ELEMENTAL MAPPING: A TECHNIQUE FOR INVESTIGATING DELICATE PHOSPHATIZED FOSSIL SOFT TISSUES

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ABSTRACT. Some phosphatized soft tissues of vertebrates and invertebrates from the Santana Formation (Cretaceous) of Brazil are too delicate to withstand acid preparation despite their relative insolubility. Examination of sectioned specimens using energy dispersive analytical systems attached to scanning electron microscopes provides an alternative method. This allows the nature of such delicate fossil soft tissues to be seen for the first time, and the relationship of fossil soft tissues to skeletal elements, to other diagenetic mineral phases and to sedimentary structures to be examined.

THE fossil-bearing concretions of the Santana Formation (Lower Cretaceous, ?Lower Albian) are now well-known for their exceptionally well-preserved fossils, which include three-dimensional fishes complete with soft tissues preserved in calcium phosphate (Martill 1988, 1990*a*, 1990*b*; Martill and Unwin 1989; Wenz and Brito 1990; Maisey 1991). Whilst they more usually contain fishes, the concretions rarely yield pterosaurs (Campos and Kellner 1985), turtles, and crocodiles (Price 1959). Invertebrates are common, but not diverse (Mabesoone and Tinoco 1973; Martill 1988). Apart from the fish, soft tissues have been reported from ostracodes (Bate 1972), and pterosaurs (Martill and Unwin 1989).

The soft tissues of the Santana Formation fauna are especially noteworthy because of their highly detailed preservation. Martill (1990*a*) considered the resolution of detail to be at sub-micrometre levels in some samples, and demonstrated the preservation (by replacement) of cell membranes and inclusions, including nuclei, in fish muscle fibres. Wilby and Martill (1991) have even shown that soft tissues are preserved in arthropods contained within the guts of fishes (in some cases the arthropods too have their gut contents preserved).

The soft tissues are replaced by calcium phosphate and are preserved in a variety of styles (Wilby in prep.), some of which may withstand development using dilute acetic and formic acids. However, where soft tissues have been observed in hand specimens prior to development, it has been noted that a substantial amount has been lost during acid preparation. This led Schultze (1989) to suppose that the soft tissues are preserved in calcium carbonate, and not calcium phosphate. However, X-ray diffraction analyses demonstrate unequivocally that the soft tissues are preserved in calcium phosphate (Martill 1990*b*). A petrographic analysis of the fossil soft tissues shows that it is the preservational fabric which determines how much of the soft tissue survives acid treatment.

These preservational fabrics include aggregates of calcium phosphate microspheres, 1–3 μm in diameter (see Martill 1988, pl. 2, figs 2, 4, 6); coalesced hollow spheres, 3–10 μm in diameter (Text-fig. 1A–B); and irregularly distributed minute crystallites approximately 300 nm or less in length (Text-fig. 1C). In addition, well-ordered crystallites, possibly originally templated onto proteins (Allison 1988), occur in the most exceptional material (Text-fig. 1D). In all cases there is considerable void space left within the former soft tissues, which in unprepared specimens is usually filled with ferroan and non-ferroan calcites of late diagenetic origin syntaxial on bone (Text-fig. 2A). Removal of this calcite renders the calcium phosphate delicate, and contacts between adjacent grains may be broken by the slightest agitation. In addition many phosphate grains are not in contact, possibly

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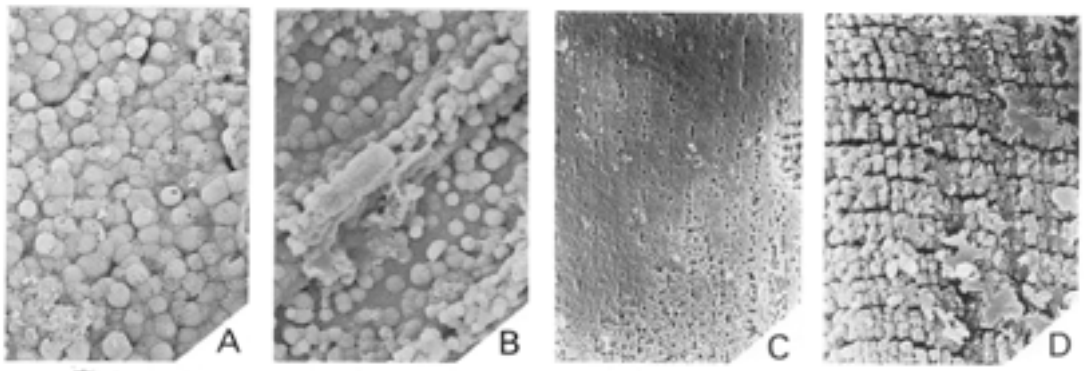


TEXT-FIG. 1. Scanning electron micrographs of preservational fabrics in phosphatized soft tissues from fishes in the Santana Formation. All have been prepared in 10 per cent acetic acid. A, spherical bodies from a portion of phosphatized gill lamella, coalesced in a 'robust' structure, × 2000. B, a similar fabric but with a more open texture; this has survived acid treatment, but if the texture were even more open it would probably have fallen apart, × 1500. C, muscle fibre with sarcolemma preserved as cryptocrystalline phosphate; it is not possible to resolve the crystallites satisfactorily on the scanning electron microscope, but they are easily resolved by transmission electron microscopy, × 1200. D, muscle fibre with crystallites probably templated onto specific protein sites, × 4000.

having grown at isolated nucleation centres within the soft tissue, and having been held in place by degraded organic material, later replaced by coarsely crystalline diagenetic calcite.

Thus, soft tissues extracted by acid preparation only represent the more robust, and more heavily mineralized portion of the total preserved soft tissue. Indeed these samples may be held together by a second, slightly later diagenetic phosphate. In order to investigate that fraction lost to the acid treatment, we experimented with thin and polished sections, fracture surfaces and light acid washes. The results and preparation hints given here are based on SEM thin section petrology.

METHODS

Three-dimensionally preserved specimens of fish (mainly *Notelops* sp. and *Rhacolepis* sp.) with soft tissues were cut normal to the long axis of the fish skeleton at regular intervals along the length of the fish. Special effort was made to ensure that sections were made through the gill arches, the stomach region and the caudal peduncle. These areas offer the greatest opportunity for finding phosphatized soft tissues in these Brazilian fishes.

Polished thin sections

It was thought that back-scattered electron imaging of polished thin sections would allow accurate, high-resolution mapping of skeletal and soft tissue structures in sectioned specimens with a minimum of sample preparation. Unfortunately the contrast between phosphatized soft tissues and late calcite infills is not great, rendering detailed work difficult. Text-figure 2G, shows one such back-scattered image through a block of muscle fibres. Although it is just possible to distinguish individual fibres, the detail is not sharp. It was also found that the polishing process plucked the phosphate of the soft tissues from the surface, the resultant cavities becoming severely clogged with diamond paste. However, bone phosphate resisted plucking and was left standing proud.

Non-polished thin sections

Uncovered petrographic thin sections of standard thickness (30 μm) were examined, but images produced by elemental maps were diffuse. Thicker sections, of around 50 μm, produced much

sharper images, probably due to a lack of interference from the adhesive. Sections were lightly carbon coated for SEM analysis; carbon may still be mapped despite the carbon coating. We therefore recommend the use of thin sections slightly thicker than normal petrographic sections. However, we suggest experimentation, as we have only tried our technique on the Santana material.

Thin sections through several well-preserved specimens of *Notelops* and *Rhacolepis* were examined by light and scanning electron microscopy.

ELEMENTAL MAPPING

For these analyses we used a JEOL 820K Scanning Electron Microscope fitted with a Kevex energy dispersive X-ray microanalytical system. A Kevex Super Quantum Detector was used, and the signal was processed by the Kevex Delta 4 System.

Elemental mapping is a routine procedure for mapping the distribution of elements in petrographic sections, and is widely used by igneous, metamorphic and sedimentary petrologists. The technique has not been widely adopted by palaeontologists for identifying structures, although it has been used as an analytical tool for enigmatic fossils (e.g. Aldridge and Armstrong 1981). This method highlights areas of differing elemental composition. Samples are placed in the scanning electron microscope and positioned and focused in the normal manner. First of all, however, it is useful to examine and sketch the sections optically under transmitted light. This is especially important when looking for fine detail in large sections as extensive mapping is extremely time consuming at high magnifications. Key areas can be marked with a felt tip pen which is usually visible under SEM, even after coating. Elemental maps can be constructed at various magnifications and accelerating voltages. We found that low accelerating voltages (*c.* 10 kV) required extremely long periods before there were sufficient counts to produce a visible elemental map, and that very high voltages (*c.* 20 kV) can produce charging, and at worse may damage the section during prolonged exposure. In general we used 15 kV and worked at magnifications of 30–500 times. A more detailed account of the procedures involved in elemental mapping may be found in Anon. (1983).

Count time is difficult to quantify as it is dependent on the quantity of the element being analysed for in the sample, the accelerating voltage and the magnification. We generally allowed count time to continue until a reasonable image had been generated. Short count times of 1–2 minutes were used to make rapid assessments of the whereabouts of phosphorus-containing tissues, but in some cases count times of up to one hour were used when trying to resolve detail.

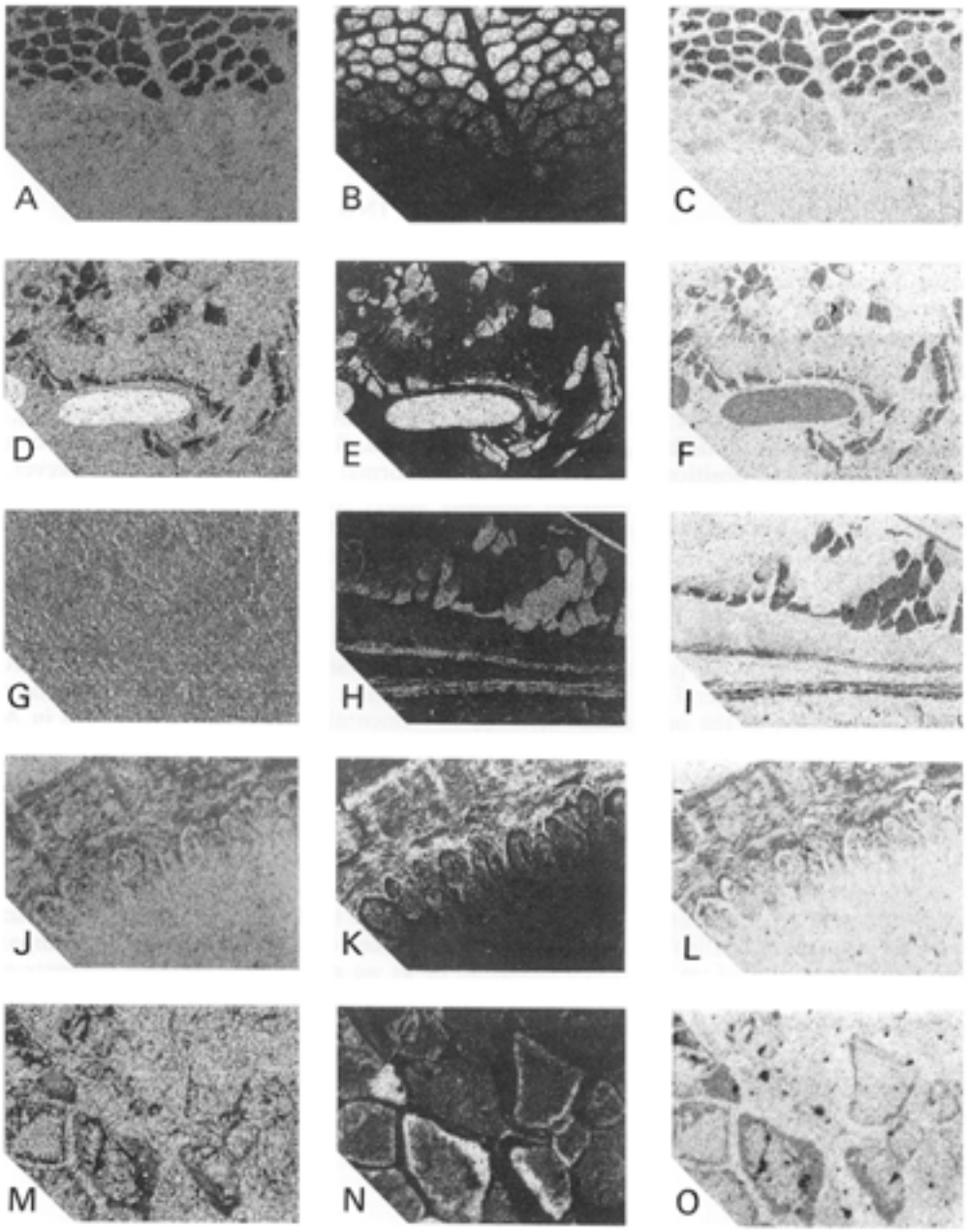
An advantage with the Kevex system is that the screen can be divided into two, four, or sixteen images. Each image can be displayed on the screen and stored on disc. This allows for comparison between the distribution of up to sixteen elements, or as we prefer, two or three elements and a secondary electron or back-scattered electron image. We routinely analysed for phosphorus, and calcium, and sometimes for iron and barium. Elements analysed will obviously vary according to circumstances.

We used elemental mapping to test if it was an appropriate technique in: (1) the identification of preservational fabrics; (2) distinguishing diagenetic mineral phases; and (3) soft tissue recognition. The technique proved to be especially useful in the latter two cases, the results of which are presented below.

RESULTS

Identifying preservational fabrics

The distribution of phosphorus not only maps out the presence of bones, phosphatized soft tissues and diagenetic or detrital phosphates, but also identifies differences between individual tissues based on the abundance of phosphorus in each. Thus it is relatively easy to distinguish different fabrics based on qualitative elemental abundances according to the brightness of the phosphorus map. Some soft tissues show greater concentrations of phosphorus at their margins, with a gradual reduction towards the centre of the tissue (Text-fig. 2M–O). These are tissues that would probably



TEXT-FIG. 2. Electron micrograph and elemental map images of fossil soft tissues from Santana Formation fishes. All photographs in the left-hand column represent secondary electron or back-scattered electron images; the centre column shows elemental phosphorus maps; the third column, elemental calcium maps. The photographs represent the same field of view horizontally at the same magnification. A–C, muscle fibres in cross-section showing clearly a layer of preserved muscle fibres with a dividing septum; A, secondary electron image; B, phosphorus map showing that almost as much muscle is preserved below that seen in A; C, calcium map highlighting the distribution of muscle fibres. D–F, bony fin rays (bright white oval object) in cross-section

not survive acid treatment. Text-figure 2A–C shows a block of muscle which is preserved in phosphate. The upper part of the figure shows heavily mineralized bright muscle fibres in cross-section, and below them are similar muscle fibres, but with a much reduced brightness, reflecting a much lower phosphorus content. These are less well-mineralized, and would also probably not withstand acid treatment.

Soft tissue recognition

By far the most important application of the technique has been in the identification of lightly phosphatized soft tissues, normally lost during acid treatment. Text-figure 2A–C, G–I, M–O shows blocks of muscle fibres as back-scattered or secondary electron images, a phosphorus map and a calcium map. The elemental maps produce an exaggerated image which allows much greater detail to be seen. This is particularly marked in Text-figure 2K where the stomach wall is shown to have complex internal structure barely visible on the secondary electron image.

In general, phosphorus is more abundant in bone than in the soft tissue, and accordingly appears much brighter and is easy to recognize (Text-fig. 2D–F). It is therefore possible to relate soft tissues to mineralized skeletal components, such as areas of muscle attachment and gill filament supports.

Distinguishing diagenetic minerals

Although the technique is essentially identifying differences in elemental composition, it highlights areas of greatest abundances of the element present. When examining specimens of known mineralogy the technique allows rough identifications to be made based on the elemental abundance. Thus in the Santana samples bright areas on calcium maps are almost always attributable to calcite. Multi-elemental mapping may confirm such *ad hoc* identifications, as does spot analysis by energy dispersive systems, e.g. EDAX.

In the Santana specimens diagenetic calcites, phosphates, baryte, celestine and pyrite frequently are associated with fossils with large voids. Elemental mapping allows for rapid approximate identifications of such phases, and for determining their diagenetic sequences and relationships.

DISCUSSION

Although our efforts have concentrated entirely on the Santana Formation concretions, we believe this technique may find wider applications. It is possible that soft tissue preservation by phosphatization is far more widespread than hitherto believed, but that many examples are destroyed because of the very delicate nature of the material (see Müller 1985). We recommend that exceptionally well-preserved fossils in concretions be examined in this way if it is suspected that soft tissues might be present. Besides phosphatized soft tissues, this technique may be suitable for pyritized and silicified soft tissues in a variety of host rocks.

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showing some enveloping muscle tissue; D, back-scattered electron image; E, phosphorus map showing greater extent of muscle tissue; notice the brightness contrast between the bone and the muscle fibres; F, calcium map of same area; notice that although it is clearly possible to identify the bony fin ray and muscle fibres, there is no contrast difference between the biomineralized and diagenetically mineralized tissues. G–I, muscle fibres and epithelium in cross-section; G, back-scattered image; almost no detail is discernible; H, phosphorus map showing two distinct layers of epithelium, each with distinct structure, overlain by a band of muscle; I, calcium map providing additional contrast. J–K, gut wall; J, secondary electron image with some vague detail visible on lower margin; K, phosphorus map of same showing structure within gut wall and presence of complex extensions into the lumen of the gut; L, calcium map of the same. M–O, several muscle fibres in cross-section; M, secondary electron image; here it is only possible to make out vague outlines of individual fibres; N, phosphorus map clearly showing boundaries of muscle fibres, some with enriched phosphate rims; O, similar detail in a calcium map. Magnifications: A–C, ×120; D–F, J–L, ×70; G–I, ×80; M–O, ×250.

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