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Phylogenetics of *Panicoideae* (Poaceae) based on chloroplast and nuclear DNA sequences

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Abstract

Phylogenetic relationships among major subfamilies in Poaceae and among major tribes within Panicoideae were evaluated using parsimony and Bayesian analyses of chloroplast *trn*L–F and *atp*β–*rbc*L DNA sequences and a nuclear ribosomal DNA sequence, ITS1–ITS2. The Panicoideae-Aristidoideae-Chloridoideae-Micrairoideae-Arundinoideae-Danthonioideae (PACMAD) clade was well resolved. A close relationship between Aristidoideae and Chloridoideae was found. The monophyly of Micrairoideae was resolved but the relationships of three tribes (Eriachneae, Isachneae, Micraireae) within Micrairoideae were unclear, only *Eriachne* and *Isachne* were monophyletic. Panicoideae *sensu stricto* were supported as monophyletic and sister to a clade of *Danthoniopsis* and *Tristachya*. Within Panicoideae, only a clade of Andropogoneae + *Arundinella* + *Garnotia* was supported. None of the analyses supported the monophyletic status of Paniceae. Within Paniceae, the bristle clade (excluding *Cenchrus*) + *Alexfloydia*, and the forest shade clade *sensu* Giussani et al. (2001), were found, but their circumscription remains ambiguous. A sister relationship between the endemic and rare Australian grasses *Homopholis* and *Walwhalleya* was also resolved. Arundinelleae were found to be polyphyletic. This study supported the separation of *Arundinella* and *Garnotia* from the remaining Arundinelleae and the inclusion of both genera in their own subtribes (Arundinellinae Honda *sensu stricto* and Garnotiinae Pilger) within the Andropogoneae. Arundinelleae should be abandoned as a taxonomic tribe within the Centothecoid + Panicoid clade. Within Andropogoneae, five out of a total of 11 subtribes (Chionachninae, Coicinae, Dimeriinae, Germainiinae, and Tripsacinae) were monophyletic. This was the first time that Dimeriinae and Germainiinae have been included in a molecular study.

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Introduction

Panicoideae are one of the biggest subfamilies in Poaceae, comprising approximately 3,000 species and 200 genera (GPWG 2001). Although Panicoideae are highly diverse, all members can be grouped on the basis of the presence of two-flowered spikelets with male or sterile lower florets (Clayton & Renvoize 1986, Kellogg 2000) and simple type starch grains in the endosperm (Tateoka 1962). These spikelet and starch grain types were described as uniquely-derived characters by Kellogg and Campbell (1987). The monophyly of Panicoideae has also been verified by many molecular studies using chloroplast and nuclear DNA (e.g., Barker et al. 1999, Gomez-Martinez & Culham 2000, Giussani et al. 2001, GPWG 2001, Aliscioni et al. 2003, Bouchenak-Khelladi et al. 2008, Christin et al. 2008, Vicentini et al. 2008, Edwards & Smith 2010). In the widely used classification system of Clayton and Renvoize (1986), Panicoideae were split into seven tribes: Andropogoneae, Paniceae, Arundinelleae, Isachneae, Hubbardieae, Steyermarkochloeae and Eriachneae. Subsequently, Panicoideae were divided into two groups using phenetic analyses: the Andropogoneae + Garnotieae group and the Paniceae + Isachneae + Arundinelleae group of Hilu and Wright (1982) and the supertribes Panicodae and Andopogonodae of Watson and Dallwitz (1992 onwards). More recent phylogenetic analyses have resolved six tribes in Panicoideae because Eriachneae were placed outside Panicoideae as *incertae sedis* (GPWG 2001). Two tribes, Eriachneae and Isachneae, were placed together with Micraireae in a new subfamily Micrairoideae (Sánchez-Ken et al. 2007).

Over the past few decades, members of Arundinelleae have been included in several phylogenetic analyses. According to the morphological phylogenetic reconstruction of Kellogg and Watson (1993), Arundinelleae (excluding *Garnotia*) were monophyletic. In contrast, several analyses of DNA sequences resolved Arundinelleae as polyphyletic and suggested that its delimitation should be reconsidered (Barker et al. 1999, Hilu et al. 1999, Spangler et al. 1999, GPWG 2001, Sánchez-Ken & Clark 2007, Sánchez-Ken et al. 2007, Bouchenak-Khelladi et al. 2008, Christin et al. 2008, Vicentini et al. 2008).

Andropogoneae are one of the two major tribes in Panicoideae. Based on morphology, this group of tropical grasses is well-defined containing more than 900 species with extensive morphological variation among its members (Clayton & Renvoize 1986, GPWG 2001). Two widely used classifications of Andropogoneae come from the studies of Clayton and Renvoize (1986) and Watson and Dallwitz (1992 onwards). Andropogoneae were found to be non-monophyletic in the morphology based phylogenetic reconstruction of Kellogg and Watson (1993). Molecular data, on the other hand, consistently supported the monophyly of Andropogoneae as circumscribed by Clayton and Renvoize (1986), and also supported the sister relationship of Andropogoneae to *Arundinella* (e.g., Spangler et al. 1999, Mathews et al. 2002, Bomblies & Doebley 2005, Sánchez-Ken & Clark 2007, Sánchez-Ken et al. 2007, Christin et al. 2008, Vicentini et al. 2008). Even though many molecular results have supported the monophyly of the tribe, the identity and relationships of its subtribes are not well understood. Recent phylogenetic analyses have suggested that the short branches along the backbone of their trees and the concentration of nucleotide changes on terminal branches in the Andropogoneae clade have been caused by a rapid evolutionary radiation near the base of the clade (Kellogg 2000, Mathews et al. 2002, Skendzic et al. 2007). Many of these studies suggested that better sampling of lineages within the tribe, or the addition of more phylogenetic characters (more nucleotides), may help to resolve the relationships.

The major objective of this study was to improve phylogenetic understanding within Panicoideae and among subfamilies in the PACMAD clade by increasing the sampling of taxa and by using plastid (*trn*L intron, *trn*L–F intergenic spacer, and the *atp*β–*rbc*L intergenic spacer) and nuclear (ITS) DNA sequences separately and in combination. The *atp*β–*rbc*L spacer was used for the first time to study inter-relationships of taxa within this group of plants. It also aimed to apply the results of the molecular phylogenies to taxonomy by testing the infra-subfamilial classification proposed by several authors. More specifically, it aimed to: (1) resolve major groupings within Panicoideae and investigate their inter-relationships, (2) investigate the monophyly of tribes of Panicoideae *sensu* Clayton and Renvoize (1986) and (3) investigate the subtribal classification of Andropogoneae.

Material and Methods

Taxonomic sample

The majority of the materials were collected during our expeditions within several regions of Thailand, South America and Australia. Leaf material was dried with silica gel to rapidly desiccate the material and reduce DNA degradation before extraction (Chase & Hills 1991) or with the alternative preservative solution of saturated CTAB to reduce degradative changes affecting the quality of DNA (Thomson 2002). A few samples were taken from herbarium specimens and some DNA was available in the Botany Molecular Laboratory, Trinity College, Dublin and the Royal Botanic Gardens, Kew, England, U.K. The sample of species presented in this study relied greatly on the Old World grasses, especially the panicoid group. Additional taxa of other subfamilies from the New World and Australasia were also included. Five out of a total of seven tribes of Panicoideae *sensu* Clayton and Renvoize (1986) were sampled. The number of panicoid species presented here is considerably larger than most molecular studies to date. However, representatives of the two small tribes, Steyermarkochloeae and Hubbardieae, were not included due to the lack of material suitable for DNA extraction. In total, 132 taxa from six subfamilies *sensu* Clayton and Renvoize (1986), including 10 other taxa of subfamilies Centothecoideae, Arundinoideae and Chloridoideae, and five tribes of Panicoideae and all subtribes of Andropogoneae were sampled. This was the first time that Dimeriinae and Germainiinae have been included in a molecular study (Appendix 1). Five grasses from Ehrhartoideae and Pooideae were chosen as outgroup taxa, according to the results of GPWG (2001) and Bouchenak-Khelladi et al. (2008). For the *trn*L–F region, 13 taxa out of a total of 129 taxa were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). For the *atp*β–*rbc*L region, we sequenced 122 taxa and downloaded five sequences from GenBank. For the ITS region, 127 taxa were sampled, including 27 sequences from GenBank. In total, 124 taxa were included in the combined dataset.

DNA extraction, amplification and sequencing

Total genomic DNA (tDNA) was extracted using a modified hot CTAB (hexadecyltrimethylammonium bromide) method of Doyle and Doyle (1987) as outlined in Hodkinson et al. (2007b). The extractions used 0.2 g of dried leaf or 0.3 g of material obtained from herbarium specimens. The extract was precipitated using isopropanol and kept at -20 ºC overnight or for three weeks to increase precipitation

in the case of herbarium specimens. The total DNA samples were then washed and purified with 70 % ethanol and further purified by using JETquick spin columns (GENOMED-GmbH, Löhne, Germany). The DNA was then transferred into a 1.5 ml micro-centrifuge tube and stored at -20°C until used or at -80 °C for longer periods. The Polymerase Chain Reaction (PCR) was used to amplify two regions of chloroplast genome DNA, *trn*L–*trn*F and *atp*β–*rbc*L, and one region of nuclear ribosomal DNA, ITS. The *trn*L intron and *trn*L–F intergenic spacer were amplified as a single fragment using the 'c' and 'f' primers of Taberlet et al. (1991), the *atp*β–*rbc*L region with the primers of Samuel et al. (1997) and the ITS region using the 17SE and 26SE primers of Sun et al. (1994). All of the amplifications were carried out in an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler. The amplification of the target fragment began with an initial pre-melt at 94° C for 1 min, followed by 29 cycles of denaturation at 95°C for 45 s, annealing at 50°C for *trn*L–F or 52°C for *atp*β–*rbc*L and ITS for 45 s, and extension at 72°C for 2 min. A final extension at 72°C for 7 min was also included. All successful PCR products were purified using the same procedure as the total DNA purification but using sterile ultra pure water as the elution buffer. DNA was sequenced using BigDye Terminator v. 1.1 cycle-sequencing kits (Applied Biosystems) and an Applied Biosystems 310 automated DNA sequencer.

Phylogenetic analyses

Full sequences of all taxa listed in Appendix 1 were obtained. DNA sequences were checked and aligned by inserting gaps manually using Se-Al v. 2.0a11 (Rambaut 1996) following the guidelines of Kelchner (2000) and Baldwin et al. (1995) for the ITS matrix. Gaps smaller than 10 bp were coded as missing data, unless they were found in regions where there was an obvious tandemly arranged duplication in one sequence that was clearly due to a single mutation (a duplication). Such duplications were scored as only one character in the subsequent phylogenetic analyses. Gaps larger than 10 bp were excluded from the analyses. For the ITS matrix, some taxa had some polymorphic nucleotide sites and at these sites the dominant peak was chosen or alternatively the site was excluded from the matrix if a single dominant peak was not present. The aligned sequences were imported into PAUP* v.4.0b10 (Swofford 2002).

Maximum parsimony (hereafter MP) analyses of the final matrix were performed using the heuristic search algorithms of PAUP* with 1,000 replicates of random addition sequence (holding 20 trees at each replication) and with tree bisection reconnection branch swapping on multiple trees. Clade support was examined using 1,000 bootstrap replicates (Felsenstein 1985) with the same settings as the initial heuristic search but with simple sequence addition instead of random sequence addition. Bayesian inference (hereafter BI) of the phylogeny was performed using MrBayes version 3.2 (Huelsenbeck & Ronquist 2001). The appropriate nucleotide substitution models for BI analysis were chosen using hierarchical likelihood ratio tests as implemented in MODELTEST 3.06 (Posada & Crandall 1998). The three datasets, two plastid and one nuclear, showed the same best-fit nucleotide substitution model (GTR + G + I). Four parallel Markov chain Monte Carlo (MCMC) chains were run for 25,000,000 generations with trees sampled every 1,000 generations, and 25% of trees were discarded as burn-in.

Results

Analyses of the combined dataset

The matrix used for the combined analysis was obtained from *trn*L–*trn*F, *atp*β–*rbc*L and ITS sequences. The final aligned matrix was 3,348 bp long; 969 characters were constant, 288 were variable but parsimony-uninformative, and 682 characters were parsimony-informative. The tree search using maximum parsimony generated 687 equally most parsimonious trees of 4,330 steps with consistency index (CI) of 0.365 and retention index (RI) of 0.626. Bootstrap percentages (hereafter BP) are described as low (50–74 BP), moderate (75–84 BP) and high (85–100 BP). The MP analysis produced a topology that was congruent with the tree obtained from the BI analysis. By this we mean that there were no strongly supported groups in one analysis that were incongruent with strongly supported alternative groupings in the other analysis (no hard incongruence; following Reeves et al. 2001). The tree illustrated in Figure 1 is the Bayesian tree with the posterior probability (PP) values obtained from the BI analysis and with bootstrap percentages from the separate MP bootstrap analysis. The tree is largely consistent with the combined plastid tree (Appendix 2, Fig. S1) and the ITS tree (Appendix 2, Fig. S2). We therefore base discussion of our results on the combined tree but occasionally refer to the supplementary information where appropriate.

The PACMAD clade was strongly supported (99 BP, 1.00 PP). *Aristida* (Aristidoideae) was sister to Chloridoideae (support for the monophyly of this subfamily was 81 BP, 1.00 PP) with 100 BP and 0.96 PP. The position of this clade was unresolved in the BI tree but it was sister to a Micraroideae clade, consisting of a monophyletic Eriachneae (100 BP, 1.00 PP) and an unresolved Isachneae (as the position of *Coelachne* was unresolved), in the strict consensus tree of the MP analysis (not shown). In the BI tree the Micraroideae clade was sister to *Arundo* with 0.92 PP, but unresolved relative to Danthonioideae, Chloridoideae and a clade represented by the rest of the grasses. The PACCMAD group (including "Centothecoideae", in contrast to PACMAD) was not supported because Centothecoideae were paraphyletic with Panicoideae. However, support for the monophyly of a *Centotheca* + *Thysanolaena* clade was high (99 BP, 1.00 PP). This reduced centothecoid clade was sister to a lineage, containing ((Panicoideae + Arundinelleae) + *Chasmanthium*) with 1.00 PP in the BI analysis, or was sister to a lineage consisting of Panicoideae + Arundinelleae, in the strict consensus tree of the MP analysis (not shown).

Arundinelleae were polyphyletic because *Arundinella* and *Garnotia* were included within Panicoideae (69 BP, 1.00 PP), while *Danthoniopsis* and *Tristachya* were grouped together with low support (55 BP, 0.94 PP) and were placed as the next most outlying branch to the rest of Panicoideae. Paniceae were not supported or retrieved in the strict consensus tree in the MP analysis, but the BI analysis resolved the monophyly of Paniceae with 0.66 PP. None of the subtribes of Paniceae *sensu* Clayton and Renvoize (1986) were found to be monophyletic.

The combined dataset analyses supported two genera of Paniceae as monophyletic: *Pennisetum* (74 BP, 1.00 PP) and *Sacciolepis* (100 BP, 1.00 PP). The *Pennisetum* clade was sister to *Setaria* (51 BP, 0.71 PP). This clade was sister to a group consisting of *Alexfloydia* and *Spinifex* (support for the monophyly of this clade was 90 BP, 1.00 PP) with strong support (100 BP, 1.00 PP). *Homopholis* was sister to *Walwhalleya* (57 BP, 1.00 PP). *Cyrtococcum*, *Pseudoechinolaena*, and *Acroceras* were grouped together (57 BP,

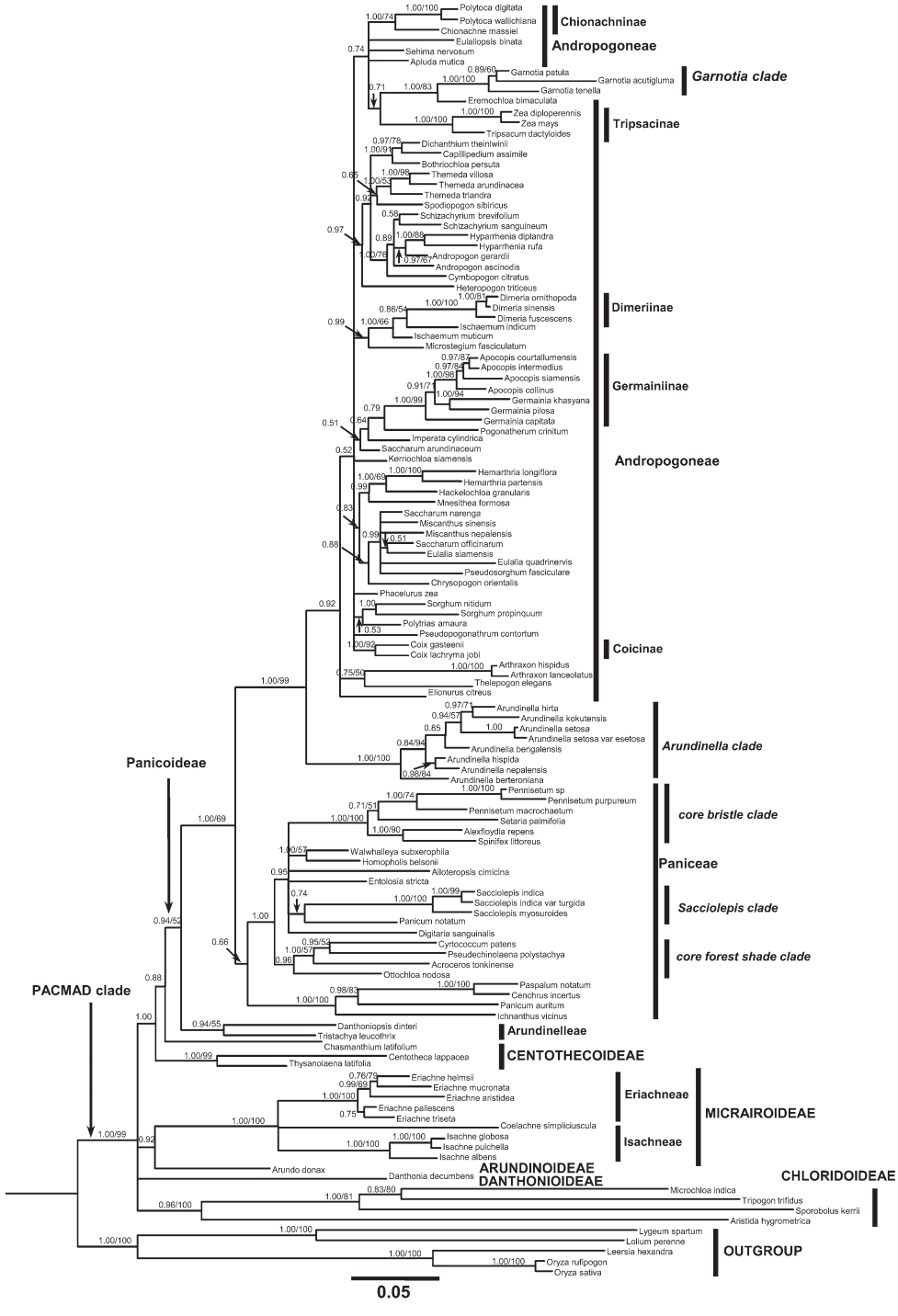

Fig. 1. Bayesian consensus tree from the combined analysis (*trn*L–F + *atp*β–*rbc*L + ITS) shown as a phylogram. Bayesian posterior probabilities and bootstrap values are shown above the branches. The PACMAD clade, and the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.

1.00 PP) and sister to *Ottochloa* (< 50 BP, 0.96 PP). The clade consisting of *Cenchrus*, *Ichnanthus, Panicum auritum* and *Paspalum* was also resolved with high support (100 BP, 1.00 PP).

Andropogoneae and a *Garnotia* clade (100 BP, 1.00 PP) were grouped together with 0.92 PP. This clade was sister to an *Arundinella* clade (100 BP, 1.00 PP) with strong support (99 BP, 1.00 PP). Within Andropogoneae, five subtribes: Chionachninae, Coicinae, Dimeriinae, Germainiinae and Tripsacinae (according to Clayton & Renvoize 1986), were monophyletic with 74, 92, 100, 99 and 100 BP, respectively (all with 1.00 PP). The relationships between the monophyletic subtribes and the rest of Andropogoneae were unclear, except Dimeriinae and Tripsacinae. Dimeriinae had *Ischaemum indicum* and *I. muticum* as its successively sister taxa with 54 BP, 0.86 PP and 66 BP, 1.00 PP, respectively. Tripsacinae were sister to the clade consisting of *Eremochloa* and *Garnotia* (83 BP, 1.00 PP) with low support (< 50 BP, 0.71 PP). Four out of a total of seven taxa from Rottboelliinae (*Hackelochloa*, *Hemarthria longiflora*, *H. partensis* and *Mnesithea*) were grouped with 0.99 PP. The monophyly of *Arthraxon* was resolved (100 BP, 1.00 PP). This clade was united with *Thelepogon* (50 BP, 0.75 PP). The monophyletic *Hyparrhenia* (88 BP, 1.00 PP) was sister to *Andropogon gerardii* (67 BP, 0.97 PP). This clade was grouped together with *Andropogon ascinodis*, *Cymbopogon*, and *Schizachyrium* (76 BP, 1.00 PP). A clade consisting of *Bothriochloa*, *Capillipedium* and *Dichanthium* was resolved (91 BP, 1.00 PP). The analysis also resolved the monophyly of *Themeda* with 53 BP and 1.00 PP.

Discussion

PACMAD Clade

It is clear that the PACMAD clade, including Panicoideae *sensu stricto* (excluding Isachneae, Eriachneae and Arundinelleae, but including *Arundinella* and *Garnotia*), Arundinoideae, Chloridoideae, Micrairoideae *sensu* Sánchez-Ken et al. (2007), Aristidoideae and Danthonioideae, is monophyletic. It is also robustly supported based on molecular data by previous studies (e.g., Giussani et al. 2001, GPWG 2001, Sánchez-Ken et al. 2007, Christin et al. 2008, Edwards & Smith 2010). Not all of these studies included representatives of each of the PACMAD subfamilies, but in combination they consistently grouped these taxa together. All members of this clade also have several apparently synapomorphic morphological or anatomical characters, such as the presence of an elongated mesocotyl internode and the loss of the epiblast (GPWG 2001). Within the PACMAD clade, the relationships among the six subfamilies were largely unresolved. The representatives of subfamily Centothecoideae (*Centotheca*, *Chasmanthium* and *Thysanolaena*) did not form a monophyletic group. Only *Centotheca* and *Thysanolaena* were grouped together with high support (99 BP, 1.00 PP). This relationship was also found in the studies of Bouchenak-Khelladi et al. (2008) and Christin et al. (2008). The grouping of members of Centothecoideae with the monophyletic Panicoideae is consistent with previous studies (Sánchez-Ken & Clark 2001, Sánchez-Ken & Clark 2007, Bouchenak-Khelladi et al. 2008). We therefore prefer to use the acronym PACMAD instead of PACCMAD, following Duvall et al. (2007). Micrairoideae, including Eriachneae and Isachneae, were monophyletic with strong support (100 BP, 1.00 PP), a finding consistent with previous studies (Duvall et al. 2007, Sánchez-Ken et al. 2007). However, the position of Micrairoideae within the

PACMAD clade was not clear. The monophyly of Micraironideae *sensu* Sánchez-Ken et al. (2007) with Eriachneae, Isachneae and Micraireae, was not well supported (< 50 BP) in the ITS analysis (S2, supplementary information). We can deduce some patterns from the MP analyses of single DNA regions. A strict consensus tree of these would reveal: (((*Eriachne* + *Isachne*) + *Coelachne*) *Micraira*).

The monophyly of *Eriachne,* as found in the previous study by Sánchez-Ken et al. (2007), was consistently resolved and well supported (100 BP, 1.00 PP). Clayton and Renvoize (1986) noted that Eriachneae, consisting of *Eriachne* and *Pheidochloa*, closely resembles Isachneae, in the number of fertile florets and in the induration of lemmas with inrolled margins, but differs in having awned lemmas and Kranz anatomy. Isachneae, represented by *Coelachne* and *Isachne*, were non-monophyletic due to the exclusion of *Coelachne* (Figs. 1, S1). However, the result supported *Isachne* as monophyletic (100 BP, 1.00 PP). Morphologically, Isachneae, including *Coelachne*, *Heteranthoecia*, *Isachne*, *Limnopoa* and *Sphaerocaryum*, are characterised by having two fertile disarticulating florets and by their non-Kranz anatomy. Clayton and Renvoize (1986) suggested that Isachneae were most likely to be derived from *Panicum* based upon close morphological similarities of spikelets between *Isachne* and *Panicum* sect. *Verruculosa*. However, these similarities were found to be homoplasies by Sánchez-Ken et al. (2007).

Panicoideae *sensu stricto* (excluding Isachneae, Eriachneae and Arundinelleae, but including *Arundinella* and *Garnotia*) were supported as monophyletic (69 BP, 1.00 PP). Within the Panicoideae only the ((Andropogoneae + *Garnotia*) + *Arundinella*) clade (99 BP, 1.00PP) can be identified. This finding is inconsistent with some previous studies in which Panicoideae were made up of three major clades, comprising Andropogoneae, Paniceae [x=10] and Paniceae [x=9] (e.g., Gomez-Martinez & Culham 2000, Giussani et al. 2001, Aliscioni et al. 2003, Vicentini et al. 2008). The failure to retrieve this topology could be due to uneven sampling among the clades.

Unfortunately, no DNA samples from two small tribes (monotypic Hubbardieae and the two genera of Steyermarkochloeae) were available in this study. Based on morphological data, *Hubbardia*, which was distinguished by the absence of paleas, was apparently derived from Isachneae (Clayton & Renvoize 1986) sharing similarities in spikelet structure, the disarticulation of the florets above the glumes and the C_3 photosynthetic pathway. Steyermarkochloeae are comprised of two extraordinary genera, *Steyermarkochloa* and *Arundoclaytonia*. Based on leaf blade anatomy, Davidse and Ellis (1984) first suggested that *Steyermarkochloa* was similar to an arundinoid grass such as *Gynerium* (now placed within the Panicoideae + Centothecoideae clade, Sánchez-Ken & Clark 2001), *Arundo*, *Phragmites* and *Thysanolaena* (now Centothecoideae, GPWG, 2001). However, it was later transferred to be under Panicoideae because *Steyermarkochloa* is morphologically distinct from arundinoid grasses in almost all characters of leaves, inflorescences, spikelets and flowers (Clayton & Renvoize 1986). Although *Arundoclaytonia* showed no anatomical resemblance with the panicoid grasses, it was included in Steyermarkochloeae on the basis of spikelet morphology (Davidse & Ellis 1987). Recent phylogenetic study showed that *Arundoclaytonia* was grouped outside the Panicoideae clade. It was placed as sister to the PACMAD clade (Sánchez-Ken & Clark 2007).

Tribes of Panicoideae

Paniceae

A clade, consisting of *Alexfloydia* and three bristle clade taxa (*Pennisetum*, *Setaria* and *Spinifex*) was found to be monophyletic with high support (100 BS, 1.00 PP). Interestingly, *Alexfloydia*, a rare Australian grass, was placed in the bristle clade. It is a non bristle-bearing genus not included in any previous analyses. Some previous studies have also found non-bristle bearing taxa in the bristle clade (Gomez-Martinez & Culham 2000, Giussani et al. 2001, Aliscioni et al. 2003, Bess et al. 2005, Doust et al. 2007; O. Morrone, Instituto de Botánica Darwinion, Argentina, 'pers. comm.'). Surprisingly, one of the bristle-bearing genera, *Cenchrus* (represented by a single taxon, *C. incertus*), was not grouped within this clade but was positioned within the clade that corresponds to x=10 Paniceae, represented in this study by *Ichnanthus*, *Paspalum* and *Panicum auritum*, with high support (100 BP, 1.00 PP). Other *Cenchrus* species were previously placed in the bristle-clade of the x=9 Paniceae (Doust et al. 2007, Christin et al. 2008, Vicentini et al. 2008), which suggests that this genus may be polyphyletic, but future studies are needed to test this hypothesis. Within the bristle clade, *Pennisetum* was monophyletic (74 BP, 1.00 PP). However, previous studies have found that *Pennisetum* was paraphyletic and always forms a monophyletic assemblage with *Cenchrus* (Giussani et al. 2001, Doust & Kellogg, 2002, Doust et al. 2007, Donadio et al. 2009).

The forest shade clade, as defined by Giussani et al. (2001), was also resolved in this study (0.96 PP) with *Acroceras*, *Ottochloa*, *Pseudechinolaena* and a new member, *Cyrtococcum*. However, the monophyly of the forest shade clade, which was supported in other recent studies (Christin et al. 2008, Ibrahim et al. 2009), remained only moderately supported and ambiguous in our analyses (Figs 1, S1 & S2).

Homopholis and *Walwhalleya*, endemic grasses from Queensland, Australia, were grouped together (57 BP, 1.00 PP). This grouping is inconsistent with the morphological phylogenetic trees of Wills et al. (2000) in which three members of *Walwhalleya* formed a monophyletic group and were sister to the clade consisting of *Digitaria* and *Panicum*, while *Homopholis* was well-supported as the most outlying member of the ingroup. The monotypic and endangered genus *Homopholis* was previously placed within section Digitariastrae under Paniceae, and considered to be closely related to *Digitaria*, but differing in its well developed lower glumes and comparatively small fertile florets (Clayton & Renvoize 1986). However, this relationship was not supported by either the morphological study of Wills et al. (2000) or by the present molecular data although the topology here was only weakly supported.

Sacciolepis was also resolved as monophyletic with high support (100 BP, 1.00 PP), but its position was uncertain. Previously, *Sacciolepis*, represented by *S. indica*, was nested within the clade consisting of *Panicum* section *Monticola,* clade-*Parvifolia* clade-*Verrucosa* (Aliscioni et al. 2003, Vicentini et al. 2008). Aliscioni et al. (2003) also suggested that the inclusion of *Sacciolepis* within this *Panicum* clade was doubtful because no apparent morphological relationship exists between *Sacciolepis* and those sections or groups. *Sacciolepis* comprising of c. 30 species is widely distributed in the tropics, especially in Africa. It is a distinctive genus and differs from the rest of Paniceae by the presence of spiciform panicle, with ribbed glumes and gibbous upper glumes (Clayton & Renvoize 1986).

Arundinelleae

Arundinelleae were polyphyletic and split into three clades: (1) an *Arundinella* clade (100 BP, 1.00 PP), which was sister to Andropogoneae, (2) a *Garnotia* clade (100 BP, 1.00 PP), which was embedded within Andropogoneae as sister to Tripsacinae (99 BP, 1.00 PP), and (3) a clade of *Danthoniopsis* + *Tristachya* (55 BP, 0.94 PP), which was sister to Panicoideae (52 BP, 0.94 PP). The grouping of *Danthoniopsis* and *Tristachya* was consistent with Sánchez-Ken et al. (2007), while the sister group relationship between *Danthoniopsis* + *Tristachya* clade and the panicoid clade can be interpreted as a novel result. However, this finding should be interpreted with care because some species of *Tristachya* have been placed within the Andropogoneae with high support (e.g., Hilu et al. 1999, Bouchenak-Khelladi et al. 2008).

Based on morphological characters, Arundinelleae taxa have the unique feature of the two-flowered spikelet with male or sterile lower floret and a bisexual upper floret. However, Arundinelleae differ from other panicoids in having a spikelet with a persistent glume (except *Garnotia*) (Clayton & Renvoize 1986, Renvoize & Clayton 1992). The non-monophyly of Arundinelleae has been reported in other phylogenetic analyses (e.g., Barker et al. 1999, Hilu et al. 1999, Spangler et al. 1999, GPWG 2001, Sánchez-Ken & Clark 2007, Sánchez-Ken et al. 2007, Bouchenak-Khelladi et al. 2008). Therefore, the unique Arundinelleae *sensu lato.* morphological characters are most likely homoplasious. The classification at the generic level of Arundinelleae *sensu lato* is complex (Phipps 1966, Clayton & Renvoize 1986) but, on the basis of anatomy, its taxa can be divided into two types, the non–Kranz type or C_3 pathway in *Chandrasekharania* and *Jansenella* and the Kranz MS type or C_4 pathway in the rest of the tribe (Clayton & Renvoize 1986). It would be interesting to include those two genera in further analysis, especially *Jansenella* which was found as a morphologically intermediate taxon between *Arundinella* and *Danthoniopsis* (Bor 1955, Clayton & Renvoize 1986, Teerawatananon & Hodkinson 2008).

Arundinella* and *Garnotia

The results from the combined dataset supported the separation of *Arundinella* and *Garnotia* from the remaining Arundinelleae, and suggested that *Arundinella* and *Garnotia* could better be placed in their own subtribes (Arundinellinae Honda *sensu stricto* and Garnotiinae Pilger) within Andropogoneae and that Arundinelleae *sensu lato* should be abandoned as a taxonomic group. Although the appearance of *Garnotia* within Andropogoneae was previously demonstrated by the cluster analysis of Hilu and Wright (1982), the inclusion of *Garnotia* in Andropogoneae is a novel result overlooked by previous phylogenetic studies (e.g., Barker et al. 1999, Hilu et al. 1999, Spangler et al. 1999, GPWG 2001, Sánchez-Ken & Clark 2007, Sánchez-Ken et al. 2007, Bouchenak-Khelladi et al. 2008) probably due to lack of *Garnotia* DNA samples. *Arundinella* was sister to Andropogoneae + *Garnotia* (99 BP, 1.00 PP). However, the position of *Garnotia* within Andropogoneae, relative to other subtribes was unclear. The *Garnotia* clade was found to be sister to *Eremochloa* (83 BP, 1.00 PP) but there is no obvious shared morphology. Morphologically, *Arundinella* differs from the rest of Arundinelleae in having a membranous ligule, a scabrid upper lemma and a punctiform hilum, while *Garnotia* is distinguished by its single-flowered spikelets that disarticulate below the glumes (Clayton & Renvoize 1986). Both genera differ from the rest of the tribe by having a punctiform hilum and a membranous ligule (Clayton & Renvoize 1986). Anatomically, *Arundinella* and *Garnotia* are C_4 taxa but have isolated vascular

bundle sheath cells (which were called distinctive cells or auxillary bundle cells), and auriculate paleas (Tateoka 1958, Clayton & Renvoize 1986, Renvoize & Clayton 1992). On the basis of our results these shared characters are homoplasious as *Arundinella* and *Garnotia* were not grouped together.

Andropogoneae and its subtribal classification

Andropogoneae were found to be monophyletic only if *Garnotia* was included (<50 BP, 0.92 PP) and this clade was united with *Arundinella* (99 BP, 1.00 PP) (Fig. 1). Within the tribe, none of the phylogenetic trees were consistent with the awned/awnless classification proposed by Clayton (1972, 1973). This hypothesis was supported by the molecular study of Mathews et al. (2002), but no strong evidence for this clade was found. It is clear that the subtribal classification of Clayton and Renvoize (1986) requires considerable revision (Kellogg 2000, Mathews et al. 2002) even though some subtribes (Chionachninae, Coicinae, Dimeriinae, Germainiinae and Tripsacinae) were supported as monophyletic in our study.

Chionachninae, Coicinae and Tripsacinae

All monoecious taxa of Andropogoneae were traditionally placed in Maydeae (Bentham 1882, Hackel 1889, Watson & Dallwitz 1992 onwards, Kellogg & Watson 1993). However, Maydeae were divided into three subtribes, Chionachninae, Coicinae and Tripsacinae by Clayton (1973) and Clayton and Renvoize (1986) using the difference of inflorescences and spikelets based mainly on the different origin of the bead-like feature of female spikelets (Clayton & Renvoize 1986). In Chionachninae this structure is formed by a lower glume, while in Coicinae it is modified from a spatheole. These two subtribes completely differ from Tripsacinae in having paired female spikelets and the inflorescence rachis is broader than their spikelets.

In this study, Chionachninae, represented by *Chionachne massiei*, *Polytoca digitata* and *P. wallichiana*, were supported as monophyletic (74 BP, 1.00 PP). *Polytoca wallichiana* was first proposed under the name *Cyathorhachis wallichiana* by Steudel (1854). It was transferred to be *Polytoca* by Bentham (1882). Recently, the name *Cyathorhachis* was reinstated by Jannink and Veldkamp (2002). However, *P. digitata* and *P. wallichiana* are morphologically similar in many respects. The low level of genetic divergence between these two taxa also confirmed that *P. wallichiana* should be placed within *Polytoca* rather than *Cyathorhachis*. Morphologically, Chionachninae were found to be polyphyletic by Kellogg and Watson (1993) in which *Polytoca* was grouped together with Tripsacinae, while *Chionachne* was placed outside this clade. Clayton and Renvoize (1986) and Renvoize and Clayton (1992) suggested that Chionachninae are linked to Rottboelliinae by the appearance of the peg and the socket callus joints of sessile spikelets. This relationship was not supported by this study.

The monophyly of *Coix* (Coicinae) was demonstrated by Bomblies and Doebley (2005) and was also resolved with high support by this study (92 BP, 1.00 PP) as the Australian *C. gasteenii* grouped with the widespread *C. lacryma-jobi*. Morphologically, the highly modified inflorescence of the monotypic Coicinae is composed of paired unisexual racemes. Female racemes are hidden in an indurated utricle which is derived from a spatheole. These extraordinary modifications confirmed the separation of Coicinae from the remaining monoecious taxa and indicated a possible link with *Apluda* and Coicinae (Clayton & Renvoize 1986, Renvoize & Clayton 1992). However, this debate remains unresolved based on molecular data.

Our study strongly supported the monophyly of Tripsacinae (100 BP, 1.00 PP). Species of this subtribe - *Zea mays* and wild species of *Zea* and *Tripsacum* - were grouped together (Clayton 1973, Clayton & Renvoize 1986, Watson & Dallwitz 1992 onwards, Kellogg 2000). Tripsacinae were also found to be monophyletic and closely related to Rottboelliinae and Chionachninae in all molecular studies to date (e.g., Spangler et al. 1999, Mathews et al. 2002, Bomblies & Doebley 2005). A sister-group relationship between Tripsacinae and Rottboelliinae was not found in our study, but our combined dataset showed that Tripsacinae were grouped together with the clade consisting of *Garnotia* clade + *Eremochloa* with low support (< 50 BP, 0.71 PP). There is no obvious morphological character to arrange these taxa together.

Germainiinae

Germainiinae, represented by *Apocopis* and *Germainia*, were highly supported (99 BP, 1.00 PP). These taxa have not been combined in phylogenetic analyses before. This novel clade is incongruent with the morphological phylogenetic trees of Kellogg and Watson (1993). Within Germainiinae, *Apocopis* was monophyletic (98 BP, 1.00 PP). The taxa with awned upper lemmas (*A. courtallumensis*, *A. intermedius* and *A. siamensis*) form a clade with moderate support (84 BP, 0.97 PP) and have the awnless upper lemma taxon (*A. collinus*) is the most outlying species to the rest of *Apocopis*. However, the monophyly of *Apocopis* was not supported by the combined chloroplast tree due to the inclusion of *G. lanipes* (Appendix 2, Fig. S1). *Germainia* was consistently paraphyletic in all analyses. Both MP and BI analyses found that *G. khasyana* was sister to *G. pilosa* (94 BP, 1.00 PP), while *G. capitata* was the next most outlying branch to the rest of Germainiinae.

Dimeriinae

The monotypic Dimeriinae (*Dimeria* spp.) was strongly supported (100 BP, 1.00 PP). A sister group relationship between Dimeriinae and Ischaeminae was demonstrated by the combined dataset in which *Ischaemum indicum* and *I. muticum* were successively sister taxa to a Dimeriinae clade. These relationships are inconsistent with the studies of Clayton and Renvoize (1986) and Kellogg and Watson (1993). Morphologically, Dimeriinae is unlike the remaining Andropogoneae in having a single pedicelled spikelet with no trace of the pairing (Clayton 1972) and hence it is presumably derived from that state (Renvoize & Clayton 1992). According to Clayton and Renvoize (1986), Dimeriinae is linked to *Pogonachne* in the Ischaeminae through *D. leptorhachis* but differs by its espatheate inflorescences, racemes with tough rachis, epaleate florets and the presence of two stamens.

Hackelochloa, Hemarthria* and *Mnesithea

Although Rottboelliinae (with *Elionurus*, *Eremochloa*, *Hackelochloa*, *Hemarthria*, *Mnesithea* and *Phacelurus*) were not found to be monophyletic in this study, three genera, *Hackelochloa*, *Hemarthria* and *Mnesithea*, often grouped together, (< 50 BP, 0.99 PP). The monophyly of *Hemarthria* was confirmed by the combined dataset (100 BP, 1.00 PP). This clade was found to be closely related to *Hackelochloa* (69 BP, 1.00 PP). However, no obvious morphological traits support this relationship. The relationship between *Hemarthria* and *Hackelochloa* is inconsistent with the morphological studies of Clayton and Renvoize (1986) and Kellogg and Watson (1993). Morphologically, both genera are placed in Rottboelliinae based on the

characters of awnless upper lemmas, thickened internodes and the fused pedicel to internode (Clayton 1973). *Hemarthria* can be distinguished from the rest of its subtribe in possessing tough rachis and an oblique basal callus, while *Hackelochloa* is the only genus in the subtribe having globose sessile spikelets with wingless lower glumes (Clayton & Renvoize 1986).

Bothriochloa, Capillipedium and *Dichanthium*

Three genera, *Bothriochloa*, *Capillipedium* and *Dichanthium*, were found to form a monophyletic group in the combined analysis (91 BP, 1.00 PP) and this clade has also been resolved by previous molecular studies (e.g., Spangler et al. 1999, Mathews et al. 2002, Skendzic et al. 2007). These three genera are known as an agamic complex and have produced a large number of interspecific and some intergeneric hybrids (Harlan & De Wet 1963, De Wet & Harlan 1970). However, this relationship was not supported by most morphological studies of Clayton and Renvoize (1986), Watson and Dallwitz (1992 onwards) and Kellogg and Watson (1993) and most studies preferred to keep these three genera separate. Morphologically, *Dichanthium* is closely related to *Bothriochloa* in having sub-digitate racemes, but can be distinguished by its pedicels and rachis internodes being solid and lacking a translucent median line. The members of *Capillipedium* are often confused with members of *Bothriochloa*, but the former differs in having paniculate inflorescences and short racemes often reduced to triads (Clayton & Renvoize 1986). Based on molecular data, this clade was previously found to be within the core Andropogoneae (Spangler et al. 1999, Mathews et al. 2002, Skendzic et al. 2007). This relationship was also resolved in this study. The core Andropogoneae (with *Andropogon*, *Coix*, *Cymbopogon*, *Heteropogon*, *Hyparrhenia*, *Schizachyrium* and *Sorghastrum*) was informally named by Spangler et al. (1999) corresponding to the chromosome number (*x*) of 20. The core Andropogoneae was later found to be non-monophyletic by Mathews et al. (2002) and Skendzic et al. (2007) due to the exclusion of *Coix*.

Other genera and unresolved topology

The results from all analyses confirmed the monophyly of *Arthraxon* (100 BP, 1.00 PP). Morphologically, *Arthraxon* is distinguished from all other Andropogoneae by its lemmas with a sub-basal awn.

Hyparrhenia was resolved as monophyletic (88 BP, 1.00 PP). Clayton and Renvoize (1986) suggested that *Hyparrhenia* is closely related to *Andropogon* and *Cymbopogon*. This study also found that a *Hyparrhenia* clade was sister to *Andropogon gerardii* (67 BP, 0.97 PP). This clade was grouped together with three other taxa, *Cymbopogon*, *Schizachyrium* and *Andropogon ascinodis* (76 BP, 1.00 PP). *Themeda* was also monophyletic (53 BP, 1.00 PP). Morphologically, *Themeda* is distinctive among the sampled Anthistiriinae in that its racemes have two large homogamous pairs at the base and upper lemmas are entire. According to Clayton and Renvoize (1986), the position of *Themeda* within Anthistiriinae should be between *Heteropogon* and *Iseilema*. The relationship between *Iseilema* and *Themeda* was also found in the study of Kellogg and Watson (1993). However, none of our analyses supported this hypothesis.

Although several well supported groups have been identified, the present matrix with three non-coding markers (*trn*L–F, *atp*β–*rbc*L and ITS) was insufficient to provide enough phylogenetic informative characters to resolve many evolutionary relationships at the intergeneric level in Andropogoneae. There are several reports

of phylogenetic analyses within the angiosperms that have encountered similar difficulties with resolution due to short lengths of internal branches relative to terminal branches (e.g., Kellogg 2000, Mathews et al. 2002, Wortley et al. 2005). These patterns have been explained by suggesting that the groups have undergone rapid phylogenetic radiation (and that the phylogenetic signal to resolve the inter-relationships of the lineages has been lost). However, the theory behind such empirical observations and deductions is not well developed or reported (Moore et al. 2007). Adding more data and more taxa are the only ways of resolving these difficult groups (Hillis et al. 2003, Hodkinson et al. 2007a, Pirie et al. 2008). Choice of gene is also critical and it would be worthwhile sequencing a large number of more slowly evolving genes to reconstruct the phylogenetic patterns inside these clades of Panicoideae (Hodkinson et al. 2007a, Moore et al. 2007, Pirie et al. 2008).

Conclusions and resulting taxonomy

In this study, 42 out of 85 genera of Andropogoneae, representing 11 subtribes, were sampled and sequenced using three non-coding markers from both chloroplast and nuclear ribosomal DNA. We present our taxonomy of Arundinellinae and Garnotiinae:

Subtribe Arundinellinae Honda, J. Fac. Sci. Univ. Tokyo Bot. 3: 303. 1930. **Type:** *Arundinella* Raddi.

Arundinellinae as treated here includes only its type genus: *Arundinella*. This subtribe was first established with three genera: *Arundinella*, *Phaenosperma* and *Thysanolaena* (Honda, 1930). However, a recent systematic treatment of Poaceae placed *Phaenosperma* in Pooideae and grouped *Thysanolaena* within Centothecoideae (GPWG, 2001).

Subtribe Garnotiinae Pilger in Engl. & Prantl, Nat. Pflanzenfam. Aufl. 14d: 167. 1956. Garnotiinae Pilger in Bot. Jahrb. 76(3): 341. 1954. nomen. **Type:** *Garnotia* Brongn.

Included genus: *Garnotia*.

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References

Aliscioni SS, Giussani LM, Zuloaga FO & Kellogg EA (2003) A molecular phylogeny of *Panicum* (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. *American Journal of Botany* 90: 796–821.

Baldwin BG, Sanderson M J, Porter JM, Wojciechowski MF, Campbell C & Donoghue MJ (1995) The *ITS* region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Gardens* 82: 247–277.

Barker NP, Linder HP & Harley EH (1999) Sequences of the grass-specific insert in the chloroplast *rpo*C2 gene elucidate generic relationships of the Arundinoideae (Poaceae). *Systematic Botany* 23: 327–350.

Bentham G (1882) Notes on Gramineae. *The Journal of the Linnean Society* 19: 14–134.

Bess EC, Doust AN & Kellogg EA (2005) A naked grass in the "bristle clade": a phylogenetic and developmental study of *Panicum* section *Bulbosa* (Paniceae: Poaceae). *International Journal of Plant Sciences* 166: 371–381.

Bomblies K & Doebley J (2005) Pleiotropic effects of the duplicate maize *FLORICAULA/LEAFY* genes *zfl1* and *zfl2* on traits under selection during maize domestication. *Genetics* 172: 519–531.

Bor NL (1955) Notes on Asiatic grasses XXIII: *Jansenella* Bor, a new genus of Indian grasses. *Kew Bulletin* 1955: 93–99.

Bouchenak-Khelladi Y, Salamin N, Savolainen V, Forest F, van der Bank M, Chase MW & Hodkinson TR (2008) Large multi-gene phylogenetic trees of the grasses (Poaceae): progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution* 47: 488–505.

Chase MW & Hills HH (1991) Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

Christin P-A, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, Savolainen V & Salamin N (2008) Oligocene CO_2 decline promoted C_4 photosynthesis in grasses. *Current Biology* 18: 37–43.

Clayton WD (1972) The awned genera of Andropogoneae: studies in the Gramineae XXXI. *Kew Bulletin* 27: 457–474.

Clayton WD (1973) Studies in the Gramineae XXXIII: the awnless genera of Andropogoneae. *Kew Bulletin* 28: 49–58.

Clayton WD & Renvoize SA (1986) *Genera Graminum. Grasses of the world. Kew Bulletin, Additional Series* 13: 1–389.

Davidse G & Ellis RP (1984) *Steyermarkochloa unifolia*, a new genus from Venezuela and Columbia (Poaceae: Arundinoideae: Steyermarkochloeae). *Annals of the Missouri Botanical Garden* 71: 994–1012.

Davidse G & Ellis RP (1987) *Arundoclaytonia*, a new genus of Steyermarkochloeae (Poaceae: Arundinoideae) from Brazil. *Annals of the Missouri Botanical Garden* 74: 479–490.

De Wet JMJ & Harlan JR (1966) Morphology of the compilospecies *Bothriochloa intermedia. American Journal of Botany* 53: 94–98.

De Wet JMJ & Harlan JR (1970) *Bothriochloa intermedia.* A taxonomic dilemma. *Taxon* 19: 339–340.

Donadio S, Giussani LM, Kellogg EA, Zuolaga FO & Morrone O (2009) A preliminary molecular phylogeny of *Pennisetum* and *Cenchrus* (Poaceae-Paniceae) based on the trnL-F, rpl16 chloroplast markers. *Taxon* 58: 392–404.

Doust AN & Kellogg EA (2002) Inflorescence diversification in the panicoid "bristle grass" clade (Paniceae, Poaceae): evidence from molecular phylogenies and developmental morphology. *American Journal of Botany* 89: 1203–1222.

Doust AN, Penly AM, Jacobs SWL & Kellogg EA (2007) Congruence, conflict, and polyploidization shown by nuclear and chloroplast markers in the monophyletic "bristle clade" (Paniceae, Panicoideae, Poaceae). *Systematic Botany* 32: 531–544.

Doyle JJ & Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin for the Botany Society of America* 19: 11–15.

Duvall MR, Davis JI, Clark LG, Noll JD, Goldman DH & Sánchez-Ken JG (2007) Phylogeny of the grasses (Poaceae) revisited. *Aliso* 23: 237–247.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Giussani LM, Cota-Sanches JH, Zuloaga FO & Kellogg EA (2001) A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C_4 photosynthesis. *American Journal of Botany* 88: 1993–2012.

Gomez-Martinez R & Culham A (2000) Phylogeny of the subfamily Panicoideae with emphasis on the tribe Paniceae: evidence from the *trnL-F* chloroplast DNA region. Pp. 136–140 in Jacobs SWL & Everett J (eds) *Grasses: Systematics and Evolution*. (Collingwood, Victoria: CSIRO Publishing)

GPWG (2001) Phylogeny and subfamily classification of the grasses (Poaceae). *Annals of the Missouri Botanical Garden* 88: 373–430.

Hackel E (1889) Andropogoneae. Pp. 1–716 in De Candolle A & De Candolle C (eds) *Monographiae Phanerogamarum,* vol. 6.(Sumptibus G. Masson: Paris)

Harlan JR & De Wet JMJ (1963) The compilospecies concept. *Evolution* 17: 497–501.

Hillis DM, Pollock DD, McGuire JA & Zwickl DJ (2003) Is sparse taxon sampling a problem for phylogenetic inference? *Systematic Biology* 52: 124–126.

Hilu KW & Wright K (1982) Systematics of Gramineae: a cluster analysis study. *Taxon* 31: 9–36.

Hilu KW, Alice LA & Liang HP (1999) Phylogeny of Poaceae inferred from *matK* sequences. *Annals of the Missouri Botanical Garden* 86: 835–851.

Hodkinson TR, Salamin N, Chase MW, Bouchenak-Khelladi Y, Renvoize SA & Savolainen V (2007a) Large trees, supertrees and diversification of the grass family. *Aliso* **23**: 248–258.

Hodkinson TR, Waldren S, Parnell JAN, Kelleher CT, Salamin K & Salamin N (2007b) DNA banking for plant breeding, biotechnology and biodiversity evaluation. *Journal of Plant Research* 120: 17–29.

Huelsenbeck JP & Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

Ibrahim DG, Burke T, Ripley BS & Osborne CP (2009) A molecular phylogeny of the genus *Alloteropsis* (Panicoideae, Poaceae) suggests an evolutionary reversion from C_4 to C_3 photosynthesis. *Annals of Botany* 103: 127–136.

Jannink TA & Veldkamp JF (2002) Revision of Chionachninae (Gramineae: Andropogoneae). *Blumea* 47: 545–580.

Kelchner SA (2000) The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* 87: 182–198.

Kellogg EA (2000) Molecular and morphological evolution in Andropogoneae. Pp. 149–158 in Jacobs SWL & Everett J (eds) *Grasses: Systematics and Evolution*. (CSIRO Publishing: Collingwood)

Kellogg EA & Campbell CS (1987) Phylogenetic nalyses of the Gramineae. Pp. 310–322 in Soderstrom TR, Hilu KW, Campbell CS & Barkworth ME (eds) *Grass Systematics and Evolution*. (Smithsonian Press: Washington, D.C)

Kellogg EA & Watson L (1993) Phylogenetic studies of a large dataset. I. Bambusoideae, Andropogonodae and Pooideae (Gramineae). *Botanical Review* 59: 273–343.

Mathews S, Spangler RE, Mason-Gamer RJ & Kellogg EA (2002) Phylogeny of Andropogoneae inferred from *phytochrome B*, *GBSSI* and *ndhF*. *International Journal of Plant Sciences* 163: 441–450.

Moore MJ, Bell CD, Soltis PS & Soltis DE (2007) Using plastid genomic-scale data to resolve enigmatic relationships among basal angiosperms. *Proceedings of the National Academy of Sciences* 104: 19363–19368.

Phipps JB (1966) Studies in the Arundinelleae III: check-list and key to the genera. *Kirkia* 5: 235–258.

Pirie MD, Humphreys AM, Galley C, Barker NP, Verboom GA, Orlovich D, Draffin SJ, Lloyd K, Baeza CM, Negritto M, Ruiz E, Cota-Sánchez JH, Reimer E & Linder HP (2008) A novel supermatrix approach improves resolution of phylogenetic relationships in a comprehensive sample of danthonioid grasses. *Molecular Phylogenetics and Evolution* 48: 1106–1119.

Posada D & Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 49: 817–818.

Rambaut A (1996) *Se-Al Sequence Alignment Editor*, vers. 2.0a11. (University of Oxford: Oxford)

Reeves G, Chase MW, Goldblatt P, Rudall P, Fay MF, Cox AV, Lejeune B & Souza-Chies T (2001) Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *American Journal of Botany* 88: 2074–2087.

Renvoize SA & Clayton WD (1992) Classification and evolution of grasses. Pp. 3–37 in Chapman GP (ed.) *Grass Evolution and Domestication*. (Cambridge University Press: Cambridge)

Samuel R, Pinsker W & Kiehn M (1997) Phylogeny of some species of *Cyrtandra* (Gesneriaceae) inferred from the *atp*β/*rbc*L cpDNA intergene region. *Botanica Acta* 110: 503–510.

Sánchez-Ken JG & Clark LG (2001) Gynerieae: a new neotropical tribe of grasses (Poaceae). *Novon* 11: 350–352.

Sánchez-Ken JG, Clark LG, Kellogg EA & Kay EE (2007) Reinstatement and emendation of subfamily Micrairoideae (Poaceae). *Systematic Botany* 32: 71–80.

Sánchez-Ken JG & Clark LG (2007) Phylogenetic relationships within the Centothecoideae + Panicoideae clade (Poaceae) based on *ndhF* and *rpl16* sequences and structural data. *Aliso* 23: 487–502.

Skendzic EM, Columbus JT & Cerros-Tlatilpa R (2007) Phylogenetics of Andropogoneae (Poaceae: Panicoideae) based on nuclear ribosomal internal transcribed spacer and chloroplast *trn*L-F sequences. *Aliso* 23: 530–544.

Spangler R, Zaitchick B, Russo E & Kellogg EA (1999) Andropogoneae evolution and generic limits in *Sorghum* (Poaceae) using *ndhF* sequences. *Systematic Botany* 24: 267–283.

Steudel EG (1854) *Synopsis plantarum glumacearum,* vol. 1. (J. B. Metzler: Stuttgart)

Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.

Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)* vers. 4.0b10. (Sinauer Associates: Sunderland)

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Tateoka T (1958) Note on some grasses VIII: on leaf structure of *Arundinella* and *Garnotia*. *Botanical Gazette* 20: 101–109.

Tateoka T (1962) Starch grains of endosperm in grass systematics. *Botanical Magazine, Tokyo* 75: 377–383.

Teerawatananon A & Hodkinson TR (2008) *Jansenella griffithiana* (Müll. Hal.) Bor (Gramineae/Poaceae): a new record for Thailand. *Thai Forest Bulletin (Botany)* 36: 109–113.

Thomson JA (2002) An improved non-crogenic transport and storage preservative facilitating DNA extraction from 'difficult' plants collected at remote sites. *Telopea* 9: 755–760.

Vicentini A, Barber JC, Aliscioni AA, Giussani LM & Kellogg EA (2008) The age of the grasses and clusters of origins of C_4 photosynthesis. *Global Change Biology* 14: 2693–2977.

Watson L & Dallwitz MJ (1992 onwards) *The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references.* (http://delta-intkey.com) (Downloaded 02 March 2009).

Wills KE, Whalley RDB & Bruhl JJ (2000) Systematic studies in Paniceae (Poaceae): *Homopholis* and *Whalleya gen*. et *sp nov*. *Australian Systematic Botany* 13: 437–468.

Wortley AH, Rudall PJ, Harris DJ & Scotland RW (2005) How much data are needed to resolve a difficult phylogeny?: case study in Lamiales. *Systematic Biology* 54: 697–709.

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Appendix 1. Species sequenced for *trn*L-*trn*F, *atp*β-*rbc*L and ITS, with vouchers and GenBank accession numbers.

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Subfamily Bambusoideae	Subfamily Bambusoideae	Subfamily Ehrhartoideae				
	Supertribe Oryzodae					
Tribe Oryzeae	Tribe Oryzeae	Tribe Oryzeae				
Leersia hexandra Sw.			Hodkinson 636/TCD	EU434093	EU434157	AF019793
Oryza rufipogon Griff.			AT&SS 164/THNHM	EU434094	EU434158	DQ355276
Oryza sativa L.			Hodkinson 46/TCD	EU434095	EU434159	AF169230
Subfamily Pooideae	Subfamily Stipoideae	Subfamily Pooideae				
Tribe Lygeeae	Tribe Lygeeae	Tribe Lygeeae				
Lygeum spartum Loefl. ex. L.			Hodkinson 18/TCD	EU434098	EU434162	AF019797
	Subfamily Pooideae					
Tribe Poeae	Tribe Poeae	Tribe Poeae				
Lolium perenne L.			Hodkinson 29/TCD	EU434099	EU434163	GQ870136
Subfamily Arundinoideae	Subfamily Arundinoideae	Subfamily Arundinoideae				
Tribe Aristideae	Tribe Aristideae	in Subfamily Aristidoideae				
Aristida hygrometrica R.Br.			SJ 9547/TCD	GQ869906	GQ870018	GQ870137
Tribe Arundineae	Tribe Arundineae	in Subfamily Arundinoideae				
Arundo donax L.			Hodkinson 131/TCD	GQ869907	GQ870019	AF019809
Danthonia decumbens (L.) DC.	Tribe Danthonieae	in Subfamily Danthonioideae	Salamin s.n.	GQ869908	GQ870020	AF019183
Tribe Micraireae	Tribe Micrairieae	in Subfamily Micrairoideae				

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Micraira subulifolia F.Muell.			AF019859	N/A	N/A	AF019859
Tribe Thysanolaeneae	Tribe Arundineae or Centothecoideae?	in Subfamily Centothecoideae				
Thysanolaena latifolia (Roxb. ex Hornem.) Honda			Hodkinson K-10/TCD	GQ869909	GQ870021	AF019854
Subfamily Chloridoideae	Subfamily Chloridoideae	Subfamily Chloridoideae				
Tribe Cynodonteae	Tribe Cynodonteae	Tribe Cynodonteae				
subtribe Chloridinae						
Microchloa indica (L.f.) P. Beauv.			AT&SS 291/THNHM	GQ869910	GQ870022	GQ870138
Tribe Eragrostideae	Tribe Eragrostideae	Tribe Eragrostideae				
subtribe Eleusininae						
Eleusine indica (L.) Gaertn.			Hodkinson 126	GU045493	GU045489	N/A
Tripogon trifidus Munro ex Hook.f.			AT&SS 771/THNHM	GQ869911	GQ870023	GQ870139
subtribe Sporobolinae	subtribe Sporobolinae	subtribe Sporobolinae				
Sporobolus kerrii Bor			AT&SS 940/THNHM	GQ869912	GQ870024	GQ870140
Subfamily Centothecoideae	Subfamily Centothecoideae	Subfamily Centothecoideae				
Tribe Centotheceae	Tribe Centotheceae	Tribe Centotheceae				
Centotheca lappacea (L.) Desv.			Hodkinson 235/TCD	GQ869913	GQ870025	AF019814
Chasmanthium latifolium (Michx.) H.O.Yates			K-15-Nicolas	GQ869914	GQ870026	AF019815
Lophatherum gracile Brongn.			AT&SS 684	GU045492	GU045488	N/A
Subfamily Panicoideae	Subfamily Panicoideae	Subfamily Panicoideae				
Tribe Isachneae	in supertribe Panicodae	in Subfamily Micraioideae				
Isachne albens Trin.			AT&SS 778/THNHM	GQ869915	GQ870027	GQ870141

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Isachne globosa (Thunb. ex Murray) Kuntze			AT&SS 130704-3/THNHM	GQ869916	GQ870028	GQ870142
Isachne gracilis C.E.Hubb.			AT&SS 84	GU045494	GU045490	N/A
Isachne pulchella Roth			AT&SS 445/THNHM	GQ869917	GQ870029	GQ870143
Isachne smitinandiana A.Camus			AT&SS 172	GU045495	GU045491	N/A
Coelachne simpliciuscula (Wight & Arn. ex Steud.) Munro ex Benth.			AT&SS 686/THNHM	GQ869918	GQ870030	GQ870144
Tribe Eriachneae	in Subfamily Arundinoideae	in Subfamily Micraroideae				
Eriachne aristidea F.Muell.			SJ 9573/TCD	GQ869919	GQ870031	GQ870145
Eriachne helmsii Domin ex Hartley			SJ 9575/TCD	GQ869920	GQ870032	GQ870146
Eriachne mucronata R.Br.			SJ 9504/TCD	GQ869921	GQ870033	GQ870147
Eriachne pallescens R.Br.			AT&SS 408/THNHM	GQ869922	GQ870034	GQ870148
Eriachne triseta Nees ex Steud.			AT&SS 120704-7/THNHM	GQ869923	GQ870035	GQ870149
Tribe Arundinelleae	in supertribe Panicodae	Tribe Arundinelleae				
Arundinella bengalensis (Spreng.) Druce			AT&SS 546/THNHM	GQ869924	GQ870036	GQ870150
Arundinella berteroniana (Schult.) Hitchc. & Chase			SL 19182/TCD	GQ869925	GQ870037	GQ870151
Arundinella hirta (Thunb.) Tanaka			DQ004957; DQ005019	DQ004957	N/A	DQ005019
Arundinella hispida (Humb. & Bonpl. ex Willd.) Kuntze			SL 20203/THNHM	GQ869926	GQ870038	GQ870152
Arundinella nepalensis Trin.			AT&SS 722/THNHM	GQ869927	GQ870039	GQ870153
Arundinella setosa Trin.			AT&SS 293/THNHM	GQ869928	GQ870040	GQ870154
Arundinella setosa Trin. var. *esetosa* Bor ex S.M.Phillips & S. L.Chen			AT&SS 792/THNHM	GQ869929	GQ870041	GQ870155

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Arundinella kokutensis Teerawat. & Sungkaew.			AT&SS 914/THNHM	GQ869930	GQ870042	GQ870156
Danthoniopsis dinteri (Pilg.) C.E.Hubb.		in Subfamily Centothecoideae	GSK s.n./Kew, MWC 22002	GQ869931	GQ870043	GQ870157
Garnotia acutigluma (Steud.) Ohwi			AT&SS 894/THNHM	GQ869932	GQ870044	GQ870158
Garnotia patula (Munro) Benth.			AT&SS 813/THNHM	GQ869933	GQ870045	GQ870159
Garnotia tenella (Arn. ex Miq.) Janowski			AT&SS 669/THNHM	GQ869934	GQ870046	GQ870160
Tristachya leucothrix Trin. ex Nees			DQ005008; DQ005088	DQ005008	N/A	DQ005088
Tribe Paniceae	in supertribe Panicodae	Tribe Paniceae				
Walwhalleya subxerophila (Domin) Wills & J.J.Bruhl			SJ 9436/TCD	GQ869935	GQ870047	GQ870161
subtribe Setarinae						
Acroceras tonkinense (Balansa) C.E.Hubb. ex Bor			AT&SS 630/THNHM	GQ869936	GQ870048	GQ870162
Alloteropsis cimicina (L.)			AT&SS 130/THNHM	GQ869937	GQ870049	GQ870163
Cyrtococcum patens (L.) A.Camus			AT&SS 984/THNHM	GQ869938	GQ870050	GQ870164
Entolasia stricta (R.Br.) Hughes			SJ 9366/TCD	GQ869939	GQ870051	GQ870165
Ichnanthus vicinus (F.M.Bailey) Merr.			AT&SS 795/THNHM	GQ869940	GQ870052	GQ870166
Ottochloa nodosa (Kunth) Dandy			AT&SS 646/THNHM	GQ869941	GQ870053	GQ870167
Panicum auritum J.Presl ex Nees			AT&SS 645/THNHM	GQ869942	GQ870054	GQ870168
Panicum notatum Retz.			AT&SS 732/THNHM	GQ869943	GQ870055	GQ870169
Paspalum notatum Flüggé			K-8-Nicolas	GQ869944	GQ870056	GQ870170
Pseudechinolaena polystachya (Kunth) Stapf			AT&SS 797/THNHM	GQ869945	GQ870057	GQ870171
Sacciolepis indica (L.) Chase			AT&SS 695/THNHM	GQ869946	GQ870058	GQ870172
Sacciolepis indica var. *turgida* (Ridl.) Gilliland			AT&SS 947/THNHM	GQ869947	GQ870059	GQ870173

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Sacciolepis myosuroides (R.Br.) Chase ex E.G.Camus			AT&SS 948/THNHM	GQ869948	GQ870060	GQ870174
Setaria palmifolia (J.König) Stapf			AT&SS 786/THNHM	GQ869949	GQ870061	GQ870175
subtribe Digitarinae						
Alexfloydia repens B.K.Simon			SJ 9391/TCD	GQ869950	GQ870062	GQ870176
Digitaria sanguinalis (L.) Scop.			Hodkinson 110/TCD	GQ869951	GQ870063	AY116268
Homopholis belsonii C.E.Hubb.			SJ 9431/TCD	GQ869952	GQ870064	GQ870177
subtribe Cenchrinae						
Cenchrus incertus M.A.Curtis			Salamin s.n.	GQ869953	GQ870065	AY116301
Pennisetum purpureum Schumach.			SJ 9394/TCD	GQ869954	GQ870066	GQ870178
Pennisetum macrochaetum Jacq.			Hodkinson 117	GQ869955	GQ870067	AY116266
Pennisetum sp.			WK 104/THNHM	GQ869956	GQ870068	GQ870179
subtribe Spinificinae						
Spinifex littoreus (Burm.f.) Merr.			AT&SS 657/THNHM	GQ869957	GQ870069	GQ870180
	Supertribe Andropogonodae					
Tribe Andropogoneae	Tribe Andropogoneae	Tribe Andropogoneae				
subtribe Andropogoninae	subtribe Andropogoninae					
Andropogon ascinodis C.B.Clarke			AT&SS 849/THNHM	GQ869958	GQ870070	GQ870181
Andropogon gerardii Vitman			Hodkinson 15	AY116263	GQ870071	AY116299
Arthraxon hispidus (Thunb.) Makino			AT&SS 661/THNHM	GQ869969	GQ870072	GQ870182
Arthraxon lanceolatus (Roxb.) Hochst.			AT&SS 720/THNHM	GQ869960	GQ870073	GQ870183
Cymbopogon citratus (DC.) Stapf			Hodkinson 42/TCD	AY116258	GQ870074	AF019823

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Schizachyrium brevifolium (Sw.) Nees ex Büse			AT&SS 750/THNHM	GQ869961	GQ870075	GQ870184
Schizachyrium sanguineum (Retz.) Alston			AT&SS 751/THNHM	GQ869962	GQ870076	GQ870185
subtribe Anthistiriinae						
Hyparrhenia diplandra (Hack.) Stapf			AT&SS 858/THNHM	GQ869963	GQ870077	GQ870186
Hyparrhenia rufa (Nees) Stapf			AT&SS 735/THNHM	GQ869964	GQ870078	GQ870187
Heteropogon triticeus (R.Br.) Stapf ex Craib			AT&SS 733/THNHM	GQ869965	GQ870079	GQ870188
Themeda arundinacea (Roxb.) A.Camus			AT&SS 739/THNHM	GQ869966	GQ870080	GQ870189
Themeda triandra Forssk.			K-2-Nicolas	GQ869967	GQ870081	AY116261
Themeda villosa (Poir.) A.Camus			AT&SS 414/THNHM	GQ869968	GQ870082	GQ870190
subtribe Dimeriinae						
Dimeria fuscescens Trin.			AT&SS 830/THNHM	GQ869969	GQ870083	GQ870191
Dimeria ornithopoda Trin.			AT&SS 685/THNHM	GQ869970	GQ870084	GQ870192
Dimeria sinensis Rendle			AT&SS 819/THNHM	GQ869971	GQ870085	GQ870193
subtribe Germainiinae						
Apocopis collinus Balansa			AT&SS 977/THNHM	GQ869972	GQ870086	GQ870194
Apocopis courtllumensis (Steud.) Henrard			AT&SS 928/THNHM	GQ869973	GQ870087	GQ870195
Apocopis intermedius (A.Camus) Chai-Anan			AT&SS 934/THNHM	GQ869974	GQ870088	GQ870196
Apocopis siamensis A.Camus			AT&SS 975/THNHM	GQ869975	GQ870089	GQ870197
Germainia capitata Balansa & Poitrass.			AT&SS 834/THNHM	GQ869976	GQ870090	GQ870198
Germainia khasyana Hack.			AT&SS 906/THNHM	GQ869977	GQ870091	GQ870199
Germainia lanipes Hook.f.			AT&SS 677/THNHM	GQ869978	GQ870092	N/A
Germainia pilosa Chai-Anan			AT&SS 886/THNHM	GQ869979	GQ870093	GQ870200

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
subtribe Ischaeminae						
Apluda mutica L.			AT&SS 415/THNHM	GQ869980	GQ870094	GQ870201
Ischaemum indicum (Houtt.) Merr.			AT&SS 650/THNHM	GQ869981	GQ870095	GQ870202
Ischaemum muticum L.			AT&SS 694/THNHM	GQ869982	GQ870096	GQ870203
Kerriochloa siamensis C.E.Hubb.			AT&SS 959/THNHM	GQ869983	GQ870097	GQ870204
Sehima nervosum (Rottler ex Roem. & Schult.) Stapf			AT&SS 678/THNHM	GQ869984	GQ870098	GQ870205
Thelepogon elegans Roth			AT&SS 697/THNHM	GQ869985	GQ870099	GQ870206
subtribe Rottboelliinae	subtribe Rottboelliinae					
Elionurus citreus (R.Br.) Munro ex Benth.			SJ 9561/TCD	GQ869986	GQ870100	GQ870207
Eremochloa bimaculata Hack.			AT&SS 899/THNHM	GQ869987	GQ870101	GQ870208
Hackelochloa granularis (L.) Kuntze			AT&SS 769/THNHM	GQ869988	GQ870102	GQ870209
Hemarthria longiflora (Hook.f.) A.Camus			AT&SS 100704-3/THNHM	GQ869989	GQ870103	GQ870210
Hemarthria partensis (Balansa) Clayton			AT&SS 892/THNHM	GQ869990	GQ870104	GQ870211
Mnesithea formosa (R.Br.) de Koning & Sosef			SJ 9526/TCD	GQ869991	GQ870105	GQ870212
Phacelurus zea (C.B.Clarke) Clayton			AT&SS 860/THNHM	GQ869992	GQ870106	GQ870213
subtribe Sorghinae	subtribe Andropogoninae					
Bothriochloa pertusa (L.) A.Camus			SJ 9427/TCD	GQ869993	GQ870107	GQ870214
Capillipedium assimile (Steud.) A.Camus			AT&SS 791/THNHM	GQ869994	GQ870108	GQ870215
Chrysopogon orientalis (Desv.) A.Camus			AT&SS 647/THNHM	GQ869995	GQ870109	GQ870216
Dichanthium theinlwinii Bor			AT&SS 699/THNHM	GQ869996	GQ870110	GQ870217
Pseudosorghum fasciculare (Roxb.) A.Camus			AT&SS 698/THNHM	GQ869997	GQ870111	GQ870218

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Sorghum nitidum (Vahl) Pers.			AT&SS 713/THNHM	GQ869998	GQ870112	GQ870219
Sorghum propinquum (Kunth) Hitchc.			AT&SS 696/THNHM	GQ869999	GQ870113	GQ870220
subtribe Saccharinae						
Eulalia quadrinervis (Hack.) Kuntze			AT&SS 706/THNHM	GQ870000	GQ870114	GQ870221
Eulalia siamensis Bor			AT&SS 979/THNHM	GQ870001	GQ870115	GQ870222
Pseudopogonatherum contortum (Brongn.) A.Camus			AT&SS 953/THNHM	GQ870002	GQ870116	GQ870223
Eulaliopsis binata (Retz.) C.E.Hubb.			AT&SS 561/THNHM	GQ870003	GQ870117	GQ870224
Imperata cylindrica (L.) Raeusch.			K. Kowarat 108/THNHM	GQ870004	GQ870118	AY116297
Microstegium fasciculatum (L.) Henrard			AT&SS 728/THNHM	GQ870005	GQ870119	GQ870225
Miscanthus nepalensis (Trin.) Hack.			66/1293	AY116252	GQ870120	AY116292
Miscanthus sinensis Andersson			Hodkinson & Renvoize 5/ Kew	GQ870006	GQ870121	GQ870226
Pogonatherum crinitum (Thunb.) Kunth			AT&SS 865/THNHM	GQ870007	GQ870122	GQ870227
Polytrias amaura (Büse) Kuntze			AT&SS 866/THNHM	GQ870008	GQ870123	GQ870228
Spodiopogon sibiricus Trin.			114	AY116257	GQ870124	GQ870229
Saccharum arundinaceum Retz.			AT&SS 864/THNHM	GQ870009	GQ870125	GQ870229
Saccharum narenga (Nees ex Steud.) Wall. ex Hack.			AT&SS 783/THNHM	GQ870010	GQ870126	GQ870230
Saccharum officinarum L.			Hodkinson & Renvoize 104/Kew	AY116253	GQ870127	AY116284
subtribe Tripsacinae	tribe Maydeae					
Tripsacum australe H.C.Cutler & E.S.Anderson			U46655	N/A	N/A	U46655

Taxon/Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Voucher/Herbarium or DNA accession number	Gene regions		
				*trn*L-*trn*F	*atp*β-*rbc*L	ITS
Tripsacum dactyloides (L.) L.			4027; U46652	GQ870011	GQ870128	DQ005086
Zea diploperennis H.Iltis, Doebley & R.Guzmán			Kew 1986-5307	AY116260	GQ870129	AY116294
Zea mays L.			WK 105/THNHM	GQ870012	GQ870130	U46613
subtribe Chionachninae						
Chionachne cyathopoda (F.Muell.) F.Muell. ex Benth.			AF019819	N/A	N/A	AF019819
Chionachne massiei Balansa			AT&SS 731/THNHM	GQ870013	GQ870131	GQ870231
Polytoca digitata (L.f.) Druce			AT&SS 844/THNHM	GQ870014	GQ870132	GQ870232
Polytoca wallichiana (Nees ex Steud.) Benth.			AT&SS 683/THNHM	GQ870015	GQ870133	GQ870233
subtribe Coicinae						
Coix gasteenii B.K.Simon			SJ 9560/TCD	GQ870016	GQ870134	GQ870234
Coix lacryma-jobi L.			AT&SS 816/THNHM	GQ870017	GQ870135	U46660

Abbreviations are as follows: AT, A. Teerawatananon; WK, W. Kowarat; N/A, not applicable; SJ, S.W.L. Jacobs; SS, S. Sungkaew; KEW, Kew Herbarium, England, U.K.; TCD, Herbarium, School of Botany, Trinity College, Dublin, Ireland; THNHM, Thailand Natural History Museum, Techno Polis, Pathum Thani, Thailand.

Appendix 2. Supplementary information.

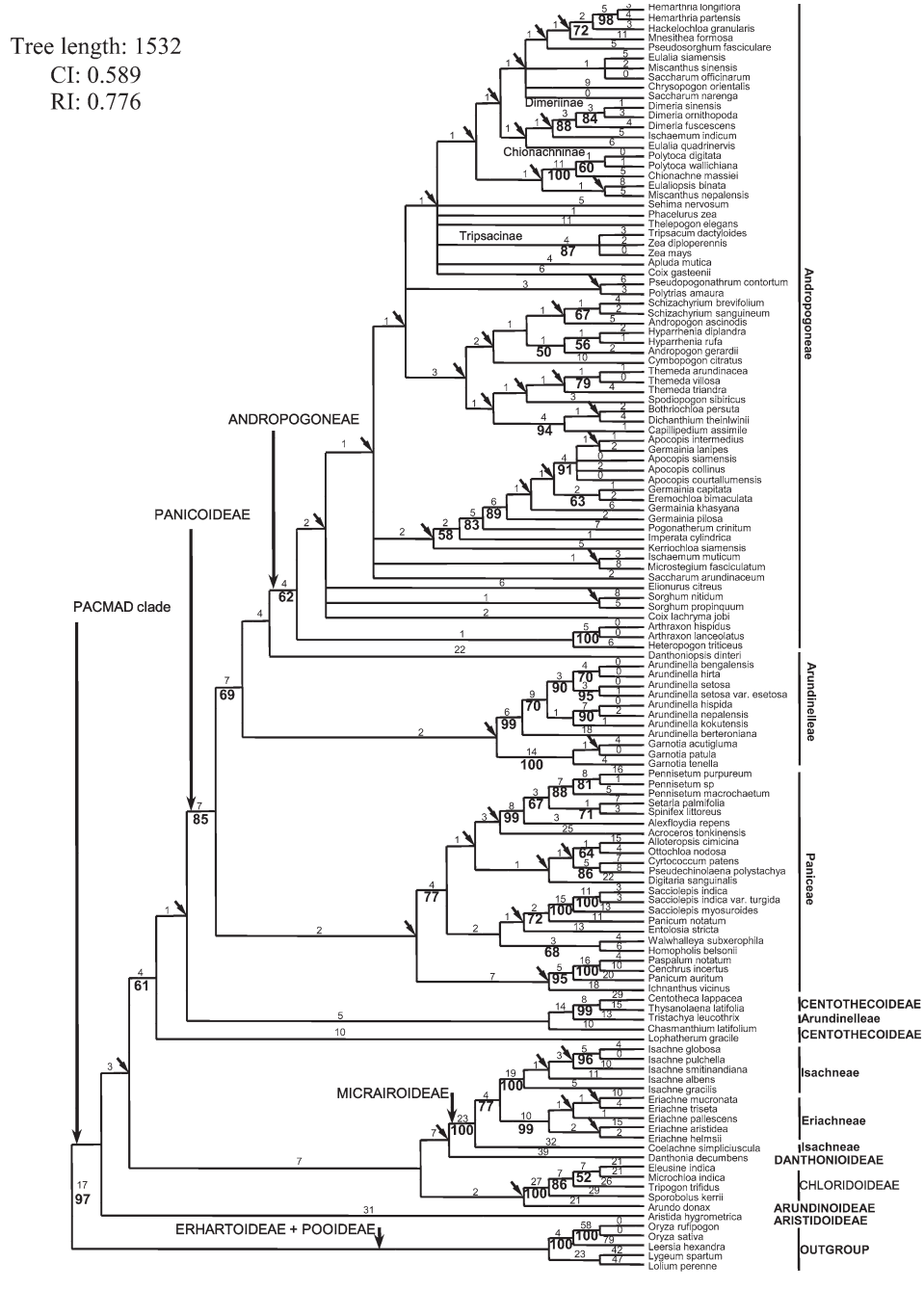

Fig. S1. One of 13,100 equally most parsimonious trees shown as a cladogram obtained from comparative sequence analysis of combined chloroplast DNA sequences. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in the strict consensus. The PACMAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.

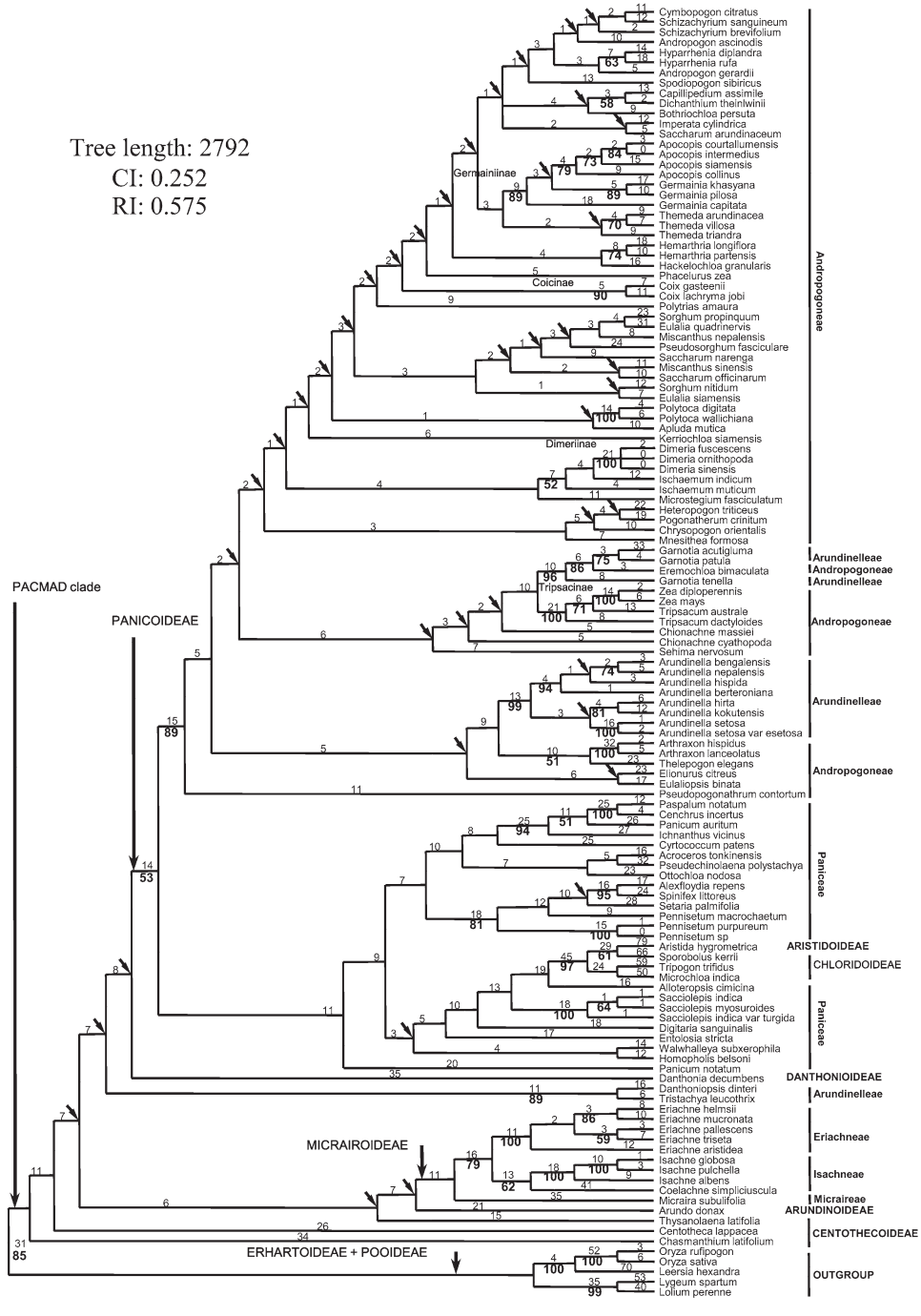

Fig. S2. One of 3,100 equally most parsimonious trees shown as a cladogram obtained from comparative sequence analysis of the ITS DNA sequences. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in the strict consensus. The PACMAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.