

## Phytolith Assemblages in Grasses Native to Central Argentina

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• **Background and Aims** Phytolith reference collections are a prerequisite for accurate interpretation of soil phytolith assemblages aimed at reconstructing past vegetation. In this study a phytolith reference collection has been developed for several grasses native to central Argentina: *Poa ligularis*, *Piptochaetium napostaense*, *Stipa clarazii*, *Stipa tenuis*, *Stipa tenuissima*, *Stipa eriostachya*, *Stipa ambigua*, *Stipa brachychaeta*, *Pappophorum subbulbosum*, *Digitaria californica*, *Bothriochloa edwardsiana* and *Aristida subulata*.

• **Methods** For each species, phytoliths present in the leaf blades were classified into 47 morphotypes, and their relative frequency determined by observing 300–400 phytoliths per sample ( $n = 5$ ). Data were analyzed by complete linkage cluster analysis, using the Morisita Index as measure of association.

• **Key Results** The results showed differentiation among phytolith assemblages at species level or at plant functional type level. Cluster analysis separated C<sub>3</sub> from C<sub>4</sub> species and palatable from non-palatable species.

• **Conclusions** This study highlights the possibility of reconstructing past vegetation in central Argentina grasslands through the analysis of soil phytolith assemblages. © 2004 Annals of Botany Company

**Key words:** Poaceae, native grasses, Argentina, phytolith assemblages, grass phytoliths.

### INTRODUCTION

Phytoliths are microscopic particles of hydrated silica deposited in intracellular and/or intercellular spaces of plant tissues, which can take on a considerable variety of forms (Rovner, 1983; Piperno, 1988). Because of their consistent shape within species, phytoliths provide significant taxonomic information (Jones and Handreck, 1965; Blackman, 1969; Raven, 1983; Piperno, 1988). Moreover, since these resistant siliceous structures can remain stable in the soil for hundreds and even millions of years after plant tissues decay (Rovner, 1983; Twiss, 1987; Piperno, 1988; Carter, 1999), they are important in palaeoecological studies.

Although the accumulation of silica occurs in various taxa of the plant kingdom, phytoliths are particularly abundant in the family Poaceae (Gramineae) (Rovner, 1971; Piperno, 1988; Mulholland, 1989; Twiss, 1992). In this family, individuals produce many types of phytolith forms (multiplicity), and a particular form may be produced by a number of different species (redundancy) (Rovner, 1983). For that reason, phytolith descriptions at species level include all morphotypes and their relative frequency, which together characterize specific phytolith assemblages. The diagnostic potential of grass phytoliths and their durability in the soil makes phytolith analysis an important microfossil technique for the reconstruction of past vegetation in grasslands (Sendulsky and Labouriau, 1966; Blackman, 1971; Bartolome *et al.*, 1986; Twiss, 1987; Fisher *et al.*, 1995; Alexandre *et al.*, 1997; Barboni *et al.*, 1999). To that end, phytolith reference collections are a prerequisite for accurate interpretation of soil phytolith assemblages.

In the grasslands of central Argentina a process of species replacement appears to have taken place since the introduction of domestic livestock at the beginning of the last century (Llorens, 1995; Distel and Bóo, 1996). However, this assumption is based on circumstantial evidence because of the lack of relict areas and historical data. The objective of this study was to examine phytolith assemblages in grasses native to central Argentina, in order to assist in the analysis of soil phytolith assemblages towards the reconstruction of past vegetation.

### MATERIALS AND METHODS

Species were collected from a site located in the south-east of La Pampa Province, Argentina (38°45'S, 63°45'W, and 80 m a.s.l.). The climate of this region is temperate and semi-arid. Mean annual temperature is 15 °C, mean annual precipitation is 400 mm, and mean annual potential evapotranspiration is 800 mm (Instituto Nacional de Tecnología Agropecuaria, 1980). The dominant soils are coarse-textured Petrocalcic Calciustolls (Castelli *et al.*, 1995). The site is located in the southern part of the Caldén Phytogeographical District (Cabrera, 1976). The physiognomy of the vegetation is grassland with isolated shrubs and trees. The more abundant herbaceous species are C<sub>3</sub>, perennial, cool-season grasses; although some C<sub>4</sub>, perennial, warm-season grasses are also present. Table 1 contains a list and description of the grass species examined in this study.

Leaf blade samples ( $n = 5$  individuals) from each of the examined species were collected at the end of their annual growing cycle, to better represent phytolith composition at the moment of incorporation into the soil (Parry and Smithson, 1964; Geis, 1978; Rovner, 1983). Cool and

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TABLE 1. Grass species examined for phytoliths. Species descriptions conform to Cabrera (1970) and Lamberto et al. (1997)

Species	Tribe	Photosynthetic pathway	Animal grazing preference
<i>Piptochaetium napostaense</i> (Speg.) Hackel	Stipeae	C <sub>3</sub>	High
<i>Stipa clarazii</i> Ball.	Stipeae	C <sub>3</sub>	High
<i>Stipa tenuis</i> Phil.	Stipeae	C <sub>3</sub>	High
<i>Poa ligularis</i> Nees	Festuceae	C <sub>3</sub>	High
<i>Stipa tenuissima</i> Trin.	Stipeae	C <sub>3</sub>	Low
<i>Stipa eriostachya</i> H.B.K.	Stipeae	C <sub>3</sub>	Low
<i>Stipa ambigua</i> Speg.	Stipeae	C <sub>3</sub>	Low
<i>Stipa brachychaeta</i> Godr.	Stipeae	C <sub>3</sub>	Low
<i>Aristida subulata</i> Henrard	Aristideae	C <sub>4</sub>	Low
<i>Pappophorum subbulbosum</i> Arech.	Pappophoreae	C <sub>4</sub>	High
<i>Digitaria californica</i> (Benth.) Henr.	Paniceae	C <sub>4</sub>	High
<i>Bothriochloa edwardsiana</i> (Gould) Parodi	Andropogoneae	C <sub>4</sub>	High

warm-season grasses were collected in December of 1998 and April of 1999, respectively.

Phytolith extraction from the leaf blades was accomplished through the calcination technique (Labouriau, 1983). The material was first washed with distilled water and dried at 100 °C. Immediately after this it was charred at 200 °C for 2 h, boiled in 5 N HCl for 30 min, filtered through ashless filter paper and washed with distilled water until no more chloride ions were detected. Finally, the material was dried and charred at 200 °C for 20 min and subsequently ignited at 800 °C for 2 h. The ashes were mounted on microscope slides in Canada balsam and observed in a Wild M11 microscope.

We observed 300–400 phytoliths per sample and calculated the percentage of each type present, following the morphological classification proposed by Twiss (1992) and Zucol (2001) (Table 2). Phytoliths were grouped into 47 morphotypes. Ten were present in associated forms (phytolith skeletons formed of more than one anatomic element), and 37 in isolated forms (phytolith formed of only one anatomic element) (Table 2 and Figs 1–4). Phytolith assemblages were subjected to complete linkage cluster analysis. The Morisita Index was used to measure the strength of the association among specific phytolith assemblages (Horn, 1966).

## RESULTS

Table 3 contains the phytolith assemblages of the examined species. The dendrogram from the cluster analysis showed distinctive phytolith assemblages for *Poa ligularis*, *Aristida subulata* and *Pappophorum subbulbosum* (Fig. 5). Phytolith assemblages in these species were characterized by a high frequency of round, elliptical and oblong shapes in

TABLE 2. Morphological classification of phytoliths (according to Twiss, 1992, and as modified by Zucol, 2001)

Isolated forms
<i>Pooide Class</i>
1. Elliptical
2. Hat
3. Biconvex
4. Crescent moon
5. Oblong
6. Round
7. Rectangular
<i>Chloridoide Class</i>
8. Normal saddle
9. Thin saddle
<i>Panicoide Class</i>
10. Cross, thick shank
11. Cross, thin shank
12. Dumb-bell with short central portion and convex end
13. Dumb-bell with short central portion and straight end
14. Dumb-bell with long central portion and convex end
15. Dumb-bell with long central portion and straight end
16. Dumb-bell with nodular central portion and convex end
17. Dumb-bell with nodular central portion and straight end
18. Dumb-bell, spiny central portion, convex end
19. Dumb-bell, spiny central portion, straight end
20. Regular, complex dumb-bell, convex end
21. Regular, complex dumb-bell, straight end
22. Irregular, complex dumb-bell, convex end
23. Irregular, complex dumb-bell, straight end
24. Crenate dumb-bell, convex end
25. Crenate dumb-bell, straight end
<i>Elongate Class</i>
26. Elongate, smooth
27. Elongate, sinuous
28. Elongate, spiny
29. Elongate, concave ends
<i>Fan-shaped and Polyhedral Class</i>
30. Fan
31. Polyhedral
<i>Point-shaped Class</i>
32. Apex of sharp-pointed shapes (hairs, prickles or hooks)
33. Macro-hairs
34. Micro-hairs
35. Prickles hairs
36. Hooks hairs
37. Triangular shapes
<b>Associated forms</b>
38. Subepidermal elements
39. Bulliform elements
40. Stomatal complex
41. Large cells
42. Short cells
43. Papillae
44. Macro-hairs
45. Micro-hairs
46. Prickles hairs
47. Hooks hairs

*P. ligularis*, dumb-bell with a short central portion and straight end in *A. subulata*, and saddle and cross in *P. subbulbosum*. The rest of the species were arranged in the following subgroups:

- (I) *Stipa tenuis* and *Piptochaetium napostaense*
- (II) *Digitaria californica* and *Bothriochloa springfieldii*
- (III) *Stipa eriostachya* and *Stipa tenuissima* associate with *Stipa clarazii*, *Stipa ambigua* and *Stipa brachychaeta*.

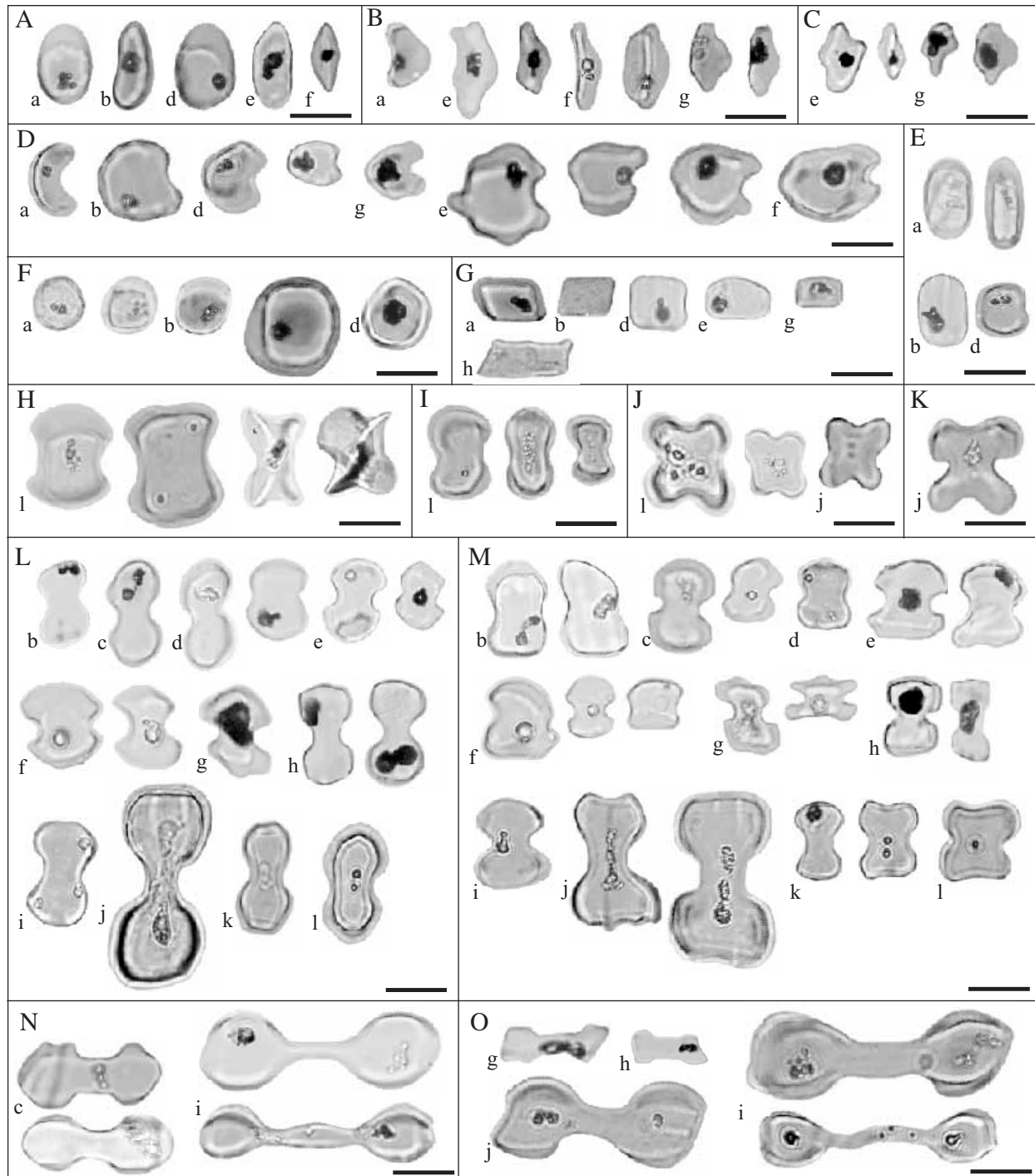


FIG. 1. Light micrographs of isolated-form phytoliths. (A) Elliptical. (B) Hat. (C) Biconvex. (D) Crescent moon. (E) Oblong. (F) Round. (G) Rectangular. (H) Normal saddle. (I) Thin saddle. (J) Cross, thick shank. (K) Cross, thin shank. (L) Dumb-bell, short shank, convex end. (M) Dumb-bell, short shank, straight end. (N) Dumb-bell, long shank, convex end. (O) Dumb-bell, long shank, straight end. a, *Poa ligularis*; b, *Stipa clarazii*; c, *Piptochaetium napostaense*; d, *Stipa tenuis*; e, *Stipa gynerioides*; f, *Stipa tenuissima*; g, *Stipa ambigua*; h, *Stipa brachychaeta*; i, *Aristida subulata*; j, *Bothriochloa edwardsiana*; k, *Digitaria californica*; l, *Pappophorum subbulbosum*. Scale bar = 10  $\mu$ m.

Phytolith assemblages in these subgroups were characterized by a high frequency of dumb-bells with a short central portion and convex ends, and isolated hooks in Subgroup I, dumb-bells with large central portion and straight end in Subgroup II, and dumb-bells with a short central

portion and straight ends, rectangular, smooth elongate, hooks and apex of sharp-pointed shapes in Subgroup III.

A detailed comparison of morphotype frequency and presence showed species differences within subgroups. In Subgroup I, *S. tenuis* was characterized by a high frequency of

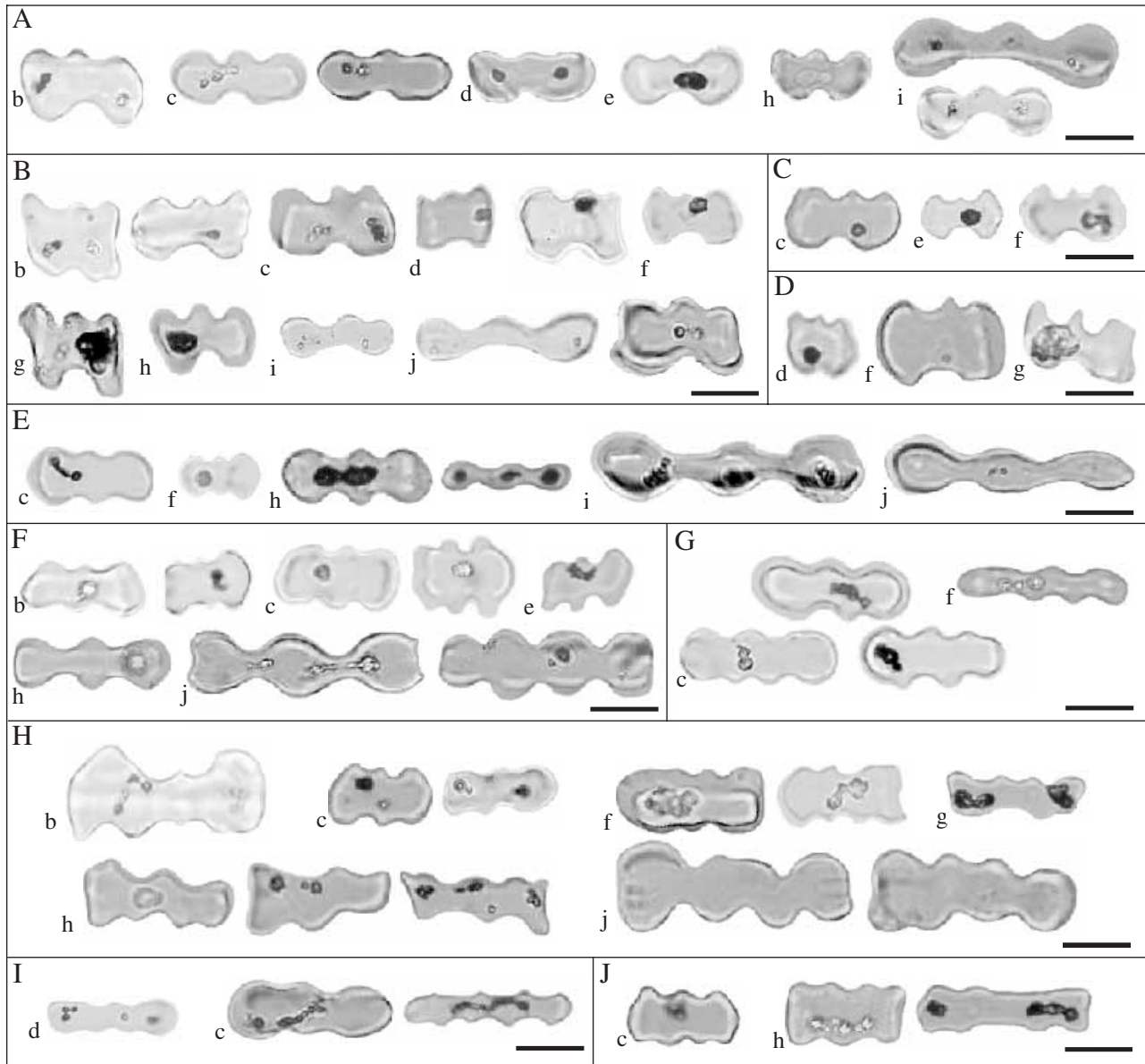


FIG. 2. Light micrographs of isolated-form phytoliths. (A) Dumb-bell, nodular shank, convex end. (B) Dumb-bell, nodular shank, straight end. (C) Dumb-bell, spiny central portion, convex end. (D) Dumb-bell, spiny shank, straight end. (E) Regular, complex dumb-bell, convex end. (F) Regular, complex dumb-bell, straight end. (G) Irregular, complex dumb-bell, convex end. (H) Irregular, complex dumb-bell, straight end. (I) Crenate dumb-bell, convex end. (J) Crenate dumb-bell, straight end. b, *Stipa clarazii*; c, *Piptochaetium napostaense*; d, *Stipa tenuis*; e, *Stipa gynerioides*; f, *Stipa tenuissima*; g, *Stipa ambigua*; h, *Stipa brachychaeta*; i, *Aristida subulata*; j, *Bothriochloa edwardsiana*. Scale bar = 10  $\mu$ m.

smooth elongate and elliptical, round and crescent moon morphotypes, whereas *P. napostaense* was characterized by a high frequency of dumb-bells with a large and nodular central portion (both with convex ends), regular-complex dumb-bells with a convex end, and prickles. In Subgroup II, *D. californica* was distinguished by a high frequency of dumb-bells with a short central portion, isolated hooks and isolated prickles, whereas a high frequency of short cells and the presence of regular-complex dumb-bells with straight end, papillae and cross shapes characterized *B. edwardsiana*. In Subgroup III, a high frequency of

sharp-pointed apex shapes characterized *S. eriostachya* and *S. tenuissima*, whereas a high frequency of rectangular, smooth elongate, and the presence of hooks characterized *S. clarazii*, *S. ambigua* and *S. brachychaeta*. Furthermore, a high frequency of elliptical, hat and associated prickles distinguished *S. eriostachya*, whereas a high frequency of isolated macro-hairs distinguished *S. tenuissima*. On the other hand, a high frequency of elliptical, short cells, stomata complex and associated hooks, and the presence of round and oblong phytoliths characterized *S. clarazii*, whereas the presence of nodular dumb-bells with a straight

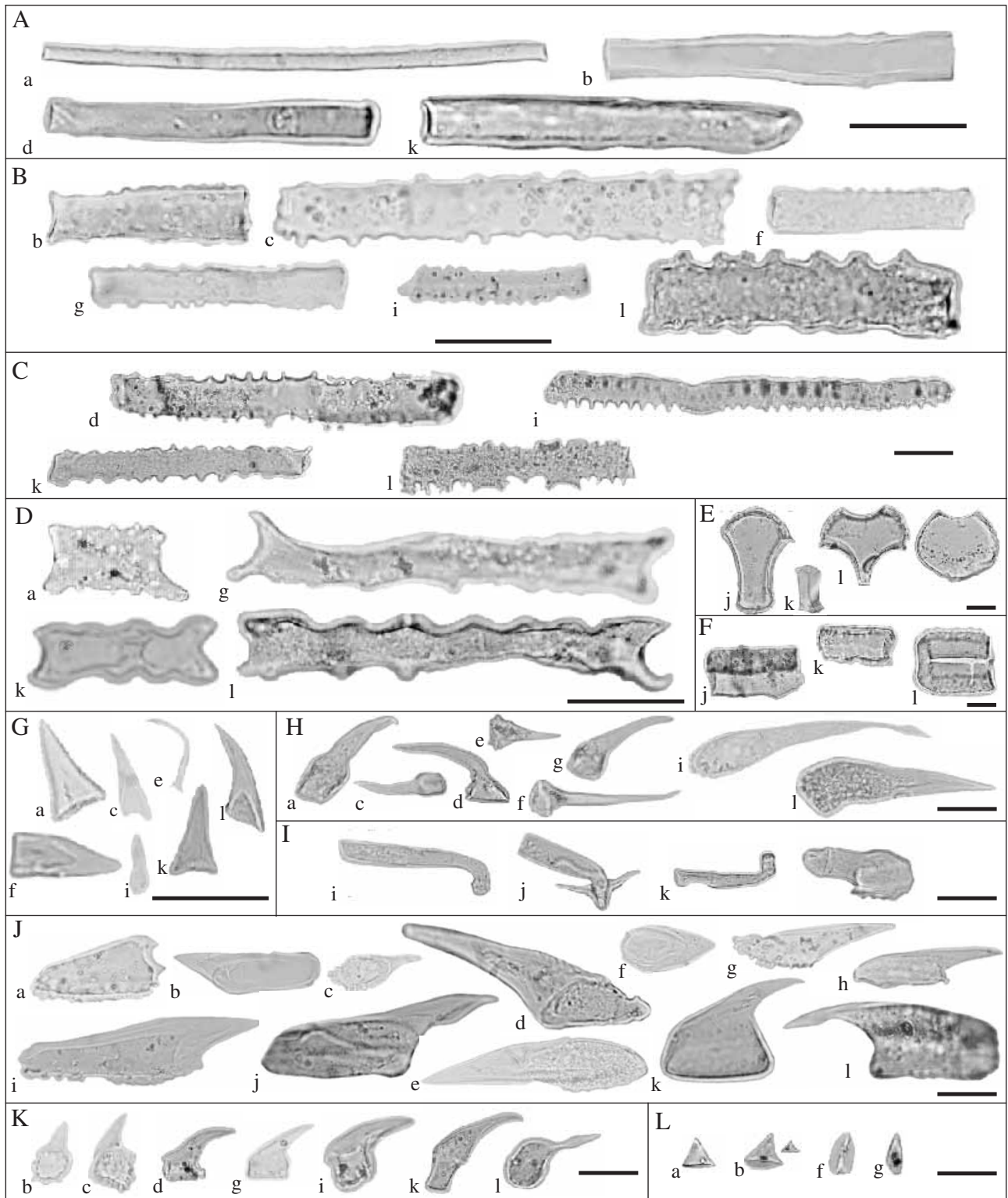


FIG. 3. Light micrographs of isolated-form phytoliths. (A) Elongate, smooth. (B) Elongate, sinuous. (C) Elongate, spiny. (D) Elongate, concave ends. (E) Fan. (F) Polyhedral. (G) Apex of sharp-pointed shapes (hairs, prickles or hooks). (H) Macro-hairs. (I) Micro-hairs. (J) Prickles hairs. (K) Hooks hairs. (L) Triangular shapes. a, *Poa ligularis*; b, *Stipa clarazii*; c, *Piptochaetium napostaense*; d, *Stipa tenuis*; e, *Stipa gynerioides*; f, *Stipa tenuissima*; g, *Stipa ambigua*; h, *Stipa brachychaeta*; i, *Aristida subulata*; j, *Bothriochloa edwardsiana*; k, *Digitaria californica*; l, *Pappophorum subbulbosum*. Scale bar = 20  $\mu\text{m}$ .

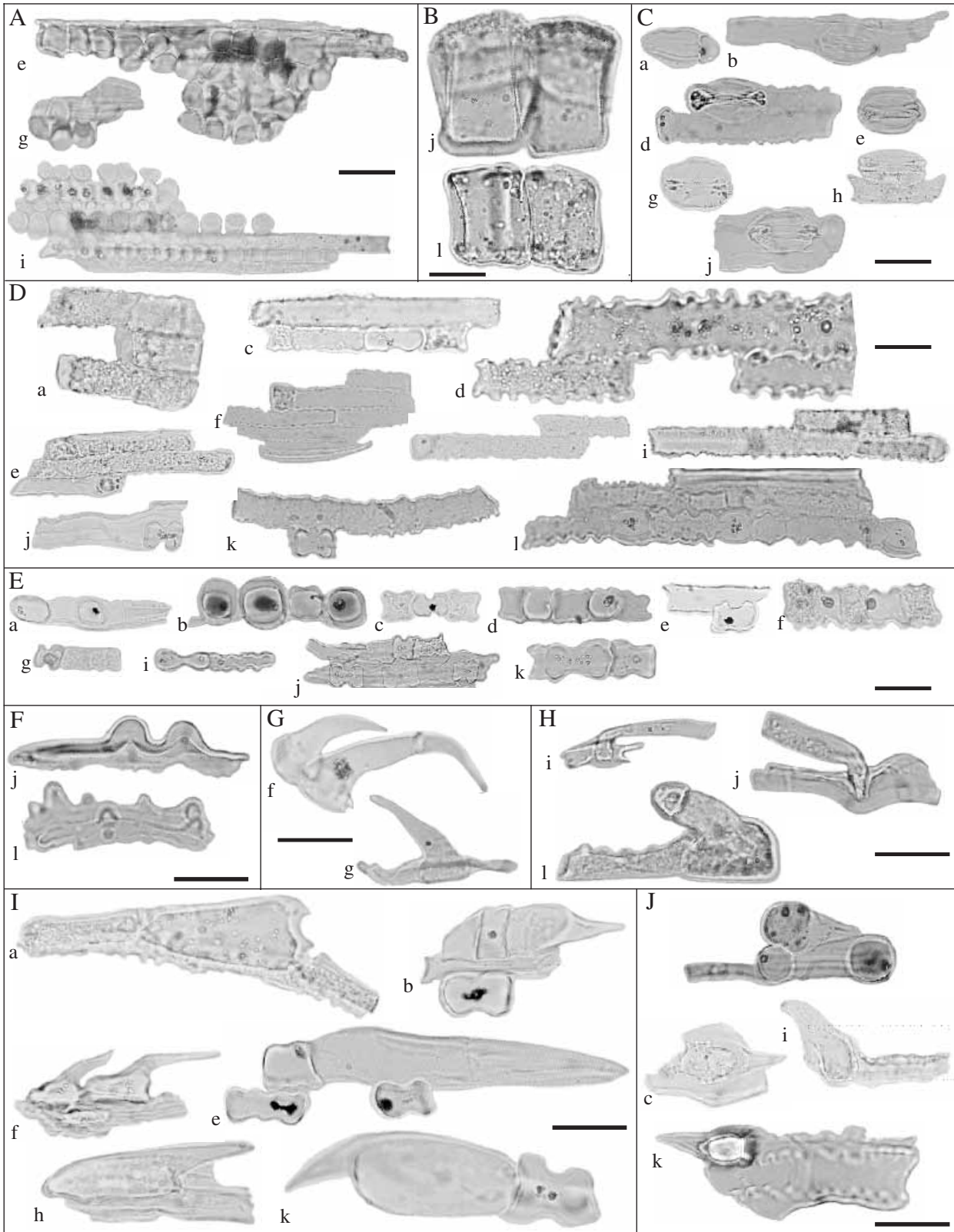


FIG. 4. Light micrographs of associated-form phytoliths. (A) Subepidermal elements. (B) Bulliform elements. (C) Stomatal complex. (D) Large cells. (E) Short cells. (F) Papillae. (G) Macro-hairs. (H) Micro-hairs. (I) Prickles hairs. (J) Hooks hairs. a, *Poa ligularis*; b, *Stipa clarazii*; c, *Piptochaetium napostaense*; d, *Stipa tenuis*; e, *Stipa gynerioides*; f, *Stipa tenuissima*; g, *Stipa ambigua*; h, *Stipa brachychaeta*; i, *Aristida subulata*; j, *Bothriochloa edwardsiana*; k, *Digitaria californica*; l, *Pappophorum subbulbosum*. Scale bar = 20  $\mu$ m.

TABLE 3. Relative frequency of phytolith morphotypes. Morphotypes are as in Table 2

Morphotypes	<i>Poa ligularis</i> <sup>a</sup>	<i>Stipa clarazii</i> <sup>a</sup>	<i>Piptochaetium napostaense</i> <sup>a</sup>	<i>Stipa tenuis</i> <sup>a</sup>	<i>Stipa eriotachya</i> <sup>a</sup>	<i>Stipa tenuissimica</i> <sup>a</sup>	<i>Stipa ambigua</i>	<i>Stipa brachychaeta</i>	<i>Batrachochloa edwardsiana</i>	<i>Digitaria californica</i>	<i>Pappophorum subulbosum</i>	<i>Aristida subulata</i>
1	18.55 ± 3.03	4.84 ± 0.67	0.87 ± 0.21	12.27 ± 1.00	7.19 ± 2.16	3.65 ± 0.80	0.99 ± 0.09	2.30 ± 0.54	—	0.23 ± 0.10	0.05 ± 0.05	—
2	—	1.19 ± 0.28	—	0.66 ± 0.19	5.82 ± 1.98	1.70 ± 0.16	0.42 ± 0.15	0.39 ± 0.25	—	—	—	—
3	—	0.17 ± 0.06	—	0.04 ± 0.04	1.24 ± 0.37	—	0.44 ± 0.17	0.10 ± 0.10	—	—	—	—
4	1.70 ± 0.39	2.08 ± 0.35	0.43 ± 0.17	4.16 ± 0.53	2.43 ± 0.64	1.23 ± 0.29	0.29 ± 0.19	0.05 ± 0.05	—	—	0.10 ± 0.10	0.05 ± 0.05
5	6.32 ± 2.09	4.55 ± 0.37	—	2.67 ± 0.56	0.05 ± 0.05	1.02 ± 0.46	—	—	—	—	0.34 ± 0.21	0.05 ± 0.05
6	34.40 ± 2.93	3.27 ± 1.34	0.34 ± 0.17	4.63 ± 0.96	0.96 ± 0.30	0.27 ± 0.12	—	0.20 ± 0.10	—	0.30 ± 0.18	0.14 ± 0.10	0.10 ± 0.06
7	4.09 ± 0.63	8.19 ± 1.07	0.82 ± 0.31	1.41 ± 0.25	1.31 ± 0.29	1.58 ± 0.22	5.64 ± 0.97	6.79 ± 1.66	0.29 ± 0.20	3.08 ± 0.69	1.46 ± 0.47	0.31 ± 0.15
8	—	—	—	—	—	—	—	—	—	—	30.59 ± 1.79	—
9	—	—	—	—	—	—	—	—	—	—	0.88 ± 0.59	—
10	—	—	—	—	—	—	—	—	1.37 ± 0.45	—	11.92 ± 1.77	—
11	—	—	—	—	—	—	—	—	0.03 ± 0.03	—	0.04 ± 0.04	—
12	1.14 ± 0.92	6.03 ± 1.14	15.13 ± 1.46	15.68 ± 2.01	1.76 ± 0.40	4.97 ± 0.84	3.87 ± 0.80	5.20 ± 1.33	0.17 ± 0.08	6.25 ± 0.50	9.54 ± 2.34	1.54 ± 0.48
13	1.10 ± 0.76	24.08 ± 1.48	11.68 ± 1.31	9.91 ± 1.32	35.59 ± 3.45	32.12 ± 1.98	29.77 ± 1.75	33.51 ± 3.42	32.19 ± 2.22	43.43 ± 1.28	10.90 ± 3.31	5.49 ± 1.72
14	—	0.06 ± 0.06	6.16 ± 1.10	0.22 ± 0.16	0.56 ± 0.26	0.21 ± 0.07	1.28 ± 0.61	—	0.10 ± 0.10	3.00 ± 0.72	1.48 ± 0.48	5.75 ± 1.67
15	—	0.50 ± 0.19	1.30 ± 0.24	—	0.96 ± 0.16	0.40 ± 0.17	0.30 ± 0.09	3.64 ± 0.93	10.22 ± 0.43	12.04 ± 1.26	—	37.96 ± 3.22
16	0.05 ± 0.05	0.09 ± 0.06	8.13 ± 0.91	2.51 ± 0.55	0.24 ± 0.07	0.13 ± 0.09	1.22 ± 0.33	0.45 ± 0.19	0.04 ± 0.04	0.32 ± 0.19	—	0.36 ± 0.10
17	0.14 ± 0.14	1.96 ± 0.41	1.15 ± 0.13	1.02 ± 0.17	1.09 ± 0.26	1.87 ± 0.49	4.48 ± 0.91	3.29 ± 0.93	3.89 ± 0.84	0.11 ± 0.07	0.20 ± 0.12	0.91 ± 0.37
18	—	—	1.07 ± 0.33	0.13 ± 0.05	0.09 ± 0.06	0.25 ± 0.15	0.07 ± 0.07	0.10 ± 0.06	—	—	—	—
19	—	0.92 ± 0.30	0.26 ± 0.14	0.27 ± 0.09	1.08 ± 0.53	2.95 ± 1.32	0.93 ± 0.24	0.98 ± 0.17	0.20 ± 0.20	0.06 ± 0.06	—	—
20	—	0.03 ± 0.03	4.84 ± 0.40	0.21 ± 0.11	0.10 ± 0.06	0.17 ± 0.08	0.25 ± 0.11	0.10 ± 0.10	—	—	0.05 ± 0.05	0.20 ± 0.09
21	—	0.37 ± 0.10	0.91 ± 0.33	0.04 ± 0.04	0.09 ± 0.06	0.63 ± 0.32	0.98 ± 0.52	0.83 ± 0.41	3.07 ± 0.70	—	—	0.20 ± 0.14
22	—	—	1.97 ± 0.21	0.09 ± 0.05	—	0.13 ± 0.09	0.07 ± 0.07	0.15 ± 0.10	—	—	—	0.05 ± 0.05
23	—	0.23 ± 0.12	0.10 ± 0.10	0.08 ± 0.08	0.28 ± 0.09	0.26 ± 0.08	0.63 ± 0.15	0.79 ± 0.23	0.27 ± 0.13	—	—	0.10 ± 0.06
24	—	—	2.11 ± 0.47	—	—	—	2.51 ± 0.88	0.64 ± 0.10	0.15 ± 0.07	0.11 ± 0.11	—	0.16 ± 0.10
25	—	—	—	—	0.05 ± 0.05	—	—	—	0.16 ± 0.12	—	—	—
26	5.71 ± 1.88	5.68 ± 1.35	4.25 ± 1.78	16.51 ± 2.57	0.64 ± 0.28	3.65 ± 1.04	10.48 ± 0.65	14.06 ± 2.90	1.76 ± 0.48	1.94 ± 0.25	0.59 ± 0.23	1.70 ± 0.12
27	0.55 ± 0.15	0.46 ± 0.13	1.43 ± 0.52	1.34 ± 0.33	0.33 ± 0.17	0.74 ± 0.16	1.88 ± 0.58	0.73 ± 0.22	0.46 ± 0.18	0.75 ± 0.15	0.69 ± 0.14	1.39 ± 0.12
28	1.49 ± 0.58	0.61 ± 0.22	3.36 ± 0.72	1.54 ± 0.31	0.77 ± 0.33	0.37 ± 0.12	2.29 ± 0.45	1.72 ± 0.63	0.16 ± 0.07	1.10 ± 0.18	2.12 ± 0.32	3.87 ± 0.72
29	0.09 ± 0.05	0.03 ± 0.03	0.05 ± 0.05	0.19 ± 0.09	0.09 ± 0.06	0.28 ± 0.14	0.69 ± 0.21	0.15 ± 0.10	0.26 ± 0.13	0.17 ± 0.11	0.29 ± 0.06	0.05 ± 0.05
30	0.04 ± 0.04	—	—	—	—	—	—	—	0.48 ± 0.19	—	0.91 ± 0.57	—
31	0.18 ± 0.13	0.06 ± 0.06	0.05 ± 0.05	0.09 ± 0.09	—	—	0.12 ± 0.07	0.30 ± 0.18	1.03 ± 0.31	0.67 ± 0.22	1.01 ± 0.21	0.31 ± 0.15
32	6.74 ± 1.17	2.82 ± 0.66	1.75 ± 0.53	0.94 ± 0.30	9.41 ± 1.26	9.47 ± 2.21	1.73 ± 0.54	4.85 ± 0.98	0.85 ± 0.33	3.35 ± 0.26	6.18 ± 0.40	6.35 ± 1.00
33	3.66 ± 0.67	0.37 ± 0.12	2.25 ± 0.26	0.44 ± 0.20	2.23 ± 0.45	16.13 ± 1.60	1.52 ± 0.55	—	—	0.05 ± 0.05	0.17 ± 0.11	—
34	—	—	—	—	—	—	—	—	0.69 ± 0.20	—	2.20 ± 1.47	—
35	1.50 ± 0.31	1.99 ± 0.07	4.87 ± 0.75	1.81 ± 0.18	1.99 ± 0.39	1.05 ± 0.07	8.41 ± 1.17	7.09 ± 0.81	0.66 ± 0.27	5.67 ± 1.06	1.12 ± 0.40	3.93 ± 0.80
36	—	2.88 ± 0.78	10.45 ± 1.32	7.93 ± 1.37	—	—	6.37 ± 1.98	3.70 ± 1.12	0.85 ± 0.21	4.71 ± 0.42	2.05 ± 0.34	4.49 ± 0.81
37	—	0.24 ± 0.14	0.10 ± 0.10	—	—	0.76 ± 0.28	0.07 ± 0.07	—	0.08 ± 0.05	—	0.04 ± 0.04	—
38	0.13 ± 0.08	0.65 ± 0.15	0.22 ± 0.10	0.37 ± 0.12	0.57 ± 0.12	0.10 ± 0.10	0.92 ± 0.08	0.10 ± 0.10	0.85 ± 0.30	0.06 ± 0.06	0.73 ± 0.11	2.03 ± 0.69
39	—	—	—	—	—	—	1.11 ± 0.25	1.18 ± 0.36	0.65 ± 0.14	0.20 ± 0.09	1.11 ± 0.12	0.10 ± 0.06
40	4.22 ± 0.94	5.89 ± 0.65	0.82 ± 0.13	1.02 ± 0.19	2.13 ± 0.51	0.66 ± 0.26	3.14 ± 0.46	2.16 ± 0.75	0.42 ± 0.14	0.32 ± 0.10	3.16 ± 0.41	5.81 ± 0.73
41	3.27 ± 0.32	4.61 ± 0.48	3.22 ± 0.16	4.87 ± 0.41	3.05 ± 0.30	2.97 ± 0.77	3.07 ± 0.65	1.98 ± 0.60	4.52 ± 0.35	5.85 ± 0.60	2.12 ± 0.43	7.39 ± 1.23
42	2.46 ± 0.62	7.49 ± 0.73	2.85 ± 0.61	1.96 ± 0.46	6.17 ± 0.56	4.93 ± 1.27	1.28 ± 0.33	0.74 ± 0.30	10.36 ± 0.99	3.47 ± 0.71	4.53 ± 0.40	4.66 ± 0.99
43	—	—	—	—	—	—	—	—	19.00 ± 1.58	—	1.51 ± 0.35	—
44	1.11 ± 0.27	0.48 ± 0.26	0.05 ± 0.05	0.22 ± 0.07	4.00 ± 1.08	2.92 ± 0.54	—	—	—	—	0.05 ± 0.05	—
45	—	—	—	—	—	—	—	—	0.86 ± 0.37	0.05 ± 0.05	1.07 ± 0.31	0.81 ± 0.28
46	1.37 ± 0.44	2.78 ± 0.29	3.63 ± 0.52	1.74 ± 0.25	7.74 ± 0.79	2.45 ± 0.62	1.67 ± 0.65	1.18 ± 0.35	2.85 ± 0.64	1.95 ± 0.19	0.46 ± 0.09	2.14 ± 0.41
47	—	4.40 ± 1.08	3.42 ± 0.70	3.03 ± 0.41	—	—	1.11 ± 0.25	0.54 ± 0.30	1.06 ± 0.31	0.73 ± 0.21	0.21 ± 0.13	1.71 ± 0.46

Values are mean ± s.e. (n = 5).

<sup>a</sup>Morphotypes 8–11, 34, 39, 43 and 45 were absent for these species.

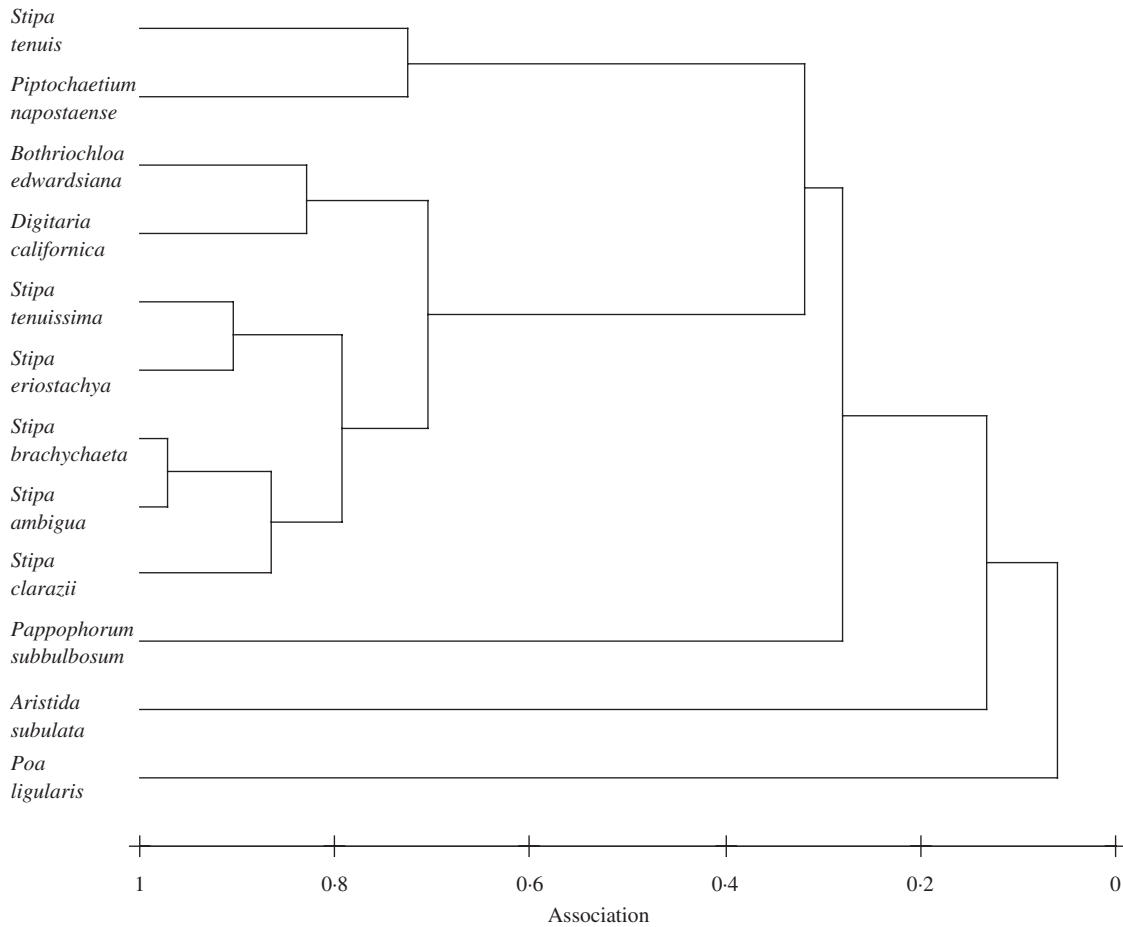


FIG. 5. Dendrogram showing the relationships among phytolith assemblages of the examined species. The Morisita Index was used to measure the strength of the association among specific phytolith assemblages (Horn, 1966).

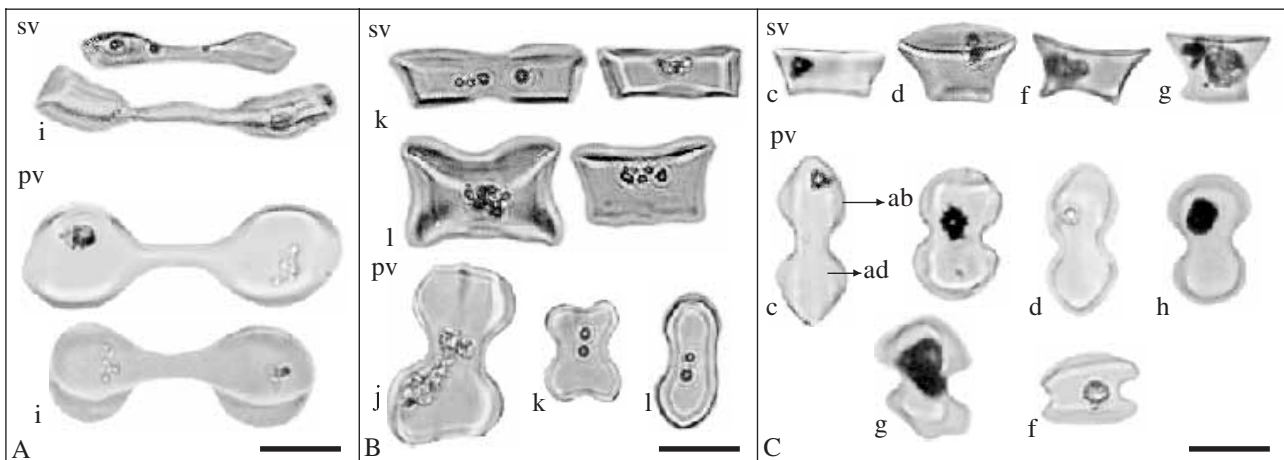


FIG. 6. Light micrographs of phytolith forms in three-dimensional view. (A) Simple-type dumb-bell, with large and narrow central portion, rounded end in planar view (pv), and lateral plane of symmetry well developed (sv). (B) Panicoid type dumb-bell, with indented end, a double outline in planar view (pv), and a tabular side view (sv). (C) *Stipa* type dumb-bell, with two opposite broad faces of different size (ad: adaxial face, ab: abaxial face) in planar view (pv), and a trapezoidal side view (sv). c, *Piptochaetium napostaense*; d, *Stipa tenuis*; f, *Stipa tenuissima*; g, *Stipa ambigua*; h, *Stipa brachychaeta*; i, *Aristida subulata*; j, *Bothriochloa edwardsiana*; k, *Digitaria californica*; l, *Pappophorum subbulbosum*. Scale bar = 10  $\mu$ m.



end, smooth elongate, isolated hooks, and isolated prickles characterized *S. ambigua* and *S. brachychaeta*. The latter phytolith assemblages differed from each other by a high frequency of dumb-bells with a large central portion and straight end, and apex sharp-pointed shapes in *S. brachychaeta*, whereas isolated hooks and large cells characterized *S. ambigua*.

## DISCUSSION

The phytolith morphotypes present in the species studied are characteristic of the *Poaceae*, as defined by Twiss (1992). Although phytolith multiplicity and redundancy can limit the differentiation between species (Rovner, 1971), our cluster analysis results show the possibility of distinguishing at the species level or between plant functional types (i.e. C<sub>3</sub> from C<sub>4</sub> grasses, preferred from avoided grasses) in the native grasslands of central Argentina.

The dominant phytolith forms described for *P. ligularis*, *P. subbulbosum* and *A. subulata* were consistent with previous descriptions for the same species obtained through microhistological analysis (Lindström *et al.*, 1998). The phytolith assemblages of these species corresponded to the subfamilies *Pooideae*, *Chloridoideae* and *Arundinoideae*, respectively, as defined by Twiss (1992). The subfamily *Pooideae* is characterized by the presence of round, rectangular, oblong, elliptical and crescent-moon shapes, and by the absence of saddle, dumb-bells and cross shapes. The subfamily *Chloridoideae* is characterized by the presence of cross, dumb-bell and, particularly, saddle shapes. The subfamily *Arundinoideae* represents a highly heterogeneous group, in which the dominant forms are rectangular, oblong, crenate and dumb-bell.

In *A. subulata* the dominant form was the dumb-bell type, with a large and narrow central portion, rounded end and lateral plane of symmetry well developed (Fig. 6A). This form corresponds to the simple bilobate type described by Mulholland (1989), Fredlund and Tieszen (1994) and Kerns (2001). These authors distinguished this type from other types of dumb-bell, denominating them *Stipa* and Panicoid types. The *Stipa*-type dumb-bell (observed in Subgroups I and III) has two opposite broad faces of different size (top or planar view showing the outline, with two lobes connected by a shaft), and a trapezoidal side view (lateral faces connecting the two opposite broad faces) (Fig. 6C). The Panicoid-type dumb-bell (observed in *P. subbulbosum*) has indented ends, a double outline in planar view, and a tabular side view (Fig. 6B).

The phytolith characterization of *D. californica* and *B. edwardsiana* corresponded to the subfamily *Panicoidae*, as defined by Twiss (1992). This subfamily is characterized by the presence of smooth and sinuous elongate, dumb-bell (Panicoid-type) and cross shapes. Forms representative of the subfamily *Arundinoideae*, with a high frequency of *Stipa*-type dumb-bells forms (Fig. ??), characterized the rest of the species. Zucol (1996) reported similar results for species of the genus *Stipa* inhabiting the Entre Ríos province, Argentina.

Our examination of phytolith assemblages in several grasses native to the central Argentina also highlights the possibility to differentiate between C<sub>3</sub> and C<sub>4</sub> species (Table 1 and Fig. 5). The C<sub>4</sub> grasses were dominated by the dumb-bell (simple bilobate and Panicoid-types), cross and saddle forms, whereas the C<sub>3</sub> grasses were dominated by the Pooide Class forms (mainly in *P. ligularis*) and *Stipa*-type dumb-bells. The distinction between C<sub>3</sub>/C<sub>4</sub> grasses based upon phytolith forms was first suggested by Twiss *et al.* (1969). Later studies have improved the differentiation between C<sub>3</sub>/C<sub>4</sub> grasses by adding a three-dimensional examination of phytoliths, to minimize problems associated with multiplicity and redundancy of phytolith forms (Brown, 1984; Fredlund and Tieszen, 1994). Our results also show that differences in phytolith assemblages allow differentiation between preferred (palatable, high forage value) and avoided (non-palatable, low forage value) grasses within photosynthetic pathway groups (Table 1 and Fig. 5). However, the distinction between preferred and avoided grasses based on phytolith analysis need to be examined further to know its generality. In summary, we conclude that the present phytolith reference collection can assist in the interpretation of soil phytolith assemblages aimed at reconstructing past vegetation in the central Argentina grasslands.

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