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EPOFIX AND VACUUM: AN EASY METHOD TO MAKE CASTS OF HARD SUBSTRATES

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ABSTRACT

An effective and fast procedure is presented for making transparent casts of hard substrates such as bones, rock, shells, and wood. The procedure is based on a two-component epoxy (Epofix) that is especially suitable for cold mounting, avoiding heat, and limiting pressure impregnation that might damage fragile substrates. Critical to successful casting is the use of a vacuum chamber or a vacuum desiccator during early hardening to eliminate air bubbles. The vacuum is interrupted and turned on again to force air out and epoxy into blind-ending cavities; this action may be repeated. Afterward, the hardening takes place at atmospheric pressure. Unlike other embedding procedures, neither infiltration phases nor acetone "boiling" are necessary to produce detailed casts that are suitable for studies at micron scale and for long-term use.

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PLAIN-LANGUAGE SUMMARY:

Being able to visualize three-dimensionally is essential when studying trace fossils. Except for their entrances, borings are often difficult to observe directly because they are situated in hard substrates such as bones, rocks, and shells. Multiple cross sections of the substrate may solve this problem. Yet, a more convenient method is casting, especially of microscopic borings. We suggest here an easy and fast way to cast. At room temperature the dried sample is embedded in Epofix (epoxy), using a vacuum chamber before hardening begins. Afterward the sample is removed chemically (e.g., by using a weak acid), leaving the cast.

The procedure was tested on shells from an infaunal bivalve, the ocean quahog (Arctica islandica Linné 1767) collected from Pleistocene deposits in northern Russia, and on recent shells of an epifaunal gastropod collected off Rhodes, Greece. Both types of shells contained micron-sized borings, which were successfully cast by the procedure.

Casts may give important knowledge on paleoecology and paleoenvironmental changes, such as the extent the tracemakers explored the substrate, and how many times colonization of the substrate occurred. Many tracemakers, e.g., fungi and algae, do not usually leave any body fossils. Without their borings and casts, it would be even more difficult to reconstruct biocoenoses.

Glossary

Araldite: Araldites are glycerol-based aromatic epoxy resins.

Boring: Biogenic structure excavated into a hard substrate, e.g., rock, shell, or wood. The structure is made in a chemical and/or mechanical way (Ekdale et al. 1984).

Epon 812: A glycerol-based aliphatic epoxy resin.

Epoxy: A thermosetting resin, having the property of becoming permanently hard and rigid when heated or cured. Syn.: epoxy resin, epoxy glue.

Resin: Any of a class of solid or semisolid viscous substances obtained either as exudations from certain plants or prepared by polymerization of simple molecules.

Trace fossil: Biologically-produced sedimentary structures that reflect behavioral patterns. Also included in trace fossils are borings in hard substrates and fossilized fecal material.

Vestopal: A polyester resin that is a copolymer between a polyester of maleic and fumaric acids, esterified with di- and tri- hydroxyalcohols and styrene.

INTRODUCTION

Taphonomy concerns the transit of material from the biosphere to the lithosphere (Efremov 1940) and involves a huge array of biological and geological processes affecting the post-mortem history of organisms. The taphonomic agents, abrasion, bioerosion, fragmentation, and disarticulation, are examples of such processes. The term bioerosion was originally suggested by Neumann (1966) as an abbreviation of "biologic erosion" and has been used to describe every form of biologic penetration into hard substrates, i.e., lithic, skeletal, or woody (see Warme 1975; Ekdale et al. 1984; Bromley 1994). Bioerosion structures, especially borings, are in many cases associated with observational problems. Fractures of a bioeroded substrate reveal random sections through the borings and tend to confuse the observer (Ekdale et al. 1984). Accurate three-dimensional casts are therefore necessary for making detailed studies of bioerosion structures.

During the 1970s different methods were developed to cast recent and fossil bioerosion structures and also recent tracemakers. The frequently used Epon-812, which originally was developed by Mollenhauer (1964) for embedding plant and animal tissue, is no longer available, but possible replacements for Epon-812 have been introduced (e.g., Embed 812 see Mascorro and Kirby 1986, 1989, 1991). Using Epon-812, Frankel (1970) described a technique designed to embed recent microfauna occurring in unconsolidated sandy sediments. Golubic et al. (1970) applied Epon-812 (at 60°C for 40-72 hours) to cast recent tracemakers within their borings. Golubic et al. (1970) tested the use of Vestopal and Araldite with comparable results. The exact kind of Vestopal and Araldite was not given. Perkins and Halsey (1971) cast recent tracemakers, i.e., in situ endolithic fungi and algae, and made plastic-impregnated thin sections and etched slabs. Following Ginsburg et al. (1966), they impregnated unconsolidated sediments with polyester resin under vacuum and pressure. The used resin was Plaskon No. 0951 mixed with a promotor and a catalyst. Later Golubic et al. (1975) suggested a double embedding method (i.e., two infiltration phases, both requiring Araldite Durcupan no. 2) that allowed the tracemaker to be examined in situ within the cast of its boring. This method was modified by Golubic et al. (1978) and Golubic (1983) to cast fossil borings applying one infiltration phase with gradual replacement of

acetone with polymerizing resin, Araldite. This is analogous to the embedding schedules suggested by Finck (1960) and Mollenhauer (1964). The infiltration phase was followed by acetone "boiling" in a sample chamber attached to an aspirator. The method by Golubic et al. (1978) and Golubic (1983) has been widely used (e.g., Vogel 1987; Vogel et al. 1987; Tavernier et al. 1992; Schmidt and Freiwald 1993; Glaub 1994).

All these methods require heating of the embedding medium, containing sample material, at about 60°C for at least 40 hours, mostly 48. In this paper we describe a simpler and less time-consuming method, excluding such aspects as pressure impregnation, gradual infiltration, and "boiling" acetone away. Our method is based on a two-component epoxy (Epofix), which originally was developed for the metallographic field. The method is especially suited for mounting samples that are sensitive to heat or pressure.

PROCEDURE

The procedure requires an Epofix kit, which is available from the Electron Microscopy Sciences (P.O. Box 251, 321 Morris Road, Fort Washington, PA 19034), and Struers (Valhøjs Allé 176, 2610 Rødovre, Denmark). The Epofix kit consists of Epofix resin (contains bisphenol-a-diglycidylether), Epofix hardener (contains triethylenetetramine), measuring syringes, wood stirers, and paper cups. Instructions are included in the kit and should be read carefully (Anonymous 1995). The procedure described here is partially based on these instructions. Suitable gloves, eye/face protection, and clothing protection must be used because potential health hazards may cause health problems (see Wells 1989; Chaney 1989; Smith and Latimer 1989).

The procedure consists of the following steps.

1. Set up the Epofix kit in a proper laboratory. Use a fume hood because of toxic fumes.
2. Dry, in an oven (e.g., 45°C for two days), the material, which will be embedded, to remove moisture before it is placed in the mould.
3. Maintain a mixing ratio of resin and hardener that is 15 and 2 parts by volume, respectively. Otherwise, mix 25 parts by weight of resin with 3 parts by weight of hardener. Mix the resin and hardener in a paper cup and stir carefully for at least 2 minutes. Pot life is about 30 minutes.
4. Pour the mixture carefully over the material in the mould so that only a few air bubbles are caught.
5. Put the mould containing the material and epoxy mixture into a vacuum chamber or vacuum desiccator for no longer than 20 minutes. The time in vacuum is critical to prevent overheating and associated boiling of the epoxy mixture (boiling point 20°C at 40 mm Hg) (see Waters 1975; Wells 1989). To ensure casting of tiny slender cavities, the vacuum may be interrupted and then turned on again. This action, which may be repeated, will force the epoxy further into the cavities and squeeze out air.
6. Let the mixture harden at room temperature (20°C) and atmospheric pressure. The timed required for hardening is dependent on a number of factors, e.g., amount of mounting material used, mixing ratio, and temperature. Hardening time for 30 g of mounting material is about 8 hours at room temperature.
7. Saw the sample into two or more pieces to produce cross sections of the embedded material. Epofix is a transparent epoxy; it is easy to monitor the cutting.
8. A weak acid (e.g., hydrochloric acid [0.5 percent by volume], soda water) may be applied for 10 to 30 minutes to remove the embedded material; depending on its amount and texture. Otherwise a buffered solution of the calcium chelator EDTA, which is a disodium salt of ethylenediamine tetraacetic acid, may be used (Carter and Ambrose 1989). Alternatively, ion-exchange water can be used (H. J. Hansen, personal commun., 1999). Removal of the embedded material has to be slow-acting as to prevent possible breakage of the delicate parts of the cast.
9. Rinse the cast with distilled water to prevent further etching and possible chemical precipitation. After drying and gold coating, the cast is ready for scanning electron microscopy (SEM) (see Carter and Ambrose 1989).

EXAMPLES OF USE

The procedure was tested on late Pleistocene fragments of the ocean quahog *Arctica islandica* (Linné 1767) (class Bivalvia, family Arcticidae) (Figure 1 and Figure 2).

The fragments were collected from a stone-rich shell conglomerate exposed along the Pyoza river (=Peza river) (locality 9801, geographic coordinates 65° 40' 40"N, 47° 32' 50"E, 49.75 meters above sea level, sample 98409), which is situated in Arkhangelsk Region, northern Russia (locality no. 25 of Devyatova and Loseva 1964). The procedure revealed several elongate borings oriented nearly perpendicular to the external shell surface. The procedure was also tested on a recent specimen of *Phalium undulatum* (Gmelin 1791) (class Gastropoda, family Cassidae), collected off Rhodes, Greece.

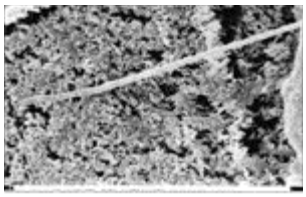

Figure 1.

Figure 2.

In this specimen, complex networks, which consist of sinuous and rarely branching borings, occur in crossed lamellar shell structure (Figure 3 and Figure 4). Micrographs obtained by using a scanning electron microscope indicate potential use in studies of bioerosion in relation to microstructures. In the present case there appears to be no such relationship.

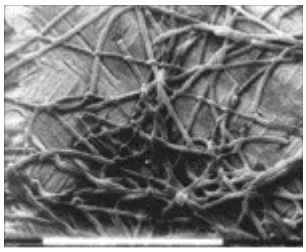

Figure 3.

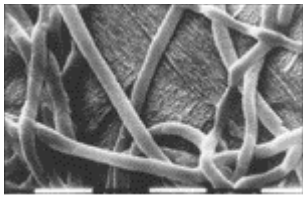

Figure 4.

COMMENTS

The Epofix mixture is slow-curing and has a low viscosity (550 cP at 20° C, 150 cP at 50° C) (Anonymous 1995), making the mixture particularly suited for vacuum impregnation. Although Epofix resin and hardener are both soluble in alcohol and acetone, these solvents should not be used to reduce viscosity (Electron Microscopy Sciences, personal commun., 1999). Instead, the Epofix mixture may be heated a little before application (max. 40°C). It was not done in the examples above. As the linear hardening shrinkage is insignificant or not existing (Anonymous 1995) it produces reliable results suitable for quantitative studies. The sectioning properties of Epofix mixture are good. A comparison with other embedding media is beyond the scope of this paper. The reader is referred to Bromage (1985), who provided a systematic evaluating procedure for casts and moulds.

The procedure described here produces adequately impregnated hard-substrate samples for studying macroscopic and microscopic scale bioerosion structures (Figure 1, Figure 3, and Figure 4). It is essential that the bioerosion structures are empty or that their fill is removed. Otherwise, the cast agent cannot enter and fill the bioerosion structures (e.g., Ekdale et al. 1984). Therefore, our procedure is not suitable for casting recent borings containing organic matter, e.g., remains of tracemarkers. Sodium hypoclorite, better known as commercial Clorox, may remove the organic matter. It should be kept in mind that excessive use of Clorox has a corrosive effect on molluscan organic matter, can alter the ultrastructure of the shell surface (Carriker 1979), and may affect bioerosion structures. This is important since relationships between microbioerosion and shell architecture may exist (e.g., Golubic et al. 1975).

The cold mounting within our procedure is particularly appropriate for heat or pressure sensitive samples (i.e., shell material to study microstructures, ultrastructures, and chemistry). Consequently, the procedure seems better than earlier suggested ones, which required warm curing for 40-72 hours.

Due to the cost of an Epofix kit (about US$ 100) a small amount as possible is used. To lower the amount of Epofix used, apply aluminum foil to casting containers. Foil, with a greasy internal surface, can be shaped to fit a given sample. The grease makes it possible to separate the hardened Epofix and the foil. The container may be stabilized by placing in a box with sand (H. Lindgård, personal commun., 1999).

The aging properties of Epofix are promising, that is minimal yellowing over time with nominal shrinking. This is concordant with our observations during the last three years, which include SEM re-examination of casts containing preserved remains of shells. Hence, it follows that the casts are suitable for long-term use.

CONCLUSIONS

Tests of the Epofix procedure show that it is useful for casting hard substrates, even those containing microscopic borings. The procedure is effective and simple, utilizing variable vacuum pressure. The cold mounting makes it suitable to heat- or pressure-sensitive samples.

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The Danish Research Council supported the fieldwork in Russia by giving a grant to the CATLINA project (Climate transitions of the last interglacial-glacial-interglacial cycle in the North Atlantic region). N.-M. Hanken (University of Tromsø) and three anonymous reviewers provided helpful comments on the manuscript.

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Figure 1. Epofix cast of a late Pleistocene fragment of *Arctica islandica* (Bivalvia), locality 9801, Pyoza river (northern Russia). The embedded fragment, which has an aragonitic homogenous shell structure, became radially sectioned and partially etched with hydrochloric acid (4.0 percent by volume). The cast of an elongated boring appeared. The external shell surface is toward right. Scanning electron micrograph, scale 0.1 mm.

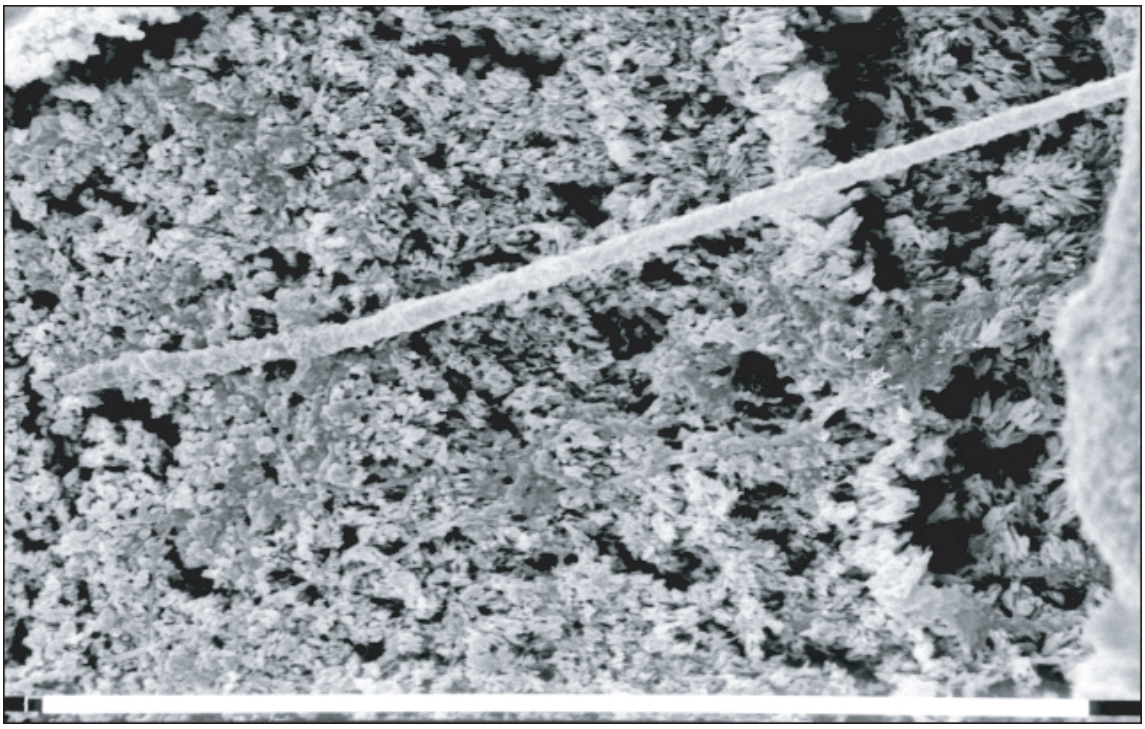

Figure 2. Cast of a fracture in connection with the external surface (toward right) of the substrate. The partial etched substrate is a late Pleistocene fragment of *Arctica islandica* (Bivalvia), locality 9801, Pyoza river (northern Russia). Scanning electron micrograph, scale 1 mm.

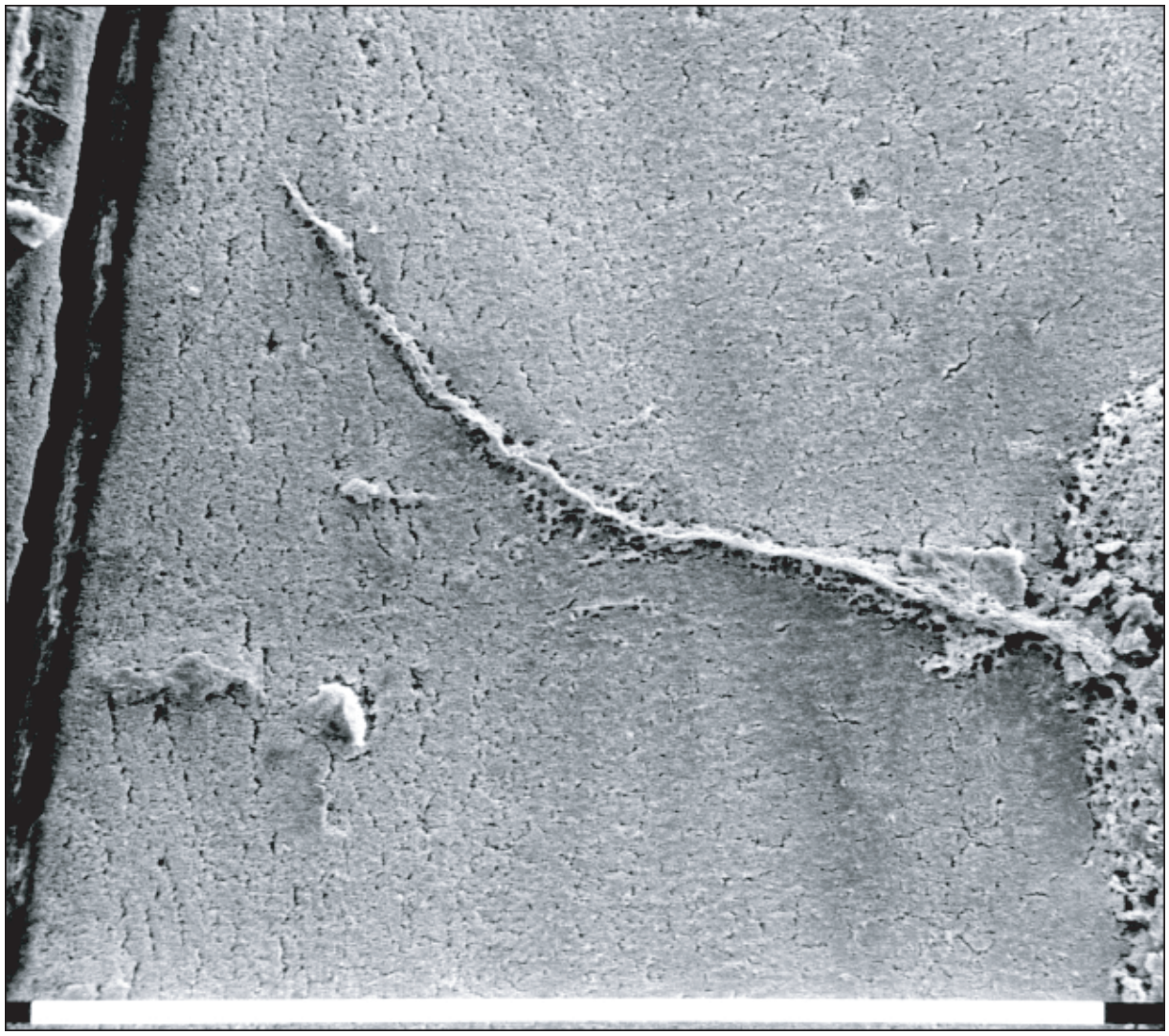

Figure 3. Fragment of a recent specimen of *Phalium undulatum* (Gastropoda), embedded in Epofix. The specimen was collected off Rhodes, Greece. The fragment, which contains crossed lamellar structure having linear-shaped first order lamellae, has partially been etched away with hydrochloric acid (0.5 percent by volume), leaving cast of borings standing out. The external shell surface is toward right. Scanning electron micrograph, scale 0.1 mm.

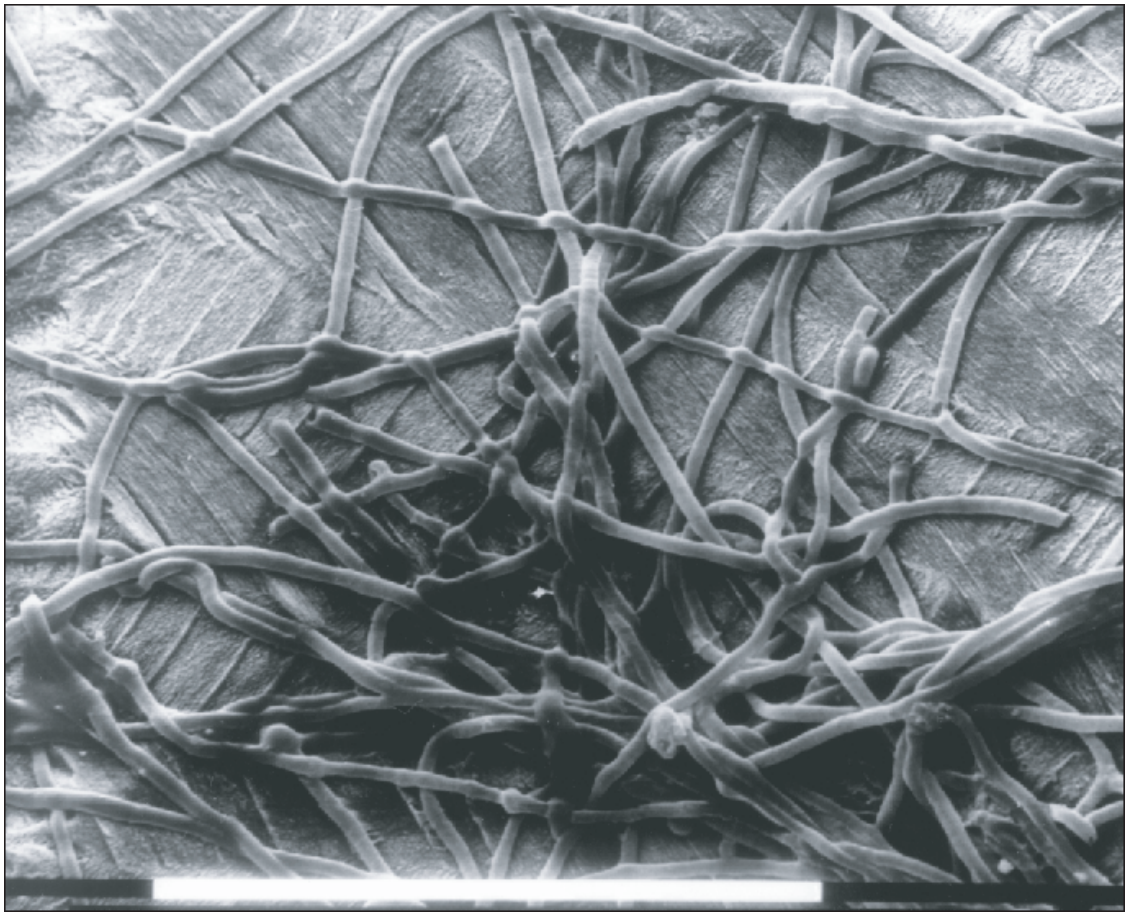

Figure 4. Close-up of Figure 3, showing details of a complex network of borings, i.e., casts. Scale 10 µm.