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Infrageneric phylogeny of Chloantheae (Lamiaceae) based on chloroplast *ndh*F and nuclear ITS sequence data

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Abstract. The tribe Chloantheae (Prostantheroideae, Lamiaceae) currently consists of over 100 species in nine genera, all of which are endemic to Australia. Generic delimitations were assessed using chloroplast 3′*ndh*F and nuclear ITS nucleotide sequence data for up to seventy species. Analyses of the two datasets, independently and in combination, used maximum parsimony and Bayesian phylogenetic inference methods. Topologies derived from each marker were broadly congruent, but better resolution and stronger branch support was achieved by combining the datasets. The monophyly of the Chloantheae was confirmed. *Brachysola* is sister to the rest of the tribe and *Chloanthes*, *Cyanostegia* and *Dicrastylis* (including *Mallophora*) are monophyletic. Although the species within *Dicrastylis* were only partially resolved, it appears likely that the current sectional classification of this genus will require revision. A clade containing *Newcastelia*, *Physopsis* and *Lachnostachys* (=Physopsideae) was recovered, but the topology indicates that the current generic circumscriptions need further investigation. A close relationship between *Hemiphora elderi*, *Pityrodia bartlingii* and *P. uncinata* was resolved and reflects their palynological and carpological similarities. The relationship between remaining species of *Pityrodia* was incompletely resolved.

Introduction

The relatively large, cosmopolitan angiosperm order Lamiales has received considerable attention concerning the phylogenetic relationships of the traditionally recognised higher taxa that comprise it (Wagstaff and Olmstead 1997; Wagstaff *et al*. 1998; Olmstead *et al*. 2000). Much of the attention has been aimed at resolving family limits, and the taxonomic depth of the analyses has been necessarily limited (Wagstaff *et al*. 1998; Spangler and Olmstead 1999; Young *et al*. 1999; Beardsley and Olmstead 2002; Schwarzbach and McDade 2002). The greatest advances in resolving the deeper branches of the order have been provided by the use of coding and non-coding nucleotide sequences, primarily from the chloroplast (Olmstead and Palmer 1994; Soltis and Soltis 1998), and these studies have provided a relatively robust indication of the familial limits within the order. The sister relationships between and within some of the constituent families still remain elusive.

The synthesis of research into the phylogeny of the Lamiales has led to the consistent recognition of Lamiaceae and Verbenaceae (Cantino 1992*a*, 1992*b*; Cantino *et al*. 1992; Rimpler and Winterhalter 1992; Wagstaff and Olmstead 1997; Olmstead *et al*. 1998; Wagstaff *et al*. 1998; Olmstead *et al*. 2000). Within Lamiaceae, seven subfamilies are currently recognised (Harley *et al*. 2004), with the subfamilial placement of ten genera remaining uncertain. Of those subfamilies, Prostantheroideae is exclusively Australian and comprises two tribes: Westringieae Bartl. and Chloantheae Benth. & Hook. *f*. (Conn 2004). The sister relationship and monophyly of each of these tribes was confirmed in several independent studies (Junell 1934; Wunderlich 1967; Cantino 1982; Olmstead *et al*. 1998).

Before the aforementioned molecular phylogenetic analyses, Chloantheae was accorded family status (for history of taxonomy of this group, refer to Munir 1977*a*). Despite its recent taxonomic reassignment, Chloantheae has largely maintained its traditional circumscription, and is considered to be morphologically separable from Westringeae. Chloantheae currently consists of over 100 species in nine genera (*Brachysola, Chloanthes, Cyanostegia, Dicrastylis, Hemiphora, Lachnostachys, Newcastelia, Physopsis* and *Pityrodia*). Generally, Chloantheae is characterised by above-ground parts having a complete cover of branched hairs, combined with a distinctive decussate phyllotaxy and branching pattern, and most species are restricted to one of two centres of species richness in Western Australia or the Northern Territory.

Like its sister taxon Westringeae (Conn 1984, 1988, 1992, 2004; and other papers by this author), Chloantheae has received considerable systematic attention aimed at delimiting its constituent genera and infrageneric taxonomy (Rye 1996, 2005, 2007; Munir 1977*a*, 1977*b*, 1978*a*, 1978*b*, 1979). Most of the generic realignment has taken place without recourse to a comprehensive phylogenetic framework. Many of the genera are currently defined by relatively superficial or labile morphological characters concerning details of the inflorescence (degree of contraction of uniflorescences, degree

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of branching), flowers (perianth and staminal merosity, extent of stylar division; position of staminal insertion on corolla) and leaves (leaf arrangement, types of hairs and density). As a result, the taxonomic limits and phylogenetic integrity of several taxa remain somewhat equivocal, particularly *Hemiphora*, *Lachnostachys*, *Newcastelia*, *Physopsis* and *Pityrodia*.

A phylogenetic analysis by Olmstead *et al*. (1998) using the chloroplast marker *ndh*F provided preliminary insights into tribal relationships, and some adjustments of generic circumscriptions resulted (for example, *Brachysola* was distinguished from *Pityrodia* – Rye 2000). The taxonomic sample used by Olmstead *et al*. (1998) contained a limited sample of species, and so only preliminary conclusions about the naturalness of the constituent genera could be drawn. In the present paper, we use a combined analysis of 3′*ndh*F from the chloroplast genome and ITS from the nuclear genome to address questions concerning the circumscription and phylogenetic relationships of the genera that comprise Chloantheae. Of particular interest are the soundness of the recent transfer of *Mallophora* to *Dicrastylis* (Rye 2007), the sister relationship of *Brachysola* with the rest of the tribe as proposed by Olmstead *et al*. (1998), the phylogenetic integrity of the large genus *Pityrodia* and the taxonomic relationship between *Newcastelia, Lachnostachys* and *Physopsis*.

Material and methods

Taxon sampling

In total, 64 species, representing 62% of described species, of Chloantheae were used for the ingroup (Table 1). In selecting ingroup taxa, we included the type species of each Chloantheae genus and representatives of all previously proposed higher-level subdivisions (Munir 1978*a*, 1979) within the tribe. In addition, we sampled species that have had varied generic placements in the past. The ingroup was further supplemented with geographically widespread and taxonomically unproblematic species so that morphological variability and geographic range of the tribe were captured in the analyses. *Prostanthera calycina* and *P. rotundifolia* from the sister tribe Westringieae were used as the outgroup for all analyses. Separate analyses of *ndh*F and ITS were augmented by additional outgroup taxa (*Westringia fruticosa* and *W. rigida*; *W. sericea* and *W. longifolia* respectively).

Selection of molecular markers

The 3′ region of the *ndh*F gene was sequenced for this study because it is reasonably long (>1000 bp) and is known to have moderately high levels of base substitutions (Olmstead *et al*. 1998, 2000). The two internal transcribed spacer (ITS) regions of the 18S–26S nuclear rDNA are somewhat shorter than *ndh*F (~600 bp of aligned sequence) but are commonly used for comparative sequence studies because they are faster evolving than some coding regions. Despite potential difficulties with ITS concerning incomplete concerted evolution (Soltis *et al*. 2008), this marker has proven to be informative when inferring phylogenetic relationships within Lamiaceae (El Oualidi *et al*. 1999; Steane *et al*. 1999). Therefore, it was considered appropriate to sequence ITS for the Chloantheae.

Acquisition of sequences

Plant cellular DNA was extracted out of fresh, dried or cetyltrimethylammonium bromide (CTAB)-preserved leaf material (Thomson 2000) and processed using the protocol for the Qiagen DNeasy Plant Mini Kit (Qiagen, www.qiagen.com). The 3′end of *ndh*F was amplified using the forward primers 1F new or *ndh*F-PCR-mid and the reverse primers 2112R new or *ndh*F-PCR-end (Fig. 1). Most nucleotides of the two ITS regions and the 5.8S rDNA were amplified using the forward primers ITS 5 or ITS-Forw-PCR and the reverse primers ITS 4 or ITS-Rev-PCR (White *et al*. 1990; Baldwin 1992; Fig. 2). PCR products were purified using the Concert Rapid PCR Purification System (Life Technologies, Melbourne). The sequencing reactions were performed by Sydney University and Prince Alfred [Hospital] Molecular Analysis Centre (SUPAMAC) using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits and a Gene Amp 9700 cycle sequencer (both ABI Biosystems, www.appliedbiosystems.com) using the following cycling conditions according to manufacturer's specifications: 25 cycles of (10 s at 96°C, 5 s at 50°C and 4 min at 60°C). A consensus sequence of double-stranded DNA for each species was assembled using the electropherograms of at least four successful sequencing reactions. Electropherograms were visually checked, edited and aligned with the computer software Sequencher 3.1.1 (Genes Codes Corporation, www.genecodes.com), and alignments were refined manually in MacClade Version 4.05 (Maddison and Maddison 2001). Insertions/deletions (indels) were positioned so as to best conform to the indel types of Golenberg *et al*. (1993). Parsimony-informative indels were coded according to the 'simple' scheme of Simmons and Ochoterena (2000) and added to the alignment as binary characters. All nucleotide sequences have been deposited into GenBank and the accession numbers are listed in Table 1.

Data exploration

The partition homogeneity test was applied to the 3′*ndh*F–ITS pair of datasets using 10 000 replicates and the 'TWOregions' character partition option in PAUP* version 4.0b10 (Swofford 2002). Parsimony-uninformative characters were excluded from the comparison, as recommended by Lee (2001).

Maximum parsimony analyses

Maximum parsimony (MP) analyses of the 3′*ndh*F, ITS and combined datasets were performed using PAUP* version 4.0b10 (Swofford 2002) with all characters given equal weight. Full heuristic searches and bootstrap (BS) analyses exceeded available computer memory. Searches were performed using 10 000 full heuristic replicates (unless otherwise stated) and were set for TBR (tree-bisection-reconnection) branch swapping with random taxon addition to search for multiple islands of trees. A strict consensus of all equally parsimonious trees was produced after each analysis. Branching confidence was assessed by using bootstrap (Felsenstein 1985) and decay (Eriksson 1998) values obtained with 1000 resampling replicates, using the same tree search procedure described above. Bootstrap values $\geq$95% are interpreted as strong support, values between 75 and 95% are

Table 1. Taxa of Prostantheroideae (Lamiaceae) used in the present study

The total number of species in each genus is given in parentheses after the name and authority. The classification presented here is based on Rye (1996, 2005, 2007), and Rye and Trudgen (1998). GenBank accessions are given for each marker

Taxa	Voucher	3′*ndh*F	ITS
***Brachysola* (F.Muell.) Rye (2 species)**			
B. coerulea (F.Muell. & Tate)Rye	Lepschi 2933, PERTH	GQ381200	
B. coerulea (F.Muell. & Tate)Rye	Streiber 8 (NSW480339)		GQ381134
B. halganiacea (F.Muell.)Rye	ANBG 602366	GQ381201	GQ381135
***Chloanthes* R.Br. (4 species)**			
C. coccinea Bartl.	Streiber 56, NSW	GQ381202	GQ381136
C. glandulosa R.Br.	Streiber 1, SYD	GQ381203	GQ381139
C. parviflora Walp.	Lally 186, PERTH	GQ381204	
C. parviflora Walp.	NSW 435805		GQ381137
C. stoechadis R.Br.	Streiber 2, SYD	GQ381205	GQ381138
***Cyanostegia* Turcz. (5 species)**			
C. angustifolia Turcz.	Streiber 49, NSW	GQ381206	GQ381194
C. corifolia Munir	Streiber 50, NSW	GQ381206	GQ381149
C. lanceolata Munir	Streiber 57, NSW	GQ381208	GQ381148
C. microphylla S.Moore	CBG602380	GQ381209	GQ381145
***Dicrastylis* J.Drumm. ex Harv. (33 species)**			
Sect. *Dicrastylis* (9 species)			
D. fulva J.R.Drumm. ex Harv.	Craven 9426, PERTH	GQ381214	
D. fulva J.R.Drumm. ex Harv.	KP 19883398		GQ381140
D. incana Munir	KP 19893163	GQ381217	GQ381158
D. linearifolia Munir	KP 19920838	GQ381219	GQ381159
D. maritima Rye & Trudgen	Streiber 33, NSW	GQ381220	GQ381141
D. micrantha Munir	Streiber 42, NSW	GQ381221	GQ415410
D. parvifolia F.Muell.	ANBG 9810122	GQ381223	GQ381137
D. soliparma Rye & Trudgen	ANBG 9809799	GQ381225	GQ381155
Sect. *Corymbosae* (5 species)			
D. corymbosa (Endl.)Munir	ANBG 9810051	GQ381212	GQ381157
D. globiflora (Endl.)Rye	Streiber 25, NSW	GQ381235	GQ381143
D. reticulata Harv.	Streiber 55, NSW	GQ381224	GQ381142
D. rugosifolia (Munir)Rye	Smith 1103, PERTH	GQ381236	
D. rugosifolia (Munir)Rye	ANBG 602350		GQ381144
Sect. *Pyramidatae* (10 species)			
D. brunnea Munir var. *brunnea*	KP 19940485	GQ381211	GQ381156
D. cordifolia Munir	Pilbarra 7297 60, NSW	GQ381207	GQ381151
D. exsuccosa (F.Muell.)Druce	KP 20000486	GQ381213	GQ381150
D. flexuosa (Price)C.A.Gardner	Streiber 70, NSW	GQ381222	GQ381153
D. gilesii F.Muell. var. *gilesii*	Brown s.n., NSW	GQ381216	GQ381152
D. lewellinii (F.Muell.)F.Muell.	Mt. Annan 20001266	GQ381218	GQ381146
D. nicholasii F.Muell.	Streiber 69, NSW	GQ381227	GQ381161
Sect. *Spicatae* (6 species)			
D. beveridgei F.Muell.	Wilson 750, NSW	GQ381210	GQ381154
D. cundeeleensis Rye	Streiber 67, NSW	GQ381228	GQ381147
Sect. *Verticillatae* (1 species)			
D. verticillata J.M.Black	Streiber 72, NSW	GQ381226	GQ381163
***Hemiphora* F.Muell. (1 species)**			
H. elderi (F.Muell.)F.Muell.	Lepschi 3847, PERTH	GQ381229	
H. elderi (F.Muell.)F.Muell.	Streiber 11, NSW		GQ381180
***Lachnostachys* Hook. (5 species)**			
L. albicans Hook.	Streiber 51, NSW	GQ381230	GQ381164
L. coolgardiensis S.Moore	Streiber 4, NSW	GQ381231	GQ381165
L. eriobotrya Druce	Lyne 904, PERTH	GQ381232	
L. eriobotrya Druce	ANBG 9708412		GQ381166
L. ferruginea Hook.	Streiber 24, NSW	GQ381233	GQ381167
L. verbascifolia F.Muell.	KP 19930950	GQ381234	GQ381168
***Newcastelia* F.Muell. (9 species)**			
N. bracteosa F.Muell.	Streiber 71, NSW	GQ381237	GQ381169
N. cephalantha F.Muell.	Brown s.n., NSW	GQ381238	GQ381172
N. cladotricha F.Muell.	Telford 11584, PERTH	GQ381239	GQ381174

Table 1. (*continued*)

Taxa	Voucher	3′*ndh*F	ITS
N. hexarrhena F.Muell.	KP 19893441	GQ381240	GQ381170
N. insignis E.Pritz.	Streiber 5, NSW	GQ381241	GQ381171
N. spodiotricha F.Muell.	Lazarides & Palmer 234, CANB	GQ381242	
N. spodiotricha F.Muell.	Albrecht s.n., NSW497490		GQ381173
***Physopsis* Turcz. (5 species)**			
P. chrysophylla (C.A.Gardner)Rye	KP 19883381	GQ381243	GQ381176
P. lachnostachya C.A.Gardner	Streiber 62, NSW	GQ381244	GQ381175
P. spicata Turcz.	Smith 1396, PERTH	GQ381245	
P. spicata Turcz.	ANBG 9809765		GQ381177
***Pityrodia* R.Br. (38 species)**			
P. atriplicina (F.Muell.)Benth.	Craven 9422, CANB	GQ381260	
P. atriplicina (F.Muell.)Benth.	Streiber 36, NSW		GQ381178
P. axillaris (Endl.)Druce	Streiber 27, NSW	GQ381246	GQ381179
P. bartlingii (Lehm.)Benth.	Craven 9379, CANB	GQ381247	
P. bartlingii (Lehm.)Benth.	Davis s.n., NSW		GQ381179
P. cuneata (Gaudich.)Benth.	Streiber 38, NSW	GQ381261	GQ381183
P. cuneata (Gaudich.)Benth.	Streiber 40, NSW	GQ381264	
P. dilatata (F.Muell.)Benth.	ANBG 9300388	GQ381248	GQ381193
P. hemigenioides (F.Muell.)Benth.	Streiber 47, NSW	GQ381249	GQ381184
P. lepidota (F.Muell.)E.Pritz.	Streiber 15, NSW	GQ381250	GQ381185
P. loxocarpa (F.Muell.)Druce	Craven 9458, CANB	GQ381251	
P. oldfieldii (F.Muell.)Benth.	KP 19930430	GQ381262	GQ381195
P. pungens Munir	Barrow 4, NSW	GQ381252	
P. quadrangulata Munir	Short 5098, NSW	GQ381253	GQ381186
P. salviifolia R.Br.	Holmes 221, NSW	GQ381254	GQ381190
P. scabra A.S.George	KP 19930336	GQ381255	GQ381187
P. teckiana E.Pritz.	Streiber 20, NSW	GQ381256	GQ381188
P. terminalis (Endl.)A.S.George	CBG 9809809	GQ381257	GQ381182
P. terminalis (Endl.)A.S.George	Streiber 10, NSW	GQ381258	
P. ternifolia (F.Muell.)Munir	Short 5084, NSW	GQ381259	GQ381191
P. uncinata Benth.	Streiber 32, NSW	GQ381265	GQ381192
P. verbascina (F.Muell.)Benth.	Davis 9868, NSW	GQ381263	GQ381189
Outgroups			
Westringieae			
***Prostanthera* Labill.**			
Prostanthera calycina Benth.	RBGK386.86.08142	GQ381198	
Prostanthera calycina Benth.	de Kok 43, CANB		GQ381132
Prostanthera rotundifolia R.Br.	Wagstaff s.n., BHO	GQ381199	
Prostanthera rotundifolia R.Br.	de Kok 72, CANB		GQ381133
***Westringia* Sm.**			
Westringia fruticosa Druce	Wagstaff s.n., BHO	GQ381196	
Westringia longifolia R.Br.	de Kok 15, CANB		GQ415409
Westringia rigida R.Br.	Lepschi 2832, PERTH	GQ381197	
Westringia sericea B.Boivin	de Kok 18, CANB		GQ381131

interpreted as moderate support and values $\leq$74% are considered as weak support. Constraint trees were constructed in MacClade and imported into PAUP, and the analyses conducted as above.

Bayesian inference

The program MrBayes (Huelsenbeck *et al*. 2003) was used to compute Bayesian estimates of the phylogeny for the each of the 3′*ndh*F and ITS datasets, as well as the combined dataset. The searches were conducted with a general likelihood model of DNA substitutions (general time reversal model – GTR) (Hall 2001), as indicated by Modeltest (http://darwin.uvigo.es/software/modeltest.html). Rate variation was assumed to be gamma-distributed across sites. Three tree space searches were run for a total of 5 000 000 generations for five simultaneous runs (beginning with a randomly chosen tree) while running four simultaneous Monte Carlo chains, with and without heating of 0.5, and sampling the tree file every 100 generations, for each dataset. Stationarity was reached after the first 4000 trees of each run (in which the likelihoods had converged on a steady value as assessed by exporting data and graphing it) and were imported into PAUP* to calculate a 50% majority rule consensus tree. Bayesian support is referred to as posterior probability (PP) and is considered significant when it exceeds 0.95 (Larget and Simon 1999). PP values <0.80 are not included on trees.

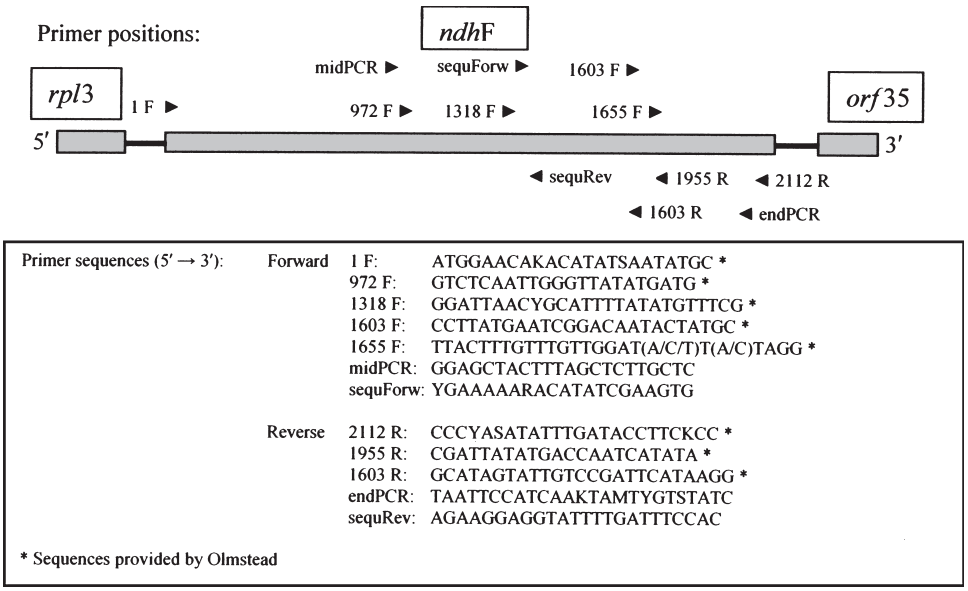

Fig. 1. Map of the chloroplast 3′*ndh*F region with primer positions and primer sequences, modified after R. G. Olmstead (pers. comm., the map is not true to scale). Grey boxes indicate reading frames and lines connecting boxes indicate non-coding DNA.

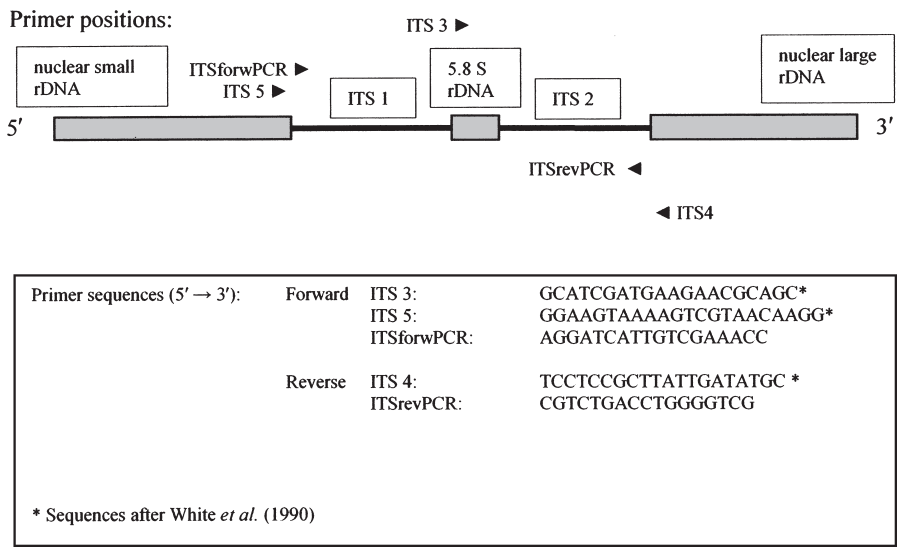

Fig. 2. Map of the nuclear ITS region with primer positions and primer sequences, modified after White *et al.* (1990, map not true to scale). Grey boxes indicate reading frames and lines connecting boxes indicate non-coding DNA.

Results

*3′*ndh*F data*

The total aligned length of the 70 3′*ndh*F nucleotide sequences was 1210 bp, including alignment gaps, with 814 characters that are constant, 169 variable characters that are parsimony-uninformative and 227 parsimony-informative characters. Nine parsimony-informative gaps, each comprising codon triplets, were identified and added at the end of the aligned file as binary characters. The ingroup consisted of 64 species.

The heuristic search of the nucleotide alignment plus the coded indels (gaps) produced 2080 maximally parsimonious trees of 691 steps, with unweighted consistency index (CI-u) = 0.62, retention index (RI) = 0.80 and rescaled consistency index (RC) = 0.57 (refer Fig. 3). The resultant

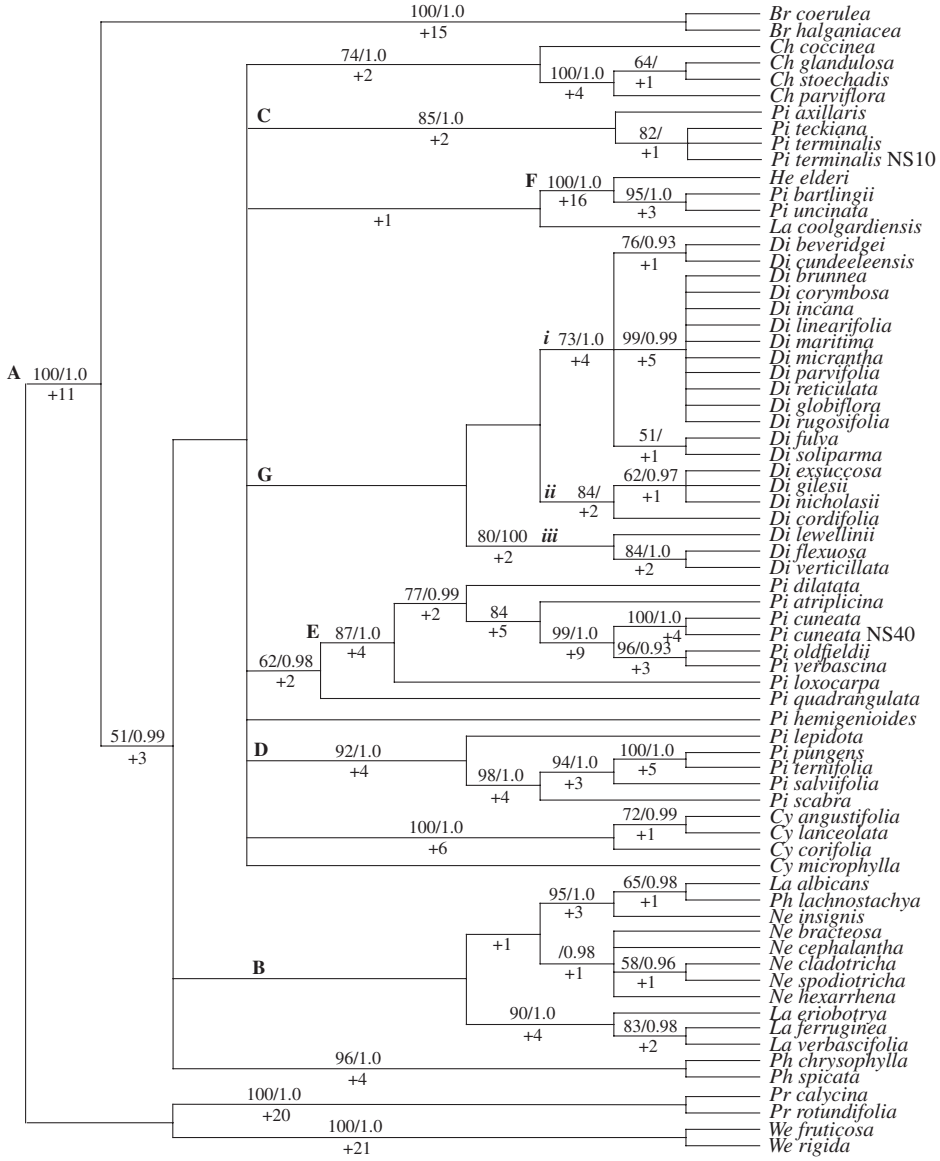

Fig. 3. Strict consensus tree of 2080 maximum parsimony (MP) trees of 691 steps based on 3′*ndh*F sequences. Bootstrap (BS) values (≥50%) followed by posterior probabilities (PP) values (≥80%) given above branches, decay values below (CI-u = 0.62, RI = 0.80, RC = 0.57). Clades discussed in the text are indicated by letters and roman numerals. The abbreviations of genera are as follows: *Br* = *Brachysola*; *Ch* = *Chloanthes*; *Cy* = *Cyanostegia*; *Di* = *Dicrastylis*; *He* = *Hemiphora*; *La* = *Lachnostachys*; *Ne* = *Newcastelia*; *Ph* = *Physopsis*; *Pi* = *Pityrodia*; *Pr* = *Prostanthera*; *We* = *Westringia*.

topology is congruent with the findings of Olmstead *et al*. (1998) that Chloantheae are monophyletic (Clade A: BS 100%, decay = 11, PP 1.0). The *ndh*F alignment proved to be somewhat inconclusive in resolving relationships between currently circumscribed genera of Chloantheae. A strongly supported *Brachysola* (BS 100%, decay = 15, PP 1.0) formed a weakly supported sister relationship to the remainder of the tribe (BS 51%, decay = 3, PP 0.99).

The majority of the species of *Lachnostachys*, all of *Newcastelia* and *Physopsis lachnostachya* form a weakly supported subclade (Clade B). *Physopsis chrysophylla* and *P. spicata* form a strongly supported clade (BS 96%, decay = 4, PP 1.0), but the remaining taxa form a large, weakly supported clade.

Pityrodia species are present in several subclades (C–F): in subclade C, *P. axillaris*, *P. teckiana* and *P. terminalis* form a moderately supported clade (BS 85%, decay = 2, PP 1.0); in subclade D, *P. pungens*, *P. ternifolia*, *P. salviifolia* (type species), *P. lepidota* and *P. scabra* form a moderately supported clade (Clade C: BS 92%, decay = 4, PP 1.0); in subclade E, *P. atriplicina*, *P. cuneata*, *P. dilatata*, *P. oldfieldii*, *P. loxocarpa* and *P. verbascina* form a moderately supported

clade (BS 87%, decay = 4, PP 1.0); and in subclade F, *P. bartlingii* and *P. uncinata* form a strongly supported clade with *Hemiphora elderi* (BS 100%, decay = 16, PP 1.0).

The monophyly of *Chloanthes* is weakly supported (BS 74%, decay = 2, PP 1.0). However, within the *Chloanthes* clade, *C. glandulosa*, *C. parviflora* and *C. stoechadis* form a strongly supported subclade (BS 100%, decay = 4, PP 1.0). *Cyanostegia angustifolia, C. cordifolia* and *C. lanceolata* form a strongly supported clade (BS 100%, decay = 6, PP 1.0). The relationship of *Cyanostegia microphylla* to the other species of *Cyanostegia* is unresolved.

The monophyly of *Dicrastylis* is not conclusively supported by the analyses of the 3′*ndh*F alignment (Clade G). Species of this genus comprise three weakly to moderately supported subclades (*i–iii*). The previously recognised diatypic genus *Mallophora* (as *D. rugosifolia* and *D. globiflora*) falls within a strongly supported subclade with seven other species of *Dicrastylis* (BS 99%, decay = 5, PP 0.99) (Fig. 3).

The consensus 50% majority rule tree from a Bayesian (BI) analysis (not presented here) is broadly congruent with the strict MP consensus tree of the 3′*ndh*F data (Fig. 3).

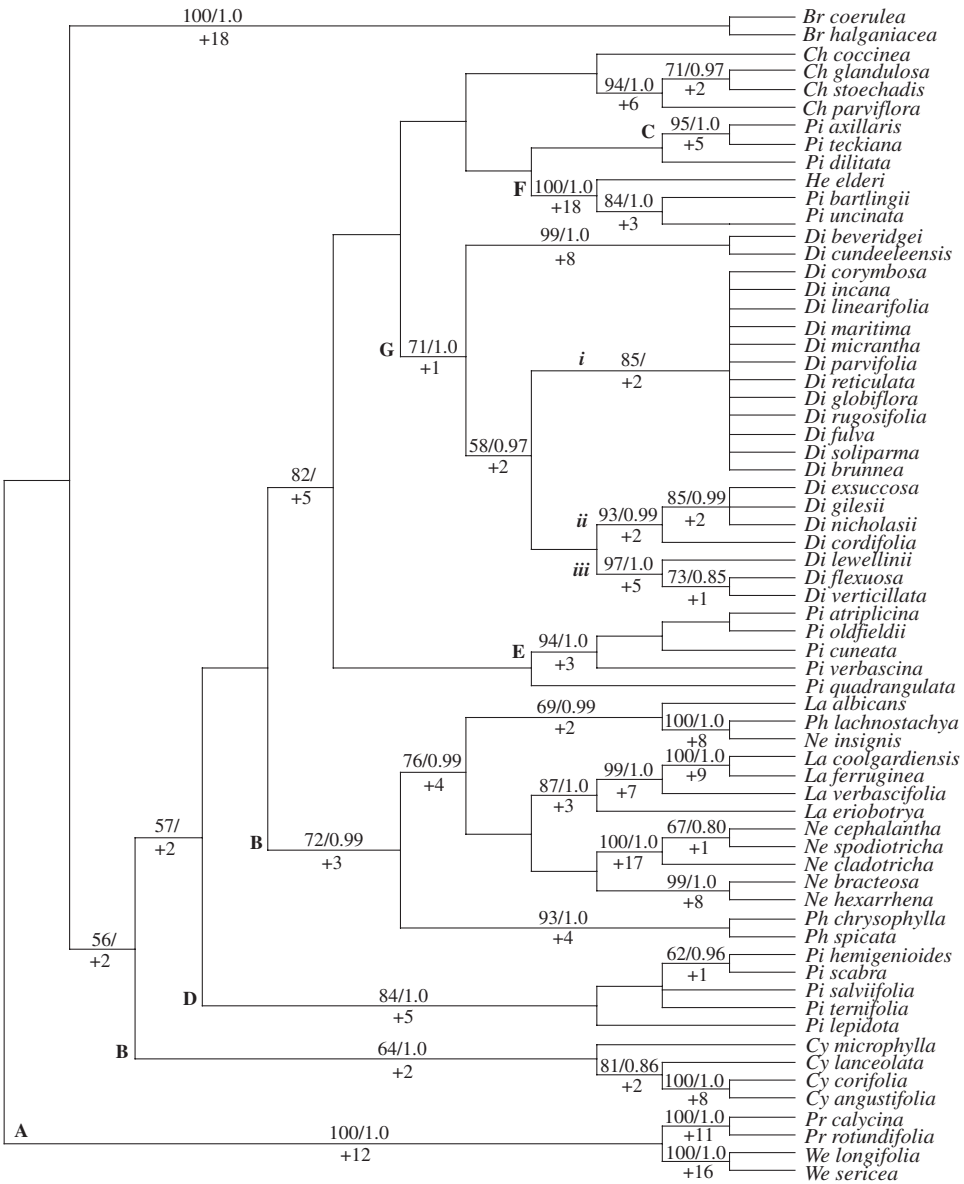

Fig. 4. Strict consensus tree of 9 maximum parsimony (MP) trees of 840 steps based on ITS sequences. Bootstrap (BS) values (≥50%) followed by posterior probabilities (PP) values (≥80%) given above branches, decay values below (CI-u = 0.49, RI = 0.72, RC = 0.40). Clades discussed in the text are indicated by letters. Generic abbreviations as listed in Fig. 3.

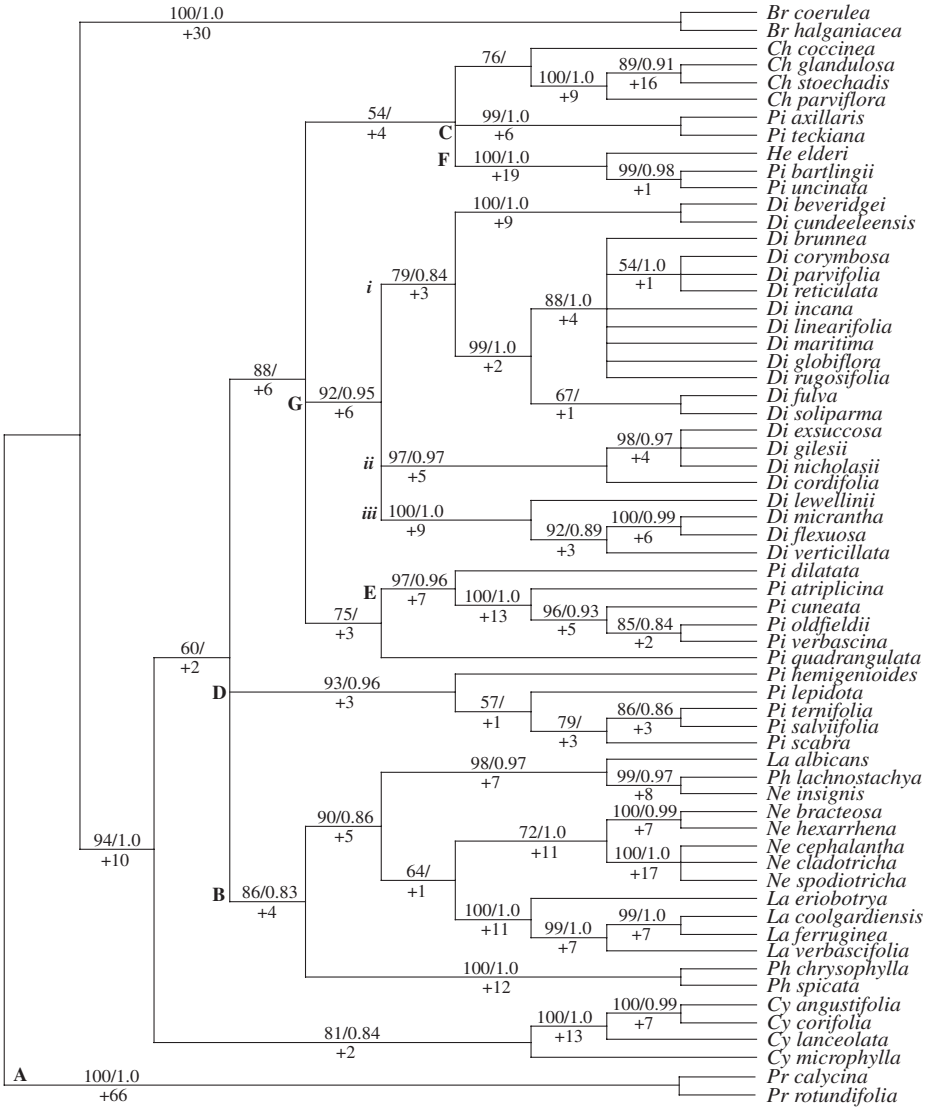

Fig. 5. Strict consensus tree of 60 maximum parsimony (MP) trees of 1654 steps based on the combined 3′*ndh*F and ITS data (including gaps) for 61 taxa of Chloantheae. Bootstrap (BS) values (≥50%) followed by posterior probabilities (PP) values (≥80%) given above branches, decay values below (CI-u = 0.47, RI = 0.63, RC = 0.34). Generic abbreviations as listed in Fig. 3.

ITS data

The ITS dataset consisted of 66 sequences with an aligned length of 643 bp, composed of 238 bp from ITS1, 167 bp from 5.8S and 238 bp from ITS2. The majority of gaps had a length of 1 or 2 base pairs and homology was uncertain. An heuristic search yielded nine equally parsimonious trees of 878 steps (CI-u = 0.47, RI = 0.72 and RC = 0.40), the strict consensus of which is shown in Fig. 4.

The topology (Fig. 4) derived from the ITS data for both MP and BI analyses (the latter not presented here) is broadly congruent with that from 3′*ndh*F data. The representatives of the Chloantheae used in this study form a strongly supported group (Clade A: BS 100%, decay = 12, PP 1.0) and, within the tribe, the diatypic genus *Brachysola* forms a strongly supported clade (BS 100%, decay = 18, PP 1.0), sister to the remaining Chloantheae. Four species of *Cyanostegia* form a weakly supported clade (Clade B: BS 64%, decay = 2, PP 1.0) that is sister to the remaining Chloantheae.

Chloanthes parviflora + *C. stoechadis* + *C. glandulosa* form a strongly supported clade (BS 94%, decay = 6, PP 1.0), but with weak support for a sister relationship with *C. coccinea* (PP 0.59).

All species of *Dicrastylis* (including *Mallophora*) are recovered in a weakly to moderately supported clade G (BS 71%, decay = 1, PP 1.0), with *D. beveridgei* and *D. cundeeleensis* (both sect. *Spicatae*) sister to all other species of *Dicrastylis* (BS 99%, decay = 8, PP 1.0). In the *ndh*F, these two species were included in subclade G*i* (Fig. 3). Subclade D*i* (Fig. 4) contains *D. corymbosa*, *D. fulva, D. globiflora, D. incana*,

D. linearifolia, *D. maritima, D. micrantha*, *D. parviflora*, *D. reticulata*, *D. rugosifolia*, *D. soliparma* (a mix of *Dicrastylis* sect. *Corymbosae* and sect. *Dicrastylis*) and *D. brunnea* (sect. *Pyramidatae*) as a moderately supported subclade (BS 85%, decay = 2, PP 0.74). Another moderately supported subclade (G*ii*) consists of *D. cordifolia*, *D.exsuccosa*, *D. gilesii* and *D. nicholasii*, all representatives of sect. *Pyramidatae* (BS 93%, decay = 2, PP 0.99). The remaining species of the genus are included in subclade G*iii* (Fig. 4), with *D. flexuosa*, *D. lewellinii* (both sect. *Pyramidatae*), plus *D. verticillata* (sect. *Verticillatae*) (BS 97%, decay = 5, PP 1.0).

The remaining species of *Pityrodia* are recovered in subclades D, E and F. Subclade D is moderately supported and consists of *P. hemigenioides*, *P. lepidota*, *P. salviifolia*, *P. scabra* and *P. ternifolia* (BS 84%, decay = 5, PP 1.0) and represents *Pityrodia sensu stricto*. Subclade E contains a strongly supported lineage of *Pityrodia atriplicina*, *P. cuneata*, *P. oldfieldii* and *P. verbascina* (BS 94%, decay = 3, PP 1.0). Subclade F is strongly supported (BS 100%, decay = 18, PP 1.0) and includes *Hemiphora elderi*, *Pityrodia bartlingii* and *P. uncinata*.

All species of *Lachnostachys*, *Newcastelia* and *Physopsis* comprise the weakly supported subclade B (BS 72%, decay = 3, PP 0.99). This subclade comprises several strongly supported lineages that were also recovered in the *ndh*F analysis (Fig. 3*B*).

*Combined 3′*ndh*F and ITS analysis*

The partition homogeneity (incongruence length difference) test for the 3′*ndh*F and ITS data indicated that the two DNA partitions are significantly different from two random partitions of the combined data ($P = 0.010$). Therefore, the observed differences between the 3′*ndh*F and ITS data must be viewed with caution. Although the incongruence length difference test is useful as a tool for exploring heterogeneity in datasets, it should not be used as an arbiter of whether datasets should be combined (Yoder *et al*. 2001; Barker and Lutzoni 2002).

An heuristic search of the combined 3′*ndh*F and ITS data matrix (including the 12 re-coded indels) for the 63 species common to both datasets (1882 characters) resulted in 310 454 trees of 1521 steps (CI-u = 0.41, RI = 0.73, RC = 0.45). The strict consensus tree is presented in Fig. 5, with the Bayesian posterior probability values included. All representatives of the Chloantheae form a strongly supported group (Clade A: BS 100%, decay = 66, PP 1.0). *Brachysola* forms a strongly supported clade (BS 100%, decay = 30, PP 1.0) that is sister to the remainder of the tribe.

The genus *Cyanostegia* is moderately supported (BS 81%, decay = 2, PP 0.84), within which there is strong support for the sister relationship of *C. angustifolia*, *C. corifolia* and *C. lanceolata* (BS 100%, decay = 13, PP 1.0).

The genus *Chloanthes* (weakly supported – BS 76%) has a strongly supported subclade (BS 100%, decay = 9, PP 1.0) containing *C. glandulosa* + *C. stoechadis* + *C. parviflora*. Subclade C is strongly supported (BS 99%, decay = 6, PP 1.0) and consists of *Pityrodia axillaris* and *P. teckiana. Hemiphora elderi* + *Pityrodia bartlingii* + *P. uncinata* form the strongly supported subclade F (BS 100%, decay = 19, PP 1.0).

Clade B is a moderately supported clade (BS 86%, decay = 4, PP 0.83) consisting of species of *Lachnostachys*, *Newcastelia* and *Physopsis*. Several strongly supported subclades recognised within this clade include: *Lachnostachys albicans* + *Newcastelia insignis* + *Physopsis lachnostachya* (BS 98%, decay = 7, PP 0.97); *Lachnostachys coolgardiensis–L. eriobotrya* (including *L. ferruginea* – type of *Lachnostachys*) (BS 100%, decay = 11, PP 1.0); *Newcastelia bracteosa* + *N. hexarrhena* (BS 100%, decay = 7, PP 0.99); *Newcastelia cephalantha* + *N. cladotricha* (type of *Newcastelia*) + *N. spodiotricha* (BS 100%, decay = 17, PP 1.0); and *Physopsis chrysophylla* + *Physopsis spicata* (BS 100%, decay = 12, PP 1.0).

Clade D is strongly supported (BS 93%, decay = 3, PP 0.96) and represents *Pityrodia sensu stricto* (including *Pityrodia salviifolia*, type species).

Dicrastylis (Clade G) is strongly supported (BS 92%, decay = 6, PP 0.95). Within *Dicrastylis*, several major lineages were recovered. Subclade *Gi* is moderately supported (BS 79%, decay = 3, PP 0.84) and includes the strongly supported *D. beveridgei* + *D. cundeeleensis* lineage (both section *Spicatae*) (BS 100%, decay = 9, PP 1.0), as well as another strongly supported lineage of 11 species of *Dicrastylis* (BS 99%, decay = 2, PP 1.0), with representatives of sections *Corymbosae*, *Dicrastylis* and *Pyramidatae. Dicrastylis exsuccosa–D. cordifolia* (all sect. *Pyramidatae*) subclade G*iii* is strongly supported (BS 97%, decay = 5, PP 0.97). Subclade G*iii* contains *D. flexuosa*, *D. lewellinii* (both sect. *Pyramidatae*), *D. micrantha* (sect. *Dicrastylis*) and *D. verticillata* (sect. *Verticillatae*) (BS 100%, decay = 9, PP 1.0).

Clade E is a strongly supported lineage (BS 97%, decay = 7, PP 0.96) consisting of *Pityrodia atriplicina*, *P. cuneata*, *P. dilatata*, *P. oldfieldii* and *P. verbascina. Pityrodia quadrangulata* is weakly supported as sister to the above species (BS 75%, decay = 3, PP 0.70).

Discussion

Phylogenetic relationships

Although the analyses of 3′*ndh*F and ITS data (Figs 3, 4, respectively) were incongruent (homogeneity partition test with $P = 0.010$), each recovered a strongly supported Chloantheae comprising a set of clades of similar taxonomic composition in both the MP and BI analyses. Likewise, the MP and BI analyses of the combined data resulted in topologically similar strict consensus trees. There are only two alignment gaps of unique origin in the *ndh*F data, both being 9-base deletions, one at position 670 supporting the *Brachysola* clade, the other (position 561) supporting the *Cyanostegia corifolia–C. lanceolata* clade. The remaining gaps of unique origin are from the ITS dataset. Of these, one gap is within *Cyanostegia* clade and the other in *Newcastelia insignis–Physopsis lachnostachya* clade. The latter clade is also supported by a 2-base deletion. *Physopsis chrysophylla–P. spicata* is supported by two gaps, one a unique 3-base insertion at position 1704, the other a 2-base deletion at position 1373; the latter is also an autapomorphy for *Physopsis*

lachnostachya. However, constraint analyses revealed that enforcing a monophyletic *Physopsis* required an extra 21 steps on the combined *ndh*F and ITS MP tree. Hence, our analyses do not support the recognition of a monophyletic *Physopsis* as currently circumscribed. However, overall, the distribution of the majority of scored gaps is a perfect fit to the strict consensus tree.

There is considerable similarity between the *ndh*F and ITS trees. A strongly supported monophyletic *Brachysola* was consistently sister to the remainder of the tribe in each analysis (Figs 3, 4). *Brachysola* can be defined by two morphological synapomorphies, the presence of stellate hairs on leaves, rather than dendritic hairs, and by anthers locules being fused throughout their length rather than being free and divergent basally (Streiber 2005). The clade consisting of all other Chloantheae has dendritic branched hairs on their leaves and calyces. *Chloanthes* and *Dicrastylis* are resolved as monophyletic by both datasets. Clades comprising components of *Cyanostegia*, *Lachnostachys*, *Newcastelia*, *Physopsis* and *Pityrodia* are also recovered in both datasets.

All analyses retrieved a *Pityrodia sensu stricto* clade comprising *Pityrodia lepidota*, *P. salviifolia* (type species), *P. scabra*, *P. ternifolia* and *P. pungens* (only in *ndh*F dataset) or *P. hemigenioides* (recovered in ITS). We are unaware of any unequivocal morphological synapomorphies that would define the above clade. Munir (1979) regarded *P. lepidota* and *P. salviifolia* as being closely related because of their shared scaly indumentum. *Pityrodia pungens* (only in *ndh*F data) and *P. scabra*, plus several other Chloantheae species, also have fringed scale-like hairs that are somewhat similar to the scaly indumentum referred to above. However, the MP and BI analyses do not support a single origin for these scales. *Pityrodia hemigenioides* has hairs with numerous short spiny branches, whereas *P. ternifolia* has hairs with fewer branches. Other species, not included in this study, that have various types of fringed scale-like hairs include *P. augustensis*, *P. byrnesii*, *P. canaliculata*, *P. chrysocalyx*, *P. gilruthiana*, *P. lanuginosa*, *P. loricata*, *P. puberula* and *P. spenceri*. Until these latter species are sampled, the phylogenetic significance of these indumentum features cannot be determined.

The monotypic *Hemiphora* is recovered in the same clade as *Pityrodia bartlingii* and *P. uncinata* (by all analyses). This relationship can be defined by morphological synapomorphies including their unique 6-colpate pollen type (El-Gazzar and Watson 1970; Mukherjee 1976; Raj and Grafstroem 1984). El-Gazzar and Watson (1970) indicated that this form of colpal arrangement was observable only before anthesis, after which the three pairs of colpi appear to unite to form a tricolpate grain as in other Chloantheae. In addition, all three species have deeply divided calyx lobes and distinct size differences between the larger fertile adaxial staminal pair and smaller abaxial pair that have reduced fertility or are sterile. Anther locules of these species lack appendages or the appendage is greatly reduced. *Hemiphora elderi* and *Pityrodia bartlingii* share longitudinally enlarged, folded seeds as described by Junell (1934), but this character has not been recorded for *P. uncinata*. Although not included in the present study, *Pityrodia exserta* is morphologically similar to the above two species of *Pityrodia* (Munir 1979). It also has an adaxial staminal pair larger than the abaxial pair, the latter with reduced fertility; all anthers either lack an appendage or the appendage is greatly reduced. The calyx of *P. exserta* is deeply divided like those of the above species. The habit, leaves and inflorescences of this species are also similar to that of *P. uncinata*. Therefore, it is expected that *P. exserta* is closely related to the above species. Based on these results and putative synapomorphies discussed above, the taxonomic circumscription of *Hemiphora* needs to include *Pityrodia bartlingii*, *P. uncinata* and probably *P. exserta*.

Taxonomic status of Pityrodia sensu lato

Three consistent lineages were recovered in all analyses for the remaining species of *Pityrodia*: (1) *Pityrodia sensu stricto* clade was strongly supported and included *P. hemigenioides*, *P. lepidota*, *P. salviifolia* (type of *Pityrodia*), *P. scabra* and *P. ternifolia*; (2) a strongly supported clade comprising *P. axillaris* (type of *Dasymalla* Endl.), *P. teckiana* and *P. terminalis* (based on *ndh*F data); and (3) a strongly supported clade including *P. atriplicina*, *P. cuneata* (type of *Quoya* Gaudich.), *P. dilatata*, *P. loxocarpa* (based on *ndh*F data), *P. oldfieldii* and *P. verbascina*. Constraint analyses revealed that enforcing a monophyletic *Pityrodia sensu lato* required an extra 48 steps on the combined *ndh*F and ITS MP tree. Based on our analyses, support for the generic status of the above three clades is strong and a monophyletic *Pityrodia sensu lato* is not supported.

The affinities of *Pityrodia quadrangulata* are unclear even though it currently has a weakly supported sister relationship with the clade containing the type species of *Quoya*. This result is in agreement with Munir (1979), who postulated that *P. quadrangulata* is close to *P. dilatata*. However, other species (not included in the present study) from the Northern Territory (namely, *Pityrodia angustisepala*, *P. megalophylla* and *P. lanceolata*) are anticipated to be closely related to *P. quadrangulata* (Munir 1979). All of these four latter species retain 4-angled branchlets, have ovaries that are longitudinally ribbed and have fruits that are 4-ridged with transverse calluses (ridges). It is here recommended that the phylogeny of *P. quadrangulata* should be evaluated together with these additional species.

Chloanthes *and* Cyanostegia

Chloanthes and *Cyanostegia* are consistently rendered as monophyletic, although the relationship of each genus with the remainder of the tribe is somewhat equivocal. Both genera are characterised by the presence of strongly zygomorphic 5-lobed flowers, and four fertile stamens. Although they share these morphological features, our data do not recover them as closely related. *Chloanthes* is morphologically recognisable by their decurrent leaves; distinctly 2-lipped corolla; stamens inserted below middle of corolla-tube and slightly exserted; anthers with shortly divergent basal lobes; and drupaceous fruit that usually separate into two 2-locular mericarps. The genus is supported by one morphological synapomorphy, leaf lamina with rounded or square protrusions forming geometrically ordered segments that are parallel to each other (Streiber 2005). In contrast, *Cyanostegia* has petiolate leaves; enlarged adaxial

corolla lobes but that are not 2-lipped; stamens inserted at middle of corolla-tube and strongly exserted; anthers with locules free over the basal half; and fruits that are dry, hard and indehiscent. The *Cyanostegia* clade is supported by one morphological synapomorphy; namely, the staminal filaments are swollen distally at the anther base.

Phylogeny of the Lachnostachys–Newcastelia–Physopsis *clade*

Lachnostachys, Newcastelia and *Physopsis* form a close association in all analyses with each genus constituting a more-or-less homogeneous subclade within the more inclusive clade. Two morphological synapomorphies support this clade; namely, the presence of condensed inflorescence-branches forming variously spike-like inflorescences and the outer surface of the corolla is glabrous (Streiber 2005). In the ITS and combined analyses, *Physopsis chrysophyla* and *P. spicata* (type of *Physopsis*) were consistently depicted as sister to a clade comprising all species of *Newcastelia* and *Lachnostachys* plus *Physopsis lachnostachya. Lachnostachys* is distinguished from *Newcastelia* and *Physopsis* by the mature ovary becoming 2-loculate at maturity with two ovules in each (whereas the mature ovary in both *Newcastelia* and *Physopsis* remains 4-loculate throughout, with one ovule in each locule) and by their lack of corolla lobes or if present, then corolla with 5−8 inconspicuous lobes, compared with both *Newcastelia* and *Physopsis*, which have distinctly lobed corollas in their distal half (5- or 6-lobed and 4- or 5-lobed, respectively) (Rye 1996; Conn 2004). *Physopsis* can be distinguished readily from *Lachnostachys* and *Newcastelia* by the smooth adaxial surface of the leaves that are covered by glandular hairs (*Lachnostachys* and *Newcastelia* have leaves covered with a dense persistent indumentum of multiple-branched and glandular hairs), and the usually distinctly lobed stigma (*Lachnostachys* and *Newcastelia* have minute stigma lobes or lobes absent) (Conn 2004).

Two strongly supported clades were recovered in *Newcastelia* (Fig. 5): (1) *N. cephalantha* + *N. cladotricha* (type of *Newcastelia*) + *N. spodiotricha*; and (2) *N. bracteosa* + *N. hexarrhena*. Although Munir (1978*a*) did not suggest any infrageneric classification for this genus, the three characters that are used as primary distinguishing features (namely, the extent of exsertion of stamens and style, plus shape of inflorescence) do not reflect potential phylogenetic groupings. The stamens and style are exserted in *N. bracteosa*, *N. cephalantha*, *N. hexarrhena* and *N. spodiotricha* (although not found to belong with the other species of *Newcastelia* in this study, *N. insignis* also has exserted stamens and style), whereas, the stamens and style are included in *N. cladotricha*. However, *N. cephalantha* has flowers arranged in globose or subglobose cymes (as does *N. insignis*), whereas, all other species have flowers arranged in elongated spike-like cymes.

An exception to the otherwise monophyletic genera was the consistent recovery of a strongly supported clade that comprised *Lachnostachys albicans*, *Newcastelia insignis* and *Physopsis lachnostachya*. Depending on the data and the type of analysis, this composite clade was either sister to *Newcastelia* (*ndh*F data), unresolved with respect to *Newcastelia* and *Lachnostachys* (ITS) or sister to a combined *Newcastelia* + *Lachnostachys* clade (combined data). The association of *L. albicans* with *P. lachnostachya* and *N. insignis* might be explicable morphologically in that the relatively conspicuous corolla lobes of *L. albicans* (lobes shallowly triangular to depressed ovate, 0.3–0.6-mm long) are more like those of *Physopsis* and *Newcastelia* than of other species of *Lachnostachys*. Constraint analyses revealed that enforcing a monophyletic *Physopsis* required an extra five steps in the combined *ndh*F and ITS MP tree. Hence, our analyses tend not to support the recognition of a monophyletic *Physopsis*.

The taxonomy of the above three genera was reviewed by Rye (1996) based on a consideration of morphological data. She modified the circumscription of each genus accordingly and recognised six species of *Lachnostachys*, nine *Newcastelia* species and nine *Physopsis* species. Our data do not recover a monophyletic *Lachnostachys*, *Newcastelia* or *Physopsis*. Her classification does not reflect the close relationship between *L. albicans*, *N. insignis* and *Physopsis lachnostachya* retrieved by our molecular analysis. A more rigorous test of the phylogeny of this group is required, based on a broader taxonomic sample of *Lachnostachys*, *Newcastelia* and *Physopsis*.

Phylogeny of Dicrastylis

All analyses recovered a monophyletic *Dicrastylis* although with weak support in the separate analyses of the *ndh*F and ITS datasets. However, the monophyly of this genus was strongly supported by the combined analyses. The genus can be defined by one morphological synapomorphy: flowers are arranged in dichasia that are condensed and head-like. In a recent revision of the sections of *Dicrastylis*, Rye (2005) formally transferred the two species of *Mallophora* (namely, *M. globiflora* Endl. and *M. rugosifolia* Munir) to *Dicrastylis* as *D. globiflora* (Endl.) Rye and *D. rugosifolia* (Munir) Rye. Brummitt (2002) recommended that the name *Dicrastylis* be conserved against the older name, *Mallophora*. Before Rye's (2005) revision, the generic status of *Mallophora* was based on its relatively condensed inflorescences, 4-merous flowers and apparently shorter style branches when compared with typical *Dicrastylis*. In segregating *Mallophora* from *Dicrastylis*, Munir (1978*a*) nominated the 4-merous flowers of *Mallophora* and *Physopsis* to indicate a close relationship. Results of the present study reject such a relationship and confirm that *Mallophora* is best considered to be congeneric with *Dicrastylis*.

As the second largest genus of Chloantheae, *Dicrastylis* (with more than 30 species) traditionally has been divided into five sections to accommodate relatively subtle, but consistent morphological differences of the inflorescence and corolla (Munir 1978*a*). Rye (2007) maintained sectional nomenclature used by Munir (1978*a*), but adjusted the taxonomic composition and morphological circumscription.

Of relevance to the current discussion is the restriction by Rye (2007) of sect. *Verticillatae* to contain only *D. verticillata*, her transferral of *D. globiflora* and *D. rugosifolia* (from *Mallophora*) to sect. *Corymbosae* and her transferral to sect. *Pyramidatae* of *D. nicholasii* (from sect. *Corymbosae*), *D. flexuosa* and

D. cordifolia (both from sect. *Verticillatae*) (Rye 2007). Our data contain representatives of all sections of *Dicrastylis* as currently defined (refer Table 1) and recovered several clades that equate with sections or combinations of sections as recognised by Rye (2007). Within *Dicrastylis*, four strongly supported clades were recovered, but none of these was consistent with previous (Munir 1978*a*) or existing (Rye 2007) sectional classifications. *Dicrastylis* sect. *Spicatae* (*D. beveridgei* and *D. cundeeleensis*) (included in Fig. 5 clade *i*) and four of the seven sampled species of sect. *Pyramidatae* (*D. cordifolia*, *D. exsuccosa*, *D. gilesii* and *D. nicholasii*) were consistently resolved (Fig. 5, clade *ii*). Rye (2007) placed the latter four species in section *Pyramidatae*, whereas Munir (1978*a*) placed *D. gilesii* in sect. *Spicatae* and *D. nicholasii* in sect. *Corymbosae*. The other strongly supported lineage included in clade *i* (Fig. 5) contains a mix of species from sections *Corymbosae* (4 species), *Dicrastylis* (6 species) and one species from sect. *Pyramidatae* (*D. brunnea*). The final clade (*iii*) also consists of a mix of sections, with two species of sect. *Pyramidatae* (*D. flexuosa* and *D. lewellinii*), *D. micrantha* (sect. *Dicrastylis*) and *D. verticillata* (sect. *Verticillatae*). Munir (1978*a*) included *D. verticillata* with *D. flexuosa* in sect. *Verticillatae*, whereas Rye (2007) transferred the latter species to sect. *Pyramidatae*. In all analyses, *D. flexuosa* and *D. lewellinii* belong together in a clade that does not include the type species of sect. *Pyramidatae* (*D. exsuccosa*) and thus is in conflict with the inclusion of both in sect. *Pyramidatae* by Rye (2005). In contrast, *D. cordifolia*, *D. exsuccosa* and *D. gilesii* always occur in a clade with *D. nicholasii*, supporting their inclusion in sect. *Pyramidatae*. The placement of *D. cordifolia* and *D. flexuosa* in sect. *Verticillatae* by Munir (1978*a*) is not supported by our data, whereas the transferral of *D. cordifolia* from sect. *Verticillatae* (*sensu* Munir 1978*a*) to sect. *Pyramidatae* (*sensu* Rye 2007) is supported (Clade *ii*). The inclusion of *D. micrantha* (sect. *Dicrastylis sensu* Munir 1978*a* and Rye 2007) and *D. lewellinii* (sect. *Pyramidatae sensu* Rye 2007; sect. *Spicatae sensu* Munir 1978*a*) in clade *iii* does not support either sectional classification. Our results provide some support for the transfer of *D. nicholasii* (from sect. *Corymbosae sensu* Munir 1978*a*) to sect. *Pyramidatae* and for the naturalness of sect. *Spicatae* (Rye 2005), but fail to resolve sections *Dicrastylis* (*sensu* Munir 1978*a*; Rye 2007), *Corymbosae*, *Pyramidatae* and *Verticillatae* (as re-circumscribed by Rye 2007). At this stage, we consider that the taxonomic sample and the resolving power of the two markers used in this study are sufficient to reject some of the sectional circumscriptions of Munir (1978*a*) and Rye (2007), but are not appropriate to unequivocally resolve taxonomic composition of sections in *Dicrastylis*.

Infra-tribal classification of Chloantheae

In his revisionary studies of Chloanthaceae (here regarded as Lamiaceae tribe Chloantheae), Munir (1978*a*, 1979) recognised tribes 'Chloantheae' and 'Physopsideae'. In the 'Chloantheae', he included *Chloanthes*, *Cyanostegia*, *Hemiphora*, *Pityrodia* and *Spartothamnella* (now included in Lamiaceae subfam. Ajugoideae; Cantino 2004), whereas his 'Physopsideae' consisted of *Dicrastylis*, *Lachnostachys*, *Mallophora* (now included in *Dicrastylis*), *Newcastelia* and *Physopsis*. *Brachysola* would be included in 'Chloantheae' (*sensu* Munir 1979). Our data do not support the recognition of infra-tribal lineages similar to those suggested by Munir (1978*a*). Constraint analyses revealed that enforcing a monophyletic 'Physopsideae' and 'Chloantheae' (both *sensu* Munir 1978*a*, 1979) required an extra 13 steps on the combined *ndh*F and ITS MP tree.

Munir regarded *Dicrastylis* as 'the most primitive type among the present-day genera' (Munir 1978*a*, p. 414). He concluded that there were 'more or less' (*loc. cit.*) three lineages within his 'Physopsideae'; namely, (1) *Dicrastylis*, (2) *Physopsis* and *Mallophora*, and (3) *Newcastelia* and *Lachnostachys*. None of these three groups is resolved as a clade in our results. However, our results suggest that there are two lineages represented by his 'Physopsideae', with *Dicrastylis* (including *Mallophora*) distinct from the *Lachnostachys* + *Newcastelia* + *Physopsis* lineage.

Conclusions

The present study has demonstrated the utility of ITS and 3′*ndh*F in resolving the broad generic relationships within Chloantheae. In the present study, the tribe comprises several clades that generally equate to the recently realigned generic concepts of Rye (2005, 2007). The segregation of *Brachysola* from *Pityrodia* and the congeneric status of *Mallophora* with *Dicrastylis* were confirmed; however, the naturalness and relationships of sections within *Dicrastylis* remained equivocal. Further evaluation of tribal limits within this genus are currently being pursued.

The analyses rejected monophyly of *Pityrodia* (as currently defined). Even though none of these clades formed strongly supported sister relationships with other Chloantheae, enforcing a monophyletic *Pityrodia sensu lato* shows strong conflict in the data for this group. Four moderately to strongly supported clades of *Pityrodia* were recovered, with *Hemiphora* congeneric with *Pityrodia pro parte*; *P. axillaris–P. teckiana* (*Dasymalla* clade); *P. atriplicina–P. dilatata* (possibly including *P. quadrangulata–Quoya* clade); and *Pityrodia sensu stricto* clade. Circumscriptions of these three generic groups within *Pityrodia sensu lato* are being prepared, together with necessary nomenclatural changes.

A close relationship between *Newcastelia*, *Physopsis* and *Lachnostachys* was confirmed by the present study. There was a strong indication that *Physopsis* as it is currently defined might be polyphyletic with *P. lachnostachya*, nesting within a clade comprising single species of *Newcastelia* and *Lachnostachys*. Four nomenclatural options are available to resolve this situation: (1) reduce all species of *Newcastelia* and *Physopsis* to the synonomy of an enlarged *Lachnostachys*, since the latter has priority (Hooker 1842) (*Newcastelia* was published by von Mueller (1857) and *Physopsis* by Turczaninow (1849)); (2) maintain *Physopsis sensu stricto* as a distinct genus, but include *Newcastelia* and *Physopsis lachnostachya* within *Lachnostachys*; (3) recognise three genera – establish a new genus consisting of *Lachnostachys albicans*, *Newcastelia insignis* and *Physopsis lachnostachya*, re-circumscribe *Lachnostachys* to include all *Lachnostachys* except *L. albicans*, plus all *Newcastelia* except *N. insignis*, and maintain *Physopsis sensu*

stricto as a third genus; or (4) each of these four clades could be recognised as distinct genera. To ensure nomenclatural stability, we recommend that a more comprehensive sample of species from each of the genera be analysed before formal changes are made.

We believe that the present study has identified several areas for future research in which the combination of rapidly evolving molecular markers with comparative morphological data should prove to be informative. In particular, we suggest that subsequent studies should aim to elucidate further the generic delimitation of *Pityrodia* (including *Hemiphora*), with particular attention to the taxonomic affinities of *P. quadrangulata*, clarify the delimitation of sections in *Dicrastylis*, and to investigate the generic limits within the *Physopsis* + *Newcastelia* + *Lachnostachys* clade.

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