Swedish nemerteans (phylum Nemertea), with description of a new hoplonemertean genus and species

PER SUNDBERG1, RAY GIBSON2 & MALIN STRAND1

1 Göteborg University, Department of Zoology, Göteborg, Sweden, and 2 School of Biological and Earth Sciences, Liverpool John Moores University, Liverpool, UK

(Accepted 23 July 2007)

Abstract
A survey of the nemertean fauna along the Swedish west coast found specimens of 20 genera and 32 species, of which one genus and species is new to science (Tetraneuronemertes lovgreni gen. et sp. n.). The proportions of the three taxa Hoplonemertea, Heteronemertea, and Palaeonemertea are 34, 55, and 11%, respectively, with respect to specimen numbers, and the proportions for species within these taxa are 53, 31 and 16%, respectively. The most common species encountered during the survey were the heteronemerteans Lineus bilineatus, Micrura fasciolata, and Micrura purpurea, the hoplonemertean Nipponnemertes pulcher, and the palaeonemertean Cephalothrix rufifrons.

Keywords: Biodiversity, marine Nemertea, taxonomy

Introduction
The marine nemertean fauna was surveyed along the Swedish west coast as part of the inventory of fauna commissioned by the Swedish Taxonomy Initiative (Miller 2005). Nemertean taxonomic research has a long tradition in Sweden and the western coastline is fairly well sampled when it comes to this taxon. Nonetheless, new species are still found in the area, and the current number of named species (around 1250 worldwide: see Gibson 1995 for the latest review) in the phylum must be regarded as an underestimate. Recent studies based on molecular information (Strand and Sundberg 2005a) furthermore indicate that in addition there are probably many cryptic species, which it is not possible to separate on purely morphological grounds. Since most nemertean species are marine we have focused on this habitat. There is only one known freshwater nemertean species presently known from Sweden (Prostoma graecense), and although not abundant it is a form found regularly in freshwater habitats (personal observation). There is also a brackish water species (Cyanophthalma obscura) commonly found in the littoral of the Baltic Sea. This species also occurs in the inner part of the Idefjord (at the border with Norway at the northern part of the west coast) where the water has reduced salinities because of river outflow (personal observation).
Ninety (Table I) species of nemerteans have been reported from Sweden (Gibson 1995; this study), comprising 17 palaeo-, 26 hetero-, and 47 hoplonemerteans. Some of these have been described from fixed material only, and it is questionable whether future observations will be able to confirm their validity. On the other hand, new surveys and expeditions undoubtedly will find new and previously undescribed species: despite the dubious validity of some of the taxa recorded, the total number found so far is probably not an overestimate. Here we report the results of our qualitative surveys and describe a new genus and species encountered during our investigations. The systematic position of this species, and the conclusion to erect a new genus, was based on morphological details in combination with phylogenetic analyses based on 18S rRNA gene sequences.

Materials and methods

Specimens and sampling

Nemerteans were sampled from several localities along the Swedish west coast (Figure 1) between latitudes 55 and 59° N. Sampling included both dredging to depths of 50 m and collecting algae from the littoral. Regular collections were made between September 2002 and June 2003, with some additional sporadic sampling in 2004 and 2005 from the upper littoral. Gibson (1995) described in detail techniques for sampling benthic nemerteans and it is thus only briefly described here. The main technique relies on placing either dredged bottom material or algae in tanks with unaerated seawater and then collecting worm specimens from the sides of the tank when they crawl out in response to increased deoxygenation. Dredgings from muddy sediments can be sieved for larger specimens, but care must be taken to avoid damaging individuals.

Specimens were observed alive, and in most cases could be identified from external characters. Specimens for histological examination were anaesthetized in 7.5% MgCl₂ following in vivo examination. Whole specimens, or individuals from which their tails had been cut off, were fixed in Bouin’s fluid, sectioned at 6 μm and stained by the Mallory trichrome technique for histological examination. Posterior tail fragments of individuals representative of each putative species were preserved in 70–80% ethanol and stored for subsequent DNA extraction using DNeasy (QIAGen Inc.) following the protocol recommended by the manufacturer. DNA was extracted from the ethanol-preserved material using either a 5–10% Chelex solution or a QIAamp DNA Mini Kit (QIAGen Inc.) following the protocol recommended by the manufacturer. Also, specimens of externally identified species were placed in 70–80% ethanol for future molecular systematic studies.

Phylogenetic analyses

The phylogenetic analysis in this study is based on 18S rRNA gene sequences from GenBank with the exception of the new species described in this paper (Table II).

Sequences of the 18S rRNA gene sequences were edited with Lasergene (DNASTAR Inc.) and aligned in MegAlign using the CLUSTAL option with gap/gap length penalties set to 15/8. Alignment of CO1 mtDNA gene sequences was straightforward given the conserved amino acid sequence-reading frame. Phylogenetic analyses of the data set were carried out using Bayesian inference performed with MrBayes version 3.06 (Huelsenbeck and Ronquist 2001). We used the default values of one cold and three heated Markov chains with model GTR+I+G, allowing sites to vary independently. Three separate analyses
Table I. List of nemertean species reported from Sweden. Sampling of present study was not quantitative and we have therefore chosen to report abundances with the subjective terms abundant, common or rare.

| ENOPLA                           |                   |
|----------------------------------|-------------------|
| **Palaeonemertea**               |                   |
| Callinera buergeri Bergendal, 1900 | Rare              |
| Callinera grandis Bergendal, 1903 | Common            |
| Carinina arenaria Hylbom, 1957a  | Rare              |
| Carinina ataia (Bergendal, 1902) |                   |
| Carinina coei Hylbom, 1957       |                   |
| Cephalothrix arenaria Hylbom, 1957|                   |
| Cephalothrix linearis (Rathke, 1799) |   |
| Hubrechtella dubia Bergendal, 1902 | Common           |
| Tubulanus annulatus (Montagu, 1804) | Common          |
| Tubulanus linearis (McIntosh, 1873–74) |   |
| Tubulanus lutescens Cantell, 2001 |                   |
| Tubulanus mimiatu (Bürger, 1892)  | Rare              |
| Tubulanus nothus (Bürger, 1892)   |                   |
| Tubulanus polymorphus Renier, 1804|                   |
| Tubulanus superbus (Kölliker, 1845)a |                   |
| Tubulanus theeli (Bergendal, 1902)a |                   |
| **Heteronemertea**               |                   |
| Cerebratulus aerugatus Bürger, 1892 |   |
| Cerebratulus fuscus (McIntosh, 1873–74) | Common       |
| Cerebratulus lacteus (Leidy, 1851) | Rare            |
| Cerebratulus marinae Renier, 1804 | Rare             |
| Lineus albocinctus Verrill, 1900  |                   |
| Lineus bilineatus (Renier, 1804)  | Abundant          |
| Lineus kristinebergensis Gering, 1912 |                   |
| Lineus longissimus (Gunnerus, 1770)a |                   |
| Lineus nigrobrunneus Bergendal, 1903 |   |
| Lineus ruber (Müller, 1774)      | Abundant          |
| Lineus viridis (Müller, 1774)    | Abundant          |
| Micrura baltica Cantell, 1975a   |                   |
| Micrura coralifila Cantell, 1975a|                   |
| Micrura fasciolata Ehrenberg, 1828|                   |
| Micrura purpurea (Dalyell, 1853) | Abundant          |
| Micruridae albopunctatus Cantell, 1988|                   |
| Nemertes assimilis Ørsted, 1843  |                   |
| Nemertoscolex parasiticusa       |                   |
| Oxypolella alba Bergendal, 1903a |                   |
| Oxypolella bergendali Cantell, 1972a|                   |
| Oxypolella punneti Bergendal, 1902a |                   |
| Ramphogordius lacteus Rathke, 1843|                   |
| Ramphogordius sanguineus (Rathke, 1799) | Rare   |
| Tarrhomyos praealbescens (Cantell, 1982) |                   |
| Tenulineus albocinctus (Bergendal, 1903) |                   |
| Vaiceninura bahusiensis Bergendal, 1902 |                   |

| **ENOPLA** |                   |
|-----------|-------------------|
| Arenonomertes minutus Friedrich, 1949 |                   |
| Annulonemertes minuscus Berg, 1985a |                   |
| Amphiporus bioculatus McIntosh, 1873–74 | Common       |
| Amphiporus dissimilans Riches, 1893 |                   |
| Amphiporus dubius Hubrecht, 1879 |                   |
| Amphiporus hastatus McIntosh, 1873–74 |                   |
| Amphiporus korschelti Friedrich, 1940 |                   |
were run to ensure convergence. In each analysis the Monte Carlo Markov chain (MCMC) length was 1,000,000 generations with sampling of every 10th generation chain. Log-likelihood values for sampled trees stabilized after approximately 100,000 generations, and burnin was set to 50,000 leaving the last 50,000 sampled trees for estimating posterior probabilities (or Bayesian support values).

Results

We found a total of 33 species (Table I), including the new species described below, among the around 1000+ specimens collected. Although sampling was not quantitative, the results still provide what we believe to be a qualitative indication of the distribution of the taxa.
Heteronemerteans constitute 31% of the sampled species, hoplonemerteans 54%, and palaeonemerteans 15%. Heteronemerteans are, however, the most abundant when it comes to the actual number of specimens (55%), followed by hoplonemertean worms (34%), and then palaeonemerteans (11%). We here follow recent phylogenetic analyses (e.g. Sundberg et al. 2001; Thollesson and Norenburg 2003) and have included the only Swedish bdellonemertean found (*Malacobdella grossa*) in the Hoplonemertea; in addition we do not consider the Archinemertea as distinct from the Palaeonemertea (Sundberg and Hylbom 1994). We also found that species abundance was very patchy—there were occasions where one small sample contained more than 50 specimens of a particular species, whilst in most cases much lower numbers were encountered. Our experience from many years of sampling nemertean is that there are also seasonal, as well as random,
variations—for example, on a particular occasion it is possible to find a large number of conspecific specimens within a very confined area, whereas at other times no examples will be found. We suggest that such differences could be accounted for by “batches” of larvae/juveniles settling at particular times, and then remaining in the area for their lifetime.

Discussion

During this survey, we encountered 33 (i.e. 37%) of the 90 species of Swedish nemerteans listed in the literature. Taking into account previous sampling (Table I) we conclude that around 60% of the recorded species can be confirmed. For the remaining 40% we do not know whether they have either become extinct or are so rare and/or ephemeral that finding them is entirely a matter of chance. There are also cases of species that may not be valid. *Oerstedia similiformis*, for example, was originally described by Friedrich (1935) as *Oerstedieilla similiformis*, but transferred to the genus *Oerstedia* by Envall and Sundberg (1993) with the comment that its morphological character states fell within variations seen in *Oerstedia dorsalis*. A somewhat similar situation applies to *Oerstedia dorsalis*; 10 colour varieties of this species are commonly encountered in Swedish waters (Envall and Sundberg 1993), but future studies are required to determine which, if any, of these varieties warrants separate specific status. Thus, this may be a case where in fact there are more species than hitherto recorded. In general, a recent molecular study (Strand and Sundberg 2005a) cast doubts on the specific status of described species in the genus *Tetrastemma*, a situation we have reasons to believe is not just confined to this particular genus. If this is a common situation, the number of nemertean species is grossly underestimated.

## Table II. List of hoplonemerteans included in the phylogenetic analysis based on 18S rDNA sequences, and the accession numbers (sequence new to this study in bold).

| Species                                      | Accession number |
|----------------------------------------------|------------------|
| *Amphiporus allucens* Bürger, 1895           | AY928343         |
| *Amphiporus hastatus* McIntosh, 1873         | AY928344         |
| *Antiponemertes novaezealandiae* (Dendy, 1895)| AY928345         |
| *Argonemertes australiensis* (Dendy, 1892)  | AY928346         |
| *Emplectonema gracile* (Johnston, 1837)      | AY928347         |
| *Emplectonema neesii* (Ørsted, 1843)         | AY928348         |
| *Malacobdella grossa* (Müller, 1776)         | AY039670         |
| *Nipponnemertes pulcher* (Johnston, 1837)    | AY928352         |
| *Oerstedia dorsalis* Abildgaard, 1806         | AY928353         |
| *Oerstedia striata* Sundberg, 1988            | AY928354         |
| *Prosorochmus* sp.                            | AY039669         |
| *Prosotoma graecense* (Böhmig, 1892)         | AY039666         |
| *Tetraneuronemertes lovgreni gen. et sp. n.*  | AY928350         |
| *Tetrastemma candidum* (Müller, 1774)         | AY928357         |
| *Tetrastemma melanoccephalum* (Johnston, 1837)| AY928370         |
| *Vulcanonemertes rangitotoensis* Gibson and Strand, 2002 | AY928379 |
| *Zygonemertes virescens* (Verrill, 1879)      | AY928381         |
| **Outgroup**                                  |                  |
| *Cerebratulus lacteus* (Leidy, 1851)          | AY145368         |
| *Lineus bilineatus* (Renier, 1804)            | DQ279932         |
| *Micrura fasciolata* Ehrenberg, 1828          | AY340436         |
| *Zygeupolia rubens* (Coe, 1895)               | AY039671         |
Systematics

During the surveys we obtained one species we conclude is new to science. The specific status was confirmed from histological sections and by comparison of its 18S rRNA sequences with those of other sequenced hoplonemertean species (Table II). This new species is named and described below, its generic placement being based on the phylogenetic analyses in Figure 2 together with an assessment of a unique nerve arrangement. The higher classification of the Nemertea is far from securely established, with many named taxa being evidently non-monophyletic (Sundberg et al. 2001; Thollesson and Norenburg 2003). We have chosen, therefore, not to place the new genus in any family, but just the more inclusive clade Monostilifera—supported both in Sundberg et al. (2001) and Thollesson and Norenburg (2003).

Clade MONOSTILIFERA
Genus *Tetraneuronemertes* gen. n.

Systematic discussion

The genus is diagnosed by the occurrence of four longitudinal nerve cords in the foregut region of the body. The paired dorsolateral nerves, which are similar in size to the main longitudinal nerve cords but are composed entirely of neurofibrous tissue, have their origin from the main nerves close behind the brain and rejoin them in the pyloric region of the foregut. There are several monostiliferous hoplonemertean taxa known to possess accessory lateral nerves, but in all these cases the accessory nerves remain intimately connected to the main nerve cords and do not separate off from them. An arrangement like this has not been reported for any other hoplonemertean taxon and is here considered to be a synapomorphy, which enables us to identify a new genus for which the name *Tetraneuronemertes* is proposed. The phylogenetic analysis (Figure 2) based on the 18S rRNA gene sequences furthermore does not place the new genus in any well-supported clade and thus does not contradict the decision to place it in a separate genus.

The genus is presently monotypic including only the type species *Tetraneuronemertes lovgreni* sp. n.

Type species

*Tetraneuronemertes lovgreni* sp. n.

Etymology

The new genus is named after the presence of the four longitudinal nerves in the foregut region of the body.

*Tetraneuronemertes lovgreni* sp. n.

(Figures 3–5)

*Hoplonemertean* sp. 2 Strand and Sundberg 2005b, Table 1, Figures 1, 2.

Type locality

Dredged from 33 m depth from a bottom consisting mainly of mixed coal slag and clinker, Humlesäcken (58°16′05″N, 11°24′52″E), 13 November 2002, two specimens.
Figure 2. Phylogeny of selected hoplonemertean taxa to show the position of the new species described here (in bold). Majority rule consensus tree from a Bayesian analysis based on 18S rDNA sequences. Numbers above branches refer to a posteriori probabilities. Outgroup species (Heteronemertea): Cerebratulus lacteus, Lineus bilineatus, Micrura fasciolata, Zygeupolia rubens.

**Holotype**

Female, series of transverse sections, deposited at the Göteborg Natural History Museum (GNM Nemertea 82), 18S rRNA sequences of the holotype are deposited with GenBank (accession number AY928350).
Etymology

The species is named after Olof Lövgren who found the specimens on which the description is based.

Figure 3. *Tetraneuronemertes lovgreni* gen. et sp. n. External features of a complete specimen. Scale bar: 1 mm.
External features (Figure 3)

Small, slender, body more or less uniform width throughout, ending in a bluntly rounded tail. When moving, head was slightly wider than remainder of body, tapering anteriorly to end in a shallow anterior notch. Body translucent white, intestine appearing grey with obvious lateral diverticula alternating with the gonads. Rhynchocoel, visible in life, extends for about 50% of body length. Four small eyes arranged to form the corners of a trapezoid shape; anterior eyes larger and more closely set together than the posterior.

Internal features (Figures 4, 5)

Body wall, musculature and parenchyma. Tall epidermis a more or less uniform appearance throughout body and presents no unusual features. Body-wall musculature moderately strongly developed consisting of outer circular and inner longitudinal layers; no diagonal layer. At the rear of the brain dorsal cephalic gland lobes separate longitudinal layer into inner and outer zones. Inner fibres running close around brain lobes and then interiorly contribute to pre-cerebral septum, other fibres radiating inwards from the undivided ventral and lateral longitudinal musculature forming remainder of septum; this is thus a
mixed-type septum. No evidence of dorsoventral muscle bundles could be found in the intestinal region. Connective tissues moderately developed throughout body.

Proboscis apparatus. Rhynchodaeum mostly thin-walled but becomes thicker after oesophagus emerging from its ventral wall, close in front of the brain. Rhynchocoel extends for about 50% of body length. Thin wall with separate outer circular and inner longitudinal muscle layers. Proboscis comparatively slender and divided into the typical three regions. Measured central stylet 50 μm long, basis 47 μm long and 23 μm in maximum diameter. Basis with shallow but obvious “waist” about half way along its length and posteriorly rounded. Two accessory stylet pouches, each containing three or four accessory stylets. So far as could be determined, eight small proboscis nerves.

Alimentary system. Oesophagus opens from ventral wall of rhynchodaeum close in front of brain. Moderately thick-walled and ciliated, but lacking gland cells. As it passes below the
brain it begins to expand and, close behind ventral cerebral commissure, merges into anterior portion of stomach; this short region is characterized by possessing only acidophilic gland cells, whereas main stomach wall, which is deeply folded, also possesses basophilic glands. Main stomach about half body width in diameter. Posteriorly, stomach gradually becomes reduced in size as it merges into pyloric region of foregut. Long intestinal caecum projects interiorly below the pylorus as far forward as stomach. Caecum with neither anterior pouches nor lateral diverticula. Intestine itself bears fairly deep lateral diverticula alternating with the gonads.

**Nervous system.** Dorsal and ventral brain lobes of similar size, with dorsal and ventral cerebral commissures located in the same plane. Longitudinal nerve cords with single fibrous core; no evidence of either accessory lateral nerves or neurochords. An unusual feature of the nervous system is a pair of large dorsolateral nerves in the foregut region, similar in cross-sectional size to the main ventrolateral nerve cords. These additional nerves branch off from the ventrolateral nerve cords close behind the brain, pass back alongside the rhynchocoel, and then rejoin the main nerve cords in the pyloric region of the foregut.

**Blood vascular system.** Blood system difficult to distinguish, but for most of body length appears to consist of the typical hoplonemerteen arrangement of three longitudinal vessels, with no evidence of pseudometameric transverse connectives. In head a pair of cephalic vessels continues through cerebral ring, each forming a small ventrolateral vascular plug close behind ventral cerebral commissure. Posterior to these two plugs the pair of vessels appears to join to give rise to the single mid-dorsal blood vessel, continuing for remaining body length.

**Excretory system.** No certain evidence of excretory tubules, although small, dark-staining structures located dorsoventrally in foregut region of body may represent slender collecting tubules. Neither efferent ducts nor nephridiopores could be traced.

**Sense organs.** Well-developed frontal organ consisting of a ciliated pit opening into cephalic notch at tip of head, just above proboscis pore. Cephalic glands well developed and exhibit a typical vacuolate appearance. In anterior half of head, glands are dispersed, with longitudinal muscle fibres running between them, but as the brain is approached they become concentrated into two glandular lobules, one dorsal and one ventral. Ventral lobe terminates just in front of where oesophagus emerges from ventral wall of rhynchodaeum, a short distance in front of ventral cerebral commissure, but dorsal lobe passing back over the brain, where it divides body--wall longitudinal muscle layer into inner and outer layers before ending close behind brain; glands appear to discharge via frontal organ, and no evidence of improvised ducts could be distinguished. Ciliated cerebral canals open from extremely short and shallow cephalic furrows situated about 70 μm behind tip of head. Cerebral sensory organs are small and simple and do not reach brain.

**Reproductive system.** The specimen examined histologically is a female with ovaries between and alternating with the intestinal diverticula along the sides of the body. Each ovary contains one to a few large eggs possessing distinct nuclei.

**Systematic discussion.** The presence of four longitudinal nerve cords in the foregut region of the body (Figure 5) is considered a species unique character. See also systematic discussion for the genus.
Acknowledgements

We are grateful to staff at Helsingör Marine Biological Laboratory, Kristineberg Marine Research Station, and the Tjärnö Marine Biological Laboratory for assistance during the fieldwork. We are furthermore grateful for technical assistance by Inger Holmquist. This research was supported by the Swedish Taxonomy Initiative project and the Swedish Research Council.

References

Envall M, Sundberg P. 1993. Intraspecific variation in nemerteans (Nemertea): synonymization of the genera Paroerstedia and Oerstediella with Oerstedia. Journal of Zoology (London) 230:293–318.
Friedrich H. 1935. Studien zur Morphologie, Systematik und Ökologie der Nemertinen der Kieler Bucht. Archiv für Naturgeschichte 4:293–375.
Gibson R. 1995. Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. Journal of Natural History 29:271–562.
Huelsenbeck JP, Ronquist F. 2001. MR BAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.
Miller G. 2005. Taxonomy: Linnaeus’s legacy carries on. Science 307:1038–1039.
Strand M, Sundberg P. 2005a. Delimiting species in the hoplonemertean genus Tetrastemma (phylum Nemertea): morphology is not concordant with phylogeny as evidenced from mtDNA sequences. Biological Journal of the Linnean Society 86:201–212.
Strand M, Sundberg P. 2005b. Genus Tetrastemma Ehrenberg, 1831 (phylum Nemertea)—a natural group? Phylogenetic relationships inferred from partial 18S rRNA sequences. Molecular Phylogenetics and Evolution 37:144–152.
Sundberg P, Hylbom R. 1994. Phylogeny of the nemertean subclass Palaeonemertea (Anopla, Nemertea). Cladistics 10:347–402.
Sundberg P, Turbeville JM, Lindh S. 2001. Phylogenetic relationships among higher nemertean (Nemertea) taxa inferred from 18S rDNA sequences. Molecular Phylogenetics and Evolution 20:327–334.
Thollesson M, Norenburg JL. 2003. Ribbon worm relationships: a phylogeny of the phylum Nemertea. Proceedings of the Royal Society of London, Series B, Biological Sciences 270(1513):407–415.