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First morphological and phylogenetic data on *Ligophorus kaohsianghsieni* (Platyhelminthes: Monogenea) from the Black Sea and the Sea of Japan and molecular evidence of deep divergence of sympatric *Ligophorus* species parasitizing *Planiliza haematocheilus*

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Abstract

Ligophorus kaohsianghsieni (Gusev, 1962) Gusev, 1985 was collected from the so-iuy mullet *Planiliza haematocheilus* (Temminck & Schlegel, 1845) from the Black Sea and the Sea of Japan. DNA sequences data for *L. kaohsianghsieni*, as well as its morphological characters from the Sea of Japan were obtained for the first time. Significant morphometric and genetic diversity between specimens of *L. kaohsianghsieni* from the Black-Azov Sea region and the Sea of Japan were not found. For the first time, the molecular phylogeny of *L. kaohsianghsieni* based on three fragments of the nuclear DNA ribosomal cluster (18S, internal transcribed spacer 1 and 28S) was reconstructed. Molecular analysis of *Ligophorus* species from the Atlantic and Pacific Oceans revealed a significant phylogenetic distance between *L. kaohsianghsieni* and two others species (*Ligophorus pilengas* and *Ligophorus llewellyni*) from the same host (*P. haematocheilus*) and region. This result indicates the lack of correspondence between the phylogenetic and geographical closeness of the hosts and the relation of their parasites from the genus *Ligophorus*.

Introduction

Monogeneans of *Ligophorus* Euzet et Suriano, 1977 are specific gill parasites of fish from the family Mugilidae Jarocki, 1822. The genus currently includes 66 nominal species (Euzet & Suriano, 1977; Dmitrieva *et al.*, 2007, 2012, 2013a; Abdallah *et al.*, 2009; Soo & Lim, 2012, 2013; El Hafidi *et al.*, 2013a, b; Kritsky *et al.*, 2013; Sarabeev *et al.*, 2013; Marchiori *et al.*, 2015; Rodríguez-González *et al.*, 2015a, 2015b; Khang *et al.*, 2016; Pakdee *et al.*, 2018). Identification of *Ligophorus* species is based mainly on the morphology of hard structures of the haptor and the distal parts of the female and male reproductive systems (Euzet & Suriano, 1977; Sarabeev *et al.*, 2013). Many species are very morphologically similar to each other, creating difficulties for delimitation of species (Euzet & Suriano, 1977; Dmitrieva *et al.*, 2007, 2013a). Some of them were distinguished on the basis of DNA sequence data (Marchiori *et al.*, 2015; Pakdee *et al.*, 2018). However, these data are discrete or insufficient, representing 127 sequences of the different parts of the nuclear DNA ribosomal cluster for only 32 species (https://www.ncbi.nlm.nih.gov/nuccore), including 12 species from the Mediterranean Sea and two species from the Azov Sea (Mollaret *et al.*, 2000; Plaisance *et al.*, 2005; Blasco-Costa *et al.*, 2012; Rodríguez-González *et al.*, 2015a), two species from the West Atlantic Ocean off Brasilia (Marchiori *et al.*, 2015), 14 species from the East Indian Ocean off Malaysia (Soo *et al.*, 2015; Khang *et al.*, 2016) and for three species from the South China Sea (Wu *et al.*, 2006, 2007; Pakdee *et al.*, 2018). Data on DNA sequences for *Ligophorus* species from the Black Sea and the Sea of Japan are still lacking.

Ligophorus kaohsianghsieni (Gusev, 1962) Gusev, 1985 was described from the so-iuy mullet *P. haematocheilus* (Temminck & Schlegel, 1845) from the Tumen-Ula River flowing into the Sea of Japan and the Liao River flowing into the Yellow Sea (Gusev, 1962, 1985), but its native range includes the Sea of Japan as such (Sarabeev *et al.*, 2013), as well as the East China and South China Seas (Zhang *et al.*, 2003; Dmitrieva *et al.*, 2013b). In the Black Sea, this monogenean was first found on *P. haematocheilus* off the coast of Crimea (Dmitrieva, 1996). Subsequently, this parasite was repeatedly registered on the same fish species in the Black Sea, off Bulgaria, and in the Sea of Azov (Pankov, 2011; Sarabeev *et al.*, 2013), where

Table 1. Sampling data, sequenced material, voucher and GenBank accession numbers of *Ligophorus kaohsianghsieni*.

Locality	Data	Specimens	Voucher	28S	18S	internal transcribed spacer 1
t Black Sea, off Karadag	June 2016	10	760.M.ce.v18	KY979156	MZ646034	MZ648433
			761.M.ce.v19	KY979157	MZ646035	–
			762.M.ce.v20	KY979158	MZ646036	MZ648434
			763.M.ce.v21	KY979159	MZ646037	–
			764.M.ce.v22	KY979154	MZ646039	MZ648432
			765.M.ce.v23	KY979155	MZ646038	MZ648435
			–	MZ648420	MZ646033	–
Black Sea, Kerch channel	July 2018	8	1237.M.ce.v25	MZ648423	MZ646031	–
			1236.M.ce.v24	MZ648422	MZ646032	–
Sea of Japan, Tavrichan Bay, mouth of River Razdolnaya	October 2018	9	1239.M.ce.v27	MZ648424	MZ646042	MZ648429
			–	MZ648425	MZ646041	MZ648428
Sea of Japan Tavrichan Bay, mouth of River Kievka	October 2018	13	1240.M.ce.v28	MZ648426	–	MZ648430
			1241.M.ce.v29	MZ648427	–	MZ648431
			–	MZ648421	MZ646040	–

it was introduced from the Sea of Japan. Morphological descriptions of *L. kaohsianghsieni* have been published based on specimens from the Tumen-Ula and Liao rivers (Gusev, 1985) and from the Black and Azov seas (Dmitrieva, 1996; Sarabeev *et al.*, 2013), but with no data on its morphology from the Sea of Japan, the region from which the host was introduced. This study presents the molecular characterization of *L. kaohsianghsieni* using 28S, 18S and internal transcribed spacer 1 (ITS1) (rDNA) gene clusters and provides new morphological data for this species across its native and introduced distribution.

Materials and methods

Sampling

Monogeneans were collected from the gills of *P. haematocheilus*, caught in the Tavrichan Bay of the Sea of Japan, near the mouth of the River Razdolnaya (43°19′48″N, 131°46′19″E) and mouth of the River Kievka (42°51′27.8″N 133°38′39.3″E), and off the coast of Crimea near Sevastopol (44°36′58.4″N, 33° 30′14″E) and Karadag (44°54′41″N, 35°12′07″E), and in the Kerch Strait (45°07′52.0″N 36°25′31.1″E), Northern Black Sea (table 1). All monogeneans were collected alive, some of them were immediately mounted in glycerine jelly (prepared with 0.5 g carbolic acid) after Gusev (1983), and parts of others were stored in absolute ethanol and kept at 5°C for DNA analysis. Additional materials of 15 specimens of *L. kaohsianghsieni* collected in the Black Sea near Crimea from the Marine Parasites Collection of the A. O. Kovalevsky Institute of Biology of the Southern Seas, Sevastopol, Russia (IBSS collection, http://marine-parasites.org) were reinvestigated for morphometry.

Table 2. Primers used for amplification.

Gene	Primers	Reference
28S	U178: 5′ – GCACCCGCTGAAYTTAAG – 3′ LSU1200R: 5′ – GCATAGTTCACCATCTTTCGG – 3′	Lockyer *et al.* (2003) and Littlewood *et al.* (2000)
ITS1	Lig18endF: 5′ – GTCTTGCGGTTCACGCTGCT – 3′ Lig5.8R: 5′ – GATACTCGAGCCGAGTGATCC – 3′	Blasco-Costa *et al.* (2012)
18S	WormA: 5′ – GCGAATGGCTCATTAAATCAG – 3′ new930F: 5′ – CCTATTCCATTATTCCATGC– 3′	Littlewood & Olson (2001) and Khang *et al.* (2016)

Morphology analyses

Measurements and light micrographs were made with Olympus CX41 microscopes (Olympus Corporation, Tokyo, Japan), at magnifications of ×800–1000, using phase-contrast optics and CellSense digital image analysis software (Olympus Corporation, Tokyo, Japan). The measuring scheme mainly followed that suggested for the Dactylogyridae by Gusev (1985) with some configurations according to Dmitrieva *et al.* (2013b). Abbreviations of the linear measurements are presented in table 2. All dimensions are given in micrometres. The mean, standard deviation and range were used to describe the linear measurements. Morphological analysis of 41 specimens was carried out using principal component analysis based on the correlation matrix (30 measurements of hamulus and bars were $\log_{10}$-transformed) using the Statistica 6 for Windows software package.

DNA extraction

Prior to DNA analysis, the voucher slides from the haptor of the specimens used for sequencing were prepared and deposited in the IBSS collection, then identified based on the haptoral structures (Gusev, 1985; Dmitrieva, 1996; Sarabeev *et al.*, 2013). DNA extraction was carried out using DNK-EXTRAN Kit (Syntol, Moscow, Russia). Single animals were incubated in 100 µl of lysis buffer (Syntol, Moscow, Russia) with 5 µl of Syntol Proteinase K and 1 µl of 2-mercaptoethanol at 56°C overnight. After lysing, animals were vortexed for 20 s and DNA

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Table 3. GenBank accession numbers of 28S rRNA, 18S rRNA and internal transcribed spacer 1 (ITS1) sequences of the *Ligophorus* species used in the phylogenetic analyses.

Ligophorus species	Host species	Locality	28S	ITS1	18S	Reference
L. llewellyni	*Planiliza haematocheilus*	Sea of Azov, Utlyuksky Estuary	JN996822 JN996823	JN996858	–	Blasco-Costa *et al.* (2012)
L. pilengas			JN996824 JN996825 JN996826	JN996859 JN996860 JN996861	–	
		Black Sea, off Karadag	KY979153	–	–	present study
L. bantingensis	*Planiliza subviridis*	Indian Ocean, Straits of Malacca, Carey Island, Selangor	KM221909	KM221922	KM221934	Soo *et al.* (2015) and Khang *et al.* (2016)
L. belanaki			KM221910	KM221923	KM221935	
L. careyensis			KM221911	KM221924	KM221936	
L. chelatus			KM221912	KM221925	KM221937	
L. funnels			KM221914	–	KM262663	
L. navjotsodnii			KM221920	KM221932	KM221944	
L. parvicopulatrix			KM221921	–	KM221945	Khang *et al.* (2016)
L. szidati	*Chelon auratus*	Mediterranean Sea, Ebro Delta	JN996806	JN996841	–	Blasco-Costa *et al.* (2012)
L. vanbenedenii			JN996801 JN996802	JN996836 JN996837	–	
L. angustus	*Chelon labrosus*	Mediterranean Sea, off Cullera	JN996803 JN996805	JN996838 JN996839 JN996840	–	Blasco-Costa *et al.* (2012)
L. confusus	*Chelon ramado*	Mediterranean Sea, off Cullera, Ebro Delta	JN996807 JN996808 JN996810	JN996842–JN996847	–	Blasco-Costa *et al.* (2012)
L. imitans			JN996814	JN996849 JN996850 JN996851	–	
L. acuminatus	*Chelon saliens*	Mediterranean Sea, Ebro Delta	JN996816	JN996852	–	Blasco-Costa *et al.* (2012)
L. heteronchus			JN996812	JN996848	–	
L. macrocolpos			JN996819 JN996820 JN996821	JN996855 JN996856 JN996857	–	
L. minimus		Mediterranean Sea, Ebro Delta	JN996817 JN996818	JN996853 JN996854	–	Blasco-Costa *et al.* (2012)
L. fenestrum	*Crenimugil buchanani*	Indian Ocean, Strait of Malacca, Langkawi Island	KM221913	–	KM221938	Soo *et al.* (2015) and Khang *et al.* (2016)
L. kedahensis			KM221917	–	KM221941	
L. kederai			KM221918	–	KM221942	
L. grandis		Indian Ocean, Strait of Johor, Malaysia	KM221915	–	KM221939	
L. johorensis			KM221916	–	KM221940	
L. liewi			KM221919	–	KM221943	
L. cephali	*Mugil cephalus*	Mediterranean Sea, off Cullera, Albufera	JN996830	JN996865 KP294376 KP294383	–	Blasco-Costa *et al.* (2012) and Rodríguez-González *et al.* (2015a)
L. mediterraneus		Mediterranean Sea, off Cullera	JN996827 JN996828 JN996829	JN996862 JN996863 JN996864	–	
L. chabaudi		Mediterranean Sea, Ebro Delta	JN996831 JN996832 JN996833 JN996834	JN996866 JN996867 JN996868 JN996869	–	

(*Continued*)

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Table 3. (*Continued.*)

Ligophorus species	Host species	Locality	28S	ITS1	18S	Reference
L. leporinus	*Mugil cephalus*	South China Sea, off Guangdong, China	DQ537380	–	–	Wu *et al.* (2007)
L. saladensis	*Mugil liza*	Atlantic Ocean, off Brazil	KF442628 KF442629	KF442627	–	Marchiori *et al.* (2015)
L. uruguayensis			KF442630	KF442626	–	

extraction was carried out according to the DNK-EXTRAN Kit protocol. The elution volume was 30 µl. The DNA was stored at −20°C.

Polymerase chain reaction (PCR) amplification and sequencing

The PCR was performed in a total volume 20 µLmix, consisting of 5xPCR ScreenMix with magnesium chloride (Evrogen, Moscow, Russia), 0.5 µM of each primer and 2 µL template DNA. The primers for amplification of 28S, ITS1 and 18S of ribosomal DNA are presented in table 2.

The 28S, ITS1 and 18S were amplified using the same following conditions: initial denaturation at 95°C for 3 min, followed by 38 cycles of denaturation at 94°C for 40 s, annealing at 56°C for 30 s and extension at 72°C for 45 s, the final extension at 72°C for 4 min. Amplicons were separated with horizontal electrophoresis on 1% agarose/Tris-Borate-Ethylenediaminetetraacetic acid buffer gel with ethidium bromide and visualized using an ultraviolet transilluminator. PCR products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, USA), as recommended by the manufacturer, with the internal sequencing primers described by Tkach *et al.* (2003) for 28S rDNA. PCR product sequences were analysed using an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, USA) at the Federal Scientific Center of the East Asia Terrestrial Biodiversity Far Eastern Branch of the Russian Academy of Sciences. Molecular analyses were performed on a total of 14 samples. All nucleotide sequences obtained during this study were deposited in the international National Center for Biotechnology Information GenBank database (table 1).

Molecular taxonomy analyses

Ribosomal DNA sequences were assembled with SeqScape v.2.6 software (Applied Biosystems, Waltham, USA). The obtained fragments of rDNA were aligned in the BioEdit software program (Hall, 1999) and then the alignment was manually refined. The multiple alignment was run by ClustalW (Thompson *et al.*, 1994) in the MEGAX software (Kumar *et al.*, 2018). Sequence datasets for phylogenetic analysis include original data and all available rDNA sequences in the GenBank database (table 3). As *Ergenstrema mugilis* Paperna, 1964 occurred as the sister group to *Ligophorus* spp. within the marine Ancyrocephalinae (Blasco-Costa *et al.*, 2012), it was chosen as the outgroup (GenBank accession number JN996800). Phylogenetic analysis was performed on the basis of each rDNA fragment separately with the Bayesian and the maximum likelihood (ML) algorithms using MrBayes v. 3.1.2 (Huelsenbeck *et al.*, 2001) and PhyML v. 3.1 software (Guindon & Gascuel, 2003), respectively. The best nucleotide substitution models, the GTR + G, TIM3ef + I + G and TPM2uf + G (Posada, 2003) were estimated with jModeltest v. 2.1.5 software (Darriba *et al.*, 2012) for ribosomal 28S rDNA, 18S rDNA and ITS1 rDNA fragments data set, respectively, using Bayesian information criterion for Bayesian inference (BI). For ML analysis, the best nucleotide substitutions, GTR + I + G, TIM3 + I + G and GTR + G (Posada, 2003), were chosen for ribosomal 28S rDNA, 18S rDNA and ITS1 rDNA, respectively, using Akaike's information criterion (Akaike, 1974). Bayesian analyses were performed using 10,000,000 generations with two independent runs. Summary parameters and the phylogenetic tree were calculated with a burn-in of 25% of generations. The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck *et al.*, 2001). Estimation of ML phylogenetic relationships' significance was performed with the help of the approximate likelihood ratio test with eBayes support (Anisimova & Gascuel, 2006). Estimates of average evolutionary divergence over sequence pairs within groups and between groups were conducted in MEGAX (Kumar *et al.*, 2018). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Results

A comparison of the shape of dorsal and ventral anchors, dorsal and ventral bars, the male copulatory organ and the vagina of *L. kaohsianghsieni* specimens collected in different seas showed no obvious differences (fig. 1).

A comparative analysis of 45 newly obtained measurements of *L. kaohsianghsieni* from the Black Sea and Sea of Japan revealed no significant differences; the ranges of all corresponding measurements overlapped between samples from different seas (table 4). A small difference in the total length of the marginal hook was observed between specimens from the rivers of the Russian Far East and the Black Sea and Sea of Japan. In addition, two dimensions of the ventral bar anterior processes in the present study were smaller than in the previous studies (table 4). The latter is most likely due to some differences in the method of measurement Thirty measurements describing the main parameters of the anchors and bars were reduced to three principal components (Factors) describing 62.5% of their overall variance, and there was no clear distinction between specimens from different seas at these plots (fig. 2).

No intraspecific differences for *L. kaohsianghsieni* for each DNA marker were revealed. Maximum ML and BI showed identical topologies regarding major lineages based on each molecular marker (figs 3–5). Due to the discrete sequence data for *Ligophorus* species we were unable to reconstruct the representative phylogeny for these worms. Data on the 28S rRNA gene are available for most of the analysed species,

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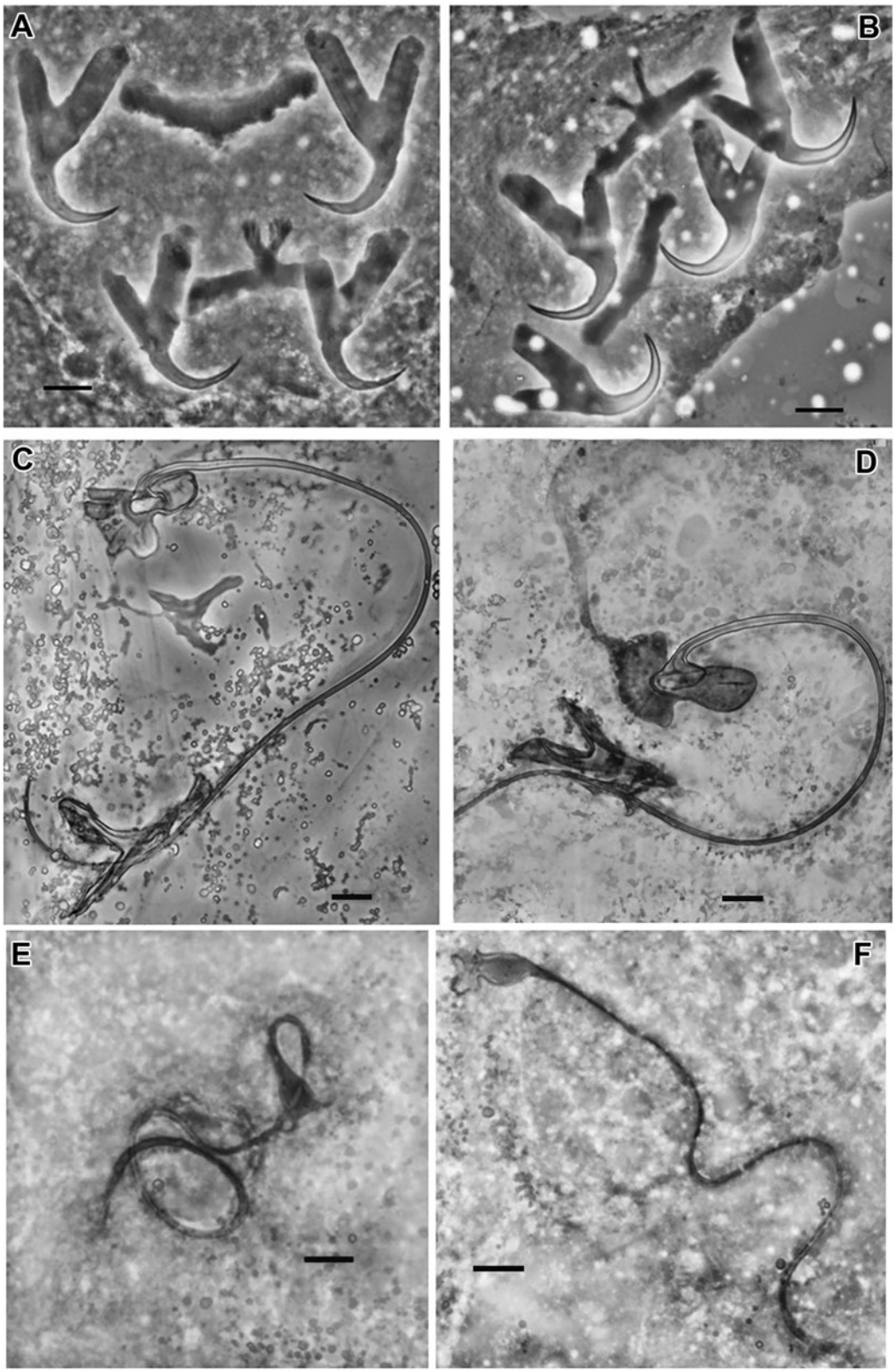

Fig. 1. Haptoral structures (A, B), male copulatory organ (C, D) and vagina (E, F) of *Ligophorus kaohsianghsieni* ex *Planiliza haematocheila* from the Black Sea (A, C, E) and the Sea of Japan (B, D, F). Scale bar = 10 μm.

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Table 4. Comparison of the dimensions of the body, haptoral and copulatory hard-parts of *Ligophorus kaohsianghsieni* from *Planiliza haematocheila* from the Black Sea and the Sea of Japan as the range followed by mean ± standard deviation; number of measurements in parentheses.

Source	Present study		Gusev (1962)	Sarabeev *et al.* (2013)
sea	Black Sea	Sea of Japan	Basin of Sea of Japan	Black Sea, Azov Sea
region	Sevastopol, Karadag, Kerch Strait	Tavrichan Bay, the mouths of the Razdolnaya and Kievka rivers	Tumen-Ula River, Liao River	Kerch Strait, Sivash Lake
body length	800.0–1500 (10)	680.0–1100 (861.4, 7)	1500	– (1249 ± 152, 18)
body width	125.0–450.0 (10)	133.0–208.0 (172.7, 7)	400	200–350 (256 ± 45.9, 16)
haptor length	88.0–115.0 (10)	88.0–110.0 (99.3, 7)	–	70–113 (91 ± 13.2, 12)
haptor width	110.0–200.0 (10)	100.0–165.0 (122.1, 7)	–	95–250 (153 ± 53, 18)
ventral anchor inner length	33.0–43.0 (38.5 ± 2.6, 31)	33.4–43.0 (39.0 ± 2.4, 20)	37–40	34–40 (38 ± 2, 12)
length of main part	21.5–26.5 (24.0 ± 1.4, 31)	20.5–25.3 (24.0 ± 1.2, 20)	25–26	22–25 (24 ± 1, 12)
length of distal part	17.5–22.0 (20.0 ± 1.3, 31)	17.0–22.0 (20.2 ± 1.4, 20)	–	–
length of shaft	12.0–16.0 (14.0 ± 1.2, 31)	11.0–18.0 (14.0 ± 1.6, 20)	–	12–17 (14 ± 1.8, 11)
length of point	10.5–13.0 (11.9 ± 0.6, 31)	11.0–12.7 (11.9 ± 0.5, 20)	12–13	8–12 (10 ± 1.3, 14)
inner length of proximal part	26.3–37.8 (29.9 ± 2.3, 31)	26.0–33.7 (30.2 ± 1.9, 20)	–	–
outer length of proximal part	17.5–24.5 (21.0 ± 1.6, 31)	17.7–24.0 (21.5 ± 1.6, 20)	–	–
span between roots	17.6–21.5 (19.6 ± 1.0,31)	16.5–28.6 (20.5 ± 2.6, 20)	–	–
outer length	30.0–41.3 (33.8 ± 2.6, 31)	30.3–41.0 (35.6 ± 2.5, 20)	–	29–34 (33 ± 1.4, 12)
length of base	11.0–14.0 (12.5 ± 0.8, 31)	9.5–14.0 (12.0 ± 1.2, 20)	–	–
length of inner root	16.4–22.5 (19.7 ± 2.0, 31)	15.6–23.0 (20.4 ± 1.9, 20)	19–21	17–22 (20 ± 1.4, 11)
length of outer root	7.5–13.8 (10.4 ± 1.6, 31)	9.7–15.0 (11.6 ± 1.3, 20)	9–10	7–10 (9 ± 0.9, 13)
dorsal anchor inner length	35.4–46.0 (39.5 ± 2.8, 31)	35.8–43.5 (40.4 ± 2.4, 20)	39–41	35–42 (39 ± 2.3, 12)
length of main part	22.0–28.5 (24.0 ± 1.4, 31)	22.0–26.5 (24.6 ± 1.4, 20)	25–28	22–29 (25 ± 2.2, 12)
length of distal part	17.0–22.5 (19.8 ± 1.4, 31)	17.3–22.5 (20.4 ± 1.4, 20)	–	–
length of shaft	13.0–17.5 (15.3 ± 1.1, 31)	13.0–17.2 (15.6 ± 1.1, 20)	–	13–19 (16 ± 2, 12)
length of point	11.0–12.8 (11.7 ± 0.6, 31)	11.0–13.0 (12.0 ± 0.6, 20)	10–12	10–13 (11 ± 1.2, 12)
inner length of proximal part	25.0–34.5 (27.4 ± 2.1, 31)	25.0–33.3 (28.9 ± 2.2, 20)	–	–
outer length of proximal part	19.0–26.0 (21.8 ± 1.6, 31)	19.0–25.0 (22.3 ± 1.6, 20)	–	–
span between roots	14.0–21.0 (18.6 ± 1.7, 31)	15.5–23.0 (20.5 ± 1.7, 20)	–	–

(*Continued*)

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Table 4. (*Continued.*)

Source	Present study		Gusev (1962)	Sarabeev *et al.* (2013)
outer length	33.8–44.5 (37.4 ± 2.8, 31)	32.0–41.5 (38.3 ± 2., 6 20)	–	30–41 (36 ± 3.1, 12)
length of base	11.0–15.0 (12.3 ± 1.0, 31)	10.0–14.2 (12.2 ± 1.0, 20)	–	–
length of inner root	14.3–24.0 (18.8 ± 2.7, 31)	14.5–22.3 (20.2 ± 2.2, 20)	18–19	15–22 (18 ± 2.3, 12)
length of outer root	9.4–17.5 (12.0 ± 1.8, 31)	8.7–14.0 (12.6 ± 1.2, 20)	13–15	8–13 (11 ± 1.5, 12)
marginal hook total length	12.0–13.0 (12.7 ± 0.3, 24)	12.5–13.0 (12.9 ± 0.2, 11)	15	11–14 (13 ± 0.9, 12)
sickle length	5.0–5.5 (5.2 ± 0.2, 24)	5.0–5.7 (5.4 ± 0.3, 11)	–	–
handle length	7.0–8.0 (7.4 ± 0.2, 24)	7.3–7.7 (7.5 ± 0.1, 11)	–	–
ventral bar height	6.0–10.3 (8.6 ± 1.0, 25)	8.0–11.0 (9.4 ± 1.0, 16)	9	–
ventral bar width	35.0–46.0 (40.2 ± 3.9, 25)	32.0–46.0 (39.0 ± 4.6, 16)	45	34–40 (37 ± 1.8, 12)
length of anterior processes	6.0–10.7 (8.2 ± 1.1, 25)	7.0–12.0 (9.1 ± 1.4, 16)	–	13–19 (16 ± 2, 12)
span between processes	3.5–7.8 (4.5 ± 0.9, 25)	4.0–6.6 (4.7 ± 0.7, 16)	–	8–12 (9 ± 1.4, 13)
dorsal bar height	6.3–13.3 (8.2 ± 1.3, 25)	6.0–11.0 (7.9 ± 1.4, 16)	15	–
dorsal bar width	38.2–53.0 (43.4 ± 4.0, 25)	38.0–52.5 (45.1 ± 4.4, 16)	43	38–45 (42 ± 2.4, 12)
copulatory organ tube length	198.0–289.0 (252.8 ± 22.4, 15)	243.0–270.0 (256.0, 6)	250–265	180–250 (209 ± 24.6, 16)
copulatory organ tube width	1.2–1.5 (1.4 ± 0.1, 15)	1.5–1.7 (1.6, 6)	–	1–1.5 (1.2 ± 0.2, 15)
length of accessory piece	37.0–48.0 (41.3 ± 2.9, 15)	38.5–44.0 (41.1, 6)	–	33–50 (37 ± 4.6, 15)
width of accessory piece proximal part	7.5–11.0 (9.0 ± 1.1, 15)	9.0–11.0 (9.8, 6)	–	4–6 (5 ± 0.8, 14)
length of distal part upper lobe	14.0–22.0 (19.0 ± 2.2, 15)	14.0–21.0 (18.9, 6)	–	–
length of distal part lower lobe	11.0–21.0 (16.0 ± 2.8, 12)	12.0–20.0 (17.2, 6)	–	–
span between tips of lobes	9.0–22.0 (15.1 ± 4.0, 12)	9.0–20.0 (16.3, 6)	–	–
vagina length	150.0–180.0 (161.0 ± 9.6, 12)	133.0–150.0 (141.5, 3)	100–110	95–150 (122 ± 18.7, 15)

so phylogenetic reconstruction based on this molecular marker is considered in detail. Additionally, a matrix of genetic distances between species was counted (online Supplementary 1). Four well-supported clades of *Ligophorus* containing different species, without agreement with geographical regions or hosts, were identified (fig. 3). Clade I consisted of two subclades (A and B), each with high nodal support and 5% average evolutionary divergence. *Ligophorus kaohsianghsieni* belonged to subclade A, whereas other species infecting *P. haematocheilus* (*Ligophorus pilengas* and *Ligophorus llewellyni*) were within subclade B. Clade II was poorly supported in general, but included well-supported subclade C and several species from the Mediterranean Sea, namely the closely related *Ligophorus minimus*, *Ligophorus acuminatus*,. *Ligophorus imitans* and *Ligophorus heteronchus*, which appeared as separate lineages. *Ligophorus vanbenedeni*

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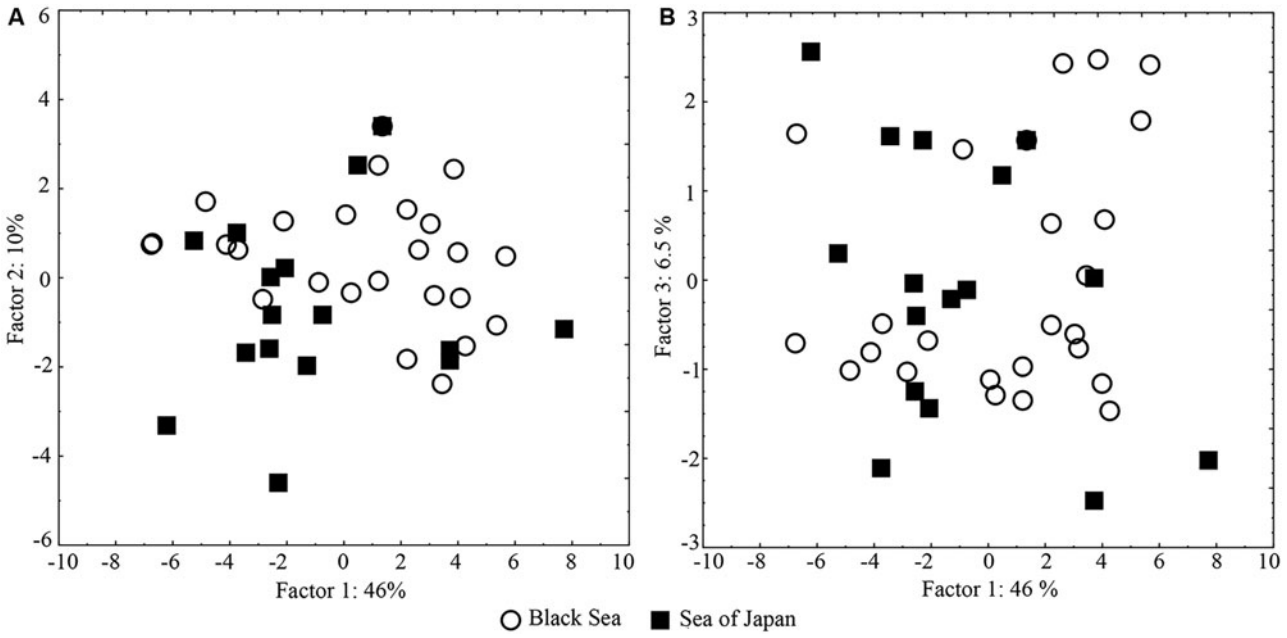

Fig. 2. Plots of 41 specimens of *Ligophorus kaohsianghsieni* from the Black Sea and the Sea of Japan according to their scores in the first (A) and second (B) principal component analysis planes, run on metric data for log-transformed 30 characters of haptor structures.

represented a sister lineage to clades I and II with high support (fig. 3). Clade III was also highly supported and encompassed three species, namely *Ligophorus confusus*, *Ligophorus szidati* and *Ligophorus angustus*. Clade IV was poorly supported with the ML algorithm and highly supported with BI and consisted of five *Ligophorus* species from mullets from the Indian Ocean.

Intra-group and inter-group genetic divergence for each clade is presented in table 5. The highest intra-group sequences divergence was observed for clade IV (10%) and subclade C (12%). These two patterns consist of species from the Indian Ocean. Intra-group sequences divergences of subclades A and B, as well as for clade III ranged from 2% to 5%.

The topologies of the phylogenetic trees based on 18S and ITS1 rDNA were similar to that based on 28S in respect of mean clades, except some species, which were out of their clades. Probably the lack of ITS1 sequence data for species from the Indian Ocean, which formed clade C in the tree based on 28S, led to the exclusion of *L. minimus* from clade II (fig. 4). The reduction in the number of species in this analysis also resulted in a lack of support for subclade A. A similar situation is observed for phylogeny based on 18S (fig. 5). *Ligophorus careyensis* dropped out of clade IA, although this species is closer to clade I than to clade II in genetic distances. Nevertheless, both phylogenetic trees based on 18S and ITS1 rDNA keep a tendency of species clustering on the 28S rDNA-based tree, indicating the basal position of *Ligophorus* species from mullets of the Indian Ocean and terminal position of species ex hosts from the Black Sea and the Sea of Japan, including the position of *L. kaohsianghsieni* in subclade B. Obviously, 18S rDNA and ITS1 rDNA have good potential for more active use for phylogenetic studies of *Ligophorus* species in the future.

Discussion

Based on the present data and taking into account the previously published information (Gusev, 1962; Sarabeev *et al.*, 2013), morphometric characters that allow to clearly distinguish specimens of *L. kaohsianghsieni* from the Black-Azov Sea region compared to rivers of the Russian Far East and the Sea of Japan has not been found.

The obtained sequences of three fragments of the ribosomal cluster of nuclear DNA (18S, ITS1 and 28S) from 14 individuals of *L. kaohsianghsieni* from the different regions are identical. This is consistent with the previously obtained data, since no mutations were observed in the 28S rRNA gene fragment between four individuals of *L. pilengas*, five individuals of *L. confusus*, four individuals of *L. chabaudi*, and in ITS1 between nine individuals of *L. cephali* and six individuals of *L. confusus* (Blasco-Costa *et al.*, 2012, figs 3 and 4). This confirms that this DNA region is highly conserved for *Ligophorus* at the intraspecific level.

At the same time, high interspecific genetic divergence is observed between the analysed *Ligophorus* species (figs 3–5). Even those species which formed monophyletic groups on the phylogenetic tree based on 28S rRNA gene sequences and parasitizing the same host species in the same region (fig. 3: subclade C and clade IV) are genetically significantly different from each other. The question arises, what contributes to this deep divergence between sympatric and synxenic species?

Specimens of *L. kaohsianghsieni* cluster with species parasitizing fish of the genera *Planiliza* and *Chelon* from the Atlantic and Pacific Oceans (clade I) in both phylogenetic reconstructions based on 28S and ITS1 rRNA (figs 3 and 4). Whereas *L. kaohsianghsieni* is significantly distant from *L. pilengas* and *L. llewellyni* occur on the same host (*P. haematocheilus*) in the same seas. Moreover, the latter two species have merged into a monophyletic group with species of *Ligophorus* infecting fish of the genus *Mugil* in the Atlantic and Pacific Oceans (fig. 3: subclade A vs. subclade B), and *L. chabaudi*, found in both oceans, occupies a basal position in this clade.

Previously, Blasco-Costa *et al.* (2012) obtained a similar result in reconstructing the phylogenetic relationships between 14 species of *Ligophorus* from the Mediterranean and Azov Seas based on 28S and ITS1, where two species (*L. pilengas* and *L. llewellyni*)

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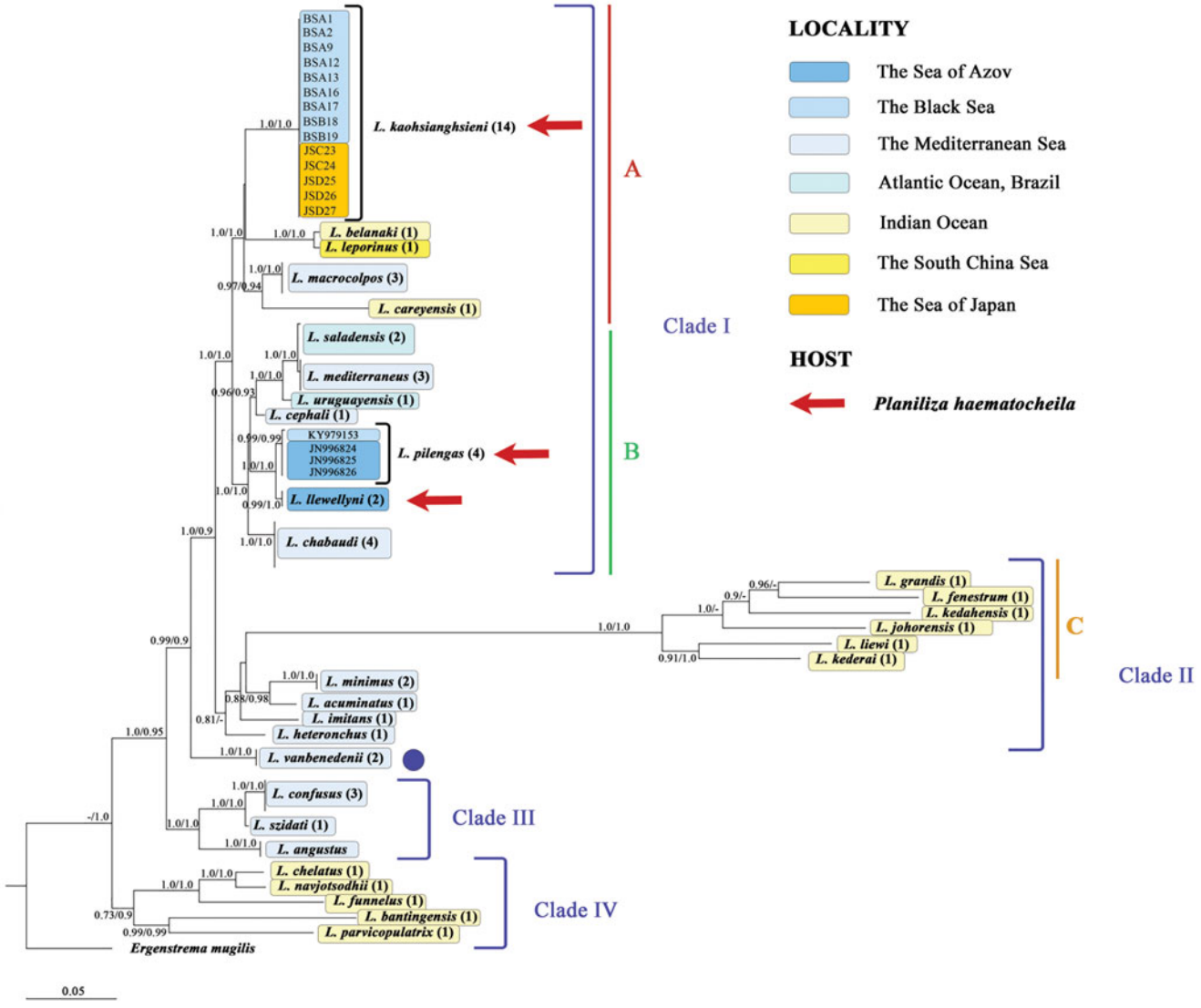

Fig. 3. Phylogenetic tree derived from the 28S rRNA gene sequences using Bayesian analysis. The alignment length was 719 positions. Nodal numbers – posterior probabilities for Bayesian inference/maximum likelihood phylogenetic algorithms (only significant values (0.9–1.0) are provided). The number of available nucleotide sequences in GenBank is noted in parentheses next to each species. The branch length is drawn to scale, with the scale bar indicating the number of nucleotide substitutions. The species with a different position in phylogeny based on different genes are marked with the dark blue spot.

from *P. haematocheilus* of north-western Pacific Ocean origin and *Ligophorus* spp. from widespread *Mugil cephalus* formed one group, distancing themselves from species parasitizing only hosts with Mediterranean Sea and north-east Atlantic Ocean distribution.

However, the addition of more representatives of *Ligophorus* from the Pacific Ocean and the north-west Atlantic Ocean into the phylogenetic analysis (fig. 3) revealed that some species from different host species and oceans were closer and included in one monophyletic lineage than species from the same host and region, for example, *Ligophorus belanaki* and *L. careyensis* entered clade I, and *Ligophorus chelatus*, *Ligophorus navjotsodhii*, *Ligophorus parvicopulatrix*, *Ligophorus funnelus* and *Ligophorus bantingensis* into clade IV (fig. 3), even though they all infected *Planiliza subviridis* from Malaysia. While *Ligophorus macrocolpos*, which parasitizes *Chelon saliens* in the Mediterranean and Black Seas, clustered with species distributed in the north-west Pacific Ocean (fig. 3: subclade A), and was significantly separated from other species occurring on the same host and in the same region, namely *L. minimus*, *L. acuminatus*, *L. imitans*, *L. heteronchus*, *L. szidati* (fig. 3: clades II and III), and *L. vanbenedeni*. Thus, there is no correspondence between the phylogenetic and geographical proximity of hosts and relation of *Ligophorus* species parasitizing them. Previously, the absence of relatedness between about half of the *Ligophorus* species infecting the same host species was suggested based on the analysis of morphological similarity (Sarabeev & Desdevises, 2014).

As a whole, the results of our study demonstrate the main vector of *Ligophorus* phylogeny, showing a constant basal position for certain species from Indian Ocean mullets, a middle position of other certain species from Mediterranean mullets and terminal position of *Ligophorus* species from mullets of different seas, including the Indo-West Pacific Ocean, Mediterranean Sea and Atlantic Ocen fauna. It cannot be excluded that the fauna of the Mediterranean Sea and Indo-Pacific Ocean *Ligophorus* species from terminal clades has secondary origin in these regions,

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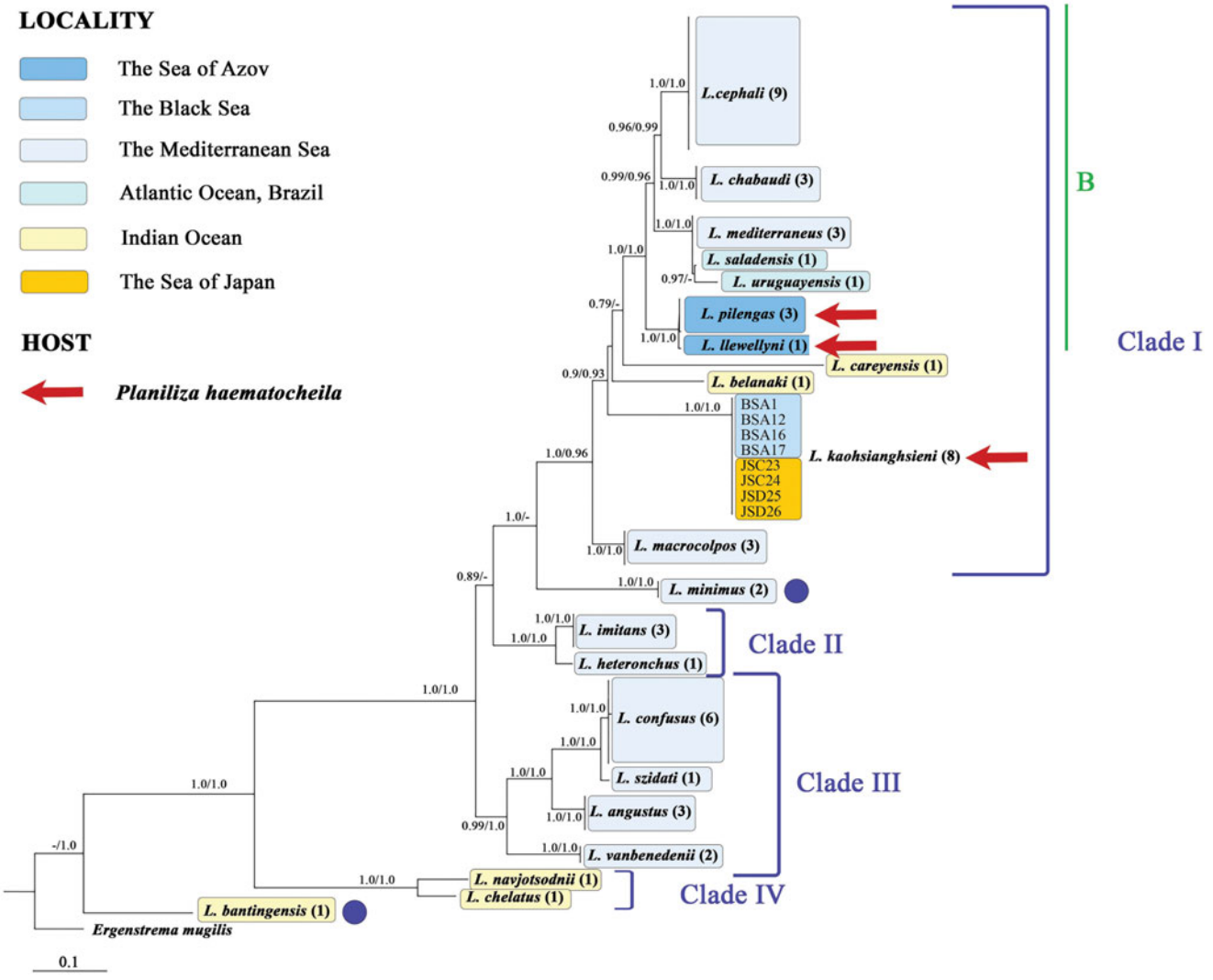

Fig. 4. Phylogenetic tree derived from the internal transcribed spacer 1 rDNA sequences using Bayesian analysis. The alignment length was 667 positions. Nodal numbers – posterior probabilities for Bayesian inference/maximum likelihood phylogenetic algorithms (only significant values (0.9–1.0) are provided). The number of available nucleotide sequences in GenBank is noted in parentheses next to each species. The branch length is drawn to scale, with the scale bar indicating the number of nucleotide substitutions. The species with a different position in phylogeny based on different genes are marked with the dark blue spot.

occurring through possible host-switching processes using different mullet fish species after long-term spatial isolation. We suppose that some representatives of the ancestral form of the studied monogeneans, inhabiting in the Indian Ocean, could have migrated to other regions, for example, to the Mediterranean Sea (according to the results of phylogenetic analysis), using host-switching, where deep divergence and speciation occurred. Later, these new species could secondarily settle Indian Ocean territories using different mullet fish species. This hypothesis partially explains the deep genetic diversity of sympatric species; it should be studied in more detail in the future. Additionally, the widespread species complex *M. cephalus* can be considered as a key host species in the secondary distribution of monogeneans throughout different zoogeographical areas.

It was previously shown that differences in the morphology of attachment (haptoral) structures between *Dactylogyrus* species (related *Ligophorus* to taxon) occurring on the same host contribute to niche segregation and increased reproductive isolation of related species to prevent hybridization, just as monogeneans occupying the same niche differ greatly in shape and size of copulatory organ (Šimková *et al.*, 2002). Thus, the morphology of both of these structures is of great evolutionary importance. Khang *et al.* (2016), analysing the haptoral morphology of 13 species from Malaysia, which formed two different clusters on a phylogenetic tree constructed based on 28S, ITS1, 18S rRNA sequences, obtained good agreement with the clustering of these species by these morphological characters. Similarly, Sarabeev & Desdevises (2014), comparing reconstructions of the relatedness of 14 species from the Mediterranean and Black Seas based on 28S and ITS1 rRNA sequences with the results of morphological analysis, mainly related to the characters of haptoral structures and copulatory organs, concluded that morphological and molecular phylogenetic trees are congruent.

However, it should be noted that the species analysed in both studies (Sarabeev & Desdevises, 2014; Khang *et al.*, 2016) were from the same region, respectively, the Mediterranean and Black Seas in the first article and Malaysia in the second. The closest species to *L. kaohsianghsieni* according to all phylogenetic

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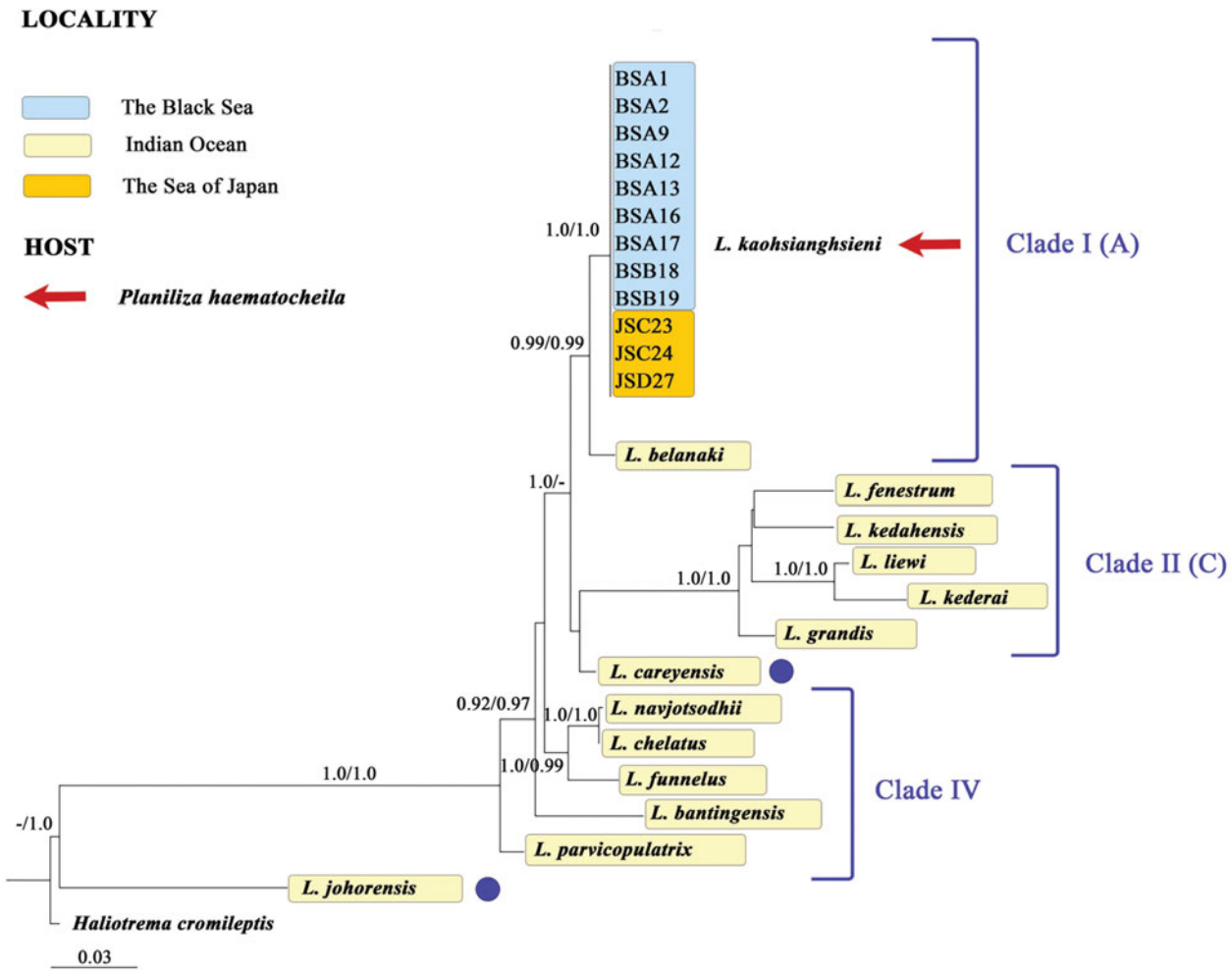

Fig. 5. Phylogenetic tree derived from the 18S rRNA gene sequences using Bayesian analysis. The alignment length was 758 positions. Nodal numbers – posterior probabilities for Bayesian inference/maximum likelihood phylogenetic algorithms (only significant values (0.9–1.0) are provided). The number of available nucleotide sequences in GenBank is noted in parentheses next to each species. The branch length is drawn to scale, with the scale bar indicating the number of nucleotide substitutions. The species with a different position in phylogeny based on different genes are marked with the dark blue spot.

reconstructions (based on 28S, ITS1 and 18S) in the present study is *L. belanaki*, also parasitizing mullet of the genus *Planiliza* in the coastal seas of the western Pacific Ocean.

At the same time, the two species differ greatly in the morphology of their haptoral structures and copulatory organ: in *L. kaohsianghsieni* anchors have a relatively short distal part (blade) compared to their proximal part, ventral bar with closely spaced anterior processes, copulatory organ with a long tube and distally bifurcated accessory part (fig. 1), whereas *L. belanaki* anchors with a slender blade that is much longer than their proximal part, the ventral bar has rather widely spaced anterior processes, and the copulatory organ tube is rather short and its accessory part is not bifurcated distally (Soo & Lim, 2013). It can be said that these species are opposite in most morphological characters of haptor and male copulatory organ, while in molecular data they are closely related species. Similarly, species infecting

Table 5. Estimates of average evolutionary divergence over sequence pairs within groups (boldface type, in the diagonal) and between groups (above the diagonal) based on 28S variability.

Group name	IA	IB	IIC	IID	III	IV
IA	**0.030**	0.050	0.178	0.060	0.084	0.119
IB		**0.020**	0.173	0.058	0.077	0.110
IIC			**0.120**	0.175	0.187	0.196
IID				**0.040**	0.080	0.111
III					**0.030**	0.115
IV						**0.100**

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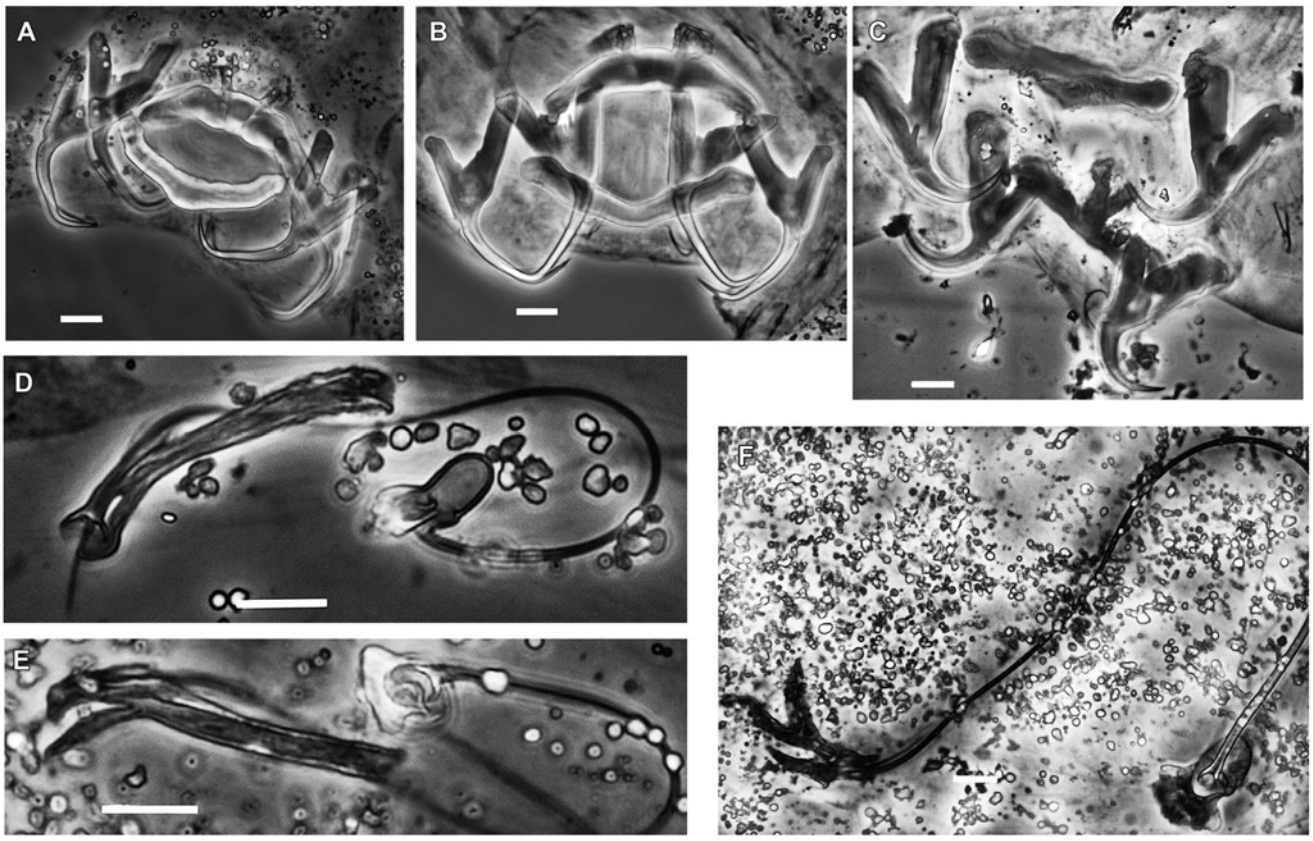

Fig. 6. Comparison of the morphology of haptoral structures and the copulatory organ of *Ligophorus kaohsianghsieni* (C, F), *Ligophorus llewellyni* (A, D) and *Ligophorus pilengas* (B, E) ex *Planiliza haematocheila* from the Black Sea: A–C, haptoral structures; and D–F, male copulatory organ. Scale bar = 10 μm.

P. subviridis, which form clade IV, are quite diverse in size and shape of haptoral structures and copulatory organ (Soo & Lim, 2012). Thus, it should be taken into account that closely related species may differ significantly in morphology.

Morphological differences in haptor structures of phylogenetically closely related species may be the result of adaptation to different hosts or to a specific attachment site on the gills. On the other hand, the marked differences in morphology of both haptor and copulatory organ between *L. kaohsianghsieni* and two other species from *P. haematocheilus* (*L. pilengas* and *L. llewellyni*) (fig. 6) are consistent with significant genetic divergence between them.

Overall, at least two groups of species of different origin parasitize *P. haematocheilus* in its natural range, the Sea of Japan, as well as in the region of introduction, the Black Sea.

Supplementary material. To view supplementary material for this article, please visit http://doi.org/10.1017/S0022149X22000724.

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Conflicts of interest. None.

Author contributions. Vodiasova E. A.: conceptualization, writing – original draft, review and editing, visualization, funding acquisition, genetic data curation. Atopkin D.: investigation, genetic analysis, software, writing – original draft, review and editing. Plaksina M.: sampling, investigation, morphology analysis, visualization. Chelebieva E.: investigation, genetic analysis, writing – review and editing. Dmitrieva E.: conceptualization, morphology data curation, writing – original draft, review and editing, project administration.

Ethical standards. All applicable institutional, national and international guidelines for the care and use of animals were followed. All studied fishes are listed as a 'Least Concern' species by the International Union for Conservation of Nature.

References

Abdallah VD, De Azevedo RK and Luque J (2009) Four new species of *Ligophorus* (Monogenea: Dactylogyridae) parasitic on *Mugil liza* (Actinopterygii: Mugilidae) from Guandu River, Southeastern Brazil. *Journal of Parasitology* **95**(4), 855–864.

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19(6)**, 716–723.

Anisimova M and Gascuel O (2006) Approximate likelihood-ratio test for branches: a fast, accurate and powerful alternative. *Systematic Biology* **55 (4)**, 539–552.

Blasco-Costa I, Míguez-Lozano R, Sarabeev V and Balbuena JA (2012) Molecular phylogeny of species of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities within the Dactylogyridae. *Parasitology International* **61(4)**, 619–627.

Darriba D, Taboada GL, Doallo R and Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9(8)**, 772.

Dmitrieva EV (1996) Fauna of Monogenea of the far-east *Mugil soiuy* in the Black Sea. *Vestnik Zoologii* **4–5**, 95–97. [In Russian.]

Dmitrieva EV, Gerasev PI and Pron'kina NV (2007) *Ligophorus llewellyni* n. sp. (Monogenea: Ancyrocephalidae) from the redlip mullet *Liza*

haematocheilus (Temminck & Schlegel) introduced into the Black Sea from the Far East. *Systematic Parasitology* **67(1)**, 51–64.

Dmitrieva EV, Gerasev PI, Gibson DI, Pronkina NV and Galli P (2012) Descriptions and a morphological grouping of eight new species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) from Red Sea mullets. *Systematic Parasitology* **81(3)**, 203–237.

Dmitrieva EV, Gerasev PI and Gibson DI (2013a) *Ligophorus abditus* n. sp. (Monogenea: Ancyrocephalidae) and other species of *Ligophorus* Euzet and Suriano, 1977 infecting the flathead grey mullet *Mugil cephalus* L. in the Sea of Japan and the Yellow Sea. *Systematic Parasitology* **85(2)**, 117–130.

Dmitrieva EV, Gerasev PI, Kolpakov NV, Nguen VH and Ha DN (2013b) On monogeneans (Plathelminthes, Monogenea) fauna of marine fishes in Vietnam. III. *Ligophorus* spp. from three species of mullets (Pisces, Mugilidae). *Izvestiya TINRO* **172**, 224–236.

El Hafidi F, Rkhami OB, De Buron I, Durand J-D and Pariselle A (2013a) *Ligophorus* species (Monogenea: Ancyrocephalidae) from *Mugil cephalus* (Teleostei: Mugilidae) off Morocco with the description of a new species and remarks about the use of *Ligophorus* spp. as biological markers of host populations. *Folia Parasitologica* **60(5)**, 433–440.

El Hafidi F, Diamanka A, Rkhami OB and Pariselle A (2013b) New species of *Ligophorus* (Monogenea, Ancyrocephalidae), parasite of *Liza* spp. (Teleostei, Mugilidae) off the Northwestern African coast. *Zoosystema* **35** (2), 83–93.

Euzet L and Suriano DM (1977) *Ligophorus* n. g. (Monogenea, Ancyrocephalidae) parasite des Mugilidae (Téléostéens) en Méditerranée [*Ligophorus* n. g. (Monogenea, Ancyrocephalidae) parasite of Mugilidae (Teleosteans) in the Mediterranean]. *Bulletin du Muséum d'Histoire Naturelle Série 3, Zoologie* **472(329)**, 799–821. [In French.]

Guindon S and Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52 (5)**, 696–704.

Gusev AV (1962) Order Dactylogyridea. pp. 204–341. In Bychovsky BE (Ed.) *Keys to the parasites of freshwater fishes of the USSR fauna*. Leningrad, Nauka, USSR. [In Russian.]

Gusev AV (1983) *Methods of collection and processing material on monogeneans parasitizing fish*. 48 pp. Leningrad, Nauka [In Russian].

Gusev AV (1985) Order Dactylogyridea. pp. 15–251. In Bauer ON (Ed.) *Keys to the parasites of freshwater fishes of the USSR fauna. Vol. 2. Metazoan parasites*. Leningrad, Nauka, USSR. [In Russian.]

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* **41 (1)**, 95–98.

Huelsenbeck JP, Ronquist F, Nielsen R and Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294(55500)**, 2310–2314.

Khang TF, Soo OYM, Tan WB and Lim LHS (2016) Monogenean anchor morphometry: systematic value, phylogenetic signal, and evolution. *PeerJ* **4(1)**, e1668.

Kritsky DC, Khamees NR and Ali AH (2013) *Ligophorus* spp. (Monogenoidea: Dactylogyridae) parasitizing mullets (Teleostei: Mugiliformes: Mugilidae) occurring in the fresh and brackish waters of the Shatt Al-Arab River and Estuary in southern Iraq, with the description of *Ligophorus sagmarius* sp. n. from the greenback mullet *Chelon subviridis* (Valenciennes). *Parasitology Research* **112**(12), 4029–4041.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA x: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**(6), 1547–1549.

Littlewood DTJ and Olson PD (2001) Small subunit rDNA and the Platyhelminthes: signal, noise, conflict and compromise. pp. 262–278. In Littlewood DTJ and Bray RA (Eds) *Interrelationships of the Platyhelminthes*. New York, Taylor & Francis.

Littlewood DTJ, Curini-Galletti M and Herniou EA (2000) The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution* **16(3)**, 449–466.

Lockyer AE, Olson PD and Littlewood DTJ (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society* **78(2)**, 155–171.

Marchiori NC, Pariselle A, Pereira J-J, Agnèse J-F, Durand J-D and Vanhove MPM (2015) A comparative study of *Ligophorus uruguayense* and *L. saladensis* (Monogenea: Ancyrocephalidae) from *Mugil liza* (Teleostei: Mugilidae) in southern Brazil. *Folia Parasitologica* **62(1)**, 024.

Mollaret I, Jamieson BGM and Justine JL (2000) Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *International Journal of Parasitology* **30(2)**, 171–185.

Pakdee W, Ogawa K, Pornruseetriratn S, Thaenkham U and Yeemin T (2018) The first record of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Dactylogyridae) on *Crenimugil buchanani* (Teleostei: Muglidae) from Thailand based on morphological and molecular analyses. *Journal of Helminthology* **93**(6), 752–762.

Pankov P (2011) *Helminths and helminth communities in mullets from the Bulgarian Black Sea coast*. Phd thesis, 33 pp. Bulgarian Academy of Sciences, Sofia. [In Bulgarian.]

Plaisance L, Littlewood DTJ, Olson PD and Morand S (2005) Molecular phylogeny of gill monogeneans (Platyhelminthes, Monogenea, Dactylogyridae) and colonization of Indo-West Pacific butterfly fish hosts (Perciformes, Chaetodontidae). *Zoologica Scripta* **34(4)**, 425–436.

Posada D (2003) Using MODELTEST and PAUP* to select a model of nucleotide substitution. *Current Protocols in Bioinformatics* **6(1)**, 6.5.1–6.5.14.

Rodríguez-González A, Míguez-Lozano R, Llopis-Belenguer C and Balbuena JA (2015a) A new species of *Ligophorus* (Monogenea: Dactylogyridae) from the gills of the flathead mullet *Mugil cephalus* (Teleostei: Mugilidae) from Mexico. *Acta Parasitologica* **60**(4), 767–776.

Rodríguez-González A, Miguez-Lozano R, Llopis-Belenguer C and Balbuena JA (2015b) Phenotypic plasticity in haptoral structures of *Ligophorus cephalic* (Monogenea: Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric approach. *International Journal of Parasitology* **45**(5), 295–303.

Sarabeev V and Desdevises Y (2014) Phylogeny of the Atlantic and Pacific species of *Ligophorus* (Monogenea: Dactylogyridae): morphology vs. molecules. *Parasitology International* **63(1)**, 9–20.

Sarabeev V, Rubtsova N, Yang TB and Balbuena JA (2013) Taxonomic revision of the Atlantic and Pacific species of *Ligophorus* (Monogenea, Dactylogyridae) from mullets (Teleostei, Mugilidae) with the proposal of a new genus and description of four new species. *Vestnik Zoologii* **28(1)**, 1–112.

Šimková A, Ondračková M, Gelnar M and Morand S (2002) Morphology and coexistence of congeneric ectoparasite species: reinforcement of reproductive isolation? *Biological Journal of the Linnean Society* **76(1)**, 125–135.

Soo OYM and Lim LHS (2012) Eight new species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) from mugilids off Peninsular Malaysia. *Raffles Bulletin of Zoology* **60(2)**, 241–264.

Soo OYM and Lim LHS (2013) A description of two new species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) from Malaysian mugilid fish using principal component analysis and numerical taxonomy. *Journal of Helminthology* **89(2)**, 131–149.

Soo OYM, Tan WB and Lim LHS (2015) Three new species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) from *Moolgarda buchanani* (Bleeker) off Johor, Malaysia based on morphological, morphometric and molecular data. *Raffles Bulletin of Zoology* **63(1)**, 49–65.

Thompson JD, Higgins DG and Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22(22)**, 4673–4680.

Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM and Swiderski Z (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* **56(1)**, 1–15.

Wu XY, Zhu XQ, Xie MQ and Li AX (2006) The radiation of *Haliotrema* (Monogenea: Dactylogyridae: Ancyrocephalinae): molecular evidence and explanation inferred from LSU rDNA sequences. *Parasitology* **132(5)**, 659–668.

Wu XY, Zhu XQ, Xie MQ and Li AX (2007) The evaluation for generic-level monophyly of Ancyrocephalinae (Monogenea, Dactylogyridae) using ribosomal DNA sequence data. *Molecular Phylogenetic and Evolution* **44(2)**, 530–544.

Zhang JY, Yang TB, Liu L and Xuejuan D (2003) A list of monogeneans from Chinese marine fishes. *Systematic Parasitology* **54(2)**, 111–130.

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