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VERSITA

Ligophorus uruguayense sp. nov. (Monogenea, Ancyrocephalidae), a gill parasite from *Mugil platanus* (Mugiliformes, Mugilidae) in Uruguay

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Abstract

The present study describes a new species, *Ligophorus uruguayense*, parasitizing the gills of *Mugil platanus* Günther, 1880 from the coast of Uruguay. It differs from all other species of the genus mainly in the shape of the ventral bar, the thick process at the distal end of the inner root of ventral anchors, the J-shaped penis accessory piece and the vaginal tube showing transverse annulations at its distal end, the host species, and geographical distribution. This is the first description of a species of *Ligophorus* from a mullet in the South Atlantic Ocean.

Keywords

Monogenea, *Ligophorus*, gill parasites, *Mugil platanus*, mullets, Uruguay, South Atlantic Ocean

Introduction

The fish species of the family Mugilidae have a wide geographic distribution and are mainly restricted to coastal estuarine waters of tropical and subtropical regions. The taxonomic status of *Mugil platanus* Günther, 1880 is still controversial, with some authors considering it as a distinct population of *Mugil cephalus* Linnaeus, 1758 (see Fraga *et al*. 2007), and others as a different species (Castro *et al*. 2008). The parasites of mullets have been thoroughly studied because of their importance for human consumption (Paperna and Overstreet 1981, Juárez-Arroyo and Salgado-Maldonado 1989, Valles-Ríos *et al*. 2000). The species of the monogenean genus *Ligophorus* Euzet et Suriano, 1977 have received considerable attention in the past few years. The genus *Ligophorus* was established for species recovered from Mediterranean mugilids. In the Northern Hemisphere, new species of *Ligophorus* were found parasitizing mugilid species from the Black Sea, Azov Sea, Mediterranean Sea, North Atlantic Ocean, and Pacific Ocean (Dmitrieva and Gerasev 1996; Sarabeev and Balbuena 2004; Sarabeev *et al*. 2005; Rubtsova *et al*. 2006, 2007; Dmitrieva *et al*. 2007). In the Southern Hemisphere the only species so far described is *L. huitrempe* Fernández Bargiela, 1987 from a mugilid in the Chilean coast, Pacific Ocean.

The present study describes a new species of *Ligophorus*, and is the first report of this genus parasitizing *Mugil platanus* from the South Atlantic Ocean.

Materials and methods

Mugil platanus specimens were collected from the coastal lagoon Laguna de Rocha (34°33′–34°41′S and 54°02′–54°22′W), Uruguay, in 1993 and 1994. Fish were dissected in the field, and their gills and other organs were immediately removed and fixed in 4% formalin. In the laboratory, gills were examined under a stereoscopic microscope. Ancyrocephalid monogeneans were cleared in lactophenol (Amman) and mounted in glycerine jelly or stained with chlorhydric carmine (Langeron 1942), dehydrated in an ethanol series and mounted in Canada balsam. To study live monogeneans additional mugilids of the same species were captured in 2007 from Las Flores (34°50′S–55°25′W), Department of Maldonado, Uruguay.

A total of twenty-five characters of sclerotized structures were measured, of which twenty-two were defined by Mariniello *et al*. (2004), Sarabeev and Balbuena (2004), and Rubtsova *et al*. (2007), and the remaining three are defined here, namely distance between upper and lower lobe of the penis ac-

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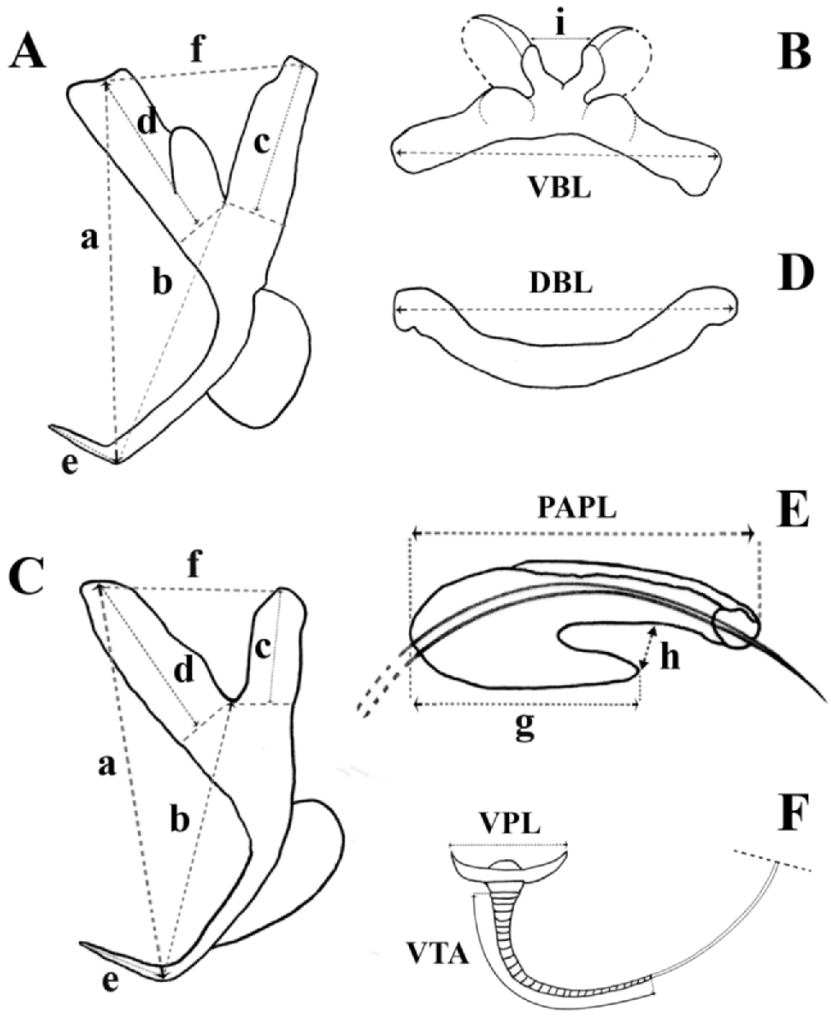

Fig. 1. Diagram showing measurements of the sclerotized structures: **A** – ventral anchor, **B** – ventral bar, **C** – dorsal anchor, **D** – dorsal bar, **E** – J-shaped accessory piece of the male copulatory complex, **F** – vaginal armament (see Table II for abbreviations of metric variables)

cessory piece; the vaginal plate (corresponding to the saucer-shaped opening); and the vaginal ring (corresponding to transverse annulations at the distal end). All these structures were measured on fixed and live specimens, except the vaginal plate and the vaginal ring, which were measured on live specimens only. The abbreviations of the characters used throughout the text, and the measurements in micrometers, with the mean followed by the standard deviation (SD) within parentheses, range and number of specimens measured (n), are given in Table II. A diagram showing measurements of the sclerotized elements is presented in Figure 1. Drawings were made with the aid of a camera lucida attached to an Olympus BX-50 microscope. Micrographs were taken with a Sony Cyber-shot 6.0 DSC-S600 digital camera. Representative specimens were mounted in Canada balsam and deposited in the Helminthological Collection of the Laboratorio de Zoología de Invertebrados, Facultad de Ciencias, Montevideo, Uruguay, and in the Helminthological Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina. Data were analyzed with χ^2 test and Mann-Whitney U-test.

Results

Thirty-one out of 123 fish collected in 1993 and 1994 were found infected with a new species of *Ligophorus*. Specimens were recovered from both the right and left gill arches, with a higher number of parasites on the first and second gill arches

Table I. Distribution of parasites on gill arches from 123 specimens of *Mugil platanus*

Site	*Ligophorus uruguayense* sp. nov.	
	n	%
Total	411	100
Left side	204	49.6
Right side	207	50.4
Gill arch 1	149	36
Gill arch 2	115	28
Gill arch 3	98	24
Gill arch 4	49	12
External hemibranches	247	60
Internal hemibranches	164	40

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Table II. Comparative morphometrical data of specimens of *Ligophorus uruguayense* sp. nov., with *L. mugilinus* (Hargis, 1955), according to Sarabeev *et al.* (2005), and *L. huitrempe* Fernández Bargiela, 1987; mean (SD) = mean, standard deviation; n = number of measures

Species	***L. uruguayense* sp. nov.** **present study**						***L. huitrempe* J. Fernández Bargiela (1987)**		***L. mugilinus* Sarabeev *et al.* (2005)**		
Characters	glycerine mean (SD)	range	n	live mean (SD)	range	n	glycerine range	n	glycerine mean (SD)	range	n
BL	433 (78.4)	2689–650	34	781 (105.6)	614–1000	30	650–800	15	699 (96)	537–806	9
BW	125 (25.8)	72–227	31	106 (24.4)	71–161	30	70–130	15	157 (37)	114–237	9
VAA	36 (3.0)	30–42	32	32 (2.0)	28–35	22	35–38	15	36 (3)	30–38	9
VAB	25 (2.8)	20–31	32	25 (1.4)	22–27	22	24–27	15	25 (1)	24–27	9
VAC	9 (1.7)	7–14	35	10 (1.4)	7–12	22	9–11	15	10 (1)	8–9	9
VAD	15 (2.6)	10–20	35	11 (1.6)	8–14	22	13–15	15	18 (1)	16–20	9
VAE	9 (1.2)	7–11	32	8 (0.8)	6–9	22	8–10	15	10 (0.4)	9–10	9
VDIOR	–	–	–	14 (1.9)	10–16	22	–	–	–	–	–
DAA	37 (4.2)	31–46	22	31 (1.6)	28–34	25	32–35	15	39 (1)	37–41	9
DAB	26 (3.5)	19–33	19	24 (1.2)	21–26	25	25–27	15	27 (2)	25–29	9
DAC	10 (2.1)	7–15	19	7 (1.1)	6–11	25	6–8	15	9 (1)	8–10	9
DAD	15 (4.0)	7–22	19	11 (1.3)	8–13	25	9–11	15	17 (1)	16–18	9
DAE	9 (1.2)	8–12	23	8 (0.8)	6–9	25	6–8	15	10 (1)	8–11	9
DDIOR	–	–	–	14 (1.7)	8–16	25	–	–	–	–	–
UL	10 (1.3)	8–12	9	13 (0.7)	11–13	21	12–15	15	12 (0.3)	12–13	9
VBL	41 (5.3)	33–56	41	37 (1.9)	34–41	24	36–40	15	44 (7)	37–57	9
VBAP	–	–	–	7 (1.1)	5– 9	22	4– 5	15	9 (3)	6–13	9
DBL	41 (4.9)	34–55	42	35 (1.8)	31–38	24	36–40	15	41 (7)	32–51	9
PAPL	17 (1.4)	15–18	18	18 (1.1)	15–20	35	15–23	15	30 (2)	27–33	9
PL	97 (5.2)	87–106	27	104 (5.2)	92–115	42	100–110	15	82 (6)	73–92	9
LWL	–	–	–	10 (0.1)	8–12	35	–	–	–	–	–
DBULL	–	–	–	3 (0.6)	2–4	31	–	–	–	–	–
VL	18 (5.4)	12–30	13	54 (8.5)	37–71	21	55–63	15	49 (11)	29–69	9
VPL	–	–	–	9 (1.1)	8–12	22	–	–	–	–	–
VTA	–	–	–	25 (2.5)	20–30	23	–	–	–	–	–
VAD/VAC	2 (0.3)	1–2	35	1 (0.2)	1–2	22	–	–	1.8 (0.11)	1.67–2	9
DAD/DAC	2 (0.6)	0.5–3	18	2 (0.2)	1–2	25	–	–	1.9 (0.28)	1.56–2.42	9

BL – body length; BW – body width; DAA (a) – dorsal anchor total length; DAB (b) – dorsal anchor main part length; DAC (c) – dorsal anchor outer root length; DAD (d) – dorsal anchor inner root length; DAE (e) – dorsal anchor point length; DBL – dorsal bar length; DBULL (h) – distance between upper and lower lobe of penis accessory piece; DDIOR (f) – distance between inner and outer root of dorsal anchors; PAPL – penis accessory piece total length; LWL (g) – penis accessory piece lower lobe length; PAPUL – penis accessory piece upper lobe [in contrast to Rubtsova *et al.* (2007), where this abbreviation refers to the distal portion of upper lobe length of the penis accessory piece, absent in the present species]; PL – total length of penis; UL – uncinulus (marginal hook) total length; VAA (a) – ventral anchor total length; VAB (b) – ventral anchor main part length; VAC (c) – ventral anchor outer root length; VAD (d) – ventral anchor inner root length; VAE (e) – ventral anchor point length; VBAP (i) – distance between membranous anterior processes of ventral bar; VBL – ventral bar length; VDIOR (f) – distance between inner and outer root of ventral anchors; VL – vagina length; VPL – vaginal plate (corresponding to the saucer-shaped opening); VTA – vaginal transverse annulations of distal end.

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(Table I). Prevalence of infection was 25.2% and mean intensity 13.7 (24.0) worms per fish.

***Ligophorus uruguayense* sp. nov.** (Figs 2–4)

Description: Worms with characters of the genus as defined by Euzet and Suriano (1977) and supplemented by Sarabeev and Balbuena (2004). Table II shows the morphometric measurements of fixed and live specimens of the studied species and those of *L. huitrempe* (Southern Pacific Ocean) and *L. mugilinus* (Hargis 1955) (North Atlantic Ocean) for comparison.

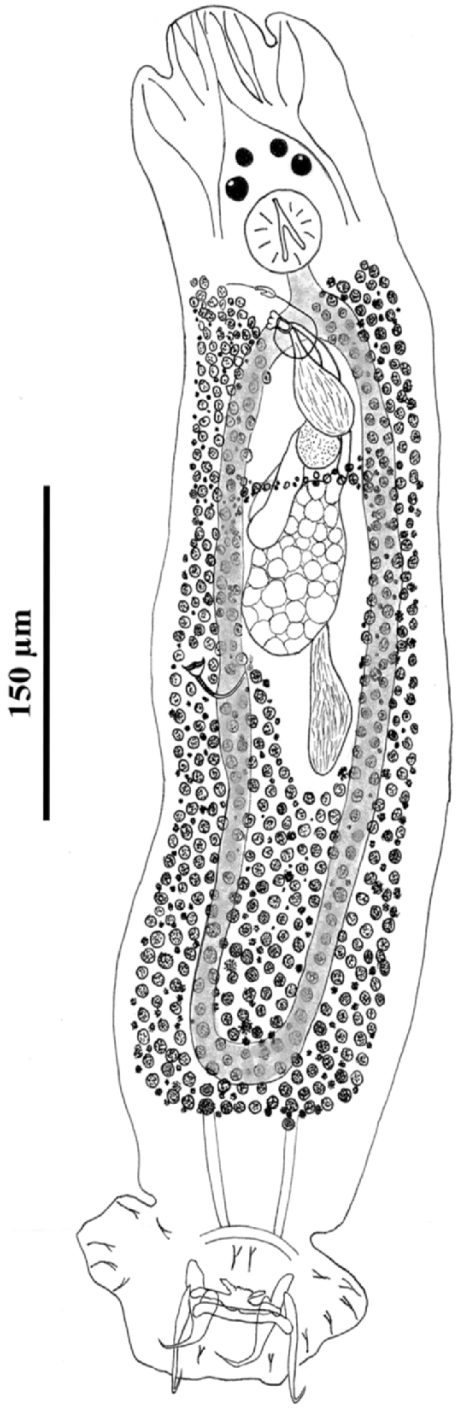

Fig. 2. *Ligophorus uruguayense* sp. nov.: Adult, ventral view

Body fusiform, with two pairs of pigmented eye-spots, posterior one with lens. Posterior haptor armed with 14 uncinuli (Fig. 2). One pair of ventral anchors connected by ventral transverse bar (Figs 3A, 4A), one pair of dorsal anchors connected by dorsal transverse bar (Figs 3B, 4A). Ventral anchors with elongate thin blade and recurved point, forming obtuse angle (about 100°). Base of anchor markedly thicker than blade, separated by a notch. Inner root (guard) somewhat longer than outer root (shaft), VAD/VAC = 1.2 ± 0.2 (1.0–1.5; n = 22). Inner root shows thick process at distal end where ventral connecting bar articulates with anchor. Angle between roots about 50°. Filaments present. Ventral connecting bar massive, slightly curved, with two sclerotized thickenings at base of two membranous anterior medial processes; heavily sclerotized median process between open V-shaped membranous processes (not always seen in stained specimens) (Figs 3A, 4A). Dorsal anchors similar in shape to ventral anchors (except for thick process at distal end of inner root (Fig. 4B). Inner root much longer than outer root, DAD/DAC = 1.5 ± 0.2 (1.0–2.0; n = 25). Base of dorsal anchors somewhat thinner than that of ventral anchors. Dorsal transversal bar slightly V-shaped, with rounded ends (Figs 3B, 4A). All 14 uncinuli similar in shape and size, with slightly curved handle and sickle (Fig. 3C). Male copulatory complex consisting of tubular penis about 1.0 µm in diameter and with J-shaped accessory piece, showing short lower lobe joined to longer, simple and tubular upper lobe at proximal end, separated by short distance (Figs 3E, 4C, 4D). Accessory piece supports distal end of penis. Vaginal armament constituted by convoluted, sclerotized tube; two proximal thirds thin, distal third thick; transverse annulations at distal end; with wide saucer-shaped opening (Figs 3D, 4E) placed submidventrally.

Type host: *Mugil platanus* Günther, 1880.

Site of infection: Gill lamellae.

Type locality: Laguna de Rocha (34°33′–34°41′S and 54°02′–54°22′W), Department of Rocha, Uruguay.

Other localities: Las Flores (34°50′S–55°25′W), Department of Maldonado, Uruguay.

Prevalence and intensity range: P = 25.2%; intensity range 1–113 worms per fish

Specimens deposited in the Helminthological Collection of the Laboratorio de Zoología de Invertebrados, Facultad de Ciencias, Montevideo, Uruguay, AP/12192-12196; and in the Helminthological Collection of the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina, MACN-Pa 477/1-4.

Etymology: The specific name refers to the geographic location where the worm was found.

Remarks: There were significant differences in the measurements of characters between live and glycerine- or balsam-mounted specimens (Table II), except in the following: VAB (U = 0.54; p = 0.58; n = 22), DAB (U = 1.67; p = 0.09; n = 25), VBL (U = 0.96; p = 0.33; n = 24) and PAPL (U = 0.75; p = 0.45;

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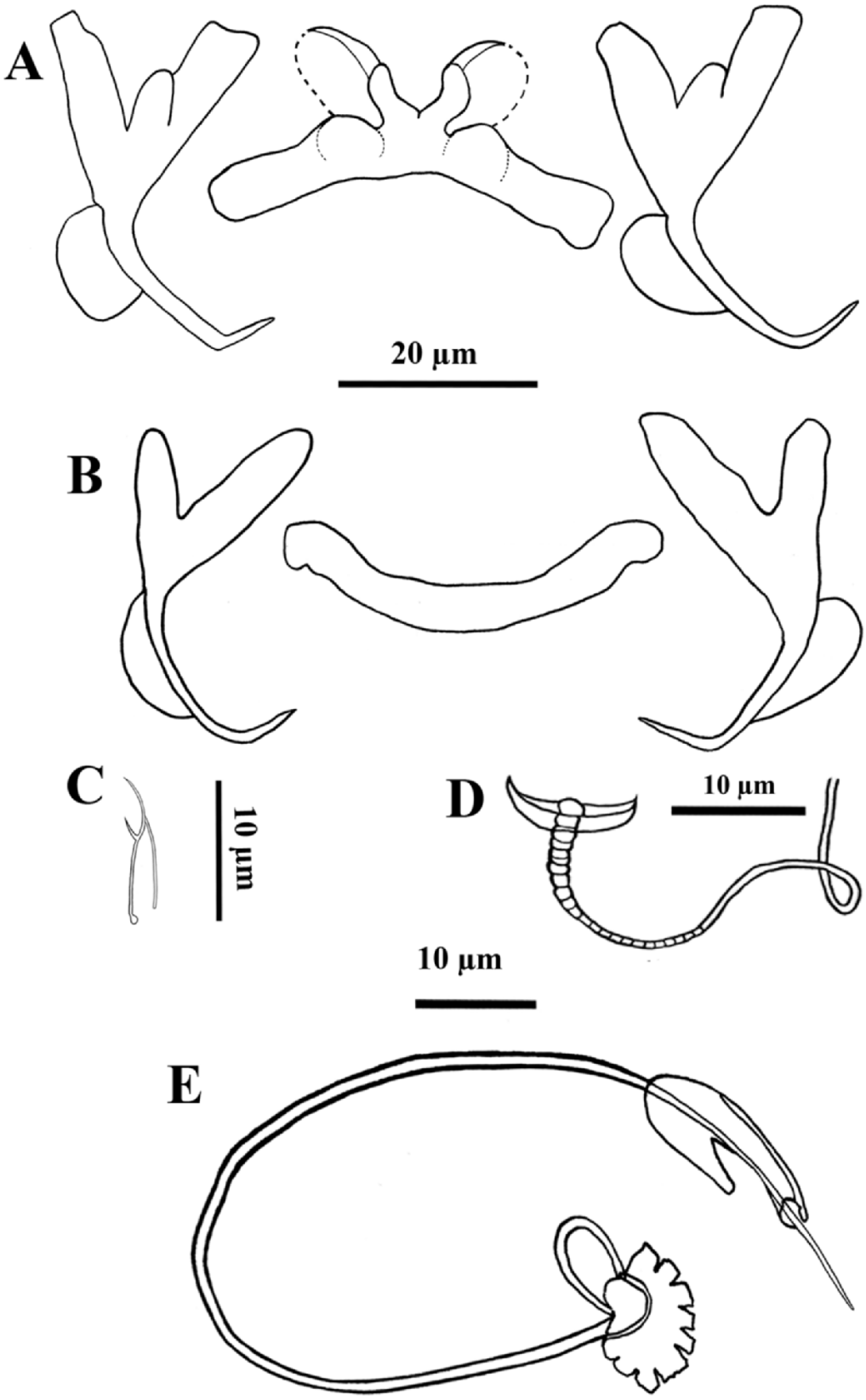

Fig. 3. Sclerotized pieces of *Ligophorus uruguayense* sp. nov.: **A** – ventral bar and anchors, **B** – dorsal bar and anchors, **C** – marginal hook, **D** – vaginal armament, **E** – male copulatory complex: penis and accessory piece

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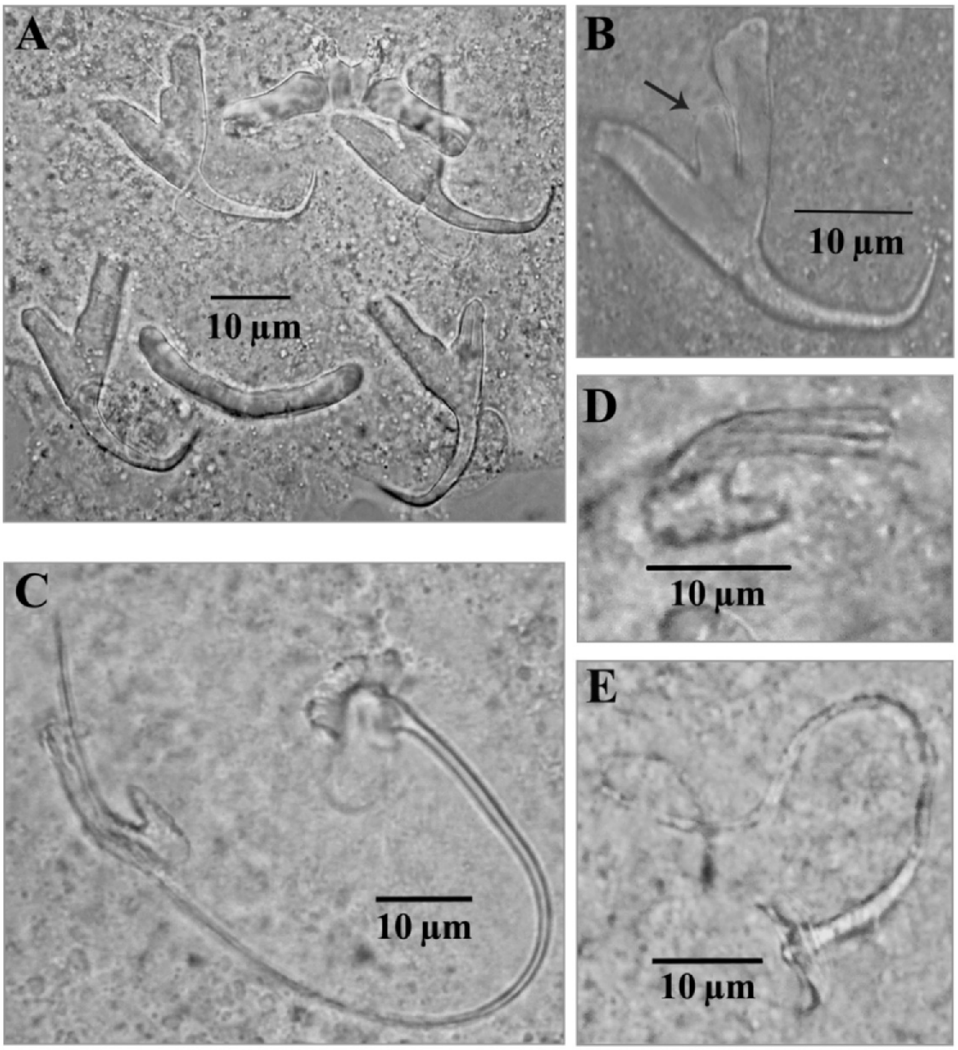

Fig. 4. Photomicrographs of sclerotized elements of haptor, male copulatory complex and vaginal armament of *Ligophorus uruguayense* sp. nov.: **A** – dorsal bar, ventral bar and anchors, **B** – detail of the distal thick process (arrow) of the inner root (guard), **C** – male copulatory complex, **D** – detail of the J-shaped accessory piece of the male copulatory complex, **E** – vaginal armament

n = 35). Thus, the characters that were not affected by the mounting methods used are the ventral bar (VBL), the ventral (VAB) and dorsal (DAB) anchor main part lengths and the accessory piece (PAPL) of the male copulatory complex. The ventral bar is a heavily sclerotized piece rather than a slightly curved piece and its morphology remained unchanged. On the contrary, the dorsal bar is less sclerotized, and its shape was modified by the pressure of the coverslip. No significant differences were found in the distance between shaft and guard of both ventral and dorsal anchors in live specimens (VDIOR, DDIOR: U = 0.46; p = 0.64; n = 22). The distance between the membranous processes of the ventral bar is not a reliable measurement because it differed between mounted and live specimens or the membranous processes could not be seen clearly. In contrast to Rubtsova *et al*. (2006), no difficulty was found in obtaining an accurate measurement of the whole length of the penis either for live or mounted specimens.

Discussion

This study reports for the first time the occurrence of a *Ligophorus* species in the host *Mugil platanus* and of this genus in the South Atlantic Ocean.

The similarity among species has led to erroneous identifications. In many cases, a single species was split into different species based on detailed morphological and morphometric analyses of the sclerotized structures of the haptor (dorsal and ventral bars, dorsal and ventral anchors) and of the genital organs (terminal genitalia of male copulatory complex, vagina), and on the occurrence of *Ligophorus* specimens in mullets from different geographic areas. This is illustrated in the following examples: (1) the separation of *L. gussevi* Miroshnichenko et Maltsev, 2004 (synonymous of *L. pilengas* Sarabeev et Balbuena, 2004, according to Balbuena *et al*. 2006) from *L. chabaudi* Euzet et Suriano, 1977; (2) the separation of

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L. mediterraneus Sarabeev, Balbuena et Euzet, 2005 cited for the Mediterranean Sea, from specimens formerly assigned to *L. mugilinus* by Euzet and Suriano (1977); and (3) *L. vanbenedenii* Parona et Perugia, 1890 cited for the Japan Sea, was first transferred to *L. chabaudi* by Euzet and Suriano (1977), and then described as a new species, *L. domnichi* Rubtsova, Balbuena et Sarabeev, 2007 (see Sarabeev and Balbuena 2004; Sarabeev *et al.* 2005; Balbuena *et al.* 2006; Rubtsova *et al*. 2006, 2007).

Based on the features mentioned above, *L. uruguayense* differs from the following Mediterranean species described by (1) Euzet and Suriano (1977): *L. szidati*, *L. chabaudi*, *L. macrocolpus*, *L. acuminatus*, *L. minimus*, *L. heteronchus*, *L. angustus*, *L. imitans*, *L. confusus*; (2) Euzet and Sanfilippo (1983): *L. parvicirrus*; (3) Sarabeev *et al*. (2005): *L. mediterraneus*; and (4) Rubtsova *et al*. (2006): *L. cephali*, on the basis of the shapes of the ventral bar and anchors, the accessory piece of the penis and the vaginal aperture, the host species and the geographic distribution. Additionally, *Ligophorus uruguayense* differs from *L. euzeti* Dmitrieva et Gerasev, 1996, *L. gussevi*, *L. llewellyni* Dmitrieva, Gerasev et Pron'kina, 2007, and the three recently described species from the Japan Sea by Rubtsova *et al*. (2007): *L. domnichi*, *L. cheleus* and *L. pacificus*, mainly in the shape of the accessory piece of the penis, the host species and the geographic distribution.

Several species of *Ligophorus* have been described from Chinese mullets. *Ligophorus hamulosus* Pan et Zhang, 1999, *L. chenzhenensis* Hu et Li, 1992 and *L. chongmingensis* Hu et Li, 1992 differ from *L. uruguayense* in the shape of the dorsal or ventral bars and the penis accessory piece (Hu and Li 1992, Pan 1999). Although the descriptions of three species cited by Zhang *et al*. (2003) (*L. ellochelon* Yang, 2001, *L. kaohsianghienis* (Gussev, 1962) and *L. leporinus* (Zhang et Ji, 1981) were not available for comparison, they may not be identical to *L. uruguayense*, as suggested by their different hosts and distant location.

Ligophorus uruguayense is similar to *L. mugilinus* from the North Atlantic Ocean, as redescribed by Sarabeev *et al*. (2005), by having the ventral bar larger than the dorsal bar, but can be distinguished from it by the shape of the sclerotized median process, the membranous anterior process, and the absence of a sclerotized thickening at its base. *Ligophorus mugilinus* has a claw-shaped penis accessory piece and a vaginal tube of uniform diameter, while *L. uruguayense* has a J-shaped penis accessory piece and a convoluted vaginal tube of variable diameter along its length. In *L. uruguayense* the guard and shaft of the ventral anchor differ slightly in length, and the guard shows a thick process at the distal end where the ventral connecting bar articulates with the anchor. In contrast, the guard and shaft of *L. mugilinus* differ considerably in length, and the guard is only slightly thickened at the distal end in some specimens (Sarabeev *et al*. 2005).

Ligophorus huitrempe is morphometrically similar to *L. uruguayense* but differs from this species in its ventral bar with two anterior thickenings; in lacking the median sclerotized and the membranous processes; in the absence of a thick process at the distal end of the guard; in its U-shaped penis accessory piece, which shows a thickening at the distal end of the upper lobe; and in that its vagina is uniformly sclerotized and opens mid-laterally. Although both *L. mugilinus* and *L. huitrempe* parasitize *Mugil cephalus*, they were found in geographically distant areas (the former in the North Atlantic and the latter in the South Pacific).

It is concluded that *L. uruguayense* should be erected as a new species based on its morphological differences (ventral bar, ventral anchors, penis accessory piece and vagina), distinct host species and a dissimilar geographical distribution.

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