



# Towards the resolution of the *Microcotyle erythrini* species complex: description of *Microcotyle isyebi* n. sp. (Monogenea, Microcotylidae) from *Boops boops* (Teleostei, Sparidae) off the Algerian coast

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Received: 12 December 2018 / Accepted: 14 March 2019 / Published online: 26 March 2019  
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## Abstract

The monogenean *Microcotyle erythrini* is atypical because it has been recorded from several fish host species in the Mediterranean Sea and Atlantic Ocean, in contrast to many species which are considered strictly specific. This could indicate a true lack of specificity or that several cryptic species are involved. This paper is a partial attempt to solve this problem. Specimens of a monogenean resembling *M. erythrini* were collected from bogues, *Boops boops*, caught off Algeria. A comparison with published descriptions and with museum specimens of *M. erythrini* did not yield any clear morphological difference. However, sequences of cytochrome c oxidase subunit I (COI) differed by 16.3% from that of *M. erythrini* (from GenBank, material collected from the type-host *Pagellus erythrinus*), indicating that the species was different. The species from *B. boops* is therefore described here as *Microcotyle isyebi* n. sp. and differential diagnoses with *Microcotyle* species from the Mediterranean and from sparids are provided. These results suggest that a molecular re-evaluation of other *M. erythrini*-like specimens from various fish hosts could reveal the existence of additional parasite biodiversity.

**Keywords** Cryptic species · New species · Monogenea · Mediterranean Sea · Integrative taxonomy

## Introduction

*Microcotyle erythrini* was described by Van Beneden & Hesse (1863) from the sparid *Pagellus erythrinus* collected off Brest (Brittany, Atlantic Ocean). The species has been since recorded from three other hosts (*Pagellus acarne*, *Boops boops* and *Dentex dentex*) in several localities in the Mediterranean (Table 1). This situation is somewhat puzzling since most

monogeneans are considered to be highly host specific. Therefore, two hypotheses can be proposed: (a) *M. erythrini* is actually a monogenean with unusually wide specificity or (b) there are several species, each specific to a single host, which could not be distinguished by morphology. Molecular systematics is the tool of choice for resolving species complexes and recognising cryptic species (Galimberti et al. 2012; Glennon et al. 2008; Jousson et al. 2000; Miller et al. 2010; Vilas et al. 2005).

In this study, we used morphology and molecules to identify a species of *Microcotyle* found on *B. boops* off Algeria, a fish on which *M. erythrini* was previously recorded in various parts of the Mediterranean Sea and Atlantic Ocean. We found that morphology alone did not allow us to differentiate the species but that cytochrome c oxidase subunit I (COI) barcoding revealed wide differences with *M. erythrini* collected from its type-host. The species from *B. boops* is herein described as a new species. This paper is a part of an ongoing effort to characterise the parasite biodiversity of fish of the southern shores of the Mediterranean Sea (Ayadi et al. 2017; Boudaya and Neifar 2016; Bouguerche et al. 2019; Chaabane et al. 2015, 2016a, b, 2017; Chaari et al. 2016; Kheddami et al. 2016; Kouider El Ouahed-Amine 1998).

Section Editor: Stephen A. Bullard

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**Table 1** Previous record of *Microcotyle erythrini*

Host and locality	Source
<i>Pagellus erythrinus</i> (type-host)	
France, Atlantic	Van Beneden and Hesse (1863)
Montenegro, Mediterranean Sea	Radujkovic and Euzet (1989)
France, Mediterranean Sea	Euzet (1957), Jovelin and Justine (2001)
Italy, Mediterranean Sea	Parona and Perugia (1890), Ulmer and James (1981)
Algeria, Mediterranean Sea	Kaouachi et al. (2010), Kouider El Ouahed-Amine (1998)
<i>Boops boops</i>	
France, Mediterranean Sea	Renaud et al. (1980)
Italy, Mediterranean Sea	Parona and Perugia (1890), Strona et al. (2010)
Spain, Atlantic Ocean	Pérez-del Olmo et al. (2007b)
Spain, Mediterranean Sea	Fernandez-Jover et al. (2010), López-Román and Guevara Pozo (1973), Marzoug et al. (2012)
Spain, Atlantic Ocean and Mediterranean Sea	Pérez-del Olmo et al. (2007a, 2008), Power et al. (2005)
Turkey, Mediterranean Sea	Akmirza (2013)
Algeria, Mediterranean Sea	Benhamou et al. (2017), Marzoug et al. (2012), Ramdane et al. (2013)
<i>Pagellus acarne</i>	
Turkey, Mediterranean Sea	Akmirza (2013)
Italy, Mediterranean Sea	Parona and Perugia (1890)
<i>Dentex dentex</i>	
Spain, Mediterranean Sea	González et al. (2004)

## Material and methods

### Fish

From 2015 through 2017, 2004 specimens of *B. boops* were obtained dead from fishermen in Bouharoun, Algerian coast (36° 37' N, 2° 39' E). 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 were observed under a microscope for the presence of monogeneans.

### Monogeneans

#### Morphological methods

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. Drawings were made with the help of an Olympus BH-2 microscope with Differential Interference Contrast and a drawing tube. Drawings were scanned and redrawn on a computer with Adobe Illustrator. Measurements are in micrometres and indicated as means  $\pm$  standard deviation if  $n > 30$  and between parentheses the range and number of measurements. Measurements of holotype are indicated.

#### Molecular methods

To ensure that hosts and monogenean were labelled with respect of host-parasites relationships, we followed a precise protocol (Ayadi et al. 2017; Bouguerche et al. 2019; Justine et al. 2013). One specimen of *Microcotyle* sp. was extracted, and a tissue sample from the gill of the fish harbouring it was taken. The monogenean was cut in three parts using a scalpel blade. The anterior part (which includes the genital atrium) and the posterior part (which contains the testes and the haptor) were mounted, on a single slide, for deposition in a museum; the middle part was preserved in absolute ethanol then subjected to molecular analyses. This protocol ensures that the sequenced monogenean specimen actually corresponds to the species described and that the sequenced host is precisely identified.

#### Molecular barcoding of fish

Total genomic DNA was isolated with a QIAamp DNA Mini Kit (Qiagen) as per the manufacturer's instructions. The 5' region of the mitochondrial COI gene was amplified with the primers FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGA CTTCTGGGTGGCCAAAGAATCA-3') (Ward et al. 2005). PCR reactions were performed in 20  $\mu$ l, containing 1 ng of DNA, 1 $\times$  CoralLoad PCR buffer, 3 mM MgCl<sub>2</sub>, 66  $\mu$ M of each dNTP, 0.15  $\mu$ M 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 the sequence, which was 637 bp in length, compared it to the GenBank database content with BLAST and deposited it in GenBank under accession number MK317921. Species identification was confirmed with the BOLD identification engine (Ratnasingham and Hebert 2007).

## COI sequences of monogeneans

Total genomic DNA was isolated using the QIAamp DNA Micro Kit (Qiagen). The specific primers JB3 (=COIASmit1) (forward 5'-TTTTTTGGGCATCCTGAGGT TTAT-3') and JB4.5 (=COI-ASmit2) (reverse 5'-TAAA GAAAGAACATAATGAAAATG-3') were used to amplify a fragment of 396 bp of the COI gene (Bowles et al. 1995; Littlewood et al. 1997). PCR reaction was performed in 20  $\mu$ l, containing 1 ng of DNA, 1 $\times$  CoralLoad PCR buffer, 3 mM MgCl<sub>2</sub>, 0.25 mM dNTP, 0.15  $\mu$ M 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. The sequence was edited with CodonCode Aligner software version 3.7.1, compared to the GenBank database content with BLAST and deposited in GenBank under accession number MK317922.

## Trees and distances

All available sequences of *Microcotyle* spp. available in GenBank and one sequence of *Microcotyle isyebi* n. sp. generated in this study were included in the phylogenetic analyses (Table 2). A sequence deposited in GenBank as '*Paramicrocotyle* sp.' was included since this genus is now considered a junior synonym of *Microcotyle* (Bouguerche et al. 2019). Sequences of *Bivagina pagrosomi* (Murray, 1931) and *Polylabris halichoeres* Wang & Zhang, 1998 were used as outgroup. All these species belong to the Microcotylidae. The trees were inferred using the neighbour-joining method (Saitou and Nei 1987) and the maximum likelihood method 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). Bootstrap numbers were 10,000 for NJ and 100 for ML. Genetic distances (p-distance and Kimura-2 parameter (Kimura 1980) distance) were estimated with MEGA7. All codon positions were used.

## Results

### Molecular identification of fish

The provisional identification of fish species using morphological characteristics was confirmed by DNA barcoding approach. BLAST analysis of the COI sequence of the fish specimen of the present study with NCBI and BOLD database showed sequence similarity values of 100% for *B. boops*, thus confirming the identification. The BOLD database (Ratnasingham and Hebert 2007) includes many sequences

of *B. boops* with published information, and thus, we are confident that the identification is valid.

### Molecular characterisation of monogeneans

The COI sequence of *M. isyebi* n. sp. was aligned with other microcotylid sequences. For trees, the neighbour-joining and maximum likelihood methods led to slightly different topologies; we show only the former in Fig. 1. The analysis involved 14 nucleotide sequences, and there were a total of 294 positions in the final dataset. *M. isyebi* n. sp. differed from a sequence of *M. erythrini* from the type-host *P. erythrinus* (Jovelin and Justine 2001) and two distinct sequences of *Microcotyle visa* from *Pagrus caeruleostictus* by 16.3%, 9.5% and 10.7%, respectively (Table 3). The high divergence strongly suggests that the new species is distinct from *M. erythrini* as well as from all the other species of *Microcotyle* from sparid hosts with available COI sequences. The sequence of *M. isyebi* n. sp. was different from sequences of *Microcotyle algeriensis* and *Microcotyle* sp. from Scorpaenidae off the Algerian coast by 14.7% and 13%, respectively.

### *Microcotyle isyebi* n. sp.

Type-host: *B. boops* (Linnaeus, 1758) (Sparidae).

Type-locality: Off Bouharoun, Algerian coast (36° 37' 24.17" N, 2° 39' 17.38" E).

Site on host: Gills.

Prevalence and intensity: 15% (298/2004), up to 3 monogeneans/fish.

Type-specimens: Holotype (MNHN HEL918) and 31 paratypes on 28 slides (MNHN HEL919-946) deposited in the collections of the Muséum National d'Histoire Naturelle, Paris.

Paratype of specimen with molecular analysis: Slide MNHN HEL919, COI barcode GenBank MK317922; this specimen has 237 spines in main group of atrium, 35 in pockets, total number 272; 23 testes; 95 clamps. COI barcode of individual host fish harbouring this paratype, GenBank MK317921.

Etymology: The species is named after ISYEB, an acronym for 'Institut de Systématique, Évolution, Biodiversité' as an acknowledgement of the help offered to the first author.

Material examined for comparison: 15 specimens of *M. erythrini* from type-host *P. erythrinus* from Sète, French Mediterranean Coast, from the collection of Louis Euzet deposited in the Muséum National d'Histoire Naturelle (MNHN 306HG, Box 12, slides 3, 6–18, 25).

### Description

Based on 31 specimens; measurements of holotype indicated as h. Body elongate, anterior end narrow, length of body

**Table 2** Species of the Microcotylidae used in the present molecular study

Parasite species	Host species	Origin	GenBank	Source
<i>Microcotyle isyebi</i> n. sp.	<i>Boops boops</i>	Algeria	MK317922 <sup>a</sup>	Present study
<i>Microcotyle visa</i>	<i>Pagrus caeruleostictus</i>	Algeria	MK27652	Bouguerche et al. (2019)
<i>Microcotyle visa</i>	<i>Pagrus caeruleostictus</i>	Algeria	MK27653	Bouguerche et al. (2019)
<i>Microcotyle visa</i>	<i>Pagrus caeruleostictus</i>	Algeria	MK27654	Bouguerche et al. (2019)
<i>Microcotyle sebastis</i> <sup>b</sup>	<i>Sebastes schlegeli</i>	South Korea	NC009055	Park et al. (2007)
<i>Microcotyle erythrini</i>	<i>Pagellus erythrinus</i>	France	AY009159	Jovelin and Justine (2001)
<i>Paramicrocotyle</i> sp. <sup>c</sup>	<i>Pinguipes chilensis</i>	Chile	KJ794215	Oliva et al. (2014)
<i>Microcotyle</i> sp.	<i>Helicolenus dactylopterus</i>	Algeria	KX926446	Ayadi et al. (2017)
<i>Microcotyle</i> sp.	<i>Helicolenus dactylopterus</i>	Algeria	KX926447	Ayadi et al. (2017)
<i>Microcotyle algeriensis</i>	<i>Scorpaena notata</i>	Algeria	KX926443	Ayadi et al. (2017)
<i>Microcotyle algeriensis</i>	<i>Scorpaena notata</i>	Algeria	KX926444	Ayadi et al. (2017)
<i>Microcotyle algeriensis</i>	<i>Scorpaena notata</i>	Algeria	KX926445	Ayadi et al. (2017)
<i>Bivagina pagrosomi</i>	<i>Sparus aurata</i>	Australia	Z83003	Littlewood et al. (1997)
<i>Polylabris halichoeres</i>	<i>Halichoeres nigrescens</i>	China	JF505509	Zhang et al. (2011)

<sup>a</sup> New sequence

<sup>b</sup> The taxonomic identity of this species has been questioned (Ayadi et al. 2017)

<sup>c</sup> *Paramicrocotyle* sp. included with species of *Microcotyle* in this study (Bouguerche et al. 2019)

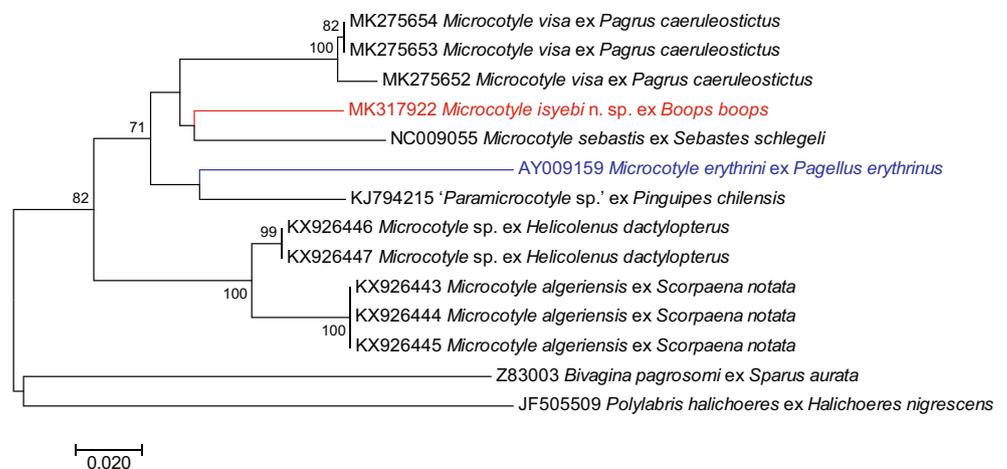
proper h 3810, 2647 ± 514 (1750–3810, n = 32), length of haptor h 870, 809 ± 164 (500–1250, n = 32), total length including haptor h 4680, 3480 ± 600 (2370–4750, n = 31); maximum width at level of ovary h 750, 598 ± 159 (310–830, n = 32). Haptor triangular, subsymmetrical or symmetrical, h 870, 809 ± 164 (500–1250, n = 32) long. Haptor armed with h 92, 83 (54–102, n = 25) clamps. Clamps of microcotylid type, arranged in two equal or sub-equal rows. Clamps dissimilar in size: posterior and newly formed anterior clamps smallest. Length of largest clamps 42 ± 11 (25–62, n = 41), width 17 ± 4 (10–31, n = 40) (Figs. 2 and 3).

Two oval prohaptor suckers 59 ± 8 (40–75, n = 34) length × 44 ± 9 (30–65, n = 34) width; row of tiny papillae visible on ventral rims. Pharynx small, subspherical 40 ± 7

(25–50, n = 34) length × 38 ± 5 (30–48, n = 34) width, behind oral organs. Intestine caeca beginning at level of genital atrium, with numerous lateral and medial diverticula. Caeca probably not confluent. Right caecum terminates near beginning of haptor, left branch extends into haptor for a short distance.

Testes post-ovarian, intercaecal, limited to posterior half of body proper, subspherical to oval, h 21, 20 ± 5 (13–29, n = 30) in number. Vas deferens, runs along body midline to genital atrium. Genital atrium at h 335, 259 ± 45 (175–375, n = 31) from anterior end of body, length h 110, 96 ± 21 (65–170, n = 31), width h 90, 90 ± 18 (45–120, n = 31). Genital atrium comprises anterior atrium proper and two posterior ‘pockets’. Atrium proper roughly shaped as

**Fig. 1** Tree inferred using the neighbour-joining method; only bootstrap values higher than 70 are indicated. The new species, *Microcotyle isyebi* n. sp. and *Microcotyle erythrini* (from its type-host), are in different colours (blue and red)—the two species are clearly different and are not sister groups



**Table 3** Distances between taxa

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 MK317922 <i>Microcotyle isyebi</i> n. sp. ex <i>Boops boops</i>													
2 AY009159 <i>Microcotyle erythrini</i> ex <i>Pagellus erythrinus</i>	16.3												
3 MK27652 <i>Microcotyle visa</i> ex <i>Pagrus caeruleostictus</i>	9.5	15.9											
4 MK27653 <i>Microcotyle visa</i> ex <i>Pagrus caeruleostictus</i>	9.5	15.9	0.0										
5 MK27654 <i>Microcotyle visa</i> ex <i>Pagrus caeruleostictus</i>	10.7	16.7	1.4	1.4									
6 KJ794215 <i>Paramicrocotyle</i> sp. ex <i>Pinguipes chilensis</i>	13.1	13.8	12.3	12.3	13.0								
7 KX926443 <i>Microcotyle algeriensis</i> ex <i>Scorpaena notata</i>	14.7	20.7	14.7	14.7	15.6	16.2							
8 KX926444 <i>Microcotyle algeriensis</i> ex <i>Scorpaena notata</i>	14.7	20.7	14.7	14.7	15.6	16.2	0.0						
9 KX926445 <i>Microcotyle algeriensis</i> ex <i>Scorpaena notata</i>	14.7	20.7	14.7	14.7	15.6	16.2	0.0	0.0					
10 KX926446 <i>Microcotyle</i> sp. ex <i>Helicolenus dactylopterus</i>	13.0	19.8	12.2	12.2	13.5	13.6	3.9	3.9	3.9				
11 KX926447 <i>Microcotyle</i> sp. ex <i>Helicolenus dactylopterus</i>	13.0	19.8	12.2	12.2	13.5	13.6	3.9	3.9	3.9	0.0			
12 NC009055 <i>Microcotyle sebastis</i> ex <i>Sebastes schlegelii</i>	10.2	19.5	11.1	11.1	12.6	10.7	15.7	15.7	15.7	13.9	13.9		
13 Z83003 <i>Bivagina pagrosomi</i> ex <i>Sparus aurata</i>	23.0	29.0	25.8	25.8	26.8	25.8	23.4	23.4	23.4	21.1	21.1	25.3	
14 JF505509 <i>Polylabris halichoeres</i> ex <i>Halichoeres nigrescens</i>	26.7	27.2	25.7	25.7	26.2	23.4	26.7	26.7	26.7	23.8	23.8	27.2	28.8

Distances shown here are percentages, according to Kimura two-parameter; *p* distances (not shown) were similar but generally slightly lower

inverted heart, bearing numerous conical spines, of similar sizes, spines apparently more dense in centre than in bilateral parts. Lumen with two lateral expansions visible only after variation in focus. Posterior pockets lined with spines similar to that of atrium proper. Number of spines in atrium proper 186 (136–230,  $n = 4$ ), in pockets 31 (18–38,  $n = 4$ ), total 216 (154–267,  $n = 4$ ). Genital pore middorsal. Vas deferens relatively wide, visible from middle of body, conspicuous in midline dorsal to uterus and opening into genital atrium.

Vaginal pore well visible in some specimens, mediodorsal, posterior to genital atrium; distance from vagina to anterior extremity 551 (410–700,  $n = 11$ ). Vaginal duct not seen. Ovary complex begins at level of anteriormost testes. Proximal section irregularly shaped, often overlapping anteriormost testis; ovary 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. Oviduct joined by vitelline reservoir in midline. Genito-intestinal canal (visible only in certain specimens) runs from oviduct ventrally across proximal end of ovary and enters right intestinal caecum. Ootype spindle-shaped, with posterior Mehlis' glands. Uterus in midline. Seminal receptacle well visible, lateral. Vitellarium co-extensive with intestinal caeca reaching up to haptor region. Vitelline ducts paired, united anteriorly and posteriorly; anterior junction in inverted Y well visible in some specimens; posterior junction Y-shaped, conspicuous in most specimens, at level of ovary and ventral to it. Egg fusiform, with two filaments; length of egg 223 (125–260,  $n = 23$ ), width 70 (45–95,  $n = 23$ ). Egg filaments, often coiled, more than 223 (125–260,  $n = 23$ ) in length.

## Discussion

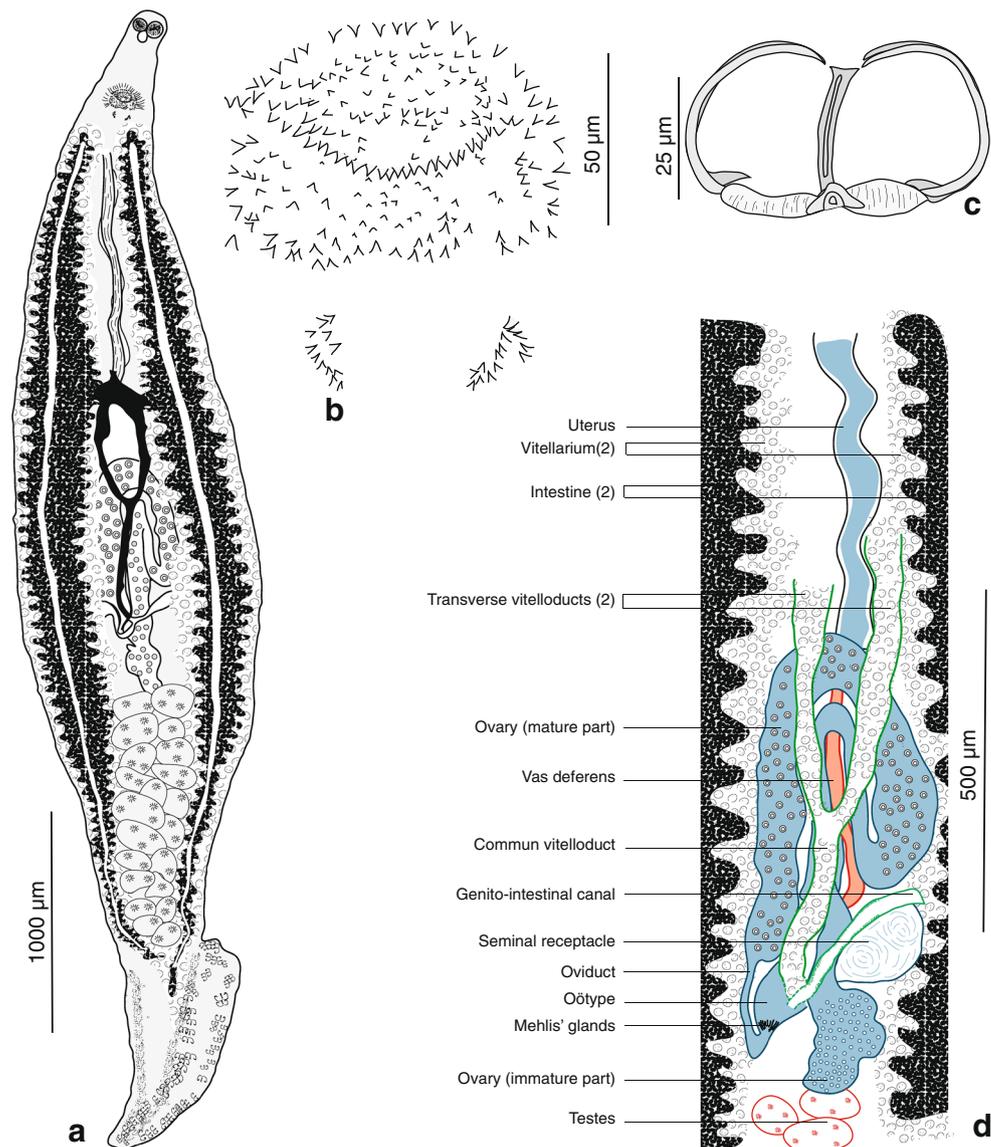
### Molecular differentiation

Although this limit is not absolute, it is generally considered that a difference of 4% in COI sequences suggests that species are different (Aquaro et al. 2012; Ward 2009; Ward et al. 2005); intraspecific differences of COI sequences ranging 0.2–5.6% have been reported in various polyopisthocotylean monogeneans (Aquaro et al. 2012; Ayadi et al. 2017; Bouguerche et al. 2019; Du Preez et al. 2007; Héritier et al. 2017; Li et al. 2011; Mladineo et al. 2009; Oliva et al. 2014; Sepúlveda and González 2015; Shi et al. 2014; Tambireddy et al. 2016; Yan et al. 2016; Yoshinaga et al. 2009). With a difference of 16.3% between the species from *B. boops* and the sequence of *M. erythrini* from the type-host, our molecular analysis strongly suggests that the species described here, *M. isyebi* n. sp., is different from *M. erythrini*. Similar high interspecific differences have also been reported for other platyhelminths such as triclads (Justine et al. 2018). The new species also shows a clear COI divergence from all other species of *Microcotyle* from sparid hosts, but the database does not include all species.

### Differential diagnosis for *M. isyebi* n. sp.

*M. isyebi* is not distinguishable from *M. erythrini* in various measurements and counts (Table 4): clamp number (54–102 vs 86–110), number of spines in the main group of atrium (136–230 vs 201–255) and in pockets (18–38 vs 31–34), clamp length (25–62 vs 50–75) and testis number (13–29 vs 16–17). The specimens of *M. erythrini* from the collection of Louis Euzet that we examined were clearly

**Fig. 2** *Microcotyle isyebi* n. sp. from *Boops boops*. **a** Holotype, whole body, ventral view. **b** Holotype, spines of genital atrium. **c** Clamp, holotype. **d** Detail of anatomy of reproductive organs in the region of ovary, paratype



smaller than specimens of *M. isyebi*, but differences in counts and other measurements were not obvious. The two species could only be distinguished by their COI distance (16.3%); they have different type-hosts (*B. boops* vs *Pagellus erythrinus*).

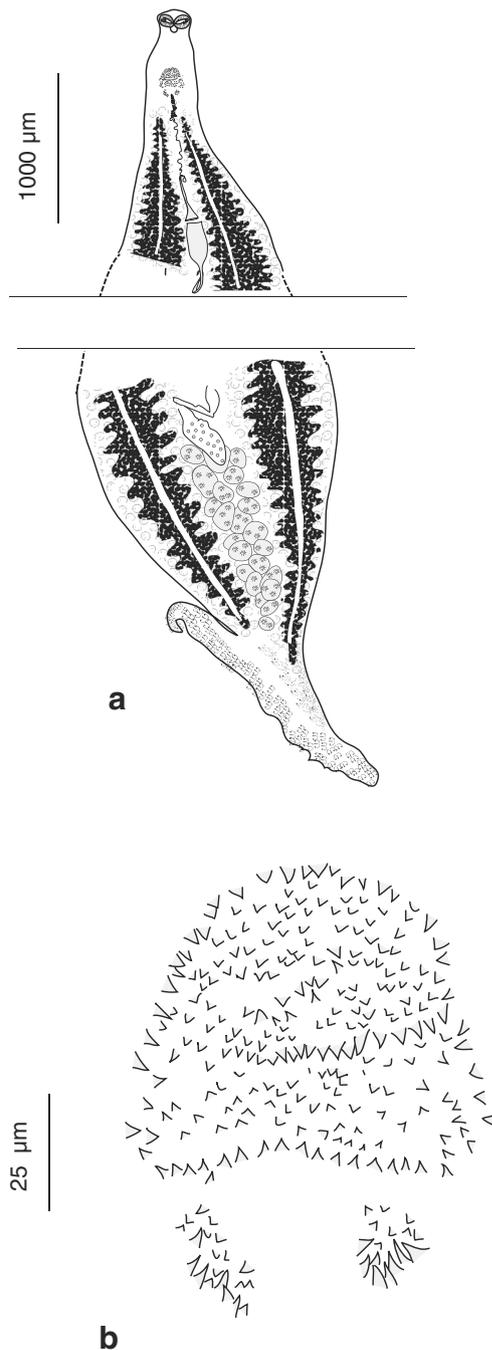
*M. isyebi* is similar to *M. visa* from *P. caeruleostictus* in clamp number (54–102 vs 59–126), number of spines in pockets (18–38 vs 18–39), number of testes (13–25 vs 14–29), size of clamps, oral organ and pharynx (Table 5). They share also the same type-locality (Algeria, Mediterranean Sea). However, hosts are different (*B. boops* vs *P. caeruleostictus*) and the COI divergence is high, 9.5–10.7%.

*M. isyebi* resembles *Microcotyle omanae* in clamp number (54–102 vs 94–120), length of genital atrium (65–170 vs 25–214) and testis number (13–25 vs 10–17). However, they differ in testis number (13–25 vs 35–55). The hosts are different

(*B. boops* vs *Cheimerius nufar*) and from distant localities (Mediterranean Sea vs Indian Ocean).

*M. isyebi* has the same type-locality as *M. algeriensis* (Algeria, Mediterranean Sea). They differ in clamp number (54–102 vs 20–39), number of spines in genital atrium (265–267 vs 76–174) especially in pockets (18–38 vs 7) and testis number (13–25 vs 9–20). They differ also by host (*B. boops* vs *Scorpaena notata*) and the COI divergence is high (14.7%).

*M. isyebi* has the same locality as *Microcotyle* sp. of Ayadi et al., 2017 from *Helicolenus dactylopterus* (Algeria, Mediterranean Sea). These species also resemble in testis number (13–25 vs 10–17); however, they differ in clamp number (54–102 vs 49–58) and total number of spines in genital atrium (265–267 vs 122–333) and in pockets (18–38 vs 12). Hosts are different (*B. boops* vs *H. dactylopterus*) and COI divergence is high (13%).



**Fig. 3** *Microcotyle isyebi* n. sp. from *Boops boops*. **a**, **b** Paratype with molecular information. **a** Anterior and posterior parts of body mounted on a slide (MNHN HEL919); the middle part was used for sequencing the COI gene (GenBank MK317922). **b** Spines of genital atrium

*M. isyebi* resembles *Microcotyle archosargi* in clamp number (54–102 vs 106) and length (42–62 vs 80). It can be distinguished from it by the shape and armature of the genital atrium since lateral expansions and pockets are absent in *M. archosargi*. Hosts are different (*B. boops* vs *Archosargus probatocephalus*) and localities are distant (Mediterranean Sea vs New York).

*M. isyebi* differs from *Microcotyle lichiae* by clamp number (83 vs 52), oral organ length (59 vs 220) and the arrangement of spines in the atrium (five concentric rows in *M. lichiae*). The host is also different (*Lichia amia*, Carangidae).

*M. isyebi* resembles *Microcotyle donavini* by clamp number (54–102 vs 86), length (25–62 vs 33–50) and genital atrium length (65–170 vs 250). However, it differs from it by the general arrangement of ovary, host (*B. boops* vs *Labrus bergylta*) and locality (Mediterranean Sea vs Atlantic).

*M. isyebi* differs from *Microcotyle pomatomi* by clamp number (54–102 vs 104–148), genital atrium length (65–170 vs 109–134), arrangement of genital atrium (absence of pockets in *M. pomatomi*) and testis number (13–25 vs 26–40). Hosts (*B. boops* vs *Pomatomus saltatrix*) and localities (Mediterranean Sea vs Pacific) are different.

*M. isyebi* can be differentiated from all other species mentioned above, except species from sparid hosts, by the presence of two lateral expansions in the genital atrium.

### *M. erythrini* as a cryptic species

After the early record of Parona and Perugia (1890), there are several records of *M. erythrini* in *B. boops* in the literature (Table 1). These authors, in the absence of molecular information, were correct in ascribing the species found to *M. erythrini* and we would have done the same in a similar situation. We could not distinguish our new species from specimens of *M. erythrini* from the type-host from Louis Euzet's collection; body length was greater in *M. isyebi*, but such measurements of 'soft' body parts are generally not good characteristics for the differentiation of microcotylids (Williams 1991). However, our paper demonstrates that the species of *Microcotyle* found on *B. boops* in Algeria, here described as *M. isyebi*, is a distinct species, based on an analysis of molecular data but still with major morphological resemblances. We note that *M. isyebi* n. sp. closely resembles *M. erythrini* in body shape, general armament of genital atrium and in clamp number (54–102 vs 86–110) which are generally considered the best characteristics to distinguish *Microcotyle* species (Ayadi et al. 2017). We conclude that it is likely that previous reports of *M. erythrini* in *B. boops* were, in fact, findings of *M. isyebi*.

It remains that *M. erythrini*, in addition to its type-host *P. erythrinus*, has also been recorded from two other host fish, *P. acarne* and *D. dentex* (Table 1). It would be interesting to obtain COI sequences of the *Microcotyle* specimens from these host fish and possibly detect the presence of additional new species.

**Table 4** Measurements of *Microcoptyle isyebi* n. sp. from *Boops boops* off Algeria, compared with *M. erythrini* from different spard hosts (other than those in Table 5)

	<i>Microcoptyle isyebi</i> n. sp.	<i>M. erythrini</i> Van Beneden & Hesse, 1863	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>M. erythrini</i>
Hosts	<i>Boops boops</i>	<i>P. erythrini</i>	<i>P. erythrini</i> , <i>P. acarne</i> , <i>B. boops</i>	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>M. erythrini</i>	<i>P. erythrini</i>
Locality	Bouharoun, Cap Djinet, Algeria, Mediterranean Sea	Brest, Atlantic	Brest (Atlantic), Genova, Italy, Mediterranean Sea.	Spain, Mediterranean Sea.	Boka Kotorska, Montenegro, Adriatic	Ain Taya, Algeria, Mediterranean Sea	Sète, France, Mediterranean Sea	
Source	Present study	Van Beneden and Hesse (1863)	Parona and Perugia (1890)	López-Román and Guevara Pozo (1973)	Radujkovic and Euzet (1989)	Kouider El Ouahed-Amine (1998)	Present study, collection of Louis Euzet	
Body length	2647 ± 514 (1750–3810, n = 32)	4000	4000–5000		2300–2700	1900–2800	658 (400–860, n = 12)	
Haptor length	809 ± 164 (500–1250, n = 32)						384 (280–580, n = 12)	
Total length	3480 ± 600 (2370–4750, n = 31)						1042 (720–1390, n = 12)	
Body width	598 ± 159 (310–830, n = 32)		500		200–400	270–530	178 (110–260, n = 12)	
Number of clamps	83 (54–102, n = 25)		90		100–110	86–110	101 (82–132, n = 9)	
Clamp length	42 ± 11 (25–62, n = 41)		70 (diameter)		60 <sup>a</sup>	50–75	58 (48–80, n = 13)	
Clamp width	17 ± 4 (10–31, n = 40)				25 <sup>a</sup>	25–40	30 (25–37, n = 13)	
Buccal organ length	59 ± 8 (40–75, n = 34)		71	50–61	45	35–56	62 (40–85, n = 12)	
Buccal organ width	44 ± 9 (30–65, n = 34)		45	33–43	40	30–35	50 (35–65, n = 12)	
Pharynx length	40 ± 7 (25–50, n = 34)			24–27 <sup>a</sup>		24–30*	44 (30–55, n = 9)	
Pharynx width	38 ± 5 (30–48, n = 34)						36 (25–50, n = 9)	
Genital atrium length	96 ± 21 (65–170, n = 31)						111 (85–140, n = 5)	
Genital atrium width	90 ± 18 (45–120, n = 31)						87 (75–110, n = 5)	
Number of spines in main group	186 (136–230, n = 4)						229 (201–255, n = 6)	
Number of spines in pockets	31 (18–38, n = 4)						33 (31–34, n = 6)	
Total number of spines	216 (154–267, n = 4)						262 (232–287, n = 6)	
Distance genital atrium- anterior end	259 ± 45 (175–375, n = 31)			250	250		262 (232–287, n = 6)	
Testis number	20 ± 5 (13–29, n = 30)		16	16–19	16–19	16–17	17 (9–24, n = 10)	
Distance vagina-anterior end	551 (410–700, n = 11)			450				
Egg length	223 (125–260, n = 23)			207–220				
Egg width	70 (45–95, n = 23)			85–90				

<sup>a</sup>Measurements are provided as diameters



**Acknowledgements** The authors are indebted to Amina Boutellis, Zouhour El Mouna Ayadi and Samia Terkmani for their continued support. Our thanks are also due to the fishermen from Bouharoun especially Mohamed Kayrouz. This work was funded by FSB, USTH, Algeria, and ISYEB, MNHN, France. The authors thank the three anonymous reviewers for their constructive suggestions and comments.

## Compliance with ethical standards

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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