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# MARSUPIAL ANATOMY OF THE DEMIBRANCH OF *MARGARITIFERA MARGARITIFERA* (LIN.) IN NORTHEASTERN NORTH AMERICA (PELECYPODA: UNIONACEA)

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## ABSTRACT

On morphological, biological and behavioral characters the freshwater mussel family Margaritiferidae generally is accepted as the most primitive of the Unionacea. Microscopic examination of the marsupial demibranch further supports this assumption, in that the interlamellar junctions lack elastic properties necessary for lateral hyperextension of the gravid demibranch. A temporary modification of marsupial anatomy in the form of a fibrous elastin accessory material is produced during the final phase of glochidial incubation. Its appearance coincides with the enlargement of epithelial cells that line the marsupium and interlamellar junctions. The material, which is situated between the glochidial mass and the inner lamellar surfaces, may provide increased aeration and support for the resting glochidia.

## INTRODUCTION

The freshwater mussel family Margaritiferidae stands apart from all other members of the Unionacea and Mutelacea primarily by its unique demibranch structures. In contrast to the Unionacea and Mutelacea, the Margaritiferidae lack true vertical, permanent, demibranch septa. Instead, they possess discontinuous, block-like interlamellar connections that are arranged in vaguely linear rows which run diagonally from a posterior dorsal position to a more anterior ventral position. In one species, *Cumberlandia monodonta* (Say), the individual interlamellar connections are joined forming solid diagonal rows. Ortmann (1911, 1912a) pointed out these differences and used them, among other morphological characters, to diagnose members within the group and to justify the distinction of the entire group as a separate family. Furthermore, Ortmann (1911) considered the family primitive in relation to other freshwater mussels. Subsequent authors (Parodiz & Bonetto, 1963; Heard & Guckert, 1970; Heard, 1974), building on Ortmann's anatomically-based system, also relegated the family to a primitive position in freshwater mussel phylogeny. Unfortunately, however, no anatomical investigations, except those concerning hermaphroditism (Heard, 1970; van der Schalie, 1970), have accompanied contemporary classificatory assessments of the family. Consequently systematists must still rely on Ortmann's studies (1911, 1912a, 1912b, 1913) for interpretation of the various components of margaritiferid anatomy.

In addition to morphological characters which supposedly establish the family's systematic position, behavioral and biological features further suggest a primitive standing for the group. These include a short-term breeding period (tachytictic strategy) and use of all demibranchs as non-elastic marsupia. Classically, strict adherence to these characteristics has been assumed for the Margaritiferidae, presumably based on the morphological homogeneity of the species within the family. However, modifications in reproductive patterns in certain populations of *Margaritifera margaritifera* (Lin.) in northeastern North America have recently been shown (Smith, 1976) wherein animals deposit eggs into demibranchs in the late summer, rather than the spring. The latter strategy is supposedly typical of tachytictic species (Heard & Guckert, 1970). In addition the animals retain mature glochidia within the demibranchs until some unknown environmental condition is met, thereby initiating release. This is suggestive of an abbreviated long-term breeding period (bradytictic strategy) as discussed by Heard & Guckert (1970). These adjustments to the reproductive biology of *M. margaritifera* have been accompanied by physical changes within the marsupial demibranch during the reproductive period.

The present paper attempts to provide a detailed analysis of the morphological characters of the marsupium of northeastern North America *M. margaritifera* during the various reproductive periods, and in so doing, indicates further modifications that have evolved within the populations studied.

Prior studies complementing the present research include descriptions of anatomical features of the gravid demibranch and its contents in various species of Margaritiferidae (Harms, 1907; Ortmann, 1913; Howard, 1915; Murphy, 1942; Smith, 1976). These are supplemented by a single cursory microscopic examination of the barren demibranchs of *M. margaritifera* by Ortmann (1911).

## METHODS AND MATERIALS

All preserved material relevant to this study is maintained in the invertebrate division of the Museum of Zoology, University of Massachusetts at Amherst. Samples of animals from which descriptions of the gravid demibranch were taken represent nine populations within the Connecticut River system in Hampshire and Hampden Counties, Massachusetts.

Three phases of reproductive activity were selected for examination: the barren phase when the demibranchs were devoid of embryos, an intermediate gravid phase, when the glochidia were in the demibranchs and well developed morphologically but were still provided with embryonic tissue and encased in egg shells, and an advanced gravid phase indicated by maturity of the glochidia and the loss of much egg shell material.

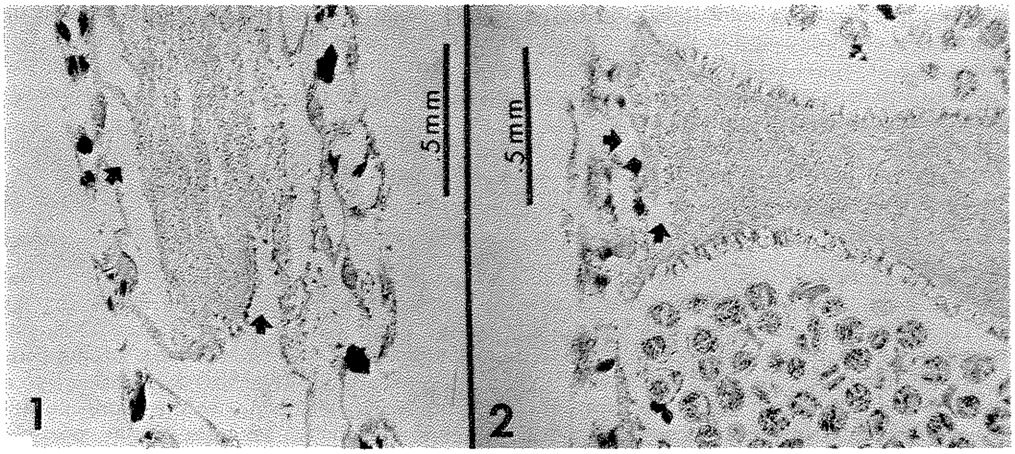
Each phase was studied by means of serial cross-sections through the demibranch of selected characteristic individuals. The specimens used in analysis were as follows: UMA //MO. 683, Cushman Brook, Amherst, Hampshire Co., 14 July 1973, length 92 mm, barren phase; UMA //MO. 683, same locality, 3 Sept. 1974, length 105 mm, intermediate gravid phase; UMA //MO. 895, Fort River, Amherst, Hampshire Co., 29 Sept. 1976, length 101 mm, advanced gravid phase; UMA //MO. 745, Joe Wright Brook, Northampton, Hampshire Co., 30 Sept. 1974, length 81 mm, advanced gravid phase.

Samples were removed from the central portion of the inner demibranchs, imbedded in paraffin and sectioned at 8µm. All barren and intermediate demibranch sections were stained with Harris's hematoxylin and eosin, and advanced gravid demibranch sections were stained with Harris's hematoxylin and eosin or Mallory's triple connective tissue stain following Pantin's formula (in Humason, 1962). Additionally, several advanced gravid sections were stained with orcein dye (1.0 and 0.4%). Sections were studied using light and phase contrast microscopy. Scales for histological sections were determined by a calibrated ocular micrometer.

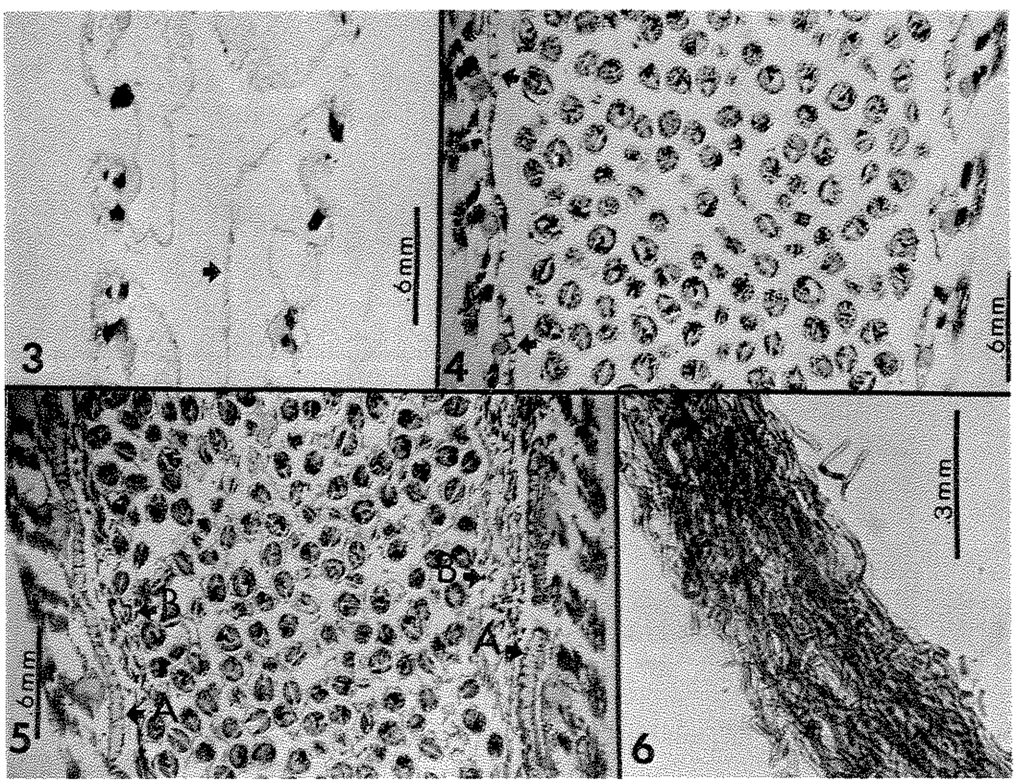
## RESULTS

The interlamellar junctions are solid, cellular extensions of the lamellae that vary in size and shape. Each junction is composed of a vascularized matrix of variously-shaped connective parenchymal cells. Dispersed throughout the tissue (except in some internal portions) are blood lacunae and fibrocytes. The entire outer margin is lined with a layer of epithelial cells. Just internal to the basal lamina of the epithelium, and variably abundant depending on reproductive phase, are bundles of parallel collagenous connective fibers which generally run laterally along the axis of the junction and which functionally provide supplemental strength for the tissue. Their degree of development is most probably regulated by the adjoining fibrocytes.

In the barren demibranch the interlamellar junctions assume a 'bellows''-like shape created by serial folding of the relaxed tissue (Fig. 1). During this period the matrix cells are spacious and thin-walled while the epithelium is reduced to a thin squamosal layer. The collagenous fibers, as shown by staining, are contracted and aggregated along the tissue margin into wavy bundles that barely extend into the cell mass. However, during the incubation period, when the demibranchs are full of developing glochidia, the junctions are elongated and unfolded, except in areas adjacent to the lamellae where some pocket-like folds persist (Fig. 2). Even at maximum extension, the junctions create constrictions at the point of connection with the lamellae. The swollen lamellae surrounding the areas of junction attachment account for the "quilted" appearance of the gravid demibranch (Smith, 1976). Corresponding with the extension of the junctions, an overall increase in the




Figs 1-6. 1-5, cross sections of demibranchs of *M. margaritifera*. 1, barren demibranch showing folded condition of relaxed interlamellar junction (arrow). 2, gravid demibranch with expanded interlamellar junction. Residual pockets shown by arrows. 3, barren demibranch with reduced epithelial cells (arrow). 4, intermediate gravid demibranch with epithelial cells (arrow) commencing to enlarge. 5, advanced gravid demibranch with enlarged epithelial cells (A) and accessory material (B). 6, Isolated portion of accessory material.



cytoplasmic density of the internal connective cells is seen, as evidenced by the deep staining properties. Contrasted against the dense connective cells are many small clear, blood lacunae. The collagenous fibers are enlarged and elongated, extending far into the matrix where they become less organized and somewhat crisscrossed. Along the margins they lose their bundled, wavy appearance and become separated and straightened.

During the gravid period, the epithelial cells lining the junction undergo a change from the simple, thin squamosal form to a much expanded, thick, pseudostratified columnar form. In the latter condition they appear to be secretory, as their apices are covered with mucoid-like granules or droplets.

The inner surfaces of the lamellae of each demibranch are lined with a single layer of epithelial cells. In the barren reproductive phase, these cells are squamous and thin-walled, as observed in the interlamellar junctions (Fig. 3). However, during the intermediate and advanced gravid phases (Figs 4-5) the lamellar epithelium becomes transformed in both shape and apparent function. These cell changes are concordant with those of the interlamellar junctions, but become most pronounced during the advanced gravid phase, when the glochidia have matured. The epithelial cells swell to a cuboidal form but remain in a single, uniform layer. The nucleus and cytoplasmic components are generally restricted to the basal region and the remaining cell portion becomes opaque, possibly from the accumulation of fluid. During this phase a mucoid-like granular coating is distributed over the cell apices which suggests that these cells also perform a secretory function.

Also coincident with the transformation of the epithelial cells is the appearance of a brown accessory material that is laid down between the inner lamellar surface of both lamellae and the

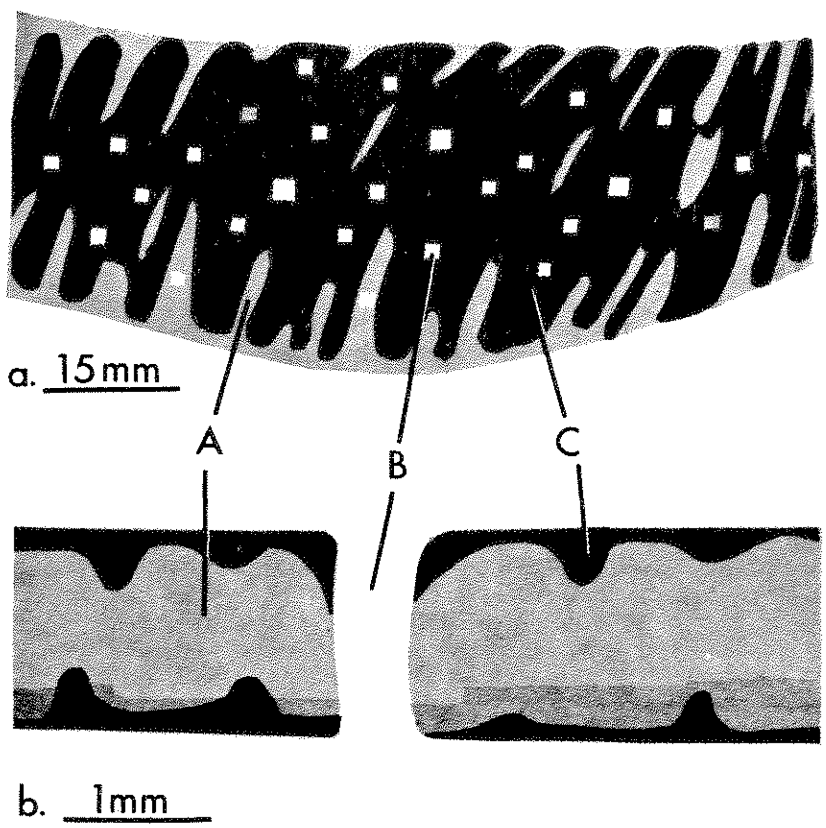



Fig. 7. Portions of glochidial mass removed from demibranch. a, lateral view; b, longitudinal view. A, glochidial mass; B, space for interlamellar connection; C, accessory material.

glochidial mass (Fig. 5). The presence of accessory material within the gravid demibranch was noted by Smith (1976) but without elaboration. The material is distributed only throughout areas of the lamellar surface that are in contact with the glochidia. In its entirety, an individual "plate" of material is composed of a connected series of oblique thickened portions of a fibrous mesh-like tissue. In lateral aspect these thickened sections are joined by thinner sheets of the same material, but in certain areas clefts and gaps are evident due to the total absence of material (Fig. 7a). In longitudinal plane, the thickened sections of the material extend into the glochidial mass, collectively simulating true demibranch septa (Fig. 7b).

Gross examination of the brownish material reveals a matrix of entwined, sinuous and variously anastomosing fibers (Fig. 6). Mallory's triple stain produced a blue color within the material indicating the presence of either collagenous or elastic connective tissue. Histochemical analysis was not performed and the exact nature of the material is unknown. However, staining with orcein dye at various strengths showed a strong color reaction implying that the material is probably composed of elastin fibers.

## DISCUSSION

Examination of the anatomical properties of the demibranch of *M. margaritifera* during three reproductive phases reveals modifications undertaken as the marsupial tissues assume incubative responsibilities. Egg deposition accounts for an approximate three fold increase in the width of the demibranch. This enlargement is controlled by the interlamellar junctions which expand from a relaxed condition to a finite state of maximum extension. Prevention of over-extension is most likely assisted by the collagenous fibers within the junction. The junctions, therefore, act in a bellows fashion with cellular elements of the tissue adjusting their linear dimensions rather than actually stretching. This apparent lack of elasticity is contrasted to its presence in all other freshwater unionacean mussels. Increased elastic properties of the marsupial demibranch have been considered an indication of advancement in freshwater mussels: Ortmann (1911) and Fuller (1972, 1973) suggest that elasticity in complete, vertical demibranch septa increases the marsupial capacity by permitting the gills to stretch considerably beyond their normal size. The inability of *M. margaritifera,* and possibly other margaritiferids, to do this may reflect the overall weakness of separate, patchy interlamellar junctions which are probably incapable of structurally sustaining a much larger glochidial mass.

The sequence of changes in epithelial cell morphology and, particularly, the appearance of accessory materials during the advanced reproductive phase suggest evolutionary alterations to increase marsupial incubative capabilities. Transformation of epithelial cells presumably coincides with the adoption of a secretory function. This is evidenced by the production of mucoid substances which would facilitate expulsion of the glochidial mass and accessory material by forming a functional viscous coating reducing resistance along the cell surfaces. However the connection between the epithelial cells and the production of the accessory material remains unclear. Unfortunately, due to the increment method of analysis, gaps are present that may have shown transitional stages including ground substance formation. It is, therefore, impossible at present to determine the actual derivation of the material. However, it is known to be present only in the final stages of incubation during the short glochidial "withholding" period (Smith, 1976). In light of its short tenure within the demibranch it is presumed that the function of the material is in response to the needs of the mature glochidia. These may include increased aeration, provided by the material's porous nature, and partial separation of the glochidial mass into subgroups as occurs in freshwater mussels with permanent, continuous interlamellar septa. In consideration of the animal's upright orientation in the substratum, separation of the glochidial mass into subgroups would diminish settling and resultant crowding of the glochidia.

The material may also provide a structural support acting as a connective brace between the glochidial mass and the lamellar surface. As demonstrated in other animals, loose elastin connectives often lie between tissues and assist in maintaining their proper relative position to each other (Bloom & Fawcett, 1975). Structural integrity in the gravid demibranch of *M. margaritifera* would become crucial later in the advanced reproductive phase when the glochidia have completed shell formation. The added weight of concentrated glochidial shell material would subsequently increase stress on the enclosing lamellae.

## SUMMARY

1. The interlamellar junctions of the margaritiferid demibranch are solid cellular structures composed of parenchymal cells and collagenous fibers and fibrocytes, and lined with a layer of epithelial cells.
2. The interlamellar junctions of the barren demibranch are relaxed and serially folded into a "bellows" shape. The collagenous fibers are wavy and grouped into bundles.
3. In the gravid demibranch the interlamellar junctions are elongated and unfolded. The collagenous fibers straighten and elongate and lose their bundled appearance. The epithelial cells enlarge to columnar form and appear to become secretory as mucoid substances accumulate at their apices.
4. Epithelial cells line the inner lamellar walls. As in the interlamellar junctions these cells transform from a squamosal shape in the barren phase to a columnar shape in the gravid phase and appear to become secretory.
5. During the advanced gravid phase accessory material is laid down between the glochidial mass and the lamellar wall. The material is composed of meshed fibers which aggregate into a plate with vertical thickened portions extending into the glochidial mass.
6. Staining with Mallory's triple stain and orcein indicates that the material is probably elastin.
7. The margaritiferid demibranch expands laterally during incubation of glochidia, the interlamellar junctions, however, are incapable of stretching as in other unionacean mussels.
8. The exact origin of the accessory material is unknown, but it is probably derived from epithelial secretions.
9. The accessory material may assist in separating patches of glochidia thus facilitating aeration, as in unionaceans with true vertical demibranch septa, and may further provide additional support for the gravid demibranch.

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