Comparative phytolith analysis of *Festuca* (Pooideae: Poaceae) species native to Tierra del Fuego, Argentina

M.G. Fernández Pepi, A.F. Zucol, and M.O. Arriaga

Abstract: *Festuca* L. is one of the most representative native grasses of Tierra del Fuego in the southernmost area of South America. In several patches, however, domestic cattle have overgrazed, causing loss or replacement by exotic and adventitious species. We have carried out a comparative phytolith analysis of Patagonian fescue present in the Province of Tierra del Fuego. Fescue leaves are characterized by the presence of truncated cones, crescents, and sinuate trapezoid elements, predominantly short ones. Fan-shaped, small elongated prismatic, and point-shaped phytoliths, as well as articulated phytoliths originating in vascular and epidermal tissues, were observed less frequently. The most common articulated elements in leaf ashes are long cells and a combination of sinuate trapezoid and long cells or crescents. In inflorescence ash assemblages, long cells with undulate borders in association with crescents or emerging hooks are predominant. We have analyzed the diagnostic and differential characteristics of each species of phytolith assemblages to provide information for further studies on the presence of this species in different past soil samples.

Key words: phytolith assemblages, *Festuca*, Tierra del Fuego, modern plants.

Résumé : Les *Festuca* L. constituent le genre le plus représentatif des graminées indigènes de la Tierra del Fuego, dans les régions les plus au sud de l'Amérique du Sud. Cependant, sur plusieurs surfaces, les animaux domestiques les ont surpâturées causant la perte ou leur remplacement par des espèces exotiques ou adventices. Les auteurs ont conduit une analyse comparative des phytolithes des fétuques patagoniennes dans la province de la Tierra del Fuego. Les feuilles de fétuques se caractérisent par la présence d'éléments en forme de cônes tronqués, de croissants et de trapézoïdes sinués, dominés par des formes courtes. On a moins souvent observé les phytolithes en forme d'éventail, petits de forme prismatique allongée et en forme de pointe, ainsi que des phytolithes allongés articulés prenant naissance dans les tissus vasculaires et épidermiques. Les éléments articulés les plus communs dans les cendres des feuilles sont de longues cellules et une combinaison de trapézoïdes sinués avec de longues cellules ou en croissants. Dans les assemblages provenant de cendres d'inflorescences, on observe une prépondérance de cellules longues avec bordures ondulées en association avec des formes en croissants ou de crochets émergents. Les auteurs ont analysé le diagnostic et les caractéristiques différentielles de chaque type de phytolithe dans le but de fournir des informations sur la présence de cette espèce dans des échantillons de sols du passé.

Mots-clés : assemblages de phytolithe, *Festuca*, Tierra del Fuego, plantes modernes.

[Traduit par la Rédaction]

Introduction

Festuca L. comprises around 450 species (Clayton and Renvoize 1986; de Nova et al. 2006) distributed in temperate and cold temperate climates. Forty of these species live in South America, from the Andes to sea level, in meadows, swampy soils, and even dry land, often giving rise to extensive grassland. Most of the plants are large and well established. Because their tender foliage provides good livestock fodder, some species are cultivated for this purpose (Correa 1978). As reported by Dubcovsky and Martínez (1988), native Patagonian *Festuca* comprise 16 species, 14 of which were grouped into five distributional groups (Fig. 1) according to their anatomical and exomorpho-logical characteristics: group 1, *F. tunicata* E. Desv. and *F. acanthophylla* E. Desv.; group 2, *F. monticola* Phil., *F. thermarum* Phil., and *F. scabriuscula* Phil.; group 3, *F. pallescens* (St.-Yves) Parodi, *F. pallescens* var. *scabra* (St.-Yves) Parodi, and *F. kurtziana* St.-Yves; group 4, *F. gracillima* Hook. f. and *F. ventanicola* Speg.; and group 5, *F. contracta* Kirk, *F. simpliciuscula* (Hack.) E.B. Alexeev, *F. pyrogea* Speg., and *F. magellanica* Lam. *Festuca argentina* (Speg.) Parodi, *F. purpurascens* Banks & Sol. Ex Hook. f., *F. cirrosa* (Speg.) Parodi, and *F. pampeana* Speg. were not included in any of these groups. Later, Dubcovsky and Martínez (1992) conducted cytotaxonomic studies and found that some elements of these groups had different phylogenetic origins.
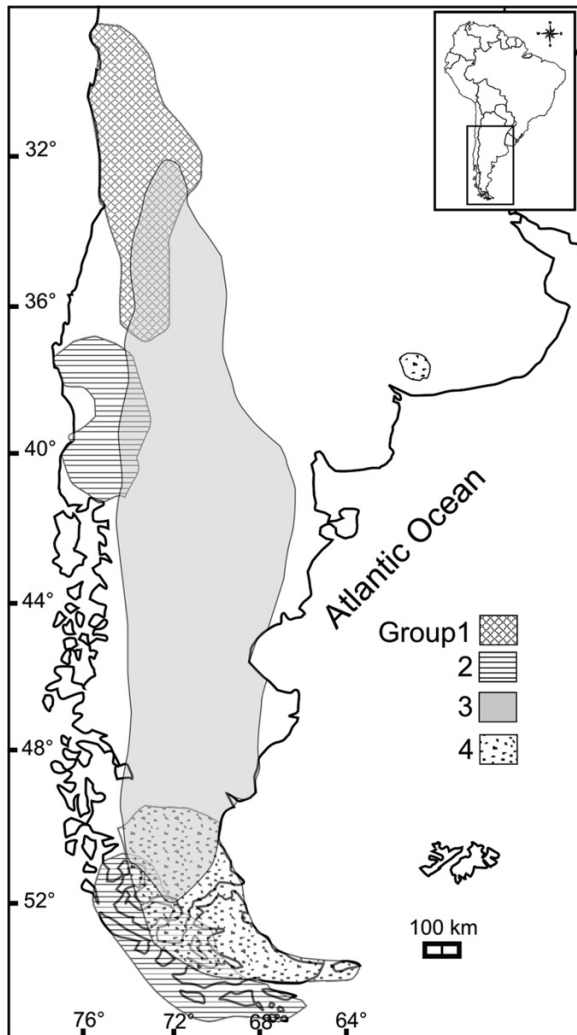
Fig. 1. Distribution of Patagonian *Festuca* groups: group 1, *F. tunicata* and *F. acanthophylla*; group 2, *F. monticola*, *F. thermarum*, and *F. scabriuscula*; group 3, *F. pallescens*, *F. pallescens* var. *scabra*, and *F. kurtziana*; group 4, *F. gracillima* and *F. ventanicola*. Group 5 is not included to avoid an overlap with the rest of the groups.

Festuca gracillima is the main component of the so-called "coironales" in the Patagonian steppe, whose communities are found in open, undulating reliefs of valleys lying between permanent or temporary creeks. It covers plains, terraces, hills, plateaus, and basal mantles between Rio Coyle (in the Province of Santa Cruz) and the Magellan Strait. It then reappears in Tierra del Fuego, where it covers the northern sector of the island (Roig et al. 1985; Roig 1998), with the presence of *F. cirrosa*, *F. contracta*, *F. gracillima*, *F. magellanica*, *F. monticola*, *F. purpurascens*, *F. pyrogea*, and *F. thermarum* among the native species within the genus (Zuloaga et al. 2008) and *F. rubra*, which was introduced as forage (Correa 1978). It is well known that grazing by domestic cattle is commonly associated with changes in the composition of grassland species (Archer 1992; Noy-Meir and Walker 1986; Westoby et al. 1989; Milchunas and Lauenroth1993; Milton et al. 1994), where selective grazing appears as the dominant

mechanism in species replacement (Anderson and Briske 1995). A study on the Fuegian ecotone suggests changes in natural grasslands due to overgrazing (Antonijevic and Faggi 2003). These changes are similar to those described by several authors (Boelcke 1957; Boelcke et al. 1985; Cingolani et al. 1998; León et al. 1988; Collantes and Faggi 1999; Cipriotti and Aguiar 2005) regarding other regions from the continental extra-Andean Patagonian or Fuegian steppe. The diet analysis of native herbivores (geese and guanaco) and introduced herbivores (sheep, cow) in the region of Tierra del Fuego shows that the latter prefer to feed on grassland with a predominance of *Festuca* sp., while the former prefer grasslands dominated by *Poa* sp. (Arriaga et al. 2004; Fernández Pepi et al. 2007, 2009a, 2009b; Alvarenga et al. 2009). *Poa pratensis* is one of the adventitious species that proliferated upon overgrazing of grassland by sheep, and it curiously became the main dietary component of native herbivores such as geese (*Cloephaga picta* and *Cloephaga poliocephala*; Arriaga et al. 2004).

Silica deposits (silica bodies or silicophytoliths) occur in many plant taxa and are particularly abundant in grasses (Poaceae) (Rovner 1971; Piperno 1988; Mulholland 1989; Twiss 1992). Given their durability, grass phytoliths provide an outstanding tool for the reconstruction of vegetation belonging to certain environments in the past (Metcalfe 1960; Bertoldi de Pomar 1975; Ellis 1979; Palmer and Tucker 1981; Brown 1984; Ollendorf et al. 1988; Mulholland 1989; Twiss 1992; Runge 1999). As their physical and chemical characteristics allow them to remain largely unchanged in soil deposits, they are therefore a valuable tool for the reconstruction of grass-dominated ecosystems and tree–grass cover ratios, as well as for information on the development and change of large-scale vegetation communities (Sendulsky and Labouriau 1966; Blackman 1971; Bartolome et al. 1986; Twiss 1987; Fisher et al. 1995; Alexandre et al. 1997; Barboni et al. 1999, 2007; Zucol 1996, 2001; Scott 2002; Abrantes 2003; Gallego and Distel 2004; Gallego et al. 2004; Bremond et al. 2005a, 2005b, 2008a, 2008b; Fernández Honaine et al. 2006; Fernández Honaine 2007; Barboni and Bremond 2009).

The aim of our study has been to characterize the phytolith assemblages of the eight species of *Festuca* native to Tierra del Fuego to provide a reference tool for future environmental studies in areas that have been modified by antropic action and overgrazing.

Material and methods

### Selection of species and phytolith extraction

Fuegian fescue was the first endemic species and is the most widely distributed one, according to Dubcovsky and Martínez (1992). Regarding endemic species of group 4, *F. gracillima* is distributed in the Province of Tierra del Fuego and *F. ventanicola* is distributed along the Ranges of Tandilia and Ventana in the Province of Buenos Aires. Species of groups 2 and 5 are distributed mostly in Patagonia. Species of Patagonian fescue of Tierra del Fuego were analyzed, especially those corresponding to the Fuegian ecotone, an area where the Patagonian steppe extends into the Andean forests (Fig. 1). We selected three species to carry out a detailed study (*F. gracillima*, *F. magellanica*, and *F. monticola*) and sampled 10 specimens of each, while two to five herbarium specimens were sampled for the other Fuegian species (*F. cirrosa*, *F. contracta*, *F. pyrogea*, *F. purpurascens*, and *F. thermarum*) (see Appendix A). All samples were collected at the end of their annual growth

Table 1. Phytolith morphotypes found in the analyzed *Festuca* species, name and acronym used in this paper, and equivalent names, following the various classifications by different authors.

Group	Morphotype	Acronym	Equivalent names	Scheme A no.
Point-shaped	Point-shaped	Ps	Silification of hairs and hair bases (*a*); aculeolita (*b*); unciform (*d*); aguzado (*e*)	1
Cylindrical vascular element	Cylindrical sulcate	Ce	Cylindrical vascular element (*f*), elementos de conducción (*e*)	2
Brief elongate	Brief elongate	Be	Braquiolita (*b*), prismático corto (*e*)	3
Fan-shaped	Fan-shaped	Fs	Flabelolita (*b*); silicified bulliform cells (*a*); fan-shaped phytolith (*c*); flabelolithum (*g*); en abanico (*e*)	4
Micro-elongate	Smooth contour	Me1	Braquiolita (*b*); elongate smooth (*d*); prismático corto (*e*)	5
	Undulate contour	Me2		6
Elongate	Thin elongate	El1	Prismatolita (*b*); elongate phytolith (*c*); elongado (*e*)	7
	Undulate contour	El2		8
	Smooth contour	El3		9
Truncated cone	Flattened, nondemarcated shank	Ct1	Estrobilolita (*b*); conos truncados (*e*)	10
	Funnel shape, one basal side acuminate	Ct2		11
	Funnel shape, slightly shanked	Ct3		12
	Very flattened, nonshanked	Ct4		13
	Flattened, one basal side bilobate	Ct5		14
Stomatal complex	Stomatal complex	Sc	Stomatal complex (*h*)	15
Hook	Hook	Ho1	Hook (*h*)	16
	Incipient hook	Ho2	Hooked papilla (*i*)	17
Hair	Hair	Ha	Hair (*h*)	18
Crescent moon	Crescent	Cr	Crescent (*c*); crescent or kidney shape (*h*)	19
Sinuate trapezoid or festucoid boat	Long sinuate trapezoid	Fb o St1	Horizontally elongate with nodular outline (*h*); festucoid boat (*j*); sinutate trapezoid (*f*)	20
	Trapezoidal short element	Fb o St2		21
Polyhedral bulliforms	Cuboids		Parallepipedal (*d*); poliédricos (*e*)	
	<30 µm length	Po1		22
	>30 µm length	Po2		23

Note: (*a*) Parry and Smithson 1964; (*b*) Bertoldi de Pomar 1971; (*c*) Twiss 1992; (*d*) Madella et al. 2005; (*e*) Patterer et al. 2011; (*f*) Zucol et al. 2010; (*g*) Zucol and Brea 2005; (*h*) Ellis 1979; (*i*) Mejía-Saules and Bisby 2003; (*j*) Kondo et al. 1994.

Scheme A.
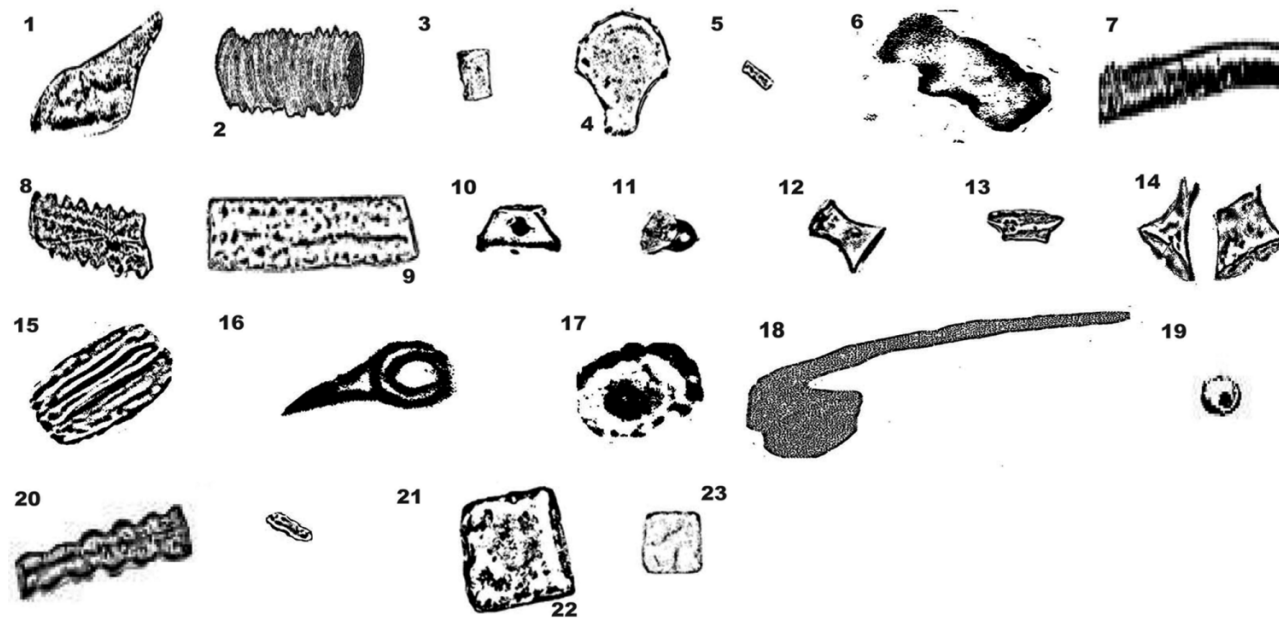
cycle to better represent phytolith composition at the moment of incorporation into the soil (Parry and Smithson 1964; Geis 1978; Rovner 1983). Specimens were collected during 2007–2010 field trips to Tierra del Fuego, while complementary specimens were from BA and SI herbaria (see Appendix A). Phytolith extraction from leaf blades and inflorescences was accomplished through calcination (Labouriau 1983). The material was first washed with pure distilled water and then with nonionic detergent to remove contaminating particles, and then it was dried at 100 °C. Immediately afterwards, it was charred at 200 °C for 2 h, boiled in 5 mol·L^{-1} HCl, and washed with distilled water until no chloride ions were detected. Finally, the material was ignited at 800 °C for 2 h. Silica content was calculated as the percentage of dry mass, according to the following procedure:

Silica content (%) = (ash mass/dry matter mass) × 100

Ashes were mounted on liquid (oil immersion) and solid (Canada Balsam) media and were observed with a Hokenn WP-5-02096 optical microscope. Photomicrographs were obtained with a Panasonic DMC-LS85 camera. SEM observations (Phillips XL30 Electronic Microscopy Service from Museo Argentino de Ciencias Naturales) were made after coating with carbon and gold–palladium. For phytolith assemblage quantification, 350–400 phytoliths per specimen were counted following the various classifications by different authors (Table 1). To understand the origin of the tissue of the isolated phytoliths observed, epidermal and transversal sections of each species were analyzed from samples obtained through traditional cutting, peeling, and mounting techniques (Arriaga 1983; D'Ambroggio de Argüeso 1986). To quantify frequency of epidermal elements, we counted the times that they appeared in each 40× optical field. Descriptions were made in the following order: phytoliths in leaf epidermis, phytolith assemblages in leaf ashes, phytoliths in anthoecia epidermis, and phytoliths present in anthoecia ashes. The remaining ashes and specimen slides were added to the reference collection of the Plant Anatomy Laboratory (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"; Appendix A).

Data analysis

Correlation analysis was carried out to differentiate the groups of species analyzed using PAST software (Hammer et al. 2007) to evaluate the relevance of the quantitative and qualitative characters of the phytolith assemblages compared.

Results

Silica content

Percentages of plant silica content did not exceed 6% (Table 2), with *F. magellanica* and *F. monticola* presenting the highest values. Our comparative analysis shows that leaf biomineralization percentages were markedly higher than those observed in inflorescences, with the only exception of *F. magellanica*, which had an extremely high percentage in inflorescences (Fig. 2; Table 2).

Phytolith description

Epidermises of the species studied have some common features: broadly, the leaf abaxial surface presents long cells with

Table 2. Silica percentage (mean ± standard deviation) from the analyzed *Festuca* species taking into account the dry matter mass of leaves, inflorescences, and the whole plant.

|	Silica percentage by dry matter mass		
	Leaves	Inflorescences	Whole plant
F. cirrosa	1.65±0.61	1.13±0.58	1.39±0.47
F. contracta	5.17±2.08	2.09±1.29	2.72±1.74
F. gracillima	3.74±1.62	2.19±1.87	3.16±1.83
F. magellanica	5.08±2.54	5.35±3.07	5.17±2.62
F. monticola	6.42±2.10	4.49±0.82	5.68±1.73
F. purpurascens	2.86±2.25	1.47±0.97	2.17±1.79
F. pyrogea	7.06±1.91	1.15±0.86	4.11±2.94
F. thermarum	6.26±2.20	2.44±0.61	4.35±2.39

Fig. 2. Dry mass percentage of silica content of leaves, inflorescences, and the whole plant of native *Festuca* species. Species abbreviations: Fci, *F. cirrosa*; Fco, *F. contracta*; Fgr, *F. gracillima*; Fma, *F. magellanica*; Fmo, *F. monticola*; Fpu, *F. purpurascens*; Fpy, *F. pyrogea*; and Fth, *F. thermarum*.
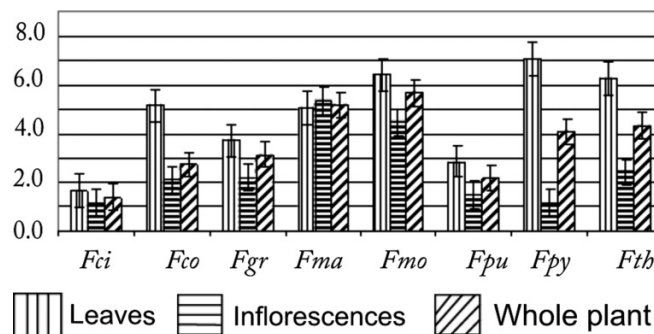
sinuous edges, interspersed with rectangular to irregularly contoured short cells associated with crescent silica bodies in the intercostal zone (Figs. 3A–3B). In the limits of the costal zone, there may be unicellular macrohairs, hooks, and prickles in quantities varying according to the species, all of them with silicified walls and free lumen. In the costal zone, there are sinuate trapezoid elements of variable size with a predominance of short ones (Fig. 3C). In some species, sinuate trapezoid elements alternate with truncate cones. On the adaxial leaf surface, stomatal complexes vary from rectangular to elliptical and are distributed mainly in the intercostal zone. In leaf ashes, there are more abundant disarticulated morphotypes associated with truncated cones (in all their variants), crescents, and sinuate trapezoids of different lengths, predominantly short ones in leaf ashes. Fan-shaped elements (Fig. 4P), short prismatic elements (Fig. 4J), hooks and prickles, cylindrical vascular elements (Fig. 4O), and stomatal complexes are seen less frequently (Table 3). More frequent articulated elements in leaf ashes are primarily composed of long cells and sinuate trapezoid elements (Fig. 4R) or crescent silica bodies (Fig. 4S).

As in all of the species studied, the anthoecia epidermis also presents common features such as unicellular macrohairs, prickles or hooks on edge contours with silicified walls, and free lumen (Figs. 3G–3H). There are small siliceous bodies such as emerging hooks among long cells of sinuous walls, the abundance of which varies according to the species. In inflo-

Fig. 3. Distribution of silica bodies in epidermis of leaves and inflorescences: (A) abaxial surface of *F. contracta*; (B) abaxial surface of *F. gracillima*; (C) adaxial surface of *F. monticola*; (D) adaxial surface of *F. cirrosa*; (E) palea of *F. gracillima*; (F) palea of *F. purpurascens*; (G) palea of *F. pyrogea*; and (H) lemma of *F. thermarum*. Scale bars: 20 μm.
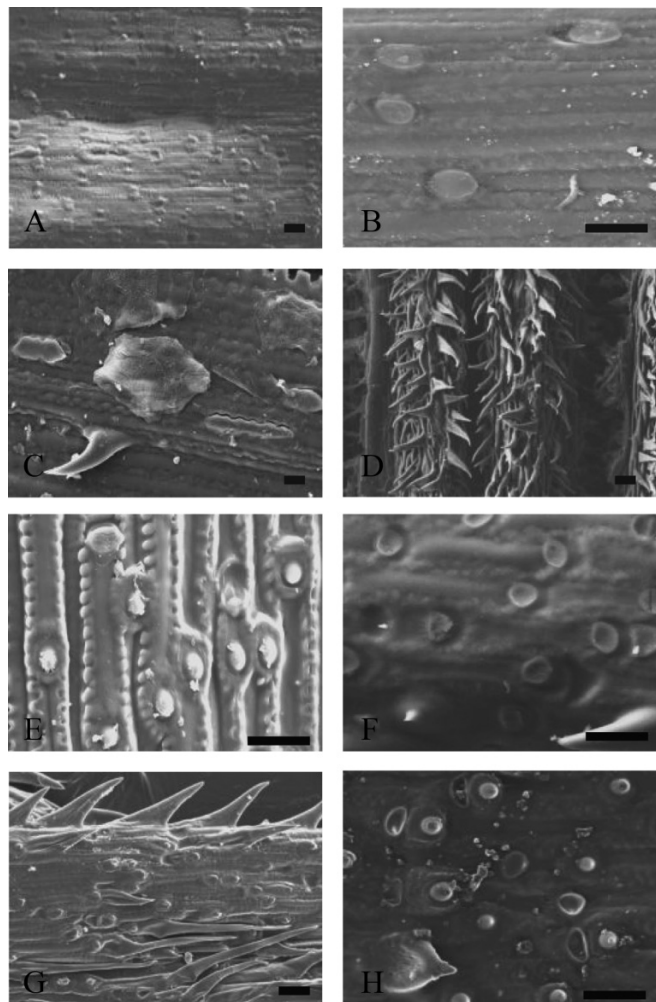
rescences, long cells with undulate contour associated with crescents and (or) emerging hooks (Ho2) are predominant (Figs. 3E–3H). Crescents (Cr) and prismatic elements (El2) predominate in inflorescence ashes. Articulated morphotypes are observed more frequently in inflorescence ashes than in leaf ashes. These morphotypes consist of long cells associated with stomatal complex (Fig. 4U) and hooks (Ho1) – emerging hooks (Ho2) or point-shaped (Ps) elements. We could characterized sinuate trapezoid and conical elements present in each of the species studied (Table 1). Sinuate trapezoid elements may be classified into two major groups, long (longer than 50 μm; Figs. 4D, 4M) or short (shorter than 50 μm), with one acute extreme end and the other end straight, or both straight. Nodules and constrictions are present in an asymmetric, non-conspicuous way (Figs. 4E, 4L). Truncated cones varied in the species studied. We found that Ct5 is also present here. Although morphotypes Ct1–Ct4 have been described and mentioned by other authors, morphotype Ct5 presents characteris-

tics of its own, distinguishable from the rest, and lacks any previous description or classification (Table 1). It is a short element of conic lateral shape and a bilobate end resulting from a constriction in the middle sector of the flat end that causes a bilobate aspect (Fig. 4C). In all of the species studied, one end of the truncated cone is flat while the other presents a height–width ratio of around 1:2–3. From the general descriptions, we determined specific variations resulting from the particular characteristics of phytolith assemblages and epidermal leaf features of each species.

Festuca cirrosa (Speg.) Parodi

On the abaxial epidermal surface, this species presents long cells of variable lengths and alternating quadrangular short cells. In the intercostal zone, there are three types of epidermical emergencies (hooks, prickles, and macrohairs), all with strongly silicified walls and free lumen. Hooks are predominant in leaf borders. Stomata are of rectangular contour (Fig. 3D). Foliar phytolith assemblage is abundant in crescent (Cr) and truncated cone morphotypes, especially in Ct3 and Ct5 (Table 3). Elongated and micro-elongated phytoliths with smooth contours (El3 and Me2, respectively) are less frequent. Anthoecia epidermis presents prickles (Ps) and emerging hooks (Ho2) with silicified walls and free lumen. Prickles and stomata are more abundant in lemma, while emerging hooks are only present in palea, interspersed among prickles and long cells.

Festuca contracta Kirk

On the abaxial surface, short cells from the intercostal zone range from square to rectangular, and in some cases, they have wavy edges. This surface also presents unicellular macrohairs. It has short sinuate trapezoid and truncated cones, more abundant than in other species. Stomata are located in the intercostal area and are elliptical. Unicellular macrohairs (Figs. 4H, 4I) and short sinuate trapezoid morphotypes are predominant in leaf ashes (Figs. 4E, 4L; Table 3). Anthoecia epidermis, palea, and lemma present prickles (Ps), with silicified walls and free lumen, and emerging hooks (Ho2), the latter interspersed among prickles and long cells.

Festuca gracillima Hook. f.

On the abaxial surface, short cells from the intercostal zone range from square to rectangular and are interspersed among long cells (Fig. 3B). Short cells are scarce. On the adaxial surface, unicellular macrohairs and elliptical stomata predominate in the intercostal zone. Macrohairs are distributed between stomata but are more abundant near the costal zone. Short sinuate trapezoid morphotypes in the costal zone are less frequent than in other species. In leaf ashes, truncated cones such as Ct1 and Ct5 (with bilobate base; Fig. 4C) and point-shaped phytoliths are predominant. Truncated cone-shaped elements are less variable than in other species, Ct1 and Ct5 morphotypes being the only ones as abundant as in *F. magellanica* and *F. monticola*. Short sinuate trapezoid morphotypes are more abundant than long sinuate trapezoid ones (Figs. 4E, 4L; Table 3). Anthoecia epidermis, palea, and lemma present hooks (Ho1) with silicified walls, free lumen, and emerging hooks (Ho2) interspersed among long cells (Fig. 3E). Hooks are less frequent in lemma than in palea, but some crescent elements (Cr) are also present in the former.

Fig. 4. Phytolith morphotypes present in ashes. Non-articulated morphotypes: (A–C, F–G) truncated cones; (D–E, L–M) sinuate trapezoid or festucoid boat; (H–I) macrohairs; (J) micro-elongate; (K, Q) polyhedral bulliforms; (N) elongate; (O) cylindrical vascular element; (P) fan-shaped. Articulated morphotypes formed by long cells and the following elements: (R) sinuate trapezoid element; (S) crescent; (T) only long cells; (U) stomata complex. Scale bars: 20 μm.
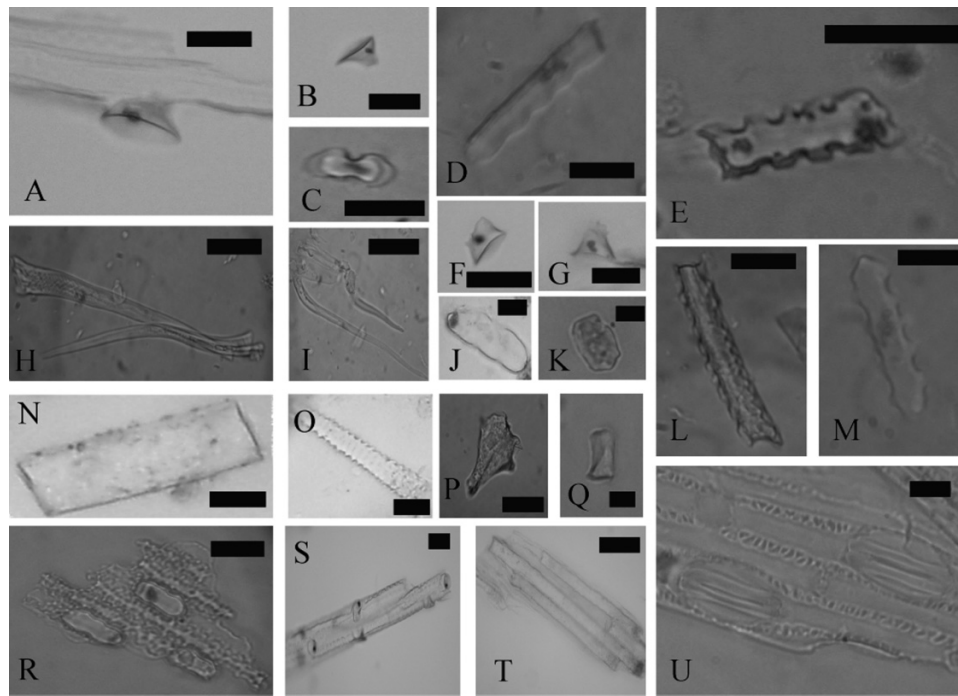

Table 3. Morphotype percentages for native *Festuca* species.

Morphotype	Fci	Fco	Fgr	Fma	Fmo	Fpu	Fpy	Fth
Ps	0.00	0.71	0.00	2.60	0.00	8.70	0.56	4.15
Ce	0.00	0.00	0.00	0.26	0.00	0.00	0.56	0.38
Be	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.75
Fs	2.86	1.41	1.73	0.98	3.45	7.25	2.79	4.90
Me1	5.71	0.71	0.86	3.77	1.48	0.00	0.56	3.77
Me2	0.00	0.00	0.00	0.00	0.00	0.00	1.68	4.52
El1	0.00	0.00	0.00	0.33	3.94	0.00	0.00	2.64
El2	0.95	3.18	5.19	5.46	5.91	5.80	5.03	10.68
El3	4.76	2.83	6.05	3.71	7.88	0.00	3.35	14.32
Ct1	1.90	0.00	20.75	6.24	11.82	14.49	19.55	10.56
Ct2	0.00	0.00	0.00	0.00	0.00	0.00	6.15	5.65
Ct3	15.24	1.77	0.00	0.00	0.00	2.90	3.35	4.52
Ct4	0.00	0.00	0.00	0.00	0.00	2.90	4.47	3.02
Ct5	12.38	1.06	1.15	0.00	18.72	4.35	2.79	2.64
Sc	0.00	1.06	1.73	1.63	1.97	4.35	0.00	0.00
Ha	0.00	20.85	4.90	4.62	1.48	0.00	1.68	2.26
Cr	46.67	47.01	44.05	57.80	18.75	2.90	35.75	9.05
St1	2.86	1.05	2.35	2.67	2.61	21.05	1.68	2.26
St2	4.76	17.31	5.76	8.65	18.08	2.14	3.35	3.39
Po1	1.90	0.71	0.00	0.26	1.48	4.35	0.56	9.80
Po2	0.00	0.35	0.00	0.00	1.48	0.00	0.00	0.75

Note: Values are the relative frequency of phytolith morphotype of each species. Morphotype acronyms are defined in Table 1. Species abbreviations: Fci, *F. cirrosa*; Fco, *F. contracta*; Fgr, *F. gracillima*; Fma, *F. magellanica*; Fmo, *F. monticola*; Fpu, *F. purpurascens*; Fpy, *F. pyrogea*; and Fth, *F. thermarum*.

Festuca magellanica Lam.

On the abaxial surface, this species presents irregular short cells associated with crescents. In the costal zone, through the edges, there are variously sized sinuate trapezoid morphotypes and truncated cones, though less abundantly than in other species. On the adaxial surface, unicellular macrohairs are distributed in the intercoastal zone, while hooks are in the marginal zones. Stomata complexes are of elliptical shape on both surfaces. Crescents are the most frequent morphotype observed in leaf ashes (with the highest degree of abundance in this species), followed by truncated cones (flat or high ones), stomatal complexes, and unicellular macrohairs, although the latter are less abundant than in the other species. Anthoecia epidermis, palea, and lemma have hooks (Ho1), emerging hooks (Ho2), and some macrohairs interspersed among long cells.

Festuca monticola Phil.

On the abaxial surface, rectangular short cells from the intercostal zone are interspersed among long cells. This surface presents crescents and sinuate trapezoid (St1 and St2) elements, as well as abundant small hooks, fewer hairs, and elliptical stomata in the adaxial intercostal zone (Fig. 3C). Truncated cones predominate in leaf ashes, with type Ct5 being the most frequent (Figs. 4A–4C, 4F–4G; Table 3). Among sinuate trapezoid morphotypes, the smaller ones are more abundant, a characteristic also present in *F. contracta*. Both species display the highest frequency of this morphotype. Ash analysis shows that fan-shaped frequency is medium compared with the other species (Table 3). Anthoecia epidermis, palea, and lemma show the presence of hooks (Ho1) and

emerging hooks (Ho2) interspersed among long cells. Hooks are smaller in lemma than in palea.

Festuca purpurascens Banks & Sol. ex Hook. f.

On the abaxial surface, irregular short cells are abundant. In the costal area, we observed sinuate trapezoid morphotypes of variable sizes (25–115 μm), more abundant than in other species. On the adaxial surface, small hooks characterize the costal zone. Macrohairs were not observed at all. In leaf ashes, this species presents less frequent crescent elements (Cr) and the highest abundance of short sinuate trapezoid elements (Table 3). We observed a high frequency of truncated cones, predominantly of intermediate height (Ct1), and short square-shaped cells (Po1) (Table 3). Silicified cells associated with stomatal complexes are also present (St). On the anthoecia epidermis, there are long cells associated with crescents, hooks (Ho1), and emerging hooks (Ho2) (Fig. 3F). We observed crescents in palea and lemma but hooks only in palea.

Festuca pyrogea Speg.

On the abaxial surface, short cells present a more or less square shape. Rectangular and crescent short cells are found in the intercostal zone. Unicellular macrohairs are shorter and are interspersed among prickles. There are rectangular stomata and prickles in the intercostal zone of the adaxial surface and sinuate trapezoid and truncated cone elements in the costal areas (Fig. 3). In leaf ashes, truncated cones are abundant (mainly type Ct1) associated with very scarce sinuate trapezoid elements. Specific characters of this taxa are the presence of triangular-shaped truncated cones (Ct2) and elongated rather than flat elements (Ct4), the highest frequency of prickles, and the presence of short cells with wavy edges (Me2) (Table 3). Anthoecia epidermis, palea, and lemma have crescents, emerging hooks (Ho2), and macrohair (Ha) interspersed among long cells, whereas point-shaped elements appear only in palea (Fig. 3G).

Festuca thermarum Phil.

The abaxial surface shows abundant marginal macrohairs. Crescent short cells are scarcely present on this surface. On the adaxial surface, the intercostal zones have elliptical stomata, abundant unicellular macrohairs, and hooks. In leaf ashes, this species presents the highest frequency of silicified hair (Ha) and short cells with wavy edges (Me2), as well as scarce crescent elements (Cr). Truncated cones are predominant in all of the analyzed varieties, with those of medium height (Ct1) and triangular shape (Ct2) being the most frequent (Table 3). Anthoecia epidermis, palea, and lemma display hooks (Ho1) and emerging hooks (Ho2) interspersed among long cells. Hooks are less abundant in lemma than in palea, and emerging hooks are more frequent in lemma (Fig. 3H).

Comparative analysis of phytolith assemblages

We arranged phytolith assemblages according to morphotype frequency for each species to characterize and differentiate them. *Festuca thermarum* presents the highest morphotype variability, with similar frequencies for all types and predominance of truncated cones, long prismatic elements, and crescents. *Festuca cirrosa* and *F. purpurascens* were the only ones that lacked macrohairs. These two glabrous species can be distinguished by their abundance of point-shaped, truncated cone, long prismatic elements, and long sinuate trape-

Fig. 5. Correspondence analysis based on qualitative and quantitative phytolith morphotypes. Components 1 and 2 are represented in A, and components 2 and 3 are represented in B. Species abbreviations: Fci, *F. cirrosa*; Fco, *F. contracta*; Fgr, *F. gracillima*; Fma, *F. magellanica*; Fmo, *F. monticola*; Fpu, *F. purpurascens*; Fpy, *F. pyrogea*; and Fth, *F. thermarum*.
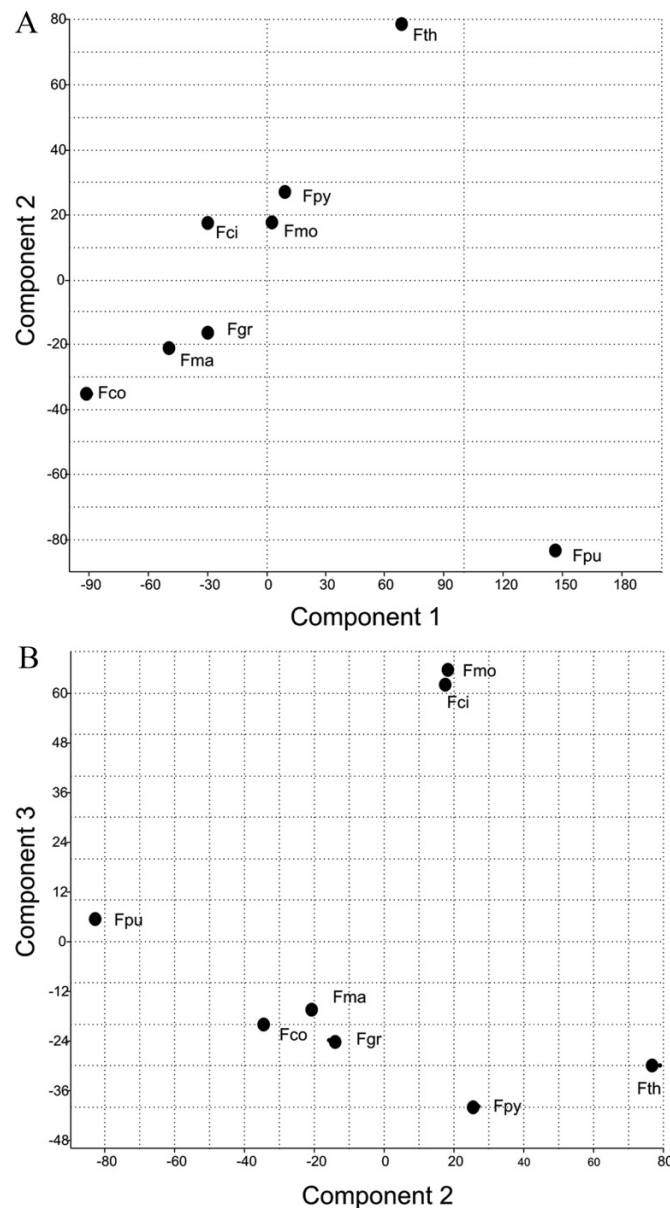
zoids. Unlike other species, *F. gracillima* is represented mainly by the presence of truncated cones (Ct1), crescents, and long sinuate trapezoids. Phytolith assemblages were studied by correspondence analysis to determine relationships among species (Fig. 5). This allowed the identification of phytolith morphotypes and the associations relating these species. The first three components of our correspondence analysis showed a variability of 61.18% of the total variability analyzed (eigenvalue 1, 31.07%; eigenvalue 2, 16.60%; and eigenvalue 3, 13.51%) and clearly differentiate the phytolith assemblages of *F. purpurascens* (through the contribution of

component 1) and *F. thermarum* (through the contribution of component 2). On the other hand, component 2 also links the phytolith assemblages of *F. monticola*, *F. cirrosa*, and *F. pyrogea*, with the first two also being linked by component 3. In all cases, the phytolith assemblage of *F. gracillima* was closely linked, particularly through the contribution of component 2, to *F. contracta* and *F. magellanica* assemblages, two of the most widely distributed species of Patagonia. Regarding morphotype variability, the main contribution to component 1 was made by Ps, St1, Ct4, Po1, Ha, Me2, and Fs; the main contribution to component 2 was made by Me2, Ct2, St1, Sc, El1, Ce, Be, and Po2; and the main contribution to component 3 was made by morphotypes Ct5, Ct2, Me2, Ce, Po2, Be, El1, and Ct3. Our analysis revealed abundant contributions to the differential variabilities of the phytolith morphotypes selected and has enabled the distinct discrimination of members of the fescue groups that inhabit this region (sensu Dubcovsky and Martínez 1988). Our results will be instrumental for future regional paleobotanical applications and illustrate the need of further studies to verify if the diagnostic value of the characters described here also applies to the other fescue species and to Pooideae.

Discussion

Among all of the species studied, biomineralization percentage versus dry mass is higher in leaves than in inflorescences. This fact is in accordance with descriptions by other authors (Zucol and Brea 2005), who pointed out that leaves are the main source of phytolith production, with culms and inflorescences contributing less.

Several studies on biogenic silica have been carried out in grasses, and there is extensive literature on phytolith morphology of these plants (e.g., Mulholland and Rapp 1992). However, quantitative data on the content of biogenic silica are available for only a few species (Bartoli and Souchier 1978; Geis 1978). Carnelli et al. (2001) present biomineralization percentages of some *Festuca* species from Alpine and sub-Alpine zones in Switzerland. These species show biomineralization percentages between 0.7% and 3.27%, whereas in species inhabiting Tierra del Fuego, this percentage varies between 1.39% and 5.68%. Total biomineralization percentages obtained in *F. cirrosa*, *F. contracta*, *F. gracillima*, and *F. purpurascens* are similar to those of the Alpine and sub-Alpine species, whereas the biomineralization percentage of the remaining Fuegian species is higher. Species herborized during the same collection, under the same environmental conditions and the same phenological state, do not present significantly different biomineralization percentages, unlike herbaria samples, even though they have all been collected during summer, as shown in their labels. According to Piperno (1988), there are ecological factors such as climate, soil variability, moisture, and plant age that affect the development of silica bodies, as they regulate the dissolution of silica concentration available to the plant. This would explain the differences found. Phytolith concentration in the plant varies according to its phenological state and to the organ of the plant under consideration. Silica concentration varies according to metabolic processes related to plant life and to the localization of many of these processes in certain organs (Bertoldi de Pomar 1975; Zucol 2001).

We found many morphotypes considered by Piperno (1988), Delhon et al. (2003), and Strömberg (2004) as typical of Festucoid or Pooid grass subfamilies, e.g., fan-shaped and parallepipedal bulliform cells, with higher frequencies of the trapeziform short cells and polylobate and elongated elements (from epidermal long cells). We observed and mentioned morphotypes considered by other authors as representative of Poaceae subfamilies such as square and truncated cone short-cell phytoliths (Metcalfe 1960; Brown 1984; Twiss 1987; Mulholland 1989). The morphotypes described by Mulholland (1989) and Twiss et al. (1969) as Pooid-type dominant in the subfamily Pooideae, which are C3 grasses abundant in cool climate, are the ones that we describe for the abaxial surfaces of leaf epidermis, where irregular rectangular shapes are more common. Our results agree with those described for the Pooideae subfamily, as we have found rondels or truncated cones (both flat and equidimensional), articulated and isolated phytoliths, prickles, and rectangular, elongated, oblong, and crenated, smooth phytoliths (Brown 1984; Mulholland 1989; Twiss 1992; Fernández Honaine et al. 2006).

Morris et al. (2009) described morphotypes of *F. idahoensis* with some phytolith morphotype descriptions and frequencies similar to our results, e.g., the long sinuate trapezoid and truncated cones. In contrast, some species included in our study present abundant macrohairs, and all of the taxa that we considered have a high frequency of crescent elements not mentioned by Morris et al. (2009). Among truncated cones, the mentioned work described only keeled rondel elements, whereas we observed more varied forms with frequencies dependent on the species. Some authors consider that "sinuate" or "crenate" elements are very common Pooid phytoliths (Fredlund and Tieszen 1994). However, in the groups that we analyzed, we found that these morphotypes are less common than others such as truncated cones and crescents. Sinuate trapezoid elements are distributed in the costal zone of the abaxial surface of leaf epidermis and are mostly medium- to short-sized. These morphotypes have sharp ends and asymmetrical contours.

Thus, the presence or absence and shape of silica bodies can be reliably used as taxonomic characters (Ellis 1979). Species of the same group differ in frequency and morphotype, e.g., *F. thermarum* and *F. monticola*. On the other hand, other species belonging to different groups become associated, as in the case of *F. pyrogea* with *F. monticola* and *F. cirrosa*. They all show phytolith presence of components 1 and 3, point-shaped, long sinuate trapezoid, truncated cones without demarcated shank, extremely flattened, bulliform polyhedrical elements, macrohairs, micro-elongated, and cylindrical vascular elements. These phytolith assemblages allowed us to separate groups, thus complementing the group differentiation of Patagonian *Festuca* species proposed by Dubcovsky and Martínez (1992). *Festuca gracillima* was associated with *F. magellanica* and *F. contracta* by characteristics of components 2 and 3. Our phytolith assemblages further allowed us to characterize the species in group 5 and in *F. gracillima* (group 4) present in Tierra del Fuego and to distinguish them from the rest of Patagonian species. These three species differ from the rest because of the predominance of short sinuate trapezoid elements and crescents, less frequent fan-shaped elements, and rare or absent truncated cones with one basal side acuminate, funnel-shaped, micro-elongated, with undulate contour, and cylindrical vascular elements.

Our work has contributed to a detailed study of the characteristic morphotypes, expanding the description of sinuate trapezoids and cones according to their variations, as well as the description of their distribution on the epidermis. We have also for the first time observed short conical elements with bilobated bases, a fact that has never been mentioned before, and have contributed with elements that can be used to establish interspecific differences.

Conclusion

This paper adds to the knowledge of species not only through its description of phytolith assemblages, but also through the characterization and description of the distribution of silica bodies on foliar epidermis. It also includes a description of phytolith assemblages of anthoecium not previously described in *Festuca*. Furthermore, it describes a new morphotype, namely the conical element with a bilobate base, never before mentioned. Our description of phytolith assemblages and our detailed study of morphotypes, especially of sinuate trapezoid and truncated cone elements, provide further characteristics to differentiate species and groups of species of Patagonian *Festuca*. This study provides a tool for the identification of native Fuegian *Festuca* in macro- and micro-remains in sediments, which may serve in paleoenvironmental reconstructions as indicators of environmental changes due to anthropic factors such as overgrazing by domestic livestock.

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References

Abrantes, F. 2003. A 340,000 years continental climate record from tropical Africa — news from opal phytoliths from the equatorial Atlantic. Earth Planet. Sci. Lett. **209**(1–2): 165–179. doi:10.1016/S0012-821X(03)00039-6.

Alexandre, A.J., Meunier, D., Lézine, A.M., Vincens, A., and Schwartz, D. 1997. Phytoliths: indicators of grassland dynamics during the late Holocene in intertropical Africa. Palaeogeogr. Palaeoclimatol. Palaeoecol. **136**(1–4): 213–229. doi:10.1016/S0031-0182(97)00089-8.

Alvarenga, E.C., Fernández Pepi, M.G., Stampacchio, M.L., Moretto, A.S., Escobar, J., and Arriaga, M.O. 2009. Estudio comparativo de dieta de herbívoros según los recursos del ecotono fueguino. *In* Congreso de Ciencias Ambientales COPIME 2009, 7–9 de octubre, Buenos Aires, Argentina. COPIME, Buenos Aires, Argentina. Available at http://www.conicet.gov.ar/new_scp/detalle.php?keywords=Fernandez%20Pepi&id=35503&congresos=yes&detalles=yes&congr_id=652879 [accessed 17 September 2012]

Anderson, V.J., and Briske, D.D. 1995. Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance? Ecol. Appl. **5**(4): 1014–1024. doi:10.2307/2269351.

Antonijevic, V., and Faggi, A.M. 2003. La vegetación de la estancia Buenos Aires en el ecotono fueguino. Contribuciones Científicas GAEA. pp. 37–43.

Archer, S. 1992. Woody plant encroachment into southwestern grasslands and savannas: rates, patterns and proximate causes. *In* Ecological implications of livestock herbivory in the west. *Edited by* M. Vavra, W. Laycock, and R. Pieper. Society for Range Management, Denver, Colorado, USA.

Arriaga, M.O. 1983. Metodología adaptada al estudio de hábitos alimentarios en insectos herbívoros. Comunicaciones del Museo Argentino de Ciencias Naturales, Botánica, Tomo II, No. 15. pp. 103–111.

Arriaga, M.O., Antonijevic, V., Stampacchio, M., and Faggi, A.M. 2004. Cauquenes: competidores o comensalistas ovinos? Un estudio de caso para Tierra del Fuego, Argentina. *In* II Reunión Binacional de Ecología. 31de octubre – 5 de noviembre, Mendoza, Argentina. Available at http://www3.cricyt.edu.ar/eco2004/resumenes_aceptados.htm [accessed 17 September 2012].

Barboni, D., and Bremond, L. 2009. Phytoliths of East African grasses: an assessment of their environmental and taxonomic significance based on floristic data. Rev. Palaeobot. Palynol. **158**(1–2): 29–41. doi:10.1016/j.revpalbo.2009.07.002.

Barboni, D., Bonnefille, R., Alexandre, A., and Meunier, J.D. 1999. Phytoliths as paleoenvironmental indicators, West Side Middle Awash Valley, Ethiopia. Palaeogeogr. Palaeoclimatol. Palaeoecol. **152**(1–2): 87–100. doi:10.1016/S0031-0182(99)00045-0.

Barboni, D., Bremond, L., and Bonnefille, R. 2007. Comparative study of modern pytholith assembleges from inter-tropical Africa. Palaeogeogr. Palaeoclimatol. Palaeoecol. **246**(2–4): 454–470. doi:10.1016/j.palaeo.2006.10.012.

Bartoli, F., and Souchier, B. 1978. Cycle et role du silicium d'origine végétale dans les écosystèmes forestiers tempérés. Ann. Sci. For. **35**(3): 187–202. doi:10.1051/forest/19780302.

Bartolome, J., Klukkert, S.E., and Barry, W.J. 1986. Opal phytoliths as evidence for displacement of native Californian grassland. Madrono, **33**: 217–222.

Bertoldi de Pomar, H. 1975. Los silicofitolitos: sinopsis de su conocimiento. Darwiniana, **19**: 173–206.

Blackman, E. 1971. Opaline silica bodies in the range grasses of southern Alberta. Can. J. Bot. **49**(5): 769–781. doi:10.1139/b71-116.

Boelcke, O. 1957. Comunidades herbáceas del Norte de la Patagonia y sus relaciones con la ganadería. Revista de Investigaciones Agronómicas, **XI**: 5–98.

Boelcke, O., Moore, D.M., and Roig, F.A. (*Editors*). 1985. Transecta Botánica de Patagonia Austral. CONICET, Royal Society, Instituto de la Patagonia, Buenos Aires, Argentina.

Bremond, L., Alexandre, A., Peyron, O., and Guiot, J. 2005*a*. Grass water stress estimated from phytoliths in West Africa. J. Biogeogr. **32**(2): 311–327. doi:10.1111/j.1365-2699.2004.01162.x.

Bremond, L., Alexandre, A., Hély, C., and Guiot, J. 2005*b*. A phytolith index as a proxy of tree cover density in tropical areas: calibration with Leaf Area Index along a forest–savanna transect in southeastern Cameroon. Global Planet. Change, **45**(4): 277–293. doi:10.1016/j.gloplacha.2004.09.002.

Bremond, L., Alexandre, A., Peyron, O., and Guiot, J. 2008*a*. Definition of grassland biomes from phytoliths in West Africa. J. Biogeogr. **35**(11): 2039–2048. doi:10.1111/j.1365-2699.2008.01944.x.

Bremond, L., Alexandre, A., Wooller, M.J., Hély, C., Williamson, D., Schäfer, P.A., Majule, A., and Guiot, J. 2008*b*. Phytolith indices as proxies of grass subfamilies on East African tropical moun-

tains. Global Planet. Change, **61**(3–4): 209–224. doi:10.1016/j.gloplacha.2007.08.016.

Brown, D.A. 1984. Prospects and limits of a phytolith key for grasses in the central United States. J. Archaeol. Sci. **11**(4): 345–368. doi:10.1016/0305-4403(84)90016-5.

Carnelli, A.L., Madella, M., and Theurillat, J.P. 2001. Biogenetic silica production in selected alpine plant species and plant communities. Ann. Bot. (Lond.), **87**(4): 425–434. doi:10.1006/anbo.2000.1355.

Cingolani, A., Anchorena, J., and Collantes, M.B. 1998. Landscape heterogeneity and long-term animal production in Tierra del Fuego. J. Range Manage. **51**(1): 79–87. doi:10.2307/4003568.

Cipriotti, P.A., and Aguiar, M.R. 2005. Effects of grazing on patch structure in a semi-arid two-phase vegetation mosaic. J. Veg. Sci. **16**(1): 57–66. doi:10.1111/j.1654-1103.2005.tb02338.x.

Clayton, W.D., and Renvoize, S.A. 1986. Genera Graminum: grasses of the world. Kew Bulletin Additional Series 8.

Collantes, M.B., and Faggi, A.M. 1999. Los humedales del sur de Sudamérica. *In* Tópicos sobre humedales subtropicales y templados de Sudamérica. *Edited by* A.I. Malvares. UNESCO. Available at unesdoc.unesco.org/images/0015/001502/150270s.pdf. pp. 15–25.

Correa, M. 1978. Flora Patagónica. Parte III, Gramineae. Colección Científica del INTA, Buenos Aires, Argentina.

D'Ambroggio de Argüeso, A. 1986. Manual de Técnicas en Histología Vegetal. Editorial Hemisferio Sur S.A, Buenos Aires, Argentina.

de Nova, P.J.G., de la Cruz, M., Monte, J.V., and Soler, C. 2006. Genetic relationships within and among Iberian fescues (*Festuca* L.) based on PCR-amplified markers. Genome, **49**(9): 1170–1183. doi:10.1139/g06-077. PMID:17110997.

de Pomar, B. 1971. Ensayo de clasificación morfológica de los silicofitolitos. Ameghiniana, **8**: 317–328.

Delhon, C., Alexandre, A., Berger, J.-F., Thiébault, S., Brochier, J.-L., and Meunier, J.-D. 2003. Phytolith assemblages as a promising tool for reconstructing Mediterranean Holocene vegetation. Quat. Res. **59**(1): 48–60. doi:10.1016/S0033-5894(02)00013-3.

Dubcovsky, J., and Martínez, A. 1988. Phenetic relationships in the *Festuca* spp. from Patagonia. Can. J. Bot. **66**(3): 468–478. doi:10.1139/b88-072.

Dubcovsky, J., and Martínez, A.J. 1992. Cytotaxonomy of the *Festuca* spp. from Patagonia. Can. J. Bot. **70**(6): 1134–1140. doi:10.1139/b92-140.

Ellis, R.P. 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view. Bothalia, **12**(4): 641–671.

Fernández Honaine, M. 2007. Análisis fitolítico del pastizal de *Paspalum quadrifarium* y su relación con la evolución pedológica en el sudeste de la provincia de Buenos Aires. Ph.D. thesis, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Buenos Aires Province, Argentina.

Fernández Honaine, M., Zucol, A., and Osterrieth, M. 2006. Phytolith assemblages and systematic associations in grassland species of the south-eastern Pampean Plains, Argentina. Ann. Bot. (Lond.), **98**(6): 1155–1165. doi:10.1093/aob/mcl207. PMID:17030553.

Fernández Pepi, M.G., Stampacchio, M., Rauber, R., Scartín, C., Collantes, M., Cipprioti, P., and Arriaga, M. 2007. Dieta de ovinos en pastizales frio-oceánicos de la estepa fueguina. *In* III Reunión Binacional de Ecología La Serena, Chile. Available at http://www.conicet.gov.ar/new_scp/detalle.php?keywords=Fernandez%20Pepi&id=35503&congresos=yes&detalles=yes&congr_id=1364262 [accessed 17 September 2012].

Fernández Pepi, M.G., Stampacchio, M.L., Alvarenga, E.C., Moretto, A.S., Escobar, J., and Arriaga, M.O. 2009*a*. Estudios de dieta de guanaco (*Lama guanicoe*) en el ecotono fueguino: posibilidades de su uso en el manejo sustentable. Revista Argentina de Producción Animal **29**(Suppl. 1): 175–176.

Fernández Pepi, M.G., Stampacchio, M., Rauber, R., Collantes, M., and Arriaga, M. 2009*b*. Uso de pastizales naturales y artificialmente mejorados por ganado ovino, en la estepa fueguina. Biocell, **33**(2): A206. Available at http://www.conicet.gov.ar/new_scp/detalle.php?keywords=Fernandez%20Pepi&id=35503&congresos=yes&detalles=yes&congr_id=652870 [accessed 17 September 2012].

Fisher, R.F., Bourn, C.N., and Fisher, W.F. 1995. Opal phytolith as an indicator of floristics of prehistoric grasslands. Geoderma, **68**(4): 243–255. doi:10.1016/0016-7061(95)00044-9.

Fredlund, G.G., and Tieszen, L.T. 1994. Modern phytolith assemblages from the North American Great Plains. J. Biogeogr. **21**(3): 321–335. doi:10.2307/2845533.

Gallego, L., and Distel, R.A. 2004. Phytolith assemblages in grasses native to central Argentina. Ann. Bot. (Lond.), **94**(6): 865–874. doi:10.1093/aob/mch214. PMID:15514027.

Gallego, L., Distel, R.A., Camina, R., and Rodríguez Iglesias, R.M. 2004. Soil phytoliths as evidence for species replacement in grazed rangelands of central Argentina. Ecography, **27**(6): 725–732. doi:10.1111/j.0906-7590.2005.03964.x.

Geis, J.W. 1978. Biogenetic opal in three species of Gramineae. Ann. Bot. (Lond.), **42**: 1119–1129.

Hammer, O., Harper, D.A.T., and Ryan, P.D. 2007. PAST — PAlaeontological STatistics, 1.75. Available at http://folk.uio.no/ohammer/past [accessed 17 September 2012].

Kondo, R., Childs, C., and Atkinson, I. 1994. Opal phytoliths of New Zealand. Manaaki Whenua Press, Lincoln, New Zealand.

Labouriau, L.G. 1983. Phytolith work in Brazil: a minireview. The Phytolitharien, **2**: 6–10.

León, R.J.C., Bran, D., Collantes, M.B., Paruelo, J.M., and Soriano, A. 1988. Grandes unidades de vegetación de la Patagonia extraandina. Ecología Austral. **8**(2): 125–144.

Madella, M., Allexandre, A., and Ball, T. 2005. International code for phytolith nomenclature 1.0. Ann. Bot. (Lond.), **96**(2): 33–123.

Mejía-Saules, T., and Bisby, F.A. 2003. Silica bodies and hooked papillae in lemmas of *Melica* species (Gramineae: Pooideae). Bot. J. Linn. Soc. **141**(4): 447–463. doi:10.1046/j.1095-8339.2003.00152.x.

Metcalfe, C.R. 1960. Anatomy of the monocyledons. I. Garmineae. Oxford University Press, Oxford, UK.

Milton, S.J., Dean, W., du Plessis, M., Siegfried, W., and du Plessis, M.A. 1994. A conceptual model of arid rangeland degradation. Bioscience, **44**(2): 70–76. doi:10.2307/1312204.

Milchunas, D.G., and Lauenroth, W.K. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. Ecol. Monogr. **63**(4): 327–366. doi:10.2307/2937150.

Morris, L.R., Baker, F.A., Morris, C., and Ryel, R.J. 2009. Phytolith types and type-frequencies in native and introduced species of sagebrush steppe and pinyon–juniper woodlands of the Great Basin, USA. Rev. Palaeobot. Palynol. **157**(3–4): 339–357. doi:10.1016/j.revpalbo.2009.06.007.

Mulholland, S.C. 1989. Phytolith shape frequencies in North Dakota grasses: a comparison to general patterns. J. Archaeol. Sci. **16**(5): 489–511. doi:10.1016/0305-4403(89)90070-8.

Mulholland, S.C., and Rapp, G. 1992. A morphological classification of grass silica-bodies. *In* Phytolith systematics: emerging issues. *Edited by* G. Rapp and S. Mulholland. Plenum Press, New York. pp. 65–99.

Noy-Meir, I., and Walker, B. 1986. Stability and resilience in range-lands. *In* Rangelands: a resource under siege. *Edited by* P.J. Joss, P.W. Linch, and O.B. Williams. Australian Acemic of Science, Canberra, Australia.

Ollendorf, A., Mulholland, S.C., and Rapp, G. 1988. Phytolith analysis as a means of plant identification: *Arundo donax* and *Phragmites communis*. Ann. Bot. (Lond.), **61**: 209–214.

Palmer, P.G., and Tucker, A.E. 1981. A scanning electron microscope survey of the epidermis of East African grasses. I. Smithsonian Contributions to Botany, **49**: 1–84. doi:10.5479/si.0081024X.49.

Parry, D.W., and Smithson, F. 1964. Opaline silica in the inflorescences of some British grasses and cereals. Ann. Bot. (Lond.), **28**: 169–185.

Patterer, N.I., Passeggi, E., and Zucol, A.F. 2011. Aanálisis fitolíticos de suelos del sudoeste de la Provincia de Entre Ríos (Argentina) como una herramienta para comprender sus procesos pedológicos. Revista Mexicana de Ciencias Geológicas, **28**(1): 132–146.

Piperno, D.R. 1988. Phytolith analysis: an archeological and geological perspective. Academic Press, San Diego, California, USA.

Roig, F.A. 1998. La Vegetación de la Patagonia. *In* Flora Patagónica. *Edited by* M.N. Correa. Tomo VIII, Vol I. Colección Científica del INTA, Buenos Aires, Argentina.

Roig, F.A., Anchorena, J., Dollenzo, O., and Mendez, E. 1985. La vegetaci6n del Area continental. *In* Transecta Botánica de la Patagonia Austral. *Edited by* O. Boelcke, D.M. Moore, and F.A. Roig. Consejo Nacional de Investigaciones Cientificas y Ttcnicas (Argentina), Instituto de la Patagonia (Chile), and the Royal Society (United Kingdom), Buenos Aires, Argentina.

Rovner, I. 1971. Potential of opal phytolith for use in paleoecological reconstruction. Quat. Res. **1**(3): 343–359. doi:10.1016/0033-5894(71)90070-6.

Rovner, I. 1983. Plant opal phytolith analysis: major advances in archaeobotanical research. *In* Advances in archaeological method and theory. *Edited by* M. Schiffer. Academic Press, New York. pp. 225–260.

Runge, F. 1999. The opal phytolith analysis: major advances in archaeological research. *In* Advances in archaeological method and theory. *Edited by* M. Schiffer. Academic Press, New York.

Scott, L. 2002. Grassland development under glacial and interglacial conditions in southern Agrecia: review of pollen, phytolith and isotope evidence. Palaeogeogr. Palaeoclimatol. Palaeoecol. **177**: 45–57. doi:10.1016/S0031-0182(01)00351-0.

Sendulsky, T., and Labouriau, L.G. 1966. Corpos silicosos da gramineas dos Cerrados. An. Acad. Bras. Cienc. **38**: 159–170.

Strömberg, C.A.E. 2004. Using phytolith assemblages to reconstruct the origin and spread of grass-dominated habitats in the Great Plains of North America during late Eocene to early Miocene. Palaeogeogr. Palaeoclimatol. Palaeoecol. **207**(3–4): 239–275. doi:10.1016/j.palaeo.2003.09.028.

Twiss, P.C. 1987. Grass-opal phytoliths as climatic indicators of the Great Plains Pleistocene. *In* Quaternary environments of Kansas. *Edited by* W.C. Johnson. Kansas Geological Survey, Lawrence, Kansas. pp. 179–188.

Twiss, P.C. 1992. Predicted world distribution of C3 and C4 grass phytolith. *In* Phytoliths systematic. Emerging issues. Advances in archaeological and museum science. 1st ed. *Edited by* G. Rapp, Jr., and S.C. Mulholland. Plenum Press, New York. pp. 113–128.

Twiss, P.C., Suess, E., and Smith, R.M. 1969. Morphological classification of grass phytoliths. Soil Science Society of America Proceedings, **33**(1): 109–115. doi:10.2136/sssaj1969.03615995003300010030x.

Westoby, M., Walker, B., and Noy-Meir, I. 1989. Opportunistic management for rangelands not at equilibrium. J. Range Manage. **42**(4): 266–274. doi:10.2307/3899492.

Zucol, A.F. 1996. Estudios morfológicos comparativos de especies del género *Stipa*, *Panicum* y *Paspalum* (Poaceae), de la prov. de Entre Ríos. Ph.D. thesis, Universidad Nacional de La Plata, La Plata, Buenos Aires Province, Argentina.

Zucol, A.F. 2001. Fitolitos. 3. Una nueva metodología descriptiva. Asociaciones fitolíticas de Piptochaetium montevidense (Stipeae: Poaceae). Bol. Soc. Argent. Bot. **36**: 69–85.

Zucol, A.F., and Brea, M. 2005. Sistemática de fitolitos, pautas para un sistema clasificatorio. Un caso en estudio en la Formación Alvear (Pleistoceno inferior), Entre Ríos, Argentina. Ameghiniana, **42**(4): 685–704.

Zucol, A.F., Brea, M., and Bellosi, E.S. 2010. Phytolith studies in Gran Barranca (central Patagonia, Argentina): the middle–late Eocene. *In* The paleontology of Gran Barranca. Evolution and environmental change through the Middle Cenozoic of Patagonia. Chap. 22. *Edited by* R.H. Madden, A.A. Carlini, M.G. Vucetich, and R.F. Kay. Cambridge University Press, Cambridge, UK. pp. 317–340.

Zuloaga, F., Morrone, O., and Belgrano, M. (*Editors*). 2008. Catálogo de Plantas Vasculares del Cono Sur (Argentina, sur de Brasil, Chile, Paraguay y Uruguay). Monogr. Syst. Bot. Missouri Bot. Gard. 107. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

Appendix A. Material studied

Festuca cirrosa

Argentina. Isla de los Estados. Puerto San Juan. 28/XII/1933, Castellanos, BA 12855. Isla Bayly. Costa Canal Washington. 21/II/1980, Pisano, SI 5102. Isla Hornos. Archipiélago Cabo de Hornos. 17/XI/1981, Pisano, SI 5259; *Ibidem*, III/1980, Pisano, SI 5156. Isla Grey. Archipiélago Cabo de Hornos. 15/I/1982, Pisano, SI 5566.

Festuca contracta

Argentina. Georgias del Sur. Puerto Jason. 21/IV/1902, Skattsberg, BA 235. Isla de los Estados. Puerto Cook. 13/I/1934, Castellanos, BA 12867. Puerto San Juan. 31/XII/1933, Castellanos, BA 12823; *Ibidem*, 29/XII/1933, Castellanos, BA 12856; *Ibidem*, 08/I/1934; *Ibidem*, Castellanos, BA 12866.

Festuca gracillima

Argentina. Chubut. Lago Fontana. 14/XII/1963, Lahitte, BA 82435. Santa Cruz. Ea. Frank, cerca del Lago San Martín. 10/I/1909, Skottsberg, BA 40195. Tierra del Fuego. Beagle. Campos de Tierra Mayor. 23/XII/1932, Castellanos, BA 7551. Remolino, 21/XII/1932 Castellanos, BA 7568. Castillo. 14/I/1933, Castellanos, BA 7602. 29/I/1942; *Ibidem*, Castellanos, BA 45572. Lago Carmi. 09/I/1933, Castellanos, BA 7575. Monte Redondo, San Sebastián, 05/XII/1962, Luna et al. BA 5412. Río Grande. 13/I/1933, Castellanos, BA 7602. Segunda Argentina. 27/XII/1918, Pico, BA 40196.

Festuca magellanica

Argentina. Tierra del Fuego. Ea. Buenos Aires, II/2010, Fernández Pepi, Alvarenga, Moretto y Escobar, BA 92285. *Ibidem*, II/2009, Fernández Pepi, Moretto y Escobar, BA 92284. *Ibidem*, II/2009, Fernández Pepi, Moretto y Escobar, BA 92283. Ea. San José. III/2008, Fernández Pepi, Escobar y Moretto, BA 92286. *Ibidem*, III/2008, Fernández Pepi, Moretto y Escobar, BA 92280. Ea. Ushuaia. XI/2007, Fernández Pepi, Moretto y Escobar, BA 92282. Parque Nacional Tierra del Fuego, 12/II/1962, Correa Luna, Razza, BA 5316. Reserva Corazón de la Isla. 11/II/2010, Fernández Pepi et al. BA 92137. *Ibidem*, XI/2007, Fernández Pepi, Moretto y Escobar, BA 92281. Ushuaia. 22/XII/1999, Biganzoli, SI 752.

Festuca monticola

Argentina. Chubut. Chushamen, 14/I/1947, Soriano, SI 2365. Languiño, Bosque de Lenga, 22/I/1948, Soriano, SI 3182. *Ibidem*, 17/I/1947, Soriano, SI 2417. Neuquén. Lago Roca, Cerro Malo. 08/III/1961, Schapossmoii, BA 82444. Parque Nacional Lanin. 27/I/1962. Lahitte et al. BA 82443. Minas, 03/II/1992, Seijo, SI 1701. Tierra del Fuego. Cerro Castor, 22/II/2007, Fraga Vila, BA 92278. Isla Grande.Estancia de los Cerros, 22/II/2007, Fraga Vila, BA 92279. *Ibidem*, 22/II/2007, Fraga Vila, BA 92277. *Ibidem*, 22/II/2007, Fraga Vila, BA 92276.

Festuca purpurascens

Argentina. Tierra del Fuego. Isla de los Estados. Bahía Flinders. 5/II/1934, Castellanos, BA 12826. Puerto Crosley. 23/I/1934, Castellanos, BA 12850. Puerto Roca. 15/II/1934, Castellanos, BA 12858; *Ibidem*, 26/II/1934, Castellanos, BA12863.

Festuca pyrogea

Argentina. Chubut. Lago Fontana. 13/XII/1963, Lahitte, BA 8024; *Ibidem*, 14/XII/1963, Lahitte, BA 8021; *Ibidem*, 14/XII/1963, Lahitte, BA 8022; *Ibidem*, 16/XII/1963, Lahitte, BA 8023. Santa Cruz. Ea. San Lorenzo Este, 04/III/1966, Roquero, BA 8901.

Chile. Fuegia Occidentalis. Fiordo Almirante Martinez, Bahía Córdoba. 18/II/1929, Roivainen, BA 66311.

Festuca thermarum

Argentina. Neuquén. 07/II/1963, Dimitri et al. BA 82474. Cordillera del Viento. Paso de Atrenco. 25/I/1935, Ragonese, BA 14311. Laguna Malbarco Tapia, 23/I/1935, Ragonese, BA 14330. Paso Pino Hachado. 10/II/1941, Perez Moreau, BA 37713.