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Poaceae pollen grains from southern Brazilian grasslands: Pollen grain size in species from dry and humid environments

Grãos de pólen de Poaceae dos Campos do sul do Brasil: tamanho do grão de pólen em espécies de ambientes secos e úmidos

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Abstract

Southern Brazil has a high diversity of grassland Poaceae species in the biomes of the region. However, few data on the morphology and the uniformity of Poaceae pollen grains cause taxonomic low resolution in pollen records. In this study, pollen samples of grassland Poaceae species from different regions of southern Brazil were analyzed. The pollen diversity of 70 grassland Poaceae species (59 genera, 15 tribes, and 6 subfamilies) of the Pampa and Atlantic Forest biomes is presented. Morphometric measurements indicated that pollen grains of humid grasslands species have a larger size than those of dry grasslands species. Though the average of measurements shows a small difference in pollen grain sizes between the Pampa and the Atlantic Forest, such differences cannot be applied to fossil records because non-significant differences occurred between samples. Regarding pollen size, non-significant differences also occurred for the C_3 and C_4 samples, probably because C_3 species had high variance in size. These records provide relevant ecological inferences of Poaceae pollen grains, providing valuable information for reconstruction of vegetation. Furthermore, regarding taxonomic relationships, results suggest the trend of decrease of the size of Poaceae pollen grains towards derived tribes.

Keywords: pollen records, Palynology, Pampa biome, grasses, diporate pollen grains.

Resumo

A uniformidade dos grãos de pólen de Poaceae aliada aos poucos dados sobre a morfologia polínica no sul do Brasil ocasionam baixa resolução taxonômica em registros polínicos. Neste estudo, foram realizadas análises em grãos de pólen de espécies campestres de Poaceae de diferentes regiões do Sul do Brasil, e apresenta-se a diversidade polínica de 70 espécies (59 gêneros, 15 tribos e 6 subfamílias) dos biomas Pampa e Mata Atlântica. As análises morfométricas dos grãos de pólen indicaram que espécies de campos úmidos apresentaram tamanho maior que os de espécies de campos secos. Apesar das médias das medidas apresentarem pequenas diferenças entre os tamanhos dos grãos de pólen dos biomas, esses dados não podem ser aplicados aos registros fósseis, uma vez que diferenças não significativas ocorreram entre as amostras, as quais também foram verificadas no tamanho de pólen de espécies C_3 e C_4, devido à alta variação do tamanho dos grãos de pólen de espécies C_3. Esses registros fornecem relevantes inferências ecológicas de grãos de pólen de Poaceae para a aplicação em reconstruções da vegetação. Além disso, quanto às relações taxonômicas, os resultados sugerem a tendência de diminuição no tamanho do grão de pólen nas tribos mais derivadas.

Palavras-chave: registros polínicos, Palinologia, bioma Pampa, gramíneas, grãos de pólen diporados.

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Introduction

Poaceae pollen grains are major pollen indicators used to analyze the grassland dynamics in South America during the Quaternary. Furthermore, they also indicate the modification of natural vegetation by the introduction of exotic species (corn and others cultivate) by the human (Behling *et al*., 2005; Macedo *et al*., 2009, 2010).

Poaceae pollen frequency was high during the late Pleistocene and early Holocene in southern South America. Poaceae pollen and pollen of Asteraceae and Fabaceae species shows a dominance of grassland vegetation (Campos) during these periods (Bauermann, 2003; Behling *et al*., 2004; Tonello and Prieto, 2010; Mourelle *et al*., 2015). However, the floristic diversity of Poaceae in South America cannot be observed in the pollen records due to the uniformity of the pollen grains of this family. The existence of few studies about the morphology of modern Poaceae in South America increases the difficulty to obtain better pollen taxonomic resolution (Heusser, 1971; Salgado-Labouriau, 1973; Markgraf and D'Antoni, 1978; Salgado-Labouriau and Rinaldi, 1990; Corrêa *et al*., 2005; Dórea, 2011; Bauermann *et al*., 2013; Radaeski *et al*., 2014).

In recent years, studies aiming to overcome the inaccessibility of ecological information of Poaceae pollen grains showed good results. Patterns in the size of the fossil Poaceae pollen grains could be obtained from different South American grassland ecosystems (Shüler and Behling, 2011). In relation to the photosynthetic metabolism of modern taxa, it was possible to observe trends in sizes of Poaceae pollen grains (Jan *et al*., 2014). Analyzing modern pollen grains was also possible to distinguish the pollen grains of grassland and forest Poaceae species of the southern Brazil (Radaeski, 2015; Radaeski *et al*., 2016). Even with these advances, interpretations about vegetation or climate change based on Poaceae pollen grains that predominated in the Quaternary fossil record of South America are hampered, especially in regions dominated by the grassland (Campos) vegetation as the Pampa biome.

About 400 species, 77 genera and 15 tribes of Poaceae are distributed in grasslands in southern Brazil (Boldrini *et al*., 2008; Boldrini and Longhi-Wagner, 2011). Poaceae, Asteraceae and Fabaceae are families that dominate natural grassland of the Rio Grande do Sul (Pillar *et al*., 2009; Pillar and Lange, 2015). This grassland vegetation distributed mainly in the Pampa biome, the southern half of Rio Grande do Sul, where these families colonized it when the climate was different from today (Bauermann, 2003; Behling *et al*., 2004; Bauermann *et al*., 2008). The permanence of the grassland for a long time in the south of Brazil allowed the distribution of floristic diversity of grassland in the region in response to different environmental and geological conditions in Rio Grande do Sul (Hasenack *et al*., 2010). However, the diversity of floristic grassland is not reflected in the pollen records due to the high concentration of different Poaceae species that may not have their pollen grains distinguished in palynological samples. To overcome these problems, more knowledge about the diversity of modern pollen grains of different grassland vegetation is required for better paleoecological inferences of fossil Poaceae pollen grains. Thus, more information about the pollen composition of fossil sediments based on the comparison of palynomorphs with their modern analogs will be available.

The aim of this study is to record the pollen diversity of Poaceae species of grasslands from Pampa and Atlantic Forest biomes in Rio Grande do Sul, determining the types of modern Poaceae pollen in southern Brazil. Furthermore, we will evaluate if there are differences between pollen grains from Atlantic Forest and Pampa biome species, as well as of humid and dry species and C_3 and C_4 species.

Material and methods

Study area

According to the Köppen climate classification, the state of Rio Grande do Sul (27º-34º S and 50º-58º W) has the predominant climate of the "*Cfa*" temperate type and annual precipitation with regular distribution (Nimer, 1989). The maximum annual average temperature is 28ºC recorded in the northwestern region of Rio Grande do Sul and the minimum annual average temperature is 10ºC located in the northeast region with high altitudes (Figure 1). The minimum annual rainfall of Rio Grande do Sul is 1100 mm located to the extreme west of the state and the maximum annual rainfall is 2000 mm in the north region (Rio Grande do Sul, 2012). The higher intensity of the winds in the Rio Grande do Sul is located in the east, in the coastal region, with winds that predominate in the northeast direction in the summer and south-west in winter (Atlas eólico: Rio Grande do Sul, 2014).

The high diversity of grassland species in Rio Grande do Sul is due to different environmental factors in the region, such as altitude, diversity of soils, rainfall and temperature (Boldrini and Longhi-Wagner, 2011). Altitude has a high influence to the distinction between the grassland of the Pampa and Atlantic Forest biomes of Rio Grande do Sul. The Campos de Altitude of the Atlantic Forest biome is located in the northeast of the state in the altitude above 800 m. The vegetation is a mosaic of grassland and *Araucaria* forest (Oliveira, 2009). The grassland of the Pampa biome are distributed in the southern half of Rio Grande do Sul at lower altitudes with gallery forests along the rivers in the region (Pillar *et al*., 2009; Boldrini and Longhi-Wagner, 2011).

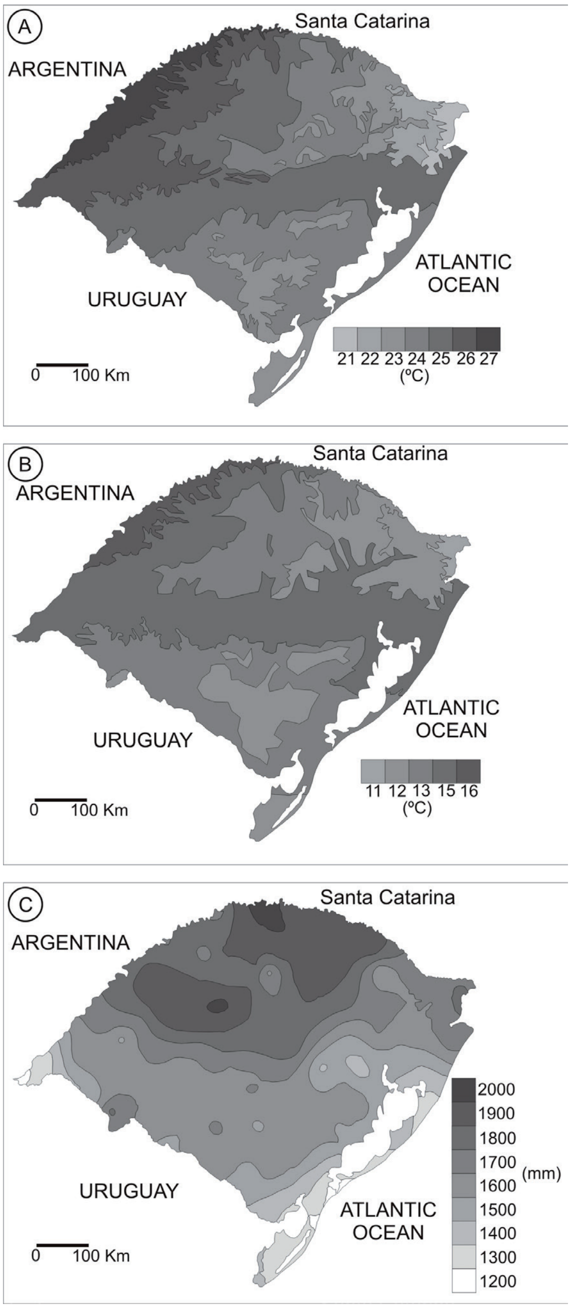

Figure 1. Temperatures in Rio Grande do Sul, southern Brazil. (A) Maximum annual average temperatures in the region. (B) Minimum annual average temperatures in the region. (C) Average annual rainfall in the region.

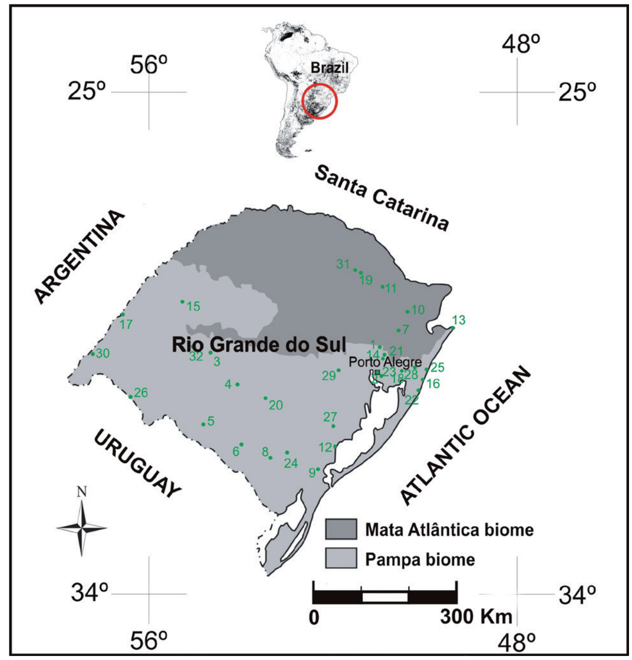

Figure 2. Collection localities of Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil (see Table 1 for more details).

Data collection and analysis

Both fresh and herbarium material may be used for the analysis of pollen morphology (Erdtman, 1952; Salgado-Labouriau, 1973). The collection of pollen material was held in the herborized deposited material in the herbaria of HERULBRA of the Universidade Luterana do Brasil – ULBRA and ICN of Universidade Federal do Rio Grande do Sul – UFRGS (Figure 2, Table 1). The pollen material was chemically processed by the acetolysis technique proposed by Erdtman (1952) and slides with glycerin gelatin were deposited in the Laboratório de Palinologia of Universidade Luterana do Brasil.

Pollen morphological description followed the terminology proposed by Barth and Melhem (1988) and Punt *et al*. (2007). Species were organized in evolutionary order according to the classification of Soreng *et al*. (2015). Twenty-five pollen grains were measured for each species with optical microscopy under magnification of 1000x. In the equatorial view, the following measures were taken from pollen grains: polar diameters, equatorial diameters and thickness of the exine. Furthermore, the pore diameters and annulus diameters and thickness of the pollen grains were also measured.

The graphs showing the pollen grain size variation with correlation to the types of vegetation were generated BioEstat 5.0 and Statistix 8 softwares. Histograms were applied in order to know the distribution of grain sizes. An analysis of variance (ANOVA), followed by Tukey, was applied when the samples showed Gaussian distribution.

Results and discussion

Pollen diversity of 70 Poaceae species of Rio Grande do Sul grasslands distributed in Pampa and Atlantic Forest

Table 1. Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil, examined in the present work. Note: (*) Numbers are the same used in Figure 2.

Number*	Collection site	Species
1	Dois Irmãos	*Leersia* sp. Sol. ex Sw.
2	Porto Alegre	*Bothriochloa laguroides* (DC.) Herter*, Catapodium rigidum* (L.) C.E.Hubb.*, Cenchrus equinatus* L.*, Dactylis glomerata* L.*, Echinochloa polystachya* (Kunth) Hitchc.*, Elionurus candidus* (Trin.) Hack.*, Eustachys distichophylla* (Lag.) Nees*, Luziola peruviana* Juss. ex J.F.Gmel., *Microchloa indica* (L.f.) P. Beauv.*, Oplismenopsis najada* (Hack. & Arechav.) Parodi
3	General Vargas	*Zizaniopsis bonariensis* (Balansa & Poitr.) Speg.
4	São Gabriel	*Chascolytrum subaristatum* (Lam.) Desv., *Eragrostis neesii* Trin.*, Melica* sp. L., *Piptochaetium montevidense* (Spreng.) Parodi Rupr., *Sporobolus indicus* (L.) R. Br.
5	Dom Pedrito	*Glyceria multiflora* Steud, *Hordeum stenostachys* Godr
6	Bagé	*Stipa filifolia* Nees, *Stipa papposa* Nees, *Stipa setigera* J.Presl
7	São Francisco de Paula	*Agrostis* sp. L., *Amphibromus quadridentulus* (Döll) Swallen*, Eriochrysis cayennensis* P. Beauv.*, Sorghastrum* cf. *nutans* (L.) Nash*, Stipa melanosperma* J. Presl
8	Pinheiro Machado	*Aira elegans* Schur
9	Pelotas	*Calamagrostis viridiflavescens* (Poir.) Steud.
10	Jaquirana	*Festuca fimbriata* Nees
11	Vacaria	*Digitaria ciliares* (Retz.) Koeler, *Poa bonariensis* (Lam.) Kunth
12	São Lourenço do Sul	*Phalaris angusta* Nees ex Trin., *Sacciolepis indica* (L.) Chase
13	Torres	*Polypogon elongatus* Kunth
14	Cachoeirinha	*Bromus catharticus* Vahl, *Cynodon dactylon* (L.) Pers.*, Eleusine tristachya* (Lam.) Lam.*, Setaria parviflora* (Poir.) Kerguélen
15	Itacurubi	*Aristida* sp. L., *Axonopus* sp. P. Beauv.
16	Cidreira	*Dichanthelium sabulorum* (Lam.) Gould & C.A. Clark var. *sabulorum*, *Spartina ciliata* Brongn
17	Itaqui	*Eriochloa montevidensis* Griseb.
18	Capivari do Sul	*Panicum aquaticum* Poir.
19	Passo Fundo	*Hymenachne grumosa* (Nees) Zuloaga
20	Caçapava do Sul	*Andropogon lateralis* Nees*, Paspalum nicorae* Parodi, *Paspalum notatum* Flüggé, *Paspalum plicatulum* Michx., *Schizachyrium microstachyum* (Desv. ex Ham.) Roseng.
21	Gravataí	*Eragrostis bahiensis* Schrad. ex Schult.*, Ischaemum minus* J.Presl*, Paspalum pauciciliatum* (Parodi) Herter, *Steinchisma hians* (Elliott) Nash
22	Palmares do Sul	*Andropogon cf. lindmanii* Hack.*, Paspalum urvillei* Steud.
23	Viamão	*Arundinella híspida* (Humb. & Bonpl. ex Willd.) Kuntze
24	Piratini	*Agenium villosum* (Nees) Pilg.
25	Tramandaí	*Imperata brasiliensis* Trin.
26	Quaraí	*Trachypogon filifolius* (Hack.) Hitchc*, Tripogon spicatus* (Nees) Ekman
27	Cristal	*Danthonia montana* Döl
28	Osório	*Leptochloa fusca* (L.) Kunth
29	Cachoeira do Sul	*Tridens brasiliensis* (Nees ex Steud.) Parodi
30	Uruguaiana	*Bouteloua megapotamica* (Spreng.) Kuntze*, Chloris canterae* Arechav
31	Lagoa Vermelha	*Gymnopogon spicatus* (Spreng.) Kuntze
32	São Francisco de Assis	*Pappophorum philippianum* Parodi

biomes are presented (Tables 2 and 3, Figures 3 and 4). The 70 analyzed species belonged to 59 genera representing 15 tribes and six subfamilies. All species have monad pollen grain, with radial symmetry, heteropolar, circular amb, spherical shape, sexine and nexine of the same thickness.

The pollen grains of species varied from small (10-25 µm) to medium-sized (25-50 µm) with Gaussian distribution. The number of apertures in some species was only monoporate and other species showed monoporate and diporate apertures. The microechinate exine with thin thickness (1 to 1,24 µm) was observed in all species using a 1600x magnification. The microechinate ornamentation of some pollen grains can be often not observed in magnifications 400x or 1000x. Thus, this microechinate ornamentation is often described as psilate or scabrate (Radaeski *et al*., 2016).

Poaceae pollen grains of grasslands of the Pampa and Atlantic Forest biomes

The pollen sample of grasslands vegetation formations of Pampa and Atlantic Forest biomes were diverse in terms of pollen size (Figure 6A). The Poaceae species of the

Table 2. Average diameter, range diameter and type and number of aperture of the pollen grains of 70 Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil.

Subfamily	Tribe	Species	Pollen grain diameter (μm)	Aperture
Oryzoideae	**Oryzeae**	*Leersia* sp.	27 μm (23-34)	1-porate
		Luziola peruviana	26 μm (24-30)	1-porate
		Zizaniopsis bonariensis	45 μm (42-48)	1 and 2-porate
Pooideae	**Meliceae**	*Melica* sp.	30 μm (25-33)	1-porate
		Glyceria multiflora	36 μm (33-39)	1-porate
	Stipeae	*Piptochaetium montevidense*	27 μm (23-29)	1-porate
		Stipa filifolia	30 μm (27-35)	1-porate
		Stipa melanosperma	38 μm (34-39)	1-porate
		Stipa papposa	28 μm (24-35)	1-porate
		Stipa setigera	35 μm (27-39)	1-porate
	Poeae	*Agrostis* sp.	29 μm (26-34)	1-porate
		Aira elegans	22 μm (16-25)	1-porate
		Amphibromus quadridentulus	35 μm (32-38)	1-porate
		Calamagrostis viridiflavescens	28 μm (24-32)	1-porate
		Catapodium rigidum	24 μm (22-27)	1-porate
		Chascolytrum subaristatum	29 μm (21-32)	1-porate
		Dactylis glomerata	33 μm (29-37)	1-porate
		Festuca fimbriata	35 μm (28-39)	1-porate
		Poa bonariensis	28 μm (25-32)	1-porate
		Phalaris angusta	35 μm (32-39)	1-porate
		Polypogon elongatus	32 μm (27-37)	1-porate
	Bromeae	*Bromus catharticus*	37 μm (32-43)	1-porate
	Triticeae	*Hordeum stenostachys*	37 μm (33-40)	1-porate
Aristidoideae	**Aristideae**	*Aristida* sp.	31 μm (26-33)	1-porate
Panicoideae	**Paniceae**	*Axonopus* sp.	29 μm (22-37)	1-porate
		Cenchrus equinatus	39 μm (32-43)	1-porate
		Dichanthelium sabulorum var. sabulorum	34 μm (28-39)	1 and 2-porate
		Digitaria ciliares	37 μm (34-40)	1 and 2-porate
		Echinochloa polystachya	40 μm (35-44)	1 and 2-porate
		Eriochloa montevidensis	34 μm (29-41)	1-porate
		Panicum aquaticum	35 μm (30-40)	1-porate
		Sacciolepis indica	25 μm (20-28)	1-porate
		Setaria parviflora	34 μm (30-37)	1-porate
	Paspaleae	*Paspalum nicorae*	34 μm (29-42)	1-porate
		Hymenachne glumosa	35 μm (29-41)	1-porate
		Oplismenopsis najada	33 μm (29-37)	1-porate
		Paspalum notatum	34 μm (32-39)	1-porate
		Paspalum pauciciliatum	42 μm (37-46)	1 and 2-porate
		Paspalum plicatulum	33 μm (27-37)	1-porate
		Paspalum urvillei	33 μm (29-36)	1-porate
		Steinchisma hians	24 μm (18-27)	1-porate
	Arundinelleae	*Arundinella hispida*	25 μm (21-30)	1-porate
	Andropogoneae	*Agenium villosum*	35 μm (31-38)	1-porate
		Andropogon lateralis	32 μm (28-36)	1-porate
		Andropogon cf. *lindmanii*	38 μm (34-41)	1-porate
		Bothriochloa laguroides	36 μm (33-38)	1-porate
		Elionurus candidus	35 μm (28-39)	1-porate
		Eriochrysis cayennensis	33 μm (29-37)	1-porate
		Imperata brasiliensis	36 μm (33-39)	1-porate
		Ischaemum minus	33 μm (30-37)	1-porate
		Schizachyrium microstachyum	30 μm (24-36)	1-porate
		Sorghastrum cf. *nutans*	42 μm (39-45)	1-porate

Table 2. Continuation.

Subfamily	Tribe	Species	Pollen grain diameter (µm)	Aperture
		Trachypogon filifolius	37 µm (31-42)	1-porate
Danthonioideae	**Danthonieae**	*Danthonia montana*	28 µm (22-32)	1-porate
Chloridoideae	**Eragrostideae**	*Eleusine tristachya*	28 µm (23-33)	1-porate
		Eragrostis airoides	28 µm (25-30)	1-porate
		Eragrostis neesii	21 µm (18-25)	1-porate
		Eragrostis bahiensis	29 µm (22-33)	1-porate
		Leptochloa fusca	25 µm (21-28)	1-porate
		Tridens brasiliensis	33 µm (30-36)	1-porate
		Tripogon spicatus	25 µm (20-27)	1-porate
	Zoysieae	*Spartina ciliata*	34 µm (32-37)	1-porate
		Sporobolus indicus	22 µm (18-26)	1-porate
	Cynodonteae	*Bouteloua megapotamica*	34 µm (25-38)	1-porate
		Chloris canterae	33 µm (27-37)	1-porate
		Cynodon dactylon	28 µm (24-32)	1-porate
		Eustachys distichophylla	30 µm (25-35)	1-porate
		Gymnopogon spicatus	34 µm (29-39)	1-porate
		Microchloa indica	25 µm (22-30)	1-porate
		Pappophorum philippianum	30 µm (25-36)	1-porate

grassland of Atlantic Forest biome (Campos de Altitude) tend to have pollen grains with larger size (average size of 36 µm – Figure 5A) and monoporate aperture. On the other hand, the Poaceae species of the grassland of Pampa biome showed pollen grains with a smaller size (average size of 32 µm – Figure 5B), with monoporate aperture in some species and monoporate and diporate apertures in others species (Table 2). The variation in the average size of pollen grains of the species from the Atlantic Forest grassland was lower (33-42 µm) than the variation found in the grassland species of Pampa biome (22-45 µm). This difference may be related to high variation in the distribution of species and differences in temperature and volume of rainfall in the grasslands of the Pampa biome (Pillar *et al*., 2009; Boldrini and Longhi-Wagner, 2011; Rio Grande do Sul, 2012). Thus, the Pampa biome shows species occurring in different climatic conditions, whereas the climatic conditions in the Atlantic Forest biome are more stable (Rio Grande do Sul, 2012). These small differences in the grains sizes of Atlantic Forest and Pampa biome species cannot be applied to fossil records, because non-significant difference between the samples (ANOVA, p>

Table 3. Measurements of the pores, annulus, annulus thickness and exine thickness of the pollen grains of 70 Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil.

Specie	Pore (µm)	Annulus (µm)	Anullus thickness (µm)	Exine (µm)
Leersia sp.	2	7	2.5	1
Luziola peruviana	2	6	2	1.08
Zizaniopsis bonariensis	4	12	4	1
Melica sp.	2	7	2.5	1
Glyceria multiflora	3	10	3.5	1
Piptochaetium montevidense	3	9	3	1.04
Stipa filifolia	3	9	3	1.04
Stipa melanosperma	3	10	3.5	1
Stipa papposa	3	9	3	1.04
Stipa setigera	3	9	3	1.04
Agrostis sp.	3	9	3	1
Aira elegans	2	6	2	1.04
Amphibromus quadridentulus	3	9	3	1
Calamagrostis viridiflavescens	3	9	3	1.04
Catapodium rigidum	2	6	2	1
Chascolytrum subaristatum	3	8	2.5	1.1

Table 3. Continuation.

Specie	Pore (µm)	Annulus (µm)	Anullus thickness (µm)	Exine (µm)
Dactylis glomerata	3	9	3	1.04
Festuca fimbriata	3	9	3	1
Poa bonariensis	3	9	3	1
Phalaris angusta	3	9	3	1
Polypogon elongatus	3	8	2.5	1
Bromus catharticus	3	9	3	1.2
Hordeum stenostachys	3	9	3	1
Aristida sp.	3	8	2.5	1.08
Axonopus sp.	3	7	2	1
Cenchrus equinatus	3	9	3	1.04
Dichanthelium sabulorum var. sabulorum	3	9	3	1.04
Digitaria ciliares	3	9	3	1.04
Echinochloa polystachya	3	9	3	1
Eriochloa montevidensis	3	8	2.5	1
Panicum aquaticum	3	9	3	1.24
Sacciolepis indica	2	6	2	1
Setaria parviflora	3	8	2.5	1.2
Paspalum nicorae	3	8	2.5	1.1
Hymenachne glumosa	3	9	3	1.04
Oplismenopsis najada	3	8	2.5	1
Paspalum notatum	2.5	6	1.75	1.2
Paspalum pauciciliatum	3	8	2.5	1.04
Paspalum plicatulum	3	7	2	1.1
Paspalum urvillei	3	9	3	1
Steinchisma hians	2	6	2	1
Arundinella hispida	2	7	2.5	1
Agenium villosum	3	9	3	1
Andropogon lateralis	4	9	2.5	1.2
Andropogon cf. *lindmanii*	3	9	3	1
Bothriochloa laguroides	3	8	2.5	1.2
Elionurus candidus	3	9	3	1.04
Eriochrysis cayennensis	3	9	3	1
Imperata brasiliensis	3	9	3	1.04
Ischaemum minus	3	8	2.5	1.04
Schizachyrium microstachyum	3	9	3	1.3
Sorghastrum cf. *nutans*	4	12	4	1
Trachypogon filifolius	3	9	3	1.24
Danthonia montana	3	8	2.5	1
Eleusine tristachya	3	8	2.5	1
Eragrostis airoides	2	6	2	1
Eragrostis neesii	2	6	2	1
Eragrostis bahiensis	3	8	2.5	1
Leptochloa fusca	2	6	2	1
Tridens brasiliensis	3	8	2.5	1.1
Tripogon spicatus	2	6	2	1
Spartina ciliata	3	8	2.5	1
Sporobolus indicus	2	6	2	1
Bouteloua megapotamica	3	9	3	1
Chloris canterae	3	8	2.5	1.04
Cynodon dactylon	3	8	2.5	1.04
Eustachys distichophylla	2	7	2.5	1.08
Gymnopogon spicatus	3	9	3	1.12
Microchloa indica	2	6	2	1
Pappophorum philippianum	3	7	2	1

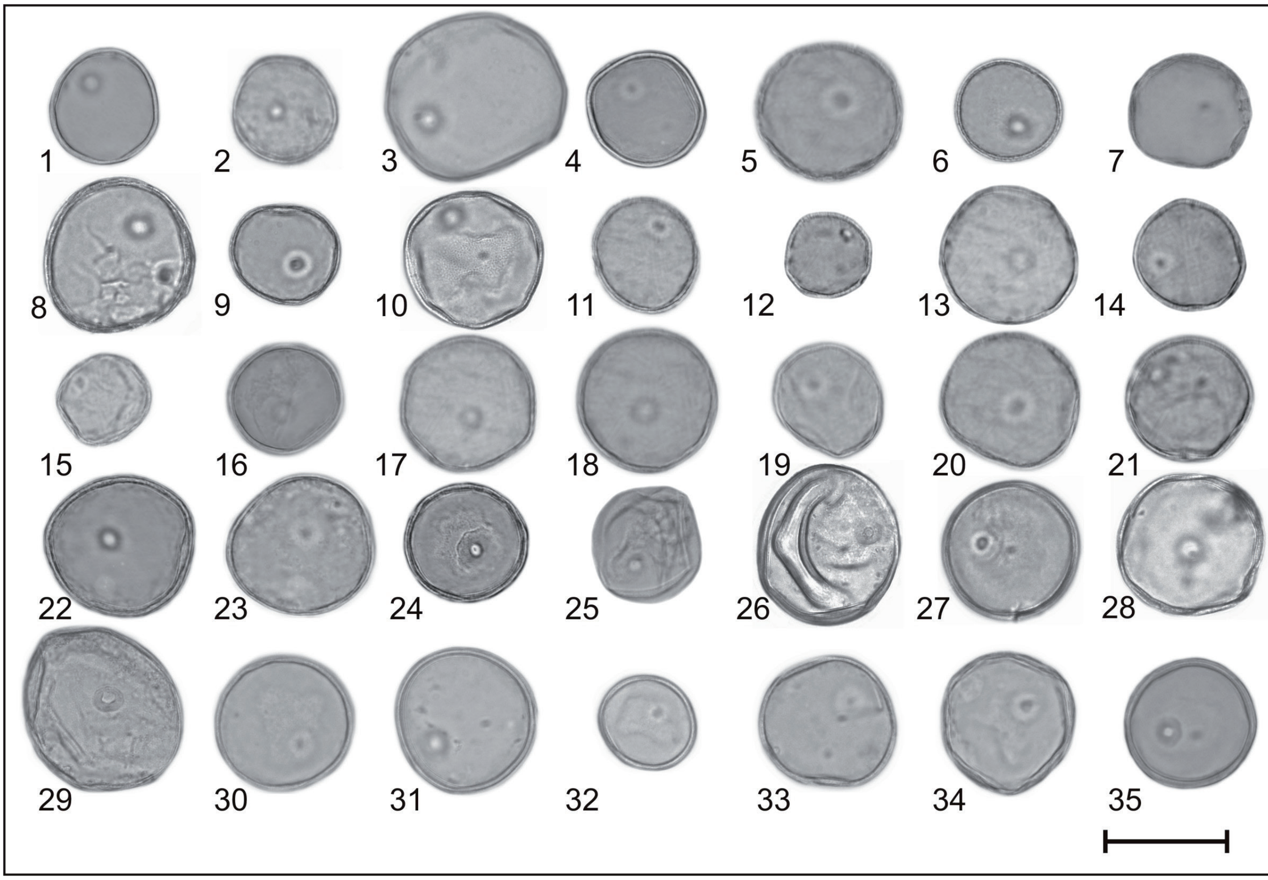

Figure 3. Pollen grains of Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil, in evolutionary order according to Soreng *et al.* (2015). (1) *Leersia* sp. (2) *Luziola peruviana*. (3) *Zizaniopsis bonariensis*. (4) *Melica* sp. (5) *Glyceria multiflora*. (6) *Piptochaetium montevidense*. (7) *Stipa filifolia*. (8) *S. melanosperma*. (9) *S. papposa*. (10) *S. setigera*. (11) *Agrostis* sp. (12) *Aira elegans*. (13) *Amphibromus quadridentulus*. (14) *Calamagrostis viridiflavescens*. (15) *Catapodium rigidum*. (16) *Chascolytrum subaristatum*. (17) *Dactylis glomerata*. (18) *Festuca fimbriata*. (19) *Poa bonariensis*. (20) *Phalaris angusta*. (21) *Polypogon elongatus*. (22) *Bromus catharticus*. (23) *Hordeum stenostachys*. (24) *Aristida* sp. (25) *Axonopus* sp. (26) *Cenchrus equinatus*. (27) *Dichanthelium sabulorum* var. *sabulorum*. (28) *Digitaria ciliares*. (29) *Echinochloa polystachya*. (30) *Eriochloa montevidensis*. (31) *Panicum aquaticum*. (32) *Sacciolepis indica*. (33) *Setaria parviflora*. (34) *Hymenachne grumosa*. (35) *Oplismenopsis najada*. Scale: 30 µm.

0,01; Table 4). Besides a significant difference between Poaceae pollen grains of forests and grasslands (Radaeski *et al*., 2016), differences of pollen size between grassland species (no forest species were considered) of Pampa and Atlantic Forest biomes are non-significant.

Humid and dry grasslands

Morphometric measurements (n=1750) of pollen grains from humid and dry grassland species in 70 Poaceae species of Rio Grande do Sul indicated that pollen grains of humid grassland species have a larger size than those of dry grassland species (Figure 6B, Table 4). The maximum pollen grain size was from species of humid grasslands, while the minimum size was from pollen grains of dry grassland species. The species of dry grasslands only presented the monoporate aperture, while pollen grains from humid grasslands had species either with monoporate aperture only or species with monoporate and diporate apertures. Thus, pollen size extreme ranges may indicate pollen grains of humid or dry grassland species on pollen records. The humid condition is determinant to the local occurrence of grassland species in Rio Grande do Sul. In this way, according to the humid conditions, Poaceae species of the humid grasslands, dry grasslands or species that can survive in both types of vegetation may occur in the region (Bond-Buckup, 2008).

C_3 and C_4 metabolism

Pollen grains of C_3 grassland species (24 taxa) show high variations of size accounting for the minimum (16 µm) and maximum (48 µm) sizes of the species studied (Figure 6B). The C_4 grassland species (46 taxa) presented

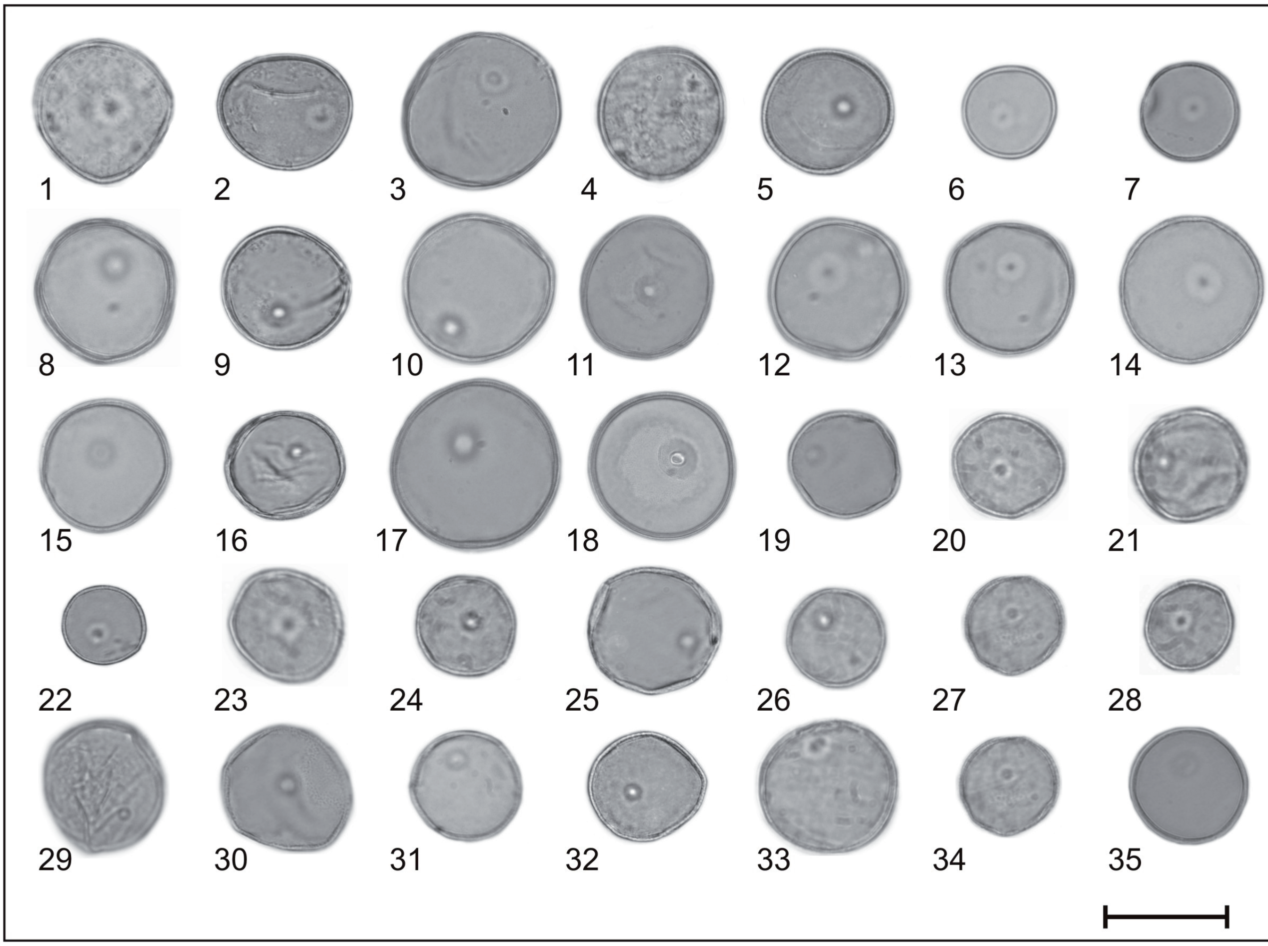

Figure 4. Pollen grains of Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil, in evolutionary order according to Soreng *et al.* (2015). (1) *Paspalum nicorae*. (2) *P. notatum*. (3) *P. pauciciliatum*. (4) *P. plicatulum*. (5) *P. urvillei*. (6) *Steinchisma hians*. (7) *Arundinella hispida*. (8) *Agenium villosum*. (9) *Andropogon lateralis*. (10) *A.* cf. *lindmanii*. (11) *Bothriochloa laguroides*. (12) *Elionurus candidus*. (13) *Eriochrysis cayennensis*. (14) *Imperata brasiliensis*. (15) *Ischaemum minus*. (16) *Schizachyrium microstachyum*. (17) *Sorghastrum* cf. *nutans*. (18) *Trachypogon filifolius*. (19) *Danthonia montana*. (20) *Eleusine tristachya*. (21) *Eragrostis airoides*. (22) *E. neesii*. (23) *E. bahiensis*. (24) *Leptochloa fusca*. (25) *Tridens brasiliensis*. (26) *Tripogon spicatus*. (27) *Spartina ciliata*. (28) *Sporobolus indicus*. (29) *Bouteloua megapotamica*. (30) *Chloris canterae*. (31) *Cynodon dactylon*. (32) *Eustachys distichophylla*. (33) *Gymnopogon spicatus*. (34) *Microchloa indica*. (35) *Pappophorum philippianum*. Scale: 30 µm.

pollen grains with a size between the minimum (18 µm) and maximum (46 µm) size of the pollen grains of C_3 species. C_4 species showed pollen grains with monoporate or diporate apertures, while that all C_3 species presented only monoporate aperture, except by *Zizaniopsis bonariensis*. Non-significant difference between the samples was showed for the C_3 and C_4 samples.

In general, species with C_4 metabolism are tropical and live in warmer and drier regions, while temperate species are C_3 and live in humid and cold conditions (Bond-Buckup, 2008; Boldrini, 2006). In Rio Grande do Sul, species with both types of metabolism may live together (Bond-Buckup, 2008; Boldrini, 2006). Thus, cold climate and high rainfall in the grassland of Atlantic Forest biome provide the predominance of C_3 species and the development of hibernal grasses. However, the megathermic species have a high frequency in the region because of the frequent use of fire by humans (Boldrini and Longhi-Wagner, 2011). In general, in the grassland of Pampa biome, the C_4 species with megathermic cycle have a high distribution, except for the region of the "campos de solos profundos" (south-west of state), that have a high presence of species with C_3 metabolism (Pillar *et al*., 2009).

Diporate apertures

Except by *Zizaniopsis borariensis* of the subfamily Oryzoideae, all species with a diporate aperture (five taxa including *Zizaniopsis borariensis*, Figure 7B) are of the

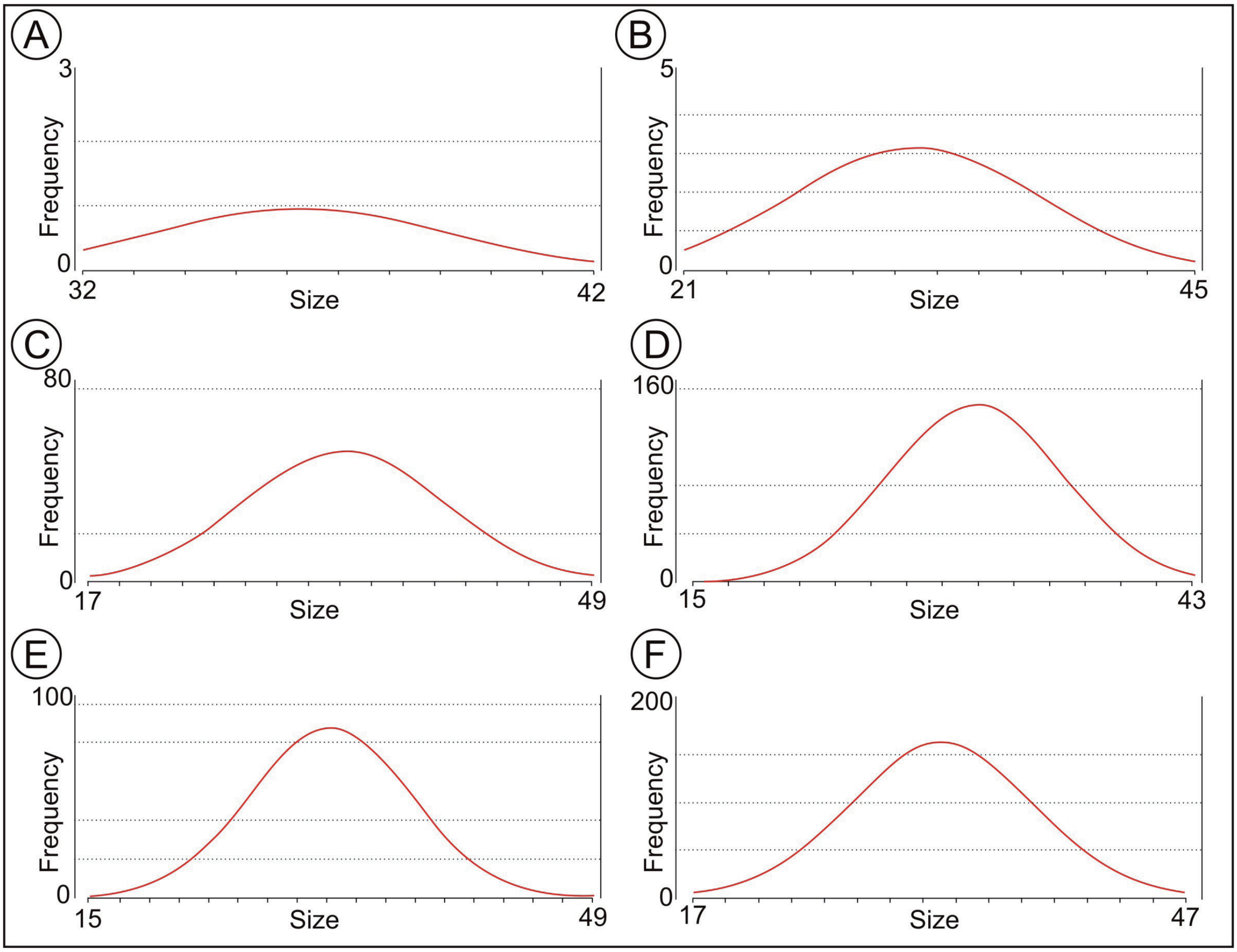

Figure 5. Frequency distribution histogram of pollen size measurements of Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil, showing Gaussian distribution. (A) Atlantic Forest biome. (B) Pampa biome. (C) Humid grassland. (D) Dry grassland. (E) C_3 species. (F) C4 species.

Table 4. Differences between the size of pollen grains of Atlantic Forest biome, Pampa biome, humid grassland, dry grassland, C_3 and C_4 species of Poaceae from grassland vegetation of Rio Grande do Sul, southern Brazil, obtained with ANOVA, followed by Tukey. Abbreviation: ns: not significant.

Sources of variation	DF	Sum of squares	Mean squares (variances)
Treatments	5	21.0 e+02	420.812
error	3094	93.7 e+03	30.296
F	13.8901		
p	< 0.0001		
Mean (Atlantic Forest biome)	36.2857		
Mean (Pampa biome)	31.9130		
Mean (humid grassland)	33.2529		
Mean (dry grassland)	30.8844		
Mean (C_3)	31.2767		
Mean (C_4)	32.1366		
Tukey:	Difference	S	p
Means (Atlantic Forest and Pampa biomes)	4.3727	2.6027	ns
Means (humid and dry grasslands)	2.3684	10.4210	< 0.01
Means (C_3 and C_4)	0.8599	4.3772	ns

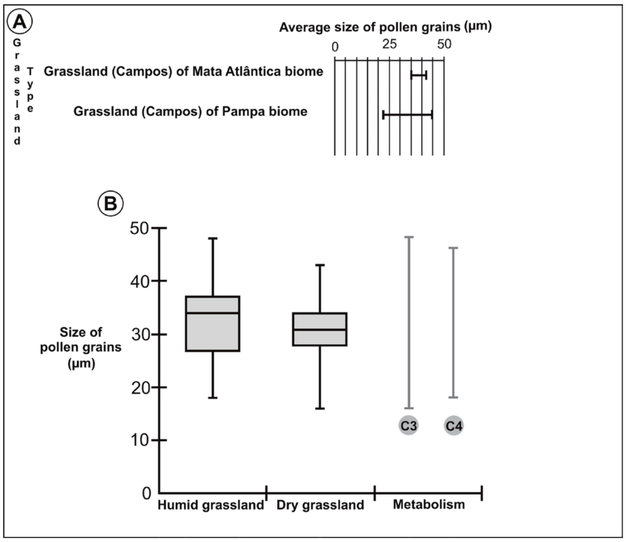

Figure 6. Pollen sizes of Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil. (A) Variation of the average size between Pampa and Atlantic Forest biomes. (B) Variation of the average size between environments (dry and humid grasslands) and metabolism (C_3 and C_4). The horizontal line in bold within the box represents the median. The box shows 50% of the interquartile range and whiskers of the total variation.

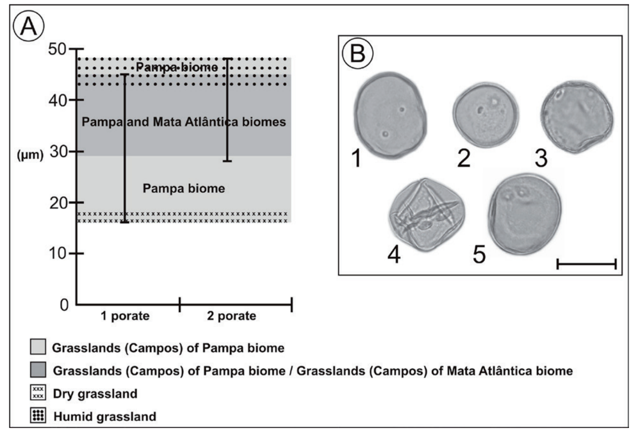

Figure 7. Pollen sizes of Poaceae species from grassland vegetation of Rio Grande do Sul, southern Brazil. (A) Variation of the average size between monoporate and diporate pollen grains. (B) Diporate pollen grains: 1 – *Zizaniopsis bonariensis*, 2 – *Dichanthelium sabulorum* var. *sabulorum*, 3 – *Digitaria ciliares*, 4 – *Echinochloa polystachya*, 5 – *Paspalum pauciciliatum*. Scale: 30 μm.

subfamily Panicoideae (*Dichanthelium sabulorum* var. *sabulorum*, *Digitaria ciliares* and *Echinochloa polystachya* – tribe Paniceae – and *Paspalum pauciciliatum* – tribe Paspaleae). The species with diporate apertures have pollen grains with medium size, preference for humid environment and a megathermic cycle with caespitose habit.

Southern Brazilian grassland species with diporate apertures showed larger size than the Forest herbaceous species *Pharus lappulaceus* with the same type of aperture (Radaeski, 2015; Radaeski *et al*., 2016). Pollen grains of grassland species have a size between 28 and 48 μm (Figure 7A), pollen grains of *Pharus lappulaceus* showed pollen grain size between 23 and 27 μm. These features allow the distinction between diporate pollen grains of the grassland and forest vegetation of southern Brazil.

Taxonomic information

Based on the recent phylogenetic classification of the Poaceae (Soreng *et al*., 2015) the results suggest a trend of decrease of the size of Poaceae pollen grains towards derived species (Figure 8). Radaeski *et al*. (2016) and Salgado-Labouriau *et al.* (1990) show that basal forest species (Bambuseae tribe) have larger pollen size (>45 μm) than species of other tribes. While species of Bambuseae tribe have larger pollen size, species of more derived tribes (Danthonieae, Eragrostideae, Zoysieae and Cynodonteae tribes) have smaller pollen size.

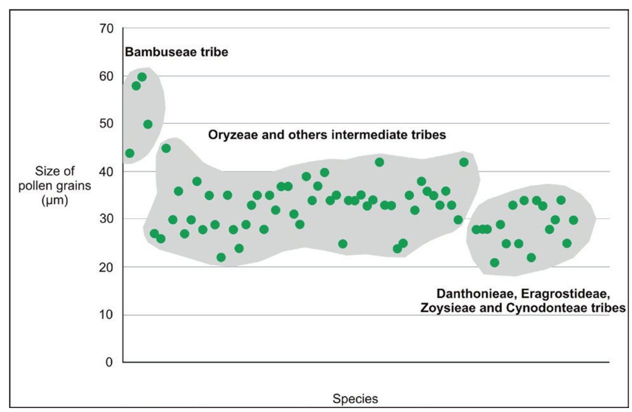

Figure 8. Average size of pollen grains of Poaceae species from grassland and forest (species of Bambuseae) vegetation of Rio Grande do Sul, southern Brazil, considering their phylogenetic classification. The size of pollen grains of species of Bambuseae tribe is according to Radaeski *et al.* (2016).

Conclusions

This work constitutes the greatest dataset of grassland Poaceae pollen diversity of South America. Although the taxonomic resolution at the species level was not obtained, the pollen database presented is important as they fill a gap about pollen morphology of the grassland of Pampa and Atlantic Forest biomes. This pollen database allows showing important information about the size of Poaceae pollen that can be used to identify species of dry and humid grasslands in Quaternary pollen samples. The pollen morphology of different groups of Poaceae species analyzed showed trends to decrease of pollen size towards more derived species. Thus, basal species have larger pollen grains while derived species have smaller pollen size. The records of diporate pollen grains are important to propose the identification of dry and humid grasslands in the two biomes of the region. Furthermore, pollen grains of humid grasslands of Atlantic Forest biome show a trend to have larger sizes than species of dry grasslands of Pampa biome. This pollen database of Poaceae species will serve as useful guide for studies of reconstruction of past vegetation in grasslands of southern Brazil.

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