Cah. Biol. Mar. (1998) 39 : 355-358

Evolutionary origins and age of vestimentiferan tube-worms

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Introduction

Vestimentiferan tube-worms are one of the dominate groups of organisms present at deep-sea hydrothermal vent habitats in the eastern Pacific Ocean. Understanding how they have evolved to thrive in such harsh environments is a subject of great interest to marine biologists. In order to assess the degree and polarity of this evolutionary change, we have used a molecular phylogenetic approach to examine the age and history of the vestimentiferans.

Considerable debate persists concerning the taxonomic status and evolutionary origins of vestimentiferans. Jones (1985) argued that the vestimentiferan body plan was sufficiently distinct to warrant placement in a unique phylum, the Vestimentifera. On the other hand, Southward (1988), among others, challenged the erection of a separate phylum for these worms. During the course of recent taxonomic debates, various authors have also referred to these worms as the Order Vestimentifera (Class Afrenulata: Phylum Pogonophora) and the Subclass Obturata (Class Pogonophora: Phylum Annelida). Although the rationale behind such changes in taxonomic rank may be legitimate, higher taxonomic categories are manmade constructs that are not meaningful for describing the diversity or the age of a monophyletic clade of organisms. To avoid assigning rank to taxonomic names throughout this manuscript, we use the terms "vestimentiferan" and "perviate pogonophoran" (i.e., the traditional non-vestimentiferan pogonophorans).

To date, our work has focused on partial sequences from the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene (Black et al., 1997), sequences from the nuclear small ribosomal subunit (18S rRNA), and a 3' region of the large ribosomal subunit (28S rRNA). The present molecular data provide support for the inclusion of perviate pogonophorans and vestimentiferans as a clade within a paraphyletic grade of traditional annelid taxa. Furthermore, extant vestimentiferans appear to be a relatively young group that radiated during the Cenozoic.

Materials and methods

Collection localities and the methods for DNA extraction, amplification, and sequencing were either the same as, or only a slightly modified version of, those in Black et al. (1997). The oligonucleotides used to collect the 18S rRNA sequences are shown in Table 1 (Halanych et al., 1995; details can be obtained from KMH, 18e and 18h are from Hillis & Dixon 1991). The oligonucleotide 28X and 28V were used for amplification and sequencing the 28S rRNA gene are given in Hillis & Dixon (1991). We have added two perviate sequences (*Polybrachia* sp. and *Spirobrachia* sp.) to the CO1 data originally published in Black et al. (1997).

Sequence alignments employed standard computer programs with subsequent corrections based either on the amino acid sequence or secondary structure models. The

Table 1. Oligonucleotides used for amplification and sequencing of the 18S rRNA gene.

Name	Sequence	Synonymous/ Complimentary	Human position
18e	5'- CTGGTTGATCCTGCCAGT - 3'	S	3-21
18h	5'- AGGGTTCGATTCCGGAGAGGGAGC- 3'	S	418-441
18i	5'- GCTCCCTCTCCGGAATCGAACCCT- 3' (complement of 18H)	C	441-418
18N	5'- GTAATTGGAATGAGTCCA- 3'	S	552-569
18L	5'- GAATTACCGCGGCTGCTGGCACC- 3'	C	632-610
18O	5'- GGAATAATGGAATAGGACC- 3'	S	859-877
18M	5'- GAACCCAAAGACTTTGGTTTC- 3'	C	1162-1142
18Mø	5'- GAAACCAAAGTCTTTGGGTTC- 3' (complement of 18M)	S	1142-1162
18Q	5'- TGTCTGGTTAATTCCGATAAC- 3'	S	1360-1380
18Qø	5'- GTTATCGGAATTAACCAGACA- 3'	C	1360-1380
18P	5'- TAATGATCCTTCCGCAGGTTCACCT- 3'	C	1870-1845

program PAUP* 4.0d59 (Phylogenetic Analysis Using Parsimony) was used for the analyses presented herein.

Results

Fig. 1 shows a phylogenetic reconstruction based on 18S rRNA sequence data of 1608 unambiguously aligned nucleotides (see legend for details). Results from the 28S rRNA data and the CO1 data (discussed below, but not shown) are consistent with the 18S rRNA results. The present data illustrates two points: 1) vestimentiferans and perviate pogonophorans cluster together within a paraphyletic annelid grade; and 2) the vestimentiferans contain very little genetic diversity. Each of these points will be discussed in turn.

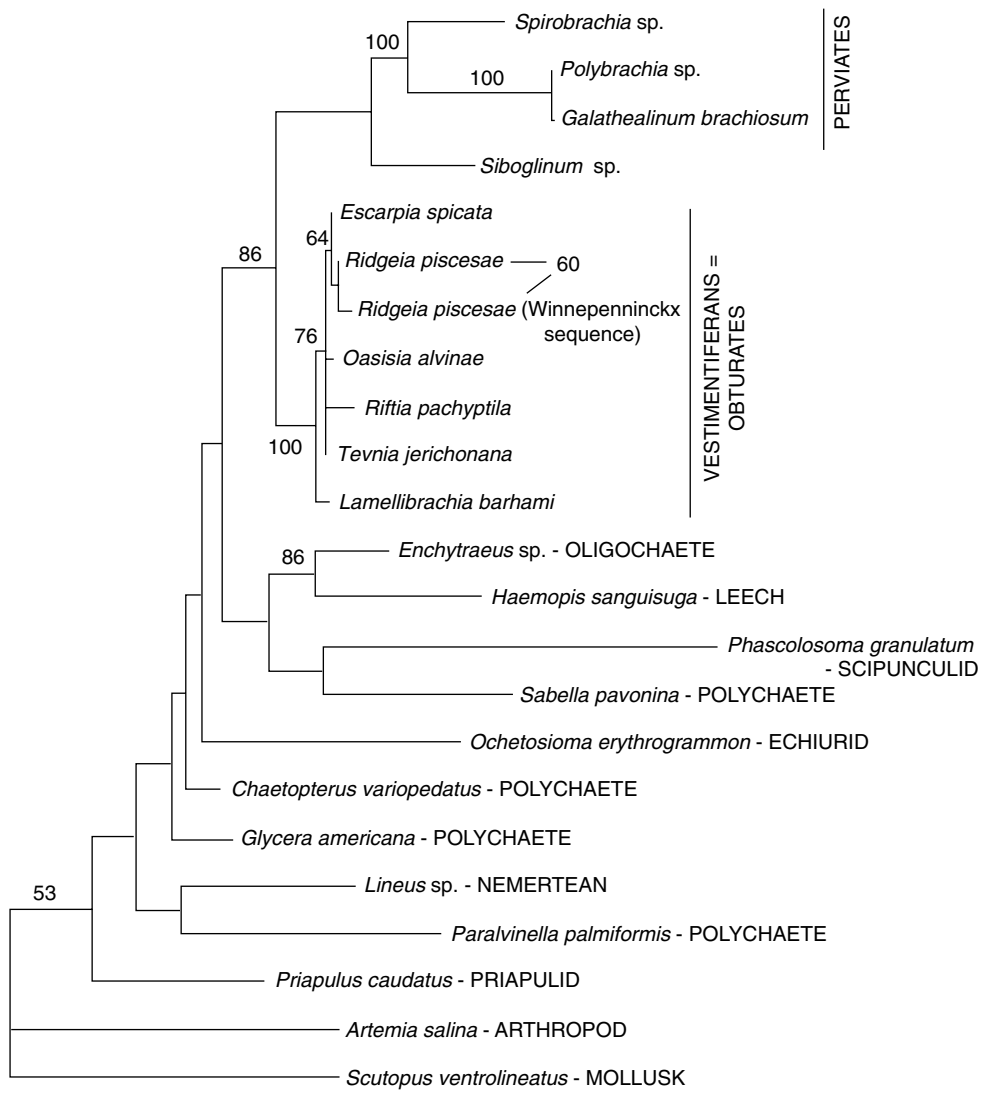

Figure 1. A phylogenetic reconstruction based on the 18S rRNA gene. The alignment consisted of 1934 nucleotide positions out of which 1608 could be unambiguously aligned. One hundred repetitions of random sequence-addition of taxa and TBR branch swapping were used for the heuristic parsimony search option in PAUP* 4.0. The tree shown is one of three most parsimonious trees produced when the empirical 1.5:1 Tv/Ti weighting was employed. Very short internal branches within the vestimentiferans accounted for the variability among the three topologies obtained. The most parsimonious trees are 772.2 steps long and contain 289 parsimony informative characters. Branch lengths are proportional to the amount of change along the branch. Heuristic bootstrap values out of 500 iterations are shown along the branch when greater than 50%. The priapulid, mollusc and arthropod sequences were used as outgroups to root the resultant topology. GeneBank accession numbers can be obtained from the authors.

Discussion

I. Vestimentiferan Origins

Although workers (e.g., Jones, 1985) have used elevated taxonomic ranks to emphasize differences in body plans between vestimentiferans and perviate pogonophorans, researchers have not disagreed on the close affinity between these two groups of worms (see Southward, 1988). The CO1 data and the 18S rRNA data (Fig. 1) both yielded high (100% and 86%, respectively) bootstrap values clustering the vestimentiferans and perviate pogonophorans as a monophyletic clade. (Bootstrap values are a commonly used measure of support for a node on a phylogenetic reconstruction.)

At present, we are not able to determine if the relationship between the two groups is one of sister taxa, or if the vestimentiferans form a clade within the "perviate" pogonophorans. This shortcoming is caused by the limited number of pogonophoran taxa available for phylogenetic analyses. However, CO1 data suggest that "Perviata" is a paraphyletic grade, that is basal to the vestimentiferans (Black et al., 1997), and supports arguments that vestimentiferans are modified pogonophorans. Throughout the remainder of the manuscript, the pogonophorans will be considered to include vestimentiferans.

For most of their known history, the placement of pogonophorans has been a contentious and unresolved issue. Nonetheless, a consensus has been recently emerging regarding the placement of this enigmatic group. Both molecular (18S rRNA from the present study, CO1 data from Black et al., 1997, and EF-1α from McHugh, 1997) and morphological (Rouse & Fauchald, 1995) cladistic analyses place the pogonophorans within the annelids. Admittedly, the present 18S rRNA data alone are not convincing on this issue, but there is a large amount of congruence among several disparate sources of data. For example, the work of C. Young and collaborators has shown that the early embryology of vestimentiferans is annelid like (including the presence of an "annelid" cross). Also, research on haemoglobins (F. Zal & collaborators) and collagens (F. Gaill & collaborators) hints at several structural similarities between vestimentiferans and traditionally recognized annelid groups.

Although annelids have traditionally been considered a well-defined group, diagnostic characters for the "Annelida" are lacking (McHugh, 1997). Recent 18S rRNA, CO1, and EF-1α sequence data suggests that other groups of protostome worms may be within the annelids. For example, McHugh (1997) argues that the annelids should be redefined to include echiurids and pogonophorans. Because the "Annelida", in the traditional sense, are not a monophyletic group, and because a rigorous phylogenetic analysis has not been done which takes into account the organismal diversity within the annelids, the placement of pogonophorans among the annelids remains unclear.

Even though the term Pogonophora is widespread and commonly used, we must agree with McHugh's assessment of the taxonomy. The original proposed name for the group, the Siboglinidae (within annelids, see McHugh (1997) for references, Rouse & Fauchald (1997)), has priority over the term "Pogonophora". The logic behind this original taxonomic term seems more consistent with the phylogenetic origins of the group than the more elevated taxonomic terms recently used.

II. Age of vestimentiferans

Within vestimentiferans, several different genes (18S rRNA, CO1, and a 3' region of the 28S rRNA) contain less genetic diversity than expected if the group radiated several hundreds of millions of years ago, as inferred from fossil evidence (Little et al., 1997). This lack of diversity, first suggested by Black et al. (1997), is depicted by the short terminal branch lengths on Fig. 1 (note: even if the 18S sites excluded here due to ambiguous alignment are included, these worms show limited diversity). However, based on this tree, one could also argue that vestimentiferans may have experienced a "slow-down" in rate of nucleotide change relative to other taxa. Although such an argument is consistent with the 18S rRNA data, relative rate comparisons for 28S rRNA and CO1 do not show a significant slow-down in vestimentiferans.

The Black et al. (1997) CO1 data show nucleotide divergence values of 0.13 to 0.20 among recognized vestimentiferan genera, and only two amino acid positions show unambiguous change. Studies on other organismal groups have shown that such divergence values (for protein coding mitochondrial genes, including CO1) are associated with taxa that are less than 50 million years old. Similarly, the 28S rRNA data show divergence values of 0.01559 to 0.00171 for intergeneric vestimentiferan comparisons, even when the rapidly evolving divergent domains (which can be easily aligned across vestimentiferans) of this ribosomal gene are included. In contrast, *Galathealinum* and *Spirobrachia* (both perviates) show 0.05065 divergence, rat and human show 0.01733 divergence, and rat and mouse show 0.02046 divergence (mice have an elevated substitutional rate compared to rats and humans). Major mammal lineages radiated in the late Paleocene to early Eocene. Thus, based on the present molecular comparisons, it would appear that extant vestimentiferans are of Cenozoic origin. This finding appears to be consistent with reports that deep ocean waters may have been anoxic in the Mesozoic.

To the contrary, Little et al. (1997) suggest that vestimentiferans existed in the Silurian, or approximately

400 MYA. However, based on the fossil evidence to date, we remain unconvinced that the ancient tubes discussed in Little et al. were produced by vestimentiferans. These fossils were ascribed to vestimentiferans because they had large flexible non-tapering tubes with conical ends and they occurred at hydrothermal vent habitats (Little et al., 1997). A diverse array of protostome worms are capable of secreting tubes of similar shape, and given the high growth rates observed in chemoautotrophic organisms at hydrothermal vents, the ability to obtain large size might not be extraordinary. Even if a separate, but now extinct, radiation from an ancestral tube worm lineage gave rise to the Silurian tubes, then large tube size has arisen at least twice. Analyses of tube ultrastructure or the discovery of fossils with preserved soft anatomy are needed to provide a convincing argument for the existence of vestimentiferans prior to the Cenozoic.

Acknowledgements

We acknowledge and thank the organizers of the First International Symposium on Deep-Sea Hydrothermal Vent Biology. Numerous people and research vessels have contributed to the collection of the organismal tissue used in this study; they are properly recognized in Black et al. (1997). Permission to use a beta version of the PAUP* program was kindly provided by D. L. Swofford, the Smithsonian Institution, Washington, D.C, USA. This work was supported by grants to RCV and RAL from the National Science Foundation (grant nos. OCE8917311, OCE9302205, OCE9633131) and the National Institutes of Health (PHS TW00735-01).

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