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Light and Scanning Electron Microscopy of *Microcotyle* sp. Nov. (Microcotylidae) from the Gills of Gilt Head Sea Bream *Sparus aurata* (Sparidae) in Egypt

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Abstract: *Microcotyle* sp. nov. was described from the gills of the gilt head sea bream *Sparus aurata* (Sparidae). Fish were collected from boot landing sites and fishermen at different water locations at Hurghada City along the Red Sea, Egypt. The morphology and morphometric characterization of the isolated worms were described by means of light and scanning electron microscopy. Thirty (42.86 %) out 70 specimens of *Sparus aurata* were infected with a *Microcotyle* sp. most of the infected fish had very pale gills and showed symptoms of anemia. The adult worm was described morphologically and measured 3.1±0.3 (2.4-3.6) mm long and 0.30±0.02 (0.27-0.34) mm width. Buccal organs, septate, without spines 0.80±0.02 (0.75-0.83) mm in width, cephalic glands in three groups; pharynx oval, esophagus inconspicuous, cecal bifurcation posterior to genital atrium; ceca penetrating first third of haptor, without posterior confluence. Haptor constituted 30% of body length, it measured 0.50±0.02 (0.46-0.55) mm wide; clamps of *Microcotyle* were typed, (40-46) in number, each once was 0.04±0.02 (0.02-0.06) mm in diameter, size uniform; midsclerite with complementary process complex, trifurcate; two lateral, coplanar branches; central branch longer, not coplanar. The new species were compared with those described previously from the same genus, it was shown that there were significant morphological and morphometric differences especially for the copulatory organ, which was a strong criteria for the placement these monogenean parasites as new species with new host and locality records in Egypt.

Key words: Monogenea · *Microcotyle* Sp. · Microcotylidae · Light Microscopy · SEM

INTRODUCTION

The name "monogenea" means born once and refers to the simple life cycle. Monogenetic trematodes or Gillworms are permanent parasites in the gills, mouths or on the bodies of fishes. Some occur in the nares, pockets in the lateral line or rarely in the gut of fish. Some species occur in the urinary bladder of fishes, frogs or turtles. They generally feed on mucus, epithelial cells or blood. Gillworms are common on fishes in all aquatic environments [1]. More than 1500 species have been described, but this is probably only a small percentage of those existing. Adults range from 30 µm to 20 mm in length and they maybe translucent, cream or pink in color. They are the type of worm parasites most frequently associated with culture problems. They seldom are a direct cause of mortality but frequently contribute to the death of their hosts due to other infectious diseases. The primary impact of monogeneans is reduced growth, stress and increased susceptibility to bacterial and fungal pathogens [1]. Members of Monogenea, Clinical signs include lethargy, anoxia, loss of appetite and scratching. Mucus excess, opacity and even ulcers or haemorrhages may appear. Gill histopathological signs include focal hyperplasia, lamellar fusion, haemorrhages and inflammatory infiltration. Monogeneans are usually very host specific, though in certain culture conditions some species can be found in unusual hosts [2]. Gillworms have a distinct attachment organ on their posterior end called a haptor (or opisthaptor) with hardened anchors or specialized clamps to pierce the epithelium and hold on to the host. Sclerotized marginal hooks often surround the haptor and bars, disks, scales or spines may occur on or near the haptor. The head sometimes has eye spots and specialized holdfast organs. Many monogenean parasites of big game fishes have large suckers or numerous clamps

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adapted for holding on to these fast moving animals. Most reproduce by laying eggs that hatch ciliated larvae (oncomiracidia) and quickly mature and attach to the host. Since no stages on intermediate hosts are necessary, they can multiply rapidly [3]. When intensive culture crowds fish together, most gillworm offspring survive and can quickly begin to kill fishes. Monogenea include two main groups, *Monopisthocotylea,* with a simple adhesive disc and *Polyopisthocotylea*, with a complex adhesive disc including clamps and hooks [4]. Among *Monopisthocotylea* the most significant species for cultured fish are *Ancyrocephalidae*, *Capsalidae*, *Dactylogyridae*, *Diplectanidae*, *Gyrodactyloidea*, *Monocotylidae* and *Furnestinidae*. *Polyopisthocotylea* include several species of pathological concern for fish cultures, most of them belonging to the family Microcotylidae and some to *Axinidae,* Heteraxinidae, *Gastrocotylidae, Heteromicrocotylidae*, *Hexabothriidae, Diclidophoroidae, Discocotylidae, Allopyragraphoridae, Hexostomatidae, Protomicrocotylidae, Chauhaneidae, Gotocotylidae*, *Mazocraeidae,* Anthocotylidae, Chimaericolidae [5]. The present study evaluated the natural prevalence of monogenetic trematodes infection with morphologic and morphometric characters of the recovered species by means of light and scanning electron microscopy.

MATERIALS AND METHODS

A total of 70 fish of Gilt head sea bream *Sparus aurata* (Sparidae) with size 14-28 cm, mean 18.5 ±7.15 cm; body weight 100-250 g, mean 205 ± 20g were caught March to July 2016 from the Red Sea, Hurghada, Egypt The fish were kept alive in aquaria filled with the same water source and examined within few hours. Skin surface, fins and gills were then examined by naked eyes and a dissecting microscope for any parasites, lesions and/or pathologic features. After removing opercula and exposing gill arches, each gill was removed carefully from the fish, immersed in normal saline to remove any excess gill mucus. Monogenean parasites were recovered with a Pasteur pipette using a dissecting binocular microscope. Worms were fixed in 4% formalin for 2 h and then washed with distilled water to remove excess fixative. Worm identification was confirmed by mounting specimens on slides in drops of ammonium picrate glycerine under cover slips and examining hard parts using light microscopy. For permanent whole mount preparation, some of the fixed and flattened specimens were stained with acid carmine followed by washing in ascending alcohol series and then cleared in clove oil, xylene and then mounted with Canada balsam [6]. For each monogenean parasite, the sclerotized parts of the haptor were measured using an ocular micrometer calibrated against a stage micrometer slide according to Gussev and Bykovskaya-Ravlovskay *et al.* [7, 8]. Ten specimens were measured for the range and the mean±standard deviation (SD). Prevalence, mean abundance and measurements followed the guidelines of Bush *et al*. [9]. For SEM, samples were fixed in 4 % glutaralde- hyde in 0.1 M sodium cacodylate buffer (pH 7.4), washed in the same buffer and dehydrated in a graded alcohol series. Samples were then processed in a critical point drier "Bomer-900" with fre- on 13, sputter-coated with gold-palladium in a Technics Hummer V and finally examined with a Jeol scanning electron microscope (Model JSM7610F).

RESULTS

Microcotyle sp.nov. (Figs. 1-9)

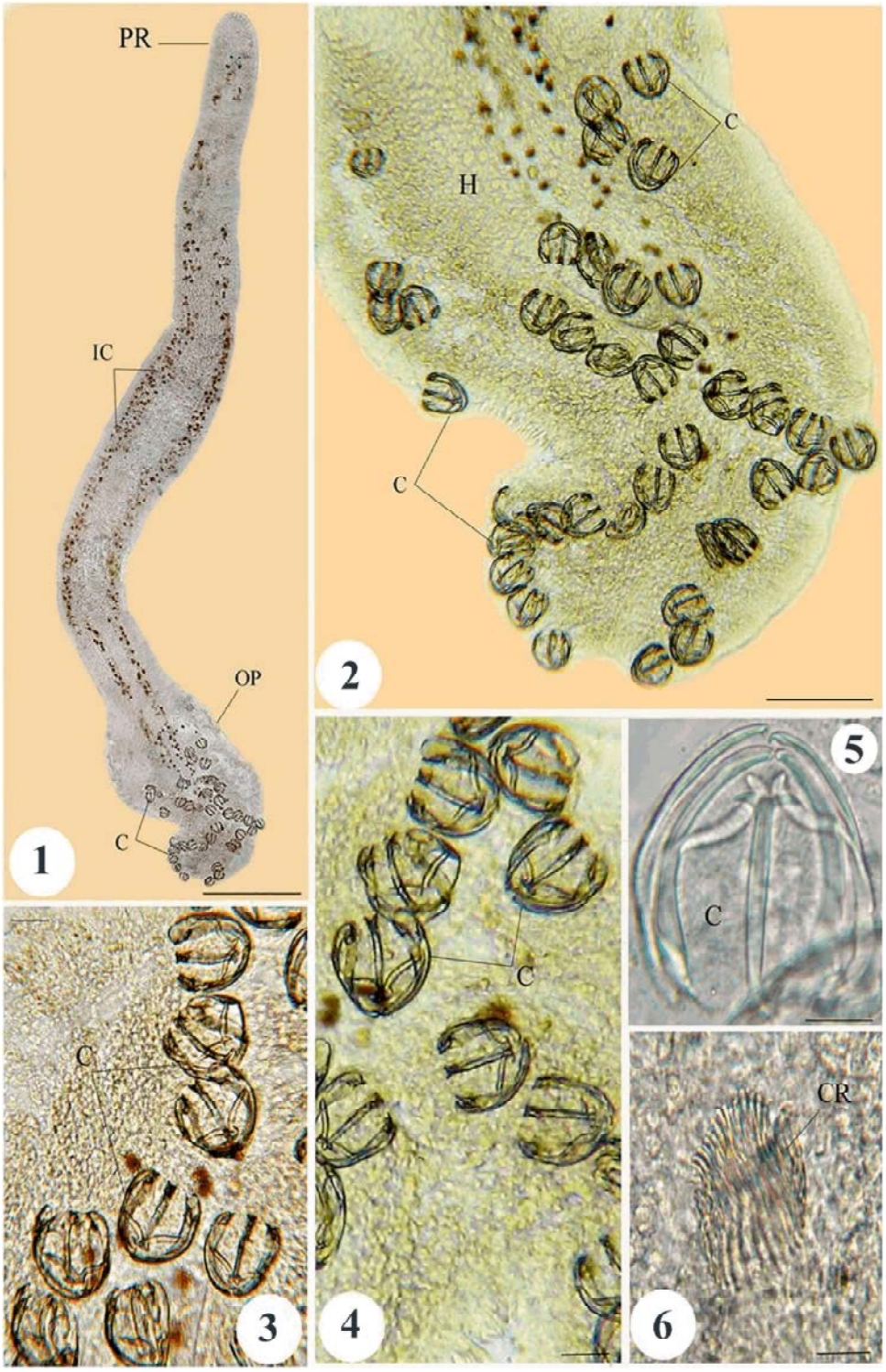
The parasite possessed a body which is elongated, with constriction at level of cecal bifurcation. Body was 3.1±0.3 (2.4- 3.6) mm long and 0.30±0.02 (0.27-0.34) mm of maximum width. Buccal organs, septate, without spines 0.80±0.02 (0.75-0.83) mm in width, cephalic glands in three groups; pharynx oval, esophagus inconspicuous, cecal bifurcation posterior to genital atrium; ceca penetrating first third of haptor, without posterior confluence. Haptor constituted 30% of body length, it measured 0.50±0.02 (0.46-0.55)mm wide; clamps of *microcotyle* were typed, (40-46) in number, each once was 0.04±0.02 (0.02-0.06)mm in diameter, size uniform; midsclerite with complementary process complex, trifurcate; two lateral, coplanar branches; central branch longer, not coplanar. Testes postovarian intercecal, not reached posterior margin of haptor, they were 20-30 (27) in number, genital atrium was kidney-shaped with central elevation. Ovary long, double inverted "U" shaped; oviduct dorsal to genito-intestinal canal; vagina middorsal immediately posterior to genital atrium.

Taxonomic Summary
Family: Microcotylidae

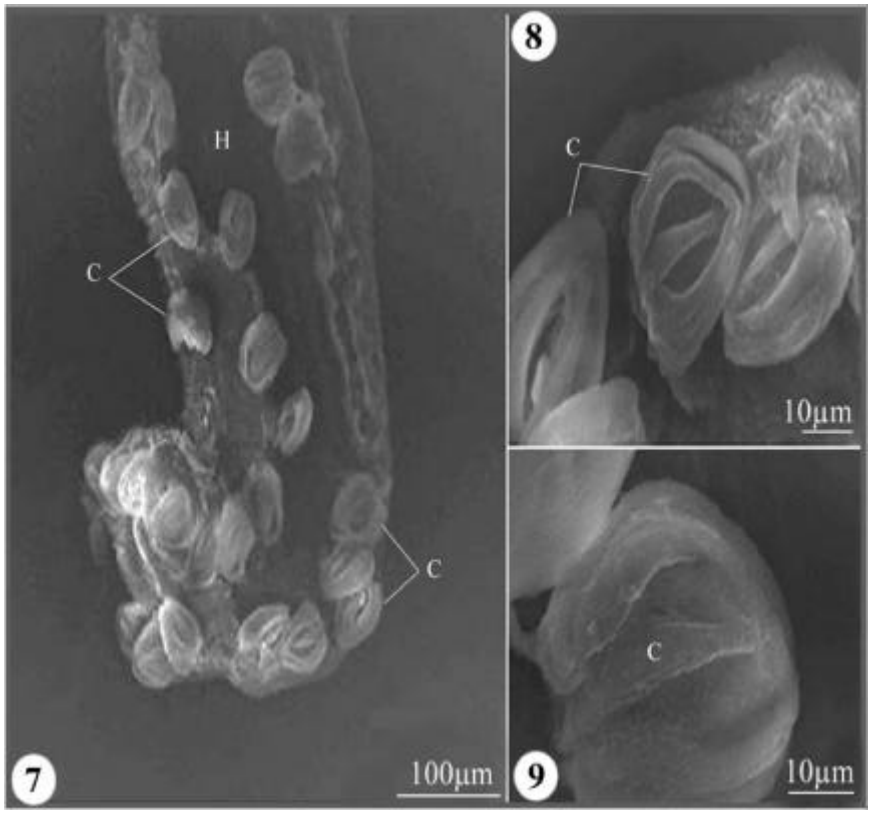
Host: Gilt head sea bream *Sparus aurata* (Family: Sparidae [11])
Infection Site: Gills of infected fish.

Type Locality: Coasts of Hughada along the Red Sea, Egypt.

Prevalence: 30 out of 70 fish with a percentage of (42.86 %) were found to be naturally infected.



Figs. 1-6: Photomicrographs of *Microcotyle* sp.nov. infecting the gills of *Sparus aurata*. 1 The adult worm in a whole mount preparation: the anterior attachment organ or prohaptor (PR) and a posterior haptor (H) which is supplied by numerous clamps (C) were observed. 2-6 High magnifications of: 2 Haptor (H) with clamps (C). 3-5 Clamps (C) and 6 Corona of hooks (CR). (Scale bars, Figs.1: 0.4 mm; 2: 0.1 mm; 3-6: 0.01mm)



Figs. 7-9: Scanning electron micrographs of *Microcotyle* sp.nov. showing the posterior attachment organ or haptor (H) and the arrangement of clamps (C).

DISCUSSION

The genus *Microcotyle* Van Beneden *et al.* [10] was divided into four subgenera *Microcotyle, Bispina, Vaginaespina* and *Aspina.* The species of the genus *Microcotyle* are *Microcotyledonavini* [10] on *Labrus donavini, L. bergyltain* Europe (Atlantic); *M.agonostomi* [12] on *Agonostomus foresteri* in W. Australia; *M.aigoi* [13] on *Siganus fuscescens* in Japa, *M.angelichthys* [14] on *Holacanthus Ciliaris* in N.Y. Aquarium; *M. angelichthys- towsendi* [15] on *Angelichthys -tow sendi* in Woods Hole; *M.archosargi* [14], *Archosargus probatocephalus* in N.Y.Market, also on *A.Oviceps*, Florida [16]; *M.arripis* [12] on *Arripis georgianus* in W.Australia; *M.australiensis* [17] on *Pomatomus saltatrix* in Sydney; *M.branchiostegi* [18] on *Branchiostegus japonicus* in Japan; *M. caballeroi* [19] on *Trachurops crumenophthalmus*, in Puerto Vallara, Mexico; *M.caudata* [20] on *Sebastodes inermis* in Japan; *M.centropristes* [21] on *Centropristes striatus* in New York Market; *M.cepholae* [18] on *Cephola schlegeli* in Japan; *M.chrysophryii* [10] on *Chrysophry aurata* in Mediterranean; *M.constricta* [22] on *Paraperes colias* in Newzealand; *M.ditrematis* [23] on *Ditrema temminchi* in Japan; *M. elegans* [20] on *Scombrops chilodipteroides* in Japan; *M.erythrini* [10] on *Pagellus erythrinus* in Brest, also on *Pagellus acarne*, *Box boops* in Genoa; *M.eueides* [14] on *Roccus Lineatus* in U.S.A, (Atlantic); *M.furcata* [24] on *Tautoga onitis* in Woods Hole; *M.fusiformis* [20] on *Centronotus rubulosus* in Japan; *M.gerres* [12] on *Gerres ovatus* in W.Australia; *M.eimpo* [25] on *Enedrias nebulosus* in Inland sea. Japan; *M.helotes* [26] on *Helotes sexlineatus* in Swan river, W.Australia; *M.hiatulae* [20] on *Hiatulaonitis* in Newport, U.S.A; *M.india* [13] on *Seriola quinaqueradiutus* in Japan; *M.labracis* [10] on *Labrax lupus* in Brest; *M.leiognathi* [27] on *Leiognathus ruconius* in Madras; *M.macroura* [14] on *Roccuslineatus* in N.America (Atlantic); *M.madrasi* [27] on *Pseudosciaena diacanthus* in Madras; *M.mormyri* [28] on *Pagellus mormyrus, Lithognathus mormyrus* in Bay of Nepal; *M.mouwoi* [13] on *Siganus fuscens*; *M.odocis* [28] on *Odax semifasciatus* in W. Australia; *M.otrynteri* [30] on *Otrynter caprinus* in Beaufort; *M. parasillaginae* [12] on *Sillaginoides punctatus* in west Australia;

Table 1: Comparative measurements (mm) of the present *Microcotyle* sp. nov. and those from previously recorded host species

Aspects	*M. arripis* [37]	*M. arripis* [38]	*M. pseudopercis* [39]	*Microcotyle* sp.nov. Present study
Host	*Arripis georgianus*	*Arripis georgianus*	*Pseudopercis numida*	
P. semifasciata	*Sparus aurata*			
Max. body length	4,347	867-2,713	4.11-6.22	3.1±0.3 (2.4- 3.6)
Max. body width	487-568	155-564	365-693	0.30±0.02 (0.27-0.34)
Buccal organ width	41-49	15-36	66-73 L	0.80±0.02 (0.75-0.83)
Opisthohaptor width	_	94-233	_	0.50±0.02 (0.46-0.55)
Clamps diameter	37-46	26-54	37-95	0.04±0.02 (0.02-0.06)
Max. number of clamps	78-96	44-96	51-69	44-60
Copulatory organ length	29-103	21-60	_	0.03±0.02 (0.01-0.05)
Copulatory corona spines	_	_	_	20 (18-22)

M. pentapodi [12] on *Pentapodus millii* in west Australia; *M. peprili* [30] on *Peprilus alepidotus* in Beaufort. N.C; *M. polynemi* [29] on *Polynemus auratus* in Java, also in *P.indicus* and *P.tetradactylum* in India; *M.pomacanthi* [21] on *Pomacanthus arcuatus*, *Choetodon ocellatus*, *Calamus arctifrons, Anisotremus viriginicus*, *Epinephalus flavolimbatus*, *Harpe-rufa* in N.Y aquarium and on *Calamus arctifrons* in Florida; *M.pomatomi* [20] on *Pomatomus saltatrix* in U.S.A (Atlantic) Black sea; *M. poronoti* [21] on *Poronotus tricanthus* in Woods Hole *M.pricanthi* [31] on *Priacanthus* sp in Galapagos Isl; *M.pseudomogilis* [32] on *Muglicephalus* in Florida, Mugilis of [33], *M.salpae* [33] (Parona and Perugia, 1890) on *Box salpa* in Genoa; *M.sargi* [33] on *Sargus rondelettii*, *S.vulgaris*, *S.annularis*, *S.salvani*, *Boxsalpa* in Italy; *M.sciaenae* [20] on *Sciaenae sin* in Japan; *M.scomberomori* [34] on *Scomberomorus maculates* in off port Aransa; *M.sebastis* [20] on *Sebastes* Spp Hokkaido Japan, also *Sebastodes* spp and *Ophiodon elongates* in Pacific coast of N.America; *M. hainanensis* [35] on *Branchiostegus auratus* in South China sea. Table (1) shows the intraspecific variations between the present described species of *Microcotyle* and those of the previous studies. From this comparison, the present species showed a morphological similarity in the general body form to *Microcotyle arripis* [28] and *Microcotyle pseudopercis* [36]. The genital atrium herein was composed of two pads each armed with a circle of spines with hooked tips; spines of similar structure, dissimilar in size. In contrast, the boundary of genital atrium of *Microcotyle arripis* was lined with large spines except posterior edge with spine points faced inwards; inner portion armed with numerous smaller, conical spines with points directed posteriorly; inner spines randomly distributed in some specimens, spaced somewhat evenly in others. *Microcotyle pseudopercis* possessed a genital atrium toke a kidney-shaped with central elevation, with spines. In the present species, clamps were 39 in number and composed of medial sclerite and paired anterolateral, posterolateral and accessory sclerites; medial sclerite bent as a U-shape, with large gun-sightlike dorsal end and flared ventral end forming 2 bilateral spines; anterolateral sclerite sickle shaped, with footlike base, truncate distal end; posterolateral sclerite a simple curved rod with truncate distal end; accessory sclerite was a sigmoid rod with folded medial end. These structures differed from those of *Microcotyle arripis* which has a chitinous skeleton of nine pieces, a central inverted U-shaped piece, one arm of U longer than the other and bifurcated at proximal end; two lateral pairs, relatively slender one pair at distal end of lateral pairs extending obliquely downward and toward center of clamp; one pair across top of clamp somewhat beaded in appearance and convex on distal borders and one small pair across the middle one. *Microcotyle pseudopercis* possessed clamps with midsclerite which was trifurcate with two lateral coplanar branches and non coplanar long central branch. Also, the present described species possessed body measurements which were generally different from the other described species except for both diameter and number of clamps which were more or less similar to the comparable spines. From the above, the present species must be considered as a new species within the genus *Microcotyle* with new host and locality records

CONCLUSION

In the present study, a new species of monogenean parasites was recorded from the gilt head sea bream Sparus aurata (Sparidae) during a general survey on parasites infectin fishes of the Red Sea. The parasite was described morphologically and morphometrically by light and SEM. One must focus for other parasitic fauna infecting fishes.

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