

P601

Phylogenetic Structure of the Sphaeriinae, a Global Clade of Freshwater Bivalve Molluscs, Inferred from Nuclear (ITS-1) and Mitochondrial (16S) Ribosomal Gene Sequences

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Although molluscs easily represent the most diverse segment of marine faunas, the number of major molluscan radiations into freshwater habitats is quite limited. Only three major groups, the basommatophoran pulmonate snails and the unionoidan and sphaeriid bivalves, have enjoyed a sufficiently long period of diversification in freshwater environments to produce taxonomically rich and globally distributed clades. This study is concerned with one of these molluscan freshwater radiations, the Sphaeriidae, and aims to construct a comprehensive molecular phylogeny of one of its primary subgroupings: the cosmopolitan subfamily Sphaeriinae. The Sphaeriidae (fingernail/nut/pill/pea clams) are ubiquitous in freshwater ecosystems. They first appeared in the Cretaceous fossil record but, at present, lack convincing marine outgroups. Recent morphological and molecular studies distinguish two primary clades: the Sphaeriinae and the Gondwanan Euperinae. The Sphaeriinae contain three cosmopolitan genera, *Pisidium*, *Sphaerium* and *Musculium*, which have maximum diversities in the Holarctic, and are diagnosed on the basis of details of the shell and soft-part morphology and of reproductive/developmental characteristics. Sphaeriinids are often the dominant benthic organisms in streams and ponds where they play a key role in energy and nutrient cycling. They have arguably the most complicated pattern of parental care in the Bivalvia, involving extraoogonial nutrition of direct-developing young, incubated as either synchronous or asynchronous clutches, within ctenidial brood sacs. Sphaeriinids exhibit a remarkable degree of genome amplification (up to 13n) and a number of North American species may share ancestral genome duplication events which predate their cladogenesis. Sphaeriinid systematics has historically been hampered by considerable ecophenotypic and allometric variation in shell shape, a factor compounded by the extensive geographic ranges of many taxa. For instance, almost half of North American sphaeriids also occur in Eurasia and the nominal taxon *Pisidium casertanum* is found on every continent except for Antarctica. Another problem has been the use of a strikingly divergent system of classification by the Russian taxonomic school. A number of sphaeriinid cladistic studies have recently been performed utilizing morphological and molecular datasets. While all three morphological studies recovered a monophyletic *Pisidium*, the molecular dataset (mt 16S RNA sequences) yielded a paraphyletic *Pisidium* in which the subgenus *Afropisidium* was sister to all the other sphaeriine taxa considered. *Pisidium* paraphyly is also apparent in preliminary trees based on nuclear gene fragments: 28S ribosomal RNA and 6-Phosphogluconate Dehydrogenase. Another difference among these cladistic studies concerns the interrelationship of the synchronous (*Pisidium*) and asynchronous brooding (*Sphaerium* and *Musculium*) taxa. Two of the morphological analyses yielded a sphaeriine topology (*Sphaerium* (*Musculium*, *Pisidium*)) in which a sister relationship for *Musculium* and *Pisidium* was supported primarily by a suite of kidney microanatomical characters. In contrast, sphaeriinid gene trees, and one morphological analysis lacking kidney characters, recovered a derived clade of asynchronous brooders, e.g., (*Pisidium* (*Musculium*, *Sphaerium*)). Intrageneric sphaeriinid relationships are poorly defined. A variety of subgeneric groupings have been proposed, although few have been widely recognized as valid taxonomic units or explicitly tested phylogenetically. The present study aims to construct comprehensive sphaeriinid gene trees, which will provide the basis for an explicit phylogenetically-based taxonomy of the group. Sampling effort has been expanded to incorporate sequence data from both nuclear (ITS1 RNA) and mitochondrial (16S RNA) genomes, 38 taxa representing all but one (*Neopisidium*) of the nominal subgeneric sphaeriinid groupings, and samples from multiple continents to test monophyly of nominally cosmopolitan taxa. Fragments of two different ribosomal genes were sequenced for each species: a ~480 nucleotide fragment of the mitochondrial large ribosomal subunit (16S) and the entire nuclear ribosomal first internal transcribed spacer (ITS1). The 16S and ITS-1 datasets were phylogenetically analyzed as either individual or combined (16S + ITS1) matrices under maximum parsimony (MP) optimality criterion. Branch support levels were estimated with both bootstrapping and Bremer decay index values. A maximum likelihood (ML) analysis was also performed on the combined matrix under the HKY model. For both MP and ML analyses, two *Eupera* species, *E. cubensis* and *E. platensis*, were designated as outgroups and sphaeriinid taxa were forced to be monophyletic and sister to monophyletic outgroups in order to root the phylogeny. Phylogenetic analyses of individual and combined (16S + ITS1) datasets all recovered a paraphyletic *Pisidium* and a derived clade of asynchronous brooding *Sphaerium*/*Musculium* taxa. Maximum parsimony as well as maximum likelihood analyses of combined data yielded largely congruent and well-resolved topologies, and robustly supported clades were utilized to revise current sphaeriine taxonomy. Instead of the traditionally accepted three cosmopolitan genera, *Pisidium s. lat.*, *Musculium*, and *Sphaerium*, five major monophyletic lineages, *Afropisidium*, *Odhneripisidium*, *Pisidium*, *Cyclocalyx* and *Sphaerium*, were recognized at the generic level. In addition, a number of subgeneric level groups were recovered in *Sphaerium*: *Herringtonium*, *Sphaerium s. str.*, *Sphaerinova*, *Amesoda*, and *Musculium*, together with one unassigned species, *S. transversum*. Although the present dataset provides valuable new insights in sphaeriinid evolution and systematics, data (including morphological characters) from additional taxa (especially *Neopisidium*, *Afropisidium* and *Odhneripisidium*), and from slowly evolving genes, are required to flesh out basal phylogenetic relationships among major sphaeriine lineages. A comprehensive understanding of sphaeriid evolution and cladogenesis awaits the incorporation of equivalent datasets from the sister Euperinae clade and the identification of convincing marine outgroup(s).