

## CHAPTER 6

### SUMMARY AND CONCLUSIONS

Although the Sphaeriidae have consistently attracted the attention of biologists, these studies have generally lacked a rigorous phylogenetic perspective. As a result, there are large gaps in our knowledge concerning the evolution of this major group of freshwater invertebrates. In addition, global freshwater biotas, including their malacofaunas, are presently under severe extinction pressure due to pollution, impoundment and the introduction of exotic competing species. Recognition of these threats has led to a renaissance in the study of some North American aquatic invertebrate taxa, especially the unionid mussels. This interest, however, has not been extended to the sphaeriids. This dissertation addresses this shortcoming by conducting evolutionary studies on the Sphaeriidae, focusing mainly on the subfamily Sphaeriinae, a major subgroup of the family.

The freshwater Sphaeriidae are small bivalves with cosmopolitan distributions and a great importance in natural food webs. Sphaeriid clams exhibit various peculiar biological features, many of which apparently represent adaptive specializations to the freshwater environment. Sphaeriids are simultaneous hermaphrodites and evidently have the ability to self-fertilize. The Sphaeriidae all brood their direct developing young within the inner demibranchs and each sphaeriid genus shows significantly different degrees of complexity in the details of how brooding is achieved. In addition, the few existing studies of chromosomes of sphaeriid clams have shown strikingly variable mitotic chromosome numbers, ranging from 36 to ca. 247. The presence of polyploids in

the family has added another dimension to the speculation about reproduction and about the methods of speciation.

Systematists currently recognize two sphaeriid subfamilies, Euperinae and Sphaeriinae. The Euperinae contains two geographically restricted genera, *Byssanodonta* and *Eupera*. On the other hand, sphaeriine genera, *Musculium*, *Pisidium* and *Sphaerium*, are cosmopolitan with maximum diversities in the Holarctic Region and each genus contains numerous species. A few subgenera have been widely recognized as valid taxonomic units within the Sphaeriinae, although a number of hypotheses have been proposed. Only a few phylogenetic analyses have been conducted on these organisms. However, analyses of different data sets, morphology and molecular sequences, resulted in incompatible sphaeriid phylogenies, and detailed subgeneric- and species-level phylogenetic relationships are still poorly understood.

In Chapter 2, I observed spermatogenetic meiosis as well as a very large number of chromosomes ( $n=76$ ,  $2n=ca. 152$ ) in a North American sphaeriid, *Sphaerium striatinum*. Sphaeriine species studied to date, except for European *S. corneum*, are all polyploids having high mitotic chromosome numbers, which range from ca. 150 to ca. 247. These results indicate that pronounced polyploidization may have played a major role in the evolution of the Sphaeriinae. My study also suggests that the basic chromosome number of the sphaeriine polyploids may be 19 and that a very significant variation in ploidy levels ( $2n$  to  $13n$ ) occur in the Sphaeriinae.

I generated single-copy nuclear gene trees in order to test hypotheses addressing the evolutionary origins of sphaeriid genome duplication (Chapter 3). Polyploid North American members of three cosmopolitan sphaeriine genera (*Sphaerium*, *Musculium* and *Pisidium*) were characterized for their expressed allelic repertoire of a 526 nt c-DNA fragment of 6-Phosphogluconate-Dehydrogenase (PGD). Pronounced levels of intra-individual genetic variation were uncovered in most of the polyploid taxa and a minority of alleles showed strong evidence of recombination. Phylogenetic analyses resolved

polyploid sphaeriine PGD alleles into two clades (A, B), each of which contained a subsample of intra-individual allelic diversity of the genus *Sphaerium*. These two clades were also recovered in *Musculium*; however one (B) was here represented by a single recombinant allele. With the exception of a divergent segment in one putatively recombinant allele, the expressed PGD repertoire of the three *Pisidium* species investigated was restricted to one of the two clades (A). Major within-clade PGD gene tree branching patterns were congruent with mitochondrial gene tree topologies for these taxa. These results are inconsistent with a pattern of recent independent attainment of a polyploid status by our *Sphaerium/Musculium* study taxa and indicate that they may share a common genome duplication event predating the Miocene appearance of these two genera in the fossil record.

Evolutionary relationships among North American sphaeriid genera were investigated based on morphological characters (Chapter 4). Thirty-two shell, soft-anatomy and brooding/life history characters were coded and a parsimony analysis was performed. The results show a sister-relationship between *Sphaerium* and *Musculium* taxa, congruent with previous molecular phylogenies but not with morphological studies. The main problem of earlier morphological analyses has been the lack of modern phylogenetic perspective and the use of micro-scale anatomical characters whose phylogenetic utility has not been fully established. The present phylogenetic analysis of morphological characters also supports the evolutionary advancement in brooding character complexity from synchronous to sequential brooding, rather than simplification due to the shell and body size reduction. However, this study is not able to provide further subgeneric-level relationships due to the shortage of informative morphological characters.

In order to better understand phylogenetic relationships of the Sphaeriinae, I attempted to encompass a large number of taxa collected from a broad geographical range and to utilize molecular sequence data obtained from multiple independent gene

fragments (Chapter 5). Sequences of three different gene fragments, mitochondrial cytochrome c oxidase subunit I (COI), mitochondrial large ribosomal subunit (16S), and nuclear ribosomal first internal transcribed spacer (ITS1), were independently characterized and phylogenetically analyzed. Although a certain degree of saturated evolution was indicated in all three data sets, they were not significantly incongruent and contributed phylogenetic signals to different levels of branches. Data sets were combined into different combinations and these partitions were simultaneously analyzed. The combined analysis of all molecular data sets yielded a well resolved phylogeny and the current sphaeriine taxonomy was revised based on this phylogeny. Instead of three cosmopolitan genera, five monophyletic lineages, *Afropisidium*, *Odhneripisidium*, *Pisidium*, *Cyclocalyx* and *Sphaerium*, were recognized within the subfamily. Although their phylogenetic relationships were not certain, several subgeneric-level groups were also recovered within *Sphaerium*: *Herringtonium*, *Sphaerium s. str.*, *Sphaerinova*, *Amesoda*, and *Musculium* together with two unassigned species, *S. rhomboideum* and *S. transversum*. Revised sphaeriine systematics were compared with current classification and with Russian systems. Intraspecific genetic variation was evident in some species. Although preliminary, genotypes obtained from geographically distant populations of *Cyclocalyx casertanum* were significantly divergent and apparently paraphyletic, suggesting the possible presence of cryptic species in the morphologically variable cosmopolitan species.

The studies on sphaeriine polyploid taxa indicate that pronounced polyploidy may be prevalent in the Sphaeriinae and ancestral patterns of polyploidization may underlay much of the present diversity of this freshwater clam radiation. Independent phylogenetic analyses of morphological and molecular characters not only provide a comprehensive phylogenetic framework for the Sphaeriinae, but also increase our understanding of the evolution of salient sphaeriine characteristics. Further studies, however, are still needed to better understand the paths of sphaeriid evolution. Although

meiosis has been observed in a polyploid species, *S. striatinum*, it is not clear whether or not polyploidy in any of the other sphaeriine species is associated with asexuality. To complete comprehensive combined phylogenetic analysis, the inclusion of *Neopisidium* representatives, thorough investigation of morphological and ecological characteristics based on newly proposed molecular phylogeny, and incorporation of those characters with DNA sequence data sets are necessary. An in-depth sampling of genotypes from geographically distant populations will determine if the ability of sphaeriid passive dispersal accounts for widespread distribution of apparent cosmopolitan species. In addition, phylogenetic study on the other sphaeriid subfamily Euperinae, whose modern distribution reflects potential Gondwanan origin, will provide valuable empirical data on the historical biogeography of the family.