Prokaryote-derived morphologies in fossil cold-seep carbonates of the Mediterranean region

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ABSTRACT

The most typical deposit related to a hydrocarbon seep is the authigenic carbonate derived from microbial activity in anoxic conditions. This activity is at the base of the chemosynthetic food chain established in seep communities. Physical evidence of the interactions between microbial metabolism and seep environment in ancient geologic bodies is present in carbonate accumulations and occurs at various scales, from the outcrop to the micrometer scale. Such evidence can be broadly included in sedimentary and organosedimentary fabrics, morphological fossils, and bioinduced minerals. Fossil prokaryotes and microbialites are mostly concentrated in and around lithofacies directly involved in the seepage, or in mineral products formed during early diagenetic phases. Seep limestone bodies from Morocco (Paleozoic) and Italy (Cenozoic) have provided a wide inventory of these evidences.

INTRODUCTION

While the discovery of cold-seep ecosystems goes back more than 20 years (Paull *et al*., 1984), surprises are not exhausted and the bacteria and clam communities reported from the antarctic deep ocean floor, in areas beneath ice cover for at least 10,000 years, are a compelling example of a recently discovered chemotrophic ecosystem sustained by seepage (Domack *et al*., 2005). Cold-seep ecosystems are common in continental margins worldwide (Campbell, 2005) and their study has significantly expanded our knowledge of extreme environments. Also, they have importance in an astrobiological perspective (Shapiro, 2004), by considering that chemosynthesis can be independent from Earth-like ambient factors, such as light and free oxygen, and methane, the main seeping gas, is abundant in the Solar System.

Cold-seep ecosystems have a long geological history. They are known since at least Silurian time (Barbieri *et al*., 2004) and probably older examples are just waiting somewhere to be discovered. A byproduct of cold-seep environments is the authigenic precipitation of calcium carbonate derived from the increased alkalinity induced by microbial metabolism in anoxic conditions (Ritger *et al*., 1987). Such a biologically induced mineral precipitation may occur at or beneath the seafloor, where the formation of thin crusts, such as those today precipitating in the Adriatic Sea (Figure 1a), may turn into volumetrically large deposits (Figure 1b). These authigenic carbonate accumulations may also form in critical environments, such as below the calcite compensation depth (see the example by Greinert *et al*., 2002). Once embedded or replaced by mineral compounds, the biological components can be preserved and become useful elements for the diagnosis of a fossil cold seep site. Because the chemosymbiotic communities of invertebrates typifying the modern seep ecosystems may be absent in fossil counterparts (see the example described by Aiello *et al*., 1999, 2001), sedimentary and bio-geochemical attributes are also important diagnostic proxies (recent review by Peckmann and Thiel, 2004). The characteristic ^{13}C-depletion of mineral (calcium carbonate) and organic compounds (lipid biomarkers) in modern and ancient methane seeps depends on the microbial ecology established by consortia of methanotrophic archaea and sulfate-reducing bacteria. Activity of methanotrophic and thiotrophic endosymbionts in soft tissues of seep invertebrate communities (clams, mussels and tubeworms), and of the sulfide-oxidizing, mat-forming filamentous bacteria (*Beggiatoa* mats) play a key role in a seep ecosystem, where they are at the base of the food chain.

Physical evidence of the interactions between microbial metabolism and seep environment in ancient geologic bodies are present in carbonate microbialites and other microbial-derived textures, which occur from the scale of the field to the scale of the electron microscopy. These evidences can be summarized in i) sedimentary and organosedimentary fabrics, ii) morphological fossils, and iii) bioinduced minerals. Evidence demonstrates that fossil prokaryotes and microbialites are mostly concentrated in and around authigenic lithologies directly involved with the seepage, such as conduit and vug fills, and products of early diagenetic phases. Seep deposits from the Silurian-Devonian of Morocco and the Neogene-Recent of Italy provide a wide inventory of such evidence, which is the subject of the present paper.

SEDIMENTARY AND ORGANOSEDIMENTARY FABRICS

Typical microbial-induced fabrics of hydrocarbon seep-carbonates from Italy and Morocco include laminated (stromatolitic) fabrics, organic/mineralized crusts and rims, microtufts, dissolution surfaces, clotted and/or peloidal textures, botryoids, rhombs and spherulitic textures. Finely laminated stromatolitic fabrics have been described in modern methane seep-carbonate crusts, at nearly 5,000 m water depth in the Aleutinian accretionary margin (Greinert *et al*., 2002), and in fossil carbonate accumulations. From remains of the El Borj body, the oldest known fossil cold-seep ecosystem located in the Moroccan Meseta (Barbieri *et al*., 2004), laminated fabrics are present in a well developed stromatolitic and stromatactoid facies (Figures 2a, b). Fine laminae arranged as a stromatolitic fabric have been found in the Early and Middle Devonian Kess-Kess mounds of Anti-Atlas, Morocco (Cavalazzi and others, unpublished data), where they line veins and sills crosscutting these spectacular conical mounds (Figure 2c). In spite of a suggested hydrothermal origin for the Early Devonian Kess-Kess mounds, low δ^{13}C values (Cavalazzi and others, unpublished data) and the mineral fluid inclusions (Belka, 1998) measured from veins and sills infill, demonstrate that they also acted as conduits for cold fluid (including methane) advection. In only one conical mound, the Middle Devonian Hollard Mound, worm-tube and bivalve fossils (Figure 3), associated with geochemical evidence suggest a seep-derived origin (Peckmann *et al*., 1999; Peckmann *et al*., 2005).

Mineralized crusts and encrusted horizons, possibly with some organic remnants, are common sedimentary features of seep carbonates (Figure 4). The nature of these features depends on the local ambient chemistry, which is largely established by microbial consortia. Crusts can derive from rapid mineral precipitation, such as the authigenic iron oxide concentrations of the El Borj body (Figure 4a), or from early mineral replacement of organic (microbial) compounds during a process of biologically induced mineralization, which is extremely common for bacteria (Lowenstam, 1981). Mineral rims of microbial origin can also coat seep megafaunal remains, such as the tubeworms encrusted by nondetrital micrite described by Campbell *et al*. (2002) from a Cretaceous seep limestone of California. Sharp changes in physico-chemical gradients can also be documented by dissolution/corrosion patterns on skeletal and sedimentary surfaces (Figure 4b). Microtufts are microbial-derived aggregates that concentrate ferric iron minerals in micritic facies. The only described paleoseep with abundant microtufts is the Silurian-aged El Borj body (Figure 4c), where they are associated with other iron concentrations.

Another type of microcrystalline aggregates leads to the formation of the “clotted micrite” (Figure 4d), which consists of irregular microaggregates cemented by calcite spar (Peckmann *et al*., 2002) or micrite, having a typical microbial origin. Clotted fabrics are largely reported from modern and ancient seep deposits, and are present in authigenic carbonates regardless of proximity to seep conduits. Peloidal fabrics would likely have the same origin of the clotted micrite.

Despite compaction and early cementation processes, a cold-seep limestone can preserve some original porosity. In the Miocene Pietralunga methane-seep deposit of northern Apennine, for example, cavities and vugs are rimmed with carbonate cements and still partially empty. These empty parts are almost totally filled with clusters of zoned spherulites and rhombs (Figures 5a, c) composed of alternations of calcium carbonate and dolomite. For these spherulites and rhombs, a microbial origin (sulfate-reducing consortia) has been suggested by different lines of evidence (Barbieri and Cavalazzi, 2005), including their association with aragonite botryoids and splays cements (Figure 5d), and the local presence of minerals that can be explained in terms of biological (microbial) intervention. Although with a different development and compositional combination, spherulites have been described from other Miocene paleoseep carbonates of Italy (Terzi *et al.*, 1994; Cavagna *et al.*, 1999; Peckmann *et al.*, 2004). The same microbial-related origin has been also hypothesized for the large dolomite rhombs of the Kess-Kess veins and sills (Cavalazzi, unpublished data). These rhombs are often associated with stromatolitic microfacies and have a characteristic zoned organization consisting of mineral and organic alternations (Figures 5e, f).

MORPHOLOGICAL FOSSILS

The expression "morphological fossil" is here used for all morphologies that can be directly traced back to their biological origin. Preserved bacterial fossils as cells or cell colonies are rare in fossil seep ecosystems. Putative cocci and bacterial rods are reported by Shapiro (2004) from the Cretaceous Tepee Buttes of Colorado, and have also been recovered from the Pliocene Stirone seep deposit of northern Apennine (Figure 6). The recognition of biogenicity for the bacterial fossils is still an issue under discussion. In general, because of the micrometer-size and simple shape of morphologies that mimic bacterial cells, a clear origin (mineral or biological) is rarely obvious (see discussion in Westall, 1999).

A spectacular finding of bacterial cell colonies is from the Pietralunga seep deposit (Barbieri *et al.*, 2001; Peckmann *et al.*, 2004; Barbieri and Cavalazzi, 2005). In this carbonate mass, dense to loose clusters of dark filaments with 1-3 mm length and about 100 µm diameter, are distributed in the micritic groundmass, associated with aragonite cements, and with a mat-forming arrangement (Figures 7a, b). These filamentous structures have been interpreted as exceptionally well preserved, giant sulfide-oxidizing bacteria belonging to the family Beggiatoaceae, that in modern seep environments, such as in the Gulf of Mexico (Larkin and Henk, 1996), develop typical bacterial mats. The Beggiatoaceae genus *Beggiatoa*, for example, is amongst the largest of prokayotes and can range in diameter up to nearly 200 µm (Larkin *et al.*, 1994). *Beggiatoa* mats originate as a consequence of the upward migration of hydrogen sulfide/hydrocarbon-rich fluids. Bio-geochemical support for interpreting filaments of the Pietralunga paleoseep as giant bacteria was convincingly provided by Peckmann *et al.* (2004). An example of non colonial bacterial fossils are the micrometer-size, filamentous morphologies (Figure 7c) recovered in the authigenic micrite of the stromatolitic textures lining veins and sills (Figure 2c) of the Kess-Kess mounds (Cavalazzi *et al.*, unpublished data).

The most common microbial morphologies recovered in cold seep deposits are mineralized bacterial sheaths or generic biofilm textures. This is because bacterial cell colonies are commonly encased in protective exopolymeric substances (EPS) biofilms, which often undergo early mineralization processes. Simple sheaths of microbial origin binding and trapping biodetrital or siliciclastic grains in the Stirone paleoseep (Figure 8) are significant examples. Complex structures interpreted as remains of mineralized bacterial mats have been recently documented in the fossil record. From the Silurian seep limestone of El Borj a three-dimensional alveolar network made up of hematite and filled by calcium carbonate (Figure 9a) has been interpreted as the fossil analogue of mats of filamentous bacteria (see discussion in Barbieri *et al.*, 2004). In this example the mineralization by hematite of partially degraded bacterial mats precedes (or co-occurs with) the precipitation of the authigenic carbonate. Similar three-dimensional structures have also been described from Mg-calcite mineral phases that fill and rim cavities and conduits

(Figure 9b) used by fluids migration in the Pliocene Stirone seep (Barbieri and Cavalazzi, 2005). In both the above examples the early mineral replacement by hematite (El Borj) or Mg-calcite (Stirone) of alveolar organic textures is a necessary prerequisite for their preservation.

MINERALS

Authigenic carbonate-group minerals, such as Mg-calcite, aragonite and dolomite, are peculiar of paleoseep sites. Mineral composition of limestone seep bodies, as well as their stable isotope values, may change significantly, depending on the nature of the fluid flow and its interaction with the local environment (Campbell *et al*., 2002). Aragonite, in the form of botryoidal splay texture, and dolomite, in the form of spherulites, abound as cement. Their relationship with microbial processes has been documented in seep environments for aragonite (Aharon, 2000), and with presence of sulfate-reducing bacteria for dolomite formation (Vasconcelos *et al*., 1995). Changes in the chemistry of the expelled fluids may also determine the precipitation of minerals other than carbonates. Barite (Figure 10a), for example, in another authigenic mineral typifying cold seep sites. Its precipitation depends on the amount of barium contained by seeping fluids and on the biogeochemical conditions established by the microbial oxidation of methane (Aloisi *et al*., 2004a).

Iron-based minerals, such as pyrite and hematite, also precipitate in seep environments under microbially controlled conditions. Pyrite is an authigenic mineral that may be abundant in zones of bacterial sulfate reduction. There, sulfide may combine with iron and precipitate pyrite as small globules (framboids, Figure 10b) or pseudomorph of foraminiferal tests (Figure 10c). The interactions between the activity of microorganisms and the hematite precipitation in a cold-seep ecosystem have been described in detail in the Silurian body of El Borj, Morocco (Barbieri *et al*., 2004), where this iron oxide replaced complex organic morphologies or accumulated via intracellular biomineralization.

CONCLUDING REMARKS

Permanent record of microbial activity in the carbonate products of seeps is largely concentrated in (although not limited to) conduit openings and other vugs, and surrounding lithofacies. This depends on the tight relationships between seeping fluids, microbiological activity, and calcium carbonate precipitation. Textures with bacterial imprints are diverse and testify to a life based on chemosynthesis for which compelling examples include the giant *Beggiatoa* of the Miocene seep of Pietralunga (Northern Apennine) and the hematitized, complex microbial networks of the Silurian seep of El Borj (Morocco). In spite of the importance of microbial activity in the formation of authigenic carbonate, biomineralized microbial textures are minor components relative to the overall volume of a seep-carbonate body.

Among the geological contexts strongly suggesting the joint occurrence of chemosynthetic communities of microorganisms and seep (or vent) settings, the Devonian Kess-Kess mounds (Morocco), for which a controversy on their origin is still waiting for a solution, deserve a special interest. In the veins and sills that crosscut these conical mounds and connected deep and seafloor paleoenvironments, geomicrobiological and geochemical (stable isotope) data suggest that the subsurface microbial communities were independent of light and free oxygen. This allows outline hypotheses based upon chemosynthetic processes developed by hydrocarbon (and other fluids)-feeding microbial consortia and able to expand views on the presence and requirements of life on Earth**.** Such adaptation to environments well below the seafloor would characterize a peculiar seep microbiotic community with considerations on the presence of microbial life on other planets. Microbiological studies conducted on modern analogues of the Kess-Kess (and similar) mounds, especially on their internal organization and the relationships between precipitating minerals and microbes, would provide clues for a proper understanding of these (present and past) ecosystems.

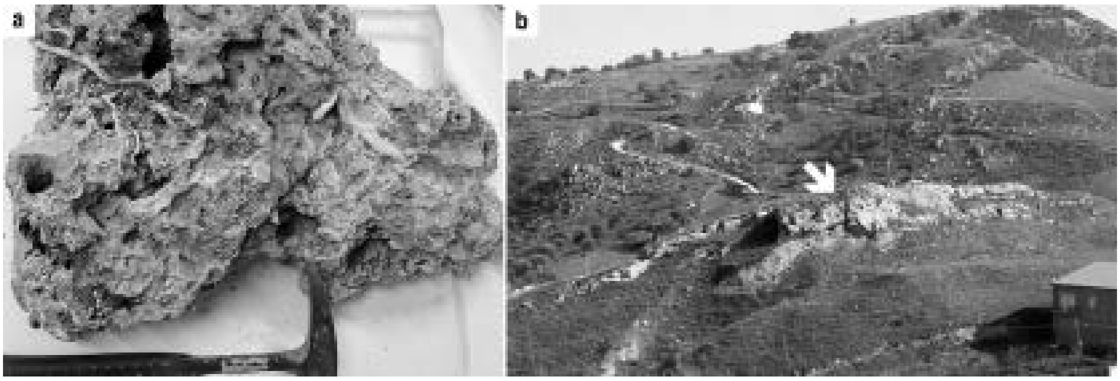

Fig. 1. **a**: Authigenic carbonate crust, Recent, northern Adriatic Sea. **b**: The calcium carbonate accumulation of Roccapalumba, Lower Miocene, Sicily.

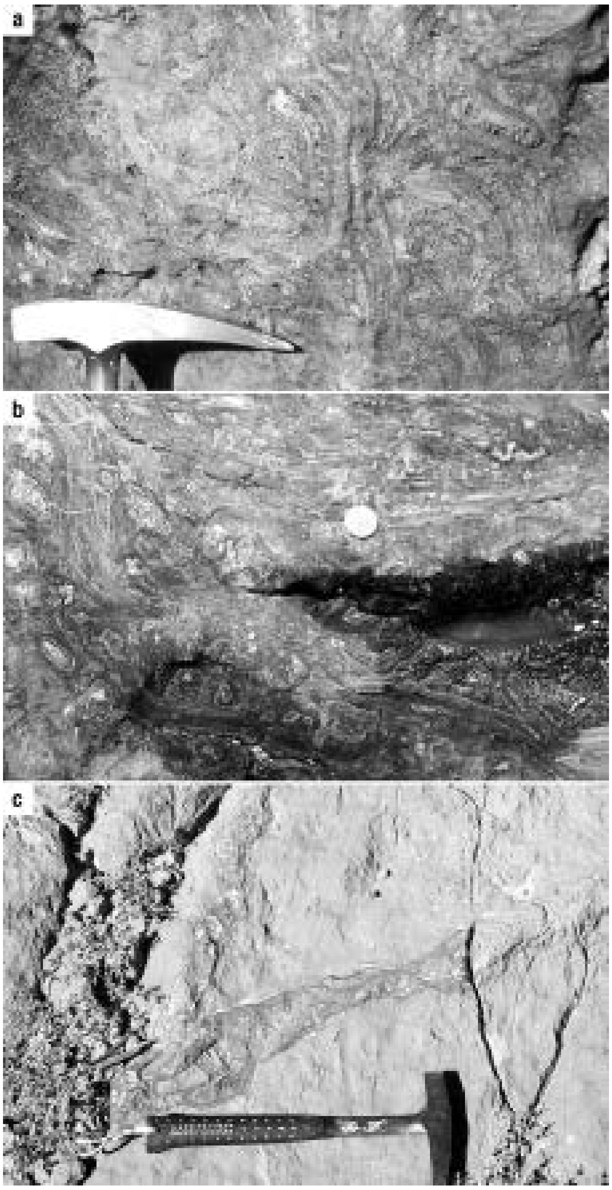

Fig. 2. Stromatolites (**a**) and stromatactoid structures (**b**) of the paleoseep at El Borj, Silurian, Middle Atlas, Morocco. **c**: veins with stromatolite infill from the side of a Kess-Kess conical mound, Devonian, Anti-Atlas, Morocco.

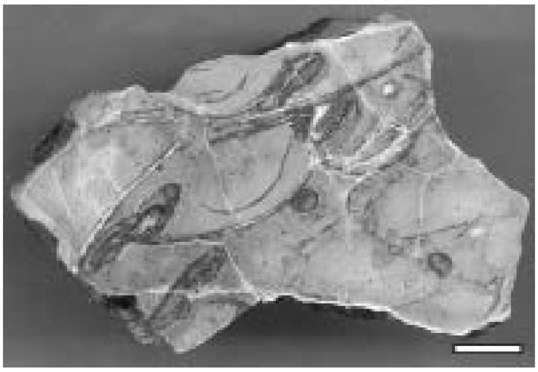

Fig. 3. Megafauna (bivalves) from the Kess-Kess mound known as Hollard Mound, Devonian, Anti-Atlas, Morocco. Scale bar = 3 cm.

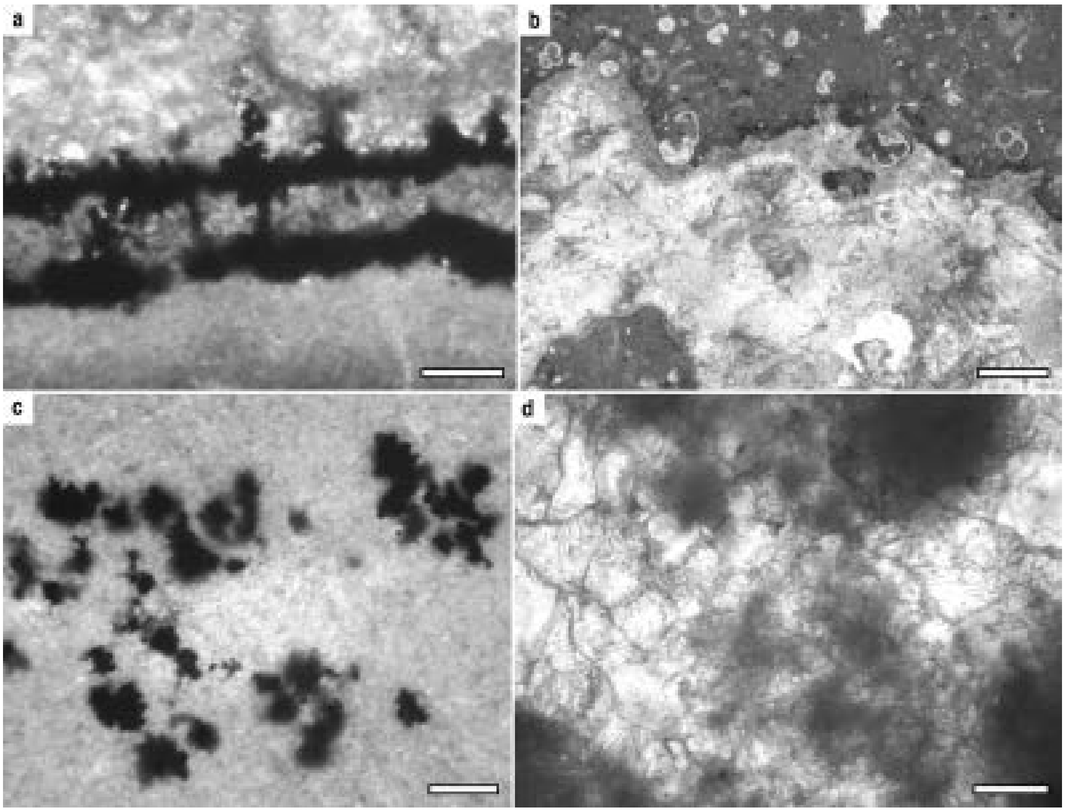

Fig. 4. Transmitted light micrographs. **a**: iron oxide crusts, El Borj paleoseep, Silurian, central Morocco. **b**: boundary (arrow) between authigenic micrite with planktic foraminiferal shells (dark area) and spar infill (light area), Roccapalumba, Lower Miocene, Sicily. **c**: microtufts, El Borj paleoseep, Silurian, central Morocco. **d**: clotted texture, Roccapalumba paleoseep, Lower Miocene, Sicily. Scale bars: **a**, **b**, **c** = 500 µm; **d** = 200 µm.

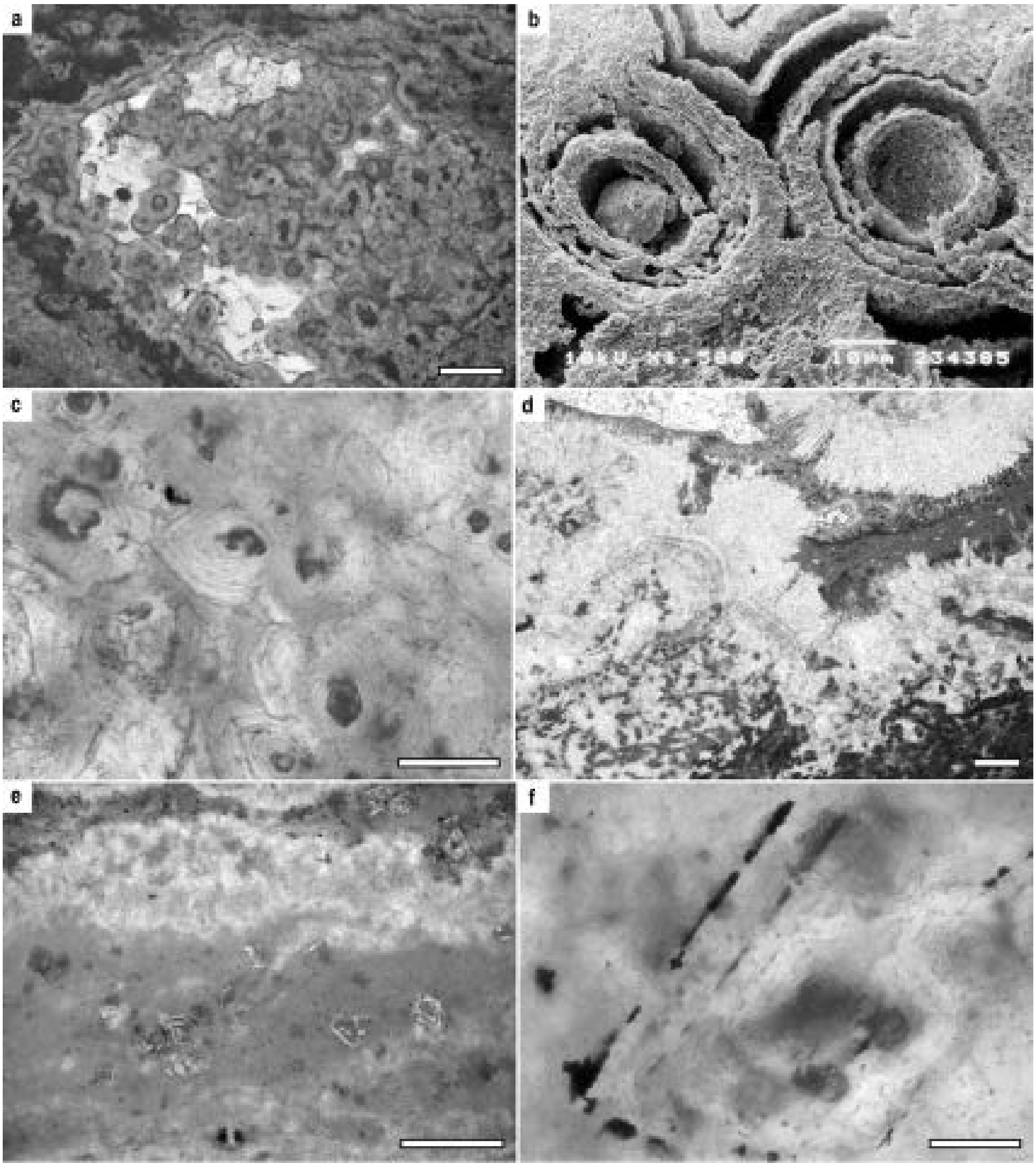

Fig. 5. Spherulite textures from transmitted light (**a**) and SEM (**b**) micrographs, and zoned dolomite rhombs (**c**), Pietralunga paleoseep, Miocene, northern Apennine. **d**: aragonite splays, Pietralunga paleoseep. **e**: dolomitic rhombs embedded in micrite from stromatolites of a Kess-Kess mound, Devonian, Morocco. **f**: detail of a zoned dolomite rhomb from a Kess-Kess mound, note the organic matter (dark) which underlines the zoned fabric. Scale bars: **a** = 50 µm; **b** = 10 µm; **c** = 50 µm; **d** = 1 µm; **e** = 500 µm; **f** = 250 µm. Transmitted light micrographs: **a**, **c**, **d**, **e**, **f**. SEM micrograph: **b**.

Fig. 6. Bacteriomorphs (putative cocci) from the Stirone paleoseep, Pliocene, northern Apennine (SEM micrograph taken by Frances Westall, CNRS, Orléans). Scale bar = 1 µm.

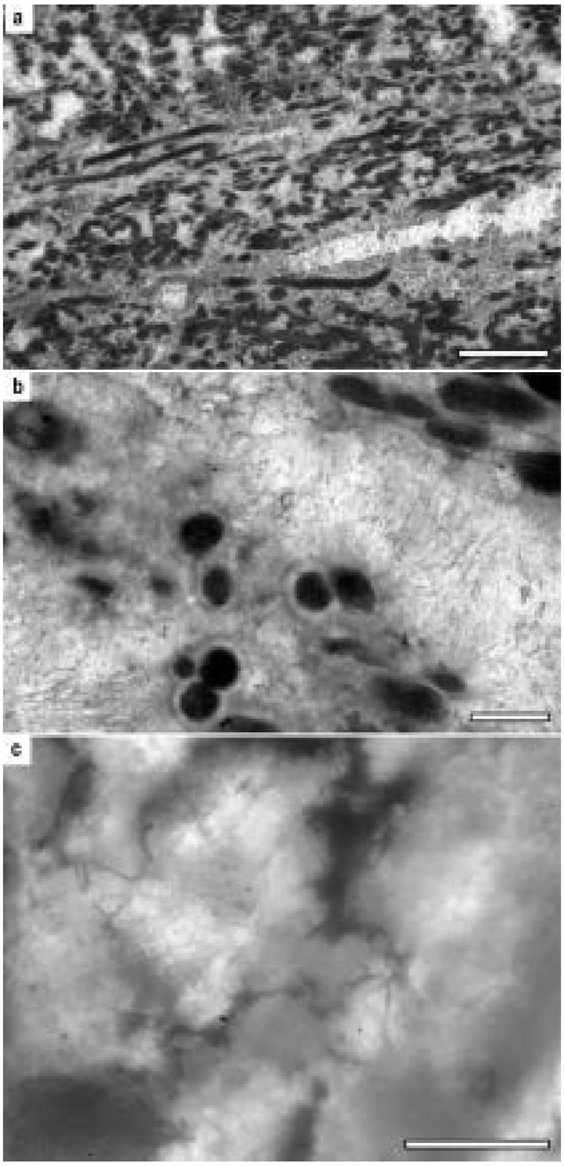

Fig. 7. Transmitted light micrographs. **a**, **b**: dense concentration of filaments arranged as microbial mats and embedded in a microsparite groundmass (**a**), with aragonite cement fringes (**b**), Pietralunga paleoseep, Miocene, northern Apennine. **c**: filamentous morphologies from the stromatolites of veins crosscutting the Kess-Kess mounds, Devonian, Morocco. Scale bars: **a** = 1 µm; **b** = 200 µm; **c** = 50 µm.

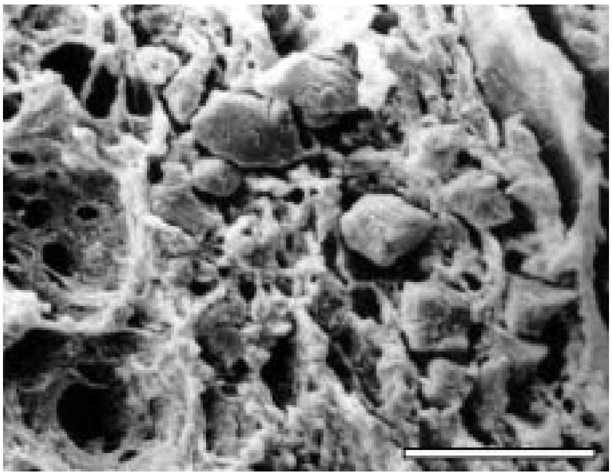

Fig. 8. Siliciclastic grains trapped by microbial sheaths, Stirone paleoseep, Pliocene, northern Apennine, SEM micrograph. Scale bar = 5 µm.

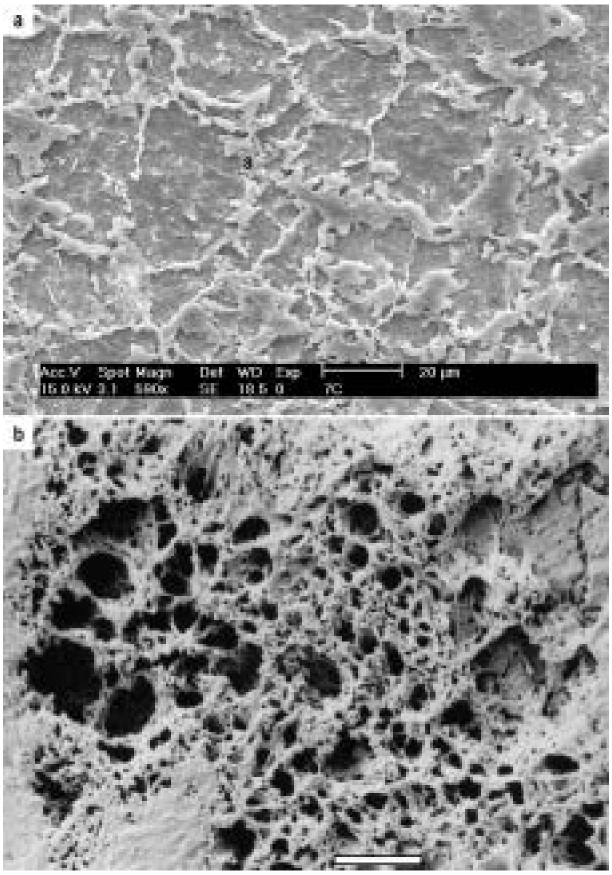

Fig. 9. SEM micrographs of alveolar morphologies described from the Devonian El Borj (a) and the Pliocene Stirone (b) paleoseeps. Scale bars: 10 µm.

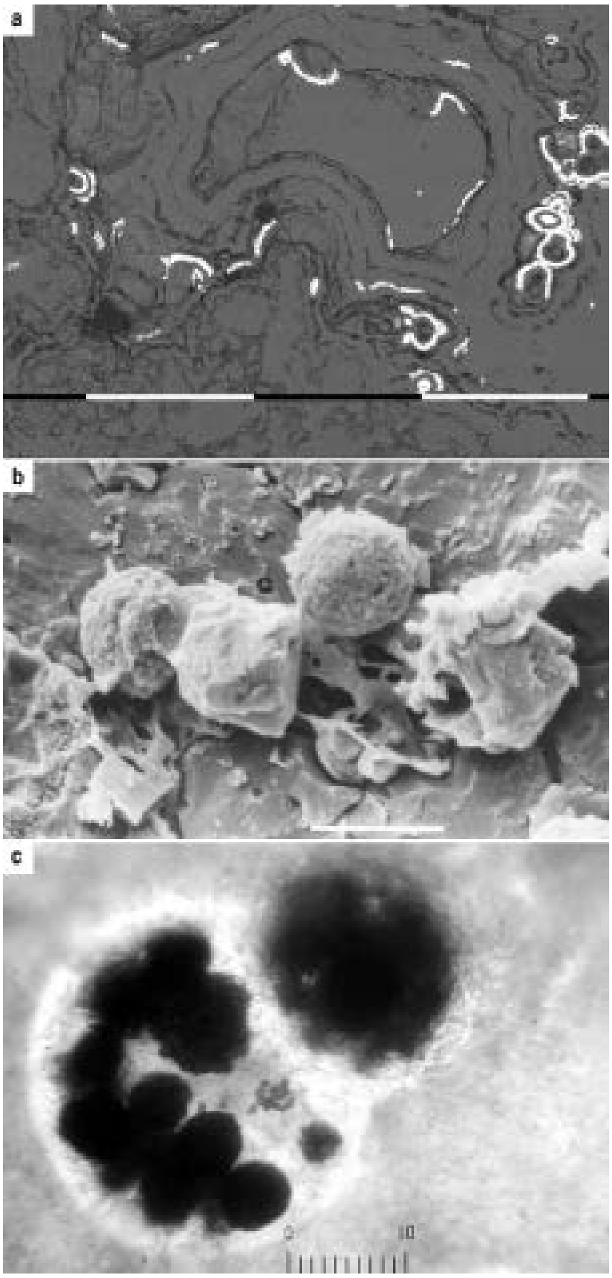

Fig. 10. **a**: SEM micrograph of precipitate barite from the Pietralunga paleoseep, Miocene. **b**: SEM micrograph of pyrite framboids, El Borj paleoseep, Silurian. **c**: Transmitted light micrograph of a planktonic foraminiferal test filled with pyrite framboids, Roccapalumba paleoseep, Lower Miocene. Scale bars: **a** = 100 µm; **b** = 5 µm; **c** = 10 µm.