Ligophorus pilengas n. sp. (Monogenea: Ancyrocephalidae) from the introduced so-iuy mullet, Mugil soiuy (Teleostei: Mugilidae), in the Sea of Azov and the Black Sea

Volodimir Leonidovich Sarabeev and Juan Antonio Balbuena*†

Department of Biology, Zaporizhzhia State University, 66 Zhukovskogo Street, 69063 Zaporizhzhia, Ukraine. e-mail: j.a.balbuena@uv.es

ABSTRACT: The monogenean Ligophorus chabaudi was originally described on the gills of the flathead mullet, Mugil cephalus, and was subsequently reported on the So-iuy mullet, Mugil soiuy. However, the morphology of sclerotized parts and multivariate statistical analyses suggest that the form from the So-iuy mullet represents a new species. This study provides a description of the new species Ligophorus pilengas n. sp. and provides additional morphological data concerning the morphology of the ventral bar that might be useful for the diagnosis of Ligophorus. Ligophorus pilengas n. sp. is the second species of Ligophorus reported on the So-iuy mullet. Zoogeographical records indicate that L. pilengas n. sp. was probably introduced to the Black Sea and the Sea of Azov from the western Pacific Ocean together with its host.

Euzet and Suriano (1977) erected Ligophorus within the Ankyrocephalidae and included 11 species parasitizing the gills of mullets (Mugilidae) from the Mediterranean Sea and the North Atlantic: Ligophorus vanbenedenii (Parona and Perugia, 1890); Ligophorus szidati Euzet and Suriano, 1977; Ligophorus mugilinus (Hargis, 1955); Ligophorus chabaudi Euzet and Suriano, 1977; Ligophorus macrocolpos Euzet and Suriano, 1977; Ligophorus acuminatus Euzet and Suriano, 1977; Ligophorus minimus Euzet and Suriano, 1977; Ligophorus heterochonus Euzet and Suriano, 1977; Ligophorus angustus Euzet and Suriano, 1977; Ligophorus imitans Euzet and Suriano, 1977; and Ligophorus confusus Euzet and Suriano, 1977. Subsequently 9 new species have been added to the genus, expanding their distribution range to the North and South Pacific: Ligophorus leporinus (Zhang and Ji, 1981); Ligophorus parvicirrus Euzet and Sanfilippo, 1983; Ligophorus kaohsianghsiieni (Gusev, 1962); Ligophorus huitrempe Fernández, 1987; Ligophorus chongmingensis Hu and Li, 1992; Ligophorus chenzenensis Hu and Li, 1992; Ligophorus euzeti Dmitrieva and Gerasev, 1996; Ligophorus hamulosus Pan, 1999; and Ligophorus ellochelon Yang, 2001 (Euzet and Sanfilippo, 1983; Gusev, 1985; Dmitrieva and Gerasev, 1996; Zhang et al. 2003).

Euzet and Suriano (1977) considered that all species of Ligophorus are oioxicen, which seemed substantiated by ensuing studies (Euzet and Sanfilippo, 1983; Radujković and Raibaut, 1989; Caltran et al., 1995; Caillot et al., 1999). However, current records show that at least some species are stenoxenic. For instance, L. kaohsianghsiieni, whose typical host is the So-iuy mullet, Mugil soiuy Basilewsky, has also been reported on the flathead mullet, Mugil cephalus L., and golden gray mullet, Liza aurata (Risso) (Dmitrieva, 1996; Miroshnichenko and Maltsev, 1998). Likewise, L. vanbenedentii, common on L. aurata, has also been recorded on M. cephalus, and L. macrocolpos, typical on the leaping mullet, Liza saliens (Risso), occurs also on L. aurata (Dmitrieva and Gerasev, 1996). However, the prevalence and abundance on such alternative hosts is much lower than those on the typical host of each species of Ligophorus. Apparently, the only exception to this pattern is L. chabaudi because it occurs typically on M. cephalus; however, according to previous studies and our own data, it also shows high prevalence (87–100%) and abundance (up to 736 individuals) on adult So-iuy mullets (Dmitrieva, 1996; Sarabeev and Domnich, 2000).

In this article, we compared the morphology of haptoral and genital sclerotized parts and conducted a linear discriminant analysis of metrical features of putative L. chabaudi from M. cephalus and M. soiuy. The results suggest that the form from M. soiuy probably represents a new species, Ligophorus pilengas n. sp., which is described in this article.

MATERIALS AND METHODS

Mullets were collected from the Sea of Azov, the Black Sea, and the Western Mediterranean Sea (Table I). Fish were examined for parasites within the day of capture and surveyed for Ligophorus infection using a stereomicroscope. Eighty-five specimens belonging to 6 species of Ligophorus (Table I) were mounted in glycerin jelly, according to Gusev (1983), and measured. To study details of their internal anatomy, 15 additional specimens of L. pilengas n. sp. were stained in alum carmine, passed through a series of increasing ethanol concentrations (from 70 to 100%), cleared in dimethyl phthalate, and mounted in Canada balsam.

Based on Gusev (1985) and Euzet and Suriano (1977), 19 characters were selected for morphometric analysis. The following abbreviations are used for the characters: BL, body length; BW, body width; VAA, ventral anchor anterior total length (dorsosapical); VAB, ventral anchor main part length; VAC, ventral anchor outer root length; VAD, ventral anchor inner root length; VAE, ventral anchor point length; DAA, dorsal anchor anterior total length (dorsosapical); DAB, dorsal anchor main part length; DAC, dorsal anchor outer root length; DAD, dorsal anchor inner root length; DAE, dorsal anchor point length; HL, marginal hook total length; VBL, ventral bar length; VBAP, distance between membranous anterior processes of ventral bar; DBL, dorsal bar length; PAPL, penis accessory piece length; PL, total length of penis; VL, vagina length. See Figure 1 for definition of measurements of sclerotized elements of haptors and genital structures.

All measurements of worms are given in micrometers as mean ± standard deviation (range). Measurements and drawings were made using a Lomo P 15 microscope with phase contrast and a Nikon Optiphot-2 microscope with interference contrast (magnification: 10× for the body and 100× 1.25× [under immersion oil] for sclerotized structures). Photographs of sclerotized structures of haptor and male copulative apparatus were taken with a Leica DMR microscope with interference contrast and a Leica DC300 camera (magnification of 100× under immersion oil).

We used stepwise linear discriminant analysis to analyze the morphometric differences between the 85 specimens of the 6 Ligophorus species studied in this work (Table I). Initially, 15 of the 19 morphometric characters, namely, VAA, VAB, VAC, VAD, VAE, DAA, DAB, DAC, DAD, DAEL, HL, VBL, VBAP, DBL, and PAPL, were chosen for

Received 5 June 2003; revised 2 September 2003; accepted 3 September 2003.
* Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, P.O. Box 22085, 46071 Valencia, Spain.
† To whom correspondence should be addressed.
The stepwise discriminant analysis selected 8 of the 15 metric variables as the best discriminating features, namely, VBL, VAA, DAE, DAB, VAE, VAB, VAC, and DAA (listed in decreasing order of importance). The discriminant analysis correctly assigned all specimens to their respective species. Incorrect allocations occurred only between L. pilengas n. sp. from the Black Sea and from the Sea of Azov: 5 specimens from the Black Sea were assigned to the Sea of Azov. Figure 2 shows the discriminant scores of each specimen plotted against the 2 first discriminant functions (accounting for 84% of variation between the groups). The specimens of L. pilengas n. sp. can be separated from those of L. chabaudi and from those of the rest of the species by their scores along the first discriminant function (Fig. 2). The variables showing high absolute values of standardized coefficients along this function were VAB (0.93), DAA (0.77), VBL (0.56), VAC (0.53), and VAE (0.51). Given the position of L. pilengas n. sp. specimens along the first function (Fig. 2), such specimens will tend to show larger VAB, VBL, and VAC and lower DAA and VAE relative to those of the other species of Ligophorus. In addition, specimens of L. pilengas n. sp. were distinguished from those of L. chabaudi by morphometric characters because the t-tests showed significant differences (P < 0.05) in 18 of the 19 metric characters (all except PAPL) (Table II).

### RESULTS

The stepwise discriminant analysis selected 8 of the 15 metric variables as the best discriminating features, namely, VBL, VAA, DAE, DAB, VAE, VAB, VAC, and DAA (listed in decreasing order of importance). The discriminant analysis correctly assigned all specimens to their respective species. Incorrect allocations occurred only between L. pilengas n. sp. from the Black Sea and from the Sea of Azov: 5 specimens from the Black Sea were assigned to the Sea of Azov. Figure 2 shows the discriminant scores of each specimen plotted against the 2 first discriminant functions (accounting for 84% of variation between the groups). The specimens of L. pilengas n. sp. can be separated from those of L. chabaudi and from those of the rest of the species by their scores along the first discriminant function (Fig. 2). The variables showing high absolute values of standardized coefficients along this function were VAB (0.93), DAA (0.77), VBL (0.56), VAC (0.53), and VAE (0.51). Given the position of L. pilengas n. sp. specimens along the first function (Fig. 2), such specimens will tend to show larger VAB, VBL, and VAC and lower DAA and VAE relative to those of the other species of Ligophorus. In addition, specimens of L. pilengas n. sp. were distinguished from those of L. chabaudi by morphometric characters because the t-tests showed significant differences (P < 0.05) in 18 of the 19 metric characters (all except PAPL) (Table II).

### DESCRIPTION

Ligophorus pilengas n. sp. (Figs. 1, 3A, B, 4A)

#### Synonyms

Ligophorus vanbenedeni (Parona and Perugia, 1890) sensu Gusev (1985).


Worms have the characters of the genus as defined by Euzet and Suriano (1977) and supplemented by Euzet and Sanfilippo (1983). Morphometric measurements of L. pilengas n. sp. specimens mounted in glycerin jelly from the Black Sea and Sea of Azov (this study) are listed in Table II.

Fifteen stained specimens mounted in Canada balsam showed smaller body sizes (565 ± 29 [538–637] long, 87 ± 7 [70–94] wide) than the 31 specimens mounted in glycerin jelly (compare with corresponding measurements in Table II). Posterior haptor armed, with 14 hooks, 2 pairs of anchors, 2 transverse bars (Figs. 1D–F, 3A, B). Ventral anchors with elongate thin blade, recurved point. Point short, constituting half the blade length, forming obtuse angle (about 100°). Base markedly thicker than blade, separated by notch. Inner root larger than outer root, VAD/VAC = 1.36 ± 0.24 (1.13–2.4). Angle between roots sharp (about 55°). Filament present. Ventral anchors connected by transverse ventral bar. Ventral bar massive, with 2 membranous anterior medial processes (Figs. 1D, 3A). In 13 of 31 specimens (42%), ventral bar with nonmembranous median process between membranous processes. Morphology of median process highly variable, ranging from small (Figs. 1D, 3A) to massive (Fig. 3B). Dorsal anchors similar in shape to ventral anchors. Inner root much larger than outer roots, DAD/DAC = 1.95 ± 0.18 (1.63–2.4), base of dorsal anchors somewhat thinner than that of ventral anchors. Dorsal transversal bar V shaped, connects dorsal anchors. All 14 marginal hooks subequal with straight handle and sickle (hooklet). Sickle formed by short base with heel, curved blade and filament loop. Male copulatory complex consists of tubular penis about 1 μm in
Figure 1. *Ligophorus pilengas* n. sp.: overall view, haptoral and genital sclerotized structures. **A.** Whole worm, ventral view. **B.** Male copulatory complex: penis and accessory piece. **C.** Vaginal armament. **D.** Ventral bar and anchors. **E.** Marginal hook. **F.** Dorsal bar and anchors. For haptoral and genital sclerotized structures, the metric variables studied are shown (see Materials and Methods for abbreviations of measurements). Lowercase letters in parentheses correspond to standard haptor measurements (Murith and Beverley-Burton, 1985).
SARABEEV AND BALBUENA—LIGOPHORUS PILENGAS N. SP. 225

Figure 2. Scores resulting from a linear discriminant analysis of metric variables of 85 specimens of Ligophorus (Table I) plotted against the first 2 discriminant functions.

Table II. Metrical data of Ligophorus pilengas n. sp. from this study and Dmitrieva’s (1996) study compared with measurements of Ligophorus chabaudi from this study. See Materials and Methods for abbreviations of metric variables.

<table>
<thead>
<tr>
<th>Character</th>
<th>This study, Ligophorus pilengas n. sp.</th>
<th>Dmitrieva (1996), L. pilengas n. sp. (=L. chabaudi)</th>
<th>This study, L. chabaudi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black Sea (n = 9)</td>
<td>Sea of Azov (n = 22)</td>
<td>Black Sea</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>BL</td>
<td>813</td>
<td>60</td>
<td>737–877</td>
</tr>
<tr>
<td>BW</td>
<td>160</td>
<td>16</td>
<td>140–187</td>
</tr>
<tr>
<td>VAA</td>
<td>41</td>
<td>2</td>
<td>37–44</td>
</tr>
<tr>
<td>VAC</td>
<td>11</td>
<td>1</td>
<td>9–13</td>
</tr>
<tr>
<td>VAD</td>
<td>15</td>
<td>2</td>
<td>13–19</td>
</tr>
<tr>
<td>VAE</td>
<td>9</td>
<td>0.5</td>
<td>8–9</td>
</tr>
<tr>
<td>DAA</td>
<td>40</td>
<td>2</td>
<td>35–44</td>
</tr>
<tr>
<td>DAC</td>
<td>9</td>
<td>1</td>
<td>8–11</td>
</tr>
<tr>
<td>DAE</td>
<td>9</td>
<td>1</td>
<td>7–9</td>
</tr>
<tr>
<td>HL</td>
<td>13</td>
<td>0.3</td>
<td>12–13</td>
</tr>
<tr>
<td>VBL</td>
<td>48</td>
<td>1</td>
<td>46–50</td>
</tr>
<tr>
<td>VBAP</td>
<td>9</td>
<td>1</td>
<td>9–10</td>
</tr>
<tr>
<td>PAP</td>
<td>36</td>
<td>3</td>
<td>32–41</td>
</tr>
<tr>
<td>PL</td>
<td>95</td>
<td>7</td>
<td>89–111</td>
</tr>
<tr>
<td>VL</td>
<td>36</td>
<td>11</td>
<td>22–60</td>
</tr>
</tbody>
</table>

diameter and nipper-shaped accessory piece (Figs. 1B, 4A). Distal end of the latter bilobed, lower lobe longer than upper lobe. Accessory piece supports distal end of penis. Vaginal armament is thin, convoluted or straight tube (Fig. 1C).

Taxonomic summary

*Type host:* So-iuy mullet, *M. soiuy* Basilewsky, 1855.
*Site of infection:* Gill rakers and lamellae.
*Type locality:* Utryukskiy Estuary (46°21′N, 35°14′E), Sea of Azov, Zaporizhzhia Region, Ukraine.
*Other localities:* Kerch Channel (Black Sea) and Molochny Estuary (Sea of Azov).

*Prevalence and intensity range:* Prevalence is 87–100% of adult fish, 2–736 worms per fish.


*Etymology:* Pilengas is the common name of the type host in Russian and is used as a noun in apposition to form the specific designation of the new species.

Remarks

*Ligophorus pilengas* n. sp. is very similar to *L. chabaudi*, but both species differ from each other in several characters in the morphology of the accessory piece of male copulatory complex and ventral bar. In the distal end of the accessory piece of *L. pilengas* n. sp., the lower lobe is longer than the upper lobe and the basal bifurcation between the lobes is situated at about one-third the distance from the distal end of the accessory piece to the penis (Figs. 1B, 4A). In *L. chabaudi*, in contrast, the upper...
Furthermore, L. pilengas processes are contiguous to the median process in latter is present) (Figs. 1D, 3A, B), whereas the membranous n. sp. are distinctly separated from the median process (if the membranous anterior processes of the ventral bar of redrawing by Dmitrieva and Gerasev [1996]). In addition, the see also original illustration of Euzet and Suriano [1977] and between them is at the middle of the accessory piece (Fig. 4B, lobe is longer than the lower lobe, and the basal bifurcation cause the accessory piece of the male copulatory complex, and the distal end of the vaginal armament, as well as in the length of the penis and the body (Hu and Li, 1992). Ligophorus pilengas n. sp. differs from L. macrocolpos and L. acuminatus in the shape and smaller length of the accessory piece of the male copulatory complex (Euzet and Suriano, 1977). The shape of the anchors and ventral bar of L. pilengas n. sp. is similar to that of L. vanbenedenii, L. mugilinus, L. minimus, L. imitans, L. heteronchus, L. euzeti, L. hamulosus, and L. huitrempe, but the new species differs from L. vanbenedenii and L. hamulosus because in these species all metrical characters are smaller (Euzet and Suriano, 1977; Pan, 1999) and from L. mugilinus because the total and main part of the anchors and the ventral bar are smaller than in the new species (Euzet and Suriano, 1977). Likewise, the outer root and point of L. heteronchus and L. euzeti are smaller than in L. pilengas n. sp. (Euzet and Suriano, 1977; Dmitrieva and Gerasev, 1996). In addition, L. heteronchus has a smaller inner root and ventral bar than in the new species (Euzet and Suriano, 1977). Finally, the outer root and ventral bar of L. imitans and L. minimus and the main part of the anchors and ventral bar of L. huitrempe are smaller than in L. pilengas n. sp. (Euzet and Suriano, 1977; Fernández, 1987). The new species can also be distinguished from the latter 8 species by the shape of the accessory piece of the male copulatory complex and also from L. vanbenedenii, L. mugilinus, L. minimus, L. heteronchus, L. hamulosus, and L. huitrempe because the accessory piece is shorter in these species.

**DISCUSSION**

Both the morphological and metric differences between L. pilengas n. sp. from the So-iuy mullet and those of other species of Ligophorus suggest that for the So-iuy mullet specimens represent a new species. Gusev (1985) studied specimens of Ligophorus on So-iuy mullets from the Liao-Ho River (Yellow Sea basin), which he tentatively identified as L. vanbenedenii. Although he did not provide body measurements of the specimens, judging from the drawings of the haptoral sclerotized elements, we think that Gusev’s (1985) specimens correspond to L. pilengas n. sp. In particular, the ventral bar of Gusev’s (1985) specimens appear to have membranous processes similar to L. pilengas n. sp. (membranous processes are absent in L.

---

**Figure 3.** Photomicrographs of sclerotized elements of haptors of Ligophorus pilengas n. sp. and Ligophorus chabaudi. A–B. Sclerotized elements of L. pilengas n. sp. haptors, showing different types of ventral bar with membranous (M) and median (MD) anterior processes. C. Sclerotized elements of a L. chabaudi haptor. Bar = 10 μm.
vanbenedenii), and the shape of the accessory piece conforms to that of L. pilengas n. sp. Likewise, the specimens from the So-iuy mullet in the Black Sea Basin and the western Pacific identified as L. chabaudi by Dmitireva (1996) should be assigned to L. pilengas n. sp. because the ventral bar lacks the median process, the copulatory complex is like that of L. pilengas n. sp., and the metric data seem closer to those of L. pilengas n. sp. than to those of L. chabaudi (Table II). After examination of the original material, we also conclude that other monogenean specimens reported from the So-iuy mullet and identified as L. chabaudi in different studies (Maltsev and Zhdamirov, 1996; Maltsev and Miroshnichenko, 1998; Domnich and Sarabev, 1999, 2000a, 2000b; Sarabev, 2000; Sarabev and Domnich, 2000) are actually L. pilengas n. sp.

This new species is the second member of Ligophorus recorded in the So-iuy mullet (the first being L. kaohsianghshieni). The So-iuy mullet was introduced in the late 1970s into the Black Sea and Sea of Azov from the western Pacific. Considering previous records of L. pilengas n. sp. in the western Pacific (Gusev, 1985; Dmitireva, 1996), it is probable that this parasite was brought to the Black Sea and Sea of Azov together with its host.

As in most monogeneans, the taxonomy of Ligophorus relies on the shape of the sclerotized structures, which are used for species characterization (Euzet and Suriano, 1977; Euzet and Sanfilippo, 1983; Gusev, 1985; Fernández, 1987; Dmitireva and Gerasev, 1996; Pan, 1999). Statistical classifiers based on these characters might be useful for classification of monogenean species, as shown for species of Gyrodactylus (Shinn et al., 2000). In fact, the results of our discriminant analysis give support to this contention because it correctly assigned all specimens to their respective species of Ligophorus. The analysis also showed that the form from the So-iuy mullet could be unequivocally and objectively distinguished from 5 species of Ligophorus known from hosts that co-occur with M. soiuy in the basin of the Black Sea, thus supporting that this form represents a new species. The discriminant analysis suggests that L. pilengas n. sp. can be characterized mostly by measurements of the main part of the ventral anchors and ventral bar.

However, the morphology of sclerotized structures should be used with caution in the taxonomy of the ancyrocephalids because of both potential intraspecific variability (Ferdig et al., 1991) and dependence of metric characters on host size (Caltran et al., 1995). In L. pilengas n. sp., we observed variability in the morphology of the ventral bar, concerning mostly the occurrence and shape of a medial process, and the discriminant analysis revealed morphometric differences between the specimens from the Sea of Azov and the Black Sea. However, these morphometric variations appeared smaller than interspecific differences and might be attributable to geographical differences in development, as proposed for other ancyrocephalids (Ferdig et al., 1991). As for the influence of host size, most specimens of L. pilengas n. sp. were from hosts of similar body length as those of L. chabaudi (Table I). Therefore, the morphometric differences between these 2 forms cannot be attributed to this factor.

The diagnosis of Ligophorus by Euzet and Suriano (1977) and supplemented by Euzet and Sanfilippo (1983) conforms to the characters present in L. pilengas n. sp. However, the comparison between L. pilengas n. sp. and L. chabaudi provided in this study suggests that the following feature should be considered in a revised generic diagnosis: ventral transversal bar can have 2 membranous anterior processes flanking and a nonmembranous median process between them.

ACKNOWLEDGMENTS

We thank Aneta Kostadinova, Bulgarian Academy of Sciences, for valuable criticism and suggestions, and Vyacheslav Maltsev, Southern Scientific Research Institute of Marine Fisheries and Oceanography (Kerch, Ukraine), for his help in collecting some of the material. V.L.S. benefited a Young Scientist Fellowship (YSF 01/1-0203) from INTAS and a North Atlantic Treaty Organization Grant for Young Scientists (71/B/02/SP) from the Ministry of Science and Technology of Spain. Funds for this study were partially provided by The Ministry of Education and Science of Ukraine.

LITERATURE CITED


Caltran, H., P. Silan, and M. Roux. 1995. Ligophorus imitans (Monogenea) ectoparasite de Liza ramada (Teleostei). II. Variabilité mor-


—, AND V. N. ZHDAMIROV. 1996. Parasitofauna of the mullet haarder (Mugil soiuy Basilewsky) of the Kerch channel. Proceedings of the Southern Scientific Research Institute of Marine Fisheries and Oceanography 44: 229–232. [In Russian.]


