Appendix 1

Preparation and Examination of Skeletal Materials for Growth Studies

Part A: Molluscs

2. Study of Molluscan Shell Structure and Growth Lines Using Thin Sections

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Early studies of molluscan shell structure, culminating in the classic work of Bøggild (1930), were based on examination of petrographic thin sections of shells. The grinding of such sections required a considerable investment of time and labor, and because of the tendency of many shells to split along growth surfaces or crystal boundaries, modern high-speed thin-sectioning equipment has not appreciably lessened this problem.

For this reason, acetate peels (see Appendix 1.A.1) have largely replaced thin sections in recent research on shell structure and growth lines. Although they provide much less information than thin sections, acetate peels can be prepared in a fraction of the time with little training.

New equipment, however, may again reverse the trend. The introduction of low-speed diamond saws, such as Buehler's Isomet 11-1180, makes it possible to prepare high-quality thin sections nearly as easily as acetate peels.

The micrographs in Plate I, which are all from a recent report on seasonal growth variations in *Mercenaria* (Clark, 1979; also see Clark, 1977), illustrate the type of information available in thin sections at relatively low magnifications. In addition to those features visible on acetate peels, these sections show variations in transparency, pigmentation, and crystallographic orientation. Higher-magnification studies can make use of the finite thickness of the thin section to make limited observations in three dimensions. Thin sections are also much easier than peels to store and study, having no surface relief to protect and no tendency to curl up.

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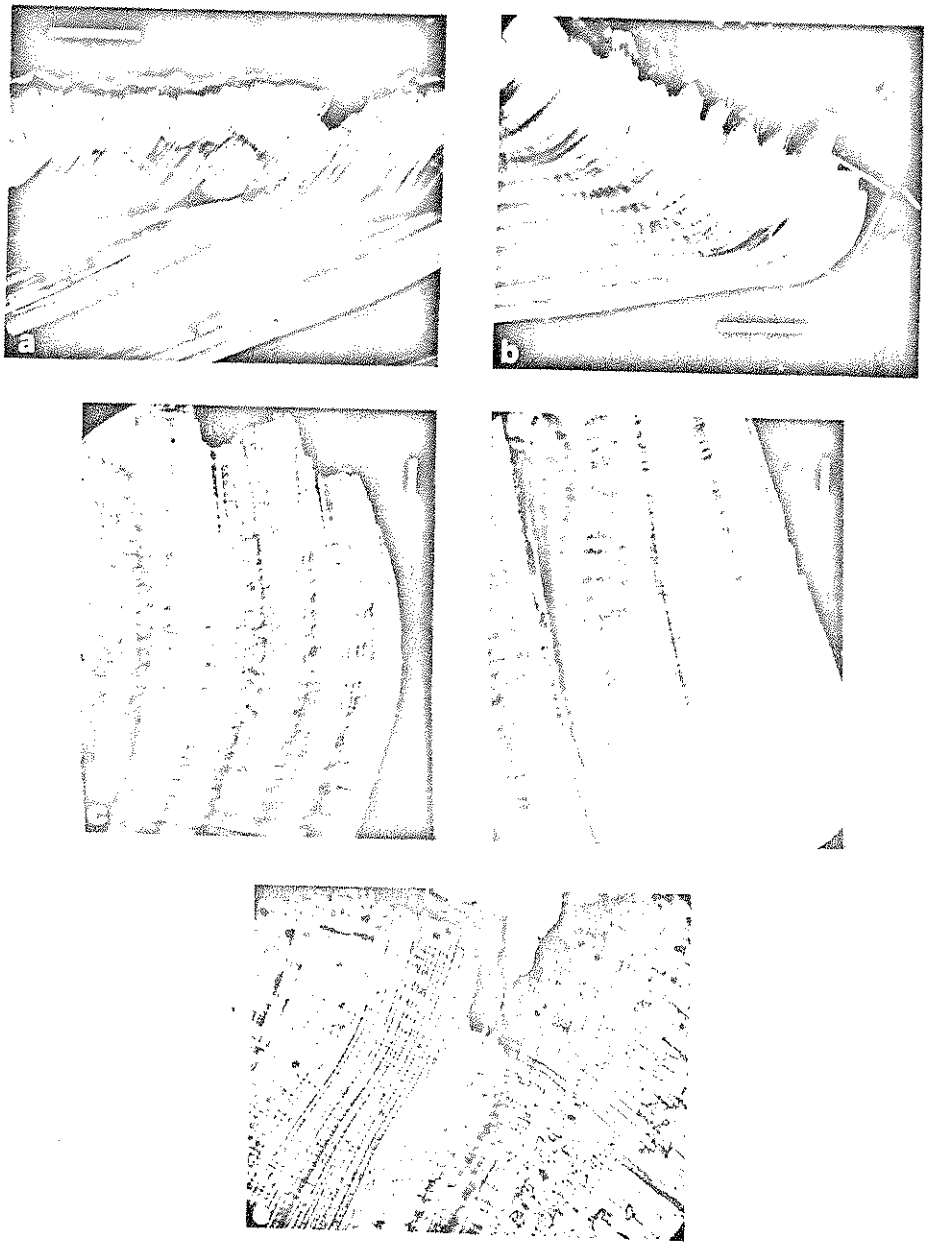
a
b

Since thin sections can now be prepared with little more effort than peels, they should become a useful supplement if not a replacement.

The first step, as in making a peel, is to cut the shell along the desired plane of section. This can be accomplished with no preliminary embedding on a low-speed saw. When the cut is completed, the sectioned surface is lightly sanded or ground using 600 or 1000 grit. Some care should be taken to ensure that only a single planar surface is present after this operation, since it is easy to develop two facets by a back-and-forth grinding motion. This surface should be thoroughly cleaned to remove grit, cutting oils, or other contaminants; ultrasonic cleaning may help if the shell is not too delicate.

After the section is dried, the sectioned surface must be cemented to a petrographic slide. I find that the bonds are improved when the slides are "frosted" beforehand with 600 grit. Both the section and the slide must be dry and free from oils or fingerprints. A variety of cements may be used, but thermoplastic cements such as Lakeside and high-temperature epoxies such as "Petropoxy" will often fail due to thermal stress on cooling. I find Ward's "Bioplastic," which cures at 70°C, satisfactory for most purposes. Only a few drops of cement should be used, for a large buildup of excess cement at the base of the section will prolong the cutting time and may warp the saw blade.

When the bond between section and slide is well developed, the slide should be mounted parallel to the saw blade and a second cut made about 200 μm (0.008 inch) from the surface of the slide. The section should be lowered carefully onto the blade to make sure the blade is not deflected by the cement.

On completion of the second cut, the slide can be ground by hand on a glass plate with 600 or 1000 grit in less than 10 min. The goal here is transparency, not a specific thickness, so progress can be readily judged and a tendency to grind too fast on one side can be compensated for before it is too late.

After being cleaned, the thin section can have a cover slip permanently attached with Canada balsam or epoxy, or it can be studied with a temporary cover slip using immersion oil as a bond. The latter method permits staining or additional grinding at a later time.

Plate I. (a) Thin section of mature region of a specimen of *Mercenaria mercenaria*. Scale bar: 1 mm. (b) Thin section of margin and senile region of a specimen of *Mercenaria mercenaria*. Rectangle indicates position of micrograph (c). Scale bar: 1 mm. (c) Thin section of the margin and outer shell layer of the senile region of a specimen of *Mercenaria mercenaria*. Scale bar: 0.1 mm. (d) Thin section of the middle shell layer and inner surface in the senile region of a specimen of *Mercenaria mercenaria*. Scale bar: 0.1 mm. (e) Thin section of the outer shell layer of the mature region of a specimen of *Mercenaria mercenaria*. Scale bar: 0.1 mm. All micrographs from Clark (1979).

References

Bøggild, O. B., 1930, The shell structure of the mollusks, *K. Dan. Vidensk. Selsk. Skr. Naturvidensk. Mathem. Afd. Ser. 9* **2**:231–326 (15 plates).

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