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A description of two new species of *Ligophorus* Euzet & Suriano, 1977 (Monogenea: Ancyrocephalidae) from Malaysian mugilid fish using principal component analysis and numerical taxonomy

O.Y.M. Soo and L.H.S. Lim*

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

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Abstract

Ligophorus belanaki n. sp. and *Ligophorus kederai* n. sp. are described from *Liza subviridis* Valenciennes, 1836 and *Valamugil buchanani* Bleeker, 1854, respectively. *Ligophorus kederai* n. sp. has fenestrated ventral anchors, while in *L. belanaki* n. sp. the ventral anchor is not fenestrated. *Ligophorus belanaki* n. sp. is similar to *L. careyensis*, one of its coexisting congeners, in the overall shape and size of hard parts, but differs in having a flat median piece in the structure of the AMP (antero-median protuberance of the ventral bar), copulatory organ with non-ornamented initial part and longer vaginal tube, compared to raised median piece in the AMP, ornamented initial part and comparatively shorter vaginal tube in *L. careyensis*. *Ligophorus kederai* n. sp. is similar to *L. fenestrum*, a coexisting congener, in having fenestrated ventral anchors, but differs in having longer points and narrower base. *Ligophorus fenestrum*, unlike *L. kederai* n. sp., also possesses fenestrated dorsal anchors. The principal component analysis (PCA) scatterplots indicate that the two new and eight known *Ligophorus* species from Malaysian mugilids can be differentiated based on the morphometries of their anchors, ventral bars and copulatory organ separately and when combined together. Numerical taxonomy (NT) analyses based on Jaccard's Index of Similarity and neighbour-joining clustering, is used to facilitate comparison of these two new species with the 50 known *Ligophorus* based on morphological and metric characters. The two new species are different from each other and the other 50 species in the overall shapes and sizes of hard parts, as indicated by the NT analyses.

Introduction

To date 50 species of *Ligophorus* Euzet & Suriano, 1977 have been described from mugilids from off the Mediterranean region, the Red Sea and off Iran (table 1) and the Gulf of Mexico to off the South American coast (Argentina, Brazil, Chile and Uruguay) and off China, Japan and Malaysia (table 2) (see Soo & Lim, 2012). Six *Ligophorus* species have been described from *Liza subviridis* (*L. navjotsodhii*, *L. chelatus*, *L. funnelus*, *L. parvicopulatrix*, *L. bantingensis* and *L. careyensis*) and two species from *Valamugil buchanani* (*L. kedahensis* and *L. fenestrum*) caught off Peninsular Malaysia (see

*Fax: + 603 7967 4178/4173
E-mail: susan@um.edu.my

Table 1. A list of *Ligophorus* species off European regions, Red Sea and off Iran.

Ligophorus species	Host species	Geographical location (Type)	References
L. vanbenedenii (Parona & Perugia, 1890) Euzet & Suriano, 1977 **(type species)** [syns. *Tetraonchus vanbenedenii* Parona & Perugia, 1890; *Ancyrocephalus vanbenedenii* Johnston & Tiegs, 1922; *Haplocleidus vanbenedenii* Palombi, 1949; *Haliotrema vanbenedenii* Young, 1968]	*Liza aurata* Risso (**type host**)	Gulf of Genoa, Italy	Euzet & Suriano, 1977
L. angustus Euzet & Suriano, 1977	*Chelon labrosus* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. szidati Euzet & Suriano, 1977	*Liza aurata* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. acuminatus Euzet & Suriano, 1977	*Liza saliens* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. heteronchus Euzet & Suriano, 1977	*Liza saliens* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. macrocolpos Euzet & Suriano, 1977	*Liza saliens* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. minimus Euzet & Suriano, 1977	*Liza saliens* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. confusus Euzet & Suriano, 1977	*Liza ramada* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. imitans Euzet & Suriano, 1977	*Liza ramada* Risso	Mediterranean Sea	Euzet & Suriano, 1977
L. mediterraneus Sarabeev *et al.*, 2005	*Mugil cephalus* Linnaeus	Mediterranean Sea	Sarabeev *et al.*, 2005
L. chabaudi Euzet & Suriano, 1977	*Mugil cephalus* Linnaeus	Mediterranean Sea	Euzet & Suriano, 1977; Rubtsova *et al.*, 2006
L. parvicirrus Euzet & Sanfilippo, 1983	*Liza ramada* Risso	Gulf of Lion	Euzet & Sanfilippo, 1983
L. llewellyni Dmitrieva *et al.*, 2007	*Liza haematocheila* Temminck & Schlegel	Black Sea	Dmitrieva *et al.*, 2007
L. euzeti Dmitrieva & Gerasev, 1996	*Liza saliens* Risso	Black Sea	Dmitrieva & Gerasev, 1996
L. cephali Rubtsova *et al.*, 2006	*Mugil cephalus* Linnaeus	Black Sea	Rubtsova *et al.*, 2006
L. bykhowskyi Dmitrieva *et al.*, 2012	*Crenimugil crenilabis* Forsskal	Red Sea	Dmitrieva *et al.*, 2012
L. zhangi Dmitrieva *et al.*, 2012	*Crenimugil crenilabis* Forsskal	Red Sea	Dmitrieva *et al.*, 2012
L. simpliciformis Dmitrieva *et al.*, 2012	*Liza carinata* Valenciennes	Red Sea	Dmitrieva *et al.*, 2012
L. bipartitus Dmitrieva *et al.*, 2012	*Liza carinata* Valenciennes	Red Sea	Dmitrieva *et al.*, 2012
L. campanulatus Dmitrieva *et al.*, 2012	*Liza carinata* Valenciennes	Red Sea	Dmitrieva *et al.*, 2012
L. mamaevi Dmitrieva *et al.*, 2012	*Liza carinata* Valenciennes	Red Sea	Dmitrieva *et al.*, 2012
L. lebedevi Dmitrieva *et al.*, 2012	*Liza carinata* Valenciennes	Red Sea	Dmitrieva *et al.*, 2012
L. surianoae Dmitrieva *et al.*, 2012	*Liza carinata* Valenciennes	Red Sea	Dmitrieva *et al.*, 2012
L. pilengas Sarabeev & Balbuena, 2004 [syn. *L. gussevi* Miroshnichenko & Maltsev, 2004]	*Liza haematocheila* Temminck & Schlegel	Sea of Azov	Sarabeev & Balbuena, 2004; Miroshnichenko & Maltsev, 2004; Balbuena *et al.*, 2006
L. fluviatilis (Bychowsky, 1949) Dmitrieva *et al.*, 2012 (syn. *Ancyrocephalus fluviatilis* Bychowsky, 1949)	*Liza abu* Heckel	Off Iran	Bychowsky, 1949; Dmitrieva *et al.*, 2012

Table 2. A list of known and new species of *Ligophorus* off South American and Asia-Pacific regions.

Ligophorus species	Host species	Geographical location (Type)	References
L. mugilinus (Hargis, 1955) Euzet & Suriano, 1977 [syn. *Pseudohaliotrema mugilinus* Hargis, 1955]	*Mugil cephalus* Linnaeus	Gulf of Mexico	Hargis, 1955; Euzet & Suriano, 1977
L. brasiliensis Abdallah *et al.*, 2009	*Mugil liza* Valenciennes	Off Brazil	Abdallah *et al.*, 2009
L. guanduensis Abdallah *et al.*, 2009	*Mugil liza* Valenciennes	Off Brazil	Abdallah *et al.*, 2009
L. lizae Abdallah *et al.*, 2009	*Mugil liza* Valenciennes	Off Brazil	Abdallah *et al.*, 2009
L. tainhae Abdallah *et al.*, 2009	*Mugil liza* Valenciennes	Off Brazil	Abdallah *et al.*, 2009
L. huitrempe Fernandez-Bargiela, 1987	*Mugil cephalus* Linnaeus	Off Chile	Fernandez-Bargiela, 1987
L. saladensis Marcotegui & Martorelli, 2009	*Mugil platanus* Gunther	Off Argentina	Marcotegui & Martorelli, 2009
L. uruguayense Siquier & Otrowski de Nunez, 2009	*Mugil platanus* Gunther	Off Uruguay	Siquier & Otrowski de Nunez, 2009
L. kaohsianghsieni (Gusev, 1962) Gusev, 1985 [syn. *Ancyrocephalus kaohsianghsieni* Gusev, 1962]	*Liza haematocheila* Temminck & Schlegel	Sea of Japan	Gusev, 1985
L. cheleus Rubtsova *et al.*, 2007	*Mugil cephalus* Linnaeus	Sea of Japan	Rubtsova *et al.*, 2007
L. domnichi Rubtsova *et al.*, 2007	*Mugil cephalus* Linnaeus	Sea of Japan	Rubtsova *et al.*, 2007
L. pacificus Rubtsova *et al.*, 2007 [syn. *L. vanbenedenii* sensu Zhang, 2001]	*Mugil cephalus* Linnaeus	Sea of Japan	Rubtsova *et al.*, 2007; in Zhang *et al.*, 2001
L. abditus Dmitrieva *et al.*, 2013a	*Mugil cephalus* Linnaeus	Sea of Japan	Dmitrieva *et al.*, 2013a
L. hamulosus Pan, 1999	*Liza macrolepis* Smith	Off Hainan Island, China	Pan, 1999
L. chenzhenensis Hu & Li, 1992	*Mugil cephalus* Linnaeus	Off Chongming Island, China	Hu & Li, 1992
L. chongmingensis Hu & Li, 1992	*Mugil cephalus* Linnaeus	Off Chongming Island, China	Hu & Li, 1992
L. ellochelon Zhang, 2001	*Liza vaigiensis* Quoy & Gaimard	South China Sea	In Zhang *et al.*, 2001
L. leporinus (Zhang & Ji, 1981) Gusev, 1985 [syn. *Ancyrocephalus leporinus* Zhang & Ji, 1981]	*Mugil cephalus* Linnaeus	East China Sea	Zhang & Ji, 1981; Zhang *et al.*, 2001
L. navjotsodhii Soo & Lim, 2012	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Soo & Lim, 2012
L. chelatus Soo & Lim, 2012	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Soo & Lim, 2012
L. funnelus Soo & Lim, 2012	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Soo & Lim, 2012
L. parvicopulatrix Soo & Lim, 2012	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Soo & Lim, 2012
L. bantingensis Soo & Lim, 2012	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Soo & Lim, 2012
L. careyensis Soo & Lim, 2012	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Soo & Lim, 2012
L. kedahensis Soo & Lim, 2012	*Valamugil buchanani* Bleeker	Off Langkawi Island, Malaysia	Soo & Lim, 2012
L. fenestrum Soo & Lim, 2012	*Valamugil buchanani* Bleeker	Off Langkawi Island, Malaysia	Soo & Lim, 2012
L. belanaki n. sp.	*Liza subviridis* Valenciennes	Off Carey Island, Malaysia	Present study
L. kederai n. sp.	*Valamugil buchanani* Bleeker	Off Langkawi Island, Malaysia	Present study

Soo & Lim, 2012) (table 2). In a recent survey (2012–2013), we discovered two new *Ligophorus* species from *Liza subviridis* off Carey Island and from *Valamugil buchanani* off Langkawi Island, which were not found in our earlier survey due to their low abundance and low prevalence. The descriptions of the two new species are given herein.

In our recent publication on *Ligophorus*, principal component analysis (PCA) and numerical taxonomy (NT) analyses were done to provide a more objective and comprehensive comparison of new species with known species and between congeneric species (see Soo & Lim, 2012). PCA and NT analyses are also used here to facilitate

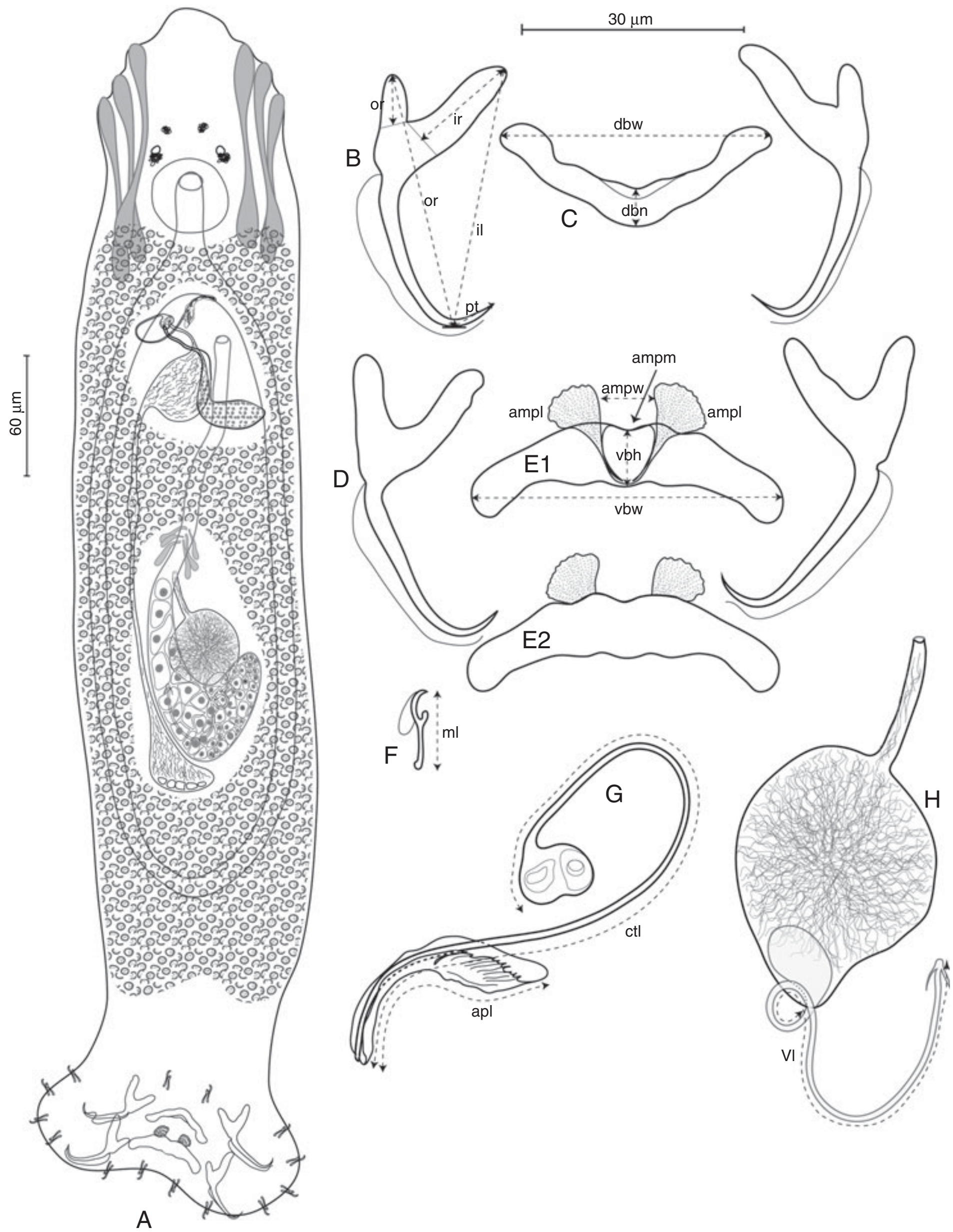

Fig. 1. Entire worm of *Ligophorus belanaki* n. sp. (dorsal view) (A) and sclerotized hard parts of dorsal anchors (B), dorsal bar (C), ventral anchors (D), ventral bar (ventral view) (E1), ventral bar (dorsal view) (E2), marginal hook (F), male copulatory organ (G) and vaginal tube and seminal receptacle (H). Parameters measured: ir, inner root; or, outer root; il, inner length; ol, outer length; pt, point; dbh, dorsal bar height; dbw, dorsal bar width; vbh, ventral bar height; vbw, ventral bar width; ampw, distance between lateral pieces of AMP (antero-median protuberance); ampl, lateral piece of AMP; ampm, median piece of AMP; ml, marginal hook length; ctl, male copulatory tube length; apl, male accessory piece length; vl, vaginal tube length. Illustrated using a digitizing tablet (WACOM) and Adobe Illustrator software.

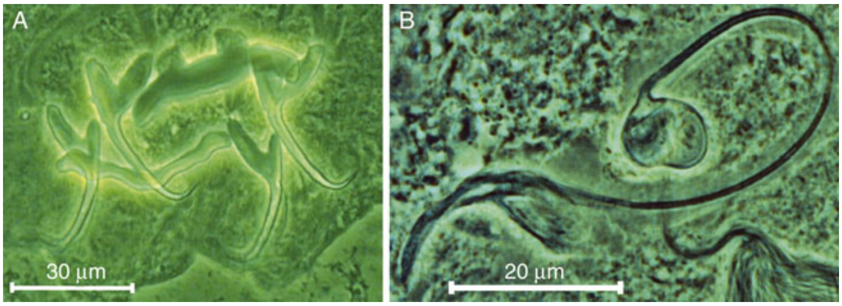

Fig. 2. Photomicrographs of sclerotized hard parts of *Ligophorus belanaki* n. sp., captured using Leica digital camera and an image analysis software (QWin Plus): (A) anchors and bars; (B) male copulatory organ.

the comparison of the present two new species with known *Ligophorus* species. PCA is used to affirm the status of the two new *Ligophorus* species based on morphometric data of these two new species and the eight known species from Peninsular Malaysia (see Soo & Lim, 2012). NT analyses are done to determine how the two new *Ligophorus* species are related to each other and to the 50 known *Ligophorus* species, based on categorized metric and morphological characters (see Soo & Lim, 2012). The NT analyses also assist in delimiting the number of species for comparison.

Materials and methods

Collection of fish hosts and monogeneans

The mullet hosts, *L. subviridis* (52 specimens) and *V. buchanani* (26 specimens) were collected in the coastal waters off Carey Island (2°51′N, 101°22′E) and off Langkawi Island (6°21′N, 99°46′E) on the west coast of Peninsular Malaysia between the years 2010 and 2013. Monogeneans were collected from freshly killed or frozen fish and processed for morphological investigation. Briefly, the monogeneans were removed from the gills, pipetted on to clean glass slides, covered with cover slips and cleared in modified ammonium–picrate–glycerin (Lim, 1991). The specimens were carefully flattened to properly expose the hard and soft anatomical structures for morphological study. These ammonium–picrate–glycerin specimens were later washed and dehydrated through a graded ethanol series and mounted in Canada Balsam without staining (Lim, 1991). Some specimens were fixed in AFA (acetic acid–formalin–alcohol) on glass slides, stained in Gomori's triple stain and mounted in Canada Balsam after dehydration in increasing ethanol series (Lim, 2006; Lim & Gibson, 2010). The stained and unstained specimens were studied under bright-field and phase-contrast microscopes. Images of the hard and soft anatomical structures of the *Ligophorus* species were captured using a Leica digital camera and image analysis software (QWin Plus). The hard and soft parts were illustrated using a digitizing tablet (WACOM) and Adobe Illustrator software. Type specimens of the two new species were deposited at the Museum of Natural History, London (BMNH) and Zoological Museum University of Malaya, Kuala Lumpur (MZUM).

Morphometrics

The diagnostic sclerotized hard parts of the haptor (anchors, bars and marginal hooks), vaginal tube and the male copulatory organ (copulatory tube and accessory piece) of 55 specimens belonging to the two new *Ligophorus* species, which had been properly flattened (stained and unstained), were measured in micrometres (μm) using the measuring option in the Leica QWin software, according to the parameters in fig. 1B, C, E1, F, G and H. It should be noted that the inverted V-shape ventral bars of *Ligophorus* species possess a highly diverse antero-median protuberance (AMP) which consists of a median piece and two lateral membranous or non-membranous pieces (see Soo & Lim, 2012). In this study, the distance between the two lateral pieces (ampw) was measured (see fig. 1E1). A total of 19 parameters of these diagnostic hard parts were measured: 10 parameters from dorsal and ventral anchors (inner root, outer root, inner length, outer length and point), one parameter from the marginal hook (hook length), three parameters from the ventral bar (length, width and distance between lateral pieces of the AMP), two parameters from the dorsal bar (length and width), two parameters from the male copulatory organ (length of copulatory tube and length of accessory piece) and one parameter from the female organ (length of vaginal tube) (fig. 1). The 'draw line' option in Leica QWin software was used to trace and measure the curves and coils of the male copulatory organ and the vaginal tube (see fig. 1G, H). The mean values and the minimum–maximum range (within parentheses) of these measurements were used in the descriptions of the new species (see figs 1, 2, 7, 8; table 3). The morphometric data were further analysed using PCA (see below).

Principal component analysis (PCA) (Pearson, 1901)

Morphometric data of the 55 specimens of the two new species and the 318 specimens of the eight *Ligophorus* species described by Soo & Lim (2012) were analysed using PCA in R (version 2.15.1; R Core Development Team, 2008). The morphometric data of all the hard parts measured excluding the length of vaginal tube (18 parameters), of only the anchors (10 parameters), ventral bars (three parameters) and copulatory organ (two parameters) were analysed separately. The length of the

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Table 3. Morphometrics (μm) of eight known and two new *Ligophorus* species obtained from *Liza subviridis* and *Valamugil buchanani*; n = number of specimens measured, and range in size given in brackets.

Host	*Liza subviridis*	*Liza subviridis*	*Liza subviridis*	*Liza subviridis*	*Liza subviridis*	*Liza subviridis*	*Liza subviridis*	*Valamugil buchanani*	*Valamugil buchanani*	*Valamugil buchanani*
Species Parameters	*L. navjotsodhii* ($n = 49$)	*L. parvicopulatrix* ($n = 60$)	*L. bantingensis* ($n = 17$)	*L. chelatus* ($n = 50$)	*L. funnelus* ($n = 28$)	*L. careyensis* ($n = 20$)	*L. belanaki* n.sp.($n = 30$)	*L. kedahensis* ($n = 67$)	*L. fenestrum* ($n = 27$)	*L. kederai* n.sp. ($n = 25$)
Body length	600 (382–801)	1078 (642–1454)	631 (418–804)	615 (402–798)	585 (381 – 790)	612 (353–825)	586 (353–782)	1181 (567–1455)	1727 (1418–2027)	701 (569–831)
Body width	97 (61–134)	166 (73–231)	103 (54–148)	94 (52–123)	114 (61–167)	118 (70–218)	108 (65–153)	199 (103–278)	270 (210–361)	160 (101–223)
Haptor length	91 (59–136)	122 (83–175)	74 (59–98)	93 (55–148)	86 (59–117)	93 (62–136)	87 (61–110)	141 (74–169)	141 (104–192)	84 (70–97)
Haptor width	109 (63–158)	150 (71–208)	78 (50–105)	118 (64–176)	106 (46–162)	118 (73–184)	106 (61–147)	139 (81–201)	129 (84–171)	106 (69–151)
Pharynx length	31 (24–34)	53 (46–59)	35 (26–41)	31 (25–37)	34 (29–39)	37 (33–45)	28 (24–34)	59 (44–71)	92 (77–105)	48 (41–51)
Pharynx width	31 (24–35)	53 (46–57)	33 (24–39)	31 (24–34)	34 (30–41)	37 (30–49)	29 (24–35)	60 (48–71)	94 (75–106)	48 (43–51)
Marginal hook	13 (11–15)	11 (9–13)	11 (10–13)	13 (12–15)	13 (9–15)	11 (9–13)	11 (9–13)	11 (10–13)	12 (11–14)	11 (10–12)
Ventral anchor:										
Inner root	14 (11–16)	13 (11–15)	8 (5–10)	14 (12– 16)	11 (9–13)	14 (10–17)	14 (10–16)	17 (12–22)	19 (14–22)	16 (15–17)
Outer root	9 (7–10)	12 (10–14)	4 (3–5)	9 (7– 11)	9 (5–11)	10 (6–13)	10 (6–13)	11 (6–15)	13 (8–16)	11 (8–13)
Inner length	36 (32–39)	29 (26–32)	14 (12–15)	37 (31–39)	29 (24–32)	36 (30–39)	36 (30–41)	34 (27–38)	38 (34– 40)	35 (34–39)
Outer length	37 (34–39)	34 (32–36)	14 (10–15)	38 (36–40)	34 (31–36)	40 (32–44)	40 (33–44)	32 (27–35)	36 (32–39)	35 (32–36)
Point	7 (5–8)	5 (4–6)	6 (4–8)	7 (5–10)	5 (4–7)	7 (5–9)	7 (5–9)	7 (5–12)	11 (9–13)	6 (5–8)
Dorsal anchor:										
Inner root	14 (12–15)	13 (10–15)	8 (7–10)	15 (12–17)	12 (10–13)	14 (11–18)	14 (10–18)	16 (12–21)	19 (16–23)	16 (14–18)
Outer root	8 (6–9)	10 (8–12)	4 (3–5)	8 (6–10)	7 (5–8)	7 (5–12)	7 (5–11)	8 (6 – 11)	12 (8–16)	9 (7–10)
Inner length	36 (33–41)	27 (23–30)	22 (20–24)	39 (36–41)	25 (22–28)	35 (31–39)	35 (31–38)	35 (31–41)	38 (33–41)	33 (32–36)
Outer length	35 (31–38)	29 (24–33)	22 (20–24)	37 (34–39)	24 (23–25)	33 (27–37)	33 (31–36)	32 (27–37)	35 (31–37)	30 (28–31)
Point	6 (4–9)	5 (3–8)	9 (7–10)	6 (4–8)	6 (4– 7)	6 (3–10)	6 (3–8)	11 (6–14)	11 (8–14)	10 (9–12)
Ventral bar:										
Width	33 (29–39)	39 (36–46)	29 (26–31)	36 (32–40)	34 (30–36)	41 (34–45)	41 (36– 44)	50 (44–57)	47 (43–52)	46 (43–50)
Height	8 (5–9)	8 (7–9)	3 (2–4)	8 (6–9)	7 (6–8)	8 (6–10)	6 (4–7)	8 (5–10)	8 (6–10)	8 (6–11)
AMP width	7 (6–10)	2 (1–4)	7 (6–8)	7 (5–11)	6 (5–7)	9 (6–11)	9 (6–11)	12 (7–15)	4 (2–6)	10 (8–12)
Dorsal bar:										
Width	33 (29–36)	49 (43–59)	28 (26–32)	36 (33–41)	38 (35–41)	37 (33–42)	37 (30–42)	51 (43–58)	50 (41–57)	46 (41–50)
Height	5 (4–6)	5 (3–6)	4 (2–5)	5 (4–7)	4 (3–6)	5 (3–6)	6 (4–7)	6 (4–8)	7 (5–10)	6 (4–8)
Copulatory organ length	71 (63–96)	48 (41–66)	67 (59–79)	69 (60–76)	77 (64–85)	94 (78–111)	95 (83–111)	65 (57–75)	86 (73–95)	83 (79–87)
Accessory piece length	27 (22–30)	21 (17–23)	23 (18–28)	26 (22–31)	24 (19–28)	25 (20–31)	25 (20–35)	33 (25–40)	34 (29–38)	24 (21–27)
Vaginal tube length*	Not observed	Not observed	37 (33–42) ($n = 10$)	34 (30–37) ($n = 10$)	37 (33–41) ($n = 10$)	36 (32–40) ($n = 10$)	70 (64–78) ($n = 10$)	Not observed	Not observed	Not observed

* Not used in the morphometric analysis due to difficulty in obtaining good specimens.

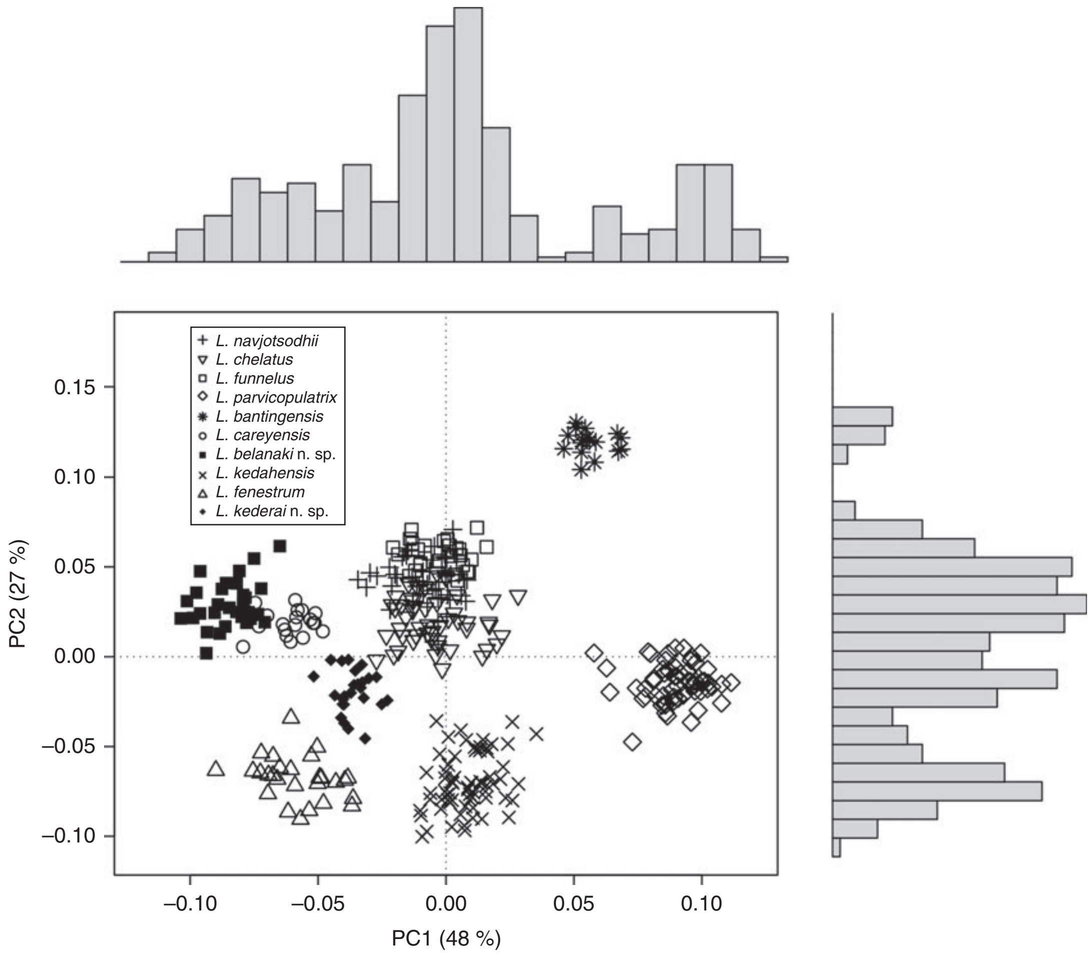

Fig. 3. Principal component analysis (PCA) scatterplot of 373 *Ligophorus* specimens based on all hard parts (marginal hook, anchors, bars and copulatory organ). Vertical and horizontal bar plots indicate one-dimensional summary of the principal component axes, PC1 and PC2 (PC1, index of total variation of overall size of hard parts; PC2, index that contrasts the copulatory tube (length); ventral anchor (outer length, inner root and outer root); dorsal anchor (inner length and outer root); and ventral and dorsal bar (width)).

vaginal tube was excluded from these analyses because the vaginal tube was only observed in five out of the ten *Ligophorus* species and the number of measurements taken was low due to poor visibility of the fine vaginal tube (table 3). The results of the four PCAs were presented in the form of scatterplots to view the important distinguishing characters as a two-dimensional PCA plot (see figs 3–6). The scattering of all the 373 individuals was indicated only in the PCA scatterplot resulting from all the 18 parameters (see fig. 3). In the scatterplots of the anchors, bars and copulatory organs, only the centroids (mean scores) for each of the ten clusters were given. This was done so that the respective diagnostic features could be included into the figures to aid in comparison (see figs 4–6). Horizontal and vertical barplots were given for the two principal component axes for all the scatterplots (see figs 3–6). The Euclidean distance between the centroids of each cluster in the four PCA scatterplots were determined in R (version 2.15.1; R Core Development Team, 2008) and tabulated in tables 4 and 5. The shortest Euclidean distance for the two new species and their nearest neighbouring species were summarized in table 6.

Numerical taxonomy (NT) analysis (Sneath & Sokal, 1973)

The procedures to collect and analyse morphological and metric data from *Ligophorus* species and to summarize the results in the form of dendrograms were given in Soo & Lim (2012). Briefly, a total of 60 character states representing the hard parts (of the haptor and copulatory organs) used in differential diagnoses have been identified, categorized and coded by Soo & Lim (2012). The two new species and previous 50 species were characterized based on the 60 character states (table available upon request). Next, a data matrix based on Jaccard's Index of Similarity was generated and a neighbour-joining method was used to summarize and cluster the 52 species in the form of dendrograms. The calculation of Jaccard's Index of Similarity and clustering were done in R (version 2.15.1; R Core Development

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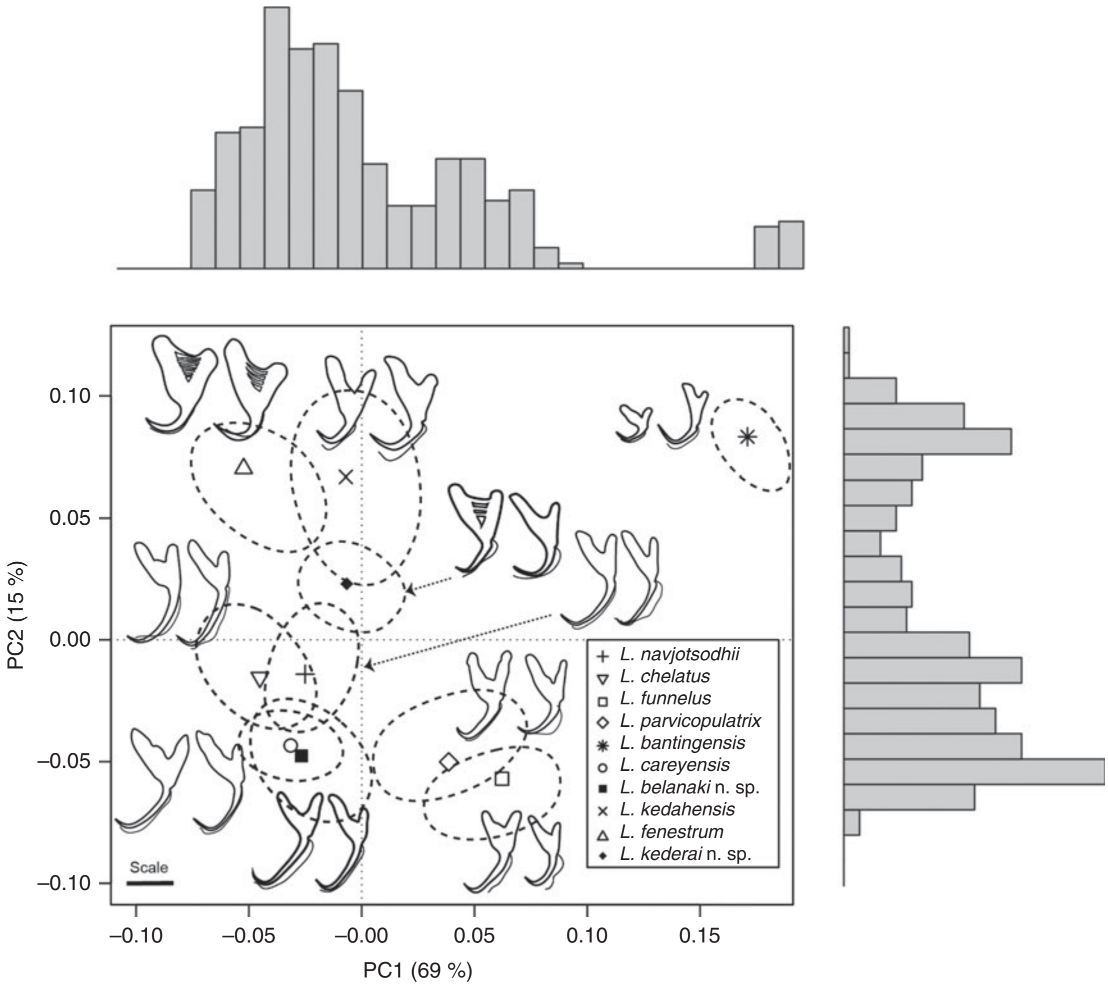

Fig. 4. Principal component analysis (PCA) scatterplot of the two new and eight known *Ligophorus* species based on the anchors, showing only the centroids for each *Ligophorus* species. Vertical and horizontal bar plots indicate one-dimensional summary of the principal component axes, PC1 and PC2 (PC1, index of total variation of overall size of anchors; PC2, index that contrasts the ventral anchor (inner length and outer length) and dorsal anchor (inner length, outer length and point)). Scale bar = 20 μm.

Team, 2008). In this study, six dendrograms have been constructed based on the characters states of all the five diagnostic features (anchors, bars, ventral bars only, AMP and copulatory organs) and on each of the five diagnostic features separately. Although six dendrograms were generated, only one dendrogram based on all the hard parts is presented in this paper (see fig. 9) for brevity. The results from this and the other five dendrograms were summarized in table 7.

Results

The results from the PCA (see figs 3–6) and from the six dendrograms generated from the NT analyses (see fig. 9) are summarized and tabulated (see tables 6 and 7). Since the results of the morphometric analyses are to facilitate differential diagnoses of the two new species, it is appropriate to discuss them prior to the descriptions of the two species to avoid excessive repetitions. In the differential diagnoses of the two new species, comparisons are only made with species which have the shortest Euclidean distance with the two new species in the PCA (see table 6) and with species that shared three or more similar characters with the new species in the NT analyses (see table 7). By doing this, we are able to limit our comparison to a manageable number of morphometrically and morphologically related species.

Clustering of Ligophorus *species using PCA*

The 373 specimens of *Ligophorus* (eight previously described and the two new species) from off Peninsular Malaysia are separated into ten clusters which correspond to the eight known and two new *Ligophorus* species in all the four PCA scatterplots (see figs 3–6). Based on the 18 parameters, the 30 specimens of *L. belanaki* n. sp. and the 25 specimens of *L. kederai* n. sp. are grouped closest to *L. careyensis* (see fig. 3; table 4). In this PCA scatterplot, the first principal component axis (PC1, *x*-axis), which accounts for 48% of the total variations, is an index of

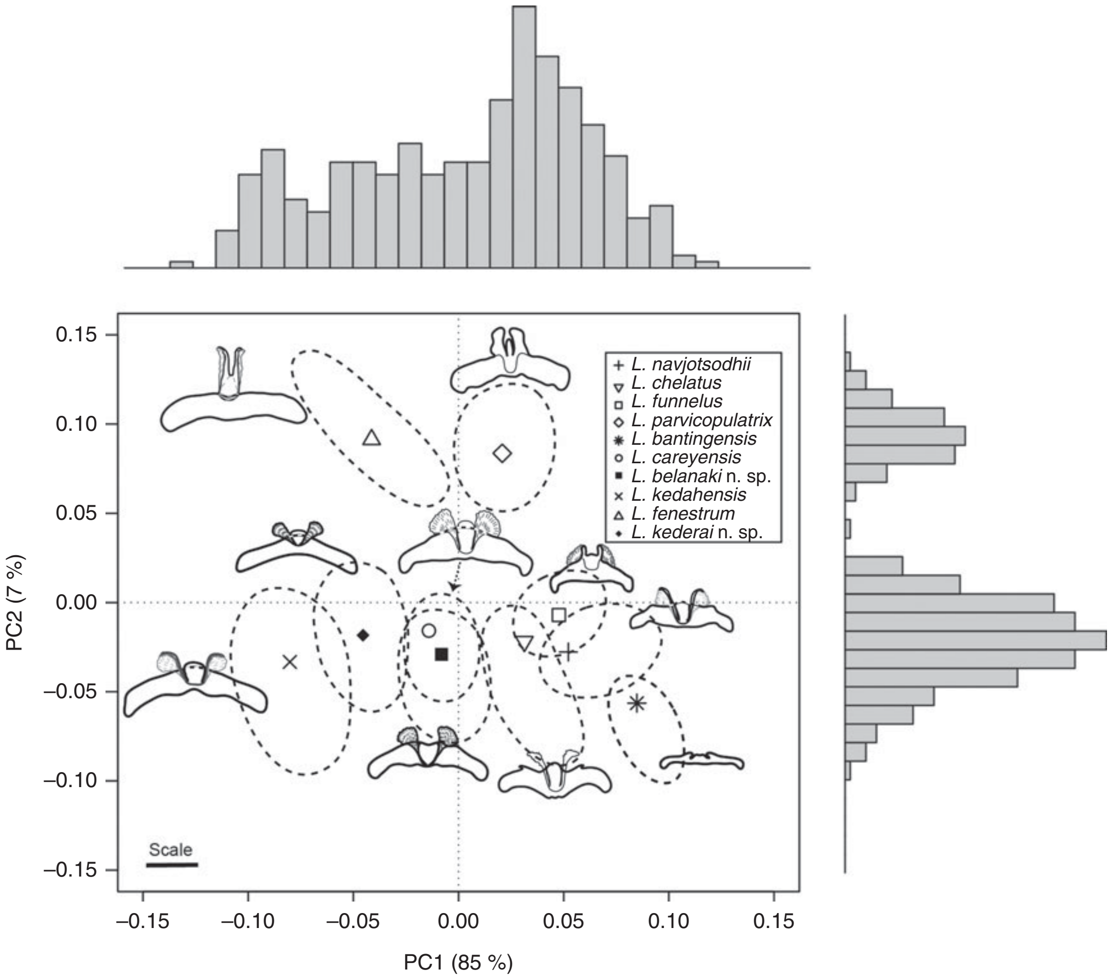

Fig. 5. Principal component analysis (PCA) scatterplot of the two new and eight known *Ligophorus* species based on the ventral bar, showing only the centroids for each *Ligophorus* species. Vertical and horizontal bar plots indicate one-dimensional summary of the principal component axes, PC1 and PC2 (PC1, index of total variation of overall size of ventral bars; PC2, index that contrasts the ventral bar width and AMP width). Scale bar = 20 μm.

the overall size of all the hard parts and separates the 373 individuals into six groups as shown in the horizontal bar plot (see fig. 3). The second principal component (PC2, *y*-axis), which explains 27% of the total variation, is an index that contrasts the copulatory tube (length); ventral anchor (outer length, inner root and outer root); dorsal anchor (inner length and outer root); ventral and dorsal bar (width) against the other parameters, separating the *Ligophorus* specimens into four groups (see fig. 3). In the three PCA scatterplots based on the anchors, ventral bar and copulatory organ, *L. belanaki* n. sp. is clustered closest to *L. careyensis* (see figs 4–6; tables 4 and 5). *Ligophorus kederai* n. sp. is clustered closest to *L. kedahensis* in the PCA scatterplot based on the anchors (see fig. 4; table 4), to *L. careyensis* in the PCA scatterplot of the ventral bar (see fig. 5; table 5) and to *L. funnelus* in the PCA scatterplot of the copulatory organ (see fig. 6; table 5). The information from the four PCA scatterplots is summarised in table 6 and will be used in the differential diagnosis of the two new *Ligophorus* species (see Descriptions and figs 1, 2, 7, 8).

Clustering of Ligophorus *species using NT analysis*

In the dendrograms generated in the NT analyses, *L. belanaki* n. sp. and *L. kederai* n. sp. are separated into different groups based on the morphology of their hard parts. Based on the dendrogram resulting from the use of all characters, the 50 known and two new *Ligophorus* species are grouped into four main clusters at 60% similarity level and into 16 groups at the 95% similarity level (see fig. 9; table 7). When only the anchors are used, 10 groups are generated, 17 groups when the two bars are used, 11 groups when only the ventral bar (inclusive of shape and size of AMP) is used, 9 groups when the shape of AMP is used and12 groups when the accessory piece is used (table 7). Although *L. belanaki* n. sp. and *L. kederai* n. sp. are similar to four and four known *Ligophorus* species,

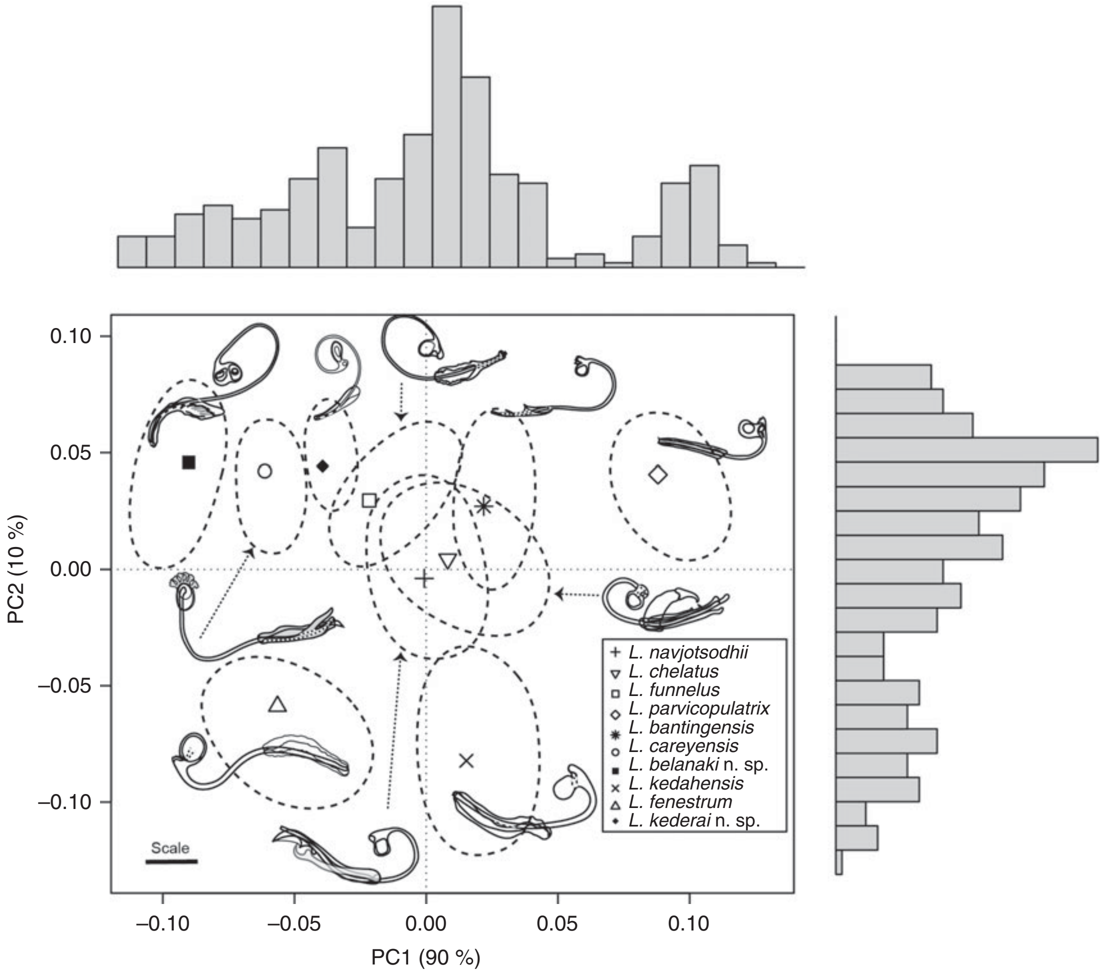

Fig. 6. Principal component analysis (PCA) scatterplot of the two new and eight known *Ligophorus* species based on the copulatory organ, showing only the centroids for each *Ligophorus* species. Vertical and horizontal bar plots indicate one-dimensional summary of the principal component axes, PC1 and PC2 (PC1, index of total variation of overall size of copulatory organ; PC2, index that contrasts the copulatory tube length). Scale bar = 20 μm.

respectively, at the 95% level of similarity using all hard parts, they are different in the detailed structures of the bars and male copulatory organ (see Descriptions, fig. 9 and table 7).

Descriptions

Ligophorus belanaki n. sp

Type host. *Liza subviridis* Valenciennes, 1836.

Type locality. Off Carey Island, Banting, Malaysia (2°51′N, 101°22′E).

Type specimens. Holotype NHMUK 2013.8.16.1 and three paratypes NHMUK 2013.8.16.2–2013.8.16.4 in the Natural History Museum, London; 28 paratypes in the University of Malaya collection MZUM(P)2013.327(P)–2013.354(P).

Materials studied. Thirty-two specimens studied and 30 specimens measured.

Etymology. This species is named after 'belanak', the Malaysian name of the fish host, *Liza subviridis*.

Description. Figures 1A–H, 2A, B. Body elongate, 586 (353–782) × 108 (65–153), 3 pairs of head organs, 2 pairs pigmented eye spots, posterior with lenses and bigger than anterior pair. Mouth subterminal, ventral; pharynx ovoid, 28 (24–34) × 29 (24–35); intestine bifurcates posterior to pharynx, rejoins posterior to testis and anterior to peduncle forming cyclocoel. Haptor well demarcated; size 87 (61–110) × 106 (61–147); 14 larval type marginal hooks, similar, length 11 (9–13); two pairs of anchors; 2 dorsal anchors, inner length 35 (31–38), outer length 33 (31–36), inner root 14 (10–18), outer root 7 (5–11), point 6 (3–8); 2 ventral anchors, inner length 36 (30–41), outer length 40 (33–44), inner root 14 (10–16), outer root 10 (6–13), point 7 (5–9); 2 connecting bars: V-shaped dorsal bar, 37 (30–42) × 6 (4–7); ventral bar inverted V-shape, 41 (36–44) × 6 (4–7), AMP consists of two membranous lateral pieces and a flattened median piece, distance between lateral pieces, 9 (6–11). Testis single, elongate, ovoid, comparatively small, postero-dorsal to ovary, spermatozoa observed in anterior region;

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Table 4. Data matrix of Euclidean distances between PCA centroids of *Ligophorus* species from Peninsular Malaysia containing (a) the Euclidean distances between PCA centroids of *Ligophorus* species based on all hard parts (see fig. 3) and (b) the Euclidean distances between PCA centroids of *Ligophorus* species based on anchors (see fig. 4); the shortest Euclidean distances are in bold.

		Euclidean distances based on all hard parts									
	Species	*L. navjotsodhii*	*L. chelatus*	*L. funnelus*	*L. parvicopulatrix*	*L. bantingensis*	*L. careyensis*	*L. belanaki* n. sp.	*L. kedahensis*	*L. fenestrum*	*L. kederai* n. sp.
Euclidean distances based on anchors	*L. navjotsodhii*	0	0.021	0.017	0.112	0.100	0.058	0.078	0.113	0.120	0.067
	L. chelatus	8.689	0	0.037	0.101	0.115	0.056	0.081	0.092	0.103	0.051
	L. funnelus	37.861	46.502	0	0.117	0.085	0.070	0.087	0.128	0.137	0.083
	L. parvicopulatrix	27.507	36.139	10.365	0	0.136	0.155	0.181	0.100	0.157	0.127
	L. bantingensis	84.928	93.575	48.654	58.509	0	0.154	0.166	0.195	0.218	0.155
	L. careyensis	4.236	5.956	40.981	30.623	88.541	0	**0.027**	0.113	0.087	**0.045**
	L. belanaki n. sp.	3.312	8.023	38.901	28.548	86.553	**2.112**	0	0.137	0.102	0.069
	L. kedahensis	11.107	18.352	32.061	22.427	76.555	15.325	14.215	0	0.066	0.069
	L. fenestrum	14.137	8.686	50.701	40.534	95.992	13.564	15.226	19.446	0	0.053
	L. kederai n. sp.	8.264	16.547	31.141	21.026	77.176	12.429	10.943	**4.285**	19.561	0

Table 5. Data matrix of Euclidean distances between PCA centroids of *Ligophorus* species from Peninsular Malaysia containing (a) the Euclidean distances between PCA centroids of *Ligophorus* species based on ventral bars (see fig. 5) and (b) the Euclidean distances between PCA centroids of *Ligophorus* species based on male copulatory organ (see fig. 6); the shortest Euclidean distances are in bold.

		Euclidean distances based on ventral bars									
	Species	*L. navjotsodhii*	*L. chelatus*	*L. funnelus*	*L. parvico-pulatrix*	*L. bantin-gensis*	*L. careyensis*	*L. belanaki* n. sp.	*L. kedahensis*	*L. fenestrum*	*L. kederai* n. sp.
Euclidean distances based on male copulatory organ	*L. navjotsodhii*	0	8.708	2.579	16.312	13.530	26.917	24.410	52.959	38.975	39.314
	L. chelatus	4.103	0	7.170	10.674	22.185	18.213	15.737	44.283	30.569	30.617
	L. funnelus	9.651	13.310	0	13.777	15.639	25.250	22.851	51.365	36.836	37.663
	L. parvicopulatrix	39.924	35.848	48.799	0	28.929	17.276	16.077	42.054	24.840	28.572
	L. bantingensis	10.610	6.520	19.298	29.524	0	40.325	37.743	66.232	52.462	52.674
	L. careyensis	27.322	31.161	17.890	66.625	37.170	0	**2.793**	26.123	14.634	**12.414**
	L. belanaki n. sp.	39.975	43.864	30.624	79.369	49.913	**12.745**	0	28.550	17.314	14.931
	L. kedahensis	10.262	8.959	19.314	34.877	11.021	35.965	48.222	0	19.837	13.736
	L. fenestrum	24.954	29.057	17.481	64.796	35.547	10.135	18.313	31.396	0	19.785
	L. kederai n. sp.	17.377	21.087	**7.778**	56.432	26.994	10.194	22.937	26.632	12.508	0

Table 6. The clustering of the two new *Ligophorus* species with known *Ligophorus* species from Peninsular Malaysia based on PCA (principal component analysis); numbers in brackets are the shortest Euclidean distances between the new species and its closest neighbouring species based on tables 4 and 5.

	Parameters used			
Species	All hard parts	Anchors	Ventral bar	Copulatory organ
L. belanaki n. sp.	*L. careyensis* (0.027)	*L. careyensis* (2.112)	*L. careyensis* (2.793)	*L. careyensis* (12.745)
L. kederai n. sp.	*L. careyensis* (0.045)	*L. kedahensis* (4.285)	*L. careyensis* (12.414)	*L. funnelus* (7.778)

vas deferens leaves anterior region of testis, to sinistral-ventral side, ascends intercaecally, distending, forming seminal vesicle, narrows as vas efferens to enter into smaller lobe of bilobed initial part of copulatory tube. Single elongate gourd-shaped prostatic reservoir with prostatic duct leaving reservoir to enter bigger lobe of bilobed initial of copulatory tube. Copulatory organ consists of copulatory tube, length 95 (83–111) with bilobed initial part and simple funnel-like groove accessory piece, 25 (20–35). Ovary J-shaped, elongate with recurved posterior region; oviduct arises from anterior part of ovary, continues anteriorly as ootype surrounded by Mehlis' gland; proceeds anteriorly as uterus to open near male copulatory organ. Sclerotized vaginal opening, median, at level of recurved portion of ovary; vaginal tube thin, long, sclerotized, 70 (64–78) ($n = 10$ stained specimens) leading to sperm-filled ovoid seminal receptacle at midbody, dorsal to ovary, duct from seminal receptacle enters oviduct. Vitellarium in lateral fields approximately co-extensive with intestinal caeca, confluent just posterior to intestinal bifurcation, just anterior to ovary and just posterior to cyclocoel.

Differential diagnosis. *Ligophorus belanaki* n. sp. is similar to *L. careyensis* in having ventral and dorsal anchors with long inner lengths and short points, narrow V-shape dorsal bars with shoulders and curved ventral bars (table 3). However, they are different in the length of the vaginal tube; 70 (64–78) μm in *L. belanaki* n. sp. and 36 (32–40) μm in *L. careyensis* (table 3) and in the morphology of the copulatory organ and median piece of AMP: ornamented bilobed initial part with two opposing accessory pieces and a raised median piece in the AMP of *L. careyensis* (Soo & Lim, 2012) compared to a non-ornamented bilobed initial part with funnel-shaped groove accessory piece and flat median piece in the AMP of *L. belanaki* n. sp. (figs 1E1, G, 2A, B). *Ligophorus belanaki* n. sp. is clustered closest to *L. careyensis* in the PCA scatterplots for all 18 parameters (fig. 3, table 4) and in the PCA scatterplot for the anchors and ventral bar (figs 4 and 5, tables 4 and 5), thus confirming our observation that the two species have similar anchors and bars (table 6). In the PCA scatterplot for the copulatory organ, *L. belanaki* n. sp. is also grouped closest to *L. careyensis* (fig. 6, table 5) based mainly on the copulatory tube length (table 3). Although *L. belanaki* n. sp., *L. funnelus* and *L. bantingensis* possess funnel-shaped accessory pieces, in the PCA scatterplot based on the male copulatory organ, *L. belanaki* n. sp. is not grouped with *L. funnelus* and *L. bantingensis* (fig. 6) because of the length of the copulatory tube, which is 95 (83–111) μm in *L. belanaki* n. sp., 77 (64–85) μm in *L. funnelus* and 67 (59–79) μm in *L. bantingensis* (table 3).

In the NT analyses, *L. belanaki* n. sp. is grouped with three species, *L. surianoae*, *L. parvicirrus* and *L. minimus*, in having similar anchors, bars and AMP with two lateral membranous pieces and flat median piece (table 7). *Ligophorus belanaki* n. sp. is different from these species in having a male copulatory organ with funnel-shaped groove accessory piece (fig. 1G), compared to the grooved tubes with expanded distal parts in *L. minimus* and *L. parvicirrus* and the grooved tube with expanded proximal part in *L. surianoae*.

Ligophorus kederai n. sp.

Type host. *Valamugil buchanani* Bleeker, 1854.

Type locality. Off Langkawi Island, Kedah, Malaysia (6°21′N, 99°46′E).

Type specimens. Holotype NHMUK 2013.8.16.5 and one paratype NHMUK 2013.8.16.6 in the Natural History Museum, London; 23 paratypes in the University of Malaya collection MZUM(P)2013.37(P)–2013.52(P), MZUM(P)2013.55(P)–2013.59(P), MZUM(P)2013.239(P)–2013.240 (P).

Materials studied. Twenty-five specimens studied and 25 specimens measured.

Etymology. This species is named after 'kedera', the Malaysian name of the fish host, *Valamugil buchanani.*

Description. Figures 7A–F, 8A, B. Body elongate, 701 (569–831) × 160 (101–223), 3 pairs of head organs, 2 pairs pigmented eye spots, posterior with lenses and bigger than anterior pair. Mouth subterminal, ventral; pharynx size 48 (41–51) × 48 (43–51). Haptor well demarcated, size 84 (70–97) × 106 (69–151); 14 larval type marginal hooks, similar, length 11 (10–12); two pairs of anchors; 2 dorsal anchors, inner length 33 (32–36), outer length 30 (28–31), inner root 16 (14–18), outer root 9 (7–10), point 10 (9–12); 2 ventral anchors with fenestration, inner length 35 (34–39), outer length 35 (32–36), inner root 16 (15–17), outer root 11 (8–13), point 6 (5–8); 2 connecting bars: dorsal bar broad inverted U, 46 (41–50) × 6 (4–8); ventral bar inverted V, 46 (43–50) × 8 (6–11), AMP consists of two membranous lateral pieces and a raised median piece, distance between lateral pieces, 10 (8–12). Testis single, comparatively small, spermatozoa observed in anterior region; vas deferens leaves anterior region of testis on sinistral side, ascends intercaecally, distending forming seminal vesicle, narrows as vas efferens to enter

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Table 7. The clustering of the two new *Ligophorus* species with 50 known *Ligophorus* species based on numerical taxonomy (NT) analyses at 95% similarity level.

Species	All hard parts	Anchors	Bars (ventral and dorsal)	Ventral bar	AMP (antero-median protuberance)	Accessory piece	Species occurring in three or more NT analyses with new species
L. belanaki n. sp.	*L. cheleus, L kaohsianghsieni, L. chenzhenensis, L. surianoae*	*L. surianoae, L. campanulatus, L. simpliciformis, L. bykhowskyi, L. careyensis, L. chelatus, L. navjotsodhii, L. uruguayense, L. saladensis, L. pilengas, L. parvicirrus, L. pacificus, L. mugilinus, L. minimus, L. mediterraneus, L. macrocolpos, L. llewelyni, L. imitans, L. huitrempe, L. heteronchus, L. euzeti, L. ellochelon, L. chongmingensis, L. cheleus, L. chabaudi, L. cephali, L. acuminatus, L. kederai* n. sp.	*L. parvicirrus, L. minimus*	*L. surianoae*	*L. surianoae, L. bipartitus, L. zhangi, L. navjotsodhii, L. vanbenedenii, L. uruguayense, L. saladensis, L. pilengas, L. parvicirrus, L. mediterraneus, L. minimus, L. macrocolpos, L. llewellyni, L. imitans, L. heteronchus, L. cheleus, L. chenzhenensis, L. euzeti*	*L. funnelus, L. bantingensis*	*L. surianoae, L. parvicirrus, L. minimus*
L. kederai n. sp.	*L. kedahensis, L. fenestrum, L. bykhowskyi, L. leporinus*	*L. surianoae, L. campanulatus, L. simpliciformis, L. bykhowskyi, L. careyensis, L. chelatus, L. navjotsodhii, L. uruguayense, L. saladensis, L. pilengas, L. parvicirrus, L. pacificus, L. mugilinus, L. minimus, L. mediterraneus, L. macrocolpos, L. llewellyni, L. imitans, L. huitrempe, L. heteronchus, L. euzeti, L. ellochelon, L. chongmingensis, L. cheleus, L. chabaudi, L. cephali, L. acuminatus, L. belanaki* n. sp.	*L. kedahensis, L. tainhae, L. guanduensis*	*L lebedevi, L. mamaevi, L. campanulatus, L. simpliciformis, L. chelatus, L. domnichi, L. chabaudi, L. careyensis*	*L. lebedevi, L. mamaevi, L. campanulatus, L. simpliciformis, L. bykhowskyi, L. kedahensis, L. chelatus, L. tainhae, L. pacificus, L. mugilinus, L. careyensis*	*L. bipartitus, L. simpliciformis, L. zhangi, L. bykhowskyi, L. fenestrum, L. kedahensis, L. parvicopulatrix, L. llewellyni, L. lizae, L. leporinus, L. hamulosus*	*L. bykhowskyi, L. simpliciformis, L. kedahensis, L. campanulatus, L. chelatus, L. careyensis*

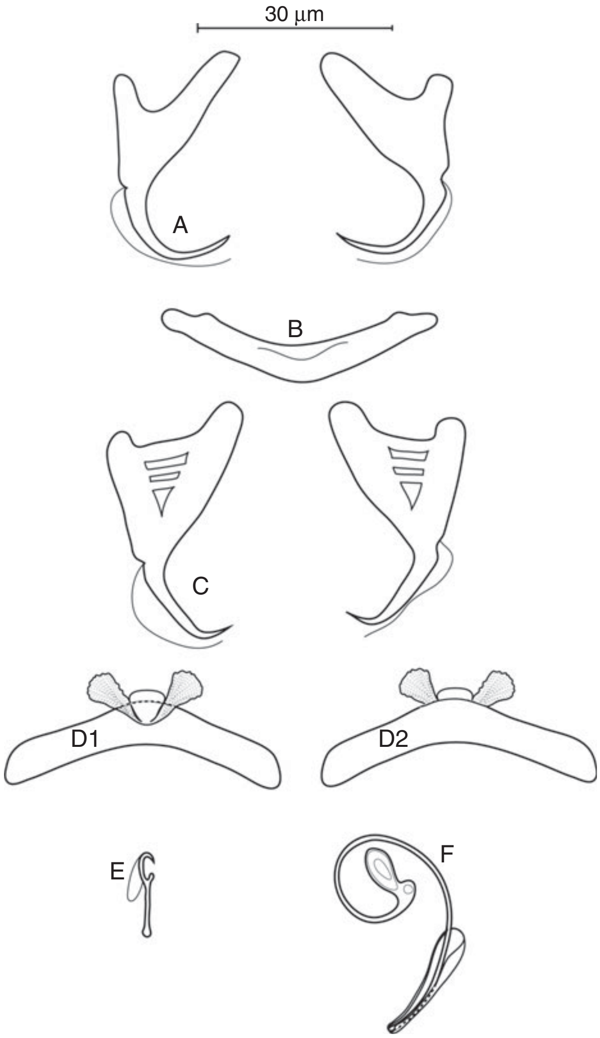

Fig. 7. Sclerotized hard parts of *Ligophorus kederai* n. sp.: (A) dorsal anchors; (B), dorsal bar; (C) ventral anchors; (D1), ventral bar (ventral view); (D2), ventral bar (dorsal view); (E) marginal hook; and (F) male copulatory organ. Illustrated using a digitizing tablet (WACOM) and Adobe Illustrator software.

into smaller lobe of bilobed initial part of copulatory tube. Single prostatic reservoir with prostatic duct entering bigger lobe of bilobed initial part of copulatory tube. Male copulatory organ consists of copulatory tube, length 83 (79–87) with bilobed initial part and a simple grooved boat-like accessory piece, 24 (21–27). J-shaped ovary elongate with recurved posterior region; oviduct arises from anterior region of ovary, continues anteriorly as ootype surrounded by Mehlis' gland; proceeds anteriorly as uterus to open near male copulatory organ. Vaginal opening and tube not observed. Sperm-filled ovoid seminal receptacle at midbody near ovary. Vitellarium in lateral fields approximately co-extensive with intestinal caeca, confluent just posterior to intestinal bifurcation, just anterior to ovary and just posterior to cyclocoel.

Differential diagnosis. *Ligophorus kederai* n. sp. has a male copulatory organ with simple grooved boat-like accessory piece (figs 7F and 8B), similar to the copulatory organ of *L. kedahensis* and *L. fenestrum*, which are from the same host, *V. buchanani* (see Soo & Lim, 2012). This new species is different in having only the ventral anchors fenestrated (figs 7C and 8A), compared to *L. fenestrum* where both the ventral and dorsal anchors are fenestrated and *L. kedahensis* with non-fenestrated anchors (see Soo & Lim, 2012). The shapes of the anchors in the two species are also different: *L. kederai* n. sp. has anchors with narrower base and longer points (fig. 7C) compared to broader base and shorter points in *L. fenestrum* (see Soo & Lim, 2012). The 25 specimens of *L. kederai* n. sp. are grouped closest to *L. careyensis* in the PCA scatterplot of all 18 parameters (fig. 3, table 4) and in the PCA scatterplot of the ventral bars (fig. 5, table 5). It differs from *L. careyensis* mainly in having fenestrated ventral anchors, slightly broader ventral bar (46 (43–50) µm) and male copulatory organ with simple boat-like accessory piece with non-ornamented initial part (fig. 7F, table 3) as compared to non-fenestrated ventral anchors, slightly narrower ventral bar (41 (34–45) µm) and male copulatory organ with two opposing accessory pieces and ornamented initial part in *L. careyensis* (Soo & Lim, 2012). In the PCA scatterplot of the anchors, *L. kederai* n. sp. is grouped closest to *L. kedahensis* (fig. 4, table 4), indicating that both these species have anchors with similar morphometries, but morphologically, *L. kederai* n. sp. is different in having fenestrated ventral anchors (fig. 7C). In the PCA scatterplot of the copulatory organ, *L. kederai* n. sp. is grouped closest to *L. funnelus* (fig. 6, table 5) based on the copulatory tube length. *Ligophorus kederai* n. sp. is different from *L. funnelus* in having a simple boat-like accessory piece compared to funnel-shape accessory piece in *L. funnelus*.

In the NT analyses, *L. kederai* n. sp. is grouped with six species, *L. bykhowskyi*, *L. simpliciformis*, *L. kedahensis*, *L. campanulatus*, *L. chelatus* and *L. careyensis*, in having similar AMP (with two lateral membranous pieces and a raised median piece) and anchors with short points ($\leq 10\,\mu m$) and long inner lengths ($\geq 30\,\mu m$) (table 6). *Ligophorus kederai* n. sp. differs from these six species in having fenestrated ventral anchors (figs 7C and 8A, table 7).

Discussion

The type species of *Ligophorus*, *L. vanbenedenii* (Parona & Perugia, 1890) Euzet & Suriano, 1977 had been assigned and reassigned to different ancyrocephalid genera (*Tetraonchus*, *Ancyrocephalus*, *Haplocleidus* and *Haliotrema*) prior to its assignment as the type species of *Ligophorus* (tables 1 and 2) (see Soo & Lim, 2012). *Ligophorus vanbenedenii* has several characteristics (single prostatic reservoir, vas deferens not overlapping the right intestinal branch and vagina opening at the medio-ventral part of the body) which prompted its reassignment into the genus *Ligophorus* by Euzet & Suriano (1977). Taxonomically, *Ligophorus* has been assigned to Ancyrocephalidae Bychowsky, 1937 by the majority of the researchers on *Ligophorus* (14 studies) and also to Ancyrocephalinae Bychowsky, 1937 in Dactylogyridae Bychowsky, 1933 by others (four studies). The 14 studies which ascribed *Ligophorus* to Ancyrocephalidae are Euzet & Suriano

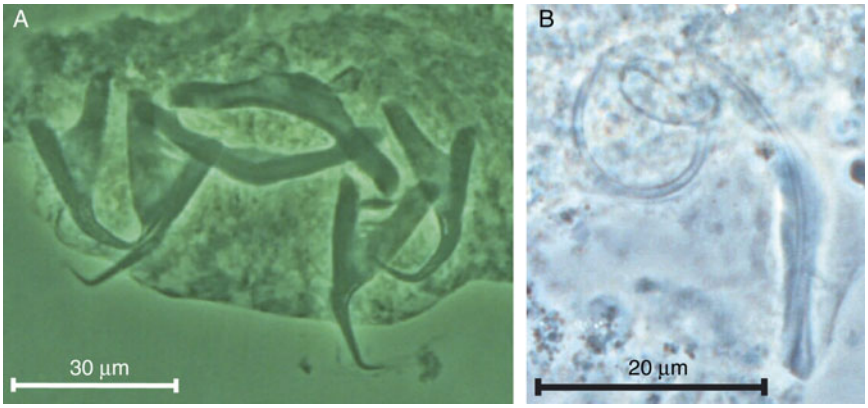

Fig. 8. Photomicrographs of sclerotized hard parts of *Ligophorus kederai* n. sp., captured using Leica digital camera and an image analysis software (QWin Plus): (A) anchors and bars (B) male copulatory organ.

(1977), Euzet & Sanfilippo (1983), Dmitrieva & Gerasev (1996), Mariniello *et al.* (2004), Sarabeev & Balbuena (2004), Sarabeev *et al.* (2005), Balbuena *et al.* (2006), Dmitrieva *et al.* (2007), Dmitrieva *et al.* (2009), Marcotegui & Martorelli (2009), Siquier & Owtrowski de Nunez (2009), Dmitrieva *et al.* (2012), Soo & Lim (2012) and Dmitrieva *et al.* (2013a). The other four studies which ascribed *Ligophorus* to Ancyrocephalinae in Dactylogyridae are Rubtsova *et al.* (2006), Rubtsova *et al.* (2007), Abdallah *et al.* (2009) and Blasco-Costa *et al.* (2012). The validity of Ancyrocephalidae and Ancyrocephalinae in Dactylogyridae is a longstanding issue and has been elaborated by Lim *et al.* (2001) and summarized herein. Briefly, to resolve the heterogeneity of the Ancyrocephalidae and the paraphyletic nature of the Ancyrocephalidae, Kritsky & Boeger (1989) proposed two options for revising the Ancyrocephalidae and favoured the one considering the Ancyrocephalidae as a junior synonym of the Dactylogyridae. Lim (1998), however, disagreed with this move, while recognizing the paraphyletic nature of Ancyrocephalidae, and proposed that the Ancyrocephalidae be left intact within the Dactylogyridea until further studies have been carried out. In this paper we will be retaining *Ligophorus* in Ancyrocephalidae until more intensive molecular and morphological studies of the different ancyrocephalid genera are done.

The two new species, *L. belanaki* n. sp. and *L. kederai* n. sp., bring the total number of *Ligophorus* species described off Peninsular Malaysia to ten species, and to 52 species globally. *Ligophorus belanaki* n. sp. and *L. kederai* n. sp. were collected from *Liza subviridis* and *Valamugil buchanani*, respectively. To date *L. subviridis* harbours seven species (six known species and *L. belanaki* n. sp.) and *V. buchanani* harbours three species (two known species and *L. kederai* n. sp.) (see Soo & Lim, 2012).

As already noted, the ventral bar of *Ligophorus* is basically an inverted V-shape bar with an antero-median protuberance (AMP) consisting of a median piece and two lateral membranous or non-membranous ear-like processes arising from the median piece (see Soo & Lim, 2012) and has been shown to be an important diagnostic feature for *Ligophorus* species (fig. 5, tables 4 and 5). The AMP of *L. belanaki* n. sp. and *L. kederai* n. sp. consists of two lateral membranous pieces but they differ in the morphology of the median piece, which is flat in *L. belanaki* n. sp. (figs 1E1, 1E2, 2A and 5) and raised in *L. kederai* n. sp. (figs 7D1, D2, 8A and 5).

To date, amongst the *Ligophorus* species, fenestrated anchors only occur in two described and one unnamed species: *L. fenestrum* (dorsal and ventral anchors) (Soo & Lim, 2012) and *L. kederai* n. sp. (ventral anchors) (figs 7C and 8A) found on *V. buchanani* off Langkawi Island, and *Ligophorus* sp. 14 (ventral anchors) from *Liza affinis* off North Vietnam (South China Sea) (Dmitrieva *et al.*, 2013b). The fenestrations in all three species are different: in *L. fenestrum* and *L. kederai* n. sp. fenestrations are made up of three or more slit-like apertures, compared to the single aperture in *Ligophorus* sp. 14. Fenestration in anchors is not limited to these *Ligophorus* species or to any particular group of monogeneans and can be found in species from different genera, such as in most *Thaparocleidus* species from pangassids (single fenestrum in ventral anchors) (see Pariselle *et al.*, 2001a, b, 2002, 2003, 2004, 2005a, b, 2006) and in *Haliotrema banana* Lim & Justine, 2007 (single fenestrum in dorsal and ventral anchors) (see Lim & Justine, 2007). The occurrences of fenestrated anchors in a few species of unrelated genera seem to suggest that fenestration in anchors is of specific importance rather than generic importance in monogeneans. Despite the presence of fenestrated anchors, *L. fenestrum* and *L. kederai* n. sp. are valid *Ligophorus* species possessing characteristics of *Ligophorus*, such as a vas deferens which does not overlap the intestinal caecum, male copulatory organ with bilobed initial part, J- to U-shaped ovary, seminal receptacle within the curve of the J- to U-shaped ovary and ventral bar with AMP. Hence there is no justification to erect a new genus to accommodate the *Ligophorus* species with fenestrated anchors. Although *Ligophorus* species are specific to mugilids, currently we have too few *Ligophorus* species with fenestrated anchors to conclude whether they are restricted to specific mugilid host species or not. The fact that *L. fenestrum* and *L. kederai* n. sp. are from *V. buchanani* might suggest close relationships of the two species caused by speciation within the host species. However, it should be noted that *V. buchanani* is also host to *L. kedahensis* which has non-fenestrated anchors (Soo & Lim, 2012). The relationship of *L. fenestrum*, *L. kederai* n. sp. and *Ligophorus* sp. 14 is

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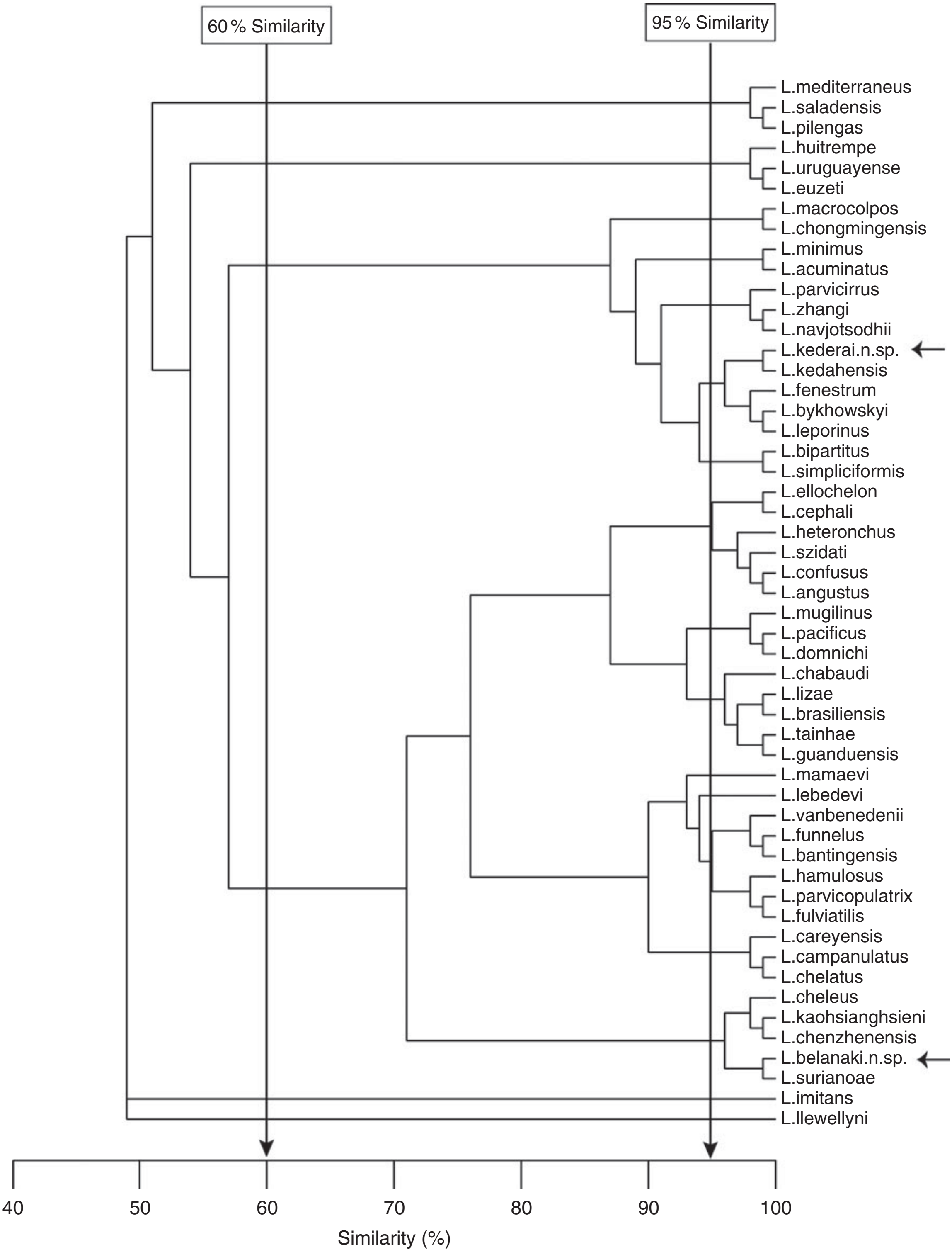

Fig. 9. Dendrogram of 52 *Ligophorus* species based on characteristics of all hard parts (anchors, bars and copulatory organ). The arrows on the far right indicate the positions of the new species (*L. belanaki* n. sp. and *L. kederai* n. sp.).

difficult to explain since the host of the latter species is *L. affinis*. The ecological advantage or disadvantage of fenestrations in anchors is difficult to speculate. Fenestration is probably caused by a 'reduction' in sclerotized materials ('scleroporosis') in a specific area, forming holes or windows or fenestrum or apertures. However, without any knowledge of the developmental history of these *Ligophorus* species, in particular that of the anchors, it is difficult even to speculate how these holes are formed and their function(s), if any.

The copulatory organ of *L. belanaki* n. sp. and *L. kederai* n. sp. consists of a copulatory tube with bilobed initial part and accessory piece (figs 1G, 2B, 7F and 8B). The bilobed initial part is a common feature in all the 52 *Ligophorus* species. The bilobed initial part of the copulatory tube of *L. belanaki* n. sp. and *L. kederai* n. sp. lacks ornamentation, bringing the number of *Ligophorus* species without ornamentation to 28 species, and 24 species with ornamentation. Of the ten *Ligophorus* species from Malaysia, only *L. careyensis* possesses ornamentation (see Soo & Lim, 2012) (fig. 6). In the majority of *Ligophorus* species, the accessory piece is not connected to the initial part of the male copulatory organ by any structure. To date only six *Ligophorus* species – *L. angustus*, *L. cephali*, *L. confusus*, *L. ellochelon*, *L. heteronchus* and *L. szidati* – were described and illustrated as having a male copulatory organ with a muscular sheath surrounding the copulatory tube, attaching at its distal end to the accessory piece and at its proximal end to the initial part of the male copulatory organ. It should be noted that such a muscular sheath is lacking in the other 46 *Ligophorus* species, including these two new species and all the eight known *Ligophorus* species on Malaysian mugilids.

Although not illustrated, the soft anatomical structures of the male and female reproductive parts of *L. kederai* n. sp. are similar to those of *L. belanaki* n. sp. and other known *Ligophorus* species (see Soo & Lim, 2012), in having the testis situated postero-dorsal to the ovary, vas deferens traversing intercaecally along the sinistral-ventral side, J-shaped ovary, vaginal opening (if observed) located at midbody near the J-ovary and a seminal receptacle located at the recurved part of the ovary. The generic characteristics of *Ligophorus* were emended in Soo & Lim (2012) to include the fact that the ovary is J- to U-shaped and that the vaginal system is not obvious in all members of the genus if not sclerotized.

Ligophorus belanaki n. sp. is observed to have a sclerotized vaginal tube (fig. 1H) which brings the number of *Ligophorus* species with sclerotized vaginal tubes to 39. A sclerotized vaginal tube was not observed in the present 25 specimens of *L. kederai* n. sp. and, to date, there are 12 other species without obvious vaginal tubes (*L. acuminatus*, *L. fluviatilis*, *L. leporinus*, *L. minimus*, *L. parvicirrus*, *L. navjotsodhii*, *L. kedahensis*, *L. fenestrum*, *L. bykhowskyi*, *L. simpliciformis*, *L. zhangi*, *L. bipartitus*). *Ligophorus parvicopulatrix* from *L. subviridis* has no obvious vaginal tube but it has a large sclerotized vaginal opening (see Soo & Lim, 2012). The vaginal system of *Ligophorus* (when observed) consists of a sclerotized vaginal pore, undulating to a coiled vaginal tube and obvious seminal receptacle. The vaginal system is not easily observed in non-stained specimens.

Previous and present studies have shown that the use of analytical methods in taxonomic studies is becoming increasingly common (Rubtsova *et al.*, 2006, 2007; Dmitrieva *et al.*, 2007, 2012; Tan *et al.*, 2010; Soo & Lim, 2012). Multivariate analyses (PCA and NT) can be used to facilitate comparative diagnoses because of rising numbers of species being described, especially between species which are morphologically and morphometrically similar (see Soo & Lim, 2012) and also to distinguish between inter- and intra-specific differences (Tan *et al.*, 2010). PCA could separate species and detect morpho-variants, but one has to bear in mind that PCA requires data from large numbers of specimens (see Tan *et al.*, 2010; Soo & Lim, 2012) for well-defined clusters to be generated. Hence metrical analyses (such as PCA and NT) are only complementary and supplementary in assisting in the clustering of species, thereby reducing the number of species for comparison to a manageable level. Such analytical tools are not obligatory tools in taxonomy, since it is highly unrealistic to require taxonomists to measure hundreds of specimens for purely taxonomic investigations. It should be noted that although metric analyses will provide a more objective comparison of new and known species, the morphometric data used do not take shape information into consideration. This is elaborated in fig. 6 where *Ligophorus* species with funnel-shape accessory piece (*L. belanaki* n. sp., *L. bantingensis* and *L. funnelus*) are clustered separately, while *L. belanaki* n. sp. and *L. careyensis* are clustered closer, based mainly on the copulatory tube length, although their accessory pieces and initial parts are morphologically different: funnel-shaped groove accessory piece and unornamented initial part in *L. belanaki* n. sp. (fig. 1G) and two opposing accessory pieces with ornamented initial part in *L. careyensis* (see Soo & Lim, 2012). The same is observed for the anchors and ventral bars (figs 4 and 5). Since morphometric data do not include morphological shapes, which are important taxonomic characteristics, geometric morphometry (Adams *et al.*, 2004; Vignon & Sasal, 2010) could be a potentially relevant analytical tool in differential diagnosis, and this is currently being explored.

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Conflict of interest

None.

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