

**BRAVOHOLLISIA GERUTI, NEW SPECIES (MONOGENEA: ANCYROCEPHALIDAE)
FROM POMADASYS HASTA (OSTEICHTHYES: HAEMULIDAE)
OF PENINSULAR MALAYSIA**

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ABSTRACT. — *Bravohollisia geruti*, new species, has been collected from *Pomadasys hasta* (Bloch) off Pulau Ketam, Peninsular Malaysia. This new species is very similar to *B. kritskyi* Lim, 1995 in the overall size of their anchors and structure of the associated net-like secretions but are different in terms of the detailed shape of anchors, and shape and size of male copulatory organ and connective bars. It also differs from the other six known *Bravohollisia* species in the shape and size of the anchors, male copulatory organ and connective bars. Principal component analysis (PCA) of the morphometric data of the male copulatory organ and connective bars of the four known and one new *Bravohollisia* species from off Peninsular Malaysia also show that this new species is different from the four known *Bravohollisia* species based on these two features.

KEY WORDS. — *Bravohollisia*, Ancyrocephalidae, *Pomadasys hasta*, Haemulidae, Malaysia

INTRODUCTION

To date there are seven species of *Bravohollisia* Bychowsky & Nagibina, 1970. Of these, five (*B. gussevi* Lim, 1995, *B. kritskyi* Lim, 1995, *B. magna* Bychowsky & Nagibina, 1970, *B. reticulata* Lim, 1995, and *B. rosetta* Lim, 1995) could be found on *Pomadasys hasta* (Bloch) obtained off Peninsular Malaysia (Lim, 1995) whilst *B. pomadasis* Bychowsky & Nagibina, 1970 and *B. tecta* Bychowsky & Nagibina, 1970 were from *P. maculatus* (Bloch) and *P. argenteus* (Forsskål), respectively, off Hainan (Table 1). *B. magna* was originally described from *P. argenteus* and *P. hasta* off Hainan. Besides these five *Bravohollisia* species, *P. hasta* also harbours four species of *Caballeria* Bychowsky & Nagibina, 1970 (*C. intermedius* Lim, 1995, *C. liewi* Lim, 1995, *C. pedunculata* Bychowsky & Nagibina, 1970, and *C. robusta* Bychowsky & Nagibina, 1970) (Lim, 1995).

When monogeneans specimens from an investigation on the distribution of monogeneans (2000 to 2002 and 2012) on *Pomadasys hasta* from off Pulau Ketam were analysed, we recovered three species of *Caballeria*, viz. *C. liewi*, *C. intermedius* and *C. pedunculata*, four known species of *Bravohollisia* (*B. rosetta*, *B. reticulata*, *B. gussevi*, *B. kritskyi*), and a new *Bravohollisia* species which is given herein. To provide an objective comparison of the new and known species of *Bravohollisia*, morphometric data

of the *Bravohollisia* species collected in this study were analysed using principal component analysis (PCA) and results incorporated in the differential diagnosis of the new species. PCA has been used to differentiate morphometric variants (Tan et al., 2010). Such analysis (PCA) is not usually incorporated in taxonomic description but it was used here because we were able to measure many specimens which we have collected for our ecological studies (see Soo & Lim, 2012).

MATERIAL AND METHODS

Forty-two specimens of *Pomadasys hasta* were obtained from a marine cage culture farm off Pulau [=Island] Ketam, Peninsular Malaysia (6°24'N; 100°7'E) (from 2000 to 2002 and 2012) and examined for monogeneans. Monogeneans were removed from the gills and prepared for investigations of their soft and hard parts (as in Lim, 1991). Briefly the specimens collected were flattened to varying degrees to best expose the soft anatomical structures and fixed and cleared in modified ammonium picrate glycerine (Lim, 1991) and later made into permanent mounts after dehydration through increasing concentration of ethanol and mounted in Canada balsam (as in Lim, 1994). Stained specimens (in Gomori's triple stain) were also prepared from specimens fixed in modified ammonium picrate glycerine (as in Lim, 2006) or

in alcohol formalin acetic acid (AFA) (as in Lim & Gibson, 2010). The specimens were examined using both bright field and phase contrast optics. Images of the hard parts were captured using a Leica digital camera (Leica DFC320) and drawn using a digitising tablet (WACOM) and Adobe Illustrator software (Figs. 1, 2).

Measurements of the sclerotised hard-parts (both haptoral and reproductive) were made on flattened specimens cleared in ammonium picrate glycerine. The basic measurements taken using Leica image analysis software (QWin Plus) as indicated in Fig. 1B, C, E & F, are summarised and given as the mean and range (within parentheses) in micrometres (μm) in Table 2 and used in the description of the new species. Table 2 also includes the morphometric measurements of the sclerotised hard parts of the seven known *Bravohollisia* species from Lim (1995) and Bychowsky & Nagibina (1970). The illustrations given in the original publications were measured whenever morphometric data were not available in the description of the species (see Table 2). The discrepancies noted in the point length in the present study and in Lim (1995) (see Table 2) are due to the way the point length is measured in the two studies as shown in Fig. 1B (cf. pt and ptl). The morphometric data obtained (stored in excel sheet) were analysed using principal component analysis (PCA). Type-specimens were deposited at the Zoological Reference Collection, Raffles Museum of Diversity Research, National University of Singapore, Singapore (ZRC), and Zoological Museum University of Malaya, Kuala Lumpur (MZUM).

Morphometric data analysis using principal component analysis in R. — Basic morphometric data (see Fig. 1B, C, E, F) are from the two dorsal and two ventral anchors [inner root length (ir), outer root length (or), inner length (il), outer length (ol) and point length (pt)], one dorsal and one ventral connective bar [length (bl) and width (bw)], marginal hook [length (ml)] as well as the male copulatory organ [initial length (cil), greatest width of initial part (woi) and total length (ctl)]. A total of 28 parameters are measured. These measurements are taken from 744 specimens belonging to five species of *Bravohollisia* viz. *B. rosetta* (150 specimens), *B. reticulata* (180 specimens), *B. gussevi* (150 specimens), *B. kritskyi* (150 specimens) and the new *Bravohollisia* species (114 specimens) from 42 *P. hasta*. The morphometric data of the anchors, bars and copulatory organ are analysed separately as well as together using principal component analysis (PCA) in R (Version 2.8.1; R Core Development Team, 2008). All the results from the PCA are presented as scatterplots (Figs. 3–6). Biplot is also generated to determine the main morphological character distinguishing the different species (Fig. 7). The information from analysis of morphometric data is used in the differential diagnosis of the new species.

For comparison we have included illustrations of the anchors and their associated secretory nets, the male copulatory organs and the connective bars of known *Bravohollisia* spp. in the respective PCA scatterplots (Figs. 3–5). The illustrations of the anchors, male copulatory organs and connective bars of the known species, *B. kritskyi*, *B. reticulata*, *B. rosetta*, *B. gussevi* and *B. magna* are re-drawn to scale from Lim (1995)

and *B. tecta* and *B. pomadasis* are re-drawn to scale from Bychowsky & Nagibina (1970).

RESULTS

In previous studies, the initial part of the copulatory organ of *Bravohollisia* spp. has been classified into cup-shape and bell-shape arbitrarily (see Lim, 1995; Bychowsky & Nagibina, 1970). In this paper we define the initial part as being bell-shape when the depth is 20–53 μm and width is 16–50 μm , and as cup-shape when the depth is 6–23 μm and width is 10–37 μm (see Lim, 1995; Bychowsky & Nagibina, 1970; Table 3; Fig. 3). Based on the above size definition the initial part of *B. kritskyi* which has a depth of 46 (39–53) and width of 39 (30–47) should be considered as bell-shape instead of cup-shape (see Lim, 1995; Table 2; Fig. 3). Lim (1995) described the pyriform structures in the haptor as haptoral glands, but these structures are actually reservoirs storing secretory products and was termed haptoral reservoir by Lim (2002).

TAXONOMY

Bravohollisia geruti, new species

(Figs. 1A–G, 2)

Type-host. — *Pomadasys hasta* Bloch, 1790

Type-locality. — Off Pulau Ketam, Straits of Malacca, Peninsular Malaysia

Type specimens. — Holotype: ZRC.PAR.31

Paratypes: 2 paratypes ZRC.PAR.32–33 and 111 paratypes MZUM(P)2013.468(P) – 2013.578(P).

Material examined. — 114 specimens studied; 114 measured

Etymology. — The specific appellation is derived from the local name of the host species “gerut-gerut”.

Description. — Body 682 (583–766) \times 130 (114–148). Four eye-spots of pigmented granules, lenses not observed; anterior pair smaller than posterior pair. Alimentary system with subterminal mouth, muscular pharynx, oesophagus and intestinal caeca unite posterior to testis. Peduncle 157 (120–186) \times 88 (68–109). Haptor small, 65 (51–84) \times 93 (77–114). Four pyriform haptoral reservoirs observed associated with each of the four anchors. Lace-like net structure observed near tip of anchors. Four anchors with lateral external grooves extending from shaft to point; dorsal anchors with inner length 22 (19–25), outer length 21 (18–24), inner root 11 (9–13), outer root 5 (4–6), point 11 (10–13); ventral anchors with inner length 24 (22–27), outer length 22 (19–24), inner root 13 (11–14), outer root 5 (4–7), point 12 (10–13). Dorsal connective bar 33 (27–38) \times 8 (6–9) with two anterior protuberances. Ventral connective bar 29 (23–34) \times 7 (6–10). Fourteen marginal hooks of larval type, small, 11 (10–12). Ovary ovoid; oviduct arises from anterior margin of ovary; uterine pore near copulatory organ. Vaginal pore

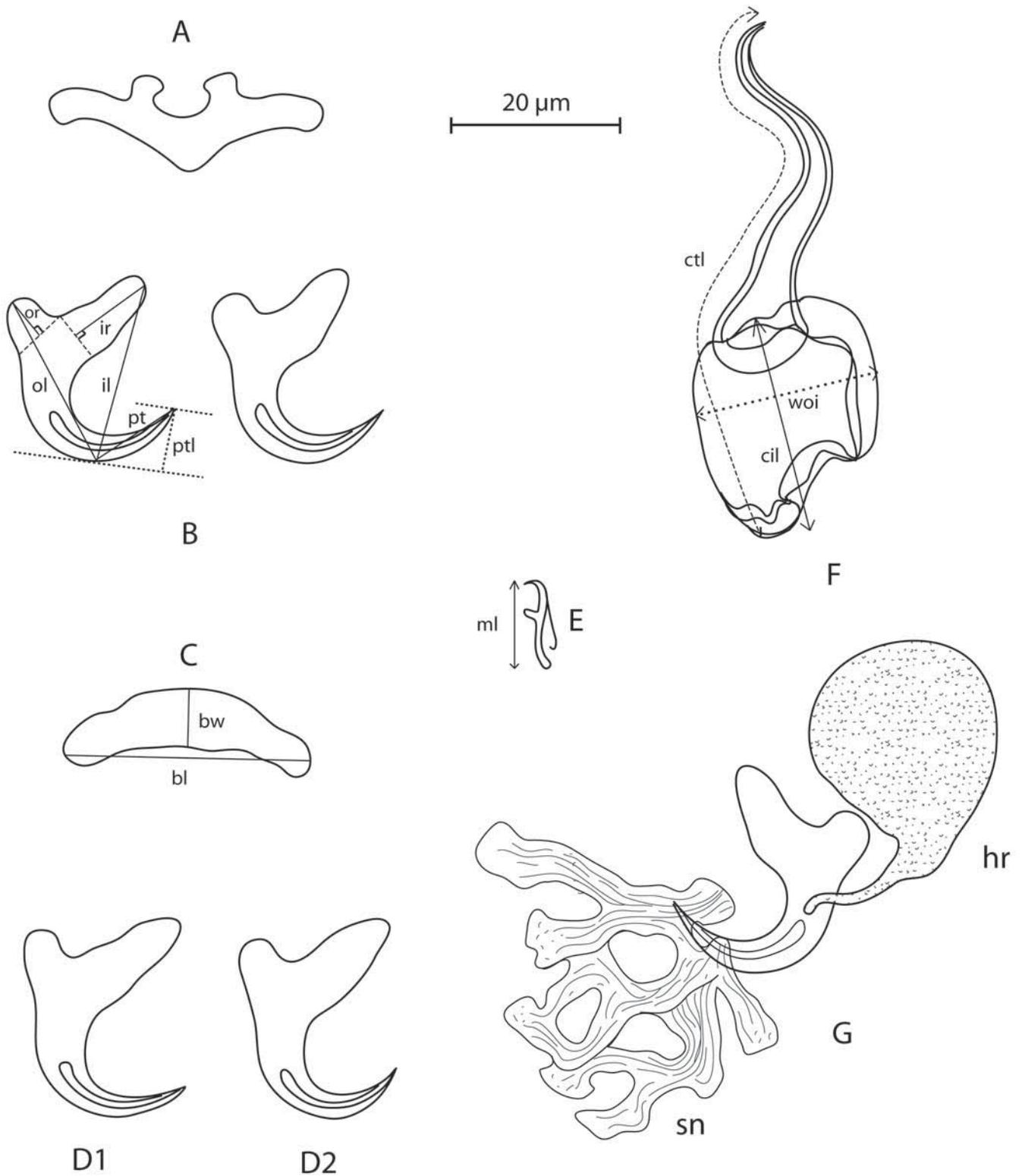


Fig. 1. Sclerotised parts of *Bravohollisia geruti*, new species: A, dorsal bar; B, dorsal anchors; C, ventral bar; D1, D2, ventral anchors (slight variations); E, marginal hook; F, copulatory organ; G, anchor with associated haptoral reservoir (hr) and secretory nets (sn). Abbreviations: parameters measured: il, inner length; ir, inner root length; ol, outer length; or, outer root length; pt, point length; ptl, point length taken by Lim (1995) (see Table 2); bw, bar width; bl, bar length; ml, marginal hook length; cil, copulatory organ initial length; ctl, copulatory organ total length; woi, greatest width of initial part.

Table 1. List of *Bravohollisia* Bychowsky & Nagibina, 1970 and *Caballeria* Bychowsky & Nagibina, 1970 and their *Pomadasy*s hosts. (Type hosts and type localities always listed first)

Monogenean	<i>Pomadasy</i> s spp.	Locality	References
<i>Bravohollisia</i> Bychowsky & Nagibina, 1970			
<i>B. magna</i> (Type species)	<i>P. argenteus</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
	<i>P. hasta</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995
<i>B. geruti</i> , new species	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
<i>B. gussevi</i>	<i>P. hasta</i>	off Sungai Buloh, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Sungai Sementa, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Matang, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. hasta</i>	off Sungai Buloh, Straits of Malacca	Lim, 1995
<i>B. kritskyi</i>	<i>P. hasta</i>	off Sungai Sementa, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Matang, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. hasta</i>	off Sungai Buloh, Straits of Malacca	Lim, 1995
<i>B. reticulata</i>	<i>P. hasta</i>	off Sungai Sementa, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Matang, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. hasta</i>	off Sungai Buloh, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Sungai Sementa, Straits of Malacca	Lim, 1995
<i>B. rosetta</i>	<i>P. hasta</i>	off Matang, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. hasta</i>	off Sungai Buloh, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Sungai Sementa, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Matang, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995
<i>B. pomadasis</i>	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. maculatus</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
<i>B. tecta</i>	<i>P. argenteus</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
<i>Caballeria</i> Bychowsky & Nagibina, 1970			
<i>C. pedunculata</i> (Type species)	<i>P. hasta</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
	<i>P. argenteus</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995
	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
<i>C. liewi</i>	<i>P. hasta</i>	off Sungai Buloh, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Sungai Sementa, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Matang, Straits of Malacca	Lim, 1995
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995
	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995
<i>C. intermedius</i>	<i>P. hasta</i>	off Pulau Ketam, Straits of Malacca	Present study
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995
<i>C. robusta</i>	<i>P. argenteus</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
	<i>P. hasta</i>	off Hainan, South China Sea	Bychowsky & Nagibina, 1970
	<i>P. hasta</i>	off Kuantan, Pahang	Lim, 1995

sclerotised, located dextrally submedial at midbody; proximal region sclerotised cup-shaped with pouch-liked appendix; distal section an elongate tube. Testis dorsal, posterior to ovary; vas deferens arises from anterior part of testis, loops around left caecum to ventral side, ascends, dilates forming first seminal vesicle; ductus ejaculatorius enters bell-shaped initial part of copulatory tube. Copulatory organ without accessory piece; bell-shaped initial part, depth 25 (20–28), width 21 (18–25); tapering tube, 50 (47–54). One prostatic reservoir enters copulatory tube.

Differential diagnosis. — This new species is observed to be different from previously described *Bravohollisia* spp. mainly in the shapes and sizes of the male copulatory organ

and connective bars and in the detailed structure of anchors. Comparative examinations of the specimens of the known and new species show that the new species possesses male copulatory organ with bell-shape initial part and tapering copulatory tube which is similar to that of *B. kritskyi*, *B. rosetta* and *B. magna*. However it differs from them in terms of size and length of copulatory tube: in the new species the initial part is 25 (20–28) × 21 (18–25), and short tapering tube 50 (47–54); in *B. kritskyi* the initial part is 39 (30–47) × 46 (39–53) and tapering tube 115 (109–128) with kink prior to ending; in *B. rosetta* the initial part is 27 (22–28) × 19 (16–20) and tapering tube with a hook-like distal tip is 87 (78–95) (Lim, 1995) whilst in *B. magna* the initial part is 50 × 45 (40–50) and the length of tapering tube with long

whip-like ending is 130 (Table 2). *B. reticulata*, *B. gussevi*, *B. tecta*, and *B. pomadasis* are different from the new species in having cup-shape initial part and copulatory tube of different length (cf. Table 2 & Fig. 3).

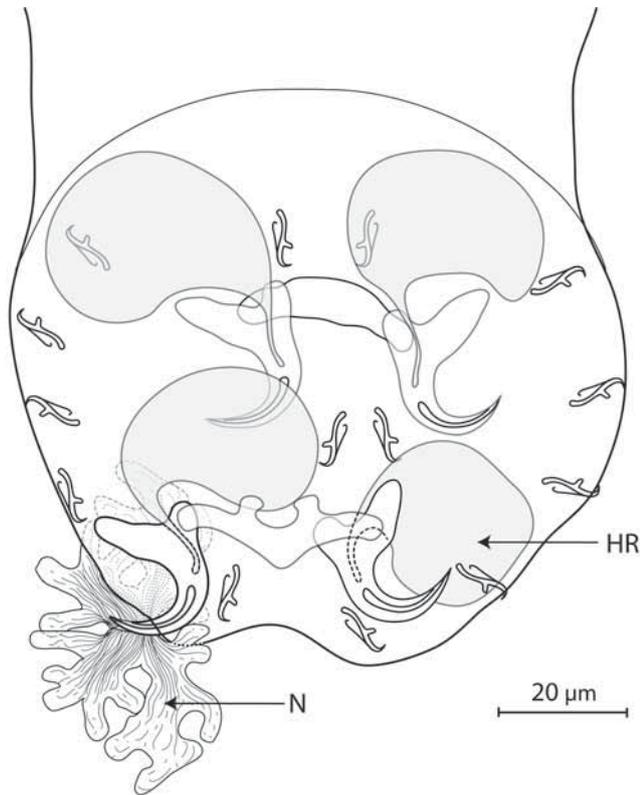


Fig. 2. Haptor of *Bravohollisia geruti*, new species showing net-like secretory product (N), 4 pyriform haptoral reservoirs (HR), 4 anchors, 2 bars and 14 marginal hooks.

In this study, the scatterplot based on the morphometric data of the male copulatory organ separates the 744 *Bravohollisia* individuals into five distinct groups corresponding to the five species, *B. rosetta*, *B. reticulata*, *B. gussevi*, *B. kritskyi*, and *Bravohollisia geruti*, new species (Fig. 3). This scatterplot confirms the observed morphological differences of the male copulatory organ of the present new species from that of *B. rosetta*, *B. reticulata*, *B. gussevi* and *B. kritskyi* (Fig. 3; Table 2).

Although the anchors of this new species are different in terms of detailed shape to the anchors of *B. kritskyi*, they are both metrically similar (Table 2). This metrical similarity between the anchors of this new species and that of *B. kritskyi* is supported by the scatterplot resulting from the PCA of the morphometric data of the anchors, which separates the 744 specimens into four groups which correspond to *B. kritskyi*—*Bravohollisia geruti*, new species (overlapping), *B. reticulata*, *B. rosetta*, and *B. gussevi* (Fig. 4). The anchors of this new species are bigger than those of *B. tecta* and *B. pomadasis* (cf. Table 2 & Fig. 4). *B. magna* differs from the present new species in the shape and size of anchors (cf. Table 2 & Fig. 4).

Morphologically the connective bars of the new species are different in terms of sizes and shapes from all the known species (cf. Table 2 & Fig. 5). The bars of this new species are bigger than those of *B. tecta* but are smaller than the bars of *B. magna*, *B. rosetta*, *B. kritskyi*, and *B. gussevi* (cf. Table 2 & Fig. 5). The bars of *B. pomadasis* and *B. reticulata* are almost similar in size as the bars of the new species but differ in terms of shape (cf. Table 2 & Fig. 5). The scatterplot resulting from PCA of the morphometric data of dorsal and ventral connective bars shows the new species is distinct from

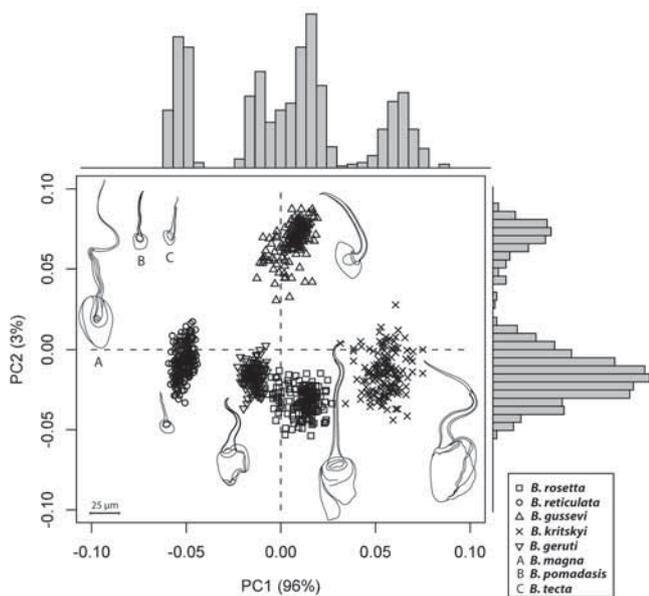


Fig. 3. PCA scatterplot of 744 specimens of five *Bravohollisia* spp. from off Peninsular Malaysia based on morphometric data of the copulatory organ. Horizontal and vertical barplots indicate one dimensional summary of the PC (PC1 and PC2) axes. Male copulatory organs re-drawn to scale from original publications. (A–C not included in PCA).

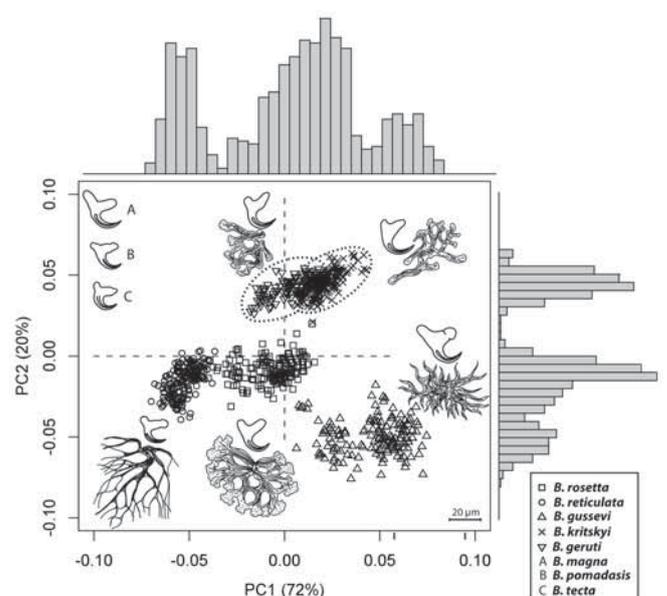


Fig. 4. PCA scatterplot of 744 specimens of five *Bravohollisia* spp. from off Peninsular Malaysia based on morphometric data of the dorsal and ventral anchors. Horizontal and vertical barplots indicate one dimensional summary of the PC (PC1 and PC2) axes. Anchors and associated net-like structures re-drawn to scale from original publications. (A–C not included in PCA).

Table 2. Morphometric data of anchors, bars, male copulatory organ and marginal hook of known and new *Bravohollisia* spp.

Parameters	<i>B. geruti</i> , new species		<i>B. kritskyi</i>		<i>B. reticulata</i>		<i>B. rosetta</i>		<i>B. gussevi</i>		<i>B. magna</i>	<i>B. tecta</i>	<i>B. pomadasis</i>
	Present data (n=114)	Lim (1995) (n=10)	Present data (n=150)	Lim (1995) (n=10)	Present data (n=180)	Lim (1995) (n=10)	Present data (n=150)	Lim (1995) (n=10)	Present data (n=150)	Lim (1995) (n=10)	Bychowsky & Nagibina (1970)	Bychowsky & Nagibina (1970)	Bychowsky & Nagibina (1970)
Dorsal anchor:													
Inner root	11 (9-13)	9 (8-13)	10 (8-13)	11 (9-11)	10 (8-11)	11 (9-11)	10 (7-13)	11 (10-11)	12 (8-14)	12 (11-14)	10**	10**	10**
Outer root	5 (4-6)	4 (3-6)	5 (4-7)	5 (4-6)	5 (4-6)	5 (4-6)	7 (4-9)	7 (6-8)	11 (8-14)	12 (11-14)	10**	4**	5**
Inner length	22 (19-25)	22 (19-22)	23 (20-25)	22 (19-22)	15 (13-19)	14 (13-15)	19 (15-22)	19 (18-22)	21 (18-24)	20 (18-22)	19**	15**	15**
Outer length	21 (18-24)	22 (19-22)	23 (20-25)	22 (19-22)	16 (14-18)	15 (13-17)	21 (17-24)	21 (18-24)	28 (21-32)	26 (22-28)	22**	19**	21**
Point*	11 (10-13)	5 (4-6)	12 (10-14)	5 (4-6)	10 (7-13)	6 (4-6)	10 (8-13)	6 (5-7)	12 (8-14)	6 (4-7)	10**	7**	6**
Ventral anchor:													
Inner root	13 (11-14)	11 (8-13)	12 (10-14)	11 (10-11)	10 (8-12)	11 (10-11)	11 (8-14)	11 (8-13)	12 (8-15)	11 (8-14)	13**	9**	9**
Outer root	5 (4-7)	5 (4-6)	6 (4-7)	5 (4-6)	5 (4-6)	5 (4-6)	7 (5-9)	7 (6-11)	11 (8-13)	11 (8-14)	12**	4**	4**
Inner length	24 (22-27)	25 (22-27)	26 (23-29)	25 (22-27)	15 (12-18)	16 (14-17)	20 (18-23)	21 (19-25)	22 (18-25)	21 (18-22)	22**	14**	14**
Outer length	22 (19-24)	24 (21-27)	24 (22-27)	24 (21-27)	16 (14-19)	16 (14-19)	22 (18-25)	22 (19-27)	29 (23-33)	26 (21-31)	27**	18**	20**
Point*	12 (10-13)	6 (4-8)	13 (10-15)	6 (4-8)	10 (8-13)	5 (4-6)	11 (7-13)	5 (3-6)	11 (8-14)	6 (5-7)	10**	7**	7**
Dorsal bar:													
Length	33 (27-38)	38 (36-39)	38 (33-45)	38 (36-39)	35 (29-40)	35 (33-36)	44 (35-52)	46 (41-50)	56 (42-67)	59 (50-64)	68-78	23-30	29-33
Width	8 (6-9)	6 (6-7)	7 (5-9)	6 (6-7)	5 (3-7)	5 (3-6)	7 (5-9)	8 (6-11)	8 (5-13)	8 (7-8)	6-15**	4**	5**
Ventral bar:													
Length	29 (23-34)	39 (36-42)	39 (33-47)	39 (36-42)	34 (30-41)	34 (30-36)	41 (33-48)	43 (36-45)	51 (39-65)	53 (47-58)	63-73	23-30	29-33
Width	7 (6-10)	6 (6-8)	6 (4-8)	6 (6-8)	5 (3-7)	5 (3-6)	8 (5-10)	8 (6-8)	11 (6-17)	10 (8-11)	8-16**	4**	5**
Copulatory organ:													
Total length	75 (67-82)	142 (121-162)	142 (121-162)	161 (148-181)	41 (33-48)	51 (44-56)	101 (82-116)	114 (100-123)	98 (78-110)	100 (92-109)	180**	48**	51**
Initial part:													
Depth	25 (20-28)	43 (31-51)	43 (31-51)	46 (39-53)	10 (6-14)	11 (8-14)	29 (22-35)	27 (22-28)	17 (11-23)	17 (14-20)	50**	8**	11**
Width	21 (18-25)	31 (22-39)	31 (22-39)	39 (30-47)	14 (10-19)	14 (11-14)	19 (15-22)	19 (16-20)	33 (28-37)	36 (33-36)	45 (40-50)	12	10
Marginal hook:													
Length	11 (10-12)	11 (9-12)	11 (9-12)	11 (10-11)	10 (9-11)	11 (10-11)	11 (9-12)	11 (10-11)	11 (9-12)	11 (10-11)	11-13	10-11	10-12

*Discrepancies in point length in present data and in Lim (1995) due to differences in methods of measurements (see Fig. 1B). **Data obtained by measuring illustrations in original publication.

B. reticulata, *B. kritskyi*, *B. rosetta*, and *B. gussevi* (Fig. 5). The morphometric measurements of the bars of *B. reticulata*, *B. kritskyi*, *B. rosetta*, and *B. gussevi* tend to overlap (Fig. 5). The lace-like nets of this new species resemble that of *B. kritskyi* but differ from the reticulate-like, rosette-like and stellate-like nets of *B. reticulata*, *B. rosetta*, and *B. gussevi*, respectively (see Lim, 1995; Fig. 4). The difference between the rosette-like nets and the lace-like nets is in the endings of

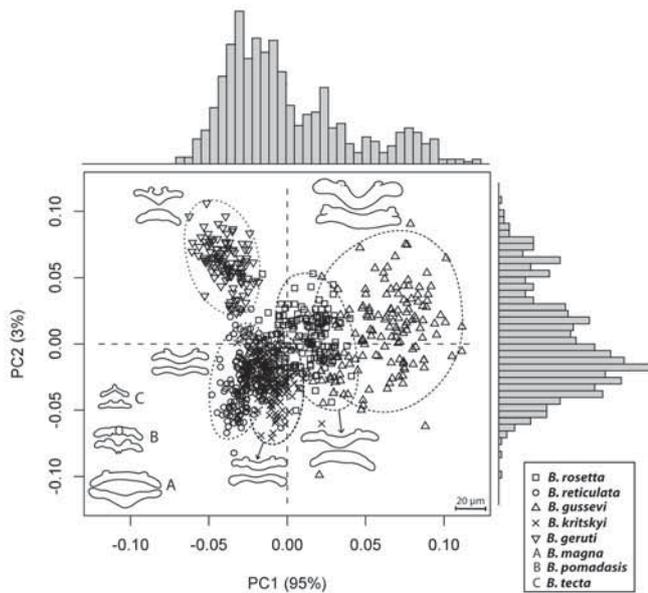


Fig. 5. PCA scatterplot of 744 specimens of five *Bravohollisia* spp. from off Peninsular Malaysia based on morphometric data of the dorsal and ventral bars. Horizontal and vertical barplots indicate one dimensional summary of the PC (PC1 and PC2) axes. Bars re-drawn to scale from original publications. (A–C not included in PCA).

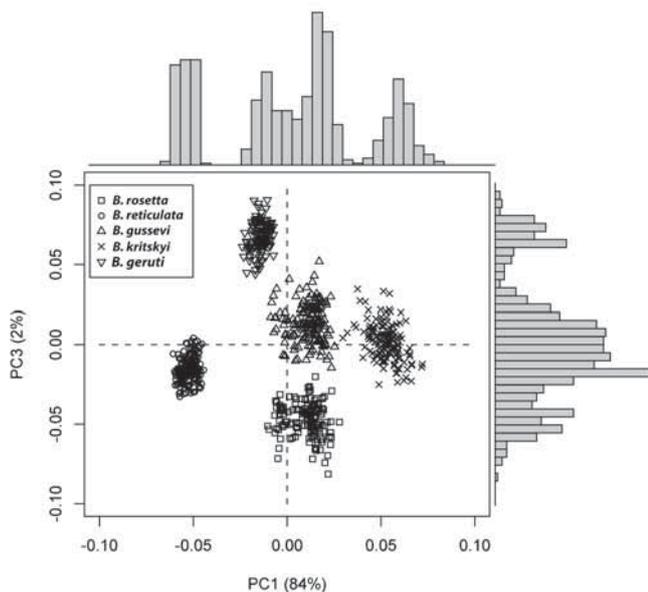


Fig. 6. PCA scatterplot of the 744 specimens of the five *Bravohollisia* spp. from off Peninsular Malaysia based on all morphometric data of anchors, copulatory organs, bars and marginal hooks. The horizontal and vertical barplots indicate one dimensional summary of the PC (PC1 and PC3) axes.

the nets which is expanded in the former and not expanded in the latter.

The validity of this new species is also confirmed by the morphometric analysis (PCA) using all morphometric data from anchors, connective bars, copulatory organ and marginal hook (Fig. 6). The combination of PC1 and PC3 provide the best separation of the 744 specimens into the five groups which correspond to the four known and one new species of *Bravohollisia*. The biplot indicates that the main character in differentiating the four known and the new species is mainly in the length of the male copulatory organ (Fig. 7) supporting our observation (see above).

DISCUSSION

This is the eighth species of *Bravohollisia* to be described from *Pomadasyss* spp. and the sixth species from *P. hasta* (Table 1). It should be noted that *B. magna* is not present in the collection of monogeneans from *P. hasta* off Pulau Ketam, although it was found on *P. hasta* off Kuantan (Lim, 1995). This new species can be differentiated from known species mainly by the sizes and shapes of the male copulatory organ (Fig. 3), connective bars (Fig. 5) and by the detailed shape of the anchors (Fig. 4). To facilitate comparison we have included in the PCA scatterplots illustrations of the anchors, connective bars and copulatory organs (Figs. 3–5). Two protuberances are observed on the anterior surface of the dorsal connective bar of all the known *Bravohollisia* species (Fig. 5). Similar protuberances can also be found on the dorsal bar in all four *Caballeria* species (Lim, 1995). Protuberances (known as antero-median protuberances) are observed on the ventral bar of *Ligophorus* species but these protuberances can range from very simple structure as in *L. bantingensis* Soo & Lim, 2012 to complex structures as in *L. fenestrum* Soo & Lim, 2012 (see Soo & Lim, 2012). The significance of the protuberances as generic feature of

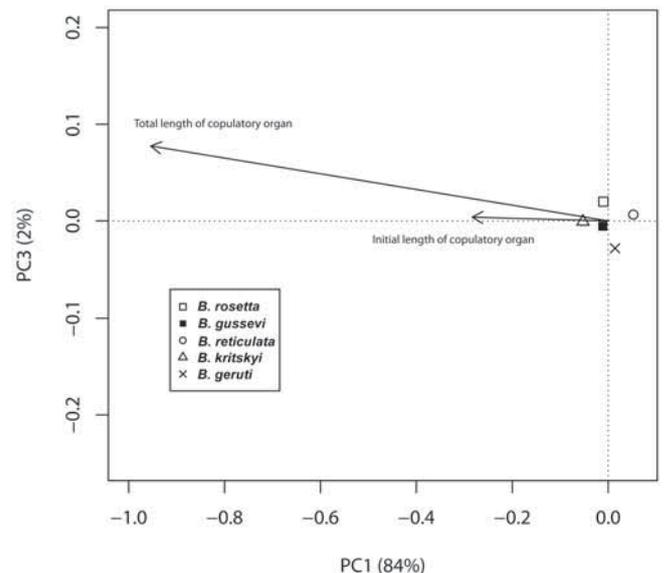


Fig. 7. Biplot of the first and third principal components for the five *Bravohollisia* species with mean coordinates of the species indicated.

Table 3. Categorisation of initial part of male copulatory organs of *Bravohollisia* spp. as cup and bell-shape based on metric criteria (in μm) (*= ranges not given in the original descriptions)

Metric criteria	Cup-shape		Bell-shape		References
	Depth	Width	Depth	Width	
	(6–23)	(10–37)	(20–53)	(16–50)	Present study
<i>B. geruti</i> , new species			25(20–28)	21(18–25)	Present study
<i>B. kritskyi</i>			46(39–53)	39(30–47)	Lim, 1995
			43(31–51)	31(22–39)	Present study
<i>B. rosetta</i>			27(22–28)	19(16–20)	Lim, 1995
			29(22–35)	19(15–22)	Present study
<i>B. magna</i>			50*	45(40–50)	Bychowsky & Nagibina, 1970
<i>B. gussevi</i>	17(14–20)	36(33–36)			Lim, 1995
	17(11–23)	33(28–37)			Present study
<i>B. reticulata</i>	11(8–14)	14(11–14)			Lim, 1995
	10(6–14)	14(10–19)			Present study
<i>B. tecta</i>	8*	12*			Bychowsky & Nagibina, 1970
<i>B. pomadasis</i>	11*	10*			Bychowsky & Nagibina, 1970

Bravohollisia can only be evaluated when more species of *Bravohollisia* are available for study.

As in the previously described species, the anchors of this new species are also associated with four pyriform haptoral reservoirs (Fig. 2; see Lim, 1995). As for the previous five *Bravohollisia* spp., the necks of the pyriform reservoirs in the present species are connected to one side of the anchor only and not as in *Lethrinitrema* spp. where the necks of the reservoirs bifurcates prior to entering into the anchor on both sides separately (see Lim & Justine, 2011). In *Bravohollisia* species the necks of the haptoral reservoirs do not bifurcate outside of the anchors as in *Lethrinitrema* spp. It has been proposed and shown that in *Bravohollisia* spp. the haptoral reservoir enters the anchor at one entry point and the secretions are probably channelled into a bifurcated channel within the anchor and the secretions are exudated via concealed exit pore and drain into external grooves on either side of the anchors of *B. rosetta* and *B. gussevi* (see Wong et al., 2008). The possible explanation for the non-observation of the entry and exit pores could be that the pores at the point of entry and exit are concealed (Wong et al., 2008) probably by a flap.

Haptoral reservoirs and net-like secretions have been described in the present species and in the other five *Bravohollisia* species, viz. *B. reticulata*, *B. kritskyi*, *B. rosetta*, *B. gussevi*, *B. magna* (see Lim, 1995) as well as in four species of *Caballeria*, viz. *C. intermedius*, *C. liewi*, *C. pedunculata* and *C. robusta* (see Lim, 1995) and in two species of *Ancyrocephaloides*, viz. *A. triacanthi* Yamaguti, 1938 and *A. chauhani* Bychowsky & Nagibina, 1975 (see Lim & Gibson, 2008). The secretory nets of the present new species are similar to that of *B. kritskyi* but different structurally from those of *B. reticulata*, *B. rosetta* and *B. gussevi* (Fig. 4). The nets of these *Bravohollisia* spp. are also different from those of *Caballeria* spp. (see Lim, 1995) and *Ancyrocephaloides* spp. (see Lim & Gibson, 2008). The nets of *Caballeria* spp. are not as extensive as that observed for *Bravohollisia* and *Ancyrocephaloides* (see Lim, 1995; Lim &

Gibson, 2008). The presence of haptoral reservoirs without nets have been reported in several monogenean species such as *Chauhanellus youngi* (see Paperna, 1960), *Haliotrema chrysostrami* (see Young, 1968), *Tetraonchus monenteron* (see Yamaguti, 1942) and *Lethrinitrema* species (see Lim & Justine, 2011). It is highly probable that secretions from the haptoral reservoirs of these monogenean species could also produce net-like structures which are not observed due to dislodgement or to net-like structures being not extensive and inconspicuous. In fact, the presence of net-like structures in *A. triacanthi* and *A. chauhani* are reported by Lim & Gibson (2008) but not noted in their original descriptions (Yamaguti, 1938; Bychowsky & Nagibina, 1975). Lim (1995) proposed that the nets are used for attachment and Lim & Gibson (2008) elaborated that the nets function as ‘safety belt’ or ‘belay device’ during the locomotion of the worm.

Lim (1995) suggested that the differences in the structure of the nets could be due to differences in the biochemical properties of the secretion and/or shape of the anchors and the location and structural differences of the exudation pores could also play a role in the structure of the nets. Wong et al. (2008) observed that at the ultra-structural level, the cores of the dual electron-dense (DED) secretory bodies in the secretions of *B. rosetta* and *B. gussevi* are different (oval and concave cores in *B. rosetta* and oval cores in *B. gussevi*), which could explain their rosette-like nets and stellate nets, respectively. There also seems to be some correlations between the structure of the nets and the shapes of the anchors (see Fig. 4) but this needs more investigation. The anchors of *B. rosetta* and *B. gussevi* are morphometrically different, while that of *B. kritskyi* and the present new species are comparatively similar (Fig. 4). Currently it is difficult to ascertain if the biochemical properties or the physical shape of the anchors in particular the shape of the point and pore or both are responsible for the differences in net-like structures of the secretions of the *Bravohollisia*, *Caballeria* and *Ancyrocephaloides* spp. More studies are needed to ascertain the factors affecting the net structures.

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