



Siboglinid evolution shaped by habitat preference and sulfide tolerance

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Abstract

Siboglinids are tube-dwelling annelids that inhabit marine reducing habitats such as anoxic mud bottoms, seeps and hydrothermal vents. As adults, they lack a functional digestive system and rely on chemoautotrophic microbial endosymbionts. Based on morphological analyses, Siboglinidae form a clade with the Sabelliariidae, Serpulidae and Sabellidae within the Annelida. The sister group to this clade is the Oweniidae. Three subgroups constitute the Siboglinidae: Frenulata typically inhabit anoxic sediments, *Sclerolinum* (a.k.a., Monilifera) live on decaying organic matter or reduced sediments and Vestimentifera are mostly found at hydrocarbon seeps and hydrothermal vents. Recent studies suggest that *Sclerolinum* is the sister group to the Vestimentifera. Within the Vestimentifera, the species inhabiting bare-rock hydrothermal vents represent a derived clade. The seep-inhabiting genus *Lamelli-brachia* forms a basal branch within the Vestimentifera. Trends in siboglinid evolution are most notable with regard to the level of sulfide tolerance and type of substrate. Basal groups inhabit soft substrate with only slightly elevated sulfide levels, whereas more derived species colonize hard substrate and tolerate elevated temperatures and high levels of sulfide. The type of substrate correlates with tube morphology and the function of the opisthosome. The role of the symbionts in habitat selection needs further investigation.

Introduction

The Siboglinidae, also known as Pogonophora, are tube-dwelling annelids that rely on endosymbiotic nourishment. Representatives of the groups are found throughout the world's oceans in reducing environments such as anoxic mud bottoms, hydrocarbon seeps and hydrothermal vents. The group has a colorful taxonomic history in that they have been placed in both deuterostomes and protostomes and have held all taxonomic ranks from family to phylum (illustrating a major problem with the use of ranks). Jones (1985) even suggested splitting Siboglinidae into two separate phyla. Interestingly, this unique viewpoint, which was not supported by others (e.g., van der Land & Norrevang, 1975; Southward, 1988), was picked up and

perpetuated by authors of invertebrate textbooks. Ironically it appears that an early suggestion about their phylogenetic position within the annelid radiation was correct (e.g., Uschakov, 1933). More recently, the annelid connection has been further substantiated by molecular, morphological and embryological studies (Suzuki et al., 1989, 1993; Kojima et al., 1993; Bartolomaeus, 1995; Rouse & Fauchald, 1995, 1997; Young et al., 1996; Black et al., 1997; McHugh, 1997; Boore & Brown, 2000).

Siboglinids received increased attention when researchers discovered that they were the dominate fauna of Eastern Pacific hydrothermal vent environments (reviewed in Tunnicliffe, 1988). In particular, several recent papers have examined the evolutionary history of siboglinids living at vents and seeps (i.e.,

vestimentiferans). A consensus of published phylogenetic evidence suggests interesting ecological trends in the evolution of siboglinids (Black et al., 1997; Halanych et al., 2001). The purpose of this communication is to examine the role of these trends in the evolutionary origin of hydrothermal vent and hydrocarbon seep fauna. Specifically, we further explore Halanych et al.'s (2001) hypothesized trend of increasing habitat specialization in siboglinid evolution, including adaptation to high sulfide levels.

Evolutionary history

The Siboglinidae form a clade with the polychaete taxa Sabelliidae, Serpulidae and Sabellidae (Rouse & Fauchald, 1997; Schulze, *in press*). The sister group to this clade is the Oweniidae. As adults, all siboglinids have a reduced digestive system that harbors chemoautotrophic bacterial endosymbionts in a long trunk region. The posterior end, called the opisthosoma, is short with clearly delineated segments. The manner in which these body regions are organized has been used to distinguish between the subgroups within siboglinids (e.g., Jones, 1985).

The two major subgroups are the Frenulata (a.k.a., Perviata *sensu* Jones, 1985 or Pogonophora *sensu* Ivanov, 1963) and the Vestimentifera (a.k.a., Afrenulata *sensu* Webb, 1969 or Obturata *sensu* Jones, 1985). A third, relatively little studied group is represented by the genus *Sclerolinum*, including only seven described species (previously referred to as the monogeneric Monilifera). How these three groups are related to each other has been the subject of recent studies (Halanych et al., 1998, 2001; Rouse, 2001; Schulze, *in press*). 18S and 16S rDNA data indicate that *Sclerolinum* is the sister group to vestimentiferans (Halanych et al., 2001), not to frenulates as previously hypothesized (Southward, 1999). Morphological cladistic analyses, however, are ambiguous: whereas Rouse's (2001) analysis supports the sister group relationship of *Sclerolinum* and Vestimentifera, Schulze's (*in press*) analysis finds support for *Sclerolinum* and Frenulata as sister groups.

Frenulata, the most speciose siboglinid clade (> 130 species), was divided into the Athecanephria and Thecanephria by Ivanov (1963) based on excretory structures. According to Rouse's (2001) analyses, the Thecanephria are a clade nested within the paraphyletic Athecanephria. This is confirmed by the analysis of 16S rDNA (Halanych et al., 2001), but only five

representatives of frenulates (out of a total of more than 130 species, Southward, 2000) were included and bootstrap support is relatively low. According to another morphological analysis (Schulze, *in press*) both Thecanephria and Athecanephria are monophyletic. Clearly, additional studies of frenulate phylogeny are needed.

Relationships among vestimentiferans were initially studied using molecular sequence data (e.g., Williams et al., 1993; Black et al., 1997), but morphological analyses have been completed (Rouse, 2001; Schulze, *in press*). Several studies suggest that vestimentiferans are a relatively young group that radiated within the past 100 My (Suzuki et al., 1993; Black et al., 1997; Halanych et al., 2001). Thus, relationships within the Vestimentifera have been difficult to resolve. Several molecular studies support *Lamellibrachia* as the most basal extant vestimentiferan (Williams et al., 1993; Black et al., 1997; Halanych et al., 1998, 2001), but Rouse (2001) places it in a clade with *Escarpia*.

Figure 1 depicts a tree which results from a combination of published phylogenetic analyses cited herein. In developing this tree, we have been cognizant of the fact that various markers and analyses have provided different amounts of resolution for distinct regions of the siboglinid tree. Although more data is needed to confirm or refute relationships shown in Figure 1, this tree is intended to represent our current understanding of siboglinid phylogeny. As such it provides an illustrative example of how habitat preference and siboglinid evolution may be intertwined. Available data suggests vestimentiferans are a younger clade than frenulates (Halanych et al., 1998, 2001) consistent with the hypotheses of a phylogenetic trend in sulfide requirements/tolerance.

Habitat preference

All siboglinids inhabit reducing environments, mostly in the deep sea, although the level of sulfides, methane, and type of substrate varies. Frenulata are generally found in anoxic sediments with the anterior ends of the tubes extending into the oxygenated bottom water. They have been found in hydrocarbon seeps, continental slopes, and the bottom of fjords. Vestimentifera are typically found at hydrocarbon seeps and hydrothermal vents, but they have been found in low abundance at whale falls and, in one case, a wooden shipwreck (Dando et al., 1992). Interestingly,

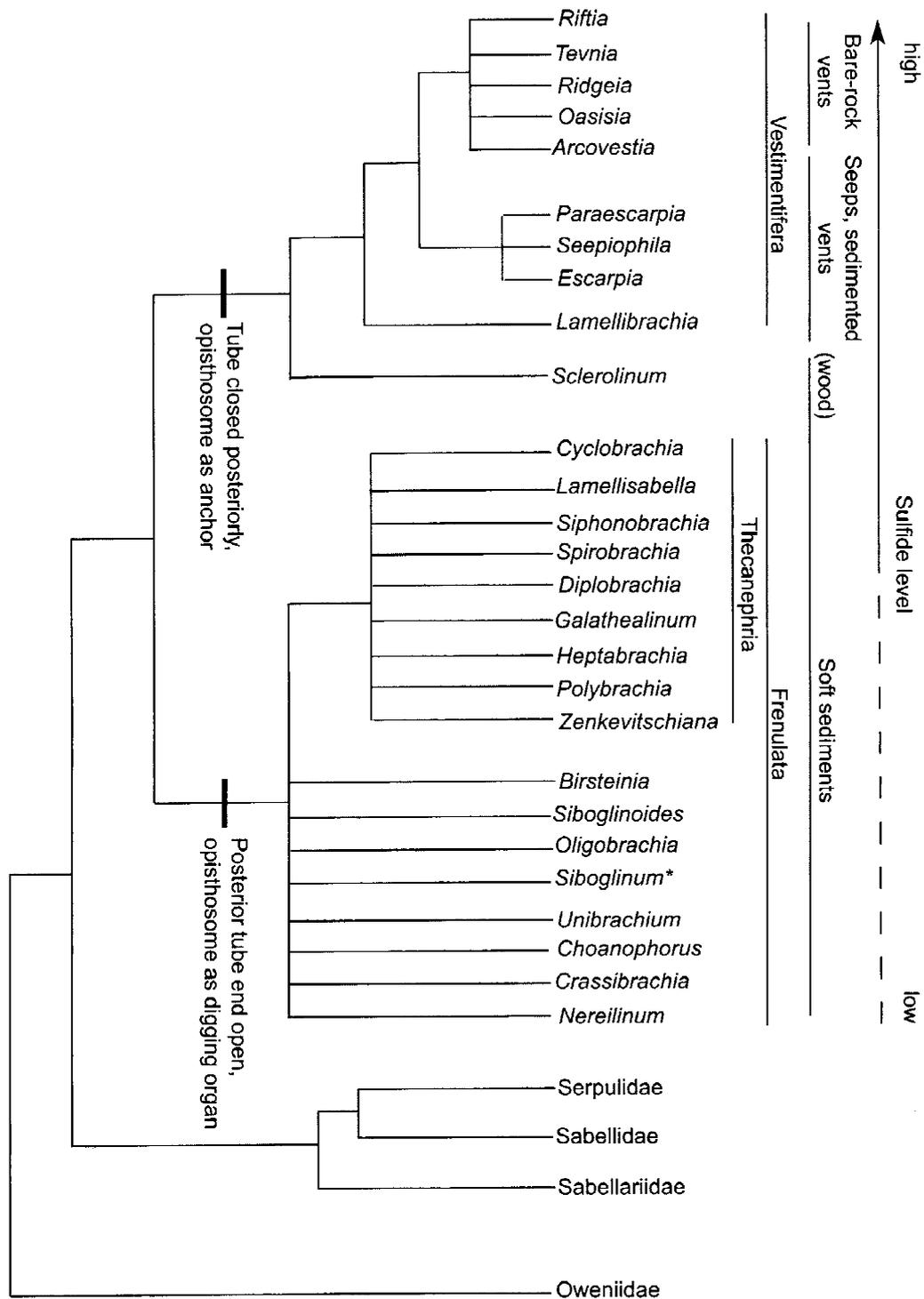


Figure 1. A summarized phylogenetic tree based on molecular and morphological sources (e.g., Suzuki et al., 1993; Williams et al., 1993; Black et al., 1997; Halanych et al., 1998, 2001; Rouse, 2001, Schulze, *in press*). Although different studies have emphasized different aspects of the tree, we have taken a conservative approach presenting resolution only for well-supported nodes. Clade names, substrate preference and the need for sulfide are indicated. The hypothesized evolution of sulfide requirements/tolerance are based in part, on the relative ages of the taxa (see text) which is not indicated on the figure. * Note that *Siboglinum* is probably a polyphyletic taxon.

the basal vestimentiferans (e.g., *Lamellibrachia*, *Escarpia*) inhabit seeps, and more derived taxa (e.g., *Riftia*, *Ridgeia*, *Tevnia*) inhabit vents (see Fig. 1). *Sclerolinum* species have been found on decaying wood and other decaying organic material, but may also occur free-living in mud (Webb, 1964; Southward, 1972, 2000) and in seeps (Sibuet & Olu, 1998). Because of biases in oceanographic studies, much more information is available for vents and seeps than other siboglinid habitats.

Sulfide tolerance

Siboglinids generally depend on sulfide in their environment due to their symbiotic relationship with chemoautotrophic bacteria. (An exception is *Siboglinum poseidoni* which hosts methanotrophic symbionts.) To most animals, even low levels of sulfide are toxic (Smith et al., 1979). Thus, a prerequisite to symbiosis with a sulfide oxidizer is sulfide tolerance.

In invertebrates, sulfide tolerance is usually correlated with tolerance to low oxygen levels, and may be enhanced by extracellular haemoglobin in the blood (Weber, 1980). Although extracellular haemoglobin is widespread in annelids, its presence in taxa closely related to siboglinids may provide clues to the evolution of sulfide-dependence in this group. Both Sabellariidae and Oweniidae share the presence of extracellular hemoglobin with siboglinids (Warren & Dales, 1980; Wells et al., 1981; Hutchings, 2000). *Phragmatopoma lapidosa* (Sabellariidae) can withstand at least short periods of burial, turbidity and increased sulfide levels (Main & Nelson, 1988). The mechanism by which they regulate sulfide toxicity is unknown.

Although the sulfide tolerance of *Owenia fusiformis* is unknown, this worm has been reported to survive periods of anoxia of up to 21 days (Warren & Dales, 1980). Wells et al. (1981) report an unusually high Bohr shift for *Owenia fusiformis* haemoglobin, indicating a strong pH gradient between gills and tissue. By contrast, both vestimentiferan and frenulate haemoglobin show only moderate to low Bohr shifts (Childress & Fisher, 1992). If tolerance of low oxygen levels and possibly elevated sulfide levels is a prerequisite to metabolic utilization of sulfide, significant additional changes in haemoglobin properties must have occurred in the siboglinid lineage in order to sustain their metabolism.

Three types of haemoglobin have been identified in the vestimentiferan *Riftia pachyptila* (Zal et al., 1996). One of them is a hexagonal bilayer haemoglobin (HBL), common in many annelid groups (Green et al., 2001). The vestimentiferan HBL is capable of binding oxygen and sulfide simultaneously and reversibly (Arp et al., 1987; Zal et al., 1996), enabling the animals to transport and store both substances in large quantities while keeping autoxidation and toxic effects at a minimum (see Powell & Somero, 1983). Frenulate blood, on the other hand, contains no HBL and sulfide binding has not been demonstrated (Terwilliger et al., 1987; Yuasa et al., 1996).

Sulfide-concentrations in siboglinid habitats vary by at least factor 10. Whereas frenulates inhabit sediments at the lower end of the spectrum, the vent-inhabiting vestimentiferan *Riftia pachyptila* was first discovered at a site with a sulfide concentration of 160 μM (Jones, 1981).

Frenulates inhabiting reducing sediments are able to absorb organic compounds through their thin tubes and their body-wall epidermis (Southward, 2000). This enables them to supplement the nutritional input by the endosymbionts. Vestimentifera, on the other hand, completely depend on their symbiotic bacteria for organic compounds (Cavanaugh et al., 1981; Felbeck, 1981; Rau, 1981). Vent-inhabiting vestimentiferans which represent a derived clade within the Vestimentifera (Black et al., 1997; Rouse, 2001, Schulze, *in press*) are generally exposed to higher sulfide concentrations than seep-inhabiting species. Sulfide levels and energetic inputs have not been measured for *Sclerolinum* species.

Sulfide uptake and tube morphology

Whereas all known frenulates inhabit soft substrate, vestimentiferans settle on hard substrate (e.g., rocks, wood, bone, other tubeworms, bivalves, etc; Southward, 2000).

The frenulate tube is open on the posterior end and the opisthosome is used as a digging organ. In *Sclerolinum* and vestimentiferans, the opisthosome is enclosed inside the tube and serves as an anchor rather than a digging organ (Southward, 1988, 1993). The differences in functions of the opisthosome and tube morphology may be adaptive to different habitats. Frenulates live with a significant portion of their tube buried in the sediment. Because dissolved organic matter can be transported across their body wall

(Southward & Southward, 1981), it is conceivable that sulfide may be transported across the opisthosome or the thin tube to the trunk region. Interestingly, in the basal vestimentiferans the opisthosome may serve both an anchoring and respiratory function. This appears to be the case in both *Lamellibrachia* and *Escarpia*, and may be true of *Paraescarpia* and *Seepiophila* as well. Vestimentiferans inhabiting seeps and sedimented vents may have approximately half of their tube length (with a closed posterior) buried in the sediment. They obtain sulfide across the thin walls of their buried tubes and have access to a relatively stable source of energy (Scott & Fisher, 1995). The water above the sediment into which the plumes extend, often has no significant sulfide levels and the plumes are the sites of oxygen uptake.

Vestimentiferans in bare-rock hydrothermal vent environments experience high sulfide levels and elevated temperatures. They extend their plumes into the mixing zone of vent fluids and ambient seawater. In this zone, temperature and fluid composition fluctuate within the scale of seconds to hours, so that the vestimentiferan tubes are alternately exposed to high oxygen and high sulfide (Fisher, 1996). Both compounds are readily absorbed through the branchial filaments. For vent tubeworms, the opisthosome appears to aid mainly in attachment and may have a more limited function in sulfide uptake.

Symbionts as habitat-determining factors in vestimentiferans?

The endosymbionts of vestimentiferans can be grouped into two or possibly three clades within which DNA sequences are nearly identical (Feldman et al., 1997). One endosymbiont clade is found in species inhabiting basaltic vents in the East Pacific whereas a second one is associated with hosts that colonize sedimented vent or seep sites. A possible third type was found in *Escarpia spicata* from a whale skeleton. Symbionts are of nearly identical genetic makeup over vast geographic distances.

The results by Feldman et al. (1997) leave room for two contrasting hypotheses. In a first scenario, endosymbiont evolution is completely uncoupled from host evolution and the hosts can live with whichever type of symbiont they encounter at their settlement site. The ability to colonize a certain habitat is regulated by environmental factors other than the presence of a species-specific symbiont. This hypothesis

would be supported if two or more phylogenetically distant vestimentiferan species inhabiting the same area shared the same type of symbiont. Examples for such sites are sedimented vent areas such as Middle Valley on the Juan de Fuca Ridge (inhabited by *Lamellibrachia barhami* and *Ridgeia piscesae*), the Lau Basin in the Eastern Pacific (inhabited by *Alaysia spiralis* and *Lamellibrachia columna*) and the Guaymas Basin vents (inhabited by *Riftia pachyptila* and *Escarpia spicata*). However, Feldman et al. (1997) only sequenced one species from each of these sites.

In a second scenario, species differ in their preference for symbiont types. The ability to host a specific symbiont (among other factors) enables a species to colonize either vent or seep habitats. Sedimented vent sites are an intermediate between cold seeps and basaltic vents; both types of symbionts may occur in these areas and allow colonization by different host species. This hypothesis is supported by the fact that *Riftia pachyptila* from the sedimented vents in Guaymas Basin hosts the same symbionts as its conspecifics from basaltic vents of the East Pacific Rise, whereas *Lamellibrachia columna* at the sedimented Lau Basin vents hosts the same symbionts as other *Lamellibrachia* and *Escarpia* species from cold seeps.

Distinguishing between these hypotheses will require additional studies of host-symbiont relationships. In particular, studies will need to be undertaken that explore multiple individuals from multiple species from multiple localities. Because observing uptake of the symbionts by larval tubeworms in a natural setting is difficult, determining the role of endosymbiont uptake during larval settlement has not been possible. This knowledge will help elucidate (1) the degree to which the presence of endosymbionts or other environmental factors, influence larval recruitment, and (2) the specificity of host-endosymbiont relationships on an ecological timescale.

Conclusions

Siboglinidae, Sabellariidae and Oweniidae share the presence of extracellular hemoglobin, but physiological properties of the hemoglobin molecule differ. This common feature may represent a propensity to tolerate elevated levels of sulfide. Interestingly, vestimentiferans are able to utilize sulfide more efficiently than frenulates. Their unique hemoglobin with its ability to bind oxygen and sulfide simultaneously is highly adapted to environmental conditions and nutritional

requirements of endosymbionts. Taking advantage of high sulfide concentrations, vent-inhabiting vestimentiferans achieve unusually high growth rates. The importance of sulfide in the biology of the organism may be coupled with phylogeny (Fig. 1). Siboglinid evolution probably originated in soft substrate that promoted organic and sulfide uptake through the posterior of the organism. Over the course of evolutionary time, more derived species inhabited hard substrate and altered their physiology to more efficiently sequester sulfide.

Such a trend in organismal evolution may not be exclusive to siboglinids. Relationships between vent and seep fauna have been discussed in the literature ever since biological communities have been discovered in these areas (Newman, 1985; Tunnicliffe et al., 1998; Van Dover, 2000). Newman (1985) suggested seeps as sources for new immigrants into the hydrothermal habitat. Apart from vestimentiferans, this trend has also been detected in mytilids (Craddock et al., 1995) and vesicomyids (Peek et al., 1997). Other taxa, such as bresiliid shrimp, also show a close relationship between vent and seep species (Shank et al., 1999).

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