

1 **APPLICATION OF RAMAN SPECTROSCOPY IN COMPARISON BETWEEN CRYPTIC**
2 **MICROBIALITES OF RECENT MARINE CAVES AND TRIASSIC PATCH REEFS**

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13
14 **ABSTRACT:**

15 Microbialites are common carbonate structures in cryptic niches of marine environments throughout
16 geological time. In this research we compare the microbialites of small bioconstructions
17 (biostalactites) of modern submarine caves of Sicily with those developed in small crypts of
18 Carnian patch reefs of the Dolomite Mountains (Heiligkreuz Formation, Alpe di Specie), using
19 Raman spectroscopy, a method that allows *in situ* determination of the organic content of microbial
20 components. **This methodology partly solves the uncertainty of geomicrobiological studies that use**
21 **bulk measurements (i.e. biomarker analyses), which make it difficult to associate mineral**
22 **precipitates with a specific microbial process.** In the modern marine caves, the complex biotic
23 relationships among skeletal organisms (mainly serpulids) and microbial communities produced
24 biostalactites in which microbially-induced biomineralization is the consequence of autotrophic and
25 chemoheterotrophic bacterial activities. Sulfate-reducing bacteria, fed by metazoan organic matter,
26 flourish in millimetric oxygen-depleted cavities of the skeletal framework, and induce

27 autochthonous micrite deposition and early stabilization of the biostalactites. Similar processes have
28 been interpreted to induce the deposition of the microbialites in the Upper Triassic patch reefs of the
29 Dolomites. These small shallow water reefs, made up mainly of scleractinian corals, sponges and
30 red algae, hold a skeletal framework rich in millimeter to centimeter size cavities, ideal cryptic
31 niches for growth of microbial communities. The *in situ* characterization of organic compounds
32 through micro-Raman spectroscopy, following prior identification of specific sulfate-reducing
33 bacteria biomarkers using bulk measurements obtained by solvent extraction, indicate the same
34 biogeochemical signatures of the microbialites within the cryptic cavities of the biostalactites of
35 modern marine caves as those inside the skeletal framework of Carnian patch reefs. These data,
36 showing the same processes in Triassic and modern cryptic microenvironments, is evidence that the
37 microbially-mediated precipitation in confined environments is a process independent of geological
38 time, that further investigation may be able to test.

39 **Keywords:** bioconstructions, cryptic cavities, microbialites, sulfate-reducing bacteria, recent,
40 Triassic.

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INTRODUCTION

43 Several organisms contributed to reefal structures in shallow marine systems, beginning with
44 stromatolites of 3.5 Ga. Ecological and environmental features led the changes of reefal
45 communities during geological time and the global distribution of reefal carbonate bodies varied
46 considerably following the diverse trophic demands of building organisms (Wood, 1999). Skeletal
47 frameworks of the reefs were often stabilized by autochthonous micrite deposition induced by
48 metabolic activities of bacteria (Reitner et al., 2000; Riding 2011b). Generally, abundance of
49 microbial sediments characterized episodes of environmental change, and some are associated with
50 mass extinction events, which allowed the huge and sudden development of microbial communities
51 into vacant ecological niches (Fischer and Arthur 1977; Schubert and Bottjer 1992; Bottjer et al.

52 1995; Wood 2000a; Heindel et al., 2018). The ratio of microbialites to skeletal metazoans, as
53 primary components of carbonate bioconstructions, is characterized by phases in which
54 microbialites greatly prevail, as in stressed environments or in the immediate aftermath of
55 biological crises (Russo et al. 1997; Webb 1996; Russo et al., 2000; Kiessling et al. 2002; Russo
56 2005) and periods of high levels of carbonate saturation in sea water during geological time (Riding
57 2002).

58 Several authors recognized the importance of microbial carbonate production in carbonate
59 platforms and reefal environments throughout Earth history (Flügel et al. 1993; Grotzinger 1994;
60 Kruse et al. 1995; Webb 1996; Feldmann and McKenzie 1997; Wood 2000a, b; Reolid and Molina,
61 2010; Riding 2011a; [Jakubowicz et al., 2014](#)). Microbialites are present in reefal carbonate,
62 independent of type of metazoan builders and geological time (Kiessling 2002; Riding 2005;
63 Rasmussen et al., 2009; Homann et al., 2016). In modern environments, marine microbialites were
64 described in different sedimentary environments, from the Great Barrier Reef to deep water in the
65 Red Sea (Macintyre 1977, 1985; Marshall 1986; Zankl 1993; Webb 1996; Brachert and Dullo 1991;
66 Braga et al., 2019). Micritic crusts, generally microbial in origin, were located within the walls of
67 forereefs (Moore et al. 1976; James and Ginsburg 1979; Land and Moore 1980; Brachert and Dullo
68 1991; Dullo et al. 1998) and inside reefal cavities (Macintyre 1984; Reitner 1993; Zankl 1993;
69 Reitner et al. 1995; Heindel et al., 2012). In general, it must be emphasized that in reef
70 environments microbialites are common components within the cryptic microenvironments of the
71 skeletal framework (Zankl 1993; Gast et al., 1998; Reitner et al., 1995, 2000; Riding, 2011b).
72 Against this background, marine cave systems have been acknowledged as ideal natural laboratories
73 to investigate the carbonate-generating role of microorganisms in confined environments and to
74 clarify how microbial processes develop under stressed conditions (Guido et al., 2013; Gischler et
75 al., 2017a). Submarine caves are cryptic habitats characterized by low light intensity, marked
76 oligotrophy and low water circulation (Harmelin et al., 1985, Fichez 1990, 1991). They are
77 colonized mainly by sponges, corals, serpulid polychaetes and bryozoans (Harmelin et al., 1985).

78 These organisms may form different types of bioconstructions, from small biogenic crusts to large
79 bioconstructions named “biostalactites” (Onorato et al., 2003; Belmonte et al., 2009; Guido et al.,
80 2013, 2017a; Sanfilippo et al., 2015; Gischler et al., 2017a, b). These structures are strengthened
81 and lithified by microbialites at an early stage in their development (Guido et al., 2013; Gischler et
82 al., 2017a,b).

83 Here we compare the microbialites developed inside biostalactites of submarine caves in the
84 Plemmirio Marine Protected Area (eastern Sicily, Italy) with those within cryptic microcavities of
85 skeletal framework of Carnian patch reefs (Heiligkreuz Formation, Alpe di Specie, Dolomites,
86 Italy). These small Triassic bioconstructions were chosen for their exceptional preservation state
87 (Scherer 1977; Russo et al. 1991; Senowbari-Daryan et al. 2001) that allowed biogeochemical
88 investigations on such ancient carbonates. The biogeochemical analyses show similar microbial
89 processes for microbialites of the modern marine caves and Triassic patch reefs. However,
90 biomarker techniques, based on the extraction of organic matter from the mineral matrix, reflect
91 average values of the study sample, and do not take into account its **heterogeneity**.

92 In this study, for the first time, micro-Raman spectroscopy was utilized for *in situ*
93 characterization of microbialites allowing a detailed comparison among the microbial activity in
94 confined cavities developed in bioconstructions, **representative of** different macro-environments
95 (modern cave *vs* Triassic reef). In geomicrobiological studies, the lack of knowledge of the exact
96 original position of the biomarkers makes it difficult to associate mineral precipitates with the
97 presence of particular microbes and specific biogeochemical pathways. Raman spectroscopy
98 enables **areal** detection of organic **matter** preserved among the complex mineral matrix at
99 microscopic level without extraction: this technique represents a unique tool to distinguish
100 microbialites from precipitates not directly mediated by microorganisms (Leefmann et al. 2014).
101 For this reason, we applied Raman spectroscopy to localize and characterize *in situ* the organic
102 compounds following the approach described by Leefmann et al. (2014), Greco et al. (2018) and
103 Guido et al. (2018).

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SAMPLE LOCATIONS

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Biostalactites from modern submarine caves

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The studied biostalactites from modern environments were sampled in submarine caves (Granchi-

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GR and Mazzere-MZ) located within the Plemmirio Marine Protected Area (Sicily). The caves have

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a karstic origin and formed during Quaternary low-stand phases, in gently dipping Miocene

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limestones. The caves were submerged by Holocene sea-level rise and colonized by marine

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communities, forming the biostalactites studied here. Caves exhibit a nearly horizontal orientation

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and a flat floor with the openings situated at about 20 m below sea level along the steep and rocky

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coastline. Cave vaults and walls expose the bedrock, which is coated to varying degrees by biogenic

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crusts formed by a skeletal fauna largely represented by sponges, serpulids and bryozoans, whereas

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scleractinians are only locally present (Pitruzzello and Russo 2008; Rosso et al. 2012). Nodular to

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pillar-like bioconstructions, here termed biostalactites, project obliquely from the walls and

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perpendicularly from the vaults toward the cave floor. The biostalactites vary in size from a few

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centimeters up to 20 cm in height and up to 10 cm in diameter (Fig. 1). They are smaller and less

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common close to the cave entrance but become more abundant, larger and more closely spaced in

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the innermost sectors. Samples used in this study were previously examined by Guido et al. (2013),

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for petrographic and biomarker analyses, and here they are used for micro-Raman spectroscopic

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analysis. The samples were collected from the vault in the innermost recess (about 53 m

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horizontally from the mouth of the cave) of the Granchi cave, and the other detached from the wall

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of the Mazzere cave about 10 m from the opening (Fig. 1).

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Alpe di Specie Patch Reefs

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The Cassian fauna from Alpe di Specie (Seelandalpe) in the northeastern Dolomites (South Tyrol,

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Italy), used here to compare with the modern Sicily biostalactites, has been extensively studied

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(Loretz 1875; Ogilvie 1893; Dieci et al. 1970; Fürsich and Wendt 1977). It is one of the most

130 famous Carnian faunas in the world (Russo et al. 1991) and represents a very important source of
131 knowledge about Upper Triassic reef building organisms, due to its unusually good preservation
132 that allowed detailed study of skeletal organisms and the associated microbialite (Fig. 2). The Alpe
133 di Specie area is located at the western end of the slope of the Cassian platform of the Picco di
134 Vallandro-Specie Mt. In the Prato Piazza (Platzwiesen) - Alpe di Specie area, on the west of the
135 slope, the Heiligkreuz Dolomite directly overlies the S. Cassiano Fm. The interpretation of these
136 carbonate deposits is hindered by poor exposure. They have been regarded as small patch reefs that
137 are either *in situ* (Ogilvie 1893) or allochthonous (Fürsich and Wendt 1977; Wendt 1982; Russo et
138 al. 1991). Russo et al. (1991) regarded Alpe di Specie patch reefs as the lower part (Member A) of
139 the Heiligkreuz Formation. Heiligkreuz deposition marked a change from the prograding high-rise
140 rimmed carbonate platforms of the upper Ladinian–lower Carnian (Bosellini 1984; De Zanche et al.
141 1993) to carbonate ramp (Preto and Hinnov 2003) and restricted, locally anoxic, basin conditions
142 (Keim et al. 2006).

143 The samples processed in this research were collected from boulders in the meadows of the
144 Alpe di Specie. They were loose blocks derived from the patch reefs, that during the Triassic were
145 deposited within the fine-grained basinal sediments of the S. Cassian Fm. that prevented their
146 dolomitization and allows detailed micromorphological and geochemical study (Fig. 2).

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148 MICROBIALITES FROM MODERN MARINE CAVES AND TRIASSIC PATCH REEFS

149 Cryptic and sciaphilic (shade-loving) microbialites inside biostalactites in marine caves have been
150 studied in several locations: the Plemmirio Peninsula in southeast Sicily (Guido et al. 2012, 2013,
151 2014, 2016; Sanfilippo et al. 2015); Cyprus (Guido et al. 2017a); karst caves of the Belize Barrier
152 Reef (Gischler et al. 2017a); and the Blue Hole off Belize (Gischler et al. 2017b). Microbial
153 mediation in carbonate precipitation has been recently suggested for cementation of biotic crusts
154 made mainly of serpulids and bryozoans in recent marine caves in the Aegean Sea (Sanfilippo et al.
155 2017; Rosso et al. 2018) and for similar bioconstructions formed in a Pleistocene marine cave in

156 NW Sicily (Guido et al. 2017b). These previous studies demonstrated that metazoan-microbial
157 associations are more common in caves and in other confined environments than previously thought
158 and that the autochthonous micrite is the evidence for abundant microbial activity in marine caves.
159 The confined conditions of these environments allows growth of numerous microorganisms,
160 including heterotrophic and phototrophic bacteria, which were described also inside various
161 terrestrial caves. Microbial mats and biofilms, dominated by bacteria or cyanobacteria, were
162 documented within oxic (Abdelahad 1989; Jones 1995) and anoxic limestone or dolostone caves
163 (Sarbu et al. 1996; Mattison et al. 1998). Recent studies of organic matter preserved in biostalactites
164 showed the presence of lipid biomarkers and their isotopic composition, indicating the bacterial
165 community was dominated by sulfate-reducing bacteria, as suggested by mono-*O*-alkyl glycerol
166 ethers, branched fatty acids (10-Me-C_{16:0}, *iso*- and *anteiso*-C_{15:0} and -C_{17:0}) and possibly
167 bishomohopanol (Guido et al. 2013; Gischler et al. 2017).

168 The small corallal patch reefs of Alpe di Specie, which are made up mainly of sponges,
169 scleractinian corals, and calcareous algae, developed in a relatively muddy, low-energy
170 environment. The framework of these bioconstructions has little early cement so that the cavities
171 represent 35% of the overall patch reef texture (Russo et al. 1991; Guido et al. 2018). Biomarker
172 analyses revealed the presence of mainly even-numbered, straight-chain, saturated carboxylic acids
173 preserved in microbialites (Tosti et al. 2014). Among these, *iso*- and *anteiso*- C_{15:0} and C_{17:0}-
174 branched fatty acids are indicative of sulfate-reducing bacteria (Heindel et al. 2010, 2012; Guido et
175 al. 2013). Sulfate-reducing bacteria are consistent with oxygen-depleted conditions, which
176 developed within microcavities of the corallal framework; indeed, these microorganisms typically
177 occur in oxygen-poor environments, such as restricted cavities in which organic matter accumulates
178 and decays. A positive Ce anomaly in rare earth element (REE) patterns, observed in the
179 autochthonous micrite, is consistent with oxygen-poor conditions of the cryptic niches of patch
180 reefs (Tosti et al. 2014).

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METHODS

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183 Samples were cut into small blocks (5 x 3 x 1cm), which were subdivided into two parts: one was
184 utilized to prepare a polished slab and the other the corresponding thin section. In this way it was
185 possible to select on the polished slab the areas with high epifluorescence and verify in the
186 counterpart thin section the micrite type (detrital/autochthonous) to be analyzed with Raman
187 Spectroscopy.

188 Uncovered thin sections (48mm × 28mm) and polished slabs were studied with an optical
189 microscope (Zeiss Axioplan 2 Imaging), up to 40 x magnification. The samples were examined for
190 fluorescence to reveal the distribution of the organic matter (Neuweiler and Reitner, 1995; Russo et
191 al., 1997). Residual organic matter as well as Mn²⁺ appear to be the most abundant and important
192 activators of fluorescence in calcite and dolomite. Organically activated luminescence is interpreted
193 to be caused mainly by aromatic and certain conjugated organic molecules. Fluorescence was
194 induced by a Hg vapour lamp linked to an Axioplan II imaging microscope (Zeiss) equipped with
195 high performance wide band pass filters (BP 436/10 nm/LP 470 nm for green light; BP 450–490
196 nm/LP 520 nm for yellow light).

197 Micro-Raman analyses were performed using a Thermo Fisher DXR Raman microscope
198 (Waltham, MA, USA), equipped with OMNICxi Raman Imaging software 1.0, an objective of 50x,
199 a grating of 900 ln/mm (full width at half maximum, FWHM), and an electron multiplying charge-
200 coupled device (EMCCD). The 532 nm line (solid state laser) was used at an incident power output
201 ranging from 1.8 to 7 mW. The spatial resolution of the laser beam was about 3–5 μm. The
202 acquisition time of the spectra varied from 5 to 40 s. Data were collected in the 50–3360 cm⁻¹ range
203 to capture the first-order and second-order Raman bands. The measurements were collected on
204 randomly oriented grains, with a fixed orientation of the polarized laser beam.

205 Even though the occurrence of high intensity fluorescence complicates the identification of
206 individual Raman-active vibrational modes, making the corresponding Raman bands more difficult
207 to detect, we were able to identify the main organic matter peaks within the microbialite mineral

208 phases. Published data obtained from similar samples using coupled gas chromatography-mass
209 spectrometry (GC-MS) were used for reference and comparing the analytical capabilities of both
210 techniques (Guido et al. 2013; Tosti et al. 2014).

211

212 Raman spectroscopy in Earth Sciences studies

213 Some background of the use of Raman spectroscopy is given here to place this work into the
214 context of this technique. Crystalline materials produce diagnostic Raman bands at characteristic
215 wavelengths with specific relative intensities allowing the identification of the mineral matrix in
216 samples (Greco et al. 2018). Raman spectroscopy can be used on both bulk polished specimens and
217 standard petrographic thin sections. This method can be used to identify mineral grains and crystals
218 independently of their orientation in the sample (Danise et al. 2012). It provides a fast, non-
219 destructive and efficient way of identifying materials. Large databases of Raman spectra aid the
220 identification of minerals (Giarola et al. 2018; Bloise et al. 2018; Miriello et al. 2018). Raman
221 spectroscopy can be used to evaluate various phases of the same or very similar chemical
222 composition. In addition, confocal Raman micro-spectroscopy is valuable also to characterize solid,
223 liquid, or gaseous inclusions within samples (Frezzotti et al. 2012); this technique has been utilized
224 to characterize amorphous materials and non-crystalline molecules allowing discrimination between
225 different types of carbonaceous material and other non-crystalline organic or inorganic compounds
226 within rocks (Greco et al. 2018). In particular, Raman spectroscopy has proved useful for analysis
227 of: 1) carbonaceous microfossils and their organic remains (Schopf and Kudryavtsev 2009;
228 Cavalazzi et al. 2011; Calça et al. 2016); 2) stromatolites and microbial mats (Allwood et al. 2006;
229 Ferretti et al. 2012); 3) acritarchs and fossil algae (Arouri et al. 2000; Javaux and Marshall 2006); 4)
230 fossil plants and pollen (Witke et al. 2004); and 5) vertebrates (Thomas et al. 2011). Greco et al.
231 (2018) recognized and characterized ancient microbial remains within Archean meta-sedimentary
232 rocks utilizing Raman data. Recently Guido et al. (2018), utilizing this technique, detected the role
233 of microbialites in dolomitization processes in Carnian carbonates from Dolomites.

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MICROBIALITE CHARACTERIZATION WITH RAMAN SPECTROSCOPY

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Microbialites examined in this study, inside the biostalactites of submerged marine caves, occur

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mainly as clotted peloidal and aphanitic (structureless) textures (Fig. 3). Autochthonous micrite fills

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the microcavities inside the skeletal framework (Fig. 3A) and commonly occludes the serpulid tubes

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and spaces between adjacent individual serpulids (Fig. 3B, 3C), contributing to cementation of

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bioclasts. This micrite type is highly fluorescent in UV observation, suggesting a high organic

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matter content trapped within the fine calcite crystals. Similar texture and epifluorescence behavior

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characterize the microbialites formed in the skeletal framework of coralgal patch reefs of Alpe di

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Specie, made up mainly of sponges, scleractinian corals, and calcareous algae (Fig. 4A-C).

244

In previous studies the organic matter recorded in recent biostalactites and Triassic patch

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reefs was characterized through biomarker analyses after extraction of organic compounds from the

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mineral matrix (Guido et al., 2013; Tosti et al., 2014). **This procedure does not allow determination**

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of the precise biomarker locations, leading to speculative interpretation of microbial activity as an

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explanation of autochthonous micrite formation. In this study, in contrast, using micro-Raman

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spectroscopy, we identified the exact location of organic compounds in the microbialites and

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provide evidence of the possible provenance of sulfate-reducing bacteria biomarkers from this

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fraction (Figs. 5, 6). Unlike XRD or GC-MS analyses, which require the extraction of carbonaceous

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material, Raman micro spectroscopy allows *in situ* measurements on polished slabs and thin

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sections. The micro-Raman system is capable of analyzing the organic and inorganic features of the

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samples with a resolution at the micrometer scale.

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The band positions of all the Raman spectra obtained on the cave biostalactites and Triassic

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patch reefs match the values of calcite reference bands (Figs. 5C, 5D, 6C, 6D). The detected peaks

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are located in the range between 50 cm^{-1} and 3360 cm^{-1} . The main calcite peaks correspond to the

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symmetric stretching (ν^1) of the CO_3 group at 1092 cm^{-1} , asymmetric stretching (ν^3) at 1437 cm^{-1}

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and symmetric deformation (ν^4) at 715 cm^{-1} . The lower wavenumbers of calcite (285 cm^{-1}) arise

260 from the external vibration of the CO₃ group that involve translatory oscillations of the group. The
261 four prominent absorption bands were recorded in the analyzed samples around 150, 275, 710, and
262 1085 cm⁻¹ (Figs. 5, 6). Minor shifts in the positions of the calcite bands between the analyzed
263 samples and the spectra published in literature may be due to the effects of natural impurities
264 present in the sample (Buzgar and Apopei 2009; Miriello et al. 2018). The studied samples show
265 distinctive peaks around 1585 cm⁻¹ and 1320 cm⁻¹ related to the presence of G and D bands of the
266 amorphous carbon (AM) (Figs. 5C, 6C). These bands were recorded in the fluorescent
267 autochthonous micrite (Figs. 5B, 6B) of biostalactites and patch reefs, regardless of the texture type
268 (peloidal, laminated or aphanitic). The organic G and D bands were not recorded in the spectra of
269 non-fluorescent allochthonous fractions (Figs. 5B, 6B). In summary, the presence of G and D bands
270 of amorphous carbon in the microbial autochthonous micrite, where high epifluorescence was
271 observed, confirmed the presence of organic matter relics in this component, **suggesting that the**
272 **biomarkers, detected with GC-MS analyses, originate from the same areas.** In our opinion Raman
273 Spectroscopy is a sound tool to detect, *in situ*, the presence and types of organic molecules. The
274 combination of epifluorescence and Raman data prove the localization of the biomarkers, which
275 Guido et al. (2018) studied with destructive procedure, in the microbialite fabric.

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DISCUSSION

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Comparison between the studied Recent and Triassic small bioconstructions

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Despite biological evolution that led to profound changes of reefal communities in time and space, a

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universal feature of many bioconstructions is the presence of an intricate skeletal framework rich in

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oxygen-depleted cryptic cavities favorable to the development of microbial communities. Modern

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biostalactites developed in submarine caves, in contrast to **the** Triassic patch-reefs, which developed

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in open shallow water. This difference allows comparison of the micromorphology and

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biogeochemical nature of microbialites in **markedly** different sedimentary contexts. The Recent

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biostalactites are composed mainly of serpulids and bryozoans whereas **the** patch reefs comprise

286 calcified demosponges, scleractinian corals and calcareous algae (Fürsich and Wendt 1977; Wendt
287 1982; Russo et al. 1991; Flügel 2002). Despite their different sizes, morphologies and depositional
288 environments, both bioconstruction types have a skeletal framework and small primary cryptic
289 cavities that host microbialites consisting of autochthonous micrite showing microbial textures:
290 peloidal to clotted peloidal and aphanitic (Figs. 3, 4). Microbialite filling the intra- and inter-skeletal
291 cavities contributes to the syndepositional cementation and stabilization of the bioconstructions.
292 The autochthonous micrite, formed in confined environments with anoxic conditions, has been
293 commonly associated with anaerobic bacteria. Organic matter enrichment helps to feed sulfate-
294 reducing bacteria in these cavities that, in turn, induce autochthonous micrite deposition in cryptic
295 environments (Monty 1976; Zankl 1993; Gast et al., 1998; Reitner et al., 1995; Riding, 2011b).

296 Guido et al. (2013, 2016) and Sanfilippo et al. (2015), through the analysis of bacterial lipid
297 biomarkers, recognized microbial activity inducing biomineralization processes in the biostalactites
298 of the modern submarine caves of Plemmirio area. As mentioned earlier, bacterial involvement in
299 the autochthonous micrites deposition was also recently documented in the biostalactites developed
300 within a submerged karst cave of the Belize Barrier Reef (Gischler et al. 2017a) and within the Blue
301 Hole off Belize (Gischler et al. 2017b). In these carbonates the autochthonous micrite have similar
302 contents and isotopic composition, presence of nonisoprenoidal *sn*-1-mono-*O*-alkyl glycerol
303 monoethers (MAGEs) and terminally-branched fatty acids (10-Me-C_{16:0}; *iso*- and *anteiso*-C_{15:0} and -
304 C_{17:0}). These organic markers, with high specificity for sulfate-reducing bacteria, are indicative of a
305 single microbial process mediating microbialite precipitation in the marine caves. Similar microbial
306 processes were inferred for the deposition of autochthonous micrite inside the small cavities of the
307 Triassic patch reefs. These coralgall patch reefs contain biomarkers for sulfate-reducing bacteria,
308 lack specific molecules typical of cyanobacteria, and have REE patterns indicative of oxygen-
309 depleted conditions (Tosti et al. 2011, 2014). These signatures are indicative of low energy
310 depositional conditions, and low-oxygen micro-habitats inside the skeletal framework. Sulfate-
311 reducing bacteria biomarkers are consistent with oxygen-depleted conditions (sulfate reduction

312 typically occurs in oxygen-poor environments), and the organic matter decaying into cryptic
313 cavities, which **generally favors** the deposition of clotted peloidal micrite (Monty 1976; Chafetz
314 1986; Buczynski and Chafetz 1991; Reitner 1993; Kazmierczak et al. 1996; Folk and Chafetz 2000;
315 Riding 2002; Riding and Tomas 2006).

316

317 Utility of Raman Spectroscopy in the microbialite characterization

318 **As described in this paper, the similarity of biogeochemical signatures within the two types of**
319 **microbialites studied was previously revealed with destructive methodologies and the data referring**
320 **to bulk sediments. Here, to prove the origin of sulfate-reducing bacteria biomarkers from the**
321 **microbialites, these fractions have been further checked for specific organic matter bands with**
322 **micro-Raman spectroscopy.** The two wavelength intervals at 1100–1800 cm^{-1} and 2500–3100 cm^{-1}
323 have been referred to as the first- and the second-order regions of Raman spectra of organic matter
324 (carbonaceous material) (Beysac et al. 2002; Pasteris and Wopenka 2003; Wopenka and Pasteris
325 1993; Yui et al. 1996; Hu et al. 2015). In the first-order region, well-organized graphite has a
326 detectable vibration mode at 1580 cm^{-1} , which is an in-plane mode (Pasteris and Wopenka 1991;
327 Wopenka and Pasteris 1993). In disordered or poorly-organized CM, it splits into two peaks: the G
328 band at 1600 cm^{-1} , and the D band at 1355 cm^{-1} (Hu et al. 2015). The G and D bands, attributed to
329 organic matter, were recorded only in the microbialite components of both bioconstructions (Figs.
330 5C, 6C) whereas they are absent in the allochthonous micrite (Figs. 5D, 6D). The presence of both
331 G and D in the fresh samples from submarine caves is in agreement with the immature nature of the
332 organic compounds of these bioconstructions. The same bands in the Triassic samples confirm the
333 extraordinary preservation of the Alpe di Specie patch reefs that were protected from alteration and
334 regional dolomitization by the surrounding shaly sediments (Scherer 1977; Wendt 1977, 1982;
335 Russo et. al. 1991).

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CONCLUSIONS

- 338 1. **Conditions unfavorable for metazoans are commonly ideal for rich** development of
339 microbial communities. Bioconstructions in this study are made up of a complex skeletal
340 framework with small primary cavities where decaying organic matter create oxygen-
341 minimum zones. Bacteria grow in these cryptic niches inducing biomineralization processes.
342 Mediterranean submarine caves hold one of the best examples for studying microbialites in
343 the cryptic environments, and comparison with those of Triassic patch reefs of the
344 Dolomites show that similar metabolic pathways led to autochthonous micrite deposition in
345 stressed niches **despite the different geological time and general sedimentary contexts.**
- 346 2. Micro-Raman spectroscopy allows *in situ* recognition of similar organic G and D bands on
347 the autochthonous micrite of both bioconstructions. The wavelength pattern suggests
348 disordered carbonaceous material preserved in the microbialites. Raman data used in
349 conjunction with biomarker analyses strengthen the hypothesis of an analogous microbial
350 metabolic mediation for the microbialite deposited in the modern submarine caves and
351 Triassic patch reefs. Comparisons with published extract-based studies (GC-MS) confirm
352 the role of sulfate-reducing bacteria in the deposition of microbialite in both the
353 bioconstructions.
- 354 3. This study indicates that microbially-mediated precipitation in cryptic environments is a
355 biological process that may be independent of geological age. **Further work is needed to**
356 **demonstrate whether this relationship may be confirmed by other cases through the fossil**
357 **record.**

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682

FIGURE CAPTIONS

683

684

685 Fig. 1. - A) Map of Mazzere cave, Sicily, from which biostalactites were collected (modified from
686 Rosso et al., 2012). B) Biostalactite from Mazzere cave. C-D) Cut slabs of biostalactites showing
687 the skeletal framework made of serpulids (Se) encrusted by microbialites (white arrows); rare corals
688 (Co) and detrital micrite (black arrow) are also observable. Dashed square in fig. 1c represents the
689 area illustrated in UV fluorescence and Raman spectroscopy in Fig. 5. Scale bars: 1 cm.

690

691 FIG. 2. - A-B) Blocks of patch reefs from Alpe di Specie, dominated by sponges *Sestrostomella*
692 *robusta*, *Stellinspongia*, *Colospongia*, *Amblisyphonella*, and colonial corals. C-D) Polished slabs of
693 Alpe di Specie blocks showing the skeletal framework made of scleractian corals (*Margarosmia*
694 *zieteni*) (Co) encrusted by solenoporacean algae (Al) and microbialites (white arrows); detrital
695 micrite is also observable (black arrow). Dashed square in Fig. 2d represents the area analyzed by
696 UV fluorescence and Raman spectroscopy in Fig. 6. Scale bars: 1 cm.

697

698 FIG. 3. - A-C) Skeletal/microbialite boundstone made of serpulids (Se), bryozoans (Br), aphanitic
699 (white arrow) and clotted peloidal micrite (black arrows); d) detrital micrite with foraminifers and
700 other small bioclasts.

701

702 FIG. 4. - A-C) Skeletal/microbialite boundstone. A) Chaetetid (Ch), calcareous sponge (Cs) and
703 clotted to aphanitic micrite (white arrow); B) Coral and autochthonous aphanitic micrite; C)
704 *Dendronella articulata* with peloidal micrite. D) Calcareous sponge and detrital micrite.

705

706 FIG. 5. - Polished slab of autochthonous and detrital micrite of a modern biostalactite from Sicily
707 (dashed square in figure 1C). A) plane-polarized light; B) ultraviolet light. The bright
708 epifluorescence of autochthonous micrite (aut. micr.) corresponds to high organic matter content, on

709 the contrary the allochthonous (detrital) micrite (all. micr.) appears dark under ultraviolet light. C)
710 Raman spectrum obtained on the autochthonous micrite (black star on image B) showing the G and
711 D bands of the organic matter and the typical calcite picks (Cal 1085, 715, 282, 148 cm^{-1}). D)
712 Raman spectrum obtained on the allochthonous micrite (white star on image b) showing only the
713 peaks of calcite minerals (Cal 1088, 718, 289, 145 cm^{-1}).

714

715 FIG. 6. - Polished slab of autochthonous micrite with stromatolitic texture from a Triassic patch reef
716 from northern Italy (dashed square in figure 2D). A) normal light; B) ultraviolet light. The bright
717 epifluorescence of autochthonous micrite (aut. micr.) corresponds to high organic matter content, on
718 the contrary the allochthonous (detrital) micrite looks dark under ultraviolet light. C) Raman
719 spectrum obtained from the autochthonous micrite (black star on image B), showing the G and D
720 bands of the organic matter and the typical calcite peaks. D) Raman spectrum obtained on the
721 allochthonous micrite (white star on image B) showing only the peaks of calcite minerals (Cal 1085,
722 721, 279, 143 cm^{-1}).











