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Systematics of the tribe Podalyrieae (Fabaceae) based on DNA, morphological and chemical data

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Phylogenetic relationships among nine genera and 28 species of the southern African tribe Podalyrieae were estimated from sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA as well as morphological and chemical data. Morphological and ITS sequence data produced cladograms with similar topologies, both supporting the monophyly of Podalyrieae (excluding *Hypocalyptus*). The combined data sets indicate that subtribe Xiphothecinae are monophyletic, but embedded within Podalyriinae. The high degree of congruence between previous taxonomic hypotheses and those based on DNA data provides further evidence for the utility of ITS sequences in studying phylogeny. © 2002 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2002, **139**, 159–170.

ADDITIONAL KEYWORDS: internal transcriber spacer – molecular phylogeny – South Africa – successive weighting – combined analysis.

INTRODUCTION

The legume family, Fabaceae, is the third largest family of flowering plants with approximately 18000 species from 650 genera. They are commonly divided into three subfamilies: Caesalpinioideae, Mimosoideae and Papilionoideae, the last comprising 30 tribes, one of which is Podalyrieae (Polhill, 1994). Polhill (1976; 1981a) expressed uncertainty about the status of Podalyrieae and suggested that it might be sensible to amalgamate it with Liparieae. Podalyrieae comprise the genera *Calpurnia* E.Mey, *Cyclopia* Vent., *Podalyria* Willd., *Virgilia* Poir. and were recently broadened to include most genera of Liparieae; *Amphithalea* Eckl & Zeyh., *Coelidium* Vogel ex Walp. and *Liparia* L. (Schutte & Van Wyk, 1998a). Van Wyk & Schutte (1995) transferred *Calpurnia* to Podalyrieae, and two subtribes, Xiphothecinae A.L. Schutte and Podalyriinae, are recognized (Schutte & Van Wyk, 1998a). According to a molecular study of *rbcL* and internal transcribed spacer (ITS) (Käss & Wink, 1996) *Podalyria*, *Cyclopia*, *Virgilia* and *Liparia* belong to a monophyletic clade, which should be recognized as a single tribe, Podalyrieae. The tribe is endemic to the Cape fynbos region of South Africa, except for *Calpurnia*, which is centred in southern Africa but has one species that extends to eastern Africa and India (Schutte & Van Wyk, 1998a).

The taxonomy of Podalyrieae and Liparieae has been the subject of intensive research over the last 30 years and especially the past 4–5 years (Hutchinson, 1964; Polhill, 1976; Polhill, 1981a, 1981b; Yakovlev, 1991; Schutte, 1995; Van Wyk & Schutte, 1995; Schutte & Van Wyk, 1998a,b). Several genera have recently been revised for the first time since Harvey's (1862) treatment in *Flora Capensis* (*Virgilia* – Van Wyk, 1986; *Liparia* – Schutte & Van Wyk, 1994; *Podalyria* and *Priestleya* – Van Wyk & Schutte, 1995; *Cyclopia* – Schutte, 1997a; *Xiphotheca* – Schutte, 1997b; *Amphithalea* and *Coelidium* – Schutte & Van Wyk, 1998a; *Hypocalyptus* – Schutte & Van Wyk, 1998b). Van Wyk & Schutte (1995) found *Priestleya* to be parapheletic and raised *Priestleya* section *Aneisothea* to generic level under the reinstated name *Xiphotheca*

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Eckl. & Zeyh., whereas *Priestleya* section *Priestleya* was found to be congeneric with *Liparia* and included therein. *Coelidium* was reduced to synonomy under *Amphithalia* by Schutte & Van Wyk (1998a). A new genus, *Stirtonanthus* B.-E. van Wyk & A.L. Schutte (= *Stirtonia* B.-E. van Wyk & A.L. Schutte), was described to accommodate three rare yellow-flowered species, which were formerly included in *Podalyria* (Van Wyk & Schutte, 1994). These species are known only from a few localities in the southern Cape. These authors also proposed a transfer of *Calpurnia* (tribe Sophoreae) to Podalyrieae because of a hypothesized close relationship with *Virgilia* (Van Wyk & Schutte, 1995). *Hypocalyptus* Thunb. was excluded because its morphology, chemical constituents and cytology were not consistent with other members of the group, and it was therefore described as a monogeneric tribe, Hypocalyptieae (Schutte & Van Wyk, 1998b) of speculative position within Papilionoideae.

We undertook this study of Podalyrieae with two broad goals in mind: to evaluate the usefulness of nuclear ribosomal DNA ITS sequences in Podalyrieae *sensu lato*, and to evaluate the earlier hypotheses based on morphological and chemical data. The nuclear ribosomal RNA genes (rDNAs) of higher plants are organized in long tandem repeating units (Appels & Honeycutt, 1986), each of which consists of a single transcribed region for the 18S, 5.8S, 26S ribosomal RNA genes, two small internal transcribed spacers (ITS1 and ITS2), and a large external non-transcribed intergenic spacer (IGS). The numerous copies of rRNA genes (typically thousands per cell in plants) are highly homogeneous (Arnheim, 1983) and exhibit differential rates of evolution among component subunits and spacer regions (reviewed in Hamby & Zimmer, 1992; Baldwin *et al.*, 1995). Sequences of the two internal transcribed spacers have proved useful for resolving relationships within and among closely related plant genera because, in general, these sequences evolve more rapidly than their flanking coding regions (e.g. Baldwin, 1992, 1993; Savard, Michard & Bousquet, 1993; Suh *et al.*, 1993; Wojciechowsk *et al.*, 1993; Sang *et al.*, 1994; Baldwin *et al.*, 1995; Hsiao *et al.*, 1995; Soltis & Kuzoff, 1995; Cox *et al.*, 1997; Pridgeon *et al.*, 1997).

MATERIAL AND METHODS

The taxa selected for this study included representatives of all genera of Podalyrieae and some additional outgroup taxa (Table 1).

MORPHOLOGICAL AND CHEMICAL ANALYSES

A cladogram was generated with the software PAUP for Macintosh, version 4.0b1 (Swofford, 1998). A tree search was carried out using a heuristic search with 1000 random sequence additions and TBR (tree bisection-reconnection) branch swapping with MULPARS 'on' and all character transformations treated as equally likely (Fitch parsimony; Fitch, 1971). A limit of ten trees per replicate was set so that less time was spent swapping on each replicate. Successive approximations weighting (SW; Farris, 1969) was then used to down-weight base positions that change excessively. Successive weighting was carried out on using the 'reweight characters' command according to the RI index, using the maximum value (best fit) criterion and a base weight of one. Using the Fitch trees as the basis for calculating the initial weights, the search-reweighting process was repeated until the same tree length was obtained twice in succession. Internal support was assessed with 1000 bootstrap replicates (Felsenstein, 1985) with both Fitch and SW weights using TBR swapping, MULPARS 'on' holding only ten trees per replicate (with simple addition of taxa). Only groups of >50% frequency were reported, and we apply the following scale for support percentages: 50–74%, weak; 75–84%, moderate; and 85–100%, strong. *Hypocalyptus* was selected as outgroup because it was the most divergent ITS sequence among the taxa studied. The morphological and chemical data are presented in Tables 2 and 3.

ITS-SEQUENCE ANALYSES

The sources of plant material and voucher specimens for taxa in this analysis are listed in Table 1. DNA was extracted from 1.0 g frozen leaf material using the 2X CTAB method of Doyle & Doyle (1987). The amplification of the ITS region (including the 5.85 gene) was carried out using the White *et al.* (1990) ITS 5 and ITS 4 primers. Amplified products were purified using Magic minicolumns (Promega, Southampton, UK), following protocols provided by the manufacturer. Data were analysed using the same procedure as described above. At the end of each round, we carried out another search with no tree number limit so that all trees at that shortest length were collected and swapped to completion. Internal support was assessed as above. From other studies (A. Hulme, unpubl. data), ITS data were available for the following taxa, which were not present in the morphological/chemical analysis: *Sophora microphylla, S. prostrata, S. tetraphylla, S. toromiro* and *Styphnolobium japonicum*.

COMBINED ANALYSIS

In the combined analysis we made use of the morphological, chemical and ITS data. There has been much controversy regarding how different data sets should be combined in phylogenetic analyses (Bull *et al.*,

Table 1. Collections of the tribe Podalyrieae studied

No.	Species	Source	GenBank No.	Voucher Specimen
	Aspalathus			
1	*A. linearis* (N.L. Burm.) R.Dahlgr.	Pakhuis Pass	AJ 409916	*Van Wyk 3630*, RAU
	Hypocalyptus			
2	*H. colutioides* (Lam.) R.Dahlgren	Witelbos	AJ 409917	*Schutte 730*, RAU
3	*H. sophoroides* Druce	Swartberg Pass	AJ 409919	*Van Wyk 3012, 3319*, RAU
4	*H. oxalidiifolius* (Sims) Phillips	Betty's Bay	AJ 409918	*Schutte 468*, RAU
	Xiphotheca			
5	*X. fruticosa* (L) A.L.Schutte & B.-E.van Wyk	Montagu, Pypsteelfontein	AJ 310726	*Schutte 673–675*, RAU
6	*X. tecta* (Thunb.) A.L.Schutte & B.-E.van Wyk	Du Toit's Kloof	AJ 310727	*Schutte 714, 738*, RAU
	Amphithalea			
7	*A. ericifolia* R.Granby	Cape Point	AJ 310728	*Schutte 620*, RAU
8	*A. micrantha* Walp.	Swartberg Pass	AJ 310729	*Vlok & Schutte 325*, RAU
	Coelidium			
9	*C. vlokii* A.L.Schutte & B.-E.van Wyk	Uniondale Koppie	AJ 310730	*Schutte 661–665, 729*, RAU
10	*C. muraltioides* Benth	Swartberg Pass	AJ 310731	*Vlok & Schutte 26*, RAU
11	*C. parvifolium* Druce	Swartberg Pass	AJ 310732	*Vlok & Schutte 123, 190*, RAU
	Cyclopia			
12	*C. burtonii* Hofmeyr & Phillips	Swartberg Pass	AJ 310733	*Vlok & Van Wyk 189*, RAU
13	*C. genistoides* Vent.	Rooiels	AJ 409895	*Schutte 614, 707*, RAU
14	*C. maculata* (Andrews) Kies	Garcia Forest Station	AJ 409896	*Schutte 609–611*, RAU
15	*C. pubescens* Eckl. & Zeyh.	Port Elizabeth	AJ 409897	*Schutte 685–689*, RAU
16	*C. subternata* Vog.	Prince Alfred Pass	AJ 409898	*Schutte 638, 639, 651, 672*, RAU
	Liparia			
17	*L. racemosa* A.L.Schutte	Swartberg Pass	AJ 409908	*Van Wyk 2970*, RAU
18	*L. parva* Vog. ex Walp.	Cape Point	AJ 409909	*Van Wyk 3149, 3243*, RAU
	Podalyria			
19	*P. cuneifolia* Vent.	Port Elizabeth	AJ 409904	*Van Wyk 2888, 3177*, RAU
20	*P. buxifolia* Willd	Prince Alfred Pass	AJ 409905	*Schutte 728*, RAU
21	*P. intermedia* Eckl. & Zeyh.	Franschhoek Pass	AJ 409899	*Van Wyk 3003*, RAU
22	*P. rotundifolia* (Berg.) A.L.Schutte	Du Toit's Kloof	AJ 409900	*Schutte 475–479*, RAU
23	*P. myrtillifolia* Eckl. & Zeyh.	Franschhoek Pass	AJ 409901	*Van Wyk 2995, 3004*, RAU
24	*P. leipoldtii* L.Bolus ex A.L.Schutte	Paleisheuwel	AJ 409902	*Van Wyk 3128*, RAU
25	*P. sericea* R. Br.	Du Toit's Kloof	AJ 409903	*Vlok & Schutte 63b*, RAU
	Stirtonanthus			
26	*S. insignus* (Compton) B.-E.van Wyk & A.L.Schutte	Montagu	AJ 409906	*Schutte & Van Wyk 721*, RAU
27	*S. taylorianus* (L. Bolus) B.-E.van Wyk & A.L.Schutte	Swartberg Pass	AJ 409907	*Van Wyk & Schutte 3248*, RAU
	Virgilia			
28	*V. divaricata* Adamson	The Craggs	AJ 409910	*Van Wyk 879–888*, RAU
29	*V. oroboides* ssp. *ferruginea* B.-E.van Wyk	Ruitersbos	AJ 409911	*Van Wyk 956, 957*, RAU
30	*V. oroboides* ssp. *oroboides* (Berg.) Salter	Betty's Bay	AJ 409912	*Van Wyk 802–806*, RAU

Table 1. *Continued*

No.	Species	Source	GenBank No.	Voucher Specimen
	Calpurnia			
31	*C. aurea Benth.*	Living collection, RBG, Kew	AJ 409913	*RBG, Kew 1991–1626*, K
32	*C. intrusa* E.Mey.	Meiringspoort	AJ 409914	*Van Wyk 3006*, RAU
33	*C. sericea* Harv.	Platberg	AJ 409915	*Van Wyk 4000*, RAU
	Sophora			
34	*S. microphylla* ssp. *macnabiana* (Meyen.)	Chile	AJ 409923	*MF Gardner & SG Knees 4703*, K
35	*S. mirophylla* (Meyen.)	Living collection, RBG, Kew	AJ 409924	*RBG, Kew 1969–16092*, K
36	*S. toromiro Skottsb.*	Living collection, RBG, Kew	AJ 409921	*RBG, Kew 1994–2331*, K
37	*S. prostrata* J. Buch.	Living collection, RBG, Kew	AJ 409922	*RBG, Kew 1988–2824*, K
38	*S. tetraphylla* J.S.Muell.	Living collection, RBG, Kew	AJ 310734	*RBG, Kew 1977–1212*, K
	Styphnolobium			
39	*S. japonicum* Schott	Living collection, RBG, Kew	AJ 409920	*RBG, Kew 1972–10834*, K

1993; Kim & Jansen, 1994). We made use of the most frequently used method, namely the direct combination of different data sets so that all the characters are considered in tree construction (i.e. total evidence, Kluge, 1989; Donoghue & Sanderson, 1992; review and recommendations in Wiens, 1998; which we have followed here). 'Congruence tests' such as ILD are unreliable (Reeves *et al.*, 2001; Yoder *et al.*, 2001), and we did not use any of these methods. Search strategies were as described above. Taxa not presented in the morphological/chemical matrix were scored as missing for these data.

RESULTS

Morphological and chemical analysis

The morphological and chemical data set (Tables 2, 3) yielded ten equally parsimonious trees with length 57, a consistency index (CI) of 0.65 and a retention index (RI) of 0.90. Successive weighting gave one tree (tree length = 49.20686 steps, CI = 0.70, RI = 0.92; Fitch length = 57 steps, therefore the SW tree is one of the Fitch trees). The SW trees can be seen in Figure 1. Subtribe Xiphothecinae form a monophelytic group (93%, SW 95%) with *Aspalathus linearis* (Crotalarieae) sister to it.

ITS-sequence analyses

Complete sequences of the ITS region were generated for 39 taxa, including 28 species of Podalyrieae and four outgroup genera. The boundaries of the internal transcribed spacers (ITS 1 and ITS 2) were determined by comparison with several published sequences obtained from a range of angiosperms (Yokota *et al.*, 1989; Baldwin, 1992; Wojciechowsk *et al.*, 1993; Manos, 1997). The length of ITS 1 varied from 231 to 238 bp, whereas that of ITS 2 varied from 210 to 216 bp for representatives of Podalyrieae. The 5.8S subunit was 161 bp long, which is consistent with most angiosperms. Some variation was detected forthe outgroup taxa containing a 5.8S of 165 bp. The length of the aligned ITS matrix for 39 taxa was 718 bp after ambiguously aligned regions were excluded (the complete matrix was 968 bp), of these, 339 positions (47%) were variable, but only 211 (29%) were potentially informative. All sequences were submitted to GenBank. The aligned data matrices are available from the authors (MVDB & MWC; mvdb@na.rau.ac.za; m.chase@rbgkew.org.uk).

The Fitch analysis resulted in 119 equally most-parsimonious trees, tree length = 647 steps, CI = 0.74 and RI = 0.82 (Fig. 2). Successive weighting produced 15 trees (tree length = 524.73236 steps, CI = 0.78, RI = 0.88; the Fitch length of these trees was 647 steps, i.e. they are a subset of the Fitch trees). Podalyrieae are monophyletic (99%, SW 100%) and within it three clades can be identified. The first clade contained the species from subtribe Xiphothecinae. The second clade contained all the species of Podalyriinae, except for the representatives of *Cyclopia* which formed a third clade, which is also sister to the rest of Podalyrieae.

Table 2. Morphological and chemical characters used

No.	Character
Morphological characters	
1	habit: trees (0); shrubs (1)
2	secondary xylem: vessels arranged in small tangential and/or radial groups (0); vessels arranged in large confluent groups (1)
3	leaf type: pinnate (0); digitate (1); simple (2)
4	stipules: present, sometimes small (0); absent (1)
5	filament: not hairy (0); hairy (1)
6	inflorescence type: not geminate (0); geminate (1)
7	bracteoles: present or strongly reduced (0); absent (1)
8	hypanthium: not prominent (0); prominent (1)
9	calyx base: not intrusive (0); intrusive (1)
10	wing petal: not lobed towards the inside (0); thickly lobed towards the inside (1)
11	keel apex: obtuse (0); slightly beaked (1); strongly beaked (2)
12	stamen grouping: monadelphous (0); diadelphous (1)
13	ovule number: several (0); 1 or 2 (1)
14	seed aril shape: not extended towards the lens (0); extended towards the lens (1)
15	seed micropyle type: ypsaloid (0); punctate (1)
16	seed micropyle position: outside the hilum (0); inside the hilum (1)
17	antipodals: not persistent (0); persistent (1)
18	bract: bract fused with base of pedicel (0); bract not fused with base of pedicel (1)
19	leaf venation: single main vein (0); main vein with lateral longitudinal veins (1)
20	inflorescence axis: without terminal extension (0); with terminal extention (1)
21	sterile bracts: absent (0); present (1)
22	bracts: single (0); paired (1)
23	aril: fleshy (0); non-fleshy, rim arillate (1)
24	aril: continuous around hilum (0); interrupted at micropylar end (1)
25	seed lens: straight (0); oblique (1)
Chemical characters	
26	piperidyl alkaloids: low concentration or absent (0); present as a major compound (1)
27	tetracyclic quinolizidine alkaloids: absent (1); present (0)
28	bicyclic quinolizidine alkaloids: absent (0); present (1)
29	carboxylic acid esters of alkaloids: absent (0); present (1)
30	esterification of monohydroxylated lupanines: absent (0); present (1)
31	ammodendrine: absent (0); present as a major compound (1)
32	canavanine: present (0); absent (1)
33	anthocyanins: absent (0); present (1)
34	anthocyanin esters: absent (0); present (1)
35	seed flavonoids: absent (0); present (1)

Resolution within Xiphothecinae and Podalyriinae (excluding the genus *Cyclopia*) was poor, largely due to low levels of divergence. *Podalyria* is paraphyletic, but none of these branches except for *P. rotundifolia* and *P. intermedia* (94%, SW 96%) has high support.

The ITS data clearly show that *Aspalathus linearis* is sister to the rest of Podalyrieae and *Sophora* (100%, SW 100%), this is in contrast with the morphological results in which *A. linearis* was embedded in Podalyrieae.

Table 3. Morpological and chemical character states used for the cladistic analysis of 31 species of Podalyrieae, using *Hypocalyptus* as outgroup. The cladogram generated from this data set is shown in Figure 1

No.	Species	Character states		
		1 0	2 0	3 0
	Aspalathus			
1	*A. linearis*	1 1 2 1 0 0 0 0 0 0 1 0 1 0 1 1 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 1 0 0 1		
	Hypocalyptus			
2	*H. colutioides*	1 0 1 0 0 0 0 0 1 0 2 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0		
3	*H. sophoroides*	1 0 1 0 0 0 0 0 1 0 2 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0		
4	*H. oxalidiifolius*	1 0 1 0 0 0 0 0 1 0 2 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0		
	Xiphotheca			
5	*X. fruticosa*	1 1 2 1 0 1 1 0 0 1 0 0 0 0 1 1 1 1 0 0 0 0 0 1 0 1 1 1 0 0 0 1 0 0 1		
6	*X. tecta*	1 1 2 1 0 1 0 0 0 1 0 0 0 0 1 1 1 1 0 0 0 0 0 1 0 1 1 1 0 0 0 1 0 0 1		
	Amphithalea			
7	*A. ericifolia*	1 1 2 1 0 1 1 1 0 1 0 0 1 1 1 1 1 0 0 0 0 0 0 1 0 1 1 0 0 0 1 1 1 1 1		
8	*A. micrantha*	1 1 2 1 0 1 1 1 0 1 0 0 1 1 1 1 1 0 0 0 0 0 0 1 0 1 1 0 0 0 1 1 0 0 1		
	Coelidium			
9	*C. vlokii*	1 1 2 1 0 1 1 1 0 1 0 0 1 1 1 1 1 0 0 0 0 0 0 1 0 1 1 0 0 0 1 1 0 0 1		
10	*C. muraltioides*	1 1 2 1 0 1 1 1 0 1 0 0 1 1 1 1 1 0 0 0 0 0 0 1 0 1 1 0 0 0 1 1 1 1 1		
11	*C. parvifolium*	1 1 2 1 0 1 1 1 0 1 0 0 1 1 1 1 1 0 0 0 0 0 0 1 0 1 1 0 0 0 1 1 1 1 1		
	Cyclopia			
12	*C. burtonii*	1 1 1 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 0 0 0 1 0 1 0 0 1 0 0 0 0 1 0 0 1		
13	*C. genistoides*	1 1 1 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 0 0 0 1 0 1 0 0 1 0 0 0 0 1 0 0 1		
14	*C. maculata*	1 1 1 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 0 0 0 1 0 1 0 0 1 0 0 0 0 1 0 0 1		
15	*C. pubescens*	1 1 1 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 0 0 0 1 0 1 0 0 1 0 0 0 0 1 0 0 1		
16	*C. subternata*	1 1 1 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 0 0 0 1 0 1 0 0 1 0 0 0 0 1 0 0 1		
	Liparia			
17	*L. racemosa*	1 1 2 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 1 1 1 0 0 1 0 0 0 0 0 1 0 1 0 0 1		
18	*L. parva*	1 1 2 0 0 0 1 0 1 0 2 0 0 0 1 1 1 0 1 1 1 0 0 1 0 0 0 0 0 1 0 1 0 0 1		
	Podalyria			
19	*P. cuneifolia*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
20	*P. buxifolia*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
21	*P. intermedia*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
22	*P. rotundifolia*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
23	*P. myrtillifolia*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
24	*P. leipoldtii*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
25	*P. sericea*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 1 1 1 1		
	Stirtonanthus			
26	*S. insignus*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 1 0 0 1 1 1 0 0 0 1 1 0 1 0 0 1		
27	*S. taylorianus*	1 1 2 0 0 0 1 0 1 0 1 0 0 0 1 1 1 0 0 1 0 0 1 1 1 0 0 0 1 1 0 1 0 0 1		
	Virgilia			
28	*V. divaricata*	0 0 0 0 1 0 1 0 1 0 2 0 0 0 1 1 1 0 0 0 0 0 1 1 0 0 0 1 1 1 0 1 1 1 1		
29	*V. oroboides* ssp. *ferruginea*	0 0 0 0 1 0 0 0 1 0 2 0 0 0 1 1 1 0 0 0 0 0 1 1 0 0 0 1 1 1 0 1 1 1 1		
30	*V. oroboides* ssp. *oroboides*	0 0 0 0 1 0 0 0 1 0 2 0 0 0 1 1 1 0 0 0 0 0 1 1 0 0 0 1 1 1 0 1 1 1 1		
	Calpurnia			
31	*C. aurea*	0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 1 1 0 0 0 0 0 1 1 0 0 0 1 1 0 0 1 0 0 1		
32	*C. intrusa*	0 0 0 0 1 0 0 0 1 0 1 0 0 0 1 1 1 0 0 0 0 0 1 1 0 0 0 1 1 1 0 1 0 0 1		

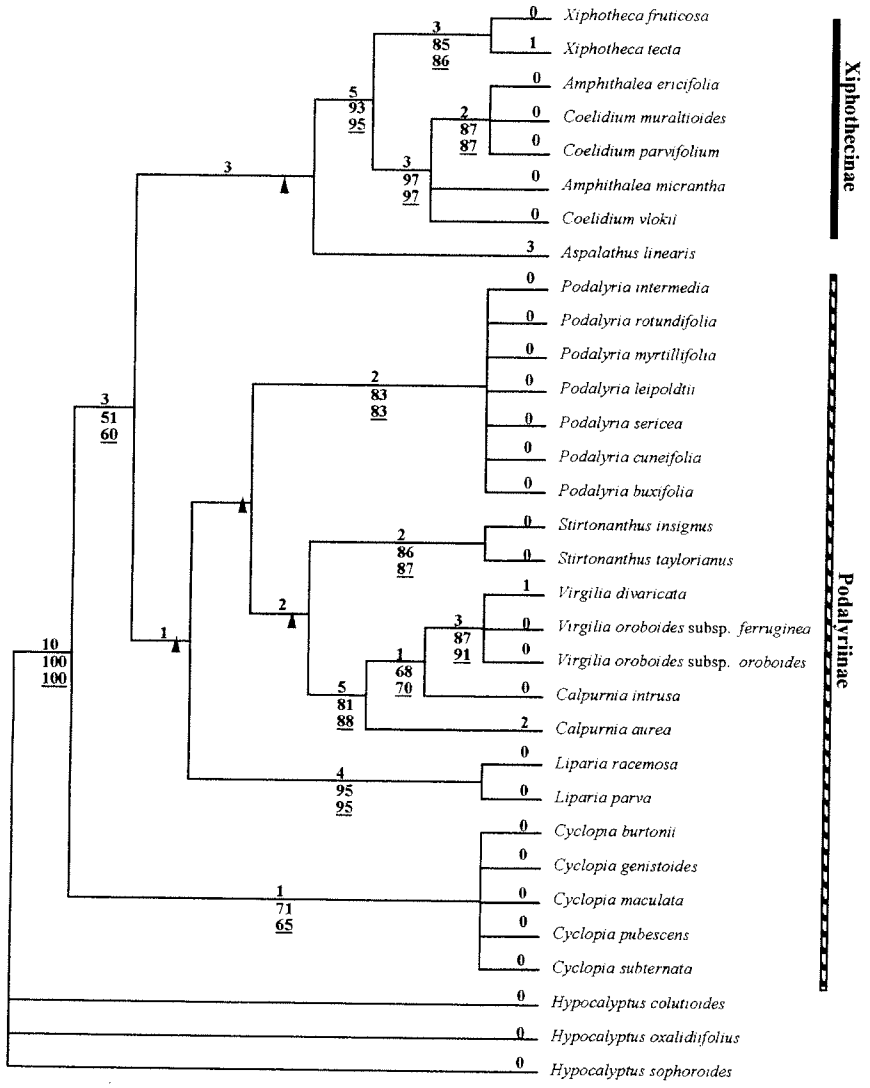

Figure 1. The single SW tree (Fitch tree length = 57) based on morphological and chemical characters of Schutte & Van Wyk (1998a) modified and reanalysed (Tables 2, 3) (CI = 0.65; RI = 0.90). Numbers above the branches are Fitch lenghts, and those below the branches are bootstrap percentages over 50% (SW bootstrap are underlined). Solid arrowheads indicate branches not present in the Fitch consensus tree.

COMBINED ANALYSIS

Much has been made of apparent disagreements between molecular and morphological data (Huelsenbeck *et al.*, 1996; Wiens, 1998; Chase *et al.*, 2000). It has been pointed out that some cases of incongruence are not due to any fundamental differences in patterns of molecular and morphological variation, but rather a result of the divergent methods used to interpret them (Kim & Jansen, 1994). Whatever the situation elsewhere in the legumes, our DNA studies produced results that are interpretable within the framework of taxonomic schemes based on characters other than DNA, and because of the high degree of congruence (Wiens, 1998), we combined all data in one analysis. There appears to be potential incongruence between morphology, which indicates that *Podalyria* is monophyletic (83%, SW 83%) and ITS with *Podalyria* polyphyletic in many trees, but not with bootstrap greater than 50%. In spite of this potential, we analysed all data directly, in accord with Wiens' (1998) recommendation.

The combined morphological, chemical and ITS data produced 68 most parsimonious trees, length of 713 steps, CI = 0.72 and RI = 0.84. Successive weighting gave 17 trees (tree length = 581.84147 steps, CI = 0.73, RI = 0.88; these had the same Fitch length and were therefore a subset of the Fitch trees). The

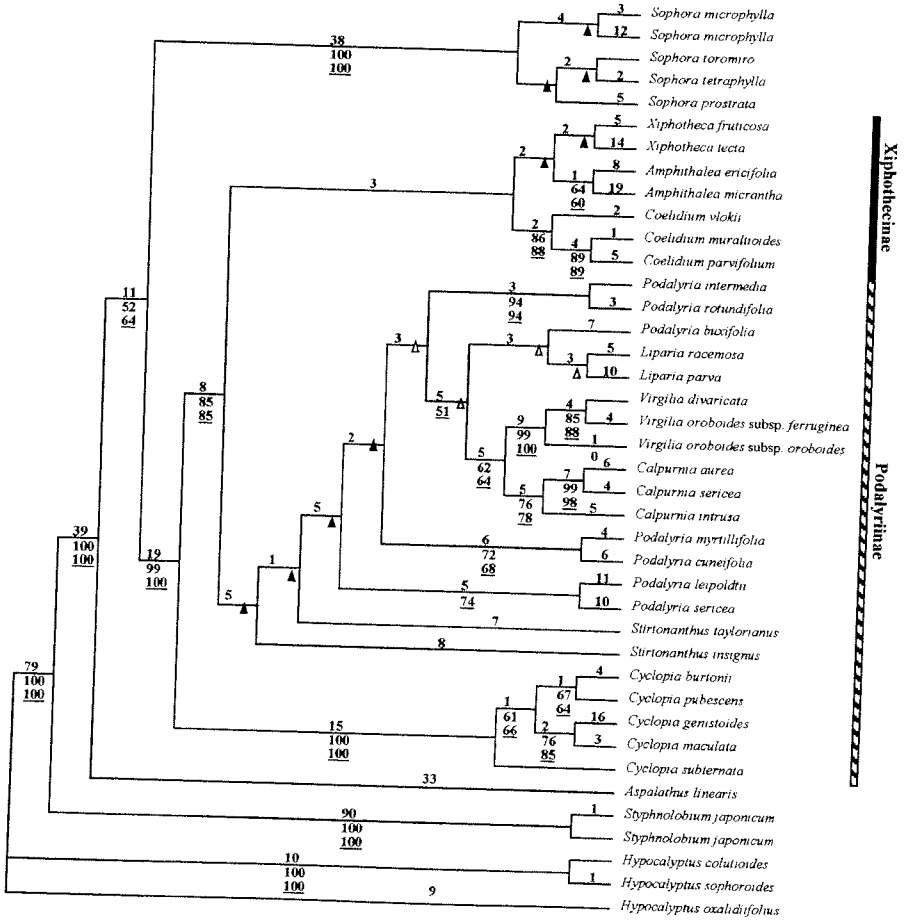

Figure 2. One of the 119 most parsimonious trees (tree length = 647) from the analysis of the ITS region (CI = 0.74, RI = 0.82). Numbers above the branches are Fitch lengths, and those below the branches are bootstrap percentages over 50% (SW bootstrap are underlined). Solid arrowheads indicate branches not present in the Fitch consensus tree. Open arrowheads indicate branches not found in the SW consensus trees.

three clades resolved in the ITS and morphological phylogenies are clearly present (Fig. 3). The strict consensus tree was well resolved, and the three clades consisted of species from Xiphothecinae, all the species of Podalyriinae (except *Cyclopia*) and species of the genus *Cyclopia* could be recognized. Support associated with the first clade (Xiphothecinae) is high (99%, SW 100%), whereas the second clade has a 75%, SW 83% bootstrap. There is high bootstrap support (98%; SW 99%) for the sister relationship between *Amphithalea* and *Coelidium*. The second clade, with the exception of all the representatives of *Cyclopia*, comprised species from Podalyriinae. Unlike the morphological/chemical trees, *Podalyria* is paraphyletic. A sister relationship is evident between *Virgilia* and *Calpurnia* (97%; SW 97%). *Liparia* is also shown to be embedded within the Podalyriinae. Exclusion of *Cyclopia* from Podalyriinae is strongly supported (91%; SW 94%).

DISCUSSION

Several conclusions can be drawn regarding the relationships within Podalyrieae based on the morphological, chemical, ITS and combined analyses. The trees produced by each independent analysis were almost identical, and this permitted us to combine them all in a 'total evidence' analysis, which revealed a largely well-resolved Podalyrieae. The monophyly of Podalyrieae is supported by a minimum of three synapomorphies, namely the general absence of bracteoles; the presence of carboxylic esters of alkaloids and the presence of persistent antipodal cells in the embryo sacs. Podalyrieae can then be separated into three clades. *Amphithalea* (which could include *Coelidium*) and *Xiphotheca* make up the first clade in the combined phylogeny, which is congruent with the previous systematic treatment by Schutte & Van Wyk (1998a). Representatives of Xiphothecinae all possess a non-

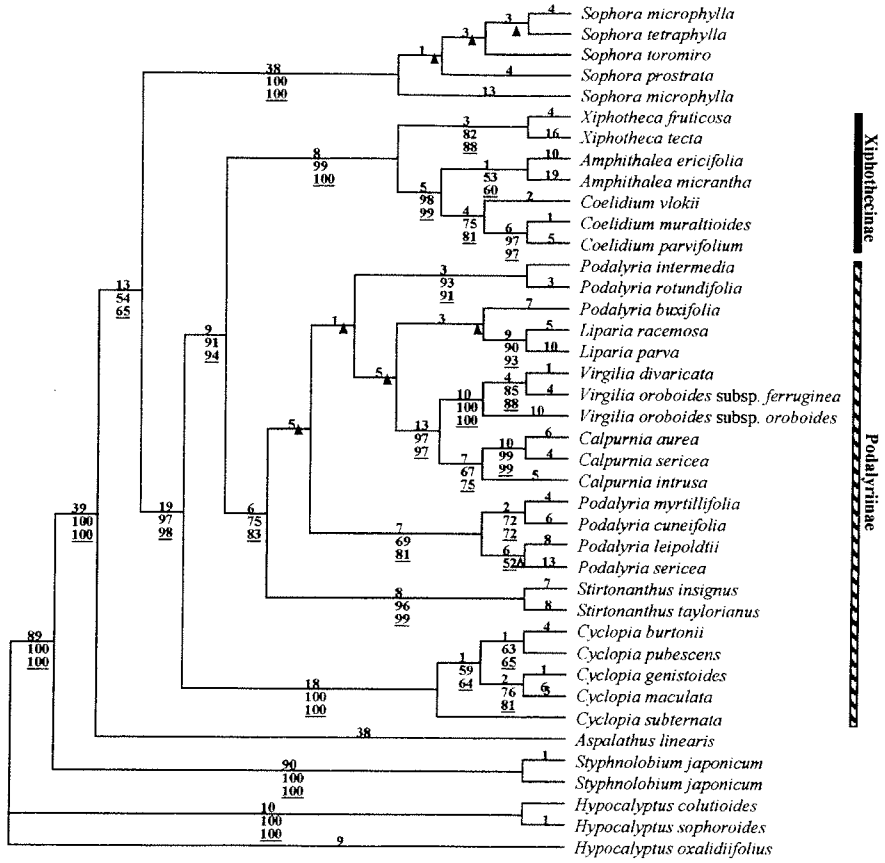

Figure 3. One of the 68 most parsimonious trees (tree length = 713) from the analysis of the ITS region, morphological and chemical data combined (CI = 0.72, RI = 0.84). Numbers above the branches are Fitch lengths, and those below the branches are bootstrap percentages over 50% (SW bootstrap are underlined). Solid arrowheads indicate branches not present in the Fitch consensus tree.

intrusive calyx base, an obtuse keel apex, a reduced number of ovules, and wing petals that have a thickened lobe on the abaxial surface. According to Schutte & Van Wyk (1998a) a sister relationship between *Amphithalea* and *Coelidium* is supported by three shared derived characters: hypanthium prominent; reduced number of seeds; and a seed aril extended towards the lens.

The second clade is composed of species from Podalyriinae. They all possess an intrusive calyx base and a beaked keel tip, as well as the presence of tetracycle quinolizidine alkaloids and esterification of monohydroxylated lupanines (Schutte & Van Wyk, 1998a). The genus *Podalyria* is paraphyletic in many trees in the combined analysis. Only two chemical characters, anthocyanins and anthocyanin esters absent, are responsible for the monophyly of *Podalyria* in the morphological analyses. When these two characters are reduced to a single character, bootstrap support for the monophyly of *Podalyria* dropped from 83% to 64%. According to Schutte (1995) *Podalyria* has a unique combination of characters namely: (1) simple, distinctly petiolate leaves (shared with *Stirtonanthus* and *Xiphotheca*); (2) pink, purple or white flowers (shared with *Virgilia*, *Amphithalea* and *Coelidium*); (3) few-flowered racemose inflorescences (shared with some species of *Liparia*); (4) caducous bracts (shared with *Virgilia*) and (5) characteristic combination of alkaloids, but none of these optimized as synapomorphies on the combined trees. Schutte (1995) described four sections in *Podalyria*, *Villosae* (Benth.) Harv. *emend.* A.L.Schutte *emend. nov.*, *Glabrescens* A.L. Schutte *sect. nov.*, *Nitidae* (Benth.) Harv., and *Podalyria* [*Podalyria* series *Sericea* Benth., *Podalyria* sect. *Sericea* (Benth.) Harv.]. Our data indicate that *Podalyria* is likely to be paraphyletic, with at least three groupings. These groupings correlate with the sections described by Schutte (1995): *Podalyria* section *Villosae* (*P. intermedia* and *P. rotundifolia*; 93%, SW 91%) is supported by two apomorphies, the reflexed calyx lobes and the presence of a split between the upper two calyx lobes at anthesis. *Podalyria buxifolia* forms

part of *Podalyria* section *Nitidae* with one apomorphy, pods not inflated; its position is not resolved. There is weak to moderate support (69%, SW 81%) for *Podalyria* section *Podalyria* (*P. myrtillifolia*, *P. cuneifolia*, *P. leipoldtii*, *P. sericea*). This group possesses a single apomorphy, namely the leaf apices are reflexed (Schutte, 1995). On the basis of morphology and chemistry (alkaloids, flower anthocyanins), *Podalyria* appears to be a natural genus, and the lack of molecular evidence for monophyly is rather unexpected. Our overall assessment is that there is little clear evidence, molecular or otherwise, for the status of *Podalyria*. However, there is an interesting diversity in the optical rotation of the main alkaloids in the genus (see Van Wyk & Verdoorn, 1995): some species produce the combination (+)-sparteine and (−)-lupanine, whereas others have (−)-sparteine and (+)-lupanine. This chemical dichotomy was equally unexpected and seems to suggest that *Podalyria* may have a more complicated evolutionary history than would appear to be the case from its morphological uniformity. In spite of this apparent discrepancy the modifications to generic limits (e.g. the reinstatement of *Xiphotheca*, broadening of *Liparia* and description of *Stirtonanthus*), as well as the transfer of *Calpurnia* from Sophoreae to Podalyrieae and the exclusion of *Hypocalyptus* from the tribe Liparieae are well supported by the ITS results. This shows that a thorough knowledge of the pattern of character state changes in morphological and chemical characters can lead to a fairly accurate estimate of phylogeny, but that DNA sequence data add a new dimension to our understanding of these patterns.

The third clade comprises all the representatives of the genus *Cyclopia*. Bentham (1837; 1839) placed *Cyclopia* in Podalyrieae on account of its free stamens and trifoliate leaves. According to Schutte (1997a), *Cyclopia* is a well-defined genus with several autapomorphies (trifoliolate leaves; unifloral inflorescences; paired, fused bracts; and a total absence of alkaloids); it shares a number of characters with *Liparia*, *Podalyria*, *Stirtonanthus*, *Virgilia* and some species of *Calpurnia* (rostrate or beaked keel; intrusive calyx base and dimorphic anthers). *Cyclopia* has decurrent leaf bases and sterile bracts at the base of the inflorescence, as does *Liparia*. It generally has the free stamens typical of *Podalyria*, *Stirtonanthus* and *Virgilia*, and the presence of distinct pockets on the keel and wing petals found in the *Xiphotheca* group of genera (Schutte, 1997a). *Cyclopia* is well supported (BP 100%) and sister to the rest of Podalyrieae. *Cyclopia* was previously included in the subtribe Podalyriinae, but it is evident that the latter is paraphyletic. The question of paraphyletic groups and whether we should accept them has been much debated in recent years. In view of the relatively small size of the tribe Podalyrieae, it seems unnecessary to erect a monotypic subtribe to accommodate the genus *Cyclopia*. It is more practical to simply accept a broader concept of Podalyrieae, which includes Xiphothecinae, Podalyriinae and *Cyclopia*.

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