



## Occurrence and evolutionary inferences about Kranz anatomy in Cyperaceae (Poales)

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*Manuscript received on March 5, 2015; accepted for publication on May 13, 2015*

### ABSTRACT

Cyperaceae is an angiosperm family with the greatest diversity of species with Kranz anatomy. Four different types of Kranz anatomy (chlorocyperoid, eleocharoid, fimbristyloid and rhynchosporoid) have been described for this angiosperm family, and the occurrence and structural characteristics of these types are important to trace evolutionary hypotheses. The purpose of this study was to examine the available data on Cyperaceae Kranz anatomy, emphasizing taxonomy, geographic distribution, habitat and anatomy, to infer the potential origin of the Kranz anatomy in this family. The results showed that the four types of Kranz anatomy (associated with C<sub>4</sub> photosynthesis) in Cyperaceae emerged numerous times in unrelated phylogenetic groups. However, the convergence of these anatomical types, except rhynchosporoid, was observed in certain groups. Thus, the diverse origin of these species might result from different environmental pressures that promote photorespiration. Greater variation in occurrence of Kranz anatomy and anatomical types was observed in *Eleocharis*, whose emergence of the C<sub>4</sub> pathway was recent compared with other genera in the family, and the species of this genus are located in aquatic environments.

**Key words:** anatomy, ultrastructure, taxonomy, phylogeny, Cyperaceae.

### INTRODUCTION

The Kranz anatomy represents a structural modification of photosynthetic tissues, generally two distinct concentric layers of chlorenchyma cells, associated with C<sub>4</sub> metabolism, the main function in plants with suppressed photorespiration, induced through decreased levels of CO<sub>2</sub> and

increased levels of O<sub>2</sub> (Sage 2004, Lundgren et al. 2014). Several factors, such as water stress, high temperature and lightness, and the salinity and high density of plants, influence the reduction of CO<sub>2</sub> (Sage et al. 2012).

The structural modification (Kranz anatomy) was initially reported in Cyperaceae species (Haberlandt 1914). Since then, several studies using different approaches have examined the Kranz structure and the C<sub>4</sub> pathway in this family (e.g.

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Carolin et al. 1977, Takeda et al. 1985, Bruhl et al. 1987, Ueno and Samejima 1989, Bruhl 1995, Bruhl and Perry 1995, Soros and Dengler 2001, Martins and Alves 2009, Martins and Scatena 2011). The Kranz structure varies in the Cyperaceae family, and four anatomical types have been described: chlorocyperoid, eleocharoid, fimbristylloid and rhynchosporoid. These types anatomically differ in the number and continuity of the vascular bundle sheath and in the presence of chloroplasts in the bundle sheath (Carolin et al. 1977, Soros and Dengler 2001, Martins and Scatena 2011).

Representatives of the Cyperaceae family with Kranz anatomy are restricted to four of the 13 tribes of the Cyperoideae subfamily: Abildgaardieae, Cypereae, Eleocharideae and Rhynchosporae (Goetghebeur 1998). Among these, only Abildgaardieae and Eleocharideae are closely related in phylogenetic studies (Ghamkhar et al. 2007, Muasya et al. 2008). The Kranz Cypereae species present a Kranz anatomy of the chlorocyperoid type, while the Abildgaardieae species present a Kranz anatomy of the fimbristylloid type (Bruhl and Wilson 2007). Chlorocyperoid, eleocharoid and fimbristylloid types have been described in the Eleocharideae tribe (Murphy et al. 2007, Martins and Scatena 2015), and chlorocyperoid and rhynchosporoid types have been reported in Rhynchosporae (Martins and Scatena 2011, Ueno 2013).

Therefore, it has been suggested that the origin of Kranz anatomy emerged four times in Cyperaceae, one type in each tribe (Soros and Bruhl 2000). In subsequent studies using molecular markers, five potential appearances of Kranz anatomy were reported: two types in Eleocharideae and one type in each of the remaining Kranz tribes (Besnard et al. 2009). Phylogenetic studies of Abildgaardieae (Ghamkhar et al. 2007) and Eleocharideae (Roalson et al. 2010) indicated multiple origins of  $C_4$  photosynthesis.

The variations of Kranz anatomy observed in this family have generated important taxonomic, ecological and evolutive interpretations. Therefore, the aim of the present study was to review taxonomic, ecological, structural and phylogenetic data associated with the structural analyses performed with Cyperaceae species of the four Kranz types (Martins and Scatena 2011, Martins and Scatena 2015, Martins et al. 2015) to develop potential hypotheses concerning the origins of these plants.

#### MATERIALS AND METHODS

The species used to illustrate anatomical and ultrastructural features are listed in Table I. The voucher materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB). The data collected here added to the literature are given in Table II.

To anatomically illustrate the different Kranz types, portions of at least three fully expanded leaf blade or scapes were fixed in FAA 50 and stored in 70% ethanol (Johansen 1940) and subsequently dehydrated in an ethyl alcohol series and embedded in Historesin (Leica Historesin Embedding Kit, Nussloch, Germany) (Feder and O'Brien 1968). Transverse sections of the mid-region were obtained using a microtome, and the sections were stained with periodic acid-Schiff's reagent and toluidine blue (Feder and O'Brien 1968) and mounted in Entellan (Merck Darmstadt, Germany). The images were obtained using Leica DFC 290 digital camera on a Leica DM LB microscope using IM50 software.

To illustrate the ultrastructural features of the four Kranz types, samples from the mid-region of at least three fully expanded leaf blades or scapes were fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.3, for 24 h at 5°C, post-fixed with 1% osmium tetroxide in the same buffer for 1 h at 25°C, dehydrated with an acetone

series and embedded in Araldite resin (Machado and Rodrigues 2004). Ultrathin transverse sections were obtained using a Diatome diamond knife, and the sections were subsequently stained with uranyl acetate and lead citrate (Reynolds 1963). The samples were examined using a Philips EM 301 transmission electron microscope (MET).

To illustrate the occurrence of the Kranz anatomical types in Cyperaceae, these data were plotted onto cladograms from previously published phylogenetic analyses for each tribe: *Abildgaardieae* (Ghamkhar et al. 2007), *Cypereae* (Muasya et al. 2009), *Eleocharideae* (Roalson et al. 2010) and *Rhynchosporae* (Thomas et al. 2008).

## RESULTS

**Abildgaardieae tribe** – This tribe has six genera with Kranz anatomy, *Abildgaardia*, *Bulbostylis*, *Crosslandia*, *Fimbristylis*, *Nelmesia* and *Nemum* (Tab. II), and two non-Kranz ( $C_3$ ) species,

*Abildgaardia hygrophila* and *Fimbristylis variegata*. This tribe is non-monophyletic, and representatives of the non-Kranz *Arthrostylideae* tribe emerge as the sister group of the clade *Fimbristylis-Abildgaardia-Crosslandia* (Fig. 1e). The Kranz taxa of *Abildgaardieae* possess the fimbriostyloid type, characterized by vascular bundles surrounded by three sheaths, an outer sheath (Os), a continuous middle sheath (Ms), and a discontinuous inner sheath (Is) in the major bundles, interrupted by only one or two metaxylem vessels (Fig. 1a-b). The outer bundle sheath originates from the ground meristem, while the middle and inner sheaths develop from the procambium. The chloroplasts in the outer-sheath cells present well-developed grana, similar to the mesophyll cells. The middle-sheath cells do not show chloroplasts, and the inner-sheath cells show centrifugal chloroplasts without grana (Fig. 1c) and with convoluted and parallel thylakoids (Fig. 1d).

TABLE I  
Cyperaceae species used to illustrate the anatomical and ultrastructural features with Kranz types and vouchers, respectively.

Tribes and species	Kranz anatomical type	Voucher
<b>Abildgaardieae</b>		
<i>Bulbostylis conifera</i> L.	fimbriostyloid	<i>S. Martins 329</i>
<i>Bulbostylis scabra</i> (J. Presl. & C. Presl.) C.B. Clarke	fimbriostyloid	<i>S. Martins 408</i>
<i>Fimbristylis autumnalis</i> L.	fimbriostyloid	<i>V.L. Scatena 343</i>
<i>Fimbristylis complanata</i> (Retz.) Link	fimbriostyloid	<i>V.L. Scatena 344</i>
<i>Fimbristylis dichotoma</i> (L.) Vahl	fimbriostyloid	<i>S. Martins 398</i>
<b>Cypereae</b>		
<i>Cyperus compressus</i> Jacq.	chlorocyperoid	<i>S. Martins 228</i>
<i>Cyperus ligularis</i> L.	chlorocyperoid	<i>S. Martins 330</i>
<i>Cyperus maritimus</i> Poir.	chlorocyperoid	<i>S. Martins 226</i>
<i>Kyllinga brevifolia</i> Rottb.	chlorocyperoid	<i>S. Martins 288</i>
<i>Pycurus flavescens</i> (L.) Rchb.	chlorocyperoid	<i>S. Martins 327</i>
<b>Eleocharideae</b>		
<i>Eleocharis minima</i> Kunth	eleocharoid	<i>S. Martins 405</i>
<b>Rhynchosporae</b>		
<i>Rhynchospora barbata</i> (Vahl) Kunth	chlorocyperoid	<i>S. Martins 313</i>
<i>Rhynchospora globosa</i> Lindl.	rhynchosporoid	<i>S. Martins 305</i>
<i>Rhynchospora terminalis</i> Kunth	rhynchosporoid	<i>S. Martins 302</i>

TABLE II

**Cyperaceae genera with Kranz anatomy (C<sub>4</sub> photosynthesis), detach to taxonomical classification, Kranz anatomical types, biochemical subtypes, habitat and distribution (K-C = chlorocyperoid; K-E = eleocharoid; K-F = fimbristylloid; K-R = rhynchosporoid).**

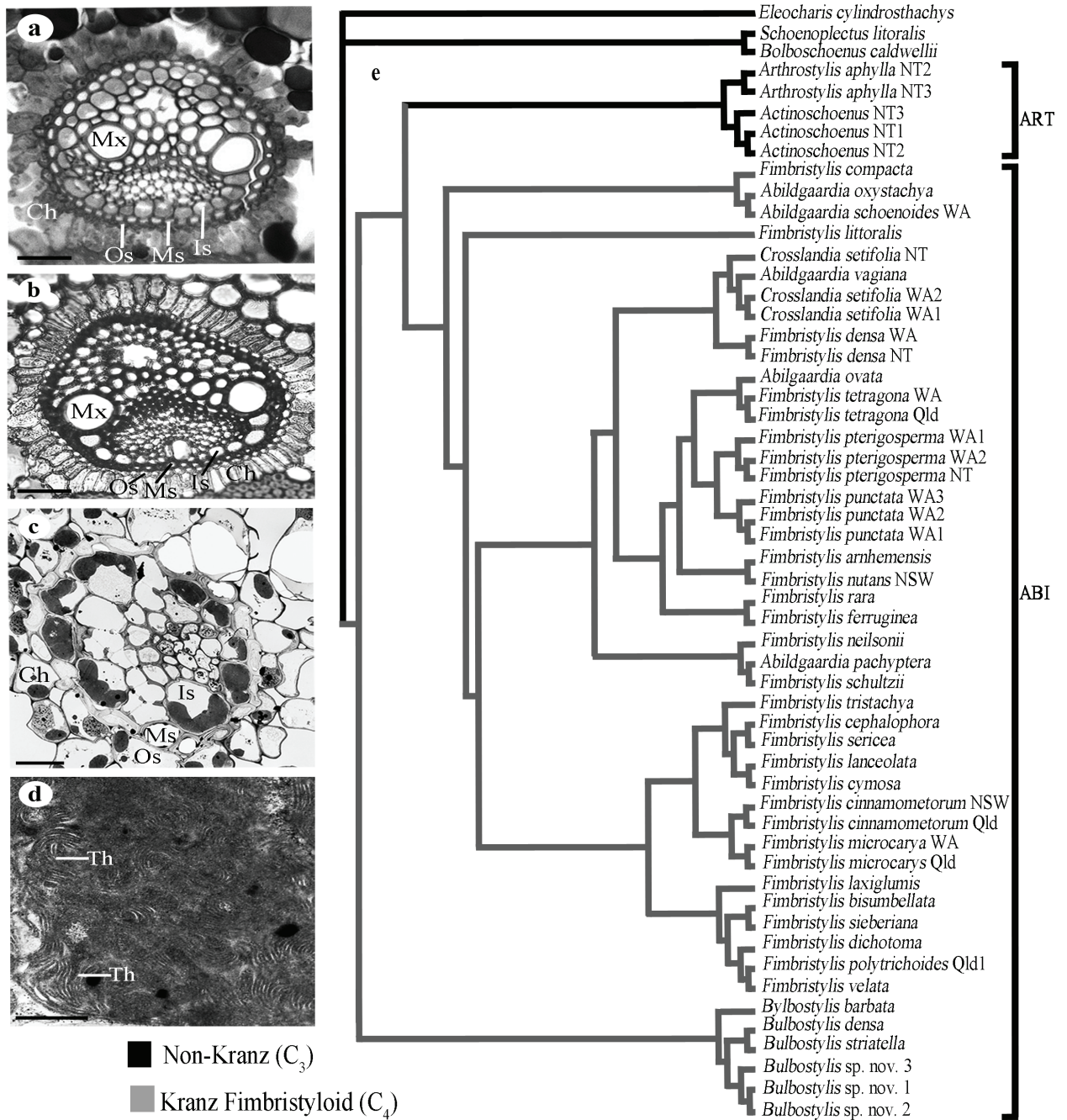
Tribes and genera (number of C <sub>3</sub> and C <sub>4</sub> species)	Kranz type	Biochemical type	Habitat	Distribution
<b>Abildgaardieae</b>				
<i>Abildgaardia</i> (1 C <sub>3</sub> /16 C <sub>4</sub> ) <sup>1,2,3,5,6</sup>	K-F	NADP-ME	Dry field and disturbed area	Tropical and subtropical
<i>Bulbostylis</i> (150 C <sub>4</sub> ) <sup>1,2,3,4,5,6</sup>	K-F	NADP-ME	Dry field, rare moist area	Tropical
<i>Crosslandia</i> (4 C <sub>4</sub> ) <sup>2,4,6</sup>	K-F	NADP-ME	Dunes and dry field	Tropical (Australia)
<i>Fimbristylis</i> (1 C <sub>3</sub> /300 C <sub>4</sub> ) <sup>1,2,3,4,5,6</sup>	K-F	NADP-ME	Dry fields and disturbed area	Tropical (Asia e Australia)
<i>Nelmesia</i> (1 C <sub>4</sub> ) <sup>2,4,6</sup>	K-F	NADP-ME	Field moist	Tropical (Africa)
<i>Nemum</i> (10 C <sub>4</sub> ) <sup>2,4,6</sup>	K-F	NADP-ME	Field moist	Tropical (Africa)
<b>Cypereae</b>				
<i>Alinula</i> (4 C <sub>4</sub> ) <sup>2,4,6</sup>	K-C	NADP-ME	Field moist	Tropical (Africa)
<i>Ascolepis</i> (20 C <sub>4</sub> ) <sup>2,4,6</sup>	K-C	NADP-ME	Field moist	Tropical
<i>Cyperus</i> subg. <i>Cyperus</i> (260 C <sub>4</sub> ) <sup>1,2,3,4,5,6</sup>	K-C	NADP-ME	Field moist and disturbed area	Tropical, rare subtropical
<i>Kyllinga</i> (75 C <sub>4</sub> ) <sup>1,2,3,4,5,6</sup>	K-C	NADP-ME	Field moist and disturbed area	Tropical, rare subtropical
<i>Lipocarpa</i> (35 C <sub>4</sub> ) <sup>2,4,6</sup>	K-C	NADP-ME	Field moist	Tropical, rare subtropical
<i>Pycreus</i> (120 C <sub>4</sub> ) <sup>1,2,3,4,5,6</sup>	K-C	NADP-ME	Field moist and disturbed area	Tropical
<i>Queenslandiella</i> (1 C <sub>4</sub> ) <sup>2,4,5,6</sup>	K-C	NADP-ME	Dunes	Tropical (Africa, Asia and Australia)
<i>Remirea</i> (1 C <sub>4</sub> ) <sup>2,4,5,6</sup>	K-C	NADP-ME	Dunes	Tropical
<i>Sphaerocyperus</i> (1 C <sub>4</sub> ) <sup>2,4,5,6</sup>	K-C	NADP-ME	Dry field	Tropical (Africa)
<i>Volkiella</i> (1 C <sub>4</sub> ) <sup>2,3,4,5</sup>	K-C	NADP-ME	Dunes	Tropical (Africa)
<b>Eleocharideae</b>				
<i>Eleocharis</i> (244 C <sub>3</sub> /8 C <sub>4</sub> ) <sup>1,2,3,4,5,6,8</sup>	K-E; K-C; K-F	NAD-ME	Border of river and lakes	Tropical (America)
<b>Rhynchosporae</b>				
<i>Rhynchospora</i> grupo <i>Capitatae</i> (5 C <sub>3</sub> /28 C <sub>4</sub> ) <sup>1,2,3,4,5,7</sup>	K-C; K-R	NADP-ME	Dry and moist fields	Neotropical

<sup>1</sup>Bruhl and Perry (1995), <sup>2</sup>Bruhl and Wilson (2007), <sup>3</sup>Bruhl et al. (1987), <sup>4</sup>Goetghebeur (1998), <sup>5</sup>Martins and Scatena (2011), <sup>6</sup>Soros and Bruhl (2000), <sup>7</sup>Ueno (2013), <sup>8</sup>Martins and Scatena (2015).

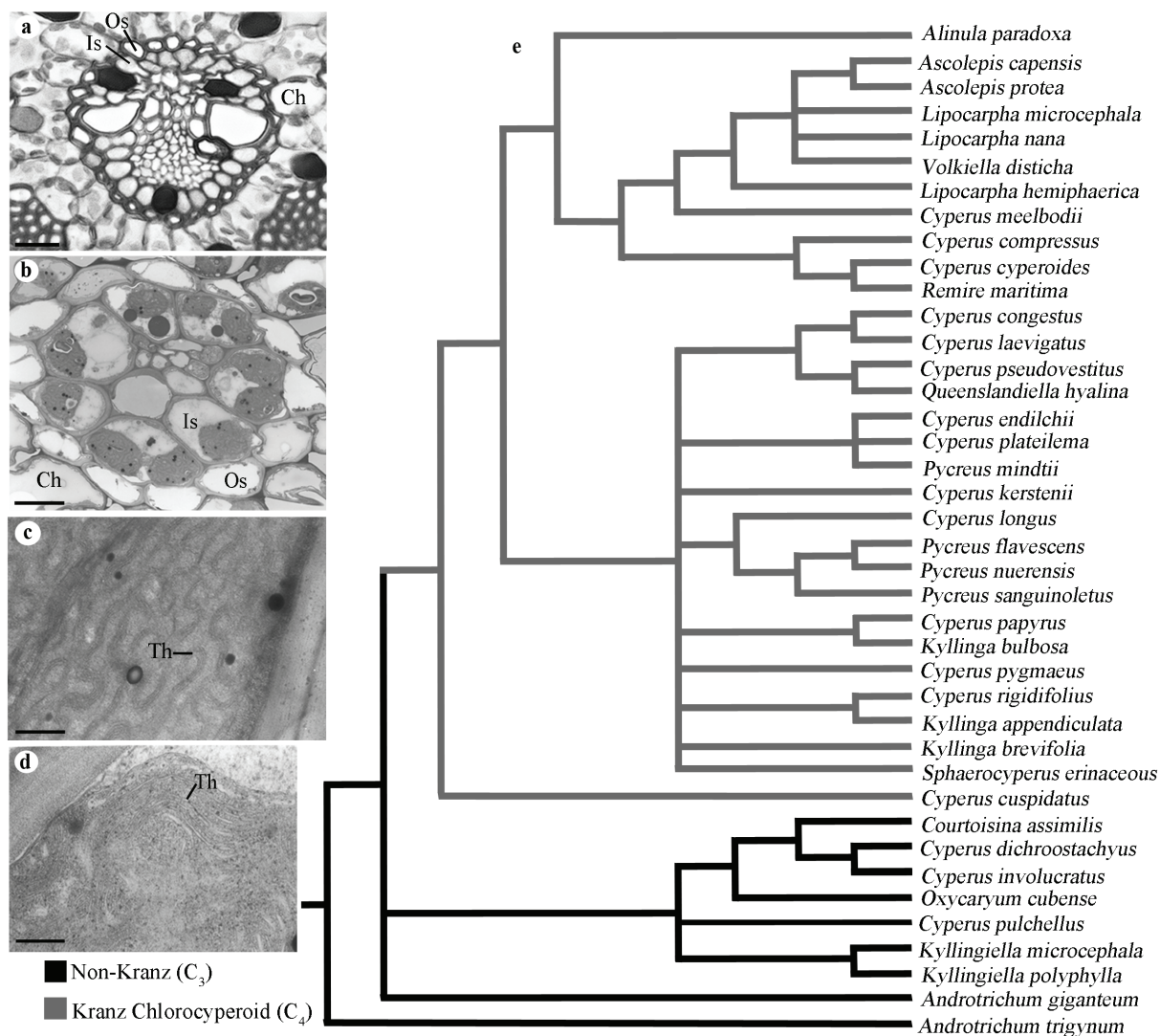
**Cypereae tribe** – This tribe possesses 19 genera, 10 of which are Kranz species, including nine exclusively Kranz (C<sub>4</sub>) species (*Alinula*, *Ascolepis*, *Kyllinga*, *Lipocarpa*, *Pycreus*, *Queenslandiella*, *Remirea*, *Sphaerocyperus* and *Volkiella*) and in *Cyperus* restricted to *Cyperus* subg. *Cyperus* (Tab. II). This tribe is monophyletic, and the Kranz taxa emerge in a separate clade from non-Kranz taxa (Fig. 2e). Cypereae present chlorocyperoid type

Kranz, characterized by two sheaths surrounding the vascular bundles: a continuous outer sheath (Os) and a discontinuous inner sheath (Is) in the major bundles (Fig. 2a). Both bundle sheaths develop from the procambium. The outer-sheath cells do not have chloroplasts (Fig. 2b), and the inner-sheath cells are generally centrifugal (Fig. 2b), with inconspicuous grana and convoluted and contorted thylakoids (Fig. 2c-d).





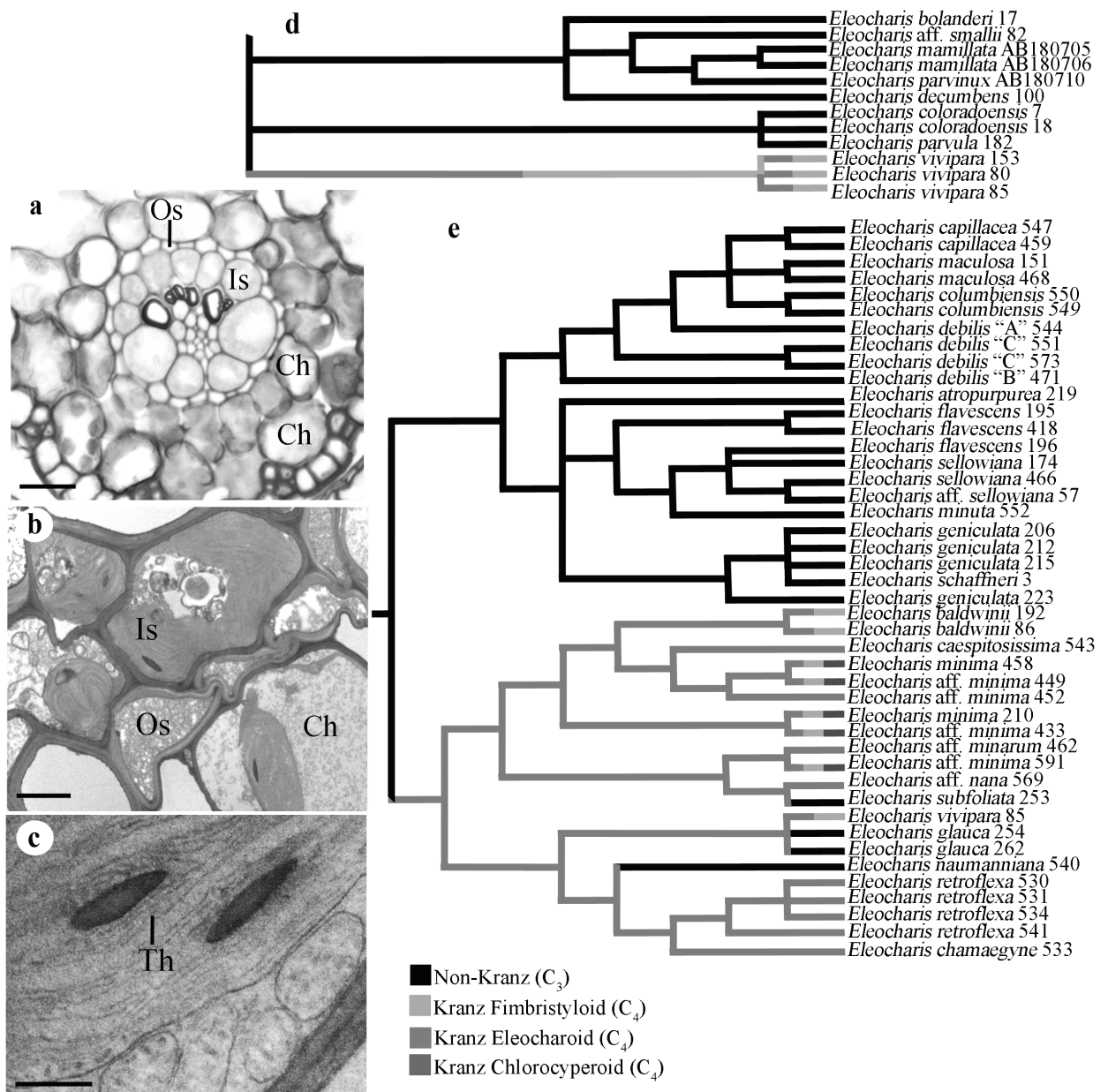
**Figure 1** - Anatomical and ultrastructural features of the Kranz-fimbristyloid type and distribution of this type in the phylogeny of the Abildgaardieae and Arthrotylidaeae tribes. **a-b.** *Bulbostylis scabra* and *Fimbristylis complanata*, respectively – leaf in transverse section showing the vascular bundle. **c-d.** *Fimbristylis autumnalis*. **c.** Vascular bundle, highlighting centrifugal distribution of chloroplasts. **d.** Chloroplast of the inner bundle sheath cell with contorted and parallel thylakoids. **e.** Phylogeny of Abildgaardieae and Arthrotylidaeae tribes, adapted from Ghamkhar et al (2007). ART = Arthrotylidaeae; ABI = Abildgaardieae; Ch = chlorenchyma; Is = inner bundle sheath; Ms = middle bundle sheath; Mx = metaxylem; Os = outer bundle sheath; Th = thylakoids. Bars: (**a-b**) = 20 µm; (**e**) = 10 µm, (**d**) = 0.2 µm. For interpretation of the references to color in this figure, the reader is referred to the web version of this article.



**Figure 2** - Anatomical and ultrastructural features of the Kranz-chlorocyperoid type and distribution of this type in the phylogeny of the Cyperaceae tribe. **a.** *Kyllinga brevifolia* – leaf in transverse section showing the vascular bundle. **b.** *Cyperus compressus* – vascular bundle, highlighting centrifugal distribution of chloroplasts. **c-d.** *Cyperus maritimus* and *C. ligularis*, respectively – chloroplast of the inner bundle sheath cell with convoluted and contorted thylakoids, respectively. **e.** Part of phylogeny of Cyperaceae tribe, detach to Kranz species, adapted from Muasya et al. (2008). Ch = chlorenchyma; Is = inner bundle sheath; Os = outer bundle sheath; Th = thylakoids. Bars: (a) = 20  $\mu\text{m}$ ; (b) = 5  $\mu\text{m}$ ; (c-d) = 0.5  $\mu\text{m}$ . For interpretation of the references to color in this figure, the reader is referred to the web version of this article.

**Eleocharideae tribe** – This tribe only includes the genus *Eleocharis* (250 spp.), presenting Kranz ( $C_4$ ) and non-Kranz species ( $C_3$ ) (Tab. II) and facultative  $C_3/C_4$  and intermediate  $C_3-C_4$  species. These species possess leaf lamina reduced to a tubular basal sheath, and the scape is the principal photosynthetic organ. The Kranz species of *Eleocharis* present eleocharoid, fimbristyloid and chlorocyperoid types (Fig. 3d-e). The eleocharoid

type shows two continuous vascular bundle sheaths (Fig. 3a): an outer sheath (Os) without chloroplasts and an inner sheath (Is) with chloroplasts that do not show a constant location and have parallel thylakoids, occasionally forming grana (Fig. 3b-c). The fimbristyloid and chlorocyperoid types in *Eleocharis* are anatomically similar to those previously described for Abildgaardieae and Cyperaceae.



**Figure 3** - Anatomical and ultrastructural features of the Kranz-elocharoid type and distribution of this type in the phylogeny of the Eleocharideae tribe. **a-c.** *Eleocharis minima*. **a.** Scape in transverse section showing the vascular bundle. **b.** Vascular bundle, detach to chloroplast without Constant location in the inner bundle sheath. **c.** Chloroplast with parallel thylakoids in the inner bundle sheath. **d-e.** Part of phylogeny of the Rhynchosporeae tribe, highlighting the Kranz species, adapted from Roalson et al. (2010). Ch = chlorenchyma; Is = inner bundle sheath; Os = outer bundle sheath; Th = thylakoids. Bars: (a) = 20  $\mu\text{m}$ ; (b) = 2  $\mu\text{m}$ ; (c) = 0.5  $\mu\text{m}$ . For interpretation of the references to color in this figure, the reader is referred to the web version of this article.

**Rhynchosporeae tribe** – This tribe possesses two genera, *Pleurostachys* and *Rhynchospora*, and only *Rhynchospora* has species with Kranz anatomy ( $C_4$ ) restricted to *Pauciflorae* (Pau) and *Pluriflorae* (Plu) sections from the Capitatae group

(Tab. II). This species presents chlorocyperoid and the rhynchosporoid Kranz types that occur in distinct clades in the phylogenetic analysis (Fig. 4e). The chlorocyperoid has two sheaths surrounding the vascular bundles, wherein the outer (Os) is



continuous and the inner (Is) is discontinuous in the major bundles (Fig. 4a). Similar to the chlorocyperoid type in Cyperaceae, both sheaths (outer and inner) originate from the procambium. The chloroplasts are absent in the outer sheath cells (Os), whereas these organelles are centrifugal or without pattern, with convoluted and parallel thylakoids in the inner sheath cells (Fig. 4b). The rhynchosporoid present a unique continuous bundle sheath (Ss – single sheath) (Fig. 4c), originating from the procambium and presenting cells with chloroplasts located centrifugally or without pattern, with inconspicuous grana and parallel thylakoids (Fig. 4d).

#### DISCUSSION

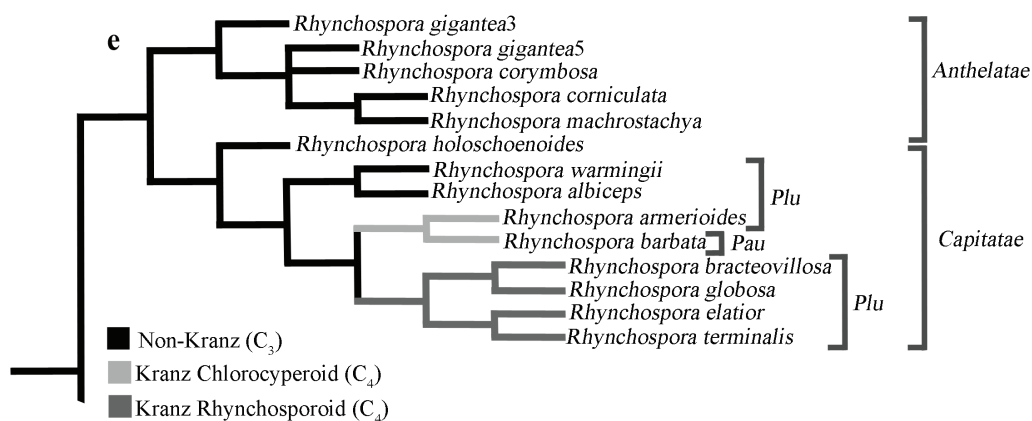
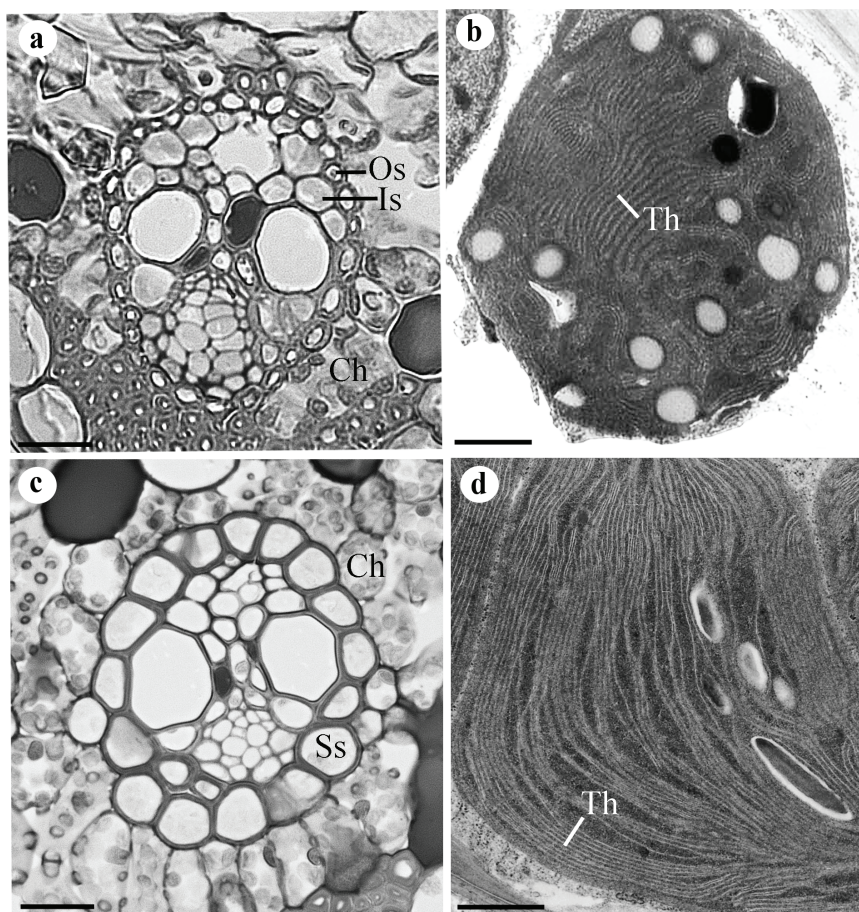
The data obtained from the present study and previously published studies suggest that multiple origins of Kranz anatomy occurred in Cyperaceae due to different environmental factors that promote photorespiration. The Kranz anatomy is uniform in Abildgaardieae Cyperaceae tribes, showing only one anatomical type, and varied in Rhynchosporae and Eleocharideae tribes, showing more than one type.

In Abildgaardieae, the Kranz anatomy ( $C_4$ ) likely had only one origin, before the occurrence of a single anatomical (fimbristylid) and biochemical (NADP-ME) type, with possible reversion to the  $C_3$  pathway (not Kranz) in *Abildgaardia hygrophila*, *Fimbristylis variegata* and representatives of the Arthrostylideae tribe. This hypothesis is confirmed by phylogenomic and phylogenetic studies (Besnard et al. 2009, Ghamkhar et al. 2007), suggesting the initial onset of  $C_4$  photosynthesis in *Bulbostylis* (ca.  $19.6 \pm 4.9$  Ma), followed by *Fimbristylis* (ca.  $12.3 \pm 3.8$  Ma). Moreover, the species of Abildgaardieae is primarily observed in xeric environments (open fields, rocky outcrops, dunes), and the  $C_4$  pathway must have arisen in this environment, where this biochemical pathway is more advantageous (Sage 2004, Sage et al. 2012).

In the Cyperaceae tribe, the Kranz anatomy likely derived from a single source, as the Kranz species in this tribe emerged in the same clade (Muasya et al. 2002, 2009) and have the same anatomical (chlorocyperoid) and biochemical (NADP-ME) types and similarities in the structure of the chloroplasts. The emergence of  $C_4$  in this tribe is indicated ca.  $10.9 \pm 3.8$  Ma (Besnard et al. 2009), initially in mesophyte species (with  $C_3$  mesophytes ancestors) and subsequently in xerophytes (with  $C_4$  mesophytes ancestors), as most of the  $C_4$  species primarily occur in mesic environments and a minority of these species are observed in xeric areas. This hypothesis has also been postulated for the  $C_4$  species of Poaceae (Osborne and Freckleton 2009). The occurrence of  $C_4$  metabolism in mesic environments with high water availability is associated with the high density, high temperature and increased luminosity, which reduce the absorption of  $CO_2$  and carbohydrate production in plants (Bowes et al 200, Sage et al. 2012).

The plasticity of the  $C_4$  pathway makes it difficult to interpret the origin of this pathway in Eleocharideae, with the possibility of two sources with reversals for  $C_3$  or multiple origins. The Kranz types in this tribe vary between individuals of the same species (Ueno 1996, 2004, Murphy et al. 2007) and even in the same individual (Martins and Scatena 2015). As eleocharoid types are considered more related to  $C_4$  metabolism, and the fimbristylid types are considered  $C_3$ - $C_4$  intermediates (Murphy et al. 2007), it is likely that the aquatic environment of these plants promotes anatomical plasticity, such as the number of vascular bundles and the presence and development of aerenchyma (Martins and Scatena 2015), as observed in other anatomical structures.

Structurally, the difference between the eleocharoid and fimbristylid types is the size and shape of the cells of the inner layer chlorenchyma (Ch) (both originate from the ground meristem), which is slightly larger and elongated in the first



**Figure 4** - Anatomical and ultrastructural features of the Kranz-chlorocyperoid and Kranz-rhynchosporoid types and distribution of these types in the phylogeny of the Rhynchosporae tribe. **a-b.** *Rhynchospora barbata*. **a.** Leaf in transverse section showing the vascular bundle. **b.** Chloroplast of the inner bundle sheath cell with parallel thylakoids. **c-d.** *Rhynchospora globosa*. **c.** Leaf in transverse section showing the vascular bundle. **d.** Chloroplast of the inner bundle sheath cell with parallel thylakoids. **e.** Part of phylogeny of Rhynchosporae tribe, detach to Kranz species, adapted from Thomas et al. (2008). Ch = chlorenchyma; Is = inner bundle sheath; Os = outer bundle sheath; Pau = *Pauciflorae*; Plu = *Pluriflorae*; Ss = single bundle sheath; Th = thylakoids. Bars: (**a, c**) = 20  $\mu$ m; (**b, d**) = 0.5  $\mu$ m. For interpretation of the references to color in this figure, the reader is referred to the web version of this article.



type, and rounded and smaller in the second type. These types also differ in the continuity of the inner sheath in the major vascular bundles, being continuous only in the eleocharoid type (Soros and Bruhl 2000). However, the continuity of the inner sheath might vary along the scape and can be interrupted in the apical region, as indicated for *Eleocharis minima* (Martins and Scatena 2015), described as Kranz eleocharoid (Murphy et al. 2007, Martins and Scatena 2011).

In Eleocharideae, the  $C_4$  pathway is considered intermediate to recent (*Eleocharis vivipara* - ca.  $10.5 \pm 3.2$  Ma; *E. baldwinii* -  $4.4 \pm 2.1$  Ma) compared with the other  $C_4$  species of this family (Besnard et al. 2009). These authors indicated that Cyperaceae species with different Kranz types do not present significant genetic modifications. Thus, before the plasticity observed in *Eleocharis*, the changing of  $C_3$  to  $C_4$  metabolism might have required a small number of genetic modifications, as indicated by Christin et al. (2007) for groups with high plasticity in present  $C_4$  photosynthesis. Plasticity in Kranz anatomy was also observed in other  $C_4$  groups, such as some species of Poaceae and Asteraceae (Araus et al. 1990, Gong et al. 2011).

In Rhynchosporae, it is likely that the Kranz anatomy arose twice, once for each anatomical type, as chlorocyperoid and rhynchosporoid occur in distinct clades (Thomas et al. 2008). Both Kranz types occur in *Pauciflorae* and *Pluriflorae* sections (Ueno and Koyama 1987), and although these types differ in the number of sheaths and chloroplast locations, similarities are observed in chloroplast distribution and thylakoid organization. Genetic markers indicate that the  $C_4$  pathway is recent in *Rhynchospora* (ca.  $7.4 \pm 2.8$  Ma) compared with the other species of this family (Besnard et al. 2009), and phylogenetic data show the occurrence of the  $C_4$  pathway in derivative gender groups (Thomas et al. 2008). The Kranz ( $C_4$ ) of the *Rhynchospora* species are primarily Neotropical, occurring in xeric environments, such as open fields and savannas

(Ueno and Koyama 1987), living sympatrically with non-Kranz ( $C_3$ ) species of the same genus. Thus, it has been suggested that the  $C_4$  pathway arose in some lineages of the genus as an adaptation to conditions of water stress, high temperatures and light from  $C_3$  ancestors potentially occurring in this type of environment. However, the occurrence of different Kranz types in closer phylogenetic groups living sympatrically is intriguing and requires more detailed studies.

The variations among the Kranz types in Cyperaceae reflect essential differences in the structure, not the origin, of the tissues. The homologous chlorophyllian cells surrounding the vascular bundles arise from the ground meristem and might have an elongated (chlorophyll parenchyma) or rounded (outer sheath) shape. In chlorocyperoid, eleocharoid and fimbristylloid types, the procambium gives rise to two vascular sheaths, with chloroplasts only in the inner sheath. In the rhynchosporoid type, only one sheath has procambial origin and presents chloroplasts. This sheath is homologous to the outer sheath, which lacks chloroplasts with procambial origin.

Herein, we showed the multiple origins of Kranz anatomy in Cyperaceae in different phylogenetic groups, confirming the results of Soros and Bruhl (2000), Muasya et al. (2002, 2008, 2009), Ghamkhar et al. (2007), Thomas et al. (2008) and Roalson et al. (2010). Thus, this study represents adaptive convergence, as phylogenetic relationships are not reflected. In addition, the anatomical types are not associated with specific environmental conditions because the Kranz species of Abildgaardieae, *Rhynchospora* and some of Cypereae occur in similar xeric environments or even live sympatrically and present different Kranz types. In addition, *Eleocharis* representatives vary in relation to the occurrence and the type of Kranz anatomy in individuals of the same species.

Thus, the Kranz types in Cyperaceae likely result from ecological pressures that promote

photorespiration, even in different environmental conditions. The Kranz species of Abildgaardieae and Rhynchosporeae tribes are xerophytes, whereas the Cyperaceae species are mesophytes or xerophytes and the Eleocharideae species are hydrophytes. Thus, independent of the environment, distinct lineages of Cyperaceae have similar evolutionary pathways for more efficient photosynthesis.

#### ACKNOWLEDGMENTS

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for a PhD grant (2008/09380-2) to S. Martins and financial support (2011/18275-0) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support to V.L. Scatena (301692/2010-6 and 471837/2011-3) and to S.R. Machado (2008/301464-1) and the Centre of Microscopy of the Universidade Estadual Paulista (Botucatu).

#### RESUMO

Cyperaceae é uma das famílias de angiospermas com maior diversidade de espécies com anatomia Kranz. Para a família foram descritos quatro diferentes tipos de anatomia Kranz (clorociperóide, eleocaróide, fimbristilóide e rincosporóide) e a ocorrência e as características estruturais desses tipos são importantes para traçar hipóteses evolutivas. Este trabalho visou examinar dados disponíveis sobre anatomia Kranz de Cyperaceae com ênfase na taxonomia, distribuição geográfica, habitat e anatomia para inferir a possível origem da anatomia Kranz na família. Verificou-se que os quatro tipos de anatomia Kranz (relacionada à fotossíntese  $C_4$ ) em Cyperaceae surgiram diversas vezes em grupos não relacionados filogeneticamente. Por outro lado, observou-se a convergência desses tipos anatômicos, exceto o rincosporóide, em determinados grupos. Assim, a origem diversa dessas espécies pode ser resultado de diferentes pressões ambientais que promovem a fotorrespiração. Maior variação da ocorrência da anatomia Kranz e de tipos anatômicos foi observada em *Eleocharis*, cujo surgimento da via  $C_4$  é recente quando comparado aos demais gêneros da

família e as espécies deste gênero vivem em ambientes aquáticos.

**Palavras-chave:** anatomia, ultraestrutura, taxonomia, filogenia, Cyperaceae.

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