



The phylogenetic position of Siboglinidae (Annelida) inferred from 18S rRNA, 28S rRNA and morphological data

Vincent Rousset¹, Greg W. Rouse², Mark E. Siddall³, Annie Tillier¹
and Fredrik Pleijel^{1,4,*}

¹Muséum national d'Histoire naturelle, Département Systématique et Evolution, CNRS UMR 7138, "Systématique, Adaptation, Evolution", 43 rue Cuvier, 75231 Paris Cedex 05, France; ²South Australian Museum, Nth Terrace, Adelaide, SA 5000 Australia and Earth and Environmental Sciences, University of Adelaide, SA 5005, Australia; ³American Museum of Natural History, Division of Invertebrates Zoology, Central Park West at 79th Street, New York, New York 10024, USA; ⁴Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Göteborg University, SE-452 96 Strömstad, Sweden

Accepted 21 October 2004

Abstract

We assess the phylogenetic position of Siboglinidae (previously known as the phyla Pogonophora and Vestimentifera, but now referred to Annelida) in parsimony analyses of 1100 bp from 18S rRNA, 320 bp from the D1 region of 28S rRNA, and 107 morphological characters, totaling 667 parsimony informative characters. The 34 terminal taxa, apart from six siboglinids, include polychaete members of Sabellida, Terbelliformia, Cirratuliformia and Spionida, plus two Aciculata polychaetes as outgroups. Our results contradict most recent hypotheses in showing a sistergroup relationship between Siboglinidae and Oweniidae, and in that the latter taxon is not a member of Sabellida. Furthermore, our results indicate that Sabellariidae is not closely related to Sabellida, that Serpulidae may be nested within Sabellidae, and that Alvinellidae is nested within Ampharetidae.

© The Willi Hennig Society 2004.

The polychaete family Siboglinidae includes the taxa Vestimentifera and Pogonophora, which were previously considered their own phyla (e.g., Jones, 1985). These marine protostome segmented worms are tubedwelling and vary greatly in size, with adults of some *Siboglinum* reaching 50 mm in length and less than 1 mm in width, and the largest, such as "the giant tubeworm" *Riftia pachyptila*, from hydrothermal vents, reaching well over 1 m in length and 5 cm in width. They have no mouth as adults, they lack a functional gut, and they receive their nutrition from endosymbiotic chemoautotrophic bacteria. There are currently nearly 150 described species, which generally live in deep-sea sediments, though exceptionally they

are found at depths of less than 100 m (Webb, 1964a; Miura et al., 1997). Some members, such as *Riftia*, are known only from the hydrothermal vent systems, while others are found in association with reducing sediments, methane seeps, with sunken plant material or whale carcasses. Today, both morphological and molecular data unequivocally indicate that Vestimentifera are nested within Pogonophora (Southward, 1988; Rouse and Fauchald, 1995; Black et al., 1997; Kojima et al., 1997; McHugh, 1997; Halanych et al., 1998; Rouse, 2001). However, the phylogenetic position of the whole of this group is highly controversial (see Rouse, 2001 for an overview). For a while, they were regarded as not being closely related to Annelida, but referred to as deuterostomes based on, among other features, a dorsally situated nerve cord (e.g., Ivanov, 1963). Even following the discovery of a segmented posterior end (Webb, 1964b), and with developmental studies indicating that the orientation

*Correspondence: Fredrik Pleijel, Tjärnö Marine Biological Laboratory, Göteborg University, SE-452 96 Strömstad, Sweden. Tel.: +46 526 686 00; Fax: +46 526 686 07.
E-mail address: fredrik.pleijel@tmbl.gu.se

of the animals had been misinterpreted such that the nerve cord was actually ventral, rather than dorsal (Nørrevang, 1970), some authors considered them to be deuterostomes instead of protostomes (e.g., Johansson, 1968; Ivanov, 1970). However, it is notable that the idea of pogonophorans being polychaetes was postulated quite early by Uschakov (1933) and Hartman (1951, 1954), and reiterated by Liwanow and Porfirjewa (1967). This phylogenetic position has subsequently been widely supported by a number of morphological studies (Bartolomaeus, 1995; Nielsen, 1995; Rouse and Fauchald, 1995) and by several molecular analyses (McHugh, 1997; Halanych et al., 1998; Boore and Brown, 2000). Recently, Rouse and Fauchald (1997) and McHugh (1997) both suggested that the name Pogonophora should revert to the family name Siboglinidae, Caullery, 1914 (note that the name Pogonophoridae, introduced by Brusca and Brusca (2003), is invalid for nomenclatural purposes). While the placement of siboglinids within the polychaetes is now generally accepted, their more precise position has not been rigorously assessed. Rouse and Fauchald (1997) found Siboglinidae to be sister to a clade comprised of the major part of Sabellida (Sabellariidae, Sabellidae and Serpulidae) in all their morphological analyses except in one, where Siboglinidae instead appeared as sister to Terebelliformia. Based on chaetal similarities, Bartolomaeus (1995) also advocated a sabellid/terebelliform relationship for Pogonophora, and showed a tree where they are sister to a restricted Sabellida clade (Sabellidae and Serpulidae). Until now, the more precise position of Siboglinidae within the polychaetes has not been properly assessed. Previous morphological analyses had suffered from a restricted number of characters, and molecular analyses from limited or uneven taxon sampling (e.g., Kojima et al., 1993; Winnepeninckx et al., 1995; Black et al., 1997; McHugh, 1997; Kojima, 1998). The objective of this study was to assess the position of Siboglinidae among polychaetes and to determine its sister group relationship. For this we used morphological data, together with sequence data from two nuclear ribosomal genes (partial 18S rRNA and the D1 region of 28S rRNA) from six siboglinids and 28 other polychaetes.

Materials and methods

Taxon sampling

The terminal taxa were selected in order to represent the two main polychaete taxa, Sabellida and Terebell-

iformia, which have previously been proposed as possible candidates for the sister group of Siboglinidae (Bartolomaeus, 1995; Rouse and Fauchald, 1997; Bartolomaeus, 1998), but also without excluding the possibility that they may be closely related to Cirratuliformia or Spionida. Sabellida and Terebelliformia are members of a major and highly diversified polychaete group, Canalipalpata. Sabellida includes sabellariids, sabellids, serpulids and oweniids, and Terebelliformia includes alvinellids, ampharetids, pectinariids, terebellids and trichobranchids (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001). Terminal taxa were further chosen in order to span as much as possible of the morphological variation within these large clades. The hesionid *Hesiospina aurantiaca* and the nephtyid *Nephtys hombergi* were selected as outgroups. They both belong within Aciculata, “errant polychaetes”, which, following Rouse and Fauchald (1997), is sister to Canalipalpata.

Sequenced specimens and related information are detailed in Table 1. The terminals are referred to by their generic names only in the text and figures; this does not imply that the characters occur uniformly across the respective genera.

DNA extraction, amplification and sequencing

Genomic DNA samples were obtained from ethanol-preserved tissues by extraction in a solution of hexadecyl-trimethyl-ammonium bromide (CTAB) buffer following a modified protocol (Winnepeninckx et al., 1993). Fragments of a portion of the 18S rRNA gene (approximately 1100 bp) were PCR-amplified in two overlapping fragments, one of 650 bp using the primer pair TimA, 5'-AMCTGGTTGATCCTGCCAG-3' (Norén and Jondelius, 1999) and 650R, 5'-CTACGAGCTTTTAACTGCA-3' (designed for this study), the other of 500 bp using the primer pair 600F, 5'-GGTGCCAGCAGCCGCGGT-3' (Norén and Jondelius, 1999) and 1100R2, 5'-CGGTATCTGATCGTCTTCGA-3' (designed for this study). Fragments of the D1 region of the 28S rRNA gene (approximately 320 bp) were amplified using the universal primer pair, 28S-C1', 5'-ACCCGCTGAATTAAAGCAT-3' and 28S-C2, 5'-TGAAGTCTCTCTTCAAAGTTCTTTTC-3' (Lê et al., 1993). Amplification reactions for 18S and 28S contained 1.5 units of QBiotaq DNA polymerase (QBiogen, Inc.), 10× buffer, 2.5 mM MgCl₂, 10 μM of each primer, 0.25 mM of each dNTP, and template, for a 25 μL total volume. PCRs for the D1 region of 28S rRNA included four steps each: 94 °C for 90 s, followed by 36 cycles of 94 °C (40 s), 52 °C (40 s), and 72 °C (45 s). PCRs for the 18S rRNA gene were carried out under the same conditions as

Table 1
 Terminals used in the phylogenetic analyses, with localities for newly sequenced taxa

Taxon	Locality	GenBank accession no.	
		18S rRNA (partial)	28S rRNA (D1 region)
Aciculata, Phyllococida, Nereidiformia, Hesionidae <i>Hesiospina aurantiaca</i>	Madang, Papua New Guinea	AY340435	AY340401
Aciculata, Phyllococida, Nephtyidae <i>Nephtys hombergi</i>		U50970	X80649
Canalipalpata, Sabellida, Oweniidae <i>Owenia</i> sp.	Japan	AY611447	X80650
<i>Myriochele</i> sp.	Japan	AY340437	AY340405
Canalipalpata, Sabellida, Sabellariidae <i>Phragmatopoma</i> sp.	Florida, USA	AY611448	AY611435
<i>Sabellaria alveolata</i>		AY340442	AY340416
Canalipalpata, Sabellida, Sabellidae <i>Amphicorina mobilis</i>	Bondi Beach, Australia	AY611449	AY611436
<i>Myxicola</i> sp.	Banyuls, France	AY611450	AY611438
<i>Pseudopotamilla reniformis</i>	Sandgerdi, Iceland	AY611451	AY611437
<i>Sabella pavonina</i>		U67144	AY340420
Canalipalpata, Sabellida, Serpulidae <i>Hydroides norvegica</i>	Trondheim, Norway	AY611452	AY611439
<i>Protula</i> sp.	Banyuls, France	AY611453	AY611440
Canalipalpata, Sabellida, Siboglinidae <i>Lamellibrachia barhami</i>		AF168742	AF185153
<i>Polybrachia</i> sp.		AF168739	
<i>Ridgeia piscesae</i>		AF315060	AY344665
<i>Riftia pachyptila</i>		AF168745	
<i>Sclerolinum brattstromi</i>		AF315061	
<i>Siboglinum fiordicum</i>		X79876	AY340418
Canalipalpata, Spionida, Magelona <i>Magelona</i> sp.	Banyuls, France	AY611454	AY611441
Canalipalpata, Spionida, Spionidae <i>Polydora</i> sp.	Banyuls, France	AY611455	AY611442
Canalipalpata, Terebellida, Cirratuliformia, Cirratulidae <i>Cirriformia tentaculata</i>	Banyuls, France	AY611456	AY611443
<i>Dodecaceria</i> sp.		AY340427	AY340389
Canalipalpata, Terebellida, Cirratuliformia, Fauveliopsidae <i>Fauveliopsis</i> sp.		AY340429	AY340393
Canalipalpata, Terebellida, Cirratuliformia, Flabelligeridae <i>Diplocirrus glaucus</i>	N. Bohuslän, Sweden	AY611457	AY611444
Canalipalpata, Terebellida, Terebelliformia, Alvinellidae <i>Paralvinella palmiformis/grasslei</i>		AF168747	X80654
Canalipalpata, Terebellida, Terebelliformia, Ampharetidae <i>Anobothrus gracilis</i>	Banyuls, France	AY611458	AF501670
<i>Melinna</i> sp.	Banyuls, France	AY611459	AY611445
Canalipalpata, Terebellida, Terebelliformia, Pectinariidae <i>Pectinaria auricoma</i>	Banyuls, France	AB106263	AF501668
Canalipalpata, Terebellida, Terebelliformia, Terebellidae <i>Artacama proboscoidea</i>		AY344666	AY344667
<i>Eupolyommia nesidensis/nebulosa</i>	Trondheim, Norway	AY611460	S53425
<i>Lanice conchilega</i>	Brittany, France	X79873	AY340403
<i>Pista cristata</i>	N. Bohuslän, Sweden	AY611461	AY611446
<i>Thelepus</i> sp./cincinnatus	Banyuls, France	AY611462	X80657
Canalipalpata, Terebellida, Terebelliformia, Trichobranchidae <i>Terebellides stroemi</i>	Banyuls, France	AY611463	X80658

described above for the 28S, except for the annealing temperature, which was set to 52 °C for the primer pair 18S TimA–650R, and 54 °C for the primer pair 18S 600F–1100R2. PCR products were purified using the MinElute PCR Purification Kit (Qiagen, Inc.) following the manufacturer's protocol. Amplification products

were sequenced in both directions. Each sequencing reaction contained 8 µL DTCS Quick Start Master Mix (Beckman Coulter Inc.), 1 µL of 10 µM primer, and 100–150 femtomoles of DNA template. The thermal cycling program was: 30 cycles of 96 °C (40 s), 50 °C (40 s) and 60 °C (4 min). Sequences were purified by ethanol

precipitation, and the sequencing products were electrophoresed in a CEQ2000 Sequencer (Beckman, Inc.).

DNA sequence alignment

Sequences of complementary strands were edited and reconciled using Sequencher™ 4.1.4 (Gene Codes, Inc.). Multiple sequence alignments were generated with ClustalX version 1.8 (Thompson et al., 1997) using its default settings, except for gap-opening penalties, for which several combinations of pair-wise and multiple alignments were used: 10/15, 15/15, 15/20 and 20/20 (pair-wise penalty/multiple penalty). We calculated ILD (incongruence length difference; see Mickevich and Farris, 1981; Farris et al., 1995) values for the two molecular sets under the four different penalties, and selected those settings that yielded the globally lowest ILD value (Wheeler, 1995).

Morphological data

The morphological data matrix of 107 characters was developed from previous polychaete studies, mainly those by Rouse and Fauchald (1997), Rouse (2000), as well as more restricted studies, including Rousset et al. (2003) on Terebelliformia, and Rouse (2001) and Schulze (2003) on the internal relationships within Siboglinidae. Character numbers in the following descriptions correspond to the character summary in Table 2 and the matrix in Table 3.

(1–2) *Prostomium*. The two prostomial characters refer to the relationship between the prostomium and the peristomium (1), and to its overall shape (2). A well delineated prostomium is found in the outgroup taxa, as well as in spioniform and most cirratuliform polychaetes, oweniids, sabellariids (both reinterpreted from Rouse and Fauchald, 1997), some siboglinids (*Sclerolinum*, *Siboglinum* and *Polybrachia*), the ampharetids *Anobothrus* and *Melinna* and the alvinellid *Paralvinella*. The prostomium can be completely fused to the peristomium, as in the flabeligerid *Diplocirrus*, *Pectinaria*, sabellariids, sabellids, serpulids and vestimentiferans, while in terebellids and trichobranchids only the frontal edge of the prostomium is fused to the peristomium. The prostomial shape in the outgroups, *Polydora*, *Myriochele*, some siboglinids and cirratuliforms, is lobed. We followed Rousset et al. (2003) in interpreting terebellids and *Terebellides* as having a prostomial ridge, and ampharetids and *Paralvinella* as having a prostomial hood over the buccal region. The prostomium of *Magelona* was scored with an autapomorphic state, flattened and shovel-shaped, and that of sabellids, serpulids and vestimentiferans as being limited to the palps only. *Owenia* was given its own state for the lobed crown,

Table 2
List of morphological characters (see text for details)

1	Prostomium: distinct 0; fused to peristomium 1; frontal edge fused to peristomium 2.
2	Shape of prostomium: lobed-ridge-shaped 0; hood-like flattened 1; shovel-shaped 2; limited to palps 3; lobes 4.
3	Peristomium: lips only 0; ring or rings 1; extended upper and lower lips 2.
4	Peristomial rings: one ring 0; two rings 1.
5	Margin of anterior peristomial ring: low external 0; ventral as triangular lobe 1.
6	Peristomial ring: smooth 0; with collar 1.
7	Palps: absent 0; present 1.
8	Appearance of palps: sensory ventral 0; grooved 1.
9	Origin of palps: prostomial 0; peristomial 1.
10	Adult location of palps: outside mouth 0; inside mouth 1.
11	Contractibility of palps: minimal 0; extensive 1.
12	Prostomial palps: paired 0; multiple 1; crown 2.
13	Radiolar lobes: separated 0; dorsally fused 1.
14	Radiole fusion: separate 0; with palmate membrane 1.
15	Radiolar flanges: absent 0; present 1.
16	Radiolar skeleton: absent 0; present 1.
17	Parallel lamellae: absent 0; present 1.
18	Peristomial palps: paired 0; single 1; multiple 2.
19	Peristomial palp pinnules: absent 0; present 1.
20	Peristomial palp pinnules: multicelled 0; single-celled 1.
21	Fused multiple peristomial palps: absent 0; present 1.
22	Obturaculum: absent 0; present 1.
23	Obturaculum size: short 0; long 1.
24	Muscular arrangement in obturaculum: parasagittal 0; frontal 1.
25	Dorsal groove in the obturaculum: grooved 0; ridged 1.
26	Retractable head: absent 0; present 1.
27	Structure of first segment: dorsally limited 0; fused to head 1; elongate 2; similar to following 3.
28	Segment 2 forming elongate trunk: absent 0; present 1.
29	Metameric papillae on segment 2: absent 0; present 1.
30	Girdle on segment 2: absent 0; present 1.
31	Uncinal girdle on segment 2: median 0; posterior 1.
32	Papillae on posterior part of segment 2: absent 0; present 1.
33	Arrangement of papillae on posterior part of segment 2: line or rows 0; scattered 1.
34	Vestimentum: absent 0; present 1.
35	Frenulum: absent 0; present as continuous ridge 1; scattered plaques only
36	Diaphragm with distinct external groove: absent 0; present 1.
37	Lateral lobes on anterior segments: absent 0; present 1.
38	Thoracic membrane on anterior segments: absent 0; present 1.
39	Ventral glandular area on anterior segments: absent 0; present 1.
40	Shape of ventral glandular area on anterior segments: ventral central pads 0; ventral annulae 1.
41	Epidermal papillae: ventral central pads 0; ventral annulae 1.
42	Building organ: absent 0; present 1.
43	Notochaetae on segment 1: absent 0; present 1.
44	Notochaetae on segment 2: absent 0; present 1.
45	Notochaetae on segment 3: absent 0; present 1.
46	Notochaetae on segment 4: absent 0; present 1.
47	Neurochaetae on segment 1: absent 0; present 1.
48	Neurochaetae on segment 2: absent 0; present 1.
49	Neurochaetae on segment 3: absent 0; present 1.
50	Neurochaetae on segment 4: absent 0; present 1.
51	Neurochaetae on segment 5: absent 0; present 1.
52	Neurochaetae on segment 6: absent 0; present 1.

Table 2
Continued

53	Neurochaetae on segment 7: absent 0; present 1.
54	Neurochaetae on segment 8: absent 0; present 1.
55	Branchiae: absent 0; present 1.
56	Shape of branchiae: simple filaments 0; branching numerous lamellae 1.
57	Arrangement of branchiae: segmentally 0; grouped on single segment 1.
58	Branchiae on segment 1: absent 0; present 1.
59	Branchiae on segment 2: absent 0; present 1.
60	Branchiae on segment 3: absent 0; present 1.
61	Branchiae on segment 4: absent 0; present 1.
62	Branchiae on segment 5: absent 0; present 1.
63	Chaetal inversion: absent 0; present 1.
64	Parapodia: projecting neuropodia 0; similar rami 1; noto- and neuropodia differing; none 2.
65	Anterior neuropodia segment 2–8: short, truncate cylinders 0; low ridges or pads 1; tori 2; with lobes or lamellae 3; chaetae from body wall 4.
66	Posterior neuropodia: short, truncate cylinders 0; low ridges or pads 1; tori 2; with lobes or lamellae 3; straight from body wall 4.
67	Dorsal half of posterior body segments: with parapodia and chaetae 0; smooth 1.
68	Hooks/uncini: absent 0; present 1.
69	Arrangement of hooks/uncini: single row 0; two rows 1; dense fields 2.
70	Hook projection: projecting beyond tip of parapodia 0; low 1.
71	Capitium: absent 0; present 1.
72	Organization of capitium: single column 0; in two or more columns 1; arched rows over rostrum 2; bidentate 3.
73	Rostrum: absent 0; present 1.
74	Size of rostrum: small tooth 0; large fang 1.
75	Subrostrum (breast of hooks/uncini): absent 0; present 1.
76	Size of breast of subrostrum: extending less than capitium or rostrum 0; extending further than capitium or rostrum 1.
77	Subrostral process of hooks/uncini: absent 0; present 1.
78	Subrostral process of hooks/uncini: appendix for muscle attachment 0; teeth in same direction as capitium teeth 1; teeth oppose capitium teeth 2.
79	Manubrium of hooks/uncini: absent 0; present 1.
80	Length manubrium of hooks/uncini: shorter than remainder of hook/uncinus 0; longer than remainder of hook/uncinus 1.
81	Uncinal hook forms: hooks only 0; found on some segments 1.
82	Hoods of hooks/uncini protection: absent 0; present 1.
83	Chaetae in posterior segments: mixed capillaries or spines 0; hooks/uncini only in notopodial position 1; hooks/uncini in noto- and neuropodial positions 2; hooks/uncini only in neuropodial position 3.
84	Companion chaetae: absent 0; present 1.
85	Notopodial spines: absent 0; present 1.
86	Notopodial spines: along major part of body 0; projecting forward as paleae 1; in few anterior segments only 2.
87	Caudal region: absent 0; present 1.
88	Opisthosoma: absent 0; present 1.
89	Length opisthosoma: short 0; long 1.
90	Opisthosomal chaetae: parapodial 0; single long rows 1.
91	Inverted faecal groove: absent 0; present 1.
92	Gular membrane: absent 0; present 1.
93	Subdermal intraepidermal nerve cord: absent 0; present 1.
94	Lateral organs: absent 0; present 1.

Table 2
Continued

95	Mouth: axial proboscis 0; simple tube 1; ventral buccal organ 2.
96	Gut: straight 0; looped 1.
97	Occluded gut: absent 0; present 1.
98	Anterior segmental organs: not differentiated from rest 0; very enlarged pair 1; absent 2; excretory series 3.
99	Single anterior pair of nephridia: two openings 0; single opening 1.
100	Genital papillae: none apparent 0; on chaetiger 5–6 1.
101	Heart bodies: absent 0; present 1.
102	Tube: absent 0; present 1.
103	Tube construction: sand and or mucus 0; chitin protein complex 1; calcareous 2.
104	Tube attachment to substrate: absent 0; present 1.
105	Sperm packaging: absent 0; present 1.
106	Sperm packaging: spermatophores 0; spermatozeugmata 1.
107	Larvae: planktotrophic trochophore 0; lecithotrophic trochophore 1; mitraria 2.

which is arguably not equivalent to palps (Gardiner, 1978); the situation in *Pectinaria* and *Diplocirrus* is at present unresolved.

(3–6) *Peristomium*. The peristomium (3) is limited to the mouth region in the outgroups and sabellariids (Rouse and Pleijel, 2001). In *Fauveliopsis*, *Polydora*, *Paralvinella*, ampharetids, oweniids, sabellids, serpulids, siboglinids and cirratuliforms, the peristomium forms a distinct ring or rings. The precise location and organization of the peristomium is unknown for *Diplocirrus*, *Magelona*, *Pectinaria* and vestimentiferans (Rouse and Pleijel, 2001). Subsidiary characters with reference to the number of peristomial rings, projections and collar, were also included (4–6). The vestimental collar in vestimentiferans was interpreted as segmental, based on Rouse (2001), and is not considered homologous with the collar in sabellids, serpulids and oweniids.

(7–25) *Palps*. We assume that all “palp” structures (6) in polychaetes are homologous as argued by Rouse and Fauchald (1997); however, see Orrhage (2001) for an alternative viewpoint with regards to the tentacles of ampharetids, pectinariids and terebellids. Of the terminals included here, palps are only lacking in oweniids and *Fauveliopsis*. *Owenia* was regarded as having palps equivalent to the radiolar crown of sabellids and serpulids by Rouse and Fauchald (1997), but the investigation by Gardiner (1978) provided no real support for this primary homology statement (Rouse and Pleijel, 2001). Further investigation of oweniids with possible palps, e.g., *Myriowenia*, is certainly warranted. All of the remaining ingroup taxa have grooved palps, as opposed to the ventral sensory palp condition found in the outgroups (8). The prostomial or peristomial origin of the palps (9) is based on whether they develop in front of (= prostomial), or behind (= peristomial) the

Table 3
Character matrix of morphological characters. “?” stands for unknown and “.” for inapplicable state

	10	20	30	40	50	60	70	80	90	100	
<i>Hesiospina</i> sp.	000	---	100000	-----	000000	-0-00-00000000000011110	-----	000000	-----	0-0-00-000000000-000--0-0?	
<i>Nephtys hombergii</i>	000	---	100000	-----	030000	-0-00-0000-001111111111110	-----	010000	-----	-0-0-00-00000000-000--0-0	
<i>Anphicorina mobilis</i>	1311111000211110	----	0	---	03000	-0-00-00100011101111110	-----	12001011B11100	-1A10100	00--100010011001010-1	
<i>Anobolus gracilis</i>	011000110111	-----	0	---	03000	-0-00-0010001110101110222101110	-----	10101010301100	-010??103-0?	1000-1	
<i>Ariacama proboscidea</i>	202	---	110011	-----	0	---	03000	-0-00-0010000010000111120011100222111121111010300	00--0?	0?2103-0?1010-?	
<i>Ciriformia tentaculata</i>	00100011100	-----	20-00	---	03000	-0-00-00111111111100111100111100	-----	0-0-1000	-----	000020010010--0-1	
<i>Diplacarrus glaucus</i>	1???	---	11?00	-----	00-00	---	13000	-0-00-00111111111101110011100	-----	0-0-00-000021010110--0-1	
<i>Dodecaceria</i> sp.	00100011100	-----	00-00	---	03000	-0-00-000110111111001110011100	-----	0-0-1000	-----	000020010010--0-1	
<i>Eupolyomia vesidensis/nebulosa</i>	202	---	110011	-----	0	---	03000	-0-00-10100000100001111100111002221111211111010300	00--01002103-011010-1		
<i>Favosites</i> sp.	0010000	-----	0	---	13000	-0-00-00101011111111110	-----	011100	-----	0-0-00-0??02107?1?0--0-?	
<i>Hydroides norvegica</i>	1311011000200000	----	0	---	03000	-0-00-01?0011101111110	-----	121001011010100	-1010100	00--100010011011210-0	
<i>Lamelibrachia barbami</i>	133???	1100	-----	21011000210111120000	000110111110	-----	04440101110	-0-121110200	0111001010111011111?		
<i>Lanice conchilega</i>	202	---	110011	-----	0	---	03000	-0-00-101000001000011111001110022211121111010300	00--01002103-011000-1		
<i>Megalona</i> sp.	02???	---	1?00	-----	00-0	---	0?000	-0-00-00?11?1111110	-----	01330100??110-0-1101200	00--00012000-010--0-0
<i>Melina</i> sp.	011000110111	-----	0	---	03000	-0-00-00110000110010111010111102421101100	-----	101010300	00--01002103-011000-1		
<i>Myriochele</i> sp.	0010010	-----	0	---	03000	-0-00-000-0011100111110	-----	02440121130	-100-1100300	00--0???	00700?1000-2
<i>Muxicola</i> sp.	1311111000211110	----	0	---	03000	-0-00-00100011101111110	-----	121001012111A0	-1A10100	00--100010011001000-1	
<i>Owenia</i> sp.	0410010	-----	0	---	03000	-0-00-0011100011110	-----	02440121130	-100-1100300	00--001200200?1000-2	
<i>Paralymella palmiformis/grasslei</i>	011000110111	-----	0	---	03000	-0-00-00?00001100000001110111102	-----	1010101110	-1010301200	00--000?2103-011010-1	
<i>Pectinaria auricoma</i>	1???	---	11?01?	-----	??-0	---	01000	-0-00-00101000000001300001102	-2010110	0-0-111010301110	00--01012103-011000-0
<i>Phragmatopoma</i> sp.	000	---	11100	-----	00-00	---	01000	-0-00-00111111111100011112000101110	-10100	-1010110	00--0000100100?1010-0
<i>Pista cristata</i>	202	---	110011	-----	0	---	03000	-0-00-101000001000011110011000222111211110110300	00--0?	0?2103-0?1010-1	
<i>Polybrachia</i> sp.	00100011100	-----	21100	---	02111010011000	000110111110	-----	0444011110	-0-121110200	010000101100111010?	
<i>Polydora</i> sp.	0010001110	-----	00-0	---	03000	-0-00-001111111111000000013301000	-----	110-0-1101200	00--00012000	-00101100	
<i>Pronia</i> sp.	1311011000200000	----	0	---	03000	-0-00-01?0011101111110	-----	12100101010100	-1010100	00--10001001101210-?	
<i>Pseudopotamilla reniformis</i>	1311011000210011	----	0	---	03000	-0-00-00100011101111110	-----	1210010121110	-1A10110	00--100010011001010-1	
<i>Ridgeia piscesae</i>	133???	1100	-----	21011110210111120000	000110111110	-----	04440101110	-0-121110200	01110010101100111111		
<i>Riftia pachyptila</i>	133???	11100	-----	210111110210111120000	000110111110	-----	04440121110	-0-121110200	01110010101100111111		
<i>Sabella pavonina</i>	1311011000211011	----	0	---	03000	-0-00-00100011101111110	-----	1210010121110	-1A10110	00--100010011001000-1	
<i>Sabellaria alveolata</i>	000	---	11100	-----	00-00	---	01000	-0-00-00111111111100011112000101110	-10100	-1010110	00--000010010011010-0
<i>Sabellaria brattstromii</i>	00100011100	-----	00-00	---	0211111010000	000110111110	-----	04440101110	-0-121110200	01010070101?111111	
<i>Siboglinum fordicum</i>	00100011100	-----	111-0	---	02111010011000	000110111110	-----	0444011110	-0-121110200	01000010111000010101	
<i>Terebellides stroemi</i>	202	---	110011	-----	0	---	03000	-0-00-00110000010000000113001100022211011211100	-1A10300	00--01002103-011010-1	
<i>Thelepus</i> sp.	202	---	110011	-----	0	---	03000	-0-00-0010000011100001111200110002221101121111101010300	00--01002103-0?1010-1		

larval prototroch. The former is the outgroup condition and is also seen in sabellids, serpulids and terebellids. Even though the palps are found inside the buccal cavity in adults (10), we also scored ampharetids and *Paralvinella* as having palps of prostomial origin, based on ontogenetic arguments in Rouse and Pleijel (2001). Peristomial palps are seen in all other ingroup taxa with these structures, except for *Pectinaria*, where further investigation is required. Strongly contractile palps (11) are known in terebelliform polychaetes, such as alvinellids, ampharetids, pectinariids, terebellids and trichobranchids, although investigation of other taxa, such as *Siboglinum*, would be worthwhile.

Prostomial palps (12) can be paired as in the outgroup, multiple as in terebelliforms, or form a crown as in sabellids and serpulids. A series of characters (13–17) based on the prostomial crown found in sabellids and serpulids are included, based on Fitzhugh and Rouse (1999). Peristomial palps (18) may be paired as in *Polydora*, *Magelona*, sabellariids, *Sclerolinum* and *Dodecaceria*. Only a single palp is present in *Siboglinum*, while in vestimentiferans and *Polybrachia* there are multiple palps. The situation in *Cirriformia*, where a single pair of palps subsequently divides into many, is scored as the multiple condition. Characters 19–25 concern the features of the palps of Siboglinidae, particularly vestimentiferans, and are derived from Rouse (2001) and Schulze (2003). Several of those based on the obturaculum are uninformative in this analysis, but are included for use in future studies.

(26–42) *Segmental features*. A retractable head (26), where the prostomium, peristomium and, possibly, some anterior segments can be withdrawn into a segmental invagination, is found in a number of polychaetes, and it is seen here in *Diplocirrus* and *Fauveliopsis*. We accept the arguments by Filippova et al. (2003) that the head of these taxa is only comprised of the prostomium and peristomium. The outgroups showed two states for the basic shape of the first segment (27), dorsally limited (*Hesiospina*), or similar to those following (*Nephtys*). The latter condition is also seen in *Polydora*, cirratuliforms, oweniids, sabellids, serpulids, and Terebelliformia (except *Pectinaria*). In sabellariids and *Pectinaria* the first segments are fused with the prostomium and peristomium to form complex head structures. The first segment in siboglinids is interpreted as vestimentum (in Vestimentifera) and the forepart (in the rest), and it is elongate (Rouse and Pleijel, 2001). The situation in *Magelona* is currently unresolved with respect to the nature of the first segments (Rouse and Pleijel, 2001). Characters 28–36 are all relevant with respect to Siboglinidae and the features are fully discussed in Rouse (2001), whose scoring is followed here, except for the occurrence of metameric papillae on the anterior trunk of *Sclerolinum* (28), which is scored as present, in accordance with Schulze (2003). Lateral lobes (37) are

characteristic of Terebellidae and the thoracic membrane (39) is an apomorphic feature of Serpulidae. Ventral glandular areas are a feature of terebelliforms, and these characters (39, 40) are taken from Rousset et al. (2003). The glandular areas are also seen in sabellids in the form of central pads (Rouse pers. obs.) and also in *Fauveliopsis* (Petersen, 2000). Epidermal papillae (41) also occur in *Fauveliopsis*, a feature otherwise found only in *Diplocirrus* of the terminals used in this study (see Rouse and Pleijel, 2001). Immediately behind the sabellariid mouth is a U-shaped structure called the building organ, which is used to construct the sandy tube, and is regarded as part of segment 1. A mid-ventral structure termed the “cementing organ” by Watson (1928) appears to lie on segment 2 of *Pectinaria*. This organ, also used for tube construction, is very similar to the building organ of Sabellariidae, and character 42 reflects this.

(43–54) *Notochaetae and neurochaetae on segment 1–8*. These are characters used by Rousset et al. (2003), as is the scoring for terebelliforms, except for *Pista*, which is based on Kritzler (1984). For most other terminals Rouse and Pleijel (2001) provided justifications, while the scoring for *Diplocirrus* and *Fauveliopsis* follows Filippova et al. (2003). There is uncertainty as to the position of the initial segments in *Magelona*, so they are scored with “?”. Segments 1–8 are regarded as present in siboglinids, with 3–8 as part of the opisthosoma, and there are both noto- and neurochaetae present in all terminals of this taxon for segments 2–8. We regard segment 1 as bearing both noto and neurochaetae in *Cirriformia* and *Dodecaceria*, though further investigation is required (see Rouse and Pleijel, 2001).

(55–62) *Branchiae*. These are extensions of the segmental body wall with a loop of the vascular system; usually well equipped with blood-vessels. Branchiae can be simple filaments as in *Polydora*, sabellariids, ampharetids, cirratulids and *Diplocirrus*; branching structures as in *Paralvinella*, and terebellids except for *Artacama* and *Thelepus* (see Kritzler, 1984). In *Artacama* and *Thelepus* numerous filamentous branchiae emerge from the body wall, in *Eupolymnia*, *Lanice*, *Paralvinella* and *Pista* they emerge singly, but then branch, while in *Pectinaria* and *Terebellides* they form lamellae. Branchiae are absent in the outgroups, *Fauveliopsis*, *Magelona*, oweniids, sabellids, serpulids and siboglinids. Branchiae may be arranged (57) as pairs on a segment (or series of segments), or a segment may apparently have a group of branchiae, as in the ampharetids, *Paralvinella* and *Diplocirrus*. There is good evidence of migration of the branchiae in the former two groups (see Rouse and Pleijel, 2001 and references within). We also regard the branchiae of flabelligerids as segmental structures that have migrated to be in an anterior position. This is argued in Rouse and Pleijel (2001), though it should be

noted that Filippova et al. (2003) suggested that the branchiae of *Diplocirrus* are peristomial, meaning that they are completely new structures. The appearance of branchiae on segments 1–5 is coded as five separate characters, further arguments regarding these features in terebellomorphs can be found in Rousset et al. (2003). Branchiae do not appear in *Polydora* until after segment 5, and for *Cirriformia* and *Dodecaceria* we regard segment 1 as bearing branchiae (and chaetae), though further investigation is required. Branchiae begin on the 2nd thoracic segment in Sabellariidae (see Rouse and Pleijel, 2001).

(63) *Chaetal inversion*. Sabellariids, sabellids and serpulids are the only polychaetes to have some segments with notopodial uncini and neuropodial capillary chaetae. This is usually referred to as chaetal inversion, and whereas this is an appropriate name for the condition in sabellids and serpulids, the situation in sabellariids is more complex. Nevertheless, we scored all these terminals as present for this feature.

(64–67) *Parapodial shapes*. We used a general parapodial character regarding the similarity of noto- and neuropodia, where projecting neuropodia are found in the outgroup *Hesiospina* and similar sized rami are found in *Nephtys*. This second condition is also found in *Polydora*, *Magelona* and cirratuliforms. Notopodia and neuropodia can differ markedly and this is found in all other terminals except for siboglinids, where we regard parapodia as absent (i.e., chaetae emerge directly from the body wall). Anterior segments (2–8) with neuropodia (65) (where present) may be short cylinders as seen in the outgroups and sabellariids. These segments have neuropodia that form very low pads in sabellids, serpulids, siboglinids and cirratuliforms. When they are present in terebelliforms, anterior neuropodia are lobe-like with chaetae on the tips, while in *Magelona* and *Polydora* there are ridges bearing the chaetae and lamellae (see Rouse and Pleijel, 2001). The organization of posterior neuropodia (66) is similar to anterior ones for most terminals, except for sabellids and serpulids where they are small cylinders. In many terebelliforms (except *Paralvinella* and *Pectinaria*) the posterior region of the body (67) has segments that are completely smooth dorsally, i.e., they lack parapodia. In all other terminals this region has notopodia.

(68–82) *Hooks/uncini*. We regard dentate hooks and uncini as homologous structures, following the studies of Bartolomaeus and colleagues, and the various hooks/uncini characters defined here are based largely on their work (see Bartolomaeus, 1998 and references within). Hooks or uncini (68) are present in some segments of all ingroup taxa except cirratuliforms (though the spines of cirratulids deserve further study). Hooks/uncini are arranged (69) in single rows in most ingroup terminals where they are found. These chaetae

are arranged in two or more rows in some terebellids, and this is also seen in the girdle uncini of *Siboglinum* and *Polybrachia*. In *Owenia* and *Riftia* they occur in dense fields. The hook shafts (70) can project well beyond the parapodial lobe in *Polydora* and *Magelona*, while in the others with hooks only the teeth tend to be emergent. A fundamental feature of these structures is the capitium (71), which is a series of teeth, each of which is formed by a single microvillus that lies over a rostrum. Hausen and Bartolomaeus (1998) showed that the spine over the rostrum in spionids is not a capitium, and we use this information to score the structure as absent in *Polydora* (though Bartolomaeus, 1998, p. 357, suggested that a capitium may be present in some spionids). The situation in *Magelona* is not known. We further regard all other ingroup terminals as having a capitium based on Bartolomaeus (1998). The teeth of the capitium (72) can be arranged in a single column, as in *Hydroides* and *Protula* (though other serpulids have several columns), *Paralvinella* and *Melinna* (a feature of Melinninae). The teeth can also appear in two or more columns, as in sabellariids, some sabellids (see Rouse, 1990), siboglinids, *Anobothrus* (Hilbig, 2000) and *Pectinaria*. The teeth of the capitium are arranged in arched rows above the rostrum in most sabellids, terebellids and in *Terebellides*. Meyer and Bartolomaeus (1996) showed that the two teeth found in oweniids do not represent a capitium and rostrum, as previously argued, and are both part of a bidentate capitium. The rostrum (73), or main fang lying below the capitium, is not found in oweniids (Meyer and Bartolomaeus, 1996), or siboglinids (Bartolomaeus, 1998). We interpret the condition in ampharetids (and sabellariids), where the capitium teeth are indistinguishable from the basal-most tooth, as seen in siboglinids, as also meaning that a rostrum is absent in these terminals. A rostrum is present in spionids (Meyer and Bartolomaeus, 1996), and we scored it as present in *Magelona*. It is also found in sabellids, serpulids (as the basal “peg”) and most terebelliforms. Bartolomaeus (1998) noted that it may be absent in some of these taxa, but this does not apply to any of the terminals used here, except for *Pectinaria* (Bartolomaeus, 1995) and ampharetids. In the latter terminals a feature regarded as the rostral point by most authors (Holthe, 1986) is here termed the subrostral process (see below), and provides further support for regarding a rostrum as absent in ampharetids such as *Anobothrus* and *Melinna*. The size of the rostrum (74) may be a small tooth, as in serpulids, or a large fang, as in all other terminals.

A subrostrum (76), or breast, may be present below the rostrum, and among the ingroup taxa we regard this as absent in *Magelona* and *Polydora*, siboglinids and *Pectinaria* (Bartolomaeus, 1998). The subrostrum can extend beyond the teeth, as in some sabellids, terebellids and *Paralvinella*. A subrostral process (77),

often discussed in terms of muscle attachments, was found all terebelliforms except *Paralvinella*. It was also regarded as present in siboglinids by Bartolomaeus (1998), and we interpret the muscle attachment point in sabellariid uncini as being a subrostral process as well. The organization of the subrostral process (78) in siboglinids and *Pectinaria* is in the form of a series of teeth (Bartolomaeus, 1998), though they point in the same direction as the capitulum teeth in the latter. The subrostral process is as an appendix for muscle attachment in other terminals. The manubrium of hooks and uncini (79) appears to be absent in sabellariids. Where present, the handle can be shorter than the remaining apical section that bears teeth, or much longer (80). In some terminals, especially sabellids, there is polymorphism in the same individual for this character. Uncini (81), which can be distinguished from hooks by having a much shorter (or absent) manubrium, are found in ampharetids, *Pectinaria*, sabellariids, sabellids, serpulids, siboglinids and terebellids. Hooks may be protected by lateral guards (82), and this condition is found in *Magelona* and *Polydora*.

(83–86) *Other chaetal characters.* The chaetae in posterior segments (83) of the terminals in this study may have a mixture of capillary or spine-like chaetae, as seen in the outgroups and cirratuliforms. There may be uncini in a notopodial position as in sabellariids, sabellids and serpulids, or in a neuropodial position as in terebelliforms, or both as seen in siboglinids. Companion chaetae (84) are only founding in certain sabellids, while stout notopodial spines (85) are present in ampharetids, cirratulids, sabellariids, *Paralvinella* and *Pectinaria*. These can be distributed (86) in many segments, or found only as a projecting group of protective paleae as in *Anobothrus*, sabellariids and *Pectinaria*. In *Paralvinella* these spines are only found in segment 9.

(87–90) *Posterior region.* The caudal region (87) of *Pectinaria* and sabellariids is a small appendix; in the latter taxon it is achaetous and recurved along the body for evacuating waste. We coded the two conditions as homologous as with the condition for paleae. An intact opisthosoma (88) is present in all siboglinids that have been recovered, where it constitutes a series of small chaetigerous segments at the posterior end. The opisthosoma is elongate (89) and has uncini only (90) in vestimentiferans (Schulze, 2001).

(91–101) *Other anatomical features.* An inverted faecal groove (91) is found only in sabellids and serpulids (e.g., Fitzhugh, 1989). A gular membrane, or prominent diaphragm, separating the anterior trunk from the remainder of the body is found in many terebelliforms. We do not regard the diaphragm between segments one and two of siboglinids as homologous to a gular membrane (*contra* Rouse and

Fauchald, 1997). A gular membrane is also not scored as present in *Fauveliopsis* or *Diplocirrus* (see Rouse and Pleijel, 2001; Filippova et al., 2003). An intra-epidermal nerve cord (93) is present in *Owenia* and siboglinids (Ivanov, 1963). Lateral organs (94) are segmental sensory organs, and occur in *Polydora*, *Magelona* and *Pectinaria*. The buccal organs (95) of the terminals in the study ranges from a muscular axial proboscis, as seen in the outgroups, to a ventral buccal organ, as seen in cirratuliforms and terebelliforms. There is no buccal organ in sabellariids, sabellids, serpulids and siboglinids (see Rouse and Pleijel, 2001). The gut (96) may be straight, as in most terminals, or looped, as seen in terebelliforms, *Diplocirrus* and *Fauveliopsis*, whereas the gut lumen (97) is completely occluded in siboglinids. The anterior segmental organs (98) of the outgroups *Magelona* and *Polydora* are basically the same as along the rest of the body. However, there is a single anterior enlarged pair that has an excretory purpose only in cirratuliforms, sabellariids, sabellids, serpulids and siboglinids. There is a pair in segment 6 only in *Owenia*, but this does not appear to be the same feature (Rouse and Pleijel, 2001). In terebelliforms there are a series of large nephridia in the anterior segments (Rousset et al., 2003). The single pair of anterior nephridia may have one or two exterior openings (99). A pair of genital papillae (100) is known in segment 5–6 in *Diplocirrus* and *Fauveliopsis* (Rouse and Pleijel, 2001). The heart body, or intravasal body (101), is known in larval *Magelona*, *Sabellaria*, serpulids (Hanson, 1950), and many cirratuliforms and terebelliforms. Schulze (2002, 2003) argued that a similar structure in some siboglinids is homologous. It is not known in sabellids or in *Polydora*.

(102–104) *Tube.* All terminals, with the exception of *Magelona* and cirratuliforms, spend their lives in tubes (102). The tube is usually constructed (103) from sediment and mucus, but in siboglinids it is a chitin–protein complex, and in serpulids it is largely calcareous. The tube is attached (104) to a substrate in terminals such as *Polydora*, sabellariids, some sabellids, serpulids, some siboglinids and terebelliforms, whereas the tube is unattached to the sediment in oweniids, some sabellids and siboglinids, *Pectinaria* and *Lanice*.

(105–106) *Sperm.* Sperm are packaged (105) into bundles for transfer to the female in siboglinids and *Polydora*. The packaging (106) can be as spermatophores or spermatozeugmata (Rouse and Pleijel, 2001).

(107) *Larvae.* We use only one larval character, and this involves larval feeding (107). Larvae may occur as trochophores, feeding in the plankton prior to metamorphosis, as seen in *Hydroides*, *Magelona*, *Nephtys*, *Pectinaria*, *Polydora* and sabellariids. Oweniids are also

Table 4

Tree length for separated (18S rRNA; 28S rRNA; Mor, Morphology) and combined (Mol, Molecular [18S + 28S]; Tot, Combined [18S + 28S + Mor]) analyses and incongruence values for the 16 combined analyses

Alignment parameters for 18S	Alignment parameters for 28S	18S	28S	Mol	ILD (Mol)	Mor	ILD Tot	(Tot)
10/15	10/15	2217	905	3204	0.02559	241	3487	0.03556
10/15	15/15	2217	883	3185	0.02669	241	3458	0.03383
10/15	15/20	2217	911	3214	0.02676	241	3486	0.03356
10/15	20/20	2217	915	3213	0.02521	241	3495	0.03491
15/15	10/15	2202	905	3190	0.02602	241	3472	0.03571
15/15	15/15	2202	883	3170	0.02681	241	3443	0.03398
15/15	15/20	2202	911	3198	0.02658	241	3470	0.03343
15/15	20/20	2202	915	3197	0.02502	241	3475	0.03367
15/20	10/15	2274	905	3256	0.02365	241	3546	0.03553
15/20	15/15	2274	883	3239	0.02532	241	3524	0.03575
15/20	15/20	2274	911	3273	0.02689	241	3553	0.03574
15/20	20/20	2274	915	3263	0.02268	241	3547	0.03299
20/20	10/15	2259	905	3234	0.02165	241	3534	0.03650
20/20	15/15	2259	883	3222	0.02483	241	3514	0.03728
20/20	15/20	2259	911	3254	0.02581	241	3547	0.03834
20/20	20/20	2259	915	3245	0.02188	241	3542	0.03586

planktotrophic but have a unique mitraria larval form. All other terminals (where known) have non-feeding lecithotrophic larvae (see Rouse, 2000).

Phylogenetic analyses

The four pair-wise and multiple gap-opening penalties in the multiple alignments (10/15, 15/15, 15/20 and 20/20) were combined differentially for each of the two molecular data sets, yielding a total of 16 different possible combinations that were generated and analyzed (Table 4). Congruence between the two molecular partitions, and between the molecular and the morphological data, was measured by ILD metrics (Mickevich and Farris, 1981; Farris et al., 1995), and we retained only those trees that were based on the data set with a minimal overall ILD value.¹

Many of the morphological features were scored as binary characters, either in the form of absent/present statements, or with both states specified. For more compound features, where the whole feature may also be absent, the absence/presence of the compound feature is treated as one character, different expres-

sions of the feature are specified in separate, subsidiary characters, and taxa lacking the feature are scored as inapplicable for the subsidiary characters (“C-coding”, *sensu* Pleijel, 1995). The combined data set consists of 667 parsimony informative characters. Phylogenetic analyses were performed using NONA ver. 2.0 (Goloboff, 1999), with heuristic searches and 200 replicates of the parsimony ratchet (Nixon, 1999). All characters were left non-additive and indels were treated as missing data. Nodal support was estimated with parsimony jackknifing (Farris et al., 1996), 1000 replicates, branch swapping, and five random addition sequences each run.

Results

Morphological data analysis

Phylogenetic analysis of the morphological characters resulted in 24 multiple parsimonious trees (MPTs), 241 steps long, and with a retention index (RI) of 0.81. The strict consensus of the 24 trees (Fig. 1A) indicates monophyly among all primary trees for Cirratulidae (Jac < 50), Oweniidae (Jac 98), Sabellariidae (Jac 99), Sabellidae (Jac 63), Serpulidae (Jac 95), Siboglinidae (Jac 100), Terebellidae (Jac < 50) and Terebelliformia (i.e., the clade containing Alvinellidae, Ampharetidae, Pectinariidae, Trichobranchidae and Terebellidae). The sister group relationship between the sabellids and serpulids is well supported (Jac 96), as is that between *Diplocirrus* and *Fauveliopsis* (Jac 88). The morphological data provide weak support for the clades (Oweniidae,

¹Whereas we recognize the arbitrary choice of gap penalty settings, we nevertheless considered it an advantage to explore several values within ranges that we considered reasonable, as seen from examination of the actual alignments and from other studies. The selection among these initial settings was made in order to maximize the congruence among the partitioned data sets, such that we selected the penalty settings that yielded the smallest overall ILD value (Table 4) (see Wheeler, 1995 and Giribet et al. 2000, but also Grant and Kluge, 2003, for criticism of the method).

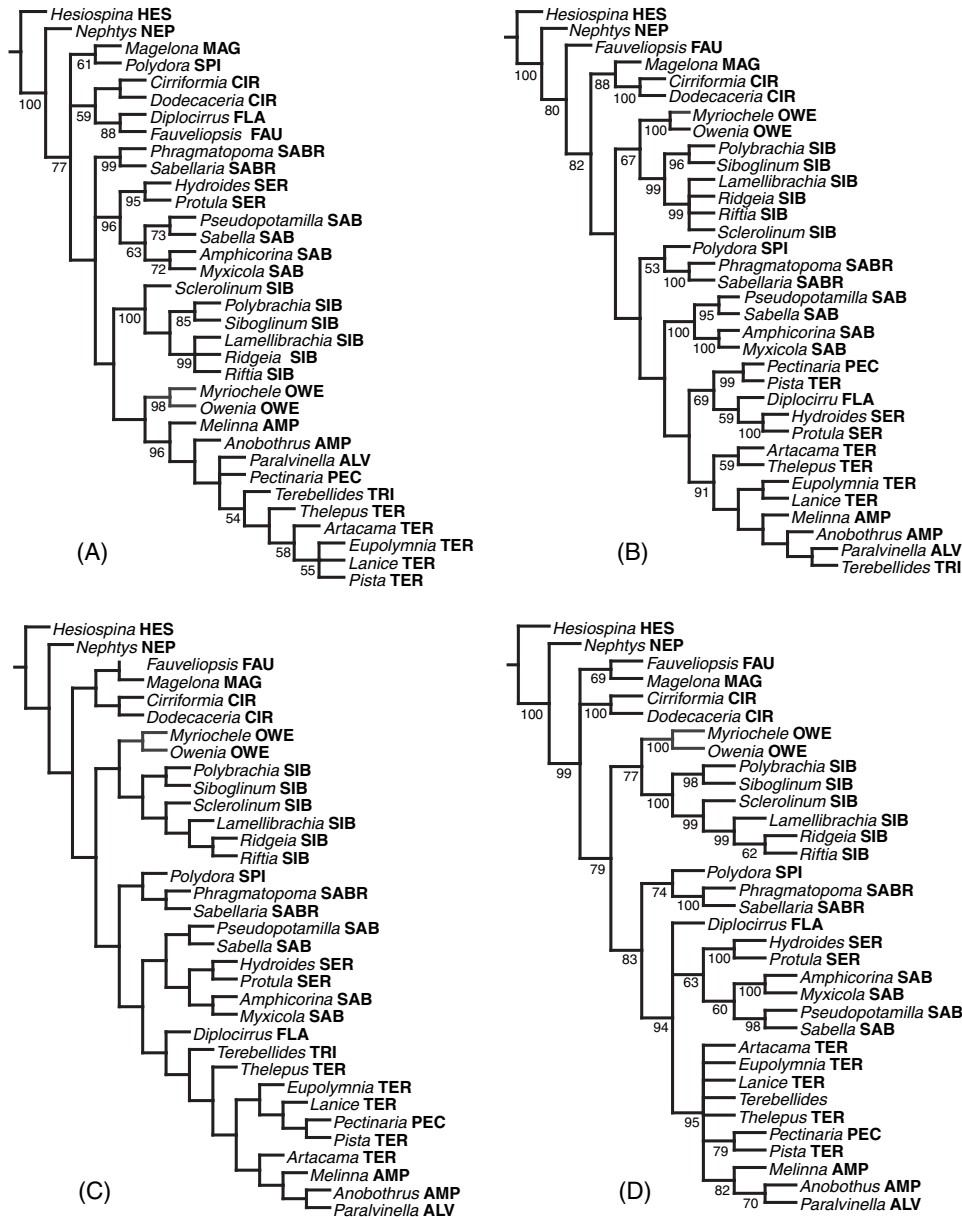


Fig. 1. (A) Strict consensus of the 24 trees obtained from the morphological data. (B) Strict consensus of eight trees obtained from the 18S and 28S rRNA data. (C) One of the two most parsimonious trees based on molecular and morphological data analyzed together. (D) Jackknife majority rule tree based on molecular and morphological data analyzed together. Values in (A), (B) and (D) are jackknife support. Abbreviations following the names of the terminals refer to their families: HES–Hesionidae, NEP–Nephtyidae, FAU–Fauveliopsidae, MAG–Magelonidae, SPI–Spionidae, CIR–Cirratulidae, FLA–Flabelligeridae, SABR–Sabellariidae, SER–Serpulidae, SAB–Sabellidae, SIB–Siboglinidae, OWE–Oweniidae, AMP–Ampharetidae, ALV–Alvinellidae, PEC–Pectinariidae, TRI–Trichobranthidae, TER–Terebellidae.

Terebelliformia) (Jac < 50), and for (Siboglinidae (Oweniidae, Terebelliformia)) (Jac < 50).

Molecular data analysis

The results of the congruence analysis of the two molecular partitions (18S rRNA and 28S rRNA) are shown in Table 4. The ILD (overall incongruence) was

minimized (ILD 0.02165), with the data sets aligned with the gap-opening penalties (pair-wise penalties/multiple penalties) setting at 20/20 for 18S and 10/15 for 28S.

Combined analysis of the molecular data sets without morphology yielded eight MPTs, 3234 steps long, and with an RI of 0.46. The eight trees differ only in the resolution within Siboglinidae, and a strict

consensus tree is illustrated in Fig. 1B. The main consistent results between the morphological and molecular analyses include the monophyly of Cirratulidae (Jac 100), Oweniidae (Jac 100), Sabellariidae (Jac 100), Sabellidae (Jac 100) and Siboglinidae (Jac 100). Well supported clades which differs from the morphological analysis include the non-monophyly of Terebellidae, owing to the unexpected position of *Pista* as sister to *Pectinaria* (Jac 99), the sister group relationship between Cirratulidae and *Magelona* (Jac 86), the basal position of *Fauveliopsis* as sister to the remaining ingroup taxa (Jac 84), the clade ((*Pectinaria*, *Pista*) (*Diplocirrus* (*Hydroides*, *Protula*))) supported in 69% of parsimony jackknife replicates, and the close relationship between Oweniidae and Siboglinidae (Jac 67).

Combined data analysis

The results of the congruence analysis between the two molecular and the morphological partitions are shown in Table 2. The overall ILD was minimized with the gap-opening penalties (pair-wise penalties/multiple penalties) set to 15/20 for 18S and 20/20 for 28S. Combined analysis of all data (18S + 28S + morphology) yielded two MPTs, 3547 steps long, and with an RI of 0.50. One of the two MPTs is illustrated in Fig. 1C. The topologies of the two trees differ only in the position of the Cirratulidae clade, which is sister either to (*Fauveliopsis*, *Magelona*), or to a clade containing all ingroup terminals except *Fauveliopsis* and *Magelona*. There is a minor difference between the jackknife tree (shown in Fig. 1D) and the two MPTs: in the jackknife tree, Serpulidae are sister to Sabellidae, whereas in the two MPTs the Serpulidae are instead nested within the Sabellidae.

The jackknife tree resulted in a topology containing 20 nodes with jackknife frequencies > 70%, and 14 > 95%. The monophyly of Cirratulidae, Oweniidae, Sabellariidae, Serpulidae, Siboglinidae, and Terebelliformia all have support values between 95% and 100%. The clade ((*Cirriformia*, *Dodecaceria*) (*Fauveliopsis*, *Magelona*)) appears in a basal position as sister to the remaining ingroup taxa (Fig. 1C), however, this clade lacks significant support. The support value for the sister group relationship between Oweniidae and Siboglinidae is 77%. *Polydora* and the Sabellariidae form a group (Jac 77), sister to a well-supported clade formed by *Diplocirrus* and three more inclusive groups: Sabellidae, Serpulidae and Terebelliformia.

The placement of *Paralvinella* close as sister to *Anobothrus*, supported by a jackknife support value of 70%, makes Ampharetidae paraphyletic. Owing to the unexpected position of *Pectinaria* as a nested sister to *Pista* (Jac 79), Terebellidae in its traditional delimitation is indicated as being non-monophyletic.

Discussion

Our results provide evidence for a sister group relationship between Siboglinidae and Oweniidae, and that Oweniidae is not closely related to Sabellida. Rouse and Fauchald (1995) suggested that Pogonophora is probably most closely related to Sabellida or Terebelliformia. Bartolomaeus (1995) also advocated a sabellid/terebelliform relationship for Pogonophora, and showed a tree with Pogonophora sister to Sabellida (containing Sabellidae and Serpulidae) based on the presence of a pair of anterior nephridia, and also placed Terebelliformia as sister to the Pogonophora/Sabellida clade, based on the shared presence of uncinigerous chaetae. Rouse and Fauchald (1997, Fig. 73), in their polychaete reclassification, placed Siboglinidae in the clade Sabellida, together with Oweniidae, Sabellariidae, Serpulidae and Sabellidae. In five of the six analyses they performed, Siboglinidae was sister group to a clade comprising Sabellariidae, Serpulidae and Sabellidae, and in two of these Oweniidae was also the successive sister to that clade. In the other analysis, Siboglinidae was sister to the terebelliforms. However, the support for all these topologies was weak, and in further analyses by Rouse (1999, 2000) Oweniidae did not group with the remaining Sabellida.

Our results, that Siboglinidae and Oweniidae are sister taxa, and that Oweniidae is not closely related to Sabellida, goes against most recent suggestions in the literature. There are a few exceptions, though. On morphological grounds, it was argued by Liwanow and Porfirjewa (1967), who suggested that Oweniidae was closely related to Pogonophora. It is also notable that a sistergroup relationship between *Owenia fusiformis* (Oweniidae) and Siboglinidae (represented by *Lamelli-brachia* and *Escarpia*) was recently reported in molecular analyses based on elongation factor 1-alpha sequences, focusing on the position of Myzostomida (Eeckhaut et al., 2000).

The combined analysis of the present study rejects both the grouping of Siboglinidae with Sabellida, and with Terebelliformia. A constrained sister-group relationship between Siboglinidae and a restricted Sabellida clade (including only Serpulidae and Sabellidae) requires 28 additional steps, 32 for a sister-group relationship between Siboglinidae and Sabellidae, and 17 for a sister-group relationship between Siboglinidae and Terebelliformia.

Unambiguous morphological characters that support (Siboglinidae, Oweniidae) in our combined analysis include chaetae emerging from the body wall in the posterior segments (character 66, state 4), and the presence of an intra-epidermal nerve cord (character 93, state 1).

The internal relationships within the Siboglinidae have been addressed based on a larger number of

terminals and in greater detail elsewhere (Halanych et al., 1998; Halanych et al., 2001; Rouse, 2001; Schulze, 2003). Common to our combined analysis and these studies is that the siboglinids, the included frenulates (i.e., *Siboglinum* and *Polybrachia*) and the vestimentiferans (i.e., *Lamellibrachia*, *Ridgeia* and *Riftia*) are each monophyletic. Our combined and molecular analyses also corroborate the relationship between the moniliferan *Sclerolinum* and the vestimentiferans, a previous result supported by morphological (Rouse, 2001) and molecular data (Halanych et al., 2001), but contradicted in the morphological analysis by Schulze (2003), where *Sclerolinum* was sister to the frenulates.

On the basis of their major morphological cladistic analyses of the polychaetes, Rouse and Fauchald (1997) recognized a major polychaete clade named Canalipalpata (referring to the presence of grooved palps), consisting of three subclades: Sabellida, Spionida and Terebelliformia. Our study involved a total of 16 representatives of the Sabellida clade, including two oweniids, two sabellariids, four sabellids, two serpulids, and six siboglinids. The apomorphy for Sabellida, as formulated by Rouse and Fauchald (1997), was the fusion of the prostomium with the peristomium. Nevertheless, this group was weakly supported, and it is not surprising that Sabellida is non-monophyletic in our hypotheses. Terebelliformia has a derived position within a paraphyletic Sabellida grade, whereas representatives of Sabellida are scattered in three separate clades in the trees: (Oweniidae, Siboglinidae) as sister to remaining Sabellida and Terebelliformia, plus the spionid *Polydora* and the flabelligerid *Diplocirrus* (Fig. 1B–D).

All our analyses support the close relationships between Sabellidae and Serpulidae, a result which is highly expected, based on the morphology of the animals (e.g., Dales, 1962; Fauchald, 1977; Pettibone, 1982; Fitzhugh, 1989; Rouse and Fauchald, 1997; Rouse and Pleijel, 2001). However, the relationships between these two groups are more ambiguous. In certain combined analyses of the sensitivity analysis, including that with the optimal alignment parameters, Sabellidae is not recovered as monophyletic owing to the nested position of Serpulidae (as shown in Fig. 1C), whereas in others analyses, Serpulidae is sister to Sabellidae (as showed in Fig. 1D). Although our taxon sampling is too sparse to address this issue appropriately, it certainly warrants further study.

Our results also contradict previous hypotheses with regard to the position of Sabellariidae. In more recent studies they are placed either close to taxa such as Serpulidae and Sabellidae (Knight-Jones, 1981; Fitzhugh, 1989; Rouse and Fauchald, 1997), based on the presence of uncini in a notopodial position (chaetal inversion), or close to Pectinariidae (Fauchald, 1977), based on the similarities in larval metamorphosis and of the head structures. In our trees *Polydora* (Spionidae) is

sister to Sabellariidae, and actually supports a suggestion of Dales (1962), who argued that Sabellariidae is closely related to spiomorphs, and placed them within the taxon Spionida. The current results are far from conclusive on this issue, considering the weak support and the incomplete taxon sampling, but it merits further examination. Furthermore, the placement of *Magelona* (a taxon considered part of Spionida) as sister to *Fauveliopsis*, makes Spionida polyphyletic, a result which certainly appears counter-intuitive.

The name Terebellida was first applied by Dales (1962) to include Ampharetidae, Pectinariidae and Terebellidae, all polychaetes having multiple grooved palps, and was subsequently expanded by Rouse and Fauchald (1997) to refer to a clade including other members with grooved palps (e.g., Acrocirridae, Cirratulidae, Flabelligeridae). Although Rouse and Fauchald (1997) identified several synapomorphies for this group, such as the presence of a first segment without chaetae, and the presence of gular membrane and heart bodies, the monophyly of Terebellida was never recovered in any of our analyses. Our results indicate that both the presence of chaetae on segment 1 (character 42 for notochaetae and character 46 for neurochaetae), and the presence of heart bodies (101), are highly homoplastic, whereas the presence of a gular membrane is a less general condition and may be a synapomorphy for a more restricted group, Terebelliformia (although subsequently lost in *Paralvinella*). The strong support for the position of the clade (Sabellidae, Serpulidae), as nested within a group containing the flabelligerid *Diplocirrus* and Terebelliformia, and the position of Cirratulidae forming a basal clade with (*Fauveliopsis*, *Magelona*), sister-group to the remaining ingroup taxa, constitute major differences from Rouse and Fauchald's (1997) delineation of Terebellida.

Rouse and Pleijel (2001), while accepting Terebellida as formulated by Rouse and Fauchald (1997), divided this clade into two subgroups: Cirratuliformia and Terebelliformia. Cirratuliformia was further delineated to include taxa with a single pair of palps (with the exception of some Cirratulidae with seemingly multiple palps, and Ctenodrilidae and Fauveliopsidae without palps). This group contains Acrocirridae, Cirratulidae, Ctenodrilidae, Fauveliopsidae, Flabelligeridae, Poeobiidae (and *Flota*) and *Sternaspis*. It is represented in our study by two cirratulids (*Cirriiformia* and *Dodecaceria*), one fauveliopsid (*Fauveliopsis*) and one flabelligerid (*Diplocirrus*). However, in none of the combined data analyses of the sensitivity analysis do Cirratuliformia appear as monophyletic. This result is not surprising because it was poorly supported in the cladistic analysis of Rouse and Fauchald (1997). We conclude that the status of Cirratuliformia merits further study and suggest that the preliminary morphological analysis of

Cirratuliformia by Rouse and Pleijel (2003) may not have had sufficient taxon sampling to address this issue.

Terebelliformia, as treated by most recent authors, includes Alvinellidae, Ampharetidae, Pectinariidae, Terebellidae and Trichobranchidae (sometimes treated as a subgroup of Terebellidae). All these family ranked-taxa belonging to Terebelliformia are represented in our study. The monophyly of this group has strong support (Jac 95), and it is also present in all combined analyses in the sensitivity analysis. In the combined analysis, it was unambiguously supported by the presence of strongly contractile palps (11), multiple prostomial palps (12), the presence of ventral glandular areas (39), and a series of large nephridia in anterior segments (97). Rouse and Pleijel (2001) pointed out the current lack of evidence for separating Trichobranchidae and Terebellidae, and the risk that recognition of the former would result in the latter being paraphyletic. Nevertheless, and with due consideration to our limited taxon sampling, our results instead corroborate the previous findings of Rousset et al. (2003) and Colgan et al. (2001), that the two groups are non-nested. However, in contrast to Rousset et al. (2003), our study Trichobranchidae is not sister to Alvinellidae, but is instead sister to the remaining terebelliform taxa, although this topology is only weakly supported. Perhaps more surprising, Terebellidae was never found to be monophyletic, mainly owing to the sister group relationship between the terebellid *Pista* and *Pectinaria* (Pectinariidae), a result which is unexpected and appears counter-intuitive, but nevertheless is well supported; this relationship also warrants further study.

Another interesting result concerns the Alvinellidae relationships with the other Terebelliform taxa, an issue that has previously been addressed in a number studies (Féral et al., 1994; Rouse and Fauchald, 1997; Colgan et al., 2001; Rousset et al., 2003). The combined data (Fig. 1C,D) contradict the result of Colgan et al. (2001) and Rousset et al. (2003), which indicated that Alvinellidae is closely related to Trichobranchidae, and instead indicates that Ampharetidae is made paraphyletic by the recognition of Alvinellidae as a family ranked-taxon. Accordingly, Desbruyères and Laubier's (1980) original allocation of *Alvinella*—as part of Ampharetidae—is not contradicted by our results. However, although our study is based on more data, the taxon sampling was not made to address this particular issue, and we consider relationships among these taxa as unsettled.

The combined analyses of the sequence data of the two nuclear ribosomal genes (part of 18S rRNA and D1 region of 28S rRNA) and the morphological data provide strong support that the former deuterostomian phyla Pogonophora and Vestimentifera, recently renamed as the polychaete family Siboglinidae by Rouse and Fauchald (1997), are monophyletic, and, although weaker, that they have a sister group relationship with

Oweniidae. The present analysis also corroborates the monophyly of Terebelliformia as currently delineated, but demonstrates that the delineation of Terebellida and Sabellida (both *sensu* Rouse and Fauchald, 1997) requires further attention.

Acknowledgments

We wish to thank Ken Halanych for providing specimens, Laetitia Plaisance for support and advice during the lab work, and two anonymous reviewers for valuable comments. Collection of polychaetes at Kristineberg Marine Research Station by FP was supported by the European Community ARI-programme, and for FP and VR at Trondhjem Biological Station by the European Community ARI-programme Trondheim Marine Systems Research Infrastructure. Financial for FP support was provided by Formas, dnr 2004-0085, and to VR by La Fondation des Treilles (Paris), and an Annette Kade Fellowship for work at the American Museum of Natural History.

References

- Bartolomaeus, T., 1995. Structure and formation of the uncini in *Pectinaria koreni*, *Pectinaria auricoma* (Terebellida) and *Spirorbis spirorbis* (Sabellida): implications for annelid phylogeny and the position of the Pogonophora. *Zoomorph.* 115, 161–177.
- Bartolomaeus, T., 1998. Chaetogenesis in polychaetous Annelida—significance for annelid systematics and the position of the Pogonophora. *Zoology, Anal. Complex Systems*, 100, 348–364.
- Black, M.B., Halanych, K.M., Maas, P.A.Y., Hoeh, W.R., Hashimoto, J., Desbruyères, D., Lutz, R.A., Vrijenhoek, R.C., 1997. Molecular systematics of vestimentiferan tubeworms from hydrothermal vents and cold-water seeps. *Mar. Biol.* 130, 141–149.
- Boore, J.L., Brown, W.M., 2000. Mitochondrial genomes of *Galatheidinium*, *Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. *Mol. Biol. Evol.* 17, 87–106.
- Brusca, R., Brusca, G.J., 2003. *Invertebrates*. Sinauer Associates, Sunderland, MA.
- Colgan, D.J., Hutchings, P.A., Brown, S., 2001. Phylogenetic relationships within the Terebellomorpha. *J. Mar. Biol. Ass. UK*, 81, 765–773.
- Dales, R.P., 1962. The polychaete stomodeum and the inter-relationships of the families of Polychaeta. *Proc. Zool. Soc. Lond.* 139, 389–428.
- Desbruyères, D., Laubier, L., 1980. *Alvinella pompejana* General sp. nov., Ampharetidae aberrant des sources hydrothermales de la ride Est-Pacifique. *Oceanol. Acta*, 3, 267–274.
- Eeckhaut, I., McHugh, D., Mardulyn, P., Tiedemann, R., Monteyne, D., Jangoux, M., Milinkovitch, C., 2000. Myzostomids: a link between trochozoans and flatworms? *Proc. R. Soc. Lond. Ser. B*, 267, 1383–1392.
- Farris, S.J., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, 12, 99–124.
- Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. *Syst. Biol.* 44, 570–572.

- Fauchald, K., 1977. The polychaete worms. Definitions and keys to the orders, families and genera. Nat. Hist. Mus. Los Angeles County Sci. Series, 28, 1–188.
- Féral, J.P., Philippe, H., Desbruyères, D., Laubier, L., Derelle, E., Chenuil, A., 1994. Molecular phylogeny of the active Pacific Ocean hydrothermal vents alvinellid Polychaetes. C. R. Acad. Sci., Paris, 317, 771–779.
- Filippova, A.V., Tzetlin, A.B., Purschke, G., 2003. Morphology and ultrastructure of the anterior end of *Diplocirrus longisetosus* Marenzeller, 1890 (Flabelligeridae, Polychaeta, Annelida). Hydrobiol. 496, 215–223.
- Fitzhugh, K., 1989. A systematic revision of the Sabellidae-Caobangidae-Sabellonidae complex (Annelida: Polychaeta). Bull. Am. Mus. Nat. Hist. 192, 1–104.
- Fitzhugh, K., Rouse, G.W., 1999. A remarkable new genus and species of fan worm (Polychaeta: Sabellidae: Sabellinae) associated with some marine gastropods. Invertebr. Biol. 118, 357–390.
- Gardiner, S.L., 1978. Fine structure of the ciliated epidermis on the tentacles of *Owenia fusiformis* (Polychaeta, Oweniidae). Zool. Morph. 91, 37–48.
- Giribet, G., Distel, D.L., Polz, M., Sterrer, W., Wheeler, W.C., 2000. Triploblastic relationships with emphasis on the Acoelomates and the position of Gnathostomulida, Cyliophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. Syst. Biol. 49, 539–562.
- Goloboff, P., 1999. NONA (NO NAME), Version 2. Published by the author.
- Grant, T., Kluge, A.G., 2003. Data exploration in phylogenetic inference: scientific, heuristic, or neither. Cladistics, 19, 379–418.
- Halanych, K.M., Feldman, R.A., Vrijenhoek, R.C., 2001. Molecular evidence that *Sclerolinum brattstromi* is closely related to Vestimentifera, not to frenulate pogonophorans (Siboglinidae, Annelida). Biol. Bull. 201, 65–75.
- Halanych, K.M., Lutz, R.A., Vrijenhoek, R.C., 1998. Evolutionary origins and age of vestimentiferan tube-worms. Cah. Biol. Mar. 39, 355–358.
- Hanson, J., 1950. The blood system in the Serpulimorpha (Annelida, Polychaeta). I. The anatomy of the blood system in the Serpulidae. Q. J. Microsc. Sci. 91, 111–129.
- Hartman, O., 1951. Fabricinae (featherduster polychaetous annelids) in the Pacific. Pacif. Sci. 5, 379–391.
- Hartman, O., 1954. Pogonophora Johansson, 1938. Syst. Zool. 3, 183–185.
- Hausen, H., Bartolomaeus, T., 1998. Setal structure and chaetogenesis in *Scolecopsis squamata* and *Malacoceros fuliginosus* (Spionidae, Annelida). Acta Zool. Stockh. 79, 149–161.
- Hilbig, B., 2000. 8. Family Amparetidae Malmgren, 1867. In: Blake, J.A., Hilbig, B., Scott, P.H. (Eds.), Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel. Santa Barbara Museum of Natural History, Santa Barbara, pp. 169–230.
- Holthe, T., 1986. Evolution, systematics, and distribution of the Polychaeta Terebellomorpha, with a catalogue of the taxa and a bibliography. Gunneria, 55, 1–236.
- Ivanov, A.V., 1963. Pogonophora. Academic Press, London.
- Ivanov, A.V., 1970. Verwandtschaft und Evolution der Pogonophoren. Z. Zool. Syst. Evol.-Forsch. 8, 109–119.
- Johansson, K.E., 1968. Pogonophora. Handb. Zool., Gegr. Willy Kükenthal 3(2) Lief. 18, 1–50.
- Jones, M.L., 1985. On the Vestimentifera, new phylum: Six new species, and other taxa, from hydrothermal vents and elsewhere. Bull. Biol. Soc. Wash. 6, 117–158.
- Knight-Jones, P., 1981. Behaviour, setal inversion and phylogeny of Sabellida (Polychaeta). Zool. Scr. 10, 183–202.
- Kojima, S., 1998. Paraphyletic status of Polychaeta suggested by phylogenetic analysis based on the amino acid sequences of elongation factor 1-alpha. Mol. Phyl. Evol. 9, 255–261.
- Kojima, S., Hashimoto, T., Hasegawa, M., Murata, S., Ohta, S., Seki, H., Okada, N., 1993. Close phylogenetic relationship between Vestimentifera (Tube Worms) and Annelida revealed by the amino acid sequence of Elongation Factor 1-alpha. J. Mol. Evol. 37, 66–70.
- Kojima, S., Segawa, R., Hashimoto, J., Ohta, S., 1997. Molecular phylogeny of vestimentiferans collected around Japan, revealed by the nucleotide sequences of mitochondrial DNA. Mar. Biol. 127, 507–513.
- Kritzer, H., 1984. Chapter 52. Terebellidae Grube 1850. In: Uebelacker, J.M., Johnson, P.G. (Eds.), Taxonomic guide to the Polychaetes of the Northern Gulf of Mexico, Vol. 7. Barry A. Vittor & Associates, Inc., Mobile, Alabama, pp. 52–1 to 52–7.
- Lê, H.L.V., Lecointre, G., Perasso, R., 1993. A 28S rRNA based phylogeny of the Gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. Mol. Phyl. Evol. 2, 31–51.
- Liwanow, N.A., Porfirjewa, N.A., 1967. Die Organisation der Pogonophoren und deren Beziehungen zu den Polychäten. Biol. Zentralbl. 86, 177–204.
- McHugh, D., 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. Proc. Natl Acad. Sci. USA, 94, 8006–8009.
- Meyer, K., Bartolomaeus, T., 1996. Ultrastructure and formation of the hooked setae in *Owenia fusiformis* delle Chiaje, 1842: Implications for annelid phylogeny. Can. J. Zool. 74, 2143–2153.
- Mickevich, M.F., Farris, J.S., 1981. The implications of congruence in *Medidia*. Syst. Zool. 30, 351–370.
- Miura, T., Tsukahara, J., Hashimoto, J., 1997. *Lamellibranchia satsuma*, a new species of vestimentiferan worms (Annelida: Pogonophora) from a shallow hydrothermal vent in Kagoshima Bay, Japan. Proc. Biol. Soc. Wash. 110, 447–456.
- Nielsen, C., 1995. Animal Evolution. Oxford University Press, Oxford.
- Nixon, K.C., 1999. Winclada (BETA), Version 0.9.9. Published by the author, Ithaca, NY.
- Norén, M., Jondelius, U., 1999. Phylogeny of Proleceuthophora (Platyhelminthes) inferred from 18S rDNA sequences. Cladistics, 15, 103–112.
- Nørrevang, A., 1970. On the embryology of *Siboglinum* and its implications for the systematic position of the Pogonophora. Sarsia, 42, 7–16.
- Orrhage, L., 2001. On the anatomy of the central nervous system and the morphological value of the anterior end appendages of Ampharetidae, Pectinariidae and Terebellidae (Polychaeta). Acta Zool. Stockh. 82, 57–71.
- Peterson, M.E., 2000. A new genus of Fauveliopsidae (Annelidae: Polychaeta), with a review of its species and redescription of some described taxa. Bull. Mar. Sci. 67, 491–515.
- Pettibone, M.H., 1982. Annelida. In: Parker, S.J. (Ed.), Synopsis and Classification of Living Organisms. 2. McGraw-Hill, New York, pp. 1–43.
- Pleijel, F., 1995. On character coding for phylogeny reconstruction. Cladistics, 11, 309–315.
- Rouse, G.W., 1990. New species of *Oropsis* and a new record for *Augeneriella* cf. *dubia* Hartmann-Schröder, 1965 from eastern Australia (Polychaeta: Sabellidae). Rec. Aust. Mus. 42, 221–235.
- Rouse, G.W., 1999. Trochophore concepts: ciliary bands and the evolution of larvae in spiralian Metazoa. Biol. J. Linn. Soc. 66, 411–464.
- Rouse, G.W., 2000. Bias? What bias? The evolution of downstream larval-feeding in animals. Zool. Scr. 29, 213–236.
- Rouse, G.W., 2001. A cladistic analysis of Siboglinidae Caullery, 1914 (Polychaeta, Annelida): formerly the phyla Pogonophora and Vestimentifera. Zool. J. Linn. Soc. 132, 55–80.
- Rouse, G.W., Fauchald, K., 1995. The articulation of annelids. Zool. Scr. 24, 269–301.

- Rouse, G.W., Fauchald, K., 1997. Cladistics and polychaetes. *Zool. Scr.* 26, 139–204.
- Rouse, G.W., Pleijel, F., 2001. *Polychaetes*. Oxford University Press, Oxford.
- Rouse, G.W., Pleijel, F., 2003. Problems in polychaete systematics. *Hydrobiol.* 496, 175–189.
- Rousset, V., Rouse, G.W., Féral, J.-P., Desbruyères, D., Pleijel, F., 2003. Molecular and morphological evidence of Alvinellidae relationships (Terebelliformia, Polychaeta, Annelida). *Zool. Scr.* 32, 185–197.
- Schulze, A., 2002. Histological and ultrastructural characterization of the intravasal body in Vestimentifera (Siboglinidae, Polychaeta, Annelida). *Cah. Biol. Mar.* 43, 355–358.
- Schulze, A., 2001. Ultrastructure of ophistosomal chaetae in Vestimentifera (Pogonophora, Obturata) and implications for phylogeny. *Acta Zool. Stockh.* 82, 127–135.
- Schulze, A., 2003. Phylogeny of Vestimentifera (Siboglinidae, Annelida) inferred from morphology. *Zool. Scr.* 32, 321–342.
- Southward, E.C., 1988. Development of the gut and segmentation of newly settled stages of *Ridgeia* (Vestimentifera): implications for relationship between Vestimentifera and Pogonophora. *J. Mar. Biol. Ass. UK*, 68, 465–487.
- Thompson, J.D., Gibson, D.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 24, 4876–4882.
- Uschakov, P.V., 1933. Eine neue Form aus der Familie Sabellidae (Polychaeta). *Zool. Anz.* 104, 205–208.
- Watson, A.T., 1928. Observations on the habits and life-history of *Pectinaria (Lagis) koreni*, Mgr. Proc. Trans. Lpool Biol. Soc. 42, 25–60.
- Webb, M., 1964a. Additional notes on *Sclerolinum brattstromi* (Pogonophora) and the establishment of a new family, Scleroliniidae. *Sarsia*, 16, 47–58.
- Webb, M., 1964b. The posterior extremity of *Siboglinum fiordicum* (Pogonophora). *Sarsia*, 15, 33–36.
- Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Syst. Biol.* 44, 321–331.
- Winnepenninckx, B., Backeljau, T., De Wachter, R., 1993. Extraction of high molecular weight DNA from molluscs. *Trends Genet.* 9, 407.
- Winnepenninckx, B., Backeljau, T., De Wachter, R., 1995. Phylogeny of protostome worms derived from 18S rRNA sequences. *Mol. Biol. Evol.* 12, 641–649.