

# Convergence in the Feeding Apparatuses of Lophophorates and Pterobranch Hemichordates Revealed by 18S rDNA: An Interpretation

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*Homology of the feeding apparatus of pterobranch hemichordates and the lophophore of bryozoans, brachiopods, and phoronids has been postulated but never rigorously tested. I employ Patterson's (1, 2) three criteria of conjunction, similarity, and congruence to test this putative homology. Although the conjunction and similarity criteria are satisfied, congruence is not. The congruence test is based on a phylogeny derived from 18S rDNA sequence data which show that pterobranchs and lophophorates are in different metazoan subkingdoms and are not closely related. This finding indicates that a lophophore-like apparatus has evolved at least twice in metazoans even though the gross morphology, feeding mechanics, ultrastructure, and ciliary patterns of these organisms are very similar. The high degree of morphological convergence presumably results from similar selective regimes acting on these taxa. These findings indicate that major clades of organisms can evolve in a corresponding fashion despite independent origins.*

The lophophorates (consisting of the phoronids, brachiopods, and bryozoans) and the pterobranch hemichordates are sessile suspension-feeding marine organisms that use ciliated tentacles to capture algae. These tentacles are unique among metazoans because they have similar ciliation patterns, are invaded by the mesocoelomic cavity, and surround the mouth but not the anus. Several workers have postulated that the lophophores of brachiopods, bryozoans, and phoronids are homologous to the tentaculate arms of pterobranch hemichordates (3–8), leading to phylogenetic hypotheses that support the notion of homology among these structures (8, 9). However, most tra-

ditional phylogenies show these taxa to be unrelated (3, 10), suggesting convergence.

In this research, a phylogeny based on 18S rDNA sequence data is used to test the putative homology of the feeding apparatuses found in the Lophophorata and Pterobranchia. Patterson's (1, 2) three criteria of conjunction, similarity, and congruence constitute the framework for the test. For these purposes, a homologous structure is defined as a feature in two or more groups of organisms that was derived from a single common feature present in a shared ancestor. That is, Patterson's notion of homology, which equates homology and synapomorphy, is adopted here. To be considered homologous, the morphological feature or pattern in question must satisfy all three of Patterson's criteria (described in turn).

If the structures in question are "anatomical singularities," then the conjunction criterion has been met. The main utility of this criterion is that it distinguishes homology by descent from homology due to serial repetition (see 1, 2). An examination of the tentacles in the lophophorate and pterobranch feeding apparatus provides clear evidence that serial repetition has occurred: (i) closely related species have different numbers of tentacles, (ii) the internal structure of the tentacles within an organism is the same, and (iii) the tentacles develop in a progressive series (3, 11; pers. obs.).

The issue, however, is whether the feeding apparatus as a whole has been serially repeated. Although phoronids, bryozoans, brachiopods, and rhabdopleurid pterobranchs each have a single bilateral tentacular feeding structure that surrounds the mouth but not the anus, the cephalodiscid pterobranchs have multiple arms bearing tentacles (6, 12). Given that the males of *Cephalodiscus sibogae* and all *Rhabdopleura* species have a single pair of arms (there are only two pterobranch genera; *Atubaria* is most likely a form of *Cephalodiscus*), the ancestral condition

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for pterobranchs is assumed, by parsimony, to be a single pair of arms because a single evolutionary event can account for the multiple arms in *Cephalodiscus*. Therefore, the feeding apparatus in lophophorates and pterobranchs satisfies the conjunction test.

Similarity is perhaps the most obvious criterion for homology. The similarity between the feeding apparatus of pterobranchs and the lophophore of bryozoans, brachiopods, and phoronids has long been known; the earliest reports on the pterobranchs considered them either related to, or included in, the Bryozoa (13–15). In both the pterobranchs and the lophophorates, the feeding apparatus is a ciliated tentacular structure that is invaded by the mesocoelomic cavity and surrounds the mouth but not the anus. (Although in some pterobranchs the mesosome completely surrounds the mouth, tentacles are not present between the mouth and the region in which the cephalic shield attaches.) This arrangement is fundamentally different from that of other suspension-feeding organisms such as entoprocts (in which both the mouth and anus are surrounded by tentacles) or some polychaetes (*e.g.*, serpulids, in which several segments of the organism are devoted to the tentacular apparatus). Except in bryozoans and some brachiopods, blood vessels are usually found in close association with the tentacular coelom (3, 7, 16, 17).

Moreover, the resemblance between these structures extends to ultrastructure and functional morphology (7, 17 and refs. therein, 18). In all of these taxa, the arrangement of the cilia on the tentacles is identical. Three ciliated bands (lateral, frontolateral, and frontal) are present along the length of the tentacle. The lateral cilia generate a current that draws water toward the frontal surface of the arm between and perpendicular to the tentacles (see fig. 2 in 18). In the suspension-feeding entoprocts, the direction of water flow is reversed (3). As the water passes through the suspension-feeding apparatus of lophophorates and pterobranchs, food particles are captured with an upstream particle-collection mechanism by a local reversal in beat of the lateral cilia (8, 17, 18). Once a particle has been captured, frontolateral and frontal ciliary bands transport it down the length of the tentacle without the aid of mucus. Other suspension-feeding organisms (*e.g.*, serpulid polychaetes and crinoids) use mucus during particle transport. These taxa all reject particles by using the frontolateral and frontal cilia to transport particles distally until they fall off the tentacle (in addition to other rejection methods). Unfortunately, information on the development of pterobranch arms and tentacles is so limited that a meaningful comparison to lophophorate tentacular development cannot be made. In essence, however, the similarity criterion supports the putative homology among these structures.

Although the conjunction and the similarity criteria of homology are often met, congruence is the most discrim-

inating criterion of the three. Congruence refers to correspondence of the homology in question to other putative or known homologies. In the case of lophophorate and pterobranch feeding morphology, an *a priori* assessment of which morphological features are evolving independently cannot be accurately made. The sessile nature of lophophorates and pterobranchs has undoubtedly caused evolutionary influences on their reproduction, feeding, and morphology. The question is which features have evolved relatively independently. For example, two potential phylogenetic characters, a U-shaped gut and a circumoral ciliated ring of tentacles, might both be consequences of a single feature (a sessile existence) because they are found in several unrelated sessile organisms. Lophophorates, pterobranchs, and urochordates have U-shaped guts. Sessile polychaetes, lophophorates, pterobranchs, and entoprocts all have ciliated circumoral tentacles. Thus, in this case, we cannot accurately test the homology of the feeding apparatus by using a phylogeny based on morphological data because we do not understand the relationships among various morphological features and different biological aspects (*e.g.*, feeding, reproduction, locomotion). The disagreement among workers as to which nonmolecular features are phylogenetically important (see 19) is a testament to this uncertainty.

To avoid the problem of nonindependence, I have examined metazoan evolutionary relationships based on sequence data from the 18S nuclear ribosomal gene. With molecular sequence data, the determination of which characters are phylogenetically informative (as well as the specification of the character state) is much less subjective (see 20), and the characters can more easily be treated as independent. Even when characters (*i.e.*, nucleotide sites) are clearly not independent, the relationship and covariation between them are well characterized and can be compensated for (21).

Recent studies using 18S rDNA data have shown that either some (22, 23) or all (24) of the traditional lophophorate taxa are protostome organisms, and that the pterobranchs are hemichordates within the deuterostome clade (25, 26). Although these investigations suggest that the feeding apparatus is not congruent with known molecular homologies, I have combined the pterobranchs and the lophophorates into a single study (Table I) to allow a more rigorous examination of the issue. The entire 18S rDNA gene was aligned for all of the taxa with the exception of the *Rhabdopleura normani* sequence for which only the 5' third of the 18S gene has been obtained. The remaining two-thirds was coded as missing (*i.e.*, as "N"), which allows these sites to be used for determining the relationships among the other taxa. Regions that could not be unambiguously aligned were excluded from subsequent analyses, and nucleotide sites were considered as independent characters (20). The

Table I

Taxa used in phylogenetic analysis

Species	GenBank acc. no.	Taxon
<i>Plumatella repens</i>	U12649	Bryozoan
<i>Terebratalia transversa</i>	U12650	Articulate
<i>Glottidia pyramidata</i>	U12647	Inarticulate
<i>Phoronis vancouverensis</i>	U12648	Phoronid
<i>Rhabdopleura normani</i>	U15664	Pterobranch
<i>Balanoglossus carnosus</i>	D14359	Enteropneust
<i>Antedon serrata</i>	D14357	Crinoid
<i>Asterias amurensis</i>	D14358	Seastar
<i>Artemia salina</i>	X01723	Crustacean
<i>Placopecten magellanicus</i>	X53899	Bivalve
<i>Branchiostoma floridae</i>	M97571	Cephalochordate
<i>Xenopus laevis</i>	X04025	Vertebrate
<i>Acanthopleura japonica</i>	X70210	Chiton
<i>Tenebrio molitor</i>	X07801	Insect
<i>Anemonia sulcata</i>	X53498	Cnidarian
<i>Sycon calcaravis</i>	D15066	Sponge

alignment can be obtained from the author at internet address *Halanych@mail.smu.edu*.

The alignment of the 18S rDNA sequences produced 1608 unambiguously aligned positions of which 295 were phylogenetically informative characters (*i.e.*, parsimony sites or shared derived characters). The general heuristic search algorithm of PAUP (*v.* 3.1.2d5; 27) and equal character weighting generated six equally parsimonious trees (1041 steps). The consistency index (C.I.) was 0.667 (C.I. = 0.561 when excluding uninformative characters), indicating that there was not a large amount of homoplasy among the data. Of the six trees, the one shown in Figure 1A conforms most closely to the traditional phylogeny (3, 10) because the deuterostomes are monophyletic. A maximum likelihood analysis (using PHYLIP *v.* 3.5; 28) revealed that none of these topologies are significantly worse than the best tree (likelihood score of  $-7753.43979$ ). Figure 1B shows the strict consensus of these six trees.

The phylogenies recovered are consistent with the finding that bryozoans, brachiopods, and phoronids are protostomes, and that the pterobranchs are deuterostomes. The pterobranchs fall outside both the inclusive bryozoan-brachiopod-phoronid node and the protostome node; these have bootstrap values of 84% and 71%, respectively (Fig. 1B). Bootstrap values  $\geq 70\%$  usually correspond to a probability of  $\geq 95\%$  that the given clade is real (29). When the empirically derived transition-transversion ratio (1.4:1) is used to weight character changes accordingly, the bootstrap analysis yields values of 90% for the bryozoan-brachiopod-phoronid node and 76% for the protostome node. Transition-transversion ratios of 2:1, 3:1, and 10:1 give similar results. Additional analyses in which only the first third of the 18S gene (which is available for all taxa) was used also show that the bryozoans, brachiopods, phoronids, and molluscs form a monophyletic clade

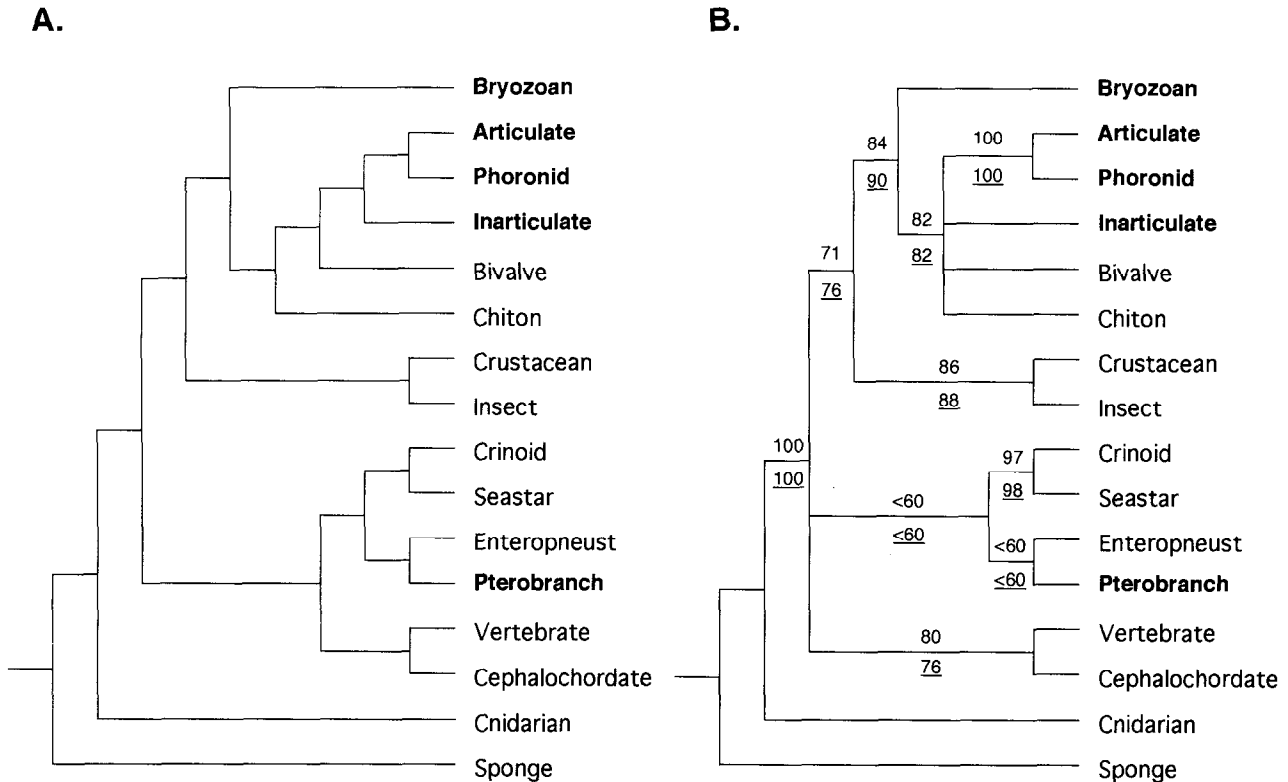
to the exclusion of the pterobranchs (bootstrap value of 88% for equal weighting and 94% for weighting transversions 1.4:1 over transitions). The phylogenetic analysis of the 18S rDNA data is therefore inconsistent with the putative homology of the feeding apparatuses.

Not only do the molecular data argue against homology, but recent fossil evidence also suggests that the lophophorates and pterobranchs are not closely related. Conway Morris and Peel (30) propose that extinct metazoans known as halkieriids are actually the common ancestor of annelids and brachiopods, and are closely related to molluscs. More evidence is needed to determine the exact position of the halkieriids, but the paleontological evidence (30) definitely places at least one of the lophophorate groups, the brachiopods, within the protostome lineage. The pterobranchs, on the other hand, are closely related to graptolites (31) and are clearly deuterostomes.

As for the morphological data based on extant taxa, the position of the lophophorates relative to pterobranchs is dependent upon which features are considered phylogenetically informative (32). Rigorous cladistic analyses (9, 33) suggest that lophophorates and pterobranchs form a basal paraphyletic grade within the Deuterostomia; *i.e.*, these taxa delineate a group which includes some, but not all, descendants of a common ancestor near the root of the deuterostome clade. Some of the characters employed in these analyses may, however, be ecological, and not phylogenetic, in nature; *e.g.*, the chaetognaths have been shown not to be in the Deuterostomia despite the fact that their blastopore is retained to form an anus (34, 35), and some potentially important characters—for example, the similar setae of annelids and brachiopods—are occasionally overlooked. Ideally, a phylogeny based on morphology should be developed by reanalyzing the existing data as more information becomes available. In reality, this is a somewhat subjective undertaking because it is difficult to agree on which morphological features are phylogenetically informative. Although morphological data are definitely an important phylogenetic tool, their application is not always useful or appropriate (36).

To date, no rigorous phylogenetic analysis has produced results suggesting that the pterobranchs, brachiopods, phoronids, and bryozoans form a monophyletic taxon rather than a paraphyletic grade. On the basis of the molecular and paleontological data, the criterion of congruence clearly is not satisfied. Thus, Patterson's three-criterion test for homology is failed. The most parsimonious explanation is that a lophophore-like apparatus has evolved at least twice in metazoans, and the homology, advocated by previous workers (3–8), between the lophophorate and the pterobranch feeding apparatuses is refuted.

Given this result, the degree of convergence in the feeding apparatus of these taxa is particularly striking. These organisms are ultrastructurally and mechanically very



**Figure 1.** (A) The most traditional (in that the deuterostomes are monophyletic) of the six trees produced from the general heuristic search of PAUP. The trees had a length of 1041 with a consistency index of 0.667 (C.I. = 0.561 excluding uninformative characters). The  $g_1$  statistic ( $g_1 = -1.096090$ ) indicates that there is significant phylogenetic signal in this data set. (B) The strict consensus tree produced from the six topologies obtained in the heuristic search. The pterobranchs and lophophorates are several nodes apart. The values along the branches are bootstrap values (plain numbers are equal weighting and underlined numbers are transversions weighted 1.4 times transitions) produced from 500 iterations of a heuristic bootstrap analysis. All collapsed branches in the strict consensus topology were supported in less than 60% of the bootstrap iterations.

similar, which presumably reflects convergence due to comparable selective pressures. Bryozoans, brachiopods, phoronids, and pterobranchs are all sessile organisms that feed on unicellular algae about 7–20  $\mu\text{m}$  in length. Conceivably, physical characteristics (*e.g.*, viscosity) that affect the capture and manipulation of particles in this size range, combined with a sessile existence, exert a strong selective influence toward a similar morphology. Obviously, however, a lophophore-like apparatus is not the only approach to suspension-feeding for a sessile animal. Bivalves, polychaetes, and urochordates use completely different mechanisms.

Despite the amazing similarity among the feeding apparatuses of the traditional lophophorates and pterobranchs, there are a few differences. For example, the feeding apparatus in pterobranchs does not surround the mouth as completely as it does in the lophophorates. Also, monociliated cells make up the tentacular ciliary bands in pterobranchs, brachiopods, and phoronids, but bryozoans have multiciliated cells. Thus a closer inspection

of the feeding apparatus in different lineages may reveal that the similarity is more superficial than believed. Unfortunately, because the information is not complete for some groups (*e.g.*, rhabdopleurid pterobranchs), a more rigorous analysis at the electron microscopy level is necessary to determine the degree of similarity. Ideally, this analysis would consider all the taxa simultaneously.

The hypothesis that a lophophore-like apparatus has arisen at least twice in metazoan evolution raises some interesting evolutionary questions. How is the pterobranch feeding apparatus and ciliation similar to an enteropneust's proboscis and collar? Given that the lophophorates are protostomes, why is their ciliation pattern so different from that of other sessile suspension-feeding protostomes? Was a lophophore a primitive protostome character? Before many of these questions can be answered, we need a more complete understanding of protostome relationships. In view of the recent placement of the lophophorates suggested by molecular and fossil data, the relationships between annelids, molluscs, lophophorates, and other

protostome taxa are not clear. Thus, to answer the above questions, to determine whether the traditional lophophorates are polyphyletic or paraphyletic, and to determine if brachiopods are polyphyletic (as indicated by the molecular data in Fig. 1), additional taxa must be sampled for both molecular and morphological data.

Finally, the convergence in feeding morphology in pterobranchs and lophophorates suggests that the evolutionary trajectory of major metazoan lineages, such as subkingdoms or phyla, can be very similar despite distinct phylogenetic origins.

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### Literature Cited

- Patterson, C. 1982. Morphological characters and homology. Pp. 21–74 in K. A. Joysey and A. E. Friday, eds. *Problems of Phylogenetic Reconstruction*. Academic Press, London.
- Patterson, C. 1988. Homology in classical and molecular biology. *Mol. Biol. Evol.* 5: 603–625.
- Hyman, L. H. 1940–1968. *The Invertebrates*. McGraw-Hill, New York.
- Gilmour, T. H. J. 1979. Feeding in pterobranch hemichordates and the evolution of gill slits. *Can. J. Zool.* 57: 1136–1142.
- Hoverd, W. A. 1985. Histological and ultrastructural observations of the lophophore and larvae of the brachiopod, *Notosaria nigricans* (Sowerby 1846). *J. Nat. Hist.* 19: 831–850.
- Lester, S. M. 1985. *Cephalodiscus* (Hemichordata: Pterobranchia): observations of functional morphology, behavior and occurrence in shallow water around Bermuda. *Mar. Biol.* 85: 263–268.
- Dilly, P. N., U. Welsch, and G. Rehkamper. 1986. Fine structure of tentacles, arms, and associated coelomic structures of *Cephalodiscus gracilis* (Pterobranchia, Hemichordata). *Acta Zool. (Stockh.)* 67: 181–191.
- Nielsen, C. 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zool. (Stockh.)* 68: 205–262.
- Schram, F. R. 1991. Cladistic analysis of metazoan phyla and the placement of fossil problematica. Pp. 35–47 in A. M. Simonetta and S. Conway-Morris, eds. *The Early Evolution of Metazoa and Significance of Problematic Taxa*. Cambridge University Press, New York.
- Barnes, R. D. 1987. *Invertebrate Zoology*. 5th ed. Saunders, Philadelphia.
- Ridewood, W. G. 1907. On the development of the plumes in buds of *Cephalodiscus*. *Q. J. Microsc. Sci.* 51: 221–252.
- Harmer, S. F. 1905. The Pterobranchia of the Siboga Expedition. Siboga-Expedition, Monograph XXVI. E. J. Brill, Leyden.
- Allman, G. 1869. On *Rhabdopleura*. *Q. J. Microsc. Sci.* IX: 57–63.
- Sars, G. O. 1874. On *Rhabdopleura mirabilis*. *Q. J. Microsc. Sci.* 14: 23–44.
- MacIntosh, W. 1882. *Cephalodiscus*. *Ann. Mag. Nat. Hist.*, ser 5 vol 10.
- Reed, C. G., and R. A. Cloney. 1977. Brachiopod tentacles: ultrastructure and functional significance of the connective tissue and myoepithelial cells in *Terebratalia*. *Cell Tissue Res.* 185: 17–42.
- Halanych, K. M. 1993. Suspension feeding and the lophophore-like apparatus of the pterobranch hemichordate *Rhabdopleura normani*. *Bio. Bull.* 185: 417–427.
- Strathmann, R. R. 1973. Function of lateral cilia in suspension feeding lophophorates (Brachiopoda, Phoronida, Ectoprocta). *Mar. Biol.* 23: 129–136.
- Willmer, P. 1990. *Invertebrate Relationships; Patterns in Animal Evolution*. Cambridge University Press, New York.
- Swofford, D. L., and G. J. Olsen. 1990. Phylogeny reconstruction. Pp. 411–501 in D. M. Hillis and C. Moritz, eds. *Molecular Systematics*. Sinauer Associates, Sunderland, MA.
- Dixon, M. T., and D. M. Hillis. 1993. Ribosomal RNA secondary structure: compensatory mutations and implications for phylogenetic analysis. *Mol. Biol. Evol.* 10: 256–267.
- Field, K. G., G. J. Olsen, D. J. Lane, S. J. Giovannoni, M. T. Ghiselin, E. C. Raff, N. R. Pace, and R. A. Raff. 1988. Molecular phylogeny of the animal kingdom. *Science* 239: 748–753.
- Lake, J. A. 1991. Origin of the Metazoa. *Proc. Natl. Acad. Sci. USA* 87: 763–766.
- Halanych, K. M., J. Bachelor, A. M. Aquinaldo, S. Liva, D. M. Hillis, and J. A. Lake. 1995. 18S rDNA evidence that the lophophorates are protostome animals. *Science* 267: 1641–1643.
- Halanych, K. M. 1995. The phylogenetic position of the pterobranch hemichordates based on 18S rDNA sequence data. *Mol. Phylo. Evol.* 4: 72–76.
- Turbeville, J. M., J. R. Schultz, and R. A. Raff. 1994. Deuterostome phylogeny and the sister group of the chordates: evidence from molecules and morphology. *Mol. Biol. Evol.* 11: 648–655.
- Swofford, D. L. 1993. *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.2d5*. Illinois Natural History Survey, Champaign, IL.
- Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package) Version 3.5c*. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42: 182–192.
- Conway Morris, S., and J. S. Peel. 1995. Articulated halkieriids from the Lower Cambrian of North Greenland and their role in early protostome evolution. *Phil. Trans. R. Soc. Lond. B* 347: 305–358.
- Lehmann, U., and G. Hillmer. 1983. *Fossil Invertebrates*. Cambridge University Press, New York.
- Backeljau, T., B. Winnepeninckx, and L. DeBruyn. 1993. Cladistic analysis of metazoan relationships: a reappraisal. *Cladistics* 9: 167–181.
- Eernisse, D. J., J. S. Albert, and F. E. Anderson. 1992. Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan morphology. *Syst. Biol.* 41: 305–330.
- Telford, M. J., and P. W. H. Holland. 1993. The phylogenetic affinities of the Chaetognaths: a molecular analysis. *Mol. Biol. Evol.* 10: 660–676.
- Wada, H., and N. Satoh. 1994. Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proc. Natl. Acad. Sci. USA* 91: 1801–1804.
- Hillis, D. M. 1987. Molecular versus morphological approaches to systematics. *Annu. Rev. Ecol. Syst.* 18: 23–42.