2) (reverse 5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') were used to amplify a fragment of 424 bp of the *cox1* gene (Bowles et al., 1995; Littlewood et al., 1997). PCR reaction was performed in 20 µl,

containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM MgCl<sub>2</sub>, 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 the accession numbers MK27652-MK27654.

#### *Trees and distances*

All available sequences for *Microcotyle* spp. available on GenBank, and one sequence of *Paramicrocotyle* sp., were included in the phylogenetic analyses (Table 1). Sequences of *Bivagina pagrosomi* (Murray, 1931) and *Polylabris halichoeres* Wang & Zhang, 1998 were used as the outgroup. The trees were inferred using the neighbour-joining (NJ) and maximum likelihood (ML) methods using MEGA7 (Kumar et al., 2016). For the latter, the best model, estimated by MEGA7, was the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with discrete Gamma distribution (HKY + G). Genetic distances [p-distance and Kimura 2-parameter distance (Kimura, 1980)] were estimated with MEGA7. All codon positions were used.

## Results

### **Molecular identification of fish**

The provisional identification of fish species using morphological characteristics was challenged by DNA barcoding approach. BLAST analysis of the *cox1* sequences of fish specimens examined in the present study with sequences in the GenBank and BOLD databases showed sequence similarity values of 99% for *Pagrus caeruleostictus*, thus confirming the identification of the hosts.

### **Molecular characterisation of monogeneans**

The *cox1* sequences of *Microcotyle visa* n. sp. were aligned with 10 other microcotylid sequences. The

**Table 1** Species of the Microcotylidae used in the present molecular study. All species belong to the Microcotylinae except for *Polylabris halichoeres* (Polylabrinae)

Parasite species	Host species	Origin	GenBank ID	Source
<i>Microcotyle visa</i> n. sp.	<i>Pagrus caeruleostictus</i> (Valenciennes)	Off Algeria	MK275652 MK275653 MK275654	Present study
“ <i>Microcotyle sebastis</i> Goto, 1894” <sup>a</sup>	<i>Sebastes schlegeli</i> Hilgendorf	Off South Korea	NC009055	Park et al. (2007)
<i>Microcotyle erythrini</i> van Beneden & Hesse, 1863	<i>Pagellus erythrinus</i> (Linnaeus)	Off France	AY009159	Jovelin & Justine (2001)
<i>Paramicrocotyle</i> sp.	<i>Pinguipes chilensis</i> Valenciennes	Off Chile	KJ794215	Oliva et al. (2014)
<i>Microcotyle</i> sp.	<i>Helicolenus dactylopterus</i> (Delaroche)	Off Algeria	KX926446 KX926447	Ayadi et al. (2017)
<i>Microcotyle algeriensis</i> Ayadi, Gey, Justine & Tazerouti, 2017	<i>Scorpaena notata</i> Rafinesque	Off Algeria	KX926443 KX926444 KX926445	Ayadi et al. (2017)
<i>Bivagina pagrosomi</i> (Murray, 1931)	<i>Sparus aurata</i> Linnaeus	Off Australia	Z83003	Littlewood et al. (1997)
<i>Polylabris halichoeres</i> Wang & Zhang, 1998	<i>Halichoeres nigrescens</i> (Bloch & Schneider)	Off China	JF505509	Zhang et al. (2011)

<sup>a</sup>Specific identity of this species was questioned (Ayadi et al., 2017)

neighbour-joining and maximum likelihood methods led to similar tree topologies and thus only the NJ tree is shown (Fig. 1). All species included in *Microcotyle* and *Paramicrocotyle* formed a monophyletic clade separated from the outgroups (84% bootstrap in NJ, 75% in ML) and the species of *Paramicrocotyle* was nested within the *Microcotyle* + *Paramicrocotyle* clade. The three sequences for the new species grouped as a robust clade (100% bootstrap support in NJ, 98% in ML) within the *Microcotyle* + *Paramicrocotyle* clade. Another clade with high support included the two species of *Microcotyle* from scorpaeniform fishes, *Microcotyle algeriensis* ex *Scorpaena notata* Rafinesque and *Microcotyle* sp. ex *Helicolenus dactylopterus* (Delaroche) (100% bootstrap support in NJ). The support for other branches was generally low.

Distances were computed using p-distance and Kimura 2-parameter distance. The differences were minor so only p-distances are commented here. Two sequences for *M. visa* n. sp. were identical differed by 1.4% from the third. Distances between these

sequences of *M. visa* n. sp. and the other species ranged between 14.3–15.0% (*Microcotyle erythrini*) and 10.2–13.9% (remaining species) (Table 2). These distances, and the tree, clearly indicate that the new species is distinct from all species of *Microcotyle* and *Paramicrocotyle* for which sequence data are available, and especially that it is distinct from *M. erythrini*. Intraspecific distances within *M. visa* n. sp. were an order of magnitude inferior to the interspecific distances (1.4% vs 10.2–15.0%).

### Family Microcotylidae Taschenberg, 1879

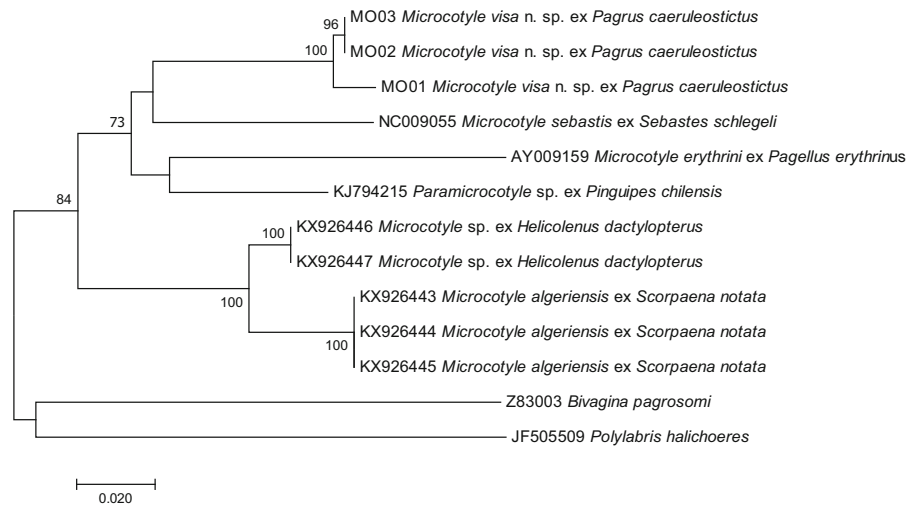
#### Genus *Microcotyle* van Beneden & Hesse, 1863

#### *Microcotyle visa* n. sp.

*Type-host*: *Pagrus caeruleostictus* (Valenciennes) (Perciformes: Sparidae), blue-spotted seabream.

*Type-locality*: Off Zemmouri el Bahri (36°48′4.58″N, 3°34′7.01″E), Algerian coast of the Western Mediterranean.

*Site on host*: Gills.



**Fig. 1** Neighbour-Joining tree (p-distance method) based on an analysis of *cox1* sequence data for *Microcotyle* spp. Bootstraps percentages (1,000 replicates) are indicated next to the branches (only values > 70% are shown). There were a total of 294 positions in the final dataset. The Maximum Likelihood tree had similar topology and is not presented

**Table 2** Estimate of evolutionary divergence between sequences. There were a total of 294 positions in the dataset. Distances are p-distances, shown as percentages

Sequence	1	2	3	4	5	6	7	8	9	10	11	12
1 MK275654 (MO03) <i>Microcotyle visa</i> n. sp. ex <i>Pagrus caeruleostictus</i>												
2 MK275653 (MO02) <i>Microcotyle visa</i> n. sp. ex <i>Pagrus caeruleostictus</i>	0.0											
3 MK275654 (MO01) <i>Microcotyle visa</i> n. sp. ex <i>Pagrus caeruleostictus</i>	1.4	1.4										
4 AY009159 <i>Microcotyle erythrini</i> ex <i>Pagellus erythrinus</i>	14.3	14.3	15.0									
5 KJ794215 <i>Paramicrocotyle</i> sp. ex <i>Pinguipes chilensis</i>	11.2	11.2	11.9	12.6								
6 KX926443 <i>Microcotyle algeriensis</i> ex <i>Scorpaena notata</i>	13.3	13.3	13.9	18.0	14.3							
7 KX926444 <i>Microcotyle algeriensis</i> ex <i>Scorpaena notata</i>	13.3	13.3	13.9	18.0	14.3	0.0						
8 KX926445 <i>Microcotyle algeriensis</i> ex <i>Scorpaena notata</i>	13.3	13.3	13.9	18.0	14.3	0.0	0.0					
9 KX926446 <i>Microcotyle</i> sp. ex <i>Helicolenus dactylopterus</i>	11.2	11.2	12.2	17.3	12.2	3.7	3.7	3.7				
10 KX926447 <i>Microcotyle</i> sp. ex <i>Helicolenus dactylopterus</i>	11.2	11.2	12.2	17.3	12.2	3.7	3.7	3.7	0.0			
11 NC009055 <i>Microcotyle sebastis</i> ex <i>Sebastes schlegeli</i>	10.2	10.2	11.6	17.0	9.9	13.9	13.9	13.9	12.6	12.6		
12 Z83003 <i>Bivagina pagrosomi</i> (outgroup)	21.8	21.8	22.4	23.8	21.8	20.1	20.1	20.1	18.4	18.4	21.4	
13 JF505509 <i>Polylabris halichoeres</i> (outgroup)	21.8	21.8	22.1	22.8	20.1	22.4	22.4	22.4	20.4	20.4	22.8	23.8

*Type-specimens:* Holotype (MNHN HEL850) and 50 paratypes (MNHN HEL851–HEL900), including three with molecular information, are deposited in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN). Paratypes with molecular information: anterior and posterior parts of specimens mounted together on slide, median part used for molecular analysis: specimen MO01, slide MNHN HEL851; specimen MO02, slide MNHN HEL852; specimen MO03, slide MNHN HEL853.

*Representative DNA sequences:* *cox1* gene: GenBank MK27652 (paratype MO01, MNHN HEL851); MK27653 (paratype MO02, MNHN HEL852); GenBank MK27654 (paratype MO03, MNHN HEL853).

*ZooBank registration:* To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Microcotyle visa* n. sp. is urn:lsid:zoobank.org:act:EA3F6DB0-1DBB-4C9C-9A01-50BB4EE666AA.

*Etymology:* The species name, *visa*, refers to the joy of the first author when she obtained her visa from the French administration after a long period of uncertainty. Invariable, treated as a noun in apposition.

## Description (Figs. 2, 3)

[Based on 31 specimens] Body elongate, anterior end narrow, length of body proper 1,300–4,000 (1,998) (n = 31) (holotype: 2,200), total length 1,910–4,620 (2,737 ± 668) (n = 31) (holotype: 3,000); width at level of ovary 270–1,000 (500 ± 182) (n = 31) (holotype: 600). Haptor subsymmetrical or symmetrical, 250–1,250 (739) (n = 31) (holotype: 800) long, armed with 59–126 (100) (n = 20) (holotype: 94) clamps. Clamps of microcotylid type, arranged in 2 equal or sub-equal rows; length of clamps 25–60 (35) (n = 31), width 12–35 (21) (n = 31).

Pair of oval septal organs septate, elongate-oval, 30–85 × 20–60 (47 × 32) (n = 31) (holotype: 45 × 35). Pharynx subspherical, 20–55 × 20–45 (30 × 27) (n = 31) (holotype: 30 × 30). Intestine bifurcates at level of genital atrium. Caeca with numerous lateral and medial diverticula, apparently fuse just anterior to haptor, left branch extends into haptor.

Testes 14–29 (23) (n = 29) (holotype: 29) in number, post-ovarian, occurring in 2 rows generally,

intercaecal, in posterior half of body proper. Vas deferens conspicuous, runs in midline to genital atrium. Genital atrium at 120–600 (220) (n = 31) (holotype: 205) from anterior extremity of body, length 50–130 (74) (n = 31), width 45–95 (63) (n = 31). Genital atrium comprises anterior atrium proper and two posterior “pockets”. Atrium proper roughly shaped as inverted heart, bearing numerous [142–224 (189) (n = 12)] conical spines of similar sizes; spines apparently more dense in centre than in lateral parts. Variation in focus shows that the lumen of the atrium proper has two lateral expansions. Posterior pockets lined with spines similar to that of atrium proper, 18–39 (28) (n = 12). Total number of spines 172–262 (218) (n = 12). Genital pore middorsal.

Vaginal pore visible in most specimens, mediodorsal, posterior to genital atrium; distance from vagina to anterior extremity 320–780 (553) (n = 30). Canal from vagina to posterior part of female organs not seen. Ovary complex, begins at level of anteriormost testes, continues anteriorly in midline, reflexes at level of confluence of vitelline ducts, reflexes again toward anterior extremity, forms large anterior curve and reflexes a last time posteriorly and ends as oviduct. Oötype spindle-shaped, with posterior Mehlis' gland. Seminal receptacle well visible, lateral. Canal from vagina not seen. Genitointestinal canal visible in some specimens. Precise junctions between oviduct, oötype, vitelline duct and genitointestinal canal not elucidated.

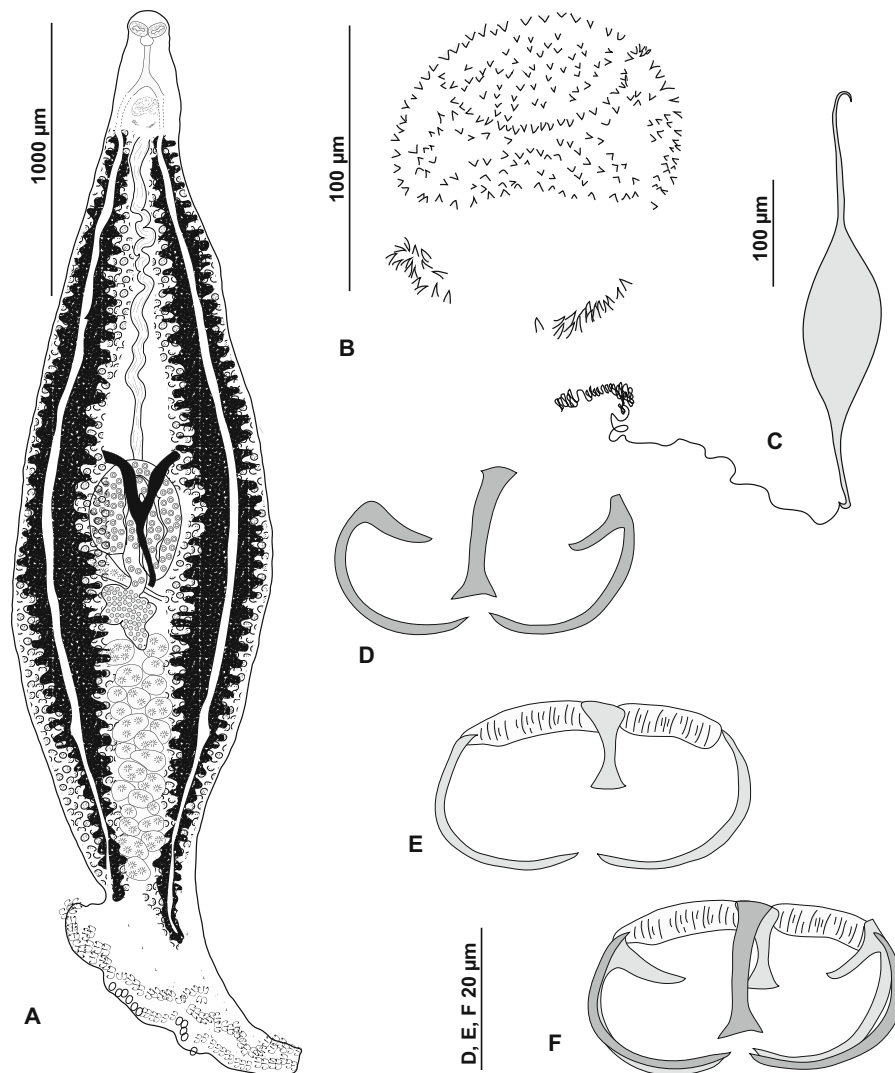
Vitellarium extends in most part of body from genital atrium to haptor. Vitelline ducts pair, united anteriorly and posteriorly; anterior junction visible only in some specimens; posterior junction conspicuous as an Y in most specimens, at level of ovary and ventral to it.

Egg fusiform, 157–260 × 60–100 (195 × 71) (n = 12), with 2 filaments, often coiled, 157–260 (195) (n = 12) long.

## Discussion

Differential diagnosis for *Microcotyle visa* n. sp.

In the following paragraphs, we compare the new species with *Microcotyle* spp. previously recorded from the Mediterranean (Table 3), then with



**Fig. 2** *Microcotyle visa* n. sp. ex *Pagrus caeruleostictus*. A, Holotype, whole body; B, Holotype, spines of genital atrium; C, Egg; D–F, Clamp (D, lower part, E, upper part, F, both parts superposed)

*Microcotyle* spp. from sparid hosts (Table 3); to lighten the text, references in tables are not repeated here.

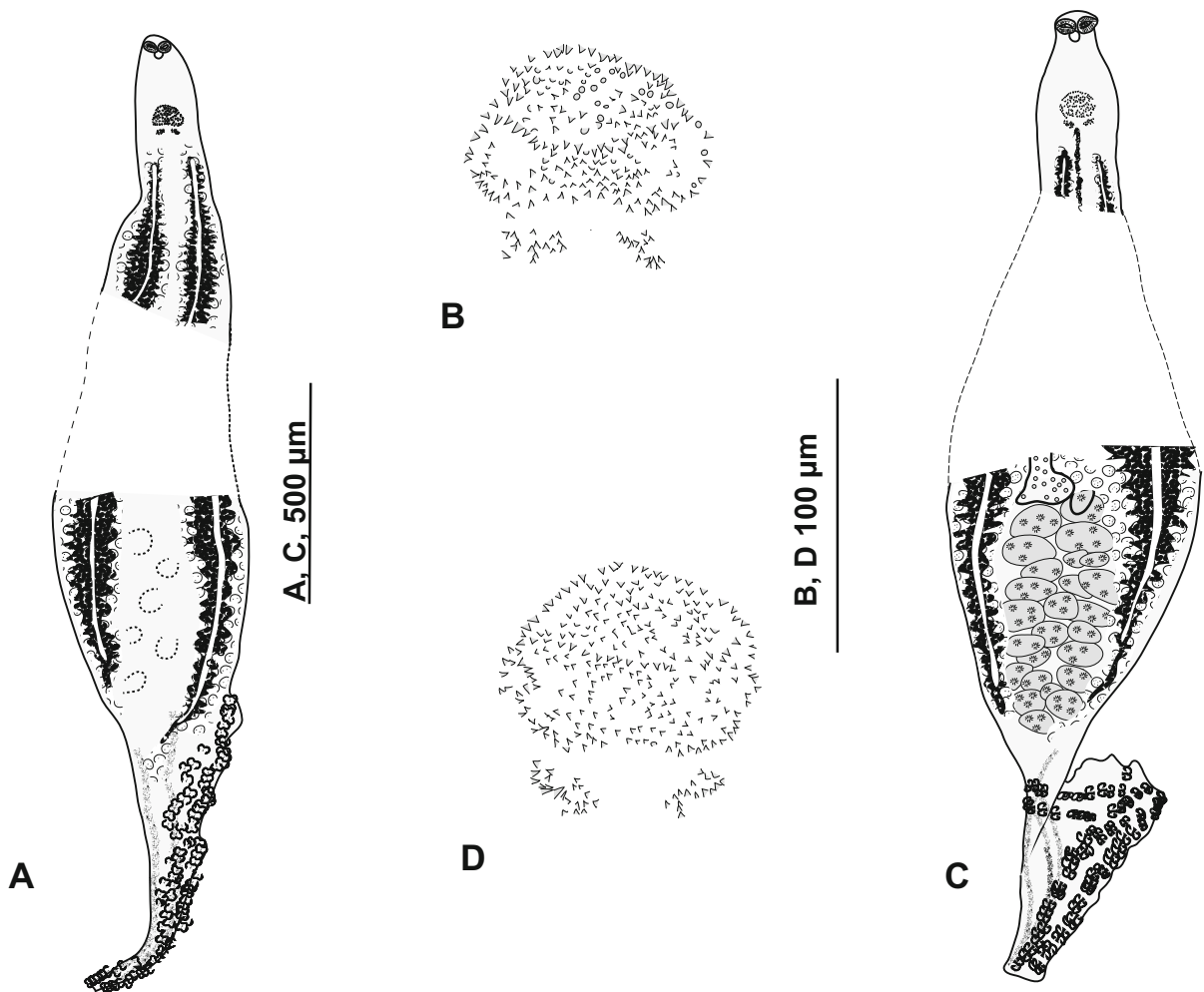
#### Comparison with species from the Mediterranean

*Microcotyle visa* n. sp. has in common with *M. algeriensis* the same shape of the genital atrium with two posterolateral “pockets” but differs in possessing a greater number of clamps which are also smaller, smaller genital atrium, greater number of testes and greater number of atrial spines (Table 3). Although the two species were described from the same type-

locality, the hosts are different (*Pagrus caeruleostictus* vs *Scorpaena notata*, Scorpaenidae).

*Microcotyle visa* n. sp. has in common with *Microcotyle* sp. of Ayadi et al. (2017) the general organisation of the genital atrium but differs in having a greater number of clamps and a greater number of atrial spines and testes (Table 3). The hosts are also different (*Helicolenus dactylopterus* (Sebastidae) for *Microcotyle* sp.).

Apart from the above, the new species can be differentiated from the other species described in the Mediterranean by the presence of two lateral



**Fig. 3** *Microcotyle visa* n. sp. ex *Pagrus caeruleostictus*, paratypes with associated molecular information. A, B, Paratype MNHN HEL852 (GenBank: MK27653); C, D, Paratype MNHN HEL851 (GenBank: MK27652)

expansions in the genital atrium (*vs* not mentioned in other species).

*Microcotyle visa* n. sp. differs from *M. lichiae* Ariola, 1899 by the arrangement of spines in the atrium (5 concentric rows in *M. lichiae*; unfortunately, never redescribed since the original description). The host is also different (*Lichia amia*, Carangidae) (Table 3).

*Microcotyle visa* n. sp. differs from *M. pomatomi* Goto, 1899 by the smaller number of clamps, clamp size, larger genital atrium and a smaller number of testes (Table 3). This species is allegedly present in the Mediterranean (Euzet et al., 1993) but measurements are available only for specimens from the

Atlantic (Table 3). The host is *Pomatomus saltatrix* (Pomatomidae), a fish with circumglobal distribution.

*Microcotyle visa* n. sp. differs from *M. donavini* van Beneden & Hesse, 1863 from *Symphodus mediterraneus* (Linnaeus) by the presence of two lateral expansions in the genital atrium, the larger number of clamps, smaller clamps size, smaller pharynx and genital atrium and a greater number of testes. *Microcotyle donavini* is allegedly present in the Mediterranean (Euzet et al., 1993) but measurements are available only for specimens from the Atlantic (Table 3) from the host *Labrus bergylta* (Labridae).

Distinguishing *Microcotyle visa* n. sp. from *M. erythrini* van Beneden & Hesse, 1863, a parasite of sparids, was a more difficult task. The two species



**Table 3** Measurements of *Microcoyle visa* n. sp. from *P. caeruleostictus* off Algeria and *Microcoyle* spp. recorded from the Mediterranean

Species	<i>M. visa</i> n. sp.	<i>Microcoyle</i> sp.	<i>M. algeriensis</i>	<i>M. erythrinii</i>	<i>M. lichiae</i>	<i>M. donavini</i> <sup>a</sup>	<i>M. pomatomi</i>
Host	<i>Pagrus caeruleostictus</i>	<i>Helicolenus dactylopterus</i>	<i>Scorpaena notata</i>	<i>Pagellus erythrinus</i>	<i>Lichia amia</i>	<i>Symphodus mediterraneus</i>	<i>Pomatomus saltatrix</i>
Locality	Off Zemmouri, Algeria	Off Bouharoune, Algeria	Off Bouharoune, Algeria	Off Ain Taya, Algeria	Off Genova, Italy	Off Roscoff, English Channel (NE Atlantic)	Off Perth, Western Australia, (Pacific)
Source	Present study	Ayadi et al. (2017)	Ayadi et al. (2017)	Kouider El Ouahed-Amine (1998)	Ariola (1899)	Euzet & Marc (1963)	Williams (1991)
Body proper length	1,300–4,000 (1,998 ± 644; n = 31)						
Haptor length	250–1,250 (739 ± 200, n = 31)	570–1,200 (962, n = 20)	450–1,040 (781 ± 153, n = 35)				464–1,680 (976)
Total length	1,910–4,620 (2,737 ± 668, n = 31)	410–3,800 (3,092, n = 20)	1,900–4,300 (3,298 ± 592, n = 35)	1,900–2,800	8,000	4,000–5,000	3,040–4,080 (3,416)
Body width	270–1,000 (500 ± 182, n = 31)			270–530	500	400–500	400–416 (408)
No. of clamps	59–126 (100, n = 20)	49–58 (54, n = 20)	20–39 (31 ± 4, n = 32)	86–110	52	86	104–148 (130)
Clamp length	25–60 (35 ± 7, n = 31)	42–74 (64 ± 8, n = 32)	48–85 (70 ± 9, n = 51)	50–75	100–300 <sup>b</sup>	33–50	45–56 (52)
Clamp width	12–35 (21 ± 4, n = 31)	40–69 (44 ± 7, n = 42)	40–78 (48 ± 7, n = 51)	25–40		2–87	59–64 (61)
Buccal organ length	30–85 (47 ± 11, n = 31)	47–73 (61, n = 20)	40–85 (59 ± 10, n = 28)	35–56	220	65	34–61 (48) <sup>b</sup>
Buccal organ width	20–60 (32 ± 8, n = 31)			30–35	64	40	
Pharynx length	20–55 (30 ± 7, n = 31)	40–77 (61, n = 20)	50–100 (74 ± 13, n = 28)	24–30 <sup>b</sup>	70	50	32–42 (36) <sup>b</sup>
Pharynx width	20–45 (27 ± 6, n = 31)	50–69 (58, n = 20)	46–90 (69 ± 12, n = 28)		51	40	
Genital atrium length	50–130 (74 ± 19, n = 31)	95–160 (131, n = 5)	77–175 (115, n = 11)			250	109–134 (122) <sup>b</sup>

Table 3 continued

Species	<i>M. visa</i> n. sp.	<i>Microcotyle</i> sp.	<i>M. algeriensis</i>	<i>M. erythrini</i>	<i>M. lichiae</i>	<i>M. donavini</i> <sup>a</sup>	<i>M. pomatomi</i>
Host	<i>Pagrus caeruleostictus</i>	<i>Helicolenus dactylopterus</i>	<i>Scorpaena notata</i>	<i>Pagellus erythrinus</i>	<i>Lichia amia</i>	<i>Symphodus mediterraneus</i>	<i>Pomatomus saltatrix</i>
Locality	Off Zemmouri, Algeria	Off Bouharoune, Algeria	Off Bouharoune, Algeria	Off Ain Taya, Algeria	Off Genova, Italy	Off Roscoff, English Channel (NE Atlantic)	Off Perth, Western Australia, (Pacific)
Source	Present study	Ayadi et al. (2017)	Ayadi et al. (2017)	Kouider El Ouahed-Amine (1998)	Ariola (1899)	Euzet & Marc (1963)	Williams (1991)
Genital atrium width	45–95 (63 ± 12, n = 31)	102–150 (133, n = 5)	83–130 (106, n = 11)			175	
No. of spines in main group	142–224 (189, n = 12)	104–307 (184, n = 5)	68–162 (102, n = 12)				
No. of spines in pockets	18–39 (28, n = 12)	12–38 (24, n = 5)	8–18 (14, n = 12)				
Total no. of spines	172–262 (218, n = 12)	122–333 (210, n = 5)	76–174 (116, n = 12)				
Distance genital atrium-anterior end	120–600 (220 ± 96, n = 31)	270–520 (397, n = 5)	110–400 (225, n = 2)				
No. of testes	14–29 (23 ± 4, n = 29)	10–17 (13, n = 11)	9–20 (13, n = 9)	16–17		18–22	
Egg length	157–260 (195, n = 12)		215–257 (236, n = 10)			200–225	26–40 (28)
Egg width	60–100 (71, n = 12)		50–85 (68, n = 10)			75–80	

<sup>a</sup>Except *M. donavini* and *M. pomatomi* which were recorded from the English Channel and Pacific, respectively, all localities are in the Mediterranean. Note that these species have been recorded from the Mediterranean, but measurements are only available for specimens from elsewhere. <sup>b</sup>Length and width are given as diameter

**Table 4** Measurements of *Microcotyle* spp. from sparid hosts (with localities other than those in Table 3)

Species	<i>Microcotyle visa</i> n. sp.		<i>M. erythrina</i> van Beneden & Hesse, 1863		<i>M. omanae</i> Machkewskyi et al., 2013	<i>M. archosargi</i> MacCallum, 1913
	<i>Pagrus caeruleostictus</i> Off Zemmouri, Algeria (Mediterranean)	<i>Pagellus erythrinus</i> Off Brest (NE Atlantic)	<i>P. erythrinus, Pagellus acarne, Boops boops</i> Off Brest (NE Atlantic); Genoa, Italy (Mediterranean)	<i>P. erythrinus</i> Off Boka Kotorska, Montenegro (Adriatic Sea)		
Host	Present study	van Beneden & Hesse (1863)	Parona & Perugia (1890)	(Radujkovic & Euzet 1989)	<i>Cheimereus nufar</i>	<i>Archosargus probatocephalus</i>
Locality					Off Shuweymiyah and Sharbithat, Arabian Sea (Indian Ocean)	New York fish market
Source					Machkewskyi et al. (2013)	MacCallum (1913)
Body length	1,300–4,000 (1,998 ± 644; n = 31)	4,000			1,125–3,225	1,500
Haptor length	250–1,250 (739 ± 200, n = 31)					
Total length	1,910–4,620 (2,737 ± 668, n = 31)		4,000–5,000	2,300–2,700	3,500–11,000 (6,020)	8,000
Body width	270–1,000 (500 ± 182, n = 31)		500	200–400		800
No. of clamps	59–126 (100, n = 20)		90	100–110	94–120	106
Clamp length	25–60 (35 ± 7, n = 31)		70 (diameter)	60	26–55	80
Clamp width	12–35 (21 ± 4, n = 31)			25	70–100	40
Buccal organ length	30–85 (47 ± 11, n = 31)		71	45	60–120	10
Buccal organ width	20–60 (32 ± 8, n = 31)		45	40	28–75	70
Pharynx length	20–55 (30 ± 7, n = 31)					
Pharynx width	20–45 (27 ± 6, n = 31)					
Genital atrium length	50–130 (74 ± 19, n = 31)				25–214	100
Genital atrium width	45–95 (63 ± 12, n = 31)				127–193	80
No. of spines in main group	142–224 (189, n = 12)					
No. of spines in pockets	18–39 (28, n = 12)					
Total no. of spines	172–262 (218, n = 12)					
Distance genital atrium-anterior end	120–600 (220 ± 96, n = 31)			250		

Table 4 continued

Species	<i>Microcotyle visa</i> n. sp.	<i>M. erythrini</i> van Beneden & Hesse, 1863	<i>M. omanae</i> Machkewskyi et al., 2013	<i>M. archosargi</i> MacCallum, 1913
Host	<i>Pagrus caeruleostictus</i> Off Zemmouri, Algeria (Mediterranean)	<i>P. erythrinus, Pagellus erythrinus</i> Off Brest (NE Atlantic)	<i>P. erythrinus</i> Off Boka Kotorska, Montenegro (Adriatic Sea)	<i>Archosargus probatocephalus</i> New York fish market
Locality		Genoa, Italy (Mediterranean)		
Source	Present study	van Beneden & Hesse (1863)	Parona & Perugia (1890)	MacCallum (1913)
No. of testes	14–29 (23 ± 4, n = 29)	16	16–19	20–35
Egg length	60–100 (71, n = 12)			260–300
Egg width	20–55 (30 ± 7, n = 31)		34–55	70

share similarities such as locality (Algeria, Mediterranean Sea), host family (Sparidae), shape of genital atrium (especially the presence of two lateral expansions) and maximum number of clamps (Table 3). However, *Microcotyle visa* n. sp. can be differentiated from *M. erythrini* by the smaller clamps size, a larger pharynx and a greater number of testes. Our molecular analysis showed that the distance between the *cox1* sequences of both species was 14%, strongly suggesting that the species are distinct.

Comparison with species from sparids not from the Mediterranean

*Microcotyle visa* n. sp. resembles *M. omanae* in the armament of the genital atrium and the number of clamps as well as their size. However, *Microcotyle visa* n. sp. differs from *M. omanae* by its distant location (Indian Ocean vs Mediterranean Sea), the presence of two lateral expansions in the atrium, and the smaller number of testes. Hosts are also different (*Cheimerius nufar* for *M. omanae*) (Table 4).

*Microcotyle visa* n. sp. resembles *M. archosargi* MacCallum, 1913 in the number of clamps and testes as well as the size of the genital atrium but differs by its locality (Off Algeria, Mediterranean Sea vs off New York, Western Atlantic) and host (*P. caeruleostictus* vs *Archosargus probatocephalus* (Walbaum)) as well as by the smaller clamp size and the shape and armature of genital atrium as the two lateral expansions and pockets are absent in *M. archosargi* (Table 4).

Species of *Microcotyle* from sparids

Sparid hosts are known to be parasitised with microcotylids. Eleven species recovered from sparids have been originally described as members of *Microcotyle* but have been transferred to other genera: *Bivagina alcedinis* (Parona & Perugia, 1889) Yamaguti, 1963; *Sparicotyle chrysophrii* (van Beneden & Hesse, 1863) Mamaev, 1984; *Bivagina pagrosomi* (Murray, 1931) Dillon & Hargis, 1965; *Neobivagina canthari* (van Beneden & Hesse, 1863) Dillon & Hargis, 1965; *Pagellicotyle mormyri* (Lorenz, 1878) Mamaev, 1984; *Atrispinum salpae* (Parona & Perugia, 1890); and *Atrispinum sargi* (Parona & Perugia, 1890) Euzet & Maillard, 1974 (see van Beneden & Hesse, 1863;

**Table 5** Intraspecific variations of the *cox1* gene within species of the Polyopisthocotylea

Species	Family	Sequence divergence (%)	Source
<i>Allodiscocotyla diacanthi</i> Unnithan, 1962	Allodiscocotylidae	3.7	Tambireddy et al. (2016)
<i>Neoheterobothrium</i> sp.	Diclidophoridae	“0–2 bases”	Yoshinaga et al. (2009)
<i>Neoheterobothrium hirame</i> Ogawa, 1999	Diclidophoridae	“0–2 bases”	Yoshinaga et al. (2009)
<i>Parapedocotyle prolatili</i> Oliva, Sepulveda & González, 2014	Diclidophoridae	0.4	Oliva et al. (2014)
<i>Pedocotyle bravoii</i> Luque-Alejos & Iannacone-Oliver, 1990	Diclidophoridae	0.6	Oliva et al. (2014)
<i>Gotocotyla sawara</i> Ishii, 1936	Gotocotylidae	0.13–3.65	Shi et al. (2014)
<i>Zeuxapta seriola</i> (Meserve, 1938)	Heteraxinidae	0.5–0.9	Sepúlveda & González (2015)
<i>Mazocraeoides gonialosae</i> Tripathi, 1959	Mazocraeidae	0.01–2.08	Li et al. (2011)
<i>Pseudokuhnia minor</i> (Goto, 1984)	Mazocraeidae	0.1–4.8	Yan et al. (2016)
<i>Kuhnia scombri</i> (Kuhn, 1829)	Mazocraeidae	0.1–5.6	Yan et al. (2016)
<i>Polystoma</i> sp.	Polystomatidae	0.64–1.29	Du Preez et al. (2007)
<i>Polystoma dawiekoki</i> Du Preez, Vaucher & Mariaux, 2002	Polystomatidae	1.92	Du Preez et al. (2007)
<i>Bilaterocotylodes madrasensis</i> Radha, 1966	Protomicrocotylidae	0.3	Tambireddy et al. (2016)
<i>Microcotyle</i> sp.	Microcotylidae	2.56	Li et al. (2011)
<i>Microcotyle algeriensis</i> Ayadi, Gey, Justine & Tazerouti, 2016	Microcotylidae	0	Ayadi et al. (2017)
<i>Microcotyle</i> sp.	Microcotylidae	0	Ayadi et al. (2017)
<i>Microcotyle visa</i> n. sp.	Microcotylidae	1.4	Present study
<i>Sparicotyle chrysofritii</i> (van Beneden & Hesse, 1863)	Microcotylidae	4.6	Mladineo et al. (2009)
	Microcotylidae	0.3	Mladineo et al. (2009)

Parona & Perugia, 1890; Yamaguti, 1963; Dillon & Hargis, 1965; Mamaev, 1984).

Only three monogenean species described from sparid hosts are still considered members of *Microcotyle*: *M. omanae* from *Cheimerius nufar*; *M. archosargi* from *Archosargus probatocephalus*; and *M. erythrini* from *Pagellus erythrinus* (see van Beneden & Hesse, 1863; MacCallum, 1913; Machkewskyi et al., 2013). Only the latter species occurs in the Mediterranean. Our study increases the number of *Microcotyle* spp. infecting sparid hosts up to four.

*Microcotyle erythrini* is exceptional by its wide host range and relatively large geographical distribution ranging from the Mediterranean to the Atlantic. Records in the Atlantic include off France (van Beneden & Hesse, 1863) and off Spain (Pérez-del-Olmo et al., 2007a, b); records in the Mediterranean include a number of localities (Parona & Perugia, 1890; Ulmer & James, 1981; Kouider El Ouahed-

Amine, 1998; Jovelín & Justine, 2001; Pérez-del Olmo et al., 2007a, b; Fernandez-Jover et al., 2010; Strona et al., 2010; Marzoug et al., 2012; Akmirza, 2013; Papoutsoglou, 2016), from different host species, mainly sparids. Monogeneans are often considered to be strictly host-specific and this situation is puzzling. It is likely that the specific status of the *M. erythrini*-like specimens infecting sparid hosts would change if a detailed morphological and molecular study is performed.

Intraspecific morphometric and molecular variability in polyopisthocotylean monogeneans

In the course of our study, we found some variability in several measurements of the “soft” parts within our new species, but measurements of “hard” parts (clamps) and counts of organs, either hard (clamps, atrium spines) or soft (testes) did not differ

**Table 6** Diagnostic characters of the genus *Paramicrocotyle* Caballero & Bravo-Hollis, 1972 and the genus *Microcotyle* van Beneden & Hesse, 1863. Features used by Caballero y Caballero & Bravo-Hollis (1972) to distinguish the two genera are shown in bold

Genus Source	<i>Paramicrocotyle</i> Caballero & Bravo-Hollis, 1972 Caballero y Caballero & Bravo-Hollis (1972)	<i>Microcotyle</i> van Beneden & Hesse, 1863 Yamaguti (1963)
Body shape	Sagittiform, front end slightly truncated	Lanceolate
<b>Haptor</b>	Pyramidal, symmetrical, without larval anchors	Triangular, symmetrical or subsymmetrical without larval anchors. Numerous clamps commencing at or behind level of testes
Cephalic glandular system	Present	May or may not be present
Oral organs	Paired, oblong and septate	Paired, septate each, and with a single or double row of minute spines
Oesophagus	Short and diverticulate	Simple or diverticulate laterally
Intestinal branches	Diverticulate, extends into haptor and without anastomosis	Diverticulate may or may not extend into haptor, not united posteriorly
<b>Genital atrium</b>	Large, post-bifurcal, elliptical in shape	Variouly armed, bifurcal or rarely post-bifurcal
Genital pore	Opens in the bottom of atrium	Genital pore oesophageal
Cirrus	Cirrus bulb muscular	Cirrus may or may not be differentiated, sometimes bulbous
Testes	Few	Usually numerous
Genito-intestinal canal	Present	Present
Egg	Elongated, filamented at one pole	Egg filamented at one pole or both poles
Vagina	Single mid-dorsal, papillated	Usually single mid-dorsal
<b>Vaginal duct</b>	Usually shaped like an inverted Y	Usually shaped like an inverted Y, occasionally as an inverted V

significantly. A molecular study of the *cox1* gene, unfortunately limited to a small number of samples, showed that the intraspecific distance was low (1.4%), especially when compared with distances between established species (10.2–15.0%). Intraspecific variations in body size in polyopisthocotylean monogeneans are well known, and the literature contains many examples of authors discussing artefacts of fixation and effect of flattening on the size of organs (Goto, 1894; Sproston, 1946; Justine, 2011; Machkewskyi et al., 2013). In Table 5, we compiled published results concerning the intraspecific differences of *cox1* sequences. The difference found in the present specimens (1.4%) falls well within the intraspecific range found by various authors, thus confirming that a single species, here described as *M. visa* was present in our specimens.

The genus *Paramicrocotyle* Caballero & Bravo-Hollis, 1972

Caballero & Bravo-Hollis erected *Paramicrocotyle* Caballero & Bravo-Hollis 1972 for *P. atriobursata* Caballero & Bravo-Hollis, 1972 and *P. tampicensis* Caballero & Bravo-Hollis, 1972 (see Caballero y Caballero & Bravo-Hollis, 1972). They distinguished *Paramicrocotyle* from *Microcotyle* by the following features: structure of the haptor, structure and shape of the genital atrium and the presence of two vitello-vaginal pouches. They placed in their newly erected genus 16 species previously belonging to the genus *Microcotyle*. Later, Mamaev (1986) synonymised *Paramicrocotyle* with *Microcotyle*, and *Paramicrocotyle* was considered *taxon inquirendum* by Mendoza-Garfias et al. (2017).

Based on morphological similarities between the two genera (Table 6), we follow Mamaev (1986) and consider *Paramicrocotyle* a junior synonym of

*Microcotyle*. Moreover, our molecular study included a member of *Paramicrocotyle*, which was placed amongst several species of *Microcotyle* without having a distinct branch, and the *Microcotyle* + *Paramicrocotyle* clade was well supported. Mamaev (1986) did not change the generic status of *P. danielcarrioni* Martinez & Barrantes, 1977 (probably omitted) nor that of *P. moyanoi* Villalba & Fernandes, 1986 (described simultaneously to his study) (see Martinez & Barrantes, 1977; Villalba & Fernández, 1986). After examining the original descriptions, we transfer these species as *Microcotyle danielcarrioni* (Martinez & Barrantes, 1977) n. comb. and *Microcotyle moyanoi* (Villalba & Fernandes, 1986) n. comb.

**Acknowledgements** The authors are grateful to the French Consulate in Algiers. We thank members of the administrative staff of ISYEB, MNHN, for help for the visa, namely Sabrina Permall, Guzide Selcuk-Shahine, Sylvianne Babin and Nora Tagnit-Hamou. The authors are indebted to the staff of LBEIG, Amina Boutellis, Zouhour El Mouna Ayadi and Samia Terkmani for their continued support of our research. Our thanks are also due to fishermen from Regaia and especially Mohamed Kayrouz. We thank Marcelo Enrique Oliva and John D. Chero for providing references.

**Funding** This work was funded by ISYEB, MNHN.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable institutional, national and international guidelines for the care and use of animals were followed.

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