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CONCHIOLIN LAYERS AMONG THE UNIONIDAE AND MARGARITIFERIDAE
(BIVALVIA): MICROSTRUCTURAL CHARACTERISTICS
AND TAXONOMIC IMPLICATIONS

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ABSTRACT

The purposes of this paper are to determine the function of the conchiolin layers deposited within the shell of the freshwater bivalve families Unionidae and Margaritiferidae, whether they contain species-specific characteristics, and whether the microstructure of these layers supports inclusion of the margaritiferids within the family Unionidae, as proposed by Davis & Fuller (1981). Scanning electron microscope observations were conducted on 23 unionids and three margaritiferids. In addition, fossil unionids from the Eocene and Late Jurassic of Wyoming were examined to determine whether the microstructure of such layers is preserved and can be used as a taxonomic character among fossils.

The results indicate that unionids possess two types of conchiolin layers within the shell: thin, homogeneous and thick, more complex layers. The latter attain their highest degree of complexity within the Unionidae: Ambleminae, especially *Elliptio*. Unionid complex layers can be divided into three distinct regions. Margaritiferids, on the other hand, possess only one type of layer, also divisible into three distinct regions, which closely resembles the periostracum in ultrastructure.

Species examined possess highly individualistic conchiolin layer characteristics, with the exception of some members of the recently radiating *Elliptio*, among which overlaps in both reticulate region characteristics and inter-population variability occur. Placement of the margaritiferids within the family Unionidae is considered incompatible with the differences observed among the taxa.

The conchiolin layers prevent rapid shell dissolution in acidic or poorly buffered waters. Bivalves collected from habitats in which dissolution is severe often show significantly greater numbers of conchiolin layers per millimeter of shell thickness than do conspecific populations from habitats where dissolution is less severe. This indicates that these bivalves exercise control over the frequency of layer deposition, but the mechanism that actuates formation of layers other than damage-response layers remains obscure.

Unionid fossils from the Eocene of Wyoming have remarkably well preserved conchiolin layers. Overall characteristics of these layers are highly consistent with those that occur among Recent taxa examined. When preserved, this feature should allow discrimination of relationships among fossil taxa, and estimation of some environmental parameters, such as water acidity.

Key words: conchiolin layers; microstructure; Unionidae; Margaritiferidae; taxonomy.

INTRODUCTION

The occurrence of conchiolin layers within the shells of freshwater bivalves of the families Unionidae and Margaritiferidae has been documented for some time (e.g. Gray, 1833; Tolstikova, 1974), and recent studies suggest that possession of such conchiolin layers increases resistance of these bivalves to shell dissolution after the periostracum has worn away (Tevesz & Carter, 1980; Kat, 1982). Examination of specimens of *Elliptio complanata* indicated that two types of con-

chiolin layers are present within the shell of this species: thin, undifferentiated and thick, differentiated layers which can be subdivided into three regions. I proposed (Kat, 1982) that the microstructure of the thick conchiolin layers, especially characteristics of the central reticulate region, might be useful as a taxonomic character at the species level. The purpose of this paper is to test that hypothesis with a number of Atlantic Slope unionids representing two subfamilies, the Anodontinae and the Ambleminae (sensu Davis & Fuller, 1981).

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In addition, Tolstikova (1974) indicated a substantial difference between the Unionidae and the Margaritiferidae with respect to both shell and conchiolin layer microstructure. Davis & Fuller (1981) recently included North American margaritiferids within the family Unionidae, which seems to contradict Tolstikova's evidence. Conchiolin layer microstructure of three margaritiferids is compared to that of a number of unionids to determine whether the differences observed by Tolstikova (1974) are compatible with subfamilial status of the margaritiferids.

Taxonomic problems among the Unionacea are especially pronounced among fossil forms. Loss of all soft-part characteristics, as well as evidence of ecophenotypic plasticity has led to much taxonomic uncertainty among these fossil taxa (Haas, 1969). If microstructure of conchiolin layers is preserved, discrimination among fossil species should be simplified. Three fossils, one from the Late Jurassic and two from the Eocene of Wyoming, were examined to study the taxonomic value of conchiolin layer microstructure in fossils.

METHODS

The classification and collection locations of the specimens used in this study are listed in the Appendix. All shells were embedded in clear plastic, radially sectioned with a circular rock saw, polished with carborundum grit, and etched for 5 seconds with 5% HCl in preparation for scanning electron microscopy (SEM). During SEM, the specimens were tilted to reveal clearly the microstructure of the reticulate portions of the conchiolin layers; the relative thickness of the uppermost homogeneous region of the conchiolin layers thus is slightly distorted.

Variability within a species and within a population of a species was studied by examining seven populations of *Elliptio complanata* and four individuals within each of four populations of this species (Ellenwood, French Lake, Norwich, Bull Run). *E. complanata* was chosen for this survey of variability because it is one of the most variable unionid species in terms of shell shape, soft-part characteristics such as stomach anatomy and siphonal papillation, and molecular genetics of the Atlantic Slope unionid assemblage (Davis et al., 1981; Kat & Davis, in press). Variability of conchiolin layer charac-

teristics occurring in this species thus might similarly represent an upper limit to that occurring in other species.

RESULTS

The results of this study indicate the existence of two separate groups within the bivalves examined; as indicated by Tolstikova (1974), the conchiolin layers among the Margaritiferidae have a different structural organization from those in the Unionidae.

A) Margaritiferidae (*Margaritifera margaritifera*, Fig. 1C; *M. falcata*, Fig. 1E; *Cumberlandia monodonta*, Fig. 1F)

The shell of the margaritiferids contains only one type of layer, a thick (35 to 80 μm) conchiolin band (Fig. 1A) which is composed of three distinct regions (Fig. 1C). An outermost homogeneous region of approximately 10 to 16 μm in thickness surmounts a vacuolated region approximately 10 to 20 μm thick. The third region is composed of rather widely spaced organic lamellae, between which fit blocks of subprismatic shell material. This innermost region is the thickest of the three and varies from about 15 to 40 μm . The general appearance of the conchiolin layers of the margaritiferids is very similar to that of the periostracum among these bivalves (Tolstikova, 1974; see also Tevesz & Carter, 1980).

Among the Margaritiferidae examined, *Margaritifera margaritifera* (Fig. 1C) has the thickest layers, which show the best definition of the three regions. *Cumberlandia monodonta* (Fig. 1F) also has good definition of each region, but the layers are only about half as thick as those deposited in the shell of *M. margaritifera*. Finally, *M. falcata* (Fig. 1E) has layers equally thick to those of *C. monodonta*, but the vacuolated region generally is poorly developed in most individuals, and the lamellae seem more randomly placed.

B) Unionidae

Anodontinae (*Anodonta gibbosa*, Fig. 2A; *A. c. cataracta*, Fig. 2B; *A. c. fragilis*, Fig. 2C; *A. implicata*, Fig. 2D; *Strophitus undulatus*, Fig. 2E; *Alasmidonta undulata*, Fig. 2F)

Ambleminae: Lampsilini (*Lampsilis radiata*, Fig. 2G, H; *L. splendida*, Fig. 2I; *L. sp.*, Fig. 2J; *L. teres*, Fig. 3A; *L. dolabraeformis*, Fig. 3B)

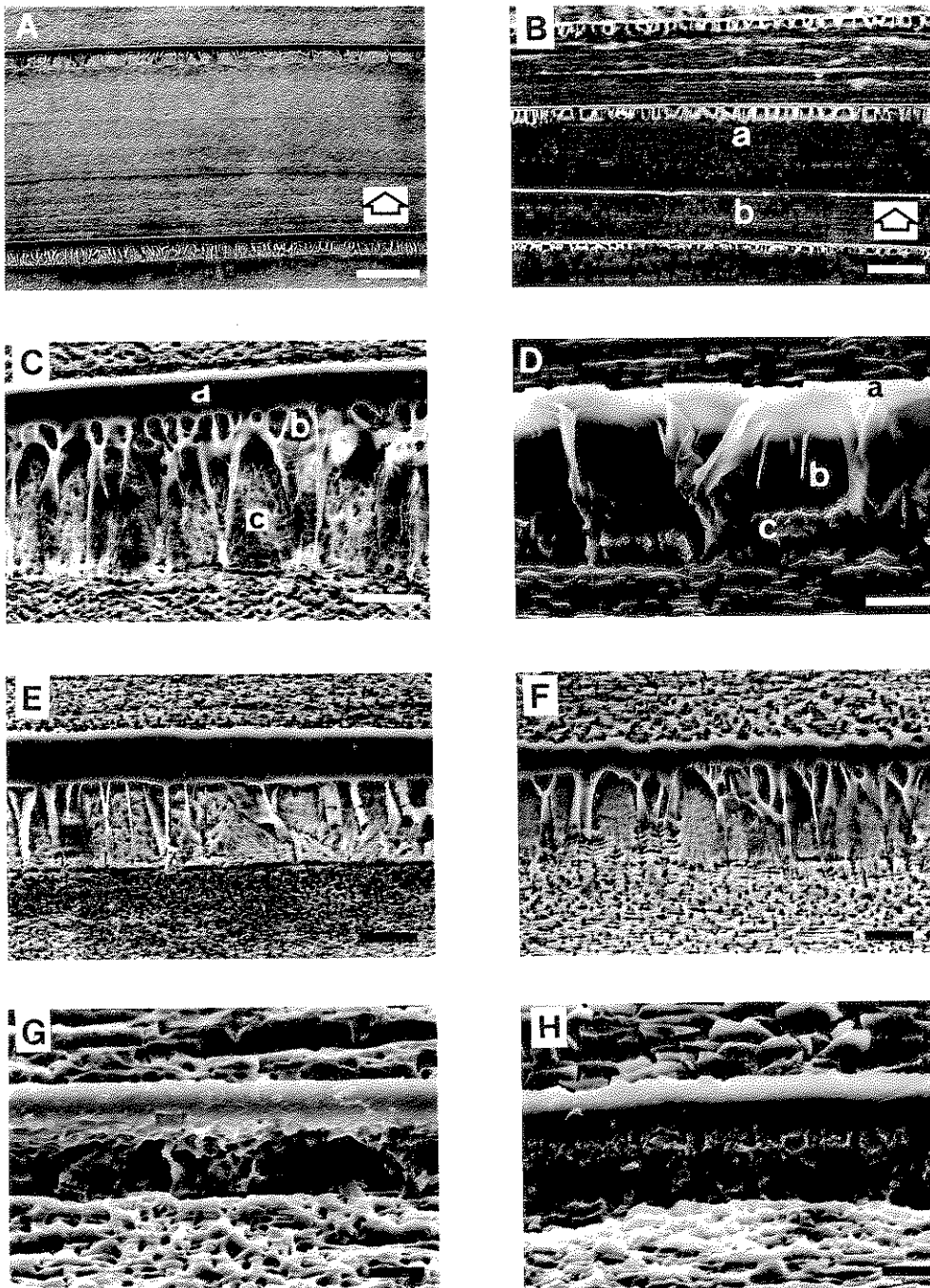


FIG. 1. A. Shell of *Margaritifera falcata* illustrating the exclusive presence of thick conchiolin layers in the shell. The arrow indicates the direction to the outside of the shell; scale bar = 100 μm . B. Shell of *Elliptio complanata* illustrating the presence of thick (a) and thin (b) conchiolin layers characteristic of the unionids. The arrow indicates the direction to the outside of the shell; scale bar = 50 μm . C. Thick conchiolin layer of *Margaritifera margaritifera* illustrating the three regions present in each layer: upper homogeneous (a),

mis, Fig. 3B; *Ligumia nasuta*, Fig. 3C; *Villosa delumbis*, Fig. 3D)
 Ambleminae: Pleurobemini (*Elliptio hope-tonensis*, Fig. 3E; *E. shephardiana*, Fig. 3F; *E. spinosa*, Fig. 3G; *E. lanceolata*, Fig. 3H; *E. fisheriana*, Fig. 3I; *E. folliculata*, Fig. 3J; *E. dariensis*, Fig. 4A; *E. arctata*, Fig. 4B; *E. complanata*, Fig. 4C-I)

The conchiolin layers of the Anodontinae are among the simplest observed in this study. The shell contains two types of layers; simple, thin (2.5–5 μm), homogeneous layers and slightly more complex layers composed of at most three partially defined regions: an outermost homogeneous region (about 3 μm thick), a reticulate region composed of rather poorly defined chambers, and an innermost, thin, homogeneous sheet. The reticulate region in particular is not very well developed; for example, in *Anodonta gibbosa* (Fig. 2A) thin lamellae form only partial chambers, and in *Strophitus undulatus* (Fig. 2E) the chambers are small and highly irregular in shape. The reticulate region varies in thickness from about 7 μm in *A. gibbosa* to about 5 μm in *S. undulatus*.

The Ambleminae form a cohesive group characterized by possession of two types of layers: thin, homogeneous (2–5 μm) and thick, complex (5–18 μm). Thick and thin layers commonly alternate, but thin shells, such as those of *Lampsilis splendida*, *Elliptio fisheriana*, and *Ligumia nasuta*, frequently have only one thick layer among several thin ones. Formation of these two layers seems rather similar; thick layers could result from elaboration of thin layers. The dimensions of the homogeneous upper portions of the thick layers are certainly similar to those of the thin layers, and thin layers sometimes have an incomplete reticulate region.

In the Ambleminae thick conchiolin layers consist of three regions: an outermost, homogeneous portion, which varies in thickness from about 2 to about 8 μm ; a central, reticulate portion (ranging in thickness from

about 3 to about 14 μm) composed of chambers of various shapes formed by sheet-like lamellae; and a lowermost, thin homogeneous region. The reticulate region contains calcareous material within the chambers formed by the lamellae ("irregularly shaped polyhedra" according to Tolstikova, 1974), which largely dissolved when the specimens were etched with HCl, but remains visible, for example, in Fig. 4H. The reticulate region of the conchiolin layers seems best developed in the genus *Elliptio*. The various features of a thick conchiolin layer characteristic of the Unionidae are illustrated in Fig. 5.

The fossil specimen of Late Jurassic age had a highly altered shell in which neither conchiolin layer microstructure nor shell microstructure was preserved. In contrast, both Eocene specimens (Fig. 1G, H) were exceptionally well preserved in that they seemed to retain the original conchiolin and contained both thin, undifferentiated and thick, differentiated layers. These conchiolin layers are similar to those of Recent Ambleminae, especially the Lampsilini, but are quite plesiomorphic in that the lamellae and chambers are poorly developed, and the entire reticulate region presents a disorganized appearance.

Based on the observed variability of conchiolin layer microstructure, it is possible to define a set of characters that determine plesiomorphic and apomorphic conditions among the Unionidae (Table 1). Highly plesiomorphic characters appear mainly among the Anodontinae; the Lampsilini and some Pleurobemini have some plesiomorphic characters; and most other Pleurobemini have apomorphic characters. This division agrees in a general fashion with the previously proposed times of origin of these taxa: The Anodontinae appeared during the Upper Cretaceous, the Lampsilini appeared during the Oligocene, and the Pleurobemini appeared during the Pleistocene (Haas, 1969; Davis *et al.*, 1981).

Tolstikova (1974) noted no differences among the microstructures of *Unio tumidus*

central vacuolated (b), and lowermost lamellar (c). Scale bar = 20 μm . D. Thick conchiolin layer of *Elliptio waccamawensis* illustrating the presence of three regions: uppermost homogeneous (a), central lamellar (b), and lowermost thin homogeneous (c). Scale bar = 10 μm . E. Thick conchiolin layer of *Margaritifera falcata*. Scale bar = 15 μm . F. Thick conchiolin layer of *Cumberlandia monodonta*. Scale bar = 15 μm . G. Thick conchiolin layer of a *Pseudelliptio* from the Eocene of Wyoming. Scale bar = 7 μm . H. Thick conchiolin layer of a *Pseudelliptio* from the Eocene of Wyoming. Scale bar = 5 μm .

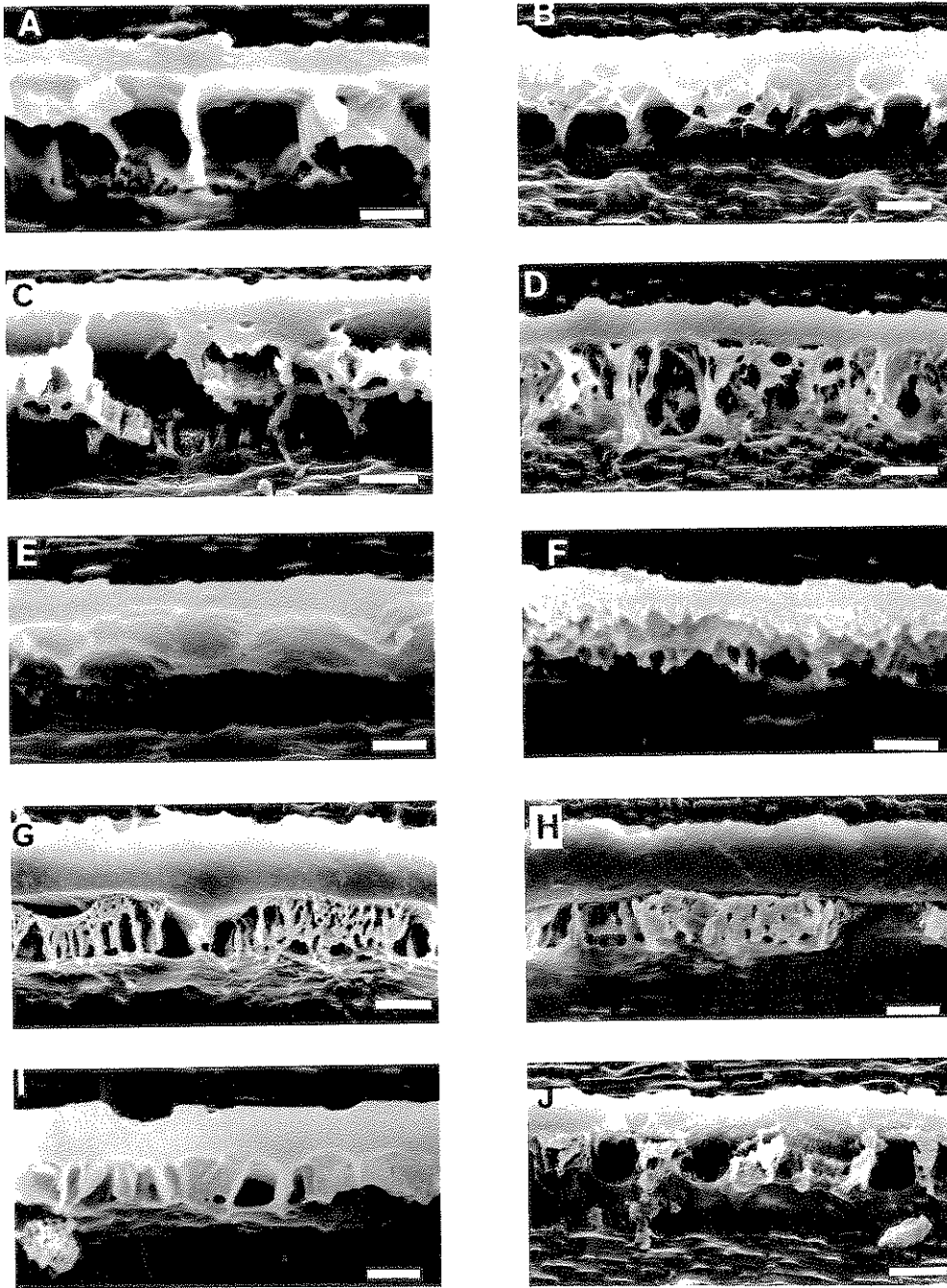


FIG. 2. A. Thick conchiolin layer of *Anodonta gibbosa*. Scale bar = 4 μm . B. Thick conchiolin layer of *Anodonta cataracta cataracta*. Scale bar = 4 μm . C. Thick conchiolin layer of *Anodonta cataracta fragilis*. Scale bar = 6 μm . D. Thick conchiolin layer of *Anodonta implicata*. Scale bar = 5 μm . E. Thick conchiolin layer of *Strophitus undulatus*. Scale bar = 3 μm . F. Thick conchiolin layer of *Alasmidonta undulata*. Scale bar = 5 μm . G. Thick conchiolin layer of *Lampsilis radiata* (Locality 1). Scale bar = 4 μm . H. Thick conchiolin layer of *Lampsilis radiata* (Locality 2). Scale bar = 3 μm . I. Thick conchiolin layer of *Lampsilis splendida*. Scale bar = 3 μm . J. Thick conchiolin layer of *Lampsilis* sp. Scale bar = 4 μm .

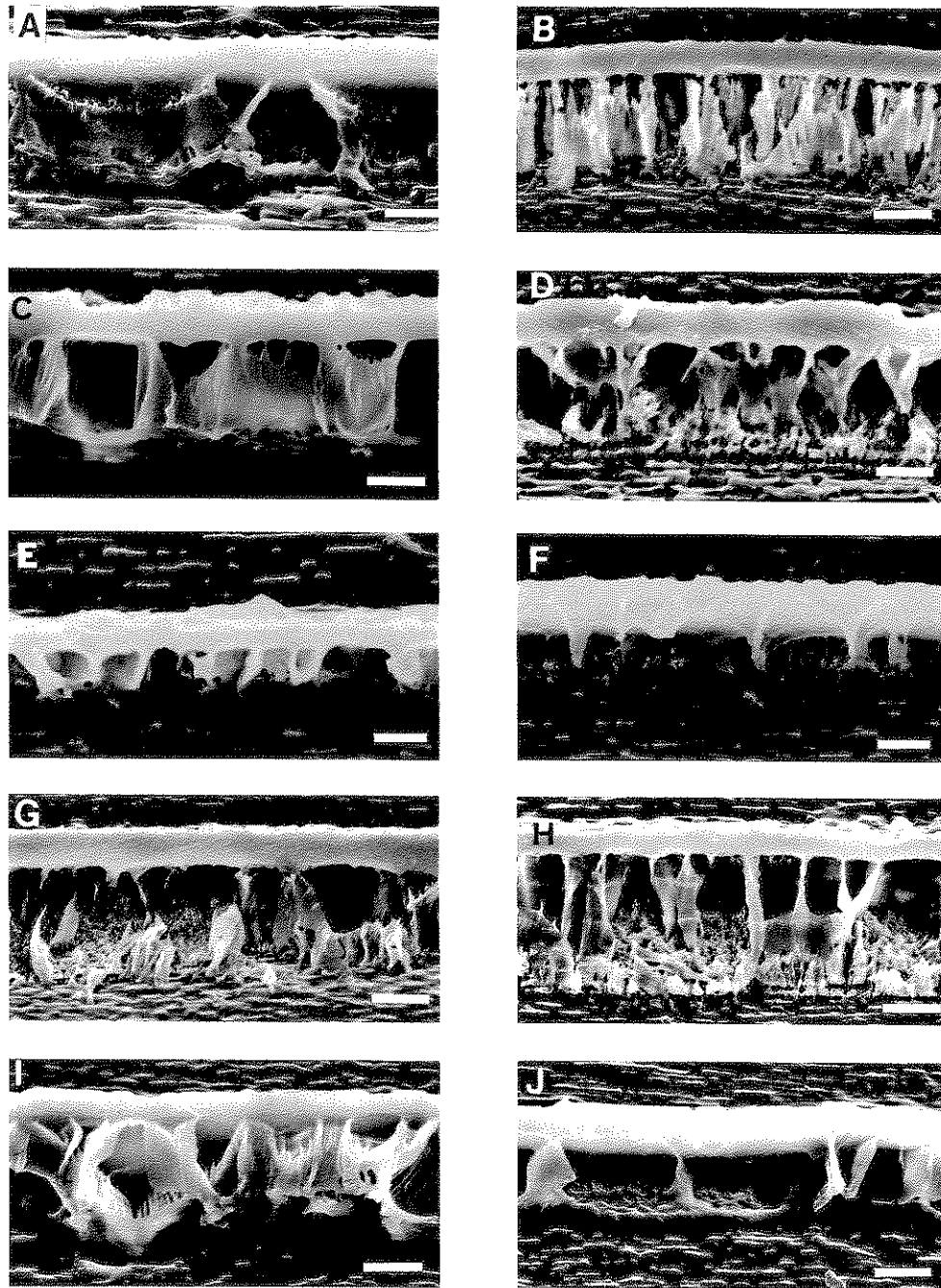


FIG. 3. A. Thick conchiolin layer of *Lampsilis teres*. Scale bar = 3 μm . B. Thick conchiolin layer of *Lampsilis dolabraeformis*. Scale bar = 7 μm . C. Thick conchiolin layer of *Ligumia nasuta*. Scale bar = 5 μm . D. Thick conchiolin layer of *Villosa delumbis*. Scale bar = 5 μm . E. Thick conchiolin layer of *Elliptio hopetonensis*. Scale bar = 3 μm . F. Thick conchiolin layer of *Elliptio shephardiana*. Scale bar = 3 μm . G. Thick conchiolin layer of *Elliptio spinosa*. Scale bar = 7 μm . H. Thick conchiolin layer of *Elliptio lanceolata*. Scale bar = 7 μm . I. Thick conchiolin layer of *Elliptio fisheriana*. Scale bar = 5 μm . J. Thick conchiolin layer of *Elliptio folliculata*. Scale bar = 5 μm .

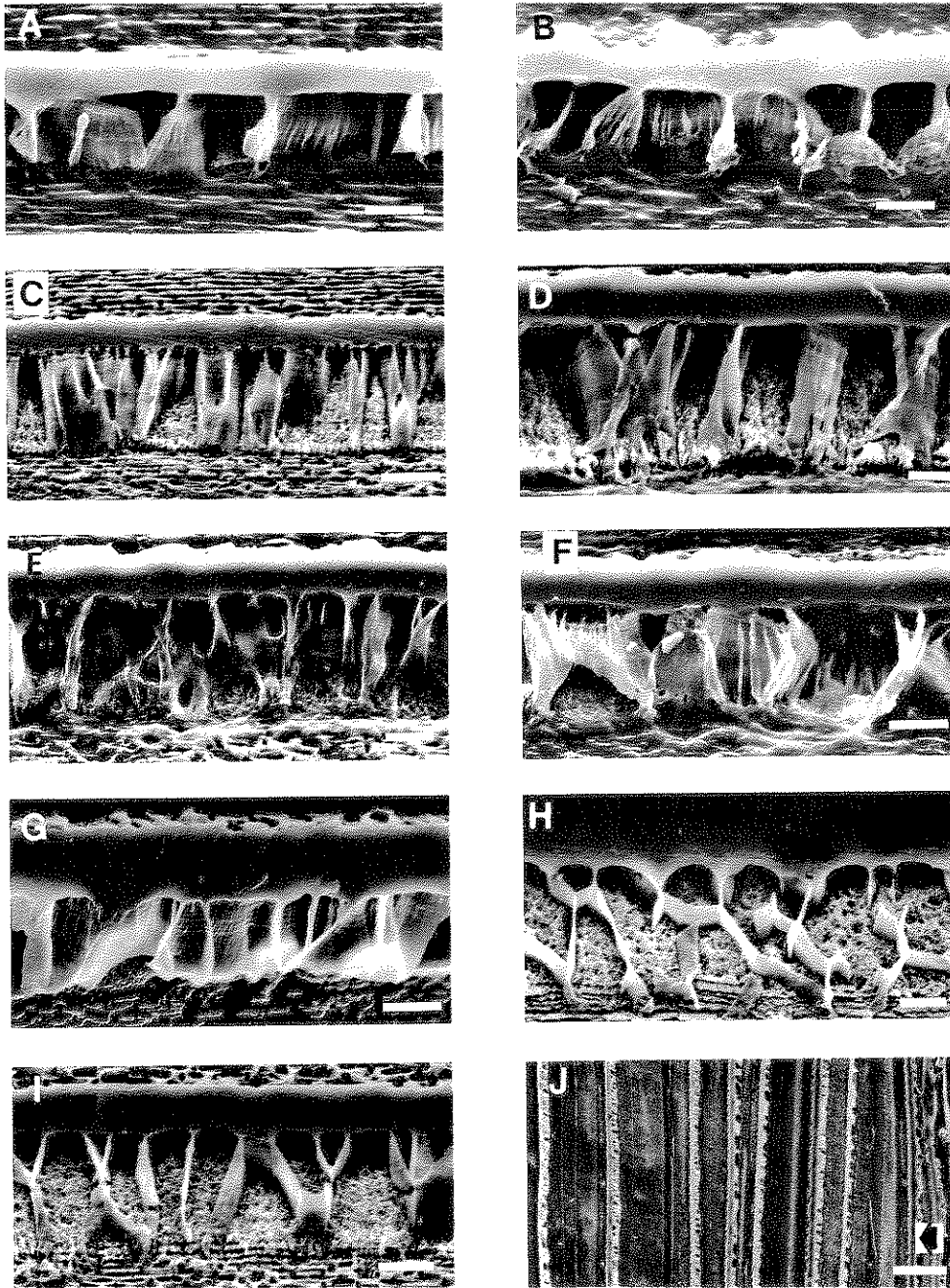


FIG. 4. A. Thick conchiolin layer of *Elliptio dariensis*. Scale bar = 5 μm . B. Thick conchiolin layer of *Elliptio arctata*. Scale bar = 5 μm . C. Thick conchiolin layer of *Elliptio complanata* (Locality 1). Scale bar = 7 μm . D. Thick conchiolin layer of *Elliptio complanata* (Locality 2). Scale bar = 5 μm . E. Thick conchiolin layer of *Elliptio complanata* (Locality 3). Scale bar = 6 μm . F. Thick conchiolin layer of *Elliptio complanata* (Locality 4). Scale bar = 7 μm . G. Thick conchiolin layer of *Elliptio complanata* (Locality 5). Scale bar = 7 μm . H. Thick conchiolin layer of *Elliptio complanata* (Locality 6). Scale bar = 6 μm . I. Thick conchiolin layer of *Elliptio complanata* (Locality 7). Scale bar = 7 μm . J. Presence of many conchiolin layers in shells from

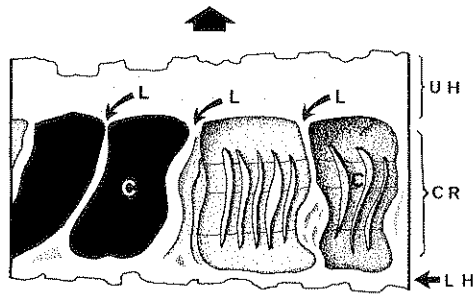


FIG. 5. Semi-diagrammatic representation of a unionid thick conchiolin layer. Illustrated are the upper (or outermost) homogeneous region (UH), the central reticulate region (CR), and the lower (or innermost) homogeneous region (LH). The reticulate region is composed of chambers (C), which are separated by lamellae (L). The back walls of the chambers are illustrated on the right; each chamber was filled with calcareous material which dissolved during HCl etching. The arrow at the top indicates the direction to the outside of the shell.

from different provinces in the U.S.S.R., but such differences are evident when populations of the phenotypically plastic and genetically variable *Elliptio complanata* are compared. In eastern Canada, for example, populations from the Sydney and Nine Mile Rivers and from French Lake have variously spaced, approximately vertical lamellae (with respect to the homogeneous layer) within the reticulate portion of the conchiolin layer (Fig. 4C, D, E), while the populations from Placide and Ellenwood Lakes possess curved lamellae (Fig. 4F, G). In contrast, Bull Run (Virginia) and Norwich Creek (Maryland) populations are characterized by lamellae that are interwoven in a variably complex network (Fig. 4H, I). Individuals in a population, however, resemble each other closely.

Resemblance in conchiolin layer microstructure can occur among different species of *Elliptio*. For example, *E. arctata* is similar to *E. dariensis* (Fig. 4A, B). Despite such similarities, pronounced differences usually occur. For example, the lanceolate forms *E. lanceolata*, *E. folliculata*, *E. fisheriana*, *E. shephardiana*, and *E. arctata* are clearly different (Fig. 3H, J, I, F, and 4B, respectively).

Variability among populations of *Elliptio*

TABLE 1. Plesiomorphic and amorphic characteristics of the reticulate portion of unionid conchiolin layers.

A. Plesiomorphic characters.	
1.	Absence of lamellae, or presence of coarse lamellae which vary in width or length.
2.	Absence of chambers, presence of poorly defined chambers formed by partial lamellae, or presence of variously sized chambers.
3.	Reticulate portion only slightly thicker than the upper homogeneous portion of the conchiolin layer.
B. Apomorphic characters.	
1.	Presence of well-developed lamellae of rather uniform length and appearance.
2.	Presence of well-defined chambers of generally equal dimensions.
3.	Reticulate portion considerably thicker than the upper homogeneous portion of the conchiolin layer.

complanata and overlap of characteristics among species of this genus contrasts with conservatism among the Lampsilini and Anodontinae. For example, *L. sp.*, an undescribed species from Lake Waccamaw, North Carolina, is different in reticulate region characteristics from both *L. radiata* and *L. splendida*, the species found in the drainages around Lake Waccamaw and from which *L. sp.* presumably was derived (Fig. 2G, H, I, J) (Kat, in press a).

Despite the high degree of plesiomorphy among the Anodontinae, it is possible to discriminate among anodontine species. For example, *Anodonta c. cataracta* differs in reticulate region characteristics from *A. c. fragilis*, hypothetically a closely related anodontine from Nova Scotia (Fig. 2B, C) (Clarke & Rick, 1963). *A. implicata* (Fig. 2D) is an exception to the plesiomorphic trend among the anodontines; while the reticulate portion still presents a somewhat disorganized picture, the lamellae are thin and the chambers complete.

DISCUSSION

Functional Significance

The presence of thick conchiolin layers in bivalve shells usually has been associated

habitats in which dissolution is severe. Specimen is *Elliptio complanata* from Locality 5. The arrow indicates the direction to the outside of the shell; scale bar = 50 μ m.

(e.g. by Lewy & Samtleben, 1979) with resistance to both predation by boring gastropods and shell dissolution. In the limited case of the unionids and margaritiferids the function of the shell layers has been proposed to fall in the latter category; after the periostracum has been worn away from the umbonal region (which is the oldest part of the bivalve shell), these successive organic layers might retard shell dissolution sufficiently to permit compensatory shell deposition under most conditions (Kat, 1982). For instance, as a result of possession of such layers, unionids can survive with minimal shell damage in habitats where *Corbicula* experience considerable mortality due to excessive shell dissolution because their shells lack conchiolin layers (Kat, 1982). In addition, when the unionid shell is damaged, similar conchiolin layers are deposited to seal off the affected area (Beedham, 1965; Tevesz & Carter, 1980; personal observations). These damage-response layers can be distinguished from other conchiolin layers by the presence of an underlying prismatic layer (Tevesz & Carter, 1980).

With the exception of damage-response layers, it is uncertain what actuates formation of these conchiolin layers. Layers are deposited mainly in small patches in the region of the umbo, and usually end abruptly (Fig. 1B). There is a suggested relationship between frequency of conchiolin layers within the shell and water acidity, although confirmation of this trend requires measurement of inter-population variability in conchiolin layer abundance with both the averages and standard deviations of environmental parameters such as water pH and hardness. Nevertheless, bivalves from habitats in which shell dissolution is extensive (such as Lake Waccamaw, North Carolina; various mesotrophic and oligotrophic lakes in Nova Scotia; and certain small creeks in Georgia and Florida) often have more bands per millimeter of shell (Fig. 4J) than do conspecifics in habitats where minimal shell dissolution occurs (Fig. 1B); it thus appears that these bivalves exercise some degree of control over frequency of layer deposition. There is no evidence that these layers correspond to growth stops as proposed by Tolstikova (1974).

Russell-Hunter *et al.* (1981) found no relationships between total organic content and water hardness in several populations of freshwater limpets from habitats in which hardness varied by an order of magnitude.

Their methods, however, are open to some criticism: the variance of environmental parameters such as water pH and hardness apparently was not taken into account, limpets of various size classes were lumped (large limpets could have had eroded shells), and tissues were not extracted from the shells prior to determination of organic content. It is not known whether any freshwater gastropods deposit conchiolin layers within their shells to counter dissolution. Various other relationships among shell calcium and water hardness were presented by Russell-Hunter *et al.* (1981); it is apparent that freshwater molluscs have a variety of responses in terms of shell components and their relationships to environmental parameters.

Taxonomic Significance

Species-level Discrimination

SEM examinations of conchiolin layer microstructure reveal that features of the reticulate region, in particular, can often be used to discriminate among species within a genus. Exceptions to this trend occur among some species of the genus *Elliptio*, which vary considerably in characteristics among geographic subgroups and some overlap of characteristics among species. Conchiolin layer discriminants among anodontine species could be fewer and/or more equivocal because the Anodontinae examined here usually lack a well-defined reticulate region.

The overlap of characteristics among some species of *Elliptio* and the variability among geographic subgroups of *E. complanata* are not surprising. The genus apparently is undergoing a Recent radiation, and levels of molecular genetic resemblance among some species are not different from those that characterize different populations of a wide-ranging species. For example, species within the *E. complanata* "group" defined by Davis *et al.* (1981) are genetically cohesive with identity values (Nei, 1972) ranging from 0.90 to 0.99. Populations of morphologically defined *E. complanata* from various locations, on the other hand, show genetic identity values ranging from 0.82 to 0.99 (Kat & Davis, in press), and I propose elsewhere (Kat, in press b) that *E. complanata* should be regarded as a highly polytypic species presently distributed as a Rassenkreis, or ring species, around the Appalachian mountain chain. This pattern of close interspecific and variable intraspecific resemblances generally

has been considered to be indicative of rapid speciation and little time since divergence of species within a genus (Avisé *et al.*, 1975; Avisé, 1976; Davis *et al.*, 1981; Kat, in press a). Morphological characters such as conchiolin layer microstructure therefore could show overall similarity among species unless those genetic changes that accompanied speciation directly or indirectly (through pleiotropy) affected loci that regulate pattern and process of conchiolin layer deposition or unless such differences accumulated since divergence. The latter process seems to be responsible for accumulated differences among widely-separated (and presumably reproductively isolated) populations of *E. complanata*, but it is not possible to determine which process is responsible for observed differences between species which are well separated genetically. For example, *E. folliculata* and *E. fisheriana* are distantly related ($I = 0.64$; Davis *et al.*, 1981) and exhibit considerable differences in conchiolin layer microstructure (Fig. 3I, J), but it is not possible to determine by which pathway (directly associated with a punctuated process of speciation or as a result of divergence over time) the differences arose.

Three species within *Elliptio* (*E. shephardiana*, *E. hopetonensis*, *E. spinosa*; Fig. 3E, F, G) show considerable plesiomorphy in conchiolin layer microstructure when compared with other members of the genus examined here. All three species are endemic to the ancient Altamaha River drainage (Johnson, 1970) and could represent a group of species that diverged from ancestral *Elliptio* early in time and thus retained some plesiomorphic characters.

Members of the Anodontinae and Ambleminae: Lampsilini do not show such variability. Both groups are characterized by rather plesiomorphic conchiolin layers, but there are some exceptions to this overall trend. *Lampsilis dolabraeformis* (Fig. 3B), for instance, has the most apomorphic conchiolin layers among the lampsilines examined. This species can be characterized as "advanced" with respect to other features as well; for example, the mantle flap and marsupium show highly derived conditions. *Anodonta implicata* (Fig. 2D) is an exception to the plesiomorphic trend among the anodontines; the lamellae in this species are thin and complete, and the chambers are quite regular. I have proposed separation of *A. implicata* from the subgenus *Pyganodon* (which includes all other *Anodonta*

TABLE 2. Characteristics of the conchiolin layers which differentiate the Unionidae and Margaritiferidae.

Unionidae

1. Presence of two types of conchiolin layers within the shell: thin, undifferentiated, and thick, differentiated.
2. Thick, differentiated layers can always be divided into three or more or less distinct layers: an uppermost homogeneous portion; a central reticulate portion, and a lowermost thin homogeneous portion.
3. Chambers of various sizes are present within the reticulate portion of the conchiolin bands, which are filled with unconsolidated, chalky shell material.

Margaritiferidae

1. Presence of one type of conchiolin layer within the shell: thick, differentiated.
2. Differentiated layers can be divided into three layers: an uppermost homogeneous portion, a central vacuolated portion, and a lowermost lamellar portion. The appearance of the conchiolin layers is similar to that of the periostracum.
3. Chambers of various sizes are formed within the lamellar portion, which are filled with blocks of subprismatic shell material.

examined here) because of electrophoretic and soft-part characteristic differences (Kat, 1983); the differences observed in conchiolin layer microstructure support this separation. I also described a substantial difference between *A. c. cataracta* and *A. c. fragilis*; the latter "subspecies" differs considerably from the former in electrophoretic and soft-part characters (in fact, *A. c. fragilis* shows close affinity to the European *A. cygnea* with respect to stomach morphology); conchiolin layer characteristics again positively correlate with other discriminants (Fig. 2B, C).

Taxonomic concepts based on conchiolin layer similarities, however, sometimes disagree with relationships suggested by electrophoresis: for example, *Lampsilis* sp. seems more closely related to *L. splendida* than to *L. radiata* with respect to overall conchiolin layer microstructure (Fig. 2H, I, J) while the reverse relationship is suggested by electrophoretic and soft-part similarities (Kat, in press a).

Higher-order Taxonomy

Two very distinct categories can be defined on the basis of characteristics of the

conchiolin layers among the species examined (Table 1). The Unionidae show varying degrees of elaboration of the conchiolin layers, which appear to be the most complex among the Ambleminae. Also, all unionids possess thin, undifferentiated bands, which could be indicative of plesiomorphy. The margaritiferids show very different, periostracum-like bands, which are unlike any encountered among the unionids examined. Tolstikova (1974) was equally convinced of the differences between unionids and margaritiferids with respect to this character and described some additional significant differences in shell microstructure.

I propose two hypotheses to explain these differences:

(1) Recent margaritiferids show conchiolin layers that are as highly derived from an ancestral state as those of the Pleurobemini are; in other words, the fact that these layers are now very different does not mean that they could not once have been very similar. This hypothesis is rejected for two reasons. First, while the conchiolin layers of the Pleurobemini can be regarded as apomorphic, the simultaneous presence of thin, undifferentiated layers within the shell, as well as the presence of features that link differentiated and undifferentiated bands among the unionids, points to common ancestry. This is supported by close resemblance of conchiolin layer microstructure between Recent species and Eocene taxa. A similar argument cannot be constructed for the margaritiferids; they lack all but differentiated layers, and these layers exhibit few features in common with those of the unionids. Second, conchiolin layers of the margaritiferids resemble the periostracum in ultrastructure, while those of the unionids do not.

(2) Recent margaritiferids have conchiolin layers that are derived from a very different ancestral condition; in other words, they arose in a lineage different from that of the Unionidae. This hypothesis is most compatible with the data.

It appears that there have been two distinctly different pathways taken by different unionacean lineages towards the solution of the common problem of shell dissolution. Ancestral unionids likely laid down simple to slightly elaborated conchiolin layers, similar to those encountered among Recent anodontines. Elaboration of such bands is en-

countered among Recent *Lampsilis* and *Elliptio*. Ancestral margaritiferids likely formed layers similar to the periostracum. Recent margaritiferids continue to have this ancestral condition. This hypothesis should be testable in the fossil record.

The classification of Davis & Fuller (1981), which relegates the margaritiferids to subfamilial status within the Unionidae, is therefore rejected; the margaritiferids have conchiolin layer microstructural differences suggestive of an early divergence from the unionid lineage, and on the basis of this character I propose restoration of familial status. On the other hand, similarities among the Lampsilini and Pleurobemini with respect to conchiolin layer microstructure should constitute additional evidence to support including them in the same subfamily (Ambleminae) as proposed by Davis & Fuller (1981).

Placement of the margaritiferids within the Unionidae was based on molecular genetic and some morphologic data (Davis & Fuller, 1981). First, genetic similarity was found to be higher than expected if the taxa belonged to different families (comparative data were derived from comparisons of different gastropod families), especially because fossil evidence indicated that the divergence could have begun before the Cretaceous (Haas, 1969). With respect to electrophoretically determined genetic distances, remaining similarities among distantly related taxa generally are found among slowly evolving loci (Sarich, 1977). Divergence times based on genetic distances that include such loci must include a large margin of error, because rates of differentiation for such loci are unknown, because it is unknown to what extent natural selection maintains similarities among such loci, and because it is not intuitively obvious why genetic distances between distantly related taxa should continue to increase in a regular fashion. In fact, the strict applicability of the molecular clock has recently been questioned: sea urchin species pairs separated by the Isthmus of Panama reveal radically different genetic distances, even though they have presumably been isolated for exactly the same amount of time (Lessios, 1979, 1981). Some taxa thus might diverge more rapidly than others. Cluster-ordination analysis of immunoelectrophoretic distances also indicates high levels of similarity between the Ambleminae and *Margaritifera* (Davis & Fuller, 1981). In sum, the combined electrophoretic and immunoelectrophoretic data indi-

cate that the taxa probably are not of polyphyletic origin, but the distinct differences observed between the Margaritiferidae and the Unionidae in conchiolin layer microstructure must place the taxa in different families, which I propose shared a common ancestor before the Cretaceous (Fig. 6).

Second, similarities exhibited by the margaritiferids to the unionid morphological groundplan, including the glochidial larval type, could imply similarity of response to similar selective pressures. Margaritiferids and unionids share identical habitats, and parasitize identical hosts (fishes); it is thus entirely likely that, given a similar ancestral bivalve groundplan, the taxa now resemble each other in a general fashion. It must be noted that margaritiferid glochidia are much smaller than those found among the unionids, and that careful comparative observations have not been made to support contentions of their similarity. An initial examination of soft-part characteristics of margaritiferids and unionids reveals significant differences between the taxa. Siphonal papillae among the margaritiferids are both muscular and arborescent, a condition not encountered among unionids thus far examined (although some Ambiemini apparently possess arborescent papillae; see Davis & Fuller, 1981); margaritiferids possess no true septa or water tubes in their lamellae; and examination of characteristics of the margaritiferid stomach reveals that it is simple in structure, resembles those of the anodontines, but differs from the unionids thus far examined in possession of a sorting pouch beneath the minor typhlosole fold (Kat, 1983; personal observations).

Fossils

According to Haas (1969), loss of all soft-part characters, evidence of a high degree of phenotypic plasticity of unionacean shell shape, and gaps in knowledge of Recent forms contribute to render classification of fossil forms an extremely difficult endeavor. In addition, fossil specimens often are fragmentary, or preserved only as casts or molds, which further reduces the amount of information that can be deduced from them. Preservation of microstructural characteristics of the conchiolin layers within shells that have undergone little replacement, or replacement with little deformation of the original microstructure of the shell, would allow identification of species as well as degree of pheno-

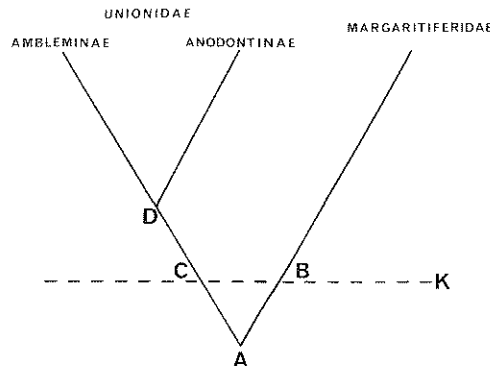


FIG. 6. Relationships among North American Unionacea based on conchiolin layer microstructure. In the diagram, a generalized unionacean ancestor A gives rise to descendant groups B and C, present during the Cretaceous (K). Group B is characterized by deposition of conchiolin layers which resemble the periostracum in ultrastructure, and is ancestral to the Margaritiferidae. Group C is characterized by deposition of simple, non-differentiated layers within the shell, and is ancestral to the Unionidae. This group then diverges (D) into the Ambleminae, which has differentiation of conchiolin layers into separate portions, and the Anodontinae, which largely retain simple layers.

typic plasticity shown by such species, even from fragmentary material. Such information is important in that it allows assessment of fossil species diversity, determination of relationships between fossil and Recent taxa, and because the number of layers deposited in the shell seem related to water acidity, some environmental reconstruction. Unfortunately, preservation of conchiolin layer microstructure requires rather exceptional conditions, which might only seldom be met; permineralization which faithfully replicates the conchiolin layer could be extremely rare. The Eocene fossils studied were exceptionally well preserved, and appear to have retained the original components of the conchiolin layers (Fig. 1G, H). In contrast, a fossil specimen from the Late Jurassic had been extensively altered. Preservation of taxonomically valuable characters might thus be limited to specimens of Tertiary or younger age. Nevertheless, good preservation of conchiolin layer microstructure observed in this preliminary study of fossil forms is extremely encouraging, and should provide a useful way to discriminate among previously problematic fossil taxa.

SUMMARY

Thick conchiolin layers within the shells of the Unionidae and the Margaritiferidae seem to serve a common purpose: prevention of rapid shell dissolution in the region of the umbo once the protective periostracum has worn away. Most species examined, with the exception of some recently diverging taxa within the genus *Elliptio*, seem to possess highly individualistic characteristics of especially the reticulate regions of the layers. Placement of the margaritiferids within the Unionidae as proposed by Davis & Fuller (1981) is considered incompatible with the significant differences observed in the microstructure of the conchiolin layers of these taxa; there seem to have been two distinctly different pathways taken by the different lineages toward the solution of the common problem of shell dissolution. The margaritiferids deposit layers that resemble the periostracum, while the unionids lay down simple and variably complex chambered conchiolin layers, which do not resemble the periostracum.

Preservation of conchiolin layer microstructure among Eocene taxa suggests the existence of a powerful tool to discriminate among fossil forms, and should facilitate fossil classification. Such discriminatory ability should additionally allow estimates of fossil assemblage diversity, relationships among fossil and Recent taxa, and reconstruction of some environmental parameters such as water pH.

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REFERENCES CITED

- AVISE, J. C., 1976, Genetic differentiation during the speciation process. In AYALA, F. J. (ed.), *Molecular Evolution*. Massachusetts, Sinauer Press, p. 106–122.
- AVISE, J. C., SMITH, J. J. & AYALA, F. J., 1975, Adaptive differentiation with little genetic change between two native California minnows. *Evolution*, 29: 411–426.
- BEEDHAM, G. E., 1965, Repair of the shell in species of *Anodonta*. *Proceedings of the Zoological Society of London*, 145: 107–124.
- CLARKE, A. H. & RICK, A. M., 1963, Supplementary records of Unionacea from Nova Scotia with a discussion of the identity of *Anodonta fragilis* Lamarck. *National Museum of Canada Bulletin* 199.
- DAVIS, G. M. & FULLER, S. L. H., 1981, Genetic relationships among Recent Unionacea (Bivalvia) of North America. *Malacologia*, 20: 217–253.
- DAVIS, G. M., HEARD, W. H., FULLER, S. L. H. & HESTERMAN, C., 1981, Molecular genetics and speciation in *Elliptio* and its relationships to other taxa of North American Unionidae (Bivalvia). *Biological Journal of Linnean Society*, 15: 131–150.
- GRAY, J. E., 1833, Some observations on the economy of molluscos animals, and on the structure of their shells. *Philosophical Transactions of the Royal Society of London*, 123: 771–819.
- HAAS, F., 1969, Superfamily Unionacea. In MOORE, R. C. (ed.), *Treatise on Invertebrate Paleontology*, Part N. Mollusca, 6: Vol. 1 (of 3): N411–N470.
- JOHNSON, R. I., 1970, The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the Southern Atlantic Slope region. *Bulletin of the Museum of Comparative Zoology*, 149: 263–447.
- KAT, P. W., 1982, Shell dissolution as a significant cause of mortality for *Corbicula fluminea* (Bivalvia: Corbiculidae) inhabiting acidic waters. *Malacological Review*, 15: 129–134.
- KAT, P. W., 1983, Genetic and morphological divergence among nominal species of North American *Anodonta* (Bivalvia: Unionidae). *Malacologia*, 23: 361–374.
- KAT, P. W., in press a, Genetics, morphological divergence, and speciation in *Lampsilis* (Bivalvia: Unionidae). *Journal of Molluscan Studies*.
- KAT, P. W., in press b, Patterns of electrophoretic and morphologic variability among widely distributed species. I. An initial survey of variability in *Elliptio complanata* (Bivalvia: Unionidae). *Netherlands Journal of Zoology*, 33.
- KAT, P. W. & DAVIS, G. M., in press, Genetic divergence of peripheral populations of Nova Scotian Unionidae. *Biological Journal of the Linnean Society*.
- LESSIOS, H. A., 1979, Use of Panamanian sea urchins to test the molecular clock. *Nature*, 280: 599–601.
- LESSIOS, H. A., 1981, Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the Isthmus of Panama. *Evolution*, 35: 618–634.

- LEWY, Z. & SAMTLEBEN, C., 1979, Functional morphology and palaeontological significance of the conchiolin layers in corbulid gastropods. *Leuthaia*, 12: 341-351.
- NEI, M., 1972, Genetic distance between populations. *American Naturalist*, 106: 283-292.
- RUSSELL-HUNTER, W. D., BURKY, A. J. & HUNTER, R. D., 1981, Interpopulation variation in calcareous and proteinaceous shell components in the stream limpet, *Ferrissia rivularis*. *Malacologia*, 20: 255-266.
- SARICH, V. M., 1977, Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature*, 265: 24-28.
- TEVESZ, M. J. & CARTER, J. C., 1980, Environmental relationships of shell form and structure of Unionacean bivalves. In RHOADS, D. C. & LUTZ, R. A. (EDS.), *Skeletal Growth of Aquatic Organisms*. Plenum Press, New York, p. 295-322.
- TOLSTIKOVA, N. V., 1974, Microstructural characteristics of freshwater bivalves (Unionidae). *Paleontological Journal* [translation of *Paleontologicheskii Zhurnal*], 8: 55-60.

APPENDIX. Classification and collection localities of the species studied

Recent Taxa

Unionidae

Anodontinae

- Anodonta gibbosa* Say, 1824
Ocmulgee River, Ben Hill Co., Georgia
- Anodonta cataracta cataracta* Say, 1817
Pickering Creek, Chester Co., Pennsylvania
- Anodonta cataracta fragilis* Lamarck, 1819
Nowlans Lake, Digby Co., Nova Scotia, Canada
- Anodonta implicata* Say, 1829
Fletcher Lake, Halifax Co., Nova Scotia, Canada
- Strophitus undulatus* (Say, 1817)
Norwich Creek, Talbot Co., Maryland
- Alasmidonta undulata* (Say, 1817)
Norwich Creek, Talbot Co., Maryland

Ambleminae

Lampsilini

- Lampsilis radiata* (Gmelin, 1791)
1. Shubenacadie Grand Lake, Halifax Co., Nova Scotia, Canada
2. Newville Lake, Cumberland Co., Nova Scotia, Canada
- Lampsilis splendida* (Lea, 1838)
Ocmulgee River, Ben Hill Co., Georgia
- Lampsilis dolabraeformis* (Lea, 1838)
Ocmulgee River, Ben Hill Co., Georgia
- Lampsilis teres* (Say, 1834)
Withlacoochee River, Lacoochee, Pasco Co., Florida

Lampsilis sp.

Lake Waccamaw, Columbus Co., North Carolina

Ligumia nasuta (Say, 1817)

Norwich Creek, Talbot Co., Maryland

Villosa delumbis (Conrad, 1834)

Fountain Mill, Pulaski Co., Georgia

Pleurobemini

Elliptio complanata (Lightfoot, 1786)

1. Sydney River, Cape Breton Co., Nova Scotia, Canada

2. French Lake, Sunbury Co., New Brunswick, Canada

3. Nine Mile River, Halifax Co., Nova Scotia, Canada

4. Ellenwood Lake, Yarmouth Co., Nova Scotia, Canada

5. Placide Lake, Digby Co., Nova Scotia, Canada

6. Norwich Creek, Talbot Co., Maryland

7. Bull Run, Prince William Co., Virginia

Elliptio fisheriana Ortmann, 1919

Norwich Creek, Talbot Co., Maryland

Elliptio waccamawensis (Lea, 1863)

Lake Waccamaw, Columbus Co., North Carolina

Elliptio hopetonensis (Lea, 1838)

Ocmulgee River, Ben Hill Co., Georgia

Elliptio spinosa (Lea, 1836)

Ocmulgee River, Ben Hill Co., Georgia

Elliptio shephardiana (Lea, 1834)

Ocmulgee River, Ben Hill Co., Georgia

Elliptio lanceolata (Lea, 1820)

Fountain Mill, Pulaski Co., Georgia

Elliptio dariensis (Lea, 1842)

Bowens Mill, Ben Hill Co., Georgia

Elliptio arctata (Conrad, 1834)

Mosquito Creek, Gadsden Co., Florida

Elliptio folliculata (Lea, 1858)

Lake Waccamaw, Columbus Co., North Carolina

Margaritiferidae

Margaritifera margaritifera (Linnaeus, 1758)

Maccan River, Cumberland Co., Nova Scotia, Canada

Margaritifera falcata (Gould, 1850)

Deschutes River, Deschutes Co., Oregon

Cumberlandia monodonta (Say, 1829)

Clinch River, Hancock Co., Tennessee

Fossil Taxa

Genus?

Jurassic: Lower Morrison Formation, East Como Bluff, Albany Co., Wyoming. Johns Hopkins University M-COMO-CLM-1

Pseudelliptio sp.

Eocene: Willwood Formation, Elk Creek, Big Horn Co., Wyoming. Johns Hopkins University W-KC-PSE-1

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