Journal of the Marine Biological Association of the United Kingdom, 2008, 88(3), 509–513. ©2008 Marine Biological Association of the United Kingdom
doi:10.1017/S0025315408000982 Printed in the United Kingdom

The phylogenetic relationships between Amphinomidae, Archinomidae and Euphrosinidae (Amphinomida: Aciculata: Polychaeta), inferred from molecular data

HELENA WIKLUND[1], ARNE NYGREN[1], FREDRIK PLEIJEL[2] AND PER SUNDBERG[1]

[1]Department of Zoology, Göteborg University, PO Box 463, SE-405 30 Göteborg, Sweden, [2]Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Göteborg University, SE-452 96 Strömstad, Sweden

Amphinomida is an 'isolated' clade within the polychaete group Aciculata and traditionally includes the families Amphinomidae, Archinomidae and Euphrosinidae. Archinomidae were erected for a single species, the hydrothermal vent polychaete Archinome rosacea. *Originally,* A. rosacea *was assigned to Euphrosinidae although it shares more morphological similarities with Amphinomidae. In this study we assess the position of Archinome, Euphrosinidae and Amphinomidae by using molecular data from nuclear 18S rDNA and 28S rDNA. Parsimony, maximum likelihood and Bayesian analyses are performed on the nucleotide datasets covering in total 19 terminals from Amphinomidae, Euphrosinidae, Archinomidae and outgroups. Our results conclusively show that Euphrosinidae and Amphinomidae are sister taxa and that Archinome is sister to Chloeia within Amphinomidae. Based on these results the family name Archinomidae is treated as a junior synonym of Amphinomidae.*

Keywords: 18S rDNA, 28S rDNA, phylogeny, Amphinomida, Amphinomidae, Archinomidae, Euphrosinidae

Submitted 25 December 2006; accepted 15 November 2007

INTRODUCTION

The most well known examples of Amphinomida (to the careless diver at least) are the fireworms, common in shallow warm and temperate water, which easily break off their chaetae when touched, causing pain and sometimes rather serious wound infections (e.g. Fauchald, 1977). Amphinomida is a well delineated clade within aciculate polychaetes, although its position within Aciculata has not yet been resolved. The clade is identified by a series of morphological apomorphies: nuchal organs situated on a caruncle, a ventral muscular eversible proboscis with thickened cuticle on circular lamellae, and calcareous chaetae (Fauchald & Rouse, 1997; Pleijel *et al.*, 2004). Amphinomida comprises approximately 200 described species and 25 genera (Glasby *et al.*, 2000; Rouse & Pleijel, 2001). The worms, from what is known, are mainly scavengers or predators on sessile prey, feeding by rasping with their muscular chitinized lip (Fauchald & Jumars, 1979), although some, such as *Archinome rosacea* and *Pherecardia* sp. have been shown to feed on more active prey (Day, 1967; Ward *et al.*, 2003).

Amphinomida is currently divided into three families: Amphinomidae, Archinomidae and Euphrosinidae, with Amphinomidae exhibiting most of the morphological diversity. The bulk of the species in Amphinomidae live in shallow warm and temperate waters. In contrast, Euphrosinidae are more common in cold waters, while a single member of Archinomidae has been recorded from hydrothermal vents both in the Indo-Pacific and Atlantic Oceans (Ward *et al.*, 2003). Although no apomorphies have been identified for the three different groups, morphological differences between Amphinomidae and Euphrosinidae include the shape of the notopodia, the shape of the branchiae, chaetae and orientation of the prostomium (Kudenov, 1991; Fauchald & Rouse, 1997). To date, the relationships between Amphinomidae and Euphrosinidae have not been addressed and Rouse & Pleijel (2001) suggested that one of them might be paraphyletic with respect to the other. In 1991, Kudenov erected the family Archinomidae for the hydrothermal vent species *Archinome rosacea* (Blake, 1985). This taxon was originally placed in Euphrosinidae as *Euphrosine rosacea*, but has also a number of features that suggest an amphinomid affinity (Kudenov, 1991; Fauchald & Rouse, 1997). In this study, we use molecular data from the nuclear genes 18S rDNA and 28S rDNA to investigate the phylogenetic position of *Archinome* and the delineations of Euphrosinidae and Amphinomidae.

MATERIALS AND METHODS

Taxa

Included taxa are listed in Table 1 with GenBank accession numbers, vouchers, and sampling areas/sites. Voucher specimens are deposited at the Swedish Museum of Natural

Corresponding author:
Helena Wiklund
Email: helena.wiklund@zool.gu.se

https://doi.org/10.1017/S0025315408000982 Published online by Cambridge University Press

Table 1. Taxa, collection site, NCBI GenBank accession numbers, and voucher specimen numbers.

Taxon	Source	18S	28S	Voucher
Amphinomidae				
Chloeia flava (Pallas, 1766)	Tanabe Bay, Japan	EF076780	EF076781	SMNH95025
Eurythoe complanata (Pallas, 1766)	NCBI GenBank (1)	AY040685	–	–
Eurythoe complanata	NCBI GenBank (2)	AY364851	AY364849	–
Hermodice carunculata (Pallas, 1766)	NCBI GenBank	AY495948	–	–
Hermodice sp.	NCBI GenBank	DQ779653	DQ779691	–
Hipponoe gaudichaudi Audouin & Milne-Edwards, 1830	NCBI GenBank	AY577888	–	–
Paramphinome jeffreysii (McIntosh, 1868)	Gullmarsfjord, Sweden (1)	AY176299	EF076786	SMNH95027
Paramphinome jeffreysii	NCBI GenBank (2)	DQ779664	DQ779702	–
Pareurythoe borealis (M. Sars, 1862)	Trondheimsfjord, Norway	EF076787	EF076788	SMNH95015
Archinomidae				
Archinome rosacea (Blake, 1985)	Oasis Vent 17°25.384′S, 113°12.279′W, expedition BIOSPEEDO, Nautile dive 1590	EF076777	EF076778	SMNH95029
Eunicidae				
Eunice pennata (O.F. Müller, 1776)	NCBI GenBank	AY040684	AY340391	–
Euphrosinidae				
Euphrosine armadillo M. Sars, 1851	Trondheimsfjord, Norway	EF076782	EF076783	SMNH95017
Euphrosine foliosa Audouin & Milne-Edwards, 1834	Banyuls, France	EF076784	EF076785	SMNH95028
Euphrosine sp.	NCBI GenBank	DQ779649	DQ779687	–
Maldanidae				
Maldane sarsi Malmgren, 1865	NCBI GenBank	AY612617	AY612628	–
Opheliidae				
Ophelina acuminata Ørsted, 1843	NCBI GenBank	AY176296	AY612630	–
Pholoidae				
Pholoe baltica Ørsted, 1843	Koster Area, Sweden	AY839573	EF076779	SMNH73634
Phyllodocidae				
Eulalia viridis (Linnaeus, 1767)	NCBI GenBank	AY340428	AY340392	–
Sabellidae				
Sabella pavonina Savigny, 1822	NCBI GenBank	U67144	AY612632	–

History. *Eunice pennata* was included as an outgroup since previous morphology-based analyses have indicated a close relationship between Eunicida and Amphinomida (Rouse & Fauchald, 1997). Additionally, two other members of Aciculata, two from Scolecida and one from Canalipalpata were included in the study. Some previously published sequences were obtained from NCBI GenBank, although for three of the species only 18S was available.

DNA sequencing

Extraction of DNA was done with the DNAeasy Tissue Kit (Qiagen), following the protocol supplied by the manufacturer. About 1800 base pairs (bp) of 18S were amplified in two overlapping fragments of around 1100 bp each, using for the 5′-fragment the primers 18SA–1115R, and for the 3′-fragment 18SB–620F. About 800 bp of the D1/D2 region of 28S was amplified, using the primers C1′ and D2 (Table 2). Polymerase chain reaction (PCR) mixtures contained ddH2O, 10X Buffer, 25 mM $MgCl_2$, 2, 5 mM dNTP, 2, 5 µl of each primer (10 mM), 0, 25 µl TaKaRa LA Taq DNA Polymerase (5units/ µl) and 3 µl template DNA in a mixture of total 50 µl. The temperature profile was as follows: 96°C/240s -(94°C/30s-46°C/30s-72°C/60s)*45cycles-72°C/480s. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen). The 18S gene was sequenced using for the 5′-fragment the primers 18SA, 620F, 584R and 1115R, while for the 3′-fragment the primers 18SB, 860F, 1324F and 1324R were used (Table 2). 28S was sequenced using C1′ and D2 (Table 2). Each sequence mixture contained 1 µl primer (5 mM), 4 µl DTCS Quick Start Mix, purified amplification product and ddH_2O. The sequence reaction profile was as follows: (96°C/20s-50°C/20s-60°C/240s)*29 cycles for the 18S primers, while the 28S primers had an annealing temperature of 55°C. Sequences were obtained from a BeckmanCoulter CEQ8000.

Alignment and analyses

Overlapping sequence fragments were merged into consensus sequences using SeqMan 4.0 (DNAStar), and aligned with Clustal X (Thompson *et al.*, 1997) with default settings, 10/0.2 for gap/gap length penalties. All regions that could not be unambiguously aligned in the alignments were excluded, totalling 257 characters. Alignments are available at TreeBase, http://www.treebase.org. PAUP* 4.0b10 (Swofford, 2002) was used for the parsimony (PA) and maximum likelihood (ML) analyses, with heuristic search and TBR (tree bisection and reconnection) branch swapping. Clade support was assessed using non-parametric bootstrapping with 5000 replicates and ten random additions for the PA, and with 100 replicates for ML. Bayesian phylogenetic analyses (BA) were conducted with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Analyses were run three times for each dataset with four chains for 1,000,000 generations. 250,000 generations were discarded as burn-in. The results from each dataset

https://doi.org/10.1017/S0025315408000982 Published online by Cambridge University Press

Table 2 Polymerase chain reaction and sequencing primers.

Primer	Sequence 5′-3′	Position	References
18SA	AYCTGGTTGATCCTGCCAGT	1–20	Medlin et al. (1988)
18SB	ACCTTGTTACGACTTTTACTTCCTC	1776–1800	Nygren & Sundberg (2003)
620F	TAAAGYTGYTGCAGTTAAA	618–636	Nygren & Sundberg (2003)
584R	ACGCTATTGGAGCTGGAAT		Persson, pers comm
860F	GAATAATGGAATAGGA	821–836	Turbeville et al. (1992)[a]
977R	AACCTCTGACTTTCGTTCTT		Persson, pers comm
1324F	GGTGGTGCATGGCCG	1284–1298	Cohen et al. (1998)
1324R	CGGCCATGCACCACC	1284–1298	Cohen et al. (1998)
28SC1'	ACCCGCTGAATTTAAGCAT		Lê et al. (1993)
28SD2	AACTCTCTCMTTCARAGTTC		Lê et al. (1993)

Note. Position numbers refer to the *Autolytus prolifer* (O.F. Müller, 1784) 18S sequence (GenBank Accession No. AF474295); F, Forward; R, Reverse.
[a]Modified from original primer by Nygren and Sundberg (2003).

analysis were compared, and when values approached similar mean values for all parameters they were considered to have converged. Models used for the molecular data were obtained by running the datasets in MrModelTest (Nylander, 2004) for the BA, and in ModelTest (Posada & Crandall, 1998) for the ML. MrModeltest suggested GTR + I + G for 18S, and GTR + G for 28S. The datasets were tested for incongruence using the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) in PAUP*, with RELL (resampling estimated log-likelihood) 1000 bootstrap replicates, and using the incongruence length difference (ILD) test (Farris *et al.*, 1995) implemented in PAUP* (as the partition homogeneity test) using 100 replicate heuristic searches. The 18S and 28S trees within the 95% confidence interval from the Bayesian analyses were used in the SH test. Both tests failed to demonstrate incongruence between the data sets. ModelTest suggested GTR + I + G for the combined dataset, which was used in ML, while in MrBayes, the combined dataset was partitioned into 18S and 28S, and each partition had its respective model according to MrModelTest.

RESULTS AND DISCUSSION

The combined dataset consists of 2552 characters, of which 677 are variable and 325 are parsimony-informative. The three BAs converged on similar log-likelihood values, mean values for all parameters, and clade probabilities. The 50%-majority rule consensus tree from the BA supports 14 nodes, of which ten have clade credibilities >95%. The ML tree supports 11 nodes, all of which were recovered by the BA, and five of these have bootstrap values above 95%. The bootstrap of the PA provided >50% support for nine nodes, all of which are present in the BA and ML. There are no topological incongruencies between the three analyses, and the differences between them relate solely to weaker or no support in ML and PA for some groups that were recovered in the BA. All three analyses provide strong support to the nodes of interest for the position of *Archinome* and the relationships between Amphinomidae and Euphrosinidae.

Amphinomida is a well supported clade within Aciculata based on a number of morphological apomorphies (e.g. Fauchald & Rouse, 1997), but both the position of Amphinomida within Aciculata and the delineation of Archinomidae, Amphinomidae and Euphrosinidae remains uncertain (Fauchald & Rouse, 1997; Rouse & Pleijel, 2001). Whereas the first issue is outside the scope of this study and requires a much larger taxon sampling outside Amphinomida, our data permit us to assess the position of *Archinome* and the interrelationships of the three families.

When *Archinome rosacea* was originally described, it was assigned to Euphrosinidae as *Euphrosine rosacea* by Blake (1985). He remarked on the similarities to amphinomids in the distribution pattern of notochaetae and the lack of ringent chaetae, but referred to these similarities as 'superficial'. Kudenov (1991) rejected the original placement in Euphrosinidae but also a position within Amphinomidae and instead introduced the new family name Archinomidae, together with the generic name *Archinome* for the single species *A. rosacea*. Subsequently, it has been suggested that both Archinomidae and Euphrosinidae may be nested within Amphinomidae (e.g. Fauchald & Rouse, 1997; Rouse & Pleijel, 2001; Pleijel *et al.*, 2004). Kudenov (1994) in an abstract referred to a morphology-based phylogenetic analysis of the three families. He stated that minimally one of these families was paraphyletic but did not specify any further results, and the full study has not been published.

Our topologies provide strong support for Amphinomidae and Euphrosinidae as sister groups, and we can therefore reject earlier suggestions that the two taxa have a nested relationship. A number of morphological characters separate the two families, but in the absence of knowledge about the closest relatives to Amphinomida it is not obvious which of these are apomorphic for either group. Our conclusion is therefore at present founded solely on molecular data.

In contrast to both Blake (1985) and Kudenov (1991), the molecular data used in this study unequivocally also show that *Archinome* belongs within Amphinomidae. Among the included taxa, *Archinome* is most closely related to *Chloeia flava* and these two taxa form a sistergroup to the remaining amphinomids (Figure 1). Kudenov (1991) pointed out a series of similarities between *Archinome* and *Chloeia* (plus *Notopygos* which is not present among our terminals), including the number of ciliated tracts on the caruncle, the position of the branchiae, the presence of dorsal cirri, the limited chaetal kinds, and the body-shape. Whereas he interpreted these similarities as homoplastic ('superficial'), our tree topology instead suggests that their occurrences in *Archinome*, *Chloeia* and, possibly, *Notopygos*, are actually homologies. Seen in this light, Kudenov's diagnosis of the family Archinomidae actually consisted of a mixture of autapomorphies for *Archinome rosacea* and more general features, some of which

https://doi.org/10.1017/S0025315408000982 Published online by Cambridge University Press

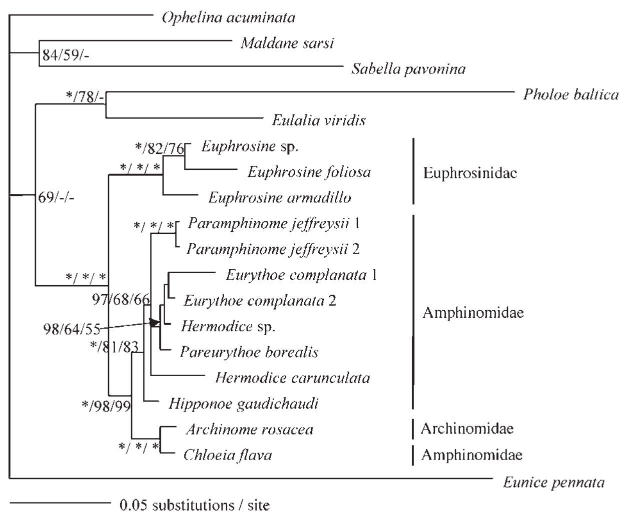

Fig. 1. Majority rule consensus tree from the Bayesian analyses (BA) of the combined data. The first values for each node represent clade credibility support from the Bayesian analyses, the second are bootstrap support from the maximum likelihood analyses, and the third are bootstrap support from the parsimony analyses. *, support value of 100; -, no support. The nodes *Eurythoe complanata* ↔ *Hermodice* sp. (support values not shown) have low support in BA and no support in the other analyses.

are present in Amphinomidae and some in the whole of Amphinomida.

From a nomenclatural viewpoint this leaves us with two choices: either transfer *Chloeia* to Archinomidae (possibly followed by other taxa that were not included in our analysis), or treat Archinomidae as a junior synonym to Amphinomidae. We opt for the second alternative. Furthermore, our study also provides strong support for a sister-group relationship between Euphrosinidae and Amphinomidae, and thus that both these clades can be retained as family-level taxa in line with traditional classifications.

ACKNOWLEDGEMENTS

We thank Stéphane Hourdez and Didier Jollivet for specimens of *Archinome*, Greg Rouse for valuable comments on the manuscript, and the following persons and institutions for providing working facilities: Michel Bhaud, Laboratoire Arago, France; Kristineberg Marine Research Station, Sweden; Yoshihisa Shirahama, Seto Marine Biological Laboratory, Japan; and Jon-Arne Sneli, Trondhjem Biological Station, Norway. Financial support for F.P. was provided by Formas (grant #2004-0085), the Japan Society for the Promotion of Science, Kristineberg Marine Research Station (European Community ARI-programme) and Trondheim Marine Systems Research Infrastructure (European Community ARI-programme). The research of P.S. is financially supported by the Swedish Research Council.

REFERENCES

Blake J.A. (1985) Polychaeta from the vicinity of deep-sea geothermal vents in the eastern Pacific. I. Euphrosinidae, Phyllodocidae, Hesionidae, Nereididae, Glyceridae, Dorvilleidae, Orbiniidae, and Maldanidae. *Bulletin of the Biological Society of Washington* 6, 67–101.

Cohen B.L., Gawthrop A. and Cavalier-Smith 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, 2039–2061.

Day J.H. (1967) *A monograph on the Polychaeta of southern Africa. Part 1. Errantia*. London: Trustees of the British Museum (Natural History).

Farris J., Källersjö M., Kluge A.G. and Bult C. (1995) Constructing a significance test of incongruence. *Systematic Biology* 44, 570–572.

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.

Fauchald K. and Jumars P.A. (1979) The diet of worms: a study of polychaete feeding guilds. *Oceanography and Marine Biology: an Annual Review* 17, 193–284

Fauchald K. and Rouse G. (1997) Polychaete systematics: past and present. *Zoologica Scripta* 26, 71–138.

Glasby C.J., Hutchings P.A., Fauchald K., Paxton H., Rouse G.W., Watson Russell C. and Wilson R.S. (2000) Class Polychaeta. In Beesley P.L. *et al.* (eds) *Polychaetes and allies: the southern synthesis*. Melbourne: CSIRO Publishing, pp. 1–296.

Kudenov J.D. (1991) A new family and genus of the order Amphinomida (Polychaeta) from the Galapagos hydrothermal vents. In Petersen M.E and Kirkegaard J.B. (eds) *Proceedings of the 2nd International Polychaeta Conference, Copenhagen, 1986. Systematics, Biology and Morphology of World Polychaeta. Ophelia* 5, Supplement, pp. 111–120.

Kudenov J.D. (1994) The order Amphinomida: a revision of the families (Annelida, Polychaeta). In Dauvin J.-C. *et al.* (eds), *Actes de la 4ème Conférence Internationale des Polychètes. Mémoires du Muséum National d'Histoire Naturelle*, pp. 627–628.

Lê H.L.V., Lecointre G. and Perasso R. (1993) A 28S rRNA based phylogeny of the gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. *Molecular Phylogenetics and Evolution* 2, 31–51.

Medlin L., Elwood H.J., Stickel S. and Sogin M.L. (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 71, 491–499.

Nygren A. and Sundberg P. (2003) Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida). *Molecular Phylogenetics and Evolution* 29, 235–249.

Nylander J.A.A. (2004) MrModeltest v2. Program distributed by the author. Uppsala University: Evolutionary Biology Centre.

Pleijel F., Rouse G.W. and Vannier J. (2004) Carboniferous fireworms (Amphinomida: Annelida), with a discussion of species taxa in palaeontology. *Invertebrate Systematics* 18, 693–700.

Posada D. and Crandall K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.

Ronquist F. and Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.

Rouse G.W. and Fauchald K. (1997) Cladistics and polychaetes. *Zoologica Scripta* 26, 139–204.

Rouse G.W. and Pleijel F. (2001) *Polychaetes*. Oxford: Oxford University Press.

Shimodaira H. and Hasegawa M. (1999) Multiple comparisons of Log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16, 1114–1116.

Swofford D.L. (2002) *PAUP**. *Phylogenetic Analysis Using Parsimony (*and other methods). Version 4*. Sunderland, MA: Sinauer Associates.

https://doi.org/10.1017/S0025315408000982 Published online by Cambridge University Press

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. and Higgins D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876–4882.

Turbeville J.M., Field K.G. and Raff R.A. (1992) Phylogenetic position of phylum Nemertini, inferred from 18S-ribosomal-RNA sequences—molecular data as a test of morphological character homology. *Molecular Biology and Evolution* 9, 235–249.

and

Ward M.E., Jenkins C.D. and Van Dover C.L. (2003) Functional morphology and feeding strategy of the hydrothermal vent polychaete *Archinome rosacea* (family Archinomidae). *Canadian Journal of Zoology* 81, 582–590.

Correspondence should be addressed to:
Helena Wiklund
Göteborg University
Department of Zoology
PO Box 463
SE-405 30 Göteborg, Sweden
email: helena.wiklund@zool.gu.se

https://doi.org/10.1017/S0025315408000982 Published online by Cambridge University Press

https://doi.org/10.1017/S0025315408000982 Published online by Cambridge University Press