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Barbara Cavalazzi, Frances Westall, Roberto Barbieri. (Crypto-)Endoliths from vesicular pillow lavas, Coral Patch Seamount, North Atlantic Ocean. *Studi Trentini di Scienze Naturali. Acta geologica*, 2008, 83, pp.177-182. hal-00527444

**HAL Id: hal-00527444**

**<https://hal.science/hal-00527444>**

Submitted on 17 Jun 2020

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# (Crypto-)Endoliths from vesicular pillow lavas, Coral Patch Seamount, North Atlantic Ocean

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**SUMMARY** - (*Crypto-*)Endoliths from vesicular pillow lavas, Coral Patch Seamount, North Atlantic Ocean - Microbial endoliths, such as bacteria, fungi/lichens and algae, occupy distinct ecological niches inside rocks and can colonize a wide variety of substrates, especially in extreme environments. Recently, *in situ* bioerosion of the glassy rinds of volcanic rocks by euendoliths, leaving characteristic microbial textures as micro-channels and microborings, has been described. The shape and distribution patterns of the fossil borings reveal the behavior of the boring-makers and this information can be used to predict their environmental and trophic requirements. Comparison of the preserved trace fossils with structures produced by modern microorganisms can provide information about the nature of the original trace maker. In this study, we characterise the evidence for microbial activity within degassing vesicles in the outer alteration rinds of pillow lavas, Coral Patch Seamount, eastern North Atlantic Ocean, in order to elucidate the nature of metabolic pathways of their trace maker and their environmental context.

**RIASSUNTO** - (*Cripto-*)Endoliti in vescicole di lave a cuscino, Coral Patch Seamount, Oceano Atlantico Settentrionale - Endoliti microbici, come ad esempio batteri, funghi/licheni e alghe, occupano distinte nicchie ecologiche all'interno delle rocce e possono colonizzare un'ampia varietà di substrati rocciosi, soprattutto in ambienti estremi. Recentemente sono state descritte caratteristiche strutture microbiche, come ad esempio micro-canali e *microboring*, generate da attività bioerosiva prodotta in vetri vulcanici ad opera di euendoliti. La forma e la distribuzione dei *boring* fossili rivelano il comportamento dei *boring-maker* e queste informazioni possono essere utilizzate per ipotizzare le loro necessità ecologico-ambientali e trofiche. Il confronto tra tracce fossili e analoghi prodotti da microrganismi attuali può fornire informazioni preziose circa la natura e l'interpretazione del relativo *trace maker*. In questo lavoro, sono state descritte evidenze di attività microbica osservate all'interno delle vescicole di degassazione presenti nella crosta di alterazione superficiale delle lave a cuscino del Coral Patch Seamount (Oceano Atlantico Settentrionale), con l'obiettivo di interpretare il metabolismo e ricostruire il contesto paleo-ambientale dei relativi *trace maker*.

**Key words:** microbial morphologies, cryptic-habitat in pillow lavas, Atlantic Ocean

**Parole chiave:** morfologie microbiche, habitat criptici in lave a cuscino, Oceano Atlantico

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## 1. INTRODUCTION

Endolithic microorganisms can colonize a wide variety of terrestrial, freshwater and marine substrates, especially in extreme environments, and may produce typical structures and bioalteration textures, such as microborings with high preservation potential (McLoughlin *et al.* 2007). Microborings and the well-preserved fossil remains of endolithic microorganisms occur throughout the terrestrial geological record, dating back to the Early Archaean (Zhang & Golubic 1987; Furnes *et al.* 2004). The oldest putative traces of borings are microtubules occurring in ~3.5 Ga pillow basalt rinds from the Barberton Greenstone Belt in South Africa (Furnes *et al.* 2004; Banerjee *et al.* 2006).

Sedimentary rocks are the most common rock type hosting endolithic colonisation (Friedmann & Koriem 1989),

which is mainly performed by fungi, lichens and algae. The deep subsurface is an important habitat for microbial life (Parkes *et al.* 2005), and microbial processes are known to continue at considerable depths (>800 m) in sub-seafloor sediments (Schippers *et al.* 2005). Microorganisms have even been found at depths of >3000 m within the crust in the deep gold mines of South Africa (Onstott *et al.* 1997). Recent studies, however, show that a number of textural, geochemical and microbiological indications of bioalteration/biomediation occur along the fractures and cracks of volcanic glass rinds of modern and ancient oceanic crust. They are suggestive of intense microbial activity in the uppermost submarine oceanic crust (Thorseth *et al.* 1992, 1995; Fisk *et al.* 1998; Banerjee & Muehlenbachs 2003; Furnes *et al.* 2007). Cryptoendoliths from vesicles represent a poorly studied niche for life (Peckmann *et al.* 2008). General-

ly, only the shape and distribution patterns of fossil borings reveal the behavior of their makers; this can be used to predict some of their environmental and trophic requirements. In this study, we examine the evidence for microbial activity within degassing vesicles in the alteration rinds of pillow lavas (cryptic-habitat) from the Coral Patch Seamount, eastern North Atlantic Ocean, in order to elucidate the nature of metabolic pathways of the trace makers and their environmental context.

## 2. GEOLOGICAL SETTING

The studied samples were collected at Coral Patch Seamount, in the Gulf of Cadiz off Morocco, (Fig. 1). The Coral Patch Seamount is a drowned ocean island volcano and represents the intermediate phases of a  $\geq 72$  Ma old hotspot (Geldmacher & Hoernle 2000). Basaltic samples from Madeira hotspot track including the Coral Patch Seamount, range from transitional tholeiites to basanites, whereas more evolved samples range from hawaiites to trachytes. The volcanic rocks at Coral Patch Seamount are alkali (hawaiite) basalts (see Geldmacher & Hoernle 2000: p. 79, tab. 1b).

## 3. MATERIAL AND METHODS

Samples were collected during the scientific expedition SWIM 2004 (PI: N. Zitellini, ISMAR-CNR, Bologna) of the R/V URANIA. We examined samples dredged from station 29 (SWIM04-29: Lat  $34^{\circ}58.3106'$  N, Long  $-11^{\circ}57.3238'$  W, depth 1011.8 m below sea level) along the cruise track across Ampère-Coral Patch Seamount region (Fig. 1). The sampled material consists of massive, altered and reddish-black pillow lava characterized by several micrometer-scale vacuoles and encrusted by a white carbonate crust. Material was subsampled (drilled) from the outer rinds of the pillow lava. Uncovered standard petrographic thin sections were prepared and investigated with a multi-analytical approach. Samples were characterized by examination of petrographic thin sections with an Olympus BX51 TH-200 optical microscope, an LSM 510/3 META Zeiss confocal laser scanning (CLSM) available at the Centre de Biophysique Moléculaire - CNRS, Orléans, a FEI Environmental Scanning Electron Microscope Quanta 200 equipped with an X-ray energy dispersive spectrometer system (ESEM-EDX) at the Centro Interdipartimentale Grandi Strumenti, Università di Modena, and a Scanning Electron Microscope Philips 512B fitted with an EDAX DX4 microanalytical device (SEM-EDX) at the Dipartimento di Scienze della Terra e Geologico-Ambientali, Università di Bologna. ESEM-EDX analyses were performed on slightly etched (1% HCl for a few seconds) and non-coated thin sections. The ESEM observations were conducted in low vacuum (LV: 1 and 0.5 Torr). SEM-EDX observations were conducted on the same samples after C-coating and using a 15Kv accelerating voltage.



Fig. 1 - Location map of studied sample (star). The sample was obtained from Station 29 of the SWIM2004 scientific cruise at the Coral Patch Seamount, Gulf of Cadiz, Atlantic Ocean.

*Fig. 1 - Ubicazione geografica del campione studiato (stella). Il campione è stato prelevato dalla stazione 29 in corrispondenza del Coral Patch Seamount durante la crociera scientifica SWIM2004 nel Golfo di Cadice, Oceano Atlantico.*

## 4. DESCRIPTION

### 4.1. Rock Textures

The outer rinds of the glassy pillow lava exhibit an extensive alteration (palagonitization) (Fig. 2A). Palagonitization, a continuous diagenetic process during which amorphous glass is replaced by crystalline clay-like materials, leads to rinds characterized by a diffuse system of cooling fractures and ovoid to elongate degassing vesicles, and include altered/fractured fragments of olivine, pyroxene and Cr-spinel crystals (Fig. 2A). The vesicles have an average diameter of 250  $\mu\text{m}$  (maximum diameter: less of 2 mm) and are filled by fibrous, radiating and porous authigenic K-zeolite minerals, a common authigenic mineral forming in submarine volcanic rocks. The vesicles are isolated from the altered glassy pillow lava surface and the yellowish porous K-zeolites contain disseminated structures with tubular or filamentous microbial-like morphologies (Fig. 2). The outermost edge of the altered pillow lava is a 100-150  $\mu\text{m}$  Mn-Fe-Ti-enriched film encrusted by yellowish-white material with abundant planktonic foraminiferal tests.

### 4.2. Microbial-like morphologies

Tubular and filamentous structures occur throughout the porous K-zeolite filling of vesicles of the outer altered glassy basaltic pillow lava (Figs 2-5). They occur as individuals with a sinuous to straight shape and an average diame-

Fig. 2 - Transmitted light micrographs of petrographic thin sections. a. The texture of outer rind of altered (palagonite), basaltic oceanic crust is characterized by cooling fractures and degassing vesicles filled with fibrous and porous zeolite (black arrows). b. Detail of a porous-fill zeolite vesicle showing tubular to filamentous microbial-like morphologies (black arrows).

Fig. 2 - Microfotografie a luce trasmessa di sezioni sottili petrografiche. A. La tessitura della parte esterna della lava a cuscino alterata (palagonite) è caratterizzata da fratture da raffreddamento e vescicole di degassazione occluse da zeolite fibrosa e porosa (freccie nere). B. Dettaglio di una vescicola occlusa da zeolite porosa cui sono associati biomorfi con strutture tubulari e filamentose (freccie nere).

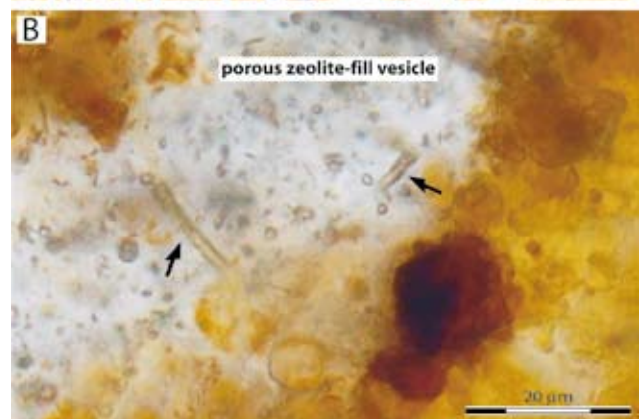
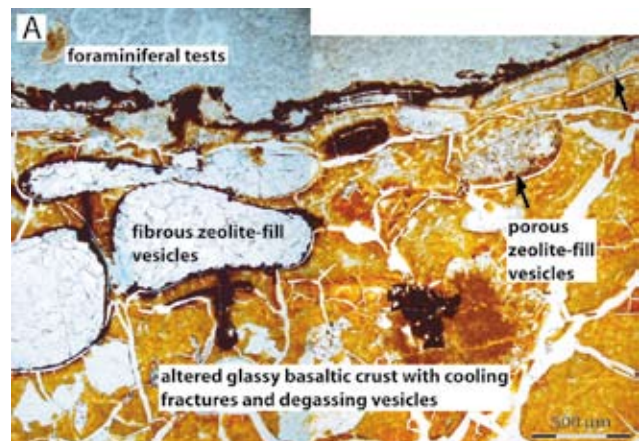
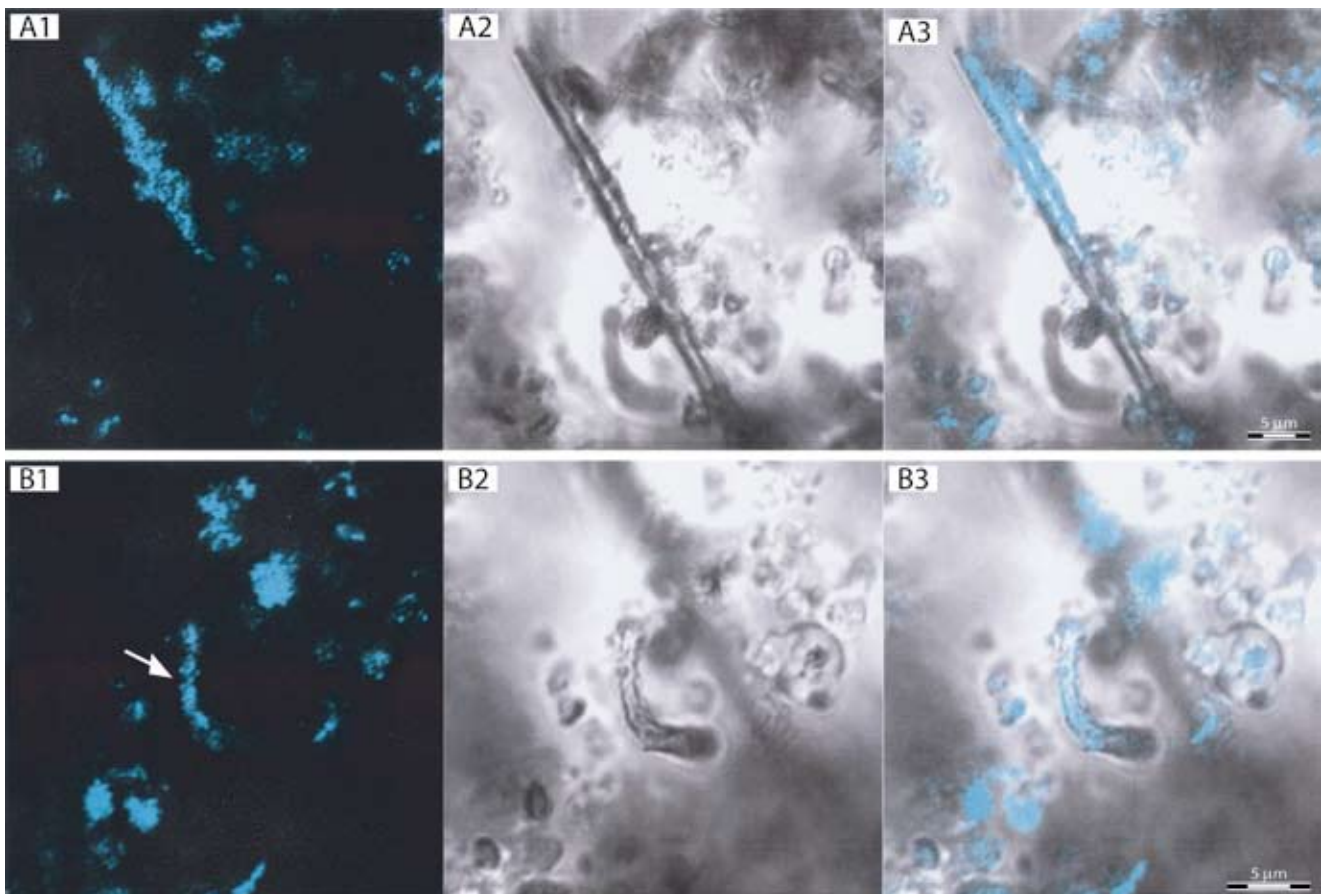


Fig. 3 - Stacked CLSM images showing auto-fluorescence along the tubular structures within the outer vesicles of altered glassy pillow lava. Note the microbial morphologies, sinuous and straight tubes (A2, B2). This auto-fluorescence contrast mode highlights the presence of micro-spheres forming short chains within tubular cast (arrow in B1).

Fig. 3 - Immagini CLSM illustranti l'auto-fluorescenza distribuita lungo le strutture tubulari presenti all'interno delle vescicole della lava a cuscino alterato. Sono evidenti le morfologie microbiche, rappresentate da tubuli sinuosi e dritti (A2, B2). Questa modalità di contrasto in autofluorescenza mette in luce la presenza di micro-sfere che formano delle corte catene all'interno della struttura tubulare (freccia in B1).



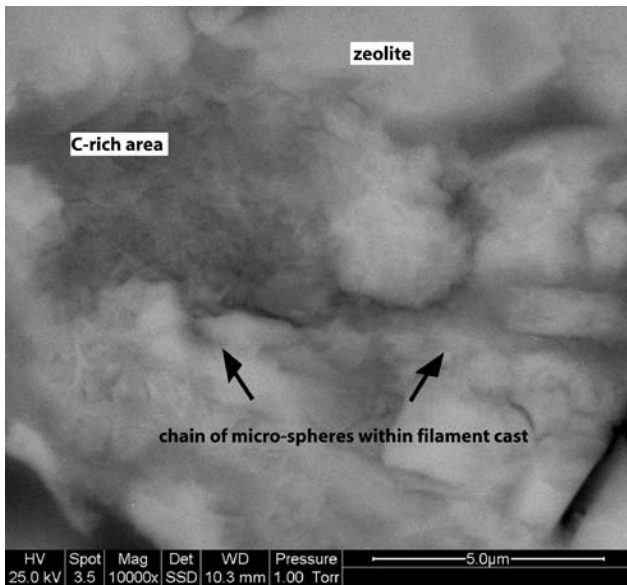


Fig. 4 - ESEM image of putative cell-like structures (micro-spheres) arranged in a short chain within a tubular cast. Note the C-enrichment of the zeolite walls of tubular cast.

*Fig. 4 - Immagine all'ESEM di presunte strutture cellulari (micro-sfere) organizzate in catena all'interno di una struttura tubulare.*

ter of 2  $\mu\text{m}$  and  $\sim 1 \mu\text{m}$ , respectively. The tubular structures exhibit smooth surfaces (Fig. 2B). As observed by CLSM, these structures show a characteristic auto-fluorescent signal (Fig. 3) and the micro-tubes are surrounded by an aureole of relatively low density material (Fig. 3, A2 and B2). CSLM and ESEM images show some filamentous structures consisting of short chains of micro-spheres with a K-zeolite composition (Figs 3B and 4). ESEM-EDX observations document void space between the tubular structures and their internal casts (Fig. 5A). Locally, micro-tubular structures are surrounded by amorphous, C-rich membrane-like envelopes, less than 500 nm thick (Fig 5B). The walls of the micro-channels and microtubes are enriched in C, as is the zeolite matrix in the vicinity of these structures (Fig. 5).

## 5. DISCUSSION AND CONCLUSIONS

The micro-tubes and filamentous structures are interpreted as being of microbial origin, and their biogenicity is suggested by (i) morphologies having the size and shape range of typical prokaryote filaments (Madigan *et al.* 2000), (ii) the low degree of morphological variability, (iii) their elemental and mineral composition, and (iv) their environmental context, i.e. vesicles in altered submarine pillow lava (cf. Schopf 1999; McLoughlin *et al.* 2007).

Laser microscopic techniques are commonly used in cell biology for detecting biomolecules within natural environment (Halbhuber & König 2003) and the auto-fluorescence together with the C-Fe-Ti-enrichment of the aureoles

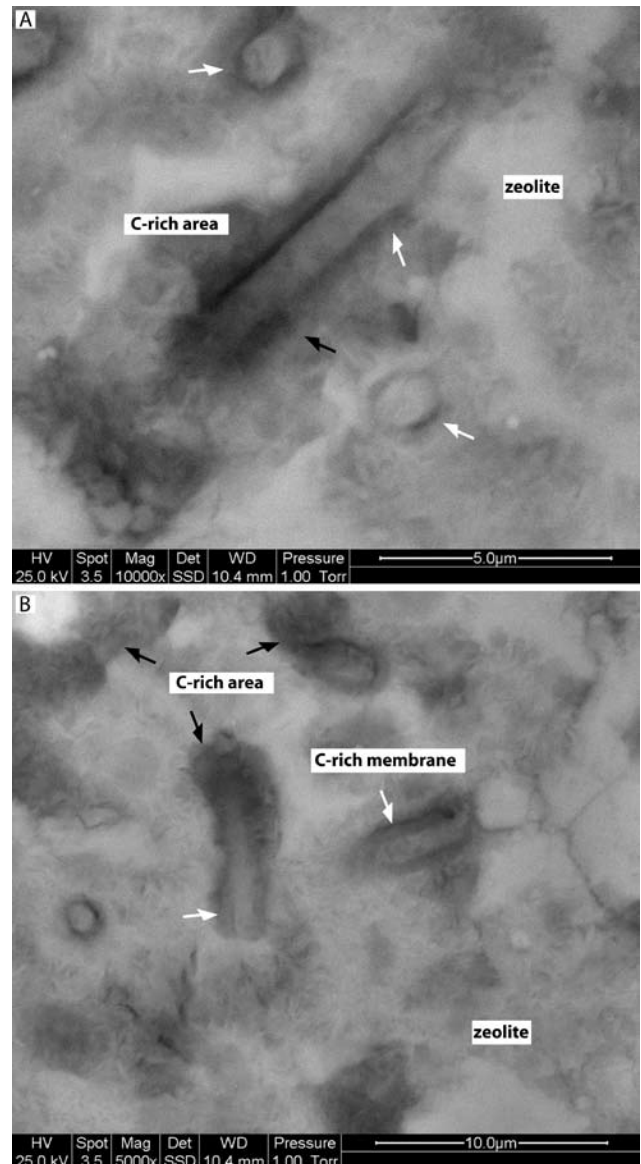


Fig. 5 - ESEM images showing a tubular cast and micro-tubular structures within K-zeolite fill vesicles from the outer altered glassy basalt. The tubular structures and the matrix in the immediate vicinity of the tubes are typically C-enriched. White arrows show the presence of a void space and a membrane-like aureole around the tubular structure.

*Fig. 5 - Immagini all'ESEM che mostrano differenti strutture tubulari all'interno delle vescicole riempite di K-zeolite nella parte esterna del basalto vetroso alterato. Le strutture tubulari e la matrice nelle immediate vicinanze dei tubuli sono tipicamente arricchite in C. Le frecce bianche indicano la presenza di uno spazio vuoto e un'aureola tipo membrana intorno alla struttura tubulare.*

surrounding the fossil tubular structures in the palagonitized pillow lava rinds suggest that the aureoles are the remnants of membrane-like structures, such as sheaths (cf. Halbhuber & König 2003; Furnes *et al.* 2005, 2007). Preserved organic matter and presence of nucleid acids have previously been described from endolithic microbial structures (such

as microborings, micro-channels and pits) in modern ocean basalts (Giovannoni *et al.* 1996; Banerjee & Muehlenbachs 2003). The biologic nature of this membrane or sheath is also suggested by its resemblance to microbial extracellular polymeric substances (EPS). Sheathed bacteria, characterised by a sheath of extracellular material, are primarily distinguished on the basis of morphological criteria (Mulder & Deinema 1992). They may be filamentous or consist of chains of cells within the sheath. Experimental and natural observations show that biological surfaces such as microbial cell and biofilms (EPS) can concentrate bio-elements and/or act as nucleation sites for mineral crystals (Westall *et al.* 1995; Frankel & Bazylinski 2003). Bacteria have the ability to selectively accumulate metal ions (Schultze-Lam *et al.* 1995; Westall *et al.* 2001), leading to their cell surface mineralization, first step in the fossilization process (Ferris *et al.* 1988; Southam & Donald 1999). Oxidized and fixed-iron reduced compounds located around/on the cell walls and within the EPS of modern and ancient filamentous microorganisms have been reported (Fortin & Langely, 2005). The observed Fe-Ti-enrichment of C-rich membrane of the micro-tubes is therefore not unusual and may represent the first stage in the progressive encrustation of microbial cell walls. In particular, Fe-oxidizing bacteria with encrusted sheaths have been documented from volcanic deep-sea environments (Emerson & Moyer 2002; Edwards *et al.* 2004; Little *et al.* 2004; Cavalazzi 2007). The C-Fe-Ti-rich sheathed tubular structures described in this study strongly suggest that they were originally sheathed Fe-oxidizing bacteria with the K-zeolite tubes and chains of micro-spheres enclosed in tubular casts with C-rich wall, representing empty Fe-encrusted sheaths filled by mineral precipitates (K-zeolite). The iron and titanium enrichment in this micro-habitat is probably the product of microbial leaching from the silicate glass (Rogers & Bennett 2004). Iron driven processes represent an abundant energy source for chemolithotrophic microorganisms in volcanic rocks (Edwards *et al.* 2003). The enrichment of Fe-Ti in the fossilized sheath-like membranes further supports their interpretation as the remains of Fe-oxidizing microorganisms. Fe-oxidizing bacteria are microaerophilic: they are therefore capable to live within basalt vesicles where levels of dissolved oxygen are low (Edwards *et al.* 2003). Chemolithotrophs are common in relatively young volcanic deposits (King 2007). Moreover, phylogenetic analysis of 16rDNA sequences revealed that microbes from basalt oceanic rocks are mainly endoliths (Mason *et al.* 2007). The bacteriomorph tubular and filamentous structures described in this study are therefore most likely to be the remains of chemotrophic/-lithoautotrophic fossil prokaryotes. Whereas the endolithic microorganisms previously recognized in modern and very ancient volcanic rocks are euendolithic microorganisms occurring along the fractures of the basaltic glass that they inhabit (Furnes *et al.* 2007 and therein references), those described here in the cryptic-habitat of degassing vesicles (=“fossilized” bubbles, Peck 1978) are cryptoendolithic microaerophilic mi-

croorganisms, Fe-oxidizing bacteria that proliferate in habitats where levels of dissolved oxygen are low.

## ACKNOWLEDGEMENTS

Authors would like to thank Nevio Zitellini, Lucilla Capotondi and Alessandro Ceregato, ISMAR-CNR Bologna, for donating samples used in this investigation. Thanks are due to Gilles Breuzard, Chantal Pichon, CBM-CNRS Orléans, and Massimo Tonelli, CIGS-University of Modena and Reggio Emilia, for their support during the CLSM and ESEM-EDX observations. This paper benefited from discussion with Roberto Braga and Giorgio Gasparotto (University of Bologna). Financial to perform the analytical work was given by the GDR-Exobiologie (to F.W.) and MUR-PRIN 2006 (to R.B.) research funds, a CNR/Italy-CNRS/France International Cooperation Program 2006-2007 sponsored the mobility at CBM-CNRS Orléans of B.C.

A. D’Alessandro and A. Rosso provided helpful reviews of the manuscript.

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