

## FOUR NEW SPECIES OF *LIGOPHORUS* (MONOGENEA: DACTYLOGYRIDAE) PARASITIC ON *MUGIL LIZA* (ACTINOPTERYGII: MUGILIDAE) FROM GUANDU RIVER, SOUTHEASTERN BRAZIL

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**ABSTRACT:** Four species of *Ligophorus* (Monogenea: Dactylogyridae), i.e., *L. tainhae* n. sp., *L. brasiliensis* n. sp., *L. guanduensis* n. sp., and *L. lizae* n. sp., are described. The specimens were collected from the gills of *Mugil liza* (Mugilidae) from the Guandu River (22°48'32"S, 43°37'35"W), State of Rio de Janeiro, Brazil, between January 2008 and March 2008. The male copulatory organ of *L. tainhae* n. sp. differs from the all known species of this genus in having the largest accessory piece, the length of accessory piece exceeding the length of the copulatory organ tube, and the distal tip of the lower lobe crossing the upper lobe. *Ligophorus brasiliensis* n. sp. and *L. guanduensis* n. sp. have a similar shape of the accessory piece, but in *L. guanduensis* n. sp. the lower lobe is larger than the upper lobe (as opposed to *L. brasiliensis* n. sp.), the ratio between length of upper lobe and the length of the proximal part of the accessory piece before the bifurcation is shorter and the distal tip of the lower lobe extends to the level of the upper lobe (in *L. brasiliensis* n. sp. the distal tip of lower lobe crossing the upper lobe). In *L. lizae* n. sp., the terminal bifurcations of the accessory piece are equal in length and unequal in the other 3 new species. Species of *Ligophorus* are recorded for the first time from Brazil.

*Ligophorus* Euzet & Suriano, 1977 comprises 29 species parasitic on mugilid fishes. The currently known species of *Ligophorus* have been recorded from an area restricted to north Atlantic, the Mediterranean Basin, off the coasts of northwestern Pacific, and the Pacific coast of South America (Bychowsky, 1949; Euzet and Suriano, 1977; Zhang and Ji, 1981; Euzet and Sanfilippo, 1983; Fernández, 1987; Wu et al., 1991; Hu and Li, 1992; Dmitrieva et al., 1996; Pan, 1999; Yang, 2001; Miroshnichenko and Maltsev, 2004; Sarabeev and Balbuena, 2004; Sarabeev et al., 2005; Rubtsova et al., 2006, 2007; Dmitrieva et al., 2007). According to Dmitrieva et al. (2007), this area is spatially smaller than the natural distribution of the mugilid hosts. Because all mugilids studied to date have been infected with more than 1 species of *Ligophorus*, it is natural to assume that this genus is far more diverse than currently described. Therefore, investigations of other geographical regions and mugilid species should further increase provide additional information regarding species diversity in this genus. Here, we described four 4 new species of *Ligophorus* of *Mugil liza* Valenciennes, 1836 from the Guandu River, State of Rio de Janeiro, Brazil.

### MATERIALS AND METHODS

Fifteen specimens of *M. liza* were collected from the Guandu river (22°48'32"S, 43°37'35"W), State of Rio de Janeiro, Brazil, between January 2008 and March 2008. The fish measured 37.3 (29.0–48.0) cm in standard length. The monogeneans were removed from the gills of the hosts and placed in vials containing a 1:4,000 (0.025%) solution of formalin. After 1 hr, the vials were shaken and additional formalin was added to increase the concentration to 5%. Some parasites were stained with Gomori's trichrome and mounted in Canada balsam; other specimens were mounted in Gray and Wess' medium (Humason, 1979) for study of sclerotized structures.

Sclerotized structures were measured by 25 characters as defined Euzet and Suriano (1977) and Rubtsova et al. (2007). Dimensions of soft internal organs and body represent their largest measurement. The following abbreviations for the characters are used throughout the text: BL, body length; BW, body width; VAA, ventral anchor total length; VAB, ventral anchor main part length; VAC, ventral anchor outer root length; VAD, ventral anchor inner root length; VAE, ventral anchor point length; VAF,

ventral anchor shaft length; DAA, dorsal anchor total length; DAB, dorsal anchor main part length; DAC, dorsal anchor outer root length; DAD, dorsal anchor inner root length; DAE, dorsal anchor point length; DAF, dorsal anchor shaft length; UTL, uncinulus total length; USHL, uncinulus shaft length; USL, uncinulus sickle length; VBL, ventral bar length; VBAP, distance between membranous anterior processes (protuberances) of ventral bar; DBL, dorsal bar length; PAPL, penis accessory piece total length; PAPUL, penis accessory piece distal portion of upper lobe length; PAPUW, penis accessory piece distal portion of upper lobe width; PAPSHL, penis accessory piece upper lobe shaft length; PAPLL, penis accessory piece lower lobe length; PL, total length of penis; VL, vagina length; PhL, pharynx length; PhW, pharynx width; TL, testis length; TW, testis width; OL, ovary length; OW, ovary width; HL, haptor length; HW, haptor width.

Measurements are given in micrometers as mean  $\pm$  standard deviation, with the range in brackets and the number of specimens measured for each character in parentheses. The illustrations were made with the aid of a drawing tube mounted on a Hund Wetzlar H-600 phase contrast microscope (magnification  $\times 10\times 20$  for the body and  $\times 10\times 100$  [under immersion oil] for sclerotized structures and internal organs). Measurements were made with the use of software Motic Images Plus<sup>®</sup> 2.0. Photographs of the sclerotized structures were made using an Olympus BX-51 phase contrast microscope. Type specimens are deposited in the Helminthological Collection of the Institute Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

### RESULTS

#### DESCRIPTIONS

##### *Ligophorus tainhae* n. sp.

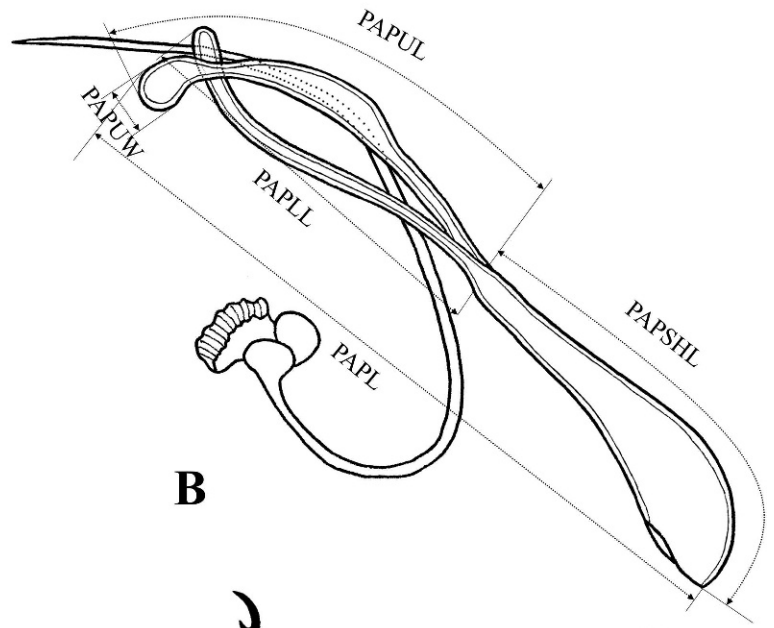
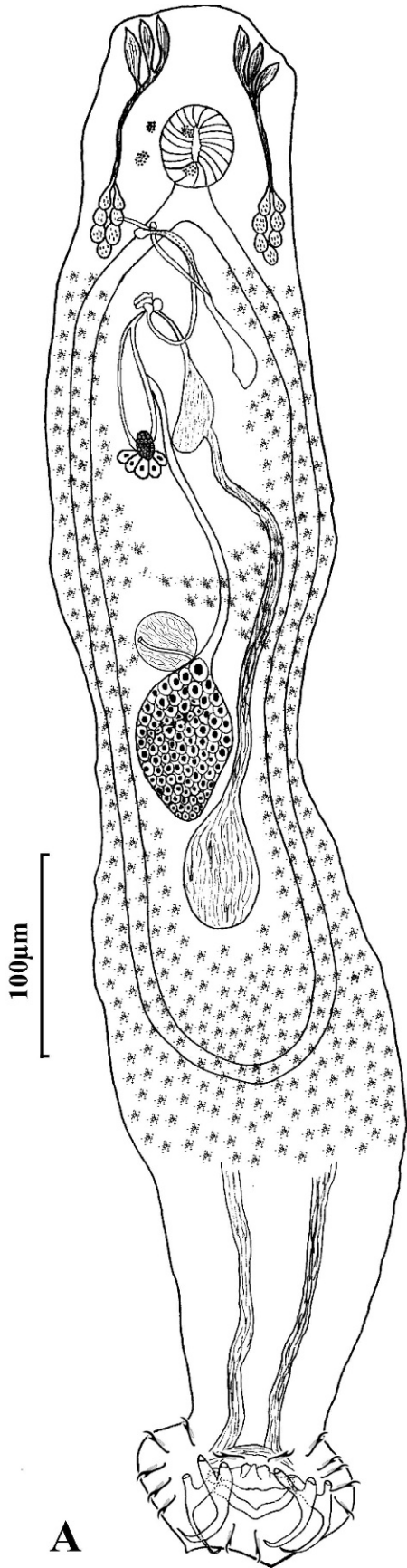
(Figs. 1, 2A–C)

*Diagnosis* (based on 20 whole mounts; morphometric measurements of sclerotized structures are presented on Table I): Body 748.2  $\pm$  87.8 [620–810] (20) long; maximum width 127.9  $\pm$  18.2 [105–145] (20). Cephalic region moderate; 1 terminal and 2 bilateral cephalic lobes; cephalic glands indistinct, posterolateral to pharynx. Each eye with conspicuous lens. Pharynx sub-spherical 42.3  $\pm$  1.7 [40–45] (10) long, 39.6  $\pm$  0.6 [38–41] (10) wide. Testis 56.5  $\pm$  7.6 [43–70] (5) long, 48.4  $\pm$  2.8 [30–56] (5) wide, ovate to pyriform; seminal vesicle tapered, posteromedial to penis; prostatic reservoir pyriform. Male copulatory organ consists of tubular penis with well-developed heel, ratio between length of tube and total length of accessory piece = 1.25  $\pm$  0.02 [1.23–1.26] (10), ratio between length of upper lobe and length of proximal part of accessory piece before bifurcation = 0.9  $\pm$  0.01 [0.8–1.0] (10). Lower lobe elongate, bowed, concave, along upper

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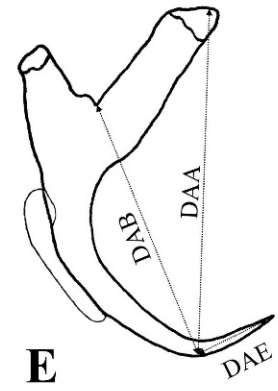
**B**



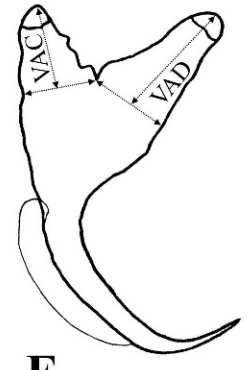
**C**



**D**

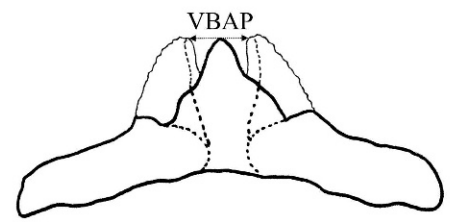


**E**

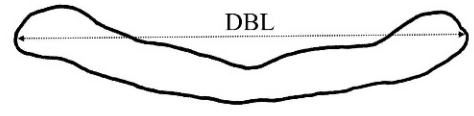


**F**

20µm



**G**



**H**

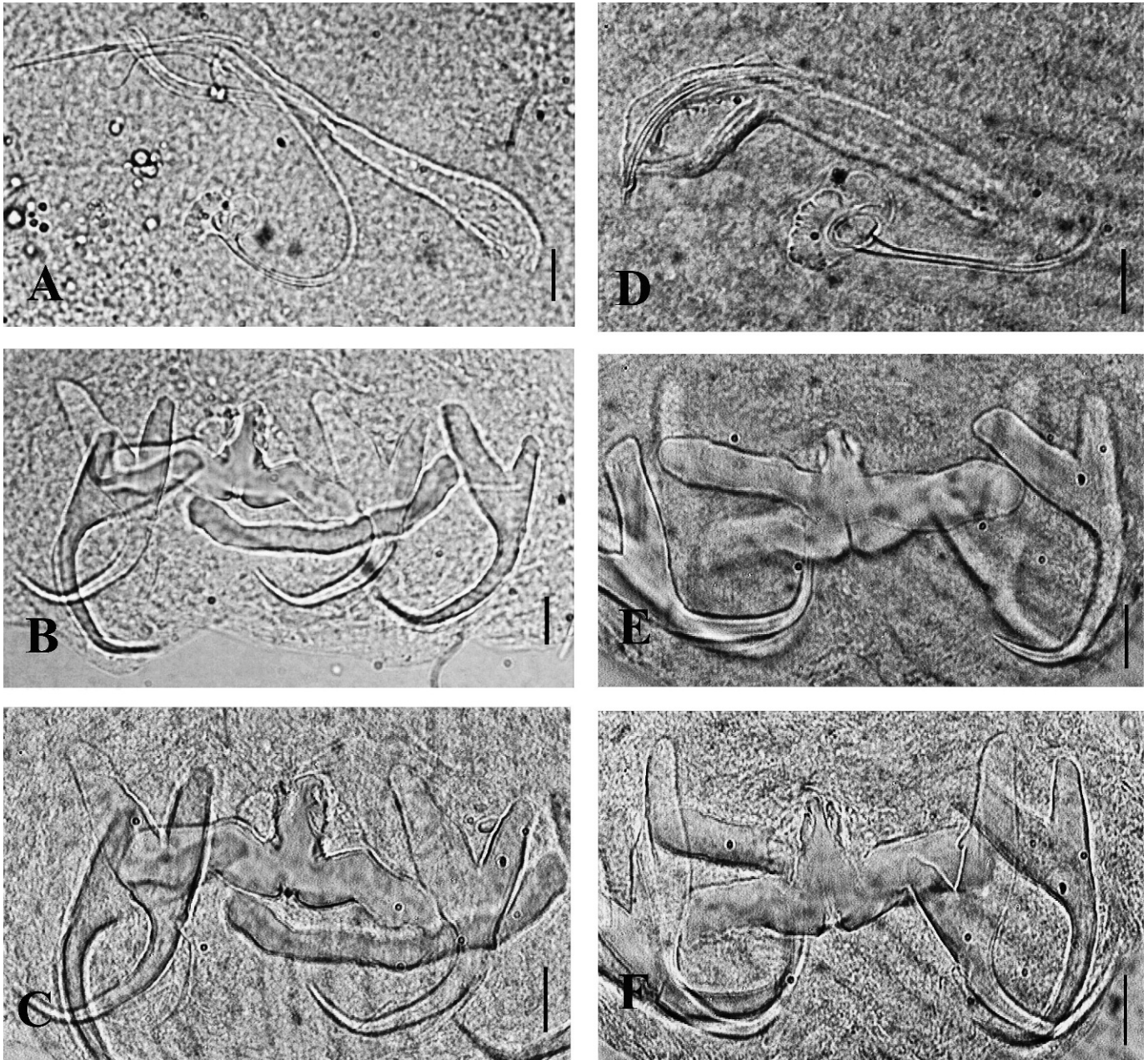


FIGURE 2. Photomicrographs of sclerotized structures of haptor and male copulatory complex of *Ligophorus tainhae* n. sp. (A–C) and *Ligophorus brasiliensis* n. sp. (D–F). A, D. Male copulatory complex. B, E. Dorsal bar and anchors. C, F. Ventral bar and anchors. Bar = 10  $\mu$ m.

lobe, shorter than latter, distal tip of lower lobe crossing upper lobe. Upper lobe tubular, thin walled, with notch at distal part, ratio between penis accessory piece distal portion of upper lobe length and penis accessory piece lower lobe length =  $1.08 \pm 0.02$  [1.06–1.1] (10). Ovary  $80.7 \pm 8.1$  [70–91] (10) long,  $60.5 \pm 8.4$  [50–72] (10) wide, U-shaped. Vaginal aperture midventral, funnel shaped; vaginal canal long, thin, convoluted to straight, sclerotized, leading to ovate seminal receptacle. Vitelline follicles dense. Peduncle broad, long, tapering posteriorly; haptor sub-

hexagonal  $69.5 \pm 11.5$  [56–84] (15) long,  $110.9 \pm 14.1$  [94–129] (15) wide, armed with 14 uncinuli, 2 pairs of anchors, 2 transverse bars. Ventral anchor with inner root longer and heavier than outer root (VAD/VAC =  $1.8 \pm 0.1$  [1.6–2.2] [15]); point and outer root subequal in length; arched blade bent at distal third. Base markedly thicker than blade, separated by notch; filament present. Ventral anchors connected by transverse ventral bar. Ventral bar slightly bowed with 2 membranous protuberances closely located medially, distance between 2 membranous process

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FIGURE 1. *Ligophorus tainhae* n. sp. (A) Whole worm, ventral view. (B) Male copulatory complex: penis and accessory piece. (C) Hook. (D) Vaginal armament. (E) Dorsal anchor. (F) Ventral anchor. (G) Ventral bar. (H) Dorsal bar. See Materials and Methods for abbreviations of measurements.

TABLE I. Comparison of sclerotized metrical characters of 4 new species of *Ligophorus* from Guandu River, Brazil. See Materials and Methods for abbreviations of metric variables.

Characters	Species															
	<i>Ligophorus tainhae</i> n. sp.				<i>Ligophorus brasiliensis</i> n. sp.				<i>Ligophorus guanduensis</i> n. sp.				<i>Ligophorus lizae</i> n. sp.			
	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N
VAA	43	1.4	41.2-44.5	15	44.4	0.3	44.1-44.8	12	44.3	1.5	42.7-46.6	13	48.7	0.6	48-49.3	14
VAB	34.6	2.1	32.8-37.2	15	29.4	0.7	28.7-30.2	12	34.2	0.6	33.6-35.2	13	34.3	1.5	32.7-35.7	14
VAC	9.8	1.8	7.7-11.5	15	9.1	0.5	8.7-9.6	12	12.1	1.1	10.4-13.3	13	7.2	1	6.2-8.2	14
VAD	17.9	1.9	15.5-19.8	15	24.9	0.2	24.7-25.1	12	18.2	1.2	16.2-19.1	13	21.8	0.6	21.1-22.4	14
VAE	10.6	0.2	10.3-10.8	15	13.4	0.3	13-13.9	12	10.9	0.6	10.3-11.7	13	13.4	0.1	13.3-13.6	14
VAF	24.9	0.7	24.3-25.8	15	21.3	1	20.1-22.4	12	23.7	1.9	22.1-26.4	13	22.5	0.8	21.6-23.1	14
DAA	44.1	1.2	42.9-45.6	15	41.2	0.5	40.7-41.8	12	42.1	0.5	41.4-42.6	13	40.6	11.6	27.3-48	14
DAB	36.2	0.8	35.2-37.2	15	31.5	0.3	31-31.8	12	30.8	0.6	30.1-31.7	13	37.3	0.9	36.3-38.1	14
DAC	6.9	0.2	6.7-7.2	15	10	0.5	9.5-10.5	12	10.5	0.4	10.1-11.1	13	10.6	0.7	9.9-11.3	14
DAD	17.3	1.3	15.2-18.2	15	17.2	0.7	16.5-18	12	18.5	2.2	15.7-21.2	13	18.7	1.1	17.5-19.7	14
DAE	10.4	0.4	10-11	15	12.3	0.4	12-12.8	12	10.2	0.5	9.4-10.7	13	11.4	0.2	11.2-11.7	14
DAF	26.2	0.7	25.6-27.3	15	23.9	0.3	23.5-24.3	12	22.7	0.8	21.9-23.7	13	27.2	0.9	26.1-28	14
UTL	15.1	0.4	14.7-15.7	15	13.7	0.7	12.9-14.5	12	14.1	0.6	13.3-14.8	13	14.5	0.3	14.2-14.8	14
USHL	9.1	0.2	8.9-9.5	15	7.3	0.3	7-7.6	12	8.4	0.5	8-9.3	13	8.7	0.9	7.9-9.8	14
USL	6.1	0.4	5.6-6.7	15	6.6	0.2	6.3-6.9	12	5.7	0.3	5.3-6.1	13	6.1	0.1	6-6.3	14
VBL	53.6	2.6	50.4-56.2	15	55.6	1	54.3-56.8	12	56.4	1.1	55.2-57.8	13	54.4	0.8	53.6-55.2	14
VBAP	5.1	0.2	4.9-5.3	15	2.7	0.2	2.5-2.9	12	5.5	0.2	5.3-5.7	13	5.5	0.3	5.1-5.7	14
DBL	55.2	1.8	53.4-57.7	15	60.3	0.5	59.8-60.9	12	59.2	3.6	56-63.2	13	66.6	0.6	65.9-67.2	14
PAPL	101.8	0.9	100.7-102.9	12	54.8	0.3	54.4-55.2	10	60.1	3.3	55.8-63.6	10	48.5	0.3	48.2-48.9	11
PAPUL	49.1	1.1	47.7-50	12	30.5	0.4	30-31	10	26.3	0.7	25.2-27.1	10	0	0	0-0	11
PAPUW	7.8	0.3	7.3-8.1	12	4.6	0.2	4.5-4.8	10	7.4	0.3	6.9-7.9	10	4.4	0.2	4.2-4.6	11
PAPSHL	54.2	1.5	52.4-55.7	12	39.7	0.4	39.3-40.3	10	38.4	0.6	37.7-39.4	10	0	0	0-0	11
PAPLL	45.4	0.6	44.9-46.3	12	21.1	0.4	20.6-21.6	10	26.9	1	25.8-28.2	10	0	0	0-0	11
PL	127.4	0.8	126.6-128.3	12	116.4	0.4	116-117	10	127.1	1.1	125.9-128.8	10	121.4	0.7	120.9-122.2	11
VL	29.1	0.7	28.2-30	11	41.6	0.2	41.3-41.9	10	66.7	9.4	57.2-77.3	12	52.5	1.3	51.2-53.9	9

=  $5.1 \pm 0.2$  [4.9–5.3], sclerotized median process with upper border conical between them. Dorsal anchor similar to ventral, but with total length slightly longer and larger ratio between inner and outer roots (DAD/DAC =  $2.5 \pm 0.1$  [2.3–2.7] [15]). Dorsal transversal bar bowed, connects dorsal anchors. All 14 uncinuli similar in shape and size with straight shaft and sickle. Filament loop close to distal third of shaft.

### Taxonomic summary

*Type host:* *Mugil liza* Valenciennes, 1836, Liza.

*Site of infection:* Gill lamellae.

*Type locality:* Guandu River (22°48'32"S, 43°37'35"W), State of Rio de Janeiro, Brazil.

*Specimens deposited:* Holotype CHIOC 37179a, Paratypes CHIOC 37179b-d.

*Etymology:* The specific name *tainhae* refers to Brazilian vernacular name (tainha) of mugilid fishes.

### Remarks

The male copulatory organ of *Ligophorus tainhae* n. sp. differs from those of all known species of this genus, in having the largest accessory piece, with the length of the accessory piece exceeding the length of the copulatory organ tube and the distal tip of the lower lobe crossing the upper lobe. Among the 29 species of *Ligophorus*, *L. domnichi* Rubtsova et al., 2007, which parasitizes *Mugil cephalus*, seems the most similar to *L. tainhae* in the shape of both the copulatory organ and the haptoral structures. In addition to the above-mentioned characteristics, the latter can be distinguished by the following: greater length of the main part (VAB, DAB), greater length of the shaft of both anchors, greater lengths of both bars, greater total length of the penis accessory, greater length of the distal portion of the upper lobe of the penis accessory, greater width of the upper lobe of the distal portion of the penis accessory, greater length of the upper lobe shaft of the penis accessory piece, and greater lower lobe length of the penis accessory. *Ligophorus pacificus* Rubtsova et al., 2007, *L. cephalis* Rubtsova et al., 2006, *L. pilengas* Sarabeev & Balbuena, 2004, *L. gussevi* Miroshchichenko & Maltsev, 2004, and *L. chenzhenensis* Hu & Li, 1992, have accessory pieces shaped with unequal distal bifurcations, and a long shaft of the upper lobe resembling that of *L. tainhae*. The latter differs from all these species in the larger main part length and the shaft length of both anchors (VAB, DAB) and both bars. Additionally, in *L. pacificus* and *L. cephalis*, the upper lobe of the accessory piece is longer than the lower lobe, and expanded. Moreover, in *L. pilengas*, *L. gussevi*, and *L. chenzhenensis*, there is a shorter distance between membranous anterior processes. The new species also differs greatly from other species in the shape and size of both the haptoral structure and the copulatory organ.

### *Ligophorus brasiliensis* n. sp.

(Figs. 2D–F, 3A–G)

*Diagnosis (based on 15 whole mounts; morphometric measurements of sclerotized structures are presented on Table I):* Body  $710.9 \pm 117.9$  [699–854] (15) long; maximum width  $140.6 \pm 31.8$  [110–185] (15) at level of gonads. One terminal and 2 bilateral cephalic lobes. Each eye with lens. Pharynx subspherical  $38.9 \pm 5.1$  [33–45] (12) long,  $35.6 \pm 5.4$  [31–42] (12) wide. Testis  $50.2 \pm 4.1$  [45–

55] (5) long,  $35.0 \pm 3.5$  [30–40] (5) wide. Male copulatory organ consists of tubular penis with well-developed heel, opening on top of distal part of accessory piece, ratio between length of tube and total length of accessory piece =  $2.12 \pm 0.02$  [2.10–2.14] (10), ratio between length of upper lobe and length of proximal part of accessory piece before bifurcation =  $0.77 \pm 0.01$  [0.75–0.78] (10). Lower lobe moderately elongate, bowed, concave, along upper lobe, shorter than latter, distal tip of lower lobe bifurcate, crossing upper lobe. Upper lobe tubular, thin walled, with triangular expansion at proximal part, ratio between penis accessory piece distal portion of upper lobe length and penis accessory piece lower lobe length =  $1.45 \pm 0.02$  [1.43–1.47] (10). Ovary  $53.5 \pm 8.9$  [43–65] (8) long,  $38.2 \pm 6.1$  [34–48] (8) wide, U-shaped. Vagina long, thin, winding, sclerotized, leading to ovate seminal receptacle. Vitelline follicles dense. Peduncle broad, long, tapering posteriorly; haptor sub-hexagonal  $67.0 \pm 5.1$  [59–71] (12) long,  $95.5 \pm 13.8$  [82–116] (12) wide armed, with 14 uncinuli, 2 pairs of anchors, 2 transverse bars. Ventral anchor with inner root longer and heavier than outer root (VAD/VAC =  $2.7 \pm 0.1$  [2.6–3.0] [12]); point longer than outer root; arched blade bent at middle. Base markedly thicker than blade, separated by notch; filament present. Ventral anchors connected by transverse ventral bar. Ventral bar straight with 2 membranous protuberances closely located medially, distance between 2 membranous process =  $2.7 \pm 0.2$  [2.5–2.9], sclerotized median process with upper border conical between them. Dorsal anchor similar to ventral, but with total length smaller, much smaller ratio between inner and outer roots (DAD/DAC =  $1.7 \pm 0.07$  [1.6–1.8] [12]) and different in shape of blade, which bent at distal half. Dorsal transversal bar slightly bowed, connects dorsal anchors. All 14 uncinuli similar in shape and size with straight shaft and sickle. Filament loop close to distal third of shaft.

### Taxonomic summary

*Type host:* *Mugil liza* Valenciennes, 1836, Liza.

*Site of infection:* Gill lamellae.

*Type locality:* Guandu River (22°48'32"S, 43°37'35"W), State of Rio de Janeiro, Brazil.

*Specimens deposited:* Holotype CHIOC 37180a, Paratypes CHIOC 37180b-d.

*Etymology:* The specific name *brasiliensis* refers to the country that includes the type-locality.

### Remarks

Among the 29 species of *Ligophorus*, *L. cheleus* Rubtsova et al., 2007, which parasitizes *Mugil cephalus*, seems to be the most similar to *L. brasiliensis* n. sp. in the shape of both the copulatory organ and the haptoral structures. The latter can be distinguished by the larger total length of the ventral anchor, the length of the ventral anchor point, the length of the main part of the dorsal anchor (DAB), the length of both bars, the total length of the penis accessory piece, the length of the distal portion of the upper lobe of the penis accessory piece, the length of the upper lobe of the penis accessory piece, the length of the lower lobe of the penis accessory, and the shape of the ventral bar. *Ligophorus angustus* Euzet and Suriano, 1977 and *L. szidat* Euzet and Suriano, 1977 have an accessory piece with practically equal distal bifurcations resembling that of *L. brasiliensis*. The latter differs from these species in its greater total ventral anchor length, greater length of the outer and inner root of the ventral anchor, greater length of

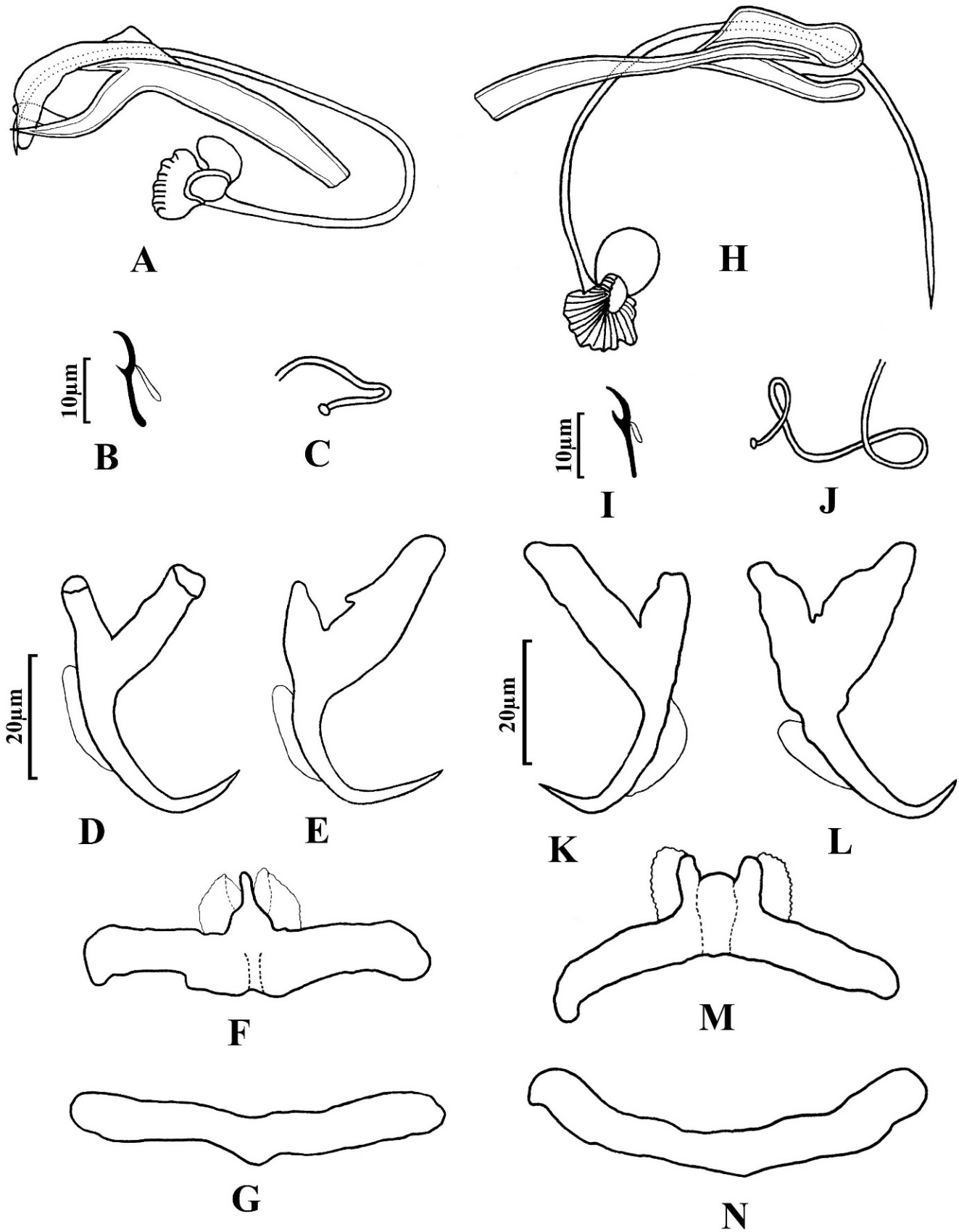


FIGURE 3. Haptor and genital sclerotized structures of *Ligophorus brasiliensis* n. sp. (A–G) and *Ligophorus guanduensis* n. sp. (H–N). A, H. Male copulatory complex: penis and accessory piece. B, I. Uncinulus. C, J. Vaginal armament. D, K. Dorsal anchors. E, L. Ventral anchors. F, M. Ventral bars. G, N. Dorsal bars.

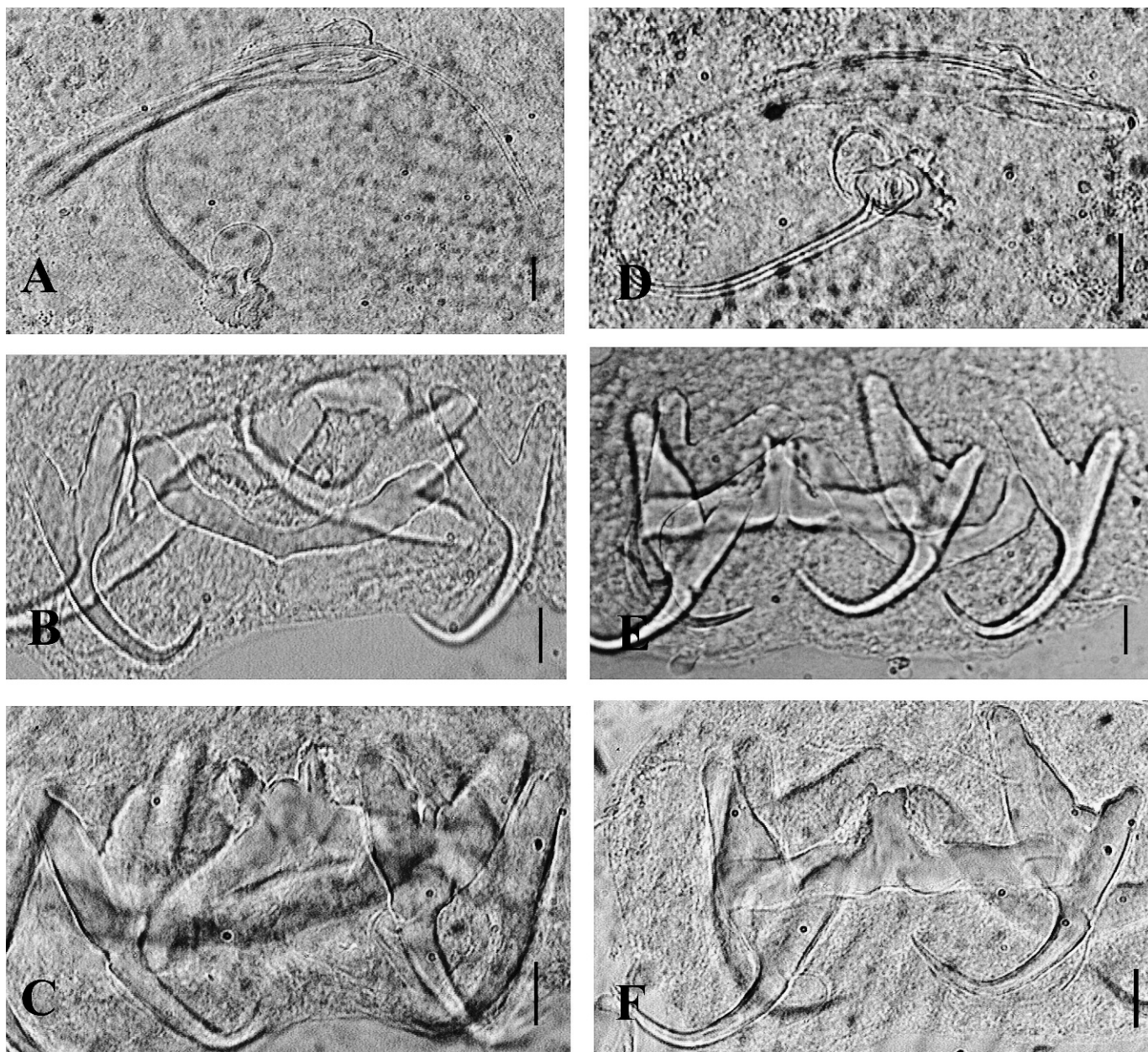


FIGURE 4. Sclerotized structures of haptor and male copulatory complex of *Ligophorus guanduensis* n. sp. (A–C) and *Ligophorus lizae* n. sp. (D–F). A, D. Male copulatory complex. B, E. Dorsal bar and anchors. C, F. Ventral bar and anchors. Bar = 10  $\mu$ m.

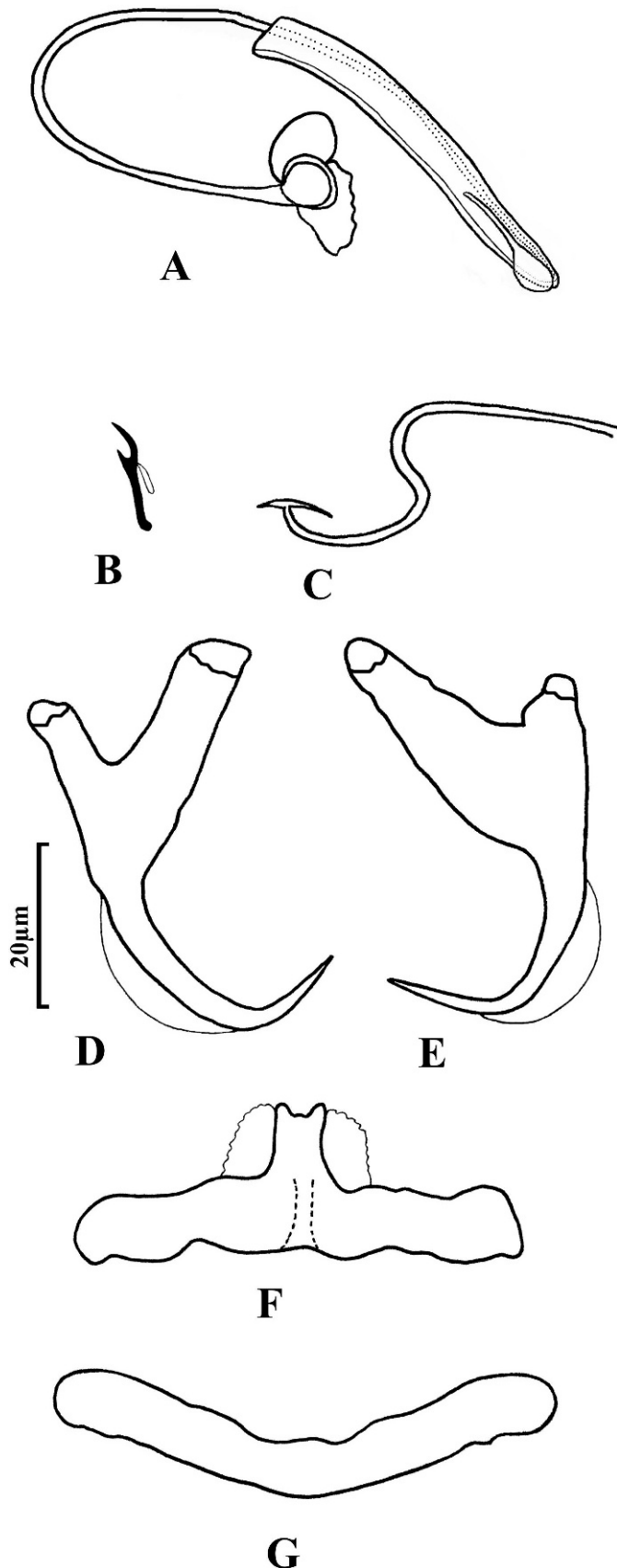
the outer root of the dorsal anchor, greater length of both bars, greater total length of the penis and vagina, and in the shape of the ventral bar. *Ligophorus brasiliensis* differs greatly from the remaining species in the shape and size of both the haptoral structure and the copulatory organ.

***Ligophorus guanduensis* n. sp.**

(Figs. 3H–N, 4A–C)

*Diagnosis* (based on 15 whole mounts; morphometric measurements of sclerotized structures are presented on Table I): Body 662.8  $\pm$  157.7 [500–840] (15) long; maximum width 133.9  $\pm$  23.9 [114–168] (15) at gonads level. One terminal and 2 bilateral cephalic lobes. Each eye with lens. Pharynx spherical 27.5  $\pm$  4.2

[25–35] (10) long, 27.6  $\pm$  3.9 [20–30] (10) wide. Testis 47.2  $\pm$  4.7 [40–51] (5) long, 34.0  $\pm$  2.8 [29–37] (5) wide. Male copulatory organ consists of tubular penis with well-developed heel, ratio between length of tube and total length of accessory piece = 2.11  $\pm$  0.01 [2.09–2.13] (10), ratio between length of upper lobe and length of proximal part of accessory piece before bifurcation = 0.68  $\pm$  0.01 [0.67–0.69] (10). Lower lobe moderately elongate, bowed, concave along upper lobe, larger than latter, distal tip of lower lobe extending to level of upper lobe. Upper lobe tubular, thin walled, ratio between penis accessory piece distal portion of upper lobe length and penis accessory piece lower lobe length = 0.98  $\pm$  0.01 [0.97–0.99] (10). Ovary 50.1  $\pm$  5.4 [42–55] (6) long, 34.6  $\pm$  2.5 [30–38] (6) wide, U-shaped. Vagina long, thin, winding or coiled, sclerotized, leading to ovate seminal receptacle. Vitelline



follicles dense. Peduncle broad, long, tapering posteriorly; haptor sub-hexagonal  $63.2 \pm 4.7$  [58–71] (13) long,  $116.2 \pm 6.2$  [111–127] (13) wide armed, with 14 uncinuli, 2 pairs of anchors, 2 transverse bars. Ventral anchor with inner root longer and heavier than outer root (VAD/VAC =  $1.5 \pm 0.05$  [1.3–1.6] [13]); arched blade bent at distal third. Base markedly thicker than blade, separated by notch; filament present. Ventral anchors connected by transverse ventral bar. Ventral bar bowed with 2 membranous protuberances, distance between 2 membranous process =  $5.5 \pm 0.2$  [5.5–5.7], 2 sclerotized anterior processes widely spread apart. Dorsal anchor similar to ventral, but with total length smaller and with larger ratio between inner and outer roots (DAD/DAC =  $1.8 \pm 0.2$  [1.5–2.0] [13]). Dorsal transversal bar bowed, connects dorsal anchors. All 14 uncinuli similar in shape and size with straight shaft and sickle. Filament loop close to proximal third of shaft.

#### Taxonomic summary

*Type host:* *Mugil liza* Valenciennes, 1836, Liza.

*Site of infection:* Gill lamellae.

*Type locality:* Guandu River (22°48′32″S, 43°37′35″W), State of Rio de Janeiro, Brazil.

*Specimens deposited:* Holotype CHIOC 37181a, Paratypes CHIOC 37181b-d.

*Etymology:* The specific name *guanduensis* refers to the type locality.

#### Remarks

*Ligophorus cephalis* Rubtsova et al., 2006, which parasitizes *Mugil cephalus*, seems the most similar to *L. guanduensis* n. sp. in the shape of both the copulatory organ and haptor structures. The latter can be distinguished by the greater length of main part of the ventral anchor (VAB), greater length of both bars, greater total length of the penis accessory piece, and greater total length of the penis and vagina. In *L. domnichi* Rubtsova et al., 2007, *L. pacificus* Rubtsova et al., 2007, *L. gussevi* Miroshnichenko & Maltsev, 2004, *L. pilengas* Sarabeev & Balbuena, 2004, and *L. chenzhenensis* Hu & Li, 1992, the accessory pieces have unequal distal bifurcations and the long shaft of the upper lobe resembles that of *L. guanduensis*. The latter differs from all these species in the greater length of the dorsal bar and the greater total length of the penis. The new species differs from *L. domnichi*, *L. pacificus*, and *L. gussevi* in the lower lobe of the accessory piece, which is longer than the upper lobe, from *L. pilengas* in the shape of the upper lobe, and from *L. chenzhenensis* in the shape of the median process between membranous anterior processes. *Ligophorus guanduensis* differs greatly from remaining species in the shape and size of both the haptor structure and the copulatory organ.

*Ligophorus brasiliensis* and *L. guanduensis* have a similar shape of the accessory piece. However, in *L. guanduensis*, the lower lobe is larger than the upper lobe (as opposed to *L. brasiliensis*), the ratio between the length of upper lobe and the length of proximal part of accessory piece before bifurcation is shorter, and the distal

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FIGURE 5. Haptor and genital sclerotized structures of *Ligophorus lizae* n. sp. (A) Male copulatory complex: penis and accessory piece. (B) Uncinulus. (C) Vaginal armament. (D) Dorsal anchor. (E) Ventral anchor. (F) Ventral bar. (G) Dorsal bar.



tip of the lower lobe extends to the level of the upper lobe (in *L. brasiliensis*, the distal tip of the lower lobe crosses the upper lobe).

### *Ligophorus lizae* n. sp.

(Figs. 4D–F, 5)

*Diagnosis* (based on 15 whole mounts; morphometric measurements of sclerotized structures are presented on Table 1): Body  $756.2 \pm 72.2$  [677–821] (15) long; maximum width  $152.7 \pm 13.5$  [138–165] (15) at level of gonads. Two bilateral cephalic lobes. Each eye with lens. Pharynx spherical  $34.3 \pm 10.6$  [24–45] (12) long,  $30.3 \pm 8.3$  [22–38] (12) wide. Testis  $58.9 \pm 5.9$  [54–66] (8) long,  $28.9 \pm 1.9$  [26–31] (8) wide. Male copulatory organ consists of tubular penis with well-developed heel, opening at distal end of accessory piece, ratio between length of tube and total length of accessory piece =  $2.5 \pm 0.02$  [2.3–2.7] (10), accessory piece bifurcates into 2 equal parts. Ovary  $70.6 \pm 5.9$  [65–78] (8) long,  $31.8 \pm 6.0$  [27–39] (8) wide, U-shaped. Vagina long, thin, winding, sclerotized, leading to ovate seminal receptacle. Vitelline follicles dense. Peduncle broad, long, tapering posteriorly; haptor sub-hexagonal  $66.8 \pm 10.4$  [56–77] (15) long,  $92.0 \pm 15.7$  [77–109] (15) wide armed, with 14 uncinuli, 2 pairs of anchors, 2 transverse bars. Ventral anchor with inner root longer and much heavier than outer root (VAD/VAC =  $3.0 \pm 0.3$  [2.7–3.6] [14]); point longer than outer root; arched blade bent at middle. Base markedly thicker than blade, separated by notch; filament present. Ventral anchors connected by transverse ventral bar. Ventral bar straight with 2 membranous protuberances closely located medially, distance between two membranous process =  $5.5 \pm 0.3$  [5.1–5.7], sclerotized median process with 2 anterior protuberances between them. Dorsal anchor similar to ventral, slightly smaller in total length, smaller ratio between inner and outer roots (DAD/DAC =  $1.8 \pm 0.07$  [1.6–1.9] [14]) and different in shape of blade, which bends at distal half. Dorsal transversal bar bowed, connects dorsal anchors. All 14 uncinuli similar in shape and size with straight shaft and sickle. Filament loop close to middle of shaft.

### Taxonomic summary

*Type host*: *Mugil liza* Valenciennes, 1836, Liza.

*Site of infection*: Gill lamellae.

*Type locality*: Guandu river (22°48'32"S, 43°37'35"W), State of Rio de Janeiro, Brazil.

*Specimens deposited*: Holotype CHIOC 37178a, Paratypes CHIOC 37178b.

*Etymology*: The specific name *lizae* refers to the specific name of the type-host species.

### Remarks

*Ligophorus llewellyni* Dmitrieva et al., 2007, which parasitizes *Liza haematocheilus*, seems the most similar to *L. lizae* n. sp. in the shape of both the copulatory organ and the haptoral structures. The latter can be distinguished by its greater total length of the ventral anchor, greater length of the ventral and dorsal anchors, greater length of the ventral and dorsal anchor points, greater total length of the uncinulus, greater length of the uncinulus shaft, greater length of the uncinulus sickle, greater length of both bars, greater total length of the penis accessory piece, greater width of

the distal portion of the upper lobe of the penis accessory piece, greater total length of the penis, and by the shape of the median process between the membranous anterior processes. None of the other species have the same shape of the accessory piece as the new species, with equal distal bifurcations. *Ligophorus lizae* differs greatly from other species in the shape and size of both the haptoral structure and the copulatory organ. As opposed to the other 3 new species described herein, the terminal bifurcations of the accessory piece are equal in length in *L. lizae*.

### DISCUSSION

According to Mariniello et al. (2004), the discrimination of species of *Ligophorus* can be achieved on the basis of differences in the shape and size of sclerotized parts of the haptor, copulatory organ, and vagina. In the present study, main morphological differences between *L. tainhae*, *L. brasiliensis*, *L. guanduensis*, and *L. lizae* are based on the shape of the accessory piece of the male copulatory organ. These 4 species are the first records of *Ligophorus* in *Mugil liza* and the first record from Brazilian waters. Currently, 2 species of *Ligophorus* has been recorded in South America, i.e., *L. huitrempe* in *M. cephalus* from Pacific Ocean (Chile) by Fernández (1987) and *L. mugilinus* in *M. curema* from Atlantic Ocean (Venezuela) by Fuentes and Nasir (1990). Both species possessed several morphological and metric differences from the new species described here. *Ligophorus huitrempe* can be distinguished from the 4 new species in shape of accessory piece and ventral bar, smaller ventral anchor, smaller dorsal anchor main part, outer root and point length, and penis total length. *Ligophorus mugilinus* is different from the 4 new species in the shape of accessory piece and ventral bar, a much larger distance between membranous anterior processes (protuberances) of the ventral bar, smaller ventral anchor total length, dorsal anchor main part length, and penis accessory piece total length. However, the new species are similar to several species in other geographic regions: *L. tainhae* seems similar to *L. domnichi* and *L. cheleus* from the Japan Sea; *L. guanduensis* to *L. cephalis* from the Mediterranean Sea and Black Sea; and *L. lizae* to *L. llewellyni* from the Black Sea. Results presented herein show that new species of *Ligophorus* most closely resemble species from Mediterranean Basin and off the coasts of northwestern Pacific than the species described in South and North America.

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