

1 **The influence of light attenuation on the biogeomorphology of a marine karst cave:**
2 **a case study of Puerto Princesa Underground River, Palawan, the Philippines.**

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11
12 **Abstract**

13 Karst caves are unique biogeomorphological systems. Cave walls offer habitat for
14 microorganisms which in-turn have a geomorphological role via their involvement in rock
15 weathering, erosion and mineralisation. The attenuation of light with distance into caves is
16 known to affect ecology, but the implications of this for biogeomorphological processes
17 and forms have seldom been examined. Here we describe a semi-quantitative microscopy
18 study comparing the extent, structure, and thickness of biocover and depth of endolithic
19 penetration for the Puerto Princesa Underground River system in Palawan, the
20 Philippines, which is a natural UNESCO World Heritage Site.

21 Organic growth at the entrance of the cave was abundant (100% occurrence) and
22 complex, dominated by phototrophic organisms (green microalgae, diatoms,
23 cyanobacteria, mosses, and lichens). Thickness of this layer was 0.28 ± 0.18 mm with
24 active endolith penetration into the limestone (mean depth = 0.13 ± 0.03 mm). In contrast,
25 phototrophs were rare 50 m into the cave and biofilm cover was significantly thinner (0.01
26 ± 0.01 mm, $p < 0.000$) and spatially patchy (33% occurrence). Endolithic penetration here

27 was also shallower (< 0.01 mm, $p < 0.000$) and non-uniform. Biofilm was found 250 m into
28 the cave, but with a complete absence of phototrophs and no evidence of endolithic
29 bioerosion.

30 We attribute these findings to light-induced stress gradients, showing that the influence of
31 light on phototroph abundance has knock-on consequences for the development of
32 limestone morphological features. In marine caves this includes notches, which were most
33 well-developed at the sheltered cave entrance of our study site, and for which variability in
34 formation rates between locations are currently poorly understood.

35

36 **Key-words:** biogeomorphology; marine cave; microbiology; bioerosion; karst; Palawan.

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38

39 **1. Introduction**

40 Caves provide a unique habitat for rock-dwelling microorganisms that in turn are thought to
41 have a geomorphological role via their involvement in weathering, erosion and
42 mineralisation (Barton, 2006; Barton and Jurado, 2007; Riquelme and Northup, 2013).

43 Cave characteristics such as dimension, morphology, location, orientation and lithology
44 have an important influence on the structure of the biological communities found in these
45 environments (Lamprinou et al., 2012). This appears to be especially important for
46 limestone caves, where biogeomorphological interactions between rock and biota are
47 strongest (e.g., Cañveras et al., 2001; Jones, 2010; Pasic et al., 2010).

48 The ecology of cave walls (including marine caves) is characterised by a decrease in
49 biomass towards the interior, resulting from the presence of marked environmental
50 gradients, including light, oxygen, temperature and nutrient availability. Light attenuation is
51 particularly critical for phototrophs (Gili et al., 1986). In terrestrial caves in Spain, for
52 example, Roldán and Hernandez-Mariné (2009) found that biofilms comprised of

53 cyanobacteria, green microalgae, diatoms, mosses and lichens on the walls and floors
54 were thinner farther from the entrance, and had lower species diversity. Similarly, Cuezva
55 et al. (2009) found that microbial colonies on rock surfaces are concentrated at the
56 entrance of Altamira Cave, Spain, and that these microorganisms are involved in
57 biomineralisation and CaCO₃ deposition. While the influence of light attenuation on cave
58 microbial communities is clear, there has been very little attempt to relate these patterns to
59 biogeomorphological processes. This is an important research gap because the relative
60 contribution of biological processes (i.e., bioweathering, bioerosion, and bioprotection) to
61 the formation of distinct morphological features associated with cave systems is relatively
62 poorly understood. For marine caves this includes notches, which are very common and
63 well developed on Mediterranean and tropical limestone coasts (e.g., Trudgill, 1976; De
64 Waele et al., 2009; Furlani et al., 2011; Moses, 2012; Pirazzoli and Evelpidou, 2013).
65 The mechanisms involved in notch formation have been debated (Furlani et al., 2011), but
66 probably involve a combination of chemical, biochemical, biomechanical and physical
67 processes (i.e., waves) (see De Waele and Furlani, 2013, and references therein). Some
68 researchers suggest that bioerosion is the key parameter controlling the rate of marine
69 notch formation (e.g., Evelpidou et al., 2012; Pirazzoli and Evelpidou, 2013; Boulton and
70 Stewart, in press), yet direct quantitative evidence of bioerosion is scarce (but see Furlani
71 and Cucchi, 2013).

72 Here we describe a study aimed at addressing this knowledge gap using the Puerto
73 Princesa Underground River cave system as a case study. The study had three main
74 aims: (1) to examine the presence and characteristics of ecology (focusing on
75 microorganisms) on cave walls in relation to distance from the entrance and therefore
76 availability of light; (2) to determine the biogeomorphological significance of these
77 organisms in a context of bioweathering and bioerosion, and thereby; (3) contribute to

78 understanding of biogeomorphological processes in the development of marine cave
79 morphological features, including notches.

80

81 **2. Study Site**

82 Palawan is located between 11°50' and 12°20' latitude north, and 117°00' and 120°20'
83 longitude east, in the south-western part of the Philippines archipelago. Climate is
84 characterised by a dry season (November to May) and a wet season (June to October),
85 with stable temperatures throughout the year ranging from about 26°C to 28°C (Piccini and
86 landelli, 2011). The island is narrow and elongated, mostly mountainous, and divided into
87 three geologic sectors by two north–south oriented tectonic depressions. The Saint Paul
88 Dome karst ridge divides the northern from the central sector, located east of Ulugan Bay
89 about 50 km northeast of Puerto Princesa (Figure 1). The ridge covers an area of about 35
90 km² (10 km long and roughly 4 km wide) and is formed of massive to roughly bedded (400
91 m thick) light to dark grey Oligocene-Miocene limestone rich with fossils (Hashimoto,
92 1973). The site is a National Park and an UNESCO natural World Heritage Site.

93 Structurally the area consists of a multiple northwest dipping homoclinal relief, limited by
94 northeast–southwest oriented faults that control the general morphology of the karst
95 landscape, including the orientation of dolines and the development of major caves.

96 One such cave system is the Puerto Princesa Underground River (PPUR) complex, a 32
97 km long cave that consists of an active branch developing at the present sea-level, and
98 two relict levels of huge tunnels and large breakdown chambers at approximately 5–10
99 and 60–80 m above present sea-level. The cave has had a long and multiphase evolution
100 coupled with uplift phases and sea level fluctuations. Several morphological features
101 suggest that the lowest and presently active level of the cave is inherited (e.g., Blanco
102 Chao et al., 2003), probably formed during the Middle-Late Pleistocene (Piccini and

103 landelli, 2011). These features include notches at +12.4 m above mean sea level, and
104 large and corroded speleothem masses that are interbedded with alluvial deposits.
105 Features that indicate former water levels are present up to 5–6 km upstream from the
106 coastal spring, where a notch is evident at +7–8 m that is correlated to a notch on the
107 coastal cliff about +7 m above present sea level (Maeda et al., 2004; Omura et al., 2004).
108 These notches date back to the MIS 5e interglacial phase (about 125,000 years BP)
109 (Linsley, 1996).

110 At the present sea-level a combination of physical erosion, bioweathering, bioerosion and
111 chemical dissolution are thought to have contributed to the development of a deeply-
112 carved notch (up to 2 to 3 m) along the coastline (Figure 2a). The notch is present in areas
113 both exposed to the direct action of waves and more sheltered alcoves, which in itself
114 indicates that processes other than wave action are important for formation. In the
115 entrance zone of the cave, where wave energy is greatly reduced, the notch is about 0.8–
116 1.2 m deep (Figure 2b). In the inner part of the cave the present-day notches are rarely
117 deeper than 0.4–0.6 m, except where freshwater tributaries occur.

118

119 **3. Materials and Methods**

120 Samples of limestone rock from the PPUR were collected for examination to compare
121 biological and biogeomorphological features. Drill core samples (1 cm diameter x 2 cm
122 deep) and chippings of similar dimensions were obtained by boat from the cave walls at
123 three distances from the entrance (SP0 = cave entrance with ‘full light’, SP1 = 50 m from
124 the entrance with ‘semi-light’, SP2 = 250 m from the entrance in ‘darkness’) and at three
125 different heights from the mean water level (suffix 1 = 20–30 cm above mean waterline, 2
126 = 40–50 cm above mean waterline, 3 = 70–80 cm above the mean waterline) (Table 1,
127 Figure 2c–e). All samples were therefore taken from within the tidal zone, where organic
128 activity in the form of biofilms and entrusting organisms was visually abundant in

129 comparison to areas above high water. Although grazing organisms (including limpets and
130 chitons) may contribute to limestone bioerosion, these were rare in this study location and
131 never abundant.

132

133 **3.1. Microscopic analyses**

134 Light microscopy and scanning electron microscopy (SEM) were used to analyse the
135 samples. A light microscope (Leica MZ 10 F) was first used to characterise and compare
136 the nature (abundance, structure, types and diversity) of biological cover on the top
137 surface of core samples taken from the three distances from the entrance (0 m, 50m and
138 250 m) and the three heights above the mean waterline (20–30 cm, 40–50 cm, 70–80 cm).
139 In total 43 cores were examined, 15 from both the entrance at 50 m depth, and 13 at 250
140 m depth. Each core was observed with artificial lighting from above (using an optic fiber) at
141 increasingly higher magnification and photographs were taken using DeltaPix Insight
142 computer software.

143 For comparison between samples, the organisms forming the organic layer (where
144 present) were broadly classified into the following morphological groups: green
145 microalgae, filamentous cyanobacteria, coccoid cyanobacteria, diatoms, mosses, lichens,
146 actinobacteria and invertebrates. The entire surface of each core was examined and the
147 presence/absence of each group was recorded in each case. Presence/absence of
148 morphological groups was compared as a percent occurrence (e.g., Taylor and Viles,
149 2000) for each set of cores from each distance/height combination (Table 1).

150 The nature (extent, structure and thickness/depth) of interactions between surface
151 microorganisms and the limestone substratum was further examined using SEM. As the
152 light microscopy revealed consistent patterns in microbial cover for all tide levels (see
153 Section 4 and Section 5) SEM observations were made for samples from one height (40–
154 50 cm above mean water) at each of the distances into the cave (i.e., SP0/2, SP1/2 and

155 SP2/2). For these observations core samples were fractured using a hammer and chisel.
156 This was done from the side, with cores placed on a piece of foam so as to minimize
157 disruption of the surface of interest. Prior to SEM observation the air-dry samples were
158 mounted on aluminium stubs using carbon cement, with the upper colonized surface to the
159 perpendicular (i.e., a cross-section view of the rock–biota interface) (e.g., Viles, 1987;
160 Viles et al., 2000). Samples were then gold sputter coated and observed using a
161 Cambridge Stereoscan 90 SEM.
162 To characterise and measure the rock–biota interface, the top edges of three randomly
163 selected and fractured cores from each distance into the cave were observed.
164 Observations were first made at low magnification (x50) and then at progressively higher
165 magnifications until distinction could be made between any modified zones (typically
166 x200–500). For each core sample five micrographs were taken with roughly equal spacing
167 along their top edge (i.e., 3 full turns of the SEM navigation wheel at x50, equivalent to
168 around 1 mm spacing). Micrographs were then scaled using ImageJ computer software to
169 enable semi-quantitative measurements of: (a) surface organic cover thickness, and (b)
170 depth of organism (endolithic) penetration, where present, measured parallel to the rock
171 surface (e.g., Coombes et al., 2011).

172

173 **4. Results**

174 ***4.1. Biological characterisation: light microscopy***

175 Using the classification of microorganisms adopted here, we found little variation in
176 occurrence in relation to height above the waterline. Therefore, data from the three heights
177 were collapsed into one group per distance from the cave entrance. For these three
178 groups (0 m, 50 m and 250 m), microbiological communities varied significantly as
179 indicated by a visible colour change of the rock surface (e.g., Figure 2c–e); cores from the
180 cave entrance were visibly greener, those 50 m from the entrance were green-grey, and

181 those 250 m from the entrance were dark brown. Microscope observations showed that
182 these colour differences are attributed to marked variations in the presence/absence of
183 phototrophs (Figure 3).

184 As expected, biofilm at the cave entrance was dominated by a range of phototrophic
185 organisms (filamentous and coccoid cyanobacteria, green microalgae, diatoms, mosses,
186 and lichens) forming a photosynthetic layer (Figure 3). The structure of this organic layer
187 was complex and stratified (Figure 4a–c). Biofilm was also observed on samples from
188 farther within the cave, but this was simple in structure and spatially-patchy in comparison
189 to those at the cave entrance (Figure 4d–f). Importantly, phototrophs were rare at 50 m
190 and completely absent 250 m depths (Figure 3). Supplementary qualitative SEM
191 observations of the top surfaces of cores confirmed that there were markedly more
192 biological cells (especially filamentous algae and cyanobacteria) on samples taken from
193 the cave entrance (e.g., Figure 5a). Microbial cover was notably less clear for 50 m and
194 250 m samples, although there were some crusting forms of possible biochemical origin
195 (e.g., Figure 5b–c) similar to those observed to develop on limestone exposed to intertidal
196 conditions in the UK (Coombes et al., 2011).

197 In addition to microorganisms, some invertebrate species were found including those from
198 the phyla Mollusca and Arthropoda (Figure 3, Figure 6). In contrast to phototrophic
199 microorganisms, which showed little variation in relation to height, invertebrates were only
200 found on samples originating closest to the waterline (20–30 cm), irrespective of distance
201 from the entrance.

202

203 **4.2. Rock-biota interactions: SEM**

204 Figure 7 shows characteristic cross-section views for fractured rock cores taken at the
205 three distances from the cave entrance. Figure 8 shows measurements of biocover
206 thickness on top of the rock surface, and depth of active penetration of microorganisms

207 into the substrate. At the cave entrance, a complex and complete biocover exists (100%
208 occurrence) of biological filaments and single-celled algae and diatoms (Figure 7a),
209 corresponding well to top-surface observations. There was evidence that some of the
210 filaments within this layer were heavily mineralised. The thickness of the epilithic layer
211 averaged 0.28 ± 0.18 mm (Figure 8), although this was spatially variable as indicated by a
212 relatively high standard deviation. It was noted that biofilm thickness was typically greatest
213 in association with surface irregularities/depressions on the rock. Observation at higher
214 magnifications (x300+) showed that microorganisms are actively penetrating the limestone
215 at the cave entrance, where *in situ* organic filaments were visible in a relatively uniform
216 zone of microbial boring (Figure 7a). This bioeroded zone had an average depth of $0.13 \pm$
217 0.03 mm (Figure 8).

218 In comparison, the thickness of biocover 50 m into the cave was significantly thinner than
219 at the entrance (0.01 ± 0.01 mm, Student's $t(14) = 5.71$, $p < 0.000$, Figure 8) and spatially
220 patchy (present in only 33% of observations at this location). There was some evidence of
221 bioerosion of these samples but this was less distinct and markedly less uniform (e.g.,
222 Figure 7b). The depth of this altered zone was significantly shallower than at the entrance
223 (a mean depth of 0.01 ± 0.01 mm, Student's $t(16) = 13.46$, $p < 0.000$, Figure 8), and may
224 be attributed to chemical weathering (evidenced by disaggregation and increased pore
225 space, Figure 7b) alongside any biogeomorphological alteration. No surface biocover of
226 measureable thickness was observed for samples taken from 250 m into the cave (Figure
227 8). Equally, there was no evidence of endolithic organisms in the 250 m samples, although
228 there was some morphological evidence of alteration of the very top surface, possibly via
229 chemical means (Figure 7c).

230

231 **5. Discussion**

232 **5.1. Height above the waterline**

233 Height above the waterline did not have a significant influence on the composition and
234 structure of phototrophic biofilms; at this scale of observation, few differences were found
235 between respective groups of samples from the three heights. The tidal range in this cave
236 is 50–120 cm, suggesting that the influence of rising and falling tides may preclude any
237 observable difference in microbiological communities associated with tidal height for our
238 samples. This can be explained by the fact that microorganisms forming intertidal biofilms
239 are relatively tolerant to desiccation given that the matrix of extracellular polymeric
240 substances in which they are embedded can retain moisture (Decho, 2000). Furthermore,
241 the high moisture content of the air near the entrance and farther into the cave (almost
242 100%) limits evaporation and maintains the walls wet during the low tide. Further
243 investigations to compare microbial communities occurring above and below the tide line
244 would help clarify whether the kinds of biogeomorphological processes observed are
245 contingent on tides, especially at the entrance where water and desiccation stress (rather
246 than light) are likely to limit growth. In contrast to microbial communities, height was found
247 to be an important for the presence of motile marine invertebrates, which were only found
248 on samples taken closest to the waterline (20–30 cm). This indicates that these organisms
249 are more sensitive to variations in moisture (and desiccation) resulting from tidal
250 fluctuations (e.g., Gosselin and Qian, 1997).

251

252 ***5.2. Distance from the cave entrance***

253 Overall, there was a significant decrease in taxa richness and biomass with distance into
254 the cave system. At the cave entrance, a complete cover of biofilm composed of several
255 phototrophic groups was present, with a well-stratified structure and a maximum thickness
256 of 0.49 mm. Barton (2006) suggests that the two most common bacterial forms in cave
257 communities are bacilli (in chains, as streptococci) and cocci (sometimes in pairs, as
258 diplococci), which agrees well with our observations. Some encrusting and epilithic

259 organisms were also present 50 m into the cave, but the biofilm here was significantly
260 thinner and there were few phototrophic forms. Deeper into the cave (250 m from the
261 entrance) biofilm cover was patchy and poorly stratified, with no phototrophic organisms.
262 These observations conform to previous studies that have observed a reduction in the
263 presence of microalgae and cyanobacteria species in association with light attenuation in
264 cave systems (Roldàn et al., 2004; Roldàn and Hernandez-Mariné, 2009). Notably,
265 phototrophic microflora are restricted to the vicinity of the cave entrance (e.g., Pantazidou,
266 1996; Albertano and Urzi, 1999; Mulec, 2008). Light intensity also appears to influence the
267 ratio of different organism groups (e.g., algae to bacteria, Figure 3), probably in
268 combination with variations in other environmental factors such as the availability of
269 nutrients and organic material, and the efficiency of gaseous exchange (Ohki and Gantt,
270 1983). The availability of light is nevertheless the critical limiting factor for autotroph
271 occurrence and abundance, even where other environmental conditions may be
272 favourable.

273

274 ***5.3. The biogeomorphological significance of light attenuation in caves***

275 SEM cross-section observations showed greatest evidence of organic modification of the
276 limestone substratum (i.e., bioerosion) at the cave entrance, corresponding to the location
277 where lithic organisms were most abundant. Here, a bored zone averaging 0.13 mm deep
278 was observed below the upper phototrophic biofilm layer. In this bored zone, mineral rock
279 and organic structures were closely associated, with filaments visibly penetrating into the
280 rock alongside boreholes and tunnelling artefacts (e.g., Viles et al., 2000; Naylor and Viles,
281 2002; Coombes et al., 2011). There was also evidence of mineralisation of calcite in
282 association with organic structures, which has been noted as an important modifying
283 process in karst caves (e.g., Cuezva et al., 2009). The extent to which such processes
284 represent possible bioprotective mechanisms in marine caves requires much more

285 investigation. On the other hand, organic breakdown of the rock (i.e., microbial penetration
286 into the surface) was particularly apparent in association with topographic depressions,
287 where the surface biocover was also usually thickest. This indicates that bioerosion is at
288 least partly responsible for creating (or enhancing) the marked mm–cm scale physical
289 complexity of the rock surface at the cave entrance compared to smoother, fluted rock in
290 the interior part of the cave (Figure 2c–e). A positive feedback mechanism likely exists
291 here, whereby topographic depressions created by bioerosion offer favourable microsites
292 (i.e., wetter and cooler) for subsequent microbial growth, but such causal relationships are
293 difficult to corroborate.

294 Phototrophic organisms, particularly cyanobacteria, are known to be effective bioeroders
295 of limestone rock, and although the precise mechanisms involved are debated, this
296 probably occurs via chemical means including acidulation during photosynthesis (Garcia-
297 Pichel, 2006). Beyond the cave entrance, light limits the presence of these phototrophs,
298 and consequently the relative importance of bioerosion by endolithic microbes is markedly
299 reduced. This transition between bioerosive biofilms dominated by phototrophs and
300 epilithic, non-photosynthetic biofilms may be relatively abrupt; our sampling indicates that
301 a distance of 50 m is sufficient to give rise to a significant reduction in phototroph
302 abundance and associated bioerosion, although this will likely vary depending on cave
303 entrance morphology and aspect. Other studies have similarly found that microorganisms
304 in the transition zone between well-illuminated parts of caves (i.e., at the entrance) and
305 areas of darkness (the ‘twilight zone’) only occupy the epilithic niche (e.g., Jones, 1993)
306 and as such are not involved in rock boring.

307 Microbes deeper in caves may also contribute to rock breakdown via other means (such
308 as biochemical etching), but this could not be easily distinguished based on these
309 microscope observations. Our findings do clearly show, however, that the extent (and
310 probable rate) of rock breakdown and topographic development is greatest at the cave

311 entrance, where phototrophic organisms are able to dominate. In contrast, the relative
312 geomorphological significance of endolithic microorganisms is markedly reduced in the
313 cave interior. Here, chemical processes such as mixing corrosion are likely to be more
314 important than biogeomorphological process. For example, in their broad-scale survey of
315 submerged notches along the Adriatic coast, Furlani et al. (2014) conclude that freshwater
316 mixing (from groundwater and fluvial inputs) is a major factor in their development
317 alongside bioerosive processes. The relative importance of notch formative processes is
318 significant given that notches are commonly used as indicators of relative sea-level
319 change, particularly in carbonate rocks (e.g., Pirazzoli and Evelpidou, 2013; Boulton and
320 Stewart, in press) and in tectonically active areas where changes may be gradual and/or
321 abrupt (Evelpidou et al., 2012). Our results demonstrate that, where present, phototrophs
322 are probably significant in their contribution to notch formation in this area, especially in the
323 controlled setting of cave entrances where the direct influence of physical wave action is
324 markedly reduced.

325

326 **6. Conclusions**

327 This study demonstrates how the known influence of light attenuation on the microbial
328 ecology of cave systems has important, but largely unstudied, biogeomorphological
329 consequences. This occurs via the maintenance of a gradient of lithic niche occupation,
330 from biofilms dominated by bioerosive endolithic phototrophs at cave entrances (alongside
331 biomineralising epiliths) to non-photosynthetic epilithic biofilms in cave interiors. The
332 transition between these types of growths was in the order of 50 m for the Puerto Princesa
333 Underground River cave complex. Further work is now required to determine the
334 consistency of this spatial transition between different locations.

335 Based on these observations we conclude that bioerosion of these cave walls (in the tidal
336 zone) is spatially controlled by light availability; bioerosion was only active at the current

337 cave entrance where there is sufficient light for photosynthetic microorganisms to exist,
338 and as such it is here that microbes are most directly involved in the morphological
339 development of the limestone surface. Whilst bioerosion was found to be negligible
340 beyond 50 m from the cave entrance, other biologically-mediated processes may be
341 operating. These probably include biocorrosion and possibly bioprotection via
342 mineralisation, but this requires further investigation particularly in a marine context. More
343 broadly, our observations have demonstrated the utility of geomorphological process
344 studies for elucidating controls on the morphological development of marine dissolutional
345 cave systems. This includes the processes contributing to the formation of marine notches,
346 and how the relative contribution of biological, chemical and physical weathering is
347 complicated by spatial variations in the abundance and niche occupation of microbial
348 biofilms. Our observations further suggest that relationships between notch lateral depth
349 and distance from the entrance of caves probably exist as a function of relative phototroph
350 abundance. This is certainly appears the case for the Puerto Princesa Underground River
351 cave, where bioerosion artefacts are present to significantly greater depths in the rock at
352 the entrance. This research demonstrates that current process biogeomorphological
353 studies can aid understanding of marine notch formation rates and mechanisms, which is
354 critical for employing notches in sea-level reconstruction (e.g., Furlani and Cucchi, 2013;
355 Pirazzoli and Evelpidou, 2013).

356

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474 **Table 1.** Rock sampling in relation to distance into the Puerto Princesa Underground River
475 system and height above mean water level.

	20–30 cm above waterline	40–50 cm above waterline	70–80 cm above waterline
Cave entrance	SP0/1	SP0/2	SP0/3
50 m from entrance	SP1/1	SP1/2	SP1/3
250 m from entrance	SP2/1	SP2/2	SP2/3

476

477 **Figure Captions:**

478 **Figure 1.** Location map of the Saint Paul karst area. Location of the Puerto Princesa
479 Underground River system (PPSE) indicated.

480 **Figure 2.** Photographs showing: **(a)** well-developed notches of the Saint Paul karst area; **(b)**
481 notch development at the cave entrance; **(c)** core sampling at the cave entrance; **(d)** core
482 sampling 50 m into the cave, and; **(e)** core sampling 250 m into the cave (scale bars indicate
483 10 cm).

484 **Figure 3.** Occurrence (%) of phototrophic groups of microorganisms and invertebrates in
485 light microscope observations of samples taken at three distances from the cave entrance
486 (0 m $n = 15$, 50 m $n = 15$, 250 m, $n = 13$).

487 **Figure 4.** Varying structure of biofilm on the surface of samples from different locations in
488 the cave: **(a)** thick and stratified biofilm at the cave entrance; **(b and c)** complex biofilm
489 with visible filamentous algae at the cave entrance; **(d)** simple epilithic biofilm
490 characteristic of surfaces 50 m from the entrance, and; **(e and f)** 250 m from the cave
491 entrance (scale bars = 1 mm).

492 **Figure 5.** SEM micrographs of top surfaces of samples taken at: **(a)** 0 m, **(b)** 50 m, and **(c)**
493 250 m from the cave entrance (all samples are 40–50 cm above the mean waterline,
494 magnification and scale as shown).

495 **Figure 6.** Invertebrates present on samples sampled 20–30 cm from the mean waterline:
496 **(a)** Mollusca (bivalves) at the cave entrance; **(b)** Mollusca (gastropods) at the cave
497 entrance; **(c)** Mollusca (bivalves) 250 m from the cave entrance; **(d)** Arthropod crustacean
498 at the cave entrance (bars = 1 mm).

499 **Figure 7.** SEM observations of surfaces in cross-section for samples taken at: **(a)** 0 m
500 from the entrance ([i] thickness of biocover, [ii] zone of bioerosion, [iii] close-up view of *in*
501 *situ* filaments penetrating the rock); **(b)** 50 m from the entrance ([i] possible zone of
502 biochemical alteration); **(c)** 250 m from the cave entrance ([i] amorphous structures, of

503 possible chemical rather than organic origin) (all samples were from 40–50 cm above the
504 mean waterline, magnification and scale as shown).

505 **Figure 8.** Indicative thickness of biocover (mean + SD) and depth of active penetration into
506 the substratum (mean + SD) by microorganisms at different distances within the cave (all
507 samples from 40–50 cm above mean waterline, $n = 15$).

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