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Reconstructing the phylogeny of the Sipuncula

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Abstract

Sipunculans are marine spiralian worms with possible close affinities to the Mollusca or Annelida. Currently 147 species, 17 genera, 6 families, 4 orders and 2 classes are recognized. In this paper we review sipunculan morphology, anatomy, paleontological data and historical affiliations. We have conducted cladistic analyses for two data sets to elucidate the phylogenetic relationships among sipunculan species. We first analyzed the relationships among the 45 species of Phascolosomatidea with representatives of the Sipunculidea as outgroups, using 35 morphological characters. The resulting consensus tree has low resolution and branch support is low for most branches. The second analysis was based on DNA sequence data from two nuclear ribosomal genes (18S rRNA and 28S rRNA) and one nuclear protein-coding gene, histone H3. Outgroups were chosen among representative spiralians. In a third analysis, the molecular data were combined with the morphological data. Data were analyzed using parsimony as the optimality criterion and branch support evaluated with jackknifing and Bremer support values. Branch support for outgroup relationships is low but the monophyly of the Sipuncula is well supported. Within Sipuncula, the monophyly of the two major groups, Phascolosomatidea and Sipunculidea is not confirmed. Of the currently recognized families, only Themistidae appears monophyletic. The Aspidosiphonidae, Phascolosomatidae and Golfingiidae would be monophyletic with some adjustments in their definition. The Sipunculidae is clearly polyphyletic, with *Sipunculus nudus* as the sister group to the remaining Sipuncula, *Siphonosoma cumanense* the sister group to a clade containing *Siphonosoma vastum* and the Phascolosomatidea, and *Phascolopsis gouldi* grouping within the Golfingiiformes, as suggested previously by some authors. Of the genera with multiple representatives, only *Phascolosoma* and *Themiste* are monophyletic as currently defined. We are aiming to expand our current dataset with more species in our molecular database and more detailed morphological studies.

Introduction

The Sipuncula are in several respects an ideal group for systematic studies: 1. The taxon has only 147 recognized species (see Cutler, 1994 for the current taxonomy), theoretically enabling researchers to include every single one instead of exemplars, 2. The majority of species (ca. 90%) are relatively large (i.e. >5 mm), facilitating examination, 3. Approximately 64% of the species are, at least in some locations, shallow-water inhabitants (<20 m) and easy to collect. On the other hand, morphological uniformity within the Sipuncula restricts the number of phylogenetically informative characters for cladistic analyses.

Keferstein (1863, 1865a,b, 1866, 1867), Selenka (1875, 1885, 1888, 1897) and Selenka et al. (1883) laid the groundwork for the understanding

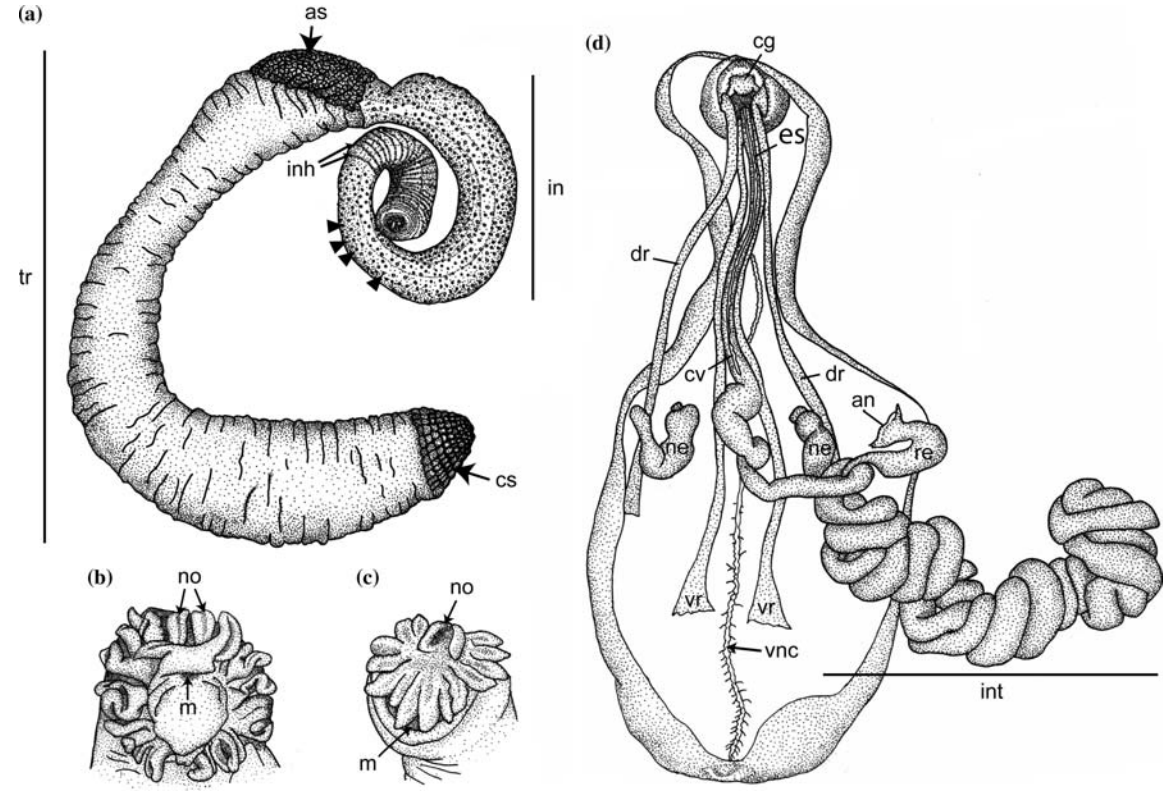

Figure 1. Morphology and anatomy of representatives of the Sipuncula. (a) External morphology of *Aspidosiphon fischeri*, lateral view. Note introvert retractor muscles and esophagus shining through body wall of introvert; tentacles visible at tip of introvert; arrowheads: introvert papillae. (b) Tentacular arrangement in *Golfingia margaritacea*, representative of the Sipunculidea. (c) Tentacular arrangement in *Phascolosoma nigrescens*, representative of the Phascolosomatidea. (d) Anatomy of *Golfingia margaritacea*. an – anus; as – anal shield; cg – cerebral ganglion; cs – caudal shield; cv – contractile vessel; dr – dorsal introvert retractor muscle; in – introvert; inh – introvert hooks; int – intestine; m – mouth; ne – nephridium; no – nuchal organ; es – esophagus; re – rectum; tr – trunk; vnc – ventral nerve cord; vr – ventral introvert retractor muscle.

of the morphology and internal anatomy of sipunculans. Several detailed accounts have been published in the past decade (see Rice, 1993; Cutler, 1994; Edmonds, 2000). We will therefore keep our review of morphology and anatomy short.

The sipunculan body is divided into trunk and retractable introvert (Fig. 1a). The ratio between introvert and trunk length varies among species and also depends on the state of relaxation of a specimen. Introvert length is only meaningful when measuring specimens with a fully extended introvert. The mouth, at the anterior end of the introvert, is surrounded by an array of tentacles in the Sipunculidea (Fig. 1b). In the Phascolosomatidea, the tentacles are arranged in an arc around the nuchal organ, also located at the tip of the introvert (Fig. 1c). The anus lies dorsally, usually at the anterior end of the trunk, except in *Onchnesoma* and four *Phascolion* species where it is shifted anteriorly onto the introvert. The nephridiopores lie ventrolaterally, typically at the level of the anus.

Hooks are often present on the distal part of the introvert. These are proteinaceous, non-chitinous specializations of the epidermis (Voss-Foucart et al., 1977) which are either arranged in rings or scattered. They are usually curved posteriorly and can have a variety of shapes and internal structures (Fig. 2). In *Aspidosiphon*, *Lithacrosiphon* and *Cloeosiphon* the epidermis forms specializations in the form of an anal shield (Fig. 1a). In *Aspidosiphon* and *Lithacrosiphon* the anal shield is restricted to the dorsal side, causing

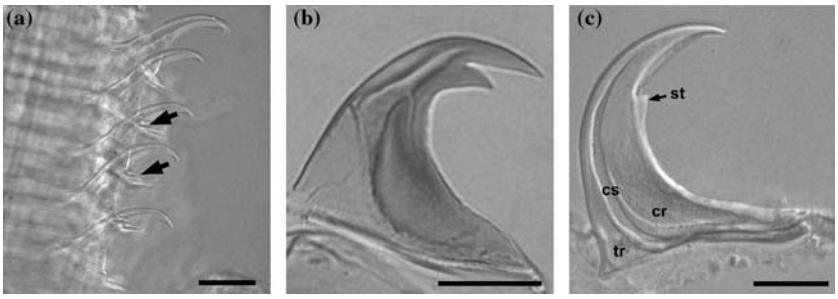

Figure 2. Shape and internal structure of sipunculan hooks, LM. (a) Partial ring of hooks in *Apionsoma pectinatum*; arrows point to basal spinelets. (b) *Aspidosiphon steenstrupi*, typical bidentate hook. (c) *Phascolosoma stephensoni*. cl – clear streak; cr – posterior crescent; st – secondary tooth; tr – clear anterior clear triangle; scale bars 20 μm in all cases.

the introvert to emerge at an angle, whereas it surrounds the anterior trunk in *Cloeosiphon* with the introvert emerging from its center. In *Aspidosiphon* the shield is a hardened, horny structure; in *Lithacrosiphon* it is a calcareous cone; in *Cloeosiphon* it is composed of separate plates. At the posterior end, a hardened caudal shield is sometimes present in *Aspidosiphon*.

Numerous papillae, associated with epidermal organs (combination of glandular and sensory organ) may be present on the trunk and introvert. *Phascolion* has specialized holdfast papillae. These are large papillae on the mid to posterior parts of the trunk with a hardened margin, shaped as a letter U, V or broken O (Åkesson, 1958; Cutler, 1994).

The body wall consists of a non-ciliated epidermis, overlain by a cuticle, an outer layer of circular and an inner layer of longitudinal musculature. In some larger species, oblique muscle fibers may be present between the longitudinal and circular muscle layers (Rice, 1993). A coelomic cavity fills most of the body and encloses the interior organs. Dybas (1981) distinguishes five types of coelomic cells: haemocytes, granulocytes, large multinuclear cells, ciliated urns and immature cells.

The alimentary canal starts with the esophagus, located between the introvert retractor muscles. In the trunk the intestine runs posteriorly, forms a loop and turns anteriorly again. The downward and upward sections of the gut are coiled around each other, forming a double helix (Fig. 1d). At the anterior end of the gut coil the rectum emerges and ends in the anus. A rectal caecum, present in most species, is a blind ending sac at the transition between intestine and rectum with unknown function.

Apart from the body wall musculature, the two other muscle systems are the introvert retractor muscles and intestinal fasteners. There are usually two pairs of introvert retractor muscles which may be fused to various degrees. Some species have a single pair. The spindle muscle inserts anteriorly near the anus, runs through the gut coil and is posteriorly attached either to the body wall near the posterior end or inside the gut coil. In addition, thin filamentous muscles often attach the esophagus and the anterior intestine to the body wall.

A pair of metanephridia is usually present, except in *Phascolion* and *Onchnesoma* which have only a single nephridium. The nephridia have the shape of elongated sacs and are orange or light brown in live or freshly fixed material. A ciliated funnel, or nephrostome, opens into the coelomic cavity at the anterior end, close to the nephridiopore (Ocharan, 1974; Rice, 1993).

The central nervous system consists of an anterior cerebral ganglion with a circumesophageal connective and a ventral nerve cord. The cerebral organ is a non-ciliated structure at the anterior margin of the cerebral ganglion (Purschke et al., 1997) and has been interpreted as a larval vestige (Åkesson, 1958). Purschke et al. (1997), however, suggested a sensory function because the epithelium contains bipolar sensory cells. The nuchal organ, located posterior to the cerebral organ, has fewer sensory cells. Both organs may function as a unit for chemoreception. Based on ultrastructural evidence the nuchal organ is probably not homologous to the nuchal organ in polychaetes (Purschke et al., 1997).

Sipuncula lack a blood vascular system. Fluid transport and gas exchange are instead accomplished by the coelom and the tentacular system. The latter connects the tentacles to a ring canal at their base, from which a contractile vessel that runs along the esophagus and ends blindly posteriorly arises (Edmonds, 2000). Pilger & Rice (1987) found evidence that the contractile vessel might also serve as a site for ultrafiltration.

Evolutionary origin of the Sipuncula

The phylogenetic position of the Sipuncula has long been subject to controversial opinions. While they were regarded as close relatives to holothurians in the early 1800s (Lamarck, 1816; Cuvier, 1830), Quatrefages (1847) erected the group Gephyrea, which he regarded as an intermediate between 'worms' and holothurians and which also contained echiurans, sternaspids and priapulids. Nichols (1967) revived the idea of echinoderm affinities, but found little acceptance in the scientific community. Today, there is general agreement that Sipuncula are protostomes and belong in the Lophotrochozoa, with affinities to annelids and/or molluscs (Cutler, 1994; Winnepenninckx et al., 1995; Zrzavý et al., 1998; Giribet et al., 2000), although their precise position remains unresolved.

The only unambiguous fossil record of sipunculans has recently been revealed by Huang et al. (2004) who describe three species from the Lower Cambrian Maotianshan Shale in southwest China, suggesting that sipunculan morphology has changed little over the past 520 million years. *Ottoia* has been proposed as another fossil sipunculan (Banta & Rice, 1976), but is now presumed to be a primitive 'aschelminth' or Priapulida (Conway Morris, 1989). The paleozoic *Hyolitha* has a mix of attributes of sipunculans and molluscs, suggesting a close phylogenetic relationship with both (Runnegar et al., 1975; Conway Morris, 1998; Marti Mus & Bergstrom, 2001). Fossilized burrows thought to be created by sipunculans in soft sediments are known from early and mid-Paleozoic times (Pemberton et al., 1980; Brett et al., 1983), but the inhabitants of such burrows are hard to determine. More recent Mesozoic and Cenozoic fossil burrows have also been attributed to sipunculan worms (Wetzel & Werner, 1981; Frey et al., 1984; Romero-Wetzel, 1987; McBride & Picard, 1991). Other sipunculans appear to have lived in association with corals and in vacated mollusc shells since the mid-Paleozoic, throughout the Mesozoic, and Cenozoic (Hyman, 1959; Gill & Coates, 1977; Brett & Cottrell, 1982; Pisera, 1987).

Embryological evidence for the origin of the Sipuncula is ambiguous. A trochophore larva in sipunculan development confirms affinities with Mollusca and Annelida. Scheltema (1993) regards the presence of a molluscan cross during cleavage as an indication to place Sipuncula as the sister taxon to the Mollusca. However, in recent years cell lineage studies have shown that the concept of the molluscan cross vs. the annelidan cross is oversimplified and of limited phylogenetic significance (Guralnick & Lindberg, 2001; Guralnick, 2002). Scheltema (1993) further suggests homologies in the head regions of sipunculan and mollusc larvae. She argues that the sipunculan lip shows similarities to the molluscan foot, the sipunculan lip glands to the molluscan pedal glands and the sipunculan buccal organ to the molluscan radular sac.

Rice (1985), on the other hand, lists certain similarities between sipunculan and annelid development. She notes the resemblance of the prototrochal and metatrochal bands in the larvae of both taxa, but Nielsen (1987) suspects that the posterior ciliary band in the sipunculan pelagosphera is an accessory ciliary band and not a true metatroch. Rice (1985) further mentions the retention of the egg envelope in some species to form the larval cuticle. She notes that, although in most sipunculan species examined to date, the nerve cord develops as a single tract, there are two known exceptions that may point to annelidan affinities: in *Phascolosoma agassizii* the nerve cord is double in early larval stages and in *Nephasoma pellucidum* it is partially split in the young pelagosphera larva. A notable difference between polychaete and sipunculan larvae is that there is no opposed-band feeding in sipunculans.

Even less conclusive with regard to phylogenetic affinities is evidence from comparative biochemistry, such as carbonic anhydrase activity (Henry, 1987), actin/myosin control of muscle contraction (Lehman & Szent-Györgyi, 1975), chromatin subunit structure in erythroid cells (Wilheim & Wilheim, 1978), the presence/absence

of different pyruvate oxireductases (Livingstone et al., 1983), phospholipids (Kostetskii, 1984), hemerythrin biochemistry (Florkin, 1975) and properties of the immune system (Ionescu-Varo & Tufesco, 1982). The study of ultrastructural evidence such as septate junctions (Green & Bergquist, 1982), ciliary bands (Nielsen, 1987) and sperm (Klepal, 1987) gives no clear answer either.

Previous cladistic analyses of morphological, molecular and, recently, gene order data (Boore & Staton, 2002) have rendered a number of hypotheses relating Sipuncula: to an unresolved clade containing the Mollusca, Echiura, and a clade grouping the annelid taxa with the Arthropoda (Brusca & Brusca, 1990; Backeljau et al., 1993); sister group to Echiura (Meglitsch & Schram, 1991); sister group to Annelida (Erber et al., 1998); sister group to Mollusca (Brusca & Brusca, 2003); derived Annelida (Boore & Staton, 2002); sister group to Echiura + Annelida (Eernisse et al., 1992); sister group to an unresolved clade containing Mollusca, Annelida and the Panarthropoda (Nielsen et al., 1996), or sister group to a clade as follows: (Echiura (Mollusca (Annelida (Onychophora (Tardigrada + Arthropoda))))) (Sørensen et al., 2000); sister group to Mollusca (Zrzavý et al., 1998; Giribet et al., 2000); sister group to Mollusca + Annelida (Peterson & Eernisse, 2001); or within an unresolved clade also containing Mollusca, Annelida and Echiura (Zrzavý et al., 2001). The number of molecular or combined morphological/molecular hypotheses is even greater since the monophyly of Mollusca or Annelida is often not recovered. In summary, little agreement is found about the exact position of Sipuncula within the protostome worms, perhaps due to the lack of structure for resolving some of those animal phyla (see Giribet, 2002).

The Sipuncula constitutes the first protostome phylum in which the ParaHox cluster has been fully characterized, presenting the three expected genes (Gsx, Xlox, Cdx) in two sipunculan species (Ferrier & Holland, 2001). This confirms the hypothesis that protostomes and deuterostomes shared a common ancestor whose ProtoHox cluster duplicated into ParaHox and Hox clusters that were conserved in both bilaterian lineages (Holland, 1998).

Sipuncula have been ranked as a family, order, sub-class or class at various times until Sedgwick (1898) proposed the name Sipunculoidea for the group, which he considered a phylum. However, this ranking did not gain much acceptance until Hyman (1959) proposed the spelling Sipunculida as she 'obliterated' the biologically meaningless construct Gephyrea. The present name, Sipuncula, and the use of 'sipunculan' for the vernacular name (not sipunculid) was proposed by Stephen (1964) and restated by Stephen & Edmonds (1972). Prior to this latter work there had been only two 20th century informal proposals regarding the arrangement of genera into unnamed family-like sets (Pickford, 1947; Åkesson, 1958). This void of intermediate taxa was partially filled when Stephen & Edmonds (1972) erected four families. Cutler & Gibbs (1985) set forth a more complete arrangement of the 17 genera into two classes, four orders, and six families. This arrangement has been followed by subsequent authors.

Phylogeny of sipunculan species

The first attempts to reconstruct the internal phylogeny of the Sipuncula were made by Cutler & Gibbs (1985), Gibbs & Cutler (1987) and Cutler (1994) (Fig. 3). The three studies relied on the same character set, however the polarities of several characters were changed since they were linked to the newly revised version of the hypothetical ancestral sipunculan (RHAS) in Cutler (1994). These analyses did provide some forward momentum, but currently they fall short of more rigorous standards for phylogenetic analyses. There are too few characters and too many unresolved branch points. Polarizing characters using a hypothetical ancestor can be accepted as an act of creative synthesis, or rejected as something less than objective science.

Some of the elements used in Cutler (1994) cannot be incorporated into a strict phylogenetic analysis based on a matrix of character states due to the incompleteness of the data set and thus some potentially meaningful information gets lost. In the present case this includes the number and shape of chromosomes, type of epidermal glands, or which of the four types of developmental patterns is exhibited. Additionally, interpretations of current patterns of distribution set against the

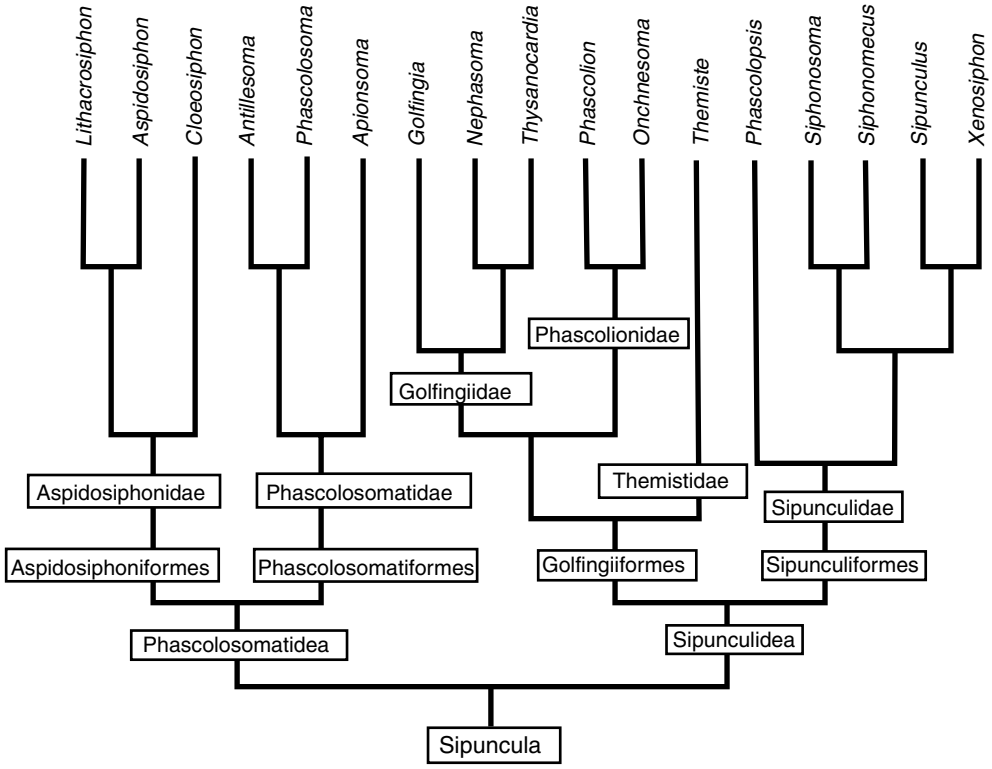

Figure 3. Phylogenetic relationships among sipunculan genera as proposed by Cutler & Gibbs (1985).

background of paleo-oceanography used by Cutler (1994: 360–374) in the construction of his evolutionary scenario, are difficult to incorporate into more restrictive analytical approaches.

Maxmen et al. (2003) recently tested Cutler's (1994) phylogenetic hypothesis. They analyzed molecular sequence data of the nuclear ribosomal genes 18S rRNA and 28S rRNA and the nuclear protein-coding gene histone H3 for 24 sipunculan species distributed in 13 genera, using a direct optimization approach with parsimony as the optimality criterion. This study showed polyphyly of the family Sipunculidae, with the genus *Sipunculus* being the sister taxon to the remaining sipunculans, *Siphonosoma* grouping with the Phascolosomatidea and *Phascolopsis* with the Golfingiidae (*Xenosiphon* and *Siphonomecus* were not sampled). Apart from the polyphyly of the Sipunculidae, the phylogenetic scheme mostly agreed with Cutler's (1994) system, with the exception of the problematic genus *Apionsoma* for which only partial sequences for the 18S rRNA were obtained (see Maxmen et al., 2003: their Fig. 3).*

Cladistic analysis of Phascolosomatidea based on morphology

To study the relationships among members of the Phascolosomatidea we assembled a morphological data matrix for the 45 species currently classified in the group, with representatives of all of the families in the Sipunculidea as outgroups (Appendix A). Thirty-five characters (31 parsimony informative) were included in the analyses. Data were analyzed using parsimony as an optimality criterion in

*Note added in proof: Another analysis of sipunculan phylogenetic relationships has been published recently (Staton, J.L. 2003, Inv. Biol. 122: 252–264), based on cytochrome *c* oxidase subunit I and encompassing thirteen sipunculan genera. This analysis is largely congruent with Cutler's 1994 analysis, except for the position of *Phascolopsis*. Sipuncula were found to be most closely related to annelids.

PAUP* 4.0b10 (Swofford, 2000). All characters were treated as unordered and no differential weighting was applied. Searches for the shortest trees were performed with the heuristic search option, for 1000 replicates of random addition sequence. Tree-bisection-reconnection (tbr) was chosen for branch-swapping, saving no more than 10 trees at each step per replicate. All trees were unrooted. Bremer support indices were calculated in AutoDecay 4.0.2 (Eriksson, 1998) in conjunction with PAUP. For each of the constraint trees generated by AutoDecay, ten random addition replicates were performed.

After the 1000 replicates, 980 equally most parsimonious trees of length 132 were retained. The strict consensus of all most parsimonious cladograms is presented in Figure 4. Resolution in the strict consensus is low and the ingroup does not appear as monophyletic.

Due to the relatively small size of the phylum a cladistic analysis including all species would be possible. However, when including all Sipuncula in the analysis, rooting becomes impracticable when relying on morphology alone. Other than in the molecular analyses, the inclusion of representatives of a number of other phyla as outgroups is problematic because morphological homologies are unclear and most characters of Sipuncula are inapplicable to other taxa. Therefore we preferred to explore the resolution of morphological features within the Sipuncula by selecting a subset of sipunculan taxa (the Phascolosomatidea) as ingroup and using other representatives of the phylum to polarize the phascolosomatidean tree. Morphologically, the Phascolosomatidea are well characterized by the presence of nuchal tentacles.

The low number of phylogenetically informative characters is obviously a problem and one would not expect full resolution with a character/taxon ratio of 0.58 (it is said that a ratio of three is desirable to obtain well resolved nodes, if there are no contradictory characters). Yet another problem seems to be the proportional representation of the various organ systems. Of the 31 phylogenetically informative characters included, 15 refer to hooks, leading to an unproportionately high weight of hook-associated characters in the analysis. The first 10 taxa in the tree in Figure 4 have no hooks and the presence of hooks appears as a synapomorphy for the clade including the remaining species. In light of all our previous data on sipunculan phylogeny, considering both morphological and molecular studies, we find this result questionable. It is possible that the morphological dataset contains more homoplasy than phylogenetic signal. This could be tested by combining the morphological data with a molecular data matrix. This has not been attempted here due to the lack of molecular data for a large proportion of the morphologically represented species.

Analysis of molecular data

For the molecular sequence analyses we used the sipunculan and outgroup sequences generated by Maxmen et al. (2003). Additional sequences were obtained for the following species:

Siphonosoma vastum (Selenka & Bülow, 1883): Bath, Barbados; June 24, 2002; Schulze, Saiz-Salinas & Cutler; MCZ DNA100625

Siphonosoma cumanense (Keferstein, 1867): Bath, Barbados; June 24, 2002; Schulze, Saiz-Salinas & Cutler; MCZ DNA100622

Aspidosiphon (Paraspidosiphon) fischeri ten Broeke, 1925: Martin's Bay, Barbados, June 21, 2002; Schulze, Saiz-Salinas, Cutler; MCZ DNA 100620

Lithacrosiphon cristatus (Sluiter, 1902): Bank Reef, Barbados, June 25, 2002; Schulze & Saiz-Salinas, MCZ DNA100623

Apionsoma (Edmondsius) pectinatum (Gibbs & Cutler, 1987): Six Mens Bay, Barbados, June 27, 2002; Schulze, Saiz-Salinas & Cutler; MCZ DNA100624

Phascolosoma nigrescens Baird, 1868: Six Mens Bay, Barbados, June 27, 2002, Schulze, Saiz-Salinas & Cutler; MCZ 100622

DNA sequences were deposited in GenBank (accession numbers in Table 1). Outgroup representatives were chosen among the spiralian phyla Nemertea, Mollusca, Entoprocta and Annelida (Table 1).

Methods for DNA extraction, amplification and sequencing are outlined in Maxmen et al. (2003). DNA electropherograms were edited in Sequencher^{TM} 4.0. Complete sequences were

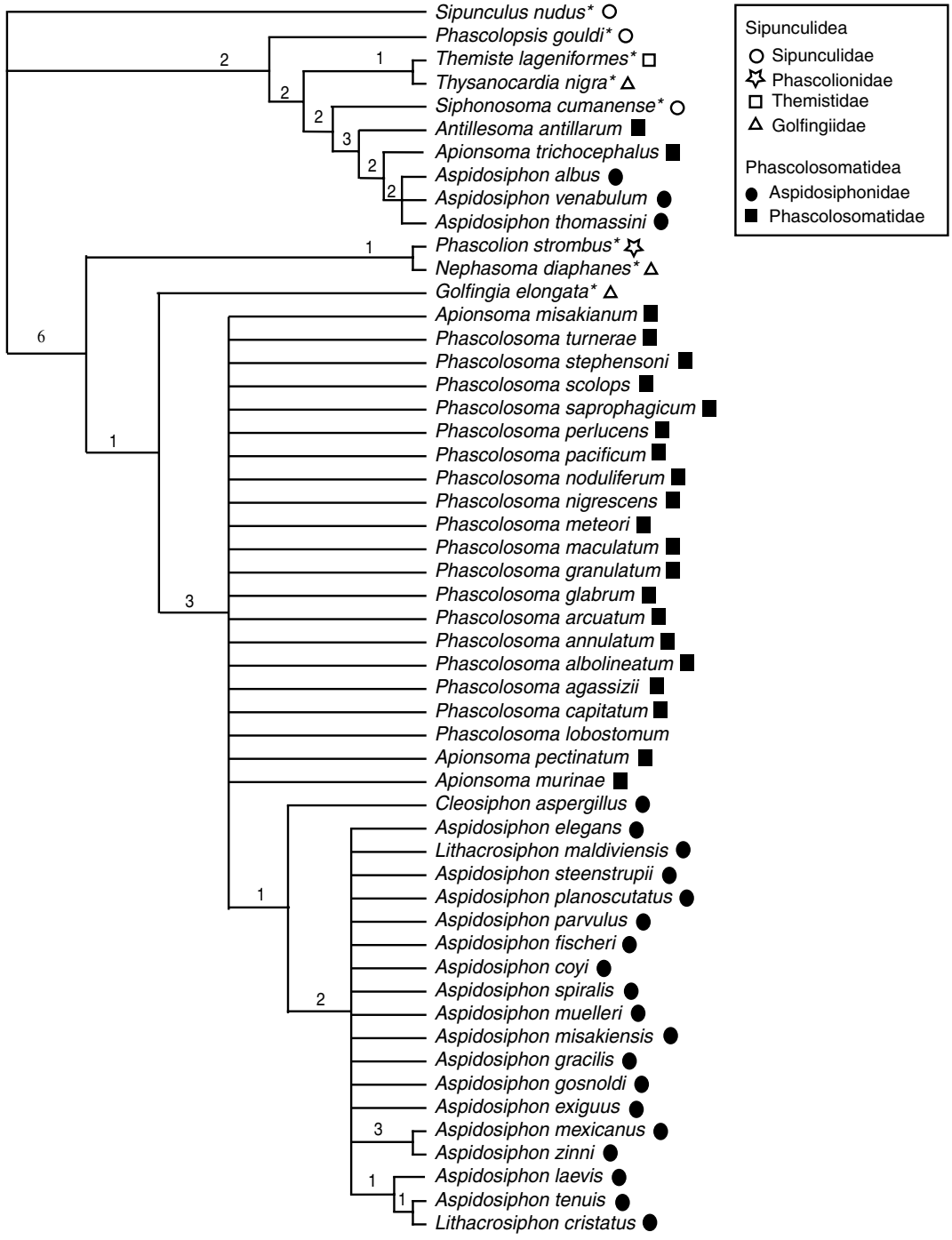

Figure 4. Strict consensus tree of 980 most parsimonious trees, generated in PAUP* using the morphological character matrix in Appendix A. Numbers above branches indicate Bremer support values. Asterisks indicate outgroup taxa.

edited in Genetic Data Environment (GDE) (Smith et al., 1994). All sequence data were analyzed simultaneously, using the program POY (Wheeler et al., 2002) assuming equal weights for all transformations (indels and base substitutions), as this was the parameter set that minimized

Table 1. Taxon sampling and accession codes to GenBank for the loci used in the analyses

		18S rRNA	28S rRNA	Histone H3
Phylum Nemertea				
Lineus sp.		X79878		
Argonemertes australiensis		AF519235	AF519264	AF519293
Amphiporus sp.		AF119077	AF519265	AF519294
Phylum Mollusca				
Lepidopleurus cajetanus		AF120502	AF120565	AY070142
Rhabdus rectius		AF120523	AF120580	AY070144
Haliotis tuberculata		AF120511	AF120570	AY070145
Yoldia limatula		AF120528	AF120585	AY070149
Phylum Entoprocta				
Barentsia hildegardae		AJ001734		
Pedicellina cernua		U36273		
Phylum Annelida				
Polyophthalmus pictus		AF519236		AF185259
Paralepidonotus ampulliferus		AF519237	AF519266	AF185247
Lumbrineris latreilli		AF519238	AF519267	AF185253
Chaetopterus variopedatus		U67324*		U96764
Lamellibrachia spp.		AF168742		AF185235
Urechis caupo		AF119076	AF519268	X58895
Lumbricus terrestris		AJ272183*		AF185262
Phylum Sipuncula Sipunculidae				
Sipunculus nudus	DNA100245	AF519239	AF519269	
Sipunculus nudus	DNA100246	AF519240[a]	AF519270	AF519295
Siphonosoma cumanense	DNA100235	AF519241	AF519271	AF519296
*Siphonosoma cumanense**	DNA100622	AY326291	AY445139	AY326296
*Siphonosoma vastum**	DNA100625		AY445137	AY326297
Phascolopsis gouldi	DNA100199	AF123306	AF519272	AF519297
Golfingiidae				
Golfingia elongata	DNA100466	AF519242[b]		AF519298
Golfingia vulgaris	DNA100207	AF519244[c]	AF519273	
Nephasoma flagriferum	DNA100439	AF519243		AF519299
Nephasoma diaphanes	DNA100443	AF519245[d]		
Nephasoma diaphanes	DNA100445	AF519246[e]		
Thysanocardia nigra	DNA100606	AF519247[f]	AF519274	AF519300
Phascolionidae				
Phascolion strombus	DNA100101	AF519248	AF519275	AF519301
Themistidae				
Themiste lageniformis	DNA100229	AF519249[g]	AF519276	AF519302
Themiste minor	DNA100210	AF519250[h]	AF519277	AF519303
Phascolosomatidae				
Phascolosoma albolineatum	DNA100396	AF519251[i]	AF519278	
Phascolosoma granulatum	DNA100201	AF519252[j]	AF519279	AF519304
Phascolosoma granulatum		X79874		
*Phascolosoma nigrescens**	DNA100622	AY326292	AY445140	AY326299
Phascolosoma noduliferum	DNA100208	AF519253[k]	AF519280	AF519305
Phascolosoma perlucens	DNA100228	AF519254	AF519281	AF519306
Phascolosoma scolops	DNA100373	AF519255[l]	AF519282	AF519309

Continued on p. 286

Table 1. (Continued)

		18S rRNA	28S rRNA	Histone H3
Phascolosoma stephensoni	DNA100469	AF519256	AF519283	AF519310
Phascolosoma stephensoni	DNA100209	AF519257[m]	AF519284	AF519307
Phascolosoma stephensoni	DNA100485	AF519258[n]	AF519285	AF519308
Antillesoma antillarum	DNA100390	AF519259	AF519286	AF519311
Apionsoma (*A.*) *misakianum*	DNA100231	AF519260[o]	AF519287	
Apionsoma (*E.*) *pectinatum**	DNA100624	AY326293[p]	AY445142	AY326300
Aspidosiphonidae				
Aspidosiphon (*P.*) *fischeri**	DNA100620	AY326294		AY326301
Aspidosiphon (*A.*) *misakiensis*	DNA100205	AF119090	AF519288	AF519312
Aspidosiphon (*P.*) *laevis*	DNA100467	AF519261[q]	AF519289	AF519313
Aspidosiphon (*P.*) *parvulus*	DNA100202	AF119075	AF519290	AF519314
Aspidosiphon (*P.*) *steenstrupii*	DNA100232	AF519262	AF519291	AF519315
Cloeosiphon aspergillus	DNA100393	AF519263	AF519292	AF519316
Lithacrosiphon cristatus	DNA100623*	AY326295	AY445141	AY326302

*Asterisks after species names indicate new sequences for this study. For incomplete 18S rRNA sequences, the number of bp sequenced (excluding primers) is indicated.
[a] 376 bp sequenced; [b] 381 bp; [c] 943 bp; [d] 383 bp; [e] 383 bp; [f] 988 bp; [g] 460 bp; [h] 923 bp; [i] 394 bp; [j] 1282 bp; [k] 1429 bp; [l] 1064 bp; [m] 394 bp; [n] 602 bp; [o] 378 bp; [p] 1432 bp; [q] 1389 bp.

overall incongruence (Maxmen et al., 2003). As there is little conflict among the three genes (Maxmen et al., 2003), separate analyses of the three datasets were not attempted here.

Due to length differences, fixed homology statements (alignments) were not implied for the two ribosomal genes which were analyzed using the direct optimization method (Wheeler, 1996). For the protein-coding gene histone H3 no insertions/deletions had to be inferred and the sequences were treated as prealigned. Hundred replicates of spr, tbr and tree fusing and tree drifting were performed.

Combined analysis of molecular and morphological data

The morphological data matrix used in the analysis of the Phascolosomatidea was combined with the molecular data but only those taxa for which sequence data were available were included. The analysis was performed with the same parameter set as in the analysis of the molecular data alone. All data files, batch file and results can be downloaded from the following website: http://www.mcz.harvard.edu/Departments/InvertZoo/giribet_data.htm.

The results of the molecular data only (Fig. 5) and of the combined analysis (Fig. 6) were very similar. We will therefore base our discussion on the results of the combined analysis and mention discrepancies wherever appropriate.

While showing support for the monophyly of the Sipuncula, there is low branch support for the relationships among outgroup taxa, and the sister group to the Sipuncula could not be determined. With respect to the ingroup, *Sipunculus* is the sister group to the remaining genera (*Xenosiphon*, *Siphonomecus* and *Onchnesoma* not studied). This second clade includes two main groups: the first one comprises *Themiste*, *Phascolopsis*, *Golfingia*, *Thysanocardia*, *Nephasoma*, and *Phascolion*; the second one includes *Siphonosoma cumanense* as the sister group to a clade containing *Siphonosoma vastum* and the Phascolosomatiformes.

Overall, our results are very similar to those of Maxmen et al. (2003). The main difference between both analyses is the placement of *Apionsoma misakianum*: in our combined analysis its position is unresolved whereas in the previous analysis it is the sister taxon to a clade comprising the remaining Phascolosomatidea and *Siphonosoma cumanense*. This placement is also

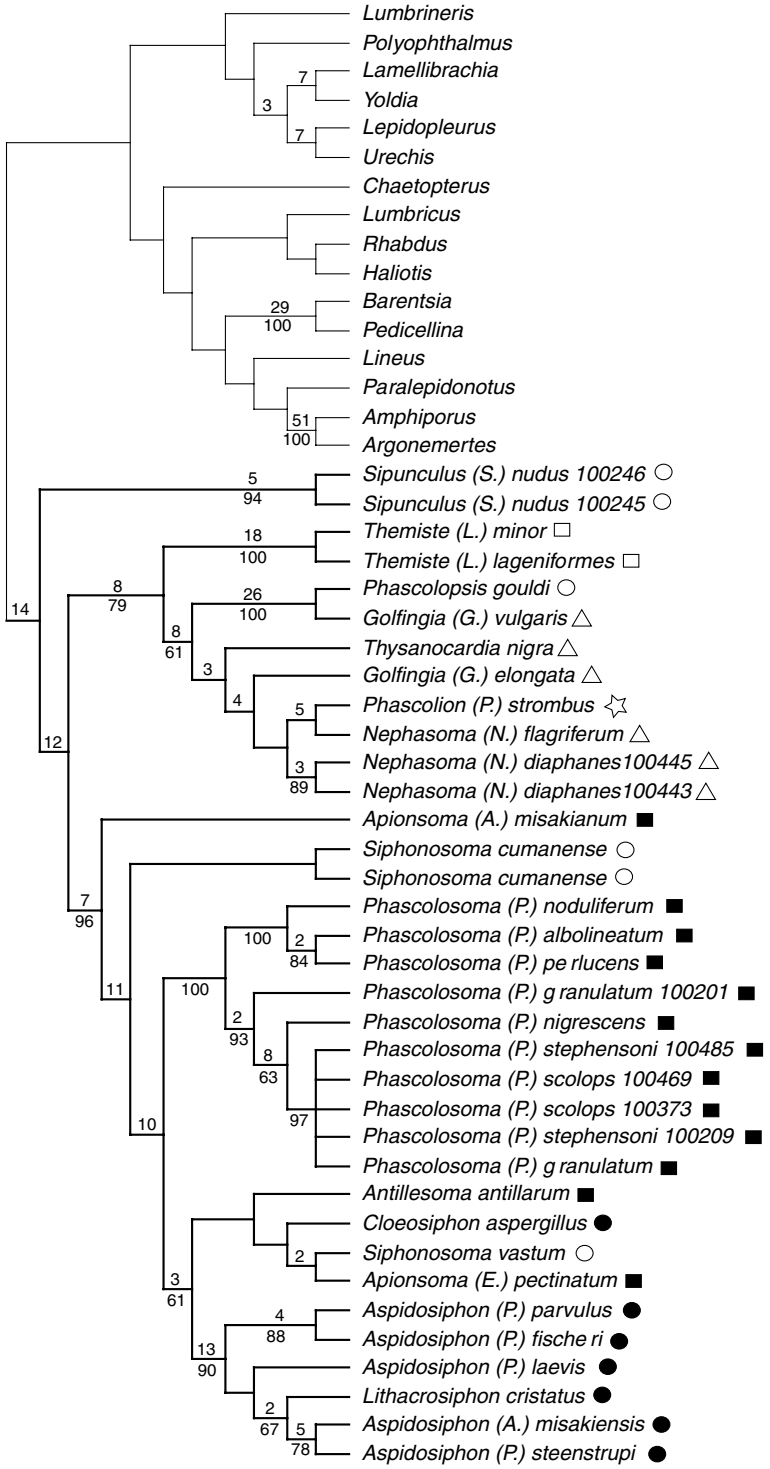

Figure 5. Strict consenus tree of five most parsimonious trees of length 4098, based on the analysis of DNA sequence data (18S rRNA, 28S rRNA and histone H3) alone. Branch support: jackknife proportions (36% deletion) underneath branches, Bremer support values on top of branches. Symbols after taxon names as in Figure 4.

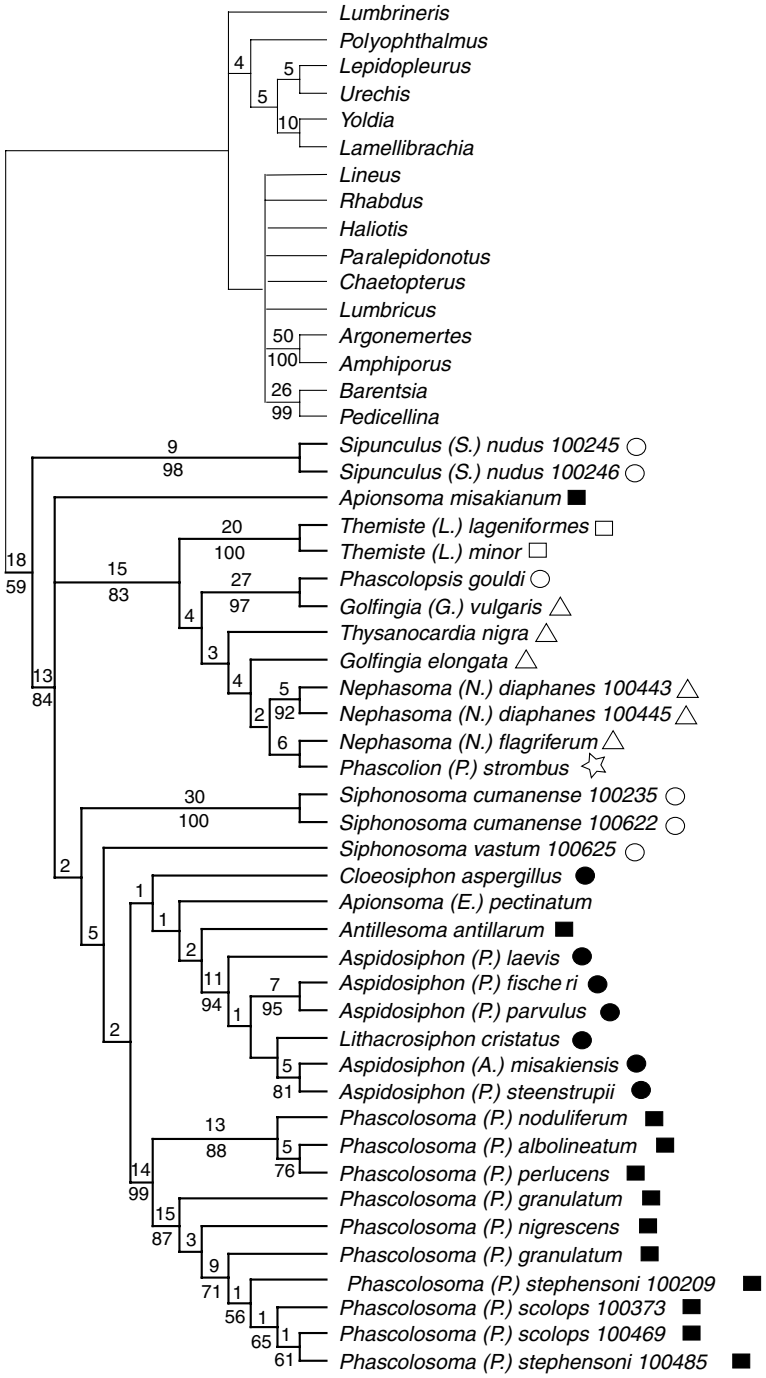

Figure 6. Strict consensus tree of three most parsimonious trees of length 4186, based on the combined analysis of 18S RNA, 28S rRNA, histone H3 and morphological data. Branch support: jackknife proportions (36% deletion) underneath branches, Bremer support values on top of branches. Symbols after taxon names as in Figure 4.

supported in our analysis of the molecular data alone (Fig. 5) and in one of the three shortest trees in the combined analysis. These results apparently indicate that nuchal tentacles may have evolved more than once in the Sipuncula. It should be noted, however, that the placement of

Apionsoma misakianum might be an artifact. The 18S rRNA sequence is currently incomplete for this species and histone H3 has not been successfully sequenced. Once full sequences are available, the phylogenetic affinities of *Apionsoma misakianum* will probably become less ambiguous.

Of the currently recognized families, only the Themistidae is monophyletic according to our analysis, but only 2 out of 10 species have been analyzed, both belonging to the same subgenus. Morphologically, Themistidae are characterized by stem-like extensions of the oral disk which bear the tentacles (Cutler, 1994).

The Phascolosomatidae and Aspidosiphonidae could be re-defined with slight modifications in their diagnosis: if *Antillesoma antillarum* and *Apionsoma pectinatum* were excluded from the Phascolosomatidae and moved to the Aspidosiphonidae, both families would be monophyletic. This would result in a monogeneric Phascolosomatidae, morphologically defined by two pairs of retractor muscles and laterally compressed, posteriorly directed hooks, arranged in rings and with a characteristic clear streak (Fig. 2c). Within *Phascolosoma*, species for which several representatives were included do not necessarily form clades. This is the case for *P. granulatum* and for *P. stephensoni*. One of the sequences for *P. granulatum* is from GenBank and we cannot rule out a misidentification. Species within *Phascolosoma* show similar morphology and intraspecific variability. A more comprehensive study of the genus, including multiple representatives of each species from a variety of locations would shed more light on species boundaries and intrageneric phylogeny. Morphological synapomorphies for the Aspidosiphonidae would be less obvious.

The tree topology within the Aspidosiphonidae differs between the combined analysis and the analysis of molecular data alone. In the combined analysis *Cloeosiphon aspergillus* is the sister taxon to a clade comprising the *Aspidosiphon* species, *Antillesoma antillarum*, *Apionsoma pectinatum* and *Lithacrosiphon cristatus*. The same result, with the exception of *L. cristatus* and *Apionsoma pectinatum* which were not sampled, was obtained by Maxmen et al. (2003). In our analysis of molecular data alone, the Aspidosiphonidae comprise two clades (Fig. 5). One of them contains *Antillesoma antillarum*, *Cloeosiphon aspergillus*, *Siphonosoma vastum* and *Apionsoma pectinatum*. With the exception of Cloeosiphon, none of these species has previously been assiociated with the Aspidosiphonidae. The 18S rRNA sequences are currently lacking for *Siphonosoma vastum* and its placement might be preliminary. The grouping of *Antillesoma antillarum* and *Apionsoma pectinatum* with the Aspidosiphonidae in both the molecular and the combined analysis is also surprising because both species do not have an anal or caudal shield as typical for the Aspidosiphonidae. This could imply that the anal shield in *Cloeosiphon* is not homologous to the anal shield in *Aspidosiphon* and *Lithacrosiphon*. Considering that the anal shield shows quite a different morphology among these genera (see section on external morphology) this interpretation could be plausible.

The Golfingiidae would be monophyletic if *Phascolion strombus* and *Phascolopsis gouldi* were incorporated. Again, morphological synapomorphies are not obvious. More drastic re-arrangements would be required to accommodate the taxa Sipunculidea, Sipunculiformes, and Sipunculidae. The Sipunculidae are clearly polyphyletic with *Sipunculus* as the sister group to the remaining Sipuncula, *Siphonosoma cumanense* at the base of the Phascolosomatidea clade and *Phascolopsis gouldi* the sister group to *Golfingia vulgaris*. *Siphonosoma* does not appear as monophyletic but, again, this might be due to the lack of 18S rRNA data in *Siphonosoma vastum*.

Maxmen et al. (2003) showed that the rooting between *Sipunculus* and the remaining Sipuncula is not dependent on the choice of outgroups, and therefore the results obtained here are interpreted the same way. It would be interesting to include more species of *Sipunculus* as well as *Siphonomecus* and *Xenosiphon* in the analysis. *Xenosiphon* might group with *Sipunculus* whereas *Siphonomecus* might group with *Siphonosoma* (Fig. 3). *Phascolopsis gouldi* has had a confusing nomenclatural history, but prior to Stephen (1964) who moved it into its own monotypic genus and Cutler & Gibbs (1985) who shifted the genus into a different family, it was associated with species that are currently members of the genus *Golfingia*.

Conclusions

Our molecular database currently includes 28 out of the 147 recognized sipunculan species, a rough 20% of the known diversity for the entire phylum. These cover 14 out of the 17 genera, except *Xenosiphon*, *Siphonomecus* and *Onchnesoma*. For most genera, except *Phascolion* and *Thysanocardia* we have more than one representative, enabling us to conduct initial tests of monophyly, although in a few cases we were not able to obtain samples of the different subgenera. Some genera, especially *Nephasoma* and *Onchnesoma* are difficult to obtain because most or all of the species occur beyond Scuba diving depth and often only in waters deeper than 500 m. Scientific cruises aimed for benthic deep-sea fauna are rare today and even rarer are dredging activities, making it difficult to obtain fresh and appropriately fixed material for molecular studies.

The main difficulties we are facing with regard to morphological data are the problems in determining the sister taxon of the Sipuncula and the paucity of phylogenetically informative characters. We plan to conduct more detailed morphological studies in the future, employing histological, ultrastructural and immunohistochemical techniques, to address these problems. For example, the arrangement of the body wall musculature might provide more characters than we are currently using. Another potential source of characters are developmental data which need to be explored thoroughly in a phylogenetic context.

Eventually our results should lead to a revision of sipunculan taxonomy and systematics. This would include elimination of taxon names that are nested within others, for example *Lithacrosiphon*, which appears to be nested in *Aspidosiphon* or *Phascolopsis gouldi*, nested within *Golfingia*. Most of the family names could be retained as long as a few species are moved from one family to another with slight changes in family definitions as required.

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Morphological characters

Tentacles

Nuchal tentacles (nuc): tentacles arranged in an arc around the nuchal organ (Fig. 1c); 0 = absent; 1 = present.

Peripheral tentacles (per): tentacles arranged in a circle around the mouth (Fig. 1b); 0 = absent, 1 = present.

Branched tentacles (bra): tentacles arising from stemlike outgrowths; 0 = absent, 1 = present.

Nephridia

Number of nephridia (npn): 0 = paired, 1 = single.

Nephridial shape (nps): 0 = unilobed, 1 = bilobed.

Nephridial attachment (nat): 0 = unattached, 1 = (partially) attached to body wall.

Body wall

Coelomic extensions in body wall (coe): in several sipunculan genera the coelom extends into the body wall in the form of coelomic canals or sacs. The canals either run longitudinally between the longitudinal muscle bands (*Sipunculus*), or diagonally as short, subcutaneous canals (*Xenosiphon*). In *Siphonosoma* and *Siphonomecus* the extensions are more sac-like (Ruppert & Rice, 1995). 0 = absent; 1 = present.

Longitudinal muscles (lmu): 0 = continuous; 1 = in bands.

Anal shield

All currently recognized Aspidosiphonidae are characterized by a hardened shield at the ante-

rior end of the trunk. However, its chemical composition, extend and morphology are variable among the species.

Dorsal anal shield (dsh): calcareous or horny protein shield at anterior end of trunk; 0 = absent, 1 = present.

Shape of dorsal anal shield (sha): 0 = ± flat, 1 = cone-shaped.

Pineapple shield (psh): shield at anterior end of trunk, composed of calcareous plates; 0 = absent, 1 = present.

Grooves in anal shield (gro): 0 = absent, 1 = present.

Spindle muscle

Spindle muscle (spm): slender, thread-like muscle running through the intestinal coil; 0 = absent, 1 = present.

Attachment of spindle muscle (att): 0 = attached at posterior end of trunk, 1 = ends in gut coil.

Hooks

Hooks on introvert (hoo): 0 = absent, 1 = present.

Hooks in rings (rin): 0 = absent, 1 = present.

Number of rings of hooks (nrh): 0 = < 50; 1 = > 50.

Scattered hooks (sca): hooks not arranged in rings; 0 = absent, 1 = present.

Basal spinelets (spi): small pointed units found at the base of introvert hooks (Fig. 2a); 0 = absent, 1 = present.

Bidentate hooks (bid): hooks with two pointed teeth (Fig. 2B); 0 = absent, 1 = present.

Secondary tooth (sec): accessory tooth on posterior concave side of hooks (Fig. 2c): 0 = absent, 1 = present.

Shape of secondary tooth (sst): 0 = blunt, 1 = pointed.

Anterior clear triangle (tri): clear space in anterior basal position in hook (Fig. 2c): 0 = absent, 1 = present.

Clear streak (cls): tube-like hollow space extending from the base toward the tip of the hook (Fig. 2c); 0 = approximately uniform diameter, 1 = with distinct bulge.

Crescent (cre): crescent-shaped clear space posterior to clear streak; 0 = absent, 1 = present

Posterior basal structures (pbs): structures at posterior basal edge of hooks (see Cutler, 1994, Fig. 45); 0 = absent, 1 = present, Type of posterior basal structures (tbs): 0 = warts, 1 = long processes, 2 = warts.

Angle of hooks (ang): angle of hook tip relative to main axis; 0 = < 90, 1 > 90.

Pyramidal hooks (pyr): hooks with triangular bases; 0 = absent, 1 = present.

Conical hooks (con): hooks with a nearly circular cross section; 0 = absent, 1 = present.

Other

Pigmented introvert bands (pib): 0 = absent, 1 = present.

Contractile vessel villi (cvv): The contractile vessel is part of the tentacular coelomic system. It runs dorsally along the esophagus, has a coelomic lining. It contains hemocytes and is considered an analogue to a blood vascular system. In some species, in particular of the genus *Themiste*, digitiform villi are present along the length of the vessel (Rice, 1993); 0 = absent, 1 = present. Type of contractile vessel villi (tvv) (see Cutler 1994, Fig. 36): 0 = villi, 1 = tubules.

Introvert retractor muscles (irm): This set of strong muscles insert anteriorly near the brain and are posteriorly attached to the body wall (Fig. 1d); 0 = 2 pairs, 1 = 1 pair.

Introvert/trunk length (itl): 0 = introvert < 75% trunk length, 1 = 75–200%, 2 = > 200%.

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Appendix A

Morphological dataset and characters for cladistic analysis of Phascolosomatidea; ? = unknown state; x napplicable.

	nuc	per	bra	npn	nps	nat	coe	dsh	sha	gro	psh	spm	att	hoo	rin	nrh	sca	Spi	bid	sec	ssh	tri	cls	cre	pbs	tbs	ang	pyr	con	pib	lmu	cvv	tvv	irm	itl
Antillesoma antillarum	1	0	x	0	0	1	0	0	x	x	0	1	0	0	x	X	x	x	x	x	x	x	x	x	x	x	x	0	0	0	1	1	0	0	0
Apionsoma misakianum	1	0	x	0	1	?	0	0	x	x	0	1	0	1	1	1	0	1	0	0	x	0	0	0	0	x	1	0	0	0	0	0	x	0	2
Apionsoma murinae	1	0	x	0	0,1	0	0	0	x	x	0	?	0	1	1	0	0	1	0	0	x	0	0	0	0	x	0	0	0	0	0	0	x	0	2
Apionsoma trichocephalus	1	0	x	0	1	?	0	0	x	x	0	1	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	0	x	0	2
Apionsoma pectinatum	1	0	x	0	1	1	0	0	x	x	0	1	1	1	1	0	0	1	0	0	x	0	0	0	0	x	1	0	0	0	1	0	x	0	2
Aspidosiphon albus	0	0	x	0	0	?	0	1	0	0	0	1	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	0	x	1	2
Aspidosiphon mexicanus	0	0	x	0	0	?	0	1	0	0	0	1	0	1	0	x	1	0	0	0	x	0	0	0	0	x	0	0	0	0	0	0	x	1	2
Aspidosiphon thomassini	0	0	x	0	0	1	0	1	0	0	0	1	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	0	x	1	2
Aspidosiphon venabulum	?	0	x	0	0	1	0	1	0	0	0	1	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	0	x	1	2
Aspidosiphon zinni	1	0	x	0	0	?	0	1	0	0	0	1	0	1	0	x	1	0	0	0	x	0	0	0	0	x	0	0	0	0	0	0	x	1	1
Aspidosiphon elegans	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	1	1	0	1	0	x	0	0	0	0	x	0	0	1	0	0	0	x	1	2
Aspidosiphon exiguus	1	0	x	0	0	?	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	0	0	0	0	0	x	1	2
Aspidosiphon gosnoldi	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	1	0	0	0	0	x	1	1,2
Aspidosiphon gracilis	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	0	0	x	0	0	0	0	x	0	1	0	0	0	0	x	1	1
Aspidosiphon misakiensis	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	0	0	0	0	0	x	1	1
Aspidosiphon muelleri	1	0	x	0	0	1	0	1	0	1	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	1	0	0	0	0	x	1	1
Aspidosiphon spiralis	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	1	0	x	0	0	0	0	x	0	0	0	0	0	0	x	1	1
Aspidosiphon coyi	1	0	x	0	0	?	0	1	0	1	0	1	0	1	1	0,1	0	0	1	0	x	0	0	0	0	x	0	1	0	0	1	0	x	1	1
Aspidosiphon fischeri	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	1	0	0	1	0	x	1	1
Aspidosiphon laevis	1	0	x	0	0	1	0	1	0	1	0	1	0	1	1	0,1	1	0	0	0	x	0	0	0	0	x	0	0	0	0	1	0	x	1	1
Aspidosiphon parvulus	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	1	0	0	1	0	x	1	1
Aspidosiphon planoscutatus	1	0	x	0	0	?	0	1	0	0	0	1	0	1	1	0	0	0	0	0	x	0	0	0	0	x	0	0	0	0	1	0	x	1	1
Aspidosiphon steenstrupii	1	0	x	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	1	0	0	1	0	x	1	1
Aspidosiphon tenuis	1	0	x	0	0	0	0	1	0	0	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	0	0	0	1	0	x	1	1
Cloesiphon aspergillus	1	0	x	0	0	1	0	0	x	x	1	1	0	1	1	0	0	0	1	0	x	0	0	0	0	x	1	0	0	0	0	0	x	1	1
Lithacrosiphon cristatus	1	0	x	0	0	0	0	1	1	1	0	1	0	1	1	0	1	0	1	0	x	0	0	0	0	x	0	0	0	0	1	0	x	1	1
Lithacrosiphon maldiviensis	1	0	x	0	0	1	0	1	1	0	0	1	0	1	1	?	0	0	1	0	x	0	0	0	0	x	0	0	0	0	1	0	x	1	1
Phascolosoma lobostomum	1	0	x	0	0	0	0	0	x	x	0	1	0	1	1	0	0	0	0	1	1	0	0	0	0	x	0	0	0	?	0	0	x	0	0
Phascolosoma capitatum	1	0	x	0	0,1	1	0	0	x	x	0	1	0	1	1	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	x	0	1
Phascolosoma agassizii	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	0,1	0	1	0	0	1	0	0	0	0	1	1	0	x	0	1
Phascolosoma albolineatum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	1	1	0	x	0	1
Phascolosoma annulatum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	0	x	1	0	0	1	0	0	0	0	1	1	0	x	0	1
Phascolosoma arcuatum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	1	0	0	0	0	x	0	1	0	1	0	0	0	0	0	1	0	x	0	1
Phascolosoma glabrum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	1	0	0	0	0	1	2	1	0	0	1	1	0	x	0	1

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Appendix A. (Continued).

	nuc	per	bra	npn	nps	nat	coe	dsh	sha	gro	psh	spm	att	hoo	rin	nrh	sca	Spi	bid	sec	ssh	tri	cls	cre	pbs	tbs	ang	pyr	con	pib	lmu	cvv	tvv	irm	itl
Phascolosoma granulatum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	1	0	0	0	0,1	0	0	0	0	1	0	0	0	0	0	1	0	x	0	1
Phascolosoma maculatum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	1	0	x	0	1
Phascolosoma meteori	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	0	x	0	0	0	?	?	0	0	0	?	1	0	x	0	0
Phascolosoma nigrescens	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	1	0	0	0	0,1	0	0	1	0	1	0	0	0	0	1	1	0	x	0	1
Phascolosoma noduliferum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	1	0	0	0	0	x	0	0	0	0	x	0	0	0	0	1	0	x	0	1
Phascolosoma pacificum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	x	0	1
Phascolosoma perlucens	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	1	0	x	0	1
Ph. saprophagicum	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	1	0	0	0	0	0	x	1	0	0	0	1	0	x	0	1
Phascolosoma scolops	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0	0	0	0	0,1	0	1	0	0	1	0	0	0	0	1	1	0	x	0	1
Phascolosoma stephensoni	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	1	0	x	0	1
Phascolosoma turnerae	1	0	x	0	0	1	0	0	x	x	0	1	0	1	1	0,1	0	0	0	0	x	0	0	0	1	1	0,1	0	0	0	1	0	x	0	1
Sipunculus nudus	0	1	0	0	0	1	2	0	x	x	x	1	1	0	x	x	0	x	x	x	x	x	x	x	0	x	x	0	0	0	1	0	x	0	0
Golfingia elongata	0	1	0	0	0	0	0	0	x	x	x	1	1	1	1	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	x	0	0
Phascolion strombus	0	1	0	1	0	1	0	0	x	x	x	0	x	1	0	x	1	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	x	0	1
Themiste lageniformes	0	1	1	0	0	0	0	0	x	x	x	1	1	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	1	0	1	0
Siphonosoma cumanense	0	1	0	0	0	0	1	0	x	x	x	1	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	1	0	0	0
Phascolopsis gouldi	0	1	0	0	0	0	0	0	x	x	x	1	1	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	0	x	0	0
Nephasoma diaphanes	0	1	0	0	0	0	0	0	x	x	x	1	1	1	0	x	1	0	0	0	x	0	0	0	0	x	0	1	0	0	0	0	x	1	0
Thysanocardia nigra	1	1	0	0	0	0	0	0	x	x	x	1	1	0	x	x	x	x	x	x	x	x	x	x	x	x	x	0	0	0	0	1	0	1	0