Considerations on the taxonomy and morphology of *Microcotyle* spp.: redescription of *M. erythrini* van Beneden & Hesse, 1863 (*sensu stricto*) (Monogenea: Microcotylidae) and the description of a new species from *Dentex dentex* (L.) (Teleostei: Sparidae)

María Villora-Montero, Ana Pérez-del-Olmo*, Simona Georgieva, Juan Antonio Raga and Francisco Esteban Montero

**Abstract**

**Background:** *Microcotyle erythrini* van Beneden & Hesse, 1863 (Platyhelminthes: Monogenea) and other closely related species of the genus are often considered as cryptic. Records in hosts other than the type-host with no species confirmation by molecular analyses have contributed to this situation.

**Methods:** Gill parasites of five sparid fishes, *Boops boops* (L.), *Pagellus erythrinus* (L.), *P. acarne* (Risso), *Dentex dentex* (L.) and *Pagrus pagrus* (L.), from the Western Mediterranean off Spain were collected. Specimens of *Microcotyle* spp. were characterised both molecularly and morphologically. Partial fragments (domains D1-D3) of the 28S rRNA gene and the cytochrome c oxidase subunit 1 (*cox1*) gene were amplified and used for molecular identification and phylogenetic reconstruction. Principal components analysis was used to look for patterns of morphological separation.

**Results:** Molecular analyses confirmed the identity of three species: *M. erythrini* ex *P. erythrinus* and *Pa. pagrus*; *M. isyebi* Bouguerche, Gey, Justine & Tazerouti, 2019 ex *B. boops*; and a species new to science described herein, *M. whittingtoni* n. sp. ex *D. dentex*. The specific morphological traits and confirmed hosts (*P. erythrinus* and *Pa. pagrus*) are delimited here in order to avoid misidentifications of *M. erythrini* (*sensu stricto*). *Microcotyle erythrini* (*s.s.*) is mostly differentiated by the shape of its haptor, which is also longer than in the other congeners. New morphological and molecular data are provided for *M. isyebi* from the Spanish Mediterranean enlarging the data on its geographical range. *Microcotyle whittingtoni* n. sp. is described from *D. dentex* and distinguished from the remaining currently recognised species of the genus by the number and robustness of the clamps.

*Correspondence: Ana.perez-del-olmo@uv.es
Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, C/Catedrático José Beltrán 2, 46980 Paterna, Spain

© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background

*Microcotyle erythrini* van Beneden & Hesse, 1863 (Monogenea: Microcotylidae) was originally described from *Pagellus erythrinus* (L.) (Teleostei: Sparidae) off the coast of Brest (France, North-East Atlantic) and to date it has been listed and considered a valid species [1–3]. Like many of the earliest descriptions of species of *Microcotyle*, *M. erythrini* was described briefly, and only differentiated by the number of clamps and testes, and the traits of the genital atrium [4]. Since the original description, many authors have recorded and described new specimens identified as *M. erythrini* in different sparid species, mostly in the Mediterranean Sea (see Table 1 in Bouguerche et al. [3], for details on the records of *M. erythrini*). These publications sometimes offered morphological ranges based on a combination of measurements of specimens from different host species (e.g. [5, 6]). Along this process, the morphological ranges of *M. erythrini* have been enlarged abnormally, which has made it difficult to define a clear and distinguishing morphology. Recently, with the help of molecular tools (cox1 partial fragment), *M. erythrini* has been split into two species, each in a different sparid host off the Algerian coast: *M. erythrini* ex *P. erythrinus* and *M. isyebi* Bouguerche, Gey, Justine & Tazerouti, 2019 ex *Boops boops* (L.) [3]. These authors also included the most recent morphometric information on *M. erythrini* from the type-host *P. erythrinus*. Bouguerche et al. [2, 3] suggested that morphological and molecular characterization of *M. erythrini*-like specimens infecting different sparid hosts would reveal higher parasite diversity.

The aim of the present study is a revision of the taxonomy of *Microcotyle* spp. in sparids from the Western Mediterranean off Spain. The specific objectives of the study are: (i) to describe a new species of *Microcotyle* parasitic in *Dentex dentex* (L.); (ii) to redescribe *M. erythrini* with the support of molecular evidence, define the actual morphological boundaries of the species and indicate the valid historical records; and (iii) to provide new morphological and molecular data useful for the taxonomy of *Microcotyle* spp. New morphological approaches and classification tools for species discrimination are proposed for these monogeneans which are notoriously difficult to differentiate.

Methods

Sample collection

A total of 150 fishes of four sparid species were examined for microcotylid infections: 40 bogues (*Boops boops*), 40 common pandoras (*Pagellus erythrinus*), 40 common dentexes (*Dentex dentex*) and 30 red porgies (*Pagrus pagrus*). Additionally, 40 axillary seabreams (*P. acarne* (Risso)) were also examined. Fishes were caught by commercial bottom trawling vessels during July of 2012 and 2013, off Guardamar del Segura, Alicante, Spain (38°05′N, 0°39′W; Western Mediterranean Sea, FAO fishing subarea 37.1). Fishes were transported on ice to the laboratory, where they were weighed, measured (weight provided in g and standard length in cm, expressed as the range with the mean and standard deviation (SD) in parentheses; only provided for infected hosts in the taxonomic summary) and then dissected for gill examination. Each pair of gills was dissected and inspected for parasites under a stereomicroscope. All parasites were collected and washed in 0.9% saline solution. For *Microcotyle* spp. specimens, two different protocols were used. Adult and completely mature specimens in optimal conditions (not broken, contracted, stretched, wrinkled or folded) were selected for morphological analyses; these were fixed in 4% formaldehyde solution and preserved for four days, then the specimens were transferred into 70% ethanol. For molecular analyses, fresh specimens were selected; the testes and clamps were counted and photographed and then the specimens were divided into three pieces, storing the anterior and posterior parts as molecular vouchers. The middle pieces were fixed and preserved in molecular-grade ethanol. Prevalence, expressed as a percentage (infected fish and total number of analysed fish in parentheses), and mean intensity, expressed as the mean with standard deviation, in each host, were calculated according to Bush et al. [7].
**Sequence generation**

Ethanol-preserved specimens of *Microcotyle* spp. collected from the four fish species were used for genomic DNA isolation. Total genomic DNA was isolated from the excised pieces of the middle part of the worm body which was dried out at 56 °C before DNA isolation. Chelex™100 Resin (BIO-RAD) was used for extraction (see [8] for details).

Mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*, partial fragment) was amplified using primers JB3 (=COI-ASmit1) (forward: 5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) and JB4.5 (=ASmit2) (reverse: 5′-AAA GAA AGA ACA TAA TGA AAA TG-3′) [9, 10]. Partial fragment (domains D1-D3) of the 28S rRNA gene was amplified using the primer combination LSU5 (forward: 5′-TAG GTC GAC CCG CTG AAY TTA AGCA-3′) and LSU3′ (reverse: 5′-TAG AAG CTT CCT GAG GGA AAC TTC GTC CCT GAG-3′) [11]. Both genes were amplified using puReTaq Ready-To-Go-PCR beads or MiFy™ DNA Polymerase mix (Bioline Inc., Taunton, USA) and PCR amplifications were performed in a total volume of 20 μl containing 8 pmol of each primer and c.50 ng of DNA. The thermocycling profiles consisted of: (i) *cox1*: initial denaturation at 94 °C for 5 min, followed by 40 cycles of 92 °C for 30 s, 45.5 °C for 45 s, 72 °C for 90 s, and a final extension step at 72 °C for 10 min; (ii) partial 28S rDNA: initial denaturation of 94 °C for 4 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 30 s, 72 °C for 45 s, followed by a final extension step at 72 °C for 7 min.

PCR amplicons were purified using QIAquick TM PCR Purification Kit (Qiagen Ltd., Hilden, Germany). Sequencing reactions were performed using the PCR primers and two additional internal primers in the case of 28S rRNA gene, i.e. IF15 (forward: 5′-GTG GTA GTG GTA GAC-3′) and IR14 (reverse: 5′-CAT GTT AAA CTC CTT GGT CCG-3′) [12]. Cycle sequencing was carried out at Macrogen Europe Inc. (Amsterdam, the Netherlands).

**Alignment and data analyses**

Contiguous sequences were assembled in MEGA v.6 [13] and alignments with currently available sequences for *Microcotyle* spp. in the GenBank database (retrieved on 25th July 2019) were constructed using MAFFT v.7 [14] under default gap parameters on EMBL-EBL bioinformatics web platform (http://www.ebi.ac.uk/Tools /msa/mafft). The outgroup choice was based on previous phylogenies of the group [15–17]. The *cox1* alignment (381 nt) comprised a total of 12 newly generated sequences and 20 sequences for 10 species available on GenBank. *Bivagina pagrosomi* ex *Sparus aurata* (L.) (GenBank: Z83002) was used as the outgroup. The 28S alignment (823 nt) comprised 4 newly generated sequences and 10 sequences available on GenBank. *Bivagina pagrosomi* ex *S. aurata* (GenBank: Z83002) was used as the outgroup. Distance matrices (using the uncorrected p-distance model) were calculated in MEGA v. 6. Neighbour-joining analyses based on Kimura 2-parameter distances were also performed in MEGA v.6 with nodal support estimated using 1000 bootstrap resamplings. Model-based Bayesian inference (BI) and maximum likelihood (ML) analyses were carried out using MrBayes v.3.2.6 on XSEDE at the CIPRES Science Gateway v. 3.3 [18] and PhyML v.3.0 [19] as an online execution on the ATGC bioinformatics platform (http://www. atgc-montpellier.fr/) with a non-parametric bootstrap validation of 1000 pseudoreplicates, respectively. The MCMC chains were run for 10,000,000 generations with trees sampled every 1000 generation. Posterior probability and mean marginal likelihood values were calculated. The first 25% of the sampled trees were discarded as ‘burn-in’. Prior to analyses, jModelTest v.2.1.4 [20, 21] was used to select the best-fitting models of nucleotide substitution under the Akaike’s information criterion. These were the general time-reversible model with gamma distributed among-site rate variation and estimates of invariant sites (GTR+I+Γ) for the *cox1* dataset and the Hasegawa-Kishino-Yano model (HKY) for the 28S dataset. Consensus topologies and nodal supports were visualized in FigTree v.1.4.3 [22], posterior probabilities (pp) and bootstrap support (bs) values are summarised on the BI trees (as pp/bs).

**Morphological analyses**

Parasites selected for morphological analyses were stained with iron acetocarmine, dehydrated through an ethanol series, cleared in dimethyl phthalate and prepared as permanent mounts in Canada balsam. After mounting, there was a second selection of specimens suitable for morphological studies, i.e. only specimens in optimal condition (not broken, contracted, stretched, wrinkled or folded). Parasites were examined using a light microscope Nikon Optiphot-2 (Nikon Instruments, Tokyo, Japan) with differential interference contrast at magnifications of 400–1000×. A total of 86 specimens of *Microcotyle* spp. were selected and drawn (n=22 ex *B. boops*; n=21 ex *D. dentex*; n=23 ex *P. erythrinus*; n=20 ex *Pa. pagrus*). Drawings were made with the aid of a drawing tube attached to a light microscope Nikon Optiphot-2. Measurements were taken from digitalized illustrations using ImageJ v.1.48 software [23] and expressed in micrometers as the range followed by the mean in parentheses unless otherwise stated. When characters were visible, a total of 52 morphometric measurements were taken from each specimen. Clamp thickness was estimated as both the maximum width of the
distal end of the antero-lateral sclerite (‘c,’ see Fig. 1a) and its relation to the clamp length. The type-specimens were deposited in the Collection of the Natural History Museum (NHMUK), London, UK.

To look for patterns of separation between Microcotyle spp. specimens from different host species, a principal components analysis (PCA) was applied to a dataset of 86 specimens using morphometrical variables associated with body shape. Prior to the analysis, the data were divided by total body length to account for the effect of body size while visualising possible morphometric differences between species. The specimens were identified as M. erythrini (n = 23 ex P. erythrinus; n = 20 ex Pa. pagrus), M. isyebi (n = 22 ex B. boops) and Microcotyle whittingtoni n. sp. ex D. dentex (n = 21).

Results
Molecular identification
A total of 12 cox1 and four 28S rDNA sequences were generated for the newly collected specimens of Microcotyle spp. from the four fish species from the Western Mediterranean off Spain. Partial cox1 (434 nt) sequences were generated for a total of 12 isolates, i.e. 4 M. isyebi ex B. boops, 6 M. erythrini (4 ex Pa. pagrus and 2 ex P. erythrinus) and 2 M. whittingtoni n. sp. ex D. dentex. Partial 28S rDNA sequences (1238–1527 nt) were generated for a representative subset of the specimens used for cox1 sequence generation; single sequences per species were used for the reconstruction of the 28S rDNA phylogeny. The newly generated sequences for the isolates recovered in the present study were analysed in two separate datasets together with all currently available sequences in the GenBank database for Microcotyle spp. (see Table 1 for details on the ingroup taxa used in the analyses). Posterior probabilities (pp) and bootstrap support (bs) values are summarised on the BI trees (as pp/bs).

The newly generated cox1 sequences were analysed together with 19 published sequences for Microcotyle spp. (Table 1). Phylogenetic analysis revealed that the newly sequenced isolates belonged to 3 species: M. erythrini ex P. erythrinus and Pa. pagrus; M. isyebi ex B. boops; and M. whittingtoni n. sp. ex D. dentex. The tree from the BI analysis is provided in Fig. 2 together with the statistical support from the ML analysis. The four isolates recovered from B. boops clustered together with an isolate of M. isyebi from the same host species reported from the southern coast of the Western Mediterranean off Algeria [3]. The sequences for the isolates recovered from Pa. pagrus and P. erythrinus clustered together with the published sequences for M. erythrini ex P. erythrinus from the Western Mediterranean off France [15]. The two sequences for M. whittingtoni n. sp. ex D. dentex clustered together in a basal clade to the remaining representatives of Microcotyle spp. All of the above clades were strongly supported in both BI and ML analyses. Overall, the cox1 phylogeny (Fig. 2) recovered three groups of sister species within the Microcotyle although with poor support: (i) M. isyebi and M. visa Bouguerche, Gey, Justine & Tazerouti, 2019; (ii) M. caudata Goto, 1894 and an unidentified Microcotyle sp. ex Sebastiscus marmoratus (Cuvier) from the North-West Pacific off Japan; and (iii) M. algeriensis Ayadi, Gey, Justine & Tazerouti, 2016 ex Scorpæna notata Rafinesque and Microcotyle sp. ex Helicolenus dactylopterus (Delaroche) (syn. M. sebastian sensu Radujković & Euzet, (1989) [31]) (both reported from the Western Mediterranean off Algeria). The single sequence for ‘Microcotyle sebastian’ was close to the M. caudata-Microcotyle sp. from off Japan, and an isolate originally identified as “Paramicrocotyle sp.” (genus synonymised with Microcotyle [30]) ex Pinguipes chilensis Velenciennes from the South-East Pacific off Chile, was recovered as sister species to the major clade comprising the previously reported representatives from the Mediterranean, North-East Atlantic, Indian Ocean and the North-West Pacific. Microcotyle erythrinus was recovered apart from the above-mentioned main multi-taxon clade albeit with low nodal support.

The intraspecific sequence divergence (see Additional file 1: Table S1) within the newly generated cox1 sequences ranged between 0.2–1.4% (1–6 nt difference) for M. erythrini (ex P. erythrinus and Pa. pagrus); 0.2–0.5% (1–2 nt difference) for M. isyebi and 1.4% (6 nt difference) for M. whittingtoni n. sp. ex D. dentex. The newly generated sequences for the isolates of M. isyebi from off Spain differed by 1.4–1.7% (4–5 nt) from M. isyebi from off Algeria; these for M. erythrini differed by 2.1–2.8% (6–8 nt) from the published isolate from off Corsica (GenBank: AY009159); and the two isolates of M. whittingtoni n. sp. ex D. dentex differed substantially from both M. isyebi and M. erythrini, i.e. by 14.4–15.2% (43–56 nt) and by 10.8–13.5% (41–44 nt), respectively. The overall sequence divergence among the species of Microcotyle ranged between 4.5–18.5% (17–62 nt difference).

Both, ML and BI analyses for the 28S dataset yielded congruent tree topologies (Fig. 3) and high nodal support for most of the clades. Most of the species of Microcotyle clustered in a single multi-taxon clade with the unpublished sequence for an isolate identified as Microcotyle sp. ex Nemipterus japonicus (Bloch) from the Indian Ocean as a distinct, basal species. The newly generated sequences for M. erythrini ex P. erythrinus and Pa. pagrus, clustered in a strongly supported clade together with a previously published sequence for M. erythrini ex P. erythrinus off Corsica, France and a sequence for “Microcotyleidae sp.” M11 ex Argyrosomus japonicus (Temminck & Schlegel) from off Australia. Microcotyle
whittingtoni n. sp. and M. isyebi clustered together as close relatives of M. erythrini + "Microcotylidae sp." M11. Microcotyle arripis Sandars, 1945 from the South-West Pacific off Australia and an isolate provisionally identified as Microcotyle sp. 2 from off China clustered together in a strongly supported subclade.

The novel 28S sequences for M. erythrini recovered from the two fish host species differed by a single base.
Table 1  Summary data for the isolates of *Microcotyle* spp. used in the phylogenetic analyses

| Parasite species | Host species | Isolate | FAO Fishing Area | GenBank ID | Source |
|------------------|--------------|---------|------------------|------------|--------|
| *M. algeriensis* Ayadi, Gey, Justine & Tazerouti, 2016 | Scorpaena notata Rahn- esque | MO-01 WM | KX926443 | Ayadi et al. [24] |
| | Scorpaena notata | MO-02 WM | KX926444 | Ayadi et al. [24] |
| | Scorpaena notata | MO-03 WM | KX926445 | Ayadi et al. [24] |
| *M. archosargi* MacCallum, 1913 | Archosargus rhomboidalis (L.) | 81 WCA | MG586867 | Mendoza-Franco et al. [25] |
| *M. arrisis* Sandars, 1945 | Arripis georgianus (Valenciennes) | SA | GU263830 | Catalano et al. [26] |
| *M. caudata* Goto, 1894 | Sebastes inermis Cuvier | MC06 NWP | LC472527 | Kamio & Ono (unpublished data) |
| | “Sebastes inermis species complex” | MC12 NWP | LC472528 | Kamio & Ono (unpublished data) |
| | “Sebastes inermis species complex” | MC18 NWP | LC472529 | Kamio & Ono. (unpublished data) |
| | “Sebastes inermis species complex” | MC20 NWP | LC472530 | Kamio & Ono (unpublished data) |
| | “Sebastes inermis species complex” | MC24 NWP | LC472531 | Kamio & Ono (unpublished data) |
| *M. erythrinii* van Beneden & Hesse, 1863 | Pagellus erythrinus (L.) | MePe1 WM | MN814848 | Present study |
| | Pagellus erythrinus | MePe2 WM | MN816012 | Present study |
| | Pagellus erythrinus | MePe3 WM | MN816013 | Present study |
| | Pagellus erythrinus | MePe4 WM | AM157221 | Jovelin & Justine [15] |
| | Pagrus pagrus (L.) | MePp1 WM | MN816014 | Badets et al. [12] |
| | Pagrus pagrus | MePp2 WM | MN816015 | Present study |
| | Pagrus pagrus | MePp3 WM | MN816016 | Present study |
| | Pagrus pagrus | MePp4 WM | MN816017 | Present study |
| *M. isyebi* Bouguerche, Gey, Justine & Tazerouti, 2019 | Boops boops (L.) | MiBb1 WM | MN816018 | Present study |
| | Boops boops | MiBb2 WM | MN816019 | Present study |
| | Boops boops | MiBb3 WM | MN816020 | Present study |
| | Boops boops | MiBb4 WM | MN816021 | Present study |
| | Boops boops | MO01 WM | MK317922 | Bouguerche et al. [3] |
| *M. sebastianis* Goto, 1894 | Sebastes sp. | NSP | AF382051 | Olson & Littlewood [16] |
| *Microcotyle* sp. AKV-2016 | Nemipterus japonicas (Bloch) | VII37_12 EAS | KU926692 | Verma & Agrawal (unpublished data) |
| *Microcotyle* sp. DG-2016 | Helicolenus dactylopterus (Delaroche) | MO-04 WM | KX926446 | Ayadi et al. [24] |
| | Helicolenus dactylopterus | MO-06 WM | KX926447 | Ayadi et al. [24] |
| | Sebastes schilegii Hilgendorf | NWP | DQ412044 | Park et al. [27] |
| *Microcotyle* sp. YK-2019 | Sebastiscus marmoratus (Cuvier) | MK02 NWP | LC472525 | Kamio & Ono (unpublished data) |
| *Microcotyle* sp. YK-2019 | Sebastiscus marmoratus | MK01 NWP | LC472526 | Kamio & Ono (unpublished data) |
| *Microcotyle* sp. 1 SC-2018 | – | – | MH700256 | Chou (unpublished data) |
| *Microcotyle* sp. 2 SC-2018 | – | – | MH700266 | Chou (unpublished data) |
| *Microcotytidae* sp. M10 | Sebastes sp. | M10 NWA | EF653385 | Aiken et al. [28] |
| *Microcotytidae* sp. M11 | Argyrosomus japonicus | M11 SA | EF653386 | Aiken et al. [28] |
| *M. visa* Bouguerche, Gey, Justine & Tazerouti, 2019 | Pagrus caeruleostictus (Valenciennes) | PacoerMO001 WM | MK275652 | Bouguerche et al. [2] |
| | Pagrus caeruleostictus | PacoerMO002 WM | MK275653 | Bouguerche et al. [2] |
| | Pagrus caeruleostictus | PacoerMO003 WM | MK275654 | Bouguerche et al. [2] |
The novel sequence for *M. erythrina* from *P. erythrinus* was identical with the published sequence *ex* *P. erythrinus* (GenBank: AM157221) in the Western Mediterranean (see Additional file 2: Table S2). The 28S rDNA sequences for *M. isyebi* and *M. whittingtoni* n. sp. differed from the *M. erythrina* isolates by 1 and 3 nt (0.1 and 0.4%), respectively, and by 2 nt (0.2%) between themselves. *Microcotyle* sp. AKV-2016 (KU926692) differed substantially from the remaining *Microcotyle* spp., i.e. by 80–116 nt (12.8–14.7%) corresponding to intergeneric-level differences. The overall sequence divergence among the species of *Microcotyle* ranged between 1–8 nt (0.1–1.0%).

**Morphological data**

**Microcotyle erythrina** van Beneden & Hesse 1863 (*sensu stricto*)

**Hosts:** *Pagellus erythrinus* (L.) (type-host), common pandora [weight: 98.9–160.0 g (123±17.3 g); standard length: 15.8–23 cm (17.5±1 cm)] off Guardamar del Segura, Spain; *Pagrus pagrus* (L.), red porgy [weight: 84.2–289.3 g (175.0±44.5 g); standard length: 13.4–20.5 cm (17.12±1.5 cm)], off Guardamar del Segura, Spain (both Perciformes: Sparidae).

**Locality:** Off Guardamar del Segura, Western Mediterranean off Spain. Other localities with valid records: off Brest, France (type-locality); Boka Kotorska Bay and off Montenegro coast, Montenegro; off Sète, France.

**Voucher material:** Specimens from *P. erythrinus* (*n* = 3) and *P. pagrus* (*n* = 3) from off Guardamar del Segura are deposited in the Natural History Museum, London, UK (NHMUK.2019.12.10.6-8 and NHMUK.2019.12.10.9-11, respectively); the remaining material from Guardamar del Segura is deposited in the Parasitological Collection of the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

**Infection parameters:** *P. erythrinus* (*n* = 40); prevalence, 51% (21 out of 40); mean intensity, 2.2±3.8; *Pa. pagrus* (*n* = 30); prevalence, 45% (14 out of 30); mean intensity, 2.2±1.7.

**Site on host:** Gill filaments.

**Representative DNA sequences:** GenBank accession numbers: MN816012 and MN816013 *ex* *P. erythrinus*; MN816014, MN816015, MN816016 and MN816017 *ex* *Pa. pagrus* (*cox1*); MN814848 *ex* *P. erythrinus* and MN814849 *ex* *Pa. pagrus* (28S).

**Description**

[Based on 43 mature adults (23 *ex* *P. erythrinus* and 20 *ex* *Pa. pagrus*), except where otherwise indicated; data in the description are reported as mean±SD for specimens *ex* *P. erythrinus* [mean±SD *ex* *Pa. pagrus*]; ranges are provided in Table 2; Fig. 4]. Body fusiform, elongate, slender, 3532±918 [3630±835] long, 182±38 [182±37] wide at level of genital atrium and 249±92 [258±57] wide at level of testes, tapered anteriorly up to 566±107 (*n* = 18) [513±141 (*n* = 13)] from anterior extremity of body; body laterally narrowed at posterior end of anterior tapered region, 204±55 (*n* = 18) [190±39 (*n* = 13)] wide, often posteriorly delimited by lateral notches. Haptor dorsoventrally bi-lobed, elongated (haptor length/total body length ratio: 38–62% (44%) [35–60% (42%)]), well differentiated from body, sometimes with peduncle 448±103 (*n* = 7) [414±127 (*n* = 4)] long, with minimum width 174±36 (*n* = 7) [158±47 (*n* = 4)]; haptor laterally symmetric, ventrally projected in anterior (ventral) lobe and longer posterior (dorsal) lobe (anterior/posterior haptoral lobe length ratio 60–90% (72%) (*n* = 20).
[52–81% (66%) (n = 19)]. Haptor armed with two rows of sessile clamps, 90–124 in number for specimens from both host species, in two lateral frills, joining at anterior and posterior extremities of haptor; with slightly smaller clamps at anterior and posterior margins of haptor. Clamps of “microcotylid” type, slender, “c” sclerite maximum width 3 ± 1 for specimens from both host species and 0.084 ± 0.015 (n = 25) [0.068 ± 0.019 (n = 25)] corrected by clamp length; with trident-shaped accessory sclerite (‘e’, see Fig. 1a) formed by thick central bar reaching to distal tips of antero-lateral sclerites ‘c’ and two thin short sclerites directly branched from basis of ‘e’.

Mouth subventral, within conical vestibule with pair of septate buccal suckers. Pharynx subspherical; oesophagus short; intestinal bifurcation posterior to genital atrium, sometimes at level of atrium. Caeca extend into haptor or peduncle, with inner and intricate external lateral ramifications.

Testes numerous, 12–20 [14–22] in number, dorso-ventrally flattened, subelliptical to irregular, most anterior located at 1536 ± 360 [1922 ± 585] from anterior extremity, post-germinal and pre-haptoral, partially extending into haptor peduncle, arranged in clusters of 1 or 2 rows, with some testes dorso-ventrally overlapped. Vas deferens relatively straight, dorsal to uterus; copulatory organ muscular, 68 ± 22 (n = 12) [62 ± 7 (n = 8)], located in posterior part of genital atrium. Genital atrium at 216 ± 40 [267 ± 63] from anterior extremity of body, with wide medial muscular chamber, armed with small conical spines, 216–408 [275–363] in number, communicating with 2 lateral posterior small chambers (“pockets” sensu Mamaev, 1989 [44]) armed with longer spines, 20–33 [21–41] in number.

Bayesian inference (BI) phylogram based on the mitochondrial cox1 dataset for Microcotyle spp. Bivagina pagrosomi was used as the outgroup. Posterior probabilities and bootstrap support values are shown at the nodes; only values > 0.90 (BI) and 75% (ML) are shown. The scale-bar indicates the expected number of substitutions per site. Sequence identification is as in GenBank, followed by a letter: A, Ayadi et al. [24]; Ba, Bouguerche et al. [2]; Bb, Bouguerche et al. [3]; J, Jovelin & Justine [15]; K, Kamio & Ono (unpublished); O, Oliva et al. [29]; P, Park et al. [27].
globular region, 599 ± 50 (n = 8) [621 ± 51 (n = 6)] long, with proximal arched section directed dextro-sinistrally, connected to wide arched section directed sinistro-dextrally; maximum width at distal section, 71 ± 8 (n = 8) [90 ± 10 (n = 6)]. Oviduct slightly sinuous, including elongated seminal receptacle, 99 ± 7 × 160 ± 12 (n = 4) [93 ± 8.3 × 163 ± 13 (n = 2)] directed postero-sinistrally; ending in oötype; Mehlis’ gland well developed.

Vaginal pore mid-dorsal, often imperceptible, at 465 ± 64 (n = 11) [473 ± 59 (n = 8)] from anterior extremity. Vitelline follicles dispersed, starting at 356 ± 81 [437 ± 82] from anterior body extremity, in 2 lateral fields surrounding caecal ramifications; vitelline follicles extending within haptor or peduncle in all specimens. Posterior extremities of vitelline fields asymmetrical in 52% [95%] of specimens, distance between fields usually short, 83 ± 54 [89 ± 46]; right field longer in 56% [61%] of specimens with asymmetrical fields; posterior extremities of vitelline fields often joined (83% [45%] of specimens with symmetrical fields). Vitelline ducts Y-shaped (Fig. 4e), with 2 separate efferent ducts, right 204 ± 67 [224 ± 91] long, left 178 ± 77 [304 ± 119] long, joining in common different duct 214 ± 79 [289 ± 67] long, ventral, at germarium level. Eggs fusiform (Fig. 4f), with 2 filaments; opercular filament long, thin, slightly thickened at posterior end; abopercular filament shorter with solid tip, capitate or pointed (Fig. 4g). Opercular end of egg narrowed to connect abruptly with tubular hollow section (1/3–1/7 of total egg length, not including filaments, for specimens for both host species) leading to opercular filament.

**Remarks**

*Microcotyle erythrinis* was described by van Beneden & Hesse [4] and mostly characterized by its specific host, *P. erythinus*, as authors provided limited morphological information (mostly at the generic level) and with no supporting drawing. Parona & Perugia [5] redescribed this species; however, the description is unreliable as these authors provided pooled morphological information from material ex *B. boops*, host of *M. isyebi* (see [3] and present study) and ex *P. acarne*, a host not confirmed for *M. erythrinis*. Morphological data with pooled information form specimens collected from more than one host species or parasites collected in fish species different from the type-host or other confirmed hosts should not be considered as suitable (see also Additional file 3: Table S3). Several new geographical records of *M. erythrinis* ex *P. erythinus*, exclusively, have been published by other authors since 1863 (see Table 1 in [3]). Among these records, only Radujković & Euzet [31] and Bouguerche et al. [3] provided morphological and morphometric data for specimens off Montenegro and Sète, respectively (see also Additional file 3: Table S3). Here, we provide metric data (Table 2) for newly collected specimens ex *P. erythrinis* and *Pa. pagrus* (new host record) from the Spanish Western Mediterranean. Specimens from these two hosts collected in the present study are genetically and morphologically indistinguishable.

Only considering the specimens reported ex *P. erythrinis* and *Pa. pagrus* by van Beneden & Hesse [4], Radujković & Euzet [31], Bouguerche et al. [3] and the present study [from here onwards *M. erythrinis* (*sensu stricto*), the diagnostic characters of *M. erythrinis* (*s.s.*) agree and measurements mostly overlap but wide ranges for some features are still observed (see also Additional file 3: Table S3), which hampers the differentiation from other congeneric species. Paying attention to the characters traditionally used in the taxonomy of *Microcotyle*, the number of clamps (82–132) and the number of testes (9–24) of *M. erythrinis* (*s.s.*), combining the information from all descriptions in confirmed hosts (see Table 2 and Additional file 3: Table S3), resemble or overlap with those of several species reported in the Mediterranean (*M. donavini* van Beneden & Hesse, 1863 and *M. pomatomi* Goto, 1899) and in other spardin hosts (*M. isyebi* and *M. visa*). Regarding the traits more recently used to differentiate the species of *Microcotyle*, such as the genital atrium armature and combining the information from all descriptions in confirmed hosts (see Table 2 and Additional file 3: Table S3), *M. erythrinis* (*s.s.*) resembles other species with large number of
Table 2: Metrical ranges for *Microcotyle erythrinii* (sensu stricto), *M. isyebi* and *M. whittingtoni* n. sp. described in this study based on collections from off Guardamar del Segura, Spain, Western Mediterranean.

| Parasite species | *M. erythrinii* (s.s.) | *M. isyebi* | *M. whittingtoni* n. sp. |
|------------------|------------------------|-------------|--------------------------|
| **Host species** | **P. erythrinus** | **P. pagrus** | **B. boops** | **D. dentex** |
| **Sample size**  | *(n = 23)* | *(n = 20)* | *(n = 22)* | *(n = 21)* |
| **Body length**  | 1998–6215 | 2042–6183 | 2355–6401 | 2719–4569 |
| **Body length without haptor** | 1376–3760 | 1520–5307 | 1757–4970 | 1916–3591 |
| **Maximum body width** | 194–647 | 189–610 | 322–966 | 314–605 |
| **Body width at level of buccal suckers** | 95–179 | 68–153 | 95–185 | 110–173 |
| **Body width at level of genital atrium** | 102–251 | 109–270 | 157–322 | 159–260 |
| **Body width at level of testes** | 156–574 | 150–357 | 237–790 | 225–468 |
| **Length of anterior tapered region** | 359–749 | 132–687 | 288–922 | 457–730 |
| **Width of anterior tapered region** | 135–378 | 124–242 | 180–388 | 147–354 |
| **Haptor length** | 1126–1840 | 761–1590 | 702–1436 | 862–1264 |
| **Anterior haptoral lobe length** | 353–735 | 307–690 | 164–339 | 187–370 |
| **Posterior haptoral lobe length** | 758–1,163 | 608–991 | 474–1,226 | 632–1,038 |
| **Peduncle length** | 339–615 | 263–574 | 178–651 | 199–476 |
| **Width of peduncle at connection with haptor** | 120–246 | 72–227 | 92–414 | 100–369 |
| **Minimum peduncle width** | 120–276 | 67–227 | 91–414 | 81–368 |
| **No. of clamps** | 90–124 | 90–124 | 80–110 | 60–78 |
| **Clamp length** | 20–50 | 22–42 | 21–40 | 22–47 |
| **Clamp width** | 55–86 | 40–64 | 46–68 | 52–75 |
| **Sclerite ‘c’ width** | 2–4 | 2–3 | 1–3 | 3–6 |
| **Buccal sucker length** | 47–80 | 38–78 | 50–82 | 57–90 |
| **Buccal sucker width** | 34–59 | 19–50 | 33–63 | 39–59 |
| **Pharynx length** | 29–47 | 20–42 | 29–55 | 27–41 |
| **Pharynx width** | 23–41 | 18–34 | 23–54 | 25–43 |
| **Oesophagus length** | 159–307 | 217–343 | 150–360 | 209–367 |
| **Testes to anterior extremity distance** | 982–2223 | 1055–4018 | 1215–3288 | 1305–2342 |
| **No. of testes** | 12–20 | 14–22 | 19–26 | 16–27 |
| **No. of testis rows** | 1–2 | 1–2 | 1–2 | 1–3 |
| **Testes length** | 32–115 | 38–82 | 32–162 | 39–112 |
| **Testes width** | 51–116 | 42–75 | 52–132 | 36–88 |
| **Testicular area length** | 298–1007 | 364–1250 | 88–301 | 68–717 |
| **Testicular area width** | 41–193 | 56–155 | 408–1558 | 393–1023 |
| **Genital atrium to anterior extremity distance** | 108–277 | 206–424 | 128–355 | 183–300 |
| **Genital atrium length** | 71–151 | 71–165 | 73–177 | 105–184 |
| **Genital atrium width** | 88–168 | 54–201 | 109–255 | 115–173 |
| **No. of spines in the main chamber of the genital atrium** | 216–408 | 275–363 | 253–356 | 272–391 |
| **Length of spines in the main chamber of the genital atrium** | 4–6 | 4–6 | 4–7 | 4–7 |
| **No. of spines in the ‘pockets’ of the genital atrium** | 20–33 | 21–41 | 19–49 | 34–47 |
| **Length of spines in the ‘pockets’ of the genital atrium** | 6–8 | 6–9 | 7–11 | 7–13 |
| **Copulatory organ length** | 43–100 | 47–70 | 52–86 | 46–107 |
| **Copulatory organ width** | 54–100 | 33–100 | 54–118 | 37–93 |
| **Germarium to anterior extremity distance** | 1093–2149 | 1381–2460 | 1141–3240 | 1240–2240 |
| **Vagina to anterior extremity distance** | 321–550 | 380–585 | 328–425 | 380–550 |
| **Germarium length** | 566–1428 | 890–1428 | 896–1792 | 730–1199 |
| **Germarium maximum width** | 37–127 | 83–96 | 47–134 | 38–86 |
| **Seminal receptacle length** | 144–171 | 140–185 | 289–316 | 182–208 |
| **Seminal receptacle width** | 89–105 | 80–99 | 96–112 | 70–87 |
| **Vitellarium to anterior extremity distance** | 204–560 | 319–613 | 244–578 | 320–566 |
spines in the main chamber (201–408) and “pockets” of the genital atrium (20–41), overlapping with *M. isyebi*, *M. pomatomi*, *M. visa*, *M. whittingtoni* n. sp. and *Microcotyle* sp. ex *H. dactylopterus* (see [24, 31]; numbers estimated from the drawing for *M. pomatomi*) (see Additional file 3: Table S3).

According to the combination of the characters listed above, *M. isyebi*, *M. pomatomi* and *M. visa* appear most similar morphologically to *M. erythrini* (s.s.). *Microcotyle pomatomi*, the only species described and reported from a non-sparid host (*Pomatomus saltatrix* (L.); Pomatomidae), is difficult to differentiate due to the numerous circumglobal records and descriptions which have increased abnormally the ranges for the metrical data of this species. Moreover, the only Mediterranean description of *Pomatomi* (off Turkey, Sezen & Price, 1967 in [32]) is particularly similar to *M. erythrini* (s.s.). Detailed morphological and molecular studies are needed to differentiate the two species. The other two species, both sparid parasites, were described as hardly morphologically distinguishable from *M. erythrini*. *Microcotyle visa* was differentiated from *M. erythrini* by the smaller clamp size, larger pharynx and greater number of testes; however, these differences are not completely sufficient to differentiate species as all they overlap (even with those of *M. erythrini* (s.s.)) [2]. No diagnostic morphological differences were provided by Bouguerche et al. [3] to distinguish *M. isyebi* from *M. erythrini*, other than body size, different hosts and large genetic divergence based on cox1 data. New evidence reported in the present study allows characterizing *M. erythrini* (s.s.) based on the size and shape of the haptor which is relatively longer in relation to body length (35–62% vs 27–34% in *M. visa* and 21–32% in *M. isyebi*) and the greater ratio of anterior/posterior haptoral lobe length (52–90% vs 34–50% in *M. visa* and 17–52% in *M. isyebi*) (data for *M. visa* estimated from figure 3A in Bouguerche et al. [2]; those for *M. isyebi* from the present study). The anterior/posterior haptoral lobe length ratio range for *M. erythrini* (s.s.) is very close to the upper range limits for these two species; however, the ratio was > 60% in some of the *M. erythrini* (s.s.) specimens examined here (11 out of 21 *P. erythrinus* and 5 out 18 *P. pagrus*). Additionally, vitelline fields always extend within the haptor in *M. erythrini* (s.s.). Bouguerche et al. [3] reported that the left caecum-vitellarium branch of *M. isyebi* extends into haptor; however, both vitellarium fields of the *M. isyebi* specimens analysed in the present study are always prehaptoral. Finally, in the new material from the Spanish Mediterranean, the tips of the abopercular filaments of the eggs are solid (capitated or pointed) in *M. erythrini* (s.s.) vs half cup-shaped to bifid in *M. isyebi*. No information on this trait is available for *M. visa*.

**Microcotyle isyebi** Bouguerche, Gey, Justine & Tazerouit, 2019

**Host:** *Boops boops* (L.) (type-host) (Teleostei: Sparidae), bogue [weight: 112.9–216.7 g (157.2 ± 22.3 g); standard length: 19.8–24.0 cm (21.7 ± 1 cm)], off Guardamar del Segura, Spain.

**Locality:** Off Guardamar del Segura, Western Mediterranean off Spain. Other localities with valid records: off Bouharoun, Algeria (type-locality) and off Granada, Spain.

**Voucher material:** Three specimens from off Guardamar del Segura are deposited in the Natural History Museum, London, UK (NHMUK.2019.12.10.12-14); the remaining material from Guardamar del Segura is deposited in the Parasitological Collection of the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

**Infection parameters:** Prevalence: 70% (28 out of 40); mean intensity, 4.96 ± 4.46 (*n* = 40).
Fig. 4 *Microcotyle erythrini* van Beneden & Hesse (1863) (*sensu stricto*) ex *Pagellus erythrinus* (L.) from off Guardamar del Segura, Spain. All drawings from the same voucher specimen. a Whole mount. b Anterior body end. c Clamp. d Genital atrium, including copulatory organ. e Germarium. f Egg. g Detail of abopercular egg filament end. *Abbreviations*: co, copulatory organ; mc, main chamber of the genital atrium; p, small posterior chambers (*pockets* *sensu* Mamaev [44]). *Scale-bars*: a, 500 μm; b, d–f, 100 μm; c, 50 μm
**Site on host:** Gill filaments.

**Representative DNA sequences:** GenBank accession numbers: MN816018, MN816019, MN816020 and MN816021 (cox1); MN814850 (28S).

**Description**

[Based on 22 mature adults (paragenophores sensu [3]); data in the description are reported as mean ± SD, ranges are provided in Table 2; Fig. 5]. Body fusiform, stout to elongate. Anterior region tapered, 585 ± 147 long, posteriorly delimited by lateral notches, which narrow body to 271 ± 57 wide. Body width 221.0 ± 45 at level of genital atrium, 411 ± 130 at level of testes. Haptor relatively short [haptor length/total body length ratio 21–32% (26%)], dorsoventrally bi-lobed, well differentiated from body by lateral arch directed sinistro-dextrally, with maximum distal width 83 ± 28 (n = 10). Oviduct directed postero-sinistral extending in ootype, sinistral to germarium, with short sinuous proximal section connected with wide elongated chamber filled with sperm (oviducal seminal receptacle 106 ± 7 × 304 ± 112 (n = 3). Mehlis’ gland well developed.

Vaginal pore medial, dorsal, unarmed, often unobserved, at 372.8 ± 62 (n = 8) from anterior extremity. Vitelline follicles dispersed, extending from 391 ± 82 from anterior extremity of body, extended in 2 lateral fields together with caeca and surrounding testes, usually pre-haptor but partly extending within peduncle. Posterior extremities of vitelline fields mostly different in length (79% of specimens with asymmetrical fields), always unjoined; distance between fields, 0–462, right field longer in 70% of the specimens. Vitelline ducts Y-shaped, with 2 unjoined ducts 339 ± 150 and 357 ± 213 long (right and left, respectively) (n = 10), joining ventral to germarium in slightly sinuous different duct, 337 ± 84 (n = 10) long. Egg fusiform (Fig. 5f), with 2 filaments; opercular filament long, thin, often with thickened internal tip; abopercular filament short, ending in thickened internal tip, half cup-shaped to bifid (Fig. 5g). Opercular extremity of egg narrowing abruptly in tubular hollow section (1/3–1/6 of total egg length, not including filaments) leading to opercular filament.

**Remarks**

Both morphological and molecular data reported in the present paper agree with the original description of Microcotyle isyebi based on material from B. boops off Algeria [3] and from the Spanish Mediterranean [33]. Parona & Perugia [5] and Akmira [6] also provided morphological data from specimens identified as M. erythrinii ex B. boops but these were not considered as species diagnostic in the present study as they represent pooled information for parasites ex B. boops and another host, P. acarne; Microcotyle spp. in sparids are highly host species-specific (see [2, 3] and the present study). In the present study, no specimens of Microcotyle spp. were found in P. acarne.

Some comments on the original diagnosis of the species can be added in light of the data from the description of López-Román & Guevara Pozo [33] and the present study. The range for the number of clamps seems too wide in the original description of Microcotyle isyebi based on material collected off Algeria (54–102) compared with that reported by López-Román & Guevara Pozo.
Fig. 5. *Microcotyle isyebi* Bouguerche, Gey, Justine & Tazerouti, 2019 ex *Boops boops* (L.) from off Guardamar del Segura, Spain. All drawings are from the same voucher specimen, except for the egg. a Whole mount. b Anterior end. c Clamp. d Genital atrium, including copulatory organ. e Germarium. f Egg. g Detail of abopercular egg filament. Abbreviations: co, copulatory organ; mc, main chamber of the genital atrium; p, small posterior chambers (“pockets” sensu Mamaev [44]). Scale-bars: a, 500 µm; b, d-f, 100 µm; c 50 µm.
The number of spines in the main chamber of the genital atrium of *M. isyebi* is also clearly lower in the original description than in the present material (136–230 vs 253–356) (see [3] and Table 2), thus enlarging the range for *M. isyebi* and making this trait almost useless in characterizing this species as it overlaps with most of the species except for *M. donavini* and *M. omanae* Machkewskyi, Dimitrieva, Al-Jufaili & Al-Mazrooei, 2013 (with lower and higher number of spines respectively, see Additional file 4: Table S4). The presence of posterior small chambers of the genital atrium (“pockets”) was also reported as diagnostic in the original description of *M. isyebi*; however, this feature requires a further comment. According to Bouguerche et al. [3], “pockets” are absent in *M. archosargi*, *M. lichiae* Ariola, 1899 and *M. pomatomi*; however, this difference seems to be valid only for *M. lichiae* as these small chambers exist in *M. archosargi* and *M. pomatomi* according to the drawings in [25] and [32], respectively.

In the original description of the species, *M. isyebi* was differentiated from *M. pomatomi* and from *Microcotyle* sp. ex *H. dactylopterus* [24] by traits with overlapping ranges (the number of clamps and spines of the genital atrium for *Microcotyle* sp. ex *H. dactylopterus*) or almost overlapping ranges (the number of clamps and testes for *M. pomatomi*). *Microcotyle pomatomi* and *Microcotyle* sp. ex *H. dactylopterus* [24] require further taxonomic research; *M. pomatomi* has numerous descriptions and synonyms worldwide which have expanded extremely the ranges for morphological features (see [32]; also the only Mediterranean record by Sezen & Price (1967) in [32]), and the morphology *Microcotyle* sp. ex *H. dactylopterus* has been only briefly described [24, 31].

Bouguerche et al. [3] reported that *M. isyebi* is almost indistinguishable from *M. erythrini*. As mentioned above, examination of mature, entire, uncontracted, unstretched and unfolded specimens of this species would be helpful to define or shorten some of the descriptive morphological ranges. Other morphological traits suggested in the present study reveal additional differences. Thus, *M. isyebi* differs from *M. erythrini* (s.s.) in having a shorter haptor in relation to body length (21–32 vs 35–62%) and a shorter anterior haptor lobe in relation to posterior haptor lobe length (17–52 vs 52–90%) and from *M. whittingtoni* n. sp. in the possession of slender clamps (ratio “c” sclerite maximum width/total clamp length, 0.027–0.88 vs 0.100–0.146; see the Remarks for *M. whittingtoni* n. sp. below).

**Microcotyle whittingtoni** n. sp.

*Synonym:* *Microcotyle erythrini* van Beneden & Hesse, 1863 of González González (2005) [36].

**Type-host:** *Dentex dentex* (L.) (Teleostei: Sparidae), common dentex [weight: 204.0–296.2 g (227.5 ± 24 g); standard length: 22.3–20.0 cm (20.8 ± 0.7 cm)], off Guardamar del Segura, Spain.

**Type-locality:** Off Guardamar del Segura, Western Mediterranean off Spain. Other locality with a valid record: off Balearic Islands, Spain.

**Type-material:** The holotype (NHMUK.2019.12.10.1) and 3 paratypes (NHMUK.2019.12.10.2-5) from off Guardamar del Segura are deposited in the Natural History Museum, London, UK; the remaining material from off Guardamar del Segura is deposited in the Parasitological Collection of the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

**Infection parameters:** Prevalence, 58% (23 out of 40); mean intensity, 4.36 ± 5.18 (n = 40).

**Site on host:** Gill filaments.

**Representative DNA sequences:** GenBank accession numbers: MN816010 and MN816011 (cox1); MN814847 (28S).

**ZooBank registration:** To comply with the regulations set out in article 8.5 of the emended 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 whittingtoni* n. sp. is urn:lsid:zoobank.org:act:5E369A8A-0EA2-4ED2-A3C6-0D6E4CC5A390.

**Etymology:** The new species is named in honour of the late Dr Ian David Whittington, eminent researcher on monogenean biology and taxonomy. His comprehensive, meticulous and brilliant studies have inspired and encouraged fish parasitologists worldwide.

**Description**

[Based on 21 mature adults, except when otherwise indicated; data in the description are reported as mean ± SD; ranges are provided in Table 2; Fig. 6]. Body fusiform, elongate, occasionally slender, 3509 ± 507 long, tapered anteriorly at 563 ± 79 (n = 20) from anterior extremity of body; anterior tapered region posteriorly delimited by lateral notches, which narrow body to 253 ± 54 (n = 20) wide. Body 212 ± 27 wide at level of genital atrium and 316 ± 69 wide at testes level. Haptor dorsoventrally bilobed, relatively long [haptor length/total body length
ratio 24–35% (30%), well differentiated, sometimes with peduncle [peduncle 309 ± 104 long, with minimum width 204 ± 77 (n = 6)]; laterally symmetric, with short ventral lobe projected anteriorly and longer posterior (dorsal) lobe [anterior/posterior haptoral lobe length ratio 21–52% (36%)]. Haptor armed with sessile clamps, 60–78 in number, in two rows in lateral frills, joining at anterior and posterior extremities of haptor; clamps slightly smaller at anterior and posterior extremities of haptor. Clamps robust, “c” sclerite maximum width 5 ± 1 and 0.120 ± 0.017 corrected by clamp length (n = 25); “microcotylid” type with trident-shaped accessory sclerite (‘e’; see Fig. 1) with long thick central bar reaching to distal tips of antero-lateral sclerites ‘c’ and 2 delicate branches ramified from basis of ‘e’.

Mouth subterminal, ventral, with 2 septate buccal suckers within funnel-shaped vestibule; oesophagus short; intestinal bifurcation at level of posterior margin of genital atrium or just posterior. Caeca with inner and profuse external lateral ramifications extending into haptor.

Testes numerous, 16–27 in number, dorso-ventrally flattened, subelliptical to irregular, arranged in clusters of 1–3 rows, with some testes overlapping dorso-ventrally; testicular field at 1807 ± 304 from anterior extremity of body, post-germarial and pre-haptoral, partially extending into haptor peduncle. Vas deferens wide, coursing dorsal to uterus, straight up to short muscular copulatory organ, 80 ± 17 × 60 ± 19 (n = 10), opening into posterior part of genital atrium. Genital atrium at 240 ± 34 from anterior extremity of body, with muscular wall, formed by wide main medial chamber, covered with tiny conical spines (272–391 in number), connected with 2 postero-lateral small chambers (“pockets”) armed with longer curved spines (34–47 in number), flanking copulatory organ.

Germarium elongated 953 ± 331 long (n = 11), question mark-shaped, at 1336 ± 167 (n = 11) from anterior extremity of body; proximal globular germinal area 58 ± 13 × 119 ± 44 (n = 11) followed by straight narrow section 311 ± 59 × 37 ± 8 (n = 11), connected with wide tubular region 642 ± 180 (n = 11) formed by 2 arches crossing first dextro-sinistrally and then sinistro-dextrally, gradually widening up to maximum width of 60 ± 16 (n = 11) at distal section. Oviduct directed postero-sinistrally ending in ootype; connected to elongated oviducal seminal receptacle 83 ± 7 × 197 ± 12 (n = 3) by sinuous narrow section. Mehlis’ gland well developed.

Vaginal pore mid-dorsal, unarmed, inconspicuous, at 487 ± 59 from anterior extremity of body (n = 10). Vitelline follicles small, scattered from 423 ± 59 from anterior extremity of haptor, with lateral fields accompanying caecal ramifications and surrounding testes; vitellarium spread along peduncle and within haptor in all specimens.

Posterior extremities of vitelline fields unjoined, always different in length (distance between fields, 308 ± 113; right field longer in 86% of the individuals). Vitelline ducts Y-shaped; efferent ducts 316 ± 81 and 331 ± 79 (right and left, respectively) long (n = 10) separated up to germarium and ventrally joined in slightly sinuous deferent duct, 228 ± 55 long (n = 10). Eggs fusiform (Fig. 6f), with 2 filaments; opercular filament very long, thin, with slightly thicker final tip; abopercular filament shorter, ending in half cup-shaped to bifid tip (Fig. 6g). Opercular end of egg narrowing gradually to connect through conical hollow section (1/6–1/8 of total egg length, not including filaments) leading to opercular filament.

**Remarks**

*Microcotyle whittingtoni* n. sp. differs from *M. erythrini* (s.s.) by the number of clamps (60–78 vs 82–132), the haptor length/total body length ratio (24–35 vs 35–62%), the anterior/posterior haptoral lobe length ratio (21–52 vs 52–90%) and the shape of the tip of the abopercular egg filament (half cup-shaped to bifid vs capitate or pointed). The number of clamps of *M. whittingtoni* n. sp. is particularly low (60–78), similar to other species reported in non-sparid hosts (*M. lichiae* and *Microcotyle* sp. ex *H. dactylopterus* [24]). The ranges for this trait also overlap with those in the original descriptions of two species described from sparids: *M. isyebi* ex *B. boops* (54–102 clamps; see [3]) and *M. visa* ex *Pa. caeruleosticus* (59–126 clamps; see [2]). These ranges are abnormally wide and should be reviewed (see also the Remarks for *M. isyebi* above). The number of testes of *M. whittingtoni* n. sp. (16–27) is a less defining character as the range overlaps the ranges for most *Microcotyle* spp. (see e.g. [34] and Additional file 3: Table S3). Considering the species reported in the Mediterranean or in sparid hosts, this trait is only useful for differentiating the new species from *M. omana* (34–55 testes) and from *Microcotyle* sp. ex *H. dactylopterus* [24], a species with lower but slightly overlapping number of testes (10–17). Regarding the genital atrium armature, the number of spines in the main chamber (272–391) in the new species overlaps with the ranges for *M. erythrini* (s.s.), *M. isyebi* ([3]; present study), *M. pomatomi* and *Microcotyle* sp. ex *H. dactylopterus* [24]. The spines in the “pockets” of the genital atrium in *M. whittingtoni* n. sp. appear to be longer and more curved than those of the other species examined in the present study (*M. erythrini* (s.s.) and *M. isyebi*). The number of spines in the “pockets” of the genital atrium in *M. whittingtoni* n. sp. (34–47) overlaps with the ranges for *M. erythrini* (s.s.), *M. isyebi, M. omana* and *Microcotyle* sp. ex *H. dactylopterus* [24].

The combination of characters for *M. whittingtoni* n. sp. previously mentioned in the remarks is also present in
Fig. 6  *Microcotyle whittingtoni* n. sp. *ex Dentex dentex* (L.) from off Guardamar del Segura, Spain. Holotype: a Whole mount. b Anterior end. c Clamp. 

**d** Genital atrium, including copulatory organ. e Germarium. f Egg. g Detail of abopercular egg filament.

*Abbreviations*: co, copulatory organ; mc, main chamber of the genital atrium; p, small posterior chambers ("pockets" sensu Mamaev [44]).

*Scale-bars*: a, 500 µm; b, d-f, 100 µm; c, 50 µm
**Discussion**

No type-species was selected for the genus Microcotyle in the original definition by van Beneden & Hesse [4], which included the descriptions of two species, *M. donavini* and *M. erythrini* (also *M. canthari* and *M. labratis*, but these species currently belong to the genera Neobivagina and Serranicotyle, respectively). Later, Sproston [37] selected *M. donavini* as the type-species for the genus but at the time of the erection of the genus, these two species were the first morphological references. First descriptions of new Microcotyle species were based on vague morphological differences (mostly the number of clamps and testes [5, 38, 39]). Many more species have been described since then worldwide and several genera of the subfamily Microcotylinae have been erected, and *M. erythrini* has continued being considered valid [2, 3]. Recently, Bouguerche et al. [2] and Bouguerche et al. [3] provided molecular evidence that despite the validity of this species, several Microcotyle spp. from different host species have been wrongly identified as *M. erythrini* because of their morphological homogeneity. These authors referred to a *M. erythrini* complex of cryptic species and suggested that a molecular re-evaluation may reveal additional parasite diversity [3]. Caution must be taken in order to select representative specimens in perfect conditions of maturity, completeness and constitution. Whittington [40] stated that to separate monogeneans of a species complex with high levels of diversity “it is vital to ensure that there is a useful trail of high quality parasite material” for taxonomic studies, also stressing the importance of supporting the results with molecular genetic analyses. The present study shows that morphological differences between *M. erythrini* (s.s.) and similar species

**Multivariate morphometric analysis**

The PCA using seven morphometric variables associated with body shape produced a plot of the 86 specimens (one extreme outlier was removed prior to analyses) in the first plane of the PCA showing the morphological variability between the species of Microcotyle from the Spanish Western Mediterranean (Fig. 7). The first two axes of the PCA explained 73.81% of the variation in the dataset. The first axis explained 55.32% of the variation and showed a separation between *M. erythrini* (s.s.) and *M. isyebi*, while *M. whittingtoni* n. sp. overlapped with the other two species. The specimens of *M. erythrini* ex *P. erythrinus* and *Pa. pagus* showed a wider variation, whereas, for *M. whittingtoni* n. sp. ex *D. dentex* and *M. isyebi* ex *B. boops* the variation was lower. The first axis was positively correlated with the maximum body width (0.844), body length without the haptor (0.736), body width at level of the genital atrium (0.726) and body width at testis area (0.777), and negatively correlated with the length of the anterior haptor lobe (−0.856) and haptor length (−0.803). The second axis which was negatively correlated with body width at the level of the buccal suckers (−0.838) showed intraspecific separation between the specimens of *M. erythrini* ex *P. erythrinus* and ex *Pa. pagus*.
can be found: a new species of *Microcotyle* is described in *D. dentex*, together with the redescription of *M. erythrini* (s.s.) (including a new host record, *Pa. pagrus*) and a new geographical record of *M. isyebi* with additional morphological information, all supported by molecular evidence.

Molecular analyses of the cox1 gene showed clear differences between *Microcotyle* spp. distinctly separating the three species described here. Previous studies have suggested levels of intraspecific variation lower than 5% for species of mazocraeids and microcotylids (up to 5.6% and up to 4.5%, respectively, Yan et al. [41]; Mladineo et al. [42]). Based on cox1 sequences, *M. whittingtoni* n. sp. appears markedly distinct, since the genetic distance from the remaining congeners was higher than 10.8%; specifically, the two isolates ex *D. dentex* differed from *M. erythrini* (s.s.) by 10.8–13.5% and from *M. isyebi* by 14.4–15.2%. The available 28S rDNA sequences for *Microcotyle* spp. are scarce as this region is not commonly used as a marker for interspecific differences.

We have delimited the valid morphological ranges of *M. erythrini* (s.s.) and similar species in sparids; in addition, we suggest the use of new diagnostic characters and morphological tools for assessment of multivariate patterns (e.g. PCA). One of the issues in defining the differential traits of similar species of microcotylids is to avoid abnormally wide morphological ranges by selecting only representative specimens: (i) not including specimens potentially belonging to other species (e.g. morphologically similar parasites from other host species not confirmed by molecular analysis); (ii) selecting morphologically optimal specimens (mature, unbroken, uncontracted, unstretched, not wrinkled and unfolded); and (iii) characterizing these specimens accurately to ensure that the diagnostic species-specific characters are properly described. More than 150 years after the original description of *M. erythrini*, numerous descriptions of this species from *P. erythrinus* and other hosts have provided extremely wide ranges of morphological information for this species, making it almost impossible to find differentiating features. By defining *M. erythrini* (sensu stricto) here, we aimed to characterize the species by much narrower morphological ranges only considering valid genetically tested specimens from confirmed hosts. Regarding the optimal specimen selection, only completely mature adults should be representative for standardized taxonomic descriptions. Worms with fully developed both male and female reproductive systems must be selected, as testes in young adults are early functional and vas deferens is full of sperm while no developed oocytes exist in the germarium. Also, the preservation and completeness of the specimens must be ensured.

Knowledge of the three-dimensional structure of the monogeneans in fresh preparations is crucial to understand the morphology of the specimens mounted in Canada balsam as under the coverslide they are represented in a two-dimensional view; knowledge of the natural shapes allows detecting possible folds and missing parts. For example, the haptor of *Microcotyle* spp. has a dorsal lobe and a ventral lobe (sometimes notched anteriorly), both with clamps; when the specimens are mounted (usually in ventral view), dorsal and ventral projections are folded and often the ventral lobe overlaps the haptor peduncle and/or the posterior end of the body (Fig. 1c). When measuring the haptor, this morphology must be considered in order to measure the body length and the haptor dimensions (see Fig. 1c, d). Moreover, when clamps are counted, possible gaps in the sequence of clamp frills (Fig. 1e, arrowhead) or possible missing pieces of haptor (Fig. 1e, arrow) must be considered, taking into account that the most distal clamps at the ends of the haptoral lobes are smaller.

In general, we recommend the revision and adequate counting of some discrete characters as the number of clamps or testes in the descriptions of several previously described species of *Microcotyle*, as some ranges are often abnormally high (e.g. 59–126 clamps for *M. visa* or 9–24 testes for *M. erythrini*, see [2, 3]). We must be particularly rigorous with this consideration as these traits are key in the species diagnoses of polyopisthocotyleans. For example, the number of clamps herein reported for *M. whittingtoni* n. sp. is 60–78, as the much higher range reported for *M. erythrini* of González González [36] in *Dentex dentex* (110–120) was not confirmed and the drawing and the photomicrograph show that their specimens had 60 clamps [36]. Regarding testes, it should be considered that they are flattened and stacked in at least
two dorsoventral levels, so they must be detected and counted at different depth levels.

Some traditionally used morphological traits are intrinsically highly variable, and must be considered with extreme caution when used for taxonomy. Total length has been considered to characterize species such as *M. archosargi* and *M. lichiae* which are, in general, much larger than *M. erythrini* (s.s.) and similar species; however this trait is uncertain as monogenean sizes are known to be highly dependent on host size [32, 43]; e.g. Mendoza-Franco et al. [25] described smaller specimens of *M. archosargi*, lowering the range of body length to numbers that overlap with most of the similar species in sparid fishes (see Additional file 3: Table S3). In the case of *M. lichiae*, the original description was based on a single specimen and there are no data for its intraspecific variability. The number of spines in the genital atrium has been used more recently to differentiate species of *Microcotyle*; this character is often highly variable (e.g. for *M. isyebi* Bouguerche et al. [3] reported 154–267 vs 272–395 in the present study) and can depend on the condition of the specimen (e.g. incorrect fixation or genital atrium more or less evaginated) or discordances related to the observers. The “pockets” of the genital atrium (*sensu* Mamaev [44]); posterior small chambers), typical of the genus, have also provided taxonomic information. Mamaev [44] already indicated that the presence or absence of spines in these “pockets” was a good diagnostic character. For example, Bouguerche et al. [3] also stated that *M. isyebi* shared the presence of genital atrium “pockets” with the other *Microcotyle* species parasitic in sparid fishes (i.e. *M. archosargi*, *M. erythrini*, *M. isyebi* and *M. visa*) and not in species parasitic in fishes of other families (i.e. *M. donavini* and *M. lichiae*; these authors also listed *M. pomatomi* and *Microcotyle* sp. ex *H. dactylopterus* [24] but “pockets” are present in these species, see Remarks to *M. isyebi* above). “Pockets” are often not described and sometimes not clearly drawn (e.g. *M. pomatomi* [32]), as sometimes they can be unarmed or armed with a few spines [44]. Moreover, when the genital atrium is evaginated, chambers often become indistinguishable in ventral view. Their absence implies a different general structure of the genital atrium, a feature used for differentiation at the generic level within the subfamily *Microcotylinae* [30, 45]. Our last considerations of the traditionally used diagnostic traits refer to the dimensions of the soft muscular organs such as the pharynx or the genital atrium, both contractile and highly variable depending on the specimen, often mentioned in species descriptions (e.g. [2, 43]). All these soft organs can entail diagnostic evidence, but reliable differences should be outstanding, mostly referred to their volume or area, and if possible, relative to the specimen size.

The use of the correct tools and procedures can allow that the currently genetically differentiated species (*M. erythrini*, *M. isyebi* and *M. whittingtoni* n. sp.) become pseudocryptic with defining diagnostic characters or combinations of characters. When the morphometric data of individual worms was integrated in the PCA, the resulting components could not be useful to separate species but provided useful information on specimen groupings based on their shape. The results of the PCA in the present study illustrated that additional diagnostic information can be extracted from the general form of the worms, particularly regarding the relative dimensions and arrangement of the haptor and the remaining of the body. In view of this evidence, we suggest new diagnostic characters revealing previously unnoticed morphological differences: (i) haptor dimensions including anterior and posterior lobes (the larger values for haptor length to body length ratio and for anterior/posterior haptor lobe length ratio differentiate *M. erythrini* (s.s.) from *M. isyebi* and *M. whittingtoni* n. sp.); (ii) thickness of the clamps (the higher ratio between “c” sclerite maximum width/total clamp length differentiates *M. whittingtoni* n. sp. from *M. isyebi* and *M. erythrini* (s.s.)); (iii) relative size and shape of spines of the “pockets” of the genital atrium (spines of the “pockets” in *M. whittingtoni* n. sp. appear to be longer and more curved than those of *M. isyebi* and *M. erythrini* (s.s.)); (iv) extension and symmetry of the posterior extremities of vitelline fields (posterior extremities of vitelline fields always asymmetrical in *M. whittingtoni* n. sp. vs occasionally symmetrical in *M. isyebi* and *M. erythrini* (s.s.)); and (v) shape of the tip of the abopercular filament of the egg: the solid (capitated or pointed) tips of the abopercular filaments differentiate *M. erythrini* (s.s.) from *M. isyebi* and *M. whittingtoni* n. sp. We propose that the region that can provide more taxonomic information is the haptor, taking into account its three-dimensional structure as an oval to fusiform (when pointed at both ends) “foot” holding a body perpendicularly inserted, directly or through a peduncle (Fig. 1c–e). In this way the total and relative haptor dimensions must include both lobes (anterior and posterior) and one of them is often unnoticed in mounted specimens because they fold over the body (see Fig. 1c, d). In fact, some authors have described the haptor of some *Microcotyle* species as triangular (e.g. [3, 26, 33, 34]) only referring to the lobe not folded over the body. In this way, *M. erythrini* (s.s.) can be defined by its relatively longer ventral lobe, the one that is usually unnoticed as it is adhered to...
the body in permanent mounts. As a note of caution, we must stress the need of examination of adult specimens only, as the relative dimensions of the haptor are known to change significantly during the development (see, for example Machkewskyi et al. [43]). The shape and size of the clamps also provides useful taxonomic information. These structures are usually described only as Microcotyle-type, and the width and length are provided (sometimes wrongly addressed, see Additional file 3: Table S3, Additional file 4: Table S4 and Fig. 1a for correct measuring). However, within this morphological description, some variations can be found. A more detailed study of clamp features can provide further taxonomic information. For example, the accessory sclerite (‘e’) is herein described as trifid or trident-shaped for all three species analysed, but it is mostly not described and not drawn, and the few authors drawing the sclerite represent it as single or lancet-shaped (e.g. [24, 46]). We also suggest that more attention should be paid to the thickness of the clamps: among the three species herein analysed, M. whittingtoni n. sp. shows noticeably thicker clamps; we

In the specimens of Microcotyle from the Spanish Western Mediterranean we observed some differences in the extension of the posterior extremities of vitelline fields (also including the extension of the caeca, as they accompany the vitellarium): extending into the haptor or peduncle in M. erythrinia (s.s.) and into the haptor in M. whittingtoni n. sp. and prehaptoral in M. isyebi. However, this trait was not here suggested to characterize M. isyebi as according to the original description the posterior extension of the left caecum (and consequently the accompanying vitelline fields) extends into haptor “for a short distance” of the specimens from off Algeria [3]. This character may be dependent on the degree of contraction of the specimen, and therefore all specimens should be fixed and mounted in a similar way to be comparable. Other aspect related to the posterior extension of the vitelline fields of the vitellarium is their symmetry. We observed that the posterior extensions of the vitelline fields were always asymmetrical in M. whittingtoni n. sp., while in the other two species we found both specimens with symmetric and asymmetric vitelline fields. Gill polypisthocotyleans show more or less distinct asymmetry related with the side of the gill filament they attach to [47, 48]; interestingly Bouguerche et al. [3] reported that left caecum (and consequently the accompanying vitelline fields) was longer in M. isyebi, while in all the species herein observed included specimens with both dextral or sinistral asymmetry.

Mamaev [44] described the eggs of Microcotyle spp. as two-filamented, with usually long opercular and shorter abopercular filament, but no further morphological details are normally provided in the species descriptions. The examination of the new specimens from the Spanish Western Mediterranean also revealed differential details regarding the eggs such as the different shapes of the end of the abopercular filament: solid (pointed or capitlate) in M. erythrinia (s.s.) (Fig. 4f) and hollow (bifid or cup-shaped) in M. isyebi and M. whittingtoni n. sp. (Figs. 5f, 6f). Other possible differential details were observed such as the type of connection between the egg and the opercular filament: abruptly connected in M. erythrinia (s.s.) and M. isyebi (Figs. 4f, 5f) and inserted through a gradual transition in M. whittingtoni n. sp. (Fig. 6f). This trait is not used for diagnosis in the present study as it requires a more standardized description. More detailed descriptions are recommended as this trait can be taxonomically useful and other authors, e.g. Sproston [37], have already reported interspecific differences regarding the egg shape. The information on this trait can be limited as the egg shape varies depending on the condition and presence of uterine eggs.

Conclusions

The present study suggests new diagnostic morphological traits to differentiate Microcotyle spp. in Mediterranean sparids and shed light on the case of M. erythrinia species complex changing its previously considered cryptic status. More detailed descriptions are recommended, including molecular data, preferably of more informative gene markers regarding the interspecific differences in the polypisthocotyleans such as cox1 [41, 42, 49], but also 28S rDNA sequences as they can provide useful
complementary information. This study also shows that *M. erythrini* (s.s.) is not species-specific (even not genus-specific) to its hosts, as it parasitizes *P. pagrus* in addition to the type-host, *P. erythrini*; therefore, although the host species must continue as referential in the taxonomy of *Microcotyle* spp., a new host record does not necessarily mean a new species. However, further studies are needed in order to establish the morphological traits defining the microcotylids, especially for genera such as *Microcotyle*, with numerous species reported worldwide.

**Supplementary information**

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s13071-020-3878-9.

**Additional file 1: Table S1.** Mean genetic divergence (uncorrected p-distance in % and number of pairwise nucleotide differences in parentheses) estimated for the partial co1 sequence pairs within (along the diagonal, emboldened) and among species of *Microcotyle* (below the diagonal).

**Additional file 2: Table S2.** Pairwise nucleotide differences among species of *Microcotyle* for the partial 28S rDNA sequences, including *Bivagina pagosomi*.

**Additional file 3: Table S3.** Metrical data for *Microcotyle erythrinei* (sensu stricto) and other species of *Microcotyle* in spined fishes in the Mediterranean Sea and North-East Atlantic. Measurements are in micrometres expressed as ranges, except where a single value was provided.

**Additional file 4: Table S4.** Metrical data from descriptions of *Microcotyle* spp. similar to *M. erythrinei* (sensu stricto) from Mediterranean non-spard or non-Mediterranean fishes. Measurements are in micrometres expressed as ranges, except where a single value was provided.

**Abbreviations**

AHL: anterior haptor lobe length; BL: body length; BH: body length without haptor; CL: clamp length; CO: copulatory organ; CPS: Central South-East Pacific; CSW: c'sclerite width; CW: clamp width; EAS: Eastern Arabian Sea; HL: haptor length; MC: main chamber of the genital atrium; NS: North Sea; NHMA: North-West Atlantic; NWP: North-West Pacific; P: small posterior chambers ("pockets" sensu Marmaea [44]); PHL: posterior haptor lobe length; SA: Southern Australia; SWP: South-West Pacific; WCA: Western-Central Atlantic; WM: Western Mediterranean.

**Acknowledgements**

The authors thank Hermanos Narejo S.L., and particularly Francisco Piedecausa, for their collaboration providing the fish smaples. We are indebted to Professor Aneta Kostadinova (Bulgarian Academy of Sciences) for her generous advice and comments. We are indebted to Rachel V. Pool (University of Valencia) for revising the English and to the anonymous reviewers for their helpful comments and suggestions. MV-M, APO and FEM also thank Dr Natalia Fraija (AZTI) for her guidance in applying molecular thechniques and analyses at the initial stage of the study.

**Authors’ contributions**

MV-M conceived the study, obtained the samples, undertook the morphocharacterisation and drawings. APO co-designed and planned the project, carried out the multivariate morphometric analysis and helped draft the manuscript. SG and MV-M carried out the sequencing, performed the phylogenetic analyses, contributed to the taxonomic discussion and drafted the corresponding parts. JAR took part in the preparation of the manuscript and discussed the results. FEM coordinated and co-designed the project, drafted the manuscript and defined the general structure. All authors read and approved the final manuscript.

**Funding**

This study was supported by the projects AGL2015-68405-R (MINECO/FEDER, Spanish Government/UE) and Promeetoe/2015/018, ReviDpqua IISC/2012/003 and GV/2019/143 (Valencian Regional Government, Spain). SG benefited from a postdoctoral fellowship Juan de la Cierva-Formacion of the MICINN (FGC-2016-29535), Spain.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article. The newly generated sequences were submitted to the GenBank database under the accession numbers MN816010-MN816021 (coxl) and MN814847-MN814850 (28S). The holotype and paratypes of *M. whittingtoni* n. sp., and vouchers of *M. erythrinei* (s.s.) and *M. isyebi* were deposited in the Natural History Museum, London, UK (NHMUK.2019.12.10.1-NHMUK.2019.12.10.14); the remaining voucher material is deposited in the Parasitological Collection of the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain (Acc. Nos. ICIBIE/PeMe2019, ICIBIE/PpMe2019, ICIBIE/ BbMl2019 and ICIBIE/DbMw2019).

**Ethics approval and consent to participate**

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

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 7 August 2019  **Accepted:** 1 January 2020

**Published online:** 31 January 2020

**References**

1. World Register of Marine Species Database. WoRMS Editorial Board; 2019. http://www.marinespecies.org/index.php. Accessed 21 June 2019.

2. Bouguerche C, Gey D, Justine JL, Tazerouti F. Towards the resolution of the *Microcotyle* species complex: description of *Microcotyle isyebi* sp. n. (Monogenea: Microcotylidae), a gill parasite of *P. pagrus* (Teleostii: Sparidae) off the Algerian coast, Western Mediterranean. Syst Parasitol. 2019;96:131–47.

3. Bouguerche C, Gey D, Justine JL, Tazerouti F. Towards the resolution of the *Microcotyle erythrinei* species complex: description of *Microcotyle isyebi* n. sp. (Monogenea, Microcotylidae) from *Bogos boops* (Teleostei, Sparidae) off the Algerian coast. Parasitol Res. 2019;118:1417–28.

4. van Beneden P-J, Hesse C-E. Recherches sur les bdellodose ou hirudinés et les trématodes marins. Brussels: Mém Acad Roy Sc Belgique; 1863. p. 112–6.

5. Parona C, Perugia A. Res ligusticae, XIV. Contribuzione per una mono-genea del genere *Microcotyle*. Ann Museo Civico Storia Nat Giacomo Doria Genoa, Ser. 2a. 1890;10:173–220.

6. Akmirza A. Monogeneans of fish near Gökçeada, Turkey. Turk J Zool. 2013;37:441–8.

7. Bush A, Lafferty K, Lotz J, Shostak A. Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol. 1997;88:575–83.

8. Georgieva S, Selbach C, Faltýnková A, Soldánová M, Sures B, Skírnisson K, et al. New cryptic species of the *revolutum* group of *Echinostoma* (Digena: Echinostomatidae) revealed by molecular and morphological data. Parasit Vectors. 2013;6:64.

9. Bowles J, Blair D, McManus DP. A molecular phylogeny of the human schistosomes. Mol Phylogenet Evol. 1995;4:103–9.

10. Littlewood D, Rohde K, Clough K. Parasite speciation within or between host species? Phylogenetic evidence from site-specific polystome monogeneans. Int J Parasitol. 1997;27:1289–97.

11. Littlewood D, Johnston D. Molecular phylogenetics of the four *Schisto‑ soma* species groups determined with partial 28S ribosomal RNA gene sequences. Parasitology. 1995;111:167–75.
12. Badets M, Whittington I, Lalubin F, Allienne J‑F, Maspimby J‑L, Bentz S, Víllora‑Montero et al. Correlating early evolution of parasitic platyhelminths to Gondwana breakup. Syst Biol. 2011;60:762–81.

13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:772–9.

14. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–8.

15. Jovelin R, Justine J‑L. Phylogenetic relationships within the polypoisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. Int J Parasitol. 2001;31:393–401.

16. Olson P, Littlewood D. Phylogenetics of the Monogenea—evidence from a medley of molecules. Int J Parasitol. 2002;32:233–44.

17. Littlewood D, Rohde K, Bray R, Henriou E. Phylogeny of the Platyhelminthes and the evolution of parasitism. Biol J Linn Soc. 1999;68:257–87.

18. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE); 2010. p. 1–8.

19. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59:307–21.

20. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate maximum-likelihood phylogenies by large data sets. Syst Biol. 2003;52:696–704.

21. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012;9:772.

22. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of development of an open-source, platform-independent image analysis software. Nat Methods. 2012;9:671–75.

23. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of development of an open-source, platform-independent image analysis software. Nat Methods. 2012;9:671–75.

24. Ayadi ZEM, Gey D, Justine JL, Tazerouti F. A new species of Microcotyle (Monogenea: Microcotylidae) from Scorpaena notata (Teleostei: Scorpaenidae) in the Mediterranean Sea. Parasitol Int. 2017;66:37–42.

25. Mendoza‑Franco EF, Tun MCR, Andechida AID, del Rio Rodriguez RE. Morphological and molecular (28S rRNA) data of monogeneans (Platyhelminthes) infecting the gill lamellae of marine fishes in the Campeche Bank, southwest Gulf of Mexico. ZooKeys. 2018;783:125.

26. Catalano SR, Hutson KS, Ratcliffe RM, Whittington ID. Redescriptions of Pisthocotylean monogeneans (Platyhelminthes) from Scorpaena notata (Teleostei: Scorpaenidae). Syst Parasitol. 2010;295:160–7.

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

28. Aiken HM, Bott NZ, Madlineo I, Montero FE, Nowak BF, Hayward CJ. Molecular evidence for cosmopolitan distribution of platyhelminth parasites of tuna (Thunnus spp.). Fish Fish. 2007;8:167–80.

29. Catalano SR, Hutson KS, Ratcliffe RM, Whittington ID. Redescriptions of two species of microcotyldyl monogeneans from three arripid hosts in southern Australian waters. Syst Parasitol. 2010;76:211–22.

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

31. Aiken HM, Bott NZ, Madlineo I, Montero FE, Nowak BF, Hayward CJ. Molecular evidence for cosmopolitan distribution of platyhelminth parasites of tuna (Thunnus spp.). Fish Fish. 2007;8:167–80.

32. Catalano SR, Hutson KS, Ratcliffe RM, Whittington ID. Redescriptions of two species of microcotyldyl monogeneans from three arripid hosts in southern Australian waters. Syst Parasitol. 2010;76:211–22.

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

34. Yamaguti S. Systema Helminthum. Monogenea and Aspidocotylea, vol. IV. New York: Interscience Publishers, Wiley; 1963.

35. Ariola V. Di alcuni trematodi di pesci marini. Boll Mus Zool Anat Comp. 1899;9:1–10.

36. González González P. Parasitofauna branquial de Dentex dentex (Linneo, 1758) (Pisces; Sparidae). PhD thesis, University of Valencia, 2005. http://roderic.uv.es/handle/10550/15041. Accessed 26 Dec 2019.

37. Sproston NG. A synopsis of the monogenean trematodes. Trans Zool Soc Lond. 1946;25:185–600.

38. Sato G. Studies on the ectoparasitic trematodes of Japan. Tokyo: Imperial University; 1894.

39. MacCallum G. Further notes on the genus Microcotyle. Zool Jahrb. 1913;3:389–402.

40. Whittington ID. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. Folia Parasitol. 2004;51:109.

41. Yan S, Wang M, Yang C‑P, Zhi T‑T, Brown CL, Yang T‑B. Comparative phylo‑geography of two monogenean species (Monogenea: Polystomidae) in the host of chub mackerel, Scomber japonicus, along the coast of China. Parasitology. 2016;143:594–605.

42. Madlineo I, Šegvić T, Grabilić L. Molecular evidence for the lack of transmission of the monogenean Spicarcotyle chrysophrii (Monogenea, Polyopisthocotylea) and isoloph Ceratothoa oestroides (Crustacea, Cymothoida) between wild bogus (Boops boops) and cage-reared sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax). Aquaculture. 2009;295:160–7.

43. Machikesskyi VK, Dmitrieva EV, Al‑Jafaili S, Al‑Mazrooei NA. Microcotyle monogenean n. sp. (Monogenea: Microcotylidae), a parasite of Cheimenis nufar (Valenciennes) (Sparidae) from the Arabian Sea. Syst Parasitol. 2013;86:153–63.

44. Mamayev YL. On species composition and morphological features of the Microcotyle genus (Microcotylidae, Monogenea). In: Lebedev BI, editor. Investigations in parasitology. Collection of papers. Vladivostok: DBNTs AN SSSR; 1989. p. 32–8 (In Russian).

45. Mamayev YL. The composition of the genera Atriastra and Atrispinus (Microcotylidae, Monogenea) and some peculiarities of their morphology. Parazitologiya. 1984;18:204–8 (In Russian).

46. Dillon WA, Hargis WJ, Harrisre AE. Monogeneans from the southern Pacific Ocean: Polyopisthocotyleids from the Australian fishes. Sub-families Polylabrini (Genus Polyplabris) and Microcotylinae (Genus Neobivagina). Parasitol. 1985;33:83–7 (In Russian).

47. Llewellyn J. The host-specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. J Mar Biol Assoc UK. 1956;35:113–27.

48. kearn G. Some aspects of the biology of monogenean (Platyhelminth) parasites of marine and freshwater fishes. Oceanography. 2014;2:1–8.

49. Shi S‑F, Li M, Yan S, Wang M, Yang C‑P, Liu Z‑R, Brown CL, Yang T‑B. Phylo‑geography and demographic history of Gotocotyla sawara (Monogenea: Gotocotylidae) on Japanese Spanish mackerel (Scomberomorus niphonius) along the coast of China. J Parasitol. 2014;100:85–93.