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Combined mitochondrial and nuclear sequences support the monophyly of forcipulatacean sea stars

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Abstract

Previous molecular phylogenetic analyses of forcipulatacean sea stars (Echinodermata: Asteroidea) have reconstructed a non-monophyletic order Forcipulatida, provided that two or more forcipulate families are included. This result could mean that one or more assumptions of the reconstruction method was violated, or else the traditional classification could be erroneous. The present molecular phylogenetic analysis included 12 non-forcipulatacean and 39 forcipulatacean sea stars, with multiple representatives of all but one of the forcipulate families and/or subfamilies. Bayesian analysis of approximately 4.2 kb of sequence data representing seven partitions (nuclear 18S rRNA and 28S rRNA, mitochondrial 12S rRNA, 16S rRNA, 5 tRNAs and cytochrome oxidase I with first and second codon positions analyzed separately from third codon positions) recovered a consensus tree with three well-supported clades (78%–100% bootstrap support) that corresponded at least approximately to traditional taxonomic ranks: the superorder Forcipulatacea (Forcipulatida + Brisingida) + *Pteraster*, the Brisingida/Brisingidae and Asteriidae + *Rathbunaster* + *Pycnopodia*. When a molecular clock was enforced, the partitioned Bayesian analysis recovered the traditional Forcipulatacea. Five of six genera represented by two or more species were monophyletic with 100% bootstrap support. Most of the traditional subfamilial and familial groupings within the Forcipulatida were either unresolved or non-monophyletic. The separate partitions differed considerably in estimates of model parameters, mainly between nuclear sequences (with high GC content, low rates of sequence substitution and high transition/transversion rate ratios) and mitochondrial sequences.

Keywords: 12S rRNA; 16S rRNA; 18S rRNA; 28S rRNA; Cytochrome oxidase subunit I; tRNA; Forcipulatida; Brisingida; Sea star

1. Introduction

Forcipulatacean sea stars (as defined by Blake, 1987) are a morphologically and ecologically diverse assemblage of two orders (Forcipulatida and Brisingida), 13 families and approximately 83 genera (Clark and Mah, 2001). The most taxon-rich forcipulate family is the Asteriidae, with approximately 45 genera that include several familiar genera with a temperate and intertidal or shallow subtidal distribution, such as *Asterias, Evasterias, Pisaster* and *Leptasterias*. Previous phylogenetic work on this group has been reviewed by Knott and Wray (2000) and Mah (2000). The monophyly of forcipulate or forcipulatacean sea stars is one of the least controversial aspects of sea star phylogeny, and has been accepted by all investigators before 2000, including Fisher (1928, 1930), Spencer and Wright (1966), Blake (1987), Gale (1987) and Clark and Downey (1992). There has been disagreement about sub-ordinal relationships. For example, although Downey (1970) gave the forcipulate family Zoroasteridae ordinal status as the Zorocallida, this decision was reversed by McKnight (1977). Blake (1987) later used a suborder Zorocallina as the sister group to the Asteriadina (Asteriidae + Heliasteridae). In addition, Gale (1987) considered the brisingids to be a family within the Forcipulatida; other treatments of the brisingids include Downey (1986) and Mah (1998a). Also, Fisher (1928) considered it likely that the Asteriidae and Labidiasterinae were each not monophyletic. In spite of those doubts, most recent investigators have either accepted the taxonomic scheme of Fisher (1928, 1930) for forcipulates, which

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recognized three families (Zoroasteridae, Heliasteridae and Asteriidae) and seven subfamilies within the species-rich Asteriidae (Pycnopodiinae, Labidiasterinae, Pedicellasterinae, Coscinasteriinae, Asteriinae, Neomorphasterinae and Notasteriinae), or else have synonymized the Coscinasteriinae and Notasteriinae with the Asteriinae and raised the Labidiasterinae, Pedicellasterinae, Pycnopodiinae and Neomorphasterinae to family level (e.g., Clark and Mah, 2001).

The first class-level molecular phylogeny of sea stars (Lafay et al., 1995) used a short region of the 28S rRNA gene (380 bp containing 19 phylogenetically informative sites) and nine taxa. Relatively little support (bootstrap values <50%) was found for basal relationships, although an asteriid clade was well-supported (no other forcipulate taxa were included). More support for basal relationships was found by combining the molecular data with 43 morphological characters. Wada et al. (1996) analyzed 600 bp of combined 12S rRNA and 16S rRNA for 17 taxa and found high bootstrap support (99%–100%) for an asteriid clade that included two of the same genera (*Asterias* and *Coscinasterias*) that were studied by Lafay et al. (1995), but included no other forcipulate taxa. Knott and Wray (2000) sampled 32 taxa representing 17 families and six orders for 1826 bp of tRNA and cytochrome oxidase subunit I (COI) sequence, including a brisingid sea star and members of four forcipulate families (Asteriidae, Heliasteridae, Labidiasteridae and Zoroasteridae). They recovered maximum parsimony and maximum likelihood phylogenies in which forcipulatacean taxa were embedded in a paraphyletic assemblage that included all sampled spinulosid taxa and some velatid taxa. Janies (2001) analyzed 18S rRNA and 28S rRNA sequences, plus 61 morphological characters, for 20 sea star taxa representing 13 families and six orders, including a brisingid, an asteriid, a heliasterid and a labidiasterid. His maximum parsimony reconstruction included a paraphyletic Forcipulatacea. The recent molecular phylogeny by Matsubara et al. (2004) also found evidence of forcipulatacean paraphyly when 20 taxa, including a brisingid and one or more representatives each of the families Asteriidae, Labidiasteridae and Heliasteridae, were analyzed for 1669 bp of 18S rRNA sequence.

The above results have led Smith et al. (2004:376) to conclude that "there is very little signal in the molecular data currently available with which to resolve asteroid relationships." Another possibility is that the poor phylogenetic resolution of recent molecular studies is due in part to sparse taxon sampling. The aim of the present study was to determine if inclusion of additional forcipulate taxa in a molecular phylogenetic analysis would provide better resolution of the relationships within this group.

2. Materials and methods

2.1. DNA extraction

DNA extraction from formalin-preserved museum specimens used a modified version of Shedlock et al.'s (1997) protocol, as described in Flowers and Foltz (2001). This method provided amplifiable DNA up to 1 kb in length. DNA extraction from non-museum material was done as in Foltz et al. (1996) or by using the GenElute kit for Mammalian DNA® (Sigma–Aldrich, St. Louis, Missouri), with final elution in sterile water instead of the manufacturer's elution buffer.

2.2. PCR and sequencing techniques

PCR reactions were set up using a scaled-down version of the protocol in Hrincevich and Foltz (1996); reactions for formalin-preserved specimens generally included Gene Releaser® (BioVentures, Mufreesboro, Tennessee) as per manufacturer's instructions. Occasionally, PCR or sequencing required the use of primers that were internal or external to the usual target region. The primers used most frequently in this study are listed in Table 1; details on other more taxon-specific primers are available from the senior author. One amplicon representing the 16S rRNA gene region for *Labidiaster annulatus* could not be sequenced directly and was first cloned using TOPO-TA® technology (Invitrogen, Carlsbad, California).

Negative and positive controls were used in all PCR amplifications, and additional validation was provided by an unrestricted BLAST search for each sequence obtained. Success rates for obtaining useable DNA sequences from museum specimens on the first attempt were from 25% to 50% (Flowers, 1999 and unpubl. data), although most specimens eventually yielded useable sequences following repeat extractions. Diluting the DNA extracts 1:10 or 1:100 before PCR increased the amplification success rate. Cycle sequencing was performed using scaled-down reactions and ABI-Prism Dye-Terminator chemistry (Hrincevich et al., 2000). Reaction products were visualized on an ABI 3100 Capillary Sequencer located in the LSU Museum of Natural Science, using standard protocols. Opposite strands were assembled and reconciled in Sequencher 4.0. Alignments were generated by ClustalX (Thompson et al., 1997); the lowest 5% of the sites in terms of quality scores from Clustal were excluded, but no other attempt was made to align sequences manually or to exclude regions of putative poor alignment.

2.3. Selection of taxa and sequences

Sequence data were available for 39 forcipulatacean species and 12 non-forcipulatacean sea star species (Electronic Appendix). Several forcipulate species were excluded because data were available only for 12S rRNA and/or 16S rRNA (*Ampheraster marianus, Distolasterias nipon, Leptasterias hylodes, Leptasterias* (*Eoleptasterias*) *squamulata* and *Zoroaster fulgens*), or else because only COI and/or mitochondrial control region sequence data were available (*Allostichaster insignis, Astrostole scabra, Coscinasterias calamaria, C. muricata, Sclerasterias mollis* and *Uniophora granifera*). Non-forcipulatacean species were included if they had at least 12S rRNA, 16S rRNA and 18S rRNA