Systematics and phylogeny of the hoplonemertean genus *Diplomma* (Nemertea) based on molecular and morphological evidence

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We provide a redescription of the poorly known distromatonemertean, *Diplomma serpentina* (Stimpson, 1855), a species that has not been reported since its original description. One of the freshly obtained topotypes is designated as a neotype. Based on examination of the new material, as well as the type specimens of three known species, we present a taxonomic revision of the so-far monotypic genus *Diplomma* Stimpson, 1857, which has been regarded as a dubious taxon since 1904. The following three nominal species are transferred to *Diplomma*: *Paramphiporus albimarginatus* Kirsteuer, 1965, *Poseidonemertes bothwellae* Gibson, 1982, and *Correanemertes polyophthalma* Gibson & Sundberg, 2001. The genus *Paramphiporus* Kirsteuer, 1965 is regarded as a junior synonym of *Diplomma*. Comparison of cytochrome *c* oxidase subunit I gene (COI) sequences indicates that *D. serpentina* is distributed in Indonesia, the Philippines, and Japan. Phylogenetic analyses based on partial sequences of nuclear 28S rRNA and 18S rRNA genes, as well as COI, from a selected number of distromatonemerteans fail to specify the sister relation to *Diplomma*, although it is suggested that the genus is more closely related to *Antarctonemertes*, *Gononemertes*, *Nemertellina*, *Tetraneuronemertes*, *Oerstedia*, and *Vulcanonemertes*, than to *Amphiporus, Cyanophthalma, Geonemertes, Malacobdella, Paranemertes, Poseidonemertes, Prostoma, Tetrastemma*, and *Zygonemertes*.

INTRODUCTION

The taxon Monostilifera within the ribbon worms (phylum Nemertea) is characterized by the presence of a single stylet in the proboscis apparatus. The stylet is used to stab the prey animals, which are mostly small crustaceans and polychaetes (e.g.

McDermott & Roe, 1985). Monostilifera contains around 570 species, accounting for about a half of the phylum (Gibson, 1995; Kajihara *et al*., 2008). This taxon, originally proposed by Brinkmann (1917), has been shown to be monophyletic in Thollesson & Norenburg's (2003) comprehensive molecular phylogenetic analysis. Some authors (e.g. Gibson, 1988; Crandall, 1993; Chernyshev, 2003) had earlier abandoned the taxon Monostilifera according to modified hoplonemertean classification schemes. These

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suggestions were not based on monophyly and have not gained general acceptance.

About 90% of the species in Monostilifera possess a bilayered rhynchocoel wall composed of inner longitudinal and outer circular muscle fibres. Thollesson & Norenburg (2003) named these monostiliferans Distromatonemertea, although their definition of this taxon was rather indirect. Systematics of Distromatonemertea has been hampered by (1) the species richness of this group (> 500 spp.), (2) the lack of type specimens in many nominal species (e.g. Kajihara, 2007), especially those that are the type species of long-established genera, and (3) that the named subtaxa (i.e. families and genera) are not always monophyletic (Thollesson & Norenburg, 2003; Strand & Sundberg, 2005). Only a few studies have addressed the monophyly of more inclusive distromatonemertean taxa. These include Envall & Sundberg's (1998) research on *Ototyphlonemertes* with the 16S rRNA gene and Strand & Sundberg's (2005) analysis of *Tetrastemma* using the 18S rRNA gene. No nemertean taxonomists have carried out revisional works based on examination of actual type specimens in this group, with the exception of Maslakova & Norenburg (2008a, b) who worked on Prosorhochmidae.

The genus *Diplomma* Stimpson, 1857 is a good starting point toward a revision of Distromatonemertea, with freshly collected material of its type species, *Diplomma serpentina* (Stimpson, 1855) from several places including the type locality. In this paper, we present a taxonomic revision of the genus *Diplomma*, based on examination of the newly obtained material, as well as the type specimens of three known species that have been classified in different genera. We investigated the phylogenetic position of *D. serpentina* amongst Distromatonemertea using the nuclear large ribosome subunit gene (28S) and small ribosome subunit gene (18S), and mitochondrial cytochrome *c* oxidase subunit I gene (COI) sequences. We provide an emended diagnosis of the genus *Diplomma*, based on possible synapomorphies inferred from the topology of the molecular phylogenetic tree. We discuss some morphological characters that have traditionally been regarded as having taxonomic importance, with respect to their intraspecific variation.

MATERIAL AND METHODS
SAMPLES
Morphological study
About 50 specimens of *D. serpentina* were obtained from five localities [Tigbauan (Panay Island, Philippines), Motobu (Okinawa Island, Japan), Gesashi (Okinawa Island, Japan), Shirahama (Honshu Island,

Japan), and Misaki (Honshu Island, Japan)] from 1996 to 2005 (Fig. 1). Some specimens obtained from Tigbauan and Okinawa were examined *in vivo* by the squeezing method (Kirsteuer, 1967a). Specimens for histological examination were anaesthetized either in 7.5% MgCl$_2$ or MS-222, fixed in Bouin's solution for 12–24 h, and preserved in 70% EtOH. These were dehydrated in 100% EtOH, cleared in xylene, embedded in paraffin wax (melting point: 56–57 °C) and sectioned at 6, 8, or 10 µm. Sections were subsequently stained by the Mallory trichrome method (Gibson, 1994). Voucher specimens have been deposited in the Zoology Section (New Series), Museum of Natural History, University of the Philippines, Los Baños, Laguna, Republic of the Philippines (UPLB-MNH), the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), and the Hokkaido University Museum, Sapporo, Japan (ZIHU).

For the taxonomic revisional work, species with external appearances similar to *D. serpentina* were selected; namely, those that have been reported to have a uniformly brown body colour. Specimens examined include the holotype of *Poseidonemertes bothwellae* Gibson, 1982 deposited in the Australian Museum, Sydney, Australia (AM), syntypes of *Paramphiporus albimarginatus* Kirsteuer, 1965 at the American Museum of Natural History, New York, USA (AMNH), and the holotype and a paratype of *Correanemertes polyophthalma* Gibson & Sundberg, 2001 at the Museum of Tropical Queensland, Townsville, Australia (MTQ). For comparison, the holotypes of *Poseidonemertes gondwanae* Kirsteuer, 1965 and *Poseidonemertes caribensis* Kirsteuer, 1974 at AMNH were also examined.

Molecular study
Specimens for molecular studies were collected from Pasir Putih (Sumatra, Indonesia), Tigbauan (Panay, Philippines), and Motobu (Okinawa, Japan) (Fig. 1). These were fixed and preserved in 70–99% EtOH. DNA was extracted using a DNeasy Tissue Kit (QIAgen Ltd, Tokyo, Japan). Additional specimens and their localities are listed in Table 1.

AMPLIFICATION, SEQUENCING, AND ALIGNMENT
Amplification of the partial sequences of genes was carried out using universal primers for 28S (Littlewood, 1994), 18S (Medlin *et al*., 1988) and COI (Folmer *et al*., 1994) by a thermal cycler (iCycler, Japan Bio-Rad Laboratories Ltd, Tokyo, Japan) with a preheating at 94 °C for 7 min before 35 cycles of 94 °C for 45 s, 50 °C for 90 s, and 72 °C for 90 s, then postheating at 72 °C for 7 min. The amplified products were purified according to Boom *et al*.'s (1990)
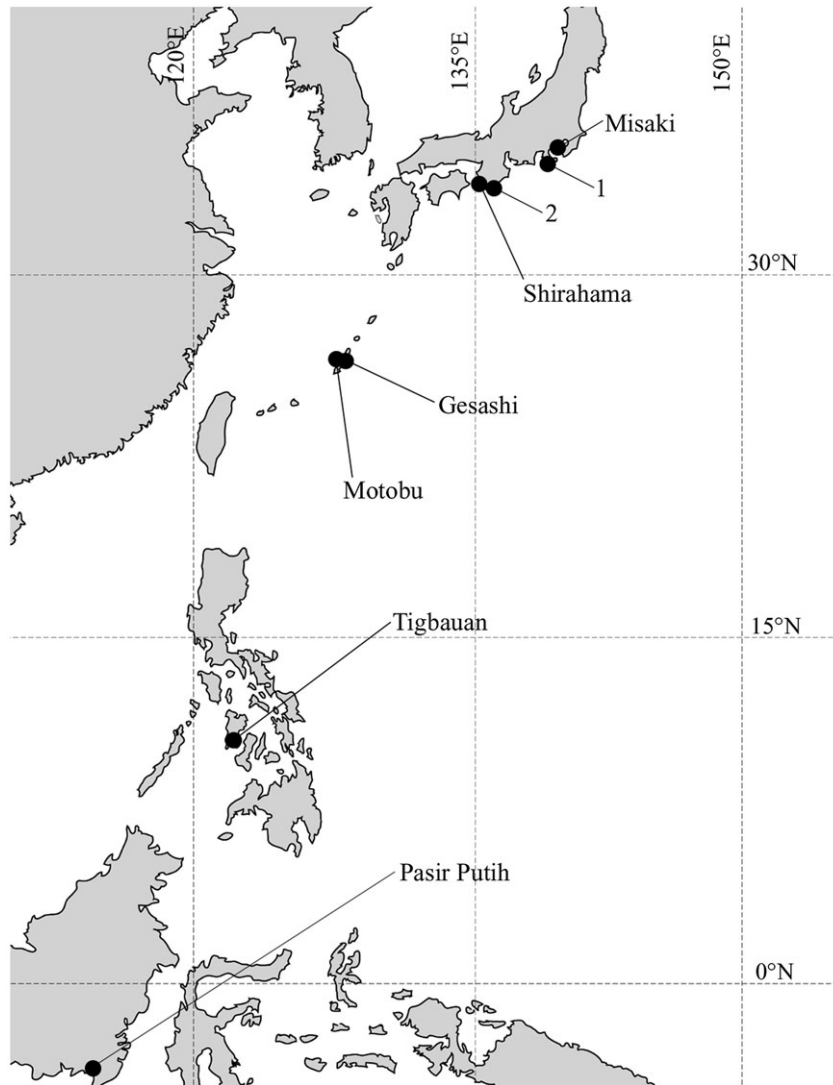
Figure 1. Collecting sites of *Diplomma serpentina* (Stimpson, 1855) in the present study. 1: Shimoda, where Yamaoka (2005) reported *Paranemertes* sp. that we regard as synonymous with *D. serpentina*. 2: Kushimoto, the type locality of *Amphiporus insolitus* Iwata, 1954a, a species that may be synonymous with *D. serpentina*.

method. Cycle sequencing was carried out with an ABI BigDye Terminator version 3.1 Cycle Sequencing Kit (25 cycles: 96 °C for 30 s, 50 °C for 15 s, and 60 °C for 4 min). The nucleotide sequences were carried out with the same primer pairs as in the initial PCR procedure for each gene, either directly determined in both directions with an ABI 3100-Avant Genetic Analyzer (Applied Biosystems Japan Ltd, Tokyo, Japan) or performed by Macrogen (Korea). Alignment was straightforward for COI following amino acid triplets, whereas for 18S and 28S, sequences were aligned with default settings (gap opening cost = 15, gap extensioncost = 6.66, and transition weight = 0.5) by CLUSTALW (Thompson, Higgins & Gibson, 1994) implemented in MEGA version 4.0 (Tamura *et al.*,

2007); regions that could not be unambiguously aligned were excluded.

PHYLOGENETIC ANALYSES

28S analysis
Eighteen partial 28S sequences were used for the analysis (Table 1), of which three were new for this study. Phylogenetic analyses were carried out using Bayesian inference (BI) and maximum likelihood (ML). The general time-reversible model (Tavaré, 1986) with invariant sites and gamma-distributed rates was selected based on the Akaike information criterion (Akaike, 1974), implemented in MrModeltest version 2.3 (Nylander, 2004) for BI and

Table 1. List of nemertean species included in the phylogenetic analyses, their GenBank accession numbers and sources

Species	Accession number (source)		
	28S	18S	COI
Acteonemertidae sp. A		EU255596 (Mateos & Giribet, 2008)	
Acteonemertidae sp. B		EU255585 (Mateos & Giribet, 2008)	
Acteonemertidae sp. C		EU255579 (Mateos & Giribet, 2008)	
Antarctonemertes varvarae Chernyshev, 1999	AJ436845 (Thollesson & Norenburg, 2003)		AJ436900 (Thollesson & Norenburg, 2003)
Antiponemertes novaezealandiae (Dendy, 1895)		AY928345 (Strand & Sundberg, 2005)	
Amphiporus allucens Bürger, 1895		AY928343 (Strand & Sundberg, 2005)	
Amphiporus lactifloreus (Johnston, 1828)	AJ436844 (Thollesson & Norenburg, 2003)	AB505814 (present study)[1]	
Amphiporus ochraceus (Verrill, 1873)		AY039668 (Sundberg, Turbeville & Lindh, 2001)	
Argonemertes australiensis (Dendy, 1892)		AY928346 (Strand & Sundberg, 2005)	
Argonemertes australiensis (Dendy, 1892)		AF519235 (Maxmen et al., 2003)	
Cyanophthalma obscura (Schultze, 1851)		AY039667 (Strand & Sundberg, 2005)	
Diplomma polyophthalma (Gibson & Sundberg, 2001) **comb. nov.**		AY062924 (Gibson & Sundberg, 2001)	AB505816 (present study)[2]
Diplomma serpentina (Stimpson, 1855)	AB505817 (present study)[3]	AB505818 (present study)[3]	AB505819 (present study)[3] AB505815 (present study)[4] AB505820 (present study)[5]
Emplectonema buergeri Coe, 1901	AJ436847 (Thollesson & Norenburg, 2003)		
Emplectonema gracile (Johnston, 1837)	AJ436848 (Thollesson & Norenburg, 2003)	AY928347 (Strand & Sundberg, 2005)	
Emplectonema mitsui Yamaoka, 1947	AB505821 (present study)[6]	AB505822 (present study)[6]	AB505823 (present study)[6]
Geonemertes pelaensis Semper, 1863		EU255578 (Mateos & Giribet, 2008)	
Gononemertes parasita Bergendal, 1900		AB505824 (present study)[7]	AB505825 (present study)[7]
Malacobdella grossa (Müller, 1774)	AJ436850 (Thollesson & Norenburg, 2003)	AY039670 (Sundberg et al., 2001)	
Nemertellina yamaokai Kajihara et al., 2000	AJ436852 (Thollesson & Norenburg, 2003)	AB505826 (present study)[8]	AJ436907 (Thollesson & Norenburg, 2003)
Nipponnemertes bimaculata (Coe, 1901)	AJ436854 (Thollesson & Norenburg, 2003)		
Nipponnemertes pulchra (Johnston, 1837)	AY210465 (Thollesson & Norenburg, 2003)	AY928352 (Strand & Sundberg, 2005)	AY791971 (Strand & Sundberg, 2005) AY791972 (Strand & Sundberg, 2005)
Oerstedia dorsalis (Abildgaard, 1806)	AJ436856 (Thollesson & Norenburg, 2003)	AY928353 (Strand & Sundberg, 2005)	AJ436911 (Thollesson & Norenburg, 2003)
Oerstedia striata Sundberg, 1988	AJ436857 (Thollesson & Norenburg, 2003)	AY928354 (Strand & Sundberg, 2005)	AJ436912 (Thollesson & Norenburg, 2003)
Oerstedia venusta Iwata, 1954b	AJ436860 (Thollesson & Norenburg, 2003)		
Oerstedia zebra (Chernyshev, 1993)	AJ436862 (Thollesson & Norenburg, 2003)		
Paranemertes peregrina Coe, 1901	AJ436864 (Thollesson & Norenburg, 2003)	AY039666 (Strand & Sundberg, 2005)	
Paranemertes sanjuanensis Stricker, 1982			
Poseidonemertes collaris Roe & Wickham, 1984			
Prostoma graecense (Böhmig, 1892)		AY928350 (Strand & Sundberg, 2005)	
Tetraneuronemertes lovgreni Sundberg et al., 2007	AB505827 (present study)[9]		EF208982 (Sundberg et al., 2007)
Tetrastemma candidum (Müller, 1774)		AY928372 (Strand & Sundberg, 2005)	
Tetrastemma robertianae McIntosh, 1874		AY928376 (Strand & Sundberg, 2005)	
Tetrastemma stimpsoni Chernyshev, 1992		AY928378 (Strand & Sundberg, 2005)	
Tetrastemma vermiculum (Quatrefages, 1846)		AY928379 (Strand & Sundberg, 2005)	
Vulcanonemertes rangitotoensis Gibson & Strand, 2002			AB505828 (present study)[10]
Zygonemertes simoneae Corrêa, 1961	AJ436867 (Thollesson & Norenburg, 2003)		
Zygonemertes virescens (Verrill, 1879)	AJ436868 (Thollesson & Norenburg, 2003)	AY928381 (Strand & Sundberg, 2005)	

[1]Anglesey, UK; [2]Queensland, Australia; [3]Okinawa, Japan; [4]Tigbauan, Philippine; [5]Pasir Putih, Indonesia; [6]Oshoro, Japan; [7]Tjärnö, Sweden; [8]Akkeshi, Japan; [9]Bukkasundet, Norway; [10]Takapuna, New Zealand.
COI, mitochondrial cytochrome *c* oxidase subunit I gene.

in MODELTEST version 3.7 (Posada, 1998–2008; Posada & Crandall, 1998) for ML, both using PAUP* version 4.0b10 (Swofford, 2002). BI was performed by MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001) with 10^7 generations of four Markov chains (one cold and three heated chains with two parallel runs), sampling every 100th generation chain. Burnin was set to 25 000 leaving the last 75 000 sampled trees for estimating posterior probabilities. Parameters were altered in the proposal mechanism so that the acceptance rates of swaps between adjacent chains were in the range of 20–60%. Four separate analyses were run starting from random trees to ensure congruence. The ML tree was obtained by tree bisection reconnection branch swapping, starting with a topology given by the neighbor-joining (NJ) method (Saitou & Nei, 1987). Bootstrap values (Felsenstein, 1985) were calculated from 1000 replicates by nearest-neighbour interchange branch-swapping algorithm with an initial tree topology given by NJ. *Nipponnemertes bimaculata* was used as the outgroup based on Thollesson & Norenburg (2003).

18S analysis

Twenty-nine partial 18S rRNA gene sequences of monostiliferous hoplonemerteans were used for the phylogenetic analysis (Table 1), of which five were newly determined in the present study. Phylogeny was inferred by the same method as in the 28S analysis, except that BI was performed with 10^6 generations, sampling every 100th generation; burnin was set to 2500, leaving 7500 sampled trees for estimating posterior probabilities. The tree was rooted with cratenemerteans as the outgroup (Thollesson & Norenburg, 2003; Sundberg & Strand, 2007).

COI analysis

Based on the resulting topology of the 28S and 18S trees, additional analyses were performed to infer further the sister relationship of *D. serpentina* with 14 COI sequences (Table 1). BI was performed with the general time reversible model and site-specific rates model (charset 1st_pos=1-.\3; charset 2nd_pos=2-.\3; charset 3rd_pos=3-.\3; partition by_codon=3: 1st_pos, 2nd_pos, 3rd_pos; set partition=by_codon; lset nst=6; prset ratepr=variable), with 10^6 generations of four Markov chains (one cold and three heated chains with two parallel runs), sampling every 100th generation chain; burnin was set to 2500, leaving 7500 sampled trees for estimating posterior probabilities. Parameters were altered so that the acceptance rates of swaps between adjacent chains were in the range of 20–60%. Four separate analyses were run starting from random trees to

ensure congruence. ML was carried out in the same way as in the 18S analysis. The tree was unrooted.

RESULTS

PHYLOGENY

After eliminating ambiguously aligned sites, the datasets contained 718 (28S), 1248 (18S), and 535 bp (COI). Aligned NEXUS files are available from TreeBase with accession numbers 10753 (28S), 10754 (18S), 10755 (COI). BI and ML resulted in almost the same topology in each dataset; differences were mostly found in the resolution of minor branches.

In the 28S and 18S trees (Figs 2, 3), a well-supported sister taxon relationship with *D. serpentina* was not found, whereas in the COI analysis (Fig. 4), the sister taxon to *D. serpentina* appeared to be *Correanemertes polyophthalma* (redescribed as **D. polyophthalma comb. nov.**, see Taxonomy), supported by a 71% bootstrap value in ML and 100% posterior probability in BI. Although the sister taxon relationship of *Diplomma* amongst Distromatonemertea remained unclear in every dataset, the genus *Diplomma* appears to be more closely related to *Antarctonemertes*, *Gononemertes*, *Nemertellina*, *Oerstedia*, *Tetraneuronemertes*, and *Vulcanonemertes* than to *Amphiporus*, *Cyanophthalma*, *Emplectonema*, *Geonemertes*, *Malacobdella*, *Paranemertes*, *Poseidonemertes*, *Prostoma*, *Tetrastemma*, and *Zygonemertes* (Figs 2, 3).

TAXONOMY

### GENUS *DIPLOMMA* STIMPSON, 1857

Diplomma Stimpson, 1857: 164 (type species: *Nareda serpentina* Stimpson, 1855, fixed by monotypy).
Paramphiporus Kirsteuer, 1965: 294 (type species: *Paramphiporus albimarginatus* Kirsteuer, 1965, fixed by monotypy), syn. nov.

Diagnosis: Distromatonemerteans with mid-dorsal blood vessel running always under rhynchocoel; dorsoventral muscles occasionally passing laterally to (= outside) lateral nerve cords; body colour uniformly brownish in life.

Composition: The following four species are here regarded as congeners: ***Diplomma albimarginata*** (Kirsteuer, 1965) **comb. nov.,** ***Diplomma bothwellae*** (Gibson, 1982) **comb. nov.,** ***Diplomma polyophthalma*** (Gibson & Sundberg, 2001) **comb. nov.,** and *Diplomma serpentina* (Stimpson, 1855).

Remarks: In addition to the diagnostic characters, the following features are found in both in *D. serpentina* and **D. polyophthalma comb. nov.**, thus may be used as a guide in identifying species in the genus: (1)
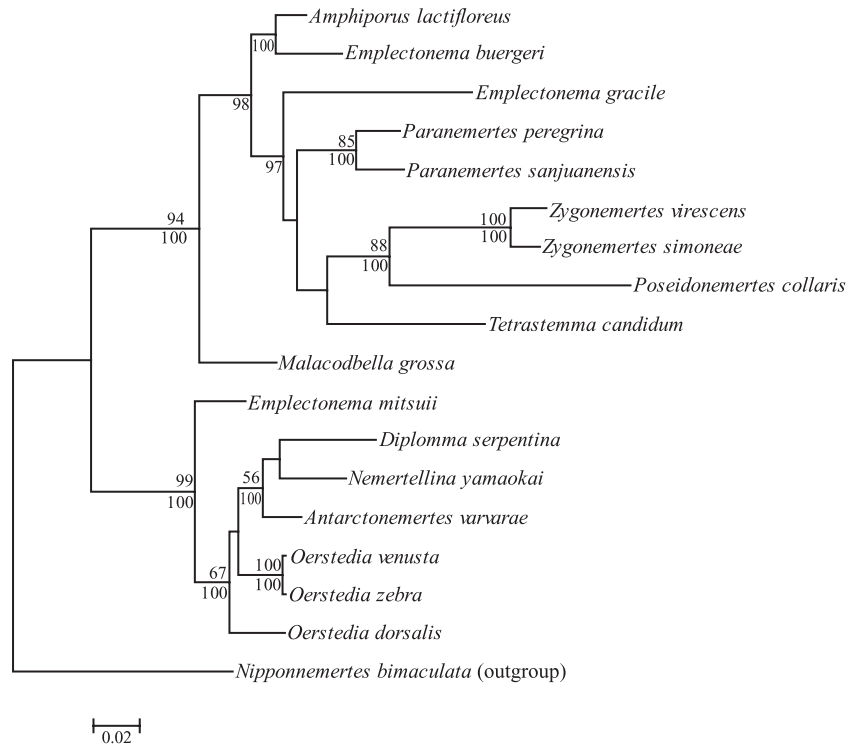
Figure 2. Bayesian tree of a selected number of morphologically known distromatonemerteans using the general time-reversible model with invariant sites and gamma-distributed rates based on 28S rRNA gene sequences (718 bp after alignment). Numbers above branches are bootstrap percentages from the maximum likelihood analysis (values > 50% are shown); numbers below are posterior probabilities (values > 95% are shown). *Nipponnemertes bimaculata* was used as the outgroup.

single neuropil in lateral nerve cord; (2) short intestinal caecum, not reaching to brain, having neither anterior nor lateral pouches; (3) eyes that tend to be difficult to observe in life; (4) rhynchocoel being almost full body length; (5) single shallow apical organ (not elaborated as those in prosorhochmids); (6) cephalic gland restricted precerebrally; and (7) post-cerebral submuscular glands absent.

Congeners have so far been known from the temperate to tropical Western and Central Indo-Pacific. Possible additional congeners, which are at present classified in other genera, include *Amphiporus insolitus* Iwata, 1954a described from Japan and *Amphiporus texanus* Coe, 1951a *sensu* Corrêa (1961) reported from Virginia Key, Miami (USA). Morphologically, congeners can be discriminated by the number of proboscis nerves (indicated in parentheses): ***D. albimarginata*** comb. nov. (15), ***D. bothwellae*** comb. nov. (10), ***D. polyophthalma*** comb. nov. (13–14), and *D. serpentina* (12).

DIPLOMMA ALBIMARGINATA (KIRSTEUER, 1965) COMB. NOV. (FIGS 5–8)

*Paramphiporus albimarginatus* Kirsteuer, 1965: 296, figures 4, 5, 12A.

Paramphiporus albomarginatus [lapsus calami]: Kirsteuer, 1974: 164.

Material examined: AMNH 277, the syntypes of *Paramphiporus albimarginatus*, four slides, serial transverse sections of two individuals; Tanikely, Madagascar; collected by Ernst Kirsteuer amongst the corals *Acropora pharaonis*, *Acropora corymbosa*, and *Seriatopora angulata*, during the Österreichische Indo-Westpazifik-Expedition 1959/60.

Distribution: Only known from the type locality, Nosy Tanikely (13°29′S, 48°14′E), Madagascar.

Diagnosis: Diplomma with proboscis having 15 nerves (Fig. 5).

*Supplementary redescription:* Dorsoventral muscles running laterally (= externally) to the lateral nerve cord were found in the syntypes (Fig. 6), conforming to the present diagnosis of the genus *Diplomma*. The morphology of the intestinal caecum differs between the two syntype specimens, although Kirsteuer (1965) described that it possessed two anterior pouches situated on both sides of the pylorus. In one syntype,
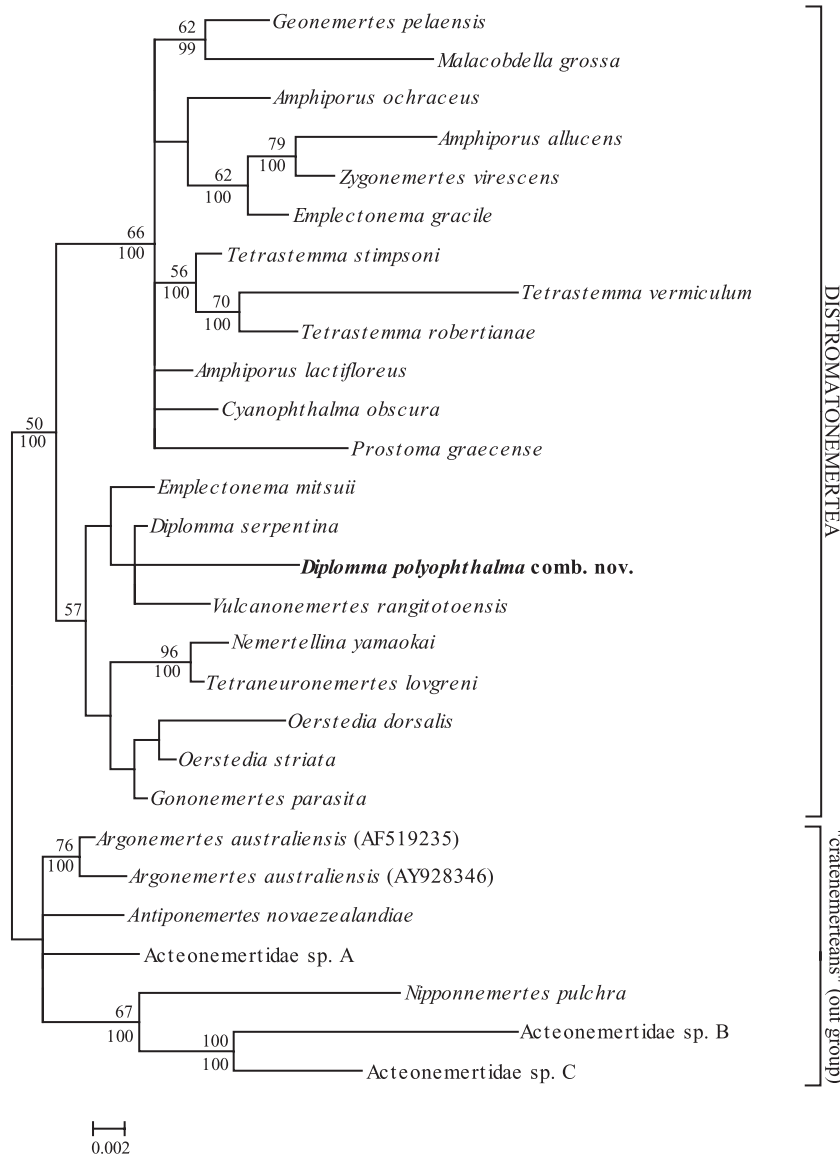
Figure 3. Bayesian tree of a selected number of distromatonemerteans, using the general time-reversible model with invariant sites and gamma-distributed rates model based on 18S rRNA gene sequences (1248 bp after alignment), rooted with cratenemerteans as the outgroup. Numbers above branches are bootstrap percentages from the maximum likelihood analysis (values > 50% are shown); numbers below are posterior probabilities (values > 95% are shown). In this paper we transfer *Correanemertes polyophthalma* to the genus *Diplomma*.

these pouches extend for 31 slices of serial sections (Fig. 7) before they merge posteriorly to form an unpaired intestinal caecum below the pylorus, stretching for six slices; i.e. supposing the serial sections were cut at 10 μm thick, the intestinal caecum would be 60 μm long, with two pouches extending for 310 μm from its anterior end. The sections that contain the pair of anterior pouches show an exceedingly expanded rhynchocoel (Fig. 7), suggesting strong contraction during fixation; the intestinal

caecum was thus probably deformed in this specimen. In the other syntype, the anterior end of the intestinal caecum appears on one side of the pylorus (Fig. 8A) for seven slices; the caecum posteriorly abuts the pylorus on its lateral and ventral sides (Fig. 8B) for 12 slices before the pylorus opens to the dorsal wall of the intestine (Fig. 8C); i.e. the intestinal caecum is 190 μm long (assuming the sections were cut at 10 μm thick), without anterior and lateral pouches.
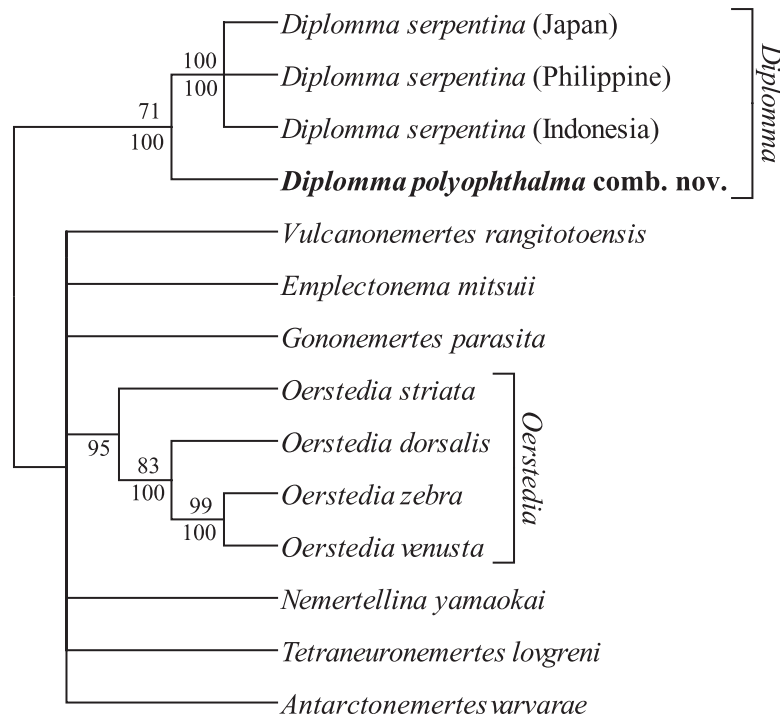
Figure 4. Bootstrap 50% majority-rule consensus unrooted tree of a selected number of distromatonemerteans, based on a maximum likelihood analysis with the general time-reversible model with invariant sites and gamma-distributed rates using mitochondrial cytochrome *c* oxidase subunit I gene sequences (535 bp after alignment). Numbers above branches are bootstrap percentages from the maximum likelihood analysis (values > 70% are shown); numbers below are posterior probabilities (values > 95% are shown). In this paper we transfer *Correanemertes polyophthalma* to the genus *Diplomma*.
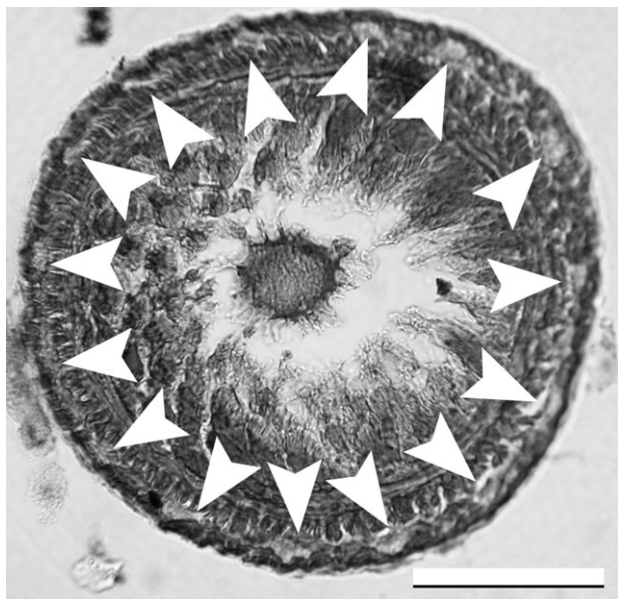
Figure 5. *Diplomma albimarginata* comb. nov. (formerly *Paramphiporus albimarginatus* Kirsteuer, 1965). Syntype (AMNH 277). Transverse section showing the proboscis nerves (arrowed). Scale bar = 50 µm.

DIPLOMMA BOTHWELLAE (GIBSON, 1982) **COMB. NOV.** (FIGS 9–11)

*Poseidonemertes bothwellae* Gibson, 1982: 270, figures 1–4.

Material examined: AM W.5890, the holotype of *Poseidonemertes bothwellae* Gibson, 1982, 26 slides, serial transverse sections prepared by Ray Gibson; obtained from a chunk of coral covered with small algae and hydroids, at an inner reef flat on western Heron Island, Capricorn Group, Great Barrier Reef, Australia, by Anne Bothwell on 15.vii.1975.

For comparison, AMNH 278, the holotype of *Poseidonemertes gondwanae* Kirsteuer, 1967b (type species of the genus *Poseidonemertes*) and AMNH 279, the holotype of *Poseidonemertes caribensis* Kirsteuer, 1974 were also examined.

Distribution: Great Barrier Reef, Australia (only known from the type locality).

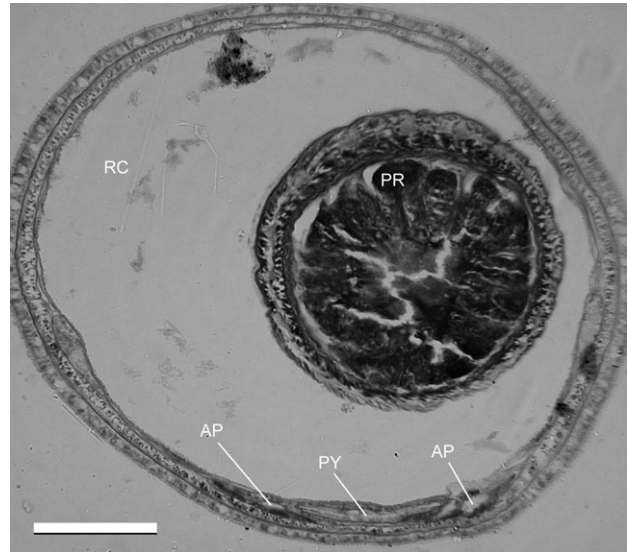*Diagnosis: Diplomma* with proboscis having ten nerves (Fig. 9); eyes four in number.
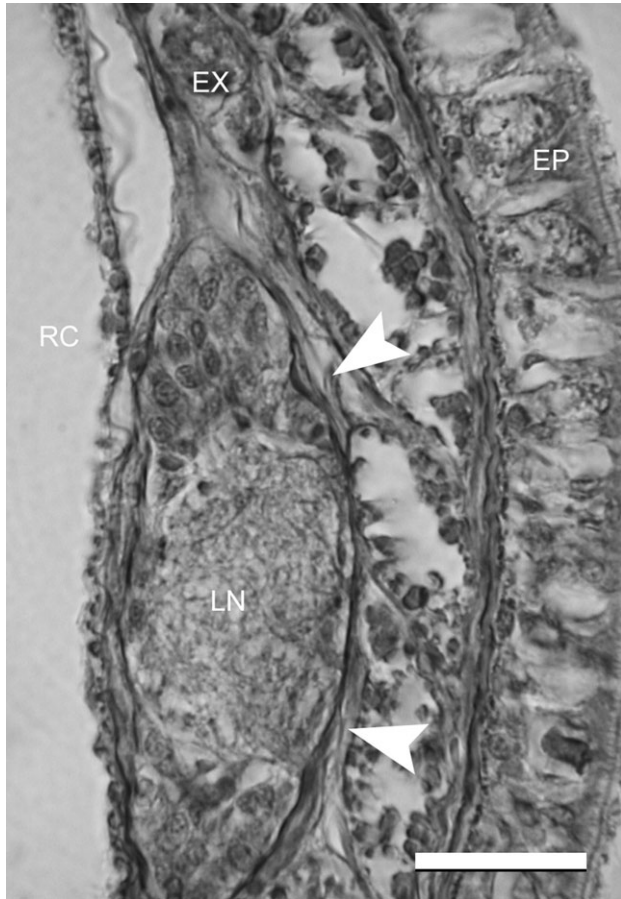
Figure 6. *Diplomma albimarginata* **comb. nov.** (formerly *Paramphiporus albimarginatus* Kirsteuer, 1965). Syntype (AMNH 277). Transverse section showing the dorsoventral muscle fibres running laterally to the lateral nerve cord (arrowed) in the stomach region. Abbreviations: EP, epithelium; EX, excretory collecting tubule; LN, lateral nerve cord; RC, rhynchocoel. Scale bar = 20 μm.

Figure 7. *Diplomma albimarginata* **comb. nov.** (formerly *Paramphiporus albimarginatus* Kirsteuer, 1965). One of the two syntypes (AMNH 277). Transverse section to show the two anterior 'pouches' (AP) situated laterally to the pylorus (PY); note the exceedingly expanded rhynchocoel (RC), compared to the body diameter and the proboscis (PR). Scale bar = 100 μm.

Supplementary redescription: Dorsoventral muscle fibres running laterally to the lateral nerve cords are found on almost every section from the stomach region through the pyloric region (Figs 10, 11), as if they form a 'body-wall inner circular muscle layer'. These fibres become sparse posteriorly, although they are still sporadically found in the intestinal region. The intestinal caecum extends for 35 slices, equivalent to 210–245 μm long.

Note on Poseidonemertes*:* Contrary to the descriptions of *Poseidonemertes gondwanae* given by Kirsteuer (1967b, 1974), our observation of the holotype revealed that the mid-dorsal blood vessel enters the rhynchocoel for a very short course soon after its origin (Fig. 12) and the diagonal muscle layer is well developed (Fig. 13A); additionally, dorsoventral

muscle fibres always run medial to the lateral nerve cord (Fig. 13B), a character state not mentioned by Kirsteuer (1967b, 1974). We also confirmed the presence of a thin thread of nerve covered by connective tissues, horizontally sent from the lateral nerve cord to the epidermis on each side (Fig. 13B) in the foregut and intestinal regions. This character has been reported at least in *Poseidonemertes collaris* by Roe & Wickham (1984) and in *Poseidonemertes buergeri* by Senz (2001); this might be used in diagnosing the genus *Poseidonemertes* or distinguishing congeners.

DIPLOMMA POLYOPHTHALMA (GIBSON & SUNDBERG, 2001) COMB. NOV. (FIGS 14–15)

Correanemertes polyophthalma Gibson & Sundberg, 2001: 1266, figures 6–8.

Material examined: MTQ G20024, the holotype of *Correanemertes polyophthalma*, 13 slides, serial transverse sections through the anterior body region; MTQ G20025, the paratype of *C. polyophthalma*, five slides, serial transverse sections through anterior body.

Distribution: North-western Australia; apart from the type locality, the species has been also reported from Rib Reef (18°28.8′S, 146°52.4′E), north-east of the Palm Islands (Gibson & Sundberg, 2001).
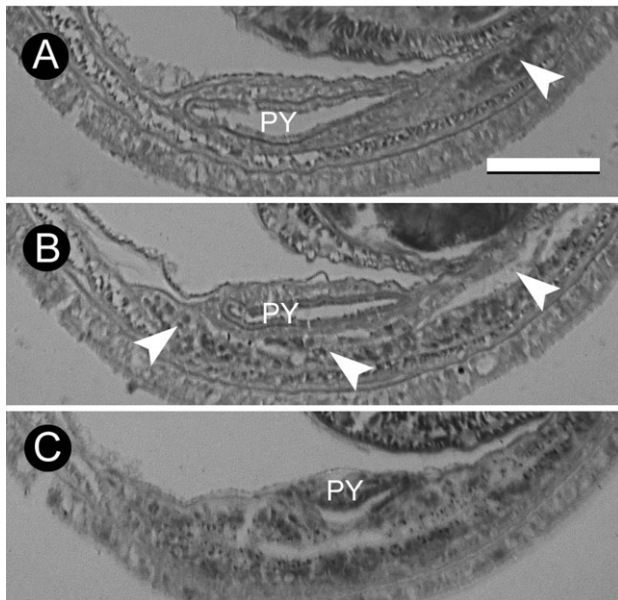
Figure 8. *Diplomma albimarginata* **comb. nov.** (formerly *Paramphiporus albimarginatus* Kirsteuer, 1965). Syntype (AMNH 277). A, transverse section showing anterior portion of unpaired intestinal caecum (arrowed) situated on one side of the pylorus (PY); B, ten sections posterior to A, showing the intestinal caecum (arrowed) lateral and ventral to the PY; C, seven sections posterior to B, showing the PY opening to the dorsal wall of the intestine. Scale bar = 50 μm.

*Diagnosis: Diplomma* with proboscis having 13 or 14 nerves (Fig. 14A, B).

Supplementary redescription: The holotype (MTQ G20024) contains 14 proboscis nerves (Fig. 14A), and the paratype (MTQ G20025) 13 (Fig. 14B). The intestinal caecum is simple, more than 500 μm long, without anterior and lateral pouches, extending anteriorly below the pylorus, terminating posteriorly to the stomach, not reaching to the brain (Fig. 15). Both the holotype and paratype everted the proboscis during fixation, which makes it difficult to find an apical organ. Although the apical organ was deemed to be absent in the original description (Gibson & Sundberg, 2001), we found the organ at least in the paratype (MTQ G20025).

Note on Correanemertes*:* The establishment of the genus *Correanemertes* by Kirsteuer (1967b) was based on the description of *Amphiporus bioculatus* McIntosh, 1874 *sensu* Corrêa (1958) from Brazil. Therefore, Kirsteuer (1967b) is deemed to have created the nominal species *Correanemertes bioculata* Kirsteuer, 1967b, although Gibson (1995) erroneously attributed the author of the specific name to Corrêa (1958).
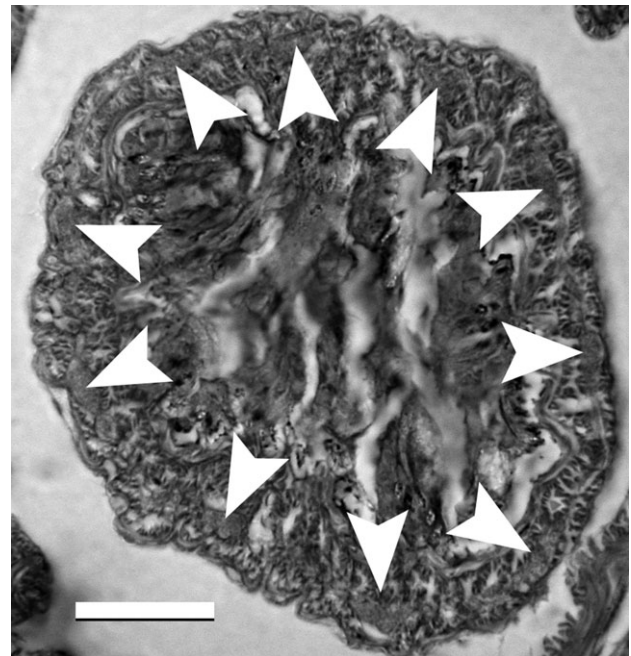
Figure 9. *Diplomma bothwellae* **comb. nov.** (formerly *Poseidonemertes bothwellae* Gibson, 1982). Holotype (AM W.5890). Transverse section through the anterior proboscis; arrows indicate the proboscis nerves. Scale bar = 20 μm.

Before the present study, the genus had contained three species: *C. bioculata* Kirsteuer, 1967b; *C. polyophthalma* Gibson & Sundberg, 2001; and *Correanemertes gordoni* Gibson, 2002. This genus, like many other genera in the phylum Nemertea, appeared nonmonophyletic. *Correanemertes bioculata* shares some characters with *Poseidonemertes gondwanae*, such as (1) the 'divided' body-wall longitudinal muscle layer (see Discussion) in the cerebral region; (2) two eyes; and (3) the horizontal nerve from the lateral nerve cord to the epidermis (Corrêa, 1958: fig. 22). Indeed, *Correanemertes* and *Poseidonemertes* are supposed to be distinguishable by a single character: whether or not the proboscis insertion receives muscle fibres from the outer portion of the body-wall muscle layer that is divided near the brain (Kirsteuer, 1967b, 1974). Meanwhile, subsequent research has indicated that the degree of the development of the proboscis insertion muscles can vary intraspecifically, as was shown by Norenburg (1986) in *Cyanophthalma obscura* and by Moore & Gibson (1988) in *Aniponemertes* and *Argonemertes*, as well as in *D. serpentina* by the present study (see below). This suggests that *Correanemertes* and *Poseidonemertes* are synonymous, although confirmed synonymization requires additional material of *C. bioculata* Kirsteuer, 1967b from Brazil.
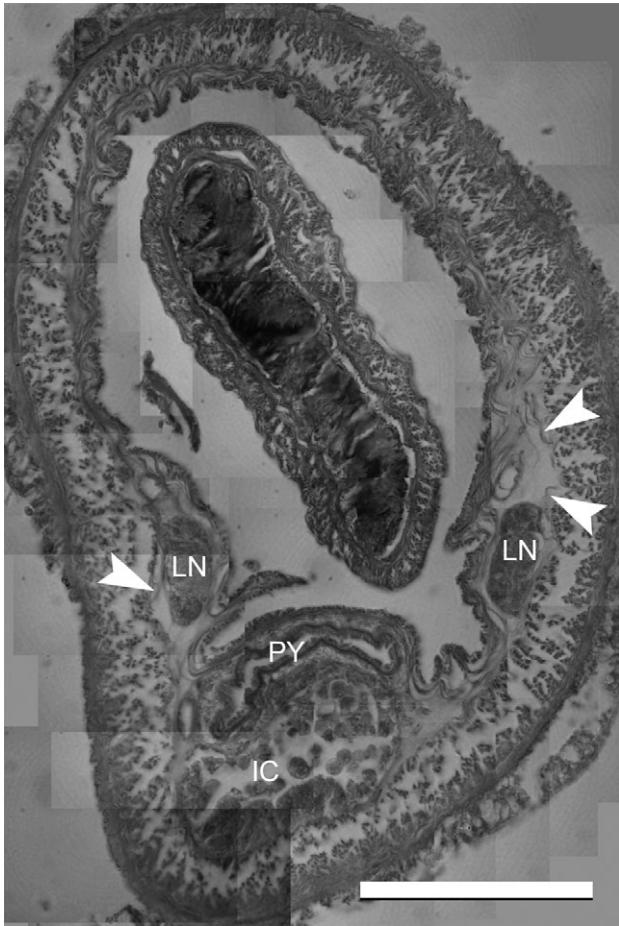
Figure 10. *Diplomma bothwellae* **comb. nov.** (formerly *Poseidonemertes bothwellae* Gibson, 1982). Holotype (AM W.5890). Transverse section through the pylorus (PY) and intestinal caecum (IC); arrows indicate dorsoventral muscle fibres running lateral to the lateral nerve cord (LN). Scale bar = 100 μm.

Correanemertes polyophthalma is here transferred from *Correanemertes* to *Diplomma*, based on both molecular and morphological evidence, as ***Diplomma polyophthalma*** **comb. nov.** *Correanemertes gordoni* is reported to have four eyes (Gibson, 2002), a feature indicating that the species is not closely related to *C. bioculata*. As the identity of *Correanemertes* is doubtful, reappraisal of the generic placement of *C. gordoni* is necessary in future studies.

DIPLOMMA SERPENTINA (STIMPSON, 1855)
(FIGS 16–25)

Nareda serpentina Stimpson, 1855: 381.
Diplomma serpentina: Stimpson, 1857: 164.
?*Amphiporus insolitus* Iwata, 1954a: 39, figures 2B, 3A, B.
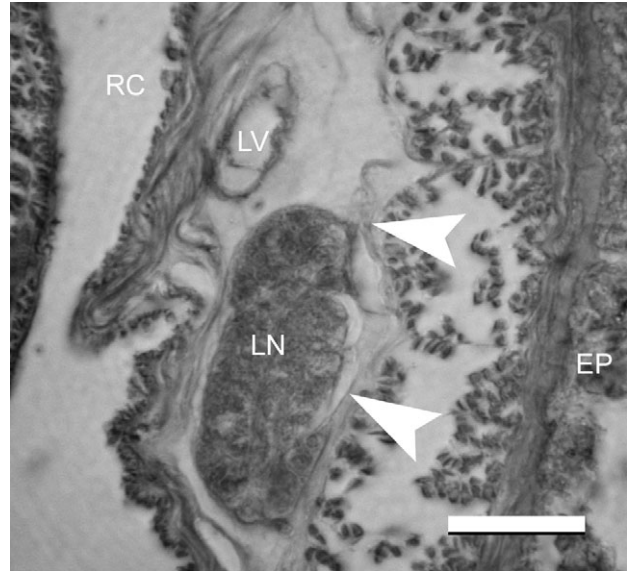
Figure 11. *Diplomma bothwellae* **comb. nov.** (formerly *Poseidonemertes bothwellae* Gibson, 1982). Holotype (AM W.5890). Magnification of Figure 10 to show the dorsoventral muscle fibres (arrowed). Abbreviations: EP, epithelium; LN, lateral nerve cord; LV, lateral blood vessel; RC, rhynchocoel. Scale bar = 20 μm.

*Paranemertes* sp. Yamaoka, 2005: 142, pl. 1, figure 6, text-figure 1.

Material examined: Fifteen sets of serial sections, consisting of the neotype and 14 paraneotypes (see below).

*Diagnosis: Diplomma* with proboscis having 12 nerves.

Neotype locality and habitat: Motobu, Okinawa, Japan (Fig. 1); amongst sessile organisms on buoys of aquaculture rafts at the Okinawa Prefectural Sea Farming Centre.

Other localities and habitats: Intertidal on rocky shore, Misaki, Kanagawa, Japan; intertidal amongst calcareous red algae on rocky shore, Shirahama, Wakayama, Japan; intertidal amongst algae, Gesashi, Okinawa, Japan; on a plastic piece sunken in an experimental tank, to which seawater is taken up from about 100 m offshore, at the Aquaculture Department, South-East Asian Fisheries Development Centre, Tigbauan, Iloilo, Philippines, 10°40′12.4″N, 122°21′10.3″E; habitat unrecorded, Pasir Putih Beach close to Condong Sulah Island, Sumatra, Indonesia, 5°32′10″S, 105°21′26″E (Fig. 1).

Stimpson's (1855) original description of *Na. serpentina* Stimpson, 1855 contains no habitat
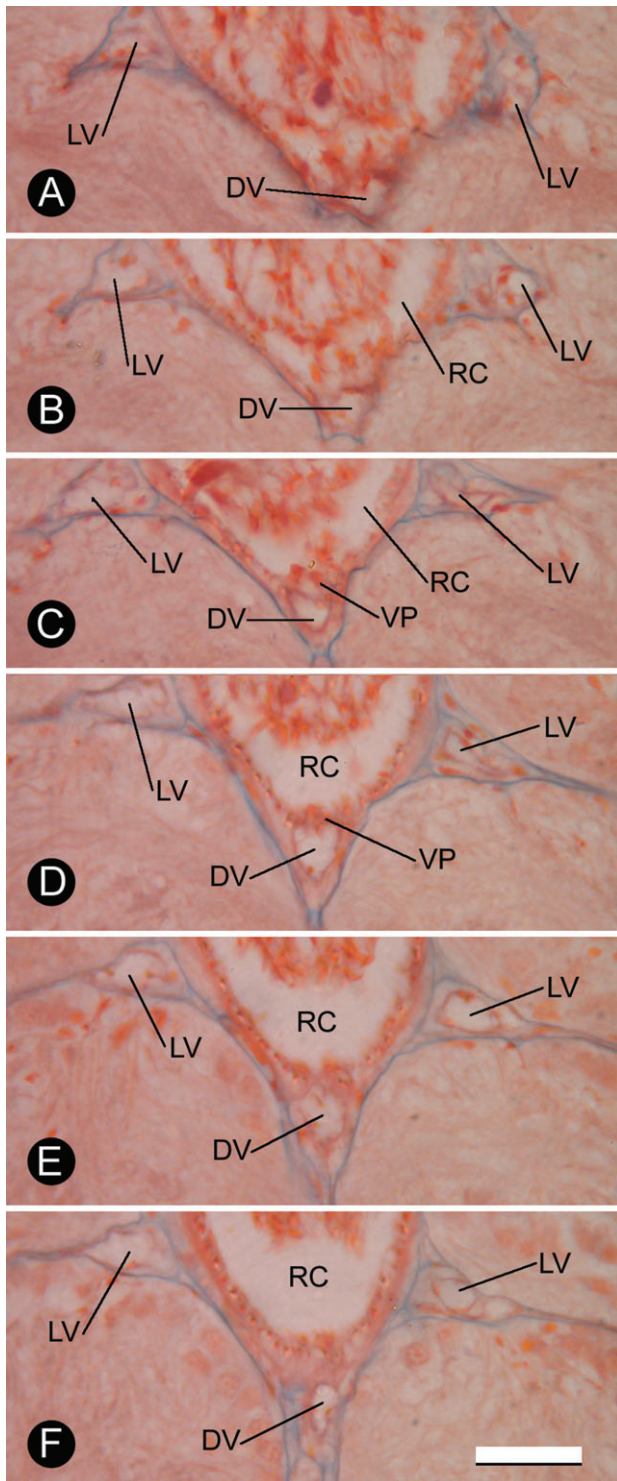
Figure 12. *Poseidonemertes gondwanae* Kirsteuer, 1965. Holotype (AMNH 278). A–F, serial transverse section to show that mid-dorsal vessel enters rhynchocoel. Abbreviations: DV, mid-dorsal vessel; LV, lateral blood vessel; RC, rhynchocoel; VP, vascular plug. Scale bar = 50 µm.
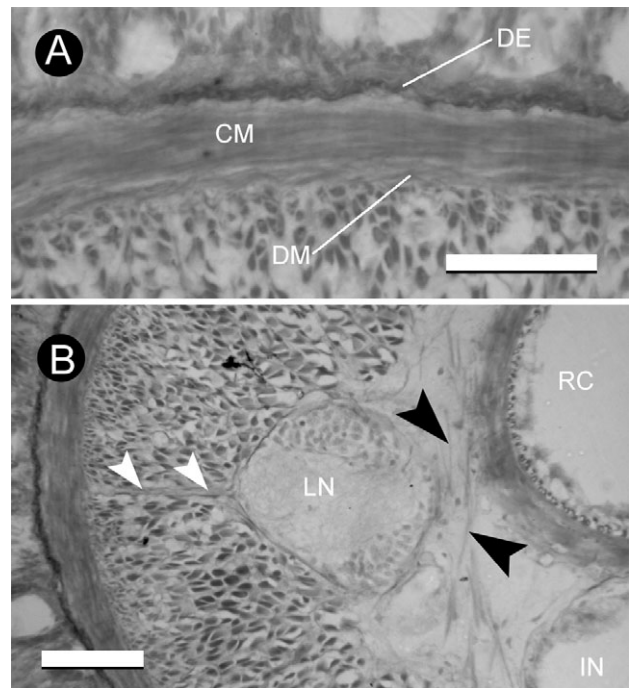

Figure 13. *Poseidonemertes gondwanae* Kirsteuer, 1965. Holotype (AMNH 278). A, diagonal muscle layer between body-wall outer circular and inner longitudinal muscle layers. B, horizontal nervous thread from lateral nerve cord to the epidermis (indicated by white arrows); black arrows indicate dorsoventral muscle fibres running inside lateral nerve cord. Abbreviations: CM, body-wall circular muscle layer; DE, dermis; DM, diagonal muscle layer; IN, intestine; LN, lateral nerve cord; RC, rhynchocoel. Scale bars: A = 30 µm; B = 50 µm.

information other than that the material was collected in Okinawa. In the following redescription, Stimpson (1857) stated that the specimen was found under stones on sandy to muddy places. Yamaoka (2005) reported a form under the name *Paramphiporus* sp., which is here considered as synonymous with *D. serpentina*, from Itado, near Shimoda, Shizuoka, Japan (Fig. 1).
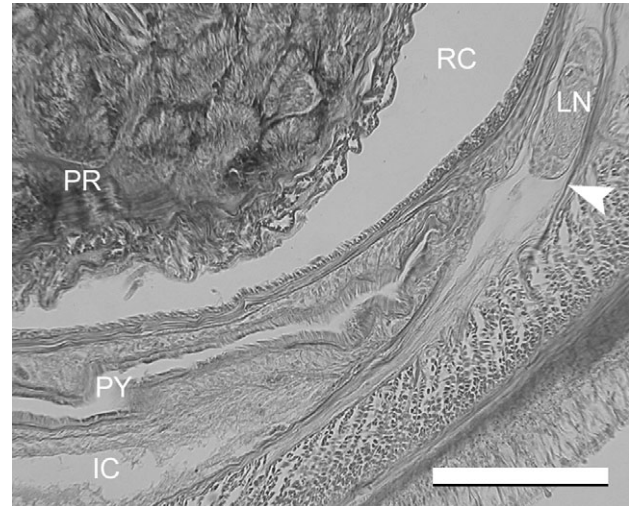
Type series: Stimpson's (1855) type material from Okinawa, as well as his drawings and illustrations were lost (Verrill, 1892: 392). For the purpose of the stability of the name, we designate the neotype and paraneotypes.

Neotype, ZIHU-1352, immature, complete series of transverse sections, 6 µm thick, 28 slides, 23.vi.1999. Paraneotypes: UPLB-NHM 0488, female, complete series of transverse sections, 8 µm, 26 slides, 28.x.2004, Tigbauan, Iloilo; UPLB-NHM 0489, female, complete series of longitudinal sections, 10 µm thick, 15 slides, 28.x.2004, Tigbauan, Iloilo;
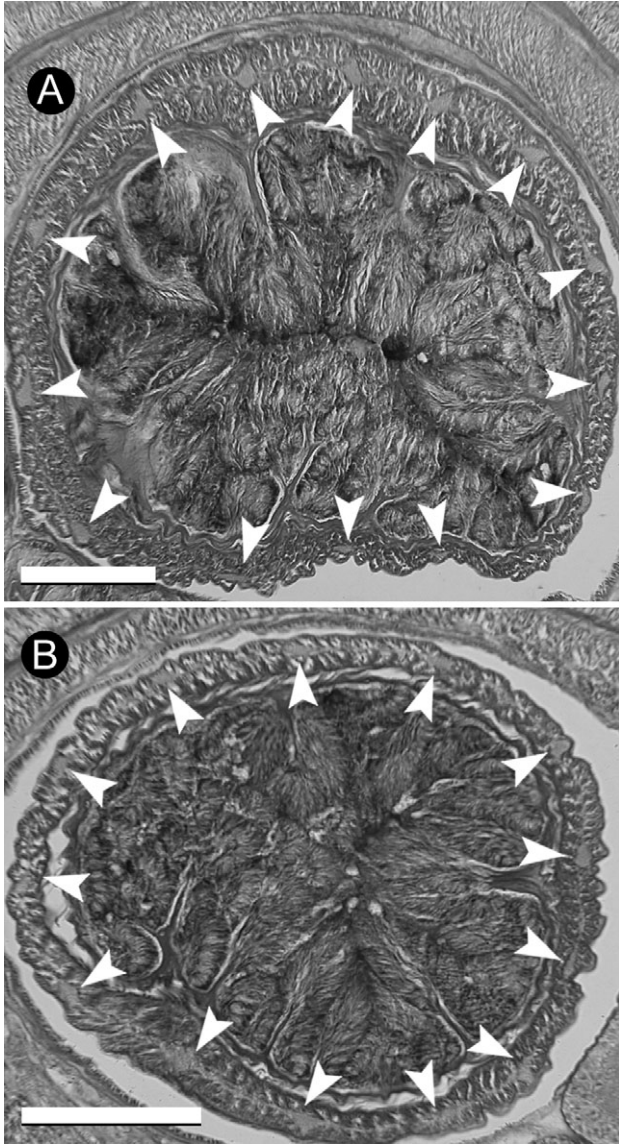
Figure 14. *Diplomma polyophthalma* **(Gibson & Sundberg, 2001) comb. nov.** (formerly *Correanemertes polyophthalma* Gibson & Sundberg, 2001). Proboscis nerves (arrowed) in the holotype, MTQ G20024 (A) and paratype, MTQ G20025 (B). Scale bars = 100 μm.

Figure 15. *Diplomma polyophthalma* **(Gibson & Sundberg, 2001) comb. nov.** (formerly *Correanemertes polyophthalma* Gibson & Sundberg, 2001). Holotype (MTQ G20024). Intestinal caecum below pylorus; arrow indicates dorsoventral muscle fibre running outside lateral nerve cord. Abbreviations: IC, intestinal caecum; LN, lateral nerve cord; PR, proboscis; PY, pylorus; RC, rhynchocoel. Scale bar = 100 μm.

UPLB-NHM 0490, female, transverse sections of anterior region, 8 μm, ten slides, 28.x.2004, Tigbauan, Iloilo; USNM-1136675, male, serial transverse sections, 6 μm thick, 14 slides, 26.vi.1999, Gesashi, Okinawa; ZIHU-1353, immature, transverse sections through anterior region, 6 μm thick, 16 slides, 26.vi.1999, Gesashi, Okinawa; ZIHU-1354, immature, horizontal sections through anterior region, 6 μm thick, four slides, 26.vi.1999, Gesashi, Okinawa; ZIHU-1355, immature, longitudinal sections through anterior region, 6 μm thick, six slides, 23.vi.1999, neotype locality; ZIHU-1356, immature, complete series of transverse sections, 6 μm thick, 13 slides, 26.vi.1999, Gesashi, Okinawa; ZIHU-1357, sex unknown, longitudinal sections through anterior region, 6 μm thick, 31 slides; 26.vi.1999, Gesashi, Okinawa; ZIHU-1358, sex unknown, longitudinal sections through anterior region, 6 μm thick, four slides, 26.vi.1999, Gesashi, Okinawa; ZIHU-3167, male, complete series of longitudinal sections, 10 μm thick, six slides, 5.vii.1999, Shirahama, Wakayama; ZIHU-3166, female, transverse sections of anterior region, 8 μm thick, 19 slides, 5.vii.1999, Shirahama, Wakayama; ZIHU-3168, sex unknown, transverse sections of anterior region, 8 μm thick, one slide, 18.v.1996, Misaki, Kanagawa; and ZIHU-3175, male, complete series of longitudinal sections, 10 μm thick, three slides, 18.v.1996, Misaki, Kanagawa.

External features: Body slender, uniform brown, paler on ventral side, 3–45 mm long, 0.3–0.8 mm wide (Figs 16A, 17A). Head wider than body, with white margin on anterior edge (Figs 16B, 17A–C). Two pairs of oblique cephalic furrows present (Fig. 17B). Anterior furrows run obliquely forward on each side of head, turn slightly backward on their ventral ends (Fig. 17C). Posterior furrows, sometimes indistinguishable in small specimens especially without anaesthetization, completely encircle body, forming
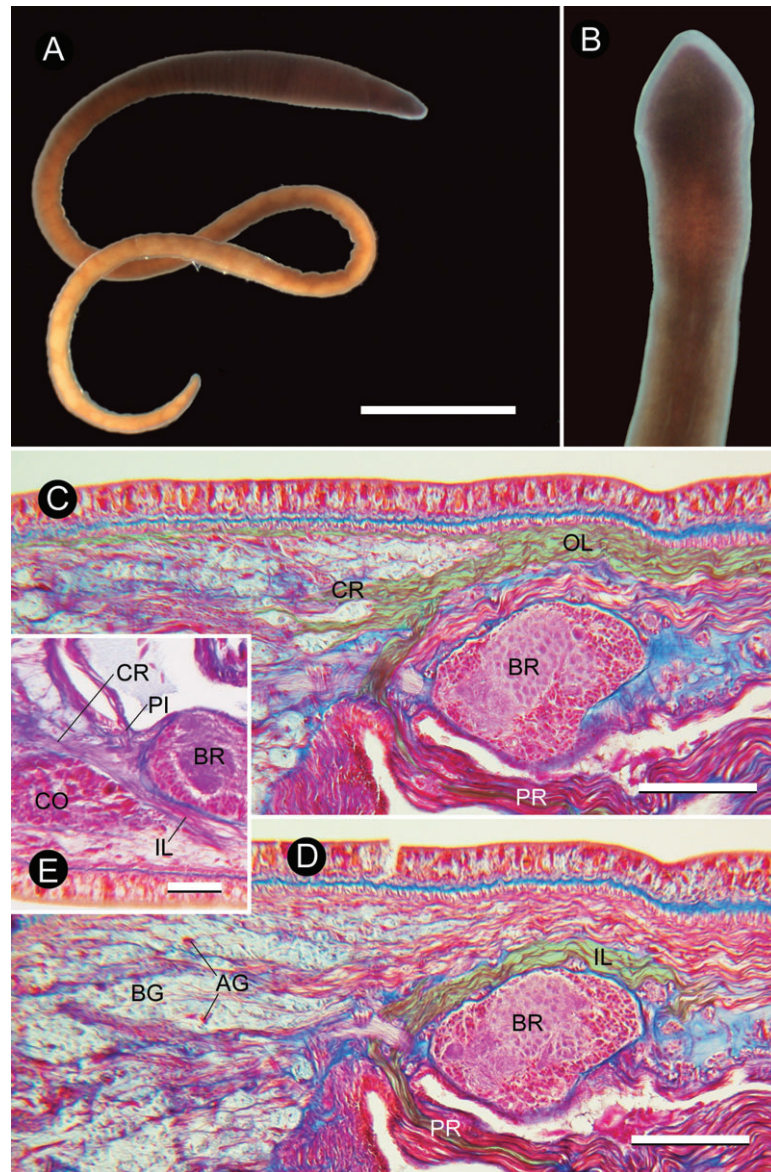
Figure 16. *Diplomma serpentina* (Stimpson, 1855). A, photograph of narcotized specimen taken in life; B, photograph of the same specimen as (A) before anaesthetization, showing natural shape of head; C, horizontal section through head, showing arrangement of outer portion of body-wall longitudinal musculature and its derivatives; D, horizontal section through head, showing arrangement of inner portion of body-wall longitudinal musculature contributing to proboscis insertion; E, sagittal section through head, showing cephalic retractor muscle fibre derived from inner portion of divided body-wall longitudinal muscle layer. A, B, E, UPLB-NHM 0489; C, D, ZIHU-1354. Abbreviations: AG, acidophilic cephalic gland; BG, basophilic cephalic gland; BR, brain; CO, cerebral organ; CR, cephalic retractor muscle; IL, inner portion of body-wall longitudinal musculature; OL, outer portion of body-wall longitudinal musculature; PI, proboscis insertion; PR, proboscis. Scale bars: A = 3 mm; C, D = 100 μm; E = 50 μm.

posteriorly pointing V-shape on dorsal surface (Fig. 17B). Eyes usually difficult to observe in living state because of body coloration (Figs 16A, B, 17A, B), randomly scattered in precerebral region, but they may be arranged more or less in two rows on each side (Fig. 18), with peripheral rows along edge of head and the medial rows running somewhat parallel with each other; the peripheral eyes tend to be larger than the medial ones and may be visible from lateral views (Fig. 17D); anteriormost pair of eyes were visible from above in living state in individuals collected at Misaki.
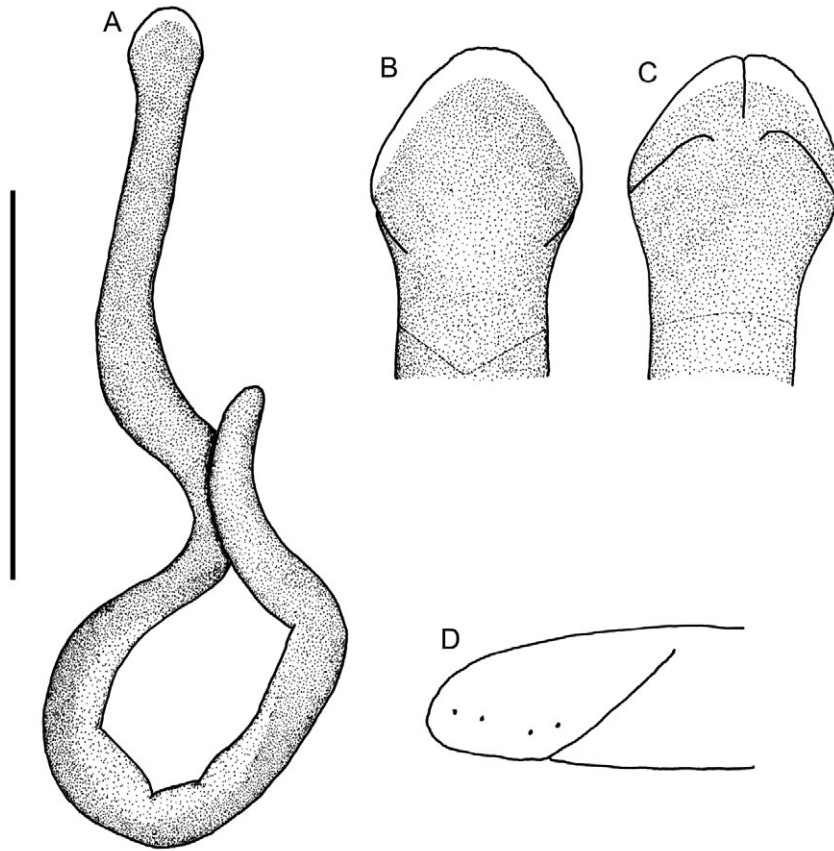
Figure 17. *Diplomma serpentina* (Stimpson, 1855). A, drawing based on neotype, showing general appearance of body in life; B, C, D, enlargements of cephalic region in dorsal (B), ventral (C), and lateral (D) aspects. Scale bar [refers to (A) only] = 3 mm.

Body wall, musculature, and parenchyma: Ciliated glandular epidermis (Figs 19A, 20C), 16–38 µm thick in brain region, generally thinner posteriorly, 9–35 µm thick in intestinal region. Connective tissue dermis (Figs 20C, 21B) thin but distinct, 5 µm in maximum thickness. Body wall muscle layers generally better developed in anterior half of body. Circular muscle layer (Fig. 20C) extending full length of body, about 2–9 µm deep in stomach region, reduced to 1–7 µm in intestinal region. From the region near the posterior end of dorsal ganglia forward, internal portions of body-wall longitudinal layer become surrounded by thin connective tissue membranes to become isolated bundles, which partially fuse anteriorly to form an incomplete and discontinuous layer to divide the body-wall longitudinal muscles into inner and outer layers (Figs 16C, D, 19A); in a smaller specimen (ZIHU-1356); however, the separation of the body-wall longitudinal layer into inner and outer portions was not obvious. When the division of the body-wall longitudinal muscle layer is apparent, all fibres from the inner layer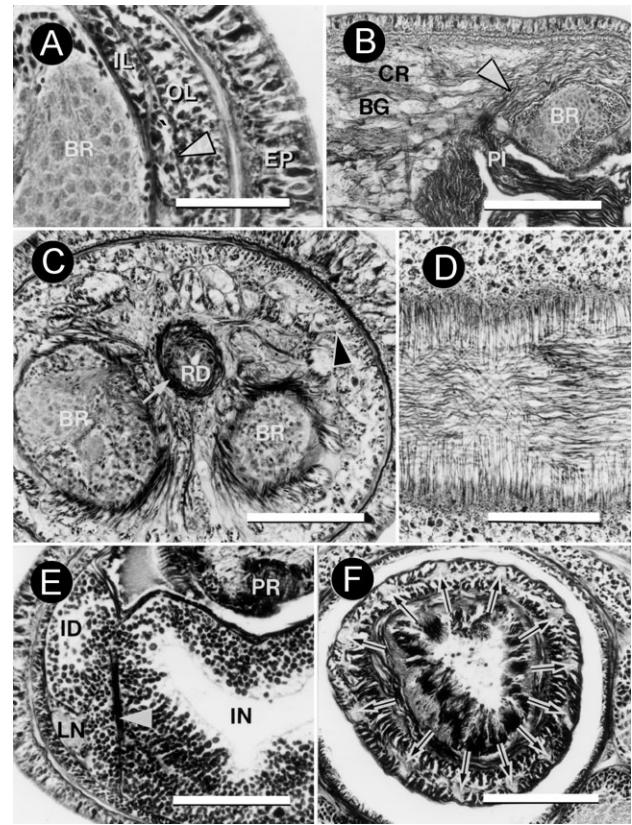 appear to lead into proboscis insertion (Fig. 16D) in most of the specimens; in a few instances, part of the fibres from the inner portion seem to run forward beyond proboscis insertion (Fig. 16E). In some specimens (including the neotype), a few fibres from the outer longitudinal layer also contribute to proboscis insertion (Figs 16C, 19B). In other specimens, participation of outer portion of the longitudinal musculature to the proboscis insertion may be subtle (ZIHU-3166) or impossible to confirm (ZIHU-3167, UPLB-NHM 0940). Diagonal muscle layer present between body-wall outer circular and inner longitudinal muscle layers (Fig. 19D), although it could not be observed in smaller specimens. Dorsoventral muscles variously developed; in the neotype they may run outside the lateral nerve cord in the foregut region (Fig. 20A) and between intestinal lateral diverticula in intestinal region (Fig. 19E); in some paraneotypes they occur in foregut region just inside body-wall longitudinal muscle layer (Figs 20B, C, 21A) or some distance inside from the longitudinal layer with parenchymatous connective tissue between them (Fig. 21D), sometimes even outside excretory
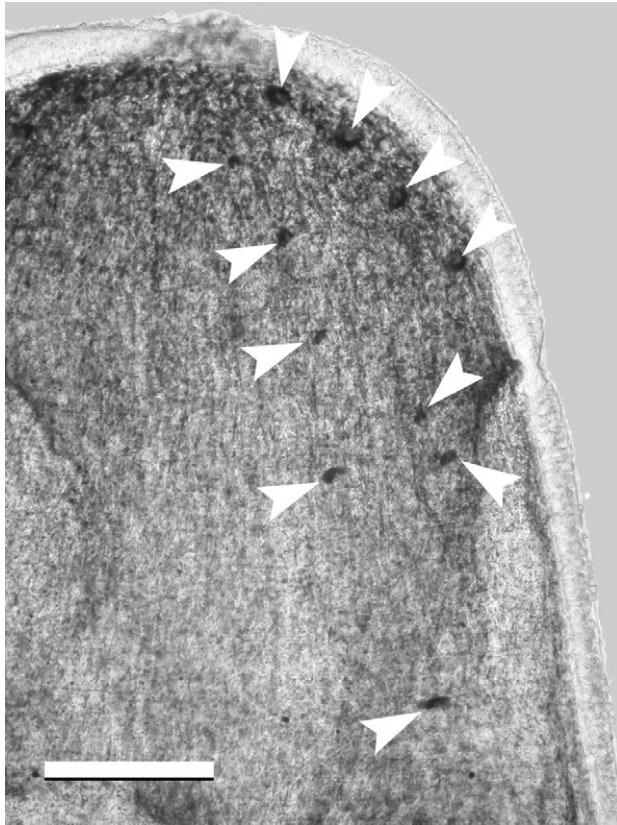
Figure 18. *Diplomma serpentina* (Stimpson, 1855). Photomicrograph of squeezed specimen collected at Tigbauan, showing arrangement of eyes indicated by arrowheads; the specimen was destroyed after *in vivo* examination. Scale bar = 30 μm.

Figure 19. *Diplomma serpentina* (Stimpson, 1855). A, transverse section through brain region, showing epidermis and divided longitudinal musculature; arrowhead indicates connective tissue layer that divides longitudinal musculature into two layers; B, horizontal section through brain region; arrowhead indicates fibres from outer longitudinal muscle layer; C, transverse section through proboscis insertion; white arrow indicates rhynchodaeal sphincter; black arrowhead indicates fibre from outer longitudinal muscle layer; D, tangential section of body wall, showing lattice-type diagonal muscles; E, transverse section through intestinal region, showing dorsoventral muscle (indicated by arrowhead) running between intestinal lateral diverticula; F, transverse section through anterior portion of proboscis, showing 12 proboscis nerves (indicated by arrows). A, E, F, neotype (ZIHU-1352); B, paraneotype (ZIHU-1354); C, paraneotype (ZIHU-1353); D, paraneotype (ZIHU-1356). Abbreviations: BG, basophilic cephalic gland; BR, brain; CR, cephalic retractor muscle; EP, epidermis; ID, intestinal lateral diverticulum; IL, inner portion of divided body-wall longitudinal muscle layer; IN, intestine; LN, lateral nerve cord; OL, outer portion of divided body-wall longitudinal muscle layer; PI, proboscis insertion; PR, proboscis; RD, rhynchodaeum. Scale bars: A, C, D, F = 50 μm; B, E = 100 μm.
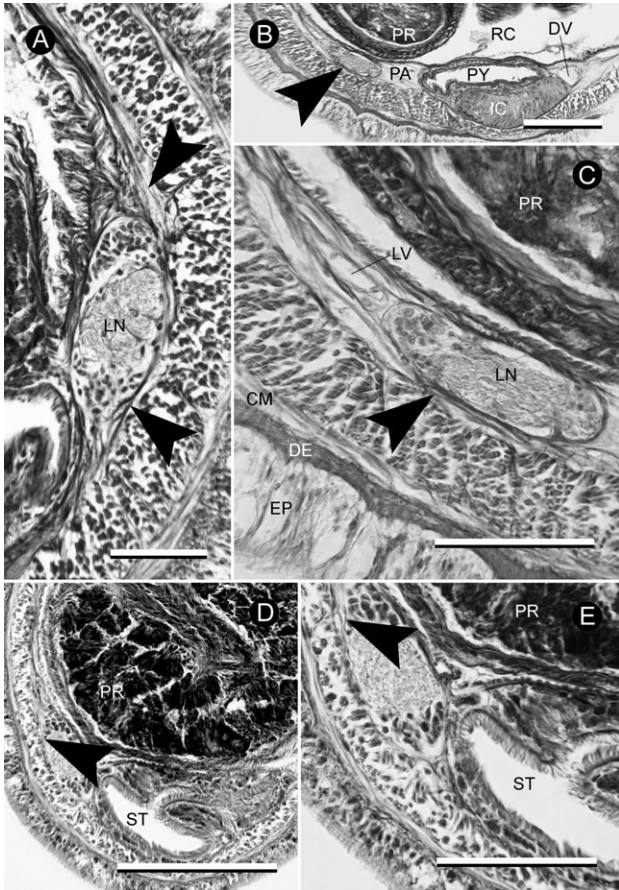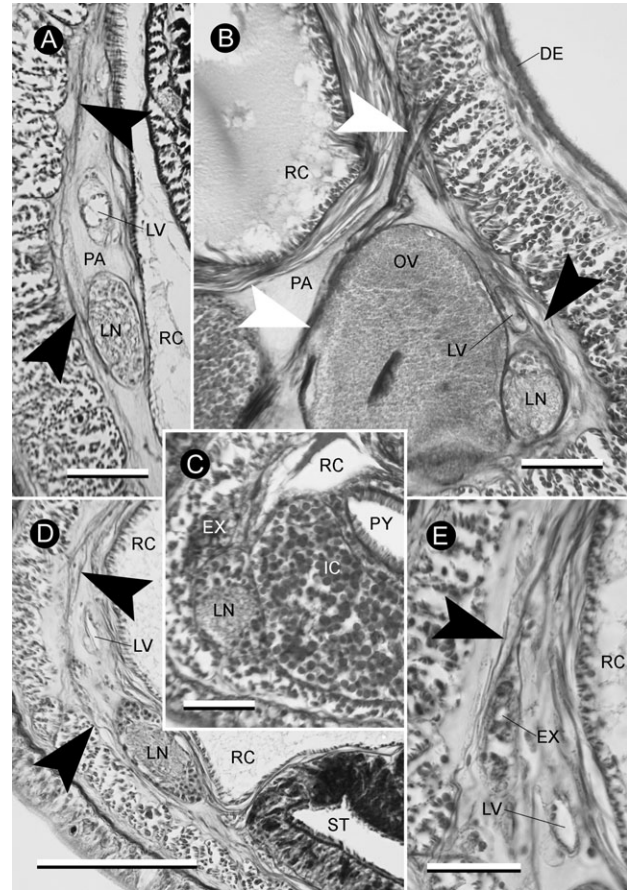
collecting tubules (Fig. 21E); in intestinal region they may occur mediad to the gonads as well as laterad to the lateral nerve cords (Fig. 21B); in smaller specimens, however, the dorsoventral muscles are not apparent (Fig. 20D, E) or not found (Fig. 21C); when present, dorsoventral muscle fibres may appear as if they constituted an inner circular 'layer' (Fig. 21D), but these do not line body-wall longitudinal muscle layer completely, and they do not occur below alimentary canal. Parenchymatous connective tissues not well developed in neotype (Figs 19E, 20A) and also in smaller paraneotypes (Figs 20D, E, 21C), but moderately developed in foregut and intestinal regions in other paraneotypes (Figs 20B, 21A, 22D).

Proboscis apparatus: Proboscis pore subterminal, leading into thin-walled rhynchodaeum that is neither ciliated nor glandular. Just in front of proboscis insertion, rhynchodaeal epithelium thicker (5–12 μm) and enclosed by circular muscle layer (Fig. 19C). Anterior proboscis region, 23–48% of the body diameter, consisting of three muscle layers
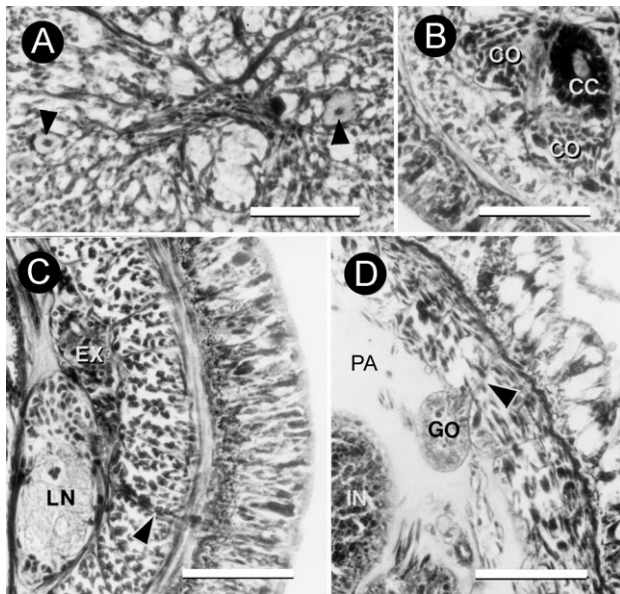
Figure 20. *Diplomma serpentina* (Stimpson, 1855). A, transverse section through foregut region, showing dorsoventral muscles running outside lateral nerve cord (indicated by arrowheads); B, C, transverse section through pyloric region, showing dorsoventral muscles (indicated by arrowhead); D, E transverse section through foregut region, showing dorsoventral muscle fibre (indicated by arrowhead). A, neotype (ZIHU-1352); B, C, paraneotype (ZIHU-3168); D, E, paraneotype (USNM-1136675). Abbreviations: CM, circular muscle layer; DE, dermis; DV, mid-dorsal vessel; EP, epidermis; IC, intestinal caecum; LN, lateral nerve cord; LV, lateral blood vessel; PA, parenchyma; PR, proboscis; PY, pylorus; RC, rhynchocoel; ST, stomach. Scale bars: A = 30 μm; B, D = 100 μm; C, E = 50 μm.
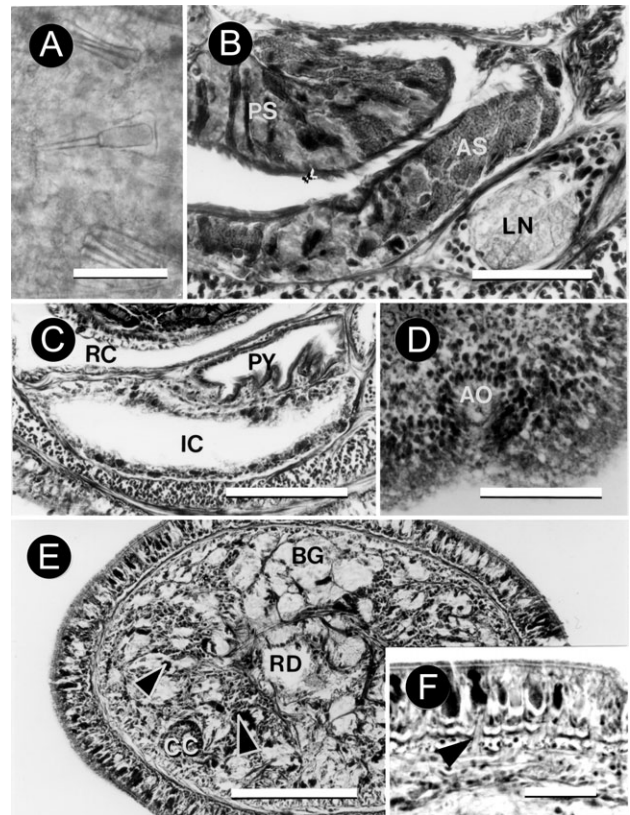
Figure 21. *Diplomma serpentina* (Stimpson, 1855). A, transverse section through foregut region, showing dorsoventral muscles (indicated by arrowheads); B, transverse section through intestinal region, showing dorsoventral muscles running outside lateral nerve cord (indicated by black arrowhead) and inside ovary (indicated by white arrowheads); C, transverse section through pyloric region – dorsoventral muscles were not evident in this individual; D, transverse section through foregut region, showing dorsoventral muscles (indicated by arrowheads) running outside lateral nerve cord and lateral blood vessel; E, transverse section through foregut region, showing dorsoventral muscles (indicated by arrowhead) running outside excretory tubule. A, B, paraneotype (ZIHU-3166); C, paraneotype (ZIHU-1356); D, E, paraneotype (UPLB-NHM 0488). Abbreviations: DE, dermis; EX, excretory collecting tubule; IC, intestinal caecum; LN, lateral nerve cord; LV, lateral blood vessel; OV, ovum; PA, parenchyma; PY, pylorus; RC, rhynchocoel; ST, stomach. Scale bars: A, B = 50 μm; C, E = 30 μm; D = 100 μm.

(outer circular, middle longitudinal, inner circular); in all sectioned specimens 12 proboscis nerves run in longitudinal muscle layer (Fig. 19F). Central stylet smooth, without sculpting (Fig. 23A); measurements taken on 14 specimens collected from the Philippines (Tigbauan) and Okinawa, Japan (Motobu and Gesashi) are summarized in Table 2. Central stylet (S), 73 μm long in neotype, 60 μm long on average, longer than basis (B), 52 μm long in neotype, 40 μm

long on average; S/B ratio 1.40 in neotype, 1.51 on average. Two accessory stylet pouches, each containing two to four accessory stylets. Posterior proboscis region about 12–22% of body diameter, possessing glandular epithelium.
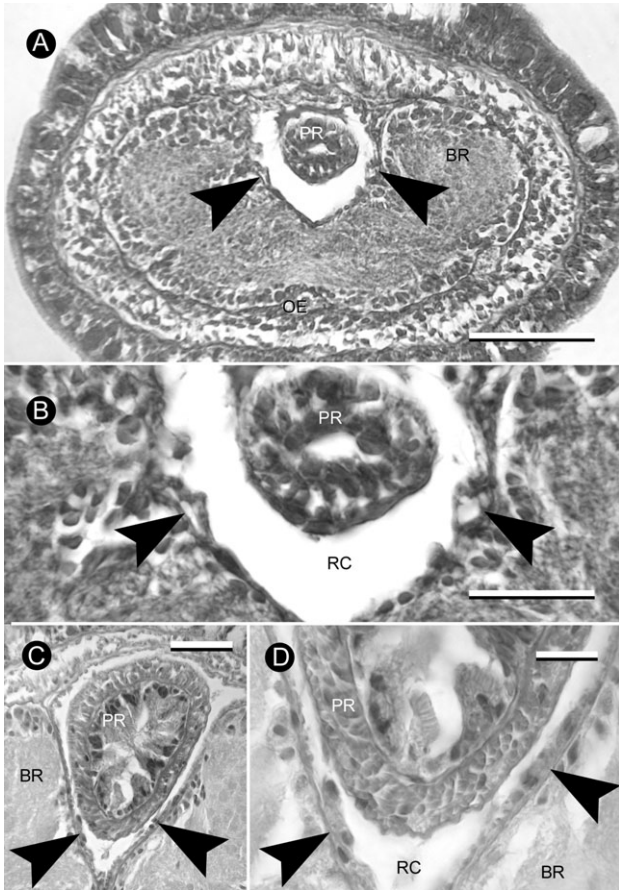
Figure 22. *Diplomma serpentina* (Stimpson, 1855). A, transverse section showing ocelli (indicated by arrowheads); B, transverse section through cerebral organ; C, transverse section through excretory collecting tubule; arrowhead indicates efferent duct; D, transverse section through intestinal region, showing immature gonad; arrowhead indicates gonoduct. A–C, neotype (ZIHU-1352); D, paraneotype (ZIHU-1353). Abbreviations: CC, cerebral sensory organ canal; CO, cerebral sensory organ; EX, excretory collecting tubule; GO, gonad; LN, lateral nerve cord; IN, intestine; PA, parenchyma. Scale bars: A–D = 50 μm.

Alimentary canal: Oesophagus separating from rhynchodaeum just in front of rhynchodaeal sphincter. Oesophageal epithelium, 7–9 μm thick near ventral cerebral commissure, lacking cilia and gland cells. Oesophagus leading below ventral cerebral commissure into short transitional zone where its epithelium contains acidophilic glands but remains unciliated. Stomach histologically divisible into two distinct regions (Fig. 23B). In the anterior stomach region acidophilic gland cells interspersed amongst those with granular contents that stain brown or orange with Mallory. In the posterior stomach region cells with granular contents replaced with typical basophilic glands. Basophilic glands decrease in pylorus, where epithelial height reduces (Figs 20B, 21C, 23C). Stomach seemingly possesses an appendage in neotype (Fig. 25A, B), most probably a fixation artefact; no appendage or diverticula in the rest of all sectioned material. Intestinal caecum present (Figs 20B, 21C, 23C), extending forward below pylorus, but not reaching brain, without anterior pouches or lateral diverticula. Main intestinal canal provided with shallow lateral diverticula throughout its length (Fig. 19E).

Figure 23. *Diplomma serpentina* (Stimpson, 1855). A, photomicrograph of stylet apparatus, taken from life; B, transverse section through junction between anterior and posterior stomach regions; C, transverse section through pyloric region; D, transverse section to show apical organ; E, transverse section through precerebral region, showing various components of cephalic glands; arrowheads indicate coarsely granular acidophilic glands; F, horizontal section through precerebral region, showing improvised duct (indicated by arrowhead). A, paraneotype (ZIHU-1357); B, C, E, neotype (ZIHU-1352); D, paraneotype (ZIHU-1353); F, paraneotype (ZIHU-1354). Abbreviations: AO, apical organ; AS, anterior portion of stomach; BG, basophilic cephalic gland; CC, cerebral sensory organ canal; IC, intestinal caecum; LN, lateral nerve cord; PS, posterior portion of stomach; PY, pylorus; RC, rhynchocoel; RD, rhynchodaeum. Scale bars: A, C, E = 100 μm; B, D = 50 μm; F = 30 μm.

Blood system: Pair of cephalic vessels in head, meeting anteriorly above rhynchodaeum as simple cephalic loop, and extending backward through cerebral ring without connecting with each other; no extra-cerebral vessels. When passing through cerebral ring, vessels become compressed between rhynchocoel wall and brain (Fig. 24A, B); in most cases, including the neotype, vessels so flattened that their lumens are indiscernible, which makes it difficult to trace them in the cerebral region. In one specimen

Figure 24. *Diplomma serpentina* (Stimpson, 1855). A, transverse section through cerebral ring, showing cephalic vessels protruded into rhynchocoel (indicated by arrowheads); B, higher magnification of (A); C, putative position of compressed and flattened cephalic vessels (indicated by arrowheads); D higher magnification of (C). A, B, paraneotype (ZIHU-1356); C, D, paraneotype (USNM-1136675). Abbreviations: BR, brain; OE, oesophagus; PR, proboscis; RC, rhynchocoel. Scale bars: A = 50 μm; B = 20 μm; C = 30 μm; D = 10 μm.

(ZIHU-1356), the two cephalic vessels are protruded into the rhynchocoel when they pass through the cerebral ring (Fig. 24C), although the vessel epithelium exposed to the rhynchocoel does not appear to be histologically differentiated (Fig. 24D). Soon after vessels pass through cerebral ring, either left or right lateral vessel bifurcates to give rise to single middorsal vessel (Fig. 20B), which does not enter rhynchocoel or form median vascular plug. Postcerebrally, the three longitudinal vessels do not meet until they join in the supra-intestinal commissure near hind end of body.

Nervous system: Brain moderately well developed (Figs 16C, D, 19A–C), ensheathed by thin but distinct outer neurilemma, but no inner neurilemma. Dorsal
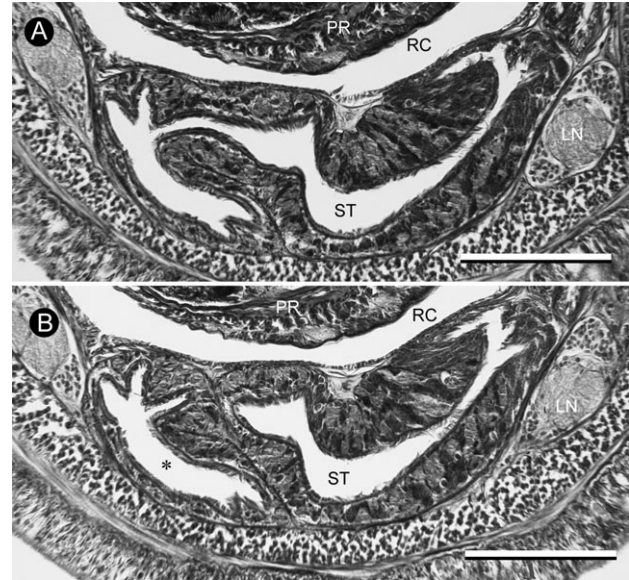
Figure 25. *Diplomma serpentina* (Stimpson, 1855). A, B, transverse section through stomach, showing seemingly appendage-like appearance of the stomach (indicated by asterisk). A, B, neotype (ZIHU-1352). Abbreviations: LN, lateral nerve cord; PR, proboscis; RC, rhynchocoel; ST, stomach. Scale bars: A, B = 100 μm.

and ventral cerebral commissures 12–19 μm and 25–35 μm thick, respectively. Neurochord cells not found. Lateral nerve cord containing single myofibrilla, posteriorly joined by supra-intestinal commissure. Neither accessory lateral nerves nor neurochords present.

Apical organ and cephalic glands: Small, single apical organ (Fig. 23D), 12–25 μm in diameter, opening into shallow ciliated pit situated just above the proboscis pore. Cephalic glands consisting of basophilic lobules with vacuolate appearance and coarsely granular acidophilic glandular cells (Figs 16D, 23E); basophilic lobules, less distinct in smaller specimens, extending posteriorly but never beyond cerebral ring; coarsely granular acidophilic glands rather sparsely scattered throughout head. Basophilic glands discharge to exterior via apical organ, as well as through numerous independent ducts passing through dermis (Fig. 23F).

Sense organs: Three to 16 eyes on each side of the head. Pigment cup ocelli (Fig. 22A), 7–23 μm in diameter. Cerebral sensory organs moderately large and well developed, opening ventrolaterally from anterior cephalic furrows. Each ciliated canal, 12–23 μm in diameter at its opening, extends posteriorly to terminate in a chamber flanked dorsally and ventrally by acidophilic gland cells (Fig. 22B). These glands continue posteriorly as posterior cap of cerebral organs,

Table 2. Measurements of stylet apparatus of *Diplomma serpentina* (Stimpson, 1855) based on specimens collected at Tigbauan (Panay, Philippines), Motobu (neotype locality, Okinawa, Japan), and Gesashi (Okinawa, Japan)

Specimen	Locality	Body length (mm)	Body width (mm)	Stylet length (S, μm)	Basis length (B, μm)	Basis diameter (μm)	S/B ratio
Nontype	Tigbauan	15	0.5	66	41	20	1.61
Nontype	Tigbauan	26	0.7	62	42	21	1.48
Nontype	Tigbauan	16	0.5	55	35	16	1.57
Nontype	Tigbauan	10	0.4	51	30	14	1.7
Nontype	Tigbauan	13	0.5	54	38	17	1.42
Nontype	Motobu	3	0.3	44	29	16	1.52
Nontype	Gesashi	5	0.3	46	32	16	1.44
ZIHU-1352*	Motobu	15	0.5	73	52	23	1.4
ZIHU-1353	Gesashi	13	0.4	73	55	23	1.33
ZIHU-1354	Gesashi	20	0.5	41	31	14	1.32
ZIHU-1355	Motobu	15	0.5	58	52	26	1.12
ZIHU-1356†	Gesashi	4	0.3	–	–	–	–
ZIHU-1357	Gesashi	13	0.4	84	52	23	1.62
ZIHU-1358	Gesashi	6	0.3	62	35	17	1.77
USNM-1136675	Gesashi	4	0.3	64	35	17	1.83
Average		12	0.4	60	40	19	1.51
SD		6.57	0.12	12.18	7.83	3.46	0.16
Min.		3	0.3	41	52	14	1.12
Max.		20	0.5	84	55	26	1.77

*Neotype.
†Proboscis apparatus absent.

reaching in front of brain; in one paraneotype (ZIHU-1356) they extend to just below anterior borders of cerebral ganglia. Cerebral organs attain 34–70 μm wide and 47–96 μm tall.

Excretory system: Excretory system consists of thick-walled collecting tubules (Figs 21C, 22C), 9–23 μm in maximum diameter, distributed in stomach region, situated above lateral nerve cords; in one specimen (ZIHU-1356), they reach backward some distance beyond pyloric-intestinal junction (Fig. 21C). They open to exterior through thin efferent ducts, 5–7 μm wide, passing ventrolaterally or laterally above lateral nerve cords, exiting through one to six (two in the neotype) small nephridiopores on each side (Fig. 22C). No flame cells found.

Reproductive system: Sexes separate. No mature gonads were found in all sectioned specimens obtained from Okinawa in June (Fig. 22D); sexually mature individuals collected from Tigbauan in October, Misaki in May, and Shirahama in July (Fig. 21B). Gonads are arranged in a row on each side of intestine. A single gonoduct from each gonad passes mostly above, but sometimes below, lateral nerve cord to open at a gonopore. Mature ovary contains one to

three eggs, each about 100 μm in diameter, with germinal vesicle about 35 μm in diameter.

Genetic difference: The genetic distances of COI based on the Kimura 2-parameter model (Kimura, 1980) were 0.9–13.1% amongst three localities, Pasir Putih (Indonesia), Tigbauan (Philippines), and Okinawa (Japan); *D. serpentina* differed from **D. polyophthalma comb. nov.** by 12.9–13.1% (Table 3).

DISCUSSION

SPECIES IDENTIFICATION

We identified the newly collected material as *D. serpentina* based on: (1) the similarity of the external characteristics given in the original description; (2) the concordance of the locality; and (3) the common occurrence of the species in the eastern Pacific tropical to subtropical waters observed in the present study, which was implied in Stimpson's (1855) original article. The present material agrees with the original description (Stimpson, 1855) and subsequent complement (Stimpson, 1857) of *D. serpentina* in the shape of the head and the body coloration. That is, our specimens are brownish, and the head is oval-shaped and broader than the body, with a constricted neck. However, the present material differs from

Table 3. Infra- and intraspecific variation of mitochondrial cytochrome *c* oxidase subunit I gene sequence (615 bp) from *Diplomma* species

	D. serpentina (Japan)	*D. serpentina* (Philippine)	*D. serpentina* (Indonesia)	***D. polyophthalma* comb. nov.** (Australia)
D. serpentina	–	5	9	63
D. serpentina	0.9	–	8	63
D. serpentina	1.7	1.5	–	64
***D. polyophthalma* comb. nov.**	12.9	12.9	13.1	–

Numbers above diagonal are base-pair differences; below diagonal are Kimura 2-parameter percentage genetic distances.

Stimpson's (1855, 1857) description in body size and 'eye' arrangement. The body length was originally recorded as 6.35 cm (2.5 inches) in Stimpson (1855), later as 7.62 cm (3 inches) in Stimpson (1857), thus much longer than the maximum length observed in the present study (= 4.5 cm). Stimpson (1855) mentioned that *Na. serpentina* possessed a pair of large bilobed eyes in the middle of the head. Such a condition was not encountered in the present study; this could have been embodied by two ocelli closely situated to each other, but it is unlikely because the ocelli in our samples were generally difficult to ascertain in the living state. Verrill (1892: 403–404) postulated that what Stimpson (1854) interpreted as 'eyes' in *Nareda superba* might have actually represented the cerebral ganglia. If this was the case, Stimpson (1855) might have also overlooked the true 'eyes' in *Na. serpentina*.

The original locality of *D. serpentina* (Stimpson, 1855) was given as 'Loo Choo' (which is spelled as 'Ryukyu' in the current Japanese orthography, referring to Okinawa Island) that is covered by the present faunal survey, from which we did not see any other uniformly brownish monostiliferous hoplonemerteans. *Diplomma serpentina* is the sole species of Hoplonemertea amongst 76 species of the marine invertebrates reported by Stimpson (1855) from China and Japan, consisting of cnidarians, turbellarians, amphipods, gastropods, and ascidians. This implies that this species occurred commonly in this area at that time, and coincides with the results of our faunal survey.

Yamaoka's (2005) material from Shimoda (close to Misaki, one of the sampling localities in the present study, Fig. 1), identified as *Paranemertes* sp. based on features of the external appearance and the stylet apparatus, conforms with the present material in the uniformly brown body coloration and the head with characteristic anterolateral white margins. The S/B ratio of Yamaoka's specimen (= 1.2) falls within the range of variation observed in the present study (= 1.12–1.77). Yamaoka (2005) found two large ocelli

at the tip of the head, a condition similar to *Am. insolitus*, as well as to the present material from Misaki. This, together with the geological closeness of the localities, further supports our view that these represent the same species.

ASSESSMENT OF TAXONOMIC CHARACTERS – INTRASPECIFIC VARIATION

Protrusion of cephalic vessels into rhynchocoel

The protrusion of the pair of cephalic vessels into the rhynchocoel lumen while passing through the cerebral ring was regarded as one of the key characters for Plectonemertidae *sensu lato*, including *Acteonemertes*, *Antiponemertes*, *Argonemertes*, *Campbellonemertes*, *Plectonemertes*, and *Potamonemertes* (Moore & Gibson, 1972, 1973, 1985, 1988; Gibson, 1990a). A similar character state has also been reported in *Halimanemertes slacksmithae* Gibson, 1990b (*q.v.*) and *Tetraneuronemertes lovgreni* Sundberg, Gibson & Strand, 2007 (*q.v.*). In freshwater and terrestrial plectonemertids, however, the wall of the protruded blood vessel is histologically differentiated to contain rod-shaped cells (Pantin, 1969) or sphere-shaped cells (Moore & Gibson, 1972), thus differing from the condition found in *Halimanemertes* and *Tetraneuronemertes*, as well as the present material of *D. serpentina*, in which notable histological specialization of vessel walls was not observed. Of the eight series of transverse sections examined in the present study, one specimen (ZIHU-1356) showed the two cephalic vessels projected into the rhynchocoel lumen while passing through the cerebral ring (Fig. 24A, B). This was one of the smallest specimens obtained (Table 2), about one-fifth of the length of the largest individual collected within the same locality, and less than one-tenth of the longest individual recorded in another locality. In another individual of the same size from the same locality (USNM-1136675), no obvious protrusion was observed, although the vessels become extremely compressed and flattened when they pass through the cerebral ring (Fig. 24C, D) as in

the other larger specimens. Therefore, the protruded condition does not appear to have direct correlation to the body size. Whether the protruded state represents an artefact during preparation of the specimen or reflects the natural condition remains unclear. In either case, the present study shows that whether the cephalic vessels protrude or not may vary intraspecifically. To our knowledge, this intraspecific variation has never been reported amongst Monostilifera, although a similar variation is confirmed in *Nemertellina yamaokai* Kajihara *et al.*, 2000 and *Gononemertes parasita* Bergendal, 1900 (H. Kajihara, pers. observ.). In *Ne. yamaokai*, the protrusion of the cephalic vessels is observed in three out of six transversely sectioned specimens; it is also observed in one of the three cross-sectioned individuals of *G. parasita*. Specialization of the blood vessel walls associated with the rhynchocoel lumen was not obvious at the light microscopy level in *D. serpentina* and *Ne. yamaokai*. In *G. parasita*, however, the walls of the cephalic vessels protruded into the rhynchocoel were apparently thick and contained sphere-shaped bodies.

'Divided' body-wall longitudinal musculature

The present study shows that the following character states vary intraspecifically in *D. serpentina*, and thus should be used with care in identifying and defining genera and species in Monostilifera: (1) whether or not the body-wall longitudinal muscle layer is 'divided' near the brain; (2) which layer of the 'divided' longitudinal layer contributes to the proboscis insertion; and (3) which layer of the 'divided' longitudinal layer forms the origin of the cephalic retractor muscles. Many of the genera established on the fact that they possessed a 'divided' body-wall longitudinal muscle layer must be reappraised with regard to their monophyly.

Functionally, the body-wall longitudinal muscle layer must be always 'divided' in all the monostiliferous hoplonemerteans near the brain. In monostiliferous hoplonemerteans, the proboscis is attached to the body (between the rhynchodaeum and rhynchocoel) in front of the brain. The attachment of the proboscis to the body is achieved by muscle fibres derived from the body-wall longitudinal muscle layer, and the position where the proboscis is attached to the body is called the proboscis insertion. Part of the body-wall longitudinal muscles, running from behind the brain forward, branches off medially then runs in front of the brain and eventually passes into the proboscis insertion; therefore, the body-wall longitudinal muscle layer is theoretically 'divided' into inner and outer portions near the brain. Confusingly, the outer portion of the 'divided' body-wall longitudinal muscle layer may send off fibres medially to the proboscis insertion. The muscle fibres running medially in front

of the brain have been regarded as forming a 'precerebral septum'. After the body-wall longitudinal muscle layer medially branches fibres off to the proboscis insertion, either the inner or outer (or both) portion(s) of the layer run(s) anteromedially in front of the brain; these fibres are called cephalic retractor muscles.

Confusion exists as to the interpretation of the character state and terminology of the 'divided' nature of the body-wall longitudinal muscle layer, dating from the time when this character state was first used in creating a new genus, *Paramphiporus* Kirsteuer, 1965, without giving any terminological definition.

The genus *Paramphiporus*, which we synonymize with *Diplomma* in this paper, was created for *Paramphiporus albimarginatus* Kirsteuer, 1965 from Madagascar, primarily based on the nature of the body-wall longitudinal musculature, which is divided into inner and outer portions near the brain (Kirsteuer, 1965, 1974). Indeed, there were some species in three genera that possessed a similar condition of the body-wall longitudinal musculature, including *Amphiporus* Ehrenberg, 1832, *Nemertes sensu* Johnston (1837) [renamed as *Tetranemertes* by Chernyshev (1992)], and *Paranemertes* Coe, 1901. Species in *Tetranemertes* Chernyshev, 1992 clearly differed from *Paramphiporus albimarginatus* in possessing a short rhynchocoel, whereas Kirsteuer (1965) considered that *Amphiporus* and *Paranemertes* were ill-circumscribed and heterogeneous, and that classifying his new species into either genus would make these genera more nonmonophyletic. The establishment of the genus *Paramphiporus* Kirsteuer, 1965 can be regarded as the commencement of a taxonomic splitting trend, in which many new genera have been later created for those species with anteriorly 'divided' body-wall longitudinal musculature (e.g. Kirsteuer, 1967b, 1974). This trend largely stems from Friedrich's (1955) revision of Monostilifera, where he pointed out the nonhomogeneity of the long-established genus *Amphiporus* that contains approximately 150 nominal species (Gibson & Crandall, 1989). Friedrich (1955) set apart four species from what he regarded as '*Amphiporus sensu stricto*'. The four species, *Am. bioculatus* McIntosh, 1874, *Am. hastatus* McIntosh, 1874, *Am. nebulosus* Coe, 1901, and *Am. korschelti* Friedrich, 1940, were considered as possessing anteriorly 'divided' longitudinal musculature, and referred to as the '*hastatus* group'. Friedrich (1955) discussed that out of the four species *Am. bioculatus* should be removed from the group because it has a simple cephalic blood vascular system, whereas the other three species possess extracerebral vessels, a condition that is quite unusual amongst monostiliferous hoplonemerteans.

Friedrich (1955) did not venture to separate *Am. bioculatus* from the group, because descriptions of *Am. bioculatus* available at that time, including those of McIntosh (1873–1874), Joubin (1890), and Bergendal (1903), were considered as inadequate as to their internal anatomy. After Corrêa (1958) reported a detailed muscular morphology in the cephalic region in a form under the name *Am. bioculatus* from Brazil, Kirsteuer (1967b) considered it to be different from *Am. bioculatus s.s.* and established the genus *Correanemertes* Kirsteuer, 1967b for it. Later, more than ten genera were created for those species with anteriorly 'divided' body-wall longitudinal musculature, based on the combination of the characters variously including the cephalic vessel morphology, presence or absence of diagonal muscle layer, origin of the cephalic retractor, nature of the proboscis insertion, rhynchocoel length, or presence or absence of neurochord cells in the central nervous system (e.g. Sánchez & Cancino, 1980; Gibson, 1990b, 2002; Kajihara, Gibson & Mawatari, 2001; Iwata, 2006, 2007).

At least three problems are conceivable with this trend. One is that no attempt has been made to examine the characters and their states in the type species of the genus *Amphiporus*, namely *Amphiporus lactifloreus* (Johnston, 1828) [for the replacement of the type species, see Gibson & Crandall (1991) and International Commission on Zoological Nomenclature (1992)]. Friedrich (1955) implied that in *Amphiporus s.s.* the longitudinal musculature was not divided. However, observation of a specimen of *Am. lactifloreus* (ZIHU-3276, collected in Anglesey, UK), revealed that in this species the body-wall longitudinal muscle layer is indeed divided into inner and outer portions near the brain (H. Kajihara, pers. observ.).

The second problem is that intraspecific variation of the characters used in creating these genera has been seldom studied. It should be stressed that in the present material of *D. serpentina*, the division of the body-wall longitudinal muscle layer may be obscure in smaller specimens, and that the participation of the outer portion of the divided longitudinal muscle layer into the proboscis insertion is not always apparent. Similar problems have been already pointed out by Norenburg (1986) and Moore & Gibson (1988). Although various types of 'precerebral septum' have been proposed and categorized (e.g. Kirsteuer, 1974; Chernyshev, 2002), the usage of this term and categorization of its state into one of these proposed types were intentionally avoided in the present redescription.

The last problem is the semantic one; the criterion of applying the term 'divided' was unclear from its first usage by Kirsteuer (1965). The character state, anteriorly divided longitudinal muscle layer, can be embodied in both qualitatively and quantitatively different states; however, these states appear to be more or less mutually continuous either intragenerically or even intraspecifically. The longitudinal muscle layer can be divided variously by connective tissues, by posteriorly extended cephalic glands, or even by the combination of them (e.g. dorsally by connective tissues, ventrally by cephalic glands). The layer can even also be divided partially (e.g. only ventrally in the brain region). The layer may be divided only in the brain region or further backward in the foregut-pylorus region. Qualitative assessment of their character states may be difficult in some cases, because the implementation of the divided longitudinal musculature is directly influenced by the degree of the posterior extent of cephalic glands, which may vary intraspecifically or by contraction during fixation. Quantifying the extent of the area where the longitudinal muscle layer is divided is also difficult, although some measures can be carried out by using other internal organs (brain, foregut, pylorus, etc.) as landmarks.

In conclusion, detailed description of the body-wall longitudinal muscular complex in the proboscis insertion is preferably to be given in taxonomic descriptions for some time to come, until we can reasonably categorize these character states. Additionally, in a case where the layer is 'divided' only near the brain in an observed specimen, this character state, together with the combination of its associated characters such as the origin of the proboscis insertion, should not be used to identify it into one of the existing genera.

Body-wall inner circular muscle 'layer'

Although commonly found in anoplans, a circular muscle layer inside the body-wall longitudinal muscles is rare amongst hoplonemerteans, as Crandall (1993) pointed out. Monostiliferans thus far reported to possess a complete inner circular muscle layer include *Am. insolitus* Iwata, 1954a, four species of *Ischyronemertes* (Gibson, 1990b; Senz, 1997), and *Parischyronemertes mathesonensis* Gibson, 2002; the genera *Ischyronemertes* and *Parischyronemertes* have been mainly diagnosed by the possession of the inner circular muscle layer. Weakly developed, more or less discontinuous inner circular muscles have also been reported in *Digononemertes australiensis* Gibson, 1990b, *Amphiporus mortonminelli* Gibson, 2002 and *Vulcanonemertes rangitotoensis* Gibson & Strand, 2002.

In *D. serpentina*, the dorsoventral muscle fibres inside the body-wall longitudinal muscle layer were generally well developed especially in the foregut region, except in one of the smallest specimens (Fig. 21C). When present, these fibres often appeared to be an incomplete 'layer' (e.g. Figs 20A, 21C), and

similar to those in *Ischyronemertes* or *Parischyrone-mertes*. In all the sectioned material of *D. serpentina*, however, this 'layer' was not connected below the foregut, and the degree of the development of these muscles varied amongst various body regions within a single individual.

As Crandall (1993) noted, interpretation of the inner circular muscle 'layer' is difficult with respect to the homology with those in other nemertean higher taxa. We can at least conclude that it is important in distromatonemertean species descriptions to record whether or not the dorsoventral (or circular) muscle fibres run outside the lateral nerve cord, as this character has not routinely been described in the previous literature.

Diagonal muscle layer
This character was once used in discriminating genera, in combination with other characters (e.g. Kajihara *et al*., 2001). As already noted by some nemertean systematists (e.g. Maslakova & Norenburg, 2008a, b) and confirmed by the present study, the presence or absence of the diagonal muscles between the body-wall circular and longitudinal muscle layers may vary intraspecifically.

POSSIBLE CONGENERS

Amphiporus insolitus Iwata, 1954a, may be congeneric, or even conspecific, with *D. serpentina*. *Amphiporus insolitus* was described from Kushimoto, close to Shirahama, one of the sampling localities in the present study (Fig. 1). Iwata (1954a) reported that *Am. insolitus* possessed a uniformly dark brown body, without any marking, 4 cm long and 0.7 mm wide, features common with (or falling within the range of the variation in) the present material. In *Am. insolitus*, similarly to the present material, the eyes were reported to be difficult to observe in life, although 'a pair of small ocellus-like spots is situated at the lower portion of the lateral side of the rhynchodeal opening' (Iwata, 1954a), a condition also observed in specimens collected from Misaki in the present study. Although Iwata (1954a) stated that an intestinal caecum was absent in *Am. insolitus*, the drawing of the transverse section through the portion of the foregut-intestine junction in the original description (Iwata, 1954a: fig. 3) clearly indicates the presence of the intestinal caecum below the pylorus, as is present in our material. The presence of a body-wall inner circular muscle 'layer' in *Am. insolitus*, which was regarded to be rare in monostiliferous hoplonemerteans (Crandall, 1993), is also indicative of congenericity and/or conspecificity. However, *Am. insolitus* differs from the present material in that the number of the proboscis nerves is reported to be 14

(12 in all the sectioned material in the present study) and the S/B ratio is 1.0. We refrain from synonymizing *Am. insolitus* and *D. serpentina* before observing the holotype of *Am. insolitus*.

Amphiporus texanus was described based on a single, preserved specimen collected from Port Aransas, Texas, USA, and thus the external appearance of the living state is unknown (Coe, 1951a, b). In the holotype of *Am. texanus* the number of the proboscis nerves is ten and the S/B ratio is below 1.0. Later, Corrêa (1961) attributed specimens from Miami, USA, in which the S/B ratio is more than 1.0 to *Am. texanus*. As Kirsteuer (1965) pointed out, *Am. texanus sensu* Corrêa (1961) resembles *Paramphiporus albimarginatus* in body coloration in life. The other aspects of the internal anatomy of *Am. texanus* are poorly known (Coe, 1951a, b; Corrêa, 1961), and thus a detailed comparison between *Am. texanus* and *D. serpentina* is currently impossible.

PHYLOGENY

In the present molecular phylogenetic analyses amongst Distromatonemertea, *D. serpentina* formed a clade with *Antarctonemertes varvarae*, *Emplectonema mitsuii*, *Ne. yamaokai*, *Oerstedia dorsalis*, *Oerstedia venusta*, and *Oerstedia zebra* in the 28S analysis (Fig. 2), and **D. polyphthalma** comb. nov., *Emplectonema mitsuii*, *Gononemertes parasita*, *Ne. yamaokai*, *Oe. dorsalis*, *Oe. striata*, *Tetraneuronemertes lovgreni*, and *Vulcanonemertes rangitotoensis* in the 18S analysis (Fig. 3). These species commonly possess a mid-dorsal vessel that does not enter the rhynchocoel (Chernyshev, 1999; Kajihara, Gibson & Mawatari, 2000; Gibson & Sundberg, 2001; Gibson & Strand, 2002; Sundberg, Gibson & Strand, 2007; H. Kajihara, pers. observ.). Therefore, this type of vessel morphology might be synapomorphic for this clade.

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