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Redescriptions of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 and *L. chabaudi* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae), with notes on the functional morphology of the copulatory organ

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Abstract Redescriptions of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 and *L. chabaudi* Euzet & Suriano, 1977 based on original material from the Black Sea, the Mediterranean Sea and the Sea of Japan are presented. A comparison of samples of these two species from different regions was carried out with the aid of principal components analysis. The occurrence of *L. chabaudi* on *Mugil cephalus* in the Sea of Japan was confirmed. The functional morphology of the male copulatory organ was examined, and the use of the shape of this structure in the taxonomy of *Ligophorus* Euzet & Suriano, 1977 is discussed.

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Introduction

The history of *Ligophorus* Euzet & Suriano, 1977 goes back to 1871 when van Beneden published a brief description of a gill parasite of the mugilid fish *Chelon labrosus* (Risso) under the name *Gyrodactylus* sp. (van Beneden, 1871; see also Parona & Perugia, 1890). Until the middle of the last century this monogenean was considered as a species [recorded by the most authors under the name *Ancyrocephalus vanbenedenii* (Parona & Perugia, 1890) Johnston & Tiegs, 1922] with an exceptionally wide geographical distribution and host range, exhibiting a high level of intraspecific variability (e.g. Bychowsky, 1949; Gusev, 1955; Ergens, 1960). After Euzet & Suriano (1977) established *Ligophorus* for *L. vanbenedenii*, *L. mugilinus* (Hargis, 1955) and nine newly-described species, the number of species in this genus increased considerably. Euzet & Suriano showed that each investigated species of the Mugilidae was infected by a range of host-specific species. To date, several species of *Ligophorus* are known from every mullet species which has been examined.

The *Ligophorus* spp. parasitising the cosmopolitan host *Mugil cephalus* L. from the Mediterranean Basin and the North-western Pacific are the most thoroughly studied. Even from this host in these locations, new species are still being described, and two new species from the Mediterranean Sea and three from the Sea of Japan have recently been reported (Sarabeev, Balbuena & Euzet, 2005; Rubtsova et al., 2006a; Rubtsova, Balbuena & Sarabeev, 2007).

One of these is *L. cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006, which was distinguished from the Mediterranean *L. chabaudi* Euzet & Suriano, 1977. Dmitrieva & Gerasev (1996), describing specimens of *L. chabaudi* collected from *M. cephalus* in the Black Sea, noted differences in some of the characters of this species compared with those reported by Euzet & Suriano (1977). Miroshnichenko & Maltsev (2004), when comparing *L. chabaudi* from the Black Sea with its description from the Mediterranean Sea by Euzet & Suriano (1977), also pointed out differences in some details. The comparison of our material, which was used for descriptions of *L. chabaudi* from *M. cephalus* in the Black Sea (Dmitrieva, 1996; Dmitrieva & Gerasev, 1996; Dmitrieva et al., 2007), with the type-material of *L. cephali* confirms that *L. chabaudi* of Dmitrieva & Gerasev (1996) is identical with the latter species. However, the original description of *L. cephali* contains significant inaccuracies and mistakes, which have already been noted in our redescription of *L. mediterraneus* Sarabeev, Balbuena & Euzet, 2005 (see Dmitrieva et al., 2009). As in the case of *L. mediterraneus,* the point to shaft angle of both anchors is highly variable, with a variation of 21° in *L. cephali* and 26° in *L. chabaudi* (Rubtsova et al., 2006a, pp. 488, 492). The impossibility of using the differences in the shape of the dorsal and ventral sides of the ventral bar for differentiating species of *Ligophorus*, and the determination of intrageneric groups of species using the degree of expression (heavily sclerotised, massive, prominent, etc.) of the median knoll (=median process) of the ventral bar, have already been discussed by us in a previous paper (Dmitrieva et al., 2009). We showed that a median knoll on the dorsal side of the ventral bar is present in all species of *Ligophorus* and is necessary for the proper functioning of this bar. This structure exhibits intraspecific variability in the degree of its development, so it is essential to view comparative material from the same side for an accurate comparison of the shape of the ventral bar in different species.

Neglecting the function of the haptoral structures when describing their morphology has lead to the use of the position of the proximal end of the marginal hooklet filament loop relative to its shaft as a character useful for differentiating *L. cephali* and *L. chabaudi* (see Rubtsova et al., 2006a, p. 488 and fig. 1D, I). Firstly, all representatives of *Ligophorus* have larval-type marginal hooklets (Gusev, 1985), with a similar shape in different species, since they have not been subjected to adaptive pressures in terms of attachment to the host gills in these four-anchored monogeneans. Moreover, as shown for different species of the Dactylogyridea (e.g. Gerasev, 1981, fig. 20), the filament loop of the marginal hooklets is attached by a tab to the aperture in the body-wall through which the point of the sickle passes and thus appears to move along the point, dependent upon the functional state of these hooklets. When the marginal hooklet is fully extruded from the haptor, the filament loop is close to the tip of the point and holds the hooklet in this position, and, when the hooklet is withdrawn into the haptor, the proximal end of the filament loop moves closer to the proximal end of the shaft. This apparent sliding movement and correspondingly position of the filament loop depends on the contraction of small muscle bundles which attach to the proximal end of the loop. Thus the position of the filament loop relative to the shaft can in no way be used for species differentiation.

The description of the male copulatory organ in *L. cephali* and *L. chabaudi* (see Rubtsova et al., 2006a) does not agree with its function, as originally described by Llewellyn & Anderson (1984) and confirmed by the present study. As a result of the inaccurate description of the shape of the accessory piece of the copulatory organ, some species of this genus were wrongly grouped by the position of the entrance of the copulatory organ tube into the accessory piece, and *L. chabaudi* was erroneously considered as a species morphologically closely related to both *L. cephali* and *L. pilengas* Sarabeev & Balbuena, 2004.

In view of the above-mentioned comments, redescriptions of *L. cephali* and *L. chabaudi* are presented below. In addition, the occurrence of *L. chabaudi* in the Sea of Japan is confirmed and data on this species from this region are reported for the first time.

Materials and methods

The redescription of *Ligophorus cephali* was based on data from the article of Dmitrieva, Gerasev & Pron'kina (2007) and on the re-examination of the specimens used in that work. Measurements and drawings of *L. chabaudi* are based on 20 specimens collected from the gills of four individuals of *Mugil cephalus*, 29–39 cm long, captured in the Mistras

Lagoon (39°54′N, 8°28′E), Sardinia, western Mediterranean Sea, and on 18 specimens collected from the gills of four specimens of *M. cephalus*, 35–40 cm long, captured in the Zaliv Pos'yeta (42°42′N, 130°49′E), Sea of Japan. For comparison, 10 specimens of *L. domnichi* Rubtsova, Balbuena & Sarabeev, 2007, 10 specimens of *L. pacificus* Rubtsova, Balbuena & Sarabeev, 2007 and eight specimens of *L. cheleus* Rubtsova, Balbuena & Sarabeev, 2007 from the same host specimens in the Japan Sea were used. All monogeneans were collected from freshly caught fish and then immediately mounted in glycerine-jelly (prepared with 0.5 g carbolic acid). Some of the type-material of *L. cephali* from the British Museum Natural History Collection (BMNH) at the Natural History Museum, London (nos 2004.11.4.1-7) was also examined. Drawings and light micrographs were made using a Carl Zeiss Amplival microscope (magnification 2,000×) with phase contrast illumination, using a drawing tube and an Olympus C180 digital camera. The measurement scheme of Dmitrieva, Gerasev & Pron'kina (2007) was used with minor changes (Fig. 1), and the abbreviations for the features measured are explained in Table 1; but, in order to enable a comparison with the data of Rubtsova et al. (2006a), measurements of the roots of the anchors (VIR, DIR—inner roots; VOR, DOR—outer roots) and main part of the anchor (VM, DM) were also recorded. All measurements are given in micrometres, with a resolution of 1 µm. Measurements are presented using the mean, standard error and range of variation. Principal Components Analysis (PCA) was carried out and a graphical representation of the specimen groupings was produced using the Statistica 6 for Windows (StatSoft, Inc. 2001).

Ligophorus cephali Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006

Syns *L. chabaudi* Euzet & Suriano, 1977 *sensu* Dmitrieva & Gerasev (1996), Sarabeev & Balbuena (2004) and Miroschnichenko & Maltsev (2004)

Host: *Mugil cephalus* L.
Locality: Off Sevastopol, Crimean peninsula, Black Sea (44°35′N, 33°30′E).
Site: Gills.
Material examined: 18 specimens deposited in the Institute of Biology of the Southern Seas, Sevastopol (Nos 255/23, 255/32, 256/12, 256/23) and in the Zoological Institute, St Petersburg (Nos 12182–12184, 12187).

Redescription (Figs. 1–5; Table 1)

Body flattened, 838 ± 33 (700–1,000) × 162 ± 10 (110–200). Haptoral armament consists of 2 pairs of anchors, 14 marginal hooklets and 2 bars, characteristic of genus (Euzet & Suriano, 1977). For measurements of anchors, bars and sclerotised parts of reproductive system, see Table 1. Both pairs of anchors elongate, with similar shape and length (Fig. 1A, B; Table 1: VI, DI); inner length of proximal part greater than outer length (Table 1: VIP vs. VOP and DIP vs. DOP); proximal part longer than distal (Table 1: VIP vs. VD and DIP vs. DD); proximal and distal parts form obtuse angle of ca. 102° (Fig. 1: angle II). Distal part of anchors consists of shaft and point; latter is at angle of ca. 95° (Fig. 1: angle I). Marginal hooklets of larval type, consist of sickle and shaft without widened handle; filament loop (Fig. 2A) attached by tab to aperture in body-wall through which hooklet sickle passes, its position in relation to hooklet varying dependent on degree of protrusion of sickle. Bars equal in length (Table 1: VBW, DBW). Dorsal bar equal in width along its entire length, bowed in middle, with ends down-turned. Ventral bar with 2 long, digitiform anterior processes, which are positioned closely together (Table 1: VBP, VBS; Figs. 1D, E, 3); dorsal side of ventral bar with 2 wide, wing-shaped laminae attached to each anterior process; relatively narrow median knoll, with cupola-shaped anterior margin which is, in most cases, surmounted by Λ-shaped prominence, situated between laminae (Fig. 3B, C).

Male copulatory organ (MCO) consists of tube and accessory piece (Figs. 1F, 4). Copulatory tube C-shaped. Accessory piece forms gutter, U-shaped in cross-section, within which copulatory tube slides, bifurcates into 2 terminal lobes close to its middle; upper[1] lobe and proximal part (prior to bifurcation) are similar in width; lower lobe is much thinner and shorter than upper lobe (Table 1: APLL vs. APUL)

[1] We use the designations 'upper' and 'lower' with respect to the attitude of different parts of the accessory piece based on its position in the figures, as its orientation along longitudinal or transverse axes in live worms was not determined.

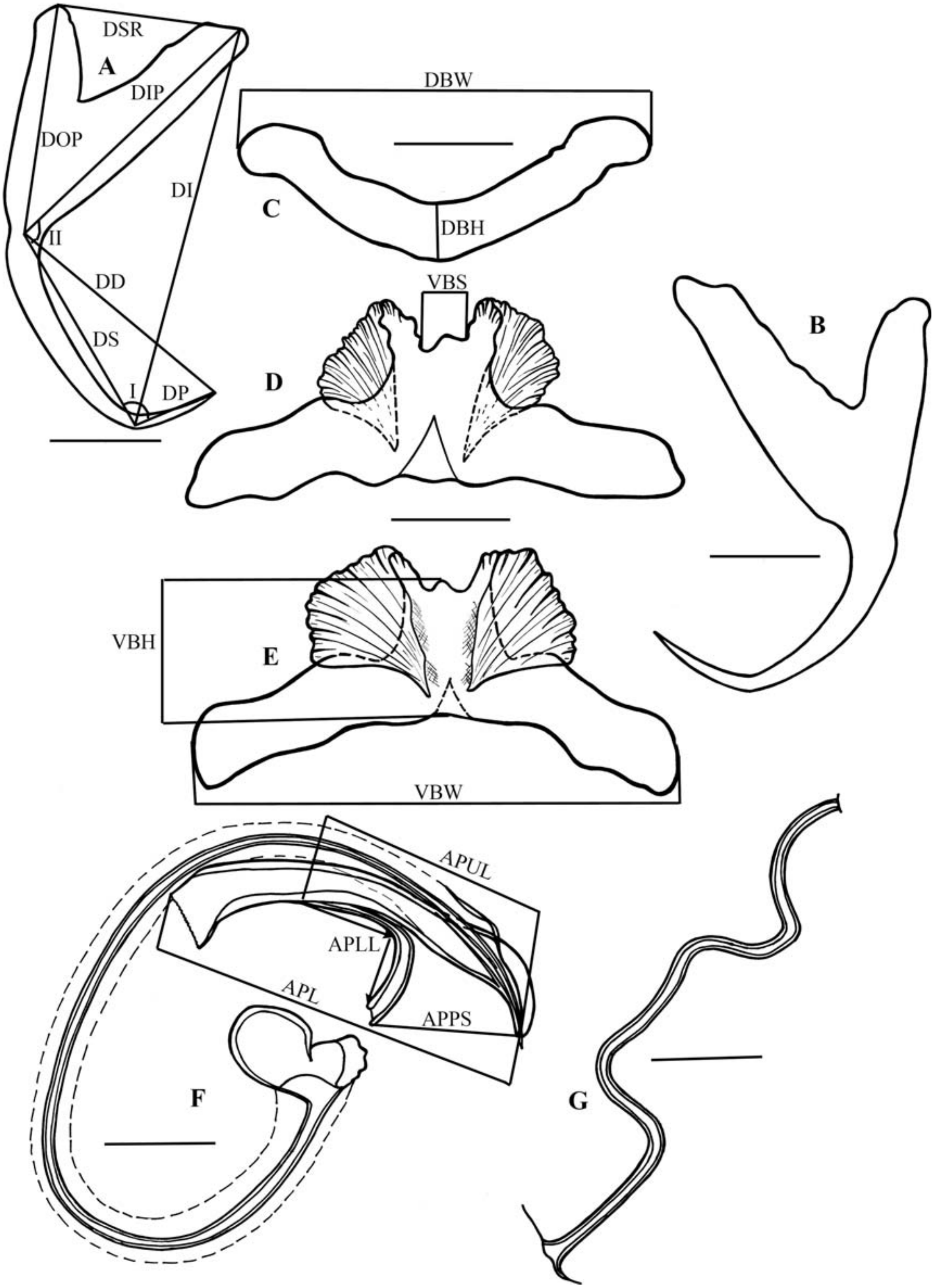


Fig. 1 *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 ex *Mugil cephalus* from the Black Sea. A, dorsal anchor; B, ventral anchor; C, dorsal bar; D, ventral bar (ventral view); E, ventral bar (dorsal view); F, male copulatory organ; G, vagina. *Scale-bars*: 10 µm. See Table 1 for abbreviations

Table 1 Dimensions, as the range (mean ± standard error), of the anchors, bars, male copulatory organ and vagina of *Ligophorus cephali* Rubtsova et al., 2006a from the Mediterranean and Black Seas and *L. chabaudi* Euzet & Suriano, 1977 from the Mediterranean Sea and the Sea of Japan

Sp. of *Ligophorus*	*L. cephali*				*L. chabaudi*		
Source of data	Present study		Rubtsova et al. (2006a)		Present study		
Material Sea No. of specimens	Type Black 5	New Black 18	 Black 27	 Med. 16	Type Med. 10	New Med. 20	New Japan 18
Ventral anchor:							
Inner length (VI)[a]	39–40	35–43 (38.1 ± 0.5)	35–39	35–43	38–43	38–42 (39.8 ± 0.2)	37–43 (39.9 ± 0.5)
Length of main part (VM)	28	26.5–28 (27 ± 0.2)	26–30	25–32	26–29	25–31 (28.1 ± 0.4)	25–28 (26.4 ± 0.3)
Length of shaft (VS)	19	18–21 (19.4 ± 0.3)			17–19	17–20 (18.2 ± 0.1)	17–18 (17.4 ± 0.1)
Length of distal part (VD)	22	20–24 (21.8 ± 0.3)			21–22	20–22 (21.1 ± 0.1)	19–22 (20.3 ± 0.2)
Length of point (VP)	10–11	10–11 (10.6 ± 0.1)	9–11	9–12	10–11	9–10 (9.3 ± 0.1)	9
Inner length of proximal part (VIP)	28–29	25–27 (26 ± 0.3)			28–30	26–31 (29.0 ± 0.3)	29–34 (31.4 ± 0.4)
Outer length of proximal part (VOP)	20–22	20–22 (21 ± 0.2)			20–24	22–29 (25.1 ± 0.3)	24–27 (25.9 ± 0.3)
Span between roots (VSR)	20	17–21 (19.2 ± 0.3)			19–23	20–25 (21.6 ± 0.3)	21–24 (23 ± 0.3)
Length of inner root (VIR)	18–19	12–18 (15.1 ± 0.4)	17–19	15–21	17–21	17–21 (18.5 ± 0.3)	19–23 (21.1 ± 0.3)
Length of outer root (VOR)	9–10	10–11 (10.8 ± 0.1)	9–13	8–12	7–11	12–17 (14.1 ± 0.3)	13–19 (15.8 ± 0.4)
Dorsal anchor:							
Inner length (DI)	39–44	35–40 (38.2 ± 0.4)	36–41	37–43	37–43	36–43 (39.1 ± 0.6)	39–44 (41.3 ± 0.5)
Length of main part (DM)	29–32	26.5–30 (28.5 ± 0.3)	27–32	28–34	28–34	27–31 (29.1 ± 0.3)	28–30 (29.1 ± 0.2)
Length of shaft (DS)	20–23	19–23 (20.8 ± 0.3)			19–24	17–20 (18.8 ± 0.2)	17–19 (17.9 ± 0.2)
Length of distal part (DD)	23–25	21–25 (22.8 ± 0.3)			22–26	21–23 (21.9 ± 0.2)	20–22 (21 ± 0.2)
Length of point (DP)	8–9	9–10 (9.1 ± 0.1)	7–9	8–10	8–9	9–10 (9.8 ± 0.1)	9–10 (9.7 ± 0.1)
Inner length of proximal part (DIP)	27–29	25–30 (28 ± 0.4)			25–29	24–31 (27.2 ± 0.4)	28–32 (30.4 ± 0.3)
Outer length of proximal part (DOP)	19–22	21–23 (22.7 ± 0.2)			17–22	19–23 (20.6 ± 0.2)	20–23 (21.4 ± 0.3)
Span between roots (DSR)	18–20	13–18 (15.4 ± 0.3)			16–20	17–24 (19.4 ± 0.4)	20–23 (21.9 ± 0.2)

Table 1 continued

Sp. of *Ligophorus*	*L. cephali*				*L. chabaudi*		
Source of data	Present study		Rubtsova et al. (2006a)		Present study		
Material	Type	New			Type	New	New
Sea	Black	Black	Black	Med.	Med.	Med.	Japan
No. of specimens	5	18	27	16	10	20	18
Length of inner root (DIR)	17–19	14–18 (15.8 ± 0.4)	15–19	14–18	15–20	14–21 (16.5 ± 0.4)	16–21 (18.6 ± 0.4)
Length of outer root (DOR)	9–10	6–12 (8.9 ± 0.3)	8–11	8–12	7–11	8–12 (9.3 ± 0.2)	9–11 (10.1 ± 0.2)
Marginal hook:							
Total length	13	12–13 (12.5 ± 0.1)	13–15	13–15	13–14	12	11–12 (11.9 ± 0.1)
Sickle length	5	5	6–7	5–6	5–6	5	5
Shaft length	8	7–8 (7.5 ± 0.1)	7–9	7–9	7–8	7	6–7 (6.9 ± 0.1)
Ventral bar:							
Height (VBH)	11–17	5–10 (7.3 ± 0.3)			10–15	10–15 (13.3 ± 0.3)	8–15 (12.2 ± 0.5)
Width (VBW)	41–46	35–40 (37.3 ± 0.4)	35–40	34–43	32–40	46–58 (50.7 ± 0.7)	48–62 (55 ± 1.2)
Length of anterior processes (VBP)	8–11	6–8 (6.8 ± 0.1)			5–8	8–12 (9.8 ± 0.3)	10–15 (11.2 ± 0.4)
Span between processes (VBS)	2–5	3–7 (5.1 ± 0.2)	7–8	5–8	2–4	7–12 (8.9 ± 0.4)	7–13 (9.4 ± 0.3)
Dorsal bar:							
Height (DBH)	5–6	4–6 (5.2 ± 0.1)			4–6	5–9 (7.5 ± 0.3)	6–12 (8.5 ± 0.4)
Width (DBW)	38–55	32–41 (35.7 ± 0.7)	34–38	32–43	32–42	46–57 (51.5 ± 0.8)	47–67 (56.2 ± 1.5)
Copulatory tube:							
Length (CTL)	102–107	93–115 (106 ± 2.2)	60–102	55–105	100–115	100–116 (110.3 ± 1.1)	112–120 (115.2 ± 0.5)
Accessory piece of MCO:							
Length (APL)	35–40	35–43 (38 ± 0.7)	35–37	33–49	34–40	29–40 (35.9 ± 0.7)	33–39 (36.2 ± 0.4)
Width (APW)	5–7	3–5 (4.3 ± 0.2)			5–7	4–5 (4.8 ± 0.1)	4–5 (4.7 ± 0.1)
Length of upper lobe (APUL)	20–25	19–23 (21 ± 0.4)			15–25	12–17 (14.8 ± 0.5)	12–18 (14.7 ± 0.6)
Length of lower lobe (APLL)	16–20	11–18 (15.1 ± 0.5)			12–20	7–10 (9.5 ± 0.4)	9–10 (10 ± 0.2)
Span between upper and lower lobes (APPS)	6–14	3–10 (5.5 ± 0.5)			6–12	3–10 (6.7 ± 0.4)	2–7 (4.7 ± 0.6)

Table 1 continued

Sp. of *Ligophorus*	*L. cephali*				*L. chabaudi*		
Source of data	Present study		Rubtsova et al. (2006a)		Present study		
Material	Type	New			Type	New	New
Sea	Black	Black	Black	Med.	Med.	Med.	Japan
No. of specimens	5	18	27	16	10	20	18
Vagina:							
Length (VL)	58[b]	60–90 (73.3 ± 2.6)	41–66	29–73	57–65[b]	50–66 (55.5 ± 2.7)	48–70 (57.1 ± 2.1)

[a] For measurements see Fig. 1. Additional abbreviations: anchors (VIR, DIR—inner roots; VOR, DOR—outer roots); main part of anchor (VM, DM)—see Euzet & Suriano (1977)

[b] The vagina was visible along the full length only in one specimen from the Black Sea and in three specimens from the Mediterranean Sea, and no more than two-thirds of its length was visible in the remainder

and their extremities may be greatly separated (by as much as 14 µm) (Fig. 4D); upper lobe is connected with neighbouring tegument of ventral surface of worm body by crimped ligament (Fig. 3B); 2 processes arise above and below upper lobe close to its distal end (Fig. 4C), to which muscular sheath surrounding copulatory tube attaches; proximal end of latter attaches to sclerotised flange of expanded base of MCO; lower of these processes is rod-shaped; upper process is widened, scoop-shaped (Fig. 4A).

Vaginal armament is typical of genus, forming hollow, narrow tube with solid walls. Distal end of vagina expanded, funnel-shaped, resembling nail-head in profile (Figs. 1G, 5).

Comments

Three of the seven slides of the type-material of *Ligophorus cephali* deposited in the BMNH collection were examined. There were: no. 2004.11.4.1-3, inscribed as bearing five syntypes from the Kerch Channel; no. 2004.11.4.4 said to bear 12 syntypes from the Gulf of Valencia; and no. 2004.11.4.5-7 labelled as bearing two syntypes from the Mouth of

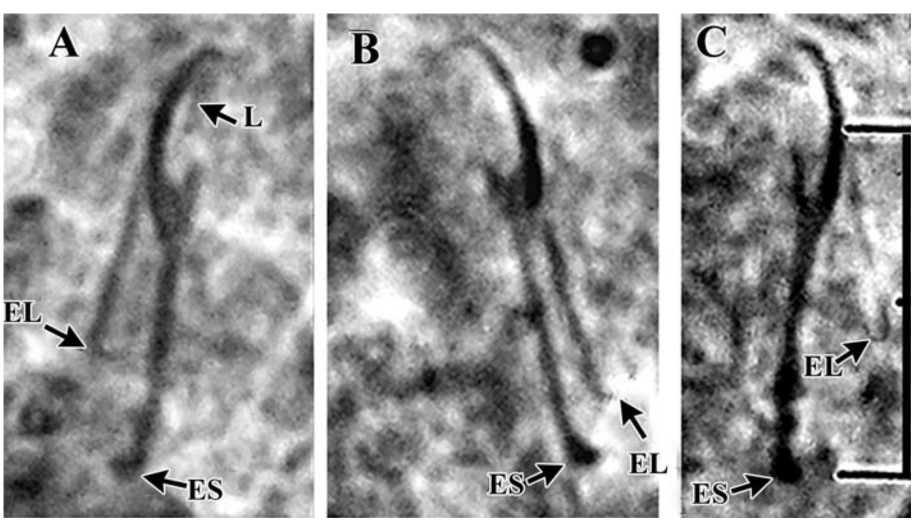


Fig. 2 Marginal hooklets of *Ligophorus chabaudi* ex *Mugil cephalus* from the Mediterranean Sea (A—with proximal end of the filament loop at mid-shaft level; B—with proximal end of the filament loop at a quarter of the shaft length from its proximal end) and *L. cephali* ex *Mugil cephalus* from the Black Sea (C). *Abbreviations*: EL, proximal end of the filament loop; ES, proximal end of the shaft; L, distal part of the filament loop. *Scale-bars*: 10 µm

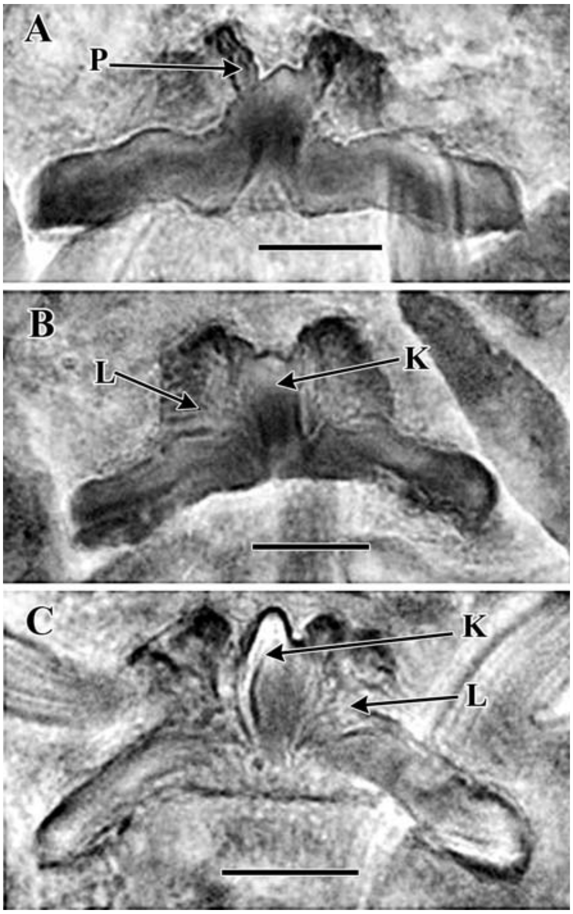


Fig. 3 Ventral bar of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 ex *Mugil cephalus* from the Black Sea. A, ventral view; B–D, dorsal views. *Abbreviations*: L, wing-shaped laminae; P, anterior processes; K, median knoll. *Scale-bar*: 10 μm

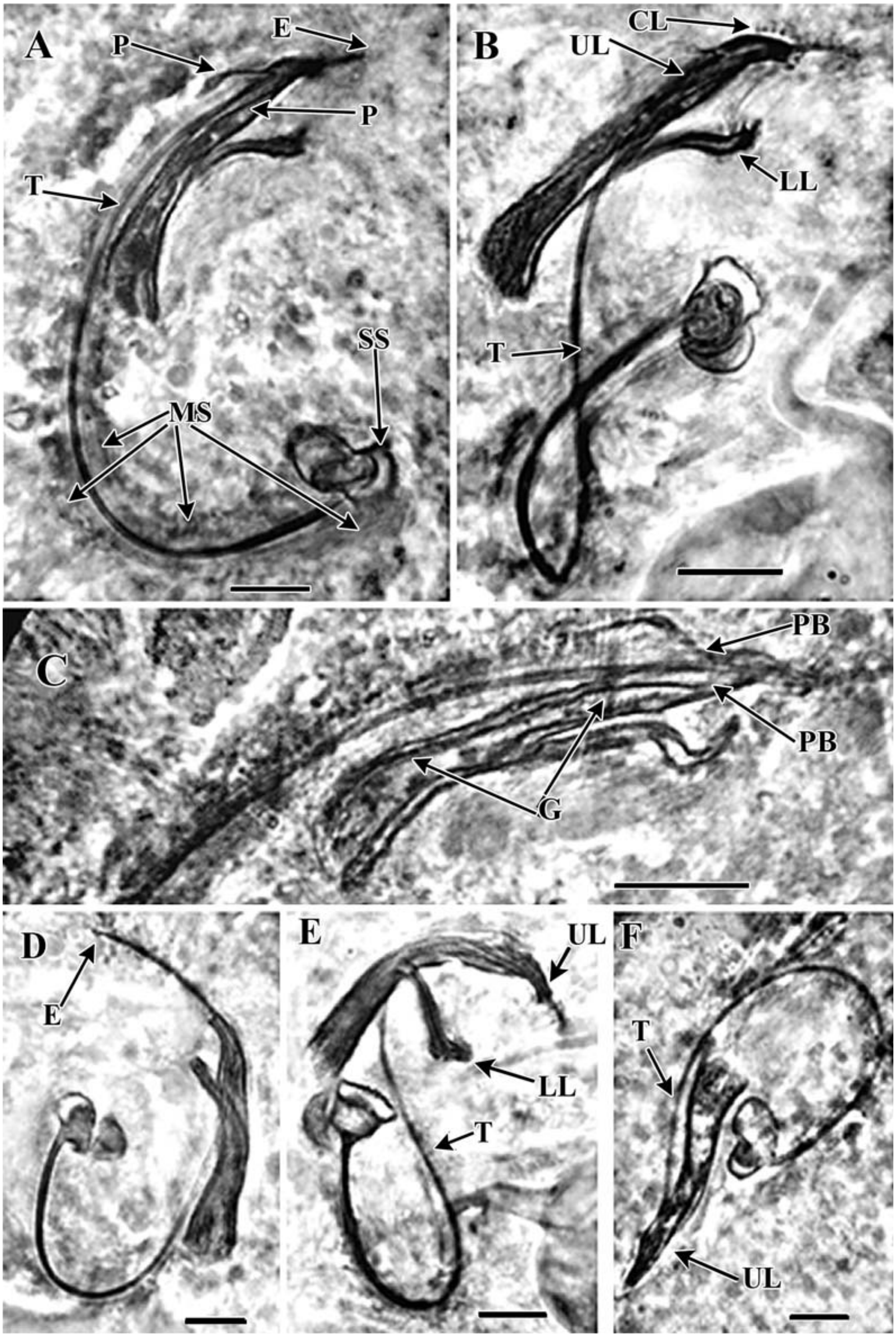
Fig. 4 Male copulatory organ of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 ex *Mugil cephalus* from the Black Sea showing different positions of the copulatory tube relative to the accessory piece (A—"above"; B, F—"below"; D—protruded distally) and with strongly deflected lower lobe (E). *Abbreviations*: CL, crimped ligament attaching the accessory piece to the tegument; E, distal end of the copulatory tube; G, gutter-like main part of the accessory piece; LL, lower lobe; MS, muscular sheath surrounding the copulatory tube; P, processes to which the distal end of the muscular sheath attaches; PB, origin of the processes; SS, sclerotised structure which serves for the attachment of the proximal end of the muscular sheath; T, copulatory tube; UL, upper lobe. *Scale-bars*: 10 μm ▶

the Ebro. On the first slide there are 11 worms, but only five of them are suitable for measuring; the next slide has 10 worms, one of them lacking a haptor; and on the final slide only one of the two worms present is suitable for measuring. Examination of the 15 specimens in adequate condition for investigation revealed that the morphology of the haptoral structures, MCO and vagina agrees with the above redescription in all details. However, the upper limit of the length of the copulatory tube in these syntype-specimens, including one specimen marked 'holotype', is 115 μm (and not 105 μm, see Rubtsova et al., 2006a; Table 1). The lower limits for the length of the copulatory tube and the vagina reported by these authors are about half the size of those observed in both the type- and our own material (Table 1); however, the vagina was visible along its full length (e.g. Fig. 5) in only four type-specimens, no more than two-thirds of its length being visible in the remainder.

Differential diagnosis and remarks

Taking into consideration the measurements presented above and the new details of the morphology of *Ligophorus cephali*, we propose new diagnostic characters for differentiating this taxon from related species of *Ligophorus*.

L. cephali differs from *L. chabaudi* (Fig. 4; Table 1), which also infects *Mugil cephalus* in the Mediterranean, in: (1) as previously pointed by Rubtsova et al. (2006a), the smaller width of the ventral bar (32–45 vs. 46–62 μm); (2) the shape of the anchors, the proximal and distal parts of which form a more acute angle (100–103° vs. 110–112°); (3) the ventral bar, which has wider, wing-shaped laminae, a more closely positioned anterior processes and a narrower median knoll (Figs. 1E and 3B, C) with a cupola-shaped anterior border in most cases surmounted by a Λ-shaped prominence, whereas *L. chabaudi* has a median knoll with, in most cases, a V-shaped hollow on its anterior margin (Figs. 5E, 6B); (4) the accessory piece of the MCO, which has longer terminal lobes (upper 19–25 and lower 11–20 vs. 12–18 and 7–10 μm in *L. chabaudi*) and a narrower upper lobe of the same width as the proximal part (prior to the bifurcation), whereas in *L. chabaudi* the upper lobe is 1.5–2 times wider than proximal part; and (5) the distal end of the muscular sheath of the tube attaches to two processes, but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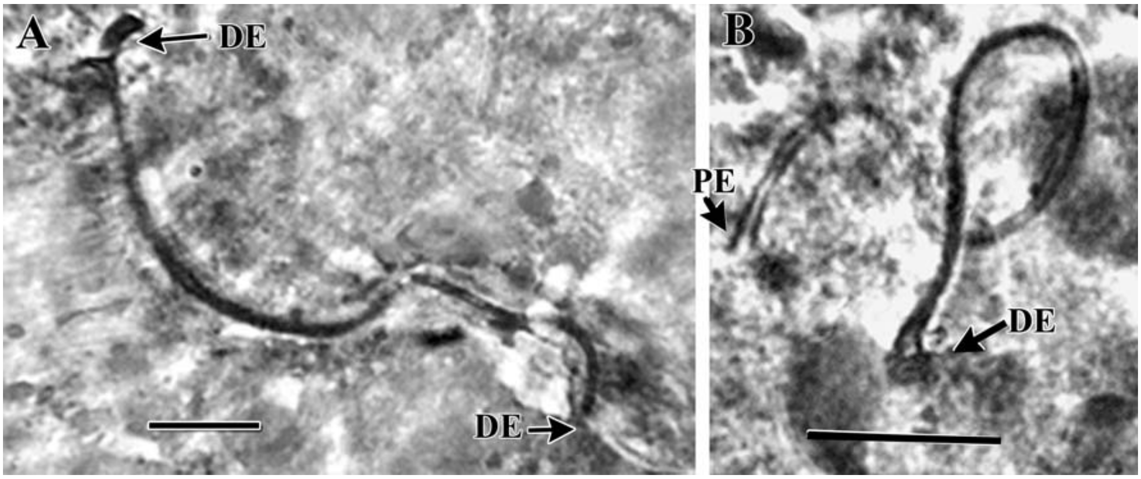


Fig. 5 Vagina of *Ligophorus cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006 ex *Mugil cephalus* from the Black Sea (A) and *L. chabaudi* Euzet & Suriano, 1977 ex *M. cephalus* from the Mediterranean Sea (B). *Abbreviations*: DE, distal end; PE, proximal end opening into receptaculum seminis. *Scale-bar*: 10 μm

arises from an oval dilatation attached to the upper lobe in *L. chabaudi*.

L. cephali can be distinguished from specimens identified as *L. mediterraneus* by Dmitrieva et al. (2009), which parasitise the same host in the same region, as follows: (1) the proximal part of the dorsal anchors have a greater outer length (DOP 21–23 vs. 15–20 μm in '*L. mediterraneus*'[2]); (2) the accessory piece of the MCO has a greater size (APL 35–43, APUL 19–25, APLL 11–20 vs. 23–34, 15–18 and 4–6 μm in '*L. mediterraneus*'); and (3) the shape of the accessory piece, which in *L. cephali* bifurcates in the middle, and the tip of the upper lobe is straight and no more than twice as long than the lower one, whereas in '*L. mediterraneus*' it bifurcates at two-thirds of its length from the distal end, the upper lobe is three or four times as long than the lower one and the tip of the upper lobe is strongly in-turned; and (4) the distal vagina is funnel-shaped, whereas in '*L. mediterraneus*' it is oval.

Seven species of *Ligophorus* have been described from *Mugil cephalus* from other regions: *L. mugilinus* (Hargis, 1955) Euzet & Suriano, 1977 sensu Sarabeev, Balbuena & Euzet (2005) from the Gulf of Mexico; *L. leporinus* (Yang & Ji, 1981) from the East China Sea; *L. chongmingensis* Hu & Li, 1992 and *L. chenzhenensis* Hu & Li, 1992 from the Yellow Sea; and *L. domnichi*, *L. pacificus* and *L. cheleus* from the Sea of Japan.

L. mugilinus has similarities with *L. cephali* in the shape and size of the haptoral hard-parts (Dmitrieva et al., 2009: Fig. 5), but the latter differs in: (1) the size of the MCO, which has greater lengths for all of its parts (CTL 93–115, APL 35–43 and APLL 11–20 vs. 73–92,[3] 25–33 and 4[4] μm in *L. mugilinus*); and (2) the distal end of the vagina is funnel-shaped, whereas it is oval in *L. mugilinus*.

Among the species described from the Northwest Pacific, *L. leporinus* and *L. chongmingensis* differ greatly from *L. cephali* in most taxonomic characters, and *L. domnichi* appears to be the most similar. *L. cephali* can be distinguished from the latter by the facts that: (1) the ventral anchors have a relatively greater outer length of their proximal part (VOP 20–24 vs. 17–18 μm); and (2) the upper lobe of the accessory piece tapers distally but is dilated to form a trumpet-shape in *L. domnichi*. *L. cephali* also has some similarity with *L. chenzhenensis,* but differs by having: (1) ventral anchors with a shorter main part (26.5–29 vs. 32–35 μm in *L. chenzhenensis*);[5] (2) an MCO with a longer tube (93–115 vs. 59–74 μm); and (3) the lower lobe of the accessory piece shorter than

[2] Measurements of '*L. mediterraneus*' from Dmitrieva et al. (2009).

[3] Measurements of '*L. mugilinus*' from Sarabeev, Balbuena & Euzet (2005).

[4] Measurements of one specimen of *L. mugilinus* from the Gulf of Mexico.

[5] Measurements of *L. chenzhenensis* from Hu & Li (1992).

the upper lobe rather than significantly longer as in *L. chenzhenensis*. *L. cephali* can be distinguished from *L. pacificus* by: (1) the bifurcation of the accessory piece of the MCO being in the middle, whereas in *L. pacificus* this is at only one-third of its length from the distal end; and (2) the upper lobe of the accessory piece tapers distally and has the same width as the proximal region (before the bifurcation) rather than having a medial dilation which is 2–2.5 times wider than the proximal part. Finally, *L. cephali* can be distinguished from *L. cheleus* as: (1) the accessory piece of the MCO bifurcates in the middle rather than at one-third of its length from the distal end; and (2), as previously pointed by Rubtsova et al. (2007), the curved lower lobe of the accessory piece which is significantly narrower than the upper, rather than both lobes being straight and equal in width and shape, as in *L. cheleus*.

Among those species infecting hosts ecologically related to *M. cephalus*, *L. pilengas* Sarabeev & Balbuena, 2004 (syn. *L. gussevi* Miroshnichenko & Maltsev, 2004), a parasite of *Liza haematocheilus* (Temminck & Schlegel) in the Black Sea, appears the most similar to *L. cephali*. The latter differs from *L. pilengas* in that: (1) as previously pointed by Rubtsova et al. (2007), the accessory piece of MCO bifurcates in the middle and the upper lobe is longer (APUL 19–25 vs. APLL 11–20 µm) and wider than the lower, whereas in *L. pilengas* bifurcation begins at two-thirds of its length from the distal end and the distal lobes are of almost equal length and width, or the lower lobe may be slightly narrower and longer than the upper (APUL 7–14[6] vs. APLL 8–16 µm); and (2) the distal end of the muscular sheath of copulatory tube attaches to two processes which are different in shape in *L. cephali* (the upper process is scoop-shaped) (Figs. 1F, 4A), whereas they are both rod-shaped in *L. pilengas*.

Ligophorus chabaudi Euzet & Suriano, 1977

Host: *Mugil cephalus* L.
Locality: Coast of Sardinia, western Mediterranean Sea (Mistras Lagoon, 39°54′N, 8°28′E); Zaliv Pos'yeta, Sea of Japan (42°42′N, 130°49′E).

[6] Measurements of 26 specimens of *L. pilengas* from the Black Sea.

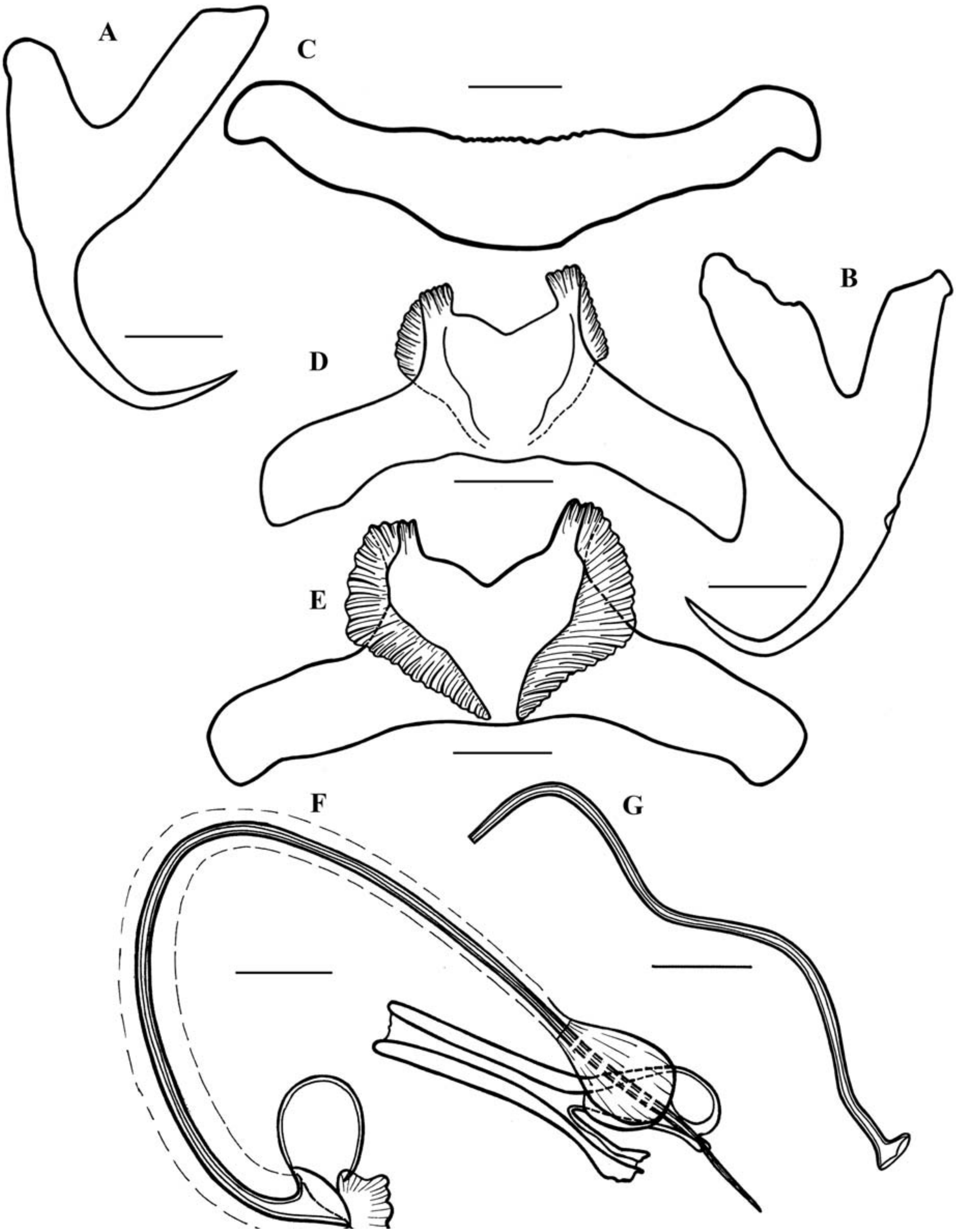
Fig. 6 *Ligophorus chabaudi* Euzet & Suriano, 1977 ex *Mugil cephalus* from the Sea of Japan. A, dorsal anchor; B, ventral anchor; C, dorsal bar; D, ventral bar (ventral view); E, ventral bar (dorsal view); F, male copulatory organ; G, vagina. *Scale-bars*: 10 µm

Site: Gills.
Material examined: 38 specimens deposited in the Zoological Institute, St Petersburg (Nos 12188–12195) and in the Institute of Biology of the Southern Seas, Sevastopol (Nos 6JS/2, 6JS/1, 6JS/4, 1MS/1, 1MS/2, 1MS/3, 1MS/5, 1MS/6).

Redescription (Figs. 2B, 5–8, Table 1)

Body flattened, 905 ± 34 (750–1,200) × 175 ± 9 (140–250). Haptoral armament characteristic of genus (Euzet & Suriano, 1977). For measurements of anchors, bars and sclerotised parts of reproductive system, see Table 1. Shape of both pairs of anchors (Fig. 6A, B) as for *L. cephali*; elongate, of similar length; inner length of proximal part greater than outer length; proximal part longer than distal (Table 1); proximal and distal parts form obtuse angle of ca. 110–112° (Fig. 1: angle II); distal part with point at angle of ca. 95° (Fig. 1: angle I). Marginal hooklets of larval-type. Bars equal in length (Table 1: VBW, DBW). Dorsal bar slightly widened in middle, with central part of anterior margin flattened and ends down-turned. Ventral bar (Figs. 6D, E, 7) with 2 long, digitiform, widely separated anterior processes (Table 1: VBP, VBS); dorsal side of ventral bar (Fig. 7B, C) with 2 narrow, wing-shaped laminae attached to each anterior process and wide median knoll, in most cases with V-shaped hollow on anterior margin, situated between them (Fig. 7B).

MCO consists of tube and accessory piece which forms U-shaped gutter, inside of which tube can move freely (Figs. 6F, 8). Accessory piece bifurcates into 2 terminal lobes at third of its length from distal end; lower lobe thinner and shorter than upper lobe (Table 1: APLL vs. APUL); upper lobe widens towards distal end where there is short, rectangular, gutter-shaped projection (Fig. 8A). Distal end of muscular sheath surrounding tube arises from oval dilatation attached to upper lobe of accessory piece from above; visual texture of this dilatation differs from that of other parts of accessory piece (Fig. 8B)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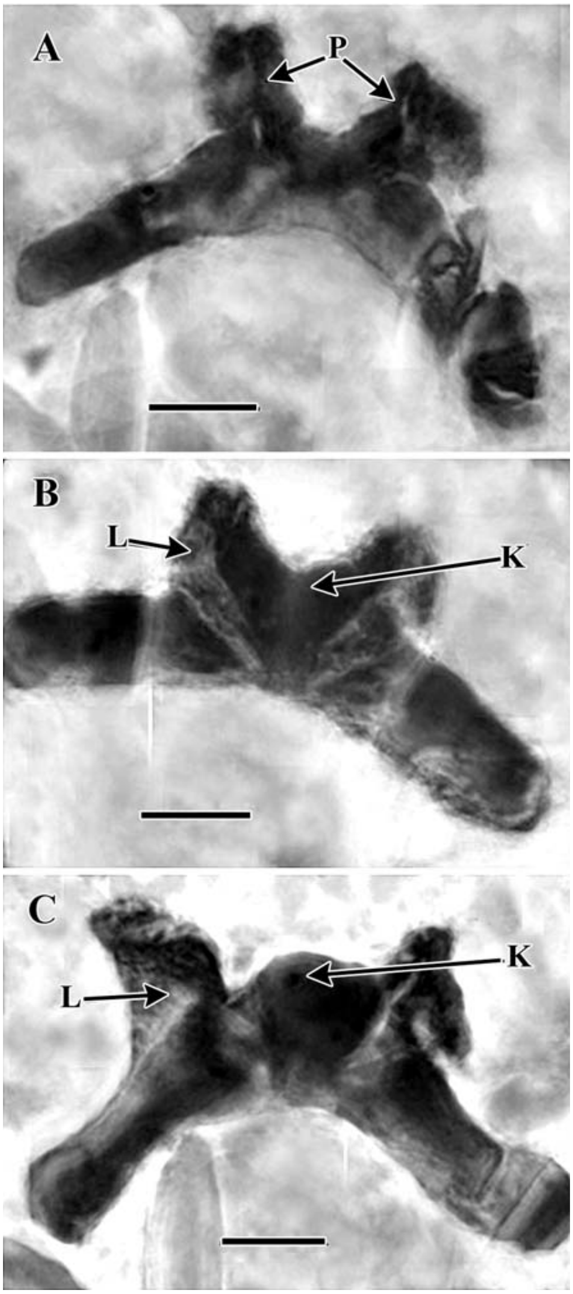

Fig. 7 Ventral bar of *Ligophorus chabaudi* Euzet & Suriano, 1977 ex *Mugil cephalus* from the Mediterranean Sea (A, C) and the Sea of Japan (B). A, ventral view; B, C, dorsal view. *Abbreviations*: P, anterior processes; L, wing-shaped laminae; K, median knoll. *Scale-bars*: 10 μm

and it probably represents widened, compact distal part of muscular sheath.

Vaginal armament as for *L. cephali* (Fig. 6G).

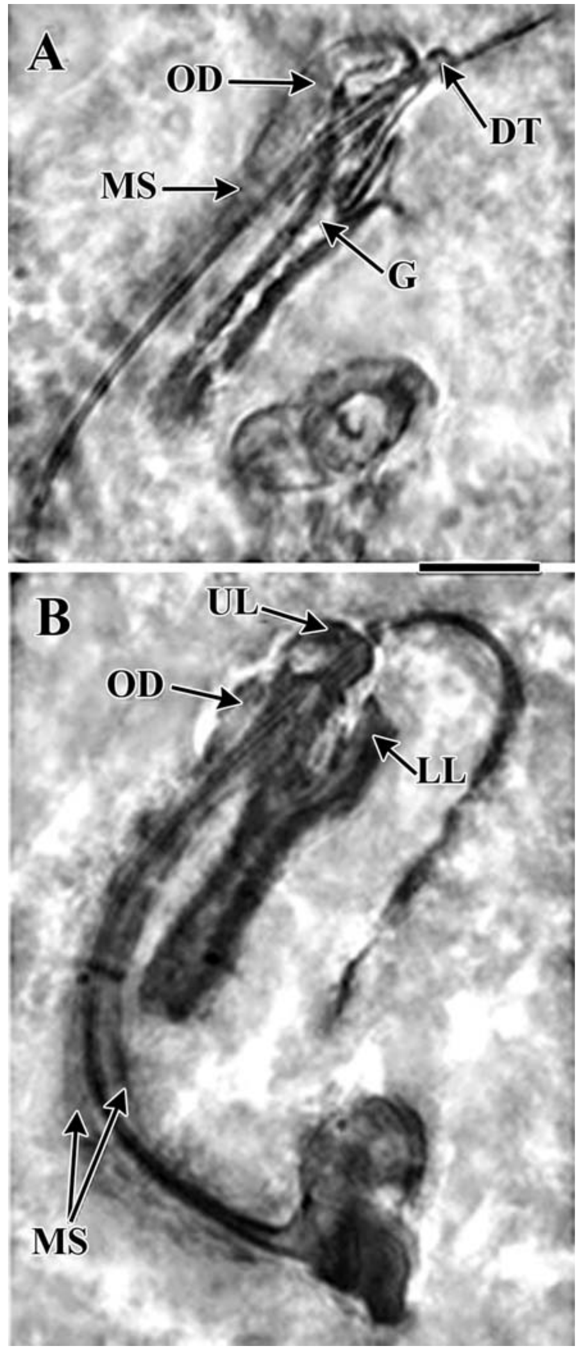

Fig. 8 Male copulatory organ of *Ligophorus chabaudi* Euzet & Suriano, 1977 ex *Mugil cephalus* from the Sea of Japan (A) and Mediterranean Sea (B). *Abbreviations*. DT, short rectangular gutter-shaped tip of upper lobe; G, gutter-like main part of the accessory piece; LL, lower lobe; MS, muscular sheath surrounding the copulatory tube; OD, oval dilatation by which the muscular sleeve attaches; UL, upper lobe. *Scale-bars*: 10 μm

Differential diagnosis and remarks

Taking into consideration the new details of the morphology of *Ligophorus chabaudi* presented above, the principal taxonomic character which clearly distinguishes this species from all known species infecting *Mugil cephalus* (listed below) is the shape of the MCO, namely the presence of an oval dilatation (Figs. 6F, 8) connected to the upper lobe of the accessory piece and to which the distal end of the muscular sheath surrounding tube attaches. This dilatation is not present in any other species, which all have the muscular sheath attached to two processes, which in most cases are rod-shaped. In addition to this feature, other characters that can be used to differentiate *L. chabaudi* from related *Ligophorus* spp. are presented below.

Compared to *L. pacificus*, a parasite of *M. cephalus* from the Sea of Japan and a closely related species based on the morphology of the anchors and the MCO, *L. chabaudi* differs in that: (1) the ventral bar has narrower wing-shaped laminae and a wider median knoll, in most cases with a V-shaped hollow on its anterior margin, whereas in *L. pacificus* the narrow median knoll has a cupola-shaped anterior margin; and (2) the upper lobe of the accessory piece of the MCO widens smoothly towards the distal end, whereas it is arched and dilated medially in *L. pacificus*.

In relation to *L. domnichi* and *L. cheleus*, which also infect *M. cephalus* in the Sea of Japan, *L. chabaudi* differs in that: (1) the outer length of the proximal part of the ventral anchor is larger (22–29 vs. 17–18 in *L. domnichi* and 20–21 µm in *L. cheleus*); and (2) the anterior processes of the ventral bar are set more widely apart (VBS 7–13 vs. 2–5 in *L. domnichi* and 3–5 µm in *L. cheleus*), and the median knoll is wider than in *L. domnichi* and *L. cheleus*. Moreover, *L. chabaudi* can be distinguished from *L. domnichi* as: (1) the accessory piece of the MCO bifurcates at one-third of its length from its distal end, whereas in *L. domnichi* this bifurcation is in the middle of the accessory piece; and (2) the proximal region of the accessory piece is straight, but in *L. domnichi* it is in-turned at the proximal end almost at a right angle for a distance of 4–6 µm. It can also be distinguished from *L. cheleus* by the upper lobe of the accessory piece being wider than the lower lobe and by a difference in the shape of these lobes, which in *L. cheleus* are equal in width and the same shape.

In the Northwest Pacific, *M. cephalus* is also infected by *L. leporinus*, *L. chongmingensis* and *L. chenzhenensis*. These species differ greatly from *L. chabaudi* in the shape and size of both the haptoral hard-parts and the MCO.

In the Mediterranean Sea, *L. cephali* and *L. mediterraneus* have also been described from *M. cephalus*. Characters which allow the differentiation of *L. chabaudi* from *L. cephali* have been mentioned above. *L. chabaudi* can be distinguished from *L. mediterraneus* by: (1) the ventral bar having a wider median knoll, narrower, wing-shaped laminae and anterior processes set further apart (VBS 7–13 vs. 2–5[7] µm in *L. mediterraneus*); (2) a longer copulatory tube (100–116 vs. 85–98 µm); (3) the bifurcation of the accessory piece of the MCO is at one-third of its length from its distal end, rather than at two-thirds as in *L. mediterraneus*, the lower lobe is longer (APLL 7–10 vs. 4–6 µm in *L. mediterraneus*) and the distal end of upper lobe is represented by a short rectangular projection, rather than being in-turned as in *L. mediterraneus*; and (4) the distal end of the vagina is funnel-shaped rather than being oval.

L. chabaudi differs from *L. mugilinus,* a parasite of *M. cephalus* in the Gulf of Mexico, in that: (1) the ventral bar has a wider median knoll and narrower wing-shaped laminae; (2) the copulatory tube is longer (100–120 vs. 73–92[8] µm in *L. mugilinus*); (3) the lower lobe of the accessory piece of the MCO is longer (7–10[9] vs. 4 µm); and (4) the distal end of the vagina is funnel-shaped rather than being oval as in the case of *L. mediterraneus.*

Principal Components Analysis of the haptoral structures of *Ligophorus cephali* and *L. chabaudi*

Most measurements of the haptoral hard-parts of *Ligophorus cephali* and *L. chabaudi* overlap, apart

[7] Measurements of '*L. mediterraneus*' from Dmitrieva et al. (2009).

[8] Measurements of '*L. mugilinus*' from Sarabeev, Balbuena & Euzet (2005).

[9] Measurements of one specimen of *L. mugilinus* from the Gulf of Mexico.

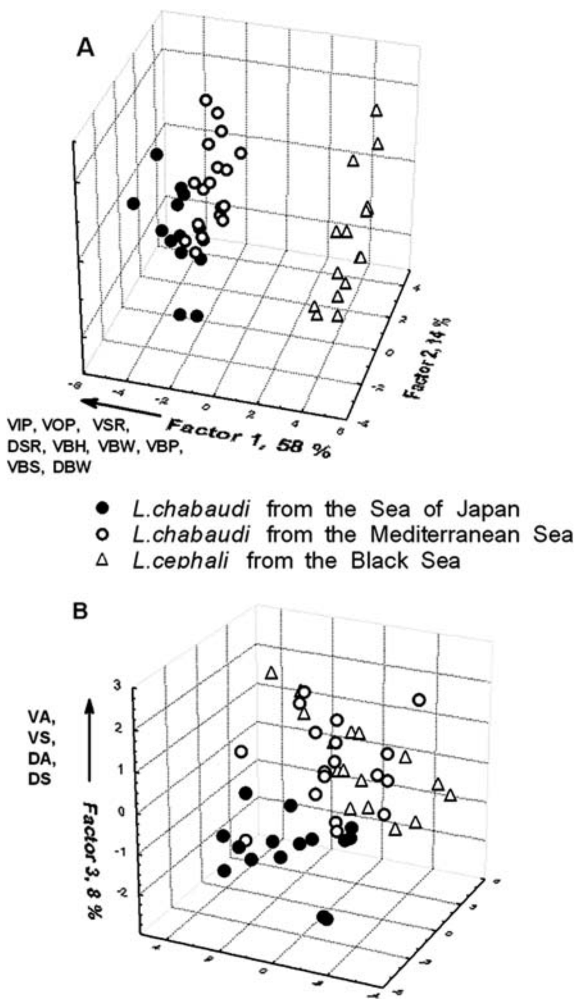

Fig. 9 PCA plots of the scores of the first three factors calculated from 20 characters of the haptoral hard-parts for 56 specimens belonging to two species of *Ligophorus* from three localities. A, B, different projections of the plot (→ = direction of increasing measurements separating the specimens)

from the width of the bars (Table 1). Twenty characters included in the measuring scheme of Dmitrieva et al. (2009) were reduced to three Principal Components (Factors) describing 80% of the overall variance (Fig. 9). All specimens of *L. chabaudi* from both localities were clearly separated from *L. cephali* by PC Factor 1, which explained 58% of the total variance (Fig. 9A). Measurements of the bars (VBW, VBH, VBP, VBS and DBW), the proximal part of the ventral anchor (VIP, VOP and VSR) and the span between the dorsal anchor roots (DSR) contributed most to this dimension. The specimens of *L. chabaudi* from the Mediterranean Sea and Sea of Japan formed one cluster. However, most specimens of this species from the Mediterranean Sea were set apart from those from the Sea of Japan along axis 3 (Fig. 9B). Specimens of *L. cephali* from the Black Sea occupied the same position along this axis as specimens of *L. chabaudi* from the Mediterranean Sea. Thus, most of the specimens of both species from the Mediterranean region were separated from *L. chabaudi* from the Sea of Japan along axis 3, which, however, explains only 8% of the total variance. Measurements of distal part of both anchors (VA, VS, DA and DS) contributed most to Factor 3 and consequently, at least partly, to the separation of specimens from the Mediterranean region and the Sea of Japan (Fig. 9B).

Discussion

Of all the characters used for the differentiation of *Ligophorus* spp., the shape of the MCO is the most species-specific and, therefore, most significant in terms of the taxonomy of this genus. A precise description of this structure is very important. In the description of the accessory piece of the MCO of *L. cephali*, Rubtsova et al. (2006a, p. 488) wrote: "Bowed upper lobe tubular, thin-walled with membranous funnel-shaped opening on top of distal end, …… Penis enters membranous funnel-shaped mouth at distal end of accessory piece" and in the legend to their figure 3 (p. 491): "… opening (O) on top of distal end of accessory piece". This description suggests that the accessory piece is in the form of a duct with an O-shaped cross-section, closely surrounding the copulatory tube. The interpretation of the position of the copulatory tube relative to its accessory piece as being above or below apparently results from a notion that the latter holds the copulatory tube fast. This concept of a rigid connection between all parts of the MCO was developed by Rubtsova et al. (2006b, p. 252), who considered the shape of the copulatory tube as "C-shaped or forming coil" and the position of the entrance of the tube into the accessory piece as a taxonomic characters.

According to Llewellyn & Anderson (1984) and our own data, the copulatory tube can move freely inside the accessory piece, which forms a U-shaped gutter only partly enclosing the tube. The accessory piece is rigid and its position in the worm body is determined by crimped ligaments (Fig. 4B) which fasten it to the tegument surrounding the male genital pore and through which the tube can be extended to the exterior. The copulatory tube and the accessory piece are not directly connected, but a muscular sheath ("sleeve" of Llewellyn & Anderson, 1984) surrounding the tube is attached to the accessory piece. In most examined *Ligophorus* spp., the distal end of the muscular sheath attaches to two rod-shaped processes arising from above and below the accessory piece, while its proximal end attaches to the sclerotised flange of the expanded base of the tube (Euzet & Suriano, 1977; Llewellyn & Anderson, 1984). Apparently, only the uppermost of these rod-shaped processes, which is scoop-shaped in *L. cephali* (Fig. 4A), and the distal part of the muscular sheath arising from it, were recognised as a "funnel-shaped mouth" by Rubtsova et al. (2006a). When the muscular sheath relaxes, the copulatory tube is completely withdrawn into the body. Conversely, contraction of this muscle causes the accessory piece to approach the proximal end of the MCO, and, as a consequence, the copulatory tube is extended outside the body, because the accessory piece is connected to the tegument surrounding the aperture. Llewellyn & Anderson (1984) likened the mode of functioning of the MCO to that of a Bowden cable.

On slides of *L. cephali* and *L. chabaudi* in our collection, there are specimens with the copulatory tube at different stages of twisting and protrusion in relation to the accessory piece (e.g. Figs. 4, 8). This is due, on the one hand, to the relative flexibility of the connection between the muscular sheath and the accessory piece and, on the other hand, to deformation caused by coverslip pressure during slide preparation.

Thus, it is obvious that in living worms the copulatory tube does not coil, either proximally (which would hinder the functioning of the MCO in the same way as a curved piston would in a syringe), or especially distally, where it protrudes from the body only during copulation. At all other times the tube lies entirely within the body and, moreover, within the accessory piece. In the 12 species of *Ligophorus* examined live, the copulatory tube has the same C-shaped bend and its distal region traverses the main part of the accessory piece. As a consequence, both the degree of convolution of the copulatory tube and its position in relation to the accessory piece ('above', 'below', 'under top' and other positions visible in slide preparations; for example, see Fig. 4) cannot be used as taxonomic characters for distinguishing *Ligophorus* spp.

The muscular sheath surrounding the copulatory tube attaches to the processes of the accessory piece, which arise from different regions of the latter and may have various shapes. For example, in *L. mediterraneus* these processes arise from the proximal end of the accessory piece (Dmitrieva et al., 2009, fig. 1). In *L. gussevi* and *L. cephali* they originate above and below the upper lobe of the accessory piece (Fig. 4C). Both processes are rod-shaped in the former two species, but the upper process is scoop-shaped in *L. cephali* (Fig. 4A). The attachment of the muscular sheath to the accessory piece looks more complicated in *L. chabaudi* (Figs. 6, 8), where it forms by an oval dilatation attached to the upper lobe. Therefore, the point of attachment of the muscular sheath surrounding the tube to the accessory piece, and its shape, can be used as characters for differentiating species.

In contrast to the variation in shape of the accessory piece of the MCO, which is highly diverse in *Ligophorus* spp., the haptoral structures are practically indistinguishable in different species, especially in synxenic species. Nevertheless, after careful analysis using multivariate statistics, it is possible to find differences, even in the shapes of anchors and bars of such similar species. Thus, despite the similarity of the haptoral hard-parts in *L. cephali* and *L. chabaudi*, and the overlap of most measurements (Table 1), specimens can be clearly divided into two clusters according to their species by using PCA (Fig. 9). The differences between the species can be explained not so much by the linear measurements of their hard parts but by their proportions, which matter for the identification of species. Anchor shape is the most constant feature for specimens belonging to the same species, whereas linear measurements are more variable and depend, for example, on the size of the host (e.g. Gusev & Kulemina, 1971a, b).

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