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ORIGINAL PAPER

Ligophorus llewellyni n. sp. (Monogenea: Ancyrocephalidae) from the redlip mullet *Liza haematocheilus* (Temminck & Schlegel) introduced into the Black Sea from the Far East

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Abstract *Ligophorus llewellyni* n. sp. (Ancyrocephalidae: *Ligophorus* Euzet & Suriano, 1977) is described from the gills of *Liza haematocheilus* (Temminck & Schlegel) introduced into the Black Sea from the Far East. *Ligophorus llewellyni* is closely related to *L. pilengas* Sarabeev & Balbuena, 2004, which parasitises the same host species. The two species differ in the morphology of the accessory piece of the copulatory organ and in some of the characters of the haptoral hard-parts. The morphometric variability of *L. llewellyni* and in its morphologically most similar congeners from the Black Sea is studied. Correlations between 30 morphometric characters of the haptoral hard-parts and the significance of each for species differentiation are examined. It is suggested that only 22 characters are useful as diagnostic criteria permitting the differentiation of morphologically similar species of *Ligophorus*.

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Introduction

Ligophorus Euzet & Suriano, 1977 comprises 23 species (Bychowsky, 1949; Dmitrieva & Gerasev, 1996; Euzet & Sanfilippo, 1983; Euzet & Suriano, 1977; Fernandez, 1987; Gusev, 1985; Miroshnichenko & Maltsev, 2004; Sarabeev & Balbuena, 2004; Sarabeev, Balbuena, & Euzet, 2005; Zhang, Yang, Liu & Ding, 2003), which parasitise only fishes of the family Mugilidae. Thomson (1997) identified 62 valid species in this family, but to date only 11 are known as hosts of *Ligophorus* spp. Species of *Ligophorus* have been described from an area limited to parts of the North Atlantic (especially the Mediterranean Basin) and off the coasts of the North-Western Pacific and the Pacific coast of South America, an area that is spatially considerably smaller than the natural distribution of their mugilid hosts. As all studied mugilids have been infected with more than one species of *Ligophorus* (for example, at least six species are known as common parasites of *Mugil cephalus*), it is natural to presume that this genus is far more diverse than currently described and investigations of other geographical regions and mugilid species will further increase our knowledge of the species diversity of the genus. *Ligophorus* is of particular interest for clarifying the phylogeography of mugilid fishes.

There is an urgent need for a taxonomic revision of *Ligophorus*. Taking into account the fact that many species of the genus are morphologically similar, a more precise knowledge of the taxonomic characters of this monogenean is of particular importance. For example, the examination of *Ligophorus* spp. from *Liza haematocheilus* in the Black Sea revealed that a taxon reported by Dmitrieva (1996) from this host as a host-variant of *Ligophorus chabaudi* Euzet & Suriano, 1977 (described from *Mugil cephalus*) represents a distinct species. This species has been recently described as *L. pilengas* Sarabeev & Balbuena, 2004, which is a senior synonym of *L. gussevi* Miroshnichenko & Maltsev, 2004 (Balbuena, Rubtsova & Sarabeev, 2006).

This paper presents the description of *L. llewellyni* n. sp. from *Liza haematocheilus*, which has been introduced into the Black Sea from the Far East. This host has previously been regarded as *Mugil soiuy* Basilewsky, but recent work (Dr I.J. Harrison, personal communication) suggests that this fish should be regarded as *Liza haematocheilus*. A description of the new species and discrimination from its most morphologically similar congeners, *Ligophorus pilengas* and *L. chabaudi* Euzet & Suriano, 1977 *sensu* Dmitrieva & Gerasev (1996) are presented below, along with an analysis of the characters used in the description of species of *Ligophorus*.

Materials and methods

The new species is described based on 16 specimens collected from the gills of two specimens of *Liza haematocheilus*, 28 and 42 cm long, captured in coastal waters of the Black Sea near Sevastopol (44°35′N, 33°30′E) during July, 2002. For comparison, 17 specimens of *Ligophorus pilengas* from the same fish, and 15 specimens of *L. chabaudi* (*sensu* Dmitrieva & Gerasev, 1996) from two specimens of *Mugil cephalus*, 33 and 35 cm long, captured at the same locality were also studied. Monogeneans were collected from the freshly caught fish and then immediately mounted in glycerine-jelly (prepared with a trace of carbolic acid).

Drawings and light micrographs were made using a Carl Zeiss Amplival microscope fitted with a drawing tube and an Olympus C180 digital camera.

The measuring scheme included 36 characters (Fig. 1) and is based on that suggested for the Dactylogyridea by Gussev (1985). The names and abbreviations of the characters measured are presented in Table 1. All measurements are given in micrometres, and the smallest division of the graticule used for measuring was 1 μm. The mean, standard error and the coefficient of variation (calculated as a percentage of the standard deviation of the mean) are used.

Data analysis was carried out using independent t-tests, Pearson correlations and Principal Component Analysis (StatSoft Inc., 2001). The statistical analyses and their graphical representation were produced using the Statistica 6 for Windows software package.

The nomenclature of fish species is given according to Harrison (2004) and Bogutskaya & Naseka (2004).

Ligophorus llewellyni n. sp.

Type-host: Redlip mullet *Liza haematocheilus* (Temminck & Schlegel) [syns *Mugil soiuy* Basilewsky; *L. haematocheila* (Temminck & Schlegel)].
Site on host: Gills.
Type-locality: Off Sevastopol, Crimean peninsula, Black Sea (44°35′N, 33°30′E).
Type-specimens: Holotype and several paratypes deposited in the Institute of Biology of the Southern Seas, Sevastopol (holotype: No. 509, paratypes: No. 509/1–10). Additional paratypes are in the Zoological Institute, St. Petersburg (paratypes: No. 8195–98) and the Natural History Museum, London (paratypes: BMNH No. 2006.5.23.1).
Etymology: The species is named for the late Prof. J. Llewellyn, an outstanding expert on monogeneans.

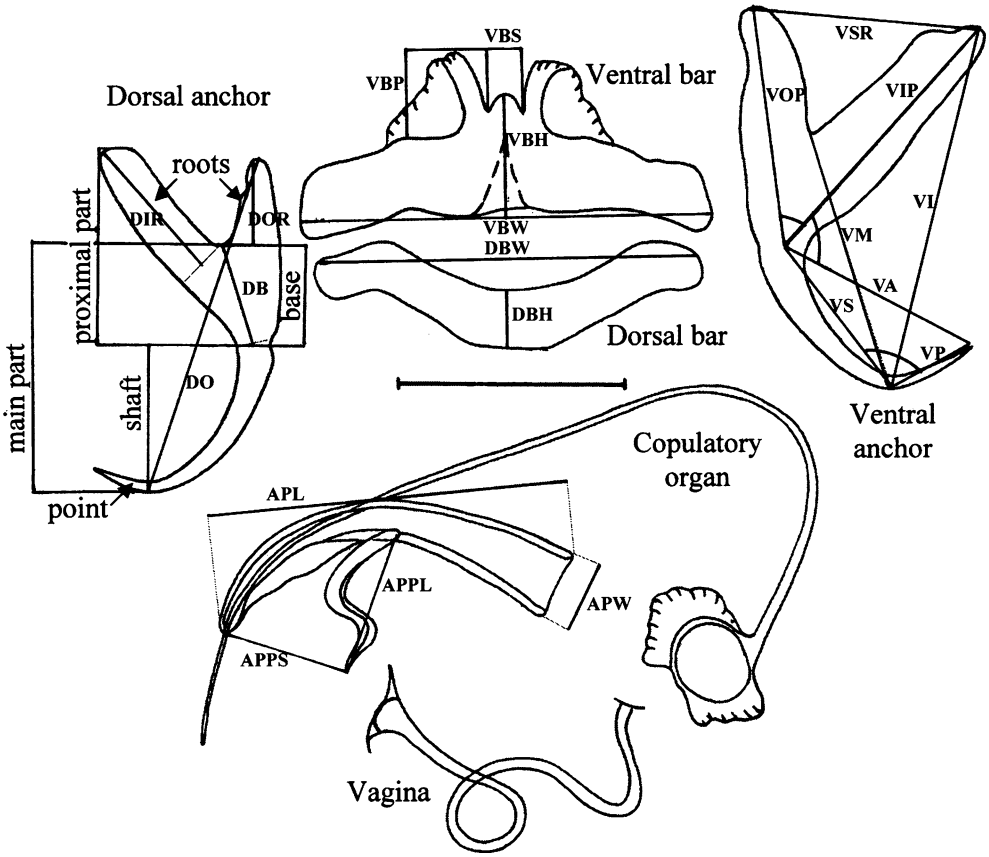

Fig. 1 Scheme of measurements for the anchors, bars, copulatory organ and vagina of *Ligophorus* spp. illustrated using, as an example, features of *L. chabaudi* Euzet & Suriano, 1977 *sensu* Dmitrieva & Gerasev (1996) from *Mugil cephalus* in the Black Sea. *Scale-bar*: 25 μm

Description (Figs. 2, 3a)

Flattened specimens with body-size 715 ± 29 (592–978) × 118 ± 8 (80–176). Haptoral armament conforms to descriptions of Euzet & Suriano (1977). Size characteristics of anchors, bars and parts of reproductive system are given in Table 2. Both anchors elongate; shaft and proximal part of similar length; shaft at obtuse angle of *c*.120° (angle between VIP and VS, see Fig. 1). Points of both anchors and their shafts form right-angle (angle between VS and VP, see Fig. 1). Proximal part of ventral anchor with roots of equal length; inner root of dorsal anchor twice as long as outer root. Ventral bar has 2 anterior processes widely spread apart and attached to main part of bar with lateral membranes on either side. Dorsal bar strongly curved. Marginal hooklets typical for genus in shape and size (Euzet & Suriano, 1977); total length 12.5 ± 0.1 (12–13), shaft length 7 ± 0.1 (6–7) and sickle length 6.5 ± 0.1 (5–6).

Copulatory organ consists of copulatory tube and accessory piece. Accessory piece forms deep gutter, with U-shaped cross-section partly enclosing copulatory tube, bifurcates into 2 equal parts 1/3 of distance from its distal end; terminal bifurcations also have gutter-like form and closely abut each other along open faces to form closed canal; consequently, rounded aperture of distal end of accessory piece is composed of 2 well-defined half-rings through which copulatory tube can move.

Vaginal armament is typical for genus, forming hollow, narrow tube with solid walls.

Table 1 List of characters and abbreviations

I. Ventral anchor (V), Dorsal anchor (D):	
1, 2	outer length of anchor (VO, DO)
3, 4	inner length of anchor (VI, DI)
5, 6	length of main part (VM, DM)
7, 8	span between roots (VSR, DSR)
9, 10	length of outer root (VOR, DOR)
11, 12	length of inner root (VIR, DIR)
13, 14	length of base (VB, DB)
15, 16	inner length of proximal part (VIP, DIP)
17, 18	outer length of proximal part (VOP, DOP)
19, 20	length of shaft (VS, DS)
21, 22	distance from point tip to shaft end (VA, DA)
23, 24	length of point (VP, DP)
II. Ventral bar (VB), Dorsal bar (DB):	
25, 26	height (VBH, DBH)
27, 28	width (VBW,DBW)
29	height of anterior processes (VBP)
30	span between anterior processes (VBS)
III. Copulatory organ:	
31	length of accessory piece (APL)
32	width of accessory piece (APW)
33	length of "lower lobe" of accessory piece (APPL)
34	span between "lower lobe" and main part of accessory piece (APPS)
35	copulatory tube length (CTL)
IV. Vagina:	
36	vaginal length (VL)

Differential diagnosis

Compared with the closely related *Ligophorus pilengas*, which also parasitises *Liza haematocheilus* (Figs. 3b, 4), *Ligophorus llewellyni* n. sp. can be distinguished by four characters. These are: (1) larger dorsal anchors (Table 2); (2) the inner length of the proximal part (VIP) and the length of the main part (VM) of the ventral anchor are both significantly shorter; (3) the copulatory tube is shorter in overall length; and (4) the terminal bifurcations of the accessory piece of the copulatory organ are equal in length and width, whereas in *L. pilengas* their length and width differs and they can be distinguished as an 'upper lobe' and 'lower lobe' (Sarabeev & Balbuena, 2004), and the ends of the bifurcations are separate in *L. pilengas* and do not closely enclose the copulatory tube (Fig. 3).

Among other species of *Ligophorus*, *L. chabaudi* (*sensu* Dmitrieva & Gerasev, 1996) (Fig. 1), which parasitises *Mugil cephalus*, appears the most similar to *L. llewellyni* n. sp. The latter differs from *L. chabaudi* in: (1) the overall larger size of the haptoral hard-parts (Table 2); (2) the different proportions of the ventral anchor differ – in *L. chabaudi* the length of the shaft (VS) and the distance from the point tip to the end of the shaft (VA) are shorter and non-overlapping; (3) the anterior processes of the ventral bar are more closely positioned in *L. chabaudi*, but are set widely apart in *L. llewellyni*; and (4) the morphology of the accessory piece of the copulatory organ of *L. chabaudi* resembles that of *L. pilengas* (Fig. 3) more than it does that of *L. llewellyni*.

Black Sea specimens of *L. llewellyni* n. sp. and *L. pilengas* were repeatably found to differ in 14 of the 36 size characters, while *L. llewellyni* and *L. chabaudi* differed in 21 size characters (Table 2).

Remarks

In the Black Sea, in addition to *Ligophorus llewellyni* n. sp. and *L. pilengas, Liza haematocheilus* is also parasitised by *Ligophorus kaohsianghsieni* (Gusev, 1962), which was originally described from the Far East. This differs greatly from the new species in the shape and size of both the haptoral hard-parts and the copulatory organ. All three species were found in samples collected from *Liza haematocheilus* from the Far East (the collection of the Zoological Institute of the RAS, St Petersburg and the Institute of Biology and Soil Sciences of the Far East Branch of the RAS, Vladivostok). Moreover, *Ligophorus leporinus* (Yang & Ji, 1981), originally described from Far Eastern *Mugil cephalus*, was also identified in these samples. This species resembles *L. kaohsianghsieni* but differs from *L. llewellyni* in all characters of taxonomic importance.

In the Azov and Black Seas, as in its Far Eastern habitats, *Liza haematocheilus* often occurs in mixed schools with *Mugil cephalus* (see Popov, 1930). This is presumably the reason for the overlap of their *Ligophorus* fauna with that recorded on these fishes from the North-Western Pacific region (Zhang et al., 2003).

In addition to the above-mentioned *Ligophorus chabaudi*, *L. leporinus* and *L. kaohsianghsieni*, *Mugil cephalus* in the Northwest Pacific is also

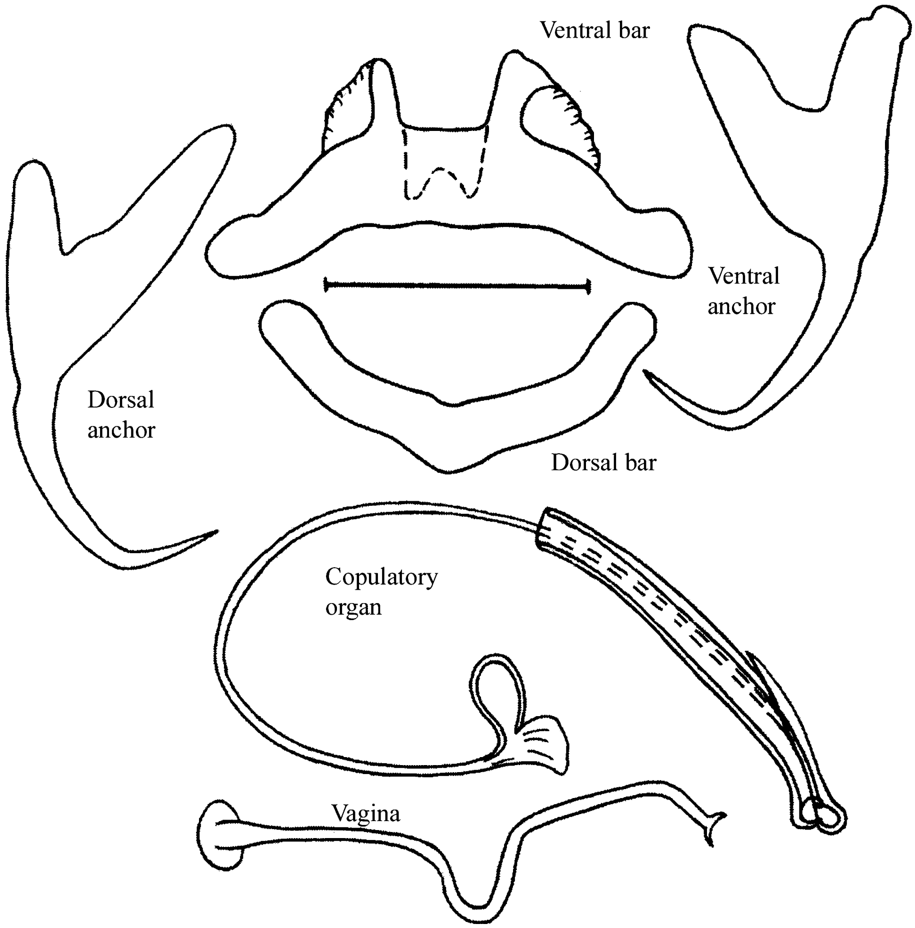

Fig. 2 Anchors, bars, copulatory organ and vagina of *Ligophorus llewellyni* n. sp. from *Liza haematocheilus* in the Black Sea. *Scale-bar*: 25 µm

parasitised by *L. vanbenedeni* (Parona & Perugia, 1890), *L. chenzhenesis* Hu & Li, 1992, *L. chongmingensis* Hu & Li, 1992 and *L. mugilinus* (Hargis, 1955). *L. chongmingensis* differs greatly from the new species in most morphological characters. The other three species have some similarity with *L. llewellyni* in the shape of the haptoral hard-parts but differ in the structure of the copulatory organ. Moreover, *L. mugilinus* and *L. vanbenedeni* have smaller haptoral structures and accessory piece of the copulatory organ, while *L. chenzhenesis* has a considerably shorter copulatory tube.

In the Azov and Black Seas, *Mugil cephalus* is parasitised by *L. chabaudi* and *L. mediterraneus* Sarabeev, Balbuena & Euzet, 2005. The latter has been recently distinguished from *L. mugilinus* but closely resembles it. Additionally, *L. mediterraneus* differs from *L. llewellyni* in the shape of the dorsal bar.

In the native habitat of *Liza haematocheilus, Ligophorus ellochelon* Zhang, Yang & Liu, 2001 and *L. hamulosus* Pan & Zhang, 1999 from *Liza vagiensis* and *L. macrolepis*, respectively, have also been described. Both can be distinguished from *Ligophorus llewellyni* by the morphology of both the haptoral hard-parts and the copulatory organ.

To summarise, *L. pilengas*, the only one of four *Ligophorus* species recorded on *Liza haematocheilus* in both its original Far Eastern habitats and in its recently introduced Black Sea habitats, is most similar to *L. llewellyni*. The other species all differ greatly in most characters of taxonomic

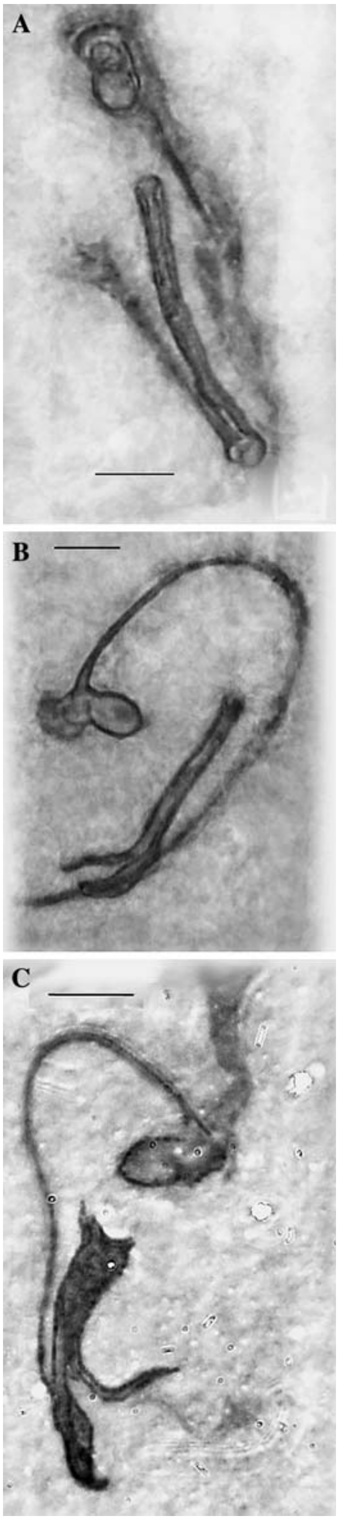

Fig. 3 Copulatory organs of: A. *Ligophorus llewellyni* n. sp.; B. *L. pilengas* Sarabeev & Balbuena, 2004; C. *L. chabaudi* Euzet & Suriano, 1977 *sensu* Dmitrieva & Gerasev (1996). *Scale-bars*: 10 µm

significance. Among the species parasitising the ecologically related host *Mugil cephalus* in both regions, only *L. chabaudi* resembles the new species. Finally, *L. llewellyni* also differs considerably from other *Ligophorus* spp. parasitising other mullets in the NW Pacific region.

Within the new Azov and Black Seas habitats, *Liza haematocheilus* is in contact with two indigenous mullets (*L. aurata* and *L. saliens*) and can become infected with their specific species of *Ligophorus*. In these seas, *Liza aurata* is parasitised by *Ligophorus vanbenedeni* and *L. szidati* Euzet & Suriano, 1977 (see Dmitrieva & Gerasev, 1996; Miroshnichenko & Maltsev, 1998); the latter can be distinguished from *L. llewellyni* in all major characters. *Liza saliens* is parasitised by *Ligophorus euzeti* Dmitrieva & Gerasev, 1996 and *L. acuminatus* Euzet & Suriano, 1977 (see Dmitrieva & Gerasev, 1996). Both possess similar haptoral hard-parts which are clearly different from those of *L. llewellyni*. Thus, *L. llewellyni*, which apparently has a Far Eastern origin, differs considerably from its congeners parasitising other native mullet species within the Black/Azov Sea region.

Morphometric analysis of *Ligophorus llewellyni* n. sp., *L. pilengas* and *L. chabaudi*

The coefficients of variation (CV) calculated for most haptoral dimensions are consistently low in each of the three examined species (Table 2). Those for anchor root dimensions have a higher CV, probably because of high individual variability in the shape of the proximal part of the anchors at the point where the roots bifurcate (Fig. 5).

Fifteen haptoral dimensions are positively correlated with the length of the worm (Table 3). The ventral anchor outer length (VO) and the width of the bars (VBW, DBW) are significantly correlated with the length of the worm. Measurements of the total length of the anchors, such as the inner and outer lengths (VO, VI, DO, DI) and the length of its main part (VM, DM), are positively correlated with many of the anchor dimensions, and coefficients of correlation between anchor dimensions are greater than any for correlation between anchor dimensions and

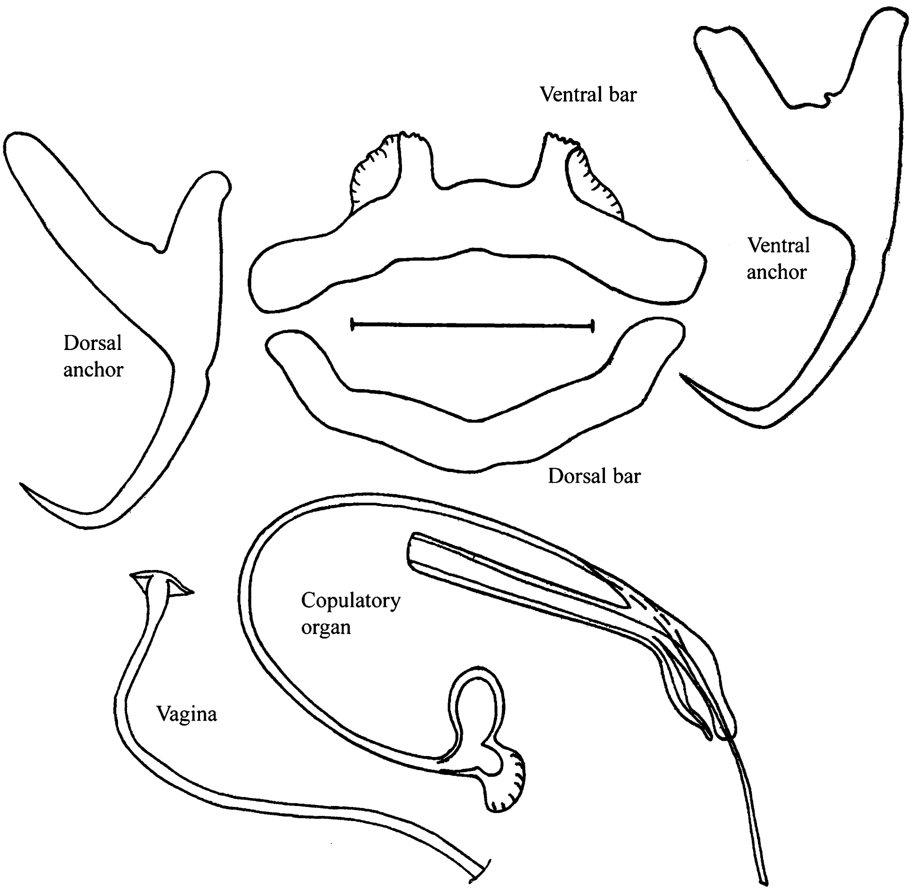

Fig. 4 Anchors, bars, copulatory organ and vagina of *Ligophorus pilengas* Sarabeev & Balbuena, 2004 from *Liza haematocheilus* in the Black Sea. *Scale-bars*: 25 μm

the length of the worm. The lengths of the anchor roots (VOR, DOR, VIR, DIR) are significantly correlated with the largest number of other anchor dimensions.

Principal Component Analysis has shown that the 30 characters describing the haptoral hard-parts contribute differently to the differentiation of the compared species. The first two principal components (PCs) describe more than half of the total variance observed between these species. Based on the component loadings for the first two PCs (Fig. 6) and correlations between the 30 measurements (Table 3), 22 characters were selected: VI, DI, VM, DM, VSR, DSR, VOP, DOP, VIP, DIP, VS, DS, VA, DA, VP, DP, VBW, DBW, VBH, DBH, VBS and VBP. Fig. 1 shows the measurements taken, including these 22 characters, which are indicated for the bars and the ventral anchor. The selected characters were used to discriminate between *L. llewellyni* n. sp. and the morphologically similar *L. pilengas* and *L. chabaudi*. The first three PCs based on these 22

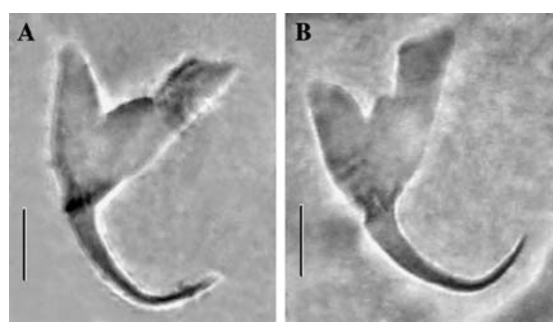

Fig. 5 Variation of the shape of the proximal part of the ventral anchor illustrated, as an example, for *L. chabaudi* Euzet & Suriano, 1977 *sensu* Dmitrieva & Gerasev (1996). *Scale-bar*: 10 μm

Table 2 Size characteristics of the anchors, bars, copulatory organ and vagina of *Ligophorus llewellyni* n. sp., *L. pilengas* Sarabeev & Balbuena, 2004 and *L. chabaudi* Euzet & Suriano, 1977 *sensu* Dmitrieva & Gerasev (1996) from the Black/Azov Sea region

Statistical characteristic	$\bar{X} \pm SE/CV$ min-max*			T-test		
Species of *Ligophorus* Host	*llewellyni* n. sp. *Liza haematocheilus*	*pilengas*	*chabaudi* *Mugil cephalus*			
No. of specimens Sample index	16 A	17 B	15 C	A-B	A-C	B-C
Worm length	715 ± 29/15 592–978	683 ± 33/18 432–896	464 ± 29/20 288–592	0.7	**6**	**4.7**
Ventral anchor:						
outer length	43.7 ± 0.5/4 41–47	44.3 ± 0.3/3 42–47	38.7 ± 0.5/5 36–42	1.1	**8.3****	**10.8**
inner length	40.8 ± 0.5/4 39–45	40.5 ± 0.3/3 38–44	38.1 ± 0.5/6 35–43	0.5	**3.2**	**3.1**
length of main part	32.6 ± 0.3/3.5 31.5–34.5	35.5 ± 0.2/3 33–36.5	27 ± 0.2/3 26.5–28	**8**	**16**	**29**
span between roots	17.8 ± 0.2/5 16–19	17.2 ± 0.3/7 15–19	19.2 ± 0.3/6 17–21	1.6	2.2	**3.7**
length of outer root	9.8 ± 0.3/10 8–11	10.6 ± 0.2/8 8–11	10.8 ± 0.1/4 10–11	2.3	**3.4**	0.6
length of inner root	14.2 ± 0.3/9 13–17	12 ± 0.3/9 10–13	14.5 ± 0.3/8 12–16	**5.2**	1.5	**7.2**
length of base	15.2 ± 0.3/7 14–17	15.7 ± 0.3/8 14–18	15.8 ± 0.3/8 14–17	1.3	2.4	0.1
outer length of proximal part	22 ± 0.2/3 21–23	22 ± 0.1/3 21–23	21 ± 0.2/4 20–22	1	**4.2**	**3.7**
inner length of proximal part	25 ± 0.4/6 23–27	32 ± 0.2/3 30–33	26 ± 0.3/4 25–27	**15**	0.8	**17.5**
length of shaft	21.9 ± 0.2/4 21–23	22.8 ± 0.2/4 21–24	19.4 ± 0.3/5 18–21	2.8	**8.5**	**12.2**
distance from point tip to shaft end	23.4 ± 0.3/9 22–25	23.9 ± 0.2/3 23–25	21.8 ± 0.3/6 20–24	1.8	**4.5**	**6.8**
length of point	9.8 ± 0.1/4 9–10	9.9 ± 0.1/3 9–10	10.6 ± 0.1/5 10–11	1.2	**4.7**	**4.5**
Dorsal anchor:						
outer length	40.9 ± 0.6/6 36–45	38.7 ± 0.1/2 38–40	38.7 ± 0.4/4 35–42	**3.6**	**3**	0.9
inner length	44.2 ± 0.7/6 40–48	40.7 ± 0.2/2 40–42	38.2 ± 0.4/4 35–40	**5.4**	**7.4**	**3.6**
length of main part	30.8 ± 0.3/3 30–33	28.7 ± 0.2/3 28–30	28,5 ± 0,3/5 26.5–30	**6**	**5.5**	0.6
span between roots	16.9 ± 0.3/7 14–18	18.2 ± 0.2/5 17–20	15.4 ± 0.3/8 13–18	**3.3**	3	**7.5**
length of outer root	10 ± 0.4/14 7–12	9.2 ± 0.3/14 6–12	8.9 ± 0.3/14 6–12	1.7	2.2	0.2
length of inner root	19.5 ± 0.5/9 15–22	17.4 ± 0.2/6 16–19	15.8 ± 0.4/10 14–18	**4.3**	**6.6**	**3.2**
length of base	12.3 ± 0.2/7 11–13	12.4 ± 0.2/8 11–14	11.3 ± 0.3/9 10–13	0.1	**3.1**	**3.2**
outer length of proximal part	22.4 ± 0.2/3 21–23	22.5 ± 0.2/3 21–23	22.7 ± 0.2/5 21–23	0.3	1.2	1
inner length of proximal part	30.8 ± 0.3/3 30–33	28.2 ± 0.2/3 27–30	28 ± 0.4/5 25–30	**8**	**5.9**	0.1
length of shaft	20.7 ± 0.2/4 20–22	20.1 ± 0.2/4 19–21	20.8 ± 0.3/5 19–23	2.4	0.1	2

Table 2 Continued

Statistical characteristic	$\overline{\mathbf{X}} \pm$ SE/CV min-max*			T-test		
Species of *Ligophorus* Host	*llewellyni* n. sp. *Liza haematocheilus*	*pilengas*	*chabaudi* *Mugil cephalus*			
No. of specimens Sample index	16 A	17 B	15 C	A-B	A-C	B-C
distance from point tip to shaft end	22.3 ± 0.3/5 21–24	21.8 ± 0.2/4 21–23	22.8 ± 0.3/5 21–25	1.4	1.1	2.7
length of point	9.7 ± 0.1/5 9–10	9.3 ± 0.1/5 9–10	9.1 ± 0.1/4 9–10	2.8	**4.5**	1.5
Ventral bar:						
width	47.1 ± 0.4/4 44–50	47.7 ± 0.5/4 44–51	37.3 ± 0.4/4 35–40	0.9	**15.4**	**16.4**
height	9.9 ± 0.3/13 8–12	8.5 ± 0.2/7 8–10	6.1 ± 0.1/7 5–7	**3.9**	**12.8**	**13.9**
length of anterior processes	5.1 ± 0.2/13 4–6	5.2 ± 0.1/8 5–6	4.8 ± 0.1/12 4–6	0.5	1	1.6
span between processes	7.7 ± 0.2/12 6–9	8.4 ± 0.2/8 7–9	5.1 ± 0.2/20 3–7	2.6	**5.9**	**8.3**
Dorsal bar:						
width	41.7 ± 0.6/6 38–47	43.5 ± 0.4/4 40–46	35.7 ± 0.7/8 32–41	2.4	**3.4**	**5.4**
height	7.4 ± 0.3/17 6–9	6.1 ± 0.2/13 5–8	5.2 ± 0.1/10 4–6	**3.5**	**7.5**	**4.4**
Accessory piece of copulatory organ:						
length	39.5 ± 0.4/4 35–42	36.1 ± 0.6/7 32–40	38.1 ± 0.7/7 35–43	**4.6**	1	**3.5**
width	3.1 ± 0.1/11 3–4	3.2 ± 0.1/12 3–4	4.3 ± 0.2/14 3–5	0.3	**3.4**	**3.6**
length of 'lower lobe'	0	10.1 ± 0.2/10 8–12	15.1 ± 0.5/14 11–18	–	–	**9.1**
span between 'lower lobe' and main part	0	2.2 ± 0.2/42 1–4	5.5 ± 0.5/35 3–10	–	–	**6.5**
Copulatory tube:						
length	102.5 ± 2.3/6 91–110	114 ± 1.2/19 108–120	106 ± 2.2/13 93–115	**4.7**	0.3	**3.5**
Vagina:						
length	66.4 ± 1.2/6 60–75	57.2 ± 2.6/19 45–75	73.3 ± 2.6/13 60–90	**3.6**	2.1	**4.6**

* $\overline{\mathbf{X}}$, mean, µm; ± SE, standard error, µm; CV, coefficient of variation, %; min-max, range, µm; T-test, independent t-test;
** T-test: significant differences at the 5% level are given in bold

characters, which explain 72% of the overall variance, were calculated from 48 specimens of the three species of *Ligophorus* (Fig. 7). *L. pilengas* and *L. llewellyni*, from *Liza haematocheilus*, were separated from *Ligophorus chabaudi*, from *Mugil cephalus*, by Factor 1 (which explains 44% of the total variance), and specimens were ranked mainly according to the sizes of the ventral anchors and bars (Fig. 7A). *L. pilengas* was separated from two other species by Factor 2 (17%), the specimens being ranked mainly by the size of the dorsal anchor. *L. llewellyni* was separated from *L. pilengas* and *L. chabaudi* by Factor 3 (9%), with specimens ranked primarily according to the inner length of the proximal part of ventral anchor (VIP) and by the distance between the roots of the dorsal anchor (DSR; Fig. 7B).

Table 3 Indices of correlation between anchors and bars measurements (n = 48): coefficient of determination (r^2) and regression coefficient for significant level of correlation (r^2 / b)

	Worm length	VO	VI	VM	VSR	VOR
VO	**0.45***/0.014					
VI	**0.25**/0.007	**0.75**/0.601				
VM	**0.37**/0.015	**0.77**/1.070	**0.52**/1.260			
VSR	0.10	**0.11**/– 0.141	0.01	**0.24**/–0.170		
VOR	0.02	0.00	0.02	0.00	0.03	
VIR	0.08	0.08	0.00	**0.25**/– 0.200	**0.27**/0.605	0.03
VB	0.01	0.00	0.02	0.00	0.02	0.00
VOP	**0.33**/0.003	**0.68**/0.228	**0.47**/0.272	**0.44**/0.150	0.10	0.01
VIP	0.08	**0.38**/0.694	**0.21**/0.745	**0.54**/0.675	**0.12**/–0.918	**0.16**/1.491
VS	**0.30**/0.006	**0.77**/0.480	**0.54**/0.578	**0.79**/0.398	0.08	0.01
VA	**0.18**/0.004	**0.56**/0.318	**0.46**/0.412	**0.51**/0.248	0.00	0.00
VP	**0.33/**–0.002	**0.25**/–0.075	**0.18**/– 0.094	**0.24/**–0.061	0.03	0.00
	VIR	VB	VOP	VIP	VS	VA
VB	**0.12**/– 0.259					
VOP	0.06	0.00				
VIP	**0.37**/–1.404	0.02	**0.19**/1.746			
VS	**0.15**/–0.433	0.00	**0.45**/1.332	**0.41**/0.313		
VA	0.04	0.00	**0.28**/0.816	**0.30/**0.206	**0.81**/0.696	
VP	0.00	0.00	**0.15**/–0.212	0.03	**0.16/–**0.110	0.08
	Worm length	DO	DI	DM	DSR	DOR
DO	0.09					
DI	**0.32**/0.011	**0.66**/1.239				
DM	**0.27**/0.005	**0.38**/0.481	**0.63**/0.408			
DSR	**0.33**/0.006	0.08	**0.18**/0.234	**0.13**/0.387		
DOR	0.04	**0.51**/0.506	**0.30**/0.255	**0.29**/0.483	0.10	
DIR	**0.35**/0.008	**0.49**/0.804	**0.77**/0.661	**0.58**/1.118	**0.30**/0.747	**0.40**/1.013
DB	0.01	0.00	0.03	0.00	0.00	**0.11**/–0.250
DOP	0.01	**0.30**/0.188	0.05	0.02	0.01	**0.21**/0.223
DIP	0.09	**0.55**/0.612	**0.60**/0.415	**0.48**/0.725	0.00	**0.30**/0.632
DS	0.00	**0.30**/0.253	0.07	0.05	0.01	**0.18**/0.273
DA	0.01	**0.30**/0.297	0.04	0.03	0.00	**0.21**/0.35
DP	0.15	0.06	**0.21**/0.078	**0.31**/0.186	0.08	0.06
	DIR	DB	DOP	DIP	DS	DA
DB	0.00					
DOP	0.03	0.05				
DIP	**0.39**/0.445	0.01	0.10			
DS	**0.12**/0.137	0.03	0.05	**0.16**/0.223		
DA	0.08	0.04	0.08	0.07	**0.82**/1.07	
DP	**0.30**/0.125	0.00	0.02	**0.18**/0.136	0.00	0.00
	Worm length	VBW	VBH	DBW		
VBW	**0.51**/0.022					
VBH	**0.39**/0.006	**0.37**/0.178				
DBW	**0.41**/0.015	**0.74**/0.661	**0.29**/1.403			
DBH	**0.18**/0.002	**0.33**/0.118	**0.36**/0.422	**0.31**/0.15		

* r^2 values corresponding to significant level of correlation for $p < 0{,}05$ are given in bold

Discussion

In describing the new species, we were faced with the problem that previous authors examining species of *Ligophorus* have used different measurement systems (Euzet & Suriano, 1977; Gusev, 1985; Dmitrieva & Gerasev, 1996; Mariniello, Ortis, D'Amelio & Petrarca, 2004; Miroshnichenko & Maltsev, 2004; Sarabeev & Balbuena, 2004). This makes comparison of different species difficult, especially as some are morphologically similar.

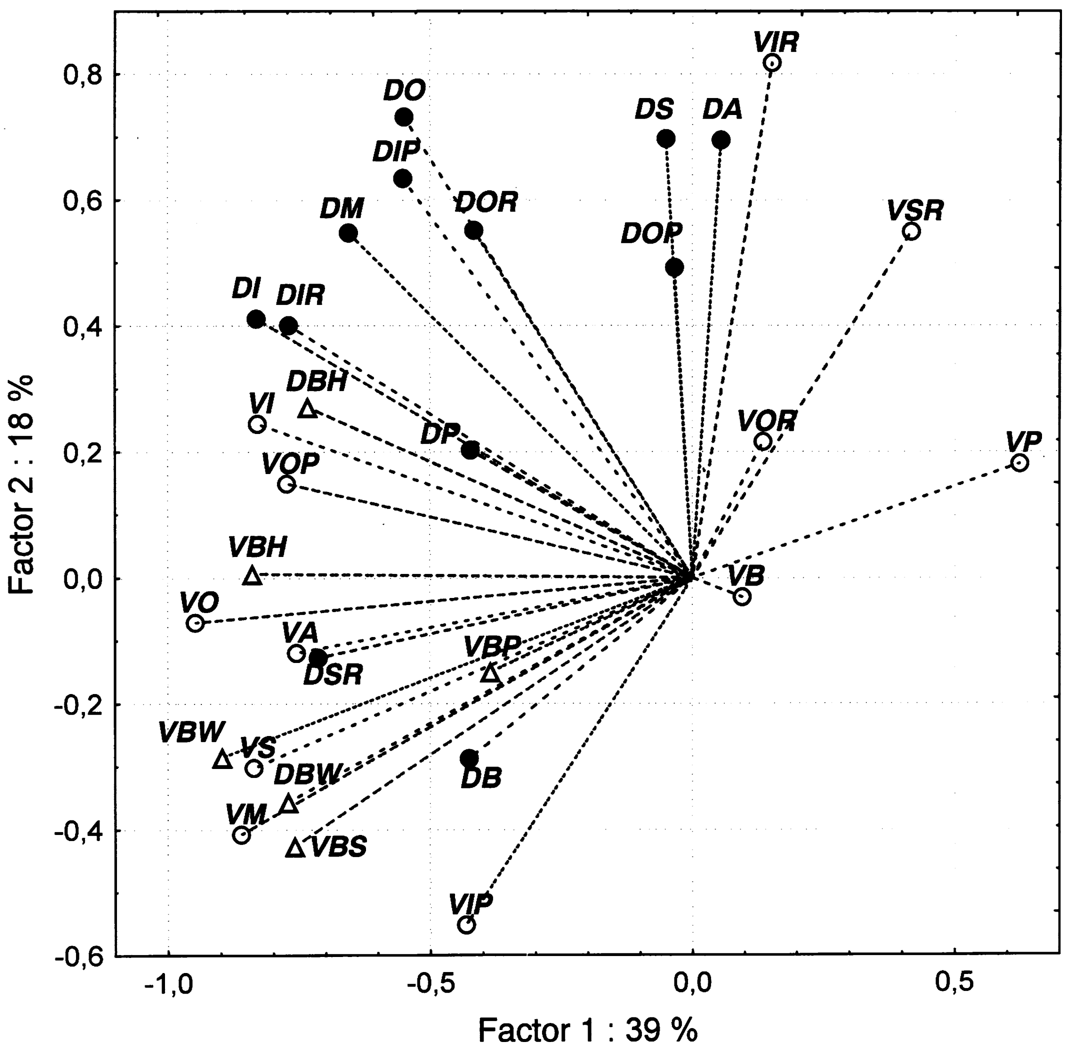

Fig. 6 PCA plot of the contribution made by 30 characters taken from the haptoral hard-parts for the first two factors calculated from 48 specimens of three species of *Ligophorus* from the Black Sea. The projection of the vector on the axis is the value of the factor loadings of the corresponding variable. The angle between any two variables is inversely proportional to the correlation between them. *Key*: ○ ventral anchor; • dorsal anchor; Δ bars

The analysis presented here has utilised all 36 characters that have been used by previous researchers in describing the morphology of the haptoral hard-parts, copulatory organ and vagina. It was found that some of these characters partly overlapped or duplicated each other and that they were highly correlation with each other. Thus, it seemed reasonable not to use measurements of the highly variable inner and outer lengths of the anchor roots (VOR, DOR, VIR and DIR, see Fig. 1) and, instead, to concentrate on more stable measurements, such as the inner and outer lengths of proximal part of the anchors (VIP, DIP and VOP, DOP), as previously proposed for lower monogeneans by Pugachev (1988). These latter dimensions are of greater importance for differentiating between the species (Fig. 6). The outer length of the anchors (VO and DO) is significantly correlated with the length of the main part (VM and DM) and with the outer length of the proximal part (VOP and DOP), essentially duplicating them. Moreover the choice of VOP, VIP, DOP and DIP was made based on the position at the margin between the shaft and base of the anchor from which these characters were measured. This position is functionally important

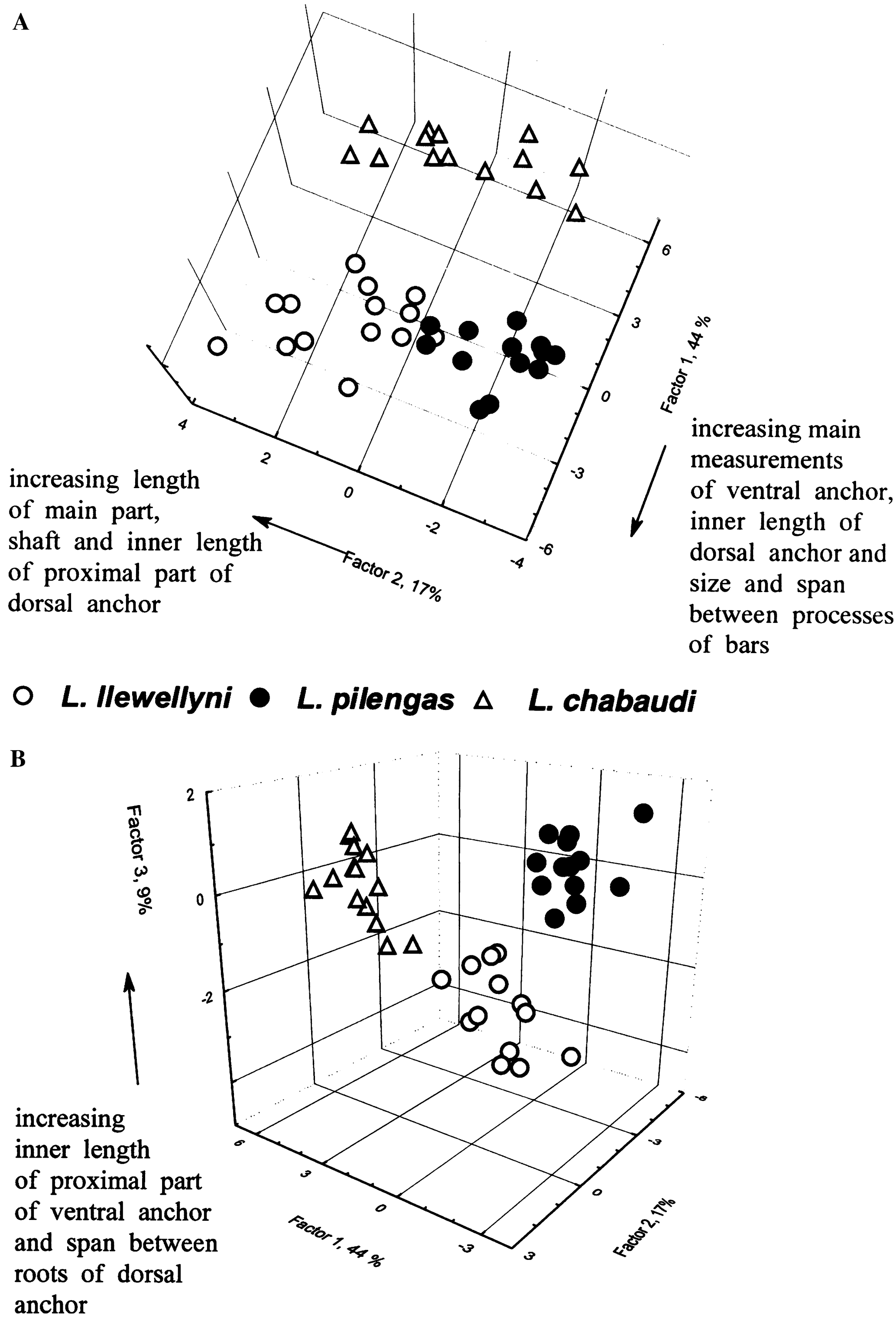

Fig. 7 PCA plot of the scores of the first three factors calculated from 22 characters of the haptoral hard-parts for 48 specimens belonging to three species of *Ligophorus* from the Black Sea. A, B, different projections of the plot; →, direction of increasing characters size separating the specimens

as the centre of rotation of the anchor as it penetrates or is removed from the gills (Gerasev, 1977, 1981; Pugachev, 1988). Apart from the common measurements of shaft and point length, we also proposed the measurement of the distance from the point tip to the end of the shaft (the above-mentioned centre of rotation of the anchor) used previously by Gusev (1985, fig. 7.3) to describe the dactylogyrid anchor which lacks a distinct boundary between the shaft and the point and is termed a 'blade'. This measurement defines the 'straightness' of the anchor. Finally, the length of the anchor base (VB, DB, see Fig. 1) is of minor significance in species discrimination.

Hence, only eight (Fig. 1: the included characters are shown for the ventral anchor) of the 12 analysed characters (Fig. 1: the excluded characters are shown for the dorsal anchor) are considered useful for describing the anchors of *Ligophorus* species.

In describing anchor shape, some researchers (Pugachev, 1988; Sarabeev & Balbuena, 2004) have used angles, but accurate measurement is difficult. The suggested scheme of measurement consists of three triangles (Fig. 1: ventral anchor: VA-VP-VS, VI-VS-VIP and VIP-VOP-VSR), which are firmly associated with and therefore strictly determine the angles between the shaft and the base of anchor (VS to VIP), the point and the shaft (VP to VS) and between the roots (VOP to VIP). This system of measurement has been successfully employed to discriminate *L. llewellyni* from its morphologically most similar congeners.

The considerable reduction in the number of measurements suggested here may lead to an underestimation of the differences when comparing morphologically similar species. For example, comparing our data on the morphology of *Ligophorus* spp. parasitising *Liza haematocheilus* with the description of *Ligophorus pilengas* given by Sarabeev & Balbuena (2004), we have presumed that these authors have probably examined a mixture of the two species, including specimens of *L. llewellyni* n. sp. This assumption is based on the observation that the majority of the reported characters of *L. pilengas* (see Sarabeev & Balbuena, 2004) had a very wide range of values, while others (e.g. the length of the copulatory tube) were more similar to that of *L. llewellyni*. This failure to separate both species might be due to an insufficient number of measurements.

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