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Pollen morphology of Poaceae (Poales) in the Azores, Portugal

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Abstract
The objective of this study was to expand knowledge on the Poaceae and morphologically describe pollen grains of species occurring in the Azores Archipelago, Portugal. Pollen grains from each species under study were subjected to standard acetolysis, mounting of at least three permanent slides of each species for observations and measurement of pollen grains under light and scanning electron microscope. With regard to pollen morphology, all pollen grains were arranged as monads, with a single pore-type aperture – monoporate, featuring annulus and operculum; and outline in polar view circular, sub-circular and ovalate. Most species featured pollen size small, except for *Agrostis stolonifera* with pollen size very small, *Brachypodium sylvaticum* with pollen size medium, *Zea mays* with pollen size large. Species endemic to the archipelago show ornamentation pattern ranging between areolate-microechinate, microechinate, microrugulate-microechinate and verrucate-microechinate, most of which feature exine surface areolate-microechinate. Among endemic species, *Rostraria azorica* stood out for its oblate-spheroidal shape, whereas most species showed prolate-spheroidal shape.

Keywords: *Azores archipelago, grass, palynology, stenopollinic*

Grasses belong to the class of Monocotyledons, order Poales and family Poaceae, with worldwide distribution, grouped into 823 genera and 12 096 identified species (Clayton 1993; Simon et al. 2011). Based on morphological and molecular data, this group is organised into 12 subfamilies, the largest of which is Panicoideae, featuring 220 genera and close to 3300 species (Sánchez-Ken & Clark 2010). A total of 121 species are described in the archipelago of the Azores, distributed by 53 genera. Of these species, 12 are endemic to the archipelago (Silva et al. 2010). Some Poaceae species are known for their wide use in human and animal diets and for their ecological value. Estimates indicate that approximately one-third of the planet's plant cover consists of these plants (Clayton 1993; Fray 2000; Simon et al. 2011).

Poaceae plants are characterised basically as annual or perennial, herbaceous, subshrubs or shrubs, featuring culms with solid or hollow nodes. Leaves feature open sheath, blade and ligule, sometimes pseudopetiolate. Flowers are arranged in spikelets, grouped in inflorescences with quite varied morphologies; stigma plumose and anthers that cluster a large number of pollen grains anemophilous – dry and lightweight (Chase & Sendulsky 1991; Longhi-Wagner et al. 2001; Boldrini et al. 2005).

Aeropalynological studies indicate Poaceae pollen grains as possible allergens, due to the high concentration of this pollen type suspended in the atmosphere at any given time (Vieira & Negreiros 1989; Minero et al. 1998; Vergamini et al. 2006; D'Amato et al. 2007; Kasprzyk & Walanus 2010; Matyasovszky et al. 2011; Dąbrowska 2012; Piotrowska 2012).

In palynology, the Poaceae family is regarded by several authors as stenopollinic, with species featuring

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morphologically similar pollen grains (Wodehouse 1935; Fægri & Iversen 1964; Erdtman 1966; Salgado-Labouriau 1973; Melhem et al. 1983; Corrêa et al. 2005; Perveen 2006). Pollen from this family is usually monad, outline in polar view circular, prolate-spheroidal to oblate-spheroidal, pore-type aperture, with the presence of annulus and operculum (Corrêa et al. 2005).

The scientific knowledge on the Poaceae family found in the Azores is still limited (Henderson & Schäfer 2003; Schäfer 2005; de Sequeira & Castroviejo 2007; Prieto et al. 2008; de Sequeira et al. 2009; Silva et al. 2010), and this is the first palynological study of these plants in the archipelago. Our research aims to expand the knowledge on this family and morphologically describe the pollen grains of the species occurring in the Portuguese Azores Archipelago.

Material and methods

The pollen material used in the morphology study was obtained from 45 species of Poaceae, of which 34 were classified as naturalised, seven were endemic, three casual and one native, all occurring in the archipelago of the Azores, Portugal, located in the north-eastern Atlantic Ocean between 37° and 40° North and 25° and 31° West. The archipelago consists of nine islands and a few islets, all of volcanic origin (Borges et al. 2010; Silva et al. 2010). Samples were obtained from fertile anthers of specimens found in the field and at the AZU herbarium belonging to the University of the Azores, located on Terceira Island. The specimens collected in the field were deposited in the AZB herbarium of the Biology Department of the University of the Azores. Examined material: *Agrostis stolonifera* L. (AZB3463), *Anthoxanthum odoratum* L. (AZB3469), *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl. et C. Presl subsp. *bulbosum* (Willd.) Schübl. et Mart (AZB3483), *Avena barbata* Pott ex Link (AZB3474), *Axonopus fissifolius* (Raddi) Kuhlm. (AZB3465), *Brachypodium distachyon* (L.) P. Beauv. (AZB3477), *Brachypodium sylvaticum* (Huds.) P. Beauv. (AZB3478), *Briza maxima* L. (AZB3470), *Briza minor* L. (AZB3458), *Bromus catharticus* Vahl (AZB3454), *Bromus diandrus* Roth (AZB3466), *Cortaderia selloana* (Schult. et Schult.f.) Asch. et Graebn. (AZB3452), *Cynodon dactylon* (L.) Pers. (AZB3475), *Dactylis glomerata* L. (AZB3455), *Deschampsia foliosa* Hack. (AZB3449), *Digitaria sanguinalis* (L.) Scop. (AZB3443), *Eleusine indica* (L.) Gaertn. (AZB3460), *Elymus repens* (L.) Gould (AZB3481), *Festuca arundinacea* Schreb. (AZB3462), *Festuca francoi* Fern. Prieto, C. Aguiar, E. Dias et M. I. Gut (AZB3485), *Festuca petraea* Guthnick (AZB3484), *Gaudinia coarctata* T. Durand et Schinz (AZB3468), *Holcus azoricus* M. Seq. et Castrov. (AZU555), *Holcus lanatus* L. (AZB3451), *Holcus rigidus* Hochst. (3450), *Hordeum marinum* Huds. (3464), *Lagurus ovatus* L. (AZB3456), *Lolium multiflorum* Lam. (AZB3445), *Lolium perenne* L. (AZB3473), *Paspalum dilatatum* Poir. (AZB3447), *Paspalum urvillei* Steud. (AZB3471), *Pennisetum villosum* R. Br. ex Fresen. (AZB3453), *Phalaris minor* Retz. (AZB3476), *Poa annua* L. (AZB3457), *Poa pratensis* L. (AZB3152), *Polypogon viridis* (Gouan) Breistr. (AZB3467), *Rostraria azorica* S. Hend. (AZU1015), *Rostraria cristata* (L.) Tzvelev (AZB3459), *Setaria palmifolia* (Koenig) Stapf (AZB3479), *Setaria parviflora* (Poir.) Kerguélen (AZB3448), *Setaria pumila* (Poir.) Roem. et Schult. (AZB3444), *Setaria verticillata* (L.) P. Beauv. (AZB3446), *Sporobolus africanus* (Poir.) Robyns et Tournay (AZB3442), *Stenotaphrum secundatum* (Walter) Kuntze (AZB3461) and *Zea mays* L. (AZB3482).

Pollen grains from each studied species were subjected to standard acetolysis as described by Erdtman (1952, 1966), and were mounted on at least three permanent slides for each species. The pollen samples were adhered to glycerol jelly, then placed between slide and cover slip and sealed with paraffin. The slides were used for observations and measurements of pollen grains under a light microscope (Zeiss Primo Star) using a 40× lens, measuring pollen grains over an interval of seven days following preparation.

To measure the polar and equatorial axis, 25 undamaged pollen grains were measured (in micrometres) for each species, taken at random. Descriptive statistical analysis of pollen grain morphology used arithmetic mean (x), standard deviation of the mean ($x \pm SD$) and coefficient of variation (CV%). The dimensions (in micrometres) of exine layers, aperture and annulus size were obtained by measuring 10 undamaged pollen grains taken at random.

Acetolysed pollen grains were photo-micrographed under light microscopy (LM) at 100× magnification. Non-acetolysed samples were metalised in gold/palladium using a metal evaporator, and later electromicrographed using a scanning electron microscope model JEOL JSM-5410. The terminology used to describe the pollen grains was based on Barth and Melhem (1988) and Punt et al. (2007).

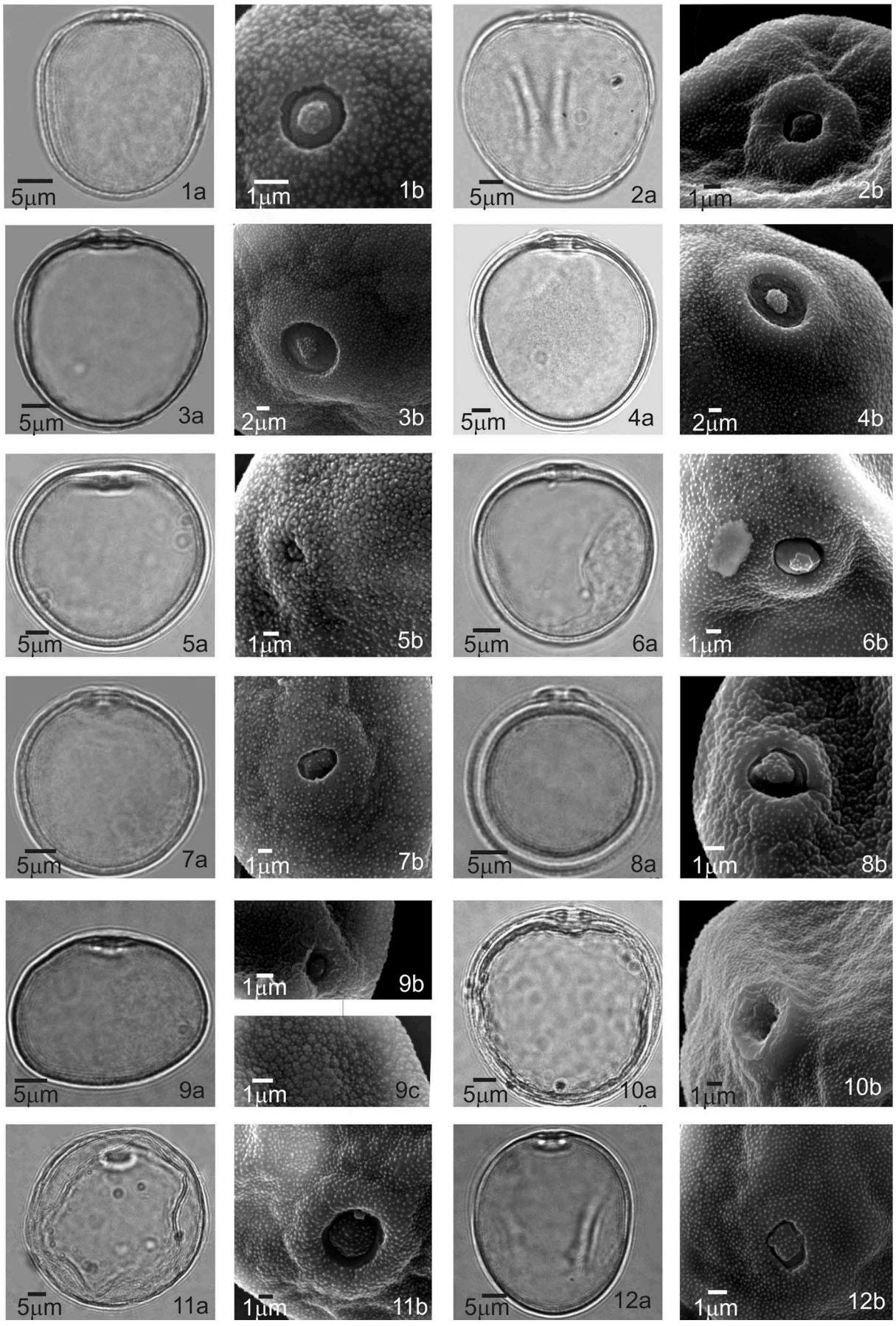
Results

The study of the pollen morphology of the 45 species showed that all pollen grains were monads, with a single pore-type aperture (monoporate), featuring annulus and operculum, and outline in polar view circular, sub-circular and ovalate (Figures 1–4)

The shapes of pollen grains from the specimens ranged between prolate-spheroidal (n = 34),

Table I. Arithmetic mean (x), standard deviation of the mean ($x \pm SD$), coefficient of variation (CV%) and shape of pollen grains of Poaceae species occurring in the Azores, Portugal ($n = 25$).

Species	Polar view (μm)			Equatorial view(μm)			Shape
	Range	$x \pm SD$	CV%	Range	$x \pm SD$	CV%	
Agrostis stolonifera L.	8.0–12.0	9.76 ± 0.21	10.79	8.0–11.0	9.62 ± 0.15	7.84	Prolate-spheroidal
Anthoxanthum odoratum L.	12.5–19.0	15.12 ± 0.26	8.55	13.0–16.2	14.75 ± 0.18	6.13	Prolate-spheroidal
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl. et C. Presl subsp. *bulbosum* (Willd.) Schübl. et Mart	13.0–18.0	16.02 ± 0.18	5.66	13.0–16.0	15.3 ± 0.158	5.17	Prolate-spheroidal
Avena barbata Pott ex Link	14.0–19.0	16.64 ± 0.24	7.33	15.5–19.0	16.64 ± 0.19	5.92	Oblate-spheroidal
Axonopus fissifolius (Raddi) Kuhlm.	13.0–17.0	15.1 ± 0.26	8.55	11.0–18.0	14.80 ± 0.34	11.37	Prolate-spheroidal
Brachypodium distachyon (L.) P. Beauv	12.5–15.0	14.0 ± 0.15	5.44	11.0–16.0	13.95 ± 0.23	8.43	Prolate-spheroidal
Brachypodium sylvaticum (Huds.) P. Beauv	28.2–33.9	30.9 ± 0.26	4.27	26.1–34.2	30.08 ± 0.41	6.85	Prolate-spheroidal
Briza maxima L.	9.0–12.0	10.48 ± 0.13	6.23	9.0–11.0	10.52 ± 0.13	6.05	Spherical
Briza minor L.	10.0–13.0	11.2 ± 0.17	7.84	10.0–12.0	10.52 ± 0.13	6.05	Prolate-spheroidal
Bromus catharticus Vahl	15.0–23,0	18.42 ± 0.41	11.13	15.0–22.0	17.96 ± 0.34	9.57	Prolate-spheroidal
Bromus diandrus Roth	16.0–21.0	18.84 ± 0.27	7.297	16.0–20.0	18.1 ± 0.22	5.97	Prolate-spheroidal
Cortaderia selloana (Schult. et Schult. f.) Asch. et Graebn.	11.0–15.0	12.9 ± 0.21	8.07	10.5–15.0	12.5 ± 0.25	10.07	Prolate-spheroidal
Cynodon dactylon (L.) Pers.	10.0–12.0	10.84 ± 0.14	6.48	9.0–12.0	10.5 ± 0.14	6.59	Prolate-spheroidal
Dactylis glomerata L.	13.5–18.0	15.32 ± 0.21	6.91	13.0–17.0	14.88 ± 0.22	7.53	Prolate-spheroidal
Deschampsia foliosa Hack.	12.0–14.5	12.96 ± 0.16	6.39	11.0–15.0	12.66 ± 0.20	8.12	Prolate-spheroidal
Digitaria sanguinalis (L.) Scop.	11.0–17.0	14.54 ± 0.25	8.71	13.0–17.0	14.88 ± 0.21	7.21	Oblate-spheroidal
Eleusine indica (L.) Gaertn.	11.0–13,0	12.16 ± 0.11	4.55	11.0–13.0	12.16 ± 0.14	5.66	Spherical
Elymus repens (L.) Gould	20.0–24.0	22.16 ± 0.21	4.67	19.8–26.3	22.26 ± 0.25	5.68	Oblato-esferoidais
Festuca arundinacea Schreb.	13.0–18.0	15.06 ± 0.21	6.86	13.0–17.0	14.54 ± 0.21	7.15	Prolate-spheroidal
Festuca francoi Fern. Prieto, C. Aguiar, E. Dias et M. I. Gut	12.0–14.5	13.18 ± 0,15	5.88	10.0–14.0	12.5 ± 0.21	8.25	Prolate-spheroidal
Festuca petraea Guthnick	12.0–17.0	13.68 ± 0.25	9.13	12.0–15,0	13.24 ± 0.18	6.73	Prolate-spheroidal
Gaudinia coarctata T. Durand et Schinz	11.0–14.0	12.96 ± 0.17	6.77	9.0–14.0	12.02 ± 0.25	10.57	Prolate-spheroidal
Holcus azoricus M. Seq. et Castrov.	11.0–15.0	13.10 ± 0.28	10.74	11.0–16.5	12.92 ± 0.27	10.46	Prolate-spheroidal
Holcus lanatus L.	10.0–14.0	12.52 ± 0.17	6.96	11.0–14.0	12.44 ± 0.13	5.23	Prolate-spheroidal
Holcus rigidus Hochst.	11.0–13.5	12.2 ± 0.12	4.88	11.0–14.0	12.04 ± 0.11	4.63	Prolate-spheroidal
Hordeum marinum Huds.	15.0–20.0	18.14 ± 0.35	9.66	15.0–18.0	16.80 ± 0.19	5.70	Prolate-spheroidal
Lagurus ovatus L.	13.0–16.0	15.1 ± 0.15	4.97	11.0–17.0	14.96 ± 0.23	7.65	Prolate-spheroidal
Lolium multiflorum Lam.	13.0–16.0	14.36 ± 0.17	5.82	12.5–15.0	14.10 ± 0.13	4.69	Prolate-spheroidal
Lolium perenne L.	13.0–16.0	14.30 ± 0.15	5.34	13.0–16.0	14.24 ± 0.13	6.09	Spherical
Paspalum dilatatum Poir.	13.0–20.0	15.36 ± 0.28	9.18	13.0–16.0	14.96 ± 0.18	5.94	Prolate-spheroidal
Paspalum urvillei Steud	13.0–16.0	14.60 ± 0.12	4.31	12.0–16.0	14.50 ± 0.18	6.22	Prolate-spheroidal
Pennisetum villosum R. Br. ex Fresen.	17.0—24.0	20.78 ± 0.31	7.42	18.0–23.0	20.22 ± 0.29	7.10	Prolate-spheroidal
Phalaris minor Retz.	15.0–20.0	18.32 ± 0.25	6.82	16.0–20.0	17.92 ± 0.19	5.26	Prolate-spheroidal
Poa annua L.	10.0–12.0	11.56 ± 0.12	5.04	11.0–13.0	12.04 ± 0.11	4.47	Oblate-spheroidal
Poa pratensis L.	12.0–17.0	14.36 ± 0.22	7.75	14.0–17.0	15.24 ± 0.21	6.90	Oblate-spheroidal
Polypogon viridis (Gouan) Breistr.	9.0–12.0	10.56 ± 0.14	6.88	8.0–12.0	10.32 ± 0.17	8.49	Prolate-spheroidal
Rostraria azorica S. Hend.	11.0–17.0	14.76 ± 0.29	9.73	13.2–17.0	15.19 ± 0.17	5.72	Oblate-spheroidal
Rostraria cristata (L.) Tzvelev	10.0–15.0	11.86 ± 0.22	9.47	10.0–14.0	11.06 ± 0.17	7.53	Prolate-spheroidal
Setaria palmifolia (Koenig) Stapf	16.0–20.0	17.48 ± 0.22	6.26	15.0–19.0	17.14 ± 0.20	5.74	Prolate-spheroidal
Setaria parviflora (Poir.) Kerguélen	17.0–25.0	21.14 ± 0.40	9.52	18.0–24.0	20.06 ± 0.25	6.28	Prolate-spheroidal
Setaria pumila (Poir.) Roem. et Schult.	19.5–25.0	21.46 ± 0.32	7.43	18.0–23.0	19.90 ± 0.27	6.80	Prolate-spheroidal
Setaria verticillata (L.) P. Beauv.	10.0–15.0	12.10 ± 0.27	11.32	10.0–15.0	12.53 ± 0.27	10.89	Oblate-spheroidal
Sporobolus africanus (Poir.) Robyns et Tournay	11.0–15.0	13.48 ± 0.18	6.90	11.0–14.5	12.94 ± 0.22	8.52	Prolate-spheroidal
Stenotaphrum secundatum (Walter) Kuntze	15.0–20.0	18.14 ± 0.35	9.66	15.0–18.0	16.80 ± 0.19	5.70	Prolate-spheroidal
Zea mays L.	81.2–105.4	95.2 ± 1.09	5.77	73.7–112.4	95.82 ± 2.23	11.65	Oblate-spheroidal



oblate-spheroidal (n = 8) and spherical (n = 3) (Figures 1–4; Table I). With regard to pollen size, it was observed that most specimens were classified as small, while *Agrostis stolonifera* (Figure 1 (1A,B)) showed very small pollen grains, *Brachypodium* sylvaticum (Figure 1 (7A,B)) had pollen medium-sized grains, and *Zea mays* (Figure 4 (45A–C)) showed large pollen grains (Table II).

The pollen pore from the *Zea mays* specimen had the largest mean diameter (x = 6.1 µm) and smallest annulus (0.54 µm), whereas the other species had mean pore size ranging from 0.98 µm to 2.9 µm, and annuli varying between 0.57 µm and 1.27 µm in size (Table II).

Exine in pollen grains ranged in thickness from thinnest in species *Bromus diandrus* with 0.5 µm to thickest in *Paspalum urvillei* with 1.59 µm. Among the 45 species studied, 29 species showed sexine larger than nexine, whereas ten featured pollen grains with nexine larger than sexine. *Arrhenatherum elatius, Avena barbata, Briza maxima, Bromus catharticus, Dactylis glomerata, Deschampsia foliosa* and *Rostraria azorica* featured sexine as thick as nexine (Table II).

The patterns of ornamentation for sexine are divided into five different types: areolate-microechinate – presence of areolae and microspines (< 1 µm); microechinate – presence of small spines (< 1 µm); verrucate-microechinate – presence of small verrucae and spines (< 1 µm) and microrugulate-microechinate – presence of small rugae and spines (< 1 µm). Based on these patterns of sexine ornamentation, it was observed that 25 species showed pollen wall ornamentation pattern type areolate-microechinate, 16 species had ornamentation pattern microechinate, two species with sexine verrucate-microechinate and two species with sexine ornamentation microrugulate-microechinate (Figures 1–4; Table II).

Discussion

As reported in other palynological studies on Poaceae, pollen grains from this plant group are morphologically similar, making it a stenopollinic family (Melhem et al. 1983; Salgado-Labouriau & Rinaldi 1990; Salgado-Labouriau et al. 1993; Corrêa et al. 2005; da Dórea 2011). In this work, the species also remained stenopollinic, with pollen grains monads, monoporate, featuring annuli and opercula, with small differences in size, shape and sexine ornamentation.

A majority of species showed small pollen size, with the exception of *Agrostis stolonifera* with very small pollen, *Brachypodium sylvaticum* with medium size pollen, and *Zea mays* with large pollen grains. Three species – *Briza maxima* (Figure 1 (8A)), *Eleusine indica* (Figure 2 (17A)) and *Lolium perenne* (Figure 3 (29A)) – featured shape spherical, while most were prolate-spheroidal and oblate-spheroidal. This is in agreement with Melhem et al. (1983), Salgado-Labouriau and Rinaldi (1990), Salgado-Labouriau et al. (1993) and Corrêa et al. (2005), who in their studies found Poaceae pollen grains

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Figure 1. Photomicrographs and electromicrographs of pollen grains from the family Poaceae. **1A, B.** *Agrostis stolonifera* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **2A, B.** *Anthoxanthum odoratum* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **3A, B.** *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl. et C. Presl subsp. *bulbosum* (Willd.) Schübl. et Mart. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **4A, B.** *Avena barbata* Pott ex Link. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **5A, B.** *Axonopus fissifolius* (Raddi) Kuhlm. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **6A, B.** *Brachypodium distachyon* (L.) P. Beauv. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **7A, B.** *Brachypodium sylvaticum* (Huds.) P. Beauv. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **8A, B.** *Briza maxima* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **9A–C.** *Briza minor* L. Outline in equatorial view (**A**); detail of pore (**B**) and ornamentation (**C**). **10A, B.** *Bromus catharticus* Vahl. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **11A, B.** *Bromus diandrus* Roth. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **12A, B.** *Cortaderia selloana* (Schult. et Schult.f.) Asch. et Graebn. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**).

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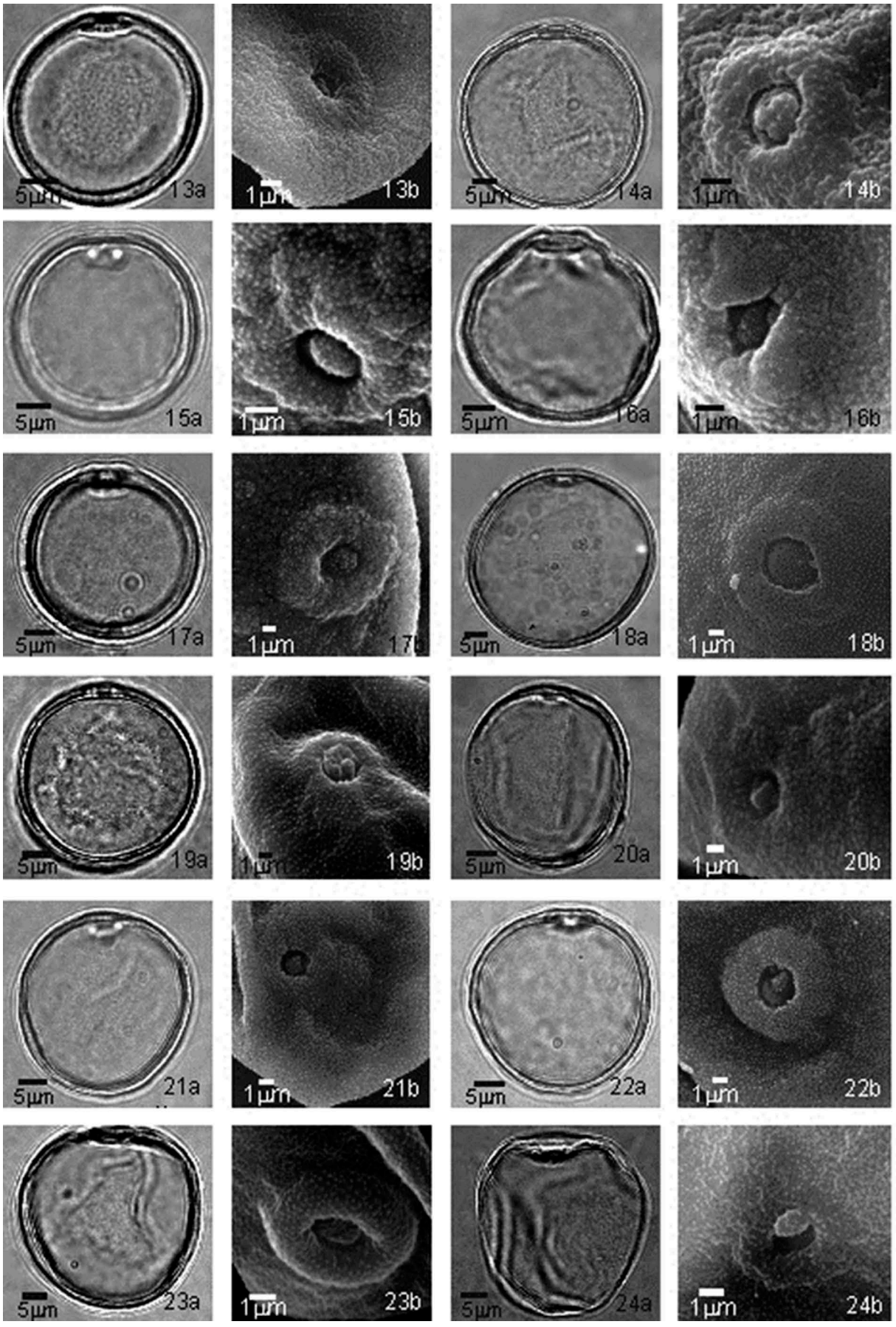
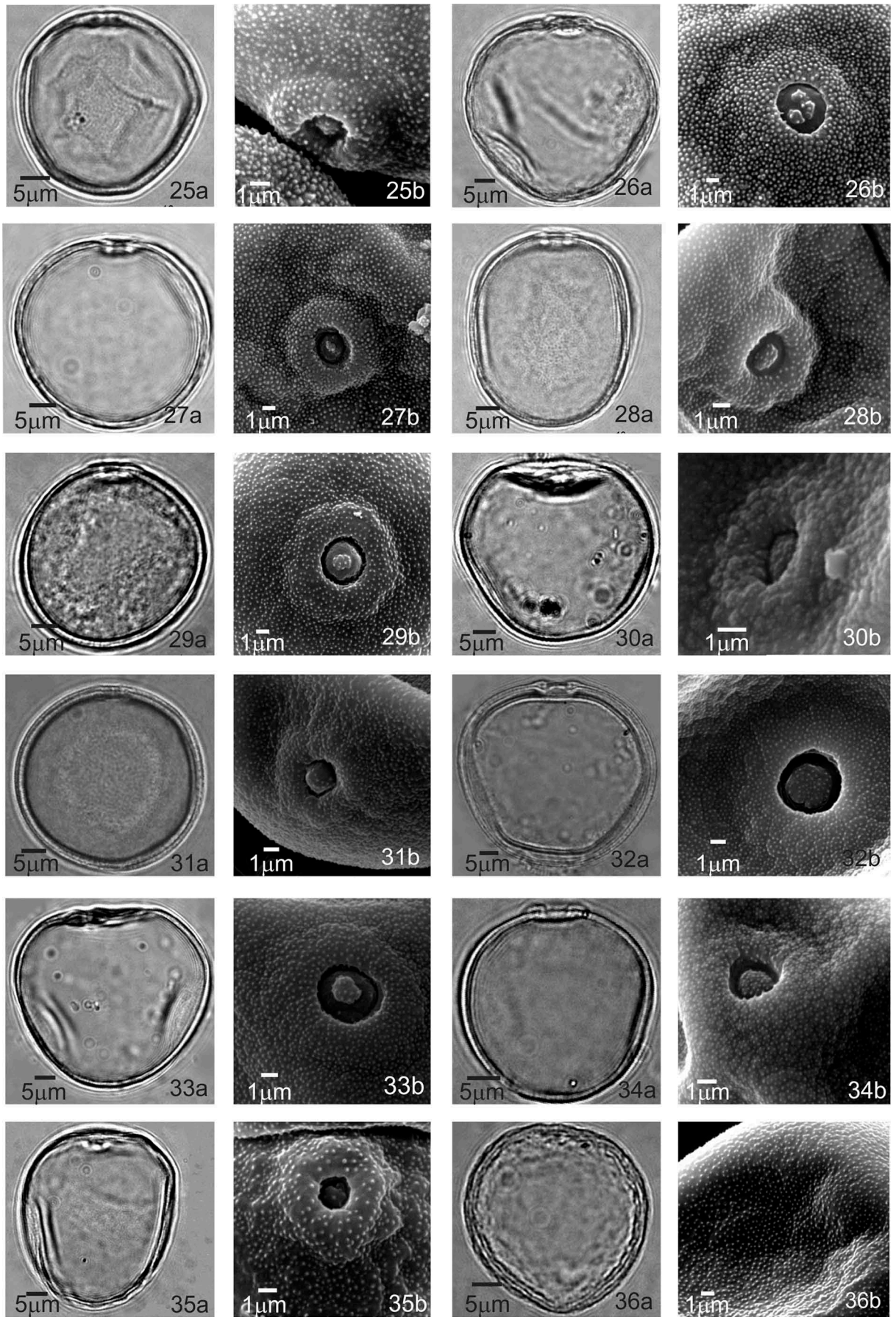
Figure 2. Photomicrographs and electromicrographs of pollen grains from the family Poaceae. **13A, B.** *Cynodon dactylon* (L.) Pers. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **14A, B.** *Dactylis glomerata* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **15A, B.** *Deschampsia foliosa* Hack. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **16A, B.** *Digitaria sanguinalis* (L.) Scop. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **17A, B.** *Eleusine indica* (L.) Gaertn. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **18A, B.** *Elymus repens* (L.) Gould. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **19A, B.** *Festuca arundinacea* Schreb. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **20A, B.** *Festuca francoi* Fern. Prieto, C. Aguiar, E. Dias et M. I. Gut. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **21A, B.** *Festuca petraea* Guthnick Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **22A, B.** *Gaudinia coarctata* T. Durand et Schinz. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **23A, B.** *Holcus azoricus* M. Seq. et Castrov. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **24A, B.** *Holcus lanatus* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**).





with shape prolate-spheroidal and oblate-spheroidal. The prolate-spheroidal shape of pollen from *Paspalum urvillei* has also been cited by Corrêa et al. (2005), but with differences regarding the dimensions found in this work.

Da Dórea (2011) analysed the pollen morphology of 86 taxa distributed into 29 genera and subordinate to four subfamilies found in Atlantic Forest remnants at an ecological reserve in the state of Bahia, Brazil. Among the species analysed by that author are *Paspalum corcovadense* Raddi and *Setaria sulcata* Raddi. These species are different from those in the present study and the results obtained here in the definition of ornamentation, were also different – that is, the authors regarded the ornamentation of both species as areolate-microechinate, whereas in the species analysed herein ornamentation ranged from areolate-microechinate to microrugulate-echinate.

For any palynological study of Poaceae it is essential to prepare material for analysis under scanning electron microscopy (SEM), obtaining images with at least 5000× magnification (5 μm), as this will make it possible to obtain detailed information on the ornamentation of sexine and other morphological structures found on the exine of pollen grains, which are undetectable under LM.

Previous works using only LM for pollen analysis (Wodehouse 1935; Erdtman 1943, 1966; Campos & Salgado-Labouriau 1962; Melhem et al. 1983) refer to the ornamentation of pollen grains in Poaceae as psilate, or with sexine featuring discrete ornamentation.

According to Silva et al. (2005), there are approximately 947 vascular taxa in the Azores among them endemic species and subspecies, native and introduced taxa. The archipelago is characterised by a flora with a small number of native species and only 7.2% of endemism of the Azores, from which 7% belong to clade Monocotiledonea. Within this clade, there are 228 taxa described in the archipelago, from which four species are endemic to the Macaronesian biogeographic region. Among the six endemic genus and 12 endemic species of the Azorean Poaceae, only the genus *Agrostis* was not represented in this study thus, our work has analysed 58% of the all the species found in this family, endemic to the Azores. The main reason for this fact is that the specimens belonging to this genus came from a herbarium collection, and had no pollen.

Species endemic to the archipelago of the Azores (*Deschampsia foliosa* [Figure 2 (15A,B)], *Festuca francoi* [Figure 2 (20A,B)], *Festuca petraea* [Figure 2 (21A,B)], *Gaudinia coarctata* [Figure 2 (22A,B)], *Holcus azoricus* [Figure 2 (23A,B)], *Holcus rigidus* [Figure 3 (25A,B)] and *Rostraria azorica* [Figure 4 (37A,B)]) showed an ornamentation pattern ranging from areolate-microechinate, microechinate, microrugulate-microechinate and verrucate-microechinate, with most having exine surface areolate-microechinate. Among all endemic species, *Rostraria azorica* stood out for its oblate-spheroidal shape, whereas most featured shape prolate-spheroidal.

Within *Rostraria*, there is a difference in the ornamentation found on the pollen of the endemic species (*R. azorica*) and of the introduced one (*R. cristata*), with the endemic species presenting an areolate-microechinate ornamentation while the

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Figure 3. Photomicrographs and electromicrographs of pollen grains from the family Poaceae. **25A, B.** *Holcus rigidus* Hochst. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **26A, B.** *Hordeum marinum* Huds. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **27A, B.** *Lagurus ovatus* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **28A, B.** *Lolium multiflorum* Lam. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **29A, B.** *Lolium perenne* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **30A, B.** *Paspalum dilatatum* Poir. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **31A, B.** *Paspalum urvillei* Steud. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **32A, B.** *Pennisetum villosum* R. Br. ex Fresen. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **33A, B.** *Phalaris minor* Retz. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **34A, B.** *Poa annua* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **35A, B.** *Poa pratensis* L. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **36A, B.** *Polypogon viridis* (Gouan) Breistr. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**).

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Figure 4. Photomicrographs and electromicrographs of pollen grains from the family Poaceae. **37A, B.** *Rostraria azorica* S. Hend. Outline in equatorial view (**A**); detail of pore (**B**). **38A, B.** *Rostraria cristata* (L.) Tzvelev. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **39A, B.** *Setaria palmifolia* (Koenig) Stapf. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **40A, B.** *Setaria parviflora* (Poir.) Kerguélen. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **41A, B.** *Setaria pumila* (Poir.) Roem. et Schult. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **42A, B.** *Setaria verticillata* (L.) P. Beauv. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **43A, B.** *Sporobolus africanus* (Poir.) Robyns et Tournay. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **44A, B.** *Stenotaphrum secundatum* (Walter) Kuntze. Outline in equatorial view (**A**); detail of pore and ornamentation (**B**). **45A–C.** *Zea mays* L. Outline in equatorial view (**A**); detail of pore (**B**) and ornamentation (**C**).

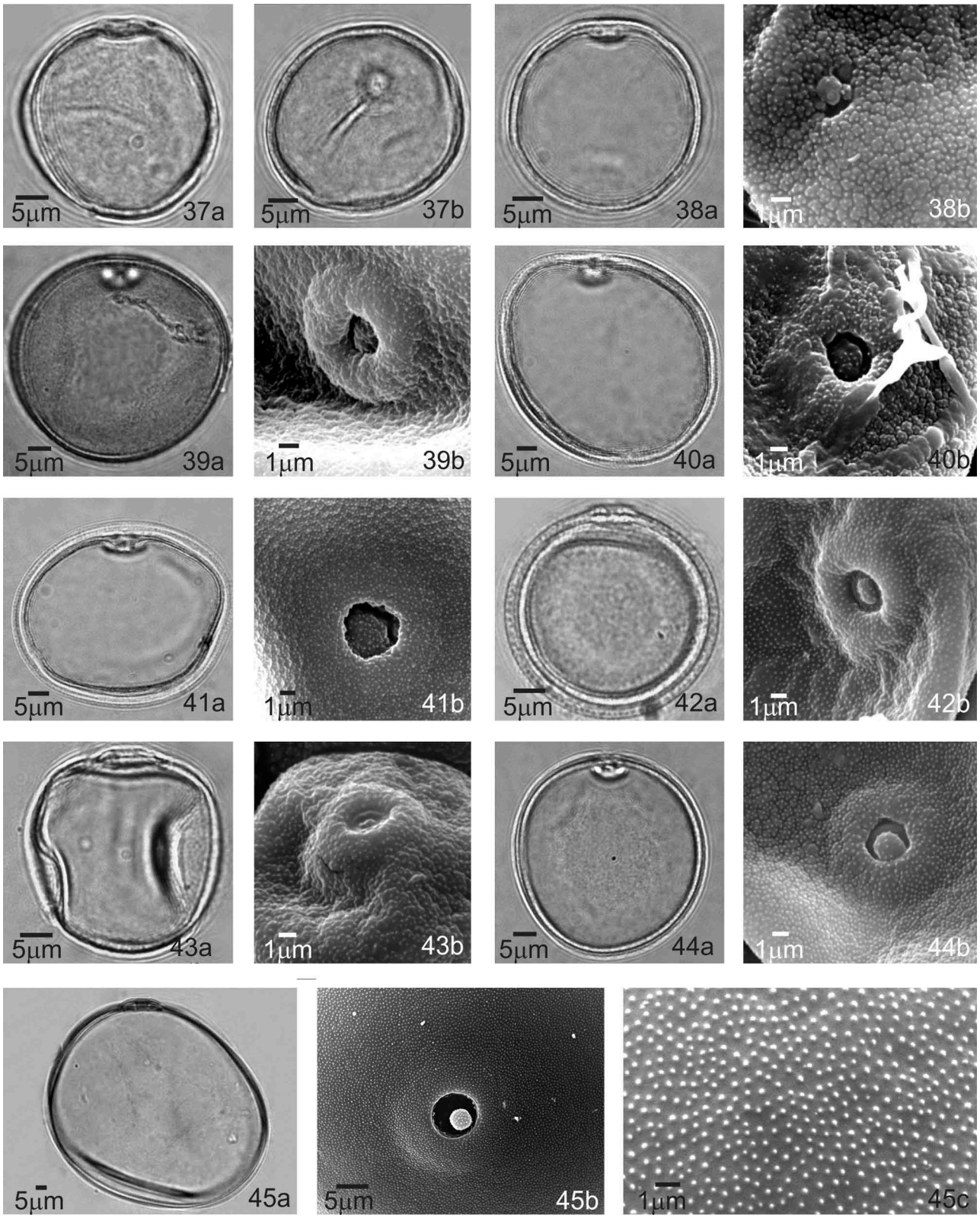

Table II. Arithmetic mean (x), standard deviation of the mean ($x \pm SD$), of the aperture (pore and annulus) and exine (sexine and nexine) of Poaceae species occurring in the Azores, Portugal ($n = 10$).

		Aperture (μm)		Exine	(μm)	
Species	Size	Pore $x \pm SD$	Annulus $x \pm SD$	Sexine $x \pm SD$	Nexine $x \pm SD$	Ornamentation
Agrostis stolonifera	very small	1.26 ± 0.30	0.91 ± 0.50	0.30 ± 0.05	0.28 ± 0.05	Areolate-microechinate
Anthoxanthum odoratum	small	1.36 ± 0.30	0.90 ± 0.06	0.32 ± 0.03	0.30 ± 0.03	Microechinate
Arrhenatherum elatius ssp. *bulbosum*	small	1.96 ± 0.25	0.84 ± 0.04	0.45 ± 0.06	0.45 ± 0.04	Microechinate
Avena barbata	small	1.58 ± 0.40	0.66 ± 0.10	0.28 ± 0.08	0.28 ± 0.06	Microechinate
Axonopus fissifolius	small	1.45 ± 0.17	0.64 ± 0.60	0.45 ± 0.03	0.39 ± 0.03	Microrugulate-microechinate
Brachypodium distachyon	small	1.45 ± 0.20	0.89 ± 0.06	0.52 ± 0.10	0.47 ± 0.05	Microechinate
Brachypodium sylvaticum	medium	2.59 ± 0.30	0.96 ± 0.05	0.44 ± 0.09	0.34 ± 0.10	Microechinate
Briza maxima	small	1.01 ± 0.07	0.86 ± 0.10	0.44 ± 0.07	0.44 ± 0.09	Areolate-microechinate
Briza minor.	small	1.18 ± 0.25	0.76 ± 0.05	0.43 ± 0.03	0.40 ± 0.04	Areolate-microechinate
Bromus catharticus	small	1.68 ± 0.28	0.77 ± 0.03	0.45 ± 0.06	0.45 ± 0.05	Microechinate
Bromus diandrus	small	2.23 ± 0.40	0.83 ± 0.06	0.26 ± 0.02	0.24 ± 0.04	Microechinate
Cortaderia selloana	small	1.65 ± 0.40	0.94 ± 0.05	0.47 ± 0.03	0.44 ± 0.05	Microechinate
Cynodon dactylon	small	1.0 ± 0.06	1.02 ± 0.04	0.71 ± 0.20	0.49 ± 0.01	Microechinate
Dactylis glomerata	small	1.5 ± 0.47	0.57 ± 0.08	0.48 ± 0.03	0.47 ± 0.03	Areolate-microechinate
Deschampsia foliosa	small	1.22 ± 0.19	0.63 ± 0.10	0.26 ± 0.03	0.25 ± 0.01	Areolate-microechinate
Digitaria sanguinalis	small	1.44 ± 0.30	0.89 ± 0.06	0.52 ± 0.14	0.43 ± 0.06	Areolate-microechinate
Eleusine indica	small	1.75 ± 0.26	0.97 ± 0.08	0.45 ± 0.05	0.50 ± 0.00	Areolate-microechinate
Elymus repens	small	1.81 ± 0.13	1.27 ± 0.14	0.33 ± 0.07	0.37 ± 0.05	Microechinate
Festuca arundinacea	small	1.62 ± 0.30	0.87 ± 0.050	0.61 ± 0.20	0.43 ± 0.06	Microechinate
Festuca francoi	small	1.16 ± 0.16	0.81 ± 0.04	0.35 ± 0.07	0.39 ± 0.08	Areolate-microechinate
Festuca petraea	small	1.39 ± 0.30	0.82 ± 0.05	0.29 ± 0.04	0.34 ± 0.06	Areolate-microechinate
Gaudinia coarctata	small	1.18 ± 0.15	0.75 ± 0.08	0.38 ± 0.08	0.37 ± 0.08	Areolate-microechinate
Holcus azoricus	small	1.34 ± 0.29	1.03 ± 0.08	0.46 ± 0.04	0.44 ± 0.03	Microechinate
Holcus lanatus	small	2.10 ± 0.30	0.68 ± 0.10	0.47 ± 0.03	0.43 ± 0.05	Microechinate
Holcus rigidus	small	1.24 ± 0.20	0.93 ± 0.05	0.41 ± 0.06	0.47 ± 0.03	Verrucate-microechinate
Hordeum marinum	small	1.50 ± 0.47	0.88 ± 0.07	0.49 ± 0.02	0.45 ± 0.05	Microechinate
Lagurus ovatus	small	1.42 ± 0.26	0.67 ± 0.15	0.52 ± 0.10	0.47 ± 0.19	Microechinate
Lolium multiflorum	small	1.65 ± 0.30	0.96 ± 0.05	0.43 ± 0.06	0.38 ± 0.05	Microechinate
Lolium perenne	small	1.28 ± 0.34	0.71 ± 0.05	0.39 ± 0.09	0.41 ± 0.08	Microechinate
Paspalum dilatatum	small	1.40 ± 0.29	0.68 ± 0.10	0.32 ± 0.06	0.28 ± 0.06	Areolate-microechinate
Paspalum urvillei	small	1.15 ± 0.20	0.93 ± 0.06	1.09 ± 0.50	0.50 ± 0.09	Microechinate
Pennisetum villosum	small	2.90 ± 0.20	0.95 ± 0.05	0.45 ± 0.05	0.47 ± 0.03	Microrugulate-microechinate
Phalaris minor	small	1.38 ± 0.30	0.74 ± 0.05	0.43 ± 0.08	0.47 ± 0.05	Microechinate
Poa annua	small	2.10 ± 0.70	0.93 ± 0.06	0.50 ± 0.02	0.48 ± 0.02	Areolate-microechinate
Poa pratensis	small	1.95 ± 0.15	0.78 ± 0.05	0.48 ± 0.02	0.42 ± 0.07	Verrucate-microechinate
Polypogon viridis	small	1.18 ± 0.20	0.58 ± 0.10	0.46 ± 0.02	0.42 ± 0.05	Microechinate
Rostraria azorica	small	1.62 ± 0.40	0.90 ± 0.05	0.45 ± 0.06	0.45 ± 0.07	Areolate-microechinate
Rostraria cristata	small	1.30 ± 0.30	0.89 ± 0.05	0.43 ± 0.05	0.41 ± 0.05	Rugulate-microechinate
Setaria palmifolia	small	1.75 ± 0.30	0.64 ± 0.09	0.41 ± 0.08	0.40 ± 0.07	Rugulate-microechinate
Setaria parviflora	small	1.79 ± 0.30	0.89 ± 0.06	0.77 ± 0.30	0.54 ± 0.10	Areolate-microechinate
Setaria pumila	small	1.75 ± 0.30	0.87 ± 0.05	0.78 ± 0.20	0.47 ± 0.04	Areolate-microechinate
Setaria verticillata	small	0.98 ± 0.04	0.85 ± 0.08	0.41 ± 0.07	0.40 ± 0.06	Microechinate
Sporobolus africanus	small	1.65 ± 0.30	0.99 ± 0.06	0.44 ± 0.04	0.40 ± 0.04	Areolate-microechinate
Stenotaphrum secundatum	small	1.50 ± 0.47	0.93 ± 0.06	0.49 ± 0.02	0.45 ± 0.05	Areolate-microechinate
Zea mays	large	6.10 ± 0.30	0.54 ± 0.05	0.63 ± 0.18	0.68 ± 0.15	Microechinate

other presents a rugulate-microechinate. Since the introduced species has become invasive, these differences on their pollens can be important to indicate possible hybridisation between the two species.

However, and based on morphological characters and chromosome number, *Holcus azoricus* may have a hybrid origin possibly from the species *H. lanatus* and *H. rigidus* (de Sequeira & Castroviejo 2007). According to the morphological characteristics of the pollen grains of these three species, one can observe differences in the ornamentation: *H. azoricus* and *H. lanatus* have a microechinate standard while *H. rigidus* has a verrucate-microechinate one, which can be an indication of the hybrid origin of *H. azoricus*.

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No potential conflict of interest was reported by the authors.

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