ELSEVIER

Anatomy and reproduction of viviparous *Pisidium* (*Parapisidium*) *reticulatum* Kuiper, 1966: Implications for the phylogeny of Sphaeriidae (Mollusca: Bivalvia: Heterodonta)

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Abstract

The pea clams Sphaeriidae represent a major molluscan freshwater radiation with cosmopolitan distribution in all kinds of lotic and lentic habitats. Their phylogenetic relationships are still controversial, with comprehensive taxonomic sampling and examination of morphological characters still challenging. Here, based on rare and rediscovered original material, we study in detail the anatomy and aspects of brood protection of the African *Pisidium reticulatum* Kuiper, 1966. Representing the monotypic subgenus *Parapisidium* Kuiper, 1966, this species is characterized by its peculiar combination of shell and anatomical features of potentially high phylogenetic relevance. While similar to other congeners in several anatomical characters (e.g. reduction of inhalant siphon and descending lamella of outer demibranch, simplified structure of intestine coil and nephridium), *P. reticulatum* differs from other *Pisidium* species in retaining both pairs of retractor muscles of the inhalant siphon, and particularly in its peculiar mode of brooding. The yolky eggs are relatively large (160–170 µm in diameter) and are incubated in the gill, albeit in the absence of the formation of brood pouches. During later stages of incubation the larvae are surrounded by large cells similar to nourishing cells in other sphaeriids and probably with similar function. This unique combination of reproductive features is hypothesized to represent an intermediate stage between the typical ovoviviparity of Euperidae and euviviparity (i.e. nourishment by the parent animal) as found exclusively in Sphaeriidae, the latter being characterized by the possession of closed brood pouches. Phylogenetic analyses based on a comprehensive set of morphological characters reveal *Parapisidium* as the most basal lineage within a clade *Pisidium*. Evaluating the phylogenetic reconstructions based also on available molecular data for Sphaeriidae, we discuss alternative scenaria of (parallel) evolution of brood pouches and viviparity in this group.
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Keywords: Sphaeriidae; Reproduction; Brooding; Ovoviviparity; Euviviparity; Phylogeny

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Introduction

The pea or pill clams Sphaeriidae represent a major and diverse molluscan freshwater radiation with

presently cosmopolitan distribution in all kinds of lotic and lentic habitats. Though the group comprises the smallest bivalves in freshwater, these mostly mud-dwelling clams are important ecosystem components and often constitute a large portion of the benthic biota. Regarding their reproduction sphaeriids appear as specialized freshwater molluscs, being simultaneous hermaphrodites and viviparous with either synchronous or sequential brooding (Kuiper 1983; Hetzel 1993; Brunckhorst 1998; Cooley and Ó Foighil 2000; Guralnick 2004; Lee 2004).

In an attempt to understand adaptations of these molluscs to diverse freshwater environments, several recent studies aimed at reconstructing the origin and main features of the evolutionary history of pea clams by means of phylogenetic analyses on different sets of morphological characters (Dreher Mansur and Meier-Brook 2000; Korniushin and Glaubrecht 2002; Lee 2004) or molecular genetic data (Park and Ó Foighil 2000; Cooley and Ó Foighil 2000; Lee and Ó Foighil 2003), with a special focus on the peculiarities of sphaeriid reproductive biology. Underscoring a strong correlation between the evolution of brooding and colonization of freshwater habitats, the Sphaeriidae, alongside Unionoidea and Corbiculidae, were found to represent one of the three independent invasions of viviparous freshwater bivalve lineages (see discussion in, e.g., Graf and Ó Foighil 2000). However, with sphaeriid phylogenetic relationships still unresolved the evolution of brooding remains controversial, since comprehensive and balanced taxonomic sampling and re-examination of morphological data are equally challenging in this case; for a more detailed discussion see Lee (2004).

There is general agreement on the monophyly of the Sphaeriidae and on the sister group relationships of its two constituent clades, which traditionally have been classified at subfamily rank (as Euperinae and Sphaeriinae). However, based on phylogenetic analysis of morphological characters, and with a focus on differences in reproductive features, Korniushin and Glaubrecht (2002) suggested family rank for these groups, which is followed here accordingly. Admittedly, any formal decision on this marginal problem of ranking should be postponed until the phylogenetic relationships of these and closely related taxa are reconstructed more explicitly.

Interestingly, the major division of sphaeriid clams (in the traditional broad sense) is correlated, among other characters, with different reproductive strategies and modes of brooding. While in the euperid clade, represented by *Eupera*, large eggs (with diameter up to 0.5 mm) that are rich in yolk are incubated within the intrabranchial space (Dreher Mansur and Meier-Brook 2000), in the sphaeriids s. str. much smaller eggs (about 0.05 mm in diameter) are incubated within specialized brood pouches, where the developing embryos and juveniles receive considerable parts of their nourishment from the parent animal by matrotrophy (Meier-Brook 1970; Hetzel 1993; Dreher Mansur and Meier-Brook 2000; Korniushin and Glaubrecht 2002).

However, within the Sphaeriidae s. str. the details of phylogenetic relationships are still unresolved. Particularly the monophyly of *Sphaerium*, *Musculium* and *Pisidium* remains controversial. Analyses based on morphological characters (Dreher Mansur and Meier-Brook 2000; Korniushin and Glaubrecht 2002) interprete the mode of brooding characteristic for *Pisidium* (where only one brood develops at a time, i.e. synchronous brooding) as an apomorphic state and suggest monophyly of the group. In contrast, all available molecular data (Cooley and Ó Foighil 2000; Park and Ó Foighil 2000; Lee and Ó Foighil 2003) show *Pisidium* as a paraphyletic assemblage, with the Gondwanan *Afropisidium* representing a basal lineage within Sphaeriidae, whereas a clade comprising *Sphaerium* and *Musculium* is sister to the cosmopolitan *Cyclocalyx* plus the Holarctic *Pisidium* s. str. (for more details see the discussion section below; Fig. 7A). Accordingly, the authors of these molecular studies reinterpreted synchronous brooding as the plesiomorphic condition in Sphaeriidae s. l., whereas asynchronous brooding is considered as synapomorphic characterizing *Sphaerium* and *Musculium*.

A recent re-evaluation of morphology-based phylogeny particularly for North American sphaeriid taxa (Lee 2004) found that *Sphaerium* and *Musculium* form a clade while the monophyly of *Pisidium* remained unsettled, a view congruent with previous gene trees but not with morphological studies. This analysis also suggested that synchronous brooding observed in *Eupera* and *Pisidium* is plesiomorphic and favoured the hypothesis of an evolutionary development in brooding-related character complexity from synchronous to sequential brooding as poposed earlier (Korniushin 1991; Cooley and Ó Foighil 2000). Until a comprehensive phylogenetic analysis combining morphological and all available molecular data is undertaken, this conflict between trees from morphological and molecular characters – though not a rare phenomenon in the phylogenetic literature, see e.g. Baker et al. (1998), Hillis and Wiens (2000) – remains unsettled for sphaeriid phylogeny. However, such an attempt is beyond the scope of the present paper.

Besides the lack of a simultaneous morphological and molecular analysis of Sphaeriidae, another unfortunate drawback has been that the taxonomic basis for both was incomplete. In particular, neither of these analyses included the monotypic African subgenus *Parapisidium* Kuiper, 1966. Kuiper's original description reported on some rather peculiar morphological features in this taxon, viz. a unique reticulate sculpture and external ligament of the shell in combination with the

non-reduced branchial opening and the developed outer demibranch (Kuiper 1966). However, despite the potential phylogenetic relevance of these features, our knowledge of *Pisidium reticulatum* remained very poor.

Based on the original material rediscovered in the Danish Bilharziasis Laboratory we undertook a scanning electron microscopy study and in the present paper provide new data on the anatomy of *Parapisidium*, revealing in particular its rather unusual mode of brooding. We here integrate the relevant morphological data for *P. reticulatum* with a comprehensive morphological data matrix composed recently for 57 taxa (for more details see Korniushin and Glaubrecht 2002), discuss the reconstructed phylogeny, and evaluate alternative evolutionary scenarios for the (multiple) origin of viviparity and incubatory strategies in Sphaeriidae s. l. in the light of these new data.

Material and methods

In addition to the types of *Pisidium reticulatum* present in SMF we rediscovered other specimens, also cited in the original description by Kuiper (1966) and in subsequent publications by Mandahl-Barth (1972, 1988), among the collections of the latter at the DBL – Institute for Health Research and Development (formely Danish Bilharziasis Laboratory). Unfortunately, no material particularly suitable for molecular study was available, due to the poor preservation of specimens even in the only extant alcohol lot collected in 1969 that was fixed with formalin; see below.

For comparison, specimens of *Eupera klappenbachi* Mansur & Veitenheimer, 1975 from Brazil (collection of Claus Meier-Brook, Tübingen, Germany) and *Pisidium carum* Cotton, 1953 from New South Wales, Australia (AMS) were studied.

Throughout this paper we use the following codens for museum collections in which vouchers of all material discussed here is deposited: AMS = Australian Museum, Sydney; DBL = Institute for Health Research and Development (formerly Danish Bilharziasis Laboratory), Charlottenlund near Copenhagen, Denmark; SMF = Forschungsinstitut und Naturkundemuseum Senckenberg, Frankfurt am Main, Germany.

Material examined

The type material in the SMF is from Madagascar; it is supplemented with material indistinguishable from *P. reticulatum*, on which Kuiper (1966) also based his original description and which is extant in the DBL. However, in anatomical descriptions and figures we refer to the respective origin of the corresponding specimens, as detailed below.

Madagascar: "Insel Nossi-Bé, NW Mad., Andranobé-Bach''; = Nosy Be Island (holotype, SMF 183936; 2 paratypes, SMF 183937; leg. F. Starmühlner 1958).

Zimbabwe: "Hunyami River, below dam, S Salisbury''; = Hunyani River (a tributary of the Zambezi River), S of Harare (DBL no. 155, dry; leg. C.C. Cridland 1963); "Gwebi River, 56 km W of Salisbury''; (a tributary of the Hunyani, which it joins SW of Harare) (DBL no. 28, dry; leg. C.C. Cridland 1963).

Malawi: "Zambo Bay'', Lake Malawi; 14°03′S, 34°55′E, at about 10 m (30–40 ft) depth, in algae (DBL; part of this lot is kept dry, another part in 70% alcohol; leg. C.C. Cridland, Dec. 1969). Altogether six specimens were dissected, with shell lengths 2.3–3.1 mm.

We have attempted to precisely locate the geographic origin of the studied museum specimens, evaluated using the following sources: Southern Rhodesia and Malawi Official Standard Names, approved by the United States Board on Geographic Names, prepared by the Defense Mapping Agency, Topographic Center, Washington, DC, 1973 and 1970; the Rhodesia sheet SE-36-5 Salisbury, published by the Surveyor General of Rhodesia, 1973, in connection with The Times Atlas of the World (1956), vol. 4, pl. 92.

Morphological analyses

Shells and soft parts or their remains preserved in some dry specimens were studied under a stereomicroscope and figured by means of a camera lucida. Shells were prepared for scanning electron microscopy (SEM) using routine procedures. Soft tissues utilized for SEM were processed with hexamethyldisilazane (HMDS) following the protocol provided in Nation (1983); parts removed from specimens were returned with other material to the respective collections, i.e. DBL.

Morphological data for *P. reticulatum* obtained during the study were incorporated in the data matrix compiled in Korniushin and Glaubrecht (2002), and analysed using PAUP* 4.0b10 (Swofford 2002). *Chamelea gallina* (Linnaeus, 1758), *Corbicula fluminea* (O.F. Müller, 1774) and *Cyanocyclas* (= *Neocorbicula*) *limosa* (Maton, 1809) were used as outgroups; the ingroup was set to monophyly; all other settings remained as in the previous analysis (Korniushin and Glaubrecht 2002).

Results

Pisidium reticulatum Kuiper, 1966

Morphological description

Shell (Fig. 1): Small (up to 3.5 mm long), trapezoid or pentagonal (Fig. 1A, E), rather high, moderately convex, thick-walled and solid. Umbo subcentral,
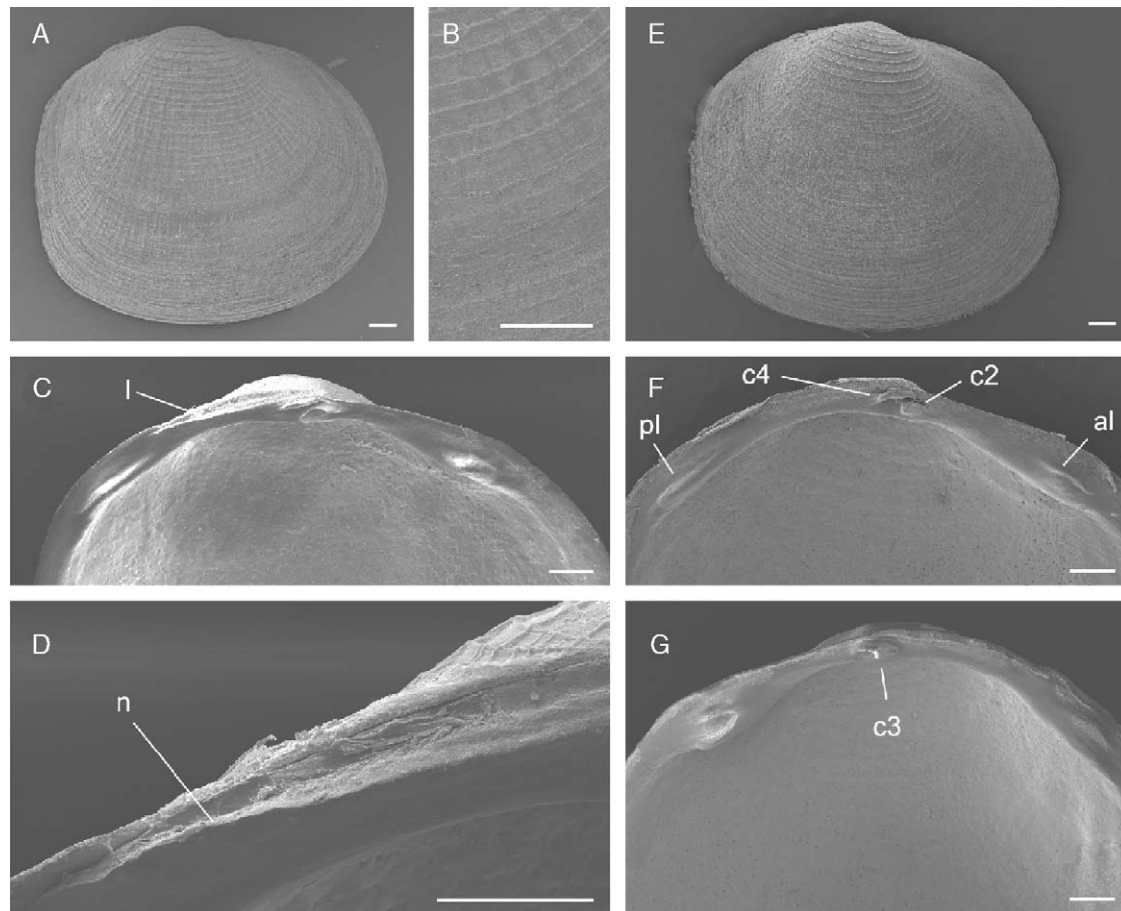
Fig. 1. Shells of *Pisidium* (*Parapisidium*) *reticulatum* Kuiper, 1966; (A–D) Hunyani River, Zimbabwe (DBL No. 155), (E–G) Lake Malawi, Malawi (DBL). (A, E) Outside view of right valve. (B) Detail of sculpture. (C, F). Hinge of left valve. (D) Posterior end of ligament pit. (G) Hinge of right valve. All scale bars = 200 µm. Abbreviations: al = anterior laterals; c2–c4 = cardinal teeth; l = ligament pit; n = nympha; pl = posterior laterals.

slightly shifted posteriorly, broad and not protruding. Sculpture well-marked, with radial and concentric ribs forming reticulate pattern. Small concentric folds of periostracum visible between ribs (Fig. 1B). Punctal canals (pores) well distinguishable on inner shell surface. Hinge plate broad (Fig. 1C, F–G). Ligament externally visible, not introverted. Ligament pit with nympha seen as pronounced step-like elevation (Fig. 1D). Cardinal teeth (Fig. 1C, F, G): c2 bent, dome-shaped or triangular; c3 also bent, markedly thickened and cleft on posterior end; c4 straight or slightly curved, placed obliquely behind c2. Lateral teeth short, thick, with blunt cusps.

Mantle edge: Only one (anal or exhalant) siphon present (Figs. 2, 3A, B), with one pair of retractors. Branchial opening present, with two pairs of retractors of which the upper one (sr2) is rudimentary (Fig. 3A, B). Presiphonal suture short or slightly elongated (up to 1/5 length of pedal slit). Inner radial mantle muscles organized in six to seven rather strong bundles, evenly arranged along pedal slit (Fig. 3D, E).
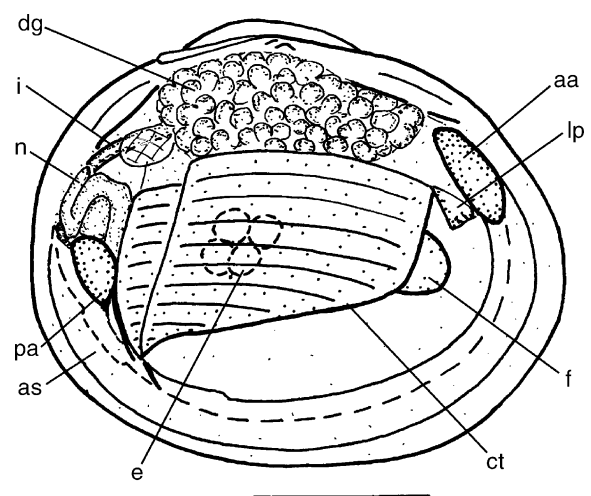

Fig. 2. Gross anatomy of *Pisidium reticulatum*, specimen from Lake Malawi, Malawi (DBL). Scale bar = 1 mm. Abbreviations: aa = anterior adductor; as = anal siphon; ct = ctenidium; dg = digestive gland; e = eggs; f = foot; i = intestine; lp = labial palps; n = nephridium; pa = posterior adductor.
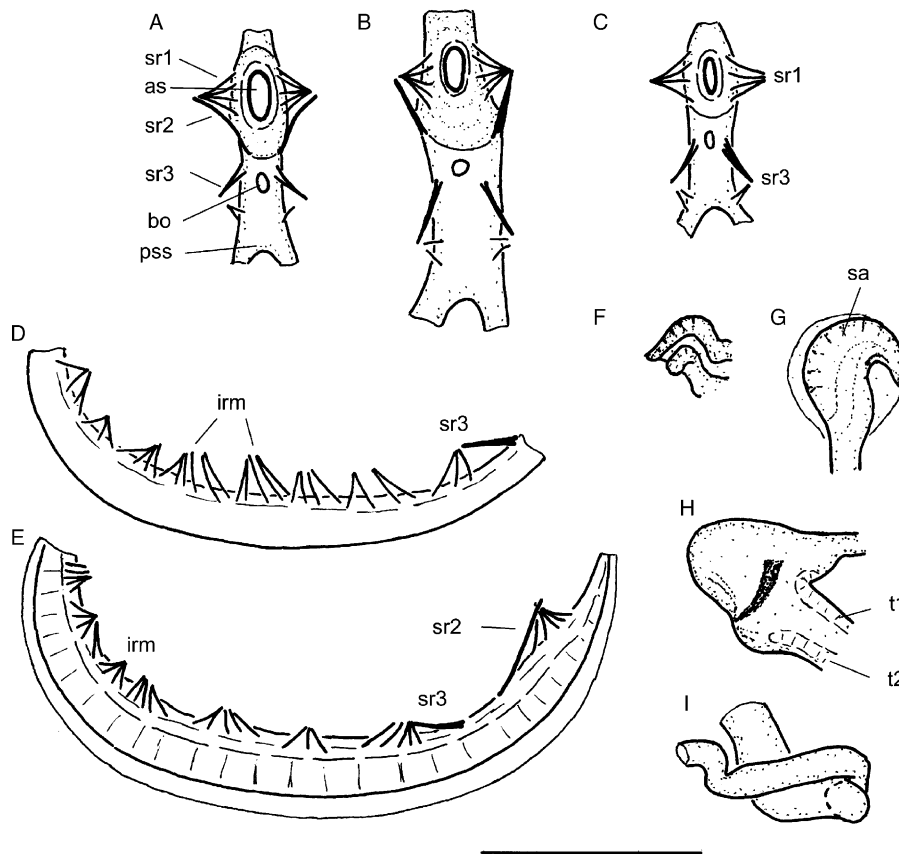
Fig. 3. Details of mantle edge and digestive system. (A, D) *Pisidium reticulatum*, Hunyani River, Zimbabwe (DBL no. 155), (B, E–I) *P. reticulatum*, Lake Malawi, Malawi (DBL), (C) *Pisidium* s. str., Lake Malawi.(A–C) Arrangement of siphonal muscles (diagrammatic). (D, E) Muscles of the mantle edge. (F) Right labial palps from inside. (G) Dorsal view of stomach. (H) Lateral view of stomach (right side). (I) Intestine coil. Scale bar = 1 mm. Abbreviations: as = anal siphon; bo = branchial opening; irm = bundles of inner radial mantle muscles; pss = presiphonal suture; sa = sorting area on stomach roof; sr1–sr3 = siphonal retractors; t1 = major typhlosole; t2 = minor typhlosole.

Gills: Outer demibranch with only one lamella, relatively large, its anterior edge placed at 7th or 8th filament of inner one (Fig. 4A–C).

Digestive system: Labial palps small. Outer palps with anteriorly projecting angle; five ridges distinguishable at their anterior edge (Fig. 3F). Stomach not separated from midgut, stretched in antero-posterior direction, with narrow sorting area on its roof (Fig. 3G). Anterior fold not elevated. Major typhlosole forming two loops directed to openings of digestive gland ducts. Minor typhlosole turning posteriorly at its proximal end (Fig. 3H). Intestine coil simple, with one loop (Fig. 3I).

Nephridia: Funnel relatively long and narrow. Pericardial tube forming two loops (Fig. 4E). Dorsal lobe relatively narrow, with medial and lateral branches tightly adjoining (Fig. 4D). Lateral loop well visible from dorsal side, separated from dorsal lobe by loop of pericardial tube. Excretory sac relatively large (as long as dorsal lobe), but not extended anteriorly (Fig. 4E).

Remarks: The specimens from Lake Malawi (DBL) differ from others by the broader hinge plate, shortened lateral teeth, more elongated presiphonal suture, and somewhat weakened bundles of inner radial mantle muscles. However, the available material is not sufficient for taxonomic distinction of the Malawian population. Variation in the form of the nephridium also deserves closer study based on a larger sample size, in order to demonstrate whether the peculiar type observed in the only available sample is restricted to Lake Malawi or a general characteristic of *Parapisidium*.

Reproduction

The gonad in *Pisidium reticulatum* is relatively small, placed at the base of the foot and not extending dorsally (Fig. 4F). Up to four relatively large eggs (160–170 μm in diameter) were recovered between the lamellae of the inner demibranch in one specimen each from Zimbawe and Lake Malawi (Figs. 2, 4C, 5A, B). The yellowish color of these eggs indicates large amounts of yolk. In *P. reticulatum* during this stage of incubation, the inner demibranch structure appears to be similar to that seen in *Eupera* (Fig. 5G); however, the eggs in the former
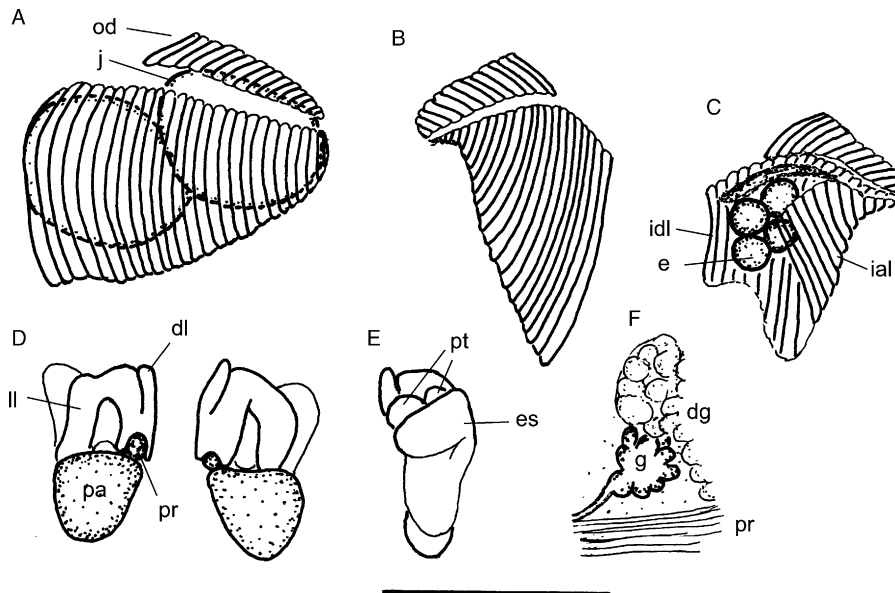
Fig. 4. Ctenidia, nephridia and gonad in *Pisidium reticulatum*; (A) paratype from Nossi-Bé (SMF 183937), (B, C) Hunyani River, Zimbabwe (DBL no. 155), (D–F) Lake Malawi, Malawi (DBL). (A, B) Ctenidia from outside. (C) Ctenidium from inner side, inner demibranch of ascending lamella partly removed. (D) Nephridia from dorsal side. (E) Nephridium from ventral side. (F) Gonad. Scale bar = 1 mm. Abbreviations: dg = digestive gland; dl = dorsal lobe of nephridium; e = eggs; es = excretory sac; g = gonad; ial = inner demibranch of ascending lamella; idl = inner demibranch of descending lamella; j = juveniles; ll = lateral loop of nephridium; od = outer demibranch; pa = posterior adductor; pr = pedal retractor; pt = pericardial tube.

taxon are apparently smaller than in the latter (280–350 μm in diameter, as measured in our mounts). Furthermore, while no transformation of gill tissue was noticeable in *Eupera,* in *P. reticulatum* thickened tissue stretched along the dorsal edge of the inner demibranch (Figs. 4C, 5A).

Three other specimens of *P. reticulatum* (one of the paratypes in SMF; two specimens from Malawi in DBL) contained two large shelled juveniles each, found in both inner demibranchs. As revealed by SEM examination (Fig. 5C, D), these juveniles are partly surrounded by thick tissue formed in both the ascending and descending gill lamellae and consisting of enlarged cells. After removal of the ascending lamella and some juveniles, this tissue is seen to form ridges along the upper edge of the inner demibranch and filaments of its middle part (Fig. 5E). The largest juvenile found in these specimens had a shell length of 0.83 mm (Fig. 5F).

None of the studied specimens, though, had a brood pouch, i.e. an incubatory structure built by the filaments of the inner demibranch that completely surrounds eggs, embryos and larvae possessing shells. We also failed to find any structures that can be interpreted as remains of the external wall of the brood pouch. It seems improbable, therefore, that such a structure exists even for a short period during incubation in *P. reticulatum*. This condition of incubation without closed pouches

(Fig. 5A–D) is distinct from the one known in all other pisidia (e.g. *Pisidium carum*, Fig. 5H).

Vela were not observed in the juveniles of *P. reticulatum* (Fig. 5D, F). However, as internal features of these juveniles remain unknown, the presence of velum rudiments cannot be ruled out.

Phylogenetic analysis

The character states for a total of 69 morphological, in particular reproductive, characters of *P. reticulatum* are provided in the Appendix A. Phylogenetic analysis of this taxon in combination with all sphaeriid species included in an earlier study (Korniushin and Glaubrecht 2002) resulted in 143 most parsimonious trees with 161 steps (CI = 0.671; RI = 0.900). The strict consensus tree (Fig. 6) shows *Pisidium (Parapisidium)* as the most basal lineage of a monophyletic clade *Pisidium*. The relationships of other sphaeriid taxa were found to remain the same as reported in our previous analysis.

Pisidium in the broad sense, i.e. including *P. (Parapisidium) reticulatum,* is supported by six synapomorphies, among them three unambiguous ones: character 14 (presence of one siphon only), character 46 (simplified coil of intestine), and character 50 (pericardial tube of nephridium with two loops). Bootstrap support of 89% for the *Pisidium* clade is relatively high. The clade consisting of the remaining pisidia (excluding *P. reticulatum*) is supported by three synapomorphies,
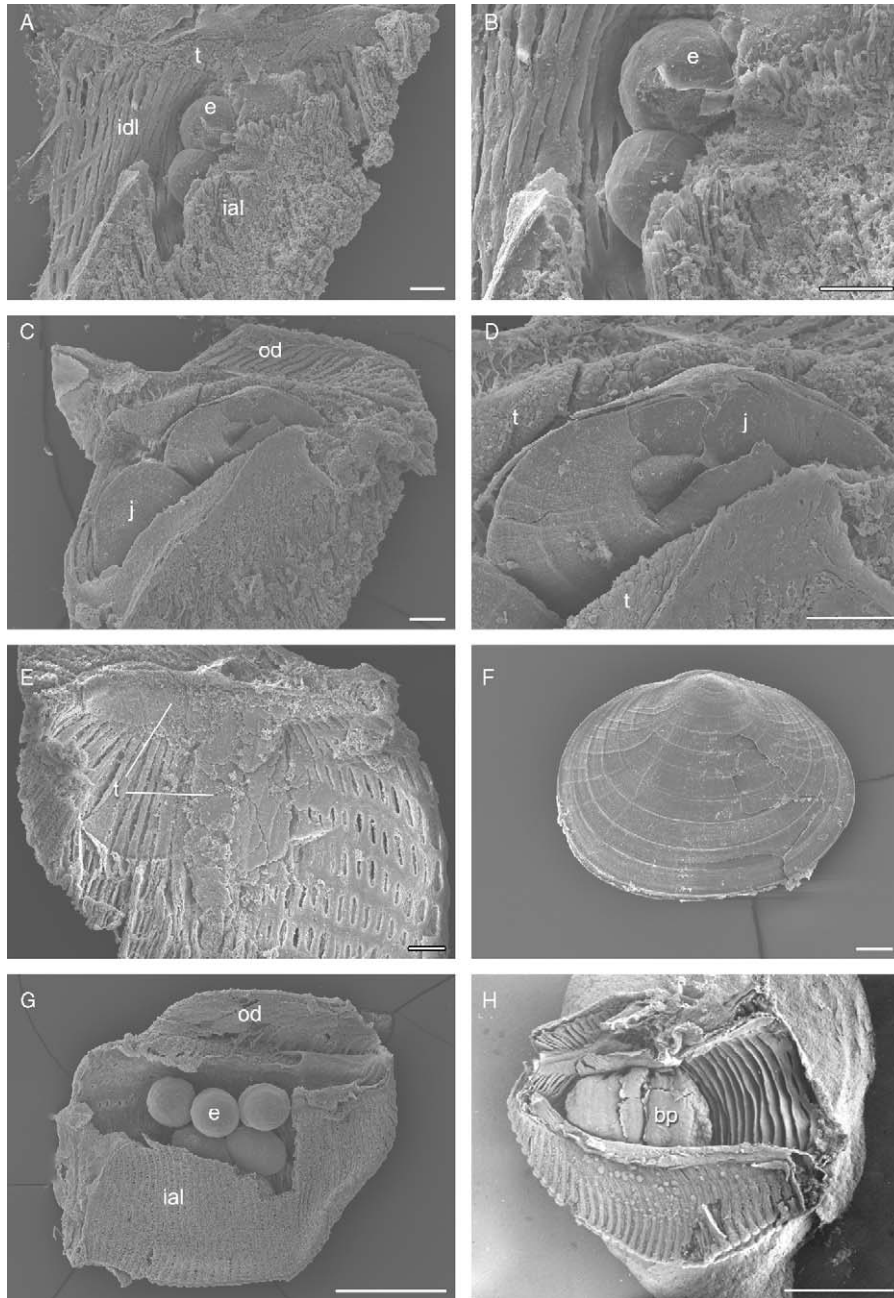
Fig. 5. Gills of brooding *Pisidium reticulatum*: (A–F) Lake Malawi, Malawi (DBL), (G) *Eupera klappenbachi*, (H) *Pisidium carum* (AMS). (A, G) Gill with eggs, ascending lamella of inner demibranch partly removed. (B) Detail of gill in A. (C) Gill with juveniles. (D) Detail of gill in C. (E) Gill of another specimen after removal of juveniles. (F) Isolated juvenile. (H) Gill with brood pouch. Scale bars: A–F = 100 μm, G–H = 500 μm. Abbreviations: bp = brood pouch; e = eggs; j = juveniles; ial = ascending lamella of inner demibranch; idl = inner demibranch of descending lamella; od = outer demibranch; t = transformed (thickened) gill tissue.

one of them unambiguous (character 18: upper retractors of inhalant siphon absent). However, bootstrap support for this clade exceeded the 50% threshold only slightly. Four character states (all ambiguous, though) represent autapomorphies of *P. reticulatum*; notably, the absence of a brood pouch in this taxon was interpreted as a secondary loss.

Discussion

Most of the morphological characters of *P. reticulatum* recovered in the present study (including shell and hinge features, mantle musculature, stomach form, and structure of nephridium) are also found in earlier studies in other members of the Sphaeriidae (cf. Dreher
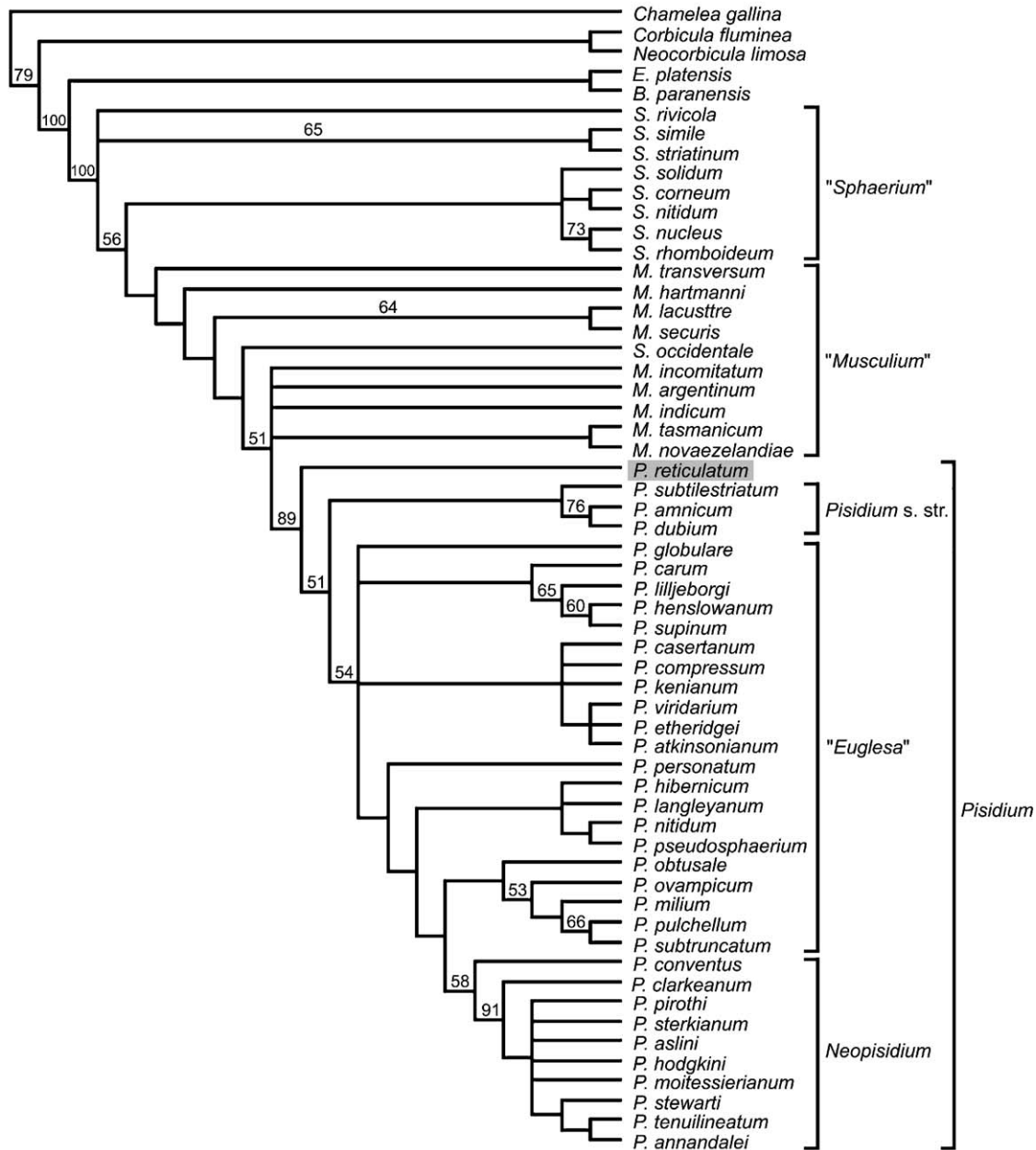
Fig. 6. Strict consensus of 143 maximum parsimony trees with 161 steps (CI = 0.671; RI = 0.900) obtained from the phylogenetic analysis of morphological characters of *Pisidium reticulatum* in combination with a data matrix on Sphaeriidae (for details see Korniushin and Glaubrecht 2002). Numbers above branches indicate bootstrap support values higher than 50%. Note that this analysis does not discover traditional monophyletic groups; the *Neopisidium* s. l. clade includes, e.g., *Afropisidium* (cf. Fig. 7).

Mansur and Meier-Brook 2000; Korniushin and Glaubrecht 2002; Lee 2004). In addition, several characters (e.g. reduced inhalant siphon, posteriorly shifted outer demibranch, simplified coil of intestine, and pericardial tube of nephridium) confirm the close relationships of *P. reticulatum* to other *Pisidium* s. l. However, this species is distinct from all other pisidia in the presence of rudimentary upper retractors of the inhalant siphon.

In contrast, the reproductive anatomy of *P. (Parapisidium) reticulatum* differs not only from other pisidia, but also from all known Sphaeriidae s. str. Interestingly, this species appears similar to the Euperidae in sharing some (presumably plesiomorphic) features, such as relatively large, yolky eggs that are incubated between the inner demibranch lamellae without being enclosed in a brood pouch (Dreher Mansur and Meier-Brook 2000).

At the same time, a similar layer of large cells as are embedding juveniles in the taxon under study was observed earlier in the internal wall of brood pouches in *Sphaerium*, *Musculium* and *Pisidium* (cf. Meier-Brook 1970; Hetzel 1993). These latter authors suggested and presented evidence, respectively, that those cells provide nourishment to the developing embryos; see also the discussion of the early literature on this matter in Meier-Brook (1970). The large size of the released embryos (greater than 0.8 mm) relative to the size of the eggs (up to 0.17 mm) is here considered as indicative of matrotrophy in *Parapisidium*, i.e. of providing nourishment other than yolk only. While in *Eupera* the size of the larvae only slightly exceeds that of the eggs (see Dreher Mansur and Meier-Brook 2002, p. 37), embryonic growth in *P. reticulatum* is more pronounced.

Therefore, we conclude that *P.* (*Parapisidium*) *reticulatum* shows a unique mode of reproduction. We hypothesize, furthermore, that this mode represents an intermediate stage between ovoviviparity which characterizes Euperidae and euviviparity which distinguishes Sphaeriidae (terminology used after Korniushin and Glaubrecht 2002).

Alternative scenarios for the evolution of viviparity

In developing hypotheses on the systematic position of *Parapisidium*, the conflicting phylogenetic reconstructions for Sphaeriidae suggested by molecular (Cooley and Ó Foighil 2000; Lee and Ó Foighil 2003) and morphological analyses (Korniushin and Glaubrecht 2002; Lee 2004) need to be taken into account. Our analyses revealed *Parapisidium* as representing the most basal lineage within a monophyletic clade *Pisidium*. However, the latter taxon itself is suggested to be a terminal clade within the Sphaeriidae s. str. that are characterized by the possession of brood pouches. Thus, the absence of a brood pouch only in *Parapisidium* can be interpreted, as one possible explanation, as secondary loss of a structure that evolved only once, i.e. in the ancestor of all proper sphaeriids.

Contrary to earlier morphological analysis, a different tree topology obtained by molecular analysis (Cooley and Ó Foighil 2000; Lee and Ó Foighil 2003) suggests, in relation to other sphaeriids such as *Sphaerium* and *Musculium,* a basal position of some *Pisidium* lineages, including *Afropisidium* (represented by *Pisidium sterkianum*) and *Odhneripisidium* (although generic status in the latter two cases needs to be verified). If, for the sake of hypothesis, we add *Parapisidium* (for which molecular sequences are lacking, though), most likely this taxon would find its place as the basal group of all Sphaeriidae s. str. based on all other available evidence (see Fig. 7A). Accordingly, the mode of reproduction in *Parapisidium* should be interpreted as the ancestral state to the
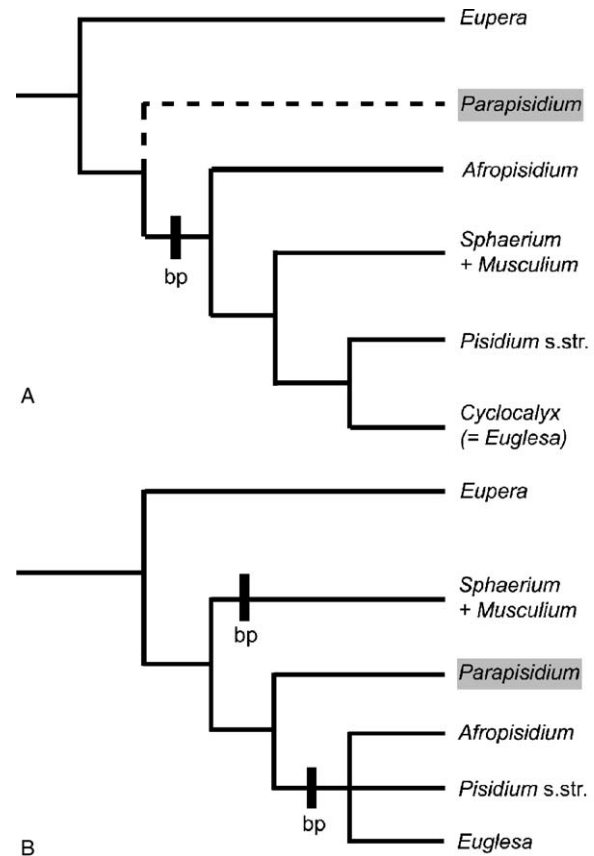

Fig. 7. Alternative phylogenetic hypotheses as discussed in the text, showing the position of *Pisidium* (*Parapisidium*) *reticulatum* (hypothetical only in Fig. 7A, since actual sequence data are missing) and the evolution of brood pouches (bp) within Sphaeriidae: (A) Hypothesis based on molecular data; adopted from Cooley and Ó Foighil (2000), with *P. reticulatum* plotted onto the tree, suggesting a single origin of brood pouches. (B) Hypothesis derived from the analysis of morphological characters in Sphaeriidae (Korniushin and Glaubrecht 2002), implying the convergent evolution of brood pouches. Note that reconstruction is simplified and that names are applied consistently to allow for comparison.

euviviparous mode found in the entire remaining clade, thus representing a model for the initial step towards the development of euviviparity that characterizes other members of the family.

Alternatively, a third scenario is suggested by suboptimal trees, i.e. those a single step longer than the most parsimonious trees in our morphological analysis, and obtained under constraints as described in Korniushin and Glaubrecht (2002). Accordingly, the enforcement of monophyly of a clade *Sphaerium* + *Musculium* (i.e. *Sphaerium* s. l.) provides a topology that also supports a monophyletic clade *Pisidium* s. l., consisting of *Afropisidium*, *Pisidium* s. str. and *Euglesa* (Fig. 7B). Consequently, when recognizing both *Sphaerium* s. l. and *Pisidium* s. l. as monophyla, the absence of a closed

brood pouch in *Parapisidium* as the most basal member of *Pisidium* could also be interpreted as an indication of multiple or parallel origin of this specific incubatory mode in two major lineages within the Sphaeriidae s.str.: once in the ancestors of *Sphaerium + Musculium*, and again in *Pisidium* sensu lato. In this scenario, the alternative modes of brooding that are exhibited by members of these two lineages, viz. asynchronous and synchronous brooding, respectively, rather support than reject this latter hypothesis.

Conclusions

The resolution of the principal problems of sphaeriid phylogeny and evolution of brooding in these freshwater bivalves is beyond the scope of the present study. However, with a unique combination of anatomical and reproductive features *Parapisidium* is certainly crucial to such a resolution. According to our cladistic analysis, the reproductive mode of *P. reticulatum* is anticipated to not only represent a potential model for an intermediate stage between those of Euperidae and Sphaeriidae, but also a potential candidate to shed new light on the evolution of brood pouch formation. Hopefully, the present study will stimulate collecting efforts in Africa to provide fresh material for more detailed morphological (in particular histological), ontogenetical and molecular studies. Analysis of sequence data and subsequent combined analysis of all available data may not only resolve the phylogenetic position of *Parapisidium*, but also facilitate an evolutionary scenario explaining the transformation of reproductive strategies in this ubiquitous but still unsufficiently known group of freshwater clams.

Acknowledgments

Appendix A

Morphological characters and character states in *Pisidium* (*Parapisidium*) *reticulatum*; see Korniushin and Glaubrecht (2002) for a detailed description of the characters and character coding for other species of sphaeriid clams used in the analyses.

1. Position of umbo: posterior (2)
2. Caps (calyculi): absent (0)
3. Position of ligament: not introverted (0)
4. Nympha: present (0)
5. Inner cardinal tooth of right valve (c1): absent (1)
6. Inner cardinal tooth of left valve (c2): bent (1)
7. Outer cardinal of right valve (c3): not divided (1)
8. Outer cardinal tooth of left valve (c4): present (0)
9. Lateral teeth: present (1)
10. Folds of periostracum: present (0)
11. Size of periostracum folds: not pronounced (0)
12. Periumbonal striae: absent (0)
13. Branchial mantle opening: present (0)
14. Siphons: only anal siphon (1)
15. Fusion of siphons: inapplicable (–)
16. Contraction of siphons: without apical part turning inside (0)
17. Retractors of anal (exhalant) siphons: separated (1)
18. Upper muscles of branchial siphon: present (0)
19. Strength of upper retractors of branchial siphon: weak (1)
20. Attachment of upper muscles of branchial siphon: tightly adjoining adductor muscles; scars not separated (1)
21. Arrangement of lower muscles of branchial siphon: forming one pair of bundles (2)
22. Presiphonal mantle fusion (suture): present (0)
23. Length of presiphonal suture: short or slightly elongate (0and1)
24. Inner radial mantle muscles: organized in bundles (1)
25. Bundles of inner radial muscles: strong (0)
26. Differentiation of mantle muscle bundles: bundles uniform (0)
27. Orientation of mantle muscle bundles: perpendicular to mantle margin (0)
28. Number of muscle bundles: six to seven (1 and 2)
29. Outer radial muscles: short (1)
30. Inner mantle fold: normally developed (0)
31. Outer demibranch: present (0)
32. Position of outer demibranch: at 7th to 8th filament (1)
33. Outer demibranch descending lamella: absent (1)
34. Inner demibranch ascending lamella: relatively low (1)
35. Interlamellar septae in inner demibranch: state unknown (?)
36. Anterior edge of outer palp: with a projecting angle (1)

37. Ridged area on palp: narrow (1)
38. Separation between stomach and midgut: absent (1)
39. Form of stomach. States: stretched in posterior direction (1)
40. Sorting area on stomach roof (SA3): narrow (1)
41. Anterior fold: not elevated (0)
42. Caeca: absent (1)
43. Anteriorly directed branch of right digestive gland duct: state unknown (?)
44. Course of major typhlosole: with two loops (0)
45. Course of minor typhlosole in stomach: turns posteriorly (1)
46. Coil of intestine: simple, with one loop (1)
47. Funnel: long (1)
48. Form of funnel: narrow (1)
49. Pericardial tube: present (1)
50. Course of pericardial tube: with two loops (1)
51. Dorsal lobe: present (1)
52. Splitting of dorsal lobe: in two sections (1)
53. Form of dorsal lobe: elongated (0)
54. Position of pericardial tube relative to dorsal lobe: covered by dorsal lobe (0)
55. Anterior extension of lateral loop: present (1)
56. Position of anterior extension: open from dorsal side (2)
57. Excretory sac: present (1)
58. Form of excretory sac: not extended (0)
59. Gonad: not extending dorsally (1)
60. Nutrition of embryos: lecithotrophic and provided by the parental animal (1 and 2)
61. Simultaneous development of several broods (asynchronous brooding): absent (0)
62. Brood pouches: absent (0)
63. Number of filaments in pouch: inapplicable (–)
64. Position of pouch: inapplicable (–)
65. Compartmentalization of brood pouches: inapplicable (–)
66. Byssus in adults: absent (1)
67. Velum: state unknown (?)
68. Outer demibranch in released young: state unknown (?)
69. Second lamella of outer demibranch in incubated larvae: state unknown (?)

References

Baker, R.H., Yu, X., DeSalle, R., 1998. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. Mol. Phyl. Evol. 9, 427–436.

Brunckhorst, D.J., 1998. Superfamily Corbiculoidea. In: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), Mollusca: The Southern Synthesis. Fauna of Australia, vol. 5. CSIRO Publishing, Melbourne, pp. 352–355.

Cooley, L.R., Ó Foighil, D., 2000. Phylogenetic analysis of the Sphaeriidae (Mollusca: Bivalvia) based on partial mitochondrial 16S rDNA gene sequences. Invert. Biol. 119, 299–308.

Dreher Mansur, M.C., Meier-Brook, C., 2000. Morphology of Eupera Bourguignat, 1854 and Byssanodonta Orbigny, 1846 with contributions to the phylogenetic systematics of Sphaeriidae and Corbiculidae (Bivalvia: Veneroida). Arch. Moll. 128, 1–59.

Graf, D.L., Ó Foighil, D., 2000. The evolution of brooding characters among the freshwater pearly mussels (Bivalvia: Unionoidea) of North America. J. Moll. Stud. 66, 157–170.

Guralnick, R., 2004. Life-history patterns in the brooding freshwater bivalve Pisidium (Sphaeriidae). J. Moll. Stud. 70, 341–351.

Hetzel, U., 1993. Reproduktionsbiologie. Aspekte der Viviparie bei Sphaeriidae mit dem Untersuchungsschwerpunkt Musculium lacustre (O.F. Müller, 1774) (Bivalvia, Eulamellibranchia). Unpubl. doctoral dissertation, University Hannover.

Hillis, D.M., Wiens, J.J., 2000. Molecules versus morphology in systematics. In: Wiens, J.J. (Ed.), Phylogenetic Analysis of Morphological Data. Smithsonian Institution Press, Washington, DC, pp. 1–19.

Korniushin, A.V., 1991. Anatomical aspects of the taxonomy and phylogeny of Pisidioidea (Bivalvia). In: Proceedings of the Tenth International Malacological Congress (Tübingen 1989). Unitas Malacologica, Tübingen, pp. 601–605.

Korniushin, A.V., Glaubrecht, M., 2002. Phylogenetic analysis based on the morphology of viviparous freshwater clams of the family Sphaeriidae (Mollusca, Bivalvia, Veneroida). Zool. Scripta 31, 415–459.

Kuiper, J.G.J., 1966. Pisidium (Parapisidium n. subg.) reticulatum n. sp. von der Insel Nossi-Bé bei Madagascar und aus Rhodesien. Arch. Moll. 95, 15–18.

Kuiper, J.G.J., 1983. The Sphaeriidae of Australia. Basteria 47, 3–52.

Lee, T., 2004. Morphology and phylogenetic relationships of genera of North American Sphaeriidae (Bivalvia, Verneroida). Am. Malacol. Bull. 19, 1–13.

Lee, T., Ó Foighil, D., 2003. Phylogenetic structure of the Sphaeriinae, a global clade of freshwater bivalve molluscs, inferred from nuclear (IST-1) and mitochondrial (16S) ribosomal gene sequences. Zool. J. Linn. Soc. 137, 245–260.

Mandahl-Barth, G., 1972. The freshwater mollusca of Lake Malawi. Rev. Zool. Bot. Afric. 86, 257–289.

Mandahl-Barth, G., 1988. Studies on African Freshwater Bivalves. Danish Bilharziasis Laboratory, Charlottenlund.

Meier-Brook, C., 1970. Untersuchungen zur Biologie einiger Pisidium-Arten. Arch. Hydrobiol. (Suppl. 38), 73–150.

Nation, J.L., 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Stain Tech. 58, 347–351.

Park, J.-K., Ó Foighil, D., 2000. Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. Mol. Phyl. Evol. 14, 75–88.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, MA.