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Calcification of the filamentous cyanobacterium *Blennothrix ganeshii* in calcareous tropical streams of central Mexico region

Calcificación de la cianobacteria *Blennothrix ganeshii* en ríos calcáreos tropicales de la región central de México

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ABSTRACT

Geochemical, mineralogical and microbiological data from four freshwater streams in central region of Mexico indicate the importance of *Blennothrix ganeshii* mats (Cyanobacteria, Oscillatoriales) in promoting the formation of calcium carbonate crystals. The streams were characterized by alkaline waters and relative physicochemical stability during three seasons (cold dry, warm dry and warm rainy). Calcification took the form of a thick, dense layer of calcium carbonate crystals surrounding the extracellular polymeric substances produced by *B. ganeshii* filaments, giving an appearance of micritic tubes (structures formed by crystallization in the spaces between filaments) along the sheath surfaces. The precipitate was analyzed using X-ray diffraction and energy dispersal X-ray spectrometry, and the calcite crystal habit was determined. The photosynthetic activity of cyanobacterial growth and the presence of abundant extracellular polymeric substances and epiphytic species promote the absorption of ions and mineral nucleation on the surface of the sediment and contribute to the formation of travertine in tropical regions.

Key words: *Blennothrix ganeshii,* calcification, cyanobacteria, micritic tubes, tropical streams.

RESUMEN

Los datos geoquímicos, mineralógicos y microbiológicos en cuatro ríos de agua dulce de la región central de México, ponen de manifiesto la importancia de las matas de *Blennothrix ganeshii* (Cianobacteria, Oscillatoriales) como promotoras de la formación de cristales de carbonato de calcio. Los ríos se caracterizaron por presentar aguas alcalinas y una relativa estabilidad fisicoquímica durante tres estaciones del año (seca fría, seca templada y lluviosa templada). La calcificación estuvo caracterizada como una densa y gruesa capa de cristales de carbonato de calcio que rodean el mucílago extracelular producido por los filamentos de *B. ganeshii*, dando la apariencia de tubos micríticos (estructuras formadas por un material cristalizado en las hendiduras existentes entre filamentos) a lo largo de la superficie de la vaina. El precipitado fue identificado como calcita por su hábito cristalino típico y por análisis de difracción de rayos-X y espectrometría de dispersión de energía de rayos X. La actividad fotosintética de los crecimientos de la cianobacteria y la presencia de abundantes sustancias poliméricas extracelulares y especies epífitas promueven la absorción de iones y nucleación de minerales en la superficie del sedimento y contribuyen a la construcción de travertino en corrientes de regiones tropicales.

Key words: *Blennothrix ganeshii,* calcificación, cianobacteria, tubos micríticos, ríos tropicales.

INTRODUCTION

The deposition of calcium salts, generally called calcification, is a common phenomenon associated with many freshwater bacteria (particularly cyanobacteria), small algae, fungi and bryophytes that contribute to the growth of microbial biofilms and mats (Riding, 2000; Pentecost, 2005; Turner & Jones, 2005). Microbial carbonates are most common in the geologic record in seas and lakes but at present they are also important in spring, cave and soil environments. Minerals originated through biologically induced mineralization (BIM) generally nucleate and grow both extracellularly and intercellularly as a result of various metabolic processes, such as photosynthetic uptake of CO_2 and/or HCO_3^- by cyanobacteria, and ammonification, denitrification and sulfate reduction by other bacteria (Riding, 2000; Couradeau *et al.,* 2012). Extracellular polymeric substances (EPS), widely produced by microbes, are important in providing nucleation sites and facilitating sediment trapping (Riding, 2000). These exopolymers have diverse compositions, depending on the biology of the individual organisms and environmental conditions, but are commonly dominated by negatively charged polysaccharides (Decho, 1990; Turner & Jones, 2005). Although many cyanobacteria have metabolic processes that stimulate calcium carbonate formation, favorable environmental conditions generally appear to be necessary for precipitation to occur (Merz-Preiß & Riding, 1999). This facultative and environmentally dependent calcification reflects the saturation state of the ambient water as well as the metabolic activities of the cyanobacteria.

Blennothrix ganeshii Kützing ex Anagnostidis et Komárek is often a major component of attached lotic communities in submerged (littoral and benthic) habitats and occurs in calcareous regions of Mexico (Montejano *et al.*, 2000; Beltrán-Magos *et al.,* 2005). This perennial cyanobacterium grows in large mats and is an important biotic component in its microhabitat (Carmona *et al.*, 2005). Studies of *B.* ganeshii have concerned taxonomic and ecological information (Anagnostidis & Komárek, 1988; Valadez-Cruz *et al.*, 1996; Komárek, 1998; Watanabe & Komárek, 1989; Montejano *et al.,* 2000; Cantoral & Aboal, 2001; Beltrán-Magos *et al.*, 2005; Carmona *et al.,* 2005) but studies of calcification processes are sparse. The purpose of this study is to evaluate the mechanisms as well as environmental and biological factors involved in *B. ganeshii* calcification in four tropical streams in central Mexico.

MATERIALS AND METHODS

Differences in water chemistry of four freshwater streams with *B. ganeshii* populations (Manantiales, Tambaque, Micos and Puente de Dios) were evaluated by sampling three times over a period of ten months, from January to November 2004 (Fig. 1). The climatic conditions include an intense summer rainy season (García, 2004). The study period covers three main seasons during the year in Mexico for regions below 800 m.s.n.m at latitudes of 18-22° N: the cold dry (February), warm dry (May) and warm rainy season (November). Various physical and chemical parameters were recorded *in situ* at each site. Water temperature, pH and specific conductivity (standardized to 25 °C, K_{25}) were measured with a PC-18 conductivity meter (Conductronic, Puebla, Mexico); dissolved oxygen was measured with an oxygen meter YSI-85 (YSI Incorporated, Ohio, USA); and current velocity and photosynthetically active radiation (PAR) were measured as close as possible to the algal growth using a 2100 current velocity meter (Swoffer Instruments, Washington, USA) and a LI-1000 quantum meter (LI-COR Biosciences, Nebraska, USA) with a flat subaquatic PAR sensor. Depth and type of substrate were also recorded for each site. Oxygen saturation percentage was calculated from dissolved oxygen data, taking into account altitude and water temperature (Wetzel & Likens, 1991).

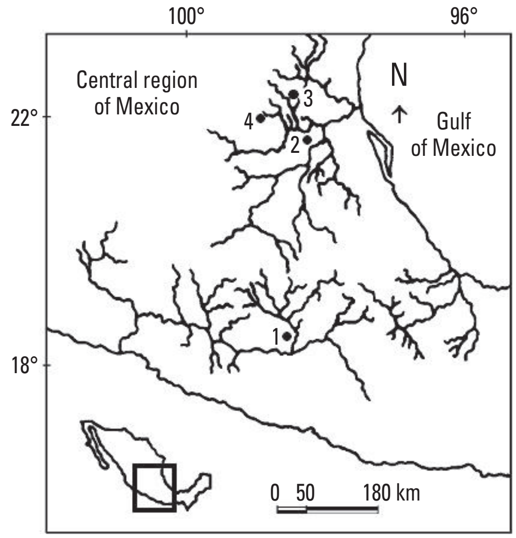

Figure 1. Sampling sites of *B. ganeshii* populations in central Mexico. Site 1: Manantiales, Site 2: Tambaque, Site 3: Micos and Site 4: Puente de Dios.

Water samples for nutrient determination were collected in duplicate. Each sample replicate was filtered *in situ* with 0.22 µm pore size membranes (Millipore, Massachusetts, USA), preserved with a few drops of chloroform and frozen for subsequent laboratory analysis with a San plus segmented-flow analyzer (Skalar Inc., Georgia, USA), following standard titration. Soluble reactive phosphorous (theoretically, the majority being orthophosphates, $P\text{-}PO_4^{3-}$), $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ were analyzed following the techniques described by ASTM (1989) and APHA (1995). Water samples for determination of anions (HCO_3^-, CO_3^{2-}, Cl^-, SO_4^{2-}), total dissolved solids (TDS) and pH were frozen (–20°C) and preserved in dark conditions, whereas samples for cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were preserved with 40% nitric acid (down to pH 2-3). Determination of carbonates was carried out using the titration method, chlorides using the selective electrode method, sulfates using the turbidimetric method and Na^+ and K^+ using the spectrophotometric atomic absorption method (APHA, 1995).

Saturation of stream water with respect to calcite was calculated according to the saturation index (SI), SI = pH - pH_s, where pH is the registered pH and pH_s is the calculated pH in equilibrium with $CaCO_3$ at the existing concentrations of Ca^{2+} and HCO_3^- (ASTM, 1989). Populations of *B. ganeshii* were collected for calcification analysis. Maximum differences in carbonate precipitates on cyanobacteria were observed and analyzed at the end of the dry season (May) and at the end of the rainy season (November). Carbonate content of samples was evaluated using the loss-on-ignition method (550 °C for 4 h; Heiri *et al.*, 2001; Boyle, 2004). Calcification morphology and mineral composition was determined using a BX51 light microscope (Olympus Corporation, Tokyo, Japan), a JSM-6380LV scanning electron microscope (SEM; Jeol, Tokio, Japan) and by INCAx-sight energy-dispersive X-ray spectroscopy (EDXS; Oxford instruments, Oxfordshire, UK). Mineralogy was confirmed by X-ray diffraction (XRD) using a powder diffraction meter Broker D8-advance (CuK radiation, graphite monochromator; Bruker AXS, Wisconsin, USA). Samples were oven dried (105 °C for 24 h; Heiri *et al.*, 2001; Boyle, 2004) and ground to a fine powder; analysis was based on the Diffplus Bs software and the International Centre for Diffraction Data (ICDD) database. Significant differences ($p < 0.05$) in chemical composition between sites and seasons were assessed using one-way analysis of variance (ANOVA), followed by a Scheffé test. Relationships between calcification and physicochemical parameters were evaluated with redundancy analysis (RDA). Statistical analyses were performed with the programs SPSS ver.12 (Levesque, 2006) and XLSTAT ver. 7.5 (XLSTAT, 2004).

RESULTS

Water conditions. *Blennothrix ganeshii* populations from central Mexico are present under particular chemical and physical conditions: alkaline waters (2.7-4.8 eq l^{-1}) dominated by SO_4^{2-}/HCO_3^- and Ca^{2+}/Mg^{2+}, warm temperature (23-29 °C), shallow depth (3-28 cm), moderate current velocity (13-27 cm s^{-1}), high percentage of oxygen saturation (96-107%) and different types of substrata (lime, sand, clay, gravel, boulder or rock). Stream segments were shaded or partly shaded (11-85 µmol photons m^{-2} s^{-1}; Tables 1 and 2).

Seasonal variations in temperature, pH, total alkalinities and concentration of Ca^{2+} are shown in Figs. 2A-D. The highest temperatures were recorded at site 1 throughout the sampling period. The pH of stream water varied over a small range of values (7.0-7.9), generally with a slight increase during the warm dry season, except for site 1. Total alkalinities showed no differences during the seasons at site 1. At sites 2, 3 and 4, lower values were detected during the warm season. Variations in Ca^{2+} and ionic concentration between sampling dates were noted at sites 2, 3 and 4, with higher concentrations during dry seasons and lower during rainy seasons, showing a seasonal dilution process. Virtually no variation in Ca^{2+} ions was detected at site 1 during the sampling period.

ANOVA showed significant differences in chemical composition between sites (F = 5.8, $p = 0.001$-0.02). Scheffé test results indicated the presence of two groups ($p < 0.05$): one containing sites 1, 2 and 4, and the other containing site 3. No significant differences ($p < 0.05$) were found in chemical composition among seasons from different sampling years at all sites.

Saturation of stream water with respect to calcite. Seasonal changes in the saturation index of calcite (SI) are presented in Fig. 2E. Stream water was generally supersaturated with respect to calcite (SI > 0). SI values at site 3 were negative in the warm rainy season when sampling took place soon after heavy rains. Average annual supersaturation index values of calcite were between 0.25 and 0.85.

Calcification. All studied populations were characterized by calcium carbonate precipitation around the sheaths and were found in shallow water conditions, even when substantial parts of the mat were outside the water column, facilitating evaporation processes and cementation of minerals. Variability in precipitation was found between populations at different sites. Samples from sites 1 and 4 had the highest mean carbonate precipitation regardless of sampling season (31 and 36%, respectively), whereas populations from sites 2 and 3 showed the lowest precipitation (13 and 16%, respectively). The eigenvalues of the first two RDA axes were high (RDA1, 0.66 and RDA2, 0.33) when compared with subsequent axes, and they accounted for 99% of the variance in carbonate precipitation (Fig. 3). Both axes accounted for 99% of the variance in the relationship between carbonate precipitation at the four sites and environmental variables. The results of the permutation tests revealed the statistical significance ($p \leq 0.05$) of the effects of current velocity and N-NO_2^- at sites 2 and 3; total alkalinity, K_{25}, SO_4^{2-}, Ca^{2+}, Mg^{2+}, temperature, P-PO_4^{3-} and SI at site 1, and pH and SI at site 4. No differences in total alkalinity among the seasons at site 1 were associated with the direction of the distribution pattern of carbonate precipitation.

Calcification occurred as dense, thick encrustation around the sheaths, creating micritic (metabolically induced, but environmentally dependent) tubes typically 10-25 µm thick enclosing *B. ganeshii* filaments (Figs. 4A, C). The calcareous tubes around the filaments were separate from each other. Distal ends of the filaments remained uncalcified, and allowing trichomes to be visible (Fig. 4E). Calcite crystals displayed a rhombohedral form with triangular surfaces and irregular disposition (Figs. 4C-D), forming the "gothic arch crystals" described by Rainey and Jones (2009). Scanning-electron-microscopic examination of the initial precipitate nucleation indicates the presence of calcium carbonate par-

Table 1. Chemical composition measured at the study sites of the rivers of the portion central of Mexico. Values expressed in mg l^{-1}, unless otherwise stated. Values represent minimum, maximum, and average + standard deviation (*n* = 5 for each site).

	Site 1	Site 2	Site 3	Site 4
pH	7.2-7.8 7.5+0.2	7.3-7.7 7.5+0.1	7.0-7.9 7.3+0.4	7.4-7.8 7.7+0.2
K_{25}[a] [µS cm^{-1}]	1703-1778 1740+31	674-2360 1423+643	701-948 810+109	571-3000 1562+937
TDS	1512-1635 1594+48	464-1647 1139+492	493-770 621+127	508-2320 1289+739
Total alkalinity [meql^{-1}]	4.7-5 4.8+0.1	3.6-4.4 3.8+0.4	2.4-3.3 2.7+0.4	1.9-3.7 3+0.9
HCO_3^-	225-305 269+37	197-245 229+21	148-286 196+57	118-286 207+64
CO_3^{2-}	0-32 11+15	0-14 5+7	0-11 3+5	0-20 9+9
Cl^-	12-28 16+7	4-6 4+1	5-10 7+2	5-47 25+18
SO_4^{2-}	735-907 859+70	223-806 471+234	196-361 294+67	270-829 588+269
Ca^{2+}	309-330 322+8	121-335 231+79	123-186 148+26	155-313 237+69
Mg^{2+}	65-73 68+3	20-61 36+17	22-33 26+4	28-85 58+25
Na^+	35-53 47+7	4-15 11+4	9-19 13+4	22-55 41+14
K^+	6-7 6+0.4	0.6-2 1+0.4	1-2 2+0.2	2-5 4+1
$P\text{-}PO_4^{3-}$ [a]	0.01-0.03 0.02+0.008	0.007-0.02 0.01+0.003	0.005-0.01 0.01+0.003	0.007-0.02 0.01+0.005
$N\text{-}NO_3^-$	0.03-237 174+98	69-344 178+107	73-196 143+49	104-195 151+35
$N\text{-}NO_2^-$	0.001-0.006 0.004+0.002	0-0.02 0.005+0.008	0.001-0.02 0.007+0.01	0.0005-0.007 0.003+0.003
$N\text{-}NH_4^+$	0.03-0.5 0.1+0.2	0.02-0.04 0.03+0.01	0.02-0.06 0.04+0.01	0.01-0.04 0.02+0.008
IC [meq l^{-1}]	46-49 48+1	17-44 30+10	18-23 20+2	23-59 36+15
Ionic dominance	$SO_4^{2-} > HCO_3^- > Cl^- > CO_3^{2-}$ $Ca^{2+} > Mg^{2+} > Na^+ > K^+$	$SO_4^{2-} > HCO_3^- > CO_3^{2-} > Cl^-$ $Ca^{2+} > Mg^{2+} > Na^+ > K^+$	$SO_4^{2-} > HCO_3^- > CO_3^{2-} > Cl^-$ $Ca^{2+} > Mg^{2+} > Na^+ > K^+$	$S04^{2-} > HCO_3^- > CO_3^{2-} > Cl^-$ $Ca^{2+} > Mg^{2+} > Na^+ > K^+$
SI	0.55-1.11 0.85+0.2	0.45-0.71 0.62+0.1	–0.28-0.74 0.25+0.4	0.08-1.0 0.73+0.4

[a] Abbreviations: K_{25} = specific conductance standardized at 25 °C, TDS = Total Dissolved Solids, IC = Ionic concentration, $P\text{-}PO_4^{3-}$ = Soluble Reactive Phosphorous, SI = Calcium Saturation Index.

ticles with diameters that range from several tenths of a micrometer to as much as 1 micrometer on the polysaccharide sheath (Fig. 4B). EDXS of these precipitates showed they contained a lower Ca^{2+} percentage than the fully formed calcite crystals (Fig. 5A-B). Inclusions contained Ca^{2+} as a major element. Around the sheaths, the calcite coating was enlarged (up to five times the diameter of the trichome) mainly by the addition of calcite crystals, which grouped themselves in cylindrical tubes (Fig. 4F).

X-ray diffraction showed a high number of minerals (carbonates, silicates and oxides) deposited on *B. ganeshii* sheaths

Table 2. Climatic and physical factors at the study sites on central Mexico. Values represent minimum, maximum, and average + standard deviation; n = 5 at each site.

Site and location	Altitude [m a.s.l.][a]	Climate[b]	Water temperature [°C]	Depth [cm]	Current velocity [cm s^{-1}]	PAR[c] [μmol photons $m^{-2} s^{-1}$]	Substrate[d]	Dissolved Oxygen [mg l^{-1}]	Oxygen Saturation [%]
Site 1 Manantiales 18° 55´ N 96° 00´ W	800	A w_0 (w)	26-29 29+1	5-10 6+2	14-26 20+4	18-276 85+108	L, S, C	7.2-7.8 7.5+0.4	107
Site 2 Tambaque 21° 41´ N 99° 02´ W	150	(A)C(m)(w)	23-24 23+1	11-60 28+19	11-41 27+12	13-69 34+25	S, G, B, R	7.6-8.4 8.1+0.4	96
Site 3 Micos 22° 05´ N 99° 09´ W	120	(A)C(m)(w)	20-27 23+2	7-13 10+3	3-43 21+16	3-14 11+5	L, S, G, B, R	8.1-8.8 8.4+0.3	100
Site 4 Puente de Dios 21° 55´ N 99° 24´ W	450	(A)C(m)(w)	23-27 25+2	2-7 3+2	0-30 13+14	4-245 73+116	L, S, C, G, B, R	7-8.2 7.8+0.7	104

[a] Meters above sea level

[b] Climate: (A)C(m)(w), Semicalid humid with abundant spring rains, mean annual precipitation 1500 mm; Aw_0(w), Calid humid with summer rains, mean annual precipitation 800-1000 mm; Bs_1kw, Semicalid dry with summer rains, mean annual precipitation 503 mm (INEGI, 1985, 1992; SPP, 1981).

[c] Photosynthetically active radiation

[d] Substrate: B = boulder, C = clay, G = gravel, L = lime, R = rock, S = sand.

(Table 3). XRD analyses indicated that calcite was the only phase of $CaCO_3$ present in readily detectable amounts at all sites. Sites 1, 3 and 4 had the highest proportion of calcite (average = 95, 81 and 88%, respectively). A temporal variation in precipitate minerals around the sheaths was recorded in all sampling sites. A comparison among seasons showed that major calcification occurred in the dry season, when higher SI values were found, except in site 3, which had a negative SI value.

DISCUSSION

The formation of travertine in tropical streams of the central region of Mexico evidently is stimulated by calcite supersaturation in the water. In particular, *B. ganeshii* populations occurred under conditions of alkaline water and the presence of abundant EPS and ephyphitic species that promote the absorption of ions and mineral nucleation.

High SO_4^{2-} concentrations occurred in the water at all sampling sites, resulting from solution of underlying gypsum deposits (Consejo de Recursos Minerales 1992, 1993, 2000). According to Carmona *et al.* (2005) sulfates are an essential component of *B. ganeshii* sheaths as well as of several other cyanobacteria. Our analysis shows that Ca^{2+}, HCO_3^- and Mg^{2+} are the prevailing ions in the studied streams, as would be expected for water in contact with limestone and dolomite (Consejo de Recursos Minerales 1992, 1993, 2000; Ferrusquía-Villafranca, 1998). Na^+, K^+ and Cl^- are present in low concentrations at the studied localities. Similar water composition has been described in thermal carbonate spring deposits in Canada and Mexican maar-crater lakes (Vilaclara *et al.,* 1993; Armienta *et al.*, 2008; Rainey & Jones, 2009). The most abundant nutrient in water from all sampling sites is nitrogen as nitrate. Phosphate concentrations at all sites are low due to the presence of high concentrations of Ca^{2+}. This condition has been observed in similar karstic systems elsewhere (Reddy, 1988) due to the co-precipitation of phosphates with calcium carbonate (Kleiner, 1990).

Mineral formation in *B. ganeshii* could be explained by the reduction of CO_2 in the water due to photosynthetic activity. As the water in which these cyanobacteria live is supersaturated with respect to calcium carbonate, CO_2 reduction induces mineralization and increases in pH values, alkalinity and CO_3^{2-} ion concentration, thus principally promoting calcite precipitation. EPS excreted by cyanobacterial cells favor calcium carbonate encrustation by providing an ideal surface for the adsorption of ions and mineral nucleation (Emeis *et al.*, 1987; Braissant *et al.*, 2003; Dittrich & Sibler, 2010). EPS possess the ability to concentrate Ca^{2+} cations from solution due to the net-negative surface charge that occurs on several cyanobacteria, including *Pleurocapsa* sp.,

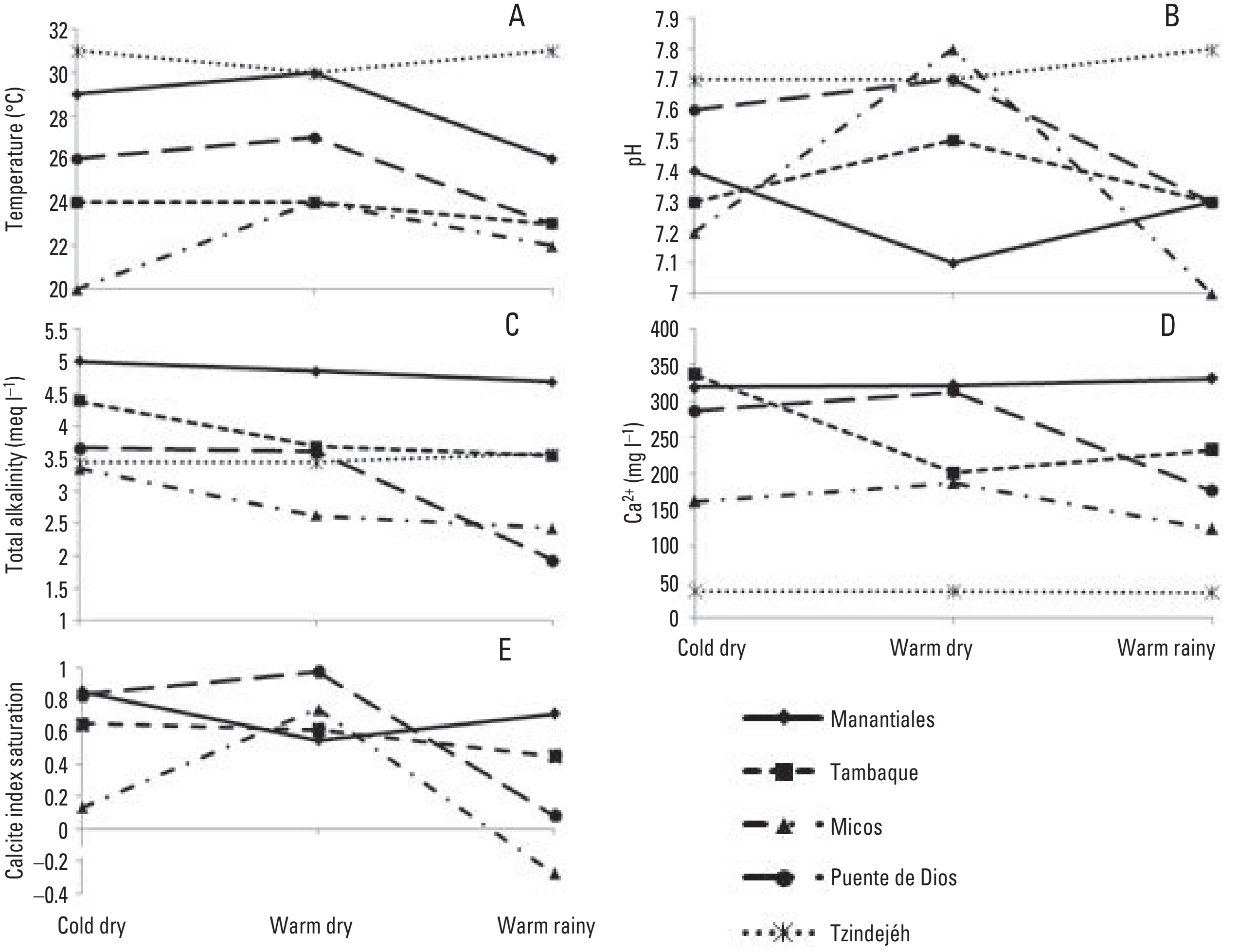

Figure 2A-E. Seasonal changes in temperature (A), pH (B), total alkalinity (C), Ca^{2+} concentrations (D) and saturation index of calcite (E) in the sampled streams in central Mexico.

Plectonema sp. and *Scytonema* sp. (Golubić, 1973; Merz-Preiß & Riding, 1999; Riding, 2000; Frankel & Bazylinski, 2003; Pentecost, 2005); possibly the same phenomenon occurs in *B. ganeshii.* Pentecost (1978) and Konhauser (2007) proposed that cyanobacterial species that produce sheaths or EPS generally precipitate more calcium carbonate than those species without such structures.

Merz-Preiß and Riding (1999) report that calcium carbonate precipitation in freshwater streams becomes conspicuous where average annual supersaturation index values exceed 0.75. However, all *B. ganeshii* populations showed extracellular mineral formation, although relatively lower values were measured in several seasons, particularly in the warm rainy season. Nucleation can be disadvantaged by the mechanical removal of minerals related to high flow velocity.

The evaporation processes occurring on exposed surfaces of benthic mats could explain the plentiful calcification. According to Schneider and Le Campion-Alsumard (1999), boundary layers are the most important environments for cyanobacterial activity during the formation of carbonates.

The *Blennothrix ganeshii* calcification process involves two main phases: first, the formation of a solid nucleus from dissolved ions and second, the addition of ions to the nucleus to form crystals of calcium carbonate. During the first phase, ions are positioned over the sheath or epiphytic species and resist rapid dissolution with a posterior crystalline phase. According to Merz-Preiß and Riding (1999), Schneider and Le Campion-Alsumard (1999), Pedley (2009), Pedley *et al.* (2009) and Rainey and Jones (2009) microbes do not contribute to the establishment of elevated supersaturation states but passively serve as substrates upon which calcite precipitates. Filaments of *B. ganeshii* were used as substrate by several cyanobacteria, diatoms and epiphytic species of red algae present throughout the entire study, including *Chamaesiphon confervicola* A. Braun, *Chamaecalyx swirenkoi* (Sirsov) Komárek et Anagnostidis, *Stichosiphon sansibaricus* (Hieronymus) Drouet

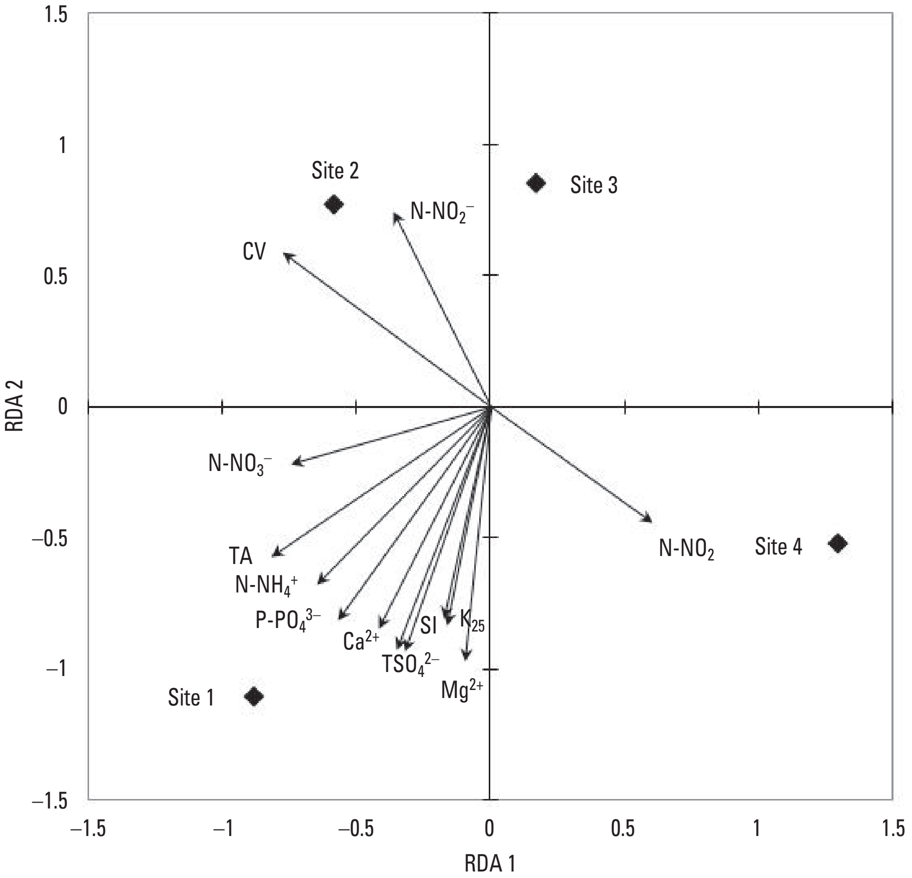

Figure 3. Redundancy analysis (RDA) biplot of the carbonate precipitation and physicochemical parameters of four sites on rivers in central Mexico.

et Daily, *Xenococcus bicudoi* Montejano, Gold et Komárek, *X. willei* Gardner, *Cocconeis placentula* Ehrenberg var. *placentula, Gomphonema gracile* Ehrenberg, *Surirella linearis* W. Smith, *Synedra ulna* (Nitzsch) Ehrenberg var. *ulna, Terpsinoë musica* Ehrenberg, *Audouinella meiospora* (Skuja) Garbary and *Compsopogon coeruleus* (C. Agardh) Montagne. Several of them have been reported in similar tropical and alkaline environments (Golubic, 1973; Prins & Elzenga, 1989; Freytet & Verrecchia, 1998; Merz-Preiß & Riding, 1999; Beltrán-Magos *et al*., 2005). According to Emeis *et al*. (1987), epiphytic diatoms excrete mucilage enriched in aspartic acid as a response to high Ca^{2+} concentrations. The mucilage traps micrite particles that are suspended in the water, which then act as crystal seeds for inorganic calcite precipitation. The second phase includes the precipitation mainly of calcium carbonate ions and the formation of micritic tubes of calcite minerals that enclose sheaths. Pentecost (1978), Tavera and Komárek (1996), Freytet and Verrecchia (1998), Schneider and Le Campion-Alsumard (1999) and Pentecost (2005) reported this type of calcification is the most common on travertine surfaces, and similar trends have been observed in several filamentous cyanobacteria: *Tapinothrix janthina* (Bornet et Flahault) Bohunická et Johansen (*H. janthina* Bornet *et* Flahault), *Lyngbya aerugineo-caerulea* Gomont, *Microcoleous vaginatus* Gomont ex Gomont, *Phormidium incrustatum* (Nägeli) Gomont ex Gomont, *Plectonema gloeophilus* Borzi, *P. gracillimum* Zopf ex Hansgirg, *P. phormidioides* Hansgirg ex Forti, *Rivularia haematites* (De Candolle) Agardh ex Bornet et Flahault, *R. varians* Obenlüneschloss, *Schizothrix calcicola* Gomont and *Scytonema myochrous* (Dillwyn) C.A. Agardh ex Bornet et Flahault. The extracellular biomineralization reported in these species and the intracellular calcification in a cyanobacterium belonging to Gloeobacterales confirm the importance of cellular control and physicochemical parameters on the formation and morphology of different types of travertine (Tavera & Komárek, 1996; Couradeau *et al.,* 2012).

Cyanobacterial mats of *B. ganeshii* in the central region of Mexico create ideal conditions for biologically induced mineralization of calcite and have presumably played a significant role in the development of these natural calcium carbonate environments. This facultative but environmentally dependent calcification reflects the saturation state of the water, as well as the oxic sediment role of *B. ganeshii* sheaths. The present investigation also highlights the significance of the study of physical and

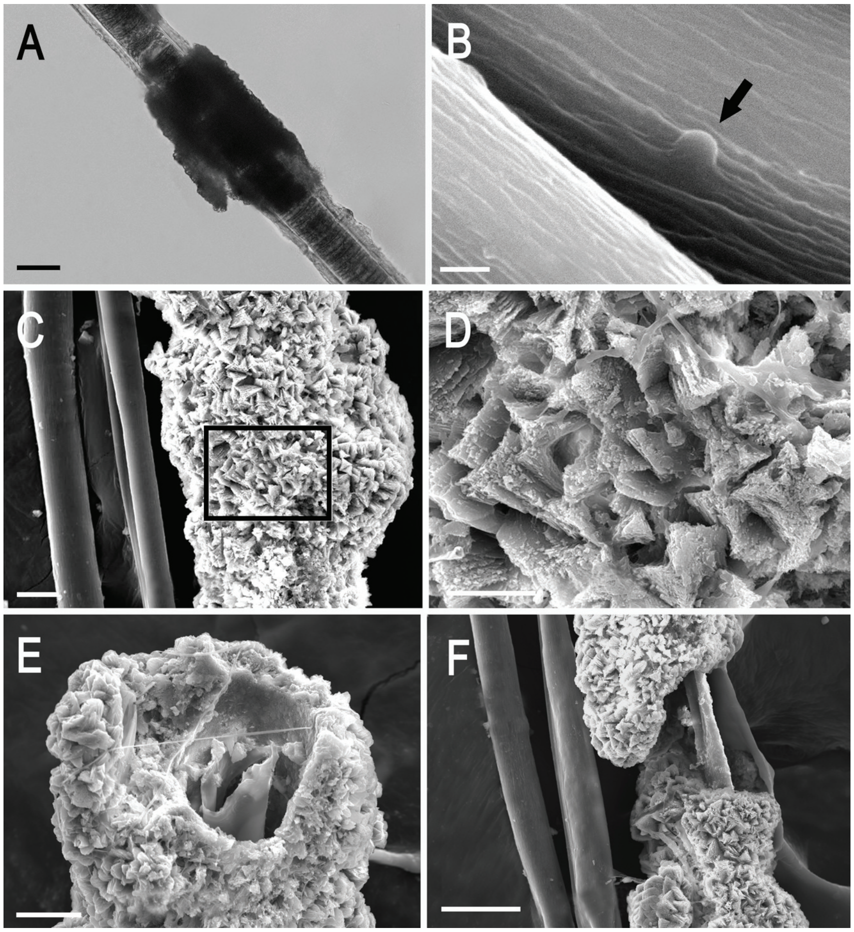

Figure 4A-F. Calcification in *B. ganeshii.* (A) Light-microscopy view of an encrusted filament segment; the scale bar represents 30 µm.(B) SEM view of nucleation of spherical particles of amorphous calcium carbonate (arrow); the scale bar represents 1 µm. (C) SEM views of without encrustation (left side) and a cluster of calcite triangles on a heavily encrusted filament (right side). The rectangular area is enlarged in (D). The scale bar represents 20 µm. (D) SEM view of lamellar triangular plan view of calcite crystals; the scale bar represents 10 µm. (E) SEM view of cross-section of a heavily encrusted filament with calcite crystals; the scale bar represents 20 µm. (F) SEM view of broken encrustation showing the trichome in the interior; the scale bar represents 50 µm.

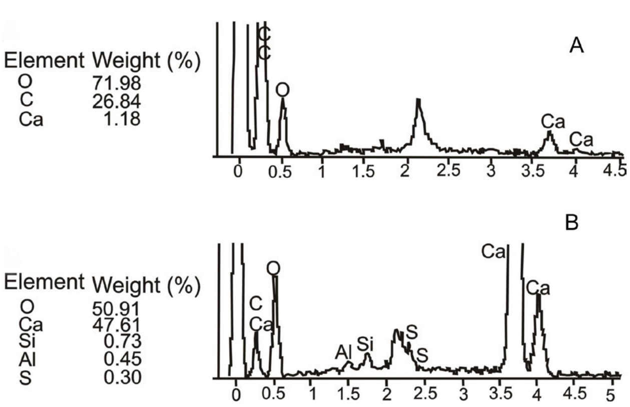

Figure 5A-B. Electron dispersive X-ray spectroscopy of *B. ganeshii* filaments: (A) nucleation and (B) calcite crystals.

chemical seasonal variations in freshwater alkaline streams and the calcification processes of cyanobacteria so as to understand their functioning and prevalence in tropical streams.

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Table 3. Minerals deposited on *B. ganeshii* sheaths from sites 1 through 4 of rivers of the central portion of Mexico. Values are expressed as percentage of total minerals present.

			Site 1		**Site 2**		**Site 3**		**Site 4**	
Class	Group	Mineral	28.v.04 [a]	18.xi.04	15.v.04	5.xi.04	15.v.04	6.xi.04	14.v.04	7.xi.04
carbonates	calcite	calcite	97	93	49	18	71	90	90	86
silicates	clays	palygorskite		2						
silicates	clays	clinochlore		2						
silicates	clays	halloysite				25				
silicates	clays	montmorillonite			16		15			
silicates	feldspars	albite		2				5		
silicates	feldspars	anorthite							3	
silicates	feldspars	microcline				8			2	
silicates	quartz	quartz		1	27	23	11	2	5	8
silicates	quartz	tridymite	1							
silicates	quartz	cristobalite	1							
silicates	micas	muscovite			8		3	3		6
silicates	micas	eastonite				8				
silicates	amphibole	richterite				12				
silicates	zeolites	laumontite				6				
oxides	hematite	hematite	1							

[a] Sampling dates.

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