Analogous biomineralization processes between the fossil coral *Calceola sandalina* (Rugosa, Devonian) and other Recent and fossil cnidarians.

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Abstract

The current work represents a distinctive study about the biomineral properties of exceptionally good preserved skeletons of *Calceola sandalina* from the Middle Devonian of Couvin (Belgium), Smara (Morocco) and (Algeria) and their relation in the evolution of biomineralization of cnidarians. Structural and crystallographic analyses of the skeletons have been done by petrographic microscopy, electron scanning microscopy (SEM), atomic force microscopy (AFM), electron backscatter diffraction (EBSD), computer-integrated polarization microscopy (CIP) and electron microprobe analysis (EMPA). *Calceola* skeletons have many similarities with other cnidarians, mainly with other Palaeozoic corals as Syringoporicae: The microcrystals are composed of co-oriented nanocrystals that remind to mesocrystals, suggesting a biocrystallization process by particle attachment (CPA). The relationship between the nanocrystals and microcrystals suggest a growth mode similar to mineral bridges. A similar model was described for Syringoporicae corals (Tabulata) and it is similar to the coordinated-growth mode described in scleractinians and molluscs. *Calceola* skeletons show also a convergent structure with scleractinian forming Rapid Accretion Deposits (RAD), which share
some structural and chemical properties. These evidences suggest analogous processes of biomineralization derived from a stem group of cnidarians. The results of this paper highlight the value of biomineralization studies in fossil organisms to understand the evolution of biomineralization mechanism through Phanerozoic.

Keywords: calcite, Rapid Accretion Deposits, stepped coordinated-growth mode, mineral bridges, evolution, biocrystallization.

Introduction

The phylum Cnidaria appears to have one of the longest fossil histories (2000 m.y.) and uncertain among the metazoan phyla (Scrutton, 1979), probably due to their morphological simplicity and gaps in the fossil record, which does difficult to recognise lineages. In fact, the mineralised record of cnidarian, although have a better understanding, is charged of controversy due to this lacks in the record (cites herein Coronado et al., 2013). During the Cambrian radiation, Tommotian onwards, appeared sporadically new organisms with mineralized skeletons that could be the ancestors of Palaeozoic Anthozoa (e.g. Hydroconozoa and Tabulaconids (Porter, 2010), uncertain Tabulata (Lafuste, 1991) and cothonids (Korde, 1959), among others). Nevertheless it was not until early Ordovician when the Palaeozoic corals appeared (Tabulata and later Rugosa), which radiating very quickly, occupying empty ecological niches and colonizing most environments in the Palaeozoic seas during more than 233 Ma.

After the Permian extinction, when Palaeozoic corals disappeared, the scleractinian dominated the seas during 247 Ma, but during 5 m.y. in early Triassic there is not record of mineralized cnidarian. However, the knowledge about their evolution, in terms of biomineralization pathways, is unresolved because the evolutionary link between Palaeozoic corals and scleractinian is actually controversial (Oliver, 1980, Sorauf, 1996, Ezaki, 1998, Cuif, 2014). Molecular studies in anemones and corals suggest an origin of Scleractinia rooted in the
Palaeozoic (at least 300 Ma. Romano & Palumbi, 1966; Medina et al., 2006; around 425 Ma.
Stolarski et al., 2011). On the other hand, Cuif et al. (2011) and Cuif (2014) have compared the
biocrystallization processes between Permian and Triassic corals with the purpose to solve the
evolutionary lineage between Rugosa and Scleractinia, as was suggested by Wang (1950),
establishing common morphological and biocrystallization processes between Polycoelidae
(Rugosa, Permian) and Pachitecalids (Scleractinian, Middle Triassic), although with marked
differences.

The phylum Cnidaria is represented in the geological record mainly by the corals. This
informal grouping refers to the skeletonized members of the Anthozoa class (Tabulata, Rugosa,
Scleractinia and Octocorallia, among others). Three mineral groups have been detected in
cnidarian (phosphates, sulphates and carbonates), being the carbonates the most abundant both
in fossil and Recent organisms (Lowenstan and Weiner, 1989; Macintyre et al., 2000).
Carbonates are ubiquitous in Anthozoa class (Cambrian to Recent), in form of spicules and
skeletons of Mg-calcite and aragonite in a genera (Octocorallia) and as skeletons of calcite and
aragonite (Rugosa, Tabulata, Heterocorallia and Scleractinia), known as stony corals, but also in
Hydrozoa skeletons such as Milleporidae and Stylasteridae (Rahman et al., 2006; Cuif et al.,
2011; Coronado et al., 2013; Janiszewska et al., 2011).

The most common biomineralization studies on cnidarian have been focused mainly in
fossil and modern Scleractinian (Cuif et al., 2011; Stolarski, 2003; Stolarski and Mazur, 2005,
Tambutté et al., 2011; Janiszewska et al., 2011, 2015) and recent octocorals (Dauphin et al.,
2006; Vielzeuf, 2010, Rahman and Yeishin. 2005; Rahman et al., 2006). Classically, the
microstructural studies in Palaeozoic corals have been driven to identify and classify
evolutionary patterns of biocalcification and their applications as taxonomical criteria (Wang,

Biominerals (sensu Marin et al., 2014) offer chemical information about the original
environmental conditions (by geochemical proxies). They also offer a possibility to understand
the growth processes that provide to organisms an evolutionary advantage to colonize different
habitats (e.g., growth patterns Barbin et al., 2008; changes in mineralogy Benedix et al., 2014;
formation of structural elements Pérez-Huerta, *et al.*, 2009, etc). The importance of biomineralization studies in fossils can help to understand the evolution of metazoan through Phanerozoic. Numerous articles have developed biomineral studies in different fossils; molluscs (Mutvei, 1997; Dauphin, 2002; Vendrasco, 2013), brachiopods (Williams and Wright, 1970; Balthasar *et al.*, 2011), cnidarians (Sorauf, 1980; Stolarski 2003; Stolarski and Mazur, 2005 Coronado *et al.*, 2013), porifera (Retiner and Engeser, 1987; Cuif *et al.* 2011) and trilobites (McAllister and Brand, 1989; Dalingwater, 1973; Lee *et al.* 2012) among others. Comparative studies of fossil and recent organisms considering their processes of biocrystallization, strategies and features, can also solve paleontological problems such as uncertain systematic affinities (Coronado *et al.*, 2015c). Likewise, these studies can help to understand the biomineralization in evolutionary terms, as common strategies of biocrystallization (Towe, 1978; Cuif et l., 2011), structures and chemical properties driven for the global geochemistry (Sandberg, 1983; Checa *et al.*, 2007a;Stolarski et al., 2007)).

The crystallo-chemical properties (*sensu* Mann, 2001; Coronado *et al.*, 2013) of biominerals that compose the fossil skeletons are not easily determined because they often are obliterated by diagenetical processes. Following this line, new advances on biomineralization of Palaeozoic corals have been focused on the biogenic origin of skeletons of Carboniferous Syringoporicae (Coronado *et al.* 2013; Coronado *et al.* 2015a, 2015b; Coronado and Rodriguez, 2015) a superfamily of tabulate corals, taking special interest in the diagenetical alteration, establishing of a biocrystallization model for this group. It is remarkable these studies are only the starting place to advance in the evolution of cnidarians from biomineralization.

The current work represents an innovative study about the biomineral (crystallo-chemical) properties preserved in the skeletons of an unusual rugose coral (*Calceola sandalina*) and their relation in the evolution of biomineralization of Palaeozoic corals regarding the aforementioned work.

The slipper coral *Calceola sandalina* is a common coral in Devonian rocks (Termier & Termier, 1948; Lafuste, 1983; Hill & Jell, 1969; Stolarski, 1993; Gudo, 1998; Galle & Ficner, 2004). This taxon belongs to Gonioophyllidae, a family of operculate Palaeozoic corals with a
distribution from Cambrian? to Devonian (Stolarski, 1993). The unusual morphology of *Calceola* and their operculum turned this taxon into a controversial topic for palaeontologists during more than a century, including it in different phyla (Kunth, 1986; Linstroem, 1882; Kowalski, 1983; Richter, 1929). *Calceola sandalina* is an ahermatipic coral that inhabited soft-bottom substrates and is typically found in moderate- to deep-water sediments (Jakubowicz *et al*., 2015). Lafuste (1983) compared the microstructure of *Calceola* (one sample of this study) and *Goniophylum* (from Silurian of Sweden), concluding that both microstructures are similar with slight differences. *Goniophylum* show a cupolar microlamellar structure similar to other rugose corals, whereas *Calceola* has ‘scutellate’ and plier-shaped microlamellae. This author suggested that although *Calceola* looks very close to *Goniophylum*, could be considered as a genus *incertae sedis*.

**Material and Methods**

Five skeletons of *Calceola* were selected in the Lafuste’s Collection from the Muséum National d’Histoire Naturelle (MNHN) of Paris (France). One sample come from the Middle Devonian (Eifelian, 393.3-387.7 Ma) of Couvin (Belgium), label A47628, were collected by Ms. D. Jacob (Lafuste, 1983); two samples from Smara (Morocco, old Spanish Sahara); Middle Devonian (Givetian, 387.7-382.7 Ma), label A47629 - A47630, were collected by a unknown collector and the two samples from Gara Djebilet (Algerian Sahara), Middle Devonian (Givetian, 387.7-382.7 Ma), label A47631 - A47632, were collected by Sr. P. Semenoff-Tian-Chansky (data of locality in Lafuste and Semenoff-Tian-Chansky, 1968). The taxonomic identification was done by Dr J. Lafuste and Dr. P. Semenoff-Tian-Chansky. Samples from various geological sites have been used to try to minimize artefacts due to the fossilization processes (diagenesis).

A multilevel study to recognize the structure, crystallographic arrangement and geochemistry by means of different techniques was achieved. The microstructure was studied
by petrographic microscopy (in all samples), electron scanning microscopy (SEM) (only at the Belgian sample, A47628), at microscale, and atomic force microscopy (AFM), at nanoscale, (only at the Belgian sample, A47628). The crystallographic properties and arrangement was studied by Computer-Integrated Polarization microscopy (CIP) at mesoscale (in all samples) and Electron Backscatter diffraction (EBSD) at microscale (at the Belgian sample, A47628 and Algerian samples, A47629-A47630). Besides the geochemical characterization was done using an electron microprobe analysis (EMPA) (at the Belgian sample, A47628). Microstructural and crystallographic analyses have not revealed differences between the different samples, reinforcing the study from a biomineral approximation.

**Structural characterization**

Ultra-thin sections from longitudinal and transverse sections were prepared using the method developed by Lafuste (1970); for correct visualization of microcrystals under petrographic microscopy the samples should be between 2 and 10 µm in thickness. The samples were uncovered and the surfaces were polished with alumina of 1 and 0.05 µm in the final process.

In addition, sample fragments, obtained by means of natural breakage of the skeleton, were prepared in order to observe the crystalline elements in three dimensions. SEM images were obtained with a scanning electron microscope JEOL JSM-6400 operated to 20 kV, equipped with an EDX system, located in the National Centre of Electron Microscopy (Universidad Complutense of Madrid, Spain). The samples were coated with a thin conducting carbon and gold coating and observed using secondary electrons.

The skeleton counterpart of thin sections, that presented better preservation, were selected to study with AFM. The samples were polished (polished with alumina of 1 µm, 0.3 µm, 0.05 µm) and cut forming thin slides and etched with a Milli-Q water solution for 7 h and observed with an Atomic Force Microscope, model Digital Instruments Nanoscope IIIA (Veeco), located at the National Centre of Electron Microscopy, Universidad Complutense de Madrid, Spain. The images were obtained at room temperature and in air using a tapping mode with a silica tip.
The images were processed with the Nanoscope software v5.30 r3 sr3 of Veeco Instruments Inc. and the WSxM v5.0 Develop 5.0 software of Nanotec (Horcas et al. 2007).

**Crystallographic characterization**

Slides were prepared by sectioning of transversal and longitudinal sections of Calceola. The slides were polished with alumina of 1 μm, 0.3 μm, 0.05 μm and finally polished with colloidal silica (0.06 μm) for EBSD analysis. Before analysis, samples were coated with a thin layer (1.5 nm) of Au/Pt (Pérez-Huerta and Cusack, 2009). The EBSD study has was carried out with an Oxford Nordlys camera mounted on a Field Emission Scanning Electron Microscope (FE-SEM) JEOL 7000 located in the Central Analytical Facility (CAF) of The University of Alabama. EBSD data were collected with Oxford Aztec 2.0 software at high vacuum, 30 kV, large probe current, and a resolution of 0.3 μm step size for crystallographic maps, and a working distance of about 10 mm. Finally, data were analysed using OIM 5.3 from EDAX-TSL.

In this study, EBSD data are represented by crystallographic maps and pole figures, which represent the stereographic projection of crystallographic planes in reference to the {0001} calcite plane. MATLAB™ toolbox MTEX (Bachmann et al. 2011) was used to plot the ODF (orientation density functions) of the EBSD maps. The GrainsSet is a tool of MTEX that has been chosen to determine grains differentiation, the analysis of misorientation and the representation of the pole figures in the plane {10\ 14}, which better represent the turbostratic distribution of crystals (Checa et al., 2007b; Coronado et al., 2015a), in the case there.

Ultra-thin sections (transverse and longitudinal) of all specimens were used for CIP (computer-integrated-polarization microscopy), independently of the preparation method of ultra-thin sections (cover or uncovered), using a petrographic Zeiss microscope with a reflex camera attached and a Kodak Wratten filter no. 25. The analysis have been performed at 10x magnifications (resolution of 3072 x 2048 pixel and the ratio pixel–μm is 1:1.14) to determine the crystallographic arrangement of coral skeleton microstructure at mesoscale. The CIP method (computer-integrated-polarization microscopy) has been described by Heilbronner & Barret (2014) as a method for texture analysis and optical orientation imaging. It determines the c-axis orientations of uniaxial minerals from optical micrographs, displaying the results in the form of
pole figures and orientation images, using a colour-code (CLUT), which represents each orientation. This method has been applied to biomineralization studies of fossil specimens with relevant results (Coronado et al., 2015b,c and Coronado and Rodríguez, 2015). The CIP analyses have been done with Image SXM software (Barret, 1997).

**Geochemical characterization**

Electron microprobe analysis was conducted on polished slides with a carbon coating, with a JEOL Superprobe JZA-8900 with five wavelength-dispersive spectrometers, located at the National Centre of Electron Microscopy (the Universidad Complutense of Madrid, Spain).

A quantification of some major, minor and trace elements (MTE) was made with EMPA in coral skeleton in random points (100 points). Nine elements were analysed (Ca, Mg, Sr, S, Ba, Na, Mn, Fe and P) at each point and two transverse sections were made sectioning Rapid Accretion Deposits (16 points), each point was analysed each 40 μm. An accelerating voltage of 15 kV with a beam current of 10 nA and a spot size of 5 μm were used. The counting time for punctual analyses was 45 s per element; given that 5 elements could be analysed simultaneously, the total time for each analysis was 90 s.

In addition, ten elements (Ca, Mg, Sr, S, Na, Mn, Fe and P) were mapped at two regions with rapid accretion deposits. The EMPA mapping enables simultaneous analysis of different elements and the generation of distribution maps for each element with 1μm resolution. An accelerating voltage of 20 kV with a beam current of 100 nA and a spot size and step interval of 1μm diameter (dwell time = 1000 ms) were used.

**Results**

**Structure of Calceola**

*Morphological description*

The specimens of *Calceola sandalina* are solitary corallum slightly curved, without rootlets, with deep calices and without tabulæ and dissepiments. The epithecal area in the
external surface has growth lines highly marked, which correspond with adult stage of ontogeny and this taxon have a characteristic thick skeleton. Slipper-like external morphology highly marked, with a sub-semicircular transversal section (Fig. 1). All the samples analysed lacked of operculum (Fig. 1), and the study is focused in the sclerenchyma area.

The structure of Calceola is composed of a flattened and straight hingeline area with protrusions (Fig. 1), formed by septa in a similar structure to articulation of strophic brachiopods, although with some differences. The hingeline area, where the operculum is attached, is formed by the amalgamation of major and minor septa, which sculpt fossae and ridges (Fig. 1). The central septum in middle of flattened side is known as K-septum (sensu Wright, 2010), the major septa are located generating ridges, with alar fossae at angles between flat and curved sides of corallum. Major and minor septa ridges are merged toward the lumen, which is almost filled by sclerenchyma. Major septa are wider than minor septa in the inner surface of lumen, and all them are separated by rows of depressions (desmocyte attachment scars, sensu Stolarski, 1993) in the interseptal space (Fig. 1). Single rows of desmocyte scars only can be observed in adult samples (Wright, 2010).

The mode of life of Calceola has been discussed in numerous papers due to their unusual opercular structure (Stolarski, 1993; Gudo, 1998, 2002; Galle and Ficner, 2004). Although some aspects of their life style, as the current orientation and feeding, are unresolved, the position of the calix over the muddy seafloor is accepted, lying on the flat side as a snowshoe (Stolarski, 1993; Gudo, 2002). Some authors suggest that the distal or cardinal tip of calix could be partially buried in sediments and the function of operculum is protecting the polyps of predators and turbulent currents, compensating the effects of sedimentation. Galle and Ficner (2004) suggest that the articulation of operculum could help to the coral to move upward in the sediments changing the gravitational centre of calix (Fig. 2A-C).

Microstructural characterization

Calceola sandalina has a complex microstructure, which is result of their complex inner structuration, probably as consequence of the opercular insertion and mobility.
The polished slides and thin-sections show that microstructure of *Calceola* is separated in three areas: K-septum, hingeline and septal apparatus (Fig. 2E-F). The different bricks that compose the microstructure are lamellae, fibres and granules.

Several areas have been distinguished by microstructure in the hingeline: a long undulated microgranular structure that separates the hingeline and the septal apparatus (Fig. 2E) crossing the semispherical section of the corallum along its transverse axis; and hemispheric microgranular zones (100 – 150 µm of radius) separated each c.a. 800 µm, which correspond with the protrusions centres of septa (Fig. 2E-F, white arrow). Surrounding these zones appear tiny microgranular lines that come from the flattened external part of the epitheca and intercept the undulated microgranular zone (Fig. 2F). These lines correspond with the fossae between septa and in some points the microgranular zones are cut in a triple point, where microgranular hemispheric areas are developed (Fig. 2F, 3A). These microgranular deposits (undulated structure, hemispheric deposits and tiny lines) are referred as Rapid Accretion Deposits (RAD) throughout the text (Fig. 2).

On the other hand, the septal apparatus is subdivided in two microstructural areas (Fig, 2E): composed-septa, which area formed by a row of major and minor septa parallels, pointing to the lumen and slightly curved to the K-septum (middle area of skeleton, Figure 2F). In polished sections an alternating change of coloration between septa can be observed. This area is shorter in calical regions than middle regions of skeleton (Fig. 2F). Moreover, the composed-septa end when the minor septa disappear, merging the major septa in a very sloped structure (Fig. 2E), and this area is referred as merged septa throughout the text.

The lamellae, *sensu* Lafuste (1983), are common microcrystals present in all the areas. They are straight to slightly wavy, with indentations at their edges, and are completely imbricated with each other showing a compact frame (Fig. 3). The most characteristic morphologies are ‘scutellate’ lamellae (Lafuste, 1983, Figure 3D), plier-shaped lamellae (Lafuste, 1983, Figure 3B,E) and cupolar (dome-shaped) lamellae (Lafuste, 1981). These structures are microlamellae, (< 25µm, *sensu* Rodriguez, 1989), having a lateral development in
two dimensions, with lengths from 3 to 15 µm (mean = 9 µm) and width from 1 to 5 µm (mean = 3 µm). In those areas where the stacking of lamellae is continuous as in the septal apparatus, the morphological axis is permanently oriented perpendicular to the lumen, parallel to growth direction, pointing the concave part of lamellae out to the lumen. In the hingeline the lamellae show a concentric appearance (Fig. 3A) around RAD, with the concave part pointing to the centre of structure.

K-septum is characterized by a lamellar sclerenchyma with dome-shaped lamellae, whereas the composed septa and merged septa show scutellate (large and undulate appearance, Fig. 3F) and plier-shaped lamellae.

The fibres (F) are located in the desmocyte scars, where the skeleton was projected forming tepee-like structures (Fig. 3B) toward the lumen area, favouring the attachment of cells. The tepee-like structures have a length of c.a. 100 µm. Fibres are crystals with irregular elongated morphologies and indentations at their edges, which occasionally converge at their apex forming needles (Fig. 3C), with a perpendicular orientation in relation to the location of the skeleton. The fibres are from 8 to 23 µm (mean = 17 µm) in length and between 3 and 5 µm (mean = 4 µm) in width.

Instead, the granules (G) are located in the Rapid Accretion Deposits (RAD) in the hingeline (Fig. 3A). The granules have a length from 2 to 6 µm (mean = 3 µm) and width between 2 and 4 µm (mean = 3 µm)

The microcrystals are structured by submicrometric lamination, which grew almost parallel to morphological axis (Fig. 3D-F). They are imbricated and their morphological shapes change gradually between the different skeletal elements (from granules to lamellae changing the sizes; from lamellae to fibres changing the morphological axis, Figures 3A-C).

Nanostructural characterization

AFM images of Calceola skeleton (Figure 4) show an intricate nanostructure composed of similar nanogranules in lamellae fibres and granules. The nanogranules are arranged forming pill-shaped morphologies with variable sizes, 63–155 nm (mean = 105 nm) in length and 24–48
nm (mean = 33 nm) in width, which depends on the section. The distribution of nanocrystals shows the long axis parallel to submicrometric lamination observed at microscale. This intralamination is composed of laminar aggregates of nanocrystals, arranged in domains co-oriented parallel to the morphological axis of microcrystals (Fig. 4C-D). In those cases where two microcrystals are co-oriented, the edge between both seems a disrupted contact. The nanocrystals are co-oriented in the contact points bridging the microcrystals (Fig. 4E-G). If the microcrystals are misoriented (Fig. 4A-D), the nanotexture between both crystals are misoriented and bridges between them cannot be observed.

In addition, the boundaries of the nanogranules show a dark colour in phase images, indicating a different chemical composition (Figure 4H, I, L), being in some cases very thin c.a. 5 nm and diffuse (Fig. 4 H, I). Groups of nanocrystals show dark envelopes around them, thicker than those observed between the nanounits (Fig. 4H). Several authors (Dauphin 2002; Baronnet et al., 2008; Cuif et al. 2008; Gorzelak et al. 2013; Coronado et al., 2015c; Coronado and Rodríguez, 2015) have studied these envelopes in recent and fossil organisms and proposed that they could be organic coatings from the original organic matrix with amorphous calcium carbonate (ACC) remaining.

Crystallography of Calceola

The location of the c-axis orientation images of (CIP) and the EBSD maps are positioned in the Figure 2.

CIP (mesoscale):

Computer-integrated-polarization microscopy (CIP) has been used with the purpose of identify the crystallographic arrangement of the skeleton at mesoscale. Three c-axis orientation images (COI) of a transversal section and their corresponding pole-figures were obtained using the CIP method from different parts: one in the composed septa and two in the hingeline area. The purpose was to analyse the relationship between the RAD undulate and the insertion of septa (Fig. 5A-B) and the relationship between the hemispherical RAD and the surrounding lamellar sclerenchyma (Fig. 5E-F).
The crystallographic data of CIP reveal a complex architecture. The c-axis is perpendicular to morphological axis in lamellae, and parallel in fibres, whereas the granules, and those transitional lamellae (more rounded) formed around of RAD, show a c-axis almost orthogonal to the structure. The c-axis orientation of the sclerenchyma is opposite in the hingeline side versus the septal apparatus. In the case of the microcrystals beyond the hingeline (septal apparatus) the c-axis points toward the lumen, but the microcrystals at the hingeline, exhibit a complex orientation constantly modified by the RAD, pointing to outside the c-axis of lamellae at the edge of coral (epitheca).

The mean inclination of c-axis is *c.a.* 35° in lamellae in the septal insertion of the hingeline area (Fig. 5A-B). Although the azimuthal dispersion is great, *c.a.* 90° as a result of the rotation around RAD area, most crystals are grouped in two pole maxima at *c.a.* 50° and the orientation and morphological axis exhibit an undulate trajectory between septa. The region I (Fig. 5) shows the punctual orientation of all pixels in the RAD zone, exhibiting a high inclination of c-axis *c.a.* 75° and a surrounded azimuthal distribution focused in two pole maxima completely opposite. The c-axis inclination of the orientation image (Fig. 5A-B) shows that RAD undulate line is discontinuous but exhibits the same inclination in all of scattered deposits.

During the advance of septa to the lumen, the composed-septa exhibit a more controlled c-axis orientation, with less azimuthal dispersion (Fig. 5C-D). The studied area shows a twist of the lamellae sclerenchyma favoured by the union of septa, showing a wavy structure and orientation (Fig. 5C-D). The azimuthal dispersion is constricted to *c.a.* 40° and the inclination varies depending on the side of septum, between *c.a.* 25° to 60°.

In the area of hemispheric RAD, the lamellae surround the microgranular zone, rotating each *c.a.* 15°. The mean inclination is *c.a.* 30°, similar to other areas, but as it approaches to the RAD the lamellae are more inclined *c.a.* 55° and an azimuthal dispersion of *c.a.* 40° in the area II, to *c.a.* 70° of inclination and azimuthal dispersion in the area III. The orientation image shows a large irregular ellipsoidal area around the RAD, where the lamellar sclerenchyma is highly inclined. This area can be observed in the polished sections with different coloration (Fig. 2E,F).
EBSD (microscale):

Six EBSD maps have been drawn in two different transversal sections of Calceola skeleton: three of them correspond with the composed septa area, one was done in the inner of K-septum, one was done in the inner of a hemispheric RAD and the last one was done in merged septa area.

The analyses with EBSD confirm that the composition of Calceola is only calcite, without traces of other carbonates, as was observed with petrographic microscopy and CIP. Each microcrystal have a pole maxima in the planes \(\{10\bar{1}0\}\) and \(\{10\bar{1}4\}\) characteristics of trigonal symmetry of calcite.

Crystallographic maps show the fibres and granules behaves like a single crystal (Fig. 6C-D, 7), the morphology of crystal is clearly defined by the index intensity images by means of the quality of diffraction of each crystal and the crystallographic orientation maps exhibit individual orientations for each microcrystal. In the case of lamellae, most of them behave as single crystals but some of them have composed-crystals features as can be seeing as in the plier-shaped lamellae (Fig. 6E-F). The index intensity images reveal different subcrystals forming the composed-crystal (fig. 6E). In misoriented crystals the none-diffraction areas are continuous and thick, whereas between co-oriented crystals and in the inner of composed-crystals are dashed.

The c-axis orientation varies with respect to the morphological axis between each crystallographic element; perpendicular in lamellae, parallel in fibres and almost vertical in granules.

Diffraction maps of composed-septa zone (Fig. 6A-B) show well defined crystals with microlamellae morphology imbricated composing a frame. The plane \(\{0001\}\) exhibits a pole maximum with a dispersion of \(c.a.\ 40^\circ\) and an inclination \(c.a.\ 30^\circ\). The planes \(\{10\bar{1}0\}\) and \(\{01\bar{1}0\}\) shows a rotation of a- and b-axes around c-axis each \(c.a.\ 15^\circ\) and an inclination of axis of \(c.a.\ 40^\circ\) (Fig. 6I-II). Orientation distribution function (ODF) of the \(\{10\bar{1}4\}\) plane confirm the rotation of a- and b-axes, showing an irregular turbostratic distribution (Fig. 6).
These features vary along the composed-septa, *e.g.* in those areas where the fibres appear, which correspond with desmocyte scars (Fig. 6C-D). These areas are characterised by a protrusion of the septum forming an attachment structure with the desmocyte cells. The fibres appear as tepee-like structures and the crystallographic orientation vary from the lamellar sclerenchyma to the tepee-like *c.a.* 85°. Two different areas have been observed: one with part of the crystals rotating *c.a.* 60° with an inclination of *c.a.* 40° and other cluster of crystals with an inclination of *c.a.* 15° and a rotation of *c.a.* 35° but with a gradual rotation of the c-axis between two clusters (Fig. 6IV-V). The pole figure and ODF figures, in the planes \{10\overline{1}0\} and \{10\overline{1}4\}, show a complex arrangement with a rotation each *c.a.* 45° of a- and b-axes.

On the other hand, the merged septa area shows a high ordered crystallography. The Figure A1A-B, shows a fusion area between two major septa (green colorations) and a minor septa (purple coloration). The c-axis shows a rotation between septa of *c.a.* 55°, and an inclination of *c.a.* 30° in lamellae (Fig. A1,I-II). The planes \{10\overline{1}0\} and \{10\overline{1}4\} show a rotation of a- and b-axes of *c.a.* 60° without turbostratic distribution (Fig. A1,III).

In the case of K-septum map, the EBSD data reveal a microstructure slightly ordered with a pole maxima in the plane \{0001\} but broadly disperse, and a variation of inclination of *c.a.* 30° (Fig.A1C-D). The planes \{10\overline{1}0\} and \{10\overline{1}4\} show a turbostratic distribution of a- and b-axes rotating *c.a.* 20° around c-axis (Fig. A1,VI).

In contrast to the previously shown, RAD show a microgranular texture (Fig. 7), composed by rounded granules with thick none-diffraction areas around them. The crystallographic grains reveal that the granules exhibit a pole maxima in the \{0001\} plane with an inclination *c.a.* 70°, and a rotation of a- and b-axes represented in the pole figures and ODF figures of *c.a.* 30° (Fig. 7I-III). The index intensity image shows that diffraction increases toward outside the RAD, and the crystallographic arrangement is more ordered (Fig. 7A).

The misorientation histograms show that the correlated and uncorrelated misorientation differs between the different zones studied, although some zones have similar distributions (Fig A2). The common features in the composed septa, merged septa and RAD have a main mode at 5°, indicating the co-orientation of microcrystals and highly ordered structures. On the contrary,
the area of composed-septa with desmocyte scars shows a less ordered crystallography, with a
distribution centred at the mode 45° and high misorientation angles (e.g. 85°). In the case of
merged septa the histogram describes a double distribution with two modes, one to 15°-20° and
other to 55°, the last one could be derived of the fusion of septa and their disorientation. K-
septum misorientation exhibit an almost normal distribution centred at 40°-45° in agreement
with the broad dispersion at the plane {0001}.

Geochemistry

The chemical composition of the rapid accretion deposits (RAD) was evaluated by EMPA
mapping (Fig. 8) in one area, and the chemical composition of skeleton thorught116 random
points of analysis of nine elements (Ca, Mg, Sr, S, Ba, Na, Mn, Fe and P) in a transversal
section. Only six elements (Ca, Mg, Sr, Na and S) and backscatter electron images (BSE) have
been represented in the Figure 8, because show representatives changes in that area.
Additionally, two transverse lines of RAD were analysed (16 points) to check the geochemical
internal variation within the structure (Fig. 9A-B, represents one of them). Table 1 summarises
the EMPA analyses for all the elements. The values of EMPA analysis were normalised to mol
% of CaCO₃, MgCO₃, SrCO₃, MnCO₃ and FeCO₃, whereas the values of S, Na, Ba and P were
given as ppm.

The BSE images (Fig. 8A, G, Fig. 9B) show a homogenous composition of the lamellar
sclerenchyma, whereas the RAD structure is clearly visible by the variation in its brightness.
The grey scale variation of the BSE image depends mainly on the atomic number of elements at
each point. Levels of Ca (Fig.8B) are decreased in the RAD, delimiting a circular structure and
some isolated spots around the structure exhibit a special decline of the Ca amount. This
variation has a co-variation with the Mg levels, which increase in these areas with regard to the
lamellae. It should be noted that some isolated points show high amounts of Mg (Fig. 8C,H).
These points match with black dots in BSE. The mol % of MgCO₃/CaCO₃ have been plotted,
exhibiting a linear correlation (R² = 0.96541). This linear correlation indicates these elements
are located at the same spaces in the lattice of CaCO₃. The MgCO₃ mean value in the corals is
2.39 mol % (Table 1), far below of the lower limit of high magnesium calcite (HMC), at 4 mol % of MgCO₃.

Sr and Na distributions are homogeneous except in some isolated areas. Sr seems to be more concentrated around RAD (Fig. 8D-E), in contrast with Na which seems to be concentrated in isolated points in the inner part of RAD, although values are lower than the detection limit (Table 1). Noteworthy the inner area of RAD show higher values of S with regard to the lamellar sclerenchyma, which exhibits a homogeneous distribution of S (Fig. 8F,I). The Ba and P value are not statistically representative and in many points its quantity is below the detection limit of the spectrometer. The Fe and Mn are elements indicative of diagenetic alteration, replacing the Mg with Fe and Mn (Barbin, 2013; Coronado et al., 2013; Coronado et al., 2015c). The values of these elements are very low in coral skeleton and homogeneous, except isolated points, not indicating diagenetic alteration (Table 1).

The analyses of transverse line (Fig. 9A) show that the values of Mg are increased with regard of Ca in the RAD area. S exhibit a slight rising in the RAD, but the maximum values not match with the Mg. Moreover, Sr exhibits a decrease in the RAD and a slight increase in the limits of structure.

Discussion and conclusions

The structural, crystallographic and geochemistry study reveals that Calceola skeletons are hierarchical structures formed by low magnesium calcite crystals with a high degree of self-assembly. The microstructural elements show gradual transition between them and the different specialized areas, which is common characteristic in Palaeozoic corals (Coronado et al., 2013, 2015a). The different crystalline bricks that form the microstructure (lamellae, fibres and granules) are imbricated and their morphological shape change gradually between the different areas of skeletal elements (hingeline, septal apparatus).

The microcrystals are composed of co-oriented nanocrystals that remind to mesocrystals (Cölfen and Antonietti, 2005). Observations at atomic scale (with AFM and TEM, Cuif and
Dauphin, 2005; Stolarski, 2003; Janiszewska et al., 2011) have demonstrated that the biocrystallization occur by particle attachment (CPA, sensu de Yoreo et al., 2015) in biominerals, including recent corals (Cuif et al., 2012). The nanoscale observations in Calceola suggest a crystallization process similar to CPA, by the attachment of nanogranules with pill-shaped co-oriented forming a microcrystal. Apparently the pathway of crystallization cannot be solved with our fossil data, but some evidences suggest that the Palaeozoic corals, including Calceola skeleton, could have grown by a stepped coordinated-growth mode as described in scleractinians and molluscs by Cuif et al. (2012) and also in Syringoporicae by Coronado et al. (2015a) and Coronado and Rodríguez (2015): 1) Attachment of nanocrystals and their arrangement in sub-micrometric laminae (Fig.3D-F,4 J-L) by CPA, controlled by by organic hydrogel. 2) Stacking of submicrometric laminae and the subsequent formation of microcrystals (Fig.3,4), controlled by the intercrystalline organic matrix. 3) Organization in higher skeletal structures as septa (Fig.5C-D), will be controlled by genetic code. The discontinuous contact of nanocrystals when two microcrystals are co-oriented (Fig.4H, 6E-F) may indicate a growth of microcrystals similar to mineral bridges (Checa, 2011), and the dashed none-diffraction areas inside of composed grains as plier-shaped microcrystals support this hypothesis (Fig. 6-F). Similar results were found in Syringoporicae (Coronado and Rodríguez, 2015).

The structure of Calceola is a dense microstructure comparable to a dendrite-like structure. Similar data have been reported in Syringoporicae (Coronado et al., 2015a), regarding to the interdigitated growth alike to described by Goetz et al. (2011) in primary layer of brachiopods, named 3D-jigsaw structure. This structure needs a complex organic structuration, which defines the dendrite-like structure by mean membranes, or by deposition of vesicles filled with the precursor of CaCO₃ in each place of biocrystallization Goetz et al. (2011); unfortunately the data presented here cannot evidence this process.

The new crystallographic evidences of Calceola show that the epithelial tissue should be almost in contact with the biocrystallization area because the skeletal structures shows high crystallographic arrangement, as result of the control exerted during biocrystallization. This is a common feature with other Palaeozoic corals, as Syringoporicae (Coronado and Rodriguez,
Like the turbostratic distribution in some of skeletal areas, this is lost in complex microstructural areas as desmocyte scars. Probably this feature is an inherited character of other cylindrical Palaeozoic corals (Coranado et al., 2015a) being more easy observable in the well organised areas as the composed-septa. However the concentrically distribution of microcrystalline domains of Syringoporicae (Coranado et al., 2013; Coronado et al., 2015a,b) is not represented in Calceola, as result of their complex inner structuration and the merging of their septal apparatus.

*Calceola* skeleton shows a great crystallographic organization being higher in the merged septa than in the hingeline area. The hingeline area is characteristic by their complex microstructure, highlighting the presence of Rapid Accretion Deposit (RAD) on it. These deposits have a diverse morphology, which depends on the position in the skeleton. The RAD in the hingeline separates two areas with opposite c-axis orientation (septal apparatus and hingeline).

As occur in Syringoporicae and other calcitic groups of animals, (brachiopods and molluscs) the crystallographic arrangement of the skeletons show a distribution and misorientation that would prevent skeleton fracture by cleavage. Calcite crystals are easily cleaved on their \{10\bar{1}4\} planes (Schmahl et al. 2004; Peréz-Huerta et al., 2007). The skeletons of *Calceola* have the c-axis oriented perpendicular to the growth direction around the lumen, except in the hingeline, where the c-axis is almost similar to growth direction. Coronado et al. (2015a) established that the circular variation of c-axis around skeleton in Syringoporicae protects against fracture from lateral currents of seawater and the turbostratic distribution could be a strategy to prevent cleavage. In the case of *Calceola* the disorientation of c-axis in the bottom-side, probably is related with the mechanical effort of hingeline area during the movement of the operculum for example when the coral was being retracted.

A remarkable structure present in *Calceola* is Rapid Accretion Deposits (RAD), which remind to RAD of scleractinian. RAD in scleractinian are deposits composed of very fine granular crystals forming occasionally hemispheric structures, which are surrounded by isometric aragonitic fibres, shorter at the beginning. A high concentration of organic phases at
the centre of RAD is common (Stolarski, 2003; Cuif et al., 2012; Janiszewska et al., 2015). On the other hand, these isometric aragonitic fibres are arranged in thin layers and individual bundles of fibres, composing a compact zone, densely banded with less organic phases present on them, forming the Thickening Deposits (TD, Stolarski, 2003; Janiszewska et al., 2015). The RAD are enriched, in contrast with the TD, in several elements as a response to ‘vital effects’; Mg, Sr, S and Ba (Cuif et al., 2003; 2012; Meibom et al., 2004, 2008), whereas several isotope ratios, such as $d^{13}$C, $d^{18}$O, and $d^{11}$B, are depleted in RAD (Holocomb et al., 2009). The enhancement in S and Mg has been associated with organic phases of organic matrix (which is composed by carbohydrates, proteins and lipids Dauphin et al., 2008, Cuif et al., 2011). The S is found mainly as sulphated polysaccharides (Cuif et al., 2003; Cuif and Dauphin, 2005; Dauphin et al., 2008, Cusak et al., 2008) and the Mg is introduced by a disordered Mg-bearing, which could accommodated in organic phases or in a highly disordered inorganic phase (Finch and Allison, 2008). On other hand, the RAD are nucleated quickly during the biocrystallization (Domart-Coulon et al., 2014), and probably the high concentrations of organic phases help to stabilise the CaCO$_3$ deposits during the fast growth.

The RAD in Calceola are enriched in Mg, S, and slightly in Sr in the edges of structure. The microstructure of these hemispheric deposits has a granular microstructure that changes to lamellae laterally, modifying the morphology and size of crystals, in a similar way to scleractinian. Besides, RAD in Calceola are located in areas of fast development, as the ridges of the hingeline area, responsible for the operculum closure. These data support the idea that the Rapid Accretion Deposits (RAD) of Calceola were a convergent structure with other cnidarians and their biocrystallization processes could be analogous.

On the other hand, Lafuste and Semenoff-Tian-Chansky (1968) described a system of tubes along of Calceola skeleton in other skeletons from Smara (Morocco, old Spanish Sahara). They found that these tubes, named ‘canalicules’, cross longitudinally the Calceola skeleton, near to external perimeter in the hingeline area. Lafuste and Semenoff-Tian-Chansky (1968) indicated a maximum diameter of 0.17 mm and a vertical spacing of generally 0.7-0.8, varying from 0.5-1 mm for the tubes. These features and their location in the structure suggest that the ‘canalicules’
described by Lafuste and Semenoff-Tian-Chansky (1968) could be part of the hemispheric RAD, which are structures of 100-150 µm of diameter and 800 µm of spacing. Lafuste and Semenoff-Tian-Chansky (1968) and Wright (2010) suggest that these tubes are filled with clear calcite. Although the biological significance of tubes is unknown, some authors have offered a hypothesis: Pedder et al. (1998) highlight that these tubes have not found in other corals and that they could be produced by commensal organisms. Wright (2010) rejects the commensalism idea and suggests that they could be part of the specialised muscles for the closing system of operculum. The new data reported here support the idea that these tubes described by Lafuste and Semenoff-Tian-Chansky (1968) could be a diagenetic artefact of the recrystallization of RAD, by dissolution of granules and sparite co-precipitation.

On the other hand, the RAD are composed of granules forming hemispheric structures in aggregates, which look opaque areas in transmitted light derived of stacking of crystals, similar to scleractinian. The Lafuste and Semenoff-Tian-Chansky (1968) descriptive observations of ‘canalicules’ system point to a similar hemispheric structures described in the RAD of septal areas of deep-water corals as Desmophyllum (Stolarski, 2003) and micrabaciids (Janiszewska et al., 2011). Although the recrystallized RAD of Lafuste and Semenoff-Tian-Chansky (1968) have a concave base instead of the convex base of recent scleractian.

Lafuste (1983) differentiated Calceola from Goniophyllidae by the microstructure, as explain above. The microstructural data provided by petrographic microscopy and EBSD in this study show dome-shaped microcrystals at the skeleton of Calceola in K-septum mainly. These microcrystals are common in Goniophyllum and Calceola suggesting that these two groups are not separated and they should be part of Goniophyllidae as grouped Hill (1991). Stolarski (1993) proposed a phylogenetic lineage between these groups and the parent group Cothoniida (Cambrian, Korde, 1963) based in their morphological similarities in early stages of growth. The crystallo-chemical features of this basal group should be analysed to enlighten the origin of Palaeozoic corals.
The features described in this study suggest common biocrystallization processes between different cnidarians (Tabulata, Rugosa and Scleractinian) at nano- and microscale. The crystallo-chemical features of Palaeozoic corals are most dissimilar to the scleractinians probably as result of their mineralogy and phylogenetic origin, but some common characteristics in the two groups of corals suggest analogous processes of biomineralization derived from a stem group of cnidarians. The results of this paper highlight the value of biomineralization studies in fossil organisms to understand the evolution of biomineralization mechanism through Phanerozoic.

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Figure captions

**Fig. 1.** Selected views, of macroscopic features, of *Calceola sandalina* from the Palaeontology Department of Universidad Complutense de Madrid. (A) Corallite with operculum (scale bar 1 cm). (B) Corallite with operculum in calical view (scale bar 5 mm). (C) View of a calix showing...
major (white arrows) and minor septa separated by desmocyte scars (white circle) and K-septum (scale bar 5 mm).

**Fig. 2.** Structural features. (A-C) Synthetic sketch of *Calceola* with the movement of the operculum and the change of gravity centre. Modify of Galle and Ficner (2004). (D) Synthetic sketch of the sections analysed in this study. (E) Transversal section where can be seeing the composed-septa and merged septa and the location of EBSD maps (scale bar 2 mm). (F) Transverse section of a calical area, where the EBSD maps and CIP areas are shown (scale bar 1 mm). White arrows point to two RAD as reference of the two sections.

**Fig. 3.** Microscopic features of studied taxa. F: Fibres, G: Granules. (A) Transverse section showing a Rapid Accretion Deposit (RAD) in the hingeline limit surrounded by lamellae (scale bar 100 µm). Inset of the RAD with a granular microstructure (scale bar 200 µm). (B) Lamellar sclerenchyma in the composed-septa area (scale bar 100 µm). (C) Fibres development in desmocyte scar in the composed-septa area (scale bar 100 µm). Inset showing the cupolar morphology of lamellae (scale bar 50 µm). (D-F) SEM images showing the microscopic features of lamellae. (D) ‘Scutellate’ lamellae from the composed septa area (scale bar 10 µm). (E) Plier-shaped lamellae from the merged septa area (scale bar 5 µm). (F) Large ‘scutellate’ undulated lamellae from the merged septa area (scale bar 10 µm). Note the wavy structure and the submicrometric structuration in the inset (scale bar 2 µm).

**Fig. 4.** AFM images of the nanostructure in *Calceola*. (A-B) Height and phase images in the contact of two lamellae (black dashed line, scale bar 500 nm). Note the arrows point to the c-axis orientation of microcrystals. (C-D) Height and phase images of the nanotexture of a microcrystal with plier-shaped (scale bar 1.25 µm). Note the intralamination that form the microcrystal and the misorientation between crystals (black dashed line). (E-G) Height, phase and amplitude images of a contact area (black dashed line) between two microcrystals co-oriented (scale bar 270 nm). (H) Phase image where the pill-shaped nanotexture can be observed in a transverse section of a microcrystal (scale bar 66 nm). Note the dark envelopes
around nanocrystals (white arrow). (I-J) Height, phase images of a longitudinal section of a
microcrystal showing the stacking of nanogranules forming a microcrystal (scale bar 400 nm).
(K-L) Height and phase image of nanotexture of a longitudinal section of a microcrystal,
showing the intralaminated structure of microcrystals (scale bar 180 nm).

**Fig. 5.** CIP analysis of a transverse section of *Calceola sandalina*. (A) Petrographic micrograph
of the insertion area of septa and the hingeline, showing the undulated RAD (scale bar 100 µm).
(B) Orientation image of the studied area (scale bar 100 µm). Inset showing the pole figure of
the entire investigated area. (C) Petrographic micrograph of the insertion area a composed septa,
showing the wavy microstructure (yellow arrows points to the septal ridges, scale bar 100 µm).
(D) Orientation image of the studied area (scale bar 100 µm). Inset showing the pole figure of
the entire investigated area. (E) Petrographic micrograph of the hemispheric RAD surrounded
by a lamellar sclerenchyma (scale bar 50 µm). (F) Orientation image of the studied area (scale
bar 50 µm). Inset showing the pole figure of the entire investigated area. Pole figures were
calculated as an orientation distribution function and provided in multiples of uniform
distribution intervals of 0.5 for c-axis orientations. Red points correspond to the punctual c-axis
orientation of each pixel of selected areas, I (RAD), II (upward lamellae) and III (RAD). Last
circle correspond with the standard colour look-up table (CLUT).

**Fig. 6.** Crystallography of composed-septa area (A-B), scale bar 60 µm; desmocyte scar (C-D),
scaling bar 35 µm and a detail of dendritic-like structure of septal area (E-F), scale bar 15 µm. (A,
C, E) Index intensity maps, showing the microstructural features of studied area. (B, D, F)
Crystallographic orientation maps, showing the main crystallographic orientations in the studied
areas. Note the gradual transition between the lamellae and the fibres of desmocyte scar in (D)
and the co-orientation in the plier-shaped lamellae in (F). Pole figures and ODF pole figures (in
normal direction view (ND) to the sample surface in a three axes reference system with
indication of the reference (RD) and transverse (TD) directions) indicating crystallographic
orientation of calcite crystals in reference to the c axes; and crystallographic key indicating
colour coding of crystallographic axes. I-III correspond with the (A-B) maps, and IV-VI corresponds with (C-D) maps.

**Fig. 7.** Crystallography of and hemispheric Rapid Accretion Deposits (RAD). (A) Index intensity maps, showing the microgranular arrangement of the deposit (scale bar 25 µm). Note the thick none-diffraction areas around microcrystals and the rise of crystallinity toward outside the RAD. (B) Crystallographic orientation maps, showing the main crystallographic orientations of the studied area (scale bar 25 µm). Note the gradual organisation of the crystals toward outside the RAD. Pole figures (in normal direction view (ND) to the sample surface in a three axes reference system with indication of the reference (RD) and transverse (TD) directions) indicating crystallographic orientation of calcite crystals in reference to the c axes; and crystallographic key indicating colour coding of crystallographic axes.

**Fig 8.** EMPA mapping of a transversal section of *Calceola* (scale bar 50 µm). (A) BSE image of the Rapid Accretion Deposits (RAD). Note the difference in colour between the RAD and the lamellar sclerenchyma. (B-F) Mapping of different elements (Ca, Mg, Sr, S).

**Fig. 9.** (A) Graph showing the distribution of the amounts the Ca, Mg, Sr, S along of a Rapid Accretion Deposit (RAD). (B) BSE image locating the analysed points (yellow dots, scale bar 100 µm). (C) Linear regressions of mol % MgCO₃ versus CaCO₃ and table showing the parameters of linear regression.

**Fig. A1.** Crystallography of merged septa area (A-B), scale bar 45 µm and K-septum (C-D), scale bar 50 µm. (A-C) Index intensity maps, showing the microstructural features of studied area. (B-D) Crystallographic orientation maps, showing the main crystallographic orientations in the studied area. Note the variation in orientation between the major septa in green coloration and minor septum in purple coloration in (B). Pole figures and ODF pole figures (in normal direction view (ND) to the sample surface in a three axes reference system with indication of the reference (RD) and transverse (TD) directions) indicating crystallographic orientation of calcite crystals in reference to the c axes; and crystallographic key indicating colour coding of...
crystallographic axes. I-III correspond with the (A-B) maps, and IV-VI corresponds with (C-D)

Fig. A2. Relative frequency (%) of the misorientation angles of different taxa studied by EBSD.
A diagram showing the distribution of elements S, Sr, Mg, and Ca across different point positions. The y-axis represents the intensity in arbitrary units (a.u.), and the x-axis represents the point position.

B is an image demonstrating uniformity across the sample with a scale of 100 μm.

C is a scatter plot depicting the correlation between MgCO₃ (%) and CaCO₃ (%). The equation is given as $y = a + bx$, with an adjusted R-squared of 0.96541. The table below lists the intercept and slope values along with their respective standard errors:

<table>
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<th>Equation</th>
<th>Value</th>
<th>Standard Error</th>
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<td>Intercept</td>
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<td>Slope</td>
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