A-PDF Merger DEMO : Purchase from www.A-PDF.com to remove the watermark

Estudo citogenético e evolutivo em espécies brasileiras de *Eleocharis* (Cyperaceae)

Tese apresentada ao curso de pós-graduação em Genética para obtenção do Título de Doutor em Genética, da Universidade Estadual Paulista

Orientador: Prof. Dr. André Luis Laforga Vanzela

2010

Carlos Roberto Maximiano da Silva

Livros Grátis

http://www.livrosgratis.com.br

Milhares de livros grátis para download.

Estudo citogenético e evolutivo em espécies brasileiras de *Eleocharis* (Cyperaceae).

Tese apresentada ao curso de pós-graduação em Genética para obtenção do Título de Doutor em Genética, da Universidade Estadual Paulista

Comissão examinadora

Prof. Dr. ANDRÉ LUIZ L VANZELA (Orientador)

Prof. Dr. CESAR MATINS

Profa. Dra. DORALICE MARIA CELLA

Prof. Dr. MARCELO DOS SANTOS G FILHO

Profa. Dra. MARIA TERCILIA V DE AZEREDO OLIVEIRA

2010

Carlos Roberto Maximiano da Silva

Agradecimentos

Aos meus pais, Paulo Roberto M. da Silva e Anna Maria dos Santos da Silva, a minha avó Janir Pererira dos Santos e meu avô José Messias dos Santos por sempre me apoiarem.

A minha noiva Renata da Rosa, em especial, por tornar meus sonhos realidades.

Ao meu amigo André L. L. Vanzela.

Ao meu grande irmão (literalmente), Fernando Roberto M. da Silva.

A todos os professores que me ajudaram em especial a Profa. Claudia Regina Bonini Domingos e a Profa. Maria Tercília Vilela de Azeredo Oliveira.

A todos do LABRE, pelo auxilio e pelo agradável ambiente de trabalho.

A CNPq, pelo auxilio financeiro.

A todos que de algum modo contribuíram para a realização deste trabalho.

Dedico este trabalho a minha Família em especial ao meu avô: José Messias dos Santos.

Resumo

A família Cyperaceae é a terceira maior entre as monocotiledôneas, com 42 gêneros encontrados no Brasil. *Eleocharis* é o quarto gênero mais diverso, com 69 espécies encontradas em áreas alagadas, margens de rios e lagos. São plantas com morfologia simples, porém, com uma grande variação morfológica. Isto tem dificultado a identificação e organização taxonômica deste grupo. Este gênero, assim como toda a família, também é conhecido por possuir cromossomos holocêntricos, meiose pós-reducional e formação de pseudomônades, além de uma grande variação intra- e interespecífica no número cromossômico. Buscando compreender a evolução cariotípica e esclarecer problemas taxonômicos neste grupo, foram estudadas 259 amostras de 10 estados brasileiros, representando cerca de 40% das espécies. Análises citogenéticas revelaram variação cromossômica interespecífica de $2n = 6$ (*E. subarticulata* e *E. maculosa*) até $2n = 60$ (*E. laeviglumis*), causada principalmente por poliploidia. Apesar desta variação, o número básico $x = 5$ é sugerido. As maiores variações intraespecíficas foram encontradas em *E. maculosa* (2n=10, 8, 7 e 6) decorrente de simploidia e no complexo de espécies formado por *E. viridans* e *E. niederleinii*, nas quais os dados citogenéticos e moleculares indicam uma origem híbrida associada à fissão e/ou fusão cromossômicas e poliploidia. A localização física dos sítios de DNAr 45S e 5S por FISH mostrou sinais de 45S sempre terminais e com múltiplos sítios (2 a 10). Esta multiplicação de sítios pode ser resultado de amplificação seguido de dispersão pelas pontas dos cromossomos com mesmo tamanho. O sítio de DNAr 5S foi menos variável em número (2 a 4) por cariótipos, contudo, variou entre as posições terminais e intersticiais. Este estudo mostra que os mecanismos responsáveis pela diversidade cariotípica em *Eleocharis* são próximos àqueles reportados para o gênero *Rhynchospora* e *Carex*, com diferenças na predominância de agmatoploidia e simploidia em *Carex* e poliploidia nos demais grupos. Em termos taxonômicos, este estudo reforçou ainda mais a grande diversidade dentro do subgênero *Eleocharis*, bem como a separação de *Limnochloa* dos demais subgêneros, como proposto em estudos de filogenia molecular.

Palavras chave: Citotaxonomia, Cromossomos holocêntricos, Poliploidia, Rearranjo cromossômico

Sumário

Introdução.. 6
Justificativa... 22
Objetivos... 13
Referências da Introdução……………..…………………………………….. 24
Capítulo 1
Karyotypic diversification and its contribution to the taxonomy of *Eleocharis* (Cyperaceae) from Brazil……………………………………………………... 37
Capítulo 2
Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis* (Cyperaceae)…………………………………………………… 50
Capítulo 3
Distribution of 45S and 5S rDNA sites in 23 species of *Eleocharis* (Cyperaceae)…………………………………………………………...…… 60
Capítulo 4
Chromosome reduction in *Eleocharis maculosa* (Cyperaceae)………………. 79
Capítulo 5
Análises citogenéticas e moleculares revelam um complexo de híbridos e poliploides em *Eleocharis* série *Tenuissimae* (Cyperaceae)............................ 86

Conclusões.. 120

1. Introdução

1.1. Família Cyperaceae

Cyperaceae Juss. é a terceira maior família das monocotiledôneas e apresenta distribuição cosmopolita. Seus representantes ocorrem em uma variedade de habitats, sendo mais frequentes em áreas úmidas como brejos, pântanos, margens de rios, charcos e ambientes de restinga (Dahlgren *et al.* 1985, Goetghebeur 1998 e Judd *et al.* 1999).

Esta família possui mais de 5.500 espécies, distribuídas em mais de 109 gêneros (Govaerts *et al.* 2007). O primeiro levantamento da flora de Cyperaceae realizado para todo o Brasil registrou cerca de 300 espécies em 65 gêneros (Ness 1842). Recentemente, foram identificadas 678 espécies em 42 gêneros, sendo que os de maior riqueza de espécies são *Rhynchospora* Vahl (157espécies), *Cyperus* L. (101espécies), *Scleria* Berg. (82 espécies) e *Eleocharis* R. Br., com 69 espécies (Alves *et al.* 2009). Algumas imagens de representantes desses grupos são apresentadas na Figura 1.

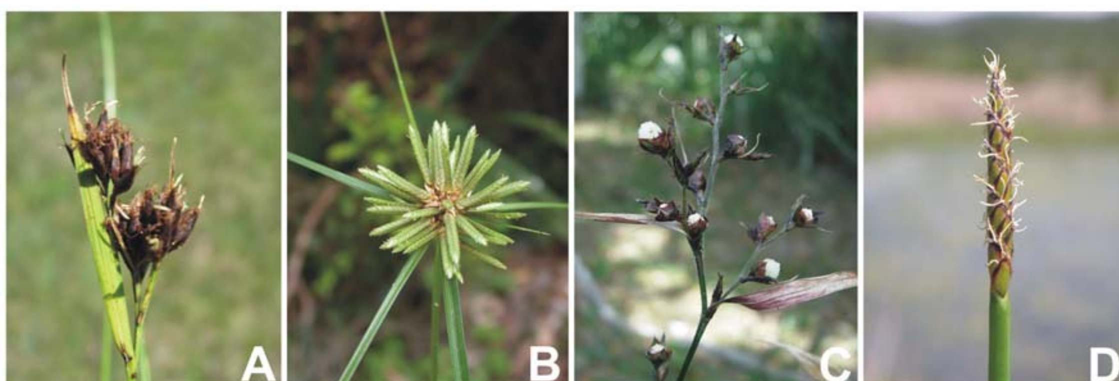


Figura 1. Diversidade da inflorescência das espécies de Cyperaceae dos quatro gêneros mais abundantes no Brasil. A) *Rhynchospora polyantha* Steud.; B) *Cyperus incomtus* Kunth; C) *Scleria panicoides* Kunth e D) *Eleocharis laeviglumis* R. Trevisan & Boldrini

Estudos filogenéticos, realizados por Muasya *et al.* (2000), sugerem que esta família compreende um grupo monofilético, próximo de Juncaceae. No entanto, a determinação das relações taxonômicas em Cyperaceae pode ser considerada difícil, devido ao tamanho reduzido das flores e às inflorescências condensadas. Segundo o estudo taxonômico realizado por Bruhl (1995), a família é dividida em duas subfamílias (Cyperoideae e Caricoideae) compostas por 14 tribos. Goetghebeur (1998) concordou com as 14 tribos, no entanto, este autor dividiu a família em quatro subfamílias (Cyperoideae, Caricoideae, Scleroioideae e Mapanoideae). Com o auxílio de dados moleculares, Simpson *et al.* (2003, 2007) e Muasya *et al.* (2009) dividiram a família Cyperaceae em duas subfamílias: Cyperoideae e Mapanoideae, composta pelas mesmas 14 tribos. Apesar das diferentes classificações, o gênero *Eleocharis* foi sempre incluído à tribo Eleocharidae, subfamília Cyperoideae.

1.2. Características morfológicas do gênero *Eleocharis*

Eleocharis possui aproximadamente 250 espécies, das quais 16 são endêmicas do Brasil (Alves *et al.* 2009). Apesar de ser um gênero cosmopolita, a maioria das espécies está nas regiões tropicais e subtropicais das Américas (Govaerts *et al.* 2007). São encontradas em solos que ficam temporariamente úmidos ou inundados, e algumas espécies vivem total ou parcialmente submersas (Trevisan e Boldrini 2008). Segundo Svenson (1929), *Eleocharis* é um gênero natural que fazia parte do gênero *Scirpus* L.. Atualmente, *Eleocharis* encontra-se separado de *Scirpus* devido à ocorrência de inflorescência única sem brácteas involucrais e estilopódio persistente, endurecido, dilatado e articulado com o ovário. *Scirpus*, de acordo com Goetghebeur (1998), possui inflorescência composta com brácteas involucrais, estilopódio pouco nítido e não espessado.

As espécies do gênero *Eleocharis* possuem: colmo fotossintetizante com bainhas na base, glumas geralmente imbricadas e espiraladas ao longo do eixo da espigueta, flores bissexuais, perigônio reduzido a cerdas ou ausente, estames geralmente trífidos e estilete 2-3-fido (Svenson 1929 e González-Elizondo 1994). Segundo Svenson (1929, 1932, 1934, 1937 e 1939), as espécies podem ter hábitos cespitoso, rizomatoso ou estolonífero. Contudo, Trevisan e Boldrini (2008) organizaram as espécies brasileiras em cespitosas com e sem formação de cáudice; cespitosas-estoloníferas com estolhos de entrenós longos; cespitosas-rizomatosas com rizomas curtos e cespitosas-rizomatosas com rizomas alongados de entrenós curtos. A Figura 2 mostra os esquemas com os hábitos propostos por estes autores.

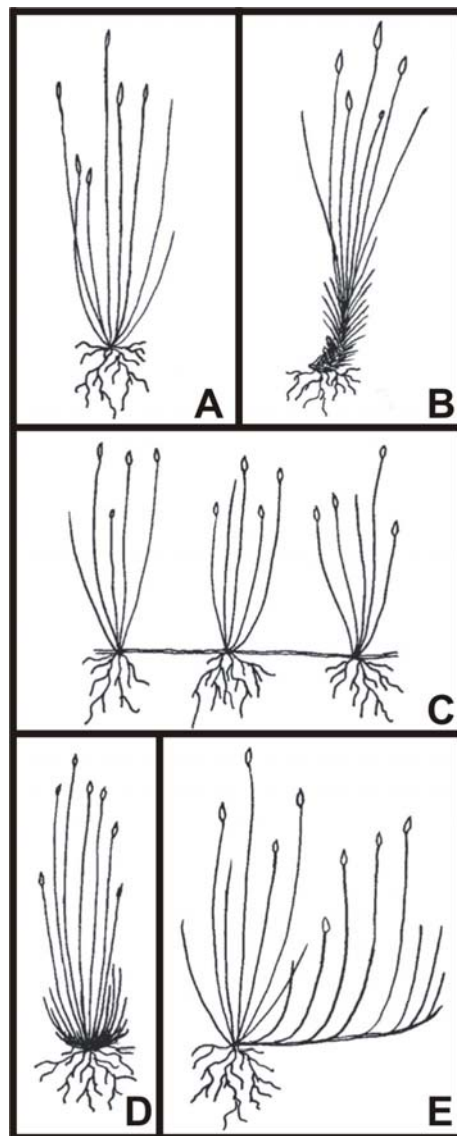


Figura 2. Esquema mostrando os hábitos em *Eleocharis*. A) Cespitoso; B) Cespitoso, com formação de cáudice; C) Cespitoso- estolonífero, com estolhos de entrenós longos; D) Cespitoso-rizomatoso com rizomas curtos e E) Cespitoso-rizomatoso com rizomas alongados de entrenós curtos. Fonte: Trevisan e Boldrini (2008).

Os caules das ciperáceas, também chamados de colmos, são geralmente sólidos, frequentemente 3-angulado, podendo ser ramificados ou não (Mohlenbrock 1976). Em *Eleocharis*, o colmo é um escapo, o qual em secção transversal pode apresentar três ou mais ângulos, ou ainda ser redondo a elíptico, e com função fotossintetizante (Ueno *et al*. 1989). Cada colmo ereto que sai do rizoma de *Eleocharis* é um ramo terminal que possui duas bainhas. Uma inferior que é frouxa, membranácea e mais fendida, enquanto que a superior é cilíndrica e envolve o

colmo (veja a Figura 3). Esta última estrutura tem valor taxonômico, sobretudo o ápice. A presença ou ausência de múcron dorsal, o ápice truncado ou oblíquo, o apêndice hialino, rugoso ou não, são alguns dos principais caracteres diagnósticos das bainhas.

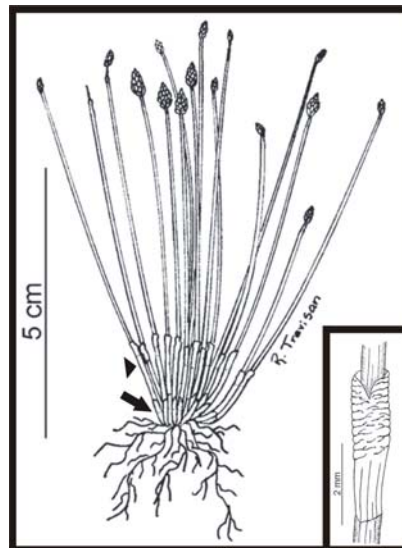


Figura 3: Desenho de *E. flavescens*. A seta aponta para a bainha inferior. A cabeça de seta aponta para a bainha superior. Em maior aumento, o detalhe do ápice hialino rugoso hialino da bainha superior. Fonte: Trevisan e Boldrini (2008).

As inflorescências das Cyperaceae são compostas por uma ou mais brácteas involucrais. *Eleocharis*, no entanto, não possuem esses invólucros, sendo isto uma das características que definem o gênero. As inflorescências são as mais simplificadas dentro da família, aparecendo como espigueta única no ápice do colmo (veja a Figura 4). A polinização é anemófila, como na maioria das Cyperaceae. As espécies apresentam redução das características de atração a visitantes. Por outro lado, produzem grande quantidade de grãos de pólen.

O número de estames em plantas anemófilas é geralmente grande, e as anteras encontram-se frequentemente expostas, elevadas para fora da flor por filetes longos e flexíveis, com pedicelo móvel. O estigma é geralmente plumoso e grande, ficando exposto para aumentar a probabilidade de contato com os grãos de pólen. Os aquênios apresentam as características morfológicas mais significativas na diferenciação das espécies de *Eleocharis* (Svenson 1929, 1939 e González-Elizondo e Peterson 1997). As mais importantes são a forma e a coloração da base do estilete, bem como o tamanho e o número de cerdas periânticas. Neste gênero, assim como em toda a família, algumas espécies possuem aquênios com superfícies lisas e outras com superfície ornamentada (Trevisan e Boldrini 2008) (veja a Figura 5). Contudo, quando observados ao

microscópio eletrônico de varredura, todos os aquênios apresentam ornamentação com valor taxonômico (Menapace 1990 e Lye 2000) (veja a Figura 6).

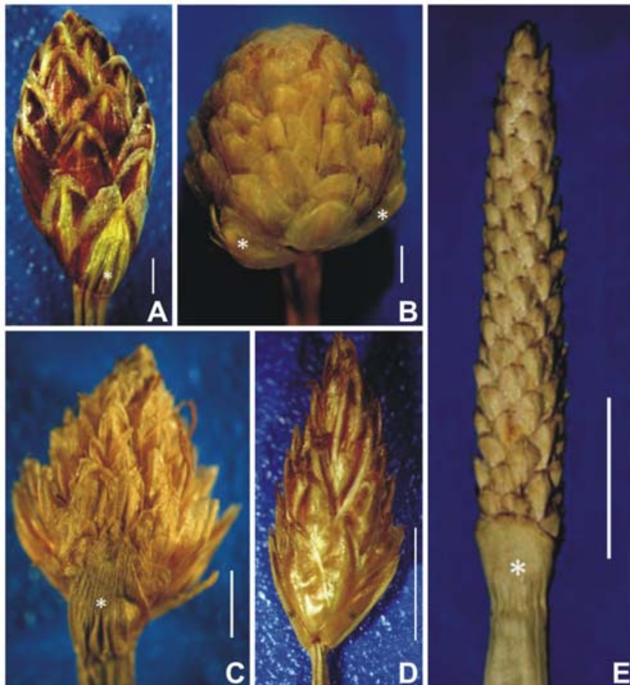


Figura 4. Diferentes formas de espiguetas em *Eleocharis*. A) Elipsóide em *E. maculosa*. B) Globosa de *E. geniculata*. C) Obovóide de *E. pachystyla*. D) Elipsóide de *E. sellowiana*. A-D) Barra = 1mm. E) Cilíndrica em *E. interstincta*. Barra = 10 mm. Os asteriscos indicam as basais. Fonte: Gil e Bove (2007)

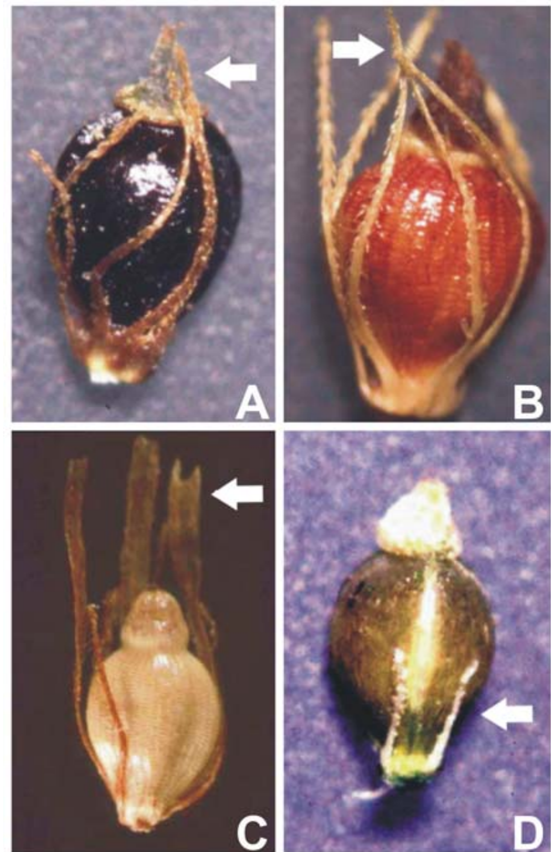


Figura 5: Aquênios de *Eleocharis*. A) Com superfície lisa em *E. debilis*. A seta aponta a cerda periântica com o mesmo tamanho do corpo do aquênio. B) Com superfície ornamentada em *E. interstincta*. A seta aponta a cerda periântica maior que o comprimento do corpo do aquênio. C) com superfície ornamentada em *E. mutata*. A seta aponta a cerda periântica muito maior que o corpo do aquênio. D) Com superfície lisa de *E. minima*. A seta aponta a cerda periântica menor que o comprimento do aquênio. Observe ainda as diferentes formas e cores dos frutos.

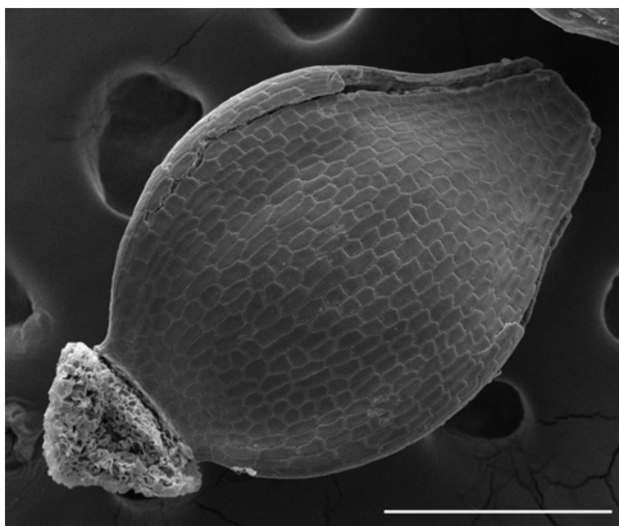


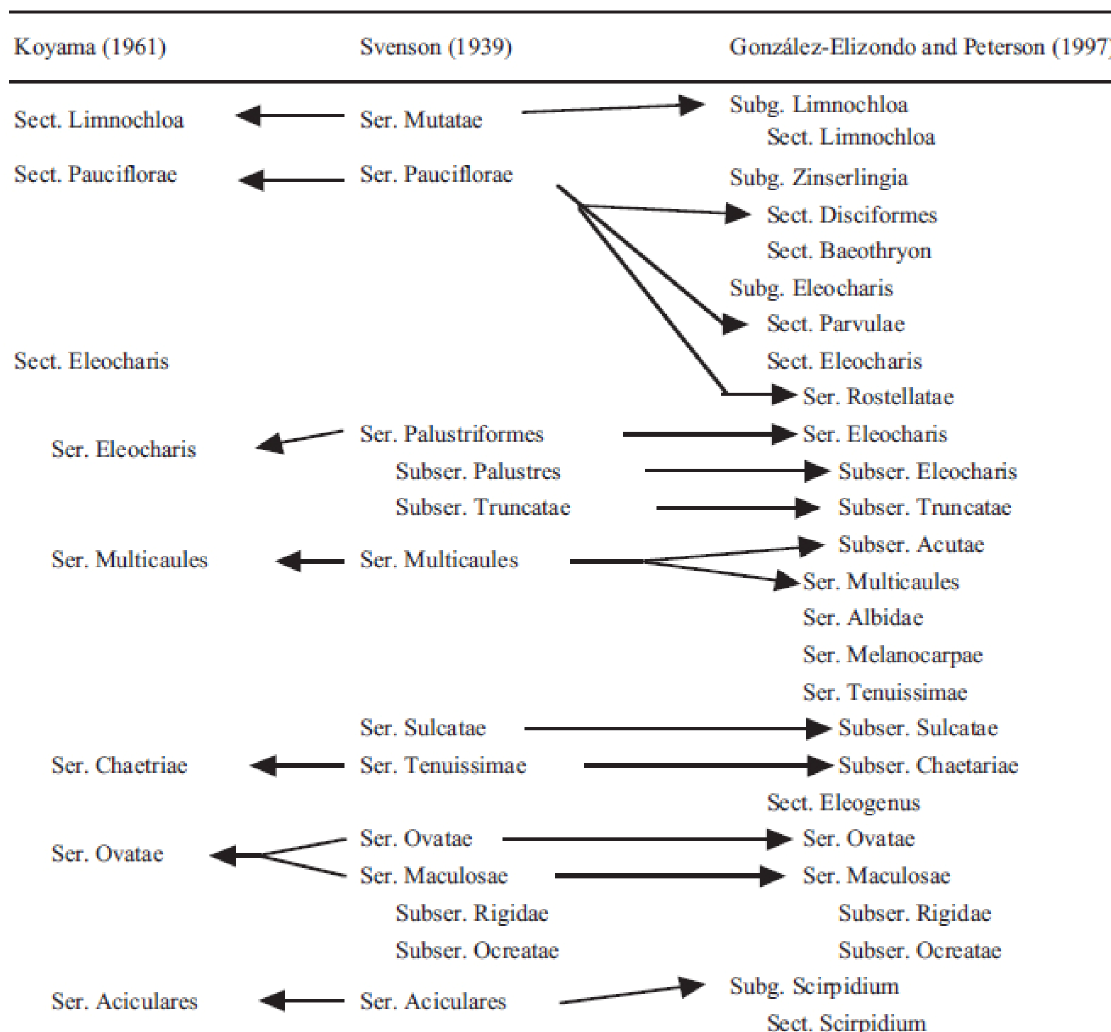
Figura 6: Aquênio de *Eleocharis minima* em microscopia eletrônica de varredura, mostrando ornamentação em sua superfície. Barra = 300µm

1.3. Relações taxonômicas e filogenéticas do gênero *Eleocharis*

Eleocharis é um grupo marcado por morfologia simples, mas com grande variação intraespecífica (Simpson 1988). Isto tem proporcionado problemas de identificação e classificação das espécies no gênero. Prova disso é que na literatura são encontrados mais de 600 nomes de espécies. Contudo, atualmente, são reconhecidas pouco mais de 250 espécies (Svenson 1939, González-Elizondo e Peterson 1997 e Govaerts *et al.* 2007). Torrey (1836) organizou o gênero *Eleocharis* em 7 subgêneros, considerando as espécies da América do Norte. Koyama (1961) moveu *E. margaritaceae* e *E. parvula* do gênero *Scirpus* para *Eleocharis* e criou a seção *Pauciflorae* em *Eleocharis*. Kunth (1937), baseado no trabalho de Nees (1834, 1835), dividiu o gênero em três grupos sem hierarquia definida: *Eleocharis Eleocharis* (incluindo *Eleocharis*, *Chaetocyperus* Nees e *Scirpidium* Nees), *E. Eleogenus* e *E. Limnocharis* Kunth.

Com o passar do tempo, várias espécies de *Eleocharis* foram descobertas e novas classificações deste gênero foram elaboradas e modificadas. Uma das mais completas foi feita por Svenson (1929, 1934, 1937 e 1939), que organizou o gênero em nove séries. Kukkonen (1990) propôs a organização de *Eleocharis* nos subgêneros: *Eleogenus* (Nees) C.B. Clarke, *Zinserlingia* T.V. Egorova, *Scirpidium* (Nees) Kukkonen, *Limnochloa* (P. Beauv. ex T. Lestib.) Torr. e *Eleocharis* R.Br. A revisão mais recente e mais aceita foi feita por González-Elizondo e Peterson (1997). Estes autores dividiram o gênero nos subgêneros *Scirpidium*, *Limnochloa*, *Zinserlingia* e *Eleocharis*, seis seções, oito séries e sete subséries (Tabela 1).

Tabela 1. Comparação entre as classificações para o gênero *Eleocharis*. Fonte: Yano *et al*. (2004)



Koyama (1961)	Svenson (1939)	González-Elizondo and Peterson (1997)
Sect. Limnochloa	Ser. Mutatae	Subg. Limnochloa
		Sect. Limnochloa
Sect. Pauciflorae	Ser. Pauciflorae	Subg. Zinserlingia
		Sect. Disciformes
		Sect. Baeothryon
		Subg. Eleocharis
		Sect. Parvulae
Sect. Eleocharis		Sect. Eleocharis
		Ser. Rostellatae
Ser. Eleocharis	Ser. Palustriformes	Ser. Eleocharis
	Subser. Palustres	Subser. Eleocharis
	Subser. Truncatae	Subser. Truncatae
		Subser. Acutae
Ser. Multicaules	Ser. Multicaules	Ser. Multicaules
		Ser. Albidae
		Ser. Melanocarpae
		Ser. Tenuissimae
	Ser. Sulcatae	Subser. Sulcatae
Ser. Chaetriae	Ser. Tenuissimae	Subser. Chaetariae
		Sect. Eleogenus
Ser. Ovatae	Ser. Ovatae	Ser. Ovatae
	Ser. Maculosae	Ser. Maculosae
	Subser. Rigidae	Subser. Rigidae
	Subser. Ocreatae	Subser. Ocreatae
Ser. Aciculares	Ser. Aciculares	Subg. Scirpidium
		Sect. Scirpidium

Tanto a classificação de Svenson (1939), quanto as demais classificações como as de Koyama (1961), Kukkonen (1990) e González-Elizondo e Peterson (1997), levaram em consideração apenas caracteres ligados a macromorfologia, como forma dos aquênios e relação da largura entre a espigueta e o colmo. Porém, trabalhos que fizeram a comparação destas classificações com dados moleculares, obtida pelo sequenciamento do "*Internal Transcribed Spacer*" (ITS) do rDNA, revelaram que não há correspondência total entre as classificações e as relações filogenéticas do grupo (Roalson e Friar 2000 e Yano *et al.* 2004). Os primeiros autores analisaram 38 espécies coletadas na América do Norte e compararam com as classificações Svenson (1939), Kukkonen (1990) e González-Elizondo & Peterson (1997). Uma das maiores

contradições envolveu o gênero *Websteria* S.H. Wrigth, o qual foi tratado como independente por alguns autores (González-Elizondo e Peterson 1997). Porém, este gênero ficou inserido no mesmo clado de *Eleocharis*. Além disso, este estudo suportou a idéia da monofilia nos subgêneros *Limnochloa* e *Zinserlingia* (*sensu* González-Elizondo & Peterson 1997) e séries parafiléticas ou polifiléticas no subgênero *Eleocharis*.

O estudo de Yano *et al.* (2004) foi feito com 24 espécies japonesas de *Eleocharis*, comparando as classificações propostas por Koyama (1961), Svenson (1939) e González-Elizondo & Peterson (1997). Os dados obtidos indicaram que a espessura da espigueta em relação à espessura do colmo é uma característica mais importante para a taxonomia do que as características encontradas no aquênio. As análises também confirmaram o que foi observado por Roalson e Friar (2000), já que os subgêneros *Limnochloa* e *Zinserlingia* (*sensu* González-Elizondo e Peterson 1997) apareceram como monofiléticos. Além disso, muitas subdivisões do subgênero *Eleocharis* apareceram como parafiléticas ou polifiléticas, como por exemplo, a série *Tenuissimae*.

Em um estudo mais recente, as análises moleculares do nrDNA ITS e cpDNA das sequências *trnC-ycf6* e *ycf6-psbM* foram realizadas em 259 amostras de 154 espécies coletadas em todo mundo (Roalson *et al. in press*). Estes dados foram comparados com a classificação mais aceita (González-Elizondo & Peterson 1997). Dentre os quatro subgêneros, os principais contrastes entre as análises filogenéticas e a classificação taxonômica apareceram no subgênero *Eleocharis*, principalmente nas séries *Tenuissimae* e *Eleocharis*. Para os subgêneros *Scirpidium, Zinserlingia* e *Limnochloa*, com exceção de algumas espécies como *E. dulcis* (subg. *Limnochloa*) e *E.*

acicularis e *E. bonariensis* (subg. *Scirpidium*) que foram paralilléticas, os três subgêneros como um todo foram monofiléticos.

1.4.Características citológicas de Cyperaceae e *Eleocharis*

A família Cyperaceae possui três características citogenéticas conjuntas que são incomuns dentre os demais grupos de organismos: 1) cromossomos com centrômero difuso; 2) meiose pós-reducional e 3) formação de pseudomonades.

1.4.1 Cromossomos holocêntricos

Estes cromossomos não possuem constrição primária (Figura 7A) e o cinetócoro se organiza de modo difuso ao longo dos cromossomos, com exceção das regiões teloméricas, como demonstrado para *Luzula nivea* (Nagaki *et al.* 2005). Tais cromossomos foram descritos primeiramente em insetos (Scharader 1935), e somente em 1954 este tipo de cromossomo foi descrito no gênero *Eleocharis*, por Bataglia e Håkansson (veja Strandhede 1965). Além da família Cyperaceae, cromossomos holocêntricos foram descritos em representantes da família Juncaceae (Malheiros *et al.* 1947), apenas no gênero *Drosera* da família Droseraceae (Sheikh e Kondo 1995), em um subgênero de *Cuscuta* (Cuscutaceae) (Pazy e Plitmann 1994), em uma única espécie da família Liliaceae, *Chionagraphis japonica* Maxim. (Tanaka e Tanaka 1977) e em outra da família Myristicaceae, *Myristica fragrans* Houtt (Flach 1966). Além das plantas, cromossomos holocêntricos são encontrados em nematóides, como *Caenorhabditis elegans* Maupas (Buchwitz *et al.* 1999), em protozoários, algas e em várias ordens de insetos, incluindo Lepidoptera, Heteróptera e Odonata (Perez *et al.* 2000, Nokkala *et al.* 2002 e Wang e Porter 2004), e em espécies de alguns grupos de

aracnídeos, como, Acari, Araneae e Scorpiones (Araujo *et al*. 2008 e Schneider et al. 2009).

Na literatura, são encontradas diferentes denominações para este tipo de cromossomo. Autores como Håkansson (1958), Hoshino (1987) e Vanzela *et al.* (1996) o denominam cromossomo holocêntrico, no entanto, há autores que denominam este cromossomo como policêntrico (Bernardini 1959). Apesar do nome holocêntrico ser erroneamente empregado, esta denominação é amplamente aceita e utilizada. O termo mais correto seria holocinético, pois não existe centrômero distribuído ao longo do cromossomo, mas sim, atividade cinética. Esta característica leva à migração paralela dos cromossomos na anáfase mitótica (Figura 7B), como já demonstrado por Harms (1968) e Guerra *et al.* (2006). Em alguns casos, são observadas constrições em alguns cromossomos. Estas são chamadas constrições nucleolares, que são relativas às constrições secundárias dos cromossomos monocêntricos (Westerman e Colle 1984, Vanzela *et al.* 2000 e Da Silva *et al.* 2008a).

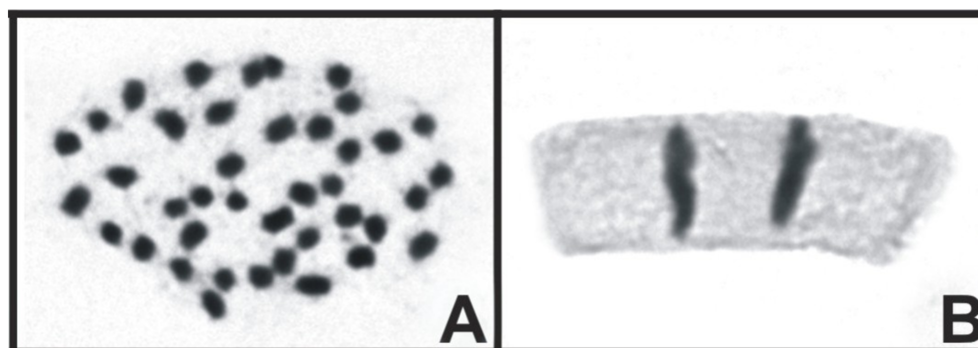


Figura 7. Cromossomos em coloração convencional com Giemsa A) Metáfase de *Eleocharis montana* (2*n* = 40), uma espécies com cromossomos holocêntrico. Note que neste tipo de cromossomo não é possível observar constrições. B) Migração paralela dos cromossomos holocêntricos durante a anáfase da mitose em *Eleocharis sellowiana* (2*n* = 20).

Cromossomos com cinetócoro difuso, quando natural ou artificialmente fragmentados e/ou fundidos, segregam normalmente na divisão celular, tanto na meiose quanto na mitose. Isto foi demonstrado por Castro *et al.* (1949), em *Luzula purpurea* (Juncaceae), por Håkansson (1954), em *Eleocharis palustris* (Cyperaceae) e em

Rhynschospora pubera, por Vanzela e Colaço (2002). Esses processos de quebra e fusão cromossômica em holocêntricos recebem a denominação de agmatoploidia (quebra) e simploidia (fusão), respectivamente (Luceño e Guerra 1997). Muito embora alguns trabalhos citem agmatoploidia tanto para quebra como para fusão cromossômica, em holocêntricos (Roalson 2008).

A permanência de cromossomos originados por fusão e fissão contribui para a extensa variação inter e intra-específica no número cromossômico em Cyperaceae, mesmo que poucas espécies tiveram seus números cromossômicos descritos. De cerca de 5.500 espécies de Cyperaceae (Govaerts *et al.* 2007), apenas 16% têm o número cromossômico conhecido, e metade destes números foram descritos para espécies de *Carex* L. (Roalson 2008). Apesar da escassez de conhecimento da evolução cromossômica nesta família (Hipp *et al.* 2009), a literatura indica que o processo de diferenciação dos cariótipos varia entre os grupos. Em *Carex*, agmatoploidia (quebra) e simploidia (fusão) são consideravelmente mais freqüentes (Hipp *et al.* 2009), quando comparado com outros gêneros, como *Rhynchospora* Vahl (Luceño *et al.* 1998 e Vanzela *et al.* 2000), *Scleria* PJ Bergius (Yano e Hoshino 2007) e *Eleocharis* (Yano *et al.* 2004, Da Silva *et al.* 2008a e Da Silva *et al.* 2010), nas quais a poliploidia é a principal causa de alterações nos números cromossômicos.

Neste contexto, não podemos descartar a possibilidade da existência de algum controle genético que favoreça a agmatoploidia e/ou simploidia em espécies diferentes. No entanto, a falta de estudos envolvendo um maior número de populações em uma mesma espécie, contribui para esta lacuna no conhecimento da evolução cromossômica em Cyperaceae. Estudos citogenéticos em diversas populações de uma determinada espécie podem revelar alterações cromossômicas que não são detectadas em amostras pequenas. As quais podem ser importantes na compreensão da evolução do cariótipo

desses grupos (Luceño e Castroviejo 1991, Hoshino e Okamura 1994, Ohkawa *et al.* 2000, Bureš *et al.* 2004 e Da Silva *et al.* 2008b).

Estudos citogenéticos em *Eleocharis* datam de antes de 1924, quando Piech descreveu o número de cromossomos de *E. prostes* (veja Nijalingappa 1973). Atualmente, a literatura tem registrado uma considerável variação no número cromossômico de espécies deste gênero, sendo de 2*n* = 6 para *E. subarticulata* (Da Silva *et al.* 2005) a 2*n* = 200 para *E. dulcis* (veja Roalson 2008). Variações intraespecíficas têm sido apontadas em algumas populações de *E. geniculata*, com 2*n* = 10 e 20 (Sanyal e Sharma 1972 e Nijalingapa 1973), *E. uniglumis* com 2*n* = 46, 78-82 e *E. palustris* com 2*n* = 14-17, 38 e 39, (Bureš 1998 e Bureš *et al.* 2004), *E. aciculares* f. *longiseta* com 2*n* = 20 e 21 (Yano *et al.* 2004) e *E. maculosa* com 2*n* = 10, 8, 7 e 6 (Da Silva *et al.* 2008b). Apesar dessa variação, a maioria das espécies possui números múltiplos de 5 (Da Silva *et al.* 2008a, Roalson 2008) tal número, *x* = 5, foi proposto como o número básico para a família por Löve *et al.* (1957).

1.4.2. Meiose nas Cyperaceae

A família Cyperaceae é conhecida pela ocorrência de meiose pós-reducional ou invertida. Este comportamento meiótico foi sugerido pela primeira vez em *Carex* por Heilborn (1928) e Wahl (1940). Este tipo de meiose, ocorre quando as cromátides irmãs apresentam orientação paralela na placa equatorial na metáfase I, migrando uma para cada lado na anáfase I. Com isso, o número de cromossomos não se reduz durante a primeira fase da meiose, já que o que se reduz é o número de

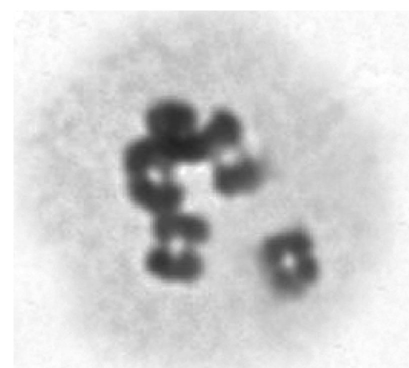


Figura 8. Cromossomos meióticos em coloração convencional com Giemsa em Metáfase I de *Eleocharis capillacea*, com cinco bivalentes formando estrutura em caixa.

cromátides de cada cromossomo. Nos cromossomos monocêntricos, as cromátides irmãs apresentarem orientação perpendicular ao eixo longo da placa equatorial antes da anáfase I, o que proporciona a separação dos cromossomos homólogos (meiose reducional).

O alinhamento dos bivalentes na meiose de holocêntricos gera uma configuração conhecida como "estrutura em caixa" (Figura 8) (Vanzela *et al.* 2000). Contudo, apenas a ocorrência desta estrutura não é prova de que a meiose é invertida. A análise de toda a meiose seria necessária para confirmar este comportamento. A meiose invertida foi bem documentada em *E. subarticulata*, na qual apesar da inexistência de bivalentes, pôde-se ver claramente que durante a primeira fase da meiose ocorre a separação das cromátides irmãs e, com isso, o número de cromossomos não é reduzido durante a anáfase I, ocorrendo a redução somente durante a segunda fase da meiose (Da Silva *et al.* 2005). Este tipo de comportamento meiótico foi sugerido para vários outros gêneros de Cyperaceae, como *Carex* (Wahl 1940), *Rhynchospora* (Vanzela *et al.* 2000) e também nos gêneros *Cuscuta*, da família Cuscutaceae (Pazi e Plitman 1994) e *Luzula*, da família Juncaceae (Nordenskiöld 1951). Além dos vegetais, alguns grupos de insetos que possuem cromossomos holocêntricos também apresentam meiose invertida; no entanto, diferentemente dos vegetais, nos quais este tipo de meiose ocorre para todos os cromossomos, em alguns grupos de insetos apenas os cromossomo sexuais, no sexo heterogamético, sofrem meiose invertida (Solari 1979).

Além de possuir cromossomos holocêntricos e meiose invertida, a família Cyperaceae exibe também a ausência de tétrades com formação de pseudomônades, vista também na família Epacridaceae (Brown e Lemmon 2000). Ao final da meiose tradicional, os núcleos se organizam em uma tétrade (McCormick 1993) e são gerados ao final quatro grãos de pólen (gametófitos). Em Cyperaceae, no entanto, três dos quatro

núcleos são degenerados e apenas um torna-se funcional (Furness e Rudall 1999, San Martins *et al.* 2009). Após o amadurecimento da pseudomônade, apenas um androgametófito é formado (Furness e Rudall 1999).

1.5 Localização de DNA repetitivo

Existe uma grande variação tanto na quantidade quanto na disposição de bandas heterocromáticas em cromossomos de angiospermas, e esta variação pode ser vista até mesmo entre espécies próximas (Rego *et al.* 2009). Apesar das técnicas de bandamento mostrarem muita eficácia para a compreensão da organização cromossômica, tais estudos ainda são escassos em espécies vegetais com cromossomos holocêntricos.

Dentre as técnicas mais empregadas, o bandamento-C tem sido usado para localizar blocos de heterocromatina, enquanto que as colorações com fluorocromos DAPI (4`-6 diamidino – 2 fenilindol) e CMA_3 (cromomicina A_3), são úteis para determinar a composição de bases de heterocromatina. O DAPI liga-se preferencialmente em regiões cromossômicas ricas em adenina e timina (A-T), enquanto CMA liga-se preferencialmente em regiões ricas em citosina e guanina (C-G). O uso sequencial destes fluorocromos permite entender a composição e a dinâmica de muitas famílias de DNA altamente repetitivas nos genomas (Fregonezi *et al.* 2006).

Alguns estudos têm demonstrado que cromossomos holocêntricos podem exibir blocos definidos de heterocromatina em regiões terminais e intersticiais (Westerman e Collet 1984; Panzera *et al.* 1992; Sheikh e Kondo 1995). De fato, o padrão de distribuição de heterocromatina em cromossomos holocêntricos parece ser similar ao encontrado em monocêntricos (Guerra 2000). Vanzela e Guerra (2000) descreveram para seis espécies de *Rhynchospora* (Cyperaceae) três tipos de heterocromatina, $CMA^+/DAPI^-$, $CMA^+/DAPI^+$ e $CMA^-/DAPI^+$, que variaram em tamanho e

posicionamento nos cromossomos das espécies analisadas. No entanto, para sete espécies de *Eleocharis* foi observado apenas blocos de heterocromatina $CMA^{-}/DAPI^{+}$ terminais (Da Silva *et al.* 2008a) nos cromossomos metafásicos mitóticos. Porém, quando estes cromossomos foram analisados em prófase, estas regiões de heterocromatina apresentaram-se dispersas ao longo dos cromossomos em pequenas porções.

Muitos segmentos repetidos de DNA têm sido precisamente detectados e mapeados fisicamente pela técnica de hibridação *in situ*. No entanto, os sítios de DNA ribossomal são, sem dúvida, os mais estudados por FISH em espécies vegetais e animais. Poucos estudos foram realizados em espécies vegetais com cromossomos holocêntricos. Em *Cuscuta approximata* (Cuscutaceae), sítios de DNAr 45S foram detectados em um par e sítios de DNAr 5S em três pares (Guerra e García 2004). Para a família Cyperaceae, vários sítios de rDNA 45S foram detectados em 8 espécies de *Rhynchospora* (Vanzela *et al.* 1998). Neste trabalho os autores encontraram uma grande variação no número de sítios; espécies com $2n = 10$ apresentaram de 4 a 8 sítios e, até 30 sítios em espécies com $2n = 50$. Apesar desta variação numérica, o posicionamento dos sítios foi sempre terminal.

Furuta e Hoshino (1999) descreveram, para *Eleocharis mamillata* var. *cyclocarpa* ($2n = 16$), 12 sinais terminais com a sonda de DNAr 18S e dois sinais intersticiais com sonda de DNAr 5S. Para outras sete espécies de *Eleocharis*, a sonda de DNAr 45S revelou sempre marcações terminais. Da Silva *et al*. (2008a) encontraram 10 marcações em *E. flavescens* ($2n = 10$), oito em *E. sellowiana* ($2n = 20$) e *E. acutangula* ($2n = 54$) e quatro em *E. maculosa* ($2n = 10$), *E. geniculata* ($2n = 20$) e *E. montana* ($2n = 40$). Para *E. subarticulata,* dos seis cromossomos do complemento diplóide, quatro revelaram sítios de DNAr 45S (Da Silva *et al.* 2005). A posição terminal destes sítios

permaneceu inalterada, até mesmo quando ocorrem rearranjos cromossômicos, como detectados em *E. maculosa*, na qual a simploidia foi responsável pela redução cromossômica de $2n = 10$ para $2n = 8$, 7 e 6. Neste trabalho também foram detectados dois sítios de DNAr 5S em cada cariótipo, porém, estes sítios foram terminais e intersticiais dependendo do cariótipo analisado (Da Silva *et al.* 2008b).

Além de sondas de DNAr, outras sondas foram utilizadas em estudos com espécies de Cyperaceae. Em *Rhynchospora tenuis*, hibridações *in situ* com a sonda telomérica (TTTAGGG) de *Arabidopsis thaliana* revelaram sinais apenas nas extremidades dos cromossomos de ambas as raças cromossômicas ($2n = 4$ e 8), e sinais intersticiais não foram encontrados (Vanzela *et al.* 2003). Sinais intersticiais também não foram detectados em *E. maculosa* que sofreram simploidia (Da Silva *et al.* 2008b). Porém, estudo realizado por Da Silva *et al.* (2005) com *Eleocharis subarticulata*, que possui um cariótipo originado por translocações múltiplas com apenas $2n = 6$, foram encontrados sítios ectópicos, adicionais aos sinais terminais.

2. Justificativa

Cyperaceae é a terceira maior família de monocotiledôneas (Trevisan e Boldrini 2008). O gênero *Eleocharis* é o quarto maior em número de espécies no Brasil. Muitas destas são endêmicas de áreas alagadas e importantes para a formação de biomassa nesses ecossistemas, como *E. interstincta* (Dos Santos e Esteves, 2005). Pelo menos duas espécies *E. acutangula* e *E. sellowiana* possuem atividade moluscicidas (Ruiz *et al.* 2005), já outras são utilizadas como ornamentação (*E. minima*) e muitas são consideradas ervas daninhas, principalmente em plantações de arroz.

O gênero *Eleocharis* apresenta inúmeras discordâncias quanto ao posicionamento das espécies em subgêneros, seções e séries, e isto tem trazido indefinições sobre os aspectos evolutivos do grupo (Roalson *et al. in press*). A escassez de informações citogenéticas em *Eleocharis*, e nas Cyperaceae de um modo geral, tem dificultado a compreensão de como tais cariótipos se organizam e se modificam. Tendo em vista isso, torna-se evidente a necessidade de ampliar tais conhecimentos neste grupo de plantas com cromossomos pouco comuns, não só com o auxílio de citogenética, mas também da taxonomia, morfologia e genética molecular.

3. Objetivos

Este trabalho tem como objetivo analisar, por parâmetros citogenéticos, moleculares e morfológicos, as relações entre as espécies do gênero *Eleocharis*, coletadas em vários estados do Brasil. Com estas análises, pretende-se esclarecer problemas taxonômicos e elaborar um modelo de diferenciação genético/cariotípica para um grupo vegetal com cromossomos especiais (holocêntricos).

3.1 Objetivos Específicos

(a) Coletar e identificar diferentes espécies e populações do gênero *Eleocharis* do território brasileiro para registrar a diversidade deste grupo;

(b) Determinar o número e a morfologia cromossômica de diferentes espécies pertencentes a diversas populações estudadas;

(c) Avaliar os mecanismos de diferenciação e evolução cariotípica em várias espécies de *Eleocharis,* pela aplicação das técnicas de coloração (i) convencional, (ii) bandamento cromossômico e (iii) hibridação *in situ*;

(d) Utilizar diferentes marcadores moleculares (RAPD) para diferenciar espécies e populações que tenham problemas taxonômicos evidentes;

(e) Elaborar um modelo evolutivo para o gênero baseado em características morfológicas e genéticas.

4. Referências

Alves MV, Araújo AC, Prata AP, Vitta FA, Hefler SM, Trevisan R, Gil AB, Martins S, Thomas SWW (2009). Diversity of Cyperaceae in Brazil. Rodriguésia 60 (4): 1-12.

Araujo D, Rhemis CA, Brescovit AD, Cella DM (2008). Extreme degree of chromosome number variability in species of the spider genus *Scytodes* (Araneae, Haplogynae, Scytodidae). J Zool Syst Evol Res 46: 89 – 95.

Barros M (1960). Las Ciperáceas del estado de Santa Catarina. Sellowia 12: 1-430.

Bernardini J (1959). Studies of the kinetochore in *Eleocharis macrostachya* Britt. Proc. Monnesota Acad. Sci. 27: 104-114.

Brown RC, Lemmon BE (2000). The cytoskeleton and polarization during pollen development in *Carex blanda* (Cyperaceae). Amer J. Bot. 87: 1–11.

Bruhl JJ (1995). Sedge genera of the world: relationships and a new classification of the Cyperaceae. Aust J Bot 8: 125-305.

Buchwitz BJ, Ahmad K, Moore LL, Roth MB, Henikoff S (1999). A histone-H3-like protein in *C. elegans*. Nature 401:547–548.

Bureš P (1998). A high polyploidy *Eleocharis uniglumis* S.L. (Cyperaceae) from central and southeastern Europe Folia Geobot 33: 429-439.

Bureš P, Rotreklová O, Stoneberg HSD, Pikner R (2004). Cytogeographical survey of *Eleocharis* subser. *Eleocharis* in Europe 1. *Eleocharis palustris*. Folia Geobot 39: 235-257.

Castro D, Camara A, Malheiros N (1949). X-rays in the centromere problem of *Luzula purpurea* Link. Gen. Iber. 1: 49-54

Dahlgren RMT, Clifford HT, Yeo PF (1985). The families of the monocotyledons: structure, evolution and taxonomy. Berlin: Springer-Verlag.

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2005). Reduction of chromosome number in *Eleocharis subarticulata* (Cyperaceae) by multiple translocations. Bot. J. Linn. Soc. 149, 457–464.

Da Silva CRM, González-Elizondo MS, Rego LNAA, Torezan JMD, Vanzela ALL (2008a). Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis*. Aust J Bot 56: 82-90.

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2008b). Chromosome Reduction in *Eleocharis maculosa* (Cyperaceae). Cytogenetic Genome Res 122, 175-180.

Da Silva CRM, Trevisan R, González-Elizondo MS, Ferreira JM, Vanzela ALL (2010). Karyotypic diversification and its contribution to the taxonomy of *Eleocharis* (Cyperaceae) from Brazil. Aust J Bot 58: 1–12

Dos Santos AM, Esteves FA (2005). Influence of Water Level Fluctuation on the Mortality and Aboveground Biomass of the Aquatic Macrophyte *Eleocharis interstincta* (VAHL) Roemer et Schults. Brazilian Archives of Biology and Technology 47: 281-290

Flach M (1966). Diffuse centromeres in a dicotyledonous plant. Nature 209: 1369–1370.

Fregonezi JN, Fernandes T, Torezan JMD, Vieira AOS and Vanzela ALL (2006). Karyotype differentiation of four *Cestrum* species (Solanaceae) based on physical mapping of repetitive DNA. Gen Mol Biol 29:97-104.

Furness CA, Rudall PJ (1999). Microsporogenesis in monocotyledons. Ann Bot 84: 75-99.

Furuta K, Hoshino T (1999). Localization of the 18S and 5S rDNA on the chromosomes non-localized centromere in Cyperaceae. XVI International Botanical congress. Abstract Number: 4331 Poster No. 1829.

Gil ASB, Bove CP (2007). *Eleocharis* R.Br. (Cyperaceae) no Estado do Rio de Janeiro, Brasil. Biota Neotropica 7: 163 – 193.

Goetghebeur P (1998). Cyperaceae. Pp. 141-190. In: Kubitzki, K. (ed.). The families and genera of vascular plant: IV. Flowering plants – monocotyledons. Berlin: Springer-Verlag.

González-Elizondo MS (1994). *Eleocharis* R. Br. (Cyperaceae) Pp. 458-464. In: Davidse, G., Souza, M. & Chater, A. O. (eds.). Flora Mesoamericana. Alismataceae a Cyperaceae 6. Mexico: Universidad Nacional Atónoma de Mexico, Missouri Botanical Garden, The Natural History Museum (London).

González-Elizondo MS, Peterson PM (1997). A classification of and key to the supraspecific taxa in *Eleocharis* (Cyperaceae). Taxon 46: 433-449.

Govaerts R, Simpson D, Bruhl J, Egorova T, Goetghebeur P, Wilson K (2007). World checklist of Cyperaceae – Sedges. Kew Gardens, Surrey.

Guerra MS (2000). Patterns of heterochromatin distribution in plant chromosomes. Genet. Mol. Biol. 23: 1029-1041.

Guerra M, García MA (2004). Heterochromatin and rDNA sites distribution in the holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae). Genome 47: 134–140.

Guerra M, Brasileiro-Vidal AC, Arana P, Puertas MJ (2006) Mitotic microtubule development and histone H3 phosphorylation in the holocentric chromosomes of *Rhynchospora tenuis* (Cyperaceae). Genetica 126: 33-41.

Håkansson A (1954). Meiosis and pollen mitosis in x-rayed and untreated spikelets of *Eleocharis palustris*. Hereditas (Lund) 15: 325–345.

Håkansson A (1958) Holocentric chromosomes in Cyperaceae. Hereditas 11: 182-192.

Harms LJ (1968). Cytotaxonomic studies in *Eleocharis* Subser. Palustres: Central united States Taxa. Amer J. Bot. 55: 966-974.

Hipp AL, Rothrock PE, Roalson EH (2009). The evolution of chromosome arrangements in *Carex* (Cyperaceae). Bot Rev 75: 96-109.

Heilborn O (1928). Chromosome studies in Cyperaceae. Hereditas 11: 182–192.

Hoshino T (1987) Karyomorphological studies on 6 taxa of *Eleocharis* in Japan. Bulletin of the Okayama University of Science 22, 305–312.

Hoshino T, Okamura K (1994). Cytological studies on meiotic configurations of intraspecific aneuploids of *Carex blepharicarpa* (Cyperaceae) in Japan. J Plant Res 107: 1–8.

Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999). Systematics: a philogenetic approach. Sinauer Associates, Inc: Massachusetts. USA.

Koyama T (1961). Classification of the family Cyperaceae. J. Fac. Sci. Univ. Tokyo, Sect. 3. Bot. 8: 37-148.

Kukkonen I (1990). On the genus *Eleocharis* (Cyperaceae) in the flora Iranica area, with revised infrageneric classification and nomenclature. Ann. Bot. Fenn. 27: 109-117.

Kunth CS (1937). Enumeration plantarum omnium hucusque cognitarum. 2. Cyperographia synoptica.

Löve A, Löve D, Raymond M (1957). Cytotaxonomy of *Carex* section *Capillares*. Can. J. Bot. 35: 715–761.

Luceño M, Castroviejo S (1991). Agmatoploidy in *Carex laevigata* (Cyperaceae): Fusion and fission of chromosomes as the mechanism of cytogenetic evolution in Iberian populations. Pl. Syst. Evol. 177: 149–160.

Luceño M, Alves M, Mendes AP (1997). Catálogo florístico y claves de identificación de lãs Cyperaceae de los estados de Paraíba y Pernambuco (Nordeste de Brasil). Anales del Jardín Botánico de Madrid 55: 67-100.

Luceño M, Guerra M (1997). Numerical variation in species exhibiting holocentric chromosomes: a nomeclatura proposal. Caryologia 49: 301-309.

Luceño M, Vanzela ALL, Guerra M (1998). Cytotaxonomic studies in Brazilian *Rhynchospora* (Cyperaceae), a genus exhibiting holocentric chromosomes. Can. J. Bot. 76: 440–449.

Lye KA (2000). Achene structure and function of structure in Cyperaceae. Pp. 615-628. In: Wilson, K. L. & Morrison, D. A. (eds.). Monocots: Systematics and Evolution. Melbourne: CSIRO.

Malheiros N, De Castro D, Câmara A, (1947). Cromosomas sem centrômero localizado. Agron. Lusitana 9: 57–74.

McCormick S (1993) Male gametophyte development. Plant Cell 5: 65-75.

Menapace FJ (1990). A preliminary micromorphological analysis of *Eleocharis* (Cyperaceae) achenes for systematic potencial. Can. J. Bot. 69: 1533-1541.

Mohlenbrock RH (1976). The illustrated Flora of Illinois. Sdges – Cyperus to Scleria. London: Southern Illinois University Prees.

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. Bot. Rev. 75:2–21

Muasya AM, Bruhl JJ, Simpson DA, Culham A, Chase MW (2000). Suprageneric phylogeny of Cyperaceae: a combined analysis. Pp. 593-601. In: Wilson, K. L. & Morrison, D. A. (eds.). Monocots: Systematics and Evolution. Melbourne: CSIRO.

Nagaki K, Kashihara K, Murata M (2005). Visualization of diffuse centromeres with centromere-specific histone H3 in the holocentric plant *Luzula nivea*. The Plant Cell 17, 1886–1893.

Nees TFL (1834). No III. Cyperaceae indicae. Praecipue juxta herbaria Wightii, Wallichii, Roylei et Lindleyi. Pp 69-129 in: Wight, R., Contributions to the botany of india. London.

Nees TFL (1835). Uebersicht der Cyperaceengattungen. Linnaea 9:273-306.

Nees C (1842). Cyperaceae. In: Martius, C. (ed.). Flora brasiliensis. Munich. Vol. 2. Pp.1-226.

Nijalingappa BHM (1973). Cytological studies in *Eleocharis*. Caryologia 26: 512-520.

Nokkala S, Laukkanen A, Nokkala C (2002). Mitotic and meiotic chromosomes in *Somatochlora metallica* (Cordulidae, Odonata). The absence of localized centromeres and inverted meiosis. Hereditas 136: 7–12.

Nordenskiöld H (1951). Cyto-taxonomical studies in the genus *Luzula* I. Somatic chromosomes and chromosomes numbers. Hereditas 37: 325-355.

Ohkawa T, Yokota M, Hoshino T (2000). Aneuploidal population differentiation in *Carex sociata* Boott (Cyperaceae) of the Ryukyu Islands, Japan. Bot. J. Linn. Soc 132: 337-358.

Panzera F, Álvarez F, Sánchez-rufas J, Pérez R, Sujas JA, Scvortzoff E, Dujardinni JP, Estramil E, Salvatella R (1992). C-heterocromatin polymorphism in holocentric cromosomes of *Triatoma infestans* (Hemíptera-Reduviidae) Genoma 35: 1068-1074.

Pazy B, Plitman U (1994). Holocentric chromosome behaviour in *Cuscuta* (Cuscutaceae). Plant Syst. Evol. 191: 105–109.

Perez R, Rufas JS, Suja JA, Page J, Panzera F (2000). Meiosis in holocentric chromosomes: Orientation and segregation of an autosome and sex chromosomes in *Triatoma infestans* (Heteroptera). Chrom Res 8: 17–25.

Prata AP (2002). Listagem florística das Cyperaceae do estado de Roraima, Brasil. Hoehnea 29: 93-107.

Rego LNAA, Da Silva CRM, Torezan JMD, Gaeta ML, Vanzela ALL (2009). Cytotaxonomical study in Brazilian species of *Solanum*, *Lycianthes* and *Vassobia* (Solanaceae). Plant Syst Evol 279: 93-102.

Roalson EH, Friar EA (2000). Infrageneric classification of Eleocharis (Cyperaceae) revisited: evidence from the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA. Syst Bot 25: 323-336.

Roalson EH (2008). A synopsis of chromosome number variation in the Cyperaceae. Bot Rev 74: 209-393

Roalson EH, Hinchliff CE, Trevisan R, Da Silva CRM (*in press*). Phylogenetic relationships in *Eleocharis* (Cyperaceae): C4 photosynthesis origins and patterns of diversification in the spikerushes. Syst Bot 35: xxx-xxx.

Ruiz ALTG, Magalhães EG, Magalhães AF, Faria AD, Amaral MCE, Serrano DR, Zanotti-Magalhães EM, Magalhães AL (2005). Avaliação da atividade tóxica em *Artemia salina* e *Biomphalaria glabrata* de extratos de quatro espécies do gênero *Eleocharis* (Cyperaceae) Braz J Pharma 15: 98-102

San Martins JAB, Andrade CGTJ, Vanzela ALL (2009). Early meiosis in *Rhynchospora pubera* L. (Cyperaceae) is marked by uncommon ultrastructural features Cell Biol Int 33: 1118-1122.

Sanyal B, Sharma AK (1972). Cytological studies in Indian Cyperaceae. I. Tribe *Scirpeae*. Cytologia 37: 13-32.

Scharader F (1935). Notes on the mitotic behavior of long chromosomes. Cytologia. 6: 422-430.

Schneider MC, Zacaro AA, Pinto-Da-Rocha R, Candido DM, Cella DM (2009). A Comparative Cytogenetic Analysis of 2 *Bothriuridae* Species and Overview of the Chromosome Data of Scorpiones. J Hered 100:545-555.

Sheikh SA, Kondo K (1995) Differential staining with orcein, giemsa CMA, and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). Amer J. Bot. 82, 1278–1286.

Simpson DA (1988). Some problems in *Eleocharis*. Notes on Brazilian Cyperaceae: III. Kew Bulletin, 43(1): 127-134.

Simpson DA, Furness CA, Hodkinson TR, Muasya AM, Chase MW (2003). Phylogenetic relationships in Cyperaceae Subfamily Mapaniodeae inferred from pollen and plastid DNA sequence data. Amer J. Bot. 90: 1071–1086.

Simpson DA, Muasya AM, Alves MV, Bruhl JJ, Dhooge S, Chase MW, Furness CA, Ghamkhar K, Goetghebeur P, Hodkinson TR, Marchant AD, Nieuborg R, Reznicek AA, Roalson EH, Smets E, Starr JR, Thomas WW, Wilson KL, Zhang X (2007). Phylogeny of Cyperaceae based on DNA sequence data—A new rbcL analysis. Aliso 23: 72–83.

Solari AJ (1979). Autosomal synaptonemal complex and sex chromosomes without axes in *Triatoma infestans* (Reduviidae, Hemiptera). Chromosoma 72:225-240.

Strandhede SO (1965) Chromosome studies in *Eleocharis*, subser. Palustres. Opera Bot 9, 1–86.

Svenson HK (1929). Monographic Studies in the Genus *Eleocharis* I. Rhodora 31: 121-242.

Svenson HK (1932). Monographic Studies in the Genus *Eleocharis* II. Rhodora 34: 193-227.

Svenson HK (1934). Monographic Studies in the Genus *Eleocharis* III. Rhodora 36: 377-389.

Svenson HK (1937). Monographic Studies in the Genus *Eleocharis* IV. Rhodora 39: 210-273.

Svenson HK (1939). Monographic Studies in the Genus *Eleocharis* V. Rhodora 41: 1-110.

Tanaka N, Tanaka N (1977). Chromosome studies in *Chionographis* (Liliaceae) I. On the holokinetic nature of chromosomes in *Chionographis japonica* Maxim. Cytologia 42: 753–763.

Torrey J (1836). Monograph of North American Cyperaceae. Ann. Lyceum Nat. Hist. New York 3: 239-448.

Trevisan R, Boldrini II (2008). O gênero *Eleocharis* R. Br. (Cyperaceae) no Rio Grande do Sul, Brasil. Revista Brasileira de Bociências 6, 7-67.

Ueno O, Samejima M, Koyama T (1989). Distribution and evolution of C4 syndrome in *Eleocharis*, a sedge group inhabiting wet and aquatic environments, based on culm anatomy and carbon isotope ratios. Ann Bot. 64: 425-438.

Vanzela ALL, Guerra M, Luceño M (1996) *Rhynchospora tenuis* Link (Cyperaceae), a species with the lowest number of holocentric chromosomes (n=2). Cytobios 88, 219–228.

Vanzela ALL, Cuadrado A, Jouve N, Luceño M, Guerra M (1998). Multiple locations of the rDNA sites in holocentric chromosomes of *Rhynchospora* (*Cyperaceae*). Chrom Res 6: 345-349.

Vanzela ALL, Guerra M (2000). Heterochromatin differentiation in holocentric chromosomes of *Rhynchospora* (Cyperaceae) Genet. Mol. Biol. 2: 453-456.

Vanzela ALL, Luceño M, Guerra M (2000). Karyotype evolution and cytotaxonomy in Brazilian species of *Rhynchospora* Vahl (Cyperaceae). Bot. J. Linn. Soc. 134: 557–566.

Vanzela ALL, Colaço W (2002). Mitotic and meiotic behavior of γ irradiated holocentric chromosomes of *Rhynchospora pubera* (Cyperaceae). Act Scientiarum 2: 611-614.

Vanzela ALL, Cuadrado A, Guerra M (2003). Localization of 45S rDNA and telomeric sites on holocentric chromosomes of *Rhynchospora tenuis* Link (Cyperaceae). Gen Mo Bio 26: 199-201.

Yano O, Katsuyama T, Tsubota H, Hoshino T (2004). Molecular phylogeny of Japanese *Eleocharis* (Cyperaceae) based on ITS sequence data, and chromosomal evolution. J Plant Res 117, 409-419.

Yano O, Hoshino T (2007). Karyomorphological Studies of Four Species of Japanese *Scleria* (Cyperaceae). Cytologia 72: 275-278.

Wahl HA (1940). Chromosome numbers and meiosis in the genus *Carex*. Amer. J. Bot. 27: 458–470.

Wang B, Porter AH (2004). An AFLP-based interspecific linkage map of sympatric, hybridizing Colias butterflies. Genetics 168: 215–225.

Westerman M, Colle C (1984). Interspersed distribution patterns of c-bands and satellite DNA in the holocentric chromosomes of *Luzula flaccida* (Juncaceae). Genetica 63: 175-179.

Capítulo 1

Karyotypic diversification and its contribution to the taxonomy of *Eleocharis* (Cyperaceae) from Brazil

Artigo publicado na revista Australian Journal of Botany, 2010, 58, 49–60

CSIRO PUBLISHING

www.publish.csiro.au/journals/ajb *Australian Journal of Botany*, 2010, **58**, 49–60

Karyotypic diversification and its contribution to the taxonomy of *Eleocharis* (Cyperaceae) from Brazil

Carlos Roberto Maximiano da Silva[A], *Rafael Trevisan*[B], *Maria Socorro González-Elizondo*[C], *Josué Maldonado Ferreira*[D] *and André Luís Laforga Vanzela*[D,E,F]

[A]Universidade Estadual Paulista, Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, (UNESP/IBILCE), 15054-000 São José do Rio Preto, SP, Brazil.

[B]Instituto de Biociências, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, RS, Brazil.

[C]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, IPN, Sigma 119 Fracc. 20 de Noviembre II, 34220 Durango, Dgo., Mexico.

[D]Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, 86051-970 Londrina, PR, Brazil.

[E]Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, Paraná, CEP 86051990, Caixa Postal 6001.

[F]Corresponding author. Email: andrevanzela@uel.br

Abstract. A karyotype analysis of 147 populations of 25 Brazilian species of *Eleocharis* (Cyperaceae) was carried out, including representatives of the three subgenera that occur in the country: *Limnochloa*, *Scirpidium* and *Eleocharis*. The analyses showed chromosomes without centromeres, but with terminal nucleolar constrictions (satellites) in some chromosomes. The chromosome numbers varied from $2n = 6$ in *E. subarticulata* and *E. maculosa* to $2n = 60$ in *E. laeviglumis*, but the chromosome basic number $x = 5$ was confirmed. Species of the subgenera *Eleocharis* and *Scirpidium* possess fewer and larger chromosomes, while those in the subgenus *Limnochloa* have small and more numerous chromosomes. These features indicate that the karyotypes of the subgenera *Eleocharis* and *Scirpidium* are more closely related, in agreement with morphological and phylogenetical data. The representatives of the section *Eleocharis* exhibited the largest differences in chromosome number and size, probably due to chromosome fission and fusion. Polyploidy was the most common event in this group. Nevertheless, most of the studied species exhibited regular meiosis with only bivalent formation, even the polyploids, such as in *E. geniculata* and *E. sellowiana*. The cytogenetic information obtained showed quite variable karyotypes with chromosomes gradually decreasing in size, and predominance of polyploidy. These results are useful in the differentiation of the subgenera.

Introduction

Eleocharis R.Br. (Cyperaceae) is a genus with a worldwide distribution, including more than 200 species with a remarkable richness in South America (González-Elizondo and Tena-Flores 2000). The relatively simple morphology associated with a great phenotypic variation make difficult the identification and delimitation of the species (Simpson 1988; González-Elizondo and Peterson 1997; Trevisan and Boldrini 2008). There are few morphological characters that have taxonomic value in *Eleocharis* and several of them may be over-weighted, for example the ornamentation of the achenes and the number of style branches, which shows a high degree of homoplasy (González-Elizondo and Peterson 1997).

The supraspecific classifications that have been proposed for *Eleocharis*, for example, Svenson (1929, 1939), Kukkonen (1990) and González-Elizondo and Peterson (1997), are based on morphological data, and can be considered preliminary due to the scarce diagnostic features. When morphology is compared with the current phylogenetic hypothesis, it seems to be clear that many morphological characters are either pleisiomorphic or have multiple origins (Roalson and Hinchliff 2007). To understand the evolutionary process in this group of plants, reviews are necessary that combine different approaches, as morphological, anatomical, cytogenetical and molecular markers, as suggested by González-Elizondo and Peterson (1997) and Roalson and Friar (2000). Molecular and cytogenetical analyses were useful to understand the evolutionary profile of two Cyperaceae genera from Japan. *Fimbritylis* Vahl was considered monophyletic and composed by three karyotype groups (Yano and Hoshino 2006*a*) and *Schoenoplectus* (Rchb.) with two clades with predominance of polyploidy (Yano and Hoshino 2005).

Eleocharis, as well as the other representatives of Cyperaceae, possess holocentric chromosomes, inverted meiosis and absence of tetrads with pseudomonad formation (Wahl 1940; Davies 1956; Strandhede 1965; Faulkner 1972). This family exhibits a wide range of chromosome numbers (Roalson 2008), originated by fusion (symploidy), fission (agmatoploidy) and polyploidy

© CSIRO 2010 10.1071/BT09185 0067-1924/10/010049

Table 1. Karyotype features of Brazilian species of *Eleocharis* R.Br. Species are grouped according to the supraspecific classification of González-Elizondo and Peterson (1997)

Vouchers were deposited at the ICN herbarium of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Species	2*n*	Total length (µm)	L/S (µm)[C]	Coordinates, localities (voucher number)
Subgenus ***Eleocharis***				
Section ***Eleogenus*** (Nees) Benth. and Hook. f. (1883)				
E. maculosa (Vahl) Roem. and Schult.	10	10.86	2.3–1.8	30°12′08′S 50°12′52′W, Balneário Pinhal, RS (158385)
	8	18.75*	4.4–2.0	19°13′20′S 43°33′32′W, Serra do Cipó, MG (158386)
	7	19.04*	4.3–1.8	19°32′52′S 43°44′29′W, Jaboticatubas, MG (158390)
	6	19.04*	4.4–1.8	28°09′20′S 48°51′78′W, Apiaí, SP (158387) 24°21′58′S 49°02′06′W, Palmitalzinho, SP (158389)
E. capillacea Kunth[A]	10	7.44	1.6–1.2	22°15′54′S 47°55′30′W, Brotas, SP (152642)22°36′45′S 50°23′57′W, Assis, SP (26)24°11′47′S 49°07′09′W, Bom Sucesso do Itararé, SP (239)19°13′20′S 43°33′32′W, Serra do Cipó, MG (152652; 152661) 19°04′13′S 43°24′58′W, Conceição do Mato Dentro, MG (133)
E. flavescens (Poir.) Urb.	10	6.70	1.4–1.1	25°50′53′S 48°32′50′W, Caiobá, PR (156144)
E. geniculata (L.) Roem. and Schult.	10	10.50	2.4–1.7	09°05′39′S 35°16′23′W, Japaratinga, AL (8)25°50′53′S 48°32′50′W, Caiobá, PR (156140)24°53′60′S 50°18′80′W, Ponta Grossa, PR (2)08°23′50′S 35°05′42′W Ipojuca, PR (10)
	20	14.77	1.7–1.2	20°34′51′S 51°36′36′W, Vestia, MS (69)07°45′10′S 34°51′38′W, Itamaracá, PE (9)23°14′03′S 44°45′28′W, Paraty, RJ (74)
E. sellowiana Kunth	10	6.70	2.0–1.2	25°17′48′S 49°54′42′W, Tibagi, PR (152018)25°28′74′S 49°52′93′W, São Luis do Purunã, PR (152019) 22°29′42′S 50°17′06′W, Echaporã, SP (4)26°16′46′S 51°03′24′W, Porto União, SC (84)29°48′18′S 50°03′45′W, Xangri-lá, RS (225)
	20	7.44	2.1–1.4	21°45′13′S 48°59′55′W, Ibitinga, SP (151980 and 3) 22°29′42′S 50°17′06′W, Echaporã, SP (151994) 22°32′51′S 50°21′18′W Assis, SP (152006)26°51′31′S 50°49′54′W, Caçador, SC (152011)26°14′56′S 49°54′27′W, Mafra, SC (152022)26°42′24′S 51°35′11′W, Irani, SC (204)25°50′53′S 48°32′50′W, Caiobá, PR (156143)25°17′48′S 49°54′42′W, Tibagi, PR (152018)24°57′40′S 53°26′12′W, Cascavel, PR (391)46°29′51′S 21°49′42′W, Poços de Caldas, MG (152645)19°04′13′S 43°24′58′W, Conceição do Mato Dentro, MG (152656)20°38′42′S 46°21′24′W, Furnas, MG (152663)19°32′52′S 43°44′29′W, Jaboticatubas, MG (152665)19°13′20′S 43°33′32′W, Serra do Cipó, MG (152669)21°34′08′S 45°46′77′W, Paraguaçu, MG (200)19°42′49′S 51°08′31′W, Paranaíba, MS (152672)20°10′30′S 51°12′54′W, Aparecida do Taboado, MS (62)30°06′18′S 50°44′33′W, Viamão, RS (191)30°12′08′S 50°12′52′W, Balneário Pinhal, RS (215)
E. debilis Kunth[A]	30	28.22	2.2–1.5	25°39′59′S 51°40′05′W, Pinhão, PR (153091)25°21′55′S 48°52′29′W, Morretes, PR (156128 and 156129)
Section ***Eleocharis***				
E. minima Kunth	20	15.66[B]	2.0–1.2	26°36′54′S 51°04′18′W, Calmon, PR (152033)
	30	22.91[B]	1.7–1.3	20°07′16′S 44°32′25′W, Itaúna, MG (152658)
	34	45.83[B]	3.8–2.1	19°42′09′S 50°35′33′W, Alexandrita, MG (152674) 19°04′13′S 43°24′58′W, Conceição do Mato Dentro, MG (152654)25°17′248′S 49°54′42′W, Caçador, SC (152010)29°54′18′S 50°06′15′W, Imbé, RS (218)
E. contracta Maury[A]	20	19.42	2.2–1.5	26°51′31′S 50°49′67′W, Caçador, MG (152014) 25°17′48′S 49°54′42′W, Tibagi, PR (152096)

Table 1. (*continued*)

Species	2*n*	Total length (µm)	L/S (µm)[C]	Coordinates, localities (voucher number)
E. nana Kunth[A]	20	18.06	2.8–1.2	25°38′37′S 51°40′01′W, Pinhão, PR (158383)
E. loefgreniana Boeck.[A]	20	16.84	2.0–1.4	19°13′20′S 43°33′32′W, Serra do Cipó, MG (152659)
Eleocharis sp. 1[A]	40	34.98	2.1–1.3	20°05′57′S 51°09′33′W, Aparecida do Taboado, MS (151987)19°10′55′S 57°42′47′W, Pantanal, MS (153095)20°38′42′S 46°21′24′W, Furnas, MG (158459)
E. niederleinii Boeck.[A]	20	13.86	1.6–1.7	25°38′37′S 51°40′01′W, Pinhão, PR (158380)19°42′09′S 50°35′33′W, Alexandrita, MG (151975)20°10′30′S 51°12′54′W, Aparecida do Taboado, MS (151991) 27°07′12′S 48°36′77′W, Rio Lavadeiras, SC (1) 30°06′18′S 50°44′33′W, Viamão, RS (192)
	30	33.10	6.5–0.5	26°16′46′S 51°03′02′W, Porto União, SC (152027) 25°41′06′S 51°32′08′W, Turvo, PR (152032)
E. viridans Kük. ex Osten[A]	20	17.88	2.2–1.3	30°06′18′S 50°44′33′W, Viamão, RS (153094)
	40	35.21	3.8–1.1	25°17′24′S 49°54′42′W, Caçador, SC (92)23°14′03′S 44°45′28′W, Paraty, RJ (75)26°49′67′S 50°21′54′W, Lebon Regis, SC (94)
E. filiculmis Kunth[A]	30	25.77	2.1–1.3	20°34′51′S 51°36′36′W, Vestia, MS (151969)19°42′49′S 51°08′31′W, Paranaíba, MS (151999)20°10′30′S 51°12′54′W, Aparecida do Taboado, MS (151986 and 151990)19°04′13′S 43°24′58′W, Conceição do Mato Dentro, MG (152657)20°00′54′S 49°03′18′W, Itapagipe, MG (151974)19°43′39′S 50°13′39′W, Alexandrita, MG (1511978)49°03′18′S 20°00′54′W, Frutal, MG (152670)20°07′16′S 44°32′25′W, Itaúna, MG (114)19°13′20′S 43°33′32′W, Serra do Cipó, MG (141)22°15′54′S 47°55′30′W, Brotas, SP (152001) 22°32′51′S 50°21′18′W, Assis, SP (152005)26°51′83′S 50°49′67′W, Caçador, SC (152012)24°57′40′S 53°26′12′W, Cascavel, PR (389)
E. montana (Kunth) Roem. and Schult.	40	41.19	2.9–1.3	23°13′73′S 51°13′71′W, Londrina, PR (151964) 25°36′47′S 49°49′55′W, Lapa, PR (152023)25°36′16′S 51°40′74′W, Pinhão, PR (152028)21°45′13′S 48°59′55′W, Ibtinga, SP (151979)22°32′51′S 50°21′18′W, Assis, SP (24 and 152002)22°46′39′S 50°22′21′W, Cândido Mota, SP (77)26°51′83′S 50°49′67′W, Caçador, SC (152015)26°49′67′S 50°21′54′W, Lebon Regis, SC (152021)26°16′46′S 51°03′24′W, Porto União, SC (152034)19°53′15′S 49°22′39′W, Itapagipe, MG (151973)21°49′42′S 46°29′51′W, Poços de Caldas, MG (152643) 20°38′42′S 46°21′24′W, Furnas, MG (152662) 20°07′16′S 44°32′25′W, Itaúna, MG (152664) 19°04′13′S 43°24′58′W, Conceição do Mato Dentro, MG (138)20°34′51′S 51°36′36′W, Vestia, MS (67) 30°12′08′S 50°12′52′W, Balneário Pinhal, RS (213)
E. subarticulata (Nees) Boeck.	6	19.10*	3.8–2.2	21°49′42′S 46°29′51′W, Poços de Caldas, MG (152647) 25°17′24′S 49°54′42′W, Tibagi, PR (152031) 24°32′16′S 49°57′11′W, Piraí do Sul, PR (156093) 25°35′17′S 51°33′34′W, Pinhão, PR (156125) 25°22′51′S 48°51′50′W, Morretes, PR (156130) 25°28′74′S 49°52′93′W, São Luis do Purunã, PR (102) 29°03′57′S 50°06′19′W, Cambará do Sul, RS (236) 28°45′05′S 50°22′09′W, Cambará do Sul, RS (230)
Subgenus ***Scirpidium*** (Nees) Kukkonen				
Eleocharis sp. 2[A]	10	8.47	1.9–1.5	25°39′59′S 51°40′05′W, Pinhão, PR (193)
E. bonariensis Nees[A]	20	20.20	2.5–1.6	28°45′05′S 50°22′09′W, Cambará do Sul, RS (229) 30°06′18′S 50°44′33′W, Viamão, RS (189)

(*continued on next page*)

Table 1. (*continued*)

Species	2*n*	Total length (µm)	L/S (µm)[C]	Coordinates, localities (voucher number)
Subgenus ***Limnochloa*** (P. Beauv. ex. Lestib.) Torr.				
E. interstincta (Vahl) Roem and Schult.[A]	40 52	22.92 23.58	1.4–0.9 1.1–0.7	08°39′40′S 35°09′57′W, Rio Formoso, PE (11) 25°48′28′S 48°33′11′W, Matinhos, PR (158384) 25°42′38′S 48°34′34′W, Matinhos, PR (374) 20°14′06′S 49°10′12′W, Fronteira, MG (35)
E. obtusetrigona (Lindl. and Nees) Steud.[A]	52	24.40	1.1–0.7	19°4′13′S 43°24′58′W, Conceição do Mato Dentro, MG (152655)19°32′52′S 43°44′29′W, Jaboticatubas, MG (119)23°28′05′S 48°39′18′W, Paranapanema, SP (284) 25°36′16′S 51°40′74′W, Pinhão, PR (354)
E. liesneri S. González and Reznicek[A]	50	24.24	1.3–0.7	21°34′08′S 45°46′77′W, Paraguaçu, MG (152648)
E. plicarhachis (Griseb.) Svenson[A]	54	30.64	1.5–0.9	19°10′55′S 57°42′47′W, Pantanal, MS (180)20°10′30′S 51°12′54′W, Aparecida do Taboado, MS (63) 09°57′76′S 67°52′98′W, Rio Branco, AC (158)
E. acutangula (Roxb.) Schult.	54	30.63	1.3–1.0	19°43′39′S 50°13′39′W, Iturama, MG (151977) 21°34′08′S 45°46′77′W, Paraguaçu, MG (152649) 19°32′52′S 43°44′29′W, Jaboticatubas, MG (152666) 20°00′54′S 49°03′18′W, Itapagipe, MG (36 and 38) 19°42′09′S 50°35′33′W, Alexandrita, MG (41) 20°38′42′S 46°21′24′W, Furnas, MG (112)19°4′13′S 43°24′58′W, Conceição do Mato Dentro, MG (134) 46°29′51′S 21°49′42′W, Poços de Caldas, MG (148) 22°29′42′S 50°17′06′W, Echaporã, SP (13)22°28′28′S 50°20′06′W, Assis, SP (19, 20 and 23)22°15′54′S 47°55′30′W, Brotas, SP (156)22°46′39′S 50°22′21′W, Cândido Mota, SP (78)20°05′57′S 51°09′33′W, Aparecida do Taboado, MS, (55 and 64)24°57′40′S 53°26′12′W, Cascavel, PR (390)25°31′35′S 49°19′45′W, Piraí do Sul, PR (158378)09°57′76′S 67°52′98′W, Rio Branco, AC (160)
Eleocharis sp. 3[A]	42	22.31	1.2–0.8	08°39′40′S 35°09′57′W, Rio Formoso, PE (12)
E. laeviglumis R. Trevis. and Boldrini[A]	60	31.67	1.2–0.8	29°54′18′S 50°06′15′W, Imbé, RS (219)

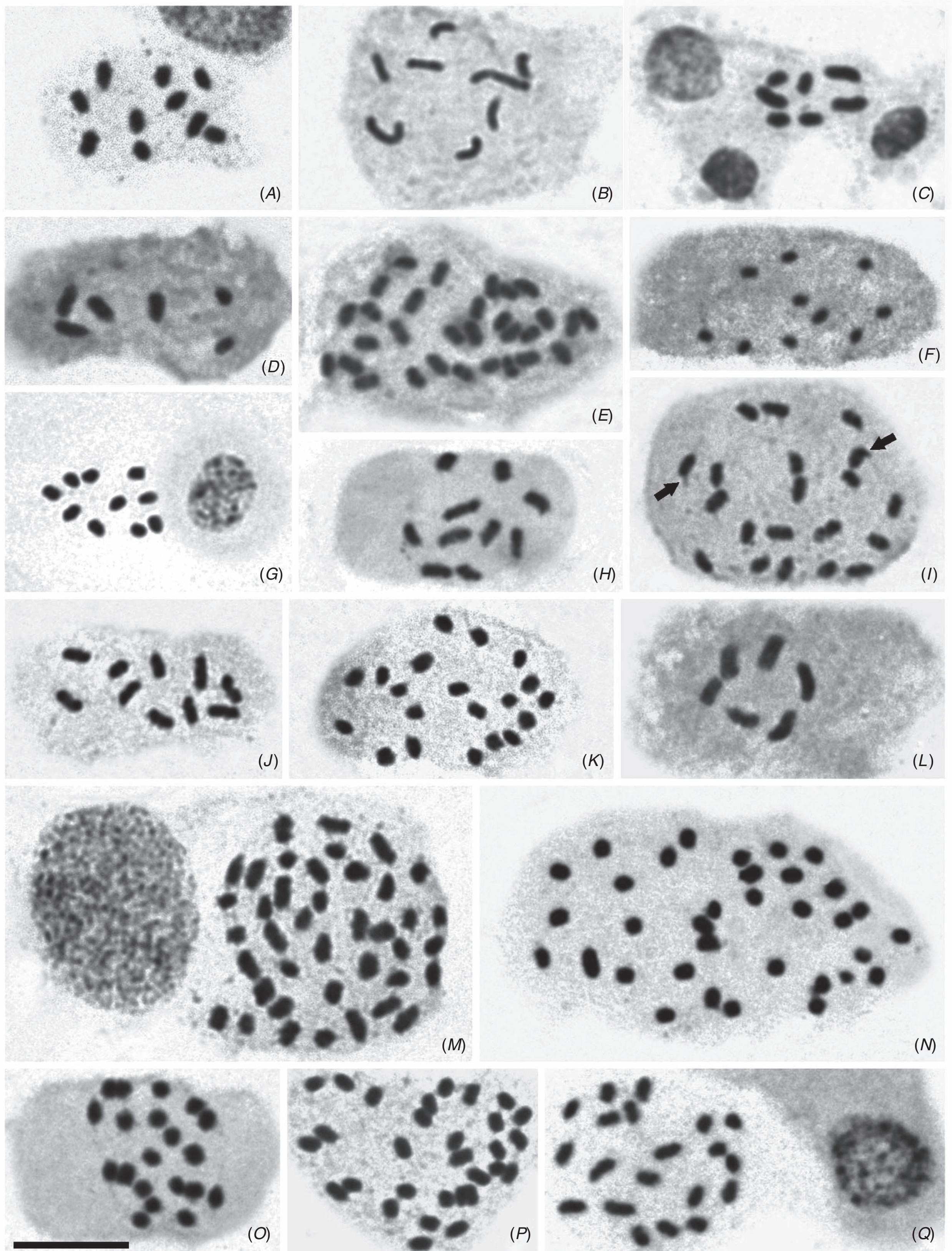
*Total length diploid.
[A]chromosome numbers recorded for the first time.
[B]the chromosomes numbers are reported for the first time.
[C]larger chromosome/smaller chromosome. All samples were collected by CRM Da Silva and ALL Vanzela.

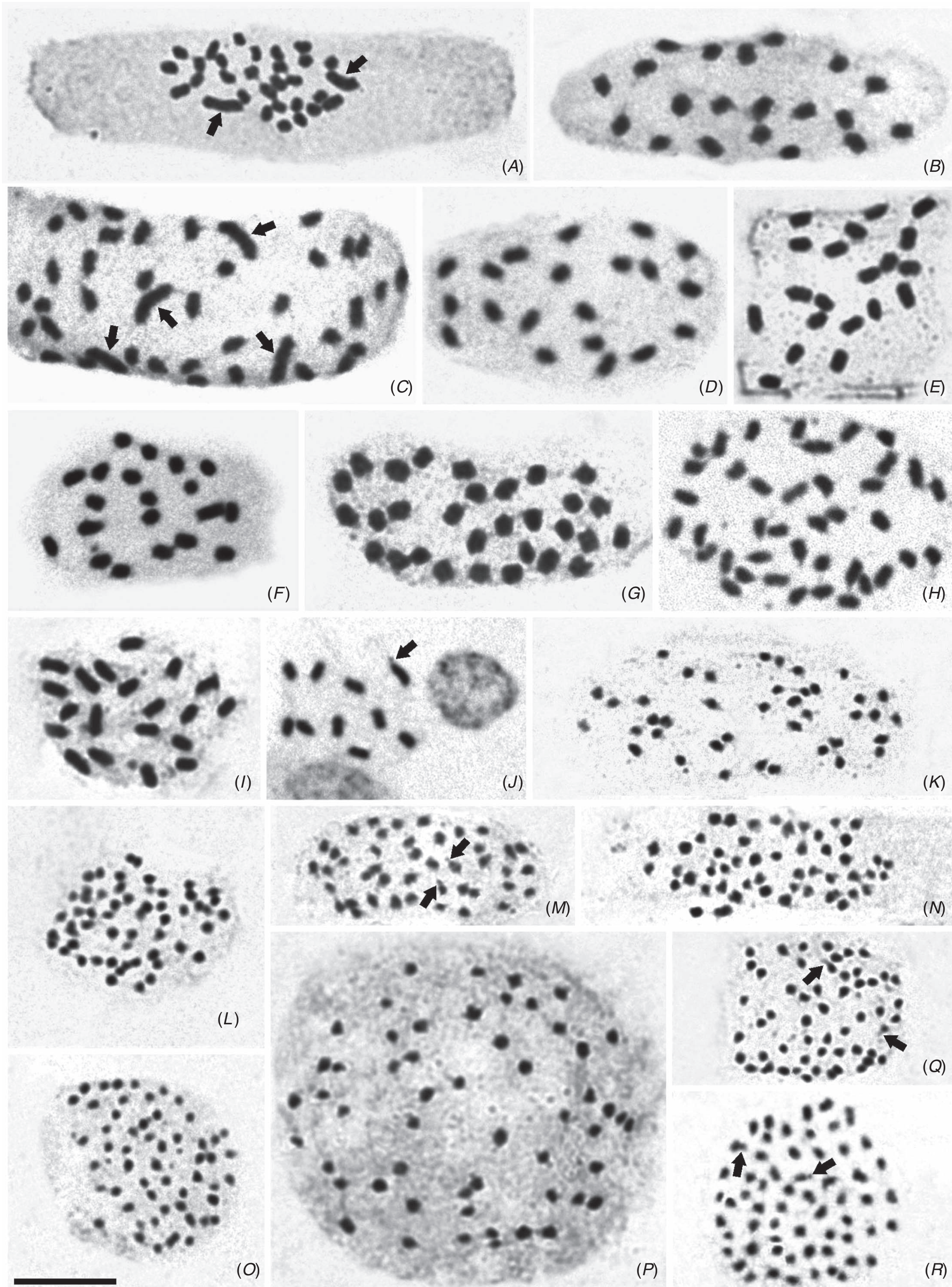
(Håkansson 1958; Strandhede 1965; Vanzela *et al*. 2000, 2003; Yano *et al*. 2004; Da Silva *et al*. 2008*a*; Hipp *et al*. 2009). In a smaller proportion, variation caused by multiple translocations and aneuploidy have been reported (Da Silva *et al*. 2005; Yano and Hoshino 2006*b*).

The more common mechanism of karyotype variation in *Eleocharis* appears to be polyploidy (Hoshino 1987; Yano *et al*. 2004; Da Silva *et al*. 2008*a*). The chromosome numbers described in the literature show a great variation, from $2n=6$ (Da Silva *et al*. 2005) to $2n=200$ (Roalson 2008). Further, some examples of intraspecific variation have been reported, such as $2n=16$, 38, 39, 46, 72–82 in the *Eleocharis palustris* complex (Bureš *et al*. 2004). Yano *et al*. (2004) proposed a grouping of the *Eleocharis* species based in karyotype composition/organisation and molecular data. The first group was monophyletic and represented by karyotypes with small, symmetrical and numerous chromosomes (<1.1 µm), which includes the subgenus *Limnochloa* (P. Beauv. ex T. Lestib.) Torr. The second group was paraphyletic and composed of species with larger chromosomes (1.4–4.3 µm) and a gradient of size or bimodal karyotypes, which includes species of subgenus *Eleocharis*.

At least 60 species of *Eleocharis* occur in Brazil, with representatives of three of the four subgenera: *Eleocharis*, *Scirpidium* (Nees) Kukkonen and *Limnochloa*, only *Zinserlingia* T. V. Egorova not being present in the country (González-Elizondo and Tena-Flores 2000; Alves *et al*. 2009; Trevisan and Boldrini 2008). *Eleocharis* is a genus with a special importance in the Brazilian flora because of a great number of species that grow in wetland areas, some of them widely distributed and others endemic. In this study we analysed the karyotypes of 25 species of *Eleocharis* by mitosis and meiosis

Fig. 1. Conventional chromosome analysis showing metaphases and prometaphases in species of the section *Eleogenus* (*A–M*): (*A–D*) *Eleocharis maculosa* with $2n=10$, 8, 7 and 6, respectively; (*E*) *E. debilis* with $2n=30$; (*F*) *E. flavescens* with $2n=10$; (*G*) *E. capillacea* with $2n=10$; (*H–I*) *E. sellowiana* with $2n=10$ and 20, respectively. Arrows indicate the nucleolar constrictions; (*J–K*) *E. geniculata* with $2n=10$ and 20, respectively; (*L*) *E. subarticulata* with $2n=6$; (*M*) *E. montana* with $2n=40$, and in the section *Eleocharis.* (*N–Q*): (*N–P*) *E. minima* with $2n=34$, 20 and 30, respectively; (*Q*) *E. niederleinii* with $2n=20$. Note the diffuse interphase nuclei without evident chromocenters in *C*, *M* and *Q* and with ones in *G*. Bar = 10 µm.


(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)
(P)
(Q)


(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)
(P)
(Q)
(R)

with the aim to compare the karyotype structure and evolution, as well as the usefulness of such information to the taxonomy of *Eleocharis*.

Materials and methods

For this study 147 populations of 25 species, with at least three specimens of each population, were collected in 10 Brazilian states. Samples were cultivated in the greenhouse of the Laboratório de Biodiversidade e Restauração de Ecossistemas (LABRE) at Universidade Estadual de Londrina, Paraná, Brazil. Vouchers were deposited at the ICN herbarium of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (Table 1).

The karyotypical analyses were performed with root tips pre-treated in 2 mM 8-hydroxyquinoline for 24 h, fixed in ethanol : acetic acid (3 : 1, v : v) for 24 h, and stored at –20°C or immediately used. Samples were washed in distilled water, softened in 4% cellulase plus 40% pectinase (w : v) at 37°C for 3 h, hydrolysed in 1 M HCl for 10 min at 60°C, washed again in distilled water, and squashed in a drop of 45% acetic acid. Slides were stained in 2% Giemsa and mounted with Entellan. The chromosome counts were made in at least 20 cells for each sample. The chromosome size and the haploid/diploid set length were measured from at least 10 metaphase cells with similar condensation, with the MicroMeasure 3.3 software (www.colostate.edu/Depts/Biology/MicroMeasure) and the data used to assemble idiograms.

For the meiotic study, spikelets of at least one species of each section were dissected and the anthers directly fixed in absolute ethanol : acetic acid (3 : 1, v : v) for 12 h, and kept at −20°C until used. Anthers were hydrolysed in 1 M HCl at 60°C for 9 min, washed in distilled water and squashed in a drop of 45% acetic acid. The coverslips were removed after freezing in liquid nitrogen, stained with 2% Giemsa and the slides mounted with Entellan. All images were acquired with a Leica DM 4500 B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software.

Results and discussion

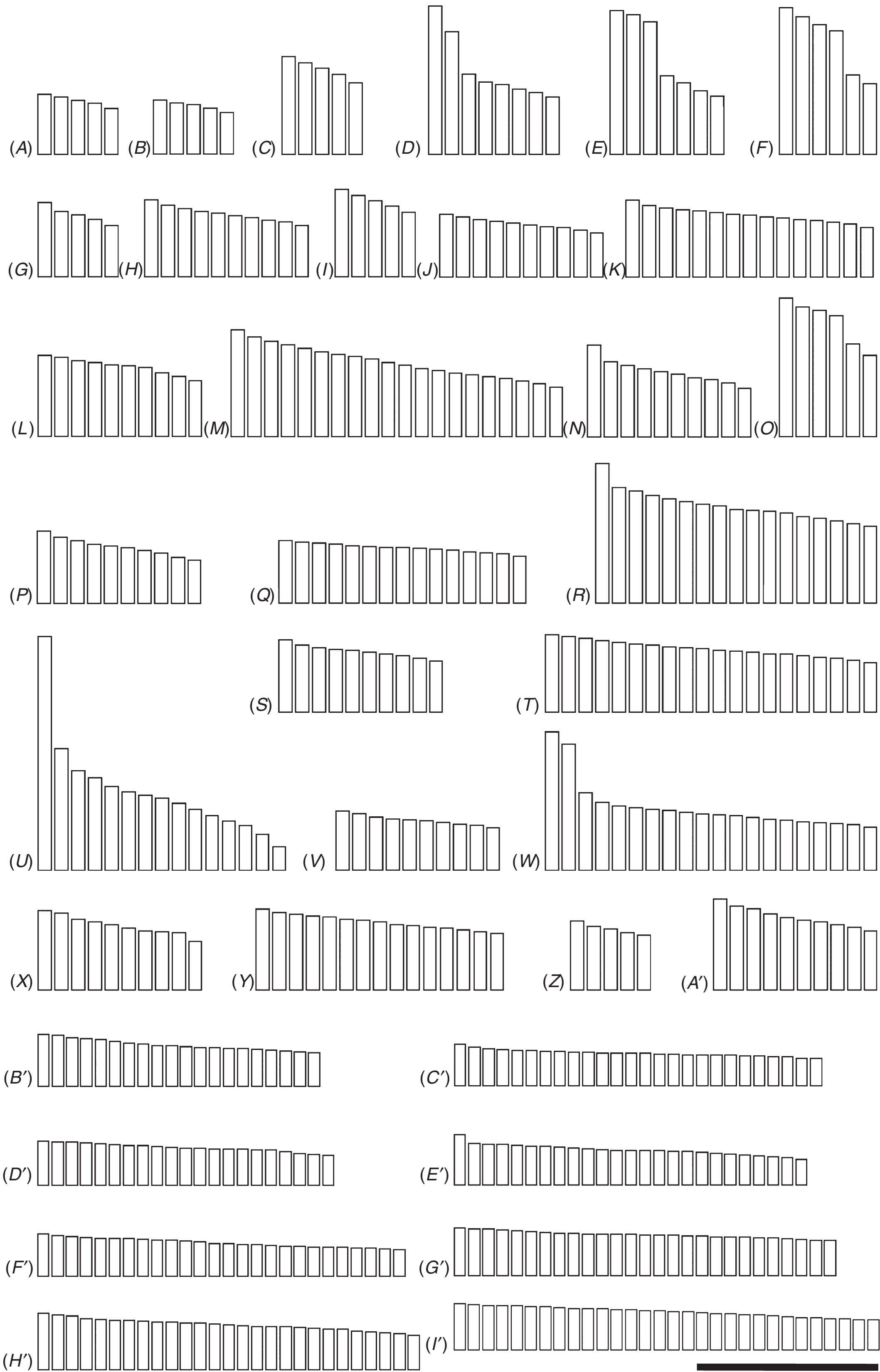
The chromosome number of 147 populations of 25 species, including representatives of all the subgenera of Brazilian *Eleocharis* (Table 1) was analysed. None of the species studied presented primary constrictions in the chromosomes and only terminal nucleolar constrictions (satellites) were visualised (Figs 1*I*, 2*J*, *M*, *Q*, *R*), as reported for other species of *Eleocharis*, as well as other Cyperaceae (Hoshino 1987; Luceño *et al*. 1998; Yano and Hoshino 2007; Da Silva *et al*. 2008*a*). For 17 species, the chromosome numbers are reported for the first time. These varied from $2n = 10$ in *E. capillacea* Kunth to $2n = 60$ in *E. laeviglumis* R. Trevis. and Boldrini. In the other eight species previously reported numbers were confirmed (Table 1). Of the more than 200 species of *Eleocharis* known worldwide, less than 25% have their karyotypes described (Davies 1956; Strandhede 1964, 1965; Harms 1968; Nijalingappa 1972, 1973; Sanyal and Sharma 1972; Schuyler 1977; Löve and Löve 1981; Hoshino 1987; Hoshino *et al*. 2000; Yano *et al*. 2004; Da Silva *et al*. 2005, 2008*b*; Yano and Hoshino 2006*b*; Roalson 2008). As for Brazil, of the 63 species listed at present (Alves *et al*. 2009), the chromosome numbers of 12 were previously described, $2n = 20$ in *E. geniculata* (Pedrosa *et al*. 1999) and six different numbers for 11 species: $2n = 6$, 10, 12, 20, 40 and 54 (Da Silva *et al*. 2008*a*).

Of ~5000 species in the family Cyperaceae (Goetghebeur 1998), only 16% have the chromosome number known, and half of these numbers have been described for species of *Carex* L. (Roalson 2008). Although there is a 'gap' in the knowledge of the patterns and processes of chromosomal evolution in this family (Hipp *et al*. 2009), the literature indicates that the karyotype differentiation process varies among groups. In *Carex*, agmatoploidy and symploidy are considerably more frequent (Hipp *et al*. 2009) when compared with other genera such as *Rhynchospora* Vahl (Luceño *et al*. 1998; Vanzela *et al*. 2000), *Scleria* P.J. Bergius (Yano and Hoshino 2007) and *Eleocharis* (Yano *et al*. 2004; Da Silva *et al*. 2008*a*). In this context, we cannot discard the possible existence of some genetic control that favours the agmatoploidy and/or symploidy in different species. However, the lack of studies involving a larger number of populations contributes to this gap in the knowledge of chromosomal evolution in Cyperaceae. Cytogenetic studies in several populations of one species, like those in *Carex laevigata* Smith (Luceño and Castroviejo 1991), *Carex conica* Boott (Hoshino and Waterway 1994), *Carex blepharicarpa* Franch. (Hoshino and Okamura 1994), *Carex sociata* Boott (Ohkawa *et al*. 2000), *Eleocharis palustris* (L.) Roem. and Schult. (Bureš *et al*. 2004) and *Eleocharis maculosa* (Vahl) Roem and Schult. Da Silva *et al*. (2008*b*) show that an increase in intraspecific sampling can reveal chromosome changes that are not detected in smaller samples, which can be important in understanding karyotype evolution of these groups.

Subgenus Eleocharis

Of the 25 studied species, 16 belong to the subgenus *Eleocharis*, representing two of the three sections recognised by González-Elizondo and Peterson (1997): *Eleogenus* (Nees) Benth. and Hook. F. and *Eleocharis*. In section *Eleogenus*, six species were studied. The chromosome numbers varied from $2n = 6$ in *Eleocharis maculosa*, which also exhibited populations with $2n = 10$, 8 and 7 (Fig. 1*A–D*), as previously reported by Da Silva *et al*. (2008*b*), to $2n = 30$ in *Eleocharis debilis* Kunth (Fig. 1*E*). *Eleocharis flavescens* (Poir.) Urb. and *E. capillacea* Kunth

Fig. 2. Conventional chromosome analysis showing metaphases in species of the section *Eleocharis*. (*A–H*): (*A*) *E. niederleinii* with $2n = 30$. Arrows point out two larger chromosomes; (*B–C*) *E. viridans* with $2n = 20$ and 40. Arrows indicate four larger chromosomes. (*D–F*) *E. loefgreniana*, *E. contracta* and *E. nana*, all with $2n = 20$. (*G*) *E. filiculmis* with $2n = 30$, (*H*) *Eleocharis* sp. 1 with $2n = 40$, and in the subgenus *Scirpidium*. (*I–J*): *E. bonariensis* with $2n = 20$ and *Eleocharis* sp. 2 with $2n = 10$, respectively. Arrows point out nucleolar constrictions. Conventional chromosome analysis in species of subgenus *Limnochloa*. (*K–R*): (*K–L*) *Eleocharis interstincta* with $2n = 40$ and 52, respectively; (*M*) *Eleocharis* sp. 3 with $2n = 42$; (*N*) *E. liesneri* with $2n = 50$; (*O*) *E. obtusetrigona* with $2n = 52$; (*P*) *E. plicarhachis* with $2n = 54$; (*Q*) *E. acutangula* with $2n = 54$ and (*R*) *E. laeviglumis* with $2n = 60$. Arrows point out nucleolar constrictions. Bar = 10 μm.


(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)
(P)
(Q)
(R)
(S)
(T)
(U)
(V)
(W)
(X)
(Y)
(Z)
(A′)
(B′)
(C′)
(D′)
(E′)
(F′)
(G′)
(H′)
(I′)

showed $2n = 10$ (Fig. 1*F*,*G*). The other two species, *Eleocharis sellowiana* Kunth and *E. geniculata* (L.) Roem and Schult., also have intraspecific variation with two chromosome numbers, both with $2n = 10$ and 20 (Fig 1*H–K*).

In section *Eleocharis*, chromosome numbers varied from $2n = 6$ in *E. subarticulata* (Nees) Boeck. (Fig. 1*L*) to $2n = 40$ in *E. montana* (Kunth) Roem. and Schult. (Fig. 1*M*). *Eleocharis minima* (Vahl) Roem. and Schult. was the only species that showed three numbers with $2n = 20$, 30 and 34 (Fig. 1*N–P*). Other species also presented multiple numbers, as *E. niederleinii* Boeck. with $2n = 20$ and 30 (Figs 1*Q*, 2*A*) and *E. viridans* Kük. ex Osten with $2n = 20$ and 40 (Fig. 2*B*,*C*). For the remaining species the numbers were $2n = 20$ in *E. loefgreniana* Boeck., *E. contracta* Maury, and *E. nana* Kunth (Fig. 2*D–F*), $2n = 30$ in *E. filiculmis* Kunth (Fig. 2*G*), and $2n = 40$ (Fig. 2*H*) in one species related to *E. loefgreniana* but with very proliferous spikelets, named here as *Eleocharis* sp. 1. Proliferous spikelets, producing new culms instead of flowers, are not characteristic of *E. loefgreniana* and these samples could belong to an undescribed taxon.

The chromosome measurements revealed that all the species of subgenus *Eleocharis* have karyotypes with chromosomes decreasing gradually in size (Fig. 3). *Eleocharis niederleinii* with $2n = 30$ (Fig. 3*U*) showed two chromosomes ~6.5 μm in length, and *E. viridans* with $2n = 40$ (Fig. 3*W*) four chromosomes varying from 3.8 μm to 2.2 μm (Table 1, Fig. 3). These bigger chromosomes could have been originated by chromosome fusion or symploidy (which is a type of disploidy). This rearrangement seems to be common in other species of section *Eleocharis*, such as *E. palustris* (L.) Roem. and Schult., *E. uniglumis* (Link) Schult. and *E. mamillata* H. Lindb. (Strandhede 1965), but was better documented in *E. maculosa*, which is a member of the section *Eleogenus* (Da Silva *et al*. 2008*b*). However, our data do not allow us to identify, in this case, the chromosomes that participated in these fusions in the process of disploidy. In another case, Yano and Hoshino (2006*b*) reported the occurrence of aneuploidy in *Eleocharis kamtschatica* (C.A. Mey.) Kom. based in chromosome length differences, but these authors do not show the non-disjunction process or the irregular meiosis to confirm the aneuploidy process.

The representatives of the sections *Eleogenus* and *Eleocharis* from Brazil share common cytogenetic features including: (i) chromosome reduction to $2n = 6$ starting from $x = 5$; (ii) polyploidy in ~80% of the species; (iii) occurrence of intraspecific variation in chromosome number; (iv) chromosome length around 2.2 μm, except for *E. niederleinii* and *E. viridans*; and (v) ~20.42 μm of haploid total length. Similarly, other species of subgenus *Eleocharis* that occur in other continents show similar karyotype variation as presented here (Håkansson 1958; Strandhede 1965; Nijalingappa 1973; Bureš *et al*. 2004; Yano *et al*. 2004; Roalson 2008). The diversity of karyotypes found in *Eleocharis* subgenus *Eleocharis* is mirrored by the tremendous morphological diversity in the group, many of whose subdivisions are para- or polyphyletic (Roalson and Friar 2000).

Subgenus Scirpidium

Only two species of subgenus *Scirpidium* were analysed, *E. bonariensis* Nees with $2n = 20$ (Fig. 2*I*) and *Eleocharis* sp. 2 with $2n = 10$ (Fig. 2*J*), a species closely related to *E. radicans* (Poir.) Kunth, which was also recorded with $2n = 10$ (Da Silva *et al*. 2008*a*). *Eleocharis* sp. 2 differs from *E. radicans* by having capillary culms, spikelets 15–30-flowered and smaller achenes, being probably a new taxon for the subgenus. Besides *E. radicans*, there are few cytogenetical data for species of *Scirpidium* in the literature, with records for only two additional species, *E. acicularis* (L.) Roem. and Schult. with $2n = 56$, 20 and *E. atricha* R.Br. with $2n = 10$ (Roalson 2008).

In spite of the low number of studied species, the intraspecific variation in *E. acicularis* and the chromosomes gradually decreasing in size that have been found, allow us to suggest that the karyotypes of *Scirpidium* and *Eleocharis* have greater similarity to each other than either is to karyotypes of subgenus *Limnochloa.* This conclusion corroborates the results of the phylogenetic studies of Roalson and Friar (2000) and Roalson and Hinchliff (2007). The morphological evidence also indicates that *Scirpidium*, although a well-delimitated group with species sharing a particular ornamentation of the achenes (with longitudinal rows of fine trabeculae), is more closely related to the subgenus *Eleocharis* than to *Limnochloa*.

Subgenus Limnochloa

All the species of subgenus *Limnochloa* exhibited karyotypes with many and small chromosomes. *Eleocharis interstincta* (Vahl) Roem. and Schult. presented $2n = 40$ and 52, being 40 the lowest found to this subgenus (Fig. 2*K*, *L*). Interestingly, we found $2n = 42$ (Fig. 2*M*) in a species (named here as *Eleocharis* sp. 3), perhaps related to *E. interstincta* due to the presence of hollow and septate culms, but with an evident central vascular bundle along the culm. Since the septate culms have multiple origins in *Limnochloa* (Roalson and Hinchliff 2007) the relations between *Eleocharis* sp. 3 and *E. interstincta* need to be explored with other studies. The other numbers found in *Limnochloa* were $2n = 50$ in *E. liesneri* S. González and Reznicek (Fig. 2*N*), $2n = 52$ in *E. obtusetrigona* (Lindl. and Nees) Steud. (Fig. 2*O*), $2n = 54$ in *E. plicarhachis* (Griseb.) Svenson and (Fig. 2*P*) and *E. acutangula* (Roxb.) Schult. (Fig. 2*Q*), and $2n = 60$ in *E. laeviglumis* R. Trevis. and

Fig. 3. Idiograms of the studied species. Note that all species exhibited chromosomes decreasing gradually in size, independent of chromosome numbers. All the idiograms represent the haploid set, except *D–F* and *O*, which indicate the diploid set. (*A–K*) Species of the section *Eleogenus*, being *E. flavescens* (*A*); *E. capillacea* (*B*); *E. maculosa* with $2n = 10$ (*C*), $2n = 8$ (*D*), $2n = 7$ (*E*) and $2n = 6$ (*F*); *E. sellowiana* $2n = 10$ (*G*) and 20 (*H*); *E. geniculata* with $2n = 10$ (*I*) and 20 (*J*); *E. debilis* (*K*). Species of the section *Eleocharis* (*L–Y*), being: *E. contracta* (*L*); *E. montana* (*M*); *E. nana* (*N*); *E. subarticulata* (*O*); *E. minima* with $2n = 20$ (*P*), 30 (*Q*) and 34 (*R*); *E. loefgreniana* (*S*); *Eleocharis* sp. 1 (*T*); *E. niederleinii* with $2n = 30$ (*U*) and 20 (*V*); *E. viridans* with $2n = 40$ (*W*) and 20 (*X*), and *E. filiculmis* (*Y*). Species of the subgenus *Scirpidium* (*Z, A′*), being: *Eleocharis* sp. 2 (*Z*) and *E. bonariensis* (*A′*). Species of the subgenus *Limnochloa* (*B′–I′*), being: *E. interstincta* with $2n = 40$ (*B′*) and 52 (*C′*); *Eleocharis* sp. 3 (*D′*); *E. liesneri* (*E′*); *E. obtusetrigona* (*F′*); *E. acutangula* (*G′*); *E. plicarhachis* (*H′*) and *E. laeviglumis* (*I′*). Bar = 5 μm.

Boldrini (Fig. 2*R*). Of all these records, only *E. acutangula* had been previously studied showing always $2n = 54$ (Nijalingappa 1972; Rath and Patnaik 1978; Hoshino 1987; Da Silva *et al*. 2008*a*). The higher chromosome numbers found in *Eleocharis* worldwide belong to species of *Limnochloa*, as in *Eleocharis kuroguwai* Ohwi with $2n = \sim 196$ (Hoshino 1987), *E. ochrostachys* Steud. with $2n = 74$ (Yano *et al*. 2004), and *E. dulcis* (Burm.f.) Trin. ex Hensch. with $2n = 200$ and ~172 (Roalson 2008).

Our cytogenetical analyses confirm that the species of *Limnochloa* are separated from the rest of the genus by their numerous, small chromosomes (<1.4 µm), although the

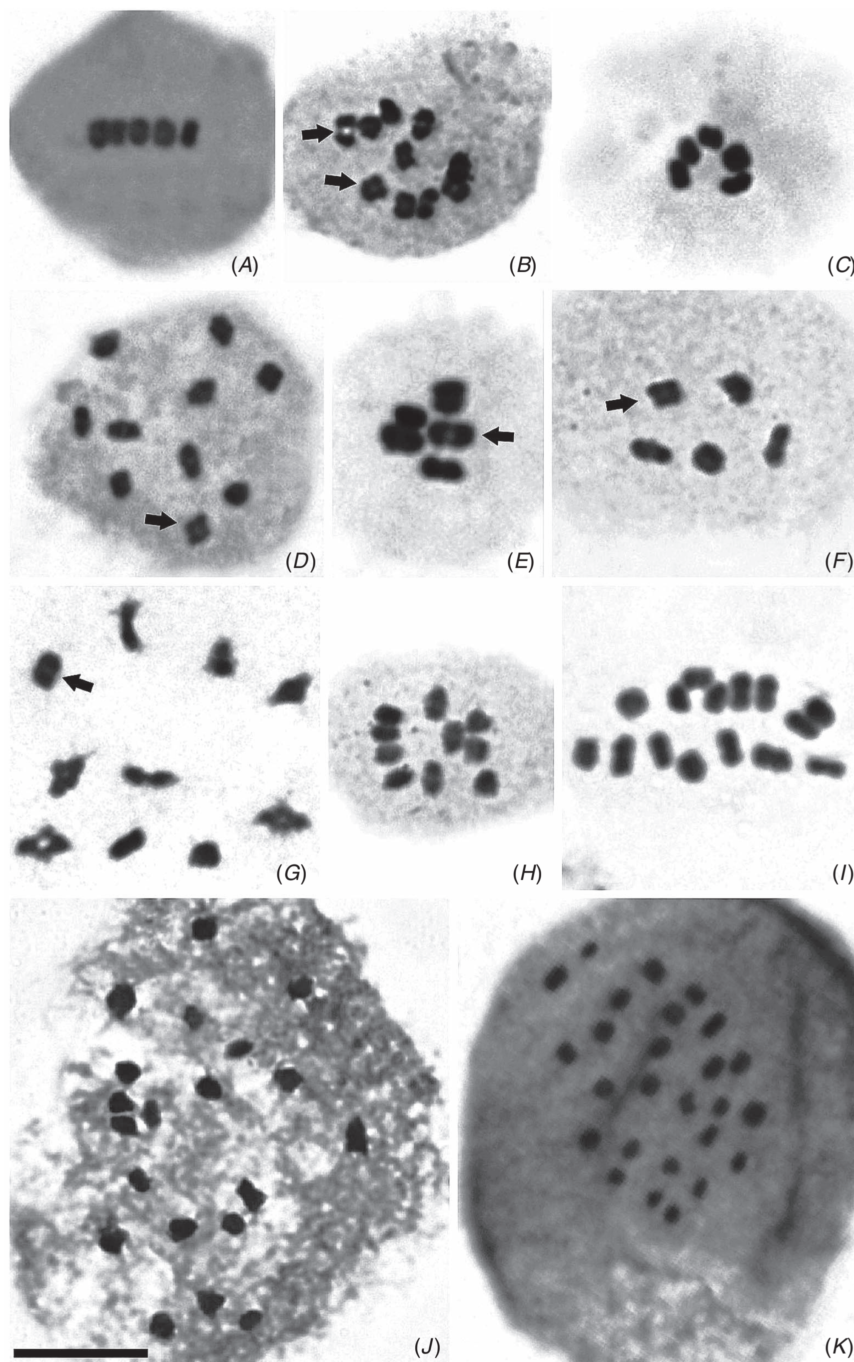


Fig. 4. Meiotic analysis in species of *Eleocharis*: (*A*, *B*) metaphase I of *E. geniculata* with 5^{II} and 10^{II}, respectively; (*C*, *D*) metaphase I of *E. sellowiana* with 5^{II} and 10^{II}, respectively; (*E*, *F*) metaphase I of *E. flavescens* and *E. capillacea*, both with 5^{II}; (*G*) diakinesis in *E. nana* with 10^{II}; (*H*) metaphase I of *E. bonariensis* with 10^{II}; (*I*) metaphase I of *E. filiculmis* with 15^{II}; (*J*) metaphase I of *E. montana* with 20^{II} and (*K*) metaphase I of *E. interstincta* with 26^{II}. Arrows point out the 'Box' arrangement of bivalents. Bar = 10 µm.

karyotypes do have chromosomes decreasing gradually in size as in the other species of the genus (see Table 1 and Fig. 3). The haploid set genome size of *Limnochloa* species seems not to exceed the sizes of those of other subgenera with lower chromosome numbers (Table 1). According to Yano *et al*. (2004), the karyotypes of *Limnochloa* can have been derived from others with $2n = 10$ and 20. Our results do not permit us to support or confirm the proposal of Yano and colleagues, but it seems clear that the karyotype differences among *Limnochloa* and the other subgenera corroborate the separation of *Limnochloa* as a group based on morphological features (Kukkonen 1990; González-Elizondo and Peterson 1997) and molecular data (Roalson and Friar 2000).

Meiotic analyses and polyploidy

The majority of the studied species presented chromosome numbers multiple of $x = 5$. *Eleocharis geniculata*, *E. sellowiana*, *E. flavescens* and *E. capillacea*, all belonging to section *Eleogenus* and all with $2n = 10$, show regular meiosis with five bivalents (Fig. 4*A*, *C*, *E*, *F*). The first two species presented also polyploid populations with $2n = 20$, but the regular meiosis with bivalent formation was also confirmed (Fig. 4*B*, *D*, respectively). In other species with chromosome numbers larger than $2n = 10$, such as *E. nana* with $2n = 20$ (Fig. 4*G*), *E. bonariensis* with $2n = 20$ (Fig. 4*H*), *E. filiculmis* with $2n = 30$ (Fig. 4*I*), *E. montana* with $2n = 40$ (Fig. 4*J*) and *E. interstincta* with $2n = 52$ (Fig. 4*K*), only bivalents were observed. Additionally, some bivalents exhibited a 'box' shape, as in *E. geniculata* (Fig. 4*B*), *E. sellowiana* (Fig. 4*D*), *E. flavescens* (Fig. 4*E*), *E. capillacea* (Fig. 4*F*) and *E. nana* (Fig. 4*G*), which are characteristic of holocentric chromosomes (Strandhede 1965; Vanzela *et al*. 2000).

Our study showed 80% polyploidy in Brazilian *Eleocharis*, with bivalent associations in both diploids and polyploids. As the bivalent or multivalent associations may depend on the neopolyploid or paleopolyploid condition (Grant 1971; Otto 2007), as well as of gene control (Evans 1988; Griffiths *et al*. 2006; Bozza and Pawlowski 2008), our results do not allow us to determine if the chromosome numbers were doubled by auto- or allopolyploidy. Some multivalent associations have been associated with natural allopolyploids, as in the *E. palustris* complex, *E. uniglumis* and *E. mamillata* (Strandhede 1965) and in hybrids of *Carex* (Luceño 1993). Similarly, multivalent associations have been found in some autopolyploids, as in *Fimbristylis falcata* (Vahl) Kunth (Nijalingappa 1977), *Carex siderosticta* Hance (Tanaka 1940) and *Rhynchospora tenuis* Link (Vanzela *et al*. 1996). In the last case, normal bivalents were only rarely observed.

The cytogenetic information obtained here shows quite variable karyotypes with chromosomes gradually decreasing in size, independently of the chromosome numbers, and the predominance of polyploidy. These results are useful in the differentiation of the subgenera, but when the comparisons are done within a subgenus, more detailed cytogenetical analyses (chromosome banding and cyto-molecular tools) are required. Future studies should combine these tools with molecular data to better understand the evolution of *Eleocharis* and related genera.

Acknowledgements

This study was supported by the Brazilian agencies Fundação Araucária, CNPq and ProPPG-UEL.

References

Alves MV, Araújo AC, Prata AP, Vitta F, Hefler S, Trevisan R, Gil ASB, Martins S, Thomas W (2009) Diversity of Cyperaceae in Brazil. *Rodriguésia* **60**, 1–12.

Bozza CG, Pawlowski WP (2008) The cytogenetics of homologus chromosome pairing in meiosis in plants. *Cytogenetic and Genome Research* **120**, 313–319. doi:10.1159/000121080

Bureš P, Rotreklová O, Stoneberg HSD, Pikner R (2004) Cytogeographical survey of Eleocharis subser. Eleocharis in Europe 1. Eleocharis palustris. *Folia Geobotanica* **39**, 235–257. doi:10.1007/BF02804780

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2005) Reduction of chromosome number in *Eleocharis subarticulata* (Cyperaceae) by multiple translocation. *Botanical Journal of the Linnean Society* **149**, 457–464. doi:10.1111/j.1095-8339.2005.00449.x

Da Silva CRM, González-Elizondo MS, Rego LNAA, Torezan JMD, Vanzela ALL (2008*a*) Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis*. *Australian Journal of Botany* **56**, 82–90. doi:10.1071/BT07017

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2008*b*) Chromosome Reduction in *Eleocharis maculosa* (Cyperaceae). *Cytogenetic and Genome Research* **122**, 175–180. doi:10.1159/000163096

Davies EW (1956) Cytology, evolution and origin of the aneuploid series in the genus Carex. *Hereditas* **42**, 349–365.

Evans GM (1988) Genetic control of chromosome pairing in polyploids. In 'Kew Chromosome Conference III. Proceedings of the Third Chromosome Conference held in the Jodrell Laboratory, Royal Botanic Gardens, Kew, England, 1–4 September'. (Ed. PE Brandham) pp. 253–260.

Faulkner JS (1972) Chromosome studies on *Carex* section *Acutae* in north-west Europe. *Botanical Journal of the Linnean Society* **65**, 271–301.

Goetghebeur P (1998) 'Cyperaceae. The families and genera of vascular plants: IV. Flowering plants – monocotyledons.' (Springer-Verlag: Berlin)

González-Elizondo MS, Peterson PM (1997) A classification of and key to the supraspecific taxa in *Eleocharis* (Cyperaceae). *Taxon* **46**, 433–449. doi:10.2307/1224386

González-Elizondo MS, Tena-Flores JA (2000) *Eleocharis* (Cyperaceae) in the New World. In 'Monocots: Systematics and Evolution'. (Eds KL Wilson, DA Morrison) pp. 637–643. (CSIRO Publishing: Melbourne)

Grant V (1971) 'Plant Speciation'. (Columbia University Press: New York).

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. *Nature* **439**, 749–752. doi:10.1038/nature04434

Håkansson A (1958) Holocentric chromosomes in *Eleocharis*. *Hereditas* **44**, 531–540.

Harms LJ (1968) Cytotaxonomic studies in *Eleocharis* subser. *Palustres*: Central United taxa. *American Journal of Botany* **55**, 966–974. doi:10.2307/2440562

Hipp AL, Rothrock PE, Roalson EH (2009) The evolution of chromosome arrangements in *Carex* (Cyperaceae). *Botanical Review* **75**, 96–109. doi:10.1007/s12229-008-9022-8

Hoshino T (1987) Karyomorphological studies on 6 taxa of *Eleocharis* in Japan. *Bulletin of the Okayama University of Science* **22**, 305–312.

Hoshino T, Okamura K (1994) Cytological studies on meiotic configurations of intraspecific aneuploids of *Carex blepharicarpa* (Cyperaceae) in Japan. *Journal of Plant Research* **107**, 1–8. doi:10.1007/BF02344523

Hoshino T, Waterway MJ (1994) Cytogeography and meiotic chromosome configurations of six intraspecific aneuploids of *Carex conica* Boott (Cyperaceae) in Japan. *Journal of Plant Research* **107**, 131–138. doi:10.1007/BF02346008

Hoshino T, Rajbhandari KR, Ohba H (2000) Cytological studies of eleven species of Cyperaceae collected from Central Nepal. *Cytologia* **65**, 219–224.

Kukkonen I (1990) On the genus *Eleocharis* (Cyperaceae) in Flora Iranica area, with revised infrageneric classification and nomenclature. *Annales Botanici Fennici* **27**, 109–117.

Löve A, Löve D (1981) In IOPB chromosome number reports LXXIII. *Taxon* **30**, 845–851.

Luceño M (1993) Cytotaxonomic studies in the sections *Spirostachyae* (Drejer) Bailey and Ceratocystis Dumort. of the genus *Carex* L. (Cyperaceae), with special reference to Iberian and North Aftrican taxa. *Botanical Journal of the Linnean Society* **112**, 335–350.

Luceño M, Castroviejo S (1991) Agmatoploidy in *Carex laevigata* (Cyperaceae). Fusion and fission of chromosomes as the mechanism of cytogenetic evolution in Iberian populations. *Plant Systematics and Evolution* **177**, 149–159. doi:10.1007/BF00937952

Luceño M, Vanzela ALL, Guerra M (1998) Cytotaxonomic studies in Brazilian *Rhynchospora* (Cyperaceae), a genus exhibiting holocentric chromosomes. *Canadian Journal of Botany* **76**, 440–449. doi:10.1139/cjb-76-3-440

Nijalingappa BHM (1972) In IOPB chromosome number reports. XXXVIII. *Taxon* **21**, 679–684.

Nijalingappa BHM (1973) Cytological studies in *Eleocharis. Caryologia* **26**, 512–520.

Nijalingappa BHM (1977) Autotetraploidy in *Fimbristylis falcata* (Vahl) Kunth (Cyperaceae). *Proceedings of the Indian Academy Science B* **85**, 21–24.

Ohkawa T, Yokota M, Hoshino T (2000) Aneuploidal population differentiation in *Carex sociata* Boott (Cyperaceae) of the Ryukyu Islands, Japan. *Botanical Journal of the Linnean Society* **132**, 337–358.

Otto SP (2007) The evolutionary Consequences of Polyploidy. *Cell* **131**, 452–462. doi:10.1016/j.cell.2007.10.022

Pedrosa A, Gitai J, Silva AEB, Felix LP, Guerra M (1999) Citogenética de Angiospermas Coletadas em Pernambuco. *Acta Botanica Brasilica* **13**, 49–60.

Rath SP, Patnaik SN (1978) Cytological studies in Cyperaceae. In 'Proceedings of the Indian Science Congress Association (III, C). Session 65, pp. 107–108, Ahmedabad, India.

Roalson EH (2008) A synopsis of chromosome number variation in the Cyperaceae. *Botanical Review* **74**, 209–393. doi:10.1007/s12229-008-9011-y

Roalson EH, Friar EA (2000) Infrageneric classification of *Eleocharis* (Cyperaceae) revisited: evidence from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. *Systematic Botany* **25**, 323–336. doi:10.2307/2666645

Roalson EH, Hinchliff C (2007) Phylogenetic relationships in *Eleocharis* R. Br. (Cyperaceae): comparisons with classification, morphology, biogeography and physiology. In '58° Congresso Nacional de Botânica'. (Eds Sociedade Botânica do Brasil) pp. 304–307. (Botânica no Brasil: Pesquisa Ensino e Políticas Públicas)

Sanyal B, Sharma AK (1972) Cytological studies in Indian Cyperaceae. I. Tribe *Scirpeae. Cytologia* **37**, 13–32.

Schuyler AE (1977) Chromosome observations on some eastern North American *Eleocharis* (Cyperaceae). *Brittonia* **29**, 129–133. doi:10.2307/2805751

Simpson DA (1988) Some problems in *Eleocharis*. Notes on Brazilian Cyperaceae III. *Kew Bulletin* **43**, 127–134. doi:10.2307/4118045

Strandhede SO (1964) Chromosome studies in *Eleocharis* subser. *Palustres*. I. Meiosis in some forms with 15 chromosomes. *Hereditas* **53**, 47–62. doi:10.1111/j.1601-5223.1965.tb01979.x

Strandhede SO (1965) Chromosome studies in *Eleocharis*, subser. *Palustres. Opera Botanica* **9**, 1–86.

Svenson HK (1929) Contributions from the Gray Herbarium of Harvard University. LXXXVI. Monographic studies in the genus *Eleocharis. Rhodora* **31**, 121–245.

Svenson HK (1939) Monographic studies in the genus *Eleocharis* V. *Rhodora* **41**, 1–110.

Tanaka N (1940) Chromosome studies in Cyperaceae, VIII: Meiosis in diploid and tetraploid forms of *Carex siderosticta* Hance. *Cytologia* **10**, 282–310.

Trevisan R, Boldrini II (2008) O gênero *Eleocharis* R. Br. (Cyperaceae) no Rio Grande do Sul, Brasil. *Revista Brasileira de Biociências* **6**, 7–67.

Vanzela ALL, Guerra M, Luceño M (1996) *Rhynchospora tenuis* Link (Cyperaceae), a species with the lowest number of holocentric chromosomes (n = 2). *Cytobios* **88**, 219–228.

Vanzela ALL, Luceño M, Guerra M (2000) Karyotype evolution and cytotaxonomy in Brazilian species of *Rhynchospora* Vahl (Cyperaceae). *Botanical Journal of the Linnean Society* **134**, 557–566. doi:10.1111/j.1095-8339.2000.tb00551.x

Vanzela ALL, Cuadrado A, Guerra M (2003) Localization of 45S rDNA and telomeric sites on holocentric chromosomes of *Rhynchospora tenuis* Link (Cyperaceae). *Genetics and Molecular Biology* **26**, 199–201. doi:10.1590/S1415-47572003000200014

Wahl HA (1940) Chromosome numbers and meiosis in the genus *Carex. American Journal of Botany* **27**, 458–470. doi:10.2307/2437081

Yano O, Hoshino T (2005) Molecular phylogeny and chromosomal evolution of Japanese *Schoenoplectus* (Cyperaceae), based on ITS and ETS 1f sequences. *Acta Phytotaxono Geobot* **56**, 183–195.

Yano O, Hoshino T (2006*a*) Phylogenetic relationships and chromosome evolution of Japanese *Fimbristylis* (Cyperaceae) using nrDNA ITS and ETS 1f sequence data. *Acta Phytotaxonomica et Geobotanica* **57**, 205–217.

Yano O, Hoshino T (2006*b*) Cytological Studies of Aneuploidy in *Eleocharis kamtschatica* (Cyperaceae). *Cytologia* **71**, 141–147. doi:10.1508/cytologia.71.141

Yano O, Hoshino T (2007) Karyomorphological Studies of Four Species of Japanese *Scleria* (Cyperaceae). *Cytologia* **72**, 275–278. doi:10.1508/cytologia.72.275

Yano O, Katsuyama T, Tsubota H, Hoshino T (2004) Molecular phylogeny of Japanese *Eleocharis* (Cyperaceae) based on ITS sequence data, and chromosomal evolution. *Journal of Plant Research* **117**, 409–419. doi:10.1007/s10265-004-0173-3

Manuscript received 21 October 2009, accepted 17 December 2009

Capítulo 2

Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis* (Cyperaceae)

Artigo publicado na revista Australian Journal of Botany, 2008, 56, 82–90

CSIRO PUBLISHING
www.publish.csiro.au/journals/ajb *Australian Journal of Botany*, 2008, **56**, 82–90

Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis* (Cyperaceae)

Carlos Roberto Maximiano da Silva[A], *Maria Socorro González-Elizondo*[B], *Letícia do Nascimento Andrade de Almeida Rego*[C], *José Marcelo Domingues Torezan*[C] *and André Luís Laforga Vanzela*[C,D]

[A]Instituto de Biociências, Letras e Ciências Exatas, UNESP, São José do Rio Preto 15054-000, SP, Brazil.
[B]Instituto Politécnico Nacional, CIIDIR and COFAA, Durango, Dgo. 34000, Mexico.
[C]Laboratório de Biodiversidade e Restauração de Ecossistemas, CCB, Universidade Estadual de Londrina, Londrina 86051-990, PR, Brazil.
[D]Corresponding author. Email: andrevanzela@uel.br

Abstract. Karyotype analysis of 21 samples of 11 species of *Eleocharis* (Cyperaceae) from 10 localities in Brazil, showed the presence of chromosomes without primary constrictions and parallel movement of chromatids at metaphase–anaphase transition. Only the terminal nucleolar constrictions (satellites) were visualised. The chromosome numbers varied from $2n = 6$ in *E. subarticulata* to $2n = 54$ in *E. acutangula*, but the chromosome basic number $x = 5$ was confirmed. Generally, C-CMA_3^+ bands appear mostly in the extremities of the chromosomes, associated to NOR, and interstitial C-CMA_3 bands were found only in *E. geniculata* and *E. acutangula*. C-$DAPI^+$ bands were not found. Fluorescence *in situ* hybridisation (FISH) with the 45S rDNA probe was performed in five species. The results showed from four to eight hybridisation signals, always terminal. The analysed species include representatives of the following three subgenera of *Eleocharis* that occur in Brazil: *Limnochloa*, *Scirpidium* and *Eleocharis*. Species from the subgenus *Limnochloa* have small and numerous chromosomes. The remaining species, belonging to subgenera *Eleocharis* and *Scirpidium*, possess fewer and larger chromosomes. In subgenus *Eleocharis*, karyotypes of the section *Eleocharis* were differentiated by symploidy, agmatoploidy and polyploidy, whereas species of the section *Eleogenus* were all polyploids. Polyploidy seems to be the most frequent event in the karyotype differentiation in *Eleocharis*, but changes in the chromosome size and repetitive DNA sites were also observed.

Introduction

Eleocharis comprises ~200 species, which are frequently found in flooded areas (Svenson 1929; González-Elizondo and Peterson 1997). *Eleocharis* is distinguished from the other genera of Cyperaceae by possessing stems with leaves reduced to a basal, tubular sheath, persistent style base, and inflorescence reduced to a simple terminal spikelet (González-Elizondo and Peterson 1997). In spite of the easy recognition of *Eleocharis* as a genus in the field and its prominent delimitation within Cyperaceae (Kukkonen 1990), the species are difficult to identify and classify because of the limited number of morphological features. The supraspecific classification of *Eleocharis* has been revised and modified in recent years (Faria 1998) and the more global classifications are those proposed by Kukkonen (1990) and González-Elizondo and Peterson (1997), on the basis of the widely accepted classification of Svenson (1929, 1932, 1934, 1937, 1939).

Most of the cytogenetical knowledge in Cyperaceae comes from studies in *Carex* and *Rhynchospora* (Davies 1956; Faulkner 1972; Luceño *et al*. 1998; Vanzela *et al*. 2000). These and other authors accept that the species of Cyperaceae possess uncommon cytogenetical features such as (i) holocentric chromosomes, (ii) post-reductional meiosis, (iii) absence of tetrads, owing to degeneration of three of the four nuclei during pollen mitosis and (iv) karyotype evolution on the basis of agmatoploidy (fission), symploidy (fusion) and polyploidy. The occurrence of holocentric chromosomes in *Eleocharis* was proposed by Håkansson (1954), after treating samples of *E. palustris* with X-ray and the observation of the normal kinetic behaviour of the chromosome fragments produced in subsequent cellular cycles. Greilhuber (1995) suggested that the occurrence of holocentric chromosomes is a synapomorphy of the Cyperaceae. Recently, Guerra *et al*. (2005) studied the behaviour of holocentric chromosomes of *Rhynchospora tenuis* (Cyperaceae) by immunodetection with anti-tubulin, and found that during the metaphase, the microtubules are attached along the whole chromosome extension, typical of holokinetic chromosomes.

Cytogenetical studies in *Eleocharis* date from the early 1920s, when Piech described the chromosome number of *E. palustris* (Nijalingappa 1973). There is considerable variation in the chromosome numbers in the genus, from $2n = 6$ (da Silva *et al*. 2005) to $2n = \sim196$ (Hoshino 1987); however, most of the species present the numbers $2n = 10$, 20, 30 and 40, associated with the possible basic number $x = 5$. Intraspecific differences in the chromosome numbers were reported in *E. geniculata* with $2n = 10$ and 20, in *E. atropurpurea*

© CSIRO 2008 10.1071/BT07017 0067-1924/08/010082

with $2n = 20$ and 21 (Nijalingappa 1973) and *E. palustris* with $2n = 15$ and 16 (Strandhede 1964), which originated by agmatoploidy/symploidy. More recently, Bureš *et al*. (2004) showed that *E. palustris* subsp. *palustris* has $2n = 16$, rarely 15, whereas *E. palustris* subsp. *vulgaris* frequently has $2n = 38$ and can range from 36 to 42. Yano *et al*. (2004) showed the occurrence of mixoploidy in the Japanese *E. acicularis* f. *longiseta* ($2n = 20$ and 21). These authors, on the basis of cytogenetical and molecular studies, have proposed a cytological division of the genus into the following two groups: (i) species with karyotypes composed of small and numerous chromosomes (*E.* section *Limnochloa*) and (ii) species with big and less numerous chromosomes (other sections).

Some studies about repetitive DNA segments in members of the Cyperaceae report the existence of numerous GC-rich heterochromatic blocks in *Carex* (Greilhuber 1995) and the occurrence of different heterochromatin types (C-Giemsa$^+$, CMA_3^+/DAPI$^-$, CMA_3^+/DAPI$^+$ and CMA_3^-/DAPI$^+$) in *Rhynchospora* (Vanzela and Guerra 2000). FISH with 45S rDNA probes in members of Cyperaceae was performed on eight species of *Rhynchospora* by Vanzela *et al*. (1998). These authors found large variation in the number of sites, but stability in localisation, since all the sites were terminal. FISH with rDNA probes was also performed in *Eleocharis* and *Carex* by Furuta and Hoshino (1999), who found that in *E. mamillata* var. *cyclocarpa* ($2n = 16$) the 18S rDNA probe hybridised in 12 of the 16 chromosomes of the complement, always in the terminal positions. Terminal 45S rDNA sites were also found in *E. subarticulata* with $2n = 6$ (da Silva *et al*. 2005). FISH with the 5S rDNA probe hybridised in the interstitial positions of two chromosomes. In *Carex pachgyna* and *C. ciliato-marginata*, both with $2n = 12$, the 5S rDNA probe localised segments in the terminal and interstitial regions and 18S rDNA probe always hybridised in the terminal regions. These examples indicate that these repetitive DNA families are dynamics and present a great potential to study the karyotype differentiation and evolution in this genus.

In the present study, the karyotypes of 21 samples of 11 species of *Eleocharis* (Cyperaceae) from 10 localities in Brazil were analysed. The aim of the study was to contribute to knowledge on the relationship between size, chromosome number and taxonomic grouping, such as those proposed by Yano *et al*. (2004). We also focused on the importance of DNA segment repeats in karyotype differentiation in *Eleocharis*.

Materials and methods

Twenty-one samples representing 11 species of *Eleocharis* were collected in 10 different localities of south-eastern and southern Brazil. Vouchers were deposited in herbarium FUEL. In a few localities, more than one species was collected (Table 1).

Table 1. Chromosome count and size in Brazilian species of *Eleocharis*
Species are grouped according to González-Elizondo and Peterson (1997)

Species	2*n*	Size (µm)	Localities (coordinates) and voucher number
Subgenus *Eleocharis* S.González & P.M.Peterson			
Section *Eleocharis* S. González & P.M.Peterson			
E. subarticulata (Nees) Boeckl.	6	3.0–1.5	Ponta Grossa, Paraná, (25°05′06″S, 50°09′57″W), 35.411.
E. minima Kunth[A]	12	2.7–0.9	Sapopema, Paraná, (23°53′06″S, 50°35′24″W), 35.403.
E. montana (Kunth) Roem. & Schult[A]	20 40	2.3–1.3 2.2–1.1	Bela Vista do Paraíso, Paraná (23°01′36″S, 51°12′36″W), 36.079; Bela Vista do Paraíso, Paraná 36.081; Rolândia, Paraná (23°19′48″S, 51°22′21″W), 35.422; Assaí, Paraná (23°22′12″S, 50°49′48″W), 36.073; Sapopema, Paraná, 36.064.
E. elegans (HBK) R & S[A]	40	2.2–1.0	Assaí, Paraná, 36.074.
Section *Eleogenus* (Nees) Benth. & Hook.			
E. flavescens (Poir.) Urban[A]	10	2.0–1.3	Rolândia, Paraná, 35.424.
E. maculosa (Vahl) Roem & Schult.[A]	10	2.4–1.6	Florianópolis, Santa Catarina (27°34′12″S, 48°31′48″W), 35.406; Tibagi, Paraná (24°32′06″S, 50°24′18″W), 35.434.
E. sellowiana Kunth.[A]	20	2.6–1.4	Ibitinga, São Paulo (21°45′11″S, 49°01′33″W), 35.429; Rolândia, Paraná, 35.426; Bela Vista do Paraíso, Paraná, 36.080.
E. geniculata (L.) Roem & Schult.	20	3.2–4.1	Ponta Grossa, Paraná (24°59′24″S, 49°59′24″W), 36.084.
Subgenus *Limnochloa* (P.Beauv. ex Lestib.) Torr.			
Section *Limnochloa* (P.Beauv. ex T.Lestib.) Benth. & Hook. f.			
E. brasiliensis Boeck[A]	~54[B]		Biological collection of Unicamp, Campinas, São Paulo (22°48′48″S, 47°04′12″W), 35.405.
E. acutangula (Roxb.) Schult.	54	1.2–0.7	Rio Claro, São Paulo (22°24′54″S, 22°24′55″W), 35.455; Rolândia, Paraná, 35.420; Assaí, Paraná, 36.075; Sapopema, Paraná, 36.068.
Subgenus *Scirpidium* (Nees) Kukk.			
Section *Scirpidium* (Nees) Benth. & Hook. f.			
E. radicans (Poir.) Kunth. Enum.[A]	10		Sapopema, Paraná, 35.408.

[A] First chromosome counts.
[B] Approximate chromosome number.

Samples were cultivated in the nursery of the Laboratório de Biodiversidade e Restauração de Ecossistemas at Universidade Estadual de Londrina, Londrina, Brazil.

Karyotype analyses were performed with root tips pre-treated in 2 mM 8-hydroxyquinoline for 24 h, fixed in ethanol : acetic acid (3 : 1, v : v) for 24 h, and stored at –20°C or immediately used. For the conventional staining, the root tips were washed in distilled water, softened in 4% cellulase plus 40% pectinase at 37°C for 3 h, hydrolysed in 1 M HCl for 10 min at 60°C, washed again in distilled water, and squashed in a drop of 45% acetic acid. Slides were stained in 2% giemsa and mounted with Entellan (Merck). The chromosome counts were made in at least 20 cells for each sample. For calculating the chromosome sizes, at least 10 cells with good chromosome morphology and similar condensation and spreading ability were measured, and the mean length was calculated for each pair. To test the parallel migration of the holocentric chromosomes in anaphases, slides were prepared with meristems obtained without a previous treatment with anti-mitotic agent and directly fixed in ethanol : acetic acid, as described above.

For chromosome banding, fixed samples were washed in distilled water, softened in 4% cellulase plus 40% pectinase at 37°C for 3 h, washed again and squashed in a drop of 45% acetic acid, without acid hydrolysis. After freezing in liquid nitrogen for coverslip removal, preparations were aged for later C-CMA_3 banding or immediately used for FISH with the 45S rDNA probe. For C-CMA_3 banding, preparations were treated according to Schwarzacher *et al.* (1980), with modifications. Slides were incubated in 45% acetic acid at 60°C for 10 min, 5% $Ba(OH)_2$ at 25°C for 10 min and 2 × SSC pH 7.0 at 60°C for 1 h and 30 min, and to finish, the samples were air-dried. After 3 days, samples were stained with a drop of 0.5 $\mu g\, \mu L^{-1}$ CMA_3 containing 2.5 mM $MgCl_2$ for 1 h 30 min, washed in distilled water and subsequently stained with 2 $\mu g\, mL^{-1}$ DAPI for 30 min. Slides were mounted in a solution of glycerol : McIlvaine buffer, pH 7.0, (1 : 1, v : v) containing 2.5 mM $MgCl_2$.

FISH was carried out with the p*Ta*71 probe containing 45S rDNA isolated from wheat (Gerlach and Bedbrook 1979). The probe was labelled with biotin-14-dATP by nick translation. Material was incubated in RNase (100 $\mu g\, mL^{-1}$), post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70% and 100% graded ethanol series for 5 min each and air-dried. A hybridisation mix (30 µL per slide), containing 100 ng of labelled probe (4 µL), 100% formamide (15 µL), 50% polyethylene glycol (6 µL), 10% SDS (1 µL), 100 ng of calf thymus DNA (1 µL) and 20 × SSC (3 µL), was denatured at 70°C for 10 min and immediately chilled on ice. Chromosome denaturation/hybridisation was performed at 90°C for 10 min, 50°C for 10 min and 38°C for 5 min with a thermal cycler, and kept at 37°C overnight in a moist chamber. Post-hybridisation washes were performed at 80% stringency in 2 × SSC, 0.1 × SSC/20% formamide, 0.1 × SSC, 2 × SSC and 4 × SSC/0.2% Tween 20, at 42°C for 5 min each. The biotin-labelled probe was detected with avidin FITC-conjugate, and slides were mounted in an antifade solution (2.3% of 1,4-diaza- bicyclo(2.2.2)-octane, 20 mM TRIS–HCl, pH 8.0, and 90% glycerol, in distilled water) containing propidium iodide (1 $\mu g\, mL^{-1}$). Images were taken with a Leica DM 4500 B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software (all from Leica Microsystems).

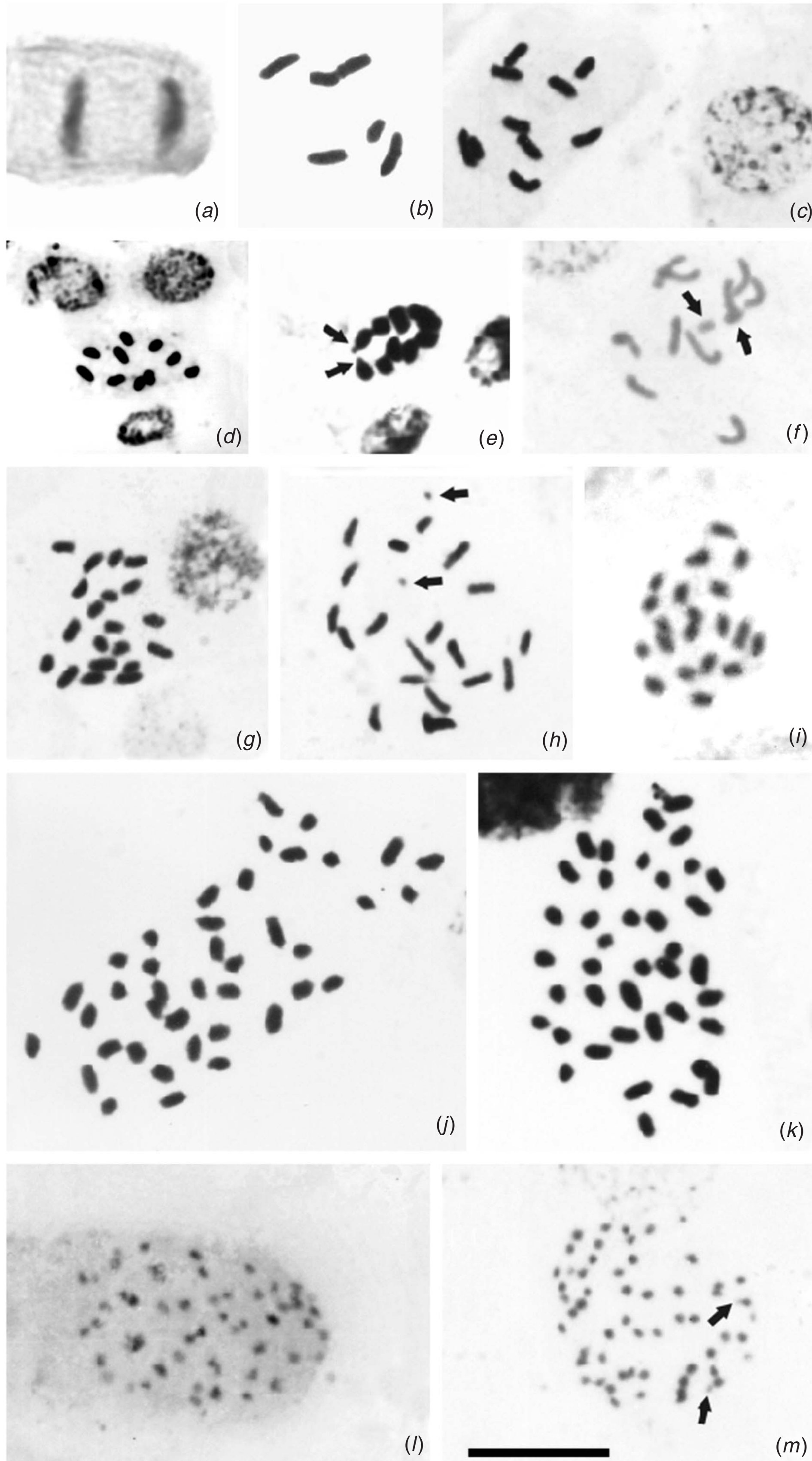
Results and discussion

Karyotype features and cytotaxonomy

The reduction in morphological structures of *Eleocharis* also reaches the chromosome level. Cyperaceae possess holocentric chromosomes, so that it is not possible to compare populations and species by the use of chromosome shape. Cytogenetical features, such as nucleolar constrictions, chromosome size and interphase nuclei types, can be useful to study many plant groups; however, this information is scarce in the literature on Cyperaceae. Hoshino (1987) reported that interphasic nuclei in *Eleocharis* possess diffuse chromatin with spherical bodies stained darkly and with differences in size. The nuclei described by Hoshino (1987), as well as those found in the present study, and those described by Vanzela *et al.* (2000) in *Rhynchospora*, are similar to the chromocentric interphase nuclei described by Kondo *et al.* (1994) in *Drosera* (Droseraceae), which also has holocentric chromosomes. Thus, the occurrence of chromocentric nuclei in all the studied species of *Eleocharis*, with minor differences in the chromocenter size (see Fig. 1*c*, *d*, *g*), suggests that this feature is not a useful cytotaxonomic tool to separate species and subgenera of *Eleocharis*; however, it indicates that besides the holokinetic chromosomes (Greilhuber 1995), the nucleus type may also be a synapomorphy of the Cyperaceae.

None of the species studied presented primary constrictions in the chromosomes and, additionally, the chromosomes migrate parallel to the equatorial plate axis at anaphase, as can be observed e.g. in *E. sellowiana* (Fig. 1*a*). The absence of Rabl organisation during separation of sister chromatids in Cyperaceae, was previously noted in *R. tenuis* (Vanzela *et al.* 1996), and confirmed recently by Guerra *et al.* (2005), after the detection of mitotic apparatus with anti-α-tubulin antibody. Several studies have shown the occurrence of holocentric chromosomes in *Eleocharis* (Strandhede 1965; Hoshino 1987; da Silva *et al.* 2005), reinforcing that the holokinetic condition of the chromosomes may be a synapomorphy of the family, as proposed by Greilhuber (1995).

Fig. 1. Conventional chromosome analysis in *Eleocharis*. (*a*) Anaphase in *Eleocharis sellowiana*, showing the parallel movement of the chromosomes at the equatorial plate; (*b*) metaphase with $2n = 6$ in *E. subarticulata*; (*c*) metaphase with $2n = 10$ in *E. maculosa*; (*d*) metaphase with $2n = 10$ in *E. flavescens* (note the chromocentric interphase nuclei in *c* and *d*); (*e*) metaphase with $2n = 10$ in *E. radicans* (arrows point to the terminal nucleolar constrictions (satellites)); (*f*) prometaphase with $2n = 12$ in *E. minima*; two small chromosomes (arrows); (*g*) metaphase with $2n = 20$ in *E. geniculata*; adjacent, a diffuse chromocentric nucleus; (*h*) prometaphase with $2n = 20$ in *E. sellowiana*; arrows point to two satellites; metaphase in *E. montana* with (*i*) $2n = 20$ and (*j*) $2n = 40$; (*k*) metaphase with $2n = 40$ in *E. elegans*; (*l*) metaphase with $2n = \sim 54$ in *E. brasiliensis*; and (*m*) metaphase with $2n = 54$ in *E. acutangula*; arrows point to two minor satellites. Scale bar = 10 µm.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)

For eight of the species studied (Fig. 1*f*, *h–l*; Table 1), the chromosome number is documented here for the first time. Chromosome number for the other three (*E. acutangula*, *E. geniculata* and *E. subarticulata*) are confirmed here (Fig. 1*b*, *g*, *m*; Table 1). The number varied from $2n=6$ in *E. subarticulata* (Fig. 1*b*) to $2n=\sim54$ in *E. brasiliensis* and $2n=54$ in *E. acutangula* (Fig. 1*l*, *m*). *E. maculosa* (Fig. 1*c*), *E. flavescens* (Fig. 1*d*) and *E. radicans* (Fig. 1*e*) exhibited $2n=10$, whereas *E. geniculata* and *E. sellowiana* showed a duplicated chromosome number, with $2n=20$ (Fig. 1*g*, *h*). An intermediate chromosome number, $2n=12$, was found in *E. minima* (Fig. 1*f*); however, in this species, two of the chromosomes are very small (arrows). Cytotypes were found in *E. montana* with $2n=20$ and 40 (Fig. 1*i*, *j*). In this case, the chromosomes of the cytotype with $2n=20$ varied from 1.3 to 2.3 µm, and those of the cytotype with $2n=40$ varied from 1.1 to 2.2 µm. The total length of the complement was twice the normal length in the populations with $2n=40$. Significant morphological differences were not found between the two cytotypes of *E. montana*, suggesting that the polyploidisation to $2n=40$ could be a recent event, since it does not yet affect the gross morphology. The chromosome number $2n=40$ was also found in *E. elegans* (Fig. 1*k*). Another interesting case involves *E. sellowiana* and *E. flavescens*, two species in which the morphological characters, in some instances, are not enough to distinguish them. Some differences are the transversally elliptic stems in *E. flavescens* but circular in *E. sellowiana* (Faria 1998). Also, the upper portion of the distal sheath is not abruptly differentiated from the lower portion in *E. flavescens*, whereas in *E. sellowiana* it is abruptly differentiated (González-Elizondo *et al*. 2005). Populations of *E. sellowiana*, when slender or depauperate, may be morphologically very similar to and difficult to differentiate from *E. flavescens*. However, the karyotype of these species can be considered an additional micromorphological feature useful to taxonomy, since *E. sellowiana* possesses $2n=20$ and *E. flavescens* $2n=10$.

Intraspecific variations, as those found here, are common in *Eleocharis* and some other Cyperaceae. Numerical variations were described by Bureš *et al*. (2004) in an extensive study of 78 European populations of *E. palustris* subsp. *palustris*. These authors found three prevalent cytotypes, namely, the diploid with $2n=16$ and, in fewer samples, the symploid ($2n=15$) and the mixoploid ($2n=15$ and 16). Ohkawa *et al*. (2000) studied different populations of *Carex sociata* from Japan and found numbers varying from $2n=40$ to 44, with prevalence of $2n=42$ and 44. Such variations have also been found in genera such as *Fimbristylis*: *F. bisumbellata* and *F. dichotoma*, both with populations with $2n=10$ and 20, and *F. falcata* with $n=11$ and 22 (Bir *et al*. 1992). In the genus *Rhynchospora*, *R. ciliata* was treated as a subspecies of *R. nervosa*; however, *R. ciliata* showed $2n=10$ and *R. nervosa* $2n=30$; thus, Luceño *et al*. (1998) proposed that they could be considered as two different species. Polyploid and diploid cytotypes have been found in *E. geniculata*, with $2n=10$ and 20, and *E. atropurpurea* with $2n=20$ and 21 (Nijalingappa 1973). The study that described the largest numerical variations among related species was published by Strandhede (1965). This author showed that in *E.* subser. *Eleocharis*, *E. mamillata* has $2n=16$, *E. palustris* subsp. *palustris* has $2n=15$ and 16, *E. palustris* subsp. *vulgaris* showed $2n=36$, 38, 39 and 42, and *E. uniglumis* $2n=46$ and $2n=74$ to 82.

Most of the chromosome numbers found in *Eleocharis* are multiples of $x=5$, which is in line with the proposition of Löve *et al*. (1957) about Cyperaceae. This basic number is common in some close genera, such as *Bulbostylis*, *Fimbristylis* (Bir *et al*. 1992) and *Abildgaardia* subser. (André L. L. Vanzela 2006, unpubl. data), but also in other, more distant genera, such as *Rhynchospora* (Luceño *et al*. 1998). Secondary basic numbers have also been detected in *Eleocharis*; however, these are probably derived from structural changes. *E. subarticulata* with $2n=6$ is a good example. In this case, there was a reduction owing to multiple translocations (da Silva *et al*. 2005). Reduction in the number by fusion of holocentric chromosomes was reported in *R. tenuis*, with $2n=4$ (Vanzela *et al*. 1996) and *Fimbristylis umbellaris* with $2n=6$ (Rath and Patnaik 1978). If we consider $x=5$ as the primary basic number of *Eleocharis*, it is possible that the two minor chromosomes found in *E. minima* with $2n=12$ appeared by fission (agmatoploidy) and the numbers $2n=\sim54$ found in *E. brasiliensis* and $2n=54$ found in *E. acutangula* (Fig. 1*l*, *m*) probably originated from agmatoploidy followed by or preceding polyploidy. It is also possible that the high chromosome number in these species has appeared by widespread chromosome fission and not only by polyploidy. A similar event was reported by Nordenskiöld (1951), who observed that in some polyploid species of *Luzula* (Juncaceae), the holocentric chromosomes seemed to be half the size of those of the diploid species. An increase in the chromosome number resulting from agmatoploidy has been described in several genera of Cyperaceae. As examples, there are numerical variations in genera close to *Eleocharis*, such as *Schoenoplectus* with $2n=42$, 44 and 78 (Jankun 1989; Löve and Löve 1981) and *Scirpus* with $2n=20$, 31, 32, 34, 37, 48, 50, 56, 60 and 62 (Heiser 1979; Harriman 1981; Löve and Löve 1981; Pignotti and Fiorini 1998; Subramanian 1988).

Chromosome sizes of the species studied are shown in Table 1. Hoshino (1987) proposed the existence of the following three karyotype variations in *Eleocharis* in relation to the chromosome size: (i) bimodal variation, with two groups of chromosomes of distinctly different sizes, (ii) gradual variation, where the chromosomal size decreases gradually and (iii) homogeneous variation, where all the chromosomes are similar, and very small. Yano *et al*. (2004) studied several Japanese species of *Eleocharis* and suggested that *Eleocharis* can be divided into two groups according to chromosome sizes. The first includes species with very small chromosomes (<1.1 µm in length), and the second includes species with chromosome sizes ranging from 1.4 to 4.3 µm. The chromosome measurements made in the present study corroborate the analyses by Hoshino (1987), and partially corroborate the results of Yano *et al*. (2004); the species of subgenus *Limnochloa* (*E. acutangula* and *E. brasiliensis*) have many and smaller chromosomes (1.2–0.7 µm), whereas size is variable in the species of the subgenera *Eleocharis* and *Scirpidium*, ranging from 4.1

to 1.2 µm (and even 0.9 µm in the small chromosomes of *E. minima*). Species of subgenera *Eleocharis* and *Scirpidium* exhibit a bimodal pattern of karyotype, where the numbers form a gradual, incremental sequence of numbers. Our findings are consistent with the classification system proposed by González-Elizondo and Peterson (1997) and with the

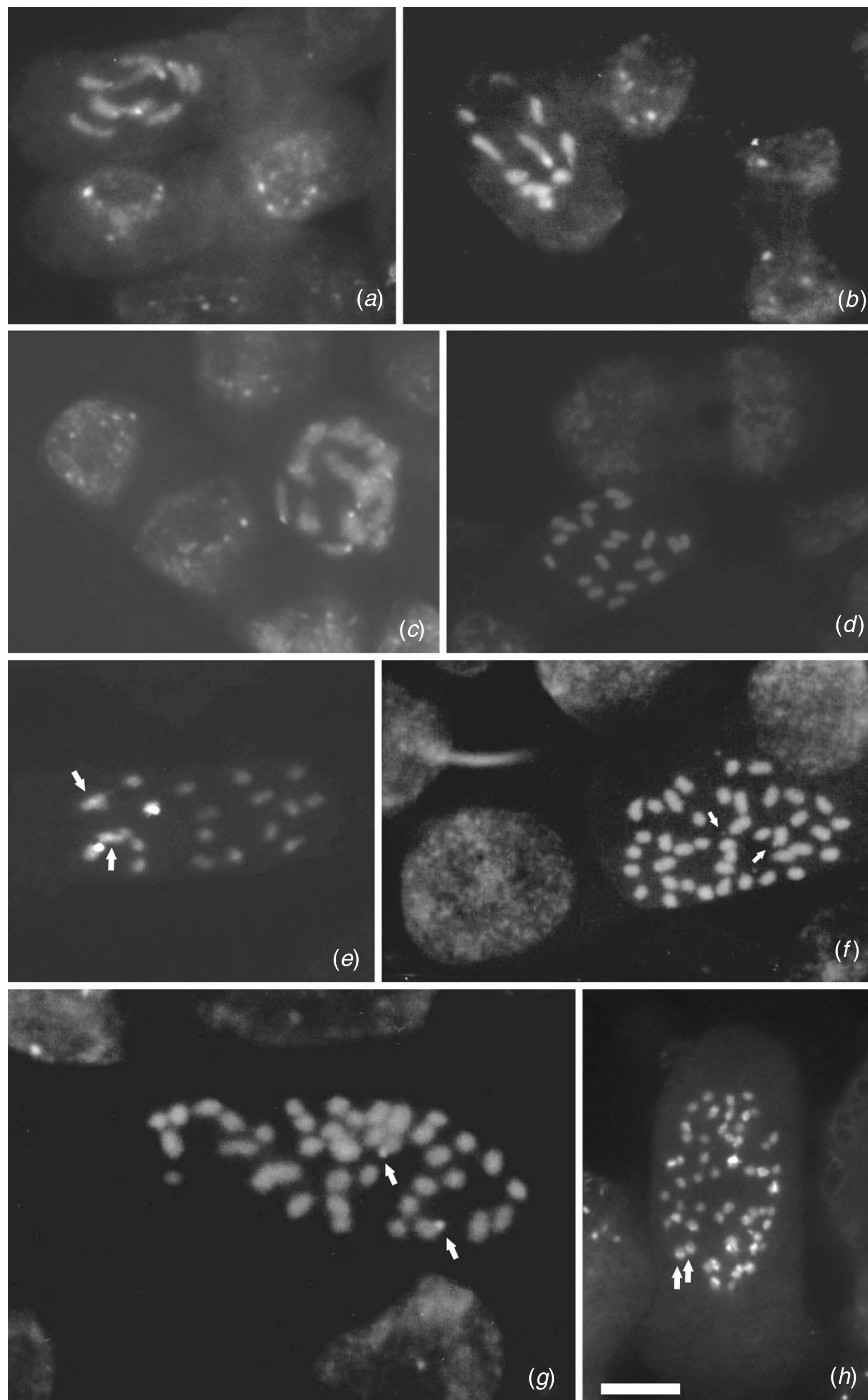


Fig. 2. C-CMA_3/DAPI banding in *Eleocharis*. (*a*) *Eleocharis flavescens* ($2n = 10$) with four terminal CMA_3 blocks; (*b*) *E. maculosa* ($2n = 10$) with four terminal CMA_3 blocks; (*c*) *E. sellowiana* ($2n = 20$) with about 10 terminal C-CMA_3 blocks; (*d*) C- DAPI banding in *E. sellowiana*; note the absence of DAPI blocks; (*e*) *E. geniculata* ($2n = 20$), showing two larger terminal C-CMA_3 blocks and two smaller and interstitial (arrows); (*f*) *E. elegans* ($2n = 40$); arrows indicate the two tenuous and terminal C-CMA_3 blocks; note the absence of signals in the nuclei; (*g*) *E. montana* ($2n = 40$) with two terminals C-CMA_3 blocks (arrows); and (*h*) *E. acutangula* ($2n = 54$) with ~20 terminal and few interstitial C-CMA_3 bands; the arrows point to two interstitial blocks. Scale bar = 5 µm.

phylogenetical analysis based on ITS proposed by Roalson and Friar (2000).

Repetitive DNA and chromosome organisation

The C-CMA_3/DAPI banding showed that the studied species have no DAPI bands (Fig. 2*d*), suggesting that AT-rich sequences seem not to be important in the karyotype organisation. However, most of the species showed fine terminal C-CMA_3 bands (GC-rich blocks), with low fluorescence intensity, but varying in the number and size of the bands. *E. flavescens* and *E. maculosa*, both with $2n = 10$, exhibited four C-CMA_3 signals, two signals being slightly larger and two smaller (Fig. 2*a*, *b*). *E. sellowiana* with $2n = 20$ exhibited ~10 C-CMA_3 blocks, eight being more conspicuous and two fine (Fig. 2*c*). *E. geniculata* with $2n = 20$ showed four C-CMA_3 blocks, two being larger and terminal and two smaller and interstitial. Interestingly, these interstitial blocks were found in the largest pair (Fig. 2*e*). *E. elegans* and *E. montana*, both with $2n = 40$, showed tenuous C-CMA_3^+ bands always in one chromosome pair (Fig. 2*f*, *g*). Interestingly, *E. acutangula*, the species with the smallest chromosomes, presented the largest number of C-CMA_3^+ bands, with ~20 interstitial and terminal bands (Fig. 2*h*). In spite of the very small number of treated species for the chromosome banding, these data indicate that heterochromatin rich in GC is very important

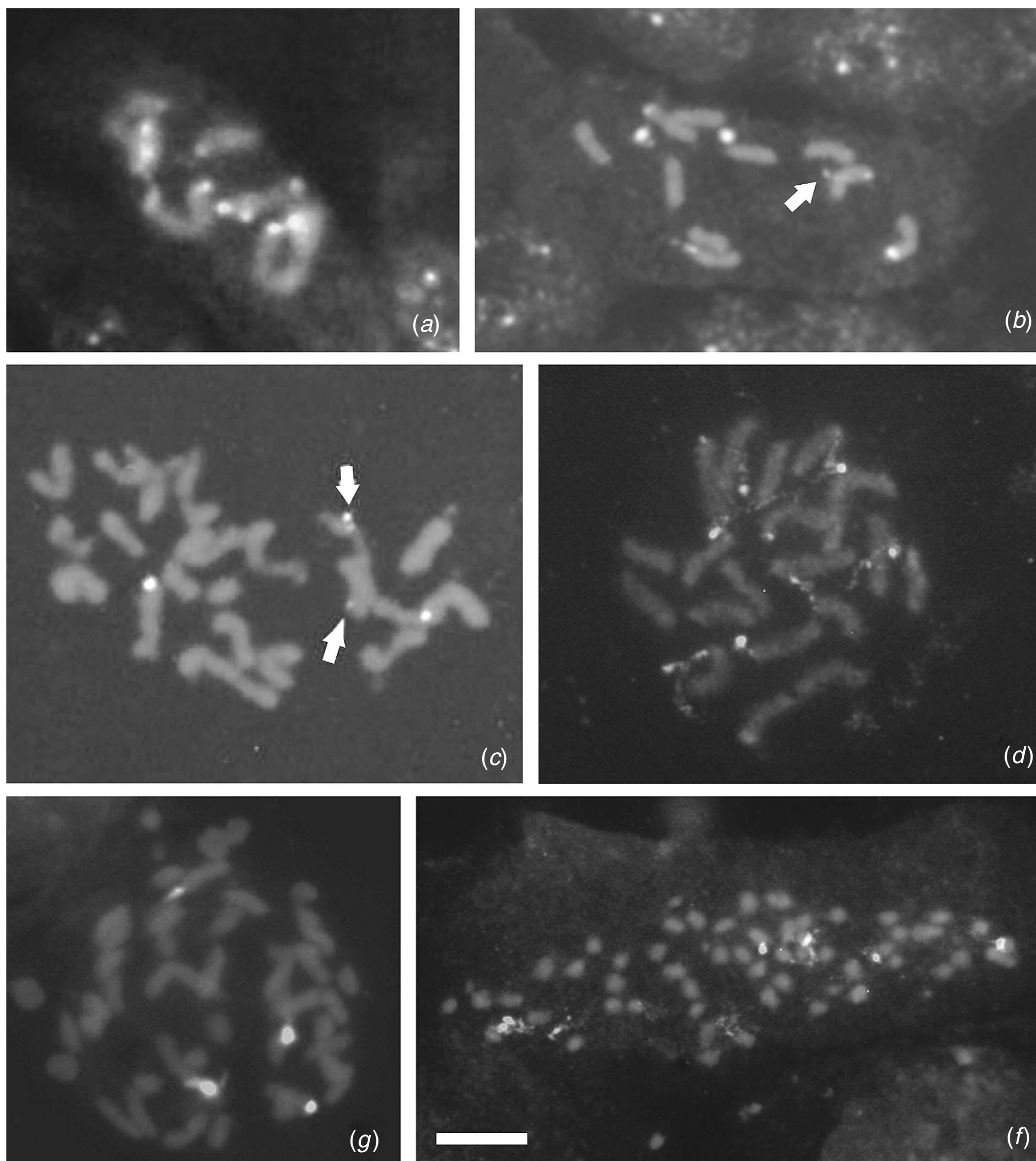


Fig. 3. FISH with 45S rDNA probe in species of *Eleocharis*. (*a*) *E. flavescens* ($2n = 10$) with 10 rDNA sites; (*b*) *E. maculosa* ($2n = 10$), showing six rDNA sites, two being minor and four major; the arrows point to the minor sites; note also the spread out aspect at prometaphases; (*c*) *E. geniculata* ($2n = 20$), showing four hybridisation sites; arrows point to the two minor rDNA sites; (*d*) *E. sellowiana* ($2n = 20$), exhibiting eight rDNA sites; note the spread out aspect of hybridisation sites; (*g*) *E. montana* with four rDNA sites; and (*f*) *E. acutangula* ($2n = 54$), showing eight 45S rDNA sites. Scale bar = 5 µm.

in chromosome organisation in *Eleocharis*, whereas AT-rich segments represent a repetitive DNA family that perhaps appears sporadically in a few species, with a punctual importance. Sheikh and Kondo (1995) used chromosome banding to study the karyotypical differences in *Drosera* (Droseraceae), another group containing holocentric chromosomes. These authors found that in 12 species, the bands appeared preferentially in the terminal chromosome positions, but with accumulation of CMA-positive and not DAPI-positive bands, as observed here in *Eleocharis*. In contrast to our results, there are two published examples of GC- and AT-rich heterocromatin accumulation. In the first case, Vanzela and Guerra (2000) described three heterochromatin types in the genus *Rhynchospora* (Cyperaceae), i.e. $CMA_3^+/DAPI^-$, $CMA_3^+/DAPI^+$, and $CMA_3^-/DAPI^+$, which varied in size and position; however, only the karyotype of *R. globosa* was differentiated owing to accumulation of AT-rich blocks. In the second case, Guerra and García (2004) described a large amount of heterochromatin (more AT- than GC-rich bands) in *Cuscuta approximata* (Cuscutaceae). In fact, the accumulation and distribution of heterochromatin in holocentric chromosomes seems to be very similar to that known for monocentric chromosomes (Guerra 2000).

The common association among terminal GC-rich blocks with nucleolar organiser regions was previously shown in other Cyperaceae, including *Carex* (Greilhuber 1995) and *Rhynchospora* (Vanzela and Guerra 2000). However, on the basis of the species investigated here, the association between GC-rich heterochromatin and NOR is not a rule. Thus, these data suggest the occurrence of at least two repetitive DNA families in *Eleocharis*: (1) C-CMA_3^+ bands NOR associated and (2) C-CMA_3^+ not NOR associated. Nucleolar constrictions are not frequently visualised on holocentric chromosomes, and following this tendency, only a few chromosomes with satellites (terminal NORs) were seen in *Eleocharis*. These terminal constrictions were found in *E. radicans* ($2n = 10$), *E. sellowiana* with $2n = 20$ and *E. acutangula* with $2n = 54$ (Fig. 1*e*, *h*, *m*). Similar constrictions were also described by Hoshino (1987) in *Eleocharis* and by Vanzela *et al*. (1996) and Luceño *et al*. (1998) in *Rhynchospora*.

To confirm the number of NORs, FISH with 45S rDNA probe was performed. The p*Ta*71 probe localised hybridisation sites always at the chromosome ends, as previously reported by Vanzela *et al*. (1998) in *Rhynchospora*, by Furuta and Hoshino (1999) in *E. mamillata* var. *cyclocarpa* and by Guerra and García (2004) in *C. approximata* (Cuscutaceae). These latter authors hybridised chromosomes with 18S rDNA probes and found 12 terminal hybridisation sites, with two small chromosomes with rDNA sites at both extremities. The results of FISH with the 45S rDNA probe, according to our study, also showed multiple and terminal hybridisation sites, and that the differences in hybridisation site number were independent of the ploidy level. The hybridisation sites appear spread out at prometaphase and metaphase, hindering determination of the number of sites. Ten 45S rDNA sites were found in *E. flavescens* with $2n = 10$ (Fig. 3*a*), in which only four are associated to the GC-rich heterochromatin. In *E. maculosa* ($2n = 10$), six rDNA sites were observed, two being minor and four major (Fig. 3*b*), and these four are associated with GC-rich blocks. *E. geniculata* ($2n = 20$) showed four hybridisation sites (Fig. 3*c*). *E. sellowiana* ($2n = 20$) showed eight rDNA sites (Fig. 3*d*), and all associated with GC-rich blocks. *E. montana* ($2n = 40$) exhibited four rDNA sites (Fig. 3*e*), with two associated with GC-rich heterochromatin. *E. acutangula* ($2n = 54$) showed eight 45S rDNA sites (Fig. 3*f*) and a larger number of GC-rich blocks. The multiplicity of 45S rDNA sites and the occurrence of two heterochromatin types (GC-rich associated and not associated with NOR) observed in *Eleocharis*, were also reported in *Rhynchospora* by Vanzela *et al*. (1998), suggesting a cytogenetical feature common for Cyperaceae.

In conclusion, these results show that despite of the reduction in the number of morphological and cytogenetical features (considering the absence of primary constrictions), the karyotypes of *Eleocharis* accumulate differences in the size and number of chromosomes between species and populations as well as in the size and number of GC-rich bands and 45S rDNA sites. Hence, cytogenetics can be considered a good tool for taxonomic and evolutionary studies in Cyperaceae.

Acknowledgements

This study was supported by Brazilian agencies CAPES, CNPq and Fundação Araucária.

References

Bir SS, Cheema P, Sidhu M (1992) Chromosomal analysis of *Fimbristylis* Vahl in Punjab, North West India. *Proceedings of the Indian Academy of Sciences B58* **1**, 63–70.

Bureš P, Rotreklová O, Holt SDS, Pikner R (2004) Cytogeographical survey of *Eleocharis* subser. *Eleocharis* in Europe. 1. *Eleocharis palustris*. *Folia Geobotanica* **39**, 235–257.

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2005) Reduction of chromosome number in *Eleocharis subarticulata* (Cyperaceae) by multiple translocations. *Botanical Journal of the Linnean Society* **149**, 457–464. doi: 10.1111/j.1095-8339.2005.00449.x

Davies EW (1956) Cytology, evolution and origin of the aneuploid series in the genus *Carex*. *Hereditas* **42**, 349–365.

Faria AD (1998) O gênero *Eleocharis* R.Br. (Cyperaceae) no Estado de São Paulo. Mestrado em Biologia Vegetal. Unicamp, Campinas, São Paulo, Brasil. p. 150.

Faulkner JS (1972) Chromosome studies on *Carex* section *Acutae* in north-west Europe. *Botanical Journal of the Linnean Society* **65**, 271–301.

Furuta K, Hoshino T (1999) Localization of the 18S and 5S rDNA on the chromosomes with non-localized centromere in Cyperaceae. In 'XVI international botanical congress, St Louis, MI, USA'. Abstract No. 4331, Poster No. 1829.

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acids Research* **7**, 1869–1885. doi: 10.1093/nar/7.7.1869

González-Elizondo MS, Peterson PM (1997) A classification of and key to the supraspecific taxa in *Eleocharis* (Cyperaceae). *Taxon* **46**, 433–449. doi: 10.2307/1224386

González-Elizondo MS, Tena-Flores J, Alarcón-Herrera T, Flores-Tavizón E, Barajas-Acosta N (2005) An arsenic tolerant new species of *Eleocharis* (Cyperaceae) from Chihuahua, Mexico. *Brittonia* **57**(2), 150–154. doi: 10.1663/0007-196X(2005)057[0150:AANSOE]2.0.CO;2

Greilhuber J (1995) Chromosomes of the monocotyledons (general aspects). In 'Monocotyledons: systematics and evolution'. (Eds PJ Randall, PJ Cribb, DF Cutler, CJ Humphries) pp. 379–414. (Royal Botanic Gardens, Kew: London)

Guerra M (2000) Patterns of heterochromatin distribution in plant chromosomes. *Genetics and Molecular Biology* **23**, 1029–1041.

Guerra M, García MA (2004) Heterochromatin and rDNA sites distribution in the holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae). *Genome* **47**, 134–140. doi: 10.1139/g03-098

Guerra M, Brasileiro-Vidal AC, Arana P, Puertas MJ (2005) Mitotic microtubule development and histone H3 phosphorylation in the holocentric chromosomes of *Rhynchospora tenuis* (Cyperaceae). *Genetica* **126**, 1–9.

Håkansson A (1954) Meiosis and pollen mitosis in X-rayed and untreated spikelets of *Eleocharis palustris. Hereditas* **40**, 325–345.

Harriman NA (1981) Chromosome number reports LXXI. *Taxon* **30**, 517.

Heiser CBJr (1979) The totora (*Scirpus californicus*) in Ecuador and Peru. *Economic Botany* **32**, 222–236.

Hoshino T (1987) Karyomorphological studies on 6 taxa of *Eleocharis* in Japan. *Bulletin of the Okayama University of Science* **22**, 305–312.

Jankun A (1989) Further studies in chromosome numbers of Polish angiosperms. Part XXII. *Acta Biologia Cracoviensia Series Botanica* **31**, 1–17.

Kondo K, Sheikh SA, Hoshi Y (1994) New finding of another $2n = 6$ species in the angiosperms, *Drosera roseana* Marchant. *Chromosome Service* **57**, 3–4.

Kukkonen I (1990) On the genus *Eleocharis* (Cyperaceae) in the Flora Iranica area, with revised infrageneric classification and nomenclature. *Annales Botanici Fennici* **27**, 109–117.

Löve A, Löve D (1981) In IOPB chromosome number reports LXXIII. *Taxon* **30**, 845–851.

Löve A, Löve D, Raymond M (1957) Cytotaxonomy of *Carex* section *Cappilares. Canadian Journal of Botany* **35**, 715–761.

Luceño M, Vanzela ALL, Guerra M (1998) Cytotaxonomic studies in Brazilian *Rhynchospora* (Cyperaceae), a genus exhibiting holocentric chromosomes. *Canadian Journal of Botany* **76**, 440–449. doi: 10.1139/cjb-76-3-440

Nijalingappa BHM (1973) Cytological studies in *Eleocharis. Caryologia* **26**, 513–520.

Nordenskiöld H (1951) Cyto-taxonomical studies in the genus *Luzula.* I. Somatic chromosomes e chromosome numbers. *Hereditas* **37**, 325–355.

Ohkawa T, Yokota M, Hoshino T (2000) Aneuploidal population differentiation in *Carex sociata* Boott (Cyperaceae) of the Ryukyu Islands, Japan. *Botanical Journal of the Linnean Society* **132**, 337–358. doi: 10.1006/bojl.1999.0303

Pignotti L, Fiorini G (1998) Mediterranean chromosome number reports 8 (899–904). *Flora Mediterranea* **8**, 214–221.

Rath SP, Patnaik SN (1978) Cytological studies in Cyperaceae. *Proceedings of the Indian Science Congress Association (III, C)* **65**, 107–108.

Roalson EH, Friar EA (2000) Infrageneric classification of *Eleocharis* (Cyperaceae) revisited: evidence from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. *Systematic Botany* **25**, 323–336. doi: 10.2307/2666645

Schwarzacher TP, Ambros P, Schweizer D (1980) Application of giemsa banding to orchid karyotype analysis. *Plant Systematics and Evolution* **134**, 293–297. doi: 10.1007/BF00986805

Sheikh SA, Kondo K (1995) Differential staining with orcein, giemsa CMA, and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). *American Journal of Botany* **82**, 1278–1286. doi: 10.2307/2446251

Strandhede SO (1964) Chromosome studies in *Eleocharis* subser. *Palustres*. I. Meiosis in some forms with 15 chromosomes. *Hereditas* **53**, 47–62.

Strandhede SO (1965) Chromosome studies in *Eleocharis*, subser. *Palustres. Opera Botanica* **9**(2), 1–86.

Subramanian D (1988) Cytotaxonomical studies in south Indian Cyperaceae. I. Species from the plains. *Cytologia* **53**, 67–72.

Svenson HK (1929) Contributions from the Gray Herbarium of Harvard University. LXXXVI. Monographic studies in the genus *Eleocharis. Rhodora* **31**, 121–245.

Svenson HK (1932) Monographic studies in the genus *Eleocharis*. II. *Rhodora* **34**, 193–230.

Svenson HK (1934) Monographic studies in the genus *Eleocharis*. III. *Rhodora* **36**, 377–408.

Svenson HK (1937) Monographic studies in the genus *Eleocharis*. IV. *Rhodora* **39**, 219–279.

Svenson HK (1939) Monographic studies in the genus *Eleocharis* V. *Rhodora* **41**, 1–110.

Vanzela ALL, Guerra M (2000) Heterochromatin differentiation in holocentric chromosomes of *Rhynchospora* (Cyperaceae). *Genetics and Molecular Biology* **23**, 453–456. doi: 10.1590/S1415-47572000000200034

Vanzela ALL, Guerra M, Luceño M (1996) *Rhynchospora tenuis* Link (Cyperaceae), a species with the lowest number of holocentric chromosomes ($n = 2$). *Cytobios* **88**, 219–228.

Vanzela ALL, Cuadrado A, Jouve N, Luceño M, Guerra M (1998) Multiple locations of the rDNA in species of *Rhynchospora* (Cyperaceae). *Chromosome Research* **6**, 345–349. doi: 10.1023/A:1009279912631

Vanzela ALL, Luceño M, Guerra M (2000) Karyotype evolution and cytotaxonomy in Brazilian species of *Rhynchospora* Vahl (Cyperaceae). *Botanical Journal of the Linnean Society* **134**, 557–566. doi: 10.1006/bojl.2000.0352

Yano O, Katsuyama T, Tsubota H, Hoshino T (2004) Molecular phylogeny of Japanese *Eleocharis* (Cyperaceae) based on ITS sequence data, and chromosomal evolution. *Journal of Plant Research* **117**, 409–419. doi: 10.1007/s10265-004-0173-3

Manuscript received 1 February 2007, accepted 9 October 2007

Capítulo 3

Distribution of 45S and 5S rDNA sites in 23 species of *Eleocharis* (Cyperaceae)

Artigo submetido a revista Genetica

Editorial Manager(tm) for Genetica
Manuscript Draft

Manuscript Number:

Title: Distribution of 45S and 5S rDNA sites in 23 species of Eleocharis (Cyperaceae)

Article Type: Original Research

Keywords: FISH, holocentric chromosomes, rDNA distribution, amplification and location

Corresponding Author: andré luís laforga vanzela, Dr.

Corresponding Author's Institution: Universidade Estadual de Londrina

First Author: andré luís laforga vanzela, Dr.

Order of Authors: andré luís laforga vanzela, Dr.

Abstract: Studies of rDNA location in holocentric chromosomes of Cyperaceae are scarce, but a few reports have indicated the predominance of 45S rDNA in terminal positions, multiple numbers of sites and a decondensed state of hybridization region in prometaphase/metaphase. To increase the knowledge of the number 45S and 5S rDNA sites and distribution in holocentric chromosomes of Cyperaceae, 23 Brazilian species of Eleocharis were studied. FISH showed 45S rDNA signals always located in terminal regions, which varied from two (E. bonariensis with 2n = 20) to ten (E. flavescens with 2n = 10 and E. laeviglumis with 2n = 60). The 5S rDNA showed less variation, with 16 species exhibiting two sites and 7 species with four sites, preferentially in terminal positions, except for 4 species (E. subarticulata, E. flavescens, E. sellowiana and E. geniculata) that showed interstitial sites. Results are discussed in order to understand the prevalence of terminal rDNA sites, the mechanisms involved in the interstitial allocation of 5S rDNA sites and the events of amplification and dispersion of 45S rDNA.

Manuscript
Click here to download Manuscript: Eleocharis FISH rDNA.doc
Click here to view linked References

1 # Distribution of 45S and 5S rDNA sites in 23 species of
2 # *Eleocharis* (Cyperaceae)
3

4 **Carlos Roberto Maximiano da Silva[1] and André Luís Laforga Vanzela[2]**

5 [1]Pós-graduação em Genética, Instituto de Biociências, Letras e Ciências Exatas,
6 UNESP, 15054-000, São José do Rio Preto, SP, Brazil.

7 [2]Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de
8 Biologia Geral, CCB, Universidade Estadual de Londrina, 86051-970, Londrina, PR,
9 Brazil. Correspondent author: andrevanzela@uel.br

10

11 **Abstract**

12 Studies of rDNA location in holocentric chromosomes of Cyperaceae are scarce, but a
13 few reports have indicated the predominance of 45S rDNA in terminal positions,
14 multiple numbers of sites and a decondensed state of hybridization region in
15 prometaphase/metaphase. To increase the knowledge of the number 45S and 5S rDNA
16 sites and distribution in holocentric chromosomes of Cyperaceae, 23 Brazilian species
17 of *Eleocharis* were studied. FISH showed 45S rDNA signals always located in terminal
18 regions, which varied from two (*E. bonariensis* with $2n = 20$) to ten (*E. flavescens* with
19 $2n = 10$ and *E. laeviglumis* with $2n = 60$). The 5S rDNA showed less variation, with 16
20 species exhibiting two sites and 7 species with four sites, preferentially in terminal
21 positions, except for 4 species (*E. subarticulata*, *E. flavescens*, *E. sellowiana* and *E.*
22 *geniculata*) that showed interstitial sites. Results are discussed in order to understand
23 the prevalence of terminal rDNA sites, the mechanisms involved in the interstitial
24 allocation of 5S rDNA sites and the events of amplification and dispersion of 45S
25 rDNA.

1 **Key words:** FISH, holocentric chromosomes, rDNA distribution, amplification and
2 location

3

4 **Introduction**

5 The rRNA genes occur as moderately repetitive DNA families on eukaryote
6 genomes. The 45S rDNA of plants, as well as in other organisms, appears as repeated
7 and clustered segments at particular chromosomal positions. These segments
8 predominantly occupy the chromosome ends (Furuta and Kondo 1999; Rego et al.
9 2009), ranging in the number of sites among and within species (Vanzela et al. 1998; De
10 Melo and Guerra 2003; Da Silva et al. 2008a; Raskina et al. 2008). The 5S rDNA is also
11 organized in repeated and clustered segments, occurring in most cases in distinct
12 chromosome positions of those occupied by 45S rDNA (Moscone et al. 1999).
13 Although these segments vary in the number of sites and chromosome pairs in different
14 organisms, they tend to occupy similar chromosome and position in closely related
15 species, as in *Aristolochia* (Berjano et al. 2009).

16 Plant holocentric chromosomes were reported in all representatives of the
17 families Juncaceae Juss. and Cyperaceae Juss. (Bokhari and Godward 1980; Roalson et
18 al. 2008), in some species of genus *Drosera* - Droseraceae and subgenus *Cuscuta* -
19 Cuscutaceae.However, there are two isolated species, *Chionographis japonica* -
20 Liliaceae and *Myristica fragrans* Myristicaceae, which present this chromosome type
21 (Flach 1966; Tanaka and Tanaka 1977; Sheikh et al. 1995; García 2001). The locations
22 of rDNA sites have only been determined in a few studies. Multiple 45S rDNA sites
23 were detected in nine species of *Rhynchospora* (Vanzela et al. 1998, 2003) and seven
24 species of *Eleocharis* (Da Silva et al. 2005, 2008a, b), both of the Cyperaceae family.
25 Hoshi (1995) and Furuta and Kondo (1999) also reported multiple and terminal rDNA

sites in *Drosera*. However, in *Cuscuta approximata* one pair of 45S rDNA and three of 5S rDNA sites were reported (Guerra and García 2004). In spite of general tendency towards a terminal location of 45S rDNA, the 5S rDNA was reported preferentially in interstitial regions in plants and animals, and in *Eleocharis* the interstitial occurrence of 5S rDNA was associated to chromosome rearrangement (Da Silva et al. 2008b).

Studies about the amount and distribution of rDNA sites in plants with holocentric chromosomes are scarce. Cyperaceae family possess approximately 5,500 species (Govaerts et al. 2007), but less than 1% of studied species possess rDNA as probed by FISH. These few reports do not discuss the location and amplification of rDNA based in the models, as is commonly done in organisms with monocentric chromosomes. The aim of this study was to localize the 45S and 5S rDNA sites using double-FISH in 23 Brazilian species of *Eleocharis* containing different chromosome number and size. Data were discussed to assess the karyotype diversity involving the rDNA sites and to understand the possible mechanisms that are operating in the rDNA amplification and distribution in organisms with holocentric chromosomes.

Material and Methods

Three individuals of each of 23 species of *Eleocharis* were collected in different Brazilian states (Table 1). Samples were cultivated in a greenhouse at the Laboratório de Biodiversidade e Restauração de Ecossistemas (LABRE) at Universidade Estadual de Londrina, Paraná, Brazil. Vouchers were deposited at the ICN herbarium of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (Table 1). Metaphase cells were obtained from root tips pre-treated with 2mM 8-hydroxyquinolin for 24 h, fixed in ethanol:acetic acid (3:1, v:v) for 24 h, and stored at -20 °C. Samples were

softened in 4% cellulase plus 40% pectinase (w:v) at 37 °C for 4 h, squashed in a drop of 60% acetic acid, and the cover slips were removed after freezing in liquid nitrogen.

Fluorescent in situ hybridization (FISH) was performed as described in Da Silva et al. (2008a). The 45S rDNA probe (p*Ta*71) from *Triticum aestivum* was labeled with digoxigenin-11-dUTP and the 5S rDNA probe from *Rhynchospora pubera* (Angeles Cuadrado, unpublished) was labeled with biotin-14-dATP, both by nick translation. Probes were simultaneously utilized for FISH in a mixture of 30 µL containing 100% formamide (15 µL), 50% polyethylene glycol (6 µL), 20× SSC (3 µL), 100 ng of calf thymus DNA (1 µL), 10% SDS (1 µL), and 200 ng of probes (4 µL each). The material was denatured at 90 °C for 10 min and hybridization was performed at 37 °C overnight in a humidified chamber. Post-hybridization washes were carried out with 70% stringency, using baths with SSC buffer.

The probes were simultaneously detected with avidin-FITC and anti-digoxigenin-rhodamine conjugates. The post-detection washes were performed in 4× SSC/0.2% Tween 20, all at room temperature. Slides were mounted with 25 µL of DABCO solution, composed of glycerol (90%), 1,4-diaza-bicyclo(2,2,2)-octane (2.3%), 20 mM TrisHCl, pH 8.0 (2%), 2.5 mM MgCl 2 (4%) and distilled water (1.7%), plus 4 µl of 2 µg.mL^{-1} DAPI.

All the chromosome images were acquired separately without color (grayscale) with a Leica DM 4500 B microscope coupled with a DFC 300FX camera and overlapped with red color for DAPI, white for rhodamine (45S rDNA) and greenish-yellow (5S rDNA) for FITC, using the Leica IM50 4.0 software. The images were optimized for best contrast and brightness with Adobe Photoshop CS3 version 10.0 software.

1

2 **Results**

3 Twenty-three species were analyzed (among these, 15 belonged to the subgenus
4 *Eleocharis*, two to *Scirpidium* and six to *Limnochloa*) according to González-Elizondo
5 and Peterson (1997) (Table 1). The number of 45S rDNA sites varied from two to ten
6 independent of chromosome numbers and subgenus. In contrast, terminal location of
7 45S rDNA was constant in all species. The 5S rDNA probe showed a minor variation
8 when compared to 45S rDNA, ranging from two to four chromosome sites which were
9 terminal in 19 species and interstitial in four others (Table 1). The rDNA hybridization
10 signals also varied in size among homologous, as observed in *E. niederleinii* (Fig. 1c),
11 *E. montana* (Fig. 1g), *E. debilis* (Fig. 2f) and *E. obtusetrigona* (Fig. 3b). In all cases, the
12 45S rDNA appeared diffused/decondensed and different of the 5S rDNA probed, even
13 when they were located in the terminal region.

14 The representatives of subgenus *Eleocharis* showed no correspondence between
15 polyploidy level and rDNA site numbers. *Eleocharis flavescens*, with 2*n* = 10, exhibited
16 signals of 45S rDNA in all chromosomes of the complement and only one interstitial 5S
17 rDNA signals located in pair 1 (Fig. 1k), while *E. nana* with 2*n* = 20, showed two
18 terminal signals of each of 45S and 5S rDNA (Fig. 1b). Two interstitial signals of 5S
19 rDNA were also found in *E. subarticulata* (2*n* = 6), *E. sellowiana* and *E. geniculata*,
20 but, in these latter two species, karyotypes with 2*n* = 10 and 20 were found. These
21 species also showed variations in the number of terminal 45S rDNA sites, being four in
22 *E. subarticulata* (Fig. 1f), in the diploid and polyploid of *E. geniculata* (Figs. 2c and d),
23 and in the diploid form of *E. sellowiana* and eight in the polyploid form of *E.*
24 *sellowiana* (Figs. 2a and b). *Eleocharis contracta* (Fig. 1a), *E. niederleinii* (Fig. 1c), *E.*
25 *loefgreniana* (Fig. 1d), *E. viridans* (Fig. 1e), *E. montana* (Fig. 1g), *E. minima* (Fig. 1h),

1 *E. filiculmis* (Fig. 1i), *E. capillacea* (Fig. 1j), *E. maculosa* (Fig. 1l) and *E. debilis* (Fig.
2 2f) exhibited distinct chromosome numbers but equal rDNA site numbers of rDNA
3 sites, being four signals to 45S rDNA and two to 5S, always in terminal position (Table
4 1).

5 The two species of the subgenus *Scirpidium* varied in number and position of
6 rDNA sites. *Eleocharis bonarienesis* with 2*n* = 20 showed four 45S rDNA sites and
7 four signals of 5S rDNA, always in terminal positions (Fig. 2g), while *Eleocharis* sp.,
8 with 2*n* = 10 (a new species cited in Da Silva et al. 2010, but yet undetermined) showed
9 six chromosomes with 45S rDNA signals and another two with 5S rDNA, also always
10 in terminal positions (Fig. 2e).

11 Six species of the subgenus *Limnochloa* were studied. These species showed
12 different chromosome number (40, 50, 52, 54 and 60) and exhibited the smallest
13 chromosomes of the genus. Four 5S rDNA terminal sites were found in all species but
14 the number of terminal 45S rDNA sites varied. *Eleocharis obtusetrigona*, *E. acutangula*
15 and *E. interstincta* presented eight hybridization signals (Figs. 3b, d and e), while *E.*
16 *plicarhachis*, *E. liesneri* and *E. laeviglumis* showed 10 sites (Figs. 3a, c and f).

17

18 **Discussion**

19 Cyperaceae is marked by a high variability in chromosome number (Roalson
20 2008), provoked mainly by events of symploidy (fusion), agmatoploidy (fission) and
21 polyploidy (Håkanson 1958; Vanzela et al. 2000; Yano et al. 2004; Hipp et al. 2009).
22 The basic chromosome number, *x* = 5, proposed by Löve et al. (1957) for Cyperaceae is
23 accepted by several authors (Vanzela et al. 1998; Yano et al. 2004; Da Silva et al.
24 2008a), even considering the high numeric variability. In the context of 45S rDNA sites,
25 it would be natural to expect that the karyotypes with few chromosomes would also

1 present few rDNA sites. However, we found species with 2*n* = 10 with four signals (the
2 majority of species), six signals (*Eleocharis* sp.) and signals in all chromosomes, such
3 as in *E. flavescens*. Multiple hybridization sites were previously reported to *Eleocharis*
4 *flavescens* (Da Silva et al. 2008a), as well as in some species of *Rhynchospora* (Vanzela
5 et al. 1998) and *Drosera* (Hoshi 1995).

6 In this sense, the polyploids should have the double the number of sites in
7 relation to their diploid representatives, as reported in *Rhynchospora tenuis* with 2*n* = 4
8 and 2*n* = 8, containing two and four 45S rDNA sites, respectively (Vanzela et al. 2003).
9 This was also observed in *E. sellowiana* (with four and eight 45S rDNA signals). On the
10 other hand, *Eleocharis geniculata* exhibited the same number of sites in both 2*n* = 10
11 and 2*n* = 20. The remainding polyploid species with 2*n* = 20, 30 and 40 showed two and
12 four 45S rDNA signals, as did some diploid species (see Table 1). Fissions could
13 explain the relationship between the reduction of 45S rDNA sites number and increase
14 of chromosome number, but, there is no a conclusive evidence of such events in these
15 mentioned cases. Thus, the most likely explanation would a loss of sites due to the
16 diploidization process, as proposed by Leitch and Bennett (1997). Similar events were
17 reported in *Passiflora* (De Melo and Guerra 2003).

18 Species of subgenus *Limnochloa* presented karyotypes with many and small
19 chromosomes and little variation in size among then. Yano et al. (2004) and Da Silva et
20 al. (2008a) have proposed that these karyotypes may have been generated by
21 chromosome fission, but it was not confirmed in the later study of Da Silva et al.
22 (2010). We found here that karyotypes of *Limnochloa* had multiple 45S rDNA sites
23 (eight and ten). When we compared the FISH data in relation to karyotype organization,
24 as chromosome number and symmetry (see Da Silva et al. 2010), regardless of
25 systematic arrangement, it was possible to detect that the species with more symmetrical

1 karyotype, as in *E. interstincta* (*Limnochloa*), *Eleocharis* sp. (*Scirpidium*), *E. flavescens*
2 and *E. sellowiana* (both of subgenus *Eleocharis*), exhibited a greater number of 45S
3 rDNA sites. The karyotypes with a greater difference in chromosome sizes, i.e. more
4 asymmetric, as *E. montana*, *E. nana* and *E. viridians* (all of subgenus *Eleocharis*),
5 showed lower 45S rDNA sites.

6 Cases of multiple 45S rDNA sites have been described in both plants with
7 holocentric chromosomes (Vanzela et al. 1998) and in plants with monocentric ones
8 (Hasterok and Maluszynska 2000; Lim et al. 2001; Kwon and Kim 2009). Some
9 mechanisms of amplification/dispersion of repetitive DNA have been proposed or
10 mentioned for the monocentric system: i) rDNA sites mobility is associated with co-
11 location and activity of transposable elements (Shubert and Wobus 1985; Raskina et al.
12 2008) and ii) equilocal dispersion of heterochromatin and rDNA (Schweizer and Loidl
13 1987; Pederson and Linde-Laursen 1994, respectively). In relation to first model, there
14 are no reports in the literature involving the distribution of transposable elements in
15 plant holocentric chromosomes. For the second model, however, Guerra (2000)
16 interpreted that the equidistribution of repetitive DNA may depend on some structural
17 or functional similarity of each chromosomal region. In this sense the polarization of
18 rDNA sites observed in the interphase some *Rhynchospora* species might be due to an
19 association of active sites building a single nucleolus (Vanzela et al. 1998). Similarly,
20 the grouping of 45S rDNA regions depending on the NOR activity could contribute to
21 the accumulation/dispersion of 45S rDNA segments to the chromosome ends with
22 similar size. This could explain the occurrence of multiple rDNA sites preferably in
23 karyotypes whose chromosomes have the same size (symmetrical). Idiograms and
24 chromosome measurements showing the karyotype symmetry/asymmetry are detailed in
25 Da Silva et al (2010).

1 The 5S rDNA probe hybridized in different regions of the 45S rDNA sites. The
2 occurrence of 45S (terminal) and 5S rDNA (interstitial) sites in the same chromosome
3 pair was found in *E. flavescens*, precisely because the 45S rDNA sites were distributed
4 in the terminal regions of all chromosomes. The 5S hybridization signals were also
5 detected in interstitial positions in *E. sellowiana*, *E. geniculata*, *E. subarticulata* and *E.*
6 *maculosa*, but, in last two species the karyotypes with $2n = 6$ and $2n = 8$, 7 and 6 were
7 originated after chromosome rearrangements (Da Silva et al. 2005, 2008, respectively),
8 and could support a reasonable explanation for the interstitial positioning of 5S rDNA.
9 The scientific advance of this article was the comparative analysis in a large
10 number of species, i. e. about 30% of the Brazilian *Eleocharis*, with representatives of
11 three subgenera. Our study showed that the 45S rDNA is more variable in sites number
12 than 5S, but it could not state why the 45S sites never appeared in interstitial positions,
13 as occurred with the 5S, since rearrangements are very common in the karyotype
14 differentiation of Cyperaceae. Besides, it was unclear why the events that favor the
15 multiplication and dispersion of 45S rDNA did not act in the same direction for 5S
16 rDNA segments. In the future, efforts should be directed towards the investigation of
17 the following aspects: (i) relationships between the location of 45S and 5S rDNA sites
18 in other Cyperaceae groups, (ii) functionality of rDNA sites and (iii) relationships
19 between rDNA segments detected by FISH, pseudogenes and transposable elements.
20
21 **Acknowledgements**
22 The authors would like to thank the Brazilian agencies Fundação Araucária and CNPq
23 by financial support.
24

References

Berjano R, Roa F, Talavera S, Guerra M (2009) Cytotaxonomy of diploid and polyploid *Aristolochia* (Aristolochiaceae) species based on the distribution of CMA/DAPI bands and 5S and 45S rDNA sites. Plant Syst Evol 280: 219-227. doi: 10.1007/s00606-009-0184-6

Bokhari FS, Godward MBE (1980) The ultrastructure of the diffuse kinetochore in *Luzula nivea*. Chromosoma 79: 125-136. doi: 10.1007/BF00328478

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2005) Reduction of chromosome number in *Eleocharis subarticulata* (Cyperaceae) by multiple translocation. Bot J Linn Soc 149: 457-464. doi: 10.1111/j.1095-8339.2005.00449.x

Da Silva CRM, González-Elizondo MS, Rego LNAA, Torezan JMD, Vanzela ALL (2008a) Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis*. Aust J Bot 56: 82-90. doi:10.1071/BT070170067-1924/08/010082

Da Silva CRM, González-Elizondo MS, Vanzela ALL (2008b) Chromosome Reduction in *Eleocharis maculosa* (Cyperaceae). Cytogenetic and Genome Res 122: 175-180. doi: 10.1159/000163096

Da Silva CRM, Trevisan R, González-Elizondo MS, Ferreira JM, Vanzela ALL (2010) Karyotypic diversification and its contribution to the taxonomy of *Eleocharis* (Cyperaceae) from Brazil. Aust J Bot 58: 1-12. doi: 10.1071/BT091850067-1924/10/010001

De Melo NF, Guerra MS (2003) Variability of the 5S and 45S rDNA sites in *Passiflora* L. species with distinct base chromosome numbers. Ann Bot 92: 309-316. doi: 10.1093/aob/mcg138

1 Flach M (1966) Diffuse centromeres in a dicotyledonous plant. Nature 209: 1369-1370.
2 doi: 10.1038/2091369b0
3 Furuta T, Kondo K (1999) Sites of 18S-5.8S-26S rDNA sequences in diffused-
4 centromeric chromosomes of *Drosera falconerii*. Chromosome Sci 3: 69-73. doi:
5 00A0408029
6 García MA (2001) A new western Mediterranean species of *Cuscuta* (Convolvulaceae)
7 confirms the presence of holocentric chromosomes in subgenus *Cuscuta*. Bot J
8 Linn Soc 135: 169-178. doi: 10.1111/j.1095-8339.2001.tb01089.x
9 González-Elizondo MS, Peterson PM (1997) A classification of and key to the
10 supraspecific taxa in *Eleocharis* (Cyperaceae). Taxon 46: 433-449. doi:
11 10.2307/1224386
12 Govaerts R, Simpson DA, Bruhl JJ, Egorova T, Goetghebeur P, Wilson K (2007) World
13 checklist of Cyperaceae, Sedges. Kew: Kew Publishing.
14 Guerra MS (2000) Patterns of heterochromatin distribution in plant chromosomes.
15 Genet. Mol. Biol. 23: 1029-1041. doi: 10.1590/S1415-47572000000400049
16 Guerra MS, García M (2004) Heterochromatin and rDNA sites distribution in the
17 holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae).
18 Genome. 47: 134-140. doi: 10.1139/G03-098.
19 Håkansson A (1958) Holocentric chromosomes in *Eleocharis*. Hereditas 44: 531-540.
20 Hasterok R, Maluszynska J (2000) Cytogenetic markers of *Brassica napus* L.
21 chromosome. J. Appl. Genet. 41: 1-9.
22 Hipp AL, Rothrock PE, Roalson EH (2009) The evolution of chromosome
23 arrangements in *Carex* (Cyperaceae). Bot Rev 75: 96-109. doi: 10.1007/s12229-
24 008-9022-8

1 Hoshi Y (1995) Chromosome studies in *Drosera*, the Droseraceae in connection with
2 possible origin of the basic chromosome number of x = 10 weel-differentiated in
3 the North-hemisphere. Dissertation. Hiroshima University.
4 Kwon J, Kim B (2009) Localization of 5S and 25S rRNA genes on somatic and meiotic
5 chromosomes in *Capsicum* species of chili pepper. Mol. Cells 27: 205-209.
6 doi:10.1007/s10059-009-0025-z
7 Leitch IJ, Bennett MD (1997) Polyploidy in angiosperms. Trend in Plant Science 2:
8 470-476. doi: 10.1016/S1360-1385(97)01154-0
9 Lim K, Wennekes J, Jong JH, Jacobsen E, Tuyl JM (2001) Karyotype analysis of *Lilium*
10 *longiflorum* and *Lilium rubellum* by chromosome banding and fluorescence in situ
11 hybridization. Genome 44: 911-918. doi:10.1139/gen-44-5-911
12 Löve A, Löve D, Raymond M (1957) Cytotaxonomy of *Carex* section *Cappilares*. Can
13 J Bot 35: 715-761.
14 Moscone EA, Klein F, Lambrou M, Fuchs J, Schweizer D (1999) Quantitative
15 karyotyping and dual-color FISH mapping of 5S and 18S-25S rDNA probes in the
16 cultivated *Phaseolus* species (Leguminosae). Genome 42: 1224-1233. doi:
17 10.1139/gen-42-6-1224
18 Pedersen C, Linde-Laursen I (1994) Chromosomal locations of four minor rDNA loci
19 and a marker microsatellite sequence in barley. Chrom Res 2: 65-71. doi:
20 10.1007/BF01539456
21 Raskina O, Barber JC, Nevo E, Belyayev A (2008) Repetitive DNA and chromosomal
22 rearrangements: speciation-related events in plant genomes. Cytogenet Genome
23 Res 120: 351-357. doi: 10.1159/000121084

1 Rego LNAA, Da Silva CRM, Torezan JMD, Gaeta ML, Vanzela ALL (2009)
2 Cytotaxonomical study in Brazilian species of *Solanum*, *Lycianthes* and *Vassobia*
3 (Solanaceae). Plant Syst Evol 279: 93-102. doi: 10.1007/s00606-009-0149-9
4 Roalson EH (2008) A synopsis of chromosome number variation in the Cyperaceae. Bot
5 Rev 74: 209-393. doi: 10.1007/s12229-008-9011-y
6 Schweizer D, Loidl J (1987) A model for heterochromatin dispersion and the evolution
7 of C-banded patterns. Chromosom T 9: 61-74.
8 Sheikh SA, Kondo K, Hoshi Y (1995) Study on diffused centromeric nature of *Drosera*
9 chromosomes. Cytologia 60: 43-47.
10 Schubert I, Wobus U (1985) In situ hybridization confirms jumping nucleolus
11 organizing regions in *Allium*. Chromosoma 92: 143-148. doi: 10.1007/BF00328466
12 Tanaka N, Tanaka N (1977) Chromosome studies in *Chionographis* (Liliaceae) I. On
13 the holocentric nature of chromosomes in *Chionographis japonica* Maxim.
14 Cytologia 42: 753-763.
15 Vanzela ALL, Cuadrado A, Jouve N, Luceño M, Guerra M (1998) Multiple locations of
16 the rDNA sites in holocentric chromosomes of *Rhynchospora* (*Cyperaceae*).
17 Chromosome Res 6: 345-349. doi: 10.1023/A:1009279912631
18 Vanzela ALL, Luceño M, Guerra M (2000) Karyotype evolution and cytotaxonomy in
19 Brazilian species of *Rhynchospora* Vahl (Cyperaceae). Bot J Linn Soc 134: 557-
20 566. doi: 10.1006/bojl.2000.0352
21 Vanzela ALL, Cuadrado A, Guerra M (2003) Localization of 45S rDNA and telomeric
22 sites on holocentric chromosomes of *Rhynchospora tenuis* Link (Cyperaceae). Gen
23 Mo Biol 26: 199-201. doi: 10.1590/S1415-47572003000200014

1 Yano O, Katsuyama T, Tsubota H, Hoshino T (2004) Molecular phylogeny of Japanese
2 *Eleocharis* (Cyperaceae) based on ITS sequence data, and chromosomal evolution.
3 J Plant Res 117: 409-419. doi: 10.1007/s10265-004-0173-3.

4

5 **Legends**

6 **Figure 1.** Metaphases of *Eleocharis* species double-probed with 45S rDNA (white) and
7 5S rDNA (greenish-yellow) probes. a) *E. contracta* (2*n* = 20) showing four
8 hybridization signals of 45S rDNA and two of 5S. b) *E. nana* (2*n* = 20) with two signals
9 of each 45S and 5S rDNA. c) *E. niederleinii* (2*n* = 20) with four 45S rDNA signals and
10 two for 5S. d) *E. loefgreniana* (2*n* = 20). e) *E. viridans* (2*n* = 20). f) *E. subarticulata* (2*n*
11 = 6). g) *E. montana* (2*n* = 40). h) *E. minima* (2*n* = 20). i) *E. filiculmis* (2*n* = 30). j) *E.*
12 *capillacea* (2*n* = 10). d, e, g, h, i, exhibited four 45S rDNA and two 5S rDNA signals in
13 terminal positions. f) *E. subarticulata*, exhibited 5S signal in interstitial positions. k) *E.*
14 *flavescens* (2*n* = 10) with ten 45S rDNA signals and two interstitial 5S signals. l) *E.*
15 *maculosa* (2*n* = 10) with four 45S rDNA and two 5S. Bar represents 10 µm.

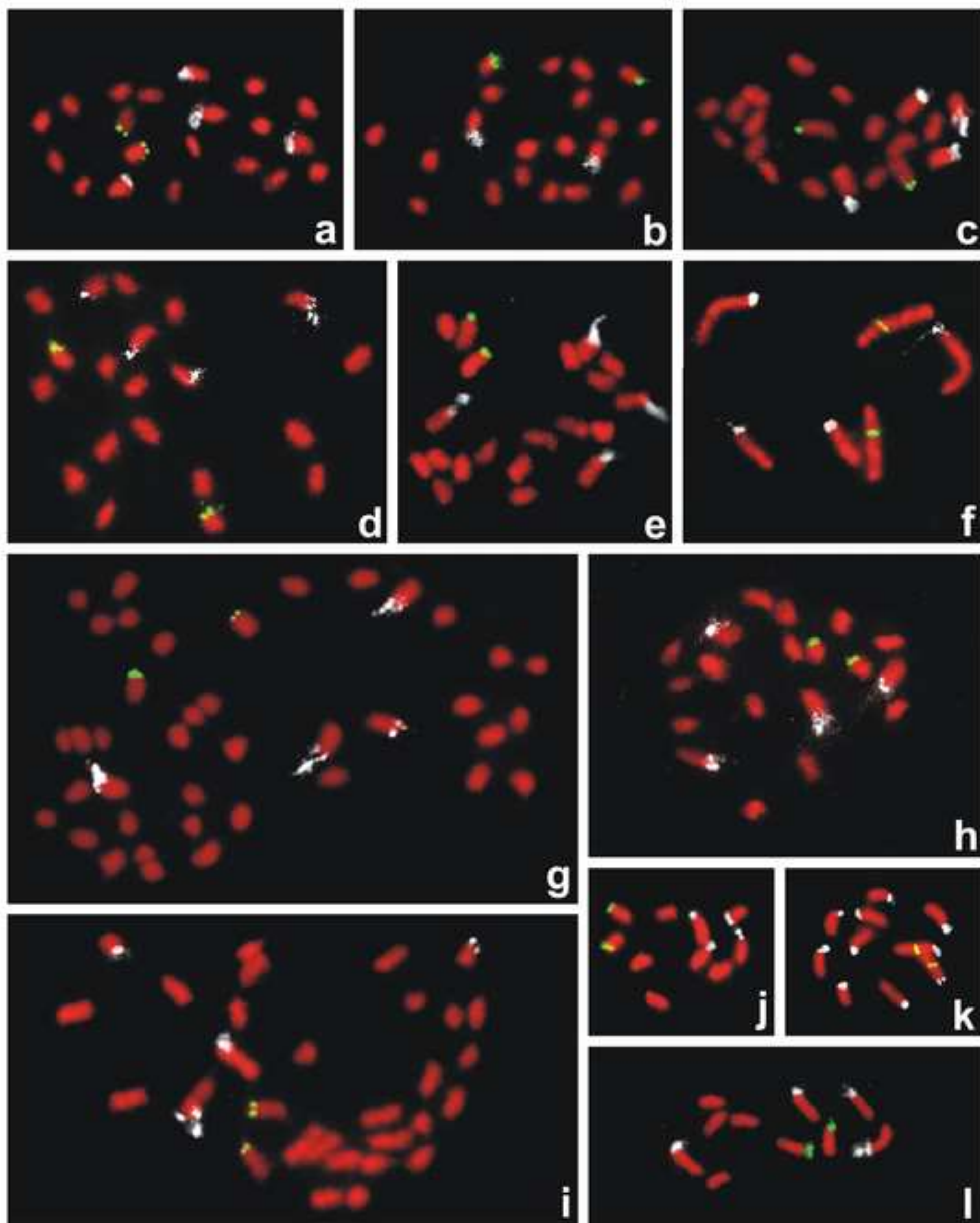
16

17 **Figure 2**. Metaphases of *Eleocharis* species double-probed with 45S rDNA (white) and
18 5S rDNA (greenish-yellow) probes. a) *E. sellowiana* (2*n* = 10) with four 45S rDNA
19 signals and two interstitial 5S signals. b) *E. sellowiana* (2*n* = 20) with eight 45S rDNA
20 signals and two interstitial 5S signals. c and d) *E. geniculata* (2*n* = 10 and 20) both with
21 four 45S rDNA signals and two interstitial 5S signals, respectively. e) *Eleocharis* sp.
22 (2*n* = 10) showing six hybridization signals of 45S rDNA and two of 5S. f) *E. debilis*
23 (2*n* = 30) with four 45S rDNA and two 5S. g) *E. bonariensis* (2*n* = 20) with four 45S
24 rDNA and four 5S. Bar represents 10 µm.

25

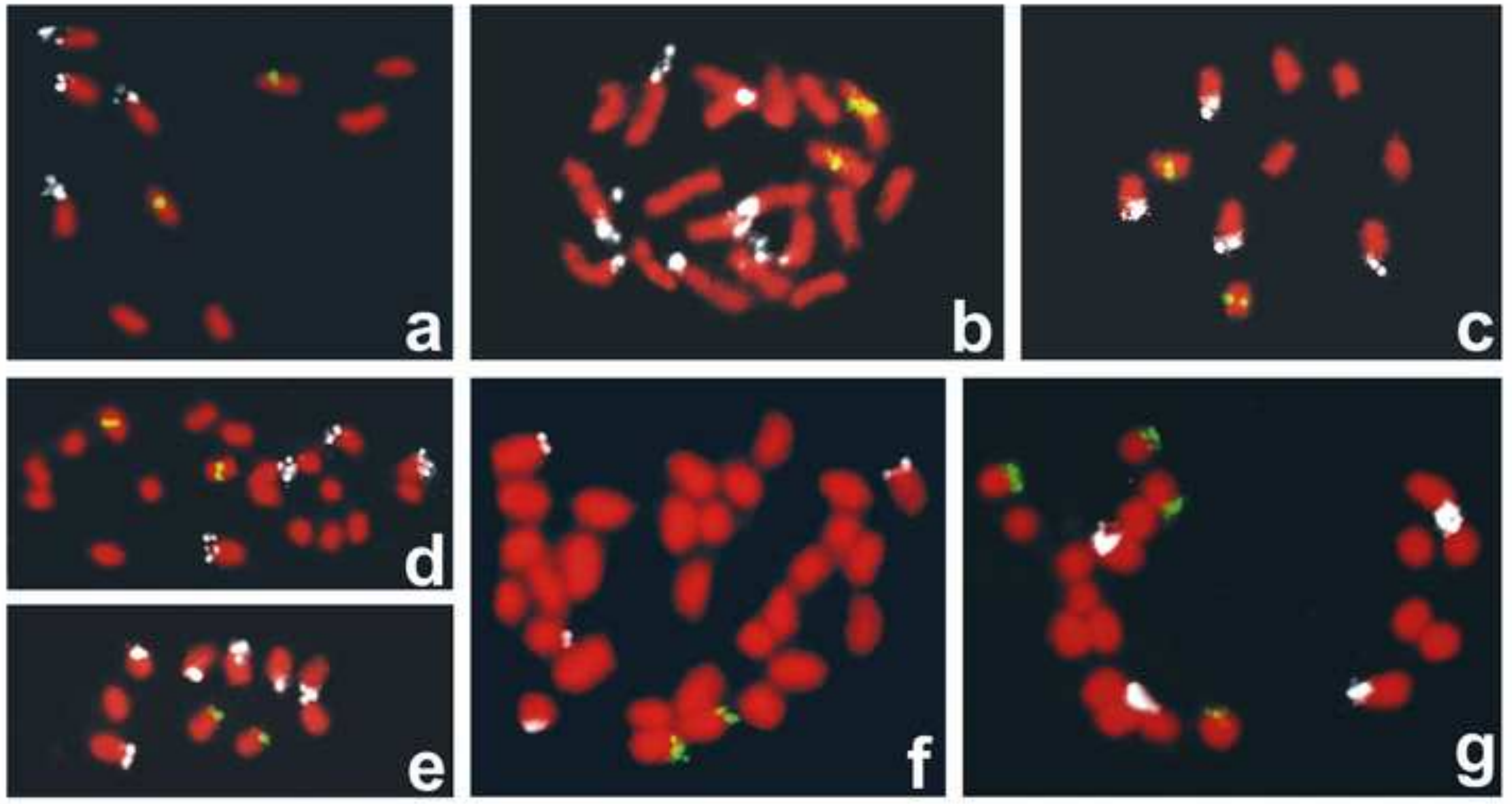
1 **Figure 3**. Metaphases of subgenus *Limnochloa* species double-probed with 45S rDNA
2 (white) and 5S rDNA (greenish-yellow) probes. a) *E. plicarhachis* (2*n* = 54) showing
3 ten hybridization signals of 45S rDNA and four of 5S. b) *E. obtusetrigona* (2*n* = 52)
4 with eight hybridization signals of 45S rDNA and four of 5S. c) *E. liesneri* (2*n* = 50)
5 with ten hybridization signals of 45S rDNA and four of 5S. d and e) *E. acutangula* (2*n*
6 = 54) and *E. interstincta* (2*n* = 40) both with eight hybridization signals of 45S rDNA
7 and four of 5S. f) *E. laeviglumis* (2*n* = 60) with ten 45S rDNA and four 5S. Bar
8 represents 10 µm.

colour figure
Click here to download high resolution image



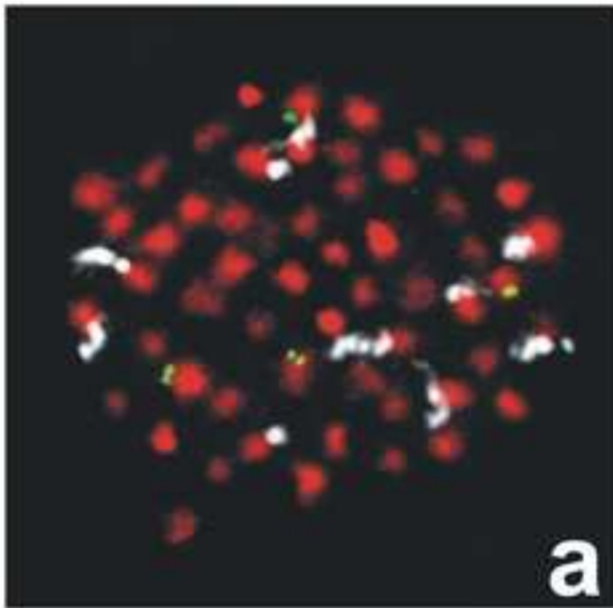
colour figure

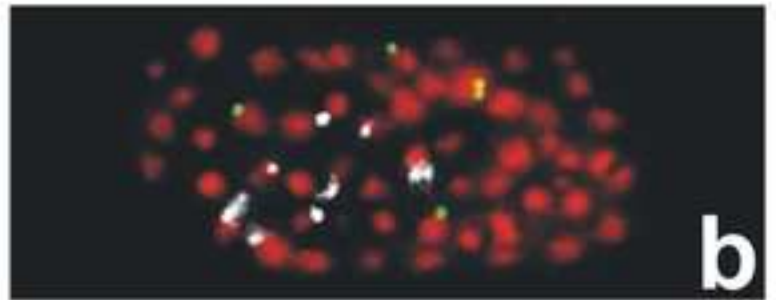
Click here to download high resolution image

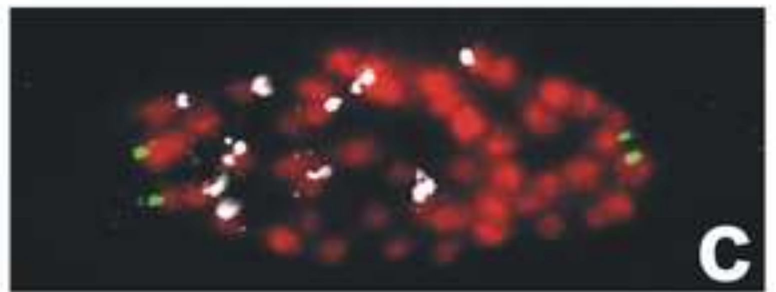


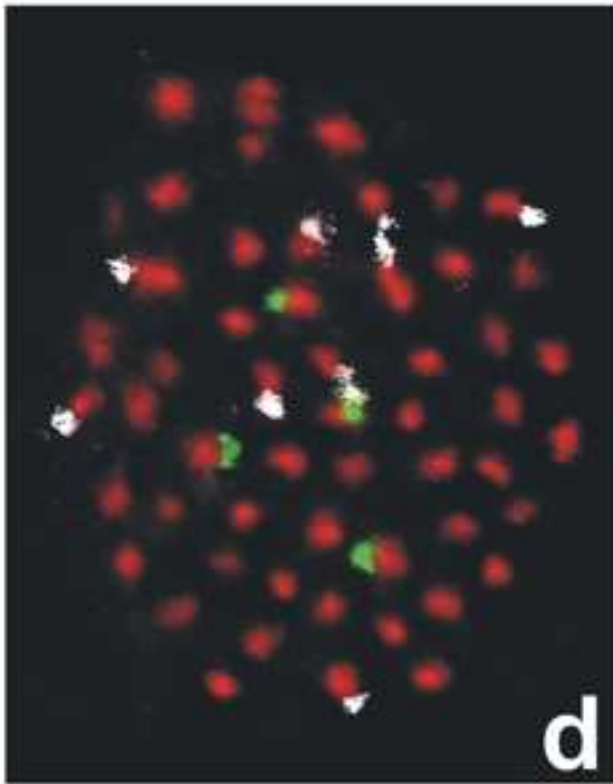
colour figure

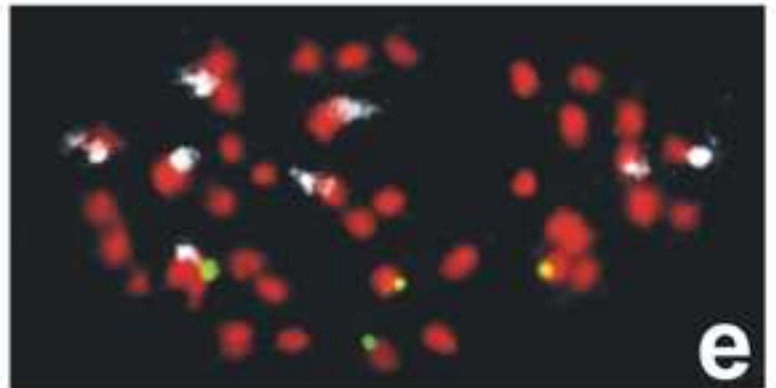
Click here to download high resolution image











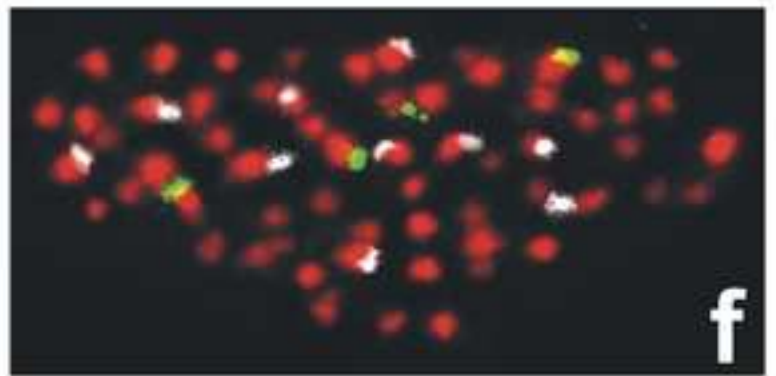


table
Click here to download table: Table 1.doc

Table 1. Amount and distribution of rDNA hybridization sites in 23 species of *Eleocharis* (Cyperaceae). Species are grouped according the classification of González-Elizondo and Peterson (1997).

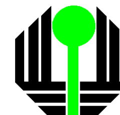
Species	2*n*	45S sites number	CP	5S sites number	CP	Coordinates, localities and voucher numbers
Subgenus *Eleocharis*						
E. contracta Maury	20	4	T	2	T	25°17'48"S 49°54'42"W, Tibagi, PR. 152096
E. nana Kunth	20	2	T	2	T	25°38'37"S 51°40'01"W, Pinhão, PR. 158383
E. loefgreniana Boeck.	20	4	T	2	T	19°13'20"S 43°33'32"W, Serra do Cipó, MG. 152659
E. niederleinii Boeck.	20	4	T	2	T	25°38'37"S 51°40'01"W, Pinhão, PR. 158380
E. minima Kunth	20	4	T	2	T	19°04'13"S 43°24'58"W, Conc. do Mato Dentro, MG. 133
E. viridans Kük ex Osten	20	4	T	2	T	30º06'18"S 50°44'33"W, Viamão, RS. 153094
E. filiculmis Kunth	30	4	T	2	T	20°34'51"S 51°36'36"W, Vestia, MS. 151969
E. montana (Kunth) Roem. & Schult.	40	4	T	2	T	23°13'73"S 51°13'71"W, Londrina, PR. 151964
E. subarticulata (Nees) Boeckl.	6	4	T	2	I	21°49'42"S 46°29'51"W, Poços de Caldas, MG. 152647
E. maculosa (Vahl) Roem & Schult	10	4	T	2	T	30°12'08"S 50°12'52"W, Balneário Pinhal, RS. 158385
E. capillacea Kunth.	10	4	T	2	T	22°15'54"S 47°55'30"W, Brotas, SP. 152642
E. flavescens (Poir.) Urban	10	10	T	2	I	25°50'53"S 48°32'50"W, Caiobá, PR. 156144
E. sellowiana Kunth.	10	4	T	2	I	25°50'53"S 48°32'50"W, Caiobá, PR. 156140
	20	8		2	I	20°34'51"S 51°36'36"W, Vestia, MS. 69
E. geniculata (L.) Roem & Schult.	10	4	T	2	I	26°16'46"S 51°03'24"W, Porto União, SC. 84
	20	4		2	I	21°45'13"S 48°59'55"W, Ibitinga, SP. 151980
E. debilis Kunth.	30	4	T	2	T	25°22'51"S 48°51'50"W, Morretes, PR. 156129
Subgenus *Scirpidium*						
E. bonariensis Nees	20	4	T	4	T	28°45'05"S 50°22'09"W, Cambará do Sul, RS. 229
Eleocharis sp.	10	6	T	2	T	25°39'59"S 51°40'05"W, Pinhão, PR. 193
Subgenus *Limnochloa*						
E. interstincta (Vahl) Roem & Schult.	40	8	T	4	T	08°39'40"S 35°09'57"W, Rio Formoso, PE. 11
E. obtusetrigona Lindl. & Nees.	52	8	T	4	T	25°36'16"S 51°40'74"W, Pinhão, PR. 354
E. liesneri S. González & Reznicek	50	10	T	4	T	21°34'08"S 45°46'77"W, Paraguaçu, MG. 152648
E. laeviglumis R. Trevis. & Boldrini	60	10	T	4	T	29°54'18"S 50°06'15"W, Imbé, RS. 219
E. acutangula (Roxb.) Schult.	54	8	T	4	T	19°43'39"S 50°13'39"W, Iturama, MG. 151977
E. plicarhachis (Griseb.) Svenson	54	10	T	4	T	19°10'55"S 57°42'47"W, Pantanal, MS. 180

T = terminal and I = interstitial rDNA positions; CP = chromosome positions

line figure
Click here to download line figure: cover letter.doc

Laboratório de Biodiversidade e Restauração de Ecossistemas
Conservação da Biodiversidade – Restauração de Ecossistemas - Genética de Plantas Nativas

Departamento de Biologia Animal e Vegetal - Departamento de Biologia Geral
Centro de Ciências Biológicas - Universidade Estadual de Londrina
Tel 43 3371 4509 - Fax 43 3371-4207 - Campus Universitário - Caixa Postal 6001 - 86.051-990-Londrina-Paraná-Brasil - http://www.uel.br/ccb/labre/



Mach 03, 2010

Dear Editor of Genetica

I am submitting the manuscript "**Distribution of 45S and 5S rDNA sites in 23 species of *Eleocharis* species (Cyperaceae)**"of Carlos Roberto Maximiano da Silva and André Luís Laforga Vanzela, to evaluation and possible publication at Genetica.

In this cover letter we cleared the following points:

1. we assured that this article was not submitted for publication in any other journal, book, conference proceedings and printed or digital circulation vehicle;
2. we assured that this study generated only this article and that similar versions were not prepared or submitted to other journals;
3. we also assured that we continued studying this theme and that other studies and articles will be accomplished in continuity to this;
4. we assured that all the consultation sources, as well as financing agency are properly mentioned, and that these do not hurt copyrights;
5. and to conclude, we assured that all of the authors have contributed substantially to the manuscript and approved the final submission.

Best regards,

Prof. Dr. André L. L. Vanzela
Laboratório de Biodiversidade e Restauração de Ecossistemas
Departamento de Biologia Geral, CCB, Universidade Estadual de Londrina
Caixa Postal 6001, CEP 86051-990, Londrina, Paraná, Brazil
Fone (Fax): +55 43 3371 4509, *E-mail:andrevanzela@uel.br*

Capítulo 4

Chromosome reduction in *Eleocharis maculosa* (Cyperaceae)

Artigo publicado na revista Cytogenet Genome Research 2008 122:175–180

Original Article

Cytogenet Genome Res 122:175–180 (2008)
DOI: 10.1159/000163096

Cytogenetic and Genome Research

Chromosome reduction in *Eleocharis maculosa* (Cyperaceae)

C.R.M. da Silva[a, b] M.S. González-Elizondo[c] A.L.L. Vanzela[a]

[a]Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, PR;
[b]Instituto de Biociências, Letras e Ciências Exatas, UNESP, São José do Rio Preto, SP (Brazil)
[c]CIIDIR, Instituto Politécnico Nacional, Durango, Dgo. (Mexico)

Accepted in revised form for publication by M. Schmid, 11 August 2008.

Abstract. Chromosome numbers in Cyperaceae lower than the typical basic number x = 5 have been described for only three species: *Rhynchospora tenuis* (n = 2), *Fimbristylis umbellaris* (n = 3) and *Eleocharis subarticulata* (n = 3). *Eleocharis maculosa* is recorded here as the fourth species of Cyperaceae that has a chromosome number lower than 2n = 10, with 2n = 8, 7 and 6. The karyotype differentiation in *E. maculosa* was studied using conventional staining (mitosis and meiosis), FISH with 45S and 5S rDNA and telomere probes. The results allow us to determine which chromosomes of the chromosome race with 2n = 10 fused to form the remaining reduced numbers, as well as to understand how the symploidy and translocation mechanisms were important in karyotype differentiation and the formation of chromosome races in *Eleocharis*.

Copyright © 2008 S. Karger AG, Basel

Some plant groups exhibit seemingly conserved karyotypes, with chromosome number, shape and size relatively constant when these are studied by conventional cytogenetics. A good example is the relative similarity among karyotypes of different species of *Lobelia,* Campanulaceae (Ruas et al., 2001), and *Solanum,* Solanaceae (Chiarini and Bernardello, 2006). However, in the family Cyperaceae, intra- and interspecific karyotype variations associated with chromosome number and size are very common. The occurrence of chromosomes with diffuse kinetochore (holocentric chromosomes) is well known in Cyperaceae. When these chromosomes are broken (agmatoploidy) or fused (symploidy) (Luceño and Guerra, 1996), they continue to be viable in the subsequent cell divisions generating different numbers for the same species. This has been demonstrated in experiments with X and γ irradiation of the chromosomes of *Eleocharis palustris* (L.) Roemer and Schultes (Håkanson, 1954) and *Rhynchospora pubera* (Vahl) Boeck (Vanzela and Colaço, 2002).

Structural rearrangements that culminate in a decrease in chromosome number have been described in different plant groups (Jones, 1998). However, few studies have demonstrated in detail the mechanisms by which such events occur. Among these few cases, we can mention the study of Ikeda (1987) who showed that n = 2 in *Haplopappus gracilis* (Nutt.) A. Gray (Asteraceae) originated by chromosome tandem fusion. Similarly, Lysak et al. (2006) showed a study of chromosome painting where a series of peri- (and para-) centric inversion, followed by reciprocal translocations and meiotic loss of the small product, caused the chromosome number reduction in *A. thaliana* and other Brassicaceae species. In Cyperaceae, numeric reduction below the more common basic number (x = 5) in the family has been reported for three species: *Fimbristylis umbellaris* (L.) Vahl with n = 3 (Rath and Patnaik, 1981), *Rhynchospora tenuis* Link with two chromosome races, n = 2 and 4 (Vanzela et al., 1996) and *Eleocharis subarticulata* (Nees) Boeck with n = 3 (da Silva et al., 2005). However, only the last two studies showed meiotic configurations compatible with reduction in chromosome number that originated by symploidy and multiple translocations, respectively.

This study was supported by the Brazilian agencies Fundação Araucária, CNPq and ProPPG-UEL.

Request reprints from André Luís Laforga Vanzela
Laboratório de Biodiversidade e Restauração de Ecossistemas
Departamento de Biologia Geral, Centro de Ciências Biológicas
Universidade Estadual de Londrina, Caixa Postal 6001
CEP 86051-990, Londrina, PR (Brazil)
telephone/fax: +55 43 3371 4509; e-mail: andrevanzela@uel.br

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2008 S. Karger AG, Basel
1424–8581/08/1222–0175$24.50/0

Accessible online at:
www.karger.com/cgr

Table 1. Localities of the analyzed populations of *Eleocharis maculosa* and karyotype features. 2n = diploid number, DSL = diploid set length, FUEL = voucher number, and GPS = global positioning system, ICN = herbarium of the Universidade Federal do Rio Grande do Sul, Brazil.

2n	Natural location in Brazil Cities, States (GPS)	DSL	FUEL
10	Matos Costa, Santa Catarina (W 51°07′52′′ – S 26°27′68′′)	20.96 μm	C.R.M. Silva & A.L.L. Vanzela 85a (ICN)
	Lebon Regis, Santa Catarina (W 50°21′51′′ – S 26°49′67′′)		C.R.M. Silva & A.L.L. Vanzela 95 (ICN)
	Viamão, Rio Grande do Sul (W 50°44′33′′ – S 30°06′18′′)		C.R.M. Silva & A.L.L. Vanzela 184 (ICN)
	Balneário Pinhal, Rio Grande do Sul (W 50°12′52′′ – S 30°12′08′′)		C.R.M. Silva & A.L.L. Vanzela 216 (ICN)
	São Luis do Purunã, Paraná (W 49°52′93′′ – S 25°28′74′′)		C.R.M. Silva & A.L.L. Vanzela 100 (ICN)
	Palmeira (BR 277), Paraná (W 50°09′49′′ – S 25°24′49′′)		C.R.M. Silva & A.L.L. Vanzela 71 (ICN)
8	Palmeira (BR 277), Paraná (W 50°09′49′′ – S 25°24′49′′)	20.95 μm	C.R.M. Silva & A.L.L. Vanzela 71a (ICN)
	Iporanga, São Paulo (W 48°43′70′′ – S 24°31′92′′)		C.R.M. Silva & A.L.L. Vanzela 76 (ICN)
7	Bom Sucesso de Itararé, São Paulo (W 49°07′95′′ – S 24°11′47′′)	20.30 μm	Gaeta M.L. & A.L.L. Vanzela 244 (ICN)
6	Curitiba, Paraná (W 49°16′40′′ – S 25°23′04′′)	20.46 μm	C.R.M. Silva 72 (ICN)
	Serra do Cipó, Minas Gerais (W 43°33′31′′ – S 19°20′28′′)		C.R.M. Silva & A.L.L. Vanzela 142 (ICN)
	Conceição do Mato Dentro, Minas Gerais (W 43°25′03′′ – S 19°02′03′′)		C.R.M. Silva & A.L.L. Vanzela 139 (ICN)
	Bom Sucesso de Itararé, São Paulo (W 49°07′95′′ – S 24°11′47′′)		Gaeta M.L. & A.L.L. Vanzela 245 (ICN)

About 60 of the more than 200 species of *Eleocharis* are found in Brazil (Trevisan and Boldrini, 2008). *Eleocharis maculosa* (Vahl) Roem. & Schult. occurs from Central America to the south of Brazil (Svenson, 1929; Trevisan and Boldrini, 2008). Chromosome counts in this species indicate the existence of four different chromosome numbers (2n = 10, 8, 7 and 6), 2n = 10 being initially described by da Silva et al. (2008). In order to understand this numeric variation, samples of 11 populations of *E. maculosa* were studied using different cytogenetic tools. The discussion shows how the findings lead to a better understanding of the evolutionary role of the symploidy in the process of karyotype differentiation in the formation of chromosome races in *E. maculosa*.

Material and methods

Representatives of *Eleocharis maculosa* were collected from eleven flooded regions in five Brazilian states (Table 1). Three to five samples of each population were cultivated in pots at the Laboratório de Biodiversidade e Restauração de Ecossistemas (LABRE) of the Universidade Estadual de Londrina (UEL) to produce new roots and anthers. Vouchers were deposited in the herbarium of the Universidade Federal do Rio Grande do Sul (ICN). For the study of somatic chromosomes, roots were pretreated with 2 mM 8-hydroxyquinoline for 24 h and fixed in absolute ethanol:glacial acetic acid (3:1, v:v) for 12 h and kept at –20 °C until used. Root tips were digested for 3 h in a mixture of 2% (w/v) cellulase and 20% (v/v) pectinase, further hydrolyzed in 1 N HCl at 60°C for 11 min, dissected in a drop of 45% acetic acid and squashed. The cover slips were removed after freezing in liquid nitrogen. The material was stained with 2% Giemsa and permanent slides mounted in Entellan. The size of the chromosomes and the length of the diploid set were measured from ten different metaphases of each population, considering each chromosome number.

For meiotic study, spikelets were dissected and the anthers immediately fixed in absolute ethanol:glacial acetic acid (3:1, v:v) for 12 h, and kept at –20°C until used. Anthers were hydrolyzed in 1 N HCl at 60°C for 5 min and squashed in a drop of 45% acetic acid. The coverslips were removed after freezing, as described above, and the samples were stained with 2% Giemsa. Slides were mounted with Entellan.

Fluorescent in situ hybridization (FISH) was performed according to Heslop-Harrison et al. (1991) and Cuadrado and Jouve (1994), with modifications. Slides were prepared with root tips pretreated as described above. Root tips were softened in 2% cellulase plus 20% pectinase at 37°C for 3 h and squashed in a drop of 45% acetic acid, without acid hydrolysis. The 45S rDNA probe from *Triticum aestivum* and the $(TTTAGGG)_n$ telomeric probe from *Arabidopsis thaliana* (Richards and Ausubel, 1988) were labeled with biotin-14-dATP and the 5S rDNA probe from *Rhynchospora pubera* (Angeles Cuadrado, unpublished) was labeled with digoxigenin-11-dUTP, both by nick translation. The probes of rDNA were simultaneously utilized for FISH and the telomeric probe was separately hybridized. For FISH, a 34 μl mixture was used composed of 100% formamide (15 μl), 50% polyethylene glycol (6 μl), 20× SSC (3 μl), 100 ng of calf thymus DNA (1 μl), 10% SDS (1 μl), and 200 ng of probes (4 μl each). The material was denatured at 90°C for 10 min and hybridization was performed at 37°C overnight in a humidified chamber. Post-hybridization washes were carried out in 2× SSC, 20% formamide in 0.1× SSC, 0.1× SSC and 4× SSC/0.2% Tween 20, all at 42°C. The probes were simultaneously detected with avidin-FITC conjugate and anti-dig-rhodamine conjugate. The post-detection washes were performed in 6× SSC and 4× SSC/0.2% Tween 20, all at room temperature. Slides were mounted with 25 μl of antifade, composed of glycerol (90%), 1,4-diaza-bicyclo(2,2,2)-octane (2.3%), 20 mM TrisHCl, pH 8.0 (2%), 2.5 mM $MgCl_2$ (4%) and distilled water (1.7%), plus 1 μl of 2 μg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI).

All the images were acquired separately without color (black and white) with a Leica DM 4500 B microscope equipped with a DFC 300FX camera and overlapped with blue colors for DAPI, green-yellowish for FITC and red for rhodamine, using the Leica IM50 4.0 software. The images were optimized for best contrast and brightness with iGrafx Image software.

Results and discussion

Morphological analysis and conventional karyotype

All individuals studied are part of a complex identified as *Eleocharis maculosa* s.l. (Svenson, 1929), belonging to the subgenus *Eleocharis,* section *Maculosae* (González-Elizondo and Peterson, 1997). Independent of the locality, individuals exhibited an apex of upper sheath membranous, rugose and hyaline without a mucron, spikelet ovoid to lanceolate, the scales ovate with scarious margins and reddish to brownish sides that become lighter at age; and obovoid, black achenes. The conventional study performed on 13

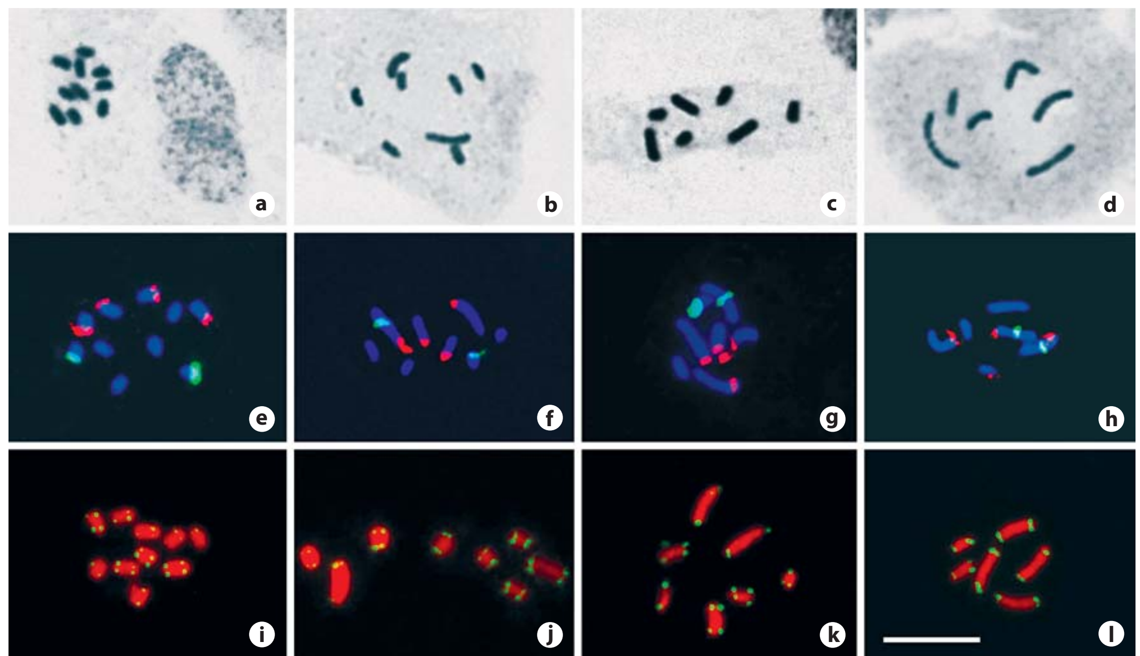


Fig. 1. Conventional staining and FISH with rDNA probes in *Eleocharis maculosa*. (**a**) Mitotic metaphase of plesiomorphic chromosome race with 2n = 10 (note the chromocentric interphase nuclei). (**b–d**) Metaphase and prometaphases in the chromosome races with 2n = 8 (**b**), 2n = 7 (**c**) and 2n = 6 (**d**). (**e–h**) Double FISH with 45S rDNA (red) and 5S rDNA (green) probes, where the chromosome races are (**e**) 2n = 10, (**f**) 2n = 8, (**g**) 2n = 7, and (**h**) 2n = 6. The chromosomes were counterstained with DAPI (blue). (**i–l**) FISH with $(TTTAGGG)_n$ probe (yellowish green), where the chromosome races are (**i**) 2n = 10, (**j**) 2n = 8, (**k**) 2n = 7, and (**l**) 2n = 6. The chromosomes were counterstained with DAPI (red). Scale bar = 10 μm.

samples of 11 populations of *E. maculosa* showed four chromosome numbers: 2n = 10, 8, 7 and 6 (Fig. 1a–d). The number 2n = 10 was recorded by da Silva et al. (2008), the other ones are described here for the first time. Given that the samples studied exhibited similar morphological features that do not enable the recognition of infraspecific taxa, we believe that the different karyotypes represent 'chromosome races' of *Eleocharis maculosa*.

The number 2n = 10 was observed in six populations, 2n = 8 in two, 2n = 7 in one and 2n = 6 in four. Most of the populations included individuals with just one chromosome number, except those of Palmeira, Paraná, with 2n = 10 and 8 and Bom Sucesso de Itararé, São Paulo, with 2n = 7 and 6 (Table 1). The karyotype with 2n = 10 was the most symmetrical, with chromosomes decreasing gradually in size, unlike 2n = 8 (two large and six smaller chromosomes), 2n = 7 (three large and four smaller) and 2n = 6 (four large and two smaller chromosomes) which were bimodals (Fig. 1a–d). In spite of differences found in the sizes of individual chromosomes among the samples, the values of diploid set sizes were similar in all the samples with 2n = 10, 8, 7 and 6 (Table 1).

Species with more than one chromosome number are common in some genera of Cyperaceae, e.g., *Carex blepharicarpa* Franchet with 2n = 26–32 and 41 (Hoshino and Okamura, 1994), *C. sociata* Boott with 2n = 40–44 (Ohkawa et al., 2000); *Rhynchospora nervosa* (Vahl) Böckeler with 2n = 20 and 30, *R. globosa* (H B K.) Roem. & Schult., 2n = 24, 37 and 48 (Luceño et al., 1998); *R. confinis* (Nees) C. B. Clarke with 2n = 10 and 20 (Vanzela et al., 2000), *Eleocharis palustris* subsp. *palustris* (L.) Roem. et Schult., 2n = 15 and 16 and *E. palustris* subsp. *vulgaris* Walters, 2n = 36, 38, 39 and 42 (Strandhede, 1965), and *Eleocharis kamtschatica* (C. A. Mey.) Kom., 2n = 41–47 (Yano and Hoshino, 2006). Several of these studies point out that symploidy and agmatoploidy are responsible for the numeric variations. However, it has not been clearly shown which chromosomes are involved in these rearrangements. The plants studied here showed similar morphological features and size of diploid complement, and so the differences in the chromosome numbers could have originated by fusion of holocentric chromosomes, or symploidy, as proposed by Luceño and Guerra (1996). This event was responsible for the reduction in chromosome number in *Rhynchospora tenuis* Link from the probable basic number x = 5 (Vanzela et al., 1996).

FISH and meiotic analysis

The samples studied here showed the same number of rDNA hybridization signals, being always four signals of 45S rDNA and two signals of 5S rDNA. The karyotypes with 2n = 10 exhibited only terminal signals, while in the karyotypes with 2n = 8, 7 and 6, 45S rDNA signals were always terminal (red color) and 5S rDNA signals were terminal and interstitial (green color) (Fig. 1e–h). Meiotic and FISH anal-

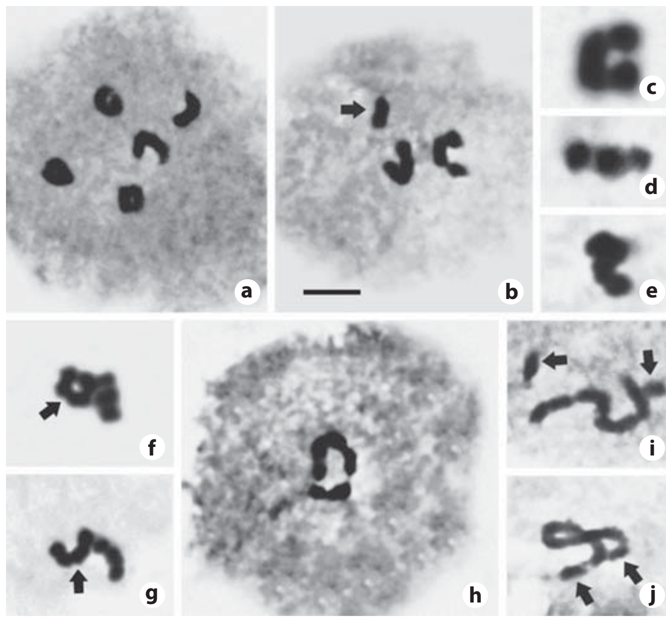


Fig. 2. Meiosis in *Eleocharis maculosa*. (**a**) Metaphase I with five bivalents in the chromosome race with 2n = 10. (**b**) Chromosome race with 2n = 8 showing one bivalent (arrow) and two heteromorphic trivalents in metaphase I. (**c–e**) Trivalents of chromosome race with 2n = 8 in a larger magnification. The smaller chromosomes on the same side of the larger (**c**), the smaller ones at both ends (**d**) and one small on each side of the larger chromosome (**e**). (**f** and **g**) Chromosome race with 2n = 7 showing one tetravalent (arrows) and one trivalent in chain. Arrows point out a ring tetravalent (**f**) and a chain tetravalent (**g**). (**h–j**) Chromosome race with 2n = 6 showing one hexavalent structure (**h**) and a linear hexavalent (**i** and **j**). The arrows point out two small chromosomes located at the ends in (**i**) and (**j**). Scale bar = 5 μm.

yses allowed us to propose the pathways in which the chromosome reduction occurred from one plesiomorphic karyotype (2n = 10) with terminal hybridization sites to different karyotypes with interstitial and terminal sites of rDNA. Meiotic analysis performed in six samples with 2n = 10 showed for all cases the presence of five bivalents at metaphase I (Fig. 2a), comprising a regular meiosis typical of a plesiomorphic karyotype in the context of *Eleocharis maculosa*. However, the samples with reduced chromosome numbers displayed different configurations in the first phase of meiosis.

The chromosome race with 2n = 8: In the two samples with 2n = 8, the formation of one bivalent was observed, composed probably of two smaller chromosomes, and two different heteromorphic trivalents, each one composed of a larger chromosome pairing with two smaller chromosomes (Fig. 2b). The trivalents appeared arranged in at least three different forms: (i) the largest chromosome with two smaller on the same side (Fig. 2c), (ii) linear with the smaller ones at both tips (Fig. 2d), and (iii) a small chromosome on each side of the larger one (Fig. 2e). FISH with rDNA probes revealed that one of the largest chromosomes had two hybridization signals. One of these was terminal (45S rDNA) and the other was interstitial (5S rDNA). According to our chromosome measurements, the largest chromosome can be the result of the fusion of a homolog of pair 1 with a homolog of pair 3 (see Fig. 3). Due to this association, these heterologous chromosomes appear as a heteromorphic trivalent at meiosis (see Fig. 2b). The second largest chromosome showed only one terminal signal with the 45S rDNA probe. This might have been the result of the fusion of a homolog of pair 2 probably with a homolog of pair 5, which is suggested also by the chromosome measurements (see Fig. 3). In this case, this association appears also as a heteromorphic trivalent at meiosis (see Fig. 2b). Three other chromosomes with similar sizes exhibited terminal signals, two with the 45S rDNA and one with the 5S rDNA probe. The other three smallest do not show hybridization signals (Figs. 1f, 3). Finally, the only bivalent may be formed by two chromosomes of pair 4 (Fig. 3).

The chromosome race with 2n = 7: FISH analysis of mitotic chromosomes with rDNA probes showed that the three largest chromosomes exhibited only one hybridization signal each. Two chromosomes showed terminal signals with the 45S rDNA probe and the other chromosome showed one interstitial signal with the 5S rDNA probe. Three of the four other smaller chromosomes displayed a terminal signal, where two were hybridized by the 45S rDNA probe and one by the 5S rDNA probe (Fig. 1g). According to our chromosome measurements, the largest chromosome is the result of the fusion of a homolog of pair 1 with a homolog of pair 4. The second largest chromosome may be the result of the fusion of a homolog of pair 2 probably with a homolog of pair 4. Similarly, the third largest chromosome can be the result of the fusion of a homolog of pair 3 with probably a homolog of pair 5. The remaining smaller chromosomes, exhibited terminal signals, two with 45S rDNA and one with 5S rDNA, except for one without signals (Fig. 3c). Mei-

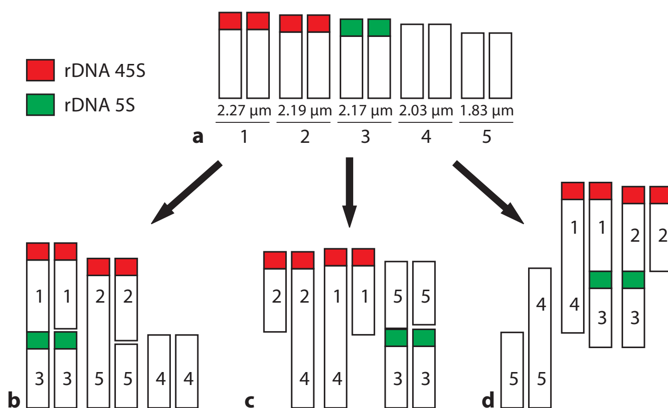


Fig. 3. Idiograms showing the symploidy pathways to formation of chromosome races in *Eleocharis maculosa*. The chromosome arrangement in the idiogram follows possible meiotic pairing. (**a**) Chromosome races with 2n = 10. The μm values correspond to the average of the size of each pair. (**b–d**) Chromosome race with 2n = 8, 7 and 6, respectively.

otic analysis done on this chromosome race always showed one tetravalent and one trivalent, both heteromorphic. The tetravalent appears forming a ring structure or in a chain, and the trivalent always appears in a chain (Fig. 2f, g). The tetravalent configuration can be a result of the pairing among fused chromosomes 1/4 and 2/4, besides the homologous chromosomes 1 and 2, which are not fused. The trivalent was composed of fused chromosome 3/5 associated with its non-fused homologs, as suggested also by chromosome measurements (Fig. 3). However, we cannot reject the possibility of translocations, because ring structures were found, as observed in *E. subarticulata* (da Silva et al., 2005).

The chromosome race with 2n = 6: Meiotic analysis of the samples with 2n = 6 showed that all chromosomes appeared as a single multivalent structure. In most cases the chromosomes formed a hexavalent structure, but not in a ring form (Fig. 2h–j). Da Silva et al. (2005) observed one hexavalent ring in *Eleocharis subarticulata,* however, it originated by multiple translocations. In the chromosome race with 2n = 6 we found a multivalent association with two chromosomes of small size located at the extremes, but not physically associated with each other (Fig. 2i, j). FISH with rDNA probes showed that in mitotic metaphases the first and second largest chromosomes, which differed in size, each exhibited two hybridization signals.

These were terminal with the 45S rDNA probe and interstitial with the 5S rDNA probe (Fig. 1h). According to our analysis, the two homologs of pair 3, containing the 5S rDNA sites, were fused with two different heterologous chromosomes, one of pair 1 and the other of pair 2 and both containing 45S rDNA sites (Figs. 1h, 3d). Continuing the reasoning for the explanation of the formation of the hexavalent structure, the two homologs of pair 4, without rDNA sites, were also fused with two different heterologous chromosomes, one from pair 1 (containing 45S rDNA site) and the other from pair 5 without rDNA sites (Fig. 3d). Thus, the two small chromosomes located at the ends of the hexavalent structure may correspond to the chromosomes of pairs 2 and 5 that were not fused (Fig. 3d).

The use of conventional and molecular techniques allows us to identify most of the chromosomes that participated in the fusion processes, as well as the different combinations of the chromosomes giving rise to each reduced karyotype. This combination of different procedures has been useful in identifying chromosomes in other plant groups that exhibit rearrangements, e.g., *Arabidopsis* (Lysak et al., 2006) and *Brachyscome* (Adachi et al., 1997). FISH with $(TTTAGGG)_n$ probes was used here to verify if there was the maintenance of ectopic segments after process of chromosome reduction in *E. maculosa*. The absence of ectopic signals in the samples with 2n = 10, 8, 7 and 6 (Fig. 1i–l) could indicate that the chromosome races appeared from translocations with terminal breakpoints and subsequent loss of small products (with telomeres), as found in *Arabidopsis* (Lysak et al., 2006). However, breaks in chromosomes with diffuse kinetochores could originate mini or micro-chromosomes with kinetic activity and be viable in cell division (see Håkanson, 1954 and Vanzela and Colaço, 2002). But, these little chromosomes were not found in our study. We suggested that the fusion process happened due to symploidy of holocentrics, as described in *Rhynchospora tenuis* with 2n = 4 and 8 (Vanzela et al., 2003).

Interstitial telomeric sites have been shown in *Haplopappus gracilis* with 2n = 4 (Hanmoto et al., 2007) and in *Eleocharis subarticulata* with 2n = 6 (da Silva et al., 2005) due to chromosome fusion and multiple translocation processes, respectively. According to Schubert (2007) telomeric sequences may occupy interstitial positions because of translocation or inversion, and can be considered to be hot spots of chromosome breakage. Chromosome fusions without the maintenance of telomeric ectopic sites have also taken place in other plant groups with monocentric chromosomes (Cox et al., 1993; Fuchs et al., 1995). The 'minimum-interaction hypothesis' of Imai et al. (1986), elaborated in monocentric systems, states that karyotype evolution has been in large part shaped by selection to reduce the occurrence of such fitness-reducing spontaneous chromosomal mutations as reciprocal translocations. Thus, we suggested symploidy as the principal chromosome reduction mechanism in *E. maculosa*. However, we cannot discard the translocation in the chromosome races formation, since a ring configuration was found in meiosis of the race with 2n = 7.

Jones (1998) mentions that most of the suspected cases of chromosome fusion and fission are records of karyotype comparisons of small numbers of individuals. Most of the records involve groups with monocentric chromosomes, whose karyotype evolution tends to increase chromosome numbers, mainly by polyploidy, where reduction in chromosome number is rarely considered (Schubert, 2007). In the context of holocentric chromosomes, the pathways of karyotype evolution also seem to be associated with increased chromosome numbers, due to agmatoploidy and polyploidy. In *Carex,* the most studied genus of Cyperaceae, a decrease in chromosome number is not common (Hipp et al., 2008). Thus, considering that most of the chromosome numbers recorded for *Eleocharis* are equal to or larger than 2n = 10, with x = 5 as the probable basic number, the chromosome races found in *E. maculosa* can be an apomorphic feature. In conclusion, our findings reinforce the suggestion of Guerra (2008) that descending disploidy, reaching very small chromosome numbers, is more frequent in organisms with holokinetic chromosomes.

Acknowledgements

We are very grateful to Dr. Rafael Trevisan for sample identification and herbarium work and to Dr. A. Leyva for English correction and editing.

References

Adachi J, Watanabe K, Fukui K, Ohmido, N, Kosuge K: Chromosomal location and reorganization of the 45S and 5S rDNA in the *Brachyscome linneariloba* complex (Asteraceae). J Plant Res 110: 371–377 (1997).

Chiarini F, Bernardello G: Karyotype studies in South American species of *Solanum* subgen. *Leptostemonum* (Solanaceae). Plant Biol 8:486–493 (2006).

Cox AV, Bennett ST, Parokonny AS, Kenton A, Callimassia MA, Bennett MD: Comparison of a plant telomere location using a PCR-generated synthetic probe. Ann Bot 72:239–247 (1993).

Cuadrado A, Jouve N: Mapping and organization of highly-repeated DNA sequences by means of simultaneous and sequential FISH and C-banding in 5*x*-Triticale. Chromosome Res 2:231–338 (1994).

da Silva CRM, González-Elizondo MS, Vanzela ALL: Reduction of chromosome number in *Eleocharis subarticulata* (Cyperaceae) by multiple translocations. Bot J Linn Soc 149:457–464 (2005).

da Silva CRM, González-Elizondo MS, Rego LNAA, Torezan JMD, Vanzela ALL: Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis* (Cyperaceae). Aust J Bot 56:82–90 (2008).

Fuchs J, Brandes A, Schubert I: Telomere sequence localization and karyotype evolution in higher plants. Pl Syst Evol 196:227–241 (1995).

González-Elizondo MS, Peterson PM: A classification of and key to the supraspecific taxa in *Eleocharis* (Cyperaceae). Taxon 46:433–449 (1997).

Guerra M: Chromosome numbers in plant cytotaxonomy: concepts and implications. Cytogenet Genome Res 120:339–350 (2008).

Håkanson A: Meiosis and pollen mitosis in X-rayed and untreated spikelets of *Eleocharis palustris.* Hereditas 40:325–345 (1954).

Hanmoto H, Kataoka R, Ohmido N, Yoshihiko Y: Interstitial telomere-like repeats in the *Haplopappus gracilis* (Asteraceae) genome revealed by fluorescence in situ hybridization. Cytologia 72:483–488 (2007).

Heslop-Harrison JS, Schwarzacher T, Anamthawat-Jonsson K, Leitch AR, Shi M, Leitch IJ: In situ hybridization with automated chromosome denaturation. Technique 3:106–109 (1991).

Hipp AL, Rothrock PE, Roalson EH: The evolution of chromosome arrangements in *Carex* (Cyperaceae). Bot Rev, in press (2008).

Hoshino T, Okamura K: Cytological studies on meiotic configurations of intraspecific aneuploids of *Carex blepharicarpa* (Cyperaceae) in Japan. J Plant Res 107:1–8 (1994).

Ikeda H: Cytogenetic studies on the chromosome complements of *Haplopappus gracilis* (2n = 4) and *H. ravenii* (2n = 8). J Sci Hiroshima Univ B 2:67–104 (1987).

Imai HT, Maruyama T, Gojobori T, Inoue Y, Crozier RH: Theoretical bases for karyotype evolution. 1. The minimum-interaction hypothesis. Am Nat 128:900–920 (1986).

Jones K: Robertsonian fusion and centric fission in karyotype evolution of higher plants. Bot Rev 64:273–289 (1998).

Luceño M, Guerra M: Numerical variation in species exhibiting holocentric chromosomes: a nomenclatural proposal. Caryologia 49:301–309 (1996).

Luceño M, Vanzela ALL, Guerra M: Cytotaxonomic studies in Brazilian *Rhynchospora* (Cyperaceae), a genus exhibiting holocentric chromosomes. Can J Bot 76:440–449 (1998).

Lysak MA, Berr A, Pecinka A, Schmidt R, McBreen K, Schubert I: Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. Proc Natl Acad Sci USA 13:5224–5229 (2006).

Ohkawa T, Yokota M, Hoshino T: Aneuploidal population differentiation in *Carex sociata* Boott (Cyperaceae) of the Ryukyu Islands, Japan. Bot J Linn Soc 132:337–358 (2000).

Rath SP, Patnaik SN: Cytological studies in Indian species of *Fimbristylis* Vahl. (Cyperaceae) with special reference to taxonomy, in Manna GK, Sinha U (eds): Perspectives in Cytology and Genetics 3:175–181 (Hindasia Publishers, New Delhi 1981).

Richards EJ, Ausubel FM: Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53:127–136 (1988).

Ruas PM, Vanzela ALL, Vieira AOS, Bernini C, Ruas CF: Karyotype studies in Brazilian species of *Lobelia* L., subgenus *Tupa* (Campanulaceae). Revta Brasil Bot 24:249–254 (2001).

Schubert I: Chromosome evolution. Curr Opin Plant Biol 10:109–115 (2007).

Strandhede SO: Chromosome studies in *Eleocharis,* subser. *Palustres.* Opera Bot 9:1–86 (1965).

Svenson HK: Contributions from the Gray Herbarium of Harvard University. LXXXVI. Monographic studies in the genus *Eleocharis*. Rhodora 31:121–245 (1929).

Trevisan R, Boldrini II: O gênero *Eleocharis* R. Br. (Cyperaceae) no Rio Grande do Sul, Brasil. Rev Bras Biociênc 1:7–67 (2008).

Vanzela ALL, Colaço W: Mitotic and meiotic behavior of y irradiated holocentric chromosomes of *Rhynchospora pubera* (Cyperaceae). Acta Scient 24:611–614 (2002).

Vanzela ALL, Guerra M, Luceño M: *Rhynchospora tenuis* Link (Cyperaceae), a species with the lowest number of holocentric chromosomes (n = 2). Cytobios 88:219–228 (1996).

Vanzela ALL, Luceño M, Guerra M: Karyotype evolution and cytotaxonomy in Brazilian species of *Rhynchospora* Vahl (Cyperaceae). Bot J Linn Soc 134:557- 566 (2000).

Vanzela ALL, Cuadrado A, Guerra M: Localization of 45S rDNA and telomeric sites on holocentric chromosomes of *Rhynchospora tenuis* Link (Cyperaceae). Gen Mol Biol 26:199–201 (2003).

Yano O, Hoshino T: Cytological studies of aneuploidy in *Eleocharis kamtschatica* (Cyperaceae). Cytologia 71:141–147 (2006).

Capítulo 5

Análises citogenéticas e moleculares revelam um complexo de híbridos e poliploides em *Eleocharis* série *Tenuissimae* (Cyperaceae)

O artigo será submetido a revista Annals of Botany

1 **Análises citogenéticas e moleculares revelam um complexo de híbridos e**
2 **poliploides em *Eleocharis* série *Tenuissimae* (Cyperaceae)**

3 Carlos Roberto Maximiano da Silva[1], Rafael Trevisan[2], Maria Socorro González-
4 Elizondo[3], Elson Felipe Sandoli Rosseto[4], Carolina Cristina Quintas[5], Rogério
5 Fernandes de Souza[5], André Luís Laforga Vanzela[5,6]
6
7 [1]Instituto de Biociências, Letras e Ciências Exatas, UNESP, 15054-000, São José do
8 Rio Preto, SP, Brazil.
9 [2]Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil.
10 [3]CIIDIR IPN Unidad Durango, Sigma s.n. Fracc. 20 de Noviembre II, 34220 Durango,
11 Dgo., Mexico.
12 [4]Departamento de Biologia Vegetal e Animal, CCB, Universidade Estadual de
13 Londrina, 86051-970, Londrina, PR, Brazil.
14 [5]Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de
15 Biologia Geral, CCB,
16 [7]Corresponding author. Email: andrevanzela@uel.br
17

18 **Resumo**

19 O gênero *Eleocharis* R. Br. possui características morfológicas bastante simples,
20 quando comparado com as demais Cyperaceae. Isto tem ocasionado problemas de
21 identificação e classificação, os quais não têm sido resolvidos com o auxílio de
22 ferramentas moleculares. Um dos grupos mais complexos do gênero é a série
23 *Tenuissimae* (C. B. Clarke) Svenson, de origem parafilética. Esta série exibe a maior
24 diversidade morfológica no gênero, incluindo complexos de espécies intimamente
25 relacionadas, como o complexo formado por *Eleocharis viridans* e *E. niederleinii*. Para
26 compreender a organização e evolução deste complexo, foram estudadas 39 amostras
27 coletadas em diversas regiões do Brasil. As análises revelaram uma série cromossômica
28 para ambas as espécies, as quais exibiram $2n$ = 20, 29, 30, 31, 35, 40, 41 e 42. Em
29 alguns cariótipos foram encontrados de um a quatro cromossomos maiores, contendo ou

não sítios de DNAr 45S. As poucas amostras que produziram flores férteis apresentaram meiose regular, apenas com bivalentes. Os dados moleculares indicam que as populações podem estar isoladas e a GISH revelou que duas destas populações têm origem híbrida. Esses híbridos possuem reprodução exclusivamente vegetativa, o que indica a ocorrência de isolamento ativo.

Palavras-chave: Alopoliploidia, DNAr 45S, *Eleocharis*, GISH, hibridação.

1 **Introdução**

2 O gênero *Eleocharis* R. Br. possui características morfológicas bastante
3 reduzidas, quando comparado com as demais Cyperaceae (Trevisan e Boldrini 2008),
4 associadas a uma grande variação morfológica intraespecífica (Simpson 1988). Isto tem
5 ocasionado problemas de identificação e classificação, os quais vêm se mantendo
6 mesmo com o auxílio de ferramentas moleculares (Roalson e Friar 2000, Yano *et al.*
7 2004 e Roalson *et al. in press*). González-Elizondo e Peterson (1997) dividiram o
8 gênero *Eleocharis* em quatro subgêneros, seis seções, oito séries e sete subséries. Dentre
9 estes, encontra-se a série *Tenuissimae* (C. B. Clarke) Svenson, de origem para e
10 polifilética (Roalson e Friar 2000). Esta série exibe a maior diversidade morfológica e
11 distribuição no gênero, incluindo complexos de espécies intimamente relacionadas,
12 especialmente na América do Sul, indicando especiação ativa (González-Elizondo e
13 Tena-Flores 2000).

14 Estudos citogenéticos em *Eleocharis*, assim como nos outros representantes de
15 Cyperaceae, mostram que há uma grande variação cromossômica numérica, desde $2n$ =
16 6 em *E. subarticulata* (Da Silva *et al.* 2005) a $2n$ = 200 em *E. dulcis* (Roalson 2008).
17 Esta variação está associada à condição holocêntrica dos cromossomos, devido a
18 eventos de agmatoploidia, simploidia, translocações e poliploidia (Luceño *et al.* 1998,
19 Da Silva *et al.* 2005, 2008a, Hipp *et al.* 2009). Há evidências de que em algumas
20 espécies, esses eventos podem levar a algum tipo de isolamento, como em
21 *Rhynchospora tenuis*, já que intermediários entre as raças cromossômicas simpátricas
22 com $2n$ = 4 e 8 nunca foram encontrados (Vanzela *et al.* 1996). Similarmente, para *E.*
23 *maculosa* foram encontradas populações com $2n$ = 10, 8, 7 e 6 (Da Silva *et al.* 2008b),
24 cujos cariótipos surgiram a partir de $2n$ = 10, sem indícios de cruzamentos entre essas
25 raças cromossômicas.

1 Estudos prévios realizados em representantes de *E. viridans* Boeck e *E.*
2 *niederleinii* Kük. Ex Osten da série *Tenuissimae*, revelaram diferentes números
3 cromossômicos e organização cariotípica, além de uma clara sobreposição de caracteres
4 diagnósticos. De acordo com Svenson (1939), *Eleocharis viridans* e *E. niederleinii*
5 podem ser sinonímias, contudo, diferentes taxonomistas consideram *E. viridans* como
6 uma espécie bem circunscrita. Essas espécies apresentam colmos quadrangulares,
7 variando de 0,2 -1,1 μm de largura, comportamento prolífero e raras vezes formam
8 espiguetas viáveis, levando a uma escassez de caracteres diagnósticos e erros na
9 identificação. Este problema pode ser bem exemplificado em Rambo (1959) na
10 separação entre *E. viridans* e *E.* filiculmis, em Trevisan e Boldrini (2008) entre *E.*
11 *viridans* e *E. dunensis*, bem como na determinação errônea de *E. niederleinii* registrada
12 em vários herbários brasileiros (Alves *et al*. 2010). Para entender a relação entre
13 *Eleocharis viridans* e *E. niederleinii*, neste estudo foram empregadas ferramentas
14 citogenéticas, como coloração convencional, para análises meióticas e mitóticas, FISH
15 com sonda de DNAr 45S e GISH e moleculares como, o RAPD, em 39 amostras dessas
16 duas entidades taxonômicas, coletadas em 18 localidades das regiões Sul e Sudeste do
17 Brasil.
18
19 **Materiais e Métodos**
20
21 As análises morfológicas dividiram as 39 amostras coletadas, em duas espécies,
22 19 foram determinadas como *E. niederleinii* e 20 como *E. viridans* (Tabela 1). Estas
23 amostras foram mantidas em casa de vegetação do LABRE (Laboratório de
24 Biodiversidade e Restauração de Ecossistemas), do Centro de Ciências Biológicas da

1 Universidade Estadual de Londrina. As exsicatas foram depositadas no herbário da
2 Universidade Federal do Rio Grande Sul (ICN).
3
4 *Citogenética convencional*
5 Pontas de raízes foram pré-tratadas com 2 mM 8-hidroxiquinoleína por 24 h e
6 fixadas em etanol absoluto:ácido acético glacial (3:1, v:v) por 12 h e mantidas a -20 °C
7 até o uso. As pontas das raízes foram digeridas por 3 h a 37 °C em uma mistura de 4%
8 celulase e 40% pectinase (w:v), posteriormente hidrolisadas em HCl 1N a 60 °C por 10
9 min e lavadas com água destilada. As amostras foram dissecadas e esmagadas em uma
10 gota de ácido acético a 60% e coradas com Giemsa 2%. O tamanho dos cromossomos e
11 o comprimento total do cariótipo foram medidos em dez diferentes metáfases de cada
12 população com o programa MicroMeasure 3.3.
13 Para o estudo das meioses, anteras foram fixadas em etanol absoluto:ácido
14 acético glacial (3:1, v:v), por 12 h, e mantidas a -20 °C até o uso. As anteras foram
15 hidrolisadas em HCl 1N a 60 °C, por 9 min, e lavadas em água destilada.
16 Posteriormente, as amostras foram dissecadas em uma gota de ácido acético a 60% e
17 coradas com Giemsa 2%.
18
19 *Análises cito-moleculares*
20 A hibridação *in situ* fluorescente (FISH) foi feita de acordo com Heslop-
21 Harrison *et al.* (1991), com modificações. A sonda de DNAr 45S obtida de *Triticum*
22 *aestivum* (p*Ta*71) foi marcada com biotina-14-dATP por *nick translation*. Entre 100 e
23 200 ng (4 μL) da sonda foi adicionada a uma solução composta por formamida 100%
24 (15 μL), polietilenoglicol 50% (6 μL), 20× SSC (3 μL), 100 ng de DNA de timo de
25 bezerro (1 μL), SDS 10% (1 μL) e H_2O (4 μL). A sonda foi desnaturada a 70 °C por 10

1 min e a hibridação feita a 37 °C por até 24 h. Os banhos pós-hibridação foram feitos
2 com 6× SSC e 4 SSC/0,2% Tween 20, à temperatura ambiente. A sonda foi detectada
3 com avidina-FITC e os banhos pós detecção foram com 4× SSC/0,2% Tween 20 a
4 temperatura ambiente. Os cromossomos foram contra-corados com DAPI e as lâminas
5 foram montadas com 25 μL de antifade, composto por glicerol (90%), 1,4-diaza-
6 bicyclo(2,2,2)-octane (2.3%), 20 mM TrisHCl, pH 8.0 (2%) e água destilada.

7 Para a hibridação genômica *in situ* (GISH) foi utilizado o mesmo protocolo da
8 FISH, exceto pelas sondas utilizadas na hibridação, as quais foram obtidas do DNA
9 genômico extraído das amostras com $2n = 20$ de *E. viridans* (amostra 186) e *E.*
10 *niederleinii* (amostra 44). Amostras de DNA genômico das duas espécies foram
11 fragmentadas por choque de temperatura, avaliadas em gel de eletroforese a 1% e em
12 seguida marcadas com biotina-14-dATP por *nick translation*. As hibridações in situ
13 foram feitas baseadas nos resultados das análises de RAPD e do coeficiente de
14 similaridade entre os genomas de cada amostra.

15 Todas as imagens, convencionais, FISH e GISH, foram capturadas em tons de
16 cinza em um fotomicroscópio Leica DM 4500 B equipado com câmera DFC 300FX. As
17 sobreposições e colorações das imagens foram feitas no programa IM50 da Leica e o
18 controle de brilho e contraste feito com o programa IGrafix.

19

20 *Extração de DNA*

21 O DNA genômico de 39 indivíduos das duas espécies foram extraídos de acordo
22 com o método CTAB (Brasileiro e Carneiro 1998), onde cerca de 3 a 4 colmos jovens
23 foram transferidos para um almofariz contendo nitrogênio líquido e macerados até a
24 obtenção de um pó fino. Cerca de 2 mL de tampão CTAB (Tris-HCl 1M pH 8, NaCl
25 5M, EDTA 0,5M pH 8, CTAB, βmercaptoetanol e PVP 40) foi adicionado ao pó e a

solução foi transferida para um microtubo. A solução foi incubada em banho-maria a 65 °C, por 50 min, tratada com Proteinase K (100 mg/mL) a 65 °C, por mais 30 min. Após este período foi adicionado o mesmo volume de fenol:clorofórmio (1:1; v:v) e a solução foi centrifugada a 10.000 rpm, por 10 min. Após a centrifugação, a fase superior foi transferida para outro tubo e tratada com RNAse (100 mg/mL) a 37 °C por 30 min. Após este período duas lavagens com clorofórmio:álcool isoamílico (24:1; v:v) foram realizadas. A fase superior foi transferida para outro tubo e a ela foi adicionado 2,5 volumes de etanol absoluto para formar um precipitado. Este foi dissolvido em 50-100 µL de água destilada. As amostras foram quantificadas em um fluorômetro DyNA Quant200 (Hoefer-Pharmacia).

Reação de RAPD

As reações de RAPD foram realizadas em apenas 39 amostras. Para estas reações, foram testados 36 *primers*, dos quais oito do kit OPA (Operon Technologies – Alameda, CA) foram selecionados. A reação de amplificação foi feita em um total de 15 µL, contendo 1 mM dNTP, 0,2 mM de primer, 2,5 mM de $MgCl_2$ e uma unidade de enzima (DNA polimerase), 1× o Tampão de reação e 15 a 25 ng de DNA e H_2O Qsp. As amplificações foram feitas em um termociclador MJ Research PTC-100 com a seguinte programação: 92 °C, por 4 min, seguido por 40 ciclos, sendo que cada ciclo consiste em 40 seg, a 92 °C, 1 min e 30 seg, a 40 °C e 2 min a 72 °C, e uma extensão final de 5 min, a 72 °C após o último ciclo. Ao término da reação de amplificação, foi adicionado 4 µL de tampão da amostra (glicerol e azul de bromofenol) e a solução aplicada em um gel de agarose 1,4%, submerso em tampão de corrida TEB 0,5× (Tris 0,045 M, EDTA 0,5 mM e Ácido Bórico 0,045 M, pH 8,3). A eletroforese aconteceu a 3

1 W/cm, o gel foi corado com brometo de etídio por 5 minutos, exposto em
2 transiluminador ultravioleta e a imagem capturada com o programa Alpha Digi Doc RT.
3 Os perfis de RAPD foram determinados por comparação das bandas produzidas
4 para cada amostra em cada um dos oito *primers* e os dados comparados na forma de
5 variáveis binárias (1 presença e 0 ausência). Para as análises de similaridade genética
6 entre os indivíduos dos diferentes pontos foi utilizado o programa NTSYS-PC. Para a
7 construção do dendograma de similaridade genética foram empregados o coeficiente de
8 Jaccard (J) e o método de agrupamento UPGMA (*Unwreighted Pair-Group Method*
9 *with Aritimetal Average*). O programa STRUCTURE 2.2 (Pritchard *et al*. 2000; Falush
10 *et al*. 2003) foi empregado para determinar, por análise Bayesiana de agrupamento, o
11 número de entidades (K) geneticamente distintas. Neste caso, foi utilizada a
12 configuração para marcadores dominantes, conforme estabelecido por Falush *et al.*
13 (2007). Para ambas as espécies, as simulações foram realizadas com 100.000 repetições
14 pelo método Monte Carlo para a Cadeia de Markov (MCMC), após um período de
15 *burning* de 20.000 repetições. Essas simulações foram baseadas no modelo de mistura,
16 usando a opção de frequências alélicas correlacionadas, sem determinar *a priori* a
17 origem dos indivíduos. Duas metodologias foram empregadas para a escolha do número
18 mais provável de populações inferidas a partir do programa STRUCTURE 2.2. Os
19 resultados foram utilizados para direcionar as reações de hibridação genômica *in situ.*
20
21 **Resultados**
22 Todas as 39 amostras exibiram proliferação, ou seja, na extremidade do colmo,
23 novos colmos foram produzidos. Contudo, 22 delas também produziram espiguetas. As
24 amostras foram classificadas em duas espécies, sendo *E. niederleinii* aquelas que
25 apresentaram colmos de 0,2–0,4 mm de largura, bainha com 1,1–3,5 cm de

1 comprimento, ápice oblíquo e bordo inconspícuo ou com pontos vináceos. Destas, oito
2 produziram espiguetas, que tiveram 3–9 × 1–2 mm de tamanho, forma lanceolada com
3 cor castanha, 10–18 flores; gluma inferior estéril, e glumas superiores frágeis e não
4 imbricadas (Tabela 1). Foram consideradas *E. viridans* as amostras mais robustas com
5 colmos de 0,3–1mm de largura, bainha com 1,5–8 cm de comprimento e ápice oblíquo.
6 Das 20 amostras consideradas *E. viridans*, sete produziram espiguetas (Tabela 1), que
7 tiveram 6–13 × 2–3 mm de tamanho, com forma ovóide e cor castanha a vinácea, com
8 30–60 flores; gluma inferior estéril, e glumas superiores imbricadas. Estas duas
9 entidades apresentaram como características sobrepostas a ausência de apêndice
10 hialino/rugoso e múcron dorsal.

11 As análises citogenéticas mostraram uma grande variação no número
12 cromossômico de ambas as espécies. As 19 amostras de *E. niederleinii* exibiram $2n$ =
13 20, 29, 30, 31, 35 e 40 (Figura 1A – I). As 20 amostras de *E. viridans* exibiram $2n$ = 20,
14 29, 31, 40, 41 e 42 cromossomos (Figura 2 A – H). Várias amostras de ambas as
15 espécies exibiram cariótipos contendo cromossomos bem maiores que os demais, os
16 quais variaram de 3.16 a 4.74 μm (Tabela 1). O comprimento total do lote diplóide foi
17 similar para os cariótipos com o mesmo número cromossômico independente da espécie
18 (Tabela 1).

19 A análise meiótica mostrou bivalentes apenas nas amostras com $2n$ = 20 de
20 ambas as espécies (Figura 3A), $2n$ = 30 de *E. niederleinii* (Figura 3B) de $2n$ = 42 para
21 *E. viridans* (Figura 3C), em todos estes casos aquênios foram produzidos. As amostras
22 de *E. niederleinii* com $2n$ = 30 coletadas em Sapopema e Porto União (veja a Tabela 1),
23 produziram poucas inflorescências e apenas 24 meiócitos puderam ser analisados. Estes
24 exibiram diferentes configurações, com $1^{IV} + 2^{III} + 9^{II} + 2^{I}$ (Figura 3D) observados em
25 11 células, $1^{IV} + 5^{III} + 4^{II} + 3^{I}$ em quatro células, $2^{IV} + 2^{III} + 6^{II} + 3^{I}$ em três células, 1^{IV}

1 $3^{III} + 7^{II} + 3^{I}$, $1^{IV} + 3^{III} + 6^{II} + 5^{I}$ e $1^{IV} + 4^{III} + 4^{II} + 6^{I}$, encontradas duas vezes para cada
2 configuração.
3 Múltiplas configurações também foram encontradas em amostras de *E. viridans*
4 com 2*n* = 31 coletadas em Caçador/SC. Dos 15 meiócitos analisados, seis mostraram 7^{III}
5 $+ 3^{II} + 4^{I}$ (Figura 3E), quatro mostraram $1^{VI} + 1^{IV} + 5^{III} + 2^{II} + 2^{I}$ (Figura 3F), duas 1^{VI} +
6 $4^{III} + 5^{II} +3^{I}$, duas $1^{V} + 5^{III} + 4^{II} + 3^{I}$ e uma exibiu $1^{V} + 1^{IV} + 4^{III} + 3^{II} + 4^{I}$. Além das
7 meioses irregulares, diferentes números cromossômicos foram encontrados na mitose
8 polínica. Das 56 células analisadas, 26 apresentaram *n* = 15, com dois cromossomos
9 muito maiores que os demais (Figura 3 H), 15 com *n* = 17 (Figura 3I), cinco com *n* =
10 16, quatro com *n* = 15 e os números *n* = 13, *n* =14 e *n* = 15, foram verificados duas
11 vezes, e em todas estas células, um cromossomo muito maior que os demais foi
12 observado.
13 Para as amostras de *E. viridans* com 2*n* = 41 coletadas em Balneário Pinhal e
14 Capivari do Sul, foram analisadas 63 meiócitos. Destes, 38 células exibiram $1^{III} + 19^{II}$
15 (Figura 3G) e 25 células apresentaram $20^{II} + 1^{I}$. Também foram encontrados diferentes
16 números cromossômicos na mitose polínica. Das 73 células analisadas, 34 exibiram *n* =
17 22 (Figura 3J), 23 mostraram *n* = 21 (Figura 3K), *n* = 20 foi encontrada em 13 células e
18 apenas três exibiram *n* = 23. Em todas estas células sempre dois cromossomos maiores
19 foram observados.
20 A hibridação *in situ* realizada com a sonda de DNAr 45S, mostrou de quatro a
21 seis sinais, sempre terminais nas duas espécies. *Eleocharis niederleinii* mostrou quatro
22 sinais nas amostras com 2*n* = 20, 29 e 40 (Figuras 4A, B e G), e seis sítios nas amostras
23 com 2*n* = 30, 31, 35 e 40 (Figuras 4C, D, E, H e I). *Eleocharis viridans* mostrou quatro
24 sítios nas mostras com 2*n* = 20, 29 e 41 (Figuras 5A, D e H), e seis sítios nas amostras

1 com $2n$ = 29, 31, 40, 41 e 42 (Figuras 5B, C, E, F e G). Variações nos tamanhos dos
2 sinais de hibridação foram encontradas em todas as amostras estudadas.

3 O dendrograma gerado pelo método de UPGMA agrupou as amostras em dois
4 grandes grupos, considerando a similaridade de 80%, contudo, houve uma mistura de
5 espécies e localidades. O primeiro grupo foi composto por amostras do Paraná (várias
6 amostras de *E. niederleinii* e apenas um indivíduo de *E. viridans*), Santa Catarina (*E.*
7 *niederleinii* e *E. viridans*) e Rio Grande do Sul (somente *E. viridans*), e o segundo por
8 amostras de Minas Gerais (*E. niederleinii*), Rio Grande do Sul (*E. viridans*) e Paraná
9 (apenas um indivíduo de *E. niederleinii*). As amostras de uma mesma região foram
10 agrupadas com alto coeficiente de similaridade (Figura 6). O dendrograma indica que
11 em um mesmo estado as duas espécies podem ser encontradas, porém, em cada ponto
12 coletado indivíduos de apenas uma espécie foram amostrados, com exceção de
13 Pinhão/PR. A análise com o *software* Structure apontou a possível existência de sete
14 grupos bem estruturados, sendo três de *E. niederleinii* e quatro de *E. viridans* (Figura.
15 6), indicando um possível isolamento para essas amostras.

16 Para a realização da GISH os DNAs genômicos de *E. niederleinii* coletada em
17 Alexandrita/MG e *E. viridans* coletada em Viamão/RS, ambas com $2n$ = 20, foram
18 utilizadas como sonda. A sonda obtida da amostra de *E. niederleinii*, foi hibridada nas
19 amostras de *E. niederleinii* com $2n$ = 40 coletadas em Pinhão/PR e São Luis do
20 Purunã/PR, $2n$ = 30 coletada em Tibagi/PR e na amostra com $2n$ = 35 coletada em
21 Conceição do Mato Dentro/MG. Em nenhum desses casos sinais de hibridação foram
22 observados. Esta mesma sonda foi hibridada também em uma amostra de *E. viridans*
23 com $2n$ = 41 de Capivari do Sul/RS, e novamente nenhum sinal de hibridação foi visto.
24 A mesma sonda foi hibridada na amostra de *E. niederleinii* com $2n$ = 40 coletada em

1 Alexandrita, neste caso sinais de hibridação foram observados em quase todos os
2 cromossomos (Figura 7A).
3 A sonda de *E. viridans* hibridou nas amostras de *E. viridans* com $2n = 42$
4 coletadas em Pinhão/PR e Lapa/SC, na amostra de $2n = 40$ de Pinhão e na amostra com
5 $2n = 29$ de Irani/SC, contudo o padrão de hibridação foi diferente entre elas. Na
6 amostra com $2n = 42$ de Pinhão, a sonda hibridou na região terminal de 13
7 cromossomos e em regiões intersticiais em dois (Figura 7B). Na amostra com $2n = 40$
8 de Pinhão, a maioria dos cromossomos exibiu sinais de hibridação (Figura 7E). Na
9 amostra de *E. viridans* com $2n = 42$, coletada na Lapa, sinais terminais foram
10 observados em 12 cromossomos e apenas 1 exibiu sinal intersticial (Figura 6C). Na
11 amostra com $2n = 29$, a maioria dos cromossomos foi detectada pela sonda, com sinais
12 terminais, intersticiais e em cromossomos inteiros (Figura 7D).
13 Esta sonda também foi hibridada em outras amostras de *E. viridans* coletadas
14 em Caçador/SC, Capivari do Sul/RS e Pinhão/PR, além das amostras de *E. niederleinii*
15 coletadas em Alexandrita, Tibagi/PR, São Luis do Purunã/PR e Biturama/PR. Em
16 nenhum destes casos foram observados sinais. Tanto para *E. viridans* quanto para *E.*
17 *niederleinii*, a sonda hibridou somente nas amostras cujas populações estavam
18 estruturadas, como pode ser observado no gráfico gerado pelo programa Structure
19 (Figura 6). Aparentemente as amostras que tiveram maior coeficiente de similaridade
20 com a amostra da sonda exibiram maior quantidade de sinais de hibridação. A única
21 diferente foi a amostra de *E. viridans* coletada na Lapa, a qual exibiu maior quantidade
22 de sinais do que esperado (Figura 7C), já que seu coeficiente de similaridade com o
23 genoma que originou a sonda foi cerca de 82% (Figura 6).
24

25 **Discussão**

1 De um modo geral, as alterações cromossômicas numéricas em *Eleocharis* são
2 provocadas por agmatoploidia, simploidia e principalmente por poliploidia, (Da Silva *et*
3 *al*. 2008a,b). Contudo, em subdivisões menores do gênero, como a Secção *Eleocharis*,
4 há um conjunto maior de espécies com cariótipos derivados por agmatoploidia e
5 simploidia, como as séries cromossômicas encontradas em *E. uniglumis* e *E. palustris*
6 (Bureš 1998 e Bureš *et al*. 2004). De acordo com Roalson e Friar (2000), esse grupo
7 pode ter uma origem para e polifilética. A série *Tenuissimae* estudada aqui também
8 pertence a esta secção e exibe uma grande variação nos números cromossômicos, como
9 $2n$ = 12 e 20 em *E. minima*, $2n$ = 10 em *E. retroflexa* (Da Silva *et al*. 2008a), bem como
10 os números $2n$ = 20, 29, 30, 31, 35, 40, 41 e 42 encontrado aqui para *E. niederleinii* e
11 *E. viridans.* A série *Tenuissimae* pode ter origem parafilética (Roalson e Friar 2000 e
12 Yano *et al*. 2004), o que poderia explicar a grande diversidade morfológica das espécies
13 desse grupo (González-Elizondo e Tena-Flores 2000).
14 As amostras mais facilmente diferenciadas, seja para *E. niederleinii* ou *E.*
15 *viridans*, produziram inflorescências com flores férteis, aquênios viáveis, e cariótipos
16 com menores números cromossômicos, $2n$ = 20 e 30 e meiose regular. Há também um
17 caso de *E. viridans* coletada em Viamão, RS, com $2n$ = 42, com a presença de dois
18 cromossomos maiores em seu cariótipo, meiose regular e aquênios viáveis. Nas demais
19 amostras prevaleceram números cromossômicos variados, a ocorrência de um a quatro
20 cromossomos duas a três vezes maiores que os demais, derivados provavelmente de
21 fissão/fusão nestes cariótipos. Em alguns poucos casos houve a produção de espiguetas,
22 porém, a meiose foi irregular com formação de multivalentes e univalentes. Meiose
23 irregular em holocêntricos, associada a rearranjos cromossômicos, foi reportada para
24 *Carex laevigata* (Luceño e Castroviejo 1991). Além das evidências citogenéticas, a

1 sobreposição de caracteres morfológicos associada à preferência por reprodução
2 assexuada, pode indicar uma origem híbrida para as duas espécies.
3 A FISH com a sonda de DNAr 45S revelou de 4 a 6 sítios terminais em todos
4 os diferentes cariótipos das duas espécies. Estes sítios aparecem nos cromossomos
5 maiores, porém não em todos eles, como observado nas amostras de *E. niederleinii* com
6 2*n* = 40 (São Luis do Purunã e Pinhão) e de *E. viridans* com 2*n* = 40 (Lebon Regis e
7 Pinhão). Múltiplos sítios terminais de DNAr 45S também foram encontrados em
8 espécies de *Rhynchospora* e *Eleocharis*, sempre na posição terminal dos cromossomos
9 (Vanzela *et al*. 1998, Da Silva *et al*. 2008a, b), mesmo quando os cariótipos sofreram
10 rearranjos.
11 A reprodução assexuada foi observada em campo para todas as amostras
12 estudadas aqui, inclusive para aquelas com meiose regular que floresceram em
13 condições de casa de vegetação. Espécies prolíferas são encontradas em vários gêneros
14 de Cyperaceae, como em *Cyperus*, *Eleocharis*, *Isolepis*, *Kyllinga* e *Schoenpplectus*, e
15 para muitos desses casos, a reprodução prolífera foi induzida por condições de
16 ambientais estressantes. Isto foi observado em várias espécies na África do Sul
17 (Gordon-gray *et al*. 2008). Nossos resultados não permitem concluir se a condição
18 prolífera (reprodução assexuada) das amostras estudadas aqui aconteceu por influência
19 do ambiente, mas é possível que este evento ocorra devido a origem híbrida, como
20 sugerido por Chapman e Burke (2007).
21 As análises moleculares indicam uma similaridade superior a 95% entre as
22 amostras de 2*n* = 20 e 40 de *E. niederleinii* coletadas em Alexandrina, isto pode
23 explicar o padrão de marcas obtidas na GISH. Esta marcação parcial sugere a presença
24 de mais de um genoma na composição de 2*n* = 40, ou seja, uma origem hibrida, para
25 esta amostra. O que poderia explicar também a ausência de inflorescências, quando

comparada com a amostra de $2n = 20$, a qual exibiu inflorescências férteis e meiose regular. Os resultados da GISH sugerem também mais de um genoma na composição dos cariótipos de *E. viridans* coletadas em Pinhão, Viamão e Irani. Alopoliplóides naturais são comuns (Soltis *et al*. 2003), e em alguns casos a GISH foi fundamental para revelar este tipo de poliploidia (Galina *et al*. 2008; Bennett *et al*. 2004), como as encontradas aqui.

Heilborn (citado em Davies 1956) considerou que a aparente falta de alopoliplóide em *Carex* pode ser atribuída à degeneração de três núcleos dos quatro formados ao final da meiose. Apesar desta relação não estar suficientemente esclarecida (Hoshino e Shimizu 1986), híbridos interespecíficos foram detectados em *Carex* (Luceño 1994).

Apesar da taxonomia identificar duas espécies (*E. niederleinii* e *E. viridans*), as análises moleculares indicaram sete genomas estruturados com algumas sobreposições, e as análise de hibridação genômica, mais de dois genomas. Essas informações nos permitem sugerir a existência de isolamento, mesmo em situação simpátrica, sobretudo para as amostras de Pinhão. Nossos resultados abrem uma excelente perspectiva para estudos futuros envolvendo análises populacionais, de marcadores de herança extragenômica, hibridação e poliploidia, e assim contribuir para o esclarecimento das relações de parentesco dentro da série *Tenuissimae*.

Agradecimentos

Os autores agradecem as agências Brasileiras Fundação Araucária e CNPq pelo suporte financeiro.

1 **Referências**
2

3 **Alves MV, Araújo AC, Prata AP, Vitta FA, Hefler SM, Trevisan R, Gil AB,**
4 **Martins S, Thomas SWW. 2010**. Diversity of Cyperaceae in Brazil
5 *Rodriguésia* **60**: 1-12

6

7 **Bennett ST, Kenton AY, Bennett MD. 2004.** Genomic *in situ* hybridization reveals
8 the allopolyploid nature of *Milium montianum* (Gramineae) *Chromosoma* **101**:
9 420 - 424

10

11 **Brasileiro ACM, Carneiro VTC 1998**. Manual de Transformação Genética de Plantas.
12 Brasileiro ACM, Carneiro VTC (eds) Brasília, *Embrapa-SPI*/Embrapa-Cenargen. 309 p.

13

14 **Bureš P. 1998**. A high polyploidy *Eleocharis uniglumis* S.L. (Cyperaceae) from central
15 and southeastern Europe *Folia Geobotanica* **33**: 429-439.

16

17 **Bureš P, Rotreklová O, Stoneberg HSD, Pikner R. 2004**. Cytogeographical survey of
18 *Eleocharis* subser. *Eleocharis* in Europe 1. *Eleocharis palustris*. *Folia*
19 *Geobotanica* **39**: 235-257.

20

21 **Chapman MA, Burke JM. 2007.** Genetic divergente and hybrid speciation. *Evolution*
22 **61**: 1773–1780

23

24 **Da Silva CRM, González-Elizondo MS, Vanzela ALL. 2005.** Reduction of
25 chromosome number in *Eleocharis subarticulata* (Cyperaceae) by multiple
26 translocations. *Botanical Journal Linnean Society* **149:** 457–464.

1

2 **Da Silva CRM, González-Elizondo MS, Rego LNAA, Torezan JMD, Vanzela ALL.**
3 **2008a.** Cytogenetical and cytotaxonomical analysis of some Brazilian species
4 of *Eleocharis*. *Australian Journal of Botany* **56**: 82-90.

5

6 **Da Silva CRM, González-Elizondo MS, Vanzela ALL. 2008b.** Chromosome
7 Reduction in *Eleocharis maculosa* (Cyperaceae). *Cytogenetic and Genome*
8 *Research* **122:** 175-180.

9

10 **Davies EW. 1956.** Cytology, evolution and origin of the aneuploid series in the genus
11 *Carex*. *Hereditas* **42**: 349–365.

12

13 **Falush D, Stephens M, Pritchard, JK. 2003.** Inference of population structure using
14 multilocus genotype data: Linked loci and correlated allele frequencies.
15 *Genetics* **164**: 1567–1587.

16

17 **Galina P, Tatjana G, Jiming J, David SM. 2008.** Allopolyploid speciation of the
18 Mexican tetraploid potato species *Solanum stoloniferum* and *S. hjertingii*
19 revealed by genomic in situ hybridization. *Genome* **51**: 714-720

20

21 **González-Elizondo MS, Peterson PM. 1997**. A classification of and key to the
22 supraspecific taxa in *Eleocharis* (Cyperaceae). *Taxon* **46**: 433-449.

23

1 **González-Elizondo MS, Tena-Flores JA. 2000.** *Eleocharis* (Cyperaceae) in the New
2 World. Pp. 637-641. In: Wilson, K. L. & Morrison, D. A. (eds.). *Monocots: Systematics*
3 *and Evolution*. Melbourne: CSIRO.
4
5 **Gordon-Gray KD, Baijnath H, Ward CJ, Wragg PD. 2008.** Studies in Cyperaceae
6 in southern Africa 42: Pseudo-vivipary in South African Cyperaceae. *South*
7 *African Journal of Botany* **75**: 167-171.
8
9 **Heslop-Harrison JS, Schwarzacher T, Anamthawat-Jonsson K, Leitch AR, Shi M,**
10 **Leitch IJ. 1991.** *In situ* hybridization with automated chromosome denaturation.
11 *Technique* **3**: 106–109.
12
13 **Hipp AL, Rothrock PE, Roalson EH. 2009.** The evolution of chromosome
14 arrangements in *Carex* (Cyperaceae). *Botanical Review* **75**: 96-109.
15
16 **Hoshino T, Shimizu T. 1986.** Cytological studies on degenerative nuclei at pollen
17 development of *Carex ciliato-marginata*. *Journal Plant Research* **99**: 185–190.
18
19 **Luceño M, Castroviejo S. 1991.** Agmatoploidy in *Carex laevigata* (Cyperaceae):
20 Fusion and fission of chromosomes as the mechanism of cytogenetic evolution
21 in Iberian populations. *Plant Systematic Evolution* **177**: 149–160.
22
23 **Luceño M. 1994.** Cytotaxonomic studies in Iberian, Balearic, North African, and
24 Macaronesian species of *Carex* (Cyperaceae): II. *Canadian Journal of Botany*.
25 **72**: 587–596.

1
2 **Luceño M, Vanzela ALL, Guerra M. 1998**. Cytotaxonomic studies in Brazilian
3 *Rhynchospora* (Cyperaceae), a genus exhibiting holocentric chromosomes.
4 *Canadian Journal of Botany* **76**: 440–449.
5
6 **Pritchard JK, Stephens M, Donnelly P. 2000.** Inference of population structure using
7 multilocus genotype data. *Genetics* **155**: 945-959.
8
9 **Rambo B. 1959.** Cyperaceae Riograndenses. *Pesquisas* **3**: 354-453
10
11 **Roalson EH, Friar EA. 2000.** Infrageneric classification of Eleocharis (Cyperaceae)
12 revisited: evidence from the Internal Transcribed Spacer (ITS) region of
13 nuclear ribosomal DNA. *Systematic Botany* **25**: 323-336.
14
15 **Roalson EH. 2008.** A synopsis of chromosome number variation in the Cyperaceae.
16 *Botanical Review* **74**: 209-393
17
18 **Roalson EH, Hinchliff CE, Trevisan R, Da Silva CRM. (*in press*)**. Phylogenetic
19 relationships in *Eleocharis* (Cyperaceae): C4 photosynthesis origins and
20 patterns of diversification in the spikerushes. *Systematic Botany* **35**: xxx-xxx.
21
22 **Simpson DA. 1988.** Some problems in *Eleocharis*. Notes on Brazilian Cyperaceae: III.
23 *Kew Bulletin* **43**: 127-134.
24

1 **Svenson HK. 1939.** Monographic Studies in the Genus *Eleocharis* V. *Rhodora* **41**: 1-
2 110.
3
4 **Soltis DE, Soltis PS, Tate JA. 2003.** Advances in the study of polyploidy since Plant
5 speciation. *New Phytologist* **161**: 173–191
6
7 **Trevisan R, Boldrini II. 2008.** O gênero *Eleocharis* R. Br. (Cyperaceae) no Rio
8 Grande do Sul, Brasil. *Revista Brasileira de Bociências* **6**: 7-67.
9
10 **Vanzela ALL, Guerra M, Luceño M. 1996** *Rhynchospora tenuis* Link (Cyperaceae), a
11 species with the lowest number of holocentric chromosomes (n=2). *Cytobios*
12 **88:** 219–228.
13
14 **Vanzela ALL, Cuadrado A, Jouve N, Luceño M, Guerra M. 1998.** Multiple
15 locations of the rDNA sites in holocentric chromosomes of *Rhynchospora*
16 (*Cyperaceae*). *Chromosome Research* **6**: 345-349.
17
18 **Yano O, Katsuyama T, Tsubota H, Hoshino T. 2004**. Molecular phylogeny of
19 Japanese *Eleocharis* (Cyperaceae) based on ITS sequence data, and
20 chromosomal evolution. *Journal of Plant Research* **117**: 409-419.

1 **Legendas**

2 **Figura 1.** Mitose em *Eleocharis niederleinii*, com diferentes números cromossômicos.
3 A) Amostra com $2n = 20$, com os cromossomos tamanhos próximos. B)
4 Amostra com $2n = 29$. Note o núcleo arreticulado à esquerda. C) Amostra com
5 $2n = 30$, com os cromossomos tamanhos próximos. D) Amostra com $2n = 30$.
6 E) Amostra com $2n = 31$. F) Amostra com $2n = 35$, com os cromossomos
7 tamanhos próximos. G - I) Amostras com $2n = 40$. Todas as setas apontam os
8 cromossomos maiores. Barra = 10µm

9

10 **Figura 2.** Mitose em *Eleocharis viridans*, com diferentes números cromossômicos. A)
11 Amostra com $2n = 20$, com os cromossomos tamanhos próximos. B) Amostra
12 com $2n = 29$. C) Amostra com $2n = 31$. D e E) Amostras com $2n = 40$. F e G)
13 Amostras com $2n = 41$. G) Amostra com $2n = 42$. Todas as setas apontam os
14 cromossomos maiores. Barra = 10µm.

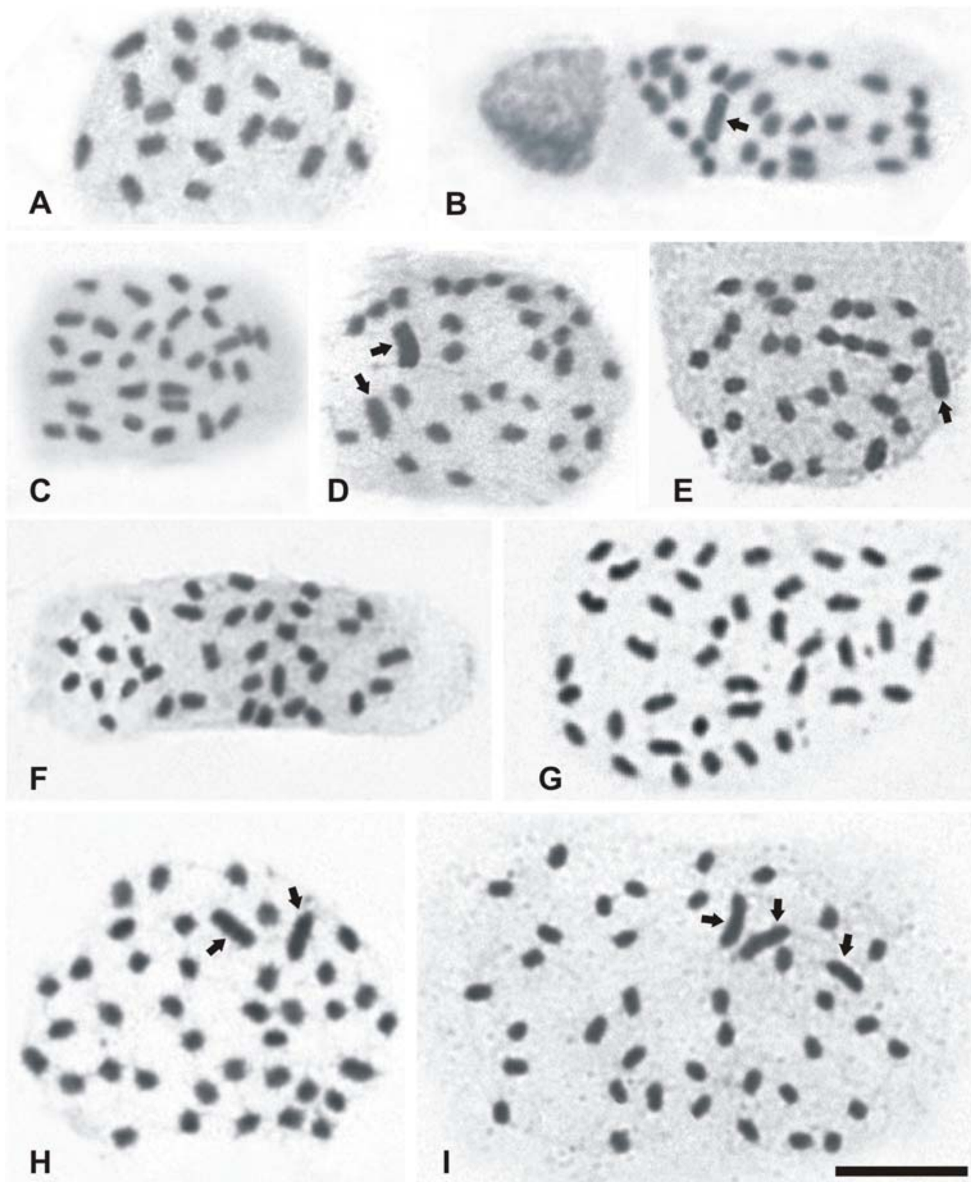
15

16 **Figura 3.** Diferentes fases da meiose para *E. niederleinii* e *E. viridans*. A) Metáfase I
17 em *E. niederleinii* com $2n = 20$ e 10 bivalentes. B) Metáfase I em *E.*
18 *niederleinii* com $2n = 30$ e 15 bivalentes. C) Metáfase I em *E. viridans* com $2n$
19 $= 42$ e 21 bivalentes. Note os dois bivalentes maiores. D) Metáfase I em *E.*
20 *niederleinii* com $2n = 30$ e com $1^{IV} + 2^{III} + 9^{II} + 2^{I}$. E) Metáfase I em *E.*
21 *viridans* com $2n = 31$ e $7^{III} + 3^{II} + 4^{I}$. F) Metáfase I em *E. viridans* com $2n =$
22 31 e $1^{VI} + 1^{IV} + 5^{III} + 2^{II} + 2^{I}$. G) Metáfase I em *E. viridans* com $2n = 41$ e $1^{III} +$
23 19^{II}. E m todas estas fotos: o "I" indica os univalentes, as setas apontas as
24 associações multivalentes e as cabeças de seta apontam as associações
25 trivalentes. H) Metáfase polínica em *E. niederleinii* com $n = 15$, note os dois

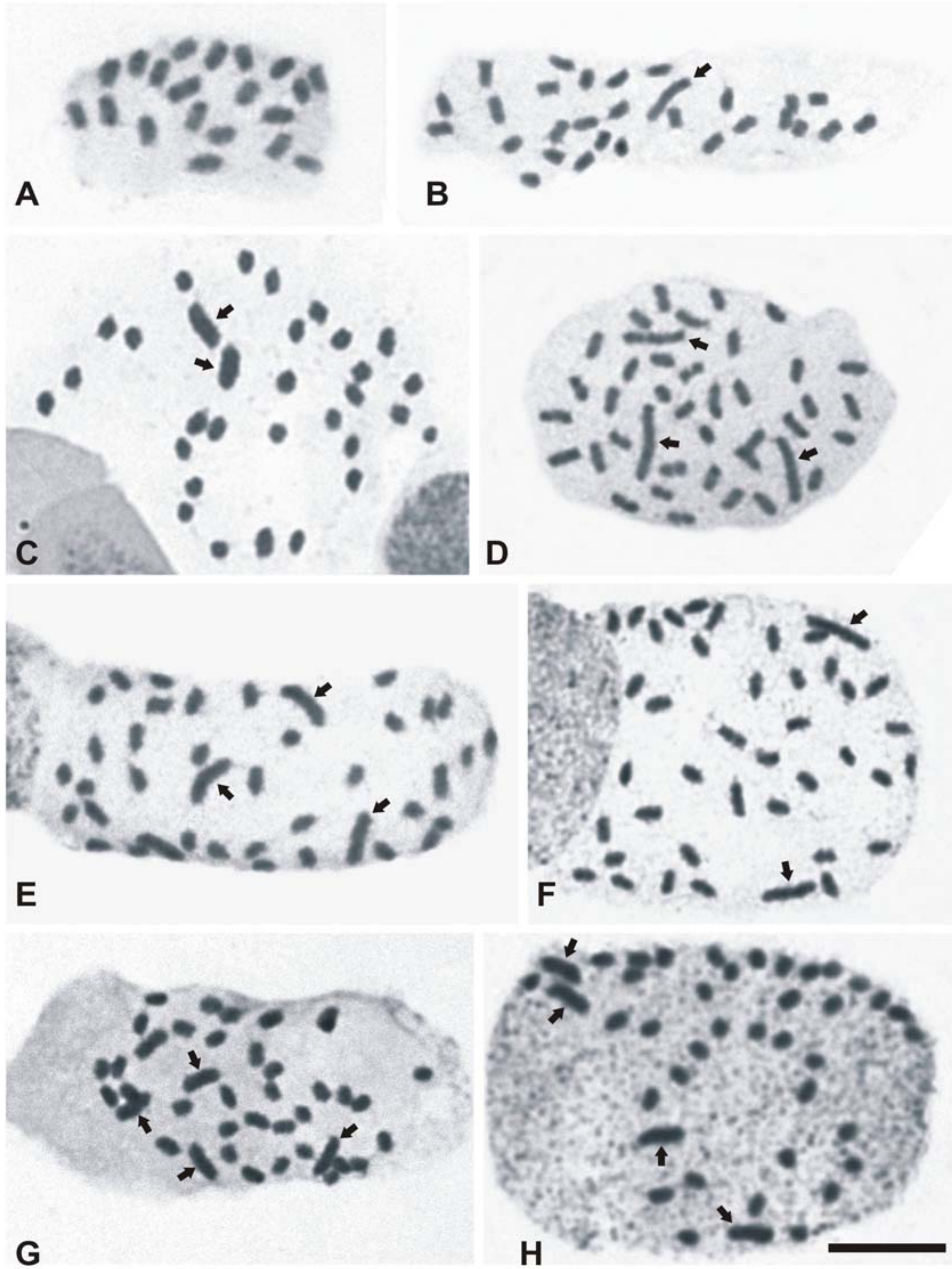
1 cromossomos maiores. I) Metáfase polínica em *E. niederleinii* com n = 17, e
2 todos os cromossomos com tamanhos próximos. J e K) Metáfase polínica em
3 *E. viridans* com n = 22 e n = 21, respectivamente, as setas apontam os
4 cromossomos maiores. Barra = 10µm.
5
6 **Figura 4.** Hibridação *in situ* com a sonda de DNAr 45S em amostras de *E. niederleinii*.
7 A) Amostra com $2n$ = 20 e quatro sinais de hibridação. B) Amostra com $2n$ =
8 29 e quatro sinais de hibridação. C) Amostra com $2n$ = 30 e seis sinais de
9 hibridação. D) Amostra com $2n$ = 30 e seis sinais de hibridação. E) Amostra
10 com $2n$ = 31 e seis sinais de hibridação. F) Amostra com $2n$ = 40 e quatro
11 sinais de hibridação. Note que nas figuras D, E e F, os dois cromossomos
12 maiores portam os sítios de DNAr 45S. G) Amostra com $2n$ = 40 e quatro
13 sinais de hibridação. H) Amostra com $2n$ = 35 e seis sinais de hibridação. I)
14 Amostra com $2n$ = 40 e seis sinais de hibridação. A seta aponta o único
15 cromossomo maior sem o sítio de DNAr 45S. Barra = 10µm.
16
17 **Figura 5.** Hibridação *in situ* com a sonda de DNAr 45S em amostras de *E. viridans*. A)
18 Amostra com $2n$ = 20 e quatro sinais de hibridação. B) Amostra com $2n$ = 29 e
19 seis sinais de hibridação. C) Amostra com $2n$ = 31 e seis sinais de hibridação.
20 D) Amostra com $2n$ = 40 e quatro sinais de hibridação. E) Amostra com $2n$ =
21 40 e seis sinais de hibridação. F) Amostra com $2n$ = 41 e seis sinais de
22 hibridação. Nas figuras D, E e F, as setas apontam os cromossomos maiores
23 sem o sítio de DNAr 45S. G) Amostra com $2n$ = 42 e seis sinais de hibridação.
24 H) Amostra com $2n$ = 41 e quatro sinais de hibridação. Barra = 10µm.
25

Figura 6. A) Mapa com os pontos de coleta. Os números representam as exsicatas de cada amostra. Os números vermelhos são de *E. niederleinii* os pretos são de *E. viridans*. Note que apenas em um ponto as duas espécies foram coletadas juntas (Pinhão). B) Dendrograma de similaridade genética obtido pelo coeficiente de Jaccard e pelo método UPGMA para as duas espécies.

Figura 7. Hibridação genômica *in situ*. A) Amostra de *E. niederleinii* com $2n = 40$ coletada em Alexandrita/MG, hibridada com a sonda da amostra de *E. niederleinii* com $2n = 20$ coletada no mesmo local. As fotos de B – E, são de diferentes amostras hibridadas com a sonda de *E. viridans* com $2n = 20$ coletada em Viamão/RS. B) Amostra de *E. viridans* com $2n = 42$ coletada em Pinhão/PR. C) Amostra de *E. viridans* com $2n = 42$ coletada na Lapa. D) Amostra de *E. viridans* com $2n = 29$ coletada em Irani/SC. Amostra de *E. viridans* com $2n = 40$ coletada em Pinhão/PR. Barra = 10µm.



1
2
3
4
5
6
7
8
9
10



1

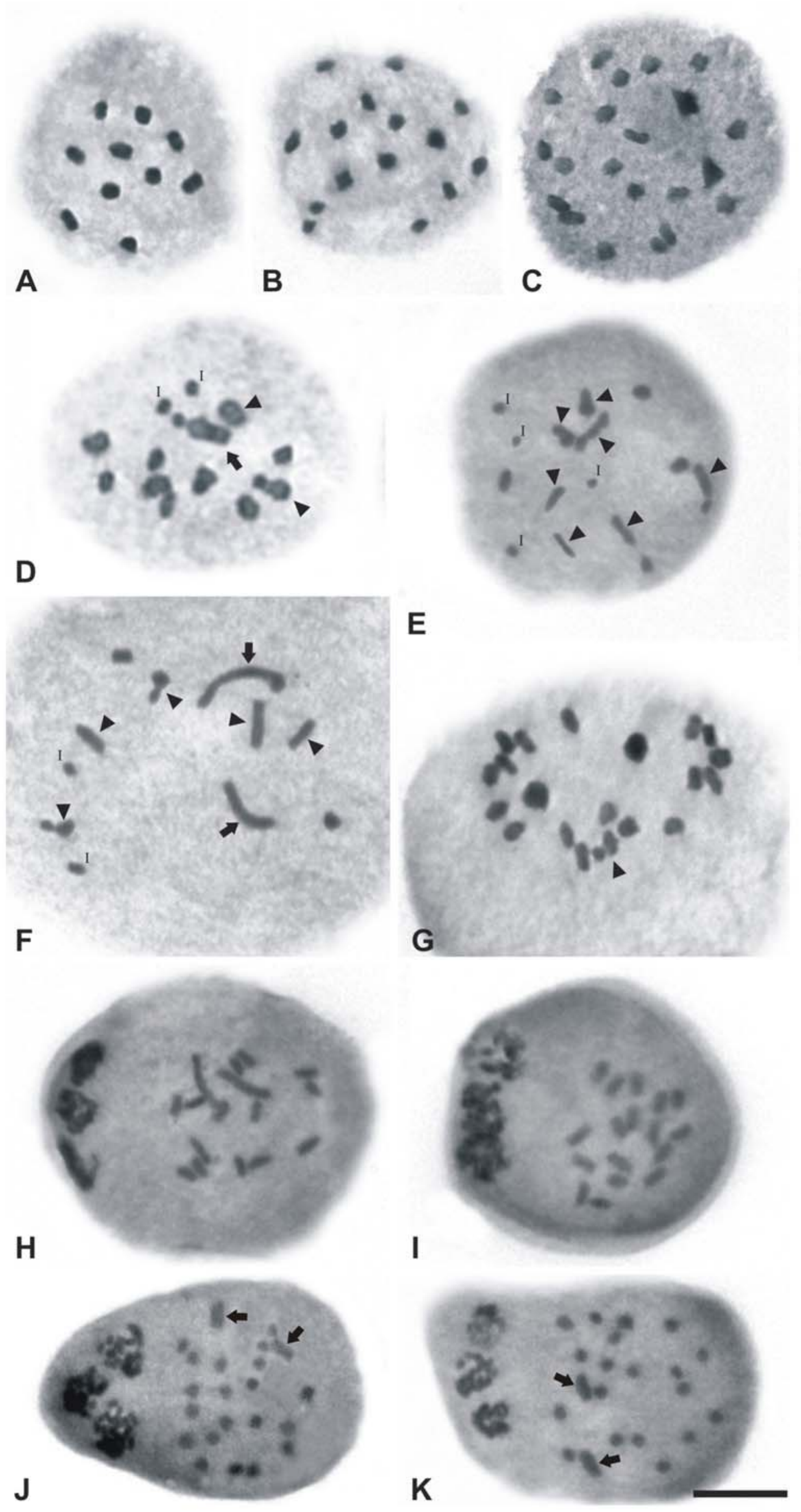
2

3

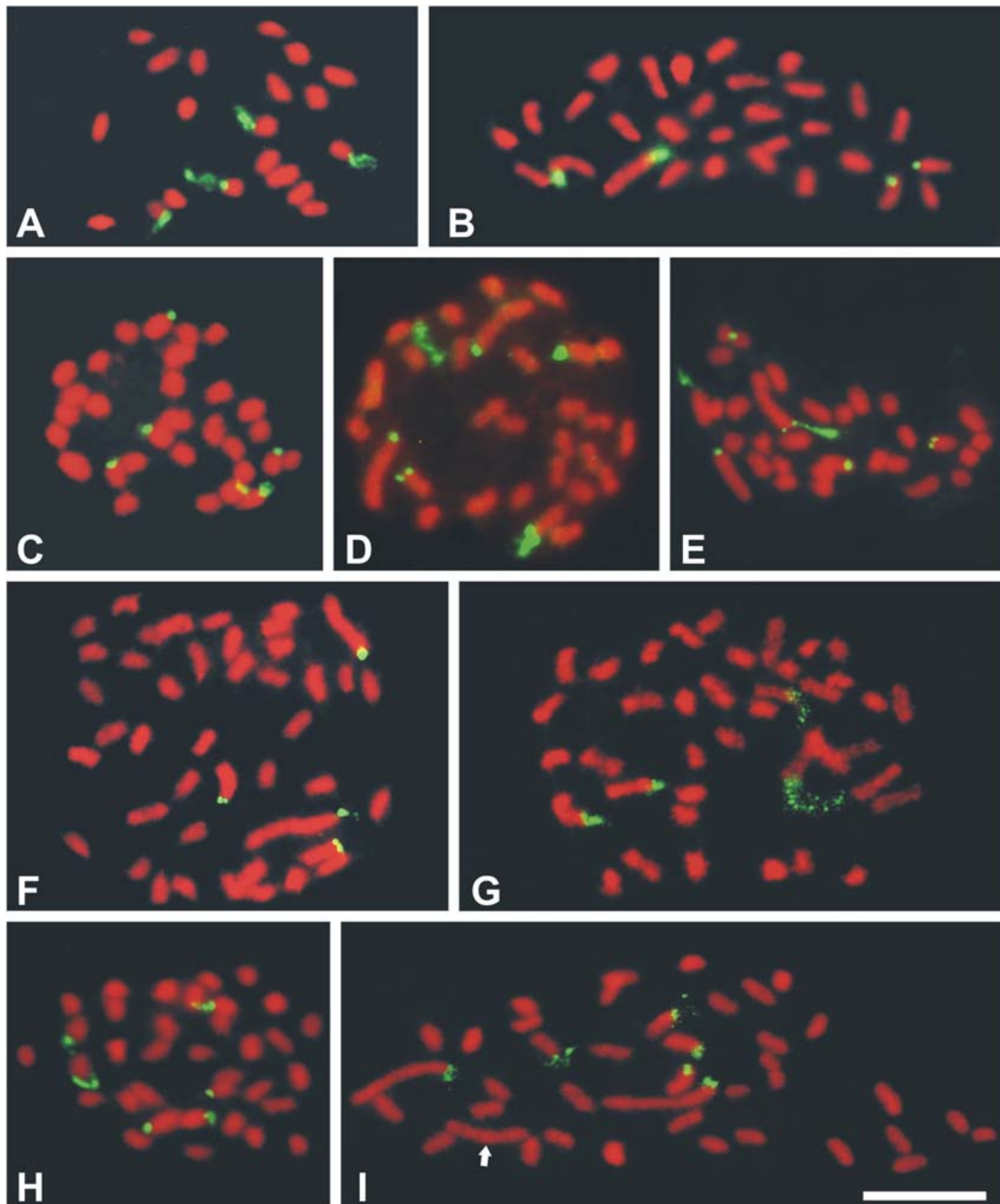
4

5

6



1



1

2

3

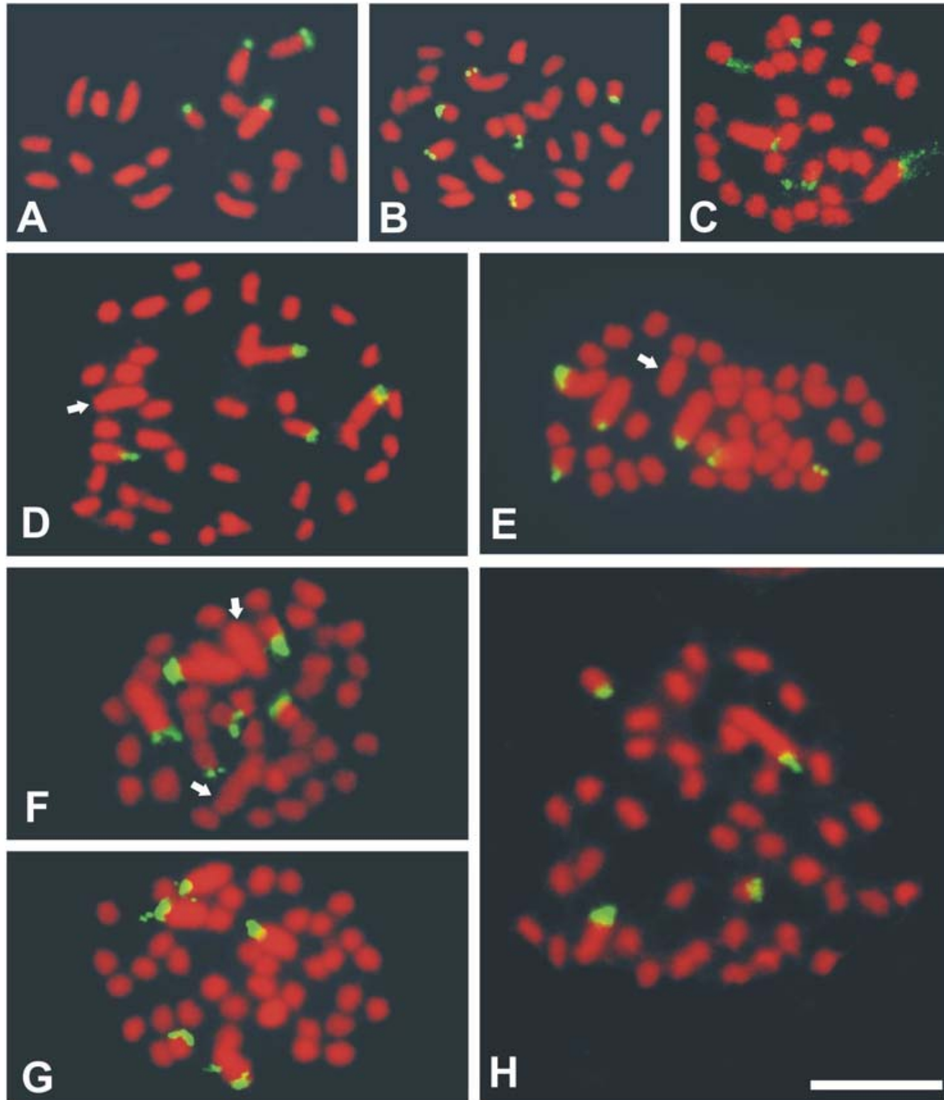
4

5

6

7

8



1

2

3

4

5

6

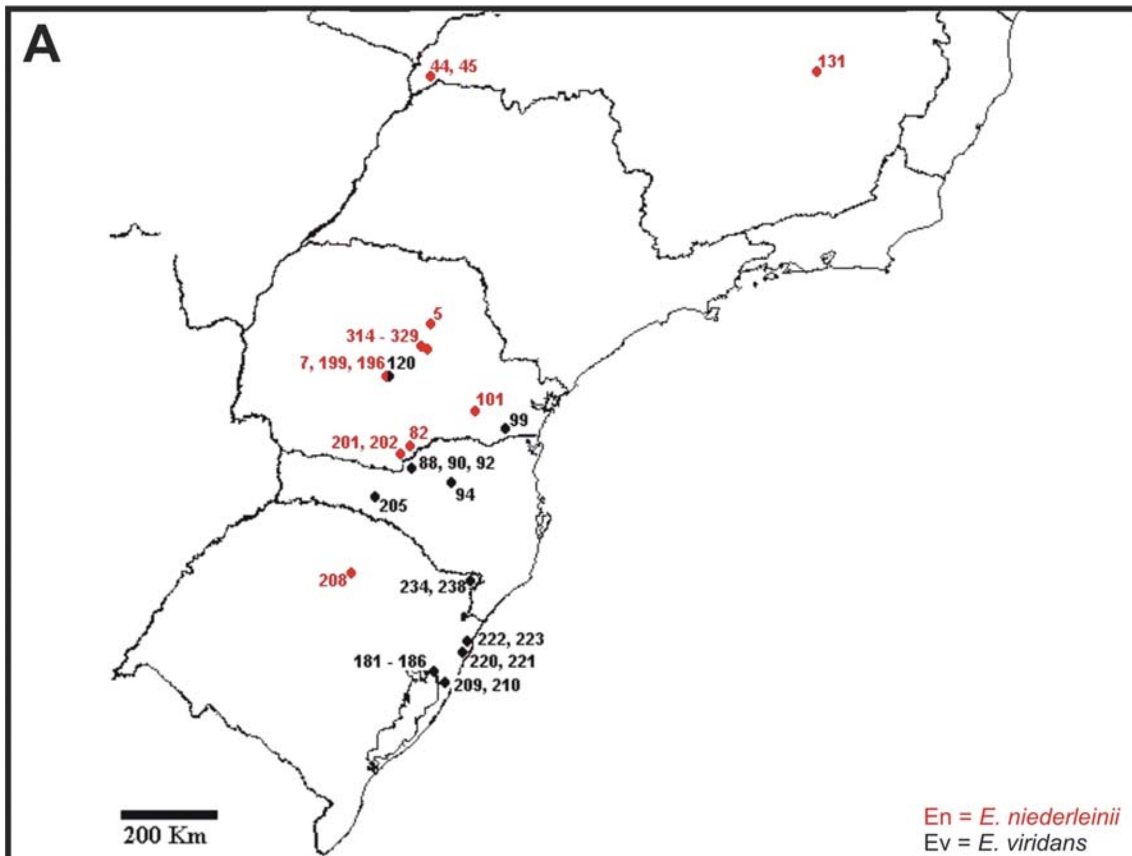
7

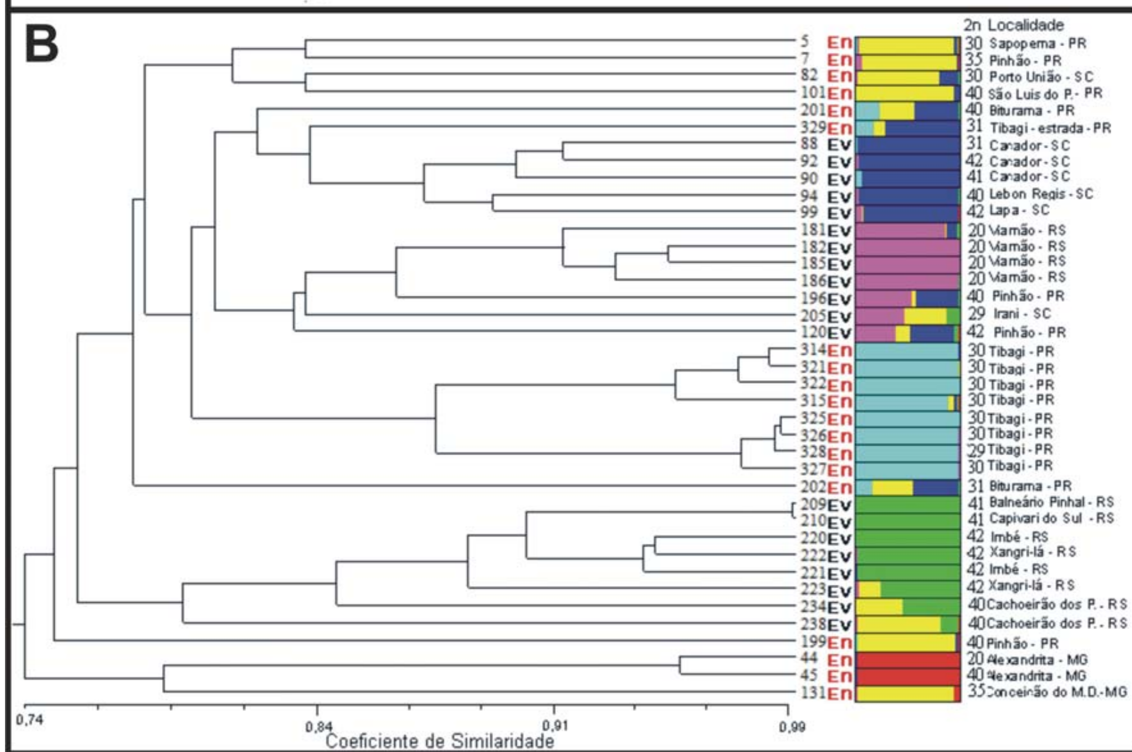
8

9

10

11



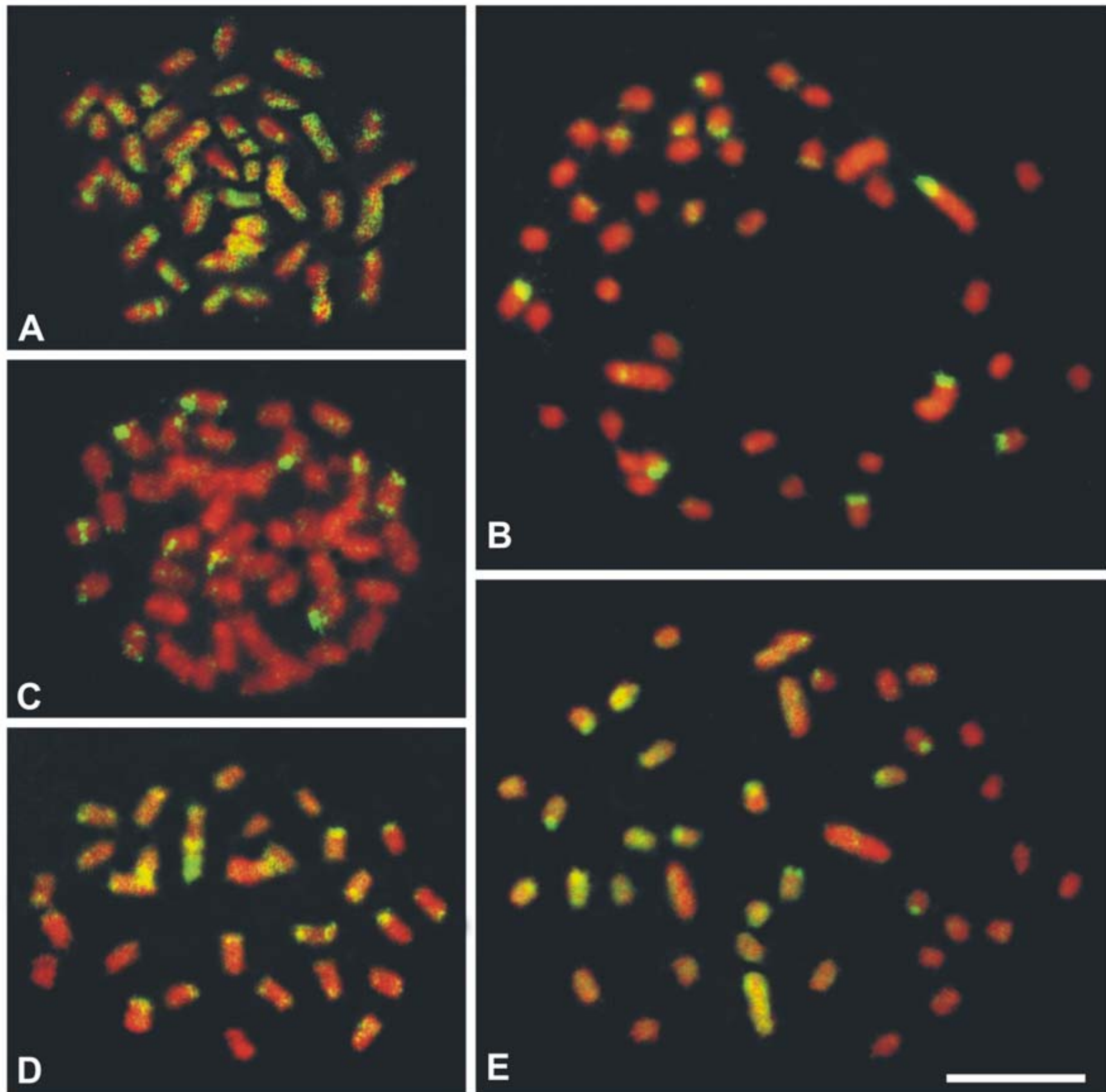


1

2

3

4



1

2

3

4

5

6

7

8

9

10

Espécies	2*n*	TL[A]	SLC[B]	Coordinates, Localities (voucher number)
E. niederleinii	20	35.03		19°42'09"S 50°35'33"W, Alexandrita, MG (44)**
	29(1)*	45.13	3.72	50°30'03" S 24°32'04" W, Tibagi, PR (328)
	30	47.25		50°29'05" S 24°28'02" W, Tibagi, PR (314)**
				50°29'05" S 24°28'02" W, Tibagi, PR (315)**
				50°29'05" S 24°28'02" W, Tibagi, PR (321)**
				50°29'05" S 24°28'02" W, Tibagi, PR (322)**
				50°30'03" S 24°32'04" W, Tibagi, PR (325)**
				50°30'03" S 24°32'04" W, Tibagi, PR (326)**
				50°30'03" S 24°32'04" W, Tibagi, PR (327)**
	30(2)*	49.08	3.20	23°53'92" S 50°36'27" W Sapopema, PR (5)
			3.16	26°16'46"S 51°03'24"W, Porto União, SC (82)
	31(1)*	50.58	3.67	50°30'03" S 24°32'04" W, Tibagi, PR (329)
	35	57.25		25º39'59"S 51º40'05"W, Pinhão, PR (7)
				19°04'13"S 43°24'58"W, Conceição do Mato Dentro, MG (131)
	40	69.01		19°42'09"S 50°35'33"W, Alexandrita, MG (45)
	40(2)*	71.90	3.95	26°21'58"S 51°21'08"W, Biturama, PR (201)
			3.89	
	40(3)*	75.16	4.05	25°28'74"S 49°52'93"W, São Luis do Purunã, PR (101)
			3.97	25°39'59"S 51°40'05"W, Pinhão, PR (199)
			3.95	
E. viridans	20	35.61		30º06'18"S 50º44'33"W, Viamão, RS (181)**
				30º06'18"S 50º44'33"W, Viamão, RS (182)**
				30º06'18"S 50º44'33"W, Viamão, RS (185)**
				30º06'18"S 50º44'33"W, Viamão, RS (186)**
	29(1)*	44.68	3.60	26°42'24"S 51°35'11"W, Irani, SC (205)
	31(2)*	51.54	3.77	26°51'31"S 50°49'54"W, Caçador, SC (88)**
			3.58	

40(3)*	76.12	4.63	26º49'67"S 50º21'54"W, Lebon Regis, SC (94)
		3.96	
		3.93	
40(4)*	80.96	4.88	25°39'59"S 51°40'05"W, Pinhão, PR (196)
		4.67	Caminho do Cachoeirão dos Padrinhos – RS (234)
		4.34	Caminho do Cachoeirão dos Padrinhos – RS (238)
		4.28	
41(2)*	79.57	4.69	26°51'31"S 50°49'54"W, Caçador, SC (90)
		4.23	
41(4)*	81.22	4.65	30°12'08"S 50°12'52"W, Balneário Pinhal, RS (209)**
		4.23	30°09'15"S 50°25'53"W, Capivari do Sul, RS (210)**
		4.22	
		4.19	
42(4)*	81.72	4.74	25º36'47"S 49º49'55"W, Lapa, PR (99)**
		4.73	26°51'31"S 50°49'54"W, Caçador, SC (92)**
		4.37	25°39'59"S 51°40'05"W, Pinhão, PR (120)**
		4.36	29°54'18"S 50°06'15"W, Imbé, RS (220)**
			29°54'18"S 50°06'15"W, Imbé, RS (221)**
			29°48'18"S 50°03'45"W, Xangri-lá, RS (222)**
			29°48'18"S 50°03'45"W, Xangri-lá, RS (223)**

1 [A] Comprimento total do lote diplóide em µm. [B]:Tamanho dos cromossomos maiores em µm.*Entre parênteses a quantidade de cromossomos
2 muito maiores que os demais. ** plantas que produziram inflorescência.
3

Conclusões

Conclusões

1) Nenhuma das 27 amostras estudadas exibiu constrição primária. Somente constrições nucleolares foram observadas em algumas metáfases para poucas espécies. Em *E. sellowiana* e *E. maculosa* a migração paralela dos cromossomos foi registrada. Para várias espécies, bivalentes formando a "estrutura em caixa" puderam ser observados. Assim, os dados resultantes deste trabalho confirmam a condição holocêntrica dos cromossomos neste gênero.

2) As medições cromossômicas feitas em 25 espécies mostram diminuição gradual no tamanho para a maioria delas. Com exceção, de *E. maculosa*, *E. niederleinii*, *E. viridans* e *E. subarticulata*, as quais exibiram cromossomos muito maiores em relação aos demais do seu cariótipo. Nas três primeiras espécies estes cromossomos grandes, surgiram por simploidia, e na última espécie, translocações múltiplas pode ser a causa da formação destes cromossomos.

3) Todas as espécies do subgênero *Limnochloa* estudadas exibiram $2n = 40$ ou mais cromossomos e estes foram menores que 1,4 µm.

4) Os números cromossômicos variaram de $2n = 6$ (*E. subarticulata* e *E. maculosa*) até $2n = 60$ (*E. laeviglumis*), com vários números intermediários, como 10, 20, 30 e 40, para a maioria das espécies. A alta frequência de espécies com números cromossômicos múltiplos de 5, concordam com trabalhos anteriores (Löve e Love 1957; Da Silva *et al*. 2008) que indica $x = 5$ como o número básico do gênero.

5) Apesar de *E. maculosa*, apresentar mais de um número cromossômico, decorrente de simploidia (fusão), e *E. niederleinii* e *E. viridans*, exibirem uma série cromossômicas causada provavelmente por poliploidia e rearranjos cromossômicos (fusão/fissão) associada a hibridação. A maioria da espécie (cerca de 80%) foi poliplóide. Porém não foi possível concluir se ocorre há predominância de auto ou alopoliplóides.

6) Regiões heterocromáticas ricas em CG ($DAPI^-/CMA^+$) foram localizadas apenas nas pontas dos cromossomos. Em várias espécies estas regiões estão associadas aos sítios de DNAr 45S.

7) Os sítios de DNAr 45S variaram em número entre as espécies, muitas vezes não acompanhando o nível de ploidia, como visto em *E. flavescens* ($2n = 10$) com 10 sítios e em *E. bonariensis* ($2n = 20$) com apenas dois. Mesmo quando os cariótipos sofreram rearranjos, como em *E. maculosa* com $2n = 10$, 8, 7 e 6, o número de sítios foram sempre quatro. Em todas as espécies analisadas os sítios foram terminais. Nas espécies que tiverem cariótipos mais simétricos, mais sítios de DNAr 45S foram encontrados. Estes dados nos permitem concluir que rearranjos cromossômicos (fusão, fissão e poliploidia) têm pouca influência na dispersão destes sítios, e que esta multiplicação de sítios pode ser resultado de amplificação seguido de dispersão pelas pontas dos cromossomos com tamanho próximos.

8) Os dados citológicos mostrados neste trabalho corroboram os de Yano *et al*. (2004) e Roalson e Friar (2000), os quais separam as espécies do subgênero *Limnochloa* dos demais subgêneros. Nossos resultados mostram que as espécies de *Lmnochloa*, possuem

cariótipos com cromossomos numerosos e pequenos (< 1,4 μm). Os demais subgêneros possuem cromossomos maiores (> 1,5 μm). Assim sendo, a análise citogenética pode ser utilizada neste caso como um caráter micromorfológico muito útil na taxonomia.

Referências

Da Silva CRM, González-Elizondo MS, Rego LNAA, Torezan JMD, Vanzela ALL (2008). Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis*. Aust J Bot 56: 82-90.

Löve A, Löve D, Raymond M (1957). Cytotaxonomy of *Carex* section *Capillares*. Can. J. Bot. 35: 715–761.

Roalson EH, Friar EA (2000). Infrageneric classification of Eleocharis (Cyperaceae) revisited: evidence from the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA. Syst Bot 25: 323-336.

Yano O, Katsuyama T, Tsubota H, Hoshino T (2004). Molecular phylogeny of Japanese *Eleocharis* (Cyperaceae) based on ITS sequence data, and chromosomal evolution. J Plant Res 117, 409-419.

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Administração
Baixar livros de Agronomia
Baixar livros de Arquitetura
Baixar livros de Artes
Baixar livros de Astronomia
Baixar livros de Biologia Geral
Baixar livros de Ciência da Computação
Baixar livros de Ciência da Informação
Baixar livros de Ciência Política
Baixar livros de Ciências da Saúde
Baixar livros de Comunicação
Baixar livros do Conselho Nacional de Educação - CNE
Baixar livros de Defesa civil
Baixar livros de Direito
Baixar livros de Direitos humanos
Baixar livros de Economia
Baixar livros de Economia Doméstica
Baixar livros de Educação
Baixar livros de Educação - Trânsito
Baixar livros de Educação Física
Baixar livros de Engenharia Aeroespacial
Baixar livros de Farmácia
Baixar livros de Filosofia
Baixar livros de Física
Baixar livros de Geociências
Baixar livros de Geografia
Baixar livros de História
Baixar livros de Línguas

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo