STANDARD PREPARATION TECHNIQUE FOR FOSSIL FISH FROM THE ROMUALDO MEMBER OF THE SANTANA FORMATION

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This standard preparation technique is a method evolved during my first year as a preparator at the American Museum of Natural History, a year spent largely in the preparation of fossil fish from the Romualdo Member of the Santana Formation. The method involves certain improvements over the Elver's technique (Maisey *et al*., 1991), but I acknowledge a great debt to Dr. Elver's for his key insight in placing the fossil face up before embedding it in resin. Fossil fish from the Romualdo Member of the Santana Formation are frequently whole skeletons, preserved in three dimensions at the centers of dense limestone nodules. The fossils are revealed by splitting the nodules open. The goal of preparation is to remove the matrix while at the same time preserving the three-dimensional nature of the fossil. The object of preparation is usually just the axial skeleton, keeping the tail, complete vertebral column, and neurocranium intact. Because fossil fish from the Romualdo Member are so abundant, no attempt is made to preserve details of the scale pattern. These scale patterns are relatively well known from unprepared specimens. Pre-embedding mechanical preparation, pre- embedding acid preparation, and embedding in clear polyester using the two-box method all precede formic acid preparation.

Specimen Selection

Virtually any specimen from the Romualdo Member can be acid prepared to some advantage. But given the abundance of fossils from Brazil, the process of selecting those specimens that will give the best preparations is of some importance. Specimens of *Tharrhias* from the Romualdo Member typically present the ideal starting point for this method: the nodule generally breaks along the length of the vertebral column, with portions of the tail visible at one end of the nodule, and portions of the skull visible at the other. Under such ideal conditions, mechanical pre-embedding preparation can be skipped, and the procedure initiated with pre-embedding acid preparation. The ideal case, then, is one in which the entire axial skeleton is visible from the outset.

Taphonomic processes and crude native collecting techniques have left most Romualdo fish in a condition that is somewhat short of this ideal condition. Specimens of *Vinctifer* never break along the vertebral column. They invariably break along one side of the body or the other. In such cases, it becomes necessary to embed only the side containing the axial skeleton and to leave the counterpart without further preparation. Specimens of *Cladocyclus* and *Enneles* usually break on the *Vinctifer* pattern. But with these larger fish, only one side of the fish is recovered. Specimens of *Rhacolepis* are frequently inflated into a cigar shape. In my experience, these cigar-shaped *Rhacolepis* are barren of internal structure. The neurocranium of *Notelops* is a particularly difficult structure to prepare, because the head is often crushed. Intact *Notelops* neurocrania are exceedingly rare.

Pre-embedding Mechanical Preparation

The primary goal of pre-embedding mechanical preparation is to modify the fossil so that it represents, as nearly as possible, the ideal condition for acid preparation. This is accomplished by assigning primary importance to the axial skeleton, and relegating any intervening structures to a secondary role. Typically, these secondary structures must be sacrificed in order to properly reveal an articulated axial skeleton. To put the case more bluntly, it is necessary to destroy some scales in order to dig out the vertebral column. In the case of *Vinctifer*, such destruction is of minimal impact, because it is possible to remove only the center of each dorsal scute. But with most fossil fish, the scales over the vertebral column must be sacrificed permanently in order to reveal to vertebral column before embedding.

Typically, Romualdo fish break along one side of the skull or the other. As a result, the dermal skeleton of the skulls of the various species are relatively well known. However, the morphology of the deeper structures, such as the branchial and pharyngeal architecture, is much more imperfectly known. By sacrificing one layer of superficial structures, it is possible to begin to expose these deeper structures.

Practically, removal of superficial structures is accomplished with an Air Scribe. I follow the vertebral column forward from the tail, removing scales and enough overlying matrix to reveal the hour-glass pattern of sectioned vertebrae. In the gill region, I simply sacrifice all visible dermal bones: the operculum, the pre- and subopercular and suborbitals. In some cases the maxilla and dentary are removed as well.

Removing the operculum from a specimen using an air scribe.

Pre-embedding acid preparation

The general goal of pre-embedding acid preparation is to fully develop the axial skeleton for embedding on clear polyester. Nominally, this involves removal of enough matrix so that the polyester can coat more than just the flat, broken surface of the vertebrae. By developing modest relief between a structure to be embedded and the matrix, fixation by the polyester becomes a tongue and groove relationship. Three-dimensional fixation is enhanced.

Pre-embedding acid preparation also has some important side benefits. This step cleans the fossil, removing both unwanted dirt and unstable bone. A short acid preparation also tends to mask messy mechanical preparation. But most importantly, acid preparation of the skull region often develops a three-dimensional branchial skeleton of such beauty that preparation may be terminated at this stage. In some cases, results of preparation are so startling at this early stage that embedding would be inappropriate.

Pre-embedding acid preparation can take between one hour and a full day, depending upon the size of the fossil and the judgment of the preparator regarding the most opportune moment to cease preparation of the branchial region. If the vertebral column develops more quickly than the branchial region, it is possible to leave the fish head down in the acid, so that the projecting body and tail do not become over developed. The progress of this period of acid preparation must be monitored closely at one to two hour intervals

Two-box embedding technique

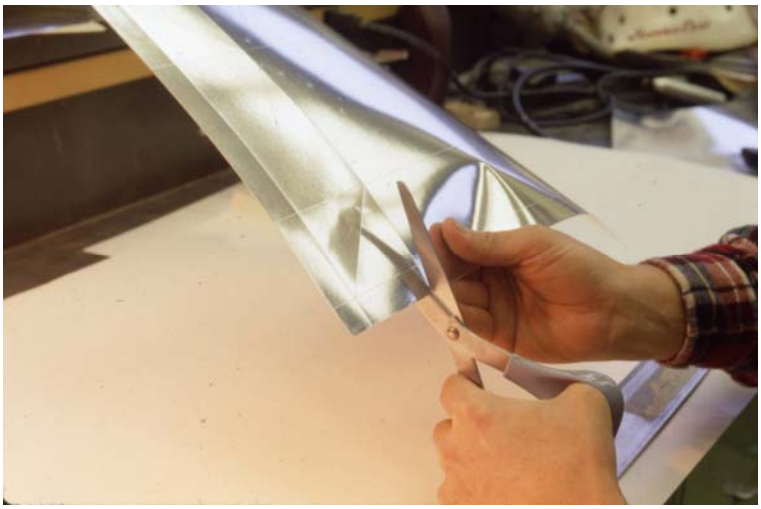
The two-box embedding technique is a modification of the Elver's method (Maisey *et al*., 1991) of transferring a fossil from its original matrix to a polyester resin. The two-box technique involves the folding of two five-sided boxes from foil-coated paper. The inner box is used to square up the shape of the fish. The outer box is a secure vessel into which the liquid polyester can be poured. When taped together, the two-boxes create a form for a thin-walled plastic box. The fossil, which is exposed to the plastic through a hole cut in the center of the inner box, becomes embedded in the bottom of the plastic box.

The two-box method is particularly economical of preparator time, requiring only about 2 man-hours per embedding. Weights of completed preparations demonstrate that the Elver's and the three-box method utilize about the same amount of polyester.

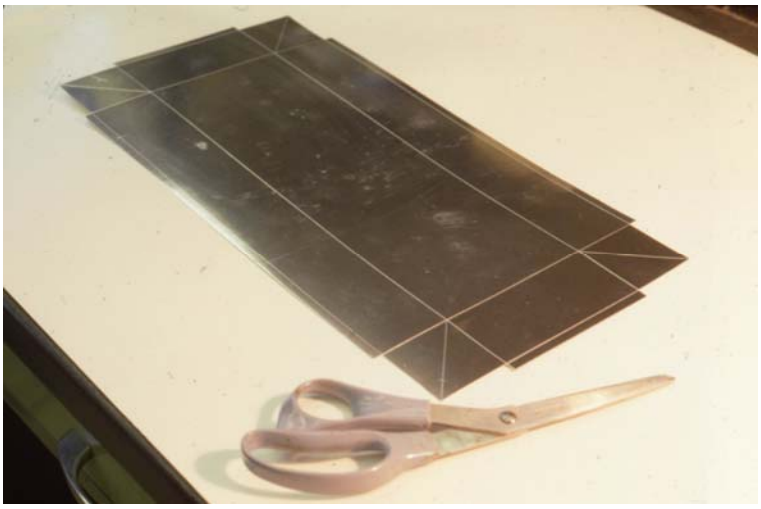
The inner box of the two-box technique is placed open-side down, with the broken surface of the concretion facing upwards through a hole in the top of the box. This box must be one-half inch longer than the length of the specimen, one-half inch wider than the width of the specimen, and deep enough to contain the greatest depth of the fossil. The box is constructed of foil- coated paper, and is folded so that the foil is on the outside of the box. A one-quarter inch phalange projects out from the perimeter of the bottom of this box.

The fossil is secured to the top of the inner box with rubber cement, then masking tape. The corners of the inner box are sealed with masking tape, and then the inner box is secured to the floor and walls of the outer box with masking tape. The joint between the fossil and the inner box is then sealed with a silicon caulk. The entire construction is inspected for possible leaks, and if any are discovered, they can be caulked as well. The outer foil surface of the inner box and the inner foil surface of the outer box are painted with a releasing agent. When this dries, the corners of the outer box are folded up, and the walls stapled into an upright position. The phalange around the circumference of the upper box is stabilized with masking tape.

Scoring the foil paper with a regular preparation needle.



Cutting excess paper from the corners.



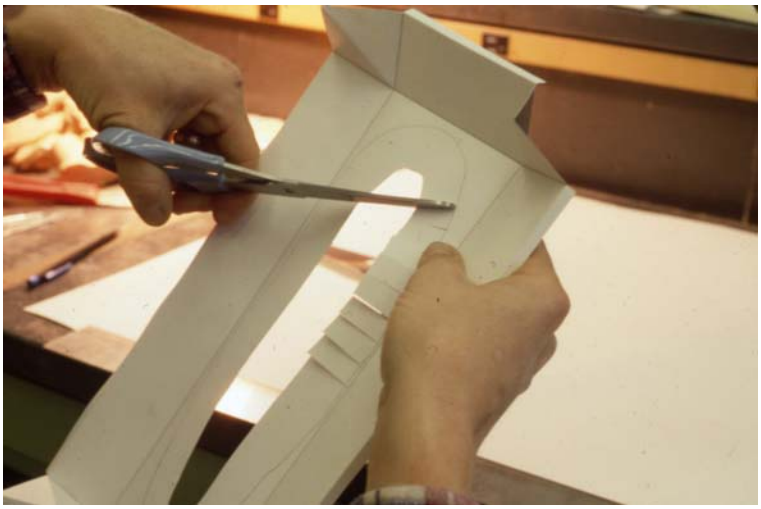
Completed sheet for the inner box.

Tracing out shape of fish on paper side of inner box.

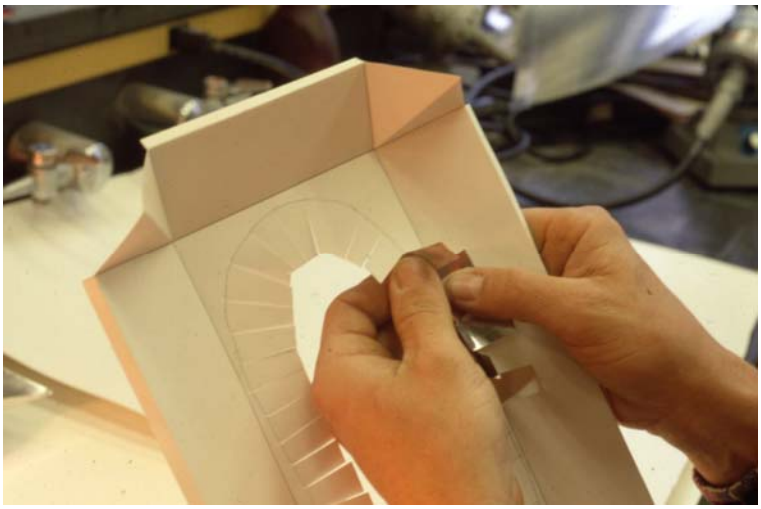
Folding inner box over edge of desk.

Folding the corners on the inner box. The corners get folded inward on the inner box.

Cutting a hole for the fish in the top of the inner box. The hole that is cut in the inner box must be smaller than the outline of the fish traced earlier.



Cutting tabs around the perimeter of the hole. The tabs are cut to the depth of the tracing of the fish the fish.



Folding the tabs inward from the top of the inner box.

Gluing the fish to the box using rubber cement.

Securing the fish against the tabs using masking tape.

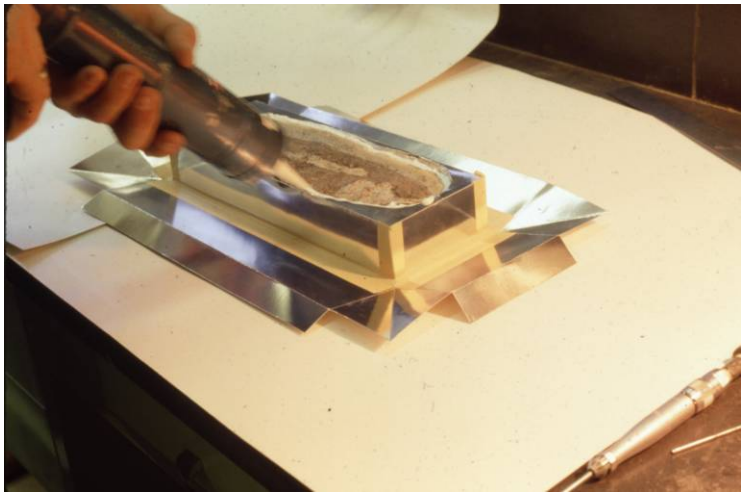
Sealing the corners of the inner box with masking tape.

Completed inner box, shown upside down. Note the pinch of clay used to insure that the fish supports itself when turned right side up, so that the weight of the fish puts no tension on the box.

The outer box of the two-box technique has an open top, and is constructed to contain the fossil, the inner box, and the phalange of the inner box. This outer box is one inch longer than the length of the specimen, and one inch wider than the width of the specimen. It is at least one-half inch deeper than the inner box. The outer box is also constructed of foil-coated paper, and is folded so that the foil is located on the inside of the box. A one inch phalange projects out from the top of this box to stabilize the structure.

Completed inner box, with fish on upper surface, placed atop folded outer box.

Securing the inner box to the outer box using masking tape. The pieces of masking tape must be long enough to seal the edge of the inner box to the outer box.



Sealing and holes between the fish and the edge of the inner box with silicon caulk. Once this seal is complete, the outer box can be taped closed, and the plastic poured.

At the American Museum of Natural History, we embed the fossil in a water-clear polyester resin. We use a water-clear resin because the sides of the protective box are then translucent, which permits better lighting of the prepared specimen. In the early 1990's while working on this project I used a product known commercially as Alplex. Although I have no experience with other resins, I have little doubt that other commercially available water-clear resins would also work.

The polyester resin is mixed and poured over the fossil, and is allowed to run down into the sidewalls between the two boxes. If the fossil being embedded is flat, then only one pouring is necessary. Otherwise, a preliminary pouring over just the fossil can be followed in about half an hour with a second pouring. In general, thicknesses of one-half inch or more of resin should be poured in stages. When complete, the entire upper surface of the fossil and the inner box should be covered with one-quarter inch of resin.

The resin casting is generally allowed to cure overnight. Occasionally, I use a short period of oven drying at 50º C to enhance hardening of the polyester. Removal of the foil paper from the casting is much easier if the polyester has been completely cured, and subsequently cooled to room temperature. After the paper is removed, the number is marked on the specimen using a grinder with a fine point. Following this interval, acid preparation can begin in earnest.

Acid preparation

Acid preparation at the American Museum is conducted using approximately 1 M formic acid. This is a 1:20 volume:volume reduction of technical grade formic acid. The acid solution is buffered with 0.025 M calcium phosphate. The calcium phosphate powder is measured out volumetrically, with about 3/4 ounce of calcium phosphate added to each liter of solution. Specimens are placed in the acid on a three-day cycle involving one full day in the acid, one full day in fresh (but not running) water, and one full day for drying and the addition of consolidants. My general prejudice is to cycle each fossil as few times as possible in the course of preparation.

I have used both Glyptol and Acryloid B-72 as consolidants, and I much favor the B-72. B-72 is more highly soluble in acetone, so that the "bathtub ring" remaining from the previous cycle of consolidants is more easily removed. It also dries to a less brittle state than glyptol. Thin bones coated with the B-72 are likely to bend when touched with a brush. I find thin bones coated with glyptol more likely to break. In this sense, the B-72 is a more forgiving consolidant. B-72 is marketed to cultural institutions by Talas (http://www.talasonline.com/) and other preservation suppliers.

I dry the specimens for treatment by a consolidant with a small fan placed at a distance of about 6 feet. I aim for a drying time of about 2 hours. If the fossils are drying too fast, I shut the fan off. My first treatment of a dry fossil is an attempt to remove the "bathtub ring" of earlier consolidants. I find this ring of consolidant relatively easy to remove with acetone alone on a small paintbrush. Once the "bathtub ring" is removed, I apply consolidant with either a brush or a dropper. The fossil is extremely fragile until it receives a couple of coats of consolidant, so throughout this process, I attempt to touch the fossil only with the drop on the end of the brush or dropper, and to avoid contact between either the brush or the dropper and the fossil itself. I find that the "bathtub ring" becomes progressively harder to remove as consolidants are added. Although I resolve the problem of the "bathtub ring" when the fossil is in its most fragile condition, it must be remembered that the ring too is much more easily destroyed at this early juncture.

Finishing the preparation

Acid preparation of fossil fish from the Romualdo Member surely reveals some of the most delicate fossil bones known to vertebrate paleontology. Some special effort must be undertaken to provide for the preservation of these fragile structures for posterity.

Standard methodology dictates the construction of a cover for the preparation. On time scales of tens of years, dust becomes a considerable hazard to tiny bones. Dust, unlike the matrix in which these fossils were discovered, is not amenable to chemical attack. Once present, it can be removed only by meticulous manual mechanical preparation. Prevention is a more appropriate solution to this problem. At the American Museum, we use a lid constructed of 1/8" clear acrylic to protect the fossil from dust accumulation.

The clear protective box in which the fossil was embedded was clearly labeled with the specimen number earlier in preparation. The plastic lid can be labeled with the number as well. We do not advise etching the generic or specific identity of the fossil in the lid because fossil taxonomy is too unstable over long periods of time. We already have specimens in our Brazilian collection that have been labeled with a generic moniker that is no longer an acceptable name. Instead, we recommend gluing a small label on each lid. This label denotes such changeable attributes as the generic and specific identity of the fossil.

Reference

Maisey, J. G., I. Rutzky, S. Blum, and W. Elvers. 1991. Laboratory Preparation Techniques. In John G. Maisey, editor. Santana Fossils: An Illustrated Atlas, pages 99 – 103.