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Ligophorus abditus n. sp. (Monogenea: Ancyrocephalidae) and other species of *Ligophorus* Euzet & Suriano, 1977 infecting the flathead grey mullet *Mugil cephalus* L. in the Sea of Japan and the Yellow Sea

Evgenija V. Dmitrieva · Pavel I. Gerasev · David I. Gibson

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Abstract As a result of the re-examination of museum slides and new material of monogeneans collected from *Mugil cephalus* L. in the Sea of Japan, the estuary of a river which flows into the Sea of Japan, the Yellow Sea (off Zhifu, at the boundary of the Bohai Sea) and the East China Sea (off the Ryukyu Islands), five species of *Ligophorus* Euzet & Suriano, 1977 were identified, one of which is new. The known species are *L. chabaudi* Euzet & Suriano, 1977, *L. cheleus* Rubtsova, Balbuena & Sarabeev, 2007, *L. domnichi* Rubtsova, Balbuena & Sarabeev, 2007 and *L. pacificus* Rubtsova, Balbuena & Sarabeev, 2007, which are reported from the Yellow Sea; in addition, *L. domnichi* is reported for the first time from the East China Sea. *Ligophorus abditus* n. sp., from the Sea of Japan, differs from its most similar congeners, *L. pacificus* and *L. domnichi*, in the shapes of the dorsal anchors and the accessory piece of the male copulatory organ. A comparison of all of the species of *Ligophorus* recovered from *M. cephalus* in the Sea of Japan was carried out using Principal Component Analysis, and their distribution and origin are discussed.

E. V. Dmitrieva (✉)
Department of Ecological Parasitology, Institute of Biology of the Southern Seas, National Academy of Sciences of the Ukraine, 2, Nakhimov Ave., 99011 Sevastopol, Ukraine
e-mail: evadmitr@optima.com.ua

P. I. Gerasev
Department of Parasitic Worms, Zoological Institute, Russian Academy of Sciences, Universitetskaya nab.1, 199034 St Petersburg, Russia
e-mail: gerasev_vermes@zin.ru

D. I. Gibson
Department of Life Sciences, Natural History Museum, London SW7 5BD, UK
e-mail: dig@nhm.ac.uk

Introduction

Representatives of *Ligophorus* Euzet & Suriano, 1977 are strictly specific parasites of mullets (Mugilidae), which makes them of great interest for the study of their host's phylogeography (Gerasev et al., 2007). Species parasitising a single host species in the same region are especially relevant to an understanding of speciation and species coexistence in monogeneans. To date, 17 species within the Mugilidae are known as hosts for about 50 species of *Ligophorus* (see Dmitrieva et al., 2012; Soo & Lim, 2012). The greatest number of *Ligophorus* spp. has been reported from the flathead grey mullet *Mugil cephalus* L., i.e. 14 species (Dmitrieva et al., 2012), and for 11 of these, this mullet species is the primary or sole host. Seven of the latter species have been described or redescribed only recently (Sarabeev et al., 2005; Rubtsova et al., 2007; Dmitrieva et al., 2009a, b) and their occurrence on this one host only has been strongly indicated. Among these are four species, namely *L. chabaudi* Euzet & Suriano, 1977, *L. cheleus* Rubtsova, Balbuena & Sarabeev, 2007, *L. domnichi* Rubtsova, Balbuena &

Sarabeev, 2007 and *L. pacificus* Rubtsova, Balbuena & Sarabeev, 2007, which have been recorded on *M. cephalus* in the Sea of Japan (Rubtsova et al., 2007; Dmitrieva et al., 2009b). A study of museum and fresh material from the Sea of Japan, the Yellow Sea (off Zhifu, which is located on the north-western coast of the Yellow Sea at the boundary with the Bohai Sea) and the East China Sea (off the Ryukyu Islands) revealed all four of these species, plus a new one which is described below.

Materials and methods

New specimens were collected from the gills of four specimens of *Mugil cephalus*, 35–40 cm in total length, caught in the Zaliv Pos'yeta (42°39′10″N, 130°44′44″E), Sea of Japan. All monogeneans were collected from freshly caught fish and then immediately mounted in glycerine-jelly (prepared with 0.5 g carbolic acid). Slides (22) deposited in the Parasite Collection of the Zoological Institute RAS, St Petersburg, and labelled as '*Ancyrocephalus vanbenedenii*', which were collected from *M. cephalus* in different parts of the Sea of Japan, including the estuary of a river which runs into it, the Yellow Sea (off Zhifu) and the East China Sea (off the Ryukyu Islands), were also examined (Table 1). Many of these slides are old and include specimens collected from formalin-fixed fishes collected in the 19th and early 20th Centuries.

Drawings and light micrographs were made using a Carl Zeiss Jena Amplival microscope at magnifications of ×2,000, using phase-contrast optics, a drawing tube and an Olympus C180 digital camera. The measurements used for various anatomical structures follow Dmitrieva et al. (2009b). Their configuration and abbreviations are presented in Fig. 1 and Table 2, respectively. All dimensions are given in micrometres, with the smallest division of the graticule used for measuring being 1 µm. The mean, standard error and range were used to describe the linear measurements. Morphological analysis of 134 specimens (20 *Ligophorus abditus* n. sp.; 22 *L. chabaudi*; 30 *L. cheleus*; 30 *L. domnichi*; and 32 *L. pacificus*) was carried out using Principal Component Analysis based on the correlation matrix (all measurements were ln-transformed) using the Statistica 6 for Windows software package.

Museum abbreviations are as follows: Zoological Institute RAS, St Petersburg, Russia (ZIN RAS); Institute of the Southern Seas, Sevastopol, Ukraine

Table 1 Investigated material and determined species of *Ligophorus*

Samples/No. of collection slides, accession numbers, sites	Coordinates	Date of collection	Collector(s)	*Ligophorus* spp. (No. of specimens)
Zaliv Pos'yeta, Russia, Sea of Japan	42°39′10″N, 130°44′44″E	22-25.ix.2006	Own material	*L. domnichi* (32) *L. cheleus* (33) *L. pacificus* (35) *L. chabaudi* (23) *L. abditus* n. sp. (22)
11 slides from ZIN RAS No. 412/12-21 Off Zhifu, Yantai, China, Yellow Sea	37°46′N, 121°45′E	05.vii.1957	Bykhowskyi & Nagibina	*L. domnichi* (14) *L. cheleus* (3) *L. pacificus* (3) *L. chabaudi* (2)
9 slides from ZIN RAS No. 16821/2-10 Tumen-Ula River estuary, Russia, Sea of Japan	42°17′37.04″N, 130°42′4.43″E	28.ix.1913	Bykhowskyi, det. A. Gusev (Gusev, 1955)	*L. domnichi* (8) *L. pacificus* (6)
1 slide, ZIN RAS No. 23206 Off Ryukyu Islands, East China Sea	-	1926	Bykhowskyi	*L. domnichi* (2)
1 slide, ZIN RAS No. 608 "m. Sondaicum" (may refer to the Sunda Sea)	-	1856	Bykhowskyi	*L. chabaudi* (2) *L. pacificus* (1)

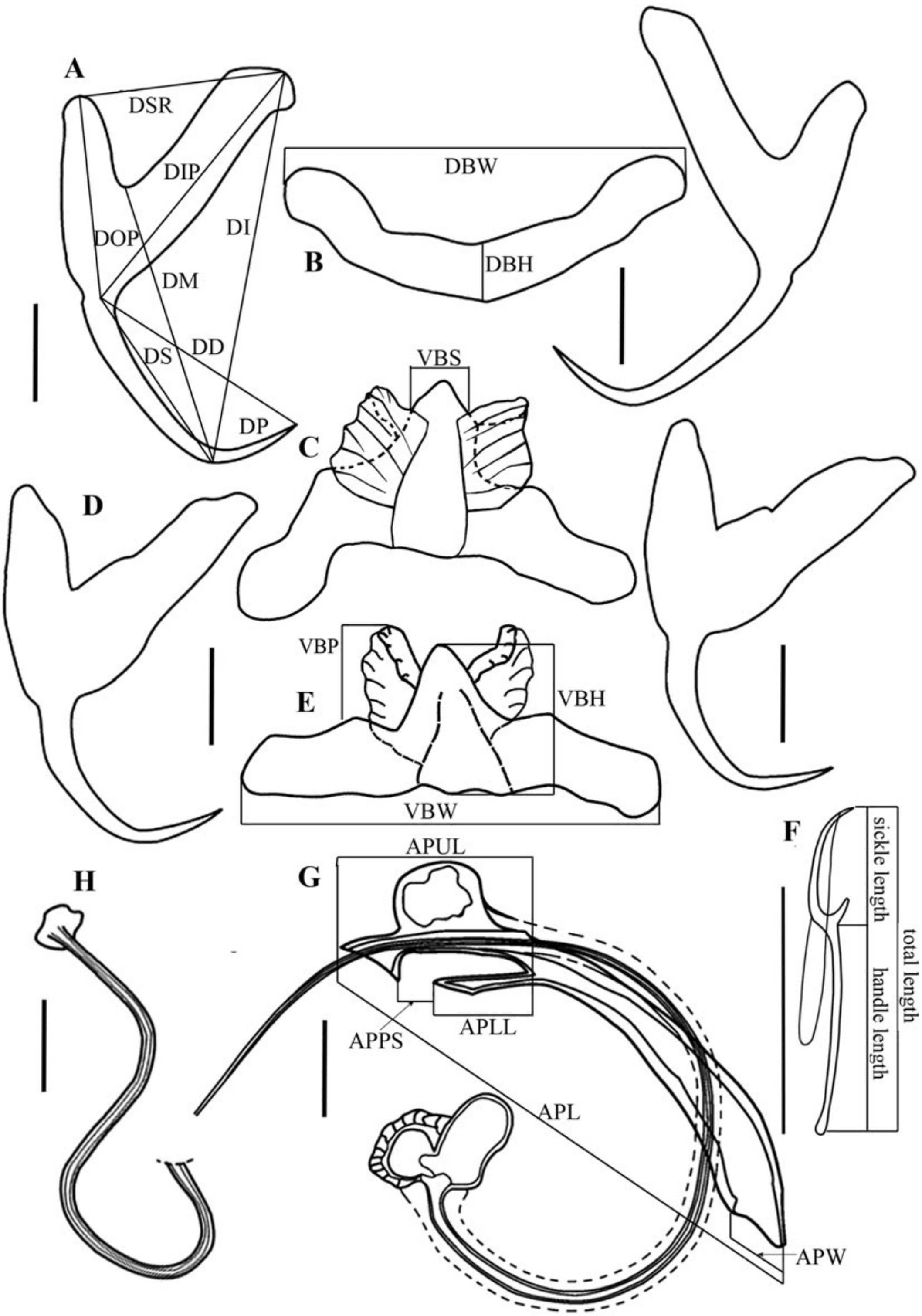

Fig. 1 The haptoral and copulatory hard-parts of *Ligophorus abditus* n. sp. from *Mugil cephalus* in the Sea of Japan, showing the measurements taken. A, dorsal anchor; B, dorsal bar; C, ventral bar (ventral view); D, ventral anchor; E, ventral bar (dorsal view); F, marginal hook; G, male copulatory organ; H, vagina. *Abbreviations:* See Table 2. *Scale-bars*: 10 µm

Table 2 Dimensions, as the range and mean ± SE, of the haptoral and copulatory hard-parts of *Ligophorus* spp. ex *Mugil cephalus* from off the Russian Far East

Ligophorus	*abditus* n. sp.	*chabaudi*	*cheleus*	*domnichi*	*pacificus*
No. of specimens	20	22	30	30	32
Ventral anchors					
Inner length (VI)	37–44	37–43	36–42	36–41	37–41
	41 ± 0.3	40 ± 0.4	39 ± 0.3	38.5 ± 0.2	39 ± 0.2
Length of main part (VM)	28–31	25–28	27–30	24–27	24–27
	29 ± 0.2	26 ± 0.2	29 ± 0.2	26 ± 0.2	25 ± 0.1
Length of distal part (VD)	21–24	19–22	22–23	20–21	19–22
	22 ± 0.2	20 ± 0.2	22 ± 0.1	20.5 ± 0.1	20 ± 0.1
Length of shaft (VS)	18–20	16–18	18–21	15–18	15–17
	19 ± 0.2	17 ± 0.1	19 ± 0.1	17 ± 0.1	16 ± 0.1
Length of point (VP)	9–10	9–10	9–10	10–11	10–11
	10 ± 0.1	9 ± 0.1	9.5 ± 0.1	10 ± 0.1	10 ± 0.1
Inner length of proximal part (VIP)	28–34	29–34	27–32	28–32	28–34
	31 ± 0.3	31 ± 0.3	29 ± 0.2	30 ± 0.2	31 ± 0.2
Outer length of proximal part (VOP)	22–24	24–27	20–23	17–20	18–24
	23 ± 0.1	26 ± 0.2	21 ± 0.2	18 ± 0.2	20 ± 0.2
Span between roots (VSR)	22–25	21–26	21–24	20–25	19–27
	23 ± 0.3	24 ± 0.3	22.5 ± 0.2	22.5 ± 0.3	23.5 ± 0.4
Dorsal anchor					
Inner length (DI)	42–50	39–44	38–47	37–43	40–45
	44 ± 0.4	42 ± 0.4	40 ± 0.3	40 ± 0.25	42 ± 0.3
Length of main part (DM)	31–34	28–30	27–31	26–30	26–30
	32 ± 0.2	29 ± 0.2	29 ± 0.2	28 ± 0.2	28.5 ± 0.2
Length of distal part (DD)	25–27	20–22	22–24	20–22	20–22
	27.5 ± 0.2	21 ± 0.1	23 ± 0.14	21 ± 0.14	21 ± 0.1
Length of shaft (DS)	21–23	14–16	19–21	17–19	16–19
	27 ± 0.2	15 ± 0.5	20 ± 0.14	18 ± 0.14	17.5 ± 0.1
Length of point (DP)	8–9	9–10	8–9	8–9	9–11
	9 ± 0.1	10 ± 0.1	8.5 ± 0.1	9 ± 0.1	10 ± 0.1
Inner length of proximal part (DIP)	30–35	27–33	26–30	26–33	29–35
	32 ± 0.3	30 ± 0.3	28 ± 0.2	29 ± 0.3	32 ± 0.3
Outer length of proximal part (DOP)	20–24	20–24	18–21	18–23	21–25
	21 ± 0.2	22 ± 0.3	19 ± 0.15	19.5 ± 0.2	22.5 ± 0.2
Span between roots (DSR)	20–24	20–24	19–23	19–22	19–26
	22 ± 0.2	22 ± 0.2	20 ± 0.2	21 ± 0.2	22 ± 0.3
Marginal hooks					
Total length	14	11–12	12–13	13–14	12–13
		12 ± 0.1	13 ± 0.1	13.5 ± 0.1	12.5 ± 0.1
Sickle length	5–6	5	5	5–6	5
	6 ± 0.1			5.5 ± 0.1	
Handle length	8–9	6–7	7–8	8	7–8
	8 ± 0.1	7 ± 0.1	8 ± 0.1		7.5 ± 0.1

Table 2 continued

Ligophorus	*abditus* n. sp.	*chabaudi*	*cheleus*	*domnichi*	*pacificus*
Ventral bar					
Height (VBH)	11–19	8–15	10–15	11–18	8–18
	16 ± 0.4	13 ± 0.5	11.5 ± 0.3	14.5 ± 0.4	14 ± 0.4
Width (VBW)	38–48	48–62	38–48	37–51	30–50
	42 ± 0.6	55 ± 1.1	41.5 ± 0.4	42 ± 0.6	43 ± 0.7
Length of anterior processes (VBP)	7–14	8–15	7–12	7–13	5–13
	10 ± 0.4	11 ± 0.4	10 ± 0.2	10.5 ± 0.3	10 ± 0.3
Span between processes (VBS)	4–7	7–13	3–6	2–5	3–7
	5 ± 0.2	9 ± 0.3	4 ± 0.14	3.5 ± 0.2	5.5 ± 0.2
Dorsal bar					
Height (DBH)	5–8	6–12	4–7	4–9	6–8
	6 ± 0.2	8 ± 0.3	5.5 ± 0.1	6 ± 0.2	7 ± 0.1
Width (DBW)	38–51	47–67	39–49	35–49	40–52
	45 ± 0.8	56 ± 1.4	44 ± 0.5	41 ± 0.7	45.5 ± 0.6
MCO					
Length (CTL)	90–120	108–120	93–110	100–122	117–130
	111 ± 2.2	115 ± 0.6	100 ± 0.7	110 ± 0.9	125 ± 0.6
Width (CTW)	1	1	1	1	1
APL	48–60	33–40	38–48	45–68	38–54
	53 ± 0.7	37 ± 0.5	42 ± 0.4	54 ± 0.9	43 ± 0.5
APW	4–6	4–5	4–6	3–5	4–7
	5 ± 0.1	5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	5 ± 0.1
Length of upper lobe (APUL)	17–22	12–18	12–18	21–30	14–21
	20 ± 0.4	14 ± 0.5	15 ± 0.2	24 ± 0.5	17 ± 0.3
Length of lower lobe (APLL)	8–12	7–10	6-12	19–26	9–16
	10 ± 0.4	9 ± 0.3	10.5 ± 0.3	21 ± 0.3	13 ± 0.3
Span between tips of lobes (APPS)	3–7	2–7	5–12	1–12	1–9
	5 ± 0.3	5 ± 0.5	8 ± 0.4	4.5 ± 0.6	4.5 ± 0.4
Vagina					
Length (VL)	60–68	50–70	56–72	45–58	60–73
	63 ± 1.7	57 ± 2.1	61.5 ± 2	52 ± 1.6	67 ± 0.6

(IBSS); British Museum (Natural History) Collection at the Natural History Museum, London (BMNH).

Results

About 180 monogenean specimens, including the museum material, were studied. Their general internal morphology and haptoral armament all agree with the diagnosis of *Ligophorus* given by Euzet & Suriano (1977). Five species were identified among these worms (Table 1), one of which is new.

Ligophorus abditus n. sp.

Type-host: *Mugil cephalus* L. (Mugilidae), flathead grey mullet.
Type-locality: Zaliv Pos'yeta, Expedition Bay (42°39′10″N, 130°44′44″E), Sea of Japan.
Site on host: Gills.
Type-specimens: 20 specimens: holotype and 9 paratypes deposited in the ZIN RAS collection (holotype: Reg. No. 12292; paratypes: Reg. Nos. 12293-12295). Additional paratypes are deposited in both the BMNH (Reg. No. 2013.3.28.1-2) and IBSS (Reg. No. 523/1-5) collections.

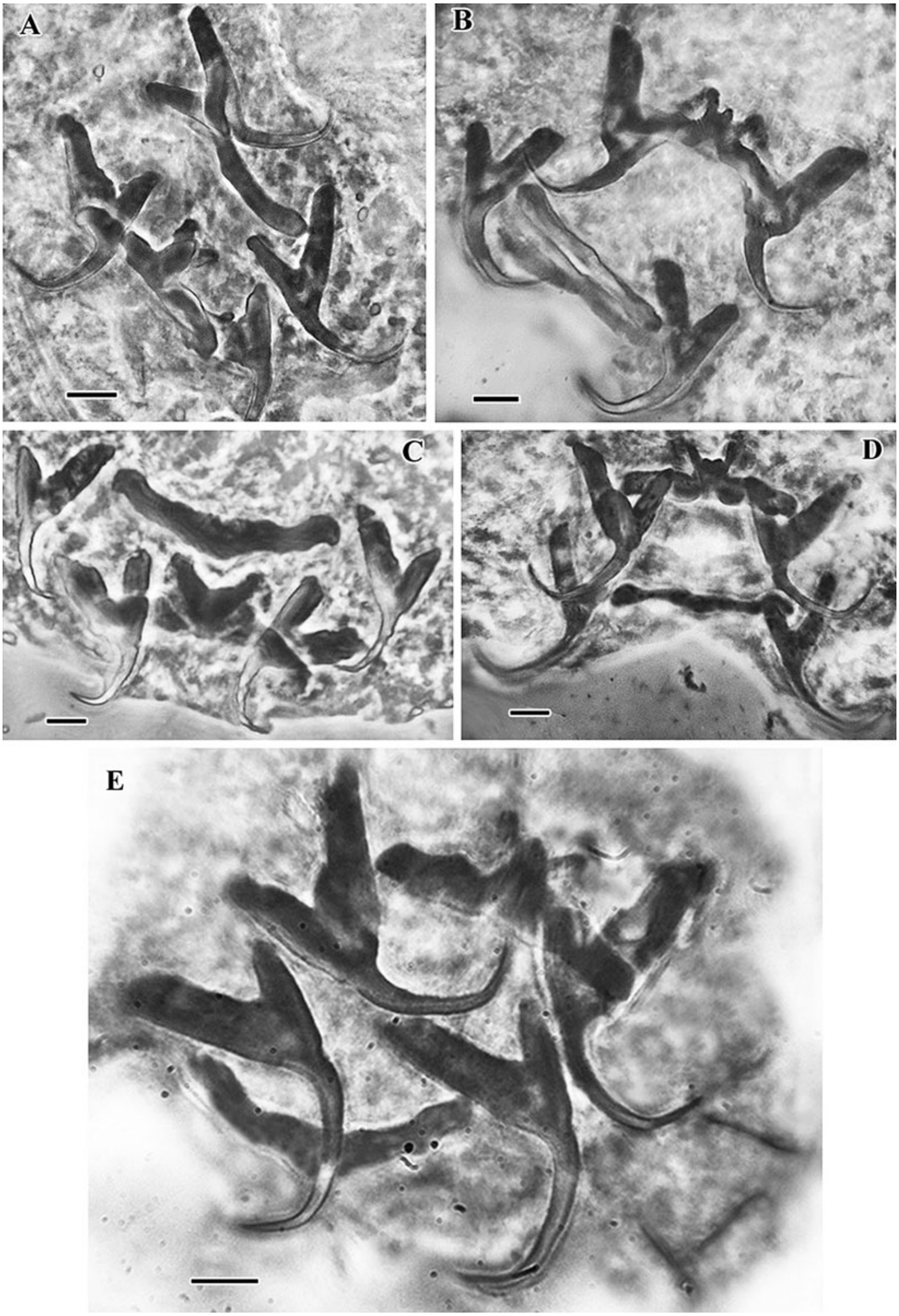

Fig. 2 Haptors of the five species of *Ligophorus* ex *Mugil cephalus* from the Sea of Japan: *L. domnichi* (A), *L. pacificus* (B), *L. chabaudi* (C), *L. cheleus* (D) and *L. abditus* n. sp. (E). *Scale-bar*: 10 µm

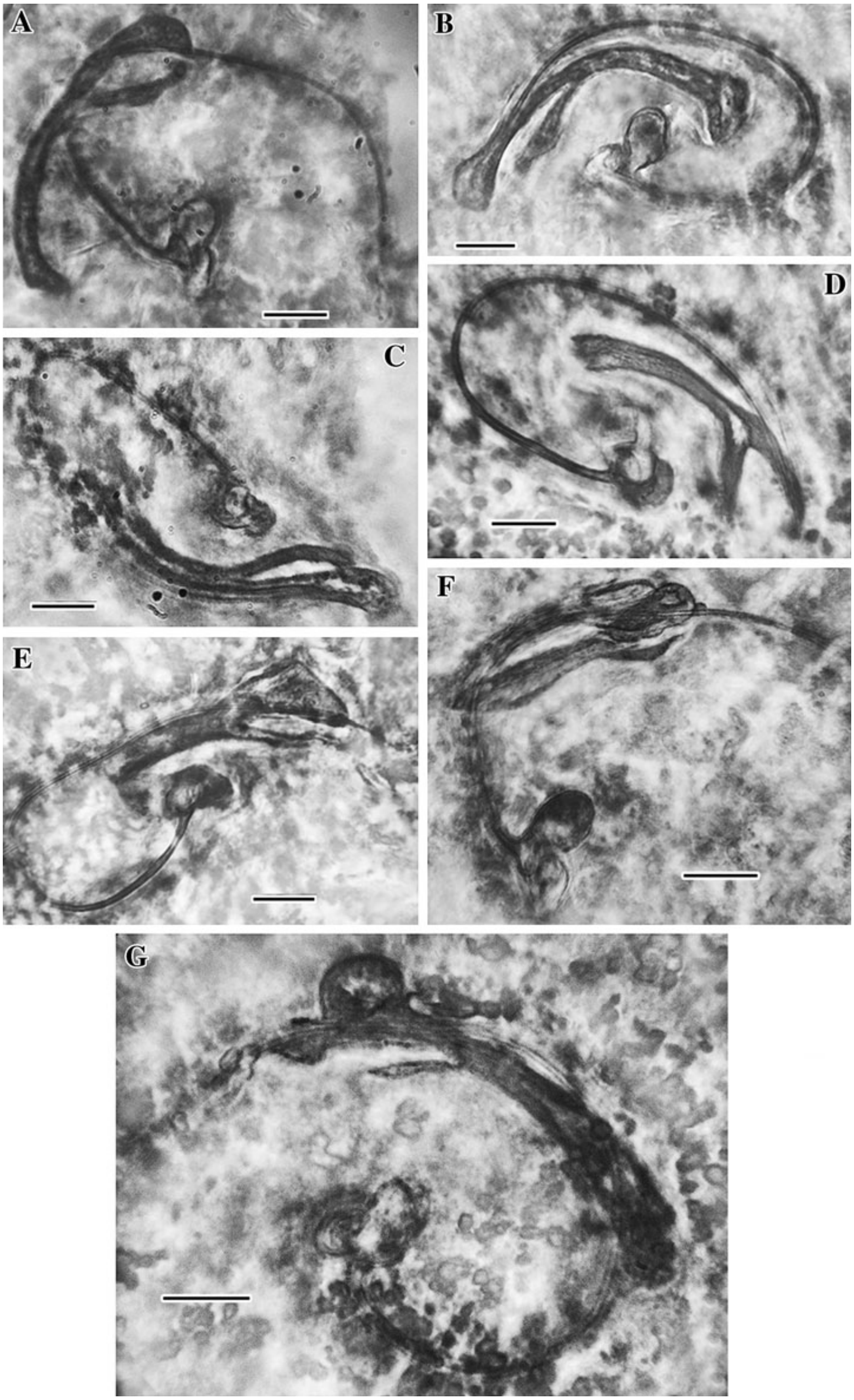

Fig. 3 Copulatory organs of *Ligophorus domnichi* (A, B, C, different views of accessory piece), *L. cheleus* (D); *L. pacificus* (E), *L. chabaudi* (F) and *L. abditus* n. sp. (G) ex *Mugil cephalus* from the Sea of Japan. *Scale-bar*: 10 µm

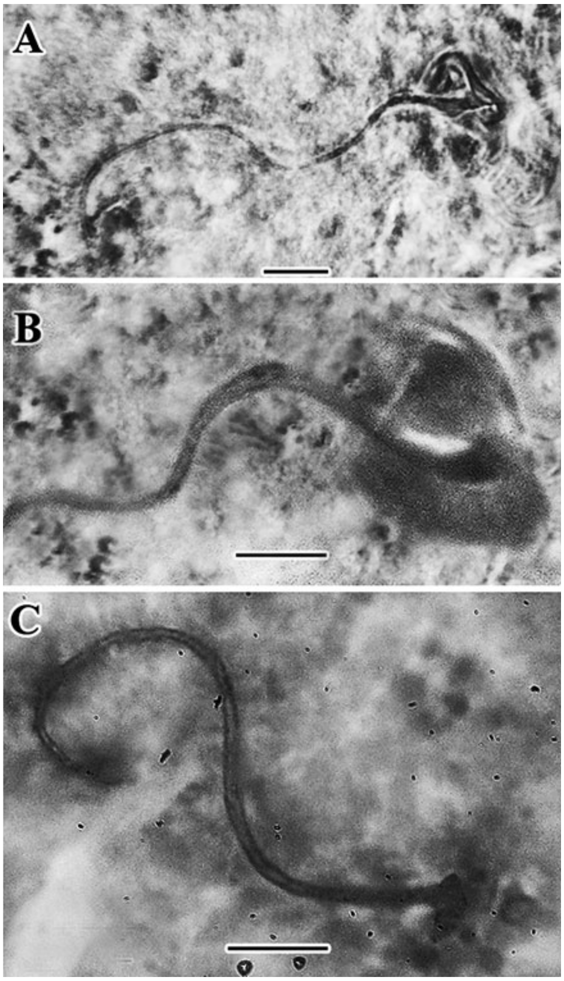

Fig. 4 Vagina of *Ligophorus pacificus* (A), *L. chabaudi* (B) and *L. abditus* n. sp. (C) ex *Mugil cephalus* from the Sea of Japan. *Scale-bars*: 10 µm

Etymology: The species name refers to its similarity to other species from this host and region; from the Latin *abditus*, hidden.

Description (Figs. 1, 2E, 3G, 4C; Table 2)

Body flattened, 420–750 × 70–100. Both pairs of anchors elongate, similar in size and shape (Figs. 1, 2E); distal parts shorter than proximal parts (Table 2: VD *vs* VIP and DD *vs* DIP); inner length of proximal part greater than outer (Table 2: VIP *vs* VOP and DIP *vs* DOP); distal part with point distinctly shorter than shaft (Table 2: VP *vs* VS and DP *vs* DS), with latter at slightly obtuse angle. Dorsal anchors have larger shaft and distal part than ventral anchors (Table 2: DS *vs* VS and DD *vs* VD); ratio VIP/VD *c.*1.5, but DIP/DD 1.2; shaft of dorsal anchor straighter than that of ventral anchor, which is slightly curved proximally. Despite ranges of inner length of both anchors overlapping (Table 2: VI, DI), length of dorsal anchor consistently 2–4 greater than that of ventral anchor. Marginal hooks of larval type, unhinged, consist of sickle formed by short base with small upright thumb, slightly curved blade and straight shaft. Bars equal in length (Table 1: DBW *vs* VBW). Dorsal bar equal in height along its entire width, slightly bowed in shallow V-shape (Fig. 1B). Ventral bar with 2 rather long, digitiform anterior processes set quite far apart (Fig. 1C, E; Table 1: VBP, VBS); ventral side with 2 wide wing-shaped laminae attached to each anterior process and to either side of median knoll; latter with prominent anterior margin, occasionally extending anterior to tip of processes (Figs. 1C, 2E).

Male copulatory organ (MCO) consists of copulatory tube and accessory piece (Figs. 1F, 3G). Copulatory tube C-shaped, thin and long (Table 1: CTL, CTW); expanded base bipartite. Accessory piece forms gutter, U-shaped in cross-section, which bifurcates into 2 unequal terminal lobes at one-third of its length from distal end; upper lobe twice length of lower one (Table 1: APUL *vs* APLL) with almost round expansion (8–10 in height and 10–12 in width) situated 4–5 from distal end of upper lobe; latter is trapezoidal with slightly widened gutter-shaped projection distally. Muscular sheath surrounding copulatory tube attaches to beginning of upper terminal lobe of accessory piece, appearing to connect with round expansion of this lobe.

Vaginal armament typical for genus, in form of hollow, narrow tube with stout walls. Distal end of vagina expanded, funnel-shaped (Figs. 1G, 4C).

Differential diagnosis

Ligophorus abditus n. sp. most closely resembles *L. pacificus* and *L. domnichi*, both described from *Mugil cephalus* in the Sea of Japan (Rubtsova et al., 2007), in the shape of the haptoral hard parts (Fig. 2A, B, E) and the MCO (Fig. 3A, E, G). However, it differs in: (i) the greater length of the distal part of the dorsal anchors (DD 25–27 *vs* 20–22 µm in *L. pacificus* and *L. domnichi*) and the shaft (DS 21–23 *vs* 16–19 in *L. pacificus* and 17–19 µm in *L. domnichi*); (ii) the shape of the MCO accessory piece, which has an upper lobe twice the length of the lower lobe and a rounded expansion 4–5 µm from the trapezoidal distal end, whereas the APUL/APLL ratio is *c.*1.3 and the expansion of the lower lobe is cupola-shaped and tapers distally in both of the compared species (Fig. 3G *vs* 3A, E); and (iii) having a longer ventral anchor shaft (VS 18–20 *vs* 15–17 µm) than *L. pacificus* and a longer proximal part of the ventral anchor (VOP 22–24 *vs* 17–20 µm) than *L. domnichi*. It can also be differentiated from *L. domnichi* by having: (iv) a shorter lower lobe of the MCO accessory piece (APLL 8–12 *vs* 19–26 µm); and (v) a greater expansion of the upper lobe of the MCO accessory piece (8–10 *vs* 5–6 µm), which occupies the middle region of the lobe, as opposed to the distal region in *L. domnichi* (Fig. 3G *vs* 3A).

L. cheleus, which also infects *M. cephalus* in the Sea of Japan, has practically the same dimensions for all morphometric characters as the new species (Table 2), but the latter can be distinguished from *L. cheleus* by: (i) the shape of the ventral bar, which is twice as high in the middle compared to its lateral parts, and has a median knoll with a prominent anterior margin, occasionally extending above the extremities of the anterior processes, rather than a median knoll with a V-shaped hollow that does not extend as far as the anterior processes, as in *L. cheleus* (Fig. 2E *vs* 2D); and (ii) the shape of the MCO accessory piece, which has longer and unequal terminal lobes, with the upper one bearing a pronounced expansion, rather than both lobes being straighten and equal in width and shape, as in *L. cheleus* (Fig. 3G *vs* 3D).

From *L. chabaudi*, another species found on the same host in the Sea of Japan, *L. abditus* differs in: (i) the greater length of the distal part of the dorsal anchor (DD 25–27 *vs* 20–22 µm in *L. chabaudi*) and shaft (DS 21–23 *vs* 14–16 µm in *L. chabaudi*); (ii) the shape of the ventral bar with a prominent anterior margin of the median knoll, rather than a V-shaped hollow on its anterior margin; (iii) the greater length of the MCO accessory piece (APL 48–60 *vs* 33–40 µm); and (iv) the shape of the MCO accessory piece, which has an expansion of the upper lobe to which the muscular sheath surrounding copulatory tube attaches at a single point and through which the copulatory tube does not pass, rather than possessing a separate oval dilatation which is only connected to the upper lobe of the accessory piece, to which the distal end of the muscular sheath attaches over its entire length and through which the copulatory tube passes.

Among the other species of *Ligophorus*, i.e. *L. leporinus* (Zhang & Ji, 1981) Gusev, 1985, *L. chongmingensis* Hu & Li, 1992, *L. chenzhenensis* Hu & Li, 1992 and *L. kaohsianghsieni* (Gusev, 1962) Gusev, 1985, reported from *M. cephalus* in the NW Pacific region (Zhang & Ji, 1981; Hu & Li, 1992; Zhang et al., 2003), only *L. chenzhenensis* appears similar to the new species in the shape of the MCO. However, *L. abditus* differs from this species in the greater lengths of: (i) the dorsal anchors (DI 42–50 *vs* 37–38 µm); (ii) both roots of the dorsal anchors (inner root 19–23, outer root 9–11 *vs* 15–17 and 6–8 µm); (iii) the inner root of the ventral anchor (18–22 *vs* 11–14 µm); (iv) the copulatory tube (CTL 90–120 *vs* 59–74 µm); (v) the MCO accessory piece (APL 48–60 *vs* 38–45 µm); and (vi) the vagina (VL 60–68 *vs* 39–57). It also differs in the shape of the MCO accessory piece, which has the lower lobe shorter than the upper lobe and the latter with a pronounced expansion, rather than a longer lower lobe which is constant in width and has a turned-in distal end (data for *L. chenzhenensis* from Hu & Li, 1992). With regard to the other species mentioned above, *L. abditus* differs significantly in relation to both haptoral and MCO structures.

Of the six species of *Ligophorus,* reported from *M. cephalus* in other regions, i.e. *L. mugilinus* (Hargis, 1955) Euzet & Suriano, 1977 in the Gulf of Mexico, *L. huitrempe* Fernandez, 1987 from off Chile, and *L. mediterraneus* Sarabeev, Balbuena & Euzet, 2005, *L. cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006, *L. vanbenedenii* (Parona & Perugia, 1890) Euzet & Suriano, 1977 and *L. szidati* Euzet & Suriano, 1977, all from the Mediterranean Sea (Hargis, 1955; Fernandez-Bargiela, 1987; Sarabeev et al., 2005; Rubtsova et al., 2006; Pankov, 2011), only *L. cephali* has similarities with *L. abditus* in the shape of the haptoral hard parts and MCO. However, *L. abditus* differs from this species in the greater: (i) inner length of the proximal part of the ventral anchor (VIP 28–34 *vs* 25–27 µm); (ii) span between the roots of both sets of anchors (VSR 22–25, DSR 20–24 *vs* 17–21 and 13–18 µm); (iii) height of the ventral bar (VBH 11–19 *vs* 5–10 µm); and (iv) length (APL 48–60 *vs* 35–43) of the MCO accessory piece. It can also be differentiated by the shape of the MCO accessory piece, which has an upper lobe with a pronounced expansion situated 4–5 µm from its trapezoid distal end, rather than an upper lobe which is equal in width along its entire length and tapers at its distal extremity.

Morphometric analysis of the five species of *Ligophorus*

Principal Component Analysis (PCA) was carried out on four separate datasets of morphological dimensions: eight characters for each anchor pair, four characters for the ventral bar and five characters for the MCO (data from 134 specimens).

Eight dimensions describing the main parameters of the anchors were reduced to two principal components (PCs, i.e. factors) describing 68% of the overall variance for the ventral anchor (Fig. 5A) and 79% for the dorsal anchor (Fig. 5C).

Generally, both pairs of anchors in all five species represent a single morphological type of anchor, with the distal part having a greater length than the proximal part (Table 2: VD *vs* VIP), a relatively long, rather straight shaft which forms an angle of >100° with the proximal part (Fig. 2), and a relatively short point with a length of about half the shaft length (VP/VS 0.5–0.6). Moreover, the ranges for most of the anchor measurements overlap in the case of all five species (Table 2). However, specimens of *L. pacificus* were separated from *L. cheleus* and *L. abditus* n. sp. on the PCA plot run on metrical data of the ventral anchor (Fig. 5A), mainly due to the shorter main part (VM), shaft (VS) and the distal part (VD) (Fig. 5B). Moreover, since the latter two measurements and point length (VP) have opposite reactions relative to the axis of Factor 1 (Fig. 5B), their combination in each specimen results in a difference in the proportion of the distal part of the ventral anchor between *L. pacificus* and the other two species.

These same three species clearly separated from each other on the plot of the PCA run on the morphometry of the dorsal anchor (Fig. 5C). In this plot, data for *L. abditus* are displaced to positions indicating an increased inner length (DI) measurements compared to *L. cheleus* and main part (DM), distal part (DD) and shaft (DS) measurements compared to *L. pacificus*. The lengths relating to the proximal part (DIP, DOP and DSR) and point (DP) contributed most to the separation of *L. pacificus* from *L. cheleus* (Fig. 5D). The other two species, *L. domnichi* and *L. chabaudi*, have an intermediate position between *L. pacificus* and *L. cheleus* on both plots (Fig. 5A, C).

The four dimensions describing the main parameters of the ventral bars were reduced to two PCs (factors) describing 79% of the overall variance (Fig. 6A). Specimens of *L. chabaudi* were clearly separated in this plot from three of the species, *L. abditus*, *L. cheleus* and *L. domnichi*, by having the largest ventral bar and span between the anterior processes (Fig. 6B). The ventral bar of the latter three species and *L. pacificus* are of the same morphological type, i.e. with a median knoll which protrudes well above the anterior margin of the remaining part of the bar and with the anterior processes positioned relatively closely together (Fig. 2A, B, D, E; Table 2: VBS), which distinguishes them from *L. chabaudi* (Fig. 2C; Table 2: VBS).

The five dimensions describing the main proportions of the MCO were reduced to two PCs (factors) which explained 77% of the overall variance (Fig. 7A). Specimens of *L. domnichi* were clearly separated in this plot from the other species by having the greatest length for the MCO accessory piece and its distal lobes (Fig. 7B).

None of the five species analysed were separated from each other by the size and shape of the dorsal bars or the length of the vagina.

Consequently, in the PCA, *L. abditus* was clearly separated from the four morphologically most similar species of *Ligophorus* from *M. cephalus* in the Sea of Japan by the proportions of the distal part of the dorsal anchor (Fig. 5C), from *L. chabaudi* by the proportions of the ventral bar (Fig. 6A), from *L. domnichi* by the proportions of the MCO accessory piece (Fig. 7A), and from the remaining two species, *L. pacificus* and *L. cheleus*, by the proportions of the anchors (Fig. 5A, C).

Discussion

Among *Ligophorus* spp., only *L. chongmingensis* and *L. chenzhenensis* have previously been recorded in the Yellow Sea (Hu & Li, 1992; Zhang et al., 2003), of which the Bohai Sea forms the innermost bay. These species have not been found in the Sea of Japan; neither has *L. leporinus*, which was described from *Mugil cephalus* in the East China Sea (Zhang & Ji, 1981). In the present study, four species (Table 1) which have been found earlier in the Sea of Japan are identified among the material in the ZIN RAS collection from *M. cephalus* caught in the Yellow Sea.

Moreover, representatives of *Ligophorus*, which were found in *M. cephalus* from the East China Sea

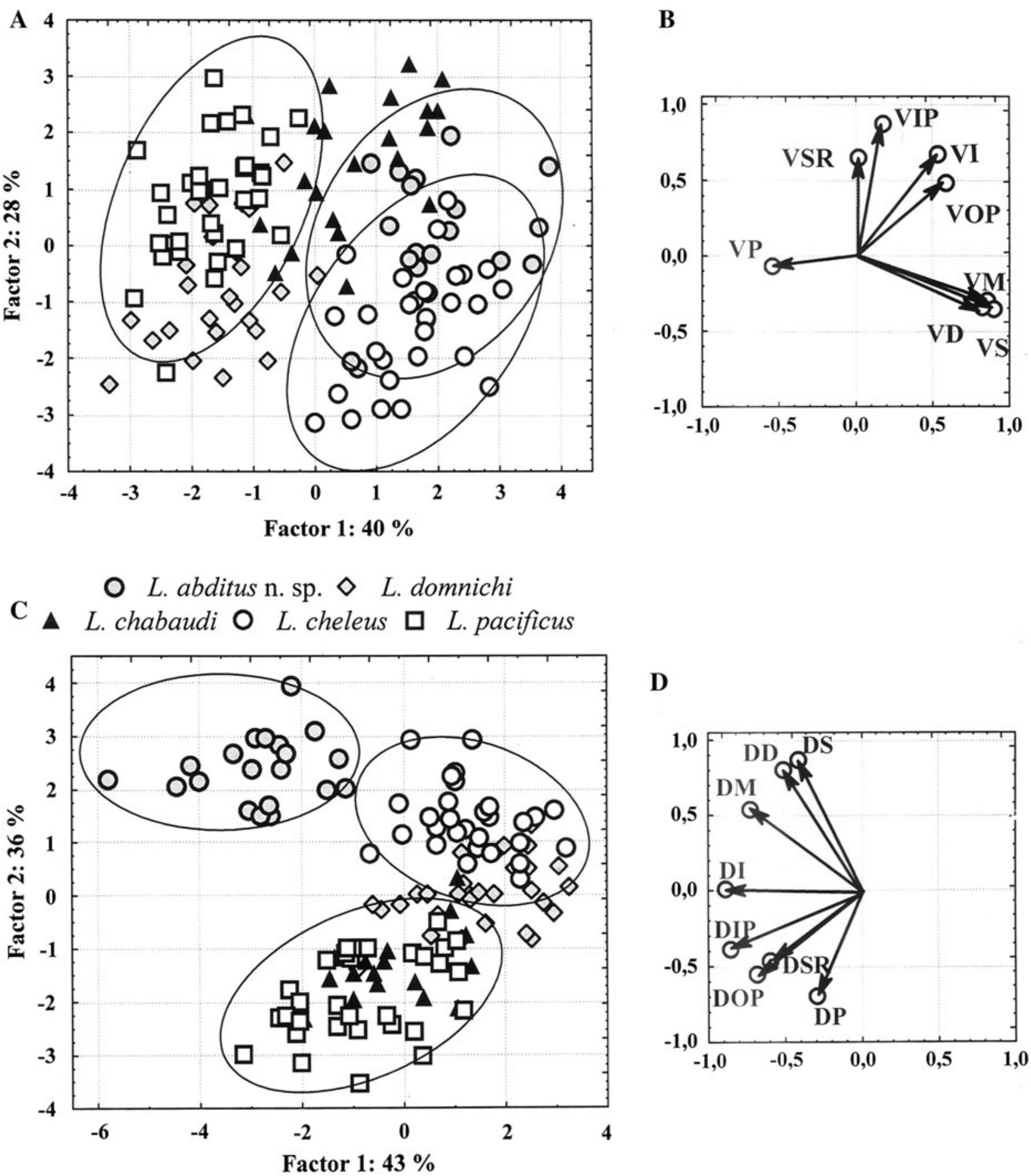

Fig. 5 A,C. PCA plots of 134 *Ligophorus* specimens based on their scores in the first plane of the PCA run on metrical data for eight characters of the each pair of anchors: *L. abditus* n. sp. (n = 20); *L. domnichi* (n = 30); *L. chabaudi* (n = 22); *L. cheleus* (n = 30); *L. pacificus* (n = 32); B,D. PCA plots of the contributions made by these characters for the first two factors. Ellipse coefficient, 90%; → , direction of increasing measurements

and designated as *L. vanbenedenii* (see Zhang et al., 2001), were probably a mix of other species, but an illustration of the MCO, typical for *L. pacificus*, is presented in their figure 10-72.4A (p. 173), and an MCO, which is very similar to that of *L. chabaudi*, is presented in their figure 10-72.4B (p. 173). The latter

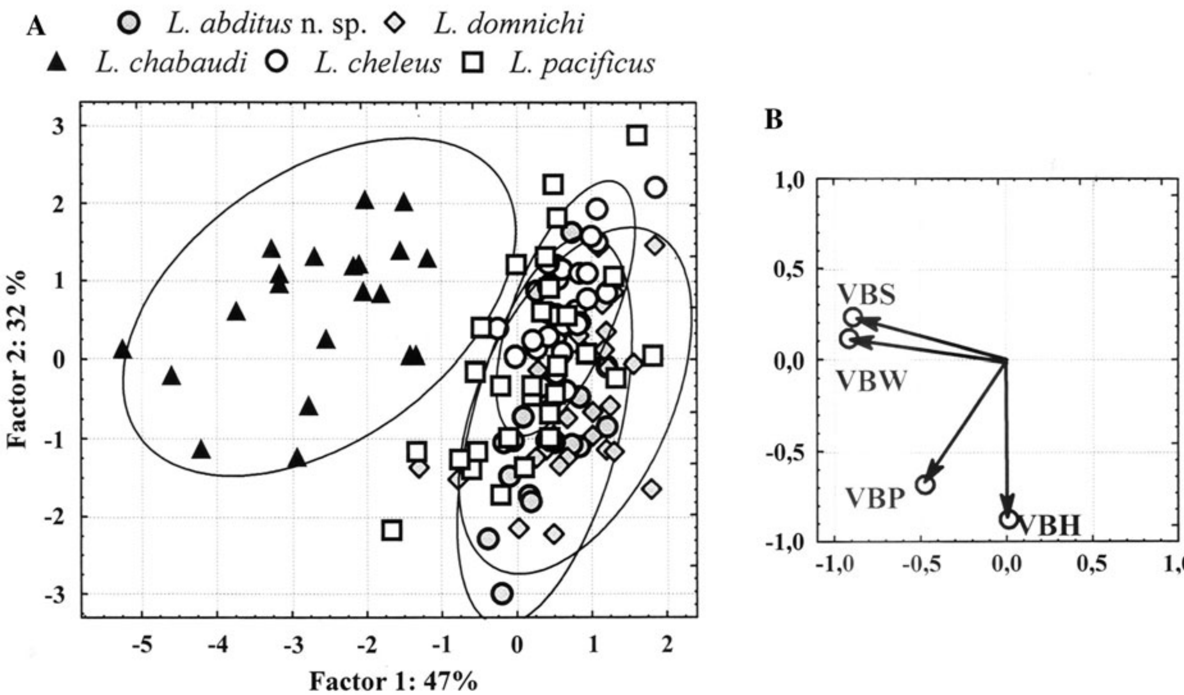

Fig. 6 A. PCA plot of 134 *Ligophorus* specimens based on their scores in the first plane of the PCA run on metrical data for four characters of the ventral bar: *L. abditus* n. sp. (n = 20); *L. domnichi* (n = 30); *L. chabaudi* (n = 22); *L. cheleus* (n = 30); *L. pacificus* (n = 32); B. PCA plot of the contributions made by these characters for the first two factors. Ellipse coefficient, 90%; →, direction of increasing measurements

species has subsequently been reported from this sea (Zhang et al., 2003).

Thus four of the five species (*L. chabaudi*, *L. cheleus*, *L. domnichi* and *L. pacificus*) recorded as parasitising *M. cephalus* in the Sea of Japan in the present work also occur in the Yellow Sea. *L. pacificus* and *L. chabaudi* have also been found in the East China Sea (Zhang et al., 2003) and *L. chabaudi* also occurs in the Mediterranean Sea (Euzet & Suriano, 1977).

All of the investigated species parasitising *M. cephalus* in the Sea of Japan exhibit a similarity in the morphology of their attachment apparatus, having the same type of anchors, although these differ slightly in their dimensions (Fig. 2; Table 2). They also have a similar reproductive hard-part, namely the MCO accessory piece, with a well-marked distal bifurcation, which also occurs in all known species from the Atlantic region but is absent in most species of this genus from the Indo-Pacific (Zhang & Ji, 1981; Dmitrieva et al., 2012; Soo & Lim, 2012).

In the present study, all five species were found on the same host specimens in a single sample from the Sea of Japan. The concurrent presence and morphological similarity of these species permits some conclusions concerning the sympatric and synxenic divergence of these apparently closely related species, which has resulted in uniform attachment structures and a relatively close similarity of the MCO.

Among the species analysed, *L. chabaudi* is morphologically the most distinctive due to the presence of a separate oval dilatation, which resembles a continuation of the muscular sheath surrounding the copulatory tube and is associated with the upper lobe of the MCO accessory piece (Fig. 3). The presence of this species in the Mediterranean Sea, as well as the morphological resemblance between *Ligophorus* spp. from *M. cephalus* in the Sea of Japan and *L. cephali*, which also occurs in this same host in the Mediterranean region (Blasco-Costa et al., 2012), suggest that these species may have had a common centre of origin. A close phylogenetic relationship between *L.*

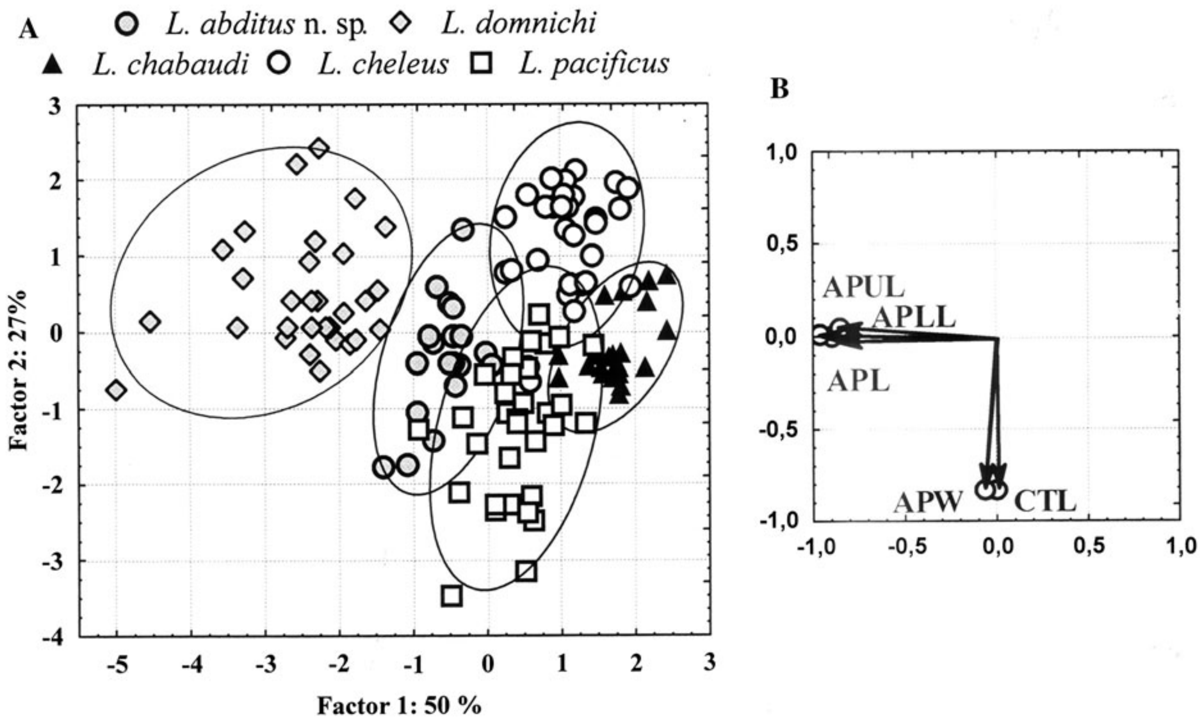

Fig. 7 A. PCA plot of 134 *Ligophorus* specimens based on their scores in the first plane of the PCA run on metrical data for five characters of the MCO: *L. abditus* n. sp. (n = 20); *L. domnichi* (n = 30); *L. chabaudi* (n = 22); *L. cheleus* (n = 30); *L. pacificus* (n = 32); B. PCA plot of the contributions made by these characters for the first two factors. Ellipse coefficient, 90%; →, direction of increasing measurements

chabaudi and *L. cephali* has recently been supported by Blasco-Costa et al. (2012) in a molecular phylogeny.

Moreover, a recent study of the molecular phylogeny of grey mullets (Durand et al., 2012) has revealed the presence of 14 mitochondrial lineages in the '*Mugil cephalus* species complex', three of which occur sympatrically off Taiwan (in the East China Sea), and one of these is related to lineages in the Mediterranean Sea and off Atlantic coast of Africa. Furthermore, a phylogenetic relationship between *M. cephalus* from the Mediterranean Sea and *Liza haematocheilus* (Temminck & Schlegel) (syn. *Mugil soiuy* Basilewsky) from the Sea of Japan has also been demonstrated (Turan et al., 2005).

The presence of three mitochondrial lineages of the *M. cephalus* complex in the East China Sea, which are apparently reproductively isolated species (Durand et al., 2012), may explain the speciation of *L. leporinus*, which is morphologically very distinct from other species infecting *M. cephalus* in this region of the Pacific.

The species of *Ligophorus* known on *M. cephalus* in the Gulf of Mexico and off the coast of Chile differ significantly in the morphology of both the haptor and the hard-parts of the reproductive system from the species parasitising the same host in the NW Pacific and have likely diverged apparently as a result of the long-term geographical isolation of their hosts.

Nevertheless, further comments on the relationships between *Ligophorus* spp. and their hosts are limited by our lack of knowledge of representatives of this genus on mugilids from the Indian, East Pacific and South Atlantic Oceans.

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