

## First record of the genus *Tropidia* Lindl. (Orchidaceae) for Brazil

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Received: 28.04.2016; accepted: 15.08.2016

**ABSTRACT** - (First record of the genus *Tropidia* Lindl. (Orchidaceae) for Brazil). The genus *Tropidia* is composed of ca. 20-30 species distributed in southern Asia, South Pacific islands and northern Australia, and a single species occurring in America, from United States to Ecuador. The first record of the genus *Tropidia* for Brazil is presented here. It consists of *Tropidia polystachya*, a species found in the Cerrado biome, Midwestern Brazil. Description, illustration, photos, and distribution map of the species are provided. Additionally, the leaf anatomy of the species was studied and it is very similar to the leaves of other species of Tropidieae described so far. DNA sequences of *T. polystachya* are presented (plastid *matK* and *rbcL* and nuclear ITS) in order to assist future phylogenetic studies with the genus.

**Keywords:** Anatomy, Cerrado biome, terricolous habit, youngpalm orchid

**RESUMO** - (Primeiro registro do gênero *Tropidia* Lindl. (Orchidaceae) para o Brasil). O gênero *Tropidia* é composto por ca. 20-30 espécies distribuídas pelo sul da Ásia, ilhas do Pacífico Sul, norte da Austrália, e uma única espécie ocorrendo também na América, desde os Estados Unidos até o Equador. O primeiro registro do gênero *Tropidia* para o Brasil é apresentado aqui. A espécie é *Tropidia polystachya*, encontrada no bioma Cerrado, no Centro Oeste do Brasil. Neste trabalho são fornecidos para a espécie uma descrição, ilustração, fotos e um mapa de distribuição. Adicionalmente, foi estudada a anatomia foliar da espécie, que apresenta morfologia muito similar a das folhas de outras espécies de Tropidieae que já foram estudadas. Sequência de DNA de *T. polystachya* são apresentadas (plastidial, *matK* e *rbcL*, e nuclear, ITS) afim de complementar futuros estudos filogenéticos com o gênero.

**Palavras-chave:** Anatomia, bioma Cerrado, hábito terrícola, orquídea *youngpalm*

### Introduction

The genus *Tropidia* Lindl (Orchidaceae) is composed of ca. 20-30 species, distributed in southern Asia, South Pacific islands and northern Australia, and a single species occurring in America, from United States to Ecuador (Pridgeon *et al.* 2005, Govaerts *et al.* 2016). Early classifications (*e.g.* Dressler 1981) placed *Tropidia* as related to Spiranthoid orchids, especially due to its erect dorsal anther and soft sectile pollinia (Pridgeon *et al.* 2005). However, cladistic analysis based on anatomical and morphological characters positioned the genus within Epidendroideae (Stern *et al.* 1993, Freudenstein & Rasmussen 1999). On the other hand, molecular analyses, of the *rbcL* plastid gene, positioned *Tropidia* as part of an unresolved

grade that is sister of most other Epidendroideae (Cameron *et al.* 1999). A recent phylogenetic study on the subfamily based on mitochondrial, nuclear and plastid regions combined (Freudenstein & Chase 2015) placed *Tropidia* as sister to *Corymborkis* Thouars, with strong support, among the other early-branching epidendroids.

*Tropidia* comprises terricolous herbs, green or holomycotrophic; with erect stem, often branched; two or many plicate leaves; inflorescences axillary or terminal, racemose or paniculate, few- to many-flowered; flowers resupinate or non-resupinate, sometimes with a spur; and column with prominent rostellum, and a dorsal and erect anther with two elongated sectile pollinia (Pridgeon *et al.* 2005).

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*Tropidia polystachya* (Swartz) Ames is commonly known in the United States as youngpalm orchid (Hammer 1997), and it is the only Neotropical species of the genus (Pridgeon *et al.* 2005). Detailed information about *T. polystachya* are scarce, however, is distributed from Southern Florida through the West Indies, Venezuela, Central America and Galapagos Islands (Pridgeon *et al.* 2005).

In this study we expand the known distribution of *T. polystachya*, registering the first record of the species for Brazil. Additionally, based on the analyses of the Brazilian specimen, we present a description, detailed illustration, photographs, distribution map, anatomical characterization of the leaves, and plastid genes (*rbcL* and *matK*) and nuclear (ITS) sequences of the species.

### Material and methods

**Sampling** - In 2012 and 2013, during field works in southwestern Mato Grosso State, in an area of the Cerrado biome, sterile individuals of *T. polystachya* were found. The population occurs in the Reserva Particular do Patrimônio Natural (RPPN) Vale do Sepotuba, located in the São Marcelo Farm (municipality of Tangará da Serra). The reserve occupies 1.200 ha of native semideciduous forest on the margins of the Sepotuba River, and presents a rhodic hapludox soil (Martins *et al.* 2010). This region is climatically classified as AW, according to the Köppen (1948) system, with an average annual temperature of 24.4 °C, annual precipitation of 1.500 mm, and a relative humidity ranging from 50% to 80%.

Collected specimens were grown in the Catasetum Orquidarium at the campus Tangará da Serra of Universidade do Estado de Mato Grosso (UNEMAT). A specimen flowered in April 2013 and a voucher was pressed and deposited at Herbarium TANG (acronym according to Thiers 2015).

**Species description** - Flower measurements were taken from pickled flowers and all other measurements were made from the dried material. General morphological terms were based on Harris and Harris (1994), Radford *et al.* (1974) and Stearn (1983). The illustration was based on voucher material and pickled flowers. Map of distribution was made using the distribution presented in genera *Orchidacearum* (Pridgeon 2005) and from compiled data available in Govaerts *et al.* (2016).

**DNA sequencing** - DNA sequences from three regions (*matK* and *rbcL* plastid genes, and the nuclear ITS)

were obtained in order to increase the knowledge of the phylogenetic placement of *T. polystachya*. Genomic DNA was extracted from fresh leaf material according to Doyle & Doyle (1987), using 2× CTAB extraction buffer, without the addition of RNase A and scaled to 2 ml tubes. Polymerase chain reaction (PCR) of all fragments was performed in 20 µl reactions (1× enzyme buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.5 µM of each primer, 10 ng BSA, 1 U of Taq DNA Polymerase (Invitrogen, Life Technologies Corporation), and 20-50 ng of genomic DNA. For the nuclear region 5 M betaine, 2% DMSO were added to the reaction. The nrITS region was amplified and sequenced with the primers described in Desfeux & Lejeune (1996); *matK* with the primers *matK 3F\_Kim f* and *matK IR\_Kim r* (Ki-Joong Kim, *pers. comm.*); and *rbcL* with the primers *rbcLa\_f* (Levin *et al.* 2003) and *rbcLa\_r* (Kress & Eriksson 2007). Thermal cycling profile consisted of an initial step of 1 min at 94 °C and a final step of 5 min at 72 °C, which was intercalated with 40 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 40 s (nrITS), 53°C (*matK* and *rbcL*), and extension at 72 °C for 40 s. The PCR products were purified with polyethylene glycol (PEG) 10%, and the sequencing reactions were performed in the ABI3739XL genetic analyzer (Macrogen Inc., South Korea), using the same primers of the PCR amplification. The sequences were superimposed and edited with the Staden Package software (Staden *et al.* 2003). Statistics of the three genomic regions of *T. polystachya* were calculated in the MEGA 6 software (Tamura *et al.* 2013). The sequences obtained were compared with those from the GenBank database using the BLASTn tool from the National Center for Biotechnology Information (NCBI) (<https://blast.ncbi.nlm.nih.gov/>) with default parameters. Sequences of plastid *matK*, *rbcL* and nrITS nucleotide obtained during this study were deposited in GenBank under accession numbers KR604798, KR604799 and KR604800.

**Anatomical characterization** - Two leaves of one individual were pickled in 70% ethyl alcohol or FAA 50 (Johansen 1940). Cross and paradermic sections from the medium third of the leaf blade were obtained. Histological sections were freehand sectioned with razor blade. Cross-sections were stained with astra blue and safranin 9:1 (Bukatsch 1972) and the paradermic sections were stained with toluidine blue as described by O'Brien *et al.* (1965). Semi-permanent slides were mounted with glycerin gelatin (Kaiser

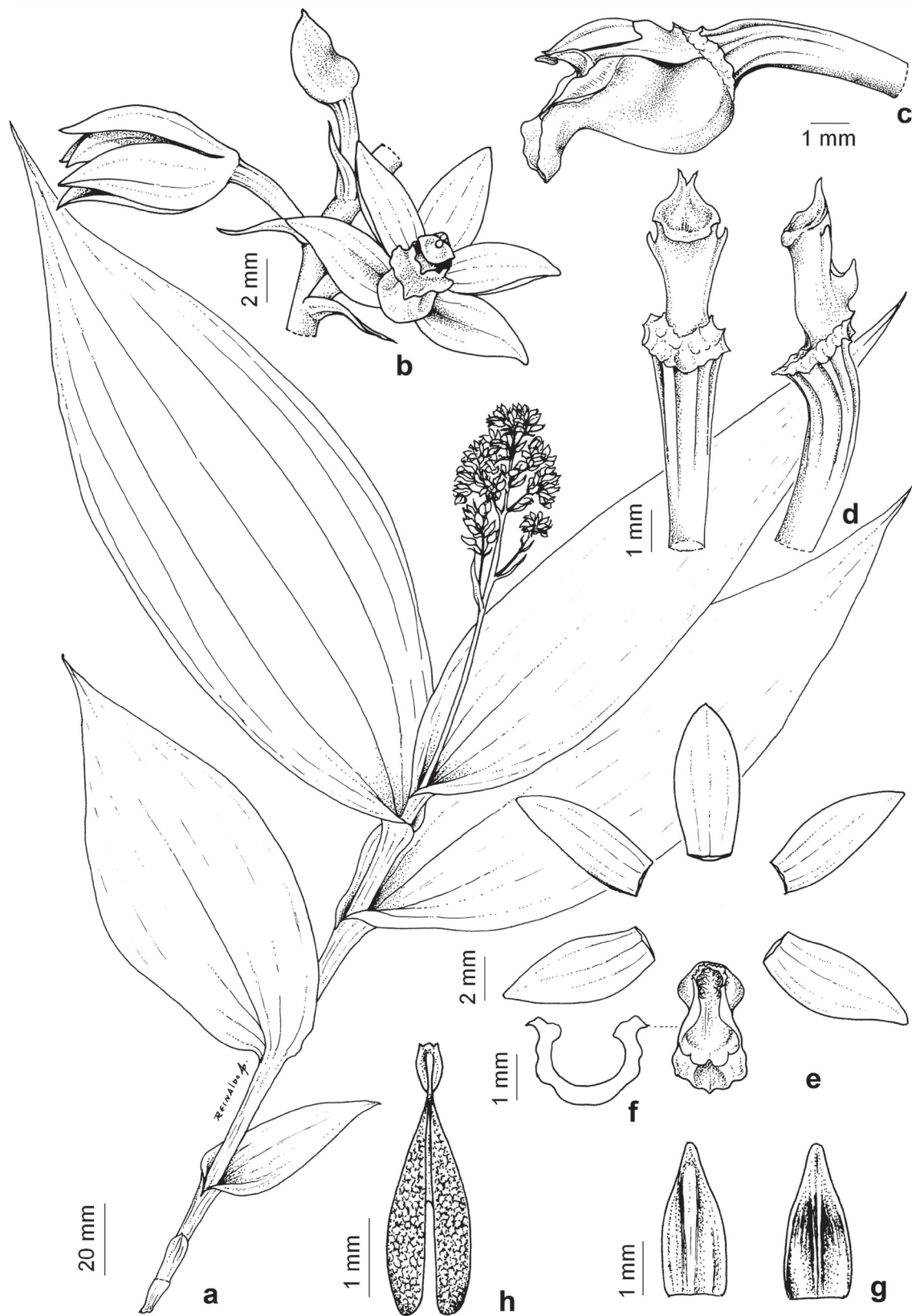


Figure 1. *Tropidia polystachya*. a. General aspect. b. Detail of the flower. c. Column and lip (lateral view). d. Column (front and lateral views). e. Floral pieces dissected. f. Cross section of the lip. g. Anther's cap (dorsal and ventral view). h. Pollinarium. Illustration by Reinaldo Aguiar from D.C. Dias 07 (TANG).

1880). Observations and photomicrographs were conducted using an Olympus microscope with a SC30 camera and analySIS getIT image processing software.

### Results and Discussion

*Tropidia polystachya* (Sw.) Ames, Orchidaceae 2: 262 (1908).

Figures 1-2

Terricolous herbs. Roots villous. Stem ca. 25 cm tall, 2-3 cm diam., erect, cylindrical, partially covered by tubular sheaths when juvenile, naked when older, distally covered by foliar sheaths. Sheaths thin, often

caducous. Leaves 10-15 × 3.6-5.2 cm, green, elliptic to lanceolate, prominently veined, glabrous, margin entire, apex acuminate. Inflorescence ca. 7 cm long, terminal, paniculate, congested, multiflowered; peduncle ca. 2.5 cm long, cylindrical; rachis ca. 4.5 cm long; bracts 2-4 × 1-2 mm, greenish, linear to lanceolate, glabrous. Flower resupinate, 8-10 mm diam., pedicel + ovary 4-5 mm long, greenish, subcylindrical; sepals greenish, 5-veined; dorsal sepal ca. 7-10 × 3 mm, oblong-elliptic, concave, apex acute to obtuse; lateral sepals ca. 7-10 × 3 mm, oblong-lanceolate, slightly concave, slightly falcate, apex acute; petals ca. 7-10 × 2 mm, greenish, lanceolate to elliptic-oblongate, subfalcate, 3-veined, apex acute;



Figure 2. *Tropidia polystachya*. a. Habit. b. Inflorescence. c. Flower. d. Column with lip (front view). e. Pollinarium. Photos by Ana Kelly Koch and Celice Alexandre Silva.

lip ca.  $7 \times 4$  mm, white with a yellow macula on the disc, oblong, constricted at the middle, embracing the column, sometimes obscurely 4-lobed, apex retuse and recurved; disc 2-lamellate, distally flabellate; column ca. 3 mm long, greenish, subcylindrical; anther 2-3 mm long, ovoid; pollinarium 3-4 mm long, viscidium ca. 1 mm long, oblongate, hamulus stipe 1 mm long, slender; pollinia 2, ca. 2 mm long, white, clavate; stigma transversally elliptic; rostellum broadly triangular, apex bifid. Capsules not seen.

Examined material: BRAZIL. MATO GROSSO: Tangará da Serra, Reserva Particular do Patrimônio Natural Vale do Sepotuba, Fazenda São Marcelo, bank of the Sepotuba River, 2-IV-2013, D.C. Dias 7 (TANG!).

Habitat and distribution - *Tropidia polystachya* has been cited for Southern Florida through the West Indies, Venezuela, Central America and Galapagos Islands (Pridgeon *et al.* 2005), and in the present study its distribution is extended to the Central Brazil (figure 3). The young palm orchid is a short plant with palm aspect, which usually grows in deep shade of forest floor, often on leaf litter, loamy soil or sandy humus (Hammer 1997, Pridgeon *et al.* 2005). In Brazil, *T. polystachya* grows on loamy soil in open areas of Cerrado. These data increase not only the area of occurrence, but also the types of habitat where the species occurs.

Conservation status - *Tropidia polystachya* has a broad distribution from Florida to northern South America

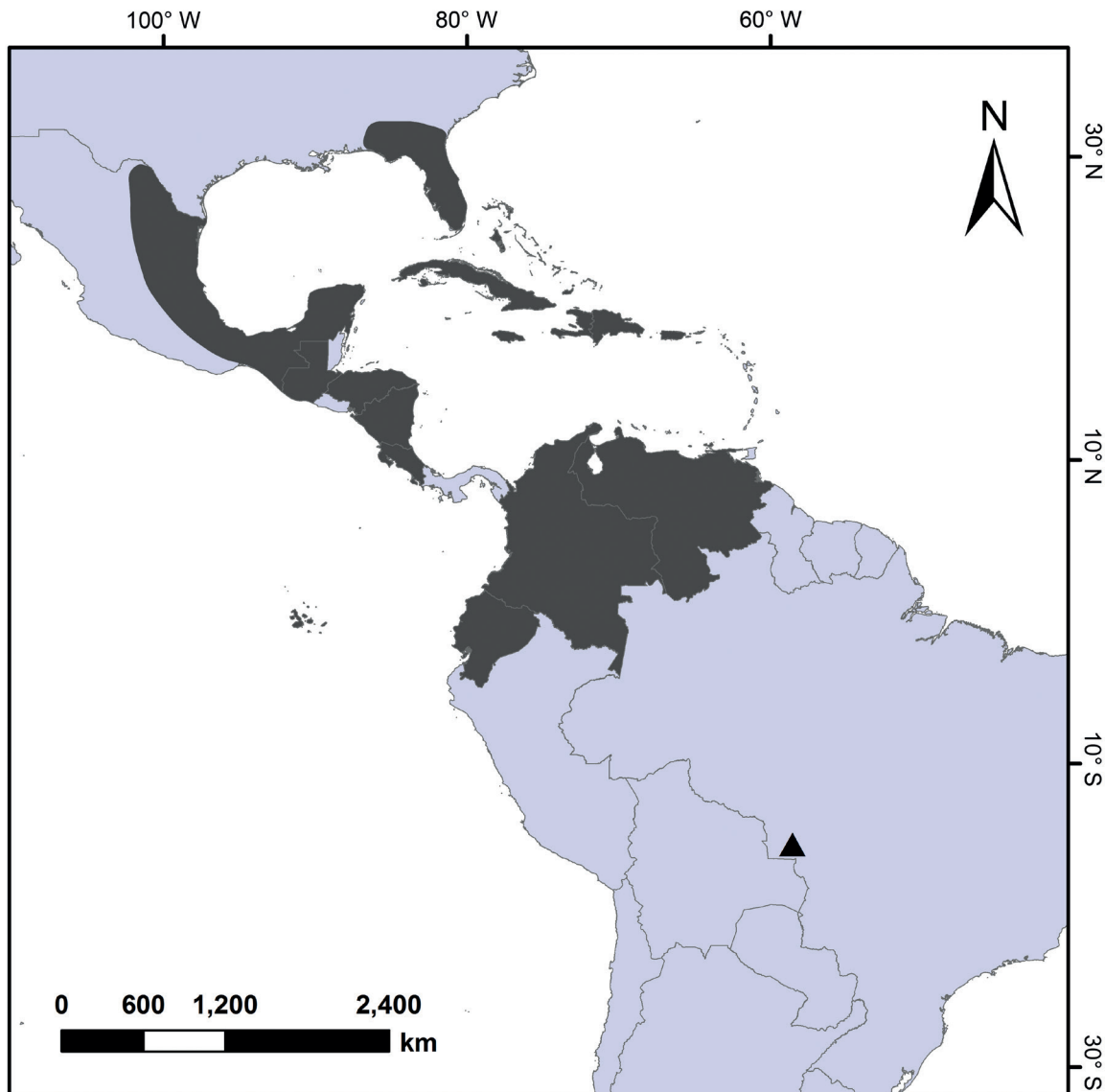


Figure 3. Distribution map of *Tropidia polystachya* including the new record for Brazil.

(B1 criteria) and a great number of known locations (B2 criteria), therefore the species is assessed as Least Concern (LC), according to the IUCN Red List Categories and Criteria (IUCN 2014). On the other hand, *T. polystachya* is known only in one locality in Brazil, and although found within a protected conservation unit, the species should be considered as Critically Endangered (CE; B1) for the country.

**DNA sequencing** - The genus *Tropidia* and *Corymborkis* are recognizable within the Tropidieae tribe, among the other early-branching Epidendroideae

(Freudenstein & Chase 2015). According to the BLASTn tool, our nrITS sequence (KR604798), with 588 base pairs (bp), presented 99.3% identity with another sequence of *T. polystachya* (EU490674.1) deposited in the GenBank database of the NCBI; *matK* plastid sequence (KR604800), with 897 bp, presented 99% identity with *T. graminea* (EF079304.1); and *rbcL* plastid sequence (KR604799), with 561 bp, presented 100% identity with *Tropidia* sp. (Chase O-211). Unfortunately, due to the lack of more sequences from species of *Tropidia* deposited in the GenBank, it is not possible to draw any picture of the

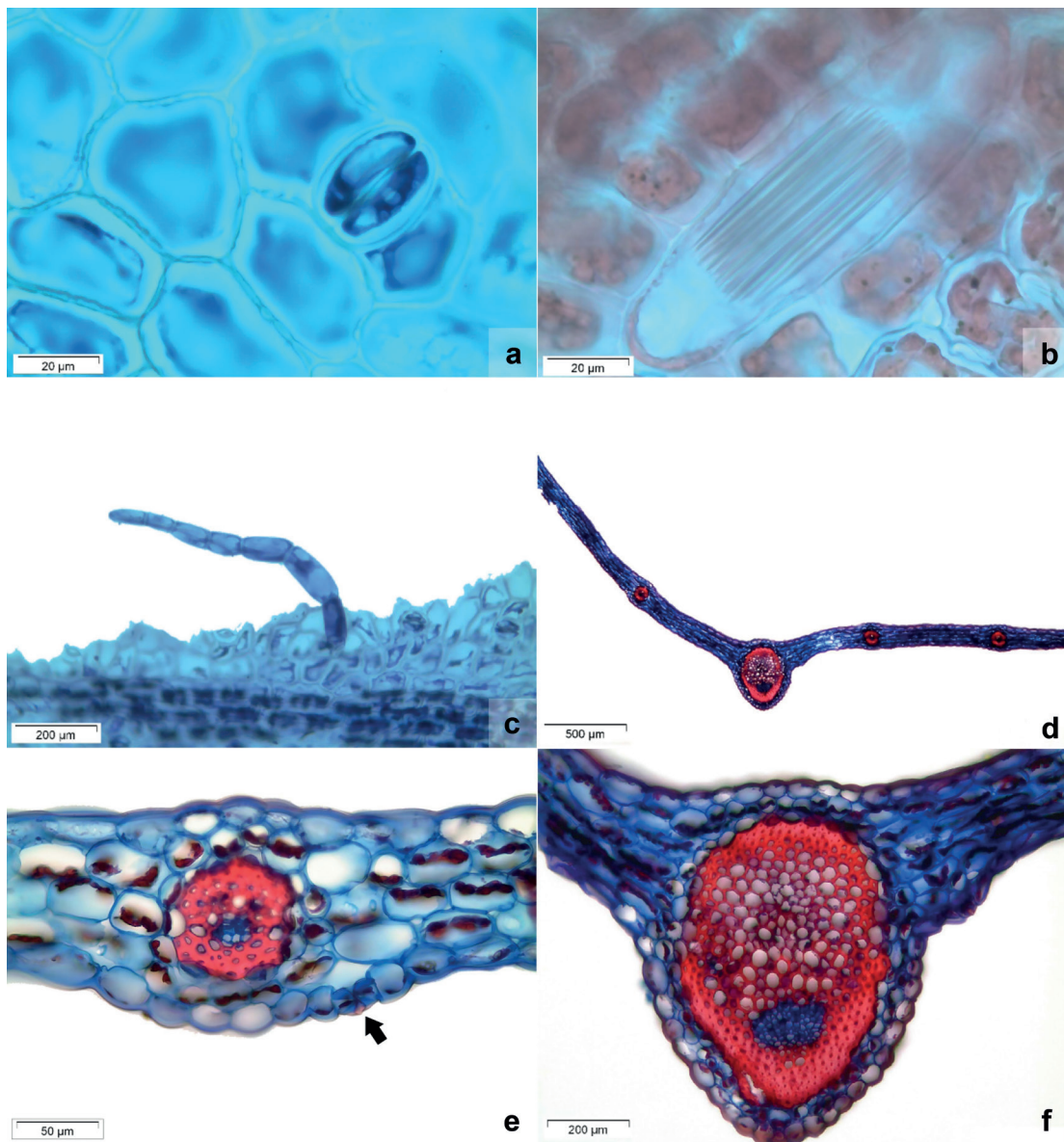


Figure 4. Anatomical characterization of the leaves of *Tropidia polystachya*. a. Abaxial epidermis with tetracytic stomata and irregular-shaped epidermal cells (front view); b. Idioblasts containing crystals in the shape of druses (front view); c. Unisseriate tector trichome (front view); d. General view of the leaf (cross section); e. Epidermal cells, homogeneous mesophyll, stomata with substomatal chamber (arrow) and collateral vascular bundle (cross section); f. Middle vascular bundle partially surrounded by sclerenchyma fibres (cross section).

phylogenetic placement of *T. polystachya* within the genus at the moment.

**Anatomical characterization** - The leaf of *T. polystachya* presents, in front view, epidermal cells irregularly shaped, slightly curved anticlinal walls and tetracytic stomata (figure 4a). Additionally, stegmatas were observed close to the vascular bundles, as well as the presence of idioblasts with raphides in epidermal tissue (figure 4b), and uniseriate glandular trichomes scattered on the leaf surface (figure 4c). In cross section, the leaf blade shows a flat surface (figure 4d). The uniseriate epidermis cells are elliptical or isodiametric in shape, and the cells of the adaxial epidermis are higher than the cells of the abaxial epidermis (figure 4e). Presence of a thin cuticle covering the leaf blade was observed. Hypostomatic leaves show stomata at the same level of the other epidermal cells and present well developed substomatal cavity. Chlorenchyma is homogeneous, with isodiametric, rectangular or elliptical cells arranged from three to four layers horizontally disposed. Presence of collateral vascular bundles surrounded by a sclerenchymatic sheath was also observed (figure 4e). Midrib is constituted by a large collateral vascular bundle, whose adaxial region is surrounded by three to four layers of fibers, whereas the abaxial region of the bundle is surrounded by seven to eight layers of fibers arranged in an U shape (figure 4f).

The vegetative morphology and anatomy have proved to be great data sources to the understanding of relationships in groups within Orchidaceae, as opposed to reproductive characters, notably prone to selective pressures by pollinator groups and therefore considered more homoplastic (Cameron 2005, Salazar & Dressler 2011). Previously, leaf anatomy of *Tropidia* was only known from *T. curculigoides* Lindl., species distributed from the Himalaya to New Caledonia (Stern *et al.* 1993). Leaves of *T. polystachya*, from the individuals found in open areas of Cerrado are anatomically very similar to the leaves of other species of Tropidieae described so far (Stern *et al.* 2013, 2014), including the genus *Corymborkis* Thou. Thus, we believe that the leaf anatomical features may have low taxonomic significance for the tribe, since they remained unchanged even in very different habitats and species. The overall similarities with others basal Epidendroideae like *Sobralia* Ruiz & Pavón, *Palmorchis* Barb. Rodr., *Xerorchis* Schltr. and *Eleanthus* C. Presl indicated a conservative leaf structure and would probably be useful for the recognition of these basal groups of the subfamily in a broader context.

## Acknowledgements

The authors acknowledges the support from the Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship of the Programa de Capacitação Institucional (PCI-DB; MPEG/MCTI) to the first and second authors, and the productivity grant CNPq-Nível 2 to the third author; Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT) process 296039/2010 for the financial support to the last author. We also thank the administration of the São Marcelo Farm; and Reinaldo Aguiar for the illustration.

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