American Journal of Botany 88(9): 1675–1687. 2001.

A MOLECULAR PHYLOGENY OF THE ENDEMIC AUSTRALIAN GENUS *GASTROLOBIUM* (FABACEAE: MIRBELIEAE) AND ALLIED GENERA USING CHLOROPLAST AND NUCLEAR MARKERS[1]

GREGORY T. CHANDLER,[2,4] RANDALL J. BAYER,[3] AND MICHAEL D. CRISP[2]

[2]Division of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia; and [3]CSIRO, Plant Industry, Centre for Plant Biodiversity Research, Australian National Herbarium, GPO Box 1600, Canberra ACT 2601, Australia

Gastrolobium (Fabaceae: Mirbelieae) is an endemic Australian genus that produces toxic sodium monofluoroacetate. A phylogenetic reconstruction of *Gastrolobium* and the related genera *Brachysema, Callistachys, Jansonia, Nemcia, Oxylobium,* and *Podolobium* is presented, using sequence data from three regions—the *psbA-trnH* intergenic spacer and the *trnK* 5′ intron from chloroplast DNA and the 3′ end of the external transcribed spacer (ETS) from nuclear ribosomal DNA. *Gastrolobium* is shown to be paraphyletic, with *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare* nesting within it, and *Nemcia* is shown to be polyphyletic within *Gastrolobium.* Past key morphological characters, such as fluoroacetate content and characters associated with pollination syndrome, are shown to be homoplastic, with fluoroacetate possibly a plesiomorphic condition lost in more derived species. *Podolobium* is also shown to be polyphyletic, with the *P. ilicifolium* group sister to *Gastrolobium* and the *P. alpestre* group sister to *Callistachys,* a member of the *Oxylobium* group. It is recommended that *Gastrolobium* be expanded to include *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare,* while further work is required to test the sister-group relationship between *Podolobium* s.s. (sensu stricto) and *Gastrolobium.*

Key words: external transcribed spacer; *trnK* intron; *matK, psbA-trnH* spacer; *Gastrolobium*; Fabaceae; molecular phylogenetics; Mirbelieae.

The tribe Mirbelieae (Fabaceae) is endemic to Australia and comprises a major component of the flora in many temperate ecosystems. This tribe is related to the ''Genistoid Alliance'' within the Fabaceae, though it is no longer regarded as part of that alliance (see Crisp, Gilmore, and Van Wyk, in press). A major component of this tribe is *Gastrolobium* R.Br., containing ~60 species. This genus is endemic to the southwest of Western Australia, except two species that are widespread throughout northern Australia (*G. brevipes* and *G. grandiflorum*; Fig. 1). Furthermore, it is one of the largest legume genera in the southwest of Western Australia, where it forms a major component of the understory in many areas, such as sandplains with their accompanying vegetation (''Kwongan''), which is usually heath or mallee (shrubby eucalypt woodland).

Species of *Gastrolobium* are simple-leaved shrubs that have terminal racemose inflorescences with yellow, orange, and red flowers. The coloration of the flower is typical of the tribe Mirbelieae, the standard petal is generally orange or yellow, with a central red ring on the front of the standard petal surrounding a yellow center. These orange and yellow standard petals are considered an indicator of insect pollination, while a red standard (present in only one species, *G. grandiflorum*) indicates bird pollination (for example, Keighery, 1982). Members of *Gastrolobium* accumulate sodium monofluoroacetate (e.g., Aplin, 1971; Twigg et al., 1996), which makes them highly toxic, and severe stock losses have occurred in the past due to fluoroacetate poisoning, which led to an eradication program, particularly in the wheat-belt region of southwestern Western Australia. As a consequence, many species are now rare or threatened with extinction, making *Gastrolobium* both ecologically and economically important.

Taxonomic history of Gastrolobium—Throughout its taxonomic history, the circumscriptions of *Gastrolobium* and its allied genera, particularly *Oxylobium* Jackson, have changed considerably. As a result, species have been transferred from one genus to another on several occasions. A major component of the problem of the circumscription of *Gastrolobium* is due to the fact that morphological data have, to date, failed to fully resolve the relationships within the tribes Mirbelieae and Bossiaeeae (Crisp and Weston, 1987, 1995), especially the *Gastrolobium*/*Oxylobium* generic group.

Gastrolobium was described by Brown (1811) as a monotypic genus, diagnosed by a stipitate ovary with two ovules, which distinguished it from *Oxylobium* (below), though Brown (1811) did not mention this fact explicitly. Species were added to *Gastrolobium* over time by various authors, and Bentham (1864) provided the first revision of this group in *Flora Australiensis.* Again, it was primarily ovule number that separated *Gastrolobium* from *Oxylobium,* with *Gastrolobium* having two ovules and *Oxylobium* four or more ovules (Bentham, 1864). Both genera contained species that produced fluoroacetate, and

[1] Manuscript received 20 October 2000; revision accepted 13 February 2001.

The authors thank the Centre for Plant Biodiversity Research, Australian National Herbarium (CANB) for housing of material, loans assistance, and use of laboratory facilities, and the Australian Biological Resources Study (ABRS) for providing funds for field work, without which this project would not have been possible; Mike O'Donoghue from CALM in Perth, Western Australia, for providing permit assistance and follow up collections from geographically difficult species; and Mark Clements, Edward Cross, Rogier de Kok, and Brenda Wingfeld for providing critical evaluations on this manuscript. The first author thanks the Australian National Botanic Gardens, Canberra, Living Collections, in particular Stuart Donaldson for field assistance during three field trips and Lindy Cayzer, Wes Keys, and Anna Monro for field assistance during one trip each.

[4] Author for reprint requests, current address: Department of Biology, Virginia Commonwealth University, 816 Park Ave, P.O. Box 842012, Richmond, Virginia, 23284-2012 USA (email: the.lizard@prontomail.com).

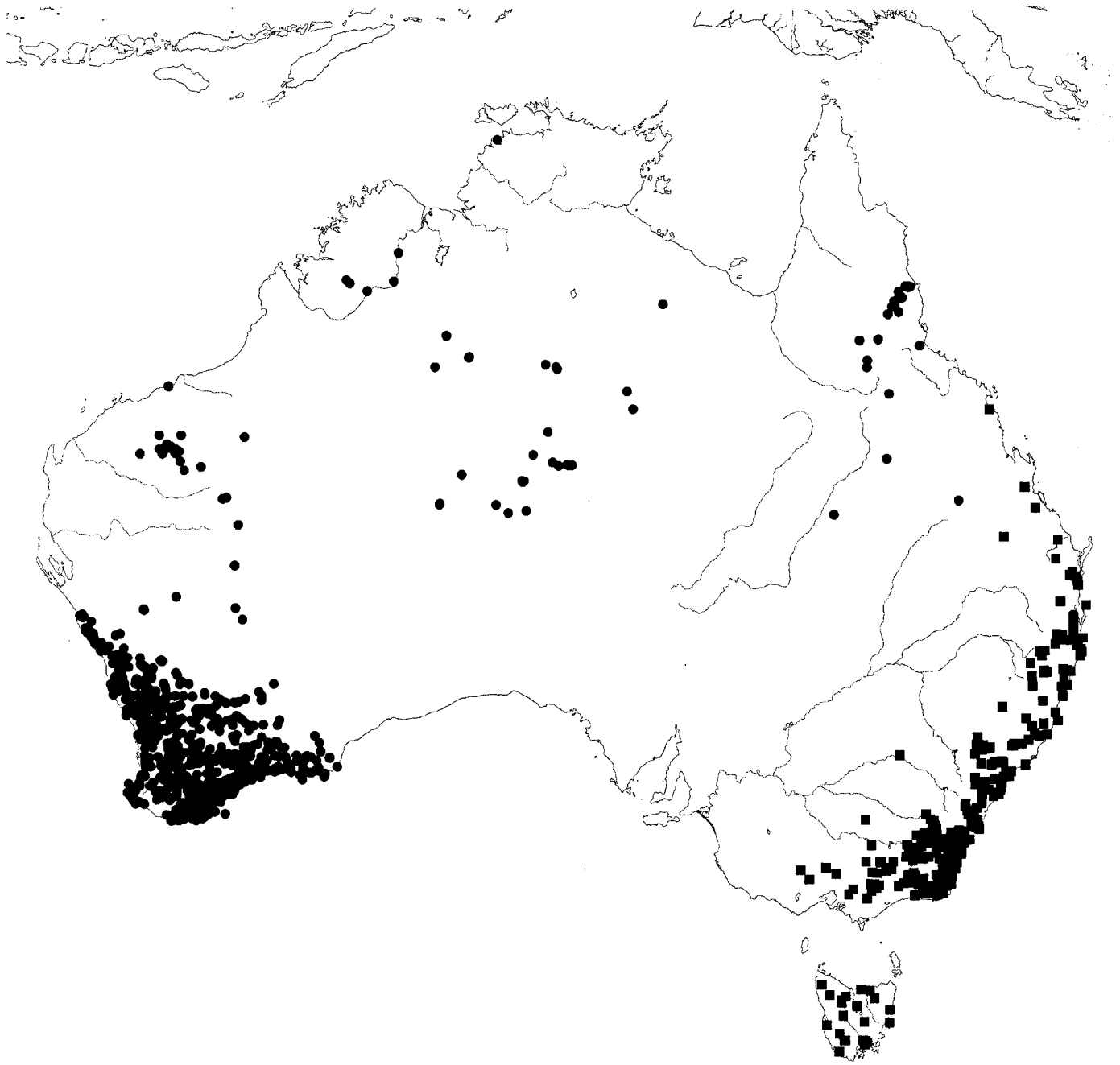

Fig. 1. Distribution of *Gastrolobium* and related genera in Australia. *Gastrolobium* is marked with circles, and *Oxylobium* and *Podolobium* occur in the area delimited by squares. The genera *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare* occur in southwestern Western Australia, in the area densely covered by circles.

Oxylobium contained species from both eastern and western Australia (Fig. 1).

Kuntze (1891) subsumed *Oxylobium* and *Gastrolobium* into the earlier genus *Callistachys* Vent. However, *Oxylobium* was later conserved against *Callistachys. Nemcia* was described by Domin (1923), including 12 species characterized by 4–6 ovules, trifid bracts, and condensed racemose inflorescences. This work was largely ignored, and the concepts of *Gastrolobium* and *Oxylobium* remained as they had been since Bentham (1864).

Gardner and Bennetts (1956) provided a revision of the toxic plants of Western Australia, which included a number of species of *Gastrolobium* and *Oxylobium.* However, this was not a complete revision of the group, because it did not include the nontoxic species of either genus. Furthermore, these toxic species were interleaved in this artificial key, the authors apparently being unable to distinguish easily between the two genera, and again the concept of Bentham (1864) was used as the division between *Gastrolobium* and *Oxylobium,* relying on ovule number as the main character to separate the two genera.

Crisp and Weston (1987) published the first major review of *Gastrolobium* since Bentham (1864). They presented a phy-

logeny of the tribe Mirbelieae based on morphology and reinstated and expanded both *Nemcia* and *Podolobium* F.Muell., the latter of which is an eastern Australian genus closely aligned with *Oxylobium. Gastrolobium* fell into the 'Callistachys' group, which consisted of *Brachysema* R.Br., *Callistachys, Jansonia* Kipp., *Gastrolobium, Nemcia, Podolobium,* and *Oxylobium lineare.* The analysis of Crisp and Weston (1987), however, was done at a higher level to resolve tribal relationships within the Mirbelieae, using either genera or subgeneric groups as terminals.

Crisp and Weston (1987) changed the circumscription of *Gastrolobium* to include all toxic species of *Gastrolobium* and *Oxylobium,* so that for the first time, species with more than two ovules were included within *Gastrolobium.* This left only one species of *Oxylobium* occurring in Western Australia (*O. lineare*), which required further work to determine its generic affinities. Their reduced concept of *Oxylobium* comprises five species endemic to eastern Australia, mostly along the central and southern coastal plain and the adjacent escarpment of the Great Dividing Range (Fig. 1). The nontoxic species of *Gastrolobium* and *Oxylobium* were removed into *Nemcia* (but see Twigg et al., 1996).

Nemcia, as defined by Crisp and Weston (1987), contained species with axillary racemes often reduced to one or two flowers (though some had condensed, terminal racemes with many flowers), and included the nontoxic species transferred from *Gastrolobium* and *Oxylobium* (see Aplin, 1971), thereby using secondary metabolites as an aid in the resolution of this taxonomically difficult group.

Genera such as *Brachysema, Jansonia,* and *Leptosema* Benth. were distinguished by floral characteristics that have been interpreted by later authors as indicative of bird pollination (e.g., Keighery, 1982). These characters include red petals, a reduced standard petal and enlarged keel petals, and copious nectar. *Gastrolobium* and *Oxylobium* are primarily bee pollinated, except *G. grandiflorum,* which has large, red flowers, but lacks the ''bird–flower'' modifications of genera such as *Brachysema,* like a reduced standard petal. However, most of the assumptions of bee- or bird-pollination are largely inference based on floral structure, which often came from empirical data, such as sightings of birds visiting flowers (e.g., Keighery, 1980, 1982, 1984).

The evolution of bird-pollination in some Australian legumes was discussed by Crisp (1994), where a phylogeny of *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare* was presented, but did not include *Gastrolobium.* Crisp (1994) also tested the monophyly of these genera with a species-level phylogeny using morphology. Even though this analysis did not include *Gastrolobium, Nemcia* was shown to be paraphyletic, while *Brachysema* was demonstrated to be monophyletic.

Crisp, Gilmore, and Van Wyk (in press) provide a molecular phylogeny of the genistoid legume tribes, though only two species of the 'Callistachys' group are used in this tribal phylogeny, so nothing can be deduced about the relationships within this group. A sound, well-resolved phylogeny of *Gastrolobium* and its close relatives is therefore required in order to resolve the taxonomic dilemmas surrounding this group and bring stability to these genera.

Molecular phylogeny of Gastrolobium and related genera—As morphological data alone have been insufficient in resolving the relationships of the *Gastrolobium* group, molecular data were the obvious choice to try to find a robust phylogeny on which to base future taxonomic classifications. This study has used the *psbA-trnH* intergenic spacer, the *trnK* 5′ intron (both from chloroplast DNA), and the 3′ end of the external transcribed spacer (ETS, from nuclear ribosomal DNA) in an attempt to resolve the relationships of *Gastrolobium* and its close relatives.

The *psbA-trnH* intergenic spacer region lies in the inverted repeat region of the chloroplast genome, near the boundary with the large single-copy region, adjacent to the *trnK* gene (Sugiura, 1992). The *psbA* chloroplast gene belongs to the Photosystem II (PSII) protein complex and codes for the PSII D1-protein; the $trnH^{His}$ (GUG) gene belongs to the transfer RNA gene system and transfers for the amino acid histidine. Phylogenetic studies reported this spacer to be of more use at higher taxonomic levels, particularly intergeneric levels (for example, Aldrich et al., 1988; Sang, Crawford, and Stuessy, 1997; Asmussen and Liston, 1998; Kim et al., 1999), though Kim et al. (1999) did find it somewhat useful at the infrageneric level.

The *trnK* intron, which includes the *matK* coding region, has been used to reconstruct phylogenies in a number of different families, such as the Apiaceae (Plunkett, Soltis, and Soltis, 1996, 1997), Cornaceae (Xiang, Soltis, and Soltis, 1998), Cupressaceae (Gadek et al., 2000), Fabaceae (Hu et al., 2000), Juglandaceae (Stanford, Harden, and Parks, 2000), Nymphaeaceae (Les et al., 1999), Orchidaceae (Jarrell and Clegg, 1995), Pinaceae (Wang et al., 1999), Poaceae (Liang and Hilu, 1996; Hilu and Liang, 1997; Hilu and Alice, 1999), Polemoniaceae (Steele and Vilgalys, 1994; Johnson and Soltis, 1995; Johnson et al., 1996), and Saxifragaceae (Johnson and Soltis, 1994, 1995). Hilu and Liang (1997) evaluate the rate, patterns, and types of nucleotide substitutions in the *matK* gene, functional constraints, and phylogenetic utility of the gene, using data from a number of different plant families, and report that the 5′ end of the *trnK* intron is larger and contains more informative characters than the 3′ end. Accordingly, the 5′ section of the *trnK* intron was selected for use in this study.

The external transcribed spacer has recently been shown to be much larger and contain more phylogenetically informative characters than the internal transcribed spacer (ITS), providing a large number of characters for use in phylogenetic analyses (Baldwin and Markos, 1998; Bena et al., 1998). To date, the ETS has been used mainly to study phylogeny within the Asteraceae (Baldwin and Markos, 1998; Linder et al., 2000), and the Fabaceae (Bena et al., 1998). However, sequences have been generated from the ETS region in a number of families, sometimes covering the entire intergenic spacer (IGS) of ribosomal DNA, including the Asteraceae (Baldwin and Markos, 1998; Linder et al., 2000), Brassicaceae (Rathgeber and Capesius, 1990), Cucurbitaceae (King et al., 1993), Fabaceae (Rogers and Bendich, 1987a, b; Schiebel et al., 1989; Bena et al., 1998), and Solanaceae (Schmidt-Puchta, Gunther, and Sänger, 1989; Borisjuk et al., 1994; Volkov et al., 1996).

METHODS

Fieldwork—Extensive fieldwork was undertaken throughout the southwest of Western Australia, involving several trips made during different times of the year, in summer and early, mid-, and late spring. This allowed the collection of material in the best condition for both DNA extraction and for subsequent morphological analysis (not presented here). Leaves for DNA extraction were preserved in liquid CTAB/NaCl solution at ambient temperature and stored later at −20°C (Rogstad, 1992). Table 1 shows the 94 taxa used in this analysis including their authorities, along with GenBank accession

TABLE 1. Taxa used in the analysis, with the nearest named place given in the locality, and with GenBank accession numbers for each sequence. All voucher specimens are housed at the Australian National Herbarium (CANB). Taxa appear in alphabetical order, and the nearest named place is followed by the state of collection. WA = Western Australia, NSW = New South Wales (eastern Australia), QLD = Queensland, ACT = Australian Capital Territory.

Taxon	Collector	Number	Locality	*trnK* 5′ intron accession[a]	*psbA-trnH* accession	ETS accession
Brachysema bracteolosum F. Muell.	G. T. Chandler	426	Bremer Bay, WA	GBAN-AF298424	GBAN-AF298330	GBAN-AF298236
Brachysema celsianum Lem.	M. D. Crisp	9009	Mogumber, WA	GBAN-AF298425	GBAN-AF298331	GBAN-AF298237
Brachysema latifolium R. Br.	G. T. Chandler	365	Lort River, WA	GBAN-AF298426	GBAN-AF298332	GBAN-AF298238
Brachysema melanopetalum F. Muell.	M. D. Crisp	8470	Manjimup, WA	GBAN-AF298427	GBAN-AF298333	GBAN-AF298239
Brachysema minor Crisp	M. D. Crisp	8922	Mt Barker, WA	GBAN-AF298428	GBAN-AF298334	GBAN-AF298240
Brachysema modestum Crisp	M. D. Crisp	8465	Busselton, WA	GBAN-AF298429	GBAN-AF298335	GBAN-AF298241
Brachysema praemorsum Meisn.	G. T. Chandler	729	Mt Barker area, WA	GBAN-AF298430	GBAN-AF298336	GBAN-AF298242
Brachysema sericeum Domin	J. M. Taylor	1959	Hay River, WA	GBAN-AF298431	GBAN-AF298337	GBAN-AF298243
Brachysema subcordatum Benth.	M. D. Crisp	8511	Porongorup Range	GBAN-AF298432	GBAN-AF298338	GBAN-AF298244
Callistachys lanceolata Vent.	G. T. Chandler	474	Albany area, WA	GBAN-AF298433	GBAN-AF298339	GBAN-AF298245
Gastrolobium acrocaroli ms	G. T. Chandler	778	Peak Charles, WA	GBAN-AF298434	GBAN-AF298340	GBAN-AF298246
Gastrolobium appressum C. A. Gardner	G. T. Chandler	208	Gunyidi area, WA	GBAN-AF298435	GBAN-AF298341	GBAN-AF298247
Gastrolobium bennettsianum C. A. Gardner	G. T. Chandler	556	Bodallin area, WA	GBAN-AF298436	GBAN-AF298342	GBAN-AF298248
Gastrolobium bilobum R. Br.	G. T. Chandler	724	Two People Bay, WA	GBAN-AF298437	GBAN-AF298343	GBAN-AF298249
Gastrolobium brownii Meisn.	G. T. Chandler	726	Denmark area, WA	GBAN-AF298438	GBAN-AF298344	GBAN-AF298250
Gastrolobium callistachys Meisn.	G. T. Chandler	678	Dingo Rock, WA	GBAN-AF298439	GBAN-AF298345	GBAN-AF298251
Gastrolobium calycinum Benth.	G. T. Chandler	544	Wongan Hills, WA	GBAN-AF298440	GBAN-AF298346	GBAN-AF298252
Gastrolobium carinatum ms	G. T. Chandler	427	Bremer Bay, WA	GBAN-AF298480	GBAN-AF298386	GBAN-AF298292
Gastrolobium congestum ms	G. T. Chandler	404	Fitzgerald River National Park, WA	GBAN-AF298441	GBAN-AF298347	GBAN-AF298253
Gastrolobium crassifolium Benth.	G. T. Chandler	419	Jerramungup area, WA	GBAN-AF298442	GBAN-AF298348	GBAN-AF298254
Gastrolobium cuneatum Henfr.	M. D. Crisp	8937	Blackwood River, WA	GBAN-AF298443	GBAN-AF298349	GBAN-AF298255
Gastrolobium densifolium C. A. Gardner	G. T. Chandler	532	Tarin Rock, WA	GBAN-AF298444	GBAN-AF298350	GBAN-AF298256
Gastrolobium diablophyllum ms	G. T. Chandler	559	Bodallin area, WA	GBAN-AF298445	GBAN-AF298351	GBAN-AF298257
Gastrolobium floribundum S. Moore	G. T. Chandler	553	Carrabin, WA	GBAN-AF298446	GBAN-AF298352	GBAN-AF298258
Gastrolobium glaucum C. A. Gardner	G. T. Chandler	543	Wongan Hills, WA	GBAN-AF298447	GBAN-AF298353	GBAN-AF298259
Gastrolobium grandiflorum F. Muell.	G. T. Chandler	598	Aust Nat Botanic Gardens	GBAN-AF298448	GBAN-AF298354	GBAN-AF298260
Gastrolobium graniticum (S. Moore) Crisp	G. T. Chandler	567	Bullabulling, WA	GBAN-AF298449	GBAN-AF298355	GBAN-AF298261
Gastrolobium hamulosum Meisn.	G. T. Chandler	845	Wongan Hills, WA	GBAN-AF298450	GBAN-AF298356	GBAN-AF298262
Gastrolobium heterophyllum (Turcz.) Crisp	G. T. Chandler	918	Fitzgerald River National Park, WA	GBAN-AF298451	GBAN-AF298357	GBAN-AF298263
Gastrolobium hians ms	G. T. Chandler	868	Norseman area, WA	GBAN-AF298452	GBAN-AF298358	GBAN-AF298264
Gastrolobium involutum ms	G. T. Chandler	805	Mt. Buraminya, WA	GBAN-AF298453	GBAN-AF298359	GBAN-AF298265
Gastrolobium laytonii J. White	G. T. Chandler	664	Mt. Gibson area, WA	GBAN-AF298454	GBAN-AF298360	GBAN-AF298266
Gastrolobium microcarpum Meisn.	G. T. Chandler	686	Clackline, WA	GBAN-AF298455	GBAN-AF298361	GBAN-AF298267
Gastrolobium nutans ms	G. T. Chandler	817	Lake King area, WA	GBAN-AF298456	GBAN-AF298362	GBAN-AF298268
Gastrolobium oxylobioides Benth.	G. T. Chandler	654	Geraldton area, WA	GBAN-AF298457	GBAN-AF298363	GBAN-AF298269
Gastrolobium parviflorum (Benth.) Crisp	G. T. Chandler	760	Narrogin, WA	GBAN-AF298458	GBAN-AF298364	GBAN-AF298270
Gastrolobium polystachyum Meisn.	G. T. Chandler	627	Badgingarra area, WA	GBAN-AF298459	GBAN-AF298365	GBAN-AF298271
Gastrolobium propinquum C. A. Gardner	G. T. Chandler	652	Port Gregory, WA	GBAN-AF298460	GBAN-AF298366	GBAN-AF298272
Gastrolobium pusillum Crisp & P. H. Weston	M. D. Crisp	8921	Mt Barker, WA	GBAN-AF298461	GBAN-AF298367	GBAN-AF298273
Gastrolobium pycnostachyum Benth.	G. T. Chandler	337	Mt Ragged, WA	GBAN-AF298462	GBAN-AF298368	GBAN-AF298274
Gastrolobium reflexum ms	G. T. Chandler	645	Arrino area, WA	GBAN-AF298463	GBAN-AF298369	GBAN-AF298275
Gastrolobium revolutum ms	G. T. Chandler	524	Lake King, WA	GBAN-AF298464	GBAN-AF298370	GBAN-AF298276
Gastrolobium rigidum (C. A. Gardner) Crisp	G. T. Chandler	531	Tarin Rock, WA	GBAN-AF298465	GBAN-AF298371	GBAN-AF298277
Gastrolobium rotundifolium Meisn.	G. T. Chandler	658	Watheroo, WA	GBAN-AF298466	GBAN-AF298372	GBAN-AF298278
Gastrolobium semiteres ms	G. T. Chandler	694	Boorabbin Rock, WA	GBAN-AF298467	GBAN-AF298373	GBAN-AF298279
Gastrolobium spectabile (Endl.) Crisp	G. T. Chandler	821	Kunanoppin, WA	GBAN-AF298468	GBAN-AF298374	GBAN-AF298280

TABLE 1. Continued.

Taxon	Collector	Number	Locality	*trnK* 5′ intron accession[a]	*psbA-trnH* accession	ETS accession
Gastrolobium spinosum Benth.	G. T. Chandler	548	Mt O'Brien, WA	GBAN-AF298469	GBAN-AF298375	GBAN-AF298281
Gastrolobium stenocarpum ms	G. T. Chandler	406	Mt Desmond, WA	GBAN-AF298470	GBAN-AF298376	GBAN-AF298282
Gastrolobium stenophyllum Turcz.	G. T. Chandler	735	Marningarup area, WA	GBAN-AF298471	GBAN-AF298377	GBAN-AF298283
Gastrolobium stowardii S. Moore	G. T. Chandler	950	Lake Grace, WA	GBAN-AF298503	GBAN-AF298409	GBAN-AF298315
Gastrolobium tenue ms	G. T. Chandler	688	Belka area, WA	GBAN-AF298472	GBAN-AF298378	GBAN-AF298284
Gastrolobium tergiversum ms	G. T. Chandler	344	Mt Ragged, WA	GBAN-AF298473	GBAN-AF298379	GBAN-AF298285
Gastrolobium tetragonophyllum (E. Pritzel) Crisp	G. T. Chandler	706	Mt Short, WA	GBAN-AF298474	GBAN-AF298380	GBAN-AF298286
Gastrolobium tomentosum C. A. Gardner	G. T. Chandler	756	Williams area, WA	GBAN-AF298475	GBAN-AF298381	GBAN-AF298287
Gastrolobium triangulare Domin	G. T. Chandler	655	Geraldton area, WA	GBAN-AF298476	GBAN-AF298382	GBAN-AF298288
Gastrolobium trilobum Benth.	G. T. Chandler	536	Bindi Bindi area, WA	GBAN-AF298477	GBAN-AF298383	GBAN-AF298289
Gastrolobium truncatum Benth.	M. D. Crisp	8919	Bokal, WA	GBAN-AF298478	GBAN-AF298384	GBAN-AF298290
Gastrolobium villosum Benth.	G. T. Chandler	542	Callingiri area, WA	GBAN-AF298479	GBAN-AF298385	GBAN-AF298291
Isotropis cuneifolia Heynh.	M. D. Crisp	8459	Mogumber, WA	GBAN-AF298481	GBAN-AF298387	GBAN-AF298293
Jacksonia horrida DC.	M. D. Crisp	8934	Scott River, WA	GBAN-AF298482	GBAN-AF298388	GBAN-AF298294
Jansonia formosa Kipp.	M. D. Crisp	8933	Scott River, WA	GBAN-AF298483	GBAN-AF298389	GBAN-AF298295
Latrobea hirtella Benth.	M. D. Crisp	8478	Stirling Range, WA	GBAN-AF298484	GBAN-AF298390	GBAN-AF298296
Mirbelia depressa E. Pritzel	M. D. Crisp	9020	Perenjori, WA	GBAN-AF298485	GBAN-AF298391	GBAN-AF298297
Mirbelia dilatata R. Br.	M. D. Crisp	8491	Stirling Range, WA	GBAN-AF298486	GBAN-AF298392	GBAN-AF298298
Nemcia alternifolia ms	M. D. Crisp	8512	York area, WA	GBAN-AF298487	GBAN-AF298393	GBAN-AF298299
Nemcia coriacea Domin	G. T. Chandler	723	Nanarup area, WA	GBAN-AF298488	GBAN-AF298394	GBAN-AF298300
Nemcia crenulata (Turcz.) Crisp	G. T. Chandler	490	Stirling Range, WA	GBAN-AF298489	GBAN-AF298395	GBAN-AF298301
Nemcia emarginata (S. Moore) Crisp	M. D. Crisp	8963	Stirling Range, WA	GBAN-AF298490	GBAN-AF298396	GBAN-AF298302
Nemcia hookeri (Meisn.) Crisp	M. D. Crisp	8907	York area, WA	GBAN-AF298491	GBAN-AF298397	GBAN-AF298303
Nemcia leakeana (Drumm.) Crisp	M. D. Crisp	8481	Stirling Range, WA	GBAN-AF298492	GBAN-AF298398	GBAN-AF298304
Nemcia luteifolia Domin	M. D. Crisp	9407	Stirling Range, WA	GBAN-AF298493	GBAN-AF298399	GBAN-AF298305
Nemcia obovata (Benth.) Crisp	G. T. Chandler	657	Watheroo, WA	GBAN-AF298494	GBAN-AF298400	GBAN-AF298306
Nemcia plicata (Turcz.) Crisp	G. T. Chandler	623	Badgingarra area, WA	GBAN-AF298495	GBAN-AF298401	GBAN-AF298307
Nemcia pulchella (Turcz.) Crisp	M. D. Crisp	8480	Stirling Range, WA	GBAN-AF298496	GBAN-AF298402	GBAN-AF298308
Nemcia pyramidalis (T. Moore) Crisp	G. T. Chandler	488	Stirling Range, WA	GBAN-AF298497	GBAN-AF298403	GBAN-AF298309
Nemcia reticulata Domin	G. T. Chandler	540	Seabird, WA	GBAN-AF298498	GBAN-AF298404	GBAN-AF298310
Nemcia retusa Domin	G. T. Chandler	535	South Stirling, WA	GBAN-AF298499	GBAN-AF298405	GBAN-AF298311
Nemcia rubra Crisp	G. T. Chandler	489	Stirling Range, WA	GBAN-AF298500	GBAN-AF298406	GBAN-AF298312
Nemcia spathulata (Benth.) Crisp	M. D. Crisp	8448	Bindoon, WA	GBAN-AF298501	GBAN-AF298407	GBAN-AF298313
Nemcia vestita Domin	M. D. Crisp	8489	Stirling Range, WA	GBAN-AF298502	GBAN-AF298408	GBAN-AF298314
Oxylobium arborescens R. Br.	G. T. Chandler	616	Gibraltar Range, NSW	GBAN-AF298504	GBAN-AF298410	GBAN-AF298316
Oxylobium ellipticum R. Br.	G. T. Chandler	603	Aust Nat Botanic Gardens	GBAN-AF298505	GBAN-AF298411	GBAN-AF298317
Oxylobium lineare (Benth.) Benth.	M. D. Crisp	8471	Tonebridge, WA	GBAN-AF298506	GBAN-AF298412	GBAN-AF298318
Oxylobium pulteneae DC.	M. D. Crisp	9046	Howes Valley, NSW	GBAN-AF298507	GBAN-AF298413	GBAN-AF298318
Oxylobium robustum Joy Thomps.	I. R. Telford	4294	Lake Cootharaba, QLD	GBAN-AF298508	GBAN-AF298414	GBAN-AF298320
Phyllota phylicoides Benth.	M. D. Crisp	9048	Morgans Gully, NSW	GBAN-AF298509	GBAN-AF298415	GBAN-AF298321
Podolobium aciculiferum F. Muell.	G. T. Chandler	606	Wyong State Forest, NSW	GBAN-AF298510	GBAN-AF298416	GBAN-AF298322
Podolobium aestivum Crisp & P. H. Weston	G. T. Chandler	612	Gibraltar Range, NSW	GBAN-AF298511	GBAN-AF298417	GBAN-AF298323
Podolobium alpestre (F. Muell.) Crisp & P. H. Weston	G. T. Chandler	1039	Brindabella Range, ACT	GBAN-AF298512	GBAN-AF298418	GBAN-AF298324
Podolobium ilicifolium (Andrews) Crisp & P. H. Weston	G. T. Chandler	308	Nelligen, NSW	GBAN-AF298513	GBAN-AF298419	GBAN-AF298325
Podolobium procumbens (F. Muell.) Crisp & P. H. Weston	B. Hadlow	461	Verneys Range, NSW	GBAN-AF298514	GBAN-AF298420	GBAN-AF298326
Podolobium scandens DC.	G. T. Chandler	309	Nelligen, NSW	GBAN-AF298515	GBAN-AF298421	GBAN-AF298327
Pultenaea dentata Labill.	M. D. Crisp	9053	Boonoo Boonoo Falls, NSW	GBAN-AF298516	GBAN-AF298422	GBAN-AF298328
Pultenaea reticulata Benth.	G. T. Chandler	953	Mt Barker area, WA	GBAN-AF298517	GBAN-AF298423	GBAN-AF298329

[a] The prefix GBAN- has been added to link the online version of the *American Journal of Botany* to GenBank but is not part of the actual accession number.

TABLE 2. List of primer sequences and references used in this study.

Primer name	Primer sequence	Reference (if applicable)
psbA f	GTTATGCATGAACGTAATGCTC	Sang, Crawford, and Stuessy (1997)
trnH r	CGCGCATGGTGGATTCACAAATC	Sang, Crawford, and Stuessy (1997)
3914 f	GGGGTTGCTAACTCAACGG	Johnson and Soltis (1994)
1110 r	TATTCTGTTGATACATTCG	Previously unpublished
Gast11	GTGCTTGGTGTGGTAAAGGC	Previously unpublished
Gast12	CAACGGATTCTCTCACCTCGC	Previously unpublished
18SIGS	CACATGCATGGCTTAATCTTTG	Baldwin and Markos (1998)
26S	CTGCCACGATCCACTGAGSTCC	Baldwin and Markos (1998)
Gast1	CGGTTGCGGCTCTGGTGTTC	Previously unpublished

numbers for the sequences obtained. Vouchers of all specimens used in the analysis are deposited at the Australian National Herbarium (CANB), and the collector name and number for each accession are provided in Table 1, along with a brief locality description.

Outgroup selection—Outgroups were selected using the analysis of the genistoid legume tribes by Crisp, Gilmore, and Van Wyk (in press), following the work of Crisp and Weston (1995), and sampled throughout the 5-nucleate embryo sac clade. *Isotropis cuneifolia* was used to root the tree, as this genus occurs at the base of the 5-nucleate embryo sac group. Other outgroups used were *Jacksonia horrida, Latrobea hirtella, Mirbelia depressa, M. dilatata, Phyllota phylicoides, Pultenaea dentata,* and *P. reticulata.* Outgroup genera that appeared closely related to *Gastrolobium* were sampled more extensively, including *Callistachys* (1/1 species), *Oxylobium* (5/6 species), and *Podolobium* (6/6 species).

Ingroup sampling—A pilot study suggested that *Brachysema, Jansonia,* and *Nemcia* were nesting within *Gastrolobium,* so these genera were sampled more extensively than originally planned. The study included 9/10 species of *Brachysema,* 1/1 species of *Jansonia,* and 16/39 species of *Nemcia,* and one undescribed species (all undescribed taxa are marked in Table 1 with 'ms'). Species of *Nemcia* were added to the sample as it became clear that this genus is polyphyletic, and were chosen to represent the diversity of this group. Within *Gastrolobium* s.s. (sensu stricto), 48/60 species were sampled, including 13 undescribed species. Of the 12 species of *Gastrolobium* not sampled, six were unavailable recent discoveries. For the other six, fresh or CTAB-preserved material was unavailable and herbarium material of these failed to amplify. It was felt that the final sample size was sufficient to test the monophyly (or nonmonophyly) of each group and to resolve relationships within them.

DNA isolation, amplification, and sequencing—Total DNA was isolated as outlined in Bayer, Hufford, and Soltis (1996). Methods outlined in Gilmore, Weston, and Thompson (1993) were used to isolate DNA from herbarium tissue and to purify recalcitrant DNAs. When these methods failed, DNAs were run through a QIAquick™ PCR Purification Kit (QIAGEN, Hilden, Germany).

All three regions were amplified by the polymerase chain reaction (PCR) using *Taq* DNA polymerase and the following conditions. The PCR samples were heated to 94°C for 3 min prior to the addition of DNA polymerase to denature unwanted proteases and nucleases. The double-stranded PCR products were produced via 30 cycles of denaturation (94°C for 1 min), primer annealing (48°C for 1 min), and extension (72°C for 1 min). A 7-min final extension cycle at 72°C followed the 30th cycle to ensure the completion of all novel strands. See Table 2 for all primer sequences and references. Double-stranded PCR products were cleaned using QIAquick™ PCR Purification Kits (QIAGEN, Hilden, Germany) prior to sequencing.

psbA-trnH intergenic spacer sequences—The PCR reaction mixture consisted of 70 μL of sterile water, 10 μL of 10× reaction buffer, 6 μL of 25 mmol/L magnesium chloride solution, 4 μL of 40 mmol dNTP solution in equimolar ratio, 20 pmol of each primer (*psbA*f and *trnH*r), 10–50 ng of template DNA, and 0.5 μL of *Taq* polymerase in a total volume of 100 μL.

trnK 5′ intron sequences—The PCR reaction mixture consisted of 35 μL of sterile water, 5 μL of 10× reaction buffer, 3 μL of 25 mmol/L magnesium chloride solution, 2 μL of 40 mmol dNTP solution in equimolar ratio, 10 pmol of each primer (3914f and 1110R), 5–25 ng of template DNA, and 0.25 μL of *Taq* polymerase in a total volume of 50 μL. (The 1110r primer was designed by R. Bayer for use in the Asteraceae, which worked well in the legumes in this study. The 1110r primer lies 1110bp from the 3′ end of the *trnK* gene in the tobacco chloroplast genome and within the *matK* coding region.) Some taxa required the use of four primers (3914f and Gast12; Gast11 and 1110r) to amplify this region, particularly when herbarium material was used. Gast11 and Gast12 were designed in a conserved part of the *trnK* intron and provide overlapping sequences (Table 2).

External transcribed spacer sequences—Specific primers were developed by initially using a long-range PCR amplification of the entire intergenic spacer region between the 18S and 26S subunits of rDNA, using the universal primers of Baldwin and Markos (1998). The 18S-IGS primer was then used to sequence the 3′ end of the ETS region. The 5′ end of this region yielded a conservative site suitable for the design of another primer, Gast1 (Table 2), which allowed the amplification of ~350 bp of sequence. The PCR reaction mixture consisted of 70 μL of sterile water, 10 μL of 10× reaction buffer, 6 μL of 25 mmol/L magnesium chloride solution, 4 μL of 40 mmol dNTP solution in equimolar ratio, 20 pmol of each primer (18SIGS and Gast1), 10–50 ng of template DNA, and 0.5 μL of *Taq* polymerase in a total volume of 100 μL.

Sequencing of PCR products—The double-stranded PCR products were used as templates in cycle sequencing reactions, which employed the same primers that were used for PCR amplification to sequence both strands. The double-stranded PCR products were sequenced using the dideoxy chain termination method (Sanger, Nicklen, and Coulson, 1977) with the use of the Big Dye Terminator RR Kit® (Perkin Elmer Applied Biosystems, Norwalk, Connecticut, USA) at CSIRO, Plant Industry. An annealing temperature of 60°C was used for both primers. The cycle sequencing protocol followed manufacturer's instructions.

Sequences were assembled using Sequencher™ 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA), then manually adjusted following the principles of noncoding sequence alignment using secondary structure (Kelchner and Clark, 1997). Indels were placed where they minimized the number of inferred length mutations, unless clear evidence was seen for nonhomologous length mutation events. Unambiguous indels have been coded as additional characters (Golenburg et al., 1993).

Sequence data analysis—Sequence data were analyzed using parsimony as implemented in PAUP 4.0b3a (Swofford, 1997) on a Macintosh G3 computer. The data matrix contained 75 ingroup taxa, taken from the 'Callistachys' group, and 19 outgroup taxa. Phylogenetic reconstruction was performed on unweighted characters by heuristic searches with simple addition of taxa. An

TABLE 3. Sequence characteristics of the *psbA-trnH* spacer, the *trnK* 5′ intron, and the external transcribed spacer (ETS) sequenced in this study. NA = not applicable.

Sequence characteristic	*psbA-trnH* spacer	*trnK* 5′ intron	ETS	Combined (all 3 sequences)
Length range (bp)	180–414	816–1016	315–345	1592–1931
Length mean (bp)	356	897	339	1781
Aligned length (bp)	603	1352	355	2310
G + C content mean	29.5%	33%	58%	37%
Sequence divergence (%)	0.00–21.36%	0.00–20.20%	0.00–28.44%	0.44–21.45%
No. variable sites	328/603 (54%)	466/1352 (34%)	233/355 (65%)	1027/2310 (45%)
No. potentially informative sites	192/603 (32%)	237/1352 (18%)	142/355 (40%)	571/2310 (25%)
No. constant sites	275/603 (46%)	887/1352 (66%)	123/355 (35%)	1285/2310 (55%)
No. autapomorphic sites	136/603 (22%)	229/1352 (16%)	91/355 (25%)	456/2310 (20%)
No. unambiguously coded indels	5	11	0	16
Coded indel size range (bp)	2–241	5–20	NA	2–241
Ratio of coded indels to potentially informative sites	1 : 38	1 : 22	NA	1 : 36

island search was employed to search for further most parsimonious trees, with a random addition sequence of 100 replicates using a heuristic search (Maddison, 1991). The three sets of sequences were analyzed individually and together. A partition homogeneity test was conducted to test the compatibility of the three data sets.

The robustness of clades was tested using two methods: bootstrapping (Felsenstein, 1985) and decay analysis (Bremer, 1988). One thousand replicates were used for the bootstrap. The decay analysis was facilitated by the program AutoDecay (Eriksson, 1998). The decay values were then extracted using AutoDecay and visualized using the tree-drawing package, TreeView (Page, 1996).

RESULTS

Sequence characteristics—We summarize here statistics for the sequences used, including length variation, proportion of nucleotide differences, G/C content, sequence divergence, informative characters, and indel information (Table 3). Combined sequence lengths vary from 1392 bp (base pairs) in *Isotropis cuneifolia* to 1731 bp in *Nemcia alternifolia* ms; the *psbA-trnH* spacer ranges from 180 bp in *Isotropis cuneifolia* to 414 bp in *Gastrolobium tenue* ms and *G. oxylobioides*; the *trnK* 5′ intron ranges from 816 bp in *G. villosum* to 1016 bp in *Nemcia alternifolia* ms; and the ETS ranges from 315 bp in *Pultenaea reticulata* to 345 bp in *Mirbelia depressa.*

The G/C content in the combined analysis ranges from 35.84% in *Mirbelia dilatata* to 38.74% in *Podolobium procumbens*; that in the *psbA-trnH* spacer ranges from 25.68% in *Gastrolobium laytonii* to 32.12% in *G. appressum*; that in the *trnK* 5′ intron varies from 31.63% in *Nemcia rubra* to 35.47% in *Isotropis cuneifolia;* and that in the ETS ranges from 51.45% in *Mirbelia depressa* to 64.13% in *Pultenaea reticulata.*

In the combined matrix, sequence divergence varies from 0.435% between *Nemcia hookeri* and *N. obovata* to 21.451% between *Pultenaea reticulata* and *Isotropis cuneifolia*; in the *psbA-trnH* spacer it varies from 0% between *Podolobium alpestre* and *P. procumbens* to 21.364% between *Gastrolobium parviflorum* and *Isotropis cuneifolia*; in the *trnK* 5′ intron it ranges from 0% between *Gastrolobium heterophyllum* and *G. nutans* to 20.2% between *Isotropis cuneifolia* and *Nemcia* sp. nov. A; and in the ETS it ranges from 0% between several sets of taxa (*N. leakeana*/*N. luteifolia*/*N. rubra*; *N. coriacea*/*N. hookeri*/*N. obovata*/*N. plicata*; *Gastrolobium stowardii*/*G. carinatum* ms; *G. revolutum*/*G. tetragonophyllum*/*G. parviflorum*; *G. floribundum*/*G. propinquum*; and *G. appressum*/*G. oxylobioides*) to 28.444% between *Callistachys lanceolata* and *Isotropis cuneifolia.*

The number of unambiguous indels in each sequence varies considerably, with numerous indels present in the *psbA-trnH* spacer (ranging in size from 2 to 241 bp) to very few in the ETS (all of which were autapomorphic and, therefore, phylogenetically uninformative). Only the numbers of unambiguously coded indels are given in Table 3, which range in size from 2 to 241 bp in the *psbA-trnH* spacer, 5 to 20 bp in the *trnK* 5′ intron, and none in the ETS.

Phylogenetic reconstruction—A heuristic search of all potentially phylogenetically informative nucleotide characters from the total combined data matrix, including indels, revealed 360 trees of 2327 steps, with confidence index (CI) = 0.404, retention index (RI) = 0.631. A 50% majority-rule tree also shows the decay and bootstrap values calculated for each clade (Fig. 2). Only five branches in the majority-rule tree collapse in the strict consensus. These are shown with dashed lines (Fig. 2). A phylogram shows the number of synapomorphies supporting each branch (Fig. 3) in one of the equally most parsimonious trees. The partition homogeneity test indicated the data sets are not significantly different ($P = 0.08$) and can therefore be combined into one analysis.

Topology of major clades—This analysis shows *Gastrolobium* (clade C, Fig. 2) to be paraphyletic, with *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare* nesting within it. *Nemcia* is shown to be polyphyletic (clades I, J, K, and L, Fig. 2), as is *Podolobium* (Clades A and B). The *Podolobium ilicifolium* group (clade B) is sister to *Gastrolobium.* The major clades, as indicated in Fig. 2, are described below.

Clade A (decay [D] = 3, synapomorphies [SYN] = 16)—The 'Oxylobium' group contains *Oxylobium* (excluding *O. lineare*), *Mirbelia, Callistachys,* and three species of *Podolobium* (*P. alpestre, P. procumbens,* and *P. scandens*). *Oxylobium* and *Podolobium* both occur in eastern Australia (Fig. 1), *Mirbelia* occurs in both eastern and western Australia, and *Callistachys* is endemic to the southwest of Western Australia.

Clade B (D = 27, SYN = 45)—The *Podolobium ilicifolium* group contains three species of *Podolobium, P. aciculiferum,*

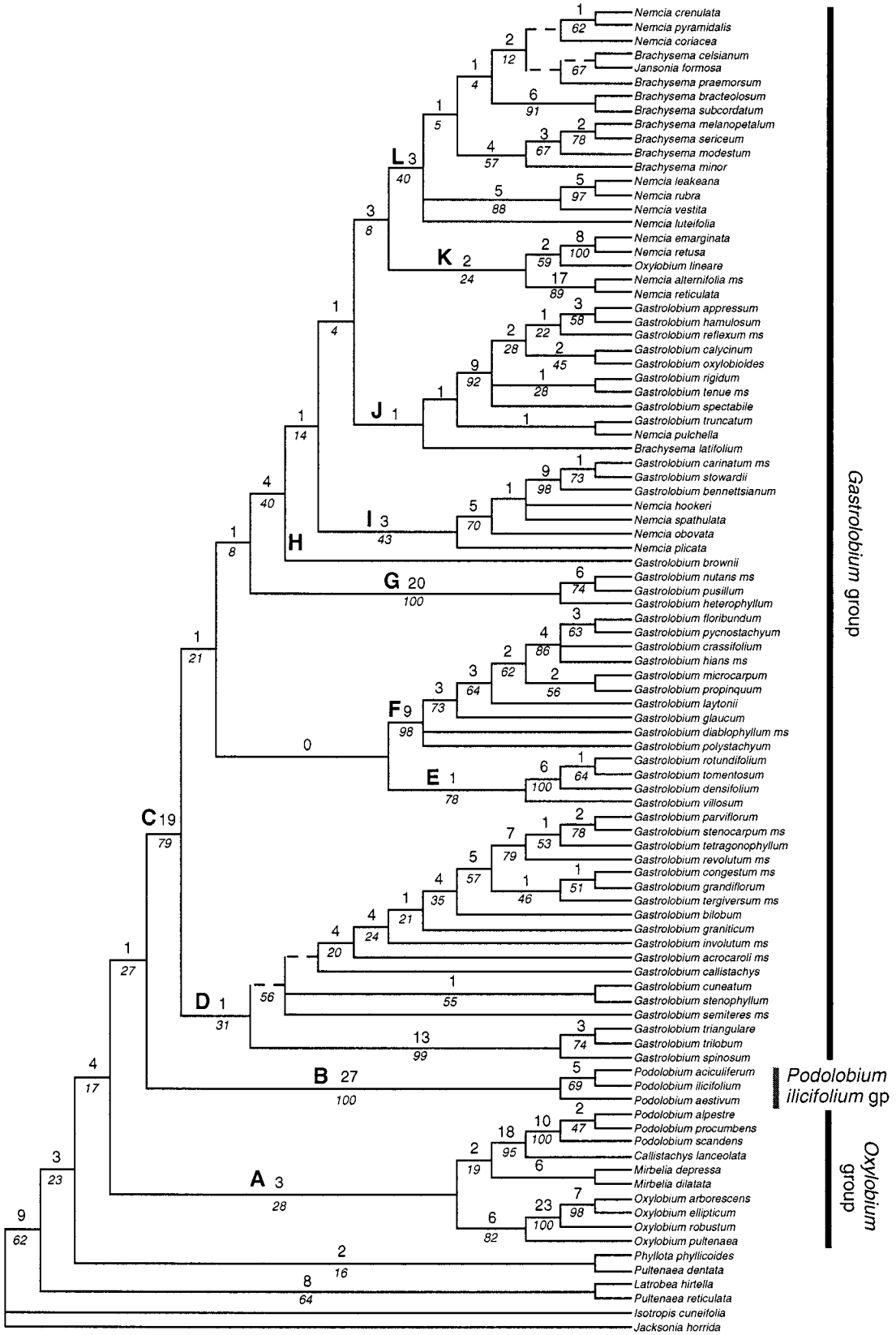

Fig. 2. Majority-rule consensus tree of 360 trees. The major clades, marked A to L, are discussed in the text. Decay values are given above the line, and bootstrap values are given below the line. Manuscript names have been designated by the placement of ms after a name.

Fig. 3. Phylogram of one tree of 360. Synapomorphies for each branch are given, and the major clades are marked A to L. Branch lengths are proportional to the amount of change, with a scale provided.

P. aestivum, and *P. ilicifolium.* These species all have prickly leaves and recurved calyces, and occur on the east coast and associated escarpment of Australia (Fig. 1).

Clade C (D = 19, SYN = 30)—The 'Gastrolobium' group contains all species of *Gastrolobium*, as well as the genera *Brachysema*, *Jansonia*, and *Nemcia*, plus *Oxylobium lineare*, a doubtful species of *Oxylobium* that Crisp and Weston (1995) made clear belongs in another genus and the only one occurring in Western Australia.

Clade D (D = 1, SYN = 11)—This clade contains a number of species, including some that form smaller clades, including the *Gastrolobium spinosum* group (*D* = 13, SYN = 23; *G. spinosum, G. triangulare,* and *G. trilobum*); the *G. bilobum*/*G. parviflorum* group (*D* = 4, SYN = 14; *G. parviflorum,* G. 'revolutum' ms, G. 'stenocarpum' ms, *G. tetragonophyllum, G. bilobum, G. congestum* ms, *G. grandiflorum,* and *G. tergiversum* ms). There are also a number of species that form a grade at the base of Clade D and occur only on or on the margins around granite outcrops, including *G. acrocaroli* ms, *G. callistachys, G. graniticum, G. involutum* ms, *G. semiteres* ms, and *G. stenophyllum.*

Clade E (D = 1, SYN = 10)—The ''tomentose-leaved'' group comprises *G. densifolium, G. rotundifolium, G. tomentosum,* and *G. villosum.*

Clade F (D = 9, SYN = 12)—The ''sandplain'' group. This clade contains a number of species of *Gastrolobium* that occur throughout the sandplains of middle and northern southwest Western Australia, and includes *G. crassifolium, G. floribundum, G. diablophyllum, G. glaucum, G. hians* ms, *G. laytonii, G. microcarpum, G. polystachyum, G. propinquum,* and *G. pycnostachyum.*

Clade G (D = 20, SYN = 34)—Three morphologically disparate species make up this clade, *G. heterophyllum, G. nutans,* and *G. pusillum.*

Clade H (autapomorphies = 20)—This clade contains a single species only, *G. brownii,* situated directly between clade G and clade I.

Clade I (D = 3, SYN = 12)—This group contains a number of species of *Nemcia* that are intermediate in morphology between *Gastrolobium* and *Nemcia* (*N. hookeri, N. obovata, N. plicata,* and *N. spathulata*), plus *Gastrolobium bennettsianum, G. stowardii,* and *G. carinatum* ms (aff. *bennettsianum*). These species have shortly racemose inflorescences, generally in the axils of the leaves.

Clade J (D = 1, SYN = 7)—Brachysema latifolium (the type species of *Brachysema*), *Nemcia pulchella,* and *Gastrolobium truncatum* are contained in this clade. There is also a group of *Gastrolobium* s.s. species (*D* = 9, SYN = 13), including *G. appressum, G. calycinum, G. hamulosum, G. oxylobioides, G. reflexum* ms, *G. rigidum, G. spectabile,* and *G. tenue* ms, that share glaucous leaves with strongly reticulate venation and an intramarginal vein.

Clade K (D = 2, SYN = 8)—This clade includes a number of *Nemcia* species, *N. alternifolia* ms, *N. emarginata, N. reticulata,* and *N. retusa,* as well as *Oxylobium lineare.* These species all have strongly tomentose calyces, which may be two-tone in color, and generally have inflorescences reduced to a few flowers in the leaf axils.

Clade L (D = 3, SYN = 8)—This group contains all bird-pollinated species within the greater *Gastrolobium* group except two (*Brachysema latifolium* and *Gastrolobium grandiflorum*), as well as three bee-pollinated species. This includes all but one species of *Brachysema* (*B. bracteolosum, B. celsianum, B. melanopetalum, B. minor, B. modestum, B. praemorsum, B. sericeum,* and *B. subcordatum*), *Jansonia formosa,* and the red-flowered *Nemcia* group, *N. leakeana* (the type species of *Nemcia*), *N. luteifolia, N. rubra,* and *N. vestita.* The bee-pollinated species of *Nemcia* included within this clade are *N. coriacea, N. crenulata,* and *N. pyramidalis.*

DISCUSSION

Resolution of the dilemma in circumscribing Gastrolobium—Whereas morphological analyses of *Gastrolobium* and its close relatives have provided unsatisfactory resolution within this group (e.g., see Crisp and Weston, 1987, 1995; Crisp, 1994), molecular data have clarified relationships. It is clear from this analysis that *Gastrolobium* s.s. is paraphyletic, with strong support for the inclusion of *Brachysema, Jansonia, Nemcia,* and *O. lineare* within *Gastrolobium. Nemcia* itself is polyphyletic within this clade.

Past classifications have circumscribed various genera primarily using floral characters that appear related to pollination syndrome (e.g., bird pollination vs. bee pollination), inflorescence structure, and fluoroacetate content (see Crisp and Weston, 1987). Thoughts on the homology of such characters need to be reconsidered, especially in light of this analysis, which shows these to be homoplastic. More care needs to be taken when choosing morphological characters for phylogenetic reconstruction. Many characters in the past were more relevant to phenetic analyses rather than cladistic analyses, yet were used in cladistic analysis (e.g., Crisp and Weston, 1987, 1995). This is not to say that morphology does not provide important phylogenetic information in the *Gastrolobium* group, simply that it does not provide enough resolution exclusive of other data. Indeed, this analysis gives some support for a large, mostly bird-pollinated lineage within *Gastrolobium* (clade L), showing that the floral characters that appear related to bird pollination are indeed phylogenetically informative. However, morphological characters were unable to satisfactorily work out the broader relationships of this lineage, which the molecular data more satisfactorily resolve.

The red-flowered species (Clade L, including *Brachysema, Jansonia,* and some species of *Nemcia*) form a well-supported clade (*D* = 3, SYN = 8), although three other species of *Nemcia* which have short, dense, many-flowered racemes with large, orange flowers are also within this clade (*N. coriacea, N. crenulata,* and *N. pyramidalis*). This conflicts with previous morphological work (Crisp, 1994), which showed that bird pollination in the genera *Brachysema, Jansonia,* and *Nemcia* was due to convergence, and had arisen twice. This study shows that there is one main lineage within the greater *Gastrolobium* that appears to be bird pollinated, with only two other species (*Brachysema latifolium* and *Gastrolobium grandiflorum*) occurring singly outside this group, with bird pollination originating three times within *Gastrolobium.*

Morphology can be selected to change rapidly in response to change in pollination syndrome when a plant moves towards bird pollination (see review in Crisp, 1994), so convergence among a number of different lineages is quite possible. In fact, only one or two genes may be responsible for flower color and shape (Gottleib, 1984; Coen, 1991; Coen and Meyerowitz, 1991), such that minimal genetic change may dramatically alter floral morphology. In the *Brachysema* and red-flowered *Nemcia* clade (clade L), a variety of floral shapes and colors are found. For example, the red-flowered *Nemcia* species do not have a reduced standard petal or enlarged keel

petals, whereas the *Brachysema* species do. The colors range from white (e.g., *B. modestum*) through green (*B. bracteolosum*), red (e.g., *B. subcordatum, Jansonia formosa,* and *Nemcia rubra*) to black or very dark purple (*B. melanopetalum*). It is possible that these species shared a common ancestor that evolved towards bird pollination and then underwent an adaptive radiation, expanding into many shapes and colors. This may have been facilitated by the release of developmental constraints on the ancestral, yellow and red, bee-pollinated flowers in the rest of the *Gastrolobium* clade. This may also be true for other genera within the Mirbelieae and Bossieeae, as red flowers with elongated keels are found in species of *Bossiaea* Vent., *Chorizema* Labill., *Daviesia* Sm., *Gompholobium* Sm., *Leptosema, Mirbelia* Sm., and *Sphaerolobium* Sm.

Fluoroacetate is found in a number of clades within the *Gastrolobium* clade (clades D–J), but not in clades K or L (see, Aplin, 1971; Twigg et al., 1996). It is possible that production of fluoroacetate is the plesiomorphic condition in this group (acquired in the ancestor of clade C), which was then lost from some lineages, most notably in the common ancestor of the red-flowered group (clade L) and a group of yellow-flowered *Nemcia* species (clade K). Toxin strength does not otherwise appear to decline in derived clades in the tree (except in the species of *Nemcia* intermediate with *Gastrolobium* in clade I, where trace levels have been recorded). Usually, fluoroacetate is either present or absent in these groups, implying that a mutation in the fluoroacetate metabolic pathway to interrupt production could have occurred, which could have led to a drastic reduction in fluoroacetate production, as found in *N. spathulata* by Twigg et al. (1996), or even a complete absence of fluoroacetate in some of the more derived clades.

Ovule number has been shown to be homoplastic throughout the tribe Mirbelieae (Crisp and Weston, 1987, 1995). This study has shown this character to be equally homoplastic throughout *Gastrolobium* and related groups, and no support can be found for its use in past classifications to distinguish among various genera in this group, such as *Gastrolobium, Nemcia,* and *Oxylobium.*

Characteristics of the major clades—Most major clades (Figs. 2, 3) show consistency in morphology and ecology among their included species as described in detail below. Groups contained within clade D, which consists entirely of species from *Gastrolobium* s.s. and is sister to the rest of *Gastrolobium* s.l. (sensu lato; clade C), include the *G. parviflorum* complex and members of the *G. bilobum* group (the type of the genus), which all share condensed, many-flowered racemes and have cuneate, emarginate leaves, plus the strongly supported *G. spinosum* clade ($D = 13$, SYN = 23), which all have spinose leaves and short, few-flowered racemes. Clade D also contains a number of species occurring solely on granite outcrops and their immediate margins, though these do not form a clade (*G. acrocaroli, G. callistachys, G. graniticum, G. involutum, G. semiteres,* and *G. stenophyllum*). In fact, the only species occurring in the same habitat that is not within clade D is *G. spectabile,* which occurs within clade J. These granite-inhabiting species all share a similar inflorescence and floral structure (long, open racemes with long internodes, relatively large flowers, and strongly recurved calyx lobes) in addition to habitat.

Clade E contains the tomentose-leaved *Gastrolobium* species. These four species all share details of the inflorescence structure (strongly hairy, with short floral internodes and with large, lanceolate bracts that persist longer than in most species of *Gastrolobium,* that are caducous), and all except *G. densifolium* have leaves that are tomentose on the abaxial surface. These species are the sister group to the ''sandplain'' group (clade F), though this is weakly supported ($D = 1$, SYN = 10). These sandplain species are open, spreading shrubs that have tough, often glaucous leaves and long, open racemes, and generally have widespread distributions, occurring throughout the sandplains of southwestern Western Australia.

There is strong support for clade G ($D = 20$, SYN = 31), though the composition of this group is somewhat puzzling. Two of the three species, *G. heterophyllum* and *G. nutans* ms, share similar leaves and inflorescences, but the placement of *G. pusillum* there is interesting, although this species does not strongly resemble any other species of *Gastrolobium* s.l. It could be that this small group of species are well differentiated, with a number of morphological autapomorphies making them appear quite different.

The position of *Gastrolobium brownii* is interesting, as it was one of three species out of 22 transferred to *Nemcia* by Crisp and Weston (1987), along with *G. pusillum* and *G. truncatum,* only to be transferred back to *Gastrolobium* by Crisp and Weston (1995) because of uncertainties in relationships based on morphology. Like most of *Gastrolobium* sens. str., *G. brownii* is well known to accumulate fluoroacetate. *Gastrolobium brownii* and *G. truncatum* both have inflorescences similar to many of those in the *Nemcia* group (short, few-flowered axillary racemes), and both sit with or near these species in the phylogeny presented here. *Gastrolobium brownii* is closely related to a group of species intermediate in morphology between *Gastrolobium* s.s. and *Nemcia* (clade I), which includes *N. hookeri, N. obovata, N. spathulata,* and *N. plicata.* These species of *Nemcia* have more in common with *Gastrolobium* s.s., such as short racemes, recurved calyx lobes, stipitate ovaries, and ovoid fruits, than with the circumscription of *Nemcia* provided by Crisp and Weston (1987). *Gastrolobium truncatum* is sister to *Nemcia pulchella* (with which it shares details of inflorescence structure such as short racemes and petal coloration) and also appears related to *Brachysema latifolium* (which has racemes of red, bird-pollinated flowers). Thus, the molecular data agree with the morphology in placing this group of species intermediate between *Gastrolobium* s.s. and *Nemcia.*

Within the rest of clade J, a group of *Gastrolobium* s.s. species form a strongly supported group ($D = 9$ and SYN = 13). These species have similar morphology, including glaucous leaves with strongly reticulate venation and an intramarginal vein, inflorescences with long floral internodes, very pubescent calyces, and deep orange standard petals.

Clade K contains a group of *Nemcia* species, plus *Oxylobium lineare. Nemcia alternifolia* and *N. reticulata* are sister species sharing standard petals that are almost entirely maroon on the back, an identical inflorescence type (solitary or paired flowers in the axils) and strongly tomentose calyces. Similarly tomentose calyces are shared with *N. emarginata* and *N. retusa,* however the latter two species have cuneate and emarginate leaves, two-toned hairs on the calyces (silver at the base, and golden brown at the top), and inflorescences clustered at the branchlet terminus with numerous flowers. *Oxylobium lineare* has similar leaves to those of *Nemcia reticulata,* but has a long raceme with many flowers that have uniformly colored hairs on the calyces, and may be a reversion to the typical, long *Gastrolobium*-type raceme as seen in the

more basal *Gastrolobium* groups. Many species of *Nemcia,* and a few in *Gastrolobium* (such as *G. heterophyllum*), have short axillary shoots with a short, terminal raceme, so many developmental changes may not be required to further reduce this to a solitary flower.

The red-flowered group (clade L) includes *Brachysema, Jansonia,* and species of *Nemcia* with red flowers, and some *Nemcia* species with orange flowers in condensed, dense racemes. There is some support for a sister relationship between *Brachysema celsianum* and *Jansonia formosa,* though this is not found in all trees, and they share riverine habitats and straggly habits. The other *Brachysema* species have similar floral architecture. Three out of four red-flowered *Nemcia* species group together strongly and consistently ($D = 5$, SYN = 7), and all four group together in some of the most parsimonious trees. The three presumably bee-pollinated *Nemcia* species share a condensed terminal raceme with many, large flowers that are strongly orange in color and have large, crenulate leaves.

Phylogenetic utility of the various loci—Together, the three sequence regions used in this analysis provide a robust phylogeny. In a data set this large (94 taxa), numerous characters are required to obtain much resolution, and any DNA region alone is unlikely to yield a sufficient number of informative characters. For example, *trnK* 5′ intron, which has the greatest number of informative characters (Table 2), only has 2.5 informative characters, on average, per taxon. In contrast, the combined analysis has 587 informative characters (including coded indels), or 6.3 characters, on average, per taxon.

Some regions appear more phylogenetically useful than others, however. The trees produced from only the *trnK* 5′ intron and ETS data sets (not presented) more closely resemble the tree from the combined analysis than the *psbA-trnH* spacer data set. This may be due to the large number of indels (particularly deletions that can be large) present in the *psbA-trnH* spacer, most of which are not phylogenetically informative. These large deletions leave a many potential characters unavailable for many taxa. The analyses performed with and without coded indels produced trees with identical topologies, but support for individual clades increased slightly.

Different combinations of data sets also appear more phylogenetically informative than others, with the combined chloroplast data set most closely resembling the tree from the combined data. It also has the largest number of informative characters. The combined *trnK*/ETS data set is next most similar, with the topology of the *psbA-trnH*/ETS data set the most different from the combined analysis. There is no strong disagreement between nuclear and chloroplast sequences, however, with all topologies being quite similar, and the result of the partition homogeneity test showed no significant difference between the data sets, justifying the combination of the data. We believe that the best phylogeny is one with all data present, because the more characters added, the more robust the phylogeny becomes, though caution is recommended, and we believe that conflict between data sets must be taken into consideration.

Implications for taxonomy—This study has important implications for the taxonomy of the *Gastrolobium* group. The analysis provides strong support for the inclusion of *Brachysema, Jansonia,* and *Nemcia* within *Gastrolobium* s.s., so the continued recognition of these genera as currently circumscribed is untenable. There are two options for the taxonomy of this group. One would be to lump all species into *Gastrolobium,* and the other would be to split the *Gastrolobium* clade into different genera. The internal support of many branches within *Gastrolobium* (Figs. 2, 3) is quite low (decay = 1 for many of them), with many of these branches forming a ladder. In light of this low support and the overall shape of the tree, even though some individual groups have very strong support, the further splitting of *Gastrolobium* would not be the optimal solution. In addition, some of the genera would be difficult to distinguish morphologically, such as *Brachysema* and *Jansonia,* and *Gastrolobium* and *Nemcia,* due to the similar combinations of characters present in these genera. The lumping of *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare* appears to have the strongest support from this analysis.

Conclusions—Molecular data have an important role to play in estimating phylogenies in the Mirbelieae/Bossiaeeae. This study has shown that a taxonomically difficult group can be resolved using molecular data where morphology has achieved only partial success and is an important step forward in the systematics of Australian legumes. The circumscription of *Gastrolobium* may need to be expanded to include *Brachysema, Jansonia, Nemcia,* and *Oxylobium lineare,* pending further analysis using additional DNA markers. A future paper will present a systematic revision of the greater *Gastrolobium,* with descriptions of all species. Further investigation is required to test the possible sister relationship of the *Podolobium ilicifolium* group to *Gastrolobium* and of *Gastrolobium* to other genera within the tribe.

LITERATURE CITED

ALDRICH, J., B. W. CHERNEY, E. MERLIN, AND L. CHRISTOPHERSON. 1988. The role of insertions/deletions in the evolution of the intergenic region between *psbA* and *trnH* in the chloroplast genome. *Current Genetics* 14: 137–146.

APLIN, T. E. H. 1971. Poison plants of Western Australia: the toxic species of *Gastrolobium* and *Oxylobium. Western Australian Department of Agriculture Bulletin* 3772: 1–66.

ASMUSSEN, C. B., AND A. LISTON. 1998. Chloroplast DNA characters, phylogeny, and classification of *Lathyrus* (Fabaceae). *American Journal of Botany* 85: 387–401.

BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S nrDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.

BAYER, R. J., L. HUFFORD, AND D. E. SOLTIS. 1996. Phylogenetic relationships in Sarraceniaceae based on *rbc*L and ITS sequences. *Systematic Botany* 21: 121–134.

BENA, G., M.-F. JUBIER, I. OLIVIERA, AND B. LEJEUNE. 1998. Ribosomal external and internal transcribed spacers: combined use in the phylogenetic analysis of *Medicago* (Leguminosae). *Journal of Molecular Evolution* 46: 299–306.

BENTHAM, G. 1864. Flora Australiensis, vol. 2. Reeve and Co., London, UK.

BORISJUK, N., L. BORISJUK, G. PETJUCH, AND V. HEMLEBEN. 1994. Comparison of nuclear ribosomal RNA genes among *Solanum* species and other Solanaceae. *Genome* 37: 271–279.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BROWN, R. 1811. *Gastrolobium*. *In* W. T. Aiton [ed.], Hortus Kewensis, 2nd ed., part iii, 16. Longman, London, UK.

COEN, E. S. 1991. The role of homeotic genes in flower development and evolution. *Annual Review of Ecology and Systematics* 42: 241–279.

———, AND E. M. MEYEROWITZ. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature* 353: 31–37.

CRISP, M. D. 1994. Evolution of bird-pollination in some Australian legumes

(Fabaceae). *In* P. Eggleton and R. Vane-Wright [eds.], Phylogenetics and ecology, 281–309. Academic Press, London, UK.

———, S. R. GILMORE, AND B.-E. VAN WYK. 2000. Molecular phylogeny of the genistoid tribes of papilionoid legumes. *In* P. S. Herendeen and A. Bruneau [eds.], Advances in legume systematics, part 9, 249–276. Royal Botanic Gardens, Kew, UK.

———, AND P. H. WESTON. 1987. Cladistics and legume systematics, with an analysis of the Bossiaeeae, Brongniartieae and Mirbelieae. *In* C. H. Stirton [ed.], Advances in legume systematics, part 3, 65–130. Royal Botanic Gardens, Kew, UK.

———, AND ———. 1995. Mirbelieae. *In* M. D. Crisp, and J. J. Doyle [eds.], Advances in legume systematics, part 7, Phylogeny, 245–282. Royal Botanic Gardens, Kew, UK.

DOMIN, K. 1923. *Nemcia*, a new genus of the Leguminosae. *Preslia* 2: 26–31.

ERIKSSON, T. 1998. AutoDecay version 4.0 (program distributed by the author). Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm. Available on the web from http://www.bergianska.se/personal/TorstenE/

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

GADEK, P. A., D. L. ALPERS, M. H. HESLEWOOD, AND C. J. QUINN. 2000. Relationships within Cupressaceae sensu lato: a combined morphological and molecular approach. *American Journal of Botany* 87: 1044–1057.

GARDNER, C. A., AND H. W. BENNETTS. 1956. The toxic plants of Western Australia. West Australian Newspapers Ltd., Periodicals Division, Perth, Australia.

GILMORE, S., P. H. WESTON, AND J. A. THOMPSON. 1993. A simple, rapid, inexpensive, and widely applicable technique for purifying plant DNA. *Australian Systematic Botany* 6: 139–148.

GOLENBURG, E. M., M. T. CLEGG, M. L. DURBIN, J. DOEBLEY, AND D. P. MA. 1993. Evolution of a noncoding region of the chloroplast genome. *Molecular Phylogenetics and Evolution* 2: 52–64.

GOTTLIEB, L. D. 1984. Genetics and morphological evolution in plants. *American Naturalist* 123: 681–709.

HILU, K. W., AND L. A. ALICE. 1999. Evolutionary implications of *matK* indels in Poaceae. *American Journal of Botany* 86: 1735–1741.

———, AND H. LIANG. 1997. The *matK* gene: sequence variation and application in plant systematics. *American Journal of Botany* 84: 830–839.

HU, J.-M., M. LAVIN, M. F. WOJCIECHOWSKI, AND M. J. SANDERSON. 2000. Phylogenetic systematics of the tribe Millettieae (Leguminosae) based on chloroplast *trnK*/*matK* sequences and its implications for evolutionary patterns in Papilionoideae. *American Journal of Botany* 87: 418–430.

JARRELL, D. C., AND M. T. CLEGG. 1995. Systematic implications of the chloroplast-encoded *matK* gene on the tribe Vandeae (Orchidaceae). *American Journal of Botany* 82: 137.

JOHNSON, L. A., J. L. SCHULTZ, D. E. SOLTIS, AND P. S. SOLTIS. 1996. Monophyly and generic relationships of Polemoniaceae based on *matK* sequences. *American Journal of Botany* 83: 1207–1224.

———, AND D. E. SOLTIS. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany* 19: 143–156.

———, AND ———. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and Gilia (Polemoniaceae) using *matK* sequences. *Annals of the Missouri Botanic Gardens* 82: 149–175.

KEIGHERY, G. J. 1980. Bird pollination in south Western Australia: a checklist. *Plant Systematics and Evolution* 135: 171–176.

———. 1982. Bird-pollinated plants in Western Australia. *In* J. A. Armstrong, J. M. Powell, and A. J. Richards [eds.], Pollination and evolution, 77–89. Royal Botanic Gardens, Sydney, Australia.

———. 1984. Pollination of *Jansonia formosa* Kipp. ex Lindl. (Papilionaceae). *Western Australian Naturalist* 16: 21.

KELCHNER, S. A., AND L. G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast rpl16 intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* 8: 385–397.

KIM, S.-C., D. J. CRAWFORD, R. K. JANSEN, AND S.-G. ARNOLDO. 1999. The use of a non-coding region of chloroplast DNA in phylogenetic studies of the subtribe *Sonchinae* (*Asteraceae: Lactuceae*). *Plant Systematics and Evolution* 215: 85–99.

KING, K., R. A. TORRES, U. ZENTGRAF, AND V. HEMLEBEN. 1993. Molecular evolution of the intergenic spacer in the nuclear ribosomal RNA genes of Cucurbitaceae. *Journal of Molecular Evolution* 36: 144–152.

KUNTZE, O. 1891. Revisio generum plantarum vascularium omnium atque cellularium multarum secundum leges nomeclaturae internationales: cum enumeratione plantarum exoticarum initinere mundi collectarum: mit Erläuterungen. Part 1. A. Felix, Leipzig, Germany.

LES, D. H., E. L. SCHNEIDER, D. J. PADGETT, P. S. SOLTIS, D. E. SOLTIS, AND M. ZANIS. 1999. Phylogeny, classification and floral evolution of water lilies (Nymphaeaceae; Nymphaeales): a synthesis of non-molecular, *rbc*L, *matK*, and 18S rDNA data. *Systematic Botany* 24: 28–46.

LIANG, H., AND K. W. HILU. 1996. Application of the *matK* gene sequences to grass systematics. *Canadian Journal of Botany* 74: 125–134.

LINDER, C. R., L. R. GOERTZEN, B. V. HEUVEL, J. FRANCISCO-ORTEGA, AND R. K. JANSEN. 2000. The complete external transcribed spacer of 18S–26S rDNA: amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. *Molecular Phylogenetics and Evolution* 14: 285–303.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most parsimonious trees. *Systematic Zoology* 40: 315–328.

PAGE, R. D. M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357–358.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1996. Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. *Systematic Botany* 21: 477–495.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *matK* and *rbcL* sequence data. *American Journal of Botany* 84: 565–580.

RATHGEBER, J., AND I. CAPESIUS. 1990. Nucleotide sequence of the intergenic spacer and the 18S ribosomal RNA gene from mustard (*Sinapsis alba*). *Nucleic Acids Research* 18: 1288.

ROGERS, S. O., AND A. J. BENDICH. 1987a. Heritability and variability in ribosomal RNA genes of *Vicia faba*. *Genetics* 117: 285–295.

———, AND ———. 1987b. Ribosomal DNA genes in plants: variability in copy number and in the intergenic spacer. *Plant Molecular Biology* 9: 509–520.

ROGSTAD, S. H. 1992. Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analyses. *Taxon* 41: 701–708.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.

SANGER, F., S. NICKLEN, AND A. R. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* 74: 5463–5467.

SCHIEBEL, K., G. WALDBURG, J. GERSTNER, AND V. HEMLEBEN. 1989. Termination of transcription of ribosomal RNA genes of mung bean occurs within a 175 bp repetitive element of the spacer region. *Molecular and General Genetics* 218: 302–307.

SCHMIDT-PUCHTA, W., I. GUNTHER, AND H. L. SÄNGER. 1989. Nucleotide sequence of the intergenic spacer (IGS) of the tomato ribosomal DNA. *Plant Molecular Biology* 13: 251–253.

STANFORD, A. M., R. HARDEN, AND C. R. PARKS. 2000. Phylogeny and biogeography of *Juglans* (Juglandaceae) based on *matK* and ITS sequence data. *American Journal of Botany* 87: 872–882.

STEELE, K. P., AND R. VILGALYS. 1994. Phylogenetic analysis of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. *Systematic Botany* 19: 126–142.

SUGIURA, M. 1992. The chloroplast genome. *Plant Molecular Biology* 19: 149–168.

SWOFFORD, D. 1997. PAUP: phylogenetic analysis using parsimony, pre-release version 4.0b3a. Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D.C., and Sinauer, Sunderland, Massachusetts, USA.

TWIGG, L. E., D. R. KING, L. H. BOWEN, G. R. WRIGHT, AND C. T. EASTON. 1996. Fluoroacetate content of some species of the toxic Australian genus, *Gastrolobium*, and its environmental persistance. *Natural Toxins* 4: 122–127.

VOLKOV, R., S. KOSTISHIN, F. EHRENDORFER, AND D. SCHWEIZER. 1996. Molecular organization and evolution of the external transcribed rDNA spacer region in two diploid relatives of *Nicotiana tabacum* (Solonaceae). *Plant Systematics and Evolution* 201: 117–129.

WANG, X.-R., Y. TSUMURA, H. YOSHIMARU, K. NAGASAKA, AND A. E. SZMIDT. 1999. Phylogenetic relationships of Eurasian pines (*Pinus*, Pinaceae) based on chloroplast *rbcL*, *matK*, *rpl20-rps18* spacer, and *trnV* intron sequences. *American Journal of Botany* 86: 1742–1753.

XIANG, Q.-Y., D. E. SOLTIS, AND P. S. SOLTIS. 1998. Phylogenetic relationships of Cornaceae and close relatives inferred from *matK* and *rbcL* sequences. *American Journal of Botany* 85: 285–297.