Phylogenetic analysis of the Sphaeriidae (Mollusca: Bivalvia) based on partial mitochondrial 16S rDNA gene sequences

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Abstract. We have constructed molecular phylogenetic trees for members of the Sphaeriidae in order to test proposed generic level relationships, and to reconstruct the evolutionary pattern of parental care, in this exclusively freshwater family of heterodont bivalves. An ~480 nucleotide fragment of the mitochondrial large ribosomal subunit (16S rDNA) was sequenced for 4 corbiculid outgroups in addition to 19 sphaeriid ingroup taxa. Ingroup species were obtained from North and South America, Europe, and Australasia and included representatives of the main sphaeriid genera. Our analyses support four primary conclusions: 1) the Sphaeriinae are robustly monophyletic with respect to *Eupera platensis*; 2) the genus *Pisidium* is paraphyletic and *P. sterkianum* is sister to the 17 other sphaeriine taxa in our dataset; 3) synchronous brooding is the ancestral reproductive pattern in the Sphaeriinae; 4) the sequential brooders form a clade in which *Musculium* taxa are monophyletic and nested among lineages of *Sphaerium*. Our gene trees reveal an evolutionary progression in parental care complexity from the relatively simple pattern in the Euperinae, to the origin of brood sacs and of extraoogonial embryonic nutrition in the common ancestor of the Sphaeriinae, and ultimately to the development of sequential brooding in *Sphaerium/Musculium* taxa.

Additional key words: brooding, systematics, Heterodonta, freshwater, evolution, fingernail clams

The Sphaeriidae (fingernail, pill, and pea clams) are one of the few groups of bivalve molluscs to have undergone a successful radiation into freshwater habitats (McMahon 1991). This exclusively freshwater family has a cosmopolitan and ubiquitous distribution in seasonal pools, rivers, streams and lakes (Burch 1975; Kuiper 1983; Morton 1985). Sphaeriids are often the numerically dominant benthic macroorganisms in streams and ponds (Eckblad et al. 1977) where they may reach densities of $<50-100 \times 10^3/m^2$ (Hinz 1977; Sandusky & Sparks 1979) and play a key role in energy and nutrient cycling (Hornbach & Wissing 1984; Lopez & Holopainen 1987).

Systematists have traditionally assumed that sphaeriids and the brackish/freshwater corbiculid bivalves represent sister taxa, stemming from late Mesozoic marine veneroid ancestors (Keen & Casey 1969; Taylor et al. 1973; Nutall 1990; Morton 1996). Recent morphological (Dreher-Mansur & Meier-Brook 1992, 2000) and molecular (Park & Ó Foighil 2000) studies

indicate that these two heterodont families resulted from independent heterodont radiations into freshwater habitats, although a convincing outgroup for the sphaeriids is still lacking. Sphaeriid systematics have historically been hampered by the considerable ecophenotypic and allometric variation in shell shape exhibited by many taxa (Holopainen & Kuiper 1982; Bailey et al. 1983; Dyduch-Falinowska 1988), which contributed to a dramatic overestimation of their species diversity by early alpha taxonomists (Kuiper 1983). Another problem has been the use of an alternative system of classification by Russian taxonomic school systematists (Korniushin 1998a, b). The general consensus among non-Russian school malacologists has been that the Sphaeriidae contain five genera: *Pisidium, Sphaerium, Musculium, Eupera,* and *Byssanodonta*. These genera have been diagnosed on the basis of details of the shell and soft-part morphology and of reproductive/developmental characteristics (Burch 1975; Kuiper 1983; Heard 1977; Ituarte 1988, 1989). *Pisidium, Sphaerium,* and *Musculium* are cosmopolitan genera, placed in the subfamily Sphaeriinae by Dreher-Mansur & Meier-Brook (1992, 2000), with maximum diver-

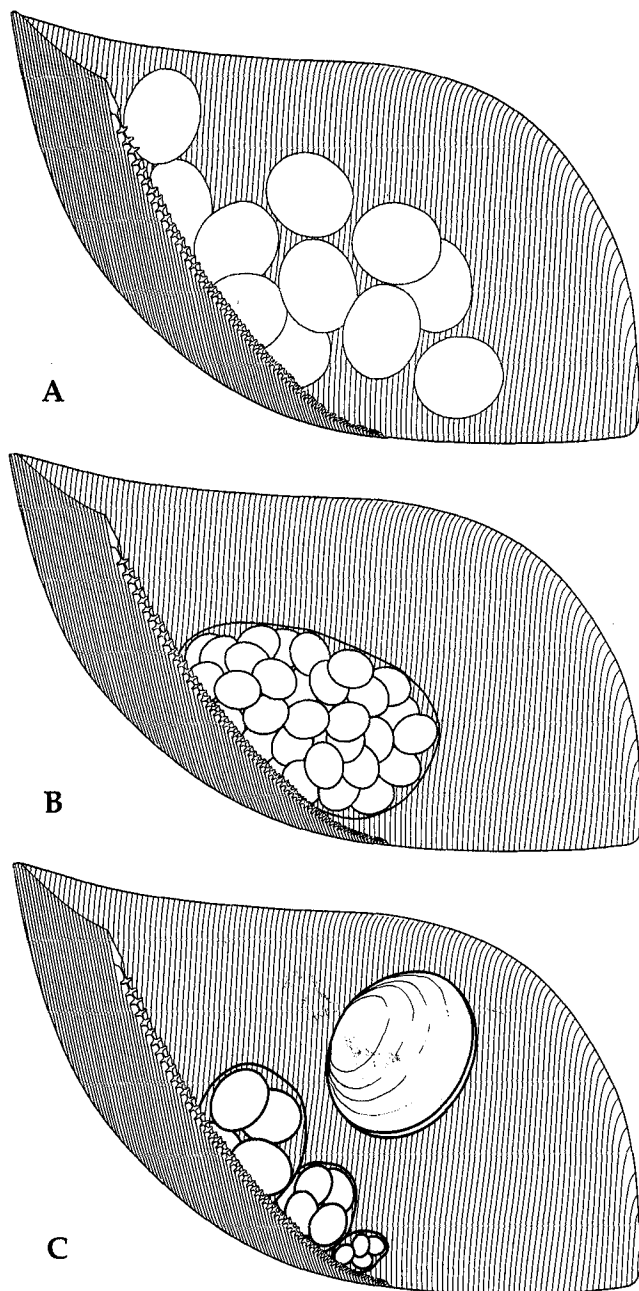[a] Author for correspondence. E-mail: diarmaid@umich.edu.

Fig. 1. Diagrammatic representation of the ctenidial brood mass organization found in species of *Eupera* (A, synchronous, no brood sac), *Pisidium* (B, synchronous, with brood sac) and *Sphaerium* (C, sequential, with ontogenetically staggered brood sacs). The late-stage shelled *Sphaerium* individual depicted has exited its brood sac but has yet to be released to the external environment.

sities in the Holarctic Region. *Eupera* and *Byssanodonta* are members of the subfamily Euperinae and have more restricted geographic ranges; the former occurring in Central and South America and Africa (although secondarily introduced to the southern United

States) and the latter is a monotypic genus restricted to the Rio Parana, Argentina (Ituarte 1989).

Generic-level relationships among the Sphaeriidae have undergone numerous, and frequently contradictory, reinterpretations over the past four decades (Klappenbach 1960; Herrington 1962; Alimov & Starobogatov 1968; Meier-Brook 1970; Zeissler 1971; Ellis 1978; Kuiper 1981; Dyduch-Falinowska 1983; Dreher-Mansur & Meier-Brook 1992, 2000; Korniushin 1991, 1994, 1995). Dreher-Mansur & Meier-Brook (1992, 2000) performed the first cladistic analyses of sphaeriids. They found a sister relationship between a euperine clade (containing *Eupera* and *Byssanodonta*) and a sphaeriine clade which had the following topology: (*Sphaerium* (*Musculium*, *Pisidium*)). A sister relationship for *Musculium* and *Pisidium* was supported primarily by a suite of kidney characters (Dreher-Mansur & Meier-Brook 1992, 2000). This result is inconsistent with earlier studies in which one of the more contentious issues centered on whether *Sphaerium* and *Musculium* taxa were sufficiently distinct to warrant separate generic status (Herrington 1962; Kuiper 1962; Clarke 1973; Heard 1977; Ellis 1978; Hornbach et al. 1980a). It is also inconsistent with Park & Ó Foighil's (2000) molecular phylogeny (28S rDNA) of freshwater heterodont lineages, which incorporated a small number of sphaeriid taxa. Their gene trees yielded a robustly monophyletic Sphaeriidae containing a sphaeriine clade with the following topology: (*Pisidium sterkianum* (*Pisidium dubium* (*Musculium lacustre, Sphaerium corneum*))). Intrageneric sphaeriid relationships are still poorly understood but a number of systematic hypotheses have been proposed (Kuiper 1962, 1983; Clarke 1973; Hornbach et al. 1980a, b; Ituarte 1989; Korniushin 1991, 1994).

Sphaeriids have complex reproductive modes that have value as systematic characters (Burch 1975; Kuiper 1983; Heard 1977; Ituarte 1988, 1989). All taxa are hermaphroditic and young are brooded within the inner demibranch of the ctenidia (gills) until their release as benthic juveniles (Odhner 1922; Heard 1965a, Ituarte 1988; Korniushin 1991, 1996). Euperine eggs are much larger and yolkier than those of the Sphaeriinae (Heard 1977; Dreher-Mansur & Meier-Brook 1992, 2000; Ituarte 1997) and there is evidence for extraoogonial gill-embryo nutrient transfer during development in the latter subfamily (Okada 1935; Mackie et al. 1974; Mackie 1978; McMahon 1991; Dreher-Mansur & Meier-Brook 1992, 2000; Hetzel 1994). Brooding structures vary in their ctenidial placement, degree of elaboration, and tempo of production (Figs. 1A-C). The simplest condition is found in the Euperinae where synchronously developing brooded embryos, resulting from a single spawning event, lie freely

between the gill lamellae (Heard 1965b; Mackie & Huggins 1976; Ituarte 1988). Members of *Pisidium* species are also synchronous brooders; however, developing embryos are sequestered within a distinct suprabranchial brood sac/marsupium that is formed by an outgrowth of the ctenidial lamellae (Heard 1965a, 1977; Korniushin 1991, 1996; McMahon 1991; Hetzel 1994). Members of *Sphaerium* and *Musculium* species are sequential brooders, i.e., the products of multiple spawning events co-exist within the inner demibranch. Their brood masses contain developmentally discrete subsets of embryos, each subset being encapsulated in a separate brood-sac attached to the gill lamellae (Heard 1965a, 1977; Mackie et al. 1974; Morton 1985; Korniushin 1991, 1996; Hetzel 1994). According to the Dreher-Mansur & Meier-Brook (1992, 2000) cladistic studies, asynchronous brooding is the ancestral condition in the Sphaeriinae and synchronous brooding has been secondarily reacquired in *Pisidium* lineages. Korniushin (1991) initially interpreted asynchronous brooding as the derived condition in broodsac-forming sphaeriids but later (Korniushin 1998c) concluded that it was likely to be ancestral.

The primary aim of our study was to test hypotheses of generic level relationships among the Sphaeriinae by constructing a sphaeriid molecular phylogeny. We additionally aimed to reconstruct the evolutionary pattern of sphaeriine parental care evolution by tracing reproductive characters on our phylogenetic trees. Our dataset [480 aligned nucleotides (nt) of mitochondrial 16S rDNA] encompasses taxa from North and South America, Europe and Australasia which include representatives of all four main sphaeriid genera and most of the primary subgeneric groupings of *Pisidium* and *Sphaerium* (Kuiper 1962; Burch 1975; Clarke 1973; Korniushin 1991, 1994).

Methods

Samples were collected from several locations in North America (Table 1) by the first author (LRC), and by T. Lee. Michigan specimens of *Sphaerium striatinum* exhibit considerable variation in shell phenotype (T. Lee, pers. comm.) and we included 5 conchologically diverse individuals from 4 locations in our dataset. Samples from outside North America were obtained by loan from generous colleagues worldwide. Species identifications were performed by the respective collectors, confirmed by the first author and by T. Lee, and voucher specimens have been deposited in the Museum of Zoology, University of Michigan (see Table 1). Specimens were either collected 95% ethanol, or transported alive to the laboratory and subsequently frozen at −70°C.

DNA extractions were performed using 20–30 mg of mantle tissue in larger specimens or the whole animal in smaller specimens. Details of the molecular techniques used for generating 16S rDNA sequencing templates are available in Jozefowitz & Ó Foighil (1998). The ~480 nt target fragments were amplified with *Taq* DNA Polymerase (Gibco BRL) and both strands were cycle-sequenced (BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, PE Applied Biosystems Inc.) prior to being electrophoresed on an ABI 377 automated DNA sequencer.

Initial alignments were constructed with the default parameters of the Clustal option in Sequence Navigator 1.0.1 [(Kececioglu & Myers 1994), which is based on Clustal V (Higgins et al. 1992)] and then manually adjusted to minimize mismatches. The alignment is available upon request from the corresponding author and the individual sequences have been deposited in GenBank (Table 1). Sequences were analyzed using a parsimony approach (PAUP* 4.0b2a, Swofford 1998). Convincing sister taxa for the Sphaeriidae are presently unknown (Dreher-Mansur & Meier-Brook 1992, 2000; Park & Ó Foighil 2000) and we utilized corbiculids (*Corbicula fluminea, C. madagascariensis, C. australis,* and *Neocorbicula limosa*) as outgroups. Analyses were performed as replicated (N = 10) unweighted heuristic searches using random stepwise addition, all of which retrieved topologically identical shortest trees. Inferred sequence gaps were considered either as character states or else coded as missing data. Consensus trees were made using the strict consensus option in PAUP. Successive character weighting as a function of the rescaled CI (Farris 1969, 1989) was employed to discriminate among equally parsimonious trees. Removing the inferred gaps from the analyses did not change the consensus topology of the most parsimonious trees. Branch support levels were estimated by bootstrapping (200 heuristic iterations using random stepwise addition with 10 replications) and also with decay index values (Bremer 1988, 1994), calculated using TreeRot (Sorenson 1996). TreeRot was utilized to establish a constraint statement for each node in the strict consensus tree. Support indices were calculated by subtracting the number of steps in the shortest unconstrained tree from the number of steps found in each of the constrained searches. MacClade 3.0 (Maddison & Maddison 1992) was used to investigate treelengths for alternative topological arrangements.

Results

Twenty seven different 16S rDNA haplotypes were generated for the 4 outgroup and 19 ingroup taxa. Of

Table 1. Locality data, voucher specimen information (University of Michigan Museum of Zoology, Mollusk Division catalog number) and GenBank Accession number for each of the taxa used in this study. Specimens sampled by [1] John Sparks, [2] Maria Byrne, [3] Louanne Reich, [4] Cristián Ituarte, [5] Taehwan Lee, [6] Olaf Hetzmeyer, [7] Sheryl Roa and [8] Alexei Krnuishin are respectively identified in the Collection locality column.

| Taxon	Collection locality
Class Bivalvia	
Subclass Heterodonta	
Order Venerodia	
Superfamily Corbiculoidea	
Family Corbiculidae	
Corbicula madagascariensis (SMITH 1882)	[1] Madagascar
C. australis (DESHAYES 1830)	[2] Australia
C. fluminea (MÜLLER 1774)	[3] Michigan USA
Neocorbicula limosa (MATON 1809)	[4] Argentina
Family Sphaeriidae	
Subfamily Euperinae HEARD 1965	
Genus *Eupera* BOURGUIGNAT 1854	
E. platensis DOELLO JURADO 1921	[4] Argentina
Subfamily Sphaeriinae DREHER-MANSUR & MEIER-BROOK 1992	
Genus *Pisidium* PFEIFFER 1821	
Pisidium s.s.	
P. dubium (SAY 1817)	[5] Michigan, USA
Subgenus *Cyclocalyx* DALL 1903	
P. adamsi STIMPSON 1851	[5] Michigan, USA
P. compressum PRIME 1852	[5] Michigan, USA
P. milium HELD 1836	[5] Michigan, USA
P. variabile PRIME 1852	[5] Michigan, USA
Subgenus *Afropisidium* KUIPER 1962	
P. sterkianum PILSBRY 1897	[4] Argentina
Genus *Musculium* LINK 1807	
M. lacustre (MULLER 1774)	[6] Germany
M. argentinium (D'ORBIGNY 1835)	[4] Argentina
M. partumeium (SAY 1822)	[5] Michigan, USA
M. securis (PRIME 1852)	[5] Michigan, USA
Genus *Sphaerium* SCOPOLI 1777	
Sphaerium s.s.	
S. corneum (LINNAEUS 1758)	[6] Germany
S. fabale (PRIME 1852)	[3] Michigan, USA
S. rhomboidium (SAY 1822)	[5] Michigan, USA
S. simile (SAY 1816)	[3] Michigan, USA
S. striatiinum (LAMARCK 1818)	
(A)	[3] Ore Creek, MI, USA
(B)	[5] Au Sable R., MI, USA
(C)	[5] Au Sable R., MI, USA
(D)	[5] Maple R., MI, USA
(E)	[3] Battle Creek, MI, USA
Subgenus *Herringtonium* CLARKE 1973	
S. occidentale (LEWIS 1856)	[3] Michigan, USA
Subgenus *Sphaerinova* IREDALE 1943	
S. novaezealandia DESHAYES 1854	[7] New Zealand
S. tazmanicum (TENISON WOODS 1876)	[8] NSW, Australia
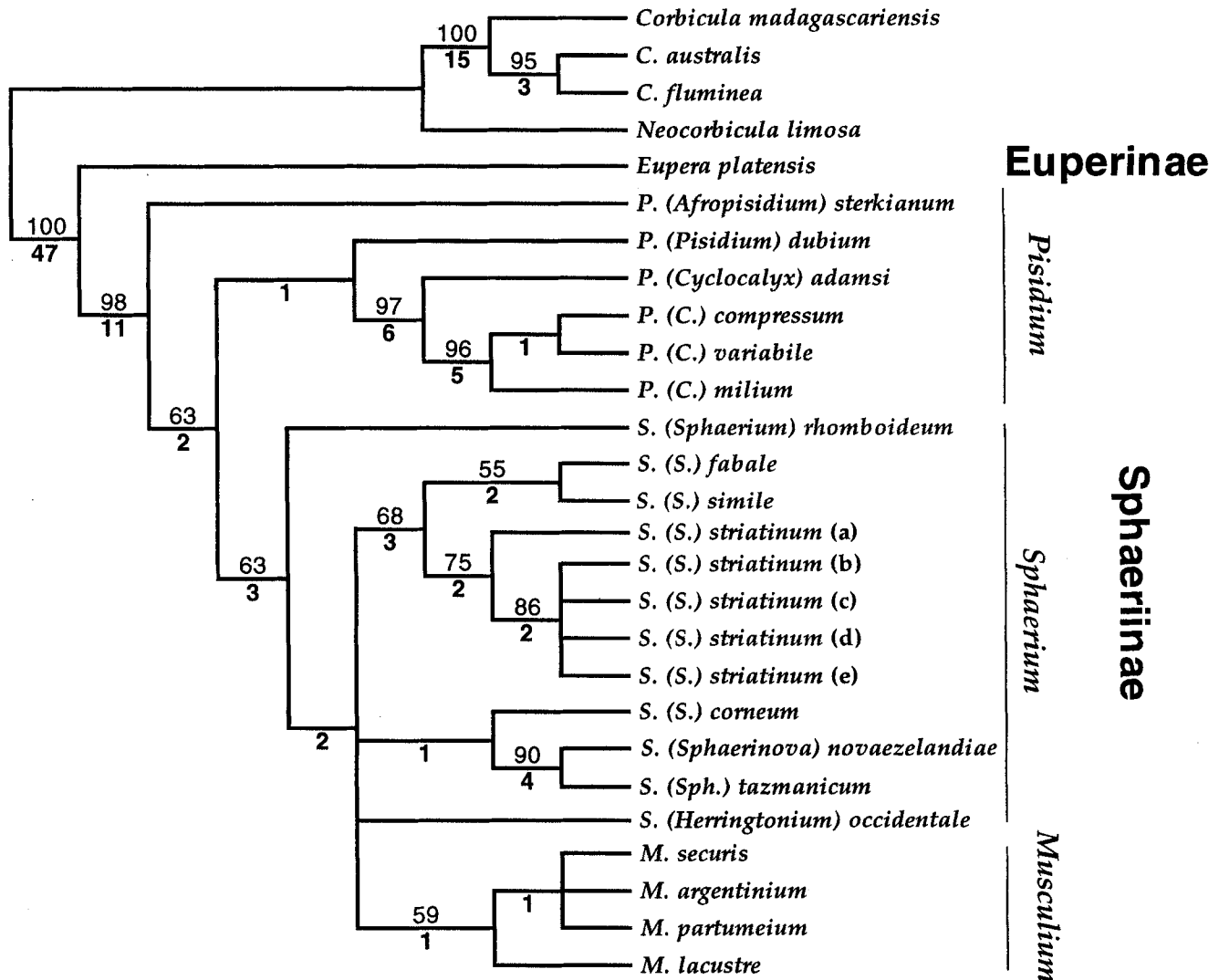
Fig. 2. Strict consensus of the 3 most parsimonious trees (length 476; CI: 0.6429, RI: 0.7595) based on heuristic analysis of the 16S rDNA mitochondrial rDNA data set in which the corbiculid taxa were designated as outgroups and inferred gaps were treated as missing characters. Numbers above the branches represent bootstrap values, numbers below indicate decay index values.

the 480 characters in the aligned dataset, 221 were variable and 170 of these were informative under conditions of parsimony when inferred gaps were coded as missing data. For ingroup (Sphaeriidae) comparisons only, 149 and 84 of the sites were variable and parsimoniously informative respectively, and there were 5 minor inferred insertions/deletions in the ingroup alignment. When inferred gaps were considered as character states, the number of variable sites was 237 (entire dataset) and 159 (ingroup) and the number of parsimoniously informative sites was 195 (entire dataset) and 85 (ingroup). A highly skewed distribution ($g_1 = -1.8946$) was obtained for 10^5 randomly sampled trees, generated in PAUP, indicating that significant cladistic information (p<0.01) exists in the

data set (Hillis & Huelsenbeck 1992). Among the ingroup taxa, sequence divergence levels (Jukes-Cantor corrected) between the sole euperine taxon, *Eupera platensis,* and the 18 sphaeriine taxa ranged 17.4–21.4% and a transition bias was maintained in a minority of pairwise comparisons. A transition bias was maintained among most among-sphaeriine pairwise comparisons and the maximum corrected pairwise genetic divergence value (16.6%) was recorded between two species of *Pisidium: P. sterkianum* and *P. milium.* Corrected genetic distance maxima among the sampled species of *Pisidium* (excluding *P. sterkianum*) was 12.6% and the respective intrageneric values for *Sphaerium* and *Musculium* were 6.4% and 2.3%.

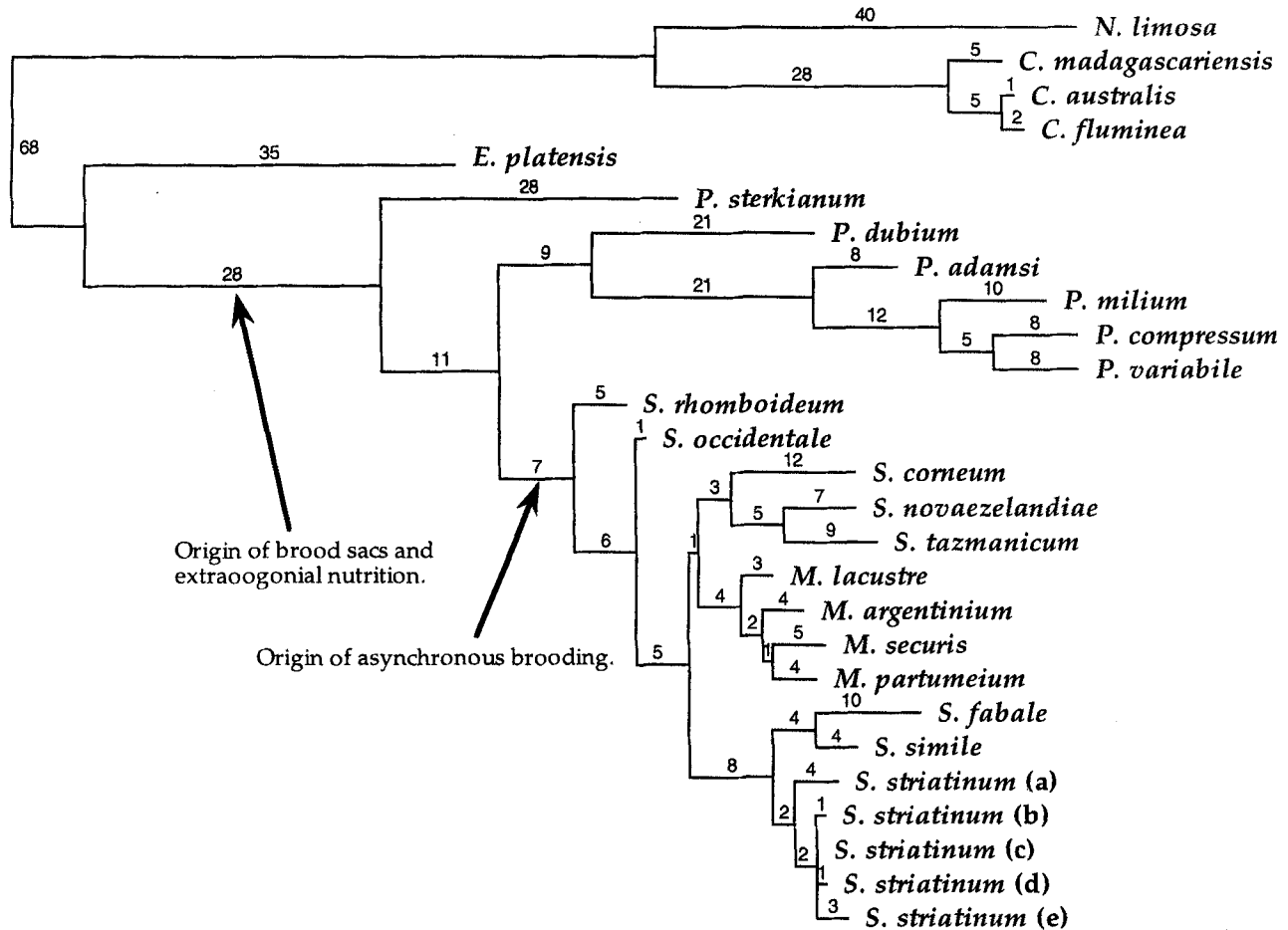Figure 2 shows the strict consensus of the 3 most

Fig. 3. The most-parsimonious tree generated by successive character weighting as a function of the rescaled CI. The inferred evolutionary origins of sphaeriine reproductive specializations (brood sacs, extraoogonial embryonic nutrition, and sequential brooding) are indicated Numbers denote branch lengths.

parsimonious trees (476 steps; CI = 0.643, RI = 0.760) obtained by a heuristic tree search of the 16S rDNA data set in PAUP. Robust support (Decay Index = 11) is evident for a monophyletic Sphaeriinae. *Pisidium* taxa form a paraphyletic assemblage in which the South American species *P. sterkianum*, our sole representative of the subgenus *Afropisidium*, is sister to all other sampled members of the Sphaeriinae. The remaining *Pisidium* species form a clade, sister to a *Sphaerium/Musculium* clade, in which *P. dubium* (the sole representative of *Pisidium s.s.*) is sister to the remaining four *Pisidium* species (placed in the subgenus *Cyclocalyx*) which are robustly (Decay Index = 6) monophyletic. The sequential brooders (*Sphaerium* and *Musculium* taxa) formed a single clade in which *S. rhomboideum* (*Sphaerium s.s.*) was basal to an unresolved polytomy encompassing four branches, one supporting *Musculium* terminal taxa and three supporting *Sphaerium* terminal taxa. The four *Musculium* species sampled were monophyletic. *S. corneum*, the

type species of *Sphaerium*, grouped with the antipodean sister lineages *S. tasmanicum* and *S. novazelandiae*, both of which were attributed to the subgenus *Sphaerinova* by Kuiper (1983). The position of *S. occidentale*, placed in the monotypic subgenus *Herringtonium* by Clarke (1973), was unresolved in the strict consensus topology (Fig. 2) and the three remaining *Sphaerium s.s.* species formed a clade containing the sister taxa *S. fabale* and *S. simile* and a monophyletic cluster of all five *S. striatinum* haplotypes.

One tree (Fig. 3) was obtained by 3 cycles of successive character weighting (Farris 1969, 1989) and it had a topology identical to one of the three most parsimonious trees obtained by the unweighted analysis. Resolution of the consensus tree polytomy (Fig. 2) involved the basal placement of *S. occidentale* and a sister status for *Musculium* lineages and the *S. corneum/Sphaerinova* taxa (Figure 3). Placement of sphaeriine reproductive/developmental characters on the resolved topology (see Fig. 3), and on the consensus

topology (Fig. 2, not shown), indicated that synchronous brooding is the plesiomorphic condition in the Sphaeriinae and that asynchronous brooding originated in the common ancestor of the *Sphaerium/Musculium* clade.

Discussion

Our dataset supports four primary conclusions: 1) the Sphaeriinae are robustly monophyletic with respect to *Eupera platensis*; 2) the genus *Pisidium* is paraphyletic and *P. sterkianum* is sister to the 17 other sphaeriine taxa in our dataset; 3) synchronous brooding is the ancestral reproductive pattern in the Sphaeriinae; 4) the sequential brooders form a clade in which *Musculium* taxa are monophyletic and nested among lineages of *Sphaerium*. A number of caveats necessarily attend our results. Our sampling of this cosmopolitan family is by no means exhaustive and, in particular, we have no representatives of the *Pisidium* subgenera *Neopisidium* and *Odhnerpisidium*. Taxa within the *Sphaerium/Musculium* clade exhibit moderate-low levels of genetic divergence for the target 16S rDNA gene fragment and some of the internal nodes are supported by relatively few characters. Supplementary data are required to more firmly establish the topological positioning of taxa such as *S. corneum*, *S. occidentale*, and *P. dubium*. Nevertheless, we are confident that the principal conclusions (outlined above) are well supported by the available data.

Our results are fully concordant with a preliminary 28S rDNA phylogeny of the Sphaeriidae (Park & Ó Foighil 2000). They partially corroborate the cladistic analyses of Dreher-Mansur & Meier-Brook (1992, 2000) by demonstrating robust support for a monophyletic Sphaeriinae. However, reconstructing our tree topology to produce a monophyletic *Pisidium-Musculium* clade sister to *Sphaerium* (Dreher-Mansur & Meier-Brook 1992, 2000) adds ≥18 additional steps to our shortest trees. It is difficult to directly compare, and/or combine, the molecular and morphological datasets because the 48 morphological characters utilized (surprisingly, sequential/synchronous brooding patterns were omitted) were not coded to allow for intrageneric variation. The phylogenetic analysis was "done up to the genus level" (Dreher-Mansur & Meier-Brook 1992, 2000) thereby enforcing generic monophyly and precluding the possibility of discovering evidence for *Pisidium* paraphyly.

One shell (absence of a cross-lamellar layer) and three kidney synapomorphies support a sister relationship for *Musculium* and *Pisidium* in the morphological dataset (Dreher-Mansur & Meier-Brook 1992, 2000), however perusal of the associated literature under-

mines the case for two of the four. Dyduch-Falniowska (1983) is cited in support of the shell character. In contrast to Mackie (1978), Dyduch-Falniowska (1983) concluded that crossed lamellar layers did not occur in *Musculium* and *Pisidium*. However, internal shell layers of some *Pisidium* species shown in her study (Figs. 76, 190, 191) clearly show crystal formations that conform to her depiction (Fig. 1) of crossed lamellar structure (Dyduch-Falniowska 1983). One of the kidney synapomorphies is listed as "kidney funnel very long" (Dreher-Mansur & Meier-Brook 1992, 2000), however according to Korniushin's (1998b) recent review of sphaeriid kidney anatomy, the pericardial duct (the proximal ciliated part of which represents the kidney funnel) of both *Sphaerium* and *Musculium* taxa are classified as "long" and those of *Pisidium* spp. as "shortened". Indeed, Zeissler (1971) utilized pericardial tube similarities to group *Sphaerium* and *Musculium* together. It is difficult to evaluate the significance of the two remaining nephridial characters supporting Dreher-Mansur & Meier-Brook's (1992, 2000) *Musculium/Pisidium* clade ("distal duct bent upward before reaching the excretory sac; slit-like opening in the excretory sac") due to the absence of information on intrageneric variation for these features. Korniushin (1998b) downplayed the pronounced levels of morphological variation in the nephridial anatomy of some *Sphaerium* and *Musculium* taxa and proposed apomorphic and plesiomorphic conditions for each kidney character. However, the inherent morphological plasticity of kidneys in some sphaeriid lineages, together with the lack of congruence between his nephridial transformation scheme (Korniushin 1998b, Fig. 33) and available gene trees (this study; Park & Ó Foighil 2000), suggest that the phylogenetic utility of nephridial characters in this family remains to be established.

Although our mitochondrial gene trees are incongruent with the morphologically based topology of inter-generic sphaeriine relationships (Dreher-Mansur & Meier-Brook 1992, 2000), we consider that they are likely to represent a more accurate reconstruction of phylogenetic relationships in this subfamily. This conclusion is based on the availability of characters from terminal sphaeriine taxa in our mitochondrial dataset and on the corroboration of salient features of the mitochondrial tree topology by an independently acquired set of nuclear gene characters (Park & Ó Foighil 2000).

Our results also have relevance for intrageneric sphaeriid classification. Both mitochondrial and nuclear (Park & Ó Foighil 2000) gene trees concur on *Pisidium* paraphyly and on the basal positioning of the South American taxon *P. sterkianum*. These results highlight this *Afropisidium* lineage as a candidate for

promotion to generic status, however such a promotion is premature, pending characterization of additional *Afropisidium* and *Pisidium s.s.* taxa and the inclusion of *Neopisidium* and *Odhnerpisidium* representatives. Our results confirm the systematic affinity of *Sphaerium* and *Musculium* taxa long proposed by sphaeriid systematists (Herrington 1962; Clarke 1973; Ellis 1978). We also find monophyly of *Musculium* lineages sampled from three continents, a result pertinent to contrasting views on the systematic validity of this genus (Herrington 1962; Kuiper 1962; Clarke 1973; Heard 1977; Ellis 1978; Hornbach et al. 1980b). Hornbach et al. (1980b) constructed a genetic distance dendogram based on allozyme data for North American *Sphaerium* (*S. fabale, S. striatinum, S. simile* and *S. occidentale*) and *Musculium* (*M. securis, M. partumeium*) taxa. Unrooted analysis of our mitochondrial 16S rDNA genotypes for these taxa yielded a topology which was broadly congruent with the allozyme dataset except that *S. fabale*, not *S. striatinum*, was sister to *S. simile*.

Phylogenetic trees represent inferred historical relationships and our gene trees reveal an evolutionary progression in parental care complexity from the relatively simple pattern in the Euperinae, to the origin of brood sac/extraoogonial embryonic nutrition in *Afropisidium* sphaeriine lineages, and ultimately to the origin of sequential brooding in *Sphaerium/Musculium* taxa. Early maturation is characteristic of many sphaeriid taxa (Heard 1977; Burky 1983; Holopainen & Hanski 1986) and the sequence of parental care character evolution implied by our gene trees may reflect selection for accelerated oogenic cycles. For instance, before an euperine can initiate brooding it must sequester enough reserves to synthesize an entire clutch of large, energy rich, yolky eggs. The oogenesis lag time until first brooding in species of *Pisidium* is potentially much shorter because embryological development can commence once the parent has produced a clutch of small, non-yolky eggs. The energy reserve threshold, and associated oogenesis time lag, necessary for commencement of brooding in species of *Sphaerium* and *Musculium* may be minimal due to the reduced number of small, non-yolky eggs in their sequential clutches. This hypothesis could be tested by running controlled laboratory experiments in which the relative temporal and energetic characteristics of sphaeriid oogenic cycles (with appropriate allometric/ fecundity corrections) were determined. Animals that live in ephemeral bodies of freshwater typically have accelerated reproductive/developmental cycles (Denver 1997) and it is tempting to view the inferred phylogenetic sequence of sphaeriid brooding patterns as an evolutionary response to the selective pressures associated with colonization of such habitats. However, many sphaeriid taxa occur in a wide variety of freshwater habitats, ranging from temporary ponds to profundal lakes (Burky 1983; Mackie 1984; Holopainen & Hanski 1986; Way 1988) and specific exceptions are available for every generality drawn concerning the ecological basis for sphaeriid life history evolution (McMahon 1991).

In summary, we have constructed the first mitochondrial gene trees of the Sphaeriidae and have utilized them to test previous phylogenetic hypotheses and to infer patterns of parental care evolution. Our results provide a preliminary phylogenetic framework for this ecologically prominent freshwater family that reveals a monophyletic Sphaeriinae, a paraphyletic *Pisidium* and a derived clade of sequentially brooding *Sphaerium* and *Musculium* lineages. Completion of a comprehensive phylogeny of the Sphaeriidae will require a more comprehensive taxonomic sampling, data from more rapidly evolving genes, and the incorporation of salient morphological characters.

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