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Phylogenetic relationships within Pappophoreae s.l. (Poaceae: Chloridoideae): Additional evidence based on ITS and *trnL-F* sequence data

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Abstract

Historically, Pappophoreae included the genera *Cottea*, *Enneapogon*, *Kaokochloa*, *Pappophorum* and *Schmidtia*. Some authors consider this tribe as a well-supported monophyletic group; while other evidences reveals Pappophoreae as polyphyletic, with *Pappophorum* separated from the rest of the tribe. When the latter happens, it can form a clade with *Tridens flavus*. Molecular phylogenetic analyses of the subfamily Chloridoideae have included few species of Pappophoreae; therefore, further research involving more representatives of this tribe is needed. With the aim of providing new evidence to help clarify the phylogenetic position of *Pappophorum* and its relationships with other genera of the tribe and the subfamily Chloridoideae, eight new sequences of ITS and *trnL-F* regions of Pappophoreae species were generated. These sequences were analyzed together with other available sequence data obtained from GenBank, using maximum parsimony and Bayesian inference, for individual (*trnL-F* or ITS) or combined *trnL-F*/ITS data sets. All analyses reveal that Pappophoreae is polyphyletic, with *Pappophorum* separated from the rest of the tribe forming a well-supported clade sister to *Tridens flavus*.

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1. Introduction

The tribe Pappophoreae s.l. comprises five genera (Nicora and Rúgolo de Agrasar, 1987), of which *Cottea* Kunth (1 specie) and *Pappophorum* Schreber (8 species) are American; *Kaokochloa* Winter (1 specie) and *Schmidtia* Steud. (2 species) are African; and *Enneapogon* Desv. ex P. Beauv. (30 species), with only one specie distributed worldwide, is mainly widespread in tropical and subtropical regions of Africa, Asia and Australia (Van den Borre and Watson, 1997; Watson and Dallwitz, 1994).

Although some authors consider this tribe as a well-supported monophyletic group (Roodt-Wilding and Spies, 2006; Van den Borre and Watson, 1997), other evidences reveal that Pappo-

phoreae is polyphyletic within subfamily Chloridoideae (Columbus et al., 2007; GPWG, 2001; Hilu and Alice, 2001; Ingram and Doyle, 2007).

Characters such as many-nerved glumes, scabrous rachilla, many-awned and many-nerved lemmas, and the presence of elongated, bulbous-tip microhairs have been used to delimit Pappophoreae (Clayton and Renvoize, 1986; Renvoize, 1985). However, although included in the tribe, *Pappophorum* appears as an atypical genus in which distinctive microhairs are absent and glumes are 1-nerved instead of having several nerves (Reeder, 1965). Based on these characters, Reeder (1965) divided the tribe Pappophoreae in two subtribes: Cotteinae to include *Cottea*, *Enneapogon*, *Kaokochloa* and *Schmidtia*, and Pappophorinae, in which he placed *Pappophorum*.

Although this division is not reflected in other studies involving Pappophoreae (Van den Borre and Watson, 1997), the differentiation between Cotteinae (sensu Reeder, 1965) and Pappophorinae (*Pappophorum*) was pointed out by Tivano and

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Vegetti (2004) based on morphology, and by Hilu and Alice (2001), Columbus et al. (2007), Ingram and Doyle (2007), and Peterson et al. (2010) based on molecular phylogenetic studies. In the molecular studies, the tribe Pappophoreae always appeared as polyphyletic, although inconsistencies were only found in the location of *Pappophorum*, while the remaining taxa of the tribe were always gathered in a monophyletic group. *Pappophorum vaginatum* Buckley and *Tridens flavus* were paired as a well-supported clade (Columbus et al., 2007); or *Pappophorum pappiferum* (Lam.) O. Kuntze was placed with *Neesiochloa barbata* (Nees) Pilg. by Peterson et al. (2010). Hilu and Alice (2001) placed it within a clade of *Eragrostis* species but this is undoubtedly the result of a misidentified sample. Based on *rps16* and GBSSI (*waxy*) sequence data, Ingram and Doyle (2007) provided additional evidence supporting species of *Pappophorum* separated from *Eragrostis*.

While molecular phylogenetic analyses of the subfamily Chloridoideae included a few representatives of Pappophoreae (Columbus et al., 2007; Hilu and Alice, 2000, 2001; Ingram and Doyle, 2007; Peterson et al., 2010; Roodt-Wilding and Spies, 2006), further research involving more representatives of this tribe is needed. In this study, we sequenced the nuclear ribosomal internal transcribed spacer region (ITS1+5.8 S+ITS2) and a chloroplast region comprising the *trnL* intron, the *trnL* partial exon, plus the intergenic spacer between *trnL* and *trnF* genes (*trnL-F*) for eight taxa including species of *Cottea*, *Pappophorum* and *Enneapogon*. The aim of this study is to clarify the phylogenetic position of *Pappophorum* and its relationships with other genera in the subfamily Chloridoideae.

2. Materials and methods

Fresh material was collected and preserved in silica gel or, in a few cases, plant material was obtained from herbarium specimens. We sequenced the *trnL-F*/ITS regions of one species of *Cottea*, three species of *Enneapogon* and three species of *Pappophorum*. *Kaokochloa* was not included in this study due to lack of samples. All sequences were analyzed together with other sequences of Pappophoreae available from GenBank (Appendix A). A total of 12 species belonging to four out of the five genera of the tribe Pappophoreae were included in the analyses. Additionally, sequences from 38 genera of the subfamily Chloridoideae were incorporated to test the monophyly of the tribe [Columbus et al., 2007; Roodt-Wilding and Spies, 2006 (Appendix A)]. As outgroup, we used the same taxa used by Columbus et al. (2007).

2.1. DNA extraction, PCR amplification and sequencing

Silica-gel-dried leaves and herbarium samples were ground in liquid nitrogen. From silica-dried material, DNA was isolated using modified CTAB protocols as described in Giussani et al. (2001). From samples taken from herbarium specimens, DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's

instructions. The ITS region was amplified by polymerase chain reaction (PCR), using the primers "ITS-5" and "ITS-4" of White et al. (1990). The PCR amplification was performed in a 25 µl reaction with 50 and 100 ng of DNA, 1.25–3 units of Taq polymerase (Invitrogen Life Technologies, São Paulo, Brazil), a final concentration of 1× PCR buffer minus Mg, 5 mM MgCl₂, 0.025 mM dNTP each, 0.24 µM of each primer, and 0.5× Q of the Taq PCR Core Kit (Qiagen) used as a PCR additive. PCR was carried out using the following parameters for the ITS: 1 cycle of 94 °C for 5 min, 39 cycles of 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1 min 30 s, and a final extension cycle of 72 °C for 10 min. The *trnL-F* region was PCR amplified using the primers C and F as described in Taberlet et al. (1991). Amplification conditions were similar to those used for the ITS region, except that 0.8X DMSO was used instead of the Q additive. PCR parameters for the *trnL-F* were similar to those of ITS, only the number of cycles was reduced to 34, and the final extension to 7 min. From *Enneapogon cylindricus*, in which primers C and F failed to amplify, the *trnL-F* region was amplified in two fragments using primers D and E of Taberlet et al. (1991), in combination with primers Cii and Fdw of Giussani et al. (2009), respectively. In order to test the concentration and quality of the amplified DNA, PCR products were electrophoresed using a 1% agarose gel in a 1× TBE buffer and visualized under UV light. Sequencing reactions were performed by MacroGen Inc. using the ABI PRISM BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, Korea), following the protocols supplied by the manufacturer. Sequencing primers were similar to the amplification ones.

2.2. Data analyses

Sequenced fragments were assembled and edited using Chromas Pro ver. 1.34 (Technelysium Pty, Ltd, Tewantin, Australia). The alignments were constructed with CLUSTAL W and then manually corrected using BioEdit version 7.0.5.3 (Hall, 1999). Sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>); GenBank accession numbers are provided in Appendix A. Alignments and phylogenetic trees were submitted to TreeBASE: Journal Peer Reviewer's PIN Number: XXXX).

Phylogenetic analyses were conducted for individual (*trnL-F* or ITS) or combined *trnL-F*/ITS data sets under Maximum Parsimony (MP), as implemented in TNT ver. 1.1 (Goloboff et al., 2003). All characters were equally weighted, treated as unordered, and gaps were scored as missing data. Prior to heuristic searches, all uninformative characters were deactivated. The searches involved 1000 replicates, each of which generated a Wagner tree using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection) and retaining a maximum of 2 trees in each replicate. Subsequently, all optimal trees were swapped using TBR, holding a maximum of 10,000 trees, except for the analysis of *trnL-F* sequences, which required holding 20,000 trees in memory.

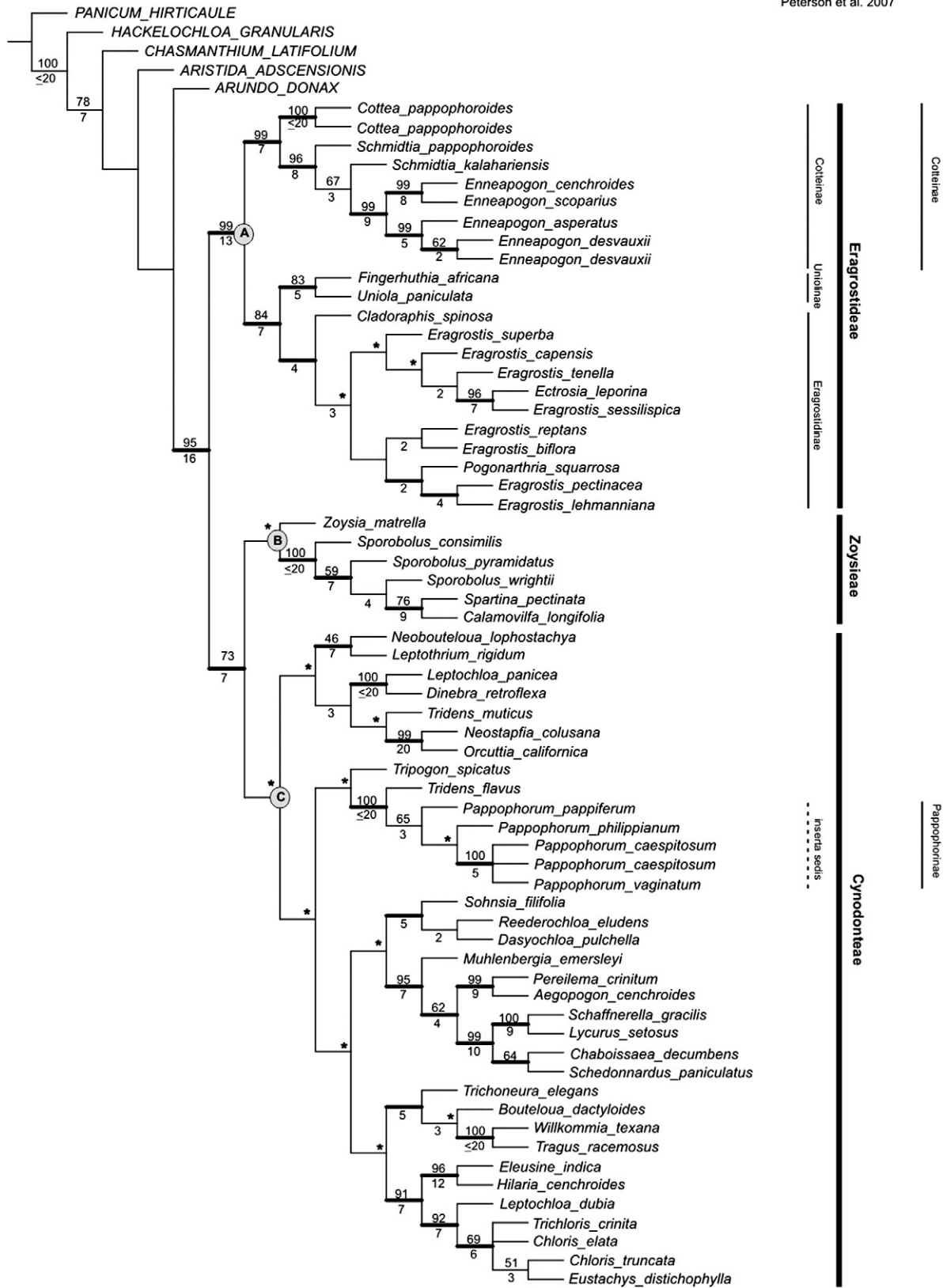
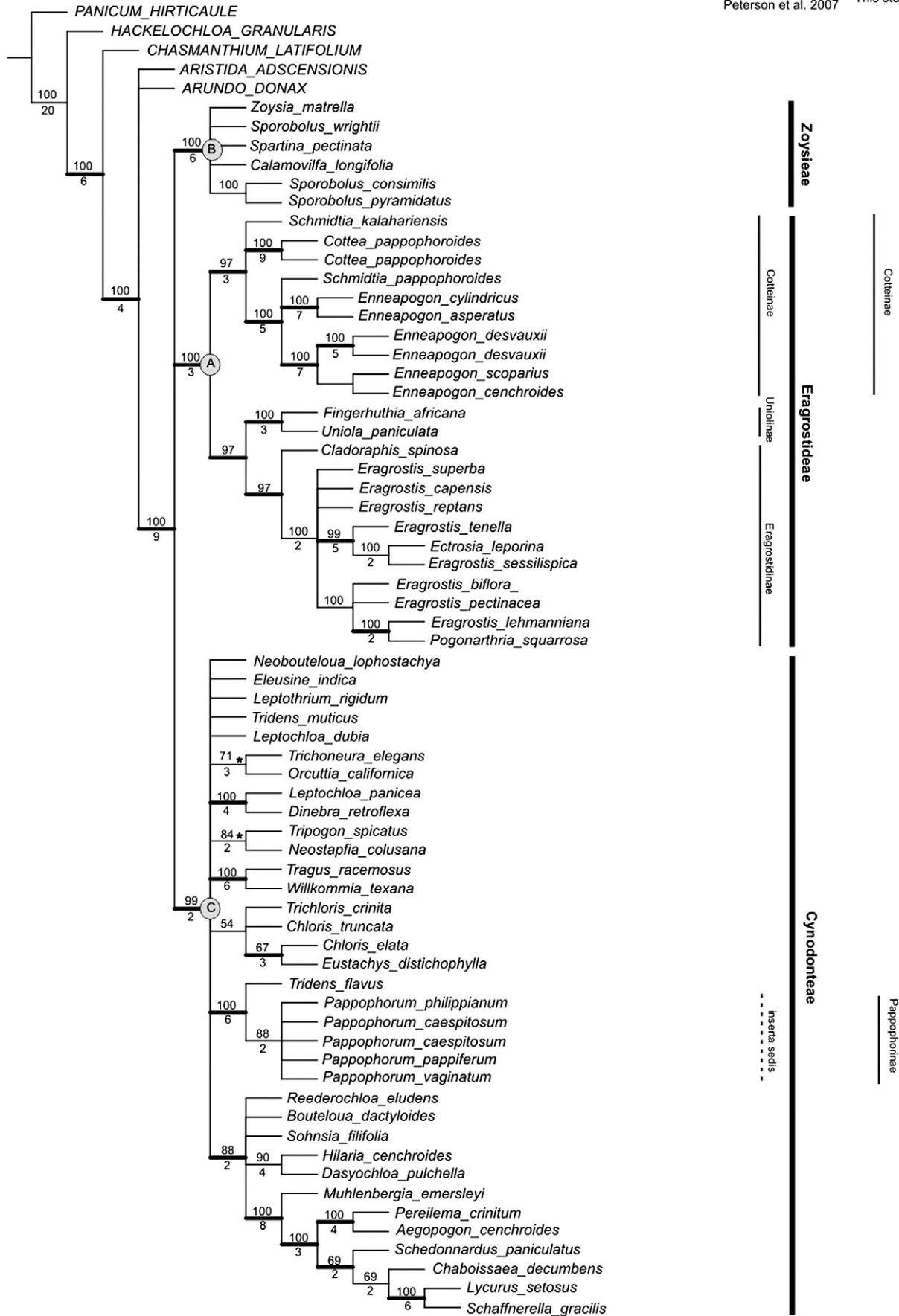


Fig. 1. Strict consensus of the ITS data set, produced from 2 most-parsimonious trees with a length of 2542, CI of 0.317 and RI of 0.613. Numbers above and below branches are bootstrap percentages ($>50\%$) and Bremer values (>2), respectively. The thick branches represent $\geq 94\%$ Bayesian posterior probability, and "*" indicates the nodes not found in the majority rule consensus tree obtained using Bayesian inference. Letters A, B and C represent the three clades observed in Hilu and Alice (2001) and Columbus et al. (2007) for Chloridoideae. The bars indicate the tribes and subtribes recognized by Columbus et al. (2007) and Peterson et al. (2007), and the subtribes supported in this work, as discussed in the text.



To evaluate the relative support for individual clades, bootstrap analysis (Felsenstein, 1985) was performed using a total of 10,000 replicates. Each replicate was analyzed using a Wagner tree as a starting point followed by TBR branch-swapping, saving only one tree per replicate. Bootstrap values are reported over 50%. Absolute Bremer support values (Bremer, 1994) were also calculated searching sub-optimal trees of 1, 2, 3 and up to 20 steps longer than the shortest trees, saving a maximum of 10,000 trees in each step.

We also conducted a phylogenetic analysis under Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), for *trnL-F* and ITS partitions, and for the combined *trnL-F*/ITS data sets. The optimal evolutionary model was chosen using the Akaike Information Criterion (AIC) as implemented in jModeltest 0.1 (Posada, 2008). For the combined data set (*trnL-F*/ITS) and for the ITS partition, GTR+I+G model was selected by the AIC, and TVM+I+G for the *trnL-F* partition; both models were set as Nst=6 for MrBayes. For the three data sets, we ran four chains of the Markov Chain Monte Carlo, for 1 million generations, and one tree per 100 generations was sampled in two independent runs (four MCMC, nrun=2; chain temperature=0.1; sample frequency=100), until the standard deviation of the split frequencies of these two runs dropped below 0.01. We estimated the 50% majority rule consensus of the remaining trees and used posterior probability (PP) to evaluate nodal support. Nodes with PP values $\geq 94\%$ were considered well-supported.

The results of both, parsimony and inference phylogenetic methods within each data set, were similar; hence only trees obtained from MP analysis are presented here, and main differences as much as support and high probabilities as shown in the Bayesian results are indicated onto these figures.

3. Results

Sixty-eight aligned sequences of the entire ITS region of 68 specimens (63 ingroup and 5 outgroup specimens) resulted in 675 characters, of which 373 (55.25%) were phylogenetically informative. Parsimony analyses yielded 2 equally parsimonious trees with a length of 2542 (CI=0.317; RI=0.613). Fig. 1 shows the strict consensus tree.

Sixty-nine aligned sequences of the *trnL-F* region (64 ingroup and 5 outgroup specimens) contained 1383 positions, of which 168 (12.15%) were phylogenetically informative. The analyses produced 15360 shortest trees of 450 steps (CI=0.510; RI=0.725). The strict consensus tree was used to summarize the results (Fig. 2).

Although the *trnL-F* sequences are longer than the ITS ones, the proportion of informative characters was remarkably lower in the *trnL-F* data set. However, both sets of data

provided enough resolution among species and clades obtained from the ITS and *trnL-F* were largely congruent (Figs. 1 and 2). Major differences are due to a higher resolution of the ITS consensus tree, and the addition of the *trnL-F* sequence of *Enneapogon cylindricus*, which was included in the *Cottea-Enneapogon-Schmidtia* clade (but not considered in ITS). In the strict consensus of both markers analyzed separately, *Cottea-Enneapogon-Schmidtia* appears as a well-supported monophyletic group, while species of *Pappophorum* are separated from the rest of the tribe, although forming a well-supported clade with *T. flavus* (Figs. 1 and 2). Within the *Cottea-Enneapogon-Schmidtia* clade, the genus *Schmidtia* is paraphyletic in both analyses, while species of *Enneapogon* form a monophyletic group nested within *Schmidtia* (ITS), or included in a polytomy together with *Schmidtia pappophoroides* (*trnL-F*).

The combined data set (ITS+*trnL-F*) includes all specimens from both matrices except *Enneapogon cylindricus*, from which only *trnL-F* sequences were obtained. The combined data matrix consisted of 2058 characters of which 539 (26.19%) were phylogenetically informative. Parsimony analyses resulted in 12 equally parsimonious trees with a length of 3021 (CI=0.343; RI=0.625). Fig. 3 represents the strict consensus tree obtained under MP. Tribe Pappophoreae, as traditionally considered, is polyphyletic. The *Cottea-Enneapogon-Schmidtia* group appears as monophyletic but separated from the monophyletic *Pappophorum*; the latter closely related to *T. flavus* (Fig. 3). The combined analyses showed a highly resolved consensus tree with high support in most branches. Relationships among other members of the subfamily Chloridoideae are congruent with results by Hilu and Alice (2001), Columbus et al. (2007), and Peterson et al. (2010), hence the relationships are briefly discussed under the scope of this study.

4. Discussion

With regard to recent molecular phylogenies which comprise representatives of the tribe Pappophoreae s. l. in this work we add *Pappophorum* and *Enneapogon* species/sequences (Table 1). Our study agree in the delimitation of major clades within subfamily Chloridoideae (Columbus et al., 2007; Hilu and Alice, 2001; Peterson et al., 2010), being the three major clades “A”, “B” and “C” [corresponding to the Eragrostidae, Zoysieae and Cynodonteae tribes respectively, of Columbus et al. (2007) and Peterson et al. (2007)] also represented in our study (Figs. 1, 2 and 3). Members of the Triraphideae clade of Peterson et al. (2010) were not included in this work. In our study clade A is basal and sister to B+C for the ITS partition and ITS+*trnL-F* under MP and BI (Figs. 1

Fig. 2. Strict consensus of the *trnL-F* data set, produced from 15360 most-parsimonious trees with a length of 450, CI of 0.510 and RI of 0.725. Numbers above and below branches are bootstrap percentages ($>50\%$) and Bremer values (>2), respectively. The thick branches represent $\geq 94\%$ Bayesian posterior probability, and “*” indicates the nodes not found in the majority rule consensus tree obtained using Bayesian inference. Letters A, B and C represent the three clades observed in Hilu and Alice (2001) and Columbus et al. (2007) for Chloridoideae. The bars indicate the tribes and subtribes recognized by Columbus et al. (2007) and Peterson et al. (2007), and the subtribes supported in this work, as discussed in the text.



Table 1
Representatives of the tribe Pappophoreae s. l. included on recent molecular phylogenies.

| Reference | Data sets | Taxon |
|--------------------------------|--|--|
| GPWG (2001) | ndhF rbcL | <i>Pappophorum bicolor</i> E. Fourn. <i>Enneapogon scaber</i> Lehm. |
| Hilu and Alice (2001) | matK | <i>Cottea pappophoroides</i> Kunth <i>Enneapogon glaber</i> N.T. Burb. <i>Enneapogon scoparius</i> Stapf <i>Pappophorum bicolor</i> E. Fourn. <i>Schmidtia pappophoroides</i> Steud ex J.A. Schmidt |
| Roodt-Wilding and Spies (2006) | trnL-F and ITS | <i>Enneapogon cenchroides</i> (Roem. & Schult.) C.E. Hubb <i>Enneapogon scoparius</i> Stapf <i>Schmidtia pappophoroides</i> Steud <i>Schmidtia kalahariensis</i> Stent |
| Ingram and Doyle (2007) | rps16 and waxy | <i>Enneapogon scoparius</i> Stapf <i>Pappophorum bicolor</i> E. Fourn. <i>Pappophorum mucronulatum</i> Nees <i>Schmidtia pappophoroides</i> Steud ex J.A. Schmidt |
| Columbus et al. (2007) | trnL-F and ITS | <i>Cottea pappophoroides</i> Kunth <i>Enneapogon desvauxii</i> P. Beauv. <i>Pappophorum vaginatum</i> Buckley <i>Enneapogon desvauxii</i> P. Beauv. |
| Peterson et al. (2010) | ndhF, rpl32-trnL, rps16-trnK, rps3, rps16 intron, ndhA, ITS | <i>Cottea pappophoroides</i> Kunth <i>Pappophorum pappiferum</i> (Lam.) O.Kuntze <i>Enneapogon scoparius</i> Stapf <i>Enneapogon desvauxii</i> P. Beauv. <i>Enneapogon cylindricus</i> N.T. Burb. <i>Enneapogon cenchroides</i> (Roem. & Schult.) C.E. Hubb <i>Enneapogon asperatus</i> C.E. Hubb. <i>Pappophorum caespitosum</i> Fries R.E. <i>Pappophorum pappiferum</i> (Lam.) O.Kuntze <i>Pappophorum philippianum</i> Parodi <i>Pappophorum vaginatum</i> Buckley <i>Schmidtia pappophoroides</i> Steud. <i>Schmidtia kalahariensis</i> Stent |
| This study | trnL-F and ITS | <i>Cottea pappophoroides</i> Kunth <i>Enneapogon scoparius</i> Stapf <i>Enneapogon desvauxii</i> P. Beauv. <i>Enneapogon cylindricus</i> N.T. Burb. <i>Enneapogon cenchroides</i> (Roem. & Schult.) C.E. Hubb <i>Enneapogon asperatus</i> C.E. Hubb. <i>Pappophorum caespitosum</i> Fries R.E. <i>Pappophorum pappiferum</i> (Lam.) O.Kuntze <i>Pappophorum philippianum</i> Parodi <i>Pappophorum vaginatum</i> Buckley <i>Schmidtia pappophoroides</i> Steud. <i>Schmidtia kalahariensis</i> Stent |

and 3). Within clade A, two more clades appear in all analyses (Figs. 1, 2 and 3): one includes *Cottea pappophoroides* and the species of *Enneapogon* and *Schmidtia* (“Cotteinae clade”), and the other includes all the species of *Eragrostis*, with *Cladoraphis*, *Ectrosia*, *Fingerhuthia*, *Pogonarthia* and *Uniola* (“Eragrostidinae-Uniolinae clade”).

Within the “Cotteinae clade”, relationships are well-resolved and highly supported by ITS and combined analyses (Figs. 1 and 3). In these analyses, *Cottea pappophoroides* appears as sister to the rest of Cotteinae, the genus *Schmidtia* is paraphyletic and closely related to species of *Enneapogon*, while genus *Enneapogon* is monophyletic and well-supported. In the ITS and ITS+trnL-F analyses (Figs. 1 and 3), *E. asperatus* and *E. desvauxii* are closely related and may be supported by the presence of cleistogamy; cleistogamous

spikelets are present in the basal region of the plant (*E. desvauxii*) or cleistogamous inflorescences are hidden in the upper leaf sheaths (*E. asperatus*). When *E. cylindricus* is included in the analysis, (trnL-F tree, Fig. 2), *E. desvauxii* forms a clade with *E. scoparius* and *E. cenchroides*, while *E. asperatus* is sister to *E. cylindricus*, a species that bears cleistogamous spikelets near the base of the culms and has cleistogamous inflorescences hidden in the upper leaf sheaths.

Pappophorum is monophyletic in all analyses, and separate from the rest of Pappophoreae (Figs. 1, 2 and 3). As Columbus et al. (2007) have observed, *Pappophorum* is located in the so-called clade C, forming a well-supported monophyletic group with *T. flavus*. Although species of *Pappophorum* and *Tridens* bear hairs along the central and marginal nerves of the lemma

Fig. 3. Strict consensus of 12 most-parsimonious trees with a length of 3021, CI of 0.343 and RI of 0.625, based on the ITS+trnL-F data sets. Numbers above and below branches are bootstrap percentages (>50%) and Bremer values (>2), respectively. The thick branches represent $\geq 94\%$ Bayesian posterior probability. “*” Indicates the nodes not found in the majority rule consensus tree obtained using Bayesian inference; “-” indicates the supported clades in the Bayesian tree, absent from the MP tree. Letters A, B and C represent the three clades observed in Hilu and Alice (2001) and Columbus et al. (2007) for Chloridoideae. The bars indicate the tribes and subtribes recognized by Columbus et al. (2007) and Peterson et al. (2007), and the subtribes supported in this work, as discussed in the text.

(Columbus et al., 2007), both genera show significant differences in aspects related to glumes, lemmas, rachilla ends as well as the anatomical leaf characters (Watson and Dallwitz, 1994). When excluding *T. flavus* from the analyses (data not shown), *Pappophorum* remains a monophyletic group within clade C, but no sister relationships can be identified. In Peterson et al. (2010) the alignment of Pappophorinae (*Pappophorum* and *Neesiochloa*) in the Cynodonteae is interesting since it appears as a grade between the Tripogoninae (includes *Tripogon* and *Eragrostiella*) and incertae sedis genera consisting of *Dignathia*–*Gymnopogon* sister to *Mosdenia*–*Perotis* in the ITS derived phylogram; and appears as a grade between the Tripogoninae (includes *Melanocenchris*, *Tripogon*, and *Eragrostiella*) and the Tragineae (includes *Monelytrum*, *Polevansia*, *Tragus* and *Willkommia*). The only species of *Tridens* [*T. muticus* (Torr.) Nash] investigated by Peterson et al. (2010) appears in a moderately supported Tridentinae clade with *Gouinia*, *Triplasis* and *Vaseyochloa*. *Gouinia*, *Tridens muticus*, *Triplasis*, and *Vaseyochloa* were also found in moderately supported clade in the combined *trnL*-F-ITS tree in Columbus et al. (2007). The association of *T. flavus* with *Pappophorum* should be further confirmed with additional accessions in future studies.

Contrary to observations made by Hilu and Alice (2000 and 2001) that *Pappophorum bicolor* is aligned within an *Eragrostis* clade, no species of *Pappophorum* in our study appears to be related to species of *Eragrostis*. There are serious suspicions that the *matK* sequence of *Pappophorum bicolor* used by Hilu and Alice (2000 and 2001) is misidentified or misplaced with other *Eragrostis* spp. (Ingram and Doyle, 2007). Analyses carried out with *rps16* and *waxy* sequences placed *Pappophorum bicolor* sister to *P. mucrunulatum*, separated from *Eragrostis* (Ingram and Doyle, 2007).

Within the *T. flavus*–*Pappophorum* clade, analyses based on ITS and ITS+*trnL*-F (Figs. 1 and 3) show *Pappophorum pappiferum* and *P. philippianum* sister to a monophyletic group consisting of *P. caespitosum* and *P. vaginatum*. In *Pappophorum pappiferum* fewer number of awns originating from the lemmas than other species in the genus, while in *P. philippianum* the lemma is larger than 3 mm in length, with its inner surface pubescent or densely pubescent near the apex. In *P. caespitosum* and *P. vaginatum* the lemma body is smaller than 3 mm long, with its inner surface scabrous or slightly scabrous near the apex (Pensiero, 1986).

4.1. Taxonomic treatment of the tribe Pappophoreae s. l.

According to phylogenetic analyses, some uncertainty exists concerning the taxonomic status of the Pappophoreae. According Reeder (1965), *Pappophorum* unique when compared to other genera of the tribe because it lacks the typical pappophoroid leaf epidermal microhairs, it possesses 1-nerved instead of multi-nerved glumes, and the margins of the embryonic leaf do not overlap, whereas in the other genera, the embryonic leaf has distinctly overlapping margins. Based on these differences, Reeder (1965) distinguished two sub-

tribes: Cotteinae to include *Cottea*, *Enneapogon*, *Kaokochloa* and *Schmidtia* and Pappophorinae, with *Pappophorum*.

Peterson et al. (2007), based on previous morphological and molecular studies, and primarily based on clades A, B, and C from Columbus et al. (2007), proposed changes to the classification of Chloridoideae recognizing three tribes: Eragrostideae, Zoysieae and Cynodonteae, respectively. Peterson et al. (2007) included *Cottea* and *Enneapogon* [*Schmidtia* is included in the Cotteinae in Peterson et al. (2010)] in subtribe Cotteinae (tribe Eragrostideae); leaving *Pappophorum* (the type genus of the tribe) as insertae sedis together with other 24 genera with unknown affinities, within tribe Cynodonteae. In Peterson et al. (2010), the Pappophorinae (including *Neesiochloa* and *Pappophorum*) is firmly embedded in the tribe Cynodonteae with equivocal alignment. Based on a larger sampling of *Pappophorum* and in the light recent of molecular phylogenetic evidence, we support the division of Pappophoreae s. l., maintaining the subtribe Pappophorinae in the tribe Cynodonteae, and subtribe Cotteinae in the tribe Eragrostideae. Additionally, data from the geographical location, is consistent with the dissolution of Pappophoreae s. l. in: a) a group of species probably originated in Africa or Australia (with the exception of *Cottea pappophoroides*) corresponding to the subtribe Cotteinae; and b) a group of exclusively American species possibly derived from the above, and included in the subtribe Pappophorinae (Peterson et al., 2010; Watson and Dallwitz, 1994). On the other hand, Cytology data do not provide evidence to support the distinction between Cotteinae and Pappophorinae; $x=10$ base chromosome number is common in all chloridoids, including *Pappophorum* (Peterson et al., 2010). A reduction of the base chromosome number can be found in *Enneapogon* ($x=9, 10$) and *Schmidtia* $x=9$, increasing to $x=11$ in *Kaokochloa* (Reeder and Singh, 1968; Watson and Dallwitz, 1994), all these numbers being derived states within major clades. Diploids were found in *Cottea*, *Enneapogon*, and *Kaokochloa*, although polyploidy is also frequent within Chloridoideae: *Pappophorum* with 4, 6 and 10-ploid (Watson and Dallwitz, 1994), *Enneapogon* with 4-ploid, and *Schmidtia* being 4-ploid (Reeder and Singh, 1968; Watson and Dallwitz, 1994). Evidences for hybridization or autopolyploidy should be further investigated for the group.

The position of *Pappophorum* as sister to *T. flavus* should be further investigated by incorporating other vouchers of *Tridens*. In addition, *Kaokochloa* has not been included in molecular analyses and its relationship to other genera in the Cotteinae is still in question (Peterson et al., 2010).

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Appendix A

| Taxon | Voucher | Source | Genbank accession number | |
|--|------------------------|--------------------------------|--------------------------|----------|
| | | | trnL-F | ITS |
| <i>Cottea pappophoroides</i> Kunth | Tivano et al. 782 (SF) | This study | xxxxxxx | xxxxxxx |
| <i>Enneapogon asperatus</i> C.E.Hubb. | Lazarides 4049 (CANB) | This study | xxxxxxx | xxxxxxx |
| <i>Enneapogon cylindricus</i> N.T.Burb. | R.A. Perry 3265 (CANB) | This study | xxxxxxx | – |
| <i>Enneapogon desvauxii</i> P. Beauv. | Tivano et al. 780 (SF) | This study | xxxxxxx | xxxxxxx |
| <i>Pappophorum caespitosum</i> Fries R.E. | Tivano et al. 797 (SF) | This study | xxxxxxx | xxxxxxx |
| <i>Pappophorum caespitosum</i> Fries R.E. | Tivano et al. 801 (SF) | This study | xxxxxxx | xxxxxxx |
| <i>Pappophorum pappiferum</i> (Lam.) O.Kuntze | Tivano et al. 776 (SF) | This study | xxxxxxx | xxxxxxx |
| <i>Pappophorum philippianum</i> Parodi | Tivano et al. 799 (SF) | This study | xxxxxxx | xxxxxxx |
| <i>Cladoraphis spinosa</i> (L.f.) S.M. Phillips | Spies 6335 | Roodt-Wilding and Spies (2006) | DQ655889 | DQ655833 |
| <i>Enneapogon cenchroides</i> (Roem. & Schult.) C.E.Hubb | Spies 7506 | Roodt-Wilding and Spies (2006) | DQ655894 | DQ655841 |
| <i>Enneapogon scoparius</i> Stapf | Spies 7487 | Roodt-Wilding and Spies (2006) | DQ655895 | DQ655843 |
| <i>Eragrostis biflora</i> Hack. ex Schinz | Spies 6705 | Roodt-Wilding and Spies (2006) | DQ655879 | DQ655824 |
| <i>Eragrostis capensis</i> (Thunb.) Trin. | Spies 6551 | Roodt-Wilding and Spies (2006) | DQ655881 | DQ655826 |
| <i>Eragrostis lehmanniana</i> Nees var. <i>lehmanniana</i> | Swart 20 | Roodt-Wilding and Spies (2006) | DQ655875 | DQ655819 |
| <i>Eragrostis superba</i> Peyr. | Spies 7470 | Roodt-Wilding and Spies (2006) | DQ655880 | DQ655825 |
| <i>Schmidtia kalahariensis</i> Stent | Spies 7490 | Roodt-Wilding and Spies (2006) | DQ655897 | DQ655845 |
| <i>Schmidtia pappophoroides</i> Steud. | Spies 6334 | Roodt-Wilding and Spies (2006) | DQ655896 | DQ655844 |
| <i>Sporobolus consimilis</i> Fresen. | HJTV 9354 | Roodt-Wilding and Spies (2006) | DQ655900 | DQ655848 |
| <i>Aegopogon cenchroides</i> Humb. & Bonpl. Ex Wild. | 4380 | Columbus et al. (2007) | EF156669 | EF153020 |
| <i>Bouteloua dactyloides</i> (Nutt.) Columbus (syn. <i>Buchloë dactyloides</i> (Nutt.) Engelm) | 2329 | Columbus et al. (2007) | EF156675 | EF153026 |
| <i>Calamovilfa longifolia</i> (Hook.) Hack. Ex Scribn. & Southw. var. <i>longifolia</i> | 3917 | Columbus et al. (2007) | EF156677 | EF153028 |
| <i>Chaboissaea decumbens</i> (Swallen) Reeder & C. Reeder | 3653 | Columbus et al. (2007) | EF156678 | EF153029 |
| <i>Chloris elata</i> Desv. (syn. <i>C. dandyana</i> C. D. Adams) | 3068 | Columbus et al. (2007) | EF156680 | EF153031 |
| <i>Chloris truncata</i> R. Br. | 3203 | Columbus et al. (2007) | EF156681 | EF153032 |
| <i>Cottea pappophoroides</i> Kunth | 3183 | Columbus et al. (2007) | EF156682 | EF153033 |
| <i>Dasyochloa pulchella</i> (Kunth) Willd. ex Rydb. (syn. <i>Erioneuron pulchellum</i> (Kunth) Tateoka) | 2577 | Columbus et al. (2007) | EF156687 | EF153038 |
| <i>Dinebra retroflexa</i> (Vahl) Panz. var. <i>retroflexa</i> | Clarke s.n. | Columbus et al. (2007) | EF156688 | EF153039 |
| <i>Ectrosia leporina</i> R. Br. var. <i>leporina</i> | Bell 171 | Columbus et al. (2007) | EF156690 | EF153041 |
| <i>Eleusine indica</i> (L.) Gaertn. | 2875 | Columbus et al. (2007) | EF156691 | EF153042 |
| <i>Enneapogon desvauxii</i> P. Beauv. | 3133 | Columbus et al. (2007) | EF156692 | EF153043 |
| <i>Eragrostis amabilis</i> (L.) Wight & Arn. ex Nees (= <i>E. tenella</i>) | 4317 | Columbus et al. (2007) | EF156695 | EF153046 |
| <i>Eragrostis pectinacea</i> (Michx.) Nees var. <i>pectinacea</i> | 2704 | Columbus et al. (2007) | EF156696 | EF153047 |
| <i>Eragrostis sessilisipica</i> Buckley (syn. <i>Acamptocladus sessilisipicus</i> (Buckley) Nash) | 3328 | Columbus et al. (2007) | EF156698 | EF153049 |
| <i>Eustachys distichophylla</i> (Lag.) Nees | 3090 | Columbus et al. (2007) | EF156700 | EF153051 |
| <i>Fingerhuthia africana</i> Nees ex Lehm. 7207 (MO) | Snow and Burgoyne | Columbus et al. (2007) | EF156701 | EF153052 |
| <i>Hilaria cenchroides</i> Kunth | 3758 | Columbus et al. (2007) | EF156704 | EF153055 |
| <i>Leptochloa dubia</i> (Kunth) Nees (syn. <i>Diplachne dubia</i> (Kunth) Scribn.) | 3155 | Columbus et al. (2007) | EF156707 | EF153058 |
| <i>Leptochloa panicea</i> (Retz.) Ohwi subsp. <i>brachiata</i> (Steud.) N. W. Snow | 2700 | Columbus et al. (2007) | EF156709 | EF153060 |
| <i>Leptothrium rigidum</i> Kunth | 3429 | Columbus et al. (2007) | EF156710 | EF153061 |
| <i>Lycurus setosus</i> (Nutt.) C. Reeder | 3286 | Columbus et al. (2007) | EF156711 | EF153062 |
| <i>Muhlenbergia emersleyi</i> Vasey | 3275 | Columbus et al. (2007) | EF156715 | EF153066 |
| <i>Neeragrostis reptans</i> (Michx.) Nicora (= <i>Eragrostis reptans</i> (Michx.) Nees) | Hill 22450 | Columbus et al. (2007) | EF156697 | EF153048 |
| <i>Neobouteloua lophostachya</i> (Griseb.) Gould | 3144 | Columbus et al. (2007) | EF156719 | EF153070 |
| <i>Neostapfia colusana</i> (Burt Davy) Burt Davy | Reeder and Reeder 6198 | Columbus et al. (2007) | EF156720 | EF153071 |
| <i>Orcuttia californica</i> Vasey | 2687 | Columbus et al. (2007) | EF156721 | EF153072 |
| <i>Pappophorum vaginatum</i> Buckley | 2540 | Columbus et al. (2007) | EF156722 | EF153073 |
| <i>Pereilema crinitum</i> J. Presl | 3621 | Columbus et al. (2007) | EF156723 | EF153074 |
| <i>Pogonarthria squarrosa</i> (Roem. & Schult.) Pilg. | Snow et al. 7023 (MO) | Columbus et al. (2007) | EF156724 | EF153075 |
| <i>Reederochloa eludens</i> Soderstr. & H. F. Decker | Bell 250 | Columbus et al. (2007) | EF156726 | EF153077 |
| <i>Schaffnerella gracilis</i> (Benth.) Nash | 4040 | Columbus et al. (2007) | EF156727 | EF153078 |
| <i>Schedonnardus paniculatus</i> (Nutt.) Branner & Coville | Reeder and Reeder 9431 | Columbus et al. (2007) | EF156728 | EF153079 |

(continued on next page)

Appendix A (continued)

| Taxon | Voucher | Source | Genbank accession number | |
|---|---------|------------------------|--------------------------|----------|
| | | | <i>trnL-F</i> | ITS |
| <i>Sohnsia filifolia</i> (E. Fourn.) Airy Shaw | 4038 | Columbus et al. (2007) | EF156730 | EF153081 |
| <i>Spartina pectinata</i> Link | 3210 | Columbus et al. (2007) | EF156731 | EF153082 |
| <i>Sporobolus pyramidatus</i> (Lam.) Hitchc. | 4264 | Columbus et al. (2007) | EF156733 | EF153084 |
| <i>Sporobolus wrightii</i> Munro ex Scribn. | 2507 | Columbus et al. (2007) | EF156734 | EF153085 |
| <i>Tragus racemosus</i> (L.) All. | 2228 | Columbus et al. (2007) | EF156736 | EF153087 |
| <i>Trichloris crinita</i> (Lag.) Parodi | 3109 | Columbus et al. (2007) | EF156737 | EF153088 |
| <i>Trichoneura elegans</i> Swallen | 4299 | Columbus et al. (2007) | EF156738 | EF153089 |
| <i>Tridens flavus</i> (L.) Hitchc. var. <i>flavus</i> | 3212 | Columbus et al. (2007) | EF156739 | EF153090 |
| <i>Tridens muticus</i> (Torr.) Nash var. <i>muticus</i> | 3254 | Columbus et al. (2007) | EF156740 | EF153091 |
| <i>Tripogon spicatus</i> (Nees) Ekman | 3108 | Columbus et al. (2007) | EF156743 | EF153094 |
| <i>Uniola paniculata</i> L. | 4206 | Columbus et al. (2007) | EF156745 | EF153096 |
| <i>Willkommia texana</i> Hitchc. var. <i>texana</i> | 4143 | Columbus et al. (2007) | EF156747 | EF153098 |
| <i>Zoysia matrella</i> (L.) Merr. s.l. | 3985 | Columbus et al. (2007) | EF156748 | EF153099 |
| Outgroup | | | | |
| <i>Aristida adscensionis</i> L. | 2991 | Columbus et al. (2007) | DQ172196 | DQ171972 |
| <i>Arundo donax</i> L. | 3201 | Columbus et al. (2007) | DQ172302 | DQ172077 |
| <i>Chasmanthium latifolium</i> (Michx.) H. O. Yates | 3211 | Columbus et al. (2007) | DQ172304 | DQ172079 |
| <i>Hackelochloa granularis</i> (L.) Kuntze | 2624 | Columbus et al. (2007) | DQ172306 | DQ172081 |
| <i>Panicum hirticaule</i> J. Presl var. <i>hirticaule</i> | 2536 | Columbus et al. (2007) | DQ172307 | DQ172082 |

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