Annals of Botany **98**: 1155–1165, 2006
doi:10.1093/aob/mcl207, available online at www.aob.oxfordjournals.org

Phytolith Assemblages and Systematic Associations in Grassland Species of the South-Eastern Pampean Plains, Argentina

MARIANA FERNÁNDEZ HONAINE[1,*,†], ALEJANDRO F. ZUCOL[2,†]
and MARGARITA L. OSTERRIETH[1]

[1]*Centro de Geología de Costas y del Cuaternario, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CC 722, Correo Central, (7600) Mar del Plata, Buenos Aires, Argentina,* [2]*Laboratorio de Paleobotánica, Centro de Investigaciones Científicas (CICYTTP-CONICET), Dr. Matteri y España (E3105BWA) Diamante, Entre Ríos, Argentina*

Received: 16 June 2006 Returned for revision: 13 July 2006 Accepted: 16 August 2006 Published electronically: 9 October 2006

• *Background and Aims* Phytolith descriptions of South American plant species are scarce. This knowledge is crucial for the interpretation of the fossil phytolith record of a region. In this study phytolith assemblages and systematic relationships of the main grasses and Asteraceae species of *Paspalum quadrifarium* grassland are described.
• *Methods* Phytoliths from leaves of Poaceae and Asteraceae species were extracted by using a calcination technique. For each species, 350–400 phytoliths were counted and the relative frequency of each morphotype was calculated. Phytolith assemblages were subject to principal components analysis (PCA) and cluster analysis (Morisita index).
• *Key Results* PCA and cluster analysis showed family (Poaceae vs. Asteraceae) and subfamily (within Poaceae) differentiation. Exceptions to general trends described for other species were detected. Floristic variants of *Paspalum quadrifarium* grassland can be separated by their phytolith assemblages.
• *Conclusions* The study provides a reference collection of phytolith assemblages of the main Poaceae and Asteraceae species of *Paspalum quadrifarium* grassland and describes some phytolith morphotypes/systematic relationships useful for the analysis of fossil phytolith assemblages of the Pampean region.

Key words: *Paspalum quadrifarium*, Asteraceae, Poaceae, phytolith assemblages, Argentina, Southern Pampa.

INTRODUCTION

Phytoliths are biominerals deposited in plant tissues, most of them compounded by hydrated SiO_2 (Parry and Smithson, 1964; Blackman, 1971; Piperno, 1988). Following degradation of the tissue, the phytoliths are incorporated into the soil, where they can be preserved for thousands of years in depositional environments that do not have high alkalinity. Thus, it is possible to use these microfossils as indicators of the plant communities of the past, in palaeoenvironmental, palaeobotanical or archaeological studies (Twiss *et al*., 1969; Rovner, 1971; Piperno, 1988; Fredlund and Tieszen, 1994; Alexandre *et al*., 1997; Barboni *et al*., 1999; Carter, 2000).

Many studies relate phytolith morphotypes with plant families, and within the Poaceae, with subfamilies and genera (Twiss *et al*., 1969; Brown, 1984; Mulholland, 1989; Mulholland and Rapp, 1992; Fredlund and Tieszen, 1994). In addition, it has been possible to establish a relationship between morphotypes and the C_3 and C_4 photosynthetic pathways, which allows the interpretation not only of the changes in plant communities through time, but also the interpretation of climatic changes (Twiss, 1992; Fredlund and Tieszen, 1994).

These relationships between phytolith morphologies and plant families have seldom been studied in South America, where those studies that do exist generally only describe the phytolith assemblages of some groups of plants. These are confined to the research of Campos and Labouriau (1969) and Sondahl and Labouriau (1970) in Brazil and Zucol (1996, 1998, 1999, 2000, 2001) and Gallego and Distel (2004) in Argentina. Although phytolith studies of Northern Hemisphere species are numerous, in some cases extrapolating these phytolith morphology/plant family relationships is difficult owing to differences in the species that form the plant communities. This means that a detailed, exhaustive description of the phytolith assemblages of local species is crucial for interpretation of the regional fossil phytolith record.

The *Paspalum quadrifarium* grassland (‘pajonal de paja colorada’) remains one of the few relictual communities of the Pampean Plains; therefore, its study is of great ecological importance in understanding the characteristics of the grasslands of Buenos Aires Province (Argentina) (Laterra *et al*., 1998). In the southern Pampas, this grassland is mixed with the ‘flechillar’, a community dominated by species of the genera *Piptochaetium* and *Stipa*, among other grasses. In some sectors, where these communities are developed, agricultural practices of winter burning or grazing are frequent employed. These human activities generate new floristic variants of the typical communities (Laterra *et al*., 1998; L. Herrera, Universidad Nacional de Mar del Plata, unpubl. res.).

The aim of this study was to identify the phytolith morphotypes of the ‘pajonal’ and ‘flechillar’ species and

---

* For correspondence. E-mail fhonaine@mdp.edu.ar
† These authors are employed by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).

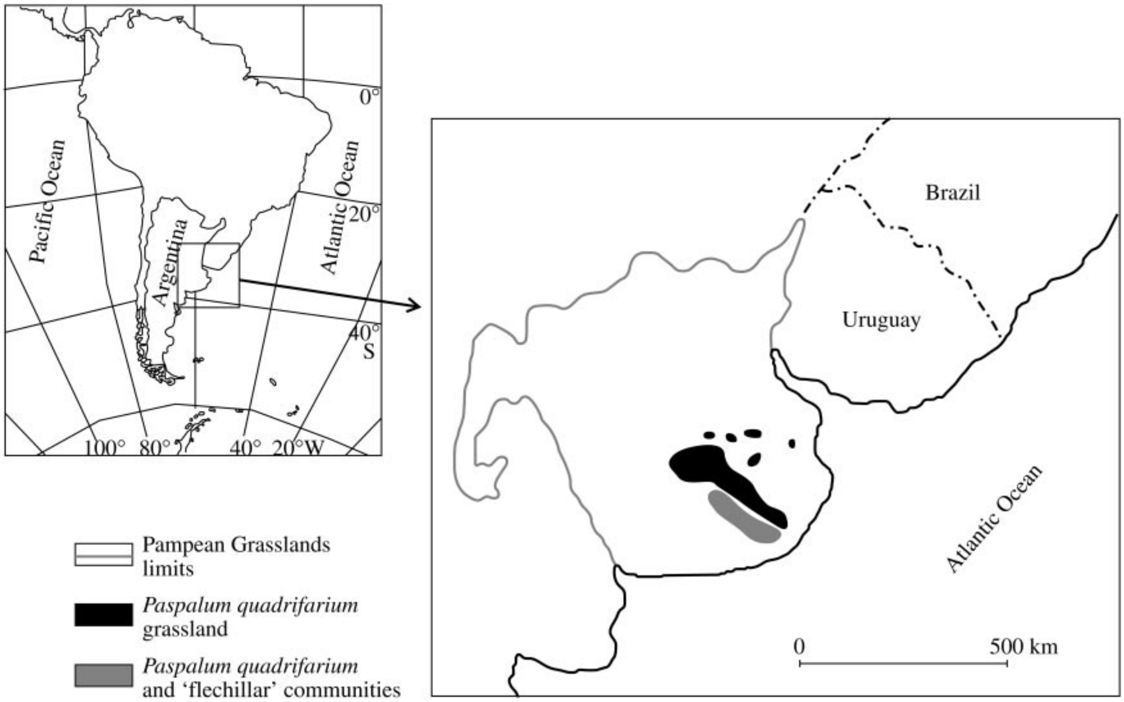

FIG. 1. Distribution of *Paspalum quadrifarium* and 'flechillar' communities within the Argentine Pampean region (Soriano *et al*., 1991). Black shading: distribution of *Paspalum quadrifarium* grassland in Flooding Pampa as defined by Vervoorst (1967); grey shading: distribution of *Paspalum quadrifarium* grassland mixed with 'flechillar' community along the Tandilia System as given by Frangi (1975) and Herrera and Laterra (2005).

their variants, so that they can be used in palaeoecological studies of the region. The results will be compared with studies of phytolith assemblages and systematics from Northern Hemisphere species.

MATERIALS AND METHODS

Study area and plant community features

The *Paspalum quadrifarium* grassland is a monospecific community that mixes with the 'flechillar'. Its distribution in the southern Pampas is largely restricted to the foothills of the Tandilia System, where the poorly developed soil has prevented its replacement by crops (Frangi, 1975; L. Herrera, unpubl. res.) (Fig. 1). However, some areas of the region are grazed, or subject to winter burnings to increase forage quality (Laterra *et al*., 1998). As a consequence, the floristic characteristics have been modified, with a noticeable increase in the number of Asteraceae species at the expense of native Poaceae species. At burned sites, there is a predominance of *Achillea* sp., whereas at grazed sites the main species found are *Carduus acanthoides*, *Cirsium vulgare* and *Taraxacum officinale*, accompanied by grasses such as *Dactylis glomerata* (L. Herrera, unpubl. res.).

Species selection and phytolith extraction

The most abundant species that characterize the community variants were selected according to previous floristic data (L. Herrera, unpubl. res.). Specimens from 23 species at flowering or seedling stage were collected from the Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria, Balcarce, Buenos Aires Province (EEA-INTA Balcarce-BAL herbarium) and the south-eastern hills of Buenos Aires Province (37°19′S, 59°15′W and 37°50′S, 58°30′W) (Table 1). For each specimen, two leaves below the leaf flag (in the Poaceae) and the five oldest leaves (in the Asteraceae) were sampled. The leaves 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 hours, and charred at 200 °C for 2 h. Later, it was boiled in a 5 N HCl solution for 10 min, washed with distilled 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 (1992) and Zucol (1996, 2001), and some descriptors used by Madella *et al*. (2005) (Table 2). The relative frequency of each phytolith morphotype was calculated.

Data analysis

Cluster analysis was carried out with the purpose of analysing the discriminate and taxonomic value of phytolith morphotypes in the species and subfamilies. The Morisita association index and unweighted pair-group

TABLE 1. *Species examined for phytoliths, which are dominant in burned sites (B), grazed sites (G), flechillar community in non-management sites (F) and pajonal community in non-management sites (P)*

Family	Subfamily (Watson and Dallwitz, 1992)	Species	Abbrev.		C_3/C_4
Asteraceae		*Achillea* sp.	Ach	B	
		Carduus acanthoides L.	Ca	B, G	
		Cirsium vulgare (Savi) Ten.	Cv	G	
		Taraxacum officinale Web.	To	G	
Poaceae	Arundinoideae	*Danthonia montevidensis* (D.C.) et Lam.	Dm	F	C_3
	Panicoideae	*Bothriochloa laguroides* (D.C.) Pilger	Bl	F, G	C_4
	Panicoideae	*Paspalum quadrifarium* Lam.	Pq	P	C_4
	Panicoideae	*Sorghastrum pellitum (*Hack.) Parodi	Sop	F	C_4
	Pooideae	*Briza subaristata* Lam.	Bs	F	C_3
	Pooideae	*Bromus auleticus* Trin.	Ba	F	C_3
	Pooideae	*Bromus catharticus* Vahl.	Bc	F	C_3
	Pooideae	*Dactylis glomerata* L.	Dg	F, G	C_3
	Pooideae	*Festuca arundinacea* Schreb.	Fa	F	C_3
	Pooideae	*Hordeum pusillum* Nutt	Hp	F	C_3
	Pooideae	*Melica brasiliana* Ard.	Mb	F	C_3
	Pooideae	*Vulpia dertonensis* (All.) Gola	Vd	F, G	C_3
	Stipoideae	*Piptochaetium bicolor* (Vahl) Desv.	Pb	F	C_3
	Stipoideae	*Piptochaetium hackelii* (Arech.) Parodi	Ph	F	C_3
	Stipoideae	*Piptochaetium lasianthum* Gris.	Pl	F	C_3
	Stipoideae	*Piptochaetium medium* (Speg.) M. (A) Torres	Pm	F	C_3
	Stipoideae	*Stipa neesiana* Trin. et Rupr	Sn	F	C_3
	Stipoideae	*Stipa papposa* Nees	Sp	F	C_3
	Stipoideae	*Stipa trichotoma* Nees	St	F	C_3

method, arithmetic average linkage (UPGMA) were used. To establish the importance of morphotypes in the segregation of species, principal component analysis (PCA) was performed, on the basis of a variance–covariance matrix. In both analyses, only the relative frequencies of the isolated morphotypes were used; they were re-calculated without including the articulated morphotypes in the total sum.

RESULTS

Phytoliths were observed in all but one (*Taraxacum officinale*) of the 23 species studied. The relative frequencies of the phytolith morphotypes in the remaining species are detailed in Table 3.

All the species produced articulated and isolated phytoliths (Figs 2–5). *Achillea* sp., *Carduus acanthoides* and *Cirsium vulgare* produced phytolith morphologies that are different from those of Poaceae species. *Achillea* sp. was characterized by jigsaw phytoliths (Fig. 2I), whereas *Ca. acanthoides* and *Ci. vulgare* produced abundant epidermal polygonal phytoliths (Fig. 2 J). Silicifications from cell walls of some epidermical cells were also observed in *Achillea* sp. (Fig. 2L).

Paspalum quadrifarium was characterized by crosses and dumb-bells with convex ends (Fig. 3E, G). Dumb-bells with concave/straight ends were the dominant morphology in *Bothriochloa laguroides*, *Sorghastrum pellitum* and *Danthonia montevidensis*, the last of which also produced abundant dumb-bells with convex ends (Fig. 3G, J). Oblong and crenate shapes were common in *Bromus* spp., *Hordeum pusillum* and *Dactylis glomerata* phytolith assemblages (Fig. 3D). *Briza subaristata* and *Festuca arundinacea* phytolith assemblages were characterized by cones or rondels, and to a lesser extent by oblong and crenate phytoliths (Figs 3D and 4I, J). *Melica brasiliana* produced some dumb-bells with concave ends and oblong and crenate phytoliths (Fig. 3D, G). *Vulpia* type macro hairs, dominant in the *Vulpia dertonensis* assemblage, differ from the other macro hairs on the basis of barb length, which could be up to three times longer than the base (Fig. 5B). This morphology can also be observed in *Melica brasiliana* (Fig. 5B). *Stipa* and *Piptochaetium* species produced abundant *Stipa*-type dumb-bells, but dumb-bells with convex ends were also common in some species (Fig. 3). Bulliform elements and elongate phytoliths were found abundant in many of the species (Fig. 4).

Cluster analysis revealed five main groups: Group 1, consisting of *Sorghastrum pellitum*, *Bothriochloa laguroides* and *Danthonia montevidensis*; Group 2, consisting of the three species belonging to the Asteraceae; Group 3, consisting of the three species of the genus *Stipa* and the four species of the genus *Piptochaetium*; Group 4, consisting of *Paspalum quadrifarium*; and Group 5, consisting of *Bromus auleticus*, *Bromus catharticus*, *Dactylis glomerata*, *Hordeum pusillum*, *Melica brasiliana*, *Festuca arundinacea*, *Briza subaristata* and *Vulpia dertonensis* (Fig. 6).

The first three axes of the PCA amount to 66 % of the total variance (24 % for axis 1, 22 % for axis 2, 20 % for axis 3). In Fig. 7, where axes 1 and 2 are represented, six groups can be noted: Group I (*Sorghastrum pellitum*, *Bothriochloa laguroides* and *Danthonia montevidensis*); Group II (*Stipa* and *Piptochaetium* species and *Paspalum quadrifarium*); Group III (*Melica brasiliana*, *Festuca*

TABLE 2. *Morphological classification of phytoliths, following schemes given by Twiss (1992) and Zucol (1996, 2001) and descriptors used by Madella* et al. *(2005)*

ARTICULATED FORMS
1 Subepidermal elements
2 Bulliform elements
3 Large cells: only includes long cells articulated
4 Short cells: includes only short cells, or long cells with short cells articulated
5 Prickle hairs
6 Hook hairs
7 Macro hairs
8 Macro hairs, *Vulpia*-type
9 Jigsaw (puzzle forms)
10 Epidermal polygonal
11 Stomatal complex
12 Others
ISOLATED FORMS
13 Crescent moon
14 Round
15 Rectangular and smooth (less than 35 µm)
16 Rectangular and crenate (less than 35 µm)
17 Oblong and crenate (less than 35 µm)
18 Normal saddle
19 Thin saddle
20 Cross, thick shank
21 Cross, thin shank
22 Dumb-bell with long central portion and convex end
23 Dumb-bell with short central portion and convex end (Simple Lobate)
24 Dumb-bell with short central portion and convex end (*Stipa*-type dumb-bell)
25 Dumb-bell with long central portion and concave/straight end
26 Dumb-bell with short central portion and concave/straight end (panicoid-type dumb-bell)
27 Dumb-bell with nodular central portion
28 Dumb-bell with spiny central portion
29 Regular complex dumb-bell
30 Irregular complex dumb-bell
31 Crenate dumb-bell
32 Elongate, smooth
33 Elongate, crenate
34 Oblong and crenate (greater than 35 µm)
35 Elongate, spiny
36 Elongate with concave ends
37 Fan, smooth
38 Polyhedrical, smooth
39 Polyhedrical, crenate
40 Macro hairs middle portions
41 Apex of sharp-pointed shapes (hairs, prickles or hooks)
42 Macro hairs
43 Macro hairs *Vulpia*-type
44 Prickles
45 Hooks
46 Micro hairs
47 Intercellular spaces infillings
48 Equidimensional cones or rondels
49 Elongate cones or rondels
50 Flat cones or rondels
51 Jigsaw (puzzle forms)
52 Epidermal polygonal
53 Others

arundinacea, *Briza subaristata* and *Vulpia dertonensis*); Group IV (*Bromus auleticus*, *Bromus catharticus*, *Dactylis glomerata* and *Hordeum pusillum*); and Group V (*Achillea* sp., *Carduus acanthoides* and *Cirsium vulgare*). The morphotypes that mainly contribute to axis 1 are oblong crenate phytoliths (less than 35 µm long) and short-shank, concave/straight-ended dumb-bells; *Stipa*-type dumb-bells and oblong crenate phytoliths contribute to axis 2.

DISCUSSION

These descriptions of leaf phytolith assemblages represent the first contribution at the level of genera for the study of *Achillea* sp., *Carduus acanthoides*, *Cirsium vulgare*, *Briza subaristata* and at the level of species for the study of *Bothriochloa laguroides*, *Bromus auleticus*, *Danthonia montevidensis*, *Festuca arundinacea*, *Hordeum pusillum*, *Melica brasiliana*, *Paspalum quadrifarium*, *Piptochaetium bicolor*, *Piptochaetium lasianthum*, *Piptochaetium medium*, *Piptochaetium hackelii*, *Sorghastrum pellitum* and *Vulpia dertonensis*. The other species have been described by other authors, using specimens from other regions of Argentina (Zucol, 1996) or elsewhere (Parry and Smithson, 1964; Brown, 1984; Mulholland, 1989).

The production of phytoliths in the Asteraceae is very variable (Piperno, 1988; Bozarth, 1992; Wallis, 2003; Thorn, 2004). Phytoliths were not observed in one species (*Taraxacum officinale*) out of the four analysed. *Carduus acanthoides* and *Cirsium vulgare* have similar phytolith assemblage composition, characterized by tracheid elements, and epidermal polygonal and rectangular phytoliths. The phytolith assemblage of *Achillea* sp. differs in the abundance of jigsaw phytoliths (*sensu* Kondo *et al*., 1987). The phytolith assemblages of Asteraceae species are compounded by several morphotypes described previously for others species of the family (Bozarth, 1992; Kondo *et al*., 1994; Blinnikov, 2005). Nevertheless, their particular composition allows differentiation of these phytolith assemblages from those of the Poaceae and characterizes burned and grazed sites of the *Paspalum quadrifarium* grassland.

The subfamily Pooideae (comprising eight species) is characterized by rondels or truncated cones (both flat and equidimensional), articulated and isolated, prickles, rectangular, elongate oblong and crenate, and elongate smooth phytoliths. *Vulpia dertonensis* phytoliths are slightly different from those of the other pooids, in the absence of predominant morphotypes of this group such as rectangular phytoliths and oblong crenate phytoliths. The results match and corroborate the descriptions of pooid species made by other authors, who noted the predominance of rondels, rectangular smooth and rectangular crenate phytoliths (Brown, 1984; Mulholland, 1989; Twiss, 1992). Only in one species (*Melica brasiliana*) were short-shank, convex-ended dumb-bells noted, and at a minor proportion, *Stipa*-type dumb-bells. These morphotypes are usually absent from this subfamily, even though some authors have described them for some genera. Mejía Saules and Bibsy (2003) described dumb-bells on the lemma epidermis of *Melica californica*, *Melica cepacea*, *Melica nutans*, *Melica onoei* and *Melica picta*, but not in *Melica brasiliana*. Mulholland (1989) observed the presence of less than 5 % dumb-bells in *Koeleria cristata*. In *Melica brasiliana* the values found are slightly higher and

TABLE 3. *Relative frequency of phytolith morphotypes (P.M.) from (A) Poaceae and (B) Asteraceae species*

P.M.	Bl (10)	Bs (9)	Ba (10)	Bc (10)	Dg (11)	Dm (6)	Fa (10)	Hp (2)	Mb (9)	Pq (11)
(A) Poaceae										
1 to 12	49·39 ± 3·11	40·92 ± 2·68	40·21 ± 4·56	32·69 ± 4·64	16·38 ± 2·23	44·3 ± 2·85	50·28 ± 3·5	4·95 ± 0·09	38·05 ± 2·88	30·28 ± 3·07
13, 14	0·00	5·09 ± 1·15	0·51 ± 0·3	0·00	0·00	0·26 ± 0·17	0·00	0·12 ± 0·12	0·00	0·02 ± 0·02
15, 16	3·27 ± 0·53	2·55 ± 0·65	2·03 ± 0·45	2·65 ± 0·89	6·57 ± 1·32	1·25 ± 0·59	7·37 ± 1·55	0·24 ± 0·24	2·16 ± 0·58	9·07 ± 0·95
17, 34	0·00	9·05 ± 1·95	30·92 ± 4·38	38·69 ± 4·46	43·46 ± 3·45	0·00	0·78 ± 0·43	26·46 ± 19·86	18·56 ± 4·07	0·00
20, 21	2·18 ± 0·94	0·00	0·00	0·00	0·00	0·26 ± 0·11	0·00	0·00	0·00	15·97 ± 1·36
22, 23	1·29 ± 1·16	0·00	0·00	0·00	0·00	11·21 ± 1·52	0·00	0·00	6·11 ± 1·96	23·34 ± 2·59
24	0·00	0·00	0·00	0·00	0·00	4·3 ± 1·9	0·00	0·00	1·93 ± 0·49	0·00
25, 26	31·24 ± 2·09	0·00	0·00	0·00	0·00	25·25 ± 3·41	0·00	0·00	0·3 ± 0·2	2·44 ± 0·66
27, 31	1·15 ± 0·15	0·00	0·00	0·00	0·00	1·33 ± 0·75	0·00	0·00	5·89 ± 1·53	2·15 ± 0·55
32, 33, 34, 36	4·93 ± 1·1	6·82 ± 2·29	5·85 ± 1·09	6·87 ± 1·77	8·46 ± 1·05	6·43 ± 1·36	14·98 ± 1·42	2·13 ± 0·23	7·87 ± 2·13	11·1 ± 2·3
37 to 39	2·68 ± 0·8	0·6 ± 0·16	0·71 ± 0·35	0·75 ± 0·2	0·18 ± 0·07	0·37 ± 0·08	0·78 ± 0·19	0·12 ± 0·12	0·24 ± 0·09	3·01 ± 0·64
40, 41	0·38 ± 0·15	0·52 ± 0·21	1·36 ± 0·53	6·63 ± 1·97	1·59 ± 0·51	0·00	1·15 ± 0·94	0·35 ± 0·35	0·58 ± 0·24	1·75 ± 0·55
42, 44, 45, 46	1·54 ± 0·42	6·73 ± 2	10·05 ± 2·05	7·99 ± 1·62	20·86 ± 3·34	0·51 ± 0·18	3·17 ± 0·7	9·34 ± 3·4	11·8 ± 2·88	0·18 ± 0·09
43	0·00	0·00	0·22 ± 0·11	0·00	0·00	0·00	0·85 ± 0·47	2·36 ± 2·36	6·12 ± 2·07	0·00
47	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·02 ± 0·02
48 to 50	0·00	23·8 ± 6·01	7·92 ± 1·89	3·14 ± 0·75	2·32 ± 0·94	4·48 ± 1·78	19·83 ± 2·82	17·23 ± 12·96	0·00	0·02 ± 0·02

P.M.	Pb (9)	Ph (5)	Pl (6)	Pm (8)	Sop (9)	Sn (10)	Sp (10)	St (5)	Vd (10)
(A) Poaceae									
1 to 12	57·31 ± 2·91	45·64 ± 5·83	61·46 ± 3·21	49·75 ± 1·74	51·57 ± 1·69	64 ± 2·53	49·35 ± 3·02	66·06 ± 3·45	52·17 ± 4·76
13, 14	0·03 ± 0·03	0·49 ± 0·14	0·00	0·03 ± 0·03	0·11 ± 0·08	0·05 ± 0·03	0·1 ± 0·05	0·1 ± 0·1	0·00
15, 16	2·83 ± 1·49	1·22 ± 0·39	4·23 ± 1·66	2·31 ± 1·25	2·84 ± 1·04	2·74 ± 0·7	3·17 ± 1·14	6·32 ± 1·69	6·95 ± 1·16
17, 34	0·00	0·05 ± 0·05	0·00	0·00	0·00	0·00	0·00	0·00	0·00
20, 21	0·03 ± 0·03	0·15 ± 0·1	0·04 ± 0·04	0·06 ± 0·06	0·55 ± 0·28	0·37 ± 0·12	0·00	0·69 ± 0·28	0·00
22, 23	9·01 ± 2·74	1·18 ± 0·87	2·09 ± 1·18	7·98 ± 1·3	2·38 ± 0·9	10·94 ± 1·96	4·85 ± 1·6	0·15 ± 0·1	0·00
24	12·2 ± 1·4	15·7 ± 2·57	7·53 ± 1·44	20·88 ± 2·25	0·00	10·58 ± 2·12	9·21 ± 2·06	13·63 ± 2·85	0·00
25, 26	0·15 ± 0·08	0·2 ± 0·09	0·12 ± 0·08	0·00	24·97 ± 3·03	0·8 ± 0·4	0·02 ± 0·02	0·68 ± 0·32	0·00
27, 31	3·07 ± 0·5	0·2 ± 0·09	9·85 ± 3·92	3·45 ± 1·26	0·03 ± 0·03	1·94 ± 0·55	0·37 ± 0·19	2·5 ± 0·29	0·00
32, 33, 34, 36	3·58 ± 0·75	4·67 ± 2·11	10·31 ± 2·82	5·48 ± 2·91	2·24 ± 0·63	4·51 ± 1·35	4·86 ± 1·98	5·82 ± 1·66	15·18 ± 2·24
37 to 39	8·45 ± 2·14	1·08 ± 0·57	0·24 ± 0·09	4·42 ± 0·9	1·22 ± 0·54	0·57 ± 0·2	0·34 ± 0·12	0·05 ± 0·05	0·36 ± 0·15
40, 41	0·00	2·06 ± 0·78	0·45 ± 0·3	0·00	2·71 ± 0·85	0·22 ± 0·13	0·00	1·46 ± 0·92	2·47 ± 1·16
42, 44, 45, 46	1·49 ± 0·66	11·28 ± 4·24	3·14 ± 0·82	2·84 ± 1·37	8·1 ± 2·19	2·09 ± 0·82	2·09 ± 0·72	1·37 ± 0·39	0·07 ± 0·07
43	0·00	0·00	0·00	0·00	0·00	0·07 ± 0·07	0·00	0·2 ± 0·2	14·58 ± 3·17
47	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
48 to 50	1·71 ± 0·92	15·94 ± 6·28	0·24 ± 0·17	2·77 ± 1·57	1·4 ± 0·78	0·82 ± 0·21	25·37 ± 5·87	0·98 ± 0·75	8·12 ± 1·73

P.M.	Ach (2)	Ca (5)	Cv (5)
(B) Asteraceae			
3	0	11·6 ± 2·91	21·97 ± 3·27
9	54·94 ± 24·14	0	0
10	0	43·47 ± 6	42·04 ± 4·43
11	1·46 ± 1·46	0·07 ± 0·07	0·46 ± 0·29
12	35·15 ± 27·20	0	0
32	2·89 ± 2·13	12·33 ± 2·71	11·6 ± 1·24
42	0	0·07 ± 0·07	0
44	0	0·4 ± 0·4	0·07 ± 0·07
51	2·99 ± 0·06	0	0
52	1·37 ± 0·53	11·79 ± 3·14	19·18 ± 3·01
53	1·2 ± 0·05	20·29 ± 3·12	4·7 ± 2·26

Values shown are the means ± s.e. Number in parentheses is the number of samples for each species. Similar phytolith morphotypes are grouped together in the table of Poaceae species. Only morphotypes with relative frequencies greater than zero in at least one species are shown.

they represent a dominant morphotype in its phytolith assemblage.

The subfamily Stipoideae (comprising seven species) is characterized by the presence of *Stipa*-type dumb-bells, both articulated and isolated. The phytolith assemblage also features a high percentage of short-shank, convex-ended dumb-bells (simple lobate *sensu* Fredlund and Tieszen, 1994), rondels and elongate smooth phytoliths. The presence of dumb-bells in this subfamily has been recorded several times, and many authors have placed them in separate categories (Blackman, 1971; Mulholland, 1989; Fredlund and Tieszen, 1994; Gallego and Distel, 2004). The difference between *Stipa*-type dumb-bells and the simple lobate dumb-bells is crucial, especially when the dumb-bells are used to discriminate between C_3 and C_4 species. The *Stipa*-type dumb-bells would indicate the presence of species of the subfamily Stipoideae, which have a C_3 photosynthetic pathway and microthermal environmental requirements (Burkart, 1975). Some authors have considered the simple lobate dumb-bells as typical of

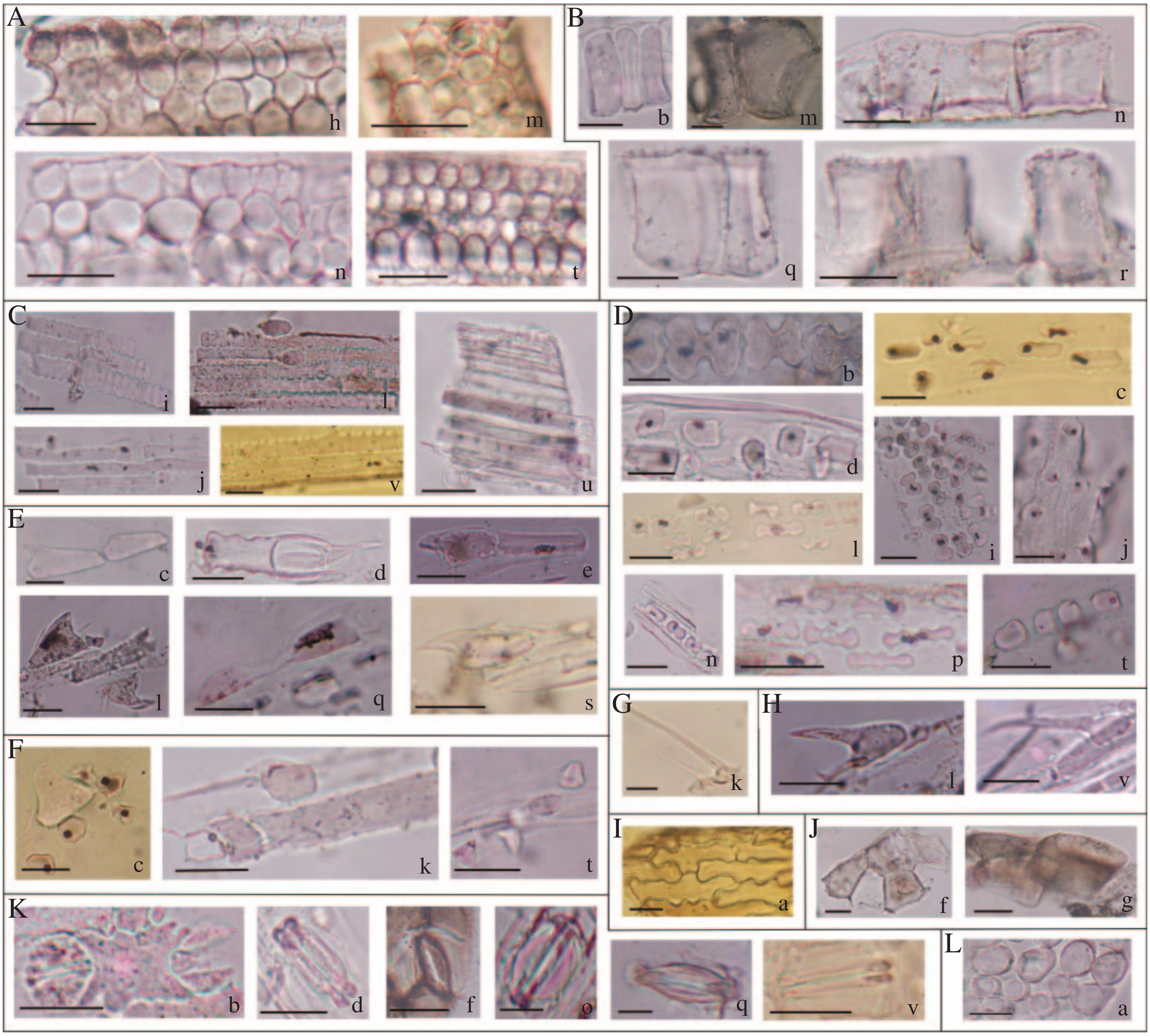

FIG. 2. (A) Subepidermal elements. (B) Bulliform elements. (C) Long cells. (D) Short cells. (E) Prickle hairs. (F) Hook hairs. (G) Macrohairs. (H) *Vulpia*-type macrohairs. (I) Puzzle forms. (J) Epidermal polygonal. (K) Stomatal complex. (L) Not identified. a, *Achillea* sp.; b, *Bothriochloa laguroides*; c, *Briza subaristata*; d, *Bromus auleticus*; e, *Bromus catharticus*; f, *Carduus acanthoides*; g, *Cirsium vulgare*; h, *Dactylis glomerata*; i, *Danthonia montevidensis*; j, *Festuca arundinacea*; k, *Hordeum pusillum*; l, *Melica brasiliana*; m, *Paspalum quadrifarium*; n, *Piptochaetium bicolor*; o, *Piptochaetium hackelii*; p, *Piptochaetium lasianthum*; q, *Piptochaetium medium*; r, *Sorghastrum pellitum*; s, *Stipa neesiana*; t, *Stipa papposa*; u, *Stipa trichotoma*; v, *Vulpia dertonensis*. Scale bar = 25 µm. Figures Bm, Kq and Db, scale bars = 10 µm.

C_4 species (Fredlund and Tieszen, 1994). However, stipoid species also produce this morphotype, and in some cases they are even the dominant form.

The phytolith assemblages of the subfamily Panicoideae show two tendencies: the first characterizes the panicoid species (*Paspalum quadrifarium*) and is dominated by the presence of short-shank, convex-ended dumb-bells; the other is present in andropogonoid species (*Sorghastrum pellitum* and *Bothriochloa laguroides*) and is characterized by short-shank, concave/straight-ended dumb-bells. The latter, according to Fredlund and Tieszen (1994), correspond to panicoid dumb-bells. The phytolith assemblages of these species also feature other dumb-bells, except of the *Stipa* type, elongate smooth phytoliths and micro-hairs. Crosses are dominant in the *Paspalum quadrifarium* phytolith assemblage, but they are less common in the other species.

Danthonia montevidensis, the only specimen of the subfamily Arundinoideae that has been analysed, shows a phytolith assemblage similar to those of *Bothriochloa laguroides* and *Sorghastrum pellitum*. It is characterized by the predominance of short-shank, concave/straight-ended dumb-bells, together with, in minor proportions, short-shank, convex-ended dumb-bells and *Stipa*-type dumb-bells. Several authors who have studied species of the subfamily Arundinoideae describe the wide range of morphotypes present in their phytolith assemblages (Brown,

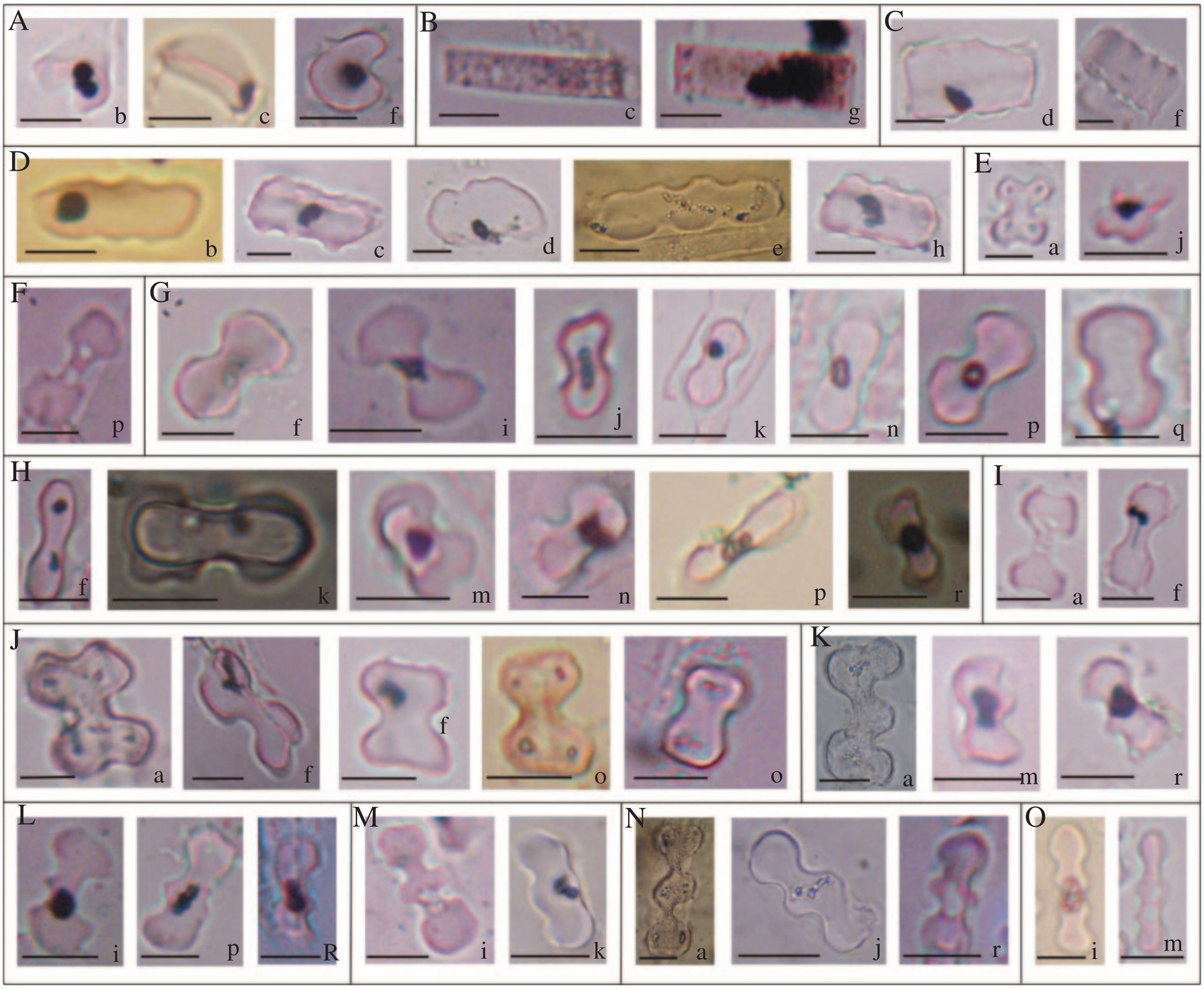

FIG. 3. (A) Crescent moon. (B) Rectangular and smooth (less than 35 µm). (C) Rectangular and crenate (less than 35 µm). (D) Oblong and crenate (less than 35 µm). (E) Cross, thick shank. (F) Dumb-bell with long central portion and convex end. (G) Dumb-bell with short central portion and convex end. (H) Dumb-bell with short central portion and convex end (*Stipa*-type dumb-bell). (I) Dumb-bell with long central portion and concave/straight end. (J) Dumb-bell with short central portion and concave/straight end. (K) Dumb-bell with nodular central portion. (L) Dumb-bell with spiny central portion. (M) Regular complex dumb-bell. (N) Irregular complex dumb-bell. (O) Crenate dumb-bell. a, *Bothriochloa laguroides*; b, *Briza subaristata*; c, *Bromus auleticus*; d, *Bromus catharticus*; e, *Dactylis glomerata*; f, *Danthonia montevidensis*; g, *Festuca arundinacea*; h, *Hordeum pusillum*; i, *Melica brasiliana*; j, *Paspalum quadrifarium*; k, *Piptochaetium bicolor*; l, *Piptochaetium hackelii*; m, *Piptochaetium lasianthum*; n, *Piptochaetium medium*; o, *Sorghastrum pellitum*; p, *Stipa neesiana*; q, *Stipa papposa*; r, *Stipa trichotoma*. Scale bar = 10 µm.

1984; Mulholland, 1989). This subfamily is cited as one of the most heterogeneous grass groups, as they do not have diagnostic characteristics (Ellis, 1987; Twiss, 1992).

Although the detailed phytolith descriptions given here coincide with features observed for other species, there are some exceptions that should be taken into account in studies in which these species predominate. The main differences lie in the linkage of dumb-bells to specific subfamilies and photosynthetic pathways.

Short-shank, convex-ended dumb-bells not only characterize species of the Panicoideae subfamilies, and therefore mostly of C_4 species, but are also present in species of the Pooideae (*Melica brasiliana*), Arundinoideae (*Danthonia montevidensis*) and Stipoideae (*Stipa* spp. and *Piptochaetium* spp.) subfamilies, which are all C_3 species. By contrast, short-shank, concave/straight-ended dumb-bells have been described not only for the C_4 panicoid species but also for *Danthonia montevidensis* (Arundinoideae, C_3 species). Lastly, although *Stipa*-type dumb-bells are characteristic of the stipoid species (C_3), they are also found, at low proportions, in *Melica brasiliana* (Pooideae) and *Danthonia montevidensis* (Arundinoideae), both C_3 species. Several of these exceptions have been noted for Northern Hemisphere species (Brown, 1984; Fredlund and Tieszen, 1994). Given these exceptions, it is recommended that local species present in the study area are taken into account when dumb-bells are used for photosynthetic pathway identification. In addition, a more detailed morphometric study of the dumb-bells *sensu lato* could lead to better differentiation among the subfamilies noted above.

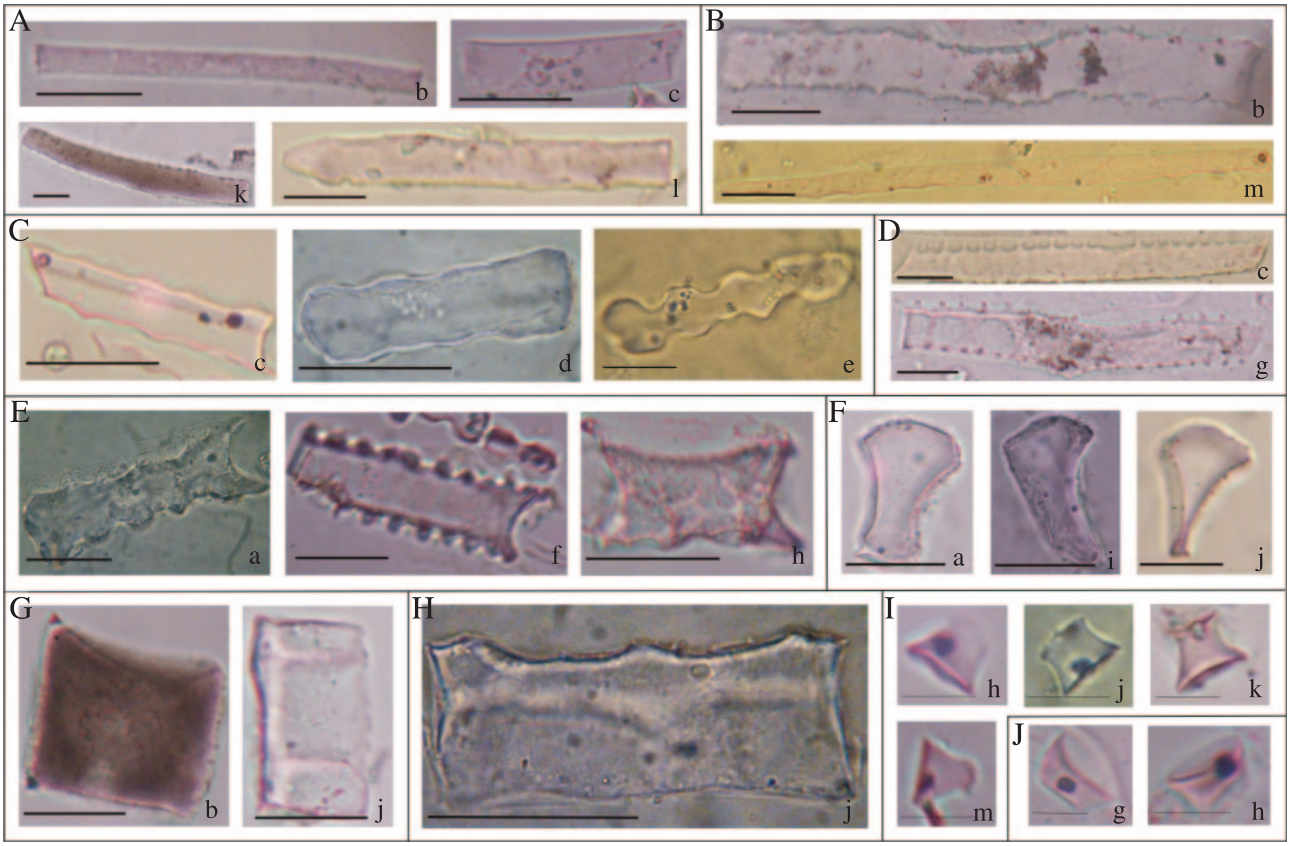

FIG. 4. (A) Elongate, smooth. (B) Elongate, crenate. (C) Oblong and crenate (greater than 35 µm). (D) Elongate, spiny. (E) Elongate with concave ends. (F) Fan, smooth. (G) Polyhedrical, smooth. (H) Polyhedrical, crenate. (I) Equidimensional cones/rondels. (J) Flat cones/rondels. a, *Bothriochloa laguroides*; b, *Briza subaristata*; c, *Bromus auleticus*; d, *Bromus catharticus*; e, *Dactylis glomerata*; f, *Danthonia montevidensis*; g, *Festuca arundinacea*; h, *Hordeum pusillum*; i, *Paspalum quadrifarium*; j, *Piptochaetium bicolor*; k, *Piptochaetium hackelii*; l, *Piptochaetium lasianthum*; m, *Vulpia dertonensis*. Scale bar = 25 µm. Figure I scale bar = 10 µm.

Rondels (or truncated cones) have been assigned generally to the subfamily Pooideae. However, there are some pooid species (*Melica brasiliana*) that do not produce this morphotype, whereas species from other subfamilies produce them in great quantities (*Stipa* spp. and *Piptochaetium* spp.).

The cluster analysis and PCA reflect quite clearly the systematics of the group of species under study. First, it is possible to distinguish between the two families analysed: Poaceae and Asteraceae. The differences lie not only in the phytolith morphotypes but also in the morphotype diversity (reduced in the Asteraceae). This difference at family level is useful in discriminating between sites that have had no agricultural management and sites that have been burned or grazed, both characterized by the presence of the Asteraceae species studied here. The phytolith assemblage composition allows the differentiation of *Achillea* sp. (present in the burned sites) from *Carduus acanthoides* and *Cirsium vulgare* (present in the grazed sites). In addition, within the family Poaceae, it is possible to discriminate among the different subfamilies, which would allow differentiation between 'flechillar' (characterized mainly by species of the subfamilies Pooideae and Stipoideae) and 'pajonal' (dominated by *Paspalum quadrifarium*) species. The panicoid species (*Sorghastrum pellitum*, *Bothriochloa laguroides* and *Paspalum quadrifarium*) do not form a homogeneous group in the multivariate analyses. The andropogonoid species show a phytolith assemblage composition more similar to that of *Danthonia montevidensis* (Arundinoideae) than to *Paspalum quadrifarium*, possibly due to the presence of short-shank, concave/straight-ended dumb-bells. *Paspalum quadrifarium*, instead, is more associated with stipoid species, a group formed by the combination of *Stipa* and *Piptochaetium* species. Within this group, similarity between species of different genera is of note. The genera *Stipa* and *Piptochaetium* have been included in diverse subfamilies, for instance in the Pooideae (Clayton and Renvoize, 1986), Arundinoideae (Barworth and Everett, 1987) or Bambusoideae (Dahlgren *et al*., 1985). However, the results drawn from this study support the separation of these genera within a different group, as with Watson and Dallwitz (1992), who placed them in the subfamily Stipoideae. Finally, the subfamily Pooideae is subdivided into two groups. In the first, species belonging to the supertribe Triticodae (*Bromus auleticus*, *Bromus catharticus* and *Hordeum pusillum*) and *Dactylis glomerata* are included. This group is characterized by the dominance of oblong

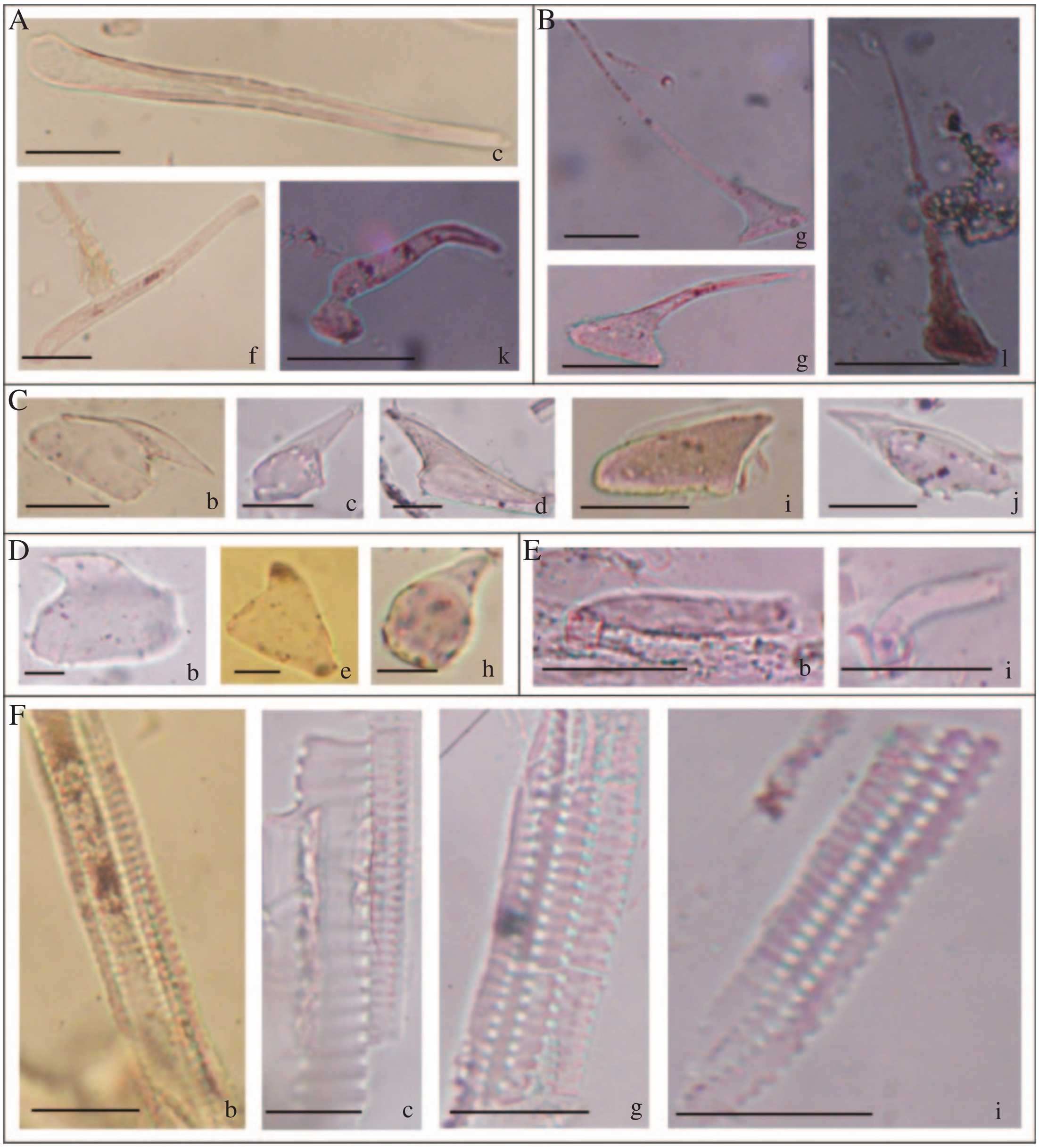

FIG. 5. (A) Macro hairs. (B) Macro hairs, *Vulpia*-type. (C) Prickles. (D) Hooks. (E) Microhairs. (F) Tracheary elements. a, *Bothriochloa laguroides*; b, *Briza subaristata*; c, *Bromus auleticus*; d, *Bromus catharticus*; e, *Festuca arundinacea*; f, *Hordeum pusillum*; g, *Melica brasiliana*; h, *Piptochaetium hackelii*; i, *Sorghastrum pellitum*; j, *Stipa neesiana*; k, *Stipa trichotoma*; l, *Vulpia dertonensis*. Scale bar = 25 µm. Figure D scale bar = 10 µm.

crenate phytoliths and, to a lesser degree, hooks and rondels or truncated cones. The other group comprises species of the supertribe Poodae (*Festuca arundinacea*, *Briza subaristata* and *Vulpia dertonensis*). Rondels or truncated cones are the main morphotypes present in their phytolith assemblages. *Melica brasiliana* is the most distinct species of the pooid group, probably owing to the presence of dumb-bell phytoliths.

CONCLUSIONS

This paper presents the first phytolith descriptions of some grasses and Asteraceae species from Argentina. Although the results do match the general patterns of phytolith assemblages within the grass subfamilies as described by other authors, it is necessary to consider the exceptions found here, especially when linking the relative frequencies of morphologies to plant systematics and/or photosynthetic pathways.

The results of the multivariate analyses confirm the usefulness of phytolith assemblages for the differentiation of families and subfamilies for these Pampean grassland communities. Within the Poaceae, the phytoliths corresponding to short-cell silicifications (dumb-bells, truncated cones or rondels, oblong and crenate phytoliths) are the best indicators for the discrimination between subfamilies, as reported previously by other authors.

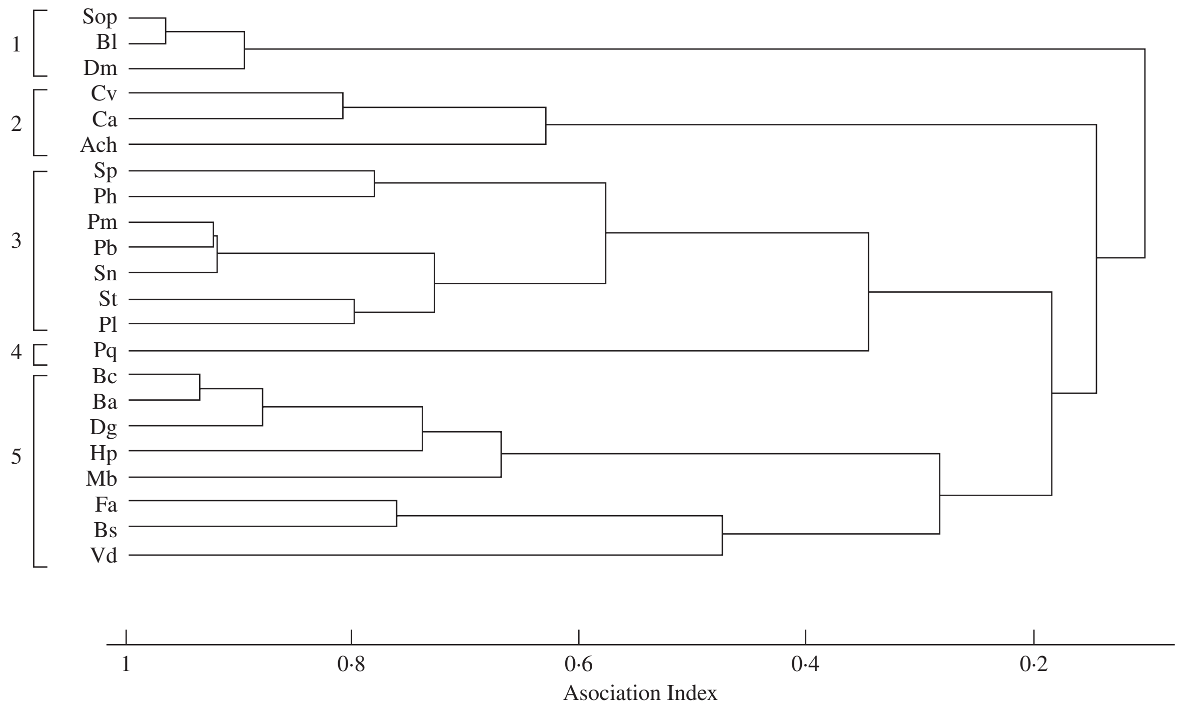

FIG. 6. Dendrogram showing species grouping based on their phytolith assemblage.

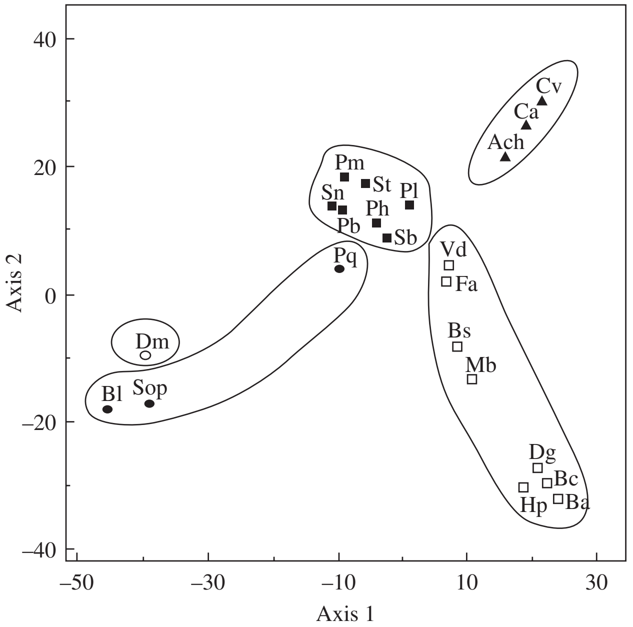

FIG. 7. PCA of species based on their phytolith assemblage. Species are grouped based on family or subfamily affinity.

Finally, the results indicate that the phytolith assemblages studied can be used to differentiate between the *Paspalum quadrifarium* ('pajonal') and 'flechillar' communities, and the variants generated by fire or range management in the Argentine Pampas.

These findings contribute to the description of the phytolith assemblages of Argentine Pampean species, which is crucial for interpretation of the regional fossil phytolith record.

ACKNOWLEDGEMENTS

This research forms part of the doctoral thesis of M.F.H. at the Universidad Nacional de Mar del Plata. Financial support for this work was provided by the project EXA 292/04 from the Universidad Nacional de Mar del Plata. A fellowship from CONICET to M.F.H. is acknowledged. We thank Ing. Agr. Sara Alonso for herbarium specimens.

LITERATURE CITED

Alexandre A, Meunier J, Lezine A, Vincens A, Schwartz D. 1997. Phytoliths: indicators of grassland dynamics during the late Holocene in intertropical Africa. *Palaeogeography*, *Palaeoclimatology*, *Palaeoecology* **136**: 213–229.

Barboni D, Bonnefille R, Alexandre A, Meunier JD. 1999. Phytoliths as paleoenvironmental indicators, West Side Middle Awash Valley, Ethiopia. *Palaeogeography*, *Palaeoclimatology*, *Palaeoecology* **152**: 87–100.

Barworth ME, Everett J. 1987. Evolution in the Stipeae: identification and relationships of its monophyletic taxa. In: Soderstrom TR, Hilu KW, Campbell CS, Barworth ME, eds. *Grass systematics and evolution*. Washington DC: Smithsonian Institution Press, 251–264.

Blackman E. 1971. Opaline silica bodies in the range grasses of southern Alberta. *Canadian Journal of Botany* **49**: 769–781.

Blinnikov MS. 2005. Phytoliths in plants and soils of the interior Pacific Northwest, USA. *Review of Palaeobotany and Palynology* **135**: 71– 98.

Bozarth SR. 1992. Classification of opal phytoliths formed in selected dicotyledons native to the Great Plains. In: Rapp G, Mulholland SC, eds. *Phytolith systematics*. New York: Plenum Press, 193–214.

Brown DA. 1984. Prospects and limits of a phytolith key for grasses in the Central United States. *Journal of Archaeological Science* **11**: 345–368.

Burkart A. 1975. Evolution of grasses and grasslands in South America. *Taxon* **24**: 53–66.

Campos AC, Labouriau LG. 1969. Corpos silicosos de gramineas dos cerrado. II. *Pesquisa Agropecuaria Brasileira* **4**: 143–151.

Carter JA. 2000. Phytoliths from loess in Southland, New Zealand. *New Zealand Journal of Botany* **38**: 325–332.

Clayton WD, Renvoize SA. 1986. *Genera Graminum. Grasses of the world*. Kew Bulletin (additional ser) 12. London: The Stationery Office.

Dahlgren RMT, Clifford HT, Yeo PF. 1985. *The families of monocotyledons*. Berlin: Springer Verlag.

Ellis RP. 1987. A review of comparative leaf blade anatomy in the systematics of the Poaceae: the past twenty-five years. In: Soderstrom TR, Hilu KW, Campbell CS, Barkworth ME, eds. *Grass systematics and evolution*. Washington, DC: Smithsonian Institution Press, 3–10.

Frangi J. 1975. Sinopsis de las comunidades vegetales y el medio de las Sierras de Tandil (Provincia de Buenos Aires). *Boletín de la Sociedad Argentina de Botánica* **15**: 293–319.

Fredlund GG, Tieszen LT. 1994. Modern phytolith assemblages from the North American Great Plains. *Journal of Biogeography* **21**: 312–335.

Gallego L, Distel RA. 2004. Phytolith assemblages in grasses native to Central Argentina. *Annals of Botany* **94**: 1–10.

Kondo R, Sase T, Kato Y. 1987. Opal phytolith analysis of aridisols with regard to interpretation of paleovegetation. In: Kinloch DI, Shoji S, Beinroth FH, Eswaran H, eds. *Proceedings of the 9th International Soil Classification Workshop*, Japan. Japanese Committee for 9th International Soil Classification Workshop and Soil Management Support Services, Washington, DC, 520–534.

Kondo R, Childs CW, Atkinson IAE. 1994. *Opal phytoliths of New Zealand*. Lincoln: Manaaki Whenua Press.

Herrera L, Laterra P. 2005. Relaciones entre la riqueza y la composición florística con el tamaño de fragmentos de pastizales en la Pampa Austral, Argentina. *Actas 1° Jornadas Argentinas de Ecología del Paisaje*: 39–40.

Labouriau LG. 1983. Phytolith work in Brazil: a mini review. *The Phytolitharien Newsletter* **2**: 6–10.

Laterra P, Vignolio OR, Hidalgo LG, Fernández ON, Cauhépé MA, Maceira NO. 1998. Dinámica de pajonales de paja colorada (*Paspalum spp*) manejados con fuego y pastoreo en la Pampa Deprimida Argentina. *Ecotrópicos* **11**: 41–149.

Madella M, Alexandre A, Ball T. 2005. International Code for Phytolith Nomenclature 1·0. *Annals of Botany* **96**: 253–260.

Mejía Saules T, Bibsy FA. 2003. Silica bodies and hooked papillae in lemmas of *Melica* species (Gramineae: Pooideae). *Botanical Journal of the Linnean Society* **141:** 447–463.

Mulholland SC. 1989. Phytolith shape frequencies in North Dakota grasses: a comparison to general patterns. *Journal of Archaeological Science* **16**: 489–511.

Mulholland SC, Rapp G. 1992. A morphological classification of grass silica bodies. In: Rapp G, Mulholland SC, eds. *Phytolith systematics*. New York: Plenum Press, 65–89.

Parry W, Smithson F. 1964. Types of opaline silica depositions in the leaves of British grasses. *Annals of Botany* **28**: 169–185.

Piperno DR.1988. *Phytolith analysis: an archaeological and geological perspective*. San Diego: Academic Press.

Rovner I. 1971. Potential of opal phytolith for use in paleoecological reconstruction. *Quaternary Research* **1**: 343–359.

Sondahl MR, Labouriau LG. 1970. Corpos silicosos de gramineas dos cerrado. IV. *Pesquisa Agropecuaria Brasileira* **5**: 183–207.

Soriano A, León RJC, Sala OE, Lavado RS, Deregibus VA, Cauhépé MA, *et al*. 1991. Río de la Plata Grasslands. In: Coupland RT, ed. *Natural grasslands. Intoduction and Western Hemisphere. Ecosystem of the world*. New York: Elsevier, 367–407.

Thorn VC. 2004. Phytoliths from subantarctic Campbell Island: plant production and soil surface spectra. *Review of Palaeobotany and Palynology* **132**: 37– 59.

Twiss PC. 1992. Predicted world distribution of C_3 and C_4 grass phytoliths. In: Rapp G, Mulholland SC, eds. *Phytolith systematics*. New York: Plenum Press, 113–128.

Twiss PC, Suess E, Smith RM. 1969. Morphological classification of grass phytoliths. *Soil Science Society of America Proceedings* **33**: 109–115.

Vervoorst FB. 1967. *La vegetación de la República Argentina. VII. Las comunidades vegetales de la Depresión del Salado (Prov. Bs. As).* Serie fitogeográfica 7. Buenos Aires: INTA.

Wallis L. 2003. An overview of leaf phytolith production patterns in selected northwest Australian flora. *Review of Palaeobotany and Palynology* **125**: 201–248.

Watson L, Dallwitz MJ. 1992. *Grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references.* http://delta-intkey.com/grass. [Last accessed December 2005.]

Zucol AF. 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.

Zucol AF. 1998. Microfitolitos de las Poaceae Argentinas: II. Microfitolitos foliares de algunas especies del género *Panicum* (Poaceae, Paniceae), en la provincia de Entre Ríos. *Darwiniana* **36**: 29–50.

Zucol AF. 1999. Fitolitos de las Poaceae argentinas: IV. Asociación fitolítica de *Cortaderia selloana* (Danthonieae: Arundinoideae), de la provincia de Entre Ríos. *Natura Neotropicalis* **30**: 25–33.

Zucol AF. 2000. Fitolitos de poaceae de argentinas: III. Fitolitos foliares de especies del género *Paspalum* (Paniceae), en la Provincia de Entre Ríos. *Darwiniana* **38**: 11–32.

Zucol AF. 2001. Fitolitos III. Una nueva metodología descriptiva. Asociaciones fotolíticas de *Piptochaetium montevidense* (Stipeae, Poaceae). *Boletín de la Sociedad Argentina de Botánica* **36**: 69–85.