Bivagina pagrosomi Murray (1931) (Monogenea: Polyopisthocotylea), a microcotylid infecting the gills of the gilt-head sea bream *Sparus aurata* (Sparidae) from the Red Sea: morphology and phylogeny

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Abstract

Introduction: Monogenea is a class of ectoparasitic flatworms on the skin, gills, or fins of fish. Microcotylidae is a family of polyopisthocotylean monogeneans parasitising only marine fishes. This work describes and taxonomically determines a microcotylid polyopisthocotylean monogenean in an important fish in Saudi aquaculture. **Material and Methods:** Thirty gilt-head sea bream captured alive from the Red Sea of Saudi Arabia were examined for monogenean infection. Worms were described morphologically and morphometrically by light microscopy and multiple sequence alignments and phylogenetic trees were also constructed after maximum likelihood analysis of the 28S rRNA sequences. **Results:** Seventeen fish were infected by a monogenean parasite in the gill lamellae. It showed a bilobed anterior extremity, two rows of numerous unequal clamps of microcotylid type, and paired muscular vaginae crowned by differently sized spines. The vaginal number and its relative armature suggested the species' affiliation to group D; the parasite possessed large, muscular vaginae with a full corona of spines over almost the entire width resembling *Bivagina pagrosomi* Murray (1931). The molecular analysis of the parasite 28s rRNA revealed 97% homology with *B. pagrosomi* (AJ577461.1). **Conclusion:** The results confirmed the taxonomic status of the parasite recorded. On the basis of morphology and molecular data, we consider that several conclusions on the systematic status of microcotylids from Red Sea fishes in Saudi Arabia should be discussed.

Keywords: Monogenea, Microcotylidae, *Bivagina pagrosomi,* morphology, molecular study.

Introduction

The gilt-head sea bream, *Sparus aurata* (*Sparidae*), is one of the most popular food fishes from the Red Sea, which makes it attractive for aquaculture (21). For successful aquaculture, the effective control of parasitic diseases infecting fish should be carefully attended to, not least also because parasites can be spread from farmed fish to the wild population (23). Due to their direct life cycles, monogenean parasites constitute one of the most dangerous parasites infecting cultured fish (22, 23). Microcotylidae (24) represent the large monogenenoid family and comprise about 8 subfamilies,

39 genera, and 150 species (1). The pathology and mortality associated with invasions of representatives of this family in cultured and wild fish have previously been reported (10, 29, 32). The genus *Bivagina* was recovered by Yamaguti (30) from the gills of the Red Sea bream *Pagrus major* (*Sparidae*) with the type species *B. tai*. These parasites were unique within the Microcotylidae family as they possess a pair of large, muscular armed or unarmed vaginae. *B. pagrosomi* (18) was isolated from the gills of *Chrysophrys aurata* (synonym: *P. aurata*) and it possess a pair of armed vaginae with a full corona of spines opposing each other and occupying almost the entire width of the worm. The

importance of molecular analysis has recently increased for the rapid and efficient phylogenetic study of parasites (17), and specifically the partial sequences of the 28S rRNA gene have been used for the phylogenetic study of monogeneans (17). During a recent parasitological survey on marine fishes of the Jizan coasts of the Red Sea, Saudi Arabia, a microcotylid polyopisthocotylean monogenean was found in the gills of gilt-head sea bream, *S. aurata* (*Sparidae*), and described. The taxonomic status of the parasites was determined according to morphological characterisation under light microscopy and confirmed by molecular analysis of their 28S rRNA.

Material and Methods

Sample collection and parasite isolation. A total of 30 specimens of the gilt-head sea bream, *Sparus aurata* (*Sparidae*), were collected alive from local Red Sea fishermen along the coasts of Jizan (16.8894° N, 42.5706° E), Saudi Arabia, between September and December 2018. The fish were transported immediately to the Parasitology Laboratory, where they were morphologically identified according to the guidelines of Randall (19). The gills were isolated and placed in Petri dishes with tap water, and then examined under a stereomicroscope for the presence of monogeneans (17). For morphological study of the sclerotised parasite structures, worms were fixed in a mixture of glycerine ammonium picrate (5), while for internal organ examinations, other specimens were fixed in hot 4% formalin then stained with Semichon's carmine and mounted in Canada balsam (5). Photomicrographs were taken with a BX53 microscope (Olympus Corporation, Japan). Drawings were made by *camera lucida*. Nomenclature of the clamp sclerites follows the key published by Kritsky and Klimpel (9) for microcotylids. Measurements are given as means followed by ranges in parentheses.

Molecular study. Genomic DNA (gDNA) was extracted from 70% ethanol-preserved samples using a DNeasy tissue kit (Qiagen, Germany) following the manufacturer's instructions. Amplification was carried out on a MJ Research PTC-150 thermocycler (Marshall Scientific, USA). PCR amplification of a partial 28S rRNA sequence was carried out using two universal primers (7):C1M13 (5′-GTAAAACGACGGCCAGA CCCGCTGAATTTAAGCAT-3′) and D2M13 (5′-CA GGAAACAGCTATGACTCCGTGTTTCAAGACGG-3′) as forward and reverse primers, respectively. The PCR was conducted in a final volume of 50 μL containing 1 × PCR buffer (20 mM Tris-HCl, pH 8.4, and 50 mM of KCl), 1.5 mM of $MgCl_2$, 0.2 mM of deoxynucleoside triphosphate mixture (dATP, dCTP, dGTP, and dTTP), 100 pmol of each primer, 2.5 units (U) of *Thermus aquaticus* (Taq) polymerase, 0.1 μg of

extracted parasite genomic DNA, and nuclease-free sterile double-distilled water up to 50.0 μL. The mixture was then subjected to a precise thermal profile through 60 cycles in a programmable thermocycler (Biometra GmbH, Germany) as follows: at 94°C for 30 s, at 50°C for 1 min, and at 72°C for 2 min (4). The amplified product (10–15 μL) was confirmed by using agarose gel electrophoresis (1.5%) and the DNA bands were stained with ethidium bromide (0.5 μg/ml) against the GeneRuler 100 bp Plus ready-to-use DNA ladder as a molecular weight marker (Fermentas, Lithuania) (4). A DNA gel purification kit (Abgene, UK) was used to purify the appropriate-sized PCR amplicons from the gel. Sequencing was carried out by the same primer sets through the Big Dye Terminator v.3.1 Cycle Sequencing Kit on a 3500 Genetic Analyser automatic sequencer (both from Applied Biosystems, USA). Sequences were aligned and compared with different Microcotylidae species previously accessed in GenBank.

Phylogenetic analysis. Sequence identity for the recovered data was checked using the Basic Local Alignment Search online Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence trimming for the congeneric species recovered was carried out by Bioedit v7.2.5, sequence alignment was done by CLUSTAL W v2.0 (13) and the phylogenetic tree was constructed using theMEGA7 (11) programme based on maximum likelihood analysis. *Allodiscocotyla diacanthi* (26) was employed as an out-group during tree construction.

Results

Diagnosis. Diagnosis was based on seven specimens. Body elongation (Fig. 1a) was dorsoventrally flattened and lanceolated. Terminal anchors were absent. The anterior extremity was bi-lobed with two septated and unarmed buccal organs (Fig. 1b, c). A prepharynx was absent and the pharynx was subglobular (Fig. 1c). The oesophagus showed no diverticula. Bifurcation between the genital pore and vaginal apertures was observed. The posterior haptor was symmetrical and weakly delineated from the body with two rows of numerous dissimilarly sized clamps of microcotylid type (Fig 1d–f). The two intestinal caeca extended into the haptoral peduncle and were largely co-extensive with vitellaria. The testes were numerous. The post-ovarian and genital atria were unarmed. Vaginae were paired and muscular, the dorsal vaginae were armed with a crown of unequally sized spines (Fig 1i–k). The germanium was U-shaped. Eggs were large, ovoid, fusiform (Fig. 1l), and operculated with extensive apical and short posterior filaments. A line diagram showing the different morphological diagnostic characteristics of the isolated *B. pagrosomi* was constructed (Fig 2a–e).
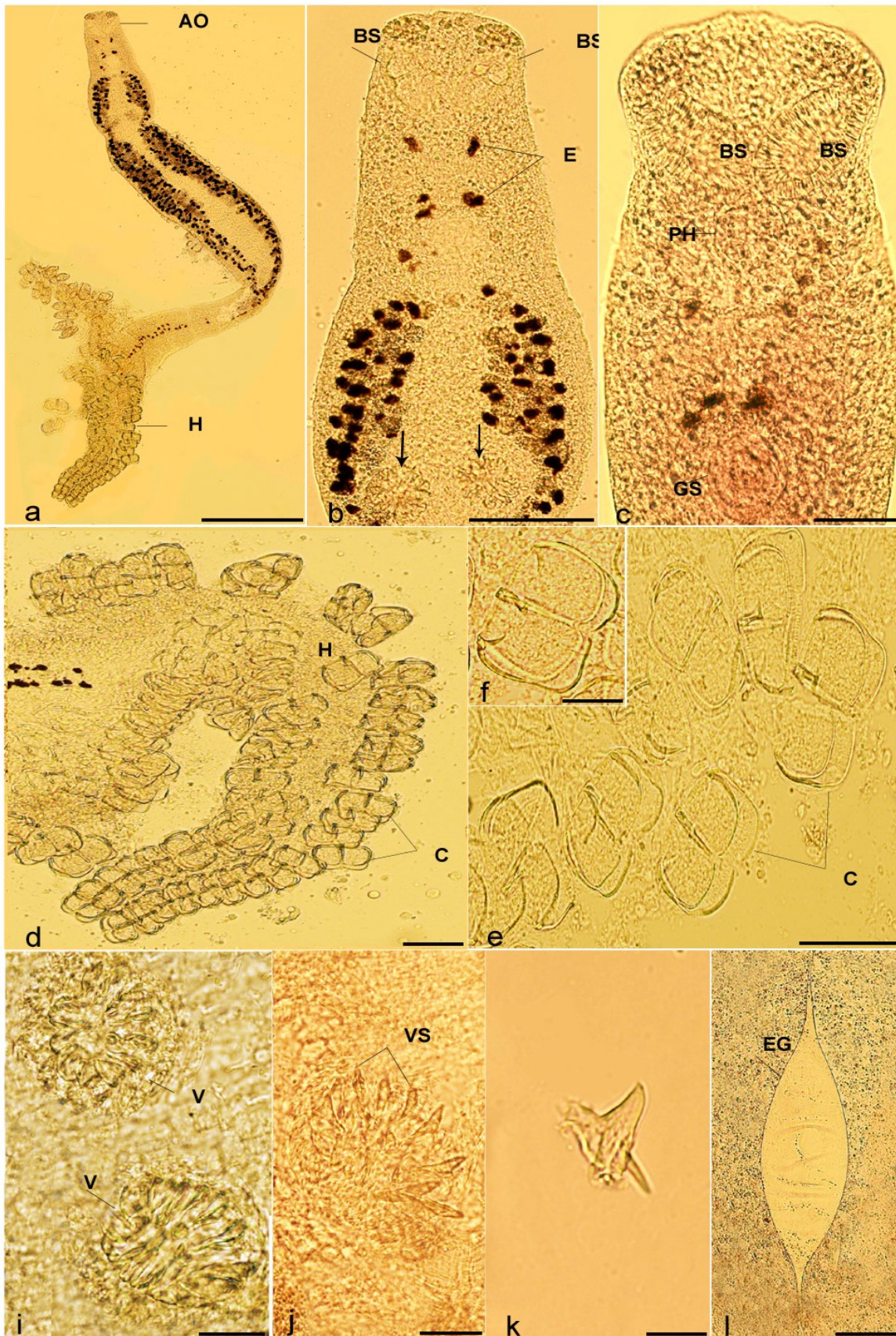
Fig. 1. Photomicrographs of *Bivagina pagrosomi* from *Sparus aurata* in the Red Sea; (a) – whole mount of a paratype adult specimen, dorsal view, AO – anterior attachment organ, H – haptor; (b, c) – anterior end of body, BS – buccal suckers, E – eyes, arrows indicate the position of the two armed vaginae, PH – pharynx, GS – genital suckers; (d) – haptor (H) with clamps (C); (e) – median clamps; (f) – anterior clamps; (i, j) – paired, armed vaginae (V) with crown of spines (VS) within each vagina; (k) – isolated vaginal spine; (l) – egg (EG). Scale bars – a = 500 μm; b = 130 μm; c = 50 μm; d = 100 μm; e = 65 μm; f, i = 20 μm; j = 4 μm; k =50 μm
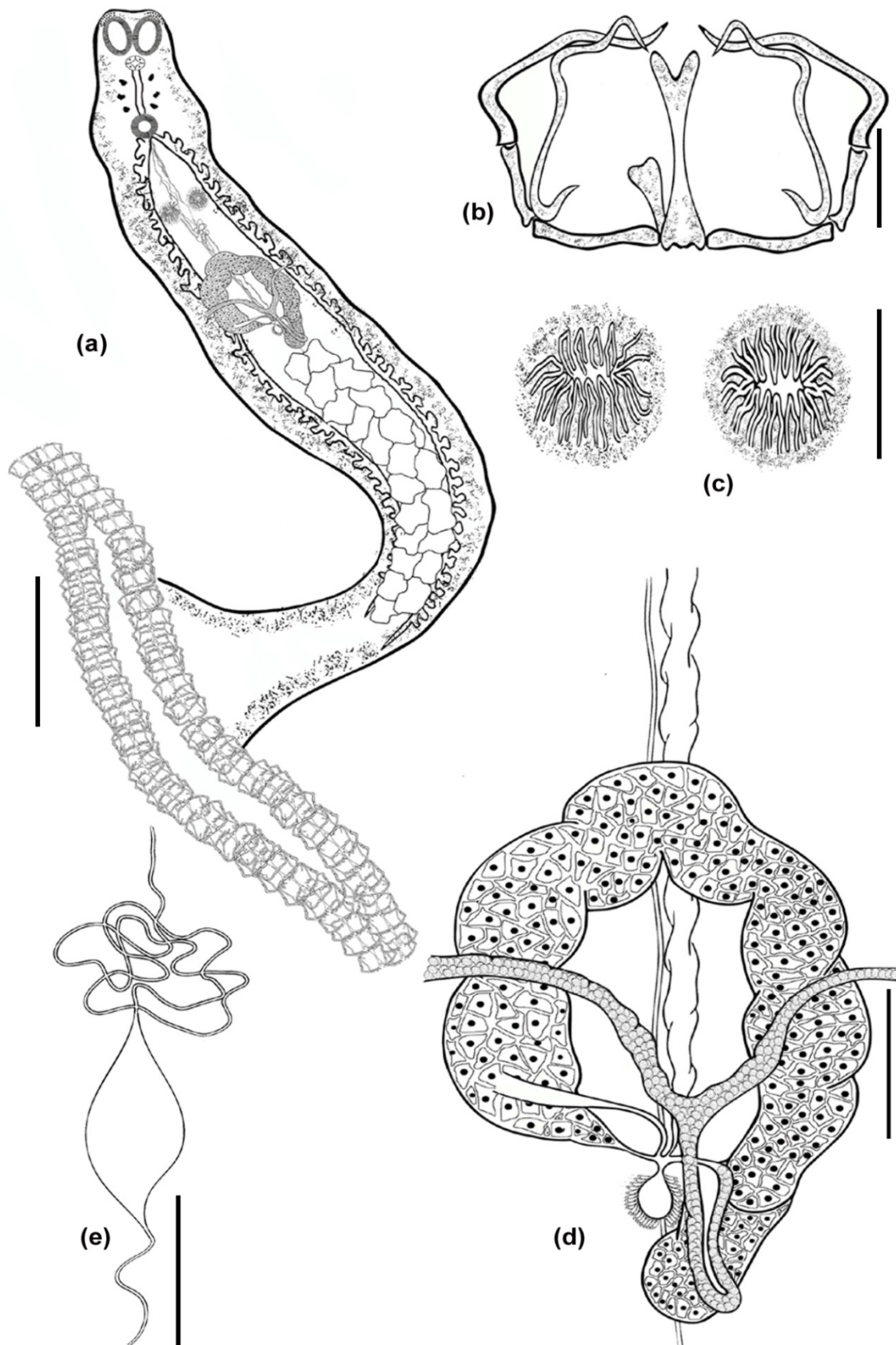
Fig. 2. *Bivagina pagrosomi.* (a) – total view; (b) – clamp, isolated median jaw, dorsal view; (c) – paired, armed vaginae; (d) – reproductive system; (e) – egg. Scale bars – a = 400 μm; b = 20 μm; c = 45 μm; d = 117 μm; e = 150 μm

Table 1. Estimates of evolutionary divergence between sequences:the number of base substitutions per site between sequences is shown. Analyses were conducted using the maximum composite likelihood model. The analysis involved 23 nucleotide sequences. There were a total of 529 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

| Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
MK439621 *B. pagrosomi*																						
Z83002.1 *B. pagrosomi*	0.01																					
MH700256.1 *Microcotyle* sp.1	0.07	0.06																				
GU263830.1 *M. arripis*	0.07	0.06	0.00																			
MH700266.1 *Microcotyle* sp.2	0.07	0.06	0.00	0.00																		
GU263831.1 *K. truttae*	0.12	0.10	0.08	0.09	0.09																	
MH700259.1 *Lutianicola* sp.	0.11	0.10	0.09	0.10	0.10	0.12																
AF382050.1 *C. branquialis*	0.13	0.12	0.12	0.12	0.12	0.13	0.11															
FJ432589.1 *D. sciaenae*	0.13	0.12	0.12	0.12	0.12	0.11	0.12	0.07														
MH700258.1 *Polylabroides* sp.	0.14	0.13	0.11	0.11	0.11	0.13	0.07	0.13	0.12													
MH700261.1 *Caballeraxine* sp.	0.16	0.15	0.12	0.12	0.12	0.13	0.14	0.15	0.15	0.13												
MH700260.1 *Metamicrocotyla* sp.	0.15	0.14	0.12	0.13	0.13	0.13	0.10	0.10	0.12	0.13	0.14											
MH700591.1 *P. mamaevi*	0.14	0.13	0.12	0.12	0.12	0.14	0.08	0.13	0.13	0.04	0.15	0.15										
GU289509.1 *P. stilaginae*	0.16	0.15	0.13	0.13	0.13	0.14	0.08	0.13	0.14	0.05	0.17	0.16	0.02									
KF804036.1 *H. heterapta*	0.14	0.13	0.13	0.13	0.13	0.13	0.16	0.14	0.15	0.17	0.17	0.16	0.17	0.18								
MH700262.1 *Intracotyle* sp.	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.14	0.12	0.12	0.13	0.13	0.15	0.16	0.16							
KF804034.1 *H. chorinemi*	0.14	0.13	0.13	0.13	0.13	0.13	0.16	0.14	0.16	0.17	0.18	0.16	0.17	0.18	0.00	0.16						
AF382043.1 *N. pacifica*	0.25	0.23	0.22	0.22	0.22	0.23	0.24	0.21	0.23	0.24	0.22	0.23	0.25	0.25	0.22	0.22	0.22					
KJ397730.1 *P. sarmientoi*	0.24	0.23	0.20	0.21	0.21	0.21	0.24	0.21	0.21	0.23	0.22	0.24	0.25	0.23	0.22	0.21	0.22	0.16				
AF382042.1 *Paradewesia* sp.	0.23	0.22	0.20	0.20	0.20	0.20	0.22	0.19	0.19	0.24	0.20	0.21	0.25	0.26	0.23	0.19	0.23	0.11	0.17			
KF378589.1 *Neomicrocotyle* sp.	0.25	0.23	0.22	0.22	0.22	0.23	0.24	0.21	0.23	0.24	0.22	0.23	0.25	0.25	0.22	0.22	0.22	0.00	0.16	0.11		
KF378588.1 *Lethacotyle* sp.	0.26	0.24	0.23	0.23	0.23	0.24	0.24	0.20	0.22	0.24	0.23	0.23	0.25	0.25	0.22	0.22	0.23	0.02	0.16	0.12	0.02	
KF804038.1 *A. diacanthi*	5.14	5.34	5.24	5.11	5.17	5.06	5.18	5.29	5.12	5.43	5.00	5.06	5.29	5.43	5.27	5.08	5.20	5.82	5.68	5.65	5.82	7.09
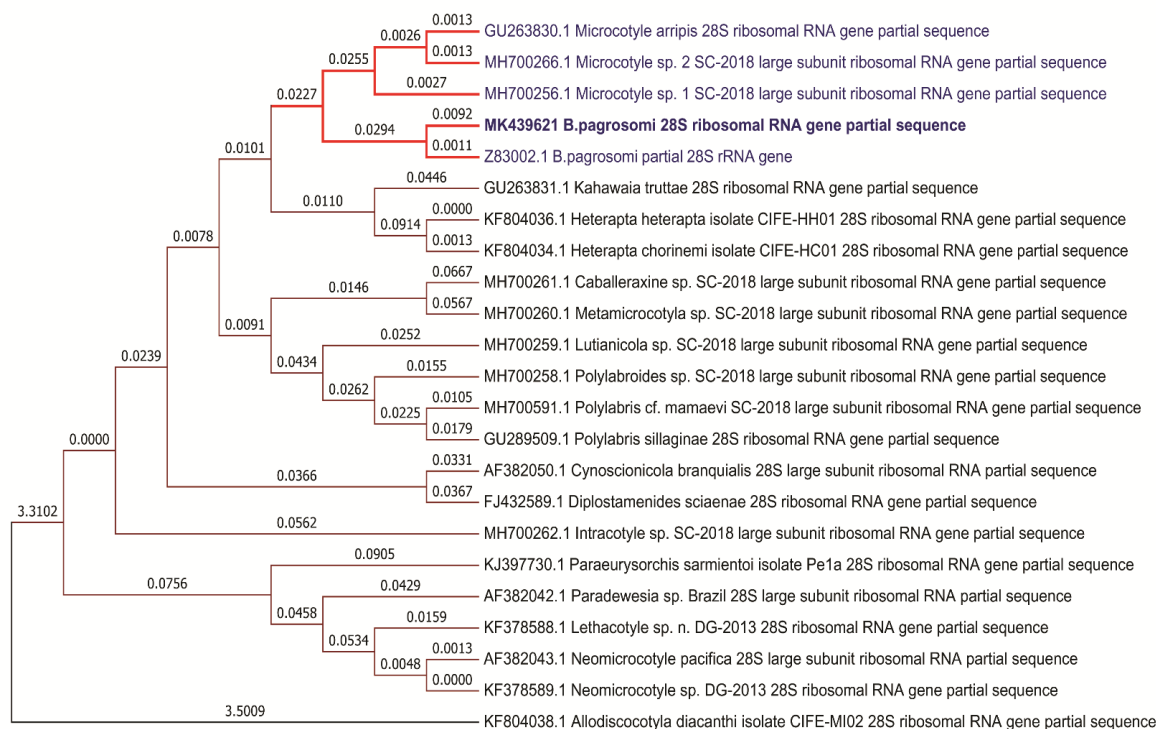
Fig. 3. Phylogenetic tree constructed by maximum likelihood method. The analysis involved 23 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 810 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

Description. The body length including the haptor was 3,266 (2,895–5,347) μm while its width at the level of the germarium measured 435 (395–544) μm. The anterior region contained the paired muscular buccal organs of 104 (90–125) μm×60 (54–83) μm. A muscular circular pharynx measured 35 (30–45) μm×37 (32–53) μm. The oesophagus was 130 (100–145) μm long. The haptor was symmetrical and delineated from the body with 43–47 pairs of clamps arranged in two equal rows. Clamps were nearly identical in shape with dissimilar sizes. Haptoral hooks were absent. The anterior clamps were 62 (58–70) μm wide and 34 (30–40) μm long; the median clamps 85 (80–88) μm long and 40 (36–45) μm wide; and the posterior clamps 58 (48–64) μm wide and 35 (30–38) μm long. Irregular vitellaria, brownish in colour, extended from the genital atrium to the haptoral peduncle. The testes, 38 (35–44) μm in size, were situated in the post-ovarian intercaecal field and did not extend into the haptoral peduncle. The germarium was median, U-shaped, pre-testicular, and divided into three parts: (i) the distal part, which measured 406 (337–550) μm; (ii) the proximal part, which extended 348 (275–475) μm; and (iii) the germinal part, which was 92 (71–110) μm long. The vitelline ducts were Y-shaped, the anterior two branches measuring 223 (210–250) μm while the posterior piece was 265 (250–280) μm long and opened into the genito-intestinal canal. *Bivagina pagrosomi* eggs had 200 (185–230) μm length and 85 (80–98) μm width and had a very long, tangled anterior apical filament measuring 955 (930–1,125) μm and a posterior filament 90 (80–130) μm long.

Molecular study. A phylogenetic tree (Fig. 3) was constructed from the sequences of the present species and available sequences of some members of the Microcotylidae family recovered after BLAST with the estimates of evolutionary divergence between sequences calculated (Table 1). The analysis used 23 species with a total of 810 bp in the final dataset. The monophyletic clade of Microcotylidae included monogenean members of the family in a separate clade with the species of *Microcotyle* and *Bivaginae* constituting the same clade, *Microcotyle* sp. (MH700256.1) and *Microcotyle* sp. 2 (MH700266.1) proving to have 92% identity with 50 bp difference, and *Microcotyle arripis* (GU263830.1) showing the same percentage identity with 55 bp difference with the query sequences. A nucleotide BLAST search showed that the SSU rRNA was most similar to the sequences of *B. pagrosomi* (accession no. Z83002.1) with a percentage of identity reaching 97% with only 5 bp difference; these species including the present parasite are assumed to be polyopisthocotylean monogeneans belonging to the Microcotylidae family. The parasite consistently grouped with *B. pagrosomi* as a strongly supported sister group to the *Microcotyle* clade. The recovered sequences were deposited in GenBank under accession number MK439621.

Taxonomic summary. Type-host: *Sparus aurata* (*Sparidae*), gilthead sea bream. Type-locality: Jizan Coasts (16.8894° N, 42.5706° E), Red Sea, Saudi Arabia. Site of infection: gills. Voucher material: ten specimens: five holotype and five paratypes (accession numbers KKU. BIO19.1–10) deposited in the parasite collection of the parasites section, Biology Department

Museum, College of Science, King Khalid University, Saudi Arabia.

Infection details: of the 30 caught fish, 17 were infected by monogeneans, each parasitised by 5–9 (mean 7 ±2) worms. Etymology: the genus name of the parasite derived from the presence of paired vaginae while the specific name derived from the generic name of the host fish *P. aurata* (synonym: *C. aurata*) from which the parasite was isolated for the first time.

Discussion

Due to the great intraspecific variability in the metrical characters used for the differentiation of Microcotylidae representatives and despite the numerous revisions of this family, it is clear that the identification and diagnosis of its species is still generally problematical (6). According to Mamaev (15), the subfamily Microcotylinae includes microcotylids that possess a sub-symmetric well-delineated haptor with no anchors, armed or unarmed genital atrium, and a single, medio-lateral vagina, or two dorsolaterally positioned vaginae. Members of the genus *Bivagina* can be differentiated from other Microcotylidae species on the basis of vaginal number (single or paired) and relative armature, and also by the armature of the genital atrium. The following groups were created according to these criteria. Group A includes the following species with a single, unarmed vagina: *Atriostella* (26), *Caballeraxine* (14), Diplostamenides (26), *Gamacallum* (26), *Jaliscia* (16), *Magniexcipula* (3), Paramicrocotyloides (20), *Paranaella* (8), *Bradyhaptorus* (26), *Polymicrocotyle* (12), *Pseudoaspinatrium* (26), *Sciaenacotyle* (15) (single opening with paired vaginal ducts), *Solostamenides* (26), and *Microcotyle* spp. (27). *Microcotyle pamae* (25) has an armed vagina. Group B includes species with single armed vagina: *Monomacracanthus* (15) and *Sebasticotyle* (16). Group C includes species with paired unarmed vagina: some species of *Bivagina* (14), *Lutianicola* (14), *Neobivaginacanthari* (15), *Pseudobivagina* (15), and *Pseudoneobivagina* (15). Finally, group D includes species with paired armed vaginae: *Bivagina* (30) (certain species within the genus), *Omanicotyle* spp. (31), *Kahawaia* (14), and *Neobivaginopsis* (28). The species isolated in the present study possesses paired armed vaginae, and by comparison with species of Group D it was observed that it is similar to *B. pagrosomi* in the large, muscular vaginae with a full corona of spines, which occupy almost the entire width of the worm. The vaginae in *Omanicotyle* are heavily muscularised and armed to a small extent with a crescent of short spines. *Kahawaia* possesses two cuticularised, pyriform pads armed with spines, interpreted as vaginae. The vaginae of *Neobivaginopsis* are large, muscular, contractile structures the openings of which have lightly sclerotised borders. The relative armature of the genital atrium also can be used as a key feature discriminating monogenean species; pertinently *Caballeraxine*,

Diplostamenides, *Lutianicola*, *Neobivagina*, *Neobivaginopsis*, *Pseudobivagina*, *Pseudoneobivagina*, *Sciaenacotyle*, *Solostamanides*, *Atriostella*, *Diplasiocotyle*, *Kahawaia*, *Jaliscia*, *Microcotyle*, *Paranaella*, *Polymicrocotyle*, and *Sebasticotyle*. The genera *Gamacallum*, *Magniexcipula*, *Monomacracanthus*, Paramicrocotyloides, *Pauciconfibula*, *Pseudoaspinatrium*, and *Bivagina* have an unarmed genital atrium. The present species have an unarmed genital atrium and no differentiated cirrus; however, it can be readily distinguished by the size and armature of their vaginae as discussed above. In addition to all of the characteristic features described above, the species isolated herein is from the same host species as *B. pagrosomi*. While two vaginae are a feature for several genera across the subfamily, the complexity and the degree of variation observed in the armament of these requires supporting molecular studies to unambiguously place species within a genus. Since construction of the phylogenetic tree has been important for monogenean taxonomy, 28S rRNA has been known to allow better phylogenetic resolution among monogenean families (22). The sequence alignment and phylogenetic tree showed that the Microcotylidae include the monophyletic *Bivagina* as a sister group to *B. pagrosomi* (18). The PCR products obtained in the present study were purified and sequenced for proper confirmation, and they expressed an 810 bp fragment of the 28S rRNA, which is a stable conserved region among microcotylid genomes (2, 29). Sequence alignment between the present parasites and those retrieved from GenBank showed a high percentage of identity with *B. pagrosomi*, confirmed by previous studies which stated that most of the causative agent worms isolated from cases of parasitic monogeneans in Sparidae fish are *Bivagina* (18). Comparative sequence analysis revealed 80% to 97% homology with sequences of microcotylid recovered from GenBank, with the most identity for *B. pagrosomi* (accession number Z83002.1) at 97%, agreeing with different studies confirming that there is uniformity in the sequence characteristics among microcotylidgenera (2, 31, 32). There are few studies regarding the morphological and molecular characteristics of *B. pagrosomi* infecting *S. aurata* worldwide and no reports of this parasite in the Kingdom of Saudi Arabia, and the present study provided data of morphological and molecular characteristics of this species as a first study with a new host and locality record in the country. Since helpful results were obtained in the present study, which confirms the taxonomic status of the parasite recorded, we consider the morphological and molecular data to compel discussion of several conclusions on the systematic status of microcotylids from Red Sea fishes in Saudi Arabia.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Ayadi Z.E.M., Gey D., Justine J.L., Tazerouti F.:A new species of *Microcotyle* (Monogenea: Microcotylidae) from *Scorpaena notata* (Teleostei: Scorpaenidae) in the Mediterranean Sea. Parasitol Int2017, 66, 37–42.

2. Bouguerche C., Gey D., Justine J.L., Tazerouti F.:Microcotyle visa n. sp. (Monogenea: Microcotylidae), a gill parasite of *Pagrus caeruleostictus* (Valenciennes) (Teleostei: Sparidae) of the Algerian coast, Western Mediterranean.Syst Parasitol 2019, doi: 10.1007/s11230-019-09842-2 (ahead of print).

3. Bravo-Hollis M.: Helmintos de peces del Pacífico Mexicano. XXXIX. Dos subfamilias nuevas de Monogeneos de la familia Macrovalvitrematidae Yamaguti, 1963 (Piscine helminths of the Mexican Pacific. XXXIX. Two novel monogenean subfamilies in the Macrovalvitrematidae Yamaguti, 1963 family). An Inst Biol Univ Nac Auton Mex 1982, 52, 27–38.

4. Catalano S.R., Hutson K.S., Ratcliff R.M., Whittington I.D.: Redescriptions of two species of microcotylid monogeneans from three arripid hosts in southern Australian waters. Syst Parasitol 2010, 76, 211–222.

5. Ergens R.: The suitability of ammonium picrate-glycerin in preparing slides of lower Monogenoidea. Folia Parasitol 1969, 16, 320.

6. Isbert W., Carrassón M., Pérez-Del-Olmo A., Montero F.E.: A new species of *Tinrovia* Mamaev, 1987 (Monogenea: Microcotylidae) from the deep-sea fish *Notacanthusbonaparte* Risso (Notacanthiformes: Notacanthidae) in the Western Mediterranean and the North East Atlantic. Syst Parasitol 2017, 94, 609–619.

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

8. Kohn A., Baptista-Farias M.F.D., Cohen S.C.: *Paranaella luquei* gen. et sp. n.(Monogenea: Microcotylidae), a new parasite of Brazilian catfishes. Folia Parasitol 2000, 47, 279–284.

9. Kritsky D.C., Klimpel S.: *Cyclocotyloides bergstadi* n. sp. (Monogenoidea: Diclidophoridae: Diclidophoropsinae) from the gills of grenadier, *Coryphaenoides brevibarbis* (Teleostei: Macrouridae), in the Northeast Atlantic Ocean. Comp Parasitol 2007, 74, 23–30.

10. Kritsky D.C., ÖktenerA.: *Solostamenides paucitesticulatus* n. sp. (Monogenoidea: Mazocraeidea: Microcotylidae) from the freshwater mullet *Liza abu* (Heckel) (Mugiliformes: Mugilidae) from Atatürk Reservoir on the Euphrates River in southern Turkey. Sys Parasitol2015, 91, 139–145.

11. Kumar S., Stecher G., Tamura K.: MEGA7: Molecular Evolutionary Genetics Analysis, Version 7.0 for Bigger Datasets. Mol Biol Evol 2016, 33, 1870–1874. doi.org/10.1093/molbev/msw054.

12. Lamothe-Argumedo R.: Monogeneos de peces V. A redescription of *Tagia ecuadori* (Meserve, 1938) Sproston, 1946. An Inst Biol Univ Nac Auton Mex 1967, 38, 35–46.

13. Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G.: Clustal W and Clustal X version 2.0. Bioinformatics 2007, 23, 2947–2948. doi.org/10.1093/bioinformatics/btm404.

14. Lebedev B.I.: Helminths of epipelagic fishes of the South China Sea. In: *Helminths of animals of southeastern Asia.* Edited by B.I. Lebedev, Y.L. Mamaev, P.G. Oshmarin, Nauka, Moscow, 1970, pp. 191–216.

15. Mamaev Y.L.: The taxonomical composition of the family Microcotylidae Taschenberg, 1879 (Monogenea). Folia Parasitol 1986, 33, 199–206.

16. Mamaev Y.L., Egorova T.P.: A description of *Sebasticotyle ochotense* n. g., n. sp., a new monogenea from scorpion fishes and the erection of Jaliscia n. g. Trudy Biologo-Poshvennogo Instituta. Novaya seriya 1977, 47, 101–105.

17. Mendoza-Franco E.F., Tun M.D.C.R., Anchevida A.J.D., Rodríguez R.E.D.R.: 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, 11, 125–161.

18. Murray F.V.: Gill trematodes from some Australian fishes. Parasitology 1931, 23, 492–506.

19. Randall J.E.: Coastal fishes of Oman. Crawford House Publishing Pty Ltd Bathurst, 1995, pp. 1–439.

20. Rohde K.: Gill Monogenea of deepwater and surface fish in southeastern Australia. Hydrobiologia 1988, 160, 271–283.

21. Shin G.H., Shin Y., Jung M., Hong J.M., Lee S., Subramaniyam S., Noh E.S., Shin E.H., Park E.H., Park J.Y., Kim Y.O., Choi K.M., Nam B.H., Park C.I.: First draft genome for Red Sea bream of family *Sparidae*. Front Genet 2018, 12, 1–5.

22. Strona G., Stefani F., Galli P.: Field preservation of monogenean parasites for molecular and morphological analyses. Parasitol Int 2009, 58, 51–54.

23. Tambireddy N., Gayatri T., Gireesh-Babu P., Pavan-Kumar A.: Molecular characterization and phylogeny of some mazocraeidean monogeneans from carangid fish.Acta Parasitol 2016, 61, 360–368.

24. Taschenberg E.O.: Zur systematik der monogenetischen Trematoden. Zeitschrift Gesammten Naturwissenschaften (Berlin) 1879, 52, 232–265.

25. Tripathi Y.R.: Studies on the parasites of Indian fishes. IV. Trematoda Monogenea, Microcotylidae. Rec Ind Mus Calcutta 1954, 52, 231–248.

26. Unnithan R.V.: On the functional morphology of a new fauna of Monogenoidea on fishes from Trivandrum and environs. Part IV. Microcotylidae *sensu stricto* and its repartition into subsidiary taxa, Am Midl Nat 1971, 85, 366–398.

27. van Beneden P.J., Hesse C.E.: Recherches sur les Bdellodes et les Trématodes marins. Mem Acad B Belg ClSci 1863, 34, 60–146.

28. Villalba C.: Nuevas especies de Monogenea en peces marinos de Chile. Parasitologia 1987, 11, 141–148.

29. Yamada F.H., Acosta A.A., Yamada P.O.F., Scholz T., da Silva R.J.: A new species of *Aphanoblastella* Kritsky, Mendoza-Franco and Scholz, 2000 (Monogenea, Dactylogyridae) parasitic on heptapterid catfish (Siluriformes) in the Neotropical region. Acta Parasitol 2018, 19, 772–780.

30. Yamaguti S.: Systema helminthum. Vol. IV. Monogenea and Aspidocotylea. Interscience Division, John Wiley & Sons, New York 1963, pp. 360–425.

31. Yoon G.H., Al-Jufaili S., Freeman M.A., Bron J.E., Paladini G., Shinn A.P.: *Omanicotyleheterospina* n. gen. et n. comb. (Monogenea: Microcotylidae) from the gills of *Argyropsspinifer* (Forsskål) (Teleostei: Sparidae) from the Sea of Oman. Parasit Vectors 2013, 6, article 170.

32. Zhang J., Wu X., Xie M., Xu X., Li A.: The mitochondrial genome of *Polylabris halichoeres* (Monogenea: Microcotylidae). Mitochondrial DNA 2011, 22, 3–5.