# **Redescription of** *Ligophorus mediterraneus* **Sarabeev**, Balbuena & Euzet, 2005 (Monogenea: Ancyrocephalidae) with some methodological notes

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**Abstract** A redescription of *Ligophorus mediterraneus* Sarabeev, Balbuena & Euzet, 2005, based on original material from the Black and Mediterranean Seas, is presented and new diagnostic characters for its recognition are proposed. The unlikely wide range of variation in the angle between the shaft and point of the anchors, reported for this species and for some others in the genus, is analysed, and the structure of the ventral bar in *Ligophorus* spp. is described and its taxonomic significance discussed.

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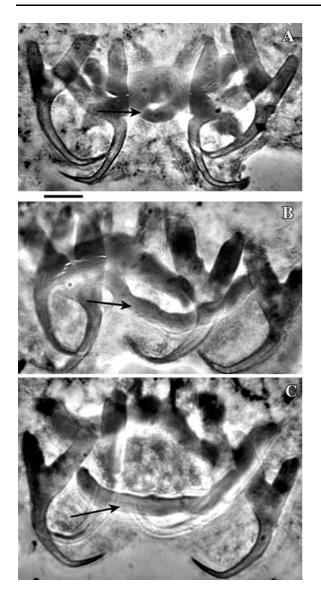
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# Introduction

To date *Ligophorus* Euzet & Suriano, 1977, originally erected by Euzet & Suriano (1977) for 11 species of monogeneans strictly specific to mugilids, now comprises 28 species described from the Atlantic and the Pacific Oceans (Dmitrieva, Gerasev & Pron'kina, 2007; Rubtsova, Balbuena & Sarabeev, 2007) plus a further 10 species reported from the Red Sea, the SE Atlantic and the NW Pacific (Dmitrieva et al., 2007b).

Many representatives of this genus are morphologically similar (Dmitrieva, Gerasev & Pron'kina, 2007), and thus accurate correct descriptions, detailed illustrations of the taxonomic characters and a careful choice of differential features are essential for correct identification at the species level. However, the absence of a modern key and the incomplete descriptions of some species make identification difficult. We consider that the main causes of inadequate description are the poor quality of the preparations, with insufficient flattening, the use of insufficient magnification (the use of a good quality  $\times 100$  oil-immersion objective is essential), and erroneous notions of the functional morphology of taxonomically significant structures.

For example, in the paper of Sarabeev, Balbuena & Euzet (2005), within which Mediterranean specimens of a taxon formerly considered to be *L. mugilinus* (Hargis, 1955) Euzet & Suriano 1977 (see Euzet & Suriano, 1977; Mariniello et al., 2004) were established as new species, *L. mediterraneus* Sarabeev,



**Fig. 1** Ligophorus mediterraneus Sarabeev, Balbuena & Euzet, 2005 ex *Mugil cephalus* in the Mediterranean Sea. Haptors of holotype (A) and paratypes (B, C), showing differences in the shape of the dorsal bar. *Scale-bar*: 10  $\mu$ m

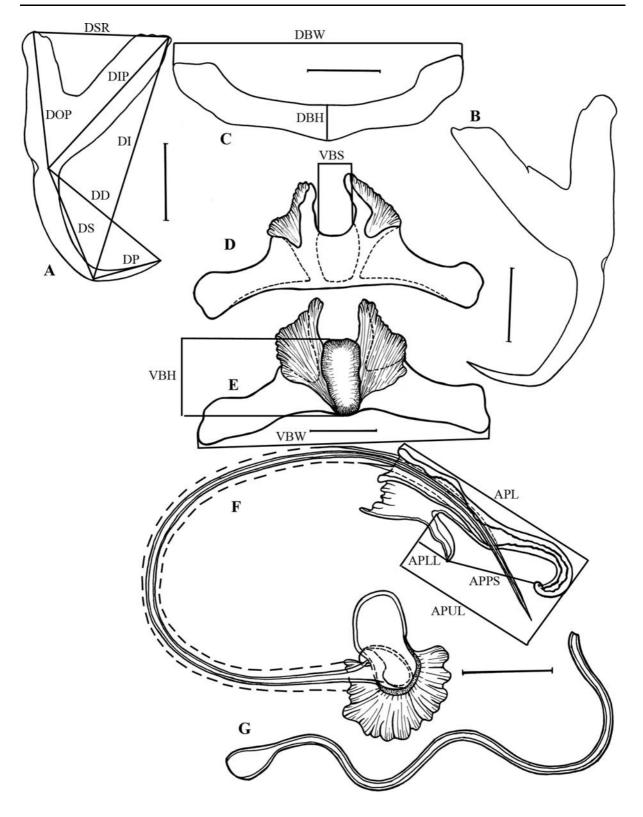
Balbuena & Euzet, 2005, the morphology of the new taxon was incorrectly presented in relation to some significant details. A precise differential diagnosis of *L. mediterraneus* is lacking, and the comparison of the new species with the related *L. mugilinus* was inadequate. The degree of curvature of the dorsal bar was proposed as the main differential character, which in *L. mugilinus* "is V-shaped, whereas it is only slightly bowed in *L. mediterraneus* n. sp." (Sarabeev et al., 2005, p.1445). This character (a slightly or hardly

**Fig. 2** *Ligophorus mediterraneus* Sarabeev, Balbuena &► Euzet, 2005 ex *Mugil cephalus* in the Black Sea. A, dorsal anchor; B, ventral anchor; C, dorsal bar; D, ventral bar (ventral view); E, ventral bar (dorsal view); F, copulatory organ; G, vagina. *Scale-bars*: 10 μm. See Table 1 for abbreviations

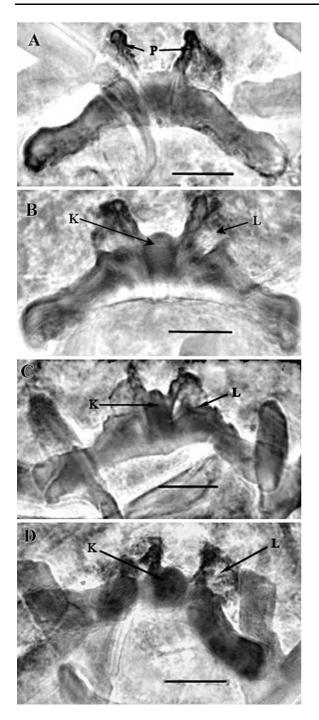
bowed dorsal bar) is, however, highly variable (Fig. 1). The second proposed differential character was the shape of ventral bar. However, the comparison of the ventral view of the ventral bar in *L. mediterraneus* with the dorsal view of this bar in *L. mugilinus* (Figures 2A and 3A of Sarabeev et al., 2005) resulted in the incorrect conclusion that the "... median process ... is absent in the new species ..." (Sarabeev et al., 2005, p. 1445). At the same time, clearly visible differences in anchor shape between *L. mediterraneus* and *L. mugilinus* were not recorded. These problems acted as the stimulus for the redescription of *L. mediterraneus* presented below.

# Materials and methods

The redescription of *Ligophorus mediterraneus* was based on 20 specimens collected from the gills of two specimens of Mugil cephalus L., 28 and 32 cm long, captured in coastal waters of the Black Sea near Sevastopol (44°35'N, 33°30'E), and on five specimens from four M. cephalus, 29-39 cm long, caught in the Cabras (39°55'N, 8°31'E) and Mistras (39°54'N, 8°28'E) Lagoons, Sardinia, western Mediterranean Sea. All monogeneans were collected from freshly caught fish and then immediately mounted in glycerine-jelly (prepared with 0.5 g carbolic acid). For comparison, one specimen of L. mugilinus collected from formalin-fixed gills of M. cephalus, caught in the Gulf of Mexico (30°12'N, 88°58'W) was used. The type-material of *L. mediterraneus*, from the British Museum (Natural History) Collection (BMNH) at the Natural History Museum, London (holotype and paratypes nos 2005.1.7.1-6), was also examined. Drawings and light micrographs were made using a Carl Zeiss Amplival optical microscope (magnification  $\times 2000$ ) fitted with phase contrast, a drawing tube and an Olympus C180 digital camera. The measurement scheme of Dmitrieva, Gerasev & Pron'kina, (2007) was used with minor changes (Fig. 2). For comparison with previous descriptions (Euzet & Suriano, 1977; Sarabeev et al., 2005), measurements



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**Fig. 3** Ventral bar of *Ligophorus mediterraneus* Sarabeev, Balbuena & Euzet, 2005 ex *Mugil cephalus* in the Black Sea (A-C) and in the Mediterranean Sea. (D; paratypes BMNH No. 2005.1.7.2–6). A, ventral view; B-D—dorsal views. *Abbreviations*: L, wing-shaped laminae; P, anterior processes; K, median knoll. *Scale-bar*: 10 μm

of the anchor roots (VIR, DIR – inner roots and VOR, DOR – outer roots) and the main part of the anchors (VM, DM) were also taken. All measurements are given in micrometres (with measurement precision of 1  $\mu$ m). The mean, standard error and range of variation were used to describe the measurements. See Table 1 for the abbreviations.

# Ligophorus mediterraneus Sarabeev, Balbuena & Euzet, 2005

# Host: Mugil cephalus L.

*Localities*: Off Sevastopol, Crimean Peninsula, Black Sea (44°35′N, 33°30′E); Sardinia, Western Mediterranean Sea (Cabras Lagoon, 39°55′N, 8°31′E and Mistras Lagoon, 39°54′N, 8°28′E).

# Site: Gills.

*Material examined*: 25 specimens deposited in the Institute of Biology of the Southern Seas, Sevastopol (Nos 256/22, 286/12, 286/13, 286/42, 286/23, 1C2MC1, 1C6MC3, 1M2MC7) and in the Zoological Institute, St Petersburg (Nos 12182–12186).

Redescription (Figs. 2–4, Table 1)

Body flattened 660  $\pm$  75 (580–800)  $\times$  115  $\pm$  16 (95-135). Haptoral armament conforms to descriptions of Euzet & Suriano, (1977). For measurements of anchors, bars and parts of reproductive system see Table 1. Both pairs of anchors elongate, with similar shape and length (Fig. 2A, B; Table 1: VI, DI); inner length of proximal part larger than its outer length (24-28 vs 20-24 for ventral anchor and 22-27 vs 15-20 for dorsal anchor); proximal part longer than distal (VIP 24-28 vs VD 20-22 for ventral anchor and 22-27 vs 19-22 for dorsal anchor); proximal and distal parts form obtuse angle of  $c.115^{\circ}$  (angle between VIP and VS, DIP and DS). Distal part of anchors (blade according to Gusev, 1983, 1985) consists of shaft and point; latter is at angle of  $c.95^{\circ}$ (i.e. angle between VS and VP). Marginal hooklets of larval form, typical for genus; total length 11-12. Bars equal in length (Table 1: VBW, DBW). Dorsal bar bowed and widened in middle and at ends. Ventral bar with 2 long (6–10), finger-shaped anterior processes, which are closely positioned (2-5), usually

**Table 1** Dimensions, as the range (mean  $\pm$  standard error), of the anchors, bars, copulatory organ and vagina of Ligopho-rus mediterraneusSarabeev, Balbuena & Euzet, 2005 from the Black and Mediterranean Seas

Locality Source of data No. of specimens	Black Sea	Mediterranean Sea				
	Present study 20	Sarabeev et al. (2006) 11	Present study		Sarabeev et al.	Euzet &
			New material	Type- material	- (2006)	Suriano (1977)
			5	6	12	20
Ventral anchor:						
inner length (VI)*	33–39 (36 $\pm$ 0.35)	32–38 (36 $\pm$ 2)	36–38	32–39	$33-39 (36 \pm 2)$	32–34
length of main part (VM)	25–28 (26.4 $\pm$ 0.2)	25–28 (26 $\pm$ 1)	24–26	24-28	24–28 (26 $\pm$ 1)	23–25
length of distal part (VD)	20–22 (20.6 $\pm$ 0.15)		20-22	20-22		
length of shaft (VS)	17–20 (18.4 $\pm$ 0.18)		17–19	17-20		
length of point (VP)	$8 – 9 \; (9.0 \pm 0.07)$	9–10 (9 ± 1)	8–9	9	9	8–9
inner length of proximal part (VIP)	24–28 (25.8 ± 0.26)		26–28	24–27		
outer length of proximal part (VOP)	20-24 (21.9 ± 0.24)		22–24	20–23		
span between roots (VSR)	17–21 (18.9 $\pm$ 0.21)		19–21	18-20		
length of inner root (VIR)	15–18 (16.6 $\pm$ 0.22)	15–18 (17 ± 1)	16-18	15-17	16–20 (17 ± 1)	15-17
length of outer root (VOR)	12–14 (12.5 $\pm$ 0.20)	$11-14 (13 \pm 1)$	12-13	11-15	12–15 (13 $\pm$ 1)	12-13
Dorsal anchor:						
inner length (DI)	33-38 (34.8 ± 0.31)	30-38 (35 ± 2)	34–37	32–38	$32-39~(36\pm2)$	34–36
length of main part (DM)	24–28 (25.9 $\pm$ 0.27)	24–28 (26 $\pm$ 1)	25-27	25-28	25-28 (27 ± 1)	24–26
length of distal part (DD)	19–22 (20.5 $\pm$ 0.17)		19–21	20-22		
length of shaft (DS)	16–20 (17.9 $\pm$ 0.26)		17–18	16–19		
length of point (DP)	9–10 (9.2 $\pm$ 0.09)	9–10 (10 ± 0.3)	9	9–10	$9-10~(9~\pm~0.4)$	7–8
inner length of proximal part (DIP)	22-26 (23.9 ± 0.29)		24–26	22–27		
outer length of proximal part (DOP)	17-20 (17.9 ± 0.20)		19–20	15–20		
span between roots (DSR)	15–19 (16.8 ± 0.26)		14–16	16–18		
length of inner root (DIR)	13–17 (15.2 $\pm$ 0.21)	13–19 (15 ± 2)	11-15	14–17	13–18 (16 ± 1)	15-18
length of outer root (DOR)	$8-10~(9.0\pm0.15)$	$8-11 (9 \pm 1)$	8–9	8-10	$8-11 (9 \pm 1)$	8-10
Marginal hook:						
total length	11–12 (11.8 $\pm$ 0.09)	$11-13 (12 \pm 0.4)$	12	12	$12-13 (12 \pm 0.3)$	
sickle length	5-6 (5.1 $\pm$ 0.07)		5	5		
shaft length	6–7 (6.7 $\pm$ 0.10)		7	7		
Ventral bar:						
height (VBH)	7–11 (8.7 $\pm$ 0.23)		9–10	8-11		
Width (VBW)	40–46 (42.6 $\pm$ 0.33)	$37-47 (41 \pm 3)$	41-42	36-44	36-44 (41 ± 2)	40-42
length of anterior processes (VBP)	6-10 (8.6 ± 0.22)		9–10	6–8		
span between processes (VBS)	2-5 (3.7 ± 0.16)	$3-7 (5 \pm 1)$	4–5	5	4-9 (7 ± 2)	
Dorsal bar:						
height (DBH)	$4-6~(4.8\pm0.15)$		5–6	4–6		
Width (DBW)	$37-46 (42.2 \pm 0.52)$	37-45 (41 ± 2)	42–44	38–44	$37-44 \ (40 \pm 2)$	38–40

#### Table 1 continued

Locality Source of data No. of specimens	Black Sea		Mediterranean Sea			
	Present study 20	Sarabeev et al. (2006) 11	Present study		Sarabeev et al.	Euzet &
			New material 5	Type- material 6	- (2006) 12	Suriano (1977) 20
length (CTL)	85–98 (92.2 $\pm$ 0.8)	80-103 (92 ± 6)	85–95	90–95	79–98 (89 ± 6)	80–90
Accessory piece of copulatory	organ:					
length (APL)	27–34 (31.0 $\pm$ 0.38)	26-32 (28 ± 2)	23-27	23-28	26-32 (30 ± 2)	30
Width (APW)	6–8 (6.7 $\pm$ 0.15)		5-8	6–7		
length of upper lobe (APUL)	16-18 (17.0 ± 0.19)		15–17	15–18		
length of lower lobe (APLL)	4-6 (5.2 ± 0.14)		6	4–5		
span between tips of upper and lower lobes (APPS)	9–13 (11.3 ± 0.24)		8	8–12		
Vagina:						
length (VL)	48-60 (54.6 ± 0.9)	28-60 (36 ± 9)	45-48	47-48**	$25-44 (35 \pm 5)$	40-45

\* For measurements see Fig. 2

\*\* The vagina was visible along the full length only in two specimens and no more than two-thirds of its length was visible in the remainder

with ends turned inwards, together making lyriform shape; on dorsal side of ventral bar 2 wing-shaped laminae are attached to each anterior process. These laminae are directed towards different ends of bar and positioned at angle to main axis; situated between laminae on dorsal side of this bar is median knoll (Fig. 2E), which is more (Fig. 3C) or less (Fig. 3B) expressed; in ventral view median knoll is not visible, but anterior processes and upper parts of both wingshaped laminae, which are prominent above anterior border of bar, are clearly seen (Figs. 2D, 3A).

Copulatory organ consists of tube and accessory piece (Figs. 2F, 4A). Tube originates from dilate ampulla, which receives ducts from vesicula seminalis and prostatic reservoir. Accessory piece forms gutter, U-shaped in cross-section, within which copulatory tube slides, and bifurcates into 2 terminal lobes at 2/3 of its length from its distal end; upper<sup>1</sup> lobe 3–4 times as long as lower lobe (16 –18 *vs* 4–6) and with distal end strongly curved downwards; lower lobe slightly

deflected. There are also 2 rod-shaped processes, which arise above and below proximal end of accessory piece, to which muscular sheath surrounding copulatory tube attaches; proximal end of latter attaches to expanded base of copulatory organ.

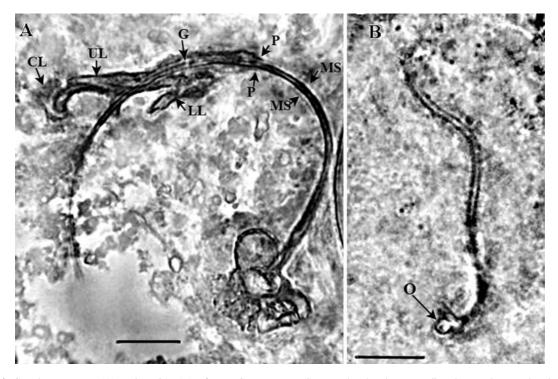
Vaginal armament is typical of genus, forming hollow, narrow tube with solid walls. Distal end of vagina oval, expanded (Fig. 2G, 4B).

The examination of BMNH slide No. 2005.1.7.1– 6, which is inscribed as the holotype and paratypes of *Ligophorus mediterraneus* Sarabeev, Balbuena, Euzet, 2005 ex *Mugil cephalus* from the Mediterranean Sea, revealed that morphology of the haptoral structures, copulatory organ and vagina in all six specimens agrees with the above redescription in all details, including the shapes of the dorsal and ventral bars (Fig. 1, 3D).

## Differential diagnosis and remarks

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

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



**Fig. 4** Copulatory organ (A) and vagina (B) of *Ligophorus mediterraneus* Sarabeev, Balbuena & Euzet, 2005 ex *Mugil cephalus* in the Black Sea. *Abbreviations*: CL, crimped ligament attaching the accessory piece to the tegument; G, gutter-like main part of the accessory piece; LL, lower lobe;

Compared with the very similar *L. mugilinus* (Hargis, 1955) Euzet & Suriano, 1977 (Fig. 5), *L. mediterraneus* can be distinguished by four features: (1) the ventral anchors (Fig. 6A,B) have a greater outer length of the proximal part (VOP 20–24 vs  $19^2$  in *L. mugilinus*) and a shorter span between the roots (VSR 17–21 vs 23 in *L. mugilinus*); (2) the dorsal anchors (Fig. 6C, D) have a smaller inner length of the proximal part (DIP 22–27 vs 28 in *L. mugilinus*); (3) the ventral bar (Fig. 3, 7) has the anterior processes more closely positioned (VBS 2–5 vs 9 in *L. mugilinus*); and (4), as previously pointed by Sarabeev et al. (2005), the accessory piece of the copulatory organ has the upper lobe with a distinctly curved distal tip, whereas in *L. mugilinus* (Fig. 5) this tip is much straighter.

*L. mediterraneus* differs from *L. cephali* Rubtsova, Balbuena, Sarabeev, Blasco-Costa & Euzet, 2006, which parasitises the same host in the same region of the Mediterranean, in: (1) the proportions of the dorsal

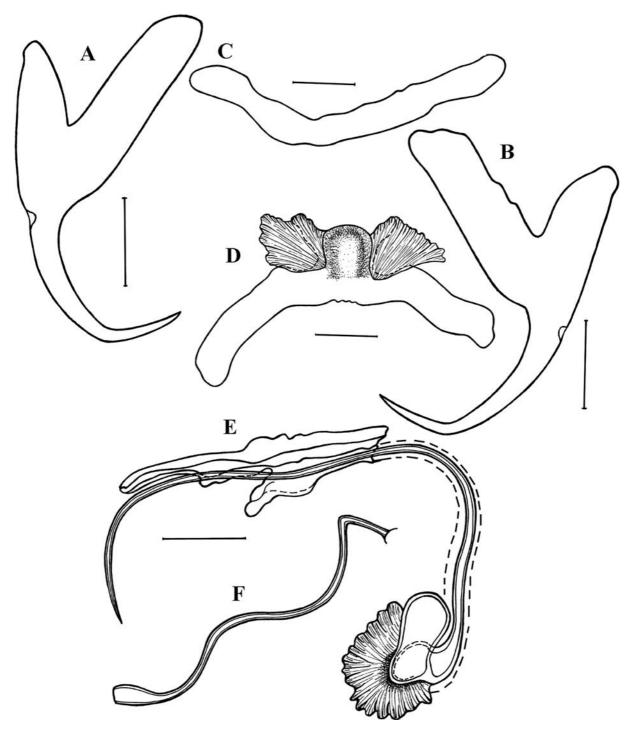
MS, muscular sheath surrounding the copulatory tube; O, outer opening of the vagina; P, processes to which muscular sheath attaches; T, copulatory tube; UL, upper lobe. *Scale-bars*: 10  $\mu$ m

anchors, which have a shorter outer length of the proximal part (VOP 17–20 vs  $21–23^3$  in *L. cephali*); (2) the length of the accessory piece of the copulatory organ, which has a shorter total length (APL 27–34 vs 35–43 in *L. cephali*) and lower lobe (APLL 4–6 vs 11–18); (3) the shape of the accessory piece, which in *L. mediterraneus* bifurcates at two-thirds of its length from the distal end, and the tip of the upper lobe is distinctly curved, whereas in *L. cephali* the bifurcation takes place in the middle and the tip of the upper lobe is not curved; and (4) the distal end of the vagina is oval in *L. mediterraneus* but funnel-shaped, resembling a nail-head in profile, in *L. cephali*.

*L. chabaudi* Euzet & Suriano, 1977 from the Mediterranean Sea (Euzet & Suriano, 1977) and *L. vanbenedenii* (Parona & Perugia, 1810) Euzet & Suriano, 1977 from the Black Sea (Dmitrieva & Gerasev, 1996) have also been reported as parasites

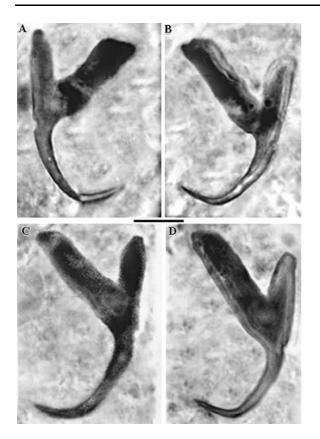
 $<sup>^2</sup>$  Measurements of one specimen of *L. mugilinus* from the Gulf of Mexico.

<sup>&</sup>lt;sup>3</sup> Measurements of *L. cephali* [as *L. chabaudi*] from Dmitrieva et al. (2007: Table 2, pp. 58–59)].



**Fig. 5** Ligophorus mugilinus (Hargis, 1955) Euzet & Suriano, 1977 ex Mugil cephalus from the Gulf of Mexico. A, dorsal anchor; B, ventral anchor; C, dorsal bar; D, ventral bar; E, copulatory organ; F, vagina. Scale-bars: 10 μm

of *M. cephalus*. However, the record of *L. vanbenedenii* on *M. cephalus* in the Black Sea is erroneous. Following a reinvestigation, this material has been reassigned as *L. mediterraneus*. We originally misidentified it as a host-variant of *L. vanbenedenii*, a specific parasite of *Liza aurata*. Thus *L. chabaudi* 



**Fig. 6** Anchors of *Ligophorus mediterraneus* Sarabeev, Balbuena & Euzet, 2005 ex *Mugil cephalus* in the Black Sea (A, ventral anchor, C, dorsal anchor) and *L. mugilinus* (Hargis, 1955) Euzet & Suriano, 1977 ex *Mugil cephalus* from the Gulf of Mexico (B, ventral anchor; D, dorsal anchor). *Scale-bar*: 10 µm

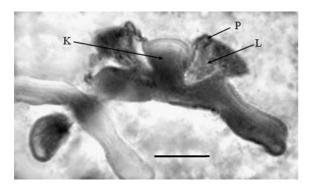


Fig. 7 Ventral bar (dorsal view) of *Ligophorus mugilinus* (Hargis, 1955) Euzet & Suriano, 1977 ex *Mugil cephalus* from the Gulf of Mexico. *Scale-bar*: 10  $\mu$ m

from the Mediterranean is the only other species of the genus recorded from *M. cephalus*.

*L. mediterraneus* can be distinguished from *L. chabaudi* by: (1) the anterior processes of the

ventral bar, which are more closely set (VBS 2-5 vs  $7-10^4$  in L. chabaudi); (2) a shorter copulatory organ tube (CTL 85-98 vs 100-116); (3) the bifurcation of the accessory piece of the copulatory organ at twothirds of its length from its distal end, whereas in L. chabaudi this is at only one-third of the distance from the same end, the lower lobe of the accessory piece is shorter (APLL 4-6 vs 7-10 in L. chabaudi) and the upper lobe has a strongly curved tip rather than having the form of a short rectangle; (4) the muscular sheath surrounding the copulatory tube attaches to two rod-shaped processes which arise from proximal end of the accessory piece, whereas in L. chabaudi it arises from an oval dilatation which is attached to the upper distal lobe of the accessory piece; and (5) the distal vagina is oval, whereas in L. chabaudi it is funnelshaped, resembling that of L. cephali.

Although our record of *L. vanbenedenii* on *M. cephalus* was erroneous, of all the Mediterranean species of *Ligophorus*, this appears the most similar to *L. mediterraneus*. However, *L. mediterraneus* differs from *L. vanbenedenii* in that: (1) both roots of the ventral anchor are longer (VIR 15–18 and VOR  $12-14 vs 10-12^5$  and 8–10 in *L. vanbenedenii*); (2) the tube of the copulatory organ is shorter (CTL 85–98 vs 100-140); and (3) the accessory piece bifurcates at two-thirds of its length from its distal end, and the upper lobe is longer and its tip distinctly curved downwards, whereas in *L. vanbenedenii* bifurcation begins at one-third from the distal end, and the upper lobe is short and its tip curves upwards.

# Discussion

As indicated above, the lack of accuracy in some descriptions of *Ligophorus* spp. was due to methodological errors in processing and analysis. In particular, inadequate flattening results in the anchors being situated at planes which are not at right angles to the axis of observation and all parts of anchor are not then clearly visible at once. The use of such slides for measuring and drawing results in a variation in the

<sup>&</sup>lt;sup>4</sup> Measurements of 20 specimens of *L. chabaudi* based on our own material from the Mediterranean Sea.

<sup>&</sup>lt;sup>5</sup> Measurements of 15 specimens of *L. vanbenedenii* based on our own material from *Liza aurata* in the Black Sea plus data from Euzet & Suriano, (1977) from the Mediterranean Sea.

angle between the point and shaft of up to  $12^{\circ}$  in *L. mediterraneus* (see Sarabeev, Balbuena & Euzet, 2005), up to  $21^{\circ}$  in *L. cephali* and up to  $26^{\circ}$  in *L. chabaudi* (see Rubtsova et al., 2006). Such variability has not been recorded previously, either for dactylogyrideans (Gusev, 1985) or gyrodactylideans (Ergens 1985). Nevertheless, this angle is very constant, it is laid down early in morphogenesis and it is a species-specific character, as has been shown for the anchors of a range of monogeneans (e.g. Bychowsky, 1957).

Another error lies in the inaccurate description of the structure of the ventral bar. Differences in the shape of the ventral and dorsal sides of this bar have been either considered to be discontinuous intraspecific variations (Sarabeev & Balbuena, 2004) or proposed as diagnostic characters for differentiating species of *Ligophorus* (see Sarabeev et al., 2005; Rubtsova et al., 2006). Thus for L. pilengas Sarabeev & Balbuena, 2004, it was reported that only part of examined material has a ventral bar with a 'nonmembranous median process<sup>'6</sup> (Sarabeev & Balbuena, 2004). In Fig. 3 of this same paper (Sarabeev & Balbuena, 2004, p. 226), which illustrates this character, a ventral view of the ventral bar without a 'nonmembranous median process' was shown (see Fig. 3A), along with a dorsal view of the ventral bar with this median process (see Fig. 3B,C). The description of the ventral bar structure, as it occurs in species of Ligophorus, has been presented above for L. mediterraneus. It follows from the present account, and from the description of ventral bar given by Bychowsky (1949), that the occurrence of a 'non-membranous median process' in 42% of examined specimens of L. pilengas was due to nothing more than the orientation of almost half of the worms on slides with the dorsal surface of the bar directed towards the observer. This feature (the absence of the median process in L. mediterraneus or presence of a 'heavily sclerotized median process', for example, in L. cephali and L. chabaudi), has been used as character enabling the distinction of groups of taxonomically related species. At the same time, in their figures illustrating this condition, the ventral bars representing these species were depicted on opposite sides: in L. mediterraneus it is shown ventrally (Fig. 2 of Sarabeev et al., 2005), whereas in L. cephali and L. chabaudi it is illustrated dorsally (Figs. 3 and 4 of Rubtsova et al., 2006). Our observations make it clear that, for L. mediterraneus and L. mugilinus (Figs. 3, 7) and for L. cephali and L. chabaudi (see Dmitrieva et al., 2009), as well as for the other 22 examined species (Dmitrieva et al., 2007a; Gerasev et al., 2009), the median knoll and wing-shaped laminae are always present on the dorsal side of the ventral bar. In fact, the median knoll does exhibit some diversity in form (height, width and shape of the anterior border) in different species of Ligophorus, and it sometimes shows some intraspecific variability (which varies in its expression; for example, compare Figs. 3B and C). The shape and size of the median knoll, when precisely described, but not its absence or the degree of its expression, can be used for the differentiation of Ligophorus spp. Moreover, for an accurate comparison of the shape of the ventral bar in different species, it is essential to view them from the same side.

The taxonomy of *Ligophorus* spp. is based on the morphology of the hard parts of the haptor and reproductive system. It is necessary to have a good understanding of the function of these organs for the proper description of their morphology. For example, differences in the shape of the dorsal and ventral sides of the ventral bar are associated with the attachment of muscle bundles and the functional interaction with other haptoral structures. Thus the functional approach is recommended for the description of the anchors (Dmitrieva et al., 2007), bars (present study), marginal hooklets, copulatory organ and vagina (Dmitrieva et al., 2009).

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<sup>&</sup>lt;sup>6</sup> Taking into account shape of this structure, we have named it as the 'median knoll' in our description.

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