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Complex evolutionary history of two ecologically significant grass genera, *Themeda* and *Heteropogon* (Poaceae: Panicoideae: Andropogoneae)

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Running title: Evolution of *Themeda* and *Heteropogon*

ABSTRACT

Themeda and *Heteropogon* are closely related grass genera which are common and frequently dominant in tropical C_4 grasslands. Evolutionary relationships between these genera are poorly resolved, impeding ecological study, particularly of *Themeda triandra* with a broad distribution from Africa to East Asia, and *Heteropogon contortus* that have pantropical distributions. Our analyses of plastome and nuclear genomes with comprehensive sampling of *Themeda* and *Heteropogon* demonstrate that neither genus is monophyletic as currently circumscribed. Plastome and nuclear data place *H. melanocarpus* and *H. ritchiei* within *Themeda*. Nested within *Themeda triandra* is *T. quadrivalvis* and *T. unica* demonstrating this widespread species is more morphologically diverse than previously recognised. *Heteropogon fischerianus* is nested within *H. contortus*. The picture is more complex for *H. triticeus* that is sister to *H. contortus* in the nuclear analysis and sister to *Cymbopogon* in the plastome analysis. Taken together, the incongruence between nuclear and plastid phylogenies suggests recurrent hybridisation between potential genome donors related to *Cymbopogon* and *H. contortus*. Plastome dating estimates the *Themeda–Heteropogon* crown age at c. 7.6 Ma, in line with the Miocene C_4 grassland expansion, while the widespread *T. triandra* and *H. contortus* both diversified 1–2 Ma ago in the Pleistocene. These results establish a foundation for studying the history of these ecologically significant widespread grasses and the ecosystems they form.

ADDITIONAL KEYWORDS: Andropogoneae – Anthistiriinae – C_4 grassland expansion – dating – phylogenomics – plastomes – Pleistocene.

INTRODUCTION

The emergence of C_4 grasslands in the Late Miocene, between 3 and 8 Ma ago, is considered one of the major global vegetation transformations (Cerling *et al.*, 1997; Edwards *et al.*, 2010). This change was facilitated by adaptation of the dominant C_4 grasses to fire, grazing, lower precipitation, and increased seasonality (Strömberg, 2011; Linder *et al.*, 2018). In the Poaceae (grass) subfamily Panicoideae, diverse grasses from the C_4 tribe Andropogoneae form the main understorey in the tropical savannas of South America, Africa, and Asia (Arthan *et al.*, 2016; Lehmann *et al.*, 2019). The genera *Heteropogon* Pers. and *Themeda* Forssk. are two important grass genera that make a significant contribution to present-day ecosystems, dominating many ecosystems (Snyman, Ingram & Kirkman, 2013; Ratnam *et al.*, 2016; Fig. 1E–G). Due to the ecological importance of these genera, understanding their evolutionary history is crucial for documenting the origin and dynamics of the savanna biome in the tropics.

Heteropogon and *Themeda* were both placed in the Andropogoneae subtribe Anthistiriinae by Clayton & Renvoize (1986). Later, the Anthistiriinae were included in the subtribe Andropogoninae, based on molecular data (Kellogg, 2015; Soreng *et al.*, 2017). Currently, five *Heteropogon* and 29 *Themeda* species are accepted and distributed mainly in the tropics (Clayton *et al.*, 2006). Two species, *Heteropogon contortus* Pers. and *Themeda triandra* Forssk. are distributed throughout the tropics, and from Africa to East Asia, respectively, and are keystone species in many grasslands where they are found (Goergen & Daehler, 2001; Wang *et al.*, 2016). The remaining species have variable range distributions from restricted to regionally widespread, mainly distributed throughout South Asia, Southeast Asia and Australia (Deshpande, 1988; Veldkamp, 2016; POWO, 2019).

Heteropogon and *Themeda* are morphologically distinct, especially in inflorescence structure which has been used to defined them (Clayton & Renvoize, 1986; Fig. 1). Both genera have sterile homogamous spikelets in the lower part of the inflorescence and fertile spikelets in the upper part. In most species an appendage called an awn emerges from the fertile lemma (Clayton & Renvoize, 1986). The term "inflorescence" is used here informally to indicate a unit at the apex of a single flowering culm traditionally named a "raceme", even though developmentally the correct term for these complex structures is a synflorescence (Kellogg, 2015; Fig. 1A–D). All *Heteropogon* species have solitary inflorescences with awns converging and twisting around one another to form a group of stiff appendages. At least three or more pairs of well-developed homogamous spikelets appear in the lower two-thirds of the inflorescence (Deshpande, 1988). In *Themeda*, a single inflorescence is subtended by a spatheole (bract) and has a compact cluster of only two pairs of homogamous spikelets, a few fertile spikelet pairs and a triad, a group of one sessile and two pedicellate spikelets (Clayton & Renvoize, 1986). Some species of *Themeda* have an aggregation of several inflorescence branches which forms a more complex structure.

Heteropogon and *Themeda* are closely related, but to date their evolutionary relationships have been investigated with only limited taxon sampling. Early single-locus phylogenetic trees (ITS and *trn*L-F) placed *Heteropogon contortus* and *Themeda triandra* as sister groups (Skendzic, Columbus & Cerros-Tlatilpa, 2007; Teerawatananon, Jacobs & Hodkinson, 2011). The broadly sampled phylogenetic tree by GPWG II (2012) showed that *Heteropogon* and *Themeda* had strong affinities with the genus *Iseilema* Andersson, as might be expected given the morphological similarities between *Themeda* and *Iseilema*. Nuclear single-copy genes suggested that *Heteropogon* and *Themeda* are closely related to *Bothriochloa* Kuntze, *Capillipedium* Stapf and *Dichanthium* Willemet (the BCD clade; Bianconi *et al*., 2020; Estep *et al*., 2014; Welker *et al*., 2015). The first plastome analysis also retrieved the *Heteropogon–Themeda* sister relationship (Arthan *et al.*, 2017). The plastome analysis also revealed an unexpected position of *Heteropogon triticeus* (R. Br.) Stapf ex Craib close to *Cymbopogon flexuosus* (Nees) Will.Watson. Phylogenetic relationships within the genus *Themeda* were investigated by Dunning *et al.* (2017) who identified seven clades but did not fully sample *Heteropogon*. Dating analyses by Dunning *et al.* (2017) showed that *Themeda* originated around the same time as the global C_4 grassland expansion. They hypothesized that the pantropical species *T. triandra* dispersed from its southeast Asian origin during the last 1.5 Ma. Similar age estimates for the sister genus *Heteropogon*, and the likewise widespread *H. contortus*, are lacking.

Previous studies on *Themeda* and *Heteropogon* were based on limited species sampling was and in most cases limited genomic information. This hinders our ability to draw conclusions on the origin, diversification and polyphyly of these important grass genera. tThe aims of this study are: (1) to reconstruct the evolutionary history of *Heteropogon* and *Themeda* using both plastome and nuclear data; (2) to estimate divergence times among species of both genera; and (3) to discuss the taxonomic implications of the relationships.

MATERIALS AND METHODS

TAXON SAMPLING

Taxon sampling (Table 1) was based on previous phylogenetic studies (Estep *et al.*, 2014; Arthan *et al.*, 2017; Dunning *et al.*, 2017; McKain *et al.*, 2018) with further samples selected on the basis of published taxonomic and morphological data (Clayton & Renvoize, 1986; Kellogg, 2015). All accessible species of *Heteropogon* and *Themeda* were sampled; samples of *H. contortus* and *T. triandra* were selected to cover the whole of their distribution ranges.

Leaf material was collected from 59 herbarium specimens from the Royal Botanic Gardens, Kew (K), the Natural History Museum, London, UK (BM) and Chinese National Herbarium, Institute of Botany, Chinese Academy of Sciences (PE) and used to produce genome skims. Genome-skimming data for 13 accessions from Arthan *et al*. (2017) and Dunning *et al*. (2017) were also included in this study. Ingroups included 27 *Heteropogon* and 38 *Themeda* accessions, representing 5 and 14 accepted *Heteropogon* and *Themeda* species, respectively. Different accessions of *H. contortus* and *T. triandra* were sampled to cover their distribution ranges and morphological variation. We added 20 specimens from the closely related genera *Agenium* Nees, *Bothriochloa*, *Capillipedium*, *Cymbopogon* Spreng., *Dichanthium*, *Eremopogon* Stapf, *Iseilema* and *Pseudanthistiria* (Hack.) Hook.f. to test the monophyly of *Heteropogon* and *Themeda*. In the plastome analyses, other Andropogoneae besides these genera from GenBank were included (Table 1), and *Arundinella deppeana* Nees*, Garnotia tenella* (Arn. ex Miq.) Janowski and *G. thailandica* Gould were selected as the outgroup. For the nuclear analysis, only *A. deppeana* was available as outgroup.

SEQUENCING AND ASSEMBLY

Fifty-nine new genome-skimming datasets were generated (Table 1). DNA extraction was performed as described in Besnard *et al*. (2013). At least 100 ng of DNA per sample was sent to the Get-PlaGe core facility (Castanet-Tolosan, France). Libraries were prepared with the Truseq Nano DNA sample prep kit. Paired-end reads of 150 bp were produced by bridge amplification with the high-throughput Illumina HiSeq 3000 sequencing platform.

Plastome assembly was performed from genome-skimming data (0.85 to 5.85 Gb of 150 bp paired-end reads per sample), using the GetOrganelle version 1.6.4d tool (https://github.com/Kinggerm/GetOrganelle) (Jin *et al*., 2019) which used SPAdes

(Bankevich *et al.*, 2012), Bowtie2 (Langmead & Salzberg, 2012), BLAST+ (Camacho *et al.*, 2009) and Bandage (Wick *et al.*, 2015). GetOrganelle was run with a search for *k*-mers of 21, 45, 65, 85 and 105 bp and extending contigs for 15 rounds. Complete circular sequences were produced for all datasets except from *Themeda sabarimalayana* Sreek. & V.J.Nair due to low sequencing coverage (ca. 10x). All sequences for each accession were annotated using GeSeq (Tillich *et al.*, 2017) with reference plastomes of *C. flexuosus*, *H. contortus*, *Iseilema macratherum* Domin and *T. triandra* from NCBI. The gene arrangement of annotated plastomes was examined in Geneious R9 (Biomatters Ltd., Auckland). Plastomes were rearranged manually by using the reference plastomes as mentioned above. For *T. sabarimalayana*, partial plastid sequences were extracted from the reads by using methods described in Dunning *et al.* (2019). In short, the reads were mapped to a *T. triandra* reference plastome with Bowtie2 (Langmead & Salzberg, 2012), and filtered with SAMtools (Li *et al.*, 2009) to discard low quality reads ($Q < 20$). The resulting mpileup file was then used to manually call nucleotide positions using custom bash scripts from Olofsson *et al.* (2016). For each position the minimum read depth was three and the SNP call depth was two. All plastomes have been deposited in NCBI (accession numbers in Table 1).

NUCLEAR GENE EXTRACTION

The raw data for the genome-skimming datasets was then cleaned as in Dunning *et al.* (2019) before extracting nuclear genome information. In brief, NGS QC Toolkit v.2.3.3 (Patel & Jain, 2012) was used to remove sequencing adaptors, low quality reads (<80% of bases with $Q < 20$), reads with ambiguous bases and low-quality bases ($Q < 20$) from the 3’ end of reads. Bowtie2 v2.3.5.1 (Langmead & Salzberg, 2012) was then used to map the cleaned paired-end reads to the *Sorghum bicolor* reference genome obtained from Ensembl Plants

(Bolser *et al.*, 2017). For each sample, we then generated sequences for 3,269 single-copy genes identified in the *Sorghum* genome using BUSCO v.2 (Simão *et al.*, 2015). Finally, we extracted sequence information using the same methods as described above for the *T. sabarimalayana* plastid genome, removing sequences with <200 bp and discarding genes with <50% of samples represented. All raw sequence reads were deposited in the Sequence Read Archive (SRA) with accession numbers SAMN15683809–SAMN15683869.

ALIGNMENT AND PHYLOGENETIC ANALYSES

MAFFT v.7.402 (Katoh & Standley, 2013) was used through the CIPRES Science Gateway v. 3.3 (Miller, Pfeiffer & Schwartz, 2010) to align the set of 136 whole plastome sequences. jModelTest2 v. 2.1.6 (Darriba *et al.*, 2012) estimated GTR+Γ+I as the best-fit model of nucleotide substitution based on BIC. The plastid alignment was then analyzed with Maximum Likelihood (ML) and Bayesian Inference (BI) by using RAxML v. 8.2.12 (Stamatakis, 2014) and MrBayes v. 3.2.7a (Ronquist *et al.*, 2012), respectively. Under ML, 500 bootstrap pseudoreplicates were specified to test node support. For BI, two independent Markov Chain Monte Carlo runs with 4 chains, 50,000,000 generations each, 1,000 generation sampling and 10 percent burnin were set. Consensus trees were visualized with FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

The 2,464 single-copy nuclear genes were aligned in reference to the *Sorghum* genome and were subsequently analyzed using a coalescent based species tree approach. ML gene trees were generated using PhyML v.21031022 (Guindon & Gascuel, 2003) with 100 bootstrap pseudoreplicates, using the best substitution model as selected by Smart Model Selection SMS v.1.8.1 (Lefort, Longueville & Gascuel, 2017). Nodes with less than 10%

bootstrap support were collapsed prior to inferring a multigene coalescence species tree from the individual gene trees using ASTRAL-III v.5.6.2 (Zhang *et al.*, 2018).

DATING ANALYSIS

The plastome alignment was analyzed with BEAST2 v. 2.6.1 (Bouckaert *et al.*, 2014) in the CIPRES Science Gateway. Due to the scarcity of fossils in our group, we used divergence of *Zea mays* from the rest of Andropogoneae, estimated at 15.26 Ma by Christin *et al.* (2014) under a younger, non-phytolith scenario as calibration point, implemented as a normal prior with a standard deviation of 0.0001. A GTR+Γ model with 4 gamma categories, a relaxed log-normal clock (Drummond *et al.*, 2006), and a Yule model were set. Four independent runs with 100,000,000 generations and sampling every 1,000 generations were executed in BEAST2. The four runs were assessed for convergence and effective sample size (ESS) by Tracer v.1.7.1 (Rambaut *et al.*, 2018). ESS values for most parameters were >200. Tree files were combined using LogCombiner v.2.6.1 implemented in the BEAST2 package. The maximum clade credibility tree was searched with TreeAnnotator v.2.6.0 and annotated with median node heights, after discarding a burn-in of 40%.

RESULTS

PLASTOMES

The newly sequenced plastomes, including the first plastomes for *Agenium*, *Eremopogon* and *Pseudanthistiria,* range in length from 134,885 bp in a *H. contortus* accession to 141,765 bp in *Iseilema hubbardii* Uppuluri, with mean coverage ranging from 57.13 to 563.82 when excluding *T. sabarimalayana*. Inverted repeat (IR) boundaries are conserved across the dataset, i.e. *rps19-psbA* and *rpl22-rps19* for the IR–LSC and *rps15-ndhF* and *ndhH-rps15* for the IR–SSC boundary (Arthan *et al.*, 2017). For *T. sabarimalayana*, only 26,905 bp of plastome sequence could be assembled.

PHYLOGENETIC ANALYSES

The original alignment of 136 plastomes has a total length of 166,902 bp. After discarding one inverted repeat, the alignment has a final length of 137,619 bp, which includes 15.5% gaps and 0.6% Ns. Both ML and BI yielded nearly identical, well-resolved tree topologies as shown in Figs 2, S1, and S2. Bootstrap (BS) values of 100 and posterior probabilities (PP) of 1 supported most nodes, although low support was observed among some early diverging lineages, such as the split of the *Mnesithea* Kunth–*Eremochloa* Buse clade from the rest of Andropogoneae (BS = 66, PP = 0.95). Maximum support was observed where the DASH clade (an acronym for the clade comprising *Diheteropogon* (Hack.) Stapf, *Andropogon* L., *Schizachyrium* Nees and *Hyparrhenia* Andersson ex E.Fourn. (Arthan *et al.*, 2017) splits from the Anthistiriinae, Fig. 2). A clade of *Agenium*, *Bothriochloa*, *Capillipedium*, *Dichanthium*, *Eremopogon*, *Iseilema* and *Pseudanthistiria* was highly supported as the sister lineage of *Heteropogon* and *Themeda*.

Our study retrieved a close relationship between most species of *Heteropogon* and *Themeda*. *Heteropogon* is polyphyletic (Fig. 2). The sister relationship of *H. triticeus* and *Cymbopogon* (BS = 96, PP = 1) was recovered as in Arthan *et al.* (2017). *Heteropogon melanocarpus* (Elliott) Benth. is nested in *Themeda*; *H. ritchiei* (Hook.f.) Blatt. & McCann shows strong affinity with *T. huttonensis* Bor, *T. minor* L. Liu and *T. mooneyi* Bor. (BS = 96, PP = 0.97). *Heteropogon fischerianus* Bor is nested in the *H. contortus* clade (BS = 100, PP

= 1). *Heteropogon contortus* is sister to the *Themeda* clade (Fig. 2; BS = 85, PP = 0.99). Most relationships within *H. contortus* have short branch lengths and poor support.

Themeda is paraphyletic, including some nominal *Heteropogon* species. The tree topology conforms to that of Dunning *et al*. (2017). A *T. arundinacea* (Roxb.) A.Camus –*T. villosa* (Lam.) A.Camus clade is sister to the other *Themeda* species (Fig. 2; BS = 100, PP = 1) plus *H. melanocarpus*. Most relationships within *Themeda* are well resolved except some recent divergences within *T. triandra.*

For the nuclear coalescent analysis, 2,464 gene trees were used, most of which (2,460) have missing taxa. As taxon sampling of the nuclear tree was not the same as that of the plastome topology, only *Heteropogon* and *Themeda* and their closely related clades (e.g. *Iseilema* and BCD clades) can be compared (Fig. 3). Most parts agree with the plastome tree, but there are a few discordances. The sister relationship between *H. triticeus* and the *Cymbopogon* clade was not recovered in the nuclear tree, and *H. triticeus* was instead placed as sister to *H. contortus* with a local posterior probability (LPP) of 1, but low quartet support (QS) of 44.89 (only 44.89% of concordant gene tree quartets supporting the branch; Fig. 3). The coalescent analysis placed *H. ritchiei* with *H. melanocarpus* inside *Themeda* rather than as isolated lineage in *Themeda* in the plastome tree (Fig. 2). The nuclear tree also places *Themeda arguens* (L.) Hack. sister to *T. triandra* (LPP = 1 and QS = 55.58) while the plastome tree places *T. tremula* (Nees) Hack. as sister species (Fig. 2).

DATING ANALYSES

A maximum clade credibility tree of plastome data (Fig. 4) found a topology nearly identical to the ML and BI trees (Fig. 2). *Heteropogon triticeus* diverged from *Cymbopogon* at 8.84

Ma (95% highest posterior density interval: 7.4–10.3 Ma) and the divergence between *H. contortus* and *Themeda* occurred at approximately 7.6 (6.1–8.8) Ma. *Heteropogon contortus* populations started to diverge from 1.6 (1.1 –2.3) Ma. The *T. arundinacea–T. villosa* lineage diverged from other *Themeda* at 5.80 (4.8–7.2) Ma, but the split between the two species occurred very recently, at 0.2 (0.04–0.3) Ma. Among *T. triandra* accessions, African populations diverged from populations from Asia and Australia at 1.15 (0.82–1.58) Ma.

DISCUSSION

With comprehensive sampling of plastome and nuclear genome-wide trees, we show that *Themeda* and *Heteropogon*, as currently defined, are not monophyletic (Figs 2, 3). Both genera therefore need revision to represent natural groups. *Themeda* is paraphyletic but forms a monophyletic taxon supported by both plastome and nuclear data if *H. ritchiei* and *H. melanocarpus* are included. *Heteropogon* is clearly polyphyletic; in a strict sense, it would only include the type species *H. contortus* and – pending further study – *H. triticeus*.

The position of *H. ritchiei* differs between the nuclear and plastome trees, although there is nuclear and plastome agreement that *H. melanocarpus* and *H. ritchiei* are embedded within *Themeda*. The result is a morphologically diverse group: both *H. melanocarpus* and *H. ritchiei* have *Heteropogon*-type inflorescences, in which 2 to 4 pairs of non-involucral homogamous spikelets are present. Such inflorescence structure is not seen in a typical *Themeda* species which have a strict arrangement of 2 pairs of involucral homogamous spikelets. However, the number of homogamous spikelets in both species is fewer than the one observed in other *Heteropogon* species (usually more than 4 pairs; Deshpande, 1988). A decrease in the number of homogamous spikelets may follow inflorescence reduction trends documented in the Poaceae (Vegetti & Anton, 1995) and may be an early stage of a transition towards a *Themeda*-type inflorescence. The arrangement of homogamous and fertile spikelets could become denser to form a *Themeda*-type inflorescence.

For *Themeda* as a whole, trends in the number of spikelet pairs can be interpreted. Only a triad is retained within the racemes of *T. arguens*, *T. quadrivalvis* (L.) Kuntze, *T. sabarimalayana*, *T. tremula* and *T. triandra*. This is also true for *T. novoguineensis* (Reeder) Jansen which Dunning *et al.* (2017) placed close to that clade. In contrast, species in the rest of the genus [e.g. *T. anathera* (Nees) Hack., *T. mooneyi* Bor and *T. strigosa* (Buch.-Ham. ex Hook.f.) A.Camus] produce at least one spikelet pair and a triad in a raceme. In some species, inflorescence branches are gathered in a cluster (in *T. triandra*), but in the others a solitary inflorescence is observed (in *T. huttonensis;* Clayton *et al.,* 2006; Veldkamp, 2016). Different combinations in the numbers of spikelet pairs, the presence of triads and the complexity of inflorescence architecture among species may reflect tradeoffs in the production of spikelets and the fitness of the plants (Valladares, Skillman & Pearcy, 2002). Increase in inflorescence branches in the species lacking spikelet pairs could compensate for the low numbers of bisexual spikelets. No explanation for a potential selective advantage of triads over spikelet pairs or vice versa has been proposed to date.

A novel result in this study is that *T. unica* S.L.Chen & T.D.Zhuang belongs to the *T. triandra* clade, in addition to two other morphologically similar species, *T. laxa* (Andersson) A.Camus and *T. quadrivalvis*. These names may need to be placed in synonymy under *T. triandra*, which our data show is more diverse morphologically than previously recognised. It has been documented that *T. quadrivalvis* and *T. triandra* form a single clade and species complex encompassing extensive genetic and morphological variation (Keir & Vogler, 2006). *Themeda quadrivalvis* lacks a spikelet pair within the raceme, which is also seen in *T. triandra,* and *T. unica* is distinct by its arrangement of homogamous spikelets on different levels (Chen & Phillips, 2006). Habit is another labile character (Heidel *et al.*, 2016): while *T.*

triandra is considered perennial, *T. quadrivalvis* is annual (Clayton *et al.,* 2006). In *Themeda*, annual habit is otherwise only known in *T. helferi* Hack., whose phylogenetic position is unknown. The closest relative of *T. triandra* differs between the nuclear (*T. arguens*) and plastome (*T. tremula*) trees, indicating possible hybridization before the origin of *T. triandra*.

Discordant positions of *H. triticeus* between nuclear and plastome trees make its classification difficult. It is sister to *Cymbopogon* in the plastome trees (Fig. 2) but sister to *H. contortus* in the nuclear trees (Fig. 3). We included multiple samples of both *H. triticeus* and *Cymbopogon* species, so the discrepancy is not due to a single aberrant individual or a mix-up of samples. Although the relationships of *H. triticeus* to other species are shown in the trees, a long branch and support did not fully support sister relationships between *H. triticeus* and *Cymbopogon* clade. In nuclear coalescent tree, although almost half of nuclear genes yielded the topology where *H. contortus* and *H. triticeus* are sister group, the alternative positions of *H. triticeus* were represented by the rest of the genes (Fig. 3; the node diverging *H. contortus* and *H. triticeus*). This inconsistency pointed out that *H. triticeus* could form its own lineage. Potentially, the discordance from the results suggested genome donors related to *Cymbopogon* and *H. contortus*. Furthermore, as the genes are orthologous, this phenomenon also implies that *H. triticeus* is of high polyploid origin as shown by at least three portions in the pie chart. Such situations can be observed throughout the coalescent tree among *H. contortus* and *T. triandra* clade. It is possible that *H. triticeus* acquired its plastid genome from the *Cymbopogon* lineage Allopolyploidy is another possible explanation as a high ploidy level in *H. triticeus* (2n = 60) has been documented (Tothill & Hacker, 1976). However, retracing the events that shaped this species' genome requires further investigation with more samples and appropriate analyses and markers (Estep *et al*., 2014). In particular, phasing paralagoous gene copies, which was not possible with our sequencing approach, will be critical. Pending such analyses and given its morphology close to that of *H. contortus*, *H. triticeus* should for the moment retained in *Heteropogon*.

Both nuclear and plastome trees place *H. fischerianus* within *H. contortus* (Figs 2, 3), which also is consistent with morphology. According to Deshpande (1988), distichous leaves (dense two-ranked phyllotaxy in one plane) are the only character that distinguish *H. fischerianus* from *H. contortus*. Even though the phyllotaxy is highly conserved across the Poaceae (Kellogg, 2000), intraspecific phyllotactic variation can emerge naturally via a single mutated gene (*abph1*) (Greyson & Walden, 1972; Jackson & Hake, 1999; Fleming, 2005). Therefore, *H. fischerianus* should be considered a synonym of *H. contortus.*

A final result with implications for taxonomy is the support – both with nuclear and plastome data – for a clade corresponding to the former subtribe Anthistiriinae *sensu* Clayton & Renvoize (1986), subsumed under Andropogoninae by Kellogg (2015) and Soreng *et al.* (2017). Although several genera (e.g. *Anadelphia* Hack., *Clausospicula* Lazarides, *Elymandra* Stapf, *Exotheca* Andersson, *Hyperthelia* Clayton, *Monocymbium* Stapf, and *Parahyparrhenia* A. Camus) were previously included in the subtribe Anthistiriinae (Clayton & Renvoize, 1986; Soreng et al., 2015), most of them were phylogenetically proven to belong to different lineages (Arthan *et al.*, 2017; McAllister *et al.*, 2018; Welker *et al.*, in press). Although type species of some genera have not been included in our analyses, we propose that an emended Anthistiriinae would be composed of the genera *Agenium*, *Bothriochloa*, *Capillipedium*, *Cymbopogon*, *Dichanthium*, *Eremopogon*, *Heteropogon*, *Iseilema*, *Pseudanthistiria*, and *Themeda*, separate from the subtribe Andropogoninae. Inflorescence structure and spikelet morphology vary across these genera with no clear synapomorphy for the clade. The presence of basal homogamous spikelets is common but this character also occurs in *Diheteropogon* and *Hyparrhenia* in subtribe Andropogoninae. Most genera of Anthistiriinae, except for members of *Cymbopogon*, only have a single raceme which may be quite short as in *Iseilema* and *Themeda*. At species level, phylogenetic relationships suggest possible taxonomic changes within *Heteropogon* and *Themeda*. *H. melanocarpus* and *H. ritchiei* could be renamed to *Themeda melanocarpa* and *Themeda ritchiei*, respectively. *H. fischerianus* should be merged into *H. contortus* as a synonym. In addition, *T. unica* and *T. quadrivalvis* could be reduced to varieties of *T. triandra*, as some morphological differences can be observed in inflorescence structure and spikelet indumentum.

The divergence time of the genus *Themeda* from *H. contortus* was estimated here at 7.56 Ma, at the start of the Miocene grassland expansion (Edwards *et al*., 2010). Speciation of *Themeda* started from 5.80 Ma in the Late Miocene until the early Pleistocene at approximately 2.40 Ma. The *T. arundinacea–T. villosa* clade diverged in the Late Miocene (Fig. 4). The latter is the only clade except *T. triandra* that is distributed in Southeast Asia, and the divergence of this lineage from the rest of *Themeda* (5.4 Ma) in Southeast Asia coincides with the development of savannas in the region (Ratnam *et al*., 2016). The divergence of the endemic Indian *Themeda* species *T. anathera* and *T. strigosa* in the Early Pliocene (Figs 2, 4) could be correlated with a shift from C_3 grasslands to monsoonal C_4 ones in Northern India during the Late Miocene (Quade *et al*., 1995; Srivastava *et al*., 2018).

The diversification of the widespread species *T. triandra* and *H. contortus* was here estimated to the Pleistocene. This supports the results of Dunning *et al*. (2017) for *T. triandra* and suggests that both species became widespread only after the Miocene grassland expansion. Aridification in the Plio-Pleistocene may have shaped the Old-World savannas (Kaya *et al*., 2018) and favoured the spread of these now common savanna grass species. Further phylogeographic inferences are not possible here due to the lack of resolution within species. Another challenge is the potential confounding effect of recent anthropogenic introductions (Oviedo Prieto *et al*., 2012; Clayton *et al*., 2016; Mata *et al*., 2018). A comparative, genome-wide population genetic study of both species would be highly informative.

CONCLUSIONS

Phylogenetic analysis of the nuclear and the plastid genome show that both *Themeda* and *Heteropogon*, as currently understood, are not monophyletic. *Themeda* includes *H. melanocarpus* and *H. ritchei*, while *Heteropogon* should be restricted to *H. contortus* (which includes *H. fischerianus*) and *H. triticeus*. The analysis also supports the recognition of subtribe Anthistiriinae. The *Themeda–Heteropogon* clade, in the strict sense, diversified during the Miocene grassland expansion, while the widespread *T. triandra* and *H. contortus* spread during the Pleistocene.

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TABLES

Table 1. List of GenBank plastome sequences and newly sequenced plastomes and nuclear data used in this study. P and N columns indicate that the accessions are present (✓) or absent (✗) in plastome (P) or nuclear (N) analysis.

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Agenium leptocladum (Hack.) Clayton	Paraguay	E. Hassler	12427	K	137,726	MT504963	This study	✓	✓
Agenium villosum (Nees) Pilg.	Bolivia	M. Nees & M. Saldias	36425	K	137,731	MT504964	This study	✓	✓
Andropogon abyssinicus R.Br. ex Fresen.	Kenya	R. Pasquet	1097	MO	138,738	NC035030	Arthan *et al.* (2017)	✓	✗

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
Andropogon burmanicus Bor	Thailand	W. Arthan	071	BKF	140,898	NC035038	Arthan *et al*. (2017)	✓	✗
Andropogon chinensis (Nees) Merr.	Thailand	W. Arthan	042	BKF	138,817	NC035012	Arthan *et al*. (2017)	✓	✗
Andropogon distachyos L.	Thailand	W. Arthan	050	BKF	139,098	NC035041	Arthan *et al.* (2017)	✓	✗
Andropogon fastigiatus Sw.	Thailand	W. Arthan	009	BKF	138,658	NC035010	Arthan *et al.* (2017)	✓	✗
Apluda mutica L.	Pakistan	E. A. Kellogg	PI219568	MO	140,299	NC036684	Gallaher *et al.* (2019)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Arthraxon hispidus (Thunb.) Makino	Thailand	W. Arthan	023	BKF	140,507	NC035048	Arthan *et al.* (2017)	✓	×
Arthraxon lanceolatus Hochst.	Thailand	W. Arthan	001	BKF	140,381	NC035017	Arthan *et al.* (2017)	✓	×
Arthraxon microphyllus Hochst.	Thailand	P. Traiperm	537	BKF	139,858	NC035050	Arthan *et al.* (2017)	✓	×
Arthraxon prionodes (Steud.) Dandy	China	E. A. Kellogg	PI 659331	MO	140,494	KY596138	Arthan *et al.* (2017)	×	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Arundinella deppeana	Mexico	L. Clark *et al.*	1680	XAL	140,804	NC030620	Burke *et al.* (2016)	✓	✓
Bothriochloa alta (Hitchc.) Henrard	USA	M. R. Duvall	s.n.	DEK	137,645	NC030621	Burke *et al.* (2016)	✓	✓
Bothriochloa decipiens (Hack.) C.E.Hubb.	Australia	E. A. Kellogg	PI239153	MO	138,381	NC040131	Burke *et al.* (2016)	✓	×
Capillipedium venustum (Thwaites) Bor	Unknown	Unknown	PI11713	Unknown	138,257	KU291493	Burke *et al.* (2016)	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Chrysopogon gryllus Trin.	Republic of Macedonia	E. A. Kellogg	PI 250984	A	140,034	NC035035	Arthan *et al.* (2017)	✓	✗
Chrysopogon orientalis A.Camus	Thailand	P. Traiperm	578	BKF	140,712	NC035047	Arthan *et al.* (2017)	✓	✗
Chrysopogon zizanioides (L.) Roberty	USA	E. A. Kellogg	Vet-MRL-001	MO	139,971	NC035034	Arthan *et al.* (2017)	✓	✗
Coix lacryma-jobi L.	Thailand	W. Arthan	072	BKF	140,863	KY596160	Arthan *et al.* (2017)	✓	✗
Cymbopogon citratus Stapf	Unknown	Jeff	90Cc	Unknown		-	Dunning *et al.* (2019)	✗	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Cymbopogon densiflorus Stapf	Tanzania	M. McCallum	T73	K	140,062	MT504965	This study	✓	✓
Cymbopogon flexuosus	Thailand	W. Arthan	027	BKF	139,715	NC035040	Arthan *et al.* (2017)	✓	✓
Cymbopogon obtectus S.T.Blake	Australia	M. Lazarides	4370	K	139,704	MT504966	This study	✓	✓
Cymbopogon pospischilii (K.Schum.) C.E.Hubb.	China	R. J. Soreng, P. M. Peterson & Sun Hung	5561	K	140,219	MT504967	This study	✓	✓

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
Cymbopogon schoenanthus Spreng.	Sudan	G. E. Wickens	Unknown	K	139,648	MT504968	This study	✓	✓
Cymbopogon sp.	Australia	E. Addicott	Strictland1; AL04	Unknown	-	-	Dunning *et al.* (2017)	×	✓
Dichanthelium acuminatum (Sw.) Gould & C.A.Clark	Canada	J. M. Saarela *et al.*	666	CAN	-	-	Burke *et al.* (2016)	×	✓
Dichanthium annulatum (Forssk.) Stapf	Unknown	Unknown	SUPG011	Unknown	136,805	NC042145	Bhatt & Thanker (unpublished)	✓	×

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Dichanthium sericeum (R. Br.) A. Camus	Thailand	P. Traiperm	571	BKF	138,271	KY596128	Arthan *et al*. (2017)	✓	✗
Diheteropogon amplectens var. *catangensis* (Chiov.) Clayton	Unknown	Unknown	PI12585	Unknown	139,700	KU291497	Burke *et al.* (2016)	✓	✓
Dimeria ornithopoda Trin.	Thailand	P. Traiperm	575	BKF	140,629	NC035020	Arthan *et al.* (2017)	✓	✗
Elymandra androphila Stapf	Liberia	J. G. Adam	24797	MO	139,731	MH181166	McAllister *et al.* (2018)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Elymandra subulata Jacq.-Fél.	Guinea	H. Jacques-Felix	7306	K	139,800	MH181214	McAllister *et al.* (2018)	✓	✗
Eremochloa ciliaris (L.) Merr.	Thailand	P. Traiperm	524	BKF	138,970	NC035028	Arthan *et al.* (2017)	✓	✗
Eremochloa eriopoda C.E.Hubb.	Thailand	P. Traiperm	591	BKF	138,995	NC035023	Arthan *et al.* (2017)	✓	✗
Eremopogon delavayi (Hack.) A.Camus	China	R. J. Soreng, P. M. Peterson & Sun Hung	5236	K	139,855	MT504969	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Eremopogon foveolatus Stapf	United Arab Emirates	T. M. Heller	Unknown	K	139,797	MT504970	This study	✓	✓
Eremopogon tuberculatus (Hack.) A.Camus	India	J. F. Duthie	10595	K	139,986	MT504971	This study	✓	✓
Eriochrysis cayennensis P. Beauv.	Brazil	C. A. D. Welker	365	ICN	140,426	NC029882	Welker *et al.* (2016)	✓	✗
Eriochrysis laxa Swallen	Brazil	C. A. D. Welker	489	ICN	140,135	NC029883	Welker *et al.* (2016)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Eriochrysis pallida Munro	Zambia	R. Pasquet	1162	MO	140,382	NC042751	Welker *et al.* (2019)	✓	✗
Eulalia aurea (Bory) Kunth	Unknown	Unknown	PI12153	Unknown	140,220	KU291499	Burke *et al.* (2016)	✓	✓
Eulalia contorta Kuntze	Thailand	P. Traiperm	573	BKF	140,738	NC035026	Arthan *et al.* (2017)	✓	✗
Eulalia siamensis Bor	Thailand	P. Traiperm	557	BKF	140,642	NC035031	Arthan *et al.* (2017)	✓	✗
Eulaliopsis binata (Retz.) C.E.Hubb.	Thailand	P. Traiperm	567	BKF	141,091	NC035049	Arthan *et al.* (2017)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Exotheca abyssinica Andersson	Kenya	R. Pasquet	1102	MO	139,230	MH181196	McAllister *et al.* (2018)	✓	✗
Garnotia tenella	Thailand	P. Traiperm	552	BKF	139,629	NC035051	Arthan *et al.* (2017)	✓	✗
Garnotia thailandica	Thailand	P. Traiperm	535	BKF	139,668	NC035042	Arthan *et al.* (2017)	✓	✗
Germainia capitata Balansa & Poitr	Thailand	W. Arthan	028	BKF	139,817	NC035046	Arthan *et al.* (2017)	✓	✗
Hemisorghum mekongense	Thailand	P. Traiperm	569	BKF	140,765	NC035022	Arthan *et al.* (2017)	✓	✗

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
(A.Camus) C.E.Hubb.									
Heteropogon contortus	Angola	T. Harris & F. Murray-Hudson	781	K	135,095	MT504972	This study	✓	✓
	Australia	M. Evans	3209	K	134,979	MT504973	This study	✓	✓
	Bolivia	J. R. I. Wood	10831	K	134,991	MT504974	This study	✓	✓
	China	R. J. Soreng, P. M. Peterson & Sun Hung	5724	K	134,979	MT504975	This study	✓	✓

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
	Eritrea	S. Edwards & G. E. Tewoldeberhan	3888	K	135,078	MT504976	This study	✓	✓
Heteropogon contortus	Haiti	H. H. Bartlett	17487	K	134,987	MT504978	This study	✓	✓
	Hawaii	Degener	33424	K	134,979	MT504977	This study	✓	✓
	India	R. P. Celarier	A-3230-I	K	135,112	MT504979	This study	✓	✓
	Italy	P. Marchi & M. Iberite	15808	BM	135,092	MT504980	This study	✓	✓
	Madagascar	O. P. Nanjarisoa *et al.*	166	K	134,988	MT504982	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
	Madagascar	C. L. Solofondranohatra *et al.*	102	K	134,885	MT504983	This study	✓	✓
	Mexico	R. V. Moran	7100	K	134,991	MT504984	This study	✓	✓
	New Caledonia	H. S. McKee	41421	K	134,980	MT504985	This study	✓	✓
Heteropogon contortus	Nicaragua	R. W. Pohl	12376	K	134,979	MT504986	This study	✓	✓
	Oman	P. N. Allison	28	K	135,001	MT504987	This study	✓	✓
	Pakistan	C. Wright	99	BM	134,988	MT504988	This study	✓	✓
	Paraguay	K. Fiebrig	5769	BM	134,979	MT504989	This study	✓	✓

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
	Sierra Leone	E. A. Cole	17	K	134,983	MT504990	This study	✓	✓
	South Africa (Johannesburg)	M. S. Vorontsova	2356	K	135,073	MT504992	This study	✓	✓
	South Africa (Kromdraai)	M. S. Vorontsova	2329	K	135,098	MT504993	This study	✓	✓
Heteropogon contortus	Sri Lanka	D. Clayton	5936	K	135,040	MT504981	This study	✓	✓
	Tanzania	P. J. Greenway & Kanuri	14454	K	134,988	MT504991	This study	✓	✓

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
	Thailand	W. Arthan	035	BKF	134,982	NC035027	Arthan *et al.* (2017)	✓	✓
Heteropogon fischerianus	India	E. T. Bourne	2099	K	135,014	MT504994	This study	✓	✓
Heteropogon melanocarpus	Ecuador	W. H. Camp	3206	K	139,830	MT504995	This study	✓	✓
Heteropogon melanocarpus	Oman	T. A. Cope	619	K	139,851	MT504996	This study	✓	✓
Heteropogon ritchiei	India	H. Santapau	22007	K	139,424	MT504997	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Heteropogon triticeus	Thailand	W. Arthan	034	BKF	140,120	KY596142	Arthan *et al.* (2017)	✓	✗
	Thailand	W. Arthan	017	BKF	140,046	KY596153	Arthan *et al.* (2017)	✓	✓
	Thailand	P. Traiperm	534	BKF	140,127	KY596159	Arthan *et al.* (2017)	✓	✗
Heteropogon triticeus	Thailand	W. Arthan	046	BKF	140,091	KY596176	Arthan *et al.* (2017)	✓	✗
	Australia	E Addicott	EPA3355	Unknown	138,972	KY707769	Dunning *et al.* (2017)	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Hyparrhenia diplandra (Hack.) Stapf	Thailand	W. Arthan	012	BKF	139,175	NC035037	Arthan *et al.* (2017)	✓	✗
Hyparrhenia rufa Stapf	Thailand	W. Arthan	045	BKF	139,269	NC035011	Arthan *et al.* (2017)	✓	✗
Hyparrhenia subplumosa Stapf	Unknown	Unknown	PI12665	Unknown	139,138	NC030625	Burke *et al.* (2016)	✓	✓
Imperata cylindrica (L.) Raeusch.	Unknown	S. V. Burke	21	DEK	140,831	KU291466	Burke *et al.* (2016)	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Ischaemum afrum (J.F.Gmel.) Dandy	South Africa	A. J. Oakes	PI364924	Unknown	141,097	KU291467	Burke *et al.* (2016)	✓	✓
Iseilema anthephoroides Hack.	India	U. Satyavathi	IAU5	K	139,777	MT504998	This study	✓	✓
Iseilema hubbardii	India	U. Satyavathi	IAU3	K	139,716	MT504999	This study	✓	✓
Iseilema laxum Hack.	India	L. J. G. van der Maesen	2833	K	139,580	MT50500	This study	✓	✓
Iseilema macratherum	Australia	C. Chapman	PI257760	Unknown	139,643	NC030611	Burke *et al.* (2016)	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Iseilema membranaceum (Lindl.) Domin	Australia	M. E. Trudgen & M. Trudgen	12750	K	139,582	MT505001	This study	✓	✓
	Australia	Jeff	07lm	Unknown	-	-	Dunning *et al.* (2019)	×	✓
Iseilema prostratum (L.) Andersson	India	V. R. K. Murty	H4043/66	K	139,806	MT505002	This study	✓	✓
*Iseilema vaginiflorum*Domin	Australia	M. L.	3131	K	139,635	MT505003	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Kerriochloa siamensis C.E.Hubb.	Thailand	P. Traiperm	580	BKF	138,335	NC035009	Arthan *et al.* (2017)	✓	✗
Miscanthus sinensis Andersson	Unknown	Unknown	Unknown	Unknown	141,372	NC028721	Nah *et al.* (2016)	✓	✗
Mnesithea helferi (Hook.f.) de Koning & Sosef	Thailand	P. Traiperm	574	BKF	140,801	NC035036	Arthan *et al.* (2017)	✓	✗
Monocymbium lanceolatum	Guinea	J. G. Adam	6762	K	139,056	MH181170	McAllister *et al.* (2018)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
C.E.Hubb. ex Hutch. & Dalziel									
Parahyparrhenia siamensis Clayton	Thailand	P. Traiperm	583	BKF	140,355	KY596155	Arthan *et al.* (2017)	✓	✗
Pogonatherum paniceum (Lam.) Hack.	USA (cultivated)	L. G. Clark	s.n.	MO	139,205	NC029881	Welker *et al.* (2016)	✓	✗
Polytoca digitata Druce	Thailand	W. Arthan	060	BKF	140,892	NC035044	Arthan *et al.* (2017)	✓	✗
Pseudanthistiria umbellata Hook.f.	Sri Lanka	G. Davidse	7828	K	137,900	MT505004	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Pseudosorghum fasciculare A.Camus	Thailand	W. Arthan	067	BKF	140,474	NC035024	Arthan *et al.* (2017)	✓	✗
Rottboellia cochinchinensis (Lour.) Clayton	USA	L. G. Clark *et al.*	1698	ISC	140,336	KU291481	Burke *et al.* (2016)	✓	✓
Saccharum officinarum L.	Indonesia	Unknown	IJ76-514	Unknown	141,176	NC035224	Evans & Joshi (2016)	✓	✗
Saccharum spontaneum L.	Malaysia	Unknown	SES234B	Unknown	141,168	NC034802	Evans & Joshi (2016)	✓	✗

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
Schizachyrium brevifolium (Sw.) Nees ex Buse	Thailand	W. Arthan	010	BKF	138,027	NC035013	Arthan *et al.* (2017)	✓	✗
Schizachyrium exile (Hochst.) Pilg.	Thailand	W. Arthan	047	BKF	138,519	NC035029	Arthan *et al.* (2017)	✓	✗
Schizachyrium imberbe (Hack.) A.Camus	Argentina	C. A. D. Welker & M. C. Peichoto	564	ICN	139,759	NC035045	Arthan *et al.* (2017)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Schizachyrium sanguineum (Retz.) Alston	Thailand	W. Arthan	022	BKF	139,272	NC035015	Arthan *et al.* (2017)	✓	✗
Schizachyrium scoparium (Michx.) Nash	USA	E. A. Kellogg	V46	MO	139,274	NC035032	Arthan *et al.* (2017)	✓	✗
Schizachyrium spicatum (Spreng.) Herter	Uruguay	C. A. D. Welker	627	ICN	139,205	NC035039	Arthan *et al.* (2017)	✓	✗
Schizachyrium tenerum Nees	Argentina	C. A. D. Welker & M. C. Peichoto	604	ICN	138,555	NC035043	Arthan *et al.* (2017)	✓	✗

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Sorghastrum nutans (L.) Nash	Unknown	W. P. Wysocki	s.n.	DEK	141,061	NC030498	Burke *et al.* (2016)	✓	✓
Sorghum bicolor (L.) Moench	Unknown	Unknown	BTx632	Unknown	140,754	NC008602	Saski *et al.* (2007)	✓	×
Themeda anathera	Nepal	Stainton *et al.*	Unknown	K	138,941	MT505005	This study	✓	✓
Themeda arguens	Australia	G. Chippendale	7795	K	138,790	MT505006	This study	✓	✓
Themeda arundinacea	Thailand	W. Arthan	064	BKF	139,742	NC035014	Arthan *et al.* (2017)	✓	✓
Themeda cymbaria Hack.	Sri Lanka	F. W. Gould	13491	K	139,036	MT505007	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Themeda huttonensis	India	N. L. Bor	6717	K	139,655	MT505008	This study	✓	✓
Themeda minor	China	Qinghai-Tibet Team	654	PE	139,649	MT505009	This study	✓	✓
Themeda mooneyi	India	H. F. Mooney	4034	K	139,865	MT505010	This study	✓	✓
Themeda quadrivalvis	Madagascar	M. S. Vorontsova	350	K	138,961	NC035492	Dunning *et al.* (2017)	✓	✓
Themeda sabarimalayana	India	K. M. Matthew & K. T. Mathew	51031	K	Partial plastome sequences	-	This study	✓	✓

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
Themeda strigosa	India	H. H. Haines	4514	K	139,085	MT505011	This study	✓	✓
Themeda strigosa	India	S. Mokim	1516b	K	-	-	This study	✗	✓
Themeda tremula	Sri Lanka	D. Clayton	5686	K	138,665	MT505012	This study	✓	✓
Themeda triandra	Angola	T. Harris	867	K	138,900	MT505013	This study	✓	✓
	Australia	E. Addicott	DCRYAN1218	unknown	-	-	Dunning *et al.* (2017)	✗	✓
	Australia	P. M. Peterson, R. J. Soreng & G. Rosenberg	14430	K	138,850	MT505014	This study	✓	✓

Species	Country	Collector name	Collector number	Herbarium	Plastome size (bp)	Plastome accession number	Reference	P	N
Themeda triandra	Australia	T. Handasyde	KununurraA	WA	-	-	Dunning *et al.* (2017)	×	✓
	China	R. J. Soreng, P. M. Peterson & Sun Hung	5205	K	-	-	This study	×	✓
	Ethiopia	H. F. Mooney	4844	K	138,816	MT505015	This study	✓	✓
	India	R. P. Celarier	A-2699	K	138,998	MT505016	This study	✓	✓
	Philippines	Dionora, *et al.*	Tt-PHIL-16-02	NA	-	-	This study	×	✓
	South Africa	R. Gallagher	TheCradle1	NA	-	-	Dunning *et al.* (2017)	×	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Themeda triandra	South Africa	K. Simpson	KS	NA	-	-	Dunning *et al.* (2017)	×	✓
	South Africa	L. Smook	3023 AD	K	138,910	MT505019	This study	✓	✓
	Tanzania	P. Peterson *et al.*	23898	K	138,833	MT505017	This study	✓	✓
	Thailand	W. Arthan	070	BKF	138,865	NC035016	Arthan *et al.* (2017)	✓	✓
	Uganda	P. A. Christin & M. R. Lundgren	PAC2015-10	NA	138,891	KY707771	Dunning *et al.* (2017)	✓	✓
Themeda triandra	Yemen	J. R. I. Wood	3457	K	138,883	MT505018	This study	✓	✓
Themeda unica	China	M. B. Deng	92103	PE	138,735	MT505020	This study	✓	✓

Species	**Country**	**Collector name**	**Collector number**	**Herbarium**	**Plastome size (bp)**	**Plastome accession number**	**Reference**	**P**	**N**
Themeda villosa	Thailand	W. Arthan	065	BKF	139,589	NC035021	Arthan *et al.* (2017)	✓	✓
Tripsacum dactyloides L.	China	NA	NA	NA	141,050	NC037087	Wang *et al.* (2017)	✓	✗
Zea mays L.	Unknown	Unknown	CultivarB73	Unknown	140,384	NC001666	Maier *et al.* (1995)	✓	✗

FIGURE LEGENDS

Figure 1. Typical inflorescence structure and floral diagram of *Themeda*, exemplified by *Themeda triandra* (A and B) and *Heteropogon*, exemplified by *Heteropogon triticeus* (C and D) and the characteristics of some savanna environments in Southeast Asia in which *Heteropogon* and *Themeda* are typically found in: low-altitude pine savanna in Thung Salaeng Luang National Park, Thailand (E) broad-leaved savanna in Phu Soi Dao National Park (F) and tall-grass savanna Thung Salaeng Luang National Park (G). Abbreviations: f = fertile spikelet, h = homogamous spikelet, t = triad and aw = awn. All photos were taken by Watchara Arthan.

Figure 2. A partial maximum likelihood phylogenetic tree showing subtribes Andropogoninae and Anthistiriinae, based on plastomes under GTR+Γ+I model showing close-up the Andropogoninae (DASH clade) and Anthistiriinae clades with the clade, comprising of *Apluda mutica*, *Eulalia aurea* and *Sorghastrum* nutans, as the outgroup. Only nodes supported by ML bootstrap and posterior probability more than 70 and 0.95 were labelled in the tree. See Supporting Information for full ML and BI trees based on 136 Andropogoneae plastomes.

Figure 3. Coalescent tree based on 2,464 single-copy nuclear genes analysed with ASTRAL-III. Pie chart at the nodes represent the proportion of concordant gene tree quartets from the main topology (dark green section), the first alternative (orange section) and the second alternative (white section). Local posterior probabilities of the main topology were labelled above the pie charts.

Figure 4. Dated tree produced from plastome data with a single calibration point fixed at 15.26 Ma, based on the divergence time between *Zea mays* and the rest of the crown Andropogoneae. Posterior probabilities (PP) more than 0.95 are shown at the nodes. 95% of HPD of estimated dates are shown as error bars. Dark green boxes above the tree and dotted lines represent timeframes covering estimated grassland expansion in the Late Miocene (8–11 Ma) and the Pleistocene epoch, respectively.

SUPPLEMENTARY DATA

Figure S1. Plastome tree for 136 Andropogoneae taxa analyzed by Maximum Likelihood under GTR+Γ+I model. Phylogenetic tree showing relationships of major clades, the subtribe Andropogoninae (DASH clade) and Anthistiriinae, respectively. Only nodes supported by ML bootstrap more than 70 were labelled in the tree.

Figure S2. Plastome trees for 136 Andropogoneae taxa analyzed by Bayesian Inference analysis under GTR+Γ+I model. Phylogenetic tree showing relationships of major clades, the subtribe Andropogoninae (DASH clade) and Anthistiriinae, respectively. Only nodes supported by posterior probability more than 0.95 were labelled in the tree.