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Preparation, Imaging, and Conservation of Paleozoic Bryozoans for Study

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PART G, REVISED, VOLUME 2, CHAPTER 3: PREPARATION, IMAGING, AND CONSERVATION OF PALEOZOIC BRYOZOANS FOR STUDY

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INTRODUCTION

Various techniques have developed over the last 150 years for the study of Paleozoic bryozoans. While a number of these have become somewhat redundant with the advance of modern approaches, or may only be applied to bryozoans preserved in particular ways, this review examines all of these procedures and outlines the methodologies of such techniques and how they may be used effectively. Three useful compendia of preparation and study methods and techniques applied to fossils are those by KUMMEL and RAUP (1965); FELDMANN, CHAPMAN, and HANNIBAL (1989); and GREEN (2001). Many of the schemes described therein can be applied to the study of Paleozoic Bryozoa.

REMOVAL OF MATRIX

Bryozoans are frequently found embedded in matrix of various lithologies. Though they can be studied through thin sections, often it is desirable to extract the zoaria from the matrix. This can often be a difficult and delicate process, and different techniques have been adopted depending on the nature of the matrix.

SHALES

Paleozoic bryozoans are often preserved in shales. In some cases, natural weathering processes yield isolated zoaria over time, which can be easily collected in the field. The Ordovician bryozoans of the argillaceous Benbolt Formation, Rye Cove, Virginia, USA (BASSLER, 1952), and those from a number of Cincinnatian units in the Ohio Valley, such as the Eden Shale (ANSTEY & PERRY, 1973), were often collected loose from surfaces, as were a number of Pennsylvanian faunas from near Richmond, Yorkshire, UK (VINE, 1881; WYSE JACKSON & BANCROFT, 1994). Collections of loose material should be sieved through a stack of sieves of various mesh sizes, and small zoaria recovered. In general, it is useful to mechanically break up shales by means of soaking in water, boiling (see Key, ZAGORŠEK, & PATTERSON, 2013, p. 307), and/or alternately freezing and thawing the samples (KESLING & CHILMAN, 1978) before embarking on chemical or ultrasonic treatments.

Mechanical Separation Using Asphalt

Frequently, the reverse surface of fenestrate bryozoans is revealed when slabs are split. This is because that surface is generally smoother than the obverse, which may contain keel nodes. In the late 1870s, John Young, Glasgow, UK, developed a technique for revealing the obverse surface features of fenestrates preserved in shales of Mississippian age from some localities in Scotland (YOUNG, 1877). Specimens were heated to drive off any moisture before a 3-5 mm-thick layer of hot asphalt was applied to the surface from a heated spoon. Before it cooled, a piece of brown paper (nowadays a piece of plastic can be used) was pressed into the asphalt. Then, using a disaggregating agent, the shale adhering to the obverse surface can be removed (see below). If this is done before the asphalt is applied, the

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Wyse Jackson, Patrick N., & Caroline J. Buttler. 2015. Part G, Revised, Volume 2, Chapter 3: Preparation, imaging, and conservation of Paleozoic bryozoans for study. Treatise Online 63:1–15, fig. 1.

specimen usually disaggregates into tiny pieces. YOUNG (1877) also suggested that the asphalt layer could simply be pulled away from the shale and the obverse revealed.

Disaggregation Using Surfactants

Bryozoa in shaley, muddy sediments may be extracted using a surfactant (see PETERSON, MAPLES, & LANE, 1983). Until the 1990s, Quaternary-O (ZINGULA, 1968) was utilized for this purpose; although this material is no longer on the market, others are available. A tablespoonful of surfactant is put into a beaker of boiling water and stirred until dissolved. This is poured onto about 2.5 kg of shale in a bucket. Further boiling water is added until all the slabs are covered and the whole mixture is simmered for four hours (Peterson, Maples, & Lane, 1983). The surfactant acts on the outer portions of the slabs, loosening the bryozoans from the muddy matrix. The slabs are rarely totally broken down. The buckets should be allowed to stand for 48 hours, after which the liquid is drained off and the bryozoan-rich residue removed. Any residual mud should be washed away through stacked sieves, leaving the delicate calcified specimens behind. These are dried in the sieves overnight in an oven.

Kerosene

Shale pieces are placed in a bucket of kerosene or other mineral spirits for a number of days. The kerosene is then poured off and stored for subsequent use. Warm water is added to the shaley-residue and left for a few days. The water seeps into the pore spaces and displaces any remaining kerosene, and following this proceedure, the residue should be washed and rinsed in water to which a detergent has been added. This will remove any traces of the mineral spirits. The rinsing process should be repeated several times. This method has been usefully employed to separate out trepostomes from the Eden Shale (PACHUT & ANSTEY, 1979) and delicate Permian bryozoans from shales and mudrocks (NEWTON, 1971); it is also used for the separation of microfossils, including

ostracods (Kesling & Chilman, 1978) and conodonts (Varker, 1987).

Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) , which is a strong oxidizing agent, has been used extensively to clean and remove adhering debris from post-Paleozoic bryozoans (see BERNING, 2006, who used a 10% solution on Miocene faunas from southwest Spain). While also used for the preparation of many Paleozoic fossil groups, it has not been used extensively on Paleozoic bryozoans. RICHARDS (1974), in a study of the Devonian ctenostomes Immergentia and Ropalonaria that bored into brachiopod shells recovered from the Plum Brook Shale, Ohio, placed the specimens in hydrogen peroxide for three days. This procedure was effective in removing marcasite and other sediment from the bryozoan borings.

Hydrofluoric Acid Treatment

A method that has been used to good effect on microcrinoids (SEVASTOPULO & KEEGAN, 1980) may have applications in bryozoology, although we do not know of its utilization for such. The shales and mudstones are broken down into small fragments and then 48% hydrofluoric acid (HF) is added for up to an hour for small specimens. For larger specimens 6% HF is left to react with the material over 24 hours. Fluoridization occurs of the crinoid stereom as a result of the faithful replacement of the original calcite by fluorite. Care should be taken when using this acid method, which should be carried out under a fume-hood.

Ultrasonic Disaggregation

The use of ultrasonic tanks has been employed for some time for the disaggregration of shales (GIPSON, 1963). LAGUROS, KUMAR, and ANNAMALAI (1974), in a comparative study of natural versus artificially induced weathering of six shales from Oklahoma, demonstrated that the use of ultrasonic treatment produced physically similar results to natural weathering. However, under natural conditions, there are often mineralogical alteration of the clays, which is not reproduced in artificial conditions. Nevertheless, ultrasonic treatment can be beneficial in cleaning off small volumes of adhering matrix left behind after other treatments have been completed. Caution needs to be taken not to run the treatment for too long as there is a danger that the bryozoan zoaria may begin to fall apart if left in the ultrasonic tank for greater than 30 seconds. However, KEY, ZÁGORŠEK, and PATTERSON (2013) used ultrasonic disaggregation for 2 minutes on Miocene bryozoans with good results.

Waterblasting

Waterblasting is a recently developed technique that has been successfully used to remove surface debris from fossils liberated by acid-etching methods from Cretaceous chalk and loosely consolidated carbonates (NIELSEN & JAKOBSEN, 2004). It can also be used to clean the surfaces of limestone blocks to reveal embedded fossils. A standard water pressure washer emits a jet of water at up to 150 bars and a variety of nozzles can be fitted to produce sprays of a variety of shapes, from a fan for surface cleaning to a point for more directed work. While the method has not yet been used on Paleozoic bryozoans, it may prove to be of value.

LIMESTONES AND CHALK

Various methodologies have been developed to free calcareous and siliceous fossils from a limestone matrix. The process used is dependent on the nature of both the fossils and the porosity and degree of lithification of the limestone itself. Calcified Paleozoic bryozoans can be very difficult to separate out, whereas post-Paleozoic bryozoans from some carbonate lithologies have proved easier to liberate. Some methods successfully used on the younger carbonates are outlined here as they may have applications for Paleozoic carbonates.

Acid Treatments for Silicified Specimens in Limestone

Where bryozoans have been silicified, they can be extracted from the carbonate

matrix by acid digestion. Blocks should be broken down in a mechanical jaw-crusher into fist-sized pieces (with higher surfacearea-to-volume ratios), which etch faster than large boulders. Two kilograms of fragmented rock pieces are combined with 4 pints of 85% formic acid in plastic buckets, which are then topped up with warm water. This speeds up the etching process. After approximately 24 hours, the spent acid should be poured away and the residue containing the exhumed bryozoans should be strained through a stack of wire sieves whose mesh diameter ranges from 120 µm to 420 µm. When some large limestone pieces remain, the digestion process should be repeated. Residues should be slowly dried in the sieves over a warm hotplate or in an oven. Following this, the specimens can be picked out under a microscope using a miniature (000) brush, and stored in cavity slides.

Acid digestion of Mississippian limestones from Ireland in two studies, one by TAVENER-SMITH (1973) and one by WYSE JACKSON (1996), have yielded a bryozoan diversity of over eighty taxa. Similarly, the Permian successions of the Glass Mountains, Texas, USA, have produced numerous brachiopods (COOPER & GRANT, 1972) and important fenestrates (ELIAS & CONDRA, 1957, in which study the order Fenestrata was erected; GAUTIER, WYSE JACKSON, & MCKINNEY, 2013).

Acid Treatments for Calcified Specimens in Limestone and Chalk

In chalk and loosely lithified carbonate sediments, fossils can be liberated by digestion in almost pure acetic acid. This method relies on the fact that the acid dissolves the microcrystalline calcite matrix more rapidly than the macrocrystalline calcite comprising the fossils (ZAGORŠEK & VAVRA, 2000). Acid is added to the limestone and heated to 80°C for up to 12 hours per day. After one to twelve weeks, a precipitate forms in which the freed fossils are contained, and this is removed, washed rapidly, and sieved.



FIG. 1. Orientations for thin sectioning. *1*, Line drawing of several fenestellid fenestrate branches partially cut away to show three oriented sections (new); *2*, line drawing through trepostome showing three oriented sections (adapted from Madsen & Hakånsson, 1989).

A similar technique is that of using a mixture of acid and hot water (NIELSEN & JAKOBSEN, 2004). Highly concentrated (98%) acetic acid is added to the limestone, which has been thoroughly dried beforehand. The mixture is left for 50 minutes, allowing the acid to saturate the rock. The acid should then be decanted, and boiling water containing soda ash added. Immediately, a chemical

reaction produces carbon dioxide and this mechanically disaggregates the limestone after about 30 minutes (NIELSEN & JAKO-BSEN, 2004). Once sieved, washed in cold water, and dried, the material will be ready for examination. Care should be taken when using this acid-and-hot-water method, which should be carried out under a fume-hood.

Chalk and other calcareous sediments can also be disaggregated using a hot solution of Glauber's Salts (HERRIG, 1966), which are added to the rock. As the solution cools down, salts crystallizes in the pores which causes the rock to fall apart. This method is more time-consuming than the acid-hot water method described above. REMIN and others (2012) have developed a method utilizing liquid nitrogen (LN₂), which they have shown to produce better results than those utilizing Glauber's Salts.

THIN SECTIONS

To accurately identify Paleozoic stenolaemate bryozoans, precisely oriented thin sections have to be made. It is essential that sections are made in three orientations longitudinal, transverse, and tangential—so that the 3-D (three-dimensional) shape of autozooecial chambers and other features can be determined (Fig. 1).

HISTORY OF USE OF THIN SECTIONS IN BRYOZOAN STUDIES

The first thin section of fossil material was produced by the Scottish mineralogist William Nicol (c. 1768–1851), who sectioned petrified wood (PIRSSON, 1918; WYSE JACKSON, 2008). However, thin sections first came into general and widespread use from 1849, when Henry Clifton Sorby (1826–1908), Sheffield, UK, began his study of rock textures and structures (JUDD, 1908). He pioneered their use for petrological and metallurgical studies.

The first bryozoan thin section dates from the mid-1840s and is that of a specimen of *Diplotrypa petropolitana* NICHOLSON, 1879, from the Ordovician of the Sias River, south of Lake Ladoga, Russia. It is now in the collections of the Natural History Museum, London (Wyse Jackson, 2008).

The general adoption of thin sections for bryozoan studies can be dated back to the 1870s when Henry Alleyne Nicholson (1844–1899) began to produce them for his work on Cincinnatian (Upper Ordovician) and other bryozoans. His published illustrations of thin-section views appeared first in 1876 (NICHOLSON, 1876; CUFFEY, DAVIS, & UTGAARD, 2002), and then in two popular and important monographs on corals and bryozoans (NICHOLSON, 1879, 1881). Nicholson realized that characterization of the internal features of many bryozoans was essential for better taxonomic discrimination (NICHOLSON, 1879, p. x, 1884).

Soon afterwards in the United States, Edgar Oscar Ulrich (1857–1944) and Ray Smith Bassler (1878–1961) began to manufacture thin sections of fossil bryozoans in large numbers, and many of these were sold to augment their incomes (BOARDMAN, 2008). The thin sections were produced by hand, grinding the specimens down on a sandstone slab (BOARDMAN, 2008, p. 4), before mounting the specimen on rather thick window glass (which was cheaper than thinner glass) measuring 1 by 3 inches.

Similarly, in England at approximately the same period, the amateur George Robert Vine (1825–1893) began to manufacture thin sections, which he sold and exchanged (BUTTLER, WYSE JACKSON, & SHARPE, 2002; Wyse Jackson, Buttler, & Sharpe, 2003). J. F. James, who published on Cincinnatian bryozoans in the 1880s, argued that producing thin sections was difficult and the results unreliable (JAMES, 1887), and not necessary for bryozoan studies as "internal characters are often misleading" (JAMES, 1888, p. 50). FOERSTE (1887) countered this argument and pointed to the pioneering microscopical work of Nicholson and Ulrich, remarking that "the difficulty of making sections is a myth" (p. 226); he later noted that it was not uncommon for even a student to be able to produce between

forty and sixty bryozoan thin sections a day (Foerste, 1888).

From 1879 onwards, Ulrich made considerable use of thin sections in his studies of North American Paleozoic bryozoans, during which he erected the suborder Trepostomata. By 1890, the use of thin sections in trepostome bryozoan studies was routine, as it had been demonstrated that they provided critical internal taxonomic information of value. Thin sections were not routinely used for fenestrates until the 1960s.

In some rare studies, the size and nature of the material has allowed for serial sections to be taken. Using such a series, PERRY and HATTIN (1958) described the astogeny of a number of fistuliporoid cystoporates, while Ross (1960) determined the budding patterns in the cryptostome *Ptilodictya lanceolata*.

PREPARATION OF THIN SECTIONS

To prepare thin sections, specimens need to be cut to required orientations. McNAIR (1938) suggested mounting fragments in plaster of Paris, which allows them to be manipulated and placed in the correct orientation before being embedded in Bakelite prior to grinding. However, these mounting media proved unreliable and later workers began to use epoxy resin instead (NYE, DEAN, & HINDS, 1972).

Small, isolated specimens can be embedded in a block of resin to make cutting easier. The cut surface is ground down and then polished, using silicon carbide powders to remove any fine scratch marks remaining from the grinding process. The surface needs to be completely flat or it will not bond to the glass slide. The specimen is then cleaned ultrasonically and dried. The glass slides are polished and cleaned to provide a flat surface for the specimen. The bryozoan is attached to the slide with epoxy resin, which must be optically transparent and have the correct refractive index. (In the past, sections were made most commonly with Canada Balsam, produced from the resin of Abies balsamea, the Balsam Fir tree, but Canada Balsam will oxidize with time and turns yellow-brown in color.) Once the specimen is adhered to a glass slide, its excess is trimmed off using a thin sectioning saw. The specimen is ground and polished to the correct thickness; different preservations will require slightly different thicknesses. The slide is then cleaned and a cover slip applied. For examination in a SEM (scanning electron microscope), sections should not be covered, but need to be polished and etched. In general, standard thin sections measure three inches by one inch, although some laboratories now have facilities that can produce slides three inches by two inches in size.

In contrast to standard thin sections, carbonate petrologists often utilize ultra-thin sections between 2 µm and 12 µm thick to determine carbonate fabrics (LINDHOLM & DEAN, 1973). These sections are best prepared (following techniques described in LINDHOLM & DEAN, 1973; WASS, 1979) by polishing one side of the fossil surface to a glassy finish, mounting it on the glass slide, and then grinding the exposed surface down to a thickness where first-order gray/ yellow-orange is seen, and then polishing that surface to a glassy finish (KOLUMBAN & CUFFEY, 1993). Delicate and subtle features of skeletal ultrastructure in modern and fossil bryozoans may be revealed in such sections (WASS, 1979, fig. 1a).

Thin sections are best stored flat in purpose-built thin-section cabinets (GOODWAY, 1992). Thin-section storage boxes, in which slides are stored vertically, are a cheaper option but care should be taken if the mounting medium is in any way unstable over time. Slides can be numbered and labled using a diamond-tipped pen to etch numbers into the glass, or details can be given on labels stuck to the glass slide.

ACETATE PEELS

Acetate peels have been used to study the interiors of bryozoans as an alternative to thin sections. These are impressions of an etched polished surface in an acetate film. The advantage of acetate peels over thin sections is that they are quicker to make, do not destroy as much of the specimen, and are an easy way to examine large surface areas of rock samples. They are also valuable for population studies when large numbers of sections need to be examined.

Acetate peels were first developed by paleobotanists using a solution of nitrocellulose, butyl acetate, tricresyl phosphate or methyl phthalate, and toluene or xylol (WALTON, 1928; GRAHAM, 1933). This was painted onto a polished, etched surface and dried to a thin film, which could be peeled off the specimen. STERNBERG and BELDING (1942) refined this method by using thin cellulose acetate film, and BOARDMAN and UTGAARD (1964) developed it further in the study of bryozoans by using microslides made of cellulose acetate rather than thin film. BISSELL (1957) and KATZ and FRIEDMAN (1965) showed how the addition of various stains to the acetate peels could enhance the textural details seen in various carbonates. KOENIG (1954) was the first to use acetate peels in the study of fenestrate bryozoans, and showed how the peels obtained could be used as photographic negatives. More recently, SORAUF and TUTTLE (1988) developed a dark-field illumination methodology for producing publication-quality images from peels. With modern scanners, it is possible to scan peels at a high resolution and to inverse the image to provide good positive electronic images.

To produce an acetate peel, the rock or specimen is first cut in the required orientation. The cut surface is then polished using silicon carbide powders to remove any blemishes or marks produced by the saw blade. Polishing is undertaken with successively finer grades. After the specimen is polished, it is etched with a 10% HCl solution—stronger solutions are not recommended because they can destroy fine wall microstructure details. Sheets (in a variety of thicknesses, 180–900 μ m) or slides of cellulose acetate are used to make the peel. For thinner sheets, the cut surface of the specimen is mounted horizontally and the flat surface of the specimen is flooded with acetone. A sheet of acetate is applied to the specimen by bending it in half, pressing the fold in the center of the specimen, and gently rolling the sheet out over the specimen to evacuate any air bubbles. The specimen should be then left to dry for 30 minutes or more, after which the acetate peel can be carefully removed.

To keep the peel flat and prevent it from curling and distorting, it should be mounted between two glass slides, which can be taped together. If thicker cellulose acetate is used, the acetone can be applied to the sheet and the specimen pressed into it. The thicker sheets do not curl as much as the thinner ones do.

Acetate peels have some advantages and some disadvantages compared with thin sections. While both processes are destructive, numerous peels can be taken serially, at very close intervals, through a specimen that would usually only produce one thin section. Serial sections using acetate peels have been employed for, among many things, the reconstruction of the unusual four-sided chamber shapes developed in some trepostomes, including Rhombotrypella (BOARDMAN & MCKINNEY, 1976) and the budding of those chambers (MCKINNEY, 1977). They have also been employed for the determination of space filling in trepostomes with wide exozones, as seen in Tabulipora from the Permian of Greenland (Key, THRANE, & COLLINS, 2001); and the recognition, also in Tabulipora, of the shape and increased complexity of maculae from initial propagation to manifestation at the zoarial surface (Key, Wyse Jackson, & Vitiello, 2011). Acetate peels also allow for numerous pulls to be taken of the same surface until the best results are obtained. In addition, peels can be of a greater surface area than the largest thin-section facilities allow.

Unfortunately, acetate peels do not provide the detailed microstructure seen in a thin section (see TAYLOR & others, 2011, for a discussion of this loss of resolution in fossil plant preparations). They may not have longterm stability; cellulose is known to degrade with time and if acetate peels are not stored in stable environmental conditions, they may start to deteriorate (GOLDEN, 1995). This may result in a dimensional change that will prevent accurate morphometric measurements being taken from them. Thus, acetate peels should be digitally scanned, as discussed above, immediately after production. It is not recommended that acetate peels be used when erecting a type specimen for a new species; thin sections will have greater longevity.

Acetate peels should be stored flat, ideally in a thin-section cabinet, in a cool, dark environment. Peels can be labeled and numbered directly using Indian ink.

SERIAL SECTIONING

Since the early 1900s, serial sections have been utilized to determine the internal features of fossils and, in some studies, reconstruct their 3-D shape (SOLLAS, 1903). While much of this method relied on laboriously hand grinding or slicing specimens, equipment was developed in the 1940s that speeded up the process and allowed the user to produce consistently spaced ground surfaces 0.5 mm apart (OLSEN & WHITMORE, 1944). The details of these surfaces could be drawn, photographed, or acetate peels could be taken from them. CUMINGS (1904, 1905) provided much information on the early astogeny of fenestrate bryozoans using this method.

Over the last thirty years, new, nondestructive methods of investigating the internal features of fossils have been developed (see below), but still it is necessary in some cases to serial grind specimens in which there is little contrast between the fossil and the encasing matrix (SUTTON & others, 2001b; BUTTLER, RAHMAN, & SLATER, 2012).

NONDESTRUCTIVE METHODS FOR REVEALING INTERNAL STRUCTURE X-RAY IMAGING

Since the discovery of X-rays in the late 1800s, scientists have utilized them to image

the internal features of various materials. TRILLAT and ROGER (1947) discussed the application of X-rays in paleontological studies, and ROGER and BUGE (1947) were the first to use them in a study of bryozoans from the Pliocene of France. More recently, X-ray radiographs of the blade-shaped, modern Antarctic bryozoan *Melicerita* have revealed distinctive, annual growth banding that was not appreciably visible on the surface (BADER & SCHAFER, 2004).

THREE-DIMENSIONAL IMAGING TECHNIQUES

In the last 20 years, a number of threedimensional techniques, such as neutron tomography, optical tomography, X-ray computed tomography, and confocal laserscanning, have been developed that have found application in paleontological studies (SUTTON & others, 2001a; SUTTON, 2008; MALLISON, 2011). X-ray scanners can now capture multiple images through even the smallest of fossils. These scanners image a series of slices through the fossil taken at micron-scale intervals. The subsequent rendering of these images into 3-D images or videos of virtual fossils can now be easily achieved using most standard computers with the necessary software (SUTTON, & others, 2001a; Abel, Laurini, & Richter, 2012).

CT Microtomography

X-ray computed tomography (CT) is a relatively new tool that has been used by a number of researchers studying modern and fossil bryozoans. Images are produced by scanning the specimen using a microtomograph at a resolution as low as 2 µm (SCHMIDT, 2012). These images can either be used as stand-alone 2-D images or united together into a 3-D rendition of the fossil.

The technique was used by TAYLOR, HOWARD, and GUNDRUM (2008), in investigating a number of cyclostomes from the Cretaceous and Recent, and SCHMIDT (2012) provided a reconstruction of the internal features of the modern cyclostome *Siphonicytara occidentalis* from offshore Western Australia.VISKOVA and PAKHNEVICH (2010) were able to use this technology to illustrate pertinent taxonomic features, which were hidden from view within the host substratum, in the Middle Jurassic, boring bryozoan *Orbig-nyopora opulenta*. These included dimorphic autozooids that were previously unknown in the genus.

In Paleozoic fossils, the methodology may prove difficult to apply. Where the mineralogical composition is similar in both the matrix and fossil, CT microtomography may not distinguish between the two (SUTTON & others, 2001b). In these cases, a scan of the external features of the specimen is taken, while details of the internal features are gathered from photographs of polished surfaces serially ground down through the specimen. With a combined approach, using X-ray and physical-optical tomography, approach, BUTTLER, RAHMAN, and SLATER (2012) rendered both external and internal views to assemble a detailed 3-D image of an Ordovician trepostome bryozoan colony from the Kanosh Formation, Utah, USA. Where there are mineralogical differences between the bryozoan skeleton and the infilling cement or matrix, these contrasts may be recognized by microtomography and high-quality images and 3-D renditions produced. In such a situation, Wyse JACKSON and MCKINNEY (2013), in a study of the type material of the fenestrate bryozoan Polyfenestella from the Mississippian of Scotland, used CT microtomography to resolve the nature of the polymorphs in this taxon.

Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy utilizes a small laser beam directed onto a specimen to produce focused images at various depths through a specimen. This method has been used by WANNINGER (2007) in an examination of bryozoan larva from modern bryozoans.

REPLICATION AND CONSERVATION MOLDING AND CASTING

A variety of materials have been used to mold and cast Paleozoic bryozoans. In the 1880s, a series of Plaster of Paris casts were made of the fenestrate taxon *Ptiloporella*, erected by James HALL (in HALL & SIMPSON, 1887), from the Devonian of Ontario, Canada—these are now in the Field Museum, Chicago. Plaster was commonly used for casting, being cheap and easy to use. However, it is not very durable. PVA glue can be added to the plaster to increase its strength (WAUGH & ERICKSON, 2002).

Latex can easily produce molds and casts and has been used for many years. To produce a mold, successive layers of latex are applied to a specimen, beginning with a thin layer followed by thicker ones. The mold can be strengthened with the addition of gauze between the layers. Casts can be produced simply by applying latex directly to moldic fossils, such as was demonstrated by ENGEL (1975) in a study of Mississippian septatoporid fenestrates from Australia. Latex produces quick, cheap molds and casts that replicate delicate surface ornamentation, and these can be photographed directly using conventional photographic methods (ENGEL, 1975) or at high magnifications in a scanning electron microscope (SIVETER, 1982). However, these latex casts do not have a long life-span; they shrink (SIVETER, 1982) and become brittle with age. A more durable product for molding and casting is silicon rubber (KELLY & MCLACHLAN, 1980), which has been used in recent studies to produce casts of moldic late Paleozoic bryozoans from Antarctica (KELLY & others, 2001) and Cretaceous bryozoans from Japan (DICK, OSAWA, & NODASAKA, 2009). These casts were suitable for SEM examination.

A variety of synthetic resins have been used to make casts. ZAPASNIK and JOHNSTON (1984) used a novel method to replicate calcareous fossils or moldic fossils in a clastic rock with plastic. The technique involved three stages. The first was the dissolution of the carbonate in fossiliferous rocks with hydrochloric acid. The resulting voids were then impregnated with liquid plastic and then the clastic rock matrix was removed with hydrofluoric acid. This left a concentrate of plastic-replaced fossils. The casts replicated the delicate structures of fenestrate and cryptostome bryozoans found in the Ulladulla Mudstone (lower Permian, New South Wales, Australia), which could then be examined under the SEM. The only drawback to the ZAPASNIK and JOHNSTON (1984) method is that it replicated the external surface of the specimens only and no internal features were revealed.

Endolithic borings made by bryozoans in shells and other substrates have been successfully cast in polyester resin (POHOWSKY, 1974). The fidelity of the replication has provided considerable taxonomic resolution.

For an assessment of the paleoenvironmental hydrological dynamics that affected the deposition of colonies of the Ordovician trepostome Diplotrypa, WYSE JACKSON, BUTTLER, and KEY (2002) produced multiple resin casts of various morphologies and subjected these to analysis under different hydrological regimes.

Synthetic resin casts can also be a valuble tool in paleontology, but the type of resin used must be carefully considered. There are a great variety of resins with different properties, including, for example, polyester resin, polyurethane resin, and epoxy resin. Factors that should be considered include cost (cheaper resins may not have much longevity), stability, shrinkage, and yellowing. Attention must be paid to health and safety guidance when using resins.

MATERIALS FOR REPAIR AND CONSERVATION

Specimens, when collected in the field, may be broken and require repair, or may be friable and in need of consolidation. Care needs to be taken in the choice of adhesives and consolidants used. In the past, various products have been used, including synthetic and natural resins, some of which have been unsuited to the task.

The ideal product should age without losing its mechanical properties or discoloring and should be reversible. It should not be too strong for the specimen, so that any further breaks will occur in the same area, not in another part of the specimen.

Adhesives and consolidants need to have a suitable glass transition temperature (Tg). Tg is the temperature at which a material changes fron a solid glassy state to a softer flexible state. If the adhesive or consolidant becomes softer, the repair could fail and dust and dirt will adhere to the specimen.

Many of the bryozoan colonies preserved in the Cinncinatian sequences (Late Ordovician) in Ohio and adjacent states, are broken up. Frequently, fragmented colonies lie in heaps in situ on bedding surfaces or else fragments are close by, having not been transported far. Once collected, these specimens can be painstakingly reassembled (CUFFEY & FINE, 2005; WAUGH, ERICKSON, & CRAWFORD, 2005, who used cyanoacrylic glue), and the 3-D form and size of colonies determined. Colonies of the trepostome Heterotrypa frondosa have been found to have diameters of 63 cm (CUFFEY & FINE, 2005), and various distinctive growth forms have been resolved from such studies (WAUGH, ERICKSON, & CRAWFORD, 2005).

PHOTOGRAPHY

Over the last half century, many taxonomic studies of Paleozoic bryozoans have focused on specimens at the microscale, at zooecial and zoarial features both at the surface of specimens and in their interiors. Less attention has been paid by taxonomists to various features at the macroscale, such as zoarial shape and form, although these have been the focus of much valuable research into the environmental controls on varied colony morphologies in both Paleozoic bryozoans (REID, 2010) and modern bryozoans (STACH, 1936; SMITH, 1995; HAGEMAN & others, 1998; see review in TAYLOR, 2005).

High-quality photographs can be obtained of bryozoan colonies using standard film or digital cameras mounted on stands. Before the photograph is taken, specimens should be cleaned and any dust removed using a fine make-up brush or compressed air. Hydrogen

peroxide (H_2O_2) is frequently used to clean modern bryozoans, whereas a solution of oxalic acid $(H_2C_2O_4)$ has been effective in cleaning Paleozoic bryozoans obtained from argillaceous-bearing sedimens, such as the Trention Limestones (RASETTI, 1947). Fossils that exhibit an uneven surface color or tone may be darkened with a material such as fountain pen ink. This needs to be done with caution and the blackening material tested to ensure that it can be removedfountain pen ink can be removed using a mix of ammonia (NH₃) and hydrogen peroxide (H₂O₂). Specimens should then be whitened evenly with a light dusting of ammonium chloride (NH₄Cl) or magnesium oxide (MgO) (TEICHERT, 1948; JEFFORDS & MILLER, 1960). In much the same way, delicate moldic specimens can be whitened, photographed, and the images obtained inverted so that the fossils appear as casts; MCNAIR (1941) used this method to image Mississippian fenestrate bryozoans. Digital images can be manipulated to create the effect.

Images obtained using digital cameras can be manipulated using computer software (e.g., Adobe Photoshop®). With digital manipulation, levels of contrast and brightness can be adjusted to remove uneven tones without using darkening fluids and dusting with white powders.

To obtain images with a greater depth of field, focus-stacking software is now being used. This digitally combines multiple images taken at different focal lengths.

Overall lighting should be directed from the upper left of the specimen so that a 3-D shadow effect is obvious. Optimal lighting can be achieved for smaller specimens by using a fiber optic light source. Small beams of light can be directed onto a small part of larger bryozoan colonies, and several lamps can provide illumination of the whole colony.

Photographs of bryozoan thin sections are best taken using a digital camera specifically designed to be mounted on either the eye-piece of a petrological microscope or mounted on the photographic tube of that microscope. SIVETER (1990) provides a synopsis of macrophotographic techniques.

SCANNING ELECTRON MICROSCOPY

The advent of commercially available SEMs (scanning electron microscopes) in the mid-1960s led to major advances in the study of fossil bryozoans (TAYLOR, 1990). The ability to obtain high-quality, highmagnification images allowed for rapid advances to be made in the understanding of bryozoan surface morphologies and in the unravelling of complicated taxonomic issues.

The earliest SEMs required that specimens were mounted on aluminium stubs and coated with gold or carbon, which acted as a conductor. In the case of type specimens, many institutions prohibit preparation of their status material in this way, and so taxonomically important specimens could not be scanned. LESLIE and MITCHELL (2007) outline a methodology using potassium cyanide (KCN) or sodium cyanide (NaCN) in which gold can be effectively removed from specimens.

In the 1970s, new SEMs with environmental chambers were developed in which uncoated specimens could be examined (TAYLOR, 1986; TAYLOR & JONES, 1996). This advance allowed for the study of important historic and type material. Another drawback of early SEMs was that specimens had to be quite small in order to fit into the machine. However, the capacity of many modern SEMs is such that moderately large specimens can be accommodated. Specimens need to be cleaned (see above) prior to examination as debris can cause charging, resulting in poor images.

Aside from examination, scanning, and imaging of isolated bryozoans or those encrusting various substrates, various studies have focused on the ultrastructure of the bryozoan skeleton (WEEDON, 1999; TAYLOR & WEEDON, 2000) of Paleozoic bryozoans (BLAKE, 1971; PODELL & ANSTEY, 1979; BUTTLER, 1989) and post-Paleozoic bryozoans (TAYLOR & JONES, 1993). It is necessary to use highly polished thin sections, which should then be etched in dilute hydrochloric acid, acetic acid, or EDTA (CARTER & AMBROSE, 1989) to produce some relief that can be detected during the scanning process.

HEALTH AND SAFETY

Before any preparation technique is undertaken, the task must be assessed for risk and any appropriate action taken. Material Safety Data sheets, Heath and Safety regulations, and guidelines exist in most countries and must be adhered to. When chemicals are purchased, health and safety data sheets are supplied, and these must be brought to the attention of all users and appropriate measures and precautions undertaken (GREEN, 2001).

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