



Original Paper

Diversity and evolution of stem structure in *Eleocharis* (Cyperaceae)

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Abstract

Eleocharis (Cyperaceae) includes more than 300 species of perennial or annual herbs, frequently found on poorly drained soils. The species are morphologically similar, and the taxonomy is difficult because their vegetative and reproductive structures are very reduced. Previous study on the stem architecture in the subgenus *Limnochloa* showed that anatomical features help in the interpretation of the evolution, taxonomy and ecological aspects of the group. Our objectives were to add new characters from the stem (= culm) structure, to explore the characters in a greater number of *Eleocharis* species, representatives of the other subgenera, and add these data in a new phylogenetic analysis with molecular data. The study covered 68 species obtained from herbaria and fixed material. In addition to the stem architecture, the internal organization of the tissues, the cross-section format, the presence or absence of stretched cells in aerenchyma air gaps and the plant size were included in the morphological analysis. Our data confirm that spongy aerenchyma pattern is the ancestral condition while the mixed and septate patterns occurred independent and punctually. Only the cross-section format was variable among specimens while the other characters were uniform and relevant for taxonomic use.

Key words: anatomy of aquatic plant, culm anatomy, evolution of aerenchyma, phylogeny, reconstruction of ancestral state.

Resumo

Eleocharis (Cyperaceae) inclui mais de 300 espécies de ervas perenes ou anuais, frequentemente encontradas em solos pouco drenados. As espécies são morfologicamente semelhantes e a taxonomia é difícil devido às estruturas vegetativas e reprodutivas reduzidas. Estudo anterior sobre a arquitetura do caule no subgênero *Limnochloa* mostrou que as características anatômicas ajudam na interpretação da evolução, taxonomia e aspectos ecológicos do grupo. Nossos objetivos foram adicionar novos caracteres da estrutura do caule (= colmo), explorar os caracteres em um número maior de espécies de *Eleocharis*, com representantes de outros subgêneros, e adicionar esses dados em uma nova análise filogenética com dados moleculares. O estudo abrangeu 68 espécies obtidas de herbários e material fixado. Além da arquitetura do caule, foram incluídas na análise morfológica a organização interna dos tecidos, o formato da seção transversal, a presença ou ausência de células estiradas nos espaços de aerênquima e o porte da planta. Nossos dados confirmam que o padrão de aerênquima esponjoso é a condição ancestral, enquanto os padrões misto e septados ocorreram independentemente e pontualmente. Somente o formato da seção transversal foi variável entre as amostras, enquanto os demais caracteres foram uniformes e relevantes para o uso taxonômico.

Palavras-chave: anatomia de planta aquática, anatomia do colmo, evolução do aerênquima, filogenia, reconstrução do estado ancestral.

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Introduction

Eleocharis R. Br. (Cyperaceae) includes over 300 species of small to medium-sized herbs, perennial or annual, found in tropical and subtropical regions of the globe associated to water bodies (Svenson 1929; Goetghebeur 1998; Govaerts *et al.* 2017). In most species, the stem system is very compact in a caespitose habit, but there are rhizomatous and stoloniferous species too. All of them produce several culms with a terminal, simplified inflorescence (Svenson 1929, 1932, 1937, 1939). Leaves are reduced and culms are the mainly photosynthetic organ (Metcalf 1971). The taxonomic grouping and phylogenetic relations are difficult due the reduced structures, both in vegetative and reproductive organs.

The main characters used for phylogeny and infrageneric organization are the morphology of the leaf sheaths, the internal structure of the culm and the morphology of the achenes (Svenson 1939; Kukkonen 1990; González-Elizondo & Peterson 1997; Roalson & Friar 2000; Roalson *et al.* 2010).

A morphological analysis by González-Elizondo & Peterson (1997) recognized four subgenera, seven sections, eight series and seven subseries. Roalson & Friar (2000) used nuclear ribosomal internal transcribed spacer (ITS) region support for monophyletic *Eleocharis* subgenus *Limnochloa* and subgenus *Zinserlingia* while other subdivisions were para- or polyphyletic. Looking for new characters, Hinchliff & Roalson (2009) demonstrated that in subgenus *Limnochloa*, the stem shape (= culm) and internal compartmentalization of air cavities have a great value in phylogenetical approaches. In continuity, Roalson *et al.* (2010) explored phylogenetic relationships using molecular data (nrDNA ITS and cpDNA trnC-ycf6 and ycf6-psbM sequence data) as well as morphology and photosynthetic pathway variations (C3, C4 and intermediate), confirming the monophyly of subgenera *Limnochloa*, *Scirpidium* and *Zinserlingia*. However, the authors highlighted many persistent problems with some subgroups circumscription.

The species of *Eleocharis* are amphibians or emerging plants that inhabit temporarily submerged or permanently flooded soils (Svenson 1929; Goetghebeur 1998). These plants are dependent on oxygen transport through the aerenchyma, which guarantees the aeration of the whole plant by increasing oxygen diffusion (Shimamura

et al. 2010). In Cyperaceae species, the culms have longitudinal air cavities in the aerenchyma, transversally interrupted by diaphragms with stellate cells (cells with projections) (Metcalf 1971). In the subgenus *Limnochloa*, Hinchliff & Roalson (2009), three main types of stem structure have been described: spongy, which contain extensive networks of diverse small air cavities; transversally septate, which have a hollow culm surrounded by small numerous air cavities; and transversally septate with a central vascular bundle. The aim of this study to verify the diversity and distribution of these morphological patterns and aerenchyma structure in the culm of 68 species and determine if the characters have taxonomic or phylogenetic value for the group.

Materials and Methods

The culm structure was evaluated in 68 species of *Eleocharis*, including species from all six clades by Roalson *et al.* (2010), plus *Bulbostylis juncooides* (Vahl) Kük. *ex* Osten, *Fimbristylis miliacea* (L.) Vahl and *Fuirena umbellata* Rottb. as the outgroup. Phylogenetic analysis was performed only for the 59 species with available molecular data, (Tab. 1; Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.16879783.v1>>).

Species samples from various parts of the world were obtained from exsiccatae from the FLOR, MBM and UPCB herbaria (acronyms according to Thiers, continuously updated). Stem of eleven species were collected and the exsiccatae were deposited in the FLOR and UPCB herbaria (see Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.16879783.v1>>). Individuals from two or three different places were used in order to avoid possible environmental variation in data collection, whenever possible. The origin of each material was obtained through data available in the voucher's label (Tab. S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.16879783.v1>>).

Fresh material was fixated in FAA70 (Johansen 1940) and stored in 70% ethanol while the herborized material were rehydrated with 30% ammonium hydroxide for three hours (Toscano de Brito 1996), and later stored in 70% ethanol. For all species, transverse and longitudinal sections were made in the mid and basal regions of the culm. Permanent slides were prepared from the processed material, included in Historesin Leica®, following

Table 1 – Character matrix for *Eleocharis* species and outgroup species (*Bulbostylis juncooides*, *Fimbristylis miliacea* and *Fuirena umbellata*) based on five morphological and anatomical characters of the stem (= culm). a: stem architecture (0 = spongy; 1 = septate; 2 = solid). b: internal organization (0 = basic spongy; 1 = mixed spongy; 2 = septate; 3 = septate with central vascular bundle; 4 = without air gaps). c: cross-section format (0 = circular; 1 = elliptical; 2 = three-angled; 3 = four-angled; 4 = five-angled; 5 = six-angled or more). d: presence of stretched cells in air gaps (0 = absent; 1 = present). e: plant size (0 = small [< 17 cm]; 1 = medium [17 to 40 cm]; 2 = tall [> 40 cm]). (? = it was not possible to classify this character).

Espécie/estado	a	b	c	d	e	Espécie/estado	a	b	c	d	e
<i>B. juncooides</i>	2	4	5	-	0,1,2	<i>E. macrostachya</i>	0	0	0	0	2
<i>E. acicularis</i>	0	0	2,3	0	1	<i>E. maculosa</i>	0	0	0	0	1
<i>E. acuta</i>	0	0	0	0	2	<i>E. mamillata</i>	0	0	0	0	2
<i>E. acutangula</i>	0	1	2	0	2	<i>E. minima</i>	0	0	2,3	0	0
<i>E. albibracteata</i>	0	0	0	0	0	<i>E. montana</i>	1	2	0	0	2
<i>E. albida</i>	0	0	1	0	1	<i>E. montevidensis</i>	0	0	0	0	1
<i>E. baldwinii</i>	0	0	2,3	0	0	<i>E. multicaulis</i>	0	0	0,1	0	1
<i>E. bonariensis</i>	0	0	0	0	2	<i>E. mutata</i>	0	0	2	0	2
<i>E. capillacea</i>	0	0	3	0	0	<i>E. nana</i>	0	0	3	?	0
<i>E. carniolica</i>	0	0	3	0	1	<i>E. niederleinii</i>	0	0	3	0	1
<i>E. compressa</i>	0	0	1	0	0	<i>E. nudipes</i>	0	0	3	?	2
<i>E. congesta</i>	0	0	0	0	1	<i>E. obtusa</i>	0	0	0	0	0
<i>E. contracta</i>	1	2	0	0	2	<i>E. obtusetrigona</i>	0	1	2	0	2
<i>E. cylindrica</i>	0	0	0	0	0	<i>E. ovata</i>	0	0	0	0	2
<i>E. cylindrostachys</i>	0	0	0	0	2	<i>E. pachycarpa</i>	0	0	3	0	1
<i>E. debilis</i>	0	0	3	?	1	<i>E. pachystyla</i>	0	0	0	0	1
<i>E. densicaespitosa</i>	0	0	3,4	0	1	<i>E. pallens</i>	0	0	0	0	0
<i>E. dombeyana</i>	0	0	1	0	0	<i>E. palustris</i>	0	0	5	0	2
<i>E. dunensis</i>	0	0	4	?	1	<i>E. parishii</i>	0	0	1	0	1
<i>E. elegans</i>	1	2	0	0	2	<i>E. parodii</i>	0	1	0	0	2
<i>E. emarginata</i>	0	0	4	0	1	<i>E. plicarhachis</i>	0	1	2	0	2
<i>E. endounifascis</i>	1	3	0	0	2	<i>E. rabenii</i>	0	0	3	0	1
<i>E. engelmannii</i>	0	0	0,1	0	1	<i>E. ramboana</i>	0	0	3	0	0
<i>E. equisetoides</i>	1	2	0	0	2	<i>E. retroflexa</i>	0	0	3,4	?	0
<i>E. erythropoda</i>	0	0	0,1	0	1	<i>E. riograndensis</i>	0	0	?	1	1
<i>E. exigua</i>	0	0	1	0	0	<i>E. rostellata</i>	0	0	0,1	0	0
<i>E. filiculmis</i>	0	0	3	?	0	<i>E. schaffneri</i>	0	0	0	0	1
<i>E. flavescens</i>	0	0	0	0	0	<i>E. sellowiana</i>	0	0	0	0	2
<i>E. fuscopurpurea</i>	0	0	?	0	0	<i>E. squamigera</i>	0	0	0	0	2
<i>E. geniculata</i>	0	0	0	0	1	<i>E. subarticulata</i>	0	0	0	0	1
<i>E. hatschbachii</i>	0	0	4	0	?	<i>E. tuberculosa</i>	0	0	0,1	0	2
<i>E. intermedia</i>	0	0	0,1	0	0	<i>E. uniglumis</i>	0	0	0	1	0
<i>E. interstincta</i>	1	2	0	0	2	<i>E. viridans</i>	0	0	3	0	2
<i>E. kleinii</i>	0	1	0	0	2	<i>F. miliacea</i>	2	4	3	-	1,2
<i>E. kuroguwai</i>	1	2	0	0	2	<i>F. umbellata</i>	2	4	4	-	2
<i>E. loefgreniana</i>	0	0	4	0	1						

the manufacturer's guidelines, and stained with Toluidine Blue (O'Brien *et al.* 1964). Analysis was made in an Olympus microscope (model BX41TF) with attached camera (Olympus SC30).

For SEM analysis, samples were dehydrated until absolute ethanol, submitted to critical point method with CO₂ (BAL-TEC CPD030 Critical Point Dryer), adhered in metallic support with adhesive copper tape and metalized with gold (BALZERS SCD030). Samples were visualized in SEM (JEOL JSM-6360LV Scanning Electron Microscope) at the Electronic Microscopy Center of UFPR (CME).

Five morphological characters of the culm were evaluated: 1. stem architecture second Metcalfe (1971); 2. internal organization; 3. cross-section format; 4. presence of stretched cells in air gaps and 5. plant size. Stem internal organization and the cross-section format were classified according to Hinchliff & Roalson (2009). During the analyses, a new character was detected based on the presence or absence of stretched cell filling the air gaps observed in longitudinal sections. Plant size were defined by examining the species collected in the field or obtained from dry material in herbarium and complemented with information from the literature (Svenson 1929, 1932, 1937, 1939; Smith 2001; Blake 1939; Martins *et al.* 1999; Gil & Bove 2007; Lun-kai & Strong 2010; López 2012; Nunes *et al.* 2016). To investigate possible correlations among characters, we analyzed mirrored ancestral reconstruction trees generated by the Mesquite v. 3.03 (Maddison & Maddison 2015).

Comparative phylogenetic analyses

The analyses were made based on the ITS1, 5.8S and ITS2 sequences of nuclear DNA, and *trnC-ycf6* and *ycf6-psbM* from the DNA chloroplast. Molecular sequences of 59 *Eleocharis* species (among the 68 evaluated) were obtained from the original phylogenetic analysis of the genus (Roalson *et al.* 2010). For the external group, we obtained sequences from the GenBank® database. Overall alignment of the sequences was done by the Muscle tool in MEGA program version 6.06 (Tamura *et al.* 2013) and manually adjusted. The matrices were joined by the Sequence Matrix program version 1.7.9 (Vaidya *et al.* 2010). The analysis was made by parsimony in TNT version 1.1 (Goloboff *et al.* 2008), using the TBR algorithm, with all the characters having equal weight and considered unordered (parsimony of

Fitch (Fitch 1971). Missing data was not encoded. Heuristic searches were performed using 10,000 repetitions of random addition. A strict consensus tree was obtained with all the trees found during the searches, after the branch support was evaluated by bootstrap, through 1,000 parsimonious replicas. Consistency (CI) and retention (HI) indices were calculated using the "stats" script.

Clades were considered as having strong branch support when $\geq 90\%$; moderate support when $\geq 75\%$ to $< 90\%$ and low support when $\leq 74\%$. Bootstrap values below 50% were not reported.

In order to evaluate the evolution of the characters from the architecture of the culms aerenchyma, analysis of reconstruction of the ancestral states by parsimony was performed, from the strict consensus tree generated, optimized by ACCTRANA, using the program Mesquite v. 3.03 (Maddison & Maddison 2015). Characters were coded as discrete and their character states are present in Table 1.

Results

Morphological and anatomical analysis

Species of the outgroup (*e.g.*, *B. juncooides*, *F. miliacea* and *F. umbellata*) have a solid culm without air gaps, typical of aerenchyma (Fig. 1). The cross-section format of the culm is six-angled or more in *B. juncooides*, four-angled in *F. miliacea* and five-angled in *F. umbellata* (Fig. 1). The species of the outgroup are small-medium (*B. juncooides*) and tall (*F. miliacea*, *F. umbellata*) (Tab. 1).

The 68 analyzed *Eleocharis* species presented only two types of stem architecture: spongy stem (Fig. 2a) or septate stem (Fig. 2b). The spongy architecture is characterized by presenting numerous air cavities, and this type was registered in the great majority of species of this genus (61

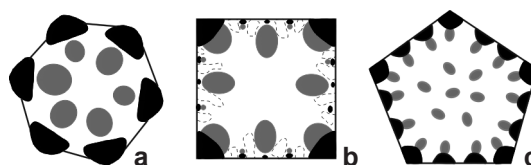


Figure 1 – a-c. Stem (= culm) cross sections diagram of the outgroup species – a. *Bulbostylis juncooides*; b. *Fimbristylis miliacea*; c. *Fuirena umbellata*. (white = parenchyma; gray = vascular bundles; black = sclerenchyma; dashed line = substomatal camera).

species). In the septate architecture, a central big lacuna (hollow) is found as well as several small peripheral lacunae. Both the spongy and septate stems present several columns of air, transversely crossed by diaphragms (Fig. 2a-b).

In cross sections, we identified the three major types of culm internal architecture previously described by Hinchliff & Roalson (2009), based on lacunae distribution and position of the vascular bundles: spongy stem (Fig. 2c-g), transversely septate stem (Fig. 2h-i) and transversely septate stem with a central vascular bundle (Fig. 2j). However, our data revealed new characteristics, and the spongy type was divided into basic spongy and mixed spongy type. The basic spongy type has vascular bundles restricted to the peripheral region of the culm underlying the chlorenchyma (Fig. 2c-f). The lacunae organization could be radially and equally distributed in the central region of the culm (Figs. 2c-e; 3a) or scattered with different formats (Figs. 2f; 3b). The mixed spongy type was included as a new character state, which has vascular bundles dispersed in the fundamental tissue and interspersed between air gaps. (Figs. 2g; 3c). The basic spongy type is the most common and occurs in 57 studied species

(Tab. 1). The mixed spongy type was observed in *E. acutangula*, *E. kleinii*, *E. obtusetrigona*, *E. parodii* and *E. plicarhachis*. Cytological observations also revealed other particularities: only *E. mamillata* presents small air spaces in the chlorenchyma cells adjacent to the vascular bundles (Figs. 2d; 3d), and in *E. acicularis*, the small air spaces are distributed around all peripheral chlorenchyma (Figs. 2e; 3e). The septate culm has a central air column, which occupies most of the organ, and several smaller and uniform lacunae alternated with the vascular bundles in the chlorenchymatic tissue (Figs. 2h-i; 3f). This type was identified in five species (*E. elegans*, *E. equisetoides*, *E. interstincta*, *E. kuroguwai*, *E. montana*). Only in *E. kuroguwai*, the vascular bundle sheath cells were well developed and protrude into the central lacuna (Figs. 2i; 3f-g). The septate culm with central vascular bundle was only recorded in *E. endounifascis*, which has several peripheral vascular bundles alternating with small gaps (Figs. 2j; 3h-i). The diaphragms were registered in all kinds of lacunae, peripheral and central ones, in all four described types of internal organization. The diaphragms cells are generally stellate and form a network with tiny triangular intercellular spaces (Fig. 3h-l). In

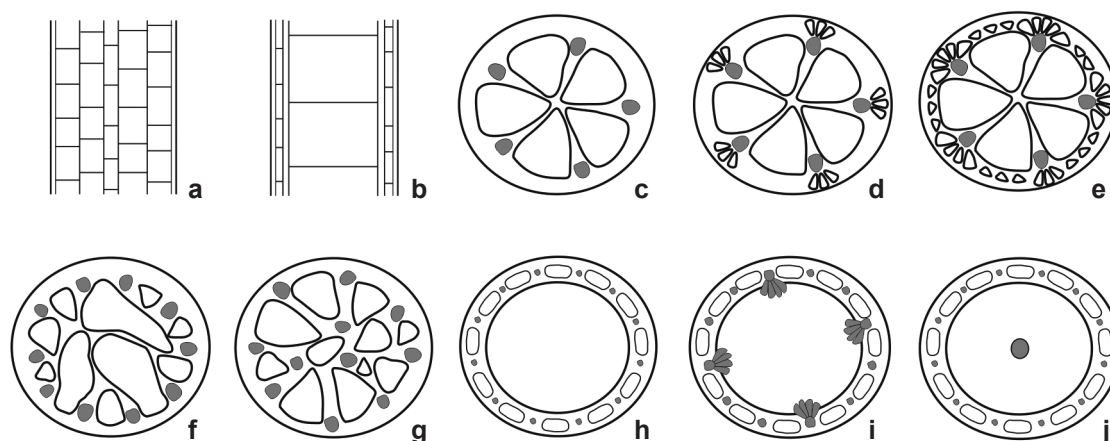


Figure 2 – a-j. Scheme of the aerenchyma patterns in the culm of the *Eleocharis* – a. longitudinal view of spongy pattern showing several lacunae, transversally divided by diaphragms (horizontal lines); b. longitudinal view of the septate pattern showing one big lacuna in the center, divided by diaphragms (horizontal lines), and small ones at the peripheral zone; c-f. morphologies of the basic spongy pattern – c. large lacunae in radial arrangement; d. large lacunae in radial arrangement and three small lacunae associated with the vascular bundles at the peripheral zone; e. similar to d but with additional small lacunae around the organ; f. several lacunae irregularly distributed; g. morphology of mixed spongy pattern showing several lacunae and vascular bundle scattered distributed; h-j. morphology of the septate patterns – h. transverse view of figure b; i. similar to figure h, but with vascular bundle sheath cells well developed and protrude into the central lacuna; j. morphology of the septate pattern with central vascular bundle. (gray = vascular bundles; white geometric shapes = air gaps).

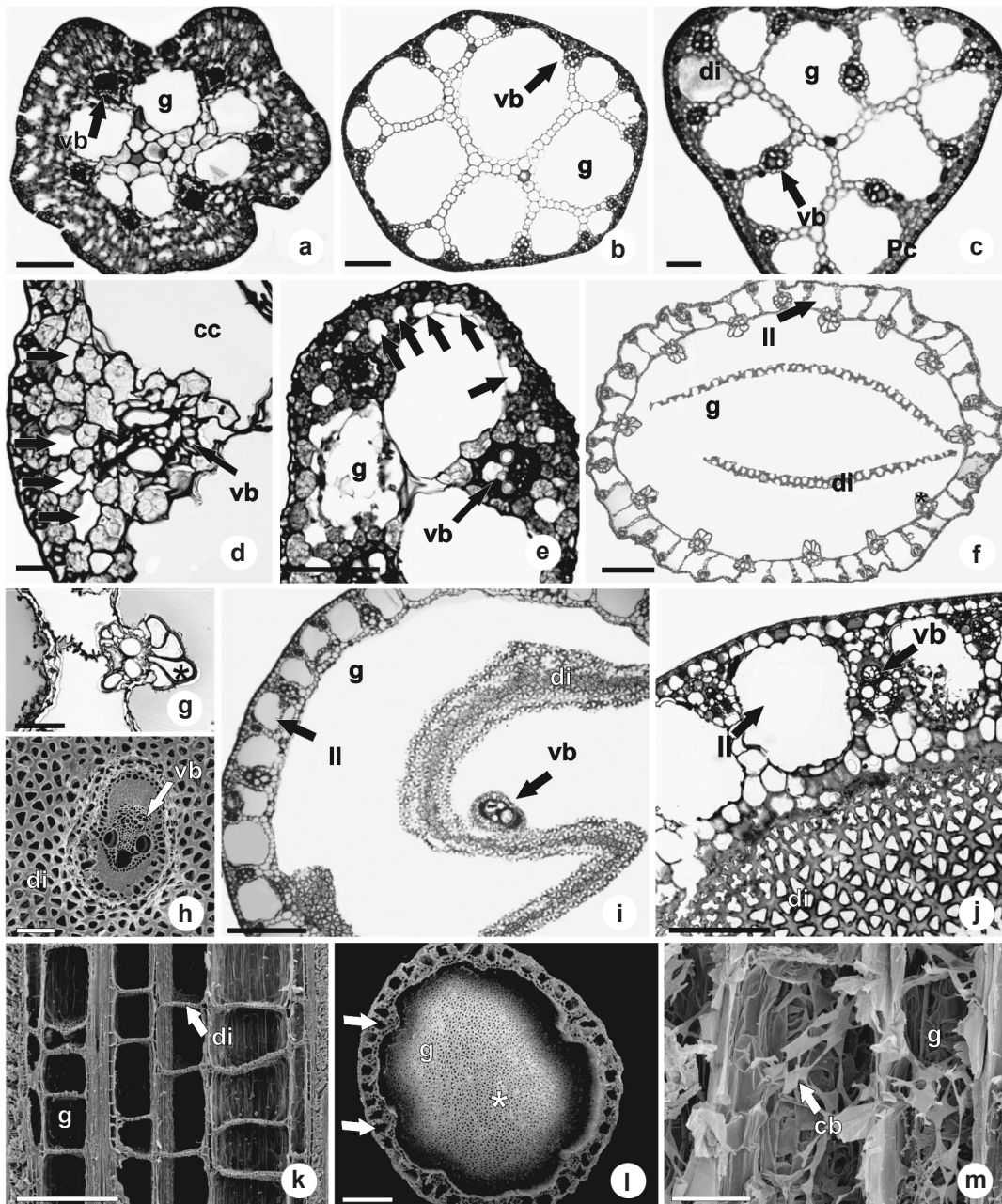


Figure 3 – a-m. Morphology of the culm aerenchyma patterns in *Eleocharis* – a. basic spongy pattern with radiated gaps, *E. albibracteata*; b. basic spongy pattern with gaps of unequal size, *E. subarticulata*; c. mixed spongy pattern, *E. plicarhachis*; d. aerenchyma with gaps between the epidermis and vascular bundles (arrow), *E. mamillata*; e. aerenchyma with smaller air gaps (arrows) surrounding the central cavities (cc) and vascular bundles (vb), *E. acicularis*; f. septate pattern with large gap in the center and lateral gaps interspersed with vascular bundles, *E. kuroguwai*; g. detail of vascular bundle sheath cells projecting into the central gap (*), *E. kuroguwai*; h. detail of central vascular bundle and diaphragm cells, *E. endounifascis*; i. septate pattern with central vascular bundle, *E. endounifascis*; j. septate pattern with lateral gaps (arrow) alternated with vascular bundles (vb) and diaphragm cells (*), *E. endounifascis*; k. detail of the diaphragms delimiting gaps in the spongy mixed pattern, *E. mutata*; l. overview of the septate culm with diaphragm (*) dividing the gap (g) transversely, *E. interstincta*; m. detail of stretched stellate-cells within the gaps, *E. uniglumis*. Bright field microscopy: a-g, i, j. Scanning Electron microscopy: h, k-m. Cross sections: a-j, l. Longitudinal sections: k, m. Scales bars: a = 20 μm ; b-e, g-j, m = 100 μm ; f, k, l = 500 μm .

E. uniglumis and *E. riograndensis* (species of distinct groups, not phylogenetically related), the internal organization was basic spongy type, and the longitudinal sections revealed that the central air spaces are filled by stretched stellate cells (Fig. 3m). This information constitutes a new character, with presence and absence states (Tab. 1).

The cross-section culm in *Eleocharis* spp. can be circular, elliptical, three-angled, four-angled, five-angled or with six or more angles. The shape of the culm was predominantly circular (44%) or four-angled (16%). Less commonly, the culm was elliptical (7,5%), three-angled (6%), or five-angled (6%). However, some species may have both circular and elliptical culms (*i.e.* *E. engelmannii* and *E. erythropoda*), three or four-angled (*i.e.* *E. baldwinii* and *E. acicularis*) or also four or five-angled (*i.e.* *E. densicaespitosa*, *E. retroflexa*). Only *E. palustris* had a culm with more six angles (Tab. 1; Fig. 4).

The species analyzed were mainly tall (35%), that is, greater than 40 cm in height, and medium size (34%), 17–40 cm in height. Small species, up to 17 cm in height, represented 24% of the total (Tab. 1).

Phylogenetic analysis

Analysis of molecular data of the 59 *Eleocharis* species, with 2,106 characters produced six more parsimonious trees of 1,654 steps, with a consistency index (CI) of 0.55 and a retention index (RI) of 0.71. Based on the clades proposed by Roalson *et al.* (2010) and rescued here, the clades one (90% bootstrap), four (100% bootstrap), five (93% bootstrap) and six (97% bootstrap) showed support of strong branches, while clade two and clade seven had weak support (> 50% bootstrap) (Fig. 4). The internal relationships of clade seven had weak, moderate and strong branch support. As the latter represents the clade with the largest number of species of the genus, even when the relationships between the species presented branches with strong support, they were not reported in this work due to possible synapomorphic states, to avoid possible future divergences for including a larger number of species.

In spite of the great diversity in the shape of the culm, this character groups some species, as is the case of the circular state that is a synapomorphy for the lineage of *E. sellowiana*, *E. flavescens*, *E. schaffneri* and *E. geniculata*, with support of 83% bootstrap, and also the lineage of *E. engelmannii*,

E. ovata and *E. obtusa* (bootstrap 79%), all with circular or elliptical cross-section culms (Fig. 4).

In relation to the culm shape and species size, the septate aerenchyma pattern occurred only in circular culm and in high plants (Figs. 5; 6). The basic spongy aerenchyma occurred in all culm formats and in all sizes of plant (Figs. 5; 6). The mixed spongy type occurred in three-angled or circular stem species with medium or high size (Figs. 5; 6).

As to the size of the *Eleocharis* species, only clades one and five presented this character as uniform (Fig. 6). In clade one, all species were tall, while in clade five they were small. The other clades presented a mosaic of these characters.

Clades one and seven were the only ones to present three of the four recorded aerenchyma patterns. The basic spongy pattern is the most frequent in *Eleocharis* species, and the only present in all clades. This shows the main pattern present in clades two, four, five and six (Fig. 5).

It was not possible to analyze the distribution of the septate pattern with central vascular bundle in the group, since the only species in which this pattern was reported in this work is not present in the phylogeny. However, morphological studies indicate that *E. endounifascis* belongs to the *Limnochoa* group, that is well resolved within the genus. The members analyzed in the work belong to clade one, a possible clade of *E. endounifascis*.

Discussion

Our study extended the analysis, of the diversity of the culm transverse format, for all clades (1–7) of *Eleocharis* spp. (Roalson *et al.* 2010) expanding the knowledge presented by Hinchliff & Roalson (2009) for the subgenus *Limnochoa*. The cross-section shape has no evidence of correlation with aerenchyma pattern types or species size. However, most species possessing the three-angled culm exhibit the spongy mixed pattern in aerenchyma, and large size. The septate pattern is related to the circular culm and larger plants, although the opposite is not verified, since the spongy type occurs in all culm formats and species size. In both the spongy and septate patterns the gaps decrease in size to the periphery improving the load capacity, since the stresses are distributed more evenly (Zhao *et al.* 2018). Due to the wide distribution in *Eleocharis*, we can assume that the mixed spongy aerenchyma pattern would be more efficient in resistance, due to the spreading of the vascular bundles, and the

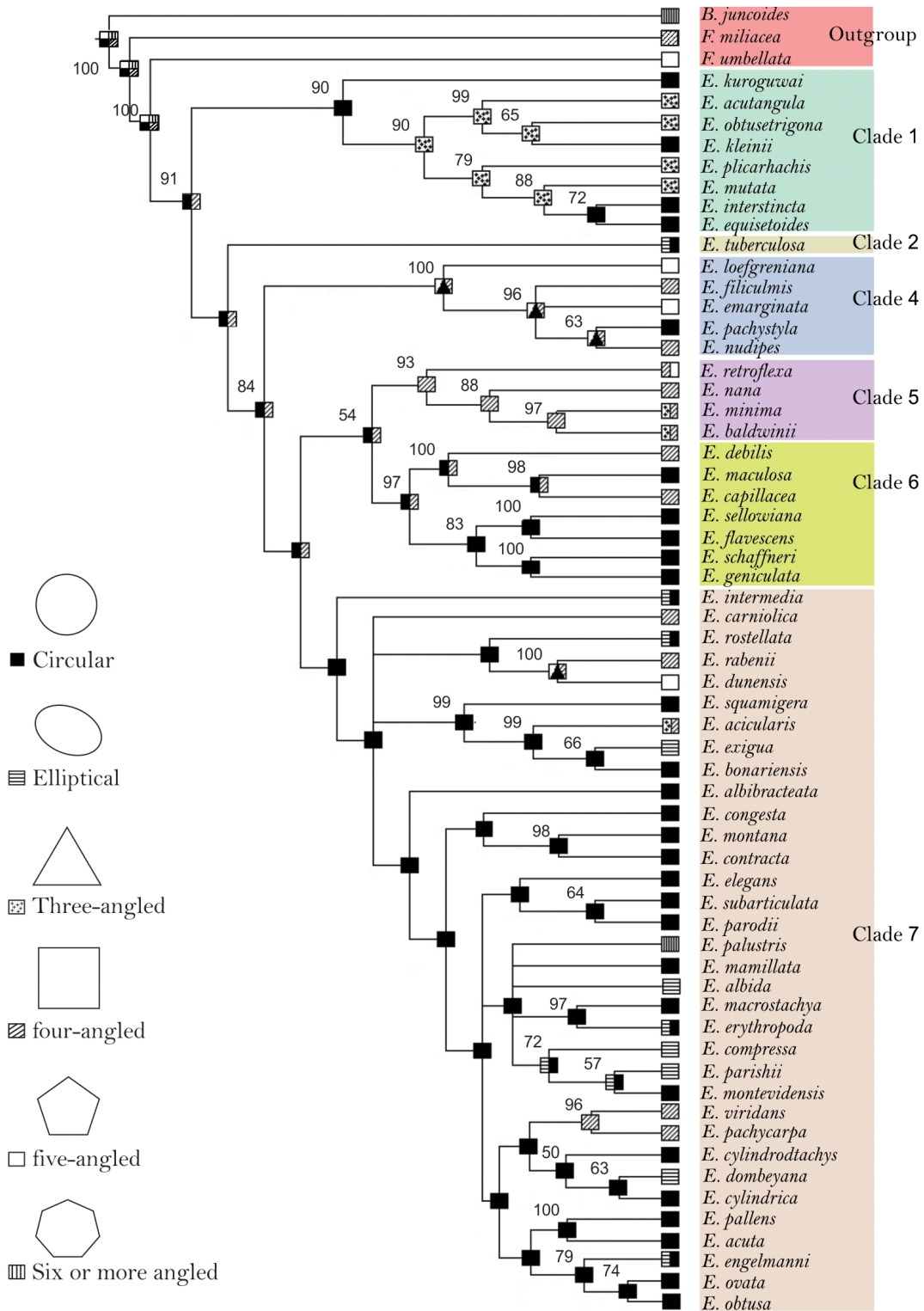


Figure 4 – Reconstruction of the ancestral state of the cross-section of *Eleocharis* stem, by parsimony method (Mesquite v. 3.03), based on the strict consensus tree obtained by the TNT program (v. 1.1). The indicated clades were previously defined based on the phylogeny of the group (Roalson *et al.* 2010). Values above the left side of the branches are the bootstrap values.

septate more efficient in transporting and reserving oxygen due to the large gaps and tissue reduction (see Williams & Barber 1961). However, further biomechanics and oxygen transport studies would be needed to support this assumption. The great diversity in shape of the *Eleocharis* spp. culm may be related to the variations of water level to which these species are subordinate. In the analyzed species, the four-angled state of the culm always presented four peripheral vascular bundles and four central lacunae intercalated. However, *E. minima* and *E. acicularis* presented state dimorphism, presenting the four-angled and three-angled shapes. The change in culm shape and reduction of vascular bundle and lacunae in *E. minima* (Martins &

Scatena 2015) and the reduction in the size of air lacunae in *E. interstincta* (Santos & Esteves 2004) evidenced the plasticity of the culm in *Eleocharis* spp. in relation to the water level. As the species are amphibians, morphological change is a feature of ecological importance. According to Santos & Esteves (2004), the reduction in height of the culm and size of the lacunae solves the problem of support derived from the reduction of the water level.

Although the transverse shape of the culm delimits small infrageneric lines in the group, this character does not present a phylogenetic signal, since it presents several state transitions, characterizing a homoplastic character.

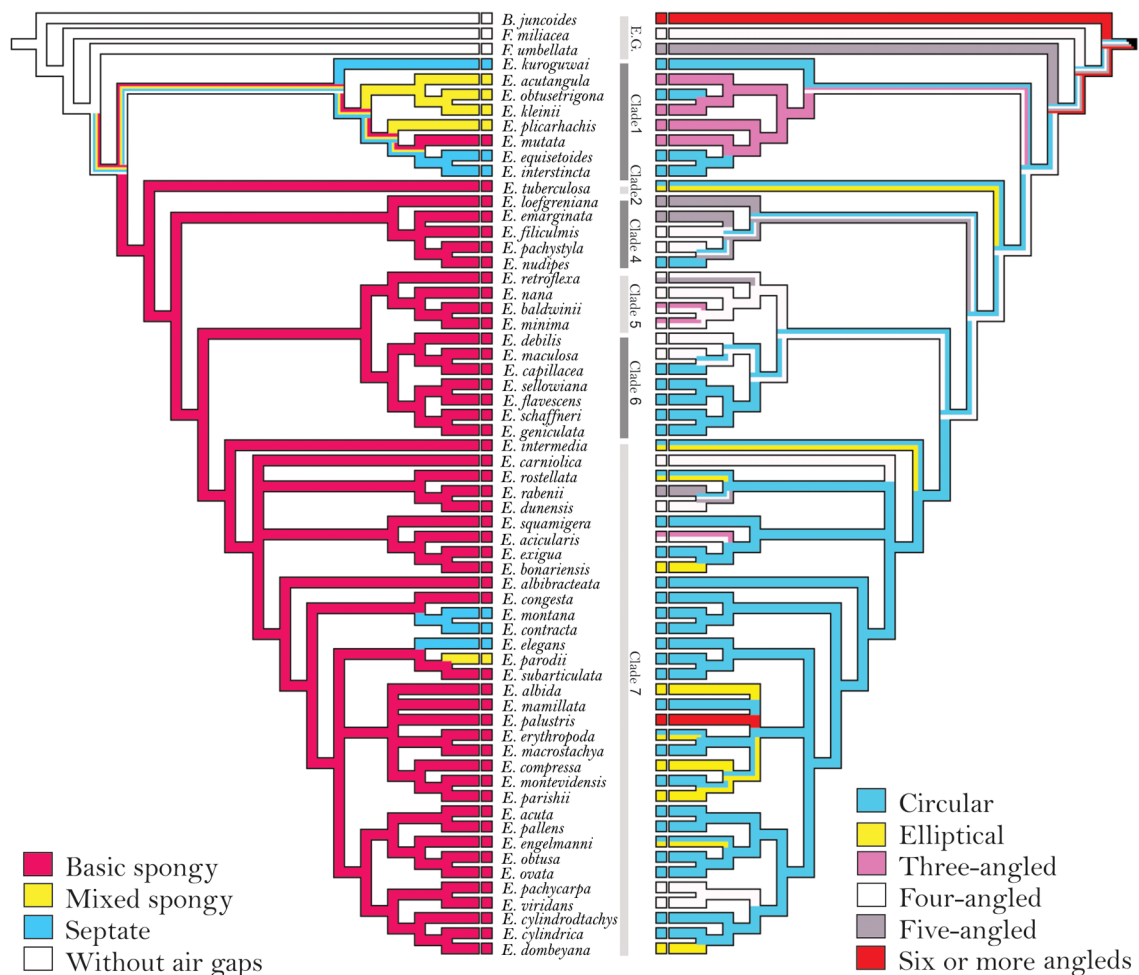


Figure 5 – Mirrored trees for the reconstruction of the ancestral state in *Eleocharis*, by parsimony method (Mesquite v. 3.03), based on the strict consensus tree by the TNT program (see 1.1). Three aerenchyma patterns of the culm and absence of aerenchyma versus culm cross-sectional shape. The indicated clades were previously defined based on the phylogeny of the group (Roalson *et al.* 2010). E.G. = outgroup.

The state of character, height of the species, is constant for the clades one and five, and is uniform among the specimens. Because of this, it can be used as a diagnostic feature, aiding in the identification and description of species, as well as in the infrageneric taxonomy of species of clades one and five.

Based on the aerenchyma, *Eleocharis* spp. presents the two structural stem categories present in Cyperaceae that had been cited for the genus (Metcalf 1971): a septate and spongy stem. The spongy stem is the ancestral condition of the genus, and the septate is the derived state. The septate structure appeared independently in the group at least three times, and there is no sign of character

reversal. Changes in the size of the lacunae, as also recorded by Martins & Scatena (2015) and Santos & Esteves (2004) and presence or absence of small lateral air lacunae do not alter the general architecture of the aerenchyma (spongy simple and mixed, septate with or without vascular bundle in the center) that can be used as a taxonomic character. The architecture of the spongy aerenchyma, present in most of the species analyzed, is considered an efficient adaptation for supporting aerial organs due to the extensive compartmentalization, which provides force and reserve of air at the same time (Williams & Barber 1961; Kaul 1971).

The arrangement of the vascular bundles in relation to air lacunae remained constant in the

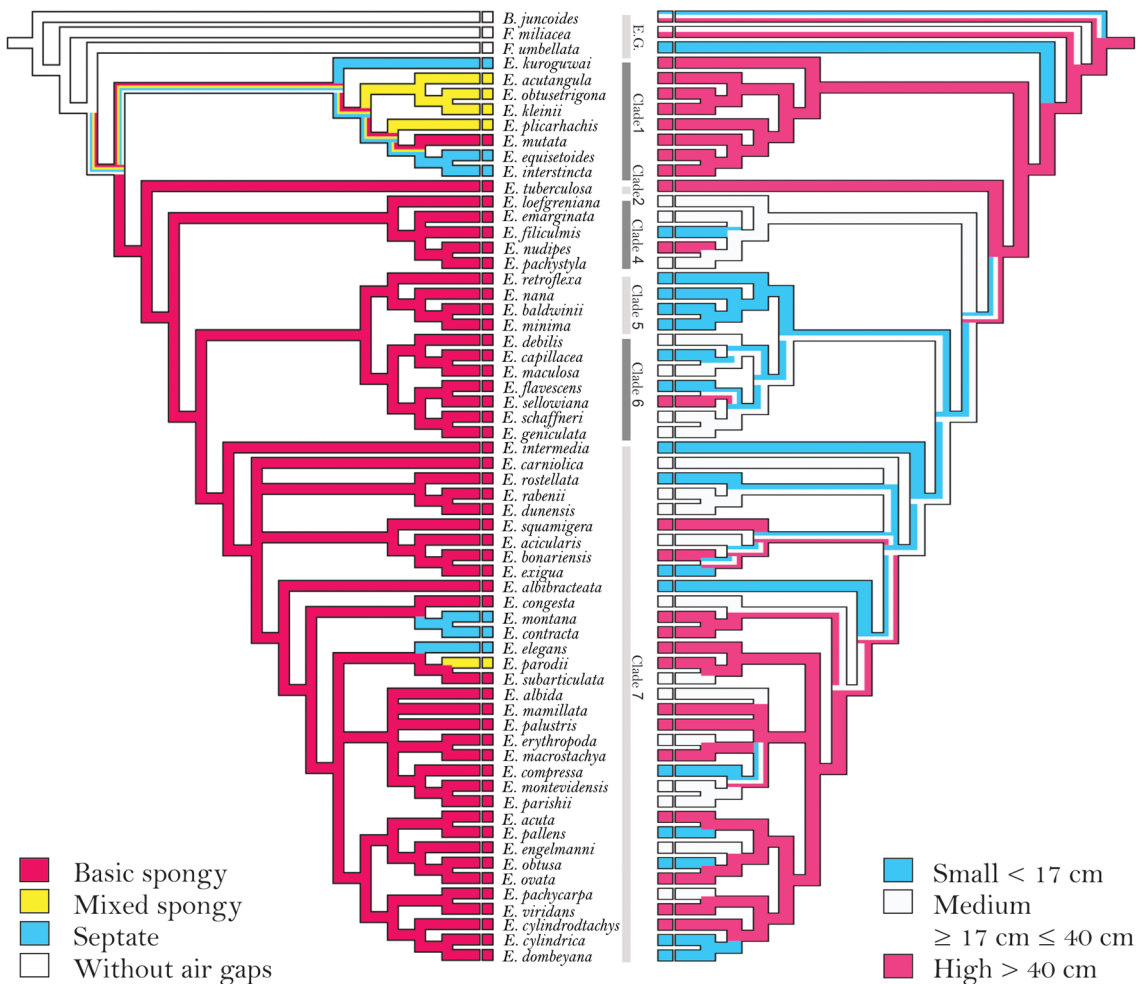


Figure 6 – Mirrored trees for the reconstruction of the ancestral state in *Eleocharis*, by parsimony method (Mesquite v. 3.03), based on the strict consensus tree by the TNT program (see 1.1). Three aerenchyma patterns and absence of aerenchyma versus species’ height. The indicated clades were previously defined based on the phylogeny of the group (Roalson *et al.* 2010). E.G. = outgroup.

culm architecture, regardless of morphological or environmental variation. This characteristic divides the aerenchyma in four patterns, one more than the proposed by Hinchliff & Roalson (2009). We chose to divide the spongy pattern described by these authors into two types (basic spongy and mixed spongy), since there was no registered change of pattern between the analyzed specimens. The mixed spongy pattern has taxonomic importance and is restricted to some species as *E. acutangula*, *E. kleinii*, *E. obtusetrigona* and *E. plicarhachis* from clade one, and *E. parodii* from clade seven, all of medium or high size, with stem three-angled or circular.

In *Eleocharis* spp., it is observed that in the four aerenchyma patterns, each air column is associated with at least one vascular bundle and, in general, the number of air lacunae coincides with the number of vascular bundles, especially in the spongy patterns. Kaul (1971) in describing the aerenchyma of the culm of *Scirpus validus* Vahl (Cyperaceae), recorded a pattern in the distribution of vascular bundles similar to the spongy mixed, here registered for *Eleocharis* spp, differing, however, by the presence of vascular bundles in the diaphragms.

The clades with the highest aerenchyma pattern are clades one, as seen by Hinchliff & Roalson (2009), and seven. Nonetheless, most species of this clade have the basic spongy pattern. For a more precise conclusion, larger studies are needed, involving a greater number of species in each clade, especially in clade seven.

We believe that the morpho-anatomical variations found in the culm cross-section format, species size, culm architecture and morphological patterns of the aerenchyma culm of *Eleocharis* spp. reflect the necessary adaptations for success of the species in their dynamic habitat. We conclude that the *Eleocharis* spp. culm presents four distinct aerenchyma patterns, which, although constant, present anatomical variations. The majority of species present the same type of aerenchyma pattern, and the other patterns were distributed punctually according to the evolutionary history of the group. The morphology of the culm (cross-section shape and size) does not show any correlation with the aerenchyma patterns found in the genus. Of the analyzed characters in *Eleocharis* spp., only the cross-section format was variable among specimens while the other characters were uniform. This makes its use in the identification and characterization of the species of the genus possible.

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