Poaceae Pollen from Southern Brazil: Distinguishing Grasslands (Campos) from Forests by Analyzing a Diverse Range of Poaceae Species

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This aim of this study was to distinguish grasslands from forests in southern Brazil by analyzing Poaceae pollen grains. Through light microscopy analysis, we measured the size of the pollen grain, pore, and annulus from 68 species of Rio Grande do Sul. Measurements were recorded of 10 forest species and 58 grassland species, representing all tribes of the Poaceae in Rio Grande do Sul. We measured the polar, equatorial, pore, and annulus diameter. Results of statistical tests showed that arboreous forest species have larger pollen grain sizes than grassland and herbaceous forest species, and in particular there are strongly significant differences between arboreous and grassland species. Discriminant analysis identified three distinct groups representing each vegetation type. Through the pollen measurements we established three pollen types: larger grains (>46 µm), from the Bambuseae pollen type, medium-sized grains (46–22 µm), from herbaceous pollen type, and small grains (<22 µm), from grassland pollen type. The results of our compiled Poaceae pollen dataset may be applied to the fossil pollen of Quaternary sediments.

Keywords: pollen morphology, grasses, pampa, South America, Atlantic forest, bamboo pollen

INTRODUCTION

Pollen grains of the family Poaceae are widely found in Quaternary sediments of southern Brazil (e.g., Behling et al., 2004; Macedo et al., 2007; Bauermann et al., 2008). However, the stenopalynous nature of the pollen from this family makes it difficult to determine subfamilies and genera using pollen data (Erdtman, 1952; Salgado-Labouriau, 1973). Consequently, low taxonomic resolution hampers paleoecological inferences. Being mainly associated with grassland vegetation (Figure 1), Poaceae pollen is usually interpreted as indicative of open formations. However in Rio Grande do Sul (RS), where more than 80% of Poaceae species occupy grasslands, a significant percentage (20%) of representatives of this family inhabit forest vegetation (Boldrini and Longhi-Wagner, 2011).

Although the Poaceae family is well represented and studied in RS, few studies have examined the pollen representatives of this family. The few descriptions or illustrations of pollen representatives presented in the study by Tedesco et al. (1999), which analyzed the diameter of the pollen grains of Hemarthria altissima under different ploidy levels, noted that the average diameters were variable, depending on the ploidy. However, the analyzed pollen grains were not acetolyzed. Medeanic et al. (2008) illustrated images of pollen grains from nine species, while Wilberger et al. (2004) presented images of pollen grains corresponding to three separate species. Nakamura et al. (2010), addressing the development of anther and pollen grains in Axonopus aureus, Chloris elata,
Eragrostis solida, Olyra humilis, Paspalum polyphyllum, and Sucrea monophylla, found similar pollen morphology between taxa. This excludes those grains that have been observed to have patterns that are important for differentiation between species of the family. Radaeski et al. (2011) described the pollen morphology of Paspalum notatum, Paspalum plicatulum, and Schizachyrium microstachyum. Later, Bauermann et al. (2013) described the pollen grains of Andropogon lateralis and Eragrostis bahiensis. Radaeski et al. (2014a) described the pollen morphology of Eragrostis neesii, and Radaeski et al. (2014b) contributed to the pollen description of six taxa of Poaceae, which showed that, in general, the pollen grains are of average size, with a monoaperture and spherical forms, and noted the identified stenopalous characteristic of the family.

In South America, studies have been conducted on Poaceae pollen grains from Venezuela, Chile, Brazil, and Argentina (Heusser, 1971; Markgraf and D’Antoni, 1978; Salgado-Labouriau and Rinaldi, 1990). In Chile, the overlap between dimensions of the pollen grains of some tribes made it difficult to differentiate between tribes or subfamilies (Heusser, 1971). Thus, further studies on the pollen grains from other taxa are needed. Likewise, the same is true of the pollen grains of Barro Colorado Island (Roubik and Moreno, 1991). However, analyses of many species that are based solely on one pollen grain cannot verify the variations in total pollen grain diameter of the species. Analyzing pollen grains from Argentina, Markgraf and D’Antoni (1978) observed—from other grassland species studied—the largest diameter of pollen grain of the bamboo Chusquea culeou. Thus, analysis of pollen grains from other forest species can provide patterns related to vegetation from this site.

The pollen grains of many species from other regions of the world (e.g., Europe, South America) have been analyzed by scanning electron microscopy (SEM). Some of these species also inhabit southern Brazil. Some studies (Table 1) have explored the surface of the Poaceae pollen grains through SEM, which has contributed to separation of taxonomic groups (Köhler and Lange, 1979; Linder and Ferguson, 1985; Chaturvedi et al., 1998; Chaturvedi and Datta, 2001; Skvarla et al., 2003; Datta and Chaturvedi, 2004; Liu et al., 2004, 2005; Perveen, 2006; Kashikar and Kalkar, 2010; Ahmad et al., 2011; Dórea, 2011;

### Table 1 | Dataset of Poaceae analyzed species, type of microscopy used and vegetation type.

| Vegetation type | Microscopy type | Number of species | Reference |
|-----------------|-----------------|-------------------|-----------|
| Grassland       | SEM             | 5                 | Ahmad et al., 2011 |
| Grassland       | LM              | 2                 | Bauermann et al., 2013 |
| Grassland       | LM/SEM          | 4                 | Chaturved and Datta, 2001 |
| Grassland       | SEM             | 2                 | Chaturved et al., 1994 |
| Grassland       | LM/SEM          | 19                | Chaturved et al., 1998 |
| Grassland/Forest| LM/SEM          | 30                | Correia et al., 2005 |
| Grassland       | LM/SEM          | 6                 | Datta and Chaturvedi, 2004 |
| Grassland/Forest| SEM             | 86                | Dórea, 2011 |
| Grassland/Forest| LM              | 16                | Heusser, 1971 |
| Grassland       | LM              | 160               | Jan et al., 2014 |
| Grassland       | LM              | 35                | Joly et al., 2007 |
| Grassland       | LM/SEM          | 2                 | Kashikar and Kalkar, 2010 |
| Grassland       | SEM             | 11                | Katsiotis and Forsberg, 1995 |
| Grassland       | LM/SEM          | 12                | Köhler and Lange, 1979 |
| Grassland       | LM/SEM          | 1                 | Linder and Ferguson, 1985 |
| Grassland       | SEM             | 57                | Liu et al., 2004 |
| Grassland       | LM/SEM          | 1                 | Liu et al., 2005 |
| Grassland/Forest| SEM             | 19                | Mander and Punyasena, 2016 |
| Grassland       | SEM             | 12                | Mander et al., 2013 |
| Grassland       | SEM             | 12                | Mander et al., 2014 |
| Grassland/Forest| LM              | 17                | Markgraf and D’Antoni, 1978 |
| Grassland       | LM              | 9                 | Medeanic et al., 2008 |
| Grassland       | LM              | 3                 | Melhem et al., 2003 |
| Grassland       | LM/SEM          | 45                | Morgado et al., 2015 |
| Grassland       | LM              | 6                 | Nakamura et al., 2010 |
| Grassland/Forest| LM/SEM          | 4                 | Nazir et al., 2013 |
| Grassland       | LM/SEM          | 31                | Needham et al., 2015 |
| Grassland       | LM/SEM          | 54                | Perveen and Qaiser, 2012 |
| Grassland       | LM/SEM          | 20                | Perveen, 2006 |
| Grassland       | LM              | 3                 | Radaeski et al., 2011 |
| Grassland       | LM              | 6                 | Radaeski et al., 2014 |
| Grassland/Forest| LM              | 49                | Roubik and Moreno, 1991 |
| Grassland       | LM              | 4                 | Salgado-Labouriau and Rinaldi, 1990 |
| Grassland       | LM              | 1                 | Schier et al., 2011 |
| Grassland       | LM              | 11                | Skvarla et al., 2003 |
| Grassland/Forest| SEM             | 1                 | Tedesco et al., 1999 |
| Grassland       | LM              | 3                 | Wilberger et al., 2004 |
Perveen and Qaiser, 2012; Mander et al., 2013, 2014; Nazir et al., 2013; Morgado et al., 2015; Needham et al., 2015; Mander and Punyasena, 2016). However, other studies using light microscopy (LM) have shown morphometric differences in the size of the Poaceae pollen grain species (Salgado-Labouriau and Rinaldi, 1990; Katsiotis and Forsberg, 1995; Joly et al., 2007; Schüler and Behling, 2011a,b; Jan et al., 2014). In addition, when analyzing large sets of palynomorphs from quaternary sediments, the use of SEM is a difficult and time-consuming task. Thus, morphometric datasets may be valuable for use in studies of fossil Poaceae pollen analysis (Schüler and Behling, 2011a,b; Jan et al., 2014).

Recently, studying pollen grains of fossil Poaceae in the grassland ecosystems of South America, Schüler and Behling (2011a) discovered potential new ways to distinguish grassland types. In their later study, Schüler and Behling (2011b) were able to differentiate the ecosystems present in South America. Moreover, Jan et al. (2014) succeeded in identifying a pattern among changes in the size of Poaceae pollen grains according to the ploidy level and C3 and C4 metabolism, thereby demonstrating that polyploid species have a larger pollen grain size. C4 species are tropical and inhabit warmer and drier regions, while temperate Poaceae species are C3 and live in humid and cold conditions (Boldrini, 2006). C3 and C4 species are important for paleoclimate studies because they indicate past variation in temperature and precipitation (Schüler and Behling, 2011a).

The aim of the study was to distinguish Poaceae pollen grains from grassland and forest vegetation of southern Brazil. The pollen grains of 68 species were analyzed to answer the following questions: (1) Can Poaceae pollen grains be separated into those of grassland species and those of forest species? (2) Do the pollen grains of forest species differ in size according to their arboreal or herbaceous habit?

**MATERIALS AND METHODS**

**Collection of Botanical Material**

During the field expeditions, 98 specimens of Poaceae were obtained, anticipating the 21 taxa representatives of this family, and some pollen material, being fertile, was selected for extraction. To obtain hibernal and estival plants in the flowering seasons, the samples were gathered using the transversal method (Filgueiras et al., 1994) in winter, autumn, spring, and summer in May, August, September, October, November, and December of 2013, as well as in January of 2014.

After collection, the plants were pressed and dehydrated. The plants were identified by a skilled taxonomist (A. A. Schneider). The collection of herbarium specimens was deposited in the "Herbário do Museu de Ciências Naturais" from the Universidade Luterana do Brasil (MCNU/HERULBRA), and duplicates were deposited in the "Herbário Bruno Edgar Irgang" from Unipampa (HBEI/UNIPAMPA). The anthers were collected for chemical treatment of the herbarium materials from other Poaceae species. Since some species have state-restricted distribution, or sporadic bloom periods, samples were collected from pollen material in accordance with information provided by the ICN Herbarium (Figure 2, Table 2).

**Treatment and Description of Pollen Grains**

The anthers were chemically processed according to the acetolysis methodology proposed by Erdtman (1952). After acetolysis, using glycerinated jelly, five permanent slides were
### TABLE 2 | Information of the examined material in the Rio Grande do Sul, Brazil.

| Species                          | Collection site–Number* | Collector                                      |
|---------------------------------|-------------------------|------------------------------------------------|
| Agrostis sp. L.                 | São Francisco de Paula–5| Z. Rügolo, H. Longhi-Wagner, S. Boechat and A.M. Molina 1435 |
| Aira elegans Willd. ex Gaudin   | Pinheiro Machado–6      | I. I. Boldrini 1143                            |
| Amphibromus quadridentatus (Döll) Swallen | São Francisco de Paula–5  | Longhi-Wagner, Boldrini, and Miotto 2654        |
| Andropogon cf. Indranali Hack.  | Palmares do Sul–4       | J. N. Radaeski                                 |
| Andropogon lateralis Nees       | Caçapava do Sul–7       | S. G. Bauermann                                 |
| Aristida sp. L.                 | Itacuruí–8              | S. G. Bauermann                                 |
| Arundinella hispida (Humbl. and Bonpl. ex Willd.) Kuntze | Viamão–9 | R. Trevisan and Boldrini 830                   |
| Axonopus sp. P. Beauv.          | Itacuruí–8              | S. G. Bauermann                                 |
| Bothriochloa lagaroides (DC.) Herter | Porto Alegre–10  | M. Marchi 97                                   |
| Bouteloua megapotamica (Spreng.) Kuntze | Uruguaiana–11  | H. S. A. 74                                   |
| Bromus catharticus Vahl         | Cachoeirinha–2          | J. N. Radaeski                                 |
| Calamagrostis viridiflavescens (Poix.) Steud | Pelotas–12 | I. Gabino 20                                   |
| Catapodium rigidum (L.) C.E.Hubb | Porto Alegre–10        | J. Valls                                       |
| Chascolytrum subaristatum(Lam.) Desv | São Gabriel–1      | J. N. Radaeski                                 |
| Chilos canterae Arechav         | Uruguaiana–11           | J. Valls and A. Barcellos 2477                 |
| Chusquea juergensii Hack.       | Passo Fundo–13          | J. Valls, H. Longhi-Wagner, and A. Barcellos 3081 |
| Colanthelia cingulata (McClure and L.B.Sm.) McClure | Araricá–14 | R. Schmidt and Ene                             |
| Cynodon dactylon (L.) Pers      | Gravatâ–3               | J. N. Radaeski                                 |
| Dactylis glomerata L.           | Porto Alegre–10         | J. Valls                                       |
| Danthonia montana Döll           | Cristal–16              | A. Guglieri, F. J. M. Caporal, S. Mochiotti, and M. Behling 533 |
| Digitaria ciliare (Retz.) Koeler | Vacaria–19              | B. Irgang and M. L. Porto                       |
| Eleusine tristachya (Lam.) Lam   | Cachoeirinha–2          | J. N. Radaeski                                 |
| Elyonurus candidulus (Trin.) Hack. | Porto Alegre–10        | R. Setubal 235                                 |
| Eragrostis bahiensis Schrad. ex Schult | Gravatâ–3              | J. N. Radaeski                                 |
| Eragrostis neesi Trin           | São Gabriel–1           | J. N. Radaeski                                 |
| Eustachys distichophylla (Lag.) Nees | Porto Alegre–10      | R. Setubal 683                                 |
| Festuca fimbriata Nees          | Jacarura–17             | I. Boldrini 1836                               |
| Glyocera multiflora Steud       | Dom Pedrito–18          | Valls, Gonçalves, Salles, and Moraes 6959      |
| Guadua trini (Neeses ex Rupr)   | Gualba–19               | N. I. Matzembacker 2293                        |
| Gymnopogon spicatus (Spreng.) Kuntze | Lagoa Vermelha–20      | Boldrini, Pillar, Kalpel, and Jacques 334      |
| Holcus lanatus L.               | Caxias do Sul–21        | K. Hagelund 3797                               |
| Hordeum stenostachys Godr        | Dom Pedrito–18          | H. Longhi-Wagner 1560                          |
| Ichnarthus pallens (Sw.) Munro ex Benth | Gualba–19 | V. Citadini 59                                 |
| Imperata brasiliensis Trin       | Tramandai–22            | Waechter 1019                                  |
| Ischaemum minus J. Presl         | Gravatâ–3               | J. N. Radaeski                                 |
| Leersia sp. Sol. ex Sw           | Dois Irmãos–23          | H. M. Longhi-Wagner                            |
| Leptochloa fusca (L.) Kunth      | Osório–24               | J. Valls et al. 4760                           |
| Lithachne pauciflora (Sw.) P.Beauv | Tenente Portela–25     | Valls, Lindeman, Irgang, Oliveira, and Pott 1782 |
| Luzola peruviana Juss. ex J.F. Omel | Porto Alegre–10      | Lacê                                          |
| Melica sp. L.                   | São Gabriel–1           | J. N. Radaeski                                 |
| Mierocystis multirameaHack       | Santa Cruz do Sul–26    | V. Kinupp                                      |
| Microchloa indica(L.) P. Beauv   | Porto Alegre–10         | H.M. Longhi-Wagner and C.A.D. Welker 9757a     |
| Muhlenbergia schreberi J.F.Omel  | Estrela Velha–27        | R. Trevisan                                    |
| Olyra istifoliol..               | Morrinhos do Sul–28    | L. C. Mancino, T. B. Guimarães, L. R. M. Batista, and G. E. Ferreira  |
| Panicum aquaticumPoir           | Capivari do Sul–29      | E. N. Garcia 892                               |
| Pappophorum philippianum Parodi | São Francisco de Assis–30 | E. Freitas 359                                |
| Paroedolophy micrantha (Kunth) Daviidsie and Zuloaga | Gravatâ–3 | J. Valls, J. Jung, and A. M. Barcellos 2151    |
| Paspalum notatum Flüggé         | Caçapava do Sul–7       | S. G. Bauermann                                 |
| Paspalum paucilatatum (Parodi) Herter | Gravatâ–3              | J. N. Radaeski                                 |

(Continued)
created for each sample and deposited in the Laboratório de Palinologia da ULBRA. The pollen grains were measured and the slides mounted on the same day to prevent any changes in pollen size (Salgado-Labouriau, 2007). Schüler and Behling (2011a) measured 60 pollen grains from fossil pollen samples, but in this paper we studied modern pollen grains, measuring 25 pollen grains according to the methodology used for the study of modern pollen grains (Erdtman, 1952; Barth and Melhem, 1988).

The morphological characteristics of the pollen grains were observed and described by LM. A Leica CME microscope was used for measurements and for recording images. Using a ×1000 magnification, we recorded the polar diameter (P), equatorial diameter (E), or only the diameter (D) of spherical pollen grains, and the thickness of the exine (Ex) in 25 randomly selected pollen grains. In addition to the above, measurements of the pore and annulus width of the studied species were recorded (Figure 3). The pollen grains were then described in regard to their pollen unit, size, symmetry, polarity, amb, type of aperture, and ornamentation, using the terminology proposed by Barth and Melhem (1988) and Punt et al. (2007).

**Statistical Analysis**

BioEstat 5.0 and PAST 3.05 software was used for the statistical analysis. BioEstat 5.0 software was used to compile a frequency distribution histogram of pollen grain sizes. The histogram was then constructed from the size of the pollen grains of species inhabiting arboreous forest, grassland, and herbaceous forest. This program (BioEstat 5.0) was also used to determine size differences among pollen grains from arboreous forest, grassland, and herbaceous forest species using One-way ANOVA followed by Tukey’s test. PAST 3.05 software was used for discriminant analysis (DA) of separate groups according to the size of the pollen grains. In DA we use the minimum, maximum, and average size of pollen grains of all species to determine whether they can be grouped. We used this program (PAST 3.05) also to show the correlation between pollen grain, annulus, and pore sizes by Pearson correlation. The Pearson’s correlation coefficient test was used to determine the average size of the pollen grains, the pore width, and the annulus width for all species (68 species studied). The PAST 3.05 software was also used to construct a box plot. The box plot shows overlapping measures and measures that

| Species                     | Collection site–Number*          | Collector                        |
|-----------------------------|----------------------------------|----------------------------------|
| Paspalum urvillei Steud    | Palmares do Sul–4                | J. N. Radaeski                   |
| Phalaris angusta Nees ex Trin | São Lourenço do Sul–31          | C. Bonilha 486                   |
| Pharus lappulaceus Aubl     | Gravatá–3                        | L. R. M. Baptista                |
| Piptochaetum montevidense (Spreng.) Parodi | São Gabriel–1        | J. N. Radaeski                   |
| Poa bonariensis (Lam.) Kunth | Vacaria–16                       | A. Kappel                        |
| Polypogon elongatus Kunth   | Torres–32                        | A. Barcelos and B. Irgang 9      |
| Schizachyrium microstachyum (Desv. ex Ham.) Roseng | Caçapava do Sul–7 | S. G. Bauermann                  |
| Setaria parviflora (Poiret) Kerguelen | Gravatá–3     | J. N. Radaeski                   |
| Spartina ciliata Brongn     | Cidreira–33                      | H. M. Longhi-Wagner and S. Leite  |
| Sporobolus indicus (L.) R.Br | Gravatá–3                        | J. N. Radaeski                   |
| Stipa filifolia Nees        | Bagé–34                          | H. M. Longhi-Wagner 5042         |
| Stipa melanosperma J. Presl | São Francisco de Paula–6         | R. L. C. Bortoluzzi 816          |
| Stipa papposa Nees          | Bagé–34                          | I. Boldrini 1177                 |
| Stipa setigera J.Presl      | Bagé–34                          | S. C. Boechat                     |
| Streptochaeta spicata Schrad. ex Nees | Torres–32        | J. F. M. Valls 1055              |
| Tridens brasiliensis (Nees ex Steud.) Parodi | Cachoeira do Sul–35 | J. Valls                        |
| Trachypogon filifolius (Hack.) Hitchc | Quaraí–36   | Boldrini and Pizz187            |
| Triopogon spicatus (Nees) Ekman | Quaraí–36       | Boldrini, Barreto, Boechat and Pillar 279 |

*Numbers corresponding to the Map of Figure 2B.
| Subfamily | Tribe | Species | Vegetation | Size | Diameter pollen grain (µm) | Exine diameter (µm) | Aperture | Portion of pore diameter | Annulus diameter (µm) | Exine thickness (µm) | Figs. |
|-----------|-------|---------|------------|------|-----------------------------|---------------------|-----------|-------------------------|---------------------|---------------------|-------|
| Anomochloideae | Streptochaeteae | Streptochaeta spicata | Forest | Medium | 28 (23–32) | monoporate | 3 (2–4) | 9 (8–10) | 1 | 3A-E | 3F-J |
| Bambusoideae | Bambuseae | Chusquea juergensii | Forest | Large | 44 (40–52) | monoporate | 5 (4-6) | 12 (11–13) | 1 | 0.8 | 3F-J |
| | | Colanthelia cingulata | Forest | Large | 58 (47–74) | monoporate | 5 (4–6) | 14 (13–15) | 1 | 1.2 | 3F-J |
| | | Guadua trinii | Forest | Large | 60 (50–77) | monoporate | 5 (4–6) | 14 (13–15) | 1 | 1.2 | 3F-J |
| | | Merostachys multiramea | Forest | Large | 50 (44–55) | monoporate | 5 (4–6) | 14 (13–15) | 1 | 1.2 | 3F-J |
| | | Olyra latifolia | Forest | Medium | 27 (23–30) | monoporate | 2 (1–3) | 6 (5–7) | 1.08 | 3K-O | 3P-Q |
| | | Parodiolyra micrantha | Forest | Medium | 30 (26–37) | monoporate | 3 (2–4) | 9 (8–10) | 1 | 1 | 3F-J |
| | | Lithachne pauciflora | Forest | Medium | 27 (25–28) | monoporate | 2 (1–3) | 7 (6–8) | 1 | 1 | 3F-J |
| | | Pharus lappulaceus | Forest | Medium | 25 (23–27) | monoporate and diporate | 3 (2–4) | 8 (7–9) | 1 | 1.08 | 3P-Q |
| | | Leersia sp. | Grassland | Medium | 27 (23–34) | monoporate | 2 (1–3) | 7 (6–8) | 1 | 1 | 3P-Q |
| | | Luziola peruviana | Grassland | Medium | 26 (24–30) | monoporate | 2 (1–3) | 6 (5–7) | 1.08 | 3P-Q | 3P-Q |
| | | Danthonia montana | Grassland | Medium | 28 (22–32) | monoporate | 3 (2–4) | 8 (7–9) | 1 | 1 | 3F-J |
| | | Eragrostis neesii | Grassland | Small | 22 (19–26) | monoporate | 2 (1–3) | 5 (4–6) | 1 | 1 | 3F-J |
| | | Eleusine tristachya | Grassland | Medium | 28 (23–33) | monoporate | 3 (2–4) | 8 (7–9) | 1 | 1 | 3F-J |
| | | Eragrostis bahiensis | Grassland | Medium | 29 (22–33) | monoporate | 3 (2–4) | 8 (7–9) | 1 | 1 | 3F-J |
| | | Leptochloa fusca | Grassland | Medium | 25 (21–28) | monoporate | 2 (1–3) | 6 (5–7) | 1 | 1 | 3F-J |
| | | Muhlenbergia schreberi | Grassland | Medium | 30 (26–35) | monoporate | 2 (1–3) | 6 (5–7) | 1.04 | 3P-Q | 3P-Q |
| | | Sporobolus indicus | Grassland | Small | 22 (18–26) | monoporate | 2 (1–3) | 6 (5–7) | 1 | 1 | 3F-J |
| | | Tridens brasiliensis | Grassland | Medium | 33 (30–36) | monoporate | 3 (2–4) | 8 (7–9) | 1.1 | 3F-J | 3F-J |
| | | Tripogon spicatus | Grassland | Medium | 25 (20–27) | monoporate | 2 (1–3) | 6 (5–7) | 1 | 1 | 3F-J |
| | Cynodonteae | Bouteloua megapotamica | Grassland | Medium | 34 (25–38) | monoporate | 3 (2–4) | 9 (8–10) | 1 | 1 | 3F-J |
| | | Chloris canterae | Grassland | Medium | 33 (27–37) | monoporate | 3 (2–4) | 8 (7–9) | 1.04 | 3P-Q | 3P-Q |
| | | Cynodon dactylon | Grassland | Medium | 28 (24–32) | monoporate | 3 (2–4) | 8 (7–9) | 1.04 | 3P-Q | 3P-Q |
| | | Eustachys distichophylla | Grassland | Medium | 30 (25–35) | monoporate | 2 (1–3) | 7 (6–8) | 1.08 | 3F-J | 3F-J |
| | | Gymnopogon spicatus | Grassland | Medium | 34 (29–39) | monoporate | 3 (2–4) | 9 (8–10) | 1.12 | 3P-Q | 3P-Q |
| | | Microchloa indica | Grassland | Medium | 25 (22–30) | monoporate | 2 (1–3) | 6 (5–7) | 1 | 1 | 3F-J |
| | | Spartina ciliata | Grassland | Medium | 34 (32–37) | monoporate | 3 (2–4) | 8 (7–9) | 1 | 1 | 3F-J |
| | | Pappophorumphilippianum | Grassland | Medium | 30 (25–36) | monoporate | 3 (2–4) | 7 (6–8) | 1 | 1 | 3F-J |
| | | Aristidaeae | Aristidaeae | Aristidaeae | Grassland | Medium | 31 (28–33) | monoporate | 3 (2–4) | 8 (7–9) | 1.08 | 3P-Q | 3P-Q |
| | | Agrostis sp. | Grassland | Medium | 26 (23–32) | monoporate | 3 (2–4) | 8 (7–9) | 1.04 | 3P-Q | 3P-Q |
| | | Acropogon speciosus | Grassland | Small | 35 (26–38) | monoporate | 3 (2–4) | 8 (7–9) | 1.04 | 3P-Q | 3P-Q |
| | | Aristidaeae | Aristidaeae | Aristidaeae | Grassland | Medium | 31 (28–33) | monoporate | 3 (2–4) | 8 (7–9) | 1.08 | 3P-Q | 3P-Q |
| | | Aristidaeae | Aristidaeae | Aristidaeae | Grassland | Medium | 31 (28–33) | monoporate | 3 (2–4) | 8 (7–9) | 1.08 | 3P-Q | 3P-Q |
| Subfamily | Tribe       | Species                     | Vegetation | Size     | Diameter pollen grain (µm) | Aperture    | Pore diameter (µm) | Annulus diameter (µm) | Exine (µm) | Figs. |
|-----------|-------------|-----------------------------|------------|---------|-----------------------------|-------------|-------------------|----------------------|------------|-------|
|           |             | Catapodium rigidum         | Grassland | Small   | 24 (22–27)                  | monoporate  | 2 (1–3)           | 6 (5–7)              | 1          |       |
|           |             | Chascolytrum subaristatum  | Grassland | Medium  | 29 (21–32)                  | monoporate  | 3 (2–4)           | 8 (7–9)              | 1.1        | 5K-O |
|           |             | Dactylis glomerata         | Grassland | Medium  | 33 (23–37)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           |             | Festuca limbrata           | Grassland | Medium  | 35 (28–39)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1          |       |
|           |             | Glycera multiflora         | Grassland | Medium  | 36 (33–39)                  | monoporate  | 3 (2–4)           | 10 (9–11)            | 1          |       |
|           |             | Holcus linatus             | Grassland | Medium  | 25 (23–28)                  | monoporate  | 2 (1–3)           | 6 (5–7)              | 1          |       |
|           |             | Poa bonariensis            | Grassland | Medium  | 28 (25–32)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1          |       |
|           |             | Phalaris angusta           | Grassland | Medium  | 35 (32–39)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1          |       |
|           |             | Polygono elongatus         | Grassland | Medium  | 32 (27–37)                  | monoporate  | 3 (2–4)           | 8 (7–9)              | 1          |       |
|           | Bromae      | Bromus catharticus         | Grassland | Medium  | 37 (32–43)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.2        | 5P-T |
|           | Meliceae    | Melica sp.                 | Grassland | Medium  | 30 (25–33)                  | monoporate  | 2 (1–3)           | 7 (6–8)              | 1          | 6A-E |
|           | Triticeae   | Hordeum stenostachys       | Grassland | Medium  | 37 (33–40)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1          | 6F-J |
|           | Stipeae     | Piptochaetium montevidense | Grassland | Medium  | 27 (23–29)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       | 6K-O; 8C |
|           | Paniceae    | Stipa filifolia            | Grassland | Medium  | 30 (27–35)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           |             | Stipa melanosperma         | Grassland | Medium  | 38 (34–39)                  | monoporate  | 3 (2–4)           | 10 (9–11)            | 1          |       |
|           |             | Stipa papposa              | Grassland | Medium  | 28 (24–35)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           |             | Stipa setgera              | Grassland | Medium  | 31 (25–34)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           | Panicoideae | Paniceae                   | Grassland | Medium  | 29 (22–37)                  | monoporate  | 3 (2–4)           | 7 (6–8)              | 1          |       |
|           | Andropogoneae | Axonopus sp.              | Grassland | Medium  | 37 (34–40)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           |             | Ichnanthus pallens        | Forest     | Small   | 23 (22–26)                  | monoporate  | 2 (1–3)           | 6 (5–7)              | 1          |       |
|           |             | Panicum aquaticum         | Grassland | Medium  | 35 (30–40)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.24       |       |
|           |             | Paspalum notatum          | Grassland | Medium  | 34 (32–39)                  | monoporate  | 2.5 (2–3)         | 6 (5–7)              | 1.2        |       |
|           |             | Paspalum pauciciliatum     | Grassland | Medium  | 42 (37–46)                  | monoporate and diporate | 3 (2–4)         | 8 (7–9)              | 1.04       |       |
|           |             | Paspalum urvillei         | Grassland | Medium  | 33 (29–36)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1          | 6P-T |
|           |             | Setaria parviflora        | Grassland | Medium  | 34 (30–37)                  | monoporate  | 3 (2–4)           | 8 (7–9)              | 1.2        |       |
|           |             | Stevinchrisa hiaris       | Grassland | Medium  | 24 (18–27)                  | monoporate  | 2 (1–3)           | 6 (5–7)              | 1          |       |
|           | Andropogoneae | Andropogon lateralis      | Grassland | Medium  | 32 (28–36)                  | monoporate  | 4 (3–5)           | 9 (8–10)             | 1.2        |       |
|           |             | Andropogon cf. Lindmani   | Grassland | Medium  | 38 (34–41)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1          |       |
|           |             | Bothriochloa laguroides    | Grassland | Medium  | 36 (33–38)                  | monoporate  | 3 (2–4)           | 8 (7–9)              | 1.2        |       |
|           |             | Elionurus candidus        | Grassland | Medium  | 35 (28–39)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           |             | Imperata brasiliensis     | Grassland | Medium  | 36 (33–39)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.04       |       |
|           |             | Iscaemum minus            | Grassland | Medium  | 33 (30–37)                  | monoporate  | 3 (2–4)           | 8 (7–9)              | 1.04       |       |
|           |             | Schizachyrium microstachyum| Grassland | Medium  | 30 (24–36)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.3        | 7A-E |
|           |             | Trachypogon filifolius    | Grassland | Medium  | 37 (31–42)                  | monoporate  | 3 (2–4)           | 9 (8–10)             | 1.24       |       |
|           | Arundinelleae | Arundinella hispida       | Grassland | Medium  | 25 (21–30)                  | monoporate  | 2 (1–3)           | 7 (6–8)              | 1          | 7F-J |
FIGURE 4 | Pollen grains of the subfamilies Anomochlooideae, Bambusoideae, and Pharoideae. (A–E) Streptochaeta spicata: PV (A), EV (B), detail of ornamentation (C), detail of the thickness of the exine (D), and detail of the aperture (E). (F–J) Chusquea juergensii: PV (F), EV (G), detail of ornamentation (H), detail of the thickness of the exine (I), and detail of the aperture (J). (K–O) Olyra latifolia: PV (K), EV (L), detail of ornamentation (M), detail of the thickness of the exine (N), and detail of the aperture (O). (P–T) Pharus lappulaceus: PV (P), EV (Q), detail of ornamentation (R), detail of the thickness of the exine (S), and detail of the aperture (T).

do not overlap. We used all the pollen grain size measurements for the box plot (i.e., 25 measures each of 68 species = total 1700 measures).

RESULTS
Measurement of Pollen Grains
Table 3 presents the measurements of the pollen grains for the 68 species, in evolutionary order according to the GPWG (Grass Phylogeny Working Group) classification (2001). Yet, differences were observed in the measurements of pollen grains, pores, and annulus. Pollen grains of species of each tribe were selected to show the Poaceae morphological characteristics of all the tribes of southern Brazil (Figures 4–8).

A frequency distribution histogram of the measurements of pollen size is shown in Figure 9A. The average measurement values were higher for arboreous forest (8% of the measurements of these species were 50 µm). Grassland and herbaceous forest species had lower average measurement values (16% of the forest herbaceous species measured 27 µm, and 8% of the grassland species measured 32 µm). Grain size distributions showed a Gaussian distribution for samples of arboreous forest, grassland, and herbaceous forest species (Figures 9B–E). The Gaussian distribution showed that ANOVA-Tukey can be applied to the data set.

Morphometric Variation in Diameters of the Pollen Grains, Pores, and Annulus
The ANOVA-Tukey test showed statistically significant differences between the size of pollen grains of arboreous forest, grassland, and herbaceous forest species (Table 4). This difference is clear in the comparison of samples that indicate
values ($p$) less than 0.01. The difference between the means of the arboreous forest and grassland samples was large (22.1719), while among the grassland and herbaceous forest samples the difference was small (4.3681).

The DA test (Figure 10) determined the separation of three groups according to the values of the variables. The DA identified the separation of arboreous forest grassland and herbaceous forest groups. The arboreous forest group showed the greatest differences, and the grassland and herbaceous forest groups were mixed.

The Pearson correlation test (Table 5) showed values that indicate a strong relationship between the size of pollen grains and the width of the pore ($r = 0.8281$). It also showed a strong relationship between the size of pollen grains and the width of the annulus ($r = 0.8565$). The diagrams of the values obtained indicate a similarity between the size of pollen grains, the pore width, and the width of the annulus (Figure 11).

All taxa showed monoporate apertures with annulus around the pores, except for *Pharus lappulaceus*, *Digitaria ciliare*, and *Paspalum pauciciliatum*, which showed diporate as well as the monoporate pollen grains. However, these three species (*P. lappulaceus*, *D. ciliare*, and *P. pauciciliatum*) showed only a few diporate pollen grains; most of their pollen grains were found to be monoporate. Nevertheless, they were unique species in terms of having diporate pollen. In the herbaceous forest species with diporate pollen grains, the grains measured 23–27 µm in width, while in the grassland species with diporate pollen, the grains measured 34–46 µm in width.

**Interpretation of, and Distinction between, the Grassland and Forest Pollen Grains**
In southern Brazil, 80% of Poaceae species are grassland species, while 20% are forest species. In our data set (68 species), 85.29% were grassland species and 14.71% were forest species. Thus,

![FIGURE 5 | Pollen grains of the subfamilies Ehrartoideae, Danthonioideae, and Chloridoideae. (A–E) Leersia sp.: PV (A), EV (B), detail of ornamentation (C), detail of the thickness of the exine (D), and detail of the aperture (E); (F–J) Danthonia montana: PV (F), EV (G), detail of ornamentation (H), detail of the thickness of the exine (I), and detail of the aperture (J); (K–O) Eragrostis neesii: PV (K), EV (L), detail of ornamentation (M), detail of the thickness of the exine (N), and detail of the aperture (O); (P–T) Chloris canteraeae: PV (P), EV (Q), detail of ornamentation (R), detail of the thickness of the exine (S), and detail of the aperture (T).](image-url)
we analyzed the appropriate proportions of species relating to grassland and forest vegetation in the region.

The box plot of data sets relating to different Poaceae vegetation (arboreous forest, grassland, and herbaceous forest) showed pollen grains of different size ranges (Figure 12). The pollen grains of arboreous forest species were larger than those of grassland and herbaceous forest species. The pollen grains of grassland species and herbaceous forest species were found to be of similar size. However, the pollen of the grassland species had a lower minimum size than that of the forest herbaceous species. Three pollen types could be separated based on pollen grain size (Table 6). The Bambuseae pollen type was found to have pollen grains larger than 46 µm in width. Pollen grains that vary in size between 22 and 46 µm are of the herbaceous pollen type; these pollen grains belong to either grassland or herbaceous forest species. The grassland pollen type has small pollen grains, measuring less than 22 µm.

**DISCUSSION**

Based on measurements of pollen grains, previous studies have allowed scholars to distinguish between Poaceae pollen grains of South American ecosystems, and also to show the trends in pollen grain size among C3 and C4 Poaceae species (Schüler and Behling, 2011a,b; Jan et al., 2014). In this work, it was possible to distinguish the Poaceae pollen grains relating to grassland and forest species of southern Brazil. Jan et al. (2014) analyzed a large data set with species from various locations around the
FIGURE 7 | Pollen grains of the subfamilies Pooideae and Panicoideae. (A–E) Melica sp.: PV (A), EV (B), detail of ornamentation (C), detail of the thickness of the exine (D), and detail of the aperture (E); (F–J) Hordeum stenostachys: PV (F), EV (G), detail of ornamentation (H), detail of the thickness of the exine (I), and detail of the aperture (J); (K–O) Piptochaetium montevidense: PV (K), EV (L), detail of ornamentation (M), detail of the thickness of the exine (N), and detail of the aperture (O); (P–T) Paspalum urvillei: PV (P), EV (Q), detail of ornamentation (R), detail of the thickness of the exine (S), and detail of the aperture (T).

world. In our work we wanted to analyze the variability within one ecosystem; therefore, we chose to analyze a large set of data relating to only one region (southern Brazil).

Studies of Poaceae pollen grains have revealed a strong correlation between size of pollen grain, pore, and annulus (Skvarla et al., 2003; Joly et al., 2007; Schüler and Behling, 2011a,b; Jan et al., 2014). The results of our own study also showed a relationship between size of pollen, pore, and annulus, as determined through correlation analysis.

Analysis of the Poaceae pollen of the plants deposited in the herbarium provided a description of the variation in pollen grain size of species that occur in different regions of the state of RS. According to the results, relating pollen data to information on the current vegetation of RS, the main variations in size of Poaceae pollen grains in the state (Figure 13) could be mapped.

Taking into account the vegetation types that are based on more representative genera from different regions (Hasenack et al., 2010), we can assign to regions the probable main pollen types occurring in different locations. Thus, the northern half of RS seems to be composed of larger pollen grains. We also found a reduction in size toward the southern half of the state, where the concentration of smaller pollen grains can be associated with the western part of RS, especially in the region of grassland with shallow soils (where the range of diameters for pollen grains is 22–34 µm).

Forest Vegetation
Pollen grains of forest Poaceae species showed distinctions between species. Arboreous species showed larger pollen grains than herbaceous species. The pollen grain size of the arboreous
species ranged from medium to large, while that of the herbaceous species ranged from small to medium. The pollen grains of arboreal Poaceae species showed a tendency toward larger sizes (Markgraf and D’Antoni, 1978; Salgado-Labouriau and Rinaldi, 1990). The differently sized grains of pollen forest species may be related to the small wind flow inside the forests and may also be influenced by pollination (Dórea, 2011). Some variations in the size of the pollen grains of modern...
Poaceae species has already been reported in South America. In Venezuela, larger pollen grains have already been reported to be related to the Bambusoideae and Pooideae subfamilies (Salgado-Labouriau and Rinaldi, 1990). However, in southern Brazil, we are able to differentiate at the level of tribes determining the Bambuseae pollen type. The Bambuseae pollen type is indicative of arboreal grasses and humid regions (Schmidt and Longhi-Wagner, 2009).
Grassland Vegetation

Pollen grains of grassland species are smaller than those of arboreal forest species and similar to those of herbaceous forest species. These results make it possible to identify the herbaceous pollen type. The smaller size of pollen grains in grassland species allows identification of the grassland pollen type. The small and medium sizes of pollen grains of grassland Poaceae species correspond to previous data relating to South American species (Heusser, 1971; Markgraf and D’Antoni, 1978; Salgado-Labouriau and Rinaldi, 1990; Melhem et al., 2003; Côrrea et al., 2005; Bauermann et al., 2013; Radaeski et al., 2014a,b) and species from other regions of the world (Joly et al., 2007; Jan et al., 2014; Morgado et al., 2015). The small size of pollen grains of grassland species can also be related to the type of dispersion involved, since grassland species produce more pollen than forest species (Radaeski and Bauermann, 2016).

Exine

Many studies have revealed differences in the exine sculpture of Poaceae pollen grains. Such differences are evident through the use of SEM, which allows adequate analysis of the surface (Köhler and Lange, 1979; Linder and Ferguson, 1985; Chaturvedi et al., 1994, 1998; Chaturvedi and Datta, 2001; Skvarla et al., 2003; Datta and Chaturvedi, 2004; Liu et al., 2004, 2005; Perveen, 2006; Kashikar and Kalkar, 2010; Ahmad et al., 2011; Dórea, 2011; Perveen and Qaiser, 2012; Mander et al., 2013, 2014; Nazir et al., 2013; Morgado et al., 2015; Needham et al., 2015; Mander and Punyasena, 2016). Light microscopy is used to study the fossil pollen of Quaternary sediments at smaller magnifications.
(×400); SEM is not suitable for such study. Thus, data pollen measures seem to be more suitable to use in comparison with fossil pollen.

The thin exine of the Poaceae pollen grains is a remarkable characteristic, not exceeding 2 µm in thickness and having equivalent sexine and nexine values. Because of this thin layer, many pollen grains—especially the larger ones—may display small changes in their spherical shape owing to the flattening of the pollen grain. This often provides the impression of pollen grains with prolate or oblate forms. However, these shapes are easily observed in crushed pollen grains, for when the non-deformed (used as parameters) pollen grains are examined, their spherical form—characteristic of the Poaceae family—is noted.

The surface of the exine of Poaceae pollen grains, when viewed by SEM, exhibits several variations among species (Dórea, 2011). However, when observed under light microscopy, the pollen surface exhibits a tectate exine with columnellae and spinulose ornamentation. The variations in the surface of the exine observed by SEM cannot be observed by light microscopy. To identify the pollen grains (from pollen records) involving smaller increases, mainly occurring in Quaternary sediments, the ornamentation is often not observed. With a magnification of ×400, much ornamentation of the studied taxa is not visible (Schüler and Behling, 2011a,b), for the ornamentation is often interpreted as psilate, scabrate, or microreticulate surfaces. However, under higher (SEM) magnifications, the sculptured grain surfaces may be evident (Chaturvedi et al., 1998; Liu et al., 2004; Dórea, 2011; Mander et al., 2013).

**CONCLUSIONS**

Using a data set of 68 species, we found that types of vegetation can be distinguished according to Poaceae pollen grains. The size of pollen grains of arboreous forest Poaceae species differs from that of grassland and herbaceous forest species. The pollen grains of forest species of arboreal habit are larger than those of forest species of herbaceous habit. Through measurements and statistical analysis, we found that these Poaceae species exhibit variation in the size of pollen grains in species inhabiting arboreal forest, grassland, and herbaceous forest. Thus, three pollen types were identified: Bambuseae, herbaceous, and grassland pollen types.

Grassland and forest vegetation may be distinguished by examining Poaceae pollen grains from southern Brazil. Thus, the dynamics of the grassland and forest vegetation during the Pleistocene and Holocene periods can be demonstrated based on Poaceae pollen grains. Also, pollen characterization by vegetation

| Size of pollen grain | Pollen type | Species included |
|----------------------|-------------|------------------|
| >46 µm               | Bambuseae   | Chusquea juergenisii, Colantheria cingulata, Guadua trinitii, Merostachys multiramea |
| 46–22 µm             | Herbaceous  | Streptocheta spicata, Lithachne pauciflora, Olyra latifolia, Parodiolyra micrantha, P翰rus lappulaceus, Leersia sp., Luziola peruviana, Danthonia montana, Eleusine tristachya, Eragrostis bahiensis, Muhlenbergia schreberi, Tidens brasiliensis, Bouteloua megapotamica, Chloris canterae, Cyrtodon dactylon, Eustachys distichophylla, Gymnopogon spicatus, Microchloa indica, Spartina ciliata, Pappophorum philopplumum, Aristida sp., Agrostis sp., Amphibromus quadridentatus, Calamagrostis viridilavescens, Catapodium rigidum, Dactylis glomerata, Festuca fimbriata, Glyceria multiflora, Holcus lanatus, Poa bonariensis, Phaliris angusta, Polygongon elongatus, Bromus catharticus, Melica sp., Hordeum stenostachys, Piptochaetium montevidense, Stipa filifolia, Stipa melanosperma, Stipa papposa, Stipa seligera, Axonopus sp., Diptaria ciliares, Khiantschius pallens, Panicum aquaticum, Paspalum notatum, Paspalum paluciculatum, Paspalum urvillei, Setaria parviflora, Andropogon lateralis, Andropogon cf. Lindmanii, Bothriochloa laguroides, Elionurus candidus, Imperata brasiliensis, Ischaemum minus, Schizachyrium microstachyum, Trachypogon filifolius |
| <22 µm               | Grassland   | Eragrostis neesi, Leptochloa fusca, Sporobolus indicus, Tripogon spicatus, Aita elegans, Chascolytrum subaristatum, Steinchisma hians, Arundinella hispida |
is of great importance, since the pollen morphology of the grasslands and forests can be used as indicators of humid or dry environments, respectively.

By determining the size of pollen grains of 68 Poaceae species in RS, it was possible to indicate previously inaccessible information for ecological inferences concerning southern Brazil grasslands. Attaining better taxonomic resolution of both vegetation types allows new opportunities to expand the pollen records beyond the family level. Further research is needed on the pollen morphology of other native genera and species of the Poaceae family for RS. It is expected that further studies will allow greater differentiation between groups and improved knowledge of pollen morphology at a family level. The presented method is being applied to the development of pollen records for southern Brazil and may favor climatic reconstruction of past environments and an evaluation of the dynamics of Quaternary grassland Poaceae vegetation.

**AUTHOR CONTRIBUTIONS**

JR provided the images of the pollen grains and pollen measurements. JR, SB, AP structured and edited the manuscript during all phases. SB and AP supervised the project. JR and SB supported the paleoecological interpretations. JR and AP developed the botanical implications.

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