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Trigonocephalotrema (Digenea : Haplosplanchnidae), a new genus for trematodes parasitising fishes of two Indo-West Pacific acanthurid genera

Daniel C. Huston[A,B], *Scott C. Cutmore*[A] *and Thomas H. Cribb*[A]

[A]The University of Queensland, School of Biological Sciences, St Lucia, Qld 4072, Australia.
[B]Corresponding author. Email: Daniel.Huston@uqconnect.edu.au

Abstract. The Great Barrier Reef is the largest coral reef ecosystem on the planet and supports a diverse community of marine fishes, as well as the organisms that parasitise them. Although the digenetic trematodes that parasitise fishes of the Great Barrier Reef have been studied for over a century, the species richness and diversity of many trematode lineages is yet to be explored. *Trigonocephalotrema*, gen. nov. is proposed to accommodate three new species, *Trigonocephalotrema euclidi*, sp. nov., *T. hipparchi*, sp. nov. and *T. sohcahtoa*, sp. nov., parasitic in fishes of *Naso* Lacepède and *Zebrasoma* Swainson (Acanthuridae) in the tropical Pacific. Species of *Trigonocephalotrema* are characterised with morphological and molecular data (*18S* rRNA, *ITS2* and *28S* rRNA). Species of *Trigonocephalotrema* are morphologically distinguished from all other haplosplanchnid lineages by having terminal, triangular, plate-like oral suckers. With the inclusion of the new molecular data, Bayesian inference and maximum likelihood analyses of the Haplosplanchnidae Poche, 1926 recovered identical tree topologies and demonstrated *Trigonocephalotrema* as a well-supported monophyletic group. Although species of *Trigonocephalotrema* are differentiated from all other haplosplanchnid lineages on the basis of morphology, species within the genus are morphologically cryptic; thus, accurate species identification will require inclusion of host and molecular data. Species of *Trigonocephalotrema* cannot be assigned to a recognised subfamily within the Haplosplanchnidae using either morphological or molecular data and would require the erection of a new subfamily to accommodate them. However, we find little value in the use of subfamilies within the Haplosplanchnidae, given that there are so few taxa in the family, and herein propose that their use be avoided.

Additional keywords: Platyhelminthes, Trematode, Great Barrier Reef, *Naso*, *Zebrasoma*.

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Introduction

The Haplosplanchnidae Poche, 1926 is the sole family in the digenetic trematode suborder Haplosplanchnata Olson, Cribb, Tkach, Bray & Littlewood, 2003 (see Madhavi 2005). Sexually mature adult haplosplanchnids are gastro-intestinal parasites of a wide range of marine teleost lineages (Nahhas *et al*. 1997; Madhavi 2005; Huston *et al*. 2017). Known life cycles for haplosplanchnids include only two hosts, with cercariae emerging from the intermediate host gastropod and encysting in the environment before ingestion by the definitive host (Cable 1954; Fares and Maillard 1975). This life-cycle pattern corresponds with definitive host utilisation, as the majority of haplosplanchnid hosts are members of herbivorous (grazing, scraping and excavating) functional groups. Key morphological features that distinguish haplosplanchnids from other digenean lineages include a single caecum, single testis and the absence of a cirrus sac (Madhavi 2005).

Four subfamilies are currently recognised in the Haplosplanchnidae: the Haplosplanchninae Poche, 1926, Haplosplanchnoidinae Yamaguti, 1971, Hymenocottinae Yamaguti, 1971 and Schikhobalotrematinae Skrjabin & Guschanskaja, 1955 (Madhavi 2005). These subfamilies include just nine genera, six of which are monotypic: *Prohaplosplanchnus* Tang & Lin, 1978, *Parahaplosplanchnus* Nahhas, Rhodes & Seeto, 1997, *Provitellotrema* Pan, 1984 (Haplosplanchninae), *Haplosplanchnoides* Nahhas & Cable, 1964 (Haplosplanchnoidinae), *Discocephalotrema* Machida, 1993 (Hymenocottinae) and *Pseudoschikhobalotrema* Yamaguti, 1971 (Schikhobalotrematinae) (Madhavi 2005). Of these monotypic genera, only *Provitellotrema* has been evaluated with molecular data (Besprozvannykh *et al*. 2016). Although the morphological distinction of *Haplosplanchnoides*, *Discocephalotrema* and *Pseudoschikhobalotrema* is convincing, it is noteworthy that *Prohaplosplanchnus* is distinguished from the other haplosplanchnid lineages by having two testes, and *Parahaplosplanchnus* is distinguished by having a cirrus sac (Tang and Lin 1978; Lu 1995; Nahhas *et al*. 1997; Madhavi 2005). These characteristics call into question the validity of

these genera as members of the Haplosplanchnidae. The most speciose genera of the family are *Hymenocotta* Manter, 1961 (Hymenocottinae), which includes three species, *Haplosplanchnus* Looss, 1902 (Haplosplanchninae), which includes 13, and *Schikhobalotrema* Skrjabin & Guschanskaja, 1955 (Schikhobalotrematinae), which includes 26 (Cribb and Gibson 2010; Huston *et al*. 2017). Although subfamily concepts have long been established in the Haplosplanchnidae, the family is relatively small and each subfamily includes only a few genera, many of which are monotypic. As each of the nine genera are readily distinguished from one another, the value of subfamily-level division in the Haplosplanchnidae is questionable.

Haplosplanchnids occur circum-globally (Bray *et al*. 2016; Cribb *et al*. 2016; Pérez-del-Olmo *et al*. 2016) and are well represented in coral reef communities in the Gulf of Mexico (Linton 1910; Manter 1947; Siddiqi and Cable 1960; Nahhas and Cable 1964; Skinner 1975), Hawaii (Pritchard and Manter 1961; Yamaguti 1970) and Fiji (Manter 1961; Nahhas *et al*. 1997). In contrast, despite sustained study of the trematodes of the Great Barrier Reef over the past three decades, a known fauna of over 300 species and an estimated fauna of up to 1800, only three named haplosplanchnids have been reported from the region (Cribb *et al*. 2014*b*; Huston *et al*. 2017).

Here we add to the known haplosplanchnid fauna of the Great Barrier Reef using an integrated morphological and molecular approach. A distinct haplosplanchnid lineage was recognised for specimens from fishes of the acanthurid genera *Naso* Lacepède and *Zebrasoma* Swainson collected from Lizard Island, northern Great Barrier Reef, and Heron Island, southern Great Barrier Reef. Our results support the proposal of a new genus and the description of three new species. Although clearly genetically distinct and host-specific, these three new species are morphologically cryptic. A revised key to haplosplanchnid genera is provided.

Materials and methods

Specimen collection

The material used in this study was collected by the authors mainly between 2015 and 2016 but was supplemented with specimens deposited into the Marine Parasitology Laboratory collection, University of Queensland, Australia, between 1998 and 2015. Fishes of the family Acanthuridae were collected by spear from off Lizard Island (14°40′S, 145°27′E) and Heron Island (23°27′S, 151°55′E), Queensland, Australia. The gut of each fish was excised and examined for trematodes following the recommendations of Cribb and Bray (2010). Trematodes collected were fixed without pressure in near-boiling saline and preserved in either 10% formalin or 70% ethanol for subsequent parallel morphological and molecular analyses.

Morphological analyses

Trematode specimens used for morphological examination were removed from their preservative, washed in fresh water, overstained in Mayer's haematoxylin, destained in a solution of 1.0% hydrochloric acid and neutralised in a 0.5% ammonium hydroxide solution. Specimens were then dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Measurements were made with cellSens standard imaging software paired with an Olympus SC50 digital camera mounted on an Olympus BX-53 compound microscope (Olympus corporation, Eagle Farm, QLD, Australia). As both laterally and dorsoventrally mounted specimens were used in this study, measurements are provided in the format 'length × width × depth', unless otherwise stated. Length is taken from both dorsoventrally and laterally mounted specimens, whereas width is taken only from dorsoventrally mounted specimens and depth is taken only from laterally mounted specimens. Measurements are provided as a range followed by the mean in parentheses. Drawings were made using an Olympus BX-53 compound microscope with attached drawing tube, and illustrations were digitised in Adobe Illustrator. All vouchers are lodged in the Queensland Museum (QM), Brisbane, Australia.

Molecular sequencing

Three rRNA markers were targeted in this study, nuclear ribosomal RNA18S (*18S* rRNA) and 28S (*28S* rRNA) and internal transcribed spacer 2 (*ITS2*). The *ITS2* gene region is the most widely used marker for the delineation of trematode species, whereas the *18S* and *28S* rRNA regions are used extensively for reconstructing phylogenetic relationships (Nolan and Cribb 2005; Blasco-Costa *et al*. 2016). Molecular data were generated from entire trematodes, or by excising a small piece of tissue from a specimen for DNA extraction and processing the remainder of the specimen for morphological study as described above to serve as both a morphological and molecular voucher (hologenophore *sensu* Pleijel *et al*. 2008). Total genomic DNA was extracted from trematodes using phenol/chloroform extraction techniques (Sambrook and Russell 2001). PCR and sequencing for the *18S* rRNA, *ITS2* and *28S* rRNA gene regions followed the protocols of Huston *et al*. (2016, 2017). For each newly generated sequence of *ITS2*, the start and end of the *ITS2* region was determined by annotation using the *ITS2* database Metazoa model (Keller *et al*. 2009; Ankenbrand *et al*. 2015). Collection data and GenBank accession numbers for taxa sequenced are presented in the taxonomic section of this manuscript.

Phylogenetic analyses

The partial *18S* and *28S* rRNA sequences generated in this study were aligned with sequences of species of Haplosplanchnidae and selected outgroup taxa available on GenBank (Table 1). Outgroup choice was based on the molecular phylogenies of Olson *et al*. (2003) and Littlewood *et al*. (2015). Alignments for the *18S* and *28S* rRNA sequences were performed separately with MUSCLE (Edgar 2004) as implemented in MEGA7 (Kumar *et al*. 2016). The resultant alignments were trimmed to match the shortest sequence length, exported in FASTA format and concatenated manually.

Phylogenetic trees for the *18S* + *28S* rRNA concatenated sequence dataset were constructed with maximum likelihood and Bayesian inference analyses. Nucleotide substitution models were selected with the Bayesian information criterion using the greedy algorithm (Lanfear *et al*. 2012) and PhyML (Guindon *et al*. 2010) as implemented in PartitionFinder v. 2.1.1

Table 1. Haplosplanchnata and outgroup taxa used in phylogenetic analyses, including host and locality information, with GenBank accession numbers and original references

Taxa	Host	Locality	*18S*	*28S*	Reference(s)
Haplosplanchnidae Poche, 1902					
Haplosplanchnus pachysomus (Eysenhardt, 1829)	*Liza ramado* (Risso) (Mugilidae)	Mediterranean coast, Spain	FJ211224	FJ211241	Blasco-Costa 2009
	Valamugil engeli (Bleeker) (Mugilidae)	Ha Long Bay, Vietnam	LK932143	LK932149	Besprozvannykh *et al*. 2016
	Mugil cephalus Linnaeus (Mugilidae)	Moreton Bay, Australia		KY852458	Huston *et al*. 2017
Haplosplanchnus purii Srivastava, 1939	*Mugil cephalus*	Anse Vata, New Caledonia	FJ211225	FJ211242	Blasco-Costa 2009
Provitellotrema crenimugilis Pan, 1984	*Liza haematocheila* (Temminck & Schlegel) (Mugilidae)	Vostock Bay south-east Russia	LK932147	LK932153	Besprozvannykh *et al*. 2016
Hymenocotta mulli Manter, 1961	*Crenimugil crenilabis* (Forsskål) (Mugilidae)	Heron Island, Great Barrier Reef, Australia	AJ287524	AY222239	Cribb *et al*. 2001; Olson *et al*. 2003
Schikhobalotrema sp.	*Scarus rivulatus* Valenciennes (Scaridae)		AJ287574	AY222238	Cribb *et al*. 2001; Olson *et al*. 2003
Schikhobalotrema huffmani Huston Cutmore & Cribb, 2017	*Tylosurus gavialoides* (Castelnau) (Belonidae)	Moreton Bay, Australia	KY852462	KY852464	Huston *et al*. 2017
	Tylosurus crocodilus (Péron & Lesueur) (Belonidae)	Lizard Island, Great Barrier Reef, Australia	KY852461	KY852463	Huston *et al*. 2017
Schikhobalotrema sparisomae (Manter, 1937)	*Liza aurata* (Risso) (Mugilidae)	Mediterranean coast, Spain	FJ211223	FJ211240	Blasco-Costa 2009
Outgroup taxa					
Pronocephalata Olson, Cribb, Tkach, Bray & Littlewood, 2003					
Pronocephaloidea Looss, 1899					
Notocotylidae Lühe, 1909					
Catatropis indicus Srivastava, 1935	*Cairina moschata* Linneaus (Anatidae)	Laboratory infection, Australia	AY222114	AY222220	Olson *et al*. 2003
Opisthotrematidae Poche, 1926					
Lankatrema mannarense Crusz & Fernand, 1954	*Dugong dugon* Lacépède (Dugongidae)	Queensland, Australia	AY222116	AY222222	Olson *et al*. 2003
Paramphistomoidea Fischoeder, 1901					
Cladorchiidae Fischoeder, 1901					
Solenorchis travassosi Hilmy, 1949[A]	*Dugong dugon*	Queensland, Australia	AY222110	AY222213	Olson *et al*. 2003
Diplodiscidae Cohn, 1904					
Diplodiscus subclavatus (Pallas, 1760)	*Pelophylax ridibundus* (Pallas) (Ranidae)	Kokaljane, Bulgaria	AJ287502	AY222212	Cribb *et al*. 2001; Olson *et al*. 2003
Echinostomata La Rue, 1926					
Echinostomatoidea Looss, 1902					
Psilostomidae Looss, 1900					
Psilochasmus oxyurus (Creplin, 1825)	*Anas platyrhynchos* Linneaus (Anatidae)	Kherson Region, Ukraine	AY222135	AF151940	Olson *et al*. 2003; Tkach *et al*. 2000
Echinostomatidae Looss, 1899					
Echinostoma trivolvis (Cort, 1914)[B]	*Mesocricetus auratus* Waterhouse (Cricetidae)	Laboratory infection, United Kingdom	AY222132	AY222246	Olson *et al*. 2003

[A]Listed as *Indosolenorchis hirudinaceus* on GenBank (see Jones 2005).
[B]Listed as *Echinostoma revolutum* on GenBank (see Georgieva *et al*. 2014).

(Lanfear *et al*. 2017). A maximum likelihood analysis was performed using RAxML (Stamatakis 2014) on the CIPRES portal (Miller *et al*. 2010*a*) with 1000 bootstrap psuedoreplicates and the GTR + G nucleotide evolution model. Bayesian inference was performed using MrBayes v. 3.2.6 (Ronquist *et al*. 2012) with the GTR + I + G nucleotide evolution model applied to both the *18S* and *28S* rRNA partitions. Four chains were sampled every 1000 generations for 10 000 000 generations with the first 3000 samples being discarded as burn-in, at which point average standard deviation of split frequencies were <0.01.

Results

Species recognition

Haplosplanchnid specimens exhibiting distinctive triangular, plate-like oral suckers were recovered from the acanthurid fishes *Naso brevirostris* (Cuvier), *Naso lituratus* (Forster), *Naso unicornis* (Forsskål), *Zebrasoma scopas* (Cuvier) and *Zebrasoma velifer* (Bloch). These unusual oral suckers, combined with other morphological characters, suggested these trematodes represented a unique lineage among the Haplosplanchnidae that did not fit into any currently recognised genus or subfamily. *ITS2* data generated for these trematode specimens indicated the presence of four distinct species, with sequences differing from one another by 8–17 bp (Table 2). Two of these putative species were recovered from just one fish species and locality each, either *N. brevirostris* from off Heron Island, or *N. lituratus* from off Lizard Island. The other two trematode species were recovered from both localities from two fish species, either *N. lituratus* and *N. unicornis* or *Z. scopas* and *Z. velifer*. Insufficient material was available for the morphological description of one of these species, which is known only from two specimens. One of these specimens was consumed during molecular analysis and is represented only by molecular data, while the other specimen is a hologenophore. The other three species are described below.

Phylogenetic analysis

The partial *18S* rRNA alignment consisted of 1789 nucleotide positions and the *28S* rRNA alignment consisted of 1091 nucleotide positions, yielding a concatenated alignment of 2880 nucleotide positions. No regions of alignment ambiguity were detected. Bayesian inference and maximum likelihood analyses of this dataset generated trees with identical topologies (Fig. 1).

The topology of the new tree was consistent with that produced previously for the Haplosplanchnata (Huston *et al*. 2017), but with higher support for the *Schikhobalotrema* clade.

Table 2. Pairwise comparison of base pair differences between *ITS2* sequences for the four species of *Trigonocephalotrema*, gen. nov.
N = number of specimens sequenced

	N	1	2	3
1. *Trigonocephalotrema euclidi*, sp. nov.	10			
2. *Trigonocephalotrema hipparchi*, sp. nov.	3	9		
3. *Trigonocephalotrema sohcahtoa*, sp. nov.	9	15	17	
4. *Trigonocephalotrema* sp.	2	8	11	14

Thus, the molecular data support the present morphological genus-level concepts accepted in the Haplosplanchnidae. Most significantly, the sequences generated from the new material in this study form a well-supported monophyletic clade sister to *Schikhobalotrema* + the clade comprising *Haplosplanchnus* + *Provitellotrema*. The phylogenetic distance between this new clade and the other haplosplanchnid lineages, along with the unique morphological characteristics of the group, warrants proposal of a new genus to accommodate the new taxa.

Taxonomy

Family **HAPLOSPLANCHNIDAE** Poche, 1926

Genus ***Trigonocephalotrema***, gen. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:3EDF8D25-F930-43C2-9C0B-E2047C39A1F6

Type species: *Trigonocephalotrema euclidi*, sp. nov.

Other species: *Trigonocephalotrema hipparchi*, sp. nov. and *Trigonocephalotrema sohcahtoa*, sp. nov.

Diagnosis

Body elongate, fusiform, distinctly constricted immediately posterior to oral sucker. Oral sucker terminal, muscular, triangular, plate-like (Figs 2–4). Mouth small, triangular, opening near centre of oral plate. Ventral sucker simple, ellipsoid. Caecum single, extending well into hindbody. Testis single, in mid to anterior hindbody. Cirrus sac absent. Seminal vesicle tubular; prostatic cells indistinct; prostatic bulb absent. Genital atrium short, canalicular. Genital pore ventral, median, at anterior margin of ventral sucker. Ovary pretesticular. Vitellarium follicular, profusely developed in fore and hindbody. Uterus sparingly coiled, in region anterior to testis to posterior forebody. Eggs unembryonated. Excretory vesicle tubular, extending at least anterior to posterior end of caecum. In intestine of herbivorous marine teleosts (Acanthuridae), Indo-West Pacific.

Remarks

The most striking morphological characteristic of *Trigonocephalotrema* is the terminal, triangular, plate-like oral sucker. No other species exhibiting such an oral sucker have been described in the Haplosplanchnidae, thus no additional taxa beyond those described here are included in *Trigonocephalotrema*. The oral suckers of species of *Trigonocephalotrema* appear comparable to those of *Hymenocotta* and *Discocephalotrema*, which also possess terminal, flattened oral suckers with a small opening for a mouth. However, the oral suckers of *Hymenocotta* and *Discocephalotrema* are modified into disc-shaped plates rather than being distinctively triangular as in *Trigonocephalotrema*. In addition, species of *Hymenocotta* and *Discocephalotrema* have vitelline follicles restricted to the hindbody, rather than extending into the forebody as in those of *Trigonocephalotrema*. Species of *Trigonocephalotrema* are similar to those of *Schikhobalotrema* and *Pseudoschikhobalotrema* in the presence of profusely developed vitelline follicles, a feature separating these genera from *Haplosplanchnus*, *Parahaplosplanchnus*, *Prohaplosplanchnus* and *Provitellotrema*. However, species

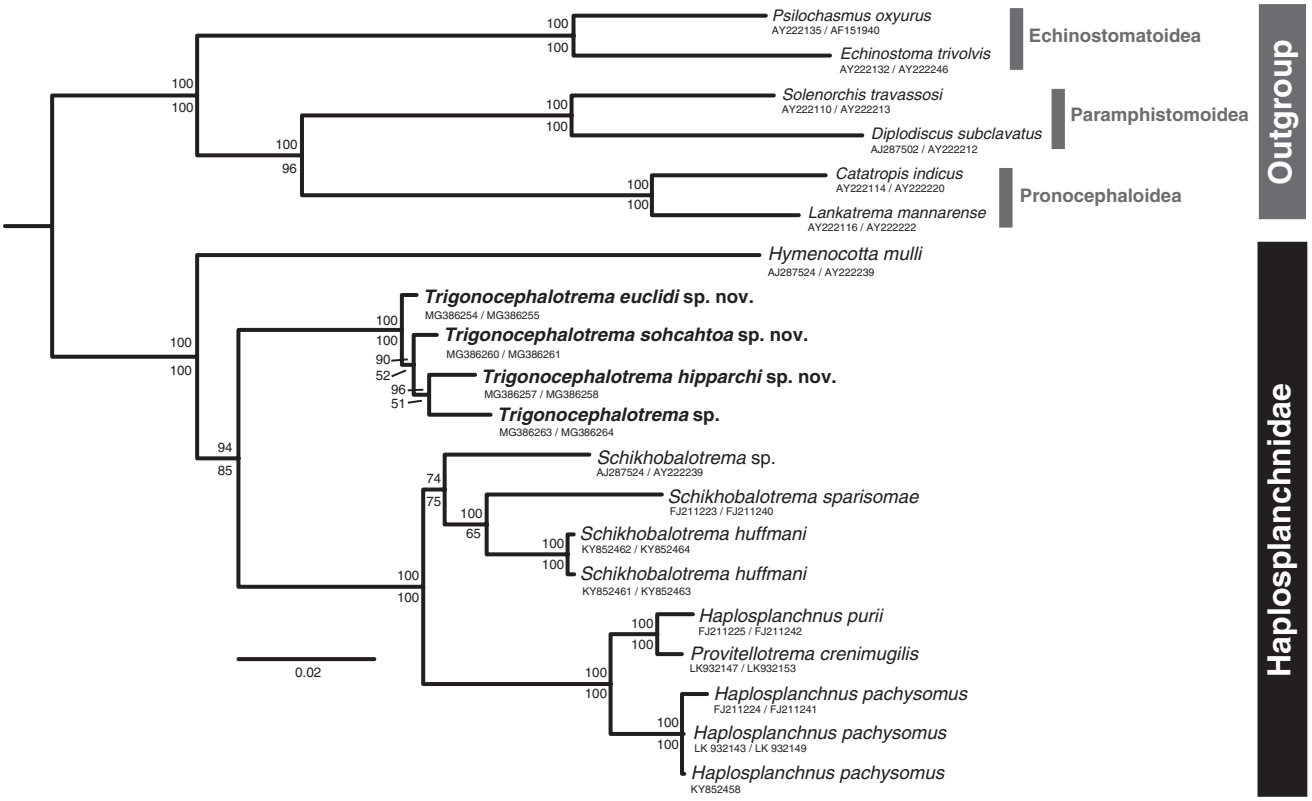

Fig. 1. Relationships of the Haplosplanchnidae based on Bayesian inference (BI) and maximum likelihood (ML) analyses of the concatenated *18S* + *28S* rRNA dataset; BI posterior probabilities are shown above the node, and ML bootstrap support values below.

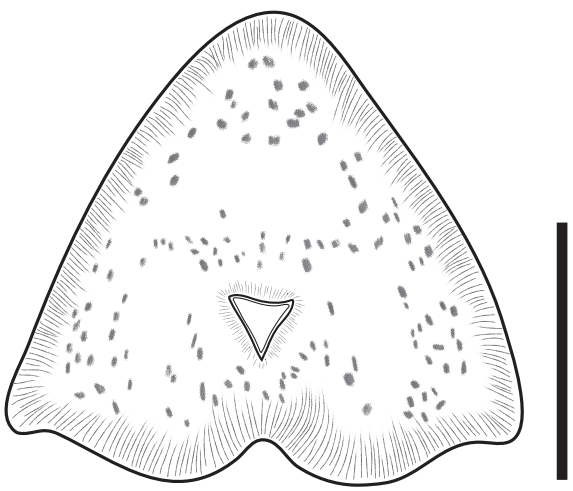

Fig. 2. Oral sucker of *Trigonocephalotrema euclidi*, sp. nov. Scale bar = 100 µm.

of *Trigonocephalotrema* are further differentiated from those of *Schikhobalotrema* by the absence of a conspicuous prostatic bulb, and from *Pseudoschikhobalotrema* by having elongate and fusiform rather than subspherical bodies, and by lacking appendages on the ventral sucker. The single known species of *Haplosplanchnoides* is easily differentiated from those of *Trigonocephalotrema* by having its ventral sucker at the posterior extremity rather than pre-median.

Many specimens of the species of *Trigonocephalotrema* described here have slightly concave dorsal surfaces (e.g. Fig. 4*A–C*), which causes some to roll from a dorsoventral to lateral position when mounted in Canada balsam on a slide. Although the triangular shape of the oral sucker is obscured in specimens mounted laterally, such specimens highlight the plate-like nature of this structure. Furthermore, laterally mounted specimens generally provide superior views of the internal anatomy and are thus useful for study of these species. To develop the most complete morphological picture of each species, we include illustrations and measurements and base the descriptions on type series including both dorsoventrally and laterally mounted specimens. We have previously expressed the view that some haplosplanchnid taxa are best mounted and studied in lateral position (Huston *et al*. 2017), as many historical workers have relied on flattening during fixation to ensure dorsoventrally mounted specimens. Such flattening should be avoided as it leads to inconsistent morphological results and complicates comparison of trematodes described by different authors (Cribb and Bray 2010).

Etymology

Trigonocephalotrema is formed from the Greek words ‘trigono’ (= triangle), ‘cephalos’ (= head) and ‘trema’ (= hole), in reference

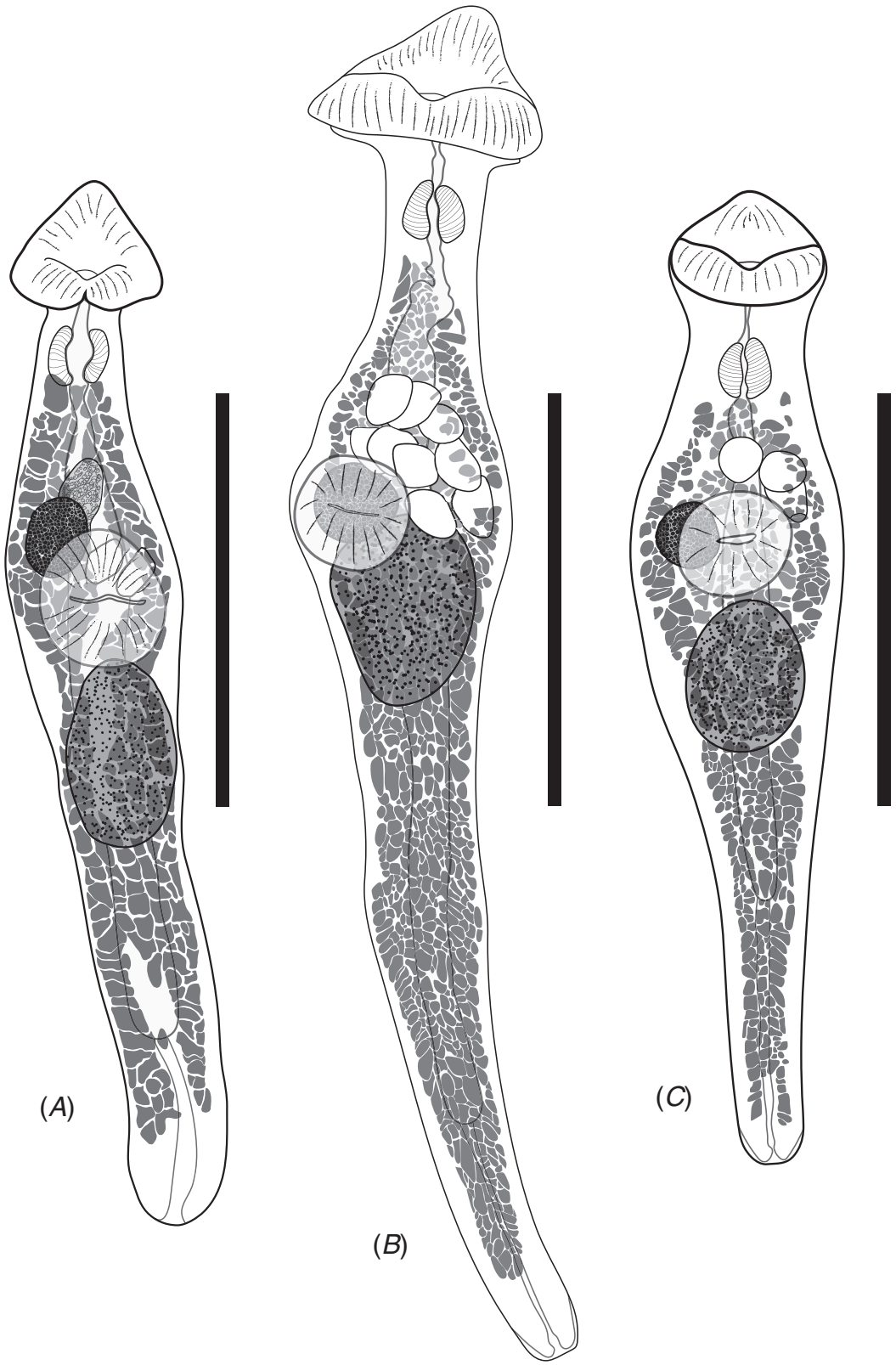

Fig. 3. (*A*) *Trigonocephalotrema euclidi*, sp. nov., holotype, dorsoventral view; (*B*) *Trigonocephalotrema hipparchi*, sp. nov., holotype, dorsoventral view; (*C*) *Trigonocephalotrema sohcahtoa*, sp. nov. holotype, dorsoventral view. Scale bars = 500 μm.

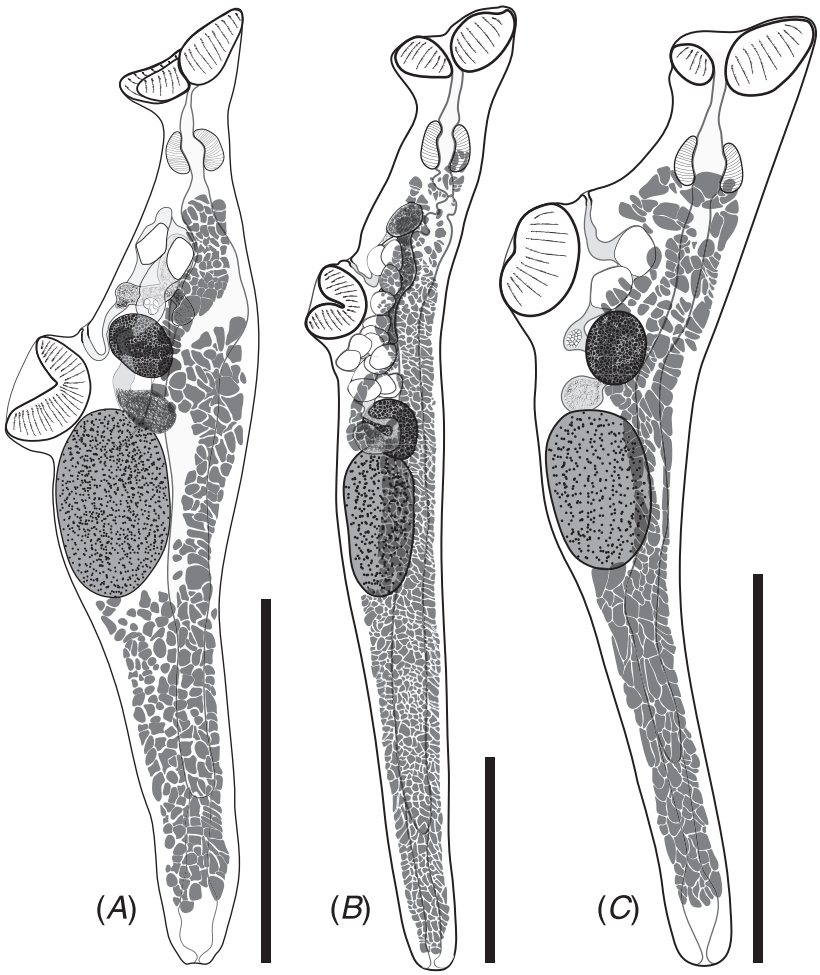

Fig. 4. (*A*) *Trigonocephalotrema euclidi*, sp. nov., lateral view; (*B*) *Trigonocephalotrema hipparchi*, sp. nov., lateral view; (*C*) *Trigonocephalotrema sohcahtoa*, sp. nov., lateral view. Scale bars = 500 µm.

to the triangular-shaped oral suckers and mouths possessed by these trematodes. The genus is treated as neuter.

Trigonocephalotrema euclidi, sp. nov.

(Figs 2, 3*A*, 4*A*, 5*A*)

http://zoobank.org/urn:lsid:zoobank.org:act:866AC1BA-739B-4204-B625-DAB1E3343961

Material examined

Holotype. Queensland: from intestine of *Naso lituratus*, off Lizard Island (LI), Great Barrier Reef (14°40′S, 145°27′E) (LI), coll. D. Huston, 2015 (QMG236459).

Paratypes. **Queensland:** two from intestine of *N. lituratus*, off LI, coll. D. Huston, 2015 (QMG236460–G236461); eight from intestine of *N. lituratus*, off LI, coll. D. Huston, 2016 (QMG236462–G236469); nine from intestine of *N. unicornis*, off Heron Island (HI), Great Barrier Reef (23°27′S, 151°55′E), coll. T. Cribb, 1999 (QMG236470–G236478).

Hologenophores. **Queensland:** two from intestine of *N. unicornis*, off LI, coll. D. Huston, 2015 (QMG236479–G236480); two from intestine of *N. unicornis*, off HI, coll. D. Huston, 2015 (QMG236481–G236482).

Representative DNA sequences. **Queensland:** Partial *18S* rRNA: five identical replicates; two from specimens from intestine of *N. unicornis* off LI, one from a specimen from intestine of *N. lituratus* off LI, two from specimens from intestine of *N. unicornis* off HI. One representative partial *18S* rRNA sequence submitted to GenBank (MG386254). *ITS2*: 10 identical replicates; four from specimens from intestine of *N. lituratus* off LI, three from specimens from intestine of *N. unicornis* off LI; three from specimens from intestine of *N. unicornis* off HI. One representative *ITS2* sequence submitted to GenBank (MG386256). Partial *28S* rRNA: four identical replicates; one from a specimen from intestine of *N. unicornis* off LI, one from a specimen from intestine of *N. lituratus* off HI, two from specimens from intestine of *N. unicornis* off HI. One representative partial *28S* rRNA sequence submitted to GenBank (MG386255).

Additional vouchers. **Queensland:** three from intestine of *N. unicornis*, off HI, coll. T. Cribb, 1999 (QMG236483–G236485). Oral suckers removed and mounted separately.

Description

Based on 20 whole mounts, 10 dorsoventral and 10 lateral. Body elongate, fusiform, 1155–1621 × 176–349 × 162–258 (1390 × 268 × 215). Body length/width 4.65–6.56 (5.45); body length/depth 5.07–7.19 (6.33). Tegument aspinose, thick

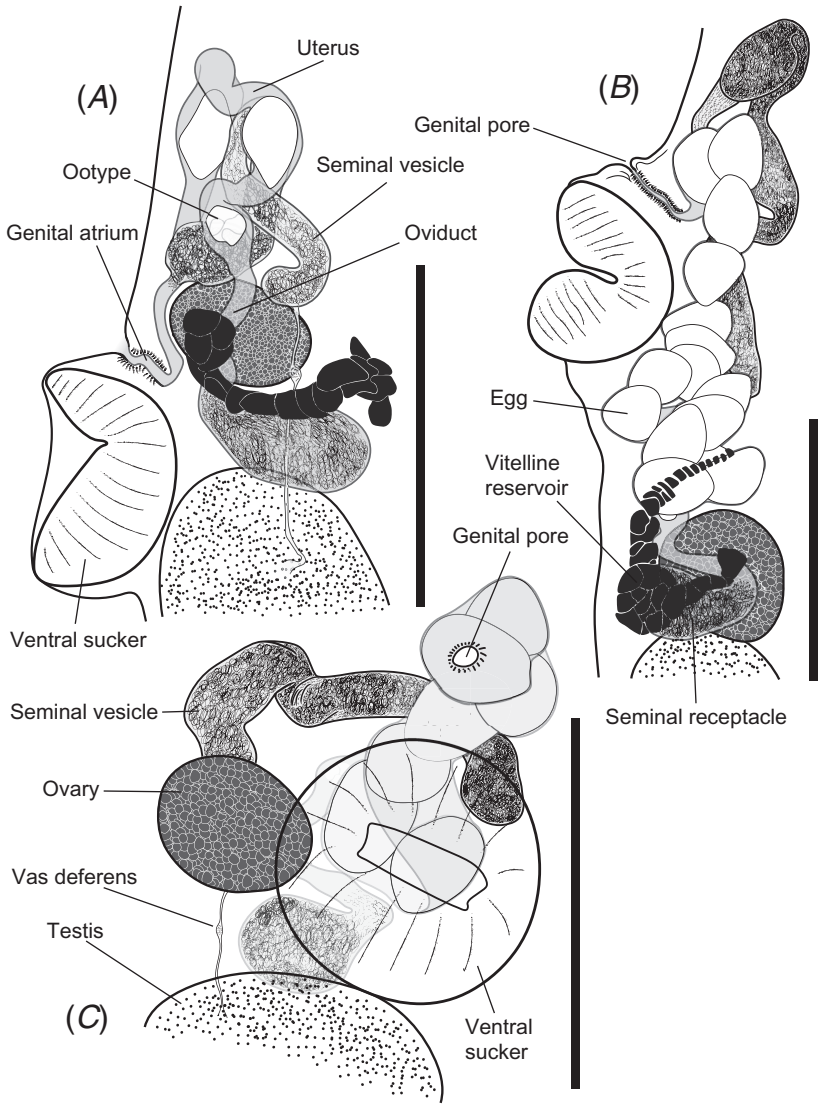

Fig. 5. (*A*) *Trigonocephalotrema euclidi*, sp. nov., ovarian complex, lateral view; (*B*) *Trigonocephalotrema hipparchi*, sp. nov., ovarian complex, lateral view; (*C*) *Trigonocephalotrema sohcahtoa*, sp. nov., ovarian complex, dorsoventral view. Scale bars = 250 μm.

with fine annulation visible in some specimens. Forebody shorter than hindbody, 407–649 (519) long, occupying 30–44% (37%) of body length, broadest at anterior margin of ventral sucker, distinctly constricted at base of oral sucker. Forebody width at level of pharynx 93–143 (122); forebody width at level of pharynx/body length 0.081–0.096 (0.084); forebody depth at level of pharynx 87–118 (103); forebody depth at level of pharynx/body length 0.069–0.088 (0.076). Hindbody 557–939 (713) long, occupying 42–58 (51)% of body length. Body pigment present, most profuse in forebody, sparsely distributed in anterior hindbody; darkly stained gland cells throughout. Oral sucker terminal, muscular, triangular, plate-like 117–202 × 170–268 × 144–213 (153 × 217 × 181). Ventral sucker pre-equatorial, subspherical, 113–180 × 127–220 × 98–144 (147 × 163 × 118); aperture horizontal. Ventral sucker length/oral sucker length 0.64–1.33 (0.98); ventral sucker width/oral sucker width 0.58–0.89 (0.75); ventral sucker depth/oral sucker depth 0.54–0.80 (0.65). Prepharynx straight, 17–86 (59) long; pharynx in mid to anterior forebody, ovoid, 52–102 × 67–87 × 55–99 (75 × 80 × 72). Oesophagus indistinct, ~58–145 (91) long. Caecum single, 639–882 (781) long, occupying 51–62 (56)% of body length. Post-caecal space 381–764 (538), representing 15–26 (20)% of body length.

Testis single, in anterior to mid-hindbody, with anterior margin usually dorsal to, though rarely up to 123 posterior to ventral sucker, ventral to caecum, ovoid, 193–296 × 90–212 × 128–161 (238 × 164 × 141), occupying 13–21 (17)% of body length. Post-testicular space 381–764 (539), representing 33–49 (39)% of body length. Vas deferens tubular, thin, arising from anterior region of testis, passing directly to posterior forebody, uniting with naked seminal

vesicle. Seminal vesicle swollen, tubular, passing into mid-forebody before coiling back into posterior forebody, uniting with uterus anterior to common genital atrium. Genital atrium short, canalicular with thickly muscled walls. Prostatic bulb absent; prostatic cells indistinct. Common genital pore ventral, median at anterior margin of ventral sucker.

Ovary usually anterodorsal to but occasionally posterior to ventral sucker, subspherical, 59–108 × 70–100 × 71–97 (89 × 85 × 88). Laurer's canal not observed. Seminal receptacle between testis and ovary, sac-like, subequal in size relative to ovary; oviduct passing just anterior to ovary, uniting with oötype. Mehlis' gland indistinct. Vitellarium follicular, profusely developed, in single field, 214–443 (289) from anterior extremity to 61–123 (80) from posterior extremity, occupying 62–79 (73)% of body length, wrapping around body from dorsal longitudinal median to dextral and sinistral ventral regions anterior to testis, wrapping around entirety of post-testicular region. Pre-vitelline region occupying 16–32 (21)% of body length; post-vitelline region occupying 4–9 (6)% of body length. Vitelline reservoir adjacent to ovary, collecting ducts curving dorsally, almost immediately indistinguishable from vitelline follicles. Uterus passing from oötype to mid-forebody, looping back to genital atrium. Eggs 1–9 in number, 62–75 (68) long, 38–53 (47) wide. Excretory vesicle a relatively straight tube, indiscernible beyond termination of caecum; excretory pore terminal.

Etymology

This species is named for the ancient Greek mathematician Euclid, in recognition of his influential geometric treatise *Elements*.

Trigonocephalotrema hipparchi, sp. nov.

(Figs 3*B*, 4*B*, 5*B*)

http://zoobank.org/urn:lsid:zoobank.org:act:A8F75073-C035-41D4-9F00-FA114CBB4F6A

Material examined

Holotype. Queensland: from intestine of *Naso brevirostris*, off HI, Great Barrier Reef (14°40′S, 145°27′E), coll. T. Cribb, 1998 (QMG236486).

Paratypes. **Queensland:** six from intestine of *N. brevirostris*, off HI, coll. T. Cribb, 1998 (QMG236487–G236492); two from intestine of *N. brevirostris*, off HI, coll. T. Cribb, 1999 (QMG236493–G236494); one from intestine of *N. brevirostris*, off HI, coll. T. Cribb, 2000 (QMG236495).

Hologenophores. **Queensland:** two from intestine of *N. brevirostris*, off HI, coll. T. Cribb, 2002 (QMG236496–G236497).

Representative DNA sequences. **Queensland:** Partial *18S* rRNA: three identical replicates; all from specimens from intestine of *N. brevirostris* off HI. One representative partial *18S* rRNA sequence submitted to GenBank (MG386257). *ITS2*: three identical replicates; all from specimens from intestine of *N. brevirostris* off HI. One representative *ITS2* sequence submitted to GenBank (MG386259). Partial *28S* rRNA: three identical replicates; all from specimens from intestine of *N. brevirostris* off HI. One representative partial *28S* rRNA sequence submitted to GenBank (MG386258).

Description

Based on 10 whole mounts, five dorsoventral and five lateral. Body elongate, fusiform, 1204–2258 × 265–343 × 205–327 (1808 × 320 × 271). Body length/width 5.09–6.53 (5.84); body length/depth 5.88–6.91 (6.42). Tegument aspinose, thick, finely annulated. Forebody shorter than hindbody, 376–685 (567) long, occupying 25–37 (32)% of body length, broadest at anterior margin of ventral sucker, narrowing, distinctly constricted at base of oral sucker. Forebody width at level of pharynx 120–173 (155); forebody width at level of pharynx/body length 0.072–0.095 (0.083); forebody depth at level of pharynx 106–182 (136); forebody depth at level of pharynx/body length 0.065–0.088 (0.079). Hindbody 794–1488 (1147) long, occupying 58–68 (63)% of body length. Body pigment sparse, restricted to forebody. Oral sucker terminal, muscular, triangular, plate-like 99–203 × 252–292 × 202–293 (165 × 281 × 248). Ventral sucker pre-equatorial, subspherical, 122–181 × 143–186 × 84–136 (152 × 167 × 109); aperture horizontal. Ventral sucker length/oral sucker length 0.81–1.23 (0.95); ventral sucker width/oral sucker width 0.50–0.67 (0.60); ventral sucker depth/oral sucker depth 0.41–0.46 (0.43). Prepharynx straight, 58–106 long; pharynx in mid to anterior forebody, ovoid to dolioform, 67–117 × 74–109 × 79–116 (89 × 94 × 97). Oesophagus distinct, winding, 55–107 (78) long. Caecum single, 718–1487 (1088) long, occupying 57–67 (62)% of body length. Post-caecal space 191–418 (300), representing 14–21 (17)% of body length.

Testis single, in mid to anterior hindbody, with anterior margin dorsal to ventral sucker or up to 246 posterior to ventral sucker, large, ovoid, 227–352 × 176–185 × 109–155 (272 × 181 × 134), occupying 14–19 (15)% of body length. Post-testicular space 437–1081 (763), representing 23–50 (42)% of body length. Vas deferens not discerned. Naked seminal vesicle swollen, tubular, arising at level of ventral sucker, passing into mid-forebody, looping back into posterior forebody, uniting with uterus just anterior to common genital atrium. Genital atrium short, canalicular, with thickly muscled walls. Prostatic bulb absent; prostatic cells indistinct. Common genital pore ventral, median at anterior margin of ventral sucker.

Ovary dorsal to ventral sucker or in anterior hindbody, subspherical to ovoid, 90–132 × 92–112 × 67–117 (106 × 99 × 100). Laurer's canal not observed. Seminal receptacle between testis and ovary, sac-like, smaller than ovary; oviduct passing just anterior to ovary, uniting with oötype. Mehlis' gland indistinct. Vitellarium follicular, profusely developed, in single field, 280–392 (319) from anterior extremity to 41–139 (76) from posterior extremity, occupying 69–83 (78)% of body length, wrapping around body from dorsal longitudinal median to dextral and sinistral ventral regions anterior to testis, wrapping around entirety of post-testicular region. Pre-vitelline region occupying 15–24 (18)% of body length; post–vitelline region occupying 2–7 (4)% of body length. Vitelline reservoir adjacent to seminal receptacle, collecting ducts pass dorsally becoming almost immediately indistinguishable from vitelline follicles. Uterus undulating from anterior of oötype to posterior forebody, then curving back directly to genital atrium. Eggs 2–33 in number, 66–79 (72) long, 49–59 (53) wide. Excretory vesicle straight, tubular, indiscernible beyond termination of caecum; excretory pore terminal.

Etymology

This species is named for the ancient astronomer and mathematician Hipparchus of Nicaea, in recognition of his great contributions to the field of trigonometry.

Trigonocephalotrema sohcahtoa, sp. nov.

(Figs 3*C*, 4*C*, 5*C*)

http://zoobank.org/urn:lsid:zoobank.org:act:8F34C1E1-2A98-402F-8642-0B09B664B7C3

Material examined

Holotype. Queensland: from intestine of *Zebrasoma velifer*, off HI, Great Barrier Reef (14°40′S, 145°27′E), coll. T. Cribb, 1994 (QMG236498).

Paratypes. **Queensland:** two from intestine of *Z. velifer*, off HI, coll. T. Cribb, 1993 (QMG236499–G236500); two from intestine of *Z. velifer*, off HI, coll. T. Cribb, 1994 (QMG236501–G236502); seven from intestine of *Z. velifer*, off HI, coll. T. Cribb, 1997 (QMG236503–G236509); two from intestine of *Z. scopas*, off HI, coll. T. Cribb, 1998 (QMG236510–G236511); one from intestine of *Z. velifer*, off LI, coll. T. Cribb, 1998 (QMG236512); four from intestine of *Z. velifer*, off HI, coll. T. Cribb, 1999 (QMG236513–G236516); four from intestine of *Z. scopas*, off LI, coll. D. Huston, 2015 (QMG236517–G236520).

Hologenophores. **Queensland:** two from intestine of *Z. velifer*, off HI, coll. R. Adlard, 2010 (QMG236521–G236522); two from intestine of *Z. scopas*, off LI, coll. T. Cribb, 2013 (QMG236523–G236524); two from intestine of *Z.* scopas, off HI, coll. T. Cribb, 2014 (QMG236525–G236526); one from intestine of *Z. scopas*, off LI, coll. D. Huston, 2015 (QMG236527).

Representative DNA sequences. **Queensland:** Partial *18S* rRNA: four identical replicates; two from specimens from intestine of *Z. scopas* off HI, two from specimens from intestine of *Z. scopas* off LI. One representative partial *18S* rRNA sequence submitted to GenBank (MG386260). *ITS2*: nine identical replicates; three from specimens from intestine of *Z. scopas* off HI, three from specimens from intestine of *Z. velifer* off HI, three from specimens from intestine of *Z. scopas* off LI. One representative *ITS2* sequence submitted to GenBank (MG386262). Partial *28S* rRNA: four identical replicates; two from specimens from intestine of *Z. scopas* off HI, two from specimens from intestine of *Z. scopas* off LI. One representative partial *28S* rRNA sequence submitted to GenBank (MG386261).

Description

Based on 23 whole mounts, 11 dorsoventral and 12 lateral. Body elongate, fusiform, 1083–1872 × 216–401 × 220–338 (1416 × 299 × 293). Body length/width 4.17–5.94 (5.03); body length/depth 3.99–5.19 (4.63). Tegument aspinose, thick. Forebody shorter than hindbody, 272–464 (357) long, occupying 18–31 (25)% of body length, broadest at anterior margin of ventral sucker, narrowing slightly then broadening before union with oral sucker. Forebody width at level of pharynx 130–208 (171); forebody width at level of pharynx/body length 0.096–0.135 (0.116); forebody depth at level of pharynx 124–197 (157); forebody depth at level of pharynx/body length 0.096–0.128 (0.116). Hindbody 677–1254 (912), occupying 58–69 (64)% of body length. Body pigment absent or sparsely dispersed in anterior forebody; darkly stained gland cells conspicuous throughout. Oral sucker terminal, muscular, triangular, plate-like 117–208 × 181–253 × 147–218 (147 × 210 × 185). Ventral sucker pre-equatorial, subspherical, 121–184 × 121–189 × 87–161 (159 × 163 × 122); aperture horizontal. Ventral sucker length/oral sucker length 0.76–1.33 (1.01); ventral sucker width/oral sucker width 0.61–0.99 (0.78); ventral sucker depth/oral sucker depth 0.50–0.83 (0.66). Prepharynx straight, 30–87 (53) long; pharynx in mid to anterior forebody, ovoid, 60–105 × 59–100 × 72–116 (81 × 86 × 91). Oesophagus indistinct, ~42–106 (69) long. Caecum single, 624–1202 (773) long, occupying 46–64 (54)% of body length. Post-caecal space 257–479 (357), representing 16–31 (25)% of body length.

Testis single, in mid to anterior hindbody, with anterior margin dorsal to ventral sucker or up to 122 posterior to ventral sucker, large, ovoid, 167–315 × 128–235 × 117–174 (219 × 161 × 145), occupying 12–19 (16)% of body length. Post-testicular space 429–874 (651), representing 36–55 (46)% of body length. Vas deferens tubular, thin, arising from anterior region of testis, passing directly to posterior forebody, uniting with naked seminal vesicle. Naked seminal vesicle swollen, tubular, arising near anterior region of testis, winding gently into posterior forebody, uniting with uterus just before common genital atrium. Genital atrium short, canalicular, with thickly muscled walls. Prostatic bulb absent; prostatic cells indistinct. Common genital pore ventral, median at anterior margin of ventral sucker.

Ovary dorsal to ventral sucker or in anterior hindbody, subspherical, 69–118 × 70–113 × 79–100 (88 × 88 × 89). Laurer's canal not observed. Seminal receptacle between ovary and testis, smaller than ovary, sac-like; oviduct passing anterior to ovary, uniting with oötype. Mehlis' gland indistinct. Vitellarium follicular, profusely developed, in single field, 183–371 (257) from anterior extremity to 38–117 (67) from posterior extremity, occupying 69–80 (77)% of body length, wrapping around body from dorsal longitudinal median to dextral and sinistral ventral regions anterior to testis, wrapping around entirety of post-testicular region. Pre-vitelline region occupying 16–25 (18)% of body length; post-vitelline region occupying 3–9 (5)% of body length. Vitelline reservoir ovoid, generally indistinct from surrounding vitelline follicles. Uterus winding gently anteriorly from oötype to common genital atrium. Eggs 1–18 in number, 50–81 (69) long, 49–70 (57) wide. Excretory vesicle straight, tubular, indiscernible beyond termination of caecum; excretory pore terminal.

Etymology

The name of this species is derived from the trigonometry mnemonic 'SOHCAHTOA', which is useful for the recollection of the sine, cosine and tangent ratios in a right triangle.

Trigonocephalotrema sp.

Material examined

*Hologenophor*e. **Queensland:** one from intestine of *N. lituratus*, off LI, coll. D. Huston, 2015 (QMG236528).

Representative DNA sequences. **Queensland:** Partial *18S* rRNA: two identical replicates from specimens from intestine of *N. lituratus* off LI. One representative partial *18S* rRNA sequence submitted to GenBank (MG386263). *ITS2*: two identical replicates from specimens from intestine of *N. lituratus* off LI. One representative *ITS2* sequence submitted to GenBank (MG386265). Partial *28S* rRNA: two identical replicates from intestine of *N. lituratus* off LI. One representative partial *28S* rRNA sequence submitted to GenBank (MG386264).

Remarks

This species occurs sympatrically with *T. euclidi* in the intestine of *N. lituratus*, but on the basis of the single hologenophore may be a much larger worm. Unmounted, however, the two specimens found resembled those of *T. euclidi* closely, and unfortunately

were not recognised as distinct before the only two specimens were consumed, whole or in part, in the molecular analyses.

Differential diagnoses

The possible presence of multiple species of *Trigonocephalotrema* was only suspected after initial molecular exploration of specimens from *N. lituratus* revealed two distinct genotypes. Additional sequencing suggested a species radiation based on host. Thus, host is the most readily available method for differentiating between the three species of *Trigonocephalotrema* described here. *Trigonocephalotrema euclidi* is known from only *N. lituratus* and *N. unicornis*, *T. hipparchi* is known from only *N. brevirostris*, and *T. sohcahtoa* is known from only *Z. scopas* and *Z. velifer*. However, host utilisation may not be a reliable character for differentiating species of *Trigonocephalotrema* in all cases, as exemplified by the undescribed species from *N. lituratus* reported here. Thus, host data should be used along with morphological and molecular data as part of a 'whole evidence' approach when diagnosing species of *Trigonocephalotrema*.

Differentiating *Trigonocephalotrema euclidi*, *T. hipparchi* and *T. sohcahtoa* on a purely morphological basis is difficult, and not possible in all cases, as the range of measurement for nearly all features falls in a continuum for these three species. This problem is further complicated by the simplicity of the ovarian complex and terminal genitalia, which provide little for comparison. In general, *T. hipparchi* is the largest of the three species; the average body length in the type series is ~400 greater than the average body length of *T. euclidi* and *T. sohcahtoa* (see Fig. 3, where holotypes are compared with scale). *Trigonocephalotrema sohcahtoa* has a robust forebody, the ratio of the width and depth of which when compared with body length appears reliable for distinguishing this species from the others. *Trigonocephalotrema euclidi* tends to have a longer forebody and shorter hindbody relative to body length than *T. hipparhi* and *T. sohcahtoa*, and has the greatest ventral to oral sucker width and depth ratios. Beyond these generalities, a combination of characters can be used to differentiate these species in some cases.

Trigonocephalotrema euclidi differs from *T. hipparchi* in having a smaller body length on average (~1300 vs ~1800), a longer forebody (30–44 vs 25–37% of body length), a shorter hindbody (42–58 vs 58–68% of body length), a greater ventral to oral sucker width ratio (0.59–0.89 vs 0.50–0.67), a greater ventral to oral sucker depth ratio (0.55–0.80 vs 0.41–0.46), a smaller ovary on average (89 × 85 × 88 vs 106 × 99 × 100) and carries far fewer eggs on average (2 vs 12). *Trigonocephalotrema euclidi* differs from *T. sohcahtoa* in having a greater body length to depth ratio on average (5.1–7.2 vs 3.9–5.1), a lesser ratio of the forebody width to body length (8–10 vs 10–13% of body length), lesser ratio of forebody depth to body length (7–9 vs 10–13% of body length), longer forebody (30–44 vs 18–31% of body length), shorter hindbody (42–58 vs 58–69% of body length) and carries fewer eggs on average (2 vs 7). *Trigonocephalotrema hipparchi* differs from *T. sohcahtoa* in having a longer body on average (~1800 vs ~1400), greater body length to width ratio (5.1–6.5 vs 4.2–5.9), greater body length to depth ratio (5.9–6.9 vs 3.9–5.2), a lesser ratio of forebody width to body length (7–10 vs 10–13% of body length), lesser ratio of forebody depth to body length (6–9 vs 10–13% of body length), a lesser pharynx width to oral sucker width ratio (0.26–0.37 vs 0.32–0.49), a lesser pharynx depth to oral sucker depth ratio (0.36–0.42 vs 0.42–0.58), less post-caecal body space (14–21 vs 16–31% of body length) and carries more eggs on average (12 vs 7).

Key to genera of the Haplosplanchnidae

1. Oral sucker disc or plate-like 2
Oral sucker unspecialised 4
2. Oral sucker a triangular plate; vitellarium profusely developed, distributed in fore and hindbody ..... *Trigonocephalotrema*
Oral sucker disc-shaped; vitellarium restricted to hindbody 3
3. Oral disc with lobes; vitellarium tubular ..... *Hymenocotta*
Oral disc without lobes; vitellarium follicular ..... *Discocephalotrema*
4. Ventral sucker near posterior extremity; gonads in forebody ..... *Haplosplanchnoides*
Ventral sucker pre or post-equatorial; gonads in hindbody 5
5. Vitelline follicles few, restricted in distribution 6
Vitelline follicles profusely developed, distributed in fore and hindbody 9
6. Testes two ..... *Prohaplosplanchnus*
Testis single 7
7. Cirrus sac present ..... *Parahaplosplanchnus*
Cirrus sac absent 8
8. Vitelline follicles arranged in arc anterior to ventral sucker ..... *Provitellotrema*
Vitelline follicles restricted to hindbody ..... *Haplosplanchnus*
9. Body subspherical; ventral sucker with two pairs of appendages ..... *Pseudoschikhobalotrema*
Body fusiform or elongate; ventral sucker simple or with one pair of appendages ..... *Schikhobalotrema*

Discussion

Because the phylogenetic topology generated here is congruent with present morphological subfamily concepts in the Haplosplanchnidae (Huston *et al*. 2017; present study), the phylogenetic distinctiveness of the new genus would require proposal of a new subfamily. However, we conclude that adding an additional subfamily would provide no additional understanding of the relationships of the haplosplanchnid lineages. We have thus chosen to not recognise subfamilies within the Haplosplanchnidae, as we see little value in subfamily-level division within a clade containing so few genera and species. Morphological identification of individual genera without the use of subfamilies is no more complex than with them. The revised key to the genera of the Haplosplanchnidae provided is as efficient as the keys provided by Madhavi (2005), even with the inclusion of *Trigonocephalotrema*. It is our opinion that a simplification of the taxonomy of the Haplosplanchnidae is the best course of action and propose that subfamilies should not be recognised within this family.

The limited morphological variation between the three new species of *Trigonocephalotrema*, which are clearly genetically distinct and utilise different hosts, exemplifies the need for an integrated whole evidence approach in modern digenean systematics. Although some morphological difference

between specimens of *Trigonocephalotrema* from different hosts does exist, in terms of metrical averages, these differences only become meaningful in light of molecular data. Without molecular data, we may have considered all the species of *Trigonocephalotrema* as one, and we certainly would have missed the undescribed species reported here.

Although the term 'cryptic' has often been loosely applied in parasite systematics (Pérez-Ponce de León and Nadler 2010; Bray and Cribb 2015), such a designation may be warranted in regard to species of *Trigonocephalotrema*. It is currently not possible to delineate all specimens without the accompanying host information, and in some cases without molecular data. Trematodes have the highest reported rate of cryptic diversity for parasitic helminths (Poulin 2011) and there has been a rapid accumulation of literature related to cryptic trematodes in recent years (e.g. Miller *et al*. 2010*b*; Razo-Mendivil *et al*. 2010; Rosas-Valdez *et al*. 2011; Hunter and Cribb 2012; Curran *et al*. 2013; Cribb *et al*. 2014*a*; McNamara *et al*. 2014; Rima *et al*. 2017; Martin *et al*. 2018). However, in many of these cases it has been found that cryptic species revealed as such by molecular analyses can actually be differentiated morphologically *a posteriori* (Bray and Cribb 2015). Although we currently consider species of *Trigonocephalotrema* morphologically cryptic, we follow the opinion of Pérez-Ponce de León and Nadler (2010) that cryptic species should be considered provisionally cryptic. Application of new techniques, statistical or otherwise, may provide methods for distinguishing these species reliably in the future.

Significant difficulty in the study of species of *Trigonocephalotrema* arises from physiological characteristics of these trematodes that frequently result in relatively poor quality morphological specimens. Some of these characteristics, such as the dark and extensive vitellarium, which can obscure most of the internal anatomy, and the lack of complex morphological structures for species delineation, are shared with *Schikhobalotrema*, a group also considered difficult to distinguish morphologically (Huston *et al*. 2017). Specimens of *Trigonocephalotrema*, like those of *Schikhobalotrema*, often react poorly to the dehydration, clearing and mounting process, regardless of the length of time specimens are kept in each solution in the series. Ultimately, some prepared slides are unsuitable for taxonomic study, and only the highest quality slides provide adequate views of the internal anatomy. Another issue arises from the lack of eggs in many specimens that otherwise appear to be sexually mature adults. Exclusion of such specimens reduces the number of prepared slides that can be used for taxonomic study. It is best to base type series on gravid trematodes so as to avoid including data from immatures, which may skew ranges and averages of certain structures (especially those of underdeveloped reproductive organs), as well as to avoid inclusion of morphologically similar immature heterospecifics. We estimate that less than 50% of mature specimens of the three *Trigonocephalotrema* species in our collection actually possessed eggs. It is possible that these species develop and lay eggs in small clutches, rather than develop and lay continually as is seen in many trematode lineages. Future work may reveal that non-gravid specimens can be assigned to species based on their morphometrics, but because of the issues with species identity discussed above, we advise caution when working with non-gravid specimens of this group.

Species of *Trigonocephalotrema* are so far restricted to acanthurid fishes in the genera *Naso* and *Zebrasoma*. Between 1991 and 2017, our research group has examined many other acanthurids from the Great Barrier Reef, including over 300 individuals of multiple species of *Acanthurus* Forsskål and over 80 individuals of two species of *Ctenochaetus* Gill. Although haplosplanchnids have been recovered from some of these fishes (unpubl. data), none of these specimens have the distinctive triangular plate-like oral suckers present in species of *Trigonocephalotrema*. Similarly, no specimens consistent with *Trigonocephalotrema* have been recovered from the many fishes from other families known to host haplosplanchnids examined on the Great Barrier Reef during the same time period (see Huston *et al*. 2017). Given that fish species of the genera *Naso* and *Zebrasoma* are restricted to the Indo-West Pacific marine region (Randall 2002), we suspect that the *Trigonocephalotrema* lineage has a similar pattern of geographic restriction. It is thus surprising that such a distinctive genus as *Trigonocephalotrema* has escaped attention until now. Several workers who have described haplosplanchnids have examined acanthurid fishes in the Indo-West Pacific (e.g. Pritchard and Manter 1961; Yamaguti 1970; Machida and Uchida 1990). Significantly, Machida and Uchida (1990) studied trematodes from fishes in the genus *Naso* collected off Japan, Palau and the Philippines, but reported only one species of haplosplanchnid, *Schikhobalotrema hawaiiensis* Pritchard & Manter, 1961. These studies pose the question of whether additional sampling of fishes of the genera *Naso* and *Zebrasoma* from other parts of the Indo-West Pacific will reveal further species richness for *Trigonocephalotrema*.

The molecular phylogeny constructed for this study demonstrates the presence of four well-supported monophyletic lineages in the Haplosplanchnidae. Inclusion of *Trigonocephalotrema* in the molecular analyses in this study added support to the monophyly of *Schikhobalotrema*, which was not well supported in the molecular phylogeny of Huston *et al*. (2017). Improved support may also relate to better outgroup choice in the present analyses. In our previous molecular phylogeny (Huston *et al*. 2017) we included a species of the superfamily Apocreadioidea Skrjabin, 1942, along with two species of Echinostomatoidea Looss, 1902 in our outgroup, based on the relationships in the molecular phylogeny of the Digenea provided by Olson *et al*. (2003). However, a more recent analysis of the higher order relationships of the Digenea suggests that species of Paramphistomoidea Fischoeder, 1901, Pronocephaloidea Looss, 1899 and Echinostomatoidea are most closely related to those of the Haplosplanchnoidea (Littlewood *et al*. 2015). The revised outgroup may have alleviated the minor alignment ambiguities observed in the *18S+28S* rRNA dataset of Huston *et al*. (2017). Besides the addition of *Trigonocephalotrema* and the increased support for *Schikhobalotrema*, the present molecular phylogeny of the Haplosplanchnidae provides no further insights beyond those previously discussed (see Huston *et al*. 2017).

The proposal of a morphologically conspicuous new genus forming a novel phylogenetic lineage in the present study highlights the diversity of digenean fauna still awaiting

discovery in coral reef communities. Proposal of taxonomic groupings above the species rank based on newly discovered taxa are becoming increasingly rare in the Digenea; they are more often a result of reorganisation in the classification of already recognised taxa (Cribb and Bray 2011). After many years of sustained study on the digenean fauna of the Great Barrier Reef, many groups are yet to be studied in detail (Cribb *et al*. 2014*b*). The lack of effort in regard to the Haplosplanchnidae is due in part to the difficulties inherent in their study, but primarily because of the vast volume of taxonomic work to be done characterising the various digenean lineages in the region. There have simply been too many trematodes, and too few taxonomists characterising them. Although the workforce tasked with characterising the remainder of the digenean fauna of the Great Barrier Reef remains small, renewed effort focusing on understudied groups will add much to our overall understanding of digenean diversity in coral reef ecosystems.

Conflicts of interest

The authors declare no conflicts of interest.

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