

# SOLOSTAMENIDES IRAQENSIS N. SP. (MONOGENOIDEA, MICROCOTYLIDAE) PARASITIZING THE FRESHWATER MULLET *LIZA ABU* (PISCES, MUGILIDAE) FROM THE TIGRIS RIVER IN IRAQ

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MONOGENOIDEA  
SOLOSTAMENIDES IRAQENSIS  
MUGILIDAE  
TIGRIS RIVER  
IRAQ

**ABSTRACT.** – *Solostamenides iraqensis* n. sp. (Microcotylidae), a monogenean parasite on the gill filaments of the freshwater mullet fish *Liza abu* (Heckel, 1843) in the Tigris River in Iraq, is described. The present specimens assigned to *Solostamenides* because they have a penis head armed with hook-like spines, atrial rim muscular unarmed, with single mid-dorsal vaginal pore. The new species is distinguished from the other four *Solostamenides* species previously recorded, namely, *Solostamenides mugilis* (Vogt, 1878), *S. pseudomugilis* (Hargis, 1956), *S. platyorchis* Zhang & Yang, 2001 and *S. paucitesticulatus* Kritsky & Öktener, 2015. Absence or presence of longitudinal septum in the ventral view of buccal organ, the size, shape and number of clamps, the arrangement, size and number of testis, the size and shape of spines of the copulatory organ, and the absence or presence and shape of the proximal filament in the egg were the main characters for differentiation between the species. All species of *Solostamenides* have been described from gills of grey mullets (Mugilidae), three of them (*S. mugilis*, *S. pseudomugilis*, *S. platyorchis*) occur on *Mugil cephalus* L. in marine habitats, whereas *S. paucitesticulatus* and *S. iraqensis* n. sp. have been found on *L. abu* in freshwater habitats. These data suggest some degree of host specificity of *Solostamenides* spp.

## INTRODUCTION

The Microcotylidae Taschenberg, 1879 is a large family of monogeneans within the subclass Oligonchoinea Bychowsky, 1937. This family includes *Solostamenides*, which was erected by Unnithan (1971) based on the following features: head of penis armed with hook-like spines and atrial rim muscular, unarmed, with single mid-dorsal vaginal pore. At present, this genus comprises four nominal species: *Solostamenides mugilis* (Vogt, 1878) Unnithan, 1971, *S. pseudomugilis* (Hargis, 1956) Unnithan, 1971, *S. platyorchis* Zhang & Yang, 2001 and *S. paucitesticulatus* Kritsky & Öktener, 2015.

During a parasitological survey of specimens of *Liza abu* (Heckel, 1843) (Osteichthyes, Mugilidae) caught in the Tigris River in Iraq, several microcotylids were found on the gill filaments. The study of their morphological and structural traits revealed that they represent a previously undescribed species of *Solostamenides*, which is erected and described herein.

## MATERIALS AND METHODS

Between March and October 2014, 425 specimens of *L. abu* were caught in the Tigris River passing through Salah Al-Deen

Province, between 34°32'N, 43°44'E and 34°44'N, 43°38'E. Specimens were identified according to Coad (2010). Fish were killed by severing the spinal cord and kept frozen until parasitological examination, during which gills were removed, placed in dishes with distilled water and checked for parasites using a dissecting microscope. Monogeneans were collected, fixed in 70 % ethanol, stained with acetocarmine, differentiated with HCl in 70 % ethanol, dehydrated through a graded ethanol series, cleared with dimethyl phthalate and mounted in di-n-butyl phthalate in xylene (DPX media). For studying and drawing the sclerotized structures, some specimens were cleaned after fixation in 200-300 µl TE9 Buffer (10 mM Tris-HCl, 10 mM EDTA, 125 mM NaCl, PH = 8) for 15-30 minutes. They were subsequently transferred into 200-300 µl of lysis buffer (Proteinase K, 100 µg/ml with TE9 Buffer), incubated at 50 °C for 15-30 minutes, refrigerated to inactivate the Proteinase K, re-cleaned with TE9 Buffer, and finally mounted in jelly-glycerin. Monogeneans were examined under a Leica DMR microscope with phase contrast (at 100× to 1000×) and drawn with the aid of a drawing tube on a Nikon compound microscope. Diagnostic morphometric measurements were obtained using LAS v. 4.3.0. [Build: 600] Leica Microsystems. All measurements are given in micrometers (µm) as average, followed by the range and sample size (n) in parentheses. For comparative purposes, 13 paratypes of *S. paucitesticulatus* Kritsky & Öktener, 2015, USNM 1273675, 1-13, were examined.

**RESULTS**

***Solostamenides iraqensis* n. sp.**

*Type-host:* *Liza abu* (Heckel, 1843) Mugilidae.

*Site on host:* Gill filaments.

*Type-locality and date:* Tigris River at Salah Al-Deen Province, between 34°32'N, 43°44'E and 34°44'N, 43°38'E, Iraq; March 23, 2014.

*Prevalence:* 6.4 % (27 of 425 fish examined).

*Mean intensity:* 2 parasites per infected fish (intensity range: 1-7).

*Type-specimens:* Holotype (NHMUK 2016.1.25.1) and four paratypes (NHMUK 2016.1.25.2-5) have been deposited in the Natural History Museum, London, UK; additional voucher specimens can be requested to the authors.

*Etymology:* The specific name refers to the geographic area where this monogenean was found (Iraq).

**Description (Fig. 1A-K)**

Body fusiform, elongate, total length 2,923 (2,240-3,796; 14); trunk 2,444 (1,913-3,177; 15); maximum width at level of ovary 616 (392-837; 17). Body passes

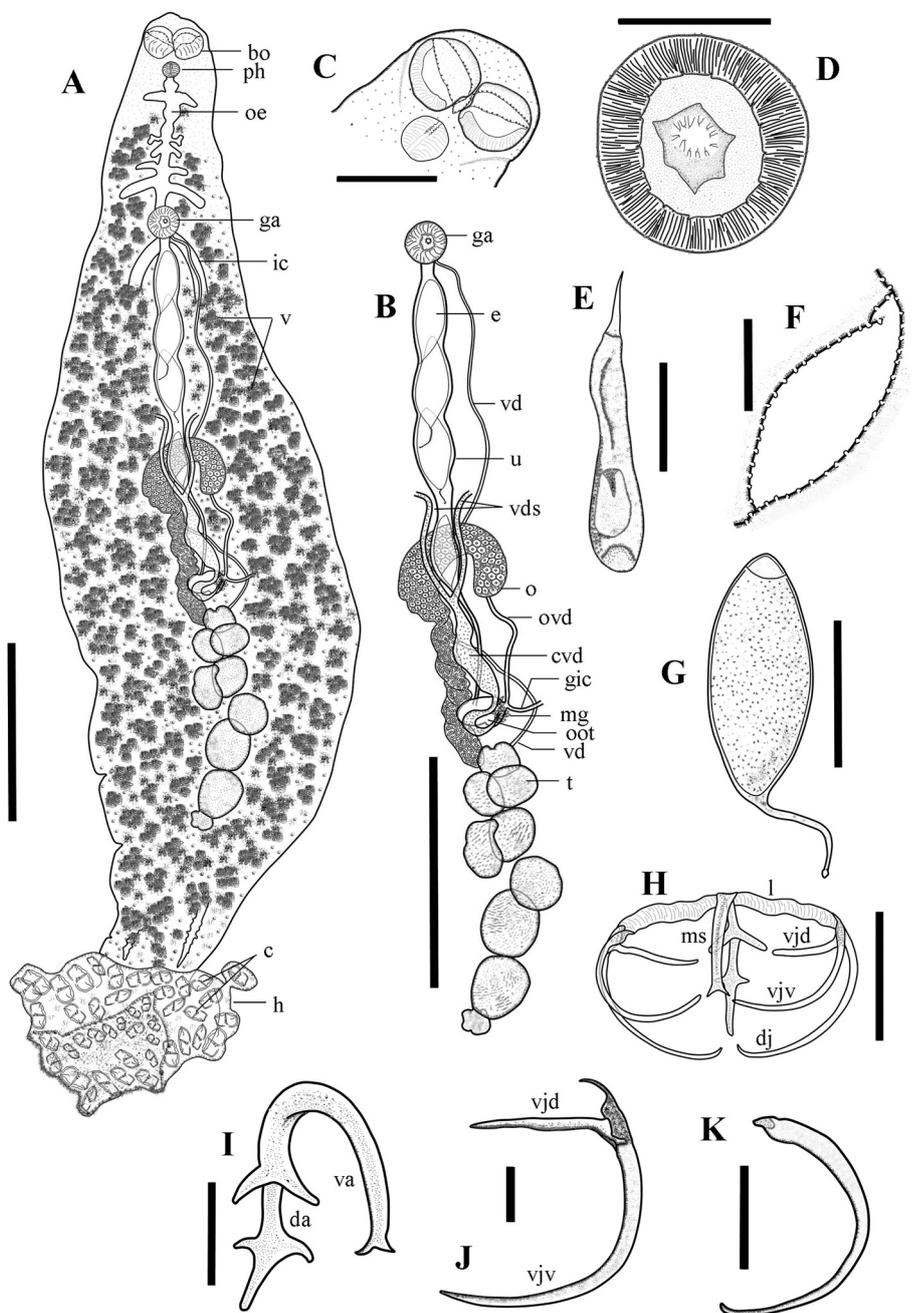


Fig. 1. – *Solostamenides iraqensis* n. sp. from the gills of the abu mullet *Liza abu* in the Tigris River, Iraq. **A:** Whole mount, ventral view; **B:** Male and female reproductive systems, ventral view; **C:** Anterior part of body, ventral view; **D:** Genital atrium and male copulatory organ, ventral view; **E:** Spine of male copulatory organ, dorso-lateral view; **F:** Vaginal pore; **G:** Egg; **H:** Haptoral clamp, ventral view; **I:** Median sclerite of haptoral clamp; **J:** Ventral jaw of haptoral clamp; **K:** Dorsal jaw of haptoral clamp. Abbreviations: bo, buccal organ; c, clamp; cvd, common vitelloduct; da, dorsal arm; dj, dorsal jaw; e, egg; ga, genital atrium; gic, genitointestinal canal; h, haptor; ic, intestinal caeca; l, ligament; mg, Mehlis gland; ms, median sclerite; o, ovary; oe, oesophagus; oot, ootype; ovd, oviduct; ph, pharynx; t, testis; u, uterus; v, vitellaria; va, ventral arm; vd, vas deferens; vds, vitelloducts; vj, ventral jaw; vjd, dorsal transverse sclerite of ventral jaw; vdv, ventral sclerite of ventral jaw. Scale bars (µm): A, B = 500; C, G = 100; D = 50; E = 7; F, J = 10; H = 40; I, K = 20.

smoothly into sub-symmetrical haptor, which is clearly differentiated from body proper, moderately tapering posteriorly. Tegument smooth, sometimes with moderately large curved parts in contracted worms. Lateral margins of anterior part of haptor well developed in its anterior part. Haptor moderately short, 612 (413-914; 14) long, consisting of two unequal parts, anterior short part and posterior long part. Anterior part and posterior part occupy 0.28 and 0.72, respectively of total length of haptor. Haptor armed with two parallel, subequal, rows of 40 (37-43; 9) ventro-lateral clamps. Clamps dissimilar in size, those in middle of haptor largest than anterior and posterior ones (measurements in Table I). Clamps similar in shape, with structure typical of Microcotylidae. Clamp bilaterally symmetrical with single median sclerite, dorsal and ventral clamp jaws. Base of clamp with thin, narrow, heavily sclerotized hinge ligament on each side, connecting median sclerite with region of articulation of jaw sclerites. Median sclerite narrow, with long ventral and short dorsal arms. Both arms V-shaped distally, with branches conspicuously larger in dorsal arm. The latter joins distally a cross-shaped sclerite projection. Dorsal and ventral jaw sclerites bilaterally symmetrical. Ventral jaw on each side consisting of dorsal, short transverse sclerite articulated with long, ventral, C-shaped sclerite, whereas dorsal jaw consists of one long C-shaped sclerite. Dorsal sclerite of ventral jaw reaching level of bifurcation of dorsal arm of median sclerite; ventral sclerite of ventral jaw reaching bifurcation of ventral arm of median sclerite; whereas dorsal jaw reaches level of cross-shaped sclerite projec-

tion of dorsal arm of median sclerite. Ventral jaw on each side articulating with dorsal jaw at base of ligament.

Anterior region of body contains paired buccal organs, pharynx, oesophagus, vagina and genital atrium. Buccal organ elliptical to subcircular; surrounded by minute papillae; 72 (61-89; 25) long, 69 (61-85; 25) wide. Mouth subterminal, ventral, opening within haptoral cavity. Buccal cavity leading to a muscular subcircular pharynx 56 (46-70; 14) long, 49 (35-68; 14) wide. Oesophagus with 4-5 pairs of lateral branches, bifurcated into two intestinal caeca immediately anterior to genital atrium in most specimens (13 out of 16), or slightly posterior to genital atrium (3 out of 16 specimens). Distance from anterior end of body to pharynx 144 (65-203; 16); to intestinal bifurcation 384 (256-510; 16); to anterior margin of genital atrium 404 (269-530; 16). Distance between posterior margin of pharynx and intestinal bifurcation 240 (110-345; 16). Intestinal caeca obscured by vitelline follicles; subequal in length, extending into peduncle before haptor. Vitellaria irregular, distributed throughout body, extending from oesophagus to mid-way of haptoral peduncle. Pigment granules dispersed throughout body, extending posteriorly to haptor region, concentrate at regions occupied by vitellaria.

Genital atrium subcircular, unarmed, 80 (67-95; 15) × 72 (60-84; 15), with well-developed radial musculature. Male copulatory organ muscular; head armed with crown of 11 (10-14; 14) spines subequal in shape, arranged in single ring. Spines 20 (19-21; 6) long, having straight (to moderately curved, depending of status of copulatory

Table I. – Morphometric comparison between *Solostamenides iraqensis* n. sp. and *Solostamenides paucitesticulatus*. Measurements in  $\mu\text{m}$ .

Character	<i>Solostamenides iraqensis</i> n. sp. (present study)	<i>Solostamenides paucitesticulatus</i> (Kritsky & Ökter, 2015)
Body length	2923 (2240-3796)	2500 (1360-3770)
Buccal organs	Undivided	Divided with longitudinal septum
Haptoral lappet and anchors	Absent	Absent
Haptoral clamps:		
Number	37-43	31-47
Size: Anterior	43 (38-46) × 81 (56-95)	99 (88-118) Wide
Median	50 (46-58) × 88 (71-97)	–
Posterior	34 (27-39) × 49 (43-64)	59 (52-72) Wide
Dorsal sclerite projection of median sclerite	Cross-shaped	Y-shaped
Ventral long arm of median sclerite	V-shaped termination	T-shaped termination
Dorsal jaw	Regular C-shaped curvature	Irregular C-shaped curvature forming protuberant angle
Ventral jaw	Regular C-shaped curvature	Regular C-shaped curvature
Testes:		
Number	7-10	5-9
Size:	Unequal	Subequal, 94 (59-170) wide
Anterior	51 (43-59)	
Central	78 (62-98)	
Posterior	33 (26-39)	
Spine of male copulatory organ:		
Number	10-14	11-14
Length	20 (19-21)	13 (11-14)
Shape and structure	Long straight blade with an ovoid bulbous base and being hooked distally	Curved blade and bulbous base
Egg filament	Present with bulbiform proximal end	Present with flared proximal end

organ) blade, with ovoid bulbous bases and hooked distal points.

Testes mostly spheroid; generally arranged in two intermittent rows; situated in postovarian, intercaecal field occupying posterior half of body and not extending to haptoral peduncle. Distance from anterior end of body to testicular area 1,462 (1,122-1,677; 16); length of testicular area 442 (287-639; 17); distance from posterior end of testicular area to haptor 426 (174-560; 17). Number and measurements of testes provided in Table I. Vas efferens not observed; vas deferens conspicuous, moderately coiled anteriorly; extending forward from testicular area along body midline to open in genital atrium. In some specimens, vas deferens runs left to longitudinal axis in its anterior part before ovary.

Ovary long, tubular, question-mark shaped, in pre-testicular field, intercaecal, dorsal to vitelline ducts and uterus; originating on right side of body as coiled germinal ovarian branch (germarium), extending anteriorly before traversing vitelline ducts leftwards, then looping and directing posteriorly as mature ovarian part. Ovary located at 1,041 (764-1,413; 16) from anterior margin of body. Distance from top of ovary (anterior looping) to end of germarium 455 (300-644; 16). Ovary 771 (620-1,182; 16) long, 72 (39-125; 16) wide in mature part, 72 (41-

125; 17) wide in germinal part. Oviduct, long, tubular, arising from mature ovarian branch, running posteriorly, joining genito-intestinal canal before turning towards smooth-walled swelling ootype, surrounded in their proximal portion by small fusiform cells forming shell gland (Mehlis' gland). Uterus wide, 1,062 (905-1,368; 16) long, with 2 (1-4; 17) eggs, arising from ootype anteriorly and running antero-medially as uncoiled tube, opening into genital atrium. Two vitelloducts run posteriorly and join at level of mature ovarian part to form large median common vitelloduct that opens into oviduct near opening of ootype. Two vitelloducts with common vitelloduct represent Y-shaped dense structure. Vaginal canal not observed. Vaginal pore single, spindle-shaped, with tinny papillae at their edges, middorsal, slightly posterior to level of genital atrium. Eggs (in uterus) fusiform, operculated, 181 (162-196; 17) long, excluding filament, 75 (64-93; 14) wide, with single short posterior filament; 96 (72-116; 13) long, having bulbiform proximal end.

#### Remarks

Specimens with characters of Microcotylidae as per Mamaev (1986). They can be ascribed to *Solostamenides*, as originally defined by Unnithan (1971) and recognized

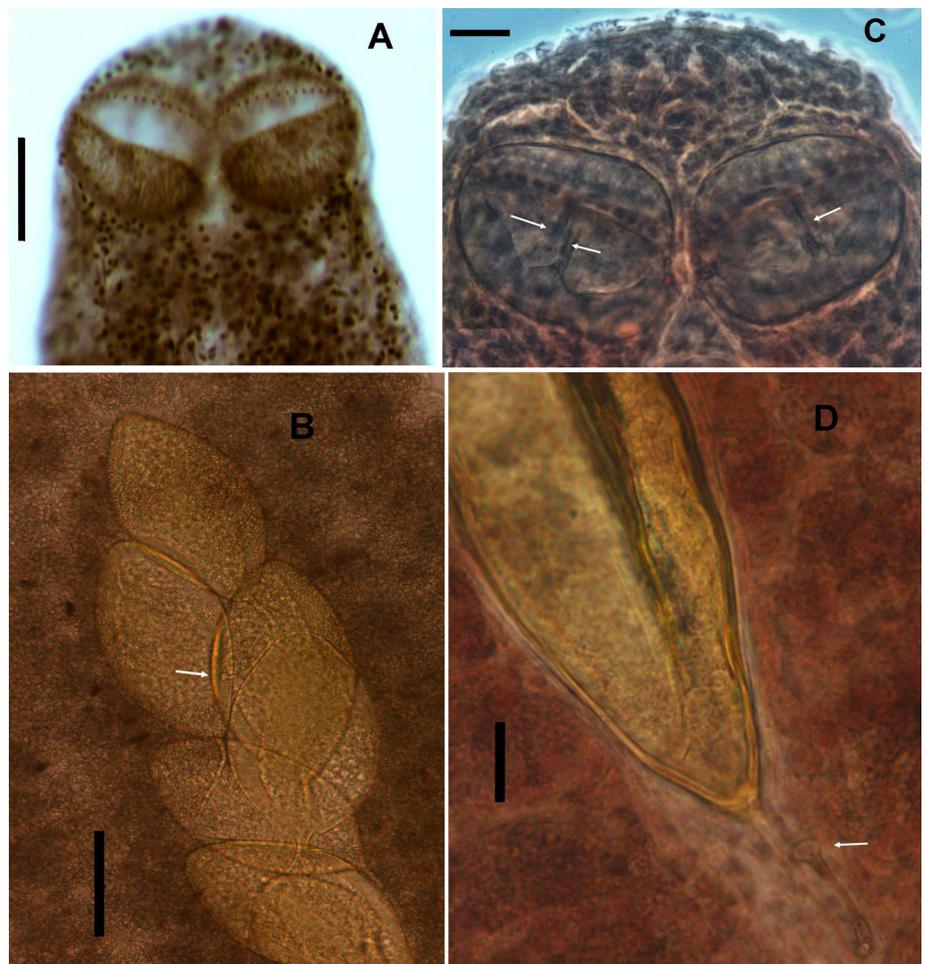


Fig. 2.— Anterior region (ventral view) and ovum of *Solostamenides iraqensis* n. sp. (A, B respectively) and *S. paucitesticulatus* paratype (C, D respectively). Arrows in C indicate the septum in the buccal organs; arrows in B and D indicate the bulbiform proximal end of filament in *S. iraqensis* n. sp. and flared proximal end in *S. paucitesticulatus*. Scale bars ( $\mu\text{m}$ ): A = 50; B = 100; C, D = 20.

by Mamaev (1986), because they have a penis head armed with hook-like spines, atrial rim muscular unarmed, with single mid-dorsal vaginal pore.

Currently, *Solostamenides* contains four valid species: *S. mugilis*, *S. pseudomugilis*, *S. platyorchis* and *S. paucitesticulatus*. *Solostamenides iraqensis* n. sp. most closely resembles *S. paucitesticulatus* described by Kritsky & Öktener (2015) from *L. abu* in the Atatürk Reservoir, Euphrates River, Turkey. The new species is quite similar to *S. paucitesticulatus* in the general shape of the genital atrium, shape of the egg and in the arrangement of the genital organs. In addition, the number of haptor clamps, testes and spines on copulatory organ in *S. iraqensis* n. sp. is subsimilar to those of *S. paucitesticulatus* (Table I). However, comparison with the paratypes of *S. paucitesticulatus* and the illustration in Kritsky & Öktener (2015) revealed several differences with *S. iraqensis* n. sp. Whereas the buccal organs of *S. paucitesticulatus* exhibits in its ventral view a longitudinal septum (Fig. 2C), that of *S. iraqensis* n. sp. is undivided (Fig. 2A). The testes are unequal in size and are smaller in *S. iraqensis* n. sp. (Fig. 1B), whereas they are subequal in *S. paucitesticulatus*. In addition, those situated anteriorly and posteriorly in *S. iraqensis* n. sp. are substantially smaller than those of *S. paucitesticulatus* (Table I). The spines of the male copulatory organ are longer in *S. iraqensis* n. sp., exhibiting a long straight blade with an ovoid bulbous base and being hooked distally (Fig. 1E). In contrast, the spines of *S. paucitesticulatus* are shorter and exhibit a curved blade and bulbous base (Table I). The egg filament exhibits a bulbiform proximal end in *S. iraqensis* n. sp. (Fig. 2B), whereas the proximal end of the egg filament is flared in *S. paucitesticulatus* (Fig. 2D). The clamps of *S. iraqensis* n. sp. are somewhat smaller (Fig. 3A-D, Table I) and the distal sclerite articulating with the dorsal short arm of the median sclerite of the clamp is cross-shaped (Fig. 3A-C), whereas it is Y-shaped in *S. paucitesticulatus* (Fig. 3E-F). The ventral long arm of the median sclerite of the clamp has a V-shaped termination in *S. iraqensis* (Fig. 3D) whereas it is T-shaped in *S. paucitesticulatus* (Fig. 3H). The curvature of the dorsal jaw of the clamp of *S. paucitesticulatus* is irregular forming a protuberant angle (Fig. 3F-H), whereas in *S. iraqensis* n. sp. the curvature is smooth forming a C-shaped sclerite without apparent protuberances (Fig. 3A-D).

*S. iraqensis* n. sp. can be readily distinguished from the other three species of the genus. Although it resembles *S. platyorchis* in the general shape of the genital atrium and in the spines on the copulatory organ, *S. iraqensis* n. sp. can be clearly differentiated by possessing an egg with proximal filament, which is absent in *S. platyorchis*. Moreover, it differs by having fewer testes (7-10 in *S. iraqensis* n. sp., vs. 16-22 in *S. platyorchis*) and fewer spines on the copulatory organ (10-14 in *S. iraqensis* n. sp., vs. 15-17 in *S. platyorchis*) (see Zhang & Yang 2001).

*Solostamenides iraqensis* n. sp. clearly differs from *S. mugilis* and *S. pseudomugilis* in having fewer clamps (37-43), testes (7-10) and spines (10-14) on the copulatory organ, (60-80, 83-113, 25-28 in *S. mugilis*; 72-78, 65-66, 16-20 in *S. pseudomugilis*; 56-62, 60-69, 16-19 in *S. pseudomugilis sensu* Williams 1991, respectively) (see Euzet & Combes 1969, Hargis 1956, Williams 1991, respectively). In addition, *S. iraqensis* n. sp. differs from *S. pseudomugilis sensu* Williams (1991) and from *S. mugilis* (see Euzet & Combes 1969) in having a cross-shaped projection sclerite articulating distally with the dorsal arm of the median sclerite, whereas it is V-shaped in *S. pseudomugilis* and Y-shaped in *S. mugilis*. In addition, the dorsal jaw of the clamps of *S. pseudomugilis* differs from those of *S. iraqensis* n. sp. in having an irregular curvature forming a protuberant angle as shown in Williams (1991). This irregular shape of curvature may represent a feature to discriminate species of microcotylids, because it is observed in a fraction of species. For example, it appears in the clamps of *Microcotyle arripis* but not in those of *Microcotyle helotes* (as shown in Williams 1991). Therefore, this feature needs to be further evaluated in future studies.

In the light of these morphological differences, we conclude that our specimens belong to a new species, *S. iraqensis* n. sp., which represent the fifth described species of *Solostamenides*.

## DISCUSSION

To date *Solostamenides* included four nominal species: *S. mugilis*, *S. pseudomugilis*, *S. platyorchis* and *S. paucitesticulatus*. *Solostamenides mugilis* was originally described as *Microcotyle mugilis* from *Mugil cephalus* L. by Vogt (1878). The species was later redescribed by Euzet & Combes (1969) and eventually transferred to *Solostamenides* by Unnithan (1971). This author designated *S. mugilis* as the type species of the genus. Unnithan (1971) also transferred *Microcotyle pseudomugilis* to *Solostamenides*, which was described originally by Hargis (1956) from *M. cephalus* in the Western Atlantic. Williams (1991) redescribed *S. pseudomugilis* from *M. cephalus* in Western Australia. According to Hargis (1956), *S. pseudomugilis* can be distinguished from *S. mugilis* on the basis of the copulatory spines occurring in the genital atrium and not on the "cirrus", since the copulatory spines occur on the "cirrus" and not in the genital atrium in the *S. mugilis* described by Vogt (1878). Thus Hargis (1956) suggested that *S. mugilis* of Parona & Perugia (1890) (originally described as *Microcotyle mugilis* by these authors) is synonymous with *S. pseudomugilis* based on this distinction. However, due to the presence of spines in the head of penis, Unnithan (1971) considered *S. pseudomugilis* of Hargis (1956) identical to *S. mugilis* of Parona and Perugia (1890). In addition,

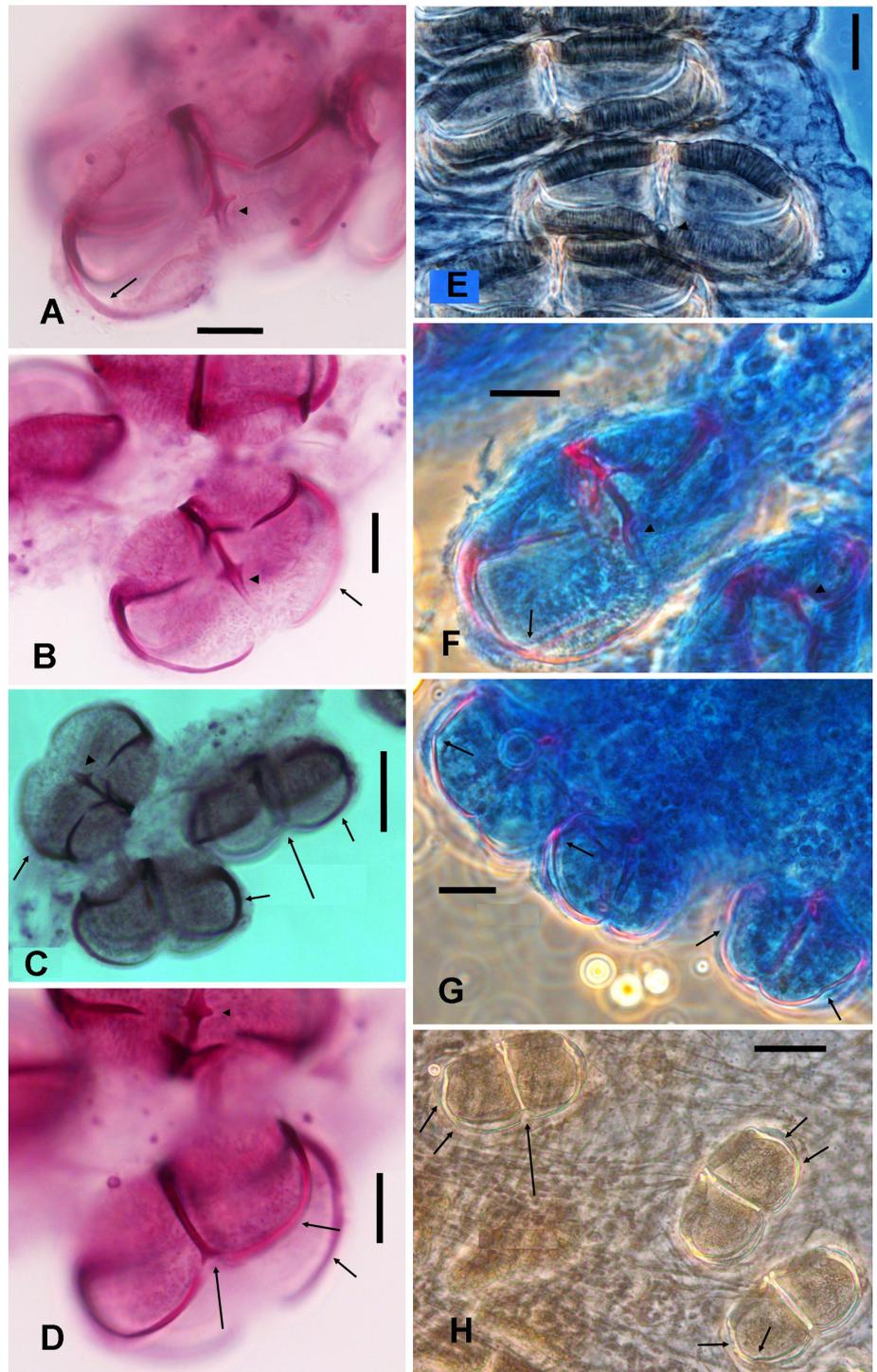


Fig. 3.— Clamps of *Solostamenides iraqensis* n. sp. (A–D) and *S. paucitesticulatus* paratype (E–H); showing: cross-shaped of the distal sclerite of median sclerite in *S. iraqensis* n. sp. (pointed arrow ◄ in A, B, C) and Y-shaped in *S. paucitesticulatus* (pointed arrow ◄ in E, F), V-shaped termination of the ventral long arm of the median sclerite in *S. iraqensis* n. sp. (long arrow in C, D) and T-shaped termination in *S. paucitesticulatus* (long arrow in H), smooth C-shaped sclerite of dorsal and ventral jaws of clamp in *S. iraqensis* (short arrow in A, B, C, D) and irregular curvature of dorsal jaw forming a protuberant angle in *S. paucitesticulatus* (short arrow in F, G, H). Scale bars ( $\mu\text{m}$ ): A–G = 20; H = 50.

Williams (1991) reported specimens of *S. pseudomugilis* having a male muscular copulatory organ with a crown of spines. Therefore, the differentiation between *S. mugilis* and *S. pseudomugilis* described from different regions of the world is confused, which calls for a re-examination of the type specimens of these forms in order to re-define their diagnosis characters.

*Solostamenides platyorchis* was described from *M. cephalus* in the South China Sea by Zhang & Yang

(2001) and was ascribed to *Solostamenides* by having spines on the copulatory organ. In addition, Kritsky & Ökter (2015) indicated that *S. paucitesticulatus* also possesses spines on the copulatory organ. Accordingly, the circle of spines around the male copulatory organ is a primarily characteristic for identification of *Solostamenides* spp.

In the original description of *S. pseudomugilis*, Hargis (1956) indicated that the haptor hooks and haptor

“lappet” were absent in the specimens. By contrast, Williams (1991) observed haptor hooks in his specimens of *S. pseudomugilis*. Therefore, there has been some confusion about the identification of this species using the presence or absence of haptor hooks. Kritsky & Öktener (2015) suggested that the Australian species of *S. pseudomugilis* were either misidentified or possibly represented a new species of *Solostamenides*. Zhang & Yang (2001) also reported the absence of haptor hooks in their specimens of *S. platyorchis*, but they suggested that this was probably attributable to curling of the posterior region of haptor. Nevertheless, the haptor hooks and haptor “lappet” are absent in *S. paucitesticulatus* (Kritsky & Öktener 2015) and in *S. iraqensis* n. sp. The later data reflect the confused results about the presence or absence of this haptor hooks and the ambiguity in its role in the identification of *Solostamenides* spp.

However, the presence and absence of haptor hooks and the haptor “lappet” probably depend on the age of the worm. The hooks are observed in the larval stages of Microcotylidae and apparently fall off as the clamps develop in the adult stage (see Repullés-Albelda *et al.* 2011). Likewise, Mamaev (1986) indicated that, in Microcotylidae, the hooks are situated on the posterior apex of the haptor and they can be observed in the youngest specimens, whereas they are usually absent in adult worms. Therefore, studies about the ontological changes in haptor hooks of *Solostamenides* are needed to clarify the discrepancies about this character.

All species of *Solostamenides* have been recorded in grey mullets (Mugilidae). Three of them (*S. mugilis*, *S. pseudomugilis*, *S. platyorchis*) occur in *M. cephalus* in marine habitats, whereas *S. paucitesticulatus* and *S. iraqensis* n. sp. have been found on *L. abu* in freshwater habitats. This suggests some degree of host specificity of *Solostamenides* spp. Further parasitological surveys of grey mullets in marine, brackish and freshwater bodies in different geographic areas may reveal additional species of the genus and would improve our understanding of the range and specificity of these monogeneans.

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