Taxonomy, palynology, and seed morphology of *Paepalanthus decumbens*, a new species of Eriocaulaceae from Minas Gerais, Brazil

Marcelo Trovó1,*, Wellerson L. Picanço2 & Vânia Gonçalves-Esteves2

1Departamento de Botânica, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
2Departamento de Botânica, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
*Author for correspondence: martrovo@gmail.com

INTRODUCTION

Morphological analysis of pollen grains and seeds are regarded as useful tools to clarify the evolutionary patterns and relationships among Eriocaulaceae (Stützel 1984, Scatena & Bouman 2001, Borges et al. 2009). However, the information available on these topics is scarce and limited to a few taxa. The vast majority of the descriptions of Eriocaulaceae species do not include data on the pollen grains and seeds, and knowledge of these structures is usually a result of isolated efforts (e.g. Kraus et al. 1996, Santos et al. 2000, Coan & Scatena 2004, Coan et al. 2007a, 2007b). A comprehensive compendium on Eriocaulaceae palynology was provided by Borges et al. (2009), while the last broad review of seed morphology in Eriocaulaceae was provided by Scatena & Bouman (2001). Among these studies, both Scatena & Bouman (2001) and Borges et al. (2009) embraced an evolutionary and taxonomic context, highlighting the relevance of detailing the species of *Paepalanthus* Mart.

*Paepalanthus* is the largest genus of Neotropical Eriocaulaceae and the largest genus of Brazilian monocots. Much of its taxonomic and morphological diversity remains unknown. A new species is described, with details on pollen and seed morphology.

Methods – The morphological description of the vegetative and floral characters was made using a stereoscopic microscope with camera. Pollen and seed samples from the new species and from two related taxa were studied under light microscopy and scanning electron microscopy.

Key results – *Paepalanthus decumbens* is described and illustrated, including details of pollen grains and seeds. The species is restricted to a small area in the campos rupestres of Serra de São José (Minas Gerais, Brazil), and therefore should be considered as critically endangered. The decumbent stem, falciform leaves with long trichomes along the margins, the numerous and sericeous scapes of roughly the same size of the leaves, and the dark castaneous involucral bracts are useful features for the recognition of the new species. *Paepalanthus decumbens* is placed in *P.* subsect. *Polyactis* and compared with *P. microphyllus* and *P. stannardii*, which have their pollen grains and seeds also herein described for the first time.

Key words – Flower morphology, Minas Gerais, Poales, pollen grains, seeds, taxonomy.
Trovó, Picanço & Gonçalves-Esteves, Taxonomy, palynology, and seed morphology of *Paepalanthus decumbens*

Sauthier 2017, Barbosa-Silva et al. 2018). However, as most of the *Paepalanthus* species are micro-endemic, usually restricted to areas within a single mountain or few adjacent mountains (Giulietti et al. 2005), some well-documented areas still host undescribed species (e.g. Trovó et al. 2011, 2013b, 2015, 2017).

In this article, a new species of *Paepalanthus* subsect. Polyactis is described and illustrated from Serra de São José, a small quartzite mountain range in the south of Minas Gerais (Brazil). The flora of Serra de São José is relatively well known, with historical collections and a recent checklist of 1144 vascular plants available (Alves & Kolbek 2009). A few detailed floristic treatments for families occurring in the area were previously published, such as for Lentibulariaceae and Melastomataceae (Drummond et al. 2007, Silva et al. 2011). Recent taxonomic novelties from Serra de São José are significant in different plant groups, as for example in Euphorbiaceae (Medeiros et al. 2009, Medeiros et al. 2011), Melastomataceae (Alves et al. 2008), Orchidaceae (Alves 1991a, 1991b, 1992a, 1992b, 1992c), and Velloziaceae (Alves 1992c). Besides the description of a new species of Ericaceae for the area, a comparison with the morphologically similar species *Paepalanthus microphyllus* (Guill.) Kunth and *Paepalanthus stannardii* Giul. & L.R.Parra is provided. Details on the morphology of pollen grains and seeds of the three species are also presented and discussed.

**MATERIAL AND METHODS**

The morphological description of the vegetative and floral characters was made using a stereoscopic Leica EZ4 microscope with a camera, with which seeds and other reproductive characters were measured. The description and measurements are representative of both herbarium and living specimens. The morphological terminology follows Radford (1974), Weberling (1989), and Stützel & Trovó (2013). The taxonomic species concept adopted follows Stuessy (1990). Relevant herbaria were consulted and herbarium acronyms are cited according to Thiers (continuously updated).

For the morphometric studies of the seeds, the length, width, and thickness were measured from 30 mature released seeds from 2 specimens per species. The morphology of the seed testa was described according to Barthlott (1981) and Radford (1974) and measurements were performed using a digital caliper. Pollen samples were prepared for study under light microscopy according to the acetylation method by Erdtman (1952) modified by Melhem et al. (2003), mounted in glycerol jelly. Subsequently, the following pollen characters were measured: diameters of the polar and equator axes from 25 grains; thickness of exine layers, width of mesocolpium, and dimensions of spines. Terminology followed Punt et al. (2007).

For the scanning electron microscopy (SEM) analysis, both non-acetylation pollen grains and dry seeds were arranged over a metal stub previously covered with carbon tape and subsequently sputter coated with a thin palladium-gold layer and examined using a Jeol Model JSM 6390 SEM at the Department of Invertebrates of the National Museum / Federal University of Rio de Janeiro (UFRJ) and a Jeol Model JSM 6510 SEM from the Biology Institute of UFRJ.

The analysis of pollen grains and seeds of *Paepalanthus decumbens* was made directly from the type specimens. For the analysis of pollen grains (*) and seeds (**) of *P. microphyllus* and *P. stannardii*, the following herbarium sheets are cited as examined specimens: *P. microphyllus*: Brazil, Minas Gerais, 30 Apr. 1981, Giulietti et al. CFSC 7359 (SPF**); ibid., 22 Jul. 1985, Zappi et al. CFSC 9355 (SPF**); ibid., 30 Apr. 1989, Giordano & Toscano 687 (RB*); ibid., 2 May 1993, Sonza & Sakuragui 3421 (SPF); ibid., 9 Jun. 2002, Pirani et al. 5092 (SPF); ibid., 22 May 2007, Trovó 395 (SPF); ibid., 29 May 2007, Penna et al. 113 (SPF); ibid., s.d., Joly et al. CFCR 2453 (SPF); *P. stannardii*: Brazil, Bahia, 19 Mar. 1977, Harley 19725 (SPF); ibid., 24 Jul. 1979, Mori et al. 12539 (RB*); ibid., 20 Feb. 1987, Harley et al. 24476 (SPF**); ibid., 13 Nov. 1988, Harley et al. 26146 (SPF); ibid., 9 Aug. 1993, Ganev 2076 (SPF); ibid., 13 Jul. 2006, Trovó et al. 283 (SPF); ibid., 5 Jul. 2009, Echternacht & Giaconim 2057 (SPF).

**RESULTS AND DISCUSSION**

*Paepalanthus decumbens* Trovó & Picanço, sp. nov.

**Diagnosis** – The following character combination is useful to recognize *Paepalanthus decumbens*: ramified and decumbent stem, falciform leaves with long trichomes along the margin, numerous and sericeous scapes of roughly the same size of the leaves, and dark castaneous involucral bracts. The species differs from *P. microphyllus*, a similar species, by being of a more robust habit, with longer and wider leaves with trichomes, wider capitula, and by the simple stigmatic branches of the gynoeicum. – Type: Brazil, Minas Gerais, Mun. Prados, Serra de São José, 21°04′6.597″S, 44°08′14.182″W, 6 Oct. 2016, Picanço et al. 69 (holo-: R; iso-: B, BHCB, P, NY, SPF, RB).

Perennial herbs, rhizome absent. Stem elongate, decumbent, branched, 15–75.0 cm long; branches arising from the elongate stem, c. 10–25 per flowering event, persistent, decumbent to ascending, 4.0–22.0 cm long, usually branching below the inflorescence. Leaves along the elongate stem and branches, persistent, deciduous only at the stem base, amplexicaul, falciform, chartaceous, not markedly veined, 1.0–3.0 × 0.1–0.3 cm, green, pilose on both surfaces to glabrous, margin densely ciliate with long trichomes at the base to sparsely ciliate with short trichomes toward the acuminate apex. Sheaths lax, membranaceous, 0.4–0.5 cm long, whitish, glabrous, apex truncate, lacerate, ciliate. Scapes free, 5–22 terminating the branch, surrounded by foliaceous bracts, 1.5–2.5 cm long, multicoate, sericeous. Capitula c. 4.0 mm diam., hemispheroïd, whitish. Involucral bracts in 2–3 series, delicate to ovate, c. 3 mm long, dark castaneous, whitish at the base, sparsely pilose to glabrescent, ciliate toward the acute to acuminate, tufted apex. Flowers 3-merous, c. 15–40 per capitula; 2 × more staminate than pistillate flowers; floral bracts linear to oblanceolate, c. 3.0 mm long, castaneous, pilose on the distal part of the abaxial surface, ciliate toward the acute, apex tufted. Staminate flowers c. 2.5 mm long; pedicel c. 0.5 mm long, with long trichomes; sepals fused only at the base, navicular, castaneous, c. 2.0 mm long, pilose on the upper part of the abaxial surface to glabrescent, ciliate toward the acute, tufted apex; corolla tubular, slightly 3–lobed,
membranaceous, glabrous, cream-colored and hyaline, when old with margin rolled inward; stamens c. 2.0–2.5 mm long, anthers hyaline; pistillodes 3, reddish, papillose. **Pistillate flowers** c. 2.5 mm long, sessile to shortly pedicellate, pedicel c. 0.2 mm long, with long trichomes; sepals fused only at the base, navicular, c. 2.5 mm long, castaneous, pilose on the distal part of the abaxial surface, ciliate toward the acute, tufted apex; petals membranaceous, oblong to obovate, c. 2.0 mm long, whitish and hyaline, pilose on the distal part of the abaxial surface, ciliate toward the acute, tufted apex; gynoeceum c. 2.0 mm long, ovary dark brown, stigmatic branches simple, longer than the whitish and plumose nectariferous branches; staminodes 3, scale-like. **Fruit** a loculicidal capsule. Figs 1 & 2.

Figure 1 – Habitat, habit, and details of *Paepalanthus decumbens* (from Picanço et al. 69, R). A, habitat, the campos rupestres in Serra de São José, Minas Gerais; B, general habit, lateral view; C, general habit, apical view; D, branching pattern detail; E, terminal inflorescences, view from the side; F, terminal inflorescences, view from above. Photographs: W.L. Picanço.
Pollen morphology – The pollen grains are monads, medium sized, spheroidal (fig. 3A & B). The aperture shows a spiral pattern, which turns one time on the pollen grain, with an ornamented membrane (fig. 3C). However, as artifacts of the acetolysis method, other arrangements can also be found (fig. 3D & E). The nexine is thicker than the sexine in Paepalanthus stannardii, as thick as the sexine in P. decumbens, and less thick than the sexine in P. microphyllus (table 1); the collumelae are just visible in P. decumbens. The sexine is tectate and its main ornamentation is echinate, also with microspines and (micro-)granula as secondary supractal elements (fig. 3B–K); spines are in general acute
Figure 3 – Pollen morphology of *Paepalanthus decumbens* and related species. A–E, *P. decumbens* (from Picanço et al. 69, R); F–H, *P. microphyllus* (from Giordano & Toscano 687, RB); I–K, *P. stannardii* (from Mori et al. 12539, RB). A, optical section; B, grain, showing the spiraled aperture (SEM); C, detail of aperture and sexine surface (SEM); D, high focus of microreticule and supratectal microelements; E, low focus of microreticulate sexine surface and supratectal microelements; F, low focus showing the apertural pattern and ornamented membrane; G, general view showing the spiroaperture and the ornamentation (SEM); H, detail of the ornamentation; I, low focus showing the microreticule and a random conformation of the aperture; J, general view of the whole grain with an incomplete loop of the aperture in the middle of the grain (SEM); K, detail of the ornamentation and the aperture with an ornamented membrane. Scale bars: A, B, D–G, I & J = 5 µm; C, H & K = 1 µm.
in *P. decumbens*, but spines with the same dimensions as in *P. microphyllus* and *P. stannardii* (table 1) were also found; the spine walls are straight and concave in *P. stannardii*, and straight in the other species (fig. 3B, C, G, J & K). The testa surface is microreticulate and very irregular (fig. 3C–E, H, I & K), showing a rugulate pattern. Supratectal structures, mainly (micro-)granula, are positioned at the edges of the apertures (fig. 3B–K).

**Seed morphology** – The largest seeds were found in *Paepalanthus decumbens* and the smallest in *P. decumbens* (table 2). Seed shape is elliptic in frontal, dorsal, and lateral views (fig. 4A) and circular to elliptical when observed in apical and basal views (fig. 4B). The apex of the seed varied among the species: it is mucronate in *P. decumbens* (fig. 4A), acuminate in *P. microphyllus* (fig. 4D), and mucronulate in *P. stannardii* (fig. 4G). The base is truncate in *P. decumbens* and *P. microphyllus* (fig. 4A); and varied from truncate to slightly rounded in *P. stannardii* (fig. 4G). The operculum is circular and prominent (fig. 4E & H), but in *P. decumbens* it is within a cavity (fig. 4B).

The primary sculpture of the seeds is composed of the external periclinal cell walls of the outer tegument. These projections are more stretched in the transversal boards than in the longitudinal boards. Cells are arranged in rows, from the base until the apex, showing a reticulate pattern over the seed surface, mainly due to the highlighted anticlinal projections in the outline of the cells (fig. 4A, F & G). The external cell wall outline varied from rectangular to hexagonal among the species (fig. 4). Furthermore, its surface is convex in *Paepalanthus microphyllus* (fig. 4D–F) and straight in the other species (fig. 4A–C & G–I). Along the longitudinal cell borders, the anticlinal wall is thicker, forming conspicuous longitudinal lines, called ribs (fig. 4B & H), which varied in number: 16 were found in *P. microphyllus*, 15 to 18 in *P. decumbens*, and 18 in *P. stannardii*. The ribs of *P. microphyllus* are composed of more thickened and prominent anticlinal cell walls than those found along the transversal axis, but along the ribs it is not possible to recognize where each cell ends (fig. 4F). However, *P. decumbens* and *P. stannardii* have bristle-like anticlinal projections along the ribs, which make them highlighted, building together the secondary sculpture of the external tegument (fig. 4A–C & G–I). These bristles have lateral projections towards the area between the ribs and towards the longitudinal axis, fused with the wall of the next column (like a membrane) as seen in *P. stannardii* seeds (fig. 4I), or not, as in *P. decumbens*, which has free columns (fig. 4C). The anticlinal projections are very prominent, extending 35 µm in *P. decumbens* and 25 µm in *P. stannardii* ending in a patelliform apex, which can appear like a continuous strip in *P. stannardii* (fig. 4C & I). They are positioned at each corner of the periclinal cell wall outline (fig. 4C, F & I).

**Paratype** – Brazil: Minas Gerais, Tiradentes, Serra de São José, 25 Sep. 2014, Couto 2971 (R).
Figure 4 – Scanning electron photomicrographs of seed testa ornamentation of *Paepalanthus decumbens* and allied species. A–C, *P. decumbens* (from Picanço et al. 69, R); D–F, *P. microphyllus* (from Giulietti et al. CFSC7359, SPF); G–I, *P. stannardi* (from Harley et al. 24476, SPF). A, lateral view of the seed making evident the apex turned to the distal face of the seed; B, base of the seed with the operculum in the middle of a cavity; C, detail of the secondary sculpture of the external tegument; D, seed apex; E, detail of the inconspicuous operculum; F, detail of the primary sculpture of the external tegument; G, lateral view of the seed; H, base of the seed showing an evident operculum bordered by a ring of fused cell walls; I, detail of the secondary sculpture of the external tegument where it is possible to see the anticlinal cell wall projections in the corners of the external cell wall outline. Scale bars: A, B, G & H = 100 µm; C–F & I = 20 µm.
**Table 3 – Main morphological differences of** Paepalanthus decumbens, P. microphyllus, and P. stannardii.**

All measurements for *P. microphyllus* and *P. stannardii* are cited from Koernicke (1863), Ruhland (1903), and Giulietti & Parra (1995), combined with the herbarium specimens.

<table>
<thead>
<tr>
<th></th>
<th><em>P. decumbens</em></th>
<th><em>P. microphyllus</em></th>
<th><em>P. stannardii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall size (cm)</strong></td>
<td>15–75</td>
<td>10–45</td>
<td>100–300</td>
</tr>
<tr>
<td><strong>Leaf size (cm)</strong></td>
<td>1.0–3.0 × 0.1–0.3</td>
<td>0.2–0.3 × 0.05–0.1</td>
<td>1.0–1.5 × 0.5–0.7</td>
</tr>
<tr>
<td><strong>Leaf margin</strong></td>
<td>long-ciliate</td>
<td>short-ciliate</td>
<td>long-ciliate</td>
</tr>
<tr>
<td><strong>Sheath length (cm)</strong></td>
<td>0.4–0.5</td>
<td>0.3–0.5</td>
<td>2.5–3.5</td>
</tr>
<tr>
<td><strong>Scape length (cm)</strong></td>
<td>1.5–2.5</td>
<td>1.0–3.0</td>
<td>10.0–22.0</td>
</tr>
<tr>
<td><strong>Capitulum diameter (mm)</strong></td>
<td>c. 4</td>
<td>c. 2</td>
<td>c. 5</td>
</tr>
</tbody>
</table>

**Etymology** – The epithet “decumbens” is related to the prostrate stem with the apex turned upward holding the inflorescences.

**Distribution and conservation status** – *Paepalanthus decumbens* is known from two recent collections gathered from the same small subpopulation in Serra de São José, Minas Gerais, Brazil. This subpopulation is not protected in any conservation unit, and various anthropic activities are common in the area, affecting the quality of its habitat. The species occurs in the campos rupestres, growing in argillaceous soils among small shrubs and rock outcrops. The AOO can be estimated as 4 km² and the species is known from one IUCN location. According to the Criteria B of the IUCN (2011), the species should be considered as Critically Endangered (CR B2ab(iii)).

**Notes** – The new species is described from a mountain range relatively well explored by botanists. However, as most of the Eriocaulaceae species, *Paepalanthus decumbens* is micro-endemic, being collected for the first time only in 2014, and recollected in 2016. The species possesses trimerous flowers, a ramified stem with fasciculate-umbellate inflorescences arising at the branch apex. It is therefore placed within *P.* subsect. *Polyactis*. The decumbent stem, the falciform leaves with long trichomes along the margin, the numerous sericeous scapes of roughly the same size of the leaves, and the dark castaneous involucral bracts are useful characters to distinguish *P. decumbens* from the remaining species of this subsection.

*Paepalanthus microphyllus* and *P. stannardii* are both species morphologically similar to *P. decumbens*, despite their more erect habit. From *P. microphyllus*, which is restricted to the Serra do Cipó (Minas Gerais), *P. decumbens* may be distinguished by its more robust size, leaf size and pilosity, wider capitula, and by the simple stigmatic branches. From *P. stannardii*, which is restricted to the Chapada Diamantina (Bahia), *P. decumbens* is easily differentiated by its more gracile habit and shorter sheaths and scapes. Detailed comparisons are given in Table 3.

*Paepalanthus campothyllus* Ruhland and *Paepalanthus bongardii* Kunth, two very similar species from the mountains in the vicinities of Belo Horizonte and Ouro Preto (Minas Gerais) are, at first glance, also similar to *P. decumbens*. These two species are however distinguished from *P. decumbens* by the following set of characters: fewer glabrescent scapes per compound inflorescence, scapes more than two times longer than the leaves (vs. numerous sericeous scapes of roughly the same length as the leaves), greenish, sheaths with acute apex, the same length as the leaves (vs. whitish sheaths with truncate apex, shorter than the leaves), and the pale straw-colored to light castaneous involucral bract with rounded apex (vs. dark castaneous involucral bracts with acute to acuminate apex). Some floral characters may be also useful to distinguish these species, such as the pilosity of floral bracts and sepals, but such characters need to be better investigated in *P. campothyllus* and *P. bongardii*.

Regarding the pollen grains, *Paepalanthus decumbens* can be differentiated from the other two species by its psilate apertural membrane, acute spines closer distributed, and by the nexine and sexine of equal thicknesses (Table 1). The apertures of the species analyzed are described as spiroaperturate, turning only one complete loop in the grain, in agreement with previous results (Kuprianova 1948, Thanikaimoni 1965, Borges et al. 2009). Borges et al. (2009) cite that other apertural arrangements may also be found in *Paepalanthus*. We suggest that the described pattern is in fact an artifact of acetylation, which breaks some fragile areas of the exine, when the cytoplasm leaves the pollen interior (fig. 3). However, transmission electron microscopy analysis is required to test this hypothesis. Borges et al. (2009) also cite the ornamented apertural membrane as a character that differs *Eriocaulon* from other genera, except for *Syngonanthus arenarius* (Gardner) Ruhland. As it was herein described for two species, such trait may be more frequent in Paepalanthoideae, since as of yet, only a few *Paepalanthus* species have been evaluated.

In terms of seed morphology, *Paepalanthus decumbens* has unique characteristics in comparison to morphologically similar species, like the mucronate apex, a secondary testa sculpture with isolated anticlinal bristle-like cell wall projections, and a truncate base with the operculum in a cavity. Between *P. stannardii* and *P. microphyllus*, the main difference is the presence of secondary sculpture, where the bristle-like projections are interconnected in *P. stannardii* while *P. microphyllus* lacks any secondary sculpture. As noticed by Giulietti et al. (1988), and Barreto et al. (2013), the shape and the testa...
of the seeds were of taxonomic significance, useful for species delimitation and identification. Studies in seed morphology in Paepalanthus are still scarce and deserve further, and broader investigations.

ACKNOWLEDGMENTS

The authors would like to thank Renato Mello Silva, Rafaela Forzza, Ruy José Valka Alves, and Dayvid Couto for their curatorial and field assistance. Financial support: MT, Alexander von Humboldt Foundation, FAPERJ (E-26/203.269/2016-JCNE) and CNPq (proc. 301832/2016-1-Pq2); WPL, CAPES; VGE, (Pq2).

REFERENCES


