## Fossil Bacteria in an Upper Silurian Limestone from the Cellon Section (Carnic Alps/Austria)

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3 Plates

fossil bacteria framboid-like structure SEM Upper Silurian Cellon section

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#### Fossile Bakterien in einem obersilurischen Kalk aus dem Cellon-Profil (Karnische Alpen/Österreich)

#### Zusammenfassung

In einem dunkelgrauen laminierten Kalzisiltit aus dem unteren Pridoli (oberes Silur) der Karnischen Alpen, der im offshore-Bereich eines mäßig tiefen Schelfs abgelagert worden war, enthüllten detaillierte SEM-Untersuchungen das Vorhandensein von Resten fossiler Bakterien.

Diese liegen in zwei unterschiedlichen Erhaltungsformen vor: einerseits als kugeliges Aggregat von ca. 1 µm großen hohlen Kugeln mit dünnen Wänden aus Fe-reichem Karbonat, das sich in einer Gasblase oder in der nun nicht mehr vorhandenen kugeligen Schale eines Einzellers gebildet haben dürfte. In den Räumen zwischen aneinandergrenzenden Kugeln treten winzige Glaukonit-Kristalle auf. Andererseits ist dieses Gebilde umgeben von Anhäufungen meist xenomorpher Fluorapatit-Kristalle, von denen einige runde bis ovale Umrisse zeigen und einen Durchmesser von ca. 1 µm aufweisen. Auch sie werden als fossile Bakterien gedeutet, die in Mattenform den Abbau toter organischer Gewebe betrieben.

Die Bakterien scheinen während der frühen Diagenese in einem reichlich organische Substanz enthaltenden, anaeroben Environment autolithifiziert worden zu sein. Auffallend ist die enge Nachbarschaft sehr unterschiedlicher Mikroenvironments, in denen die Bakterien aktiv waren.

#### **Abstract**

SEM-investigations of a dark grey laminated calcisiltite from the lower Pridoli (upper Silurian) of the Carnic Alps, deposited in an offshore setting of a moderately deep shelf, revealed remains of fossil bacteria.

The microbes appear in two forms at a single site: As a spherical aggregate of hollow spheres with diameters of  $\sim 1~\mu m$  and thin walls of Fe-rich carbonate. Within the interspaces of the hollow spheres, tiny glauconite crystals formed. Around this structure, accumulations of anhedral crystals of fluorapatite occur. Some apatite grains, however, show rounded or ovoid outlines and diameters of about 1  $\mu m$ , and are thus also interpreted as being the remains of bacteria.

The bacteria are presumed to have been autolithified during early diagenesis in an anaerobic environment, where abundant dead organic matter was present and microbial activity was high. The close neighborhood of two completely different microenvironments, in which the bacteria metabolized, is remarkable.

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#### Introduction

SEM-investigations at high magnifications sometimes lead to astonishing results. In the present case, a dark grey laminated calcisiltite from the upper Silurian of the Cellon section (Karnic Alps, Austria) was subjected to a detailed palaeontological SEM-examination at magnifications of up to 50,000 x. EDX-analyses were also carried out on numerous objects (cf. PRIEWALDER, 2013).

During these studies, a spherical structure that at low magnifications looked like a pyrite-framboid sitting in a hemispherical cavity in the limestone turned out to be an aggregation of hollow spheres with delicate walls of Ferich carbonate. This was surrounded by clusters of anhedral fluorapatite crystals that were interspersed by lumps of amorphous quartz. Some apatite grains, however, exhibited circular to ovoid outlines (Pl. 1, Fig. 2).

The spherical casings, as well as the circular apatite granules, are interpreted as remains of lithified microbes as have been previously documented from marine and terrestrial rocks since the Precambrian (LIEBIG, 1998).

A bacterial origin is indicated not only by their morphology but also by the sedimentary environment (anaerobic conditions in sediments with abundant decaying organic matter and high microbial activity), in which the autolithification of the cells could take place.

It is remarkable that the completely different microenvironments in which the microbes were autolithified in different ways and by different mineral phases are situated so close to each other (Pl. 1, Fig. 2).

#### **Material and Methods**

The studied sample comes from the upper Silurian of the Cellon section [Pridolian part of the Alticola Limestone: *M. parultimus* graptolite biozone (Brett et al., 2009); *O. remscheidensis* conodont biozone (HISTON & SCHÖNLAUB, 1999); *U. urna* chitinozoan biozone (Priewalder, 1997, 2000); sample number 149A in Priewalder (1987, 1997, 2000)] in the central Carnic Alps (Carinthia, Austria) (Walliser, 1964; Jaeger, 1975; Priewalder, 1987, 1997, 2000, 2013, cum lit.; Kreutzer, 1992, 1994; HISTON, 1997; SCHÖNLAUB, 1997; Kreutzer & Schönlaub, 1997; HISTON & Schönlaub, 1999; HISTON et al., 1999; Brett et al., 2009).

Small pieces of black limestone layers in a dark grey laminated calcisiltite that was deposited in an offshore-setting of a moderately deep shelf with changing hydrodynamic regimes and oxygen contents (HISTON & SCHÖNLAUB, 1999; BRETT et al., 2009; PRIEWALDER, 2013) were subjected to detailed investigations in the SEM and EDX.

The black layers are composed of calcite, fluorapatite, amorphous silica, clay minerals, Fe-oxide framboids, and a few pyrite framboids and idiomorphic pyrite crystals. The fossil content is made up of Chitinozoa and Muellerisphaerida (Pl. 1, Fig. 1; PRIEWALDER, 2013).

For examinations in the SEM and EDX, small pieces of rock were immersed in diluted HCl for 2 minutes, then thoroughly washed in water, dried on a heating plate and coated with gold (**Cressington 108auto**).

The samples were investigated in a SEM (**Tescan – Vega 2 XL**) at magnifications of up to 50,000 x and with an EDX (**Oxford Instruments – INCA 4.15**) at about 20,000 x.

#### **Results and Discussions**

The framboid-like aggregate, which rests in a hemispherical depression in the fine-grained limestone, has a diameter of 10 µm and is composed of numerous thin-walled spherical to sub-spherical hollow bodies with diameters of 0.9–1.3 µm. They are tightly packed and appear to form a three-dimensional structure (Pl. 2, Fig. 1). The spherical casings are very thin (0.01 µm or less) and consist of finely granulated matter. Most are broken or deformed and only at a few places, mainly in marginal positions, they are more-or-less completely preserved. At sites where they are destroyed, it is clear that the granulate material of the lower half of the former spherical wall continues into the cavities (Pl. 2, Figs. 1–4). These entities are interpreted as mineral incrusted bacteria.

Within the interstices between the spheres, growth of authigenic minerals occurred, obviously at a time when the casings were still forming. This led to deformation of their initial spherical outlines and to a honeycomb-like appearance of the central part of the framboid-like aggregate (Pl. 2, Fig. 1).

The textural difference between the granular casings and the tiny platy minerals in between is distinct (Pl. 2, Figs. 3-4), as is their chemistry. EDX-analyses of the platy mineral grains showed them to be glauconite. The EDX results of the casings are less easily to interpret. Both, the casings and the mineral grains contain the same elements; the thin walls, however, always show distinctly lower peaks of Al, Si and K, and slightly higher peaks of Ca and Fe. As the walls of the spheres are very thin it seems likely that the EDX-measurements also record the glauconite underneath. Hence the wall material is considered to be Fe-rich carbonate. This is consistent with numerous reports on the calcification of bacteria in the rock record, as well as in laboratory experiments (BRAISSANT et al., 2007; CASTANIER et al., 2000; DOUGLAS & BEVERIDGE, 1998; GAB-BOTT, 1998; KNORRE & KRUMBEIN, 2000; KONHAUSER, 1998; LIEBIG, 1998; NOVITSKY, 1981; VAN LITH et al., 2003).

The fine grained material of the spherical casings is restricted to the cavity in the limestone with its framboid-like aggregate (Pl. 1, Fig. 2). Also iron is not present outside the hole.

The spherical aggregate is surrounded by clusters of tiny anhedral crystals of fluorapatite and amorphous lumps of quartz (Pl. 1, Fig. 2). Some apatite-grains, however, show circular to oval outlines. These are interpreted to be either the remains of phosphatised bacteria or the internal moulds of bacteria. Their diameters are between 0.8 and 1.0  $\mu$ m, a bit smaller than the hollow spheres (Pl. 3, Figs. 1–3).

Both, the abundance of microfossils such as Chitinozoa and Muellerisphaerida (Pl. 1, Fig. 1) and the black colour of the studied sample indicate that a considerable amount of organic matter was present in the mud at the time of sedimentation. Under such conditions, the number of bacteria in the uppermost soft layers of the sediments is very high (SCHIEBER, 2002). Heterotrophic microbes rapidly decompose the organic material of the dead organisms which lived in the water column or on and within the sediment, thus contributing to the creation of an anaerobic depositional environment. Usually bacteria disintegrate after death and are not preserved. Under favourable con-

ditions, however, they can undergo autolithification (GABBOTT, 1998; KONHAUSER, 1998; LIEBIG, 1998; PINHEIRO et al., 2012; POLLASTRO, 1981; SCHIEBER, 2002; SCHIEBER & ARNOTT, 2003). The presence of organic matter is essential for the genesis of apatite, as well as glauconite and Fe-rich carbonates. Only by bacterial degradation of the organic tissues during early diagenesis is the proper anoxic geochemical environment for the precipitation of these minerals generated (GREENSMITH, 1988; KONHAUSER, 1998; MARSHALL & COOK, 1980; MEUNIER, 2005; O'BRIAN et al., 1990).

Microbes appeared in the Precambrian. Over a long period of time they were the only organisms on Earth and today they are ubiquitous. From the beginning, they were involved in the enzymatic decomposition of dead organisms (SCHIEBER, 2002). Modern classification schemes divide the procaryotes (organisms without nuclei) into Archaea (former Archaebacteria) and Bacteria (former Eubacteria). Although these groups are not related, it is not possible to distinguish them from each other by their morphology when fossilised (LIEBIG, 1998). Therefore, the terms "microbes" and "bacteria" in this paper include both groups.

The classification of living bacteria is based on their cell structure and metabolism. As they show a very simple morphology (the main forms are cocci, bacilli and filaments) and a pronounced pleomorphism (according to their life conditions they can appear in different shapes and sizes), their shapes are not suitable distinguishing features. Therefore, it is impossible to give a precise taxonomic determination of fossil microbes (LIEBIG, 1998). The sizes of modern bacteria range from 0.15 to 100  $\mu$ m (DUDA, 2011); mostly, however, they measure 0.5–3  $\mu$ m with a mean diameter of about 1  $\mu$ m (FOLK, 1993).

In almost all environments, bacteria are associated with the generation of minerals (CASTANIER et al., 2000; DOUG-LAS & BEVERIDGE, 1998; FOLK & LYNCH, 1997; KONHAUSER, 1998). Both, the Archaea and Bacteria have complex cell walls and some of them also have an outer layer of biofilm (the glycocalix), which are essential for their fossilisation: here, the mineralisation of the cells starts. As the cell walls/glycocalix are negatively charged, due to their chemical composition, metal ions from the surrounding water accumulate on the cell surfaces. These metals, in turn, tie in negative ions, such as carbonate-, phosphate-, silicate-, sulfate- and sulfide-ions (BARKER & HURST, 1992; COSMIDIS et al., 2013; DOUGLAS & BEVERIDGE, 1998; FOLK & LYNCH, 1997; FOLK & RASBURY, 2007; KONHAUSER, 1998; KONHAUSER & URRUTIA, 1999; LEVEILLE & LUI, 2009; LIEBIG, 1998; Novitsky, 1981; Salama et al., 2013; Toporski et al., 2002; VAN LITH et al., 2003; YEE et al., 2001). Moreover, bacteria are able to change the chemistry of their environment considerably by excretion (KNORRE & KRUM-BEIN, 2000; KONHAUSER, 1998).

Thus various mineral phases can become embedded into the cell walls or the glycocalix. At first these form crusts, but, if the mineral precipitation continues, the protoplasm can also become mineralised (LIEBIG, 1998). Permineralisation is another kind of lithification, occurring soon after death; in such cases, the bacteria are preserved as internal moulds (LIEBIG, 1998). Both processes have also been observed under laboratory conditions (KONHAUSER & URRUTIA, 1999; LIEBIG, 1998; TOPORSKI et al., 2002; VAN LITH et al., 2003; YEE et al., 2001). This lithification is not controlled by the bacteria themselves, but is exclusively

a matter of interaction between the cell surface chemistry and the chemistry of the environment (DOUGLAS & BEVERIDGE, 1998).

The generation of apatite, which in the present sample is very common (Pl. 1, Fig. 1), is often linked to the bacterial decay of organic matter during early diagenesis (Douglas & Beveridge, 1998; Gabbott, 1998; Liebig, 1998; PINHEIRO et al., 2012). Organic tissues are subjected to rapid enzymatic degradation by bacteria, leading to anoxic environmental conditions in the sediment. In such an environment, authigenic minerals like apatite, pyrite and carbonate may form by chemical reactions of dissolved compounds in the surrounding area (GABBOTT, 1998; LIEBIG, 1998; SCHIEBER & ARNOTT, 2003). Laboratory experiments show that the phosphatisation of bacteria takes place in closed systems and under specific conditions that in nature may be provided by metabolising microbial mats (LIEBIG, 1998). The numerous accumulations of tiny apatite crystals on the bedding planes of the studied sample (Pl. 1, Figs. 1-2; PRIEWALDER, 2013) are interpreted as lithified remains of such microbial mats.

Later, the phosphatised bacteria seem to have been partly or completely dissolved, and subsequently apatite was precipitated again, forming anhedral crystals and probably also irregular overgrowths on apatite microbes. IZOTOV & SITDIKOVA (2008) and SCHIEBER (2002) report observations of bacteria within minerals like pyrite. In the studied sample, only a few remains of the former, presumably numerous, fossil bacteria are present.

The near-by framboid-like aggregates of hollow spheres obviously formed in a special and, at least at the beginning, closed microenvironment of a gas bubble or in the, now gone, sheath of a spherical protozoa. This environment was completely different from the physicochemical conditions in the bacterial mats in the near vicinity. In this restricted habitat, the spherical to sub-spherical bacteria were densely packed, decomposing some organic matter and thus producing anaerobic conditions. Consequently, amorphous Fe-rich carbonate started to precipitate on the cell surfaces (cf. Castanier et al., 2000; Knorre & Krumbein, 2000; Konhauser, 1998; Van Lith et al., 2003).

The supply of nutrients plays a leading role in the relationship between microbes and mineralisation. If there is an enrichment in organic matter, bacterial activity will also be intensified. According to Castanier et al. (2000), in such cases the initially generated minerals are amorphous, because at that time the biologic processes dominate over the inherent crystal structures. This is obviously the case for the studied object, with its finely granulated casings. However, amorphous material may subsequently crystallise. The fact that here only thin carbonate walls developed around the microbes instead of complete mineralisation of the cells, may be explained by the assumption that in this minute cavity the required elements were soon depleted (GABBOTT, 1998) and/or that the physio-chemical conditions changed at an early stage.

In most interstices between the hollow spheres, tiny glauconite grains occur (Pl. 2, Figs. 2–4). These could point to a biofilm (EPS: extracellular polymeric substances) that was secreted by the bacteria in the cavity. As biofilms, like the bacterial surfaces, are negatively charged, they can be mineralised too. Relatively high C-peaks in the EDX-spectra may be an indication of this. The mineralisation of EPS

has been described by Braissant et al. (2007); Cavalazzi et al. (2012); Konhauser & Urrutia (1999); Liebig (1998); Salama et al. (2013).

Presumably, the thin-walled casings of the bacterial cells were formed at the beginning of the lithification process. The crystallisation of glauconite started later and, when mineral growth continued, the casings in the middle part of the aggregation became slightly compressed. This led to their regular honeycomb-like shape. Common triangular crystallites fit exactly in the voids between adjoining spherical casings (Pl. 2, Fig. 3).

The peculiarity of the environment in this 10  $\mu m$  wide spherical cavity is indicated by two characteristics. First, the presence of thin granulated spherical casings composed of Fe-rich carbonate which was not observed elsewhere in the sample. Second, the restriction of iron to this small area, as none of the numerous EDX-analyses of the vicinity of the framboid-like aggregate indicated Fe.

Possibly at the time of the assumed dissolution and reprecipitation of apatite, proximal fluids seem to have infiltrated the casings in the lower left of the aggregate (Pl. 2, Figs. 1, 3 arrow), leading to the growth of apatite grains. EDX-analyses from these locations showed that anhedral apatite crystals are covered by a thin Fe-containing granulate layer.

Several papers have documented aggregates of spherical bodies which resemble pyrite-framboids but are made up of small hollow pyritic spheres. Gong et al. (2008) described pyrite-framboids that exclusively occurred in laminated Zoophycos spreiten from the Middle Permian Westley Park Sandstone Member in south-eastern Australia. The aggregates were 6-12 µm in diameter and consisted of numerous tightly packed hollow spheres 0.5-0.8 µm wide. Some of the framboids were covered with thin membranes which gave them an appearance of bacterial colonies encased in biofilm. BAILY et al. (2010), however, inferred that these microbial colonies were pseudofossils. In Californian methane seep carbonates of Pleistocene age, they found framboid-like structures of hollow spheres which were originally made of Fe-sulfides and resembled modern syntrophic archaeal-bacterial consortia. Detailed studies, however, revealed that the casings were the remains of greigite or pyrite crystals which had altered to Fe-oxides and had been formed exclusively by dissolution and reprecipitation processes during early diagenesis. They noted that the straight lines bordering some of the hollow spheres in Gong et al. (2008) were reminiscent of former euhedral crystals and would thus also suggest genesis by dissolution processes. However, these straight lines could also be interpreted as growth of pyrite crystals within microbial cells, as was reported in several papers (cf. Schieber, 2002; Folk, 2005).

CAVALAZZI et al. (2012) described Fe-rich framboids with diameters of about 7 µm in Middle Devonian hydrocarbon-seep carbonates of the Anti-Atlas (Morocco). The hollow spheres were 0.5–0.7 µm wide, showed an irregular morphology and a disordered arrangement within the aggregates. These characteristics distinguished them from framboids which had been synthesised abiotically and exhibited euhedral crystallites in a regular arrangement. According to CAVALAZZI et al. (2012), further evidences for a

biogenic origin of the hollow spheres was the common occurrence of organic matter in the rocks and an environment consistent with the identified chemical and biological processes.

The framboid-like aggregation of spherical casings documented here differs in some significant aspects from the above presented pyrite and Fe-rich framboids with hollow spheres:

- Different morphology: The thin-walled tightly-packed hollow spheres are made up of finely granulated material, which presumably was originally amorphous (Pl. 2, Figs. 2–4). Later, tiny crystallites with smooth faces and straight edges grew in the interstices between adjacent spheres (Pl. 2, Figs. 2–4), which led to a slight compression of the casings and the formation of a honeycomb-like pattern (Pl. 2, Fig. 1); triangular crystallites fit perfectly into the triangular interstices between adjacent casings (Pl. 2, Figs. 3–4).
- Different chemistry: The spherical granulate casings are composed of Fe-rich carbonate, the smooth crystals in between are glauconite.

In summary there are several reasons that confirm a biogenic origin for the spherical casings in the described framboid-like structure:

- The objection of BAILY et al. (2010) that aggregates of hollow spheres may be generated abiotically by dissolution and reprecipitation, does not apply to the here presented casings. All the spheres are of about the same size and shape and show delicate and finely granulated walls of homogeneous thickness. This is not consistent with dissolution and reprecipitation. Furthermore, the other framboids in the sample show no evidence of dissolution leading to the generation of hollow crystallites (Pl. 3, Fig. 4).
- The generation of Fe-rich carbonate crusts around bacteria cells is well established (Castanier et al., 2000; KNORRE & KRUMBEIN, 2000; KONHAUSER, 1998; VAN LITH et al., 2003).
- Remains of bacteria are also present in the surroundings of the framboid-like structure which points to strong bacterial activity in the sediment.
- Microbially controlled mineral precipitation occurred in microenvironments like a spherical cavity or bacterial mats, which degraded the organic matter (the clusters of apatite crystals are considered as their lithified remains). The existence of such microenvironments was noted in several papers (DOUGLAS & BEVERIDGE, 1998; LIEBIG, 1998; MEUNIER, 2005).
- The environments postulated here for the autolithification of the bacteria are similar to previously reported examples: The presence of abundant organic matter, which was decomposed by bacteria, led to anaerobic conditions, where, depending on the microenvironment, the precipitation of apatite, Fe-rich carbonate and glauconite could take place (GABBOTT, 1998; GREENSMITH, 1988; KONHAUSER, 1998; LIEBIG, 1998; MARSHALL & COOK, 1980; MEUNIER, 2005; O'BRIAN et al., 1990; PINHEIRO et al., 2012; SCHIEBER, 2002; SCHIEBER & ARNOTT, 2003).

#### **Conclusions**

The framboid-like aggregate of thin-walled hollow spheres is interpreted to be fossilised microbial colony, the metabolism and mineralisation of which took place in a microenvironment of a gas bubble around organic matter or in the spherical sheath of a protozoa. The common clusters of apatite crystals on the bedding planes of the studied sample, however, point to fossil bacterial mats that decomposed the dead organic matter that had either sunk to the sea floor or formerly lived in the sediment. All these

processes took place during early diagenesis, in the uppermost layers of mud, not far from the water/sediment-interface, and in an anaerobic environment.

The lithification of bacterial remains from the upper Silurian appears to have been a matter of chance; notably the preservation of the delicate casings in the framboid-like structure. Despite further SEM-examination of the sample, fossil microbes were observed only at the one site.

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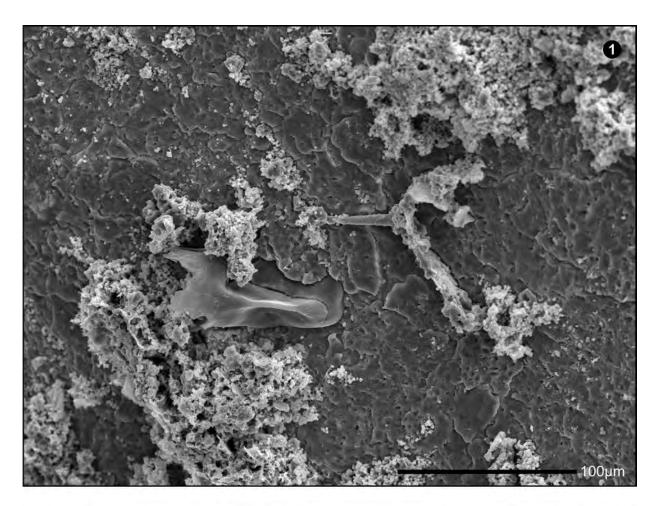
### Plate 1

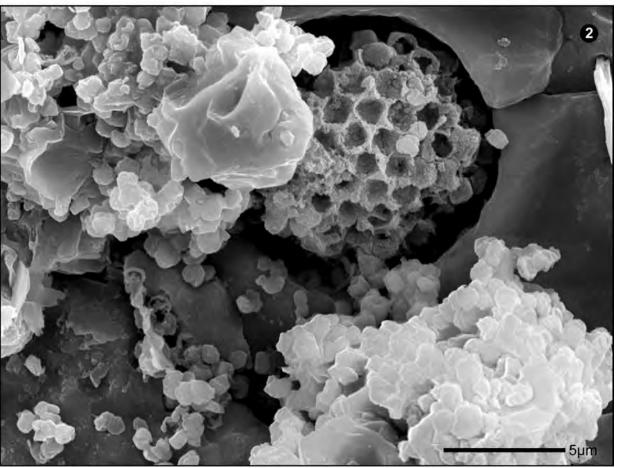
Fig. 1: Overview of the bedding plane of a piece of black layer of a laminated dark grey calcisiltite.

The fossil at the left is a Chitinozoa (*Eisenackitina krizi* PARIS & LAUFELD, 1980), embedded in black limestone; to its right, a fragment of a Muellerisphaerida shell is present. The bright clusters in the upper right and lower left corner are accumulations of tiny fluorapatite crystals.

Note: This photograph comes from a different piece of black layer than the others presented here, but from the same sample (same piece of limestone as in Pl. 3, Fig. 4).

Fig. 2: Overview of the surroundings of a framboid-like aggregation of hollow spheres.
The framboid-like aggregate is sitting in a hemispherical cavity in the limestone.
To the upper left and lower right, it is surrounded by accumulations of fluorapatite crystals, some of which still showing circular to sub-circular outlines. On and within the apatite clusters, lumps of amorphous quartz are present.





# $\mathcal{C}$ Plate

Overview of a framboid-like aggregation of hollow spheres.

Most of the thin walled spheres of Fe-rich carbonate, which are considered as casings around former bacterial cells, are destroyed; only a few in marginal positions are more or less well preserved. In most interstices between adjoining spheres, glauconite crystals formed. Where they are very small or missing, the outlines of the impressions are still almost circular. However, where they are a little larger, as for example in the centre of the aggregate, the mineral growth has formed a honeycomb-like pattern. Fig. 1:

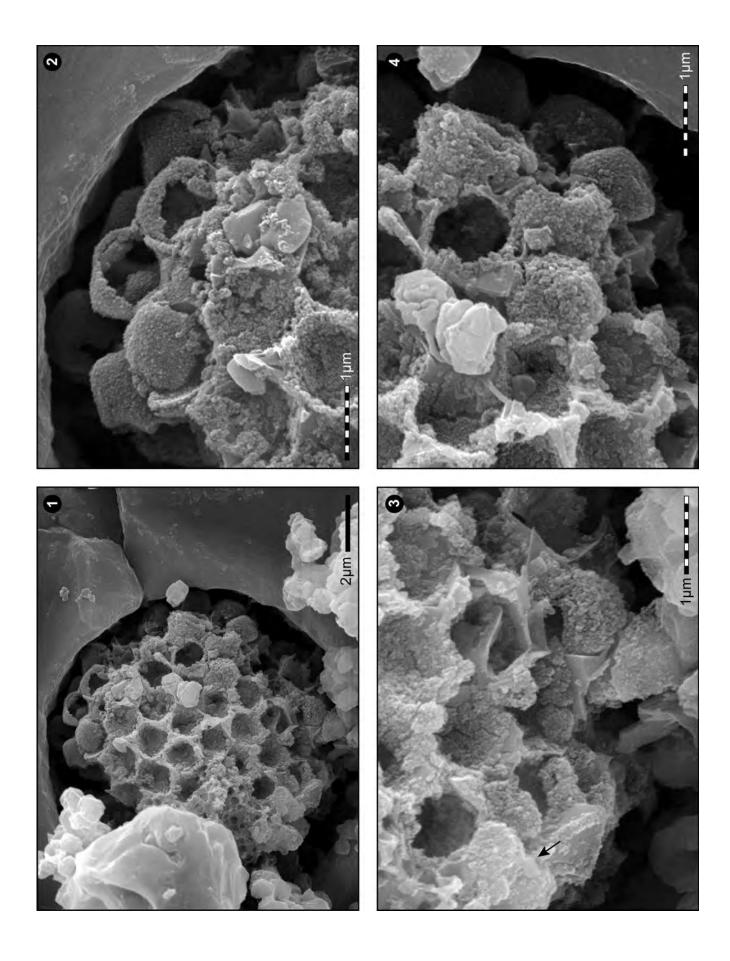
Details of Fig. 1. Figs. 2-4:

Fig. 2 (top right in the framboid-like aggregate):
The sub-spherical casings are broken, their walls are finely granulated, very thin and of uniform thickness. The broken specimens show very well that the lower halves of the casings
The sub-spherical casings are broken, their walls are finely granulated, very thin and of uniformities of glauconite. Where they are very small or missing, sub-circular outlines still are still sticking in the hemispherical impressions. The interstices are mostly infilled with tiny crystallites of glauconite. Where they are very small or missing, sub-circular outlines still

Fig. 3 (down left in the framboid-like aggregate):
The lower halves of the broken spherical casings still remain in the cavities; the glauconite crystals here are more distinctly developed. They often have triangular shapes with acute edges that fit into the interstices between three adjacent spheres. At the left margin, a few anhedral apatite crystals are covered by thin granulate casings (arrow).

Fig. 4 (down right in the framboid-like aggregate):

Some of the casings here are almost completely preserved or only slightly deformed. The different development of the glauconite crystals in size and shape is clearly visible, varying from tiny platelets at the left to more robust ones at the right.



Figs. 1-3: Accumulations of fluorapatite grains (cf. Pl. 1, Fig. 2).

Fig. 1: Some of the grains have rounded outlines (arrows) and are interpreted as remains of internal casts of bacteria.

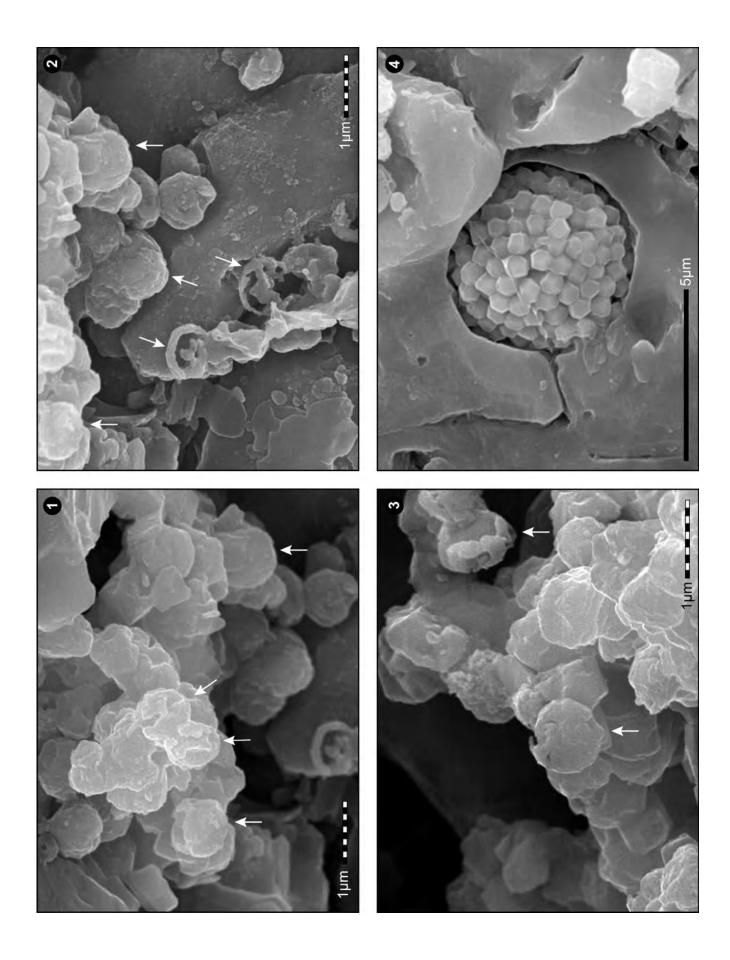
Fig. 2: Some of the internal moulds show considerable signs of dissolution.

Fig. 3: Most of the grains are anhedral apatite crystals, but some of them have circular to ovoid outlines (arrows).

Overview of a pyrite-framboid.

The framboid is – like the aggregation of hollow spheres – sitting in a cavity in the limestone, but here the crystallites are idiomorphic and their sizes and shapes vary. Their arrangement is irregular.

Note: This photograph comes from a different piece of black layer than the others presented here, but from the same sample (same piece of limestone as in Pl. 1, Fig. 1). Fig. 4:



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