**Multilocus phylogeny and phylogenomics of *Eriochrysis* P. Beauv. (Poaceae –
Andropogoneae): taxonomic implications and evidence of interspecific hybridization**

Cassiano A.D. Welker^{a,*}, Tatiana T. Souza-Chies^a, Hilda M. Longhi-Wagner^a, Myriam Carolina
Peichoto^b, Michael R. McKain^c, Elizabeth A. Kellogg^c

^aUniversidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Botânica, Av. Bento
Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brazil

^bInstituto de Botánica del Nordeste (UNNE-CONICET), Facultad de Ciencias Agrarias (UNNE),
Sargento Cabral 2131, Corrientes, 3400, Argentina

^cDonald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, U.S.A.

*Corresponding author.

E-mail addresses: cassiano_welker@yahoo.com.br (C.A.D. Welker), tatiana.chies@ufrgs.br
(T.T. Souza-Chies), hmlwagner@gmail.com (H.M. Longhi-Wagner),
mcpeichoto@agr.unne.edu.ar (M.C. Peichoto), mrmckain@gmail.com (M.R. McKain),
ekellogg@danforthcenter.org (E.A. Kellogg).

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 phylogeny of the grass genus *Eriochrysis* is presented here, focusing on the New World species, 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 (*apo1*, *d8*, *ep2-ex7* and *ep2-ex8*, *kn1*) 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 polyphyletic, as is the genus *Eulalia*. Based on nuclear and plastome sequences 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*. Natural hybrids occur between *E. laxa* and *E. villosa*. The hybrids are probably tetraploids, based on the number of paralogues in the nuclear gene 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 sufficient to resolve phylogenetic relationships and define the circumscriptions of other species complexes in the grass family and relatives, even in the presence of polyploidy and reticulate evolution. Complete plastome sequencing is also a promising tool for phylogenetic inference.

44 **Keywords:** Saccharinae; species delimitation; low-copy nuclear genes; plastome; polyploidy;
45 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 phenotypic convergence as well as in striking morphological differences among related species adapting to different conditions (Koopman et al., 2008). Cryptic species may also occur (Bickford et al., 2007). 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 in *Eriochrysis* P. Beauv. and related taxa (Poaceae – Andropogoneae) is presented here. *Eriochrysis* is a grass genus typical of marshlands and wet grasslands, with ca. 7–12 species mainly from the New World, with a few species in Africa and India (Clayton and Renvoize, 1986; Kellogg, 2015; The Plant List, 2016; Watson and Dallwitz, 1992). The circumscription of its species is complex and contentious, as shown by the discrepancy in the number of species accepted by different authors. Plants with intermediate morphology also suggested the occurrence of natural hybridization, although this had not yet been tested (Killeen, 1990; Welker et al., 2012). The delimitation of *Eriochrysis* species has been 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 Andropogoneae (Estep et al., 2014; Welker et al., 2015). Therefore, we wished to reconstruct the 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 C₄ 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*, however, is placed outside the “core Andropogoneae” (Estep et al., 2014).

Based on morphological characters, *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. *Apocypis* 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). Chromosome counts are available for only two species of *Eriochrysis* and indicate they are diploid, with $2n=20$ (Dujardin, 1979; Pohl and Davidse, 1971). No polyploid species has been documented for the genus so far, although polyploidy is frequent in the tribe Andropogoneae and in Poaceae as well (Estep et al., 2014).

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). Some of the morphological diversity within *Eriochrysis* is shown in Fig. 1. *Eriochrysis cayennensis* P. Beauv., the species of the genus with the broadest geographical distribution in the Americas, has a 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 (BFG, 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 the lower glume with a 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.) Kuhl. is also unclear. The two species are accepted as distinct taxa by several authors (BFG, 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 of 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 co-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 may be 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 and biogeography (Carstens et al., 2013; Queiroz, 2007). Although molecular studies are extremely important for species delimitation, inferring species boundaries based on DNA data is not easy due to the low variability at this taxonomic level of the DNA loci currently used in phylogenetic analyses, especially those from the plastid genome such as *rbcL*, *ndhF* and *matK* (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 their ability for 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. Although polyploidy has not been reported 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 reconstruction (Burke et al., 2014; McCormack et al., 2013; Steele et al., 2012; 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. In higher plants, 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 those within 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). Therefore, in addition to low-copy nuclear genes, complete plastome sequences were considered to have the potential to increase 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*). Multiple accessions of the New World species were included in the analyses in order to investigate their taxonomic circumscriptions. Samples were collected across the geographical range of the species, especially in Southern Brazil, where possible hybrids between these species have been reported (Welker and Longhi-Wagner, 2012; Welker et al., 2012). A map showing the geographical origins of these samples is presented in Fig. 2. The geographical distributions of the New World species of *Eriochrysis* are presented in Table 1. 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 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 Table S1 (see Supplementary Data with the online version of this article).

Whole plastomes of four taxa of *Eriochrysis* were also sequenced, as well as those for *Chrysopogon serrulatus* Trin. and *Pogonatherum paniceum* (Lam.) Hack., the latter both also 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 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 organization1* (*apo1*), *Dwarf8* (*d8*), two exons of *Erect panicle2* (*ep2-ex7* and *ep2-ex8*), and *Knotted1* (*kn1*). These genes are known to influence grass morphology and inflorescence architecture, important traits generally used by botanists to define groups and species (Estep et al., 2012). Previous

works showed 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). These loci are distributed on different chromosomes, based on genomic studies of grass crop species, suggesting they are unlinked and each of them provides an independent estimate of phylogeny (Estep et al., 2012, 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 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 loci over 1,000 bp long (*d8* and *ep2-ex7*), according to 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 *d8* and *ep2-ex7* 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. Phylogenetic reconstructions, however, were performed based on the nucleotide sequences.

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. Library preparation and sequencing methods used for each sample are found in Table S2 (see Supplementary Data with the online version of this article).

Reads were trimmed using Trimmomatic v.0.32 (Bolger et al., 2014) and initial assemblies were made using SPAdes v.3.1.0 (Bankevich et al., 2012). Each SPAdes assembly was blasted against the *Zea mays* chloroplast (GenBank, NC001666.2) and mitochondrial (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.). 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. More information about the plastome sequencing and assembly, including the parameters used, are found in the Supplementary Information of this article.

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 to 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. 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. In all, 234 out of 247 alignments were used and concatenated 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 resulting 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. 3). 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 *Apocopsis* and *Germainia* (subtribe Germainiinae) (Fig. 3). 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 de Saint-Vincent) 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. 3).

Within the *Eriochrysis* clade, the African *E. pallida* Munro is sister to all New World species (0.99 PP, 96% ML, 80% MP) (Fig. 4). *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. 4).

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. 4). 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 the lower glume with a subacute apex and inconspicuous lobes (labeled here as *Eriochrysis* sp.) fell within the *E. cayennensis* clade (Fig. 4).

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. 4, 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 repeat (IR) regions of 22,638 to 22,783 bp (Table 3). The chloroplast genome map of *E. cayennensis* is presented in Fig. 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

resulting 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. 5). Unlike the phylogenies of nuclear loci (Fig. 3), *Pogonatherum* is more closely related to *Saccharum* and *Miscanthus* than to *Eriochrysis* in the plastome phylogenies (Fig. 5). Within the *Eriochrysis* clade, the relationships among species are also somewhat different in the plastome trees comparing to the nuclear trees (Fig. 6). *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. 5).

4. Discussion

4.1. Phylogenetics of *Eriochrysis* and related genera

The pattern of very short branches along the backbone of the nuclear trees, contrasting with long external branches, supports the hypothesis that 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. 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. 3). However, *Pogonatherum* is not closely related to *Eriochrysis* in our plastome phylogeny (Fig. 5). 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. 3). 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 is polyphyletic in our trees.

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). 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 sampling 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 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 African species *Eriochrysis pallida* was found to be sister to the clade including the New World species of the genus. Other species from the Old World not included in the present phylogenetic analyses (i.e., *E. brachypogon* (Stapf) Stapf, *E. purpurata* (Rendle) Stapf, and *E. rangacharii* C.E.C. Fisch.) are morphologically similar to *E. pallida*, differing in characters such as the apex of lower glumes and the length of the inflorescences and spikelets (Clayton et al., 2006), and are expected to group together with *E. pallida* as sister to the clade including the New World species. Future studies should also include the South American species *Eriochrysis filiformis* (Hack.) Filg., previously placed in the monospecific genus *Leptosaccharum* (Hack.) A. Camus (Camus, 1956; Watson and Dallwitz, 1992), to investigate whether it is a distinct genus or a synonym of *Eriochrysis*, as currently accepted based on morphology (BFG, 2015; Filgueiras, 1997; Welker et al., 2012).

Our nuclear gene analyses also suggest that *Eulalia* is polyphyletic (Fig. 3). 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 (*Eulalia aurea*) was confirmed as closely related to *Saccharum* and relatives in nuclear

analyses, but the other species analyzed (*Eulalia 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.

4.2. Taxonomic circumscription of New World species of *Eriochrysis*

All specimens of *Eriochrysis* 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. 4) reinforce their acceptance as distinct taxa.

Eriochrysis laxa is clearly distinct from *E. warmingiana*, as accepted by several authors (BFG, 2015; Morrone et al., 2008; Swallen, 1966; Welker et al., 2012). 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. 4). Therefore, the morphological treatment of Filgueiras (2003), which considered *E. laxa* a probable synonym of *E. warmingiana*, is not supported by our molecular analyses. We find that 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 (Fig. 4). 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. 4): either (1) they should be considered as two distinct species, as accepted by several authors (BFG, 2015; Smith et al., 1982; Swallen, 1966; Welker and Longhi-Wagner, 2012; Welker et al., 2012); or (2) they should be considered 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. 5). *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 (Fig. 4) (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 non-lobed apex (see Table 1 and Fig. 4). Based on our molecular analyses, plus morphological and biogeographical aspects of the plants, we consider *E. cayennensis* and *E. villosa* as two distinct species.

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 a lower glume with a subacute 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. 4). Thus, they simply represent morphological variation within *E. cayennensis*. Plastome data also support this treatment (Fig. 5). 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 between trees based on nuclear genes and those based on plastomes (Fig. 6). 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 morphologically distinct from the other two species 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. 4). 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) (Fig. 2).

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 chromosome duplication 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, since we did identify hybrids between *E. laxa* and *E. villosa*.

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 increasing data, such as the sequences of numerous DNA loci and even complete genome sequences. A common challenge in such large sets of data is that conflicting genealogical histories often exist for different genes throughout the genome, and for the three different genomes from the organism (Degnan and Rosenberg, 2009; Maddison, 1997; Pelser et al., 2010). Although individual gene trees of 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 Fig. 3 and 5) and the position of *Eriochrysis laxa* and *E. villosa* within the *Eriochrysis* clade (Fig. 6).

Incongruence among phylogenetic trees inferred from different molecular markers may result from biological phenomena as well as from 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, that may result in incongruence between gene trees and species trees (Maddison, 1997; Pelser et al., 2010). It is well known 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 radiate rapidly 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 underwent a rapid radiation (Estep et al., 2014; Mathews et al., 2002; Teerawatananon et al., 2011). Additionally, although the actual effective population size is not known for most grasses, 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).

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. An allopolyploidization event in *Eriochrysis*, from interspecific hybridization 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. Other hybridization events not involving duplication of whole genomes could have occurred in the evolutionary history of Andropogoneae, and thus may not have been 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 differences in taxon sampling may also have contributed to incongruence between the trees. Since our nuclear gene dataset includes many more taxa than the plastome dataset, the different sampling combined with the short branches along the backbone of the trees could lead to different topologies just because some species were not included. To test this, we reduced the concatenated nuclear gene dataset to include only those taxa represented in our plastome phylogeny. This analysis resulted in trees with the same topology as those from the complete nuclear dataset – and not of the plastome phylogeny – concerning the position of *Pogonatherum* and of *Eriochrysis laxa* and *E. villosa* (trees not shown). This result suggests that differences in

taxon sampling is not the main cause of incongruence between our nuclear and plastome phylogenies. Further plastid phylogenomic studies are warranted to increase the number of taxa to test the results of 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 indicate that subtribe Saccharinae (sensu Clayton and Renvoize, 1986) is polyphyletic, as is the genus *Eulalia*. Based on molecular markers plus morphology, we can define the circumscriptions 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 found. These interspecific hybrids are probably tetraploid, based on the number of paralogues in the nuclear gene trees; all other *Eriochrysis* species are probably diploid. This is the first record of a polyploid taxon in the genus *Eriochrysis*. Incongruence between nuclear gene and plastome phylogenetic analyses is 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 the presence of polyploidy and reticulate evolution. Complete plastome sequencing was also a valuable tool for phylogenetic inference.

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763 **Table 1**
764 Comparison of morphological and biogeographical characters of New World taxa of *Eriochrysis* discussed in the text. Illustrations of the lower glume
765 of the spikelet of these taxa are presented in Fig. 4.
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	<i>E. cayennensis</i>	<i>E. holcoides</i>	<i>E. laxa</i>	<i>E. aff. laxa</i>	<i>E. villosa</i>	<i>E. warmingiana</i>	<i>Eriochrysis</i> 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, non-lobed	rounded to obtuse, non- lobed	rounded to obtuse, sometimes with inconspicuous lobes	acute, non- lobed	acute to acuminate, non- 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

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Table 2

Voucher specimens and collection localities of the samples included in the phylogenetic analyses based on nuclear genes. Herbarium acronyms according to Index Herbariorum (Thiers, 2016) except THNHM (Thailand Natural History Museum), not included in that directory.

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

Species	Voucher	Locality
<i>Eriochrysis cayennensis</i> 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
<i>Eriochrysis holcoides</i> (Nees) Kuhlman	Welker 338 (ICN)	Brazil, Rio Grande do Sul, São Francisco de Paula
	Welker 391 (ICN)	Brazil, Santa Catarina, Irani
	Welker 505 (ICN)	Brazil, Santa Catarina, Caçador
<i>Eriochrysis laxa</i> 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
<i>Eriochrysis</i> aff. <i>laxa</i> 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
<i>Eriochrysis pallida</i> Munro	Malcomber et al. 3086 (MO)	Tanzania, Iringa, Njombe
<i>Eriochrysis villosa</i> 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
<i>Eriochrysis warmingiana</i> (Hack.) Kuhlman	Neves & Monteiro 406 (ICN, RB)	Brazil, Mato Grosso do Sul, Rio Verde de Mato Grosso

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

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Species	Voucher	Locality
<i>Schizachyrium sanguineum</i> (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	Thailand, Chiang Mai, Muang
<i>Setaria italica</i> (L.) P. Beauv.	Strain Yugu1 (genome sequence)	unknown
<i>Sorghastrum elliottii</i> (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	U.S.A., Texas, Anderson County
<i>Sorghum bicolor</i> (L.) Moench	Kellogg PI 156549 (A/GH)	Zimbabwe
<i>Thelepogon elegans</i> Roth	Teerawatananon & Sungkaew 697 (THNHM)	Thailand, Tak, LanSang
<i>Themeda arundinacea</i> (Roxb.) A. Camus	Teerawatananon & Sungkaew 739 (THNHM)	Thailand, Chiang Mai, Mae Rim
<i>Tripsacum dactyloides</i> (L.) L.	Kellogg 1261 (A/GH)	U.S.A., Missouri, Pettis County
<i>Zea mays</i> L.	Cultivar B73 (genome sequence)	unknown

774 **Table 3**
775 Chloroplast genome size, presence of gaps in assembly, voucher specimens, and GenBank accession numbers of the samples included in the plastome
776 phylogenetic analyses. Herbarium acronyms according to Index Herbariorum (Thiers, 2016). *Indicates gap present.
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Species	Total Size (bp)	LSC Size (bp)	IR Size (bp)	SSC Size (bp)	Gaps in Assembly	Voucher	GenBank Accession	Source
<i>Eriochrysis cayennensis</i>	140380	82368*	22770	12742*	4	Welker 519 (ICN)	XXXXXXX	This Study
<i>Eriochrysis laxa</i>	140135	82366*	22638	12493	1	Welker 489 (ICN)	XXXXXXX	This Study
<i>Eriochrysis villosa</i>	140404	82362*	22783	12476	1	Welker 481B (ICN)	XXXXXXX	This Study
<i>Eriochrysis</i> sp.	140426	82393	22770	12493	0	Welker 365 (ICN)	XXXXXXX	This Study
<i>Chrysopogon serrulatus</i>	140700	82656	22761	12522	0	Kellogg PI 219580 (A/GH)	XXXXXXX	This Study
<i>Pogonatherum paniceum</i>	138441	81656	22173	12439	0	Clark s.n. (MO)	XXXXXXX	This Study
<i>Miscanthus sinensis</i>	141293	83122	22798	12575	0	Strain IGR-2011-003	SRA SRR559246	This Study
<i>Saccharum officinarum</i>	141182	83048	22795	12544	N/A	Cultivar NCo 310	AP006714	GenBank
<i>Sorghum bicolor</i>	140754	82685	22783	12503	N/A	Cultivar BTx623	EF115542	GenBank
<i>Zea mays</i>	140384	82353	22748	12536	N/A	N/A	NC001666	GenBank
<i>Setaria italica</i>	138833	81916	22194	12529	N/A	N/A	KJ001642	GenBank

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780 **Supplementary Information**

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782 **Plastome sequencing and assembly**

783 Whole genomic DNA Illumina libraries from the DNA isolations were made using either the
784 NEBNext Ultra DNA Library Prep Kit (New England Biolabs, Inc., Ipswich, Massachusetts,
785 U.S.A.) or the Nextera DNA Sample Preparation Kit (Illumina, San Diego, California, U.S.A.)
786 following the manufacturer's protocol. For libraries made using the NEBNext kit, DNA was
787 sheared at the University of Missouri–DNA Core Facility using a Covaris S220/E220 focused-
788 ultrasonicator (Covaris, Woburn, Massachusetts, U.S.A.) to obtain an average size of 300–350 base
789 pairs (bp). For those made with the Nextera kit, incubation time for the tagmentation step was 5
790 minutes at 55°C, providing a broad fragment distribution. Samples and their library preparation
791 methods are found in Table S2. Libraries were size-selected using Agencourt AMPure XP beads
792 (Beckman Coulter, Inc., Indianapolis, Indiana, U.S.A.) to obtain an average fragment size of 300–
793 350 bp for libraries sequenced using single-end Illumina and 400–500 bp for libraries sequenced
794 using paired-end Illumina. Libraries were sequenced for 100 bp single-end reads at the University
795 of Missouri–DNA Core Facility on a HiSeq 2000 (Illumina, San Diego, California, U.S.A.) and for
796 150 bp paired-end reads at the New York University School of Medicine using a HiSeq 2500 Rapid
797 Run (Illumina, San Diego, California, U.S.A.). Table S2 provides information for sequencing
798 methods used for each sample. A dataset of paired-end Illumina reads for *Miscanthus sinensis*
799 Andersson (SRA SRR559246) was downloaded from the SRA database of GenBank.

800 Reads were trimmed using Trimmomatic v.0.32 (Bolger et al., 2014) using the following
801 parameters: ILLUMINACLIP:appropriate_adpater_file:3:30:10 SLIDINGWINDOW:10:20
802 MINLEN:40. Initial assemblies were made using SPAdes v.3.1.0 (Bankevich et al., 2012) using
803 either single-end or pair-end when appropriate with the “only-assembler” option and k-mer sizes of
804 33, 55, and 77. Each SPAdes assembly was blasted against the *Zea mays* chloroplast (GenBank,

805 NC001666.2) and mitochondrial (GenBank, NC007982.1) genomes using blastn with an e-value
806 cutoff of $1e^{-10}$ (Camacho et al., 2009) and assembly contigs were filtered into chloroplast-like and
807 mitochondrial-like pools based on e-values and overlap lengths. Chloroplast-like contigs were
808 meta-assembled in Sequencher v.5.0.1 (Gene Codes, Ann Arbor, Michigan, U.S.A.). Annotated *Zea*
809 *mays* chloroplast genes were assembled with the meta-assembly of SPAdes contigs to provide
810 context for contigs. Gaps between contigs were closed by using an iterative plastome-walking
811 procedure. This was done by searching the trimmed reads for sequences with similarity to the ends
812 of contigs and assembling those reads to the contigs within Sequencher. To check final assemblies
813 for accuracy, counts for 20-mers from the trimmed reads were estimated using jellyfish v.2.1.3
814 (Marçais and Kingsford, 2011). Counts from the reads for all 20-mers found within the plastome
815 assemblies were recorded in a sliding window. Assemblies were considered acceptable if no sudden
816 drops in coverage were detected, aside from the expected variation at the inverted repeat
817 boundaries. If any cases were found, they were investigated by assembling reads matching the
818 regions surrounding the drop. In all cases, the reads supported the final assembly and demonstrated
819 regions of relatively lower coverage in the plastomes. Plastomes were uploaded to Verdant
820 (verdant.iplantcollaborative.org) and automatically annotated. A Circos graph (Krzywinski et al.,
821 2009) of the plastome structure was created for each species using the built-in features of Verdant.
822 Additional whole plastomes for Andropogoneae (*Saccharum officinarum*, *Sorghum bicolor*, and
823 *Zea mays*) and an outgroup (*Setaria italica*) were downloaded from GenBank and added to Verdant
824 for further analysis (Table 3).

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Table S1

Voucher specimens and GenBank accession numbers for sequences included in the phylogenetic analyses based on nuclear genes. Herbarium acronyms according to Index Herbariorum (Thiers, 2016) except THNHM (Thailand Natural History Museum), not included in that directory.

Species	Voucher	<i>apo1</i>	<i>d8</i>	<i>ep2-ex7</i>	<i>ep2-ex8</i>	<i>kn1</i>
<i>Andropogon eucomus</i> Nees	Malcomber et al. 3089 (MO)	KM578363	KM578119	KM577921	KM577706	N/A
<i>Andropogon virginicus</i> L.	Kellogg 1240 (MO)	KM578449	KM578209	KM578001	KM577789	N/A
<i>Andropterum stolzii</i> (Pilg.) C.E. Hubb.	Malcomber et al. 3091 (MO)	KM578417	KM578168	KM577978	KM577764	N/A
<i>Apocopis courtallumensis</i> (Steud.) Henrard	Teerawatananon & Kritsanachandee 928 (THNHM)	KM578499	KM578282	KM578054	N/A	N/A
<i>Apocopis intermedius</i> (A. Camus) Chai-Anan	Teerawatananon & Kritsanachandee 934 (THNHM)	KM578502	KM578283, KM578286	KM578055, KM578057	KM577851	N/A
<i>Apocopis siamensis</i> A. Camus	Teerawatananon & Sungkaew 975 (THNHM)	KM578503	KM578287	KM578058	KM577852	N/A
<i>Arthraxon lanceolatus</i> (Roxb.) Hochst.	Teerawatananon & Sungkaew 720 (THNHM)	N/A	KM578290	KM578061	KM577854	N/A
<i>Arthraxon prionodes</i> (Steud.) Dandy	Kellogg PI 659331 (MO)	N/A	KM578256	KM578036	KM577831	N/A
<i>Capillipedium assimile</i> (Steud.) A. Camus	Teerawatananon & Sungkaew 791 (THNHM)	KM578504	KM578291	KM578062	KM577855	N/A
<i>Chasmopodium caudatum</i> (Hack.) Stapf	Kellogg Kew MSB 184054 (MO)	KM578498	KM578275	KM578046	KM577842	N/A
<i>Chionachne koenigii</i> (Spreng.) Thwaites	Kellogg Chio-6-D-93 (MO)	KP243072	KP233123	KP242922	KP242976	N/A
<i>Chrysopogon gryllus</i> (L.) Trin.	Kellogg PI 250984 (A/GH)	KM578372, KM578373	N/A	KM577928, KM577929	KM577714, KM577715	N/A
<i>Chrysopogon serrulatus</i> Trin.	Kellogg PI 219580 (A/GH)	KM578434	KM578184	KM577988	KM577769	N/A
<i>Cymbopogon distans</i> (Nees ex Steud.) Will. Watson	Kellogg PI 271552 (MO)	KM578378	KM578126	KM577939	N/A	N/A
<i>Dichanthium annulatum</i> (Forssk.) Stapf	Kellogg PI 240155 (A/GH)	N/A	KM578135	KM577945	KM577725	N/A
<i>Diheteropogon amplexans</i> (Nees) Clayton	Kellogg RF 1819 (MO)	KM578432	KM578183	KM577987	KM577768	N/A
<i>Diheteropogon hagerupii</i> Hitchc.	Kellogg Kew MSB 254456 (MO)	KM578548	KM578355	KM578110	KM577907	N/A
<i>Dimeria fuscescens</i> Trin.	Teerawatananon & Sungkaew 830 (BKF, THNHM)	KM578511, KM578513	KM578300, KM578301	KM578069, KM578070	KM577862, KM577863	N/A
<i>Dimeria ornithopoda</i> Trin.	Teerawatananon & Sungkaew 685 (BKF, THNHM)	KM578514	KM578305	N/A	KM577864	N/A
<i>Eriochrysis cayennensis</i> P. Beauv.	Welker 395 (ICN)	KT449430	KT449461	N/A	KT449514	KT449545
	Welker 468 (ICN)	KT449431	KT449462	KT449492	KT449515	KT449546
	Welker 486 (ICN)	KT449432	N/A	N/A	KT449516	N/A
	Welker 536 (ICN)	KT449433	KT449463	N/A	KT449517	KT449547
	Welker & Peichoto 597 (CTES, ICN)	KT449434	KT449464	KT449493	KT449518	KT449548

Species	Voucher	<i>apo1</i>	<i>d8</i>	<i>ep2-ex7</i>	<i>ep2-ex8</i>	<i>kn1</i>
<i>Eriochrysis holcoides</i> (Nees) Kuhlman.	Welker 338 (ICN)	KT449435	KT449465	N/A	KT449519	N/A
	Welker 391 (ICN)	KT449436	KT449466	N/A	KT449520	N/A
	Welker 505 (ICN)	KT449437	KT449467	KT449494	KT449521	KT449549
<i>Eriochrysis laxa</i> Swallen	Neves & Alvarenga 493 (RB)	KT449447	KT449479	KT449502	KT449530	KT449559
	Welker 481A (ICN)	KT449442	N/A	KT449497	N/A	N/A
	Welker 488-7 (ICN)	KT449443	KT449474	KT449498	N/A	KT449554
	Welker 488-9 (ICN)	KT449444	KT449475	KT449499	KT449526	KT449555
	Welker 488-11 (ICN)	N/A	KT449476	KT449500	KT449527	KT449556
	Welker 488-12 (ICN)	KT449445	KT449477	N/A	KT449528	KT449557
	Welker 489 (ICN)	KT449446	KT449478	KT449501	KT449529	KT449558
	Welker 487-10 (ICN)	KT449438	KT449468, KT449469	N/A	KT449522	KT449550, KT449551
<i>Eriochrysis</i> aff. <i>laxa</i> Swallen	Welker 487-11 (ICN)	KT449439	KT449470, KT449471	KT449495, KT449496	KT449523, KT449524	KT449552, KT449553
	Welker 544 (ICN)	KT449440, KT449441	KT449472, KT449473	N/A	KT449525	N/A
	Malcomber et al. 3086 (MO)	KM578393	KM578137	KM577947	KM577728	N/A
<i>Eriochrysis pallida</i> Munro						
<i>Eriochrysis villosa</i> Swallen	Welker 460 (ICN)	KT449448	KT449484	KT449507	KT449536	KT449563
	Welker 481B (ICN)	KT449449	KT449485	KT449508	KT449537	KT449564
	Welker 490 (ICN)	KT449450	KT449486	KT449509	KT449538	KT449565
	Welker 652 (ICN)	KT449451	KT449487	KT449510	KT449539	KT449566
	Welker 655 (ICN)	KT449452	KT449488	KT449511	KT449540	KT449567
	Neves & Monteiro 406 (ICN, RB)	KT449458	KT449489	N/A	KT449541	KT449568
<i>Eriochrysis warmingiana</i> (Hack.) Kuhlman.						
<i>Eriochrysis</i> sp. (“inconspicuous lobes”)	Longhi-Wagner & Welker 10863 (ICN)	KT449457	N/A	KT449506	KT449535	KT449562
	Welker 342 (ICN)	KT449453	KT449480	KT449503	KT449531	KT449560
	Welker 365 (ICN)	KT449454	KT449481	KT449504	KT449532	KT449561
	Welker 617 (ICN)	KT449455	KT449482	KT449505	KT449533	N/A
	Welker 621 (ICN)	KT449456	KT449483	N/A	KT449534	N/A
	Kellogg PI 249139 (MO)	N/A	KM578247	KM578027	KT449542	N/A
<i>Eulalia aurea</i> (Bory) Kunth						

Species	Voucher	apo1	d8	ep2-ex7	ep2-ex8	kn1
<i>Eulalia quadrinervis</i> (Hack.) Kuntze	Teerawatananon & Sungkaew 706 (THNHM)	N/A	KM578308, KM578310	KM578072, KM578074	KM577867, KM577869	N/A
<i>Eulalia villosa</i> Nees	Malcomber et al. 3088 (MO)	N/A	KM578158	KM577972	KM577759	N/A
<i>Germainia capitata</i> Balansa & Poitr.	Teerawatananon & Sungkaew 834 (THNHM)	KM578515	KM578312	KM578075	KM577870	N/A
<i>Heteropogon triticeus</i> (R. Br.) Stapf ex Craib	Teerawatananon & Sungkaew 733 (THNHM)	KM578516	KM578314	KM578076	KM577874	N/A
<i>Hyparrhenia rufa</i> (Nees) Stapf	Kellogg PI 206889 (A/GH)	KM578396	KM578140	KM577948	KM577732	N/A
<i>Imperata brasiliensis</i> Trin.	Longhi-Wagner & Welker 10848 (ICN)	KT449459	KT449490	KT449512	KT449543	N/A
<i>Imperata cylindrica</i> (L.) P. Beauv.	Kowarat 108 (THNHM)	KM578524	KM578321	KM578082	KM577877	N/A
<i>Imperata tenuis</i> Hack.	Lerina & Silveira 95 (ICN)	KT449460	KT449491	KT449513	KT449544	N/A
<i>Ischaemum rugosum</i> Salisb.	Kellogg Kew MSB 183574 (MO)	KM578551	KM578356	KM578113	KM577910	N/A
<i>Iseilema macratherum</i> Domin	Snow et al. 7239 (A/GH)	KM578440	KM578192	KM577992	KM577775	N/A
<i>Microstegium vimineum</i> (Trin.) A. Camus	Kellogg VA-2 (MO)	N/A	N/A	KM578051	KM577846	N/A
<i>Miscanthus sinensis</i> Andersson	Kellogg PI 668403 (MO)	KM578443, KM578444	KM578199, KM578201	KM577993, KM577994	KM577779, KM577781	N/A
<i>Pogonatherum crinitum</i> (Thunb.) Kunth	Teerawatananon & Sungkaew 865 (THNHM)	N/A	N/A	KM578088	KM577885	N/A
<i>Pogonatherum paniceum</i> (Lam.) Hack.	Clark s.n. (MO)	KM578448	KM578204	KM577998	KM577785	N/A
<i>Polytoca wallichiana</i> (Nees ex Steud.) Benth.	Teerawatananon & Sungkaew 683 (THNHM)	KP243073	KP233124	KP242923	KP242977	N/A
<i>Polytrias indica</i> (Houtt.) Veldkamp	Kellogg 1264 (MO)	N/A	KM578208	KM578000	KM577788	N/A
<i>Pseudosorghum fasciculare</i> (Roxb.) A. Camus	Teerawatananon & Sungkaew 698 (THNHM)	N/A	KM578329	KM578089	KM577886	N/A
<i>Saccharum giganteum</i> (Walter) Pers.	Layton & Zhong 161 (MO)	KP243052, KP243053	KP233111, KP233112	N/A	KP242953, KP242954	N/A
<i>Saccharum officinarum</i> L.	Welker s.n. (MO)	KP243055, KP243056	N/A	KP242907, KP242908	KP242956, KP242957	N/A
<i>Schizachyrium brevifolium</i> (Sw.) Nees ex Buse	Teerawatananon & Sungkaew 750 (THNHM)	KM578530	N/A	KM578097	KM577893	N/A
<i>Schizachyrium sanguineum</i> (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	KM578532, KM578533	KM578339, KM578340	KM578099, KM578100	KM577894	N/A
<i>Setaria italica</i> (L.) P. Beauv.	Strain Yugu1 (genome sequence)	XM012845 284	XM004981 974	XM004958 247	XM004958 246	EF189799
<i>Sorghastrum elliottii</i> (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	KM578552	N/A	KM578114	KM577911	N/A
<i>Sorghum bicolor</i> (L.) Moench	Kellogg PI 156549 (A/GH)	KM578410	KM578151	KM577964	KM577752	N/A
<i>Thelepogon elegans</i> Roth	Teerawatananon & Sungkaew 697 (THNHM)	KM578539	N/A	KM578103	KM577895	N/A

Species	Voucher	<i>apo1</i>	<i>d8</i>	<i>ep2-ex7</i>	<i>ep2-ex8</i>	<i>kn1</i>
<i>Themeda arundinacea</i> (Roxb.) A. Camus	Teerawatananon & Sungkaew 739 (THNHM)	N/A	KM578349	KM578104	KM577897	N/A
<i>Tripsacum dactyloides</i> (L.) L.	Kellogg 1261 (A/GH)	KM578413	KM578154	KM577967, KM577968	KM577755, KM577756	N/A
<i>Zea mays</i> L.	Cultivar B73 (genome sequence)	N/A	GRMZM2 G360081, GRMZM2 G109966	GRMZM2 G024973, GRMZM2 G144744	GRMZM2 G098859, GRMZM2 G414043	N/A

829 **Table S2**
830 Plastome library preparation and sequencing methods used in this study.
831

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

832

FIGURE LEGENDS

Fig. 1. Morphological diversity in *Eriochrysis* species. A–B. *E. cayennensis*. A. Habit. B. Portion of the inflorescence. C–D. *E. holcoides*. C. Inflorescences. D. Portion of the inflorescences. E. Inflorescences of *E. cayennensis* (left), *E. villosa* (middle), and *E. laxa* (right). F. *E. laxa*, portion of the inflorescence. G. *E. villosa*, portion of the inflorescence. H–I. *Eriochrysis* sp. (“inconspicuous lobes”). H. Habit. I. Inflorescences. (Photos by H.M. Longhi-Wagner (A–D, H–I) and C.A.D. Welker (E–G)).

Fig. 2. Map with the collection localities of the New World specimens of *Eriochrysis* included in this study. Countries: Argentina (ARG), Brazil (BRA), Uruguay (URU).

Fig. 3. Bayesian phylogeny of *Eriochrysis* and other genera of Andropogoneae based on the combined dataset of low-copy nuclear loci (*apo1*, *d8*, *ep2-ex7*, *ep2-ex8*, and *kn1*), 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. 4.

Fig. 4. Bayesian phylogeny of *Eriochrysis* based on the combined dataset of low-copy nuclear loci (*apo1*, *d8*, *ep2-ex7*, *ep2-ex8*, and *kn1*). 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.

859

860 **Fig. 5.** Bayesian phylogeny of *Eriochrysis* and other genera of Andropogoneae based on whole
861 chloroplast sequences (the same tree shown as phylogram is presented on the left). Bayesian PP >
862 0.85 are shown above branches, and ML bootstrap values > 50 are shown below. Representatives
863 from subtribe Saccharinae (sensu Clayton & Renvoize, 1986) are indicated (SAC).

864

865 **Fig. 6.** Representation of the incongruence between nuclear and plastome trees concerning the
866 phylogenetic position of *Eriochrysis cayennensis*, *E. laxa*, and *E. villosa*.

867

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

Figure 1 (color)

Figure 1 (black and white)

Figure 2

Phylogenetic tree of the Poaceae family, showing relationships between various genera and species. The tree is rooted with *Arthraxon prionodes* and *Arthraxon lanceolatus*. Major clades are labeled on the right: SAC (Saccharum), SAC (Sorghum), SAC (Eulalia), SAC (Themeda), SAC (Imperata), and SAC (Eriochrysis spp.). Bootstrap values are provided at many nodes. A scale bar of 0.01 is shown at the bottom left.

Species included in the tree:

- Arthraxon prionodes*
- Arthraxon lanceolatus*
- Chasmopodium caudatum*
- Chionachne koenigii*
- Polytoca wallichiana*
- Tripsacum dactyloides* 1261
- Zea mays* B73
- Tripsacum dactyloides* 1261
- Zea mays* B73
- Thelepogon elegans*
- Chrysopogon gryllus* 250984
- Chrysopogon gryllus* 250984
- Chrysopogon serrulatus*
- Andropterum stolzii*
- Ischaemum rugosum*
- Dimeria fuscescens* 830
- Dimeria ornithopoda*
- Dimeria fuscescens* 830
- Microstegium vimineum*
- Sorghastrum elliptii*
- Polytrias indica*
- Eulalia aurea*
- Miscanthus sinensis* 668403
- Saccharum officinarum* Welker s.n.
- Saccharum officinarum* Welker s.n.
- Saccharum giganteum* 161
- Saccharum giganteum* 161
- Pseudosorghum fasciculare*
- Miscanthus sinensis* 668403
- Sorghum bicolor*
- Eulalia quadrinervis* 706
- Eulalia villosa*
- Eulalia quadrinervis* 706
- Cymbopogon distans*
- Themeda arundinacea*
- Heteropogon triticeus*
- Iscilema macrathetrum*
- Dichanthium annulatum*
- Capillipedium assimile*
- Andropogon eucomis*
- Andropogon virginicus*
- Schizachyrium sanguineum* 751
- Schizachyrium brevifolium*
- Schizachyrium sanguineum* 751
- Hyparrhenia rufa*
- Diheteropogon amplexans*
- Diheteropogon hagerupii*
- Imperata cylindrica*
- Imperata brasiliensis*
- Imperata tenuis*
- Pogonatherum panicum*
- Pogonatherum crinitum*
- Apocopsis intermedius* 934
- Germainia capitata*
- Apocopsis siamensis*
- Apocopsis courtallumensis*
- Apocopsis intermedius* 934
- Eriochrysis* spp.

Figure 4

Figure 5

Figure 6