Pollen and spores from surface samples in the *campos* region of Uruguay and their paleoecological implications

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ABSTRACT

In this study we describe and illustrate pollen and spores that have been identified as significant in modern and fossil samples from the *campos* region of Uruguay. We provide new information about modern pollen assemblages and their relationship to the vegetation types of this region. We discuss the taxonomic limitations of pollen and spores and their representation in both modern and fossil samples. We highlight the importance of identifying both silent and key indicator taxa for making accurate paleoecological interpretations. We also emphasize the importance of knowing the pollination strategies of parental plants because many of them are zoophilous, and so small changes in the proportions of their pollen grains in fossil assemblages could reflect important changes in vegetation. This study presents a practical approach to paleoecological research, which not only produces robust results in studies of the *campos* region, but can be applied to other grassland ecosystems, including those in temperate regions.

Keywords: Holocene sequences, key indicator taxa, modern pollen-vegetation relationships, pollen and spores descriptions, silent taxa

Introduction

Pollen analysis, the study of fossil pollen and spores, is the principal technique for long-term vegetation reconstructions (Seppä 2007), and it has provided fundamental advances in the understanding of the processes and mechanisms of ecological change (e.g. Jackson 2007; Rull 2010; Willis *et al*. 2010). However, pollen records do not directly reflect plant abundance due to several factors such as differences in pollen production, dispersal and preservation of taxa (Prentice 1988), which cause that taxa may be either over-represented or under-represented in pollen samples. Species with anemophilous pollination, which produce high quantities of pollen grains, are frequently over-represented in pollen assemblages while zoophilous, with low pollen productivity, are under-represented (Fægri & Iversen 1989). Palynologist must work with an incomplete representation of taxa because many floristically important families contribute little or no pollen to pollen rain (Bush *et al*. 2001).

One of the principles on which pollen analysis is based is the possibility of identifying different pollen and spores according to their morphology (Fægri & Iversen 1989). There are some difficulties in studying modern pollen-vegetation relationships in Río de la Plata grasslands (RPG) (Southern South America) related to the limited pollen taxonomic resolution of their most abundant parental plants, such as Poaceae, Cyperaceae, Asteraceae subf. Asteroideae and some genera of the Apiaceae family (Tonello & Prieto 2008; Mourelle & Prieto 2012). However, there are a great number

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of minor taxa that allow the qualitative and quantitative analysis of pollen-vegetation relationships.

In particular, palynological analyses in the *campos* region of Uruguay revealed that pollen and spores in surface samples (Mourelle & Prieto 2012) and Holocene records (Iriarte 2006; García-Rodríguez *et al*. 2010; Mourelle *et al*. 2015a; b) are very diverse. However, even though several pollen and spores atlas (e.g. Bauermann *et al*. 2013; Radaeski *et al*. 2014) and catalogues (e.g. Neves & Bauermann 2004; Macedo *et al*. 2009; Evaldt *et al*. 2013; Masetto & Lorscheitter 2014) in the *campos* region of Rio Grande do Sul have been published during the last decades, there are no morphological descriptions and illustrations for the most frequent and abundant pollen and spores that appeared in surface and fossil samples from the *campos* region of Uruguay.

For this reason we propose the following aims in this study: (1) to describe and illustrate the main pollen and spores from surface samples from the *campos* region of Uruguay; (2) to provide new information about modern pollen assemblages and their relationship to the vegetation types of this region; and (3) to discuss the taxonomic limitations of pollen and spores and their representation in both modern and fossil samples, and provide a practical approach to obtaining robust results for paleoecological research in the *campos* region.

Study area

RPG extend between 28° and 38°S latitude, covering approximately 700,000 km² of eastern Argentina, Uruguay and southern Rio Grande do Sul (Brazil) (Fig. 1) and is characterized by the dominance of grasses and other herbs. Based on geomorphological, hydrological and edaphic features and their link with natural vegetation and land use, the RPG were divided by León (1991) into the *pampas* in the eastern Argentina, and the *campos* in Uruguay and southern Rio Grande do Sul (Brazil). The presence of woody species and grasses with C4 metabolism gradually diminishes from north to south, one of the facts that allowed the division of this sub-region in southern and northern *campos* (León 1991) (Fig. 1). Most of these shrubby and arboreal species in Uruguay reach their southern distribution range and so they are absent in the *pampas*. These RPG are climatically determined (Bilenca & Miñarro 2004). The climate is temperate, with less extreme conditions than those in other regions located at similar latitudes due to the Atlantic Ocean moderating effect (Soriano 1991).

Natural grasslands are the predominant vegetation type of the *campos* region (Fig. 1) and are extended all over Uruguay (ca. 77% of the territory), in flat and gently-rolling relief, hills, gentle hills and low mountains (Puerto
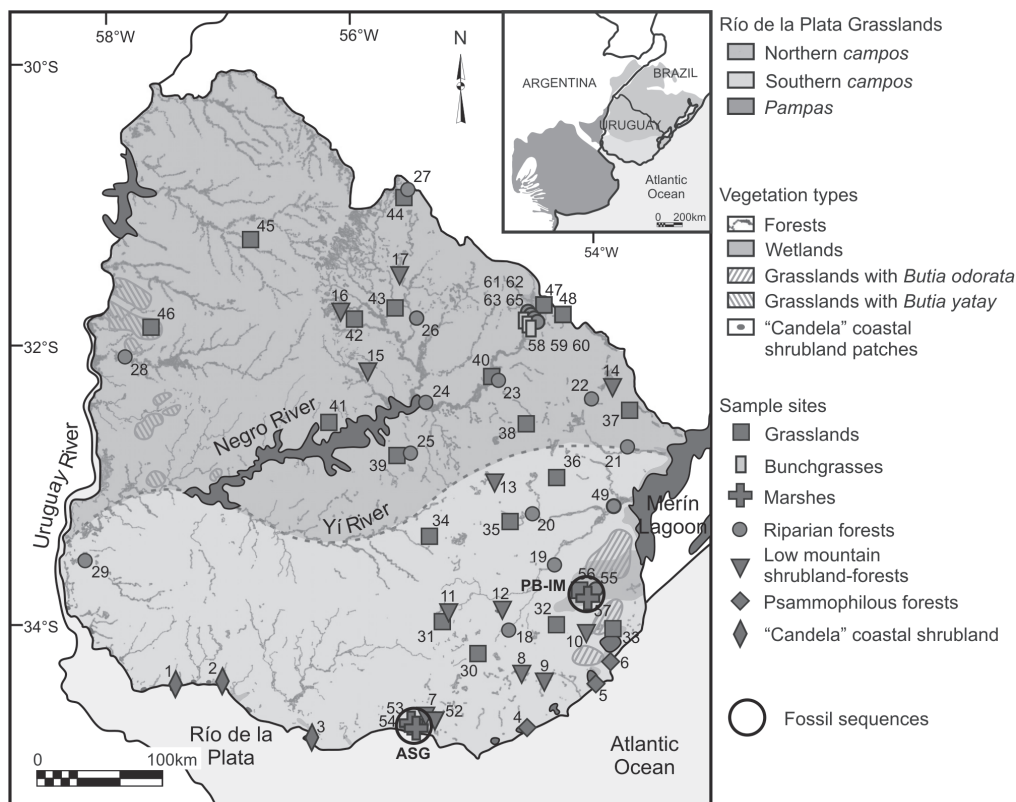
Figure 1. Uruguay map showing the main vegetation types and the location of the 62 surface samples (modified from Mourelle & Prieto 2012) and the Holocene sequences (ASG, Arroyo Solís Grande, Mourelle *et al*. 2015a; PB-IM, Paso Barranca-India Muerta, Mourelle *et al*. 2015b). The map scale does not allow the representation of bunchgrasses. Inset map: Río de la Plata grasslands (León 1991).

1987; Lezama *et al.* 2011) (Tab. 1). Woody vegetation covers approximately 4.3% of the territory (Petraglia & Dell'Acqua 2006) and is composed of about 300 species of trees and shrubs, characteristic elements of forests and shrublands, respectively (Brussa & Grela 2007; Haretche *et al.* 2012). Forests, shrublands and other herbs-dominated vegetation (not grasslands) develop locally in environments with particular edaphic conditions that define each different vegetation type (Tab. 1).

After the European settlement, the *campos* region of Uruguay has progressively become one of the most important areas of livestock and grain production in the world (Bilenca & Miñarro 2004). Furthermore, the expansion of cropland areas, the introduction of exotic grass species and their associated weeds, and more recently the substitution of grasslands for exotic forest plantations, deeply modified the original landscape.

Materials and methods

Selection of pollen and spores taxa

Described and illustrated taxa were selected from 62 surface samples from the *campos* region of Uruguay based upon two criteria: (1) their abundance and frequency in surface samples and (2) their occurrence in fossil records previously published (Mourelle *et al.* 2015a; b) (Fig. 1). A total of 46 surface samples were previously studied (Mourelle & Prieto 2012) and 16 further samples were collected and incorporated in this study (additional data are provided in Mourelle 2015). All samples were collected following Adam & Mehringer (1975) sampling strategy and they correspond to seven different vegetation types: grasslands, riparian forests, low-mountain shrublands-forests; psammophilous forests, "Candela" coastal shrublands, bunchgrasses and marshes (Fig. 1). The sample sites are principally located in the eastern part of Uruguay because: (1) it concentrates a larger number of different vegetation types than the western part; (2) it has minor human disturbance; and (3) it is where the fossil pollen sequences studied are located (Fig. 1).

Laboratory work

Surface samples were dried at 60°C for 24 h. Subsamples of 5 to 15g dry sediment were used for pollen analysis. Sediment samples were sieved trough a 110 mm screen to remove coarse particles and then processed according to standard palynological techniques including warm KOH, HCl, heavy-liquid separation with $ZnCl_2$, HF and acetolysis (Fægri & Iversen 1989).

Pollen and spores descriptions

Standard morphological descriptions of palynomorphs

(pollen and spores) follow Punt *et al.* (2007) and Hesse *et al.* (2009). Pollen shape classes are described according to ratios of their polar and equatorial axes (P:E ratios) in agreement with Erdtman (1971). Descriptions were checked using the pollen and spores reference collection of the Laboratory of Palaeoecology and Palynology, Universidad Nacional de Mar del Plata, Argentina. Identifications were supplemented by published atlas and photographs (Markgraf & D'Antoni 1978; Prieto & Quattrocchio 1993; Pire *et al.* 1998; 2001; 2006; Bauermann *et al.* 2013).

Under the light microscope, at least 20 pollen grains or spores per each taxonomic entity were measured to elaborate each morphological description, using a 100x oil immersion objective. Measurements were made using an ocular micrometer in the microscope. All images were taken by a Nikon Coolpix P3 digital camera. Pollen slide preparations have been deposited in the palynological collection at the Laboratory of Paleoecology and Palynology, Universidad Nacional de Mar del Plata, Argentina.

For pollen and spores nomenclature we followed some conventions recommended by Birks & Birks (1980): (1) if pollen or spores types are known to be produced by only one species they are named after this species (e.g. PHYLLANTHUS SELLOWIANUS); (2) if a complete family produces pollen or spores that cannot be morphologically distinguished, the type is named after the family (e.g. POACEAE); (3) if the pollen or spores morphological category is known to be produced by two taxa, then it is named after these two taxa (e.g. LITHRAEA/SCHINUS); (4) if three or more plant taxonomical entities are known to produce the same morphological pollen or spore type, the suffix "type" is added after the taxonomical name (e.g. ALTERNANTHERA-TYPE). Since there are significant differences between the concepts of "plant taxa" and "palynomorphological types" and in order to provide the necessary clarity on the nomenclature, names of pollen and spores were put in SMALL CAPITALS and refrain from using *italics* (Klerk & Joosten 2007).

Pollen were classified according to the classification system APG III (2009) and spores in accordance with the Missouri Botanical Garden (MOBOT 2016). The palynological terminology follows Punt *et al.* (2007) and Hesse *et al.* (2009).

On the other hand, pollen and spores from surface samples were counted using a microscope with 1000x magnification. A minimum of 300 pollen grains were counted, including pollen from herbs and aquatic herbs (herbaceous pollen), shrubs (shrubby pollen), trees (arboreal pollen) and climbers, and they represent the total pollen sum. Due to the over-representation of POACEAE in grassland samples, 300 pollen grains excluding POACEAE were counted in order to obtain a good representation of the subordinated taxa. Pteridophytes and bryophytes were counted and expressed as percentages of the total palynomorphs sum. Palynomorph percentages were calculated and diagrams were drawn using TGView 2.0.4 program (Grimm 2004).

Table 1. Description of the vegetation types (Chebataroff 1960; Puerto 1969; 1987; León 1991; Alonso-Paz & Bassagoda 1999; 2002; Fagúndez & Lezama 2005; Brussa & Grela 2007; Lezama *et al.* 2011) and the characteristic pollen and spores from modern surface samples in the *campos* region of Uruguay.

Vegetation type	Habitat	Vegetation frequent taxa	Surface samples: characteristic taxa
REGIONAL			
Grasslands	Flat and gently-rolling relief, hills, gentle hills and low mountains	*Stipa* spp., *Aristida* spp., *Andropogon* spp., *Briza* spp., *Erianthus* spp., *Piptochaetium* spp., *Paspalum* spp., *Axonopus* spp., *Panicum* spp. (Poaceae), *Carex* spp., *Cyperus* spp., *Eleocharis* spp., *Kyllinga* spp., *Bulbostylis* spp. (Cyperaceae), *Baccharis* spp., *Eupatorium* spp. (Asteraceae), *Eryngium* spp. (Apiaceae), *Trifolium polymorphum*, *Adesmia bicolor* (Fabaceae), *Dichondra sericea* (Convolvulaceae), *Oxalis* spp. (Oxalidaceae), *Glandularia* spp. (Verbenaceae). *Butia odorata* and *Butia yatay* (Arecaceae) in some grasslands.	POACEAE, ASTERACEAE SUBF. ASTEROIDEAE, CYPERACEAE, APIACEAE, MONOCOTYLEDONEAE. POACEAE and BUTIA in grassland samples with *Butia* palm individuals. PHAEOCEROS, ANTHOCEROS, ISOETES, OPHIOGLOSSUM, RICCIACEAE.
LOCAL			
Marshes	Freshwater: frequently inland, in soils with permanent high groundwater	Emergent herbs: *Echinodorus grandiflorus*, *Sagittaria montevidensis* (Alismataceae), *Canna glauca* (Cannaceae), *Schoenoplectus californicus*, *Scirpus giganteus* (Cyperaceae), *Thalia geniculata*, *T. multiflora* (Marantaceae), *Typha domingensis*, *Zizaniopsis bonariensis* (Poaceae). Floating herbs: *Pistia stratiotes*, *Lemna* spp. (Araceae), *Ludwigia peploides* (Onagraceae), *Azolla* spp., *Salvinia* spp. (Salviniaceae). Submerged herbs: *Myriophyllum* spp. (Haloragaceae), *Potamogeton* spp. (Potamogetonaceae).	POACEAE, CYPERACEAE, ASTERACEAE SUBF. ASTEROIDEAE.
	Brackish: at the lower reaches of major rivers that flow into the Atlantic Ocean and the Río de la Plata estuary	*Juncus acutus* (Juncaceae), *Spartina densiflora* (Poaceae).	
Bunchgrasses	Grassland-forest or grassland-marsh boundary	*Cortaderia selloana*, *Erianthus angustifolius*, *Panicum prionitis*, *Paspalum quadrifarium* (Poaceae).	POACEAE APIACEAE ASTERACEAE SUBF. ASTEROIDEAE CYPERACEAE.
Riparian forests	Margin of rivers and water streams	Hydrophilous trees: *Sebastiania commersoniana* (Euphorbiaceae), *Erythrina crista-galli* (Fabaceae), *Phyllanthus sellowianus* (Phyllanthaceae), *Cephalanthus glabratus* (Rubiaceae), *Salix chilensis* (Salicaceae), *Acanthosyris spinescens* (Santalaceae), *Pouteria salicifolia* (Sapotaceae). Mesophilous trees: *Lithraea molleoides*, *Schinus longifolia* (Anacardiaceae), *Syagrus romanzoffiana* (Arecaceae), *Celtis ehrenbergiana* (Cannabaceae), *Ocotea acutifolia*, *O. pulchella* (Lauraceae), *Myrsine laetevirens* (Primulaceae), *Blepharocalyx salicifolius*, *Myrceugenia glaucescens*, *Myrcianthes cisplatensis* (Myrtaceae), *Scutia buxifolia* (Rhamnaceae), *Acanthosyris spinescens* (Santalaceae), *Allophylus edulis* (Sapindaceae).	SEBASTIANIA, SALIX CHILENSIS, PHYLLANTHUS SELLOWIANUS, MYRTACEAE, CEPHALANTHUS GLABRATUS, MYRSINE, MYRIOPHYLLUM, POLYGONUM, NYMPHOIDES INDICA. PHAEOCEROS, ISOETES, POLYPODIACEAE.
Low mountain shrublands-forests	Hills, rocky slopes	Trees: *Lithraea molleoides*, *Schinus lentiscifolia*, *S. longifolia*, *S. molle* (Anacardiaceae), *Celtis ehrenbergiana* (Cannabaceae), *Myrsine coriacea*, *M. laetevirens* (Primulaceae), *Blepharocalyx salicifolius*, *Myrcianthes cisplatensis* (Myrtaceae), *Scutia buxifolia* (Rhamnaceae). Shrubs: *Baccharis* spp., *Heterothalamus alienus* (Asteraceae), *Croton* spp. (Euphorbiaceae), *Mimosa* spp. (Fabaceae), *Colletia paradoxa* (Rhamnaceae), *Dodonaea viscosa* (Sapindaceae), *Daphnopsis racemosa* (Thymelaeaceae).	ASTERACEAE SUBF. ASTEROIDEAE, POACEAE, LITHRAEA/SCHINUS, MYRTACEAE. MYRSINE and RHAMNACEAE in some samples. PHAEOCEROS.
Psammophilous forests	Sandy soils in coastal zones	Trees: *Lithraea molleoides*, *S. lentiscifolia*, *S. longifolia*, *S. molle* (Anacardiaceae), *Celtis ehrenbergiana* (Cannabaceae), *Myrsine coriacea*, *M. laetevirens* (Primulaceae), *Scutia buxifolia* (Rhamnaceae). Shrubs: *Baccharis* spp., *Heterothalamus alienus* (Asteraceae), *Croton* spp. (Euphorbiaceae), *Mimosa* spp. (Fabaceae), *Colletia paradoxa* (Rhamnaceae), *Dodonaea viscosa* (Sapindaceae), *Daphnopsis racemosa* (Thymelaeaceae).	POACEAE, CYPERACEAE, LITHRAEA/SCHINUS, MYRSINE, RHAMNACEAE, EPHEDRA TRIANDRA, TRIPODANTHUS ACUTIFOLIUS.
"Candela" coastal shrublands	Mobile and semi-fixed dunes in coastal zones	*Dodonaea viscosa* (Sapindaceae), *Baccharis* spp., *Eupatorium buniifolium* (Asteraceae).	DODONAEA VISCOSA, POACEAE, ASTERACEAE SUBF. ASTEROIDEAE.

Numerical analyses

Numerical analyses were performed in order to determine whether the vegetation types are associated with characteristic pollen spectra from which the parent vegetation can be identified. Pollen types were selected if the mean value of each type in percentage was higher than 2% in at least one sample (spores were excluded due to their local over-representation). The percentage data were square root transformed prior to numerical analyses in order to stabilize their variances. Pollen samples were classified into groups using Cluster Analysis unconstrained (Birks & Gordon 1985) with Euclidean distance (Overpeck *et al.* 1985), performed with TGView 2.0.4 program (Grimm 2004).

In order to evaluate the woody pollen dispersal from riparian forests to grasslands, seventeen grassland surface samples were analyzed considering (1) their distance to the riparian forests and (2) their woody pollen content. Grassland samples were selected only if a riparian forest represented the closest vegetation type. Woody pollen dispersal was assessed by plotting woody pollen content in grassland surface samples (as percentages) versus the distance to the nearest riparian forest (in meters). The power trend line has been added to the scatter plot.

Results

From the 62 surface samples, the total palynomorph sum reached a total of 86 pollen and spores related to the natural vegetation of the *campos* region of Uruguay. Only 35 pollen grains and 8 spores were the most representative in terms of abundance and frequency (Figs. 1, 2; Tab. 1). They are described in Tables 2 and 3 and illustrated in figures 3, 4 and 5.

Unconstrained Cluster Analysis shows distinctive palynological assemblages which characterized the main vegetation types of the *campos* region of Uruguay (Fig. 6). Seven vegetation types in tree groups of pollen assemblages were identified. Group I represents grasslands (Ia), bunchgrasses (Ib) and marshes (Ic). Group II comprises samples from "Candela" (*Dodonaea viscosa*) coastal shrublands (IIa) and from low-mountain shrublands-forests and psammophilous forests (IIb). Group III represents riparian forests.

The power trend line clearly demonstrates that the woody pollen from riparian forests shows very low dispersion capacity to grasslands. R-squared value of 0.86 indicates a very good fitness of the curve to the data set (Fig. 7).

The 43 palynomorph from surface samples described in this study were also the most abundant and frequent in the Arroyo Solís Grande (ASG) and Paso Barranca-India Muerta (PB-IM) fossil sequences (Mourelle *et al.* 2015a; b) (Fig. 8). They represent 52% and 58% of the pollen types that compose the total palynomorphs identified in ASG

and PB-IM sequences, respectively (additional data are provided in Mourelle 2015).

Discussion

The importance of these pollen and spores, in both modern and fossil samples, is discussed below and represents the basis of this study, as well as it provides a practical approach to obtaining reliable results.

Modern pollen-vegetation relationship

Pollen analyses from modern surface samples revealed the good relation between modern pollen assemblages and the principal vegetation types of the *campos* region of Uruguay, mainly related to either the dominance of some taxa (e.g. Poaceae in grasslands; Dodonaea viscosa in "Candela" coastal shrublands) or to the presence of key indicator taxa (e.g. hydrophilous woody taxa in riparian forests) (Figs. 2, 6). However, the low taxonomic resolution of some pollen taxa (e.g. Asteraceae subf. Asteroideae, Fig. 5U-V; Myrtaceae, Fig. 5A; Rhamnaceae, Fig. 4V) and the absence of pollen from some species that develop exclusively in some vegetation types (e.g. *Sideroxylon obtusifolium*, *Rollinia maritima* and *Varronia curassavica* in psammophilous forests; *Juncus acutus* in brackish marshes) make it difficult to differentiate some vegetation types only by the palynological assemblages. As a consequence, a loss of ecological information is expected.

Grassland pollen assemblages are dominated by herbs (ca. 80%; Group Ia, Fig. 6) while riparian forests are dominated by woody pollen taxa (ca. 70%; Group III, Fig. 6). Because riparian forests are dense, pollen dispersed from grasslands cannot easily enter into these forests. Consequently, grasslands pollen signal, regionally dominant, is under-represented in the pollen spectra from samples taken inside the riparian forests, although they consist mostly of trees and shrubs with zoophilous pollination (Tab. 3). Since other forests and scrublands of the *campos* region are less dense, the grassland regional pollen signal is better represented in such pollen assemblages, which are co-dominated by herbs, trees and shrubs (Group II, Fig. 6).

Grasslands

Poaceae (Fig. 4F) and Cyperaceae (Fig. 4E) dominate grassland pollen assemblages (Fig. 2; Tab. 1). Both families have anemophilous pollination and they represent the most abundant taxa in this vegetation type such as *Stipa*, *Aristida*, *Paspalum*, *Piptochaetium* and *Briza*, and *Carex*, *Cyperus*, *Eleocharis*, *Kyllinga* and *Bulbostylis*, respectively. Despite Asteraceae family is insect pollinated, Asteraceae subf. Asteroideae accompanies the herbs dominance in these pollen assemblages and reflects the high frequency of this
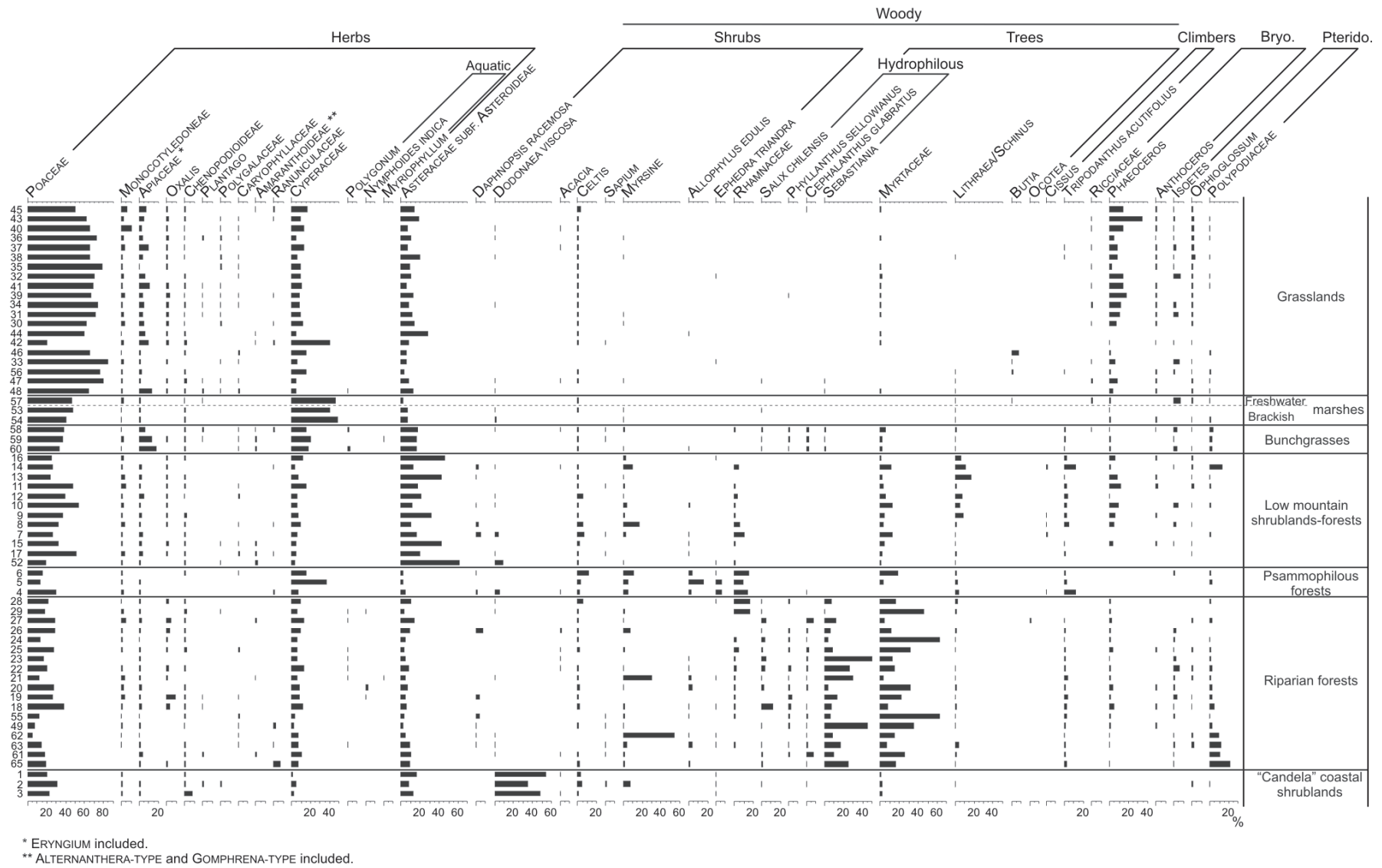
Figure 2. Pollen diagram of the 62 surface soil samples in percentages, ordered according to their vegetation types. Bryo.: Bryophytes; Pterido.: Pteridophytes.

* ERYNGIUM included.
** ALTERNANTHERA-TYPE and GOMPHRENA-TYPE included.

Table 2. Morphological descriptions of spores from the *campos* region of Uruguay. DF: distal face; PF; proximal face. PV: polar view; EqV: equatorial view. PD: polar diameter; EqD: equatorial diameter.

FAMILY/Genus or species/ SPORE TYPE	Symmetry	Polarity	Size (μm)	Aperture	Laesura	Exospore	Shape	Observations
RICCIACEAE Rchb.								
Type 01. RICCIACEAE [Fig.3A]	radiosymmetric	heteropolar	EqD: 71(91)102	trilete	straight, indistinct	reticulate	Amb subcircular	
ANTHOCEROTACEAE Dumort. *Anthoceros* L.								
Type 02. ANTHOCEROS [Fig.3B]	radiosymmetric	heteropolar	EqD: 50(58)62	trilete	narrow, straight, extremes bifurcated	psilate (PF); reticulate-echinate (DF)	Amb circular to subtriangular	echinus frequently bi(tri)furcate
PHAEOCEROS Prosk.								
Type 03. PHAEOCEROS BULBICULOSUS [Fig.3C]	radiosymmetric	heteropolar	EqD: 39(47)52	trilete	narrow, straight, extremes bifurcated; broad margo	psilate	Amb circular to subtriangular	exospore with circular area thickened at the distal face
Type 04. PHAEOCEROS LAEVIS [Fig.3D]	radiosymmetric	heteropolar	EqD: 38(45)50	trilete	narrow, straight or slightly sinuous, extremes bifurcated; granules on margo	echinate	Amb circular to subtriangular	2-3 echinus frequently joined at their bases
Type 05. PHAEOCEROS TENUIS [Fig.3E]	radiosymmetric	heteropolar	EqD: 39(49)59	trilete	narrow, straight or slightly sinuous, extremes bifurcated; amalgamated granules on margo	scabrate, with some verrucae (PF); granulate, with sinuous rugulae (DF)	Amb circular to subtriangular	
ISOËTACEAE Dumort. *Isoetes* L.								
Type 06. ISOETES [Fig.3F]	bilateral	heteropolar	with perispore: EqD: 30(34)44; PD: 22(25)31 without perispore: EqD: 25(28)35; PD: 14(16)24	monolete	straight	psilate, with scabrate perispore	eliptic (PV); plano-convex to concavo-convex (EqV)	microspore
OPHIOGLOSSACEAE Martinov *Ophioglossum* L.								
Type 07. OPHIOGLOSSUM [Fig.3G]	bilateral	heteropolar	EqD: 60(65)68	trilete	narrow, straight	Sparsely fossulate to faveolate, with secondary folds	Amb circular to subtriangular	
POLYPODIACEAE J. Presl & C. Presl								
Type 08. POLYPODIACEAE [Fig.3H]	bilateral	heteropolar	EqD: 60(65)72; PD: 38(45)48	monolete	straight	verrucate	eliptic (PV); plano-convex to concavo-convex (EqV)	

Table 3. Morphological descriptions of pollen from the *campos* region of Uruguay. PV: polar view; EqV: equatorial view. PD: polar diameter; EqD: equatorial diameter. Pollination strategy: A, anemophilous; Z, zoophilous.

| FAMILY / Genus or species/ POLLEN TYPE	Pollen unit	Symmetry	Polarity	Size (µm)	Aperture	Exine	Shape	Pollination strategy	Observations
EPHEDRACEAE Dumort. *Ephedra triandra* Tul.									
Type 01. EPHEDRA TRIANDRA [Fig.4A]	monad	radiosymmetric	isopolar	EqD: 55(60)62; PD: 15(19)25	polyplicate	psilate	peroblate	A	
LAURACEAE Juss. *Ocotea* Aubl.									
Type 02. OCOTEA [Fig.4B]	monad	radiosymmetric	apolar	26(27)29	inaperturate	echinate	spheroidal	Z	
AMARYLLIDACEAE J. St.-Hil.									
Type 03. AMARYLLIDACEAE [Fig.4C]	monad	heteropolar	bilateral	Very variable. Longer EqD: 28(48)82; shorter EqD: 18(26)39 (PV)	monosulcate	reticulate, mostly homobrochate	elliptical (PV)	Z	
ARECACEAE Bercht. & J. Presl *Butia odorata* (Barb. Rodr.) Noblick									
Type 04. BUTIA [Fig.4D]	monad	heteropolar	bilateral	Longer EqD: 52(63)79; shorter EqD: 23(32)44 (PV)	monosulcate	perforate	elliptical to pyriform (PV)	Z	
CYPERACEAE Juss.									
Type 05. CYPERACEAE [Fig.4E]	monad	radiosymmetric	isopolar or heteropolar	Very variable. Length: 33(42)47; width: 28(33)42 (EqV)	inaperturate or with rudimentary pores	psilate to scabrate	spheroidal to pyriform (EqV); sub-circular (PV)	A	
POACEAE Barnhart									
Type 06. POACEAE [Fig.4F]	monad	radiosymmetric	heteropolar	Very variable. 19(36)62	monoporate	psilate to scabrate, rarely granulate	spheroidal	A	pores usually with annulus and sometimes operculated
RANUNCULACEAE Juss.									
Type 07. RANUNCULACEAE [Fig.4G]	monad	radiosymmetric	isopolar	EqD: 22(25)28 PD: 21(24)28	tricolporate	microechinate	oblate spheroidal (EqV); circular (PV)	Z	
HALORAGACEAE R. Br. *Myriophyllum* L.									
Type 08. MYRIOPHYLLUM [Fig.4H]	monad	radiosymmetric	isopolar	EqD: 20(24)28	zonoporate (4 pores)	psilate to scabrate	suboblate to oblate (EqV); subcuadrangular (PV)	A	circular-elliptical pores with annulus
VITACEAE Juss. *Cissus* L.									
Type 09. CISSUS [Fig.4I-J]	monad	radiosymmetric	isopolar	EqD: 29(33)38; PD: 50(57)64	tricolporate	reticulate, heterobrochate	prolate (EqV); circular (PV)	Z	
OXALIDACEAE R. Br. *Oxalis* L.									
Type 10. OXALIS [Fig.4K]	monad	radiosymmetric	isopolar	EqD: 44(48)53; PD: 55(59)63	tricolporate	reticulate, heterobrochate	prolate to subprolate (EqV); subcircular (VP)	Z	
EUPHORBIACEAE Juss. *Sapium* Jacq.									
Type 11. SAPIUM [Fig.4L-M]	monad	radiosymmetric	isopolar	EqD: 34(38)42; PD: 51(64)69	tricolporate	microreticulate	prolate to perprolate (EqV); circular (VP)	Z	endocingulum

Table 3. Cont.

| FAMILY / Genus or species/ POLLEN TYPE	Pollen unit	Symmetry	Polarity	Size (µm)	Aperture	Exine	Shape	Pollination strategy	Observations
Sebastiania Spreng. Type 12. SEBASTIANIA [Fig.4N-O]	monad	radiosymmetric	isopolar	EqD: 30(41)49; PD: 55(58)66	tricolporate	reticulate	prolate (EV); circular (EqV)	Z	
PHYLLANTHACEAE Martinov *Phyllanthus sellowianus* (Klotzsch) Müll. Arg. Type 13. PHYLLANTHUS SELLOWIANUS [Fig.4P]	monad	radiosymmetric	apolar	16(17)20	pantoporate (12-15 pores)	clypeate	spheroidal	Z	
SALICACEAE Mirb. *Salix chilensis* Molina Type 14. SALIX CHILENSIS [Fig.4Q-R]	monad	radiosymmetric	isopolar	EqD: 20(25)32; PD: 19(21)25	tricolporate	Reticulate, heterobrochate	spheroidal to prolate (EqV); circular (PV)	Z	
FABACEAE Lindl. *Acacia* Mill. Type 15. ACACIA [Fig.4S]	polyad			38(42)45	indistinguishable pores	psilate to slightly scabrate		Z	16 pollen grains
POLYGALACEAE Hoffmanns. & Link Type 16. POLYGALACEAE [Fig.4T]	monad	radiosymmetric	isopolar	EqD: 16(17)19; PD: 21(25)33	zonocolporate (10-12 colpori)	psilate	prolate (EqV); circular to multilobate (VP)	Z	endocingulum
CANNABACEAE Martinov *Celtis* L. Type 17. CELTIS [Fig.4U]	monad	radiosymmetric	isopolar	EqD: 25(29)34; PD: 16(17)18	triporate	psilate to scabrate	suboblate (EqV); circular (PV)	A-Z	pores with annulus
RHAMNACEAE Juss. Type 18. RHAMNACEAE [Fig.4V]	monad	radiosymmetric	isopolar	EqD: 17(20)24; PD: 17(21)25	tricolporate	vaguely reticulate, heterobrochate	prolate spheroidal (EqV); subtriangular (PV)	Z	
MYRTACEAE Juss. Type 19. MYRTACEAE [Fig.5A]	monad	radiosymmetric	isopolar	EqD: 17(24)27; PD: 11(12)15 P	syntricolporate	psilate to slightly scabrate	peroblate (EqV); triangular to subtriangular (PV)	Z	rarely syntetracolporate
THYMELAEACEAE Juss. *Daphnopsis racemosa* Griseb. Type 20. DAPHNOPSIS RACEMOSA [Fig.5B-C]	monad	radiosymmetric	apolar	29(35)39	pantoporate (12-15 pores)	croton pattern	spheroidal	Z	
ANACARDIACEAE R. Br. Type 21. LITHRAEA/SCHINUS [Fig.5D-E]	monad	radiosymmetric	isopolar	EqD: 23(27)31; PD: 27(40)45	tricolporate	striate to reticulate	prolate to subprolate (EqV); circular (VP)	Z	
SAPINDACEAE Juss. *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Hieron. ex Niederl.									

Table 3. Cont.

| FAMILY / Genus or species/ POLLEN TYPE	Pollen unit	Symmetry	Polarity	Size (μm)	Aperture	Exine	Shape	Pollination strategy	Observations
Type 22. ALLOPHYLUS EDULIS [Fig.5F]	monad	radiosymmetric	isopolar	EqD: 27(30)32; PD: 18(19)21	zonoporate (4 pores)	microreticulate	oblate (EqV); quadrangular (PV)	Z	rarely triporate
Dodonaea viscosa Jacq.									
Type 23. DODONAEA VISCOSA [Fig.5G-H]	monad	radiosymmetric	isopolar	EqD: 25(28)33; PD: 24(27)31	tricolporate	verrucate	prolate spheroidal (EqV); subcircular (PV)	Z	rarely tetracolporate
LORANTHACEAE Juss.									
Tripodanthus acutifolius (Ruiz & Pav.) Tiegh									
Type 24. TRIPODANTHUS ACUTIFOLIUS [Fig.5I]	monad	radiosymmetric	isopolar	EqD: 23(25)28; PD: 10(12)15	syntricolporate	psilate, baculate at mesocolpium	peroblate (EqV); subtriangular (PV)	Z	rarely syntetracolporate
AMARANTHACEAE Juss.									
Amaranthoideae									
Type 25. ALTERNANTHERA-TYPE [Fig.5J]	monad	radiosymmetric	apolar	15(19)24	pantoporate (12-16 pores)	reticulate	spheroidal-polyhedral	Z	
Type 26. GOMPHRENA-TYPE [Fig.5K-L]	monad	radiosymmetric	apolar	15(19)23	pantoporate (>20 pores)	reticulate	spheroidal	Z	
Chenopodioideae									
Type 27. CHENOPODIOIDEAE [Fig.5M]	monad	radiosymmetric	apolar	20(24)26	pantoporate (>35 pores)	psilate	spheroidal	A	pores with thin annulus
CARYOPHYLLACEAE Juss.									
Type 28. CARYOPHYLLACEAE [Fig.5N-O]	monad	radiosymmetric	apolar	26(32)36	pantoporate (~15 pores)	psilate and slightly perforate	spheroidal	Z	pores with annulus
POLYGONACEAE Juss.									
Polygonum L.									
Type 29. POLYGONUM [Fig.5P-Q]	monad	radiosymmetric	apolar	40(54)64	pantoporate (~10 pores)	reticulate	spheroidal	Z	
PRIMULACEAE Batsch ex Borkh.									
Myrsine L.									
Type 30. MYRSINE [Fig.5R]	monad	radiosymmetric	isopolar	EqD: 16(20)23; PD: 19(22)25	zonocolporate (4 colpori)	psilate	prolate spheroidal (EqV); circular to tetrangular (PV)	A-Z	rarely 3-5 colpori; pore mostly indistinguishable
RUBIACEAE Juss.									
Cephalanthus glabratus (Spreng.) K. Schum.									
Type 31. CEPHALANTHUS GLABRATUS [Fig.5S]	monad	radiosymmetric	isopolar	EqD: 18(23)28; PD: 20(22)23	tricolporate	Reticulate, homobrochate	sub-spheroidal (EqV); circular to sub-triangular (PV)	Z	pores with annulus
PLANTAGINACEAE Juss.									
Plantago L.									
Type 32. PLANTAGO [Fig.5T]	monad	radiosymmetric	apolar	28(39)32	pantoporate (~7 pores)	verrucate	spheroidal	A	faint irregular pores
ASTERACEAE Bercht. & J. Presl									
Type 33. ASTERACEAE SUBF. ASTEROIDEAE [Fig.5U-V]	monad	radiosymmetric	isopolar	EqD: 15(28)40; PD: 20(33)43	tricolporate	echinate or subechinolophate	prolate spheroidal to subprolate (EqV); circular to sub-triangular (PV)	Z	
MENYANTHACEAE Dumort.									
Nymphoides indica (L.) Kuntze									
Type 34. NYMPHOIDES INDICA [Fig.5W]	monad	radiosymmetric	isopolar	EqD: 39(41)43; PD: 22(24)28	syntricolporate	microbaculate	oblate (EqV); triangular (PV)	Z	
APIACEAE Lindl.									
Eryngium L.									
Type 35. ERYNGIUM [Fig.5X-Y]	monad	radiosymmetric	isopolar	EqD: 14(18)23; PD: 32(42)48	tricolporate	psilate	perprolate (EqV); circular (PV)	Z	
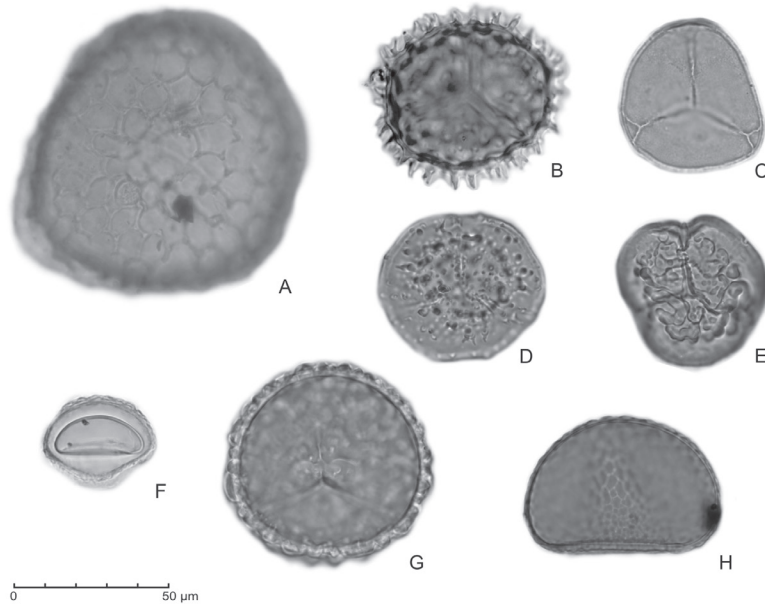
Figure 3. Photomicrographs of spores from the *campos* region of Uruguay. A – Type 01 Ricciaceae; B – Type 02 Anthoceros; C – Type 03 Phaeoceros bulbiculosus; D – Type 04 Phaeoceros laevis; E – Type 05 Phaeoceros tenuis; F – Type 06 Isoetes; G – Type 07 Ophioglossum; H – Type 08 Polypodiaceae.
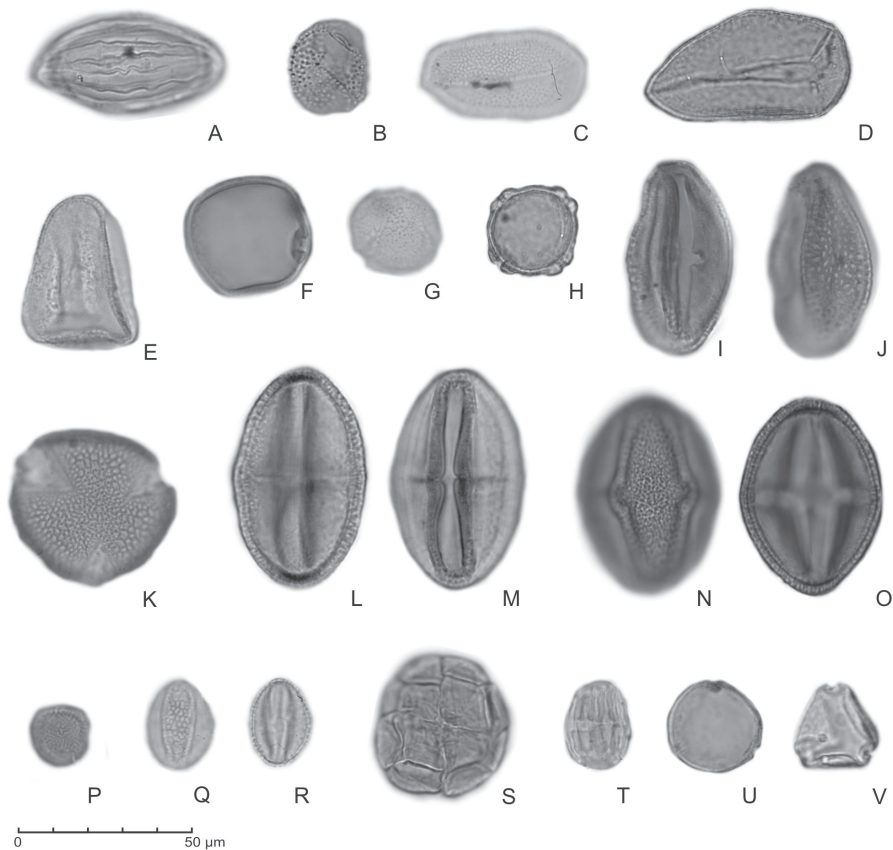
Figure 4. Photomicrographs of pollen from the *campos* region of Uruguay. A – Type 01 Ephedra triandra; B – Type 02 Ocotea; C – Type 03 Amaryllidaceae; D – Type 04 Butia; E – Type 05 Cyperaceae; F – Type 06 Poaceae; G – Type 07 Ranunculaceae; H – Type 08 Myriophyllum; I-J – Type 09 Cissus; K – Type 10 Oxalis; L-M – Type 11 Sapium; N-O – Type 12 Sebastiania; P – Type 13 Phyllanthus sellowianus; Q-R Type 14 Salix chilensis; S – Type 15 Acacia; T – Type 16 Polygalaceae; U – Type 17 Celtis; V – Type 18 Rhamnaceae.
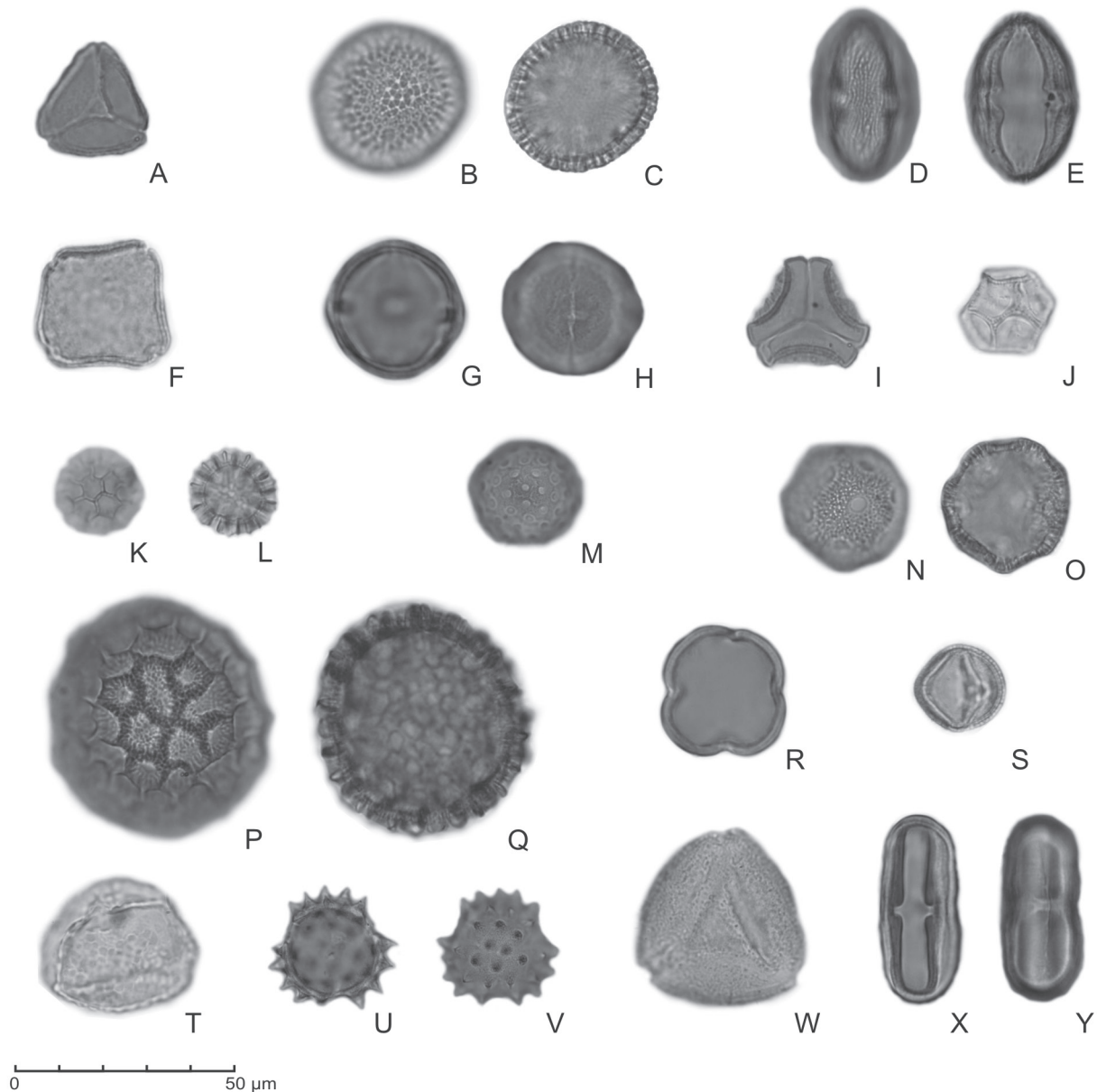
Figure 5. Photomicrographs of pollen from the *campos* region of Uruguay. A – Type 19 Myrtaceae; B-C – Type 20 Daphnopsis racemosa; D-E – Type 21 Lithraea/Schinus; F – Type 22 Allophylus edulis; G-H – Type 23 Dodonaea viscosa; I – Type 24 Tripodanthus acutifolius; J – Type 25 Alternanthera-type; K-L – Type 26 Gomphrena-type; M – Type 27 Chenopodioideae; N-O – Type 28 Caryophyllaceae; P-Q – Type 29 Polygonum; R – Type 30 Myrsine; S – Type 31 Cephalanthus glabratus; T – Type 32 Plantago; U-V – Type 33 Asteraceae subf. Asteroideae; W – Type 34 Nymphoides indica; X-Y – Type 35 Eryngium.

family in natural grasslands, mainly represented by genera such as *Baccharis* and *Eupatorium* (Tab. 1). Cyperaceae is over-represented in some samples where small local communities related to higher substrate water availability are observed (samples 5, 42, 53, 54, 57; Fig. 2) (Mourelle & Prieto 2012). Apiaceae is also well represented in pollen assemblages, principally due to *Eryngium* sp. (Eryngium, Fig. 5X-Y), which is commonly recorded in grasslands.

Other graminoid herbs are also frequent in the pollen assemblages. For instance, Amaranthaceae family is represented by pollen grains of the genera *Alternanthera*

spp. (Alternanthera-type, Fig. 5J), *Gomphrena* spp. (Gomphrena-type, Fig. 5K-L) and *Chenopodium* spp. (Chenopodioideae, Fig. 5M). Monocotyledoneae corresponds to various families such as Juncaceae, Amaryllidaceae (Amaryllidaceae, Fig. 4C) and Iridaceae. Ranunculaceae (Fig. 4G) mainly represents the common species *Anemone decapetala*, and Caryophyllaceae (Fig. 5N-O) mainly *Cerastium* spp. and *Silene* spp. Other taxa widely dispersed in the *campos* are represented by Oxalis (Fig. 4K), Plantago (Fig. 5T) and Polygalaceae (Fig. 4T). Bryophytes are dominated by Phaeoceros (Fig. 3C-E)
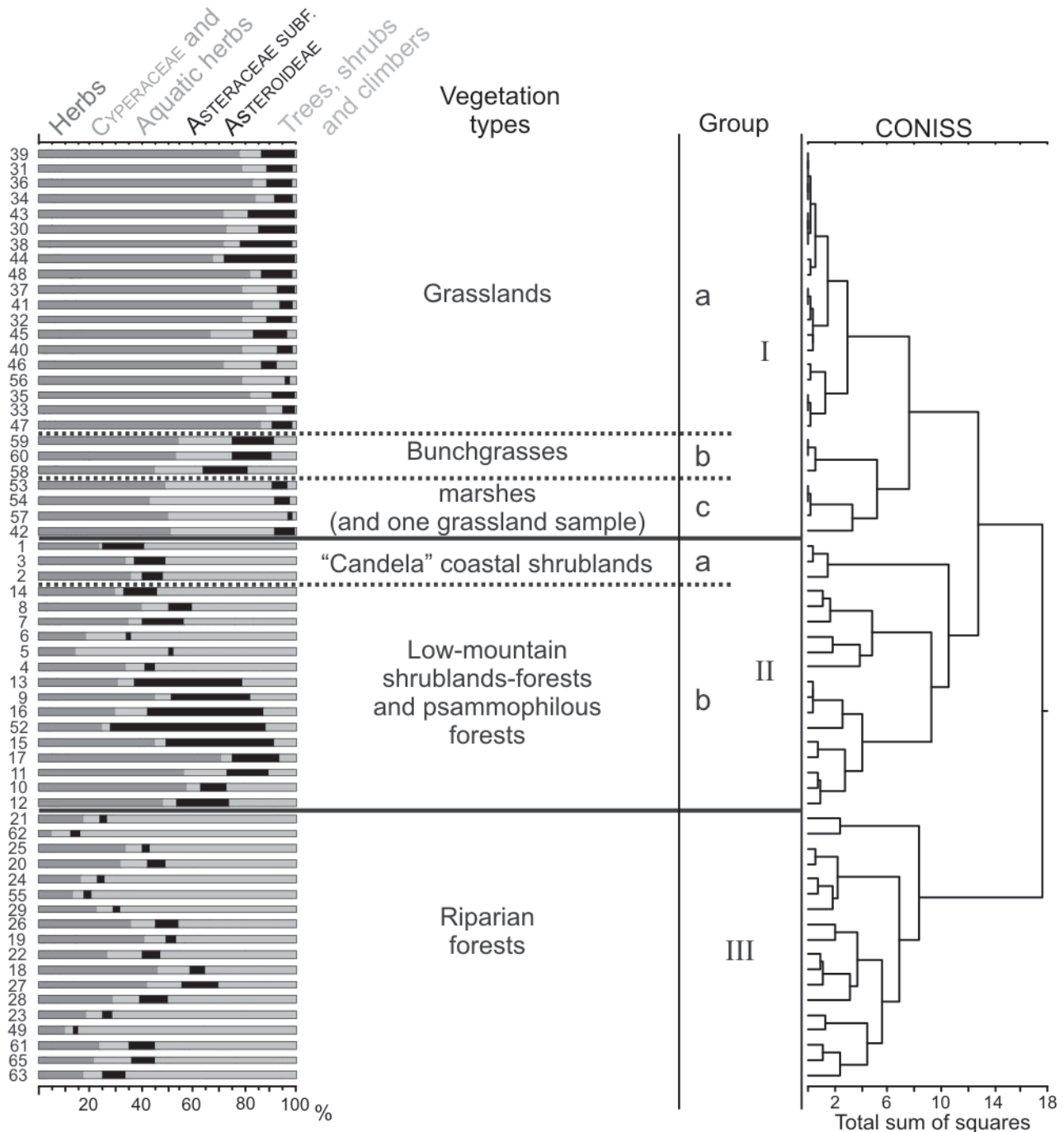
Figure 6. Summary percentage pollen diagram of the 62 surface soil samples ordered according to the Cluster Analysis unconstrained results.

and Anthoceros (Fig. 3B), frequent in South American moist soils (Villarreal *et al.* 2010); and pteridophytes by Isoetes (Fig. 3F) and Ophioglossum (Fig. 3G) spores from small plants that grow between grasses and graminoids. Ricciaceae (Fig. 3A) is present in some samples and could represent muddy areas.

In grassland surface samples shrubby and arboreal pollen is present in traces, mainly represented by mesophilous taxa (Myrtaceae; Myrsine, Fig. 5-R and Celtis, Fig. 4U). *Blepharocalyx salicifolius* (Myrtaceae) and *Myrsine laetevirens* trees reach up to 10-15 m height and constitute the forest canopy, over which *Celtis iguanaea* climbs. As a result, pollen of these mesophilous taxa, besides falling gravitationally into the forest, can be more easily dispersed into grasslands than pollen from hydrophilous taxa, which only reach up to 4 m height and grow restricted to water body margins

(e.g. *Phyllanthus sellowianus* and *Cephalanthus glabratus*). In addition, the frequent presence of Celtis and Myrsine in grassland samples could be related to wind dispersal (Otegui & Cocucci 1999; Gassmann & Pérez 2006; Torretta & Basilio 2009).

Uniform morphology of Poaceae pollen presents problems in the reconstruction of past grassland ecosystems and, therefore, many efforts have been made in order to elaborate a method to differentiate grassland ecosystems based on measurable Poaceae pollen parameters. Jan *et al.* (2015) observed that neither the scores of C3/C4 Poaceae species nor those diploid/polyploid species are clearly separable, despite there are clear trends showing that pollen sizes are larger in C4 as compared with C3 species, buy also polyploids are larger than diploid species. Grasslands dominated by C3 and C4 Poaceae species from different regions and habitats could then be separated by studying the pattern of trends in their pollen size (Jan *et al.* 2015). In this regard, Schüler & Behling (2011) used Poaceae pollen size as a tool for distinguishing past grasslands in South America, demonstrating that a distinction between grassland ecosystems is possible based on measurable Poaceae pollen grain parameters. They observed that the RPG Poaceae pollen was considerably smaller compared to the one from other grassland regions, but they did not find significant differences between Poaceae pollen from the *campos* and the *pampas* regions. In particular, for many taxa from different genus or tribes present in the *campos* region, only the extreme forms are readily separable, but there is a continuum of forms blurring the distinction between the extremes (Radaeski 2014). A false separation of forms is statistically more misleading than a lumping of types, although this involves losing ecological information for paleoecological reconstructions. In consequence, we still suggest considering Poaceae pollen grains at the family level in combination with minor taxa of grasslands. This also applies to Asteraceae subf. Asteroideae which, despite differences between their pollen grains are clearer (e.g. Punt & Hoen 2009; Cancelli *et al.* 2010), the difficulty classifying all Asteraceae subf. Asteroideae pollen present in modern and fossil assemblages might generate misleading paleoecological interpretations. Therefore, we propose considering them at the subfamily level. Therefore, the limited taxonomic resolution of the most abundant pollen in grassland samples (Poaceae, Cyperaceae and Asteraceae subf. Asteroideae) makes it impossible to differentiate samples from *northern* and *southern campos* at a regional scale since this division is based on different Poaceae genera (Mourelle & Prieto 2012). However, some divisions at a local scale are possible (see below).

The presence of shrubby and arboreal pollen in the *campos* differentiates their pollen assemblages from those of the *pampas* where these taxa are absent, taken into account that forest and shrubland hardly develop in the *pampas* region. Samples belonging to grasslands where palm

individuals grow could be differentiated from the others by the presence of Butia pollen (Fig. 4D). However, not all surface samples from grasslands where palm individuals develop exhibited Butia pollen. This absence prevents us to interpret this as a lack of palms in the vegetation (Mourelle & Prieto 2012).

Marshes and bunchgrasses

Pollen assemblages of brackish and freshwater marshes are co-dominated by Poaceae and Cyperaceae. Most Poaceae pollen comes from the regional grasslands, while Cyperaceae comes from plants from soil depressions where water accumulates, promoting the local growth of species such as *Schoenoplectus californicus* and *Scirpus giganteus* (Rodríguez-Gallego *et al.* 2012) (Fig. 2; Tab. 1). The absence of Juncus acutus in brackish marshes surface samples does not allow the differentiation between them and the freshwater marshes, and highlights the importance of identifying the silent taxa in order not to misinterpret the fossil pollen spectra. Furthermore, on the Uruguayan coast there are not salt marshes dominated by Chenopodioideae, as observed in other South American areas (Isacch *et al.* 2006) where Chenopodioideae pollen often reaches a strong local representation (up to 80%) in Bahia Samborombón (Vilanova & Prieto 2012) and Laguna Mar Chiquita (Stutz & Prieto 2003). This fact must be taken into account when interpreting coastal fossil sequences, as undertaken in ASG, where the development of salt marshes dominated by Chenopodioideae during the mid Holocene was inferred (Mourelle *et al.* 2015a). Bunchgrasses are dominated by Poaceae species that develop together with some Cyperaceae in soils with high substrate water availability, and hence Poaceae and Cyperaceae also co-dominate their pollen assemblages (Fig. 2; Tab. 1). Relatively dense *Eryngium pandanifolium* clumps, locally known as "caraguatales", are associated to bunchgrasses and are also represented in their pollen spectra (Eryngium). In addition to grasslands, both marshes and bunchgrasses are observed in open environments, which favored the deposition of woody pollen dispersed from the canopy of the neighboring riparian forests (Fig. 2).

Forests

The different forest types that develop in the *campos* region of Uruguay (Tab. 1) share most of their species, and are mainly composed of mesophilous trees and shrubs belonging to the families Myrtaceae (*Blepharocalyx salicifolius, Myrceugenia glaucescens, Myrcianthes cisplatensis*), Rhamnaceae (*Scutia buxifolia*) and Primulaceae (*Myrsine coriacea, M. ferruginea*), which are represented in the pollen samples by Myrtaceae, Rhamnaceae and Myrsine. Other pollen types from mesophilous trees are Allophylus edulis (Fig. 5F), Lithraea/Schinus (Fig. 5D-E) and

Sapium (Fig. 4L-M). All of these taxa constitute the core zone of the forests, and some of them reach the canopy on which top other species such as *Celtis iguanaea* (Celtis) and *Tripodanthus acutifolius* (Tripodanthus acutifolius, Fig. 5I) climb. On the outer zone of the forests other species, such as *Daphnopsis racemosa* (Daphnopsis racemosa, Fig. 5B-C), *Acacia bonariensis* (Acacia, Fig. 4S) and *Cissus palmata* (Cissus, Fig. 4I-J) develop. The understorey supports many herbs, bryophytes and pteridophytes, represented in the pollen spectra by Poaceae, Oxalis, Caryophyllaceae, Phaeoceros, Isoetes and Polypodiaceae (Fig. 3H). The fact that all these taxa are shared by different forest types developed in the *campos* region not only makes these pollen grains and spores characterize and define them in the palynological spectra but it also means that they are not useful to differentiate between forests.

For palynologists interpreting pollen diagrams is important to understand which groups of taxa are likely to be silent in the pollen record. For instance, pollen from the characteristic species of the psammophilous forest vegetation (*Sideroxylon obtusifolium, Rollinia maritima* and *Varronia curassavica*) were absent in the pollen assemblages (Fig. 2; Tab. 1). In consequence, such silent taxa prevent the psammophilous forest to be clearly identified from other forests in the pollen assemblages, mainly from low-mountain shrublands-forests (Fig. 6). However, it is important to note that pollen spectra from psammophilous forest have higher values of Ephedra triandra (Fig. 4A) and Rhamnaceae, and minor of Asteraceae subf. Asteroideae, related to the abundance of their parental plants on each vegetation type (Figs. 2, 6; Tab. 1).

In contrast, some different vegetation types can be identified and statistically separated from others by a few key indicator taxa (e.g. Bush 1991; Rodgers III & Horn 1996; Ortuño *et al.* 2011). Pollen samples from riparian forests are dominated by woody taxa, represented by the mesophilous taxa described above, but also by hydrophilous ones that develop exclusively in these forests (Tab. 1), represented in pollen spectra by Sebastiania (Fig. 4N-O), Salix chilensis (Fig. 4Q-R), Phyllanthus sellowianus (Fig. 4P) and Cephalanthus glabratus (Fig. 5S). These pollen types are riparian forest key indicator taxa. Aquatic herbs such as Myriophyllum (Fig. 4H), Polygonum (5P-Q) and Nymphoides indica (Fig. 5W) reflect plants that develop in the margin of the water bodies and in the floodplains. In addition, riparian forests understorey is more humid than in the other forest types, and that favored the development of *Isoetes* (Isoetes) and many pteridophytes of genera such as *Campyloneurum, Pleopeltis* and *Microgramma* (Polypodiaceae).

In riparian forest samples, some pollen types are markedly over-represented (e.g. Myrtaceae, Myrsine, Sebastiania), while others are under-represented (Ocotea, Fig. 4B) or absent (Erythrina crista-galli), even though these plants are frequent in the vegetation.

Ocotea pollen grains are very fragile (Behling & Negrelle 2006) and *Erythrina crista-galli* is pollinated by birds and so their flowers have stamens hidden deep in the corolla tube (Hidalgo 2008), two facts that make that these grains are not usually found in pollen assemblages. However, this does not generate problems concerning the correct identification of riparian forest pollen assemblages, both qualitatively (Fig. 2) and quantitatively (Fig. 6), principally due to the presence of the key indicator pollen types from hydrophilous taxa that grow exclusively in these forests.

Therefore, when we analyze the pollen spectra of a fossil sequence we must take into account that the presence of key pollen types from woody hydrophilous taxa indicate the development of riparian forests, whereas the presence of pollen types from mesophilous taxa do not allow us to distinguish what type of forest these pollen grains might be coming from.

Shrublands

There exists a considerable difference between shrubland and forest soils. Shrublands develop in soils with coarse sand, low humidity and water availability for plants, as well as low organic matter and nitrogen levels (Bartesaghi 2007). These conditions hinder the development of many taxa, mainly trees, bryophytes and pteridophytes.

Dodonaea viscosa (Fig. 5G-H) dominates the pollen spectra and vegetation from "Candela" coastal shrublands (Fig. 6; Tab. 1). Asteraceae subf. Asteroideae pollen is also well represented, mainly due to shrubs such as *Baccharis* spp. and *Eupatorium buniifolium*, and herbs such as *Senecio* spp.
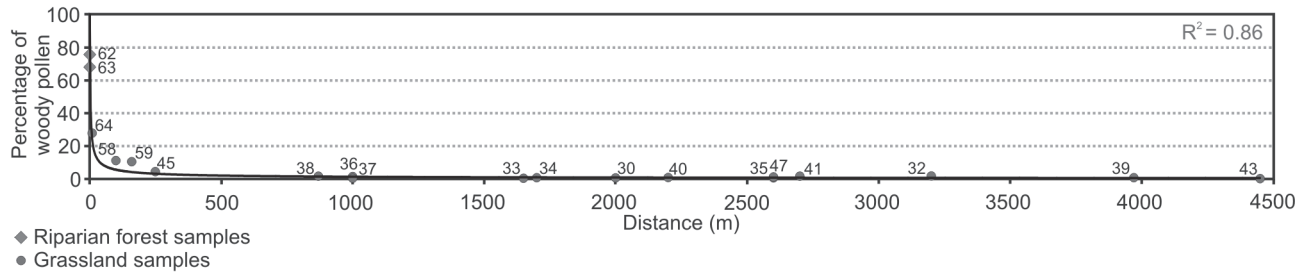
Woody pollen dispersal

Woody taxa from riparian forests have mostly zoophilous pollination (Tab. 1) and so they produce low quantities of pollen grains poorly dispersed. As a consequence, most of the woody pollen grains produced by riparian forests drop directly into the ground and have strong local effect (Figs. 2, 7). Inside the riparian forests, woody pollen represents ca. 70%, while at distances >250 m woody pollen does not reach up to 2% of the pollen assemblage, represented by mesophilous taxa (Myrtaceae, Myrsine and Celtis). *Blepharocalyx salicifolius* (Myrtaceae), *Myrsine laetevirens* and *Celtis iguanaea* reach riparian forest canopy and so some of their pollen grains can be dispersed away to grasslands. This could also be related to Celtis and Myrsine wind dispersal (Otegui & Cocucci 1999; Gassmann & Pérez 2006; Torretta & Basilio 2009).

Pollination mechanisms will predictably bias the fossil record: pollen grains arriving to lake sediments are most likely to be from anemophilous taxa or from zoophilous ones exhibiting "messy" pollination syndromes, in the sense that excess pollen is spilled into the air, for example, due

Figure 7. Woody pollen dispersal curve from riparian forests to grasslands. Two riparian forest samples were considered as pollen sources (samples 62 and 63). Numbers correspond to surface samples (see Fig. 1).

to vibrations from wings of a visiting bee (Bush & Rivera 2001). Our results reflect that just very small amounts (or even traces) of key pollen types from hydrophilous taxa found in the fossil spectra from sequences taken in open environments (such as lagoons or marshes) indicate the development of riparian forests in the surroundings. However, further detailed investigations are required to obtain more robust results.

Application to interpret Holocene sequences from the campos region

Paleoecological investigations based on pollen and spores content in fossil sediments from *campos* region (ASG and PB-IM, Fig. 8) brought information about environmental and climatic changes impact in vegetation during the last ca. 8000 cal. yr BP. (1) Palynomorph analyses carried on ASG sequence from the marsh in the lower reach of Arroyo Solís Grande (34°45'35"S - 55°25'55"W; Figs. 1, 8) allowed the reconstruction of vegetation history in the Río de la Plata northeastern coast (Uruguay) during the mid and late Holocene in relation to the relative sea-level fluctuations and the geomorphologic evolution (Mourelle *et al*. 2015a; Prieto *et al*. 2016). (2) Palynomorph analyses carried on PB-IM sequence from the wetland Paso Barranca-India Muerta at Laguna Merín basin (33°40'36"S - 53°49'15"W; Figs. 1, 8) allowed the reconstruction of vegetation history during the late Holocene in relation to edaphic and climatic conditions (Mourelle *et al*. 2015b). Despite fossil pollen assemblages from *campos* region showed considerable pollen and spores diversity, for which it is indispensable to have a good palynomorph morphology knowledge, many taxa could only be identified at genus or family level, causing a loss of ecological information that had to be taken into account while reconstructing paleovegetation. However, the good relationship between modern pollen and spores assemblages and the principal vegetation types of the *campos* region of Uruguay provided useful data for the interpretation of Holocene pollen sequences from this area.

The dominance of Poaceae in the fossil pollen spectra makes it imperative to analyze the other variables in great detail, paying special attention to the pollen types

representing small herbs and woody taxa. The knowledge of the pollination strategies of parental plants allows the evaluation of the significance of their pollen grains in the fossil spectra. Then, small changes in their proportions in fossil assemblages have been really relevant for making accurate paleoecological interpretations.

The pollen types that dominate the pollen assemblages of the two Holocene sequences studied (ASG and PB-IM, Fig. 8) represent anemophilous plants (Poaceae accompanied by Cyperaceae) associated to zoophilous ones (Asteraceae subf. Asteroideae, Monocotyledoneae, Apiaceae and pollen from small herbs) (Fig. 2). These pollen assemblages represent the regional dominance of grasslands in this region during mid and late Holocene, with characteristics similar to the current ones.
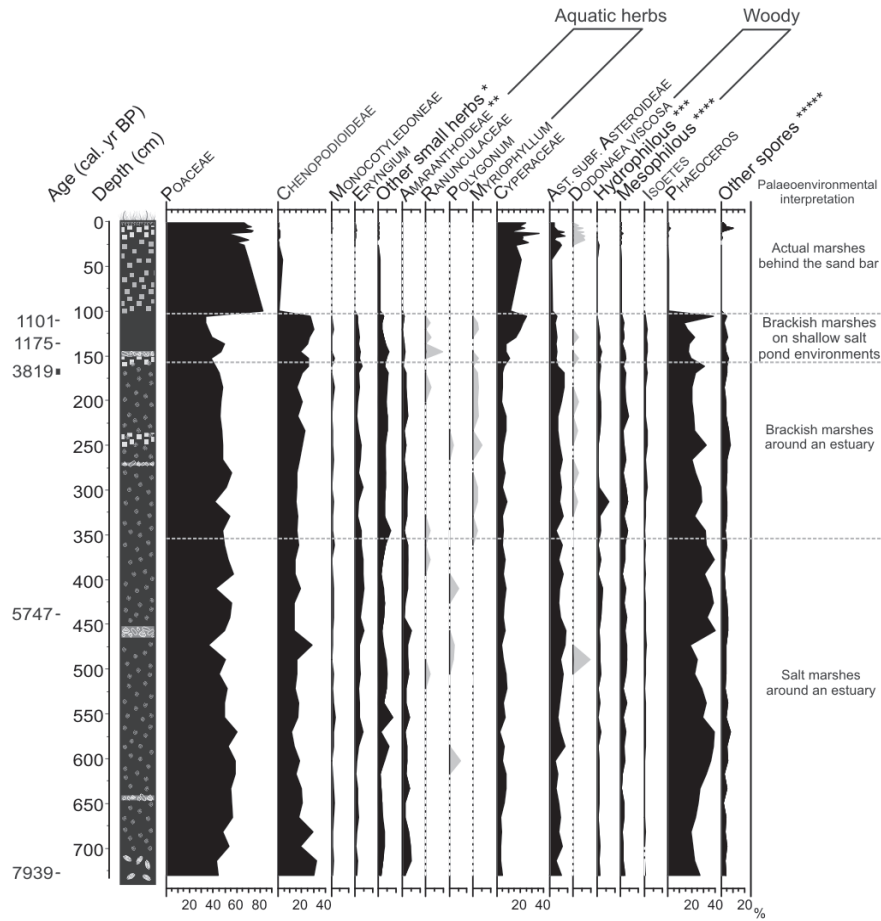
Despite Poaceae high proportions in ASG sequence are related to the dominance of grasslands all over the region, their grains may also have a local origin. Its co-dominance with Chenopodioideae and the geomorphological characteristics of the coastal area revealed the development of salt marshes around an estuary during the sea-level rise and highstand (between ca. 8000 and 2900 cal. yr BP). In consequence, Poaceae pollen probably also corresponds to *Spartina* sp., a dominant taxa in these environments. This scenario is comparable with the salt marsh vegetation that currently occurs in some coastal areas of the *pampas* (Mourelle *et al.* 2015a).

Amaranthoideae (Gomphrena-type and Alternanthera-type) and Ranunculaceae represent taxa that are very common in grasslands. However, they also represent plants that develop in humid environments and near water bodies, such as *Alternanthera philoxeroides* and *Ranunculus* spp. Other herbs that also develop there are *Polygonum punctatum, Nymphoides indica*, *Myriophyllum aquaticum* and *M. quitense*, and so their pollen grains (Polygonum, Nymphoides indica and Myriophyllum) were all found in the fossil assemblages. These associations represent freshwater areas that locally developed during the Holocene, but also suggest the occurrence of runoff that transported pollen from the hinterland source area to the estuary (Fig. 8). Relatively high Eryngium proportions in the fossil sequences reflect the *Eryngium pandanifolium* dense clumps that frequently occur in wetlands (PB-IM)
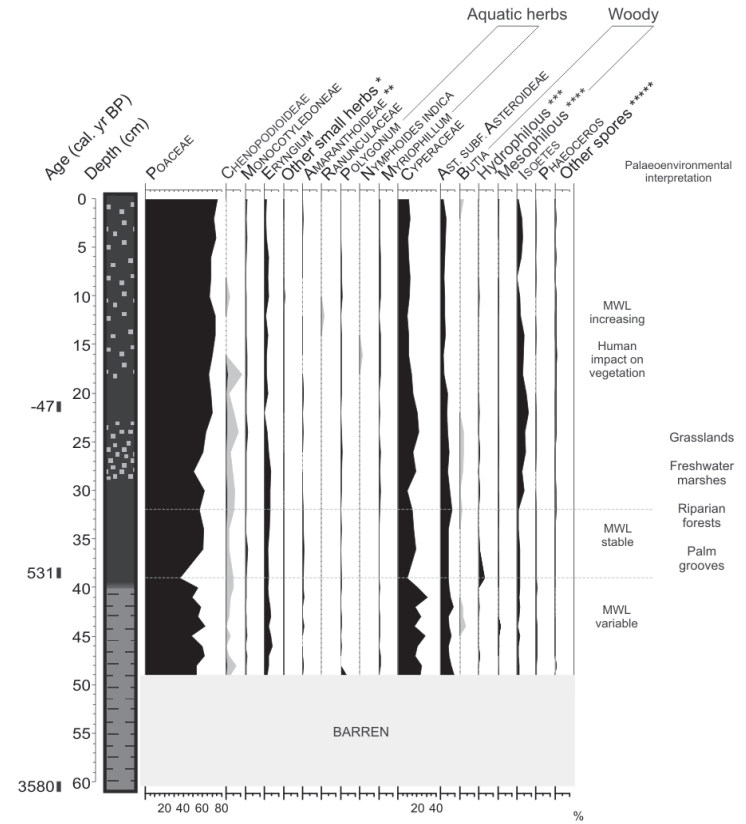
Figure 8. Calibrated ages, sediment description, percentage palynomorph diagrams and paleoenvironmental interpretation from (A) Arroyo Solís Grande (Mourelle *et al.* 2015a) and (B) Paso Barranca-India Muerta (Mourelle *et al.* 2015b) sequences, plotted against depth. MWL: marsh water level. Exaggeration 10x.

and coastal areas (ASG) (Fig. 8).

The occurrence of certain pollen types in the fossil spectra, even in low proportions, turns out to be good indicators of paleoenvironmental changes. For instance, the appearance of the low salinity-tolerant Myriophyllum in ASG sequence at around ca. 5100 cal. yr BP reflect an increase in freshwater input and a decrease in salinity derived from the transition from brackish to freshwater conditions during sea-level fall (Fig. 8). At the same time, Dodonaea viscosa grains also appeared, indicating the development of psammophilous coastal vegetation in coastal sand bars (Fig. 8). On the contrary, Butia pollen was found in low proportions and only in some PB-IM samples, disappearing towards the top of the core (Fig. 8). This initially suggests the development of palms interspersed among grasslands, close to the sampled site, at least since ca. 1300 cal. yr BP. However, as Butia pollen has not been found in all surface samples where palm individuals develop, its absence in the fossil assemblages prevents us to interpret this as an absence of palms in the past vegetation. Nevertheless, Butia disappearance in PB-IM assemblages corresponds to the last decades and probably reflects the palm vegetation decline due to human activities, such as pasture burning. This practice also reduces *Eryngium pandanifolium* clumps and *Eupatorium* spp. and *Baccharis* spp. shrubs, and this was also observed in the fossil spectra (Fig. 8).

Regarding the woody taxa, pollen from hydrophilous and mesophilous taxa were registered in the fossil sequences (Fig. 8). Key pollen types from hydrophilous taxa suggest the development of riparian forests. However, it is not possible to distinguish what type of forest or shrubland the pollen of mesophilous taxa comes from, since these plants are shared by all vegetation types. The knowledge of the geomorphology of the region (e.g. the proximity of the study area to hills) and its evolution (e.g. coastal progradation) is indispensable to determine if any of these forests or shrublands could have developed nearby in the past.

High spores proportions, mainly Phaeoceros, suggest the occurrence of runoff transported from the hinterland source area to the estuary in ASG, as these spores were not recorded in surface samples from coastal vegetation (Fig. 8). Very low proportions of deteriorated pollen grains and high pollen sum in the fossil spectra allowed us to rule out an over-representation of bryophyte spores due to poor preservation, which in addition amplifies the imperative value of the spores account. Isoetes relatively high proportions in PB-IM pollen assemblages probably reflect the edaphic characteristic of this wetland area that is usually covered by water.

Conclusions

This is the first catalogue that describes and illustrates pollen and spores in modern and fossil samples from the *campos* region of Uruguay. Distinctive palynological assemblages characterized the main vegetation types, mainly related to the dominance of some taxa (e.g. Poaceae in grasslands, Dodonaea viscosa in "Candela" coastal shrublands) or to the presence of key indicator taxa (e.g. hydrophilous woody taxa in riparian forests). However, the low taxonomic resolution of some pollen taxa (e.g. Asteraceae subf. Asteroideae, Myrtaceae, Rhamnaceae) and the absence of pollen from species that develop exclusively in some vegetation types (e.g. *Sideroxylon obtusifolium, Rollinia maritima* and *Varronia curassavica* in psammophilous forests; *Juncus acutus* in brackish marshes) makes it difficult to differentiate some vegetation types only by the palynological assemblages. The latter are considered silent taxa, and their identification proved to be very important in order not to misinterpret the fossil pollen spectra.

The knowledge of pollination strategies of parental plants was necessary to explain the presence of small amounts of key pollen types that result indicative of the vegetation (e.g. hydrophilous woody taxa indicate riparian forests development, aquatic herbs indicate freshwater areas, and Butia indicates palms development grasslands).

Studies like this one help and guide the palynologist to make identifications in surface or fossil samples, and thereby they are useful to focus on the taxa most likely to occur in the fossil record. It presents a practical approach to paleoecological research, which not only produces robust results in studies of the *campos* region, but can also be applied to other grassland ecosystems, including those in temperate regions.

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