Lophophorate Phylogeny

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Four new gene sequences presented in the report by Kenneth M. Halanych *et al.* (*1*) reinforce earlier conjectures (*2–5*) that the lophophorates (brachiopods, ectoprocts, and phoronids), long thought to lie either close to or within the deuterostomes (*6, 7*), cluster with other protostomes. Although largely neglected by mainstream zoology, such a conclusion actually has a long pedigree (*8, 9*). While we agree that evidence from a nuclear-encoded small subunit (SSU) ribosomal RNA gene sequence strongly supports the association of lophophorates with protostomes, the proposed phylogeny (*1*) and its expression in a new taxonomic category, the "Lophotrochozoa" (*1*) are open to at least four criticisms: (i) premature introduction of a new taxonomic category, (ii) the need for a more cautious interpretation of ectoproct molecular phylogeny, (iii) the incongruence of the proposed phylogeny with paleontological data, and (iv) questions about sequence reliability.

1) For anyone attempting to reconstruct a phylum-level phylogeny from an alignment of the many currently available protostome SSU sequences (*10*), a cautious approach is advisable. Caution is also necessary when choosing an outgroup. As no unambiguous evidence yet identifies the sister group (or groups) of protostome phyla collectively or individually, it is probably premature to root phylum-level trees, especially with distant taxa (as Halanych *et al.* have done). Because the role of molecular phylogenies as a prime basis for animal classification is controversial, and because the complex relationship between molecular phylogenies and taxonomic practice is still evolving (*11*), major taxonomic changes should not be proposed on the basis of a single gene sequence; congruent evidence from multiple, independent genealogical sources is needed.

2) The ectoproct bryozoa are a highly diverse phylum (*12, 13*), yet their phylogenetic relationships have been inferred from the SSU sequence of a single species, *Plumatella repens* (*1*). However, the class to which *Plumatella* belongs is not typical of the phylum (*14*), and the wide range of extant ectoproct diversity is not reflected in available sequences, one of which (*15*) has unusual apomorphies and might not be representative. No decision about the phylogenetic relationships of the whole phylum is yet firm enough to justify its place in a new taxonomic category (*1*).

3) Excluding some highly questionable Cambrian examples (*16*), the first convincing ectoprocts are Ordovician (*17*). Moreover, all known ectoprocts are colonial; possible solitary forms are not recognized in the fossil record until well after the first appearance of colonies (*18*), postdating by perhaps 50 million years the Lower Cambrian appearance of brachiopods, annelids (polychaetes), and mollusks. Thus, a phylogeny (*1*) that places the origin of ectoprocts basal to the origins of these phyla is inconsistent with present knowledge of the fossil record. As it is unlikely that fossils of hard-bodied, Lower Cambrian ectoprocts have been overlooked, such a phylogeny (*1*) predicts that ancestral ectoprocts were soft-bodied. There is currently no evidence from Cambrian faunas such as the Burgess Shale that such forms existed, although the possibility cannot be dismissed. This incongruence between paleontological and molecular evidence emphasizes the need for a cautious approach to the use of molecular phylogenies in classification.

4) When we compared the four new lophophorate SSU sequences (*1*) with homologous sequences from many other protostomes, including another phoronid (GenBank accession number U36271) and another inarticulate brachiopod (GenBank accession number X81631) (*15* and *19*, respectively), we found that nucleotides were missing at several sites that are otherwise completely or almost completely conserved (*20*). The *Glottidia* sequence (GenBank accession number U12647) also had an unusual deletion affecting only one strand of a helical stem region, whose general form is also widely conserved (*21–23*). These results call into question the reliability of the data in, and the conclusions of, the report by Halanych *et al.*

The ultimate value of molecular biology in understanding early metazoan evolution is not in dispute, but without data from a sufficiently wide range of genes and species and from other data sources such as the fossil record (*24, 25*), progress may be delayed or diverted. Paleontological information can throw light on apparent inconsistencies in a phylogeny or, alternatively, reveal intermediate states between what we choose to call phyla (*24*). For example, the fossil record may display unexpected, phylogenetically informative combinations of character states directly relevant to the evolution of lophophorates; in particular, the "shells" and precursors of the setae in halkieriids suggest a more direct connection between this group and brachiopods (*25*).

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Response: The purpose of our report (*1*) was to determine the phylogenetic placement of the Lophophorata. Specifically, we wanted to know whether lophophorates are protostomes, deuterostomes, or an independent lineage of metazoan evolution. Conway Morris *et al.* agree with our major conclusion that the Lophophorata are a part of the protostome radiation. However, they do raise some specific issues about our report.

To emphasize our major conclusion, we decided to define the node-based name Lophotrochozoa as the last common ancestor of the lophophorate taxa (that is, bryozoans, brachiopods, and phoronids), mollusks, annelids, and all of the descendants of that common ancestor. The utility of node-based names has been thoroughly discussed (*2*) and is advocated by systematists who believe that biological taxonomy should be based directly on evolutionary history. The naming of this clade is not premature because the 18S data provide the strongest evidence to date for the placement of the lophophorates and, as pointed out by Conway Morris *et al.*, paleontological (*3*) and morphological (reviewed in *4*) evidence supports this clade.

Conway Morris *et al.* question our choice of taxa with regard to outgroup and the bryozoan representative. Because we were analyzing the placement of lophophorates within triploblast metazoans, diploblast metazoans are the most appropriate outgroup. The use of the protostome sister-taxon as the outgroup would only be useful for a study that focused solely on protostome taxa. We chose the most closely related outgroup taxa whose placement outside the study group (triploblast metazoans) could be considered unambiguous. The resulting rooting of protostomes and dueterostomes would appear not to be controversial. Concerning the choice of a bryozoan, the issue of bryozoan diversity should be kept separate from that of bryozoan monophyly. Furthermore, the presence of morphological diversity within a taxon is not sufficient grounds for assuming genetic diversity (in this case 18S ribosomal (rDNA) diversity), or vice versa. The 18S rDNA data for two of the three recognized bryozoan classes (the phylactolaemate *Plumatella repens* and the gymnolaemate *Alcyonidum gelatinosum*) have been examined in independent studies (our report and *5*) which reach similar conclusions about the evolutionary relationships of Bryozoa. Given these congruent results, we are inclined to follow the data, rather than hypothesize that two bryozoan sequences from different classes are "not typical" and yet yield similar results.

Although the placement of the bryozoan in our original report is suggested to be inconsistent with paleontological evidence, other reports by Conway Morris (*3*) show that our results agree well with the fossil record. The fact that a solitary, soft-bodied, ancestral bryozoan fossil has not been discovered is not unexpected given the incompleteness of the fossil record.

The most serious issue raised by Conway Morris *et al.* is that of data reliability and its effect on our conclusions. The presence of indels in conserved regions of the 18S rDNA does occur across metazoan taxa (*6*). Since our publication, we have reexamined our results and have found a few minor errors in some of our sequences (*7*). The GenBank submissions have been appropriately updated. Also, Conway Morris *et al.* were correct in pointing out the mistake in the *Glottidia* helical stem region. However, this region is not conserved across triploblast metazoans, and therefore most of it was excluded from the original analysis because it cannot be aligned unambiguously. We have reanalyzed our data incorporating these corrections, and our original phylogenetic tree, bootstrap trees, and conclusions are unaffected. Additionally, an independent study (*5*) has confirmed our major conclusions.

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6. 18S rDNA sequences often exhibit indels in highly conserved regions. Below are a few examples: *Strongylocentrotus purpuratus* (echinoderm), GenBank accession number L28055, six indels in conserved regions when aligned to our original data set; *Antedon serrata* (echinoderm), D14357, five indels; *Limicolaria kambeul*, X66374, three indels; *Artemia salina* (crustacean), X01723, two indels; *Placopecten magellanicus* (bivalve), X53899, two indels; and two *Eurypelma californica* (chelicerate), X13457, one indel.
7. Updates have been reported for the *Plumatella repens* (GenBank accession number U12649), *Terebratalia and transversa* (U12650), *Phoronis vancouverensis* (U12648) sequences. In addition to minor changes, the *Glottidia pyramidata* (U12647) contained a mistake in a helical stem region. Most of these mistakes were originally reported as gaps and occurred at phylogenetically uninformative positions. Because they were originally scored as gaps, they were considered as missing information in the analyses, and therefore had no bearing on our conclusions.

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