

Shell Microstructure of the Patellid Gastropod *Collisella scabra* (Gould): Ecological and Phylogenetic Implications

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Abstract. Shell microstructure has a long history of use in both taxonomic and ecological research on molluscs. I report here on a study of the microstructure of *Collisella scabra*, also known as *Macclintockia scabra* and *Lottia scabra*. I used a combination of SEM and light microscopy of acetate peels, whole shells, and shell fragments to examine the shell layers and microstructure. Regular growth bands were not present in most shells examined. Several shells showed multiple bands of myostracum, which indicate periods of extreme rates of size change, and may be evidence of abiotic stress. This study also suggests that shell layers previously described as “modified foliated” are “irregular complex crossed lamellar,” with both fibrous and foliated second order structures. The presence of fibrous shell structures, in addition to other shared morphological characters noted by previous authors, suggests an affinity with the Lottiidae rather than the Nacellidae.

INTRODUCTION

Shell microstructure has a long history of use in both taxonomic and ecological research on molluscs. Shell growth bands are commonly used as records of individual growth (Frank, 1975; Hughes, 1986; Arnold et al., 1998) and for reconstructing environmental conditions (Rhoads & Lutz, 1980; Jones, 1981; Kirby et al., 1998). Shell microstructure has also been used for taxonomic identification (Kennish et al., 1998) and as evidence of shared ancestry in systematic work (Kool, 1993; Schneider & Carter, 2001). In revising the systematics of the patellogastropod genus *Collisella*, Lindberg (1986) used shell microstructure characters described by MacClintock (1967) to place *Collisella scabra* in a separate family from the rest of the species in the genus. Although Lindberg never formally published a new taxonomy for this species (see Lindberg, 1986), Kozloff (1987, 1996) contains Lindberg’s intended systematics and the name *Macclintockia scabra* has appeared in the published literature (e.g., Smith et al., 1993). More recently, Fuchigami & Sasaki (2005) re-examined the shell microstructure of 44 patellogastropod species, and updated several of MacClintock’s (1967) original descriptions, including *C. scabra*. In this study I reexamined the microstructure of *C. scabra* with two goals: 1) to explore the use of shell growth patterns as a way to age individual *C.*

scabra shells, and 2) to verify the earlier shell microstructure descriptions, including phylogenetic implications.

MATERIALS AND METHODS

Collection and Preparation of Shells

I collected 10–20 snails from each of the three field sites listed in Table 1. All three sites are rocky intertidal benches on semi-protected outer coast (*sensu* Ricketts et al., 1985). At each site, I collected snails from primarily wave sheltered areas, such as surge channels or the wave protected sides of outcrops or ridges. I specifically searched for snails with a minimum of shell erosion and collected a range of sizes at each site. In the lab, I dissected each snail from its shell.

Whole Shells

To examine the muscle scar and adjacent layers on the inner surface of the shell, I soaked several shells in 5% sodium hypochlorite for one hour and then rinsed them under running water. I then examined the inner surface of these shells under a dissecting microscope. To determine the mineral structure of the shell layers exposed on the inner surface, I treated two shells with Feigl Stain (Feigl, 1937; Friedman, 1959), which stains aragonitic areas black, but leaves calcitic areas unchanged. I also used three of these bleached shells in scanning electron microscopy (see below).

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Table 1
Location of samples collected for analysis.

Collection Site	Location	Collection Date	Number of snails	Protocol
Fort Bragg, California (MFB)	39.282°N, 123.803°W	05/07/01	10	Acetate
		06/06/01	12	Feigl Stain, SEM
Shelter Cove, California (SCV) Devil's Gate, California (DVG)	40.022°N, 124.074°W 40.407°N, 124.390°W	05/06/01	12	Acetate
		06/09/01	15	Acetate, SEM

Acetate Peels

Shells for acetate peels were embedded in clear epoxy resin (EPON 828 resin and DTA hardener, Miller – Stephenson, Danbury CT), and sectioned through the apex along the midsagittal plane using an Isomet low speed saw (Buehler Ltd., Lake Bluff, IL). I ground and etched the sections and made peels following Carter & Ambrose (1989). I used 2 mm thick acetate for the peels, which reduces problems with curling and tearing commonly reported with much thinner acetate (Carter & Ambrose, 1989). After 12–24 hr, I examined peels under a compound microscope at 25× to 250×. Photographs of specimens were made with a 35 mm camera body mounted onto the microscope.

Electron Microscopy

I examined whole shells, shell fragments, and epoxy embedded sections with scanning electron microscopy (SEM). Whole shells and shell fragments were previously bleached as described above and air dried for several days before use. Shell fragments were generated by gently tapping a whole shell with a hammer. Three embedded specimens were selected from those used for the acetate peels. These were polished with aluminum oxide and re-etched in 5% HCl for 15 sec before SEM. All samples were prepared for SEM following Carter & Ambrose (1989). Once thoroughly air-dried, they were mounted onto specimen support stubs using silver suspension paste (Ted Pella Inc., Redding, CA) and sputter-coated with gold (Pelco Model SC-7, Pelco International, Redding CA). Samples were viewed in a Philips XL 30 Scanning Electron Microscope (FEI Co., Hillsboro OR) operated at 10 KV.

RESULTS

I identified four layers in the shell of *C. scabra* (Figure 1). Feigl staining revealed the muscle scar (myostracum) to be aragonite and the remaining shell layers, calcite. Following MacClintock (1967), I identify these layers by their position relative to the myostracum. Layers interior to the myostracum are labeled with negative numbers, and shell layers exterior to the myostracum are labeled with positive numbers (Figure 1). Unless otherwise indicated all terms used in

describing shell structures follow the definitions in Carter et al. (1990).

The myostracum is a narrow band of irregular simple prismatic structure (Figure 2). Adjacent and exterior to the myostracum is the $m + 1$ layer (Figure 2), a very narrow band of branching crossed lamellar structure. This layer is rarely visible in acetate peels, and much more visible in the SEMs or by direct examination of the interior surface of bleached shells under a dissecting microscope.

The majority of the shell consists of the $m + 2$ and $m - 1$ layers, which contain a similar first order structure (Figures 2–5), best described as irregular complex crossed lamellar (Carter et al., 1990). SEM revealed the second order structure to be highly variable, consisting of long fibers (i.e., "fibrous prismatic," Figure 5) of varying width that grade into foliated sheets (i.e., "regularly foliated," Figure 4). MacClintock (1967) indicated a possible $m + 3$ layer, but did not describe it. In general, the shells examined

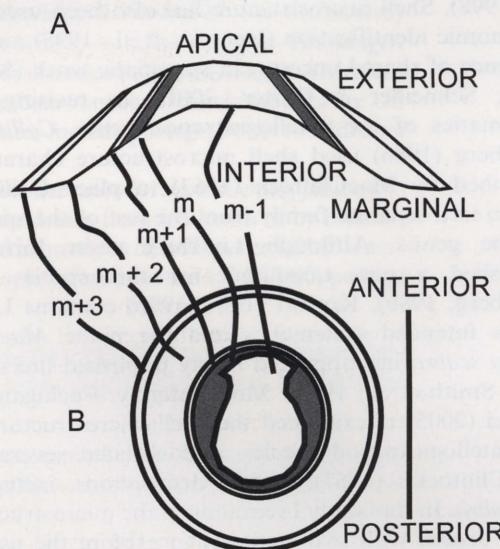


Figure 1. Diagram of shell layers in *C. scabra*. (A) Cross-section of shell along sagittal axis, after MacClintock (1967, Figure 1). (B) Shell layers as viewed on the ventral surface of the shell, after MacClintock (1967, Figure 82). The shaded area indicated by "m" is the myostracum (muscle scar).

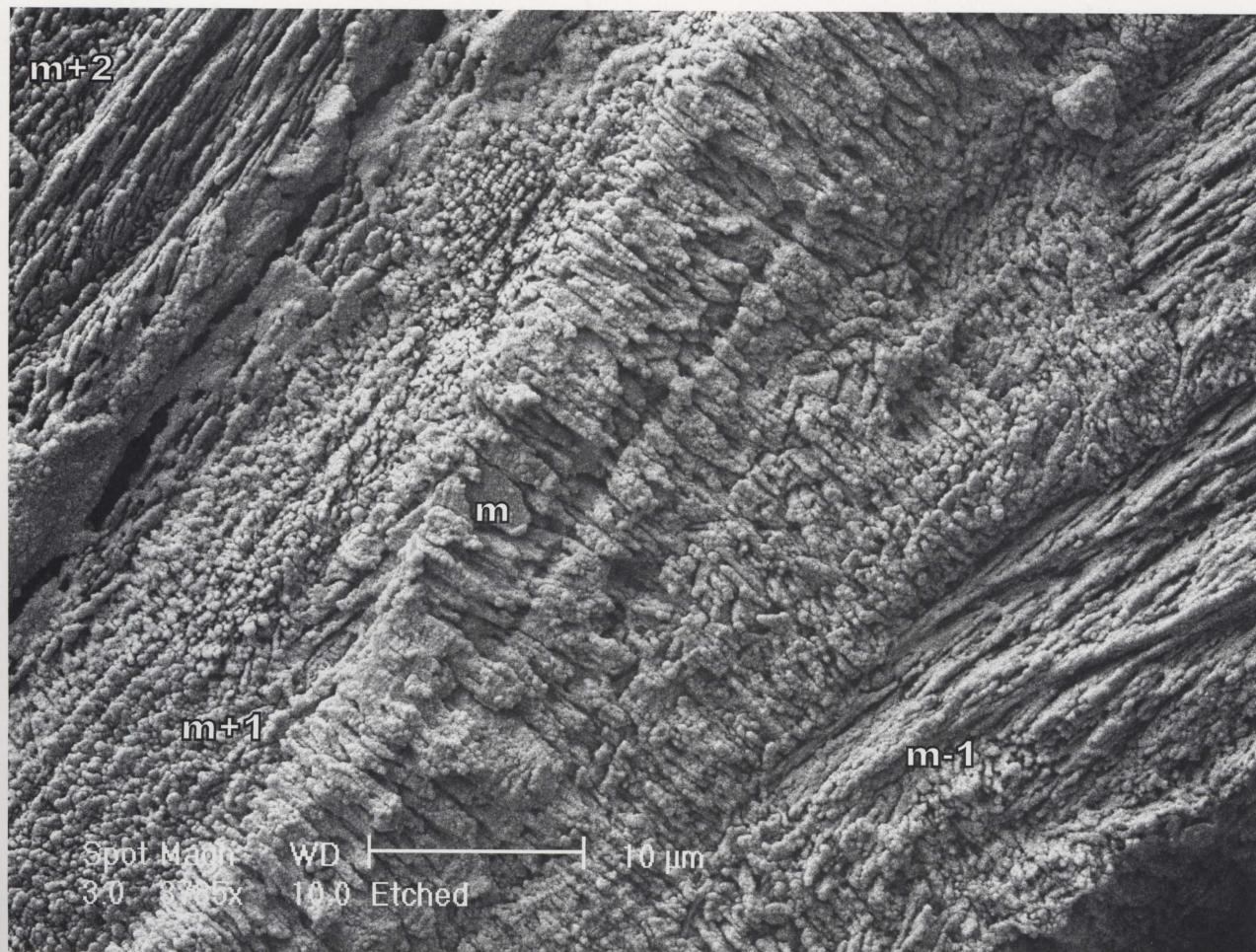


Figure 2. SEM of sagittal section of sample DVG 2A. The shell was embedded in epoxy and sectioned before SEM. Shell layers are labeled as in Figure 1. The myostracum ("m") is visible as a diagonal band of simple prismatic structure. The narrow "m + 1" layer is visible just to the left of the myostracum. "m + 2" in the upper left and "m - 1" in the lower right both are irregular complex crossed lamellar. Scale bar is 10 micron.

for this study were too highly eroded to determine if an $m + 3$ layer existed.

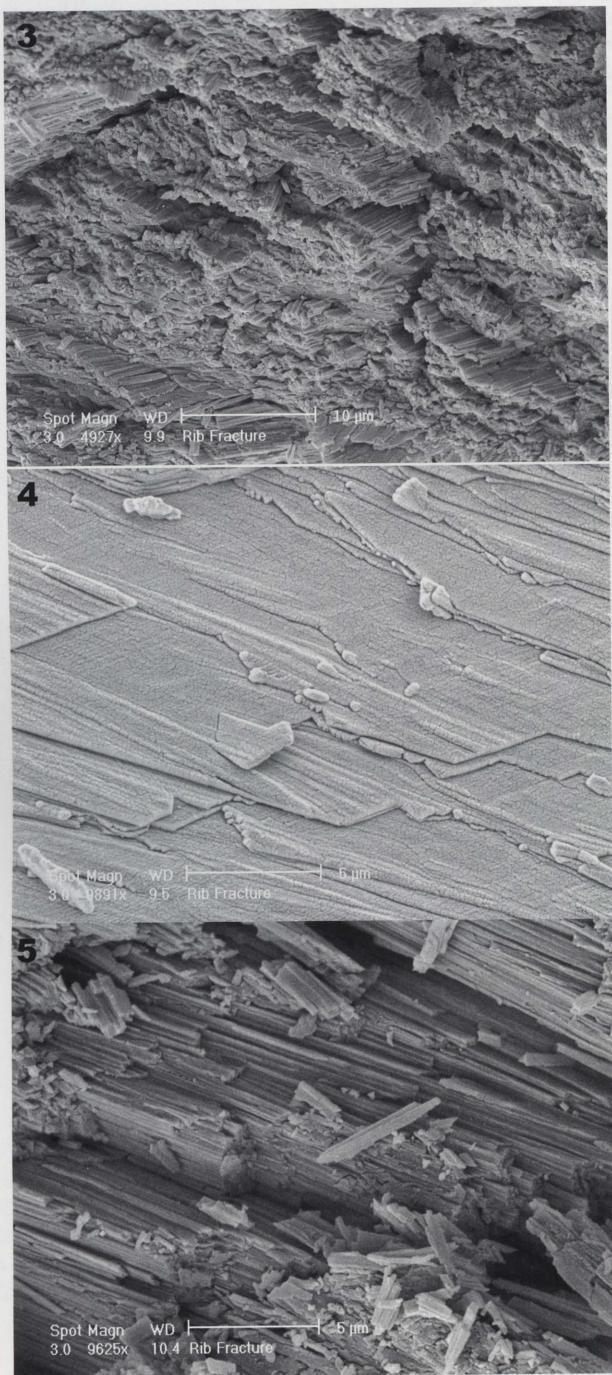
Because of the irregularity of the $m + 2$ and $m - 1$ layers, it is difficult to see consistent growth lines. Growth discontinuities (i.e., "growth bands") are occasionally observed in acetate peels (Figure 6), although they were apparent only in portions of shell cross-sections and never consistently across the entire shell. Other shells had no bands at all (e.g., Figure 7). The banding appears to be caused by changes in the orientation of the higher order structures (e.g., $m - 1$ layer of Figures 2 and 9).

Irregularities were also present in the myostracum. In most shells, the myostracum consisted of a single band that expanded with the growth of the shell; however, in some samples there appears to be more than one band of myostracum and/or the myostracum appears to double back on itself (Figures 8–9).

DISCUSSION

The goals of this study were two-fold: 1) to explore the use of shell growth patterns as a way to age individual *Collisella scabra* shells, and 2) to verify earlier descriptions and their taxonomic implications. Shell growth bands are common in other mollusks and are often used in fisheries research to age individuals (Rhoads & Lutz, 1980). Banding is occasionally evident in *C. scabra*, but generally only in larger individuals and not throughout the entire shell. Most shells showed no growth bands at all. The bands appear to be formed by changes in the orientation of the 2nd order elements of the $m - 1$ and $m + 2$ layers. It is unclear how such orientation changes might relate to annual or seasonal patterns of growth. Thus, visual shell bands are unlikely to be useful for aging individual *C. scabra*.

More intriguing is the occasional observation of



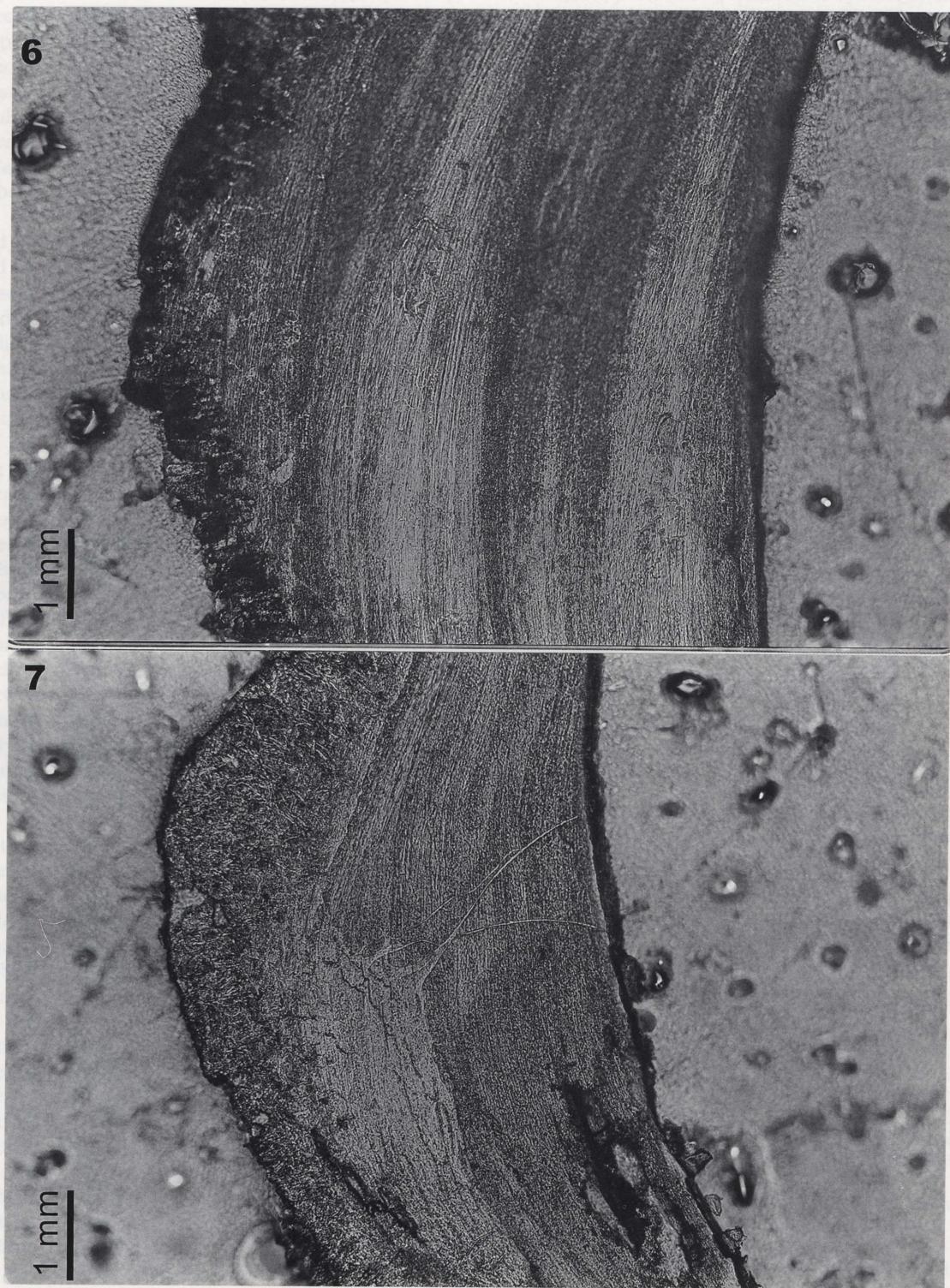
Figures 3–5. Secondary structure of the $m + 2$ and $m - 1$ layers revealed by SEM of fractured shells. (3) Fracture along a transverse axis showing cross sections of fibers. The structures vary from rod like at the upper part of the image to lath or foliated at the lower portion. Scale bar is 10 microns. (4–5) Details of secondary structure from other locations of the same shell. Scale bar is 5 microns. (4) Foliated sheets, (5) blades or laths.

multiple bands of myostracum within a single shell (Figure 5). MacClintock (1967, plate 7) reported a similar pattern in the shells of *Lottia gigantea*, which he refers to as evidence of “an earlier extended period.” The multiple bands likely occur when changes in the size of the animal (shrinkage or expansion) are rapid relative to the rate of growth of the rest of the shell; and suggest that individuals may be repositioning themselves within the shell over their lifetime, possibly as the result of starvation.

Multiple bands were usually only observed in the largest shells examined. These individuals occupy the highest vertical distribution of the species in the intertidal (Sutherland, 1970; Haven, 1973). Such areas show extreme seasonal patterns in food supply, and long periods of starvation are likely (Sutherland, 1970; Cubit, 1984). Sutherland (1970) observed a seasonal loss of body mass in these snails, which might change the position of the body within the shell and relocate the myostracum to a more apical position. This is very different from the breaks reported in other mollusks, where shell growth stops during reproduction (Rhoads & Lutz, 1980). In general, the changes in the position of the myostracum did not appear frequently enough to suggest a seasonal or annual pattern of size change; and thus are unlikely to be useful as measures of individual age. However, they might be useful as evidence of extreme environmental stress over longer time intervals.

Perhaps more importantly, the description of shell structure for *Collisella scabra* differs materially from both the original description by MacClintock (1967) and the more recent description of Fuchigami & Sasaki (2005), and the phylogenetic position of this species should be reconsidered. At the time of MacClintock’s work, *Collisella scabra* was placed within the Acmaeidae, but was removed by Lindberg (1986) as part of a larger revision of the northeastern Pacific Acmaeidae. Although Lindberg never formally published a new taxonomy for this species, he intended to place it in the Family Nacellidae, genus *Macclintockia* (Kozloff, 1987, 1996; Lindberg pers. comm.). Because Lindberg’s (1986) reclassification of the northeastern Pacific species of Acmaeidae was heavily influenced by MacClintock’s (1967) work, the redescription of *C. scabra*’s shell microstructure warrants a reexamination of its phylogenetic position.

My observations of *Collisella scabra*’s shell microstructure differ from MacClintock’s (1967) earlier description in both the number of shell layers described and their composition. I identified five of the six shell layers mentioned in MacClintock (1967); but, like Fuchigami and Sasaki (2005), I was unable to identify an $m + 3$ layer. The shells I examined may have been too highly eroded to retain the $m + 3$ layer. Within the



Figures 6-7. Acetate peels of the apex of two different samples viewed under a compound microscope. (6) Sample DVG 5A, banding is clearly visible. (7) Sample SCV 11A, no bands visible.



Figures 8–9. SEM of an etched sagittal section, posterior slope of sample CPM 5A. (8) Two separate layers of myostracum are indicated by arrows. The black box indicates the area represented in B, scale bar is 100 micron. (9) Enlargement of A, scale bar is 20 micron. Two intersecting bands of myostracum are clearly visible along the diagonal.

five layers I observed, I also identified different primary and secondary structures. In particular, MacClintock (1967 p. 76) considered the $m + 2$ and $m - 1$ layers to be "modified foliated or possibly modified fibrillar." Fuchigami & Sasaki (2005) identified the $m - 1$ layer as "irregular complex crossed foliated" and the $m + 2$ layer as "irregular fibrous foliated." I have identified both these layers as "irregularly complex crossed lamellar." Also, both MacClintock (1967 p. 76) and Fuchigami & Sasaki (2005) labeled the $m + 1$ layer as "concentric crossed lamellar," while I describe it as "branching crossed lamellar."

These differences are due to both terminological and methodological differences between the two studies. MacClintock (1967) used primarily thin sections and examination of whole shells to diagnose shell layers; whereas, acetate peels and SEMs provide much better detail of structure. Additionally, descriptive terminology of skeletal microstructure remains highly variable among researchers (Carter et al., 1990), and many of the terms used by MacClintock (1967) are not commonly in use today. In the case of the $m + 1$ layer, all three studies identified a crossed lamellar structure. MacClintock (1967) and Fuchigami & Sasaki (2005) described it as "concentric," referring to the orientation of the first order structures relative to the shell margin. This orientation is consistent with the definition of "branching crossed lamellar" (Carter et al., 1990).

MacClintock (1967) termed the primary structure of both the $m + 2$ and $m - 1$ layers "modified foliated or possibly modified fibrillar," which is not a commonly used term in shell microstructure studies. Based on MacClintock's (1967) description of these layers, Carter et al. (1990 p. 649) considered that they were likely to be either "fibrous prismatic" or "irregular complex crossed lamellar." Fuchigami & Sasaki (2005) described separate microstructures for the two layers. I have identified microstructures similar to each of Fuchigami & Sasaki's (2005) structures. Their "irregular fibrous foliated" is similar to Figure 4 of this study, and their "irregular complex crossed foliated" is similar to Figure 5. However, I observed these two structures intergrading within the same shell layer (Figure 3). I have described both layers as "irregular complex crossed lamellar" based on the definition of (Carter et al., 1990), which encompasses second order structures ranging from fibers to planar lamellae (i.e., sheets). Carter et al. (1990) consider "irregular complex crossed foliated" a variant of "irregular complex crossed lamellar" specific to foliated secondary structures. Thus I have described both the $m - 1$ and $m + 2$ layers as "irregular complex crossed lamellar" with a variable secondary structure.

MacClintock (1967) considered foliated shell structures to be characteristic of the Patellidae and absent in the Acmaeidae. Thus he concluded that *C. scabra* was

"completely unrelated to all other species of the family Acmaeidae" (MacClintock, 1967 p. 82) because of the "modified foliated" structures found in its $m + 2$ and $m - 1$ layers. This study demonstrates that, although calcitic, the second order structure of these layers varies from fibrous prismatic to foliated. Because they appear distinct from any other microstructures observed in the Lottiidae or Patellidae, they are autapomorphies of *C. scabra*, and provide no phylogenetic information.

C. scabra shares many other morphological characters, including gill and radular morphology, with the Lottiidae that are not common to other patellogastropod families (MacClintock, 1967; Lindberg, 1981). Furthermore, molecular analyses (Simison, 2000; Simison & Lindberg, 2003) also support a hypothesis of shared ancestry between *C. scabra* and members of the genus *Lottia*. Thus it is likely that *C. scabra* belongs, with its former congeners, in the genus *Lottia*, family Lottiidae.

Acknowledgments. This research was supported by NSF grant OCE-9906741 to R. Grosberg and by a DOE Graduate Research Environmental Fellowship to S. Gilman. I am indebted to J. Carter for sharing his wealth of knowledge on shell microstructure, acetate peels, and microscopy. SEM work was conducted in the Department of Medical Pathology at UC Davis, with technical support from K. Butler-DeRose. J. Carter, R. Grosberg, M. Frey, G. Herbert, and G. Vermeij provided many helpful comments on earlier drafts of this manuscript.

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