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On the So-called Mantle Muscle Scars on Shells of the Margaritiferidae (Mollusca, Pelecypoda), with Observations on Mantle–Shell Attachment in the Unionoida and Trigonioida

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Mantle attachment scars on the inner surface of shells of the Margaritiferidae have been traditionally regarded as sites of mantle muscle attachment; however, the actual occurrence of muscle tissue at points of attachment has never been verified. Mantle attachment scars occur on shells of seven examined species of margaritiferids. Gross and histological investigation of the mantle of five species reveals that mantle attachment involves modification of mantle epithelial cells and associated connective fibers within the mantle. The connective fibers within the mantle do not appear to have contractile properties, but along with the attachment cells probably provide support for the mantle. Mantle–shell attachment scars similar to those of margaritiferids also occur in shells of recent trigonids, thus strengthening arguments for a phylogentic link between the Unionoida and Trigonioida.

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Introduction

The presence of pits, muscle scars or mantle attachment scars, as they have been called, on the interior surfaces of shells of margaritiferids was noted and discussed by Pilsbry (1896), who indicated that shells of *Margaritana* (= *Margaritifera* and *Cumberlandia*) could be identified by the presence of such mantle "muscle" scars. Simpson (1896) responded by stating that muscle scars were of no value in unionid systematics; however, Simpson (1900, 1914) subsequently implied a systematic significance to the "muscle" or mantle-attachment scars by including them in his diagnosis of *Margaritana*. No further investigations, other than individual species descriptions, have been made. In the few species analyses that mention the mantle-attachment scars (in forms belonging to the Margaritiferidae) (e.g. Simpson 1914; Ortmann 1919; Brandt 1974; Ellis 1978) the scars have been regarded as sites of mantle "muscle" attachment. Since the actual presence of connective tissue in the dorso-medial area of the mantle of margaritiferids have never been verified, it was decided to determine whether or not muscle tissue, or other connective tissues, are present in the mantle in the region where mantle scars are formed on the shell.

Materials and methods

Gross examination of mantle–shell attachment scars on margaritiferid shells was performed on material maintained in four major North American museums, including the American Museum of Natural History (AMNH), New York, NY; Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, MA; Yale Peabody Museum (YPM), Yale University, New Haven, CT; Florida State Museum (FSM), Gainesville, FL. The possible presence of mantle–shell attachment scars on shells of species representing other unionoid families was investigated as well. Additionally, shells in the AMNH collections of *Neotrigonia* spp. (Trigonoida: Trigoniidae), a living genus of a largely extinct marine bivalve group alleged to be ancestral to unionoids (Newell & Boyd 1975), were examined for the presence of mantle–shell attachment scars.

Photography of mantle attachment scars on shells was performed using an ETEK autoscan scanning electron microscope (SEM).

Investigations on tissue structure were carried out using conventional light microscopy. Mantle tissue samples were taken from nine specimens representing both genera of the Margaritiferidae. All specimens examined histologically are presently housed in the Museum of Zoology (UMA), University of Massachusetts, Amherst, MA. The specimens are distributed as follows: *Margaritifera* (*Margaritifera*) *margaritifera* (L.), three specimens (UMA MO. 1161, 1220.1, 1257); *M.* (*M.*) *hembeli* (Conrad), three specimens (UMA MO. 1248); *M.* (*Margaritanopsis*) *falcata* (Gould), one specimen (UMA MO. 1249); *M.* (*M.*) *laevis* (Haas), one specimen (UMA MO. 1260); *Cumberlandia monodonta* (Say), one specimen (UMA MO. 1143). Localities of collection can be furnished upon request.

Histological techniques involved removing a portion (approximately 1 cm^2) of the mantle containing mantle attachment sites. Tissues were embedded in paraffin and serially sectioned at either six or eight microns. Serial sections were stained with any of four connective tissue stains utilized, including Masson Trichrome, Picro-Ponceau with Hematoxylin, Churg and Prado Trichrome, and Mallory–Heidenhain Rapid One-Step. Preparation of stains and staining procedures followed Humason (1962, 1979). Additionally, several sections were stained with Delafield's hematoxylin and fast green. Stained sections were dehydrated in an alcohol series, cleared and mounted using a synthetic, non-yellowing mounting medium.

Lastly, mantle portions removed from *M.* (*M.*) *margaritifera* and *M.* (*M.*) *hembeli* specimens were examined for gross morphological structure using a stereo zoom binocular dissecting microscope.

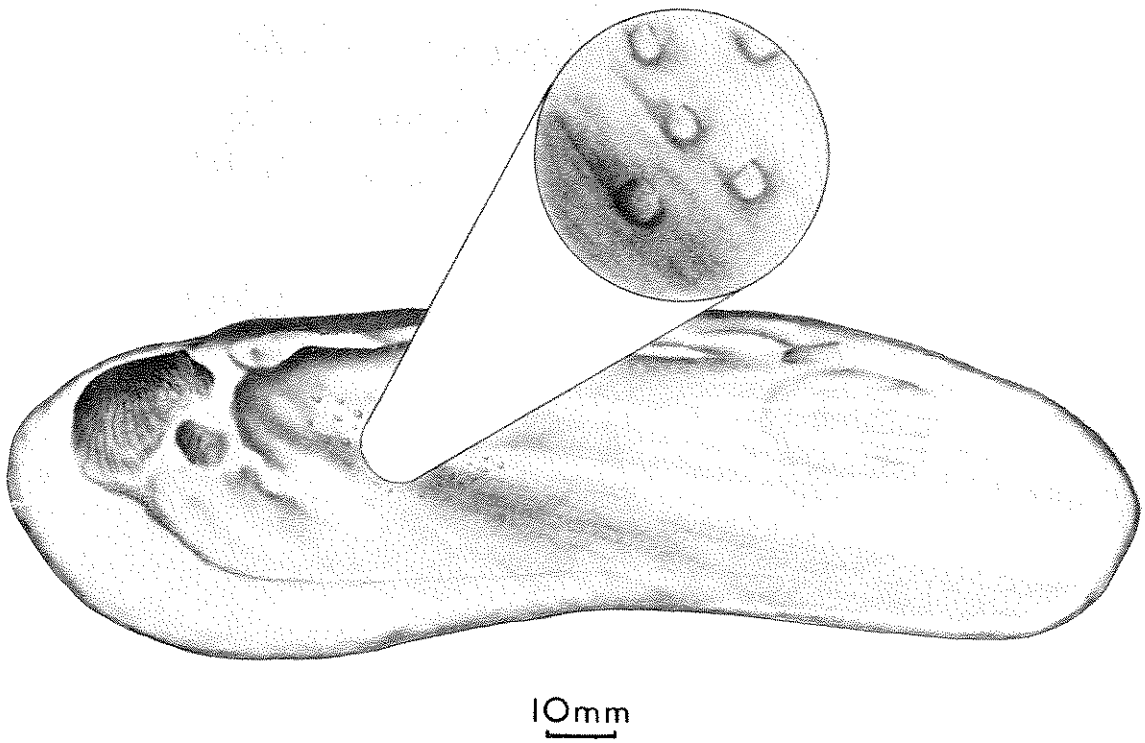

Fig. 1. Lateral view of the right valve of *Cumberlandia monodonta*. Enlargement shows outline and "trails" of individual attachment scars.

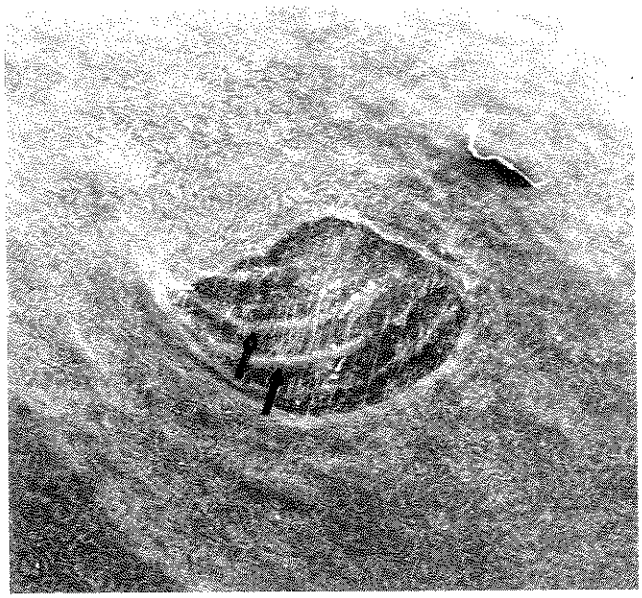

Fig. 2. SEM photomicrograph of mantle–shell attachment scar on shell of *Margaritifera margaritifera*. ×200. *Arrows* indicate ridges in scar.

Results

Mantle attachment scars in shells of examined margaritiferids are situated in an area of the shell interior (nacreous surface) approximately midway between the shell adductor muscles and range obliquely in an anterior to posterior direction away from the umbonal region (see also Pilsbry 1896). The scars appear as shallow semi-circular or circular depressions (Fig. 1). Dorsal to each depression and extending toward the umbonal cavity are faint trails, suggesting that scar position is affected by growth of the individual. Within each scar are semi-circular ridges or "annuli" (Fig. 2).

Mantle attachment scars are present on at least seven of nine known or alleged species of Margaritiferidae. Two Siberian Palearctic species, *M.* (*Margaritanopsis*) *dahurica* (Middendorff) and *M.* (*M.*) *middendorffi* (Rosen), have not been examined. The scars are well developed and numerous in Nearctic and Palearctic *M.* (*Margaritifera*) *margaritifera* and its various named races. In European Palearctic *M.* (*M.*) *auricularia* (Spengler) the scars are scarce or occasionally absent, while in the Nearctic *M.* (*M.*) *hembeli* the scars vary from being deeply impressed and abundant in Alabama River (Alabama) populations to weakly impressed and infrequent in Red River (Louisiana) populations. Shells of the Nearctic *C. monodonta* are well marked with numerous scars. Both *M.* (*Margaritanopsis*) *falcata* of the Californian Nearctic and *M.* (*M.*) *laevis* of the Siberian Palearctic typically contain scars which are often numerous; however, an occasional specimen of either species will have none. Finally, shells of the oriental *M.* (*M.*) *laosensis* (Lea), the anatomy of which remains unknown, show scars that are well impressed but few in number (but see Simpson 1896).

Examination of gross anatomy of the mantle in the region of mantle–shell attachment revealed the presence of fiber-like strands connecting the apposing walls of the mantle (Fig. 3). Connecting strands are typically singular,

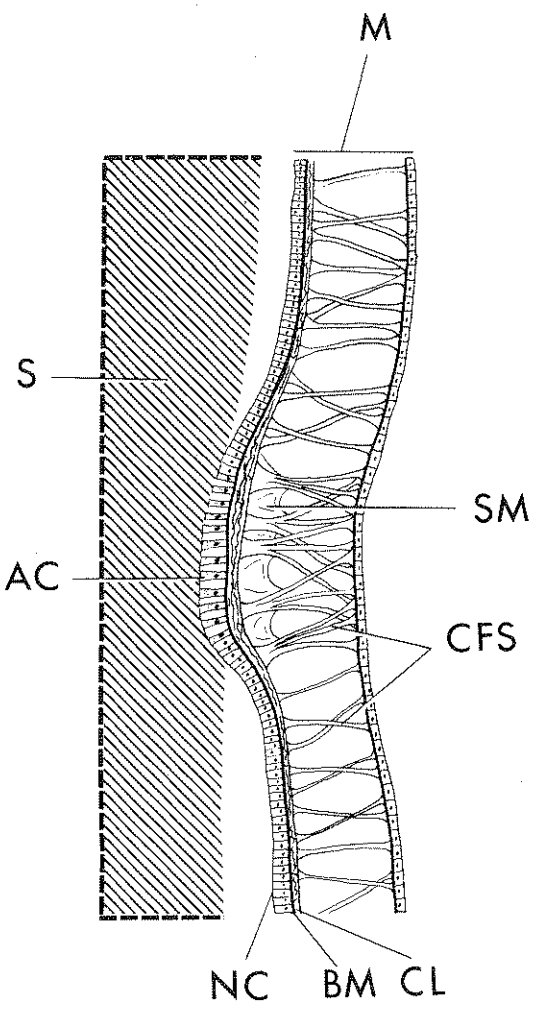

Fig. 3. Schematic diagram of mantle–shell attachment (cross section) in margaritiferids to show orientation and arrangement of tissues. Not to scale.

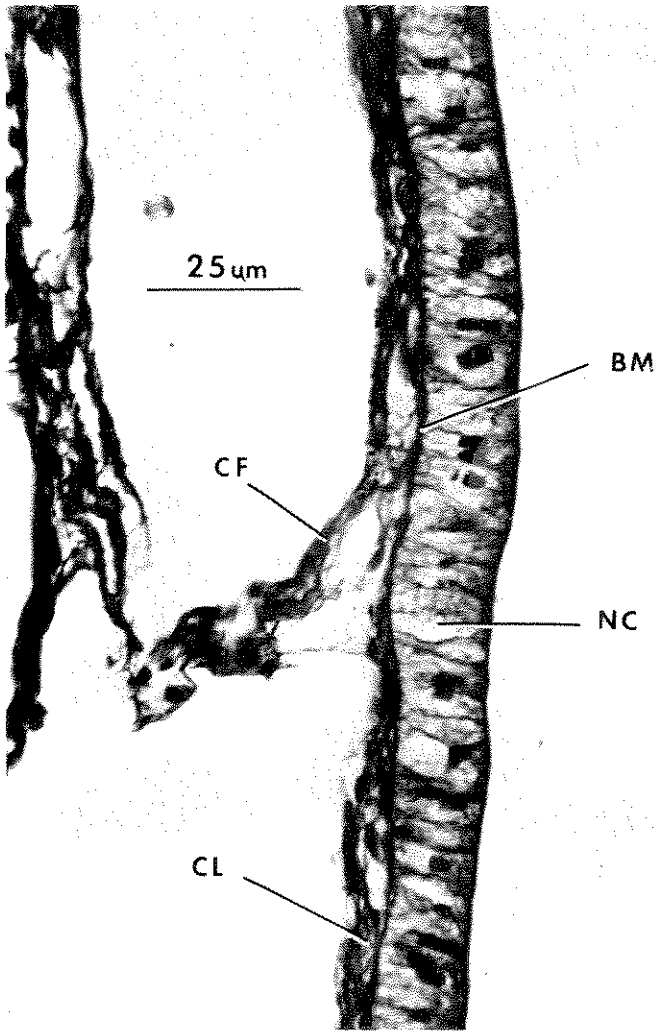

Fig. 4. Section through mantle of *Margaritifera margaritifera* in an area of non-attachment showing regular arrangement of epithelial cells and underlying connective fibers (Masson Trichrome, 8 µm).

except in the region of mantle–shell attachment sites. Here several strands unite into a common thick strand and insert at points around the periphery of the circular shell adhesion area of the mantle. Bundles of strands in the mantle–shell attachment area are enclosed in a sheath-like webbing (*SM*) that extends somewhat into the interwall space (Figs. 3 and 5). Staining shows that the strands are dense regular connective fibers. The apparent absense of nuclei in the fibers suggests that the fibers are non-muscular, an allegation supported by lack of deformation of the mantle surface during preservation without relaxation.

The crisscrossing fibrous strands insert in the basement region of the epithelial walls of the mantle. In areas of non-attachment (Fig. 4) the basement membrane (*BM*) and underlying network of longitudinal fibers (*CL*) form a strip to which the epithelial cells attach. The epithelial wall lining the mantle is composed of a single layer of tangential columnar epithelial cells (*NC*) (Fig. 4).

At sites of mantle–shell attachment, the united strands of fibers and associated sheath material insert into the connective layer beneath the basement membrane (Figs. 3 and 5). The most characteristic feature of the attachment site of the mantle is the presence of the specialized epithelial cells (*AC*) which attach to the shell. These cells have lost their tangential cohesiveness and appear as individual pillar-like cells that stain deeply with connective specifier stains (Fig. 5). Running along the longitudinal axis of the attachment cells from apex to base are intracellular fibers. The composition of these cell fibers, however, remains unknown.

Discussion

The occurrence and arrangement of mantle–shell attachment of the type described above in examined species of margaritiferids represents a unique situation within the Unionoida. Although the exact nature of attachment and fine structural details of mantle–shell adherence cells cannot be determined by the methods used in this study it is evident that certain structural modifications have been made within the mantle epithelium and underlying tissues that are associated with joining the mantle with the shell. Attachment cells show definite development for increasing longitudinal strength due to the presence of intracellular fibers. Also, fibrous strands at attachment sites on the mantle show a marked tendency for coalescence, presumably for the purpose of providing greater strength for the attachment site.

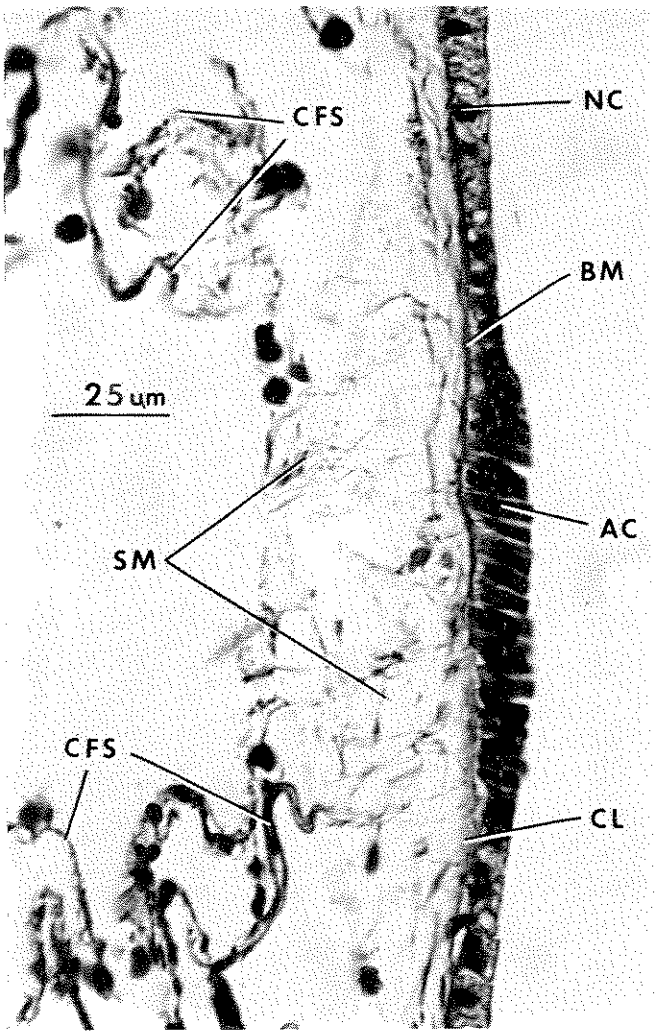

Fig. 5. Section through mantle of *Margaritifera falcata* in area of attachment. Note modified attachment cells and extensive underlying connective tissues (Churg and Prado Trichrome, 8 µm).

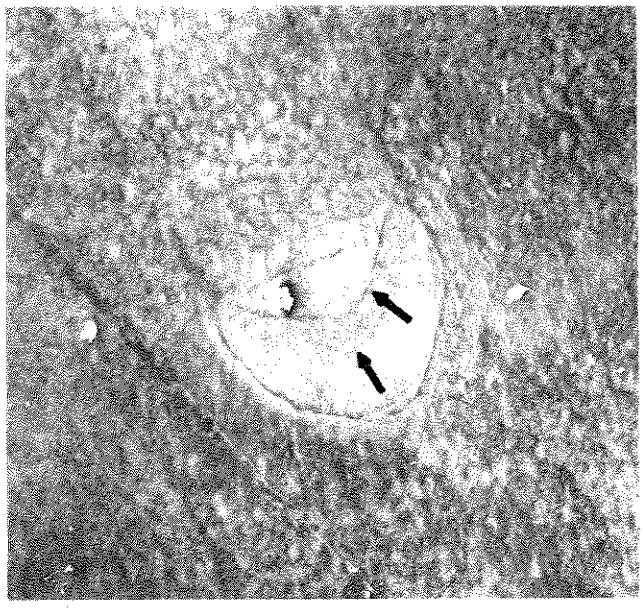

Fig. 6. SEM photomicrograph of scar on shell of *Neotrigonia* sp. ×500. Arrows indicate ridges.

Immediate questions arise, however, regarding why other unionoids lack similar mantle–shell attachments. In two genera of the Unionidae (*Amblema* spp. and *Elliptoideus* sp.) somewhat analogous attachment scars occur on the shells, but these scars differ from those in margaritiferids in their position on the shell. A single scar, which is rarely double or often absent, is closely associated with the pedal protractor muscle scar and is always located a short distance posterior to the pedal protractor scar at the distal end of the buttress supporting the anterior adductor–pedal muscle complex. In no instance do scars or scar "trails" appear on the inner (medial) disc surface of the shells of these two genera, nor any other examined unionoid species.

Although mantle–shell attachment is unique to margaritiferids among unionoid mussels, the derivation of mantle–shell attachment is probably not unique to the family. Examination of recent *Neotrigonia* spp. (Trigonioida: Trigoniidae) shows that mantle–shell attachment scars exist on the shell of these species which are very similar to mantle–shell attachment scars in margaritiferids in both position and morphology (Fig. 6). Additionally, mantle–shell attachment scars in individual shells of *Neotrigonia* spp. are much more numerous than in margaritiferid shells. If one excludes convergence, this discovery provides supplemental evidence for a phylogenetic relationship between the trigonioids and unionoids and strengthens arguments that the trigonioids constitute a bivalve stock that gave rise to early unionoids (e.g. see Cooke 1927; Newell & Boyd 1975, for discussion).

The reduction of mantle–shell attachment in margaritiferids, as evidenced by *M. auricularia* and examined species of the subgenus *Margaritanopsis*, would imply that the character is vestigial in margaritiferids. However, the complete loss of the character in other unionoids (excepting the described structures in *Amblema* spp. and *Elliptoideus* sp., which I consider to have evolved independently) suggests that, in margaritiferids, mantle–shell attachment still provides a function as a possible support mechanism for the mantle.

Conclusion

The margaritiferids are generally considered to comprise the most primitive forms of the recent Unionoida (inclusive of muteloid families) (see Smith 1979, for discussion). The persistence of mantle–shell attachment in the Margaritiferidae is interpreted here to represent a primitive character state carried over from an early evolutionary stage of the Unionoida. The absence of the character, specifically as it occurs in margaritiferids, in all other examined unionoids further evidences the primitive nature of the group.

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Abbreviations in the figures

AC	attachment epithelial cell
BM	basement membrane
CF(S)	connective fiber(s)
CL	connective layer
M	mantle
NC	normal epithelial cell
S	shell
SM	sheath material

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