

---

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS  
BIOLOGIA VEGETAL

---

# Estudos anatômicos de Cyperaceae (Poales) com ênfase na anatomia Kranz

**Shirley Martins**

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ciências Biológicas (Biologia Vegetal).

Junho - 2012

---

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS  
BIOLOGIA VEGETAL

---

# Estudos anatômicos de Cyperaceae (Poales) com ênfase na anatomia Kranz

**Shirley Martins**

**Orientadora: Profa. Dra. Vera Lucia Scatena**

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ciências Biológicas (Biologia Vegetal).

Junho - 2012

*Dedico*  
*Minha mãe Sílvia Martins por seu exemplo de luta e superação*  
*e pelo amor incondicional.*

## *Agradecimentos*

*A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela bolsa concedida no primeiro ano de doutorado (Processo: 33004137) e a Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP) pela bolsa concedida nos três últimos anos de doutorado (Processo: 2008/09380-2).*

*Ao Departamento de Botânica do Instituto de Biociências, UNESP/Rio Claro, pela infraestrutura disponibilizada para o desenvolvimento dessa pesquisa.*

*Aos funcionários da Seção de Pós-Graduação, da Biblioteca e do Departamento de Botânica, pela ajuda e atenção, especialmente a secretária Célia Hebling e ao técnico Ari Pesce, pela atenção e amizade.*

*Agradeço aos professores do Departamento de Botânica da UNESP/Rio Claro pelo enorme aprendizado durante as disciplinas e pelas importantes conversas científicas ou não científicas nos corredores.*

*Aos funcionários do Centro de Microscopia Eletrônica da UNESP/Botucatu pelo auxílio e atenção, em especial a Ligia Costa e Claudete Tardivo.*

*Ao Dr. Marccus Alves (UFPE) pelo incentivo antes e durante o doutorado, pela ajuda nas coletas, identificação das espécies, parceria e aprendizado contínuo.*

*A Dra. Silvia Rodrigues Machado (UNESP – Botucatu) pela parceria e discussões sobre ultraestrutura.*

*A Dra. Vera Lucia Scatena pelo enorme aprendizado sobre morfologia vegetal e sobre profissionalismo. Serei eternamente grata pela oportunidade concedida, pela paciência e por ter acreditado em mim e no meu trabalho.*

*Aos amigos do Curso de Pós-graduação pelas discussões científicas ou não científicas, amizade e companhia em congressos, em especial a Eliana Ramos, Graziela Dias, Paulo Roberto, Henrique Tozzi, Fernando Moraes, Felipe Daibes e as meninas do Lab. de Fenologia.*

*A todos os membros do Laboratório de Morfologia Vegetal (antigos e novos) pela troca de experiências, aprendizado, paciência, amizade, companhia nas viagens de campo e alegria, especialmente a Aline Oriani, Orlando Cavallari, Odair José, Letícia Poli, Elaine Lopes, Paula Alves, Leandro Gallo, Rita Andreota, Renata Ferrari, Angela Daltin e Blanca Dugarti.*

*Agradeço especialmente a Mayra Eichemberg “madrinha” (Lab. Morfologia Vegetal) e Camila Kjsmann (Lab. Fisiologia Vegetal) pela amizade e companheirismo que serão eternos. Obrigada pela amizade em praticamente todas as fases do meu doutorado e pelo apoio, incentivo e alegria. Vocês são verdadeiras irmãs para mim.*

*Agradeço imensamente aos amigos de Rio Claro que me apoiaram desde o início de minha chegada a cidade, facilitando muito minha vida longe da família e dos amigos, em especial a Regina Santana, uma verdadeira mãe que me acolheu como filha.*

*Agradeço aos meus familiares, especialmente a minha mãe Sílvia Martins, por aceitar minha decisão de mudar de cidade para crescer profissionalmente. Sei que não foi fácil esse período longe, principalmente diante de tantos acontecimentos que exigiam a minha presença. Obrigada por ser forte e sempre me incentivar a ir mais longe, correr atrás dos meus sonhos e apoiar sempre as minhas decisões. A senhora é meu exemplo de força e determinação. Te Amo.*

*Ao meu esposo André Andrade, pelo incentivo, apoio constante e por alegrar a minha vida durante boa parte do doutorado. A paz e a tranquilidade que você me trouxe foram fundamentais para o desenvolvimento desse trabalho. Te Amo.*

*A menos que modifiquemos nossa maneira de pensar, não seremos capazes de resolver os problemas causados pela forma como nos acostumamos a ver o mundo.*

*(Albert Einstein)*

## ÍNDICE

<b>1. RESUMO .....</b>	<b>01</b>
<b>2. ABSTRACT.....</b>	<b>02</b>
<b>3. INTRODUÇÃO GERAL.....</b>	<b>03</b>
<b>4. LITERATURA CITADA .....</b>	<b>05</b>
<b>CAPÍTULO 1. Developmental anatomy of <i>Cyperus laxus</i> (non-Nranz) and <i>Fimbristylis dichotoma</i> (Kranz) (Cyperaceae, Poales) and tissue continuity.....</b>	<b>10</b>
<b>Abstract.....</b>	<b>11</b>
<b>Introduction.....</b>	<b>11</b>
<b>Materials and methods .....</b>	<b>12</b>
<b>Results .....</b>	<b>13</b>
<b>Discussion.....</b>	<b>15</b>
<b>Resumo.....</b>	<b>19</b>
<b>References .....</b>	<b>19</b>
<b>Figures.....</b>	<b>25</b>
<b>CAPÍTULO 2. Do leaves of Cyperoideae (Cyperaceae) have a multiple epidermis or a hypodermis? .....</b>	<b>31</b>
<b>Abstract.....</b>	<b>32</b>
<b>Introduction.....</b>	<b>32</b>
<b>Materials and methods .....</b>	<b>33</b>
<b>Results .....</b>	<b>34</b>
<b>Discussion.....</b>	<b>35</b>
<b>Conclusion .....</b>	<b>37</b>
<b>References .....</b>	<b>38</b>
<b>Table.....</b>	<b>42</b>
<b>Figures.....</b>	<b>43</b>

<b>CAPÍTULO 3. Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales)</b> .....	<b>49</b>
Abstract.....	50
Introduction.....	50
Materials and methods .....	52
Results .....	52
Discussion.....	55
References .....	59
Tables .....	64
Figures.....	66
<b>CAPÍTULO 4. Ultrastructure of Kranz tissues in Cyperaceae (Poales): Emphasis on chloroplasts and mitochondria .....</b>	<b>74</b>
Abstract.....	75
Introduction.....	75
Materials and methods .....	77
Results .....	78
Discussion.....	80
Conclusion .....	82
References .....	82
Tables .....	86
Figures.....	89
<b>CAPÍTULO 5. Dimorphism and different types of Kranz anatomy in scapes of <i>Eleocharis minima</i> Kunth (Cyperaceae, Poales) .....</b>	<b>93</b>
Abstract.....	94
Introduction.....	94
Materials and methods .....	95
Results .....	96
Discussion.....	97
References .....	99
Table.....	102
Figures.....	103

<b>CAPÍTULO 6. Ocorrência e inferência evolutiva da anatomia Kranz em Cyperaceae (Poales)....</b>	<b>105</b>
<b>Resumo .....</b>	<b>106</b>
<b>Abstract.....</b>	<b>106</b>
<b>Introdução .....</b>	<b>107</b>
<b>Material e métodos.....</b>	<b>108</b>
<b>Resultados .....</b>	<b>109</b>
<b>Discussão.....</b>	<b>110</b>
<b>Referências bibliográficas .....</b>	<b>114</b>
<b>Tabelas .....</b>	<b>117</b>
<b>Figuras .....</b>	<b>120</b>
<b>5. CONSIDERAÇÕES FINAIS .....</b>	<b>128</b>



## 1. Resumo

Foram realizados estudos anatômicos e ultraestruturais de espécies com anatomia Kranz e não Kranz, de Cyperoideae (Cyperaceae) buscando ampliar o conhecimento anatômico do grupo, uniformizar a terminologia empregada para as bainhas dos feixes vasculares, levantar homologias úteis para a taxonomia e filogenia e hipóteses sobre a origem da anatomia Kranz na família. Para tanto foram utilizadas técnicas anatômicas usuais para estudos estruturais e ultraestruturais em espécies de Cyperaceae. Através do desenvolvimento de espécies Kranz e não Kranz verifica-se a continuidade dos tecidos (endoderme e periciclo) em todos os órgãos vegetativos e a uniformização dos termos empregados para as bainhas dos feixes vasculares de folhas e escapos. Estudos ontogenéticos de espécies de Cyperaceae confirmam a ocorrência de epiderme multiestratificada nas folhas, ao invés de hipoderme, e caracterizam os quatro tipos de anatomia Kranz na família: clorociperoide, eleocaróide, fimbristiloide, rincosporoide. A análise ultraestrutural da bainha dos feixes vasculares mostra a ocorrência de padrões na estrutura dos cloroplastos para os tipos eleocaróide e rincosporoide e também para *Cyperus* e *Pycnus* no tipo clorociperoide. Foi verificada variação na ocorrência e no tipo de anatomia Kranz em *Eleocharis minima*, espécie aquática (anfíbia), nas diferentes regiões do escapo emerso e submerso do mesmo indivíduo, destacando a plasticidade desse caráter nas espécies aquáticas e sua uniformidade nas espécies terrestres. Com dados desses estudos, juntamente com os demais dados disponíveis na literatura, são apresentadas hipóteses sobre a origem da anatomia Kranz em Cyperaceae.

**Palavras-chave:** anatomia, ontogenia, anatomia Kranz, ultraestrutura, Cyperaceae

## 2. Abstract

Anatomical and ultrastructural studies in Kranz and non-Kranz species of Cyperoideae (Cyperaceae) were performed searching to amplify the anatomical knowledge of the group; standardize the terminology applied to the vascular bundle sheaths, to bring up homologies useful to taxonomy and phylogeny and hypotheses about the origin of the Kranz anatomy in the family. Anatomical techniques were performed to structural and ultrastructural studies of Cyperaceae species. Through the development of the Kranz and non-Kranz species it was verified the tissues continuity (endodermis and pericycle) in all vegetative organs and the standardization of the terms employed to the vascular bundle sheaths of leaves and scapes. Ontogenetical studies of the Cyperaceae species confirmed the occurrence of multiple epidermis in leaves, instead of hypoderm, and characterized the four Kranz anatomical types: chlorocyperoid, eleocharoid, fimbristyloid, rhynchosporoid. The ultrastructural analyses of the vascular bundle sheaths showed the occurrence of pattern in the chloroplast's structure to the eleocharoid and rhynchosporoid types and also to *Cyperus* and *Pycreus* species in the chlorocyperoid type. Variations in the occurrence and in the type of Kranz anatomy was verified in *Eleocharis minima*, aquatic species (amphibious), in different regions of emerged and submerged scapes of the same individual, detaching the plasticity of this character in aquatic species and its uniformity in terrestrial species. With data obtained in these studies, along with other available data in the literature, hypotheses about Kranz anatomy origin in the family were presented.

**Keywords:** anatomy, ontogeny, Kranz anatomy, ultrastructure, Cyperaceae

### 3. Introdução Geral

Cyperaceae está inserida no clado das cyperídeas, juntamente Juncaceae e Thurniaceae na ordem Poales (Givnish *et al.* 2010). Constitui uma das maiores famílias de angiospermas com cerca de 5.500 espécies e apresenta distribuição cosmopolita (Govaerts *et al.* 2009). A família é bem representada nos diferentes ecossistemas brasileiros, principalmente em áreas de campos, bordas de mata, dunas litorâneas, margem de rios e lagos, e ambientes perturbados (Alves *et al.* 2009).

Estudos anatômicos de Cyperaceae apresentam geralmente enfoque taxonômico (Govindarajalu 1966, 1974, Metcalfe 1971, Sharma & Merha 1972, Alves *et al.* 2002, Prata *et al.* 2007, Hefler *et al.* 2010), sendo também utilizados em análises filogenéticas (Bruhl 1995, Naczi 2009). Dentre os caracteres anatômicos utilizados nessas abordagens, destacam-se aqueles da epiderme foliar, que ora é descrita como uniestratificada (Goetghebeur 1998) ora como multiestratificada (Sharma & Merha 1972) na família.

Outro caráter anatômico de destaque na família é a anatomia Kranz, caracterizada pelo arranjo radiado das células do mesofilo em torno da bainha dos feixes vasculares com cloroplastos (Brown 1975). A anatomia Kranz constitui uma resposta estrutural ao metabolismo fotossintético C<sub>4</sub>, que promove maior captação de CO<sub>2</sub> em comparação com as plantas C<sub>3</sub> e, conseqüentemente melhor aproveitamento da água, favorecendo o crescimento em ambiente com alta temperatura e luminosidade e com solos pobres em nutrientes (Sage 2004).

Além de Cyperaceae, a anatomia Kranz ocorre também em Poaceae e Hydrocharitaceae dentre as monocotiledôneas (Sage 2004) e em mais 16 famílias de eudicotiledôneas, em que Amaranthaceae e Chenopodiaceae são as mais representativas (Muhaidat *et al.* 2007).

As espécies com anatomia Kranz de Cyperaceae são restritas à Cyperoideae, que é a maior das duas subfamílias, ocorrendo em quatro das 13 tribos: Abildgaardieae, Cypereae, Elecharideae e Rhynchosporae (Goetghebeur 1998; Muasya *et al.* 2008). Apenas Abildgaardieae e Eleocharideae são próximas filogeneticamente (Ghamkar *et al.* 2007), indicando que a anatomia Kranz teve

diferentes origens na família, refletindo sua variação anatômica (Soros & Bruhl 2000). São descritos quatro tipos de anatomia Kranz em Cyperaceae: clorociperoide, eleocaróide, fimbristiloide e rincosporoide, que diferem entre si no número e na continuidade ou não das bainhas dos feixes vasculares e também na localização dos cloroplastos (Soros & Dengler 2001, Martins & Scatena 2011). A origem e a terminologia das bainhas vasculares das espécies Kranz de Cyperaceae é variada (Martins & Scatena 2011).

As espécies Kranz de Cyperaceae diferem entre si quanto à localização e à ultraestrutura dos cloroplastos, relacionadas com o tipo anatômico ou agrupamento taxonômico (Carolin *et al.* 1977, Ueno *et al.* 1988, Bruhl & Perry 1995). A quantificação de cloroplastos e mitocôndrias é importante para a caracterização da anatomia Kranz de Poaceae (Brown *et al.* 1983, Yoshimura *et al.* 2004), mas para Cyperaceae esses dados são restritos às espécies anfíbias de *Eleocharis* (Ueno 1996).

Em Cyperaceae, os gêneros *Bulbostylis*, *Eleocharis* e *Rhynchospora* apresentam mais de um tipo de anatomia Kranz (Soros & Bruhl 2000). Em *Bulbostylis* ocorrem os tipos eleocaróide e fimbristiloide (Bruhl 1995); em *Rhynchospora* os tipos clorociperoide e rincosporoide (Ueno & Koyama 1987) e em *Eleocharis* os tipos eleocaróide e fimbristiloide (Murphy *et al.* 2007). Além disso, em *Eleocharis*, algumas espécies podem desenvolver ou não a estrutura Kranz como resposta ao ambiente, sendo Kranz quando terrestre e não Kranz quando submersa (Ueno 1996).

O agrupamento de dados envolvendo taxonomia, filogenia, ocorrência e características estruturais de plantas com anatomia Kranz foi realizado para Poaceae (Giussani *et al.* 2001), Amaranthaceae (Kadereit *et al.* 2003, Sage *et al.* 2007, Muhaidat *et al.* 2007), Asteraceae (McKown *et al.* 2005) e Cleomaceae (Voznesenskaya *et al.* 2007), visando levantar hipóteses sobre a origem da fotossíntese C<sub>4</sub>. Em Cyperaceae a indicação sobre a origem da anatomia Kranz é superficial (Soros & Bruhl 2001) ou restrita numa abordagem filogenética das tribos Abildgaardieae (Gramkam *et al.* 2007) e Eleocharideae (Roalson *et al.* 2010).

Em vista do exposto, esta tese procurou estudar em Cyperaceae: 1) o desenvolvimento anatômico de espécies Kranz e não Kranz, visando levantar diferenças estruturais e verificar a continuidade dos tecidos nos órgãos vegetativos; 2) a ontogenia dos tecidos epidérmicos e subepidérmicos para confirmar ou não a ocorrência de epiderme multiestratificada; 3) a ontogenia das bainhas dos feixes vasculares de espécies Kranz e não Kranz, visando caracterizar os tipos e uniformizar a terminologia; 4) as variações ultraestruturais relacionadas aos cloroplastos e mitocôndrias de espécies Kranz para verificar se existem padrões; 5) a anatomia de escapos emersos e submersos de *Eleocharis minina* para verificar a ocorrência ou não de plasticidade da anatomia Kranz no mesmo indivíduo; 6) dados sobre a anatomia Kranz da família para levantar hipóteses sobre sua origem.

#### 4. Literatura Citada

- ALVES, M., ARAÚJO, A.C., PRATA, A.P., VITTA, F.A., HEFLER, S.M., TREVISAN, R., GIL, A.B., MARTINS, S., THOMAS, W.W. 2009. Diversity of Cyperaceae in Brazil. *Rodriguésia* 60: 771-782.
- ALVES, M.V., ESTELITA, M.E.M., WANDERLEY, M.G., THOMAS, W.W. 2002. Aplicações taxonômicas da anatomia foliar das espécies brasileiras de *Hypolytrum* Rich. (Cyperaceae). *Revista Brasileira de Botânica* 25: 1-9.
- BROWN, W.V. 1975. Variations in anatomy, associations, and origins of Kranz tissue. *American Journal of Botany* 62: 395-402.
- BROWN, R.H., BOUTON, J.H., RIGSBY, L., RIGLER, M. 1983. Photosynthesis of grass species differing in carbon dioxide fixation pathways. VIII. Ultrastructural characteristics of *Panicum* species in the *Laxa* group. *Plant Physiology* 71: 425-431.
- BRUHL, J.J. 1995. Sedge genera of the world: relationships and new classification of the Cyperaceae. *Australian Systematic Botany* 8: 125-305.

- BRUHL, J.J., PERRY, S. 1995. Photosynthetic pathway-related ultrastructure of C<sub>3</sub>, C<sub>4</sub> and C<sub>3</sub>-C<sub>4</sub> intermediate sedge (Cyperaceae), with special reference to *Eleocharis*. Australian Journal of Plant Physiology 22: 521-530.
- CAROLIN, R.C., JACOBS, S.W.L., VESK, M. 1977. The ultrastructure of Kranz cells in the family Cyperaceae. Botanical Gazette 138: 413-419.
- GIUSSANI, M.G., COTA-SÁNCHEZ, H., ZULOAGA, F.O., KELLOGG, E.A. 2001. A molecular phylogeny of the Grass subfamily Panicoideae (Poaceae) shows multiple origins of C<sub>4</sub> photosynthesis. American Journal of Botany 88: 1993-2012.
- GIVNISH, T.J., AMES, M.S., MCNEAL, J.R., MCKAIN, M.R., STEELE, P.R., DEPAMPHILIS, C.W., GRAHAM, S.W., PIRES, J.C., STEVENSON, D.W., ZOMLEFER, W.B., BRIGGS, B.G., DUVALL, M.R., MOORE, M.J., HEANEY, J.M., SOLTIS, D.E., SOLTIS, P.S., THIELE, K., LEEBENS-MACK, J.H. 2010. Assembling the tree of the monocotyledons: Plastome sequence phylogeny and evolution of Poales. Annals of the Missouri Botanical Garden 97: 584-616.
- GOETGHEBEUR, P. 1998. Cyperaceae. In: Kubitzki K., Huber, H., Rudall, P.J., Stevens, P.S., Stützel, T. (eds) The families and genera of vascular plants, Springer-Verlag, Berlin, 141-190 pp.
- GOVAERTS, R., SIMPSON, D.A., GOETGHEBEUR, P., WILSON, K.L., EGOROVA, T., BRUHL, J. 2007. World checklist of Cyperaceae. The Board of Trustees of the Royal Botanical Garden, Kew.
- GOVINDARAJALU, E. 1966. The systematic anatomy of south Indian Cyperaceae: *Bulbostylis* Kunth. Botanical Journal of the Linnean Society 59: 289-304.
- GOVINDARAJALU, E. 1974. The systematic anatomy of south Indian Cyperaceae: *Cyperus* L. subgen. *Juncellus*, *Cyperus* subgen. *Mariscus* and *Lipocarpha* R.Br. Botanical Journal of the Linnean Society 68: 235-266.

- GHAMKHAR, K., MARCHANT, A.D., WILSON, K.L., BRUHL, J.J. 2007. Phylogeny of *Abildgaardieae* (Cyperaceae) inferred from ITS and *trnL-F* data. *Aliso* 23: 149-164.
- HEFLER, S.M., LONGHI-WAGNER, H.M. 2010. A contribuição da anatomia foliar para taxonomia das espécies de *Cyperus* L. subg. *Cyperus* (Cyperaceae) ocorrentes no sul do Brasil. *Acta Botanica Brasilica* 24: 708-717.
- KADEREIT, G., BORSCH, T., WEISING, K., FREITAG, H. 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C<sub>4</sub> photosynthesis. *The International Journal of Plant Science* 164: 959-986.
- MARTINS, S., SCATENA, V.L. 2011. Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales). *Australian Journal of Botany* 59: 554-562.
- MCKOWN, A.D., MONCALVO, M.J., DENGLER, N.G. 2005. Phylogeny of *Flaveria* (Asteraceae) and inference of C<sub>4</sub> photosynthesis evolution. *American Journal of Botany* 92: 1911-1928.
- METCALFE, C.R. 1971. *Anatomy of the monocotyledons: Cyperaceae*. Clarendon Press, Oxford.
- MUASYA, A.M., SIMPSON, D.A., VERBOOM, G.A., GOETGHEBEUR, P., NACZI, R.F.C., CHASE, M.W., SMETS, E. 2008. Phylogeny of Cyperaceae based on DNA sequence data: Current progress and future prospects. *Botanical Review* 75: 2-21.
- MUHAI DAT, R., SAGE, R.F., DENGLER, N.G. 2007. Diversity of Kranz anatomy and biochemistry in C<sub>4</sub> eudicots. *American Journal of Botany* 94: 362-381.
- MURPHY, L.R., BARROCA, J., FRANCESCHI, V.R., LEE, R., ROALSON, E.H., EDWARDS, G.E., KU, M.S.B. 2007. Diversity and plasticity of C<sub>4</sub> photosynthesis in *Eleocharis* (Cyperaceae). *Functional Plant Biology* 34: 571-580.
- NACZI, R.F.C. 2009. Insight on using morphologic data for phylogenetic analysis in sedges (Cyperaceae). *Botanical Review* 75: 67-95.

- PRATA, A.P., MENEZES, N.L., MAZZONI-VIVEIROS, S.C., WANDERLEY, M.G., THOMAS, W.W. 2007. Anatomia do escapo e rizoma de espécies brasileiras de *Bulbostylis* Kunth (Cyperaceae). *Revista Brasileira de Botânica* 30: 245-256.
- ROALSON, E.H., HINCHLIFF, C.E., TREVISAN, R., SILVA, C.R.M. 2010. Phylogenetic relationships in *Eleocharis* (Cyperaceae): C<sub>4</sub> Photosynthesis origins and patterns of diversification in the spikerushes. *Systematic Botany* 35: 257-271.
- SAGE, R.F. 2004. The evolution of C<sub>4</sub> photosynthesis. *New Phytologist* 161: 341-370.
- SAGE, R.F., SAGE, T.L., PEARCY, R.W., BORSCH, T. 2007. The taxonomic distribution of C<sub>4</sub> plants in *Amaranthaceae sensu stricto*. *American Journal of Botany* 94: 1992-2003.
- SHARMA, O.P., MEHRA, P.N. 1972. Systematic anatomy of *Fimbristylis* Vahl (Cyperaceae). *Botanical Gazette* 133: 87-95.
- SOROS, C.L., BRUHL, J.J. 2000. Multiple evolutionary origins of C<sub>4</sub> photosynthesis in the Cyperaceae. In: Wilson, K.L., Morrison, D.A. (eds) *Monocots: systematics and evolution*, CSIRO Publishing, Melbourne 629-636 pp.
- SOROS, C.L., DENGLER, N.G. 2001. Ontogenetic derivation and cell differentiation in photosynthetic tissues of C<sub>3</sub> and C<sub>4</sub> Cyperaceae. *American Journal of Botany* 88: 992-1005.
- UENO, O. 1996. Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C<sub>3</sub> and C<sub>4</sub> metabolism. *Planta* 199: 382-393.
- UENO, O., KOYAMA, T. 1987. Distribution and evolution of C<sub>4</sub> syndrome in *Rhynchospora* (Rhynchosporaceae-Cyperaceae). *Botanical Magazine of Tokyo* 100: 63-85.
- UENO, O., TAKEDA, T., MAEDA, E. 1988. Photosynthetic characteristics of an amphibious plant, *Eleocharis vivipara*: expression of C<sub>4</sub> and C<sub>3</sub> modes in contrasting environments. *Proceedings of the National Academy of Sciences USA* 85: 6733-6737.
- VOZNESENSKAYA, E.V., KOTEYEVA, N.K., CHUONG, D.X., IVANOVA, A.N., BARROCA, J., CRAVEN, L.A., EDWARDS, G.E. 2007. Physiological, anatomical and biochemical



characterization of photosynthetic types in genus *Cleome* (Cleomaceae). *Functional Plant Biology* 34: 247-267.

YOSHIMURA, Y., KUBOTA, F., UENO, O. 2004. Structural and biochemical bases of photorespiration in C<sub>4</sub> plants: quantification of organelles and glycine decarboxylase. *Planta* 220: 307-317.

## CAPÍTULO 1

**Shirley Martins, Vera Lucia Scatena**

**Developmental anatomy of *Cyperus laxus* (non-Kranz) and *Fimbristylis dichotoma*  
(Kranz) (Cyperaceae, Poales) and tissue continuity**

(Submetido ao periódico *Anais da Academia Brasileira de Ciências*, corrigido e devolvido em  
abril/2012)

## Abstract

Cyperaceae are present in different ecosystems and constitute the herbaceous extract. Of the approximately 5500 species of the family, a third has Kranz anatomy, which represents an important characteristic of the taxonomy and phylogeny of the group. In *Cyperus laxus* L. (non-Kranz) and *Fimbristylis dichotoma* Vahl (Kranz), development begins with germination that is marked by the emergence of the coleoptiles, followed by the primary root, which is ephemeral. The rhizome originates from the mesocotyl and promotes the vascular connection between the roots, leaves and scapes. The continuity of the tissues is evidenced by the presence of an endodermis and pericycle in all vegetative organs. Leaves and scapes differ between the two species by the arrangement of mesophyll cells, which is regular in *Cyperus laxus* (non-Kranz) and arranged radially in *Fimbristylis dichotoma* (Kranz). Also, there are two and three bundle sheaths, respectively, in the two species. The outer bundle sheath in both species constitutes the endodermis, and the inner sheath in *Cyperus laxus* and the middle and inner sheaths in *Fimbristylis dichotoma* constitute the pericycle.

**Keywords:** pericycle, endodermis, Casparian strips, seedling

## Introduction

Cyperaceae is one of the 10 largest Angiosperm families and is the second family of the Poales order (Govaerst et al. 2007). This family is cosmopolitan (i.e., occurring in diverse ecosystems) and is located mainly in open areas (Goetghebeur 1998) and disturbed environments (Bryson and Carter 2008). The family consists of approximately 5500 species (Govaerst et al. 2007), and ca. 1500 of these have Kranz anatomy (Bruhl and Wilson 2007), which is represented by a set of structural modifications that are related to C<sub>4</sub> photosynthesis (Sage 2004).

*Cyperus laxus* L. (non-Kranz) and *Fimbristylis dichotoma* Vahl (Kranz) are present in tropical and subtropical regions, mainly at the edges of forests and in open areas of different

ecosystems (Alves et al. 2009). They are also considerate weed plants that are common in disturbed areas (Bryson and Carter 2008).

The anatomical studies of Cyperaceae usually have emphasized taxonomy (Govindarajalu 1974, Metcalfe 1971, Naczi 2009, Hefler and Longhi-Wagner 2010), and studies on anatomical development have been restricted to a few species that are of economic importance, such as *Cyperus rotundus* (Wills and Briscoe 1970), *Cyperus esculentus* (Wills et al. 1980, Gifford and Bayer 1995) and *Cyperus papyrus* (Menezes et al. 2005). In general, studies on anatomical development are restricted to a description of only one organ, such as the roots in *Cyperus giganteus* (Rodrigues and Estelita 2004); rhizomes in *Cyperus esculentus* (Bendixen 1973), *Cyperus giganteus* (Rodrigues and Estelita 2002) and *Scleria* (Lima and Menezes 2009); and leaves in *Cyperus eragrostis* (Soros and Dengler 1996) and *Cyperus giganteus* (Rodrigues and Estelita 2003).

In studies of development, only Menezes et al. (2005) and Lima and Menezes (2009) refer to the continuity of the tissues, which is marked by the presence of the endodermis and pericycle in all vegetative organs (Menezes et al. 2005). The endodermis and pericycle are structures that are often related to roots (Esau 1965); however, there are a few reports of these structures being present in other organs, such as the stem and leaf (Lersten 1997, Menezes et al. 2005).

For Cyperaceae, studies on anatomical developmental in the Kranz and non-Kranz species are important to determine the origin and distribution of the tissues in the plant as a whole. Thus, the developmental anatomy of *Cyperus laxus* (non-Kranz) and *Fimbristylis dichotoma* (Kranz) was studied to describe the origins of and verify the tissue continuity in different vegetative organs.

## Materials and Methods

*Cyperus laxus* L. (S. Martins 413, 417; P.A. Braga 14) and *Fimbristylis dichotoma* Vahl (S. Martins 414, 420; V.L. Scatena 343, 341) were collected in their natural habitat, disturbed areas in southeastern Brazil. *Fimbristylis dichotoma* is dominant in open areas, and *Cyperus laxus* is

dominant in shaded areas. Voucher materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB).

For the anatomical study, individuals at different stages of development were collected, fixed in FAA 50 (Johansen 1940) and stored in 70% ethanol. Mature achenes were allowed to germinate on filter paper that had been humidified with distilled water at 30° C in a B.O.D incubator. Monitoring was performed daily to observe the germination and the post-seminal stages of development.

Embryos and portions of young and mature vegetative organs (root, rhizome, leaf and scape) were dehydrated in an ethyl series and then embedded in historesin (Leica Historesin Embedding Kit, Nussloch, Germany) (Feder and O'Brien 1968). Paradermic sections of embryos and longitudinal and transverse sections of young and mature vegetative organs were performed on a rotary microtome. The sections were stained with periodic acid-Shiff's reagent (PAS reaction) and toluidine blue (Feder and O'Brien 1968) and mounted in Entellan (Merck, Darmstadt, Germany). Transverse sections of the medial region of all mature vegetative organs were also made, stained with basic fuchsin and Astra blue (Roeser 1972) and mounted in glycerin jelly.

Images were obtained using a Leica DFC 290 digital camera (Leica Microsystems Ltd., Heerbrugg, Germany) coupled to a Leica DMLB microscope (Leica Microsystems, Wetzlar, Germany), and the Leica IM50 image manager v. 5.0 software was used for analysis (Leica Microsystems, Wetzlar, Germany).

## Results

*Cyperus laxus* (non-Kranz) (Fig. 1A) and *Fimbristylis dichotoma* (Kranz) (Fig. 1B) are herbaceous, perennial and have rhizomes. The fruit is an achene (Fig. 1C), and the seed contains a differentiated embryo with coleoptiles (co) facing the micropyle and the radicle (ra) in a lateral position (Fig. 1D). Germination occurs within one to three days after the achene is imbibitioned, and the first structure to emerge is the coleoptiles, followed by emergence of the primary root (pr) (Fig.

1E-G). The coleoptile is tubular, photosynthetic and protects the eophylls (eo) (Fig. 1G, H). In seedling development, the primary root degenerates, and the adventitious roots (ar) originate from the pericycle in the mesocotyl (Fig. 1I). In the seedling stage, vascular connection among the mesocotyl, roots and leaves occurs (Fig. 1I). The mesocotyl then develops into the rhizome (Fig. 1J), and in the young stage present Casparian strips in the endodermal cell walls (arrows in Fig. 1K).

A mature rhizome possesses a vascular plexus that originates from the pericycle and promotes vascular connection with the roots (Fig. 2A). In this same region, the connection between the rhizome and the leaves and scapes occurs through the procambial strands and ground meristem tissues (Fig. 2A-C). The endodermis (en) and pericycle (pe) of the adventitious root is continuous with that of the rhizome (Fig. 2D-E). Detail of the tissue connections between the rhizome and the root is showed in Fig. 2E. In the young root, the precursor cells of the endodermis divide periclinaly and give rise to derivative cells of the endodermis that form radial rows on the internal cortex and part of the medial cortex (Fig. 2F). In the young root, Casparian strips are present in the endodermal cells (arrows in Fig. 2G). In the mature root, the endodermis has thick-walled cells (Fig. 2H), and the pericycle has slightly thick-walled cells (Fig. 2H). During root differentiation, occurs the formation of aerenchyma schizo-lisigenous in the median cortex; first by the separation of the cell walls (arrowheads in Fig. 2F) and after by cell lyses of the cell walls (arrows in Fig. 2H).

In the apical meristem region of the rhizome, leaves and scapes are formed (Fig. 2A-C). In this region, the continuity between the ground meristem and the procambial strands of the rhizome and the leaves and scapes is observed (Fig. 2A-C). In the young leaf of *Cyperus laxus* (non-Kranz), the mesophyll cells differentiate from the ground meristem, and the vascular bundles differentiate from the procambium (Fig. 3A-C). The mature leaf presents mesophyll cells that are distributed in a regular way (Fig. 3B-C), and the vascular bundles are collateral and surrounded by two sheaths (Fig. 3B-C). The outer bundle sheath (endodermis) originates from the innermost layer of the ground meristem and possesses thin-walled cells in the mature leaf (Fig. 3A-C). The inner bundle sheath

(pericycle) is derived from the outermost layer of the procambium and possesses thick-walled cells at maturity (Fig. 3A-C).

*Fimbristylis dichotoma* has Kranz anatomy of the fimbristylloid subtype, with vascular bundles that are surrounded by three sheaths. In the young leaf, the mesophyll precursor cells divide successively and after elongating their walls, the cells distribute radially around the vascular bundles (Fig. 3D-E). The outer bundle sheath (endodermis - en) originates from the innermost layer of the ground meristem and presents thin-walled cells at maturity (Fig. 3D-E). The middle sheath contains thick-walled cells, and thin-walled cells with chloroplasts constitute the inner sheath; both of these cell types are derived from the procambium and constitute the pericycle (pe) (Fig. 3D, E).

In the rhizome, the apical meristem also forms the scapes. In *Cyperus laxus*, the scape is triangular (Fig. 3F) with mesophyll cells that are distributed in a regular way on the periphery of the organ (Fig. 3G). The vascular bundles distribute randomly in the medullar parenchyma and are surrounded by two sheaths; the outer sheath is the endodermis (en) and the inner sheath is the pericycle (pe) (Fig. 3F-G). In *Fimbristylis dichotoma*, the scape is elliptical and possesses vascular bundles that distribute cylindrically around the organ periphery (Fig. 3H). The vascular bundles are surrounded by the mesophyll cells that are distributed radially and three sheaths; the outer sheath constitutes the endodermis (en), and the middle and inner sheaths constitute the pericycle (pe) (Fig. 3I).

## Discussion

*Cyperus laxus* (non-Kranz) and *Fimbristylis dichotoma* (Kranz) have similarities in their first stages of development and in the anatomical structures of their roots and rhizomes. These species differ only in the anatomical structures of their leaves and scapes, and these differences reflect the photosynthetic types C<sub>3</sub> and C<sub>4</sub>, respectively, which form structure non-Kranz and Kranz.

As was observed for the germination of the two species studied, the emergence of the coleoptiles before the primary root is common in other Cyperaceae species (Goethghebeur 1998, Tillich 2007). In the two species from this study, the adventitious roots originated from the pericycle in the rhizome, an occurrence that has been described to other Cyperaceae species (Leite et al. 2009, Lima and Menezes 2009) and monocotyledons (Menezes et al. 2005, Cattai and Menezes 2010). The study of the presence of the pericycle in other organs, not including the root, has often been neglected (Menezes et al. 2005). However, it has been reported that in the root, the pericycle forms lateral roots (Dubrovsky et al. 2011), and in the stem, the adventitious roots originate from the pericycle (Menezes et al. 2005, Sorin et al. 2005).

In addition to forming adventitious roots, the pericycle in the rhizome of *Cyperus laxus* and *Fimbristylis dichotoma*, as in other monocotyledons, originates vascular plexus, which promotes the vascular connection between the roots and rhizome (Simão and Scatena 2001, Alonso and Moraes-Dallaqua 2004, Menezes et al. 2005, Lima and Menezes 2009). For the species in this study, during the formation of adventitious roots, we observed that there was continuity between the root and rhizome of the vascular tissues and of the endodermis and pericycle. This formation has also been described in other Cyperaceae (Wills et al. 1980, Menezes et al. 2005) and monocotyledons, such as Agavaceae, Alismataceae, Commelinaceae, Eriocaulaceae, Heliconiaceae, Velloziaceae, Rapateceae and Zingiberaceae (Van Fleet 1942, Simão and Scatena 2001, Alonso and Moraes-Dallaqua 2004, Menezes et al. 2005, Cattai and Menezes 2010), reinforcing the presence of tissue continuity in vegetative organs.

In the adventitious roots of *Cyperus laxus* and *Fimbristylis dichotoma*, the cells of the inner cortex and part of the median cortex originate from the division of the precursor cells of the endodermis, which are termed endodermal initial cells by Menezes et al. (2005), and their derivatives. The meristematic role of the endodermis, which contributes to the formation of part of the root cortex, was demonstrated in other Cyperaceae and monocotyledons and was termed



“proendodermis” by Van Fleet (1961) and “endodermis with meristematic activity” by Menezes et al. (2005). Therefore, the results presented here confirm the meristematic activity of the endodermis in cortex formation.

The successive divisions of the precursor cells of the endodermis and their derivatives promote the radial disposition of the cortical cells, as was observed in roots of the species in this study, in other monocotyledons (Williams 1947, Rodrigues and Estelita 2004, Menezes et al. 2005). This radial arrangement favors aerenchyma formation in roots (Seago et al. 2005), corroborating what has been described in the species studied here and in other Cyperaceae and monocotyledons (Rodrigues and Estelita 2004, Menezes et al. 2005, Seago et al. 2005, Leite et al. 2009).

The endodermal cells in the studied species present Casparian strips when meristematic activity ceased, and after the cell-walls became thickened, as was described in other species of monocotyledons (Menezes et al. 2005, Leite et al. 2009, Lima and Menezes 2009). Casparian strips in the endodermis cells of young rhizomes of *Cyperus laxus* and *Fimbristylis dichotoma* were also observed, as well as, in other Cyperaceae and monocotyledons (Eiten 1969, Bendixen 1973, Govindarajalu 1974, Wills et al. 1980, Rodrigues and Estelita 2002, Alonso and Moraes-Dallaqua 2004, Menezes et al. 2005, Lima and Menezes 2009). Some authors consider the occurrence of endodermis in rhizomes as the innermost layer of the cortex, even without finding the Casparian strips (Will et al. 1980, Simão and Scatena 2001, Menezes et al. 2005). However, others authors employ other terms for the endodermis that is present in stems, such as “endodermoid sheath” (Tomlinson 1969, Metcalfe 1971, Gifford and Bayer 1995) and “starch sheath” (Esau 1965).

In the leaves of the species that have been studied, the endodermis corresponds to the vascular bundle sheath that originates from the ground meristem, as is seen in other Cyperaceae (Martins and Scatena 2011) and families of monocotyledons, such as Velloziaceae (Menezes et al. 2005), Heliconiaceae (Simão and Scatena 2001), Cannaceae (Alonso and Moraes-Dallaqua 2004) and Bromeliaceae (Proença and Sajo 2008). In leaves, as well as in stems, the occurrence of

endodermis is generally related to Casparian strips (Priestley and Radcliffe 1924, Dickison and Weitzman 1996, Lersten 1997), leading to the use of other terms, such as “parenchymatous sheath” or “endodermoid sheath”, instead of endodermis (Esau 1965).

The pericycle, like the endodermis, is continuous in all vegetative organs that have a primary structure. The pericycle in the leaves of the species that have been studied here has a procambial origin, and this origin is located inner to the endodermis. There is a consensus that the pericycle is present in roots; however, there are a few reports that suggest it is also present in stems and leaves (Altamura et al. 1998, Menezes et al. 2005, Leite et al. 2009). However, in accordance with the results presented here and those of other authors (Van Fleet 1961, Menezes et al. 2005), the endodermis and the pericycle are continuous in the vegetative organs.

The occurrence of suberin in the cell walls of the leaf vascular bundle sheath (mestome) in several monocotyledons was linked to Casparian strips that were present in the endodermis cells of the roots and subterranean stem, and therefore, were treated as endodermis (Menezes et al. 2005), endodermoid sheath (Brown 1975) and sheath like endodermis (Vecchia et al. 1999). However, in Cyperaceae and Poaceae species, it was demonstrated that this bundle sheath called mestome (Dengler et al. 1985, Soros and Dengler 2001) or outer pericycle (Martins and Scatena 2011), originates from the procambium, so, it cannot be named endodermis because it originates from the ground meristem.

The leaves and scapes of the species in this study differ structurally because of the presence of Kranz anatomy in *Fimbristylis dichotoma* and the absence of this kind of anatomy in *Cyperus laxus*. In Cyperaceae, this difference is caused by the arrangement of mesophyll cells around the vascular bundles and by the occurrence of chloroplasts in the inner bundle sheath cells (Soros and Dengler 2001). In *Fimbristylis dichotoma*, as well as in other Cyperaceae, Kranz anatomy also occurs in the scape (Estelita-Teixeira and Handro 1987, Rodrigues and Estelita 2003), which imparts to the plant a greater photosynthetic efficiency.

## Resumo

Cyperaceae ocorre em diferentes ecossistemas, constituindo o extrato herbáceo. Das cerca de 5500 espécies da família, um terço apresenta anatomia Kranz que constitui uma importante característica da taxonomia e filogenia do grupo. Em *Cyperus laxus* L. (não Kranz) e *Fimbristylis dichotoma* Vahl (Kranz), o desenvolvimento se inicia com a germinação que é marcada pela emergência do coleóptilo, seguido da raiz primária (efêmera). O rizoma se origina do mesocótilo e promove a conexão vascular entre raízes, folhas e escapos. A continuidade dos tecidos é marcada pela presença de endoderme e periciclo em todos os órgãos vegetativos. As folhas e escapos diferem entre as duas espécies estudadas no arranjo das células do mesofilo e no número de bainhas vasculares. As células do mesofilo se dispõem de forma regular em *Cyperus laxus* (não Kranz) e radialmente em *Fimbristylis dichotoma* (Kranz). *Cyperus laxus* apresenta duas bainhas vasculares e *Fimbristylis dichotoma* três. A bainha externa em ambas as espécies constitui a endoderme, e a bainha interna em *Cyperus laxus* e a mediana e a interna em *Fimbristylis dichotoma* constituem o periciclo.

## Acknowledgments

We thank the anonymous reviewers for their valuable comments and suggestions, the Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP for a PhD grant (2008/09380-2) to S. Martins, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financial support (301692/2010-6) to V. L. Scatena.

## References

ALONSO AA AND MORAES-DALLAQUA MA. 2004. Morfoanatomia do sistema caulinar de *Canna edulis* Kerr-Gawler (Cannaceae). Rev Bras Bot 29: 229-239.

- ALTAMURA MM, ZAGHI D, SALVI G, DE LORENZO G AND BELLINCAMPI D. 1998. Oligogalacturonides stimulate pericycle cell wall thickening and cell divisions leading to stoma formation in tobacco leaf explants. *Planta* 204: 429-436.
- ALVES M, ARAÚJO AC, PRATA AP, VITTA FA, HEFLER SM, TREVISAN R, GIL AB, MARTINS S AND THOMAS WW. 2009. Diversity of Cyperaceae in Brazil. *Rodriguésia* 60: 771-782.
- BENDIXEN LE. 1973. Anatomy and sprouting of yellow nutsedge tubers. *Weed Sci* 21: 501-503.
- BROWN WV. 1975. Variations in anatomy, associations, and origins of Kranz tissue. *Am J Bot* 62: 395-402.
- BRUHL JJ AND WILSON KL. 2007. Towards a comprehensive survey of C<sub>3</sub> and C<sub>4</sub> photosynthetic pathway in Cyperaceae. In: COLUMBUS JT, FRIAR EA, HAMILTON CW, PORTER JM, PRINCE LM AND SIMPSON MG (Eds), *Monocots: comparative biology and evolution*, Claremont: Rancho Santa Ana Botanic Garden, p. 99-148.
- BRYSON CT AND CARTER R. 2008. The significance of Cyperaceae as weeds. In: NACZI RF AND FORD BA (Eds), *Sedges, uses, diversity, and systematic of the Cyperaceae*, Missouri: *Monogr Syst Bot Mo Bot Gard* 108, p. 15-101.
- CATTAI M AND MENEZES NL. 2010. Primary and secondary thickening in the stem of *Cordyline fruticosa* (Agavaceae). *An Acad Bras Cienc* 82: 653-662.
- DENGLER NG, DENGLER RE AND HATTERSLEY PW. 1985. Differing ontogenetic origins of PCR (“Kranz”) sheaths in leaf blades of C<sub>4</sub> grasses (Poaceae). *Am J Bot* 72: 284–302.
- DICKISON WG AND WEITZMAN AL. 1996. Comparative anatomy of the young stem, node, and leaf of the Bonnetiaceae, including observations on a foliar endodermis. *Am J Bot* 83: 405-418.
- DUBROVSKY JG, NAPSUCIALY-MENDIVIL S, DUCLERCQ J, CHENG Y, SHISHKPVA S, IVANCHENKO MG, FRIML J, MURPHY AS AND BENKOVÁ E. 2011. Auxin minimum defines a developmental window for lateral root initiation. *New Phytol* 191: 970-983.

- EITEN LT. 1969. The vegetative anatomy of *Eleocharis interstincta* (Vahl) Roem. and Schult. Arqs Bot Est S Paulo 4: 187-228.
- ESAU K. 1965. Plant anatomy, New York: John Wiley and Sons.
- ESTELITA-TEIXEIRA ME AND HANDRO W. 1987. Kranz pattern in leaf, scape and bract of *Cyperus* and *Fimbristylis* species. Rev Bras Bot 10: 105-111.
- FEDER N AND O'BRIEN TP. 1968. Plant microtechnique: some principles and new methods. Am J Bot 55: 123-142.
- GIFFORD EM AND BAYER DE. 1995. Developmental anatomy of *Cyperus esculentus* (yellow nutsedge). I J Plant Sci 156: 622-629.
- GOETGHEBEUR P. 1998. Cyperaceae. In: KUBITZKI K, HUBER H, RUDALL PJ, STEVENS PS AND STÜTZEL T (Eds), The families and genera of vascular plants, Berlin: Springer-Verlag, p. 141-190.
- GOVAERTS R, SIMPSON DA, GOETGHEBEUR P, WILSON KL, EGOROVA T AND J BRUHL. 2007. World checklist of Cyperaceae, Kew: The Board of Trustees of the Royal Botanical Garden.
- GOVINDARAJALU E. 1974. The systematic anatomy of south Indian Cyperaceae: *Cyperus* L. subgen. *Juncellus*, *Cyperus* subgen. *Mariscus* and *Lipocarpha* R. Br. Bot J Linn Soc 68: 235-266.
- HEFLER SM AND LONGHI-WAGNER HM. 2010. A contribuição da anatomia foliar para taxonomia das espécies de *Cyperus* L. subg. *Cyperus* (Cyperaceae) ocorrentes no sul do Brasil. Acta Bot Bras 24: 708-717.
- JOHANSEN D. 1940. Plant microtechnique, New York: McGraw-Hill Book Co. Inc.
- LEITE KRB, FRANÇA F AND SCATENA VL. 2009. Anatomia de espécies anfíbias de Cyperaceae de lagoas do semi-árido, BA, Brasil. Acta Bot Bras 23: 786-796.

- LERSTEN NR. 1997. Occurrence of endodermis with a Casparian strip in stem and leaf. *Bot Rev* 63: 265-272.
- LIMA VFGAP AND MENEZES NL. 2009. Morpho-anatomical analysis of the rhizome in species of *Scleria* Berg. (Cyperaceae) from Serra do Cipó (MG). *Braz Arch Biol Technol* 52: 1473-1483.
- MARTINS S AND SCATENA VL. 2011. Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales). *Aust J Bot.* 59: 554-562.
- MENEZES NL, SILVA DC, ARRUDA RCO, MELO-DE-PINNA GF, CARDOSO VA, CASTRO NM, SCATENA VL AND SCREMIN-DIAS E. 2005. Meristematic activity of the endodermis and the pericycle in the primary thickening in monocotyledons. Considerations on the “PTM”. *An Acad Bras Cienc* 77: 259-274.
- METCALFE CR 1971 *Anatomy of the monocotyledons: Cyperaceae*, Oxford: Clarendon Press.
- NACZI RFC. 2009. Insight on using morphologic data for phylogenetic analysis in sedges (Cyperaceae). *Bot Rev* 75: 67-95.
- PRIESTLEY JH AND RADCLIFFE FM. 1924. A study of the endodermis in the Filiniae. *New Phytol* 23: 161-193.
- PROENÇA SL AND SAJO MG. 2008. Rhizome and root anatomy of 14 species of Bromeliaceae. *Rodriguésia* 59: 113-128.
- RODRIGUES AC AND ESTELITA MEM. 2002. Primary and secondary development of *Cyperus giganteus* Vahl rhizome (Cyperaceae). *Rev Bras Bot* 25: 251-258.
- RODRIGUES AC AND ESTELITA MEM. 2003. Origin and structure of the Kranz tissue in bracts of *Cyperus giganteus* Vahl (Cyperaceae). *Rev Bras Bot* 26: 445-452.
- RODRIGUES AC AND ESTELITA MEM. 2004. Anatomia da raiz de *Cyperus giganteus* Vahl (Cyperaceae) em desenvolvimento. *Rev Bras Bot* 27: 629-638.

- ROESER KR. 1972. Die nadel der schwarzkiefer - massenprodukt und kunstwerk der natur. Mikrokosmos 61: 33-36.
- SAGE RF. 2004. The evolution of C<sub>4</sub> photosynthesis. New Phytol 161: 341-370.
- SEAGO JL, MARSH LC, STEVENS KJ, SOUKUP A, VOTRUBOVÁ O AND ENSTONE DE. 2005. A re-examination of the root cortex in wetland flowering plants with respect to aerenchyma. Ann Bot 96: 565-579.
- SIMÃO DG AND SCATENA VL. 2001. Morphology and anatomy in *Heliconia angusta* Vell. and *H. velloziana* L. Emygd. (Zingiberales: Heliconiaceae) from the Atlantic forest of southeastern Brazil. Rev Bras Bot 24: 415-424.
- SORIN C, BUSSELL JD, CAMUS I, LJUNG K, KOWALCZYK M, GEISS G, MCKHANN H, GARCION G, VAUCHERET H, SANDBERG G AND BELLINI C. 2005. Auxin and light control of adventitious rooting in Arabidopsis require ARGONAUTE1. Plant Cell 17: 1343-1359.
- SOROS CL AND DENGLER NG 1996 Leaf morphogenesis and growth in *Cyperus eragrostis* (Cyperaceae). Can J Bot 74: 1753-1765.
- SOROS CL AND DENGLER NG. 2001. Ontogenetic derivation and cell differentiation in photosynthetic tissues of C<sub>3</sub> and C<sub>4</sub> Cyperaceae. Am J Bot 88: 992-1005.
- TILLICH HJ. 2007. Seedling diversity and the homologies of seedling organs in the order Poales (Monocotyledons). Ann Bot 100: 1-17.
- TOMLINSON PB. 1969. Comelinales – Zingiberales. In: Metcalfe CR (Ed), Anatomy of the monocotyledons. Vol. 3, London: Oxford University Press, p. 193-294.
- VAN FLEET DS. 1942. The development and distribution of the endodermis and an associated oxidase system in Monocotyledonous plants. Am J Bot 29: 1-15.
- VAN FLEET DS. 1961. Histochemistry and function of the endodermis. Bot Rev 27: 165-220.

- VECCHIA FD, CUCCATO F, LA ROCCA N, LARCHER W AND RASCIO N. 1999. Endodermis-like sheaths in the submerged freshwater macrophyte *Ranunculus trichophyllus* Chaix. *Ann Bot* 83: 93-97.
- WILLIAMS BC. 1947. The structure of the meristematic root tip and origin of the primary tissues in the roots of vascular plants. *Am J Bot* 34: 455-462.
- WILLS GD AND BRISCOE GA. 1970. Anatomy of purple nutsedge. *Weed Sci* 18: 631-635.
- WILLS GD, HOAGLAND RE AND PAUL RN. 1980. Anatomy of yellow nutsedge (*Cyperus esculentus*). *Weed Sci* 28: 432-437.



Fig. 1 – Morphological and anatomical aspects of Cyperaceae species: *Cyperus laxus* L. and *Fimbristylis dichotoma* Vahl. A. *Cyperus laxus*, habit; B-C. *Fimbristylis dichotoma*; B. Habit; C. Achene; D. *Cyperus laxus*, general view of the embryo in paradermic section; E-H. Morphology and anatomy of the post-seminal stages of development in *Cyperus laxus* (E, G, H) and *Fimbristylis dichotoma* (F); I-K. *Cyperus laxus*; I. General view of the mesocotyl; J. General view of young rhizome showing the radial cells originating from the precursor cells of the endodermis; K. Detail of the young rhizome showing Casparian strips in the endodermal cells (arrows). ar = adventitious root; co = coleptile; en = endodermis; eo = eophyll; pr = primary root; ra = radicle. Bars = 2 cm (A, B); 1 cm (C, E, G); 100  $\mu$ m (F, H, I, J); 50  $\mu$ m (D, K).

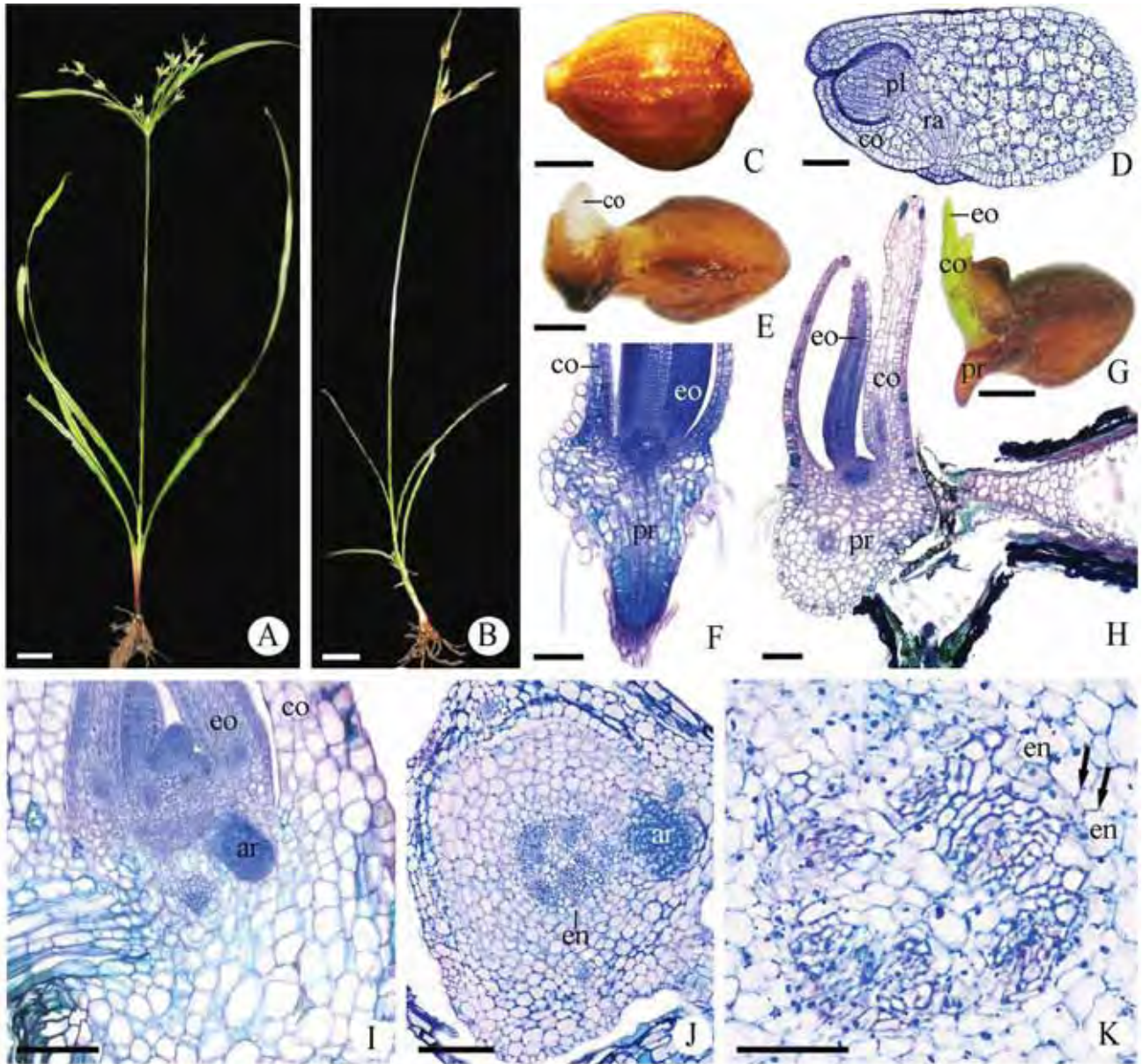


Fig. 2 – Anatomical aspects of transverse and longitudinal sections of rhizome and roots of Cyperaceae species: *Cyperus laxus* L. and *Fimbristylis dichotoma* Vahl. A. *Fimbristylis dichotoma*, longitudinal section of the mature rhizome showing root, leaf primordia and scape in protrusion; B. *Cyperus laxus*, transverse section of the mature rhizome; C-E. *Fimbristylis dichotoma*, mature rhizome; C. Longitudinal section showing leaf primordia; D. Transverse section showing roots protrusion; E. Detail of roots protrusion; F. *Cyperus laxus*, general view of the young root showing the separation of the cell walls (arrowheads); G-H. *Fimbristylis dichotoma*, roots; G. Detail of the root showing Casparian strips in the endodermal cells (arrows); H. General view of the mature root showing lyse of the cell walls (arrows) and endodermis and pericycle with thick-walled cells. en = endodermis; lf = leaf; pe = pericycle; rt = root; sc = scape. Bars = 200  $\mu\text{m}$  (A, B); 100  $\mu\text{m}$  (C, D); 50  $\mu\text{m}$  (E, F, H); 20  $\mu\text{m}$  (G).



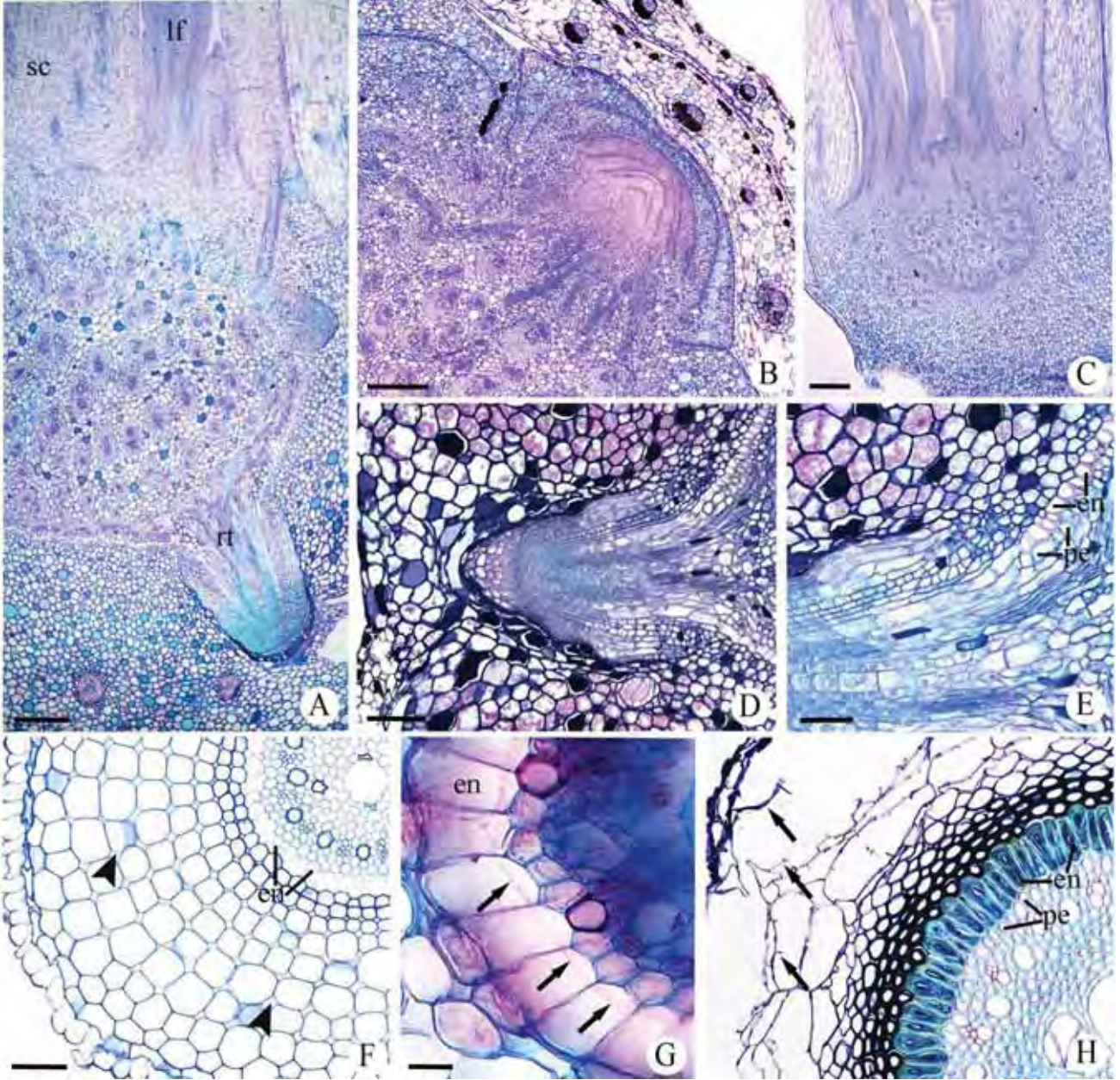
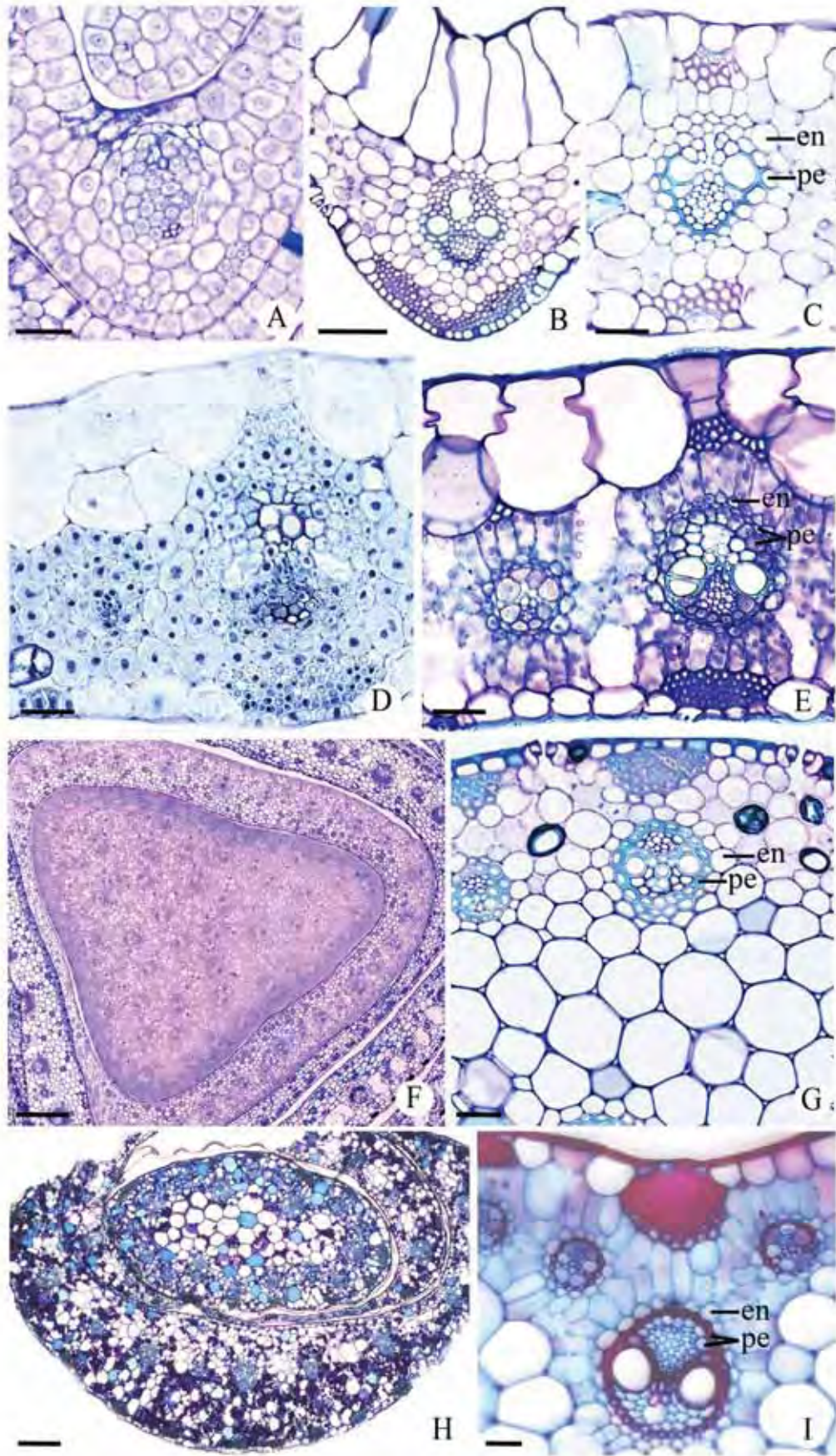


Fig. 3 – Anatomical aspects of transverse sections of leaves and scapes of Cyperaceae species: *Cyperus laxus* L. and *Fimbristylis dichotoma* Vahl. A-C. *Cyperus laxus*. A. Detail of the young leaf in the midrib region; B-C. Detail of the mature leaf; B. Midrib; C. Mesophyll; D-E. *Fimbristylis dichotoma*; D. Young leaf; E. Mature leaf; F-G. *Cyperus laxus*; F. General view of the young scape; G. Detail of the mature scape; H-I. *Fimbristylis dichotoma*; H. General view of the young scape; I. Detail of the mature scape. en = endodermis; pe = pericycle. Bars: 100  $\mu\text{m}$  (F); 50  $\mu\text{m}$  (A, B, C, H); 20  $\mu\text{m}$  (D, E, G, I).





## **CAPÍTULO 2**

**Shirley Martins, Vanesa Pilatti, Abelardo Vegetti, Vera Lucia Scatena**

**Do leaves in Cyperoideae (Cyperaceae) have a multiple epidermis or a  
hypodermis?**

(Aceito para publicação no periódico *Flora* em fevereiro/2012)

## Abstract

In Cyperaceae, leaf anatomical characters, in particular the presence of a hypodermis or of a multiple epidermis, have contributed in taxonomic and phylogenetic studies. In this family, the leaf epidermis is often described as uniseriate, and the cells of the subepidermal layers having no chloroplasts are treated as hypodermis. Both tissues have a different ontogenetic origin and hence are not homologous. The aim of the present work was to verify the origin of the subepidermal layers in eight species belonging to Cyperoideae. All species studied presented multiple epidermal that were confirmed by leaf ontogeny. In *Fimbristylis complanata*, *F. dichotoma*, *Pycneus flavescens* and *P. polystachyos* the mature leaf present multiple epidermal with cells of the distinct layers similar in shape and size; in the other species studied these cells are different. Especially in the latter case, a multiple epidermis is easily interpreted erroneously as a hypodermis, possibly leading to erroneous evolutionary conclusions. Making correctly distinction between hypodermis and multiple epidermis, and hence in case of doubt investigating the origin of the questioned tissue is compulsory in order to use both characters in a phylogenetic context. Though in the past often called 'hypodermis', our leaf ontogenetical observations show that in all species studied, the subepidermal layers constitute a multiple epidermis, originating from the protodermis.

**Keywords:** ontogeny, leaf anatomy, hypodermis, multiple epidermis, Cyperaceae

## Introduction

The family Cyperaceae has 109 genera and approximately 5500 species (Govaerts et al., 2007) and Cyperoideae is the larger of the two subfamilies of Cyperaceae. Anatomical characters are often employed in taxonomic (Holm, 1899; Plowman, 1906; Kukkonen, 1967; Metcalfe, 1971; Standley, 1990; Alves et al., 2002) and phylogenetical (Bruhl, 1995; Starr et al., 2004) studies of the family and among the leaf anatomical characteristics, those related to epidermis and hypodermis are of particular interest (Koyama, 1967; Shepherd, 1976; Goetghebeur, 1998).



The leaf epidermis is generally described as uniseriate in Cyperaceae (Metcalf, 1971; Denton, 1983; Bruhl, 1995), and the principal epidermal characters used for taxonomy are the cell shape and the presence of trichomes, papillae and silica bodies (Mehra and Sharma, 1965; Metcalf, 1969; Starr et al., 2004; Honaine et al., 2009). In the family, the subepidermal layers with cells having no chloroplasts are treated as hypodermis and present characters with taxonomic potential involving the cell shape, cell wall thickening as well as the number and continuity of the layers (Metcalf, 1971; Govindarajalu, 1974; Bruhl, 1995).

In a phylogenetic study, Bruhl (1995) used the term hypodermis for the subepidermal layers but commented that, in some groups, these layers can constitute a multiple epidermis. Sharma and Mehra (1972) in their taxonomic study of *Fimbristylis* (Cyperaceae), reported similarity between the epidermal and hypodermal layers of the leaf. These authors then indicated the presence of a multiple epidermis in *Fimbristylis* species but did not verify its origin. Correct usage of the terms hypodermis and multiple epidermis in Cyperaceae requires knowing the origin of these tissues, which are not homologous. The multiple epidermis originates from periclinal cell division of the protodermis different from the hypodermis that derives from the ground meristem (Esau, 1965).

The use of homologous structures is fundamental in phylogenetical approach and the ontogenetical studies have been important to solve problems of homology in vegetative (Martins and Scatena, 2011) and reproductive organs (Vrijdaghs et al., 2009, 2010) of Cyperaceae species.

The aim of the study was to investigate the ontogeny of the subepidermal layers of leaves in species from different tribes and genera of Cyperoideae to verify their origin.

## Materials and methods

*Cyperus ligularis* L., *Fimbristylis complanata* (Retz.) Link, *F. dichotoma* (L.) Vahl, *Pycneus flavescens* (L.) Rchb., *P. niger* (Ruiz & Pav.) Cufod., *P. polystachyos* (Rottb.) P. Beauv., *Rhynchospora globosa* Lindl. and *R. terminalis* Kunth were studied (Table 1). Species were chosen

based on the degree of similarity of the cells that constitute the subepidermal layers. Voucher materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB), and the Herbarium of the Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (SF).

For the ontogenetic study, young rhizomes with vegetative apex were collected, fixed in FAA 50 (Johansen, 1940) and stored in 70% ethanol. Portions of the rhizome apices were dehydrated in an ethyl alcohol series and then embedded in Historesin (Leica Historesin Embedding Kit, Nussloch, Germany) (Feder and O'Brien, 1968). Longitudinal and transverse sections of young leaves located in a rhizome apex were made using a microtome, stained with periodic acid-Schiff's reagent and toluidine blue (Feder and O'Brien, 1968) and mounted in Entellan (Merck). Transverse sections of the median region of mature leaves were made, stained with basic fuchsin and Astra blue (Roeser, 1972) and mounted in glycerin jelly.

## Results

The mature leaves of the species studied possess a multiple epidermis only on the adaxial surface (Fig. 1A-L – asterisk) with cells of similar shapes and sizes in *Fimbristylis complanata* (Fig. 1A), *F. dichotoma* (Fig. 1B), *Pycreus flavescens* (Fig. 1C-D) and *P. polystachyos* (Fig. 1E-F) and different shapes and sizes in *Cyperus ligularis* (Fig. 1G-H), *Pycreus niger* (Fig. 1I-J), *Rhynchospora globosa* (Fig. 1K) and *R. terminalis* (Fig. 1L). These differences in the epidermis are outlined in a diagrammatic representation (Fig. 3A-D). The number of cell epidermal layers in the multiple epidermis varies among the species studied and also intraspecifically (Table 1 and Fig. 1A-L).

In young leaves of the species studied, at the stage in which the tissues are undifferentiated, the outmost layer (protodermis) is uniseriate on both surfaces (Fig. 2A). After the tissues begin differentiation, the protodermal cells divide periclinally giving rise to their derivatives, as in *Fimbristylis dichotoma* (Fig. 2B-C, arrows), *Pycreus flavescens* (Fig. 2E-F, arrows), *Cyperus*

*ligularis* (Fig. 2H-I, arrows) and *Rhynchospora globosa* (Fig. 2K-L, arrows). In the derivative cells of the protodermis, anticlinal division can also occur (Fig. 2I, M, arrowheads), but this type of division is less frequent than periclinal division.

In the species that possess a multiple epidermis constituted by similar cells (Table 1), generally one (Fig. 2C) or two (Fig. 2F) periclinal divisions occur that result in two to three cell epidermal layers (Fig. 2D, G – asterisk). During the stage in which the tissues are totally differentiated, all the cells of the multiple epidermis increase in size (Fig. 2D, G and Fig. 3B). In the species that have a multiple epidermis constituted by different cells (Table 1), in general, more than two periclinal divisions occur (Fig. 2I, L-M, arrows), resulting in several cell epidermal layers (Fig. 2J, M – asterisk), in addition to the anticlinal divisions (Fig. 2I, M, arrowheads). In these species, at the stage in which the tissues are already differentiated, the epidermal cells increase in size, but not evenly, and the cells of the outmost epidermal layer become larger than the cells of the innermost layers (Fig. 3C), as seen in *R. terminalis* (Fig. 1L), or smaller (Fig. 3D), as seen in *Cyperus ligularis* (Fig. 1G-H, 2J), *Pycreus niger* (Fig. 1I-J) and *Rhynchospora globosa* (Fig. 1K, 2M).

## Discussion

The presence of a multiple epidermis in angiosperms in general is characterized by periclinal divisions of the outermost layer of the meristem (protodermis) (Esau, 1965; Beck, 2005), as observed in the Cyperaceae species studied in the present work and in other angiosperms taxa of the families Apocynaceae (*Nerium*) (Beck, 2005), Moraceae (*Ficus*) (Beardsell and Norden, 2004) and Piperaceae (*Peperomia*) (Kaul, 1977). To the monocotyledons it is the first indication of leaves with multiple epidermis, detaching the importance of this character to Cyperaceae in future taxonomical and phylogenetical studies, both inter and intra-family.

The same shape and parallel position of the cell walls of the outmost epidermal layer with those of subepidermal cells (Fig. 3B) led Sharma and Mehra (1972) to indicate a multiple epidermis

in *Fimbristylis*. A similar situation was observed in *Fimbristylis complanata*, *F. dichotoma*, *Pycneus flavescens* and *P. polystachyos* and it is ontogenetically demonstrated that these species have a multiple epidermis. However, in *Cyperus ligularis*, *Pycneus niger*, *Rhynchospora globosa* and *R. terminalis*, the cells of the outmost epidermal layer differ in shape and size from the cells of the other epidermal layers located internally, and their cell walls are not in a parallel position in the mature leaf (Fig. 3C-D). This non-aligned arrangement of the cells, when observed in the mature leaf, complicates the interpretation of a multiple epidermis. Such an arrangement of cells probably led many authors to call the subepidermal layers hypodermis (Metcalf, 1971; Govindarajalu, 1974, 1982; Li and Jones, 1994; Martins et al., 2008), while, in fact, they are part of a multiple epidermis, as demonstrated here.

The multiple epidermis in each of the species studied vary in the shape of the cells and in number of the layers, and can constitute useful characters for taxonomic and phylogenetic investigations in Cyperaceae. A similar set of characters was used referring to the so-called hypodermis in the taxonomic studies in Cyperaceae (Govindarajalu, 1974; Bruhl, 1995; Alves et al., 2002; Hefler and Longhi-Wagner, 2010).

The species studied here are included in the subfamily Cyperoideae and belong to three distinct tribes: Abildgaardieae (*Fimbristylis complanata* and *F. dichotoma*); Cypereae (*Cyperus ligularis*, *Pycneus flavescens*, *P. niger* and *P. polystachyos*) and Rhynchosporeae (*Rhynchospora globosa* and *R. terminalis*) sensu Muasya et al. (2009). Consequently, the character of the multiple epidermis occurs in different taxonomic categories of Cyperaceae and probably can be found in other genera of the family.

In the giant genus *Cyperus*, the subepidermal layers of several species of *Cyperus* subg. *Cyperus* have been described and termed hypodermis (Metcalf, 1971; Hefler and Longhi-Wagner, 2010). *Cyperus ligularis* studied here is included in *Cyperus* subg. *Cyperus* (Goetghebeur, 1998), but previously, this species belonged to *Cyperus* subg. *Mariscus* according to Kukenthal (1935, 1936).

Many species that belonged to this old taxonomic group have leaves with subepidermal layers on the adaxial surface (Metcalf, 1971; Hefler and Longhi-Wagner, 2010) with similar characteristics to those of *Cyperus ligularis*, and probably are homologous with a multiple epidermis. We hypothesize that also in other species with subepidermal layers of the genus, treated as ‘hypodermis’, these are a multiple epidermis.

In *Pycneus*, all species that have been anatomically described possess leaves with subepidermal layers that have also been termed hypodermis (Metcalf, 1971; Bruhl, 1995; Leite and Scatena, 2009) rather than multiple epidermis, as presented here for *Pycneus flavescens*, *P. niger* and *P. polystachyos*. Most likely, the same character occurs in other species of *Pycneus* and is common to the genus.

*Rhynchospora globosa* and *R. terminalis*, which present a multiple epidermis, belong to the subgenus *Haplostylis* section *Pluriflorae* (Thomas et al., 2009). The other 11 species from this section also have subepidermal layers (A.C. Araújo, unpublished data) that must constitute a multiple epidermis. In *Rhynchospora fascicularis* var. *distans*, which belongs to the subgenus *Rhynchospora* section *Rhynchospora*, Metcalf (1971) referred to the subepidermal layers as a hypodermis but stated that the boundary between the adaxial epidermis and the hypodermis is not very clear. The character ‘multiple epidermis’ may have appeared more than once in *Rhynchospora* because it occurs in species from distinct subgenera (Thomas et al., 2009).

## Conclusion

In all studied species, the subepidermal cell layers having no chloroplasts originate from the protodermis and hence are homologous with a multiple epidermis and not with a hypodermis. Treating the hypodermis and multiple epidermis as similar tissues is erroneous because these tissues have different origins and do hence do not constitute homologous structures. Therefore, leaf

ontogeny is necessary in all species with a putative multiple epidermis or putative hypodermis, in order to distinguish both characters and their respective character states and sub-characters.

### 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 the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financial support (301692/2010-6) to V. L. Scatena.

### References

- Alves, M., Estelita, M.E.M., Wanderley, M.G., Thomas, W.W., 2002. Aplicações taxonômicas da anatomia foliar das espécies brasileiras de *Hypolytrum* Rich. (Cyperaceae). Rev. Bras. Bot. 25, 1-9.
- Beardsell, D., Norden, U., 2004. *Ficus rubiginosa* ‘Variegata’, a chlorophyll-deficient chimera with mosaic patterns created by cell divisions from the outer meristematic layer. Ann. Bot. 94, 51-58.
- Beck, C.B., 2005. Plant structure and development. University press, Cambridge.
- Bruhl, J.J., 1995. Sedge genera of the world: relationships and new classification of the Cyperaceae. Australian Syst. Bot. 8, 125-305.
- Denton, M.F., 1983. Anatomical studies of the Luzulae Group of *Cyperus* (Cyperaceae). Syst. Bot. 8, 250-262.
- Esau, K., 1965. Plant anatomy. John Wiley and Sons, New York.
- Feder, N., O’Brien, T.P., 1968. Plant microtechnique: some principles and new methods. Am. J. Bot. 55, 123-142.
- Goetghebeur, P., 1998. Cyperaceae. In: Kubitzki, K., Huber, H., Rudall, P.J., Stevens, P.S., Stützel, T., (Eds.), The families and genera of vascular plants. Springer-Verlag, Berlin, pp. 141-190.

- Govaerts, R., Simpson, D.A., Goetghebeur, P., Wilson, K.L., Egorova, T., Bruhl, J., 2007. World checklist of Cyperaceae. The Board of Trustees of the Royal Botanical Garden, Kew.
- Govindarajalu, E., 1974. The systematic anatomy of south Indian Cyperaceae: *Cyperus* L. subgen. *Juncellus*, *Cyperus* subgen. *Mariscus* and *Lipocarpha* R.Br. Bot. J. Linn. Soc. 68, 235-266.
- Govindarajalu, E., 1982. Studies in Cyperaceae. XVII. Novelties in *Fimbristylis* Vahl and their vegetative anatomy. Proc. Ind. Acad. Sci. 91, 43-53.
- Hefler, S.M., Longhi-Wagner, H.M., 2010. A contribuição da anatomia foliar para taxonomia das espécies de *Cyperus* subg. *Cyperus* (Cyperaceae) ocorrentes no sul do Brasil. Acta Bot. Bras. 24, 708-717.
- Holm, T., 1899. Studies in Cyperaceae. VIII. On the anatomy of some North American species of *Scleria*. Am. J. Sci. 7, 5-12.
- Honaine, M.F., Zucol, A.F., Osterrieth, M.L., 2009. Phytolith analysis of Cyperaceae from the Pampean region Argentina. Aust. J. Bot. 57, 512-523.
- Johansen, D., 1940. Plant microtechnique. McGraw-Hill Book Co. Inc., New York.
- Kaul, R.B., 1977. The role of the multiple epidermis in foliar succulence of *Peperomia* (Piperaceae). Bot. Gaz. 138, 213–218.
- Koyama, T., 1967. The systematic significance of the leaf structure in the tribe Sclerieae (Cyperaceae). Mem. N. Y Bot. Gard. 16, 46-70.
- Kükenthal, G., 1935. Cyperaceae – Scirpodoideae – Cypereae. In: Dengler, A., Diels, L., (Eds.), Das Pflanzenreich IV-20. Verlag von Wilhelm Engelmann, Leipzig, pp. 1-160.
- Kükenthal, G., 1936. Cyperaceae – Scirpodoideae – Cypereae. In: Dengler, A., Diels, L., (Eds.), Das Pflanzenreich IV-20. Verlag von Wilhelm Engelmann, Leipzig, pp. 161-671.
- Kukkonen, I., 1967. Vegetative anatomy of *Uncinia*. Ann. Bot. 31, 523-544.
- Leite, K.R.B., Scatena, V.L., 2009. Anatomia de espécies anfíbias de Cyperaceae de lagoas do semi-árido, BA, Brasil. Acta Bot. Bras. 23: 786-796.

- Li, M., Jones, M., 1994. Kranzkette, a unique C<sub>4</sub> anatomy occurring in *Cyperus japonicus* leaves. *Photosynthetica* 30, 117-131.
- Martins, S., Scatena, V.L., 2011. Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales). *Aust. J. Bot.* 59, 554-562.
- Martins, S., Machado, S.R., Alves, M., 2008. Anatomia e ultra-estrutura de *Cyperus maritimus* Poir. (Cyperaceae): estratégias adaptativas ao ambiente de dunas litorâneas. *Acta Bot. Bras.* 22, 289-299.
- Mehra, P.N., Sharma, O.P., 1965. Epidermal silica in the Cyperaceae. *Bot. Gaz.* 126, 53-58.
- Metcalf, C.R., 1969. Anatomy as an aid to classifying the Cyperaceae. *Am. J. Bot.* 56, 782-790
- Metcalf, C.R., 1971. Anatomy of the monocotyledons: Cyperaceae. Clarendon Press, Oxford.
- Muasya, A.M., Simpson, D.A., Verboom, G.A., Goetghebeur, P., Naczi, R.F.C., Chase, M.W., Smets, E., 2009. Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects. *Bot. Rev.* 75, 2-21.
- Plowman, A., 1906. The comparative anatomy and phylogeny of the Cyperaceae. *Ann. Bot.* 20, 1-33.
- Roeser, K.R., 1972. Die nadel der schwarzkiefer - massenprodukt und kunstwerk der nautr. *Mikrokosmos* 61, 33-36.
- Sharma, O.P., Mehra, P.N., 1972. Systematic anatomy of *Fimbristylis* Vahl (Cyperaceae). *Bot. Gaz.* 133, 87-95.
- Sherferd, G., 1976. The use of anatomical characters in the infrageneric classification of *Carex* (Cyperaceae). *Hoehnea* 6, 33-54.
- Standley, L.A., 1990. Anatomical aspects of the taxonomy of sedges (*Carex*, Cyperaceae). *Can. J. Bot.* 68, 1449-1456.
- Starr, J.R., Harris, S.A., Simpson, D.A., 2004. Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae I: Generic relationships and evolutionary scenarios. *Syst. Bot.* 29, 528-544.



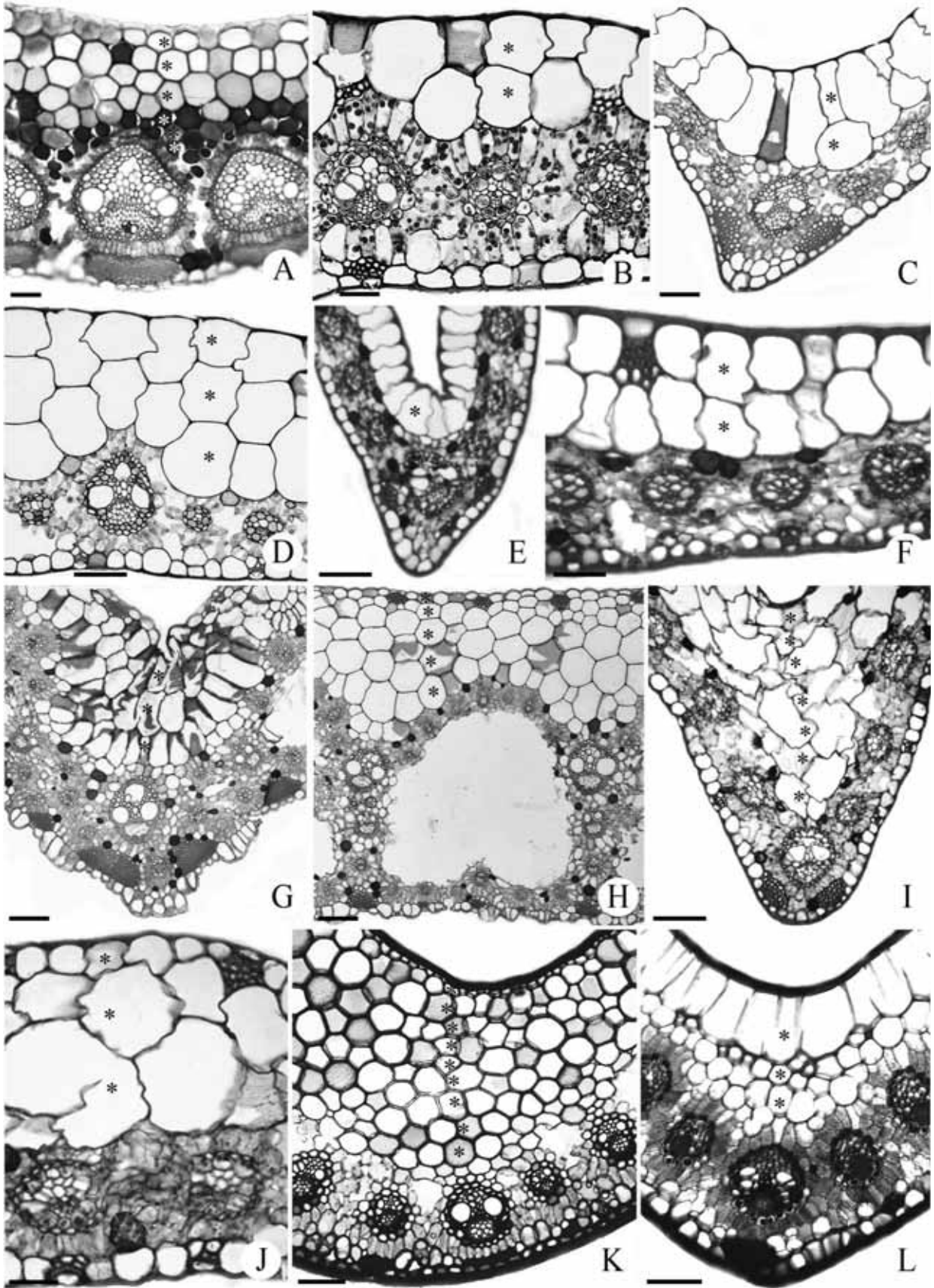
- Thomas, W.W., Araújo, A.C., Alves, M.V., 2009. A preliminary molecular phylogeny of the Rhynchosporeae (Cyperaceae). *Bot. Rev.* 75, 22-29.
- Vrijdaghs, A., Muasya, A.M.M., Goetghebeur, P., Caris, P., Nagels, A., Smets, E., 2009. A floral ontogenetic approach to homology question within the Cyperoideae (Cyperaceae). *Bot. Rev.* 75, 30-51.
- Vrijdaghs, A., Reynders, M., Larridon, I., Muasya, A.M., Smets, E., Goetghebeur, P., 2010. Spikelet structure and development in Cyperoideae (Cyperaceae): monopodial genera model based on ontogenetic evidence. *Ann. Bot.* 105, 555-571.

**Table 1**

Species of Cyperoideae studied and characteristics of the epidermis.

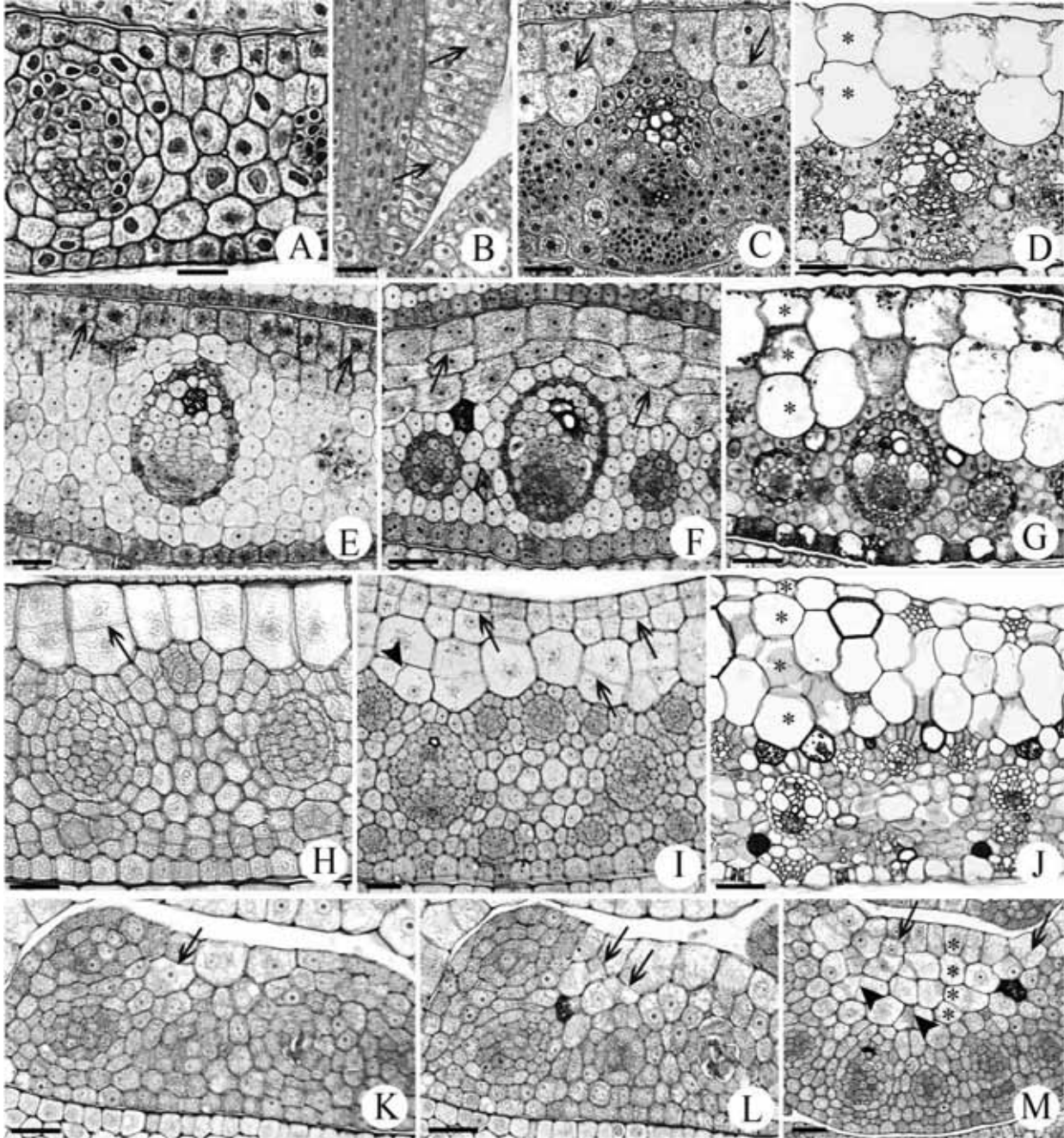
Species	Voucher	Cell size among the epidermal layers	Number of the epidermal layers
<b>Abildgaardieae</b>			
<i>Fimbristylis complanata</i>	V.L. Scatena 344	similar	3-6
<i>F. dichotoma</i>	S. Martins 398	similar	1-2
<b>Cypereae</b>			
<i>Cyperus ligularis</i>	S. Martins 330	different	3-8
<i>Pycreus flavescens</i>	S. Martins 327	similar	1-3
<i>P. niger</i>	AGR 16	different	3-7
<i>P. polystachyos</i>	N. Guarise 24	similar	1-3
<b>Rhynchosporae</b>			
<i>Rhynchospora globosa</i>	S. Martins 305	different	6-10
<i>R. terminalis</i>	S. Martins 302	different	2-5

**Fig. 1.** Anatomical aspects of mature leaves of Cyperaceae in transverse section. Note adaxial multiple epidermis (asterisk). (A) *Fimbristylis complanata*, (B) *Fimbristylis dichotoma*, (C-D) *Pycneus flavescens*, (E-F) *Pycneus polystachyos* (G-H) *Cyperus ligularis*, (I-J) *Pycneus niger*, (K) *Rhynchospora globosa*, (L) *Rhynchospora terminalis*. Bars = 30  $\mu\text{m}$  (A, J); 50  $\mu\text{m}$  (B-F, I, L); 100  $\mu\text{m}$  (G-H, K).

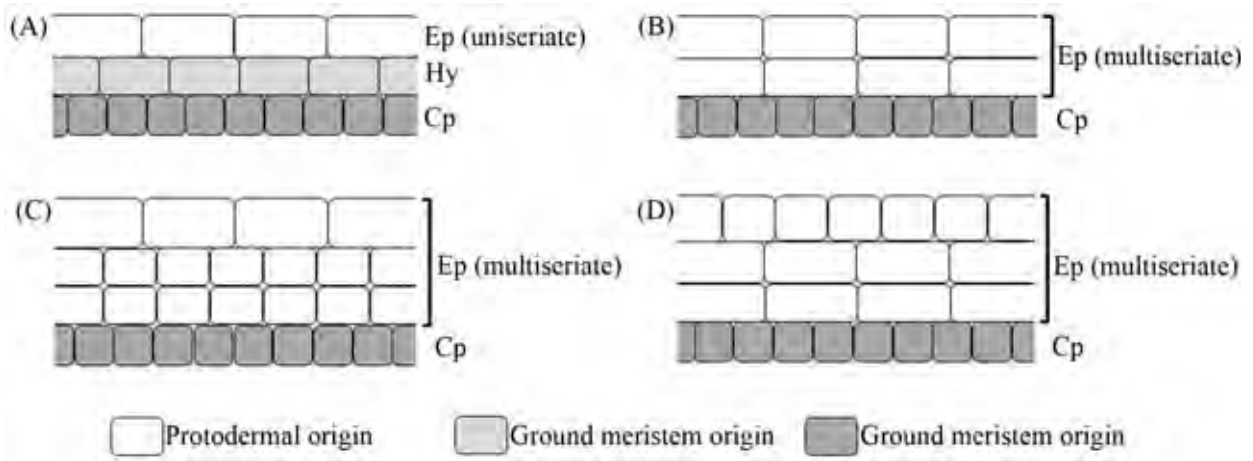


**Fig. 2.** Anatomical aspects of leaves of Cyperaceae in longitudinal (B) and transverse (A, C-N). Note different stages of development of the adaxial multiple epidermis. (A-D) *Fimbristylis dichotoma*, (E-G) *Pycreus flavescens*, (H-J) *Cyperus ligularis*, (K-M) *Rhynchospora globosa*. arrow = periclinal division of the cell; arrowhead = anticlinal division of the cell; asterisk = multiple epidermis. Bars = 10  $\mu\text{m}$  (E, H); 20  $\mu\text{m}$  (F-G, I); 30  $\mu\text{m}$  (A-B, K-M); 50  $\mu\text{m}$  (C-D, J).





**Fig. 3.** Diagrams of leaf transverse sections showing differences in the epidermis of Cyperaceae species. (A) uniseriate epidermis, (B) multiseriate epidermis with cell walls of the different layers aligned, (C-D) multiseriate epidermis with cell walls of the different layers non-aligned, (C) cells of the outmost layer larger than the inner layers, (D) cells of the outmost layer smaller than the inner layers. Ep = epidermis; Hy = hypodermis; Cp = chlorophyll parenchyma cells.





## **CAPÍTULO 3**

**Shirley Martins, Vera Lucia Scatena**

**Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales)**

(Publicado no periódico *Australian Journal of Botany* 59: 554-562 em agosto/2011)

## Abstract

Cyperaceae possess both non-Kranz and Kranz species with four subtypes that differ in the number and continuity of the bundle sheaths and by presence of chloroplasts. The ontogeny of the bundle sheaths of leaves and scapes of Cyperaceae species was studied to determine primary homologies and standardize the terminology used for its description. Two non-Kranz species and 11 Kranz species from different subtypes were studied. The non-Kranz species have two bundle sheaths, with the outer one originating from the ground meristem (endodermis) and the inner one from the procambium (pericycle). Kranz species of the chlorocyperoid and eleocharoid subtypes possess two sheaths derived from the procambium (biseriate pericycle). Kranz species of the rhynchosporoid subtype have only one bundle sheath, which develops from the procambium (pericycle). Kranz species of the fimbristyloid subtype possess three bundle sheaths; the outer one originates from the ground meristem (endodermis), while the middle and inner ones develop from the procambium (biseriate pericycle). The outer bundle sheath of non-Kranz and Kranz fimbristyloid species are homologues. The bundle sheaths inner in non-Kranz species, outer in Kranz chlorocyperoid and eleocharoid species, middle in Kranz fimbristyloid species and the single sheath in Kranz rhynchosporoid species are also homologues.

## Introduction

Cyperaceae is one of the largest families of angiosperms, comprising 109 genera and about 5500 species (Govaerst *et al.* 2007), of which 1500 are C<sub>4</sub> plants (Bruhl and Wilson 2007). The metabolism of C<sub>4</sub> plants has anatomical, ultrastructural and biochemical features that promote greater photosynthetic efficiency than C<sub>3</sub> plants (Sage 2004).

The anatomical structures related to C<sub>4</sub> photosynthesis were initially described by Haberlandt (1882, 1914), who gave the term “Kranz anatomy” to the concentric rings of chlorenchyma cells around the bundle sheaths in Cyperaceae and Poaceae species. The term “Kranz anatomy” or “Kranz

structure” was later expanded to include the entire scope of anatomical features of C<sub>4</sub> plants (Johnson and Brown 1973; Brown 1975), such as the presence of primary carbon assimilation tissue (PCA) (mesophyll cells) and of photosynthetic carbon reduction tissue (PCR) (bundle sheath with chloroplasts) (Dengler *et al.* 1985; Nelson and Dengler 1992).

The Kranz species of Cyperaceae display four anatomical subtypes: chlorocyperoid, eleocharoid, fimbristyloid and rhynchosporoid. They are structurally distinguished by the number of bundle sheaths, whether these sheaths are continuous or not and the location of the chloroplasts (Carolin *et al.* 1977; Bruhl *et al.* 1987; Perry and Bruhl 1995; Soros and Bruhl 2000). The anatomical and biochemical variations in C<sub>4</sub> species of Cyperaceae are interpreted as not always being homologous, and phylogenetic studies suggest at least four (Soros and Bruhl 2000) or six (Besnard *et al.* 2009) independent origins of C<sub>4</sub> photosynthesis in the family.

Given the anatomical variations of the Kranz anatomy in this group, knowledge of the ontogeny of the bundle sheaths is important (Nelson and Langdale 1989), mainly to indicate the primary homologies, which are fundamental to phylogenetic analyses. The origin of the bundle sheaths in Cyperaceae has been described only in *Cyperus eragrostis* Lam (non-Kranz), *Eleocharis retroflexa* (Poir.) Urban (Kranz eleocharoid), *Pycneus polystachyos* Rottb. (Kranz chlorocyperoid), *Rhynchospora rubra* (Loir.) Makino (Kranz rhynchosporoid) (Soros and Dengler 2001), and *Cyperus giganteus* Vahl (Rodrigues and Estelita 2003). These descriptions do not include information regarding Kranz species of the fimbristyloid subtype or *Rhynchospora* species of the chlorocyperoid subtype.

The bundle sheaths in the Kranz species of Cyperaceae have been given different names, as summarized in Table 1, which increases the difficulty of comparative studies and of identification of homologous structures.

Here, we studied bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae to determine the primary homologies, give subsidies to the phylogeny of Cyperaceae and propose a

standardization of the terminology. The origin of the bundle sheaths in Kranz species of the fimbristyloid type and in *Rhynchospora* species of the chlorocyperoid type is being presented for the first time.

## Materials and methods

Two non-Kranz species and 11 Kranz species from different subtypes were studied (Table 2). Voucher materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB). For the anatomical study, individuals at different stages of development were collected, fixed in FAA 50 (Johansen 1940) and stored in 70% ethanol. Longitudinal and transverse sections of young leaves and scapes located in rhizome apices were made. In *Eleocharis minima* the scape was studied because the leaf blades are reduced to sheaths.

Portions of rhizome apices were dehydrated in an ethyl alcohol series and then embedded in historesin (Leica Historesin Embedding Kit) (Feder and O'Brien 1968). The serial sections were made on a Reichert-Jung Model 2040 Microtome, stained with periodic acid-Schiff's reagent (PAS reaction) and toluidine blue (Feder and O'Brien 1968) and mounted in Entellan. Transverse sections of the median region of mature leaves were also made, stained with basic fuchsin and Astra blue (Roeser 1972) and mounted in glycerin jelly. To illustrate the successive stages of bundle sheath development, the minor veins were used. The images were obtained with a Leica DFC 290 digital camera coupled to a Leica DM LB microscope using IM50 software.

In the present study, the concept of primary homology (Pinna 1991) was adopted; this consists of a proposal of homology based on similarities and topological correspondence and needs to be legitimized in the phylogenetic approach (secondary homology). The suprageneric classification of the species studied is according Muasya *et al.* (2009).

## Results

Mature leaves of the non-Kranz species *Rhynchospora ciliolata* (Fig. 1a) and *Carex sororia* (Fig. 1g) present regularly-distributed mesophyll cells. The vascular bundles are surrounded by two sheaths: the outer one (endodermis) presents thin-walled cells containing chloroplasts, and the inner one (pericycle) presents thick-walled cells that lack chloroplasts (Fig. 1g). The precursor cells of the outer sheath develop from the ground meristem and involve the early procambial cells (Fig. 1b-f – traces). They divide in different planes, increase in size and differentiate into the outer sheath cells (Fig. 1e-f - traces, g). The early procambial cells that are located internally (Fig. 1b) arise in the middle region of the mesophyll (Fig. 1c) and divide in different planes, resulting in a peripheral layer of cells that differentiates into the inner sheath (Fig. 1c-g). The remaining central cells develop into vascular tissues (Fig. 1e-g).

In the mature scape of the Kranz species (eleocharoid subtypes) *Eleocharis minima* (Fig. 2a, g) and mature leaves of (chlorocyperoid subtypes) *Kyllinga brevifolia* (Fig. 2h), *Pycreus flavescens*, *Rhynchospora barbata* and *Cyperus ligularis* (Fig. 2m, n), the bundles are surrounded by radially-arranged mesophyll cells in relation to the veins (PCA) (Fig. 2g, h, m-n) and by two sheaths: the outer sheath (pericycle) presents slightly thick-walled cells, with reduced lumens and no chloroplasts (Fig. 2g, m), while the inner one (pericycle – PCR) has thin-walled cells with large lumens and with chloroplasts (Fig. 2g, m-n). The inner bundle sheath is continuous in all the vascular bundles of *Eleocharis minima* (Fig. 2a, g) but is discontinuous (interrupted by metaxylem elements) in the major veins of the others species (Fig. 2h). During development, the PCA precursor cells originate from the ground meristem and involve the early procambial cells (Fig. 2b, i - asterisks). These cells divide periclinally and anticlinally (Fig. 2b-f, i-l - asterisks) and the majority become elongated (Fig. 2f-g, l-n), although some cells remain rounded (Fig. 2a, h – arrows). The early procambial cells and their derivatives divide in different planes, forming the procambial strand (Fig. 2c-d, i-k), which differentiates into the peripheral layer of cells surrounding the internal cells (Fig. 2c-e, k-l). This peripheral layer develops into the outer bundle sheath (Fig. 2e-g, k-n), and the internal cells divide,

forming another layer that differentiates into the inner bundle sheath (Fig. 2e-g, l-n). The remaining central cells develop into vascular tissue (Fig. 2e-g, l-n).

In the Kranz species (rhynchosporoid subtypes) *Rhynchospora terminalis* (Fig. 3a) and *R. globosa* (Fig. 3h), mature leaves present a vascular bundle surrounded by radially-arranged mesophyll cells (PCA) and by only one sheath (pericycle – PCR) (Fig. 3a, h), which consists of thick-walled cells with chloroplasts (Fig. 3h). The precursor layer of PCA cells develops from the ground meristem and surrounds the early procambial cells (Fig. 3b - asterisks). The cells of this layer divide periclinally and anticlinally, and the majority become elongated (Fig. 3a-g – asterisks), though some cells remain rounded (Fig. 3a, h – arrows). Early procambial cells divide in different planes to form the peripheral layer of cells that develops into the single bundle sheath (Fig. 3c-h). The inner (central) procambial cells differentiate into vascular tissues (Fig. 3c-h).

Mature leaves of Kranz species (fimbristyloid subtypes) *Bulbostylis conifera*, *Fimbrisyllis autumnalis* (Fig. 3i), *F. dichotoma* and *F. complanata* (Fig. 3p) present a vascular bundle surrounded by radially-disposed mesophyll cells (PCA) and by three sheaths: the outer one (endodermis) presents thin-walled cells, with reduced lumens and with chloroplasts; the middle one (pericycle) has slightly thick-walled cells, with reduced lumens and no chloroplasts; and the inner one (pericycle – PCR) possesses thin-walled cells, with ample lumens and with chloroplasts (Fig. 3p). The inner sheath is discontinuous in the major veins (Fig. 3i) and continuous in the minor ones (Fig. 3p). The precursor cells of the PCA and of the outer bundle sheath originate from the ground meristem (Fig. 3j – asterisks and traces, respectively), divide periclinally and anticlinally and surround the early procambial cells (Fig. 3j-k – asterisks, traces). After successive divisions, the radiate parenchyma cells become elongated, while the outer sheath cells remain rounded (Fig. 3l-o, asterisks, traces). The procambial cells divide in different planes and originate the peripheral layer of cells that surrounds the central procambial cells (Fig. 3k-l). The peripheral layer differentiates into the middle bundle sheath (Fig. 3l-p), and the central procambial cells divide in different planes to form the inner layer

that develops into the inner bundle sheath (Fig. 3n-p). Within this sheath, the remaining procambial cells differentiate into vascular tissue (Fig. 3n-p).

## Discussion

The ontogeny of these bundle sheaths confirms the presence of four distinct subtypes of Kranz anatomy in Cyperaceae (Fig. 4). Differences exist in the numbers of bundle sheaths, whether these sheaths are continuous or not, and the location of the chloroplasts, as has been described for other Kranz species in the family (Carolin *et al.* 1977; Takeda *et al.* 1980; Bruhl *et al.* 1987; Ueno *et al.* 1988; Bruhl and Perry 1995; Soros and Dengler 2001; Martins and Alves 2009).

*Rhynchospora globosa* and *R. terminalis*, as studied here, present a unique bundle sheath. Previous studies have indicated that these species possess the Kranz rhynchosporoid subtype (Takeda *et al.* 1980; Ueno and Koyama 1987), which is described in the literature as presenting two bundle sheaths: one incomplete (outer) and one complete (inner) (Takeda *et al.* 1980; Ueno and Koyama 1987; Soros and Bruhl 2000). In these studies, the incomplete (outer) sheath was formed by two to five rounded cells.

The rounded cells, described by the latter authors, were observed in *Rhynchospora globosa* and *R. terminalis* in this study, and also in species of the chlorocyperoid (*Cyperus ligularis*, *Kyllinga brevifolia* and *Pycnus flavescens*) and eleocharoid (*Eleocharis minima*) subtypes. In other studied Kranz species of the chlorocyperoid and eleocharoid subtypes, the rounded cells have been treated as a partial parenchymatous sheath (Ueno and Koyama 1987; Bruhl and Perry 1995; Soros and Dengler 2001; Martins *et al.* 2008).

Based on the ontogenetic data presented, the rounded cells in the species studied here were interpreted to be part of the mesophyll layer of cells (PCA), not constituting a separate layer or sheath, because these rounded cells originated from the same mesophyll layer as the elongated cells. The data presented here suggest that the Kranz rhynchosporoid subtype should be characterized by

the presence of vascular bundles surrounded by radially-disposed mesophyll cells (PCA) and a unique bundle sheath. The same occurs in Kranz species of Poaceae, which present a single bundle sheath (Giussani *et al.* 2001). In addition, it is inappropriate to use the term “partial parenchymatous sheath” for Kranz species of chlorocyperoid and eleocharoid subtypes.

Among the Cyperaceae, the Kranz chlorocyperoid subtype occurs in various genera of tribe Cypereae and in some species of *Rhynchospora* (tribe Rhynchosporeae) (Soros and Bruhl 2000). In this study, the origins and structures of the bundle sheaths of *Rhynchospora barbata*, which possesses the Kranz chlorocyperoid subtypes, are similar to those of the species of tribe Cypereae, which are of the same subtype (*Cyperus ligularis*, *Kyllinga brevifolia*, and *Pycreus flavescens*). These data reinforce the hypothesis of two independent origins of the chlorocyperoid subtypes in Cyperaceae suggested by Soros and Bruhl (2000) and Thomas *et al.* (2009). Multiple independent origins of the same Kranz anatomical type has been indicated for some members of Poaceae (Giussani *et al.* 2001; Ueno *et al.* 2005) and some families of Eudicots (Peter and Katinas 2003; Muhaidat *et al.* 2007) as examples of evolutionary convergence.

In Cyperaceae, phylogenomic investigations carried out by Besnard *et al.* (2009) indicated that C<sub>4</sub> photosynthesis arose first in species of *Bulbostylis* and *Fimbristylis*, both of which are in tribe Abildgaardieae (fimbristylid, three bundle sheaths), and then in members of tribe Cypereae (chlorocyperoid, two bundles sheaths), *Eleocharis baldwinii* (eleocharoid, two bundles sheaths) and *Rhynchospora* (rhynchosporoid, one bundle sheath). According to this phylogenomic study and the ontogenetic data presented here, a reduction of the numbers of bundle sheaths may have occurred in the evolution of Kranz anatomy in Cyperaceae.

The outer bundle sheath of the Kranz fimbristylid subtype is homologous to the radially-disposed mesophyll cells in the Kranz chlorocyperoid, eleocharoid and rhynchosporoid subtypes. It may be that the mesophyll cells that do not elongate their walls (rounded cells) are remnants of the



outer sheath, as hypothesized for some species panicoid  $C_4$  that present one or two cells external to the single bundle sheath (Poaceae) (Giussani *et al.* 2001).

With regard to the terminology used to define these cells in the Kranz species studied, the term “endodermis” was used here to indicate the layer of cells that originates from the ground meristem and surrounds the vascular bundles in leaves, as described by Brown (1975) to parenchyma sheath. Based on this definition, in the species studied here, the endodermis constitutes the layer of radial mesophyll cells in the species with single (rhynchosporoid) and double sheaths (chlorocyperoid and eleocharoid) and the outer bundle sheath in non-Kranz species and in Kranz species of the fimbristyloid subtype.

The presence of an endodermis in roots is generally agreed upon, with or without a Casparian strip, but in stems and leaves the use of this term is controversial. In general, the endodermis is recognized in stems and leaves by the presence of starch grains (Esau 1965) or a Casparian strip (Esau 1965; Dickson and Wiltzman 1996; Lersten 1997). However, according to Van Fleet (1961) and Menezes *et al.* (2005), the endodermis occurs in all the vegetative organs; it is the innermost layer of the cortex in roots and stems, and in the leaves it is the innermost layer of the mesophyll.

The term “pericycle” was used here to define the peripheral layer of cells that develops from the procambium (Esau 1965). In mature leaves, this layer present thick-walled cells, and it is located internally in non-Kranz species, in the middle in Kranz species of the fimbristyloid subtype, and externally in Kranz species of the chlorocyperoid and eleocharoid subtypes, and it constitutes the single sheath in Kranz species of the rhynchosporoid subtype (Fig. 4; Table 2). The ontogenetic data corroborate the origin of this layer described for other Cyperaceae species with non-Kranz anatomy and with Kranz anatomy of the chlorocyperoid, eleocharoid and rhynchosporoid subtypes (Soros and Dengler 2001; Rodrigues and Estelita 2003).

The bundle sheath that is derived from the outmost layer of procambium and presents thick-walled cells in Cyperaceae and Poaceae species has been termed “mestome” (Schwendener 1890;

Esau 1965; Brown 1975; Carolin *et al.* 1977; Dengler *et al.* 1985; Ueno and Koyama 1987; Ueno *et al.* 1989), “endodermis” (Van Fleet 1950; Menezes *et al.* 2005), and “endodermoid sheath” (Sharma and Mehra 1972; Brown 1975). The use of the terms “endodermis” and “endodermoid sheath” to this sheath was related to the presence of suberin deposits in the cell walls. However, due to its procambial origin in the species studied here and in others Cyperaceae (Soros and Dengler 2001; Rodrigues and Estelita 2003) and Poaceae species (Dengler *et al.* 1985; Bosabalidis *et al.* 1994; Trivett and Evert 1998), it cannot be interpreted as an endodermis, which originates from the ground meristem, but rather should be called a pericycle. Therefore, the occurrence of suberin deposits is not restrict to endodermal cells, and can be present in other tissues as exodermis (Hose *et al.* 2001) and periderm (Meyer and Peterson 2011).

The chloroplast-containing bundle sheaths that originate from the inner layer of the procambium and are internally located in Kranz species of the chlorocyperoid, eleocharoid and fimbristyloid subtypes constitute the pericycle and are homologues (Fig. 4; Table 2). This same origin has been described in other Kranz species of the chlorocyperoid and eleocharoid subtypes (Estelita 1992; Soros and Dengler 2001; Rodrigues and Estelita 2003).

The interpretations presented here regarding the presence of only one bundle sheath in *Rhynchospora* species suggested to be of the Kranz rhynchosporoid subtype and regarding the radial mesophyll layer, which is constituted of elongated and rounded cells, expand the knowledge of Kranz anatomy in Cyperaceae. Moreover, the primary homologies presented regarding the bundle sheaths can be useful for future phylogenetic studies of the family.

## **Acknowledgements**

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 the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financial support (301692/2010-6) to V. L. Scatena.

## References

- Besnard G, Muasya AM, Russier F, Roalson EH, Salamin N, Christin PA (2009) Phylogenomics of C<sub>4</sub> photosynthesis in sedges (Cyperaceae): Multiple appearances and genetic convergence. *Molecular Biology and Evolution* **26**, 1909-1919.
- Bosabalidis MM, Evert RF, Russin WA (1994) Ontogeny of the vascular bundles and contiguous tissues in the maize leaf blade. *American Journal of Botany* **81**, 745-752.
- Brown WV (1975) Variations in anatomy, associations, and origins of Kranz tissue. *American Journal of Botany* **62**, 395-402.
- Bruhl JJ (1995) Sedge genera of the world: relationships and new classification of the Cyperaceae. *Australian Journal of Botany* **8**, 125-305.
- Bruhl JJ, Perry S (1995) Photosynthetic pathway-related ultrastructure of C<sub>3</sub>, C<sub>4</sub> and C<sub>3</sub>-C<sub>4</sub> intermediate sedge (Cyperaceae), with special reference to *Eleocharis*. *Australian Journal of Plant Physiology* **22**, 521-530.
- Bruhl JJ, Stone NE, Hattersley PW (1987) C<sub>4</sub> acid decarboxylation enzymes and anatomy in sedge (Cyperaceae): first record of NAD-Malic enzyme species. *Australian Journal of Plant Physiology* **14**, 719-728.
- Bruhl JJ, Wilson KL (2007) Towards a comprehensive survey of C<sub>3</sub> and C<sub>4</sub> photosynthetic pathway in Cyperaceae. In 'Monocots: comparative biology and evolution'. (Eds JT Columbus, EA Friar, CW Hamilton, JM Porter, LM Prince, MG Simpson) pp. 99-148. (Rancho Santa Ana Botanic Garden: Claremont)
- Carolin RC, Jacobs SWL, Vesik M (1977) The ultrastructure of Kranz cells in the family Cyperaceae. *Botanical Gazette* **138**, 413-419.
- Dengler NG, Dengler RE, Hattersley PW (1985) Differing ontogenetic origins of PCR ("Kranz") sheaths in leaf blades of C<sub>4</sub> grasses (Poaceae). *American Journal of Botany* **72**, 284-302.

- Dickison WG, Weitzman AL (1996) Comparative anatomy of the young stem, node, and leaf of the Bonnetiaceae, including observations on a foliar endodermis. *American Journal of Botany* **83**, 405-418.
- Esau K (1965) 'Plant anatomy.' (John Wiley and Sons: New York)
- Estelita MEM (1992) Origin and structure of the Kranz tissues in Cyperaceae. *Boletim de Botânica da Universidade de São Paulo* **13**, 41-48.
- Feder N, O'Brien TP (1968) Plant microtechnique: some principles and new methods. *American Journal of Botany* **55**, 123-142.
- Giussani MG, Cota-Sánchez H, Zuloaga FO, Kellogg EA (2001) A molecular phylogeny of the Grass subfamily Panicoideae (Poaceae) shows multiple origins of C<sub>4</sub> photosynthesis. *American Journal of Botany* **88**, 1993-2012.
- Govaerst R, Simpson DA, Goetghebeur P, Wilson KL, Egorova T, Bruhl JJ (2007) 'World checklist of Cyperaceae.' (The Board of Trustees of the Royal Botanic Gardens: Kew)
- Haberlandt GFJ (1882) Vergleichende Anatomie des Assimilatorischen Gewebesystems der Pflanzen. In 'Jahrbücher für wissenschaftliche Botanik'. (Ed N Pringshein) vol. 13, pp. 121-124. (Engelmann: Leipzig)
- Haberlandt GFJ (1914) 'Physiological plant anatomy.' (MacMillan: London)
- Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W (2001) The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany* **52**, 2245-2264.
- Johansen D (1940) 'Plant microtechnique.' (McGraw-Hill Book Company: New York)
- Johnson SC, Brown WV (1973) Grass leaf ultrastructural variations. *American Journal of Botany* **60**, 727-735.
- Lerman JC, Raynal J (1972) Biologie végétale – La teneur en isotopes stables du carbone chez les Cypéracées: sa valeur taxonomique. *Comptes Rendus de L'Academie des Sciences, série III* **275**, 1391-1394.

- Lersten NR (1997) Occurrence of endodermis with a Casparian Strip in stem and leaf. *The Botanical Review* **63**, 265-272.
- Martins S, Alves M (2009) Anatomical features of species of Cyperaceae from northeastern Brazil. *Brittonia* **61**, 189-200.
- Martins S, Machado SR, Alves M (2008) Anatomia e ultra-estrutura de *Cyperus maritimus* Poir. (Cyperaceae): estratégias adaptativas ao ambiente de dunas litorâneas. *Acta Botanica Brasilica* **22**, 289-299.
- Menezes NL, Silva DC, Arruda RCO, Melo-de-Pinna GF, Cardoso VA, Castro NM, Scatena VL, Scremin-Dias E (2005) Meristematic activity of the endodermis and the pericycle in the primary thickening in monocotyledons. Considerations on the “PTM”. *Anais da Academia Brasileira de Ciências* **77**, 259-274.
- Menezes NL, Silva DC, Melo-De-Pinna, GFA (2006) Folha. In ‘Anatomia Vegetal’. (Eds B Appezzato-da-Glória, S Carmello-Guerreiro) pp. 303-325. (Editora UFV: Viçosa)
- Metcalf CR (1971) ‘Anatomy of the monocotyledons: Cyperaceae.’ (Clarendon: Oxford)
- Meyer CJ, Peterson CA (2011) Casparian bands occur in the periderm of *Pelargonium hortorum* stem and root. *Annals of Botany* **107**, 591-598.
- Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MW, Smets E (2009) Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects. *The Botanical Review* **75**, 2-21.
- Muhaidat R, Sage RF, Dengler NG (2007) Diversity of Kranz anatomy and biochemistry in C<sub>4</sub> eudicots. *American Journal of Botany* **94**, 362-381.
- Nelson T, Dengler NG (1992) Photosynthetic tissue differentiation in C<sub>4</sub> plants. *International Journal of Plant Science* **153**, 93-105.
- Nelson T, Langdale JA (1989) Patterns of leaf development in C<sub>4</sub> plants. *The Plant Cell* **1**, 3-13.

- Peter G, Katinas L (2003) A new type of Kranz anatomy in Asteraceae. *Australian Journal of Botany* **51**, 217-226.
- Pinna MGG (1991) Concepts and tests of homology in the cladistic paradigm. *Cladistics* **7**, 367-394.
- Roeser KR (1972) Die Nadel der Schwarzkiefer - Massenprodukt und Kunstwerk der Natur. *Mikrokosmos* **61**, 33-36.
- Rodrigues AC, Estelita MEM (2003) Origin and structure of the Kranz tissue in bracts of *Cyperus giganteus* Vahl (Cyperaceae). *Revista Brasileira de Botânica* **26**, 445-452.
- Sage RF (2004) The evolution of C<sub>4</sub> photosynthesis. *New Phytologist* **161**, 341-370.
- Schwendener S (1890) Die Mestomscheiden der Gramineenblätter. *Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin* **22**, 405-426.
- Sharma OP, Mehra PN (1972) Systematic anatomy of *Fimbristylis* Vahl (Cyperaceae). *Botanical Gazette* **133**, 87-95.
- Soros CL, Bruhl JJ (2000) Multiple evolutionary origins of C<sub>4</sub> photosynthesis in the Cyperaceae. In 'Monocots: systematics and evolution'. (Eds KL Wilson, DA Morrison) pp. 629-636. (CSIRO Publishing: Melbourne)
- Soros CL, Dengler NG (2001) Ontogenetic derivation and cell differentiation in photosynthetic tissues of C<sub>3</sub> and C<sub>4</sub> Cyperaceae. *American Journal of Botany* **88**, 992-1005.
- Takeda T, Ueno O, Agata W (1980) The occurrence of C<sub>4</sub> species in the genus *Rhynchospora* and its significance in Kranz anatomy of the Cyperaceae. *The Botanical Magazine of Tokyo* **93**, 55-65.
- Thomas WW, Araújo AC, Alves MV (2009) A preliminary molecular phylogeny of the Rhynchosporeae (Cyperaceae). *The Botanical Review* **75**, 22-29.
- Ueno O, Koyama T (1987) Distribution and evolution of C<sub>4</sub> syndrome in *Rhynchospora* (Rhynchosporeae-Cyperaceae). *The Botanical Magazine of Tokyo* **100**, 63-85.

- Ueno O, Takeda T, Maeda E (1988) Leaf ultrastructure of C<sub>4</sub> species possessing different Kranz anatomical types in the Cyperaceae. *The Botanical Magazine of Tokyo* **101**, 141-152.
- Ueno O, Takeda T, Maeda E (1989) Distribution and evolution of C<sub>4</sub> syndrome in *Eleocharis*, a sedge group inhabiting wet and aquatic environments, based on culm anatomy and carbon isotope ratios. *Annals of Botany* **64**, 425-438.
- Ueno O, Yoshimura Y, Sentoku N (2005) Variations in the activity of some enzymes of photorespiratory metabolism in C<sub>4</sub> grasses. *Annals of Botany* **96**, 863-869.
- Van Fleet DS (1950) The cell forms, and their common substance reactions, in the parenchyma vascular boundary. *Bulletin of the Torrey Botanical Club* **77**, 340-353.
- Van Fleet DS (1961) Histochemistry and function of the endodermis. *The Botanical Review* **27**, 165-220.



**Table 1. Anatomical studies of Kranz Cyperaceae species and respective terms used to the bundle sheaths**

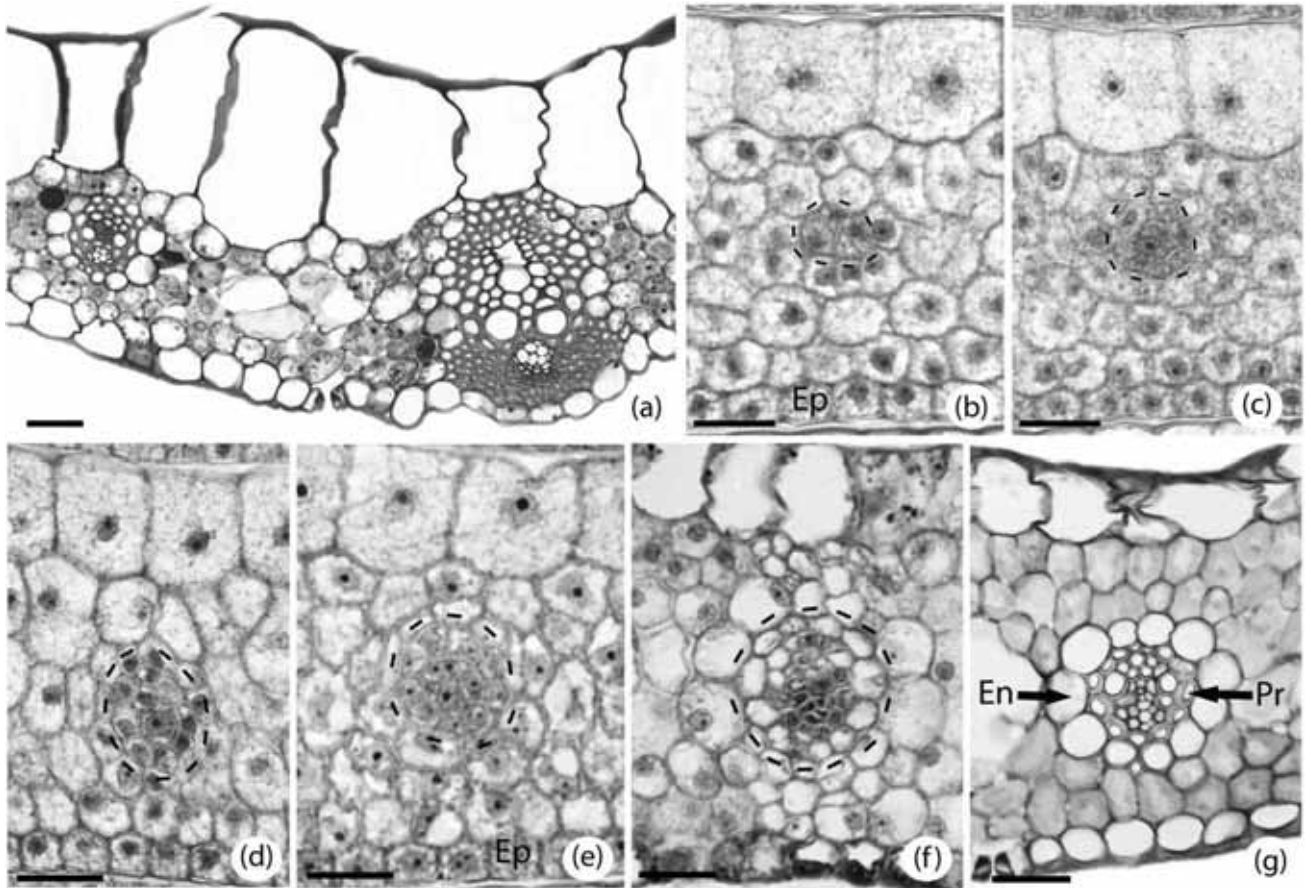
Abbreviations: \*BPC = border parenchyma cells; IBP = inner border parenchyma; MS = mestome sheath; PBS = parenchymatous bundle sheath; PCR = photosynthetic carbon reduction tissue

References	Taxa	Terms used to the bundle sheath
Metcalfe (1971)	Cyperaceae	<b>outer sheath</b> (layer of thin-walled cells or thick-walled cells), <b>inner sheath</b> (layer of thin-walled cells or thick-walled cells)
Sharma and Mehra (1972)	<i>Fimbristylis</i>	<b>outer sheath</b> (parenchymatous cells with chloroplasts), <b>middle sheath</b> or <b>endodermoid sheath</b> (thick-walled cells with no chloroplasts), <b>inner sheath</b> (parenchymatous cells with large chloroplasts).
Lerman and Raynal (1972)	Tribe Cyperae	<b>outer sheath</b> (thick-walled cells), <b>Kranz cells</b> (thin-walled cells)
Brown (1975)	Cyperaceae	<b>PBS*</b> (layer of cells surrounding the vascular bundle and derivatives from the ground meristem), <b>MS*</b> (layer of thick-walled cells located between the <b>PBS</b> or the mesophyll cell and the vascular bundles), <b>Kranz cells</b> or <b>Kranz sheath</b> (cells with large chloroplasts)
Carolin <i>et al.</i> (1977)	Tribe Cyperae; <i>Fimbristylis</i>	Chlorocyperoid subtypes (Cyperae), two sheaths: <b>MS</b> (thick-walled cells), <b>Kranz cells</b> (thin-walled cells); Fimbristyloid subtypes ( <i>Fimbristylis</i> ), three sheaths: <b>PBS</b> (thin-walled cells), <b>MS</b> (thick-walled cells), <b>Kranz cells</b> (thin-walled cells)
Takeda <i>et al.</i> (1980)	<i>Rhynchospora</i>	Rhynchosporoid subtypes, two sheaths: <b>outer sheath</b> (thin-walled cells with chloroplasts), <b>inner sheath</b> (thick-walled cells with chloroplasts)
Takeda <i>et al.</i> (1985)	Cyperaceae	Chlorocyperoid subtypes: <b>MS</b> and <b>Kranz sheath</b> ; Fimbristyloid subtypes: <b>PBS</b> , <b>MS</b> and <b>Kranz sheath</b> ; Rhynchosporoid subtypes: <b>PBS</b> and <b>Kranz sheath</b>
Ueno and Koyama (1987)	<i>Rhynchospora</i>	Fimbristyloid-chlorocyperoid subtypes: <b>partial PBS</b> , <b>MS</b> and <b>Kranz sheath</b> ; Rhynchosporoid subtypes: <b>PBS</b> and <b>Kranz sheath</b>
Bruhl <i>et al.</i> (1987)	Cyperaceae	Chlorocyperoid subtypes: <b>partial PBS</b> , <b>MS</b> and <b>PCR</b> or <b>Kranz tissue layer</b> ; Eleocharoid subtypes: <b>MS</b> and <b>PCR*</b> or <b>Kranz tissue layer</b> ; Fimbristyloid subtypes: <b>PBS</b> , <b>MS</b> and <b>PCR</b> or <b>Kranz tissue layer</b> ; Rhynchosporoid subtypes: <b>incomplete PBS</b> , <b>MS</b> (PCR tissue)
Ueno <i>et al.</i> (1988)	Cyperaceae	Chlorocyperoid subtypes: <b>MS</b> and <b>Kranz sheath</b> ; Fimbristyloid subtypes: <b>PBS</b> , <b>MS</b> and <b>Kranz sheath</b> ; Rhynchosporoid subtypes: <b>incomplete PBS</b> and <b>Kranz sheath</b>
Bruhl (1995)	Cyperaceae	<b>PBS</b> , <b>MS</b> and <b>boundary layer cells with chloroplasts</b>
Bruhl and Perry (1995)	Cyperaceae	Eleocharoid subtypes: <b>MS</b> and <b>BPC*</b> (PCR or Kranz tissue); Chlorocyperoid subtypes: <b>partial PBS</b> , <b>MS</b> and <b>BPC</b> (PCR or Kranz tissue); Fimbristyloid subtypes: <b>PBS</b> , <b>MS</b> and <b>BPC</b> (PCR or Kranz tissue); Rhynchosporoid subtypes: <b>incomplete PBS</b> and <b>MS</b> (PCR tissue);
Soros and Dengler (2001)	Cyperaceae	Chlorocyperoid subtypes: <b>partial PBS</b> , <b>MS</b> and <b>IBP*</b> ; Eleocharoid subtypes: <b>MS</b> and <b>IBP</b> ; Rhynchosporoid subtypes: <b>PBS</b> and <b>MS</b> (PCR); Fimbristyloid subtypes: <b>PBS</b> , <b>MS</b> and <b>IBP</b> . Procambial origin ( <b>MS</b> and <b>IBP</b> ) and ground meristem origin ( <b>PBS</b> )
Menezes <i>et al.</i> (2006)	<i>Fimbristylis annua</i> (All.) Roem. & Schult.	<b>endodermis</b> (thick-walled cells with suberin bands); <b>pericycle</b> (inner sheath with large chloroplasts)
Rodrigues and Estelita (2003)	<i>Cyperus giganteus</i> Vahl	<b>MS</b> or <b>endodermis</b> and <b>Kranz sheath</b>

Table 2. List of Cyperaceae species studied with respective vouchers and anatomical variations

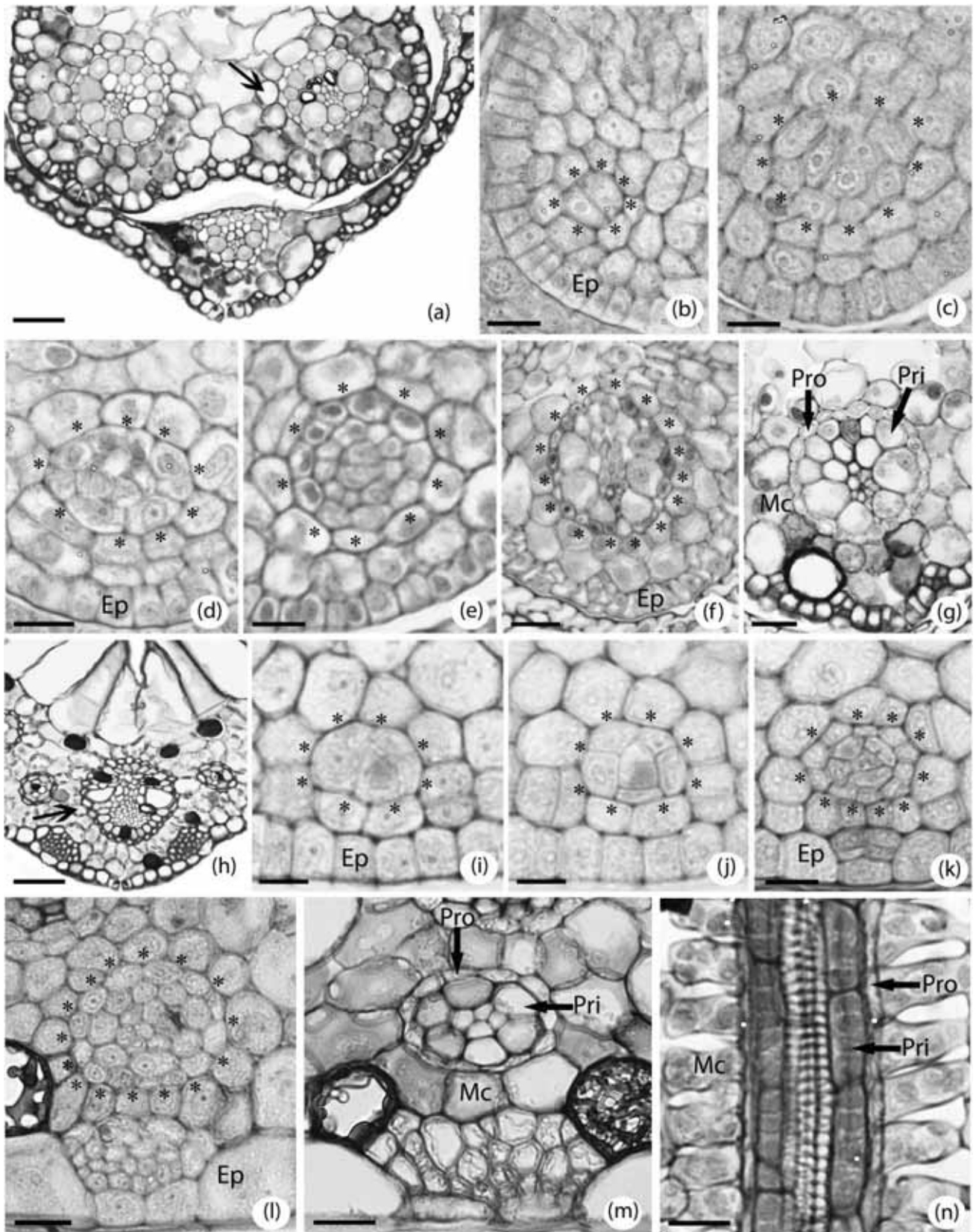
Species	Voucher	Anatomical type, subtype	Sheath – origin
<i>Carex sororia</i> Kunth	<i>S. Martins 412</i>	<b>Non-Kranz</b> – regularly-distributed mesophyll cells	<b>Endodermis</b> (outer; thin-walled cells) – the innermost layer from the ground meristem
<i>Rhynchospora ciliolata</i> Boeck.	<i>A. Souza 1730</i>	and vascular bundles with two continuous sheaths	<b>Pericycle</b> (inner; thick-walled cells) – the peripheral layer from the procambium
<i>Eleocharis minima</i> Kunth	<i>S. Martins 405</i>	<b>Kranz, electrocharoid</b> – radially-arranged mesophyll cells (PCA) and vascular bundles with two continuous sheaths, the inner one is the PCR	<b>Pericycle</b> (outer; thick-walled cells) – the peripheral layer from the procambium
<i>Cyperus ligularis</i> L.	<i>S. Martins 330</i>	<b>Kranz, chlorocyperoid</b> – radially-arranged mesophyll cells (PCA) and vascular bundles with two sheaths, the outer one continuous, and the inner one discontinuous (PCR)	<b>Pericycle</b> (inner; thin-walled cells) – the adjacent layer of the peripheral one from the procambium
<i>Kyllinga brevifolia</i> Rottb.	<i>S. Martins 288</i>		<b>Pericycle</b> (outer; thick-walled cells) – the peripheral layer from the procambium
<i>Pycurus flavescens</i> (L.) Rehb.	<i>S. Martins 327</i>		<b>Pericycle</b> (inner; thin-walled cells) – the adjacent layer of the peripheral one from the procambium
<i>Rhynchospora barbata</i> (Vahl) Kunth	<i>S. Martins 313</i>		
<i>Rhynchospora globosa</i> Lindl.	<i>S. Martins 305</i>	<b>Kranz, rhynchosporoid</b> – radially-arranged mesophyll cells (PCA) and vascular bundles with one continuous sheath (PCR)	<b>Pericycle</b> (single; thick-walled cells) – the peripheral layer from the procambium
<i>Rhynchospora terminalis</i> Kunth	<i>S. Martins 302</i>		
<i>Bulbostylis conifera</i> L.	<i>S. Martins 329</i>	<b>Kranz, fimbriatylid</b> – radially-arranged mesophyll cells (PCA) and vascular bundles with three sheaths, the outer (PCA) and middle ones continuous and the inner one discontinuous (PCR)	<b>Endodermis</b> (outer; thin-walled cells) – the innermost layer from the ground meristem
<i>Fimbristylis autumnalis</i> L.	<i>V.L. Scatena 343</i>		
<i>Fimbristylis complanata</i> (Retz.) Link	<i>V.L. Scatena 344</i>		<b>Pericycle</b> (middle; thick-walled cells) – the peripheral layer from the procambium
<i>Fimbristylis dichotoma</i> (L.) Vahl	<i>S. Martins 398</i>		<b>Pericycle</b> (inner; thin-walled cells) – the adjacent layer of the peripheral one from the procambium

**Fig. 1.** Bundle sheath ontogeny of leaves of non-Kranz Cyperaceae in transverse section. (a-f) *Rhynchospora ciliolata* and (g) *Carex sororia*. (a) Mature leaf. (b-f) Developmental stages of the bundle and bundle-sheaths. (g) Bundle detail in mature leaf. Ep, epidermis; En, endodermis; Pr, pericycle; Traces, outer bundle sheath (endodermis) cells in different stages of development. Scale: a-g = 20  $\mu\text{m}$ .



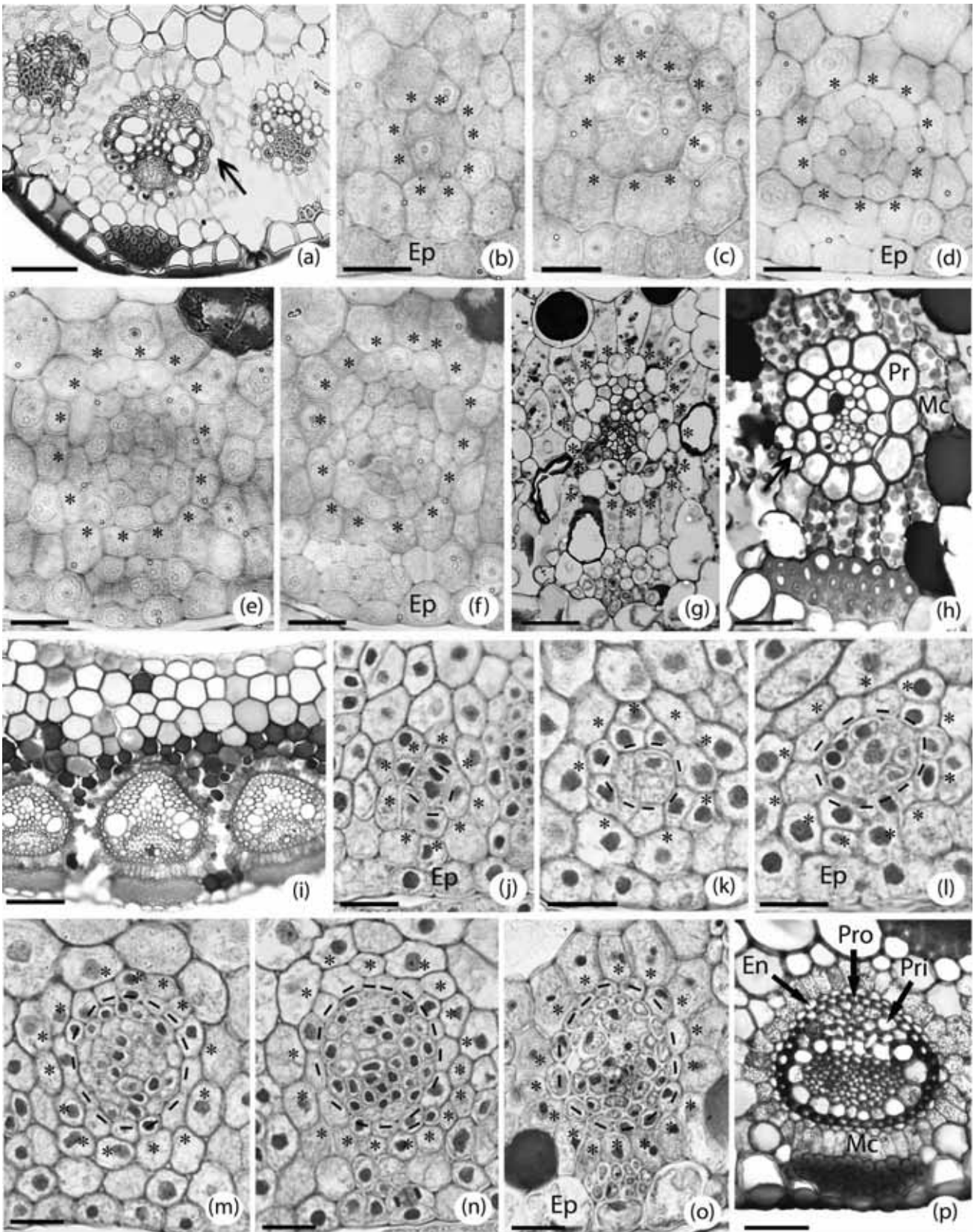
**Fig. 2.** Bundle sheath ontogeny of scape (*a-g*) and leaves (*h-m*) in transverse section and leaf (*n*) in longitudinal section of Cyperaceae. (*a-g*) *Eleocharis minima* (Kranz, eleocharoid); (*h*) *Kyllinga brevifolia* and (*i-n*) *Cyperus ligularis* (Kranz, chlorocyperoid). (*a*) Mature scape. (*b-f*) Developmental stages of the bundle and bundle-sheaths. (*g*) Bundle detail in mature scape. (*h*) Mature leaf. (*i-l*) Developmental stages of the bundle and bundle-sheaths. (*m-n*) Bundle detail in mature leaf. Arrow, rounded cell; Asterisks, mesophyll cells in different stages of development; Ep, epidermis; Mc, mesophyll cell; Pri, inner pericycle cell; Pro, outer pericycle cell. Scale: *a, h* = 50  $\mu\text{m}$ ; *b-d, i-n* = 10  $\mu\text{m}$ ; *e-g* = 20  $\mu\text{m}$ .



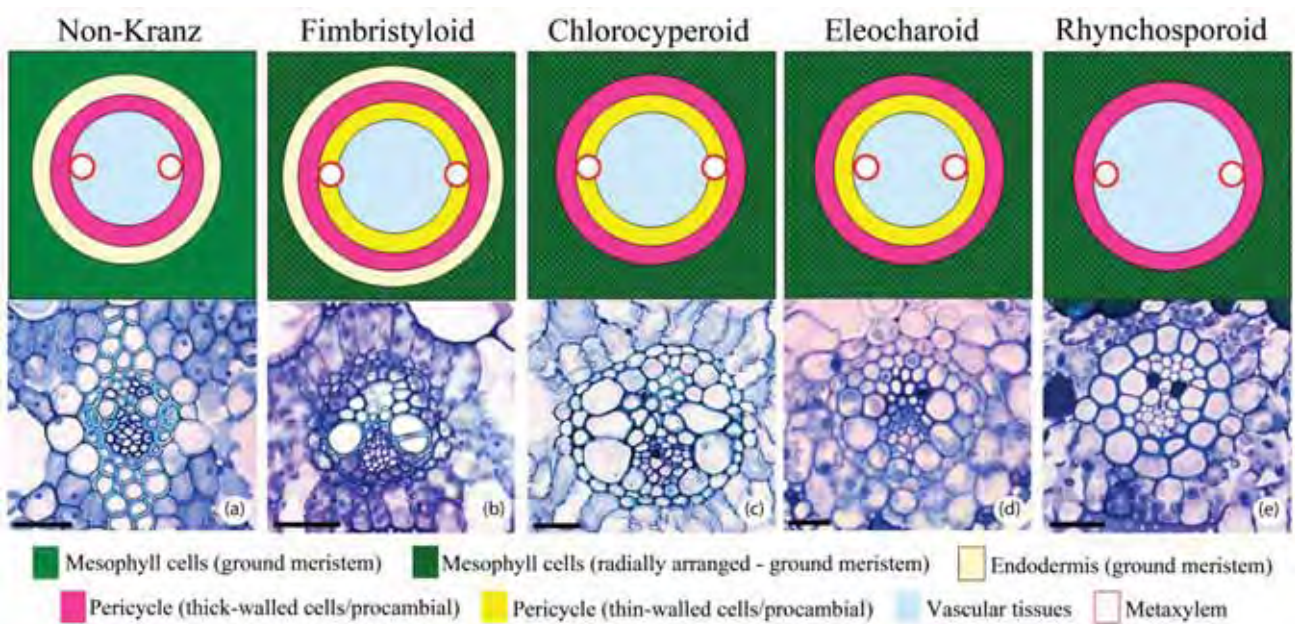


**Fig. 3.** Bundle sheath ontogeny of leaves of Cyperaceae in transverse section. (a) *Rhynchospora terminalis* and (b-h) *R. globosa* (Kranz, rhynchosporoid); (i) *Fimbristylis autumnalis* and (j-p) *F. complanata* (Kranz, fimbristyloid). (a; i) Mature leaf. (b-g; j-o) Developmental stages of the bundle and bundle-sheaths. (h; p) Bundle detail in mature leaf. Arrow, rounded cell; Asterisks, mesophyll cells in different stages of development; Ep, epidermis; Mc, mesophyll cell; Pr, pericycle; Pri, inner pericycle cell; Pro, outer pericycle cells; Traces, outer bundle sheath (endodermis) cells in different stages of development. Scale: a = 50  $\mu\text{m}$ ; b-h, j-p = 20  $\mu\text{m}$ ; i = 100  $\mu\text{m}$ .





**Fig. 4.** Diagrams (upper) and transverse section of major veins (lower) of non-Kranz species and Kranz species from different subtypes. (a) *Carex sororia*. (b) *Fimbristylis dichotoma*. (c) *Rhynchospora barbata*. (d) *Eleocharis minima*. (e). *Rhynchospora globosa*. Scale: 20  $\mu\text{m}$ . Based in Soros and Bruhl (2000) and Soros and Dengler (2001).



## **CAPÍTULO 4**

**Shirley Martins, Silvia Rodrigues Machado, Vera Lucia Scatena**

**Ultrastructure of Kranz tissues in Cyperaceae (Poales): Emphasis on  
chloroplasts and mitochondria**

(Submetido ao periódico *Micron* em março/2012 e encontra-se em análise)

## Abstract

The ultrastructure of the Kranz tissues in leaves of eight Cyperaceae species with the four types of Kranz anatomy (chlorocyperoid, eleocharoid, fimbristylloid and rhynchosporoid) were studied and compared with species already described in the literature, with the goal of establishing patterns for the Kranz subtypes and finding characters useful for the systematics of the groups. In addition, chloroplasts and mitochondria were quantified for the first time for all Kranz subtypes in the family. In the four Kranz subtypes, the chloroplast's ultrastructure is similar in the radiate parenchyma cells (PCA) and different in the bundle sheath cells (PCR). In the bundle sheath cells (PCR) of the chlorocyperoid and fimbristylloid types, the chloroplasts are centrifugal, rare and scattered, with parallel or contorted thylakoids. In the eleocharoid type, the chloroplasts are centrifugal or scattered, and in the rhynchosporoid type they are scattered; both of these types have parallel thylakoids. In the eleocharoid and rhynchosporoid types and in *Cyperus* and *Pycneus* species of the chlorocyperoid type, a pattern was observed in the chloroplasts' position in the studied species and those described of the literature. Additionally, the eleocharoid and rhynchosporoid subtypes had a distinctive pattern of thylakoid organization. Chloroplast characters that are constant in the Kranz species of *Cyperus*, *Eleocharis*, *Pycneus* and *Rhynchospora* can provide information for studies of the systematics of these groups. In most of the studied species, except for *C. ligularis*, *R. globosa* and *R. terminalis*, the number of mitochondria in the bundle sheath cells (PCR) is significantly higher than in the radiate parenchyma cells (PCA); this result differs from the results found in previous studies.

**Keywords:** C<sub>4</sub> photosynthesis, Cyperaceae, Kranz, ultrastructure

## 1. Introduction

The Cyperaceae family is one of the families with the highest numbers of species with C<sub>4</sub> photosynthesis (Sage, 2004) and includes four Kranz anatomical types (chlorocyperoid, eleocharoid,



fimbristyloid and rhynchosporoid) (Soros and Bruhl, 2000). These types differ in the number and continuity of the vascular bundle sheath and in the presence or not of chloroplasts in the bundle sheath (Soros and Bruhl, 2000; Martins and Scatena, 2011). The C<sub>4</sub> Cyperaceae species also differ in the biochemical subtype of the decarboxylation enzymes (NADP-ME or NAD-ME) (Bruhl and Perry, 1995). The species of the chlorocyperoid, fimbristyloid (Abildgaardieae Tribe) and rhynchosporoid types possess the NADP-ME subtype, and the eleocharoid and fimbristyloid (*Eleocharis vivipara*) types contain the NAD-ME subtype (Bruhl and Perry, 1995; Murphy et al., 2007).

The ultrastructural studies with C<sub>4</sub> Cyperaceae conducted in species from the four Kranz types have mainly examined the chlorocyperoid and fimbristyloid types (Carolin et al., 1977; Ueno et al., 1988; Estelita, 1992; Bruhl and Perry, 1995; Rodrigues and Estelita, 2003; Martins et al., 2008). The chloroplast characteristics in groups with C<sub>4</sub> photosynthesis might be of great relevance for taxonomy and systematic purposes (Bruhl and Perry, 1995; Jacobs, 2001). However, in Cyperaceae it is difficult to establish patterns related to the chloroplast structure because of the small number of studied species. For example, in the rhynchosporoid type, only *Rhynchospora rubra* has been described (Carolin et al., 1977; Bruhl et al., 1987; Bruhl and Perry, 1995), and no chlorocyperoid *Rhynchospora* species have been studied at the ultrastructural level (Bruhl and Perry, 1995).

Quantifying organelles is important for characterizing the cell structure of the different Kranz types, and this process has been performed with several Poaceae species (Brown et al., 1983; Yoshimura et al., 2004). However, in Cyperaceae this approach has only been performed with individuals of *Eleocharis vivipara* (fimbristyloid) growing in distinct environmental conditions (Ueno, 1996). When quantifying organelles, the proportion of mitochondria present in the radiate parenchyma cells (PCA - Photosynthetic Carbon Assimilative tissue) relative to the bundle sheath

cells (PCR - Photosynthetic Carbon Reductive tissue) is an important character for indicating the biochemical subtype (Bruhl et al., 1987; Ueno, 1996).

The aim of this study was to characterize and to quantify the chloroplasts and mitochondria of Cyperaceae species with different types of Kranz anatomy (including two new Kranz rhynchosporoid species and one *Rhynchospora* species of the chlorocyperoid type), to identify patterns and characters that may be useful for systematics studies in this family.

## 2. Materials and methods

Eight species of Cyperaceae with different types of Kranz anatomy were studied: *Cyperus ligularis* L. (*S. Martins* 330), *Kyllinga brevifolia* Rottb. (*S. Martins* 288) and *Rhynchospora barbata* (Vahl) Kunth (*S. Martins* 313) - chlorocyperoid Kranz anatomy; *Eleocharis minima* Kunth (*S. Martins* 405) – eleocharoid Kranz anatomy; *Bulbostylis scabra* (J. Presl. & C. Presl.) C.B. Clarke (*S. Martins* 408) and *Fimbristylis autumnalis* L. (*V.L. Scatena* 343) - fimbristyloid Kranz anatomy; *Rhynchospora globosa* Lindl (*S. Martins* 305) and *R. terminalis* Kunth (*S. Martins* 302) - rhynchosporoid Kranz anatomy. The species were collected in their natural habitats and the voucher materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB).

Samples of the mid-region of completely expanded leaves were fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer at a pH of 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 sections were obtained with a Diatome diamond knife and were stained with uranyl acetate and lead citrate (Reynolds, 1963). The material was examined with a Philips EM 301 transmission electron microscope (MET). The numbers of chloroplasts and mitochondria per cell were counted for 30-40 cells of the radiate parenchyma (PCA) and of the bundle sheath (PCR) in eight vascular bundles of three leaves under an electron



microscope.

Statistical analyses were performed to verify the significant differences among the number of organelles in the PCA and PCR tissues. A nonparametric test (Wilcoxon/Kruskall-Wallis) was applied using R (R Development Core Team, 2010).

### 3. Results

In leaves of all studied species, the radiate parenchymal (PCA) cells (Fig. 1A – asterisk) have lenticular chloroplasts located near the cell walls (Figs. 1B and C), with well-developed grana (Gr) (Fig. 1D), and plasmodesmata (Pl) occur between the adjacent PCA cells (Fig. 1E). The PCA cells in the studied species differ in the number of chloroplasts (Tab. 2) and in to the development of the peripheral reticulum (Pr), which is well-developed in *Eleocharis minima*, *Rhynchospora terminalis* and *R. barbata* (Fig. 1F) and which is reduced or absent in the other species. The mitochondria (Mi) in the PCA cells are oval or globular with developed cristae (Fig. 1C).

The Kranz chlorocyperoid species *Cyperus ligularis* (Fig. 1G), *Kyllinga brevifolia* (Fig. 1H) and *Rhynchospora barbata* (Fig. 1I), possess two sheaths around the vascular bundles: the outer one (Os) lacks chloroplasts, whereas the inner one (Is) has larger chloroplasts (PCR) (Figs. 1G-I). In the inner sheath cells (Is), the chloroplasts are located centrifugally in *C. ligularis* (Fig. 1G and Tab. 1) and centrifugally or scattered in *K. brevifolia* (Fig. 1H and Tab. 1) and *R. barbata* (Fig. 1I and Tab. 1). The lamellar system of the chloroplasts is composed of contorted or parallel thylakoids, and the peripheral reticulum is reduced or absent (Figs. 1J, L and Tab. 1). The mitochondria in the PCR cells are oval or elongated (Fig. 1M). In *K. brevifolia* and *R. barbata*, mitochondria are more numerous in PCR cells than in the PCA cells, while in *C. ligularis*, the PCR and PCA cells have similar numbers of mitochondria (Tab. 2). Plasmodesmata (Pl) are frequent between the anticlinal walls of the inner sheath cells (Fig. 1M), connecting them, and between the inner sheath cells and the outer bundle sheath cells (Fig. 1I).

*Eleocharis minima* possess Kranz elecharoid anatomy and have two vascular bundle sheaths: the outer bundle sheath (Os) lacks chloroplasts, whereas the inner bundle sheath (Is) has chloroplasts (PCR) (Fig. 2A). In the inner sheath cells, the chloroplasts are scattered (Fig. 2A), with a lamellar system composed of parallel thylakoids (Fig. 2B) and a reduced peripheral reticulum. Additionally, in these cells, the mitochondria (Mi) are oval or elongated (Fig. 2B) and are more numerous compared to those in the radiate parenchymal cells (PCA) (Tab. 2).

*Bulbostylis scabra* and *Fimbristylis autumnalis* have Kranz fimbristyloid anatomy (Fig. 2C), with vascular bundles surrounded by three sheaths: the outer sheath (Os) and the inner sheath (Is) (PCR) both have chloroplasts, while the middle sheath lacks chloroplasts (Fig. 2C). In the cells of the outer bundle sheath, the chloroplasts are lenticular or oval with well-developed grana (Fig. 2D) and a reduced peripheral reticulum. In the inner sheath (PCR), the chloroplasts are centrifugal or scattered (Figs. 2C and E), with contorted or parallel thylakoids (Fig. 2E) and a reduced peripheral reticulum. The mitochondria of the inner sheath cells are oval (Fig. 2E). In *Bulbostylis scabra*, the mitochondria are more numerous in inner sheath cells than in the radiate parenchymal cells, while *Fimbristylis autumnalis* has similar numbers of mitochondria in the inner sheath cells and in the radiate parenchymal cells (Tab. 2).

The rhynchosporoid species, *Rhynchospora globosa* (Fig. 2F) and *R. terminalis* (Fig. 2G), have vascular bundles surrounded by a single bundle sheath containing chloroplasts (PCR) (Fig. 2F). In the cells of this sheath, the chloroplasts are scattered (Fig. 2G), with parallel thylakoids (Fig. 2H-I) and a reduced peripheral reticulum. The mitochondria are oval and are observed in similar numbers as those in the radiate parenchymal cells (Tab. 2). Plasmodesmata are frequent between the anticlinal walls of the inner sheath cells (Fig. 2I) and between the inner sheath cells and those of the radiate parenchyma cells.

The characteristics of the organelles in the studied species (Tab. 1) were compared with those species of the literature (Tab. 3) to further the discussion.

#### 4. Discussion

The Kranz Cyperaceae species of the four anatomical types studied here and described in the literature (Carolin et al., 1977; Ueno et al., 1988; Bruhl and Perry 1995) have chloroplasts in the radiate parenchyma cells (PCA) with well-developed grana, representing a common character in the family. The chloroplasts of the outer bundle sheath cells in the Kranz fimbristylloid species also have well-developed grana, as observed in this study and in the literature (Bruhl and Perry, 1995), with the radiate parenchyma and the outer sheath interpreted to both compose PCA tissues with similar functions (Carolin et al., 1977).

In bundle sheath cells that constitute the PCR tissue, the chloroplast ultrastructure PCR cells differ among the Kranz types and also within the same type, as observed in the species studied here (Tab. 1) and previously (Tab. 3). These differences can be related to the biochemical subtype (NADP-ME or NAD-ME) and/or to the taxonomic group.

In the C<sub>4</sub> Poaceae with the NADP-ME subtype, the chloroplasts of the vascular bundle sheath (PCR) are centrifugal, with reduced or absent grana and similar ratios of mitochondria between the PCA and PCR tissues (Hatch et al., 1975; Hattersley and Browning, 1981). Therefore, in the Kranz Cyperaceae species of the chlorocyperoid, fimbristylloid (Abildgaardieae Tribe) and rhynchosporoid types, indicated as the NADP-ME subtype, the chloroplasts of the PCR cells are scattered, or centrifugal, as shown in the species studied here (Tab. 1) and in the literature (Tab. 3). In addition, in *Kyllinga brevifolia* and *Rhynchospora barbata* (chlorocyperoid) and in *Bulbostylis scabra* and *Fimbristylis autumnalis* (fimbristylloid), the number of mitochondria was significantly greater in the PCR cells relative to the PCA cells. Therefore, the centrifugal localization of the chloroplasts in the PCR cells and the similar ratio of mitochondria between PCA and PCR do not represent consistent characteristics for indicating the biochemical subtype NADP-ME in the Cyperaceae.

The species of the *Eleocharis* genus studied here (Tab. 1) and described in the literature (Tab. 3) have been indicated to possess the NAD-ME biochemical subtype and to differ from the Poaceae

species with this subtype (Hattersley and Browning, 1981; Yoshimura et al., 2004) by the localization of the chloroplasts, which is scattered in the Cyperaceae and centripetal in the Poaceae.

In the Cyperaceae of the eleocharoid, fimbristyloid and rhynchosporoid types, patterns were observed in the chloroplast features. However, for the studied Kranz chlorocyperoid species, *Cyperus ligularis* and *Kyllinga brevifolia* (Cypereae Tribe) and *Rhynchospora barbata* (Rhynchosporeae tribe) and those from the literature, it was not possible to establish a pattern because of variation in the chloroplast position, development of the peripheral reticulum and the organization of the thylakoid system (Tabs. 1 and 3). Nevertheless, the Kranz *Cyperus* and *Pycnus* species had chloroplasts in the centrifugal position (Tab. 3), representing a potential taxonomic character and reflecting similarity between the genera. This similarity was indicated by Tucker (1994), who grouped the species of these genera in *Cyperus*.

The eleocharoid Cyperaceae have a pattern in the chloroplast position and thylakoid system organization similar to that in *Eleocharis minima* and other species of the genus with this Kranz type (Tab. 3). These characteristics were also described in Kranz fimbristyloid *Eleocharis* species (Bruhl et al., 1987; Ueno et al., 1989) and can be useful to the genus taxonomy.

The species of the fimbristyloid type (Abildgaardieae tribe) studied here and in the literature have a consistent pattern in the chloroplast position in the bundle sheath cells (PCR), but the development of the peripheral reticulum and thylakoid system organization varies (Tabs. 1 and 3).

The rhynchosporoid Cyperaceae species have a pattern in the chloroplast position in the PCR cells and in the development of the peripheral reticulum and the thylakoid system organization, as observed in *Rhynchospora globosa* and *R. terminalis* studied here and in *Rhynchospora rubra* studied by Ueno et al. (1988) and Bruhl and Perry (1995); however, the chloroplast position varies (Tabs. 1 and 3). In *Rhynchospora barbata*, a Kranz chlorocyperoid species, the chloroplasts of the bundle sheath cells are similar to those of the *Rhynchospora* species of the rhynchosporoid type (Tabs. 1 and 3) and can constitute common characters for Kranz species of the genus.

The peripheral reticulum constitutes a series of anastomosed tubules located in the stroma periphery of the chloroplast (Laestch, 1974) and present taxonomic values to Kranz Cyperaceae species (Ueno et al., 1988; Bruhl and Perry, 1995). However, in the studied species here and from the literature, the degree of peripheral reticulum development is varied, even in close groups (Tabs. 1 and 2), remaining constant only in Kranz *Rhynchospora* species of the chlorocyperoid and rhynchosporoid types.

## 5. Conclusions

The characterization and quantification of chloroplasts and mitochondria amplify the knowledge about the different types of Kranz anatomy in Cyperaceae, showing that Kranz species of the eleocharoid, rhynchosporoid and fimbriatoid types, have patterns in some chloroplast characters and that in the genera *Cyperus*, *Eleocharis*, *Pycreus* and *Rhynchospora* some characters can be useful for future studies of their systematics.

## Acknowledgements

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 the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financial support (301692/2010-6) to V. L. Scatena and to S. R. Machado (2008/301464-1) and the Centre of Microscopy of the Universidade Estadual Paulista (Botucatu).

## References

Brown, R.H., Bouton, J.H., Rigsby, L., Rigler, M. 1983. Photosynthesis of grass species differing in carbon dioxide fixation pathways. VIII. Ultrastructural characteristics of *Panicum* species in the *Laxa* group. *Plant Physiol.* 71, 425-431.

- Bruhl, J.J., Perry, S. 1995. Photosynthetic pathway-related ultrastructure of C<sub>3</sub>, C<sub>4</sub> and C<sub>3</sub>-C<sub>4</sub> intermediate sedge (Cyperaceae), with special reference to *Eleocharis*. Aust. J. Plant Physiol. 22, 521-530.
- Bruhl, J.J., Stone, N.E., Hattersley, P.W. 1987. C<sub>4</sub> acid decarboxylation enzymes and anatomy in sedge (Cyperaceae): first record of NAD-Malic enzyme species. Aust. J. Plant Physiol. 14, 719-728.
- Carolin, R.C., Jacobs, S.W.L., Vesk, M. 1977. The ultrastructure of Kranz cells in the family Cyperaceae. Bot. Gaz. 138, 413-419.
- Estelita, M.E.M. 1992. Origin and structure of the Kranz tissues in Cyperaceae. Bol. Bot. Univ. São Paulo 13, 41-48.
- Estelita, M.E.M. 1993. Anatomia dos órgãos vegetativos de *Remirea maritima* Aubl. (Cyperaceae). Naturalia 18, 123-134.
- Estelita-Teixeira, M.E., Handro, W. 1987. Kranz pattern in leaf, scape and bract of *Cyperus* and *Fimbristylis* species. Rev. Bras. Bot. 10, 105-111.
- Hatch, M.D., Kagawa, T., Craig, S. 1975. Subdivision of C<sub>4</sub>-pathway species based on differing C<sub>4</sub> acid decarboxylating systems and ultrastructural features. Aust. J. Plant Physiol. 2, 111-128.
- Hattersley, P.W., Browning, A.J. 1981. Occurrence of the suberized lamella in leaves of grasses of different photosynthetic types. In parenchymatous bundle sheat and PCR (Kranz) sheaths. Protoplasma 109, 371-401.
- Kim, I.S., Pak, J.H., Seo, B.B., Song, S.D. 1999. Ultrastructure of leaves in C<sub>4</sub> *Cyperus iria* and C<sub>3</sub> *Carex siderosticta*. J. Plant Biol. 42, 213-221.
- Laetsch, W.M. 1974. The C<sub>4</sub> syndrome: a structural analysis. Annu. Rev. Plant Physiol. 25, 27-52.
- Martins, S., Machado, S.R., Alves, M. 2008. Anatomia e ultra-estrutura de *Cyperus maritimus* Poir. (Cyperaceae): estratégias adaptativas ao ambiente de dunas litorâneas. Acta Bot. Bras. 22, 289-299.

- Martins, S., Scatena V.L. 2011. Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales). *Aust. J. Bot.* 59, 554-562.
- Murphy, L.R., Barroca, J., Franceschi, V.R., Lee, R., Roalson, E.H., Edwards, G.E., Ku, M.S.B., 2007. Diversity and plasticity of C<sub>4</sub> photosynthesis in *Eleocharis* (Cyperaceae). *Funct. Plant Biol.* 34, 571-580.
- R Development Core Team. 2010. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. (<http://www.R-project.org>).
- Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17, 208–212.
- Rodrigues, A.C., Estelita, M.E.M. 2003. Origin and structure of the Kranz tissue in bracts of *Cyperus giganteus* Vahl (Cyperaceae). *Rev. Brasil. Bot.* 26, 445-452.
- Sage, R.F. 2004. The evolution of C<sub>4</sub> photosynthesis. *New Phytol.* 161, 341-370.
- Soros, C.L., Bruhl, J.J. 2000. Multiple evolutionary origins of C<sub>4</sub> photosynthesis in the Cyperaceae. In: Wilson, K.L., Morrison, D.A. (eds.), *Monocots: systematics and evolution*. CSIRO Publishing, Melbourne, pp. 629-636.
- Tucker, G.C. 1994. Revision of the Mexican species of *Cyperus* L. (Cyperaceae). *Syst. Bot. Mono.* 43, 1–213.
- Ueno, O. 1996. Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C<sub>3</sub> and C<sub>4</sub> metabolism. *Planta* 199, 382-393.
- Ueno, O. 2004. Environmental regulation of photosynthetic metabolism in the amphibious sedge *Eleocharis baldwinii* and comparisons with related species. *Plant Cell Environ.* 27, 627–639.
- Ueno, O., Samejima, M., Koyama, T. 1989. Distribution and evolution of the C<sub>4</sub> syndrome in *Eleocharis*, a sedge group inhabiting wet and aquatic environments, based on culm anatomy and carbon isotope ratios. *Ann. Bot.* 64, 425-438.



Ueno, O., Takeda, T., Maeda, E. 1988. Leaf ultrastructure of C<sub>4</sub> species possessing different Kranz anatomical types in the Cyperaceae. *Bot. Mag. Tokyo* 101, 141-152.

Yoshimura, Y., Kubota, F., Ueno, O. 2004. Structural and biochemical bases of photorespiration in C<sub>4</sub> plants: quantification of organelles and glycine decarboxylase. *Planta* 220, 307-317.

**Table 1.** Chloroplast features of the vascular bundle sheath cells (PCR) in the studied Cyperaceae species (A = absent; Fd = few developed; Md = moderately developed)

Species	Chloroplast (PCR)		
	Thylakoid system	Peripheral Reticulum	Position
<b>Chlorocyperoid</b>			
Cypereae			
<i>Cyperus ligularis</i>	contorted/parallel	A	centrifugal
<i>Kyllinga brevifolia</i>	contorted/parallel	Fd	centrifugal/scattered
Rhynchosporae			
<i>Rhynchospora barbata</i>	contorted/parallel	Fd	centrifugal/scattered
<b>Eleocharoid</b>			
Eleocharideae			
<i>Eleocharis minima</i>	parallel	Md	scattered
<b>Fimbristylid</b>			
Abildgaardieae			
<i>Bulbostylis scabra</i>	contorted/parallel	A	centrifugal
<i>Fimbristylis autumnalis</i>	contorted/parallel	A	centrifugal
<b>Rhynchosporoid</b>			
Rhynchosporae			
<i>Rhynchospora globosa</i>	parallel	A	centrifugal/scattered
<i>Rhynchospora terminalis</i>	parallel	A	centrifugal/scattered

**Table 2.** Number of chloroplasts and mitochondria in the PCA and PCR tissues of the studied Cyperaceae species. Values are given as the means  $\pm$  SD. Asterisks represent a significant difference at  $P < 0.05$  between the PCA and PCR cells and ns represents no significant difference. (CL = chloroplast; MI = mitochondria; PCA = Primary Carbon Assimilation tissue; PCR = Photosynthetic Carbon Reduction tissue)

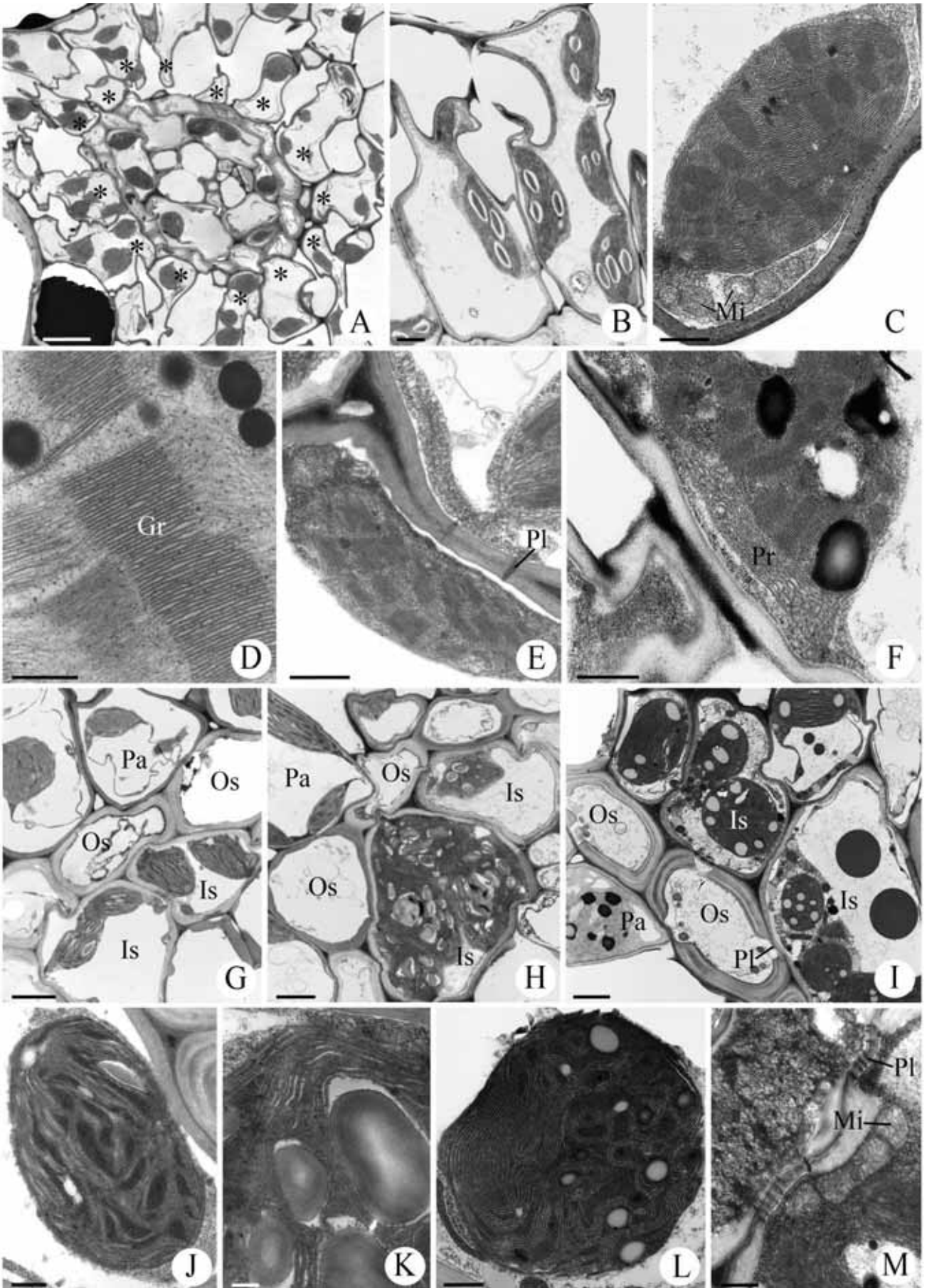
Species	Rariate Parenchyma (PCA)		Bundle sheath (PCR)		Mitochondria (PCR:PCA)
	CL	MI	CL	MI	
<b>Chlorocyperoid</b>					
Cypereae					
<i>Cyperus ligularis</i>	2.7 $\pm$ 0.9	2.2 $\pm$ 1.6	1.5 $\pm$ 0.6*	2.0 $\pm$ 0.7 <sup>ns</sup>	~1
<i>Kyllinga brevifolia</i>	2.9 $\pm$ 1.1	2.5 $\pm$ 0.8	2.2 $\pm$ 0.8	5.6 $\pm$ 1.7*	>1
Rhynchosporeae					
<i>Rhynchospora barbata</i>	3.1 $\pm$ 1.7	1.9 $\pm$ 2.2	2.9 $\pm$ 0.8 <sup>ns</sup>	5.5 $\pm$ 3.3*	>1
<b>Eleocharoid</b>					
Eleocharideae					
<i>Eleocharis minima</i>	2.5 $\pm$ 1.1	3 $\pm$ 1	2.3 $\pm$ 1.2 <sup>ns</sup>	7.7 $\pm$ 5.1*	>1
<b>Fimbristylid</b>					
Abildgaardieae					
<i>Bulbostylis scabra</i>	3.8 $\pm$ 1.2	2.9 $\pm$ 0.8	3.3 $\pm$ 0.9 <sup>ns</sup>	5.8 $\pm$ 1.4*	>1
<i>Fimbristylis autumnalis</i>	2.5 $\pm$ 0.9	1.8 $\pm$ 1.2	2.2 $\pm$ 0.5 <sup>ns</sup>	2.2 $\pm$ 1.7*	>1
<b>Rhynchosporoid</b>					
Rhynchosporeae					
<i>Rhynchospora globosa</i>	3.3 $\pm$ 0.8	1.8 $\pm$ 0.9	1.9 $\pm$ 0.7*	1.7 $\pm$ 0.9 <sup>ns</sup>	~1
<i>R. terminalis</i>	4.6 $\pm$ 2.0	3.6 $\pm$ 1.9	3.9 $\pm$ 2.0 <sup>ns</sup>	3.7 $\pm$ 2.5 <sup>ns</sup>	~1

**Table 3.** Chloroplast features of the vascular bundle sheath cells (PCR) and the ratio of mitochondria between the PCA and PCR tissues of the Cyperaceae species described in previous studies (A = absent; Fd = few developed; Md = moderately developed; PCA = Primary Carbon Assimilation tissue; PCR = Photosynthetic Carbon Reduction tissue; Wd = well developed)

Genera	Chloroplast (PCR)			Mitochondria (PCR:PCA)
	Thylakoid system	Peripheral Reticulum	Position	
<b>Chlorocyperoid</b>				
Cypereae				
<i>Cyperus</i> <sup>2,3,5,6,7</sup>	contorted/convoluted	Fd to Wd	centrifugal	~1
<i>Pycneus</i> <sup>1,7</sup>	convoluted	Md	centrifugal	~1
<i>Remirea</i> <sup>4</sup>	parallel	Wd	centripetal	?
<b>Eleocharoid</b>				
Eleocharideae				
<i>Eleocharis</i> <sup>1,8,9</sup>	parallel	Fd to Md	scattered	>1
<b>Fimbristylid</b>				
Abildgaardieae				
<i>Bulbostylis</i> <sup>7</sup>	convoluted	Fd	centrifugal	~1
<i>Fimbristylis</i> <sup>1,2,3,7</sup>	contorted/convoluted	Fd to Md	centrifugal	~1
<b>Rhynchosporoid</b>				
Rhynchosporae				
<i>Rhynchospora</i> <sup>1,7</sup>	parallel	A to Fd	centrifugal	~1

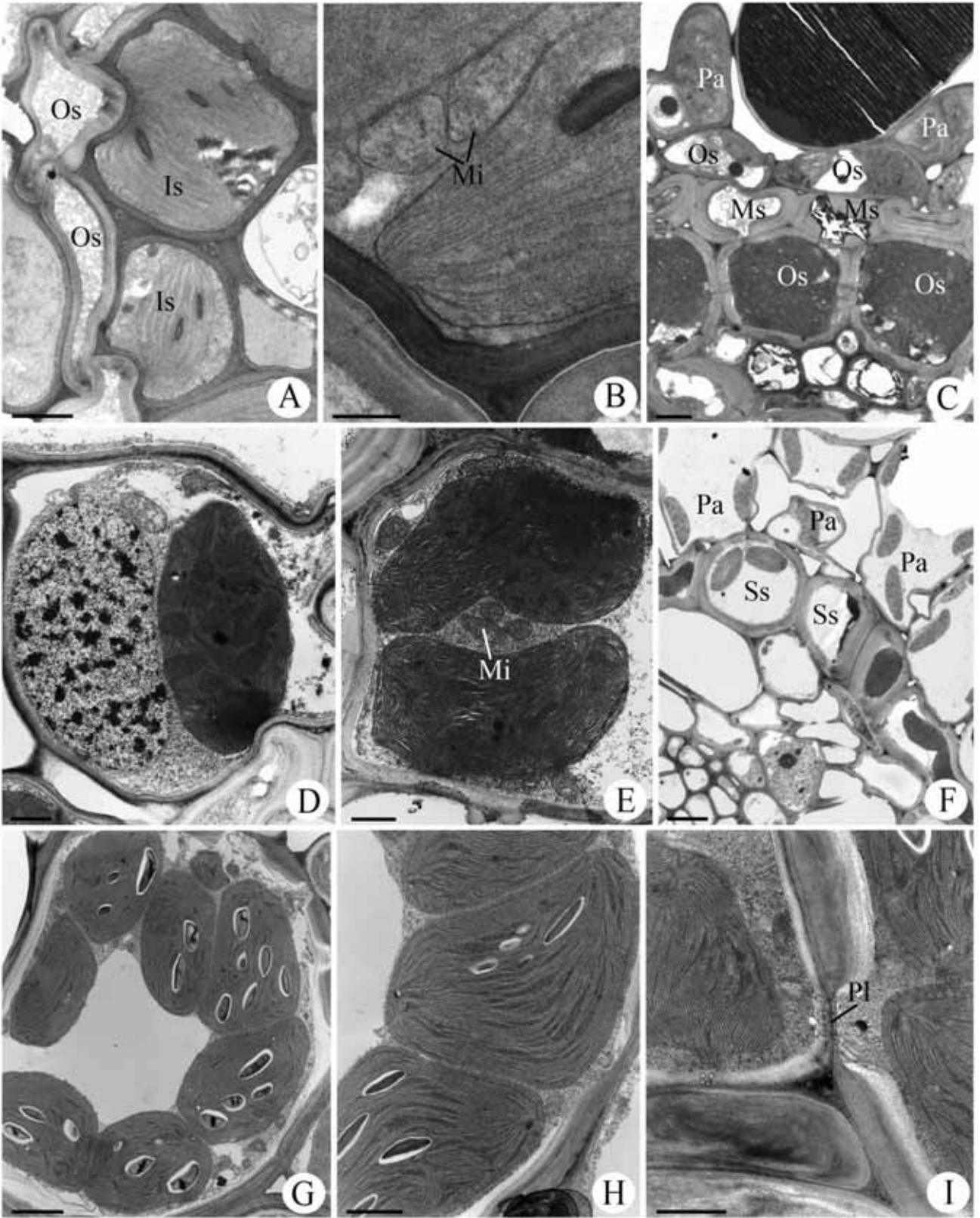
<sup>1</sup>Bruhl & Perry (1995); <sup>2</sup>Carolin *et al.* (1977); <sup>3</sup>Estelita-Teixeira & Handro (1987); <sup>4</sup>Estelita (1993); <sup>5</sup>Kim *et al.* (1999); <sup>6</sup>Rodrigues & Estelita (2003); <sup>7</sup>Ueno *et al.* (1988); <sup>8</sup>Ueno (1996); <sup>9</sup>Ueno (2004)

**Figure 1.** Transmission electron micrographs of leaves of Cyperaceae species with Kranz anatomy in transverse sections showing the radiate parenchyma cells (PCA) (A-F) and vascular bundle sheaths cells (G-M). *Cyperus ligularis* (chlorocyperoid) (A, D, G, J) – general view of the vascular bundle showing the arrangement of radiate parenchyma cells (PCA) (A); detail of the grana in the chloroplast (D); general view of the bundle sheaths (G); detail of the chloroplasts in the inner bundle sheath (J). *Kyllinga brevifolia* (chlorocyperoid) (B, E, H, K, M) – PCA cells with chloroplasts (B); plasmodesmata between the PCA cells (E); general view of the bundle sheaths (H); detail of the chloroplasts in the inner bundle sheath (K); detail of the inner bundle sheath cells showing the mitochondria and plasmodesmata (M). *Fimbristylis autumnalis* (fimbristyloid) – Chloroplasts and mitochondria of the PCA cells (C). *Rhynchospora barbata* (chlorocyperoid) (F, I, L) – detail of the peripheral reticulum of the chloroplast (F); general view of the bundle sheaths (I); detail of the chloroplasts in the inner bundle sheath (L). Asterisk, radiate parenchyma cells; Gr, grana; Is, inner bundle sheath; Mi, mitochondria; Os, outer bundle sheath; Pa, radiate parenchyma cells; Pl, plasmodesmata; Rp, peripheral reticulum. Scale bars: (A) = 5  $\mu\text{m}$ ; (B), (G-I) = 2  $\mu\text{m}$ ; (C) and (E) = 1  $\mu\text{m}$ ; (D), (F), (J), (L-M) = 0.5  $\mu\text{m}$ ; (K) = 0.2  $\mu\text{m}$ .



**Figure 2.** Transmission electron micrographs of leaves of Cyperaceae species with Kranz anatomy in transverse sections showing the vascular bundle sheaths cells. *Eleocharis minima* (eleocharoid) (**A-B**) – general view of the bundle sheaths (**A**); detail of the chloroplasts and mitochondria in the inner bundle sheath (**B**). *Bulbostylis scabra* (fimbristyloid) – general view of the bundle sheaths (**C**). *Fimbristylis autumnalis* (fimbristyloid) – detail of the chloroplasts in the outer bundle sheath and in the inner bundle sheath, respectively (**D-E**). *Rhynchospora globosa* (rhynchosporoid) – general view of the bundle sheaths (**F**). *Rhynchospora terminalis* (rhynchosporoid) (**G-I**) – general view and detail of the chloroplasts in the inner bundle sheath, respectively (**G-H**); plasmodesmata between the inner bundle sheath cells (**I**). Mi, mitochondria; Ms, middle bundle sheath; Os, outer bundle sheath; Pa, radiate parenchyma cells; Ss, single bundle sheath; Is, inner bundle sheath; Pl, plasmodesmata. Scale bars: (F) = 5  $\mu\text{m}$ ; (A), (C), (G) = 2  $\mu\text{m}$ ; (D-E), (H) = 1  $\mu\text{m}$ ; (B) and (I) = 0.5  $\mu\text{m}$ .





## CAPÍTULO 5

**Shirley Martins, Vera Lucia Scatena**

**Dimorphism and different types of Kranz anatomy in scapes of *Eleocharis***

***minima* Kunth (Cyperaceae, Poales)**

(‘Short communication’ submetida ao periódico *Aquatic Botany* em dezembro/2011, retornada em maio/2012 com 90 dias para correção)

## Abstract

In Cyperaceae family, 1/3 of the species have Kranz anatomy, and the species in the *Eleocharis* genus can either present Kranz anatomy or not according to the environmental conditions, whether terrestrial or submerged. *Eleocharis minima* has both emerged and submerged scapes in the same individual. To identify the types of Kranz anatomy and their correlation with the environment, the anatomy of these scapes was investigated in the present study. The emerged scape is quadrangular with four vascular bundles, and the submerged is triangular with three vascular bundles. Both scapes show an anatomy of the eleocharoid type in the median region, differing from the basal region by the presence of the chlorocyperoid Kranz-type anatomy in the submerged and non-Kranz anatomy in the emerged. The scapes also differ in the apical region by the occurrence of eleocharoid Kranz type in the submerged and chlorocyperoid in the emerged. This is the first report of the presence of different types of Kranz anatomy in same individual and same organ in Cyperaceae. The plasticity of the Kranz anatomy in *Eleocharis minima* and other species of this genus is probably related to their amphibious habit. Therefore, the different types of Kranz anatomy in Cyperaceae can constitute a consistent character for phylogeny only in the terrestrial species.

*Keywords:* anatomy, C<sub>4</sub>, *Eleocharis*, flooding, aquatic macrophytes, photosynthesis

## Introduction

Cyperaceae has 45 genera, of which 28 present Kranz anatomy that is related to C<sub>4</sub> photosynthesis (Bruhl and Wilson, 2007). In the family, only *Cyperus*, *Eleocharis*, *Fimbristylis* and *Rhynchospora* contain Kranz and non-Kranz species (Soros and Bruhl, 2000; Bruhl and Wilson, 2007). *Eleocharis* has approximately 250 species (Govaert et al., 2007), and 20 of them were described as having Kranz anatomy (Bruhl and Wilson, 2007). The species of the *Eleocharis* genus occur in environments with moist or flooded soil, and the plants may be partially or totally submerged (Trevisan and Boldrini, 2008). These plants are characterized by a leaf lamina reduced to

a tubular sheath that is located in the base of the scape, which is the principal photosynthetic organ (Hinchliff et al., 2010; Roalson et al., 2010).

As observed in *Eleocharis baldwinii*, *E. retroflexa* and *E. vivipara*, the *Eleocharis* genus stand out in Cyperaceae by the plasticity of having or not having Kranz anatomy in different individuals as a response to environmental conditions (Uchino et al., 1998; Murphy et al., 2007). These species present Kranz anatomy (C<sub>4</sub>) when they are terrestrial and non-Kranz anatomy (C<sub>3</sub>) or a C<sub>3</sub>-C<sub>4</sub> intermediate when they are submerged (Uchino et al., 1998; Ueno, 2004; Murphy et al., 2007).

In addition to the variable presence of Kranz anatomy, different Kranz types were observed in *Eleocharis vivipara* individuals from distinct populations (Murphy et al., 2007). To date, the plasticity of Kranz anatomy in *Eleocharis* has only been described in different individuals growing in distinct environmental conditions, either terrestrial or submerged. Therefore, because *Eleocharis minima*, which performs C<sub>4</sub> photosynthesis (Bruhl and Wilson, 2007), presents both emerged and submerged scapes in the same individual, this work aimed to study the anatomy of these scapes and to identify the possible differences that can be correlated with environmental conditions.

## Materials and Methods

Individuals of *Eleocharis minima* Kunth from different populations (*Martins 405, 406*) occurring in lakes margins from the southern Brazil were collected. Vouchers materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB).

The individuals collected were fixed in FAA 50 and stored in 70% ethanol (Johansen, 1940). Portions of the mature emerged and submerged scapes from three different individuals were dehydrated in an ethyl alcohol series and then embedded in Histo-resin (Leica Histo-resin Embedding Kit, Nussloch, Germany) (Feder and O'Brien, 1968). Transverse sections of the basal, median and apical regions were made using a microtome, stained with periodic acid-Schiff's reagent and toluidine blue (Feder and O'Brien, 1968) and mounted in Entellan (Merck). Transverse sections of

the basal, median and apical regions of mature scapes were also made by hand, stained with basic fuchsin and Astra blue (Roeser, 1972) and mounted in glycerin jelly.

Counting of number of fiber strands and cells that they have was done in 20 different individuals. The images were obtained with Leica DFC 290 digital camera clopped to the Leica DM LB microscope using the IM50 software.

## Results

*Eleocharis minima* is herbaceous, perennial and cespitous (Fig. 1A), with scapes that are approximately 10 cm high and present photosynthetic emerged (Em) (Fig. 1B) and submerged (Sb) (Fig. 1B and C) forms. In transverse section, the emerged scape is quadrangular, with four vascular bundles (Fig. 1D), and the submerged scape is triangular, with three vascular bundles (Fig. 1E).

Both scapes have a uniseriate epidermis (Fig. 1F-K), with thin-walled cells in the basal region of the emerged scape (Fig. 1F) and thick-walled cells other of the emerged scape and of all of the regions of the submerged (Fig. 1G-K). Below the epidermis, fibers occur isolated or in strands (Fi) (Fig. 1F-K); a variability in the number of strands and cells was found according to the scape region (Tab. 1). The epidermal cells associated to the fibers are papillose (Fig. 1I, detail) and have conical silica bodies (Si) (Fig. 1G and I).

The chlorophyllian parenchyma is composed of one layer of cells in the basal region of the emerged scape (Fig. 1F), of two layers in the other regions of the emerged scape and of all of the regions of the submerged scape (Fig. 1I-K). In the inner layer of the regions with two layers, the cells are arranged radially around the vascular bundles (Fig. 1G-K, asterisk). Idioblasts containing phenolic compounds are present in the chlorophyllian parenchyma (Fig. 1F-K).

The scapes possess vascular bundles of different calibers. One major, one intermediate and two small bundles occur in the emerged scapes (Fig. 1D), whereas one major and two small bundles occur in the submerged scapes (Fig. 1E). The emerged scape in the basal region exhibits non-Kranz

anatomy, and the vascular bundles are surrounded by a double sheath: the outer layer presents thin-walled cells with a few chloroplasts, constituting the endodermis (Fig. 1F, circles); and the inner layer presents thick-walled cells without chloroplasts, constituting the pericycle (P) (Fig. 1F). The median region of both scapes present Kranz anatomy of the eleocharoid type, with vascular bundles surrounded by two continuous bundle sheaths: the outer one presents thick-walled cells without chloroplasts, and the inner one presents thin-walled cells with chloroplasts (Fig. 1G and J), both constituting the biseriate pericycle (P). In the apical region of the emerged scape, Kranz anatomy of the chlorocyperoid type occurs that differs from the eleocharoid type in the discontinuity of the inner bundle sheath (Fig. 1H). In the basal region of the submerged scapes, Kranz anatomy of the chlorocyperoid type occurs (Fig. 1I), whereas Kranz anatomy of the eleocharoid type occurs in the apical region (Fig. 1K). Aerenchyma (Ae) occurs in the basal and median regions of the emerged scapes (Fig. 1D; Tab. 1) and in all of the regions of the submerged scapes (Fig. 1E; Tab. 1).

## Discussion

According to our results, the anatomical differences found between the emerged and submerged scapes of *Eleocharis minima* suggest a revision of the species characterization that has been described only with regard to quadrangular scapes (Trevisan and Boldrini, 2008). The submerged scapes are more filamentous than are the emerged scapes; this is probably due to the presence of fewer vascular bundles and cells in the fiber strands, which are both adaptive responses to the amphibious life form. The presence of aerenchyma and the reduced number of vascular bundles and support tissues is common in aquatic plants (Coan et al., 2002; Maberly and Madsen, 2002), including the *Eleocharis* species (Ueno, 1996, 2004). These hydromorphic features of the submerged scapes of *Eleocharis minima* can help the plant fluctuate, favoring its growth in an environment with variations in the water level.



This is the first report of different types of Kranz anatomy and its absence, depending on the region of the emerged and submerged scape, in the same individual of *Eleocharis minima*. In Cyperaceae, four types of Kranz anatomy (chlorocyperoid, eleocharoid, fimbristyloid and rhynchosporoid) that differ in the number and continuity of bundle sheaths and in the localization of the chloroplasts were described (Soros and Dengler, 2001; Martins and Scatena, 2011). Most of the Kranz species in *Eleocharis* present the eleocharoid type, but the fimbristyloid type also occurs in *Eleocharis baldwinii* and the eleocharoid and fimbristyloid types occur in individuals from different populations of *Eleocharis vivipara* (Ueno, 1996; Murphy et al., 2007). These two types of Kranz anatomy differ from each other in the number of bundle sheaths, with two in the eleocharoid and three in the fimbristyloid; and in the continuity of the inner bundle sheath, being continuous in the eleocharoid and discontinuous in the fimbristyloid (Soros and Dengler, 2001). Accordingly, *Eleocharis minima* differs from other species because it possesses the eleocharoid and chlorocyperoid types of Kranz anatomy and a scape region without Kranz anatomy. The chlorocyperoid type differs from the eleocharoid by the discontinuity of the inner bundle sheath and from the fimbristyloid by the presence of two bundle sheaths rather than three (Soros and Dengler, 2001; Martins and Scatena, 2011).

In *Eleocharis minima*, the continuity of the inner bundle sheath varies in accord with the scape region in the same individual, indicating the occurrence of two types of Kranz anatomy, not merely the eleocharoid type, as described by Bruhl and Wilson (2007) and Martins and Scatena (2011). With the plasticity of the Kranz anatomy in different individuals of *Eleocharis vivipara* (Murphy et al., 2007) and in the same individual of *Eleocharis minima*, the inconsistency of this character in taxonomical and phylogenetic studies of the *Eleocharis* genus is prominent.

In another species of *Eleocharis*, differences in the type of Kranz anatomy and in the photosynthetic metabolism in response to environmental conditions were verified as being related to the availability of water in the soil (Ueno et al., 1989; Uchino et al., 1998; Ueno, 2004). In these



species, which are considered facultatively C<sub>3</sub>-C<sub>4</sub>, the terrestrial individuals present Kranz anatomy, and the submerged individuals present non-Kranz anatomy or Kranz-like anatomy (Murphy et al., 2007). In *Eleocharis minima*, all of the regions of the submerged scapes possess Kranz anatomy, as characterized by the presence of chloroplasts in the inner bundle sheath. The occurrence of Kranz anatomy in aquatic plants is correlated with a low level of CO<sub>2</sub> in the water (Bowes et al., 2002), explaining its presence in the submerged scapes of *Eleocharis minima*. The absence of Kranz anatomy in the basal region of the emerged scapes can be related to the occurrence of leaf sheaths in this region, which probably reduces the photosynthetic activity in this area of the scape.

In the angiosperm groups with C<sub>4</sub> photosynthesis, including in Cyperaceae, the presence and the type of Kranz anatomy are important for phylogenetic studies (Soros and Bruhl, 2000; Giussani et al., 2001; Muhaidat et al., 2007). However, due to the plasticity of the Kranz anatomy found in *Eleocharis minima*, we suggest that these characters can only be consistent in the phylogeny of the terrestrial species.

### **Acknowledgements**

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 the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financial support (301692/2010-6) to V. L. Scatena.

### **References**

- Bowes, G., Rao, S.K., Estavillo, G.M., Reisking, J.B., 2002. C<sub>4</sub> mechanisms in aquatic angiosperms: comparisons with terrestrial C<sub>4</sub> system. *Func. Plant Biol.* 29, 379-392.
- Bruhl, J.J., Wilson, K.L., 2007. Towards a comprehensive survey of C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways in Cyperaceae. In: Columbus, J.T., Friar, E.A., Hamilton, C.W., Porter, J.M.,

- Prince, L.M., Simpson, M.G. (Eds.), Monocots: comparative biology and evolution. Rancho Santa Ana Botanic Garden, Claremont, pp. 99-148.
- Coan, A.I., Scatena, V.L., Giulietti, A.M., 2002. Anatomia de algumas espécies aquáticas de Eriocaulaceae brasileiras. *Acta Bot. Bras.* 16, 371–384.
- Feder, N., O'Brien, T.P., 1968. Plant microtechnique: some principles and new methods. *Am. J. Bot.* 55, 123-142.
- Giussani, M.G., Cota-Sánchez, H., Zuloaga, F.O., Kellogg, E.A., 2001. A molecular phylogeny of the Grass subfamily Panicoideae (Poaceae) shows multiple origins of C<sub>4</sub> photosynthesis. *Am. J. Bot.* 88, 1993-2012.
- Govaerts, R., Simpson, D.A., Goetghebeur, P., Wilson, K.L., Egorova, T., Bruhl, J.J., 2007. World checklist of Cyperaceae. The Board of Trustees of the Royal Botanical Garden, Kew.
- Hinchliff, C.E., Lliully, A.E., Carey, T., Roalson, E.H., 2010. The origins of *Eleocharis* (Cyperaceae) and the status of *Websteria*, *Egleris*, and *Chillania*. *Taxon* 59, 709-719.
- Johansen, D., 1940. Plant microtechnique. McGraw-Hill Book Co. Inc., New York.
- Maberly, S.C., Madsen, T.V., 2002. Aquatic freshwater angiosperm carbon concentrating mechanisms: processes and patterns. *Func. Plant Biol.* 29, 393-405.
- Martins, S., Scatena, V.L., 2011. Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales). *Aust. J. Bot.* 59, 554-562.
- Muhaidat, R., Sage, R.F., Dengler, N.G., 2007. Diversity of Kranz anatomy and biochemistry in C<sub>4</sub> eudicots. *Am. J. Bot.* 94, 362-381.
- Murphy, L.R., Barroca, J., Franceschi, V.R., Lee, R., Roalson, E.H., Edwards, G.E., Ku, M.S.B., 2007. Diversity and plasticity of C<sub>4</sub> photosynthesis in *Eleocharis* (Cyperaceae). *Func. Plant Biol.* 34, 571-580.

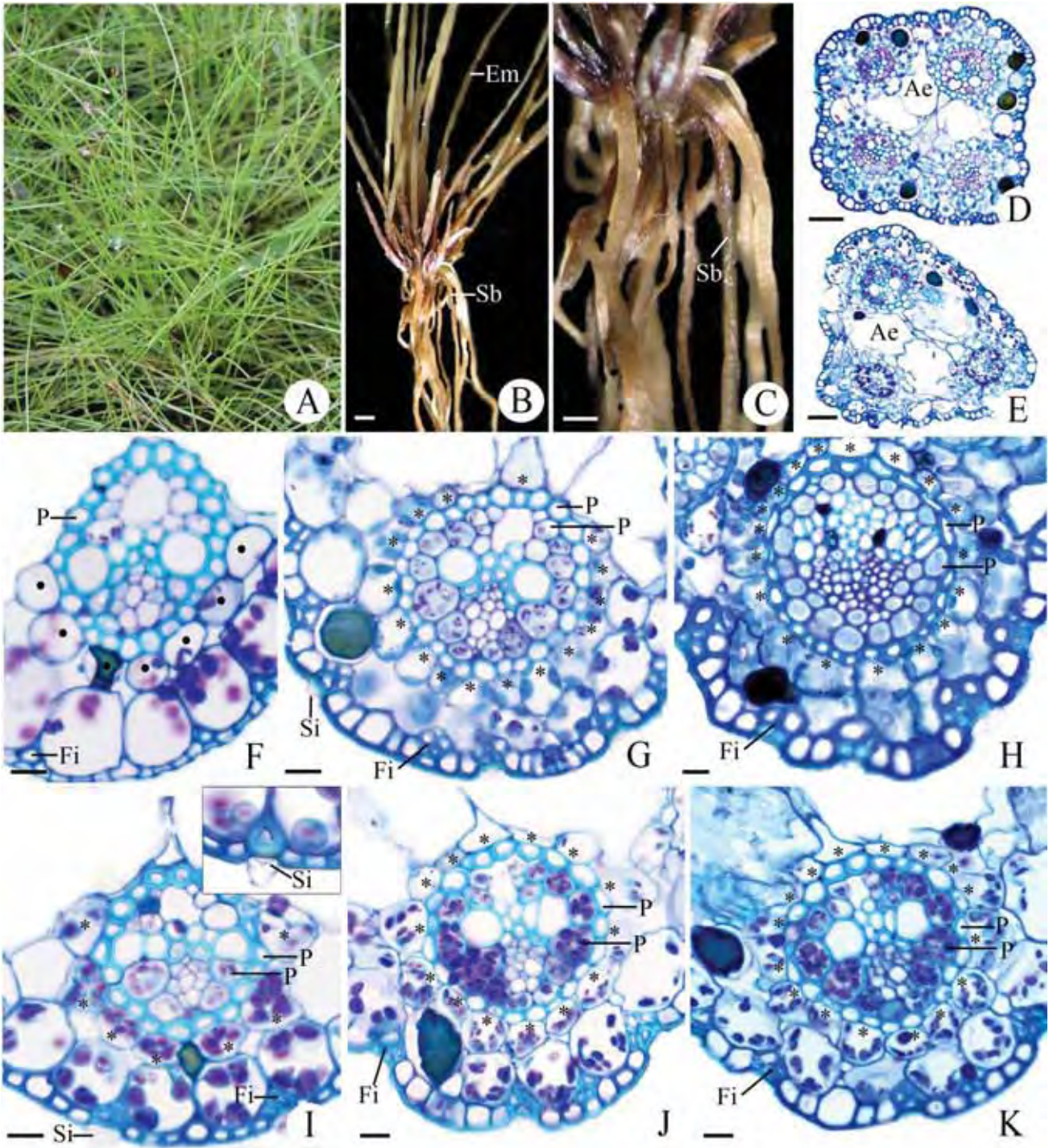
- Roalson, E.H., Hinchliff, C.E., Trevisan, R., Silva, C.R.M., 2010. Phylogenetic relationships in *Eleocharis* (Cyperaceae): C<sub>4</sub> Photosynthesis origins and patterns of diversification in the spikerushes. *Syst. Bot.* 35, 257-271.
- Roeser, K.R., 1972. Die nadel der schwarzkiefer - massenprodukt und kunstwerk der natur. *Mikrokosmos* 61, 33-36.
- Soros, C.L., Bruhl, J.J., 2000. Multiple evolutionary origins of C<sub>4</sub> photosynthesis in the Cyperaceae. In: Wilson, K.L., Morrison, D.A. (Eds.), *Monocots: systematics and evolution*. CSIRO Publishing, Melbourne, pp. 629-636.
- Soros, C.L., Dengler, N.G., 2001. Ontogenetic derivation and cell differentiation in photosynthetic tissues of C<sub>3</sub> and C<sub>4</sub> Cyperaceae. *Am. J. Bot.* 88, 992-1005.
- Uchino, A., Sentoku, N., Nemoto, K., Ishii, R., Samejima, M., Matsuoka, M., 1998. C<sub>4</sub>-type gene expression is not directly dependent on Kranz anatomy in an amphibious sedge *Eleocharis vivipara* Link. *Plant J.* 14, 565-572.
- Ueno, O., 1996. Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C<sub>3</sub> and C<sub>4</sub> metabolism. *Planta* 199, 382-393.
- Ueno, O., 2004. Environmental regulation of C<sub>3</sub> and C<sub>4</sub> differentiation in the amphibious sedge, *Eleocharis baldwinii* and comparisons with related species. *Plant Cell Environ.* 27, 627-639.
- Ueno, O., Takeda, T., Maeda, E., 1989. Distribution and evolution of C<sub>4</sub> syndrome in *Eleocharis*, a sedge group inhabiting wet and aquatic environments, based on culm anatomy and carbon isotope ratios. *Ann. Bot.* 64, 425-438.
- Trevisan, R., Boldrini, I.I., 2008. O gênero *Eleocharis* R. Br. (Cyperaceae) no Rio Grande do Sul, Brasil. *Rev. Bras. Bio.* 6, 7-67.

**Table 1.** Anatomical differences between emerged and submerged scapes of *Eleocharis minima* (K-C = Kranz clorocyperoid; K-E = Kranz eleocharoid)

Characteristics	Emerged			Submerged		
	Basal	Median	Apical	Basal	Median	Apical
Scape shape in transverse section	quadrangular	quadrangular	quadrangular	triangular	triangular	triangular
Kranz anatomy	absent	K-E	K-C	K-C	K-E	K-E
Number of fiber isolated or in strands	23-27	22-26	17-19	25-26	20-22	21-22
Number of cells in the fiber strands	1-2(3)	4-6(3)	5-9 (3)	1-2(3)	2-5(1)	2-4
Aerenchyma	present	present	absent	present	present	present

**Fig 1.** Morphological and anatomical aspects of emerged and submerged scapes of *Eleocharis minima* Kunth. (A-C) Morphological aspects; (A) Habit; (B) Mature plants with emerged (Em) and submerged (Sb) scapes (bar = 4 cm); (B) Mature plant detaching submerged scape (Sb) (bar = 2 cm); (D-K) Anatomical aspects; (D-E) General view of the median regions of emerged and submerged scapes, respectively (bar = 30  $\mu\text{m}$ ); (F-H) Detail of the basal, median and apical regions of the emerged scape, respectively (bar = 10  $\mu\text{m}$ ); (I-K) Detail of the basal, median and apical regions of the submerged scape, respectively (bar = 10  $\mu\text{m}$ ) (Ae, aerenchyma; asterisks, radiate parenchyma cells; circles, endodermis; Em, emerged scape; Fi, fibers; P, pericycle; Sb, submerged scape; Si, silica bodies).





## **CAPÍTULO 6**

**Shirley Martins, Marccus Alves, Vera Lucia Scatena**

**Ocorrência e inferência evolutiva da anatomia Kranz em Cyperaceae (Poales)**

(Manuscrito a ser submetido ao periódico *Plant Systematic and Evolution*)



## Resumo

Cyperaceae é uma das famílias de angiospermas com maior diversidade de espécies com anatomia Kranz. O estudo de sua ocorrência na família é importante para sua caracterização anatômica e ultraestrutural e também para traçar hipóteses evolutivas. Este trabalho visou levantar dados sobre anatomia Kranz de Cyperaceae, disponíveis na literatura e também de espécies aqui estudadas, reunindo taxonomia, locais de ocorrência, hábito, habitat e características estruturais procurando inferir sua possível origem na família. Verificou-se que a anatomia Kranz (relacionada à fotossíntese  $C_4$ ) surgiu múltiplas vezes em Cyperaceae e em grupos pouco relacionados filogeneticamente. As possíveis origens refletem as alterações estruturais da anatomia Kranz (quatro diferentes tipos) provavelmente como resultado de pressões ambientais.

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

## Abstract

Cyperaceae is one of the angiosperms families with the greatest diversity of species with Kranz anatomy. The study of Kranz anatomy occurrence is important to the anatomical and ultrastructural characterization and also to draw evolutive hypotheses. This work aimed to bring up data about Kranz anatomy in Cyperaceae, available in the literature and also the species studied here, gathering taxonomy, places of occurrence, habit, habitat and structural features looking infer its possible origin in the family. It was verified that the Kranz anatomy (related to  $C_4$  photosynthesis) arose multiple times in Cyperaceae and in groups few phylogenetic related. The possible origins reflect the structural changes of the Kranz anatomy (four anatomical types), probably as a result of environmental pressures.

**Keywords:** Kranz anatomy, ultrastructure, taxonomy, phylogeny, Cyperaceae

## Introdução

A anatomia Kranz, característica relacionada ao metabolismo fotossintético  $C_4$ , foi descrita inicialmente para em espécies de Cyperaceae (Haberlandt 1914). Desde então, foram realizados estudos estruturais (Carolin et al. 1977; Takeda et al. 1985; Bruhl 1995; Bruhl e Perry 1995; Soros e Dengler 2001; Martins e Alves 2009; Martins e Scatena 2011) e bioquímicos (Bruhl et al. 1987; Ueno e Samejima 1989; Bruhl e Perry 1995) com representantes Kranz da família. As variações (ou tipos) da anatomia Kranz reconhecidas em Cyperaceae como clorociperóide, eleocaróide, fimbristilóide e rincosporóide, diferem entre si no número e continuidade ou não das bainhas vasculares e na localização dos cloroplastos (Soros e Dengler 2001; Martins e Scatena 2011).

Os representantes de Cyperaceae com anatomia Kranz são restritos à Cyperoideae e ocorrem em quatro das suas 13 tribos: Abildgaardieae, Cypereae, Eleocharideae e Rhynchosporeae (Goetghebeur 1998). Dessas, apenas Abildgaardieae e Eleocharideae aparecem como grupos próximos entre si nas análises filogenéticas (Ghamkhar et al. 2007; Muasya et al. 2008).

Cypereae é a única tribo que apresenta espécies com apenas um tipo de anatomia Kranz: clorociperóide (Bruhl e Wilson 2007), enquanto que em Abildgaardieae ocorrem os tipos eleocaróide e fimbristilóide (Bruhl e Wilson 2007), em Eleocharideae os tipos clorociperóide, eleocaróide e fimbristilóide (Murphy et al. 2007; Martins e Scatena, dados não publicados) e em Rhynchosporeae os tipos clorociperóide e rincosporóide (Martins e Scatena 2011). A ocorrência da anatomia Kranz em grupos taxonômicos distintos sugere seu surgimento quatro vezes em Cyperaceae, uma em cada tribo (Soros e Bruhl 2000). No entanto, estudos desenvolvidos com marcadores genéticos nas espécies com fotossíntese  $C_4$ , propõem cinco origens para a família (Besnard et al. 2009). Além disso, filogenias de Abildgaardieae (Ghamkhar et al. 2007) e Eleocharideae (Roalson et al. 2010), mostram a possível origem múltipla da anatomia Kranz em ambas.

Diante do exposto, esse trabalho buscou reunir dados taxonômicos, ecológicos, estruturais e filogenéticos associados às espécies com os quatro tipos Kranz registrados em Cyperaceae visando possíveis hipóteses sobre sua origem.

## **Material e Métodos**

As espécies estudadas estão listadas na Tabela 1 e os vouchers estão depositados no Herbário HRCB do Departamento de Botânica – IB – UNESP – Rio Claro. Para ilustrar anatomicamente os diferentes tipos de anatomia Kranz, porções de folhas e escapos adultos foram fixados em FAA 50 e conservados em álcool 70% (Johansen 1940), desidratadas em série etílica e infiltradas em historresina (Leica Historesin Embedding Kit), seguidas da inclusão (Feder e O'Brien 1968). Foram seccionadas com o auxílio de micrótomo rotativo e as secções foram submetidas à coloração com ácido periódico-reativo de Schiff (PAS) e azul de toluidina (Feder e O'Brien 1968) e montadas em Entellan. As imagens foram obtidas com câmera digital Leica DFC 290 acoplada ao microscópio Leica DM LB, usando o programa IM50.

Para ilustrar as características ultraestruturais dos diferentes tipos de anatomia Kranz amostras da região mediana de folhas e escapos adultos foram fixadas em glutaraldeído em tampão fosfato 0,1 M, pH 7,3, pós-fixadas em tetróxido de ósmio a 0,5%, desidratadas em série crescente de acetona e incluídas em Araldite. Foram realizadas secções transversais ultrafinas, contrastadas com citrato de chumbo (Reynolds 1963) e acetato de uranila etílica (Watson 1958) e analisadas ao microscópio eletrônico de transmissão Phillips EM 100, 80kV.

Para observar a distribuição dos caracteres relacionados à anatomia Kranz em Cyperaceae, os dados estruturais das espécies aqui estudadas e das descritas na literatura foram plotados nos cladogramas previamente publicados para as tribos com espécies Kranz da família (Ghamkar et al. 2007; Muasya et al. 2008; Thomas et al. 2008; Roalson et al. 2010).

## Resultados

**Tribo Abildgaardieae** – possui seis gêneros com anatomia Kranz: *Abildgaardia*, *Bulbostylis*, *Crosslandia*, *Fimbristylis*, *Nelmesia* e *Nemum*, com apenas *Abildgaardia hygrophila* e *Fimbristylis variegata* não Kranz (C<sub>3</sub>) (Tab. 2, Fig. 1A). Apresenta o tipo fimbristilóide, com exceção de uma espécie de *Bulbostylis* descrita com o tipo eleocaróide. O tipo fimbristilóide é caracterizado por feixes vasculares envolvidos por três bainhas, a externa (Be) e a mediana (Bm) contínuas, e a interna (Bi) contínua ou descontínua nos feixes de maior calibre, interrompida por elementos de metaxilema (Fig. 1B-C). A externa origina-se do meristema fundamental, a mediana e a interna do procâmbio. Nas células da bainha externa os cloroplastos apresentam grana bem desenvolvido, semelhantes aos das células do parênquima radiado. Nas da bainha mediana os cloroplastos estão ausentes e nas da bainha interna são centrífugos ou aleatórios (Fig. 1D), com grana ausente ou reduzido e tilacóides contorcidos ou paralelos (Fig. 1E).

**Tribo Cypereae** – possui 19 gêneros dos quais 10 com espécies Kranz, sendo nove exclusivamente Kranz (C<sub>4</sub>) (*Alinula*, *Ascolepis*, *Kyllinga*, *Lipocarpha*, *Pycreus*, *Queenslandiella*, *Remirea*, *Sphaerocyperus* e *Volkiella*) e em *Cyperus*, restrito à *Cyperus* subg. *Cyperus* (Tab. 2, Fig. 2A). Apresenta o tipo clorociperóide, caracterizado por feixes vasculares envolvidos por duas bainhas: a externa (Be) contínua e a interna (Bi) descontínua nos feixes de maior calibre e ambas com origem procambial (Fig. 2B). Nas células da bainha externa os cloroplastos estão ausentes (Fig. 2C) e na interna estão presentes com distribuição centrífuga ou aleatória (Fig. 2C), grana ausente ou reduzido e tilacóides convolutos ou contorcidos (Fig. 2D-E).

**Tribo Eleocharideae** – possui apenas o gênero *Eleocharis* (250 spp.) com espécies Kranz (C<sub>4</sub>) e não Kranz (C<sub>3</sub>) (Tab. 2), além de espécies facultativas C<sub>3</sub>/C<sub>4</sub> e intermediárias C<sub>3</sub>-C<sub>4</sub>. As folhas têm lâmina reduzida à bainha sendo o escapo, o órgão fotossintetizante. Apresenta os tipos eleocaróide, fimbristilóide e clorociperóide (Fig. 3A, B). Nas do tipo eleocaróide, as duas bainhas em torno dos feixes vasculares (Fig. 3C) são contínuas e com origem procambial. Nas células da bainha externa

(Be) os cloroplastos estão ausentes e nas da bainha interna (Bi) estão distribuídos aleatoriamente e com tilacóides paralelos, às vezes formando grana (Fig. 3D-E). Os tipos fimbristilóide e clorociperóide são semelhantes aos descritas anteriormente para *Abildgaardieae* e *Cypereae*.

**Tribo Rhynchosporeae** – possui dois gêneros: *Pleurostachys* e *Rhynchospora* e apenas *Rhynchospora* apresenta espécies com anatomia Kranz (C<sub>4</sub>) e restritas às seções *Pauciflorae* (Pau) e *Pluriflorae* (Plu) do grupo *Capitatae* (Fig. 4A). Apresenta os tipos clorociperóide e rincosporóide (Fig. 4A). O tipo clorociperóide possui duas bainhas em torno dos feixes vasculares, ambas com origem procambial, sendo a externa contínua e a interna descontínua apenas nos feixes de maior calibre (Fig. 4B). Nas células da bainha externa (Be) cloroplastos estão ausentes e nas da bainha interna (Bi) estão distribuídos centrífuga ou aleatoriamente, com grana reduzido ou ausente e tilacóides contorcidos ou paralelos (Fig. 4C). O tipo rincosporóide apresenta apenas uma bainha vascular (Bs – bainha simples) que é contínua (Fig. 4D) e de origem procambial, com cloroplastos distribuídos aleatoriamente, grana ausente ou reduzido e tilacóides paralelos (Fig. 4E).

## Discussão

Os quatro tipos de anatomia Kranz indicados para *Cyperaceae* (Soros e Bruhl 2000; Soros e Dengler 2001), assim como sua distribuição nas tribos (Ueno e Koyama 1987; Bruhl 1995; Soros e Bruhl 2000; Murphy et al. 2007), foram confirmados nas espécies aqui estudadas, indicando sua possível origem múltipla.

Ghamkar et al. (2007), propõem uma filogenia na qual *Abildgaardieae*, cuja maioria das espécies apresenta anatomia Kranz, emerge como uma tribo não-monofilética. *Bulbostylis* diverge inicialmente e tem como grupo-irmão um clado formado pelo agrupamento *Abildgaardia-Crosslandia-Fimbristylis* + *Arthrostylis-Actinoschoenus* (tribo *Arthrostylideae*, exclusivamente não Kranz -C<sub>3</sub>). Isto sugere o aparecimento da anatomia Kranz (C<sub>4</sub>) inicialmente em *Bulbostylis*,

corroborando Besnard et al. (2009) que estimaram este evento em *Bulbostylis* para ca.  $19.6 \pm 4.9$  Ma, seguido de *Fimbristylis* ca.  $12.3 \pm 3.8$  Ma.

Os representantes com anatomia Kranz da tribo apresentam semelhanças estruturais no tipo, na distribuição e organização dos cloroplastos (Sharma e Mehra 1972; Carolin et al. 1977; Bruhl e Perry 1995; Martins e Scatena, dados não publicados). Assim, duas hipóteses podem ser consideradas: (1) características estruturais semelhantes da anatomia Kranz ( $C_4$ ) surgiram independentemente em linhagens distintas no agrupamento que inclui *Abildgaardieae* e *Arthrostylideae*; (2) características estruturais de  $C_4$  surgiram no ancestral que deu origem ao agrupamento que inclui *Abildgaardieae* e *Arthrostylideae*, com a reversão da via  $C_4$  para  $C_3$  em *Arthrostylideae* e em *Abildgaardia hygrophila* e *Fimbristylis variegata*. A segunda hipótese parece mais provável, não apenas por ser mais parcimoniosa, mas também porque sugere que a reversão  $C_4$ - $C_3$  em *Arthrostylideae* foi bem sucedida e apenas como eventos pontuais em *Abildgaardieae*, com uma espécie de *Abildgaardia* e uma de *Fimbristylis*. Além disto, as características anatômicas e bioquímicas semelhantes em *Bulbostylis* e demais gêneros inseridos em *Abildgaardieae* apontam para origem comum. A predileção por ambientes com estresse hídrico, altas temperaturas e luminosidade, onde o metabolismo  $C_4$  é mais vantajoso (Sage 2004), é outro fator comum às espécies Kranz de *Abildgaardieae*.

Em *Cypereae*, as espécies Kranz emergem em um mesmo clado (Muasya et al. 2002, 2009), compartilhando o tipo clorociperóide, a distribuição centrífuga ou aleatória dos cloroplastos, grana ausente ou reduzido e tilacóides convolutos ou contorcidos (Carolin et al. 1977; Bruhl e Perry 1995; Martins e Scatena, dados não publicados). Com isso, é provável que um único evento tenha dado origem à anatomia Kranz ( $C_4$ ) no grupo, a partir de ancestrais  $C_3$ . Marcadores genéticos mostram o surgimento da fotossíntese  $C_4$  em *Cyperus* ca.  $10.9 \pm 3.4$  Ma, portanto, um dos primeiros grupos da família com tal característica (Besnard et al. 2009).

Ao contrário de *Abildgaardieae*, em *Cypereae* a maioria das espécies Kranz ( $C_4$ ) ocupa ambientes abertos com solos encharcados (Goetghebeur 1998), indicando um sentido evolutivo inverso ao postulado para a tribo anterior. Pois, no caso de plantas que crescem em ambientes com alta disponibilidade hídrica, o metabolismo fotossintético  $C_4$ , segundo Bowes e Salvucci (1989) e Bowes et al. (2002), está relacionado à alta densidade das plantas, altas temperatura e luminosidade.

Na tribo *Eleocharideae*, poucas espécies apresentam anatomia Kranz ( $C_4$ ), ocorrendo ainda as intermediárias  $C_3$ - $C_4$ , as semelhantes à  $C_4$  e as facultativas  $C_3$ - $C_4$  (Murphy et al. 2007). As facultativas podem mudar seu metabolismo fotossintético dependendo do ambiente, reduzindo a expressão da anatomia Kranz no ambiente aquático (Ueno et al. 1989; Ueno 1996, 2004; Murphy et al. 2007). Os tipos indicados para a tribo ocorrem em espécies distintas (Murphy et al. 2007), em indivíduos de uma mesma espécie de ambientes diferentes (Murphy et al. 2007) e em diferentes partes do escapo do mesmo indivíduo, como *Eleocharis minima* (Martins e Scatena, dados não publicados). Diante dessa plasticidade o posicionamento das espécies nos tipos de anatomia Kranz fica duvidoso, bem como asserções sobre sua evolução no grupo. O surgimento da fotossíntese  $C_4$  em *Eleocharis* é considerado, segundo Besnard et al. (2009), como recente (*E. vivipara* – ca.  $10.5 \pm 3.2$ ; *E. baldwinii* –  $4.4 \pm 2.1$ ) quando comparado às demais espécies  $C_4$  da família e as modificações genéticas não são significativas. É possível que este evento tenha surgido inúmeras vezes em associação com cada clado do gênero onde emergem espécies Kranz, porém tal proposta ainda é frágil em virtude da complexidade estrutural detectada no gênero. Como as espécies Kranz de *Eleocharis* crescem no ambiente aquático (Murphy et al. 2007) é possível que as condições do ambiente estejam promovendo tal plasticidade, como observado para outras estruturas como diferença no número de feixes vasculares e na formação de aerênquima (Martins e Scatena, dados não publicados).

*Rhynchosporeae* apresenta espécies com anatomia Kranz ( $C_4$ ) dos tipos clorociperóide e rincosporóide restritos a *Rhynchospora* seção *Pauciflorae* e *R.* seção *Pluriflorae* (Ueno e Koyama



1987). Na filogenia da tribo (Thomas et al. 2008), ambas emergem como grupos derivados e em clados distintos indicando duas possíveis origens para a anatomia Kranz a partir de ancestrais não Kranz. No entanto, o baixo número de espécies Kranz analisadas filogeneticamente impossibilita proposta mais consistente. Marcadores genéticos indicam que a via  $C_4$  é recente no gênero (ca.  $7.4 \pm 2.8$  Ma) quando comparada com as demais da família (Besnard et al. 2009). As espécies com anatomia Kranz ( $C_4$ ) de *Rhynchospora* são principalmente neotropicais e habitam áreas campestres e savanícolas (Ueno e Koyama 1987) e, portanto, é possível que a via  $C_4$  tenha surgido em algumas linhagens do gênero como adaptação às condições de estresse hídrico, altas temperaturas e luminosidade.

Corroborar-se aqui a proposta da múltipla origem da anatomia Kranz em Cyperaceae e em grupos com baixa afinidade filogenética postulada direta ou indiretamente por Soros e Bruhl (2000), Ghamkar et al. (2007), Muasya et al. (2002, 2008, 2009), Thomas et al. (2008) e Roalson et al. (2010) e que a variação estrutural pode ser resultado das pressões ambientais. Porém, apesar das espécies Kranz de Abildgaardieae e Rhynchosporeae ocorrerem em ambientes distintos das de Cypereae e Eleocharideae todos eles promovem a fotorrespiração, que é reduzida com o metabolismo  $C_4$ , sendo apontado como principal causa do surgimento da via  $C_4$  nas espécies da família. Postula-se aqui, que independente do ambiente de ocorrência e sim da pressão metabólica, linhagens distintas de Cyperaceae tenham encontrado caminhos evolutivos similares para o surgimento de uma via fotossintética mais eficiente, tornando tais espécies mais competitivas em seu habitat.

Vale ressaltar ainda que a maior variação infragenérica da anatomia Kranz em Cyperaceae é observada nas espécies aquáticas de *Eleocharis*, diferente das espécies terrestres onde esse caráter aparentemente é bem fixado. Assim, o uso da anatomia Kranz em abordagens taxonômicas e filogenéticas parece ser mais confiável com base em grupos predominantemente terrestres.

## Referências bibliográficas

- Besnard G, Muasya AM, Russier F, Roalson NS, Christin PA (2009) Phylogenomics of C<sub>4</sub> photosynthesis in sedges (Cyperaceae): Multiple appearances and genetic convergence. *Mol Biol Evol* 26: 1909-1919.
- Bowes G, Rao SK, Estavillo GM, Reiskind JB (2002) C<sub>4</sub> mechanisms in aquatic angiosperms: comparisons with terrestrial C<sub>4</sub> system. *Func Plant Biol* 29: 379-392.
- Bowes G, Salvucci ME (1989) Plasticity in the photosynthesis carbon metabolism of submersed aquatic macrophytes. *Aq Bot* 34: 233-266.
- Bruhl JJ (1995) Sedge genera of the world: relationships and new classification of the Cyperaceae. *Aust Syst Bot* 8: 125-305.
- Bruhl JJ, Perry S (1995) Photosynthetic pathway-related ultrastructure of C<sub>3</sub>, C<sub>4</sub> and C<sub>3</sub>-C<sub>4</sub> intermediate sedge (Cyperaceae), with special reference to *Eleocharis*. *Aust J Plant Physio* 22: 521-530.
- Bruhl JJ, Stone NE, Hattersley PW (1987) C<sub>4</sub> acid decarboxylation enzymes and anatomy in sedges (Cyperaceae): first record of NAD-malic enzyme species. *Aust J Plant Physio* 22: 521-530.
- Bruhl JJ, Wilson KL (2007) Towards a comprehensive survey of C<sub>3</sub> and C<sub>4</sub> photosynthetic pathway in Cyperaceae. *In Monocots: comparative biology and evolution* (J.T. Columbus, E.A. Friar, C.W. Hamilton, J.M. Porter, L.M. Prince e M.G. Simpson, eds.). Rancho Santa Ana Botanic Garden: Claremont, p. 99-148.
- Carolin RC, Jacobs SWL, Vesik M (1977) The ultrastructure of Kranz cells in the family Cyperaceae. *Bot Gaz* 138: 413-419.
- Feder N, O'Brien TP (1968) Plant microtechnique: some principles and new methods. *Am J Bot* 55: 123-142.
- Ghamkhar K, Marchant AD, Wilson KL, Bruhl JJ (2007) Phylogeny of Abildgaardieae (Cyperaceae) inferred from ITS and *trnL-F* data. *Aliso* 23: 149-164.

- Goetghebeur P (1998) Cyperaceae. *In* The families and genera of vascular plants (K. Kubitzki, H. Huber, P.J. Rudall, P.S. Stevens e T. Stützel, eds.). Springer-Verlag, Berlin, p. 141-190.
- Haberlandt GFJ (1914) 'Physiological plant anatomy'. (MacMillan: London)
- Johansen D (1940) 'Plant microtechnique'. (McGraw-Hill Book Co. Inc.: New York)
- Martins S, Alves, M (2009) Anatomical features of species of Cyperaceae from northeastern Brazil. *Brittonia* 61: 189-200.
- Martins S, Scatena VL (2011) Bundle sheath ontogeny in Kranz and non-Kranz species of Cyperaceae (Poales). *Aust J Bot* 59: 554-562.
- Muasya AM, Simpson DA, Chase MW (2002) Phylogenetic relationships in *Cyperus* L. *s.l.* (Cyperaceae) inferred from plastid DNA sequence data. *Bot J Linn Soc* 138: 145-153.
- Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MW, Smets E (2008) Phylogeny of Cyperaceae based on DNA sequence data: Current progress and future prospects. *Bot Rev* 75: 2-21.
- Muasya AM, Vrijdaghs A, Simpson DA, Chase MW, Goetghebeur P, Smets E (2009) What is a genus in Cyperaceae: phylogeny, character homology assessment and generic circumscription in Cyperaceae. *Bot Rev* 75: 52-66.
- Murphy LR, Barroca J, Franceschi VR, Lee R, Roalson EH, Edwards GE, Ku MSB (2007) Diversity and plasticity of C<sub>4</sub> photosynthesis in *Eleocharis* (Cyperaceae). *Func Plant Biol* 34: 571-580.
- Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* 17: 208-212.
- Roalson EH, Hinchliff CE, Trevisan R, Silva CRM (2010) Phylogenetic relationships in *Eleocharis* (Cyperaceae): C<sub>4</sub> Photosynthesis origins and patterns of diversification in the spikerushes. *Syst Bot* 35: 257-271.
- Sage RF (2004) The evolution of C<sub>4</sub> photosynthesis. *New Phytologist* 161: 341-370.

- Sharma OP, Mehra PN (1972) Systematic anatomy of *Fimbristylis* Vahl (Cyperaceae). Bot Gaz 133: 87-95.
- Soros CL, Bruhl JJ (2000) Multiple evolutionary origins of C<sub>4</sub> photosynthesis in the Cyperaceae. In Monocots: systematics and evolution (K.L. Wilson, D.A e Morrison DA, eds.) CSIRO Publishing, Melbourne, p. 629-636.
- Soros CL, Dengler NG (2001) Ontogenetic derivation and cell differentiation in photosynthetic tissues of C<sub>3</sub> and C<sub>4</sub> Cyperaceae. Am J Bot 88: 992-1005.
- Takeda T, Ueno O, Samejima M, Ohtani T (1985) An investigation for the occurrence of C<sub>4</sub> photosynthesis in the Cyperaceae from Australia. Bot Mag Tokyo 98: 393-411.
- Thomas T, Araújo AC, Alves MV (2008) A preliminary molecular phylogeny of the Rhynchosporae (Cyperaceae). Bot Rev 75: 22-29.
- Ueno O (1996) Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C<sub>3</sub> and C<sub>4</sub> metabolism. Planta 199: 382-393.
- Ueno O (2004) Environmental regulation of C<sub>3</sub> and C<sub>4</sub> differentiation in the amphibious sedge, *Eleocharis baldwinii* and comparisons with related species. Plant Cell Environ 27: 627-639.
- Ueno O, Koyama T (1987) Distribution and evolution of C<sub>4</sub> syndrome in *Rhynchospora* (Rhynchosporae-Cyperaceae). Bot Mag Tokyo 100: 63-85.
- Ueno O, Samejima M (1989) Structural features of NAD-malic enzyme type C<sub>4</sub> *Eleocharis*: An additional report of C<sub>4</sub> acid decarboxylation types of the Cyperaceae. Bot Mag Tokyo 102: 393-402.
- Ueno O, Samejima M, Koyama T (1989) Distribution and evolution of C<sub>4</sub> syndrome in *Eleocharis*, a sedge group inhabiting wet and aquatic environments, based on culm anatomy and carbon isotope ratios. Ann Bot 64: 425-438.
- Watson ML (1958) Staining of tissue sections for electron microscopy with heavy metals. J Biophysic Biochem Cytol 1958: 4-475.

**Tabela 1.** Espécies de Cyperaceae estudadas com respectivos tipos anatômicos e vouchers

Tribos e espécies	Tipo anatômico	Voucher
<b>Abildgaardieae</b>		
<i>Bulbostylis conifera</i> L.	Kranz fimbristilóide	<i>S. Martins</i> 329
<i>Bulbostylis scabra</i> (J. Presl. & C. Presl.) C.B. Clarke	Kranz fimbristilóide	<i>S. Martins</i> 408
<i>Fimbristylis autumnalis</i> L.	Kranz fimbristilóide	<i>V.L. Scatena</i> 343
<i>Fimbristylis complanata</i> (Retz.) Link	Kranz fimbristilóide	<i>V.L. Scatena</i> 344
<i>Fimbristylis dichotoma</i> (L.) Vahl	Kranz fimbristilóide	<i>S. Martins</i> 398
<b>Cypereae</b>		
<i>Cyperus compressus</i> Jacq.	Kranz clorociperóide	
<i>Cyperus ligularis</i> L.	Kranz clorociperóide	<i>S. Martins</i> 330
<i>Cyperus maritimus</i> Poir.	Kranz clorociperóide	<i>S. Martins</i> 226
<i>Kyllinga brevifolia</i> Rottb.	Kranz clorociperóide	<i>S. Martins</i> 288
<i>Pycreus flavescens</i> (L.) Rchb.	Kranz clorociperóide	<i>S. Martins</i> 327
<b>Eleocharideae</b>		
<i>Eleocharis minima</i> Kunth	Kranz eleocaróide	<i>S. Martins</i> 405
<b>Rhynchosporeae</b>		
<i>Rhynchospora barbata</i> (Vahl) Kunth	Kranz clorociperóide	<i>S. Martins</i> 313
<i>Rhynchospora globosa</i> Lindl.	Kranz rincosporóide	<i>S. Martins</i> 305
<i>Rhynchospora terminalis</i> Kunth	Kranz rincosporóide	<i>S. Martins</i> 302

**Tabela 2.** Cyperaceae com anatomia Kranz destacando posição taxonômica, diferentes tipos anatômicos, hábito, habitat e locais de ocorrência (K-C = clorociperóide; K-E = eleocaróide; K-F = fimbriatilóide; K-R = rincosporóide)

Tribos e gêneros (número de espécies C <sub>3</sub> e C <sub>4</sub> )	Anatomia Kranz	Hábito	Habitat	Locais de ocorrência
<b>Abildgaardieae</b>				
<i>Abildgaardia</i> (1 C <sub>3</sub> /16 C <sub>4</sub> ) <sup>1,2,3,5</sup>	K-F	Anuais ou perenes	Campos e áreas perturbadas	Trópicos e subtropicais, concentradas na Austrália e África
<i>Bulbostylis</i> (150 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-F (K-E)	Anuais ou perenes, raramente arborescentes	Campos secos ou temporariamente úmidos	Trópicos, concentradas nas regiões tropicais da África e América do Sul
<i>Crosslandia</i> (4 C <sub>4</sub> ) <sup>2,3,5</sup>	K-F	Anuais	Dunas e campos	Norte da Austrália
<i>Fimbristylis</i> (1 C <sub>3</sub> /300 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-F	Anuais, raro perenes	Campos úmidos e ambientes perturbados	Trópicos, concentradas no sudeste asiático e nordeste da Austrália
<i>Nelmesia</i> (1 C <sub>4</sub> ) <sup>2,3,5</sup>	K-F	Anuais	Campos úmidos	África tropical
<i>Nemum</i> (10 C <sub>4</sub> ) <sup>2,3,5</sup>	K-F	Anuais ou perenes	Campos úmidos	África tropical
<b>Cypereae</b>				
<i>Alinula</i> (4 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Anuais	Campos úmidos	África tropical
<i>Ascolepis</i> (20 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Anuais ou perenes	Campos úmidos	Trópicos
<i>Cyperus</i> subg. <i>Cyperus</i> (260 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-C	Perenes, raro anuais	Campos úmidos e ambientes perturbados	Trópicos, raro nas regiões temperadas
<i>Kyllinga</i> (75 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-C	Anuais, raro perenes	Campos úmidos e ambientes perturbados	Trópicos, raro nas regiões temperadas
<i>Lipocarpus</i> (35 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Anuais, raro perenes	Campos úmidos	Trópicos, raro nas regiões temperadas
<i>Pycreus</i> (120 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-C	Anuais ou perenes	Campos úmidos, afloramentos rochosos, raro aquáticas flutuantes	Trópicos
<i>Queenlandiella</i> (1 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Anuais	Dunas	Leste da África, Madagascar, Índia, Sri Lanka, Malásia, Austrália
<i>Remirea</i> (1 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Perenes	Dunas	Trópicos
<i>Sphaerocyperus</i> (1 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Perenes	Campos secos	África tropical

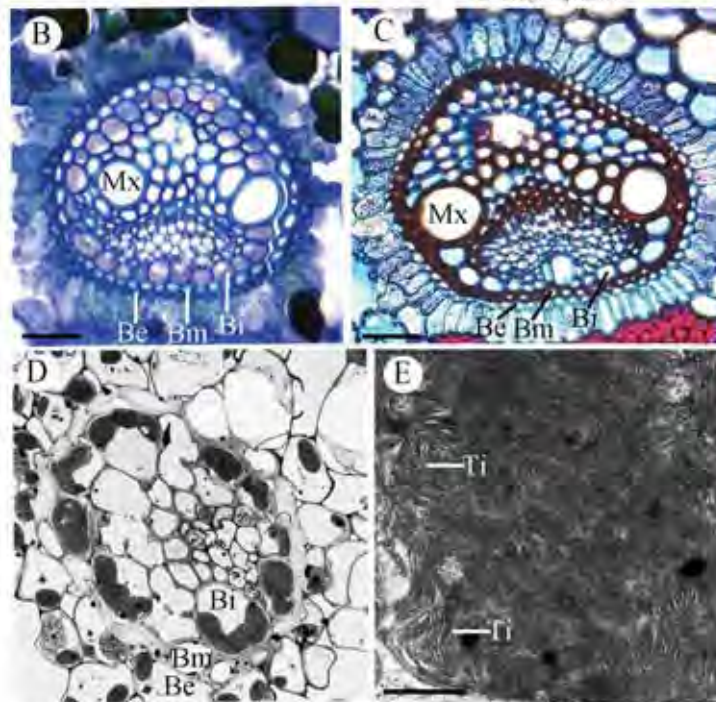
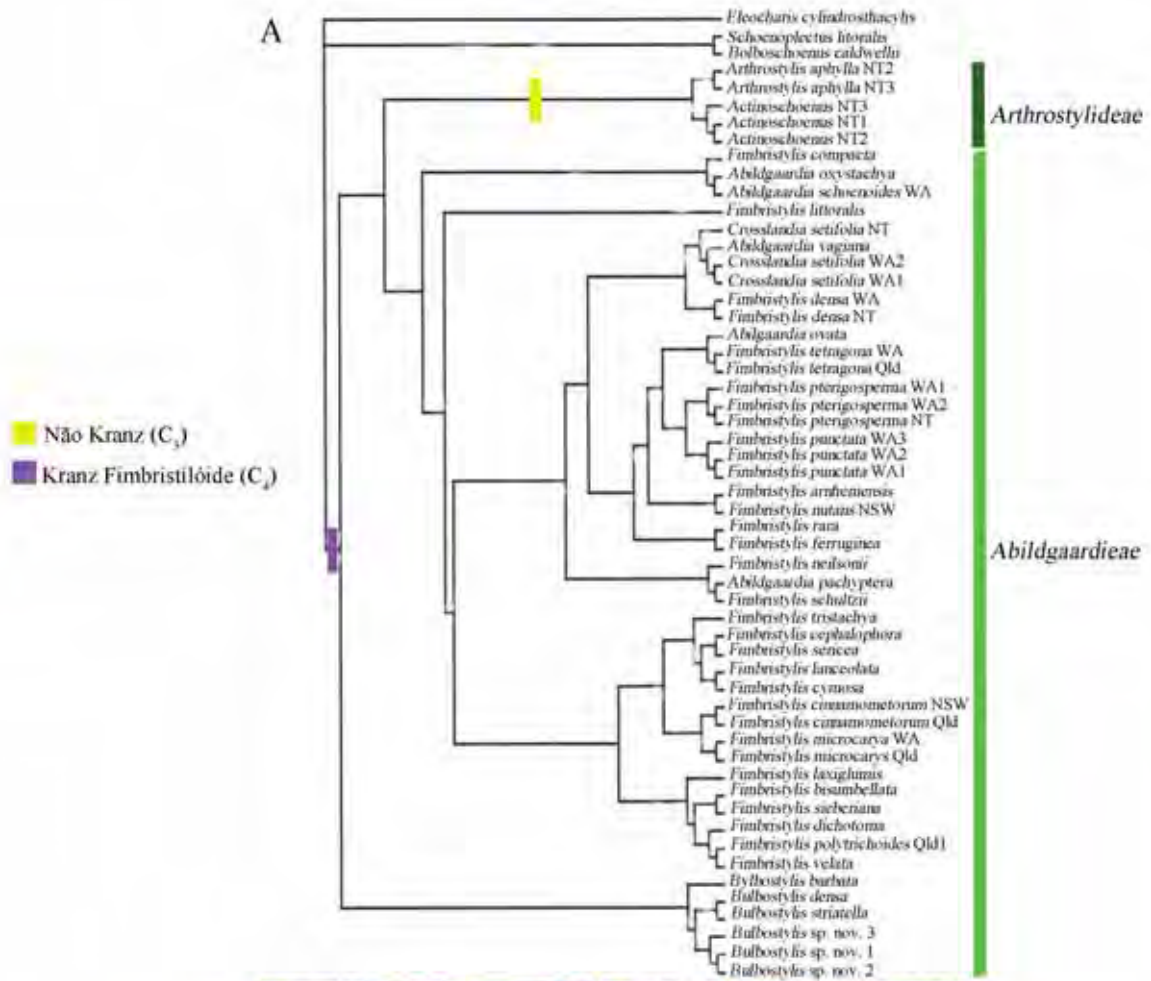
**Tabela 2.** Cont.

Tribos e gêneros (número de espécies C <sub>3</sub> e C <sub>4</sub> )	Anatomia Kranz	Hábito	Habitat	Locais de ocorrência
<i>Volkiella</i> (1 C <sub>4</sub> ) <sup>2,3,5</sup>	K-C	Anuais	Dunas	Norte da Namíbia e Zâmbia
<b>Eleocharideae</b>				
<i>Eleocharis</i> (244 C <sub>3</sub> /8 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-E (K-C; K-F)	Anuais, raro perenes	Aquáticas total ou parcialmente submersas	Cosmopolita, concentradas na América tropical
<b>Rhynchosporaeae</b>				
<i>Rhynchospora</i> grupo <i>Capitatae</i> (5 C <sub>3</sub> /28 C <sub>4</sub> ) <sup>1,2,3,4,5</sup>	K-C e K-R	Perenes, raro anuais	Campos úmidos ou secos	Neotrópicos, Austrália, Índia, Malásia e África

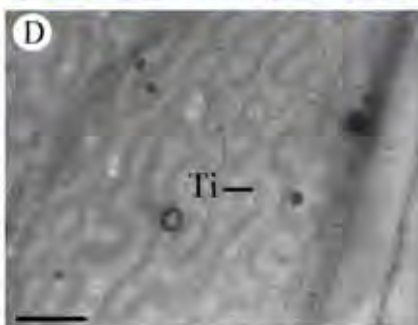
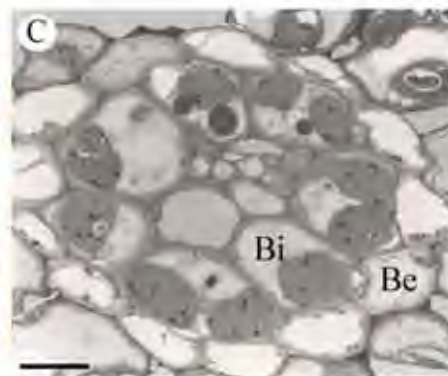
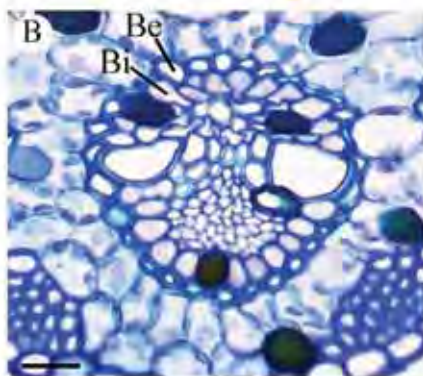
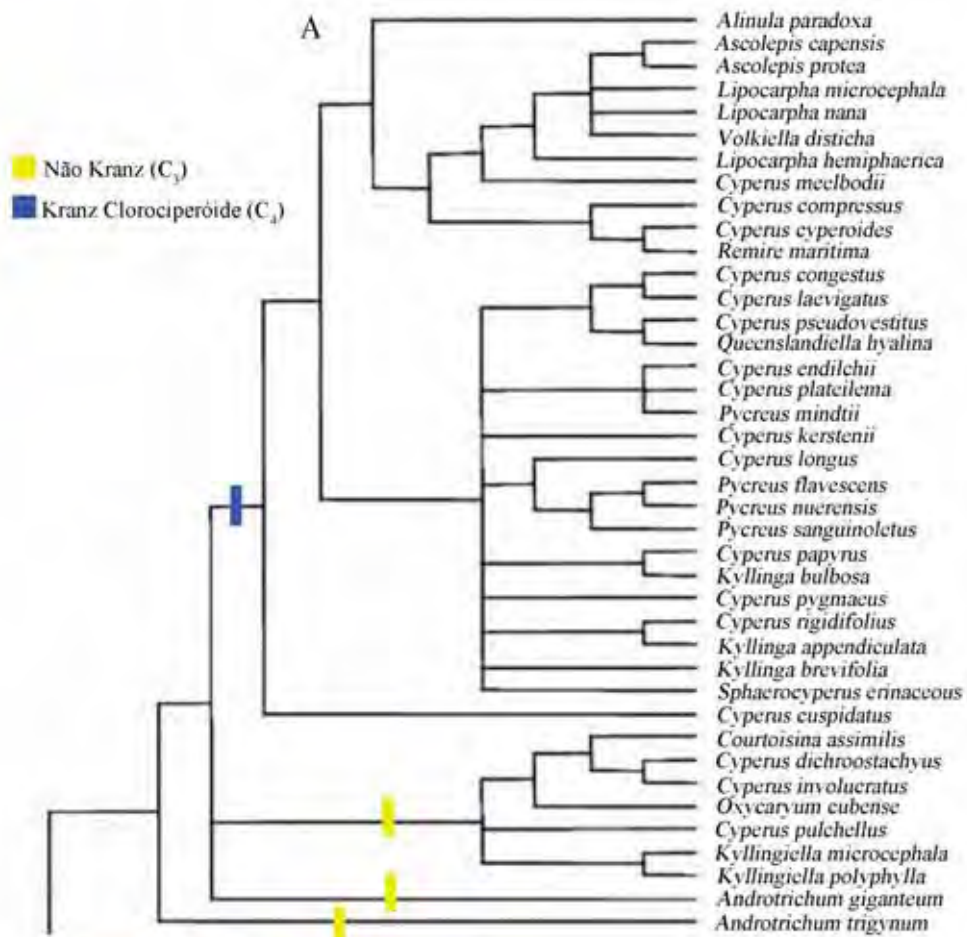
<sup>1</sup>Bruhl and Perry (1995), <sup>2</sup>Bruhl and Wilson (2007), <sup>3</sup>Goegebeur (1998), <sup>4</sup>Martins and Scatena (2011), <sup>5</sup>Soros and Bruhl (2000)



**Figura 1.** Cladograma e aspectos da anatomia Kranz de representantes da tribo Abildgaardieae. A. Cladograma da tribo, adaptado de Ghamkhar et al (2007). B-C. *Bulbostylis scabra* e *Fimbristylis complanata*, respectivamente – feixe vascular de folhas em secção transversal. D-E. *Fimbristylis autumnalis*. D. Feixe vascular destacando a distribuição centrífuga dos cloroplastos. E. Cloroplasto da célula da bainha interna com tilacóides contorcidos e paralelos (Be = bainha vascular externa; Bi = bainha vascular interna; Bm = bainha vascular mediana; Mx = metaxilema; Ti = tilacóides. Barras: (B-C) = 20  $\mu\text{m}$ ; (D) = 10  $\mu\text{m}$ , (E) = 0,2  $\mu\text{m}$ ).

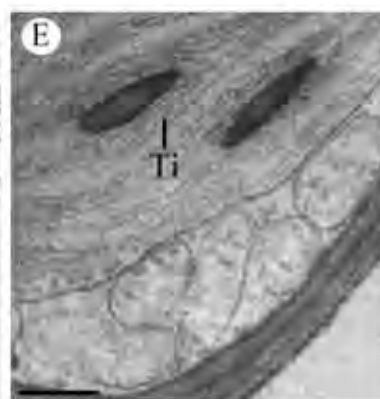
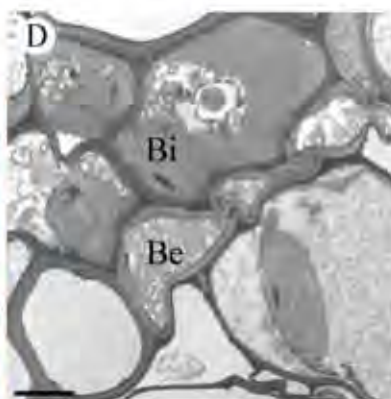
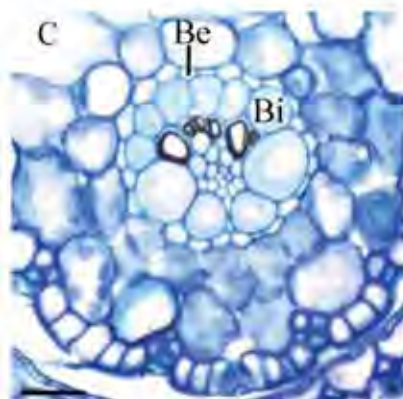
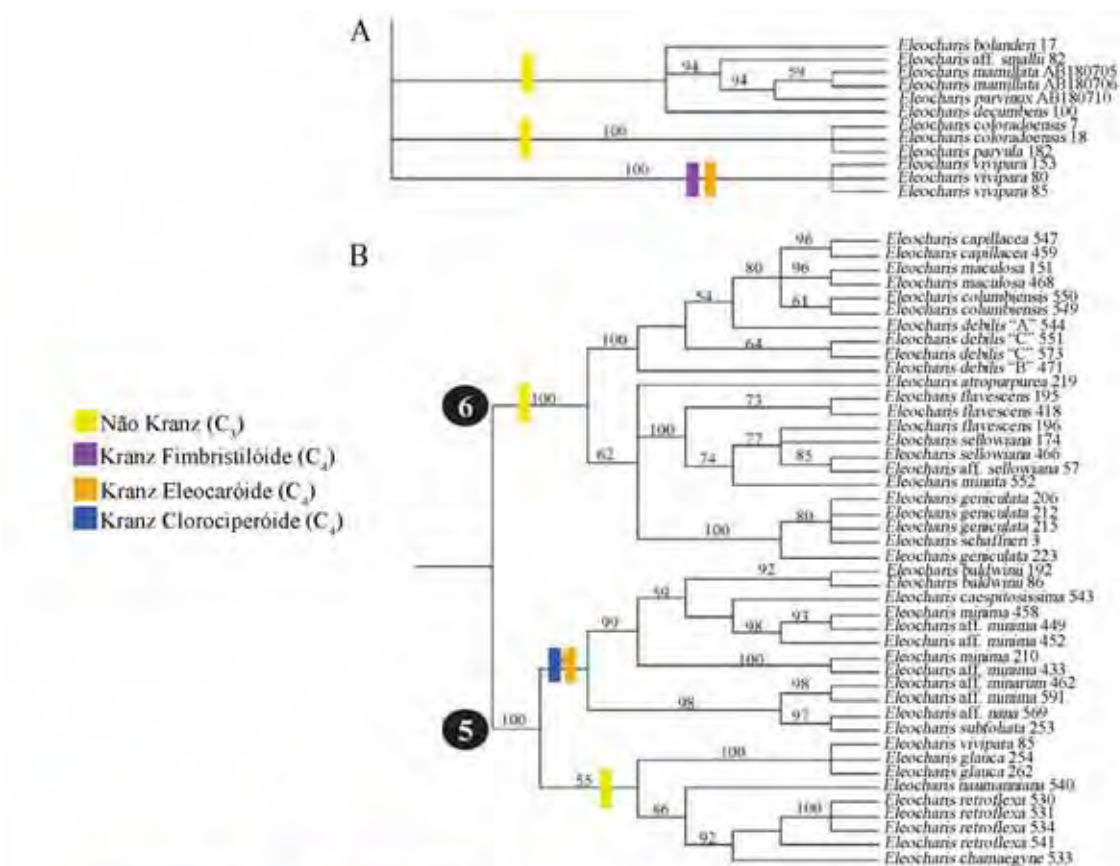


**Figura 2.** Cladograma e aspectos da anatomia Kranz de representantes da tribo Cypereae. Parte do cladograma contendo espécies Kranz da tribo, adaptado de Muasya et al. (2008). B. *Kyllinga brevifolia* – feixe vascular da folha em secção transversal. C. *Cyperus compressus* – feixe vascular destacando a distribuição centrífuga dos cloroplastos. D-E. *Cyperus maritimus* e *C. ligularis*, respectivamente – cloroplastos da bainha interna com tilacóides convolutos no primeiro e contorcidos e paralelos no segundo (Be = bainha vascular externa; Bi = bainha vascular interna; Ti = tilacóides. Barras: (B) = 20  $\mu\text{m}$ ; (C) = 5  $\mu\text{m}$ ; (D-E) = 0,5  $\mu\text{m}$ ).



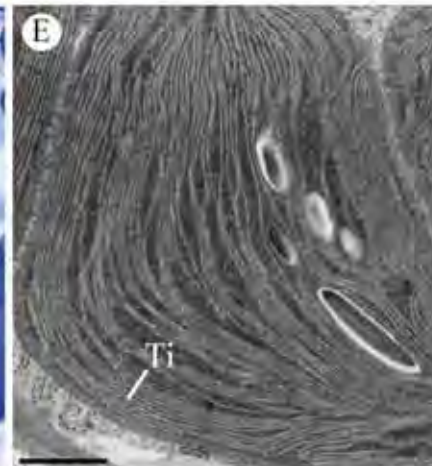
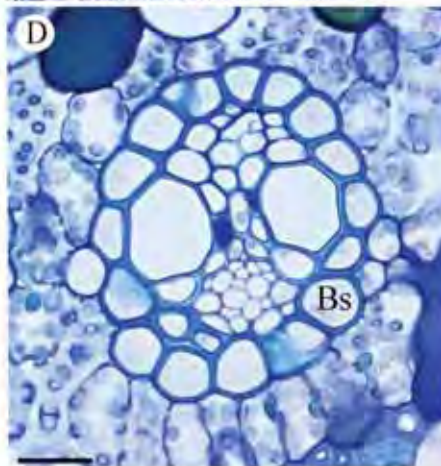
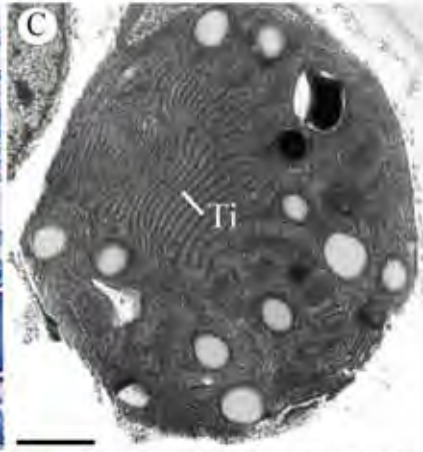
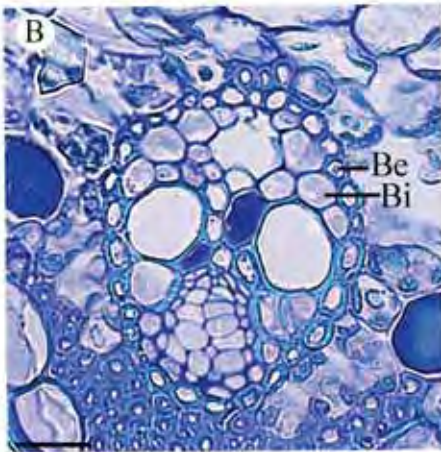
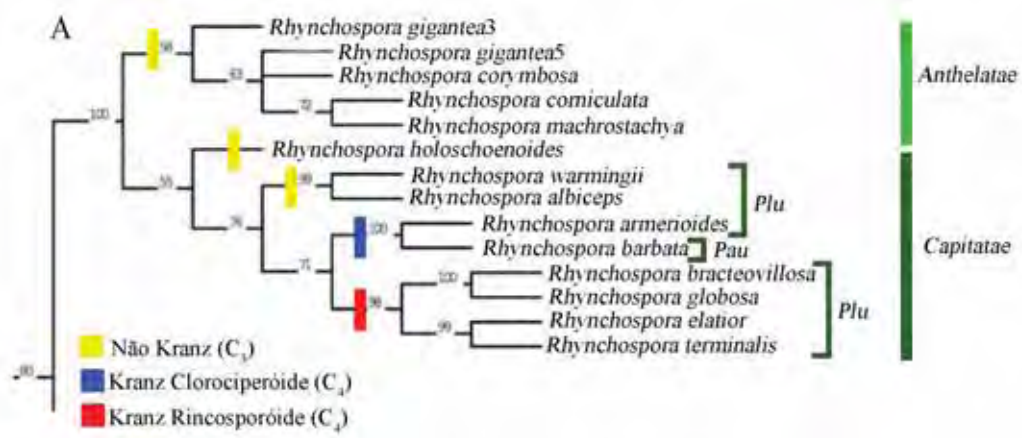
**Figura 3.** Cladograma e aspectos da anatomia Kranz de representantes da tribo Eleocharideae. A-B. Partes dos cladogramas contendo espécies Kranz da tribo, adaptado de Roalson et al. (2010). C-E. *Eleocharis minima*. C. Feixe vascular da folha em secção transversal. D. Feixe vascular destacando a distribuição aleatória dos cloroplastos na bainha interna. E. Cloroplasto da bainha interna com tilacóides paralelos (Be = bainha vascular externa; Bi = bainha vascular interna; Ti = tilacóides. Barras: (C) = 20  $\mu\text{m}$ ; (D) = 2  $\mu\text{m}$ ; (E) = 0,5  $\mu\text{m}$ ).





**Figura 4.** Cladograma e aspectos da anatomia Kranz de representantes da tribo Rhynchosporae. A. Parte do cladograma contendo espécies Kranz da tribo, adaptado de Thomas et al. (2008). B-C. *Rhynchospora barbata*. B. Feixe vascular da folha em secção transversal. C. Cloroplasto da bainha interna com tilacóides paralelos. D-E. *Rhynchospora globosa*. D. Feixe vascular da folha em secção transversal. E. Cloroplasto da bainha simples com tilacóides paralelos (Be = bainha vascular externa; Bi = bainha vascular interna; Bs = bainha vascular simples; Pau = *Pauciflorae*; Plu = *Pluriflorae*; Ti = tilacóides. Barras: (B, D) = 20  $\mu\text{m}$ ; (C, E) = 0,5  $\mu\text{m}$ ).





## 5. Considerações finais

As espécies de Cyperoideae (Cyperaceae) germinam a partir do propágulo (aquênio) embebido e a primeira estrutura que emerge é o coleóptilo seguido da raiz primária, que é efêmera. O mesocótilo formará o rizoma de onde crescem raízes adventícias, folhas e escapos. O rizoma faz a conexão vascular das raízes adventícias com as folhas e os escapos. A endoderme e o periciclo ocorrem em todos os órgãos vegetativos

. A endoderme é a camada mais interna do córtex na raiz, no rizoma, na folha e no escapo e o periciclo é a camada que limita o cilindro vascular na raiz e no rizoma e é a camada mais externa do cordão procambial de folhas e escapos, podendo ser uni ou biestratificado.

Nas folhas de Cyperaceae a epiderme pode ser uni ou multiestratificada, nesta última, ocorrem de duas a dez camadas de células com disposição semelhante ou não de suas paredes. Ainda nas folhas, pode ocorrer ou não anatomia Kranz de diferentes tipos em grupos não relacionados taxonomicamente. O tipo clorociperóide apresenta duas bainhas vasculares com origem procambial (periciclo bisseriado) e ocorre em diferentes gêneros da tribo Cypereae e em espécies de *Rhynchospora* da tribo Rhynchosporeae. O tipo eleocaróide apresenta duas bainhas vasculares (periciclo bisseriado) com origem procambial e é restrito ao gênero *Eleocharis* e não ocorre em *Bulbostylis*. O tipo fimbristilóide apresenta três bainhas vasculares, a externa (endoderme) com origem do meristema fundamental, a mediana e a interna (periciclo bisseriado) com origem procambial, e ocorre em diferentes gêneros da tribo Abildgaardieae e em *Eleocharis vivipara* da tribo Eleocharideae. O tipo rincosporóide apresenta apenas uma bainha vascular (periciclo), diferindo do encontrado na literatura (duas bainhas), e é restrito ao gênero *Rhynchospora*.

Nas espécies Kranz, os cloroplastos da bainha do feixe variam na localização (centrífuga ou aleatória) e na organização dos tilacóides (convoluta, contorcida ou paralela) entre os tipos Kranz. Só foram observados padrões para os tipos eleocaróide e rincosporóide e para as espécies de *Cyperus* e *Pycneus* do tipo clorociperóide.

Como em *Eleocharis minima* ocorre variação na anatomia Kranz em diferentes regiões de escapos emersos e submersos do mesmo indivíduo mostra-se a plasticidade desse caráter para o gênero que reduz sua importância em abordagens taxonômicas e filogenéticas.

O agrupamento de dados de taxonomia, filogenia, locais de ocorrência e características estruturais de espécies Kranz de Cyperaceae mostram origem múltipla desse caráter que provavelmente está relacionada com as pressões ambientais.