Preparing Whole Mounts of Hard Parts of Recent Bryozoa

ABSTRACT: Procedures are given for preserving, storing, cleaning and mounting calcareous Bryozoa on slides. Potassium hypochlorite digestion is preferred to incineration for cleaning because heated specimens eventually deteriorate. White glue is used instead of balsam to fix colonies to slides because balsam weakens with time. Ink or food coloring applied to dry specimens increases contrast.

Introduction

The overwhelming majority of byrozoan species deposit calcium carbonate in their body walls; bryozoologists rely heavily on these skeletal structures for taxonomic purposes. In many cases, it is possible to determine the species of the animal on the basis of the skeleton alone, but it is also frequently necessary to examine “chitinous” or cuticular structures, especially opercula, mandibles of avicularia, and setae of vibracula. This is the first of two papers describing the more important techniques used to preserve, mount, and catalogue these structures. Many of these methods are difficult to acquire for two reasons. First, many are unpublished; bryozoologists have passed on their techniques to their students by word of mouth, not troubling to set them down on paper. Second, published accounts are often too sketchy to be useful, and even these are scattered so widely in the taxonomic literature that they are difficult to locate.

Preserving Bryozoa

In the absence of the appropriate fixing fluids, calcareous Bryozoa may be preserved by drying, as long as the drying is rapid and thorough. If any trace of moisture is left, however, the animals will be damaged and sometimes destroyed by mold. It is usually adequate to set specimens in the sun for an hour or so. Once dry, spines and other delicate appendages become fragile and must be protected from crushing and abrasion. We are indebted to Mr. Russel Robinson for suggesting rolling dry material into several folds of soft toilet paper. Dry, boxed material is especially convenient to transport because it is light and easy to handle. Despite the advantages of simplicity, however, preservation by drying is to be avoided if possible. Non-calcified forms are almost always shriveled beyond recognition. Macken (1956:19) and Cook (1964:279) report some success in reviving dried ctenostomes by soaking them in 10% trisodium phosphate, but for most purposes dry ctenostomes are worthless. A second disadvantage of drying is that delicate cuticular parts of calcified Bryozoa may shrivel and become opaque; for some species, this may make identification difficult or impossible. Third, and most important, drying all but destroys cellular parts. Although soft parts may not often be essential in identifying Bryozoa, their loss forfeits the possibility of making many potentially valuable observations about the animals’ biology. It is preferable, therefore, to preserve specimens in fluid within a few hours after collection.

Acidic preserving fluids (Bouin’s, Zenker’s, Gilson’s, etc.) should be avoided because acid dissolves the calcareous skeletons. A 5–10% solution of formalin is excellent because it is inexpensive, easy to use and is a fairly good fixative. Formalin slowly oxidizes in air to formic acid, so it might be thought to attack the skeletons. Fortunately, however, the oxidation is very slow; furthermore, sea water and large amounts of calcium carbonate relative to formalin tend to buffer the fluid. Some formalin-fixed bryozoan specimens in the collections of the Allan Hancock Foundation are essentially unchanged after nearly 20 years of storage. For long storage, however, it may be wise to transfer valuable specimens to 70% alcohol.

Cataloging Specimens

For taxonomic work on calcareous Bryozoa, it is useful to dry some specimens and affix them to slides.
Slides have the advantage of being permanent, convenient, and easily stored, but they also have the disadvantages of dry material, just mentioned. For this reason, it is frequently necessary to retain several specimens of the same species, i.e. wet (alcohol or formalin) specimens and one or more dry slides. To keep track of specimens necessarily stored in two places, it is helpful to assign a number to each specimen lot. Specimens of a given species from one locality and collecting date receive a number different from all other specimens of the same species. To record specimens retained, a card for each number is filed alphabetically by genus and species. On each card is written how each specimen is preserved, the collecting locality, collector, and date. It is also a convenient place for notes on color, habitats, associations, etc. Furthermore, material removed for special treatment may be identified by only its name and a number.

Preparing Slides

Ideally, each study slide should include complete colonies or several fragments from the same colony, especially from near the growing margin. Three or four fragments should be chemically cleaned (see below); one or two others should be washed in distilled water to remove salt crystals, then dried. At least one fragment should be mounted face down to allow study of the basal surface.

Byrozoans generally encrust sturdy substrates, so it may be difficult to isolate fragments small enough and thin enough to mount conveniently on slides. There is no easy solution to this problem, but several methods are helpful. If the specimen encrusts a mollusc shell, particularly a pelecypod, a pair of heavy wire cutters may be useful to break away wanted parts. A dentist’s drill with a cutting wheel attached removes colonies from mollusc shells, but it is not of much use with really hard stone. If a lepadine shell is not available, colonies may be chopped off brittle rocks with a chisel and hammer. As a last resort, specimens attached to rock fragments too large to mount on slides may be stored dry in shell vials plugged with cotton.

Many workers use Canada balsam to glue specimens to slides. Bahamas, however, weakens with age; after about 5 years, specimens begin to drop off. “Airplane glue” (Duco cement and others) and water-soluble white glue (Elmer’s, Will-Hold and others), on the other hand, are flexible, permanent and quick-drying. Objects affixed with white glue show no change in adhesiveness after 10 years of storage. Furthermore, if it becomes necessary to free objects from slides, dry white glue may be loosened by soaking it overnight in water. Some workers prefer cardboard “foram mounts”; colony fragments may then be mounted with a drop of dilute gum tragacanth applied with a fine brush. A little formalin or clove oil in the tragacanth bottle prevents mold formation.

Cleaning the Skeleton

Byrozoa are classified largely according to the structure of the zoecium, or skeleton. For systematic purposes, it is important to see details clearly; since essential features are frequently obscured by the presence of dirt, epizoic organisms, and cuticular parts (epithecae), it is almost always useful to clean some specimens. It is important to emphasize, however, that cuticular parts are also important in systematics, and, since these are destroyed by the cleaning methods described here, unique specimens should be left alone.

There are two widely used methods of cleaning specimens of calcareous Bryozoa, incineration (“calcining”) and KOCI treatment. In the incineration method, described by Rogick (1945), dry specimens are held in a bunsen-burner or alcohol lamp flame directed with a mineralogist’s blow-pipe until organic material is burned away. Care must be taken not to heat specimens too intensely, or they will be reduced to a calcareous dust. Specimens may be incinerated directly on the substrate to which they are attached, or they may be broken away and placed in a spoon for heating. After incineration, colony fragments are usually mounted on slides, as described above.

Incineration has the following advantages: (1) it is easy and rapid; (2) specimens are rendered opaque, an advantage because details are easy to study and photograph; and (3) specimens can be cleaned directly on bulky substrates, so zoecial details may be studied without the bother of removing them to slides.

Unfortunately, the incineration method of cleaning has an important disadvantage. Incinerated specimens are at first fairly sturdy, but after a few weeks or months, depending on the intensity of heating and storage conditions, they become fragile, eventually crumbling to unrecognizable fragments. The intense heat of the blow-pipe seems to decompose the organic matrix of the skeleton and probably also causes crystal changes in the calcium carbonate as well. Many irreplaceable bryozoan preparations have been damaged beyond saving because systematists did not recognize this serious drawback. The incineration technique may still have a place in processing large collections where abundant material is available for sacrifice, but the worker must bear in mind that incinerated material is irreplaceable, and that the lifetime of heated specimens is greatly shortened.

For permanent slides, the only acceptable cleaning method seems to be treatment with a neutral or alkali chemical oxidant; the solution of choice is “eau de Javelle”, a 5% aqueous solution of potassium (or sodium) hypochlorite, KOCI (NaOCI). Commercial liquid laundry bleaches (Purex, Chlorox, etc.) are perfectly satisfactory. Specimens may be wet or dry, living or fixed. KOCI acts rapidly, so material is usually ready after only an hour or so of soaking; boiling reduces the time to a few minutes. Frequently, however, small fragments of tough cuticles persist stubbornly, so it is usually safer to allow the solution to act overnight. The bleach is poured off and the specimen is washed in several rinses of distilled water. The specimen may be blotted by tumbling it about on absorbent paper with a brush, then laid on a slide to dry (1–3 hr.).

In practice, it is convenient to isolate several colony fragments and then place about half of them in a shell vial full of KOCI solution. The remaining fragments are placed in an empty vial to dry. A labelled slide is