AN ABSTRACT OF THE THESIS OF Sara L. Persselin for the Master of Science in Biology presented May 8, 1998.

Title: The Evolution of Shell Windows within the Fraginae (Bivalvia:Cardiidae) and the Origin of Algal Symbiosis in Cardiids.

Approved: _____
Gustav Paulay, Chairman, Thesis Committee

Several cardiid clams in the subfamily Fraginae were investigated to determine the prevalence of shell windows within the Fraginae and to ascertain the origin and evolution of windows within the subfamily. The results were then used to establish an origin of photosymbiosis within the Fraginae. Only one genus, *Corculum,* was previously known to form microstructural windows. Shells of 15 species were sectioned and etched and the resulting acetate peels were observed with a light microscope. Microstructure indicative of window formation was found in *Fragum mundum, Fragum nivale,* and *Lunulicardia* sp. 1. All contain symbiotic algae, as does *Corculum.* Additionally, a concomitant reduction in shell pigmentation was often found with the microstructural windows. The windows of *F. mundum, F. nivale,* and *L.* sp. 1 are quite similar to those of *Corculum;* however, slight differences suggest that *Corculum's* windows are more highly evolved. A fourth fragine clam, *Fragum fragum,* was found to contain microstructure suggestive of window formation; however this window microstructure did not extend completely through the shell.

The results of the study suggest that windows first appeared in an ancestral species of *Fragum* which later gave rise to *Lunulicardia* and *Corculum*. The relationship between *Fragum*, *Corculum*, and *Lunulicardia* also suggests a dual origin of photosymbiosis in the Cardiidae, once in the Tridacninae and once in the *Fragum-Corculum-Lunulicardia* lineage.

TO THE OFFICE OF GRADUATE SCHOOL AND RESEARCH

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THE EVOLUTION OF SHELL WINDOWS WITHIN THE FRAGINAE (BIVALVIA:CARDIIDAE) AND THE ORIGIN OF ALGAL SYMBIOSIS IN CARDIIDS

BY

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LIST OF ABBREVIATIONS

Shell Microstructure

BCL: branching crossed lamellar

CCCL: cone complex crossed lamellar

DCP: dissected crossed prismatic

FP: fibrous prismatic

ICCL: irregular complex crossed lamellar

INTRODUCTION

Algal symbionts are not common within the phylum Mollusca but can be found in a few groups of bivalves and prosobranchs and in a variety of opisthobranchs (Berner et al., 1986b). Within the Bivalvia, zoochlorellae are found in the cockle *Clinocardium nutalli*, the giant scallop *Placopecten magellanicus* and in a few freshwater species (Jones and Jacobs, 1992). Zooxanthellae are found in the cardiid subfamilies Tridacninae* and Fraginae, and possibly in the species *Fluviolanatus subtorta* (?Trapezidae) (Morton, 1982; Janssen, 1992b).

The best-known association between zooxanthellae and bivalves occurs in the Tridacninae, the giant clams. The tridacnines have been studied extensively and all members host zooxanthellae (Yonge, 1936). The first record of algal symbiosis in the Fraginae was reported in 1950 by Kawaguti. To date, published records of photosymbiosis in the Fraginae include: *Corculum cardissa, Fragum fragum, Fragum loochooanum* and *Fragum unedo* (Kawaguti, 1950, 1983; Ohno et al., 1995). Paulay (pers. comm.) has found photosymbionts in five additional fragines: *Fragum mundum, Fragum nivale, Fragum sueziense, Fragum* sp. 11 and *Lunulicardia* sp. 1. Additionally, Ohno et al. (1995) noted the absence of zooxanthellae in *Microfragum festivum*, as did Paulay (pers. comm.) in *Ctenocardia fornicata, Ctenocardia victor* and *Americardia media*.

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* Schneider (1992) showed that giant clams are a derived group of eucardiids, thus relegating them to subfamily status.

The metabolic interactions between clams and zooxanthellae are similar to those of corals and zooxanthellae: photosynthetically fixed carbon is translocated to the host in the form of carbohydrates and amino acids (Felbeck et al., 1983). Giant clams may obtain more than 50% of their metabolic carbon requirements from their algae (Trench et al., 1981). Since the maintenance of algal symbionts is dependent on exposure of the symbionts to light, host molluscs are generally found on reefs in shallow, clear water. Accordingly, symbiotic algae usually occur in tissues directly exposed to sunlight (Berner et al., 1986a). The opaque shells of bivalves would appear disadvantageous by prohibiting light from reaching internal tissues containing photosymbionts. The tridacnines and fragines have overcome this potential disruption to sunlight through a series of adaptations.

The giant clams' adaptations of a wide gape, hypertrophied mantle, and unique anatomical shifts leading to an umbo-down, gape-up position on the reef substratum, provide zooxanthellae with exposure to sunlight when the clams' valves are open. Zooxanthellae are found mainly in the mantle (Yonge, 1936; Trench et al., 1981; Janssen, 1992a) within a complexly branched "symbiont channel system" comprised of endodermal diverticula that develop from the stomach (Norton and Jones, 1992; Norton et al., 1992). A similar and potentially homologous system of channels, as yet of unknown provenance, has been described in *Corculum cardissa* (Janssen 1992a, 1992b).

Unlike the epifaunal tridacnines, *Fragum* is infaunal, lies with anterior side facing downward and gapes only moderately. In most Fraginae, the mantle does not protrude far from the shell and only the regions of the mantle around the siphons are exposed above

the substratum (Ohno et al., 1995). Zooxanthellae of *Fragum fragum* and *Fragum unedo* are concentrated within the gills and through most of the mantle but occur in other parts of the body as well (Kawaguti, 1983; Watson and Signor, 1986; Paulay, pers. comm.). In *F. fragum* the posterior side of the shell is less opaque than the anterior side (Watson and Signor, 1986) and lies facing upward just beneath the surface of the sediment. Thus, much of the light reaching the zooxanthellae in the interior of the shell has to pass through the posterior shell surface (Janssen, 1992b). Other *Fragum* species, such as *Fragum unedo*, lack this posterior shell translucency (Kawaguti, 1983; Ohno et al., 1995; Janssen, 1992a).

Fragines are infaunal with the exception of the epifaunal genus *Corculum*. *Corculum cardissa* keeps its valves almost completely closed, is greatly compressed along its antero-posterior axis, and lies with its posterior surface facing upward (Kawaguti, 1968). As a result, the thin posterior side of the shell, which contains a series of small, translucent "window" areas, is exposed to direct sunlight (Kawaguti, 1950; Carter and Schneider, 1997). The windows, arranged in radial rows, are thought to act as lenses to condense and focus light into tissue areas containing high population densities of zooxanthellae (Carter and Schneider, 1997): the mantle, kidney, gills, labial palps, and liver (Kawaguti, 1950, 1968; Janssen, 1992a). The lens effect is created by the slightly depressed outer surface and strongly convex interior surface of the windows (Seilacher, 1974; Carter and Schneider, 1997).

Corculum is the only bivalve described in the literature as forming microstructural windows within its shell (Seilacher, 1972, 1974, 1990; Janssen, 1992a, 1992b; Norton and

Jones, 1992; Ohno et al., 1995; Kobayashi, 1996; Carter and Schneider, 1997). The non-windowed areas of *Corculum's* shell consist of three microstructural layers: an outer shell layer consisting primarily of fibrous prismatic (FP) microstructure, a middle shell layer predominantly formed of branching crossed lamellar (BCL) microstructure and an inner shell layer consisting primarily of complex crossed lamellar microstructures (Carter and Schneider, 1997) (Fig. 1). *Corculum's* windows were initially thought to be comprised of fibrous prisms and to conduct and disperse light to the clams' tissues in the same manner as fiber optics (Seilacher, 1990). Such a light-guidance system has been noted in copepods and sponges (Holloway, 1997; Gaino and Sarà, 1994); however, Carter and Schneider (1997) discounted this mechanism in *Corculum* when they showed that instead of an FP microstructure, the bulk of the windows were comprised of a dissected crossed prismatic (DCP) microstructure, a microstructure that facilitates light transmission, but does not act like fiber optics. The shell layer, identified as DCP by Carter and Schneider (1997), is referred to as trans-prismatic structure by Kobayashi (1996).

The windows of *Corculum* are created by the thinning of the FP outer shell layer, the incursion of the FP outer shell layer through the middle shell layer, and the passing of these fibrous prisms into the slightly less translucent DCP microstructure which extends the windows toward the shell interior (Carter and Schneider, 1997) (Fig. 2). The lateral portions of the DCP prisms, originating on the inner surface of the BCL middle shell layer, lack direct fiber-optic connection with the prisms in the outer shell layer (Carter and Schneider, 1997). However, the centrally located DCP prisms have a perpendicular orientation which channels light directly toward the interior of the shell (Carter and

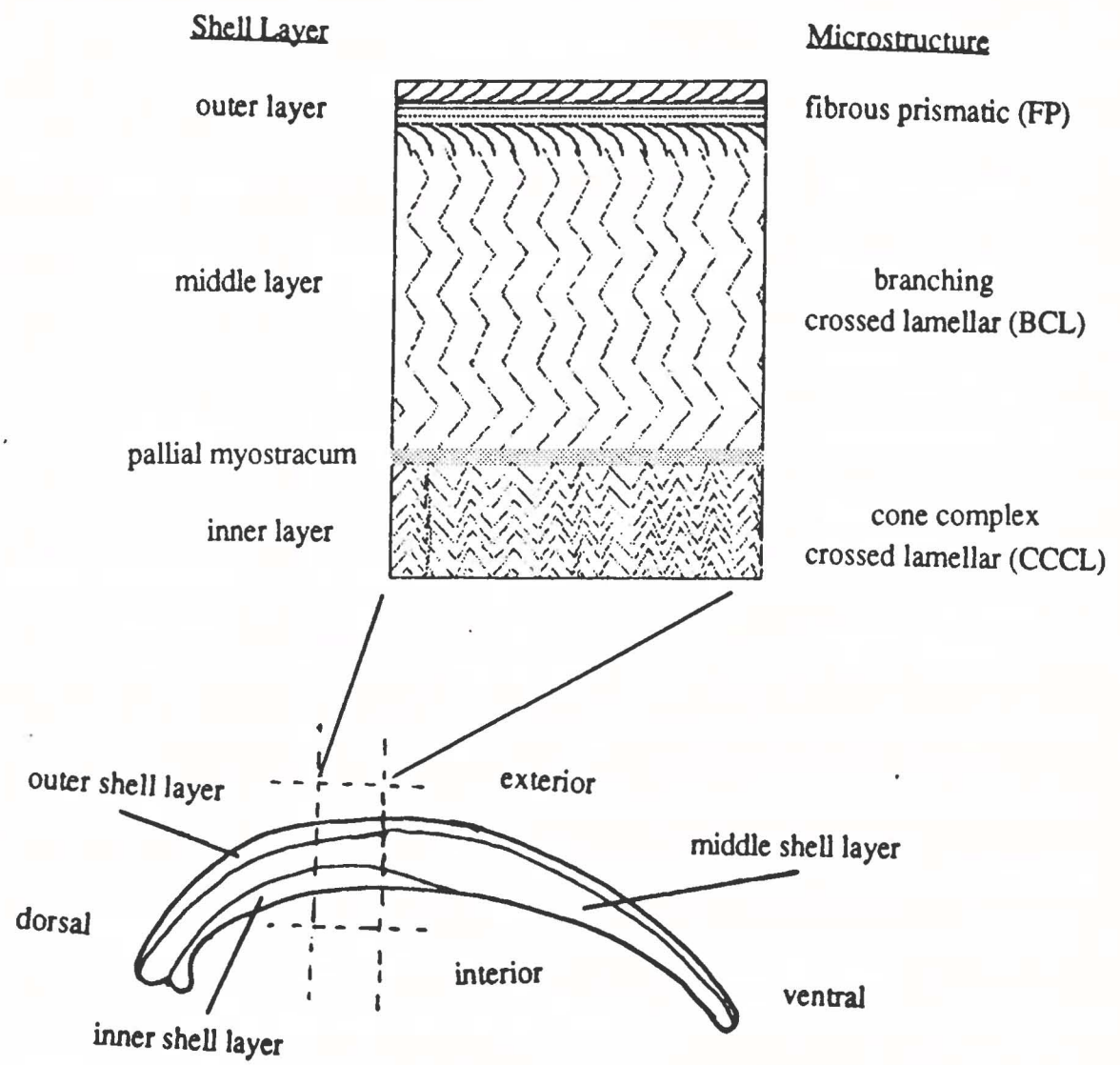

Fig. 1. Fragine shell microstructure. Shell layers and typical microstructure, as found in the non-windowed areas of *Corculum cardissa*. Not drawn to scale.

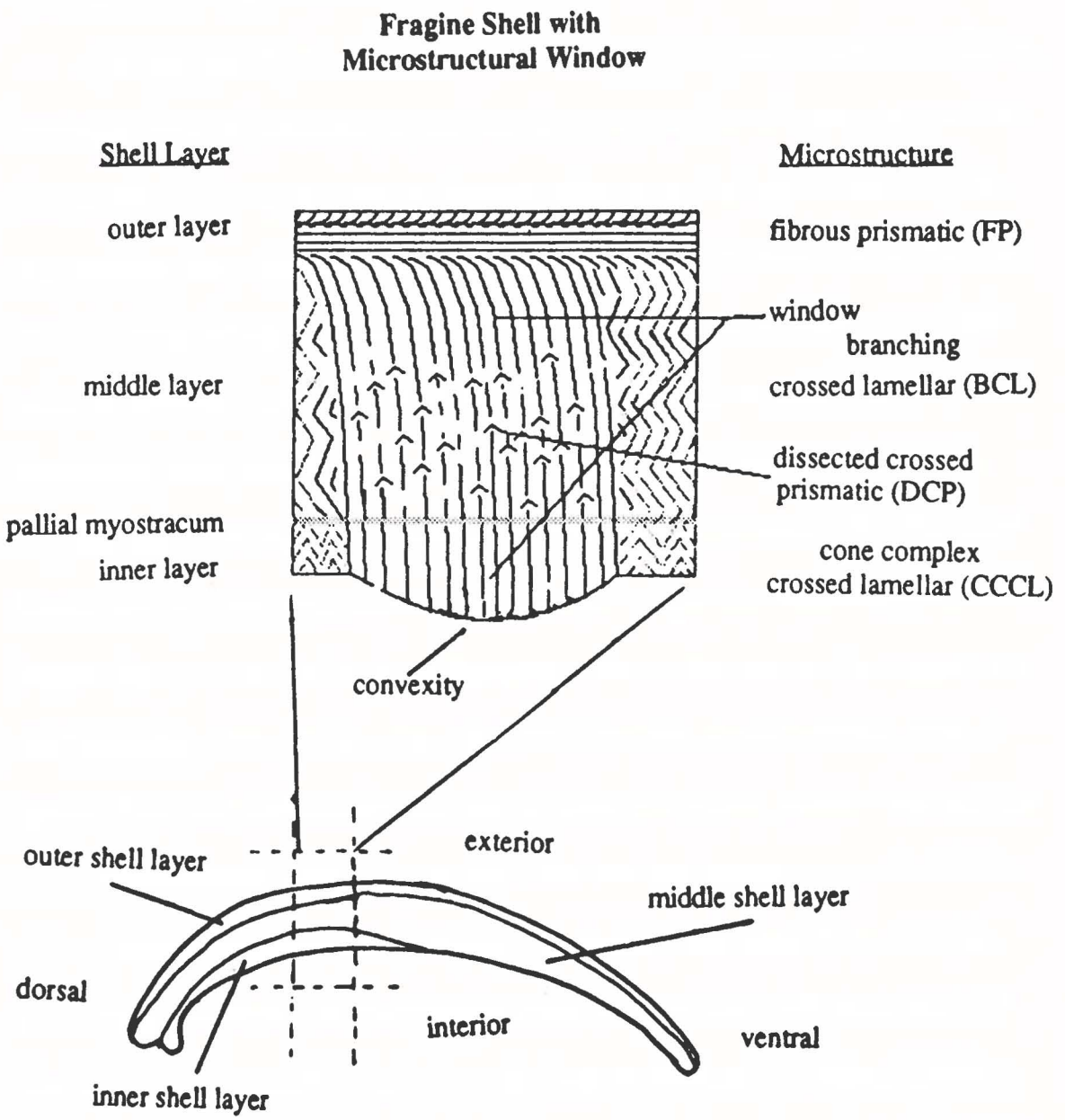

Fig. 2. Fragine shell microstructure with window. Shell layers and typical posterior microstructure of a shell with a window, as found in *Corculum cardissa*. Not drawn to scale.

Schneider, 1997). Light transmission through the shell is further enhanced by reduced shell pigmentation and shell thickness (Carter and Schneider, 1997).

Because the adaptations for exposing zooxanthellae to sunlight vary widely among the tridacnines, *Corculum,* and *Fragum*, a number of hypotheses have been proposed on their relatedness and on the origin of photosymbionts in these groups. Seilacher (1990) suggested that photosymbiosis evolved independently among the three groups. However, Janssen (1992a, 1992b) suggested that symbiotic algae were acquired only once in one common cardiid ancestor. Because *Fragum fragum* and *Fragum unedo* exhibit intermediate features between tridacnines and *Corculum*, they have been described as "missing links" between the two (Kawaguti, 1983). Norton and Jones (1992) postulated that the morphological similarities between *Corculum* and the giant clam *Hippopus* suggest that an ancestor of the genus *Hippopus* could be a link between the Fraginae and the Tridacninae. In his phylogenetic analysis of eucardiines, Schneider (1992, 1995, in press, a) found that the Tridacninae, Lymnocardiinae and Fraginae formed a monophyletic group, with the former two being sister taxa within this assemblage.

Thus, both phylogenetic affinity and morphological similarity points to a close relationship between the two photosymbiotic lineages within the Cardiidae; however, whether photosymbiosis evolved once or twice within the family is a debated question. Part of the resolution of this question lies in the relationship of the three subfamilies to each other. Schneider's results - ((Tridacninae, Lymnocardiinae) Fraginae) - cannot distinguish between these possibilities, as both alternatives: 1) two independent origins of

photosymbiosis in the Tridacninae and Fraginae, or 2) a single origin in the ancestor and subsequent loss in the Lymnocardiinae, are equally parsimonious.

One way to resolve how many times photosymbiosis originated among cardiids is to establish the origin of photosymbiosis within the Fraginae. If all fragines are photosymbiotic, the problem remains unresolved, but if basal fragines are not photosymbiotic, then the independent origin hypothesis will be favored. Schneider (in press, a) postulated that all members of the Fraginae will be found to be photosymbiotic on the basis of their simplified digestive system. Schneider (in press, a) defined the Fraginae to include the following genera and relationships: (*Papillicardium* (*Parvicardium* (*Trigoniocardia* ((*Microfragum* (*Ctenocardia* - *Americardia*)) *Fragum* (*Lunulicardia* - *Corculum*))))) (Fig. 3). Schneider (in press, a) also suggested two alternative placements for *Fragum:* ((*Fragum* (*Microfragum* (*Ctenocardia* - *Americardia*))) (*Lunulicardia* - *Corculum*)) and ((*Microfragum* (*Ctenocardia* - *Americardia*)) ((*Fragum*) (*Lunulicardia* - *Corculum*))) (Fig. 3).

To establish an origin of photosymbiosis within the Fraginae, this study attempts to clarify the relationships among the fragines by investigating the evolution of shell microstructure within the Fraginae, especially with regard to the possible origin of shell windows in *Corculum*. The study surveys many of the extant fragine genera for shell window microstructure, using this character as a key to determine relationships among the Fraginae and thus provide evidence for an origin of photosymbiosis. Additionally, since shell pigmentation effects translucency and thus a shell's ability to transmit light to photosymbionts in its interior, this study investigates the correspondence between shell

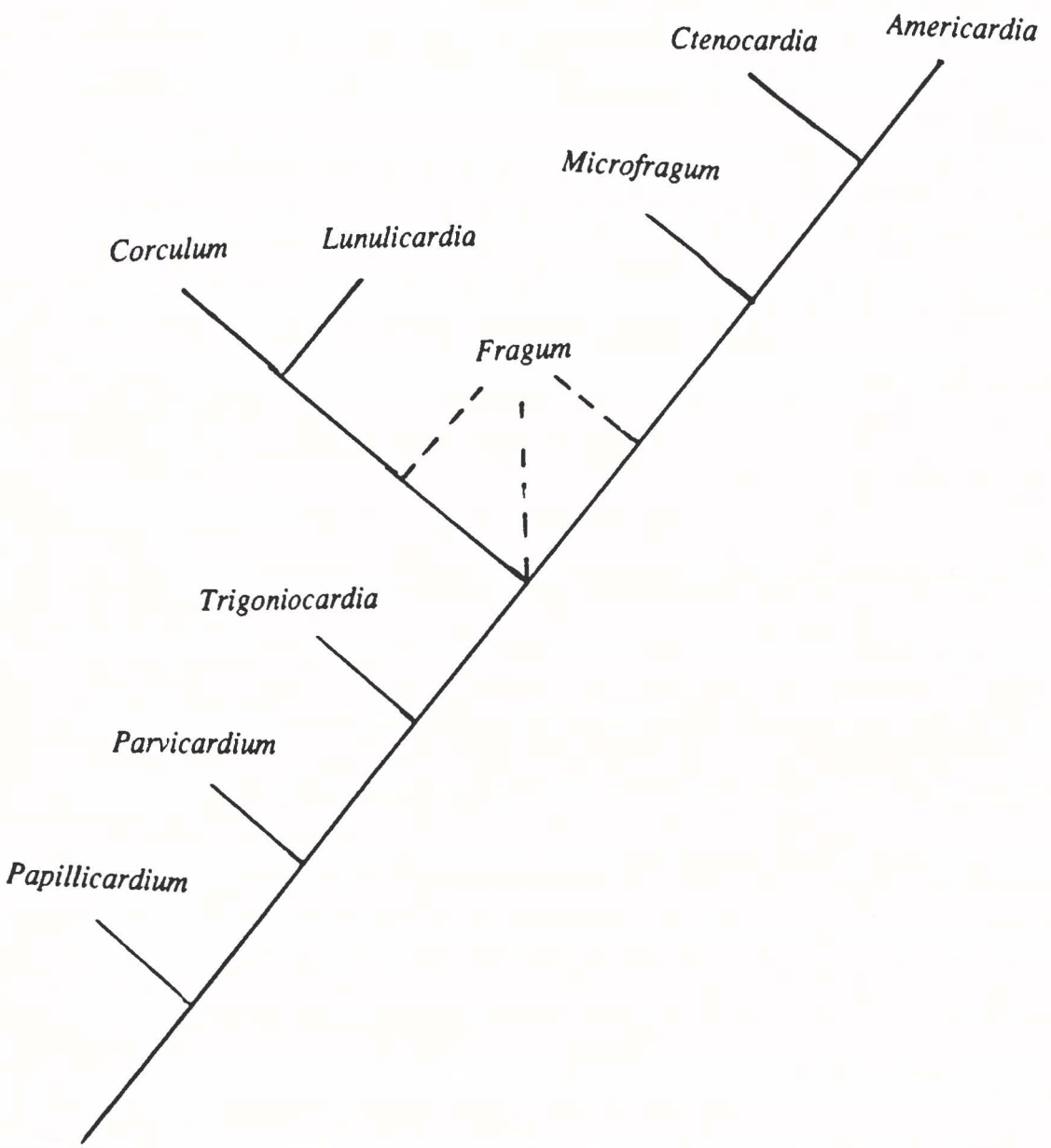

Fig. 3. Schneider's (in press, a) phylogeny of the Fraginae. Variable position of the genus *Fragum* denoted by dashed lines.

microstructure types and the presence or absence of opaque white pigmentation within the fragine shell.

MATERIALS AND METHODS

Fifteen species from three subfamilies of Cardiidae were studied: 12 Fraginae, two Laevicardiinae and one Trachycardiinae. Acetate peels from shell sections were observed with a Zeiss compound microscope and photographed with a Leitz Biomed compound microscope. Sectioned casts of shells were observed with an Electroscan model E3 environmental scanning electron microscope (SEM) and a JSM-35 SEM. Pigmentation of shell sections was examined with a Leica Wild M8 dissecting microscope.

Material Examined

Specimens were obtained from the United States National Museum of Natural History (USNM), The Natural History Museum, London (BM(NH)), the G. Paulay collection, or collected specifically for this study (those from Guam). They are listed below together with their geographic origin, their source, and the number of valves sectioned and studied are as indicated. *Lunulicardia* sp. 1 is a small (<1cm), apparently undescribed *Lunulicardia* known at present from the Philippine, Mariana and Marshall Islands.

Americardia media (Linné, 1758): location unknown, G. Paulay coll. (2).

Corculum cardissa (Linné, 1758): location unknown, G. Paulay coll. (2).

Ctenocardia fornicata (Sowerby, 1841): Fiji, G. Paulay coll. (1).

Fragum fragum (Linné, 1758): Guam (5).

F. loochooanum (Kira, 1959): Guam (6).

F. mundum (Reeve, 1845): Caroline Islands, USNM 610789 (3), Tuamotu Islands, USNM 722836 (7).

F. nivale (Reeve, 1845): Sudan, BM(NH) no catalog number (2).

F. sueziense (Issel, 1869): Guam (3).

F. unedo (Linné, 1758): Fiji, G. Paulay coll. (2).

Fulvia australis (Sowerby, 1837): Guam (2).

Laevicardium biradiatum (Bruguière, 1789): Fiji, G. Paulay coll. (1).

Lunulicardia retusum (Linné, 1767): Queensland, Australia, USNM 631219 (2); Queensland, Australia, USNM 631168 (1).

L. sp 1: Marshall Islands, USNM 582913 (2).

Microfragum festivum (Deshayes, 1855): Fiji, G. Paulay coll. (1).

Trachycardium orbita (Broderip & Sowerby, 1833): Fiji, G. Paulay coll. (1).

Acetate Peels

Acetate peels were the primary source for observation of shell microstructure. They were prepared in a manner similar to that described by Rhoads and Pannella (1970) and Kennish et al. (1980). Each shell valve or piece of shell valve was embedded in Evercoat Casting Resin (Fibre Glass-Evercoat, Co., Inc.) mixed with a methyl ethyl ketone peroxide hardener (Rocket Plastics Co.) and left to cure overnight.

Shells were sectioned with a Buehler Isomet, low-speed diamond rock saw. At least one anterior radial cut, one posterior radial cut and one transverse cut across both posterior and anterior ribs was done for each species (Fig. 4). The sectioned casts were

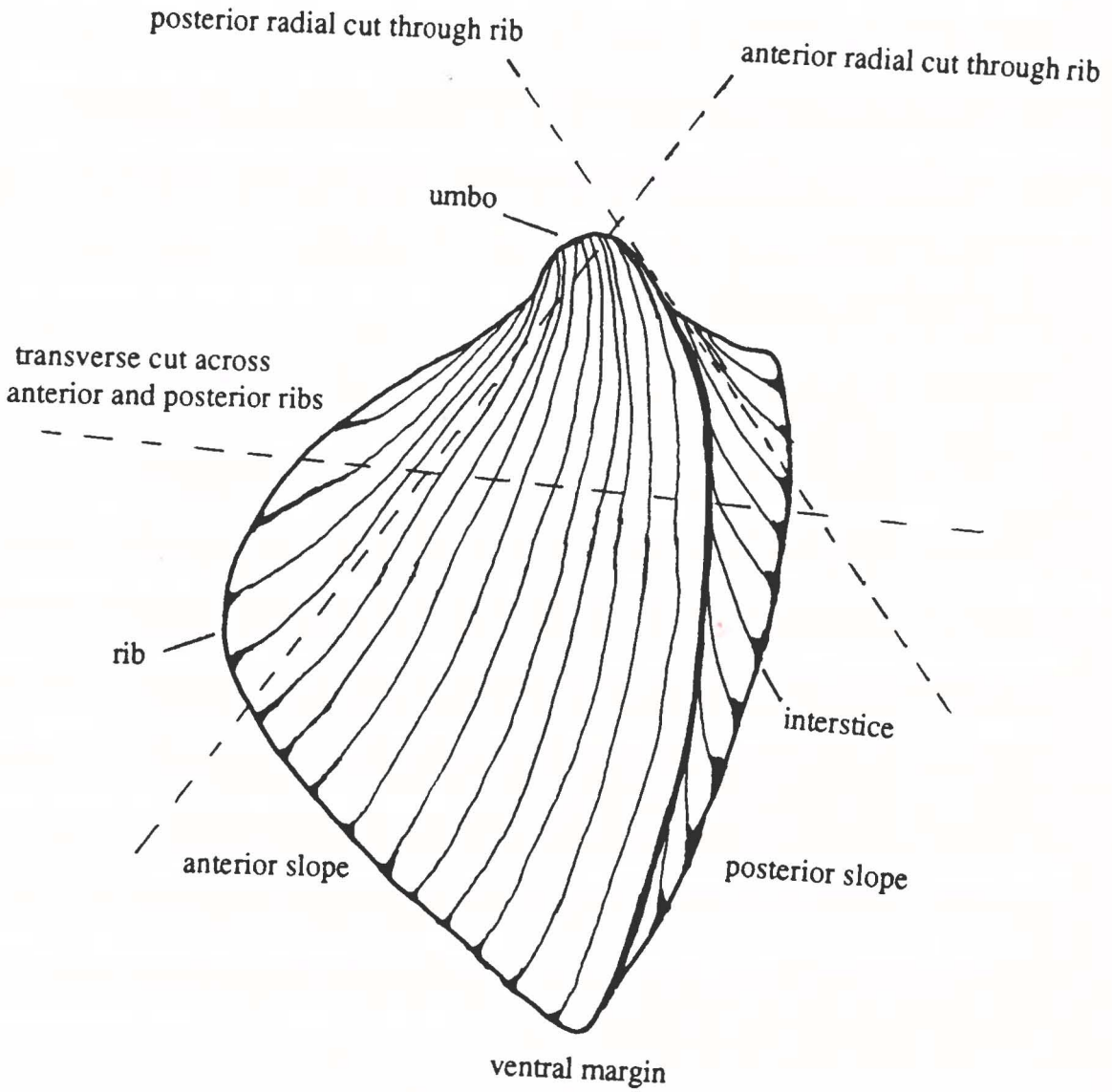

Fig. 4. Cuts used for shell sections. Exterior view of a left valve of *Fragum fragum* showing direction of cuts used in shell sectioning.

ground and polished with up to 1500 grit sandpaper, followed by a final polish with a lapidary polishing disc and 0.3 micron Buehler Alpha Micropolish II Deagglomerated Alumina. Sections were then etched in 5% hydrochloric acid for three to six seconds, rinsed with deionized water and dried with a blow dryer. The etched shell surface was flooded with acetone and immediately covered with a strip of 1.5 mm thick acetate cut to fit on a microscope stage. The acetate strip was weighted to ensure firm contact between the etching and the acetate, and left in place overnight.

Scanning Electron Microscopy

Two *Fragum mundum*, two *Fragum nivale*, one *Fragum fragum* and one *Lunulicardia* sp. 1 were observed with a SEM by Chris Meyer (Department of Integrative and Comparative Biology, University of California, Berkeley). Embedded and sectioned specimens previously used for acetate peels were repolished and re-etched as outlined above and cut to fit the SEM mounting stub.

Two additional specimens of *Fragum mundum* were observed with a SEM at the Friday Harbor Labs, University of Washington by Gustav Paulay. These shells were fractured in radial to oblique orientations.

Shell Pigmentation

The embedded and sectioned valves of *Corculum cardissa*, *Lunulicardia* sp. 1, *Fragum nivale*, *Fragum mundum*, and *Fragum fragum* were observed under the dissecting microscope with reflected light and compared to the acetate peels made from

the same casts. Window regions defined by microstructure were located on each acetate peel with the compound microscope and then the sectioned valve was observed to determine how shell pigmentation overlapped with window microstructure. Whole shells were examined for overall pigmentation and window placement.

RESULTS

Microstructure of Fraginae

All Fraginae studied share the same basic arrangement of microstructural layers and growth line curvature, but only three of the eleven species, *Fragum mundum, Fragum nivale,* and *Lunulicardia* sp. 1, exhibit window formation (Figs. 5 - 7). The posterior shell valve pigmentation and shell microstructure of the three windowed species was very similar to that of *Corculum cardissa.* All fragine shells studied were composed of three layers: an outer FP shell layer, a middle, generally BCL shell layer, and an inner complex crossed lamellar shell layer (Figs. 1, 8). Three sublayers occur in the FP outer shell layer, each defined by the orientation of the fibrous prisms: outwardly inclined in the outer sublayer, horizontal in the middle sublayer, and inwardly reclined in the inner sublayer (Figs. 9 - 11).

The extent and thickness of the FP outer shell layer varies among species. Measurements of shell thickness were taken on acetate peels at the umbo, margin and midpoint of posterior shell valves. Results from three *Lunulicardia retusum*, a species with a thick FP layer, revealed an outer shell layer with a mean of 27% of total shell thickness, whereas the shell of *Ctenocardia fornicata* had an outer shell layer with a mean of 6% of total shell thickness, with much of the shell layer appearing locally absent. The FP outer shell layer of all but three (*Fragum fragum, Fragum nivale, L. retusum*) of the eleven species investigated was found to be either occasionally invaded locally by the middle shell layer microstructure (*Fragum loochooanum, Fragum mundum, Fragum*

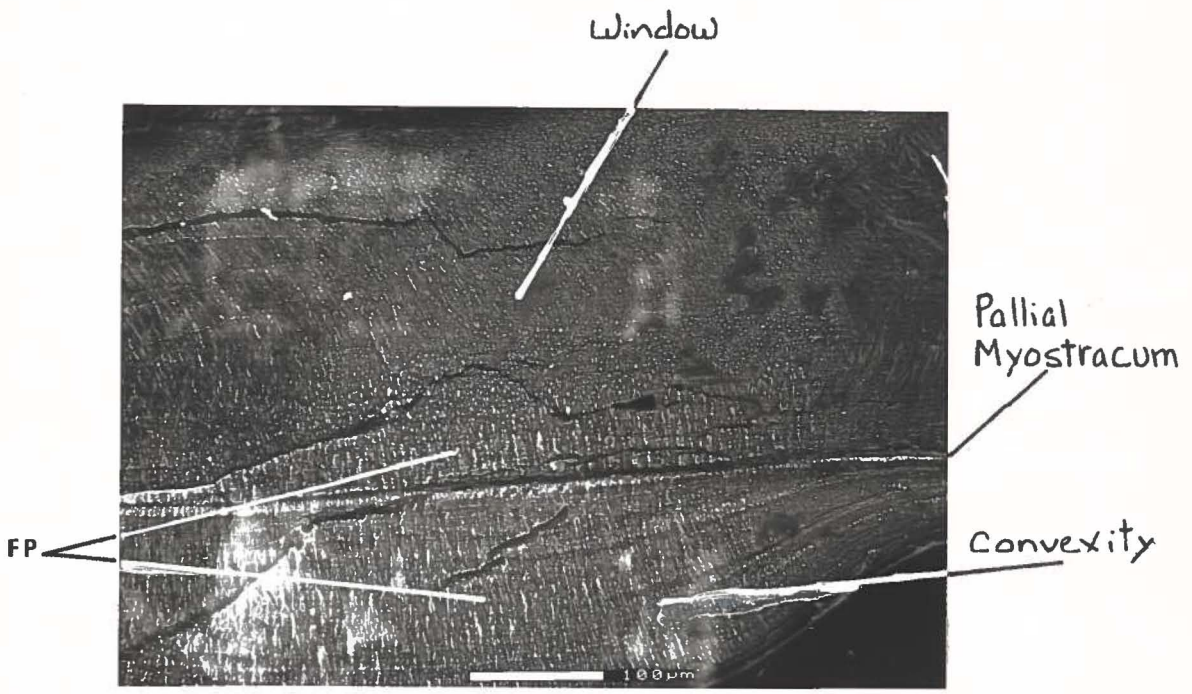

Fig. 5. Window microstructure in *Fragum mundum*. Radial section, right valve, posterior slope, exterior at top, growing edge to right. SEM.

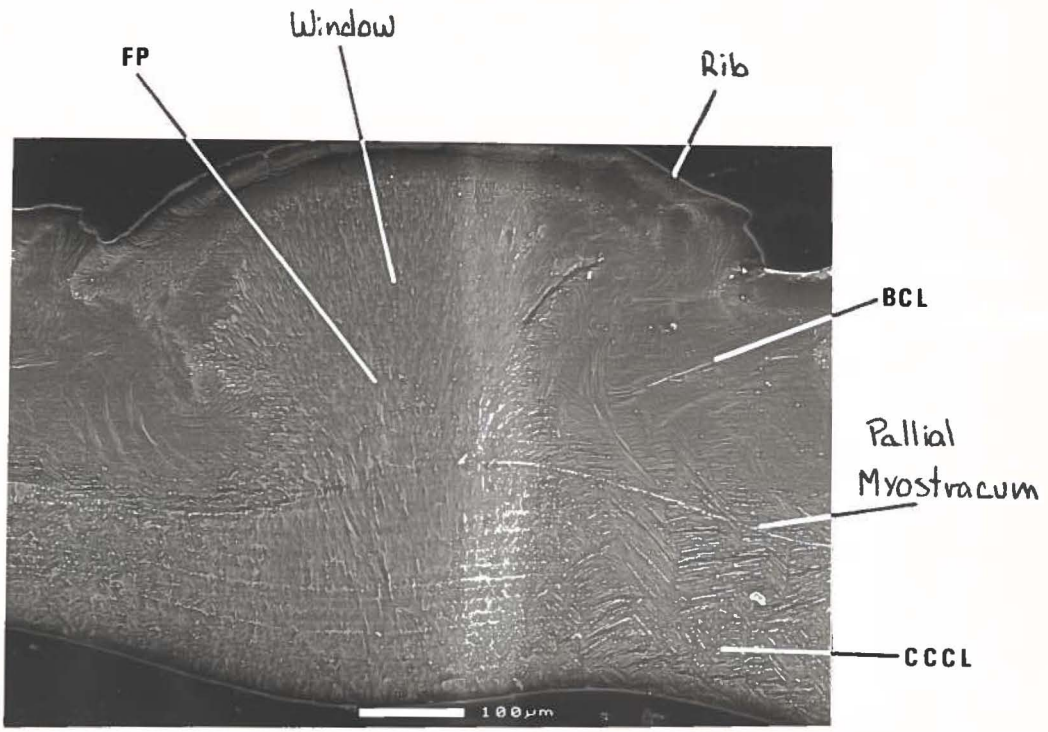

Fig. 6. Window microstructure in *Fragum nivale*. Transverse section, right valve, posterior slope, exterior at top, anterior to right, SEM.

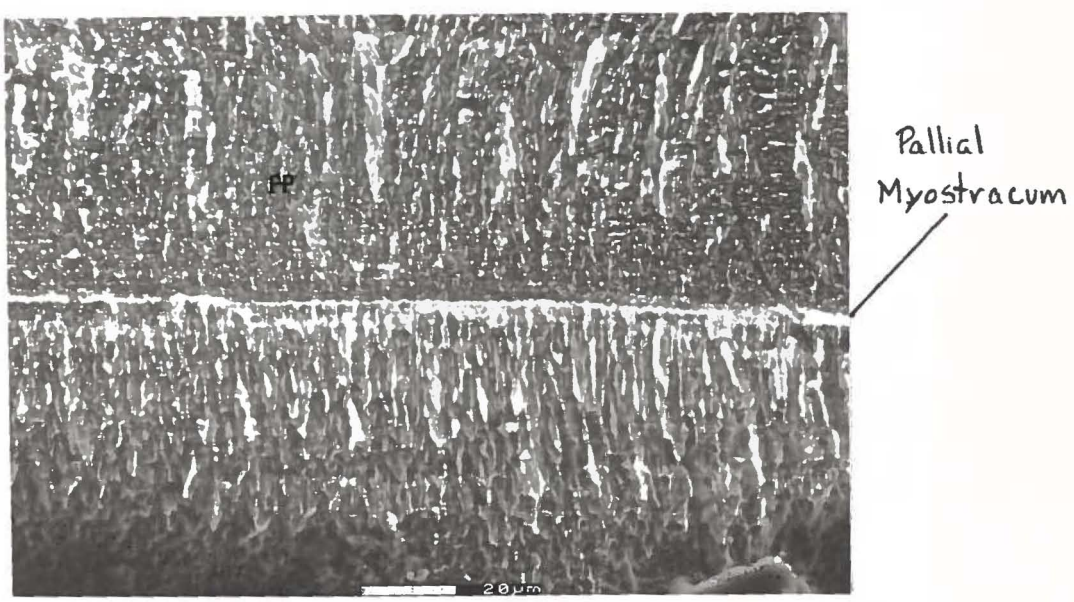

Fig. 7. Close-up view of window microstructure in *Lunulicardia* sp. 1. Radial section, left valve, posterior slope, exterior at top, growing edge to right. SEM.

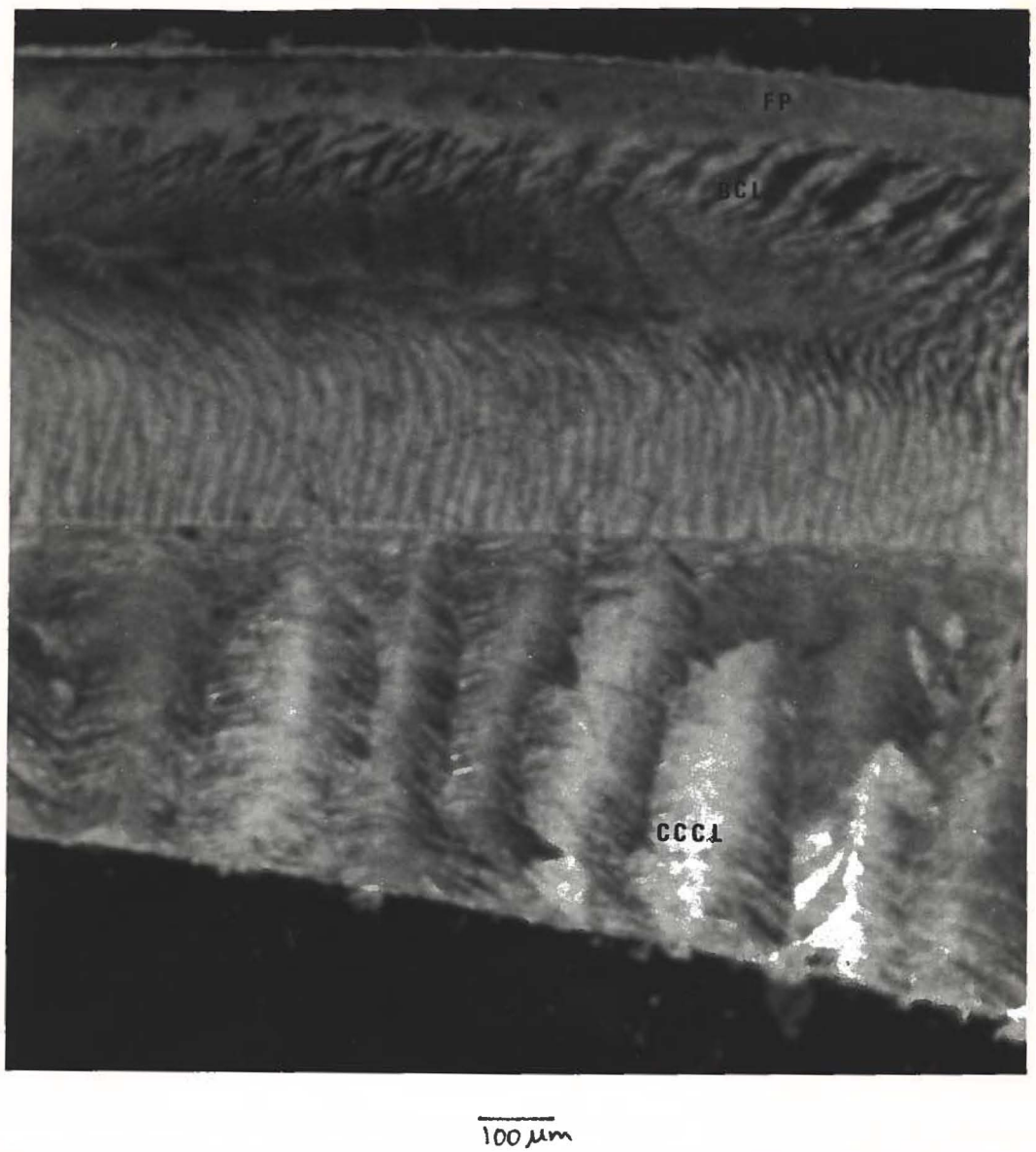

Fig. 8. Typical fragine shell microstructure. FP outer shell layer, BCL middle shell layer, CCCL inner shell layer. *Fragum loochooanum*. Radial section, left valve, posterior slope, exterior at top, growing edge to left. Acetate peel.

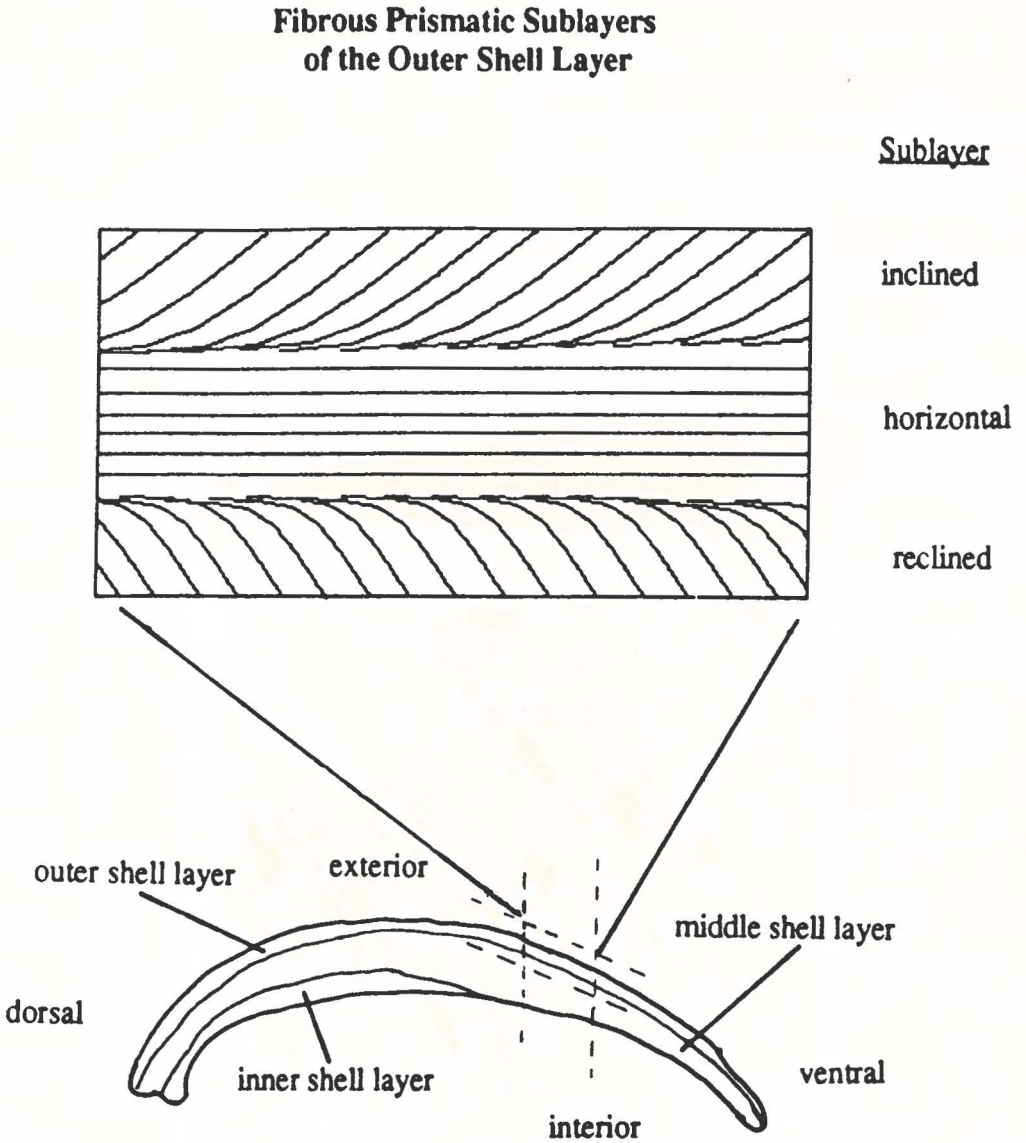

Fig. 9. Sublayers of the FP outer shell layer.

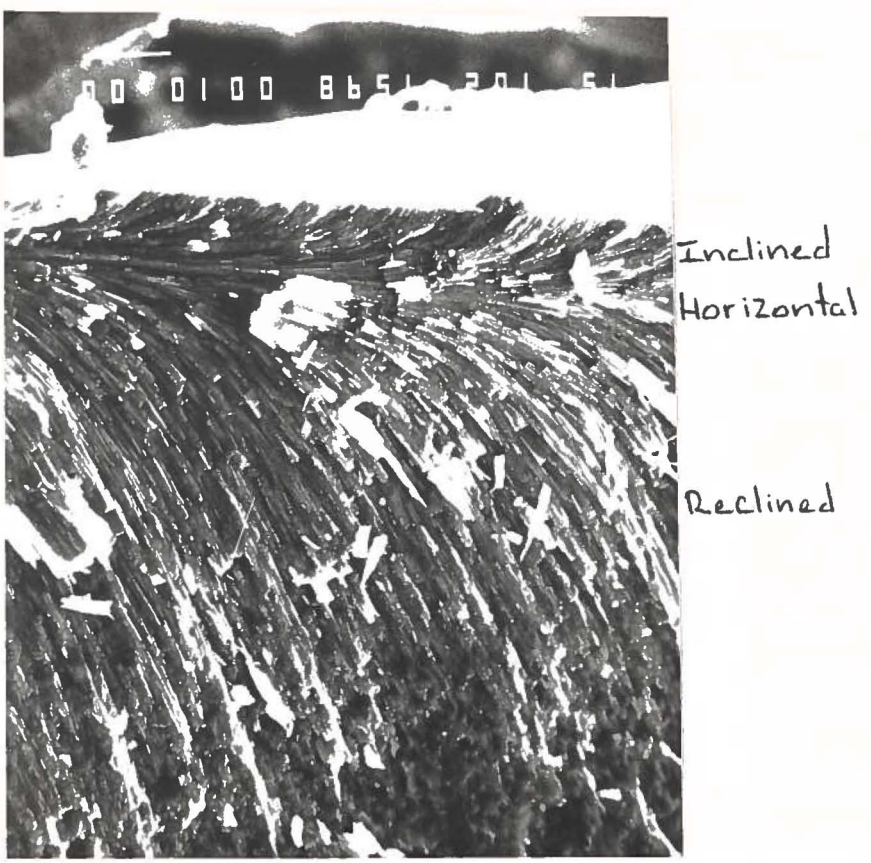

Fig. 10. FP sublayers in the outer shell layer of *Fragum mundum*. Radial section, left valve, posterior slope, exterior at top, growing edge to right. SEM.

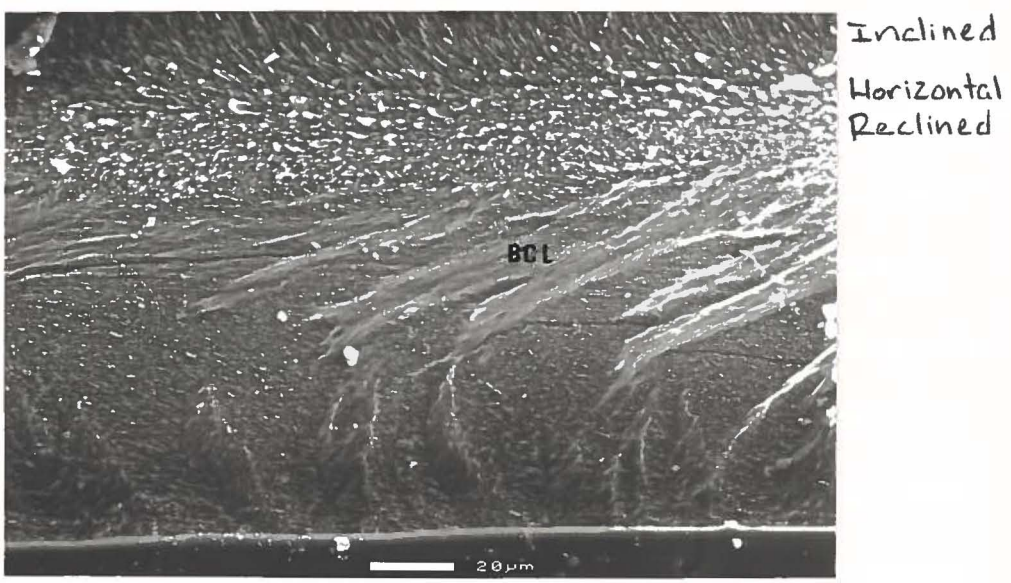

Fig. 11. FP sublayers in *Lunulicardia* sp. 1. Outer shell layer is at top third of shell section. Radial section, left valve, posterior slope, exterior at top, growing edge to left. SEM.

sueziense, *Fragum unedo*, *Lunulicardia* sp. 1, *Microfragum festivum*) or dominated by the middle shell layer microstructure with only occasional patches of FP remaining on rib tops (*C. fornicata*) or on rib tops and in interstices (*Americardia media*). The middle shell layer invades through the FP outer shell layer most often at the interstices and on the sides of ribs. Observations of *F. fragum* with the SEM revealed that its FP outer shell layer remained intact, albeit very thin, above the middle shell layer incursions even when FP was not discernible in these areas on the acetate peels (Fig. 12). Thus, the FP outer shell layer may be contiguous above middle shell layer incursions in the other species as well.

On the posterior slope of *Fragum fragum*, the FP outer shell layer locally invades the middle shell layer in some, if not all, individuals (Fig. 12). These incursions are suggestive of window formation; however no complete windows were found that extended to the interior of the shell. The FP in these "window" areas extends up to at least two thirds of the shell thickness; elsewhere FP constitutes about 15% of shell thickness. The "windows" diffuse into a possibly homogeneous microstructure which grades into a crossed lamellar microstructure at the shell interior. Thus, the outer and middle shell layer microstructure is modified in these windowed areas. It was not evident, however, whether or not the "windows" reached or surpassed the pallial myostracum because a pallial myostracum was not discernable in these sections.

The middle shell layer is the thickest layer in all fragines investigated. BCL is the dominant microstructure and is often extensively interspersed with cone complex crossed lamellar (CCCL) and occasionally irregular complex crossed lamellar (ICCL)

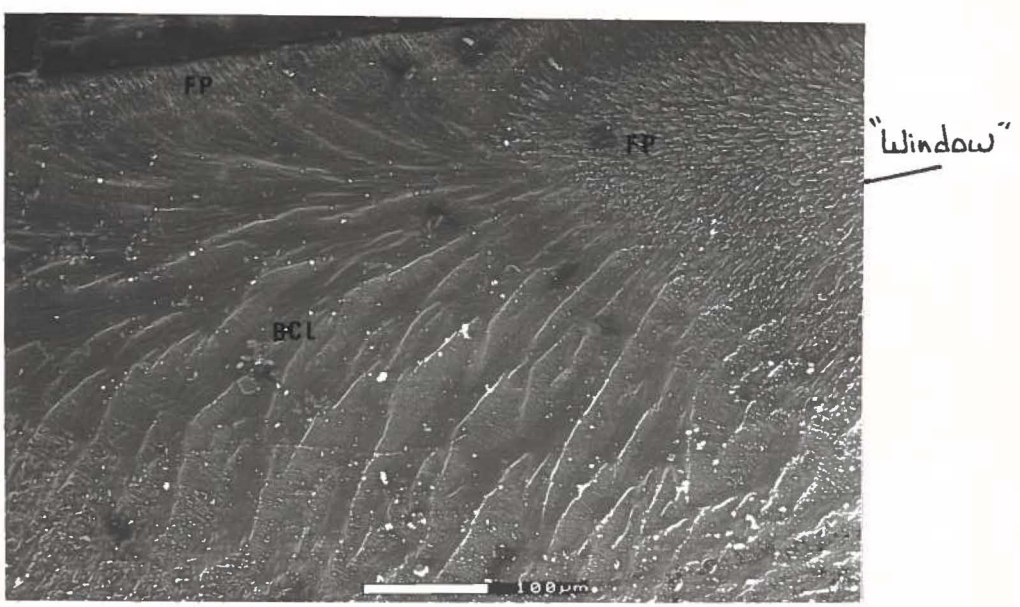

Fig. 12. Partial window in *Fragum fragum*. FP microstructure invades middle shell layer at right. Radial section, right valve, posterior slope, exterior at top, growing edge to left. SEM.

microstructures. CCCL microstructure is often found just above the pallial myostracum. The inner shell layer is dominated by CCCL and/or ICCL microstructure; BCL is also present locally in *Microfragum festivum.*

Mosaicostracal scales occur on the ribs of all the fragines studied; thin, intercostal laminae continuous with the outer shell layer occur in all but *Fragum unedo, Lunulicardia* sp. 1 and *Microfragum festivum.*

Microstructure of Windowed Species

In addition to the limited window-like structures in *Fragum fragum*, the typical microstructure of the Fraginae is interrupted by translucent windows in three of the eleven species investigated: *Fragum mundum, Fragum nivale,* and *Lunulicardia* sp. 1. In all three species, windows occur only on the posterior slope of the shell and are most common and complete towards the keel. Windows are centered on ribs.

Windows cross all three shell layers and are composed of FP, DCP and possibly homogeneous shell microstructure; the crossed lamellar microstructures (BCL, CCCL, ICCL) of typical middle and inner shell layers are absent (Fig. 13). The bulk of each window is formed from the inner sublayer of reclined prisms which change in orientation from reclined (near the shell surface) to perpendicular to the shell surface (in the shell interior). The prisms become shorter and less fibrous toward the middle of the shell, where a DCP microstructure is discernible in some electron micrographs of *Fragum mundum* (Fig. 14). A FP microstructure with relatively short fibers reestablishes below the pallial myostracum in the inner shell layer of windows (Figs. 15, 16).

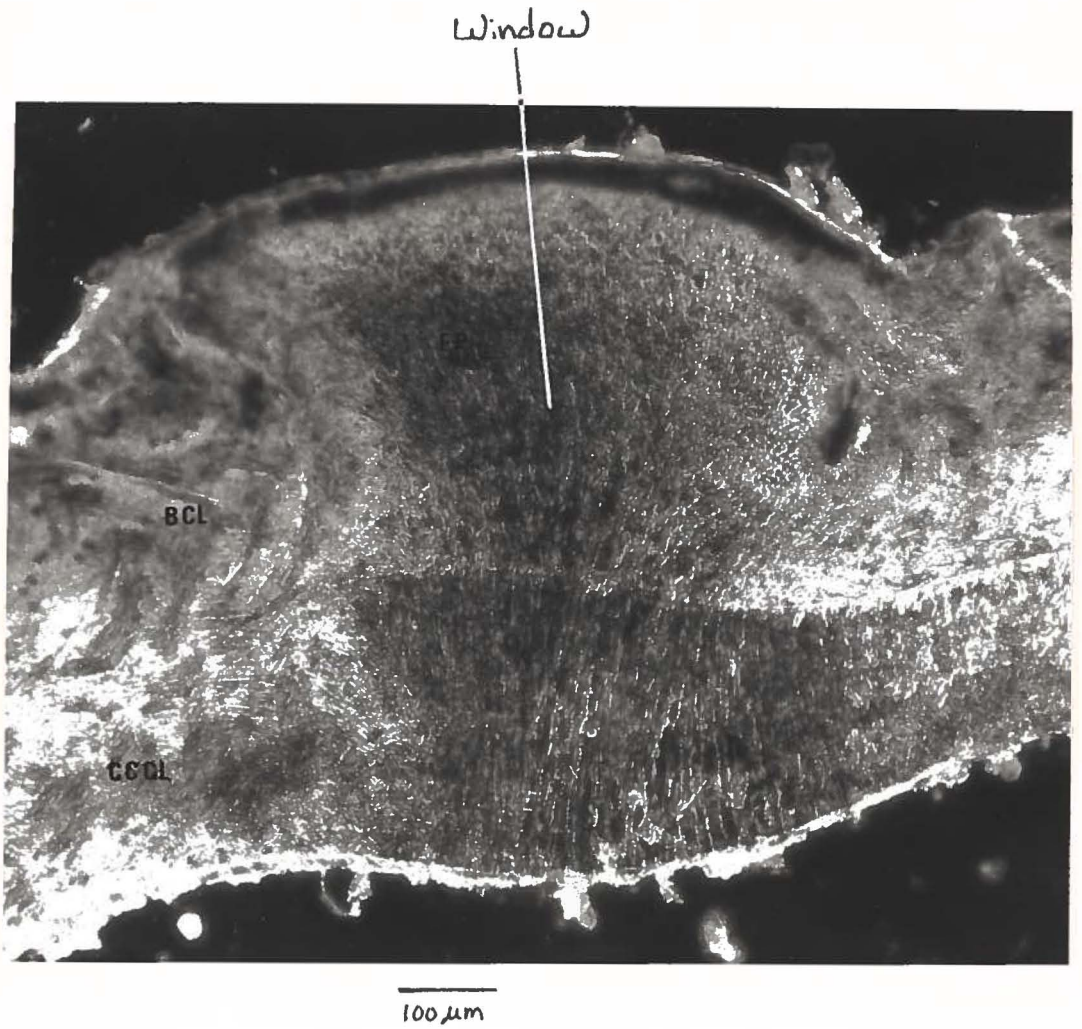

Fig. 13. Window in *Fragum nivale*. Note that windows lack the crossed lamellar microstructure found in the middle and inner shell layers of non-windowed areas. Also note the convexity towards the interior of the shell below the window. Transverse section, right valve, posterior slope, exterior at top, anterior slope to right. Acetate peel.

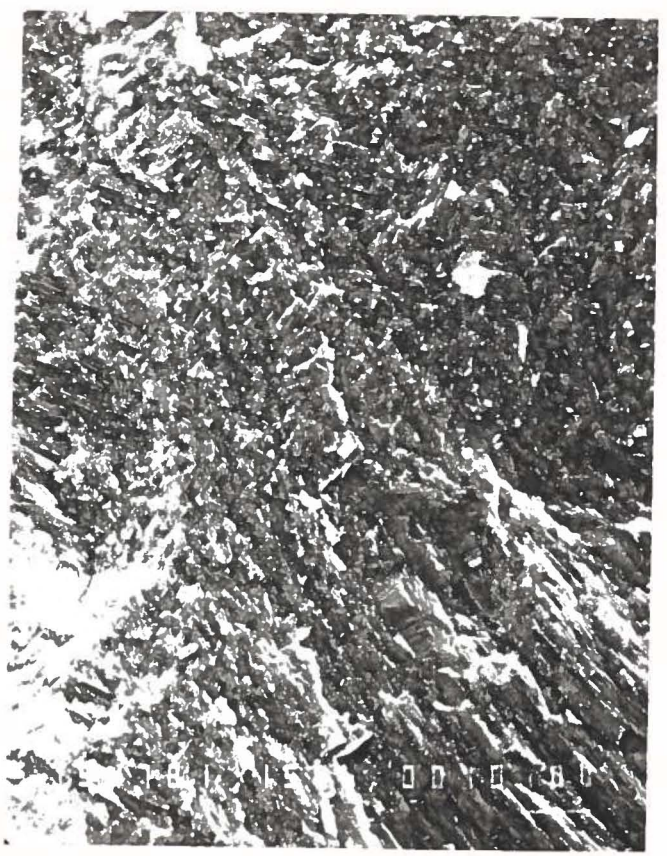

Fig. 14. DCP microstructure in the middle shell layer of a *Fragum mundum* window. Radial section, left valve, posterior slope, exterior at top, growing edge to right. SEM.

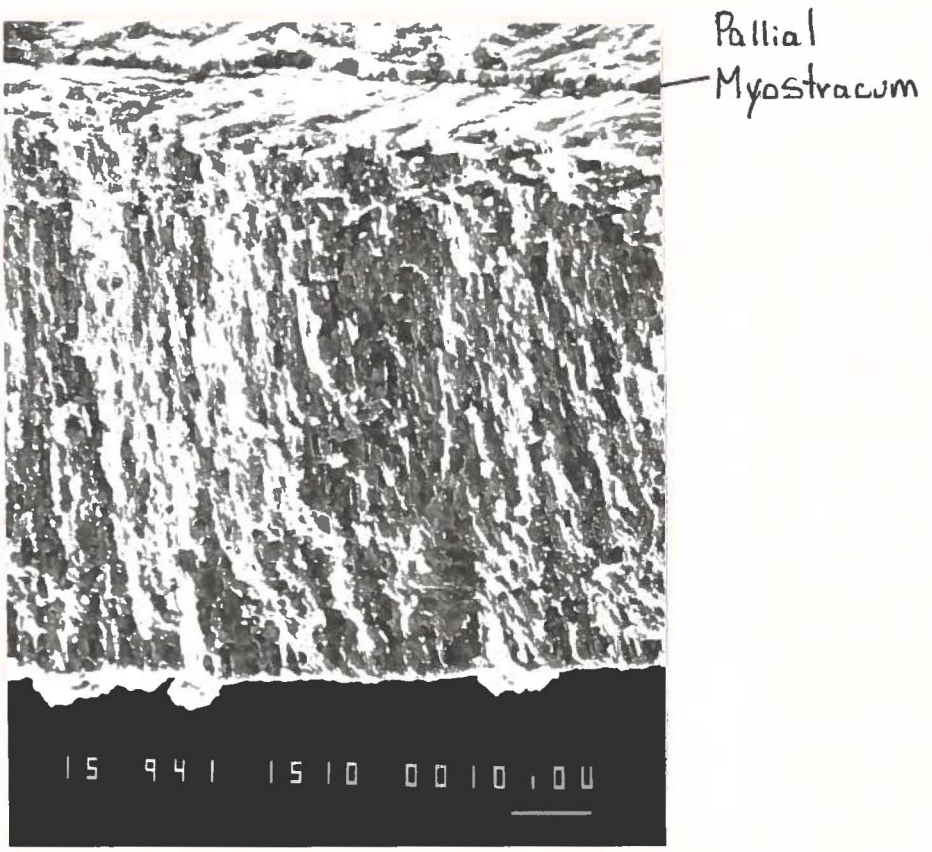

Fig. 15. FP microstructure in the inner shell layer of a *Fragum mundum* window. Radial section, left valve, posterior slope, exterior at top, growing edge to right. SEM.

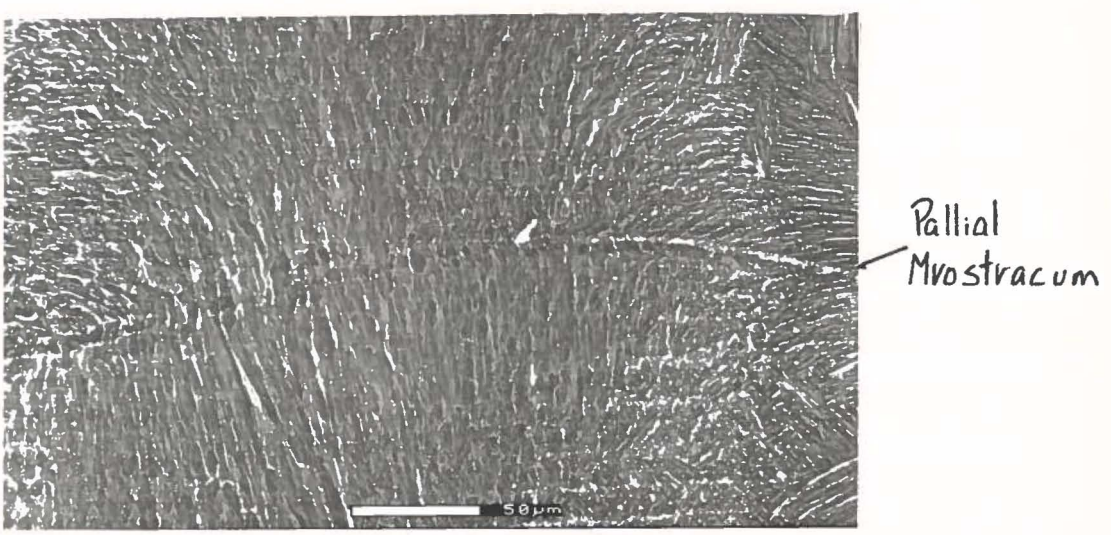

Fig. 16. FP microstructure in the inner shell layer of a *Fragum nivale* window. Transverse section, right valve, posterior slope, exterior at top, anterior slope to right. SEM.

Electron micrographs of *Fragum mundum* reveal a reduction of the inclined sublayer in the FP outer layer above the windows. This sublayer reduction is not evident in *Lunulicardia* sp. 1. The FP sublayers of *Fragum nivale* are not distinguishable in either the electron micrographs or the acetate peels.

The BCL of the middle shell layer blends with the edges of the FP window microstructure, and in some sections was seen to invade the window to the extent that it comes to dominate the microstructure within it (Fig. 17). Such incomplete windows are likely a consequence of the placement of the cut through the shell, i.e. the cut may be on the edge of a window rather than through its center, thus revealing only part of the window microstructure.

Another character unique to the windowed species is the distinctly convex areas occurring in the inner shell layer beneath each complete and partial window (Figs. 2, 5, 13, 18). The largest convexities occur near the keel and nearest the umbo, decreasing in size toward the shell margin. Within the convexities, the thickened inner shell layer consists of FP microstructure, thus differing from the CCCL and ICCL microstructures elsewhere in the inner shell layer. The FP prisms of *Fragum mundum, Fragum nivale,* and *Lunulicardia* sp. 1 are oriented vertically through these convex regions. Convexities are still evident distal to the pallial line. Carter and Schneider (1997) additionally describe a concavity in the outer shell layer above each of *Corculum's* windows. Such concavities are absent in the windowed *Fragum* and *Lunulicardia* species.

Fig. 17. Incomplete window in *Fragum mundum*. The BCL microstructure of the middle shell layer blends with the top edge of the FP window microstructure. Such incomplete windows are likely a consequence of cut placement. FP microstructure is visible in the inner shell layer and just above the pallial myostracum. Radial section, left valve, posterior slope, exterior at top, growing edge to left. Acetate peel.

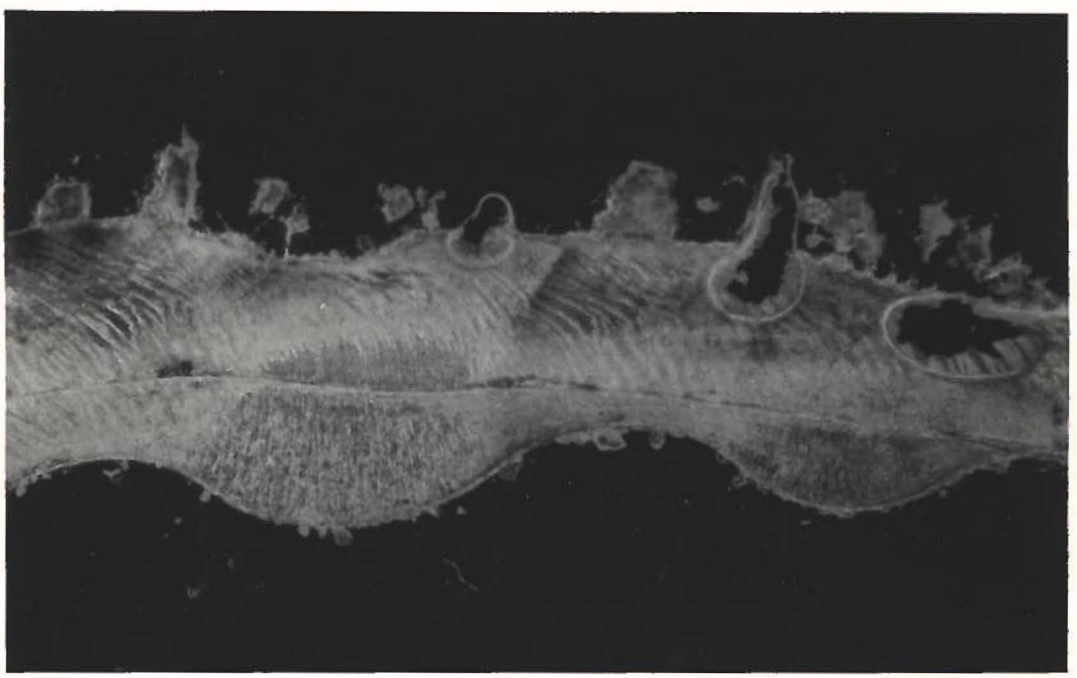

Fig. 18. Convexities in the inner shell layer beneath windows. The largest convexities occur near the keel and nearest the umbo, decreasing in size toward the shell margin. *Fragum mundum*. Radial section, left valve, posterior slope, exterior at top, growing edge to right. Acetate peel.

Microstructure of Outgroups

The shells of the three outgroup species, *Fulvia australis*, *Laevicardium biradiatum*, and *Trachycardium orbita*, exhibit no discernible FP outer shell layer in acetate peels. The shells are formed of only two discernible layers, an outer and an inner separated by the pallial myostracum. The outer layer is formed primarily of BCL interspersed with ICCL and CCCL in all three species except that *F. australis* exhibits no CCCL in the outer layer. CCCL and ICCL dominate the inner layers and BCL is locally present in the inner layer of *L. biradiatum*. The inner layers of *T. orbita*, *L. biradiatum* and *F. australis* may extend to at least half the shell thickness. Scales continuous with the outer shell layer are present on the ribs of *T. orbita* and intercostal laminae continuous with the outer shell layer are found in *L. biradiatum* and *T. orbita*.

Inner shell layer of Corculum cardissa

The inner shell layer of *Corculum cardissa* is discontinuous. Although the inner shell layer is continuous in the umbonal area, it rapidly loses its structural continuity toward the shell periphery as it thins, at least as resolved at the level of acetate peels. Over much of the shell the inner shell layer is confined to the convex areas lying at the inner margin of the shell windows, but appears to be absent between them (Fig. 19). The pallial myostracum is clearly discernible in the convex areas where the inner shell layer is expressed, but is not discernible between the convexities.

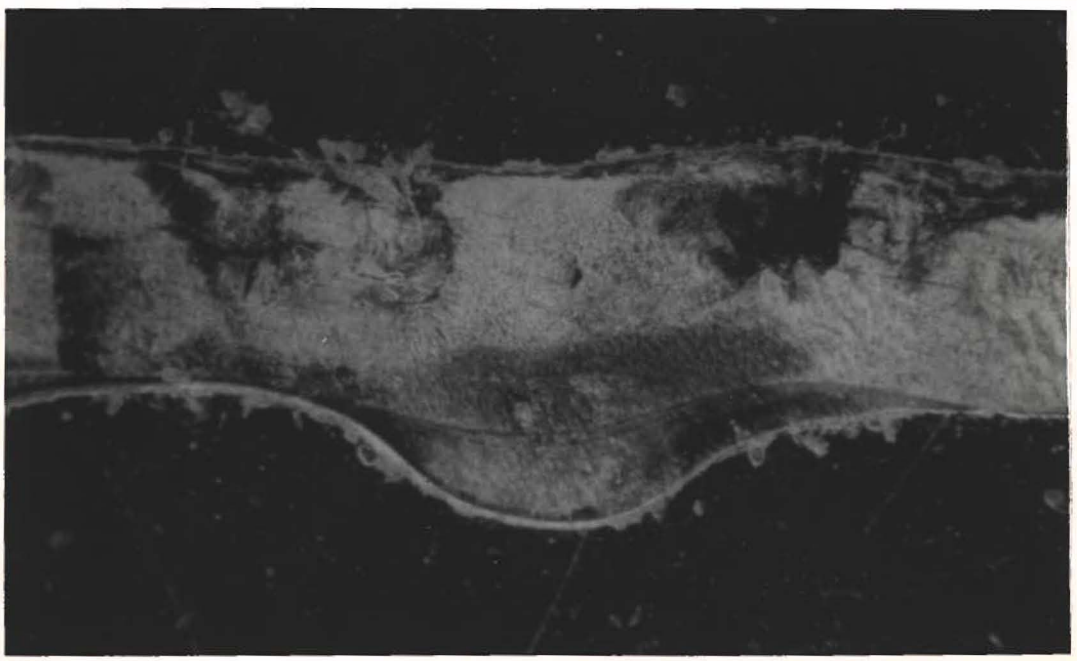

Fig. 19. The discontinuous inner shell layer of *Corculum cardissa*. Note the termination of the pallial myostracum at either side of the convexity. Transverse section, right valve, posterior slope, exterior at top, anterior slope to right. Acetate peel.

Pigmentation of Windowed Species

The shells of windowed species are characterized not only by variation in microstructure but also in shell pigmentation. While most of the shell is transfused with white pigment, like the entire shell of other fragines, some areas lack this pigment and appear translucent, both in whole shells and in shell sections. There is a general, but not perfect, correlation between the deposition of microstructural windows and areas devoid of pigment. Local patches of non-correspondence occur as microstructurally modified "windows" that are pigmented and unmodified BCL microstructure in the shell that is translucent. The FP outer shell layer is generally pigmented in the same manner as the underlying shell layers, indicating that FP microstructure in itself is not correlated with lack of pigmentation.

Growth Lines

The outer, growing edge of the shell in fragines is reflected toward the umbo so that the shell margin reaches furthest out at a level below the shell surface. This is reflected in growth lines, evident in sections, which curve toward the umbo, near the shell surface. In acetate peels where the FP sublayers are discernible, the curve in the growth line lies within the FP and corresponds to the zone where the orientation of prisms changes from outward to inward (*Fragum fragum, Fragum mundum, Fragum nivale, Fragum unedo, Lunulicardia retusum*). Hence, the fibrous prisms in the outer shell layer remain normal to the growth line. Although the sublayers of *Fragum sueziense* and *Microfragum festivum* are indiscernible on the acetate peels due to thinness of the outer

shell layer, the curve in the growth lines is visible and corresponds to the zone where the FP and BCL layers meet. Both of these growth line configurations are present in *Fragum loochooanum*, with the latter growth line configuration visible in specimens that have a reduced reclined sublayer. The FP sublayers of *Lunulicardia* sp. 1 are not discernible on the acetate peels due to thinness of the outer shell layer, and the sublayers of *Americardia media* and *Ctenocardia fornicata* are not discernible on the acetate peels due to discontinuity and paucity of the outer shell layer. As the thickness of the prismatic shell layer increases, so does the thickness of the area between the leading edge of the growth line curve and the outer shell surface.

The growth lines of *Trachycardia orbita* and *Fulvia australis* approach the exterior of the shell evenly and end at the surface with no reflection toward the umbo. *Laevicardium biradiatum* exhibits both reflected and non-reflected growth lines, perhaps corresponding to growth under ribs versus interstices.

DISCUSSION

Window Formation

Three of the fragines studied, *Fragum nivale, Fragum mundum,* and *Lunulicardia* sp. 1, have well-developed, microstructural windows in their shells, similar to those found in *Corculum*, the only genus previously known to have such features (Watson and Signor, 1986; Janssen, 1992a, 1992b; Carter and Schneider, 1997). Windows are evident in whole shells by shell transparency and associated, well-defined interior convexities. It is because of these whole-shell features that these species were selected for detailed microstructural study, confirming that the transparent areas are indeed microstructural windows. A survey of whole shells of most extant species of Fraginae revealed no other species with such clear, window-like features; thus, window development within the subfamily appears to be restricted to *F. nivale, F. mundum, L.* sp. 1 and species of *Corculum.* The only other species of Fraginae with window-like variation in shell transparency is *Fragum fragum*, which has an intermediate level of window differentiation.

Window architecture is very similar among *Corculum cardissa, Fragum nivale, Fragum mundum* and *Lunulicardia* sp. 1. Windows are formed of FP and DCP microstructures, usually with a concomitant reduction or absence of pigmentation, and are found only on the posterior portions of the shell. Windows are further denoted by slight convexities on the inner surface of the windows.

While window architecture appears to be identical among the two *Fragum* species and *Lunulicardia* sp. 1, it differs slightly from *Corculum*. The exterior of the posterior surface of *Corculum* has slight depressions over the windows, possibly increasing their lens-like nature; these depressions are lacking in the other three species. *Corculum* is also unique in having the horizontal sublayer of the FP outer shell layer reduced; this may enhance light transmission by minimally disturbing the path of light through the inclined to reclined prisms of the outer shell layer (Carter and Schneider, 1997). Unlike *Fragum nivale, Fragum mundum,* and *L.* sp. 1, the inner shell layer of *Corculum cardissa* is reduced or eliminated between convexities; this may also contribute to the lensing effect of the windows by preventing the diffusion of light away from the convex regions.

Thus, the windows of *Fragum nivale, Fragum mundum* and *Lunulicardia* sp. 1 appear to represent a stage in the evolution of windows toward the more specialized window of *Corculum*. *Fragum fragum* appears to represent a further intermediate stage in the evolution of windows, presenting a link between the windowed and non-windowed *Fragum* species. The posterior side of *F. fragum* has window-like transparent areas visible in the whole shell; however, these areas are much less translucent than the windows of *F. nivale et al.* This loss of translucency results from 1) a difference in the inner shell layer microstructure; *F. fragum* has the typical fragine CCCL inner shell layer instead of the FP microstructure found in *Corculum cardissa, F. mundum, F. nivale* and *L.* sp. 1, and 2) a usually incomplete development of FP and DCP microstructure in the middle shell layer.

Other than these variations in window development, shell microstructure was found to be conservative within the Fraginae. Typical fragine shell architecture consists of a FP outer shell layer, BCL middle shell layer, and a CCCL inner shell layer. This is similar to other cardiids except that many, but not all, lack the FP outer shell layer (Taylor, et al., 1973; Kobayashi, 1996; Schneider, in press, b; see outgroups above).

Some of my results differ from previous descriptions of window microstructure. 1.) I found the inner shell layer under the windows of *Corculum cardissa* to be FP, while Carter and Schneider (1997) reported the inner shell layer as predominantly CCCL with the DCP of the middle shell layer either terminating at the pallial myostracum or continuing a slight distance into the inner shell layer. 2.) I found a visible pallial myostracum in *C. cardissa*, as did Carter and Schneider (1997), while Watson and Signor (1986) noted the absence of a pallial myostracum. The pallial myostracum may be expressed intermittently in some species, as it was not evident in some of my sections of *C. cardissa* and *Fragum fragum.*

Carter and Schneider (1997) described the window microstructure in the middle shell layer of *Corculum cardissa* as DCP, contrary to Watson and Signor (1986) who described the windows in the middle shell layer as consisting of FP microstructure. Using SEM, I found DCP in the windowed areas of the middle shell layer in *Fragum mundum.* Since windowed areas in *Fragum nivale* and *Lunulicardia* sp. 1 appear similar to *F. mundum* in acetate peels, it is likely that they are DCP as well; however, this was not ascertained by SEM.

Watson and Signor (1986) noted that changes in shell pigmentation might enhance window transparency in *Corculum*. The windowed species in this study, including *Corculum*, show a reduction or absence of pigmentation in some, if not all, of their windows. However, the lack of perfect correlation between window microstructure and absence of pigmentation indicates that these two modes for creating translucent regions are not completely linked.

Origin of Photosymbiosis and Windows

Evidence from this study supports two origins of photosymbiosis, once in the Tridacninae and once in the Fraginae. Seilacher (1990) proposed that photosymbiosis evolved independently among the three cockle groups, Tridacninae, *Corculum*, and *Fragum*, and Janssen (1992a) proposed that photosymbiosis evolved once in a common ancestor to the fragines and the tridacnines. Schneider (in press, a) proposed that all species of Fraginae are photosymbiotic.

The Cardiidae originated in the late Triassic, the same period that witnessed the rise of the modern corals (Yonge 1975; Keen, 1980; Taylor et al., 1973; Schneider, 1995, in press, a). The Tridacninae emerged in the Eocene (Schneider, in press, a) with *Hippopus* and *Tridacna* arising in the Miocene (Cowen, 1983; Schneider, in press, a). The first Fraginae also appear in the Eocene, and *Fragum* appeared in the Miocene (Keen, 1980; Schneider, in press, a). The first *Corculum* fossils date to the late Pliocene and *Lunulicardia* to the Pleistocene (Paulay, 1996; Schneider, in press, a).

While all species of Tridacninae have been found to be photosymbiotic, zooxanthellate species of Fraginae appear to be restricted to three genera: *Corculum, Fragum,* and *Lunulicardia*; all examined species of which possess photosymbionts (G. Paulay, pers. com.). All examined species of the related genera *Americardia, Ctenocardia,* and *Microfragum* are azooxanthellate (G. Paulay, pers. com.), while the photosymbiotic status of the remaining fragine genera *Papillicardium, Parvicardium* or *Trigoniocardia* remains to be ascertained. However, it is likely that at least *Papillicardium* and *Parvicardium* lack zooxanthellae, as they are endemic to the cold waters of the north-east Atlantic, a habitat poorly suited to photosymbiosis. Furthermore, they inhabit the part of the world ocean that has received the greatest zoological scrutiny, and it is unlikely that researchers in Europe would have overlooked a photosymbiotic relationship if such existed.

Schneider (in press, a) found that *Parvicardium, Papillicardium* and *Trigoniocardia* are basal to other fragines, that *Ctenocardia, Americardia* and *Microfragum* form a well defined clade, and that *Lunulicardia* and *Corculum* are sister taxa. His results about the position of *Fragum* are ambiguous, and include several alternatives: *(Papillicardium (Parvicardium (Trigoniocardia ((Microfragum (Ctenocardia - Americardia)) Fragum (Lunulicardia - Corculum))))), ((Fragum (Microfragum (Ctenocardia - Americardia))) (Lunulicardia - Corculum)) and ((Microfragum (Ctenocardia - Americardia)) ((Fragum) (Lunulicardia - Corculum)))* (Fig. 3). The results presented here support a relationship between *Fragum* and

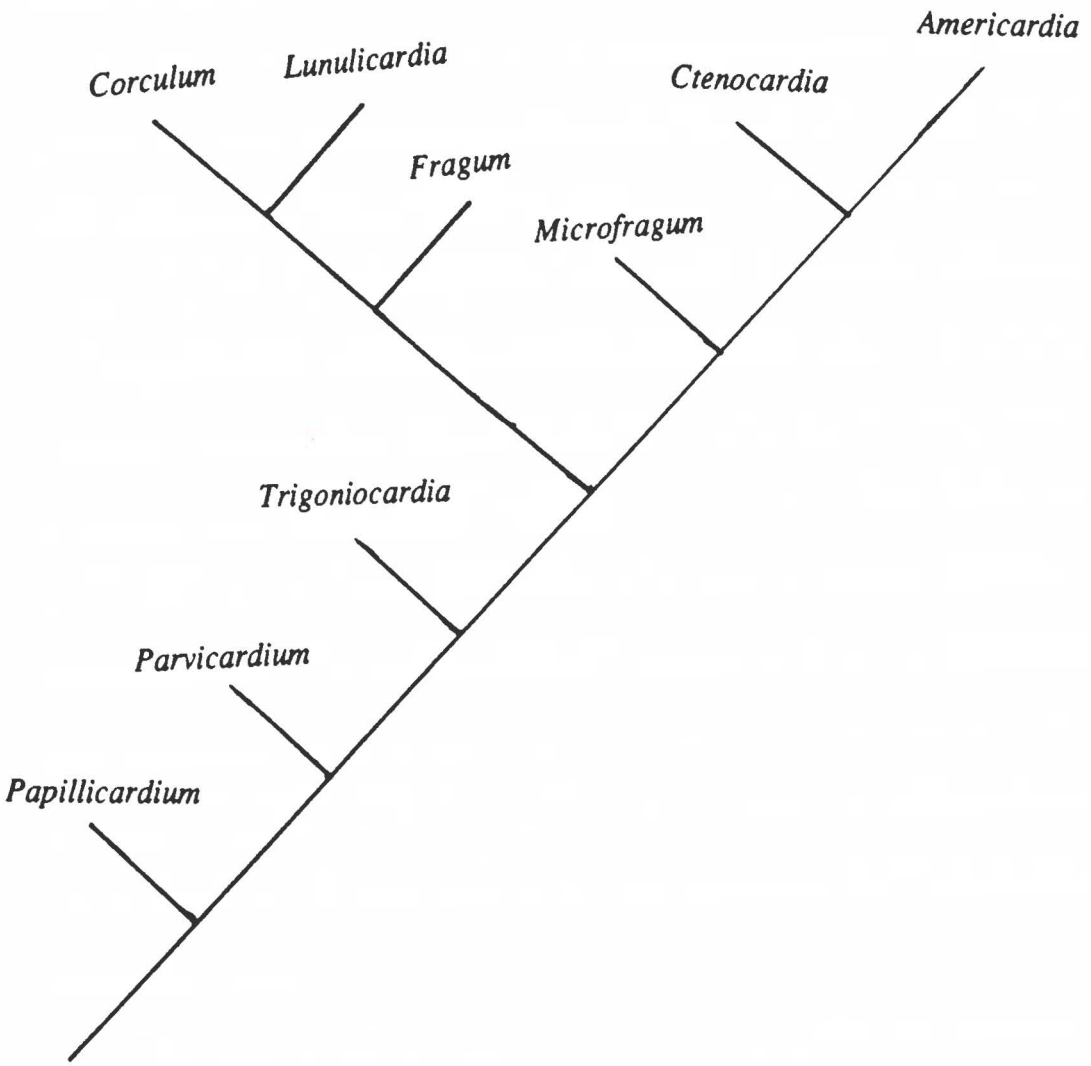

Fig. 20. Proposed phylogeny of the Fraginae based on Schneider (in press, a) and present study.

Corculum-Lunulicardia (Fig. 20); this group of genera appears to form a clade, characterized in part by a photosymbiotic lifestyle.

Fragum, Corculum and *Lunulicardia* are further united by the presence and detailed similarities of shell windows in two *Fragum* species, all *Corculum* species and one *Lunulicardia* species. *Fragum* appears to be a paraphyletic genus in which windows first appeared, and from which *Corculum* and *Lunulicardia* were derived. Evidence for the origin of windows in *Fragum* include numerous species without windows, *Fragum fragum*, a species with an intermediate level of window development, and *Fragum nivale* and *Fragum mundum*, which have a window microstructure almost as advanced as *Corculum*. Furthermore, *F. mundum* appears to live at the interface of the sediment, with its posterior above the substratum. Although I have not observed this species alive, many live-collected specimens have their posterior slope fouled by epibiota. This is an intermediate mode of life between epifaunal *Corculum* and the rest of the Fraginae, all of which are infaunal. I propose that a windowed species of *Fragum* was ancestral to *Corculum - Lunulicardia*. These latter genera share a number of synapomorphies, and have a much shallower stratigraphic record (Plio-Pleistocene) than *Fragum* (Miocene) (Schneider, in press, a).

One problem with this hypothesis is that the large species of *Lunulicardia*, *L. retusum* and *L. hemicardium*, lack windows. I suggest two possibilities for the absence of windows in the larger *Lunulicardia* species. First, windows may have been lost in the evolution of these species. Second, windows may disappear during ontogeny. In the first scenario, *Lunulicardia* evolved from *Fragum* as a windowed genus but *L. retusum* and *L.*

hemicardium lost their windows secondarily. In the second scenario, the juvenile form of *L. retusum* and *L. hemicardium* either looks similar to *Lunulicardia* sp. 1 or is in fact *L.* sp. 1, and windows are lost during ontogeny. Morphologically, *L.* sp. 1 is a small, windowed version of *L. retusum* and *L. hemicardium;* specimens with indented lunules and specimens without indented lunules occur in population samples. Since I did not examine the umbonal regions of the larger *Lunulicardia* species, I cannot confirm the occurrence of windows in their juvenile stages.

The origin and loss of windows may be a function of habitat depth: i.e. most windowed species live at very shallow depths, while species lacking windows commonly occur in moderately deep water. *Fragum fragum* is restricted to <3 m, while the windowed *Fragum nivale, Fragum mundum* and *Corculum* are all restricted to <1 m depths (Ohno et al., 1995; G. Paulay, pers. com.). All species of *Lunulicardia* (including *L.* sp. 1) are commonly found at >10 m, as are most non-windowed *Fragum* species (G. Paulay, pers. com.).

Morphological diversification among the photosymbiotic clams has been stimulated by the benefits of increasing the efficiency of light capture by zooxanthellae. The variations in shell shape, size, and transparency and exposure of tissues to light suggest a continuum of adaptations ranging from the wide gape and direct exposure of tissues to light in the Tridacninae, to the slight gape of the non-windowed infaunal *Fragum* species, to the windowed shells of *Corculum, Fragum mundum, Fragum nivale,* and *Lunulicardia* sp. 1.

Function of Windows

The modified shell microstructure and reduced shell pigmentation of *Corculum cardissa's* windows enhances light transmission through the shell, thus benefiting the photosymbiotic algae within its tissues (Watson and Signor, 1986; Ohno et al., 1995; Carter and Schneider, 1997). The fact that *Fragum nivale, Fragum mundum* and *Lunulicardia* sp. 1 host photosymbionts suggests that their windows function likewise.

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