Syst Parasitol (2010) 76:145–157
DOI 10.1007/s11230-010-9242-2

Description of *Sinodiplectanotrema malayanum* n. sp. (Monogenea: Diplectanidae), with comments on the taxonomic position of the genus

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Received: 15 December 2009 / Accepted: 23 January 2010

Abstract Monogeneans identified as *Sinodiplectanotrema malayanum* n. sp. were collected from the fish *Pennahia anea* (Sciaenidae) off the west coast of Peninsular Malaysia. The new species is recognised on the basis of morphometrical differences in the anchors, marginal hooks and eggs and apparent differences in the 28S rDNA sequence data. The new species possesses features (ovary looping the intestinal caecum, body spines, a vagina and haptoral reservoirs) not noted in the original description of the type and only other species of the genus, *S. argyrosomus* Zhang, 2001, necessitating the re-assignment of the genus to the Diplectanidae Monticelli, 1903, a move which is supported by 28S rDNA evidence. *Sinodiplectanotrema* is redefined on the basis of the observation of several features not included in the original diagnosis.

Introduction

During 2008 and 2009 we had the opportunity to collect specimens of the greyfin croaker *Pennahia anea* (Bloch) [also known as *Argyrosomus aneus* (Bloch)] off Pulau Langkawi, Malaysia, in the Andaman Sea, and off Sungai Buloh, Malaysia, in the Straits of Malacca. The Sciaenidae is a large, diverse teleost family with 70 genera and 270 species (Froese & Pauly, 2010). A literature search for monogenean species on *Pennahia* spp. and on *Argyrosomus* spp. revealed records of *Sinodiplectanotrema argyrosomus* Zhang, 2001 on *Pennahia anea* [as *Argyrosomus aneus*] (type-host). *S. argyrosomus* can also be found on *Johnius distinctus* (Tanaka) [as *Wak tingi* (Tang)], the labrid *Halchoeres nigrescens* (Bloch & Schneider) and *Nibea albiflora* (Richardson) from off the coast of China (Zhang, 2001; Wu et al., 2006, 2007). There are some discrepancies in the publication dates and the spelling of the type-species in the original description of *Sinodiplectanotrema*. As first revisors (ICZN Article 24.2.2), we propose acceptance of *Sinodiplectanotrema argyrosomus* Zhang, 2001 as the valid name of the type-species, since the species is clearly named after the generic name of the host [the epithet *argyrosomus* was used first on page 164 of Zhang (2001)], and we reject the name *argyromus* as used on pages 164, 363 and 364. Furthermore, the descriptions for the species and genus were officially published in 2001 and not in 1999, as cited on page 363 of the latter work.

Monogeneans recovered from *Pennahia anea* resembled the description of *Sinodiplectanotrema argyrosomus*. However, a close examination of these specimens revealed not only differences between them and *S. argyrosomus*, which suggests that they

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represent a new species, but also important features, not reported in the original description of the latter species, that necessitate amendments to the generic diagnosis and the re-assignment of *Sinodiplectanotrema* Zhang, 2001 to a different monogenean family.

Materials and methods

Six specimens of *Pennahia anea* were collected in Malaysian coastal waters, one off Pulau Langkawi in the Andaman Sea and five off Sungai Buloh in the Straits of Malacca. Their monogeneans were collected and some were preserved in 70% ethanol for molecular studies, whereas others were prepared for morphological studies following Lim & Gibson (2008). Briefly, they were removed from the gills and flattened using coverslip pressure in order to best expose their soft anatomical structures and hard parts. Some were mounted in modified ammonium picrate glycerine (Lim & Gibson, 2008) and later made into unstained permanent mounts in Canada balsam. Other specimens were prepared for staining in Gomori's triple stain (Humason, 1972). Both stained and unstained specimens were examined using both bright field and phase contrast optics using a Leica DMRB microscope. Images of the hard and soft body parts were captured using a Leica digital camera (3.3 MP) and QWin Plus image analysis software, and drawn on a digitising tablet (WACOM) using Adobe Illustrator software. Measurements of the sclerotised hard-parts (both haptoral and reproductive) were made on flattened stained or unstained specimens cleared in ammonium picrate glycerine using the measuring option in QWin software. The measurements are given as the mean and range (within parentheses) in micrometres, and the number of measurements (n) is given after the metric data in the description. For two-dimensional measurements, length is given before breadth. The marginal hooks are enumerated as in Gusev (1976).

DNA from three specimens preserved in 70% ethanol was extracted using a DNEasy extraction kit from Qiagen. For each specimen, 5 µl of extracted DNA were used as a template in PCR reactions to amplify the partial D1-D2 domain of the 28S rDNA using primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) (Mollaret et al., 2000). PCR reactions (50 µl) were 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 (Biometra) 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. Aliquots (10 µl) from the amplicons were examined in 1.3% agarose gels, stained with ethidium bromide and viewed under a UV illuminator. The remaining 40 µl of each amplicon were purified using a DNA purification kit (Qiagen) and subjected to automated DNA sequencing (ABI 3730 DNA Sequencer, First Base Laboratories) using the same primers as used for PCR amplification.

The 28S rDNA sequences of 26 species of diplectanids and two species of *Dactylogyrus* Diesing, 1850 (used as outgroups) from GenBank (Table 2) and the 28S rDNA sequences of the three specimens from the present study were edited and aligned with Clustal X (Thompson et al., 1997) using the default parameter and verified/edited visually using BioEdit ver. 7.0.5.3 (Hall, 1999). Phylogenetic trees were inferred using neighbour-joining (NJ), minimum evolution (ME) (Fig. 6) and maximum parsimony (MP) (Fig. 7) with MEGA ver. 4.0b (Kumar et al., 2004). The Kimura 2-parameter model was used to estimate distances for the NJ and ME analyses. The robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications for the MP, NJ and ME analyses.

The degree of similarities between the present three specimens of *Sinodiplectanotrema* and other diplectanids found in the same clade (see below; Figs. 6 and 7, i.e. *S. argyrosomus* (DQ157673), *Sinodiplectanotrema* sp. (EF100778), *Murraytrema pricei* (now *M. bychowskyi*) (DQ157672), *Lobotrema sciaenae* (EF100556), *Diplectanum umbrinum* (EF100560), *D. blairense* (also referred to as *Paradiplectanum blairense*) (AY553627) and *D. sillagonum* (AY553626) (also referred to as *P. sillagonum*) were calculated using BioEdit and the results tabulated (Table 3). (In Figs. 6 and 7, the species names are as given in GenBank and their current names are indicated in Table 2). Comments on the validity of diplectanid species in GenBank are restricted to those in the same clade as the present material.

***Sinodiplectanotrema malayanum* n. sp.**

Type-host: *Pennahia anea* (Bloch) (Sciaenidae).
Type-locality: Andaman Sea, off Pulau Langkawi, Peninsular Malaysia (6°28′N; 99°47′E).
Other locality: Straits of Malacca, off Sungai Buloh, Malaysia (3°15′N; 101°17′E).
Site: Gills.
Specimens studied: 58 specimens studied from 6 hosts; 42 specimens measured.
Type-material: Holotype BM(NH) 2010.1.27.1 and 2 paratypes BM(NH) 2010.1.27.2–3 in the Natural History Museum, London; 55 paratypes MZUM (P)1250(P)-1304(P) in the University of Malaya Collection.

Description (Figs. 1–5; Table 1)

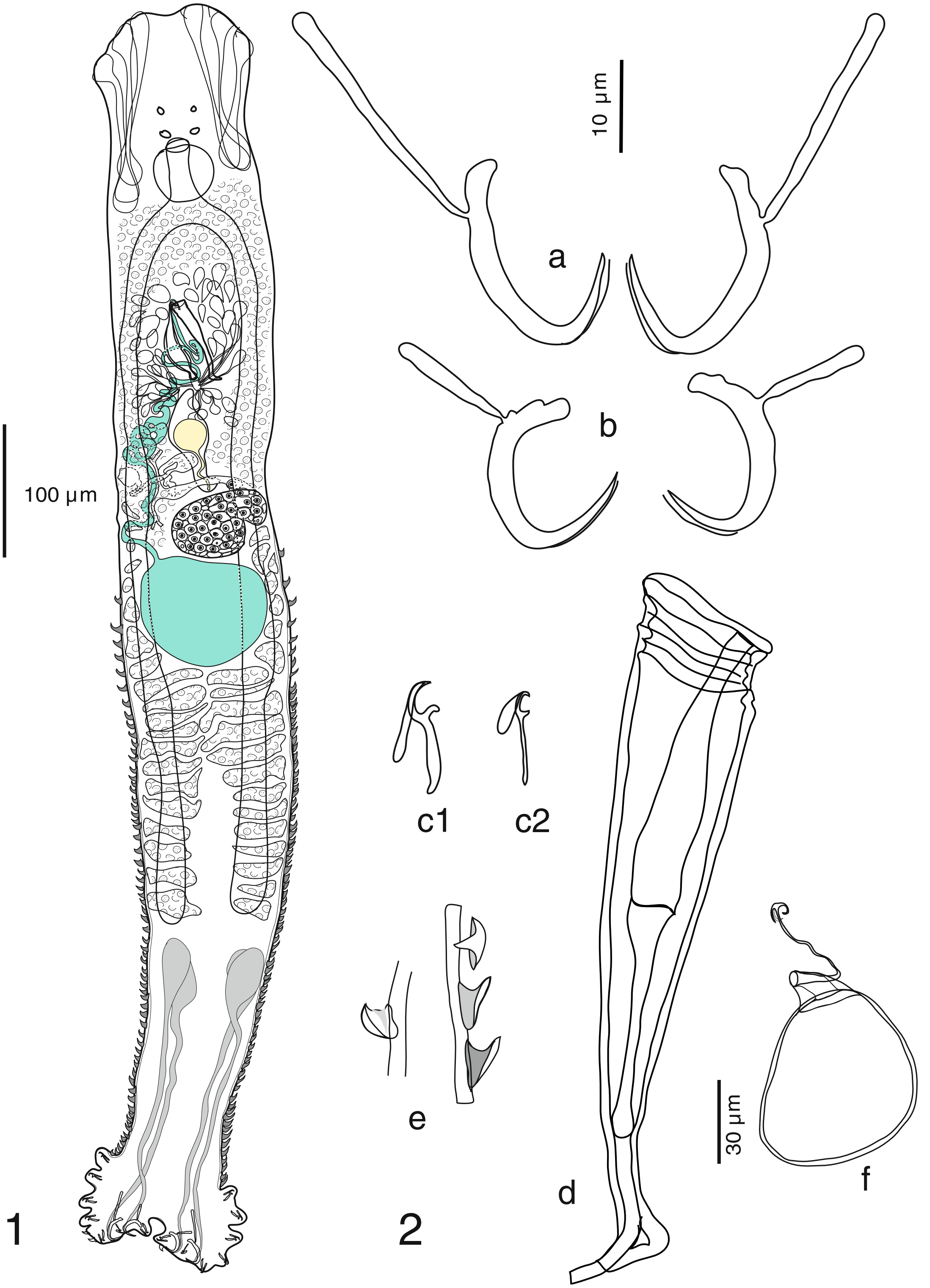
Body large, 1,481 (729–2,027) × 204 (124–320) (n = 42); peduncle long, 341 (177–610) × 135 (81–214) (n = 37), slightly tapered posteriorly, distinguishable anteriorly from body only by absence of vitelline follicles and presence of haptoral reservoirs; haptor comparatively small, 128 (61–222) × 219 (112–348) (n = 41), comprising 2 lateral digitate lobes; squamodiscs/lamellodiscs absent. Anterior region with 3 pairs of prominent head-organs and 4 pigmented eye-spots. Mouth closely posterior to eye-spots; pharynx round, 70 (44–96) × 71 (40–91) (n = 34); oesophagus short; intestine bifurcates just posterior to pharynx; caeca blind, pass posteriorly to end in middle of post-testicular field. Tegumental spines on posterior half of body, distributed sparsely at about testicular level then increasing in density more posteriorly to cover peduncle and entire haptor. Haptor without connecting bars, armed with 7 pairs of marginal hooks and 2 pairs of anchors. Marginal hook pairs 1–6 of similar length, 12 (10–13) (n = 41), located on 6 marginal projections; hooks 7 close to anchors, length 10 (9–11) (n = 38). Anchors with outer root modified as stiff rod with narrow connection ('hinge') linking it to main part of anchor, which allows it to flex; smaller ventral anchors have inner length 19 (16–21) (n = 38), inner root 6 (4–7) (n = 38), outer length 26 (20–30; variable due to hinged outer root) (n = 38), outer root 14 (10–16) (n = 38), point curved 12 (9–13) (n = 38); dorsal anchors with inner length 24 (22–26) (n = 35), outer length 39 (31–50; variable due to hinged outer root) (n = 35), inner root 6 (4–7) (n = 35), outer root 26 (17–30) (n = 35), point curved, 13 (8–15) (n = 35). Extrinsic muscles from body attach to each marginal hook. Four pyriform reservoirs in anterior peduncle, with long ducts which extend into haptor to open exteriorly at point of extrusion of anchors.

Testis globular, immediately posterior to ovary. Vas deferens leaves antero-sinistral region of testis, loops left intestinal caecum to ventral side, ascends and coils anteriorly expanding slightly, narrows just before entering initial part of male copulatory organ. Male copulatory organ consists of simple, tapered copulatory tube, with proximal (initial) part situated anteriorly and narrow, bent, bill-shaped distal end directed posteriorly, length 84 (57–97) (n = 42), without accessory piece; single prostatic reservoir, with ducts from prostatic glands entering posteriorly; duct from prostatic reservoir opens into initial part of copulatory tube.

Ovary elongate, with narrower antero-distal region looping right intestinal caecum from dorsal to ventral side, then forms oviduct; oviduct receives duct from seminal receptacle and common vitelline duct, passes through indistinct Mehlis' gland, then continues anteriorly and medially to form uterus; uterine pore opens close to distal extremity of copulatory tube. Single egg a rounded lozenge-shape, 69 (62–75) × 56 (51–65) (n = 5), with long filament, 72 (63–85) (n = 5); distal extremity of filament branched (Fig. 5), forming several thin sclerotised strands with pointed distal tips, which may be reflexed, loosely flexed or in tight bundle. Single vagina sinistral, at mid-body level, subspherical, prominent, muscular, opens via long, coiled duct into seminal receptacle. Vitellarium follicular; follicles extend from just posterior to pharynx to middle of post-testicular field, grouped into compact blocks, particularly in posterior third of body. Lateral vitelline ducts open into indistinct vitelline reservoir.

Differential diagnosis

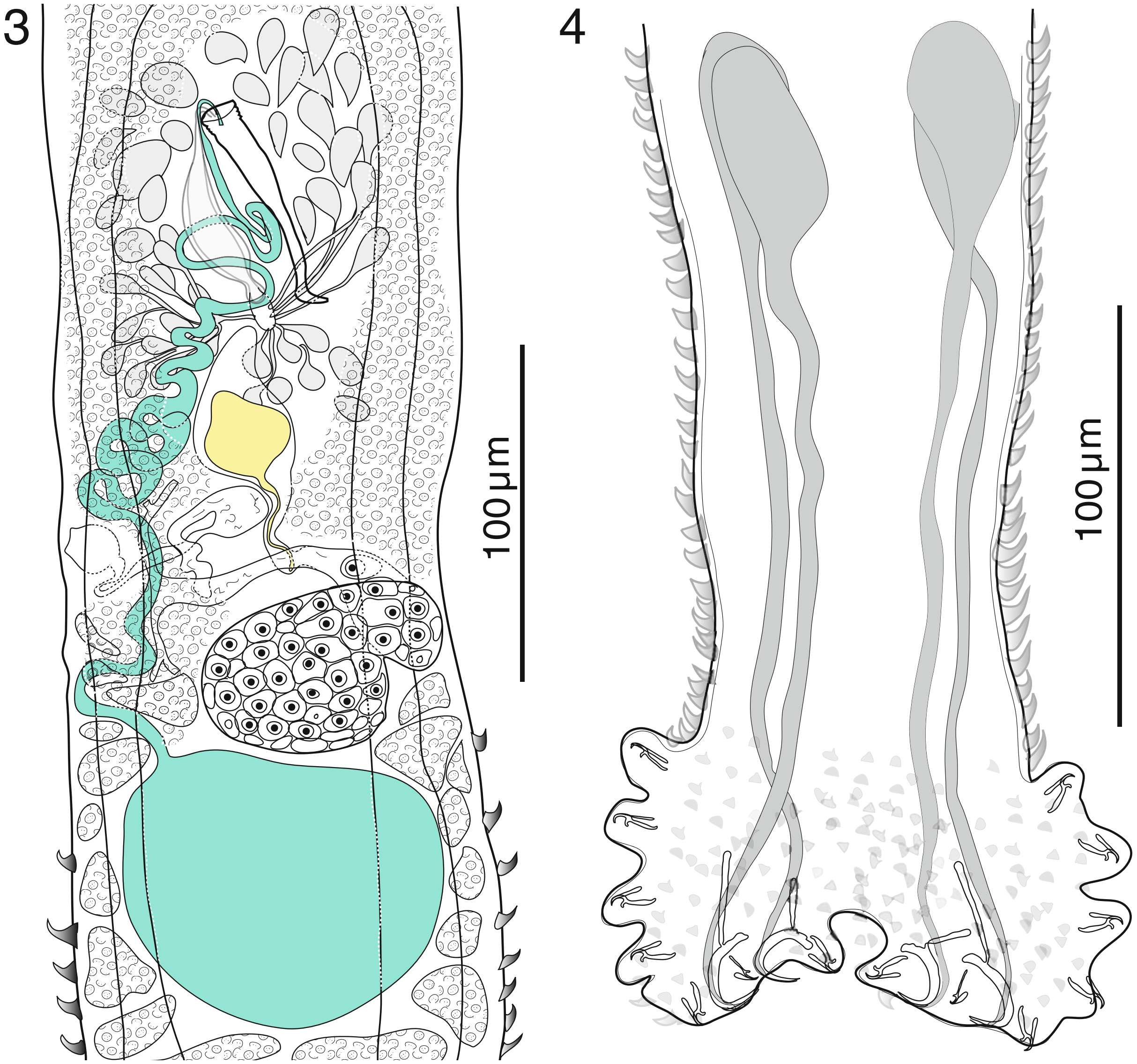
The present material resembles *Sinodiplectanotrema argyrosomus*, as described by Zhang (2001), in the unusual shape of the anchors, which have outer roots with a narrow, flexible connection (hinge) with the main part of anchor, the shape of the posteriorly directed copulatory tube, the vitellarium with follicles



Figs. 1–2 1. *Sinodiplectanotrema malayanum* n. sp. (composite dorsal view; tegumental spines omitted from haptor for clarity). 2. Hard-parts (a–e relate to upper scale-bar): a, dorsal anchor; b, ventral anchor; c1, marginal hook (1–6) from haptoral projections; c2, marginal hook (7) located near anchors; d, male copulatory organ; e, spines on body tegument; f, egg

distributed in compact blocks (particularly in the posterior third of the body), and the elongate shape of the body with a long peduncle and small, digitate haptor. In the original description of *S. argyrosomus* several features seen in the present material were not described; these are the vagina, the presence of tegumental spines (it is common for haptoral and body spines to be lost in dead specimens), the ovary looping the right intestinal caecum and the fact that the hooks of pair 7 are smaller than the others (i.e. 10 (9–11) and 12 (10–13) µm, respectively) and not on digitate projections.

The present specimens have spines covering the posterior half of the body (Fig. 1) and the haptor (Fig. 4), six pairs of marginal hooks on digitate lateral haptoral lobes and the distal region of the ovary and oviduct looping the right intestinal caecum from the dorsal to the ventral side. The latter is a diplectanid feature, suggesting that *Sinodiplectanotrema* is not an ancyrocephalid, as designated by Zhang (2001). In



Figs. 3–4 *Sinodiplectanotrema malayanum* n. sp. 3. Mid-body (dorsal view). 4. Haptor (dorsal view)

addition, body spines have not been reported for ancyrocephalids but are regularly found on diplectanids. Furthermore, the copulatory tube of this species has a typical diplectanid arrangement, with the proximal (initial) part of the copulatory tube situated anteriorly and its distal part directed posteriorly, as in species of *Diplectanum* Diesing, 1858, *Lobotrema* Tripathi, 1959 and *Murraytrema* Price, 1937 (see Oliver, 1987) but not apparently as in ancyrocephalid species (see Yamaguti, 1963; Bychowsky, 1957) (Table 2).

Unfortunately, we were unable to obtain the type-specimens of *S. argyrosomus* for comparison, despite several attempts, and are uncertain whether they are lost or misplaced. We are left only with the original description of Zhang (2001). However, the morphometric data for the anchors and marginal hooks of the present specimens do differ from those given in the latter description (cf. Table 1). The Malaysian specimens have eggs (mean 69 × 56 µm) with a longer filament (mean 72 µm), compared with 71 × 43 µm and a filament of 29 µm in the Chinese specimens. There is also a terminal branching of the filament (Fig. 5) which was not mentioned for *S. argyrosomus*. Marginal hooks present on projections of the haptor were noted in the original description of *S. argyrosomus*, but the present specimens show that only the six comparatively larger pairs are found actually on the projections; these hooks in the Malaysian material are also comparatively smaller

Table 1 Comparative morphometric data (in micrometres) for *Sinodiplectanotrema* spp.

Species Source Locality No. of specimens measured	*S. argyrosomus* Zhang (2001) Off China 14	*S. malayanum* n. sp. Present data Off Peninsular Malaysia 42
Body length	(975–1,696)	1,481 (729–2,027)
Body width	(117–184)	204 (124–320)
Pharynx length	(49–65)	70 (44–96)
Pharynx width	(69–70)	71 (40–91)
Peduncle length		341 (177–610)
Peduncle width		135 (81–214)
Copulatory tube length	(70–103)	84 (57–97)
Initial part	(14–23)	
Egg size	71 × 43	69 (62–75) × 56 (51–65)
Egg filament	29	72 (63–85)
Haptor length	(117–195)	128 (61–222)
Haptor width	(244–320)	219 (112–348)
Ventral anchor:		
Inner length	(18–21)	19 (16–21)
Inner root		6 (4–7)
Outer length		26 (20–30)*
Outer root	(10–18)	14 (10–16)
Point	(11–14)	12 (9–13)
Dorsal anchor:		
Inner length	(25–28)	24 (22–26)
Inner root		6 (4–7)
Outer length		39 (31–50)*
Outer root	(20–32)	26 (17–30)
Point	(12–15)	13 (8–15)
Marginal hooks 1–6	(13–15)	12 (10–13)
Marginal hook 7	(13–15)	10 (9–11)

* Variable due to flexible rod-like outer root

Table 2 List of the monogenean species used in this study with their host, locality and GenBank accession numbers

Monogenean species	Host species	Locality	GenBank
Diplectanidae Bychowsky, 1957			
Murraytrema pricei (=*M. bychowskyi*)	*Nibea albiflora*	Guangdong, China	DQ157672
Diplectanum penangi	*Lates calcarifer*	Hainan, China	DQ054821
Diplectanum umbrinum	*Johnius amblycephalus*	Guangdong, China	EF100560
Diplectanum grouperi	*Epinephelus coioides*	Guangdong, China	AY553628
Diplectanum blairense (=*Paradiplectanum blairense*)	*Sillago sihama*	Hainan, China	AY553627
Diplectanum sillagonum (=*Paradiplectanum sillagonum*)	*Sillago sihama*	Hainan, China	AY553626
Diplectanum veropolynemi	*Polynemus sextarius*	Guangdong, China	AY553625
Lamellodiscus japonicus	*Sparus macrocephalus*	Guangdong, China	EF100561
Lamellodiscus pagrosomi	*Pagrosomus major*	Hainan, China	EF100562
Lamellodiscus spari	*Lates calcarifer*	China	DQ054823
Lamellodiscus acanthopagri	*Lates calcarifer*	China	DQ054822
Lepidotrema longipenis	*Therapon jarbua*	Guangdong, China	EF100563
Pseudorhabdosynochus lantauensis	*Epinephelus brunneus*	Guangdong, China	AY553624
Pseudorhabdosynochus coioidesis	*Epinephelus coioides*	Guangdong, China	AY553623
Pseudorhabdosynochus epinepheli	*Epinephelus brunneus*	Guangdong, China	AY553622
*Pseudorhabdosynochus latesi****	*Lates calcarifer*	Guangdong, China	AY553621
Pseudorhabdosynochus seabassi (=*Laticola seabassi*)	*Lates calcarifer*	Guangdong, China	AY553620
Pseudorhabdosynochus shenzhenensis	*Lates calcarifer*	China	DQ054830
Calydiscoides sp.	*Nemipterus bathybius*	Guangdong, China	EF100558
Calydiscoides indianus	*Nemipterus japonicus*	Guangdong, China	EF100557
Lobotrema sciaenae	*Nibea albiflora*	Guangdong, China	EF100556
Laticola paralatesi	*Lates calcarifer*	China	DQ054826
Laticola lingaoensis	*Lates calcarifer*	China	DQ054825
Laticola latesi	*Lates calcarifer*	China	DQ054824
Sinodiplectanotrema malayanum MSIA1	*Pennahia anea*	Malaysia	GU573891
Sinodiplectanotrema malayanum MSIA2	*Pennahia anea*	Malaysia	GU573892
Sinodiplectanotrema malayanum MSIA3	*Pennahia anea*	Malaysia	GU573893
Sinodiplectanotrema argyrosomus	*Pennahia anea**/*Nibea albiflora***	Guangdong, China	DQ157673
Sinodiplectanotrema sp. HGY	*Nibea albiflora*	Guangdong, China	EF100778
Dactylogyridae Bychowsky, 1933			
Dactylogyrus lamellatus	*Ctenopharyngodon idellus*	China	EF100533
Dactylogyrus gotoi	*Lateolabrax japonicus*	China	EF100531

* Valid name following FishBase (Froese & Pauly, 2010)

** In Wu et al. (2006) two host species were given for the sequence DQ157673, but the same sequence was noted as *S. argyrosomus* from *Nibea albiflora* in Wu et al. (2007)

*** Spelled *latesis* in GenBank

(hooks 1–6 = 10–13 µm and hooks 7 = 9–11 µm *vs* hooks 1–7 = 13–15 µm in *S. argyrosomus*).

The present molecular analysis shows that the sequences for the three specimens from the present collection were identical (Table 3) and that they form a clade with GenBank data listed under *S. argyrosomus*, *Sinodiplectanotrema* sp. and *Murraytrema pricei* Bychowsky & Nagibina, 1977 nec Caballero, Bravo & Grocott, 1955 (now *M. bychowskyi* Oliver, 1987) (Figs. 6 and 7) (see below). Had all sequences been *S. argyrosomus*, then the 28S rDNA sequence of *S. argyrosomus* (DQ157673) and present three sequences should have been identical (100% similarity). However, the sequences of the present

Fig. 5 Egg-filaments of *Sinodiplectanotrema malayanum* n. sp. showing variations in the form of the tip

specimens are only 97% and 96% similar to *S. argyrosomus* and *Sinodiplectanotrema* sp., respectively (Table 3) (see below). Nevertheless, the actual identity of the Chinese sequences is questionable (see below).

When one considers the number of cryptic species and miniscule morphological differences between species in other groups (e.g. *Gyrodactylus* spp. in salmonids) and the presence of numerous congeners on the same host species (e.g. *Dactylogyrus* spp. in cyprinids) (see Shinn, Sommerville & Gibson, 1995; Gibson, Timofeeva & Gerasev, 1996), the lumping of the present material with *S. argyrosomus* could result in the loss of useful ecological and other data. Therefore, based on the morphometric (albeit small) and molecular (28SrDNA) differences outlined above, and the geographical distance between the records (Indian Ocean *vs* Chinese Pacific waters), the present species is considered as new to science (rather than as conspecific with *S. argyrosomus*) and named *Sinodiplectanotrema malayanum* n. sp.

Discussion

This is the second species of *Sinodiplectanotrema* to be described from *Pennahia anea*. Despite not being able to re-examine the type-specimens and in the absence of details of the following characters (vagina, tegumental spines, haptoral reservoirs, ovary looping the right intestinal caecum) in both the original description of the type-species, *S. argyrosomus*, and in the generic diagnosis, the present specimens are still considered to belong to *Sinodiplectanotrema* in view of the lack of squamodiscs/lamellodiscs and connecting bars, the unique anchors with 'hinged' outer roots, the shape of the posteriorly directed copulatory tube, the vitellarium with follicles distributed in compact blocks and the elongate shape of the body with a long peduncle and small, digitate haptor.

Taxonomic position of *Sinodiplectanotrema* Zhang, 2001

Sinodiplectanotrema was assigned to the Ancyrocephalidae Bychowsky, 1937 by Zhang (2001). However, Wu et al. (2006) listed it with diplectanids without comment in their molecular studies of

Table 3 Similarities (as sequence identity) of partial 28S rDNA sequences (D1–D2 domain) from *Sinodiplectanotrema* (SHGY = *Sinodiplectanotrema* sp. HGY, SAR = *S. argyrosomus*, MSIA1 = *S. malayanum* individual 1, MSIA2 = *S. malayanum* individual 2, MSIA3 = *S. malayanum* individual 3), *Lobotrema* (LSC = *L. sciaenae*), *Murraytrema* (MPR = *M. pricei* (=*M. bychowskyi*)) and *Diplectanum* (DUM = *D. umbrinum*, DBL = *D. blairense* (=*Paradiplectanum blariense*), DSI = *D. sillagonum* (=*P. sillagonum*))

	SHGY	SAR	MSIA1*	MSIA2*	MSIA3*	MPR	LSC	DUM	DBL	DSI
SHGY	1.000	0.933	0.971	0.971	0.971	*1.000*	0.842	0.813	0.791	0.800
SAR	–	1.000	0.961	0.961	0.961	0.933	0.815	0.793	0.764	0.769
MSIA1*	–	–	1.000	*1.000*	*1.000*	0.971	0.850	0.828	0.800	0.805
MSIA2*	–	–	–	1.000	*1.000*	0.971	0.850	0.828	0.800	0.805
MSIA3*	–	–	–	–	1.000	0.971	0.850	0.828	0.800	0.805
MPR	–	–	–	–	–	1.000	0.842	0.813	0.791	0.800
LSC	–	–	–	–	–	–	1.000	0.894	0.810	0.811
DUM	–	–	–	–	–	–	–	1.000	0.790	0.801
DBL	–	–	–	–	–	–	–	–	1.000	0.911
DSI	–	–	–	–	–	–	–	–	–	1.000

Italicized cells indicate 100% similarity

* Sequences of *Sinodiplectanotrema* from the current study

Haliotrema spp. and later (2007) transferred it to the Diplectanidae Monticelli, 1903 based solely on molecular evidence. Nevertheless, the genus was not included in the revision of the Diplectanidae given by Domingues & Boeger (2008). As indicated above, the present specimens of *Sinodiplectanotrema* have features, such as the anterior part of the ovary looping the right intestinal caecum, the posteriorly directed tapering copulatory tube lacking an accessory piece and the presence of spines on the posterior half of the body, all of which are consistent with this genus belonging to the Diplectanidae.

As part of the current study, we analysed the 28S rDNA sequences for three of our specimens of *S. malayanum* n. sp. and compared them with sequences for other diplectanids available in GenBank, paying particular attention to material of Wu et al. (2006, 2007) listed as *S. argyrosomus* (DQ157673), *Sinodiplectanotrema* sp. (EF100778) and *Murraytrema pricei* [now *M. bychowskyi*] (DQ157672). The latter three sequences and those from the new species form a sister group with a high bootstrap value (100%) within a clade which also includes *Lobotrema sciaenae* (Bychowsky & Nagibina, 1977), *Diplectanum umbrinum* Tripathi, 1955, *D. blairense* Gupta & Khanna, 1974 (also referred to as *Paradiplectanum blairense*) and *D. sillagonum* Tripathi, 1957 (also referred to as *P. sillagonum*) (Figs. 6 and 7).

Our morphological and molecular data confirm that *Sinodiplectanotrema*, as represented by the present species, is a diplectanid and support the molecular finding of Wu et al. (2007) that *Sinodiplectanotrema* is a diplectanid genus. However, in the Chinese study, the molecular data were from *Sinodiplectanotrema* sp. and *S. argyrosomus* apparently obtained from *Nibea albiflora* [in fact sequence DQ157673 is listed (erroneously) as being from both *Argyrosomus aneus* and *Nibea albiflora* by Wu et al. (2006) but from *N. albiflora* only by Wu et al. (2007)] and not from the type-host, *Pennahia anea* (=*Argyrosomus aneus*). The 28S rDNA sequences of *Sinodiplectanotrema* sp. (EF100778) and *M. pricei* (=*M. bychowskyi*) (DQ157672), the latter being the usual diplectanid recorded from *N. albiflora*, are identical, indicating that they are the same species. Hence, the actual identity of DQ157672, listed as *M. pricei* (=*M. bychowskyi*), is uncertain and could be *Sinodiplectanotrema* sp. based on their 28SrDNA (Table 3) or vice versa. However, the close similarity of the conserved 28SrDNA of *S. argyrosomus* and *Sinodiplectanotrema* sp. with the present three sequences of *S. malayanum* n. sp. suggests that the former two species are *Sinodiplectanotrema* spp. and that the sequence DQ157672 is that of *Sinodiplectanotrema* sp. It is therefore possible that the host species might have been misidentified, as specimens

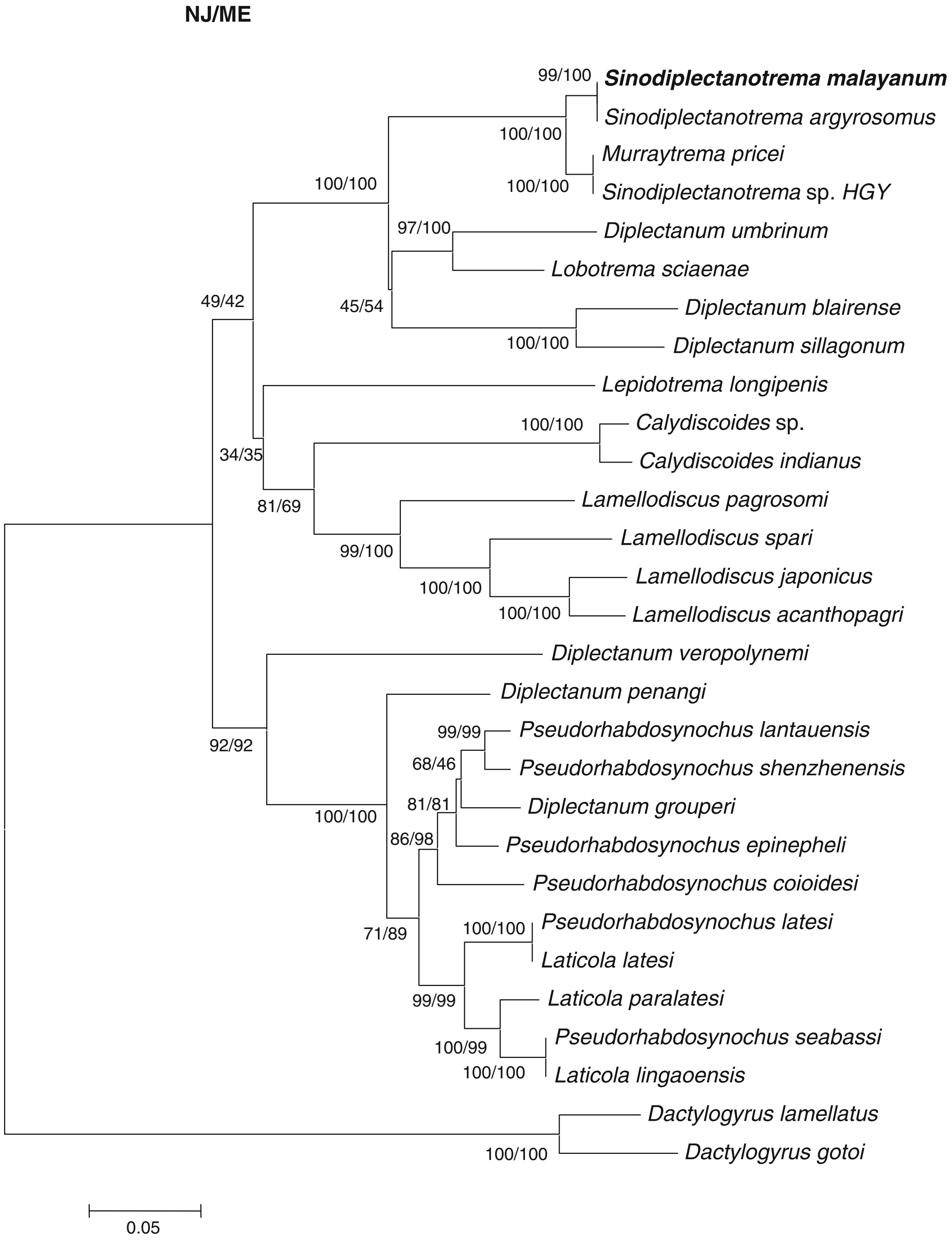


Fig. 6 Combined neighbour-joining (NJ) and minimum evolution (ME) tree for the Diplectanidae obtained using partial 28S rDNA sequences (D1–D2 domain), with *Dactylogyrus* spp. as outgroups. Bootstrap values shown along the branches are based on 1,000 replicates for the NJ and ME analysis. (See Table 2 for data on the material and GenBank accession numbers)

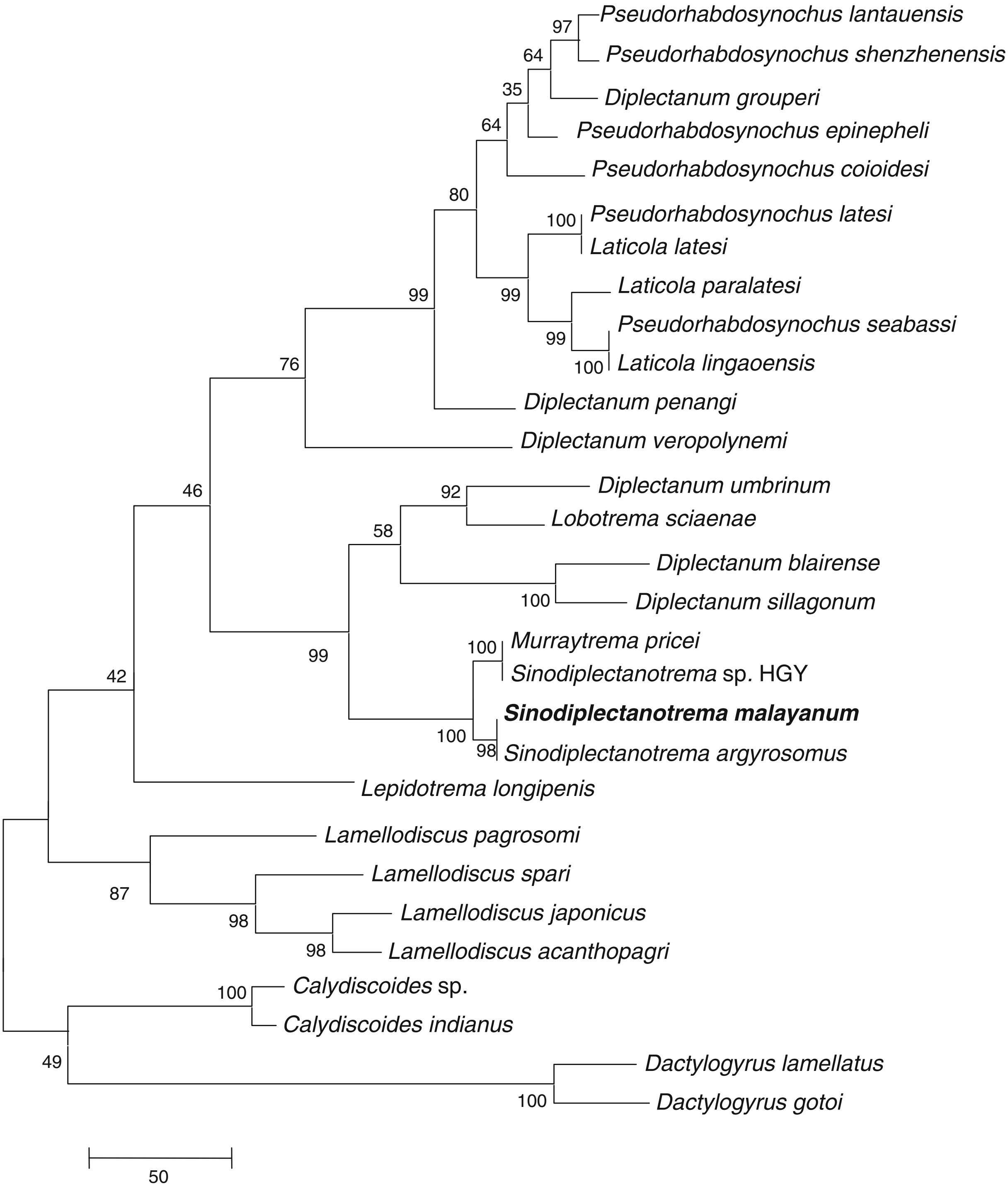


Fig. 7 Maximum parsimony (MP) tree for the Diplectanidae obtained using partial 28S rDNA sequences (D1–D2 domain), with *Dactylogyrus* spp. as outgroups. Bootstrap values shown along the branches are based on 1,000 replicates for the MP analysis. (See Table 2 for data on the material and GenBank accession numbers)

of *Sinodiplectanotrema* have not, previous to the work of Wu et al. (2006, 2007), been reported from *Nibea* spp. There is, therefore, a need to re-examine material from Chinese *N. albiflora*.

Morphologically, the present specimens of *Sinodiplectanotrema* are similar to members of the Murraytrematoidinae Oliver, 1982, i.e. *Murraytrema* Price, 1937, *Lobotrema* Tripathi, 1959 and *Murraytrematoides* Yamaguti, 1958, in lacking squamodiscs and lamellodiscs (Oliver, 1987). The anchors of species of *Murraytrema*, *Lobotrema* and *Murraytrematoides* have long outer roots which resemble the rod-like outer roots but lack the narrow, hinge-like connections with the main part of the anchors of *S. argyrosomus* and the present specimens of *S. malayanum*. The male copulatory organ of both species of *Sinodiplectanotrema* is also similar to those of species of *Lobotrema* and *Murraytrematoides* (see Oliver, 1987). Current members of the Murraytrematoidinae have either one or two connecting bars, so, as *Sinodiplectanotrema* has no bars, the diagnosis of the Murraytrematoidinae would need to be amended to accommodate this genus. However, the Murraytrematoidinae was considered to be paraphyletic by Desdevises et al. (2001) and was synonymised with the Diplectaninae Monticelli, 1903 by Domingues & Boeger (2008). The absence of squamodiscs in species of the murraytrematoidine genera and *Sinodiplectanotrema* could represent a plesiomorphic state for the Diplectanidae and the Murraytrematoidinae could be valid. An alternative, unjustified at present, would be to erect a new subfamily for *Sinodiplectanotrema*. Herein, however, we confirm the assignment of *Sinodiplectanotrema* to the Diplectanidae and refrain from a discussion on its subfamilial status until more information on the morphology and molecular biology of its constituent species become available. In order to accommodate *Sinodiplectanotrema*, the familial diagnosis of Diplectanidae, as given in Domingues & Boeger (2008), needs to be amended to read: Haptor with 3 bars (1 midventral, 2 laterodorsal), 2 bars (*Lobotrema*), 4 bars (*Diplectanocotyla*) or 0 bars (*Sinodiplectanotrema*).

The diagnosis of *Sinodiplectanotrema* is amended here to include features not given in the original generic diagnosis, such as the anterior part of the ovary looping the right intestinal caecum (a diplectanid character) and the presence of a vagina, haptoral reservoirs and spines on the posterior half of the body.

Sinodiplectanotrema Zhang, 2001 (amend.)

Diagnosis: Diplectanidae. Body elongate, 4 eye-spots and 3 pairs of head organs; alimentary system bifurcate with blind caeca; peduncle long; haptor small, set off from body, wider than long, with 12 digitate projections on lateral lobes; squamodiscs/lamellodiscs absent. Tegument armed with spines on posterior half of body and haptor. Haptor armed with 14 marginal hooks, 6 pairs located on digitate projections, 1 pair near anchors may be smaller; 4 anchors, with outer root modified as stiff rod attached by flexible hinge; dorsal anchors with longer outer root than ventral anchors; no connecting bars. Four pyriform reservoirs in anterior peduncle, with long ducts which extend into haptor and appear to open close to aperture of each anchor. Testis globular, post-ovarian; vas deferens arises from antero-sinistral side of testis, loops around left intestinal caecum to ventral side, coils and opens into male copulatory organ. Male copulatory organ inverted, lacks accessory piece; tapered copulatory tube with narrow distal end directed posteriorly; single prostatic reservoir, with prostatic glands entering posteriorly and prostatic duct opening into initial part of male copulatory organ. Ovary elongate, with anterior region and oviduct looping right caecum from dorsal to ventral side; oviduct continues antero-ventrally to form uterus; vagina sinistral, muscular, opens via thin tube into seminal receptacle; egg rounded lozenge-shaped, with filament which may terminate in distal modification. Vitellarium follicular; follicles grouped, especially posteriorly, into compact blocks, extend from just posterior to pharynx to middle of post-testicular field. On gills of sciaenid fishes.

Type-species: *S. argyrosomus* Zhang, 2001.

Other species: *S. malayanum* n. sp.

Acknowledgements The authors would like to thank Mr KS Liew (UM) for his help in collecting and staining the specimens and Dr Tim Littlewood (NHM) for useful comments. We are also grateful to Prof. Jean-Lou Justine (IRD, Noumea) for the use of his swatches. This study was funded by E-Science Research grant from the Government of Malaysia to the corresponding author.

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