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Filogenia molecular de *Saccharum* L. e *Eriochrysis* P. Beauv. (Poaceae – Andropogoneae) e resolução taxonômica de complexos de espécies

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“Quem escolhe ser cientista não escolhe uma profissão, escolhe um estilo de vida. O cientista dedica sua vida à Ciência, sem exigir nada em retorno (talvez uns bons resultados de vez em quando seriam suficientes, mas tudo bem).

O cientista não tem que trabalhar até depois do horário comercial. Não tem nada num contrato escondido que diga isso. Nem tem que ir trabalhar sábados, domingos e feriados. E se for, não vai ganhar hora extra. O cientista também não é obrigado a trabalhar em casa. Mas ele faz. O cientista faz tudo o que necessário pelo seu trabalho. Ele vai sacrificar qualquer festinha que tenha, vai chegar atrasado em eventos, porque teve que dar uma passadinha no laboratório para ver como estavam suas células. Ou seus insetos. Ou seus ratinhos. “Mas por quê?”. “É meu experimento!”, responderá, como se fosse algo normal. Mas para o mundo de fora, não é normal.

O cientista pega as malas, se desapega e vai para bem longe, para se especializar. E longe de tudo e de todos, vai se acabar de trabalhar, feliz, fazendo tudo o que podia e não podia. Mas todos acharão que ele está na farra, se esbaldando. Não, ele é cientista.

O cientista se dedica tanto ao seu trabalho, pois se ele não fizer, alguém de algum país desenvolvido, com muito mais recursos, o fará. E quando o seu trabalho atingir a glória (publicação, citação, destaque), ninguém do lado de fora dará a mínima. E o cientista estará procurando o próximo estudo. E o que o cientista ganha com isso? Se considerar a resposta para o mundo comum, nada. Lhufas. Nadica de nada! Mas esse nada, para um cientista, é tudo: ele sabe que, de um jeito ou de outro, está contribuindo para o desenvolvimento da Ciência e, consequentemente, da Humanidade. Ser cientista não é para qualquer um. Muitas pessoas acham isso um absurdo. E é. Por isso, todo cientista ama o que faz, e nunca perde aquele brilho nos olhos, quando vê algo interessante.

Portanto, mundo de fora, não se chateiem quando um cientista “esquecer” de vocês. Não é pessoal. O cientista esquece até de si mesmo.

Agora deixa eu voltar pro meu projeto.”

Cláudia Dick

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SUMÁRIO

RESUMO

A delimitação de espécies é um aspecto de fundamental importância dentro da Biologia Evolutiva, bem como para a conservação da biodiversidade. No entanto, delimitar espécies com base em morfologia é extremamente complicado, especialmente em grupos que tiveram uma radiação recente e que apresentam pouca descontinuidade morfológica entre os táxons, como a tribo Andropogoneae (Poaceae). A presente tese utiliza sequências de DNA, como genes nucleares de cópia-única (*low-copy nuclear loci*) e sequenciamento completo do plastoma, para resolver a circunscrição de complexos de espécies em Andropogoneae, particularmente dos gêneros *Saccharum* e *Eriochrysis*, bem como para investigar o posicionamento filogenético dos mesmos em relação aos demais gêneros da tribo. Aspectos filogenéticos, taxonômicos e nomenclaturais foram investigados. Do ponto de vista nomenclatural, foi possível esclarecer que Andropogoneae Dumortier é o nome correto para a tribo, e não Sacchareae Martinov, como recentemente sugerido por diversos autores. As análises filogenéticas realizadas corroboram a hipótese de uma diversificação inicial rápida em Andropogoneae e a não monofilia da subtribo Saccharinae. A origem alopoliploide do gênero *Saccharum* foi demonstrada a partir de evidências de genes nucleares. *Saccharum s.l.* é polifilético e *Tripidium* deve ser reconhecido como um gênero distinto. As análises filogenéticas também foram capazes de resolver a circunscrição interna de *Saccharum s.l.*, confirmando a ocorrência de três espécies nativas na América do Sul: *S. angustifolium*, *S. asperum* e *S. villosum*. A ocorrência de híbridos naturais entre *S. villosum* e *S. angustifolium* foi documentada. As análises filogenéticas de *Eriochrysis* confirmaram a monofilia do gênero e resolveram a circunscrição de suas espécies: *E. villosa* é um táxon distinto de *E. cayennensis*, bem como *E. laxa* é uma espécie distinta de *E. warmingiana*. Híbridos naturais entre *E. laxa* e *E. villosa* também foram documentados. *Eriochrysis villosa* é citada pela primeira vez para o Uruguai e *E. laxa* para o estado do Rio Grande do Sul. A presente tese demonstrou a eficiência dos genes nucleares de cópia única na delimitação de espécies e gêneros da tribo Andropogoneae, mesmo na presença de poliploidia, evolução reticulada e radiação recente. O sequenciamento completo do plastoma também se mostrou uma ferramenta extremamente promissora para inferências filogenéticas em Andropogoneae.

Palavras-chave: delimitação de espécies; *Eriochrysis*; evolução reticulada; genes nucleares de cópia única; plastoma; poliploidia; Saccharinae; *Saccharum*.

ABSTRACT

Species delimitation is a vital issue concerning evolutionary biology and conservation of biodiversity. However, delimiting species based on morphology is a difficult task especially in plant groups with an evolutionary history involving rapid radiation and little morphological discontinuity between taxa, as the tribe Andropogoneae (Poaceae). The present thesis uses DNA sequences, such as low-copy nuclear genes and complete plastome sequencing, to resolve the taxonomic circumscriptions of species complexes in Andropogoneae, particularly from genera *Saccharum* and *Eriochrysis*, and to investigate their phylogenetic affinities to other genera of the tribe. Phylogenetic, taxonomic, and nomenclatural aspects were investigated. We clarified that Andropogoneae Dumortier is the correct name for the tribe, rather than Sacchareae Martinov, as recently suggested by several authors. The present phylogenetic analyses support the hypothesis of an initial rapid diversification in Andropogoneae and the non-monophyly of subtribe Saccharinae. The allopolyploid origin of *Saccharum* was demonstrated using evidence from nuclear genes. *Saccharum s.l.* is polyphyletic and *Tripidium* should be recognized as a distinct genus. The phylogenetic analyses were also able to define the circumscriptions of the species of *Saccharum s.l.*, confirming the occurrence of three native species in South America: *S. angustifolium*, *S. asperum* and *S. villosum*. The occurrence of natural hybrids between *S. villosum* and *S. angustifolium* was documented. The phylogenetic analyses of *Eriochrysis* confirmed the monophyly of the genus and resolved the circumscriptions of its species: *E. villosa* is distinct from *E. cayennensis*, and *E. laxa* is distinct from *E. warmingiana*. Natural hybrids between *E. laxa* and *E. villosa* were also documented. *Eriochrysis villosa* is reported here for the first time for Uruguay and *E. laxa* for the State of Rio Grande do Sul. The present thesis has demonstrated the efficiency of low-copy nuclear genes in the delimitation of species and genera from tribe Andropogoneae, even in presence of polyploidy, reticulate evolution and recent radiation. The complete plastome sequencing is also a promising tool for phylogenetic inferences in Andropogoneae.

Key words: *Eriochrysis*; low-copy nuclear genes; plastome; polyploidy; reticulate evolution; Saccharinae; *Saccharum*; species delimitation.

INTRODUÇÃO GERAL

1. A família Poaceae

Poaceae (Gramineae) é uma das maiores famílias de Angiospermas, incluindo cerca de 800 gêneros e mais de 11.000 espécies, com distribuição cosmopolita (Watson & Dallwitz 1992, GPWG II 2012). No Brasil, ocorrem cerca de 225 gêneros e 1.500 espécies (Filgueiras *et al.* 2015). A importância ecológica e econômica dessa família é indiscutível, pela dominância em vários ecossistemas vegetais, pela utilização na alimentação dos animais e pelo uso dos cereais no regime alimentar do homem. Pertencem a essa família as quatro espécies mais cultivadas no mundo: o trigo (*Triticum aestivum* L.), o arroz (*Oryza sativa* L.), o milho (*Zea mays* L.) e a cana-de-açúcar (*Saccharum officinarum* L.), alimentos básicos para o homem (Rúgolo de Agrasar & Puglia 2004). Cerca de 70% das terras cultivadas do mundo estão cobertas por gramíneas e mais de 50% das calorias consumidas pela humanidade provêm destas (Judd *et al.* 2009).

A família Poaceae pertence à ordem Poales, juntamente com Cyperaceae, Juncaceae, Eriocaulaceae e Bromeliaceae, entre outras (APG III 2009). Poaceae está atualmente dividida em 12 subfamílias (GPWG II 2012), incluindo um grado de três linhagens (subfamílias Anomochlooideae, Pharoideae e Puelioideae) e os clados comumente denominados BEP (Bambusoideae, Ehrhartoideae e Pooideae) e PACMAD (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae e Danthonioideae).

A subfamília Panicoideae inclui cerca de um terço de todas espécies de gramíneas, sendo fortemente suportada como monofilética (GPWG II 2012). Seus representantes apresentam geralmente espiguetas com dois antécios, o superior com flor bissexuada, o inferior neutro ou com flor estaminada. Além disso, a articulação entre a ráquila e o pedicelo das espiguetas está localizada abaixo das glumas, as quais são caducas com os antécios maduros (Longhi-Wagner 2012). Panicoideae inclui três grandes tribos (Andropogoneae, Paniceae *s.s.* e Paspaleae), além de diversas outras tribos menores (Sánchez-Ken & Clark 2010, Morrone *et al*. 2012).

2. A tribo Andropogoneae

Andropogoneae apresenta cerca de 90 gêneros e 1.060 espécies, com distribuição cosmopolita (Sánchez-Ken & Clark 2010). Inclui plantas de grande importância econômica, como a cana-de-açúcar, o milho e o sorgo (*Sorghum bicolor* (L.) Moench), bem como muitas espécies ecologicamente dominantes em pastagens tropicais e temperadas. Seus representantes apresentam espiguetas dispostas aos pares em cada nó da ráquis, uma séssil e uma pedicelada

(muito raramente ambas pediceladas, então pedicelos desiguais em comprimento), geralmente caindo em conjunto com o entrenó da ráquis frágil na maturação, menos comumente as duas espiguetas caindo separadas. Além disso, as espiguetas possuem dois antécios com lemas hialinos, bem menos consistentes do que as glumas. As espécies de Paniceae e Paspaleae, por outro lado, apresentam espiguetas isoladas ou em pares, então ambas pediceladas, caindo separadamente; o lema do antécio superior é rígido e geralmente mais consistente do que as glumas, enquanto o lema do antécio inferior tem consistência semelhante às mesmas (Longhi-Wagner *et al.* 2001).

Do ponto de vista nomenclatural, havia grande controvérsia na literatura sobre o correto nome para a tribo. O nome Andropogoneae Dumortier (1824) vinha sendo tradicionalmente usado em floras do mundo inteiro (e.g., Clayton 1972, Coutinho 1974, Tsvelev 1984, Nicora & Rúgolo de Agrasar 1987, Barkworth *et al.* 2003, Jessop *et al.* 2006), bem como em artigos científicos das mais diversas áreas (e.g., Gould 1956, Brown & Emery 1957, Sánchez-Ken & Clark 2010, Scrivanti 2010, Nagahama *et al.* 2012). No entanto, Reveal (2004) propôs que o nome Sacchareae Martinov (1820) teria prioridade sobre Andropogoneae e, portanto, seria o nome correto para a tribo. Com base nisso, o nome Sacchareae foi adotado na *World-wide Phylogenetic Classification of Poaceae* (Soreng *et al.* 2013) e no banco de dados *Tropicos* (Tropicos 2014). O nome foi adotado também em diversas publicações recentes (e.g., Besnard *et al.* 2013, Peichoto 2013, Scataglini & Zuloaga 2013, Vorontsova *et al.* 2013). Já na *Flora Argentina*, por exemplo, tanto o nome Andropogoneae como Sacchareae foram usados, o primeiro na chave para as tribos de Panicoideae (Zuloaga *et al.* 2012: 250) e o segundo no restante do texto (Zuloaga *et al.* 2012: 490). Este fato exemplifica a confusão que havia sobre o correto nome da tribo. Essa questão nomenclatural foi resolvida por Welker *et al.* (2014), um dos artigos da presente tese, confirmando Andropogoneae como o nome que deve ser utilizado para a tribo.

Do ponto de vista filogenético, Andropogoneae é fortemente suportada como monofilética e *Arundinella* Raddi constitui seu grupo irmão (Sánchez-Ken & Clark 2010, Estep *et al.* 2014). A topologia das árvores filogenéticas que têm sido publicadas de Andropogoneae, com ramos internos muito curtos na base do agrupamento e ramos terminais relativamente longos, sugere que a tribo resultou de uma radiação rápida (Mathews *et al.* 2002, Teerawatananon *et al.* 2011, Estep *et al.* 2014). Segundo Kellogg (2000), esses ramos curtos não refletem homoplasia ou ambiguidade, mas sim um pequeno número de mutações, o que indica que a tribo apresentou uma rápida diversificação inicial. De acordo com Mathews *et al.* (2002), a fragilidade da ráquis, que permitiu que o par de espiguetas constituísse a unidade de dispersão mais comum em Andropogoneae (em vez da espigueta isolada, do antécio ou da cariopse, individualmente), pode

ter desempenhado um papel importante nesse rápido aparecimento de linhagens dentro da tribo.

Poliploidia e evolução reticulada são comuns na tribo Andropogoneae (Estep *et al.* 2014, Kim *et al.* 2014). Estep *et al.* (2014) demonstraram que pelo menos um terço das espécies de Andropogoneae é resultado de alopoliploidia, com um número elevado de eventos independentes de alopoliploidização ocorridos na tribo. Análises filogenéticas recentes indicam a presença de um clado "core Andropogoneae", incluindo *Andropogon* L., *Schizachyrium* Nees, *Hyparrhenia* Andersson ex E. Fourn., *Bothriochloa* Kuntze e vários outros gêneros. Por sua vez, *Saccharum* L. e *Eriochrysis* P. Beauv. estão localizados fora do clado "core Andropogoneae" (Mathews *et al.* 2002, Estep *et al.* 2014). Clayton & Renvoize (1986) dividiram a tribo Andropogoneae em 11 subtribos, baseados principalmente em caracteres morfológicos relacionados à inflorescência e às espiguetas. *Saccharum* e *Eriochrysis* foram incluídos na subtribo Saccharinae, juntamente com cerca de outros 10 gêneros (Clayton & Renvoize 1986). Análises moleculares recentes não têm suportado a maioria dessas subtribos como monofiléticas (Kellogg 2000, Hodkinson *et al.* 2002, Mathews *et al.* 2002, Teerawatananon *et al.* 2011, Estep. *et al.* 2014). No entanto, ainda não foi proposta nenhuma reorganização para as mesmas, com base nesses estudos.

3. Complexos de espécies em Andropogoneae

Delimitar espécies é uma tarefa de fundamental importância dentro da Biologia Evolutiva, bem como para a conservação da biodiversidade (Carstens *et al.* 2013). No entanto, a delimitação de espécies não é algo simples. Segundo Souza-Chies & Longhi-Wagner (2003), esta é uma das tarefas mais árduas do taxonomista devido à dificuldade em avaliar a variação existente no nível de espécie, ou seja, saber quando se está tratando de táxons distintos ou, simplesmente, acessando a variabilidade existente entre indivíduos de um mesmo táxon. Segundo Spangler (2000), descontinuidades morfológicas úteis na delimitação de táxons estão essencialmente ausentes em Andropogoneae, o que dificulta bastante a taxonomia do grupo. Devido a isso, grande parte das primeiras espécies descritas em Andropogoneae foi incluída no gênero típico, *Andropogon*, o qual foi sendo posteriormente desmembrado. Isso reflete a dificuldade em distinguir grupos discretos dentro da tribo (Spangler 2000).

Durante o levantamento florístico de alguns gêneros da tribo Andropogoneae no sul do Brasil, entre eles *Saccharum* e *Eriochrysis* (Welker 2010), constatou-se a presença de diversos complexos morfológicos de espécies, nos quais não foi possível resolver a circunscrição das mesmas apenas com a morfologia. Como exemplo destes, podem ser citados os complexos de espécies: "*Saccharum villosum–S. angustifolium*" (Fig. 1), "*Eriochrysis cayennensis–E. villosa*" (Fig. 2) e "*Eriochrysis warmingiana–E. laxa*" (Fig. 2).

O gênero *Saccharum*, em seu sentido amplo, compreende 35-40 espécies de regiões tropicais e subtropicais do mundo (Clayton & Renvoize 1986). Alguns autores consideram *Erianthus* Michx. como um gênero distinto, com cerca de 28 espécies das Américas, África, Europa e Ásia (Mukherjee 1958, Molina 1981, Watson & Dallwitz 1992). Neste caso, a principal diferença morfológica aceita entre esses gêneros é a presença (em *Erianthus*) ou a ausência (em *Saccharum s.s.*) de arista nas espiguetas (Mukherjee 1958; Cheavegatti-Gianotto *et al.* 2011). As espécies de *Erianthus* do Velho Mundo são agrupadas em uma seção diferente das americanas (*Erianthus* sect. *Ripidium* (Trin.) Henrard) ou em um gênero distinto (*Ripidium* Trin.), por diferentes autores (Grassl 1972, Besse *et al.* 1997, Hodkinson *et al.* 2002). No entanto, *Ripidium* é um nome ilegítimo e o nome *Tripidium* H. Scholz foi proposto para substituí-lo (Valdés & Scholz 2006). A circunscrição de *Saccharum* e gêneros relacionados é bastante controversa e permanece não resolvida.

Além da controvérsia taxonômica em relação aos gêneros aceitos, a circunscrição das espécies sulamericanas de *Saccharum s.l.* também é bastante complexa, sendo aceitas de três a nove espécies para a região, dependendo do autor (Swallen 1966, Molina 1981, Smith *et al.* 1982, Filgueiras 2003b). Diversas espécies tradicionalmente citadas para o sul do Brasil e países limítrofes (Swallen 1966, Molina 1981, Smith *et al.* 1982), sob o gênero *Erianthus*, têm sido tratadas, mais recentemente, como sinônimos de *Saccharum villosum* Steud. (Filgueiras 2003b, Morrone *et al.* 2008, Filgueiras & Welker 2015b). Como exemplo destas, podem ser citadas: *Erianthus balansae* Hack., *E. clandestinus* Swallen, *E. glabrinodis* (Hack.) Swallen, *E. purpureus* Swallen e *E. trinii* (Hack.) Hack. Espécimes aceitos como *Saccharum villosum*, tanto em herbário quanto nas descrições da literatura, apresentam grande variabilidade fenotípica, especialmente nas dimensões e no indumento das folhas e dos colmos, bem como no formato das lâminas foliares, sugerindo que talvez não se trate de um único táxon (Welker & Longhi-Wagner 2012).

Saccharum villosum e *S. angustifolium* (Nees) Trin. ocorrem em simpatria na América do Sul e podem ser diferenciadas, mesmo no campo, por caracteres vegetativos. *Saccharum angustifolium* apresenta lâminas foliares lineares, com 2-6 mm de largura, e com a nervura central bastante evidente em toda a extensão da lâmina. *Saccharum villosum*, por outro lado, apresenta lâminas foliares lanceoladas, com 7-20 mm de largura, e com a nervura central pouco evidente na porção superior da lâmina (Welker & Longhi-Wagner 2012). No entanto, vários espécimes coletados no sul do Brasil, na Argentina e no Uruguai apresentam morfologia intermediária entre as duas espécies, ou seja, lâminas foliares com padrão de nervura semelhante a *S. villosum*, mas estreitas como em *S. angustifolium*. Essas plantas foram denominadas *Saccharum* aff. *villosum* por Welker & Longhi-Wagner (2012). Baseado em observações

morfológicas, estes autores sugeriram que os indivíduos com morfologia intermediária poderiam ser híbridos entre *S. villosum* e *S. angustifolium*. É interessante mencionar que diversos híbridos interespecíficos e intergenéricos já foram documentados em *Saccharum* e gêneros relacionados (Hodkinson *et al.* 2002, Nair *et al.* 2005, Aitken e*t al.* 2007).

O gênero *Eriochrysis* inclui entre sete e 11 espécies distribuídas, principalmente, nas Américas, com algumas poucas espécies na África e na Índia (Clayton & Renvoize 1986, Watson & Dallwitz 1992, Clayton *et al.* 2006). A circunscrição de suas espécies é bastante controversa, o que pode ser evidenciado pelo número discrepante de espécies aceito por diferentes autores.

Eriochrysis cayennensis P. Beauv. é o tipo do gênero e a espécie que apresenta a maior distribuição nas Américas, ocorrendo desde os Estados Unidos até a Argentina, o Brasil e o Uruguai (Filgueiras 2003a). Apresenta inflorescências densamente pilosas e a gluma inferior das espiguetas com o ápice obtuso a truncado, conspicuamente trilobado (Welker & Longhi-Wagner 2012). A espécie é morfologicamente semelhante a *E. villosa* Swallen, descrita como sendo de ocorrência restrita ao sul do Brasil (Swallen 1966), a qual é diferenciada, principalmente, pelo ápice agudo da gluma inferior, o qual não apresenta lobos (Welker & Longhi-Wagner 2012). *Eriochrysis villosa* é aceita como uma espécie independente por diversos autores (e.g., Swallen 1966, Smith *et al.* 1982, Welker & Longhi-Wagner 2012, Filgueiras & Welker 2015a), porém foi considerada um provável sinômino de *E. cayennensis* por Filgueiras (2003a) e um "táxon duvidoso" por Morrone *et al.* (2008). Diversos indivíduos coletados no sul do Brasil apresentam morfologia intermediária quanto ao ápice da gluma inferior das espiguetas, que se apresenta subagudo e com lobos inconspícuos. Essas plantas foram consideradas como uma variação morfológica de *E. villosa* por Welker & Longhi-Wagner (2012), mas é possível que se tratem de híbridos naturais entre *E. cayennensis* e *E. villosa*. Killeen (1990) descreveu *Eriochrysis* × *concepcionensis* Killeen como um híbrido entre *E. cayennensis* e *E. laxa*, baseado em uma população com morfologia intermediária entre as duas espécies encontrada em Santa Cruz, na Bolívia. Uma vez que evolução reticulada é bastante comum na tribo Andropogoneae (Estep *et al.* 2014), é possível que hibridações entre outras espécies de *Eriochrysis* também ocorram no sul do Brasil.

A circunscrição de *Eriochrysis laxa* Swallen e *E. warmingiana* (Hack.) Kuhlm. também é controversa. As duas espécies são aceitas como distintas por diversos autores (e.g., Swallen 1966, Morrone *et al.* 2008, Filgueiras & Welker 2015a), mas *E. laxa* foi considerada um provável sinônimo de *E. warmingiana* por Filgueiras (2003a). As diferenças morfológicas entre as duas espécies são mais acentuadas do que entre *E. cayennensis* e *E. villosa*, discutidas no parágrafo anterior. *Eriochrysis laxa* apresenta espiguetas obovadas com a gluma inferior de ápice

arredondado a obtuso, enquanto em *E. warmingiana* as espiguetas são lanceoladas com a gluma inferior de ápice agudo a acuminado. Além disso, esta última espécie apresenta inflorescências mais longas e com os ramos basais divergentes, enquanto em *E. laxa* os ramos basais da inflorescência são adpressos ao eixo principal (Swallen 1966). No entanto, além das diferenças morfológicas, não há outras evidências disponíveis que indiquem que sejam duas espécies distintas.

O conhecimento da flora agrostológica é de fundamental importância para o embasamento de estudos fitossociológicos e ecológicos em áreas campestres. A correta identificação das espécies que compõem as pastagens é de grande interesse para a utilização desses recursos naturais. O conhecimento da família Poaceae e de suas espécies adquire especial interesse no sul do Brasil, onde a maior parte da pecuária é baseada em pastagens naturais (Boldrini *et al.* 2008).

Devido à impossibilidade de delimitar, satisfatoriamente, as espécies citadas acima apenas com evidências morfológicas, um estudo utilizando ferramentas moleculares se mostrou necessário para definir a circunscrição dos complexos de espécies observados.

4. Importância de estudos moleculares

Segundo Koopman *et al.* (2008), um dos requisitos para que um caráter seja confiável, do ponto de vista filogenético, é que o modo e a direção da evolução do mesmo sejam representativos do modo e da direção da evolução das espécies como um todo. Os caracteres morfológicos, no entanto, geralmente estão sob intensa pressão de seleção, o que pode resultar em similaridades fenotípicas entre espécies filogeneticamente distantes que estão adaptadas a condições ambientais semelhantes, bem como em diferenças morfológicas marcantes entre espécies relacionadas, mas que estão adaptadas a condições distintas (Koopman *et al.* 2008). Devido a isso, em certas situações, a morfologia pode ser um falso indicador do relacionamento entre as espécies, sendo necessária uma fonte alternativa de informação filogenética (Koopman *et al.* 2008).

A delimitação de espécies com base em evidências moleculares, no entanto, também não é uma tarefa fácil, devido, entre outras razões, à baixa variabilidade dos marcadores comumente utilizados para reconstruções filogenéticas (Sang 2002, Després *et al.* 2003). A ocorrência de hibridação e poliploidia torna a situação ainda mais complicada (McDade 1992), fatores estes comuns entre os representantes da tribo Andropogoneae (Estep *et al.* 2014, Kim *et al.* 2014).

Genes nucleares de cópia única (*low-copy nuclear loci*) têm se mostrado marcadores eficientes para resolver as relações filogenéticas entre gêneros e espécies de Andropogoneae, devido à alta variabilidade das sequências e à sua capacidade de identificar híbridos (Sang 2002, Estep *et al.* 2012, Triplett *et al.* 2012, Liu *et al.* 2014). Embora sequências plastidiais e do

espaçador interno transcrito (ITS) do DNA ribossômico nuclear sejam amplamente utilizadas, a baixa variabilidade do genoma plastidial e o elevado número de parálogos em ITS, juntamente com a evolução em concerto incompleta, as tornam inadequadas para análises filogenéticas em Andropogoneae, a não ser que sejam associadas com outros marcadores (Sang 2002, Álvarez & Wendel 2003). Árvores filogenéticas inferidas a partir de genes nucleares são úteis para entender o relacionamento entre táxons poliploides e para identificar eventos de alopoliploidização, pois elas apresentam topologia característica em que cada amostra de espécies poliploides aparece duas vezes na árvore (Sang 2002, Triplett *et al.* 2012, Estep *et al.* 2014). Nessas árvores, espécimes alopoliploides podem ser reconhecidos mesmo na ausência de contagens cromossômicas (Estep *et al.* 2014). Portanto, sequências nucleares de cópia única mostram-se altamente promissoras para inferir relações filogenéticas em *Saccharum* e *Eriochrysis*, bem como para definir a circunscrição taxonômica dos complexos de espécies citados acima.

Os avanços tecnológicos no sequenciamento de DNA nos últimos anos, como o sequenciamento de nova geração (*next-generation sequencing*) através da plataforma *Illumina*, têm permitido a inclusão de um volume cada vez maior de dados nas análises filogenéticas, como o sequenciamento completo do plastoma dos organismos (Steele *et al.* 2012, Straub *et al.* 2012, McCormack *et al.* 2013, Burke *et al.* 2014). Embora o genoma plastidial seja altamente conservado, os enormes *datasets* gerados pelo sequenciamento completo dos plastomas apresentam variação nucleotídica suficiente para inferir relações filogenéticas entre táxons, inclusive na família Poaceae (Givnish *et al.* 2010, Moore *et al.* 2010, Besnard *et al.* 2013, Burke *et al.* 2014). Análises filogenéticas baseadas em plastomas têm demonstrado potencial para aumentar significativamente a resolução e o suporte das árvores, mesmo em níveis taxonômicos baixos (Parks *et al.* 2009, Straub *et al.* 2012). De acordo com Straub *et al.* (2012), o sequenciamento de nova geração provavelmente irá revolucionar a sistemática vegetal assim como o sequenciamento pelo método de Sanger fez há mais de 20 anos atrás.

Portanto, genes nucleares de cópia única e sequenciamento completo do plastoma mostram-se como fontes valiosas de informação filogenética em grupos de espécies com uma história evolutiva complexa, incluindo poliploidia, hibridação e radiação recente, como *Saccharum*, *Eriochrysis* e demais representantes da tribo Andropogoneae.

Devido à escassez de estudos filogenéticos em *Saccharum* e à ausência destes estudos em *Eriochrysis*, que permitam um melhor entendimento da circunscrição dos mesmos e de suas espécies, e levando em conta as dificuldades encontradas na identificação destas espécies na América do Sul, foi realizado o estudo aqui apresentado com a utilização de marcadores moleculares. Aspectos filogenéticos, taxonômicos e nomenclaturais foram investigados.

A extração de DNA das amostras coletadas foi realizada no laboratório de Sistemática

Molecular de Plantas, da Universidade Federal do Rio Grande do Sul, enquanto a clonagem dos genes nucleares e a análise dos plastomas foram realizadas no laboratório da Drª. Elizabeth Kellogg (Donald Danforth Plant Science Center, St. Louis, MO, U.S.A.), durante o estágio de doutorado sanduíche do autor da presente tese.

5. Objetivos

A presente tese teve como objetivos:

- elucidar a controvérsia em relação ao correto nome para a tribo, Andropogoneae Dumortier ou Sacchareae Martinov;

- investigar a história evolutiva e o posicionamento filogenético de *Saccharum* e *Eriochrysis* em relação aos demais gêneros de Saccharinae e de Andropogoneae;

- analisar a circunscrição taxonômica de complexos de espécies dos gêneros *Saccharum* e *Eriochrysis*, como os complexos "*Saccharum villosum–S. angustifolium*", "*Eriochrysis cayennensis–E. villosa*" e "*Eriochrysis warmingiana–E. laxa*";

- investigar a identidade de espécimes com morfologias intermediárias observadas e definir se são híbridos interespecíficos;

- contribuir para a identificação das espécies de *Saccharum* e *Eriochrysis*, importantes gêneros componentes da flora campestre, e fornecer dados para embasar programas de conservação, através da definição da circunscrição dessas espécies.

6. Organização geral da tese

A presente tese é composta por quatro artigos:

(1) o primeiro deles, publicado no periódico *Taxon*, em 2014, consiste em um artigo nomenclatural que discute a controvérsia sobre o nome correto para a tribo, Andropogoneae ou Sacchareae;

(2) o segundo artigo, publicado no periódico *American Journal of Botany*, em 2015, consiste na análise filogenética do gênero *Saccharum* baseada em diversos genes nucleares de cópia única, com ênfase na circunscrição das espécies sulamericanas;

(3) o terceiro, publicado no periódico *Phytotaxa*, em 2012, relata a descoberta da ocorrência de *Eriochrysis laxa* no estado do Rio Grande do Sul, e apresenta uma chave para as espécies brasileiras do gênero;

(4) o último artigo, a ser submetido ao periódico *Molecular Phylogenetics and Evolution*, consiste na análise filogenética do gênero *Eriochrysis*, com ênfase na delimitação das espécies americanas, análise esta baseada em diversos genes nucleares de cópia única e no sequenciamento completo do plastoma.

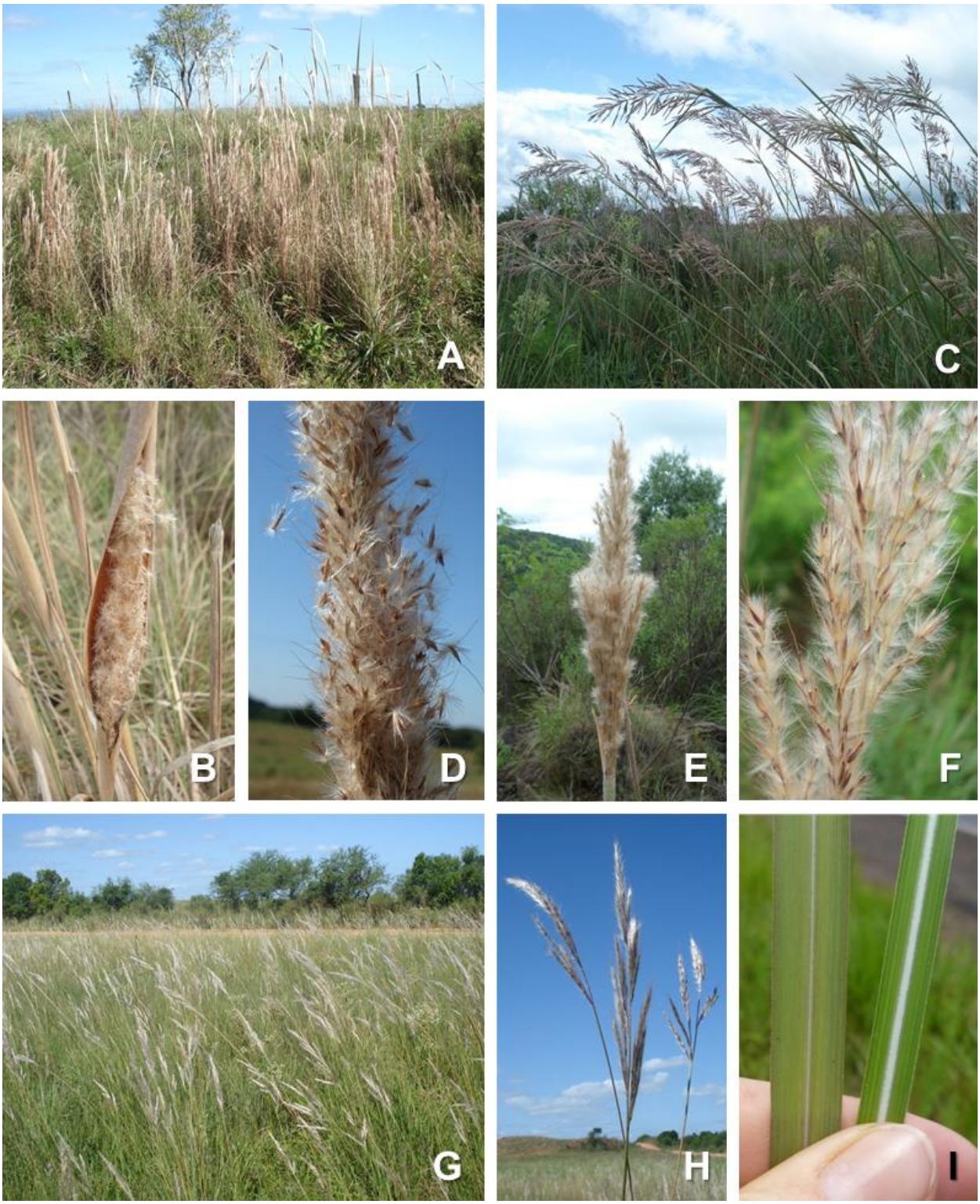

FIGURA 1. Fotos de espécies de *Saccharum* no campo. **A–B.** *S. angustifolium*. **A.** hábito. **B.** inflorescência. **C–D.** *S. asperum*. **C.** hábito. **D.** porção da inflorescência. **E–F.** *S. villosum*. **E.** inflorescência. **F.** porção da inflorescência. **G–H.** *Saccharum* aff. *villosum*. **G.** hábito. **H.** inflorescências. **I.** Porção da lâmina foliar de *S. villosum* (esq.) e *Saccharum* aff. *villosum* (dir.). Fotos: H.M. Longhi-Wagner (A–F, I) e C.A.D. Welker (G–H).

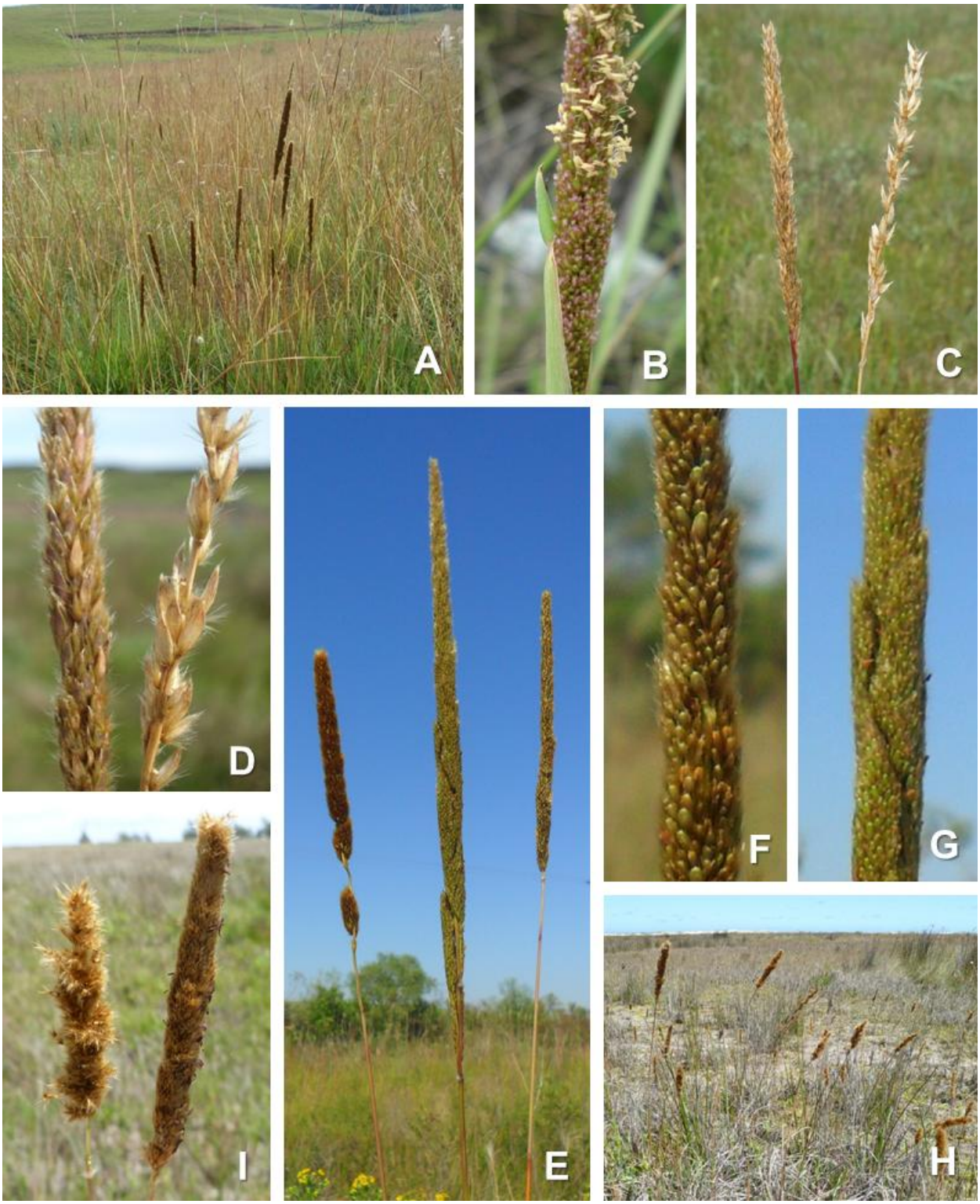

FIGURA 2. Fotos de espécies de *Eriochrysis* no campo. **A–B.** *E. cayennensis*. **A.** hábito. **B.** porção da inflorescência. **C–D.** *E. holcoides*. **C.** inflorescências. **D.** porção das inflorescências. **E.** Inflorescências de *E. cayennensis* (esq.), *E. villosa* (centro) e *E. laxa* (dir.). **F.** *E. laxa*, porção da inflorescência. **G.** *E. villosa*, porção da inflorescência. **H–I.** *Eriochrysis* sp. ("lobos inconspícuos"). **H.** hábito. **I.** inflorescências. Fotos: H.M. Longhi-Wagner (A–D, H–I) e C.A.D. Welker (E–G).

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CAPÍTULO I:

Andropogoneae versus Sacchareae (Poaceae: Panicoideae):
the end of a great controversy

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Andropogoneae versus *Sacchareae* (*Poaceae* - *Panicoideae*): the end of a great controversy

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Abstract The grass tribe *Andropogoneae* includes plants of great economic importance, such as maize, sugarcane, sorghum, and others. Recent works have questioned the use of the name *Andropogoneae* Dumort. (1824) for the tribe and have suggested erroneously that the correct name should be *Sacchareae* Martinov (1820). However, Martinov's name was not published at the rank of tribe. Therefore, it does not threaten the well-established name *Andropogoneae*. In this paper we lay out the basis for the controversy and the rationale for continued use of the name *Andropogoneae*.

Keywords *Andropogoneae*; Martinov; nomenclature; *Poaceae*; *Sacchareae*; *Saccharinea*e; tribal name

INTRODUCTION

Poaceae includes more than 11,000 species and is currently divided into 12 subfamilies (GPWG II, 2012). *Panicoideae* is one of the largest subfamilies, comprising three major tribes (*Andropogoneae*, *Paniceae* s.str. and *Paspaleae*) and several other smaller tribes (Sánchez-Ken & Clark, 2010; Morrone & al., 2012). *Andropogoneae* comprises ca. 90 genera and 1,060 species with a cosmopolitan distribution, and is strongly supported as monophyletic (Mathews & al., 2002; Sánchez-Ken & Clark, 2010). It includes some of the world's most economically important plants, such as maize (*Zea mays* L.), sugarcane (S*accharum officinarum* L.) and sorghum (*Sorghum bicolor* (L.) Moench), as well as many species of *Andropogon* L., which are dominant in several grassland vegetation formations.

Recent works have questioned the use of the name *Andropogoneae* Dumort. for the tribe, and, as outlined below, have suggested that the correct name should be *Sacchareae* Martinov. The elucidation of the correct name of this tribe is extremely important because it affects a giant user community – not just taxonomists and botanists, but all the plant genome researchers, breeders, and crop scientists who are actively working with sugarcane, sorghum, maize, and other species of the tribe. Here, we lay out the basis for the controversy and the rationale for continued use of the name *Andropogoneae*.

ANDROPOGONEAE VERSUS *SACCHAREAE*

The tribal name *Andropogoneae* Dumort. was published in 1824 based on *Andropogon* L. (Dumortier, 1824: 84, 90, 141, as "*Andropogineae*"). In this same publication, Dumortier also proposed another tribal name *Sacchareae* Dumort. (Dumortier, 1824: 83, 90, 141, as "*Saccharinea*e"), whose type is S*accharum* L. Both names were also used in his work of 1829 (Dumortier, 1829: 64). Kunth (1829: 156) adopted the name *Andropogoneae* (as "*Andropogineae*") for the tribe including *Andropogon*, *Saccharum*, and several other genera. On the other hand, Presl (1830: 325) used the tribal name *Sacchareae* (as "*Saccharinae*") for *Andropogon*, *Saccharum*, and related genera. Later, the name *Andropogoneae* was adopted in many other classical taxonomic works of the nineteenth century (e.g., Kunth, 1833: 470; Hooker, 1864: 319; Bentham & Mueller, 1878: 505; Bentham & Hooker, 1883: 1081; Hackel, 1889: 73).

Phylogenetic data show unequivocally that *Andropogoneae* is monophyletic and that *Andropogon* and *Saccharum* are in the same clade (Hodkinson & al., 2002; Mathews & al., 2002; Skendzic & al., 2007; Teerawatananon & al., 2011; GPWG II, 2012). Moreover the phylogenetic structure of the clade indicates that there is no realistic expectation that the clade would be divided into tribes that would necessitate keeping both tribal names (*Andropogoneae* Phylogeny Group, in prep.).

The name *Andropogoneae* is widely used in floras of America (e.g., Renvoize, 1984: 14; Nicora & Rúgolo de Agrasar, 1987: 495; Davidse & al., 1994: 378; Barkworth & al., 2003: 602), Europe (e.g., Merino, 1909: 230; Coutinho, 1974: 74; Tutin, 1980: 264; Cope & Gray, 2009: 51), Africa (e.g., Chippindall, 1955: 453; Stanfield, 1970: 82; Clayton, 1972: 356; Cope, 2005: 321), Asia (e.g., Tsvelev, 1984: 1032; Koyama, 1987: 388; Hsu, 2000: 521; Shouliang & al., 2006: 570), and Oceania (e.g., Guillaumin, 1948: 24; Henty, 1969: 6; Jessop & al., 2006: 509; Clayton & Snow, 2010: 80).

Andropogoneae has also been used in many papers about phylogeny (e.g., Mathews & al., 2002: 441; Skendzic & al., 2007: 530; Sánchez-Ken & Clark, 2010: 1742), morphometrics (e.g.,

Peichoto & al., 2008: 177; Nagahama & al., 2012: 114), cytogenetics (e.g., Church, 1929: 63; Gould, 1956: 395), anatomy and electron microscopy (e.g., Brown & Johnson, 1962: 110; Dávila & Clark, 1990: 499), reproductive biology (e.g., Brown & Emery, 1957: 246; Scrivanti & al., 2009: 644), biogeography (e.g., Zuloaga & al., 2007: 503; Bouchenak-Khelladi & al., 2010: 549), physiology and allelopathy (e.g., Wu & McSteen, 2007: 1745; Scrivanti, 2010: 302), among others. The name has also been used extensively in genetic studies (e.g., Bomblies & Doebley, 2005: 1082; Wang & Dooner, 2012: 212), and works of breeding and genetic improvement of the species of the tribe, especially sugarcane, sorghum, and related species (e.g., Grassl, 1980: 41; Dillon & al., 2007: 975; Vermerris, 2008: 249, 274).

Reveal (2004) recently proposed that the name *Sacchareae* had been validly published in Jul–Aug 1820 by the Russian botanist Ivan Ivanovic Martinov (sometimes spelled Ivan Ivanovich Martynov or Jean Martinoff) in his "Tekhno-Botanicheskĭĭ Slovar'" [Technical-botanical Dictionary] at the rank of tribe (Martinov, 1820: 556, as "*Saccharineae*"). According to Reveal (2004), the name was validated by a reference to the name of Kunth "Gramina Saccharina" (Kunth, 1815: 74). Therefore, according to Reveal (2004), the name *Sacchareae* Martinov would have priority over *Andropogoneae* Dumort. and would be the correct name for the tribe. Based on this claim, the name *Sacchareae* Martinov was adopted in the World-wide Phylogenetic Classification of Poaceae (Soreng & al., 2013) and in the Tropicos database (Tropicos, 2014). This name has also been adopted at tribal status in several recent publications (e.g., Zuloaga & al., 2012: 490; Besnard & al., 2013: 1063; Peichoto, 2013: 20; Scataglini & Zuloaga, 2013: 1078; Vorontsova & al., 2013: 1). In Flora Argentina, for example, both names *Andropogoneae* and *Sacchareae* were used, the former in the key for tribes of *Panicoideae* (Zuloaga & al., 2012: 250) and the latter in the remainder of the text (Zuloaga & al., 2012: 490). This exemplifies the current confusion about the correct name of this tribe.

According to Reveal (2004), *Sacchareae* and seven other grass tribal names were published by Martinov (1820): *Agrostideae*, *Bromeae*, *Chlorideae*, *Hordeeae* (as "*Hordeaceae*"), *Olyreae*, *Oryzeae*, and *Stipeae* (as "*Stipaceae*"). These names were also included in Reveal's Indices Nominum Supragenericorum Plantarum Vascularium with tribal status (Reveal, 2011). None of these names is accompanied by a description or diagnosis in Martinov (1820) but, according to Reveal (2004, 2011), they were validated by references to unranked names and descriptions published by Kunth (1815). In fact, the reference to Kunth is presented in all these names except *Agrostideae* (Martinov, 1820: 14). Reveal (2004) states that "each name was assigned the rank of 'koleno' or tribe by Martinov".

We have examined the publication of Martinov and have consulted with three Russian-speaking colleagues. The word "koleno" (колено) was used only for the name *Agrostideae*

(Martinov, 1820: 14). For the other seven names, Martinov used the word "otryad" (отрядъ), which is a general word, not meaning any specific taxonomic rank. This is completely clear in the names *Chlorideae* (Martinov, 1820: 125) and *Stipaceae* (p. 601), where Martinov used the word "otryad" and then, in parenthesis, the word "gryppa" (группа), which means simply "group".

Martinov (1820: 28) also published the name *Andropogones*, which was described as "the fourth family ['semeistvo' – семейство] of the fifth class, according to Augier [1801]". The word "semeistvo" was also used in the names *Alopecurinae* (p. 19), *Avenaceae* (p. 60), *Meliceae* (p. 390), and *Nardinae* (p. 413), for example. All these names were included in Reveal (2011) at family level. The following names published by Martinov (1820) are currently conserved and listed in Appendix IIB of the ICBN (McNeill & al., 2006) as family names with Martinov given as author: *Aizoaceae* ("*Aizoonides*"), *Cannabaceae* ("*Cannabinae*"), *Eriocaulaceae* ("*Eriocauleae*"), *Lamiaceae* ("*Lamieae*"), *Lemnaceae* ("*Lemnoides*"), *Moringaceae* ("*Moringeae*"), *Parnassiaceae* ("*Parnassiae*") *Resedaceae*, *Roridulaceae* ("*Roriduleae*"), *Staphyleaceae* ("*Staphyleae*"), and *Zingiberaceae* ("*Zinziberaceae*").

The name *Saccharineae* (Martinov, 1820: 556) was described as "the seventh 'group' ['otryad'] of the family *Gramineae*, according to Kunth [1815]; according to Augier [1801], the sixth family ['semeistvo'] of the fifth class". This sentence suggests that Martinov did not propose the name *Saccharineae* with either tribal status or family status. He simply listed the ranks in which this name was used in previous works, without concluding which rank would be correct in his opinion. Nevertheless, since Martinov provided a Latin ending and cited earlier descriptions (provided by both Augier, 1801, and Kunth, 1815), as alternative names (Art. 36.2 of the ICN, McNeill & al., 2012) the 'group' name could be considered as validly published but unranked (Art. 37, Ex. 1). By that time, publication at the alternate rank of family had already been achieved (Berchtold & Presl, Jan–Apr 1820: 265).

In a related publication, Sennikov (2006) argues that Martinov did not intend to publish new names, since his work was just a technical dictionary dealing with botanical terminology and nomenclature. According to Martinov (1820: I), the terms and names included in his work were "selected from various foreign and Russian guides, systems and dictionaries". Sennikov (2006: 144–145) claims that Martinov did not accept as correct any of these names, or we would need to assume that "he equally 'accepted' all the names that ever appeared in botanical literature", sometimes "more than one [botanical system] for the same taxon". Thus, according to Sennikov (2006), all these names should be considered not validly published based on Art. 36.1(a) of the ICN (McNeill & al., 2012). We do not feel qualified to comment on the disposition of all of

Martinov's names, but the argument of Sennikov (2006) appears relevant to the disposition of *Saccharineae* Martinov.

Based on the foregoing arguments, the name *Saccharineae* Martinov is either not validly published or is validly published but unranked. In either case, the name does not compete with any name at the rank of tribe (Art. 11.2–11.3 of the ICN, McNeill & al., 2012), such as *Andropogoneae* Dumort.

CONCLUSION

Since Martinov's name *Saccharineae* was not published at the rank of tribe, the well-established name *Andropogoneae* Dumort. is the earliest and correct name for the tribe including *Andropogon*, *Saccharum*, and related genera. Therefore, the name *Sacchareae* Martinov should not be applied to this tribe.

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CAPÍTULO II:

Phylogenetic analysis of *Saccharum s.l.* (Poaceae; Andropogoneae), with emphasis on the circumscription of the South American species

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PHYLOGENETIC ANALYSIS OF *SACCHARUM S.L.* (POACEAE – ANDROPOGONEAE), WITH EMPHASIS ON THE CIRCUMSCRIPTION OF THE SOUTH AMERICAN SPECIES[1]

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Premise of the study: Polyploidy and reticulate evolution are often a complication for discovering phylogenetic relationships between genera and species. Despite the huge economic importance of sugarcane (*Saccharum officinarum* – Poaceae, Andropogoneae), the limits of the genus *Saccharum* and its species are complex and largely unresolved, involving both polyploidy and reticulate evolution. This study aimed to assess the phylogenetic relationships of *Saccharum s.l.*, including *Erianthus* and *Tripidium*, as well as investigate the taxonomic circumscription of the South American species of the genus.

Methods: Molecular cloning and sequencing of five regions of four low-copy nuclear loci were performed, including *Aberrant panicle organization*1 (*apo*1), *Dwarf*8 (*d*8), two exons of *Erect panicle*2 (*ep*2-*ex*7 and *ep*2-*ex*8), and *Retarded palea*1 (*rep*1).

Key results: The allopolyploid origin of *Saccharum* was demonstrated using evidence from nuclear genes. The samples of *Saccharum s.l.* grouped in two distinct clades, with *S. arundinaceum* and *S. ravennae* (= *Tripidium*, or *Erianthus* sect. *Ripidium*) apart from all other species analyzed of the genus. *Saccharum angustifolium, S. asperum*, and *S. villosum* correspond to distinct clades (different species). The plants with intermediate morphology between *S. angustifolium* and *S. villosum* presented a pattern of paralogues consistent with a hybrid origin.

Conclusions: Saccharum s.l. is polyphyletic and *Tripidium* should be recognized as a distinct genus. However, no strong evidence was found to support the segregation of *Erianthus.* The taxonomic circumscription of the South American species of the genus was resolved and the occurrence of natural hybrids was documented. Better understanding of the phylogenetic relationships of *Saccharum* and relatives may be useful for sugarcane breeders to identify potential taxa for interspecific and intergeneric crosses in the genetic improvement of sugarcane.

Key words: *Erianthus*; hybridization; low-copy nuclear loci; polyploidy; *Ripidium*; species complex; species delimitation; sugarcane; *Tripidium.*

Polyploidy and reticulate evolution often complicate efforts in phylogenetic reconstruction of relationships between genera and species (McDade, 1992; Triplett et al., 2012). The two processes are common in the genus *Saccharum* L. (Poaceae – Andropogoneae), which includes one of the most important crops in the world, sugarcane (*S. officinarum* L.), whether measured by tons harvested or by dollar value (Boddey et al., 2008; Bonnett and Henry, 2011). In addition to being the major source of sugar for human consumption, sugarcane is also a source of ethanol for biofuel that powers many parts of the world, notably Brazil (Boddey et al., 2008). Despite this immense value, the limits of the genus and the species within it are complex, contentious, and largely unresolved. Species from the New World have been variously classified in *Saccharum* and *Erianthus* Michx., with different authors combining the two or keeping them separate (Mukherjee, 1958; Molina, 1981; Clayton and Renvoize, 1986; Amalraj and Balasundaram, 2006). Adding to the confusion has been difficulty in determining the limits of species (Welker and Longhi-Wagner, 2012).

Saccharum and *Erianthus* are both members of the tribe Andropogoneae, in the subfamily Panicoideae of the Poaceae. The tribe Andropogoneae, erroneously called Sacchareae by some authors (see Welker et al., 2014), comprises ca. 90 genera and 1060 species with a cosmopolitan distribution (Sánchez-Ken and Clark, 2010). It includes some of the world's most economically important plants, such as sugarcane, maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* (L.) Moench), as well as many ecologically dominant species of tropical and temperate grasslands. Andropogoneae is strongly supported as monophyletic, and *Arundinella* Raddi (tribe Arundinelleae) is its sister group (Mathews et al., 2002; Sánchez-Ken and Clark, 2010). Recent phylogenetic analyses have suggested that the very short branches along the backbone of the trees have been caused by a rapid evolutionary radiation near the base of the Andropogoneae clade (Mathews et al., 2002; Teerawatananon et al., 2011; Estep et al., 2014). Phylogenetic analyses indicate the presence of a "core Andropogoneae" clade, including *Andropogon* L.,

Schizachyrium Nees, *Hyparrhenia* Andersson ex E. Fourn., and *Bothriochloa* Kuntze, among others. *Saccharum* and *Miscanthus* Andersson are closely related genera, and are placed outside the "core Andropogoneae" (Hodkinson et al., 2002; Mathews et al., 2002; Estep et al., 2014).

The genus *Saccharum*, in the broad sense including the species of *Erianthus*, comprises 35-40 species from tropics and subtropics of the world (Clayton and Renvoize, 1986). Some authors have considered *Erianthus* as a distinct genus, with ca. 28 species from North and South America, Africa, Europe, and Asia (Mukherjee, 1958; Molina, 1981; Watson and Dallwitz, 1992). The main morphological difference between these genera is the awned spikelets in *Erianthus* and the awnless spikelets in *Saccharum s.s.* (Mukherjee, 1958). The Old World species of *Erianthus* are grouped in a different section (*Erianthus* sect. *Ripidium* (Trin.) Henrard) or a distinct genus (*Ripidium* Trin.) by different authors (Grassl, 1972; Besse et al., 1997; Hodkinson et al., 2002). However, *Ripidium* Trin. is an illegitimate name, and the name *Tripidium* H. Scholz was proposed to replace it (Valdés and Scholz, 2006). *Narenga* Bor and *Miscanthidium* Stapf. were also accepted as distinct genera by a few authors (e.g., Clayton, 1972; Watson and Dallwitz, 1992) instead of including their species in *Saccharum* or *Miscanthus*.

The circumscription of *Saccharum* and related genera is controversial. Several phenetic studies indicated strong molecular differentiation between *Saccharum* and *Erianthus* (Besse et al., 1998; Nair et al., 2005; Selvi et al., 2006). On the other hand, a phylogenetic analysis based on the internal transcribed spacer (ITS) of the nuclear ribosomal DNA (Hodkinson et al., 2002) found no support for this division, even though it suggested that *Saccharum s.l* is polyphyletic. However, only a few species of *Erianthus* were included in that analysis, and only one of them was from New World (North America). On the other hand, Hodkinson et al. (2002) suggested that *Tripidium* (under *Ripidium*) may be considered as a distinct genus, as its species grouped together but separate from other *Saccharum s.l.* species in all ITS equally most parsimonious trees (but without support in the trees). The taxonomic delimitation between *Saccharum* and

Miscanthus is also not clear, with intergeneric hybrids occurring between them (Clayton and Renvoize, 1986; Hodkinson et al., 2002).

In addition to the taxonomic controversy at the generic level, the circumscription of the South American species of *Saccharum s.l.* is also convoluted. Filgueiras (2003) recognizes three native species of *Saccharum s.l.* in the region, *Saccharum angustifolium* (Nees) Trin., *S. asperum* (Nees) Steud., and *S. villosum* Steud., in addition to the introduced sugarcane, reducing six previously recognized species of *Saccharum*/*Erianthus* (Swallen, 1966; Molina, 1981; Smith et al., 1982) to synonymy. Five of the synonyms are assigned to *Saccharum villosum* (Filgueiras, 2003; Morrone et al., 2008): *Erianthus balansae* Hack., *E. clandestinus* Swallen, *E. glabrinodis* (Hack.) Swallen, *E. purpureus* Swallen, and *E. trinii* (Hack.) Hack. Specimens identified as *Saccharum villosum s.l.* are morphologically variable, especially in the dimensions and indument of the leaves and culms, and in the shape of the leaf blades, suggesting that *S. villosum* might be more than one taxon (Welker and Longhi-Wagner, 2012).

Saccharum villosum is morphologically similar to *S. angustifolium*, a sympatric species in South America. *Saccharum angustifolium* can be distinguished mainly by the linear leaf blades, narrower than in *S. villosum*, with a conspicuous midvein. *Saccharum villosum* has lanceolate leaf blades with the midvein inconspicuous in the upper portion of the blade (Welker and Longhi-Wagner, 2012) (see Table 1 and Fig. 1). However, some specimens collected in Southern Brazil, Argentina, and Uruguay present an intermediate morphology between the two species. These specimens were identified as *Saccharum* aff. *villosum* Steud. by Welker and Longhi-Wagner (2012). Based on morphological aspects, Welker and Longhi-Wagner (2012) suggested that these specimens with intermediate leaf morphology might be natural hybrids between *S. villosum* and *S. angustifolium*. Both *S. villosum* and *S. angustifolium* have spikelets with pilose glumes, a morphological characteristic that distinguishes them from *S. asperum*, another sympatric species in South America, in which the glumes are glabrous (Table 1, Fig. 1).

Specimens of *S. asperum* do not present as much morphological variability as the species mentioned above (Welker and Longhi-Wagner, 2012).

Polyploidy and reticulate evolution are common in Andropogoneae, as well as in the clade including sugarcane and relatives (Kim et al., 2014; Estep et al., 2014). A recent study has documented that at least one third of Andropogoneae species have resulted from allopolyploidy, with a remarkably high number of independent allopolyploidization events (Estep et al., 2014). Because of this reticulate history, data from low-copy nuclear loci are required to resolve phylogenetic relationships between genera and species (Sang, 2002; Estep et al., 2012; Triplett et al., 2012; Liu et al., 2014). Although plastid markers and ITS have been widely used, the low sequence variability in the plastid genome, and the high number of paralogues plus incomplete concerted evolution in ITS make them inadequate for this purpose (Sang, 2002; Álvarez and Wendel, 2003). Phylogenetic trees inferred from nuclear genes are useful to understand the relationships of polyploid taxa and identify allopolyploidization events, because they produce characteristic double-labeled tree topologies in which the polyploid species appear twice (Sang, 2002; Triplett et al., 2012; Estep et al., 2014). In such trees, allopolyploids can be recognized even in the absence of chromosome counts (Estep et al., 2014).

The current study aimed to (1) test the monophyly of *Saccharum s.l.* and assess its phylogenetic relationships to other genera of Andropogoneae, (2) define the taxonomic circumscription of the South American species of *Saccharum s.l.*, and (3) better understand the identity of the specimens with intermediate morphology between *S. villosum* and *S. angustifolium* (*Saccharum* aff. *villosum*).

MATERIALS AND METHODS

Plant material—Twenty-nine specimens of *Saccharum s.l.* were included in the analysis, as well as 43 species belonging to 34 other genera of Andropogoneae. Two species of *Arthraxon* P.

Beauv. were used as outgroup, since it is well supported as the sister genus to the rest of the tribe (Estep et al., 2014). The sample included material from the type species of the genera *Saccharum*, *Erianthus*, and *Tripidium*: *S. officinarum*, *E. giganteus* (Walter) P. Beauv. (= *Saccharum giganteum* (Walter) Pers.), and *T. ravennae* (L.) H. Scholz. (= *Saccharum ravennae* (L.) L. / *Erianthus ravennae* (L.) P. Beauv.), respectively. (The genus *Erianthus* was described by Michaux (1803) based on *Erianthus saccharoides* Michx., which is a superfluous illegitimate name and a synonym of *E. giganteus* / *Saccharum giganteum* (Tropicos, 2014a)). Voucher specimens and collection localities are listed in Table 2. GenBank accession numbers for the sequences are listed in Appendix 1.

Molecular cloning, sequencing, and data processing—Total genomic DNA was extracted using the CTAB procedure (Doyle and Doyle, 1987), modified for microcentrifuge tubes. Five regions of four low-copy nuclear loci were PCR amplified following Estep et al. (2012): *Aberrant panicle organization*1 (*apo*1), *Dwarf*8 (*d*8), two exons of *Erect panicle*2 (*ep*2-*ex*7 and *ep*2-*ex*8), and *Retarded palea*1 (*rep*1). Previous works show that these loci are efficient markers to infer phylogenetic relationships in the tribe Andropogoneae (Estep et al., 2012; Estep et al., 2014).

The PCR products were purified via gel extraction using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, California, U.S.A.), following the manufacturer's protocol. To capture paralogous copies, purified products were cloned using pGEM-T Easy Vector and transformed into JM109 High-Efficiency Competent Cells (Promega, Madison, Wisconsin, U.S.A.), following manufacturer's protocols. Transformed cells were plated and selected via a blue-white screen on LB agar with X-Gal, isopropyl-beta-thio-galactoside (IPTG), and ampicillin. Eight to twenty-four positive clones of each PCR product were selected. Extracted DNA from the colonies was sent to Beckman Coulter Genomics (Danvers, Massachusetts, U.S.A.) for

sequencing in both directions using universal primers (T7 and M13R). Internal primers were also used for sequencing *d*8 and *ep*2-*ex*7 loci (Estep et al., 2012; Estep et al., 2014).

Chromatogram files were trimmed of vector using Geneious 6.1.8 (Biomatters, Auckland, New Zealand) and ambiguous bases from the ends of both reads were removed manually. Forward and reverse sequences (and sequences from internal primers in *d*8 and *ep*2-*ex*7 loci) were subsequently assembled for each clone. Only clones with 80% or more double-stranded sequence were used for analysis. All good quality contigs for each sample were then aligned using Geneious and primer sequences were removed. Recombinant sequences were identified by eye, comparing them with unambiguous sequences from related species, and were removed from the alignment. The redundant clones of the same gene copy were combined into a consensus sequence, to minimize the inclusion of sequencing errors and reduce the number of sequences to one per paralogue per locus. The resulting sequences were translated and aligned using MUSCLE, as implemented in Geneious.

***Phylogenetic analyses*—**Gene trees were estimated for each locus using RAxML 8.0.9 (Stamatakis, 2006; Stamatakis et al., 2008) using the Black Box setting on the CIPRES Science Gateway (Miller et al., 2010). We used the individual gene tree topologies as a guide to identify the corresponding paralogues of each genome in the five loci, for the polyploid specimens, and create concatenated sequences, according to Estep et al. (2014). The results presented here were based on the dataset with a minimum of three out of five loci per genome for each taxon, except some paralogues of the samples Welker 477, Welker 502, Welker 538, Welker & Peichoto 556, and Welker & Peichoto 584, for which we had only two loci sequenced per genome. Our dataset included 23.4% missing data (for more details, see Appendix 1). The alignment of the combined dataset is presented in Appendix S1 (see Supplemental Data with the online version of this article).

Concatenated trees were reconstructed using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses. The Parsimony Ratchet analysis (Nixon, 1999) was performed in PAUP 4.0b10 (Swofford, 2002) using the companion program PAUPRat (Sikes and Lewis, 2001). Twenty independent runs were performed with 200 iterations each. Support at each node was assessed through bootstrap analysis (Felsenstein, 1985), with a heuristic search based on 1000 replicates. Bootstrap values > 50% were recorded on the trees.

The ML analysis was performed using RAxML 8.0.9 (Stamatakis, 2006; Stamatakis et al., 2008). Models of DNA evolution were determined using jModelTest (Posada, 2008) and the GTR+G model was selected. ML support was assessed via 500 bootstrap replicates, and values > 50% were recorded on the trees. The BI analysis was conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) in parallel (Altekar et al., 2004) under the GTR+G model of evolution and six rate categories. Two independent runs of 20 million generations were performed and sampled every 1000 generations. The consensus tree was estimated after a burn-in of 25% of sampled trees. Convergence of the independent runs was confirmed using AWTY (Nylander et al., 2008). Posterior probability (PP) values > 0.85 were recorded on the trees.

RESULTS

The aligned data matrix including the five low-copy nuclear loci (and excluding specimens of *Saccharum* aff. *villosum*) was 4468 base pairs long, of which 1978 (44%) were variable and 1103 (25%) were parsimony informative. The MP analysis resulted in 1927 equally most parsimonious trees of 3290 steps (CI = 0.48, RI = 0.70).

The trees resulted from MP, ML, and BI analyses were very similar, with short branches along the backbone of the tree, with low support, in contrast to long external branches, with higher support (Fig. 2). The samples of *Saccharum s.l.* fell in two distinct strongly supported

clades (both with full support in MP, ML, and BI analyses). The first clade is formed by *Saccharum ravennae* (type species of the genus *Tripidium*) and *S. arundinaceum* Retz. The second clade includes the remaining representatives of *Saccharum s.l.*, *Miscanthus sinensis* Andersson, and *Pseudosorghum fasciculare* (Roxb.) A. Camus (Fig. 2). However, the relationship between these two clades is unclear. The type species of both *Saccharum* (*S. officinarum*) and *Erianthus* (*E. giganteus* / *Saccharum giganteum*) fell in this second clade, along with all South American species of *Saccharum s.l.* (Fig. 3, type species in bold). This clade included two paralogues per sample for all taxa except *Pseudosorghum fasciculare* (Figs. 2 and 3). Because the genetic loci that we sampled are unlinked, we infer that the two paralogous clades represent the history of two independent genomes that came together in an allopolyploidization event that preceded diversification. For convenience we call these genomes A and B.

We did not discover a paralogue from genome A in the sugarcane (*S. officinarum*) sample included in our analyses, but did find two copies from genome B (called B1 and B2). The two copies grouped closely to paralogue B of the Asian *Saccharum narenga* (Nees ex Steud.) Wall. ex Hack., with strong support in the BI analysis (1 PP), but with weak support in MP and ML analyses (73% MP and ML bootstrap). Paralogue A of *S. narenga* grouped with paralogue A of *S. ecklonii* (Nees) Steud., with strong support (1 PP, 98% ML, 94% MP) (Fig. 3).

The phylogenetic relationships of the South American specimens inferred by both genomes (A and B) were very similar (Fig. 3). All accessions of *Saccharum angustifolium* formed a strongly supported clade in both genomes (genome A: 1 PP, 100% ML and MP; B: 1 PP, 99% ML, 100% MP), as did the specimens of *S. villosum s.l.* (A: 1 PP, 99% ML, 98% MP; B: 1 PP, 96% ML, 92% MP). The *S. villosum* clade is formed by two other well-supported clades based on the paralogues of genome A. The first clade (1 PP, 98% ML, 95% MP) contains robust plants with very wide leaf blades (specimens Welker 396, Welker 477, and Welker & Peichoto 575), and is sister to the clade with the remaining accessions of *S. villosum* (1 PP, 99% ML, 97% MP),

which includes less robust plants with narrower blades. The three specimens with wide blades also grouped together in genome B, but only in the ML analysis and with moderate support (79%) (Fig. 3). On the other hand, the samples of *S. asperum* did not form a monophyletic group; two specimens formed a well-supported clade, apart from the other two specimens analyzed that formed another well-supported clade (Fig. 3).

When the specimens of *Saccharum* aff. *villosum* were included in the tree (Fig. 4), the bootstrap support for most nodes was slightly lower than in the trees without these samples. The aligned combined matrix including these specimens was 4468 base pairs long, of which 1992 (45%) were variable and 1108 (25%) were parsimony informative. The MP analysis for this dataset resulted in 2885 equally most parsimonious trees of 3348 steps (CI = 0.47, RI = 0.71).

The specimens of *Saccharum* aff. *villosum* presented three different paralogues in the trees (except the sample Welker 630, with only two) and the paralogues fell in several distinct clades, both in genomes A and B (Fig. 4). For the specimen Welker 538, for example, the paralogue from genome A grouped in the *S. villosum* clade (0.99 PP, 88% ML, 79% MP), the paralogue from genome B grouped in the *S. angustifolium* clade (0.99 PP, 81% ML, 67% MP) and the third paralogue in a distinct clade (0.96 PP, 75% ML, <50% MP), not closely related with either of the former two clades. On the other hand, the paralogues of the specimen Welker & Peichoto 584 from both genomes A and B grouped in the *S. angustifolium* clades (A: 0.99 PP, 96% ML, 94% MP; B: 0.99 PP, 81% ML, 67% MP), and the third paralogue grouped with the third paralogue of Welker 538. The opposite situation was observed with specimen Welker 502, in which all three paralogues grouped into the *S. villosum* clades, both in genome A and B (A: 0.99 PP, 88% ML, 79% MP; B: 0.99 PP, 69% ML, <50% MP). The other specimens of *Saccharum* aff. *villosum* presented a pattern similar to one of the three described above. For convenience we call the third paralogues of *Saccharum* aff. *villosum* as genomes C and D, because they seem not to be equivalent (Fig.4).

DISCUSSION

Phylogenetic analyses of Saccharum s.l.—The pattern of very short branches along the backbone of the trees indicates that the early diversification in Andropogoneae was probably rapid. Similar topologies suggesting rapid radiation were found by Mathews et al. (2002), Teerawatananon et al. (2011), and Estep et al. (2014). The phylogenetic analyses demonstrate the allopolyploid origin of *Saccharum*. They also suggest that *Saccharum s.l.* is polyphyletic, in agreement with the results of Hodkinson et al. (2002) based on ITS sequence data. The species belonging to section *Ripidium* of the genus *Erianthus* (accepted as the distinct genus *Tripidium* or *Ripidium* by some authors), did not group closely with other species of *Saccharum s.l.* (Fig. 2), indicating that *Tripidium* should be recognized as a distinct genus. However, the relationship of the *Tripidium* clade with the clade containing the remaining representatives of *Saccharum s.l.* remains unclear and requires additional investigation.

There are many distinctions between *Tripidium* and *Saccharum s.l.*, which reinforce their recognition as separate genera. Several studies based on restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) indicate that the species of *Tripidium* are genetically different from other species of *Saccharum s.l.* (Besse et al., 1997, 1998; Selvi et al., 2006; under *Erianthus* sect. *Ripidium*) but the sample of taxa and the methods of data analysis do not provide a rigorous test of the non-monophyly of the group. Biogeography and morphology also support the division. *Tripidium* includes the Old World species previously placed in *Erianthus* (*E.* sect. *Ripidium*), whereas *Erianthus*, in the strict sense, includes only the American species. According to Grassl (1972, under *Ripidium*), the Old World *Tripidium* species are distinct from New World species (*Erianthus s.s.*) in many reproductive morphological characters. The Old World species have three anthers, whereas the New World species have only two. New World species also have floral parts with strong awns and large seeds (presumably adapted for animal dispersal) whereas Old World species are apparently

adapted for wind dispersal (Grassl, 1972; Hodkinson et al., 2002). All these aspects suggest that *Tripidium* should be recognized as a distinct genus, following Grassl (1972, under *Ripidium*). Considering *Tripidium* separate from *Saccharum s.l.* was also suggested by Hodkinson et al. (2002, under *Ripidium*) but with no support in their ITS tree. Note that the name *Ripidium* Trin. is illegitimate, since it is a posterior homonym of the fern genus *Ripidium* Bernh. (Tropicos, 2014b). Therefore, the correct name is *Tripidium* H. Scholz (Valdés and Scholz, 2006).

All other species of *Saccharum s.l.* (i.e., except *S. arundinaceum* and *S. ravennae*) grouped with *Miscanthus sinensis* and *Pseudosorghum fasciculare* in a well-supported clade (Fig. 3). The genus *Miscanthus* is known to be closely related to *Saccharum*, supported by both phylogenetic analysis and documented hybridization between the genera (Clayton and Renvoize, 1986; Hodkinson et al., 2002; Kim et al. 2014). According to Kim et al. (2014), *Saccharum* and *Miscanthus* shared a whole-genome duplication before diversification of genera. *Miscanthus* is polyphyletic, according to the phylogenetic analysis of Hodkinson et al. (2002). *Pseudosorghum* A. Camus is a small genus including only two Asian species. Based only on morphological aspects, the genus was considered more closely related to *Sorghum* Moench than to *Saccharum* (Clayton and Renvoize, 1986), but this was not confirmed in the present phylogenetic analysis. In our analysis *Sorghum* grouped with *Polytrias indica* (Houtt.) Veldkamp, and this clade is the sister group of the "core Andropogoneae" clade, which includes *Andropogon*, *Schizachyrium*, *Hyparrhenia*, and *Bothriochloa* Kuntze, among others. Even though *Saccharum* and *Sorghum* are not very closely related, intergeneric hybridization between them has been documented (Nair et al., 2005).

Our phylogenetic analyses are consistent with two possible treatments of the *Saccharum* clade. The first treatment, which we favor, would consider *Saccharum* in the broad sense (excluding only the species of *Tripidium*), since the type species of both *Saccharum* and *Erianthus* fell in the same clade, closely related to the South American taxa and other species of *Saccharum s.l.* (Fig. 3, type species in bold). The phylogenetic analysis of Hodkinson et al.

(2002) also did not support the segregation of *Erianthus*; however, that work focused on species from Old World and included only *Erianthus contortus* Elliott from North America (no species from South America). The second possibility would be to consider *Saccharum s.s.* (represented by *S. officinarum* in our analyses), *Erianthus* (represented by *Saccharum giganteum*, *S. angustifolium*, *S. asperum*, *S. villosum*, and *Saccharum* aff. *villosum*), *Narenga* (represented by *S. narenga*), and *Miscanthidium* (represented by *S. ecklonii*) as distinct genera, as accepted by some authors based on morphological aspects (Clayton, 1972; Watson and Dallwitz, 1992; Amalraj and Balasundaram, 2006).

The taxonomic uncertainty rests in part on the absence of paralogue A for sugarcane in our analyses, which may have a technical or a biological explanation. The technical explanation is simply that our PCR-based approach failed to amplify the genome A paralogues. This is plausible since sugarcane has high ploidy level (Iwo and Agboire, 1992; Besse et al., 1997) presumably giving rise to many paralogues; however, we sequenced 16-24 clones per locus for this sample making it less likely that we simply missed the genome A paralogue for all loci. If the genome A is present and simply missed by our analyses then it would favor a very broad *Saccharum s.l.*

The biological explanation is complex but cannot be ruled out. In this scenario, an initial A x B hybridization event followed by allopolyploidization gave rise to *Erianthus-Miscanthidium* species, but not to *Saccharum officinarum*. Instead, the ancestor of genome B would have independently given rise to *Saccharum s.s.* In this scenario, *Narenga* might have arisen from a hybrid between *Saccharum s.s.* and *Miscanthidium*, since paralogue B of *Saccharum narenga* grouped with *S. officinarum* and paralogue A with *S. ecklonii*. This scenario would favor a more traditional circumscription of the genera (Clayton, 1972; Watson and Dallwitz, 1992) to reflect their disparate phylogenetic histories (see Fig. 5).

A larger sample is needed for a more accurate answer about the circumscription of *Saccharum* and related genera, as well as to test these hypotheses. Future studies should increase

the number of species of *Saccharum s.l.* from the Old World and North America, as well as a more representative sample of the genus *Miscanthus*, which is part of the complex evolutionary history of sugarcane and relatives. Morphological character evolution and biogeography investigations may also help to better understand the evolution of this group.

Taxonomic circumscription of South American species of Saccharum s.l.—The presence of two (or more) distinct paralogues per sample in the phylogenetic trees indicates that all analyzed South American taxa of *Saccharum s.l.* are polyploid, along with other species of *Saccharum s.l.* and *Miscanthus*. This is in agreement with published chromosome counts available for some of these species (Molina, 1981; Besse et al., 1998) and cytogenetic studies that are currently being performed with the specimens included in our phylogeny (Welker et al., in prep.).

Saccharum angustifolium and *S. villosum* are clearly distinct species, as accepted by Filgueiras (2003) and Welker and Longhi-Wagner (2012). *Saccharum villosum* can be morphologically distinguished from *S. angustifolium* mainly by its lanceolate leaf blades, which are generally pilose and 7-20 mm wide, whereas in *S. angustifolium* the leaf blades are linear, glabrous, and 2-6 mm wide. Moreover, the leaf blades of *S. angustifolium* have a conspicuous whitish midvein up to the apex of the blade, wider than or as wide as the lateral portion of the blade. In *S. villosum*, the whitish midvein is narrower than the lateral portion of the blade and is inconspicuous in the upper portion of the blade (see Table 1 and Fig. 1). These two species also differ in their habitats: *S. angustifolium* occurs in dry grasslands, whereas *S. villosum* inhabits marshlands and wet grasslands (Welker and Longhi-Wagner, 2012). The two species have a broad geographical distribution: *S. angustifolium* occurs from Colombia and Venezuela to Argentina and Uruguay, while *S. villosum* is distributed from Mexico to Argentina and Uruguay (Molina, 1981; Filgueiras, 2003).

Some specimens identified as *Saccharum villosum* from Southern Brazil and Argentina are robust plants with wider leaf blades than the type material and most members of the species (see Table 1 and Fig. 1). These specimens with wide blades represent a monophyletic group that is sister to the clade with the remaining specimens of *S. villosum*, based on sequences from genome A (but without good support based on genome B) (Fig. 3). Several binomials formerly accepted as distinct species by some authors (e.g., Swallen, 1966; Molina, 1981; Smith et al., 1982) are currently considered synonyms of *S. villosum* (Filgueiras, 2003; Morrone et al., 2008); these names include *Erianthus balansae*, *E. clandestinus*, *E. glabrinodis*, *E. purpureus*, and *E. trinii*. Although Welker and Longhi-Wagner (2012) suggested that "*S. villosum s.l.*" might include more than one taxon, investigation of the protologues and type materials of the five names cited above demonstrated that they do not correspond to the plants with very wide leaf blades, and confirmed that the five names should be considered synonyms of *S. villosum*, following Filgueiras (2003). Since the unique morphological difference between the specimens of two clades is the leaf blade width (with some overlap in some specimens), and the present phylogenetic analysis based on both genomes A and B also supports the acceptance of *S. villosum s.l.* as a single species, the specimens with wide blades are being considered here as a morphological variation of this species.

The specimens of *Saccharum asperum* did not group in the *S. angustifolium* or the *S. villosum* clades, suggesting that *S. asperum* is a distinct taxon. However, unlike the other two species, the samples of *S. asperum* did not form a monophyletic group in our analysis. The specimens formed two distinct well-supported clades in both the A and B genomes (Fig. 3). This is surprising since *S. asperum* does not present much morphological variability, and the acceptance of it as a single species was not questioned by taxonomists before (Filgueiras, 2003; Peichoto and Rúgolo, 2012; Welker and Longhi-Wagner, 2012). Molina (1981, under *Erianthus*) accepted two varieties for this species (*E. asper* var. *asper* and *E. asper* var. *brasilianus*), differentiated by the length of the spikelets and the indumentum of the axis of the inflorescence.

However, these traits are not good taxonomic characters because they vary within plants and populations, and the two varieties are currently considered synonyms of *Saccharum asperum* (Filgueiras, 2003; Peichoto and Rúgolo, 2012; Welker and Longhi-Wagner, 2012). The morphology of the specimens of the two clades from our analysis does not correspond to the two varieties accepted by Molina (1981). *Saccharum asperum* occurs from Colombia and Venezuela to Argentina and Uruguay (Molina, 1981; Filgueiras, 2003). It is morphologically distinct from the other species of the genus in South America by the entirely glabrous glumes, in both sessile and pedicelled spikelets. The other species have pilose glumes, at least in the pedicelled spikelets (Welker and Longhi-Wagner, 2012) (Table 1, Fig. 1).

Hybridization in Saccharum s.l.—Hybridization plays a significant role in the evolutionary history of sugarcane and relatives. Interspecific and intergeneric hybrids have been documented involving *Saccharum s.l.*, *Miscanthus*, and *Sorghum* (Hodkinson et al., 2002; Nair et al., 2005; Aitken et al., 2007). Welker and Longhi-Wagner (2012) suggested that the specimens identified as *Saccharum* aff. *villosum* might be natural hybrids between *S. villosum* and *S. angustifolium*, based on the intermediate morphology of those plants. The specimens of *S.* aff. *villosum* present leaves typical of *S. villosum* (lanceolate leaf blades with the midvein narrower than the lateral portion of the blade and inconspicuous in the upper portion of the leaf), but the blades are narrower than usual for the species, with width similar to those of *S. angustifolium* (Welker and Longhi-Wagner, 2012) (see Table 1 and Fig. 1). The lower bootstrap support for most nodes when these specimens were included in the phylogeny, compared to the tree without *S.* aff. *villosum*, is consistent with the hypothesis of hybrid origin for these specimens (Funk, 1985; McDade, 1992). However, as pointed out by McDade (1992), the disturbance to cladistic relationships in trees caused by hybridization is higher if the samples are hybrids between distantly related parents. This could explain the similar topologies of our trees, with and without

S. aff. *villosum*, since the probable parents of the hybrids (*S. villosum* and *S. angustifolium*) are closely related species.

The three distinct paralogues per sample of *Saccharum* aff. *villosum* in the phylogenetic tree suggest that these plants are probably hexaploid, in contrast to the two paralogues of the specimens of *S. villosum* and *S. angustifolium*, which are probably tetraploids or triploids. The ploidy level inferred by the number of paralogues is in agreement with recent cytogenetic studies of these specimens, which confirm that *S.* aff. *villosum* presents a higher ploidy level than the other two species (Welker et al., in prep.). The presence of only two paralogues for the specimen Welker 630, contrasting with the other accessions of *S.* aff. *villosum*, is not good evidence of a distinct ploidy level, since our PCR-based approach may not have uncovered all paralogues of this sample. The third paralogues (called C or D in Fig. 4) of *S.* aff. *villosum* specimens are probably recombinant copies resulted from the hybridization events, because they share some synapomorphic bases with paralogues A and some with paralogues B. Since there are not stop codons or other frameshifts that would inactivate the proteins, they do not seem to be pseudogenes (Zheng and Gerstein, 2007).

The paralogues of the specimens of *Saccharum* aff. *villosum* fell in many distinct clades along the phylogeny, both in genomes A and B, confirming the reticulate history of these plants. The topology of the paralogues of the specimen Welker 538 clearly demonstrates that it is a hybrid between *S. villosum* and *S. angustifolium*, since one paralogue grouped in the *S. villosum* clade and other paralogue in the *S. angustifolium* clade (Fig. 4). The higher ploidy level of *S.* aff. *villosum* compared to both putative parental species suggests interspecific hybridization followed by duplication of genomes (allopolyploidy), or hybridization involving an unreduced gamete. It is well known that polyploidy can restore fertility to sterile hybrid lineages after hybridization (McDade, 1992). Many independent allopolyploid events in the tribe Andropogoneae were documented by Estep et al. (2014).

The evolutionary history of the plants identified as *S.* aff. *villosum* and their parents seems to be complex, with different contributions from both *S. villosum* and *S. angustifolium* in the formation of the hybrids. Hexaploid specimens with paralogues genetically more similar to *S. villosum*, and other specimens more similar to *S. angustifolium*, are consistent with this interpretation. Our results indicate that these plants with intermediate morphology are natural hybrids, and that hybridization has probably occurred more than once; the exact mechanism of formation is unclear. Additional molecular and cytogenetic studies, including FISH and GISH analysis, may bring new insights into the evolutionary dynamics of these taxa and elucidate the different genome contribution of the parents in the formation of these hybrids.

Concluding remarks—The allopolyploid origin of *Saccharum* was demonstrated in this study using evidence from nuclear genes. Our phylogenetic analyses indicate that *Saccharum s.l.* is polyphyletic and *Tripidium* should be recognized as a distinct genus, following Grassl (1972, under *Ripidium*). However, no strong evidence was found to support the segregation of *Erianthus* from *Saccharum s.l.* The results also indicate that all South American taxa of *Saccharum s.l.* are polyploid, based on the number of paralogues in the trees. *Saccharum angustifolium, S. asperum,* and *S. villosum* proved to be distinct species. The occurrence of natural hybrids between *S. villosum* and *S. angustifolium* was also documented. Better understanding of the phylogenetic relationships of *Saccharum* and relatives may be useful for sugarcane breeders to identify potential taxa for interspecific and intergeneric crosses in the genetic improvement of sugarcane.

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TABLE 1. Comparison of morphological and biogeographical characters of South American taxa of *Saccharum s.l.*

	S. angustifolium	***S. asperum***	***S. villosum***	***S. villosum*** ("wide leaf blades")	***S.* aff. *villosum***
Leaf blades: shape, indument, width	linear (without pseudopetiole), glabrous, 2–6 mm wide	lanceolate (with pseudopetiole), glabrous or pilose, 7–23 mm wide	lanceolate (with pseudopetiole), generally pilose, 7–14 mm wide	lanceolate (with pseudopetiole), generally pilose, 14–20 mm wide	lanceolate (with pseudopetiole), glabrous or pilose, 3–6 mm wide
Leaf blades: midvein	conspicuous up to the apex of the blade, wider than or as wide as the lateral portion of the blade	inconspicuous in the upper portion of the blade, narrower than the lateral portion of the blade	inconspicuous in the upper portion of the blade, narrower than the lateral portion of the blade	inconspicuous in the upper portion of the blade, narrower than the lateral portion of the blade	inconspicuous in the upper portion of the blade, narrower than the lateral portion of the blade
Glumes of the spikelets: indument	pilose	glabrous	pilose	pilose	pilose
Habitat	dry grasslands	marshlands	marshlands and wet grasslands	marshlands	marshlands and wet grasslands
Geographical distribution	Colombia and Venezuela to Argentina and Uruguay	Colombia and Venezuela to Argentina and Uruguay	Mexico to Argentina and Uruguay	Argentina and Brazil	Argentina, Brazil, and Uruguay

TABLE 2. Species names, voucher specimens, and collection localities of the samples included in this study. Herbaria acronyms according to Index Herbariorum (Thiers, 2014) except THNHM (Thailand Natural History Museum), not included in that directory.

Species	Voucher	Locality
Andropogon eucomus Nees	Malcomber et al. 3089 (MO)	Tanzania, Iringa, Njombe
Andropogon virginicus L.	Kellogg 1240 (MO)	USA, Missouri, Saint Charles
Andropterum stolzii (Pilg.) C. E. Hubb.	Malcomber et al. 3091 (MO)	Tanzania, Iringa, Njombe
Apocopis siamensis A. Camus	Teerawatananon & Sungkaew 975 (THNHM)	Thailand, Sa Kaew, Watthana Nakhon
Arthraxon lanceolatus (Roxb.) Hochst.	Teerawatananon & Sungkaew 720 (THNHM)	Thailand, Tak, Mae Moei
Arthraxon prionodes (Steud.) Dandy	Kellogg PI 659331 (MO)	China, Xizang
Bothriochloa barbinodis (Lag.) Herter	Kellogg PI 204138 (MO)	Brazil, Rio Grande do Sul, Uruguaiana
Bothriochloa laguroides (DC.) Herter	Kellogg PI 283006 (MO)	Uruguay, San Jose, Sierra Mohonea
Capillipedium assimile (Steud.) A. Camus	Teerawatananon & Sungkaew 791 (THNHM)	Thailand, Chiang Mai, Mae Ngon
Chasmopodium caudatum (Hack.) Stapf	Kellogg Kew MSB 184054 (MO)	Burkina Faso, Houet
Chionachne koenigii (Spreng.) Thwaites	Kellogg Chio-6-D-93 (MO)	India
Chrysopogon gryllus (L.) Trin.	Kellogg PI 250984 (A/GH)	Republic of Macedonia, Skopje
Chrysopogon serrulatus Trin.	Kellogg PI 219580 (A/GH)	Pakistan, Bannu
Coix lacryma-jobi L.	Kellogg PI 320865 (MO)	India
Cymbopogon distans (Nees ex Steud.) Will. Watson	Kellogg PI 271552 (MO)	India, Pahlgam
Cymbopogon flexuosus (Nees ex Steud.) Will. Watson	Kellogg PI 209700 (A/GH)	India
Dichanthium annulatum (Forssk.) Stapf	Kellogg PI 240155 (A/GH)	Morocco
Diheteropogon amplectens (Nees) Clayton	Kellogg RF 1819 (MO)	South Africa, Gauteng
Diheteropogon hagerupii Hitchc.	Kellogg Kew MSB 254456 (MO)	Burkina Faso, Comoe
Dimeria fuscescens Trin.	Teerawatananon & Sungkaew 830 (BKF, THNHM)	Thailand, Loei, Phu Kradung
Dimeria ornithopoda Trin.	Teerawatananon & Sungkaew 685 (BKF, THNHM)	Thailand, Trat, Laem Ngob
Eriochrysis pallida Munro	Malcomber et al. 3086 (MO)	Tanzania, Iringa, Njombe
Germainia capitata Balansa & Poitr.	Teerawatananon & Sungkaew 834 (THNHM)	Thailand, Loei, Phu Kradung
Heteropogon triticeus (R. Br.) Stapf ex Craib	Teerawatananon & Sungkaew 733 (THNHM)	Thailand, Chiang Mai, Jom Thong
Hyparrhenia rufa (Nees) Stapf	Kellogg PI 206889 (A/GH)	Turkey, Antalya
	Teerawatananon & Sungkaew 735 (THNHM)	Thailand, Chiang Mai, Jom Thong
Imperata cylindrica (L.) P. Beauv.	Kowarat 108 (THNHM)	Thailand, Pathun Thani, Klong Luang
Ischaemum rugosum Salisb.	Kellogg Kew MSB 183574 (MO)	Burkina Faso, Gnagna
Iseilema macratherum Domin	Snow et al. 7239 (A/GH)	Australia, New South Wales, Moree
Microstegium vimineum (Trin.) A. Camus	Kellogg VA-2 (MO)	USA, Virginia, Fairfax
Miscanthus sinensis Andersson	Kellogg PI 668403 (MO)	Japan, Goto Islands, Nagasaki Prefecture, Osezaki
Pogonatherum crinitum (Thunb.) Kunth	Teerawatananon & Sungkaew 865 (THNHM)	Thailand, Nakhon Ratchasima, PakChong
Polytoca wallichiana (Nees ex Steud.) Benth.	Teerawatananon & Sungkaew 683 (THNHM)	Thailand, Kanchanaburi, Thong Pha Phum
Polytrias indica (Houtt.) Veldkamp	Kellogg 1264 (MO)	Philippines, Luzon
Pseudosorghum fasciculare (Roxb.) A. Camus	Teerawatananon & Sungkaew 698 (THNHM)	Thailand, Tak, Um Phang
Saccharum angustifolium (Nees) Trin. [= *Erianthus angustifolius* Nees]	Longhi-Wagner & Welker 10656 (CTES, ICN)	Brazil, Rio Grande do Sul, Jaquirana
	Welker 344 (ICN)	Brazil, Rio Grande do Sul, Caçapava do Sul
	Welker 498 (ICN)	Brazil, Santa Catarina, Caçador
	Welker 628 (CTES, ICN)	Uruguay, Rocha, Velázquez
	Welker 650 (CTES, ICN)	Uruguay, Tacuarembó
Saccharum arundinaceum Retz. [= *Erianthus arundinaceus* (Retz.) Jeswiet; *Ripidium arundinaceum* (Retz.) Grassl]	Teerawatananon & Sungkaew 864 (THNHM)	Thailand, Nakhon Ratchasima, PakChong
Saccharum asperum (Nees) Steud. [= *Erianthus asper* Nees]	Longhi-Wagner & Welker 10673 (CTES, ICN)	Brazil, Rio Grande do Sul, Bom Jesus
	Welker 366 (CTES, ICN)	Brazil, Santa Catarina, Guaruva
	Welker 435 (ICN)	Brazil, Rio Grande do Sul, São Francisco de Paula
	Welker & Peichoto 583 (CTES, ICN, K, SI)	Argentina, Misiones, Leandro Alem
Saccharum ecklonii (Nees) Steud. [= *Erianthus ecklonii* Nees; *Miscanthidium capense* (Nees) Stapf; *Miscanthus ecklonii* (Nees) Mabb.]	Kellogg PI 410159 (MO)	South Africa, Cape Province
Saccharum giganteum (Walter) Pers. [= *Erianthus giganteus* (Walter) P. Beauv.]	Layton & Zhong 161 (MO)	USA, Louisiana, Saint Tammany
Saccharum narenga (Nees ex Steud.) Wall. ex Hack. [= *Narenga porphyrocoma* (Hance ex Trimen) Bor]	Teerawatananon & Sungkaew 783 (THNHM)	Thailand, Chiang Rai, Phaya Meng Rai
Saccharum officinarum L.	Welker s.n. (MO)	USA, Missouri, St. Louis
Saccharum ravennae (L.) L. [= *Erianthus ravennae* (L.) P. Beauv.; *Ripidium ravennae* (L.) Trin.; *Tripidium ravennae* (L.) H. Scholz]	Vela s.n. (MO)	USA, Missouri, St. Louis
Saccharum villosum Steud. [= *Erianthus trinii* (Hack.) Hack.]	Longhi-Wagner & Welker 10570 (CTES, ICN)	Brazil, Rio Grande do Sul, Caçapava do Sul
	Longhi-Wagner & Welker 10611 (CTES, ICN)	Brazil, Rio Grande do Sul, Encruzilhada do Sul
	Welker 539 (CTES, ICN)	Brazil, Rio Grande do Sul, Santo Antônio das Missões
	Welker 547 (CTES, ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker & Peichoto 560 (CTES, ICN)	Argentina, Corrientes, San Roque
	Welker 651 (CTES, ICN)	Uruguay, Tacuarembó
Saccharum villosum Steud. ("wide leaf blades")	Welker 396 (CTES, ICN)	Brazil, Paraná, Aparecida do Ivaí
	Welker 477 (CTES, ICN)	Brazil, Rio Grande do Sul, São Luiz Gonzaga
	Welker & Peichoto 575 (CEN, CTES, ICN, K)	Argentina, Misiones, Apóstoles

TABLE 2. Continued.

Species	Voucher	Locality
Saccharum aff. *villosum* Steud.	Welker 502 (CTES, ICN)	Brazil, Santa Catarina, Caçador
	Welker 538 (CTES, ICN)	Brazil, Rio Grande do Sul, Santo Antônio das Missões
	Welker & Peichoto 556 (CTES, ICN)	Argentina, Corrientes, Empedrado
	Welker & Peichoto 584 (CORD, CTES, ICN)	Argentina, Misiones, Leandro Alem
	Welker 630 (CTES, ICN)	Uruguay, Rocha, Velázquez
Schizachyrium brevifolium (Sw.) Nees ex Buse	Teerawatananon & Sungkaew 750 (THNHM)	Thailand, Chiang Mai, Muang
Schizachyrium sanguineum (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	Thailand, Chiang Mai, Muang
Sorghastrum elliottii (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	USA, Texas, Anderson County
Sorghastrum nutans (L.) Nash	Kellogg PI 315744 (A/GH)	USA, West Virginia, Hampshire
Sorghum bicolor (L.) Moench	Kellogg PI 156549 (A/GH)	Zimbabwe
	Ortiz & Gomez K-1996-1544 (K)	unknown
Thelepogon elegans Roth	Teerawatananon & Sungkaew 697 (THNHM)	Thailand, Tak, LanSang
Themeda arundinacea (Roxb.) A. Camus	Teerawatananon & Sungkaew 739 (THNHM)	Thailand, Chiang Mai, Mae Rim
Tripsacum dactyloides (L.) L.	Kellogg 1261 (A/GH)	USA, Missouri, Pettis County
Zea mays L.	Cultivar B73 (genome sequence)	unknown

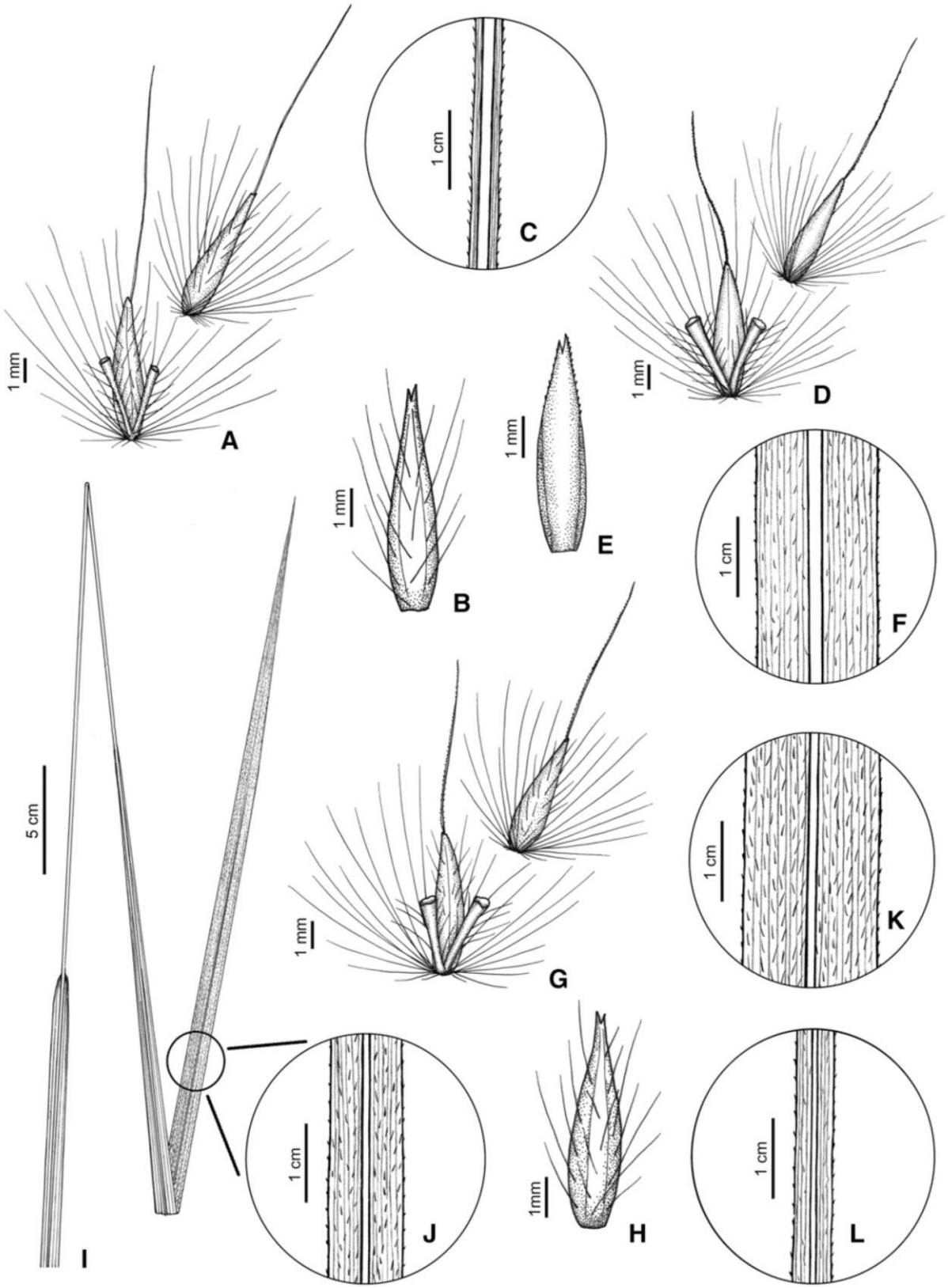

Fig. 1. Morphological details of the South American species of *Saccharum s.l.* A–C. *Saccharum angustifolium*. A. Pair of spikelets, pedicel, and rachis internode. B. Lower glume of the sessile spikelet. C. Middle portion of the leaf blade. D–F. *Saccharum asperum*. D. Pair of spikelets, pedicel, and rachis internode. E. Lower glume of the sessile spikelet. F. Middle portion of the leaf blade. G–J. *Saccharum villosum*. G. Pair of spikelets, pedicel, and rachis internode. H. Lower glume of the sessile spikelet. I. Leaf with lanceolate blade and pseudopetiole. J. Middle portion of the leaf blade. K. *Saccharum villosum* ("wide leaf blades"). Middle portion of the leaf blade. L. *Saccharum* aff. *villosum*. Middle portion of the leaf blade. Illustrations by C.A.D. Welker.

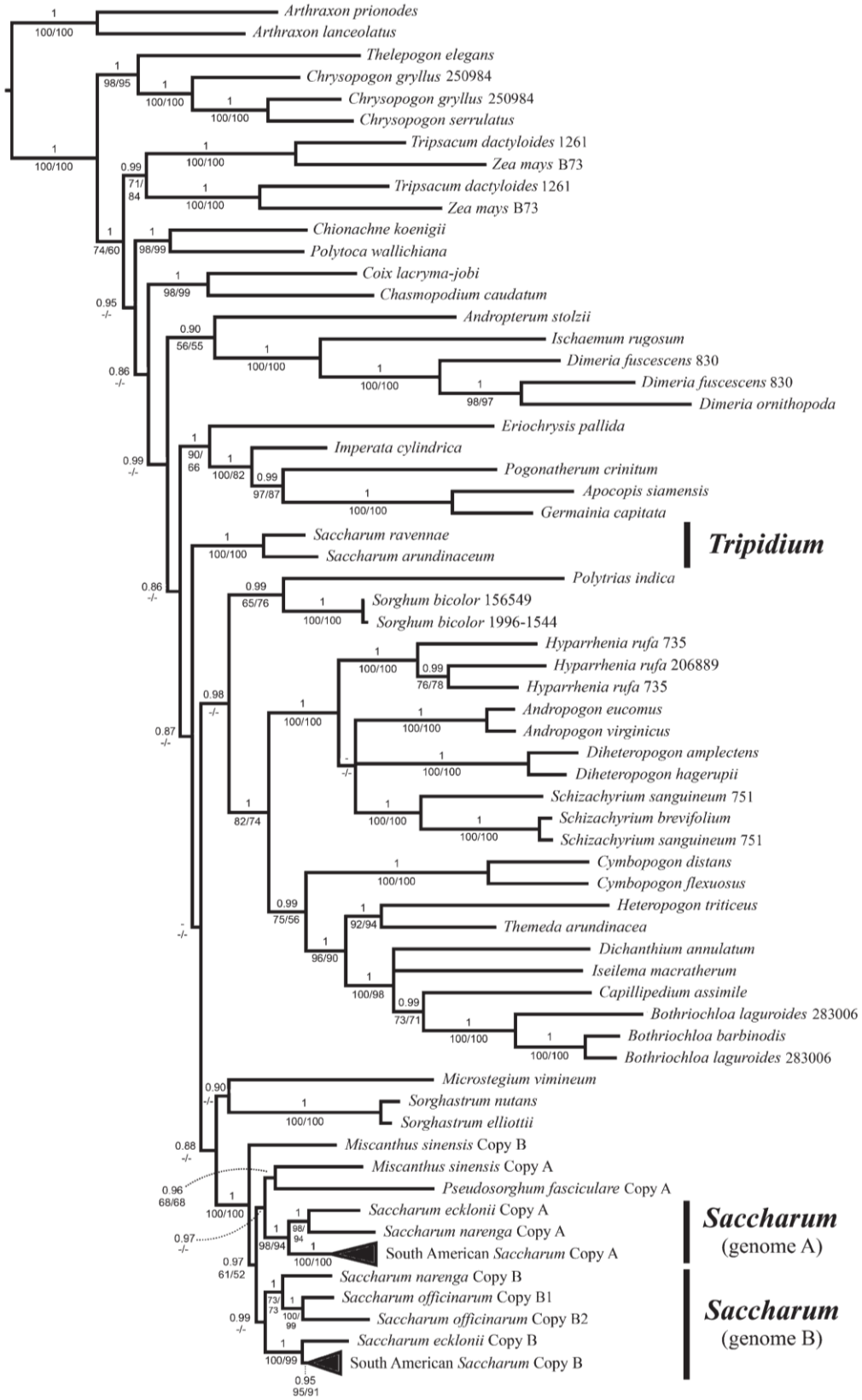

Fig. 2. Bayesian phylogeny of *Saccharum s.l.* and other genera of Andropogoneae, based on the combined data set (*apo*1, *d*8, *ep*2-*ex*7, *ep*2-*ex*8, and *rep*1), shown as a phylogram. Bayesian PP > 0.85 are shown above branches, and ML / MP bootstrap values > 50 are shown below. For species with more than one specimen or more than one paralogue in our analyses, collector number is after the binomial, according to Table 2. Clades of South American taxa of *Saccharum s.l.* were collapsed and presented in detail in Fig. 3.

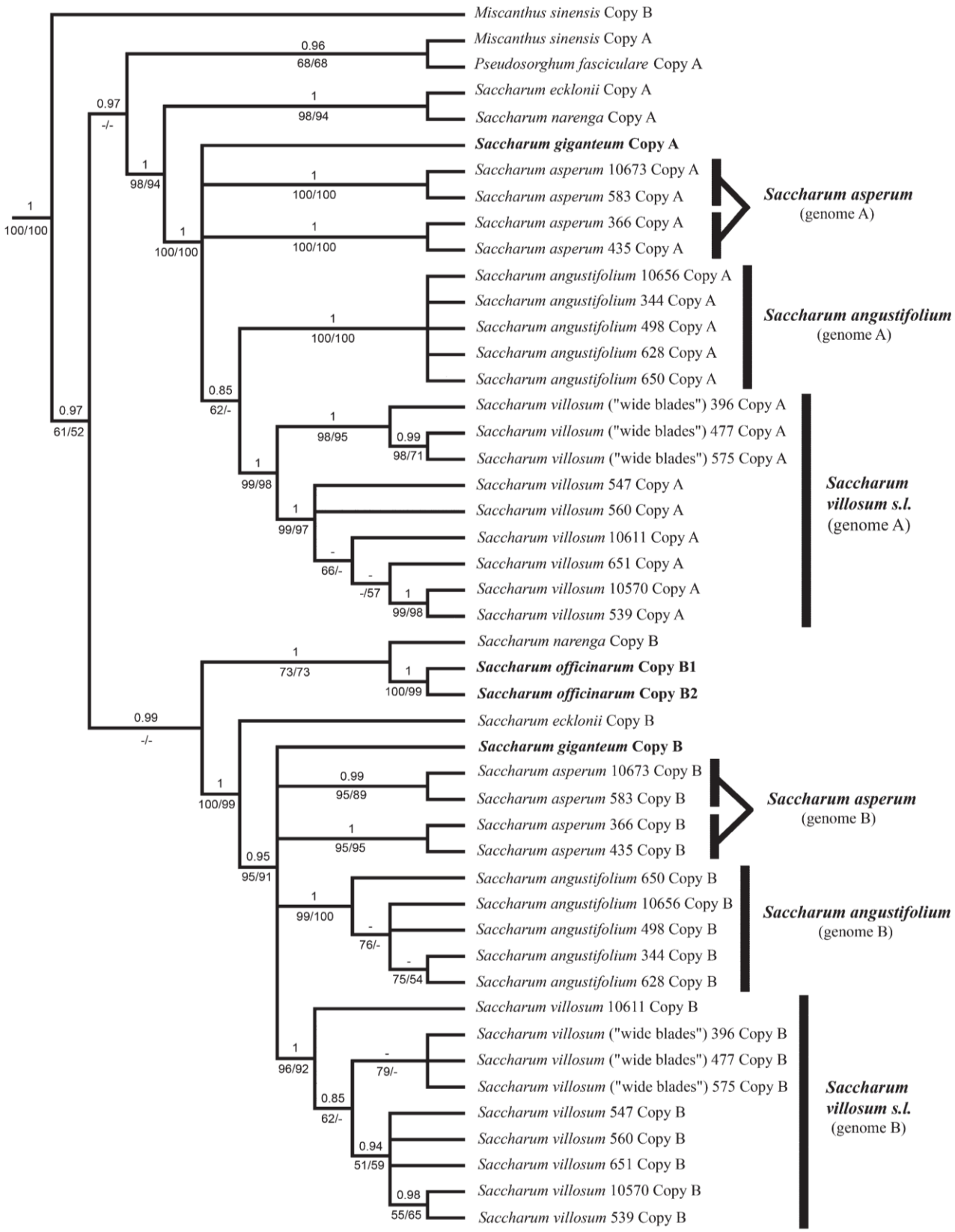

Fig. 3. Bayesian phylogeny of *Saccharum s.l.* and closely related genera, based on the combined data set (*apo*1, *d*8, *ep2-ex*7, *ep2-ex*8, and *rep*1). Bayesian PP > 0.85 are shown above branches, and ML / MP bootstrap values > 50 are shown below. For taxa with more than one specimen in our analyses, the collector number is after the binomial, according to Table 2. The type species of *Saccharum* (*S. officinarum*) and *Erianthus* (*E. giganteus* / *Saccharum giganteum*) are highlighted in bold.

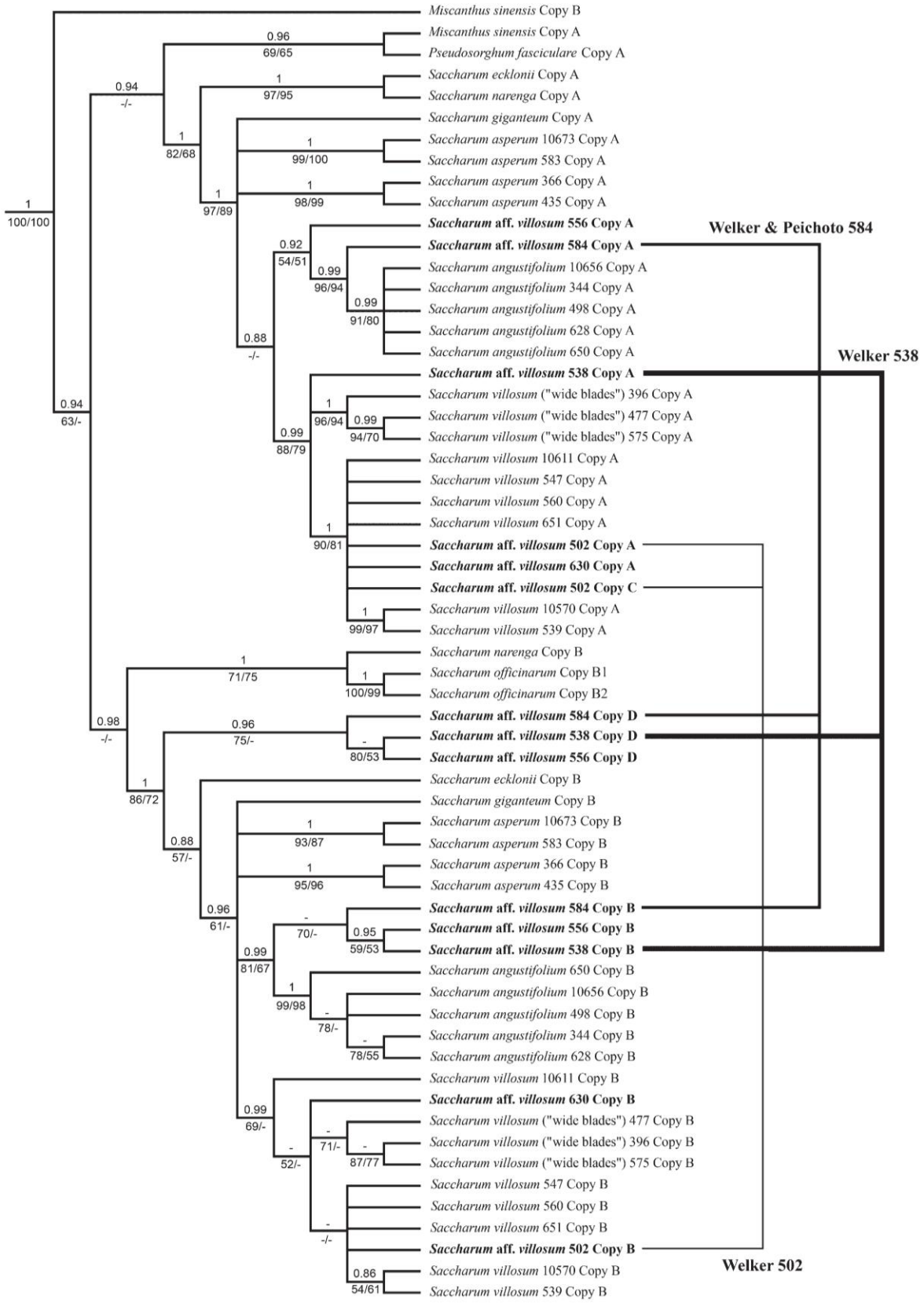

Fig. 4. Bayesian phylogeny of *Saccharum s.l.* and closely related genera, including the specimens of *Saccharum* aff. *villosum* (names in bold), based on the combined data set (*apo*1, *d*8, *ep*2-*ex*7, *ep*2-*ex*8, and *rep*1). Bayesian PP > 0.85 are shown above branches, and ML / MP bootstrap values > 50 are shown below. For taxa with more than one specimen in our analyses, the collector number is after the binomial, according to Table 2.

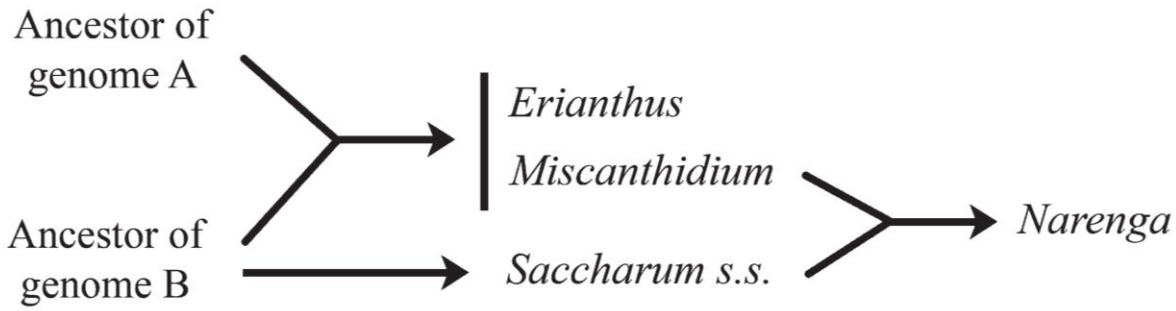

Fig. 5. Schematic representation of the possible origin of *Saccharum* and relatives.

APPENDIX 1. Species names, voucher specimens, and GenBank accession numbers for sequences included in this study. NA (not available).

Species	Voucher	*apo*1	*d*8	*ep*2-*ex*7	*ep*2-*ex*8	*rep*1
Andropogon eucomus Nees	Malcomber et al. 3089 (MO)	KM578363	KM578119	KM577921	KM577706	KM578555
Andropogon virginicus L.	Kellogg 1240 (MO)	KM578449	KM578209	KM578001	KM577789	KM578635
Andropterum stolzii (Pilg.) C. E. Hubb.	Malcomber et al. 3091 (MO)	KM578417	KM578168	KM577978	KM577764	KM578609
Apocopis siamensis A. Camus	Teerawatananon & Sungkaew 975 (THNHM)	KM578503	KM578287	KM578058	KM577852	NA
Arthraxon lanceolatus (Roxb.) Hochst.	Teerawatananon & Sungkaew 720 (THNHM)	NA	KM578290	KM578061	KM577854	KM578689
Arthraxon prionodes (Steud.) Dandy	Kellogg PI 659331 (MO)	NA	KM578256	KM578036	KM577831	KM578672
Bothriochloa barbinodis (Lag.) Herter	Kellogg PI 204138 (MO)	KM578453	KM578212	NA	KM577794	KM578638
Bothriochloa laguroides (DC.) Herter	Kellogg PI 283006 (MO)	KM578462, KM578463	KM578223, KM578224	NA	NA	KM578648, KM578649
Capillipedium assimile (Steud.) A. Camus	Teerawatananon & Sungkaew 791 (THNHM)	KM578504	KM578291	KM578062	KM577855	KM578690
Chasmopodium caudatum (Hack.) Stapf	Kellogg Kew MSB 184054 (MO)	KM578498	KM578275	KM578046	KM577842	KM578683
Chionachne koenigii (Spreng.) Thwaites	Kellogg Chio-6-D-93 (MO)	KP243072	KP233123	KP242922	KP242976	KP243025
Chrysopogon gryllus (L.) Trin.	Kellogg PI 250984 (A/GH)	KM578372, KM578373	NA	KM577928, KM577929	KM577714, KM577715	KM578561, KM578563
Chrysopogon serrulatus Trin.	Kellogg PI 219580 (A/GH)	KM578434	KM578184	KM577988	KM577769	KM578619
Coix lacryma-jobi L.	Kellogg PI 320865 (MO)	KM578425	KM578175	NA	NA	KM578613
Cymbopogon distans (Nees ex Steud.) Will. Watson	Kellogg PI 271552 (MO)	KM578378	KM578126	KM577939	NA	KM578569
Cymbopogon flexuosus (Nees ex Steud.) Will. Watson	Kellogg PI 209700 (A/GH)	KM578385	KM578128	KM577940	KM577719	KM578570
Dichanthium annulatum (Forssk.) Stapf	Kellogg PI 240155 (A/GH)	NA	KM578135	KM577945	KM577725	KM578575
Diheteropogon amplectens (Nees) Clayton	Kellogg RF 1819 (MO)	KM578432	KM578183	KM577987	KM577768	KM578616
Diheteropogon hagerupii Hitchc.	Kellogg Kew MSB 254456 (MO)	KM578548	KM578355	KM578110	KM577907	KM578735
Dimeria fuscescens Trin.	Teerawatananon & Sungkaew 830 (BKF, THNHM)	KM578511, KM578513	KM578300, KM578301	KM578069, KM578070	KM577862, KM577863	NA
Dimeria ornithopoda Trin.	Teerawatananon & Sungkaew 685 (BKF, THNHM)	KM578514	KM578305	NA	KM577864	NA
Eriochrysis pallida Munro	Malcomber et al. 3086 (MO)	KM578393	KM578137	KM577947	KM577728	NA
Germainia capitata Balansa & Poitr.	Teerawatananon & Sungkaew 834 (THNHM)	KM578515	KM578312	KM578075	KM577870	NA
Heteropogon triticeus (R. Br.) Stapf ex Craib	Teerawatananon & Sungkaew 733 (THNHM)	KM578516	KM578314	KM578076	KM577874	KM578703
Hyparrhenia rufa (Nees) Stapf	Kellogg PI 206889 (A/GH)	KM578396	KM578140	KM577948	KM577732	KM578578
	Teerawatananon & Sungkaew 735 (THNHM)	KM578523	KM578319	KM578080, KM578081	KM577875, KM577876	KM578708, KM578710
Imperata cylindrica (L.) P. Beauv.	Kowarat 108 (THNHM)	KM578524	KM578321	KM578082	KM577877	KM578712
Ischaemum rugosum Salisb.	Kellogg Kew MSB 183574 (MO)	KM578551	KM578356	KM578113	KM577910	NA
Iseilema macratherum Domin	Snow et al. 7239 (A/GH)	KM578440	KM578192	KM577992	KM577775	KM578625
Microstegium vimineum (Trin.) A. Camus	Kellogg VA-2 (MO)	NA	NA	KM578051	KM577846	KM578685

Appendix 1. Continued.

Species	Voucher	*apo*1	*d*8	*ep2-ex*7	*ep2-ex*8	*rep*1
Miscanthus sinensis Andersson	Kellogg PI 668403 (MO)	KM578443, KM578444	KM578199, KM578201	KM577993, KM577994	KM577779, KM577781	NA
Pogonatherum crinitum (Thunb.) Kunth	Teerawatananon & Sungkaew 865 (THNHM)	NA	NA	KM578088	KM577885	KM578715
Polytoca wallichiana (Nees ex Steud.) Benth.	Teerawatananon & Sungkaew 683 (THNHM)	KP243073	KP233124	KP242923	KP242977	KP243026
Polytrias indica (Houtt.) Veldkamp	Kellogg 1264 (MO)	NA	KM578208	KM578000	KM577788	KM578633
Pseudosorghum fasciculare (Roxb.) A. Camus	Teerawatananon & Sungkaew 698 (THNHM)	NA	KM578329	KM578089	KM577886	KM578716
Saccharum angustifolium (Nees) Trin.	Longhi-Wagner & Welker 10656 (CTES, ICN)	KP243042, KP243043	KP233105	KP242902, KP242903	KP242943, KP242944	KP242993, KP242994
	Welker 344 (ICN)	KP243035, KP243036	KP233100	NA	KP242935, KP242936	KP242987, KP242988
	Welker 498 (ICN)	KP243037	KP233101, KP233102	KP242896, KP242897	KP242937, KP242938	KP242989
	Welker 628 (CTES, ICN)	KP243038, KP243039	KP233103	KP242898, KP242899	KP242939, KP242940	KP242990
	Welker 650 (CTES, ICN)	KP243040, KP243041	KP233104	KP242900, KP242901	KP242941, KP242942	KP242991, KP242992
Saccharum arundinaceum Retz.	Teerawatananon & Sungkaew 864 (THNHM)	NA	KM578332	KM578090	KM577888	KM578720
Saccharum asperum (Nees) Steud.	Longhi-Wagner & Welker 10673 (CTES, ICN)	KP243050, KP243051	NA	NA	KP242951, KP242952	KP243001, KP243002
	Welker 366 (CTES, ICN)	KP243044	KP233106	KP242904	KP242945, KP242946	KP242995, KP242996
	Welker 435 (ICN)	KP243046, KP243047	KP233109	NA	KP242947, KP242948	KP242997, KP242998
	Welker & Peichoto 583 (CTES, ICN, K, SI)	KP243048, KP243049	KP233110	KP242905, KP242906	KP242949, KP242950	KP242999, KP243000
Saccharum ecklonii (Nees) Steud.	Kellogg PI 410159 (MO)	KM578467, KM578468	KM578229, KM578230	KM578012, KM578013	KM577807, KM577810	KM578654, KM578656
Saccharum giganteum (Walter) Pers.	Layton & Zhong 161 (MO)	KP243052, KP243053	KP233111, KP233112	NA	KP242953, KP242954	KP243003, KP243004
Saccharum narenga (Nees ex Steud.) Wall. ex Hack.	Teerawatananon & Sungkaew 783 (THNHM)	KM578528, KM578529	KM578334, KM578337	KM578092, KM578094	KM577891, KM577892	KM578722, KM578725
Saccharum officinarum L.	Welker s.n. (MO)	KP243055, KP243056	NA	KP242907, KP242908	KP242956, KP242957	NA
Saccharum ravennae (L.) L.	Vela s.n. (MO)	KM578491	KM578269	KM578042	KM577837	KM578681
Saccharum villosum Steud.	Longhi-Wagner & Welker 10570 (CTES, ICN)	KP243064, KP243065	KP233118	KP242917	KP242966, KP242967	KP243014, KP243015
	Longhi-Wagner & Welker 10611 (CTES, ICN)	KP243070, KP243071	NA	NA	KP242974, KP242975	KP243023, KP243024
	Welker 539 (CTES, ICN)	KP243057, KP243058	KP233113, KP233114	KP242909, KP242910	KP242958, KP242959	KP243006, KP243007
	Welker 547 (CTES, ICN)	KP243059, KP243060	KP233115	KP242911, KP242912	KP242960, KP242961	KP243008, KP243009
	Welker & Peichoto 560 (CTES, ICN)	KP243061	KP233116	KP242913, KP242914	KP242962, KP242963	KP243010, KP243011
	Welker 651 (CTES, ICN)	KP243062, KP243063	KP233117	KP242915, KP242916	KP242964, KP242965	KP243012, KP243013
Saccharum villosum Steud. ("wide leaf blades")	Welker 396 (CTES, ICN)	NA	KP233119, KP233120	KP242918, KP242919	KP242968, KP242969	KP243016, KP243017
	Welker 477 (CTES, ICN)	NA	KP233121	NA	KP242970, KP242971	KP243018
	Welker & Peichoto 575 (CEN, CTES, ICN, K)	KP243068, KP243069	KP233122	KP242920, KP242921	KP242972, KP242973	KP243021, KP243022
Saccharum aff. *villosum* Steud.	Welker 502 (CTES, ICN)	NA	NA	KP242887, KP242888	KP242924, KP242925	KP242978, KP242979, KP242980
	Welker 538 (CTES, ICN)	KP243027	KP233094, KP233095	KP242889, KP242890, KP242891	KP242926, KP242927, KP242928	KP242981, KP242982
	Welker & Peichoto 556 (CTES, ICN)	KP243028, KP243029	KP233096, KP233097, KP233098	KP242892	KP242929, KP242930, KP242931	KP242983
	Welker & Peichoto 584 (CORD, CTES, ICN)	KP243030, KP243031, KP243032	NA	KP242893, KP242894, KP242895	KP242932	KP242984
	Welker 630 (CTES, ICN)	KP243033, KP243034	KP233099	NA	KP242933, KP242934	KP242985, KP242986

APPENDIX 1. Continued.

Species	Voucher	*apo*1	*d*8	*ep*2-*ex*7	*ep*2-*ex*8	*rep*1
Schizachyrium brevifolium (Sw.) Nees ex Buse	Teerawatananon & Sungkaew 750 (THNHM)	KM578530	NA	KM578097	KM577893	NA
Schizachyrium sanguineum (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	KM578532, KM578533	KM578339, KM578340	KM578099, KM578100	KM577894	NA
Sorghastrum elliottii (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	KM578552	NA	KM578114	KM577911	KM578737
Sorghastrum nutans (L.) Nash	Kellogg PI 315744 (A/GH)	NA	NA	KM577963	KM577751	KM578591
Sorghum bicolor (L.) Moench	Kellogg PI 156549 (A/GH)	KM578410	KM578151	KM577964	KM577752	KM578593
	Ortiz & Gomez K-1996-1544 (K)	KM578412	KM578153	KM577966	KM577754	KM578595
Thelepogon elegans Roth	Teerawatananon & Sungkaew 697 (THNHM)	KM578539	NA	KM578103	KM577895	KM578728
Themeda arundinacea (Roxb.) A. Camus	Teerawatananon & Sungkaew 739 (THNHM)	NA	KM578349	KM578104	KM577897	NA
Tripsacum dactyloides (L.) L.	Kellogg 1261 (A/GH)	KM578413	KM578154	KM577967, KM577968	KM577755, KM577756	KM578596, KM578597
Zea mays L.	Cultivar B73 (genome sequence)	NA	GRMZM2G360081, GRMZM2G109966	GRMZM2G024973, GRMZM2G144744	GRMZM2G098859, GRMZM2G414043	GRMZM2G110242, GRMZM2G064628

CAPÍTULO III:

New record for *Eriochrysis* (Poaceae: Andropogoneae) in the State of Rio Grande do Sul, Brazil, and a key to the species of *Eriochrysis* in Brazil

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New record for *Eriochrysis* (Poaceae–Andropogoneae) in the State of Rio Grande do Sul, Brazil, and a key to the species of the genus in Brazil

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The genus *Eriochrysis* Beauvois (1812: 8) (Poaceae–Andropogoneae) includes ca. seven species from America, Africa, and India (Clayton & Renvoize 1986). It is characterized mainly by inflorescences with golden-brown to light-brown trichomes, and heterogamous spikelets: sessile spikelets with a bisexual flower and pedicelled spikelets with a pistillate flower. Six species of the genus occur in Brazil (Filgueiras & Welker 2012), of which three were previously reported for the State of Rio Grande do Sul: *E. cayennensis* Beauvois (1812: 8), *E. holcoides* (Nees 1829: 324) Kuhlmann (1922: 89) and *E. villosa* Swallen (1966: 90) (Welker & Longhi-Wagner 2012).

During intensive field trips in southern Brazil, we found another species of *Eriochrysis* in the State of Rio Grande do Sul, *E. laxa* Swallen (1966: 89). Here, we provide a description and illustrations of this taxon, as well as data on its geographical distribution and habitat. An illustrated key to the six species of the genus that occur in Brazil is also provided.

Eriochrysis laxa Swallen (1966: 89) (Fig. 1D–G)

Type: BRAZIL. Minas Gerais: Lavras, in wet ground near streamlet, 5 March 1925, *Agnes Chase 8729* (HT: US-1256173 photo!; IT: MO-925236 photo!).

Perennial, caespitose, (125–)140–190(–215) cm high, nodes pilose. Leaf sheaths glabrous, less commonly sparsely pilose near the apex; blades glabrous on abaxial surface, densely pilose on adaxial surface, with dense tufts of trichomes 4–8 mm long at the base of the adaxial surface behind the ligule, blades of innovations 25–50(–68) cm long, subfiliform below, 2–4 mm wide above, those of the culm (18–)35–70 cm long, 5–10 mm wide; ligule membranous-ciliate, 1–1.5 mm long. Inflorescence contracted to subcontracted, (12–)16–32 cm long, bearing numerous alternate racemes on a central axis, the lower racemes placed distantly; racemes differentiated into nodes and internodes, disarticulating at the nodes, sparsely pilose, spikelets apparent among the golden-brown trichomes. Spikelets paired at each node of the rachis, one sessile and one

pedicelled, the pedicelled spikelet falling off first at maturity, the sessile falling off together with a rachis internode and the pedicel. Sessile spikelet (2.2–)2.5–3(–3.5) mm long, obovate, awnless, with bisexual flower; glumes chartaceous, the lower glume glabrous on back, with trichomes 1–2 mm long on margins, with the apex rounded to obtuse, the upper glume ciliate in the upper half of the margins, with the apex acute to subacute; lower and upper anthecia hyaline; basal trichomes reaching 1/2 to 2/3 of the length of the spikelet. Pedicelled spikelet 1.7–2.5 mm long, similar to the sessile, but with pistillate flower. Caryopsis 0.7–1 mm long.

Distribution and habitat:—Colombia, Bolivia, Paraguay, Argentina, and Brazil (Swallen 1966). In Brazil, it occurs from the States of Goiás to Santa Catarina (Swallen 1966, Smith *et al.* 1982) and in Rio Grande do Sul, in marshlands and wet grasslands.

Eriochrysis laxa is morphologically similar to *E. cayennensis* and *E. villosa*, being distinguished mainly by the less pilose inflorescences, which result in spikelets apparent among the trichomes (Fig. 1E), and the sessile spikelets obovate (Fig. 1F–G). *Eriochrysis cayennensis* and *E. villosa* have inflorescences densely pilose with the spikelets hidden among the trichomes, and sessile spikelets ovate to elliptic. Furthermore, the apex of the lower glume is rounded to obtuse in *E. laxa* (Fig. 1G), being truncate and trilobed in *E. cayennensis* (Fig. 1A), and acute to subacute, non-lobed (Fig. 1I) or with inconspicuous lobes (Fig. 1J) in *E. villosa*.

Eriochrysis holcoides and *E. warmingiana* (Hackel 1883: 254) Kuhlmann (1922: 29) differ from *E. laxa* by the sessile spikelets lanceolate to elliptic and lower glume with apex acute or acuminate (Fig. 1C, 1K). *Eriochrysis filiformis* (Hackel 1889: 29) Filgueiras (1997: 231) differs from *E. laxa* and the other Brazilian species of the genus by having a pair of spikelets consisting of short- and long-pedicelled spikelets (Fig. 1B). Note that because of this characteristic, *E. filiformis* was initially erroneously placed in the tribe Paniceae (Filgueiras 1997). *Eriochrysis filiformis* also has narrower leaf blades than the other species.

Killeen (1990) described *Eriochrysis* × *concepcionensis* Killeen (1990: 157) as a hybrid between *E. laxa* and *E. cayennensis*, based on a single population with intermediate morphology found in Santa Cruz (Bolivia) in the same habitat as both parental species. We also found some specimens with intermediate morphology between *E. laxa* and *E. cayennensis* in a marshland in Rio Grande do Sul (Brazil) together with specimens of the two latter species. These plants, here treated as *E.* aff. *laxa* and represented by the specimen *Welker 487* (ICN), have the sessile spikelets obovate as *E. laxa* and the inflorescences densely pilose as *E. cayennensis*. The apex of the lower glume is rounded as *E. laxa*, but has inconspicuous lobes (Fig. 1H). These individuals are likely natural hybrids between these species. We are conducting molecular and cytogenetic studies to examine this issue.

Key to the *Eriochrysis* species in Brazil

1. Pair of spikelets consisting of two pedicelled spikelets, one short- and the other long-pedicelled (Fig. 1B) … *E. filiformis*

- Pair of spikelets consisting of one sessile and one pedicelled spikelet (Fig. 1F) ... 2.

2. Inflorescence sparsely pilose, spikelets apparent among the trichomes (Fig. 1E) … 3.

- Inflorescence densely pilose, with the spikelets hidden among the trichomes … 5.

3. Sessile spikelet obovate, lower glume with the apex rounded to obtuse (Fig. 1G) … *E. laxa*

- Sessile spikelet lanceolate to elliptic, lower glume with the apex acute or acuminate (Fig. 1C, 1K) ... 4.

4. Inflorescence 5–14 cm long, contracted, racemes adpressed … *E. holcoides*

- Inflorescence 17–30 cm long., subcontracted to slightly open, racemes divergent especially at the base of the inflorescence ... *E. warmingiana*

5. Lower glume of sessile spikelet with the apex truncate, trilobed (Fig. 1A). Sessile spikelet 1.8–3.5 mm long … *E. cayennensis*

- Lower glume of sessile spikelet with the apex acute to subacute, non-lobed (Fig. 1I) or with inconspicuous lobes (Fig. 1J). Sessile spikelet 3–5 mm long … *E. villosa*

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Appendix

Selected material

***Eriochrysis cayennensis* P. Beauv.:**—BRAZIL. Rio Grande do Sul: Cidreira, Lagoa da Antônia, 14 November 2008, *Welker 183* (ICN); São Borja, BR-285, 29 March 2012, *Welker 486* (ICN); São Francisco de Paula, 30 March 2009, *Longhi-Wagner & Welker 10781* (ICN). Santa Catarina: Florianópolis, Ilha de Santa Catarina, Lagoa da Conceição, 12 September 1985, *Souza 778* (HUCS). ***E. filiformis* (Hack.) Filg.:**—BRAZIL. Distrito Federal: Brasília, Área do Cristo Redentor, 21 August 1990, *Câmara & Filgueiras 30* (ICN). Goiás: Alto Paraíso de Goiás, Chapada dos Veadeiros, 7 September 1994, *Filgueiras & Fonseca 3006* (ICN, SP). Tocantins: Mateiros, 8 December 2005, *Rua et al. 691* (CEN). ***E. holcoides* (Nees) Kuhlm.:**—BRAZIL. Rio Grande do Sul: Farroupilha, 10 November 1957, *Camargo 2499* (PACA); Rio Grande, Est. Domingos Petrolini, 9 November 1945, *Swallen 7307* (PEL); São José dos Ausentes, 15 January 2009, *Longhi-Wagner & Welker 10731* (ICN); Vacaria, Estação Experimental de Forrageiras, 16 September 1971, *Valls 1593* (ICN). ***E. laxa* Swallen:**—BRAZIL. Minas Gerais: Santa Vitória, 25 January 1996, *Pietrobom-Silva 2766* (ICN). Rio Grande do Sul: São Borja, BR-285, 29 March 2012, *Welker 489* (ICN); São Luiz Gonzaga, BR-285, 28 March 2012, *Welker 481* (ICN). São Paulo: Paraguaçu Paulista, Estação Florestal, 7 February 1965, *Clayton 4569* (SP). ***E.* aff. *laxa* Swallen:**—BRAZIL. Rio Grande do Sul: São Borja, BR-285, 29 March 2012, *Welker 487*

(ICN). ***E. villosa*** **Swallen:**—BRAZIL. Rio Grande do Sul: Cidreira, 14 November 2008, *Welker 182* (ICN); São Luiz Gonzaga, BR-285, 24 March 2010, *Welker 328* (ICN); Tupanciretã, 15 November 1956, *Mohrdieck 36* (BLA). Santa Catarina: Rio Caçador, 21 January 1946, *Swallen 8237* (PEL). ***E. warmingiana*** **(Hack.) Kuhlm.:**—BRAZIL. Mato Grosso do Sul: Aquidauana, 26 February 1930, *Chase 11063* (SP); Campo Grande, 16 April 1984, *Valls et al. 7605* (CEN, SP). Minas Gerais: Formoso, Parque Nacional Grande Sertão Veredas, 5 November 1989, *Filgueiras 1902* (ICN, SP).

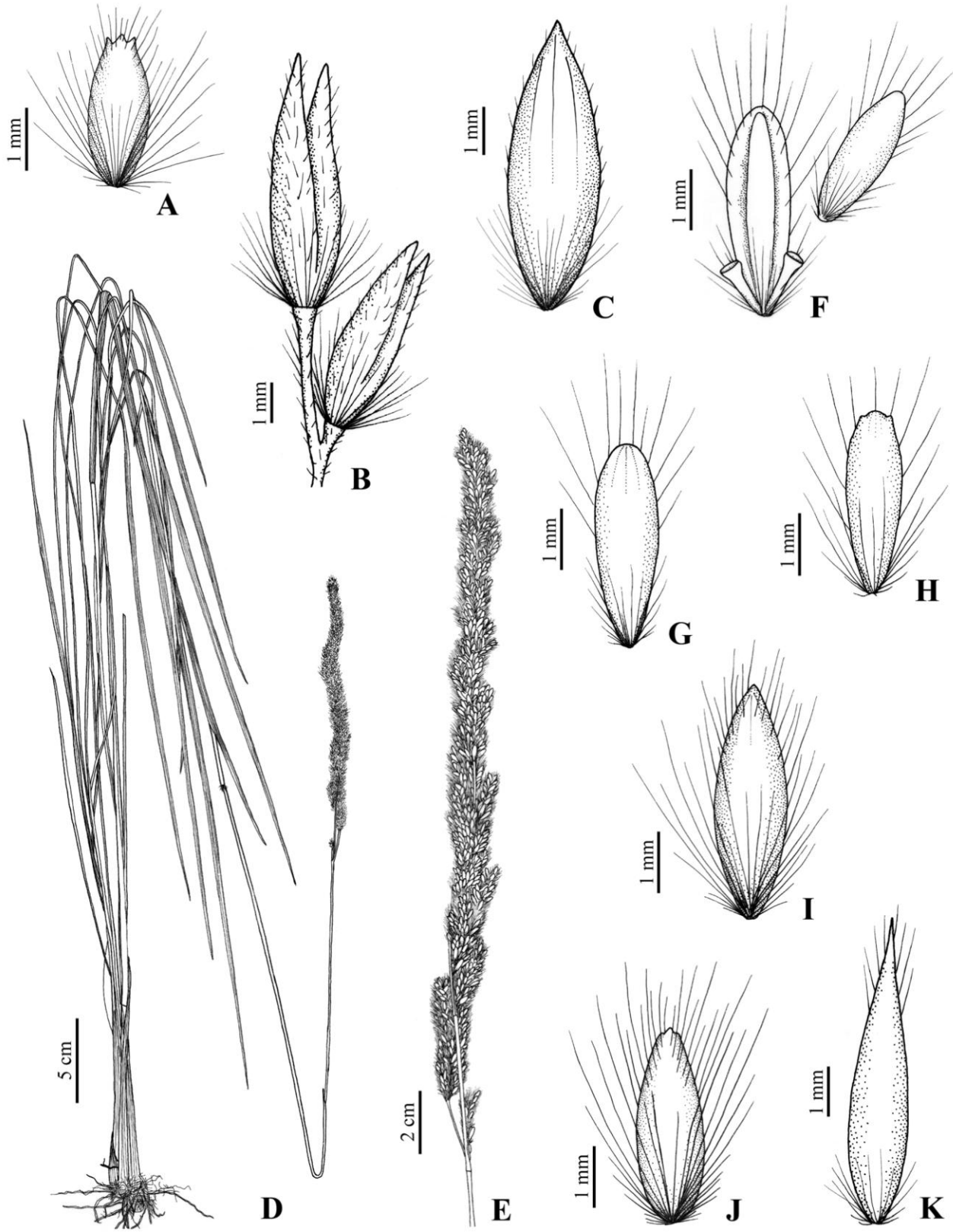

FIGURE 1. Morphological details of the *Eriochrysis* species. **A.** *E. cayennensis*, lower glume of the sessile spikelet (Longhi-Wagner & Welker 10781, ICN). **B.** *E. filiformis*, pair of spikelets (Câmara & Filgueiras 30, ICN). **C.** *E. holcoides*, lower glume of the sessile spikelet (Longhi-Wagner & Welker 10731, ICN). **D–G.** *E. laxa* (Welker 489, ICN). **D.** habit. **E.** inflorescence. **F.** pair of spikelets. **G.** lower glume of the sessile spikelet. **H.** *E.* aff. *laxa*, lower glume of the sessile spikelet (Welker 487, ICN). **I–J.** *E. villosa*. **I.** lower glume of the sessile spikelet (Welker 328, ICN). **J.** lower glume of the sessile spikelet (Welker 182, ICN). **K.** *E. warmingiana*, lower glume of the sessile spikelet (Filgueiras 1902, ICN). Illustrations by Anelise Scherer and Cassiano A. D. Welker.

CAPÍTULO IV:

Molecular phylogeny of *Eriochrysis* P. Beauv. (Poaceae – Andropogoneae) based on multiple low-copy nuclear genes and complete plastome sequences: taxonomic implications and evidence of interspecific hybridization

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Molecular phylogeny of *Eriochrysis* P. Beauv. (Poaceae – Andropogoneae) based on multiple low-copy nuclear genes and complete plastome sequences: taxonomic implications and evidence of interspecific hybridization

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Abstract

Species delimitation is a vital issue concerning evolutionary biology and conservation of biodiversity. However, it is a challenging task for several reasons, including the low interspecies variability of markers currently used in phylogenetic reconstructions and the occurrence of reticulate evolution and polyploidy in many lineages of flowering plants. The first molecular phylogeny of the grass genus *Eriochrysis* is presented here in order to examine its relationships to other genera of the subtribe Saccharinae / tribe Andropogoneae and to define the circumscriptions of its taxonomically complicated species. Molecular cloning and sequencing of five regions of four low-copy nuclear genes (*apo*1, *d*8, *ep*2-*ex*7 and *ep*2-*ex*8, *kn*1) were performed, as well as complete plastome sequencing. Trees were reconstructed using maximum parsimony, maximum likelihood, and Bayesian inference analyses. The present phylogenetic analyses indicate that *Eriochrysis* is monophyletic and the Old World *E. pallida* is sister to the New World species. Subtribe Saccharinae is not monophyletic, and the genus *Eulalia* is polyphyletic. Based on molecular markers plus morphology, we define the circumscriptions of the New World species of *Eriochrysis*: *E. laxa* is distinct from *E. warmingiana*, and *E. villosa* is distinct from *E. cayennensis*. The occurrence of natural hybrids between *E. laxa* and *E. villosa* was documented. The hybrids are probably tetraploids, based on the number of paralogues in the nuclear trees. This is the first record of a polyploid taxon in the genus *Eriochrysis*. Some incongruities between nuclear genes and plastome analyses were detected and are potentially caused by incomplete lineage sorting and/or ancient hybridization. The set of low-copy nuclear genes used in this study seems to be efficient to solve phylogenetic relationships and define the circumscription of other species complexes in the grass family and relatives, even in presence of polyploidy and reticulate evolution. Complete plastome sequencing is also a promising tool for phylogenetic inferences.

Key words: Saccharinae; species delimitation; low-copy nuclear markers; plastid phylogenomics; polyploidy; incomplete lineage sorting

1. Introduction

Species delimitation is a vital issue within evolutionary biology, and is especially important to the conservation of biodiversity (Carstens et al., 2013). However, delimiting species is not an easy task. Morphological characters are often under selective pressure, which may result in character similarity for unrelated species adapting to similar conditions, as well as in striking morphological differences between related species adapting to different conditions (Koopman et al., 2008). Species delimitation is often a challenge to molecular systematists due to the low interspecies variability of the markers currently used in phylogenetic reconstructions (Sang, 2002). The occurrence of interspecific hybridization and polyploidy makes the situation even more complicated (McDade, 1992; Welker et al., 2015). A case study of *Eriochrysis* P. Beauv. and related taxa (Poaceae – Andropogoneae) is presented here. *Eriochrysis* is a grass genus typical of marshlands and wet grasslands, with ca. 7–11 species mainly from New World, with a few species in Africa and India (Clayton et al., 2006; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992). The circumscription of its species is complex and contentious, evidenced by the discrepant number of species accepted by different authors. Plants with intermediate morphology also suggest natural hybridization, although this has not yet been tested (Killeen, 1990; Welker et al., 2012). The delimitation of *Eriochrysis* species is based exclusively on morphology, since there is no molecular phylogeny of the genus so far, with only one species included in broad phylogenies of the tribe (Estep et al., 2014; Welker et al., 2015). Therefore, it is important to reconstruct the first phylogeny of *Eriochrysis* to test its monophyly and to understand the evolution of the genus and the circumscription of its species.

Eriochrysis belongs to the tribe Andropogoneae, subtribe Saccharinae, in the subfamily Panicoideae of the Poaceae. The tribe Andropogoneae, erroneously called Sacchareae by some authors (see Welker et al., 2014), has a cosmopolitan distribution and comprises ca. 90 genera and 1,060 species (Sánchez-Ken and Clark, 2010). Andropogoneae is an ecologically and economically important group of C_4 species, including some of the most important crops in the

world, such as sugarcane (*Saccharum officinarum* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* (L.) Moench), as well as many dominant species in several grassland vegetation formations throughout the world. Polyploidy is common in most genera of Andropogoneae. A recent study has documented that at least one third of Andropogoneae species have resulted from allopolyploidy, with a remarkably high number of independent allopolyploidization events (Estep et al., 2014). The tribe is strongly supported as monophyletic, and the topology of the phylogenetic trees suggests a rapid evolutionary radiation near the base of the Andropogoneae clade, based on the short branches along the backbone of the trees (Estep et al., 2014; Mathews et al., 2002; Teerawatananon et al., 2011). Phylogenetic analyses indicate the presence of a "core Andropogoneae" clade, including *Andropogon* L., *Schizachyrium* Nees, *Hyparrhenia* Andersson ex E. Fourn., *Bothriochloa* Kuntze, and several other genera (Estep et al., 2014; Mathews et al., 2002). *Eriochrysis* is placed outside the "core Andropogoneae" (Estep et al., 2014).

Based on morphological aspects, *Eriochrysis* was included in subtribe Saccharinae by Clayton and Renvoize (1986), together with the genera *Saccharum* L., *Eulalia* Kunth, *Imperata* Cirillo, *Microstegium* Nees, *Miscanthus* Andersson, *Pogonatherum* P. Beauv., and *Polytrias* Hack., among others. *Apocopis* Nees, *Germainia* Balansa & Poitr., and *Trachypogon* Nees were included in subtribe Germainiinae, which was considered closely related to Saccharinae by Clayton and Renvoize (1986), based on morphology. The monophyly of the subtribe Saccharinae has not yet been comprehensively studied, and needs closer examination (Hodkinson et al., 2002; Mathews et al., 2002). Germainiinae seems to be monophyletic (Estep et al., 2014; Teerawatananon et al., 2011), although no species of *Trachypogon* was included in those analyses.

The genus *Eriochrysis* is characterized mainly by elongated inflorescences with golden-brown to light-brown trichomes and heterogamous spikelets in pairs on the branches of the inflorescence; each pair includes a sessile spikelet with a bisexual flower and a pedicelled

spikelet with a pistillate flower (Welker and Longhi-Wagner, 2012). Previous cytogenetic studies indicate that *Eriochrysis* species are diploid, with 2*n*=20 (Dujardin, 1979; Pohl and Davidse, 1971). No polyploid species has been documented for the genus, although polyploidy is frequent in the tribe Andropogoneae and in Poaceae as well (Estep et al., 2014). Based on morphology, *Leptosaccharum* (Hack.) A. Camus was considered a monospecific genus (*L. filiforme* (Hack.) A. Camus) by some authors (Camus, 1956; Watson and Dallwitz, 1992) or a synonym of *Eriochrysis*, with the new combination *E. filiformis* (Hack.) Filg. proposed by Filgueiras (1997).

Delimitation of the New World species of *Eriochrysis* is controversial and poorly investigated and is based on minor morphological characters such as the shape of the spikelets, the apex of the lower glume (i.e., the bract at the base of the spikelet), and the density of trichomes in the inflorescences (Swallen, 1966; Welker and Longhi-Wagner, 2012) (see Table 1). *Eriochrysis cayennensis* P. Beauv., the species of the genus with the broadest geographical distribution in the Americas, has densely pilose inflorescence and lower glume with an obtuse to truncate, trilobed apex (Welker and Longhi-Wagner, 2012). The morphologically similar species *E. villosa* Swallen, considered endemic to Southern Brazil (Swallen, 1966), is distinguished mainly by the lower glume with an acute entire apex (Welker and Longhi-Wagner, 2012) (Table 1). *Eriochrysis villosa* is accepted as a distinct species by several authors (Filgueiras and Welker, 2015; Smith et al., 1982; Welker and Longhi-Wagner, 2012; Welker et al., 2012), but considered a probable synonym of *E. cayennensis* by Filgueiras (2003) and a "dubious taxon" by Morrone et al. (2008). Plants from Southern Brazil with intermediate morphology between the two species (i.e., with lower glume with an subacute apex and inconspicuous lobes) were considered morphological variation of *E. villosa* by Welker and Longhi-Wagner (2012), but may be natural hybrids between *E. cayennensis* and *E. villosa* (see Table 1, labeled as *Eriochrysis* sp.).

The circumscription of *Eriochrysis laxa* Swallen and *E. warmingiana* (Hack.) Kuhlm. is also contentious. The two species are accepted as distinct taxa by several authors (Filgueiras and Welker, 2015; Morrone et al., 2008; Swallen, 1966; Welker et al., 2012), but *E. laxa* was

considered a probable synonym of *E. warmingiana* by Filgueiras (2003). The two species are morphologically distinct, especially in the shape of the spikelets and in the disposition of the lower branches of the inflorescence (Welker et. al., 2012) (see Table 1). Despite the morphological differences, no molecular investigation about their circumscriptions is available.

Some specimens with intermediate morphology were collected in a marshland in Southern Brazil, where the species *Eriochrysis laxa*, *E. cayennensis*, and *E. villosa* also occur. Those individuals have obovate spikelets as in *E. laxa* but densely pilose inflorescences as in *E. cayennensis* and *E. villosa*. The apex of the lower glume is rounded similar to that of *E. laxa*, but sometimes has inconspicuous lobes (Welker et al., 2012). The intermediate plants were treated as *Eriochrysis* aff. *laxa* by Welker et al. (2012) and are likely natural hybrids between *E. laxa* and *E. cayennensis* or *E. villosa*. Killeen (1990) described *Eriochrysis* × *concepcionensis* Killeen as a putative hybrid between *E. laxa* and *E. cayennensis*, based on specimens from Bolivia. It is possible that hybridization between these species also occurs in Southern Brazil.

Delimiting species is a complex task and should integrate genetic and non-genetic sources of data, such as morphology, biogeography, and others (Carstens et al., 2013; Queiroz, 2007). The difficulty in inferring species boundaries based on molecular data, among other reasons, is the low variability at this taxonomic level of the DNA markers currently used in phylogenetic analyses, especially from plastid genome (Després et al., 2003; Sang, 2002). The occurrence of interspecific hybridization and polyploidy is an additional complication for delimiting species (McDade, 1992; Welker et al., 2015), as mentioned above. Due to the high variability of the sequences and the capacity of identifying hybrids (Estep et al., 2014; Sang, 2002; Welker et al., 2015), low-copy nuclear genes are promising markers for reconstructing the phylogeny of *Eriochrysis* and circumscribing its species, which probably include interspecific hybridization. Although polyploidy has not been found in the genus, it is very common in the tribe Andropogoneae (Estep et al., 2014) and may also occur in *Eriochrysis*, which reinforces the use of low-copy nuclear markers. Nuclear genes are useful to identify allopolyploidization events

because they produce characteristic double-labeled tree topologies in which the polyploid species appear twice (Estep et al., 2014; Sang, 2002; Triplett et al., 2012). In such trees, allopolyploids can be recognized even in the absence of chromosome counts (Estep et al., 2014).

Advances in DNA sequencing technologies, such as high throughput sequencing, have increased the amount of data for phylogenetic reconstructions, such as complete plastome sequences (Burke et al., 2014; McCormack et al., 2013; Steele et al., 2012; Straub et al., 2012). In higher plants, the chloroplast genome size generally ranges from 120 to 160 kb depending on the species (Nie et al., 2012), but sometimes it is much larger (Chumley et al., 2006). Although the plastome is highly conserved, comparative analyses of large datasets of complete plastome sequences present enough variation to greatly improve our knowledge of the phylogenetic affinities of taxa, including the family Poaceae (Besnard et al., 2013; Burke et al., 2014; Givnish et al., 2010; Moore et al., 2010). Phylogenetic analyses based on plastomes have demonstrated increased resolution and support of the trees, even at low taxonomic levels (Parks et al., 2009; Straub et al., 2012). According to Straub et al. (2012), high throughput DNA sequencing is likely to revolutionize plant systematics just as Sanger sequencing did more than 20 years ago. Therefore, in addition to low-copy nuclear genes, complete plastome sequences may bring new insights to our understanding of the evolutionary history of *Eriochrysis* and relatives.

The current study aimed to (1) test the monophyly of *Eriochrysis* and assess its phylogenetic relationships to other genera of Saccharinae and Andropogoneae, (2) define the taxonomic circumscription of the New World species of *Eriochrysis*, and (3) investigate the identity of possible interspecific hybrids, apparently involving *E. laxa*, *E. cayennensis*, and/or *E. villosa*.

2. Material and methods

2.1. Plant material

For nuclear genes, we sampled 30 specimens belonging to six putative species of *Eriochrysis*, including the type species of the genus (*E. cayennensis*). Forty-seven species belonging to 33 other genera of Andropogoneae were also included in the analyses. Two species of *Arthraxon* P. Beauv. were used as an outgroup, based on Estep et al. (2014). Voucher specimens and collection localities are listed in Table 2. GenBank accession numbers for the sequences are listed in Appendix 1.

Whole plastomes of four taxa of *Eriochrysis* were also sequenced, as well as those for *Chrysopogon serrulatus* Trin. and *Pogonatherum paniceum* (Lam.) Hack., both within Andropogoneae. Additional plastomes were taken from GenBank or assembled from shotgun genome sequence data from NCBI's Short Read Archive (SRA). *Setaria italica* (L.) P. Beauv. (tribe Paniceae) was used as an outgroup. Voucher specimens and GenBank accession numbers for the plastomes are listed in Table 3.

2.2. Molecular cloning, sequencing, and data processing of nuclear loci

Total genomic DNA was extracted using the CTAB procedure (Doyle and Doyle, 1987), modified for microcentrifuge tubes. Five regions of four low-copy nuclear loci were PCR amplified following Estep et al. (2012) and Triplett et al. (2012): *Aberrant panicle organization*1 (*apo*1), *Dwarf*8 (*d*8), two exons of *Erect panicle*2 (*ep*2-*ex*7 and *ep*2-*ex*8), and *Knotted*1 (*kn*1). Previous works show that these loci are efficient markers to infer phylogenetic relationships in the subfamily Panicoideae and in the tribe Andropogoneae (Estep et al., 2012, 2014; Triplett et al., 2012; Welker et al., 2015).

The PCR products were purified via gel extraction using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, California, U.S.A.), following the manufacturer's protocol. To capture paralogous copies, purified products were cloned using pGEM-T Easy Vector and transformed into JM109 High-Efficiency Competent Cells (Promega, Madison, Wisconsin, U.S.A.), following manufacturer's protocols. Transformed cells were plated and selected via a blue-white

screen on LB agar with X-Gal, isopropyl-beta-thio-galactoside (IPTG), and ampicillin. Eight positive clones of each PCR product were selected. Extracted DNA from the colonies was sent to Beckman Coulter Genomics (Danvers, Massachusetts, U.S.A.) for sequencing in both directions using universal primers (T7 and M13R). Internal primers were also used for sequencing *d*8 and *ep*2-*ex*7 loci (Estep et al., 2012, 2014).

Chromatogram files were trimmed of vector using Geneious v.6.1.8 (Biomatters, Auckland, New Zealand) and ambiguous bases from the ends of both reads were removed manually. Forward and reverse sequences (and sequences from internal primers in *d*8 and *ep*2-*ex*7 loci) were subsequently assembled for each clone. Only clones with 80% or more double-stranded sequence were used for analysis. All good quality contigs for each sample were then aligned using Geneious, and primer sequences were removed. Recombinant sequences were identified by eye, comparing them with unambiguous sequences from related species, and were removed from the alignment. Redundant clones of the same gene copy were combined into a consensus sequence, to minimize the inclusion of sequencing errors and reduce the number of sequences to one per paralogue per locus. The resulting sequences were translated and aligned using MUSCLE, as implemented in Geneious.

2.3. Plastome sequencing and assembly

Whole genomic DNA Illumina libraries from the above DNA isolations were made using either the NEBNext Ultra DNA Library Prep Kit (New England Biolabs, Inc., Ipswich, Massachusetts, U.S.A.) or the Nextera DNA Sample Preparation Kit (Illumina, San Diego, California, U.S.A.) following the manufacturer's protocol. For libraries made using the NEBNext kit, DNA was sheared at the University of Missouri–DNA Core Facility using a Covaris S220/E220 focused-ultrasonicator (Covaris, Woburn, Massachusetts, U.S.A.) to obtain an average size of 300–350 base pairs (bp). For those made with the Nextera kit, incubation time for the tagmentation step was 5 minutes at 55°C, providing a broad fragment distribution.

Samples and their library preparation methods are found in Table 4. Libraries were size-selected using Agencourt AMPure XP beads (Beckman Coulter, Inc., Indianapolis, Indiana, U.S.A.) to obtain an average fragment size of 300–350 bp for libraries sequenced using single-end Illumina and 400–500 bp for libraries sequenced using paired-end Illumina. Libraries were sequenced for 100 bp single-end reads at the University of Missouri–DNA Core Facility on a HiSeq 2000 (Illumina, San Diego, California, U.S.A.) and for 150 bp paired-end reads at the New York University School of Medicine using a HiSeq 2500 Rapid Run (Illumina, San Diego, California, U.S.A.). Table 4 provides information for sequencing methods used for each sample. A dataset of paired-end Illumina reads for *Miscanthus sinensis* Andersson (SRA SRR559246) was downloaded from the SRA database of GenBank.

Reads were trimmed using Trimmomatic v.0.32 (Bolger et al., 2014) using following parameters: ILLUMINACLIP:appropriate_adpater_file:3:30:10 SLIDINGWINDOW:10:20 MINLEN:40. Initial assemblies were made using SPAdes v.3.1.0 (Bankevich et al., 2012) using either single-end or pair-end when appropriate with the "only-assembler" option and k-mer sizes of 33, 55, and 77. Each SPAdes assembly was blasted against the *Zea mays* chloroplast (GenBank, NC001666.2) and mitrochondrial (GenBank, NC007982.1) genomes using blastn with an e-value cutoff of 1e-10 (Camacho et al., 2009) and assembly contigs were filtered into chloroplast-like and mitochondrial-like pools based on e-values and overlap lengths. Chloroplast-like contigs were meta-assembled in Sequencher v.5.0.1 (Gene Codes, Ann Arbor, Michigan, U.S.A.). Annotated *Zea may* chloroplast genes were assembled with the meta-assembly of SPAdes contigs to provide context for contigs. Gaps between contigs were closed by using an iterative plastome-walking procedure. This was done by searching the trimmed reads for sequences with affinity to the ends of contigs and assembling those reads to the contigs within Sequencher. To check final assemblies for accuracy, counts for 20-mers from the trimmed reads were estimated using jellyfish v.2.1.3 (Marçais and Kingsford, 2011). Counts from the reads for all 20-mers found within the plastome assemblies were recorded in a sliding window.

Assemblies were considered acceptable if no sudden drops in coverage were detected, aside from the expected variation at the inverted repeat boundaries. If any cases were found, they were investigated by assembling reads matching the regions surround the drop. In all cases, the reads supported the final assembly and demonstrated regions of relatively lower coverage in the plastomes. Plastomes were uploaded to Verdant (verdant.iplantcollaborative.org) and automatically annotated. A Circos graph (Krzywinski et al., 2009) of the plastome structure was created for each species using the built-in features of Verdant. Additional whole plastomes for Andropogoneae (*Saccharum officinarum*, *Sorghum bicolor,* and *Zea mays*) and an outgroup (*Setaria italica*) were downloaded from GenBank and added to Verdant for further analysis (Table 3).

2.4. Phylogenetic analyses

Gene trees were estimated for each nuclear locus in RAxML v.8.1.11 (Stamatakis, 2006; Stamatakis et al., 2008) using the Black Box setting on the CIPRES Science Gateway (Miller et al., 2010). Individual gene tree topologies were used as a guide to identify the corresponding paralogues of each genome in the five loci, for the polyploid specimens, and create concatenated sequences, according to Estep et al. (2014). The results presented here were based on the dataset with a minimum of three out of five loci per genome for each taxon, except the samples Kellogg VA-2, Teerawatananon & Sungkaew 865, Welker 481A, Welker 486, and Welker 544, for which only two loci were sequenced.

Trees were reconstructed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses, based on the concatenated nuclear gene dataset. The parsimony ratchet analysis (Nixon, 1999) was performed in PAUP v.4.0b10 (Swofford, 2002) using the companion program PAUPRat (Sikes and Lewis, 2001). Twenty independent runs were performed with 200 iterations each. The support at each node was assessed through

bootstrap analysis (Felsenstein, 1985), with a heuristic search based on 1000 replicates. Bootstrap values > 50% were recorded on the trees.

The ML analysis was performed in RAxML v.8.1.11 using the Black Box setting on the CIPRES Science Gateway. Models of DNA evolution were determined using jModelTest 2 (Darriba et al., 2012) and the GTR+I+G model was selected. ML support was assessed via 500 bootstrap replicates, and values > 50% were recorded on the trees. The BI analysis was conducted using MrBayes v.3.2.3 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), also hosted in CIPRES, with six rate categories. Two independent runs of 20 million generations were performed and sampled every 1000 generations. The consensus tree was estimated after a burn-in of 25% of sampled trees. Posterior probability (PP) values > 0.85 were recorded on the trees.

Annotated protein-coding genes, tRNAs, rRNAs, introns, and intergenic regions of the large single copy (LSC), small single copy (SSC), and inverted repeat B (IRB) of the plastomes were compiled and then aligned individually using MAFFT v.7.215 (Katoh and Standley, 2014; Katoh et al., 2005) with the "auto" setting within the Verdant environment. Alignments of individual regions were checked for total number of samples represented and if they did not have all 11, that alignment was ignored. A total of 234 out of 247 alignments were used and concatenated together for a total alignment length of 120,281 sites. Whole plastome phylogenies were estimated using ML and BI analyses as described above for the nuclear dataset, but under the GTR+G model.

3. Results

The aligned data matrix including the five low-copy nuclear loci was 4,263 bp long, of which 1,577 (37%) were variable and 899 (21%) were parsimony informative. The MP analysis resulted in 2,947 equally most parsimonious trees of 2,554 steps (CI = 0.49, RI = 0.79). The phylogenetic trees resulted from MP, ML, and BI analyses had the same topology, with short

branches along the backbone of the tree, in contrast to long external branches (Fig. 1). No incongruence in topologies and branching patterns was found between the gene trees of the low-copy nuclear loci, although some individual trees had less resolution and support compared to the concatenated nuclear dataset.

All samples of *Eriochrysis* grouped in a strongly supported clade (1 PP, 100% ML and 95% MP bootstrap), which is sister to the strongly supported clade (1 PP, 100% ML, 99% MP) including the genera *Imperata* and *Pogonatherum* (subtribe Saccharinae sensu Clayton and Renvoize, 1986) and *Apocopis* and *Germainia* (subtribe Germainiinae) (Fig. 1). The genera *Saccharum*, *Miscanthus*, *Microstegium*, *Polytrias*, and *Eulalia* (also included in subtribe Saccharinae by Clayton and Renvoize, 1986) did not group closely to *Eriochrysis*. These five genera plus *Sorghastrum* Nash and *Pseudosorghum* A. Camus (subtribe Sorghinae) formed a clade (1 PP, 61% ML, <50% MP), which is sister to *Sorghum* Moench + "core Andropogoneae" clade (although with only moderate support in BI analysis (0.96 PP) and no bootstrap support higher than 50% in ML and MP analyses). Concerning the genus *Eulalia*, only *E. aurea* (Bory) Kunth (type species of the genus) grouped in the clade that includes *Saccharum*, *Miscanthus*, and other Saccharinae. The other *Eulalia* species analyzed (*E. quadrinervis* (Hack.) Kuntze and *E. villosa* Nees) grouped within the "core Andropogoneae" clade (Fig. 1).

Within the *Eriochrysis* clade, the African *E. pallida* Munro is sister to all New World species (0.99 PP, 96% ML, 80% MP) (Fig. 2). *Eriochrysis warmingiana* is sister to the remaining species from the New World (0.98 PP, 83% ML, 86% MP). The multiple samples analyzed from the morphological species *E. holcoides*, *E. laxa*, *E. villosa*, and *E. cayennensis* formed distinct clades, with strong support. *Eriochrysis holcoides* specimens formed a clade (1 PP, 88% ML, <50% MP) which is sister to the remaining species, and the *E. laxa* clade (0.99 PP, 99% ML, 71% MP) is sister to the *E. villosa–E. cayennensis* clade (1 PP, 99% ML, 94% MP) (Fig. 2).

Within the *E. villosa–E. cayennensis* clade, specimens morphologically identified as *E. villosa* and *E. cayennensis* formed two sister clades with strong support (0.99 PP, 82% ML, 62% MP; and 1 PP, 94% ML, 88% MP; respectively) (Fig. 2). Two specimens from Uruguay identified as *E. villosa* based on morphology (Welker 652 and Welker 655) grouped together with *E. villosa* specimens from Southern Brazil. *Eriochrysis* specimens with lower glume with a subacute apex and inconspicuous lobes (labeled here as *Eriochrysis* sp.) fell within the *E. cayennensis* clade (Fig. 2).

Specimens with intermediate morphology identified as *Eriochrysis* aff. *laxa* by Welker et al. (2012) had two distinct paralogues at each locus, in contrast to all other *Eriochrysis* samples which had only a single sequence per locus. One paralogue of *Eriochrysis* aff. *laxa* specimens fell within *E. laxa* clade and the other within *E. villosa* clade (Fig. 2, specimens highlighted in bold).

The chloroplast genome of the *Eriochrysis* samples analyzed ranges from 140,135 to 140,426 bp, including a large single copy (LSC) region of 82,362 to 82,393 bp separated by a pair of inverted repeats (IR) regions of 22,638 to 22,783 bp (Table 3). The chloroplast genome map of *E. cayennensis* is presented in Figure S1 (see Supplementary Data with the online version of this article). Other species included in this study have similar plastome size and gene content and organization (Table 3). The phylogenetic trees based on complete plastome sequences resulted from BI and ML analyses had the same topology. All samples of *Eriochrysis* grouped in a strongly supported clade (1 PP, 100% ML), which is sister to the strongly supported clade (1 PP, 100% ML) including the genera *Pogonatherum*, *Sorghum*, *Miscanthus*, and *Saccharum* (Fig. 3). Unlike the phylogenies of nuclear loci (Fig. 1), *Pogonatherum* is more closely related to *Saccharum* and *Miscanthus* than to *Eriochrysis* in the plastome phylogenies (Fig. 3). Within the *Eriochrysis* clade, the phylogenetic relationships between its species were also somewhat different in the plastome trees comparing to the nuclear trees (Fig. 4). *Eriochrysis villosa* is sister

to the clade including the remaining species analyzed. Within this clade, *E. laxa* is sister to *E. cayennensis* and *Eriochrysis* sp. (Fig. 3).

4. Discussion

4.1. Phylogenetics of Eriochrysis and related genera

The pattern of very short branches along the backbone of the nuclear trees, contrasting to long external branches, supports the hypothesis that the early diversification in Andropogoneae was rapid (Estep et al., 2014; Mathews et al., 2002; Teerawatananon et al., 2011).

The present phylogenetic analyses indicate that *Eriochrysis* is monophyletic and the Old World *E. pallida* is sister to the clade including the New World species of the genus. The phylogenetic affinities of *Eriochrysis* with the genera *Imperata* and *Pogonatherum* (also included in subtribe Saccharinae by Clayton and Renvoize, 1986) were confirmed in our analyses based on low-copy nuclear markers (Fig. 1). However, *Pogonatherum* is not very closely related to *Eriochrysis* in our plastome phylogenies (Fig. 3). Other genera included in subtribe Saccharinae by Clayton and Renvoize (1986), such as *Saccharum*, *Miscanthus*, *Microstegium*, *Polytrias*, and *Eulalia*, did not group closely to *Eriochrysis* in nuclear trees (Fig. 1). Therefore, subtribe Saccharinae (sensu Clayton and Renvoize, 1986) was not supported as monophyletic in the present analyses, in agreement with previous molecular phylogenetic studies (Hodkinson et al., 2002; Mathews et al., 2002), and seems to be polyphyletic.

Our phylogenetic analyses based on nuclear genes also indicate that *Eriochrysis*, *Imperata*, and *Pogonatherum* are closely related to the genera *Apocopis* and *Germainia*, included in subtribe Germainiinae by Clayton and Renvoize (1986). The monophyly of the subtribe Germainiinae was suggested by Teerawatananon et al. (2011) and Estep et al. (2014), and the present phylogenetic analyses show that the subtribe remains monophyletic when the taxon sample is increased substantially in *Eriochrysis*. However, representatives of *Trachypogon* (also

included in Germainiinae by Clayton and Renvoize, 1986) were not included in any of the phylogenetic studies. A larger sample of the representatives of Saccharinae, Germainiinae and other related subtribes of Andropogoneae is needed for a more accurate answer about their circumscriptions and for eventually proposing a new subtribal classification for the tribe Andropogoneae, based on molecular evidence.

The present nuclear gene analyses also suggest that *Eulalia* is polyphyletic (Fig. 1). The genus includes ca. 30 species from the tropics of the Old World and was considered closely related to *Saccharum* by Clayton and Renvoize (1986), based on morphology. The type species of the genus (*E. aurea*) was confirmed as closely related to *Saccharum* and relatives in nuclear analyses, but the other species analyzed (*E. quadrinervis* and *E. villosa*) are more closely related to representatives of the "core Andropogoneae" clade. The segregation of the latter species from the genus *Eulalia* is needed, as well as a broad phylogenetic analysis of the genus to investigate the position of the remaining species.

Unfortunately we could not include material of the South American *Eriochrysis filiformis*, previously placed in the monospecific genus *Leptosaccharum* (Camus, 1956; Watson and Dallwitz, 1992) and currently accepted in *Eriochrysis* (Filgueiras, 1997; Filgueiras and Welker, 2015; Welker et al., 2012). *Eriochrysis filiformis* has the pair of spikelets consisting of one short- and one long-pedicelled spikelet, in contrast to one sessile and one pedicelled spikelet in other species of *Eriochrysis* and in most representatives of the tribe Andropogoneae (Filgueiras, 1997; Welker et al., 2012). Because of this uncommon morphological character for Andropogoneae, *E. filiformis* was initially placed in the tribe Paniceae of the subfamily Panicoideae (see Filgueiras, 1997). *Eriochrysis filiformis* is rare in nature and the plants apparently bloom only after having been burnt (Filgueiras, 1997), which makes the collection of samples difficult. Future molecular studies should include this species to investigate whether *Leptosaccharum* is a distinct genus or a synonym of *Eriochrysis*, as currently accepted.

4.2. Taxonomic circumscription of New World species of Eriochrysis

The *Eriochrysis* specimens analyzed had only one copy of each nuclear gene (except specimens identified as *E.* aff. *laxa*, discussed below), suggesting that the *Eriochrysis* species are diploid. The ploidy level inferred by the number of paralogues is in agreement with chromosome counts performed by Pohl and Davidse (1971) and Dujardin (1979) for *E. cayennensis* and *E. brachypogon* (Stapf) Stapf, respectively. No cytogenetic analyses for other species of *Eriochrysis* are available so far.

In addition to morphological differences, the distinct strongly supported clades formed by the multiple samples analyzed of *Eriochrysis holcoides*, *E. laxa*, *E. villosa*, and *E. cayennensis* in the nuclear trees (Fig. 2) reinforce their acceptance as distinct taxa.

Eriochrysis laxa is clearly distinct from *E. warmingiana*, as accepted by several authors (Filgueiras and Welker, 2015; Morrone et al., 2008; Swallen, 1966; Welker et al., 2012). The phylogenetic analyses based on nuclear genes indicate that *E. warmingiana* is sister to the clade including all other South American species, whereas *E. laxa* is phylogenetically more closely related to *E. cayennensis* and *E. villosa* than to *E. warmingiana* (Fig. 2). Therefore, the treatment of Filgueiras (2003) based on morphology, which considered *E. laxa* a probable synonym of *E. warmingiana*, is not supported by our molecular analyses. The two species are morphologically distinct: the former has obovate spikelets and lower glume with a rounded to obtuse apex, whereas the latter species has lanceolate spikelets and lower glume with an acute to acuminate apex. In addition, *E. warmingiana* has longer inflorescences, with divergent branches at the base vs. adpressed branches in *E. laxa* (Welker et. al., 2012) (see Table 1).

Our phylogenetic analyses based on nuclear genes are consistent with two possible treatments for the *Eriochrysis cayennensis–E. villosa* clade (Fig. 2): (1) consider two distinct species, as accepted by several authors (Filgueiras and Welker, 2015; Smith et al., 1982; Swallen, 1966; Welker and Longhi-Wagner, 2012; Welker et al., 2012;); or (2) consider the two names as a single taxon, in which the name *E. cayennensis* would have nomenclatural priority, as

suggested by Filgueiras (2003). On the other hand, the analyses based on complete plastome sequences suggest that *E. villosa* should be considered a distinct taxon from *E. cayennensis*, since *E. villosa* is sister to the clade including *E. laxa*, *E. cayennensis*, and *Eriochrysis* sp. (Fig. 3). *Eriochrysis cayennensis* is the type species of the genus and has a broad geographical distribution in the Americas, from the United States to Argentina, Brazil, and Uruguay (Filgueiras, 2003). The species is characterized mainly by densely pilose inflorescences and lower glume with an obtuse to truncate, trilobed apex (Welker and Longhi-Wagner, 2012). Swallen (1966) described *E. villosa* as an endemic species from Southern Brazil, with densely pilose inflorescences but lower glume with an acute and not lobed apex (see Table 1 and Figure 2). Based on our molecular analyses, plus morphological and biogeographical aspects of the plants, we consider *E. cayennensis* and *E. villosa* as two distinct species, as currently accepted by grass taxonomists.

Specimens collected in Uruguay and identified as *Eriochrysis villosa* based on morphology grouped together with *E. villosa* specimens from Southern Brazil in the nuclear analyses, confirming the identity of those plants and the occurrence of the taxon in Uruguay. This is the first record of the species for Uruguay, expanding the known geographical distribution of the taxon. Therefore, *E. villosa* is not a narrowly endemic species, which is relevant for conservation purposes.

Some plants from Southern Brazil have densely pilose inflorescences and lower glume with an subcute apex, and with inconspicuous lobes (labeled as *Eriochrysis* sp. in Table 1). Welker and Longhi-Wagner (2012) considered those plants as morphological variants of *E. villosa*, but suggested that they could be natural hybrids between *E. cayennensis* and *E. villosa*, due to the intermediate morphology between the two species. Our phylogenetic analyses based on nuclear genes did not confirm either hypothesis, since the specimens of *Eriochrysis* sp. grouped within the *E. cayennensis* clade, without any evidence of hybridization (Fig. 2). Thus, they simply represent morphological variation within *E. cayennensis*. Plastomes data also

support this treatment (Fig. 3). Therefore, based on molecular evidence, we conclude that *E. cayennensis* is more variable than previously believed, and includes specimens with lower glumes with apices that are obtuse, truncate or subacute, trilobed or with inconspicuous lobes.

The phylogenetic position of *E. laxa*, *E. villosa*, and *E. cayennensis* within the *Eriochrysis* clade differs in the trees based on nuclear genes from those based on plastomes (Fig. 4). In the nuclear trees, *E. laxa* is sister to the *E. villosa-E. cayennensis* clade (including specimens here labeled as *Eriochrysis* sp.). In the plastome trees, *E. villosa* is sister to the clade including *E. laxa* and *E. cayennensis* (including *Eriochrysis* sp.). Phenotypically, the first (nuclear) topology makes more sense, since *E. laxa* is the most morphologically distinct species in this group by having sparsely pilose inflorescences (see Table 1). *Eriochrysis villosa* and *E. cayennensis* have densely pilose inflorescences and are differentiated only by the apex of the lower glume of the spikelets (see section 4.4 below for a discussion about incongruence between our nuclear and plastome trees).

4.3. Interspecific hybridization in Eriochrysis

Unlike all other taxa of *Eriochrysis*, the specimens from Southern Brazil identified as *Eriochrysis* aff. *laxa* by Welker et al. (2012) had two distinct paralogues in our nuclear trees, indicating they are polyploid (probably tetraploid). This is the first record of a polyploid taxon in *Eriochrysis*, although polyploidy is common in many other genera of the tribe Andropogoneae (Estep et al., 2014; Welker et al., 2015).

Welker et al. (2012) suggested that those specimens may be interspecific hybrids, based on their intermediate morphology: the spikelets are obovate and the apex of the lower glume is rounded as in *E. laxa* but the inflorescences are densely pilose as in *E. cayennensis* and *E. villosa*, and sometimes the lower glume has inconspicuous lobes (Welker et al., 2012). Our analyses confirm the hybrid origin of those specimens, since one paralogue of the samples grouped within the *E. laxa* clade and the other paralogue within the *E. villosa* clade (Fig. 2). All

three polyploid specimens (samples Welker 487-10, Welker 487-11, and Welker 544) were collected in a marshland in the State Rio Grande do Sul (Southern Brazil), occurring together with the two presumed parental species: *E. laxa* (sample Welker 489) and *E. villosa* (Welker 490).

The occurrence of polyploid hybrids (probably tetraploid) formed from two presumed diploid parental species (*E. laxa* and *E. villosa*) suggests interspecific hybridization followed by duplication of genomes (allopolyploidy). It is well known that polyploidy can restore fertility to sterile hybrid lineages after hybridization (McDade, 1992).

Killeen (1990) described *Eriochrysis* × *concepcionensis* as a hybrid between *E. laxa* and *E. cayennensis*, based on a single population with intermediate morphology found in Santa Cruz (Bolivia) in the same habitat as both putative parental species. According to Killeen (1990), pollen development and seed set in *Eriochrysis* × *concepcionensis* were abnormal compared to the two putative parental species. However, no molecular or cytogenetic evidence is available to confirm the hybrid origin of these plants. Our analyses found no hybrids between *E. laxa* and *E. cayennensis*, but such hybridization events might occur from time to time.

Cytogenetic studies of *Eriochrysis* species are scanty. Additional cytogenetic studies may bring valuable information about the ploidy level of *Eriochrysis* species, as well as the fertility of the hybrids between *E. laxa* and *E. villosa* from Southern Brazil, evidenced here. Molecular and cytogenetic investigation is also needed to confirm the hybrid origin of *Eriochrysis* × *concepcionensis* from Bolivia.

4.4. Incongruence between nuclear genes and plastomes

Molecular phylogenetic studies now include an increasing abundance of data, such as numerous DNA loci and complete genome sequences. A common challenge in such large sets of data is that conflicting genealogical histories often exist in different genes throughout the genome, and in different genomes from the organism (Degnan and Rosenberg, 2009; Maddison,

1997; Pelser et al., 2010). Although individual gene trees of the low-copy nuclear loci were not incongruent in the present study, some discordance appeared between our nuclear and plastome phylogenetic analyses. The major incongruities were the position of *Pogonatherum* (see Figures 1 and 3) and the position of *Eriochrysis laxa* and *E. villosa* within the *Eriochrysis* clade (Fig. 4).

Incongruence between phylogenetic trees inferred from different molecular markers may result from biological phenomena as well as analytical artifacts (Pelser et al., 2010). Incomplete lineage sorting (ILS) of ancestral polymorphisms seems to be a potential cause for incongruence in our analyses. ILS is the failure of ancestral polymorphisms to track speciation events accurately. Because of the stochastic nature of the coalescence process, ILS may yield gene trees with random patterns of relationships among taxa, which may result in incongruence between gene trees and species trees (Maddison, 1997; Pelser et al., 2010). It is well know that ILS can cause serious difficulties for phylogenetic inference (Degnan and Rosenberg, 2009; Maddison, 1997; Maddison and Knowles, 2006). Incomplete lineage sorting is especially likely when species rapidly radiate and effective population sizes are large (Maddison, 1997; Pelser et al., 2010). That may be the scenario for *Eriochrysis* and relatives, since Andropogoneae phylogenies suggest that the tribe resulted from rapid radiation (Estep et al., 2014; Mathews et al., 2002; Teerawatananon et al., 2011). Additionally, Andropogoneae grasses often have large populations and many of them are dominant species of tropical and temperate grasslands, covering vast areas of the world (Estep et al., 2014). However, the effective population size is not known for most grasses.

Hybridization may also explain the topological incongruence between the nuclear and plastome trees, especially concerning the position of *Pogonatherum*. Hybridization, especially followed by duplication of genomes (allopolyploidy), is common among flowering plants and particularly in grasses from tribe Andropogoneae (Estep et al., 2014; Kim et al., 2014; Welker et al., 2015). Estep et al. (2014) found a minimum of 34 independent allopolyploidization events in the tribe Andropogoneae. An allopolyploidization event in *Eriochrysis*, generating interspecific

hybrids between *E. laxa* and *E. villosa*, was also documented in the present phylogenetic analyses. However, no evidence was found that the genus itself was the result of a hybridization event. It is possible that other hybridization events not involving whole duplicating of genomes have occurred in the evolutionary history of Andropogoneae, and thus were not detected in the phylogenetic analyses based on low-copy nuclear genes of Estep et al. (2014), Welker et al. (2015), and in the present study. Hybridization events may be difficult to recognize when they result in homoploid hybrids, are ancient, or were followed by speciation or dispersal in combination with extinction in the parental distribution area (Pelser et al., 2010). Since the chloroplast genome has uniparental inheritance (Birky, 1995, 2001; Nie et al., 2012), ancient hybridization in Andropogoneae could also contribute to the incongruence between the nuclear and plastome trees, especially concerning the position of *Pogonatherum*.

In addition to the potential biological causes discussed above, analytical artifacts such as difference in taxon sample may also have contributed for incongruence between the trees. Since our nuclear gene dataset includes much more taxa than the plastome dataset, the different sample combined with the very short branches along the backbone of the trees could lead to different topologies just because some species were not included. Future plastid phylogenomic studies should increase the number of taxa in order to test the affinities between Andropogoneae taxa suggested by the present plastome analyses.

5. Conclusions

The present phylogenetic analyses indicate that *Eriochrysis* is monophyletic and the Old World *E. pallida* is sister to the New World species. The genus is closely related to *Imperata* and *Pogonatherum* (subtribe Saccharinae) and *Apocopis* and *Germainia* (subtribe Germainiinae) based on evidence from nuclear genes. Our analyses also confirm that subtribe Saccharinae (sensu Clayton and Renvoize, 1986) is not monophyletic, and the genus *Eulalia* is polyphyletic. Based on molecular markers plus morphology, we could define the circumscription of the New

World taxa of *Eriochrysis*: *E. laxa* is a distinct species from *E. warmingiana*, and *E. villosa* is a distinct species from *E. cayennensis*. *Eriochrysis villosa* is reported here for the first time for Uruguay and is not simply endemic to Southern Brazil. The occurrence of natural hybrids between *E. laxa* and *E. villosa* was also documented. These interspecific hybrids are probably tetraploid, based on the number of paralogues in the nuclear trees; all other *Eriochrysis* species are probably diploid. This is the first record of a polyploid taxon in the genus *Eriochrysis*. Incongruence observed between nuclear gene and plastome phylogenetic analyses are potentially caused by incomplete lineage sorting and/or ancient hybridization. The set of low-copy nuclear loci used in this study seems to be efficient to solve phylogenetic relationships and to define the circumscription of other species complexes in the grass family and relatives, even in presence of polyploidy and reticulate evolution. Complete plastome sequencing is also a valuable tool for phylogenetic inferences.

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Table 1
Comparison of morphological and biogeographical characters of New World taxa of *Eriochrysis* discussed in the text. Illustrations of the lower glume of the spikelet of these taxa are presented in Figure 2.

	E. cayennensis	***E. holcoides***	***E. laxa***	***E.* aff. *laxa***	***E. villosa***	***E. warmingiana***	***Eriochrysis* sp.** ("inconspicuous lobes")
Inflorescence: shape, density of trichomes, position of the lower branches	contracted, densely pilose, branches adpressed	contracted, sparsely pilose, branches adpressed	contracted to subcontracted, sparsely pilose, branches generally adpressed	contracted, densely pilose, branches adpressed	contracted, densely pilose, branches adpressed	subcontracted to slightly open, sparsely pilose, branches divergent	contracted, densely pilose, branches adpressed
Spikelet: shape	ovate to elliptic	elliptic to lanceolate	obovate	obovate	elliptic to ovate	lanceolate	ovate to elliptic
Lower glume of the spikelet: apex	obtuse to truncate, trilobed	acute to acuminate, not lobed	rounded to obtuse, not lobed	rounded to obtuse, sometimes with inconspicuous lobes	acute, not lobed	acute to acuminate, not lobed	subacute, with inconspicuous lobes
Geographical distribution	United States to Argentina, Brazil, and Uruguay	Colombia, Peru, Bolivia, Paraguay, and Brazil	Colombia, Bolivia, Paraguay, Argentina, and Brazil	Southern Brazil	Southern Brazil and Uruguay	Bolivia, Paraguay, and Brazil	Southern Brazil

Table 2
Voucher specimens and collection localities of the samples included in the phylogenetic analyses based on nuclear genes. Herbaria acronyms according to Index Herbariorum (Thiers, 2015) except THNHM (Thailand Natural History Museum), not included in that directory.

Species	Voucher	Locality
Andropogon eucomus Nees	Malcomber et al. 3089 (MO)	Tanzania, Iringa, Njombe
Andropogon virginicus L.	Kellogg 1240 (MO)	U.S.A., Missouri, Saint Charles
Andropterum stolzii (Pilg.) C.E. Hubb.	Malcomber et al. 3091 (MO)	Tanzania, Iringa, Njombe
Apocopis courtallumensis (Steud.) Henrard	Teerawatananon & Kritsanachandee 928 (THNHM)	Thailand, Phitsanulok, Khao Kho
Apocopis intermedius (A. Camus) Chai-Anan	Teerawatananon & Kritsanachandee 934 (THNHM)	Thailand, Phitsanulok, Khao Kho
Apocopis siamensis A. Camus	Teerawatananon & Sungkaew 975 (THNHM)	Thailand, Sa Kaew, Watthana Nakhon
Arthraxon lanceolatus (Roxb.) Hochst.	Teerawatananon & Sungkaew 720 (THNHM)	Thailand, Tak, Mae Moei
Arthraxon prionodes (Steud.) Dandy	Kellogg PI 659331 (MO)	China, Xizang
Capillipedium assimile (Steud.) A. Camus	Teerawatananon & Sungkaew 791 (THNHM)	Thailand, Chiang Mai, Mae Ngon
Chasmopodium caudatum (Hack.) Stapf	Kellogg Kew MSB 184054 (MO)	Burkina Faso, Houet
Chionachne koenigii (Spreng.) Thwaites	Kellogg Chio-6-D-93 (MO)	India
Chrysopogon gryllus (L.) Trin.	Kellogg PI 250984 (A/GH)	Republic of Macedonia, Skopje
Chrysopogon serrulatus Trin.	Kellogg PI 219580 (A/GH)	Pakistan, Bannu
Cymbopogon distans (Nees ex Steud.) Will. Watson	Kellogg PI 271552 (MO)	India, Pahlgam
Dichanthium annulatum (Forssk.) Stapf	Kellogg PI 240155 (A/GH)	Morocco
Diheteropogon amplectens (Nees) Clayton	Kellogg RF 1819 (MO)	South Africa, Gauteng
Diheteropogon hagerupii Hitchc.	Kellogg Kew MSB 254456 (MO)	Burkina Faso, Comoe
Dimeria fuscescens Trin.	Teerawatananon & Sungkaew 830 (BKF, THNHM)	Thailand, Loei, Phu Kradung
Dimeria ornithopoda Trin.	Teerawatananon & Sungkaew 685 (BKF, THNHM)	Thailand, Trat, Laem Ngob

Species	Voucher	Locality
Eriochrysis cayennensis P. Beauv.	Welker 395 (ICN)	Brazil, Paraná, Aparecida do Ivaí
	Welker 468 (ICN)	Brazil, Rio Grande do Sul, Arroio do Sal
	Welker 486 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 536 (ICN)	Brazil, Paraná, Sengés
	Welker & Peichoto 597 (CTES, ICN)	Argentina, Corrientes, Ituzaingó
Eriochrysis holcoides (Nees) Kuhlm.	Welker 338 (ICN)	Brazil, Rio Grande do Sul, São Francisco de Paula
	Welker 391 (ICN)	Brazil, Santa Catarina, Caçador
	Welker 505 (ICN)	Brazil, Santa Catarina, Caçador
Eriochrysis laxa Swallen	Neves & Alvarenga 493 (RB)	Brazil, Goiás, Teresina de Goiás, Chapada dos Veadeiros
	Welker 481A (ICN)	Brazil, Rio Grande do Sul, São Luiz Gonzaga
	Welker 488-7 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 488-9 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 488-11 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 488-12 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 489 (ICN)	Brazil, Rio Grande do Sul, São Borja
Eriochrysis aff. *laxa* Swallen	Welker 487-10 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 487-11 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 544 (ICN)	Brazil, Rio Grande do Sul, São Borja
Eriochrysis pallida Munro	Malcomber et al. 3086 (MO)	Tanzania, Iringa, Njombe
Eriochrysis villosa Swallen	Welker 460 (ICN)	Brazil, Santa Catarina, Bom Jardim da Serra
	Welker 481B (ICN)	Brazil, Rio Grande do Sul, São Luiz Gonzaga
	Welker 490 (ICN)	Brazil, Rio Grande do Sul, São Borja
	Welker 652 (ICN)	Uruguay, Tacuarembó, Tacuarembó
	Welker 655 (ICN)	Uruguay, Rivera, Tranquera
Eriochrysis warmingiana (Hack.) Kuhlm.	Neves & Monteiro 406 (ICN, RB)	Brazil, Mato Grosso do Sul, Rio Verde de Mato Grosso

Species	Voucher	Locality
Eriochrysis sp. ("inconspicuous lobes")	Longhi-Wagner & Welker 10863 (ICN)	Brazil, Rio Grande do Sul, Osório
	Welker 342 (ICN)	Brazil, Rio Grande do Sul, Caçapava do Sul
	Welker 365 (ICN)	Brazil, Rio Grande do Sul, São Francisco de Paula
	Welker 617 (ICN)	Brazil, Rio Grande do Sul, Cidreira
	Welker 621 (ICN)	Brazil, Rio Grande do Sul, Osório, Atlântida Sul
Eulalia aurea (Bory) Kunth	Kellogg PI 249139 (MO)	Australia, Queensland
Eulalia quadrinervis (Hack.) Kuntze	Teerawatananon & Sungkaew 706 (THNHM)	Thailand, Tak, Um Phang
Eulalia villosa Nees	Malcomber et al. 3088 (MO)	Tanzania, Iringa, Njombe
Germainia capitata Balansa & Poitr.	Teerawatananon & Sungkaew 834 (THNHM)	Thailand, Loei, Phu Kradung
Heteropogon triticeus (R. Br.) Stapf ex Craib	Teerawatananon & Sungkaew 733 (THNHM)	Thailand, Chiang Mai, Jom Thong
Hyparrhenia rufa (Nees) Stapf	Kellogg PI 206889 (A/GH)	Turkey, Antalya
Imperata brasiliensis Trin.	Longhi-Wagner & Welker 10848 (ICN)	Brazil, Rio Grande do Sul, Cidreira
Imperata cylindrica (L.) P. Beauv.	Kowarat 108 (THNHM)	Thailand, Pathun Thani, Klong Luang
Imperata tenuis Hack.	Lerina & Silveira 95 (ICN)	Brazil, Rio Grande do Sul, Rosário do Sul
Ischaemum rugosum Salisb.	Kellogg Kew MSB 183574 (MO)	Burkina Faso, Gnagna
Iseilema macratherum Domin	Snow et al. 7239 (A/GH)	Australia, New South Wales, Moree
Microstegium vimineum (Trin.) A. Camus	Kellogg VA-2 (MO)	U.S.A., Virginia, Fairfax
Miscanthus sinensis Andersson	Kellogg PI 668403 (MO)	Japan, Goto Islands, Nagasaki Prefecture, Osezaki
Pogonatherum crinitum (Thunb.) Kunth	Teerawatananon & Sungkaew 865 (THNHM)	Thailand, Nakhon Ratchasima, PakChong
Pogonatherum paniceum (Lam.) Hack.	Clark s.n. (MO)	unknown
Polytoca wallichiana (Nees ex Steud.) Benth.	Teerawatananon & Sungkaew 683 (THNHM)	Thailand, Kanchanaburi, Thong Pha Phum
Polytrias indica (Houtt.) Veldkamp	Kellogg 1264 (MO)	Philippines, Luzon
Pseudosorghum fasciculare (Roxb.) A. Camus	Teerawatananon & Sungkaew 698 (THNHM)	Thailand, Tak, Um Phang
Saccharum giganteum (Walter) Pers.	Layton & Zhong 161 (MO)	U.S.A., Louisiana, Saint Tammany
Saccharum officinarum L.	Welker s.n. (MO)	U.S.A., Missouri, St. Louis
Schizachyrium brevifolium (Sw.) Nees ex Buse	Teerawatananon & Sungkaew 750 (THNHM)	Thailand, Chiang Mai, Muang

Species	Voucher	Locality
Schizachyrium sanguineum (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	Thailand, Chiang Mai, Muang
Sorghastrum elliottii (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	U.S.A., Texas, Anderson County
Sorghum bicolor (L.) Moench	Kellogg PI 156549 (A/GH)	Zimbabwe
Thelepogon elegans Roth	Teerawatananon & Sungkaew 697 (THNHM)	Thailand, Tak, LanSang
Themeda arundinacea (Roxb.) A. Camus	Teerawatananon & Sungkaew 739 (THNHM)	Thailand, Chiang Mai, Mae Rim
Tripsacum dactyloides (L.) L.	Kellogg 1261 (A/GH)	U.S.A., Missouri, Pettis County
Zea mays L.	Cultivar B73 (genome sequence)	unknown

Table 3
Chloroplast genome size, presence of gaps in assembly, voucher specimens, and GenBank accession numbers of the samples included in the plastome phylogenetic analyses. Herbaria acronyms according to Index Herbariorum (Thiers, 2015). *Indicates gap present.

Species	Total Size (bp)	LSC Size (bp)	IR Size (bp)	SSC Size (bp)	Gaps in Assembly	Voucher	GenBank Accession	Source
Eriochrysis cayennensis	140380	82368*	22770	12742*	4	Welker 519 (ICN)	XXXXXX	This Study
Eriochrysis laxa	140135	82366*	22638	12493	1	Welker 489 (ICN)	XXXXXX	This Study
Eriochrysis villosa	140404	82362*	22783	12476	1	Welker 481B (ICN)	XXXXXX	This Study
Eriochrysis sp.	140426	82393	22770	12493	0	Welker 365 (ICN)	XXXXXX	This Study
Chrysopogon serrulatus	140700	82656	22761	12522	0	Kellogg PI 219580 (A/GH)	XXXXXX	This Study
Pogonatherum paniceum	138441	81656	22173	12439	0	Clark s.n. (MO)	XXXXXX	This Study
Miscanthus sinensis	141293	83122	22798	12575	0	strain IGR-2011-003	SRA SRR559246	This Study
Saccharum officinarum	141182	83048	22795	12544	N/A	cultivar NCo 310	AP006714	GenBank
Sorghum bicolor	140754	82685	22783	12503	N/A	cultivar BTx623	EF115542	GenBank
Zea mays	140384	82353	22748	12536	N/A	N/A	NC001666	GenBank
Setaria italica	138833	81916	22194	12529	N/A	N/A	KJ001642	GenBank

Table 4
Plastome library preparation and sequencing methods used in this study.

Species	Read Type	Read Number Trimmed	Library Type	Machine Type	Location	GenBank Accession
Eriochrysis cayennensis	2 x 150	3943771	Nextera	Illumina HiSeq 2500	NYU School of Medicine	XXXXXX
Eriochrysis laxa	2 x 150	3835275	Nextera	Illumina HiSeq 2500	NYU School of Medicine	XXXXXX
Eriochrysis villosa	2 x 150	3465715	Nextera	Illumina HiSeq 2500	NYU School of Medicine	XXXXXX
Eriochrysis sp.	2 x 150	4878784	Nextera	Illumina HiSeq 2500	NYU School of Medicine	XXXXXX
Chrysopogon serrulatus	1 x 100	4396528	NEBNext Ultra DNA	Illumina HiSeq 2000	University of Missouri	XXXXXX
Pogonatherum paniceum	1 x 100, 2 x 150	11302872	NEBNext Ultra DNA, Nextera	Illumina HiSeq 2000, Illumina HiSeq 2500	University of Missouri, NYU School of Medicine	XXXXXX
Miscanthus sinensis	2 x 100	28208330	N/A	N/A	GenBank: SRA SRR559246	SRA SRR559246

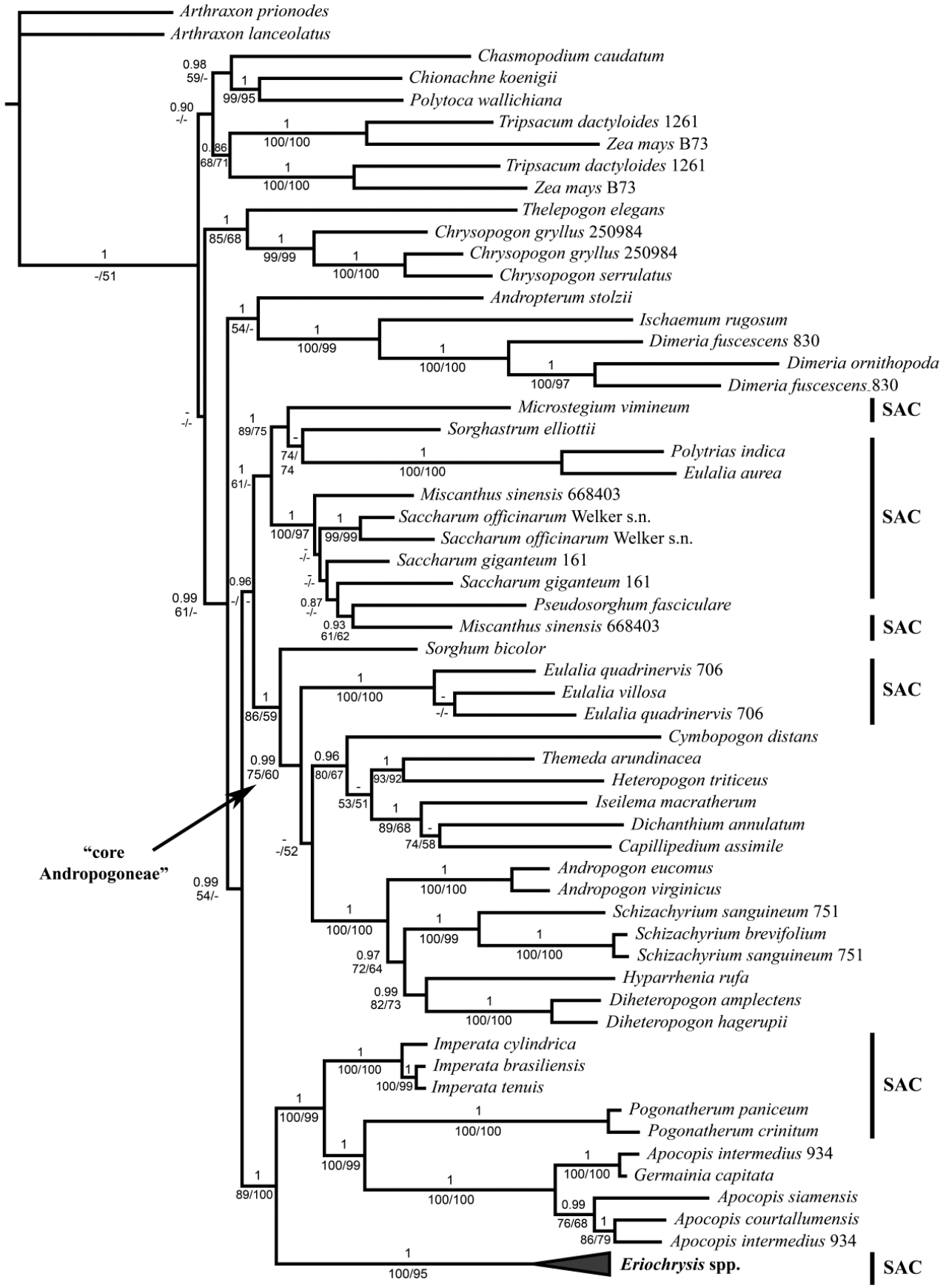

Fig. 1. Bayesian phylogeny of *Eriochrysis* and other genera of Andropogoneae based on the combined dataset of low-copy nuclear loci (*apo*1, *d*8, *ep*2-*ex*7, *ep*2-*ex*8, and *kn*1), shown as a phylogram. Bayesian PP > 0.85 are shown above branches, and ML / MP bootstrap values > 50 are shown below. For polyploid species (with two paralogues in our analyses), collector number is after the binomials, according to Table 2. Representatives from subtribe Saccharinae (sensu Clayton & Renvoize, 1986) are indicated (SAC). The clade of *Eriochrysis* taxa is presented in detail in Fig. 2.

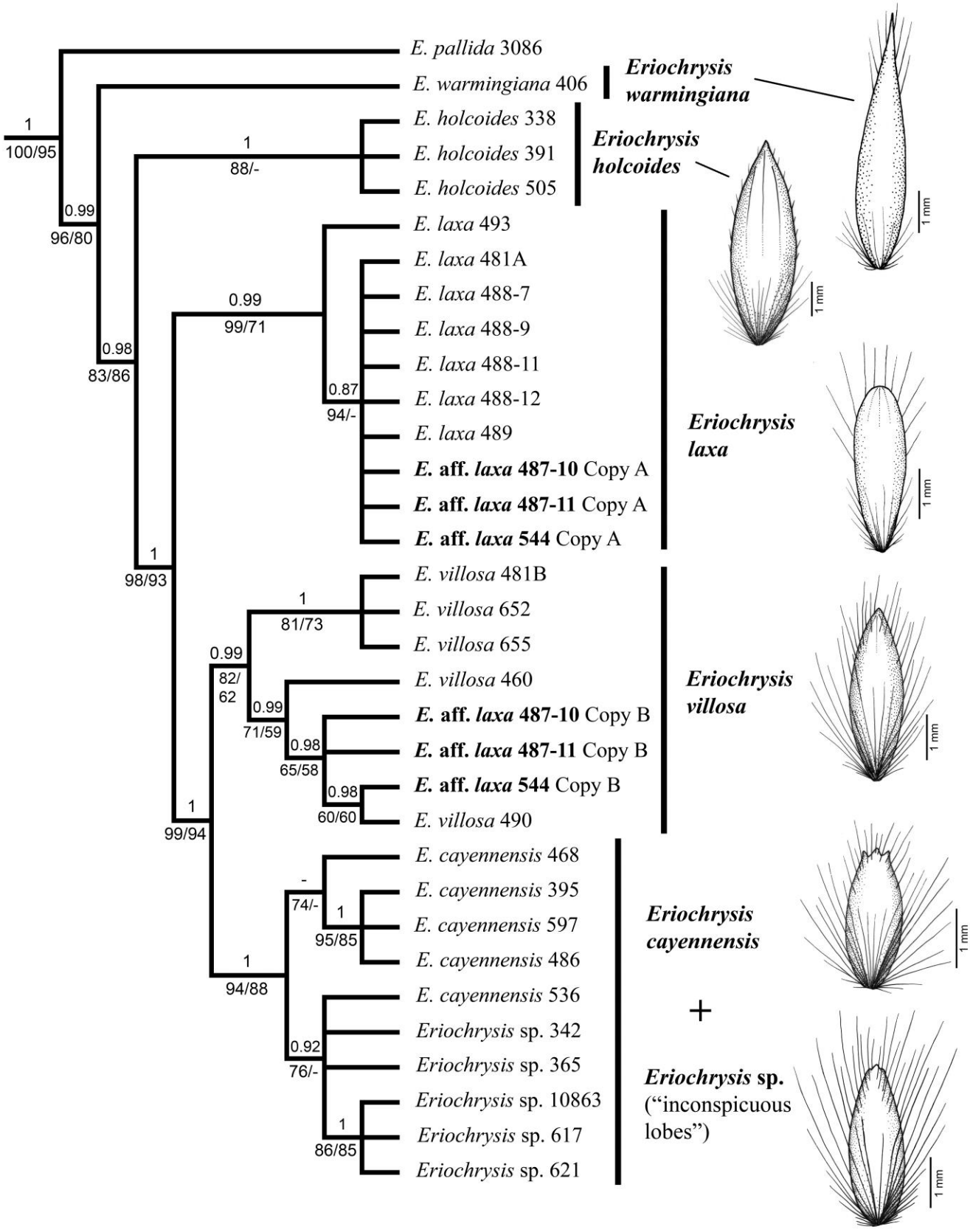

Fig. 2. Bayesian phylogeny of *Eriochrysis* based on the combined dataset of low-copy nuclear loci (*apo*1, *d*8, *ep*2-*ex*7, *ep*2-*ex*8, and *kn*1). Bayesian PP > 0.85 are shown above branches, and ML / MP bootstrap values > 50 are shown below. Collector number is after the binomials, according to Table 2. Polyploid specimens (with two paralogues in our analyses) are highlighted in bold. Illustrations of the lower glume of the spikelet of New World species of *Eriochrysis* are presented on the right of the phylogeny.

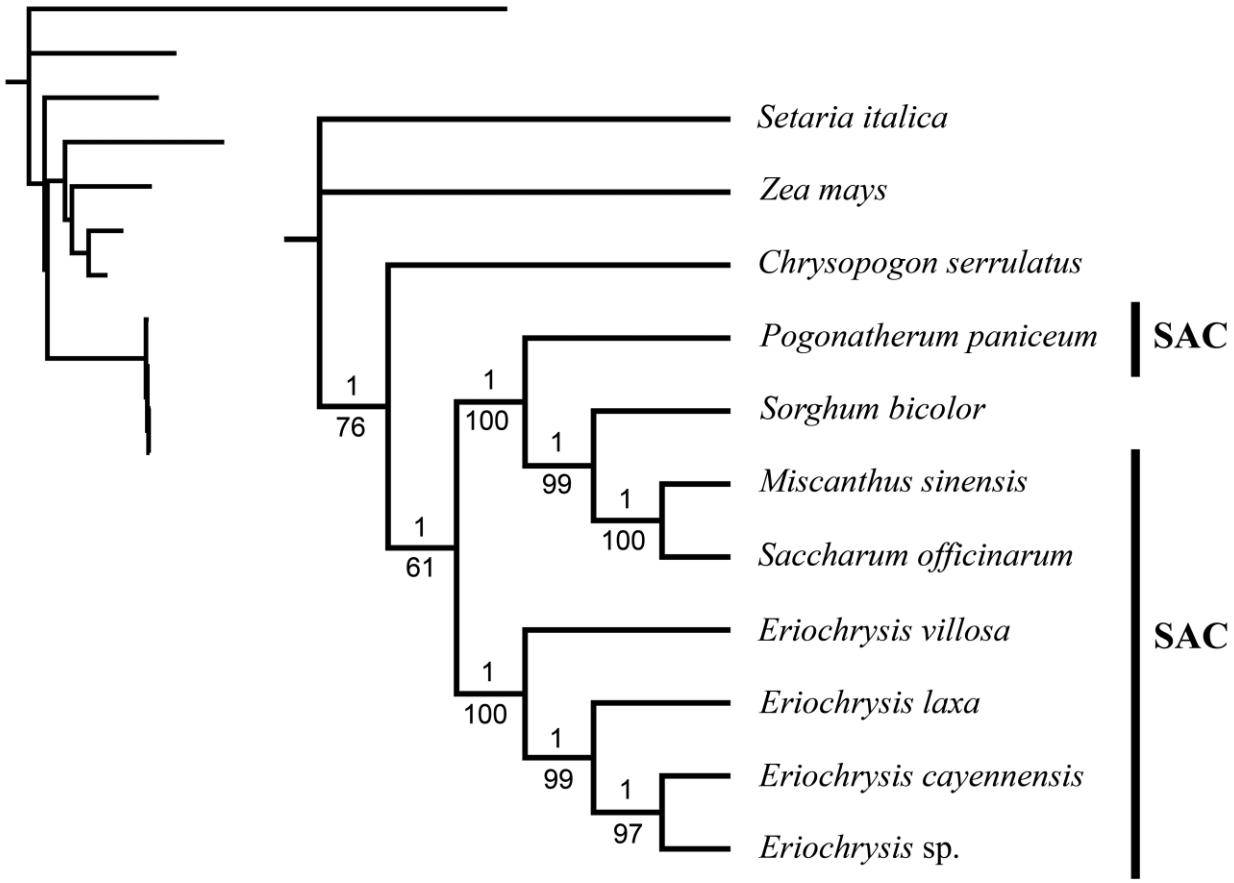

Fig. 3. Bayesian phylogeny of *Eriochrysis* and other genera of Andropogoneae based on whole chloroplast sequences (the same tree shown as a phylogram on the left). Bayesian PP > 0.85 are shown above branches, and ML bootstrap values > 50 are shown below. Representatives from subtribe Saccharinae (sensu Clayton & Renvoize, 1986) are indicated (SAC).

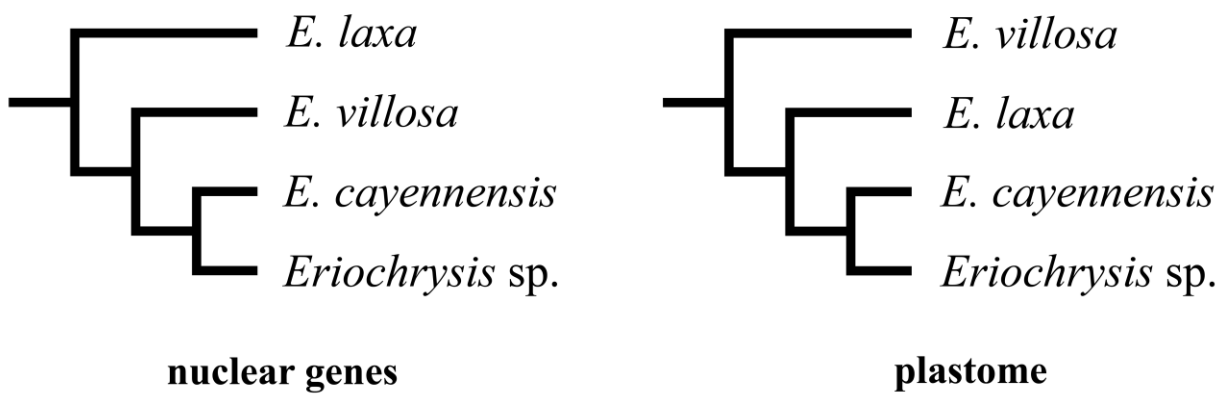

Fig. 4. Representation of the incongruence between nuclear and plastome trees concerning the phylogenetic position of *Eriochrysis cayennensis, E. laxa*, and *E. villosa*.

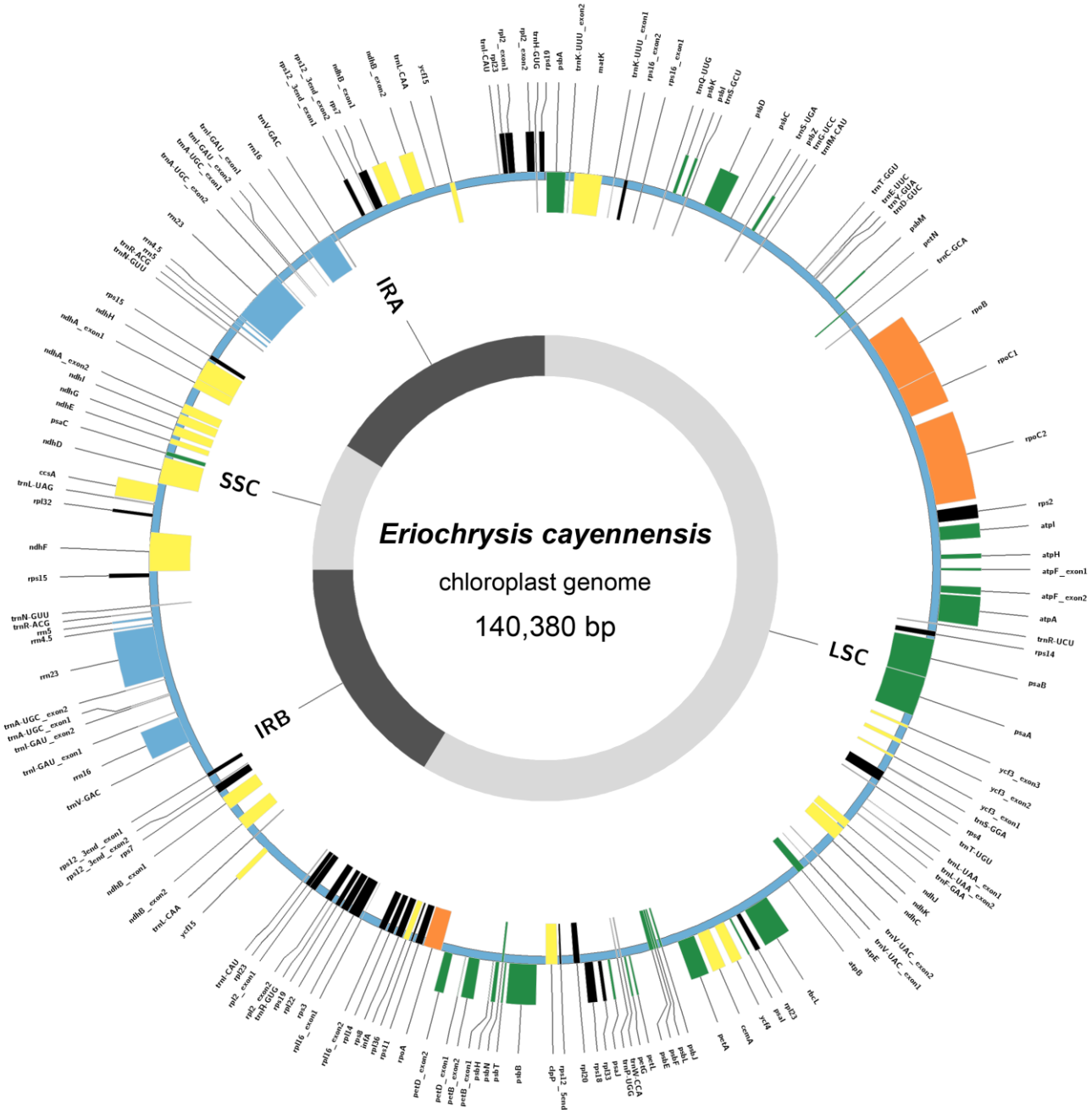

Fig. S1. Chloroplast genome map of *Eriochrysis cayennensis*. LSC (large single copy), SSC (small single copy), IRA (inverted repeat A), IRB (inverted repeat B).

Appendix 1

Voucher specimens and GenBank accession numbers for sequences included in the phylogenetic analyses based on nuclear genes. Herbaria acronyms according to Index Herbariorum (Thiers, 2015) except THNHM (Thailand Natural History Museum), not included in that directory.

Species	**Voucher**	***apo*1**	***d*8**	***ep*2-*ex*7**	***ep*2-*ex*8**	***kn*1**
Andropogon eucomus Nees	Malcomber et al. 3089 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Andropogon virginicus L.	Kellogg 1240 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Andropterum stolzii (Pilg.) C.E. Hubb.	Malcomber et al. 3091 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Apocopis courtallumensis (Steud.) Henrard	Teerawatananon & Kritsanachandee 928 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Apocopis intermedius (A. Camus) Chai-Anan	Teerawatananon & Kritsanachandee 934 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Apocopis siamensis A. Camus	Teerawatananon & Sungkaew 975 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Arthraxon lanceolatus (Roxb.) Hochst.	Teerawatananon & Sungkaew 720 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Arthraxon prionodes (Steud.) Dandy	Kellogg PI 659331 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Capillipedium assimile (Steud.) A. Camus	Teerawatananon & Sungkaew 791 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Chasmopodium caudatum (Hack.) Stapf	Kellogg Kew MSB 184054 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Chionachne koenigii (Spreng.) Thwaites	Kellogg Chio-6-D-93 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Chrysopogon gryllus (L.) Trin.	Kellogg PI 250984 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Chrysopogon serrulatus Trin.	Kellogg PI 219580 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Cymbopogon distans (Nees ex Steud.) Will. Watson	Kellogg PI 271552 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Dichanthium annulatum (Forssk.) Stapf	Kellogg PI 240155 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Diheteropogon amplectens (Nees) Clayton	Kellogg RF 1819 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Diheteropogon hagerupii Hitchc.	Kellogg Kew MSB 254456 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Dimeria fuscescens Trin.	Teerawatananon & Sungkaew 830 (BKF, THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Dimeria ornithopoda Trin.	Teerawatananon & Sungkaew 685 (BKF, THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis cayennensis P. Beauv.	Welker 395 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 468 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 486 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 536 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker & Peichoto 597 (CTES, ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX

Species	Voucher	*apo*1	*d*8	*ep*2-*ex*7	*ep*2-*ex*8	*kn*1
Eriochrysis holcoides (Nees) Kuhlm.	Welker 338 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 391 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 505 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis laxa Swallen	Neves & Alvarenga 493 (RB)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 481A (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 488-7 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 488-9 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 488-11 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 488-12 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 489 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis aff. *laxa* Swallen	Welker 487-10 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 487-11 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 544 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis pallida Munro	Malcomber et al. 3086 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis villosa Swallen	Welker 460 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 481B (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 490 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 652 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 655 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis warmingiana (Hack.) Kuhlm.	Neves & Monteiro 406 (ICN, RB)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eriochrysis sp. (“inconspicuous lobes”)	Longhi-Wagner & Welker 10863 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 342 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 365 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 617 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
	Welker 621 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eulalia aurea (Bory) Kunth	Kellogg PI 249139 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eulalia quadrinervis (Hack.) Kuntze	Teerawatananon & Sungkaew 706 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eulalia villosa Nees	Malcomber et al. 3088 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX

Species	Voucher	*apo*1	*d*8	*ep*2-*ex*7	*ep*2-*ex*8	*kn*1
Germainia capitata Balansa & Poitr.	Teerawatananon & Sungkaew 834 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Heteropogon triticeus (R. Br.) Stapf ex Craib	Teerawatananon & Sungkaew 733 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Hyparrhenia rufa (Nees) Stapf	Kellogg PI 206889 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Imperata brasiliensis Trin.	Longhi-Wagner & Welker 10848 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Imperata cylindrica (L.) P. Beauv.	Kowarat 108 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Imperata tenuis Hack.	Lerina & Silveira 95 (ICN)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Ischaemum rugosum Salisb.	Kellogg Kew MSB 183574 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Iseilema macratherum Domin	Snow et al. 7239 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Microstegium vimineum (Trin.) A. Camus	Kellogg VA-2 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Miscanthus sinensis Andersson	Kellogg PI 668403 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Pogonatherum crinitum (Thunb.) Kunth	Teerawatananon & Sungkaew 865 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Pogonatherum paniceum (Lam.) Hack.	Clark s.n. (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Polytoca wallichiana (Nees ex Steud.) Benth.	Teerawatananon & Sungkaew 683 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Polytrias indica (Houtt.) Veldkamp	Kellogg 1264 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Pseudosorghum fasciculare (Roxb.) A. Camus	Teerawatananon & Sungkaew 698 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Saccharum giganteum (Walter) Pers.	Layton & Zhong 161 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Saccharum officinarum L.	Welker s.n. (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Schizachyrium brevifolium (Sw.) Nees ex Buse	Teerawatananon & Sungkaew 750 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Schizachyrium sanguineum (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Sorghastrum elliottii (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Sorghum bicolor (L.) Moench	Kellogg PI 156549 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Thelepogon elegans Roth	Teerawatananon & Sungkaew 697 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Themeda arundinacea (Roxb.) A. Camus	Teerawatananon & Sungkaew 739 (THNHM)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Tripsacum dactyloides (L.) L.	Kellogg 1261 (A/GH)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Zea mays L.	Cultivar B73 (genome sequence)	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX

CONCLUSÕES E PERSPECTIVAS

A presente tese apresentou contribuições significativas para um melhor entendimento da taxonomia e da história evolutiva dos representantes da tribo Andropogoneae (Poaceae), especialmente dos gêneros *Saccharum* e *Eriochrysis*, através da investigação de aspectos filogenéticos, taxonômicos e nomenclaturais.

Do ponto de vista nomenclatural, foi possível elucidar a questão sobre o correto nome para a tribo. Reveal (2004) propôs que o nome Sacchareae Martinov teria sido validamente publicado, no nível de tribo, em um dicionário russo de terminologia botânica do século XIX (Martinov 1820) e, portanto, teria prioridade sobre o nome Andropogoneae Dumortier (1824), o qual vinha sendo amplamente utilizado há quase 200 anos. A análise acurada da obra de Martinov (1820), incluindo a tradução do texto original com a ajuda de três pesquisadores russos, demonstrou que aquele nome não foi publicado no nível de tribo e, portanto, não compete com qualquer nome validamente publicado para este nível hierárquico. Desta forma, fica evidente que Andropogoneae Dumortier é o nome correto para a tribo. Após a publicação desses dados (Welker *et al.* 2014), apresentados no primeiro capítulo da presente tese, Andropogoneae voltou a ser o nome aceito para a tribo na *World-wide Phylogenetic Classification of Poaceae* (Soreng *et al.* 2014) e no banco de dados *Tropicos* (Tropicos 2015). A elucidação do correto nome para a tribo é de grande importância, não apenas para taxonomistas e botânicos, mas também para pesquisadores que trabalham no melhoramento genético da cana-de-açúcar e de táxons relacionados.

As análises filogenéticas apresentadas corroboram a hipótese de uma diversificação inicial rápida em Andropogoneae (Mathews *et al.* 2002, Teerawatananon *et al.* 2011, Estep *et al.* 2014), já que as árvores filogenéticas apresentam ramos internos muito curtos na base do agrupamento e ramos terminais relativamente longos. Estas análises também indicam que a subtribo Saccharinae, a qual inclui *Saccharum*, *Eriochrysis* e cerca de outros 10 gêneros (Clayton & Renvoize 1986), não é monofilética. No entanto, uma amostragem maior, incluindo representantes de todos os seus gêneros, se faz necessária para propor uma nova circunscrição para essa subtribo.

Uma filogenia da tribo Andropogoneae baseada no sequenciamento completo do plastoma está sendo realizada pela Drª. Elizabeth Kellogg e pelo Dr. Michael McKain (Donald Danforth Plant Science Center, U.S.A.), com a colaboração do autor da presente tese e de diversos outros pesquisadores. Essa análise tem gerado resultados bastante satisfatórios, confirmando as relações

filogenéticas inferidas por genes nucleares (Estep *et al.* 2014, por exemplo) e apresentando alto suporte para nós internos não suportados nas filogenias anteriores. Essa filogenia se mostra extremamente promissora também para auxiliar na análise da circunscrição da subtribo Saccharinae.

A análise filogenética de *Saccharum s.l.*, apresentada no segundo capítulo da presente tese, demonstrou a origem alopoliploide do gênero a partir de evidências de genes nucleares (Welker *et al.* 2015). A análise também indicou que *Saccharum s.l.* é polifilético e que *Tripidium* deve ser reconhecido como um gênero distinto, como aceito por Grassl (1972, sob *Ripidium*) com base em morfologia. Novas combinações de nomes para o gênero *Tripidium* se fazem necessárias em decorrência dessa circunscrição e do fato de *Ripidium* ser um nome ilegítimo. Um artigo nomenclatural contendo as novas combinações está sendo preparado.

Dois possíveis tratamentos para o gênero *Saccharum* são consistentes com nossas análises filogenéticas: (1) considerar *Saccharum* em um sentido amplo (excluindo apenas as espécies de *Tripidium*), ou (2) considerar *Saccharum s.s.*, *Erianthus*, *Narenga* Bor e *Miscanthidium* Stapf como gêneros distintos. A incerteza de qual tratamento taxonômico deve ser adotado reside, em grande parte, da amostragem reduzida do estudo, a qual teve enfoque principal as espécies sulamericanas do grupo. Devido a isso, portanto, o estudo não constitui uma forte evidência para suportar a segregação de *Erianthus* ou dos outros gêneros envolvidos. No entanto, as análises filogenéticas sugerem (se considerarmos esses gêneros como independentes) que um evento de hibridação inicial, seguido por alopoliploidização, teria dado origem aos gêneros *Erianthus* e *Miscanthidium* (poliploides formados pelos genomas A e B, conforme denominados por Welker *et al.* 2015). *Saccharum s.s.*, porém, teria se originado diretamente do ancestral do genoma B, enquanto *Narenga* teria se originado de híbridos entre *Saccharum s.s.* e *Miscanthidium* (Welker *et al.* 2015).

As análises filogenéticas baseadas em genes nucleares de cópia única foram capazes de resolver a circunscrição das espécies sulamericanas de *Saccharum s.l.*, confirmando a ocorrência de três espécies nativas na América do Sul: *S. angustifolium*, *S. asperum* (Nees) Steud. e *S. villosum*, conforme apresentado no segundo capítulo desta tese. A delimitação das espécies coincide com a circunscrição aceita por Filgueiras (2003) e Welker & Longhi-Wagner (2012b), com base em morfologia. Além disso, o estudo documentou a ocorrência de híbridos naturais entre *S. villosum* e *S. angustifolium* (plantas com morfologia intermediária entre as duas espécies, denominadas *Saccharum* aff. *villosum* por Welker & Longhi-Wagner 2012b). A análise dos genes nucleares indicou uma história complexa de evolução reticulada, provavelmente envolvendo mais de um evento de hibridação e com diferentes contribuições de *S. villosum* e *S. angustifolium* na formação dos híbridos. O número de parálogos nas árvores filogenéticas indica

que os espécimes híbridos são provavelmente hexaploides, enquanto os seus parentais são provavelmente tetraploides (Welker *et al.* 2015). Análises citogenéticas, incluindo contagem cromossômica e caracterização citogenética molecular por FISH (*fluorescent in situ hybridization*) e GISH (*genomic in situ hybridization*), poderão trazer novas informações sobre a dinâmica evolutiva desses táxons e elucidar as diferentes contribuições genômicas dos parentais na formação desses híbridos. Parte do material para as análises citogenéticas já foi coletado pelo presente autor durante as saídas de campo de seu doutorado. Esses estudos citogenéticos serão realizados com a colaboração da Drª. Eliane Kaltchuk dos Santos (Departamento de Genética, Universidade Federal do Rio Grande do Sul), provavelmente envolvendo a orientação de um aluno de Iniciação Científica.

Devido às incertezas ainda existentes sobre a circunscrição genérica de *Saccharum s.l.*, um projeto de Pós-Doutorado Júnior (PDJ) foi submetido ao CNPq, visando à continuação dos estudos sobre a história evolutiva desse grupo. Pretendemos reconstruir a mais completa filogenia até hoje realizada de *Saccharum s.l.* e *Miscanthus* Andersson, gênero este que faz parte da complexa história evolutiva da cana-de-açúcar e dos táxons relacionados (Hodkinson *et al.* 2002, Kim *et al.* 2014). Para isso, utilizaremos genes nucleares de cópia única e sequenciamento completo do plastoma, através de *next-generation sequencing*. O projeto contará com a colaboração de diversos pesquisadores estrangeiros, que nos enviarão amostras principalmente do Velho Mundo e da América do Norte: Drª. Elizabeth Kellogg, Dr. Michael McKain, Dr. Trevor Hodkinson (Trinity College Dublin, Irlanda), Drª. Maria Vorontsova (Royal Botanic Gardens, Kew, UK) e Drª. Myriam Carolina Peichoto (Instituto de Botánica del Nordeste, Argentina). Uma análise filogenética que seja capaz de definir a circunscrição genérica de *Saccharum s.l.*, bem como testar a hipótese de surgimento desses gêneros proposta por Welker *et al.* (2015), será de grande importância não apenas para a taxonomia vegetal, mas também para programas de melhoramento genético da cana-de-açúcar, por possibilitar a identificação de potenciais táxons para cruzamentos interespecíficos e intergenéricos. Uma análise morfométrica de *Saccharum* e gêneros relacionados está sendo realizada pela Drª. Myriam Peichoto, com a colaboração do presente autor, a qual poderá também trazer novas evidências para a discussão sobre a circunscrição genérica desse grupo.

Em relação ao gênero *Eriochrysis*, as análises filogenéticas confirmaram que o mesmo é monofilético e que a espécie africana *E. pallida* Munro é irmã do clado contendo as espécies americanas estudadas, como apresentado no quarto capítulo da presente tese. Com base nos marcadores nucleares de cópia única, e com o suporte da morfologia e do sequenciamento completo do plastoma, foi possível definir a circunscrição desses táxons: *E. laxa* é uma espécie distinta de *E. warmingiana*, assim como *E. villosa* é uma espécie distinta de *E. cayennensis*.

Além disso, espécimes com gluma inferior com ápice subagudo e lobos inconspícuos, considerados como variação morfológica de *E. villosa* por Welker & Longhi-Wagner (2012b), pertencem à espécie *E. cayennensis*, com base em nossas análises filogenéticas. *Eriochrysis villosa*, até então considerada endêmica do sul do Brasil (Swallen 1966, Welker & Longhi-Wagner 2012b), é citada aqui pela primeira vez para o Uruguai.

Algumas incongruências foram observadas entre as árvores filogenéticas baseadas em genes nucleares e aquelas baseadas no sequenciamento completo do plastoma, principalmente em relação à afinidade de *Eriochrysis* com o gênero *Pogonatherum* P. Beauv. e ao posicionamento de *Eriochrysis laxa* e *E. villosa*. Essas incongruências foram causadas, provavelmente, por *incomplete lineage sorting* e/ou hibridação. A grande diferença de amostragem entre as duas análises, devido a um número reduzido de táxons na análise filogenética baseada em plastomas, também pode ter contribuído para essas incongruências.

A ocorrência de híbridos naturais entre *Eriochrysis laxa* e *E. villosa* também foi confirmada. Os espécimes híbridos (denominados *Eriochrysis* aff. *laxa* por Welker *et al.* 2012) são provavelmente tetraploides, por apresentarem dois parálogos nas árvores nucleares, enquanto as demais espécies de *Eriochrysis* são provavelmente diploides. Esses híbridos devem ter se originado de um evento de hibridação interespecífica seguido de duplicação dos genomas (alopoliploidia). Este é o primeiro registro de um táxon poliploide no gênero *Eriochrysis*, embora poliploidia seja comum em muitos outros gêneros de Andropogoneae e Poaceae (Estep *et al.* 2014). Estudos citogenéticos no gênero *Eriochrysis* são raros, sendo que a maioria de suas espécies não teve sequer o número cromossômico determinado, até o momento. Análises citogenéticas serão realizadas em colaboração com a Drª. Eliane Kaltchuk dos Santos para confirmar a origem híbrida dos espécimes anteriormente denominados *Eriochrysis* aff. *laxa*, bem como o nível de ploidia das demais espécies do gênero. Material para as análises citogenéticas de algumas dessas espécies já foi coletado pelo autor durante as saídas de campo relativas ao doutorado.

A resolução taxonômica dos complexos de espécies de *Saccharum* e de *Eriochrysis*, baseada nas análises filogenéticas da presente tese, contribuiu também para os tratamentos taxonômicos dos respectivos gêneros na *Lista de Espécies da Flora do Brasil* (Filgueiras & Welker 2015a, 2015b), através da atualização e/ou confirmação das espécies ocorrentes no país. Uma chave para as espécies brasileiras de *Eriochrysis* foi preparada, contribuindo para a identificação das espécies (Welker *et al.* 2012). Essa publicação relata também a descoberta da ocorrência de *E. laxa* no estado do Rio Grande do Sul, como apresentado no terceiro capítulo desta tese. A revisão taxonômica das espécies americanas de *Eriochrysis*, incluindo chaves de identificação, descrições e ilustrações dos táxons, está sendo preparada.

Além dos complexos de espécies dos gêneros *Saccharum* e *Eriochrysis*, discutidos acima, a circunscrição das espécies de *Schizachyrium* Nees também é bastante complexa e controversa, especialmente em relação a *S. condensatum* (Kunth) Nees e táxons relacionados. Türpe (1984) e autores que a seguiram aceitaram *S. condensatum* no sentido amplo, incluindo em sua sinonímia pelo menos cinco nomes que são aceitos como espécies distintas por outros autores (e.g., Peichoto 2010, Welker & Longhi-Wagner 2012a, Zanin 2015). As diferenças morfológicas entre esses táxons são pouco evidentes, incluindo principalmente a forma da inflorescência e as dimensões das espiguetas e de outras estruturas reprodutivas, com muita sobreposição nas medidas e com morfologias intermediárias (Peichoto 2010, Welker & Longhi-Wagner 2012a). Devido a isso, uma análise filogenética do complexo *S. condensatum* está sendo realizada para analisar a circunscrição dessas espécies. A clonagem molecular e o sequenciamento de cinco marcadores nucleares de cópia única (*apo*1, *d*8, *ep*2-*ex*7, *ep*2-*ex*8 e *kn*1) já foram realizados pelo presente autor durante seu estágio de doutorado sanduíche no laboratório da Drª. Elizabeth Kellogg, no Donald Danforth Plant Science Center (St. Louis, MO, U.S.A.). Genes nucleares de 52 indivíduos pertencentes a 13 espécies de *Schizachyrium*, principalmente do complexo *S. condensatum*, já estão sequenciados, faltando ainda editar as sequências e preparar o artigo. Além disso, uma ampla análise filogenética do gênero *Schizachyrium*, baseada em algumas sequências plastidiais e em caracteres morfométricos, está sendo realizada pela Drª. Myriam Peichoto, com a colaboração do presente autor e da Drª. María Amalia Scataglini (Instituto de Botánica Darwinion, Argentina). A revisão taxonômica das espécies de *Schizachyrium* nas Américas Central e do Norte também está sendo preparada pela Drª. Myriam, com a colaboração do autor.

A presente tese demonstrou, como mencionado acima, a eficiência dos marcadores nucleares de cópia única na circunscrição de espécies e gêneros dentro da tribo Andropogoneae, mesmo na presença de poliploidia, evolução reticulada e radiação recente. O sequenciamento completo do plastoma também se mostrou uma ferramenta extremamente promissora para inferências filogenéticas em Andropogoneae. Além disso, a presente tese propiciou diversos outros questionamentos e estudos que já estão em andamento ou serão iniciados no futuro próximo, visando um conhecimento cada vez maior da história evolutiva e da taxonomia da tribo Andropogoneae.

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ANEXO

PRODUÇÃO BIBLIOGRÁFICA DURANTE O DOUTORADO

I. Artigos publicados em periódicos

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II. Artigo aceito para publicação

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IV. Outras produções bibliográficas

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