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A new species of *Haliotrema* (Monogenea: Ancyrocephalidae (*sensu lato*) Bychowsky & Nagibina, 1968) from holocentrids off Langkawi Island, Malaysia with notes on the phylogeny of related *Haliotrema* species

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ABSTRACT

Haliotrema susanae sp. nov. is described from the gills of the pinecone soldierfish, *Myripristis murdjan* off Langkawi Island, Malaysia. This species is differentiated from other *Haliotrema* species especially those from holocentrids in having a male copulatory organ with bract-like extensions at the initial of the copulatory tube, grooved dorsal anchors and ventral anchors with longer shafts. The maximum likelihood (ML) analysis based on partial 28S rDNA sequences of *H. susanae* sp. nov. and 47 closely related monogeneans showed that *H. susanae* sp. nov. is recovered within a monophyletic clade consisting of only species from the genus *Haliotrema*. It is also observed that *H. susanae* sp. nov. forms a clade with *H. cromileptis* and *H. epinepheli* which coincides with a similar grouping by Young based on solely morphological characteristics. The morphological and molecular results validate the identity of *H. susanae* sp. nov. as belonging to the genus *Haliotrema*.

1. Introduction

The family Holocentridae (squirrelfish and soldierfish) consists of 84 species globally with 13 species recorded in Malaysia from three genera (*Sargocentron, Myripristis* and *Ostichthys*) [1]. To date, species of four genera of monogeneans have been reported from holocentrids; *Benedenia* Diesing, 1858, *Enoplocotyle* Tagliani, 1912, *Pseudohaliotrematoides* Yamaguti, 1953 (taxonomic status is *incertae sedis*) and *Haliotrema* Johnston & Tiegs, 1922, with the largest number of described species coming from the latter genus (Table 1). During a field trip in 2011, three species of holocentrid fish (*Myripristis murdjan* Forsskål, 1775, *M. hexagona* Lacépède, 1802 and *Sargocentron rubrum* Forsskål, 1775) were examined for monogeneans. A new monogenean species was discovered from *M. murdjan* and its taxonomy is described herein. This new species is not only the first described *Haliotrema* species from Malaysia but is the first study on holocentrids from Malaysian waters. Molecular information from the new species is further analysed with molecular information from related *Haliotrema* species to confirm its validity.

2. Materials and methods

The fish hosts, *Myripristis murdjan*, *M. hexagona* and *Sargocentron rubrum* were collected in the coastal waters off the island of Langkawi (6°21′N, 99°46′E) in March 2011. Monogeneans were collected from excised gills of freshly killed or frozen fish and prepared for morphological investigation. Briefly, the monogeneans were collected from the gills, pipetted onto clean glass slides, covered with cover slips and cleared in modified ammonium-picrate-glycerin [2]. These ammonium-picrate-glycerin specimens were later washed and dehydrated through a graded ethanol series and mounted in Canada balsam without staining [2] to study the sclerotized hard parts. To study the soft anatomical structures, some specimens were immediately fixed after collection, in AFA (acetic acid-formalin-alcohol) onto glass slides, then stained in Gomori's triple stain and mounted in Canada balsam after dehydration in increasing ethanol series [3]. Stained and unstained specimens were studied under bright-field and phase contrast microscopes. Images of the hard and soft anatomical structures of the new species were captured using a Leica digital camera and an image analysis software (QWin Plus). The hard and soft parts were illustrated using a digitizing tablet (WACOM) and Adobe Illustrator software (Fig. 1). The specimens were measured as indicated in Fig. 1 and measurements given in micrometres as an average with the ranges in parentheses. Type-specimens of the new species were deposited at the Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (ZRC) and Natural History Museum

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Table 1
Monogeneans from fish of the Family Holocentridae.

Monogenean	Host	Localities	Present status	Authority
Haliotrema auribaculum Zhukov, 1980	*Holocentrus adscensionis* Osbeck (formerly known as *Holocentrus ascensionis*)	Gulf of Mexico	Valid	Zhukov, 1980
Haliotrema chelicirrus Yamaguti, 1968	*Neoniphon aureolineatus* Liénard (formerly known as *Holocentrus scythrops*), *N. samara* Forsskål, *Sargocentron punctatissimum* Cuvier (formerly known as *Holocentrus lacteoguttatus*)	Hawaii	Valid	Yamaguti, 1968
Haliotrema curvicirrus Zhukov, 1980 (questionable)	*Holocentrus adscensionis* Osbeck (formerly known as *Holocentrus ascensionis*)	Gulf of Mexico	Valid	Zhukov, 1980
Haliotrema holocentri Young, 1968	*Sargocentron rubrum* Forsskål	Heron Island, Queensland, Australia	Valid	Young, 1968
Haliotrema longirectocirrus Zhukov, 1980	*Holocentrus adscensionis* Osbeck (formerly known as *Holocentrus ascensionis*)	Gulf of Mexico	Valid	Zhukov, 1980
Haliotrema myripristisi Zhukov, 1980	*Myripristis jacobus* Cuvier	Gulf of Mexico	Valid	Zhukov, 1980
Haliotrema papillibaculum Zhukov, 1980	*Myripristis jacobus* Cuvier	Gulf of Mexico	Valid	Zhukov, 1980
Haliotrema tenuihamus Zhukov, 1980	*Myripristis jacobus* Cuvier	Gulf of Mexico	Valid	Zhukov, 1980
Benedenia hawaiiensis Yamaguti, 1968	*Neoniphon aureolineatus* Liénard (formerly known as *Holocentrus scythrops*)	Hawaii	Valid	Yamaguti, 1968
Enoplocotyle hawaiiensis Yamaguti, 1968	*Neoniphon aureolineatus* Liénard (formerly known as *Holocentrus scythrops*)	Hawaii	Valid	Yamaguti, 1968
Pseudohaliotrematoides incertae sedis falcatus Yamaguti, 1968	*Sargocentron spiniferum* Forsskål	Hawaii	*Incertae sedis*	Yamaguti, 1968

London (NHMUK). The following material was examined: paratypes, *Haliotrema banana* Lim & Justine, 2007 (MZUM 578–580). The author was not able to obtain Zhukov's type specimens (from holocentrid hosts) because the slides cannot be taken out of the museum (Gerasev, pers. comm., 2017). Monogeneans collected from *S. rubrum* were found to be *Haliotrema holocentri* Young, 1968 but a redescription was not carried out as there weren't enough specimens for a revision.

For extraction of molecular information, the monogenean worms were removed from the gills of the hosts, identified and preserved in 75% ethanol. Genomic DNA was then extracted using DNEasy extraction kit from QIAGEN. The extracted DNA (5 μl) was used as a template in the PCR reaction to amplify the partial D1-D2 domain of the 28S rDNA, using forward primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′) [4] and reverse primer D2 (5′-TGGTCCGTGTTTCAAGAC-3′) [5]. The PCR reaction (50 μl) was performed in 1.5 mM $MgCl_2$, PCR buffer (Fermentas), 200 μM of each deoxyribonucleotide triphosphate, 1.0 μM of each PCR primer and 1 U of *Taq* polymerase (Fermentas) in a thermocycler (Eppendorf Mastercycler) using the following conditions: an initial denaturation at 95 °C for 4 min, followed by 35 cycles of 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. An aliquot (10 μl) from the amplicon was electrophoresed in a 1.3% agarose gel, stained with ethidium bromide and viewed under a UV illuminator. The remaining 40 μl of each amplicon was purified using a DNA purification kit (QIAGEN) and subjected to automated DNA sequencing (ABI 3730 DNA Sequencer, First Base Laboratories, KL) using the same primers used for PCR amplification. Partial 28S rDNA sequence data of the new species and selected monogeneans from the GenBank (Table 2) were analysed with *Calydiscoides indianus* Oliver, 1987 (from GenBank) as the outgroup species. It should be noted that the names of the species used for building the phylogenetic tree follow those in the World Register of Marine Species (WoRMS) [6] and not Genbank, where the species names have not been updated. These DNA sequences were edited and aligned with MAFFT v7.164 [7] and verified/edited visually using BioEdit v7.0.5.3 [8]. Maximum likelihood (ML) analysis was performed with MEGA7 [9]. Bootstrap procedure with 1000 replications was performed to assess the robustness of the inferred relationships.

3. Results

3.1. Description of new species

Monogenea (Van Beneden, 1858) Bychowsky, 1937
Dactylogyridea Bychowsky, 1937
Ancyrocephalidae (*sensu lato*) Bychowsky & Nagibina, 1968
***Haliotrema susanae* sp. nov. (Fig. 1).**

Body elongate, length 569 μm (445–708), width 159 μm (118–249), anterior region with 2 pairs of pigmented eye spots. Mouth ventral; intestine bifurcates posterior to pharynx. Haptor set off from body, almost rectangular; length 90 μm (68–113) width 123 μm (86–181), 14 similar sized marginal hooks, length 12 μm (10–14); two pairs of anchors; two dorsal anchors with inner root 24 μm (20–28); outer root 10 μm (7–12); inner length 59 μm (50–65); outer length 47 μm (43–50); point 13 μm (11–15); two ventral anchors with inner root 22 μm (18–25); outer root 11 μm (8–13); inner length 54 μm (48–61); outer length 46 μm (41–49); point 13 μm (10–16); two connecting bars; slightly bent dorsal bar, 44 μm (28–51) wide; V-shaped ventral bar with enlarged ends, 46 μm (37–56).

Testis single, roundish; vas deferens sinistral, loops around left caecal branch, ascends diagonally in intercaecal region, distends to form seminal vesicle, narrows to enter copulatory tube. An elongated prostatic reservoir, multiple prostatic gland ducts deplete into the anterior part of prostatic reservoir; prostatic duct leaves prostatic reservoir to enter initial part of copulatory tube. Copulatory organ consists of copulatory tube, no accessory piece, length 88 μm (76–101) with

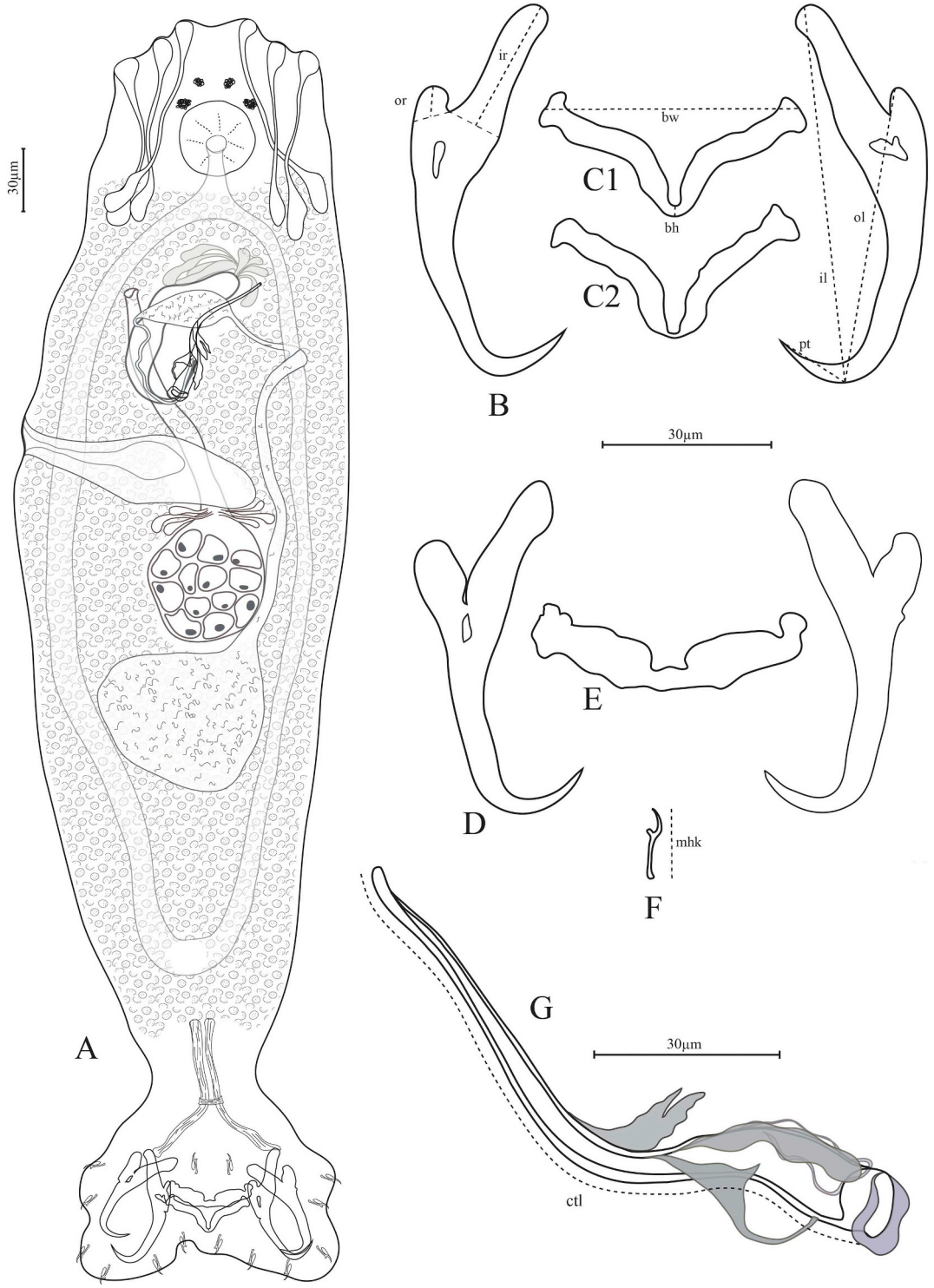

Fig. 1. *Haliotrema susanae* sp. nov. A, Whole worm (ventral view); B, Ventral anchors; C, Ventral bars (two different versions); D, Dorsal anchors; E, Dorsal bar; F, Marginal hook; G, Male copulatory organ. Abbreviations: or, outer root; ir, inner root; ol, outer length; il, inner length; pt., point; bw, bar width; bh, bar height; mhk, marginal hook length; ctl, copulatory tube length.

a short base and tapering tube, lightly sclerotized bract-like extensions at initial (proximal) region of copulatory tube. Ovary rounded, in mid body; oviduct arises from anterior of ovary, proceeds anteriorly to form ootype, receiving ducts from surrounding Mehlis' gland (not discernable in all specimens), ascends as uterus to open near male copulatory organ; vaginal duct not observed. Dextral vaginal opening with prominent large elongate gourd-shaped vaginal chamber, thick-walled, lightly sclerotized with distal end traversing into midportion of body. Vitelline system confluent anterior to haptor.

3.2. Taxonomic summary

Type-host: Myripristis murdjan Forsskål, 1775 (Beryciformes: Holocentridae: Myripristinae).
Site of infection: Gill filaments.
Type-locality: Langkawi Island, Malaysia.

Table 2
Species used for molecular analysis in this study with their host species, locality and GenBank accession numbers.

Monogenean species	Host species	Locality	GenBank number	GenBank authority
Dactylogyridea Bychowsky 1937				
Ancyrocephalidae Bychowsky, 1937				
Haliotrema susanae sp. nov.	*Myripristis murdjan*	Langkawi Island, Kedah. Malaysia	MG518632	Present study
Euryhaliotrema lisae Kritsky & Diggles, 2014	*Lutjanus johnii*	Pulau Ketam, Selangor, Malaysia	MG593832	Present study
Euryhaliotrema longibaculoides Kritsky & Diggles, 2014	*Lutjanus johnii*	Pulau Ketam, Selangor, Malaysia	MG593833	Present study
Haliotrema bilobatus (Yamaguti, 1953) Bychowsky & Nagibina, 1970	*Drepane punctata*	Off Johor, Malaysia	MG593837	Present study
Haliotrema magnihamus Bychowsky & Nagibina, 1970	*Drepane punctata*	Off Johor, Malaysia	MG593838	Present study
Haliotrema neobilobatus Bychowsky & Nagibina, 1970	*Drepane punctata*	Carey Island, Selangor, Malaysia	MG593835	Present study
Haliotrema parvihamus Bychowsky & Nagibina, 1970	*Drepane punctata*	Carey Island, Selangor, Malaysia	MG593834	Present study
Haliotrema spinnicirrus (Yamaguti, 1953) Bychowsky & Nagibina, 1970	*Drepane punctata*	Carey Island, Selangor, Malaysia	MG593836	Present study
Euryhaliotrema johnii (Tripathi, 1959) Kritsky & Boeger, 2002	*Lutjanus rhodopterus* (this host name is stated in the publication but is invalid, most likely *Lethrinus rhodopterus*)	Yangjiang, China	DQ157657.1	Wu et al., 2006
Euryhaliotrema perezponcei Garcia-Vargas, Fajer-Avila & Lamothe-Argumedo, 2008	Not available	Not available	HQ615996.1	Soler-Jiminez et al., unpublished
Euryhaliotrema annulocirrus (Yamaguti, 1968) Kritsky, 2012 [previously known as *Euryhaliotrematoides annulocirrus*]	Not available	Not available	EU836195.1	Sun & Yang, unpublished
Euryhaliotrema aspistis (Plaisance & Kritsky, 2004) Kritsky, 2012 [previously known as *Euryhaliotrematoides aspistis*]	*Chaetodon vagabundus*	Australia	AY820614.1	Plaisance et al., 2005
Euryhaliotrema berenguelae (Plaisance & Kritsky, 2004) Kritsky, 2012 [previously known as *Euryhaliotrematoides berenguelae*]	*Chaetodon citrinellus*	French Polynesia	AY820615.1	Plaisance et al., 2005
Euryhaliotrema grandis (Mizelle & Kritsky, 1969) Kritsky, 2012 [previously known as *Euryhaliotrematoides grandis*]	*Chaetodon vagabundus*	Palau	AY820616.1	Plaisance et al., 2005
Euryhaliotrema microphallus (Yamaguti, 1968) Kritsky, 2012 [previously known as *Euryhaliotrematoides microphallus*]	*Heniochus chrysostomus*	Palau	AY820617.1	Plaisance et al., 2005
Euryhaliotrema pirulum (Plaisance & Kritsky, 2004) Kritsky, 2012 [previously known as *Euryhaliotrematoides pirulum*]	*Chaetodon lunula*	French Polynesia	AY820618.1	Plaisance et al., 2005
Euryhaliotrema triangulovagina (Yamaguti, 1968) Kritsky, 2012 [previously known as *Euryhaliotrematoides triangulovagina*]	*Chaetodon kleinii*	Palau	AY820619.1	Plaisance et al., 2005
Haliotrema angelopterum Plaisance, Bouamer & Morand, 2004	*Chaetodon kleinii*	Palau	AY820620.1	Plaisance et al., 2005
Euryhaliotrema anguiformis (Zhang in Zhang, Yang & Liu, 2001) Kritsky, 2012 [previously known as *Haliotrema anguiforme*]	*Lutjanus monostigma*	Guangdong. China	DQ537375.1	Wu et al., 2007
Haliotrema bihamulatum Zhang in Zhang et al., 2001	*Upeneus quadrilineatus*	Guangdong, China	DQ537378.1	Wu et al., 2007
Haliotrema cromileptis Young, 1968	*Epinephelus coioides*	Nha Trang Bay, Vietnam	EU523146.1	Dang et al., 2010
Haliotrema ctenochaeti Yamaguti, 1968 (nec Young, 1968)	Not available	Not available	EU836199.1	Sun & Yang, unpublished
Haliotrema digyroides Zhang in Zhang, Yang & Liu, 2001	Not available	Not available	EU836200.1	Sun & Yang, unpublished
Haliotrema dongshaense Sun, Gibson & Yang, 2011	Not available	Not available	KJ571014.1	Sun et al., unpublished
Haliotrema epinepheli Young, 1968	*Epinephelus fasciatus*	Heron Island, Australia	EU836201.1	Dang et al., 2010
Lethrinitrema fleti (Young, 1968) Lim & Justine, 2011 [previously known as *Haliotrema fleti*]	*Lethrinus nebulosus*	Yangjiang, Guangdong Province, China	DQ157661.1	Wu et al., 2006
Lethrinitrema fleti (Li & Chen, 2005) Sun et al. 2014 [previously known as *Haliotrema grossecurvitubus*]	Not available	Not available	EU836204.1	Sun & Yang, unpublished
Haliotrema johnstoni Bychowsky & Nagibina, 1970	*Upeneus luzonius*	Haikou, Hainan Province, China	DQ157664.1	Wu et al., 2006
Haliotrema macassarense (Yamaguti, 1963) Bychowsky & Nagibina, 1970 [previously known as *Haliotrema macasarensis*]	Not available	Not available	EU836207.1	Sun & Yang, unpublished
Haliotrema macracantha Yamaguti, 1968	Not available	Not available	EU836208.1	Sun & Yang, unpublished
Euryhaliotrema nanaoensis (Li, Yan, Yul, Lan & Huang, 2005) Kritsky, 2012 [previously known as *Haliotrema nanaoense*]	*Lutjanus argentimaculatus*	Guangdong, China	DQ537373.1	Wu et al., 2007

(*continued on next page*)

Table 2 (*continued*)

Monogenean species	Host species	Locality	GenBank number	GenBank authority
Pseudohaliotrema platicephali (Yin & Sproston, 1948) Young, 1968 [previously known as *Haliotrema playicephali*]	Not available	Not available	FJ767866.1	Su, unpublished
Haliotrema pratasense Sun et al., 2007	Not available	Not available	EU836209.1	Sun & Yang, unpublished
Haliotrema scyphovagina Yamaguti, 1968	*Forcipiger flavissimus*	French Polynesia	AY820622.1	Plaisance et al., 2005
Haliotrematoides shenzhenensis (Wang, Liu & Zhou, 2003) Kritsky, Yang & Sun, 2009 [previously known as *Haliotrema shenzhenensis*]	*Lutjanus argentimaculatus*	Guangdong, China	DQ537372.1	Wu et al., 2007
Euryhaliotrema spirotubiforum (Zhang in Zhang, Yang & Liu, 2001) Wu, Zhu, Xie & Li, 2006 [previously known as *Haliotrema spirotubiforum*]	*Lutjanus stellatus*	Yangjiang, Guangdong Province	DQ157656.1	Wu et al., 2006
Haliotrema subancistroides Zhang in Zhang, Yang & Liu, 2001	*Gerres filamentosus, G. abbreviatus*	Dayawan, Gaungdong Province	DQ157648.1	Wu et al., 2006
Haliotrematoides guttati (Garcia-Vargas, Fajer-Avila & Lamothe-Argumedo, 2008) Kritsky, Yang & Sun, 2009	Not available	Not available	HQ615993.1	Soler-Jiminez et al., unpublished
Haliotrematoides plectridium Kritsky & Mendoza-Franco in Kritsky, Yang & Sun, 2009	Not available	Not available	HQ615994.1	Soler-Jiminez et al., unpublished
Haliotrematoides spinatus Kritsky & Mendoza-Franco in Kritsky, Yang & Sun, 2009	Not available	Not available	HQ615995.1	Soler-Jiminez et al., unpublished
Ligophorus bantingensis Soo & Lim, 2012	*Planiliza subviridis*	Carey Island, Selangor, Malaysia	KM221909	Soo & Lim, 2012
Ligophorus chelatus Soo & Lim, 2012	*Planiliza subviridis*	Carey Island, Selangor, Malaysia	KM221912	Soo & Lim, 2012
Ligophorus funnelus Soo & Lim, 2012	*Planiliza subviridis*	Carey Island, Selangor, Malaysia	KM221914	Soo & Lim, 2012
Ligophorus navjotsodhii Soo & Lim, 2012	*Planiliza subviridis*	Carey Island, Selangor, Malaysia	KM221920	Soo & Lim, 2012
Ligophorus parvicopulatrix Soo & Lim, 2012	*Planiliza subviridis*	Carey Island, Selangor, Malaysia	KM221921	Soo & Lim, 2012
Metahaliotrema scatophagi Yamaguti, 1953 [previously known as *M. geminatohamula* Pan et al., 1995]	*Scatophagus argus*	Panyu, Guangdong Province, China	DQ157646.1	Wu et al., 2006
Metahaliotrema mizellei Venkatanarasaiah, 1981	*Scatophagus argus*	Panyu, Guangdong Province, China	DQ157647.1	Wu et al., 2006
Outgroup				
Tetraonchidae Bychowsky, 1937				
Calydiscoides indianus	*Nemipterus japonicus*		EF100557.1	Wu et al., unpublished

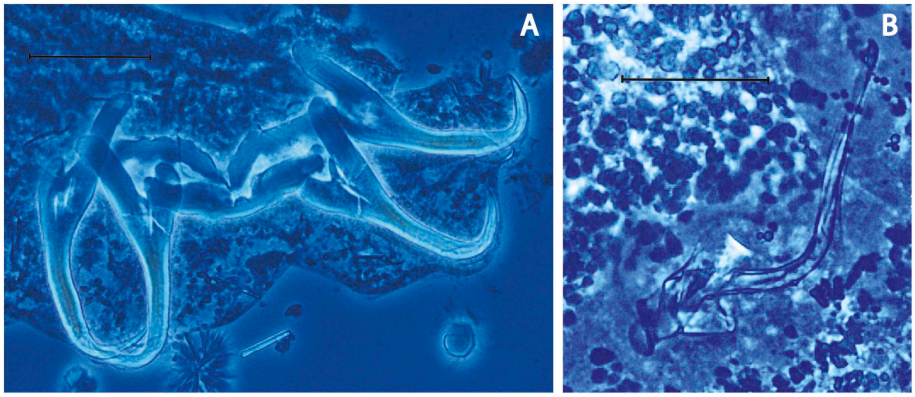

Fig. 2. Photomicrographs of sclerotized hard parts of *Haliotrema susanae* sp. nov., captured using Leica digital camera and an image analysis software (QWin Plus): (A) anchors and bars; (B) male copulatory organ. Scale = 30 μm.

Other host and locality: Myripristis hexagona Lacépède, 1802 from Langkawi Island, Malaysia.
Type-specimens: Holotype (ZRC.PLA.0367) and 1 paratype (ZRC.PLA.0368) in Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore; 48 paratypes (NHMUK 2018.9.27.1 - 48) in the Natural History Museum London (NHMUK).
Specimens studied: 50 specimens studied; 46 specimens measured.
Etymology: This new species is named in honor of the late Dr. Susan Lim Lee Hong who pioneered taxonomic studies of monogeneans from Malaysian waters.
Representative DNA sequence: MG518632 (partial 28S rDNA).

3.3. Differential diagnosis

Haliotrema susanae sp. nov. differs from previously described *Haliotrema* species from holocentrids in the morphology of the male copulatory organ and ventral bar. The general morphology of the male copulatory organ is similar for all *Haliotrema* species from holocentrids: a long copulatory tube with a broadened distal region and prominent initial. But the new species is different in having a bulbous base with bract-like expansions near the initial of the copulatory tube (Fig. 2B). Upon comparison with previous studies [10,11], it was observed that the copulatory tube of *H. susanae* sp. nov is long, similar to *H. curvicirrus* Zhukov, 1980. However, the copulatory tube of *H. susanae* sp. nov. has bract-like extensions from mid length to the initial region compared to none in *H. curvicirrus*. The ventral bar of the new species is V-shaped, similar to *H. longirectocirrus* Zhukov, 1980 and *H. myripristisi* Zhukov, 1980 but differs from both mentioned species in being enlarged at the ends (Fig. 2A). The dorsal bars of the new species are similar to *H. curvicirrus* Zhukov, 1980 in being narrow and slightly bent in the center but differs in length; 43 μm in *H. susanae* sp. nov. and 33 μm in *H. curvicirrus*. Single grooves were observed on the base of the ventral and some dorsal anchors of *H. susanae* sp. nov. The presence of grooves were observed on the dorsal anchors of *H. tenuihamus* Zhukov, 1980 and *H. myripristisi* but this feature is not a distinguishing one for *H. susanae* sp. nov. as not all of its dorsal anchors had grooves. Apart from grooves, the dorsal anchors of *H. susanae* sp. nov. has similar shaped bases, shafts and points with *H. papillibaculum* Zhukov, 1980 but differs from *H. papillibaculum* in the morphology of the copulatory organ. The copulatory tube is bent and shorter (32 μm) in *H. papillibaculum* compared to the new species where the copulatory tube is not bent and is longer (88 μm).

3.4. Molecular results

The partial 28S rDNA sequence data for *Haliotrema susanae* sp. nov. was generated and aligned alongside 47 monogeneans from closely related genera with *Calydiscoides indianus* as the outgroup species. The aligned dataset consisted of 48 sequences and comprised of 633 positions after trimming the ends to match the shortest aligned sequence. The maximum likelihood (ML) tree constructed consists of five clades (Fig. 3). The species sequences used in this analysis were specifically chosen for their relationship to the genus *Haliotrema* or those reassigned from the genus *Haliotrema* (see Table 2). Clade 1 consists of only *Euryhaliotrema* species (Fig. 3). Clade 2 and 3 consists of single genera: Clade 2 with only *Haliotrematoides* species (*H. spinatus, H. plectridium, H. guttati* and *H. shenzhenensis)* and Clade 3 with only *Ligophorus* species (*L. parvicopulatrix, L. bantingensis, L. chelatus,* L. *funnelus* and *L. navjotsodhii*). Clade 4 consists of four species: two *Metahaliotrema* species (*M. geminatohamula* and *M. mizellei*) and two *Haliotrema* species (*H. subancistroides* and *H. digyroides*). Clade 5 consists of the new species, *H. susanae* sp. nov. and other *Haliotrema* species (Fig. 3), reaffirming the designation of the new species as a member of the genus *Haliotrema*.

4. Discussion

The genus *Haliotrema* has been termed the 'waste-basket' genera because of the assignment of monogeneans with the following general characteristics: four anchors, 14 marginal hooks and two bars [12]. Forty three species, originally described as *Haliotrema* species were reassigned to different or newly-erected genera: eighteen species reassigned as *Euryhaliotrema* Kritsky & Boeger, 2002; fourteen as *Haliotrematoides* Kritsky, Yang & Sun, 2009; five as *Lethrinitrema* Lim & Justine, 2011; two as *Ligophorus* Euzet & Suriano, 1977 and one each for the following genera; *Triacanthinella* Bychowsky & Nagibina, 1968; *Tetrancistrum* Goto & Kikuchi, 1917; *Parancylodiscoides* Caballero & Bravo Hollis, 1961 and *Pseudohaliotrema* Yamaguti, 1953. After these reassignments, there are 191 species listed as *Haliotrema* species on the World Register of Marine Species [6]. These reassignments have been based on host groups and salient morphological characters, as exemplified by the reassignments of the *Haliotrema* species from mugilids to *Ligophorus* [13]; from lutjanids, caesionids, haemulids and sparids to *Haliotrematoides* [14]; from the sciaenids, sparids, haemulids, chaetodonthids and lutjanids to *Euryhaliotrema* [15] and from lethrinids to *Lethrinitrema* [16].

Haliotrema susanae sp. nov. is the most recently described *Haliotrema* species from holocentrids since Zhukov [11]. *Haliotrema susanae* sp. nov. has the 'classic' morphological characteristics of the genus *Haliotrema*; four anchors, two bars, fourteen marginal hooks and a vas deferens which loops around the intestinal caeca [17]. The new species also has a large vaginal chamber which traverses across more than half the width of the monogenean's body and is larger in comparison to the vaginal chamber of other *Haliotrema* species from holocentrids (Fig. 1). The anchors of the new species are morphologically similar to *H. papillibaculum* and *H. tenuihamus* in having long shafts and recurved points but *H. susanae* sp. nov. can be differentiated from both these species in

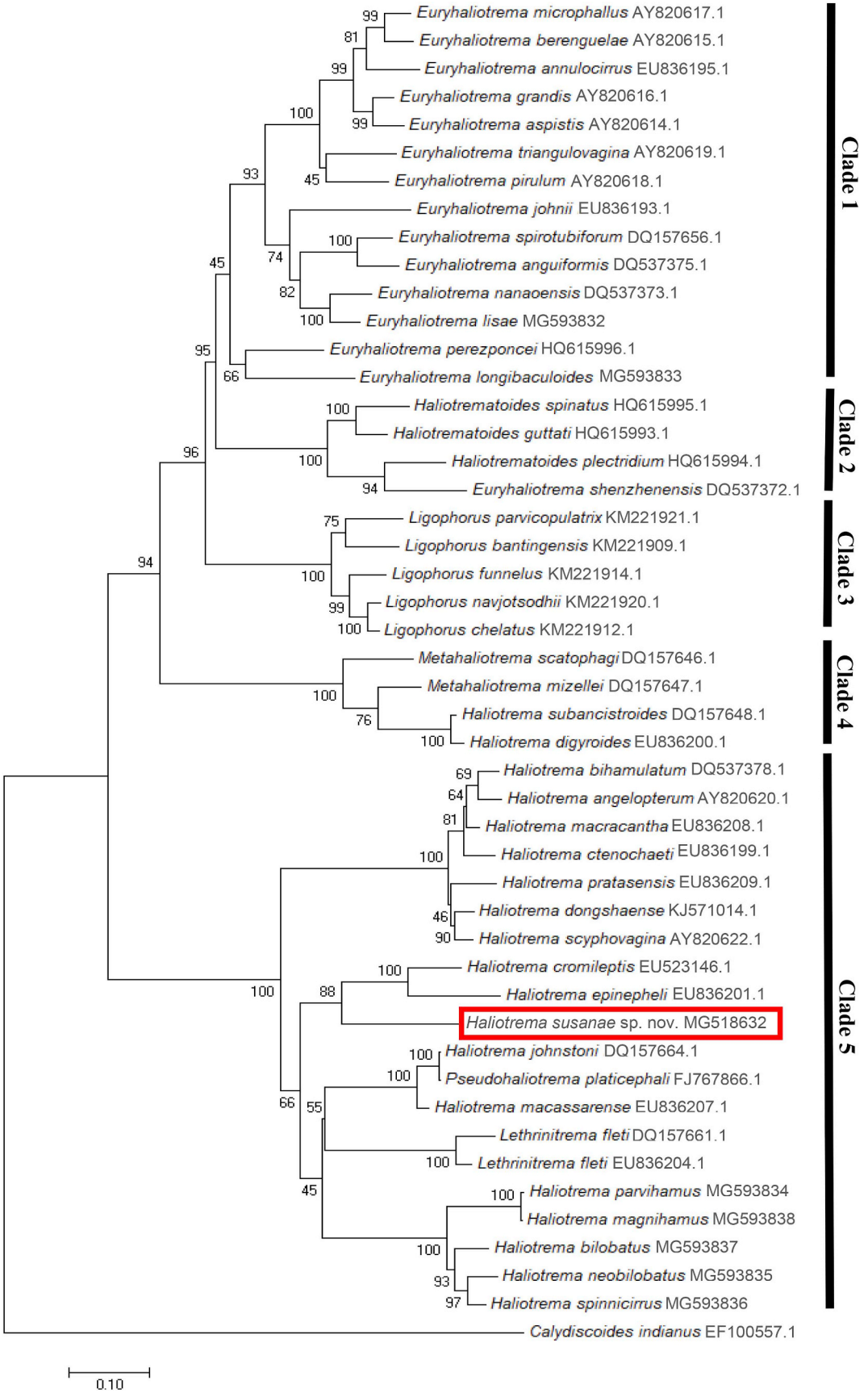

Fig. 3. Maximum likelihood (ML) tree generated from partial 28S rDNA sequence data using MEGA7 with *Calydiscoides indianus* as the outgroup. Bootstrap values are shown along the branches. The new species is indicated in the red box. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

having a morphologically different copulatory organ.

The molecular analysis consists of selected four-anchor monogeneans from previous studies and GenBank (Table 2), which serves to specifically reflect certain findings. A molecular analysis with all available *Haliotrema*-like species would have been more conclusive but this couldn't be attempted because partial 28S rDNA molecular sequence information is not available for all species on the GenBank. The present study showed that the reassignment of certain *Haliotrema*–like species based on host group and morphology is in agreement with the molecular analysis as exemplified by grouping of species in Clades 1 to 4 (Fig. 3). Clade 1 consists of only *Euryhaliotrema* species where all members of the clade have slightly overlapping gonads, a dextral vaginal pore, coiled male copulatory organ and a pretesticular germarium [15]. Clade 2 and 3 consist of single genera: Clade 2 consists only of *Haliotrematoides* species and Clade 3 only of *Ligophorus* species. Members of the genus *Haliotrematoides* were reassigned from *Haliotrema* based on a number of characteristics, some of which were marginal hook distribution, dorsal anchors lacking well-developed deep roots, ventral bar with two submedial pockets along the anterior margin and a copulatory complex lacking an accessory piece [14]. Some of the species reassigned were *Haliotrema longihamus* Zhukov, 1976 to *Haliotrematoides longihamus* (Zhukov, 1976) Kritsky et al., 2012 and *Haliotrema longitubocirrus* to *Haliotrematoides longitubocirrus* (Bychowsky & Nagibina, 1971) Kritsky et al., 2012. Two members of *Ligophorus* were reassigned from *Haliotrema* for having a long tubular copulatory organ with an accessory piece and a sclerotized vagina; *Ligophorus vanbenedenii* (Parona & Perugia, 1890) Euzet & Suriano, 1977 and *Ligophorus mugilinus* (Hargis, 1955) Euzet & Suriano, 1977, but subsequent new species description were also based on the host factor. Clade 4 consists of four species; *Metahaliotrema geminatohamula, M. mizellei, H. subancistroides* and *H. digyroides*. Reassignments are recommended for species within this clade, especially since all the species can be found in one common fish host group, *Gerres* spp. Clade 5 consists of 19 species, mostly from the genus *Haliotrema*. Three species have been reassigned: *Haliotrema fleti* Young, 1968 as *Lethrinitrema fleti* (Young, 1968) Lim & Justine, 2011 [16], *H. grossecurvitubus* Li & Chen, 2005 as L. *grossecurvitubum* (Li & Chen, 2005) Sun, Li & Yang, 2014 [18] and *H. platycephali* as *Pseudohaliotrema platicephali* (Yin & Sproston, 1948) Young, 1968 [19]. Five *Haliotrema* species (*H. bilobatus, H. magnihamus, H. parvihamus, H. neobilobatus* and *H. spinnicirrus*) collected from *Drepane punctata* off Malaysian waters formed a strongly supported clade within Clade 5. These species were proposed to be reassigned as members of *Parancylodiscoides* Caballero & Bravo-Hollis, 1961 based on possession of a dextrolateral vaginal aperture but due to uncertainty of using the position of the vaginal aperture as a main distinguishing character, reassignments have not been carried out [20].

Young (1968) found that *Haliotrema* species from the families Serranidae and Holocentridae have similar morphological characteristics, resulting in three species from his study (*H. epinepheli, H. cromileptis* and *H. holocentri*) grouped as members of Group 5 [21]. The present study supports this grouping, especially in the phylogenetic tree constructed (Fig. 3). *Haliotrema susanae* sp. nov. formed a clade (bootstrap value of 89%) with *H. epinepheli* and *H. cromileptis*, similar to Young's study [21], in which *H. holocentri* was also grouped with *H. epinepheli* and *H. cromileptis* based on only morphological features such as anchors with long roots, male copulatory organs lacking accessory pieces, a group of gland cells anterior to male copulatory organ and a large thick-walled vaginal chamber. There is no molecular sequence data for any monogeneans from the family Holocentridae in the GenBank except for *H. susanae* sp. nov. These results show that characterization of species based on morphology is in synchrony with results of the molecular analysis. Description and identification of new species are more conclusive when morphology and molecular results agree. Integrating different analytical methods during the taxonomic process (also known as integrative taxonomy) is especially useful in describing morphologically similar species. This was exemplified in the description and identification of *Ligophorus* species from Malaysia [22,23] and the Atlantic-Pacific Oceans [24]. In the current study, the integration of molecular biology into the description of *H. susanae* sp. nov. has not only reinforced its position as a *Haliotrema* species but its results is shown to agree with a morphological study done years ago.

During the course of this study, species synonymy was observed for *Haliotrema curvicirrus* whereby one record is described by Yamaguti [10] and another described by Zhukov [11]. On the WoRMS website [6], the author of *H. curvicirrus* is Yamaguti [10]. The species described by Zhukov [11] has not been reassigned or redescribed to date. Upon investigation of literature from the two authors, both synonyms of *H. curvicirrus* differ in first, having different hosts (Yamaguti's species is from *Parupeneus porphyreus* whilst Zhukov's is from *Holocentrus ascensionis*) and second, in having morphologically different hard parts. No further inferences can be made regarding the true identity of *H. curvicirrus* because both hosts are endemic to waters in the Western Atlantic Ocean and there have been no subsequent studies on both species to date. Other cases of species synonymy has also been reported for *Haliotrema* species from acanthurids; *H. ctenochaeti* Yamaguti, 1968 - *H. ctenochaeti* Young, 1968 and *H. serpenticirrus* Yamaguti, 1968 - *H. dempsteri* (Mizelle & Price, 1964) Young, 1968 [25]. Further investigation and possible reassignments are proposed for these synonymic species.

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Conflict of interest

The author declares that there is no conflict of interests.

Ethical approval

None needed as host were obtained dead from the fishermen port.

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