

Unsegmented Annelids? Possible Origins of Four Lophotrochozoan Worm Taxa¹

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SYNOPSIS. In traditional classification schemes, the Annelida consists of the Polychaeta and the Clitellata (the latter including the Oligochaeta and Hirudinida). However, recent analyses suggest that annelids are much more diverse than traditionally believed, and that polychaetes are paraphyletic. Specifically, some lesser-known taxa (previously regarded as separate phyla) appear to fall within the annelid radiation. Abundant molecular, developmental, and morphological data show that the Siboglinidae, which includes the formerly recognized Pogonophora and Vestimentifera, are derived annelids; recent data from the Elongation Factor-1 α (EF-1 α) gene also suggest that echiurids are of annelid ancestry. Further, the phylogenetic origins of two other lesser-known groups of marine worms, the Myzostomida and Sipuncula, have recently been called into question. Whereas some authors advocate annelid affinities, others argue that these taxa do not fall within the annelid radiation. With advances in our understanding of annelid phylogeny, our perceptions of body plan evolution within the Metazoa are changing. The evolution of segmentation probably is more plastic than traditionally believed. However, as our understanding of organismal evolution is being revised, we are also forced to reconsider the specific characters being examined. Should segmentation be considered a developmental process or an ontological endpoint?

The Annelida is a pivotal taxon for understanding metazoan evolution, as our interpretation of bilaterian phylogeny, development, and macroevolutionary trends are influenced by current concepts of annelid ancestry and evolution. For example, the Articulata hypothesis posits that arthropods and annelids are more closely related to each other than to other major protostome taxa (e.g., molluscs, flatworms, brachiopods). This concept is based upon the assumption that serial segmentation has arisen only once in protostome evolution. However, a growing body of morphological (e.g., Eernisse *et al.*, 1992; Peterson and Eernisse, 2001) and molecular (Halanych *et al.*, 1995; Eernisse, 1997; Aguinaldo *et al.*, 1997; Zrzavy *et al.*, 1998; de Rosa *et al.*, 1999) data are inconsistent with the Articulata hypothesis, placing annelids in the Lophotrochozoa closer to molluscs than arthropods. The major implication is that serial segmentation is more evolutionarily plastic than traditionally believed. This idea is further supported by evolutionary studies of developmental mechanisms (e.g., Seaver and Shankland, 2000; Iwasa *et al.*, 2000). Despite this paradigm shift, the phylogenetic importance and evolutionary plasticity of segmentation still has not been examined in a phylogenetically rigorous manner with a sufficient number of taxa to permit a clear understanding of its role in protostome evolution.

Moreover, synapomorphies that unite Annelida, as traditionally formulated to include the Clitellata and Polychaeta, are lacking, calling into question the con-

sensus among biologists that the Annelida is a monophyletic taxon (Rouse and Fauchald, 1995, 1997; McHugh, 1997). Retention of a traditional “Annelida” concept has resulted, in large part, from the continued reliance on serial segmentation as a useful phylogenetic character among major animal lineages. Advances in annelid systematics have shown considerable variation in segmentation patterns and suggest that plasticity of “bauplan” evolution has been underestimated.

Siboglinids (formerly pogonophorans, see McHugh, 1997; Rouse and Fauchald, 1997), echiurids, myzostomids, and sipunculans, are four taxa with enigmatic evolutionary origins. For these four groups, the interpretation of how their bodies are segmented (or not segmented) has been fundamental to judgments about their phylogenetic position. At some point, all four taxa have been considered to be derived annelids, suggesting that their body architectures are secondarily derived from a segmented ancestor. However, they have also all been elevated to phylum rank at some time in their taxonomic history. Developmental patterns within these four taxa are relatively poorly studied, and thus, interpretations of a developmental process, segmentation, have been based largely on adult morphology. Within the past few years, molecular and developmental data have been gathered for questions concerning the origins of these groups. New insights arising from these data are related here to hypotheses of segmentation.

SIBOGLINIDS

The Siboglinidae were formerly recognized as Pogonophora (including the Vestimentifera) and comprise about 150 species that inhabit marine depths from 30–10,000 m. The group has had a colorful taxonomic history that has been confounded by arguments over taxonomic rank. For example, frenalate po-

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gonophorans (*i.e.*, non-vestimentiferan pogonophorans) have been variously called Pogonophora (*e.g.*, Jones, 1985), Frenulata (Webb, 1969), Perviata (*e.g.*, Southward, 1988), and Siboglinidae (Caullery, 1914). Vestimentiferans have been called Vestimentifera (*e.g.*, Jones, 1980), Obturata (*e.g.*, Southward, 1988; Southward and Galkin, 1997) and Afrenulata (*e.g.*, Webb, 1969). Both the frenulate pogonophoran clade and the vestimentiferan clade have been referred to as phyla, classes within the Annelida, classes within the Pogonophora, and subclasses within class Pogonophora within the Annelida. The name Siboglinidae refers to the primary description of frenulate pogonophorans as a family of annelids (Caullery, 1914; see McHugh, 1997 and Rouse and Fauchald, 1997) and is used here to emphasize their phylogenetic origins within annelids. This taxonomic turmoil resulted from differences in opinion about the uniqueness of pogonophoran and vestimentiferan body plans (see Rouse and Fauchald, 1995). Such a confused taxonomic history could have been avoided with a rank-free classification (*e.g.*, de Queiroz and Gauthier, 1990, 1992).

Several sources of data now provide evidence that siboglinids are within the annelid radiation. Although Land and Nørrevang (1977) and Southward (1988) both argued for the inclusion of siboglinids within the annelids based on morphological arguments (see also Rouse and Fauchald, 1995), it was not until more rigorous phylogenetic analyses became available that the annelid nature of this group became widely accepted. At present, four different sources of molecular data are germane to this issue. Although an early 18S rDNA analysis (Winnepeninckx *et al.*, 1995) suggested that vestimentiferans and pogonophorans were closely allied to echiurids (to the exclusion of “annelids”), later studies (Eernisse, 1997; Abouheif *et al.*, 1998; Halanych, 1998) demonstrate that 18S data provide limited resolution near the base of several lophotrochozoan phyla, including the Annelida. Thus, while 18S data hint at the annelid affinities of siboglinids, they do not provide strong evidence for siboglinid origins (Halanych *et al.*, 2001). A second source of molecular data, inferred amino acid data from the mitochondrial Cytochrome c Oxidase I (CO1) gene (Black *et al.*, 1997), place siboglinids within an annelid clade, but taxon sampling among the polychaetes was limited. McHugh's (1997) study using Elongation Factor-1 α (EF-1 α) DNA sequence data was the first molecular report with adequate taxon sampling to clearly demonstrate that siboglinids fall within the annelid radiation. This work was shortly followed by a similar study (Kojima, 1998, which built on earlier work Kojima *et al.*, 1993), that confirmed siboglinids are derived annelids. More recently, analysis of the mitochondrial genomes of *Galathealinum*, a frenulate pogonophoran (Boore and Brown, 2000) and *Riftia pachyptila*, a vestimentiferan (Jennings and Halanych, unpublished) also support the placement of these animals within annelids.

At the same time molecular evidence was accumu-

lating, Rouse and Fauchald (1997) published a morphological cladistic study of polychaete worms, concluding that siboglinids appear to be allied with the annelid clade Sabellida (their fig. 73). Several additional reports on specific aspects of siboglinid biology also suggest annelid affinities. Young *et al.* (1996) showed that siboglinid early development resembles annelids. Additional developmental work by Southward (1999) highlighted some of the similarities in the larvae of siboglinids and other annelids. The morphology and arrangement of hooked setae (Bartolomeus and Meyer, 1997), as well as the amino acid sequence and structure of hemoglobins (Terwilliger *et al.*, 1985; Suzuki *et al.*, 1989; Zal *et al.*, 1997), among siboglinids are annelid-like. Lastly, limited data from the fibrillar-collagen gene shows similarity between a tubeworm, *Riftia*, and the polychaete *Arenicola* (Sicot *et al.*, 1997), but additional protostome taxa must be sampled to determine if these similarities are symplesiomorphies or apomorphies for an annelid clade. Based on the available data, both McHugh (1997) and Rouse and Fauchald (1997) recommended that the clade revert to its original nomen, Siboglinidae (Caullery, 1914), within the Annelida.

Surprisingly, one recent paper summarily rejects this abundant wealth of information (Salvini-Plawen, 2000), suggesting that all of these observed features (both morphological and molecular) placing siboglinids within the annelid clade may be the product of convergent evolution. Salvini-Plawen argues that the hypothesis that siboglinids are related to hemichordates remains viable; however, he fails to discuss the phylogenetic implications of the serially segmented opisthosome and polychaete-like chaetae, and fails to use rigorous, repeatable methods to support his arguments.

ECHIURIDS

The marine spoon worms, or echiurids, include about 160 species, most of which inhabit burrows in soft marine sediments. Early workers considered echiurids to be annelids (reviewed in Fauchald and Rouse, 1997), but Newby (1940) argued that the body wall musculature, proboscis and excretory anal vesicles are sufficiently unique to warrant “phylum” status. Newby's views have dominated most classification schemes presented in invertebrate texts (*e.g.*, Kozloff, 1990; Brusca and Brusca, 1990; Meglitsch and Schram, 1991) even though Hyman (from whom the bulk of text book information is derived) considered echiurids within the annelid radiation (Hyman, 1959, p. 611). As in siboglinids, differences in opinion concerning the uniqueness of the echiurid body plan revolve around the interpretation of segmentation. While Rouse and Fauchald (1995, 1997) score segmentation simply as being absent in echiurids, Nielsen (1995) and Eibye-Jacobsen and Nielsen (1997) consider echiurids to have secondarily lost all signs of segmentation. The latter view is supported by Purschke *et al.* (2000), who argue that treating segmentation in echiur-

ids as a primary absence rather than a secondary loss will likely confound results of phylogenetic analysis by incorrectly scoring outgroups and echiurids as same character state (in effect this pulls echiurids to a more basal position).

Despite the rapid growth of 18S rDNA sequence data in the past several years, annelids and other protostome worm groups remain severely under-sampled. At the time this paper was going to press (March 2002), only three “full-length” 18S rDNA echiurid sequences are available in GenBank (<http://www.ncbi.nlm.nih.gov>), and only a few molecular phylogenetic studies have addressed echiurid origins (*e.g.*, Winnepeninckx *et al.*, 1995; McHugh, 1997; Brown *et al.*, 1999). As with other protostome worm taxa, the 18S rDNA has limited resolution. However, EF-1 α data support the hypothesis that echiurids are derived annelids (McHugh, 1997) and indicate that segmentation was lost rather than primarily absent. Additional work on the development of the nervous system (Hessling and Westheide, 1999) also suggest an annelid origin for echiurids. Larval morphology (*e.g.*, serial ganglia on the larval nerve cord, serial mucous glands of ectodermal derivation, early reports of teloblast growth), as well as the presence of serially repeated pairs of nephridia in the adults of some species, also point towards a segmented ancestor for the echiurids (McHugh, 1997).

In contrast, Rouse and Fauchald’s (1997) morphological analysis found that echiurids were basal to a clade of segmented taxa including polychaetous and clitellate annelids; however, in this study, echiurids were scored as “segmentation” absent, a character-state judgment based on the ontological endpoint of the segmentation process in the adult. If one considers segmentation as a developmental process, then echiurids could be scored as segmented if the reported presence and pattern of repetitive teloblastic growth in the trochophore-like larvae is confirmed (Hatschek, 1881). Rouse and Fauchald recognized the potential problem of scoring secondarily absent characters and avoided this problem when dealing with parasitic or interstitial taxa, in which secondary absence of characters is generally accepted, by excluding them from their analyses. However, exclusion of these taxa can bias ancestral character-state reconstruction (see Cunningham *et al.*, 1998), coloring interpretations of how phylogenetically conservative certain features (*e.g.*, segmentation and parapodia) have been throughout annelid evolution.

MYZOSTOMIDS

Myzostomids are marine worms that are commensal or parasitic on echinoderms. Most of the ~150 species live as ecto-commensal parasites on crinoids, but exceptions (*e.g.*, endoparasites, asteroid-specific, ophiuroid-specific) are found. Myzostomids typically have a flattened dorsal surface with several paired “ventral” projections. For the most part, myzostomids have been allied with flatworms (*e.g.*, Leuckart, 1827) or variously related to annelids (reviewed in Pietsch and

Westheide, 1987). Understanding the nature of myzostomid segmentation, or lack thereof, is at the heart of the debate surrounding their affinities. Segmentation in this group is suggested by serial arrangements of lateral organs, the nervous system, appendages, and protonephridia (the latter in *Myzostoma cirriferum*). Serial patterns combined with the presence of chaetae on the appendages have been used to argue that myzostomids are derived polychaetes (*e.g.*, Westheide and Rieger, 1996; Rouse and Fauchald, 1997). Others (*viz* Jägersten, 1940; Salvini-Plawen, 1980*a, b*; Haszprunar, 1996) have found the arguments of segmentation less convincing. Lack of a divided coelom, variation in the number of lateral organs, and order of appendage development have been used to suggest that “segmented” patterns are only superficial.

Three recent studies have reached different conclusions regarding the origins of myzostomids. Eeckhaut *et al.*’s (2000) molecular analyses of 18S rDNA and Elongation Factor-1 α DNA data suggest myzostomids are more closely related to flatworms than annelids. This result is supported by analyses of the EF-1 α data alone and the combined data. The authors use simulation studies (*i.e.*, evolve DNA on different assumed trees) to examine the possible influence of long-branch attraction, a source of phylogenetic error. Although the authors conceded that long-branch attraction may be an issue with their maximum-parsimony analyses, they did not find evidence that it biased their maximum-likelihood reconstructions.

A second study (Müller and Westheide, 2000) used immunohistochemical techniques to examine the nervous system of *Myzostoma cirriferum*. Mapping fluorescently labeled nerve cells, Müller and Westheide document the presence of two ventral nerve cords connected by 12 commissures. Each paired appendage is supplied by a main nerve that splits into a dorsal and ventral process. The observed pattern of the nervous system is very similar to that found in many polychaetous annelids. Because Eeckhaut *et al.* (2000) conclude that the ancestor of flatworms, myzostomids, and trochozoans was a segmented organism with a trochophore larval stage, the results of Müller and Westheide do not directly conflict with the EF-1 α and 18S data. However, additional taxon sampling may revise Eeckhaut *et al.*’s hypotheses about this early protostome ancestor.

Zrzavy *et al.* (2001) also examined myzostomid origins using a total evidence approach. By combining 18S rDNA data with morphological data, they report that myzostomids are allied with the Cycliophora and Syndermata (*i.e.*, Rotifera & Acanthocephala) in a clade of organisms that have anterior flagella in their sperm (which they name the Prosomastigozoa). They further argue that there is no evidence for the association of myzostomes with annelids. Although Zrzavy *et al.*’s study does explore a variety of parameters associated with tree reconstruction, the underlying data deserves additional scrutiny. Jenner’s (2001) critical discussion on the recycling of morphological data sets

appears to apply to the Zrzavy *et al.* data. Not only is the data matrix filled with question marks, but several characters known to be homoplasious are included. To their credit, the authors realize some of the limitations of the morphological data (*e.g.*, in the case of brachiopods and phoronids). The molecular data of both Eeckhaut *et al.* and Zrzavy *et al.* do not support an annelid-myzostome affinity, and difference in their results (flatworms versus cyclophorans and syndermatans, respectively) may merely be a reflection of different taxon sampling in the two analyses. Clearly, more information needs to be gathered about this enigmatic group and their evolutionary origins more thoroughly studied.

SIPUNCULANS

Sipunculans, or peanut worms, have an unsegmented body with a retractable introvert. The group comprises about 150 species found exclusively in the marine realm (Cutler, 1994). Although the group was first documented in 1555, their phylogenetic affinities are obscure; they have been variously related to holothurians, echiurids, priapulids, phoronids, and annelids (Hyman, 1959; Cutler, 1994). Rice (1985) asserts that sipunculans represent an independent protostomian lineage that arose from an annelid-mollusk stem lineage. She noted a lack of segmentation in the developing nerve cord, and, similar to annelids, a double nerve cord in early development of some species. Scheltema (1993) cited three putative synapomorphies that link the sipunculans to mollusks: 1) presence of a molluscan cross during embryology, 2) a ventral, cuticular, pharyngeal (stomodaeal), protrusible invagination and attendant musculature in the pelagosphaera larva comparable to the molluscan radular sac, and 3) an anterior larval lip gland possibly homologous to the molluscan pedal gland. Based on these characters, the proposal that sipunculans are closely related to mollusks has gained acceptance.

Little molecular information bearing on sipunculan origins is available. The 18S rDNA has not been particularly informative in addressing this issue. However, a recent study examining about half of the mitochondrial genome from *Phascolopsis gouldii* (Boore and Staton, 2002) provides evidence suggesting that sipunculans have annelid affinities. Both gene rearrangement data and inferred amino acid sequences were used to show that the sipunculan consistently and significantly clustered with annelids rather than molluscs. Although synapomorphies uniting annelids and sipunculans are apparently wanting, Åkesson (1958) discussed plesiomorphic character states within the sipunculans and listed characters that indicate an annelid affinity. If Åkesson's hypothesis of annelid affinities is correct, the phylogenetic utility of a "molluscan-cross" versus an "annelid-cross" will need to be re-evaluated.

CONCLUSIONS

Over the past 100 yr, evolutionary origins of siboglinids, echiurids, myzostomids, and sipunculans have all

been related to Annelida. All four groups have strikingly distinctive body plans and each is acknowledged to be monophyletic (individually) by most workers. However, only siboglinid monophyly has been rigorously tested (Halanych *et al.*, 1998, 2001). In surveying the four groups simultaneously, some reasons for the inability to adequately understand the evolutionary origins of these groups become more apparent. Below, we briefly outline these reasons with suggestions as to how the community might resolve these problems.

For all of the groups discussed, the nature of body plan segmentation has been the central focus of discussions concerning evolutionary origins. Myzostomids and echiurids both have certain elements of the body plan (*e.g.*, nerves) that show iteration but other features do not (*e.g.*, coelom, body-wall musculature). For siboglinids, the segmented nature of the body has been accepted since the discovery of the serially-segmented opisthosome in these animals (Webb, 1964a). However, the nature of the body anterior to the short opisthosome has been debated. Given that independent data have confirmed that siboglinids are annelids, we can postulate that most of the siboglinid body is homologous to the first 3 segments of the annelid bauplan (see Webb, 1964b and Southward, 1988). Sipunculans are widely accepted as unsegmented and definitive synapomorphies with annelids appear to be lacking (but see Åkesson, 1958). However, mtDNA genomic data (Boore and Staton, 2002) offer the possibility that almost all traces of segmentation have been lost in sipunculans. This hypothesis is particularly interesting given the burrowing habitat of sipunculans, because a segmented hydrostatic skeleton is believed to offer a large selective advantage for burrowing organisms (Clark, 1969; but see Westheide *et al.*, 1999).

For all four taxa, authors disagree on which features are phylogenetically meaningful. Even when there is agreement on phylogenetically relevant characters, there may be disagreement on their importance or weight (*e.g.*, the siboglinid body plan, see Rouse and Fauchald, 1995). In several cases, a given worker may consider that the taxon in question had a fundamentally different or unique body plan when compared to other metazoans. Unfortunately, the way to signify this uniqueness currently is to elevate the taxa in question to a high taxonomic rank (*e.g.*, Phylum, Class, etc.). This convention precludes the possibility that this unique body plan is a highly derived member of a clade already recognized by the classification scheme. For example, under the current system it is difficult to reconcile the unique nature of the echiurid body plan with its evolutionary origins within the annelids. These semantic arguments over taxonomic rank have, over the years, obscured and confounded discussions of phylogenetic origins. There is a need to consider the utility of phylogenetic-based classification schemes over rank-based schemes (de Queiroz and Gauthier, 1990, 1992; Minelli, 2000). Phylogenetic-based classification schemes are advantageous because they at-

tempt to represent evolutionary history by focusing on monophyletic taxa.

To gain a more accurate understanding of the segmented or unsegmented nature of these four taxa, two types of data must be gathered. First, a well-supported phylogeny based on data independent of segmentation and encompassing these four groups must be generated. Without a comparative framework, it will not be possible to determine which features are phylogenetically conserved and which are evolutionarily labile (see Purschke *et al.*, 2000). Annelids and their allies are known to be diverse and ecologically important, yet have received surprisingly little attention by molecular systematists (reviewed in McHugh, 2000). Rouse and Fauchald (1997) offer one of the most thorough and complete morphological cladistic analyses for any major invertebrate taxon, but, as pointed out above, necessary assumptions about segmentation call the reconstructed topology into question.

Second, information on the molecular mechanisms, embryology, and selective forces (of both adults and larvae) that control and influence segmentation patterns must be gathered. Recent studies on how developmental mechanisms have evolved provide new ways to examine segmentation. Attention has been placed on understanding the mechanisms underlying annelid segmentation (*e.g.*, Shankland, 1994; Irvine and Martindale, 1996; Kourakis *et al.*, 1997; Irvine *et al.*, 1999; Seaver and Shankland, 2000), but most interpretations have compared annelids to other clearly segmented taxa, such as arthropods and chordates (*e.g.*, Valentine *et al.*, 1996; Shankland and Seaver, 2000; Iwasa *et al.*, 2000; but see Clark, 1969). Without knowing how plastic segmentation is in annelids (or the plasticity of the underlying mechanisms), the utility of comparisons across major lineages is limited. If one does not know the ancestral condition of annelids, it is hard to determine if annelid segmentation is homologous with arthropod segmentation. Applying developmental genetic techniques to explore the nature of segmentation in taxa with affinities to annelids, but that depart from traditionally recognized patterns of segmentation, will clarify the evolutionary and phylogenetic importance of segmentation in metazoan evolution.

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