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Molecular phylogeny, morphology and their implications for the taxonomy of Eriocaulaceae

Filogenia molecular, morfologia e suas implicações para a taxonomia de Eriocaulaceae

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Abstract

The pantropical family Eriocaulaceae includes ten genera and c. 1,400 species, with diversity concentrated in the New World. The last complete revision of the family was published more than 100 years ago, and until recently the generic and infrageneric relationships were poorly resolved. However, a multi-disciplinary approach over the last 30 years, using morphological and anatomical characters, has been supplemented with additional data from palynology, chemistry, embryology, population genetics, cytology and, more recently, molecular phylogenetic studies. This led to a reassessment of phylogenetic relationships within the family. In this paper we present new data for the ITS and *trnL*-F regions, analysed separately and in combination, using maximum parsimony and Bayesian inference. The data confirm previous results, and show that many characters traditionally used for differentiating and circumscribing the genera within the family are homoplasious. A new generic key with characters from various sources and reflecting the current taxonomic changes is presented.

Key words: anatomy, ITS, phylogenetics, pollen, *trnL-trnF*.

Resumo

Eriocaulaceae é uma família pantropical com dez gêneros e cerca de 1.400 espécies, com centro de diversidade no Novo Mundo, especialmente no Brasil. A última revisão da família foi publicada há mais de 100 anos, e até recentemente, as relações genéricas e infra-genéricas ainda eram pouco resolvidas. Entretanto, tem havido nos últimos 30 anos, um grande esforço por parte de pesquisadores brasileiros para preencher as lacunas existentes, utilizando caracteres morfológicos e anatômicos, complementados por dados adicionais de diferentes fontes, como palinologia, química, embriologia, genética de populações, citologia e, mais recentemente, estudos de filogenia molecular. Tal conjunto de dados tem levado a uma re-avaliação do relacionamento filogenético dentro da família. Neste trabalho são apresentados novos dados para as regiões de ITS e *trnL*-F, analisadas separadamente e em combinação, usando máxima parcimônia e inferência Bayesiana. Os dados obtidos confirmam resultados já publicados, e mostram que muitos caracteres tradicionalmente usados para diferenciação e circunscrição dos gêneros dentro da família são homoplásicos. Uma nova descrição e chave genérica para a família, utilizando caracteres de várias fontes são apresentadas, refletindo a taxonomia atual das Eriocaulaceae.

Palavras-chave: anatomia, ITS, filogenia, palinologia, *trnL-trnF*.

Introduction

The Eriocaulaceae are easily distinguished from other monocot families because most of the species have short stems and leaves in a rosette, long scapes with small unisexual flowers grouped in dense heads (capitula), a 3- or 2-locular ovary with a

single pendulous ovule per locule, and spiraperturate pollen grains (Giulietti *et al.* 1995, 2000).

Some species have considerable economic importance in Brazil, especially the genera *Syngonanthus* Ruhland and the recently re-established *Comanthera* L.B. Sm. (Parra *et al.*

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2010). These are collected when their flowers are still at anthesis and dried in the sun, to be sold as ornamental objects and are often exported to different countries as “everlasting plants” (Giulietti *et al.* 1988, 1996). Some species such as *Comanthera elegans* (Bong.) L.R. Parra & Giul. have been marketed at the rate of 40,000 kg/year (Giulietti *et al.* 1988, 1996) and some such as *C. magnifica* (Giul.) L.R. Parra & Giul. and *C. mucugensis* (Giul.) L.R. Parra & Giul. are critically endangered due to over-exploitation (Pereira *et al.* 2007). Currently, the scapes of *Syngonanthus nitens* (Bong.) Ruhland, popularly known as “golden grass”, are widely commercialized and used for the production of various local handcrafts (Schmidt *et al.* 2007). In the regions where these plants occur naturally, they are one of the main sources of income for local inhabitants, especially in the “campos rupestres” vegetation of Espinhaço Range (states of Minas Gerais and Bahia, Brazil), Serra Geral (state of Goiás, Brazil), Cerrado in Jalapão (state of Tocantins, Brazil), and western Bahia.

The Eriocaulaceae is a sub- to Pantropical family, and *Eriocaulon* L. includes 400-800 species occurring on five continents. *Paepalanthus* Mart. (>500 species) and *Syngonanthus* (c. 130 species) occur disjunctly in the Americas and Africa. *Mesanthemum* Körn. is restricted to the African continent, and *Lachnocaulon* Kunth to North America. All other genera: *Actinocephalus* (Körn.) Sano, *Comanthera*, *Leiothrix* Ruhland, *Rondonanthus* Herzog, and *Tonina* Aubl., are practically endemic to South America, whereas *Tonina* extends to Central America. The main diversity centre of the family is in Brazil with 629 species, 482 in the Cerrado, so that Eriocaulaceae is the fourth family in number of species in this biome (Giulietti *et al.* 2010; Forzza *et al.* 2010). The greatest diversity is in the “campos rupestres” (high-altitude rocky savannas) of the Espinhaço Range in the states of Minas Gerais and Bahia, where about 500 species occur. Eriocaulaceae was a very important family for the definition of endemism areas in Minas Gerais (Echternacht *et al.* 2011a).

Judd *et al.* (2002) and APG III (2009) included Eriocaulaceae within Poales, but the family was previously placed in its own order Eriocaulales (Cronquist 1981), within the Commeliniflorae (Dahlgren *et al.* 1985) or Commelinales (Judd *et al.* 1999). Although it is a morphologically well-delimited family (Dahlgren *et al.* 1985) and considered a monophyletic group (Giulietti *et al.*

2000; Davis *et al.* 2004), its inter- and infra-generic relationships are still not well resolved and the traditional generic circumscriptions were mostly based on few floral characters (Stützel 1998; Giulietti *et al.* 1995, 2000).

The last full revision of Eriocaulaceae was done by Ruhland (1903) who established the taxonomic basis of the family, which is still in use. This author recognized about 560 species and two subfamilies: Eriocauloideae with diplostemonous flowers and glandular petals, including *Eriocaulon* and *Mesanthemum*; and Paepalanthoideae with isostemonous flowers and eglandular petals, including: *Paepalanthus*, *Tonina*, *Lachnocaulon*, *Philodice* Mart., *Syngonanthus*, *Leiothrix* and *Blastocaulon*. Since then, more than twice the original number of species have been described especially in the last five years in *Comanthera*, *Eriocaulon*, *Paepalanthus* and *Syngonanthus*. Also, four new genera have been proposed: *Actinocephalus*, *Carptotepala* Moldenke, *Comanthera*, *Moldenkeanthus* P. Morat. Of all the genera described in Eriocaulaceae, six are already considered to be synonymous to existing genera: *Moldenkeanthus* in *Paepalanthus* (Stützel 1987), *Wurdackia* in *Rondonanthus* (Hensold & Giulietti 1991), *Carptotepala* and *Comanthera* both in *Syngonanthus* (Giulietti & Hensold 1991), *Philodice* in *Syngonanthus* (Giulietti *et al.* 2009) and *Blastocaulon* in *Paepalanthus* (Andrade *et al.* 2011). *Rondonanthus* and *Actinocephalus* are currently accepted and *Comanthera* was recently reestablished (Parra *et al.* 2010). Currently the genera accepted in Eriocaulaceae are: *Actinocephalus*, *Comanthera*, *Lachnocaulon*, *Leiothrix*, *Paepalanthus*, *Rondonanthus*, *Syngonanthus* and *Tonina* within Paepalanthoideae, and *Eriocaulon* and *Mesanthemum* within Eriocauloideae.

The circumscription of genera in Eriocaulaceae has been based primarily on a few floral characters including the following: level of petal union in pistillate flowers; presence of glands on petals; number of stamens; and number of microsporangia per anther (Ruhland 1903; Stützel 1985; Giulietti *et al.* 1995, 2000).

Recent studies involving more than 30 researchers from six institutions in Brazil based on morphology, anatomy, floristics, taxonomy, chemistry, population biology and genetics, economic botany, physiology and molecular phylogenetics have provided fresh data for a better understanding of the family. These studies have also

shown that, besides the characters used by Ruhland (1903), vegetative and floral parts also display many other characters, especially those relating to form and anatomy of the floral parts, embryology, type of hairs, seed testa and apertures and sculpturing of the pollen grains. The aims of this paper are to summarize the state of knowledge for the family and to present a reassessment of the relationships within Eriocaulaceae, especially in *Paepalanthus*, which is the largest genus in the Brazilian flora, with 357 species (Forzza *et al.* 2010).

Material and Methods

The material examined in the different studies is listed in Appendix 1 (morphological, and anatomical data) and Appendix 2 (molecular data). Voucher material has been deposited in the herbaria BH, C, FLAS, HRCB, HUEFS, K, MU, NY, PH and SPF.

For molecular phylogenetic analyses 71 species were sequenced. The ingroup consisted of 67 species, representing all five genera of Paepalanthoideae, including all main infrageneric divisions in *Paepalanthus*, proposed by Ruhland (1903) (Appendix 2). Four species of *Eriocaulon* were defined as the outgroup (*E. linearifolium* Körn., *E. ligulatum* (Vell.) L.B. Sm., *E. modestum* Kunth and *E. cinereum* R. Br.). The DNA was extracted mostly from fresh or silica-gel leaves using a modified version on the CTAB procedure of Doyle & Doyle (1987). The programs of PCR and methods for amplification and sequencing of DNA are described in detail by Andrade (2007) and Andrade *et al.* (2010). For amplification and sequencing of ITS, we used the primers 75 and 92 of Desfeaux *et al.* (1996) and for some samples we used the primers 17SE and 26SE (Sun *et al.* 1994). For amplification and sequencing of *trnL-F*, we used two universal primers (C, F) of Taberlet *et al.* (1991). The PCR fragments were purified by enzymatic treatment with Exonuclease I (EXO) and Shrimp Alkaline Phosphatase (SAP) (Amersham Biosciences). The cycle-sequencing reactions were performed with the Big Dye Terminator version 3.1 (Applied Biosystems). Samples were sequenced in both directions using the Spectrumedix SCI SCE9624 automated sequencer at Universidade Estadual de Feira de Santana (UEFS).

Electropherograms were edited and assembled using Staden Package (Staden *et al.* 1998) and aligned using CLUSTAL X (Thompson *et al.* 1997). The resulting alignment was corrected manually

following the guidelines in Kelchner (2000). Maximum Parsimony (MP) analyses were performed using PAUP* version 4.0b10 (Swofford 2002) with Fitch parsimony (equal weights, unordered; Fitch 1971) as the optimality criterion. We performed two separate searches for the ITS and *trnL-F* datasets. A third analysis included the combined data from both DNA regions. Each search consisted of 1,000 random taxon-addition replicates, with TBR algorithm, and limited swapping on up to 15 trees per replicate to prevent extensive swapping on islands with many trees. The resulting trees were then used as starting trees for TBR swapping with an upper limit of 30,000 trees. Nonparametric bootstrap support was estimated from 1,000 bootstrap (BP) replicates incorporating heuristic parsimony searches using addition sequence and branch-swapping options as in our MP analyses (Felsenstein 1985).

The model-based analysis was performed with Bayesian inference (Larget & Simon 1999; Lewis 2001), using MrBayes version 3.01 (Ronquist & Huelsenbeck 2003). The model used for *trnL-F* and ITS was GTR+I+G, as indicated by nested likelihood-ratio tests using MrModeltest version 2.2 (Nylander 2004). MrBayes was run for 1,300,000 generations for *trnL-F* and 1,000,000 for ITS and combined analyses; with two separate analysis with four chains each, sampling trees every 100 generations. The burn-in stage needed to reach a stable state was determined by plotting the likelihood scores against the number of generations. The trees sampled from within the burn-in stage were excluded (480 for *trnL-F*, 260 for ITS and 400 for combined analyses), and the remaining trees were assumed to be representative of the posterior probability distribution. The majority rule consensus tree was calculated in PAUP* and the resulting group frequencies estimated the posterior probabilities (PP).

For light microscopy (LM), the median portion of adult roots, stems, leaves and scapes were sectioned by hand, stained with basic fuchsin and astra blue (Roeser 1962) and mounted in glycerine jelly (Kaiser 1880). For floral anatomy and embryology, inflorescences were dehydrated, embedded in historesin, sectioned, and the sections were stained with periodic acid – Schiff's Reagent (PAS reaction) and toluidine blue (Feder & O'Brien 1968). Material for scanning electron microscopy (SEM) was dehydrated through an ethanol series; critical-point dried; coated with gold; and examined using a JEOL JSM-5410 scanning electron microscope.

For pollen analysis, chemically (acetolysis) treated and untreated pollen grains were observed using LM and SEM (LEO 1430 VP – Carl Zeiss) respectively.

Results and Discussion

Morphology and anatomy

As mentioned above, the Eriocaulaceae are easy to recognize and considered monophyletic, but different patterns in the morphology and anatomy can occur in the group, and what may help to define one subgenus or section can vary at the species or population level in another.

Vegetative organs

The roots of Eriocaulaceae either store air in the cortex, with exodermis and arm cells, as in *Eriocaulon* and a few species of *Leiothrix* and *Comanthera* (Fig. 1a), or else lack aerenchyma and exodermis, possessing instead isodiametric cortical cells, especially in *Paepalanthus* and *Actinocephalus* (Fig. 1b). The stems are erect or rhizomatous, either with primary thickening, as in various species of *Comanthera* (Fig. 1c), or without as in *Tonina fluviatilis* Aubl. (Fig. 1d). These anatomical characters of root and stem appear to depend closely on environmental factors. Because of this, they are not considered to be appropriate for establishing taxonomic groups within the family (Scatena *et al.* 2005).

The characters of leaves and scapes are also particularly useful for infrageneric taxa, species and populations (Scatena & Giulietti 1996; Scatena & Menezes 1996). The leaves (Figs. 1e-i) and scapes (Figs. 1j-l) possess epidermis with thin- (Figs. 1e-f, j) or thick-walled cells (Figs. 1i, l); stomata with (Figs. 1i, l) or without special substomatal chambers (Figs. 1e-h, j-k); presence (Figs. 1h-i, l) or absence of hypodermis (Figs. 1e-g, j-k); mesophyll formed by compact (Figs. 1g-i, k-l) or loosely aggregated chlorenchyma (Figs. 1e-f, j). These characters are closely associated with environmental factors and can be important for defining species clusters as in *Leiothrix*, *Comanthera* and *Paepalanthus* subg. *Platycaulon* from dry environments, and in *Eriocaulon*, *Tonina*, *Syngonanthus* sect. *Carphocephalus* and *S.* sect. *Syngonanthus* from wet environments (Scatena *et al.* 2005).

Except for the paraclades that define *Actinocephalus* (Fig. 2a) (Sano 2004; Costa & Sano 2006), characters related to the morphological

architecture of the plants in Eriocaulaceae (Figs. 2a-i) have been restricted largely to infra-generic categories, especially in *Paepalanthus* (see Ruhland 1903). In *Paepalanthus*, the branching patterns of the stems and the position of the inflorescences might be important tools to develop a more natural classification of the genus associated with the flowers. For example, the fused scapes that define *P.* subg. *Platycaulon* Körn. are a good example of a feature that can be used to help resolve problems seen in clades PATL and P1 of the molecular analysis (Fig. 5).

Floral organs

Floral morphology plays a central role in Eriocaulaceae classification, with subfamilies and genera defined primarily by floral features. These are mainly: number of androecial parts; presence of glands in the petals; absence or reduction of petals in the pistillate flower; form of the pistillodes in the staminate flower; fusion of the petals in the pistillate flower; and the presence or absence of stylar appendages (Körnicke 1863; Ruhland 1903; Stützel 1998). Recent re-evaluation of these characters with SEM and other anatomical tools has revealed new information (Rosa & Scatena 2003, 2007; Coan & Scatena 2004; Borges 2008; Borges *et al.* 2009) and the importance of these characters has also been evaluated here.

The staminate and pistillate flowers of Eriocaulaceae are dichlamydeous and heterochlamydeous, with free petals, united from the base or united in the middle and free at the base and apex (syngonanthoid fusion), as in *Mesanthemum* (Figs. 3a-b), *Syngonanthus* (Figs. 3c-d) and *Comanthera* (Figs. 3e-f). *Lachnocaulon* and *Tonina* have monochlamydeous flowers, and the petals are reduced to hairs, especially in the pistillate flowers (Figs. 3g-j). Most of the family has trimerous flowers (Figs. 3b, k), but in *Eriocaulon*, *Paepalanthus* and *Syngonanthus* dimerous flowers also occur (Figs. 3l). Staminate flowers are diplostemonous in *Mesanthemum* and *Eriocaulon* (Figs. 3b, m), and are isostemonous in the other genera which present antesealous scale-like staminodes (Fig. 3n), but not in *Rondonanthus roraimae* (Oliv.) Herzog with fertile stamens and nectariferous pistillodes (Fig. 3o). The staminodes in Paepalanthoideae probably indicate a reduction of the outer stamen whorl seen in Eriocauloideae (Rosa & Scatena 2003). Pistillate flowers mostly possess scale-like staminodes, except for *Rondonanthus*, which has elongated, vascularized

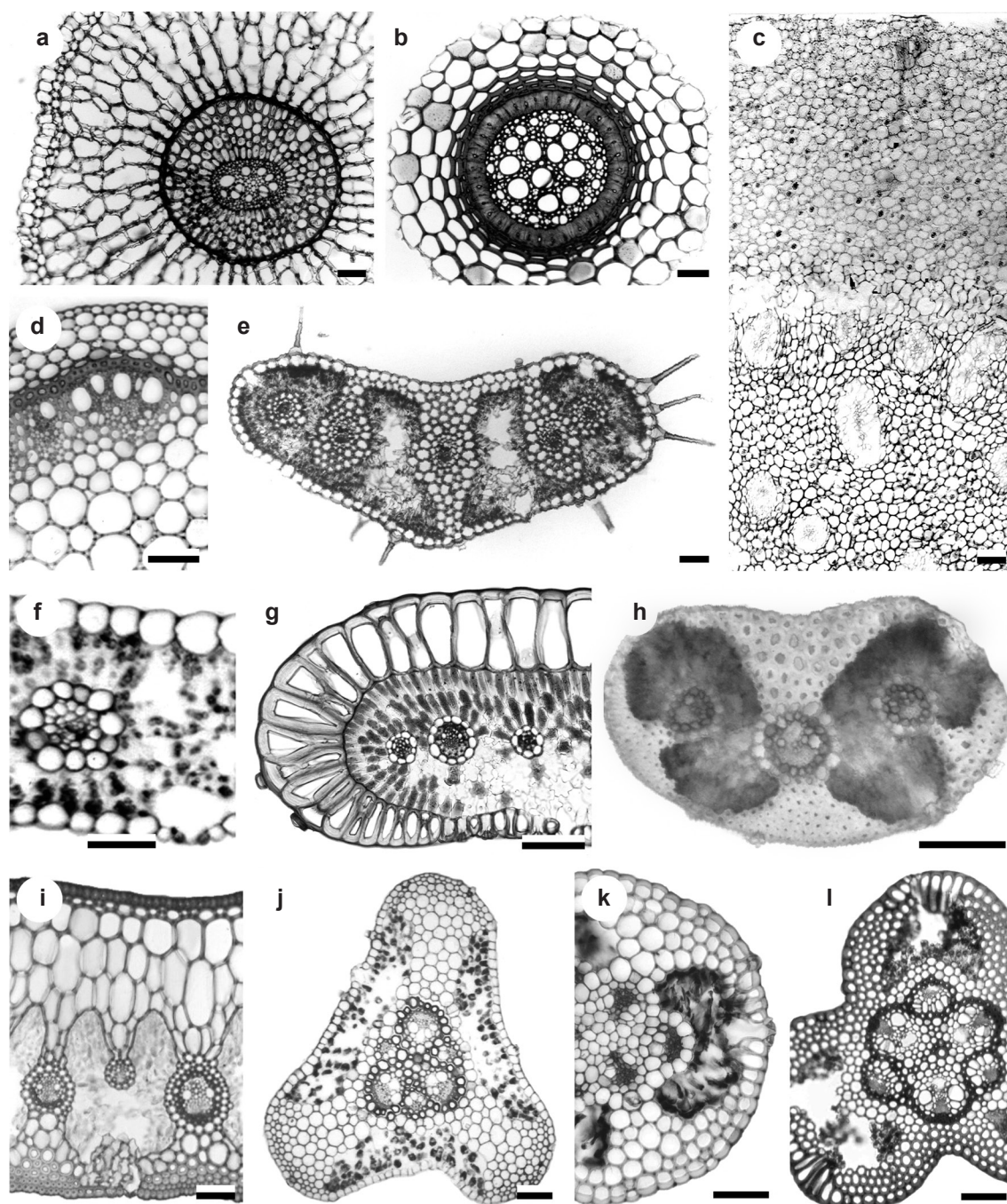


Figure 1 – Anatomical characters of the vegetative organs of Eriocaulaceae species (transverse sections) – roots: a. *Comanthera cipoensis*; b. *Actinocephalus brachypus*; stems: c. *Comanthera cipoensis*; d. *Tonina fluviatilis*; leaves: e. *Syngonanthus verticillatus*; f. *Tonina fluviatilis*; g. *Paepalanthus chlorocephalus*; h. *Comanthera aurifibrata*; i. *Comanthera cipoensis*; and scapes: j. *Syngonanthus caulescens*; k. *Actinocephalus rigidus*; l. *Leiostrix crassifolia*. Scale bars: a-e, g-l = 70 μm ; f = 30 μm .

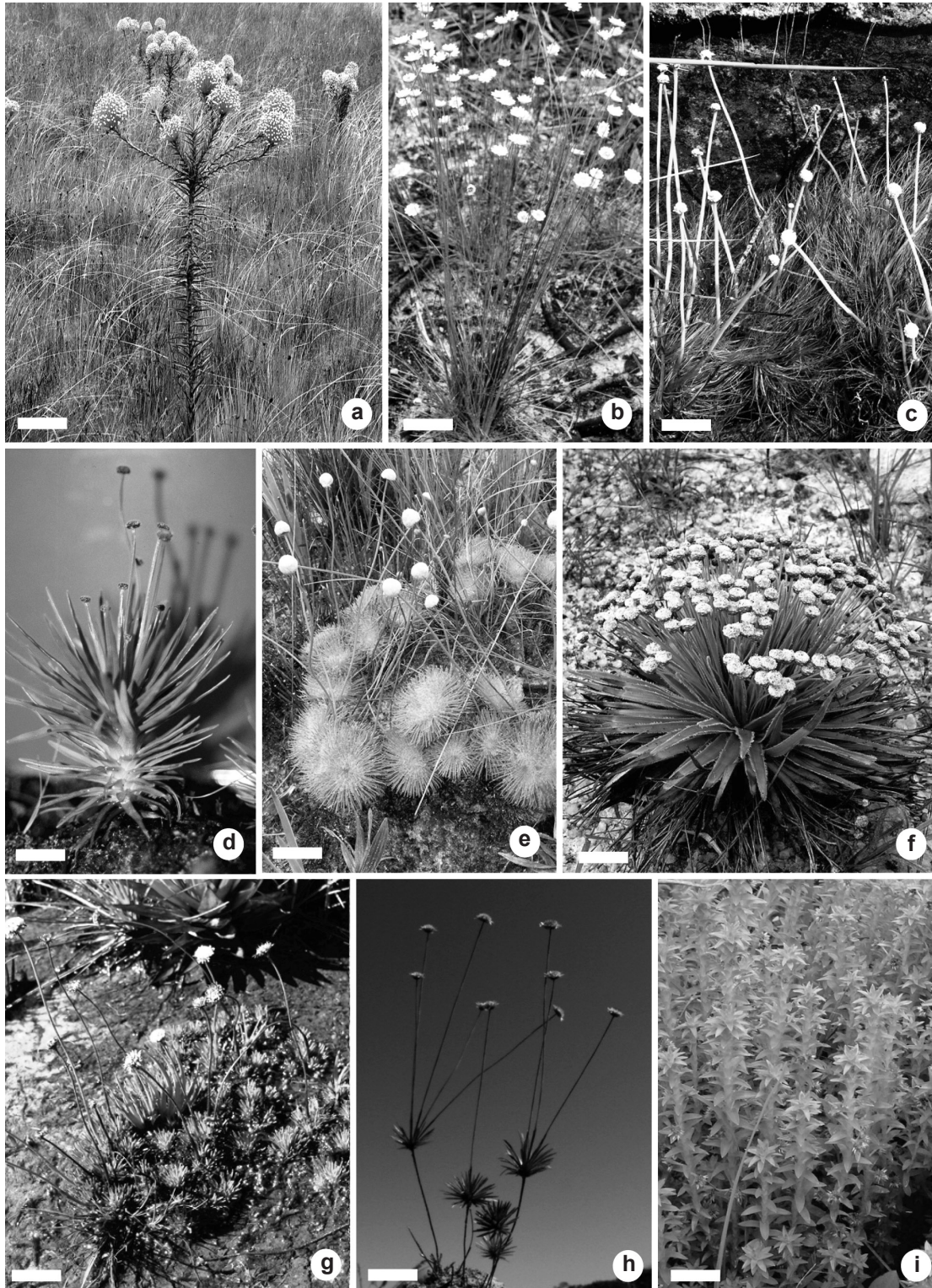


Figure 2 – Habits of Eriocaulaceae – a. *Actinocephalus bongardii*; b. *Comanthera elegans*; c. *Eriocaulon aquatile*; d. *Lachnocaulon anceps*; e. *Leiotherix curvifolia*; f. *Paepalanthus eriophaeus*; g. *Rondonanthus roraimae*; h. *Sygonanthus anthemidiflorus*; i. *Tonina fluviatilis*. Scale bars: a = 10 cm; b, f, i = 2 cm; c = 5 cm; d-e = 3 cm; g = 4 cm; h = 1 cm. Photo: a, c, f M. Trovó; b V. Scatena; d M. Unwin; e J. Lovo; g-h L. Echernacht; i S. Martins.

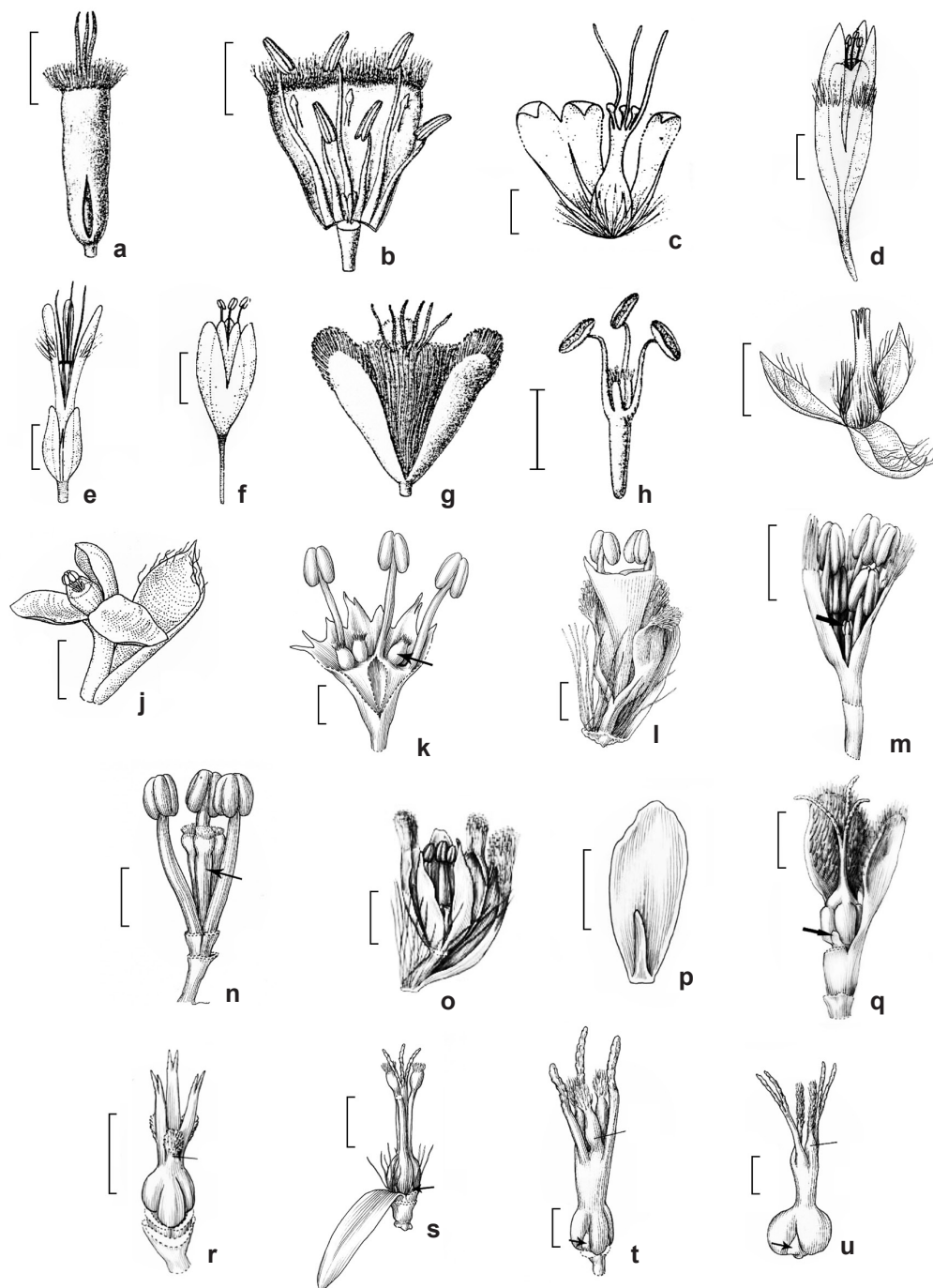


Figure 3—Flowers of Eriocaulaceae— a-b. *Mesanthemum auratum*— a. pistillate flower; b. staminate flower. c-d. *Syngonanthus nitens*— c. pistillate flower; d. staminate flower. e-f. *Comanthera nitida*— e. pistillate flower; f. staminate flower. g-h. *Lachnocaulon anceps*— g. pistillate flower; h. staminate flower. i-j. *Tonina fluviatilis*— i. pistillate flower; j. staminate flower. k. *Actinocephalus polyanthus*— staminate flower arrow indicating the pistillodes. l. *Paepalanthus flaccidus*— staminate dimerous flower. m. *Eriocaulon elichrysoides*— staminate flower. n. *Leiothrix fluitans*— androecium with stamens and pistillodes. o-p. *Rondonanthus roraimae*— o. staminate flower; p. pistillate flower, petal and staminode. q. *Eriocaulon elichrysoides*— pistillate flower, one petal removed, arrow indicating the staminodes. r. *Rondonanthus roraimae*— gynoecium. s. *Leiothrix fluitans*— pistillate flower, two petals removed, arrow indicating the staminodes. t. *Actinocephalus polyanthus*— pistillate flower, arrow indicating the staminodes. u. *Paepalanthus flaccidus*— pistillate dimerous flower, arrow indicating the staminodes. Scale bars: 0.5mm.

staminodes (Fig. 3p) and includes *R. flabelliformis* (Moldenke) Hensold & Giul., observed to have dehisced anthers, but pollen grains have not been seen inside. The gynoecium has an unbranched style with a simple stigma in Eriocauloideae (Figs. 3a, q), or a branched style with simple or bifid stigmas and the stigmatic portions alternating with nectariferous portions in Paepalanthoideae (Fig. 3r-s). In *Actinocephalus*, *Paepalanthus* and *Syngonanthus*, both portions of the style are free at the same height (Figs. 3c, t-u), but in *Leiothrix* (a monophyletic group in all morphological and molecular analyses) the two style portions diverge at different heights in the column (Fig. 3s).

In all Eriocauloideae and the majority of Paepalanthoideae the anthers are dithecous and tetrasporangiate (Fig. 4a), but bisporangiate anthers occur in few species of *Paepalanthus* (Andrade *et al.* 2011) and *Lachnocaulon* (Fig. 4b), as well as in the species of *Philodice* considered to be synonymous of *Syngonanthus*. Variation in the number of anther microsporangia in *Tonina fluviatilis* Aubl. was previously reported by Stützel (1985) using SEM, and is here confirmed by anatomical sections (Fig. 4c). The significance of the number of microsporangia as a useful character in delimiting genera has been stressed throughout the taxonomic history of Eriocaulaceae. This character has been used to delimit genera such as *Blastocaulon* from *Paepalanthus* (Giulietti 1978) and *Philodice* from *Syngonanthus* (Ruhland 1903), both now in synonymy. Molecular analysis shows that bisporangiate anthers are homoplasious in the family (Fig. 6).

Pollen grains in Eriocaulaceae are generally spherical, small- to medium-sized (22–37 µm), spiraperturate (Fig. 4d) or 2-zonasulcate in *Comanthera* subg. *Comanthera*. This is an important taxonomic character for the group (Fig. 4e). The exine surface is echinate to microechinate, sometimes with granules. *Paepalanthus* is a stenopalinological genus. A remarkable feature among pollen grains in Eriocaulaceae is presented by *T. fluviatilis* with large grooved spines, interspersed with smaller ones (Santos *et al.* 2000, Borges 2008; Borges *et al.* 2009) (Fig. 4f).

Molecular data

The earliest phylogenetic studies in Eriocaulaceae were carried out based on morphological data (Giulietti *et al.* 1995, 2000), until the theses of Unwin (2004) and Andrade (2007)

presented the first molecular analyses. Andrade *et al.* (2010) produced a broad molecular analysis of Eriocaulaceae, with 82 species representative of all recognized genera, using individual and combined analyses of sequences of the Internal Transcribed Spacers (ITS) of nuclear ribosomal DNA and regions of plastid DNA (*trnH-psbA* and *trnL-trnF*). All combined analyses show higher bootstrap values than the individual analyses. The results of those analyses suggest the need for a new delimitation of taxa within Eriocaulaceae as follows: a) recognition of Eriocaulaceae subfam. Eriocauloideae and Paepalanthoideae as well-supported monophyletic groups; b) confirmation of *Eriocaulon* and *Leiothrix* as well-supported monophyletic genera; c) synonymy of *Philodice* under *Syngonanthus* and the need for conservation of the latter name (proposed by Giulietti *et al.* 2009); d) reestablishment of *Comanthera* (Parra *et al.* 2010); e) synonymy of *Blastocaulon* under *Paepalanthus*; f) transformation of the currently polyphyletic genus *Paepalanthus* in a monophyletic group, by the inclusion of *Actinocephalus*, *Lachnocaulon* and *Tonina*. On account of the huge morphological diversity which would be encompassed in such a group, at present we prefer to await further data before embarking on such a step.

Of the five subgenera of *Paepalanthus* previously recognized by Ruhland (1903), three were sampled in the study mentioned above (Andrade *et al.* 2010) and were supported as monophyletic in at least some of the analyses: *P.* subg. *Theixinoë* Ruhland, *P.* subg. *Platycaulon* Mart. and *P.* subg. *Xeractis* Körn. Also *Actinocephalus* (*sensu* Sano 2004) was a monophyletic group as associated with *P.* subsect. *Aphorocaulon* Ruhland.

In the present study, an additional 13 taxa of Paepalanthoideae were included and sequenced for ITS and *trnL-F*. Of these, *Paepalanthus* subg. *Xeractis* (with six new species) and *P.* subg. *Platycaulon* (with five new species), have been emphasized since both are monophyletic (Andrade *et al.* 2010) and both were recently revised (Hensold 1988; Tissot-Squalli 1997).

A total of 65 taxa were sequenced for *trnL-F*, including the ingroup of Paepalanthoideae and the outgroup constituted by four species of *Eriocaulon* (Eriocauloideae). *Eriocaulon* was chosen as outgroup as shown by Andrade (2007) and Andrade *et al.* (2010) to possess an insert of about 250 bp (between positions 302 and 508) in relation to all genera of Paepalanthoideae. Maximum parsimony

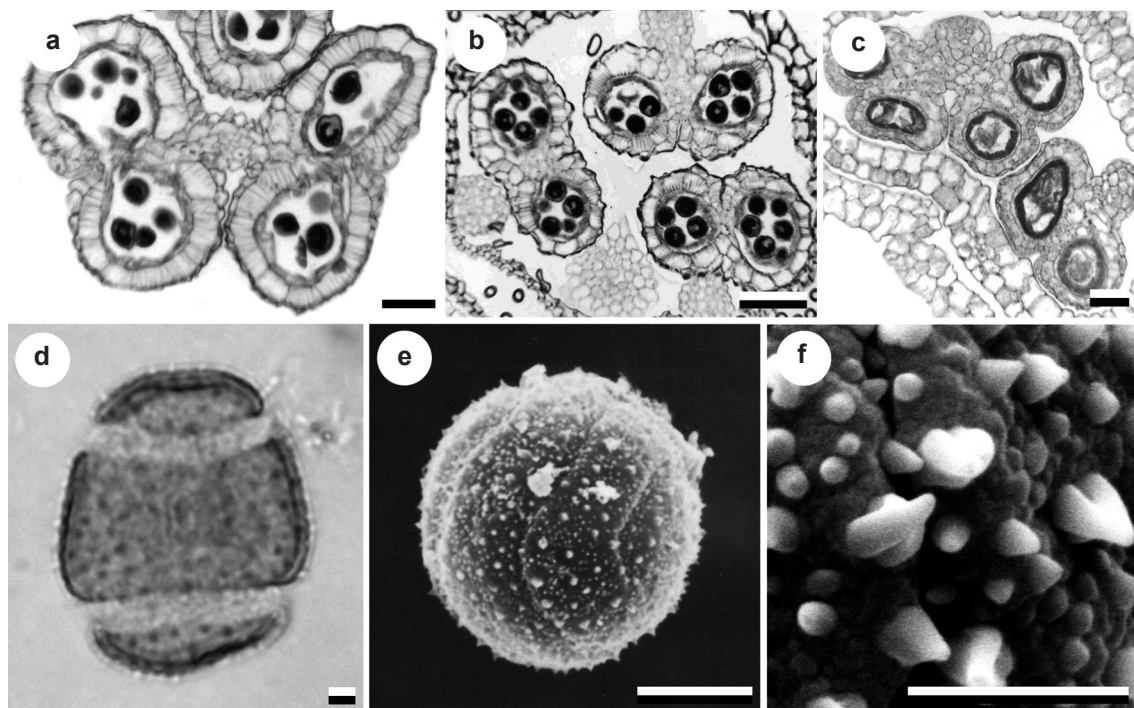


Figure 4 – Characters of anthers (transverse sections) and pollen in Eriocaulaceae – a. *Leiothrix fluitans*; b. *Lachnocaulon minus*; c. *Tonina fluviatilis*; d. *Leiothrix fluitans*; e. *Comanthera imbricatus* (optical section); f. *Tonina fluviatilis* (SEM). Scale bars: a-c = 30 µm; e, f = 3 µm; d = 10 µm.

analysis (MP) reached a pre-established limit of 30,000 of the shortest trees of 906 steps (CI 0.7064, RI 0.8525). The ITS region was sampled for 70 species. The MP analysis resulted in 39 trees with 1,938 steps, CI 0.5542, RI 0.8555. Both MP and Bayesian analyses of *trnL-F* and ITS were congruent, and the strict consensus trees of MP (with the support of bootstrap and posterior probabilities of Bayesian analyses) are plotted in Fig. 5. Analysis of the two regions resulted in similar topologies for the major groups, but differing in the internal resolution of the clades, especially within *Paepalanthus*. This appears to be due to ITS being more variable than *trnL-F*.

These MP combined analyses resulted in 4,128 trees with 2,949 steps, CI=0.5894, RI=0.8452. The topology observed in the MP analysis was very similar to that found in the Bayesian analysis except in minor details (Fig. 6). For the clades, the relationships in the combined analysis were more strongly supported than the individual analyses, in both bootstrap (PB) support values and Bayesian posterior probability (PP) (Figs. 5-6).

Using one of the most parsimonious trees of the combined analysis, the clades were named with letters that represent monophyletic groups. The bootstrap (BP) and Bayesian subsequent probability (PP) presented in the text is the result of the combined analyses (Fig. 6). However the BP obtained from individual analyses are presented in Fig. 5 for comparison.

All of the analyses support the monophyly of Paepalanthoideae (Figs. 5, 6, clade A, BP/PP 100). This group is characterized by: isostemonous flowers; eglandular petals and pistillate flowers with branched styles; stigmatic portions alternating with nectariferous portions; and simple or bifid stigmas. Except in *Rondonanthus*, which has linear and vascularized staminodes (Fig. 3p), all other genera have scale-like staminodes (Figs. 3t-u).

In Paepalanthoideae (Figs. 5, 6; Clade A), two main clades were recognized as having moderate to high bootstrap support and Bayesian posterior probability.

The Clade S (BP 93, PP 100) comprises the species of the monophyletic genus *Syngonanthus* (including *S.* sect. *Syngonanthus*,

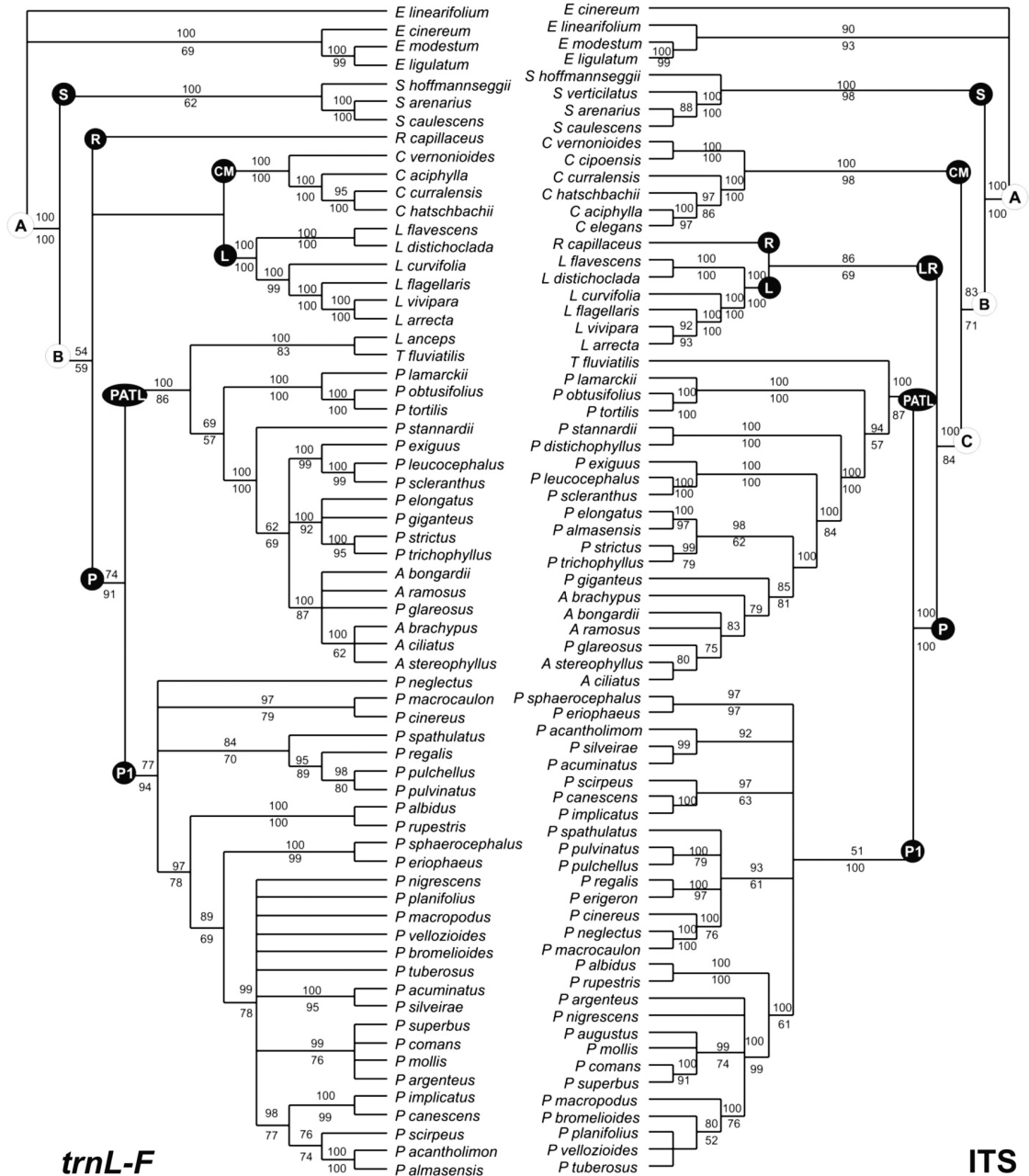


Figure 5 – Strict consensus tree of the maximum parsimony analyses (MP) of the individual region: *trnL-F* (left, 30,000 MPTs, 906 steps, CI = 0.7064, RI = 0.8525) and ITS (right, 39 MPTs, 1,938 steps, CI = 0.5542, RI = 0.8555). Numbers above lines are bootstrap support values and numbers below lines are Bayesian posterior probability.

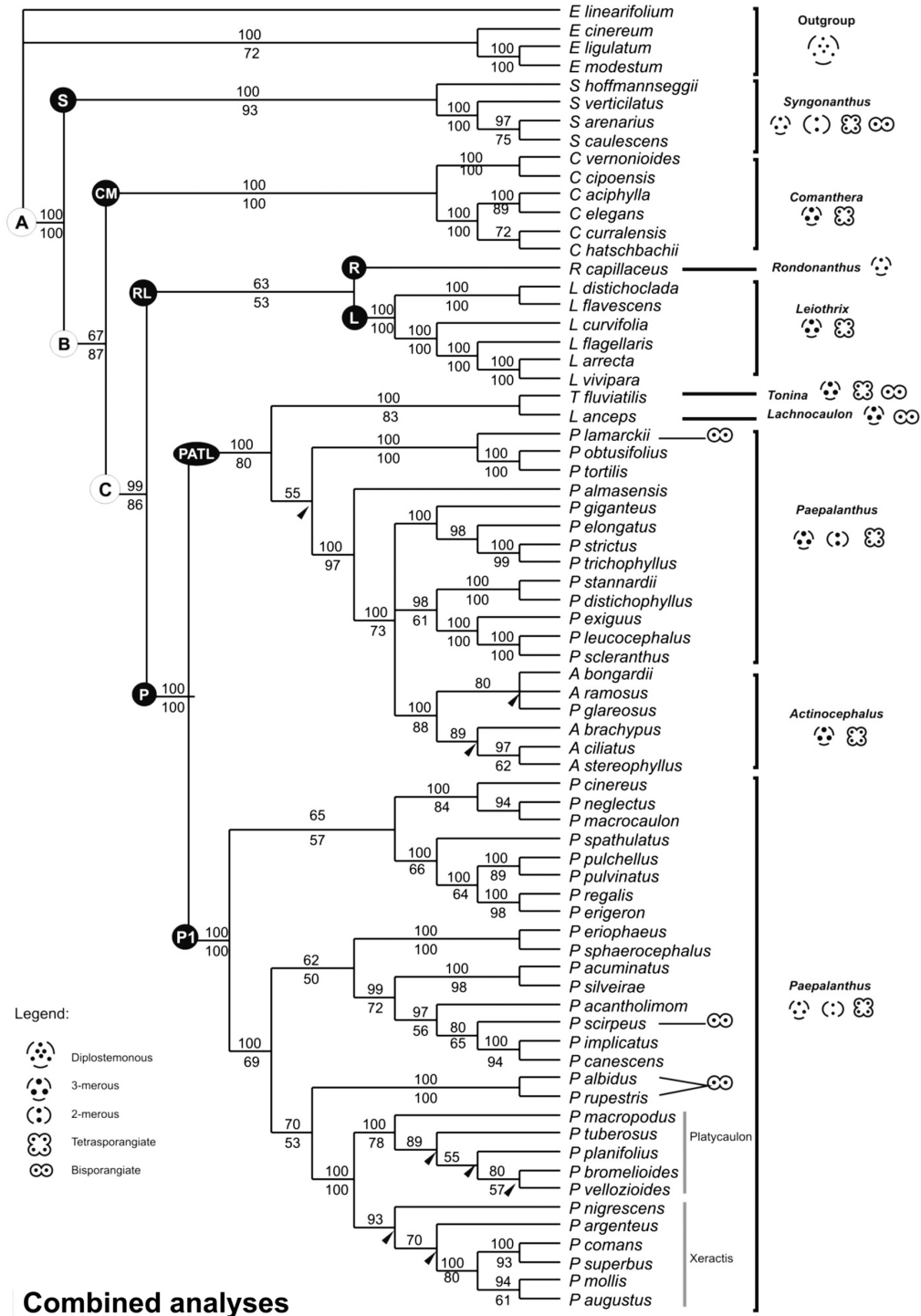


Figure 6 – Majority rule consensus tree (50%) of the Bayesian analysis of the combined *trnL-F* and ITS data. Numbers above lines are Bayesian posterior probability and numbers below lines are bootstrap support values. Arrowheads indicate clades collapsed in the strict consensus tree of the maximum parsimony analyses of the combined analyses (*trnL-F*/ITS, 4,128 MPTs, 2,949 steps, CI = 0.5894, RI = 0.8452). The generic classification indicated to the right is based on Ruhland (1903) and complemented by current data from Andrade *et al.* (2010, 2011), Giulietti *et al.* (2009), and Parra *et al.* (2010).

S. sect. *Carphocephalus* and *Philodice* sensu Ruhland). *Syngonanthus* sensu Parra *et al.* (2010) is characterized by having: staminate flowers with stamen filaments adnate to the corolla; 3 (or rarely 2) stamens; tetrasporangiate or bisporangiate anthers; and pistillate flowers with syngonanthoid fusion petals smaller than the sepals (Figs. 3c, d). The flavonoids have 6-hydroxy-luteoline derivatives (Ricci *et al.* 1996). *Syngonanthus* is strongly positioned as sister to the remainder of Paepalanthoideae in all analyses (Figs. 5, 6).

The clade B includes two main clades C and CM. Clade CM (BP 100, PP 100) is formed by the monophyletic *Comanthera* (C. subg. *Comanthera* and C. subg. *Thysanocephalus*) sensu Andrade *et al.* (2010) and Parra *et al.* (2010). This is corroborated here by the inclusion of *C. cipoensis* (Ruhland) L.R. Parra & Giul., which increases the taxa number in C. subg. *Thysanocephalus*. *Comanthera* has pistillate flowers with petals having syngonanthoid fusion that are longer than the sepals (Fig. 3e); staminate flowers (Fig. 3f) with 3 stamens and tetrasporangiate anthers, filaments free from the corolla, pistillodes with apical papillose trichomes; and seeds with rugose surfaces (Parra 2000; Parra *et al.* 2010). *Comanthera* has leaves and scapes with an epidermis having thickened cell walls, compact chlorenchyma (Figs. 1h-i), and scapes with malpighiaceus trichomes. It also has different flavonoids from *Syngonanthus* (Ricci *et al.* 1996; Coelho *et al.* 2006). Pollination is entomophilous, especially Diptera, Coleoptera, and Hymenoptera, and nectar is present (Ramos *et al.* 2005; Oriani *et al.* 2009). Two subgenera were recognized for *Comanthera*: *Thysanocephalus*, which has spiraperturate pollen grains; and *Comanthera* which has 2-zonasulcate pollen grains that have not been found in other representatives of the family (Figs. 4h-k) (Borges *et al.* 2009; Parra *et al.* 2010).

The Clade C includes two main clades: Clade RL including *Rondonanthus* and *Leiothrix* and Clade P with *Paepalanthus*. The Clade RL includes two subclades: L (*Leiothrix*) and R (*Rondonanthus*). Clade L (Figs. 5, 6; BP/PP 100) is formed by the monophyletic genus *Leiothrix*. The monophyly of *Leiothrix* confirms an earlier phylogenetic analysis based on morphology and anatomy (Giulietti *et al.* 1995). This genus includes approximately 45 species and is characterized by having pistillate flowers with styles forming a column, and with stigmatic and nectariferous portions free at different levels, basifixed anthers (Fig. 3n,s), and striate seeds. As in Andrade *et al.* (2010), the sister group of *Leiothrix* is

not still clear. *Leiothrix* (Clade L) has *Rondonanthus capillaceus* (Körn.) Hensold & Giul. (Clade R) as the sister group in both ITS (BP 69, PP 86) and combined analyses (BP 53, PP 63). However, in *trnL-F* analysis, Clade CM appears more related to Clade L (BP >50%, not shown), and in polytomy with Clades P and R, which are, on the other hand, slightly sustained. *Comanthera* (Clade CM) and *Leiothrix* (Clade L) are shown to be monophyletic and highly sustained in all of the analyses. The inclusion of more species of *Rondonanthus* in further studies might lead to a better explanation of the phylogenetic position of this genus.

Clade P in the *trnL-F* analyses (Fig. 5; BP 91, PP 74), ITS (Fig. 5; BP 100, PP 100), and combined analyses (Fig. 6; BP 100, PP 100), is formed by the genera *Paepalanthus*, *Actinocephalus*, *Lachnocaulon* and *Tonina*, and divided into two subclades, corroborating the previous data in Andrade *et al.* (2010). The Clade PATL (PB 80, PP 100) presented better resolution in combined analyses and includes a polytomy. *Tonina fluviatilis* and *Lachnocaulon anceps* Morong form a monophyletic group. They are morphologically characterized by the reduced perianth, the coalescence of the spathe with the scape and the pollen grains with grooved spines in *Tonina* (Figs. 2d, i, 3g-j, 4f), and by the geographic distribution in *Lachnocaulon* (*T. Stützel* pers. comm.). *Paepalanthus lamarckii*, with bisporangiate anthers, and *P. obtusifolius* Körn. and *P. tortilis* (Bong.) Mart., with tetrasporangiate anthers, form a well supported group (BP 100, PP 100). The other subclade includes *P. almasensis* Moldenke (dimerous flowers) as a sister group of a trichotomy. The first group is formed by species with dimerous flowers, included in *P.* sect. *Diphyomene* Ruhland and in *P.* series *Dimeri* Ruhland. The second monophyletic group includes *P. leucocephalus* Ruhland, *P. scleranthus* Ruhland (the unique species of *P.* subgen. *Thelxinoë* Ruhland), and *P. exiguus* (Bong.) Körn. All have dimerous flowers and are a sister group of *P. stannardii* Giul. & L.R. Parra and *P. distichophyllus* Mart. with elongate stems and trimerous flowers. The last group is formed by the sampled species of *Actinocephalus* and the species of *Paepalanthus* sect. *Aphorocaulon*. A new circumscription of *Actinocephalus* is being proposed by Costa & Sano (2006) for including the species of *P.* subsect. *Aphorocaulon* into *Actinocephalus* also corroborated by morphologic data. The genus is characterized by: the presence of paraclades;

the capitula arrangement; anatomical data; and chromosome size, the largest in the family (Figs. 1b, 2a, 3k,t).

The subclade P1 presented better resolution in combined analyses and includes two subclades (Fig. 6). The first is formed by many species of *P.* subgen. *Paepalanthus* (including *P. erigeron* Mart. ex Körn., the type species). The other is formed by two other groups. The first includes several sections, subsections and series of *Paepalanthus* subgen. *Paepalanthus* (*sensu* Ruhland 1903). The second includes *Paepalanthus* (*Blastocaulon*) *albidus* Gardner and *Paepalanthus* (*Blastocaulon*) *rupestris* Gardner with bisporangiate anthers as a sister group of a monophyletic, well-supported clade, formed by *Paepalanthus* subgen. *Platycaulon* and *P.* subgen. *Xeractis*. The internal resolution of the clade is a polytomy. *Paepalanthus* subgen. *Platycaulon* is a monophyletic group, but *P.* sect. *Divisi* (*P. tuberosus* (Bong.) Kunth, *P. bromelioides* Silveira and *P. vellozioides* Körn.) and *P.* sect. *Conferti* (*P. macropodus* Ruhland and *P. planifolius* (Bong.) Körn.) are not sustained. Likewise, *P.* subgen. *Xeractis* is paraphyletic and the two sections analysed (*P.* sect. *Xeractis* and *P.* sect. *Chrysostegis*) are not sustained. *Paepalanthus* sect. *Chrysostegis* (*P. argenteus* (Bong.) Körn., the only species sampled) appeared collapsed with the two other series included in *P.* sect. *Xeractis*. *Paepalanthus* series *Albidi* (*P. nigrescens* Silveira, the only species studied) appear collapsed with those of the *P.* series *Fuscati*. Hensold

(1988) considered *P.* ser. *Albidi* the most primitive group in *P.* subg. *Xeractis* and also considered that *P. uncinatus* Gardner was the most primitive species in the group. The reason is that involucre bracts are all green and leaf-like. On the other hand, *P. nigrescens* was the most specialized member in this series, mainly due to the acaulescent, thickened stem, and the leaves having a well-developed hypodermis. However, *P.* ser. *Fuscati*, including *P. comans* Silveira, *P. augustus* Silveira, *P. mollis* Kunth, and *P. superbus* Ruhland, appeared as a monophyletic group, characterized morphologically by the acaulescent habit, semi-succulent leaves with reduced buttresses and radially arranged mesophyll (Fig. 1g), and floral trichomes with rounded apices. Hensold (1988) proposed four alliances for *P.* ser. *Fuscati*, but the detection of different evolutionary lines in the series could not be determined in our study. We consider that more extensive sampling within the series should help to resolve the issue. Echternacht *et al.* (2011b) present a morphologic phylogeny of the group and the phylogenetic tree obtained agrees with many of Hensold's (1988) propositions. Firstly, all sections and series previously proposed appear to be monophyletic and the published hierarchy is also confirmed.

In spite of only c. 5% of the genus *Paepalanthus* being sampled, some lineages could be considered as subgenera: *P.* subgen. *Leptocephali*; *P.* subgen. *Paepalanthus*; *P.* subgen. *Telxinoë*, and *P.* subgen. *Platycaulon*.

Key to the genera of Eriocaulaceae

1. Flowers diplostemonous, stamens 4 or 6, petals glandular (Figs. 3b, m) 2
- Flowers isostemonous, stamens 2 or 3, petals eglandular 3
2. Pistillate flowers with petals free (Fig. 3q) *Eriocaulon*
- Pistillate flowers with pistillate petals united in the middle and free at the base and apex (Fig. 3a) *Mesanthemum*
3. Pistillate flowers, petals reduced to small lobes covered with hairs, or lacking 4
- Pistillate flowers with petals never reduced or lacking 5
4. Plants rosulate, scape subtended by a closed spathe, free from the main axis. Pistillate and staminate flowers with sepals free and petals usually lacking, anthers dithecous, bisporangiate (Figs. 2d; 3g-h) *Lachnocaulon*
- Plants long-stemmed, leaves spirally arranged, scape subtended by an open spathe, fused with the main axis. Pistillate flowers with petals reduced to small hairy lobes, staminate flowers with sepals and petals united, anthers dithecous, tetrasporangiate, rarely bisporangiate, pollen exine with grooved spines (Figs. 2i; 3i-j) *Tonina*
5. Pistillate flowers with linear staminodes or stamens present, staminate flowers with petals united or free (Figs. 2g; 3o-p,r) *Rondonanthus*
- Pistillate flowers without linear staminodes or stamens, staminate flowers with petals united 6

6. Styles with the stigmatic and nectariferous portions (*sensu* Rosa & Scatena 2007) separating at different levels, seed surface striate (Fig. 3s) *Leiothrix*
 Styles with stigmatic and nectariferous portions (*sensu* Rosa & Scatena 2007) separating at the same level, seed surface never striate 7
7. Pistillate flowers with petals free 8
 Pistillate flowers with petals united in the middle and free at the base and apex 9
8. Flowers 3-merous; staminate flowers with corolla base conspicuously fleshy, anthers dithealous, tetrasporangiate, capitula in leafy paraclades, pistillate flowers with stigmatic portions completely united (*sensu* Sano 2004; Costa & Sano 2006, including *Paepalanthus* sect. *Aphorocaulon*) (Figs. 2a; 3k,t) *Actinocephalus*
 Flowers 2 or 3-merous; staminate flowers with corolla base membranous, anthers dithealous, bi- or tetrasporangiate, capitula never in leafy paraclades, pistillate flowers with stigmatic portions completely united or not (including *Blastocaulon*) *Paepalanthus*
9. Scapes without malpighiaceae trichomes. Flowers 3-merous rarely 2-merous, unisexual rarely bisexual; staminate flowers with petals united to above the middle, anthers bi- or tetrasporangiate, filaments adnate to the corolla, pollen grains spiraperturate, apex of pistillodes without papillate trichomes; pistillate flowers with petals elliptic to obovate and short lobed, style with nectariferous portion without papillate trichomes; seed surface reticulate (including *Philodice*) (Figs. 3c-d) *Syngonanthus*
 Scapes with malpighiaceae trichomes. Flowers 3-merous, unisexual; staminate flowers with petals united up to the middle, anthers tetrasporangiate, filaments free from the corolla, pollen grains spiraperturate or 2-zonasulcate, apex of the pistillodes with papillate trichomes; pistillate flowers with petals narrowly spatulate and long lobes, style with nectariferous portion with papillate trichomes; seed surface rugose (Fig. 3e-f)
 *Comanthera*

Conclusion

Considerable advances in our knowledge of Eriocaulaceae have been made since Monocots II (Giulietti *et al.* 2000). At present we recognized ten genera in Eriocaulaceae, with *Philodice* being synonymized under *Syngonanthus* and *Blastocaulon* under *Paepalanthus*, as well as the re-establishment of the genus *Comanthera*. Despite this, much still remains to be done to establish a phylogenetic classification within the family, especially in *Paepalanthus s.l.* This will require a considerable expansion of molecular information, as well as an elucidation of the nature, ontogeny and function of a wide range of recently discovered morphological features within the family. In addition, studies exploring species evolution and phylogenetic significance within Eriocaulaceae are needed.

Based on the data presented here, we recognize four monophyletic genera in Paepalanthoideae: *Syngonanthus*, *Comanthera*, *Leiothrix* and *Rondonanthus*. As regards the polyphyletic genus *Paepalanthus*, some molecular and morphological analyses are now in progress, with a view to producing a new classification of *Paepalanthus*.

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Appendix 1 – Species and collections used in the morphological and anatomical studies of vegetative and reproductive organs.

Actinocephalus bongardii (A.St.-Hil.) Sano: *Sano CFSC13023* (SPF), Santana do Riacho-MG; *Scatena et al. 203* (HRCB). *A. polyanthus* (Bong.) Sano: *Scatena et al. 214* (HRCB), Santana do Riacho-MG; *CFCR 6349* (SPF). *A. ramosus* (Wikstr.) Sano: *Scatena & Giulietti 165*, Rio de Contas-BA; (HRCB); *L.P. Felix 9525*, Rio de Contas-BA (HUEFS). *A. robustus* (Silveira) Sano: *Scatena et al. 224* (HRCB), Rio de Contas-BA. *Comanthera aurifibrata* (Silveira) L.R. Parra & Giul.: *Rossi et al. CFCR 1079* (SPF), Joaquim Felício-MG. *C. cipoensis* (Ruhland) L.R. Parra & Giul.: *Scatena et al. CFSC 10421*, Santana do Riacho-MG; *10466, 10913* (SPF); *Scatena et al. 195* (HRCB). *Comanthera aff. circinnata* (Bong.) L.R. Parra & Giul.: *Zappi et al. CFCR 11292* (SPF), Diamantina-MG. *C. elegans* (Bong.) L.R. Parra & Giul.: *Giulietti CFCR 3789* (SPF), Diamantina-MG. *C. imbricata* (Körn.) L.R. Parra & Giul.: *Giulietti et al. 1047* (SPF), Guarapari-ES; *Andrade Lima s/n* (SPF). *C. nitida* (Bong.) L.R. Parra & Giul.: *Magalhães 4335* (SPF), Santana do Riacho-MG. *C. squarrosa* (Ruhland) L.R. Parra & Giul.: *Scatena CFCR 11309* (SPF), Santa Bárbara-MG. *C. vernonioides* (Kunth) L.R. Parra & Giul.: *Giulietti CFSC 4968*, *Scatena*, Santana do Riacho-MG; *CFSC 10912*, *Scatena et al.*; *CFSC 10387* (SPF). *C. xeranthemoides* (Bong.) L.R. Parra & Giul.: *Zappi et al. CFCR 11290* (SPF), Diamantina-MG. *Eriocaulon aquatile* Körn.: *Giulietti CFSC 5055* (SPF), Santana do Riacho-MG. *E. cipoense* Silveira: *Giulietti CFSC 5053* (SPF), Santana do Riacho-MG. *E. crassiscapum* Bong.: *Giulietti CFSC 5138* (SPF), Santana do Riacho-MG. *E. elichrysoides* Bong.: *Giulietti 5050* (SPF), Santana do Riacho-MG; *Scatena et al. 183* (HRCB). *E. ligulatus* (Vell.) L.B. Sm.: *Scatena et al. 199* (HRCB), Santana do Riacho-MG.; *Teixeira s.n. SPF 97479* (SPF), Itabirito-MG; *CFCR 1522* (SPF). *E. linearifolium* Körn.: *Giulietti et al. CFCR 6294* (SPF), Joaquim Felício-MG. *E. melanolepis* Silveira: *Hensold 582* (SPF), Santana do Riacho-MG. *E. modestum* Kunth: *Harley et al. 26224* (SPF), Rio de Contas-BA. *E. vaginatum* Körn.: *Scatena s.n. SPF 76169* (SPF), Santana do Riacho-MG. *Lachnocaulon anceps* Morong: *Unwin 201* (MU), Alabama, USA; *Harper 443* (PH), Georgia, USA. *L. engleri* Ruhland: *Unwin 237* (MU), Alabama, USA. *L. minus* Small: *Unwin 230* (MU), Alabama, USA. *Leiostrix crassifolia* (Bong.) Ruhland: *Giulietti et al. CFCR 4672* (SPF), Couto Magalhães-MG. *L. flavescens* (Bong.) Ruhland var. *distichophylla* (Silveira) Giul. & Hensold: *Cerati et al. CFCR 4287* (SPF), Datas-MG. *L. flavescens* (Bong.) Ruhland var. *flavescens*: *Scatena et al. 209* (HRCB), Rio de Contas-BA. *L. fluitans* (Mart.) Ruhland: *Giulietti CFSC 5051* (SPF), Santana do Riacho-MG; *Rosa et al. 10* (HRCB); *CFCR 8317* (SPF). *Mesanthemum auratum* Lecomte: *Chillon 1835* (C), Senegal, Africa. *Paepalanthus applanatus* Ruhland: *Silva et al. CFCR 8018* (SPF), Datas-MG. *P. (Blastocaulon) albidus* Gardner: *Arbo et al. 4362* (SPF), Diamantina-MG; *Hensold 234* (HUEFS). *P. bromelioides* Silveira: *Sano et al. CFSC 12859* (SPF), Santana do Riacho-MG. *P. bryoides* Kunth: *Giulietti et al. s.n.* (SPF), Santana do Riacho-MG. *P. chlorocephalus* Silveira: *Coan et al. 4* (HRCB), Santana do Riacho-MG. *P. flaccidus* (Bong.) Kunth: *Scatena et al. 235* (HRCB), Santana do Riacho-MG. *P. geniculatus* (Bong.) Kunth: *Hensold CFCR 4219* (SPF), Serro-MG. *P. gibbosus* Silveira: *Giulietti CFCR 57* (SPF), Diamantina-MG. *P. incanus* (Bong.) Körn.: *Zappi et al. CFCR 10527* (SPF), Diamantina-MG. *P. latipes* Silveira: *Tissot & Scatena 29* (SPF), Santana do Riacho-MG. *P. leucocephalus* Ruhland: *M.J.G. Andrade 488* (HUEFS), Grão Mogol-MG. *P. longicaulis* Silveira: *Silveira 692* (SPF), Santana do Riacho-MG. *P. macrocaulon* Silveira: *L.P. Felix 9539* (HUEFS), Rio de Contas-BA. *P. macrocephalus* (Bong.) Körn.: *Silva et al. CFCR 11126* (SPF), Serro-MG. *P. macropodus* Ruhland: *Benko-Iseppon s.n.* (SPF), Santana do Riacho-MG. *P. neglectus* Körn.: *L.P. Felix 9543* (HUEFS), Rio de Contas-BA. *P. obtusifolius* Körn.: *Harley et al. 19812* (HUEFS), Rio de Contas-BA. *P. phaeocephalus* Ruhland: *Giulietti CFCR 1073* (SPF), Alto Paraíso de Goiás-GO. *P. planifolius* (Bong.) Körn.: *Mello-Silva et al. CFCR 8023* (SPF), Santana do Riacho-MG. *P. prostratus* (Benth. & Hook.) Koern: *Hensold 496* (SPF), Santo Antonio do Itambé-MG. *P. (Blastocaulon) rupestris* Gardner: *M.J.G. Andrade 543* (HUEFS), Diamantina-MG. *P. scleranthus* Ruhland: *Scatena et al. 220* (HRCB), Santana do Riacho-MG. *Scatena et al. 247* (HRCB). *P. (Blastocaulon) scirpeus* Mart. ex Körn.: *Hensold 767* (SPF), Barão de Cocais-MG, *Scatena et al. 246* (HRCB). *P. subtilis* Miq.: *Scatena & Giulietti s.n.* (HUEFS), Rio de Contas-BA; *F. Juchum 16* (HUEFS). *P. tortilis* (Bong.) Mart.: *M.J.G. Andrade 465* (HUEFS), Subaúma-BA; *M.J.G. Andrade 472* (HUEFS), Grão Mogol-MG. *P. vellozioides* Körn.: *Martens s.n.* (SPF), Brumadinho-MG. *Rondonanthus roraimae* (Oliver) Herzog: *Huber & Alarcon 10526* (SPF), Bolívar, Venezuela. *Syngonanthus anthemidiflorus* var. *anthemidiflorus* (Bong.) Ruhland: *Hensold CFSC 5190* (SPF), Santana do Riacho-MG. *S. appressus* (Körn.) Ruhland: *Giulietti et al. CFCR 4572* (SPF), Couto Magalhães-MG. *S. caulescens* (Poir.) Ruhland: *Pereira CFCR 11310* (SPF), Serra Azul de Minas-MG; *Scatena et al. 142* (HRCB). *S. fuscescens* Ruhland: *Semir et al. CFSC 5191* (SPF), Santana do Riacho-MG. *S. (Philodice) hoffmannseggii* (Mart.) Giul. & M.J.G. Andrade: *Giulietti 1381* (HUEFS), Mato Grosso-MT. *S. nitens* (Bong.) Ruhland: *CFSC 12980* (SPF), Santana do Riacho-MG. *S. verticillatus* Ruhland: *Giulietti CFCR 3785* (SPF). *Tonina fluvialis* Aubl.: *Harley et al. 17980* (SPF), Alcobaça-BA; *Alves & Pinto s.n.* (HRCB); *Andrade 616* (HUEFS), Recife-PE.

Appendix 2 – Names, vouchers and Genbank accessions for DNA samples used for molecular phylogenetic analyses. The voucher specimens are deposited in the following Herbaria: HUEFS¹, SPF², BH³, NY⁴ and FLAS⁵. For additional information on the geographic origin of taxa see Andrade *et al.* (2010). Asterisks indicate registry number of the sample in the DNA bank (FSA) at the Universidade Estadual de Feira de Santana, Brazil. GenBank accession numbers are given in the following sequence ITS and *trnL*-T, replaced with # where not sampled.

Actinocephalus bongardii (A.St.-Hil.) Sano¹: *MJG Andrade* 501; *1927; EU924282, EU924434. *A. brachypus* (Bong.) Sano¹: *MJG Andrade* 521; *1938; EU924281, EU924433. *A. ciliatus* (Bong.) Sano¹: *MJG Andrade* 544; *1956; EU924283, EU924435. *A. ramosus* (Wikstr.) Sano¹: *BRN Araújo* 78; *1798; EU924284, EU924436. *A. stereophyllus* (Ruhland) Sano¹: *MJG Andrade* 514; *1933; EU924285, EU924437. *Comanthera aciphylla* (Bong.) L.R. Parra & Giul.¹: *MJG Andrade* 532; *1948; EU924339, EU924491. *C. cipoensis* (Ruhland) L.R. Parra & Giul.²: *Trovó 203*; *6945, GQ861448, #. *C. curralensis* (Moldenke) L.R. Parra & Giul.¹: *MJG Andrade* 595; *6890; EU924340, EU924492. *C. elegans* (Bong.) L.R. Parra & Giul.^{1,2}: *Trovó 340*; *6941; GQ861447, #. *C. hatschbachii* (Moldenke) L.R. Parra & Giul.¹: *AC Pereira* 122; *6891; EU924341, EU924493. *C. vernonioides* (Kunth) L.R. Parra & Giul.¹: *AM Giuliatti* 2185; *220; EU924343, EU924499. *Eriocaulon cinereum* R. Br.¹: *AM Giuliatti* 2582; *6875; EU924280, EU924432. *E. ligulatum* (Vell.) L.B. Sm¹: *AM Giuliatti* 2368; *298; EU924278, EU924430. *E. linearifolium* Körn.¹: *AM Giuliatti* 2366; *297; EU924277, EU924429. *E. modestum* Kunth¹: *MJG Andrade* 445; *780; EU924279, EU924431. *Lachnocaulon anceps* Morong³: *D. Goldman* s/n; *6877; #, EU924442. *Leiothrix arrecta* Ruhland¹: *AM Giuliatti* 2496; *6872; EU924296, EU924449. *L. curvifolia* (Bong.) Ruhland¹: *MJG Andrade* 553; *1964; EU924293, EU924446. *L. distichoclada* Herzog¹: *MJG Andrade* 458; *792; EU924294, EU924447. *L. flagellaris* (Guill.) Ruhland¹: *MJG Andrade* 485; *1920; EU924297, EU924450. *L. flavescens* (Bong.) Ruhland¹: *MJG Andrade* 440; *775; EU924291, EU924444. *L. vivipara* (Bong.) Ruhland¹: *AM Giuliatti* 2503; *7415; EU924298, EU924451. *P. acantholimon* Ruhland²: *Trovó 250*; *6924; GQ475232, #. *P. cf. acantholimon* Ruhland¹: *MJG Andrade* 522; *1939; #, EU924466. *P. acuminatus* Ruhland²: *Trovó 175*; *6928; GQ475234, GQ475204. *P. (Blastocaulon) albidus* Gardner¹: *MJG Andrade* 541; *1953; EU924287, EU924439. *P. almasensis* Moldenke¹: *MJG Andrade* 429; *768; EU924315, EU924468. *P. argenteus* (Bong.) Körn.¹: *MJG Andrade* 539; *1952; EU924331, EU924484. *P. augustus* Silveira²: *Borges* 178; *6963; GQ475236, #. *P. bromelioides* Silveira²: *Trovó 219*; *6905; GQ475238, GQ475207. *P. canescens* Körn.¹: *MJG Andrade* 536; *1950; EU924324, EU924477. *P. cinereus* Giul. & L.R. Parra¹: *AM Giuliatti* s/n; *1293; EU924316, EU924469. *P. comans* Silveira¹: *MJG Andrade* 540; *6820; EU924329, EU924482. *P. distichophyllus* Mart.²: *Trovó 218*; *6907; GQ475245, #. *P. elongatus* (Bong.) Körn.¹: *MJG Andrade* 572; *6884; EU924314, EU924467. *P. erigeron* Mart. ex Körn.¹: *AA Ribeiro-Filho* 107; *6882; EU924306, #. *P. eriophaeus* Ruhland¹: *MJG Andrade* 504; *6811; EU924307, EU924459. *P. exiguus* (Bong.) Körn.^{1,2}: *E Guarçoni* 710; *6885; EU924328, EU924481. *P. giganteus* Sano¹: *MJG Andrade* 527; *1943; EU924325, EU924478. *P. glareosus* Kunth¹: *MJG Andrade* 548; *1959; EU924322, EU924475. *P. implicatus* Silveira¹: *MJG Andrade* 550; *1961; EU924319, EU924472. *P. lamarckii* Kunth¹: *DS Carneio-Torres* 461; *6880; EU924303, EU924456. *P. leucocephalus* Ruhland¹: *MJG Andrade* 620; *6886; EU924334, EU924487. *P. macrocaulon* Silveira¹: *MJG Andrade* 431; *766; EU924317, EU924470. *P. macropodus* Ruhland²: *Trovó 214*; *6922; GQ475251, GQ475220. *P. mollis* Kunth²: *Trovó 376*; *6961; GQ475253, GQ475222. *P. neglectus* Körn.¹: *BRN Araújo* 85; *1805; EU924308, EU924460. *P. nigrescens* Silveira²: *Trovó 204*; *6932; GQ475254, GQ475223. *P. obtusifolius* Körn.¹: *R Harley* 54802; *1321; EU924304, EU924457. *P. planifolius* (Bong.) Körn.¹: *MJG Andrade* 526; *1942; EU924332, EU924485. *P. pulchellus* Herzog¹: *AM Giuliatti* 2423; *1409; EU924309, EU924461. *P. pulvinatus* N.E. Br.¹: *R Harley* 54634; *638; EU924313, EU924465. *P. regalis* Mart.¹: *R Harley* 54640; *660; EU924310, EU924462. *P. (Blastocaulon) rupestris* Gardner¹: *MJG Andrade* 542; *1954; EU924288, EU924440. *P. (Blastocaulon) scirpeus* Mart. ex Körn.^{1,2}: *JR Pirani* 4162; *6876; EU924289, EU924441. *P. scleranthus* Ruhland¹: *MJG Andrade* 537; *1951; EU924335, EU924488. *P. silveirae* Ruhland¹: *MJG Andrade* 568; *1976; EU924311, EU924463. *P. spathulatus* Körn.¹, *R Harley* 55476; *6883; EU924312, EU924464. *P. sphaerocephalus* Ruhland¹: *MJG Andrade* 456; *790; EU924327, EU924480. *P. stannardii* Giul. & L.R. Parra¹: *MJG Andrade* 438; *773; EU924320, EU924473. *P. strictus* Körn.¹: *MJG Andrade* 491; *1926; EU924318, EU924471. *P. superbus* Ruhland¹: *AM Giuliatti* 2504; *6858; EU924330, EU924483. *P. tortilis* (Bong.) Mart.¹: *MJG Andrade* 479; *1914; EU924302, EU924455. *P. trichophyllus* (Bong.) Körn.¹: *MJG Andrade* 439; *774; EU924326, EU924479. *P. tuberosus* (Bong.) Kunth¹: *C Van den Berg* 1364; *1641; EU924333, EU924486. *P. vellozioides* Körn.²: *Trovó 198*; *6906; GQ475261, GQ475229. *Rondonanthus capillaceus* (Körn.) Hensold & Giul.¹: *C Van den Berg* 1792; *6889; EU924338, GQ478282. *Syngonanthus arenarius* (Gardner) Ruhland¹: *MJG Andrade* 493; *6802; EU924342, EU924498. *S. caulescens* (Poir.) Ruhland¹: *MJG Andrade* 452; *786; EU924344, EU924500. *S. (Philodice) hoffmannseggii* (Mart.) Giul. & M.J.G. Andrade¹: *AM Giuliatti* 2483; *6887; EU924336, EU924489. *S. verticillatus* Ruhland²: *Trovó 222*; *6944; GQ861446, #. *Tonina fluviatilis* Aubl.¹: *MJG Andrade* 616; *6895; EU924345, EU924501.