

## The relationships of *Amphipogon*, *Elytrophorus* and *Cyperochloa* (Poaceae) as suggested by *rbcL* sequence data

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### Abstract

Nigel P. Barker<sup>1</sup> (National Herbarium of New South Wales, Royal Botanic Gardens, Sydney, Mrs Macquaries Road, Sydney, NSW, Australia 2000. <sup>1</sup>Present address: Dept. Botany, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa; email: barker@rhobot.ru.ac.za) 1997. The relationships of *Amphipogon*, *Elytrophorus* and *Cyperochloa* (Poaceae) as suggested by *rbcL* sequence data. *Telopea* 7(3): 205–213. The phylogenetic affinities of *Amphipogon*, *Elytrophorus* and *Cyperochloa* are elucidated using DNA sequence data from the chloroplast *rbcL* gene. All three genera were previously considered to belong to the subfamily Arundinoideae tribe Arundineae. However, cladistic analysis of *rbcL* sequence data obtained in this study suggests that *Cyperochloa* has no affinities with the Arundinoideae, and that *Amphipogon* and *Elytrophorus* have affinities with the Arundineae sensu stricto, but not the Danthoneae.

### Introduction

The classical subfamilial and tribal classification of the grass family has been under much scrutiny recently. Several studies have utilised DNA sequence data to resolve relationships between and within the subfamilies (Barker et al. 1995 & submitted, Clark et al. 1995, Cummings et al. 1994, Davis & Soreng 1993, Doyle et al. 1992, Duvall & Morton 1996, Hsiao et al. in press, Liang & Hilu 1996, Mathews et al. 1996, Nadot et al. 1994). These studies have modified our understanding of the relationships of the major grass lineages, and (with suitable sample sizes) can provide an indication of the composition of these lineages.

Molecular studies on the Bambusoideae (Clark et al. 1995) and Arundinoideae (Barker et al. 1995 & submitted) have indicated that these subfamilies are polyphyletic, comprising several unrelated lineages. With respect to the Arundinoideae, this merely confirms what has been long suspected, as Renvoize (1981), Campbell (1985), Clayton & Renvoize (1986), Conert (1987), Ellis (1987), Kellogg & Campbell (1987) & Watson (1990) have all considered this group to be polyphyletic.

Molecular systematic studies are, however, often restricted to taxa that are readily available. Taxa that are ephemeral, narrowly endemic or found in remote areas that require long distance collecting trips are seldom included. These taxa are nonetheless important in such studies, as they may represent unsampled or under-represented clades or biogeographic outliers, and are thus potentially valuable in clarifying phylogenetic and biogeographic relationships. Material of three such genera, *Elytrophorus*, *Amphipogon* and *Cyperochloa*, was recently collected in Australia. Coincidentally, the taxonomic position and phylogenetic affinities of these three genera are uncertain, as existing morphological and anatomical data provides unclear or conflicting indications of higher level affinities. The use of molecular (DNA sequence-based) techniques to resolve the subfamilial and possibly tribal affinities of these three genera is thus appropriate.

The choice of which particular gene to use in elucidating these relationships is important, and there are several data sets now available for the grasses. These include *rbcL* (Barker et al. 1995, Duvall & Morton 1996, Seberg & Linde-Laursen 1996), *ndhF* (Clark et al. 1995), *rpoC2* (Barker et al. submitted, Cummings et al. 1994), *rps4* (Nadot et al. 1994) and nuclear ribosomal RNA internal transcribed spacer regions (ITS; Hsiao et al. in press). Sequence data from *rbcL* are appropriate for showing the affinities of these three genera, as *rbcL* sequences are available for a wide range of arundinoid taxa as well as other grass lineages, and they are not subject to potential alignment problems associated with the more variable sequences such as *rpoC2* (Barker et al. submitted), *rps4* (Nadot et al. 1994) and ITS (Hsiao et al. in press).

### Materials and methods

DNA was extracted from dried leaf material of *Elytrophorus* and *Amphipogon* by means of the hot CTAB method (Doyle & Doyle 1987). An aliquot of DNA of *Cyperochloa* was provided by C. Hsiao. Voucher and other details of these samples are given in Table 1.

The *rbcL* gene from *Amphipogon* was amplified and sequenced according to the method published by Barker et al. (1995). The *rbcL* gene of *Elytrophorus* and *Cyperochloa* was amplified using primer 'A1' (designed by P. Gadek & S. Gilmore, University of New South Wales) and the reverse primer designed by Zurawski (Z-1375R). The amplification products were purified by means of the Promega Wizard DNA Clean-up system. The ABI PRISM dye terminator cycle sequencing kit was used to generate DNA for sequencing. Sequencing was carried out by SUPAMAC (Sydney University and Prince Alfred Macromolecular Analysis Centre) using an ABI PRISM 377 autosequencer. The two flanking primers and four internal primers were used in a total of six sequencing reactions for each template, producing a complete sequence in both directions. The primer sequences used are listed in Table 2.

Sequences were edited using Sequencher version 3.0 (Gene Codes Corporation, Inc. 1995). Once edited, the sequences were imported into DAPSA (DNA And Protein Sequence Analysis; E.H. Harley, Dept. Chemical Pathology, University of Cape Town), where they were added to the data set used in an earlier analysis (Barker et al. 1995) as well as four additional sequences of taxa of the subfamily Bambusoideae obtained from GenBank. It must be noted that not all the published grass *rbcL* sequences were used in the analysis presented here. In some instances, some of the published sequences could not be found in GenBank, and in other instances there were several species sequenced from a single genus. In the latter instance, a single taxon was selected at random to represent that particular genus. Other than four species of Bambusoideae mentioned above, all the additional sequences in GenBank were from taxa in the subfamily Pooideae.

DAPSA was used to align and manipulate these sequences and to produce data in a format suitable for phylogenetic analyses. An initial phylogenetic analysis was carried out using HENNIG86 (Farris 1988). PAUP version 3.1.1 (Swofford 1993) was used for subsequent analyses. A search to find islands of most parsimonious trees (Maddison 1991) was carried out using 100 random addition replicates, keeping a maximum of 10 trees at each replicate. The trees found from this search were then swapped to completion in a HEURISTIC search using the TBR option. Bootstrap support values (Felsenstein 1985) were estimated from one hundred bootstrap replications, and Bremer support values (Bremer 1988) for trees up to three steps longer were obtained using PAUP. As the monotypic Joinvilleaceae is the closest extant relative of the Poaceae (Campbell & Kellogg 1987; Doyle et al. 1992; Linder & Rudall 1993; Kellogg & Linder 1995), *Joinvillea* is used as the outgroup.

**Table 1. Voucher details of the specimens sequenced in this study**

Taxon	Collector	Locality	GenBank No.
<i>Amphipogon strictus</i> R.Br.	H.P. Linder 5634	Kings Tableland, NSW	U88403
<i>Cyperochloa hirsuta</i> Lazarides & L. Watson	T.D. Macfarlane 22586	Stirling Range, WA	U88404
<i>Elytrophorus globularis</i> Hack.	S.W.L. Jacobs 7964	Yelarbon, S. Qld	U88405

**Table 2. The sequences of the primers used for amplification and sequencing**

Note that the numbers associated with the names of the primers do not indicate exact nucleotide positions along the gene.

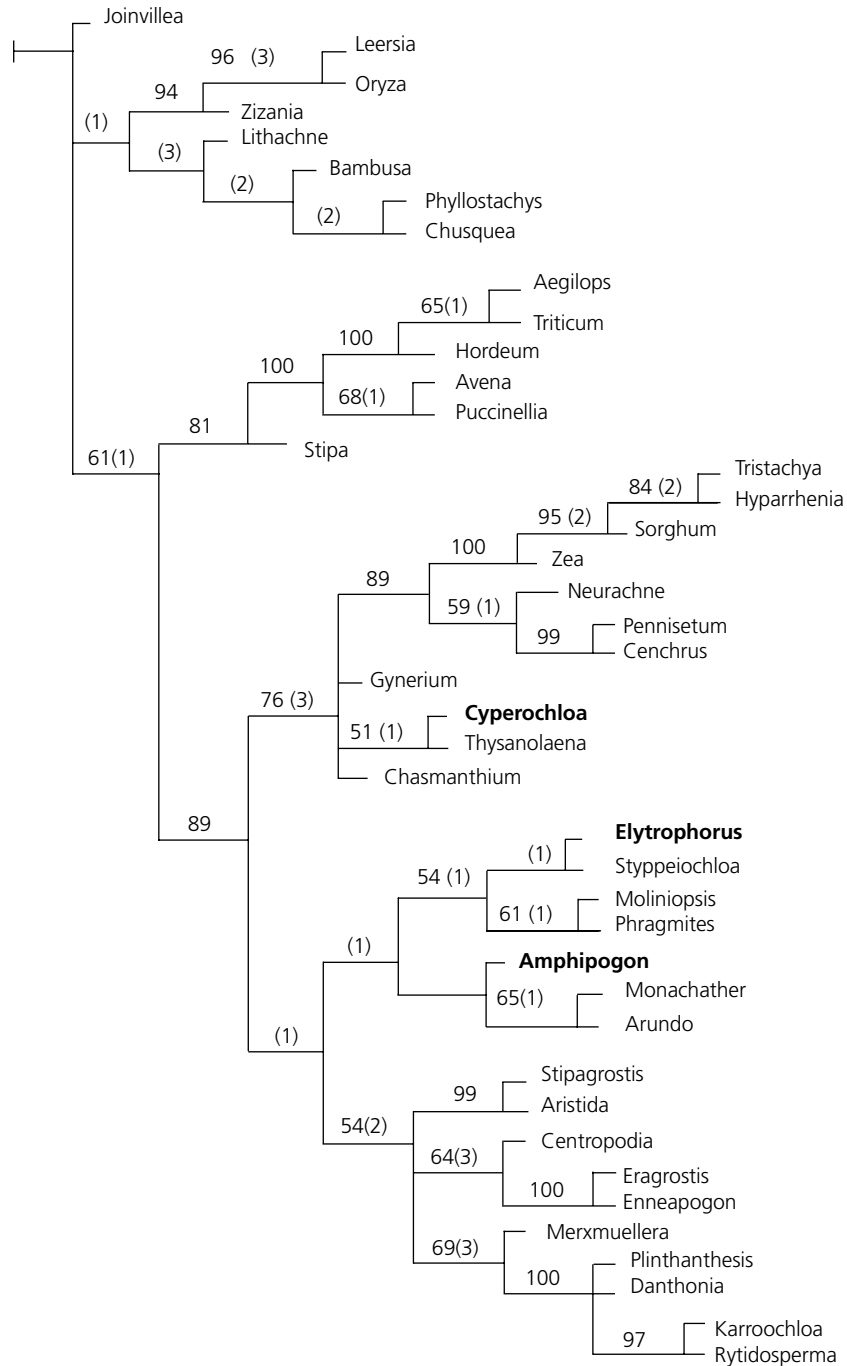
Primer	Sequence (5'-3')
A1	GGGATTATGTACCACAAACAGA (PCR and sequencing)
380F	GCTTATTCAAAAATTCCAAGGCCCGC (sequencing)
860F	ATTCACCGCGCAATGCATGC (sequencing)
670R	TGTGCTTATAAATTGCTTCGGC (sequencing)
930R	GCTAGTACACGAAAATGCATACC (sequencing)
Z1375R	AATTTGATCTCCTCCATATTCGCA (PCR and sequencing)

## Results and discussion

A preliminary analysis of the complete data set (comprising representatives of all the genera in GenBank) produced 424 trees. The strict consensus of these trees was well resolved in all areas except the subfamily Pooideae, which was reduced to a polychotomy (results not shown). For the analysis discussed here, the sequences of the pooid taxa *Elymus*, *Leymus*, *Bromus* and *Eremium* were excluded to break the pooid polytomy and reduce the number of trees and thus search time. The final data set comprised 163 phylogenetically informative characters. The random entry analysis and subsequent search found 12 equally parsimonious trees (length 516 steps, c.i. = 0.40, r.i. = 0.69), all of which were also found by the HENNIG86 analysis. The strict consensus tree is shown in Fig. 1. Bootstrap & Bremer support values are shown on this Fig.

The relationships of the major clades (subfamilies and tribes) in both the preliminary analysis and the analysis presented here are unchanged from those presented by Barker et al. (1995). However, bootstrap support is weak for some of these lineages, such as the (Aristideae, Danthoneae, Chloridoideae) clade (54% in Fig 1). The relationships of *Amphipogon*, *Elytrophorus* and *Cyperochloa* are shown to be diverse, and each genus is discussed separately below.

*Cyperochloa* is a monotypic Australian genus, first described by Lazarides & Watson (1986). It was placed in the Arundinoideae on the basis of a variety of anatomical and morphological characters, including a ligule which is a fringe of hairs, laterally flattened spikelets which disarticulate above the glume, fleshy, ciliate lodicules and 3–5-nerved lemmas. Lazarides & Watson (1986) conducted a phenetic computerised comparison that placed this genus in the tribe Danthoneae, where it was considered



**Fig. 1.** The strict consensus tree of 12 equally parsimonious trees found using the search options described in the methods section. Tree length = 516 steps, c.i. = 0.40, r.i. = 0.69. Bootstrap support values from 100 replicates are shown on nodes receiving more than 50% support. Values in parentheses indicate Bremer support values. Where no Bremer support values are given then the branch is retained in trees more than three steps longer.

to occupy an isolated position in the vicinity of *Plagiochloa* (= *Tribolium*), followed by the Australasian *Danthonia*, *Erythanthera* (= *Rytidosperma* sensu Linder & Verboom 1996), *Schismus*, *Plinthanthesis*, *Monachather* and *Spartochloa*. Watson & Dallwitz (1992) place it in its own tribe, the *Cyperochloae*, in the *Arundinoideae*.

The *rbcL* data shows that *Cyperochloa* is not an arundinoid grass, as it is placed as sister to *Thysanolaena* in a larger clade comprising members of the subfamily *Panicoideae* and *Centothecoideae*. Although bootstrap and Bremer support for the sister relationship to *Thysanolaena* is weak, support for the inclusion of these two genera in the enlarged panicoid clade is strong (76%). *Thysanolaena*, placed in the monotypic tribe *Thysanolaeneae* in the subfamily *Arundinoideae* by Clayton & Renvoize (1986), has been shown to be related to the *Centothecoideae* in other molecular studies (Barker et al. 1995 & submitted, Clark et al. 1995). The affinities of *Cyperochloa* and *Thysanolaena* (and the reedy *Gynerium*) with the *Centothecoideae* and *Panicoideae* needs to be further investigated using both molecular and morphological characters.

*Elytrophorus* and *Amphipogon* are placed in the tribe *Arundineae* as defined by Watson & Dallwitz (1992). This tribe is represented here by seven genera. As yet, no morphological characters have been found to support this clade. Nonetheless, it is interesting to note that, with the exception of *Anisopogon* (and the unsampled *Dichaetaria*), the composition of this clade of seven taxa is almost identical to the list of arundinoid taxa with atypical ligules provided by Clayton & Renvoize (1986, p. 165). A detailed comparative investigation into the ontogeny and structure of the ligule might thus be rewarding.

*Amphipogon* is an Australian genus comprising seven species (Vickery 1950). Watson & Dallwitz (1992) place this genus, along with *Diplopogon*, in its own tribe, the *Amphipogoneae* in the *Arundinoideae*. They further note that the spikelet form (especially the lemma) and microhairs (chloridoid or *Enneapogon* type) are reminiscent of *Enneapogon*, thus suggesting chloridoid affinities for this genus. Renvoize (1981, 1986) considered *Amphipogon* to have an anomalous leaf blade anatomy, in that it possessed papillate long cells and lacked microhairs, the latter character suggesting a position in the *Pooideae*. A recent survey of the genus by Linder (pers. comm.) failed to find any microhairs, supporting Renvoize's observations. Despite this apparent confusion, Clayton & Renvoize (1986) placed *Amphipogon* in the tribe *Arundineae* (*Arundinoideae*), a position also supported by the results of a phenetic analysis carried out by Hilu & Wright (1982), which placed *Amphipogon* in the *Arundinoideae* basal to *Arundo*, *Phragmites* and *Cortaderia*. In contrast, support for a pooid placing for *Amphipogon* was found by Kellogg & Campbell (1987) in their cladistic analysis of mainly morphological characters.

The results from the analysis presented here confirm that *Amphipogon* is associated with the *Arundineae* (sensu Watson & Dallwitz 1992) and is related to *Arundo* and *Phragmites*. *Amphipogon* is basal to the (*Arundo*, *Monachather*) clade, an association that is well supported (95% bootstrap support; Fig. 1).

*Elytrophorus* is a widespread genus ranging from southern and tropical Africa through to China and the Indian subcontinent to Australia. Despite this widespread distribution, it inhabits remote areas in both Africa and Australia, and is thus seldom collected. It is water-loving, being found at the edges of seasonal pans, ponds and rice fields, and is considered to be a true hydrophyte, possessing aerenchyma tissue (Schweickerdt 1942). Schweickerdt recognises four species, while Chippindall (1955), Clayton (1970) & Barker (in Gibbs Russell et al. 1994) recognise only two.

Opinions on the affinities of *Elytrophorus* have been varied. Chippindall (1955), Bor (1960) and Clifford & Watson (1977) interpreted it to be a chloridoid grass. Prat (1960) could not place it in any group with confidence, while Decker (1964) considered it to

have similarities with the Danthonieae. Jacques-Félix (1962) put it in its own tribe, the Elytrophoreae, in the Arundinoideae. Renvoize (1981, 1986) and Clayton & Renvoize (1986) place it in the Arundinoideae, tribe Arundineae, while Watson & Dallwitz (1992) place it in the Danthonieae. Schweickerdt (1942), however, considered the unusual dimorphic spikelets and membranous, unfringed to fringed ligule of *Elytrophorus* to be at odds with a position in the Danthonieae. Dimorphic spikelets are found elsewhere in the grasses, and have probably evolved several times within the family. The fruit is laterally compressed and is unlike that of any other African arundinoid genus (Barker 1994). It must be noted, however, that laterally compressed fruit is also a generic character in Andropogoneae and Chloridoideae, and is also found in some species of *Eragrostis*. There is also some confusion regarding the degree of attachment of the pericarp; Schweickerdt (1942) notes that the fruit 'show the remains of the pericarp', implying that it is separable (the fruit thus being an achene), while Clayton & Renvoize (1986) consider the pericarp to be free, but do not specify the fruit as an achene. Scanning electron micrographs do not resolve this issue with any certainty (Barker 1994). A light microscopy study of the embryology of *E. spicatus* suggests that the pericarp is represented by its outer epidermis only, which is adnate to the inner layer of the inner integument (Satyamurty 1985). Unfortunately, Satyamurty (1985) makes no mention of the presence (or absence) of haustorial synergid cells, a character that is considered to be a synapomorphy for the Danthonieae (Philipson 1977, Philipson & Connor 1984, Verboom et al. 1994). Evidence from leaf anatomical studies suggest that *Elytrophorus* has affinities with the C<sub>3</sub> panicoid taxa such as *Sacciolepis* and *Acroceras*, rather than the C<sub>3</sub> Danthonieae (Ellis 1986).

*Elytrophorus* is shown in this study to be sister to *Styppeiochloa*, a genus considered by Linder et al. (1997) to be part of the crinipoid clade, a group that includes the African genera *Alloeochaete*, *Crinipes*, *Dichaetaria*, *Leptagrostis*, *Nematopoa*, *Piptophyllum* and the Indian and Sri Lankan genera *Dichaetaria* and *Zenkeria*. Although the relationship between *Elytrophorus* and *Styppeiochloa* is not well supported by bootstrap or Bremer support measures, the relationship between *Elytrophorus* and the crinipoid clade needs to be re-examined in light of this association. In particular, the homology of the bracts and bract-like structures associated with the dimorphic spikelets of *Elytrophorus* needs to be assessed.

## Conclusion

The expanded *rbcL* sequence dataset analysed here retains the relationships between the various major lineages found in a previous study (Barker et al. 1995). With the exception of *Amphipogon*, which is well supported as the basal taxon to the (*Arundo*, *Monachather*) clade, the *rbcL* sequence data do not provide strong support for the immediate affinities of the three taxa sampled here. *Cyperochloa* is shown to be excluded from the Arundinoideae, and is placed sister to *Thysanolaena* in a panicoid — centothecoid clade that is well supported. The data suggest that both *Elytrophorus* and *Amphipogon* are members of the tribe Arundineae *sensu* Watson & Dallwitz (1992).

As these taxa may be isolated, possibly basal, members of these clades further evidence and support for their affinities may only become clearer and stronger as additional samples are added to the data set. It is therefore important that continued efforts be made to obtain material of these unusual taxa, and the *rbcL* and other data sets expanded. Finally, the caveat stated in an earlier study (Barker et al. 1995) and elsewhere (Doyle 1992) needs to be re-iterated: *rbcL* data retrieve a plastid phylogeny (as opposed to an organismal phylogeny) and relationships obtained from this source of data need to be tested against phylogenies obtained from morphological or nuclear DNA data.

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