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ORIGINAL ARTICLE

A scaleless scale worm: Molecular evidence for the phylogenetic placement of *Pisione remota* (Pisionidae, Annelida)

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Abstract

Pisionidae is a group of interstitial worms whose phylogentic affinities have been enigmatic. They have been allied to different Phyllodocida taxa. Although originally associated with Glyceridae and Phyllodocidae, they are more recently considered to be related to scale worms. Scale worms are a well-defined taxon, Aphroditiformia, within Annelida due to the unique possession of dorsal scales called elytra. Pisionidae lack elytra but they have been grouped with scale worms because they possess two pairs of jaws with venom glands, also found in Glyceridae. Determining the phylogenetic position of Pisionidae is important for understanding if features such as elytra and venomous jaws are evolutionarily labile in annelid history. Therefore, we explored 18S rDNA and Cytochrome *c* Oxidase subunit I data from several Aphroditiformia, Pisionidae, and other Phyllodocida to determine the phylogenetic placement of Pisionidae. Maximum likelihood and Bayesian inference of separate and combined data sets were conducted. All analyses support a derived position of Pisionidae within Aphroditiformia, close to Pholoidae and Sigalionidae. The loss of elytra in Pisionidae is probably due to adaptation for interstitial life. Furthermore, the results reject a monophyletic Aphroditoidea comprising Acoetidae, Aphroditidae, Eulepethidae and Polynoidae. Thus, the possession of only simple chaetae is either symplesiomorphic or convergent.

Key words: *Interstitial species, 18S, Cytochrome c Oxidase, molecular phylogeny, toxin*

Introduction

Pisionidae is a small aberrant family of Phyllodocida annelids with 40 nominal species in 4 genera (Rouse & Pleijel 2001), but recent descriptions of 16 new species from restricted areas (de Wilde & Govaere 1995; Yamanishi 1998) suggest that pisionid diversity is underestimated. Species inhabit sand in clean areas of shallow waters and intertidal zones (Åkesson 1961). However, some species have also been reported to depths of 1000 m and a freshwater species is also known (San Martin et al. 1998; Rouse & Pleijel 2001). Generally, Pisionidae are regarded as interstitial, but Rouse and Pleijel (2001) question this categorization due to their size, up to a few centimeters, relative to the size of the sand grains they live in. They consider Pisionidae as infaunal even though these worms have a long slender body like some other interstitial species.

The anterior end of Pisionidae is highly modified. The prostomium is more or less reduced and the head usually has a protruding pair of palps and a pair of antennae resembling the head of the phyllodocid *Eteone* (Aiyar & Alikunhi 1940). Only in *Pisionella* is a median antenna present. The anterior end of *Pisione* lack antennae and have a small inconspicuous prostomium positioned dorsally as well as anteriorly directed cirri of the 1st chaetiger (Aiyar & Alikunhi 1940; Siewing 1953; Stecher 1968; Rouse & Pleijel 2001). Pisionidae also possess an axial, muscular proboscis with two pairs of jaws and venom glands exhibiting similarities to scale worms (Wolf 1986). Pisionid copulation and reproduction is highly specialized and unique in annelids (Aiyar & Alikunhi 1940; Alikunhi 1951; Åkesson 1961; Stecher 1968). Male copulatory organs appear at sexual maturity on several median segments. Each organ consists of modified cirri from the parapodia and elongated protrusible tissue that is differentiated around the efferent spermioduct (Åkesson 1961; Stecher 1968). Sperm is transferred to receptacula semines of the females using the copulatory organ.

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The phylogenetic affinity of Pisionidae within Phyllodocida is uncertain. Grube (1857) in the first description of a pisionid referred them to Phyllodocidae and Glyceridae. This view was corroborated by other authors based on similarities of uniramous parapodia, nephridia, and the anterior end (Aiyar & Alikunhi 1940; Alikunhi 1951). Affinities to several other Phyllodocida taxa have also been proposed: Nephtyidae (Ehlers 1901; Alikunhi 1951; Banse 1956), Hesionidae (Grube 1857; Ehlers 1901; Hartman 1939), and Syllidae (Ehlers 1901). A close relationship to Glyceridae, Goniadidae, and Paralacydoniidae was recovered in a morphological cladistic analysis of polychaetes in general (Rouse & Fauchald 1997). However, Levinsen (1887), and later others (Ehlers 1901; Southern 1914; Hartman 1939) regarded Pisionidae as closely related to scale worms, a group treated under a variety of "formal" names including, Aphroditidae, Aphroditiformia, Aphroditacea and Aphroditoidea (see Rouse & Pleijel 2001). The best support for this hypothesis was based on larval development (Banse 1956; Åkesson 1961) and a cladistic analysis of Phyllodocida (Pleijel & Dahlgren 1998). Due to the possession of two pairs of jaws with venom glands, Rouse and Pleijel (2001) included them within Aphroditiformia without any specific affiliation. However, Glyceridae also possess two pairs of jaws with venom glands and thus their position is still controversial. Åkesson (1961) pointed out the resemblance of pisionids and species of *Pholoe* and thus was the only one to propose a placement within Aphroditiformia.

Aphroditiformia, or scale worms, is one of the best morphologically defined annelid taxa due to the possession of scales, i.e. elytra. It comprises six recognized families, Acoetidae, Aphroditidae, Eulepethidae, Pholoidae, Polynoidae and Sigalionidae (sensu Rouse & Pleijel 2001), that together include approximately 1290 species in about 220 genera (Beesley et al. 2000). Aphroditiformia is usually incorporated within Phyllodocida and thus within Aciculata and Canalipalpata (Rouse & Fauchald 1997; Pleijel & Dahlgren 1998). Species are usually epi- and infaunal on hard and soft substrate from intertidal zones to abyssal depth. Several polynoid species are commensals on a variety of hosts including echinoderms, cnidarians, polychaetes, bivalves and decapods. One of the best known genera of the group is *Aphrodita*, the "sea mouse", which was one of the first scientifically described polychaete genera. The common name alludes to the Scandinavian slang term of "mouse" for human female genitalia with the scientific etymology referring to the Greek goddess of love (Rouse & Pleijel 2001). Furthermore, Aphroditidae show iridescence along their sides due to their fine chaetae, which act like photonic crystals (McPhedran et al. 2001).

Although Aphroditiformia is well defined and long known, only the cladistic analysis of Rouse and Fauchald (1997) addressed the interrelationships to some degree. Due to this analysis and the possession of only simple chaetae, Rouse and Pleijel (2001) recognized the taxon Aphroditoidea comprising Acoetidae, Aphroditidae, Eulepethidae and Polynoidae. Within this taxon Acoetidae and Aphroditidae are proposed to be closely related. The sister group of Aphroditoidea is Sigalionidae (Rouse & Fauchald 1997). Thus, the basal group in Aphroditiformia is Pholoidae. However, the investigation is based on a data set assembled for polychaetes in general and inter-Aphroditiformia relationships are weakly supported and unambiguous synapomorphies are lacking within the group. Furthermore, a sister taxon of Aphroditiformia within Phyllodocida is not well established at all. The cladistic analysis of Pleijel and Dahlgren (1998) showed Aphroditiformia as part of a basal polytomy within Phyllodocida, whereas the analysis of Rouse and Fauchald (1997) results in a basal position. Molecular analyses have not been conducted yet addressing any of these issues as well as the position of Pisionidae.

Therefore, the purpose of this study is to examine the position of Pisionidae within the Phyllodocida using molecular markers. Based on the success of previous studies (e.g. Dahlgren et al. 2000; Struck et al. 2002), we employed molecular data of nuclear 18S rDNA and the mitochondrial Cytochrome *c* Oxidase subunit I (COI) genes. Although taxon sampling of Aphroditiformia is limited, the results indicate a different understanding of the group than is currently considered. Individual and combined analyses of the two genes were performed using Maximum Likelihood (ML) and Bayesian Inference (BI).

Material and methods

Collection of molecular data

Table I lists taxa employed in this study, museum voucher numbers, and GenBank accession numbers of 18S and COI data. Full-length 18S sequences were obtained for 37 annelid taxa and approximately 620 nucleotide fragment of the Cytochrome c Oxidase subunit I for 19 taxa. All taxa examined were members of Aphroditiformia or other Phyllodocida except one Amphinomidae (*Paramphinome jeffreysi*).

Upon collection, samples were preserved in >70% non-denatured EtOH or frozen at −80°C. Genomic DNA was extracted using DNeasy Tissue Kit (Qiagen) according to the

Table I. List of taxa, 18S data, and COI data. Accession numbers of determined sequences in bold (Smithsonian Museum voucher number and locality given for taxa from which we collected data).

Taxon	Locality	Voucher No.	Accession number	
			18S	COI
Aphroditidae				
Aphrodite negligens	Griffin Bay, off San Juan Island, WA, USA; 48° 28.260′N 122° 59.241′W	USNM 1077212	AY894294	AY894309
Aphrodite sp.	Monterey Bay, Station 1 at 30 m, CA, USA; 36° 51.433′N 121° 51.438′W	USNM 1077213	AY894295	AY894310
Glyceridae				
Glycera americana			U19519	
Hesionidae				
Kefersteinia cirrata	Banyuls-sur-Mer, France	USNM 1077228	AY527052	
Pholoidae				
Pholoe pallida	near Tautra at 200–220 m, Trondheim, Norway; 63° 36.91′N 10° 40.07′E	USNM 1077214	AY894302	AY894318
Phyllodocidae				
Anaitides sp.	Point Sur, Station 12.1 at 63 m, CA, USA; 36° 55.182′N 121° 54.279′W	USNM 1077215	AY894293	AY894308
Eteone longa			AF448155	
Eteone picta	Roscoff, France	USNM 1077229	AY525626	
Eumida sp.	Point Sur, Station 14 at 92 m, CA, USA; 36° 49.847′N 122° 01.729′W	USNM 1077216	AY894296	AY894311
Eulalia viridis			AY525627	
Phyllodoce sp.			AB106249	
Sige sp.	Point Sur, Station 9.2 at 107 m, CA, USA; 36° 23.381′N 121° 57.974′W	USNM 1077217	AY894305	AY894320
Pilargidae				
Ancistrosyllis sp.			AF474280	
Pisionidae				
Pisione remota	Cefalù, Sicilia, Italy	USNM 1077230	AY525628	AF221575
Polynoidae				
Alentia gelatinosa	Concarneau, France	USNM 1077231	AY525630	
Gattyana ciliata	San Juan Channel, WA, USA; 48° 34.231′N 123° 02.247′W	USNM 1077218	AY894297	AY894312
Halosydna brevisetosa	Munlo Cove, Griffin Bay, WA, USA; 48° 29.513′N 123° 01.099′W	USNM 1077219	AY894298	AY894313
Harmothoë impar			U50968	
Harmothoë oculinarum	Rødberg at 200 m, Trondheim, Norway; 63° 28.36′N 10° 00.04′E	USNM 1077220	AY894299	AY894314
Lepidonotus squamatus	Munlo Cove, Griffin Bay, WA, USA; 48° 29.513′N 123° 01.099′W	USNM 1077221	AY894300	AY894316
Lepidonotus sublevis	Southern New England, Station 1 at 33 m, MA, USA; 41° 09.869′N 70° 25.0405′W	USNM 1077222	AY894301	AY894317
Paralepidonotus ampulliferus			AF519237	AY583698
Nephtyidae				
Nephtys hombergii			U50970	
Nephtys hombergii	Concarneau, France	Completely used	AY527054	
Nereididae				
Ceratonereis longiceratophora			AB106251	
Nereis limbata			U36270	
Nereis pelagica			AF474279	
Platynereis dumerilii	Seahorse Key dredge, FL, USA; 29° 05.415′ N 083° 04.268′W	USNM 1077223	AY894303	NC_000931: 808756
Sigalionidae				
Leanira sp.	Southern New England, Station 4 at 96 m, MA, USA; 40° 20.410′N 70° 46.765′W	USNM 1077224		AY894315
Sigalion bandaensis			AB106254	

Table I (*Continued*)

Taxon	Locality	Voucher No.	Accession number 18S	Accession number COI
Sigalion spinosa	Point Sur, Station 9.3 at 107 m, CA, USA; 36° 22.550′N 121° 59.010′W	USNM 1077225	AY894304	AY894319
Sthenalanella uniformis	Monterey Bay, Station 1 at 30 m, CA, USA; 36° 51.433′N 121° 51.438′W	USNM 1077226	AY894306	AY894322
Sthenelais boa	Blacks Beach, MA, USA; 41° 35.191′N 70° 38.665′W	USNM 1077227	AY894307	AY894321
Syllidae				
Autolytus prolifer			AF474295	
Brania sp.	Roscoff, France	USNM 1077232	AY525633	
Exogone naidina			AF474290	
Typosyllis armillaris			AF474292	
Amphinomidae				
Paramphinome jeffreysi			AY838856	AY838875

manufacturer's instructions. Amplification and sequencing of the nuclear 18S rDNA and mitochondrial COI genes used primers shown in Table II (see also Figure 1 for position of 18S rDNA primers). Approximately 1,800 bp of 18S were amplified by a HotStart-PCR protocol in a volume of 25 μl (prerun: 3 min 94°C; application of polymerase; 1 cycle: 3 min 94°C; 40 cycles: 1 min 94°C, 1 min 30 sec 40°C, 2 min 30 sec 72°C; 1 cycle: 7 min 72°C; reaction mix: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, ~1 ng/μl genomic DNA, 0.4 mM dNTPs, 0.8 μM of each primer (18e/18R1843), 0.04 U/μl *Taq* DNA Polymerase [Promega]). A 621 bp fragment of COI was amplified using a nested approach. Primers LCO1490 and CO1r were used the first PCR and primers LCO1490 and HCO2190 in the second. Both reactions were performed as a HotStart-PCR in a volume of 25 μl (prerun: 3 min 94°C; application of polymerase; 1 cycle: 2 min 94°C; 40 cycles: 30 sec 94°C, 1 min 50°C, 2 min 72°C; 1 cycle: 7 min 72°C; reaction mix: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, ~1 ng/μl genomic DNA, 0.2 mM dNTPs, 0.8 μM of each primer, 0.04 U/μl *Taq* DNA Polymerase [Promega]).

All products were verified on a 1% agarose gel and purified with the QIAquick PCR Purification Kit (Qiagen). If necessary, PCR products were size selected on agarose gels. A CEQ^{TM} 8000 Genetic Analysis System (Beckman Coulter) using CEQ dye terminator chemistry was used for bidirectional sequencing of all products.

Phylogenetic analyses

Because placement of Pisionidae within Phyllodocida was uncertain, we first employed 18S data for which a more inclusive set of taxa was available. This was followed by a more restrictive set of taxa,

Table II. Amplification and sequencing primers. Positions correspond to residues of *Homo sapiens* (18S) and *Platynereis dumerilii* (COI).

Name	Sequence (5′→3′)	Position	Direction	Reference
18S				
18e	CTG GTT GAT CCT GCC AGT	3–21	forward	Hillis & Dixon 1991
18F509	CCC CGT AAT TGG AAT GAG TAC A	548–569	forward	Struck et al. 2002
18L	GAA TTA CCG CGG CTG CTG GCA CC	609–632	reverse	Halanych et al. 1995
18R925D	GAT CYA AGA ATT TCA CCT CT	955–974	reverse	Burnette et al. 2005
18F997	TTC GAA GAC GAT CAG ATA CCG	1044–1065	forward	Struck et al. 2002
18r	GTC CCC TTC CGT CAA TTY CTT TAA G	1191–1215	reverse	Passamaneck et al. 2004
18F1435	AGG TCT GTG ATG CCC TTA GAT	1489–1509	forward	Burnette et al. 2005
18R1779	TGT TAC GAC TTT TAC TTC CTC TA	1811–1834	reverse	Struck et al. 2002
18R1843	GGA TCC AAG CTT GAT CCT TCT GCA GGT TCA CCT AC	1843–1877	reverse	Modified from Cohen et al. 1998
COI				
LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	14–38	forward	Folmer et al. 1994
HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	697–722	reverse	Folmer et al. 1994
CO1r	CCD CTT AGW CCT ARR AAR TGT TGN GG	1270–1295	reverse	Modified from Nelson & Fisher 2000

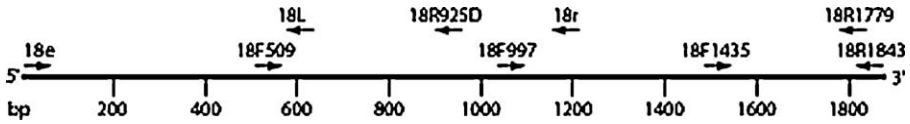

Figure 1. Relative position of 18S rDNA primers corresponding to residues of *Homo sapiens*.

focusing on Aphroditiformia, for COI and combined analyses. Based on the current understanding of annelid phylogeny (Rouse & Pleijel 2001) and data availability, the amphinomid *Paramphinome jeffreysi* was used as an outgroup taxon for the initial analysis. Subsequent analyses use *P. jeffreysi* and other (non-Aphroditiformia) Phyllodocida taxa. Limitation of taxa outside of the clade of interest, as in the COI and combined analyses, often allows for better alignments and fewer problems with noisy signal due to nucleotide saturation (Halanych 1998). Sequences were aligned with CLUSTALW using default settings (Thompson et al. 1994) and subsequently corrected by hand in GeneDoc (Nicholas & Nicholas 1997). Ambiguous positions were excluded from subsequent analyses. Alignments are available at TREEBASE (www.treebase.org).

For each data set (18S, COI, and combined), ML and BI analyses were conducted. Prior to all analyses, χ^2 tests of base frequency homogeneity across taxa were performed. Due to saturation and base frequency heterogeneity across taxa, third positions of COI were excluded from the analyses (see Results and Figure 2).

For ML analyses, appropriate models and parameters of sequence evolution for each data set were estimated by hierarchical Likelihood Ratio Tests (hLRT) using Modeltest V 3.06 (Posada & Crandall 1998, 2001) and are given under "Results". The most likely tree was reconstructed in PAUP*4.0b (Swofford 2002) using Tree-Bisection-Reconnection (TBR) branch swapping and 10 random taxon additions. The reliability of phylogenetic nodes was estimated by 100 bootstrap (BS) replicates with one random taxon addition and TBR branch swapping. To more confidently evaluate support for competing hypotheses, we used the SH test (Shimodaira & Hasegawa 1999). Two *a priori* hypotheses were compared against the best tree: first, that Pisionidae is not a taxon of Aphroditiformia, and second, that Aphroditoidea is indeed monophyletic.

MrModeltest 1.1b (Nylander 2002) was used to determine appropriate models of sequence evolution of each of the individual data sets for BI. MrBayes 3.0B4 (Huelsenbeck & Ronquist 2001) was used for BI with prior probability distributions of individual model parameters set according to the model specified by MrModeltest results. In the case of the combined data analysis, each partition was assigned its individual model and prior probability distributions. Partitions were unlinked to implement a partitioned likelihood analysis. Each Markov chain, three heated and one cold, ran simultaneously for 5×10^5 generations, with trees being sampled every

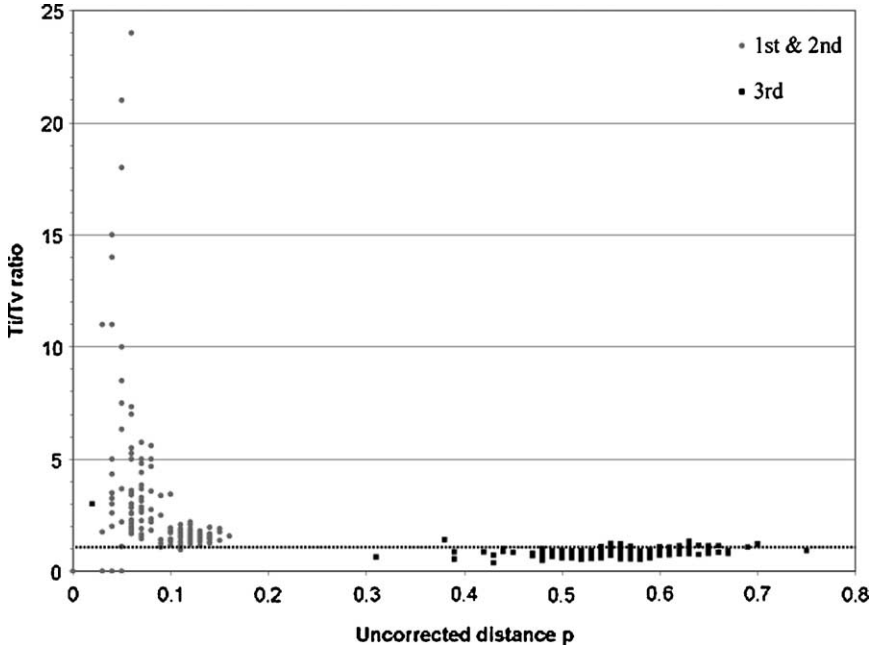

Figure 2. Plot T_i/T_v ratio against uncorrected distance p of both 1st and 2nd positions and 3rd positions of the COI data set.

100 generations for a total of 5001 trees. Based on convergence of likelihood scores the first 500 trees in each analysis were discarded as *burn in*. The majority-rule consensus tree containing posterior probabilities (PP) of the recovered topology was determined from the remaining 4501 trees.

Because PP are generally higher than BS values (see Figures 3–5 and Huelsenbeck et al. 2002) and a less reliable measurement of support than BS values (e.g. Suzuki et al. 2002), the term "significant support" refers herein to a BS $\geq$95.

Results

The aligned data set of 18S rDNA consisted of 2138 positions, from which 594 ambiguous positions were excluded. Of the 1544 unambiguously aligned positions, 307 sites were parsimony informative, 1054 constant and 183 parsimony uninformative. The χ^2 test showed homogeneity of base frequencies across taxa. The hLRT indicated the TRNef+I+Γ model for the ML analysis and the SYM+I+Γ for the BI analysis. The majority-rule consensus trees of the BI analysis was completely congruent with the best ML tree, -ln L = 8,061.57 (see Figure 3).

As expected based on morphology, monophyly of Phyllodocidae (BS: 100; PP: 1.00), Syllidae (BS: 100; PP: 1.00), Nereididae (BS: 79; PP: 0.99) and Nephtyidae (BS: 100; PP: 1.00) were all supported. Furthermore, a close relationship of Phyllodocidae and Glyceridae (BS: 80; PP: 1.00) as well as a clade of Nereididae, Pilargidae, Syllidae, Pisionidae and Aphroditiformia (BS <50; PP: 1.00) was recovered. Monophyly of Aphroditiformia, including the derived Pisionidae, was supported by a high BS value of 90 and a PP of 1.00 (Figure 3). This pisionid placement was significantly supported by the SH test over the alternative that excludes Pisionidae from

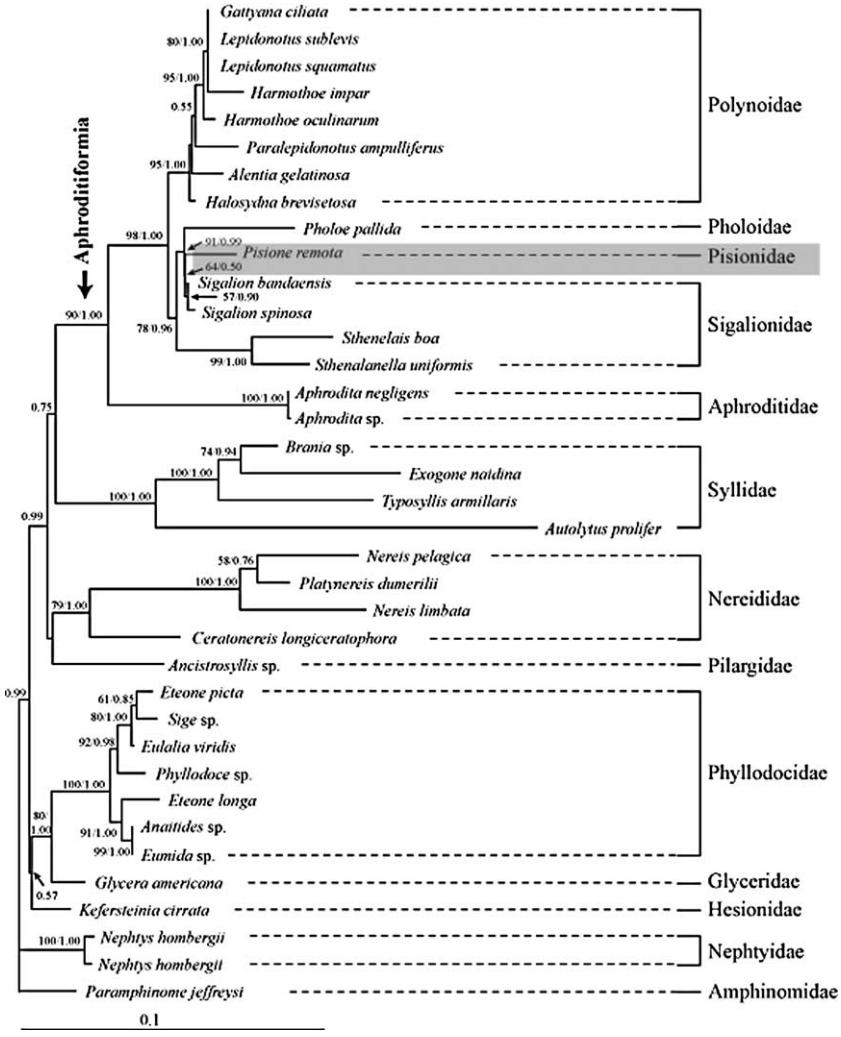

Figure 3. *18S data set.* ML phylogram based on TRNef+I+Γ (-ln L = 8,061.57). Only BS and PP above 50 or 0.50 shown, respectively. Settings-Base frequencies: A, C, G, T = 0.2500; Substitution rates: AG = 2.7990, CT = 4.1055, AC, AT, CG, TG = 1.0000; α = 0.678; pinvar = 0.4504.

Aphroditiformia ($p < 0.001$). Within Aphroditiformia, Aphroditidae was the most basal taxon and the clade comprising Polynoidae, Sigalionidae, Pholoidae and Pisionidae was significantly supported (BS: 98; PP: 1.00). Furthermore, a monophyletic Aphroditoidea was significantly rejected by the SH test ($p = 0.023$). Whereas Polynoid taxa were found to be monophyletic (BS: 95; PP: 1.00), Sigalionidae as currently recognized appears paraphyletic with two *Sigalion* species clustering with *Pholoe pallida* and *Pisione remota* (BS: 91; PP: 0.99). Based on the well-supported placement of *P. remota* within scale worms, the COI and combined analyses focused on the Aphroditiformia.

The COI data set comprised 612 positions, but 3rd positions appeared to be problematic for phylogenetic reconstruction. Namely, the T_i/T_v ratio of the 3rd position converged at a value of 1 (Figure 2) indicating saturation (e.g. Halanych & Robinson 1999). Additionally, the χ^2 test showed homogeneity of base frequencies across taxa for 1st and 2nd COI positions, but not for the 3rd position ($p < 0.00000001$). Therefore, the 204 3rd positions were excluded from the COI data set due to saturation. Of the remaining 408 sites, 74 were parsimony informative, 314 positions constant and 20 parsimony uninformative. The hLRT specified a TRN+Γ model for the ML analysis and a GTR+Γ model for the BI.

Even though ML (-ln L = 1,715.69; Figure 4A) and BI (Figure 4B) recovered different topologies, monophyly of Aphroditiformia including Pisionidae (BS: 85; PP: 1.00) was supported by both analyses (Figure 4A and 4B). Exclusion of Pisionidae was significantly rejected by the SH test ($p = 0.013$). Consistent with the 18S, results the Nereidid was placed closer to Aphroditiformia than Phyllodocidae (BS < 50; PP: 0.99). Unfortunately within Aphroditiformia, resolution was poor and neither monophyly of Sigalionidae nor Polynoidae was recovered. Whereas in the BI analysis Aphroditidae was basal (PP: 0.84; Figure 4B), in the best ML tree the polynoid *Halosydna brevisetosa* occupied this position (Figure 4A). The SH test failed to show that a

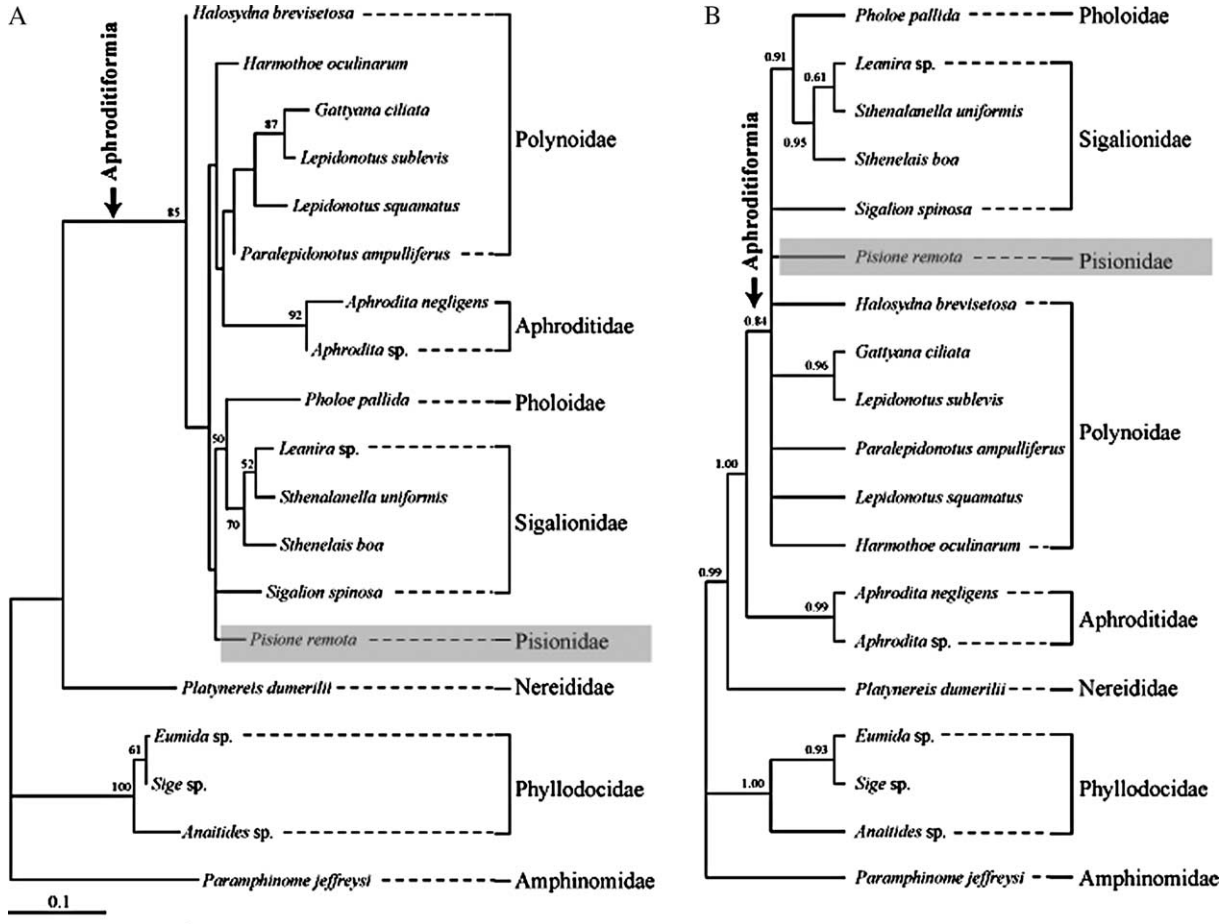

Figure 4. *COI data set.* A) ML phylogram based on TRN+Γ (-ln L = 1,715.69). Only BS above 50 shown. Settings-Base frequencies: A = 0.1997, C = 0.2650, G = 0.2350, T = 0.3003; Substitution rates: AG = 2.1121, CT = 13.5600, AC, AT, CG, TG = 1.0000; α = 0.0948. B) 50% majority rule consensus tree of BI analyis using GTR+Γ. Only PP above 0.50 shown.

monophyletic Aphroditoidea was significantly different ($p = 0.249$), but this may be due to poor resolution with Aphroditiformia. Similar to the 18S topology, the best ML tree recovered a Pisionidae/Pholoidae/Sigalionidae clade, but without bootstrap support. The BI analysis resulted in a large polytomy within Aphroditiformia.

For the ML analysis of the combined data set, the hLRT resulted in the GTR+I+Γ model. As mentioned above, BI used a partitioned analysis with the individual 18S and COI models previously employed. Again, the best tree of the ML analysis, -ln L = 9,665.87, and the BI majority-rule consensus trees were completely congruent (Figure 5). Monophyly of Aphroditiformia including Pisionidae (BS: 95; PP: 1.00) was significantly supported (Figure 5).

As in other analyses, this placement was confirmed by the SH test ($p = 0.003$). Sister taxon to Aphroditiformia was Nereididae (BS: 100; PP: 1.00), and Aphroditidae was the basal lineage of Aphroditiformia (BS: 100; PP: 1.00). Similar to the 18S analysis, the SH test of the combined data set significantly rejected a monophyletic Aphroditoidea ($p = 0.013$). Monophyly of Polynoidae was corroborated (BS: 71; PP: 1.00) and *P. remota* was closely related to Sigalionidae and Pholoidae (BS: 91; PP: 1.00), specifically to *Sigalion spinosa* and *P. pallida* (BS: 91; PP: 0.99).

Discussion

Pisionids are scaleless scale worms. 18S and COI data confirm that Pisionidae is an Aphroditiformia taxon with highly derived morphology. Both bootstrap values and SH test results showed this result was significantly supported. Only two other scale worm genera without elytrae are known. *Metaxypsamma uebelackerae*, an interstitial species in fine to coarse sands, have paired mounds of papillae very similar to structures in nectochaete I and II larval stages of *Pholoe synophthalmica*, leading to speculation of a neotenic origin (Wolf 1986). Additionally, *M. uebelackerae* shows considerable similarities to *Pholoe swedmarki* (Laubier 1975) which possesses elytra. Both species inhabit the interstitium and show similar adaptations to this habitat including: Reduced number of segments (24 or 27, respectively), reduced or absent notopodia, and reduced tentacular cirri (Wolf 1986). In *P. swedmarki*, the 2 ventral cirri are smaller and differently shaped than the dorsal pair. Comparatively in *M. uebelackerae*, the 2 ventral and 2 dorsal cirri are of similar size and shape (apparently neither Laubier 1975 nor Wolf 1986 provided measures of cirri length). The loss of elytra could have enabled *M. uebelackerae* to invade even smaller interstitial spaces than *P. swedmarki* (Wolf 1986). The other scaleless genus *Palmyra*, with up to 40 segments, was transferred from

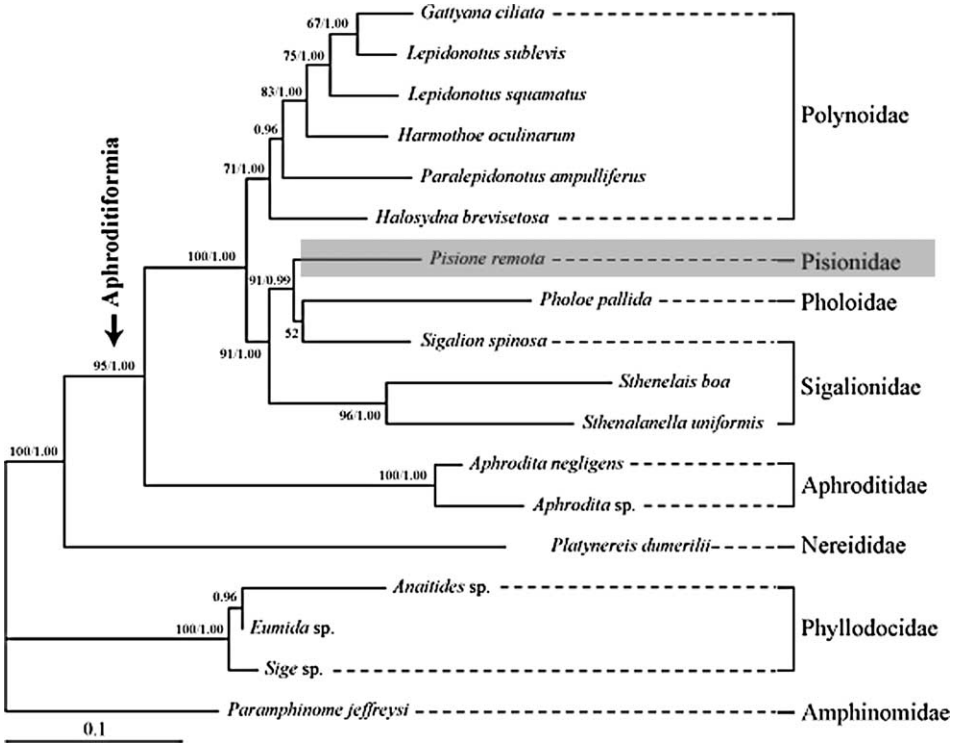

Figure 5. *Combined data set.* ML phylogram based on GTR+I+Γ (-ln L = 9,665.87). Only BS and PP above 50 or 0.50 shown, respectively. Settings-Base frequencies: A = 0.2685, C = 0.2066, G = 0.2297, T = 0.2952; Substitution rates: AC = 2.1036, AG = 5.2549, AT = 4.2558, CG = 1.7810, CT = 10.4237, TG = 1.0000; α = 0.3692; pinvar = 0.5587.

Chrysopetalidae to Aphroditidae (Watson Russell 1989). *Palmyra* can only be found in well-oxygenated water in crevices of hard substrates and, therefore, Watson Russell (1989) speculated that the loss of elytra was linked to respiration. However, selection may have also favored adaptation for the small spaces of crevices. Because of leading hypotheses suggesting scale loss due to an adaptation to an interstitial environment, and of some interstitial taxa (e.g. Polygordiidae) also possessing a long and slender body in comparison to their interstitial habitat, we favor regarding pisionids as interstitial rather than infaunal (contra Rouse & Pleijel 2001). We make this distinction because pisionids are most likely dependent upon pore water and the food availability between sediment grains, whereas infaunal organisms are less dependent on this size fraction.

The autapomorphic feature of Aphroditiformia is the possession of elytra. Even though strong morphological autapomorphies uniting Aphroditiformia and Pisionidae are lacking, several adult and developmental features support placement of Pisionidae within Aphroditiformia and thus the hypothesis of the loss of the elytra (e.g. Åkesson 1961; Pleijel & Dahlgren 1998). The possession of an axial, muscular proboscis with two pairs of jaws and venom glands is one such character (Wolf 1986). However, Glyceridae, belonging to Nereidiformia, also possess two pairs of jaws with venom glands. Therefore, studies concentrating on ultrastructure of venom glands and biochemistry of their particular toxins may further elucidate the nature of Aphroditiformia glands (including Pisionidae) relative to glycerid glands. Like other aphroditiforms, pisionids possess elongated pointed palps and anteriorly directed first segments (Pleijel & Dahlgren 1998). However, these features are likely homoplastic as similar palps are found in the hesionid *Wesenbergia* (Pleijel & Dahlgren 1998; Rouse & Pleijel 2001) and anteriorly directed first segments are present in some Onuphidae and Chrysopetalidae (Pleijel & Dahlgren 1998). The larvae of Aphroditiformia and Pisionidae resemble each other at a detailed level, for example, the gland cells of the episphere and position of the buccal ganglion (Åkesson 1961). However, there is a difference in the possession of venom glands and their styli by the pelagic stage of pisionid larvae.

None of our analyses support Aphroditoidea as proposed by Rouse and Pleijel (2001) and Rouse and Fauchald (1997). Furthermore, SH tests employing 18S and combined data sets significantly rejected monophyly of such a taxon. Based on the possession of only simple chaetae, this hypothetical taxon comprises Acoetidae, Aphroditidae, Eulepethidae and Polynoidae. All topologies based on 18S and the COI BI topology recovered Aphroditidae basal to a Pholoidae/Sigalionidae/Polynoidae/Pisionidae clade (BS: $\geq$98, PP: 1.00). Only the best ML topology based on COI data set failed to corroborate this result, but placement of the polynoid *Halosydna brevisetosa* as basal in the COI ML analysis was very weakly supported by bootstrap analysis (BS:39). It was a worse alternative than the basal placement of Aphroditidae (BS: 47) observed in all the other analyses. The present data suggest that possession of only simple chaetae is likely an Aphroditiformia symplesiomorphy, and that Aphroditoidea is not monophyletic. Admittedly, more taxa from all scale worm families including Acoetidae and Eulepethidae need to be sampled to definitely resolve whether the possession of only simple chaetae is a symplesiomorphic or convergent feature.

The recovered close relationships of Pholoidae, Sigalionidae, Polynoidae and Pisionidae is interesting given that intersegmental furrows have been described for all four groups (Wolf 1986). Furrows delineating segments externally are usually missing in Aphroditiformia. However, it has still to be determined whether these external segmental delineations can also be shown internally. Furthermore, in *Pholoides bermudensis* (Pholoidae) these furrows are faint along the entire body (Wolf 1986). Thus, it also has still to be demonstrated that such furrows are not present in the other taxa of Aphroditiformia. Nevertheless, this particular feature may represent an autapomorphy. Within this group monophyly of Polynoidae was corroborated by all analyses including 18S, but our results question either the validity of Pholoidae or the monophyly of Sigalionidae. The taxonomic position of Pholoidae Kinberg, 1858 is controversial and different pholoid species have often been considered within Sigalionidae (e.g. Åkesson 1961; Wolf 1986). Fauchald (1977) erected Pholoididae comprising the genera *Pholoides* and *Parapholoe*. Later, Pettibone (1992) re-established Pholoidae recognizing the genera *Imajinapholoe*, *Laubierpholoe*, *Metaxypsamma*, *Pholoe*, *Pholoides* and *Taylorpholoe* and thus treated Pholoididae as a junior synonym. By comparison, the present analyses suggest that Pisionidae and Pholoidae represent clades within Sigalionidae. Additionally, this close relationship is further substantiated by the brain development in Pholoidae and Pisionidae. The brain separates from the ectoderm and extends backwards as two lobes containing the corpora pedunculata (Åkesson 1961). If the molecular data are in fact correct, the Pholoidae and Pisionidae would have to be regarded as junior synonyms of Sigalionidae. Pettibone (1992) separated Pholoidae from Sigalionidae due to the following characters. Whereas

Sigalionidae have a long and slender body with up to 300 segments, Pholoidae are in general short with only up to 90 segments. Pholoidae are lacking branchiae as well as neuropodial basal bracts and distal stylodes. The compound neurosetae are falcate with short blades in contrast to the ones in Sigalionidae, which possess short and long blades and are multiarticled, flacigers or spinigers. Furthermore, the anteriorly projected tentaculophores of segment I are positioned medial to palps in Pholoidae while in Sigalionidae the position is dorsal to the palps. Whereas Sigalionidae are burrowers in sand and mud Pholoidae are crawling forms found in small spaces like under rocks, in crevices or on mud bottoms with shell and debris and even in the interstitium. Our data seem to indicate that the observed changes in Pholoidae may be due to an adaptation to smaller spaces, and thus a crawling life style, from a burrowing sigalionid ancestor. However, it has to be addressed if Pholoidae is monophyletic or if similar body types evolved several times independently due to adaptation to small spaces. To address such issues more adequately a more extensive taxon sampling is needed.

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References

Aiyar FG, Alikunhi KH. 1940. On a new Pisionid from the sandy beach, Madras. Records of the Indian Museum 42:89–107.

Åkesson B. 1961. On the histological differentiation of the larvae of *Pisione remota* (Pisionidae, Polychaeta). Acta Zoologica 42:177–225.

Alikunhi KH. 1951. On the reproductive organs of *Pisione remota* (Southern) together with a review of the family Pisionidae. Proceedings of the Indian Academy of Science, Section B 33:14–31.

Banse K. 1956. Über die Entwicklung von *Pisione remota* (Pisionidae, Polychaeta), Zoologische Jahrbücher (Abteilung für Systematik. Ökologie und Geographie der Tiere) 84:415–38.

Beesley PL, Ross GJB, Glasby CJ. 2000. Polychaetes & Allies: The Southern Synthesis. Fauna of Australia. Vol. 4A. Polychaeta, Myzostomida, Pogonophora, Echiura, Sipuncula. Melbourne: CSIRO Publishing.

Burnette, A, Struck TH, Halanych KM. 2005. Holopelagic *Poeobius meseres* (Poeobiidae, Annelida) is derived from within benthic Flabelligerid worms. Biological Bulletin. 208:213–20.

Cohen BL, Gawthrop A, Cavaliersmith T. 1998. Molecular phylogeny of brachiopods and phoronids based on nuclear-encoded small subunit ribosomal RNA gene sequences. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 353:2039–61.

Dahlgren TG, Lundberg J, Pleijel F, Sundberg P. 2000. Morphological and molecular evidence of the phylogeny of Nereidiform polychaetes (Annelida). Journal of Zoological Systematic and Evolutionary Research 38:249–53.

de Wilde CLM, Govaere JCR. 1995. On the pisionids (Polychaeta: Pisionidae) from Papua New Guinea, with a description of six new species, Bulletin de l'Institut Royal des Sciences Naturelles de Belgique. Biologie 65:53–68.

Ehlers E. 1901. Die Polychaeten des magellanischen und chilenischen Strandes. Ein faunistischer Versuch. Festschrift zur Feier des Hundertfünfzigjährigen bestehens der königlichen Gesellschaft der Wissenschaft zu Göttingen. Berlin: Weidmannsche Buchhandlung. 232 pages.

Fauchald K. 1977. The polychaete worms. Definitions and keys to the orders, families and genera. Natural History Museum of Los Angeles County. Science Series 28:1–188.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294–9.

Grube AE. 1857. Annulata Örstediana. Enumeratio Annulatorum, quae in itinere per Indiam Occidentalem et Americam centralem annis 1845–1848 suspecto legit cl. A. S. Örsted, adjectis speciebus nonnullis a cl. H. Kröyero in itineread Americam meridionalem collectis. Videnskabelige Meddelelser fra Dansk Naturhistorik Forening 1856:158–86.

Halanych KM. 1998. Considerations for reconstructing metazoan history: Signal, resolution, and hypothesis testing. American Zoologist 38:929–41.

Halanych KM, Bacheller JD, Aguinaldo AMA, Liva SM, Hillis DM, Lake JA. 1995. Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. Science 267:1641–3.

Halanych KM, Robinson TJ. 1999. Multiple substitutions affect the phylogenetic utility of cytochrome b and 12S rDNA data: Examining a rapid radiation in leporid (Lagomorpha) evolution. Journal of Molecular Evolution 48:369–79.

Hartman O.. 1939. Polychaetous annelids. Part I. Aphroditidae to Pisionidae. Allan Hancock Pacific Expeditions 7:1–156.

Hillis DM, Dixon MT. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. Quarterly Review of Biology 66:411–53.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–5.

Huelsenbeck JP, Larget B, Miller R, Ronquist F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Systematic Biology 51:673–88.

Laubier L. 1975. Adaptations morphologiques et biologiques chez un aphroditien interstitiel: *Pholoe swedmarki* sp. n. Cahiers de Biologie Marine 16:671/683.

Levinsen GMR. 1887. Kara-Havets Ledorme (Annulata). In: Lütken CF, editor. Djimphna-Togtets Zoologiske-botaniske Udbytte. Köbenhaven: J. Hagerup. p 288–303.

McPhedran RC, Nicorovici NA, McKenzie DR, Botten LC, Parker AR, Rouse GW. 2001. The Sea Mouse and the Photonic Crystal. Australian Journal of Chemistry 54:241–4.

Nelson K, Fisher CR. 2000. Speciation of the bacterial symbionts of deep-sea vestimentiferan tube worms. Symbiosis 28:1–15.

Nicholas KB, Nicholas HB jr. 1997. GeneDoc: A tool for editing and annotating multiple sequence alignments. Distributed by the author.

Nylander JAA. 2002. MrModeltest., version 1.1. http://morphbank.ebc.uu.se/MR.BAYES.

Passamaneck YJ, Schander C, Halanych KM. 2004. Investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences. Molecular Phylogenetics and Evolution 32:25–38.

Pettibone MH. 1992. Contributions to the polychaete family Pholoidae. Kinberg. Smithsonian Contribution to Zoology 532:1–24.

Pleijel F, Dahlgren TG. 1998. Position and delineation of Chrysopetalidae and Hesionidae (Annelida, Polychaeta, Phyllodocida). Cladistics 14:129–50.

Posada D, Crandall KA. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–8.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitution. Systematic Biology 50:580–601.

Rouse GW, Fauchald K. 1997. Cladistics and polychaetes. Zoologica Scripta 26:139–204.

Rouse GW, Pleijel F. 2001. Polychaetes. Oxford: University Press. 354 pages.

San Martin G, Lopez E, Camacho AI. 1998. First record of a freshwater Pisionidae (Polychaeta): description of a new species from Panama with a key to the species of *Pisione*. Journal of Natural History 32:1115–27.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of Log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16:1114–6.

Siewing R. 1953. Untersuchungen am "Kopf" der Pisioniden (*Pisone puzae* nov. spec., Annelida, Polychaeta). Zoologischer Anzeiger 150:298–313.

Southern R. 1914. Clare Island Survey. Archiannelida and Polychaeta, Proceedings of the Royal Irish Academy. Section B: Biological. Geological and Chemical Science 31:1–160.

Stecher H-J. 1968. Zur Organisation und Fortpflanzung von *Pisione remota* (Southern) (Polychaeta, Pisionidae). Zeitschrift für Morphologie der Tiere 61:347–410.

Struck TH, Hessling R, Purschke G. 2002. The phylogenetic position of the Aeolosomatidae and Parergodrilidae, two enigmatic oligochaete-like taxa of the "Polychaeta", based on molecular data from 18SrDNA sequences. Journal of Zoological Systematic and Evolutionary Research 40:155–63.

Suzuki Y, Glazko GV, Nei M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics, Proceedings of the National Academy of Sciences. USA 99:16138–43.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). version 4.0b. Sinauer Associates.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–80.

Watson Russell C. 1989. Revision of *Palmyra* Savigny (Polychaeta: Aphroditidae) and redescription of *Palmyra aurifera*. The Beagle. Records of the Northern Territory Museum of Arts and Sciences. 6:35–53.

Wolf PS. 1986. A new genus and species of interstitial Sigalionidae and a report on the presence of venom glands in some scaleworm families (Annelida: Polychaeta). Proceedings of the Biological Society of Washington 99:79–83.

Yamanishi R. 1998. Ten species of *Pisione* (Annelida: Polychaeta: Pisionidae) from Japan and evolutionary trends in the genus based on comparison of male copulatory apparatus. Publications of the Seto Marine Biological Laboratory 38:83–145.

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