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Mutvei's solution: An ideal agent for resolving microgrowth structures of biogenic carbonates

Bernd R. Schöne [a,*], Elena Dunca [b], Jens Fiebig [a], Miriam Pfeiffer [c]

[a] *Institute for Geology and Paleontology, INCREMENTS Research Group, Goethe University, Senckenberganlage 32-34, 60325 Frankfurt/Main, Germany*

[b] *Museum of Natural History, Department of Palaeozoology, Box 50007, 10405 Stockholm, Sweden*

[c] *IFM-GEOMAR, Leibniz-Institute for Marine Sciences, Wischhofstr. 1-3, 24148 Kiel, Germany*

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Abstract

Accretionary hard parts of many organisms provide excellent archives of past climate and environmental conditions or life history traits. Variable growth rates function as environmental and physiological proxies, and growth increments as calendars. Recognition of growth structures is thus a prime necessity for sclerochronological studies. Here we present a new, handy, easy-to-use and time-efficient technique that resolves annual and sub-annual growth structures in skeletons of a wide range of different organisms. Mutvei's solution simultaneously etches biogenic carbonates and calcium phosphates, fixates the soluble and insoluble organic matrices and fibers, and stains mucopolysaccharides. It produces a filigreed three-dimensional relief of etch-resistant ridges (growth lines) and etched depressions (growth increments) and stains skeletal growth structures in shadings of blue. Growth lines stand out as crisp, darker-blue stained lines. Reflected optical light microscopy (axial and oblique illumination) and scanning electron microscopy can be used to analyze the microgrowth structures. We demonstrate the use of the technique on hard tissues of various marine and freshwater bivalves, a coral, a sclerosponge, a barnacle, gastropods, a cephalopod, a fish otolith and a whale's ear bone. This technique may be of interest for paleoclimatologists, geochemists and biologists. It can significantly expand the use of biogenic hard parts as environmental and physiological indicators because it reveals microgrowth structures of biogenic skeletons that potentially form on a periodic basis and thus function as calendars.

Keywords: Biogenic skeleton; Visualization; Growth structure; Alcian blue; Glutaraldehyde; Acetic acid

1. Introduction

Marginal-growing skeletons of many organisms contain information on environmental conditions and physiological changes experienced during life. Variations in growth rates, for example, can function as

* Corresponding author. Tel.: +49 69 798 22863; fax: +49 69 798 22958.

E-mail address: B.R.Schoene@em.uni-frankfurt.de (B.R. Schöne).

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proxies for environmental variations (sclerochronology) as many organisms grow faster at optimum temperatures, better food supply or unpolluted conditions (Ansell, 1968; Kennish and Olsson, 1975; Weber et al., 1975; Hudson et al., 1976; Schöne et al., 2002a). Furthermore, numerous studies demonstrated that most such variations occur periodically (Pannella and MacClintock, 1968; Clark, 1974; Fairbanks and Dodge, 1979; Bourget, 1980; Richardson et al., 1980) and are controlled by biological clocks (Clark, 1975; Pittendrigh, 1979). Growth halts or retardations result in the formation of growth lines which segment the growth pattern into time intervals (growth increments) of approximately equal duration (Hall et al., 1974). Thus, growth structures can be used as calendars and growth increment (or line) counts can potentially be used to assign precise dates to each portion of the skeleton (Pannella and MacClintock, 1968; Crowley et al., 1997), to estimate ontogenetic ages (Jones et al., 1978; Leslie et al., 2001) or to estimate the onset of maturity (Sato, 1995). Placing temporal constraints on portions of the shell could be critical to interpreting and calibrating geochemical data taken from biogenic skeletons (Mutvei et al., 1994; Nyström et al., 1996; Schöne et al.,2004a, 2005a,b).

Deciphering environmental and physiological information from biogenic hard parts requires observation of delicate growth structures. However, existing preparation techniques are extremely time-consuming and do not always reveal growth increments and growth lines adequately, especially in sub-annual time scales. In hard tissues of many organisms such as corals, sclerosponges or freshwater bivalves, sub-annual growth structures are barely known. Accordingly, interpretations of geochemical proxies may be limited to annual averages or rely on interpolation of seasonal growth rates (Quinn et al., 1996). The accuracy of such growth models depends on various assumptions that may not necessarily be correct. Radiographs are mainly applicable to corals and do not resolve sub-annual growth increments and lines. Audioradiographs, however, have the potential for marking and interpreting sub-annual growth lines in otoliths (Yoklavich and Boehlert, 1987). Thin-section preparation is difficult and time-consuming and requires the samples to be mounted perpendicularly to the growth lines. As pointed out by Risk and Pearce (1992), transmitted light "images can be ambiguous because object space in transmitted-light microscopy is a volume rather than a surface. Details within this volume are neither easily resolved to the eye nor easily preserved on a plane film" (i.e. thin-sections or acetate peels taken from etched sections). Growth lines and growth increments can be slightly better discerned if the thin-sections are stained, e.g., with Rhodamine B (Schöne et al., 2002b). Etched polished cross-sections are commonly proposed as a time-efficient alternative that requires reflected light microscopy (Rhoads and Lutz, 1980). Slight demineralization of cross-sectioned skeletons reveals a three-dimensional relief of insoluble organic components and differentially dissolved crystals. Differential dissolution results from different crystal sizes and orientation. This technique was previously used to aid in the identification and measurement of internal growth increments in mollusks (Rhoads and Lutz, 1980). However, the fragile organic meshwork of fibers and membranes tends to collapse on the etched surface when air-dried from water thereby blurring the image. This can partly be avoided through critical point drying or immersion in hexamethyldisilazane (Schöne and Bentley, 2002) or other dehydrants after the demineralization process (Clark, 1980; Koike, 1986).

In the present paper, we present a simple technique for revealing detailed growth structures that seems superior to all previous methods in terms of quality and preparation time. The new technique combines gentle etching, preservation of water-insoluble (structural framework molecules) and water-soluble (framework-associated macromolecules) components of the organic matrix and differential staining of soluble organics in a single preparation step. Although previously mentioned (Mutvei, 1979; Carell et al., 1987; Mutvei et al., 1994), the present technique was never described in detail or the functioning explained. Here, we demonstrate that Mutvei's solution—termed after Harry Mutvei who invented the mixture—works for biogenic carbonates and calcium phosphates across taxonomic boundaries. It enables analysis of growth structures in hard parts of bivalve mollusks, gastropods, barnacles, corals, sclerosponges, fish otoliths, cephalopods and whales' tympanic bulla. We also provide an explanation on how the three components of the Mutvei's solution interact with the biogenic skeleton and with each other. Using Mutvei's solution biologists can precisely reconstruct life-history traits from growth