

Diporate Pollen Grains of Poaceae Species: High Pollen Resolution for Reconstruction of Grasslands Vegetation

Radaeski JN*, Cunha DJ and Bauermann SG

Laboratório de Palinologia da Universidade Luterana do Brasil–ULBRA, Universidade Luterana do Brazil, Brazil

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***Corresponding author:** Radaeski JN, Laboratório de Palinologia da Universidade Luterana do Brasil–ULBRA, Universidade Luterana do Brazil, Brazil, E-mail: jefferson.radaeski@gmail.com

Abstract

This study documents the relationship between the modern diporate Poaceae pollen grains and fossil diporate Poaceae pollen grains of Quaternary in the southern Brazil. Pollen grains were analyzed to know the number of the apertures of the pollen grains of 95 Poaceae species of Rio Grande do Sul region. These 95 species, five showed diporate aperture and we present the morphological descriptions of pollen grains. In the sedimentary samples of Águas Claras region, seven diporate Poaceae pollen grains were identified in all the profile. Diporate Poaceae pollen grains were absent in the basal ages (10.974-2.000 years BP) and increased in frequency from the Late Holocene (2.000 years BP to the present). The comparison between modern-fossil pollen allowed to identify three palynomorphs in the specie level (*Dichantelium sabulorum*) also indicating the metabolism type (C3) and two pollen grains indicated the vegetation type (grasslands). C3 diporate Poaceae species that reflect the occurrence of humid climate have increased the concentration since 2.000 years BP in the pollen record. Furthermore, the humid climate increased progressively until the current climate in the region of Águas Claras. The palynomorphs identified can provide important paleoecological information on the dynamics of Poaceae vegetation in the past.

Keywords: Pollen analysis; Pollen morphology; Late Holocene; Southern Brazil

Introduction

Poaceae pollen grains are easily identified by their monoporate and spheroidal shape morphology. However, the uniform pollen morphology is shown by many Poaceae species and poses problems in the separation of groups among species of the family based on pollen morphology [1,2]. Thus, the identification of the Poaceae pollen grains in the pollen records usually is limited at the family level. Many studies in the last decades have focused on improving the identification of groups of Poaceae pollen grains demonstrating satisfactory progress. These

studies were focused on the morphometric analysis on light microscopy [3-21] or observation of the surfaces of the pollen grains under scanning electron microscopy [22-42]. The use of scanning electron microscopy is difficult for studies of pollen fossil records. Thus, the light microscopy is more appropriate for the analysis of many palynomorphs.

Diporate pollen grains occur in species of many botanical families (eg. Cannabaceae, Moraceae, Acanthaceae, Urticaceae), but the pores in the pollen grains of these species are located in the equatorial region

and are distant [17,43,44]. The pores of the diporate pollen grains of the family Poaceae (Figure 1) are located in the polar region and are very close [5,45,46].

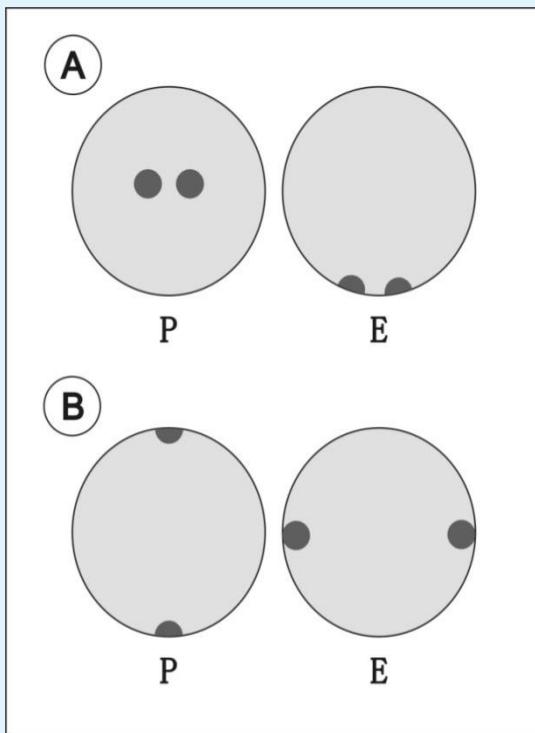


Figure 1: Representation of pollen grains in the family Poaceae (A) and diporate pollen grains of species of the others families (B).

Although the majority of Poaceae species present pollen morphology with monoporate and spheroidal pollen grains, some species present diporate or more apertures [5,21,29,45-49]. The increase in the number of apertures is related to reproduction by apomixis and in cases that sexual reproduction is not viable [45]. As only some species present this pollen morphology (diporate), these may be indicative that these species are easily identified in pollen records. In this sense, as an attempt to refine the taxonomic resolution of Poaceae pollen through diporates Poaceae pollen grains, the present work has the following objectives: (1) Identify in 95 Poaceae species the species that have diporate pollen morphology and select these pollen grains to describe their morphologies – modern pollen grains; (2) Identify diporate Poaceae pollen grains in Quaternary sediment showed the ages in which they occurred in the past – fossil pollen grains; (3) Establish relationship modern pollen – fossil pollen of diporate Poaceae de Poaceae seeking higher pollen taxonomic resolution.

Materials and Methods

Modern Pollen Grains

Pollen material of 95 Poaceae species was collected in the plants in the field and also in plants deposited in the ICN herbarium. All plants collected in the field were pressed, dehydrated and deposited in the "Herbário do Museu de Ciências Naturais" from the Universidade Luterana do Brasil (MCNU/HERULBRA). The pollen material of all species was chemically processed according to the acetolysis methodology proposed by Erdtman (1952) [50] and slides created for each sample using glycerinated jelly were deposited in the Laboratório de Palinologia da ULBRA. Six species showed diporate pollen grains from the 95 samples analyzed (Table 1). These six species were selected for pollen description according to the terminology proposed by Barth and Melhem (1988) [51] and Punt et al. (2007) [52] and measuring 25 grains of pollen. In the pollen grains was measured the diameter of the pollen grain (D), pore diameter (P) and annulus diameter (A).

Species	Aperture	
	Monoporate	Diporate
<i>Agenium villosum</i> (Nees) Pilg.	x	
<i>Agrostis</i> sp. L.	x	
<i>Aira elegantissima</i> Schur	x	
<i>Amphibromus quadridentulus</i> (Döll) Swallen	x	
<i>Andropogon cf. lindmanii</i> Hack.	x	
<i>Andropogon lateralis</i> Nees	x	
<i>Aristida</i> sp. L.	x	
<i>Arundinella hispida</i> (Humb. & Bonpl. ex Willd.) Kuntze	x	
<i>Avena sativa</i> L.	x	
<i>Avena strigosa</i> Schreb.	x	
<i>Axonopus</i> sp. P. Beauv.	x	
<i>Bothriochloa laguroides</i> (DC.) Herter	x	
<i>Bouteloua megapotamica</i> (Spreng.) Kuntze	x	
<i>Briza minor</i> L.	x	
<i>Bromus catharticus</i> Vahl	x	
<i>Calamagrostis viridiflavaescens</i> (Poir.) Steud.	x	
<i>Catapodium rigidum</i> (L.) C.E.Hubb.	x	
<i>Cenchrus clandestinus</i> (Hochst. ex Chiov.) Morrone	x	
<i>Cenchrus equinatus</i> L.	x	
<i>Chascolytrum subaristatum</i> (Lam.)	x	

Desv.		
<i>Chloris canterae</i> Arechav.	x	
<i>Chusquea juergensii</i> Hack.	x	
<i>Colanthelia cingulata</i> (McClure & L.B.Sm.) McClure	x	
<i>Cynodon dactylon</i> (L.) Pers.	x	
<i>Dactylis glomerata</i> L.	x	
<i>Danthonia secundiflora</i> subsp. <i>secundiflora</i> J.Presl	x	
<i>Dichanthelium sabulorum</i> (Lam.) Gould & C.A. Clark var. <i>sabulorum</i>	x	x
<i>Digitaria ciliares</i> (Retz.) Koeler	x	x
<i>Diplachne fusca</i> (L.) P. Beauv.	x	
<i>Echinochloa polystachya</i> (Kunth) Hitchc.	x	x
<i>Eleusine tristachya</i> (Lam.) Lam.	x	
<i>Elionurus candidus</i> (Trin.) Hack.	x	
<i>Eragrostis airoides</i>	x	
<i>Eragrostis bahiensis</i> Schrad. ex Schult.	x	
<i>Eragrostis neesii</i> Trin.	x	
<i>Eragrostis plana</i>	x	
<i>Eriochloa montevidensis</i> Griseb.	x	
<i>Eriochrysis cayennensis</i> P. Beauv.	x	
<i>Eustachys distichophylla</i> (Lag.) Nees	x	
<i>Festuca fimbriata</i> Nees	x	
<i>Glyceria multiflora</i> Steud.	x	
<i>Guadua trinii</i> (Nees) Nees ex Rupr.	x	
<i>Gymnopogon spicatus</i> (Spreng.) Kuntze	x	
<i>Holcus lanatus</i> L.	x	
<i>Hordeum stenostachys</i> Godr.	x	
<i>Hymenachne grumosa</i> (Nees) Zuloaga	x	
<i>Ichnanthus pallens</i> (Sw.) Munro ex Benth.	x	
<i>Imperata brasiliensis</i> Trin.	x	
<i>Ischaemum minus</i> J.Presl	x	
<i>Jarava megapotamica</i> (Spreng.) Peñail	x	
<i>Jarava plumosa</i> (Spreng.) S.W.L. Jacobs & J. Everett	x	
<i>Leersia</i> sp. Sol. ex Sw.	x	
<i>Lithachne pauciflora</i> (Sw.) P.Beauv.	x	
<i>Lolium multiflorum</i> L.	x	
<i>Lolium temulentum</i> L.	x	
<i>Luziola peruviana</i> Juss. ex J.F.Gmel.	x	
<i>Melica</i> sp. L.	x	
<i>Merostachys multiramea</i> Hack.	x	
<i>Microchloa indica</i> (L.f.) P. Beauv.	x	

<i>Muhlenbergia schreberi</i> J.F.Gmel.	x	
<i>Nassella melanosperma</i> (J. Presl) Barkworth	x	
<i>Olyra latifolia</i> L.	x	
<i>Oplismenopsis najada</i> (Hack. & Arechav.) Parodi	x	
<i>Oryza sativa</i> L.	x	
<i>Panicum aquaticum</i> Poir.	x	
<i>Pappophorum philippianum</i> Parodi	x	
<i>Parodiolyra micrantha</i> (Kunth) Davidse & Zuloaga	x	
<i>Paspalum leptum</i> Schult	x	
<i>Paspalum notatum</i> Flüggé	x	
<i>Paspalum pauciciliatum</i> (Parodi) Herter	x	x
<i>Paspalum plicatulum</i> Michx.	x	
<i>Paspalum urvillei</i> Steud.	x	
<i>Phalaris angusta</i> Nees ex Trin.	x	
<i>Pharus lappulaceus</i> Aubl.	x	x
<i>Piptochaetium montevidense</i> (Spreng.) Parodi Rupr.	x	
<i>Poa annua</i> L.	x	
<i>Poa bonariensis</i> (Lam.) Kunth	x	
<i>Polypogon elongatus</i> Kunth	x	
<i>Sacciolepis indica</i> (L.) Chase	x	
<i>Schizachyrium microstachyum</i> (Desv. ex Ham.) Roseng.	x	
<i>Secale cereale</i> L.	x	
<i>Setaria parviflora</i> (Poir.) Kerguélen	x	
<i>Sorghastrum cf.nutans</i> (L.) Nash	x	
<i>Spartina ciliata</i> Brongn	x	
<i>Sporobolus indicus</i> (L.) R.Br.	x	
<i>Steinchisma hians</i> (Elliott) Nash	x	
<i>Stipa setigera</i> J.Presl	x	
<i>Streptochaeta spicata</i> Schrad. ex Nees	x	
<i>Trachypogon filifolius</i> (Hack.) Hitchc.	x	
<i>Tridens brasiliensis</i> (Nees ex Steud.) Parodi	x	
<i>Tripogon spicatus</i> (Nees) Ekman	x	
<i>Triticum</i> sp. L.	x	
<i>Urochloa arrecta</i> (Hack. ex T.Durand & Schinz) Morrone & Zuloaga	x	
<i>Zea mays</i> L.	x	
<i>Zizaniopsis bonariensis</i> (Balansa & Poitr.) Speg.	x	x

Table 1: List of 95 Poaceae species indicating monoporate and diporate apertures in the pollen grains.

Fossil Pollen Grains

Sedimentary material previously analyzed by Bauermann (2003) [53] was selected. The sedimentary material is from Águas Claras region and the sediment has 2,70 m depth and 10.975 C¹⁴ AP of basal age. This sediment was selected for having demonstrated the registration of the whole Holocene and the presence of Poaceae pollen grains is confirmed (Bauermann 2003) [53]. The samples were chemically processed according to the methodology proposed by Faegri and Iversen (1989) [54] and the slides created using glycerinated jelly were deposited in the Laboratório de Palinologia da ULBRA. 60 Poaceae pollen grains were measured [16,18] at intervals of 10 cm, except in the samples with low frequency of Poaceae. In this case, pollen grains were measured until the number of measurements showed stability curve (Figure 2). Pollen grains with diporate aperture were observed and the diameters of the pollen grains, pores and annulus were measured.

Statistical Analysis

From the measurement data of the modern pollen grains the chart Box Plot were created in the Bioestat 5.0 software. The pollen percentage diagram was constructed by applying the information of pollen grains to the Microsoft Excel 2007 program.

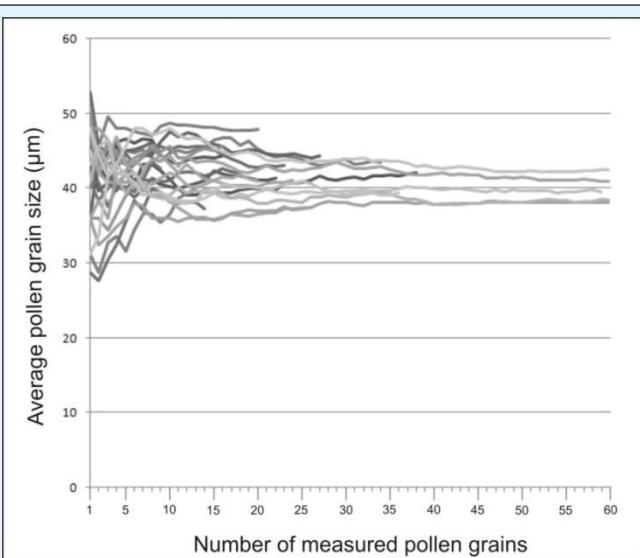


Figure 2: Stability chart of the variation of pollen grains size of the samples in relation to the number of pollen grains measured.

Results

Modern Diporate Poaceae Pollen Grains

Six species presented diporate aperture among the modern pollen grains of the 95 species analyzed for the aperture. These six species with diporate aperture have their pollen description presented along with information on vegetation type, habit, photosynthesis and life cycle according to Boldrini et al. (2008) [55] and Boldrini and Longhi-Wagner (2011) [56]. The pollen descriptions are presented in order of evolution according to Soreng et al. (2015) [57].

Subfamily: Pharoideae

Tribe: Phareae

Pharus lappulaceus Aubl.

(Figure 3A-D)

Pollen data: Monad, small and medium pollen grain, radial symmetry, heteropolar, circular amb, spheroidal, monoporate and diporate, circular pore (3 µm) with annulus located in the distal pole, annulus (8 µm) with 2,5 µm of thickness and ornamentation on the surface, tectate exine with columellae and microequinate ornamentation. Sexine with 0,54 µm and nexine of the same thickness. D: 25 µm (23-27); Ex: 1,08 µm.

Botanical informations: Forest specie, herbaceous habit, C3 metabolism, estival life cycle.

Subfamily: Oryzoideae

Tribe: Oryzeae

Zizaniopsis bonariensis (Balansa & Poitr.) Speg.

(Figure 3E-H)

Pollen data: Monad, medium pollen grain, radial symmetry, heteropolar, circular amb, spheroidal, monoporate and diporate, circular pore (4 µm) with annulus located in the distal pole, annulus (12 µm) with 4 µm of thickness and ornamentation on the surface, tectate exine with columellae and microequinate ornamentation. Sexine with 0,5 µm and nexine of the same thickness. D: 45 µm (42-48); Ex: 1 µm.

Botanical informations: Wetland specie, herbaceous habit, C3 metabolism, estival life cycle.

Subfamily: Panicoideae

Tribe: Paniceae

Dichanthelium sabulorum (Lam.) Gould & C.A. Clark var. *sabulorum*

(Figure 3I-L)

Pollen data: Monad, medium pollen grain, radial symmetry, heteropolar, circular amb, spheroidal, monoporate and diporate, circular pore (3 µm) with annulus located in the distal pole, annulus (9 µm) with 3 µm of thickness and ornamentation on the surface, tectate exine with columellae and microequinate ornamentation. Sexine with 0,52 µm and nexine of the same thickness. D: 34 µm (28-39); Ex: 1,04 µm.

Botanical informations: Grassland specie, herbaceous habit, C3 metabolism, estival life cycle.

Digitaria ciliares (Retz.) Koeler
(Figure 3M-P)

Pollen data: Monad, medium pollen grain, radial symmetry, heteropolar, circular amb, spheroidal, monoporate and diporate, circular pore (3 µm) with annulus located in the distal pole, annulus (9 µm) with 3 µm of thickness and ornamentation on the surface, tectate exine with columellae and microequinate ornamentation. Sexine with 0,52 µm and nexine of the same thickness. D: 37 µm (34-40); Ex: 1,04 µm.

Botanical informations: Grassland specie, herbaceous habit, C4 metabolism, estival life cycle.

Echinochloa polystachya (Kunth) Hitchc.
(Figure 3Q-T)

Pollen data: Monad, medium pollen grain, radial symmetry, heteropolar, circular amb, spheroidal, monoporate and diporate, circular pore (3 µm) with annulus located in the distal pole, annulus (9 µm) with 3 µm of thickness and ornamentation on the surface, tectate exine with columellae and microequinate ornamentation. Sexine with 0,5 µm and nexine of the same thickness. D: 40 µm (35-44); Ex: 1 µm.

Botanical informations: Grassland specie, herbaceous habit, C4 metabolism, estival life cycle.

Tribe: Paspaleae

Paspalum pauciciliatum (Parodi) Herter
(Figure 3U-X)

Pollen data: Monad, medium pollen grain, radial symmetry, heteropolar, circular amb, spheroidal, monoporate and diporate, circular pore (3 µm) with annulus located in the distal pole, annulus (8 µm) with 2,5 µm of thickness and ornamentation on the surface, tectate

exine with columellae and microequinate ornamentation. Sexine with 0,52 µm and nexine of the same thickness. D: 42 µm (37-46); Ex: 1,04 µm.

Botanical informations: Grassland specie, herbaceous habit, C4 metabolism, estival life cycle.

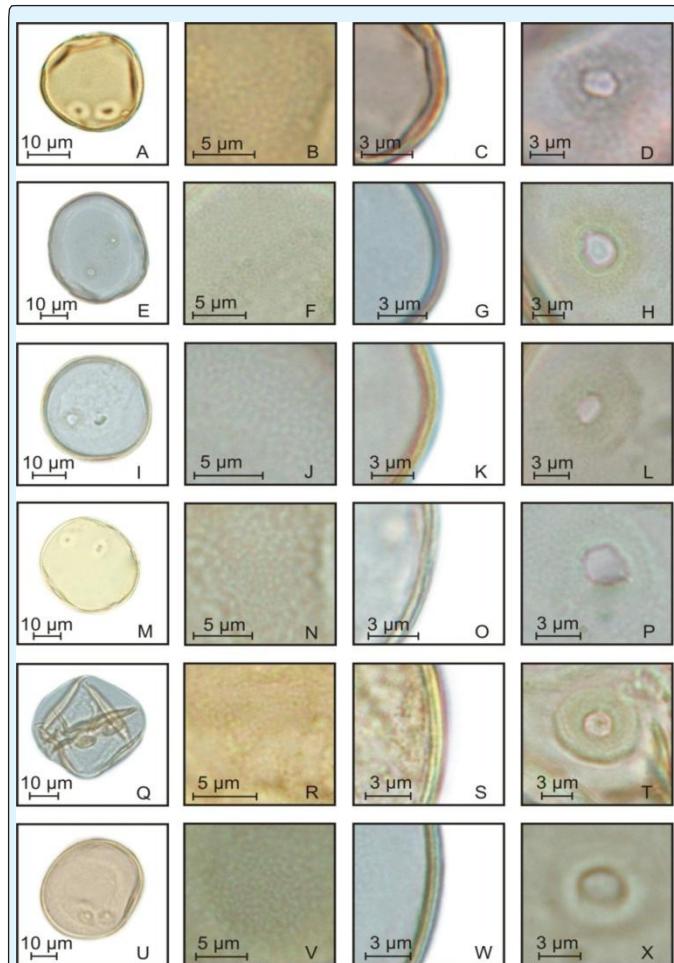


Figure 3: Modern diporate pollen grains of Poaceae species of the Rio Grande do Sul. Diporate pollen grain (A), detail of ornamentation (B), detail of exine (C) and detail of pore (D) of the *Pharus lappulaceus*; Diporate pollen grain (E), detail of ornamentation (F), detail of exine (G) and detail of pore (H) of the *Zizaniopsis bonariensis*; Diporate pollen grain (I), detail of ornamentation (J), detail of exine (K) and detail of pore (L) of the *Dichanthelium sabulorum* var. *sabulorum*; Diporate pollen grain (M), detail of ornamentation (N), detail of exine (O) and detail of pore (P) of the *Digitaria ciliares*; Diporate pollen grain (Q), detail of ornamentation (R), detail of exine (S) and detail of pore (T) of the *Echinochloa polystachya*; Diporate pollen grain (U), detail

of ornamentation (V), detail of exine (W) and detail of pore (X) of the *Paspalum pauciciliatum*.

All Poaceae species with diporate pollen grains have herbaceous habit and estival life cycle. Only *Pharus lappulaceus* is forest specie and *Zizaniopsis bonariensis* is wetland specie, the others species are from grasslands. The smaller size of the pollen grains of *Pharus lappulaceus* indicates which palynomorphs are of herbaceous forest species. On the other hand, the larger size of the pollen

grains of *Zizaniopsis bonariensis* Indicates occurrence of wetlands, while pollen grain sizes between 28-41 µm indicate grassland vegetation (Figure 4).

The exact distinction of pollen grains from C3 and C4 metabolism was not possible. The data showed that C3 species have a larger amplitude range of pollen size than C4 species and that the limits of size variation of C3 species can be used as markers of metabolism type.

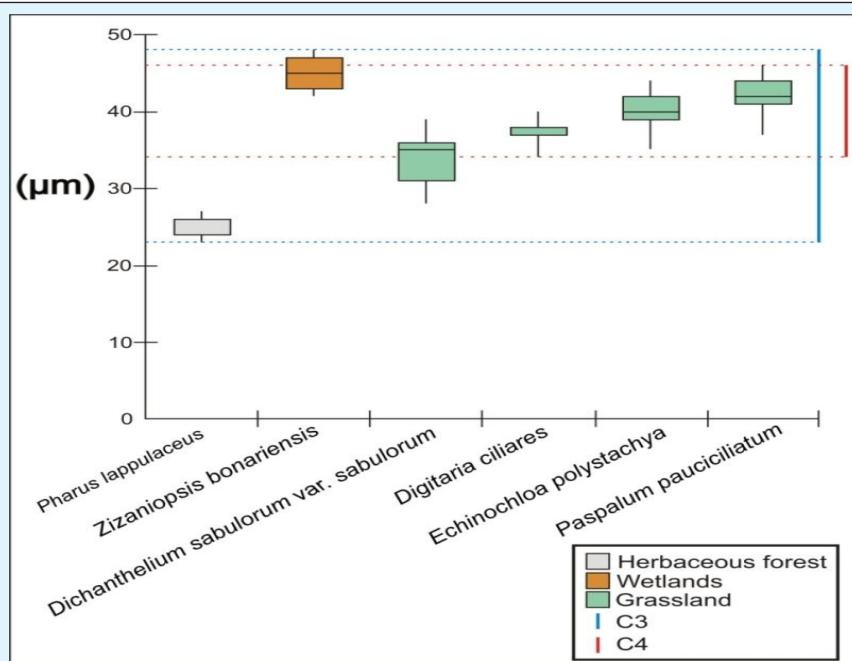


Figure 4: Chart box plot of the diameters of pollen grains of Poaceae species with diporate pollen in Rio Grande do Sul. The bold horizontal line within the box represents the median. The box shows 50% of the interquartile range, and whiskers the total variation.

Fossil Diporate Poaceae Pollen Grains

Seven fossil diporate pollen grains of Poaceae species were found (Figure 5) in the all the sediment. The pollen grains were found in recent ages related to the Late Holocene (3.420 years BP to the present). The samples showed absence of diporate Poaceae pollen grains in the lower portion of the profile between the depths 0,95-2,70 m (Table 2). There is an increase in the concentration of diporate pollen grains at more recent ages (from 1.340 years BP to the present). Some pollen grains could be

identified at the specie level (*Dichantelium sabulorum* var. *sabulorum*) and others indicated the type of environment (five pollen grains are from the grassland vegetation) and the type of photosynthesis (three pollen grains are from species with C3 metabolism). Diporate pollen grains from forest vegetation were not found. Two pollen grains could not be identified beyond the family level because their sizes are similar to the grassland and wetland species (Figure 6).

Depth	Pollen grain size	Vegetation	Metabolism	Pollen type
0,05 m	28,8 µm	Grassland	C3	<i>Dichantelium sabulorum</i>
0,05 m	31,2 µm	Grassland	C3	<i>Dichantelium sabulorum</i>
0,05 m	43,2 µm	Grassland/Wetland	C3/C4	Poaceae

0,15 m	43,2 μm	Grassland/Wetland	C3/C4	Poaceae
0,35 m	36 μm	Grassland	C3/C4	Grassland
0,65 m	36 μm	Grassland	C3/C4	Grassland
0,85 m	33,6 μm	Grassland	C3	<i>Dichantelium sabulorum</i>

Table 2: Information on the depths and ages of the samples where diporate Poaceae pollen grains were found.

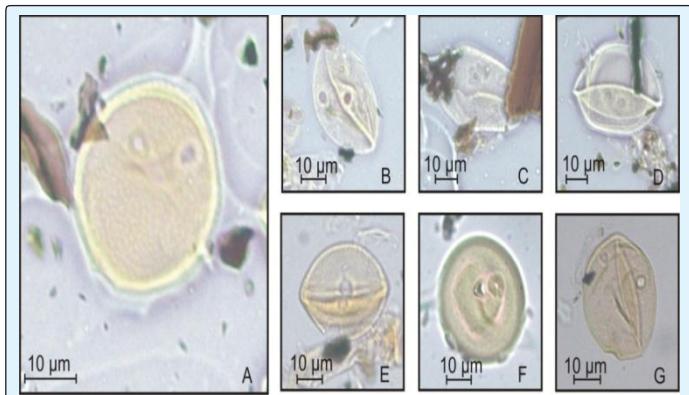


Figure 5: Fossil diporate Poaceae pollen grains from the sediment of Águas Claras region, Rio Grande do Sul.

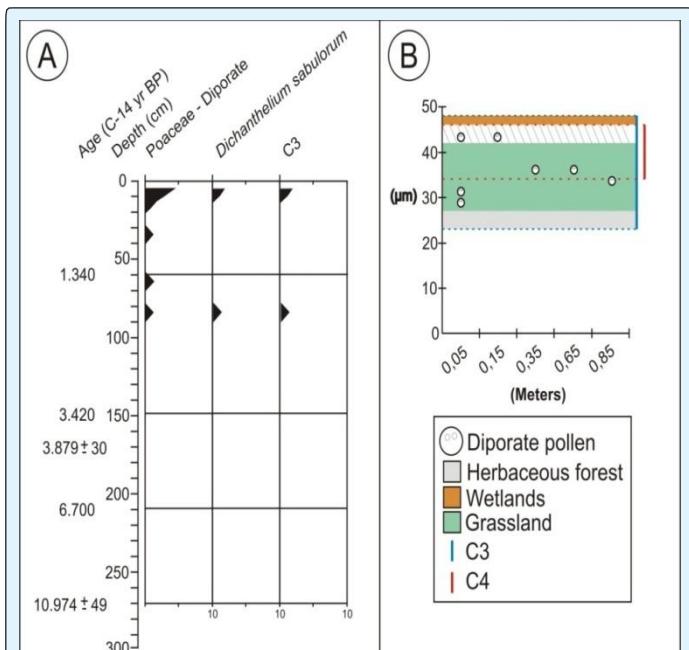


Figure 6: Pollen percentage diagram of Poaceae diporate pollen grains showing the ages and depths in which they were recorded in the profile of Águas Claras region (A). Chart of the sizes of the fossil diporate pollen grains together with the ecological information obtained by modern pollen grains (B). “\\”Overlap between pollen grain sizes of grassland and wetland species.

Discussion

Diporate pollen grains from Poaceae species had not been recorded in South America Quaternary sediments. On the other hand, some modern diporate pollen grains have been described for several regions of the world, including South America [5, 6, 30].

Some species of the genus *Agrostis*, *Bromus*, *Calamagrostis*, *Chusquea* and *Melinis* showed diporate aperture in the pollen grains described in Venezuela [5]. However, the species of these genera that we analyzed showed pollen grains with only one aperture. The diporate aperture found in *Pharus lappulaceus* in Southern Brazil was not shown in the same species described in Venezuela [5]. However, the size variation of the pollen grains of this specie in the present work is similar to the sizes described for Venezuela and São Paulo – Brazil [5,30]. *Dichanthelium sabulorum* was analyzed by Côrrea et al. (2005) [30] and showed pollen grain size similar to the present study, but the type of diporate aperture that we find in the southern Brazilian specie is not described for the specimen of São Paulo.

The species *Cenchrus brownii*, *Digitaria ciliata*, *Ischaemum rugosum*, *Paspalum conjugatum* and *Sporobolus indicus* described by Roubik & Moreno (1991) [6] showed diporate pollen grains. In our study, *Digitaria ciliata* also demonstrated diporate aperture although the size of the pollen grain that we observed is smaller than the size of pollen described in Barro Colorado. Furthermore, *Sporobolus indicus* showed only monoporate aperture in the specimen of Rio Grande do Sul. Pollen grains of some species of *Paspalum* genus, including *Paspalum urvillei*, showed diporate aperture according to Zucol (1998) [48]. However, diporate pollen grains were not observed in *Paspalum urvillei* that we analyze.

Paleoecological Implications

According to Bauermann (2003) [53] in the Águas Claras region, forest vegetation existed in higher distribution than Grassland vegetation in the period of 3.420-1.340 BP. In our analysis of pollen grains from the

profile showed that *Pharus lappulaceus* (herbaceous forest species) not lived in this vegetation. In this period, the record of species of the Atlantic Forest indicates occurrence of warm and humid climate conditions [53]. The pollen of C3 Poaceae that we have identified for this period confirms for the occurrence of humid climate.

The forest vegetation also expanded on the grasslands in the period between 1.340 BP to the present indicating that the Restinga forest occurred in mosaic with the grassland vegetation established in the drier areas [53]. The grassland vegetation that occurred in mosaic is confirmed by the diporate Poaceae pollen grains. These diporate pollen grains indicate the occurrence of grassland vegetation even though the forest vegetation has dominated. *Dichanthelium sabulorum* probably was part of this grassland vegetation since 2.000 BP. This species lives in open formations, in the natural grasslands generally forming rosetted clumps [55,56]. The increase of diporate pollen of C3 grasses in this period also reinforces that humid climate occurred as the current climate as verified by Bauermann (2003) [53].

Conclusion

The current vegetation of the Águas Claras region has C3 and C4 Poaceae species living together. The record of diporate pollen grains C3 and C4 evidences this vegetative distribution since the Late Holocene (1.340 years BP). C3 diporate Poaceae species that reflect the occurrence of humid climate have increased the concentration since 2.000 years BP. This increase is probably related to the greater humidity in the Coastal Plain of Rio Grande do Sul and in several other regions of the State where the Campos replaced by the forests in this period. Diporate pollen grains of wetlands/grasslands were found at more recent ages of the profile indicating that the humidity increased progressively until the current climate in the region of Águas Claras.

Modern diporate pollen grains of six species of the dataset (95 Poaceae species analyzed for aperture) allowed higher pollen taxonomic resolution. Statistical tests applied to modern pollen grains indicated potential information for use in fossil records. Pollen types for ecological inferences could be characterized from the differences in pollen grain sizes among forest, grassland and wetland species.

The quaternary sediment analyzed records the entire Holocene. However, the seven fossil diporate pollen grains identified were present only in samples that reflect

the Late Holocene. Three of the seven pollen grains were identified at the species level, also indicate the type of photosynthesis. Furthermore, two pollen grains suggest the type of vegetation. The integration of modern-fossil pollen data was shown to be appropriate to increase pollen refinement in the Poaceae family. Despite the low preservation of diporate pollen grains, this study provides important information to obtain pollen taxonomic resolution of Poaceae species. The data obtained by this high pollen taxonomic resolution increase paleoecological inferences about the dynamics of vegetation in the past.

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