Molecular phylogeny of siboglinid annelids (a.k.a. pogonophorans): a review

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Abstract

Siboglinid, or pogonophoran, annelids are tubicolous worms that rely on chemoautotrophic endosymbionts for nutrition. Three clades within the siboglinids are recognized: Frenulata, Vestimentifera, and Monilifera. As a group, these worms have received considerable attention from molecular phylogenetists. Most studies have focused either on the evolutionary origins of the group or on the relationships within vestimentiferans, which live at hydrocarbon seeps and hydrothermal vents. Here I review the literature to date on siboglinid molecular phylogeny and summarize the clade's evolution. The vestimentiferans have been well studied, especially in the eastern Pacific. The seep taxon *Lamellibrachia* is basal in the clade with vent species being more derived. Recent studies of seeps are finding new species and suggest that habitat depth can be correlated with species boundaries. In contrast to the vestimentiferans, frenulate evolution has been poorly studied. Despite their greater apparent diversity, frenulate specimens have not been sampled so extensively, and thus little is known about their evolution. *Sclerolinum*, also referred to as Monilifera, is a recognized genus of siboglinids that forms the sister group to Vestimentifera. Like the frenulates, little is known about the history of this group. Our present understanding of siboglinid phylogeny has, in large part, been dictated by insufficient sampling effort.

Introduction

The reliance on chemoautotrophic endosymbionts in siboglinid (a.k.a., pogonophoran) tubeworms has prompted considerable interest in their evolutionary history. Typically the group has been split into two major lineages, frenulates, and vestimentiferans. A third lesser-known group, *Sclerolinum*, also referred to as moniliferans, is important from the evolutionary perspective. Vestimentiferans are the best-known members of the clade because of their association with, and dominance at, hydrothermal vents and cool seeps. In particular, the high levels of productivity due to the endosymbionts, has drawn considerable attention from a wide range of biologists. Vestimentiferans tend to have considerably larger bodies than frenulates, which are found in numerous different types of sedimented habitats including fiords, continental

slopes, and deep sea trenches. Whereas frenulates comprise 19 nominal genera with ~136 nominal species, vestimentiferans contain about 10 genera with 15 nominal species. Thus, many vestimentiferan genera are monospecific. The seven nominal species of *Sclerolinum* live either in reducing sediments or on rotting organic material.

The focus of this manuscript is to review and summarize the molecular data relevant to the evolutionary history of siboglinids. Where do siboglinids fit into animal phylogeny? What are the clades within the siboglinids and how are they related? What are the relationships within those clades? There are several related topics that will not be discussed because they are covered elsewhere. In the case of morphology, considerable information bears on the evolution of the group.

This topic has been addressed in this volume by Southward et al. (2005) who discuss similarities and differences between the groups. Readers interested in molecular evolutionary analyses of endosymbionts are referred to Feldman et al. (1997), Di Meo et al. (2000), Nelson & Fisher, (2000), and McMullin et al. (2003) for vestimentiferans. No published analyses of frenulate endosymbionts exist, but data from *Siboglinum fiordicum* endosymbionts places these organisms in the γ-proteobacteria (Halanych et al., unpublished). Also nomenclature and higher-level taxonomy have been hotly debated and confusingly inconsistent. Recent papers cover this topic in detail (e.g., Rouse & Fauchald, 1995; Halanych et al., 2002; Southward et al., 2005) and it will not be reiterated here. The definition of the three siboglinid clades (Vestimentifera, Frenulata, and Monilifera) follows Halanych et al. (2001). As a prelude to the discussion below, Table 1 summarizes the relevant molecular studies dealing with siboglinids.

Are siboglinids annelids?

Yes! Abundant morphological (e.g., Rouse & Fauchald, 1995, 1997), embryological (e.g., Young et al., 1996; Southward, 1999) and molecular data (Kojima et al., 1993; McHugh, 1997; Black et al., 1997; Kojima, 1998; Halanych et al., 1998, 2001) place siboglinids within the annelid radiation. Much of the confusion on the status of this group is due to proposals that Pogonophora (a.k.a., Frenulata, Perviata) and Vestimentifera both be elevated to the rank of phylum. I have chosen to use the term siboglinid rather than pogonophoran to emphasize its placement within the annelid radiation. However others (e.g., Southward et al., 2005) retain the term 'Pogonophora' because of its familiarity.

Field et al. (1988) was the first molecular phylogenetic study addressing possible placement of the siboglinids within animal phylogeny. However, 18S nuclear ribosomal gene data in that study, and subsequent work by Winnepenninckx et al. (1995), failed to provide convincing support for the placement of siboglinids. Whereas Field et al. found a vestimentiferan (*Riftia pachyptila*) and a brachiopod between two annelids, Winnepenninckx et al. found siboglinids to be close to echiurans. More recent 18S studies (e.g., Halanych et al., 1998, 2001) have also suggested an annelid affinity for siboglinids, but again support for internal nodes is weak.

Molecular support showing an annelid affinity for siboglinids came from Elongation Factor-1α data by Kojima et al. in 1993. Subsequent EF-1α papers (McHugh, 1997; Kojima, 1998) included more diverse taxon sampling and provided evidence that siboglinids are within the annelid radiation. Interestingly, McHugh also found echiurans to be within annelids, suggesting a new interpretation of the Winnepenninckx et al.'s (1995) finding. Amino acid coding sequence from the Cytochrome Oxidase c subunit I gene is also consistent with the annelid placement of siboglinids (Black et al., 1997). Similarly, hemoglobin genes in siboglinids look very similar to other annelids (Suzuki et al., 1989, 1993; Zal et al., 1997; Bailly et al., 2002, 2003) and place siboglinids within annelids (Negrisolo et al., 2001). Hemoglobin studies have received less attention for phylogenetic inference due in part to taxon sample sizes, but mainly because the papers focus on the evolution of hemoglobin proteins and not the organisms. Bailly et al. (2003) did use globin sequences to build a neighbor joining tree. However, their results and conclusions must be critically examined as they did not have the taxon sampling to justify their conclusion concerning the evolution of sulfide tolerance and their tree is contra all other published phylogenetic analyses. Schulze and Halanych (2003) come to the opposite conclusion the same topic.

Two other sources of molecular data also bear on the issue. Available mitochondrial gene rearrangement data (frenulate – Boore & Brown, 2000, vestimentiferan – Jennings & Halanych, unpublished) is identical between siboglinids and clitellate annelids. Combined 28S and 18S data finds weak support for a monophyletic Annelida, including echiurids and siboglinids (Passamaneck & Halanych, submitted). In contrast to these six sources of data, molecular phylogenetic analyses reporting siboglinids to be outside the annelid radiation are lacking (except Winnepenninckx et al., 1995).

Where siboglinids fit within the annelids is not clear. Uschakov (1933) described *Lamellisabella* as a type of sabellid polychaete. Although an evolutionary link with the Sabellida is suggested

Table 1. Molecular studies of siboglinid (a.k.a. pogonophoran) evolution

References	Clade of interest					Molecular marker									
	Siboglinid origins	Within Siboglinids	Within Frenulates	Within Vestimentiferans	Intra-specific	EF-1α	Haemoglobin	Mitochondrial genome	Nuclear 18S rDNA	Nuclear 28S rDNA	Mitochondrial 16S rDNA	COI	AFLPs	RFLPs (ITS region)	Allozymes
Bailly et al. (2002)	X						X								
Boore & Brown (2000)	X							X							
Field et al. (1988)	X								X						
Jennings & Halanych (unpub.)	X							X							
Kojima (1998)	X					X									
Kojima et al. (1993)	X					X									
McHugh (1997)	X					X									
Negrisolo et al. (2001)	X						X								
Passamaneck & Halanych (submitted for publication)	X								X	X					
Suzuki et al. (1988)	X						X								
Suzuki et al. (1989)	X						X								
Suzuki et al. (1993)	X						X								
Winnepenninckx et al. (1995)	X								X						
Zal et al. (1997)	X						X								
Halanych et al. (1998)		X	X												
Halanych et al. (2001)		X	X						X		X				
Bailly et al. (2003)		X					X								
Black et al. (1997)	(X)			X								X			
McMullin et al. (2003)				X								X			
Kojima et al. (2000)				X								X			
Kojima et al. (2001)				X	X							X			
Kojima et al. (2002)				X	X							X			
Kojima (2003)				X								X			
Williams et al. (1993)				X						X					
Black et al. (1994)					X										X
Black et al. (1998)					X							X			X
Bucklin (1988)					X										X
Carney et al. (2002)					X										X
Shank & Halanych (submitted for publication)					X								X		
Southward et al. (1996)					X									X	X
Southward et al. (1995)					X									X	X

by morphological cladistic analysis (Rouse & Fauchald, 1997), recent Elongation Factor-1α data does not support this view (McHugh, pers. com.).

What are the major clades within Siboglinidae?

Webb (1969) was the first to formally recognize the evolutionary kinship between vestimentiferans and frenulates. Since then, several authors have acknowledged the close evolutionary relationship between the groups (e.g., Webb, 1969; Jones, 1981; Southward, 1988; Ivanov, 1994). As for molecular papers, at least one frenulate and one vestimentiferan were included with enough other taxa to demonstrate monophyly in studies on hemoglobin (Zal et al., 1997; Negrisolo et al., 2001; Bailly et al., 2002), CO1 (Black et al., 1997) and 18S (Halanych et al., 1998, 2001).

Major relationships within siboglinids are poorly understood because frenulate sampling has been insufficient. Specifically, because many frenulates occur in deep water, they are difficult to collect and are typically taken as bycatch whose ultimate fate is to end up in a jar of formalin limiting its utility for molecular studies. To my knowledge, Halanych et al. (1998, 2001) are the only molecular analyses to include more than two non-vestimentiferan siboglinids. Both 16S mitochondrial ribosomal data and 18S nuclear ribosomal data suggest: (1) vestimentiferans are monophyletic, (2) *Sclerolinum* (a.k.a., Monilifera *sensu* Ivanov, 1991) is sister to the vestimentiferan clade, and (3) frenulates form a monophyletic clade sister to the vestimentiferan/moniliferan clade.

Points 1 and 2 are also corroborated by CO1 data (Black et al., 1997). However, a caveat must be added to point 3. These molecular analyses (Halanych et al., 1998, 2001) include only five frenulate species representing a group with ∼136 nominal species. In contrast, vestimentiferans have been well represented (see below). From the molecular perspective, examination of more taxa is required to determine if frenulates are monophyletic or form a paraphyletic grade at the base of the Siboglinidae.

Morphological cladistic analysis (Rouse, 2001) is consistent with the molecular data on all three points. Synapomorphies (Rouse, 2001; Schulze,

2003) for the clade Vestimentifera include the presence of an obturaculum, vestimentum, and multicellular pinnules. The vestimentiferan/moniliferan clade is supported by the presence of posterior chaetae in rows on the opisthosomal segments, and a tube with the posterior end closed. The frenulate clade is supported by the presence of sparse posterior peg-like chaetae, spermatophores, and a tube with the posterior end open.

What are the relationships within the major clades?

Frenulata
As mentioned above, a maximum of five frenulate species have been included in molecular phylogenetic studies of the group. Nonetheless two interesting results have been observed in both the 16S and 18S data (Halanych et al., 2001). *Siboglinum* (*S. ekmani* and *S. fiordicum*) appears not to be monophyletic, consistent with recent morphological observations (Southward, pers. com.). Additionally, *Polybrachia* sp. and *Galathealinum brachiosum* look very similar to each other (genetic distance 0.01 for 18S and 0.02 for 16S data) calling into question the validity of separate generic status. At present the only working hypotheses of frenulate evolutionary history are based on morphology (e.g., Ivanov, 1963; Webb, 1964; Rouse, 2001). Within siboglinids, we clearly have the most to learn about frenulates.

Despite this lack of molecular knowledge about frenulates, we can look to two sources for an understanding of their evolutionary history. Given that taxonomists have grouped siboglinids in to 'genera' and 'families' based on morphological similarity, we can use the existing taxonomic framework as a working hypothesis of frenulate evolutionary history (Fig. 1); however, this framework is untested. The one morphological cladistic study that includes a diversity of frenulates is Rouse (2001). This study finds the Oligobrachiidae and Athecanephria to be paraphyletic grades. Also *Choanophorus indicus*, is placed separately from the rest of the Polybrachiidae. *Cyclobrachia auriculata* seems closer to Lamellisabellidae than Polybrachiidae (also confirmed by Southward, pers. com.). More data from both morphological and molecular sources need to be gathered to further evaluate the history of frenulates.

Monilifera

This group is represented by *Sclerolinum* which has 7 nominal species. To date, only two individuals have been sequenced. *Sclerolinum brattstromi* for 16S and 18S (Halanych et al., 2001) and an unidentified *Sclerolinum* species from the Loihi seamount for CO1 (Black et al., 1998). Because there has never been a phylogenetic analysis of the group, little can be said about their evolutionary history.

Vestimentifera

In contrast to other siboglinids, vestimentiferans have been well studied with several molecular papers focusing on within clade relationships (Table 1). Prior to the first molecular studies, Jones (1988) had provided a taxonomic framework for the group (Fig. 2) which, to some degree, implied a hypothesized phylogeny. The first molecular work to test this framework used 28S nuclear ribosomal gene data and placed *Lamellibrachia*, not *Riftia*, basal in Vestimentifera (Williams et al., 1993). This conclusion was later corroborated with mitochondrial Cytochrome Oxidase c subunit I (CO1) gene data (Black et al., 1997) and 18S rDNA data

(Halanych et al., 1998, 2001). (Note the mitochondrial 16S rDNA data places *Riftia* basal but this is not well supported; Halanych et al., 2001; also see McMullin et al., 2003). Thus, neither the Basibranchia nor the Lamellibrachiidae as proposed by Jones appear to be real entities, a result supported by morphological cladistic analyses (Rouse, 2001; Schulze, 2003).

Additionally, all molecular studies mentioned above revealed surprisingly limited diversity across vestimentiferan lineages; certainly far less than expected for a group that was purported to be a distinct phylum (see Halanych et al., 1998). The possibilities to explain this observation is that the clade is young or that there has been a slowdown in the rate of genetic change in the lineage. The available rDNA data show that vestimentiferan genes evolved more slowly than frenulate copies (Halanych et al., 2001), but even taking the slowdown into account, diversity is limited, suggesting the clade is young.

Determining if hydrothermal vent endemic species form a monophyletic clade has also been of interest to biologists. Of the six known vent-en-
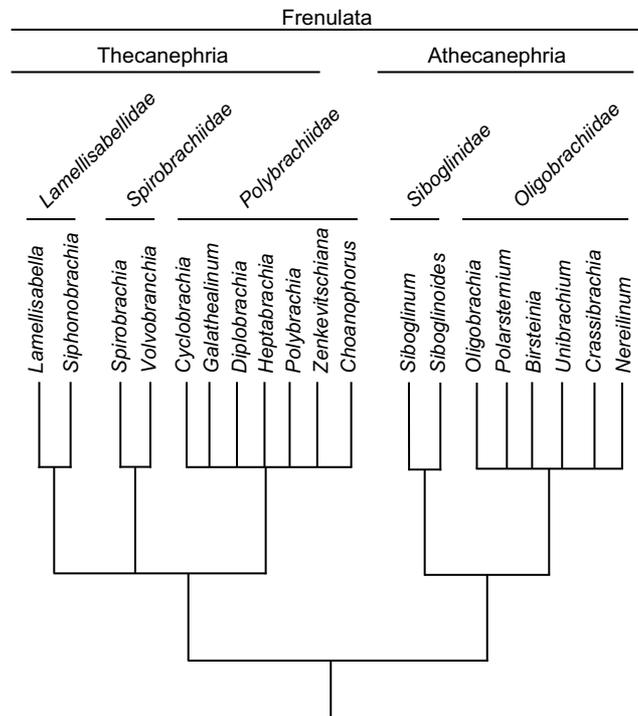

Figure 1. Phylogenetic relationships suggested by the current taxonomy for Frenulata.

demic species, four occur in the Eastern Pacific, a region that is well understood geologically (Tunnicliffe, 1988). The other species, *Arcovestia ivanovi* and *Alaysia spiralis*, occur in the western Pacific. Whereas CO1 data provides the most resolution within the vestimentiferan clade and provides marginal support for a monophyletic vent clade (Black et al., 1998, but *A. ivanovi* and *A. spiralis* were not included), other molecular data (28S – Williams et al., 1993, and 16S – Halanych et al., 2001) did not recover a vent clade. In these latter cases, support for internal branches was weak. CO1 is one of the slowest evolving genes in the mitochondrion, and perhaps a gene with a higher rate of nucleotide substitution will yield more phylogenetic signal for vestimentiferan relationships.

Even so, available data tell us something about vestimentiferan evolution in the eastern Pacific. Approximately 37 million years ago, the Juan de Fuca, Explorer and Gorda ridges (where *Ridgeia piscesae* occurs) were contiguous with the East Pacific Rise (EPR; where *Oasisia alvinae, Tevnia jerichonana*, and *Riftia pachyptila* occur). A clade consisting of all three EPR species is not found with any molecular data sets suggesting that either two separate vestimentiferan lineages have colonized EPR vents or at least two distinct lineages existed on one ridge system before it was bisected by Baja California. Given the genetic diversity of the group, the former hypothesis seems more tenable.

Despite the attention paid to vent-endemic vestimentiferans, the seep dwelling species appear to have greater phylogenetic diversity. The systematic work on seep vestimentiferans has focused on the western Pacific led by the efforts of Kojima and co-workers (Kojima et al., 1997, 2000, 2001, 2002, 2003). They have used CO1 data to explore the diversity and relationships of *Escarpia, Lamellibrachia* and *Arcovestia* populations. Although Kojima and workers were interested in the relationships between species, much of this work focuses on delineating species and population boundaries through a phylogeographic approach.

A couple of key generalizations can be drawn from this body of work. First, within a region species appear to be stratified according to depth rather than geographic proximity. In the case of *Lamellibrachia*, species 'L1' (characterized mtDNA haplotypes) was only found between 300 and 1450 m and 'L2' was restricted to below 2000 m even though they both occurred in the same geographic locality (e.g., Nankai Trough; Kojima et al., 2001). A similar story was found in *Escarpia* with one species found at 300 m and the other between 1100 and 1650 m (Kojima et al., 2002). This type of biogeographic pattern has been reported in other deep-sea animals (e.g., Etter & Rex, 1990; Etter et al., 1999). A second important finding by Kojima and co-workers was limited genetic diversity within species. Population bottlenecks are perhaps the most common explanation for limited intraspecific diversity. However, using a conservative genetic marker can produce the same effect. Similar to the situation in *Riftia pachyptila* (see below), more rapidly evolving markers may provide better insight on the patterns
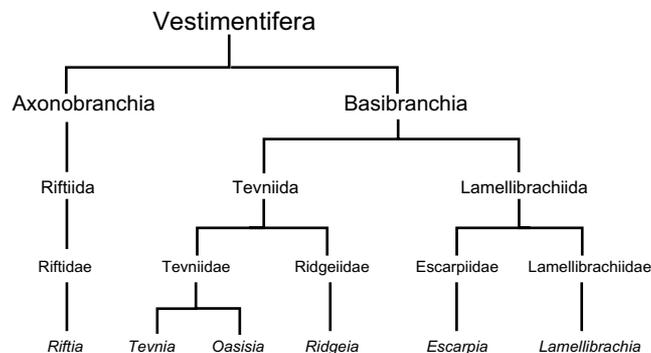

Figure 2. Taxonomic scheme for the Vestimentifera from Jones (1988). In the original diagram Jones provided a rank designation for each taxon name starting with 'phylum' for Vestimentifera. Because vestimentiferans are within Annelida, and because of the problems associated with ranks, Jones' hierarchical ranking were not included.

of genetic diversity in seep dwelling vestimentiferans.

Are populations within species genetically structured?

Although population genetic issues have never been dealt with in frenulates or moniliferans, several vestimentiferans have been studied. *Riftia pachyptila* has been the best studied with perhaps the most colorful history. Bucklin et al. (1988) conducted the first intraspecific study of *R. pachyptila*. They examined two eastern Pacific populations using 13 allozyme loci and concluded that levels of genetic diversity were low, but that there was a significant difference between the populations. In contrast, Black et al. (1994) examined six populations of *R. pachyptila* and found that levels of diversity and gene flow were high, concluding that localities were not genetically isolated. Interestingly, they also report a slight 'isolation by distance' trend. Note that these studies only had three loci in common.

Although allozymes have been useful as an inexpensive way to screen many individuals, numerous studies (e.g., Johnson et al., 1977; Sites & Davis, 1989; Reeb & Avise, 1990; Steinger et al., 1996) have repeatedly demonstrated that allozyme data often substantially underestimate genetic variation. This problem is particularly relevant when only a few polymorphic loci are used. These problems motivated Shank & Halanych (submitted) to revisit *R. pachyptila* genetics using amplified fragment length polymorphism (AFLP) analysis sampling hundreds of loci across the nuclear genome. They found genetic structure and some (but not total) genetic isolation between recognized *R. pachyptila* populations. Multidisciplinary research (Marsh et al., 2001) examined larval energetics and possible retention mechanisms that would account for along-axis retention of *R. pachyptila* larvae. Their model is compatable with the genetic observations of Shank & Halanych but more data is needed.

Black et al. (1998) examined *Oasisia alvinae, Tevnia jerichonana*, and *Ridgeia piscesae* in the eastern Pacific. The sampling of *O. alvinae* and *T. jerichonana* was limited and allozymes were mainly used to identify small juveniles. For both of these species, Black et al. report estimated rates of gene flow that were sufficient to prevent isolation between populations, but the variation observed was suggestive of a stepping-stone model of dispersal. *R. piscesae* has a very plastic morphology, leading earlier workers to suspect it was several closely related species (Jones, 1985), but subsequent allozyme, CO1 sequence data, and AFLP data revealed these different morphologies belonged to a common gene pool (Southward et al., 1995; Black et al., 1998; Carney et al., 2002). This work was further expanded (Southward et al., 1996) with additional allozyme data and restriction fragment length polymorphism (RFLP) data from the internal transcribed spacer (ITS) region of the nuclear ribosomal repeat. These data showed that *R. piscesae* experienced some disruption to gene flow between the Juan de Fuca and Gorda Ridges, but within a ridge segment, larvae were able to disperse considerable distances. Southward et al. (1996) also used these molecular tools to confirm the presence of *Lamellibrachia barhami* at sedimented vent localities.

What is our current view of siboglinid evolution?

Figure 3 shows our current understanding of siboglinid evolution based on several sources. This tree was not reconstructed based on any explicit reconstruction program, but is a summary of the relevant available data on the siboglinid phylogeny as understood by the author. Thus, this 'meta-tree', merely designed stimulate future research by highlighting unresolved nodes, should not be used as a definitive phylogenetic framework for other studies. The vestimentiferan/moniliferan clade is based on molecular works listed in Table 1. In contrast, the frenulate clade is based on a composite of morphology (Rouse, 2001) and taxonomic nomenclature. *Polybrachia* and *Galathealinum* are placed together based on molecular data (Halanych et al., 2001). Furthermore, some taxa (*Alaysia, Arcovestia, Polarsternium,* and *Volvobrachia*) have not been included in phylogenetic analyses that allow their position to be determined. The placement of these taxa in this diagram merely represents a conservative 'best guess' by the author. Nonetheless, this diagram is intended to be a useful tool representing our current knowledge of siboglinid phylogeny.

What needs to be done?

Siboglinids, or pogonophorans, are highly derived annelids whose evolution has been shaped by the environment they live in and their dependence on endosymbiotic bacteria (Schulze & Halanych, 2003). As expected, our understanding of the group has been shaped by sample availability. Because hydrothermal vents have received much attention, we know the most about the vent-endemic vestimentiferans. However, if we really want to understand the evolution of the clade and the origins of endosymbiosis in these worms, we need to examine frenulate and moniliferan evolution. At present delineating the frenulate clades and determining relationships between them is the biggest gap in our knowledge of siboglinid evolution. The limiting factor is access to specimens that can be used in molecular phylogenetic analysis. Because many siboglinids live in environments that are not easily accessible, understanding the larger picture of siboglinid evolution will require a concerted sampling effort from multiple researchers.

Note added in proof

A novel type of siboglinid called *Osedax* was reported while this work was in press (Rouse et al. 2004). *Osedax* is a unique lineage basal to the Vestimentifera/Monilifera clade that makes a living by using heterotrophic bacterial symbionts to live on whale bone.
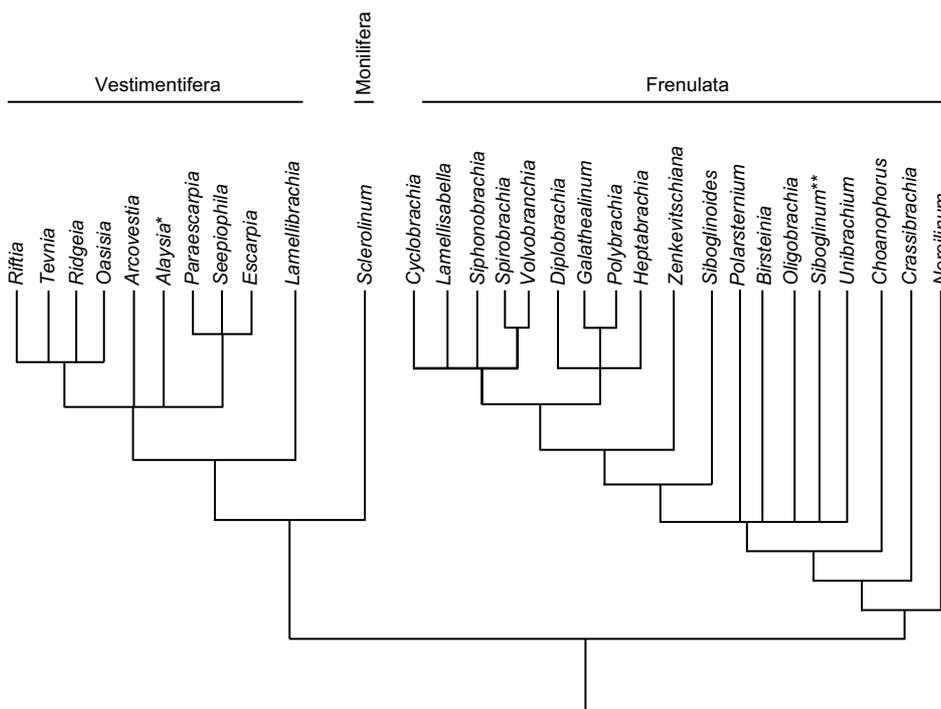

Figure 3. Current understanding of siboglinid relationships. This diagram was not produced with an explicit reconstruction algorithm, but summarizes results from several different sources. The Vestimentifera topology is a composite of the works of Kojima, Halanych, and their collaborators (see Table 1). The polytomies are shown to be conservative as many of the internal branches within the vestimentiferans are short and variously resolved in different studies (e.g., the *Riftia/Tevnia/Ridgeia/Oasisia* clade). The placement of *Sclerolinum* is based on Halanych et al. (2001). The Frenulata topology is based on the morphological work of Rouse (2001). The grouping of *Galathealinum* and *Polybrachia* and the unsolved position of *Siboglinum* relative to *Unibrachium* (see Rouse, 2001) is based on molecular data (Halanych et al., 2001). Data on the placement of some taxa (*Alaysia, Arcovestia, Polarsternium,* and *Volvobrachia*) are lacking, and their placement here are merely represents a conservative 'best guess' by the author based on their descriptions. *Currently, the validity of *Alaysia* is under debate (Southward, pers. com.) and it inclusion here is for consistency with the published literature. **Siboglinum* is probably polyphyletic.

Acknowledgements

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