

The morphology of conglomerates and conglomerate-like structures in North American freshwater mussels: a scanning-electron microscopy survey

G. Thomas WATTERS

Department of Evolution, Ecology and Organismal Biology

The Ohio State University

1315 Kinnear Road

Columbus, OH 43212 USA

Watters.1@osu.edu

KEY WORDS. Bivalvia, Unioniformes, Unionidae, North America, conglomerates, reproduction, phylogeny.

ABSTRACT. Conglomerates and conglomerate-like objects of 60 species of North American freshwater mussels were examined using scanning-electron microscopy. These structures were found to have a complexity and range of form previously unrealized. They may be organized into groups having similar morphologies. These groups approximate the currently recognized higher taxa and will serve to bolster support for them as phylogenetically coherent groups. In particular the genera *Ptychobranthus*, *Dromus*, *Obliquaria*, *Lasmigona*, and *Strophitus* have unique conglomerate-like structures. Superconglomerates most closely resemble collections of amblymine conglomerates. The results of this study may be used in conjunction with other phylogenetic databases (genetic, adult morphology, behavior, etc.) to construct phylogenetic associations based on a more whole-animal approach.

INTRODUCTION

Freshwater mussels of the Unioniformes have complex life cycles involving a parasitic larval stage, the glochidium. This parasite typically attaches to vertebrate hosts, usually fishes but occasionally amphibians (Watters, 1997; Watters & O'Dee, 1998). Reports of mussels using crustaceans (Walker, 1981; Panha, 1990) have been for non-North American taxa. Glochidia were discovered by Leeuwenhoek in 1695 and illustrated for the first time by him in 1697. Carus (1832) demonstrated that glochidia were larval freshwater mussels rather than parasites infesting freshwater mussels, an idea originally championed by Rathke (1797). But it was not until 1862 that the parasitic nature of glochidia was discovered. In that year Houghton reported finding glochidia attached to fishes and suggested that they may be parasites.

Conglomerates are packages of glochidia released by the female mussel. The concept of conglomerates as vehicles for glochidial infestation was much slower to evolve. As early as 1826 Prévost had noted the presence of an *agglomération* of *animicules* in the female mussel's gills. Lea (1834) illustrated the conglomerate of *Strophitus undulatus* without further comment. Forel (1866) proposed that glochidial threads attached to fish; these were illustrated by Schierholz (1889). Lefevre & Curtis (1910) were the first to apply the term "conglomerates" to these structures, although other authors had referred to them as "placenta" and "ovisacs." Few reports have been published since, most anecdotal (Conner, 1907;

Ortmann, 1913; Utterback, 1931) or incorrect (Lefevre & Curtis, 1911).

Watters (1999, 2002) found unprecedented complexity in the "conglomerates" of *Ptychobranthus fasciolaris* and *Strophitus undulatus*. It was clear that these reproductive structures were sufficiently diverse in form and function to harbor phylogenetic information. Although recent phylogenetic studies have focused on allozyme, genetic, gross adult morphology, or reproductive patterns, none have used conglomerate information. The purpose of this study was to examine, using scanning electron microscopy (SEM), a wide variety of conglomerate-like structures. These data should be combined with other information (genetic, morphological, etc.) in further studies to generate phylogenies using a more whole-animal approach.

METHODS

Sixty North American taxa were used in the study (Table 1). Specimens were taken primarily from the Ohio State University Museum of Biological Diversity, Columbus, Ohio, USA (OSUM). Additional samples were supplied by colleagues (see Acknowledgments and captions). Every effort was made to use fully-developed conglomerates but in some cases the glochidia had not completely matured.

Preparation of samples for SEM consisted of fixation in 4% paraformaldehyde and 2% glutaraldehyde in a 0.1 M phosphate buffer with 0.9% NaCl for 1-2 hrs. Specimens were rinsed in the same

buffer, post-fixed in 1% osmium tetroxide in buffer, and dehydrated in graded ethanol. They were critical point dried in ethanol-CO₂ in a Pelco CPD2, and sputter-coated with gold-palladium. Specimens were viewed with a Philips XL30 scanning electron microscope at an accelerating voltage of 20 kV.

RESULTS

Taxa were organized into groups having morphologically similar conglutinate-like structures (Table 2). Images of the structures are shown in alphabetical order by taxa in figures 1-64.

DISCUSSION

Morphology

Examination of the 60 species clearly indicates that an unexpected diversity of “conglutinates” exists. Closer inspection revealed possible phylogenetic associations between the taxa based on “conglutinate” features. It also was apparent that the structures were not of a single morphological type. This necessitates new terms to deal with this heterogeneous group.

Structural programmed eggs (SPE). These are unfertilized eggs that become a structural component of the conglutinate, often giving color and shape to the structure. Glochidia are embedded or attached to this core or layer of SPE. The SPE are held together by the adhesion of the egg membranes. It is possible that the animal has the ability to “turn off” certain egg cells so that they are never fertilized, but become SPE instead.

Conglutinate. The term “conglutinate” is here restricted to those structures composed solely of glochidia or a combination of glochidia and structural programmed eggs (SPE) that are held together by the adhesive properties of the egg membranes, although glochidia may be tethered by their glochidial threads. Those conglutinates lacking SPE are referred to as *simple conglutinates* and those having SPE are referred to as *composite conglutinates*. Simple conglutinates may be elastic or inelastic. In some cases simple conglutinates may be collectively assembled into *superconglutinates*.

Composite conglutinates occur in three forms: A, B, C.

In *composite A conglutinates* the glochidia are untethered to the core of SPE and are held in place by the adhesive egg membranes. The glochidia are distributed throughout the SPE core. Composite A conglutinates have been found in *Fusconaia* and *Toxolasma*.

In *composite B conglutinates* the SPE form a central ribbon to which the untethered glochidia are attached

along the lateral two margins. Composite B conglutinates have been found only in *Dromus*.

Glochidia are tethered by means of their glochidial threads to the SPE core in *composite C conglutinates*. Threads are distally fused to the inner lining of the glochidial egg membrane (Fig. 33). The SPE are smaller than the glochidia and tightly bound together by their egg membranes. This type of conglutinate has only been found in *Lasmigona*.

Amorphous mucus conglutinate. Glochidia are released in a loose mucus matrix which eventually disassociates. These conglutinates occur in the Margaritiferidae and the unionid “amblemines” – *Amblema*, *Megalonaia*, *Quadrula*, and *Quincuncina*.

Mesoconglutinate. Mesoconglutinates are composed of spongy, solid mucus bodies to which glochidia are tethered. Glochidia are originally located within individual chambers in the mesoconglutinate while within the marsupium of the female mussel. Upon release into the surrounding water the glochidia are pushed to the outside by osmotic swelling of the mucus; the glochidia then become infective. This type of “conglutinate” was described in detail by Watters (2002). It is only known from *Strophitus*.

Metaconglutinate. Metaconglutinates are the most complex conglutinate-like structures known and occur only in *Ptychobranthus*. The glochidia are contained in a central core surrounded by an acellular layer. This core is covered by a second acellular outer layer, with a fluid layer between the two. The central core extends to the surface of the outer layer at pigmented “eye spots.” The “tail” of the metaconglutinate is adhesive. Examination reveals a series of spongy pads (Figs. 48, 49). Within the cavities of this material are minute spherical objects (Fig. 50), ~ 8 μ. These objects may be “glue balls” – vesicles of some viscous or adhesive liquid. How these function is unknown, but perhaps the vesicles rupture on contact or dissolve. Metaconglutinate mimic models vary from species to species and apparently also during ontogeny, but the overall structure is consistent between all of them.

Phylogeny

No phylogenetic analysis to date has used any conglutinate information. This study indicates that there is sufficient morphological complexity to form conclusions concerning the phylogenetic relationships between the species included here. These data may be used either independently (as here) or in conjunction with existing morphological, behavioral, and genetic information to create a better picture of the evolution of this group. This study supports the recognition of suprageneric phylogenetic groups based on genetics by several recent workers. These groups and others are discussed below.

Strophitus. Simpson (1900) separated *Strophitus* from all other unionids on the basis of the placement of the marsupium (outer gills) and nature of the “ovisacs.” He placed *Strophitus* in his Diagenae, used by him in the sense of a subfamily. This study supports the uniqueness of this genus. The mesoconglutinate is found in no other genus. *Strophitus* was associated with *Pyganodon* and *Anodonta* (Hoeh et al., 2001) and with *Lasmigona* (Campbell et al., 2005) by DNA evidence. That result is supported by this study, but the uniqueness of the mesoconglutinate suggests that *Strophitus* is a distinct lineage. *Strophitus* mesoconglutinates may be functionally derived directly from the presumably ancestral amorphous mucus conglutinate by the “firming” of the mucus into a solid rod.

Dromus. The monotypic *Dromus* was placed in his Eschatigenae by Simpson (1900) based on marsupial and conglutinate characteristics. Starobogatov (1970) created the Tribe Dromini for *Dromus* in his Subfamily Medionidinae. Indeed, the conglutinate (composite B) has not been found in any other species. The elastic ribbon, composed of SPE with lateral glochidia, could be functionally derived from a *Fusconaia*-like conglutinate, in which genus the SPE form a central core. The conglutinate morphology described here supports the idea that *Dromus* is a distinct lineage.

Lasmigona. This genus is typically placed with the anodontines. The glochidium is typically anodontine and the adult shell tends to have reduced dentition. However, *Lasmigona* is unique in several respects. The glochidia are tethered to a core of SPE. All other taxa having SPE have untethered glochidia. This suggests that one of two things must have happened in the evolution of *Lasmigona*: either SPE arose independently in *Lasmigona* or *Lasmigona* “re-evolved” the tethered condition. In view of the anodontine glochidia found in *Lasmigona*, the improbability of regaining the tethered condition, and its genetic similarities to other anodontines (Lydeard et al., 1996), I suggest that SPE arose independently in *Lasmigona* (SPE may have arose a third time as well, see *Toxolasma*). Although Raley et al. (2003) suggested that *Lasmigona* was paraphyletic, no differences among the composite C conglutinates of four species was found in this study.

Cyprogenia and *Obliquaria*. Simpson (1900) also recognized the uniqueness of *Cyprogenia* and *Obliquaria* and created his Mesogenae to contain them; Starobogatov (1970) placed them in his Cyprogeniinae. Both have conglutinates comprised of glochidia bound by elastic egg membranes. These are elaborations of the typical *Elliptio/Pleurobema* inelastic conglutinates. The morphology of the marsupia suggests that *Cyprogenia* and *Obliquaria* are related; the elastic properties of the conglutinates are

probably not convergent. However, *Cyprogenia* and *Obliquaria* are not closely related in recent genetic studies (Campbell et al., 2005). *Obliquaria* has been regarded as lampsiline by some genetic studies (Graf, 2002) but as quadruline in others (Lydeard et al., 1996; Serb et al., 2003; Graf & Cummings, 2006). *Cyprogenia* glochidia have a core of SPE (Eckert & Barnhart, 2003). This condition is similar to *Dromus*; both have an elastic matrix with a central core of SPE. This supports genetic studies that suggest a close relationship between *Dromus* and *Cyprogenia* (Campbell et al., 2005).

Ptychobranchnus. Simpson’s (1900) Ptychogenae included only the genus *Ptychobranchnus* and was based on the folded condition of the gills. Starobogatov’s (1970) Ptychobranchninae also recognized the uniqueness of the genus. The metaconglutinate is very different from any other conglutinate-like object studied, both in terms of its complexity and associated structures. It was described in detail by Watters (1999). *Ptychobranchnus* is considered related to other “lampsilines” in most studies, including genetic studies (Lydeard et al., 1996; Campbell et al., 2005). But in terms of conchological and conglutinate morphology the *Ptychobranchnus* is unlike lampsiline genera such as *Villosa*, *Lampsilis*, *Ligumia*, etc. The metaconglutinate is similar to the conglutinates of *Pleurobema* or *Elliptio* but have been encapsulated in pigmented layers. *Ptychobranchnus jonesi*, not examined here, is probably not in that genus (Roe, 2000).

Superconglutinates. These structures appear to be collections of *Pleurobema*- or *Elliptio*-like conglutinates. Species having this condition were placed in the genus *Hamiota* (Roe & Hartfield, 2005).

Toxolasma. Like *Lasmigona*, *Toxolasma* has several unique and puzzling features that set it apart from other unioniformes. The adult size is quite small and the mantle is modified into “caruncles.” The latter fact has caused its inclusion with the lampsilines. However, the conglutinate is most similar to *Fusconaia* in the presence of untethered glochidia in a SPE core. Recent genetic studies place *Toxolasma* near the base of the lampsiline lineage (Campbell et al., 2005).

Margaritiferidae. Davis & Fuller (1981), using anatomical evidence and electrophoretic data, concluded that the Margaritiferidae was a subfamily of the Unionidae. Smith & Wall (1984) disagreed and reinstated the group as a family. Hoeh et al. (1998), based on Cytochrome C Oxidase Subunit I DNA (COI) sequences, found evidence for separation of the Margaritiferidae from the Ambleminae and Anodontinae - suggesting either three families or three subfamilies. This study examined the conglutinates of the monotypic *Cumberlandia*. The conglutinates are

composed of untethered glochidia in a mucus matrix, similar to the presumed ancestral condition of the non-anodontine unionids. The conglutinates differ in being more formed (or less amorphous) than in most unionids having amorphous conglutinates. It is thus different from both the anodontines and the remaining unionids ("Ambleminae" of Hoeh et al., 1998) and weakly supports the differentiation of these three groups.

Phylogenetic associations

The ancestral, pre-parasitic condition probably involved a small number of free-living larvae liberated by the female. These may have become phoretic on passing fishes and this symbiosis eventually evolved into a parasitic relationship (Watters, 2001). The earliest conglutinates may have been amorphous mucus packets or strands that attracted or entangled fishes. The Unionidae diverged into two or three groups, the Anodontinae, Ambleminae, and perhaps the Margaritiferidae. The Anodontinae have glochidia with glochidial threads, which may have been the ancestral condition. These tethers were lost in the Ambleminae (and Margaritiferidae).

The Anodontinae are represented in North America by three groups based on their conglutinates (or lack thereof). In *Strophitus* the mucus became a solid structure with embedded glochidia: the mesoconglutinate. In *Lasmigona* the mucus was lost and some eggs became structural programmed eggs forming a composite C conglutinate. In the remaining anodontines (*Pyganodon*, *Anodonta*, etc.) the mucus was retained as bundles of threads with the glochidia embedded in them. The Anodontinae are now recognized as a distinct group apart from the remaining unionids, even to the point of being considered a separate family. As a whole, the group has evolved to colonize soft-substrate habitats and are host-generalists. The results of this study support this distinction from the ambleminae.

Untethered glochidia embedded in amorphous mucus structures represent the earliest Ambleminae. These ambleminae are mainly big-river, sculptured taxa, including *Amblema*, *Megalonaias*, *Quadrula*, and *Quincuncina*, all confined to the Gulf and Interior drainages. These may be among the most primitive unioniformes (Watters, 2001).

In the remaining groups the mucus has lost its function of holding the conglutinate together. This job is now fulfilled by the egg membranes, which adhere to each other. These conglutinates are non-elastic and lack SPE. Representatives of this grade of complexity include *Elliptio*, *Cyclonaias*, *Pleurobema*, and *Plethobasis*. One group of ambleminae, characterized by *Fusconaia*, have SPE. These conglutinates are termed composite A conglutinates.

From this amblemine group several other groups arose. The combining of conglutinates into a single

structure, the "superconglutinate," occurred only in *Hamiota* (Roe & Hartfield, 2005). The formation of elastic egg membranes for greater durability occurred in the genera *Cyprogenia* and *Obliquaria*. The resemblance of these conglutinates to those of *Pleurobema* suggest a potential phylogenetic relationship to the Ambleminae. The surrounding of the amblemine-like conglutinate with multiple layers to form a metaconglutinate occurred in *Ptychobranthus*. Again, the affinities of *Ptychobranthus* may lie with the ambleminae.

In numerous groups the conglutinate has been lost, resulting in a glochidium being released individually rather than in conglutinates. In some cases glochidia may be temporarily bound together upon expulsion but are quickly dissociated. In other cases loose conglutinates may be formed at the end of a brooding season if the glochidia have not been released (Watters, unpubl.). These groups include *Actinonaias*, *Crytonaias*, *Ellipsaria*, *Epioblasma*, *Glebula*, *Lampsilis*, *Leptodea*, *Ligumia*, *Medionidus*, *Obovaria*, *Potamilus*, *Truncilla*, and *Villosa*. In most of these groups conglutinate formation has given way to other means of luring hosts – mantle flaps, papillae, tentacles, etc. Most of these genera comprise the "Lampsilini" of numerous authors. Their close relationships are born out by genetic studies (Lydeard et al., 1996; Hoeh et al., 2001; Campbell et al., 2005) and preliminary fossil analysis (Watters, 2001). However, other members of the presumed lampsiline clade, such as *Dromus*, *Cyprogenia*, and *Ptychobranthus*, all appear to be unrelated, based on conglutinate morphology, to those other Lampsilinae but related to each other. This is supported by Campbell et al. (2005) in which these genera (and others) were found to form a separate clade within their Lampsilini apart from other lampsiline genera.

Finally, at least two groups have evolved SPE: *Fusconaia* and *Toxolasma*. The great differences between these two genera (and *Lasmigona*) suggest that SPE are convergently derived. A rearranging of the *Fusconaia* composite A conglutinate yields *Dromus*, having a composite B conglutinate.

The conglutinate and conglutinate-like objects described here have a remarkable complexity and range of form. These structures are of phylogenetic interest because they may be organized into groups having similar morphologies. In general these groups approximate the currently recognized higher taxa and will serve to bolster support for them as phylogenetically coherent groups. The Anodontinae, Ambleminae, and Lampsilinae are recognizable in the conglutinate groups. The Margaritiferidae are less distinct. Support is given to the uniqueness of several genera: *Ptychobranthus*, *Cyprogenia*, *Obliquaria*, and *Dromus*. *Lasmigona* is shown to have a unique set of conglutinate characteristics and the taxonomic position of the genus is unclear. Lampsilines have a degenerate conglutinate, apparently the result of a

switch to a different host-luring strategy: mantle flaps and lures.

The presence of SPE are intriguing features. SPE have evolved independently in two lineages: *Lasmigona* and *Fusconaia*. It is apparent that some species of mussels have the ability to “turn off” egg cells such that they assume a structural rather than reproductive role. This peculiar trait deserves more study.

This study may be used in conjunction with other phylogenetic databases (genetic, adult morphology, behavior, etc.) to construct phylogenetic associations based on a more whole-animal approach.

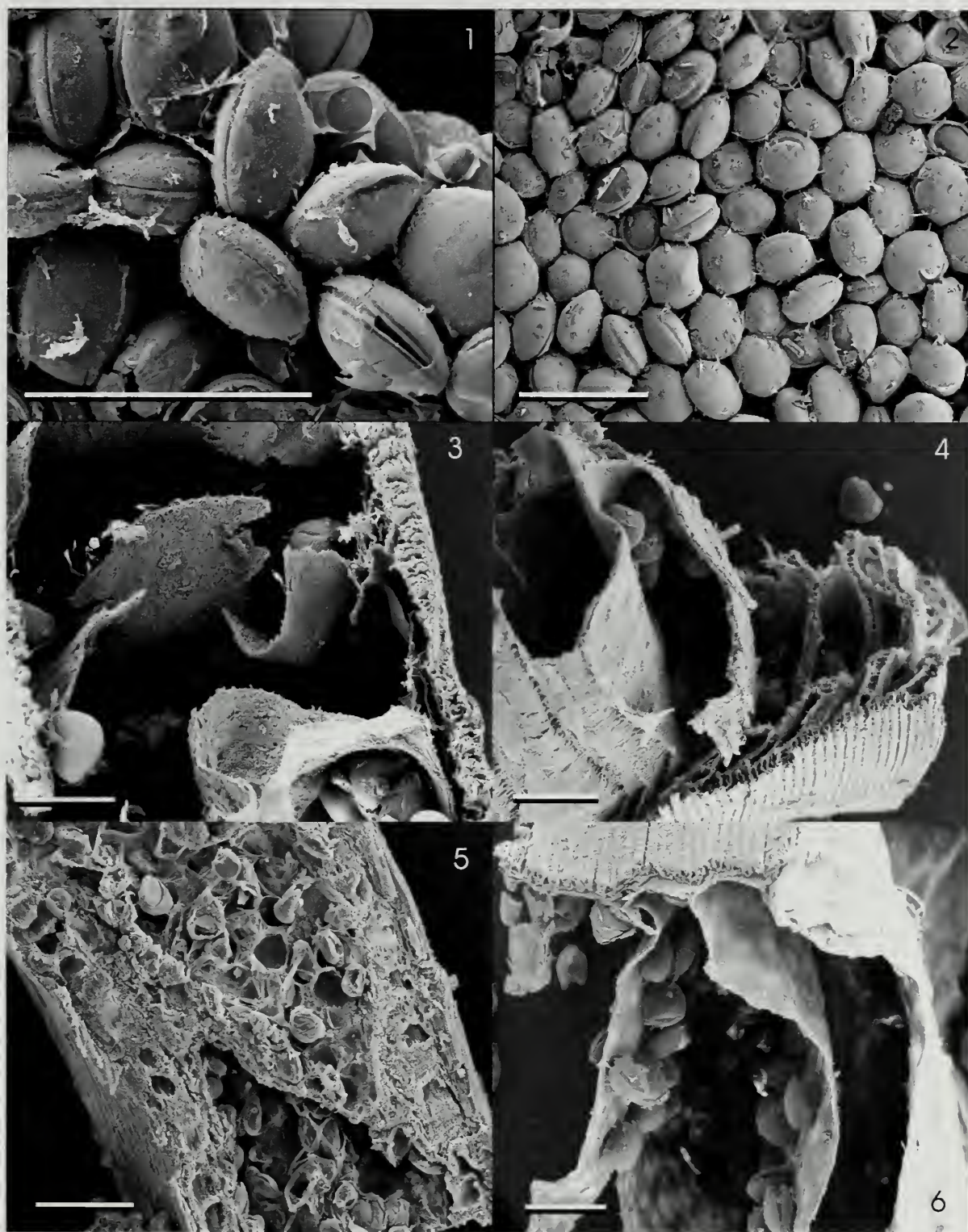
ACKNOWLEDGMENTS

I am indebted to the Ohio River Mussel Mitigation Trust for a grant to support this project. Additional specimens were supplied by M. Hove (University of Minnesota), K. Roe (then Delaware Museum of Natural History), G. Zimmerman (Enviroscience, Inc., Stow, OH), P. Morrison (USFWS, Williamstown, WV), and R. Neves (Virginia Technological Institute). Thanks also to Kathy Wolken and Brian Kemmenoe of the Ohio State University Campus Imaging Facility for assistance with preparation of specimens and use of the SEM facility. I thank Dan Graf and Art Bogan for their valuable suggestions on the manuscript.

LITERATURE CITED

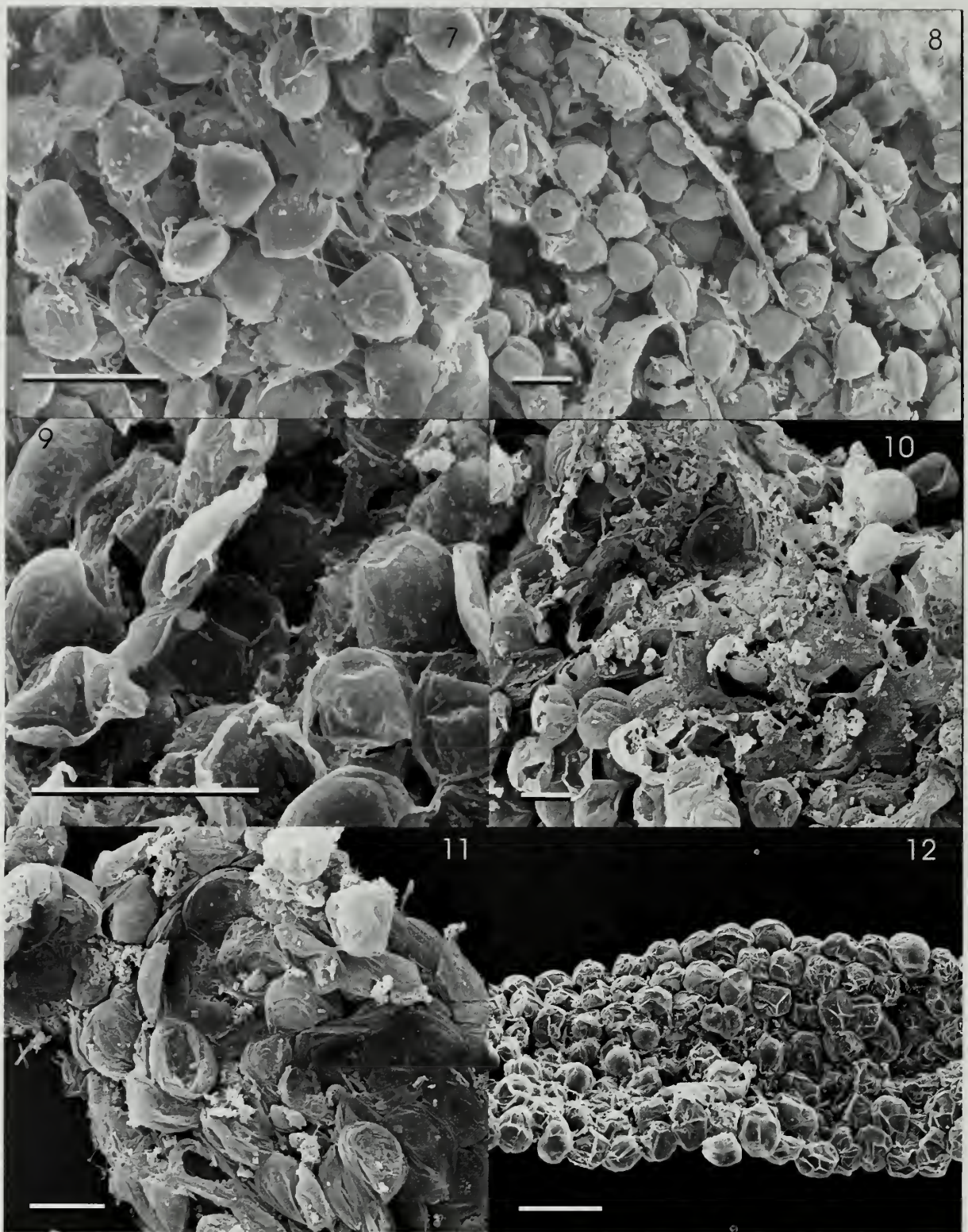
- Campbell, D.C., Serb, J.M., Buhay, J.E., Roe, K.J., Minton, R.L. & Lydeard, C. 2005. Phylogeny of North American amblymines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. *Invertebrate Biology* 124: 131-164.
- Carus, C.G. 1832. Neue Untersuchungen über die Entwicklungsgeschichte unserer Flussmuschel. *Verhandlungen der Kaiserlichen Leopoldinisch-Carolinischen Akademie der Naturforscher*, 16: 1-87.
- Conner, C.H. 1907. The gravid period of unios. *Nautilus* 22: 87-89.
- Davis, G.M. & Fuller, S.L.H. 1981. Genetic relationships among recent Unionacea (Bivalvia) of North America. *Malacologia* 20: 217-253.
- Eckert, N. & Barnhart, C. 2003. Comparison of host compatibility in two populations of western fanshell, *Cyprogenia aberti*. *3rd Biennial Symposium Freshwater Mollusk Conservation Society, Durham, NC*: 39-40 [abstract].
- Forel, F.A. 1866. Einige Beobachtungen über die Entwicklung des zelligen Muskelgewebes. Beiträge zur Entwicklungsgeschichte der Najaden. Inaugural-Abhandlung der medicinischen Facultät zu Würzburg: 40 pp.
- Graf, D.L. 2002. Molecular phylogenetic analysis of two problematic freshwater mussel genera (Unio and Gonidea) and a re-evaluation of the classification of Nearctic Unionidae (Bivalvia: Paleoheterodonta: Unionoida). *Journal of Molluscan Studies* 68:65-71.
- Graf, D.L. & Cummings, K.S. 2006. Palaeoheterodont diversity (Mollusca: Trigonioda + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. *Zoological Journal of the Linnean Society* 148: 343-394.
- Hoeh, W.R., Black, M.B., Gustafson, R., Bogan, A.E., Lutz, R.A. & Vrijenhoek, R.C. 1998. Testing alternative hypotheses of *Neotrigonia* (Bivalvia: Trigonioda) phylogenetic relationships using cytochrome C oxidase subunit I DNA sequences. *Malacologia* 40(1-2): 267-278.
- Hoeh, W.R., Bogan, A.E. & Heard, W.H. 2001. A phylogenetic perspective on the evolution of morphological and reproductive characteristics in the Unionoida. In: *Ecology and evolution of the freshwater mussels Unionoida* (G. Bauer & K. Wächtler, eds.). Ecological Studies Vol. 145, Springer-Verlag, Berlin: 257-280.
- Houghton, W. 1862. On the parasitic nature of the fry of *Anodonta cygnea*. *Quarterly Journal of Microscopical Science* (new series) 2: 162-168.
- Lea, I. 1834. Descriptions of new freshwater and land shells. *Transactions of the American Philosophical Society* 6: 1-18.
- Leeuwenhoek, A. von. 1695. *Arcana Naturae Detecta. Henricum a Kroonevelt*: 568 pp. + index.
- Leeuwenhoek, A. von. 1697. *Continuatio Arcanorum Naturae Detectorum. Henricum a-Kroonevelt*: 192 pp. + index.
- Lefevre, G. & Curtis, W.C. 1910. Reproduction and parasitism in the Unionidae. *Journal of Experimental Zoology* 9: 79-115.
- Lefevre, G. & Curtis, W.C. 1911. Metamorphosis without parasitism in the Unionidæ. *Science* 33: 863-865.
- Lydeard, C., Mulvey, M. & Davis, G.M. 1996. Molecular systematics and evolution of reproductive traits of North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16S rRNA gene sequences. *Philosophical Transactions of the Royal Society of London, B* 351: 1593-1603.
- Ortmann, A.E. 1913. Studies in najades. *Nautilus* 27: 88-91.
- Panha, S. 1990. The site survey and the study on reproductive cycles of freshwater pearl mussels in the central part of Thailand. *Venus* 49: 240-257.
- Prevost, I. 1826. De la génération chez la moule de peintres (*Unio Pictorum*). *Annales des Sciences Naturelles, Paris* 7: 447-454.
- Raley, M.E., Bogan, A.E., Harris, J.L. & Levine, J. 2003. Search for cryptic species in the paraphyletic genus *Lasmigona* Rafinesque, 1831 (Mollusc, Bivalvia, Unionidae). *3rd Biennial*

- Symposium Freshwater Mollusk Conservation Society, Durham, NC*: 51 [abstract].
- Rathke, J. 1797. Om Dammuslingen. *Naturhistorie Selskabets Skrifter (Kjöbenhavn)* 4: 139-179.
- Roe, K.J. 2000. The utility of DNA sequences to aid in the identification of rare or problematic species of freshwater mussels. In: *Freshwater Mollusk Symposia Proceedings. Part II. Proceedings of the First Freshwater Mollusk Conservation Society Symposium*. (R.A. Tankersley, D.I. Warmolts, G.T. Watters, B.J. Armitage, P.D. Johnson & R.S. Butler, eds.). Columbus, Ohio: 197-202.
- Roe, K.J. & Hartfield, P.D. 2005. *Hamiota*, a new genus of freshwater mussel (Bivalvia: Unionidae) from the Gulf of Mexico drainages of the southeastern United States. *Nautilus* 119: 1-10.
- Schierholz, C. 1889. Über Entwicklung der Unioniden. *Denkschriften der Kaiserlichen Akademie der Wissenschaften, Wien. Mathematisch-Naturwissenschaftliche Classe* 55: 183-214.
- Serb, J.M., Buhay, J.E. & Lydeard, C. 2003. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial ND1 sequences. *Molecular Phylogenetics and Evolution* 28: 1-11.
- Simpson, C.T. 1900. Synopsis of the naiades, or pearly fresh-water mussels. *Proceedings of the U.S. National Museum* 22: 501-1044.
- Smith, D.G. & Wall, W.P. 1984. The Margaritiferidae reinstated: a reply to Davis and Fuller (1981), "Genetic relationships among recent Unioniacea (Bivalvia) of North America". *Occasional Papers on Mollusks, Museum of Comparative Zoology, Harvard University* 4(64): 321-330.
- Starobogatov, Y.I. 1970. *Fauna mollyuskov I zoogeographicheskoe raionirovanie kontinental'nykh vodoemov zemnogo shara*. Akademiya Nauk SSSR, Zoologicheskii Institut Nauka, Leningrad: 372 pp.
- Utterback, W.I. 1931. Sex behavior among naiades. *Proceedings of the West Virginia Academy of Science* 5: 43-45.
- Walker, R.F. 1981. The ecology of freshwater mussels in the River Murray. *Australian Water Research Technical Paper* 63: 1-110.
- Watters, G.T. 1997. Glochidial metamorphosis of the freshwater mussel *Lampsilis cardium* (Bivalvia: Unionidae) on larval tiger salamanders, *Ambystoma tigrinum* ssp. (Amphibia: Ambystomidae). *Canadian Journal of Zoology* 75: 505-508.
- Watters, G.T. 1999. Morphology of the conglutinate of the Kidneyshell freshwater mussel, *Ptychobranchus fasciolaris*. *Invertebrate Zoology* 118: 289-295.
- Watters, G.T. 2001. The evolution of the Unionacea in North America, and its implications for the worldwide fauna. In: *Ecology and evolution of the freshwater mussels Unionoida* (G. Bauer & K. Wächtler, eds.). Ecological Studies Vol. 145, Springer-Verlag, Berlin: 281-307.
- Watters, G.T. 2002. The kinetic conglutinate of the creeper freshwater mussel, *Strophitus undulatus* (Say, 1817). *Journal of Molluscan Studies* 68: 155-158.
- Watters, G.T. & O'Dee, S.H. 1998. Metamorphosis of freshwater mussel glochidia (Bivalvia: Unionidae) on amphibians and exotic fishes. *American Midland Naturalist* 139: 49-57.



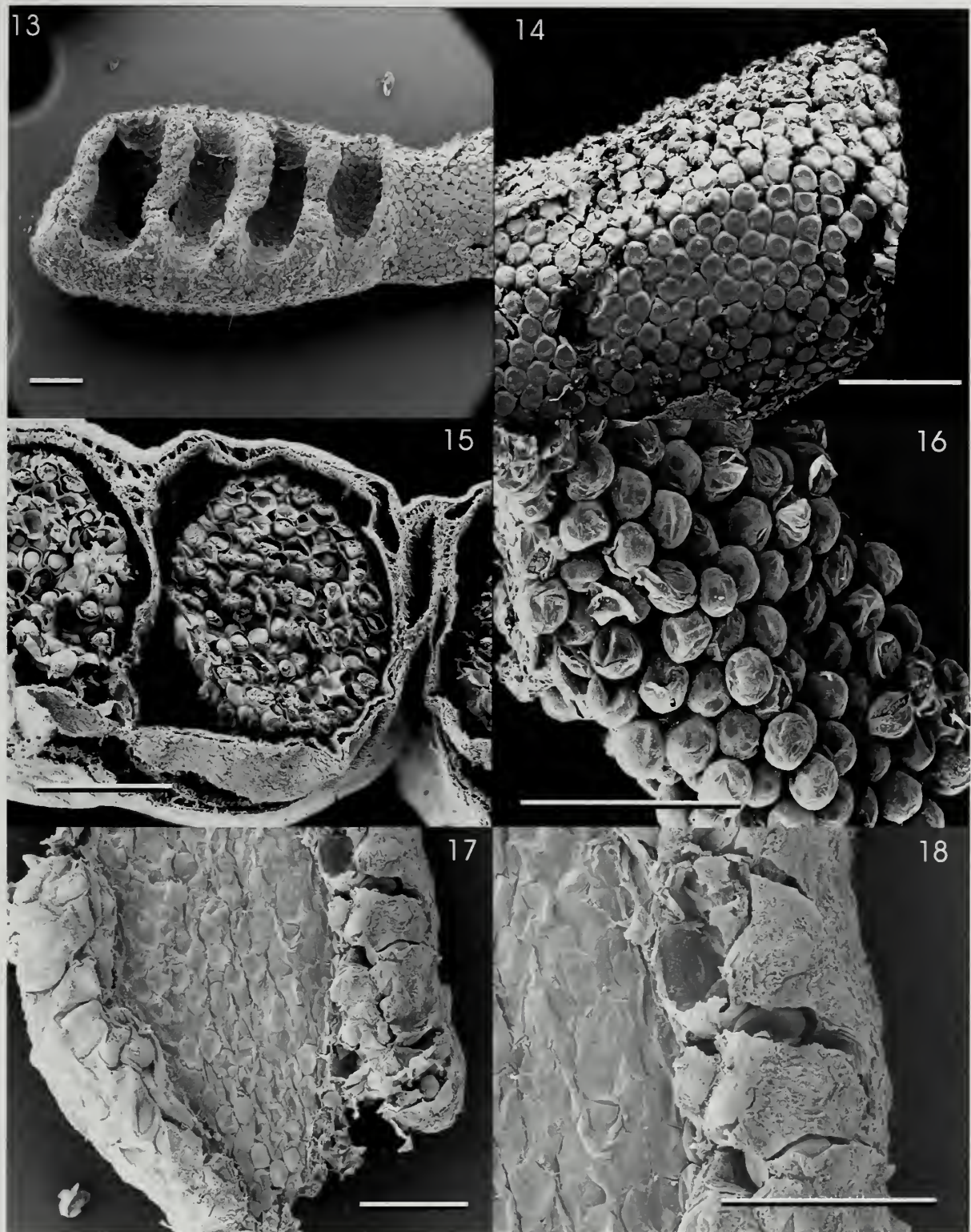
Figures 1-6. Scale bars = 500 μ unless noted otherwise

1, 2. *Actinonaias ligamentina*. OSUM 5980. St. Joseph River, OH. Glochidia with remnants of egg membranes. No conglutinate apparent. 3. *Alasmidonta marginata*. OSUM 13743. Caddo River, AK. Longitudinal section of marsupium with glochidia. No conglutinate apparent. 4. *Alasmidonta viridis*. OSUM 21560. Little Darby Creek, OH. Longitudinal section of marsupium with glochidia. No conglutinate apparent. 5. *Amblema plicata*. OSUM 48918. Muskingum River, OH. Oblique section of marsupium with glochidia imbedded in amorphous conglutinate. Two conglutinates are apparent separated by a water tube wall. 6. *Anodontoides ferussacianus*. OSUM 31265. French Creek, PA. Longitudinal section of marsupium divided into water tubes with glochidia. No conglutinate apparent.



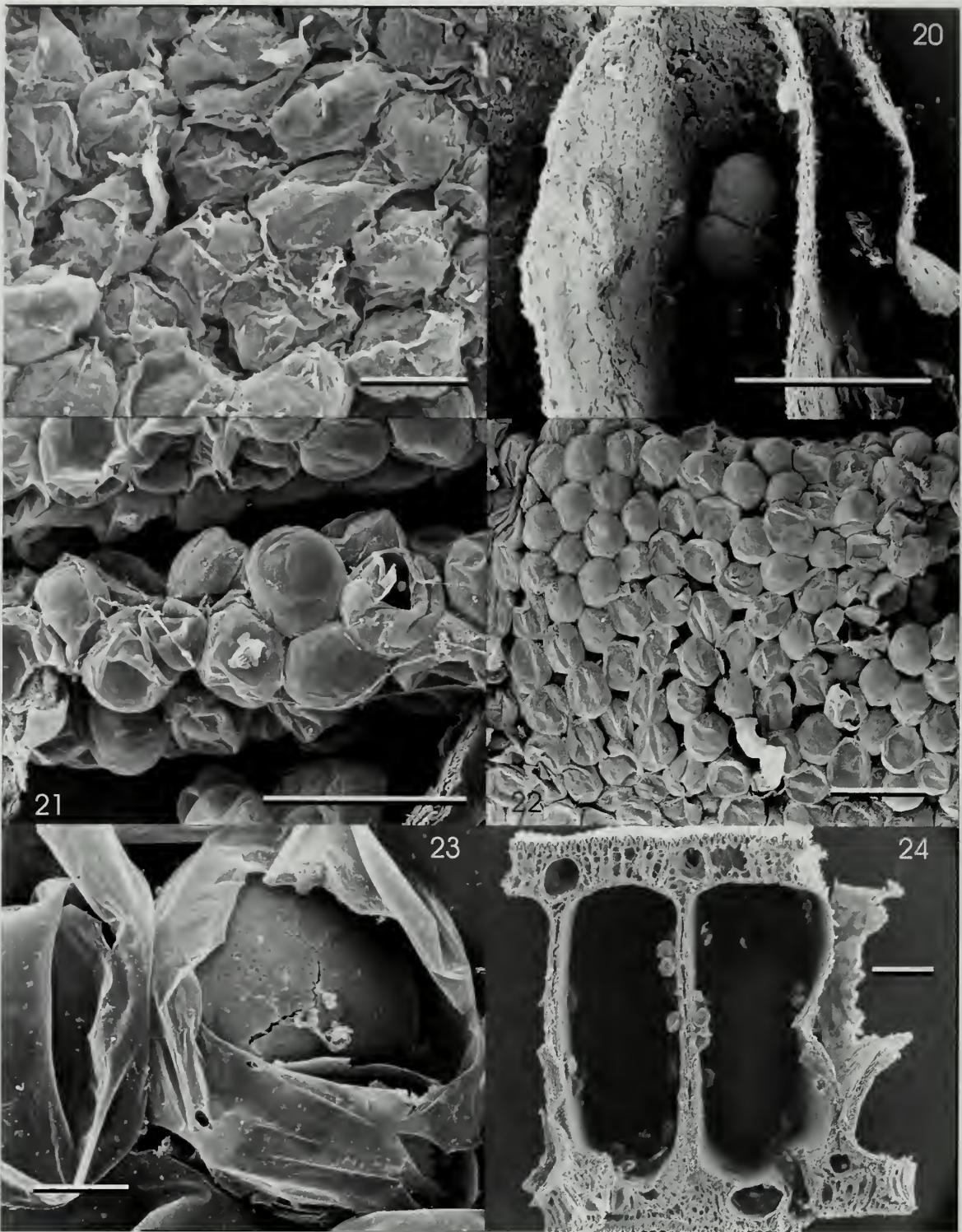
Figures 7-12. Scale bars = 500 μ unless noted otherwise

7. *Anodonta suborbiculata*. OSUM 13634. Black River, MO. Glochidia loosely bound by mucus. 8. *Arcidens confragosus*. OSUM 52015. Green River, KY. Longitudinal section of marsupium with glochidia. No conglutinate apparent. 9. *Elliptio spinosa*. OSUM 41069. Altamaha River, GA. Glochidia bound with egg membranes into conglutinate. 10, 11. *Cumberlandia monodonta*. Ex. M. Hove. St. Croix River, MN. Glochidia imbedded in amorphous conglutinate. Scale bars = 100 μ . 12. *Cyclonaias tuberculata*. OSUM 14138. Sandusky River, OH. Glochidia bound with egg membranes into conglutinate.



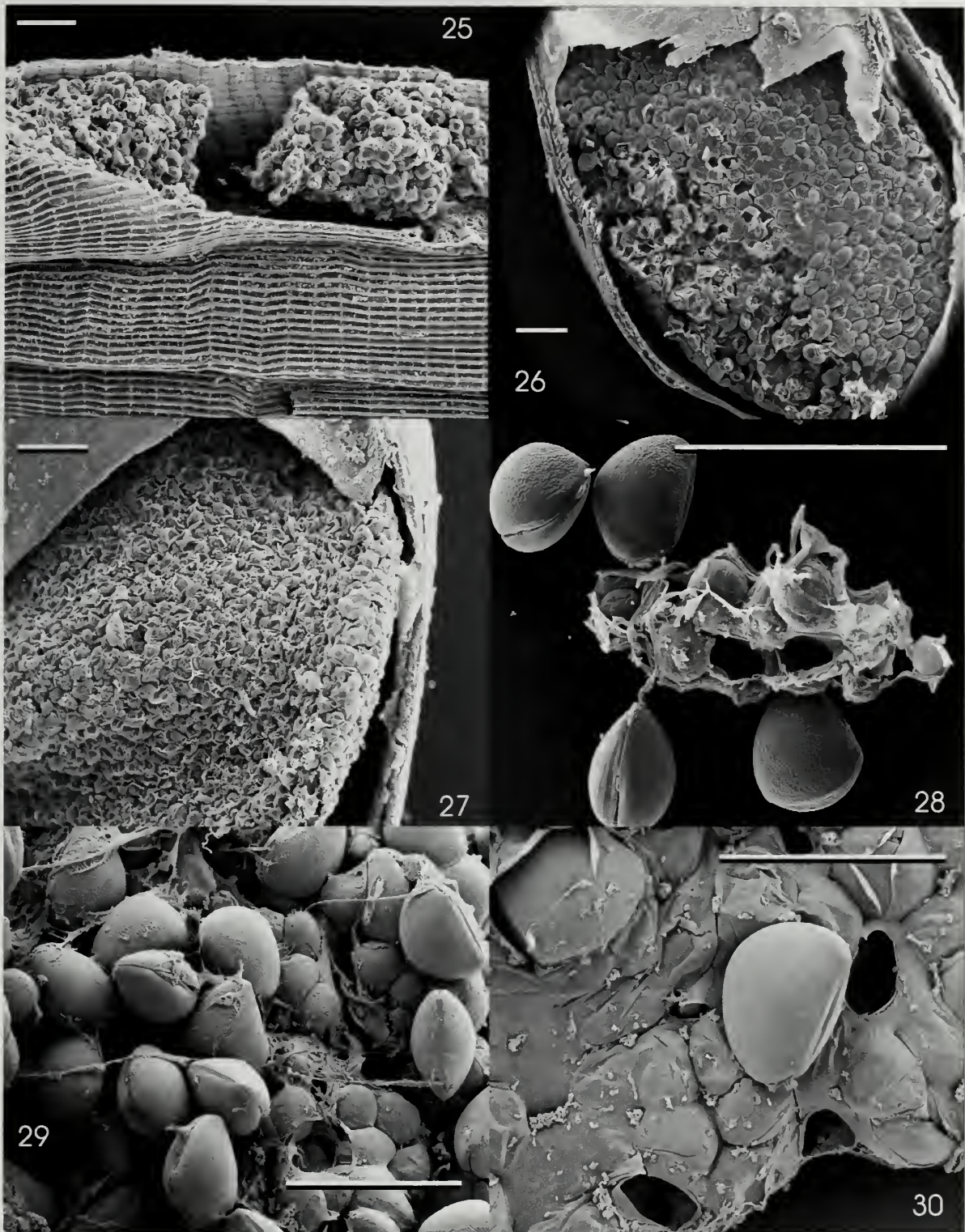
Figures 13-18. Scale bars = 500 μ unless noted otherwise

13, 14, 15. *Cyrogenia stegaria*. OSUM 16757. Clinch River, TN. Glochidia bound with elastic egg membranes into conglutinate. 13. "Head." 14. "Stalk." 15. Cross section showing that the conglutinate is composed solely of glochidia with no SPE. 16. *Crytonaias tampicoensis*. OSUM 15806. Nueces River, TX. Glochidia forming very weak conglutinate with egg membranes. 17, 18. *Dromus dromas*. Ex. R. Neves. Clinch River, VA. Composite conglutinate B formed by central ribbon of unfertilized eggs having border of glochidia in thickened membranes.



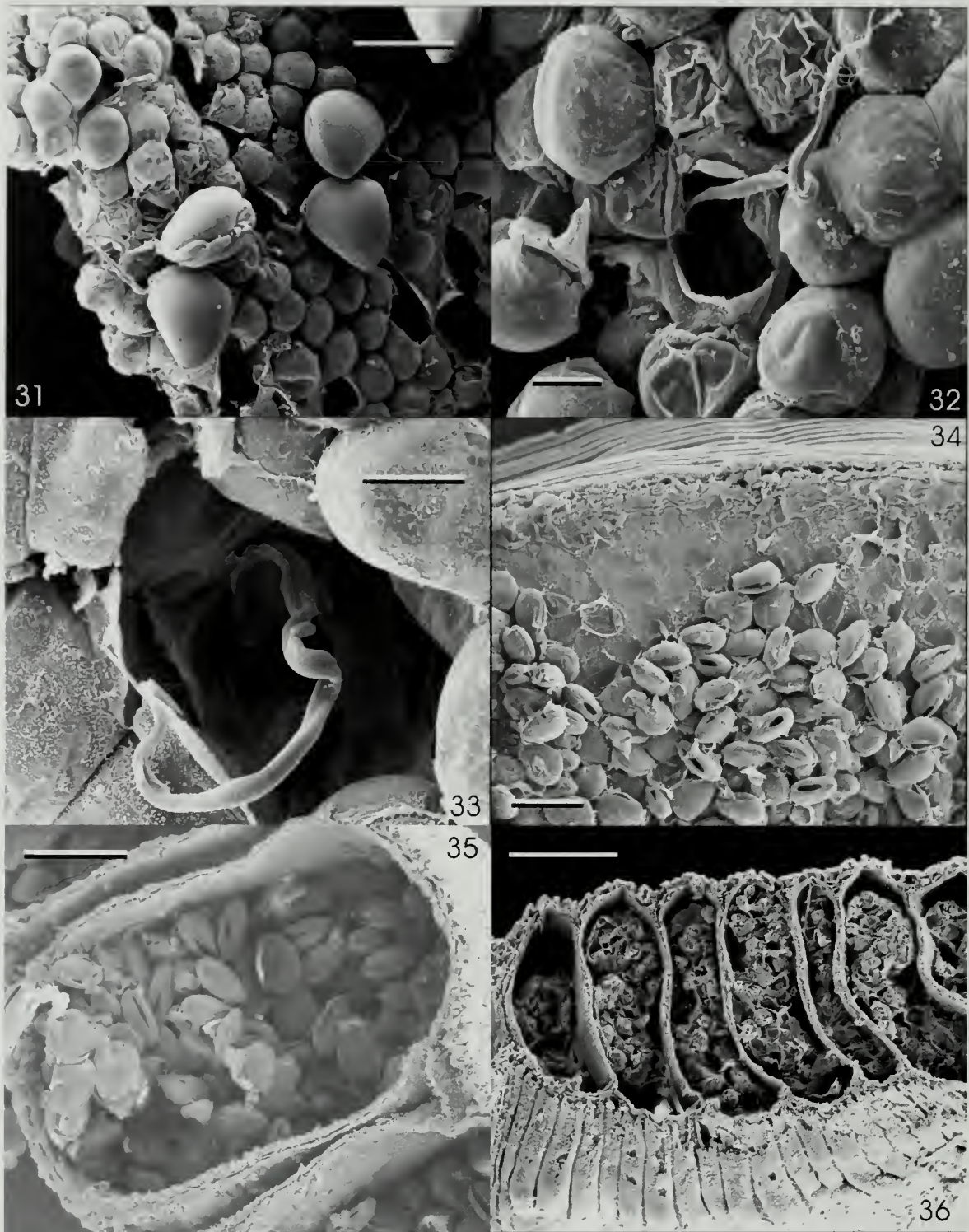
Figures 19-24. Scale bars = 500 μ unless noted otherwise

19. *Elliptio crassidens*. OSUM 15194. Tennessee River, TN. Glochidia bound with egg membranes into conglutinate. Scale bar = 100 μ . 20. *Epioblasma triquetra*. OSUM 59432. South Fork Scioto Brush Creek, OH. Longitudinal section of marsupium with glochidia. No conglutinate apparent. 21. *Fusconaia flava*. Ex. G. Watters. St. Joseph River. OH. Glochidia bound with egg membranes into conglutinate. 22. *Fusconaia maculata*. Ex. G. Zimmerman. Allegheny River, PA. Glochidia bound with egg membranes into conglutinate. Note tight adhesion between egg membranes. 23. *Fusconaia ozarkensis*. OSUM 54549. War Eagle Creek, AK. Glochidia bound with egg membranes into conglutinate. Scale bar = 50 μ . 24. *Glebulia rotundata*. OSUM 15704. Bayou Teche, LA. Longitudinal section of marsupium with glochidia. No conglutinate apparent.



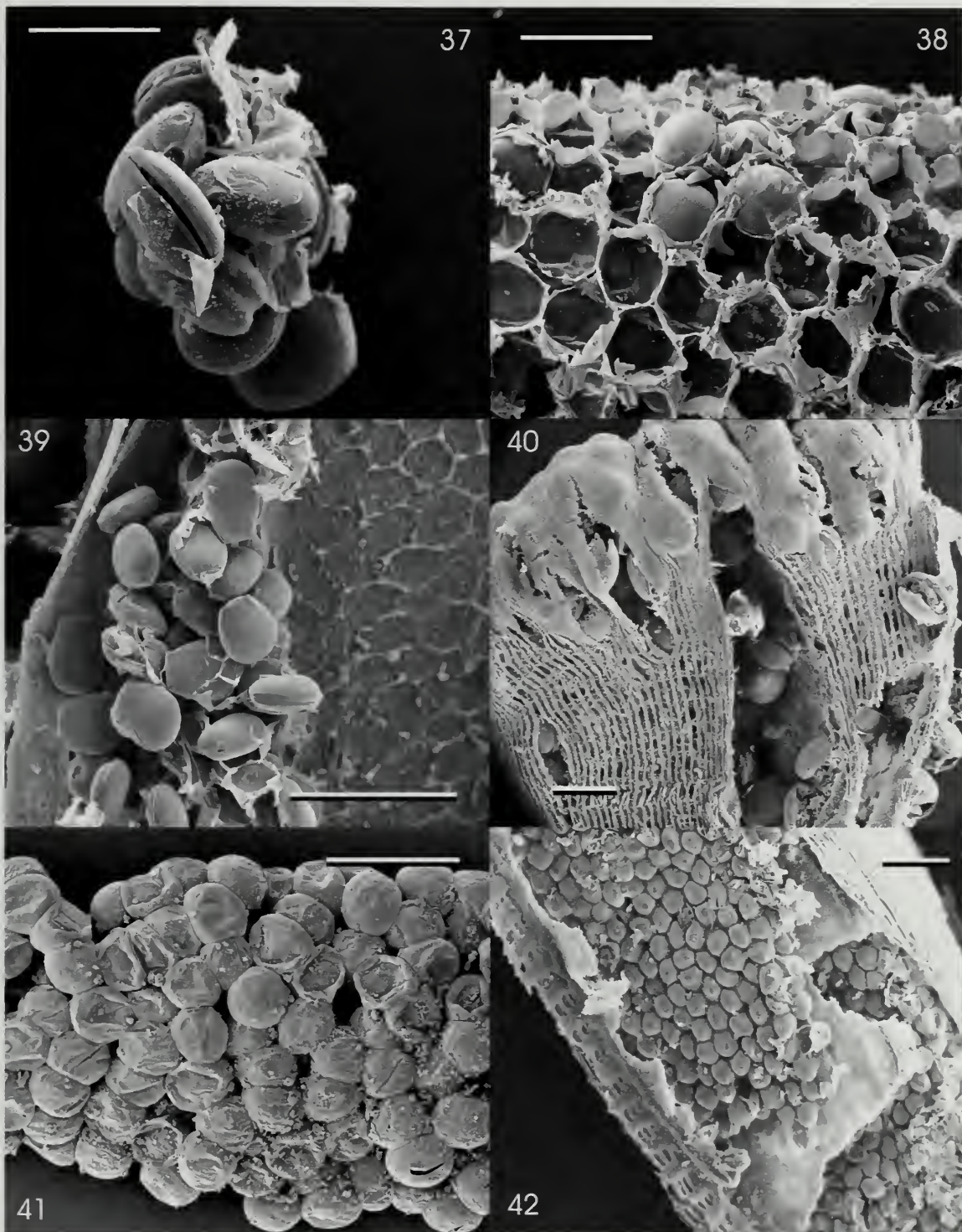
Figures 25-30. Scale bars = 500 μ unless noted otherwise.

25. *Gonidea angulata*. OSUM 8550. Trinity River, CA. Glochidia loosely bound by mucus within a water tube.
 26. *Lampsilis radiata luteola*. OSUM 63569. Lake Erie, OH . Glochidia with remnants of egg membranes in water tube. 27. *Lampsilis teres*. OSUM 43079. St. Francis, AK. Glochidia with remnants of egg membranes in water tube. 28, 29, 30. *Lasmigona compressa*. OSUM 67876. Fish Creek, OH. Composite conglomerate C formed by SPE. Glochidia are imbedded in and tethered to the conglomerate. Note the glochidial threads and smaller SPE. The SPE are tightly bound to each other by egg membranes.



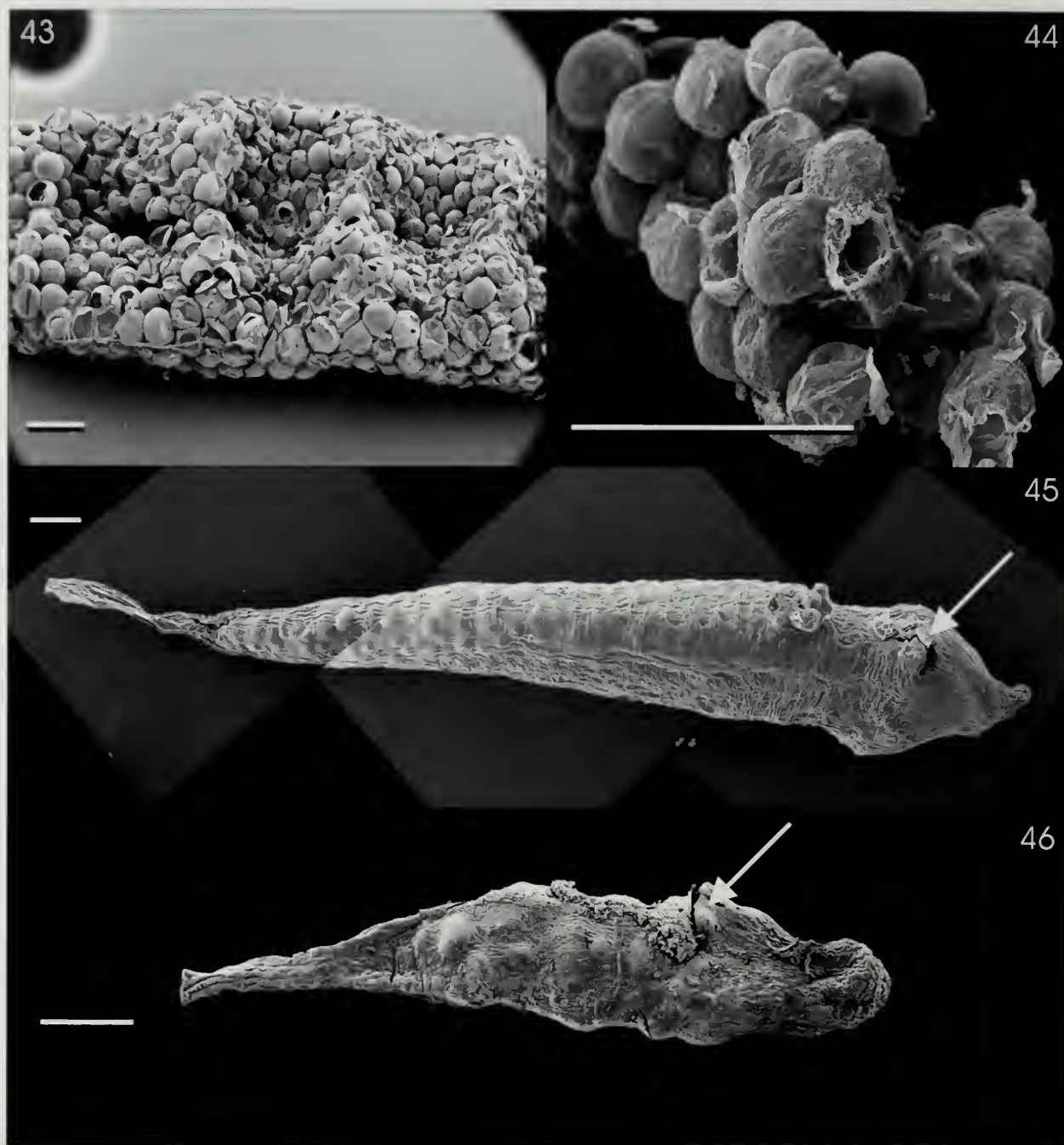
Figures 31-36. Scale bars = 500 μ unless noted otherwise.

31, 32, 33. *Lasmigona costata*. OSUM 65201. Grand River, OH. Composite conglutinate A formed by unfertilized eggs. Glochidia are imbedded in and tethered to the conglutinate. Note the glochidial thread fused with interior of egg membrane in Fig. 33. Fig. 32, 33 - Scale bars = 100 μ . 34. *Leptodea fragilis*. OSUM 63566. Lake Erie, OH. Oblique section of marsupium with glochidia with remnants of egg membranes. Glochidia lie against the wall of the water tube. No conglutinate apparent. 35. *Leptodea ochracea*. OSUM 52455. Mashpee Pond, MA. Longitudinal section of marsupium with glochidia in water tube with remnants of egg membranes. No conglutinate apparent. 36. *Lemiox rimosus*. OSUM 33955. Duck River, TN. Longitudinal section of marsupium with glochidia bound with egg membranes into conglutinates.



Figures. 37-42. Scale bars = 500 μ unless noted otherwise.

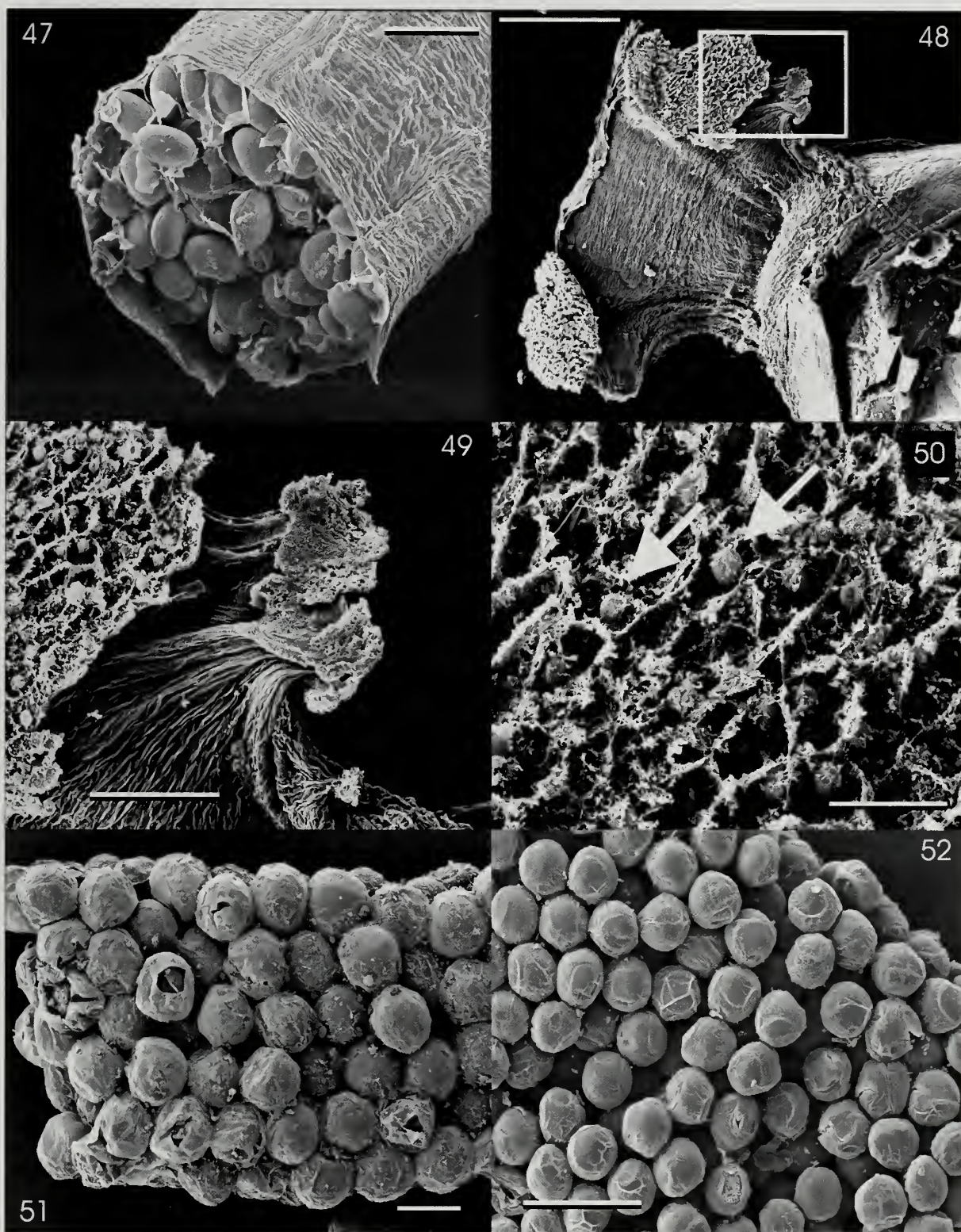
37. *Ligumia recta*. Ex. P. Morrison. Ohio River, WV. Glochidia with remnants of egg membranes. No conglutinate apparent. **38.** *Obliquaria reflexa*. Ex. G. Watters. Muskingum River, OH. Glochidia bound with elastic egg membranes into conglutinate. Note that conglutinate retains its shape even after glochidia have been removed. **39.** *Obovaria subrotunda*. OSUM 61880. Little Muskingum River, OH. Oblique section of marsupium with glochidia with remnants of egg membranes. No conglutinate apparent. **40.** *Pegias fabula*. OSUM 41309. Little South Fork Cumberland River, KY. Marsupial region of gill, split to show glochidia. No conglutinate apparent. **41.** *Plethobasus cyphus*. OSUM 47973. Muskingum River, OH. Glochidia bound with egg membranes into conglutinate. Note tightly bound egg membranes. **42.** *Pleurobema clava*. OSUM 20744. Little Darby Creek, OH. Oblique section of marsupium with glochidia very weakly bound with egg membranes into conglutinate. Two water tubes are present.



Figures 43-46. Scale bars = 500 μ unless noted otherwise.

43. *Pleurobema cordatum*. OSUM 67858. Muskingum River, OH. Glochidia bound with egg membranes into conglutinate. 44. *Pleurobema sintoxia*. OSUM 19600. Lake Erie, OH. Glochidia bound with egg membranes into conglutinate. 45. *Ptychobranchus fasciolaris*. Ex. G. Watters. Little Darby Creek, OH. Entire metaconglutinate. Adhesive "tail" to left, fluid filled "head" to right. Eyespot at arrow. Scale bar = 1 mm.

46. *Ptychobranchus greeni*. OSUM 19025. Conasauga River, GA. Entire metaconglutinate. Adhesive "tail" to left, fluid filled "head" to right. Eyespot at arrow.

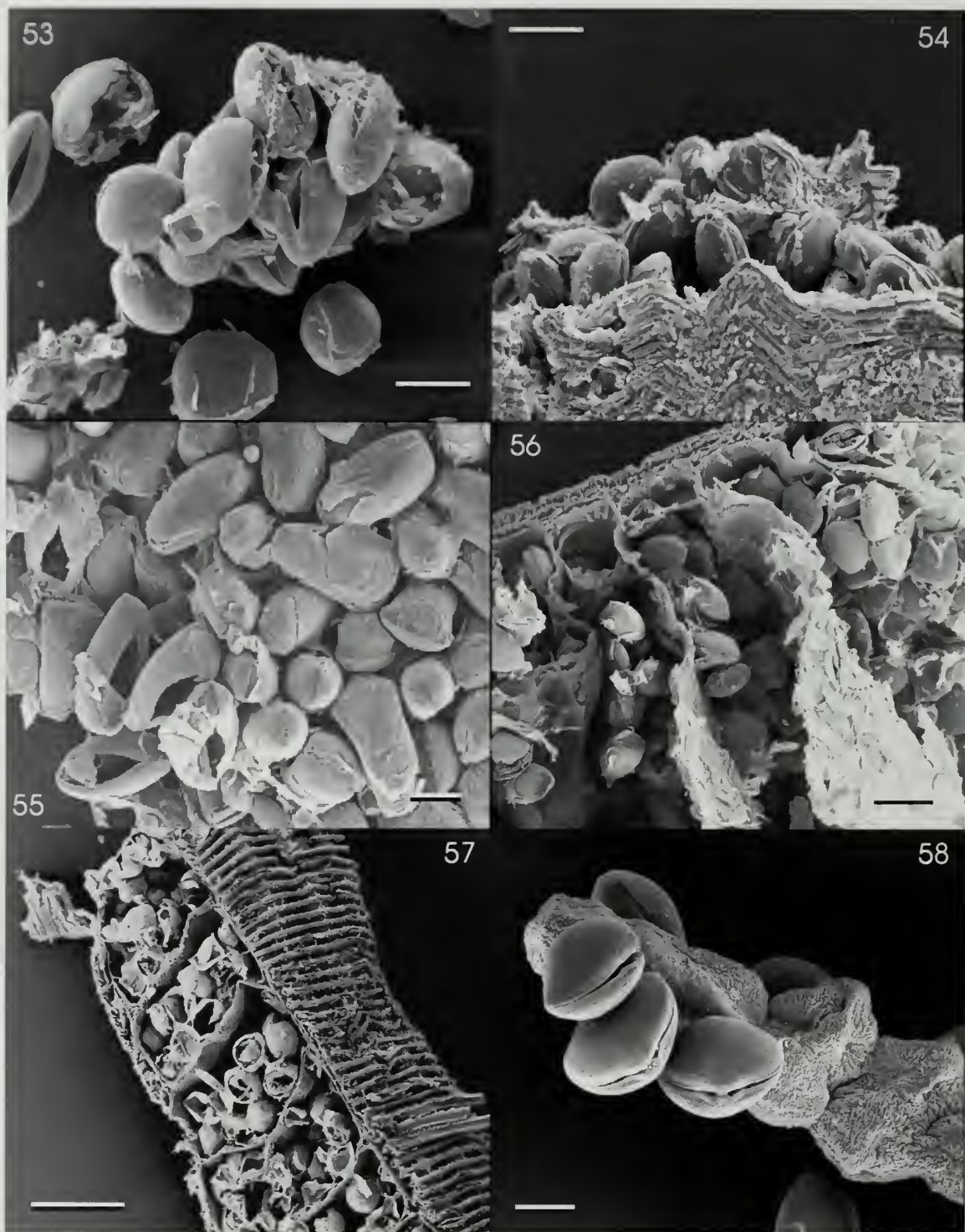


Figures 47-52. Scale bars = 200 μ unless noted otherwise. **47.** *Ptychobranchnus fasciolaris*. Ex. G. Watters. Little Darby Creek, OH. Cross section through metaconglutinate showing glochidia in central core.

48, 49, 50. *Ptychobranchnus subtentum*. OSUM 24139. North Fork Holston River, VA. Close-up of adhesive pad. Fig. 49 is the enclosed area in Fig. 48. Note spongy structure of pad and presumed "glue balls" at arrows.

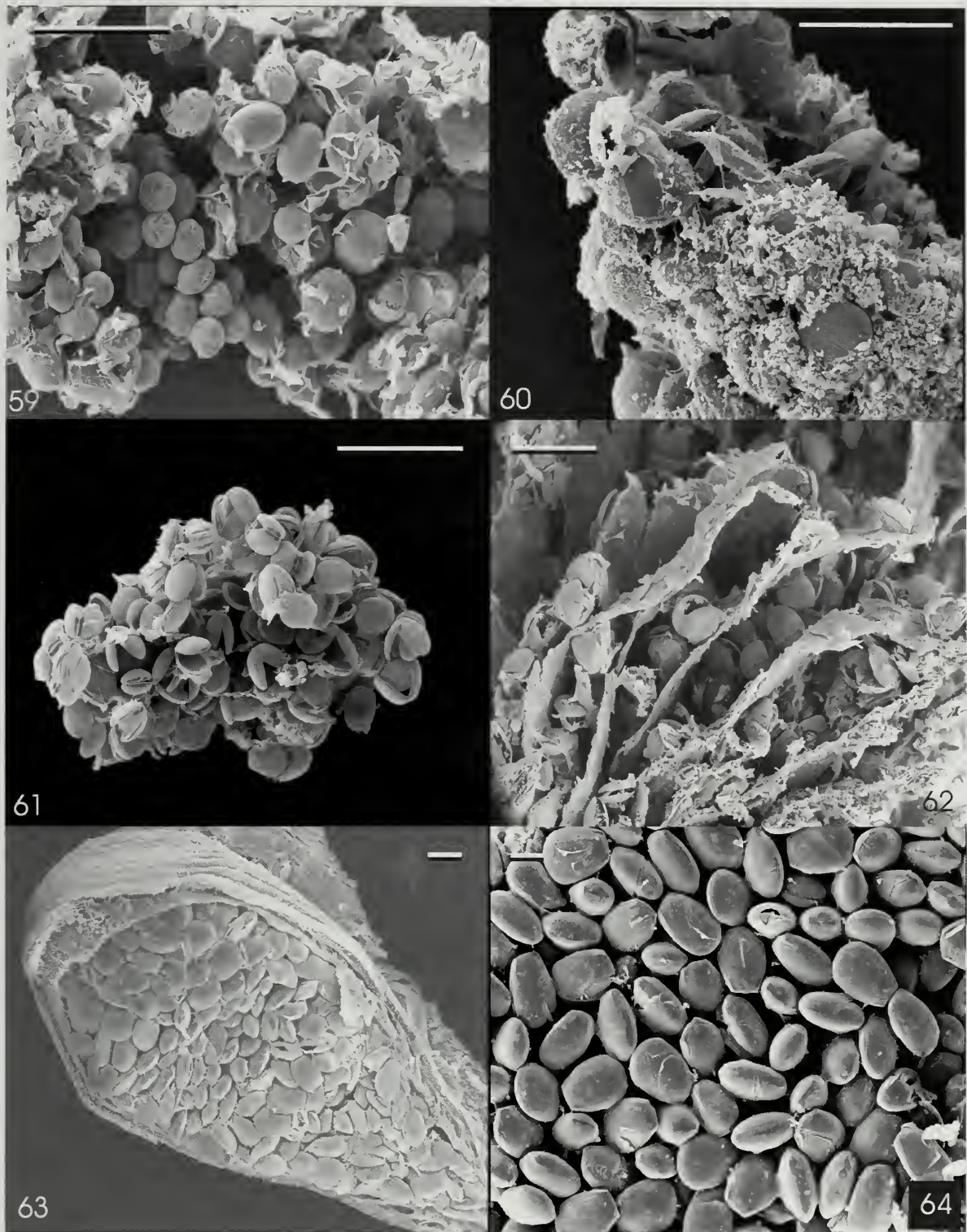
Fig. 49 - Scale bar = 50 μ . Fig. 50 - Scale bar = 20 μ . **51.** *Quadrula cylindrica*. Ex. G. Watters. Tippecanoe River, IN. Glochidia with remnants of egg membranes, weakly held together by mucus or egg membranes.

52. *Quadrula metanevra*. Ex. G. Watters. Muskingum River, OH. Glochidia with remnants of egg membranes. No conglutinate apparent.



Figures 53-58. Scale bars = 200 μ unless noted otherwise.

53. *Quadrula pustulosa*. OSUM 8367. Big Darby Creek, OH. Glochidia with remnants of egg membranes, weakly held together by mucus or egg membranes. 54. *Quincuncina kleiniana*. OSUM 48537. Suwannee River, FL. Longitudinal section through marsupium with glochidia bound in amorphous mucus conglutinate. 55. *Potamilus alatus*. OSUM 59992. Ohio Brush Creek, OH. Glochidia with remnants of egg membranes. No conglutinate apparent. 56. *Pyganodon grandis*. OSUM 48296. Ohio & Erie Canal, OH. Longitudinal section of marsupium with glochidia. No conglutinate apparent. Scale bar = 500 μ . 57. *Strophitus conasaugaensis*. OSUM 57979. Oakmulgee Creek, AL. Longitudinal section of marsupium with glochidia. No conglutinate apparent. Scale bar = 500 μ . 58. *Strophitus undulatus*. Ex. G. Watters. Little Darby Creek, OH. Partial mesoconglutinate with tethered glochidia.



Figures 59-64. Scale bars = 200 μ unless noted otherwise.

59. *Toxolasma parvum*. OSUM 12071. Olentangy River, OH. Composite conglutinate C with untethered glochidia imbedded in unfertilized eggs. 60. *Quadrula verrucosa*. OSUM 3597. Big Darby Creek, OH. Glochidia bound in amorphous mucus conglutinate. 61. *Truncilla truncata*. OSUM 18018. Ohio River, OH. Glochidia with remnants of egg membranes. No conglutinate apparent. 62. *Utterbackia imbecillis*. OSUM 8789. Scioto River, OH. Longitudinal section of marsupium with glochidia. No conglutinate apparent. Scale bar = 500 μ . 63. *Venustaconcha ellipsiformis*. OSUM 15407. Big Piney River, MO. Cross section of marsupium with glochidia. No conglutinate apparent. 64. *Villosa lienosa*. OSUM 19992. Little River, MO. Glochidia. No conglutinate apparent.

TABLE 1. Taxa, source, and general collection locale for taxa used in study. (OSUM – Ohio State University Museum of Biological Diversity).

1. *Actinonaias ligamentina* (Lamarck, 1819). OSUM 5980. St. Joseph River, OH
2. *Alasmidonta marginata* Say, 1818. OSUM 13743. Caddo River, AK
3. *Alasmidonta viridis* (Rafinesque, 1820). OSUM 21560. Little Darby Creek, OH
4. *Amblema plicata* (Say, 1817). OSUM 48918. Muskingum River, OH
5. *Anodontoides ferussacianus* (Lea, 1834). OSUM 31265. French Creek, PA
6. *Anodonta suborbiculata* Say, 1831. OSUM 13634. Black River, MO
7. *Arcidens confragosus* (Say, 1829). OSUM 52015. Green River, KY
8. *Cumberlandia monodonta* (Say, 1829). Ex. M. Hove. St. Croix River, MN
9. *Cyclonaias tuberculata* (Rafinesque, 1820). OSUM 14138. Sandusky River, OH
10. *Cyprogenia stegaria* (Rafinesque, 1820). OSUM 16757. Clinch River, TN
11. *Crytonaias tampicoensis* (Lea, 1838). OSUM 15806. Nueces River, TX
12. *Dromus dromas* (Lea, 1834). Ex. R. Neves. Clinch River, VA
13. *Elliptio crassidens* (Lamarck, 1819). OSUM 15194. Tennessee River, TN
14. *Elliptio spinosa* (Lea, 1836). OSUM 41069. Altamaha River, GA
15. *Epioblasma torulosa rangiana* (Lea, 1838). OSUM 40562. Allegheny River, PA.
16. *Epioblasma triquetra* (Rafinesque, 1820). OSUM 59432. South Fork Scioto Brush Creek, OH
17. *Fusconaia flava* (Rafinesque, 1820). Ex. G. Watters. St. Joseph River. OH
18. *Fusconaia maculata* (Rafinesque, 1820). Ex. G. Zimmerman. Allegheny River, PA
19. *Fusconaia ozarkensis* (Call, 1887). OSUM 54549. War Eagle Creek, AK
20. *Glebula rotundata* (Lamarck, 1819). OSUM 15704. Bayou Teche, LA
21. *Gonidea angulata* (Lea, 1838). OSUM 8550. Trinity River, CA
22. *Lampsilis radiata luteola* (Lamarck, 1819). OSUM 63569. Lake Erie, OH
23. *Lampsilis teres* (Rafinesque, 1820). OSUM 43079. St. Francis River. AK.
24. *Lasmigona complanata* (Barnes, 1823). OSUM 29762. West Branch St. Joseph River, OH
25. *Lasmigona compressa* (Lea, 1829). OSUM 67876. Fish Creek, OH
26. *Lasmigona costata* (Rafinesque, 1820). OSUM 65201. Grand River, OH
27. *Lasmigona holstonia* (Lea, 1838). OSUM 25294. Middle Fork Holston River, VA
28. *Leptodea fragilis* (Rafinesque, 1820). OSUM 63566. Lake Erie, OH
29. *Leptodea ochracea* (Say, 1817). OSUM 52455. Mashpee Pond, MA
30. *Lexingtonia dolabelloides* (Lea, 1840). OSUM 30251. Tennessee River, TN
31. *Lemiox rimosus* (Rafinesque, 1831). OSUM 33955. Duck River, TN
32. *Ligumia nasuta* (Say, 1817). OSUM 20398. Lake Erie, OH
33. *Ligumia recta* (Lamarck, 1819). Ex. P. Morrison. Ohio River, WV
34. *Medionidus acutissimus* (Lea, 1831). OSUM 42808. Conasauga River, TN
35. *Obliquaria reflexa* Rafinesque, 1820. Ex. G. Watters. Muskingum River, OH
36. *Obovaria subrotunda* (Rafinesque, 1820). OSUM 61880. Little Muskingum River, OH
37. *Pegiasa fabula* (Lea, 1838). OSUM 41309. Little South Fork Cumberland River, KY
38. *Plethobasus cyphus* (Rafinesque, 1820). OSUM 47973. Muskingum River, OH
39. *Pleurobema clava* (Lamarck, 1819). OSUM 20744. Little Darby Creek, OH
40. *Pleurobema cordatum* (Rafinesque, 1820). OSUM 67858. Muskingum River, OH
41. *Pleurobema sintoxia* (Rafinesque, 1820). OSUM 19600. Lake Erie, OH
42. *Ptychobranchnus fasciolaris* (Rafinesque, 1820). Ex. G. Watters. Little Darby Creek, OH
43. *Ptychobranchnus greeni* (Conrad, 1834). OSUM 19025. Conasauga River, GA
44. *Ptychobranchnus subtentum* (Say, 1825). OSUM 24139. North Fork Holston River, VA
45. *Quadrula cylindrica* (Say, 1817). Ex. G. Watters. Tippecanoe River, IN
46. *Quadrula metanevra* (Rafinesque, 1820). Ex. G. Watters. Muskingum River, OH
47. *Quadrula pustulosa* (Lea, 1831). OSUM 8367. Big Darby Creek, OH
48. *Quadrula verrucosa* (Rafinesque, 1820). OSUM 3597. Big Darby Creek, OH
49. *Quincuncina kleiniana* (Lea, 1852). OSUM 48537. Suwannee River, FL
50. *Potamnilus alatus* (Say, 1817). OSUM 59992. Ohio Brush Creek, OH
51. *Pyganodon grandis* (Say, 1829). OSUM 48296. Ohio & Erie Canal, OH
52. *Simpsonaias ambigua* (Say, 1825). OSUM 55995. Wisconsin River, WI
53. *Strophitus conasaugaensis* (Lea, 1857). OSUM 57979. Oakmulgee Creek, AL
54. *Strophitus undulatus* (Say, 1817). Ex. G. Watters. Little Darby Creek, OH
55. *Toxolasma parvum* (Barnes, 1823). OSUM 12071. Olentangy River, OH
56. *Truncilla truncata* Rafinesque, 1820. OSUM 18018. Ohio River, OH

57. *Utterbackia imbecillis* (Say, 1829). OSUM 8789. Scioto River, OH
 58. *Venustaconcha ellipsiformis* (Conrad, 1836). OSUM 15407. Pig Piney River, MO
 59. *Villosa iris* (Lea, 1829). OSUM 20013. Walhonding River, OH
 60. *Villosa lienosa* (Conrad, 1834). OSUM 19992. Little River, MO

TABLE 2. Types of conglutinate-like structures found in the examined species.

No conglutinate or temporary conglutinate weakly held together, disassociating in water

“Anodontinae”

Alasmidonta marginata
Alasmidonta viridis
Anodontoides ferussacianus
Arcidens confragosus
Pegias fabula
Pyganodon grandis
Simpsonaias ambigua

“Lampsilinae”

Actinonaias ligamentina
Crytonaias tampicoensis
Ellipsaria lineolata
Epioblasma torulosa rangiana
Epioblasma triquetra
Glebula rotundata
Lampsilis radiata luteola
Lampsilis teres
Leptodea fragilis
Leptodea ochracea
Ligumia nasuta
Ligumia recta
Medionidus acutissimus
Obovaria subtotunda
Potamilus alatus
Truncilla truncata
Villosa iris
Villosa lienosa

Amorphous mucus conglutinate

“Ambleminae”

Amblema plicata
Gonidea angulata
Megalonaias nervosa
Quadrula cylindrica
Quadrula metanevra
Quadrula pustulosa
Quadrula verrucosa
Quincuncina kleiniana

Margaritiferidae

Cumberlandia monodonta

Simple conglutinate held together by egg membranes

Nonelastic

Cyclonaias tuberculata
Elliptio crassidens
Elliptio spinosa
Lemiox rimosus
Plethobasus cyphus
Pleurobema clava
Pleurobema cordatum
Pleurobema siutoxia

Elastic

Obliquaria reflexa

Composite conglutinate A – glochidia untethered, embedded in egg core

Fusconaia flava
Fusconaia maculata
Fusconaia ozarkensis
Toxolasma parvum

Composite conglutinate B – central ribbon of eggs, lateral glochidia

Dromus dromas
 ? *Cyprogenia stegaria* (central core of eggs)

Composite conglutinate C – glochidia tethered, embedded in egg core

Lasmigona complanata
Lasmigona compressa
Lasmigona costata
Lasmigona holstonia

Mesoconglutinate – glochidia tethered in solid mucus conglutinate

Strophitus conasaugaensis
Strophitus undulatus

Metaconglutinate – multilayer conglutinate with central core of glochidia

Ptychobranchnus fasciolaris
Ptychobranchnus greeni
Ptychobranchnus subtentum