Soluble Organic Matrix of axial skeleton and sclerites of *Corallium rubrum*: partial and comparative analysis of biomineralization

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Context:

Organic matrix (OM) is the organic fraction located inside biomineral structures of various species.

OM is known to have an important role in biomineralization processes. The development of extraction techniques of OM proteins and their characterization thus represents a key step towards an improved understanding of biomineralization.

Comparative studies between different species and models are essential to a greater understanding of biomineralization.

Introduction:

The precious coral *Corallium rubrum* (Linnaeus, 1758) from the Mediterranean Sea produces 2 different biominerals: the axial skeleton and the sclerites (Fig. 1, B, C).

By comparative studies of OM proteins of its 2 skeletal structures, *C. rubrum* provides an unique opportunity to determine the different and common points in the machinery of the biomineralization process.

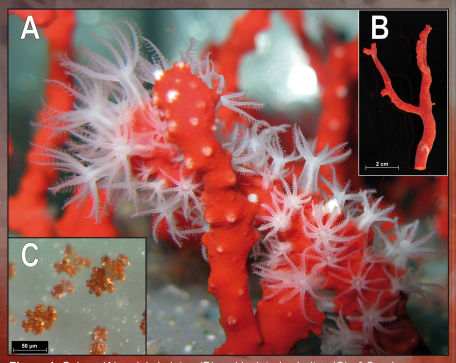

Figure 1. Colony (A), axial skeleton (B) and isolated sclerites (C) of *C. rubrum*.

Results:

Table 1. Quantification of proteins in OM extracts from axial skeleton and sclerites of *C. rubrum* (results expressed both as percentage and content relative to initial dry mass of skeletal structure used).

	Axial skeleton	Sclerites
Mean percentage	0.007%	0.008%
Mean value (µg/mg)	0.066 ±0.014	0.076 ±0.016

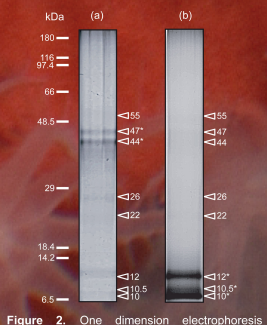

Figure 2. One dimension electrophoresis SDS-PAGE (12% poly-acrylamide gel) and silver staining of *C. rubrum* OM extracts (10µg proteins/lane). Lane (a): axial skeleton; lane (b): sclerites. Triangles show apparent molecular weight (MW in kDa) and (*) show major protein bands.

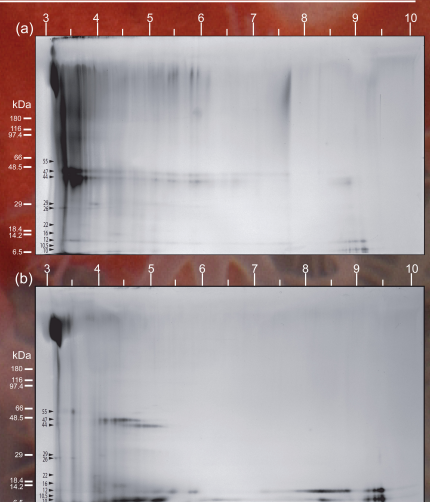

Figure 3. Two dimensions electrophoresis (IEF and SDS-PAGE electrophoresis; 4-12% poly-acrylamide gels) and silver staining of *C. rubrum* organic matrix extracts (20µg proteins/gel). (a): axial skeleton; (b): sclerites. Triangles show apparent molecular weight (MW in kDa) of major spots of proteins.

Gel electrophoresis analysis:

1. 1D gel shows protein bands of similar molecular weight (MW) both for axial skeleton and sclerites. However different intensities are observed (Fig. 2).
2. In 2D gels the protein bands are revealed as horizontal lines of protein spots with different Isoelectric points (Fig. 3).
3. 2D gels allow better discrimination of OM proteins.

Results:

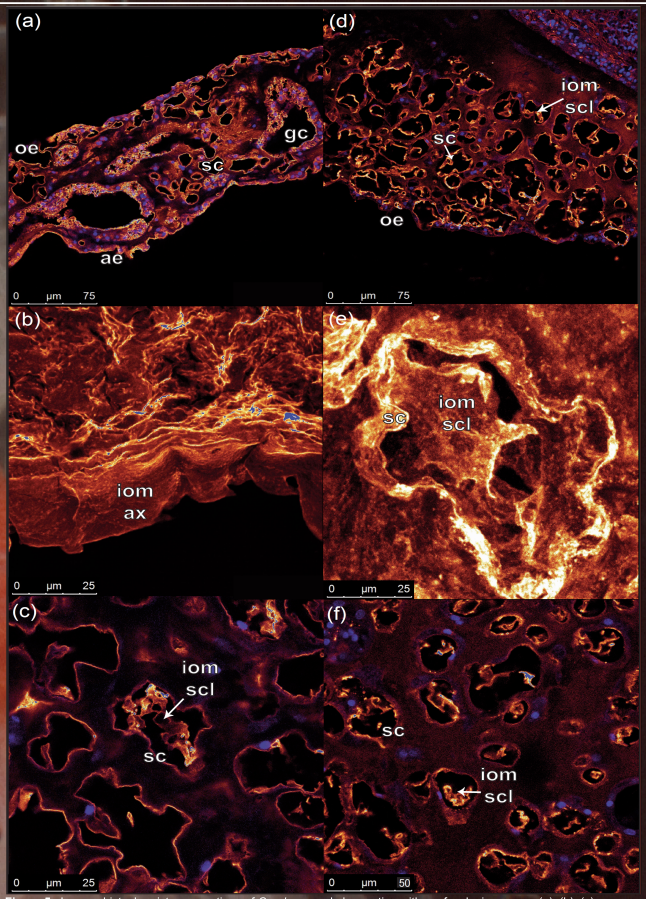

Figure 5. Immunohistochemistry on sections of *C. rubrum* and observation with confocal microscope. (a), (b), (c): orange labeling with antibodies against organic matrix of axial skeleton; (d), (e), (f): orange labeling with antibodies against organic matrix of sclerites. Nuclei labeled with DAPI appear in blue. Oral epithelium (oe); scleroblast (sc); gastrodermic canal (gc); aboral epithelium (ae); axial skeleton ghost (iom ax); sclerites ghost (iom scl).

Organic matrix localization:

1. Antibodies against axial skeleton and sclerites both label all tissues with a stronger labeling of the skeletogenic tissues (i.e aboral epithelium and scleroblasts; Fig. 4).
2. Decalcified axial skeleton and sclerites are labeled by antibodies and show ghosts of the mineral structures.

Conclusions and perspectives:

1. Axial skeleton and sclerites show similar quantity of OM proteins (Tab. 1) and seem to be composed of a set of OM proteins sharing apparent MW but differing in their biochemical properties (potential post-translational modifications).
2. Describing OM proteins opens possibilities for further investigations on sequencing and will be extended to other populations of *C. rubrum* and other *Corallium* species.
3. Potential use of OM proteins characterization as a tool for:
   i) *Corallium* species identification ?
   ii) taxonomic classification ?

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