Cleaning up the grasses dustbin: systematics of the Arundinoideae subfamily (Poaceae)

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Abstract Among the 12 subfamilies currently considered in the systematics of Poaceae, the Arundinoideae have long been considered as a dustbin group, with a diversity of forms putatively hiding *incertae sedis*. Because this subfamily has been poorly investigated using molecular markers for the last two decades, the present study provides the first complete phylogeny of the Arundinoideae based on five plastid DNA loci sequenced for 12 genera, and analysed with and without plastome data from previous studies. The refined Arundinoideae appear to be a robust evolutionary lineage of Poaceae, divided into three tribes with some biogeographical patterns: (1) tribe Arundineae, the most heterogeneous tribe, including Eurasian *Arundo*, Australian *Amphipogon* and *Monachather*, and South African *Dregeochloa*; (2) tribe Crinipedeae (described here), including *Crinipes*, *Elytrophorus*, *Styppeiochloa* and *Pratochloa* (described here), with a South and East African distribution; and (3) tribe Molinieae, including *Hakonechloa*, *Molinia* and *Phragmites*, with a Eurasian distribution. Despite reduction in size, this small subfamily conserves a high diversity of morphological forms, with several small but highly differentiated genera. Finally, the molecular dating approach provides an evolutionary framework to understand the diversification of Arundinoideae, refuting Gondwanan vicariance between genera and suggesting capability for long distance dispersal.

Keywords *Arundo*, Crinipedeae, *Phragmites,* Phylogeny, *Pratochloa,* Taxonomy

Introduction

Poaceae Barnhart is one of the most fascinating plant families by its usefulness for humans and its ecological and taxonomic diversification. Although several angiosperm families possess higher species richness, grasses exceed all others by their ecological significance (Kellogg 2000). Thanks to numerous adaptive strategies such as C_4 metabolism (GPWG II 2012), Poaceae occur in almost all terrestrial ecosystems and dominate grassland biomes covering a quarter of land surfaces (Shantz 1954). In addition, about half of the annual production of global agriculture is due to the domestication of four of the *c.* 12,000 grass species (http://faostat.fao. org/). In this context, the understanding of grass evolutionary history represents a major scientific challenge. Since the groundwork studies by Brown (1810, 1814), Poaceae have been periodically revised in several subfamilies and tribes (a synthesis in Prat 1960; Clayton and Renvoize 1986). For the last two decades, molecular studies have accelerated this systematics resolution (Clark et al. 1995; Duvall and Morton 1996 ; Hilu et al. 1999; Hsiao et al. 1999; GPWG 2001), with the current consideration of 12 subfamilies, 51 tribes and 771 genera (Soreng et al. 2015), structured in two main lineages, the BOP and PACMAD clades (Fig. 1).

Arundinoideae Kunth ex Beilschm. were dissociated from these two historical lineages early on, due to apically reduced florets and laterally compressed spikelets—specific to the 'festucoid' type—versus subtropical distribution and epidermal features—specific to the 'panicoid' type (Avdulov 1931; Jacques-Félix 1958; Stebbins and Crampton 1959; Prat 1960; Fig. 1a). Arundinoideae included *incertae sedis* taxa, which sometimes led to consider it to be a basal clade of Poaceae, or even a taxonomic 'dustbin group' (Prat 1960; Auquier 1963; Clayton and Renvoize 1986). Consequently, Arundinoideae have included up to 736 species and 72 genera (Conert 1961, 1966; Renvoize 1981; Soreng et al. 2015), but its molecular polyphyly (Barker et al. 1995, 1998; Hsiao et al. 1998) led to its drastic reduction by the description of Danthonioideae H.P.Linder & N.P.Barker (GPWG 2001) and the reinstatement of Micrairoideae Pilg. (Sánchez-Ken et al. 2007).

Following these treatments, Arundinoideae have become one of the smallest grass subfamilies, with 15 genera and about 42 species (Table 1). But these taxa show a surprising ecological and morphological heterogeneity, from helophyte reeds such as *Phragmites australis* (Cav.) Trin. ex Steud., one of the most cosmopolitan plant species, to dwarf xerophytes such as *Dregeochloa pumila* (Nees) Conert, the only succulent grass living in Southern African sandy deserts. Repeated morphological analyses of Arundinoideae provided some interesting insights but failed to clearly resolve their classification. This is partly due to the diversity of forms and few evident synapomorphies in such a small subfamily (Renvoize 1981; Linder et al. 1997). In addition, several taxa currently in the Arundinoideae may be erroneously included. In return, some apparent Arundinoideae may be misplaced in other subfamilies, such as '*Eragrostis walteri* Pilg.' previously considered as the only non-C_4 Chloridoideae, but recently placed in the Arundinoideae based on molecular

data (Ingram et al. 2011). Finally, several species and genera (*Dichaetaria* Nees ex Steud., *Leptagrostis* C.E.Hubb., *Nematopoa* C.E.Hubb. *Piptophyllum* C.E.Hubb., *Zenkeria* Trin.) of Arundinoideae have never been studied in a molecular phylogeny, due to their rarity and restricted distributions, some of them being represented only by a few herbarium specimens (Linder et al. 1997).

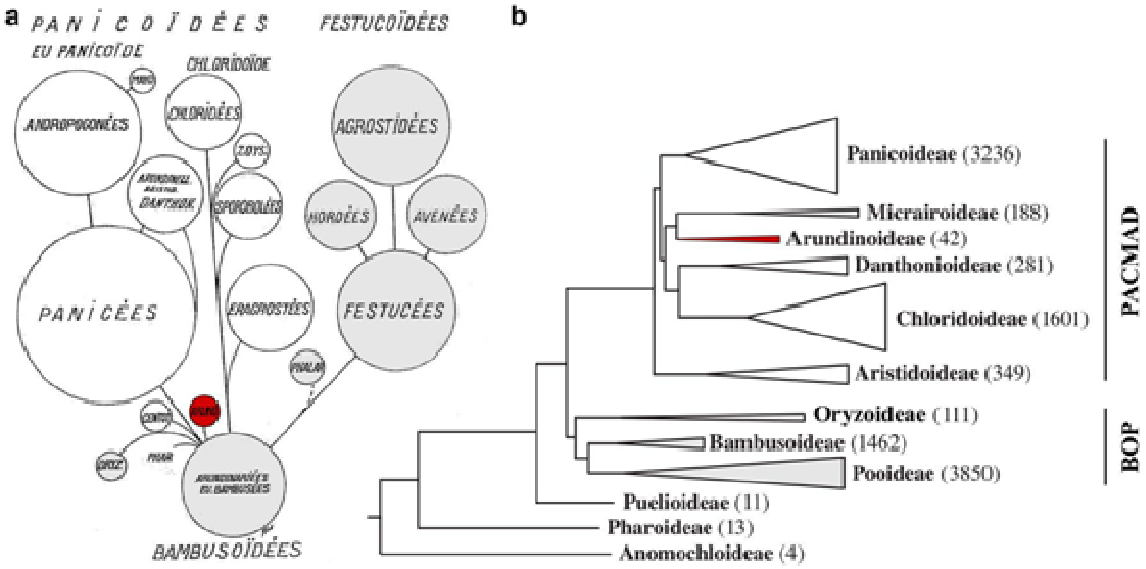

Fig. 1 Places of the Arundinoideae subfamily (in *red*) in the historical representation of grass systematics, from an old literature synthesis of morphological and anatomical studies (modified from Prat 1936; **a**) to a recent molecular phylogeny (modified from GPWG II 2012; **b**). Clade sizes are proportional to their species richness (also indicated between *brackets*). BOP clade, Bambusoideae–Oryzoideae– Pooideae (in *grey*); PACMAD clade, Panicoideae–Aristidoideae– Chloridoideae–Micrairoideae–Arundinoideae–Danthonioideae (in *white*)

Table 1 Genera, species richness (S) and geographical distribution of the subfamily Arundinoideae

Genus	S	Distribution
Amphipogon R.Br. (= *Diplopogon* R.Br.)	9	Australia
Arundo L.	5	Tropical Eurasia
Crinipes Hochst.	2	East Africa
Dichaetaria Nees ex Steud.	1	India
Dregeochloa Conert	2	South Africa
Elytrophorus P.Beauv.	2	Africa–Asia–Australia
Hakonechloa Makino ex Honda	1	Japan
Leptagrostis C.E.Hubb.	1	East Africa
Molinia Schrank (= *Moliniopsis* Hayata)	3	Eurasia
Monachather Steud.	1	Australia
Nematopoa C.E.Hubb.	1	South Africa
Phragmites Adans.	5	Cosmopolitan
Piptophyllum C.E.Hubb.	1	South Africa
Styppeiochloa De Winter	3	South Africa
**Zenkeria* Trin.	5	India
Total	42	Old World + Australia

* Genus removed from the subfamily based on the present study

The present study attempts to provide the first complete phylogeny of the Arundinoideae subfamily. The main challenges are (1) to test the monophyly of taxa currently included in Arundinoideae, (2) to resolve the phylogenetic relationships within this subfamily in order to identify putative tribes and (3) to initiate the understanding of the evolutionary history of this small but heterogeneous sub-family. To address this, we used five plastid DNA loci previously investigated to resolve the systematics of Poaceae. We also added previously published plastomes (complete plastid genomes) of several species, in order to increase the resolution of phylogenetic reconstructions and improve the calibration of molecular dating. Finally, we provide a taxonomic treatment to improve the classification of Arundinoideae.

Materials and methods

DNA extraction and plastid DNA sequencing

Sampling includes samples from BM, CANB, ETH, K, MARS, P, PRE and STR Herbaria (Online Resource 1). For each sample, about 1 cm^2 of leaf material was crushed by two metallic marbles in 2-mL tubes treated with liquid nitrogen and placed in a ball mill. Total DNA was extracted following Doyle and Doyle (1987) with a modification for herbarium material: incubation of ground material with CTAB isolation buffer (4% CTAB) for 120 min. DNA concentrations were estimated using a NanoDrop ND-1000 spectrophotometer (Labtech, Uckfield, UK) and diluted to 50 ng/µL for recent collections, or used at the initial concentration for old specimens with degraded DNA. Plastid DNA diversity was screened on five loci using primer couples as described in the literature and newly designed internal primers for specimens with degraded DNA: *trn* K–*mat*K, *mat*K, *rbc*L, *rbc*L–*psa* I, *ndh*F (Online Resource 2). Polymerase chain reactions (PCRs) were performed with 5 mL of DNA solution, 1× of GoTaq Flexi Buffer, 0.25 µM of each primer (Eurofins Scientific, Luxembourg), 1.0 mM of $MgCl_2$, 0.2 mM of dNTP and 1 unit of GoTaq G2 Flexi DNA polymerase (Promega, Fitchburg, Wisconsin, USA) in a final volume of 50 µL. The thermal cycling profiles followed indications from the previously cited literature for each locus. Purification and sequencing of PCR products were carried out by Eurofins.

Phylogenetic analyses

First, each plastid DNA locus was manually checked and aligned in MEGA 6.0 (Tamura et al. 2013). In addition, we added corresponding sequences from four plastomes of Arundinoideae, three of Micrairoideae, four of Danthonioideae and five of Chloridoideae sequenced in a previous study (Cotton et al. 2015) . Two

nucleotide datasets were used: (1) the loci dataset including the five dissociated locus alignments and (2) the loci + plastome dataset gathering every locus in one large alignment with the 16 whole plastome sequences (Online Resource 3). In the second dataset, we combined the plastid sequences of *Arundo donax* L. with transcriptomic data from a previous study (GenBank accession GBRH01000000; Barrero et al. 2015), using the mapping function in Geneious v.7 and using the *P. australis* plastome as a reference (GenBank accession KF730315). The online program MAFFT v.7 was used for loci and plastomes alignments (http://mafft.cbrc.jp/alignment/server/ ; Katoh and Standley 2013). Phylogenetic relationships and dating divergences were estimated using Bayesian inferences in Beast v.1.8.1 (Drummond and Rambaut 2007). The Arundinoideae + Micrairoideae cluster was treated as the ingroup, and its monophyly was enforced as for the remaining outgroup and for the Danthonioideae and the Chloridoideae (GPWG II 2012). The molecular dating of phylogenetic divergences was led by a secondary calibration of basal nodes using dates and uncertainties estimated in previous analyses of angiosperm-wide datasets (Christin et al. 2013). These dates were obtained using several angiosperm fossils, with additional consideration of 67 Ma fossilized phytoliths giving a lower age for the stem of Oryzeae (Prasad et al. 2011; Christin et al. 2013). Time constraints were set on the root of the tree by a normal distribution of mean 57.304 ± 5.5 and on the crown of Arundinoideae + Micrairoideae by a normal distribution of mean 47.509 ± 8.5. For each locus of the first dataset, unlink site models were estimated following substitution models indicated in jModelTest v.2.1 (Darriba et al. 2012), and unlink clock models were estimated under a log-normal relaxed clock with a normal distribution with the prior for the distribution of node ages approximated by a Yule speciation process (Table 2). The second dataset was analysed as a single alignment, following a general time-reversible substitution model with a gamma shape parameter and using a proportion of invariants (GTR + G + I), and a log-normal relaxed clock with a Yule speciation process. The MCMC tree searches were run for 10,000,000 generations, sampling a tree every 1000 generations after a burn-in period of 1,000,000 generations. The adequacy of the number of generations, sampling frequency and burn-in period was confirmed through a visual inspection of the traces and ESS for all parameters using Tracer v.1.6 (Rambaut et al. 2017). Finally, the two maximum clade credibility (MCC) trees were summarized in TreeAnnotator 17.4 (Drummond et al. 2006).

Table 2 Characteristics of the plastid DNA loci used for phylogenetic reconstructions. Site models were defined for each locus using jModel-Test 2.1.10

Locus	Length	Position	Site model	Substitution rate	% Identical sites	Pairwise % identical sites	% Indel sites
*trn*K5′–*mat*K5′	736	3342–4078	TPM3uf + G	1.28×10^{-9}	72.4	92.4	11.3
*mat*K5′(–*trn*K3′)	1788	1553–3341	GTR + G	6.44×10^{-10}	82.9	96.9	1.19
*rbc*L	1321	61,833–63,154	HKY + I + G	3.55×10^{-10}	90.8	98.0	1.74
*rbc*L–*psa*I	1202	63,155–64,357	TrN + I + G	1.18×10^{-9}	39.6	84.2	37.2
*ndh*F	2103	111,495–113,598	GTR + I + G	7.56×10^{-10}	84.8	97.1	0.57

Results

Our study presents 31 species with evidence from new plastid DNA sequences and includes two previously unsequenced genera, *Crinipes* Hochst. and *Zenkeria*. We failed to extract sufficiently preserved DNA or to find plant material in the visited herbaria for the monotypic genera: *Dichaetaria* (PRE980454), *Leptagrostis* (STR), *Nematopoa* (K) and *Piptophyllum*. The amplification of *rbc* L–*psa*I failed for *Amphipogon caricinus* F.Muell. and one sample of *D. pumila* (PRE581482).

Genetic variation of plastid DNA loci

The five loci covered 7150 bp, i.e. 4.9% of the 146,502 bp represented by the plastomes alignment. The mapping of the *A. donax* transcriptome on the plastome of *P. australis* created a partial plastome of 78,561 bp for *A. donax*, i.e. 53.6% of the plastomes alignment, but including only low variability coding regions. In the first nucleotide dataset, the five loci show different amounts of variation, from low variable genes with less than 20% of variable sites (*mat*K, *ndh*F, *rbc*L), to the intergenic spacer *rbc*L– *psa*I containing a high proportion of variable sites (60.4%), among which a large portion of indels (37.2% of sites), with a median variability for the mainly intronic *trn*K5'–*mat*K5' (Table 2). The substitution varied from 3.55 to 7.56 × 10^{-10} substitution per year for the three genes, and 1.18–1.28 × 10^{-9} for the two other loci.

Phylogenetic relationships

The MCC trees generated from the two nucleotide datasets show almost identical and well resolved topologies, with nearly all nodes supported by posterior probabilities >0.95. In the Arundinoideae subfamily, three distinct and well supported clades can be identified: (1) the first one includes the Eurasian *Arundo*, the two Australian-endemic genera *Amphipogon* R.Br. and *Monachather* Steud., and the South African *Dregeochloa* Conert; (2) the second one includes sub-Saharan genera: *Crinipes* , *Styppeiochloa* De Winter, and *Elytrophorus* P.Beauv.; (3) the third one includes mainly Eurasian genera: *Phragmites* Adans., *Molinia* Schrank, and *Hakonechloa* Makino ex Honda. The only incongruence between the topologies generated by the two nucleotide datasets is located in the first clade, where the loci dataset describes early divergence of the *Arundo–Monachather* clade from the *Amphipogon–Dregeochloa* clade, whereas the loci + plastomes dataset shows the divergence of *Amphipogon–Arundo* from *Monachather–Dregeochloa*. In both phylogenies, these four putative clades are poorly supported (pp < 0.7). The species currently known as '*E.*

walteri' is placed along a well-isolated stem as a sister group of the genus *Elytrophorus* , with a divergence estimated to 21.7 Ma (or 16.8 Ma, for the loci dataset). The Micrairoideae subfamily appears as sister to the Arundinoideae. Surprisingly, genus *Zenkeria* is not included in Arundinoideae, but rather as the nearest relative of *Micraira* F.Muell. within Micrairoideae.

The two calibration strategies, considering (1) the five loci without plastome under unlink site and clock models, or (2) the whole alignment with plastomes following unique site and clock models, show a significant difference in divergence dating, with plastome calibration inducing older node ages in the ingroup (and younger ones in the outgroup) than unlinked calibration of the five loci, with a difference of about 5 Ma (4–7 Ma) for a same node. The exception is the crown node of the whole chronogram supporting the dating calibration, remaining at 54.9 Ma (95% CI [45.4; 65]) for the loci + plastome dataset and 54.7 Ma 95% CI [44.6; 65] for the loci dataset, near the prior at 57.3 ± 5.5 Ma. The stem nodes of the three main clades previously described based on the loci + plastome dataset). Their crown nodes were estimated not so far from stem nodes, between 33 and38 Ma, expect for the crown node of clade *Molinia–Hakonechloa–Phragmites*, which is estimated around 15 Ma (95% CI [27.6; 6.3]). Despite a large 95% CI, every Arundinoideae genus started its diversification before the Quaternary, during the Neogene (23–2.6 Ma).

Discussion

Resolution of Arundinoideae phylogeny

The Arundinoideae subfamily appears here to be a robust evolutionary lineage of Poaceae, with eleven genera forming a monophyletic and structured clade. However, the present study shows that *Zenkeria* is misplaced and should reside in the Micrairoideae. We provide new molecular data for 12 genera of which two have never been sequenced but failed to analyse four other ones. These remaining four genera are monotypic, rare and have not been collected for a long time, but recent plastome sequencing data seem to place *Dichaetaria*, *Nematopoa* and *Piptophyllum* in other grass subfamilies (Teisher, pers. comm.). To date, *Leptagrostis*, known from only a few Schimper specimens collected over a century ago, has yet to be sequenced. During the present study, Kellogg (2015) mentioned three other genera in Arundinoideae: *Alloeochaete* C.E.Hubb., *Danthonidium* C.E.Hubb. and *Phaenanthoecium* C.E.Hubb., but these were placed as *incertae sedis* in the Danthonioideae by Soreng et al. (2015).

Even if the present study somewhat clarifies Arundinoideae systematics, this smaller refined subfamily remains oddly heterogeneous, with a variety of morphological forms and deep evolutionary differentiation among taxa, as suggested by the weak ratio between the numbers of species(41–42) and genera (11–14).

Indeed, most genera include only 1 or 2 (–3) species (Table 1), except *Amphipogon* (9) and the two reedy genera, *Arundo* (5) and *Phragmites* (5). Soreng et al. (2015) suggested further division into subtribes might be needed within the Arundinoideae. However, we chose to stay at a tribe level considering the limited interest in describing mono- or di-generic subtribes. With regard to the present phylogeny (Fig. 2), the structuring of the Arundinoideae into three tribes is well supported:

1. Tribe Molinieae V.Jirásek includes genera *Hakonechloa*, *Molinia*, and *Phragmites* (Jirásek 1966). Contrary to Soreng et al. (2015), we chose to remove the Crinipoid group (Barker 1997; Linder et al. 1997) from Molinieae based on the phylogenetic and morphological similarities of *Hakonechloa*, *Molinia* and *Phragmites* (supported by their stem length, recent diversification, and lemma similarity; Fig. 2). The Sino-Japanese *Moliniopsis* Hayata is sometimes considered as a distinct genus (Soreng et al. 2015) or placed as a synonym of *Molinia* based on morphological similarity (as in the present study). However, the *rpo*C2, *rbc*L and *atp*F– *atp*H sequences deposited in GenBank for *Moliniopsis japonica* (Steud.) Hayata support placement within Molinieae, and molecular data fail to resolve the monophyly of *Molinia* and *Moliniopsis* (analyses not shown). The nine species of this tribe are primarily distributed in the Old World, but each of the three genera possesses an endemic taxon from Japan and surrounding areas: *Hakonechloa macra* (Munro) Honda, *Molinia japonica* Hack. and *Phragmites japonicus* Steud. Compared to the wider distribution of the five other species, this biogeographical pattern positions the early origin of this tribe towards Eastern Asia, with the Miocene formation of the Japan Sea (since 20 Ma) progressively inducing the Pliocene isolation (5–3 Ma) of Japanese Islands (Jolivet et al. 1994; Taira 2001; Isozaki et al. 2010). This late marine isolation may explain the parallel allopatric speciation of the three Japanese endemic taxa. The presence of long hairs on the callus (but not on the lemma) may be a synapomorphy characterizing Molinieae (Fig. 2), except for the short-haired or glabrous callus found in *Molinia caerulea* (L.) Moench.
2. Tribe Crinipedeae Hardion is described here to include *Crinipes*, *Elytrophorus*, *Pratochloa* (described here), and *Styppeiochloa*. This tribe corresponds to the Crinipoid group as described by Linder et al. (1997), except *Zenkeria* which we place in the Micrairoideae, and *Dichaetaria*, *Nematopoa* and *Piptophyllum* which should be moved to other subfamilies (Teisher, pers. comm.). Our new Crinipedeae poorly matches with subtribe Crinipinae Conert that included only *Crinipes* and *Hakonechloa*(Conert 1966). Both phylogenetic data and lemma morphology support a close relationship between the East African *Crinipes* and the South African *Styppeoichloa*. *E. walteri*, recently placed in the Arundinoideae based on nuclear and plastid sequences (Ingram et al. 2011), is sister to *Elytrophorus*. However, phylogenetic divergence and deep morphological differentiation of the lemma(Fig. 2) justify placement of this Namibian species in a new monotypic genus (see taxonomic treatment). The tribe Crinipedeae is mainly sub-Saharan (including Madagascar), except for *Elytrophorus spicatus*(Willd.) A.Camus which spreads to southern Asia and Australia. This large

distribution pattern might be due to floral adaptations for long distance dispersal, e.g. by ornitochory since the lemmas have short, scabrous awns, the paleas are winged and variously notched (adherent), and this species grows in wet sites (a suitable condition for bird occurrence). Since *Crinipes–Styppeoichloa* and *Elytrophorus–Pratochloa* form clades, it might be useful to eventually have this reflected in the classification, possibly as separate subtribes.

3. Tribe Arundineae Dumort. is also strongly supported in our phylogeny, even if supports of internal nodes among the four genera are weaker (Fig. 2). Initially included in the same genus due to their eco-morphological similarities, subtropical Eurasian reed genus *Arundo* does not belong to the same tribe as cosmopolitan reed genus *Phragmites*. Surprisingly, the taxa which are the closest relatives of *Arundo* are the two Australian-endemic herbaceous genera *Amphipogon* and *Monachather* (Barker et al. 1995). The present study also places the South African genus *Dregeochloa* in Arundineae, and not in Molinieae as assumed by Soreng et al. (2015). The morphological similarities between *Dregeochloa* and *Monachather* (Fig. 2) and the biogeographical afnities between *Amphipogon* and *Monachather* might justify the use of Amphipogoneae L.Watson and T.D.Macfarl., thus isolating *Arundo* in the Arundineae. However, these assumptions need to be tested using other molecular markers, and specifically with nuclear genes that have been poorly investigated in this subfamily (Hsiao et al. 1998). The Australian genus *Amphipogon* includes the highest species richness of Arundinoideae genera, with nine species radiating in the West Mediterranean zone of Australia, with several endemic taxa and some species with a broader distribution in Eurasia.

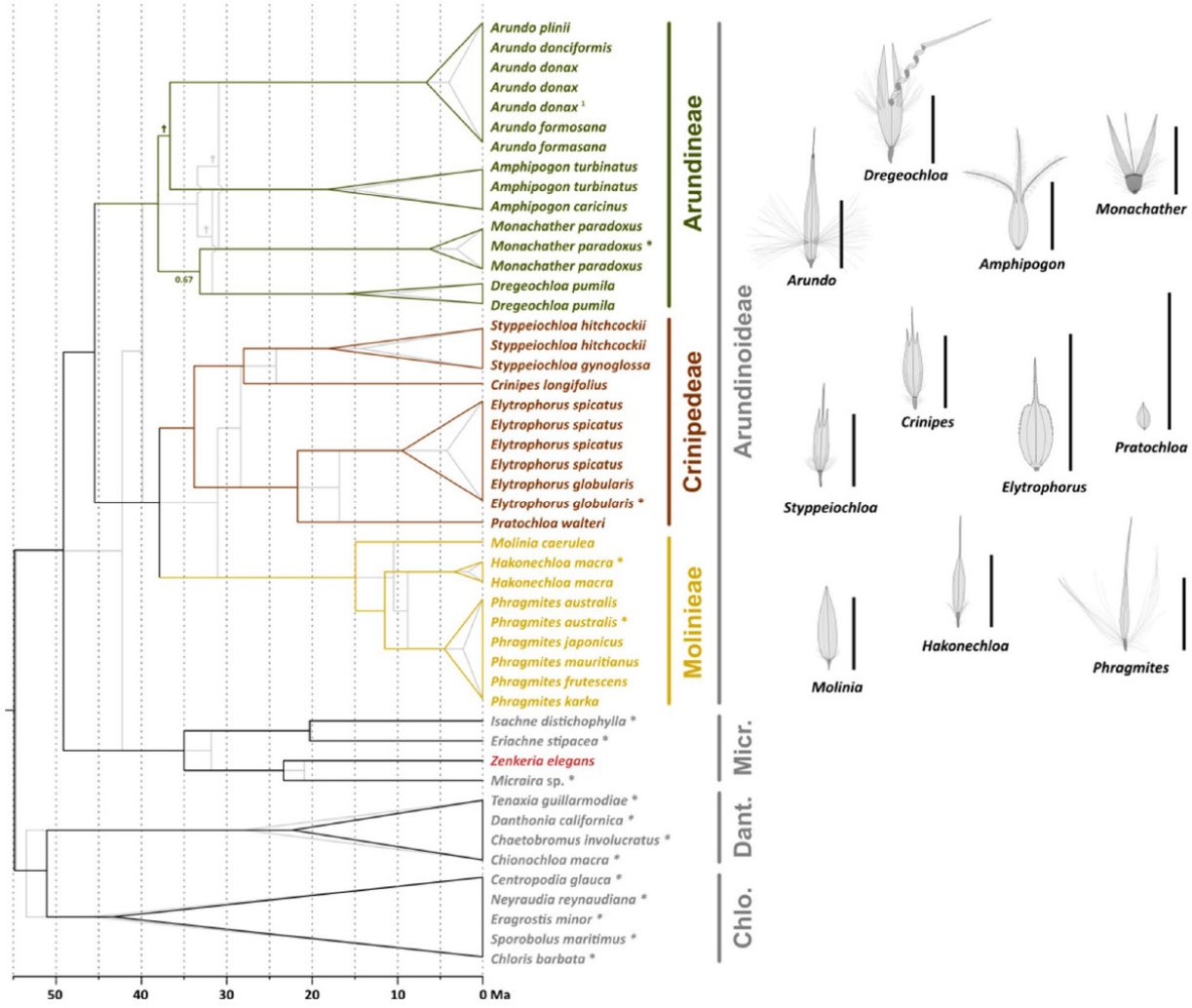

Fig. 2 Chronogram of Arundinoideae based on five plastid DNA loci (*trn*K–*mat*K, *mat*K, *rbc*L, *rbc*L–*psa*I, *ndh*F) and plastomes data (*asterisk*), and morphological diversity of lemmas within this small subfamily. Molecular clock secondarily calibrated using previous estimations from Christin et al. (2013) for the two divergence nodes with outgroup Micrairoideae (Micr.) and outgroup Chloridoideae (Chlo.) + Danthonoideae (Dant.). *Grey phylogeny* indicated the same chronogram without plastome data and considering unlinked site and clock models for each locus. Nodes without value are supported by posterior probabilities of 1.0, and nodes with cross symbols (*dagger*) are supported by posterior probabilities between 0.7 and 1.0. *Black bars*, 5 mm. [1]Partial plastome of *Arundo donax* (coding regions covering 53.6% of the plastome alignment) from transcriptome data (Barrero et al. 2015)

It is noteworthy that Arundinoideae initially described on the basis of reedy genera such as *Arundo*, *Phragmites* and *Molinia* (also called the Phragmitiformes Avdulov 1931) cannot exclusively be considered a reedy group of grasses. The high number of small herbaceous taxa and the distant position of *Arundo* and *Phragmites* argue for the homoplasy of culm height and rigidity. However, most Arundinoideae taxa are associated with humid habitats, even when they occur in semi-arid biomes. *Dregeochloa* is a counterexample of this, with its distribution in arid zones, and several xerophyte adaptations (thickened cuticle, stomata protection, succulent leaves; accurate description in Ellis 1977).

In a global consideration, except for North American native *P. australis* subsp. *americanus* Saltonstall, P.M. Peterson & Soreng (Saltonstall et al. 2004), Arundinoideae are restricted to the Old World (including Australia). The biogeography of Arundinoideae looks like a Gondwanan legacy, with South and East African, Indian and Australian species. Consequently, hypotheses of vicariance and/or land dispersal have yet to be tested (e.g. Linder et al. 1997). However, the removal of Indian endemic, *Zenkeria* and our molecular dating shed new light on the subject. Indeed, these geological zones were clearly isolated by the Indian Ocean 100 Ma ago (Crisp and Cook 2013), long before the stem node of the Arundinoideae. Consequently, the common ancestors of this subfamily had to possess some suitable traits for long distance dispersal, e.g. by anemochory, as illustrated by the long-haired lemma inclosing small caryopses and forming an efficient means for dispersal (e.g. *Phragmites*, *Arundo*, Fig. 2), or by ornitochory as suggested above for *Elytrophorus spicatus* or other species growing in wet sites; or by marine dispersal as sometimes hypothesized for *Phragmites* (Lambertini et al. 2012).

Taxonomic treatment

subfam. **Arundinoideae** Kunth ex Beilschm.

= Arundinoideae Tateoka

= Phragmitoideae Parodi ex Caro

= Arundinaceae Burmeist., unranked

tribe **Arundineae** Dumort.—TYPE: *Arundo* L.

= Amphipogoneae L.Watson & T.D.Macfarl.

= subtribe Arundininae Miq.

Genera included: *Amphipogon* R.Br. (= *Diplopogon* R.Br.), *Arundo* L., *Dregeochloa* Conert, *Monachather* Steud.

tribe **Molinieae** Jirásek—TYPE: *Molinia* Schrank

Genera included: *Hakonechloa* Makino ex Honda, *Molinia* Schrank (= *Moliniopsis* Hayata), *Phragmites* Adans.

tribe **Crinipedeae** Hardion, **trib. nov.**—TYPE: *Crinipes* Hochst.

= Crinipinae Conert pro parte.

Description: Annual or perennial erect herbs, culms 10–150 cm long. Ligule a fringe of hairs or an eciliate membrane. Leaves mostly basal or cauline, non-auriculate. Leaf blades stiff, persistent or deciduous. Leaf sheaths persistent. Panicle open or glomerate. Fertile spikelets laterally compressed, 2–12 mm long, comprising 2–15 florets, breaking up at maturity, disarticulating below each fertile floret, with diminished florets at the apex. Rachilla internodes definite. Glumes persistent, shorter than spikelet, ovate or lanceolate. Lower glume shorter than to equal to upper glume. Glumes 1-keeled and 1–3-veined. Lemma membranous, with or without keel, 3-veined, entire or dentate bifid. Lodicules 2, Stamens 3.

Genera included: *Crinipes* Hochst., *Elytrophorus* P.Beauv., *Pratochloa* Hardion, *Styppeiochloa* De Winter.

Pratochloa Hardion, **gen**. **nov**. ≡ *Eragrostis walteri* Pilg.— TYPE: *Pratochloa walteri* (Pilg.) Hardion

Pratochloa walteri (Pilg.) Hardion, **comb**. **nov**. ≡ *E. walteri* Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 15: 452. 1940. —TYPE: NAMIBIA. Kleiner Naukluftrivier, 29 Oct 1937, *Walter 458* (holotype: B [image B100272776!]; isotype: B [image B100272777!])

Etymology: This new genus is named in tribute to Henri Prat (1902–1981), professor at the Universities of Marseille (France) and Montreal (Quebec, Canada). After a thesis on the systematic study of grass epidermises, he published several works on the systematics of Poaceae with an emphasis on their morphology, anatomy and organogenesis (Prat 1932, 1936, 1960).

Acknowledgements The authors thank staffs from BM, CANB, ETH, K, MARS, P, PRE and STR Herbaria for loans and material removal. LH addresses a special thanks to Pascal-Antoine Christin (University of Sheffield) for data and support in the calibration of the molecular phylogeny, and to Jordan Teisher (Washington University in St. Louis) for useful and sporting exchange views about the systematics of Arundinoideae. The authors are also grateful to Marianick Juin (IMBE) for contribution in the lab work, to Heather Lindon (RBG Kew) for expertise in botanical Latin, and to Paul M. Peterson (US, Smithsonian Institution) for his conscientious review which greatly improved the readability of the manuscript.

Compliance with ethical standards

Confict of interest The authors declare that they have no conflict of interest.

Ethical statement The authors comply will all rules of the journal following the COPE guidelines; all authors have contributed and approved the final manuscript.

Information on Electronic Supplementary Material

Online Resource 1. Sampling information and GenBank accessions.

Online Resource 2. Primers used in PCRs and developed for the present study.

Online Resource 3. Loci + plastome alignment used for phylogenetic analyses.

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Electronic supplementary material The online version of this article (doi:10.1007/s00606-017-1451- 6) contains supplementary material, which is available to authorized users.