

Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



## Phytolith analysis of *Chusquea ramosissima* Lindm. (Poaceae: Bambusoideae) and associated soils

Lía Montti<sup>a,d,e,\*</sup>, Mariana Fernández Honaine<sup>b,e</sup>, Margarita Osterrieth<sup>b</sup>, Dalva Graciano Ribeiro<sup>c</sup>

<sup>a</sup>Laboratorio de Ecología Funcional (LEF), Departamento de Ecología, Genética y Evolución, Facultad de Ciencias, Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 4 piso, Ciudad de Buenos Aires C1428EHA, Argentina

<sup>b</sup>Centro de Geología de Costas y del Cuaternario, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata 7600, Argentina

<sup>c</sup>Laboratorio de Anatomía Vegetal, Departamento de Botánica, Universidad de Brasilia, P.O. Box 04457, Brasilia, DF, Brasil

<sup>d</sup>Asociación Civil Centro de Investigaciones del Bosque Atlántico (CeIBA), Yapeyú 23, 3370 Puerto Iguazú Misiones, Argentina

<sup>e</sup>Consejo Nacional de Investigaciones Científicas (CONICET), Argentina

Available online 11 January 2008

### Abstract

*Chusquea ramosissima* Lindm. is dominant in the understory of the Misiones province forests (Argentina); it colonizes open natural areas and gaps created by timber extraction. The aim of this study is to analyze both the phytolith assemblage produced by leaves, culms, and roots of *C. ramosissima* and the one deposited on soil surface. This information will be used in future phytolith studies of the Misiones forest soils, which will contribute to understanding the dynamics of this species in the past. Phytoliths were extracted from plant organs by using a calcination technique and morphotypes were identified in plant tissue by anatomical observations. Soil samples were collected from two sites with a different history of use and vegetal cover. The three organs produced different phytolith morphotypes; leaf was the main producer. From the soil samples, only leaf phytoliths were observed. Although concave dumb-bells are the characteristic phytoliths of *C. ramosissima*, their greater robustness and higher preservation in soil—even where this species is nowadays absent—do not help to discriminate clearly between sites with and lacking this species. On the other hand, the panicoid dumb-bell (bilobated flat/concave ends short cell) and *Chusquea* bulliform (cubic/parallelepipedal bulliform cell) phytoliths seem to be more sensitive to changes in the presence/absence of this species. Results from this research will provide useful information for future studies of late Pleistocene–Holocene reconstruction in this region.

© 2008 Elsevier Ltd and INQUA. All rights reserved.

### 1. Introduction

Bamboos, commonly called canes or takuaras, are known as the greatest grasses in the world (Judziewicz et al., 1999). Although they exist in all continents except Europe, bamboos are extremely diverse and common in America (Judziewicz et al., 1999; Londoño and Clark, 2004). The American bamboos are most diverse in the Atlantic Forest, *Chusquea* being the most diverse genus

with the widest distribution (Judziewicz et al., 1999). In Argentina, this genus is widely represented by *Chusquea ramosissima* Lindm. a very abundant bamboo in the understory of the Misiones forest, a large forest remnant at the Upper Paraná Atlantic Forest ecoregion. Although the Misiones forest is the most diverse and complex ecosystem in Argentina, it has been subjected to selective logging, even in protected areas (Campanello et al., 2005, 2006, 2007). This kind of management alters both gap size and forest dynamics, creating environmental conditions that favor the growth and development of *C. ramosissima*. At present, this bamboo is one of the most aggressive colonizers in disturbed sites of the Misiones forest (Campanello, 2004; Campanello et al., 2007; Montti, unpublished data). Nevertheless, there is no study record

\*Corresponding author at: Laboratorio de Ecología Funcional (LEF), Departamento de Ecología, Genética y Evolución, Facultad de Ciencias, Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 4 piso, Ciudad de Buenos Aires C1428EHA, Argentina. Tel.: +54 03757 491047/423176; fax: +54 11 4576 3384.

E-mail address: liamontti@yahoo.com.ar (L. Montti).

of *C. ramosissima* abundance in the past, and the botanical composition of pristine Misiones forest is not well known, due to insufficient historical data. This kind of information would be of great importance to interpret the changes in plant communities through time and to establish a clear relation between the presence of bamboo and human intervention in the forest. As Misiones Forest (like other tropical forests) constitutes a high oxidizing environment due to the predominance of ultisols, preservation of some plant microfossils such as pollen is difficult. In this case, phytolith analysis is a valuable tool for palaeo-environmental and/or palaeo-botanical studies because they are the only microfossils preserved in this type of environment (Twiss et al., 1969; Rovner, 1971; Piperno, 1988).

The Bambusoideae subfamily, as the rest of Poaceae, is one of the main phytolith producers (Twiss et al., 1969; Piperno, 1988). The high phytolith diversity of this family is a valuable taxonomic attribute and serves for its identification in the past, through soil phytolith analyses (Bertoldi de Pomar, 1975; Piperno, 1988; Mulholland, 1989; Twiss, 1992; Piperno and Pearsall, 1998; Gallego et al., 2004). Within Bambusoideae, several studies of silica content and silicification processes have been conducted especially in Asiatic species (Ueda and Ueda, 1961; Motomura et al., 2000, 2002, 2004). Piperno (1988) and Piperno and Pearsall (1998) have described some phytolith morphotypes in the *Chusquea* genus that can be used as important indicators in palaeo-environmental studies from tropical forests. Nevertheless, there is no detailed descriptive study of phytolith assemblages of complete specimens, which together with soil phytolith assemblage analyses are crucial for a correct interpretation of the regional fossil phytolith record.

The aim of this study is to analyze the phytolith assemblage produced by leaves, culms, and roots of *C. ramosissima* and the assemblage deposited on soil surface. This article also analyzes the phytolith assemblages of soil with different densities of bamboo and histories of use. This information is crucial for future studies of reconstruction of late Pleistocene–Holocene of the Misiones forest soils (Argentina), where phytolith analysis constitutes the main tool for paleo-studies in oxidizing environments.

## 2. Regional setting

The research was carried out in a native forest in the Province of Misiones, Argentina. The dominant actual vegetation corresponds to the subtropical semideciduous forest, commonly denominated as the Upper Paraná Atlantic Forest or Misiones forest (Fig. 1). The average annual precipitation is 2000 mm, evenly distributed throughout the year, and the average annual temperature is 21 °C. The soils belong to the order of the Ultisols (rodic kandiuult); developed from a basalt layer containing high concentrations of Fe, Al and Si. They are deep to very

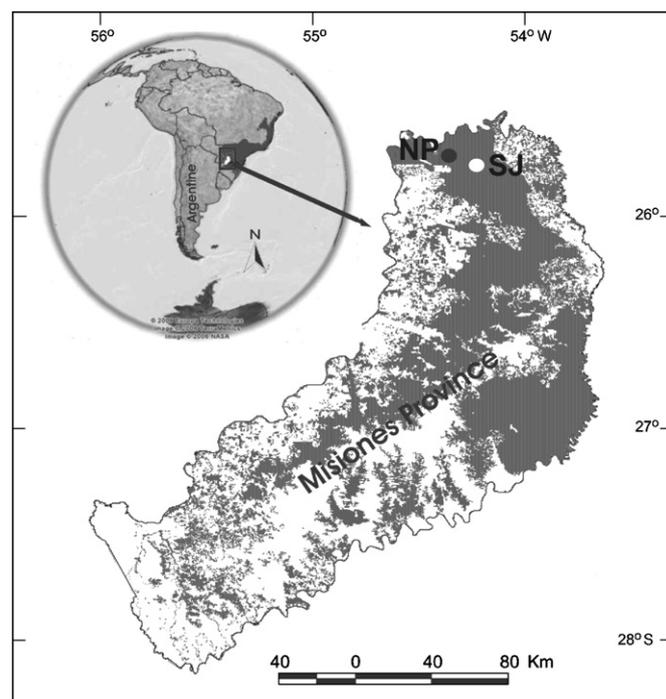


Fig. 1. Atlantic Forest distribution (dark gray shading) and localization of the study area in Misiones province (Argentina). The black point represents the study site at the Iguazú National Park (NP) and the white point represents the study site at San Jorge Property (SJ). The gray shading shows the native forest remnants in Misiones province, a large forest remnant at the Upper Paraná Atlantic Forest ecoregion. (Map modified from Google earth map.)

deep, and their color is an intense dark red (between 2.5 YR 3/4 dry and 2.5 YR 4/6 wet) (Ligier et al., 1990; Montti, unpublished data). The soils have developed three or four mineral horizons. The A horizon is approximately 10–15 cm thick, where most of the plant roots are developed, and has a blocky angular structure and a clay texture. The B1, B2, and B3 horizons are 20 cm thick approximately and have a prismatic structure. These horizons are argilic with gradual increases of clay according to the depth (Ligier et al., 1990).

## 3. Materials and methods

### 3.1. Phytolith extractions from plants and anatomical observations

Leaves, culms, and roots (rhizome and adventitious roots) were collected from specimens of *C. ramosissima* that grow in the study area. Between 0.1 and 0.3 g of leaves, 1 and 3 g of culm, and 0.2 and 0.6 g of root were first placed in an ultrasound bath for 15–20 min and washed with distilled water to remove mineral contaminants. The phytoliths were extracted by using a calcination technique (Labouriau, 1983). The material was dried at 56 °C for 24 h, and charred at 200 °C for 2 h. Later, it was boiled in a 5 N HCl solution for 10 min, washed with distilled

Table 1  
Phytolith morphotypes used in this study and the naming according to the ICPN (Madella et al., 2005)

Phytolith morphotypes	ICPN names
Panicoid dumb-bell <sup>a</sup>	Bilobate flat/concave ends short cell
Simple lobate <sup>a</sup>	Bilobate convex ends short cell
Cross	Cross
Thin chloridoid <sup>b</sup>	Long saddle
Chloridoid <sup>b</sup>	Saddle
Concave dumb-bell <sup>c</sup>	Trapeziform bilobate echinate
Trapezoid short cell	Trapeziform short cell
Equidimensional rondel	Rondel
Large rondel	Rondel
Fan, smooth <sup>d</sup>	Cuneiform bulliform cell
Polyhedral, smooth <sup>d</sup>	Parallepipedal bulliform cell
<i>Chusquea</i> bulliform	Cubic/parallepipedal sinuate bulliform cell
Tabular psilate	Tabular psilate
Tabular polygonal psilate	Tabular polygonal psilate
Tabular crenate	Tabular crenate
Tabular crenate verrucate	Tabular crenate verrucate
Elongate smooth <sup>b</sup>	Elongate psilate
Elongate, sinuous <sup>b</sup>	Elongate sinuate
Point shaped <sup>d</sup>	Hooks, prickles and macro-hairs
Spherical rugose <sup>c</sup>	Globular granulate
Spherical crenate <sup>c</sup>	Globular echinate
Rectangular, smooth <sup>b</sup>	Elongate psilate (<30 µm long)
Rectangular, crenate <sup>b</sup>	Elongate sinuate (<30 µm long)
Tracheid	Cylindric sulcate tracheid

<sup>a</sup>Sensu Fredlund y Tieszen (1994).

<sup>b</sup>Sensu Twiss et al. (1969).

<sup>c</sup>Sensu Piperno (1988).

<sup>d</sup>Sensu Zucol (1996).

water and filtered with ashless filter paper, until no more chloride ions were detected. Finally, the material was ignited at 760 °C for 2.5 h. The final ashes were mounted with immersion oil and observed by using a Leitz Wetzlar D 35780 microscope. In total, 350–400 phytoliths per slide were counted, and then classified following the schemes given by Twiss et al. (1969), Piperno (1988), Fredlund and Tieszen (1994), Zucol (1996) and the descriptors given by Madella et al. (2005) (Table 1). The relative frequency of each phytolith morphotype was calculated. Silica content was calculated as dry weight percentage.

As a complement to phytolith description, histological and anatomical observations were carried out, applying routine techniques of histological analysis. The descriptions were based on Metcalfe (1960, 1963) and Ellis (1976, 1979). Sections were observed and photographed by microscopic Olympus CX 31, with digital camera Olympus Camedia C7070 Wide Zoom.

### 3.2. Soil samples collection and phytolith extraction

After a preliminary field study, two sample sites with a different history of use were chosen. One site was in Iguazú National Park (NP, 25°31'–25°43'S; 54°08'–54°32'W), where some part of the native forest suffered selective

extraction before the creation of the park in 1934 (Dimitri, 1974). The other site was in San Jorge Property (Alto Paraná S.A. logging company) (SJ, 25°50.49'S and 54°20.32'W). This site had a small area of native forest that suffered selective timber extraction at low intensity (Fig. 1). In both sites, topsoil samples (0–5 cm) were collected from two types of vegetal cover: gaps, where *C. ramosissima* was abundant, and closed canopy, where bamboo was absent or scarce. The gaps were characterized by impenetrable thickets with bamboo 2–3 m in height, generally presenting two rooted culms per square meter median number of *C. ramosissima*. The closed canopy areas were characterized by absent or scarce bamboo (0 or less than 0.5 *C. ramosissima* rooted culms per square meter). The forest areas sampled showed basal area values between 17 and 25.8 m<sup>2</sup>/ha (Montti, unpublished data; De los Santos et al., 2006). The common dominant canopy trees are *Nectandra megapotamica* (Spreng.) Mez., *Balfourodendron riedelianum* (Engl.) Engl. *Bastardiopsis densiflora* (Hook. & Arn.) Hassler, *Cedrela fissilis* Vell., *Lonchocarpus leucanthus* Burkart. *Alchornea iricurana* Casar., *Parapiptadenia rigida* (Beth) Brenan. Common subdominant tree species, especially in closed canopy forest, are *Sorocea bonplandii* (Bailon) Burg., *Trichilia catigua* Adr. Juss. and *Trichilia elegans* A. Juss., while open areas commonly have *Solanum* spp., and *Cecropya adenopus* Mart. ex Miq inside bamboo matrix.

Four soil samples were collected from Iguazú NP (two in gaps and two in closed canopy), and six were collected from San Jorge Property (three in each situation) (Table 2). All the soil samples correspond to the same topographic position and pedological unit.

After elimination of organic matter (with H<sub>2</sub>O<sub>2</sub> and heat), carbonates (with HCl) and clays (Calgon 0.5% and centrifugation at 1000 rpm for 3 min) from the soil samples, an aliquot of the material was mounted on Canada Balsam. Five hundred particles (including phytoliths and minerales) were counted and the relative frequency of phytoliths in the total sample was calculated. Then phytoliths were concentrated with sodium polytungstate ( $\rho = 2.3$ ) and the silica fraction was mounted on immersion oil. Between 450 and 500 phytoliths for each slide were counted and classified following Twiss et al. (1969), Piperno (1988), Fredlund and Tieszen (1994), and Zucol (1996) and the descriptors given by Madella et al. (2005) (Table 1).

### 3.3. Data analysis

Cluster analysis was carried out with the purpose of analyzing the grouping of soil samples. The Morisita association index and unweighted pair-group method, arithmetic average linkages were used. To establish the importance of morphotypes in the segregation of soil samples, principal component analysis (PCA) was performed, on the basis of a variance–covariance matrix.

Table 2  
Present community plant characterized over different soil sampling points

Study sites	Soil samples	Plant community characterization and distance between soil samples
Iguazú National Park (NP)	NPg1	Open canopy (Gap), understory dominated by <i>Chusquea ramosissima</i> (100%). Scarce ferns and <i>Urera</i> sp. were present.
	NPg2	Open canopy (Gap), <i>Chusquea ramosissima</i> was very abundant (more than 95%). Scarce <i>Piper</i> spp. and ferns were observed. Located at 800 m from NPc2 and several kilometers from NPg1 y NPc1.
	NPc1	Close canopy, <i>Chusquea ramosissima</i> very scarce (between 1% and <5%) in the understory. Few herbaceous bamboos and some vines, ferns and Piperaceae were present in the understory. Moraceae and palms occurred in the overstory.
	NPc2	Close canopy, <i>Chusquea ramosissima</i> was absent (0%). Some Fabaceae and Lauraceae were the dominated trees.
	SJg1	Open canopy (Gap), <i>Chusquea ramosissima</i> was very abundant (more than 95%). Big gap (100 m <sup>2</sup> ). Localized near SJc1 (150 m approximately).
	SJg2	Open canopy (Gap), <i>Chusquea ramosissima</i> was very abundant (more than 95%). Localized 300 m from SJc2. Some palms ( <i>Syagrus romanzoffiana</i> ) were present near the soil sample.
San Jorge Property (SJ)	SJg3	Open canopy (Gap), <i>Chusquea ramosissima</i> was very abundant (more than 95%). Localized 800 m from SJc3.
	SJc1	Close canopy, <i>Chusquea ramosissima</i> was very scarce (between 1% and <5%). Abundant vines, ferns, Piperaceae and <i>Pharus</i> sp. (basal grass) in the understory. Localized near SJg1 (150 m approximately).
	SJc2	Close canopy, <i>Chusquea ramosissima</i> was absent (0%). Few vines and ferns in the understory were observed.
	SJc3	Close canopy, <i>Chusquea ramosissima</i> was very scarce (between 1% and <5%).

NP: Corresponding to Iguazú National Park soil samples and SJ: San Jorge Property soil samples. The two type of vegetal cover over the soil samples are g: gap (with dominance of bamboo *Chusquea ramosissima*) and c: closed canopy (bamboo was either absent or rare). The numbers indicate different samples for same situation.

## 4. Results

### 4.1. Silica content, phytolith assemblage, and anatomical affinities in *C. ramosissima*

#### 4.1.1. Leaves

The leaves had the greater silica content, calculated as percentage of dry weight ( $19.4 \pm 3.12\%$ ). The phytolith assemblage was characterized by both isolated and articulated phytoliths (Table 3). The concave dumb-bell phytolith (sensu Piperno, 1988) was the most abun-

Table 3  
Relative frequency (%) of phytolith morphotypes from *Chusquea ramosissima* (Poaceae: Bambusoideae)

Phytolith morphotype	<i>Chusquea ramosissima</i> 's organs		
	Leaf	Culm	Root
<i>Isolated phytoliths</i>			
1. <i>Chusquea</i> bulliform	9.01 ± 4.96	0.00	0.00
2. Saddle	2.12 ± 0.82	0.00	0.00
3. Concave dumb-bell	29.36 ± 10.46	0.00	0.00
4. Tabular crenate	3.27 ± 0.65	0.00	0.00
5. Panicoid dumb-bell	12.19 ± 1.53	0.89 ± 0.59	0.00
6. Elongate	0.31 ± 0.13	29.29 ± 5.44	0.00
7. Tabular crenate verrucate	0.00	0.31 ± 0.03	0.00
8. Poind shaped	0.60 ± 0.23	1.39 ± 0.70	0.00
9. Others	0.67 ± 0.31	1.65 ± 1.32	0.00
<i>Articulated phytoliths</i>			
10. <i>Chusquea</i> bulliform	4.12 ± 2.70	0.00	0.00
11. Saddle	0.88 ± 0.88	0.00	0.00
12. Concave dumb-bell	4.09 ± 1.37	0.00	0.00
13. Tabular crenate	2.13 ± 1.03	0.00	0.00
14. Panicoid dumb-bell	11.29 ± 2.49	0.00	0.00
15. Stomatal complex	1.05 ± 0.53	0.57 ± 0.24	0.00
16. Long and short cells	0.00	30.05 ± 10.31	0.00
17. Tabular crenate verrucate	0.00	4.69 ± 3.33	0.00
18. Elongate psilate	0.00	16.12 ± 1.95	1.00 ± 0.00
19. Short cells	0.00	5.26 ± 1.20	0.00
20. Endodermis/pericycle element	0.00	0.00	99.00 ± 0.00
21. Others	18.91 ± 4.73	10.69 ± 5.94	0.00

Values shown are the means ± S.E. ( $n = 4$ ).

dant isolated morphotype (Table 3 and Fig. 2A and B). In the tissue, these phytoliths were found along a vertical position in the intercostal zone of both epidermises (Fig. 2G).

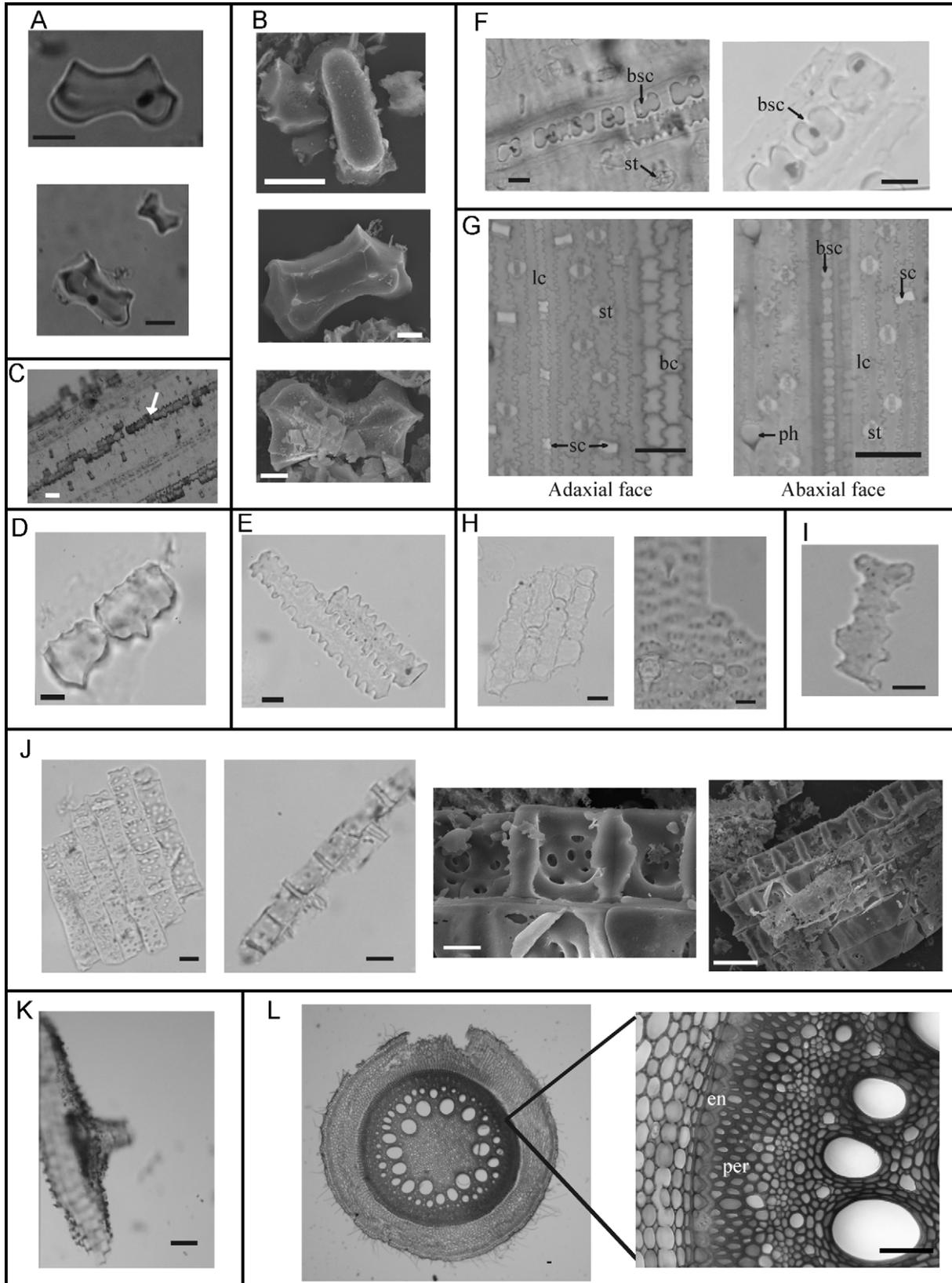
The panicoid dumb-bells (bilobate flat/concave ends short cell phytoliths) were the second most abundant morphotype (Table 3). They represent the silica cells located in the costal zone of the epidermis (Fig. 2F and G). *Chusquea* bulliform phytoliths (cubic/parallelepipedal sinuate bulliform cells) were abundant, both isolated or in articulated form (Table 3 and Fig. 2C and D). In a smaller proportion, the phytolith assemblage was characterized by the presence of saddles and tabular crenate phytoliths (Table 3 and Fig. 2E). These phytolith morphotypes correspond to the vertical silica bodies and long rectangular epidermic cells of winding walls of the costal and intercostal zone, respectively (Fig. 2G). Silicification of hairs was also observed. Within the articulated phytoliths, fragments formed by several of the morphotypes previously mentioned, were observed (Table 3, Fig. 2C and F).

#### 4.1.2. Culm

The culm had the least content of silica ( $6 \pm 0.92\%$ ). As observed in leaves, isolated and articulated phytoliths were counted (Table 3). The dominant morphotype in the phytolith assemblage were the elongate smooth phytoliths, in articulated or isolated form. These morphotypes

correspond to the epidermic cells (square cells and common epidermic cells) and also to fibers. Within the articulated phytoliths, short and long cells were the dominant morphotypes. In a lesser proportion, the phytolith

assemblage of the culm was composed of tabular crenate verrucate phytoliths (Fig. 2H and I) isolated or articulated, articulated short cells and vascular tissue with articulated short and long cells.



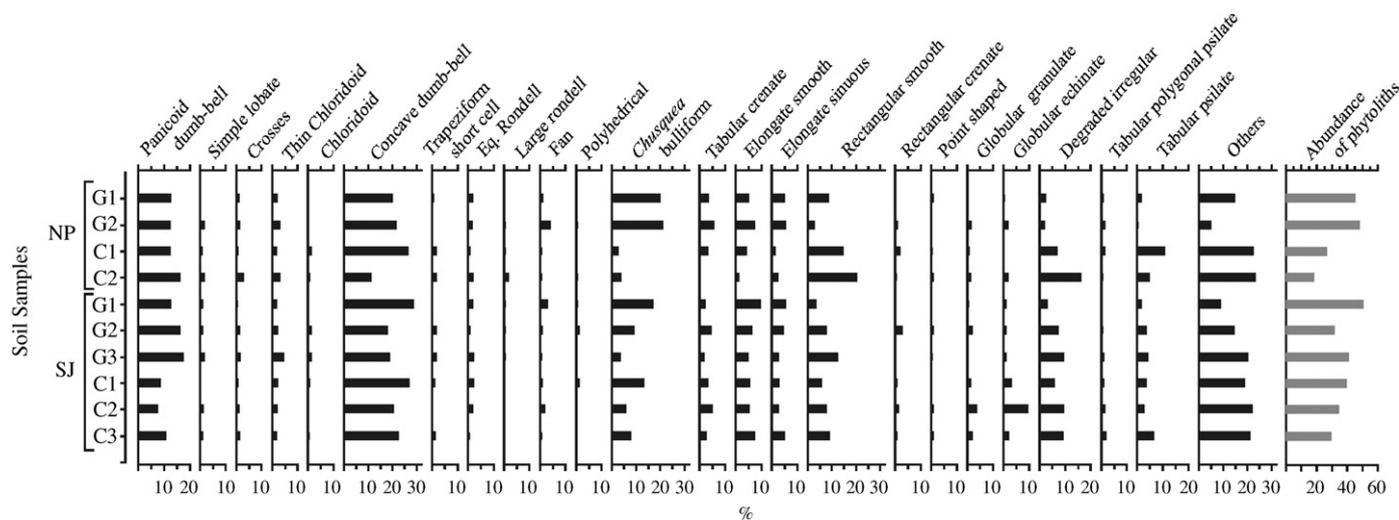


Fig. 3. Relative frequency of soil phytolith morphotypes NP: Iguazú National Park site and SJ: San Jorge Property site, g: gaps with dominance of *Chusquea ramosissima*, c: closed canopy (bamboo was either absent or rare). The numbers indicate different samples for same situation.

4.1.3. Roots

In the roots, the average silica content was between the percentage found in leaves and in culm ( $9.9 \pm 0.52\%$ ). The phytolith assemblage of this organ was characterized by articulated elements belonging to the endodermis and also, probably, to the multiseriate pericycle tissue. They consist of cylindrical elements with irregularly thickened walls, some in a U-shape (derivatives of endodermis), and others in the shape of a complete ring (possibly derived from pericycle), in both cases with perforations in the walls (Fig. 2J, K, and L). In a very small proportion (<1%) elongate smooth phytoliths (fibers) were observed (Table 3).

4.2. Soil phytolith assemblage

The abundance of phytoliths in relation to mineral particles of the soil samples was higher in gap samples than in closed canopy samples. These differences were clearer between the Iguazú National Park (NP) samples (Fig. 3).

The dominant phytolith morphotypes in all the samples were the concave dumb-bell phytoliths, except in the second sample of the Iguazú National Park close canopy (NPC2 sample), where the greater proportion of phytoliths were rectangular smooth (<30 μm long), probably broken

phytoliths, and degraded irregular phytoliths, possibly originated in trees or other dicotyledons species. The second dominant morphotype was the panicoid dumb-bells, while the *Chusquea* bulliform phytoliths predominate in some samples of gaps (Figs. 3 and 4).

In general, the differences between the gap samples (NPg1 and NPg2) and the closed canopy samples (NPC1 and NPC2) in the Iguazú National Park were due to the abundance of *Chusquea* bulliform, rectangular smooth (<30 μm long), degraded irregular phytoliths, and tabular psilate phytoliths. The first ones (*Chusquea* bulliform and rectangular smooth) were more abundant in gaps, whereas the rest were several times more abundant in closed canopy. These differences reflect the greater dominance of *Chusquea* sp. in gaps when compared with closed canopy samples. However, phytoliths (such as concave dumb-bells and panicoid dumb-bells) derived from *Chusquea* sp. or other species of bamboo were observed not only in gaps but also in closed canopy samples (Figs. 3 and 4).

In San Jorge Property, the samples corresponding to gaps had a greater abundance of panicoid dumb-bells than the closed canopy samples, where the proportion of globular phytoliths was higher. The panicoid dumb-bells are commonly associated with panicoid grasses, but they are also present in bamboo, whereas the globular

Fig. 2. Phytolith morphotypes of *Chusquea ramosissima* obtained by calcinations technique and their location in the plant tissues. (A–F) Leaf phytoliths: (A–B) Concave dumb-bells (Trapeziform bilobate echinate). (C) Articulated and isolated phytoliths. The white arrow shows articulated *Chusquea* bulliform (cubic/parallepedal sinuate bulliform cell). (D) *Chusquea* bulliform phytolith. (E) Articulated tabular crenate phytoliths. (F) Articulated panicoid dumb-bells (bilobated flat/concave ends short cell). (G) Leaf epidermal in paradermical sight (adaxial and abaxial surface). The silica cells corresponding to long cells, hairs, bulliform cells, stomatal complex, and some morphotype of silica bodies. (H–I) Culm phytoliths from calcinated material. (H) Articulated tabular crenate verrucate phytoliths from culm. (I) Isolated tabular crenate verrucate phytoliths from culm. (J–K) Root phytoliths: silicification of endodermis and/or pericycle. (L) Transversal cross section of root. General view and inner cortex in detail showing the inner walls cells of the endodermis thickened in U shape and multiseriate pericycle. lc, long cells; ph, prickle-hairs; bc, bulliform cells; st, stomatal complex; isc, intercostall silica cells in vertical position; csc, costal silica cell in horizontal position; pdb, panicoid dumb-bells phytolith; sc, silica cells; en, endodermis; per, pericycle. Scale bar = 10 μm in A, C, D, E, F, H, I, J, and K; 5 μm in B; 1 mm in G, L.

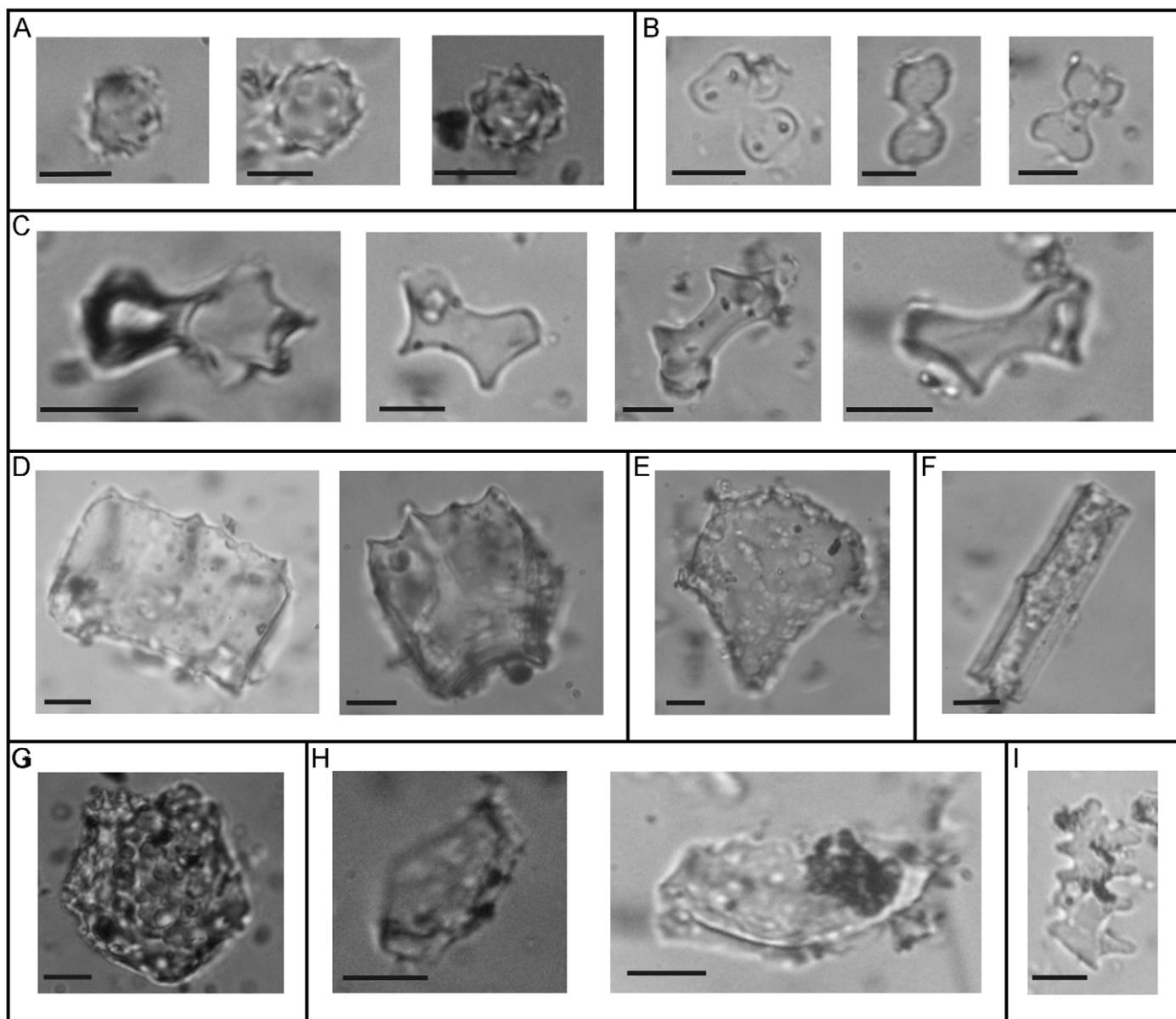


Fig. 4. Phytolith morphotypes in the soil samples: (A) Globular phytoliths. (B) Panicoid dumb-bells (bilobate flat/concave ends short cell). (C) Concave dumb-bells (Trapeziform bilobate echinate). (D) *Chusquea* bulliform (cubic/parallelepipedal sinuate bulliform cell). (E) Fan phytolith. (F) Elongate smooth (elongate psilate). (G) Degraded irregular phytoliths. (H) Tabular psilate. (I) Tabular crenate. Scale bar = 10  $\mu$ m.

phytoliths derive mainly from palms and other tropical species (Piperno, 1988; Runge, 1999) (Figs. 3 and 4).

The cluster analysis shows four groups (Fig. 5). Group 1, formed by NPC2, represents the most distinct group. This sample was characterized by the lowest percent of concave dumb-bells; tabular crenate and elongate smooth phytoliths, and the highest percent of crosses, large rondels, degraded irregular and rectangular smooth phytoliths. Group 2 was formed by SJc2, which differs from the others by having the lowest abundance of panicoid dumb-bells and the highest percent of globular phytoliths. These two groups (1 and 2) constituted unique samples without any species of bamboo or grasses at present. Group 3 was constituted by NPC1, SJc3, SJg2, and SJg3 samples and was characterized by the highest percent of panicoid dumb-bells

and elongate smooth phytoliths. This was the most heterogeneous group because it includes samples from gaps as well as from closed canopy of both sample sites. The close canopy samples (NPC1, SJc3) had some herbaceous bamboos and *C. ramosissima* in very scarce proportions (Table 2); so they were grouped with samples with a high density of bamboo (gaps). Group 4 was formed by NPg1, NPg2, SJc1, and SJg1, and was characterized by the highest values of *Chusquea* bulliform phytoliths and low values of elongate smooth and degraded irregular phytoliths. All of these samples, except SJc1, showed a very high cover of *C. ramosissima* bamboo.

The first three axes of the PCA amount to 90% of the total variance (63% for axis 1, 17% for axis 2, and 10% for axis 3). As observed in the cluster analysis, NPC2 was the

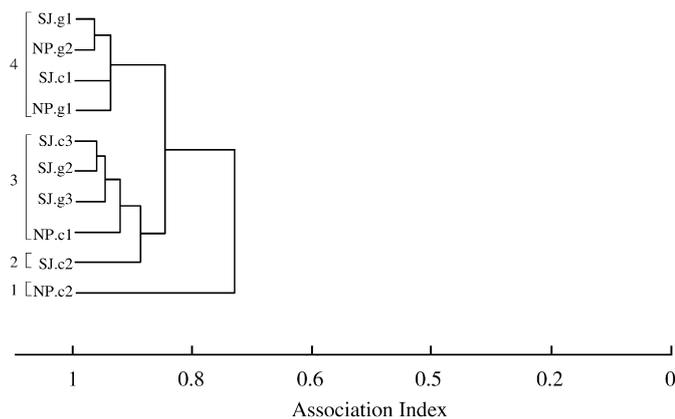


Fig. 5. Dendrogram showing soil samples grouping based on their phytolith assemblages. NP: Iguazú National Park site, SJ: San Jorge Property site, g: gap (with dominance of bamboo *Chusquea ramosissima*), c: closed canopy (bamboo was either absent or rare).

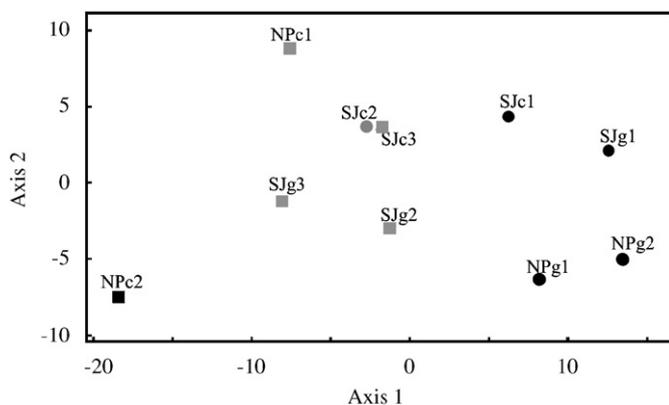


Fig. 6. Principal components analysis (PCA) of soil samples based on their phytolith assemblages. NP: Iguazú National Park site, SJ: San Jorge Property site, g: gap (with dominance of bamboo *Chusquea ramosissima*), c: closed canopy (bamboo was either absent or rare).

most distinct sample (Fig. 6). Although it was not possible to separate samples from different sampling sites (NP and SJ sites), axis 2 manages to separate gaps and closed canopy samples, except for NPc2. This separation was given mainly by concave dumb-bells, *Chusquea* bulliform, panicoid dumb-bells, and tabular psilate phytoliths. The morphotypes that contribute mainly to axis 1 were *Chusquea* bulliform, rectangular smooth (<30  $\mu\text{m}$  long), degraded irregular and concave dumb-bell phytoliths.

## 5. Discussion

The woody bamboos, like other grasses, accumulate Si in their tissues and cells in different proportions; the leaves having the highest content of this element (Twiss et al., 1969; Takahashi et al., 1981; Piperno, 1988; Motomura et al., 2000, 2004). The silica content of *C. ramosissima* leaf was higher (19%) than the values reported for other grasses (3.38–13.93% dry weight), evaluated with the same technique (Gallego and Distel, 2004; Fernández Honaine

et al., 2005a). In roots, the values found in *C. ramosissima* were similar to the *Paspalum quadrifarium* ones (9.21%), while the silica content for the culm was the smallest of the three studied organs, but fairly high compared with *P. quadrifarium* (1.19%) (Fernández Honaine et al., 2005b). The values for leaves and roots in other bamboos, found by means of other methodologies, oscillated between 2.4% and 15.9% dry weight (Ueda and Ueda, 1961; Takahashi et al., 1981; Motomura et al., 2000; Lux et al., 2003). In agreement with other studies, *C. ramosissima* presents the highest degree of silicification in the epidermic tissue of the leaves and culm (Bennet and Sanger, 1981; Motomura et al., 2000; Lux et al., 2003). In the root, however, deposition has taken place not only in the endodermis (as described in other species) (Lux et al., 2003), but also in the pericycle.

In leaves, the main phytolith morphotypes described (concave dumb-bells, panicoid dumb-bells and *Chusquea* bulliform) are the same as the ones observed by Piperno (1988) and Piperno and Pearsall (1998) for other species of the same genus or for other tropical bamboos. These authors argue that these phytolith can be diagnostic of Bambusoideae subfamily.

Although some authors have studied the silica content in different tissues in roots and rhizomes of some bamboos (Lux et al., 2003), the descriptions of phytoliths in the root and culm of *C. ramosissima* constitute the first contribution to the knowledge of phytolith morphotypes in these organs for this subfamily.

Gap soil samples, collected in areas where *C. ramosissima* was dominant (>95% of plant cover), have the highest phytolith content. These results would indicate that the main phytolith contribution by plant communities in this area comes from the mentioned species. The dominant species in closed canopy sites, trees and dicotyledons, have a phytolith production several times lower than the one in grasses, the family where bamboos belong (Piperno, 1988). The lower phytolith production of the dominant species in these sectors could account for the lower abundance of phytoliths in their soil phytolith assemblages.

When analyzing the soil samples, characterized by the presence of *C. ramosissima* in different proportions, only the morphotypes corresponding to the leaves were found. Culm phytoliths, like elongate morphotypes, were not possible to distinguish from the ones produced by other grasses. Root and other culm morphotypes (such as tabular crenate verrucate phytoliths) were not observed on soil samples. Two possible answers might explain this absence. On one hand, the leaf represents the greatest phytolith producer of the three studied organs, and constitutes the main contributor to the phytolith assemblage of the soil. On the other hand, the phytolith morphotypes of root and some phytoliths of culms are more fragile, and this is the reason why they are more susceptible to degradation, especially in these soils with a high Fe content (Piperno, 1988; Ligier 1999).

The cluster and PCA analyses did not allow a clear differentiation between samples taken in two sites with a different history of use and management (San Jorge Property and Iguazú National Park). However, it was possible to differentiate sectors with different abundance from *Chusquea* sp. based on phytolith assemblages of soil samples within these two studied sites. In San Jorge, the gaps and closed canopy soil samples could be told apart by the abundance of panicoid dumb-bells and globular phytoliths. In the Iguazú National Park, the differences were made evident by the abundance of *Chusquea* bulliform phytoliths in gaps and of tabular psilate phytoliths in closed canopy. Although concave dumb-bells (*Chusquid* body *sensu* Piperno and Pearsal 1998) are one of the diagnostic morphotypes of *Chusquea* sp., they were present in all the samples, even those in which *C. ramosissima* is actually absent, as NPc2 and SJc2. These results could indicate that concave dumb-bell is not a conclusive proof of the presence or absence of *C. ramosissima*, whereas the panicoid dumb-bells in SJ samples and *Chusquea* bulliform phytoliths in NP samples seem to be more sensitive to these variations. Concave dumb-bells are more robust morphotypes and they are probably better preserved during a longer time-span than the other two morphotypes.

The groups that include samples from gaps as well as from closed canopy (i.e. group 3) can occurred because close canopy samples presented herbaceous bamboos and *C. ramosissima* in very scarce proportion (Table 2). The separation of the NPc2 sample from the other samples was due to a total absence of *C. ramosissima* at present; nevertheless, phytoliths (such as concave dumb-bells) derived from this species or other bamboos were observed in their phytolith assemblage. This could indicate the occurrence of bamboo (especially herbaceous bamboos) at some moment previous to the sampling. It is also possible that the top 5 cm of soil analyzed represents a time span that includes changes in the forest structure, showing a shift between gaps and closed canopy. *C. ramosissima* may have occupied sectors that are actually closed canopy, reflecting a dynamic shifting, a process commonly observed nowadays. Lastly, different processes could disperse bamboo phytoliths from remote areas.

The results indicate that the soil phytolith assemblage in the study area is constituted not only by the phytoliths produced by local and current vegetation, but also by phytoliths produced by recent past vegetation (developed during the formation of the 0–5 cm of soil analyzed, which may or may not be the same as the one now present) and phytoliths derived from other sectors. For instance, phytoliths can also be brought to soil by diverse agents (water, wind, and animals) by means of assorted processes such as surface runoff and animal movement. All of these processes may explain the similarity of the phytolith assemblage of two sectors with different abundance of *C. ramosissima*, but very close in the field, such as SJg1 and SJc1.

## 6. Conclusions

The present study is one of the first contributions to the description of phytolith assemblages of whole specimens of bamboo and the relationships between phytolith morphotypes and plant tissue. This information contributes to the knowledge of phytolith morphotypes of one of the main producers of the Bambusoideae subfamily. The three studied organs (leaf, culm, and root) produce different phytolith morphotypes, the leaf being the main phytolith producer, as described in previous studies.

In the soil, only phytoliths originated in the leaf were observed. The absence of some morphotypes from culm and root could be due to their limited production in these organs, in comparison with the leaf, or to degradation due to relative fragility. Although concave dumb-bells are the characteristic phytoliths of *C. ramosissima*, their greater robustness and higher preservation in the soil—even in situations where this species is absent at the present time—does not allow clear discrimination between sites with or without this species. However, panicoid dumb-bells in SJ samples and *Chusquea* bulliform phytoliths in NP samples seem to be more sensitive to the changes in the presence/absence of this species.

Lastly, the study represents the first analysis of the phytolith assemblage in modern soils from Upper Paraná Atlantic Forest ecoregion. This information is crucial for the interpretation of fossil phytolith records in the region and for the understanding of the history and past distribution of native bamboos in this forest region of Argentina. These studies will allow interpretation of the abundance of this species in the past prior to the timber extraction and understanding in this way the role and the function of bamboo in the dynamics of the forest.

## Acknowledgments

We thank The Rufford Foundation, CONICET and a project from Universidad Nacional de Mar del Plata (EXA292/04) for supported these studies. We are also grateful to Alto Paraná S.A., to the Centro de Investigaciones Ecológicas Subtropicales (CIES) of the National Park Administration (APN). We also want to thank Paula Campanello, Fernanda Alvaréz, Diego Larraburru, Veronica Bernava, Mariana Villagra, Andrea Izquierdo, Agustin Paviolo and Genoveva Gatti by provided valuable help to conduct these studies, and two anonymous reviewers who provided valuable comments on the manuscript.

## References

- Bennet, D.M., Sangster, A.G., 1981. The distribution of silicon in the adventitious roots of the bamboo *Sasa palmata*. *Canadian Journal of Botany* 59, 1680–1684.
- Bertoldi de Pomar, H., 1975. Los silicofitolitos: sinopsis de su conocimiento. *Darwiniana* 19, 173–206.

- Campanello, P., 2004. Diversidad, crecimiento y fisiología de árboles en la Selva Misionera: efectos de los cambios en la estructura y funcionamiento del ecosistema producidos por la invasión de lianas y bambúseas. Ph.D. Thesis, Universidad de Buenos Aires, Buenos Aires, Argentina.
- Campanello, P., Montti, L., Gatti, M.G., Goldstein, G., 2005. Efectos de la tala selectiva sobre la estructura y funcionamiento del bosque nativo en Misiones: desarrollo de técnicas de manejo forestal sustentable. 3° Congreso Forestal Argentino y Latinoamericano (AFoA), 10pp.
- Campanello, P., Montti, L., Gatti, M.G., Bravo, S., Goldstein G., 2006. Forest structure and functioning in the Semideciduous Atlantic Forest of Northern Argentina: effects of native invasive bamboos and lianas on the tree regeneration and diversity. Abstracts Understanding Biodiversity Loss: A Workshop on Forest Fragmentation in South America, Argentina, 2pp.
- Campanello, P., Gatti, M.G., Ares, A., Montti, L., Goldstein, G., 2007. Tree regeneration and microclimate in a liana and bamboo-dominated semideciduous Atlantic Forest. *Forest Ecology and Management* 256, 108–117.
- De los Santos, C., Campanello, P., Montti, L., Foletto, F., Larraburu, D., 2006. Estructura arbórea y presencia de lianas en dos bosques del norte de Misiones con diferente estado de conservación. 12 Jornadas Técnicas, Forestales y ambientales. Eldorado Misiones, Argentina.
- Dimitri, M.J., 1974. La flora arbórea del Parque Nacional Iguazú. *Anales de Parques Nacionales* 12, 1–188.
- Ellis, R., 1976. A procedure for standardizing comparative leaf anatomy in the Poaceae I: the leaf blade as viewed in transversal section. *Bothalia* 12 (1), 65–109.
- Ellis, R., 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae II: the epidermis as seen in surface view. *Bothalia* 12 (4), 641–647.
- Fernández Honaine, M., Bernava Laborde, V., Zucol, A., 2005a. Silica content in native grasses from SE Buenos Aires province, Argentina. *The Phytolitharian* 17 (2), 20.
- Fernández Honaine, M., Zucol, A., Osterrieth, M., 2005b. Estudios Fitolíticos en *Paspalum quadrifarium* Lam. (Poaceae: Panicoideae). *Boletín de la Sociedad Argentina de Botánica* 40 (Suppl.), 185.
- Fredlund, G.G., Tieszen, L.T., 1994. Modern phytolith assemblages from the North American Great Plains. *Journal of Biogeography* 21, 312–335.
- Gallego, L., Distel, R.A., 2004. Phytolith assemblages in grasses native to Central Argentina. *Annals of Botany* 94, 865–874.
- Gallego, L., Distel, R.A., Camina, R., Rodríguez Iglesias, M., 2004. Soil phytoliths as evidence for species replacement in grazed rangelands of central Argentina. *Ecography* 27, 725–732.
- Judziewicz, E.J., Clark, L.G., Londoño, X., Stern, M.J., 1999. *American Bamboos*. Smithsonian Institution Press, Washington.
- Labouriau, L.G., 1983. Phytolith work in Brazil: a mini review. *The Phytolitharian Newsletter* 2, 6–10.
- Ligier, H.D., Matteio, H.R., Polo, H.L., Rosso, J.R., 1990. Provincia de Misiones. In: Atlas de suelos de la República Argentina, Tomo II. Secretaría de Agricultura, Ganadería y Pesca. Proyecto PNUD Arg.85/019. INTA, Centro de Investigaciones de Recursos Naturales, pp. 111–154.
- Ligier, H.D., 1999. Caracterización geomorfológica y edáfica de la provincia de Misiones. Fundación Vida Silvestre Argentina, Buenos Aires, Argentina.
- Londoño, X., Clark, L., 2004. Distribución, Taxonomía, Anatomía, Silvicultura y usos de los bambúes del Nuevo Mundo. III Congreso Colombiano de Botánica, Popayán, 25pp.
- Lux, A., Luxová, M., Abe, J., Morita, S., Inanaga, S., 2003. Silicification of bamboo (*Phyllostachys heterocyclus* Mitf.) root and leaf. *Plant and Soil* 255, 85–91.
- Madella, M., Alexandre, A., Ball, T., 2005. International code for phytolith nomenclature 1.0. *Annals of Botany* 96, 253–260.
- Metcalf, C.R., 1960. *Anatomy of Monocotyledons 1. Graminae*. Clarendon Press, Oxford.
- Metcalf, C.R., 1963. Comparative anatomy as a modern botanical discipline with special reference to recent advances in the systematic of monocotyledons. *Advances in Botanical Research* 1, 101–147.
- Motomura, H., Fujii, T., Susuki, M., 2000. Distribution of silicified cells in the leaf blades of *Pleioblastus chino* (Franchet et Savatier) Makino (Bambusoideae). *Annals of Botany* 85, 751–757.
- Motomura, H., Mita, N., Susuki, M., 2002. Silica acumulation in long-lived leaves of *Sasa veichii* (Carriere) Rehder (Poaceae, Bambusoideae). *Annals of Botany* 90, 149–152.
- Motomura, H., Fujii, T., Susuki, M., 2004. Silica deposition in relation to ageing of leaf tissues in *Sasa veichii* (Carriere) Rehder (Poaceae, Bambusoideae). *Annals of Botany* 93, 235–248.
- Mulholland, S.C., 1989. Phytolith shape frequencies in North Dakota grasses: a comparison to general patterns. *Journal of Archaeology Science* 16, 489–511.
- Piperno, D.R., 1988. *Phytolith Analysis: An Archeological and Geological Perspective*. Academy Press, San Diego.
- Piperno, D.R., Pearsall, D., 1998. *The Silica Bodies of Tropical Grasses: Morphology, Taxonomy and Implications for Grass Systematic and Fossil Phytolith Identification*. Smithsonian Contribution of Botany, No. 85. Smithsonian Institution Press, Washington, DC.
- Rovner, I., 1971. Potential of opal phytolith for use in paleoecological reconstruction. *Quaternary Research* 1, 343–359.
- Runge, F., 1999. The opal phytolith inventory of soils in central Africa quantities, shapes, classification and spectra. *Review of Palaeobotany and Palynology* 107, 23–53.
- Takahashi, E., Tanaka, T., Miyake, Y., 1981. Distribution of silicicolous plants in plant kingdom (5). *Journal of the Science of Soil and Manure, Japan* 52, 503–510.
- Twiss, P.C., 1992. Predicted world distribution of C3 and C4 grass phytoliths. In: Rapp, G., Mulholland, S. (Eds.), *Phytolith Systematic*. Plenum Press, pp. 113–128.
- Twiss, P.C., Suess, E., Smith, R.M., 1969. Morphological classification of grass phytoliths. *Soil Science Society of America Proceedings* 33, 109–115.
- Ueda, K., Ueda, S., 1961. Effect of silicic acid on bamboo growth. *Bulletin of the Kyoto University Forest* 33, 79–99.
- Zucol, A., 1996. Microfitolitos de las Poaceae argentinas: I. Microfitolitos foliares de algunas especies del género *Stipa* (Stipeae: Arundinoideae), de la Provincia de Entre Ríos. *Darwiniana* 34, 151–172.