Ribbons worm relationships: a phylogeny of the phylum Nemertea

Mikael Thollesson* and Jon L. Norenburg

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We present the most extensive phylogenetic analysis to date, to our knowledge, of higher-level nemertean relationships, based on sequence data from four different genes (the nuclear genes for nuclear large subunit rRNA (28S rRNA) and histone H3 (H3), and the mitochondrial genes for mitochondrial large subunit rRNA (16S rRNA) and cytochrome c oxidase subunit I (COI)). Well-supported clades are, in general, compatible with earlier, more limited, analyses, and current classification is largely in agreement with our results, although there are some notable exceptions. Bdellonemertea (represented by Malacobdella) is found to be a part of Monostilifera, and Polyplystifera is the monophyletic sister group to Monostilifera. Cranenemertidae is the sister group to the remaining monostiliferans (including Malacobdella), a group to which we apply the new name Distromatonomertea. Heteronemertea is monophyletic and forms a clade with Hubrechtella; for this clade we introduce the name Pilidiophora. Finally, Pilidiophora and Hoplonemertea (with Malacobdella) form a monophyletic group, and we introduce the name Neonomertea to refer to this group. Palaeonemertea is found to be non-monophyletic and basal among nemerteans.

Keywords: phylogenetic analysis; DNA sequence data; spiralians; phylogenetic taxonomy; Bayesian analysis

1. INTRODUCTION

Nemerteans, or ribbon worms, are unsegmented, bilaterally symmetrical and at first glance acelomate worms with separate mouth and anus. They possess a blood vascular system, however, that most probably is homologous to a coelom (Turbeville 1986). The monophyly of Nemertea is not in doubt today and is supported morphologically by, among other things, the characteristic eversible proboscis situated in a rhynchocoel, features that are unique to the phylum. Nemertea comprises about 1150 nominal species (Gibson 1995), occupying a broad spectrum of habitats, especially in the marine environment.

The current nemertean classification (e.g. table 1) is based on a limited number of morphological characters, and comprises non-monophyletic groups (Sundberg 1993; Sundberg & Svensson 1994). Higher classification is based on Stiasny-Wijnhoff (1936) who classified the nemerteans into the subclasses Palaeonemertea, Heteronemertea, Hoplonemertea and Bdellonemertea. These in turn form Anopla, nemerteans lacking armament on the proboscis (Heteronemertea and Bdellonemertea), and Enopla, nemerteans that possess a proboscis armed with one or several stylets. Some of these higher taxa may very well be monophyletic, but saronomorphies have been assigned in a post hoc fashion. At less inclusive levels, nemertean systematics is also in need of a review. Gibson (1985) noted that, at the time, 40% of the species were assigned to one of four 'mega-genera' (Cerebratulus, Lineus, Amphiporus and Tetrastemma). In a subsequent paper, Gibson (1995) estimates the number of valid genera as 250 and the number of valid species as 1150. The taxonomic resolution has increased, but the four 'mega-genera' still comprise the lion's share of nemertean species. Recent decades have also witnessed the creation of a large number of monotypic genera, rarely accompanied by informative phylogenetic evaluation.

The phylogenetic relationships within the phylum Nemertea and its position in Metazoa have recently received some attention. Studies based on molecular and combined molecular and morphological data of Metazoa have (directly or indirectly) addressed the position within Metazoa (e.g. Turbeville 1991; Turbeville et al. 1992; Sundberg et al. 1998; Zrzavy et al. 1998), but there have been relatively few published studies on the relationships within the phylum based on explicit phylogenetic analyses. A few studies have focused on specific subgroups, such as leptotyphlonemertids (Härtnin & Sundberg 1995), Otophyloneermertes (Envall & Sundberg 1998) and the palaeonemerteanid (Sundberg & Hylbom 1994). Phylogenetic studies based on sequence data were applied by Sundberg et al. to Heteronemertea (using the mitochondrial 16S rRNA gene; Sundberg & Saur 1998) and later to the phylum (using the 18S rRNA gene; Sundberg et al. 2001). Here, we report the first more extensive analysis of higher-level nemertean relationships, to our knowledge, based on sequence data from four different genes (the nuclear genes for 28S rRNA (28S) and histone H3 (H3), and the mitochondrial genes for 16S rRNA (16S) and cytochrome c oxidase subunit I (COI)). The data enable us to erect a phylogenetic hypothesis of the relationships within the phylum Nemertea, with good support for many clades, thus facilitating the evaluation of the higher taxa currently used. The result, when comparing well-supported clades, is compatible with the more limited analysis based on the 18S rRNA gene previously published (Sundberg et al. 2001). The current classification is largely

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<th>Nemertea</th>
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<td>Parborlasia carruga (McIntosh, 1876)</td>
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Table 1. Current classification (mainly after Gibson 1982) and collection sites for the species used in the present study.
Table 1. (Continued.)

<table>
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<th>Taxon</th>
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<td><em>Parvicirrus dubius</em></td>
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<td><em>Tenuilirnea bicolor</em></td>
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<tr>
<td><strong>Palaeonemertea</strong></td>
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<td>Carinomidae</td>
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<td><em>Carinoma musabili</em></td>
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<td><em>Chama nepenthoides</em></td>
<td>Fort Pierce, FL, USA</td>
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<td><em>Procepalothrix sp</em></td>
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<td>Hubrechidia</td>
<td>Fort Pierce, FL, USA</td>
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<tr>
<td><em>Hubrechella dubia</em></td>
<td>Vestok Bay, Sea of Japan, Russia</td>
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<tr>
<td><em>Hubrechella dubia</em></td>
<td>Fort Pierce, FL, USA</td>
</tr>
<tr>
<td><em>Hubrechella dubia</em></td>
<td>San Juan Island, WA, USA</td>
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<tr>
<td><strong>Outgroup taxa</strong></td>
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<td><em>Mollusca</em></td>
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<td><em>Sipunculida</em></td>
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<td><em>Terebratalia transversa</em></td>
<td>AF342802, AF331161, AF331161</td>
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*28S, 16S and COI sequences are from Phascoloposis gouldii; H3 sequence is from Sipuncula sp.

in agreement with our results, although there are some notable exceptions.

2. MATERIAL AND METHODS

(a) Specimens and DNA extraction

The species and specimens sequenced are listed in table 1, together with the collection sites. There are 10 hitherto undescribed (or unidentified) species included in the dataset. They are identified by numbers, and reference specimens are available at the National Museum of Natural History, Smithsonian Institution. These species will be described elsewhere.

Specimens sequenced in this study were either snap frozen and kept at -80 °C or preserved in 80-95% ethanol until DNA extraction. Total DNA was extracted using a protocol modified from Winnepenninckx (1993) as described in Thollesson et al. (2000). Based on this and on sequence availability, we selected two molluscs (the bivalve *Mytilus edulis* and a brachiopod *Urechis caupo*) as outgroup taxa for rooting (as discussed by Nixon & Carpenter 1993).

(c) Amplification and sequencing

Amplification of parts of the genes coding for COI, 16S rRNA, 28S rRNA and H3 was carried out using universal primers: 16sar-L ([GGCCGTTTTATCACAAAAACAT] and 16sbr-H ([CCCCGCTGAACTCAGATCAGT] from Palumbi et al. (1991) for 16S; LSU5 ([ACCCCGCTGAACTCAGATCAGT] and LSU3 ([TGTCGGGAAGAACACTCGGG] from Littlewood (1994) for 28S; LCO1490 ([GGTCACAAATCTACAAACATG] and 377 automated sequencers (Perkin-Elmer)); both strands comprised an initial 2 min denaturation at 94 °C, followed by 35 cycles of 30-45 s at 94 °C, 30-45 s at 50 °C and 4 min at 72 °C (the longer times are for the 28S fragment). The cycling ended with a 7 min extension at 72 °C.

Each PCR was performed using a 15 ng template in a 50 µl volume (50 mM Tris-HCl pH 9.1, 16 mM (NH4)2SO4, 3.5 mM MgCl2, 150 µg ml−1 bovine serum albumin (BSA), 0.5 µl of each primer, 160 µl of each dNTP and 0.25 µl of KlenTaq Tag polymerase (AB Peptides, Inc.)). Thermocycling comprised an initial 2 min denaturation at 94 °C, followed by 35 cycles of 30-45 s at 94 °C, 30-45 s at 50-55 °C (depending on the target) and 60-90 s at 72 °C (the longer times are for the 28S fragment). The cycling ended with a 7 min sequence extension at 72 °C.

The PCR product was purified with QIAquick (Qiagen Inc.) and used in cycle sequencing with dye-terminators using FS or BigDye chemistry (Perkin-Elmer) and standard cycles (4 min denaturation at 96 °C, followed by 25 cycles of 10 s at 96 °C, 5 s at 50 °C and 4 min at 60 °C). The PCR primers were used for sequencing reactions, together with two additional 28S primers: D2F ([TCTTGAGAGACATAGC] Littlewood 1994) and 28s (truncated) ([CTTGGTCCGTTTACAGCA] Hillis & Dixon 1991). The products were sequenced on ABI373 or ABI 377 automated sequencers (Perkin-Elmer); both strands were sequenced at least once. The sequences have been deposited with the European Molecular Biology Laboratory nucleotide sequence database (accession numbers AJ436785 to AJ436991).
(d) **Alignment and phylogenetic inference**

The sequences were first aligned gene by gene using MEGALIGN, v. 3.14 in the DNAStar software collection (DNAStar Inc.), using the 'CLUSTAL' option (Higgins et al. 1992; Thompson et al. 1994) with the gap-gap length penalties set to 10-5. The computer-generated alignments of the two rRNA genes were then adjusted manually based on published secondary structure models of the RNA products (e.g. Guttell et al. 1993). Stem regions where only one strand was initially aligned were adjusted so that both strands were aligned, as were other conserved regions. Between these anchor points, the bases (usually loop regions) were realigned using CLUSTALX and the original penalties. The protein coding genes (COI, H3) did not require manual intervention to produce an acceptable alignment.

Model-based phylogenetic analysis using Bayesian inference was carried out using MrBayes, v. 2.01 (Huelsenbeck & Ronquist 2001). Each Markov chain was started from a random tree and run for $10^8$ generations, sampling every hundredth generation from the chain. Each run comprised four ($1 \times 3$; temp parameter = 0.2) differently heated chains. To check that the stationary phase was reached we monitored the log likelihood values of the trees in the chain, and the analysis was done in five replicates. The first $3 \times 10^7$ generations were subsequently discarded as burn-in. The default values for priors were used: uniform priors for rate matrix (0–1), branch lengths (0–10), shape parameter for gamma distribution (0–10) and proportions of invariable sites (0–1), whereas the base frequency prior was assumed to have a Dirichlet distribution, and an uninformative prior was used for topology.

The adequate models for Bayesian inference were determined using a hierarchical likelihood ratio test (H-LRT) approach (Huelsenbeck & Crandall 1997). We used the same test hierarchy as that implemented in the program ModelTEST (Posada & Crandall 1998) together with PAUP at $p < 0.01$.

Phylogenetic analyses with parsimony as the optimality criterion were done using PAUP 4.088–10 (Swofford 2000) to enable comparisons with a method that does not use an explicit model. A heuristic search strategy (tree bisection and reconnection (TBR); random addition, 50 replicates; simple addition during bootstrap) was used with gaps treated as missing data (we had no good basis for converting them into characters). Bootstrapping (Felsenstein 1985) with 1000 replicates was used to assess sample variation and degree of support (or signal in relation to conflicting signal) in the datasets for specific clades.

Aligned sequences together with information on deleted regions and obtained trees have been deposited in TUEEBASE (Sanderson et al. 1994; Morell 1996) with the accession number S837.

### 3. RESULTS

The data subsets, after excluding sites that could not be aligned reliably (28S and 16S sequences only), comprised 1332 bp (28S), 436 bp (16S), 666 bp (COI) and 332 bp (H3), respectively. All partitions (as well as the combined data) required modelling of rate heterogeneity, and the most adequate models according to the LRT indicated a combination of invariant sites and a gamma distribution. The 28S and COI partitions both had the general time-reversible (GTR) model as the most adequate, whereas the Tamura–Nei and the transversion (TVM) models would have been sufficient for H3 and 16S, respectively. The combined dataset had GTR as the most adequate model. MrBayes does not allow the Tamura–Nei and TVM submodels, and hence the GTR (as the simplest well-fitting model) was used for all partitions as well as for the combined analysis. The values for the model parameters as estimated by the Bayesian analysis are shown in table 2.

The tree in figure 1 is the summary (majority rule consensus) tree from the Bayesian analysis of the combined datasets using the GTR model with site-specific rates (i.e. each partition has its own rate) and a rate heterogeneity modelled by a (common) gamma distribution. The result of the parsimony bootstrap analysis is topologically highly congruent with the Bayesian analysis, and the bootstrap values are additionally shown on the tree in figure 1.

Nemertea is monophyletic with the posterior probability 1.0 and with a parsimony bootstrap of 98%. Enotha, Heteronemertea and Polystilifera form highly supported clades (posterior probability/parsimony bootstrap = 1.0/100%), as does Monostilifera if Bdellonemertea is considered a sister taxon (1.0/93%). Malacobdella (Bdellonemertea) is nested firmly within Monostilifera and appears to be the sister taxon to Pantinonemertes sp. 115 (1.0/87%), although this specific position may be the result of inadequate taxon sampling. Within Monostilifera there are two major clades corresponding to Tetrastemmatidae (1.0/100%) and Amphiporidae + Emploeconematidae + Otophyphonemertidae + Prosethichidae including Malacobdella (1.0/64%).

Carcinonemertes is a sister group to these two major clades, although with insignificant support. It is worth noting that Otophyphonemertes forms a highly supported (1.0/100%) clade with Poseidonemertes, and that this clade in turn is the sister taxon to Zygonomertes with high posterior probability (1.0), albeit low bootstrap support (55%). Cristenemertidae (Nippononemertes bimaculata + N. punctatius, 1.0/100%) is the sister group to the clade comprising all other monostiliferans + Malacobdella (1.0/97%).

Within Polystilifera, the reaptants are not monophyletic in the Bayesian analysis, with reaptant species 481 being closer to the pelagics (insignificant support, 0.86). The parsimony analysis, however, renders Reptantia monophyletic with 81% bootstrap support. Pelagica is monophyletic (1.0/98%) in both analyses.

Within Heteronemertea, Risierius pugetensis is the sister to the remaining heteronemerteans (Lineidae). The high support for this (1.0) in the Bayesian analysis is lost in the parsimony analysis (57%). Furthermore, it is noted that Hubrechtia is a sister taxon to Heteronemertea sensu stricto with a posterior probability of 1.0 for the clade, but with no parsimony bootstrap support. The parsimony analysis places Hubrechtia together with Carinoma, but...
Figure 1. Summary (majority rule consensus) tree for the Bayesian analysis, using the GTR model with site-specific + gamma rates. Numbers above branches are posterior probabilities; numbers below branches are bootstrap percentages from a parsimony analysis. Asterisks indicate bootstrap support for clades (* 50-70%, ** 71-90%, *** > 90%) incompatible with the marked clades in the Bayesian tree; none of these clades in the Bayesian tree has a significant support (posterior probability > 0.95), however, and incongruence might simply be the result of insufficient character sampling. Clades indicated by A and a number are taxa hitherto placed in Amphiporidae; clades with E and a number are taxa hitherto in Emplectonematidae; and taxa in clades with P and a number were assigned to Prosorhochmidae. In this paper we introduce three new names (written vertically): Neonemertea, Pilidiophora and Distromatonemertea.
with no bootstrap support. The genus *Lineus* does not form a monophyletic group.

The palaeonemerteans, even with *Hubrechtella* excluded, do not form a monophyletic group, but are found basally on the tree as paraphyletic to the remaining nemerteans. The Bayesian analysis yields a clade corresponding to the monophyletic groups Tubulanidae and Cephalothricididae (both 1.0/100%) with a posterior probability of 0.99. In the parsimony bootstrap analysis, however, there is no support for this, but the four palaeonemertean families (Tubulanidae, Cephalothricididae, Carinomidae, Hubrechtidae) form a hexachotomy with Heteronemertea and Enopla.

A χ² test indicated that there are significant differences in base frequencies between different taxa, and thus the GTR model and parsimony may give misleading results. A minimum-evolution analysis using LogDet distances, however, gave essentially the same result as the Bayesian analyses (tree not shown), and an analysis using a non-reversible (12-parameter) model in a Bayesian analysis gave a congruent result (tree not shown). The only difference is that the clade with *Carinoma mutabilis* and *C. tremaphoros*, which form the GTR model is the sister group to Heteronemertea + Hoplonemertea (with the low probability of 0.74), is the sister group to the Cephalothricididae + Tubulanidae with a negligible 0.56 posterior probability under the non-reversible model.

Analysing the genes separately markedly reduces the number of clades with significant posterior probabilities, with 28S and 16S having the most and H3 the fewest. There are no obviously incongruent clades with significant posterior probabilities, with one exception. For the 16S gene, Monostilifera is not monophyletic: Cratenemertidae is a sister taxon to Polystilifera with Tetrastemmatidae based on the armament of the proboscis. However, cladistic analyses by Härlin & Sundberg (1995) and Härlin & Härlin (2001) indicate that neither subgroup is monophyletic. Our results for reptants are consistent with the relatively ‘unbalanced’ trees for reptants presented by Härlin & Härlin (2001). In the present study there is very strong support for a monophyletic Polystilifera as a sister group to Monostilifera. This has important bearings on the interpretation of the evolution of proboscis armament: in Sundberg’s (1990) phylogeny, the plesiomorphic state is many stylets, whereas in our phylogeny either one or many stylets can be plesiomorphic. However, the taxonomic sampling of Reptantia and Pelagica needs to be extended before a non-monophyletic Polystilifera can be ruled out.

We also note that Reptantia is not monophyletic in the Bayesian analysis, but it is in the parsimony analysis. The posterior probability for the offending species (sp. 481), placed together with Pelagica in the Bayesian analysis, is very low (0.86), whereas the bootstrap support for a monophyletic Reptantia in the parsimony analysis is moderate (81%). Thus, we treat this incongruence as the result of insufficient sampling for the time being, and see no reason to reject the hypothesis of a monophyletic Reptantia.

### 4. DISCUSSION

The nemertean classification has been relatively stable since Stiasny-Wijnhoff (1923), although some new revisions, mostly at lower levels, have been published during the last 20 years. There has been an implicit consensus about the monophyly of Heteronemertea and Hoplonemertea. The third large group, Palaeonemertea, has been regarded as a basal group and the font of nemertean diversification since Hubrecht (1879), and hence is implicitly paraphyletic. Our analysis confirms this consensus: Heteronemertea and Hoplonemertea are monophyletic with good support, the latter, however, only with the provision that the commensal *Malacocbella*, hitherto treated as a taxon separate from Hoplonemertea, is included. Furthermore, our study indicates that Palaeonemertea is non-monophyletic.

Sundberg et al. (2001) presented a phylogenetic study of Nemertea based on the 18S rRNA gene and 15 species; alas, only three of their species were included in the present study. In terms of the general pattern, their study is completely congruent with ours in supporting a monophyletic Heteronemertea and a monophyletic Hoplonemertea, including Bdellonemertea, with no support for monophyly of Palaeonemertea. It is worth noting that *Malacocbella* was found together with a prosorhochid monostilifer (Prosorhabdus sp., with 61% bootstrap support) by Sundberg et al. (2001). In the present study, it is also found with a prosorhochid, albeit another genus and species (*Pantinonemertes* sp.).

### (a) Hoplonemertea

Brinkmann (1917) divided Hoplonemertea into Monostilifera and Polystilifera based on the armament of the proboscis. He further subdivided Polystilifera into Reptantia and Pelagica. This classification was challenged by Gibson (1988) who transferred the monostiliferan family Cratenemertidae to the taxon Paramonostilifera and considered it as a sister group to the Polystilifera *sensus stricto*, comprising the reptants. This group in turn was the sister group to Pelagica. Sundberg (1990) rejected this in an analysis of morphological data, and placed Cratenemertidae as the sister group to the remaining Monostilifera. This is the exact position of found in the present study with high posterior probability, thereby affirming rejection of the taxon Paramonostilifera.

Polystilifera, however, was not monophyletic in Sundberg’s (1990) analysis, rather Pelagica was a sister group to a clade with Monostilifera and the reptants (which were paraphyletic). Stiasny-Wijnhoff (1936) divided the Reptantia Eureptantia into *Aequifurca* and *Inaequifurca* based on several implicit synapomorphies. However, cladistic analyses by Härlin & Sundberg (1995) and Härlin & Härlin (2001) indicate that neither subgroup is monophyletic. Our results for reptants are consistent with the relatively ‘unbalanced’ trees for reptants presented by Härlin & Härlin (2001). In the present study there is very strong support for a monophyletic Polystilifera as a sister group to Monostilifera. This has important bearings on the interpretation of the evolution of proboscis armament: in Sundberg’s (1990) phylogeny, the plesiomorphic state is many stylets, whereas in our phylogeny either one or many stylets can be plesiomorphic. However, the taxonomic sampling of Reptantia and Pelagica needs to be extended before a non-monophyletic Polystilifera can be ruled out.

### (b) Monostilifera

The monostiliferous hoplonemerteans are monophyletic, and there are two well-supported clades roughly corresponding to Amphiporidae and Tetrastemmatidae, respectively, but overall our results challenge the monophyly of the taxon-rich traditional families Amphiporidae,
Emplectonematidae and Tetrastemmatidae, as well as the more circumscribed Prosorhochmidae (based on the position of Oermestidae). The pairing of Amphiphorus formidabilis and A. imparipinnus, with high posterior probability but low bootstrap support, is notable in that these two sympatric forms are notoriously difficult to distinguish. They differ here by 10% and 13% for 16S and COI, respectively.

The strong support for the clade Poseidonemertes + Ootypophonemertes is intriguing, as members of both groups are recognized as morphologically specialized and conspicuously set apart from other monostiliferans by habitat. The recognized Poseidonemertes are relatively large-bodied very muscular worms with a pointed head that burrow actively through sandy habitats (unlike Paramonemertes peregrina, which appears to occupy relatively static burrows or crawl through soft surface mud). By contrast, Ootypophonemertes are among the thinnest nemerteans and occupy almost exclusively relatively coarse sediments where they are able to penetrate the aqueous pore space without burrowing.

It is also worth noting here that although the two Malacobdella cf. grossa individuals that were used in this study (table 1) are from two geographically widely separated host species these specimens were identical for the consensus tree. This begins to host species these specimens were identical for the parsimony analysis, although without bootstrap support. In the present study, we, respectively, found a clade comprising Cephalothricidae and Tubulanidae, and hence we must refute Archinemertea. Furthermore, even though Carininaeidae is the sister group to Hoplonemertea + Heteronemertea + Hubrechtidae, this has no significant probability and—without bootstrap support. The present results argue strongly that a well-developed cerebral sensory organ, a structure apparently unique to nemerteans, was present in the ancestor of the analyzed clades, unless one wishes to argue for two origins and a remarkable degree of convergence.

(d) Heteronemertea

With the exception of Riserius pugetensis, all heterone-

(c) Palaeonemertea

The prevalent notion that palaeonemertea are a basal
group in Nemertea was contested by Sundberg & Hybom
(1994). In their analysis to find an outgroup to Palaeone-
mereta, they found Heteronemertea to be this outgroup and Hoplonemertea to be their basal sister group. This is probably the result of too few characters and the use of the turbellarian Haplopharynx rensratus as the single outgroup, together with some missing data in the matrix. Our analysis moves the root, and the palaeonemertea are found basally among the nemerteans. Sundberg & Hybom (1994), furthermore, assumed monophyly of Palaeonemertea. This has been implicitly contested for a long time; for example an interpretation of Bürger's (1895) view places Hubrechtidae as a sister group to Heteronemertea, Cephalothricidae and Carininae as a sister group to Hoplonemertea, and Carinina as a sister group to all other nemerteans. Subsequent authors (e.g. Friedrich 1935; Hybom 1957; Iwata 1960) have moved parts of the Palaeonemertea around, but have essentially maintained the closer relationship between some palaeonemertea and other nemertean taxa (i.e. non-monophly).

The present study also indicates that Palaeonemertea is non-monophyletic. The Bayesian analysis shows a significant probability for Hubrechtidae dubia as the sister taxon to Heteronemertea sensu stricto. This was suggested by Norenburg (1985, 1988) because Hubrechtidae has true pilidium larvae (Cantell 1969), otherwise a unique feature of Heteronemertea, and others have considered other members of Hubrechtidae as close relatives to Heterone-

(e) Taxonomic implications

Bdellonemertea, represented here by Malacobdella cf. grossa, has traditionally been grouped together with, and with the same rank as, Hoplonemertea in the taxon Enopla; a notable exception is a lengthy treatise by Senz (1997) in which he considers it to be 'a specialized off-

Oerstedio). The pairing of Lineus viridis and Notospermus (Riser 1991) for Lineus is, however, still non-monophyletic even with the application of Riser's new genera. Riser (1994) diagnosed the new genus Myoiosphaga, later (Riser 1998) recognized as a junior synonym of Ramphogordius. Riser (1994) furthermore concluded that Lineus viridis should not be part of Lineus, although he did not place it formally in any other genus. In our analysis Ramphogordius sanguineus is the sister taxon to L. longissimus (type species of Lineus), and L. viridis is their sister taxon. We also note that L. albostratus forms a clade with Temulinae bicolos, and thus is better considered as part of Temulinae (Riser 1993), and that the erection of Notospermus (Riser 1991) for L. geniculatus seems justified. Considering the different taxon-

synonymous. Although Enopla was introduced by Schultze (1851) and is the older name, we prefer to keep Holoponemerta (Hubrecht 1879) as the name for this clade.

There are a few clades that currently lack formal names, but where names are warranted. We here name these clades in a phylogenetic framework as outlined by de Queiroz & Gauthier (1990, 1992) and others (e.g. Schander & Thollesson 1995), an approach that has already been applied within Nemertea (to Reptantia by Harlin & Sundberg 1995; Harlin & Harlin 2001).

The evident morphological discontinuity between cratennemertids and the remaining monostiliferans (reviewed by Crandall 1993) is well supported in the present study. We believe that effective communication is served by providing a name to the sister clade of cratennemertids, which we propose renaming as Cratenemertea, while applying the name Distromatonemertea (after the ill-fated Distromatoochocoeolomia of Gibson (1988), which has roughly the same composition) to its sister clade. Formally, Distromatonemertea refers to the most inclusive clade comprising all monostiliferous nemerteans except Cratenemertea.

Even though the relationships between all paleonemertean taxa are not yet resolved, we think that the clade comprising Holoponemerta and Heteronemertea (and its relatives) also needs a name. One option would be simply to include the Hubrechtella clade in Heteronemertea. However, as Heteronemertea in its present use refers to a monophyletic group, we prefer to keep the name referring to a clad with the same content. The Heteronemertea + Hubrechtella clade has a unique feature in the pilidium larva, and we thus propose the name Pilidiod (Gr. 'pilios', cap; 'phora', bearing) to refer to the least inclusive clad comprising Heteronemertea and Hubrechtella.

Finally, the clad comprising Heteronemertea and Hubrechtella (and its relatives) also needs a name. One option would be to include the Hubrechtella clade in Heteronemertea. However, as Heteronemertea in its present use refers to a monophyletic group, we prefer to keep the name referring to a clad with the same content. The Heteronemertea + Hubrechtella clade has a unique feature in the pilidium larva, and we thus propose the name Pilidiod (Gr. 'pilios', cap; 'phora', bearing) to refer to the least inclusive clad comprising Heteronemertea and Hubrechtella but not Holoponemerta or Garitoma.

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