

There is no support for Jensen's hypothesis of nemerteans as ancestors to the vertebrates

Per Sundberg¹, J. M. Turbeville² & Mikael S. Härlin¹

¹ Göteborg University, Department of Zoology, P.O. Box 463, SE-405 30 Göteborg, Sweden

² Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048, U.S.A., and Department of Biological Sciences University of Arkansas, Fayetteville, AR 72701, U.S.A.

Key words: Phylogeny, 18S rDNA, morphology, Nemertea, Chordata

Abstract

Nemerteans (phylum Nemertea) have been viewed by most zoologists as descended from, or closely related to, the flatworms. This view is based mainly on their supposedly acoelomate body. Their ancestry, however, is a point of controversy and there is evidence for a coelomate, protostomous origin. Notwithstanding these different views, most zoologists consider nemerteans to be phylogenetically distant from the chordates. Four authors (Hubrecht, Macfarlane, Jensen, Willmer), however, have postulated that nemerteans instead are closely related to the chordates and that they share a most recent common ancestor with the vertebrates. We argue that this view is based on a flawed view of homology and of seeing evolution as a series of progressions, which has no support in modern evolutionary thinking. Since there are no morphological synapomorphies supporting a Chordata-Nemertea clade, these authors instead guess what characteristics in extant nemerteans gave rise to characters observed in recent chordates. For example, they propose that the nemertean proboscis sheath has evolved into the notochord. This is mere speculation, lacking testable propositions and is hence void of information, and thus becomes futile in our view. However, the idea of a nemertean-vertebrate sister relationship as such is a testable hypothesis, and we test it by applying the parsimony criterion to a set of morphological characters, and a set of molecular (the 18S rRNA gene) characters. Both tests reject the hypothesis.

Introduction

Despite different views on whether nemerteans (phylum Nemertea) should be regarded as coelomates (e.g. Turbeville & Ruppert, 1985) or acoelomates closely related to the plathyhelminthes (e.g. Gibson, 1972) most zoologists have historically considered them as a phylum with a deep position in the metazoan phylogenetic tree. Hyman (1951), for example, concluded that 'there is little doubt of their derivation from the Plathyhelminthes'. Brusca & Brusca (1990) analysed metazoan relationships based on a set of morphological characters and also reached the conclusion that nemerteans are closely related to the flatworms, although not as sister taxa. Brusca & Brusca's phylogeny places the nemerteans as the sister taxon to the protostome coelomates, while the flatworms are considered

the sister taxon to the nemertean plus protostome 18S rRNA clade. Nielsen (1985, 1987) instead placed the nemerteans as a sister group to the flatworms. However, most of the similarities mentioned in favour of viewing nemerteans as closely related to the flatworms are sympleiomorphies (Norenburg, 1985) and a sister relationship between nemerteans and flatworms is not supported. Turbeville (1991) argued that structure and development indicate that the nemertean circulatory system is a coelom homologue, thus suggesting a closer relationship with coelomates. Turbeville & Ruppert (1983) reached a similar conclusion based on other evidence. A close relationship between Nemertea and spiralian coelomates was also found based on 18S rRNA sequences by Turbeville et al. (1992). The exact phylogenetic position of the nemerteans among metazoans is still an unsettled question, but the vast majority

of zoologists consider them as one of the basal proto-stomes in the phylogenetic tree. Figure 1, taken from Brusca & Brusca (Brusca & Brusca, 1990) illustrates the relative phylogenetic position of the nemerteans as viewed by this majority. Similar hypotheses are presented in (Eernisse et al., 1992).

Some biologists, however, have considered nemerteans more closely related to the chordates; yes, even the ancestors of the vertebrates. Hubrecht (1883, 1887) published two papers on the nemerteans as likely ancestors of the vertebrates. Hubrecht (1887: 606) concluded that ‘More than any other class of invertebrate animals, the Nemertea have (sic) preserved in their organisation traces of such features as must have been characteristic of those animal forms, by which a transition has been gradually brought about from the archicoelous diploblastic (coelenterate) type to those enterocoelous Triploblastica, that have afterwards developed into the Chordata (Urochordata, Hemichorda, Cephalochorda, and Vertebrata)’. The botanist Macfarlane (1918, 1923) developed his ideas in two books. Macfarlane (1923) devoted a chapter on the ‘evolution of fishes from invertebrates’, arguing that the most advanced nemerteans exhibit ‘remarkable fundamental agreement with the simpler chordate animals’. By going through organ system by organ system, he claimed that nemerteans have a number of characteristics that represent probable beginnings of different organs in chordates. He concluded the chapter by stating that the nemerteans ‘stand out preeminently alongside all other invertebrates as the forerunners and phylogenetic ancestors of *Amphioxus*, the cyclostomes, and the true fishes’.

The American comparative psychologist D. Jensen (first time in Jensen (1960)) suggested that it was possible to get clues to the origin of the vertebrates if ‘a group can be recognized that does possess homologs for most major vertebrate structures, including sensory and nervous features, does appear to be more closely related to the myxinoids than to other vertebrate groups . . . such a group appears to exist in the hoplonemertines’. Later Willmer (e.g. Willmer, 1974) independently proposed a hypothesis which ‘suggests likely precursor tissues from which the following vertebrate structures could plausibly have developed: olfactory organ, lateral line system, anterior and posterior pituitary, thyroid, pineal organ, chloride-secreting cells, . . .’. Following Hubrecht, he argued that such ‘precursor tissues’ were to be found among the nemerteans.

Jensen more recently has developed his ideas in a series of papers, and has also proposed (Jensen, 1988)

how it is possible to test the hypothesis. We will report on this test later in the paper, but will first discuss a major problem which all these ideas have in common – their definition of homology.

Are there homologous morphological characters for nemerteans and myxinoids?

There are many definitions of homology (see e.g. Hall, 1994); we will only discuss homology in the sense of morphological similarity since this is the concept with relevance to the nemertean-vertebrate clade hypothesis (henceforth referred to as the NV-hypothesis). Homology in the post-darwinian era is defined as similarity due to common ancestry; Darwin (1859) emphasized that it was this inherited similarity that was the clue to ancestry. It is not enough, however, to conclude that two characters are similar to support a homology statement. Homology is an historical statement which only make sense in the light of a phylogeny. Homologous traits are recognized *a posteriori* as a consequence of a global phylogenetic analysis, on basis of congruence with other characters (see Sundberg & Svensson (1994) for a discussion of this point in a nemertean context). But, and this is the crucial point in this discussion, characters have to be similar to even qualify for the hypothesis of being potential homologues. If they do not pass the similarity test (Patterson, 1982), they are rejected as potential homologues.

The reason why it is difficult to infer evolutionary relationships between distant taxa from gross morphology is because it is difficult to find shared similarities. There are no morphological similarities shared by nemerteans and vertebrates that can qualify as potential homologues. Hubrecht, Macfarlane, Jensen and Willmer instead use another approach which is based on their view of evolution as a progression series, where ‘lower’ taxa are the forerunners of more ‘advanced’ taxa. Along this line, they guess what may have been a character in a common ancestor. A citation from Hubrecht (1883: 351) exemplifies the reasoning: ‘the proboscis of the nemerteans, which arises as an invaginable structure . . . and which passes through a part of the cerebral ganglion, is homologous with the rudimentary organ which is found in the whole series of vertebrates without exception – the hypophysis cerebri. The proboscidian sheath of the nemerteans is comparable in situation (and development?) with the chorda dorsalis of vertebrates’. This is a homology concept that differs from how biologists use, and have used,

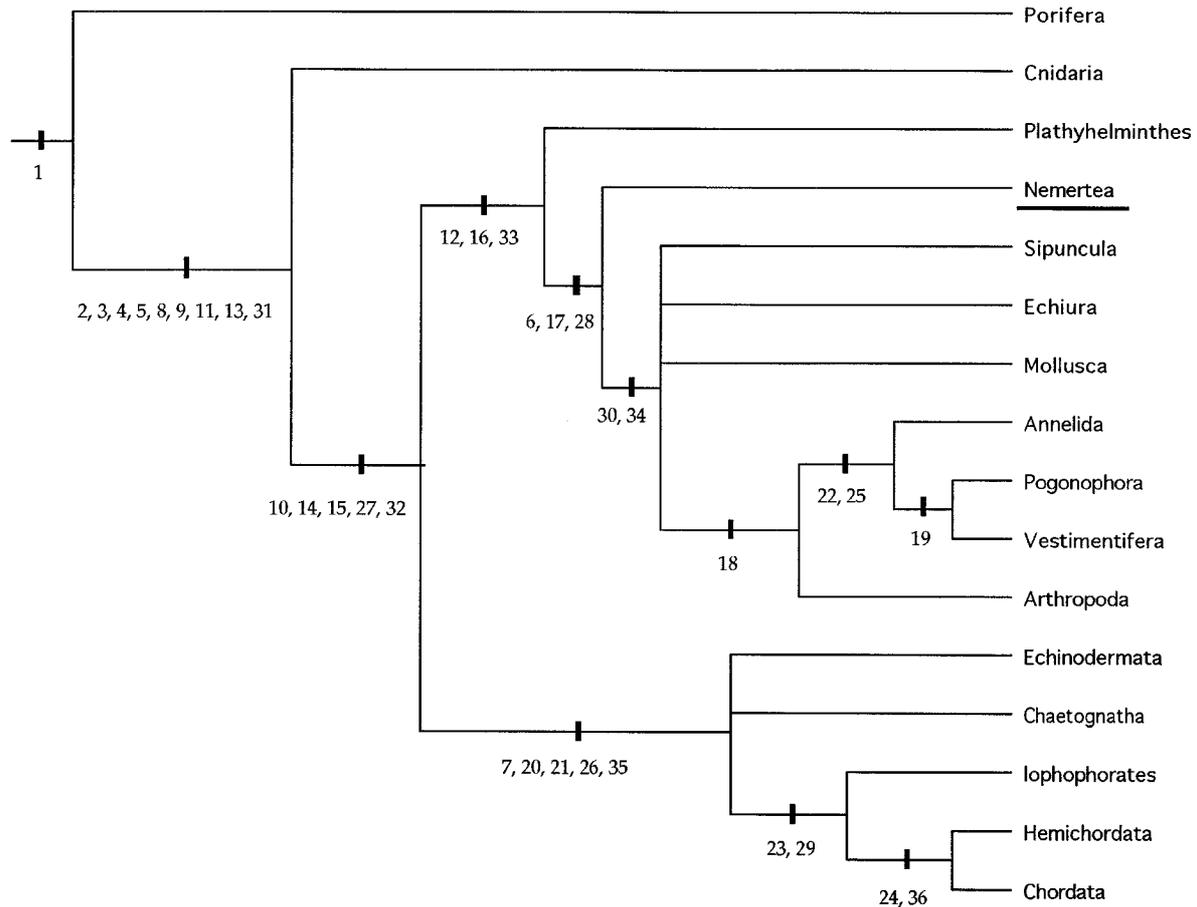


Figure 1. The phylogenetic position of the nemerteans inferred from morphological characters by Brusca & Brusca (1991). We have excluded the autapomorphies and have used the following characters (see Brusca & Brusca (1990) for details): 1 = multicellularity, with a high degree of division of labour; 2 = acetylcholine/cholinesterase system; 3 = collagen; 4 = septate/tight junction between cells; 5 = gastrovascular cavity with mouth arising from blastopore; 6 = complete gut with mouth arising from blastopore; 7 = complete gut with mouth not arising from blastopore; 8 = gastrulation; 9 = symmetrical body plan; 10 = fundamentally bilaterally symmetrical; 11 = typical radial cleavage; 12 = typical spiral cleavage; 13 = basement membrane; 14 = multiciliate/multiflagellate cells; 15 = cephalization; 16 = mesoderm arises from mesentoblast; 17 = body cavity (coelom) arises by schizocoely; 18 = true segmentation arising by teloblastic growth and resulting in serial repetition of body parts; 19 = true segmentation restricted to opisthosoma; 20 = mesoderm derived directly from archenteron; 21 = body cavity (coelom) tripartite and derived by enterocoely; 22 = serially arranged clusters of lateral epidermal setae; 23 = ciliated feeding tentacles derived from mesosome and containing extensions of the mesocoel; 24 = pharyngeal gill slits; 25 = unique annelidan head of presegmental prostomium and peristomium; 26 = sheets of subepidermal muscles derived, at least in part, from archenteric mesoderm; 27 = sheets of subepidermal muscles derived, at least in part, from 4d mesoderm; 28 = mesoderm (from mesentoblast) gives rise in part to closed circulatory system of vessels only; 29 = circularity system derived, at least in part, from archenteric mesoderm; 30 = trochophore larva; 31 = nervous system arranged in non-centralized, netlike fashion; 32 = nervous system with anterior concentration of neurons and tending toward presence of longitudinal cords; 33 = cerebral ganglion issues pairs of longitudinal cords connected by transverse commissure, with tendency to emphasize ventral or ventrolateral cords; 34 = with circumenteric nerve ring attached to one or more ventral nerve cords; 35 = longitudinal cords not ladder-like in arrangement and not emphasized ventrally; 36 = dorsal hollow nerve cord. Tree length is 36 steps, consistency index 1.0.

it. The concept of homology is older than the actual coining of the term and can be traced back to Aristotle (Russell, 1916); a common factor is that structures among recent taxa have been discussed in terms of showing homology (while not always using that term). The authors advocating the NV-hypothesis do

something different – they assume that one structure in nemerteans is the precursor of *another* structure in vertebrates, even though there is no structural resemblance between these two features.

There are two basic problems with this approach. First evolution is viewed as a series of progressions

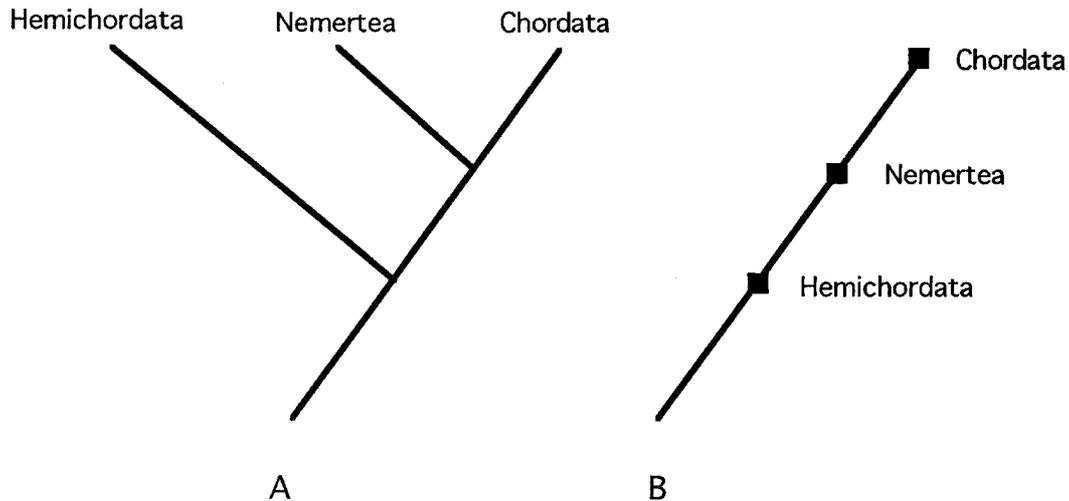


Figure 2. A. The NV-hypothesis ‘nemerteans are the ancestors to vertebrates’ interpreted as a testable hypothesis of a sister-relationship between nemerteans and vertebrates. B. Our interpretation of how Hubrecht, Macfarlane, Jensen and Willmer view the statement that nemerteans are the ancestors of the vertebrates. They suppose that the hypothetical ancestor to the recent vertebrates must have appeared in a certain way, and that these characteristics correspond to what living nemerteans look like now.

from simple to advanced, with one recent taxon derived from another. This view of evolution has little support and is rejected by contemporary evolutionary biologists. The false iconography of evolution as progress has been discussed by S. J. Gould in a number of essays and books (e.g. Gould, 1991) and we will not reiterate the arguments.

Second, this approach is fraught with mere, unsubstantiated, speculation. Only the imagination sets the limits and essentially any phylum could serve as a starting point for similar character sequences. Therefore, the evidence for the NV-hypothesis is void of information since the underlying propositions are basically untestable – they would be rejected by the similarity test at the outset if they had used homology in the normal sense (Patterson, 1982). The only information content in the NV-hypothesis lies in the phrasing that ‘nemerteans are the most recent ancestors to the vertebrates’ if it is interpreted as vertebrates and nemerteans being sistergroups (Figure 2A). Our impression is, however, that the four authors interpret it as seeing the nemerteans as the immediate ancestors to the vertebrates (Figure 2B). This assumes that nemerteans have not undergone any anagenesis, a conjecture which is falsified by the amount of variation seen among nemerteans.

If we interpret the statement as a sistergroup relationship, we agree with Jensen (1988) that his hypothesis is a scientific hypothesis in a Popperian sense. It

can thus be tested, and Jensen (1988) proposed how it can be done and what kind of characters can be used. Today, we have this information in the form of molecular characters and we will carry out the test Jensen proposed. We will also discuss his hypothesis in morphological terms and show that although we cannot find potential synapomorphies for nemerteans and vertebrates, it is possible to show that other characters must have evolved many more times in different groups if nemerteans are placed close to vertebrates. Parsimony does not support Jensen’s hypothesis.

Is there any morphological evidence for common ancestry?

There have been several suggestions for the evolutionary relationships among Metazoa as mentioned in the introduction. Take for example the phylogeny in Brusca & Brusca (reproduced in Figure 1). Using the same characters as Brusca & Brusca (excluding autapomorphies), their hypothesis requires 36 changes in characters, and the tree has a consistency index of 1.0. Moving the nemerteans to the position envisaged by the NV-hypothesis (Figure 3) would increase the tree length to 52 steps, i.e. 44% longer than the original trees, decreasing the consistency index from 1.0 to 0.69. To accept a much less parsimonious phylogenetic hypothesis, with a lower consistency index, requires

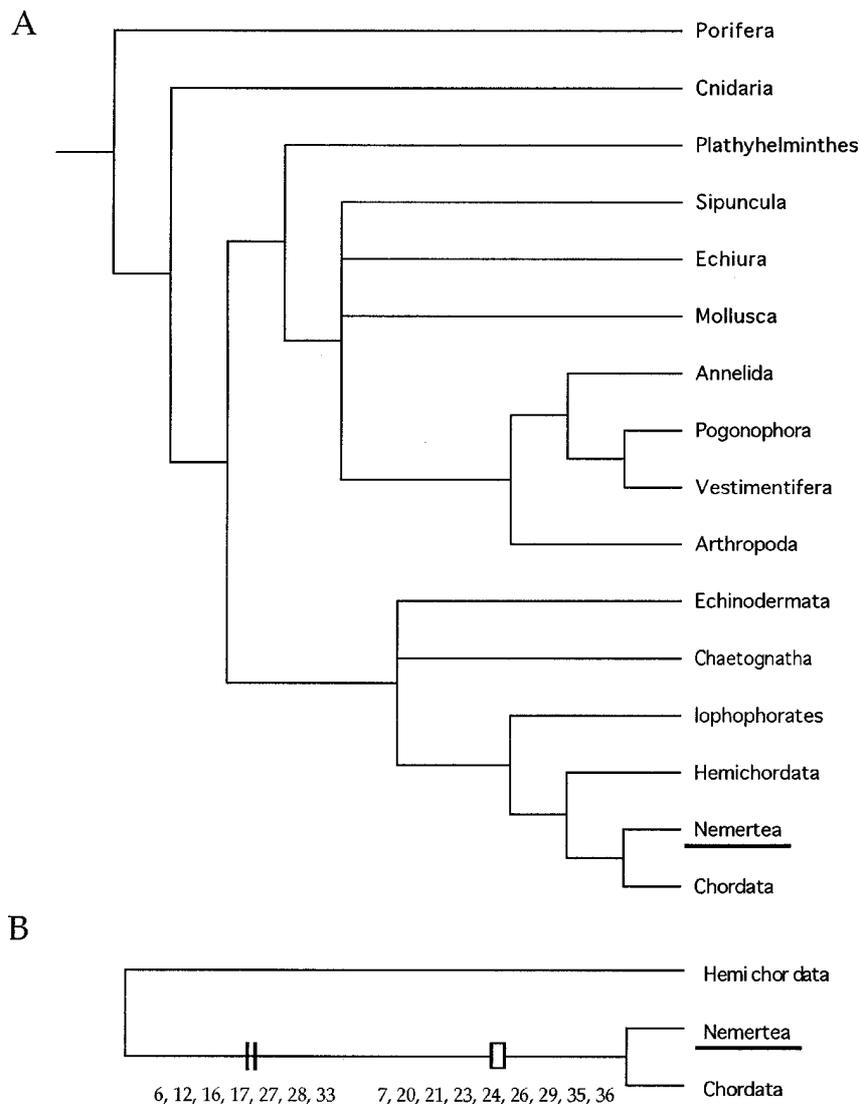


Figure 3. A. The phylogenetic position of the nemerteans according to the NV-hypothesis. This phylogeny requires 56 character changes, the consistency index changes to 0.69. B. The following characters must have arisen twice (parallelism/convergence – double bars) in the tree to account for the NV-hypothesis: 6, 12, 16, 17, 27, 28, 33; and the following characters must have reversed an extra time in the nemerteans to account for the hypothesis (reversal – open square): 7, 20, 21, 23, 24, 26, 29, 35, 36 (see legend to Figure 1).

additional information which none of the four authors behind the idea has provided. We thus conclude that there is no morphological evidence favouring the idea that nemerteans share an immediate common ancestor with the chordates.

Is there molecular evidence for a Nemertea-Chordata clade?

Gene sequences provide an additional means for estimating phylogenetic relationships among distantly related organisms assuming that sequence similarity is homologous. The nuclear 18S rRNA gene shows such a high degree of positional and compositional similarity among organisms that is considered unlikely that it would have happened by chance. It is highly probable

that the gene is homologous across phyla and thus 18S nucleotide sequence comparisons can be used to estimate phylogeny, and hence to test the NV-hypothesis.

Material and methods

Small subunit nuclear ribosomal RNA sequences (18S rRNA) from three nemertean species (Table 1) were obtained by Sundberg (unpublished data) and Turbeville (unpublished data) following standard protocols. A subset of metazoan 18S RNA sequences necessary for testing the hypothesis were retrieved from Genbank (Table 1). Sequences were aligned using the CLUSTAL-V (Higgins et al., 1992) algorithm. Alignment was improved manually where possible, with the Lasergene sequence editor (DNASTAR Inc.). Regions of major length variation that could not be unambiguously aligned were excluded. A total of 1341 positions were included in the analyses.

Phylogeny was reconstructed using maximum parsimony with PAUP 3.1.1 (Swofford, 1993). The Heuristic search option, with random addition of taxa (50 replicates), and TBR branch swapping, was used in PAUP for finding shortest trees; characters were unordered and unweighted.

Results

There were six most parsimonious trees, of length 1036 (counting only informative sites), with a consistency index CI=0.50, and retention index RI=0.54. The strict consensus tree (Figure 4) shows that the nemerteans are unambiguously placed as a sistergroup to the clade with molluscs, annelids, echiurans, vestimentiferans, and pogonophorans. The result does not place the nemerteans as the sistergroup to the chordates as suggested by Jensen et al. A tree with the nemerteans in such a position would require an extra 25 character changes and we conclude that there is no molecular support for the NV-hypothesis.

The position of the sipunculan outside of the spiralian (protostome) clade is incongruent with morphological phylogenies of the Metazoa. The placement of this taxon in the molecular phylogeny is likely incorrect and varies, depending on the ingroup and outgroup taxa included in the analysis (see Winnepenninckx et al. 1995; pers. obs.). Reasons for artifactual association of taxa in molecular analyses are discussed elsewhere (e.g., Turbeville et al., 1994; Swofford et al., 1996).

Table 1. Taxa included in the phylogenetic analysis of 18S gene sequences

Species	Source/accession #
NEMERTEA	
<i>Lineus ruber</i>	Turbeville unpublished data
<i>Amphiporus</i> sp.	Turbeville unpublished data
<i>Cephalothrix rufifrons</i>	Sundberg unpublished data
POGONOPHORA	
<i>Siboglinum</i>	X79876
ECHIURA	
<i>Ochetostoma</i>	X79875
ARTHROPODA	
<i>Androctonus</i> sp.	X77908
CNIDARIA	
<i>Anemonia</i> sp.	X53498
<i>Anthopleura</i> sp.	Z21671
<i>Tripedalia</i> sp.	L10829
ECHINODERMATA	
<i>Antedon</i> sp.	D14357
<i>Asterias</i> sp.	D14358
MOLLUSCA	
<i>Chlamys</i> sp.	L11232
HEMICHORDATA	
<i>Balanoglossus</i> sp.	D14359
UROCHORDATA	
<i>Oikopleura</i> sp.	D14360
CHORDATA	
<i>Latimeria</i> sp.	L11288
<i>Myxine</i> sp.	M97574
<i>Squalus</i> sp.	M91179
ANNELIDA	
<i>Glycera</i> sp.	U19519
CTENOPHORA	
<i>Mnemiopsis</i> sp.	L10826
PLATYHELMINTHES	
<i>Lobatostoma</i>	L16911
SIPUNCULA	
<i>Phascolosoma</i> sp.	X79874
VESTIMENTIFERA	

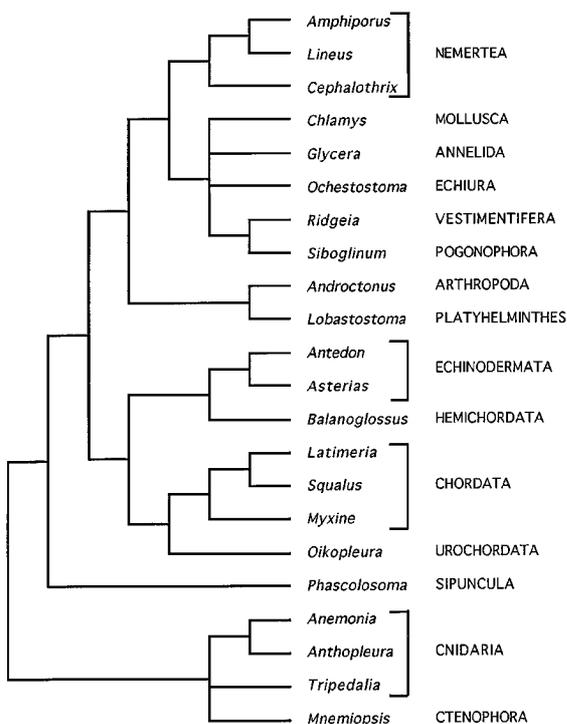


Figure 4. The strict consensus tree of the six equally most parsimonious hypotheses of relationships among metazoans based on analysis of a subset of 18S rDNA sequences.

Discussion

The scientific community should be open-minded to proposed ideas, especially to those ideas questioning knowledge considered accepted. Jensen has presented his ideas, and those of his forerunners, at several meetings and seminars without the hypothesis being critically discussed. We think that this is unfortunate, and have tried in this paper to show why we think the NV-hypothesis must be rejected. Our arguments are in short as follows.

First, we think the hypothesis is based on a flawed view of evolution as a progression series where less developed characters are progressively modified into more complex ‘higher’ characters. Even if this can be true for characters, it cannot be converted to whole taxa where one recent taxon is viewed as the precursor to another. This view has no support in modern evolutionary thinking and must be dismissed until evidence shows otherwise. Second, the four authors do not present any potentially homologous characters that indicate a sister-group relationship between vertebrates and nemerteans. Instead, they speculate about

precursor characters to organ systems found in recent vertebrates concluding that these characters are seen in extant nemerteans – hence the nemerteans represent the ancestors of the vertebrates. These are unsubstantiated speculations, which could be applied to essentially any invertebrate taxon and are therefore scientifically empty propositions. This kind of reasoning has no place in modern biology based on evolutionary theory and should be abandoned altogether. Third, to assume that nemerteans are the immediate ancestors to vertebrates would assume that a whole series of morphological characters must have evolved more than once. The characters taken as example in Figure 1 from Brusca & Brusca represent just a small fraction of possible characters – there are even more characters for which multiple origins would need to be postulated. Admittedly, this reasoning is based on the principle of parsimony and may not hold if this principle is violated. But, then Jensen must present evidence and alternatives to this idea. Fourth, molecular evidence (once again using the principle of parsimony), does not support the NV-hypothesis.

Although this study explicitly does not address the question of the phylogenetic position of the nemerteans among the protostome (spiralian) metazoans, we note that these preliminary results support Turbeville et al.’s (1992) conclusion of a closer connection between the annelids and nemerteans. Turbeville’s (1991) suggestion that the nemertean circulatory system is a coelom homologue is also supported by the analysis.

We conclude that there is currently no evidence substantiating the hypothesis that nemerteans are the ancestors of vertebrates, and it should be abandoned until evidence in favour of it is presented.

Acknowledgements

PS thanks the Swedish Natural Science Research Council, the Erna och Victor Hasselblad foundation, and the KVVS for financial support, and the Wennergren foundation for a travel grant making it possible for JMT to visit Sweden. MH gratefully acknowledges the financial support from the Royal Swedish Academy of Sciences, Göteborg Marine Research Centre, and Paul and Marie Berghaus Foundation for travel grants making it possible to attend the Fourth International Meeting on Nemertean Biology.

References

- Brusca, R. C. & G. J. Brusca, 1990. *Invertebrates*. Sinauer Ass., Inc., Massachusetts, 922 pp.
- Darwin, C., 1859. *The Origin of Species by Means of Natural Selection*. J. Murray, London, 502 pp.
- Eernisse, D. J., J. S. Albert & F. E. Anderson, 1992. Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan morphology. *Syst. Zool.* 41: 305–330.
- Gibson, R., 1972. *Nemerteans*. Hutchinson University Library, London, 224 pp.
- Gould, S. J., 1991. *Wonderful Life*. Penguin Books, Ltd, London, 344 pp.
- Hubrecht, A. A. W., 1883. On the ancestral form of the Chordata. *Quart. J. microsc. Sci.* 23: 349–368.
- Hubrecht, A. A. W., 1887. The relation of the Nemertea to the Vertebrata. *Quart. J. microsc. Sci.* 27: 605–644.
- Nielsen, C., 1985. Animal phylogeny in the light of the trochaea theory. *Biol. J. linn. Soc.* 25: 243–299.
- Nielsen, C., 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zool.* 68: 205–262.
- Norenburg, J. L., 1985. Structure of the nemertine integument with consideration of its ecological and phylogenetic significance. *Am. Zool.* 25: 37–51.
- Patterson, C., 1982. Morphological characters and homology. In K. A. Joysey & A. E. Friday (eds), *Problems of Phylogenetic Reconstruction*. Academic Press, London: 21–74.
- Russell, E. S., 1916. *Form and Function: a Contribution to the History of Animal Morphology*. John Murray, London, 383 pp.
- Sundberg, P. & M. Svensson, 1994. Homoplasy, character function, and nemertean systematics. *J. Zool., London* 234: 253–263.
- Swofford, D. L., G. J. Olsen, P. J. Waddell & D. M. Hillis. 1996. Phylogenetic inference. In D. M. Hillis, C. Moritz & B. K. Mable (eds), *Molecular Systematics*, 2nd edition. Sinauer, Sunderland, Mass: 407–514.
- Turbeville, J. M., 1991. Nemertinea. In F. W. Harrison & B. J. Bogitsch (eds), *Microscopic Anatomy of Invertebrates*, Vol. 3: Platyhelminthes and Nemertinea. Wiley Liss, Inc., New York: 285–328.
- Turbeville, J. M. & E. E. Ruppert, 1985. Comparative ultrastructure and the evolution of nemertines. *Am. Zool.* 25: 53–71.
- Turbeville, J. M., J. R. Schulz & R. A. Raff, 1994. Deuterostome phylogeny and the sister group of the chordates: Evidence from molecules and morphology. *Mol. Biol. Evol.* 11: 648–655.
- Turbeville, J. M., K. G. Field & R. A. Raff, 1992. Phylogenetic position of phylum Nemertini inferred from 18S rRNA sequences: molecular data as a test of morphological character homology. *Mol. Biol. Evol.* 9: 235–249.
- Willmer, E. N., 1974. Nemertines as possible ancestors of the vertebrates. *Biol. Rev.* 49: 321–363.
- Winnepenninckx, B., T. Backeljau & R. De Wachter, 1995. Phylogeny of protostome worms derived from 18S rRNA sequences. *Mol. Biol. Evol.* 12: 641–649.