



An archaeobotanical perspective in the study of inflorescence phytoliths of wild grasses from arid and semi-arid environments of Argentina



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ABSTRACT

Wild cereals have been harvested all over the world by hunter-gatherer with evidences as much in South America as in Australia, North America and Subsaharian Africa. In some places there are still used for human consumption. The goal of this paper is to document phytoliths in inflorescence axis, and in kernels/inflorescences/bracts of several native Argentinian wild grasses in order to get an archaeobotanical comparative referential for the analyses of residues from both food plant processing activities and consumption. We could observe typical phytolith morphotypes from specific anatomical parts of grasses, but non-taxon specific, as well as non-diagnostic phytolith morphotypes, but characteristic of the phytolith association of a certain taxa. They all allow us to get a more complete view of the phytolith production for each specific taxon. Results obtained by Morisita Test-Past Program-suggest a trend to the organization at the level of subfamilies for the phytoliths production in the 14 species studied. Species of the genus *Aristida* and *Cynodon* show an intra-generic consistency in the production of phytoliths morphotypes per organ, and tend to be grouped in cluster analysis. In some species as *Cynodon dactylon*, *Bromus catharticus*, *Aristida adscensionis*, *Sorghastrum pellitum* and *Paspalum dilatatum* it can be noted a similarity in the morphotype production as much in inflorescence axis as in kernels/inflorescences/bracts. A morphological continuum between similar morphotypes could be also established, by comparing series of shapes within fragments of silicified plant tissue. Finally, we consider phytolith production from potential useful plant parts for human consumption, in a way as their presence alone, or associated with starch grain analyses, as showed by a previous work, could be used for regional dietary reconstructions. Our results from inflorescence/kernel phytolith production give us good expectation to be able to identify them in archaeological contexts.

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1. 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, typically 5–200 µm in size (Bertoldi de Pomar, 1975; Rovner, 1983; Brown, 1984; Piperno,

2006). Because of their consistent shape within species, phytoliths provide significant taxonomic information (Twiss, 1992; Piperno, 2006). They form in plant tissues after soluble silica, in the form of monosilicic acid (H₄SiO₄), is taken up from the soil. Silica is then deposited as solid hydrogenated silicon dioxide (SiO₂ nH₂O) in fillings of cell walls, cell interiors (lumina), and intercellular spaces.

Although the accumulation of silica occurs in various taxa of the plant kingdom, phytoliths are particularly abundant in the family Poaceae. Phytoliths from Poaceae short cells are shaped actively under genetic control which representing a valuable taxonomic attribute diagnostic of subfamily, tribe and even genus level of the

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Poaceae (Rovner, 1971; Piperno, 1988; Twiss, 1992). As in other families, Poaceae individuals produce many types of phytolith forms (multiplicity), and a particular form may be produced by a number of different species (redundancy) (Rovner, 1983). Data for wild grass inflorescences are potentially useful in paleoecological studies and we sustain they can be used in addition to the information from leaves and stems.

The goal of this paper is to document phytoliths in bracts, kernels/inflorescences as well as in inflorescence axis of several native Argentinian wild grasses from arid and semi-arid environments, in order to get an archaeobotanical comparative referential for the analyses of residues from both, food plant processing activities and consumption. This paper complements previous studies on starch grains from kernels of the same taxa selected here (Musaubach



Fig. 1. Extension of arid and semi-arid biomes of Monte and Espinal in Argentine territory, according to Cabrera (1976) and *Atlas de los Bosques Nativos de Argentina* (2003).

et al. 2013), and existing information on phytoliths in aerial parts mainly stems and leaves, of native wild grasses of South America Southern Cone (Zucol, 1996, 1998, 1999, 2000, 2001; Gallego and Distel, 2004; Gallego et al. 2004; Fernández Honaine et al., 2006, 2008). On this base, the phytolith production from potential useful plant parts for human consumption, such as their presence alone, or associated with starch grain, could be used for regional dietary reconstructions based on microfossil analyses.

We deal with typical phytolith morphotypes from specific anatomical parts of grasses, but non-taxon specific, as well as non-diagnostic phytolith morphotypes, but characteristic of the phytolith association of a certain taxa. They all allow us to get a more

complete vision of the phytolith production for each specific taxon (Korstanje and Babot, 2007). Short-cell morphotypes are analyzed here by using the Jaccard Test (Past Program Version 2.17–Hammer, 1999–2012) to identify significant associations. We use the Z Brush Program to reconstruct on a 3D base, shapes in between them it can be established a *morphological continuum*. We think results from inflorescence phytolith production give us good expectation to be able to identify them in archaeological contexts. At present, the processing and use of kernels of native wild grasses in the past is an unexplored issue due in part to the lack of appropriate reference collections. The extent to which a clear differentiation between aerial Poaceae parts (i.e. from environmental or manufacturing

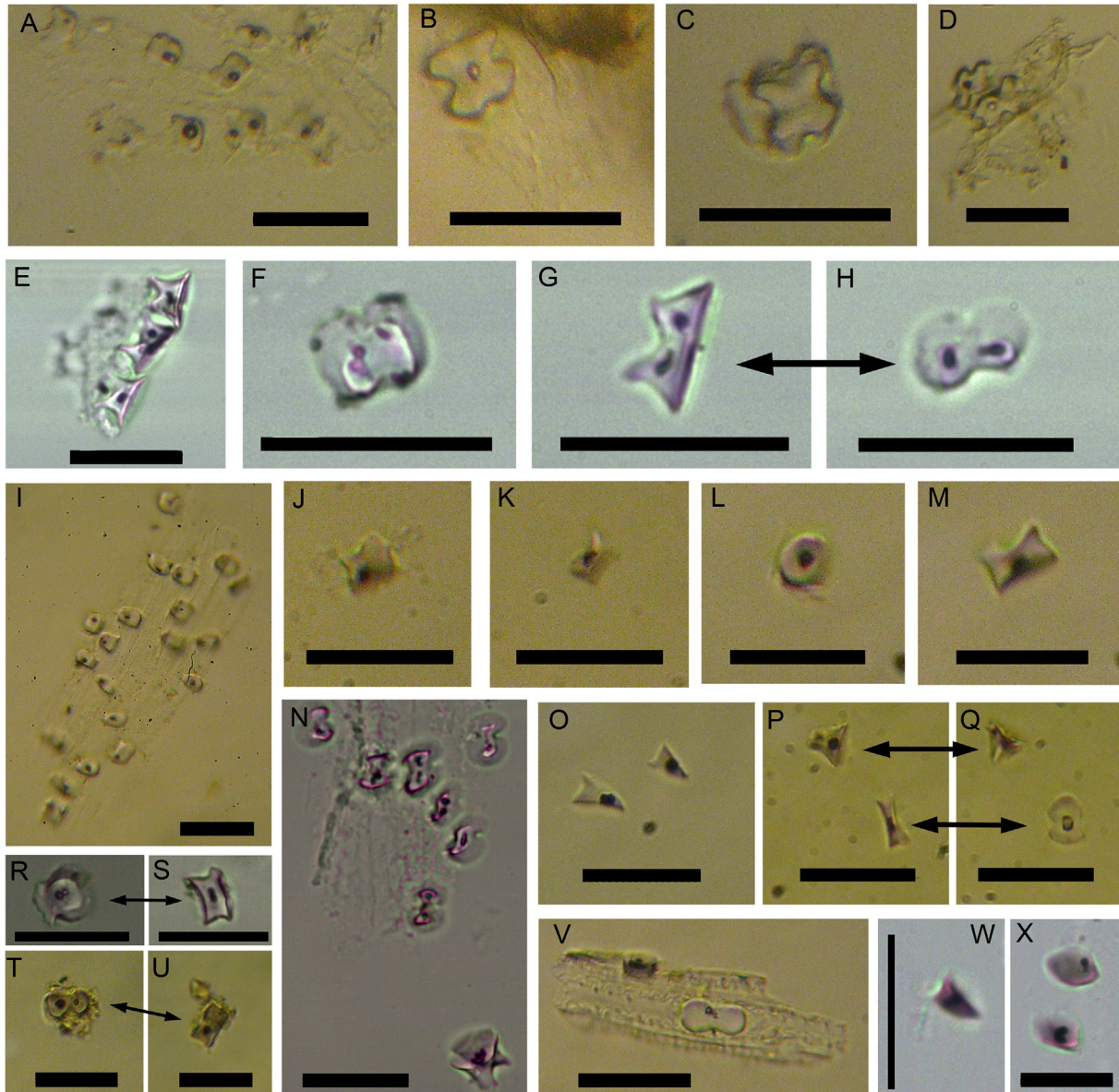


Fig. 2. Examples of short-cell phytolith variation in Poaceae species from Monte and Espinal. A: Short panicoid bilobate *in situ*. *Paspalum dilatatum* (axis); B: Quadrangular panicoid bilobate – tetralobate *Paspalum dilatatum* (kernel); C: Cross. *Paspalum dilatatum* (axis); D: Short panicoid bilobate. *Paspalum dilatatum* (axis); E: Rectangular short center panicoid bilobate, 2 projections on top. *Aristida adscencionis* (axis); F: Rectangular short center panicoid bilobate, 2 projections on top. *Aristida adscencionis* (axis); G: Rectangular short center panicoid bilobate, 2 projections on top. Lateral view. *Aristida adscencionis* (axis); H: Same phytolith top view; I: Flat rondel. *Aristida subulata* (kernel); J: *Aristida* type rondel variant 2. *Aristida adscencionis* (axis); K: *Aristida* type rondel variant 1. *Aristida adscencionis* (axis); L: Flat rondel short cell. *Aristida mendocina* (kernel); M: Waisted rondel, two projections on top. *Aristida mendocina* (kernel); N: Rectangular short center panicoid bilobate, 2 projections on top. *Sorghastrum pellitum* (kernel); O: Keeled rondel. *Amelichloa brachychaeta* (kernel); P: Short bilobate and equidimensional rondel. Lateral view. *Amelichloa brachychaeta* (kernel); Q: Short bilobate. Top view. *Amelichloa brachychaeta* (kernel); R: Waisted rondel. Top view. *Sporobolus rigens* (kernel); S: Same phytolith. Lateral view. *Sporobolus rigens* (kernel); T: Short panicoid bilobate. Top view. *Amelichloa brachychaeta* (kernel); U: Same phytolith. Lateral view; V: Short panicoid bilobate. *Aristida mendocina* (kernel). W: Keeled rondel; *Bromus catharticus* (bracts); X: Concave rondel. *Bromus catharticus* (bracts).

origin) and inflorescences is possible, it is also an irresolute issue. While it was recorded the presence of Poaceae phytoliths in residues of grinding artifacts, ceramics residues, dental tartar and archaeological sediment matrix, its interpretation has been vague and speculative (Zucol and Bonomo, 2008; Zucol et al., 2008; Babot, 2009, 2011; Musaubach et al., 2010; Musaubach, 2012, 2014, 2015; Flegenheimer et al., 2013).

The aims of this research are: 1. To contribute to characterize the morphological variation of phytolith of native wild species of grasses present in arid and semi-arid biomes of Central and Northwest Argentina; 2. To characterize the phytolith richness in inflorescence axis, and in kernels/inflorescences/bracts of native grasses in order to get an archaeobotanical comparative referential for the analyses of residues from both, food plant processing activities and consumption; 3. To establish if phytoliths assemblages can be used to discriminate between different taxa; and 4. To propose the concept of *morphological continuum* from morphotypes of close morphologies recorded *in situ* in silicified plant tissue.

2. Study area

2.1. Monte and Espinal: two phytogeographic regions from arid and semi-arid environments in Argentina

Monte and Espinal are two main phytogeographic regions defined for arid and semi-arid environments in Argentina (Cabrera, 1976) (Fig. 1). Monte is a forest region that extends along the temperate arid zone of the country. It occupies part of the hill slopes and the longitudinal broad valleys and *bolsones* located in depressions between mountainous cords of the Andes and the Precordillera (Cabrera, 1976; *Atlas de los bosques nativos de Argentina*, 2003). The typical formation of the Monte is the

xerophilous and halophilous bush or shrub steppe leaving patches of bare ground; the dominant species are the “jarillas” (*Larrea divaricata* Cav., *L. nitida* Cav. and *L. cuneifolia* Cav.). *Prosopis* sp. trees grow exceptionally in moisture places. Monte forms a broad ecotone with other forest region named Espinal (Cabrera, 1976; *Atlas de los bosques nativos de Argentina*, 2003). Espinal is a semi-arid phytogeographic region that extends in transition to more humid Pampas grasslands. It consists of open deciduous xerophytic forests with a single layer of relatively low trees, dominated by Fabaceae species such as “caldén” (*Prosopis caldenia* Burkart), and of shrub steppes. It presents rich shrub and herbaceous layers, sometimes forming savannas (Cabrera, 1976). In both biomes grow up Poaceae species of several families and tribes. Edaphic vegetation of Monte includes grasslands of *Sporobolus maximus* Hauman (hunquillo) in salt marshes, steppes of *S. rigens* (junquillo) in living dunes, and perennial *caespitose* wild grasses like *S. maximus*, from the bottom of the basin to the broken landscape of the slopes. In Caldén sub-region of Espinal, forest alternate with grass steppes of *Stipa* sp. (flechilla) and *S. rigens* (Cabrera, 1976; *Atlas de los bosques nativos de Argentina*, 2003).

2.2. Useful Poaceae species of Monte and Espinal

From the various Poaceae species recorded in these two arid and semi-arid phytogeographic regions, some highlight because of their utility to human consumption and thus, for their potential past uses as food by native prehispanic populations. According with Ragonese and Martínez Crovetto (1947), the wild grasses consumed as food are several species as *Bromus unioloides* Kunth (barley grass or cebadilla), *Chusquea culeou* Std. (colihue), *C. quila* Kunth (quila), *Merostachys clausenii* Munro (tacupá), *Oryza perennis* Moench and *O. subulata* Ness. The grains

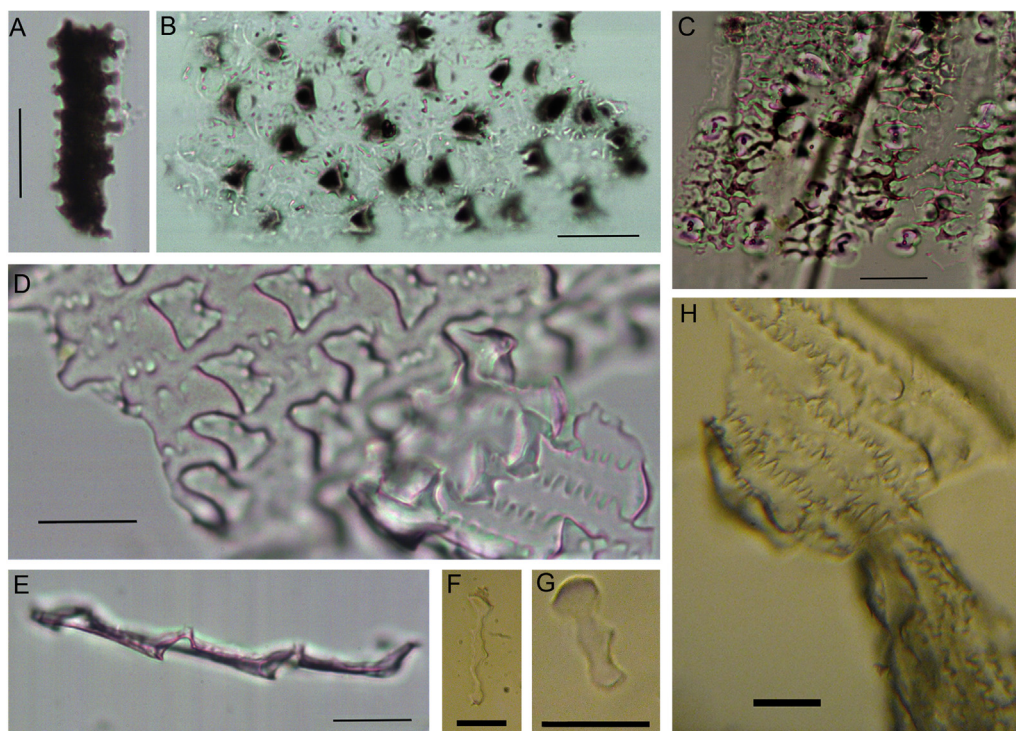


Fig. 3. Examples of long-cell phytolith variation in Poaceae species from Monte and Espinal. A: Tuberculate elongate. *Bromus catharticus* (bracts); B: Dendriform elongate, black straight end. *Sporobolus rigens* (kernel); C: Dendriform elongate. *Sorghastrum pellitum* (kernel); D: Aristida type crenate elongate. *Aristida adscencionis* (kernel); E: Aristida type elongate. Lateral view. *Aristida adscencionis* (kernel); F: Aristida type ruminant elongate. *Aristida subulata* (kernel); G: Aristida type crenate elongate. *Aristida mendocina* (kernel); H: Equinate elongate. *Paspalum dilatatum* (axis).

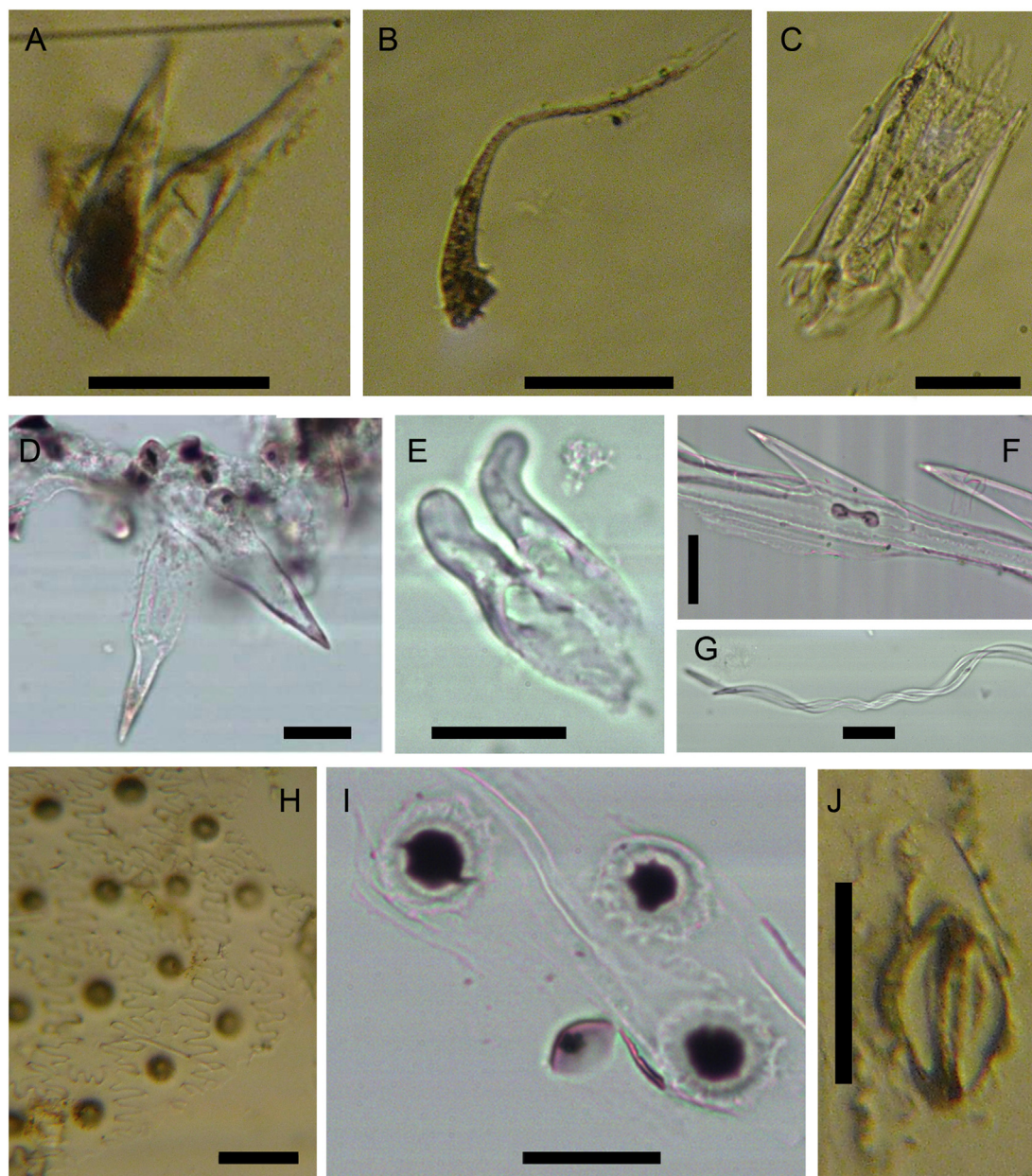


Fig. 4. Examples of dermal appendices and other phytoliths in Poaceae species from Monte and Espinal. A: Point-shaped single hair, ampliate base. *Paspalum dilatatum* (axis); B: Point-shaped single hair. *Cynodon dactylon* (axis); C: Point-shaped single hair, ampliate base, oblong internal silicification. *Aristida adscencionis* (kernel); D: *Bromus* type bicellular, point shaped hair. *Bromus catharticus* (kernel); E: Round end single hair. *Bromus catharticus* (axis); F: Point-shaped single hair, ampliate base. *Aristida adscencionis* (kernel); G: Edge hair from lemma. *Aristida adscencionis* (axis); H: Papillae in dendriform cell. *Paspalum dilatatum* (kernel); I: Papillae in dendriform cell. *Bromus catharticus* (bracts); J: Stomatal complex. *Paspalum dilatatum* (axis).

of *Bromus* sp. grass were used by societies in Argentina and Chile for preparation of food (Ragonese and Martínez Crovetto, 1947). Instead, Reiche (1938 cited in Ragonese and Martínez Crovetto, 1947) indicates that the seeds of other species of *Chusquea* were used to make flour, whose use was abandoned with the introduction of the corn (*Zea mays* L.) and grains of Old World (*Triticum aestivum* L., *Hordeum vulgare* L., among other), in southern Chile. *Oryza* species are also large recognized as food. Two different ethnic groups, “guachí” and “paraguá” harvested *O. perennis*, using grains with glumes included for the preparation of food (Métraux, 1946). In Brazil, *O. subulata* was cultivated in Pará and Alagoas states where it was consumed in the same way as rice (*O. sativa*).

Because of written records on Poaceae species usefulness for food could be fragmentary; we consider it is suitable to maintain an open mind to the possibility of past processing and consumption of a broad set of wild grasses. It is along this line we work in this research by providing a reference collection for future studies to test this expectations.

3. Material and methods

3.1. Our phytolith approach

We use the concept of phytolith assemblages to get a more complete vision of the phytolith production for each specific

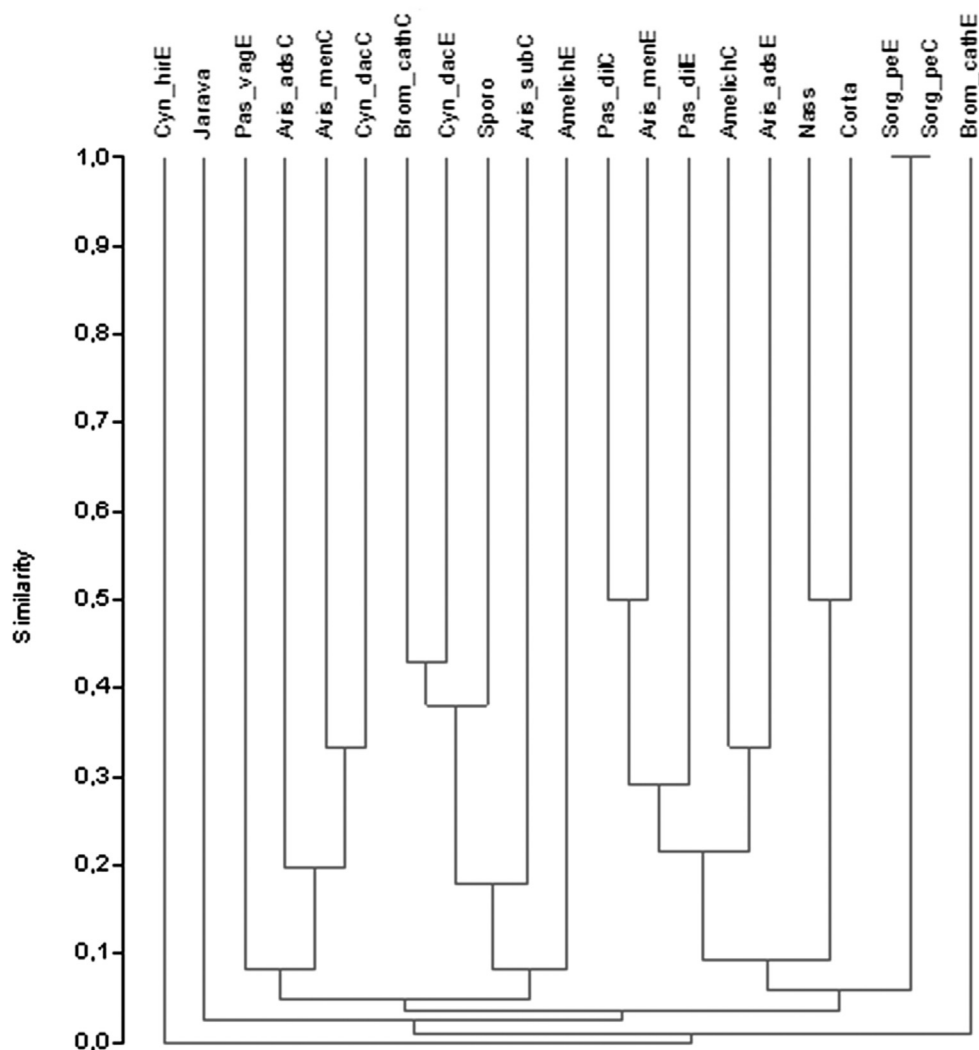


Fig. 5. Cluster tree discriminating species by their total short-cell phytolith production per organ.

taxon (Korstanje and Babot, 2007). We consider typical phytolith morphotypes from specific anatomical parts of grasses, but non-taxon specific; and non-diagnostic phytolith morphotypes, but characteristic of the phytolith association of a certain taxa. We think this procedure strengthens the data on taxa assignation particularly when deal with consumption residues in which microfossil record is of a fragmentary nature. Thus, the majorities of phytoliths we present in this study are limited to Poaceae silica short cell phytoliths (Piperno, 2006); some elongate long cell, trichomes phytoliths and other forms, are discussed and illustrated as well.

We use plant material from Herbaria in order to have certainties of its taxonomic identification and because of the ability to access the source material in future cases. Because of this, modern material samples were reduced in weight and likewise the phytoliths recovered and counted from them.

Many of the terms and nomenclature we used derived from the International Code for Phytolith Nomenclature (ICPN) 1.0 (Madella et al., 2005). However, when descriptions and names were published prior to acceptance of the ICPN, many of those descriptors were retained here in the interest of maintaining consistency (Yost and Blinnikov, 2011). In those cases, we provide equivalent notations. The broadest face of a phytolith, when present, is referred to as the base, and the opposite face, by

default, is the top. When viewed *in situ* in plant tissue, typically, the phytolith base is anatomically the top face, and protrudes slightly from the epidermis. When disarticulated from the epidermis, this “top” face typically settles face-down on the microscope slide. The morphological criteria followed to characterize phytoliths assemblages in this paper are: (1) surface and three-dimensional attributes to describe diagnostic and characteristics shapes, (2) statistical analysis which combined procedure of the short-cell morphotypes (organ of formation) and shape attributes (Jaccard Test-Past Program Version 2.17-Hammer, 1999–2012–) to discriminate production patterns by taxa, and (3) utilization of 3D design program (Z Brush © 2015 Pixologic, Inc.) to carry on geometrical comparisons between similar morphotypes identified, as an input to discuss the *morphological continuum* concept.

3.2. Sample and techniques

The material was obtained from herbarium specimens hosted at the National University of La Plata, Herbarium LP (Table 1, Appendix A). We selected fourteen species of native wild grasses according with the present occurrence of the genera on herbaceous communities belonging to Monte and Espinal phytogeographic regions (Cabrera, 1951, 1976).

Table 1
Grass species examined for phytoliths.

Scientific	Common	Subfamily	Tribe
<i>Amelichloa brachychaeta</i>	Pasto puna	Pooideae	Poeae
<i>Aristida adscensionis</i>	Pasto perro	Aristidoideae	Aristideae
<i>Aristida mendocina</i>	Flechilla crespa/Pasto crespo	Aristidoideae	Aristideae
<i>Aristida subulata</i>	Pasto crespo	Aristidoideae	Aristideae
<i>Bromus catharticus</i>	Cebadilla australiana/Cebadilla criolla	Pooideae	Poeae
<i>Cortaderia selloana</i>	Cortadera	Arundinoideae	Arundineae
<i>Cynodon dactylon longiglumis</i>	Gramilla/Gramilla rastrera/Pata de la perdiz	Chloridoideae	Cynodonteae
<i>Cynodon hirsutus</i>	Gramilla/Gramilla rastrera	Chloridoideae	Cynodonteae
<i>Jarava ichu</i>	Paja blanco/Flechilla Blanca	Pooideae	Poeae
<i>Nasella clarazzi</i>	Flechilla grande	Pooideae	Poeae
<i>Paspalum dilatatum</i> subsp. <i>dilatatum</i>	Pasto miel	Panicoideae	Paniceae
<i>Paspalum vaginatum</i>	Chepica dulce	Panicoideae	Paniceae
<i>Sorghastrum pellitum</i>	Paja colorada	Panicoideae	Andropogoneae
<i>Sporolobus rigens</i>	Unco	Chloridoideae	Sporoboleae

Table 2
Morphotypes used to classify phytoliths recorded in Poaceae species studied. Nomenclature follows ICPN (Madella et al., 2005) and equivalent notations are provided.

Code number	Shape descriptors/Texture and ornamentation descriptors/ Anatomical descriptors (ICPN 1.0)	Phytolith morphotypes following schemes given by Bertoldi de Pomar (1972, 1975) and Zucol and Bonomo (2008)
1	Flat bilobate/short central portion/2-point on top short cell	Bilobado panicoide centro corto sección rectangular con 2 prolongaciones en top (<i>Rectangular short center panicoid bilobate, 2 projections on top</i>)
2	Flat bilobate/concave end/long central portion short cell	Bilobado panicoide centro largo (<i>Long center panicoid bilobate</i>)
3	Short concave end flat bilobate short cell	Bilobado panicoide corto (<i>Short panicoid bilobate</i>)
4	Quadrangular bilobate short cell	Bilobado panicoide cuadrangular tetralobado (<i>Quadrangular panicoid bilobate – tetralobate</i>)
5	Long concave end flat bilobate short cell	Bilobado panicoide largo (<i>Long panicoid bilobate</i>)
6	Tabular polygonal psilate epidermal cell	Célula poliédrica (<i>Polyhedral cell</i>)
7	Quadra-lobate cross or Cross short cell	Cruz (<i>Cross</i>)
8	Psilate globular	Esférico liso (<i>Smooth spherical</i>)
9	Polylobate short cell	Polilobado (<i>Polylobate</i>)
10	Ruminate rectangular	Rectangular rumeado (<i>Ruminate rectangular</i>)
11	Flat rondel short cell	Rondel achatado (<i>Flat rondel</i>)
12	Concave/2-point elongate rondel short cell	Rondel <i>Aristida</i> variante 1 (<i>Aristida type rondel variant 1</i>)
13	Concave/2-point rondel short cell	Rondel <i>Aristida</i> variante 2 (<i>Aristida type rondel variant 2</i>)
14	Flat and circular rondel short cell	Rondel circular chato (<i>Flat circular rondel</i>)
15	Centrally compressed rondel short cell	Rondel con cintura (<i>Waisted rondel</i>)
16	Centrally compressed with 2-point on top rondel short cell	Rondel con cintura dos extremos aguzados en top (<i>Waisted rondel, two projections on top</i>)
17	Concave rondel short cell	Rondel cóncavo (<i>Concave rondel</i>)
18	Elongate rondel short cell	Rondel elongado (<i>Elongate rondel</i>)
19	Cylindric rondel short cell	Rondel equidimensional (<i>Equidimensional rondel</i>)
20	Keeled rondel short cell	Rondel keeled (<i>Keeled rondel</i>)
21	Elongate saddle short cell	Saddle elongado (<i>Elongate saddle</i>)
22	Saddle short cell	Saddle normal (<i>Normal saddle</i>)
23	Psilate trapeziform short cell	Trapeziforme liso (<i>Smooth trapeziform</i>)
24	Sinuate trapeziform short cell	Trapeziforme sinuoso (<i>Sinuate trapeziform</i>)
25	Prickle	Aguijón (<i>Prickle</i>)
26	Stomate, Stomatal complex/Interstomatal ground cells	Complejo estomático (<i>Stomatal complex</i>)
27	Lobulate elongate long cell	Elongado borde lobulado (<i>Elongate, lobulate sides</i>)
28	Lobulate/sinuate elongate long cell	Elongado borde sinuoso-lobulado (<i>Elongate, lobulate-sinuate sides</i>)
29	Dendriform elongate long cell	Elongado dendriforme (<i>Dendriform elongate</i>)
30	Dendriform elongate, black straight end long cell	Elongado dendriforme extremo negro recto (<i>Dendriform elongate, black straight end</i>)
31	Equinate elongate long cell	Elongado equinado (<i>Equinate elongate</i>)
32	Psilate elongate long cell	Elongado liso (<i>Smooth elongate</i>)
33	Scrobiculate psilate long cell	Elongado liso escrobiculado (<i>Scrobiculate smooth elongate</i>)
34	Ruminate elongate concave end long cell	Elongado rumeado (irregular) extremo cóncavo (<i>Ruminate elongate -irregular-, concave end</i>)
35	Ruminate elongate straight end long cell	Elongado rumeado (irregular) extremo recto (<i>Ruminate elongate -irregular-, straight end</i>)
36	Convex end sinuate/irregular elongate long cell	Elongado tipo <i>Aristida</i> crenado (<i>Aristida type crenate elongate</i>)
37	Convex end ruminate elongate long cell	Elongado tipo <i>Aristida</i> rumeado (<i>Aristida type ruminate elongate</i>)
38	Tuberculate elongate long cell	Elongado tuberculado (<i>Tuberculate elongate</i>)
39	Crenate/tuberculate elongate long cell	Elongado tuberculado-crenado (<i>Tuberculate-crenate elongate</i>)
40	Papillae in dendriform cell	Papila en célula de borde dendrítico (<i>Papillae in dendriform cell</i>)
41	Papillae	Papila en célula de borde liso (<i>Papillae in smooth cell</i>)
42	Bicelular, point-shaped hair	Pelo bicelular ápice aguzado tipo <i>Bromus</i> (<i>Bromus type bicelular, point shaped hair</i>)
43	Acicular hair cell from lemma	Pelo de arista en glumas (Edge hair from lemma)
44	Point-shaped simple hair	Pelo simple ápice aguzado (<i>Point-shaped single hair</i>)
45	Acute hair cell/Ampliate base	Pelo simple ápice aguzado base amplia (<i>Point-shaped single hair, ampliate base</i>)
46	Oblong echinate base simple hair cell	Pelo simple ápice aguzado base amplia silicificación oblonga (interna) (<i>Point-shaped single hair, ampliate base, oblong internal silicification</i>)
47	Clavate hair cell	Pelo simple ápice redondeado (<i>Round end single hair</i>)
48	Bulliform cell	Célula bulliforme (<i>Bulliform cell</i>)

The selected material was weighed to obtain the initial weight in grams. The dry ashing technique, which consists of a calcination of the samples at 600 °C for 5 h, was used for the silica concentration. The ashes were then weighted. Due to the low final weight, washing and subsequent centrifugation of the calcined samples was avoided to prevent fractures and material losses, and to maximize the recovery of poorly silicified morphotypes –accordingly to the assemblage perspective, which can already be found in archaeological samples. The amount of silica + ash relative to the initial weight of dry samples was calculated as did [Fernández Honaine et al. \(2008\)](#) by the formula: relative quantity of Si + ash = (final weight/initial weight)*100. The percentage of silica recovered in each taxon and between taxa for each organ was obtained. Ashes were mounted on cleaned slides with immersion oil. Silicophytoliths preparations were scanned in a Nikon Eclipse E200 microscope equipped with a 400× digital camera. Up to 100 phytoliths (when it was possible) per slide were counted, and classified following the schemes given by [Bertoldi de Pomar \(1972, 1975\)](#) and [Zucol and Bonomo \(2008\)](#) (Table 2). Silicified tissue fragments presenting two or more short-cell morphotypes were analyzed in order to establish the affinity between them to further discuss the concept of *morphological continuum*. This may be delimited as a number of morphologies closely related to each other, ranging from two defined ends, in this case, two traditional phytolith classes or morphotypes.

Absolute frequencies of short-cell phytolith morphotypes per species were obtained and plotted ([Appendix F, G, H, I](#)). Cluster analysis was carried out with the purpose of analyzing the discriminate and taxonomic value of phytoliths morphotypes at a certain taxa. The Jaccard index was used in the PAST Program (Version 2.17–Hammer, 1999–2012). A matrix of presence/absence for total short-cell phytolith morphotypes per species was performed ([Appendix B](#)). A second matrix was made up by grouping sub-classes within major short-cell morphotype categories ([Appendix C](#)). Presence and absence were coded as 1 and 0, respectively. Total short-cell phytoliths were analyzed by species, by plotting first inflorescence axis ([Appendix D](#)), and then kernels/inflorescences/bracts ([Appendix E](#)). Though originally discriminated, these three later were finally considered jointly based on an uniformity criteria, because they are very difficult to separate in some genera, such as *Amelichloa*, *Jarava* and *Nassella*. A Jaccard index was calculated on the base of those matrixes.

4. Results

Amount of silica and ashes recorded for Poaceae species studies indicates a low recovery of silicophytoliths in kernels/inflorescences/glumes (Table 3) when is compared with records from leaves and stems, as provided by [Gallego and Distel \(2004\)](#) and by [Fernández Honaine et al. \(2006\)](#). Nevertheless, phytoliths are still present and can be counted. Fig. 2–4 and [Appendix G to I](#) provides examples of phytoliths variation in Poaceae from Monte and Espinal.

From a qualitative point of view, and considering relative frequencies obtained highlights multiplicity and redundancy in phytolith production in analyzed species. When compared with phytolith production in leaves and stems from the same taxa, as demonstrated existing publications ([Gallego and Distel, 2004](#); [Fernández Honaine et al. 2006](#)), it can be noted that there are morphologies broadly distributed in the plant, and there are other located only in kernels/inflorescences/bracts. Between the first it can be mentioned the following morphotypes: bulliform cell, equidimensional rondel, normal saddle, *Panicoid* bilobated, polylobate short cell and smooth elongate; and between the second, *Aristida* type rondel variant 1 and variant 2, waisted rondel with

Table 3

Percentage of silica and ashes in Poaceae species studied.

	Initial weight (gr.)			Final weight (gr.)	Percentage of silica + ashes
	Bracts	Kernel/Inflorescence	Axis		
<i>Amelichloa brachychaeta</i>		0,0096	0,0011	0,0009	81,8182
<i>Aristida adscensionis</i>				0,0012	12,5
	0,0021		0,0030	0,001	33,3333
		0,0068		0,0007	33,3333
<i>Aristida mendocina</i>		0,0126		0,0015	22,0588
				0,0017	13,4921
<i>Aristida subulata</i>		0,0035		0,0033	27,2727
			0,0035	0,0007	34,2857
<i>Bromus catharticus</i>		0,0037		0,0004	20
	0,0050			0,0009	10,8108
				0,0044	18
<i>Cortaderia selloana</i>		0,0056		0,0009	20,4545
<i>Cynodon dactylon</i>		0,0018		0,001	17,8571
				0,0007	38,8889
			0,0024	0,0008	33,3333
<i>Cynodon hirsutus</i>		0,0089		0,0009	10,1123
			0,0050	0,0003	6
<i>Jarava ichu</i>		0,0025		0,0006	24
				0,0005	40
<i>Nassella clarazzi</i>		0,0156		0,0012	7,6923
				0,0025	20
<i>Paspalum dilatatum</i>				0,0014	57,1428
subsp. <i>dilatatum</i>		0,0175		0,0021	12
<i>Paspalum vaginatum</i>			0,0030	0,0002	6,6667
		0,0014		0,0005	35,7143
<i>Sorghastrum pellitum</i>		0,0117		0,0009	7,6923
			0,0011	0,0004	36,3636
<i>Sporobolus rigens</i>		0,0111		0,0004	3,6036
			0,0752	0,003	3,9894

two projections on top, rectangular short center *Panicoid* bilobated with 2 projections on top, dendriform elongate and *Aristida* type ruminate or crenate elongate and papillae in dendriform cell (Figs. 2–4).

Data on phytolith frequencies were translated into presence/absence of short-cell morphotypes by species and several cluster trees were obtained. The first and second ones discriminate species by their total short-cell phytolith production per organ (Figs. 5 and 6). They include 21 entries corresponding to inflorescence axis and kernels/inflorescences/bracts of different species in such a way may be two entries for the same species (one per each organ). Entries of Chloridoideae and Aristidoideae species dominate, followed by Pooideae entries and finally, by Panicoideae and Arundinoideae entries. The first cluster is comprised by two main groups that bring together the most entries ordered by time into several sub-groups, and by three single-entry groups separated high in the cluster (Fig. 5). This structure would indicate a high variation introduced by putting all data on axis and kernel together. In fact, by segregating entries by organ in subsequent analyzes, the number of clusters is reduced. No clear segregations are verified, by the origin of the entries in inflorescence axis versus kernel/inflorescences or by taxa. However, all entries for caryopses species of the subfamilies Chloridoideae and Aristidoideae remain grouped constituting most of the Group 1. Entries that correspond to axis of these subfamilies are the ones that scape this group. Entries corresponding to the same organ for species of *Aristida*, tend to stay in the cluster paired, showing intra-genus consistency. This does not happen in the case of *Cynodon* or *Paspalum*. Entries of different anatomical origin for a single species may also appear scattered in more than one group, but inflorescence axis and kernel/inflorescence/bract entries from *Cynodon dactylon*, *Paspalum dilatatum* and *Sorghastrum pellitum* are held together in the same group showing in this case, intra-specific consistency.

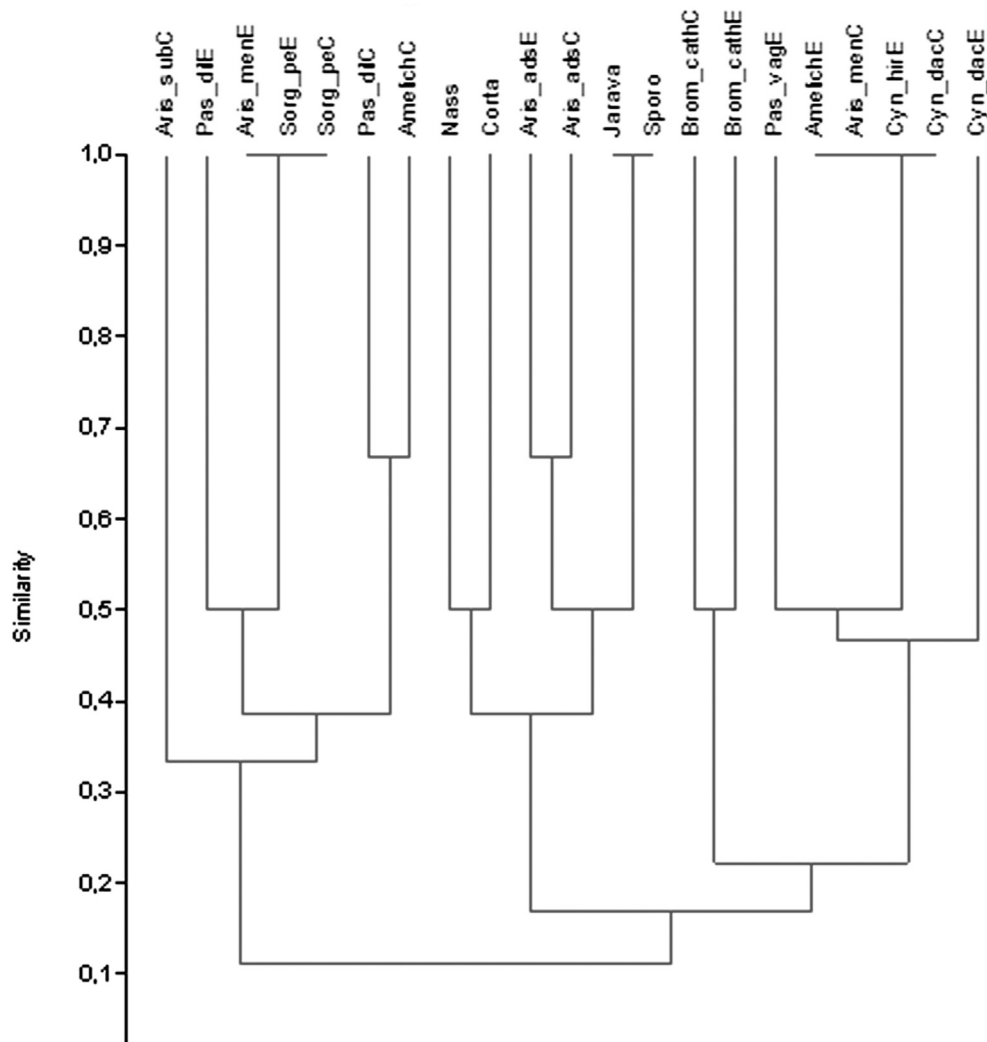


Fig. 6. Cluster tree discriminating species by their total short-cell phytolith production per organ by grouping sub-classes within major morphotypes categories.

The second cluster tree shows the previous 21 entries organized into three main groups (Fig. 6). There is also no net segregation between taxa, nor corresponding to inflorescence axis versus kernel/inflorescence/bract entries. Chloridoideae and Aristidoideae subfamilies losses the trend exposed bellow these lines. Besides, intra-specific consistency is observed by remaining paired in the same group and subgroup, entries of axis and kernel of *Cynodon dactylon*, *Bromus catharticus*, *Aristida adscensionis*, *Sorghastrum pellitum* and *Paspalum dilatatum*. On the contrary, *Aristida* misses its intra-genus consistency as showed in previous cluster.

The third cluster tree groups nine species by their inflorescence axis phytolith production (Fig. 7). They are mostly from Panicoideae subfamily, followed by Chloridoideae and Pooideae species in equal proportion. Three main groups are defined, without any apparent order by taxa except for the separation of Aristidoideae and Chloridoideae in two different groups. Nevertheless, the species of the genus *Aristida* and *Cynodon* show intra-genus consistency in the production of phytolith in axis.

The fourth cluster discriminates twelve species by their kernel/inflorescence/bract phytoliths production (Fig. 8). They are mostly of Chloridoideae Aristidoideae subfamilies, followed by Pooideae species, and finally, by Panicoideae and Arundinoideae species. A

sharp division by taxa, is neither checked here out, but Chloridoideae, Aristidoideae and Pooideae species tend to form a common group (Group 3). All Chloridoideae species are grouped together whereas some species of Aristidoideae and Pooideae subfamilies also contribute to a second related group (Group 2), in which all Panicoideae species remain together. The intra-genus consistency in the production of kernel/inflorescence phytolith is seen in the case of *Cynodon* and *Aristida*, also in the fourth cluster tree.

Finally, by using 3D reconstruction of the phytolith morphologies that can be recorded *in situ* in the same fragment of silicified plant tissue, we note a *morphological continuum* that includes several intermediate forms ranging from typical bilobated, flat circular rondel and saddle morphotypes (Fig. 9). This trend could be seen in the case of *Amelichloa brachychaeta*, *Bromus catharticus* and *Jarava ichu* in whose ashes, spodograms were found Similarities between intermediate shapes became them difficult to be assigned to a specific morphotype of the three classes previously mentioned.

5. Discussion

Not straight separation between taxa is verified, when total short-cell phytoliths or entries by organ of origin (inflorescence

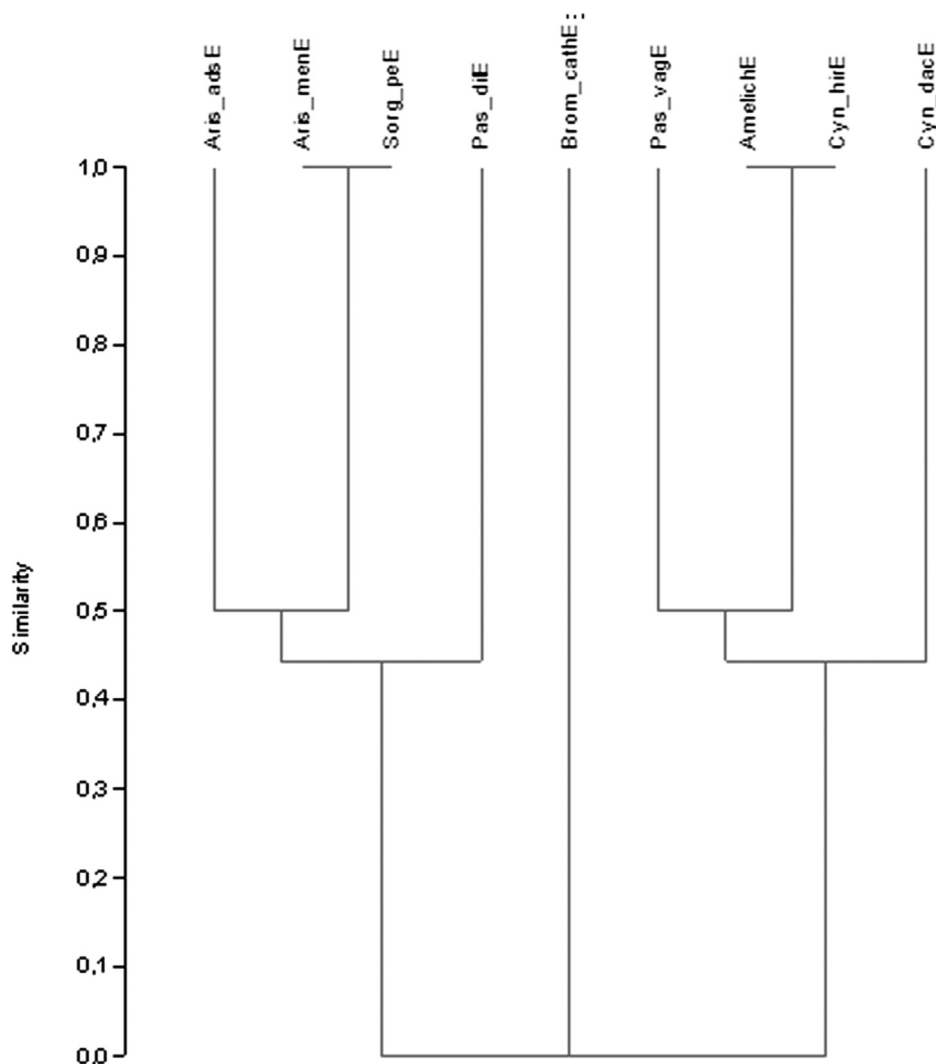


Fig. 7. Cluster tree discriminating species by their inflorescence axes short-cell phytolith production.

axis versus kernels/inflorescences and bracts) per species, are plotted. However, it is noted, a trend to data be organized into three major groups in different cluster analysis done. By considering morphological variants inside main short-cell phytolith classes as shown in Cluster 1, a dispersion of data into many sub-groups is obtained. So we considered to work at the major morphotype level, as it was done in Clusters 2–4. Furthermore, a trend to grouping in different groups the subfamilies Chloridoideae and Aristidoideae entries, is observed, when inflorescence axis are analyzed separately. When total short-cell morphotypes are plotted without discriminating by organ of origin all entries for caryopses species of the subfamilies Chloridoideae and Aristidoideae remain grouped. The majority of Chloridoideae and Aristidoideae species (*Aristida mendocina*, *A. adscensionis*, *Cynodon dactylon*, *Sporobolus rigens*) tends to occur in association with Pooideae (*Bromus catharticus*, *Jarava ichu*) species in the kernel case, because the presence of various rondel short-cell phytoliths concomitant with the absence of bilobate morphotypes. Instead, Panicoideae species tends to cluster in a different group with some Aristidoideae (*Aristida subulata*) and Pooideae (*Amelichloa brachychaeta*), because of Panicoid bilobate counting. *Nassella clarazzi* and *Cortaderia selleana* are paired solely because of the presence of saddle morphotypes whereas bilobates and rondels are absent.

Intra-genus consistency is seen in the case of *Aristida*, whose species tend to be paired in the analysis of clusters, when they are plotted as much by their organs of provenance of phytoliths as by total short-cell phytoliths without discriminating by organ of origin. It can be due to the presence of polylobate short cells and *Aristida* type rondels. This also occurs for *Cynodon* species, when inflorescence axis is analyzed, because of the presence of rondel short-cells and the absence of bilobates. By plotting total short-cell phytoliths produced by *Cynodon dactylon*, *Bromus catharticus*, *Aristida adscensionis*, *Sorghastrum pellitum* and *Paspalum dilatatum* an internal coherence in the phytolith morphotypes produced in axis and kernels/inflorescences and in bracts by those species, is noted. Finally, it is interesting that *Nassella clarazzi* and *Cortaderia selleana* tend to mate closely in all analyzes because of the presence of saddle short-cells and the absence of any other morphotypes. Beyond short-cell phytoliths, other morphotypes are of taxonomic value, such as tri-comes in the case of *Bromus-Bromus type bicellular*, point shaped hair.

Morphological analysis allows observe the occurrence of the so-called *morphological continuum* in between closely occurring phytoliths that grow in a small area comprised by a fragment of silicified plant tissue. Typically bilobated, flat circular rondel and

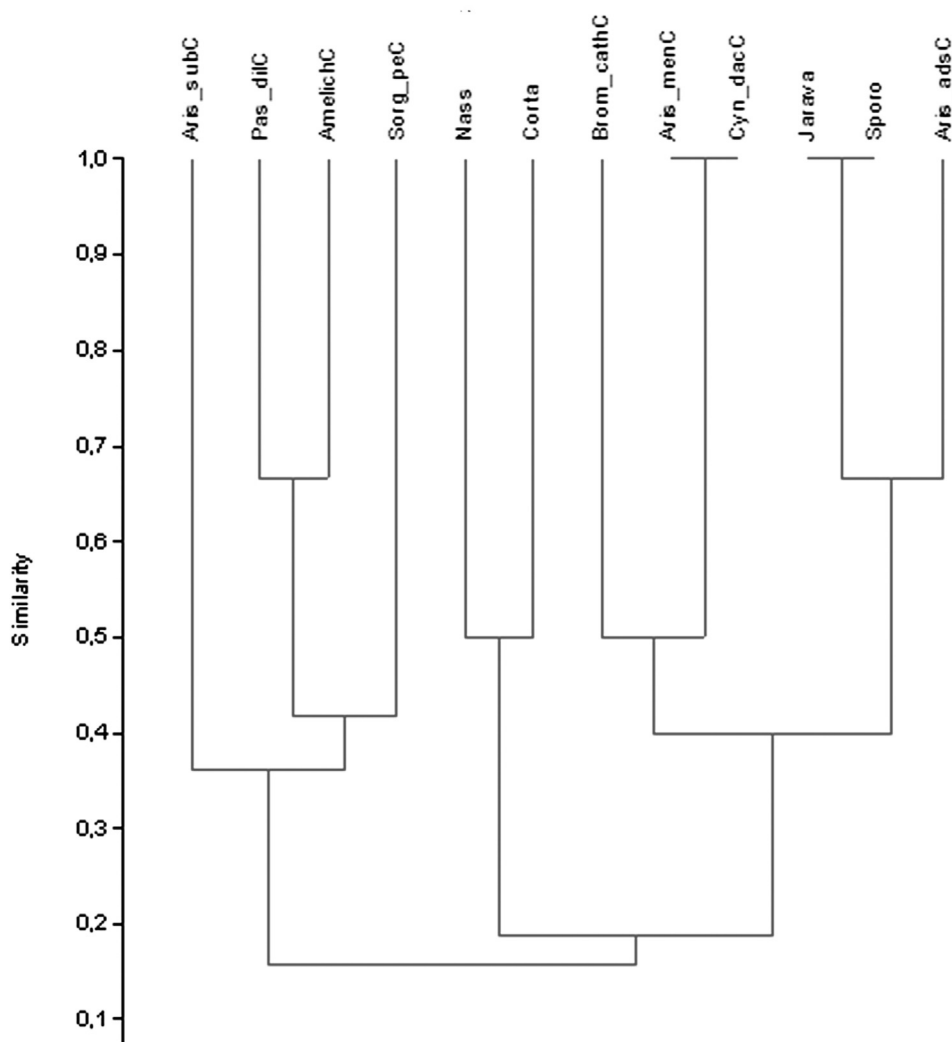


Fig. 8. Cluster tree discriminating species by their kernels/inflorescences/bracts short-cell phytoliths production.

normal saddle morphotypes show a continuous series of shapes in between them in three species in which we could recover spodograms, turning them into transitional shapes.

From a morphological point of view, though non diagnostic, some morphotypes are well indicators of grass inflorescences, because they are absent in leaves and stems. We can mention *Aristida* type rondel variant 1 and variant 2, waisted rondel with two projections on top, rectangular short center *Panicoid* bilobated with 2 projections on top, dendriform elongate, *Aristida* type ruminant or crenate elongate and papillae in dendriform cell.

In general, a significant content of silica is recorded in the native wild Poaceae analyzed, accordingly to previous works of various authors regarding production expectations of silica in the Poaceae species we studied (Fernández Honaine et al. 2008). Increased production in inflorescence axis and bracts than in kernels/inflorescences is observed. However, silica content is of great value in the latter case, because of their main interest as useful plant part with potentially food purposes. In part, this successful recovery is due to the use of a sampling technique less aggressive, especially useful under expectation of low concentration of silica. Excessive manipulation, although benefits observation of samples can particularly affect the poorly silicified fractions (Babot et al., 2014). According to the obtained values, we can expect a reasonable prospect of recovery phytoliths from grass inflorescences from

processing artifacts and consumption residues, when a less aggressive protocol is followed (Musaubach, 2014).

6. Conclusion

This study demonstrates the diagnostic value of phytoliths from the selected arid and semi-arid grasses included in this study as a proxy tool to detect its past occurrence in a variety of archaeological contexts. Results showed trends that concern subfamily groupings in producing phytoliths in inflorescences of those native wild Poaceae studied and some consistency in species and genera in the production of phytolith morphotypes. Further studies may help to refine these trends by incorporating a larger number of taxa and greater representation of subfamilies.

While phytoliths from inflorescences and caryopses are outnumbered by those from leaves and stems of native Poaceae species as provided by Gallego and Distel (2004), Fernández Honaine et al. (2006) and by our own work, we believe from an archaeobotanical perspective that should still be studied due to their role in human feeding. Moreover, even in low counts, diagnostic and characteristic phytoliths can strengthen the arguments of taxonomic assignment of past plant remains to be considered in association with other microparticles produced by the plant, such as starch grains and calcium crystals.

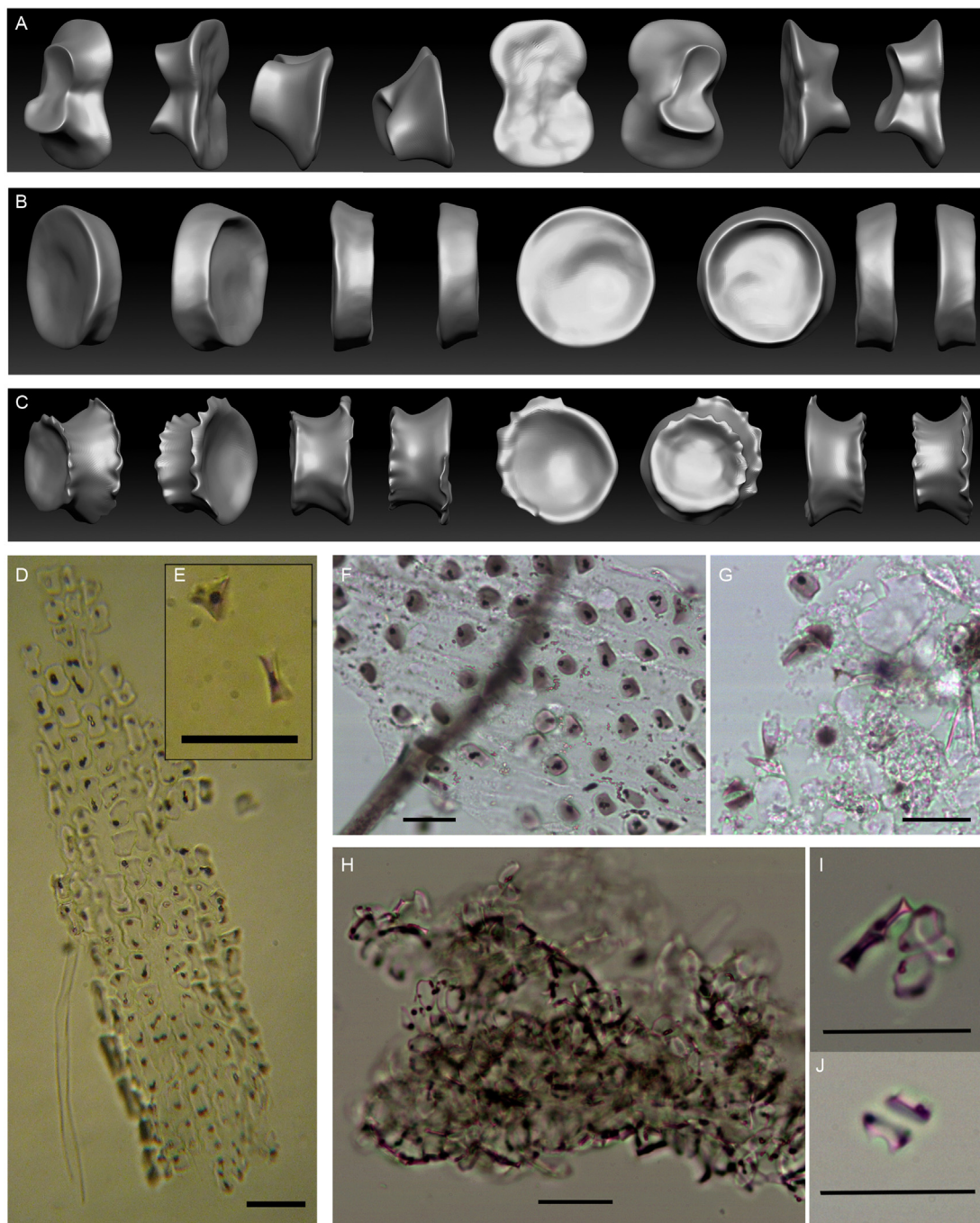


Fig. 9. 3D reconstruction of bilobate, saddle and flat circular rondel morphotypes recorded as extremes for a morphological continuum of shapes that can be found into the same fragments of silicified plant tissue. A: 3D rectangular short center panicoid bilobate, 2 projections on top; B: 3D normal saddle; C: 3D flat circular rondel; D: morphological continuum in *Amelichloa brachychaeta* (kernel); E: Detail of bilobate/rondel in D; F: morphological continuum in *Bromus catharticus* (bracts); G: Detail of rondel/saddle(?) in *Bromus catharticus* (kernel); H: morphological continuum in *Jarava ichu* (kernel); I: Detail of saddle/rondel in H; J: Detail of rondel/saddle in H.

Morphological analysis through the concept of *morphological continuum* invites to future reflections about the guidelines for building typologies on morphological basis. In addition, calls to take care to the bias that can occur when trying to fit within defined forms, intermediate variants.

This study has a global relative value and contributes to characterize the production of bioparticles in general, especially in Poaceae species and particularly in their inflorescences. But above all, has a value of specificity for studied arid and semi-arid biomes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.quaint.2016.02.045>.

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