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Multilocus phylogeny and phylogenomics of *Eriochrysis* P. Beauv. (Poaceae–Andropogoneae): 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 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 (*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 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.

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

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(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, reconstructing the phylogeny of *Eriochrysis* is required 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 1060 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*, 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. *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). 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.).

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 Fig. 4.

	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, 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

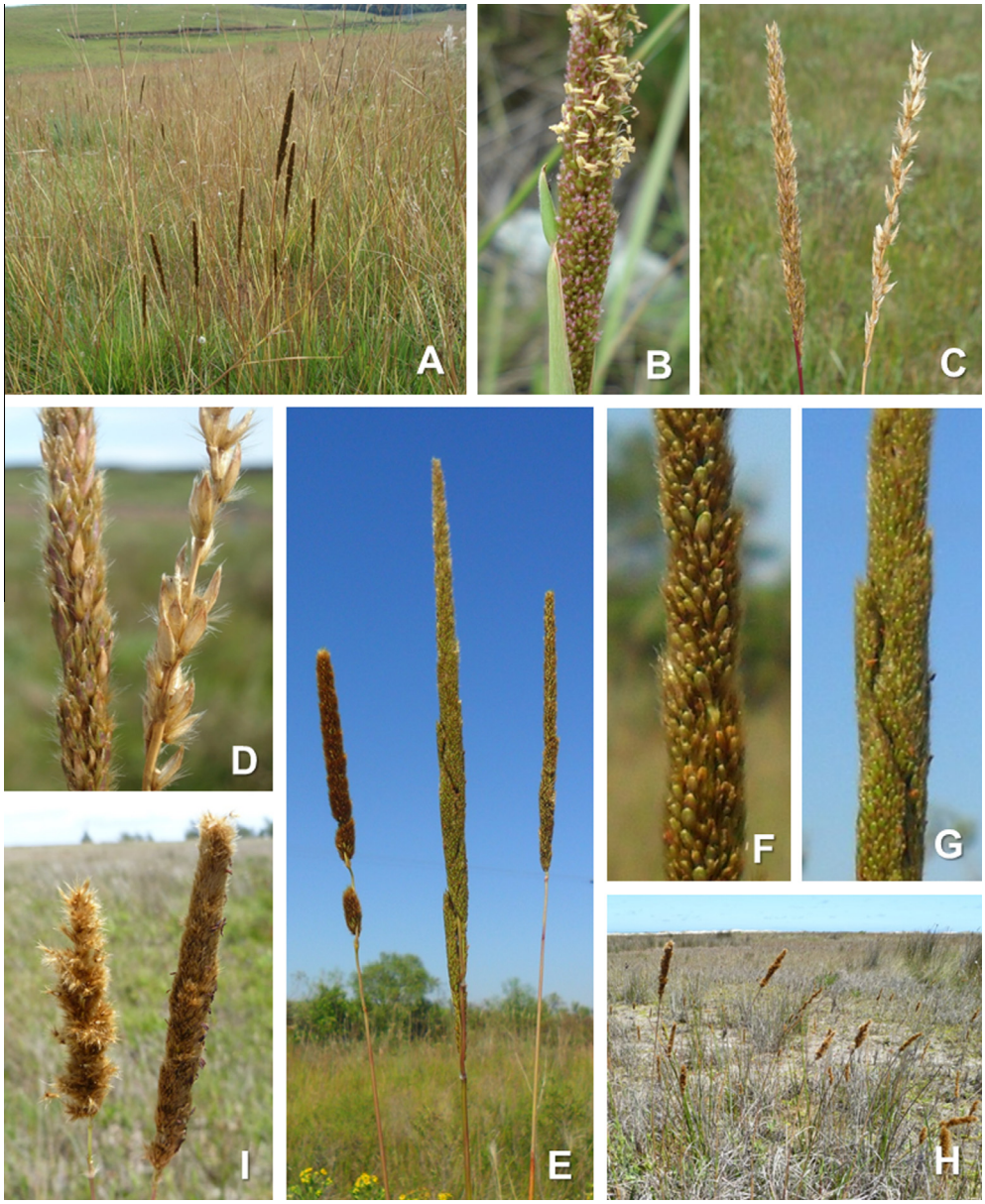

Fig. 1. Morphological diversity in *Eriochrysis* species. A and B. *E. cayennensis*. A. Habit. B. Portion of the inflorescence. C and D. *E. holcoides*. C. Inflorescences. D. Portion of the inflorescences. E. Inflorescences of *E. cayennensis* (left), *E. villosa* (middle), and *E. laxa* (right). F.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

The circumscription of *Eriochrysis laxa* Swallen and *E. warmingiana* (Hack.) Kuhlm. 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; de 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 material 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 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). These genes are known to influ-

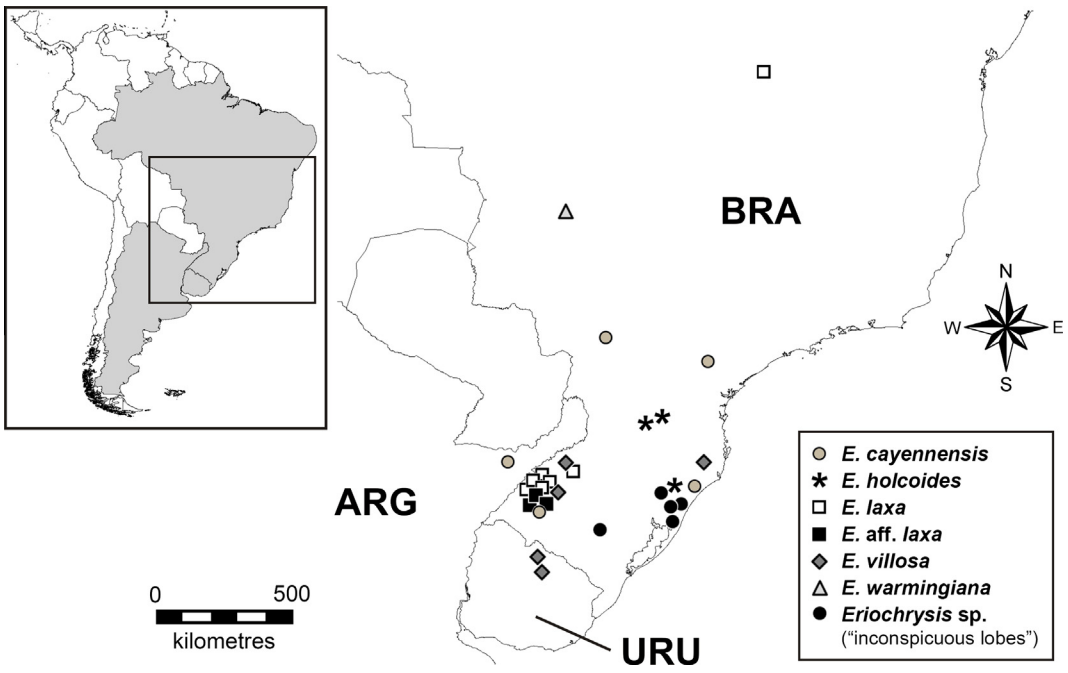

Fig. 2. Map with the collection localities of the New World specimens of *Eriochrysis* included in this study. Countries: Argentina (ARG), Brazil (BRA), Uruguay (URU).

ence 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-thiogalactoside (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 1000 bp long (*d*8 and *ep*2–*ex*7), 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 *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. 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 material 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 mitrochondrial (GenBank, NC007982.1) genomes using blastn with an e-value cutoff of 1 e^{-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 material 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 samples, 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 4263 bp long, of which 1577 (37%) were variable and 899 (21%) were parsimony informative. The MP analysis resulted in 2947 equally most parsimonious trees of 2554 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.

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
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
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, Irani
	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
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
Schizachyrium sanguineum (Retz.) Alston	Teerawatananon & Sungkaew 751 (THNHM)	Thailand, Chiang Mai, Muang
Setaria italica (L.) P. Beauv.	Strain Yugu1 (genome sequence)	Unknown
Sorghastrum elliottii (C. Mohr) Nash	Kellogg Kew MSB 491101 (MO)	U.S.A., Texas, Anderson County

Table 2 (*continued*)

Species	Voucher	Locality
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. Herbarium acronyms according to Index Herbariorum (Thiers, 2016).

Species	Total size (bp)	LSC size (bp)	IR Size (bp)	SSC Size (bp)	Gaps in assembly	Voucher	GenBank accession	Source
Eriochrysis cayennensis	140,380	82,368*	22,770	12,742*	4	Welker 519 (ICN)	KU961861	This Study
Eriochrysis laxa	140,135	82,366*	22,638	12,493	1	Welker 489 (ICN)	KU961863	This Study
Eriochrysis villosa	140,404	82,362*	22,783	12,476	1	Welker 481B (ICN)	KU961860	This Study
Eriochrysis sp.	140,426	82,393	22,770	12,493	0	Welker 365 (ICN)	KU961862	This Study
Chrysopogon serrulatus	140,700	82,656	22,761	12,522	0	Kellogg PI 219580 (A/GH)	KU961864	This Study
Pogonatherum paniceum	138,441	81,656	22,173	12,439	0	Clark s.n. (MO)	KU961859	This Study
Miscanthus sinensis	141,293	83,122	22,798	12,575	0	Strain IGR-2011-003	SRA SRR559246	This Study
Saccharum officinarum	141,182	83,048	22,795	12,544	N/A	Cultivar NCo 310	AP006714	GenBank
Sorghum bicolor	140,754	82,685	22,783	12,503	N/A	Cultivar BTx623	EF115542	GenBank
Zea mays	140,384	82,353	22,748	12,536	N/A	N/A	NC001666	GenBank
Setaria italica	138,833	81,916	22,194	12,529	N/A	N/A	KJ001642	GenBank

* Indicates gap present.

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. 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) 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 material 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).

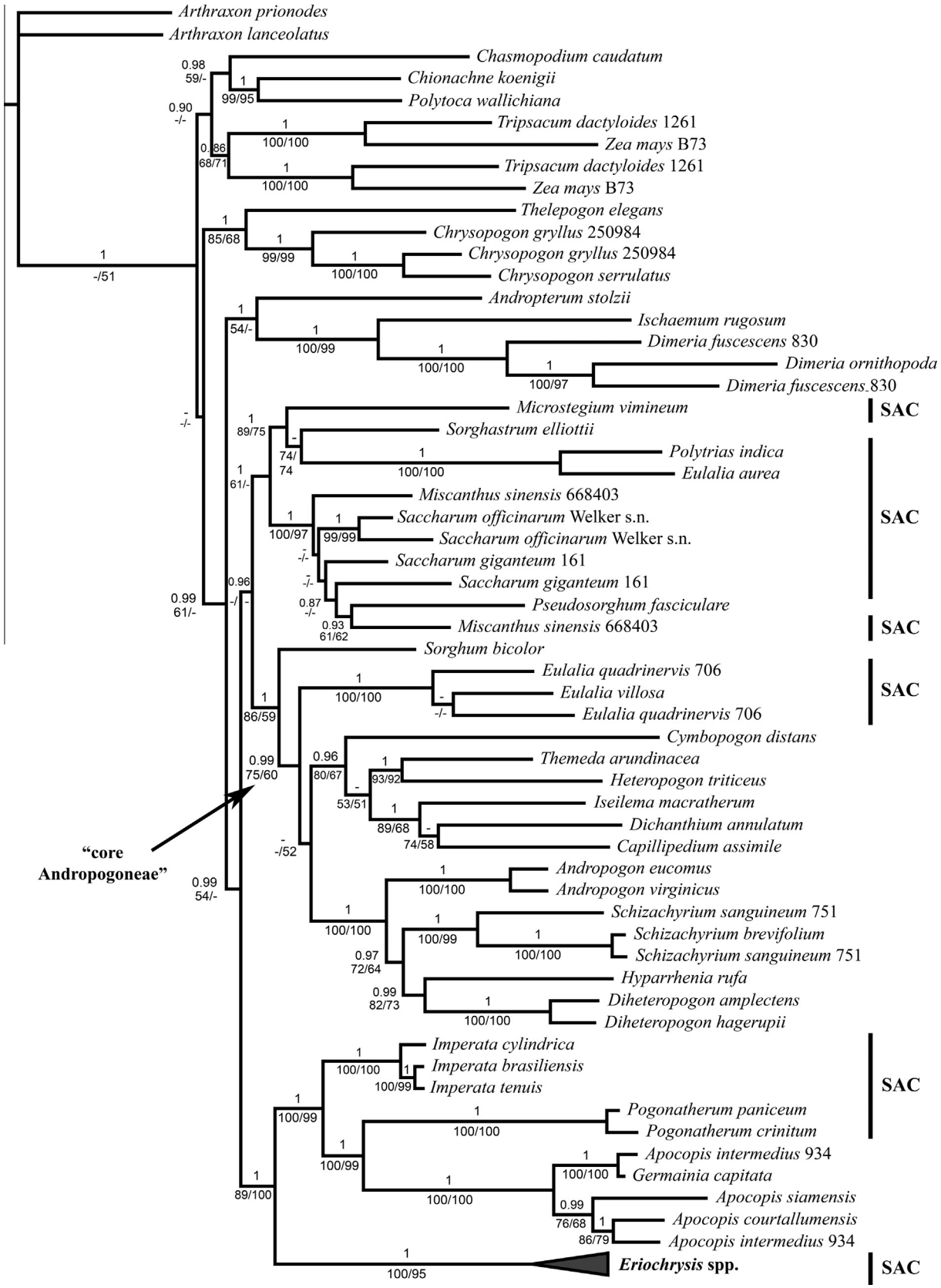

Fig. 3. Bayesian phylogeny of *Eriochrysis* and other genera of Andropogoneae based on the combined dataset of low-copy nuclear loci (*apo*1, *d*8, *ep*2–*ex*7, *ep*2–*ex*8, and *kn*1), shown as a phylogram. Bayesian PP > 0.85 are shown above branches, and ML/MP bootstrap values >50 are shown below. 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 and Renvoize, 1986) are indicated (SAC). The clade of *Eriochrysis* taxa is presented in detail in Fig. 4.

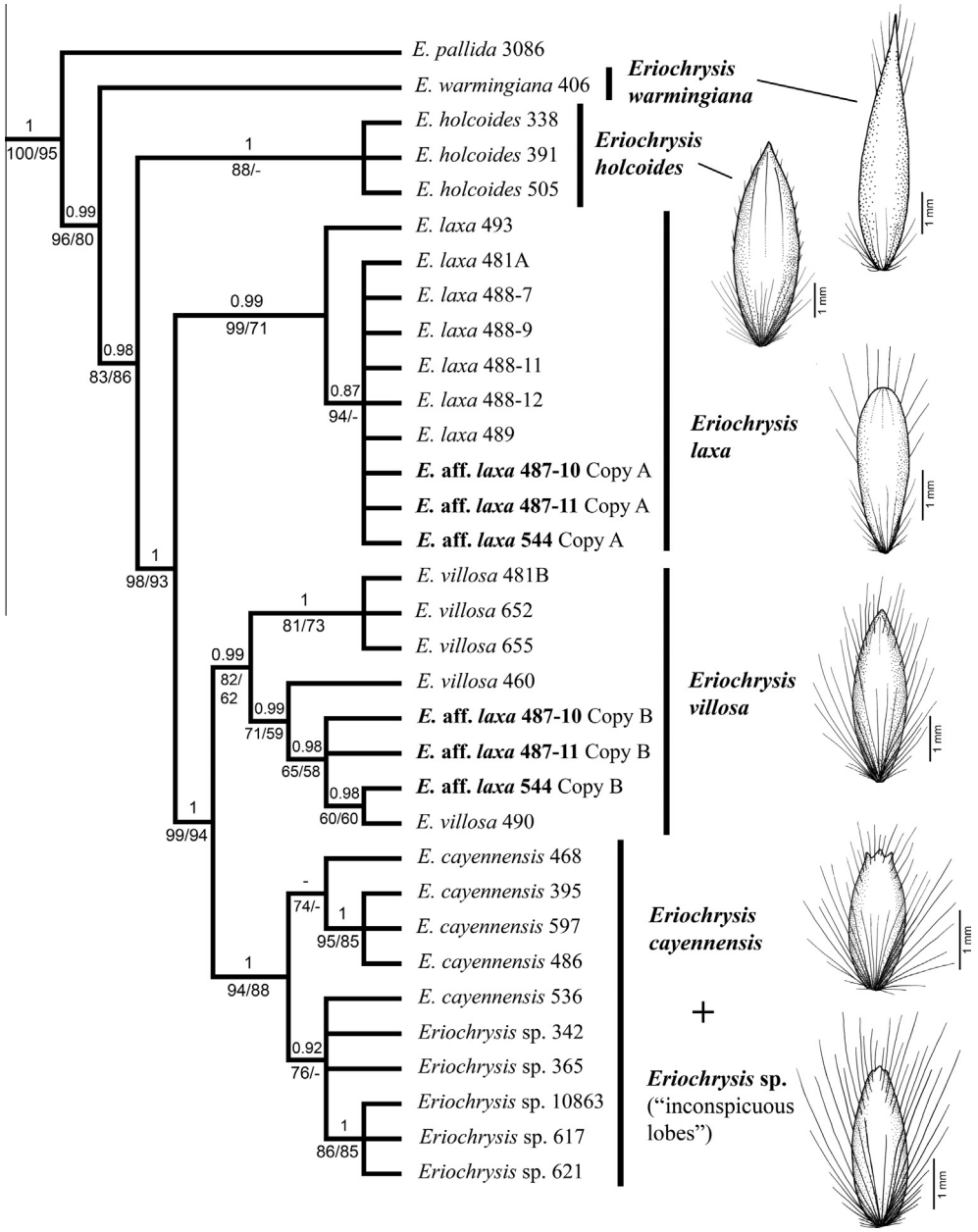

Fig. 4. Bayesian phylogeny of *Eriochrysis* based on the combined dataset of low-copy nuclear loci (*apo*1, *d*8, *ep*2–*ex*7, *ep*2–*ex*8, and *kn*1). Bayesian PP > 0.85 are shown above branches, and ML/MP bootstrap values >50 are shown below. Collector number is after the binomials, according to Table 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.

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

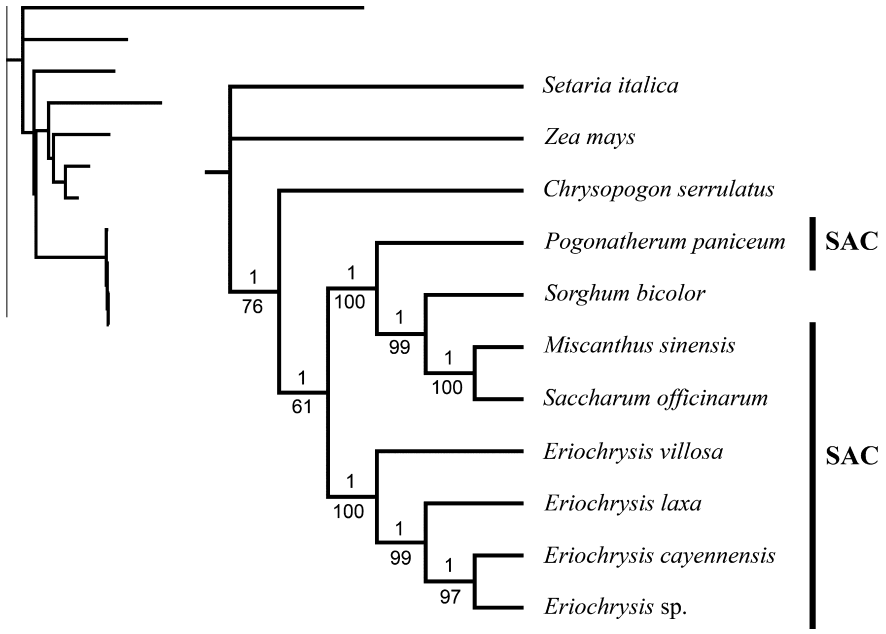

Fig. 5. Bayesian phylogeny of *Eriochrysis* and other genera of Andropogoneae based on whole chloroplast sequences (the same tree shown as phylogram is presented on the left). Bayesian PP > 0.85 are shown above branches, and ML bootstrap values >50 are shown below. Representatives from subtribe Saccharinae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

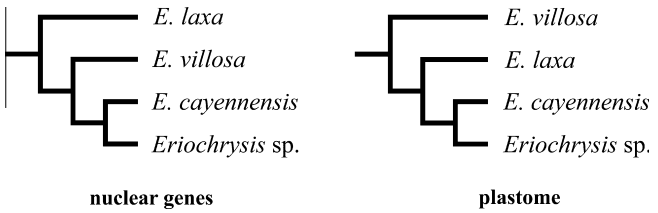

Fig. 6. Representation of the incongruence between nuclear and plastome trees concerning the phylogenetic position of *Eriochrysis cayennensis*, *E. laxa*, and *E. villosa*.

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 *E. 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 lower glume with a 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. 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 Figs. 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 are 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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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.02.022.

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