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Juan Ramón Vidal-Romaní, Laura González-López, Marcos Vaqueiro & Jorge Sanjurjo-Sánchez

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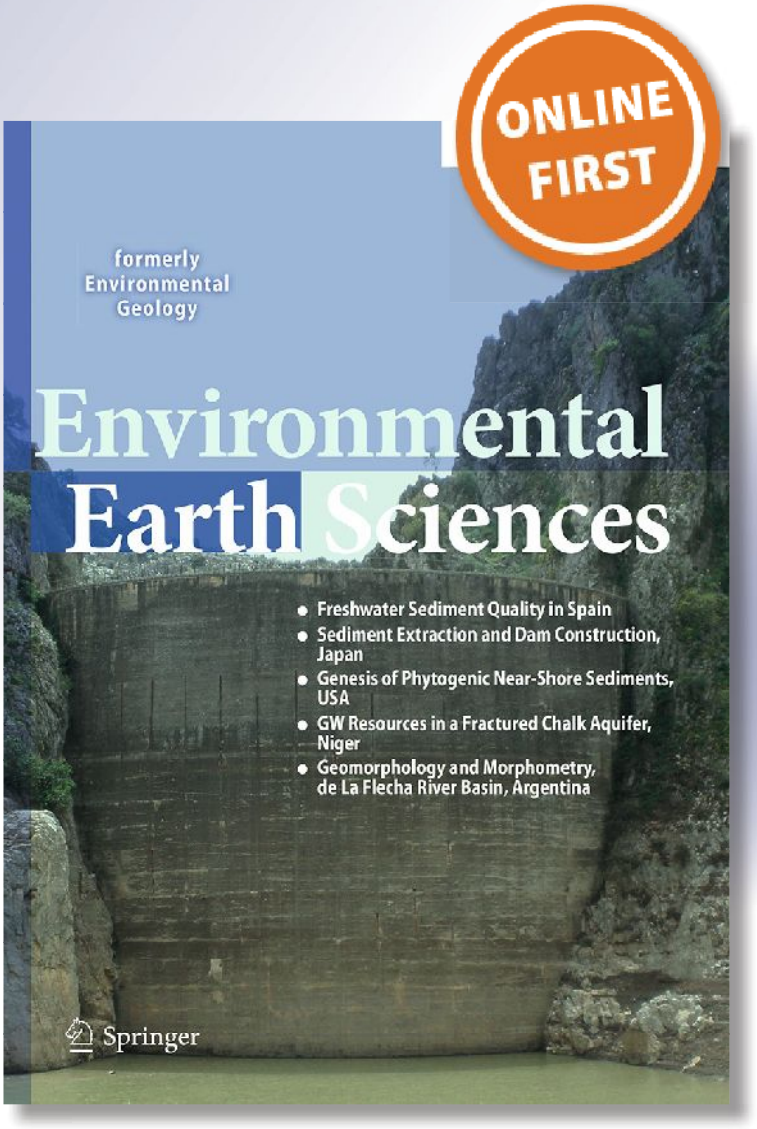

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Bioweathering related to groundwater circulation in cavities of magmatic rock massifs

Vidal-Romaní Juan Ramón[1,2], González-López Laura[1,2], Sanjurjo-Sánchez Jorge[1,2], Vaqueiro Marcos[1,2]

(1) Instituto Universitario de Geología, Campus de Elviña s/n 15071 Coruña, España.
(2) Clube Espeleolóxico Trapa. c/Manuel de Castro 8-3D. 36210 Vigo, España.
Corresponding author: juan.vidal.romani@udc.es – Tel.: 0034 981 167000

Abstract: Runoff flows not only on magmatic rocks massifs surface but also through their internal discontinuities, which define the secondary permeability of the rock. The effects, especially erosive, of the water movement on surface are well-known although it is not the same with the effects of water infiltration through the rock. Especially, when it does so at low speed (trickles or seepage) granular disaggregation of the rock is produced at small scale, associated with a specific type of sediment (speleothems) of clast-supported open fabric able to store very small volumes of interstitial water, becoming a specific subterraneous microenvironment where some organisms develop their biological cycle totally or partially. Some products derived from the metabolic activity of these organisms incorporated to the infiltration water increase their ability to attack (weathering and dissolution) the rock. This process ends during the dry season when the troglobiont organisms die and the water-transported load, either dragged or in solution (mainly silicon), is sedimented forming the speleothems. The study of these deposits using several techniques: mineralogical, sedimentological, biological (including metagenomics), indicates an influence of microorganisms on the formation of these deposits, therefore it is correct to consider them as biospeleothems.

Key words: magmatic rock caves, organic activity, biospeleothems, opal-A, whiskers, druse, micromineral, gypsum, calcite.

1. Introduction

Magmatic rocks are very susceptible to weathering with the extended contact with water enhanced by the oscillations of the water table (Twidale and Vidal Romaní 2005) in spite of their low solubility and lack of primary permeability. Weathering produces thick mantles of regolith generally associated with low relief areas (plains). Nevertheless, in all cases the greatest thicknesses of the regolith are located in the zones of the rocky massif affected by discontinuities, fractures or diaclases (secondary permeability) that allow the infiltration, and therefore the weathering penetration through the rocky massif. Subsequent erosion of regolith produces rocky landscapes which are formed by associations of specific forms similar to anywhere in the Earth with outcrops of magmatic rocks. Even though magmatic rocky landscapes are well-known on surface, there is scarce knowledge about structures inside the rocky massif (Twidale and Vidal Romaní, 2005; Vidal Romaní and Vaqueiro 2007). Inside, water circulates through fissures and hollows of variable dimensions, from some millimeters to several meters wide and metric to hectometric longitudinal developments. When water flows at high speed, it acts as erosive and transportation agent (Twidale and Vidal Romaní, 2005) as it occurs in endokarst systems. On the contrary, when the water circulates at slow velocity (trickles or seepage) through narrow conduits of capillary dimensions (Twidale and Vidal Romaní, 2005), rock weathering is strongly affected by the biological activity (bioweathering). This activity favours the formation of very specific types of small-sized speleothems (Vidal Romaní et al. 2010) directly associated with the water emergence on the ceiling, floor or walls of the cavity. At first (Caldcleugh 1829), the study of these deposits were interpreted as having been originated by the rock weathering s.l.. Later (Vidal Romaní et al. 1979; Webb and Finlaysson 1987) were exclusively focussed on identifying the mineral species that form the speleothems. However, scanning electron microscope (SEM) study of these deposits (Vidal Romaní and Vilaplana 1984; Kashima et al.1987; Vidal Romaní et al. 2010) allowed to relating the troglobiont activity to the formation of speleothems, justifying their name of biospeleothems (Forti 2001; Vidal Romaní et al. 2010). It is widely accepted that the weathering of magmatic rocks (Takaya 2014) is directly related to changes in water pH/Eh which produces a selective solution of the mineral components in contact with the water, weakening the rock cohesion and allowing its disaggregation in grains. This effect gives rise to the formation of speleothems because this granular material is first moved and then re-deposited by the water (Vidal Romaní et al., 2010) a short distance from which it was originated, forming sediments. These sediments are of open fabric, extremely porous (pores of some micrometers) and may temporarily accumulate water in small quantities. This water accumulation is sufficient for the speleothems to be a very favourable environment for the settlement of small-sized troglobiont organisms: bacteria, algae, fungi, amoebae and arthropods (collembolan, mites, isopods, thysanurans and arachnids) (González López et al. 2013; Vidal Romaní et al. 2010) which develop their biological cycle there partially or totally. The speleothems studied in this work were collected in caves developed in different magmatic rock types and situated in varied geographic-climatic environments: temperate humid to cold regions (Spain, Portugal, Açores Island, United Kingdom, Germany, Poland, Czech Republic, Sweden, Finland, Korea), tropical regions (Brazil, Venezuela, Madagascar) and arid regions (South and Western Australia, Argentina, Nigeria,

Swaziland, Mexico, U.S.A.) (**Map 1).** However, in spite of the large climatic and lithological variety of the caves from which the samples came, the final results are surprisingly consonant as to not only their sedimentological and mineralogical features but also the microorganisms associated to them, what seems to be due to a very uniform biosedimentary environment.

2. Material and methods

Speleothems studied come from cavities developed in magmatic rocks (senso lato) whose dimensions, morphology and origin (Twidale and Vidal Romaní, 2005; Vidal Romaní and Vaqueiro 2007) are much varied: simple fissures in the shelters, tafoni, block caves and fissure caves. In all these cases, the deposits are associated with trickles and seeping of water flowing inside the cavity on its walls, ceiling or floor, occasionally and apparently only depending to the pluviometric regime. Since the flows are temporal, not enduring, and always very scarce, a water analysis could not be carried out because longer stays would have been necessary (for example, it is the case of caves in arid areas). All the references used herein on the types of rocks where the studied caved are developed are based on data provided by the previous geological maps. Nevertheless, other data have been considered relevant in our work such as position of the speleothem on the ceiling, floor or walls of the cave, or lighting conditions of the cave. The samples selected for the mineralogical and sedimentological study were transported in hermetic containers to preserve the physical integrity of the samples. Once in the laboratory, they were observed under a Nikon stereographic microscope SMZ1500 interfaced with a Nikon camera DS-Fi1 to take photos of the samples that allowed obtaining the first data on the texture, structure and morphology of the speleothems. At this working scale, it was possible to identify some of the microorganisms which develop their vital cycle in the speleothems like arthropods (collembolan, mites, isopods, thysanurans and arachnids). This first examination allowed us to select the fittest specimens to be studied in a later work with a JEOL Scanning Electronic Microscope JSM 6400. For that purpose, the samples were treated in BAL-TEC critical point dryer CPD 030 and spattered with a gold coating of 50-100 Å with the BAL-TEC sputter coater SCD 004. The study of the samples under the SEM allowed a more precise observation of their texture and structure as well as a more precise identification of the microorganisms previously localized, which were studied in more detail. Moreover, other organisms not clearly distinguishable at stereomicroscope level like bacteria, algae, testate amoebae and, in some cases, spores and palynomorphs were observed. The study under the SEM also permitted us to carry out a semi-quantitative elemental analysis of the mineral fraction of the speleothems by Energy-dispersive X-ray spectrometry (EDS) with an Oxford Inca Energy 200 EDS equipment. The chemical and mineralogical composition of the fabric elements of the sediment and of the cement or matrix of the speleothems was thus obtained. Moreover, the study under the SEM allowed the determination of the morphology and symmetry of the individual crystals, twins and microcrystals. Due to their small size, these types of crystallizations, in most cases, could only be characterized with the SEM using the analysis by electron dispersive spectroscopy (EDS). The study of the samples with this technique also allowed us to establish the formation stages of the speleothem. When the size of the crystals of the speleothems was suitable, the samples were analyzed by powder X-ray diffraction (XRD) and X-ray fluorescence (XRF) to determine their exact mineralogical and chemical composition. For the XRD about 5 g of each sample was powdered and analyzed in a SIEMENS X-Ray diffractometer D5000, and the same quantity for the XRF analysis in a Fluorescence Spectrometer S4 Pioneer of wavelength dispersion Bruker-Nonius.

For the study of the not identifiable organisms of smaller size (bacteria), a genetic analysis was carried out. In this case, the sample collecting and transportation to the laboratory were made under more strict conditions. Samples were taken under sterile conditions to avoid any contamination with exogenous DNA. The samples were stored individually in sterile containers and kept on ice during transportation to the laboratory where DNA was immediately isolated upon arrival, grinding the rocks with UV-sterile pestles and using the reagents provided in the PowerSoil DNA Isolation Kit (MoBio). To carry out the 16S rRNA gene amplification a fragment of the 16S ribosomal RNA gene of around 530 bp was amplified using the primers SDBact0341bS17 (5'CCTACGGGNGGCWGCAG3') and SDBact0785aA21 (5'GACTACHVGGGTATCTAATCC3') (Herlemann et al. 2011). For each sample, a different oligonucleotide label was added to the forward primer in order to differentiate the reads corresponding to each of the samples, after sequencing. A negative control was implemented during DNA extraction in order to detect potential bacterial contamination. This control was further treated as if it was a regular sample. To carry out the FLX-454 sequencing: The purified amplicons were subjected to high-throughput pyrosequencing in a Roche FLX-454 sequencer, using one half of a plate. The selected experimental design was the one-way reads amplicon sequencing in order to increase the number of unidirectional reads per amplicon, as recommended by the manufacturer. Finally, to carry out the bioinformatic analyses the FLX-454 reads were processed using Qiime 1.7.0 (Caporaso et al. 2010) under default parameters, except when specied otherwise, and assigned to each sample. Operational taxonomic units (OTUs) were picked using cd-hit (Li & Godzik 2006; Fu et al. 2012) at the standard genetic distance value of 0.97. A prefiltering step was carried out in order to cluster sequences that were identical in their first 100 base pairs, prior to OTU picking.

Taxonomy was assigned using Blast (Altschul et al. 1990) against the Greengenes May 2013 database available at http://greengenes.lbl.gov.

3. Results

3.1 Nomenclature and classification of speleothems from caves in magmatic rocks

In order to classify these speleothems, different criteria are used in the literature. In most cases the nomenclature is based on the similarity of speleothems with either marine (coralloids) (Woo et al. 2008; Kashima et al. 1987), (stromatolites) (Konhauser et al. 2001; Aubrecht et al. 2008, 2012; Bustillo et al. 2010) or continental (terrestrial stromatolites) (Wright 1989) bioconstructions. Other nomenclatures used in the literature assimilate them to their karstic counterparts: popcorn, stalactite, stalagmite and flowstone (Anderson 1930), assuming that, like their equivalents in limestone caves, they are formed by the water dripping from the ceiling (stalactites) (Caldcleugh, 1829) to the floor of the cave (stalagmites and flowstone). As it is shown below, although occasionally speleothems from magmatic rock caves are formed by dripping, the vast majority are originated by water evaporation, and therefore the dripping has a secondary importance in the formation and growth of the speleothem. In this work, we decided to consider a more simple classification based on the morphology of the speleothem eliminating, where possible, any reference to the genesis of the speleothem which may only be known exactly by means of the examination of the speleothem with the scanning electronic microscope (SEM).

The following types of speleothems are distinguished: cylindrical or planar speleothems (Vidal Romaní et al. 2010).

3.1.1 Cylindrical speleothems

3.1.1.a. Individualized cylindrical speleothems: They are speleothems (Vidal Romaní and Vilaplana 1984) developed on the walls, ceiling or floor of the cavities. These speleothems grow by capillary movement of the water stored in the clast agglomerate mass of grain minerals coming from the previous rock weathering. They are usually thicker (up to 4 mm of diameter) and with longitudinal developments between 1 and 15 mm. Occasionally, they may develop either gypsum or calcite druses, small crystals or whiskers on the free ends of the speleothems (Fig. 1. They are cemented by opal-A in all cases.

3.1.1.b. Grass-shaped speleothems: They are numerous associations of very thin cylindrical forms (maximum 2 mm of diameter) associated with the ceiling or walls of cavities. These speleothems are formed by combination of growth of filamentous bacteria guided by capillary movements of the water from the basal agglomerate of grains soaked in water. They may develop either gypsum or calcite druses, small crystals or whiskers on the free ends of the speleothems, and they are cemented by opal-A in all cases.

3.1.1.c. Terrestrial microstromatolites: At first, these types of speleothems were described in cavities developed in sandstone (Aubrecht et al. 2008), but they have not been described in magmatic rock caves so far. They are formed by the growth of cyanobacteria that live as long as there is humidity in the cave, dying or ceasing activity during the dry season, thus becoming a mineral-organic substratum for the next generation in the cave environment in the following wet period. This mat of organisms acts as trap for sediments and is often cemented by opal-A which precipitates when the silicon hydrogel loses water by evaporation. A growth in typically "stromatolitic" rhythmic layers may be seen in cross-section. **(Fig. 2).** These terrestrial microstromatolites grow to any direction, hanging from the ceiling, on the walls, or from the floor of the cave though never related to dripping processes.

3.1.2. Planar speleothems

They are blankets on the rocky surface of variable thickness of micrograins (1 micron of diameter) formed by mineral clasts formed by the rock weathering and later water-transported a short distance from its original location as slurry. Water moves dragging rock grains produced by previous weathering, first as small films and later, when the water flow decreases and the evaporation starts, the surface tension produces the division of the water film into drops, which adhere to the ceiling, walls or floor of the cave, or even on rocky edges (edge effect), agglomerating the mineral grains around the drops. The final result is an intricate morphology of attached pools or microgour fields. The key of the sedimentation is the slow movement speed of the water film which does not overcome the adhesion force of the sand-water slurry to the rocky surface on which it moves. On steeped surfaces, microgour or pool edges are elongated in the water flow direction. Microgours fields show more irregular patterns in ceiling speleothems **(Fig 3 A and B).**

3.2. Components of speleothems

Speleothems are formed by three types of components: (1) inorganic due to physicochemical disaggregation of the rock (detritic fraction) carried out by the action of the water, (2) biological corresponding to the microorganisms which live in the underground environment using the sediment as physical substratum, and (3) biomineral (Westall and Cavalazzi 2011) which are the minerals formed by interaction between inorganic mineral substratum and metabolic products generated by troglobiontic organic activity, and incorporated in the water retained in the speleothem. From this relationship between mineral substratum and microorganisms, an increase of the weathering ability of the seeped water is produced, accelerating the

partial dissolution of pre-existing (inherited) minerals and even of the most stable ones (quartz). In magmatic rock caves, Si is the essential element of some biominerals (e.g. biogenic opal-A that forms frustules of diatoms or idiosome tests of testate amoebae), frequent organisms in these types of environments, but there are also other biominerals (gypsum, anhydrite) whose relationship with troglobionts seems to be less evident (Forti 2001). A priori, S necessary for gypsum formation may be given by the draining water that transports atmospheric aerosols of different origin (volcanic, fires, aeolian storms, sea fog) although it is more probable that they relate to the organic matter (e.g. guano, organic matter of soils, etc.) accumulated in the cavity (Forti 2001). Once the three components in dissolution, in suspension or dragged are incorporated into the circulating water, their evaporation, and not the dripping processes, triggers the subsequent speleothem formation. Other abundant elements in magmatic rocks such as Al and Fe also contribute to the formation of other types of speleothems whose origins are not related to troglobionts, thus they are not considered. This is the case for pigotite or allophane (Vidal Romaní et al. 2010). One of the aims of our research is to characterize each of the 3 components: inorganic, biological and biomineral, and to infer the relation among them, especially between microorganisms and the mineral and detritic fraction, to elaborate a proposal of formation and evolution of speleothems.

3.3. Mineralogy of speleothems

Another criterion used to classify speleothems of magmatic rock caves is their mineralogy, though it poses some difficulties due to the polymineral composition of the speleothems because of their different origins (inherited from the rocky massif and authigenic minerals). The inherited minerals are the matrix or physical base on which the authigenic minerals (of great interest to define the environmental conditions in which the speleothem was formed) are nucleated and grow.

The analysis of these minerals has great difficulties because it may be only made by scanning electronic microscope (SEM) as the authigenic minerals are dispersed in the speleothem mass, hindering their individualization to be studied with more specific techniques (X-ray diffraction).

Authigenic minerals appear either as very idiomorphic crystals or morphologically undifferentiated (amorphous minerals). The SEM study of authigenic minerals, their distribution pattern in the speleothem mass and their morphologic symmetry, when they have it, as well as their chemical composition allow us to know in which growth stage of the speleothem they were formed, and which relation they have with the microbiological activity developed in the speleothem, and therefore in the cavity. The authigenic minerals may be amorphous or crystalline.

3.3.1. Amorphous authigenic minerals

Opal-A ($SiO_2.15(H_2O)$) is certainly the most interesting one. This mineral was characterized by different analytical techniques: elemental chemical analysis, XRD, XRF and DTA-GTA (Vidal Romaní et al. 2010). It is an amorphous mineral whose formation implies the dissolution of Si enhanced by biochemical weathering of bacteria, algae, fungi and lichens (Vidal Romaní et al. 2010). The precipitation of the silicon hydrogel dissolved in water is due to oversaturation by evaporation, process that later has an important role in the nucleation and growth of the crystalline authigenic minerals (**Table 1**).

3.3.2. Crystalline authigenic minerals

There are three types: druses, whiskers and microminerals. In all of them, the nucleation and the growth of the crystals are produced from the hydrogel liquid base in physical, though not crystallographic, continuity with it. Druses, whiskers and microminerals form large sets sometimes visible to the naked-eye, and normally show multiple twins. The most frequent mineral species are gypsum, anhydrite, (Vidal Romaní et al. 2010), calcite and aragonite (Woo et al. 2008). In turn, microminerals have smaller dimensions (maximum 4 micron) than whiskers, and appear like isolated crystalline individuals or in biaxial twins. Up to now, halite, plumboaragonite, malachite have been identified, though the list of species is not closed (**Fig. 4**).

The identification of druses, whiskers and microminerals is carried out under the SEM along with their crystalline morphology combined with the semi-quantitative chemical analysis of the mineral by backscattered electrons (**Table 2).** It is not always possible to analyze them by X-ray diffraction due to the low concentration and dispersion of these crystals in the speleothem. This process was reproduced in the laboratory (García Ruíz et al. 1981), using a silicon hydrogel as growth base, under the same conditions in which the process described herein is carried out naturally.

3.3.2.a. Gypsum druses, microcrystals and whiskers. ($SO_4Ca.2H_2O$)

Some authors attribute the origin of S to the activity of microorganisms (Franklin et al. 1994; Welch and Ullman 1996) which are able to produce sulphates from oxidation of organic matter. Gypsum crystals ($SO_4Ca.2H_2O$) appear either in twins of hemisphere globular druses, individual crystals and whiskers (**Fig. 4)** outline, indistinctly either in planar or cylindrical speleothems and growing-up from the opal-A base.

In some cases, twins have a convex shape as if the crystals would have grown inside a hanging elongated drop. In other cases, the twin shape is bevelled in its upper part so that the twin seems to be cut by a flat surface. These morphologic differences seem to correspond to their relation with the form of the drop in which they develop: elongated in ceiling speleothems, attracted by gravity, and flat oblong in drops in floor speleothems.

This kind of authigenic mineral is formed in the last stage of the evolution of the speleothem, when the water contribution to the system decreases or finally stops. Its nucleation and later growth are produced from the silicon hydrogel as the losses by evaporation increase, causing the concentration of the forming elements of the crystal dissolved in water, as it is proved that the formation of gypsum crystals (monoclinic) is gradually replaced by the growth of anhydrite crystals (**Fig. 4).**

3.3.2.b. Calcite druses and microcrystals (CO_3Ca)

Calcite crystals also appear in physical, not crystallographic, continuity with the main body of the speleothem formed by opal-A (**Fig. 4**). As it occurs in gypsum crystals, the key question is the origin of C and, especially Ca. Given the relationship between the development of the speleothem and the rain water which seeps through the fissural system of the rock, the origin of C may be the CO_2 of the atmosphere, but also it may have an organic origin. Likewise, Ca may come from the minerals of the rock or have an organic origin, for example the bioclasts of aeolianites located outside the cave (Woo et al. 2008). Some authors (Stockmann et al. 2014) have experimentally established the role of silicate minerals surfaces on calcite precipitation kinetics, what allows explaining why calcite druses are invariably associated with caves developed in basic or ultrabasic magmatic rocks: diabases (Sallstedt et al. 2014), syenites (Vidal Romaní et al. 1997), granodiorites (Vidal Romaní et al. 1983; Gaal and Bella, 2008; Wojcik, 1961) or basalts (Woo et al. 2008) not knowing so far any reference of speleothems with this mineralogy for caves developed in more felsic, magmatic rock types.

3.4. Biological and genomic data of speleothems

Data obtained from the morphologic study of speleothems with the stereomicroscope and especially the SEM allow us to show (Vidal Romaní et al. 2010) that speleothems in magmatic rock caves may be considered biospeleothems, because in them there is a varied association of microorganisms which is actively involved, apparently with different strategies, in the construction of the biospeleothem, transforming it into a microsystem within the macrosystem defined by the cave.

Of all microorganisms found in caves in magmatic rocks, bacteria influence most on the development of speleothems. This is due to the relationship cause-effect of the silicon hydrogel which allows the fixation and development of filamentous bacteria and cocoids in the main body of the speleothems. This biological mat also acts as sedimentary trap fixing the detritic mineral particles. The biospeleothem thus created will later become the physical support where other organisms will be developed, creating a trophic network in the microsystem formed by each speleothem (**Fig. 5**).

Genetic studies on these types of sediments are little frequent or even scarce for magmatic rock caves, what increases the interest of the data given by our research which have allowed identifying the presence of a great variety of specific bacteria and comparing it with the usual one in genetic studies carried out on karstic or lava tube caves (Barton et al. 2001; Barton and Northup 2007; Cheeptham 2013; Engel et al. 2010; Epure 2014). Bacteria belonging to different phyla were identified. The most abundant, in decreasing order and by number of families identified, are: *Proteobacteria* (46), *Actinobacteria (33)*, *Firmicutes* (22), *Bacteroidetes* (7), *Chloroflexi* (5), *Verrucomicrobia* (5), *Acidobacteria* (4), *Planctomycetes* (4), *Chlamydiae* (4), *Nitrospirae* (2), *Fusobacteria* (2) and *Cyanobacteria* (2). The following bacteria families were also identified, belonging to the phyla *Gemmatimonadetes, OD1, AD3, Armatimonadetes, Chlorobi, Elusimicrobia, FBP, FCPU426, GAL15, GN02, OP3, Spirochaetes, TM6, TM7, Tenericutes, WPS-2, Thermi*, and of *Archaea* belonging to phyla *Crenarchaeota* (1) and *Euryarchaeota* (2).

Other microorganisms found in speleothems are testate amoebae, identified with SEM, highlighting the species: *Amphitrema wrightianum, Assulina muscorum, Physochila griseola, Centropyxis sp., Cyclopyxis sp., Corythion dubium, Difflugia minutissima, Euglypha rotunda, Euglypha strigosa, Sphenoderia lenta, Tracheleuglypha dentata, Trinema complanatum, Trinema lineare* and *Trinema enchelys.*

Diatoms are other organisms which develop in the biospeleothem, appearing in large colonies. Among the genres observed with the SEM, the highlighted ones are: *Hantzschia, Diadesmis, Orthoseira, Pinnularia, Neidium, Synedra, Sellaphora, Odontidium, Melosira, Aulacoseira, Planothidium* and *Stephanodiscus* sp.

Finally, in less quantity and only identified here in a very general way, we mention the existence of organisms belonging to upper links of the trophic chain of the microsystem of speleothems, corresponding to phylum Arthropoda like collembolan and thysanurans of the subphylum hexapode, and mites and arachnids of the subphylum Chelicerata.

4. Discussion

4.1. Genesis and development of speleothems

Speleothems of caves in igneous rocks are formed during the slow rainwater inflow through the fissural rocky system. Several compounds are added to the rainwater from its incorporation to the surficial runoff to its infiltration in the fissure system; some, for example C, are captured as CO_2 either in the same atmosphere or during runoff or infiltration through the soil. Others (Si, Al, Na, K, Ca, Mg, Fe) may be also incorporated to the seepage water while circulating through the rock fissures. Anyway, Si is one of the elements which hardly solubilises in water contrasting with the fact that it is the dominant element forming speleothems in magmatic rock caves. Low solubility and scarce velocity of the water flow also explain that speleothems, at the first stages, due to the rock disaggregation by especially moistening-drying effects, are agglomerates of angular grains of varied mineralogy in line with the rock in which the cave has been formed (inherited minerals). Under the stereomicroscope, grains of quartz, feldspar, mica, amphibole, pyroxene, etc. accumulate either as linear (cylindrical) or tabular (flowstone) speleothems growing on the walls, ceiling or floor of the cavities. The pH of fissural water of granite massifs ranges between 4.5 and 5.0, (Vidal Romaní et al. 2010) even though the pH may be higher in more basic magmatic rock caves (Woo et al. 2008; Sallstedt et al. 2014; Stockmann et al. 2014). These differences are mainly shown by the types of authigenic minerals formed in both cases: opal-A is the prevailing mineral species in acid pH environments while calcium carbonate in basic pH environments (Sallstedt et al. 2014; Vidal Romaní et al. 1983, 1997; Gaal and Bella 2008; Wojcik, 1961; Woo et al. 2008). However, another factor that also influences is the change in pH related to the microbiological activity (Cañaveras et al. 2001; Barker et al. 1997) developed inside the fissural system which releases low molecular weight organic acids (mainly oxalates) with chelant ability. These organic compounds added to the water increase their weathering ability helping Si dissolution and justifying the formation of amorphous opal. In cavities developed in more basic magmatic rocks, the prevailing authigenic mineral is calcite or aragonite (Woo et al. 2008; Sallstedt et al. 2014), similarly to what occurs in karstic environments, though less important quantitatively; it also is very significant that both the genomic studies and the samples studied with the Scanning Electronic Microscope (SEM) show a scantier spectrum of microorganisms than that of felsic rock caves. Along with these data, four stages are distinguished in the formation of speleothems: 1. Detritic accumulation of mineral clasts of the rock originated by physical weathering; 2. Colonization of the sedimentary porous fabric by microorganisms and dissolution of the mineral clasts; 3. Formation of silicon hydrogel; and 4. Growth of whiskers, druses and/or microcrystals from the silicon hydrogel substrate at the beginning of the dry period and ending with the consolidation of the silicon hydrogel as amorphous opal. In caves developed in more basic rocks, the role of the silicon hydrogel in the speleothem construction is less important. The four stages represent a micro cycle from wet to dry related to the water seeped through the rocky massif, which is repeated indefinitely while there are water contributions, even if they are very spaced-out in time.

It is estimated that the accretion of a speleothem of typical dimensions, some 1 cm long, requires at least about 100 years to be formed in a wet-warm climate (Sanjurjo and Vidal Romaní 2011). But in arid zones, the same speleothem would need 10 times more because the growth of a speleothem is only carried out when water circulates, then evaporates and finally opal-A precipitates. According to this, a very important question is: Why the sizes of the speleothems are very similar whatever the pluviometric regimen under which they develop? In arid zones, speleothems have similar sizes to those of wet-warm zones because organic activity rates are higher during wet phases due to higher temperatures (Watkins et al. 2011). In wet-warm zones with more regular and continuous rainfalls (therefore greater dilution of the solute) and lower temperatures, processes are slower. For this reason, the final result is always very similar everywhere. Another age criterion is that the amorphous opal (opal-A), at pressure and room temperature, does not form stable crystalline structures that, over time, evolve into a more stable silicon polymorph like opal-CT (Bustillo 1995). This implies a recent age for speleothems where opal-A is identified as mineral phase (Vidal Romaní et al. 2010) and an older age when the mineral phase is opal-CT. The first dating by optically stimulated luminescence (OSL) of opal-A speleothems (Sanjurjo and Vidal Romaní 2011) indicated that the growth of a speleothem may extend up to 3000 years in some cases.

Undoubtedly, the greatest interest of our study is its contribution to understand the relationship between speleothems and organic activity developed in the underground environment. The genomic studied we carried out, preferably in caves of felsic magmatic rocks, identified 29 phyla within the domain Bacteria and 2 within the domain Archea, about twice the amount described for caves in basic and ultrabasic magmatic rocks and in limestones. This implies that the remarked typical olygotrophic conditions of underground environments seem to be more restrictive for caves in felsic rocks. The most representative phyla in both cases, felsic or basic, are: *Actinobacteria, Chloroflexi, Proteobacteria, Acidobacteria, Nitrospirae, Verrucomicrobia, Bacteroidetes, Firmicutes, Planctomycetes* and *Gemmatimonadetes*, however, similar to the ones described for soils (Janssen 2006), caves in limestones (Cheeptham, 2013); thus, speleothems have been considered (Boston et al. 2009) a type of *speleosol* "sui generis". A special case is the phyla *Proteobacteria*, common in karstic caves associated with sulphurs or in volcanic lava tubes (Riquelme and Northup 2013; Engel 2010), which is the most abundant in the caves studied in this work. Though there is no relation with sulphurs in them, druses, microcrystals and whiskers of authigenic gypsum crystals normally appear on the free ends of biospeleothems. This seems to mark a key role of the Proteobacteria, extremophile

species capable of living under conditions of scarcity of resources in the formation of sulphur oxids and later gypsum crystals (Fig. 6B). Phylum *Cyanobacteria* is unusually found in caves because they need sunlight to fuel photosynthesis (Northup et al. 2012), but they frequently appear in our study caves because they are small and the light is close to the cave entrance where there is enough light. However, the special feature of these environments is given by the presence of different families belonging to the phyla, *OD1, AD3, Armatimonadetes, Chlorobi, Elusimicrobia, FBP, FCPU426, GAL15, GN02, OP3, Spirochaetes, TM6, TM7, Tenericutes, WPS-2, Thermi*, and of *Archaea* belonging to the phyla *Crenarchaeota* and *Euryarchaeota.*

If bacteria help us to understand how the construction of a speleothem is carried out and the formation of authigenic minerals, the rest of microorganisms are also essential to understand the dynamics that takes place on the speoleothem surface. Along with the mobility grade or movement autonomy on the speleothem surface, two groups are distinguished: the ones with less mobility represented by testate amoebae and diatoms, and the ones with greater movement capacity: collembolan, mites, thysanurans and arachnids. The microorganisms with less mobility (amoebae and diatoms) develop their biological cycle inside the cave and on the speleothem surface. They are strongly determined first by the presence of liquid water, at least a thin film, that covers the speleothem and also by the availability of the chemical elements, essentially Si, necessary for the construction of their exomes (amoebae) or frustules (diatoms). Thus, the slightest hint of water decrease causes a hydric stress in the microorganisms the most noticeable effect of which makes the testate amoebae, instead of building their own tests, use any alternative element available in the environment: exomes of other amoebae, fragments of diatoms, moulting fragments of collembolan or arthropods, or even mineral grains from the speleothem in order to achieve a protective cover (test) as soon as possible. The second group of microorganisms (collembolan, mites, thysanurans and arachnids) has greater mobility, and therefore search for the most suitable environment to survive in, for example mites digging their own shelters in the areas where the survival possibility is greater (bottom of microgours). It may be then said that all these microorganisms, each along with its movement capacity and apart from creating a specific microsystem, take part actively in the formation of these biospeleothems.

5. Concluding remarks

1.- Four basic stages have been defined in the formation of speleothems of magmatic rock caves: 1. Detritic accumulation of mineral clasts of the rock originated by physical weathering; 2. Colonization of the sedimentary porous fabric by microorganisms and dissolution of the mineral clasts; 3. Formation of silicon hydrogel; and 4. Growth of whiskers, druses, microcrystals and/or nanominerals from the silicon hydrogel substrate at the beginning of the dry period and ending with the consolidation of the silicon hydrogel as amorphous opal.

2.- In caves developed in the most basic rock types, the role of the silicon hydrogel in the construction of speleothems, both attracting microorganisms which look for Si availability and promoting the growth of druses or calcite-aragonite microcrystals, is less important.

3.- Although these types of environments are considered to have olygothrophic conditions, genomic studies reveal the great variety of microorganisms that exists in magmatic rock caves, about twice the amount described for basic magmatic rocks or for calcareous caves.

4.-The relationship between the formation and the evolution of these types of speleothems is established by the water availability, even in small quantities and during short time periods.

5.- Considering that all the data in this work are obtained from sediments sometimes of several thousands of years old, our work shows that it is possible to carry out the genomic studies on “fossil” material successfully.

6.- It has been proved the relationship between microbiological activity and development of these speleothems both in their fabric and structure, and mineralogy and external shape.

7.- We have to underline the greater efficiency of our working method based on studies with the scanning electronic microscope (SEM) and pyrosequencing by metabarcoding. Our method yields better and more complete results with very small samples than the normal studies with cultures of biofilm samples.

Acknowledgements

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Legend of figures, tables and geological map

Fig.1 Globular hemisphere of gypsum crystals growing from an opal-A cylindrical speleothem (Castelo da Furna, Northern Portugal)

Fig.2 Terrestrial stromatolite: general view (left) and cross-section (right) showing stromatolitic texture. A Trapa Cave, Pontevedra, Spain

Fig. 3 (A) Ceiling microgour of opal-A speleothems (Las Jaras, Córdoba, Spain). (B) Wall microgour of opal-A speleothems. (Peña del Hierro, Riotinto, Huelva, Spain)

Fig. 4 EDS (SEM) composition of autighenic crystalline minerals of speleothems. (A) Plumboaragonite (Las Jaras, Spain). (B) Halite. Boda (Grottorna, Sweden). C Calcite, (Tjuv Antas Grotta, Sweden), D Gypsum monoclinic (Dromgrotta, Sweden). E Anhidrite orthorombic, (Avila, Spain).

Fig. 5. Diversity of bacterial morphology in biospeleothems of magmatic rock caves. Scanning eletron microscope images. Escale: 50 μm (fig. A-E) (fig. F-J) 8 μm. A: Filamentous bacteria covered by opal-A (Castelo da Furna. Portugal). B: Filamentous bacteria on gypsum whiskers (Drömgrottan. Sweden) C: Testate amoebae *Euglypha strigosa* and bacterial spores (Gundarin Hill. Australia) D: Opal-A cilindric speleothem with filamentous bacteria on its surface (Skalboberget. Sweden). E: Testate amoebae Trinema complanatum and bacterial mats (Trapa. Galicia. Spain). F: fusiform bacilli (Berrocal del Rugidero. Extremadura. Spain). G: Coccoid bacteria arranged in rows (Trapa. Galicia. Spain). H: Bacterial spores germination (Skalbeberget. Sweden). I: Tetrads of coccoid bacteria (Bodagröttan. Sweden). J: Filamentous bacteria forms with pili or filamentous extracellular polymeric substances (Trapa. Galicia. Spain).

Table 1 Elemental composition by EDS (SEM) of speleothems

Table 2 X-Ray diffraction of opal-A speleothems. Ar (Argentina) (1) Anillaco, La Rioja, Argentina, (3) Pampa de Achala, Córdoba, Argentina. Au (Australia) (2) Gundarin Hill, Western Australia. Spain (Sp) (4)Las Jaras, Córdoba, Spain ,(5) Porteliña, Pontevedra, Spain, (6) Trapa Cave b Pontevedra, Spain, (7) Trapa Cave a, Pontevedra, Spain, (10) O Pindo, Coruña, Spain, (11) Albarellos, Avión, Ourense, Spain(8). Po (Portugal) Castelo da Furna b, Northern Portugal, (9) Castelo da Furna a, Northern Portugal,

Map 1. Geological map of World with the distribution (in gray) of main magmatic rocks outcrops. (Ar) Argentina, (WA) Western Australia. (Po) Portugal, (Sp) Spain, are the places of origin of the samples analyzed.

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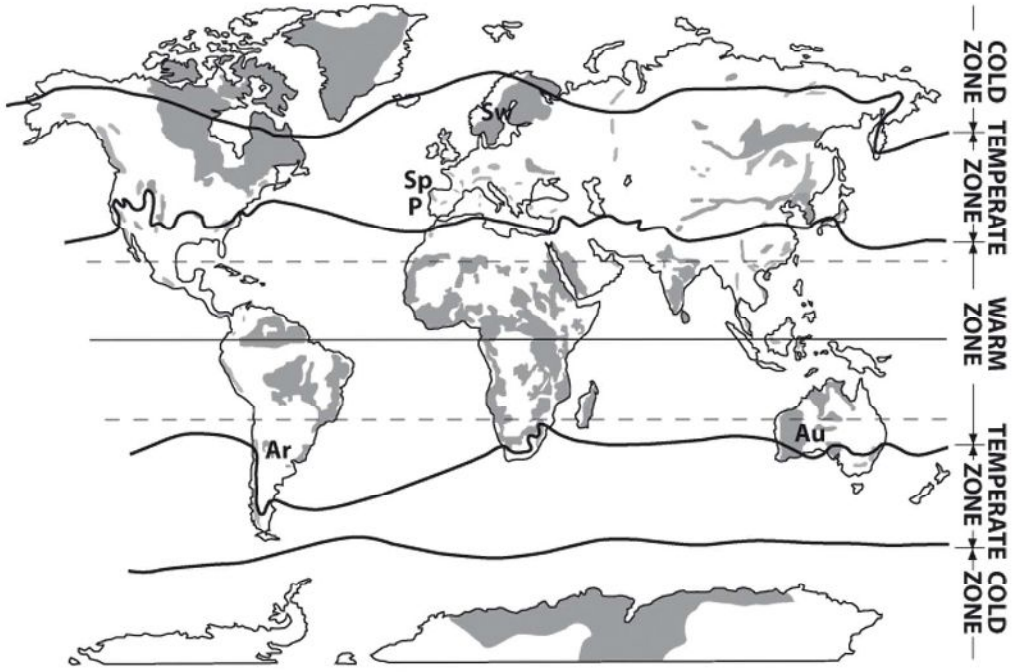
Map 1. World distribution, in gray, of magmatic rocks outcrops where the studied speleothems are located and their relation with climatic zones (Ar) Argentina, (WA) Western Australia. (Po) Portugal, (Sp) Spain, are the places of origin of the samples analyzed.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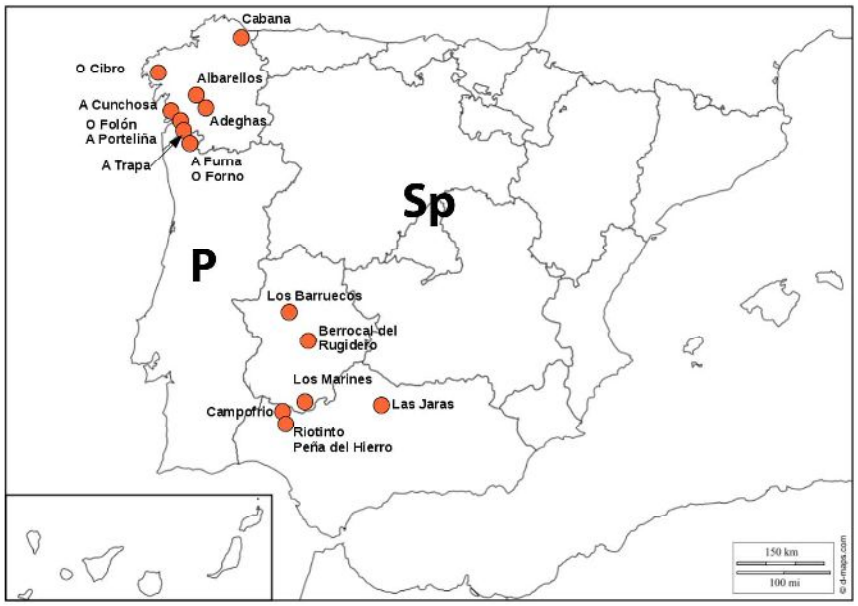
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Berrocal del Rugidero
Los Marines
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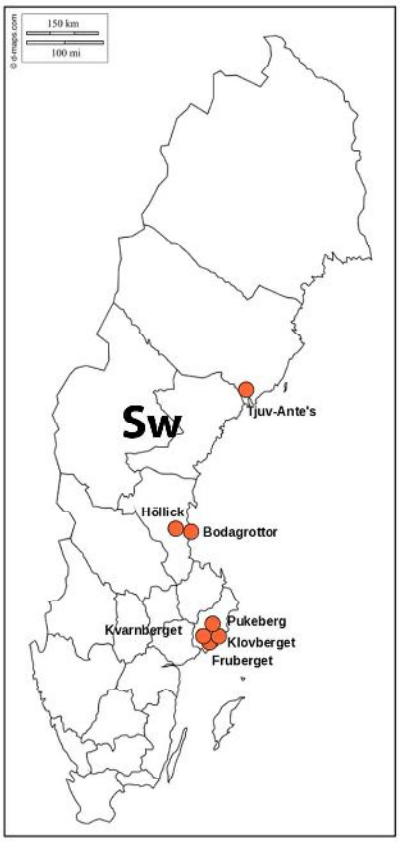
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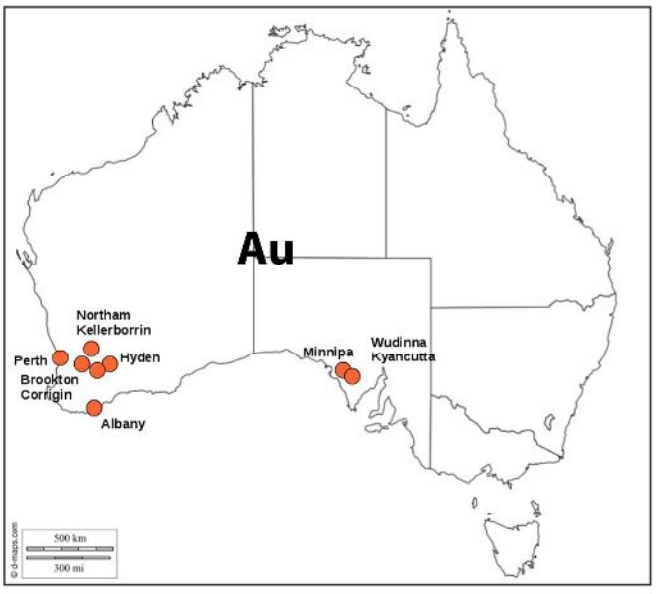
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Figure
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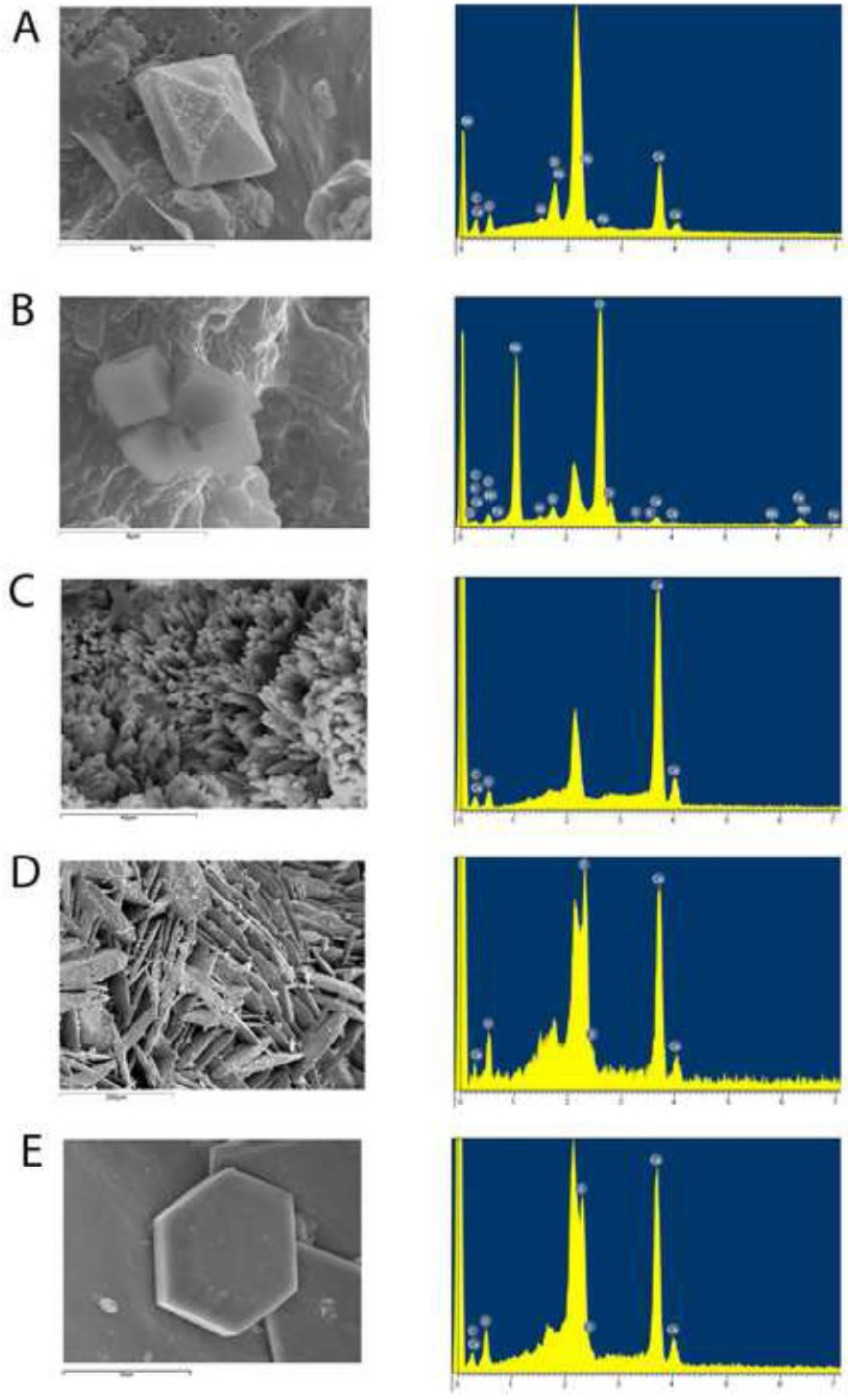

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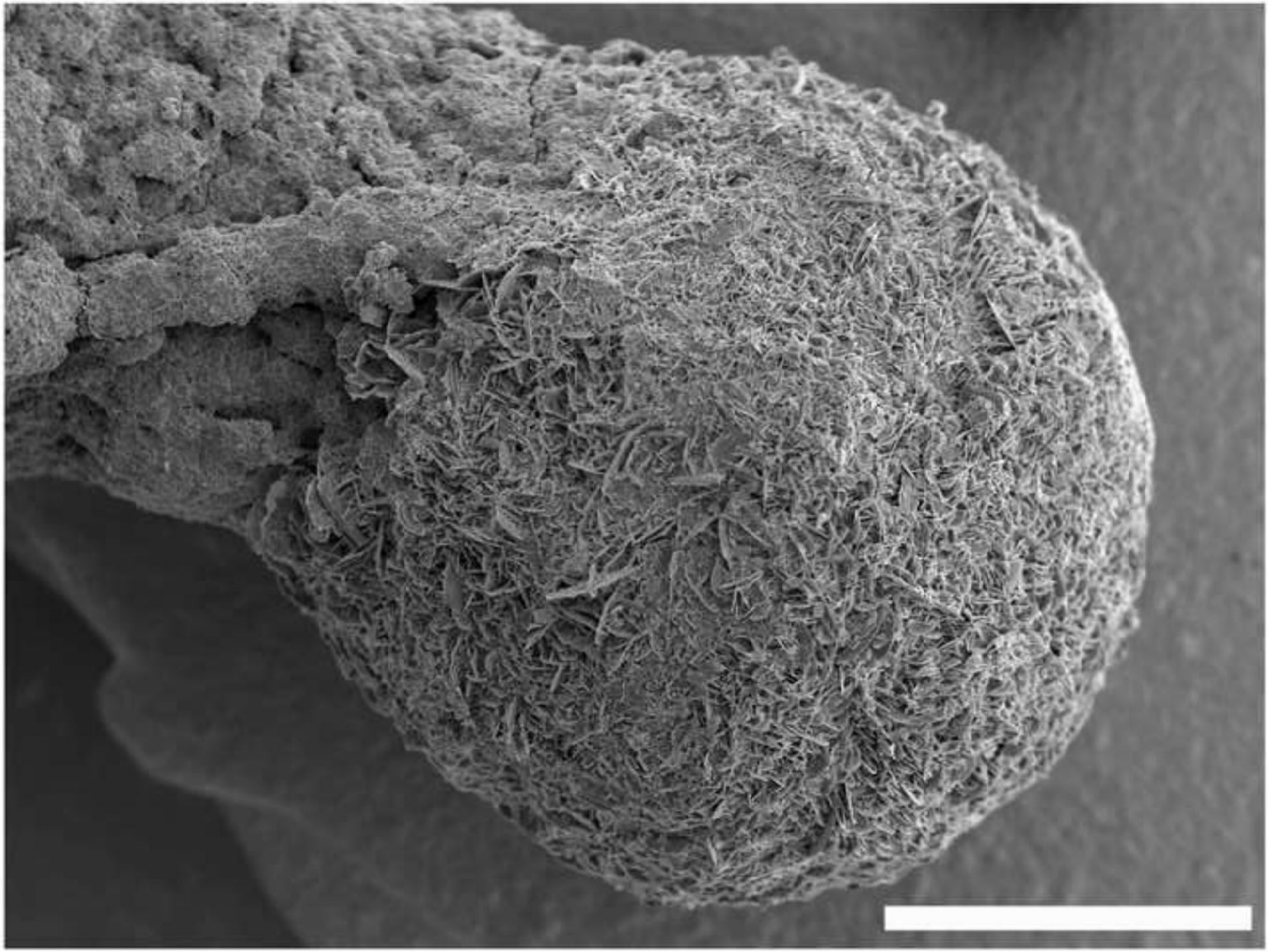

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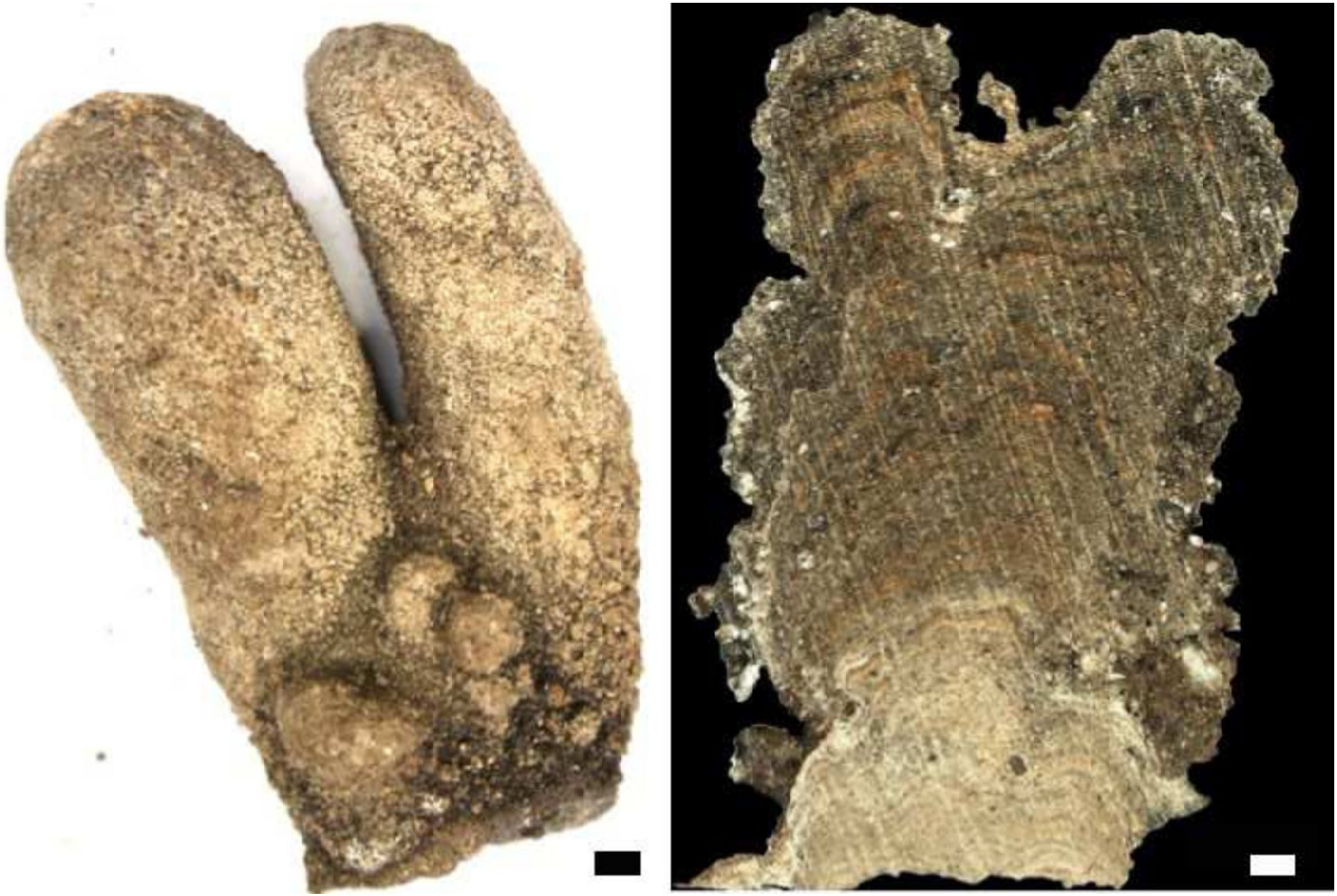

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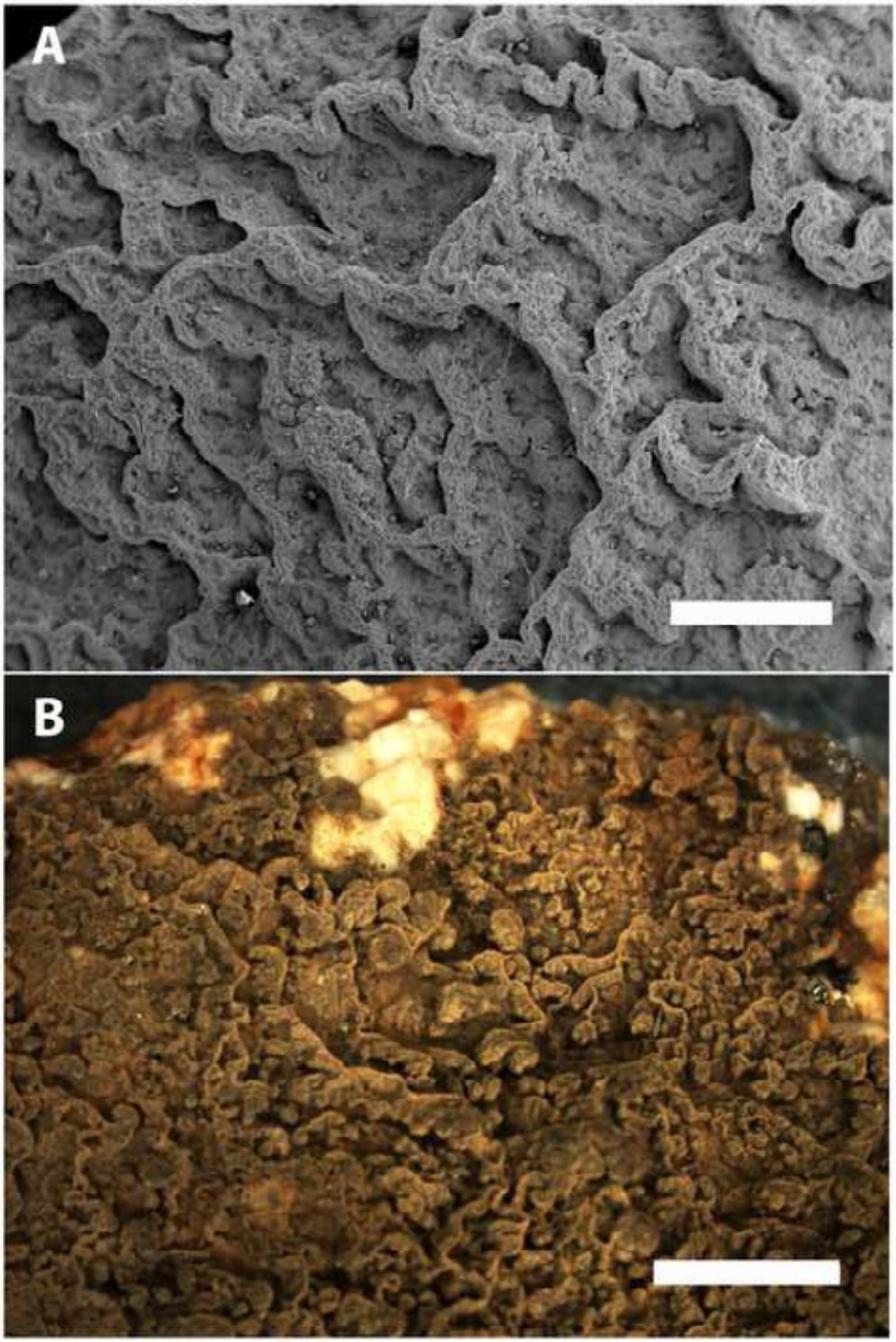

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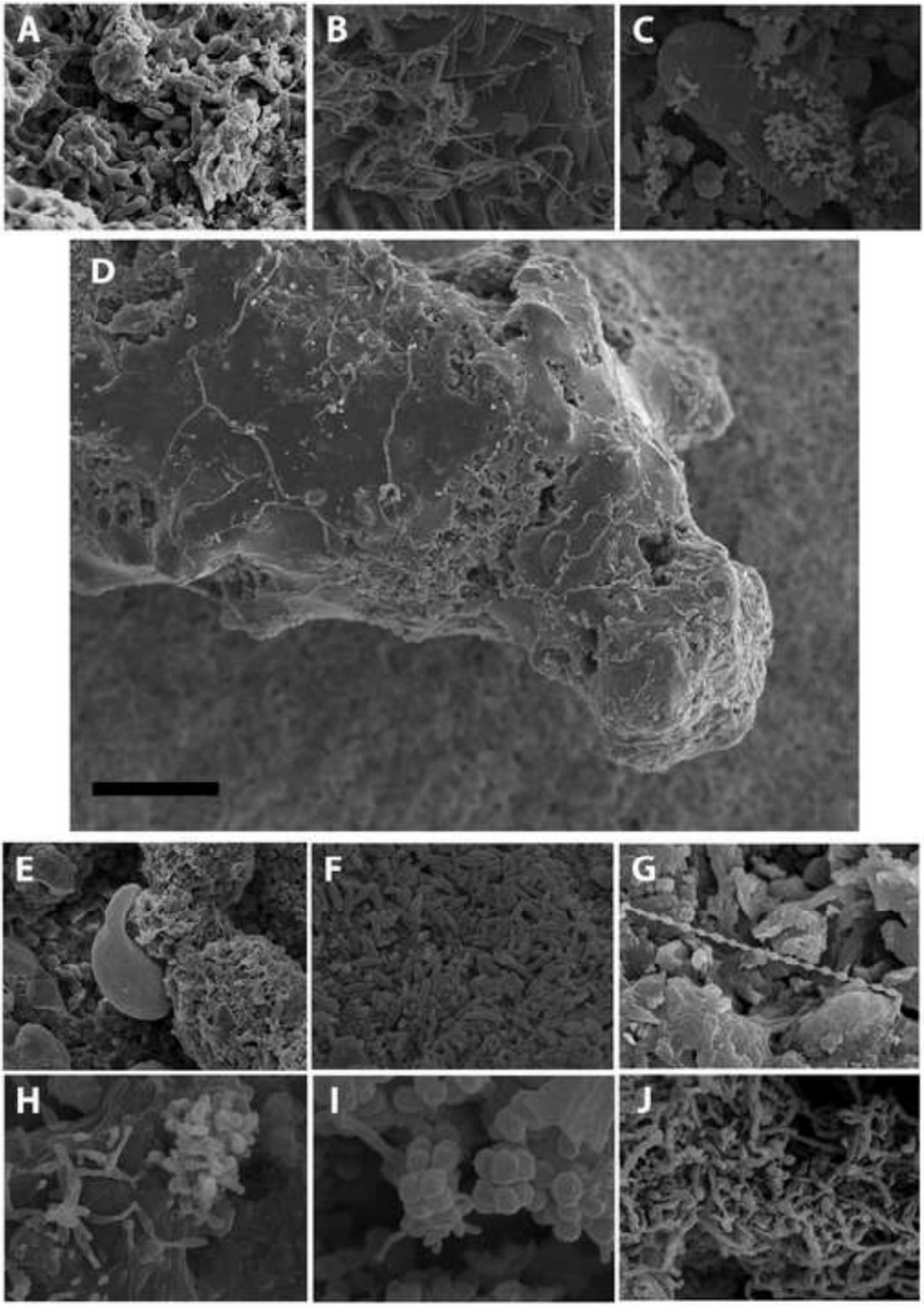

Figure

Table 1. EDS (SEM) elemental analysis of speleothems

Sample	Country	Climate	C	O	Mg	Al	Si	K	Ca	Fe	Na
Pindo	Spain	Temperate-humid	13.16	41.47		3.98	35.5		4.64		1.25
Pampa de Achala	Argentina	Semiarid	20.18	46.12	1	4.65	22.9	1.24	0.69	2.93	0.3
Anillaco	Argentina	Arid		38.9	1.35	8.14	33.4	2.95	6.21	7. 5	1.54
Trapa	Spain	Temperate-humid	18.32	26.38		3.12	37.71	1.8		12.67	
Castelo da Furna	Portugal	Temperate-humid	24.1	24.5		10.7	16.2		24.5		
Porteliña 1	Portugal	Temperate-humid	24.55	36		4.67	32.48	2.27			
Las Jaras	Spain	Semiarid	20.9	34.6		2.06	40.8		0.68		
Gundarin Hill	Australia	Arid	40.1	14.8		3.67	41.4				

Figure

Legend of figures, tables and geological map

Fig.1 Globular hemisphere of gypsum crystals growing from an opal-A cylindrical speleothem (Castelo da Furna, Northern Portugal)

Fig.2 Terrestrial stromatolite: general view (left) and cross-section (right) showing stromatolitic texture. A Trapa Cave, Pontevedra, Spain

Fig. 3 (A) Ceiling microgour of opal-A speleothems (Las Jaras, Córdoba, Spain). (B) Wall microgour of opal-A speleothems. (Peña del Hierro, Riotinto, Huelva, Spain)

Fig. 4 EDS (SEM) composition of autighenic crystalline minerals of speleothems. A Plumboaragonite,Las Jaras, Spain. B Halite Boda (Grottorna, Sweden. C Calcite, Tjuv Antas Grotta, Sweden D Gypsum monoclinic (Dromgrotta, Sweden). E Anhidrite orthorombic, (Avila, Spain).

Fig. 5. Diversity of bacterial morphology in biospeleothems of magmatic rock caves. Scanning eletron microscope images. Escale: 50 μm (fig. A-E) (fig. F-J) 8 μm. A: Filamentous bacteria covered by opal-A (Castelo da Furna. Portugal). B: Filamentous bacteria on gypsum whiskers (Drömgrottan. Sweden) C: Testate amoebae *Euglypha strigosa* and bacterial spores (Gundarin Hill. Australia) D: Opal-A cilindric speleothem with filamentous bacteria on its surface (Skalboberget. Sweden). E: Testate amoebae Trinema complanatum and bacterial mats (Trapa. Galicia. Spain). F: fusiform bacilli (Berrocal del Rugidero. Extremadura. Spain). G: Coccoid bacteria arranged in rows (Trapa. Galicia. Spain). H: Bacterial spores germination (Skalbeberget. Sweden). I: Tetrads of coccoid bacteria (Bodagröttan. Sweden). J: Filamentous bacteria forms with pili or filamentous extracellular polymeric substances (Trapa. Galicia. Spain).

Table 1 Elemental composition by EDS (SEM) of speleothems

Table 2 X-Ray diffraction of opal-A speleothems. Ar (Argentina) (1) Anillaco, La Rioja, Argentina, (3) Pampa de Achala, Córdoba, Argentina. Au (Australia) (2) Gundarin Hill, Western Australia. Spain (Sp) (4)Las Jaras, Córdoba, Spain ,(5) Porteliña, Pontevedra, Spain, (6) Trapa Cave b Pontevedra, Spain, (7) Trapa Cave a, Pontevedra, Spain, (10) O Pindo, Coruña, Spain, (11) Albarellos, Avión, Ourense, Spain(8). Po (Portugal) Castelo da Furna b, Northern Portugal, (9) Castelo da Furna a, Northern Portugal,

Map 1. World distribution, in gray, of magmatic rocks outcrops where the studied speleothems are located and their relation with climatic zones (Ar) Argentina, (WA) Western Australia. (Po) Portugal, (Sp) Spain, are the places of origin of the samples analyzed.

Figure
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