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Research note: Preliminary report on the extreme endolithic microbial consortium of 'Pailas Frías', 'Rincón de la Vieja' Volcano, Costa Rica

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SUMMARY

Flakes detached from rocks of a volcano rivulet at 'National Park Rincón de la Vieja Volcano', Guanacaste, Costa Rica, revealed a green layer 2–3 mm deep into the rock matrix. Analysis by scanning electron microscopy showed a complex assemblage of microorganisms, beginning with a thin layer of *Cyanidium* and followed by diatoms of the genus *Pinnularia* and curved and straight bacteria. To our knowledge, this is the first description of an endolithic microbial consortium dominated by diatoms, and also the first report of endolithobionts from volcanic rocks in a tropical area.

Key words: diatoms, endolithic, endolithobionts, extreme algae, extreme environment, *Pinnularia*, scanning electron microscopy.

Extreme environments are defined as seemingly uninhabitable, because their temperature, pressure, salinity, and/or pH are not permissible for life as we know it. Some are located in deep-sea hydrothermal vents and terrestrial hot springs, which are characterized by high temperatures and pH values near zero (Rothschild & Mancinelli 2001). Another example of an extreme environment are the dry valleys in the Antarctic, with winter temperatures of approximately –60°C and summer temperatures of –35 to +3°C (De la Torre *et al*. 2003). In those cold, inhospitable environments, rocks exposed to the sun attain temperatures higher than the surroundings, making them more hospitable for living organisms. The interior of such rocks are colonized by complex microbial communities called endolithobionts, which include eukaryotes, such as algae, fungi and lichens, and also prokaryotic cyanobacteria, which are the main component of the lithobiontic communities around the world. Cyanobacteria share this microhabitat with some heterotrophic bacteria, which recycle the waste products of photolithoautotrophic microorganisms (Petrisor & Decho 2004).

Endolithic ecosystems, with these kinds of communities, were first described from the cold deserts of Antarctica (Friedmann 1982). Afterwards, they were reported from different geographic and climatic areas, including hot deserts, where rock surfaces subjected to strong sunlight experience extreme temperature fluctuations that make their exterior inhospitable, whereas their interior offers conditions amenable for the development of life, resulting in the development of microbial communities (Garty 1999).

There are three kinds of endolithobionts: Chasmoendoliths are organisms that colonize cracks and fissures in the rocks. Cryptoendoliths are organisms that live in pores of rocks, which are completely enclosed; euendoliths are organisms that create their own niche by burrowing actively into the interior of rocks (Van Thielen & Garbary 1999). Photosynthetic algae are common inhabitants of the stone matrix yet obviously require light. For that reason they live only 2–3 mm under the surface of rocks, where the light is sufficient to support their physiology (Van Thielen & Garbary 1999). Therefore, the deepness of this niche is dependent on the opacity of the rock and the light available for photosynthesis (Gerrath *et al*. 1995). As a consequence, rocks with some degree of transparency are colonized by algae (Matthes *et al*. 2001), and so far approximately 100 species from 70 algal genera have been described as endolithobionts worldwide (Van Thielen & Garbary 1999).

Acidophilic and thermophilic algae belonging to the Cyanidiales (*Cyanidium, Cyanidioschyzon* and *Galdieria*) are typically found around the world in extreme environments associated with hot springs and volcanic efflux (Roberts 1999). These algae are commonly found as a green film attached to rocks (Roberts

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1999). In those extreme environments, *Galdieria* (Ciniglia *et al*. 2004) and *Cyanidium* (Walker *et al*. 2005) have been described as endolithic microorganisms of 'dead rocks': soft and fragile rocks resulting from prolonged exposure to the harsh environment. In those rocks, communities of Cyanidiales are visible as green layers covering the inner surface of detached flakes. Rocks like these from a Costa Rican volcano were collected for scanning electron microscope analysis of the Cyanidiales endolithic layer; serendipitously, however, diatoms were the predominant microorganisms observed.

The National Park, Rincón de la Vieja Volcano, located in the north-west territory of Costa Rica, comprises two contrasting environments; one is characterized by hot, acid mud and water springs, called 'Pailas Calientes', whereas 7 km away cold and acid (10°C lower than ambient temperature, at pH 1–2) mud-water springs are found forming rivulets, constantly bubbling CO_2 and sulfur gases. Because of the lower temperature these are called 'Pailas Frías' ('Pailas' is the Spanish vernacular name for vessels, and 'Calientes' and 'Frías' mean hot and cold, respectively). The rocks in these springs have a brittle surface devoid of epilithic growth, therefore it is easier to detach thin flakes in which the inner surfaces are green as a result of endolithic growth.

Rock flakes from Pailas Frías were fixed with osmium vapor, dried at 37°C, gold sputtered, and analyzed with a scanning electron microscope (SEM, Hitachi H 570). It was impossible to fix this material following the standard methodology, because these rock flakes gradually dissolve when immersed in fixative solution, buffer or alcohol gradient, altering their normal ultrastructure. Their porous matrix required a thick layer of sputtered gold (40 nm) to avoid the charge-up phenomenon observed in the SEM. Other rock flakes were mounted directly for X-ray analysis using an energy dispersive spectrometry (EDS) coupled to the SEM. Then, the green endolithic layer was removed and treated with 3% sodium hypochlorite for 5 min under microwave irradiation at 100 W to digest the organic matter, in the same way as process biological samples for SEM (Hernández-Chavarría & Guillen 2000); drops (100 μL) of that digested material was mounted on aluminum studs, air dried, covered with gold (20 nm), and observed at SEM.

The surface of the rocks has abundant microbored canals of different dimensions and forms, but no microbes or epilithic lower plants were observed in those cavities. At a depth of 2–3 mm from the surface a green layer is seen comprising a microbial community of 1–2 mm thickness. The SEM analysis of the green layer revealed that it is a complex assemblage of microorganisms, beginning with a thin layer of *Cyanidium* and at least two different rod shaped bacteria, one straight and the other curved (Fig. 1). The deepest layer of the green mat was dominated by diatoms, which appeared attached to or embedded in the rock matrix (Fig. 2). Diatoms represent a great population in this niche, such as is illustrated in Figure 3. There are at least two different morphotypes of diatoms; some are very flat and short with round ends (10–12 μm long), whereas others are thicker and longer with tapered ends (35–40 μm long). Both types of diatoms are shown with more magnification in Figure 4. The observation of these diatoms under light microscopy revealed chloroplasts, indicating their viability. The morphological characteristics of these diatoms correspond to the genus *Pinnularia* (U. Wydrzycka, pers. comm., 2005). EDS analysis of the rock surface and the rock stratum under the green layer show silicon and oxygen as the predominant elements.

Endolithic ecosystems were initially described in the deserts of Antarctica (Friedmann 1982; Hughes & Lawley 2003), and other reports later described such systems in the hot deserts of Israel (Garty 1999) and south-western USA (Chapman 1999). Also, a series of reports from Canada described communities of algae and cyanobacteria inhabiting the cliffs of the Niagara Escarpments (Gerrath *et al*. 1995). This knowledge reinforces the argument that endolithobionts are adapted to inhabit the rock matrix to escape hostile environmental conditions that prevent colonization of the external surface; however, the interior of the rock still represents a hostile environment and for this reason their inhabitants are considered extreme microorganisms.

The endolithic microbial consortia at Pailas Frías was forced to survive in a more extreme environment, because the rocks in this area are exposed to acid water and sulfurous gases, so the surface lacks any epilithic lower plants. Also, their surface appears microbored by canals that could be the result of microbial processes. Therefore, some microorganisms could begin as chasmolithobionts colonizing the microholes and then later move into the matrix.

Diatoms are defined as unicellular, photosynthetic, eukaryotic algae found throughout the world's oceans and freshwater systems (Armbrust *et al*. 2004). Prior to our observations of this endolithic consortium, some new diatom taxa were described in an acid water effluent of another Costa Rican volcano (Wydrzycka & Lange-Bertalot 2001). However, the endolithic *Pinnularia* from Pailas Frías are different from the extreme diatoms previously described by Wydrzycka and Lange-Bertalot (2001). The role of diatoms in global carbon cycling is calculated as equivalent to that of all terrestrial rain forests combined (Armbrust *et al*. 2004); therefore, the presence of diatoms inside rocks reveals a newly detected niche. This would also suggest that previous global diatom biomass calculations underestimated the true total and, therefore,

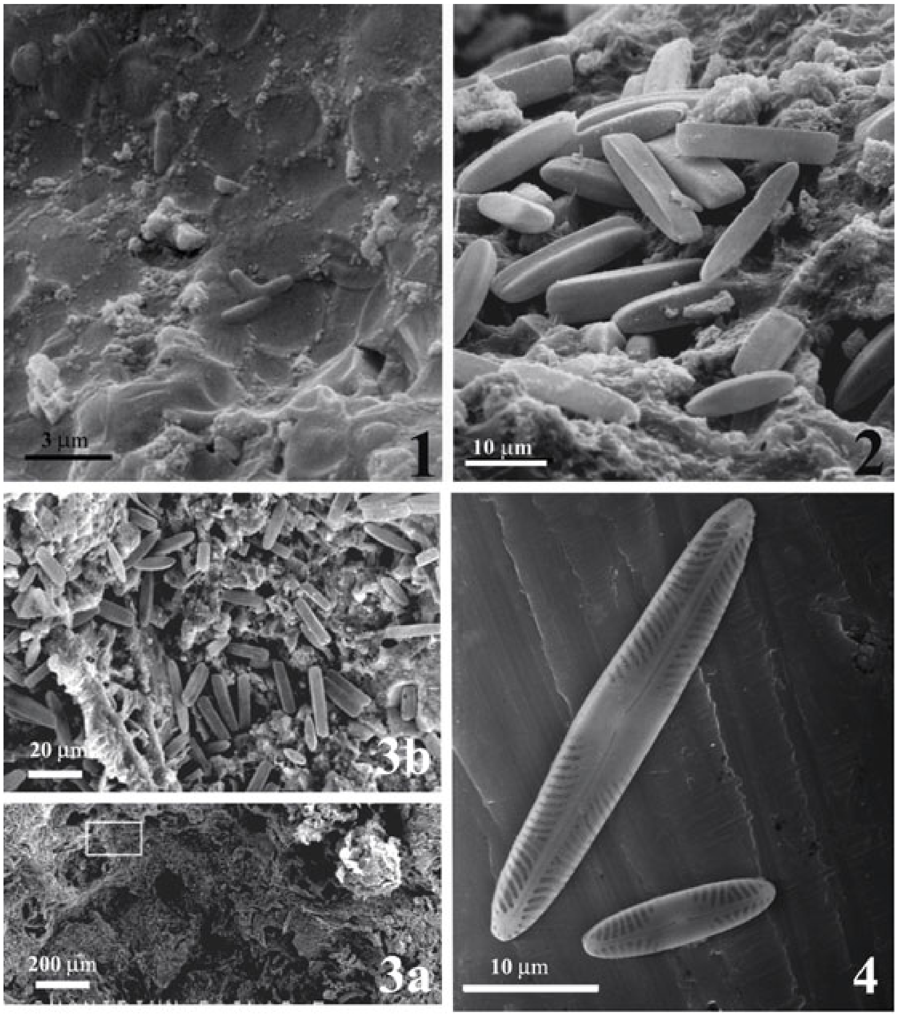

Fig. 1–4. Scanning electron micrograph of the matrix of the rock, after the detachment of a superficial flake. Two bacilli are observed in the center of the figure, one is short and straight and the other is longer and curved. Also, round marks in the background could correspond to collapsed cyanidial cells, because the specimens were air dried. 2. Abundant diatoms of different morphology inhabit the green layer inside the rock matrix. 3. (a) Panoramic view of the endolithic green layer; the frame marked is enlarged in Figure 3b and presents the great amount of diatoms in this microniche. 4. Cleaned diatoms harvested from the endolithic green layer. There are at least two morphological types as is showed in this figure.

more studies are needed to ascertain the real extent of endolithic diatoms, especially in other tropical environments. Previous reports of endolithobionts (Gerrath *et al*. 1995; Garty 1999; Hughes & Lawley 2003; Ciniglia *et al*. 2004; Walker *et al*. 2005) do not mention diatoms as inhabitants of the rock matrix. This is, to the authors' knowledge, the first time that an endolithic microbial consortium dominated by diatoms from volcanically altered rocks in a tropical area has been observed.

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