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A PHYLOGENY OF TRIODIEAE (POACEAE: CHLORIDOIDEAE) BASED ON THE ITS REGION OF nrDNA: TESTING CONFLICT BETWEEN ANATOMICAL AND INFLORESCENCE CHARACTERS.

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Abstract

A phylogeny of the predominantly arid Australian grass tribe Triodieae ('Spinifex') is presented based on the internal transcribed spacer (ITS) of nuclear ribosomal DNA. The main genus in the tribe is *Triodia* with 64 species (now also including the 16 species of *Plectrachne*). Of the two smaller genera, *Symplectrodia* has two species and *Monodia* is monotypic. Previous systematic treatments of this tribe have maintained a marked inconsistency in patterns between anatomical and inflorescence characters. This paper, which comprises part of a wider morphological and molecular phylogenetic study of Triodieae, tests whether convergence may have occurred in either one or both of these two suites of characters. Molecular results on 27 representative taxa and three outgroups strongly support the recognition of two lineages based on leaf blade anatomy contrasting with previous classifications based on glume and lemma characters. A 'soft' spinifex clade with centro-abaxial stomata and a paraphyletic 'hard' group with ambito-abaxial stomata are suggested. The previous circumscriptions of both *Plectrachne sens. str.* and *Triodia sens. str.* were based on floral morphology which this study finds strongly convergent. Similarly, five out of the nine informal infrageneric groups in *Triodia sens. lat.* are based on convergent morphological characters. *Symplectrodia* has a 'hard' leaf anatomy and is nested within that group, while the position of the monotypic 'soft' genus *Monodia* remains uncertain under the nrDNA data.

Key words: ITS, Poaceae, *Triodia*, spinifex.

INTRODUCTION

The taxonomically isolated tribe Triodieae is one of the most characteristic of all Australian arid zone plant groups. Vast tracts of central and north-western Australia are dominated by 'spinifex' grasses from the genera *Triodia*, *Monodia* and *Symplectrodia* (Poaceae: Chloridoideae). Spinifex forms a dominant vegetation community known as 'hummock grassland' on over 22% of the continent and is of key importance to the ecology of arid and semi-arid Australia (Griffin 1992).

Spinifex is abundant in all the major Australian deserts but has a much wider distribution extending into temperate and seasonally arid (monsoonal) areas, generally on poor soils such as deep siliceous sands, sandy red-earth, and skeletal soils of various origin

(Lazarides 1997). While there are several very widespread taxa, most of the species are restricted to the Hammersley, Kimberley, MacDonnell, and Musgrave mountain ranges of the arid centre and north-west of Australia (Jacobs 1982; Lazarides 1997). Despite the clear ecological significance of this tribe of grasses it has received comparatively little attention from the fields of ecology or systematics (Jacobs 1992).

The tribe is recognised by a strong synapomorphy in its C₄ Kranz tissue arrangement, which differs from that typically found in the subfamily Chloridoideae (Craig and Goodchild 1977; Renvoize 1983; Van den Borre and Watson 1997). Triodieae has been confirmed as possessing a (NAD-ME) C₄ photosynthetic pathway (McWilliam and Mison 1974; Prendergast *et al.* 1987) but has an

unusual mesophyll and outer bundle sheath structure. The stomata of all species are sunken in deep, narrow grooves which contain dense interlocking papillae (McWilliam and Mison 1974). A tightly packed mesophyll is concentrated around these stomatal grooves in a disjunct position from the associated vascular bundles. Rather than being arranged concentrically around the vascular tissue, the outer bundle sheath extends laterally to connect the displaced mesophyll with the mesome and vascular tissues (McWilliam and Mison 1974; Craig and Goodchild 1977).

There are currently three genera recognised in Triodieae of which *Triodia* R.Br. is the main genus with c. 64 species (Lazarides 1997). *Monodia* S.W.L. Jacobs is monotypic (Jacobs 1985) and *Symplectrodia* Lazarides includes only two species (Lazarides 1984). A fourth genus, *Plectrachne* Henrard (with 16 species) was synonymised into *Triodia sens. lat.* in a recent revision by Lazarides (1997).

Spikelet and other macro-morphological features have formed the basis of generic and infrageneric distinctions in Triodieae despite concerns about high levels of homoplasy in these characters (Burbidge 1946b, 1953; Jacobs 1971). The previous generic distinction between *Plectrachne sens. str.* and *Triodia sens. str.* was based on glume and lemma features, *Plectrachne* being distinguished by its long linear glumes and deeply three-lobed or three-awned lemma (Lazarides 1997). *Triodia sens. str.* typically has comparatively short glumes and emarginate to shortly three-lobed lemmas, with several other variants. In the recent revision (Lazarides 1997) nine informal infrageneric groups were erected for *Triodia sens. lat.* based on general features of the inflorescence with some contribution from geographical distribution.

Overall, the plants of these (four) genera exhibit comparatively uniform floral and gross vegetative features. Yet, previous classifications have failed to account for the presence of two quite different types of leaves in the tribe. These have been recognised previously (Burbidge 1946b; Jacobs 1971) and retain the widely used common names, 'hard' spinifex (40 species) versus 'soft' (27 species) (Gardner 1952; Mant 1998). 'Soft' species generally have a softer appearing foliage, a weakly pungent leaf apex, strongly curled senescent leaf, and a less-hummocked habit. Most, but not all, 'soft' species produce a characteristic viscous resin which is used extensively as an adhesive by indigenous Australians. Rare reports of resin in some 'hard' specimens (Burbidge 1953; Lazarides 1997) refer to droplets of a dark red exudate of a different nature to the aromatic, sticky resin of most 'soft' species (Mant 1998). Ecologically, 'soft' species are restricted to the monsoonal north of the continent, with the exception of *T. melvillei* (Lazarides 1997), whereas 'hard' species are found in both monsoonal and temperate areas.

The precise nature of the distinction between 'hard' and 'soft', however, involves the distribution of stomata and photosynthetic tissues on the outer (abaxial) face of the leaf (Burbidge 1946b). In 'hard' species the stomatal grooves are spread uniformly over the abaxial leaf face, whereas in 'soft' species the stomatal grooves and associated photosynthetic tissues are lacking from the abaxial surface except for a few that cluster around the mid-rib.

Both types of leaf occur in *Plectrachne sens. str.* and *Triodia sens. str.* and five of the nine infrageneric groups are polymorphic for

this vegetative feature (Lazarides 1997). *Monodia* has a 'soft' leaf anatomy, whereas *Symplectrodia* is 'hard' (Mant 1998). In essence then, these classification systems reflect a weighting scheme towards inflorescence and against anatomical characters. Conflict between characters or data sets is best examined by investigating a range of different data sources, both molecular and morphological (Doyle 1997). In the present paper a molecular phylogeny of the tribe based on ITS sequence data is presented to provide an independent test of these competing claims of character importance.

METHODS

Twenty-seven representative taxa (Table 1) were chosen to encompass the morphological diversity found throughout the tribe. All nine informal infrageneric groups are represented, as are the two smaller genera, *Monodia* and *Symplectrodia*. Three outgroups were selected following a preliminary analysis of taxa from among the Chloridoideae and Arundinoideae available on GenBank (Hsaio et al. 1998).

Total genomic DNA was isolated either from seedling material, fresh field samples, or herbarium specimens. DNA from fresh or CTAB preserved samples was isolated following a variation from standard procedures for small quantities of tissue (Doyle and Doyle 1990; Bayer et al. 1996). Herbarium material was isolated then purified using the diatomite method of Gilmore et al. (1993).

PCR Amplification and Sequencing

The ITS region was amplified using primers ITS1 (Hsaio et al. 1994) and ITS4 (White et al. 1990). Herbarium specimens were amplified in two fragments using the internal primers ITS2 and ITS3 (White et al. 1990) in conjunction with ITS1 and ITS4 respectively. Standard polymerase chain reaction (PCR) techniques were employed as described elsewhere (Bayer et al. 1996). Double stranded PCR products were sequenced in both directions using the dideoxy chain termination method (Sanger et al. 1977) with the use of the Big Dye Terminator RR Kit® (Perkin-Elmer Applied Biosystems) and the ABI automated sequencer at the Division of Plant Industry, CSIRO.

Phylogenetic Analysis

The analysis was conducted on PAUP* test version 4.d64 (D. Swofford unpublished). Sequences were aligned and edited using Sequencher version 3.0 and refined by eye. Minor indels of less than five base pairs were inferred in the alignment. Parsimony analyses were implemented using heuristic searches with TBR branch swapping and ten random addition sequence starting trees, with gaps treated as missing. Bootstrapping (BS) was performed with 100 replicates. T-PTP tests (Faith 1991) on individual clades were implemented. Decay indices (DI) (Bremer 1988) were calculated for the ingroup using PAUP* constraint trees and batch searches.

RESULTS

ITS Sequence Length and Nucleotide Composition

The length of ITS in Triodieae is within the range found in other studies of Poaceae (Hsaio et al. 1994; Buckler and Holtsford

Table 1. Voucher details for molecular accessions. All vouchers at CANB.

Taxon	Voucher/GenBank Accession No.	Source
<i>M. stipoides</i> Jacobs	A.A. Mitchell PRP3	h
<i>S. gracilis</i> Lazarides	C. Dunlop 4410	h
<i>S. lanosa</i> Lazarides	I.R. Telford 8029	h
<i>T. angusta</i> Burbidge	P.J. Davidson 2038A	s
<i>T. basedowii</i> E. Pritzel	P.J. Davidson 2054	s
<i>T. bitextura</i> Lazarides	G. Wells s.n.	s
<i>T. bromoides</i> (F. Muell.) Lazarides	Keighery 11, 171	h
<i>T. bynoei</i> (Hubbard) Lazarides	B.J. Carter 765	f
<i>T. danthonioides</i> (F. Muell.) Lazarides	S. Donaldson 1451	f
<i>T. epactia</i> Jacobs	P.J. Davidson 2043	s
<i>T. fitzgeraldii</i> Burbidge	M. Lazarides 3169	h
<i>T. hubbardii</i> Burbidge	G. Griffin s.n.	f
<i>T. lanigera</i> Domin	A.A. Mitchell PRP290	h
<i>T. longiceps</i> J.M. Black	S. van Leeuwen 3381	f
<i>T. melvillei</i> (Hubbard) Lazarides	P.J. Davidson 2035	s
<i>T. molesta</i> Burbidge	M. Lazarides 4393	h
<i>T. plectrachnoides</i> Burbidge	I. Cowie 5703	h
<i>T. plurinervata</i> Burbidge	D.C.F. Rentz s.n.	h
<i>T. procera</i> R.Br.	Waddy 794	h
<i>T. racemigera</i> C.A. Gardner	A. Craig s.n.	f
<i>T. rigidissima</i> (Pilger) Lazarides	P.J. Davidson s.n.	h
<i>T. scariosa</i> Burbidge	J. Mant 25	f
<i>T. schinzii</i> (Henr.) Lazarides	P.J. Davidson 2050	s
<i>T. spicata</i> Burbidge	P.J. Davidson 2057	s
<i>T. stenostachya</i> Domin	B. Rice 4388	h
<i>T. tomentosa</i> Jacobs	G. Flowers 107	f
<i>T. wiseana</i> C.A. Gardner	P.J. Davidson 2037	s
<i>Eragrostis dielsii</i> Pilger	AF019834	g
<i>Muhlenbergia richardsonis</i> (Trinius) Rydberg	AF019837	g
<i>Chloris truncata</i> R.Br.	AF019840	g

Source: (f) fresh sample; (s) seedling material; (h) herbarium specimen; (g) GenBank.

1996; Ainouche and Bayer 1997). The entire ITS region varies in length from 591 to 598 base pairs (bp) for the ingroup. Outgroup taxa vary from 596–597 bp in length. The 5.8S subunit is 164 bp in all taxa, with two variable sites in the ingroup. The ingroup (27 taxa) has 505 constant sites and 111 variable sites, 53 of these being potentially parsimony informative. The variation between ITS1 and ITS2 is comparable, with ITS2 marginally more variable (56 versus 53 variable sites).

(G+C) bias averages 59.2% throughout the ingroup and 55.2% in the three outgroup taxa, which is comparable to other studies (Hsiao *et al.* 1998). Pairwise nucleotide divergence (Kimura two-parameter distance) among Triodieae species ranges from 1.2% to 6.5%. Intraspecific variation was less than that found between species in the five taxa where this was tested (unpublished data).

Phylogenetic Results

The maximum parsimony tree shown in Fig. 1 is a strict consensus of 22 most parsimonious trees of length 303 (CI: 0.54; RI: 0.62). There is strong ITS support shown for the monophyly of

the tribe Triodieae (100% BS). This provides molecular support for the taxonomically isolated position of Triodieae that was suggested in previous morphological analyses of the chloridoids (Van den Borre and Watson 1997).

Within the ingroup the major pattern to note in the ITS trees is the split between 'hard' and 'soft' taxa. All those taxa with a 'soft' leaf anatomy, except *Monodia stipoides*, are in a strongly supported clade (BS 91%; DI 5). All other ingroup taxa have a 'hard' anatomy. *Monodia* falls outside the 'soft' clade, although not significantly so (t-tp test for non-monophyly $p=0.68$; +3 steps). *Plectrachne* is clearly not monophyletic (t-tp test for non-monophyly $p=0.01$; +21 steps).

Within the 'soft' clade there is poor resolution. Similarly, relationships among 'hard' taxa are poorly supported. However, some groups are found consistently. Of these, a 'southern temperate' clade is found although with poor support (BS 59%; DI 1). This clade comprises representative species from south-western Western Australia (*T. danthonioides*, *T. bromoides*) and southern Australia (*T. scariosa* and *T. tomentosa*). However,

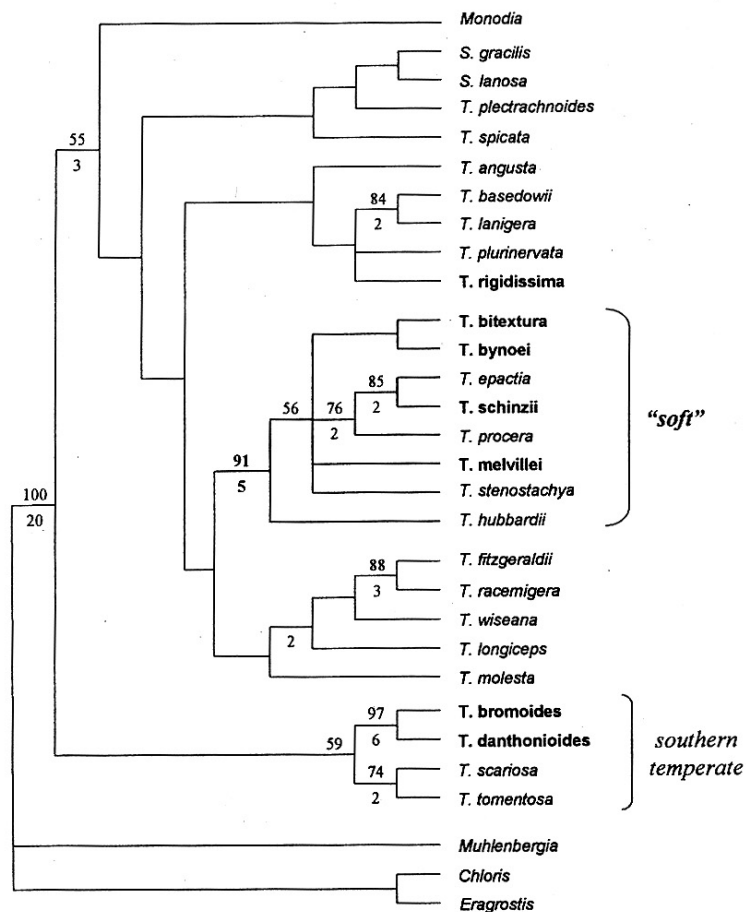


Fig. 1. ITS strict consensus of 22 most parsimonious trees of length 303 (CI: 0.54; RI: 0.62). Bootstrap (>50%) above the line, decay indices (>1) below the line. All species with a 'soft' leaf anatomy except *Monodia stipoides* form a strongly supported clade. All other ingroup species have a 'hard' leaf anatomy. Taxa in bold are former *Plectrachne* species.

T. rigidissima, which appears to be a close morphological relative of *T. danthonioides* and *T. bromoides*, is consistently found outside the southern temperate clade (t-tp test for non-monophyly $p=0.01$; +13 steps).

Outgrouping estimates the southern temperate clade as sister to the rest of the tribe. As such, the root suggests the 'hards' are paraphyletic and the 'softs' are a monophyletic group nested within the 'hards'. Given the poorly supported topologies within these two major groupings the closest sister group to the 'soft' clade remains uncertain under the ITS data.

DISCUSSION

An outstanding problem in the systematics of Triodieae has been a marked inconsistency between inflorescence and anatomical characters. This has persisted in the literature at least since the anatomical studies of Burbidge (1946a, b, 1953) in part because of an implied weighting scheme adopted towards the inflorescence characters traditionally used for taxonomic studies among closely related species of Poaceae. However, the application of molecular sequence data to this problem has provided strong evidence for a pattern of convergence in key spikelet characters (e.g. glume length and shape; lemma shape, lobing and nervation)

while supporting the hypothesis that there are two homologous leaf types in the tribe.

The molecular evidence presented strongly supports the presence of two groups within Triodieae that cut across the previous classification. A 'hard' group with ambito-abaxial stomata and a 'soft' group with centro-abaxial stomata are found. This 'hard' - 'soft' topology is strongly supported by the ITS nrDNA data and is corroborated by morphological and two cpDNA sequence data sets (Mant 1998). The monotypic *Monodia*, which has a highly apomorphic spikelet morphology, is an exception to this pattern for reasons that are not yet clear, although an origin through intergeneric hybridisation could be investigated. Overall, the floral morphology that previously circumscribed both *Plectrachne sens. str.* and *Triodia sens. str.* and which contributes to the make-up of infrageneric groups is convergent and unsuitable for generic delimitation.

While the ITS data have failed to strongly resolve relationships within these two groups, outgroup comparison suggests the 'hards' are a paraphyletic group. By extension, the root supports the suggestion of a derived 'soft' leaf anatomy. This is an intuitively appealing pattern as a loss of photosynthetic capacity from

the outer side of the leaf in the 'soft' lineage is most likely. A change from 'hard' to 'soft' may represent a specialisation to the more predictable rainfall of the monsoonal arid north of the continent to where species from the 'soft' lineage are restricted. The presence of resin among most 'soft' species, which is a relatively rare state in grasses (Burbidge 1946b), is further suggestive of a derived 'soft' condition in the tribe.

Finally, there is a suggestion of a southern temperate clade linking the south-west of Western Australia and southern Australia. This contrasts with previous interpretations of the tribe's biogeography which were based on the division between *Triodia* and *Plectrachne* (Jacobs 1982). The south-western W.A. taxa, previously 'hard' *Plectrachne* species, are not closely related to the 'soft' *Plectrachne* of the monsoonal north of Western Australia. Instead, species from the south-west of W.A. appear to share a close connection with the other southern temperate species in the tribe (*T. scariosa*, *T. irritans* and allies) despite strong dissimilarities in certain spikelet characters. The position of *T. rigidissima*, a species from the Western Desert which has morphological affinities with *T. danthonioides*, conflicts with this biogeographic pattern and requires further investigation.

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