CSIRO Publishing

AUSTRALIAN SYSTEMATIC BOTANY

VOLUME 15, 2002

An international journal devoted to the taxonomy, biogeography and evolution of all plant groups

All enquiries and manuscripts should be directed to:

Australian Systematic Botany
CSIRO Publishing
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7613
Fax: +61 3 9662 7611
Email: publishing.asb@csiro.au

Published by CSIRO Publishing
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/asb

28 February 2002 *Australian Systematic Botany* **15**, 49–62

Higher-level relationships among the eucalypts are resolved by ITS-sequence data

Dorothy A. Steane[AC], *Dean Nicolle*[B], *Gay E. McKinnon*[A], *René E. Vaillancourt*[A] *and Brad M. Potts*[A]

[A]Cooperative Research Centre for Sustainable Production Forestry and School of Plant Science, University of Tasmania, GPO Box 252-55, Hobart, Tas. 7001, Australia.
[B]School of Biological Sciences, Flinders University of South Australia, GPO Box 2100, Adelaide, SA 5001, Australia.
[C]Corresponding author; Dorothy.Steane@utas.edu.au

Abstract. This expanded survey of ITS sequences represents the largest analysis of molecular data ever attempted on *Eucalyptus*. Sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA were included in an analysis of 90 species of *Eucalyptus s.s.* and 28 species representing eight other genera (*Allosyncarpia*, *Angophora*, *Arillastrum*, *Corymbia*, *Eucalyptopsis*, *Stockwellia*, *Lophostemon* and *Metrosideros*). The results of the study indicate that *Angophora* and *Corymbia* form a well-supported clade that is highly differentiated from *Eucalyptus s.s.* *Corymbia* species are divided between two clades, one of which may be the sister to *Angophora*. *Allosyncarpia*, *Arillastrum*, *Eucalyptopsis* and '*Stockwellia*' are also highly differentiated from *Eucalyptus s.s.* If the genus *Eucalyptus* is to be expanded to include *Angophora* and *Corymbia* (*sensu* Brooker 2000), ITS data suggest that *Allosyncarpia*, *Eucalyptopsis*, '*Stockwellia*' and potentially *Arillastrum* should also be included in *Eucalyptus s.l.* The ITS data suggest that subg. *Symphyomyrtus* is paraphyletic and that subg. *Minutifructus* should be included within it. Within subg. *Symphyomyrtus*, only sect. *Maidenaria* appears to be monophyletic. Sections *Adnataria* and *Dumaria* are probably monophyletic; sections *Exsertaria* and *Latoangulatae* are very close and probably should be combined in a single section. Section *Bisectae* is polyphyletic and is divided into two distinct lineages. The phylogenetic groups depicted by ITS data are consistent with the frequency of natural inter-specific hybridisations as well as data from controlled crosses within subgenus *Symphyomyrtus*. The ITS data illustrate that subg. *Idiogenes* and western Australian monocalypts are early evolutionary lines relative to *E. diversifolia*, *E. rubiginosa* (monotypic subg. *Primitiva*) and the eastern monocalypts and that subg. *Primitiva* should be sunk into subg. *Eucalyptus*. Subgenus *Eudesmia* may be monophyletic, grouping with subgenera *Idiogenes* and *Eucalyptus*. Further work is required to confirm the phylogenetic positions of the monotypic subgenera *Alveolata*, *Cruciformes*, *Acerosae* and *Cuboidea*.

Introduction

As the dominant component of Australia's forests and woodlands, eucalypts are of great importance to the environment and economy of Australia. Despite this, many issues of the taxonomy and phylogeny of the eucalypts are still not resolved. Over the last 5 years, the classification of the eucalypts has been in a state of flux and debate continues, particularly regarding the higher-level relationships between *Eucalyptus* L'Herit., *Angophora* Cav. and *Corymbia* Hill & Johnson (Brooker 2000; Ladiges and Udovicic 2000).

A recent publication by Steane *et al.* (1999) demonstrated the potential of ITS (the internal transcribed spacer of the nuclear ribosomal DNA)-sequence data to resolve higher-level relationships among the eucalypts. The study incorporated 35 species of *Eucalyptus s.s.* and seven species representing five eucalypt-like genera (*Allosyncarpia*, *Angophora*, *Arillastrum*, *Corymbia* and *Stockwellia*). The results of that study distinguished clearly between the two major subgenera of *Eucalyptus s.s.*, *Eucalyptus* (*sensu* Brooker 2000; formerly subg. *Monocalyptus*) and *Symphyomyrtus*. ITS-sequence data demonstrated the potential to resolve relationships between sections within subg. *Symphyomyrtus*, but the sampling was insufficient to accomplish this. Subgenus *Eudesmia* appeared to be paraphyletic, a result that conflicts with some morphological data that support a monophyletic *Eudesmia* (Ladiges *et al.* 1995), as well as with data presented in the present study. At the generic level, *Corymbia* was paraphyletic, but, together

 10.1071/SB00039 1030-1887/02/01049

Table 1. Sampling of taxa used in this study
Corymbia sections follow Hill and Johnson (1995); subgenera and sections of *Eucalyptus s.s*. follow Brooker (2000). The taxon codes represent the genus (*Angophora* or *Corymbia*) or subgenus (of *Eucalyptus*) and the section of each species

Taxon code	Genus	Subgenus	Section	No. of species (no. of samples)
	Allosyncarpia			1 (2)
	Arillastrum			1 (1)
	Stockwellia			1 (2)
AL	*Angophora*		*Liberia*	6 (6)
CA	*Corymbia*		*Apteria*	1 (1)
CB	*Corymbia*		*Blakearia*	2 (3)
CO	*Corymbia*		*Ochraraia*	1 (1)
CP	*Corymbia*		*Politaria*	4 (4)
CR	*Corymbia*		*Rufaria*	8 (9)
Cb	*Eucalyptus*	*Cuboidea*		1 (1)
CC	*Eucalyptus*	*Cruciformes*		1 (1)
Ac	*Eucalyptus*	*Acerosae*		1 (2)
Al	*Eucalyptus*	*Alveolata*		1 (1)
EA	*Eucalyptus*	*Eucalyptus*	*Aromatica*	11 (17)
EAm	*Eucalyptus*	*Eucalyptus*	*Amenta*	2 (2)
ECn	*Eucalyptus*	*Eucalyptus*	*Cineraceae*	4 (4)
ECp	*Eucalyptus*	*Eucalyptus*	*Capillulus*	1 (1)
ELn	*Eucalyptus*	*Eucalyptus*	*Longistyla*	5 (5)
EPd	*Eucalyptus*	*Eucalyptus*	*Pedaria*	1 (1)
EPs	*Eucalyptus*	*Eucalyptus*	*Pseudophloia*	1 (1)
I	*Eucalyptus*	*Idiogenes*		1 (2)
MD	*Eucalyptus*	*Minutifructus*	*Domestica*	1 (1)
ME	*Eucalyptus*	*Minutifructus*	*Equatoria*	1 (4)
P	*Eucalyptus*	*Primitiva*		1 (1)
SA	*Eucalyptus*	*Symphyomyrtus*	*Adnataria*	7 (7)
SB	*Eucalyptus*	*Symphyomyrtus*	*Bisectae*	10 (10)
SD	*Eucalyptus*	*Symphyomyrtus*	*Dumaria*	4 (5)
SE	*Eucalyptus*	*Symphyomyrtus*	*Exsertaria*	8 (8)
SI	*Eucalyptus*	*Symphyomyrtus*	*Inclusa*	1 (3)
SL	*Eucalyptus*	*Symphyomyrtus*	*Latoangulatae*	3 (7)
SM	*Eucalyptus*	*Symphyomyrtus*	*Maidenaria*	16 (23)
SP	*Eucalyptus*	*Symphyomyrtus*	*Platysperma*	1 (1)
SR	*Eucalyptus*	*Symphyomyrtus*	*Racemus*	1 (1)
UCm	*Eucalyptus*	*Eudesmia*	*Complanata*	1 (1)
ULm	*Eucalyptus*	*Eudesmia*	*Limbatae*	4 (4)
UR	*Eucalyptus*	*Eudesmia*	*Reticulata*	1 (1)
	Eucalyptopsis			1 (1)
	Lophostemon			1 (1)
	Metrosideros			1 (1)

with *Angophora*, formed a monophyletic group. This finding conflicted with morphological analyses by Hill and Johnson (1995) and Ladiges *et al.* (1995), all of whom favoured the hypothesis that *Corymbia* is monophyletic.

In this second study of *Eucalyptus* phylogeny with ITS-sequence data, we have extended the data set of Steane *et al.* (1999) from 42 to a total of 120 ingroup and outgroup taxa. This paper reports on the new picture of *Eucalyptus* phylogeny that is emerging from nuclear ITS-sequence data.

Nomenclature and classification in this paper largely follows Brooker (2000) for *Eucalyptus s.s*. However, we have chosen to maintain *Corymbia* (see Hill and Johnson 1995) and *Angophora* as separate genera, rather than reducing them to subgenera of *Eucalyptus* (see Ladiges and Udovicic 2000).

Materials and methods

Plant samples

The present study included a total of 147 samples (Table 1; Appendix 1, available at http://www.publish.csiro.au/journals/asb/AccessMat.cfm) representing 118 ingroup and outgroup taxa, including 54 samples from a previous study (not shown; see Steane *et al.* 1999). The sampling encompassed nine of the 15 sections of *Symphyomyrtus* circumscribed by Brooker (2000; the excluded sections are sect. *Incognitae*, sect. *Liberivalvae* and the monotypic sect. *Sejunctae*, sect. *Bolites*, sect. *Similares* and sect. *Pumilio*). The samples included 51 species of subg. *Symphyomyrtus* (including multiple samples of the following to test for within-species variation: *E. globulus*, *E. bicostata*, *E. maidenii* and *E. pseudoglobulus*—all of which make up the *E. globulus* complex, *E. grandis*, *E. urophylla*, *E. tereticornis*, *E. nitens*, *E. diversicolor* and *E. alba*); 25 species of subg. *Eucalyptus*; two representatives of the monotypic subg. *Idiogenes*; six species from subg. *Eudesmia*; one representative each of the monotypic subgenera *Alveolata*, *Cruciformes* (formerly sect. *Tingleria*, subg. *Symphyomyrtus*), *Primitiva*, *Acerosae* and *Cuboidea* [these last two previously made up subg. *Gaubaea sensu* Pryor and Johnson (1971)]; two species of subg. *Minutifructus* (previously subg. *Telocalyptus*; sampling included four representatives of *E. deglupta* from different islands); six species of *Angophora*; 16 species of *Corymbia* (with two samples of *C. ficifolia*), two samples each of *Allosyncarpia ternata* and '*Stockwellia*' (an undescribed genus from Northern Queensland) and one of *Arillastrum* (see Steane *et al.* 1999). An ITS sequence for *Eucalyptopsis papuana* was taken from GenBank (no. AF190354). *Lophostemon confertus* and *Metrosideros collina* (GenBank no. AF172739) were included as outgroup taxa.

Molecular methods

Total cellular DNA was extracted from the new specimens (i.e. those not included in the study of Steane *et al.* 1999) with DNeasy Plant Mini Kit (QIAGEN, Germany). We extracted 0.1 g of leaf tissue, which yielded from 1 to 4 μg of PCR-quality DNA. The 5.8S nrDNA and flanking ITS-1 and ITS-2 were amplified and sequenced as described previously (Steane *et al.* 1999). Most samples were amplified at an annealing temperature of 48°C, but some required annealing temperatures of 50 or 52°C in order to yield a single PCR product. Sequences were aligned against the previous alignment (Steane *et al.* 1999) by visual inspection. All sequences are lodged in GenBank (accession numbers AF390444–AF390534 and AY039752–AY039754; and see Steane *et al.* 1999). The data set is lodged in TreeBASE (study accession number S640; matrix accession numbers M995–M997).

Sequence data analysis

There were numerous small (1–4 bp) indels (insertion or deletion events), many of which were autapomorphic and were therefore uninformative. The following three larger deletions were identified: 18 bp in ITS-1 of *Stockwellia*; 8 bp in ITS-2 of *E. obliqua*, *E. regnans* and *E. pilularis*; and 16 bp in ITS-2 of one representative of *E. cloeziana* (subg. *Idiogenes*), overlapping the 8-bp deletion of *E. obliqua*, *E. pilularis* and *E. regnans*. Some of the smaller indels made sequence alignments highly ambiguous and different alignments of these regions had significant effects on cladogram topologies. Three regions of alignment ambiguity were identified in ITS-1 (regions 1–3), one towards the 3′-end of the 5.8S nrDNA (region 4) and two in ITS-2 (regions 5 and 6); these six regions were excluded from the phylogenetic analysis, leaving 581 unambiguously aligned nucleotide positions. Indels of two or more base pairs that did not fall within these

regions of alignment ambiguity—or which overlapped them—were coded as binary characters (i.e. presence/absence of nucleotides). Eleven binary characters, of which six were autapomorphic, were scored. The nucleotide data for the potentially informative gaps of more than one base pair were retained in the analysis (rather than being treated exclusively as binary characters) in order not to lose any phylogenetic information contained in the taxa that possessed nucleotides in these regions.

Many of the sequences were identical or differed only by autapomorphies. In order to simplify the data set and accelerate analyses, taxa with identical sequences (ignoring autapomorphies) were pooled into single terminal units (DIVERSICOLOR, DUM1, EUC1, EUC2, EUC3, LATO1, SYMPH1, SYMPH2, SYMPH3, STOCKWELLIA; see figure legends).

Phylogenetic analyses

Phylogenetic analyses were done according to PAUP 4.0b3 (Swofford 1999). Maximum parsimony analyses were carried out by heuristic search strategies as described by Catalán *et al.* (1997; see below).

Parsimony analysis of the full data set (*FDS*)

Lophostemon and *Metrosideros* were chosen as outgroup taxa, on the basis of previous phylogenetic studies of *Eucalyptus* (Udovicic and Ladiges 2000) and the results of a phylogenetic analysis of Myrtaceae with *mat*K sequence data (P. G. Wilson, Royal Botanic Gardens, Sydney, pers. comm.).

First, a CLOSEST stepwise addition of taxa followed by TBR branch-swapping (MULPARS) was performed saving the highest number of trees permitted by the available computer memory. Most parsimonious trees from this analysis were used to compute the strict consensus tree. A second step consisted of 1000 random addition sequences (MULTREES on) followed by TBR branch-swapping, saving a maximum of five trees per replicate with length greater than or equal to 25. The strict consensus tree from this analysis was compared with the previous one. Next, the strict consensus tree that was obtained from former searches was used as a constraint for a search of 5000 replicates of random addition sequence (TBR, MULPARS) saving no more than five trees per replicate with length greater than or equal to 25 and setting PAUP to save only trees that did not match these constraints. This search strategy was designed to ascertain that there were no shorter trees and that the strict consensus tree reflected all most parsimonious trees, even though all equal-length trees had not been found (Catalán *et al.* 1997). The data set was bootstrapped with 10 000 replicates of the 'fast, stepwise' option of PAUP 4.0b3 (see Mort *et al.* 2000).

Parsimony analysis with Arillastrum *as a functional outgroup* (*AFOG*)

The ITS sequences of *Metrosideros* and especially *Lophostemon* were noticeably different from those of the ingroup and appeared to introduce an unnecessarily high level of homoplasy into the analysis of the ITS data set. Phylogenetic analysis of the full data set, with *Lophostemon* and *Metrosideros* as the outgroup taxa, resulted in *Arillastrum* coming out as the sister taxon to all other eucalypts and eucalypt-like genera. In order to increase the resolution of relationships among the ingroup taxa, we reanalysed the data set excluding *Lophostemon* and *Metrosideros* and with *Arillastrum* as the functional outgroup. Parsimony and bootstrap analyses were conducted as described for the FDS analysis above.

Parsimony analysis of a smaller Symphyomyrtus *data set* (*SSDS*)

A smaller '*Symphyomyrtus*' data set (SSDS) comprising 62 terminal taxa was constructed. This included all representatives of *Symphyomyrtus* plus all taxa that tended to cluster with *Symphyomyrtus* in the larger analyses (*E. deglupta* and *E. brachyandra*, subg. *Minutifructus*; *E. guilfoylei*, subg. *Cruciformes*; *E. microcorys*, subg. *Alveolata*), plus potential outgroups (*E. cloeziana*, subg. *Idiogenes*; *E. curtisii*, subg. *Acerosa*; and *E. tenuipes*, subg. *Cuboidea*). By limiting the data set to very-closely related taxa, fewer ambiguities were encountered in the sequence alignment. As a result, we were able to include all of the 'ambiguous regions' that were excluded from the analyses of the larger data set, except for 9 bp at the end of Region 3. The same search strategies and bootstrapping methods were used for this data set as for the larger data sets.

Results

Sequence analysis

The ITS region in the study group varied between 609 bp in '*Stockwellia*' and 636 bp in some species of *Eucalyptus*. The total aligned length was 663 nucleotide positions. Of these, 420 were invariant and 79 were autapomorphic. Of the 164 potentially phylogenetically informative single nucleotide characters (i.e. varied in two or more taxa), 36 were excluded due to ambiguities in the alignments. Of the additional 12 gap characters (of ≥2 bp) that were identified, one was excluded due to ambiguity in the alignments and six were autapomorphic. In all, there were 128 nucleotide characters (*c*. 19%) and five gap characters that were potentially phylogenetically informative and included in the analyses. Seventy-four autapomorphic nucleotide characters and six autapomorphic gap characters were added to phylograms of the FDS after phylogenetic analysis (five autapomorphic nucleotide characters occurred in regions of ambiguity and were therefore not added). In the AFOG analysis there were 121 potentially informative characters, of which five were gap characters; 63 autapomorphic nucleotide characters and four autapomorphic gap characters were added to the phylograms after phylogenetic analysis. The SSDS contained 65 potentially informative characters, 54 autapomorphic and 540 constant characters.

The percentage nucleotide-difference values (excluding ambiguous regions) showed a large range of sequence divergence from 0% between some congeneric species to 10% between species of *Eucalyptus s.s.* and either *Angophora* or *Corymbia*. Most pairwise distances were below 3% within the major clades discussed below, but were up to 4.5% between those clades. Percentage nucleotide-difference values between *Lophostemon* and ingroup taxa ranged from 9 to 13%; those between *Metrosideros* and ingroup taxa ranged from 7 to 12%. The ITS sequences were characterised by unequal nucleotide frequencies with a G–C bias (A = 0.19, C = 0.40, G = 0.21, T = 0.20) and a transition/transversion bias of 2.76 (estimated via maximum likelihood by the HKY-85 model; Hasegawa *et al.* 1985).

Phylogenetic analyses

Parsimony analysis of the full data set (using CLOSEST addition sequence) recovered 15 000 equally parsimonious trees [length excluding autapomorphies, (l) = 438, including autapomorphies (l_a) = 516; consistency index, excluding

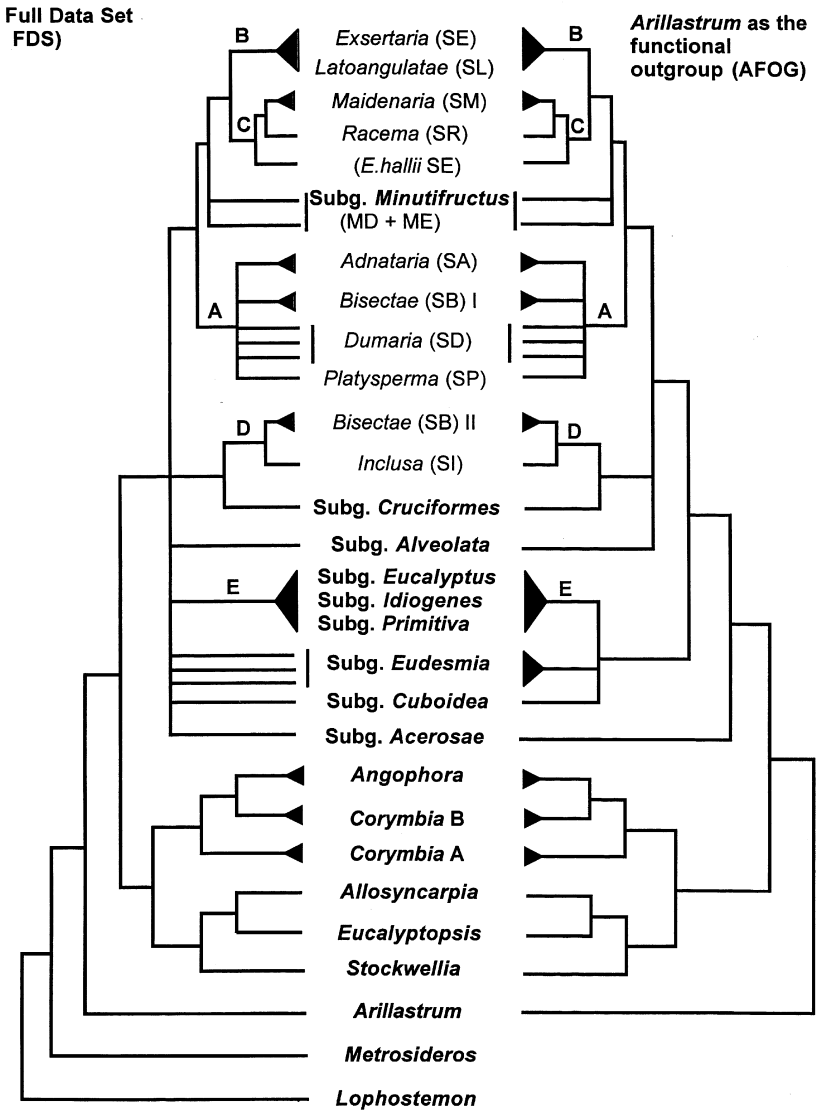

Fig. 1. Simplified strict consensus cladograms of the parsimony analyses of the full data set (FDS) and the data set that used *Arillastrum* as the functional outgroup (AFOG). The FDS consensus represents 18695 trees, (length including autapomorphies, l_a = 516; CI, excluding autapomorphies = 0.44, RI = 0.88). The AFOG consensus summarises 15920 trees (l_a = 454; CI = 0.45; RI = 0.89). Clades A–E represent groupings of species that are stable across analyses (see text). '*Corymbia* A' and '*Corymbia* B' represent two distinct lineages found within *Corymbia* (see Fig. 2). Triangles at the ends of branches represent clades comprising multiple terminal taxa. Vertical bars at terminals indicate polyphyletic or paraphyletic assemblages. The letters in parentheses represent taxon codes (Table 1).

autapomorphies (CI) = 0.438; retention index (RI) = 0.876]. A simplified strict consensus tree is shown in Fig. 1. The second analysis conducted to find additional trees yielded a total of 3695 trees of length 438 (excluding autapomorphies). The strict consensus tree of this second set of trees was the same as the former one (Fig. 1). When this consensus tree was used as a negative constraint for a third analysis of 1000 random addition sequence replicates, the search found 12650 trees of length 439 (CI = 0.437, RI = 0.875) and did not find any tree of the shortest length. Therefore, it is unlikely that there are shorter trees for the analysis.

A phylogram of one of the trees from the FDS is shown in Fig. 2. It shows the taxa divided between three main clades, although only two of these have strong bootstrap support (Fig. 2). *Eucalyptus s.s.* forms a well-supported, monophyletic group (branch length = 15, bootstrap support = 96%). Sister to *Eucalyptus s.s.* is a clade comprising *Angophora*, *Corymbia*, *Allosyncarpia*, *Eucalyptopsis* and *Stockwellia*. Within this, is a well-supported clade

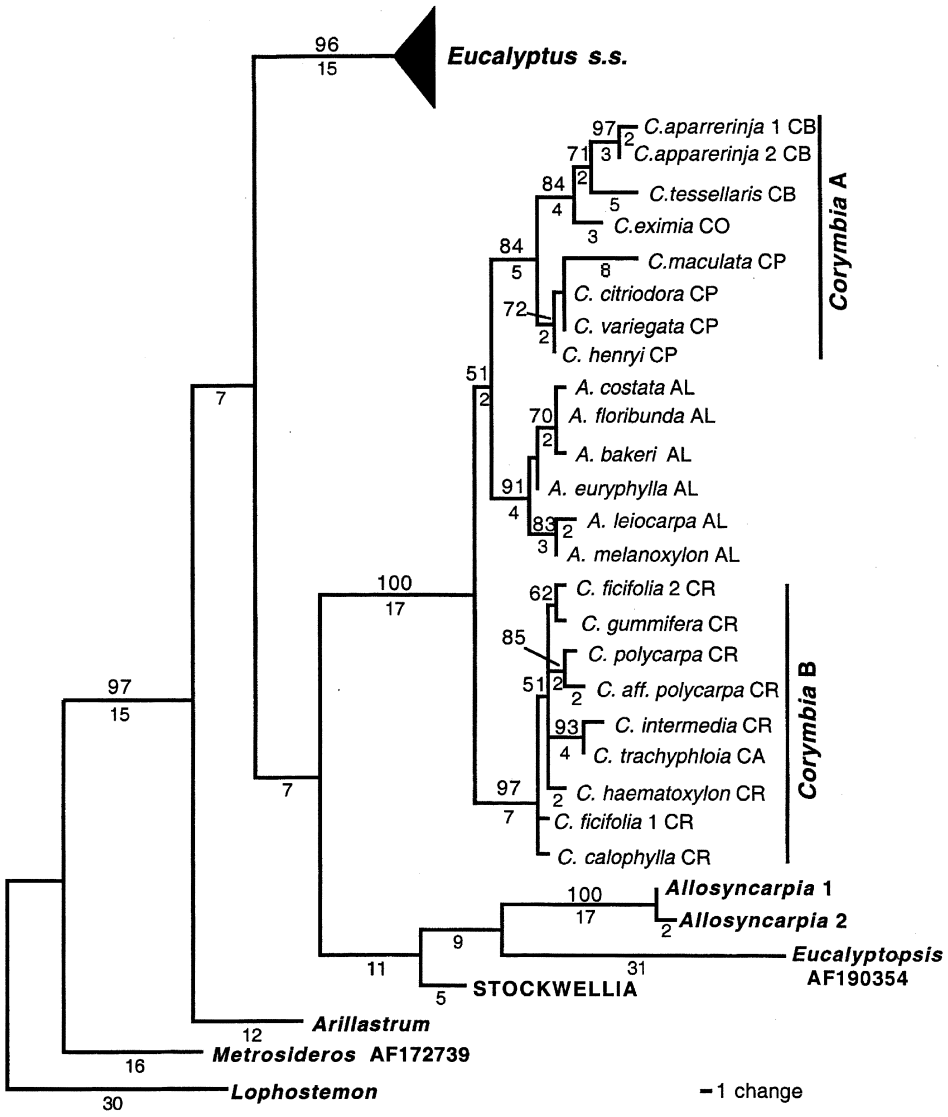

Fig. 2. Phylogram of one of the 18695 FDS cladograms, detailing relative positions of *Allosyncarpia*, *Arillastrum*, *Angophora*, *Corymbia*, *Eucalyptopsis* and '*Stockwellia*' relative to *Eucalyptus s.s.*, when rooted on *Lophostemon* (see text). Clades representing '*Corymbia* A' (Clade A) and '*Corymbia* B' (Clade B) are indicated. Branch lengths are shown below branches; numbers are not shown when branch length = 1. Bootstrap percentages greater than 50% are shown above branches. Letters following species names represent taxon codes (Table 1). The triangle leading to *Eucalyptus s.s.* represents multiple terminal taxa within that clade. STOCKWELLIA—two samples of '*Stockwellia*' had identical ITS sequences and were reduced to a single operational taxonomic unit. Tree topology, branch lengths and bootstrap support from the AFOG analysis are very similar to those in this figure.

comprising *Angophora* and *Corymbia* (branch length = 17; bootstrap support = 100%) and a clade comprising *Allosyncarpia*, *Eucalyptopsis* and *Stockwellia*. The latter clade, while it has good branch support (11 steps), has poor bootstrap support (<50%; Fig. 2). The sister group relationship between this last clade and *Angophora* + *Corymbia* has moderate branch support (seven steps) but poor bootstrap support (<50%). The analysis of the full data set places *Arillastrum* near the base of the cladogram, as sister to all other eucalypts and eucalypt-like taxa. Although bootstrap support for this placement is poor, branch support is strong (15 steps; Fig. 2).

The second analysis (AFOG) attempted to reduce the level of homoplasy of some characters by removing the relatively divergent outgroup taxa, *Lophostemon* and *Metrosideros*, and running an analysis with *Arillastrum* as the functional outgroup. Parsimony analysis of the AFOG data set yielded 15000 (CLOSEST addition sequence) and 920 (RANDOM addition sequence) equally most parsimonious trees (l = 385, l_a = 454; CI = 0.447, RI =

0.888). The constrained analysis found 13225 trees of length 386 and did not find any trees of the shortest length. The strict consensus of the AFOG analysis is shown in Fig. 1. The main difference between the results of the FDS and AFOG analyses was increased resolution within *Eucalyptus s.s.* by the latter. Whereas the FDS analysis did not resolve relationships between species of subgenera *Eudesmia*, *Cuboidea*, *Acerosae* and a clade comprising subgenera *Eucalyptus*, *Idiogenes* and *Primitiva* (Clade E; Fig. 1), the AFOG analysis identified subg. *Eudesmia* as a monophyletic group, in a clade with subg. *Cuboidea* + Clade E. The AFOG analysis found subg. *Acerosae* to be sister to the rest of *Eucalyptus s.s.* The bootstrap values, however, were essentially the same for both analyses and within *Eucalyptus s.s* these were generally very low. Because the AFOG analysis gave better resolution of clades within *Eucalyptus s.s.*, discussion will focus on those results.

The *Angophora–Corymbia* clade divides into the following three distinct groups (Fig. 2): *Angophora*; '*Corymbia* A' (the yellow bloodwoods, sect. *Ochraria*; the paper-fruited bloodwoods or ghost gums, sect. *Blakearia*; and the spotted gums, sect. *Politaria*); and '*Corymbia* B' [the red bloodwoods, sect. *Rufaria*; and the brown bloodwood (*C. trachyphloia*), monotypic sect. *Apteria*]. The results from ITS-sequence analysis suggest that *Angophora* groups with *Corymbia* A and therefore *Corymbia* is paraphyletic. However, bootstrap support (51%) and branch support (2 steps) for this are low (Fig. 2).

Within *Eucalyptus s.s.*, subg. *Acerosa* is the sister group to the rest (Figs 1, 3). Subgenera *Cuboidea* and *Eudesmia* group with subgenus *Eucalyptus*. This association, however, is supported by only a single character (Fig. 4) and bootstrap support is low (<50%; Fig. 3). The number of representatives of subg. *Eudesmia* was increased from two in our original study (Steane *et al.* 1999) to six in this study and together form a monophyletic group, but again with poor (<50%) bootstrap support.

The two major subgenera of *Eucalyptus s.s.*, *Eucalyptus* and *Symphyomyrtus*, appear to be separate from one another (Figs 1, 4), although neither subgenus appears to be monophyletic (Fig. 4). Subgenus *Eucalyptus* (taxon codes beginning with 'E') is closely associated with subg. *Idiogenes* (*E. cloeziana*) and subg. *Primitiva* (*E. rubiginosa*). The bootstrap support for this grouping is <50% (Fig. 3) but the branch support is relatively strong (Fig. 4). Therefore, the isolation by Brooker (2000) of *E. rubiginosa* in a monotypic subgenus has rendered subg. *Eucalyptus* paraphyletic. The situation with subg. *Idiogenes* is less clear, because ITS data suggest that different populations of *E. cloeziana* may not be monophyletic (Figs 3, 4). Further data are required to determine whether subgenus *Idiogenes* is monophyletic and if so, whether it is the sister group to subg. *Eucalyptus*, or whether it arises from within subg. *Eucalyptus*, as shown in Fig. 4. If both subgenera *Primitiva* and *Idiogenes* arise from within subg. *Eucalyptus*, then subg. *Eucalyptus* is polyphyletic.

Subgenera *Minutifructus*, *Cruciformes* and possibly *Alveolata* appear to arise from within subg. *Symphyomyrtus* (Fig. 1), but the bootstrap support for the clade comprising these four subgenera is low (<50%; Fig. 5). The situation is clarified by the SSDS analysis (Fig. 5). Rooting on subg. *Acerosae*, subg. *Idiogenes* or subg. *Cuboidea* (any of which could represent a sister group to the clade comprising *Symphyomyrtus* plus the three smaller subgenera) resulted in *E. microcorys* (subgenus *Alveolata*) emerging as the sister taxon to *Symphyomyrtus* + *Minutifructus*, with subgenus *Cruciformes* (*E. guilfoylei*) as the next successive sister taxon. Thus, although all analyses indicate that subg. *Minutifructus* arises from within subg. *Symphyomyrtus* (albeit with low bootstrap support) and therefore is ranked incorrectly, the other small monotypic subgenera (*Alveolata*, *Cruciformes*, *Cuboidea* and *Acerosae*) may well be justifiable at that rank (Fig. 6). Subgenus *Symphyomyrtus* is, therefore, paraphyletic.

The sections of subg. *Symphyomyrtus* form four clades (A–D; Fig. 1). Clade A consists of sections *Adnataria* (code SA), *Dumaria* (code SD) and part of sect. *Bisectae* (code SB). *Adnataria* is monophyletic in FDS and AFOG analyses (Fig. 1), but the positions of *E. microtheca* and *E. melliodora* become equivocal in analysis of the SSDS (Figs 5, 6). Relationships between the species of sect. *Dumaria* remain unresolved in all analyses. Section *Bisectae* is polyphyletic: it is divided into two groups, *Bisectae* I, in Clade A and *Bisectae* II, in clade D (Fig. 1). *Bisectae* I (*E. cornuta*, *E. dundasii*, *E. spathulata* and *E. wandoo*) is monophyletic (Fig. 5).

Clade B comprises an amalgamation of sections *Latoangulatae* (code SL) and *Exsertaria* (code SE; except for *E. hallii* which occupies an anomalous basal position in Clade C; Figs 1, 5). Neither *Latoangulatae* nor *Exsertaria* appears to be monophyletic on the basis of the ITS-sequence data (Fig. 5).

Clade C comprises sect. *Maidenaria* (code SM), monotypic sect. *Racemus* (code SR) and an anomalous placement of *E. hallii* (sect. *Exsertaria*; Figs 1, 5). Section *Maidenaria* appears to be monophyletic in all analyses, but with poor support (bootstrap values: FDS = 54%, AFOG = 53%, SSDS = 63%). There is little sequence variation within sect. *Maidenaria* and in some cases (e.g. the *E. globulus* complex and *E. nitens*) within-species (complex) divergence exceeds between-species divergence. Furthermore, neither species nor series are resolved into monophyletic groups (Figs 5, 6). A similar scenario occurs in section *Latoangulatae* (Clade B), where we included several replicates of *E. grandis* and *E. urophylla*.

Clade D consists of sections *Inclusae* (*E. diversicolor*) and *Bisectae* II (*E. balladoniensis*, *E. brockwayi*, *E. delicata*, *E. falcata*, *E. optima*, *E. pachyphylla* and *E. salmonophloia*).

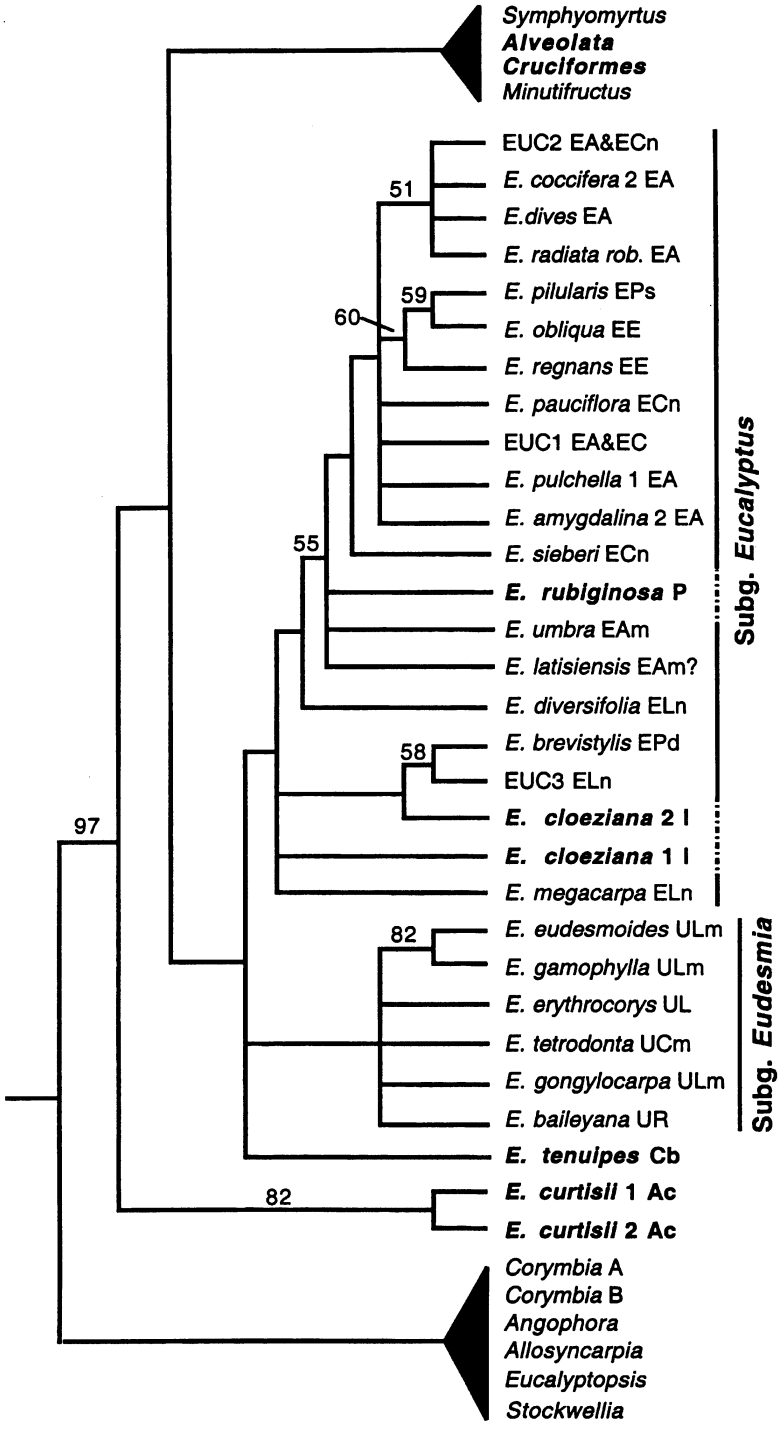

Fig. 3. Summary of strict consensus cladograms of the AFOG analysis, detailing positions of subgenera *Eudesmia*, *Eucalyptus*, *Idiogenes*, *Primitiva*, *Acerosa* and *Cuboidea*. Numbers above branches indicate bootstrap values where these are >50%. EUC1—*E. amygdalina* 1, *E. coccifera* 1, *E. coccifera* 3, *E. croajingolensis*, *E. elata*, *E. piperita*, *E. tindaliae*, *E. pulchella* 2, *E. risdonii*, *E. tenuiramis* 1 and 2, *E. willisii* ssp. *falciformis*, *E. willisii* ssp. *willisii*; EUC2—*E. delegatensis*, *E. nitida*; EUC3—*E. jacksonii*, *E. staeri*, *E. marginata*. Letters following species names represent taxon codes (Table 1). Monotypic subgenera are in bold type. The triangle represents a clade of numerous terminal taxa. Dashed line indicates taxa that are not formally classified in subg. *Eucalyptus* (Brooker 2000).

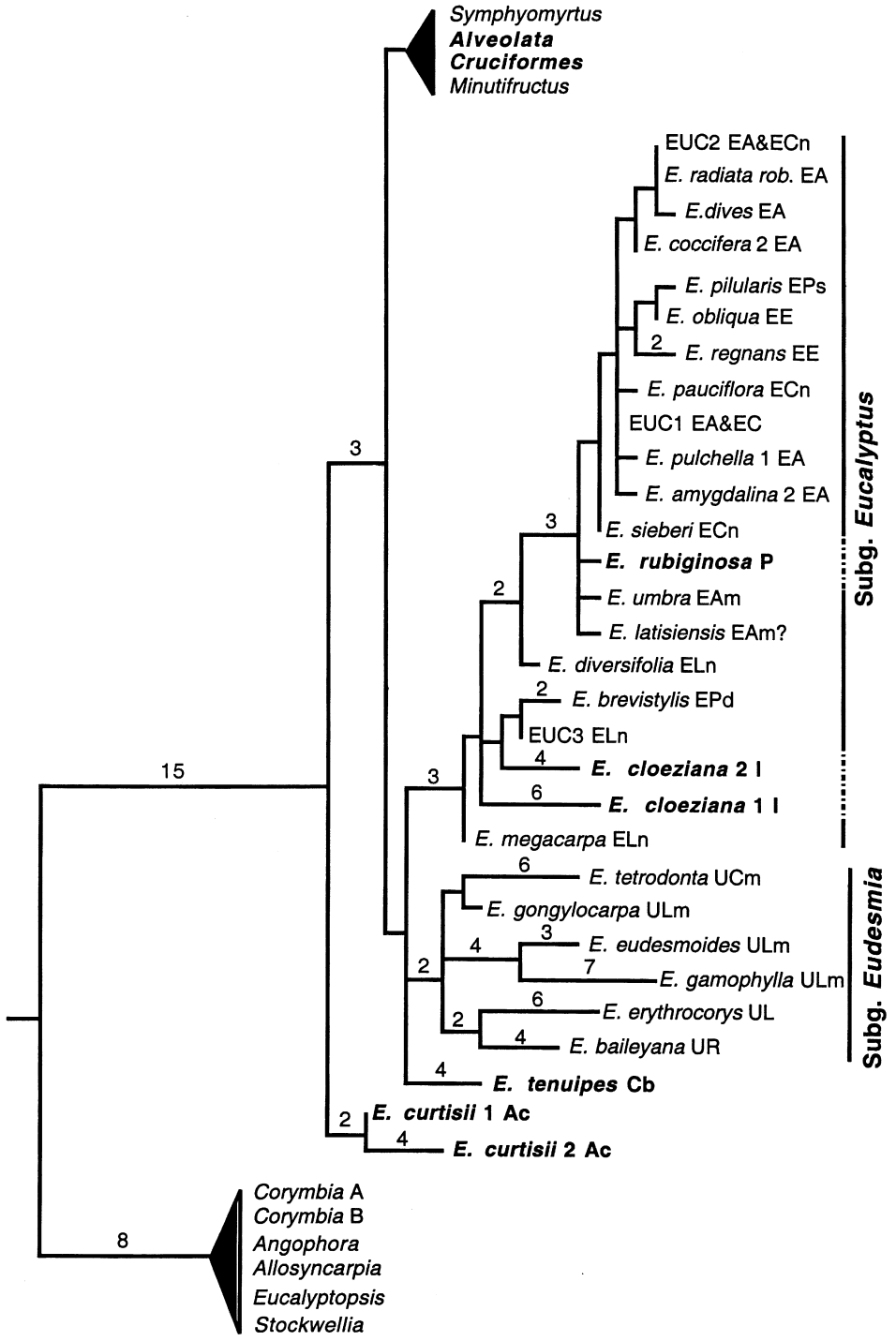

Fig. 4. Phylogram of one of the 15920 cladograms from the AFOG analysis, detailing positions of subgenera *Eudesmia*, *Eucalyptus*, *Idiogenes*, *Primitiva* and *Acerosa*. See legend to Fig. 3 for details of abbreviations and symbols. Numbers above branches represent branch lengths; numbers are not shown when branch length = 1. Letters following species names represent taxon codes (Table 1). Dashed line indicates taxa that are not formally classified in subg. *Eucalyptus* (Brooker 2000).

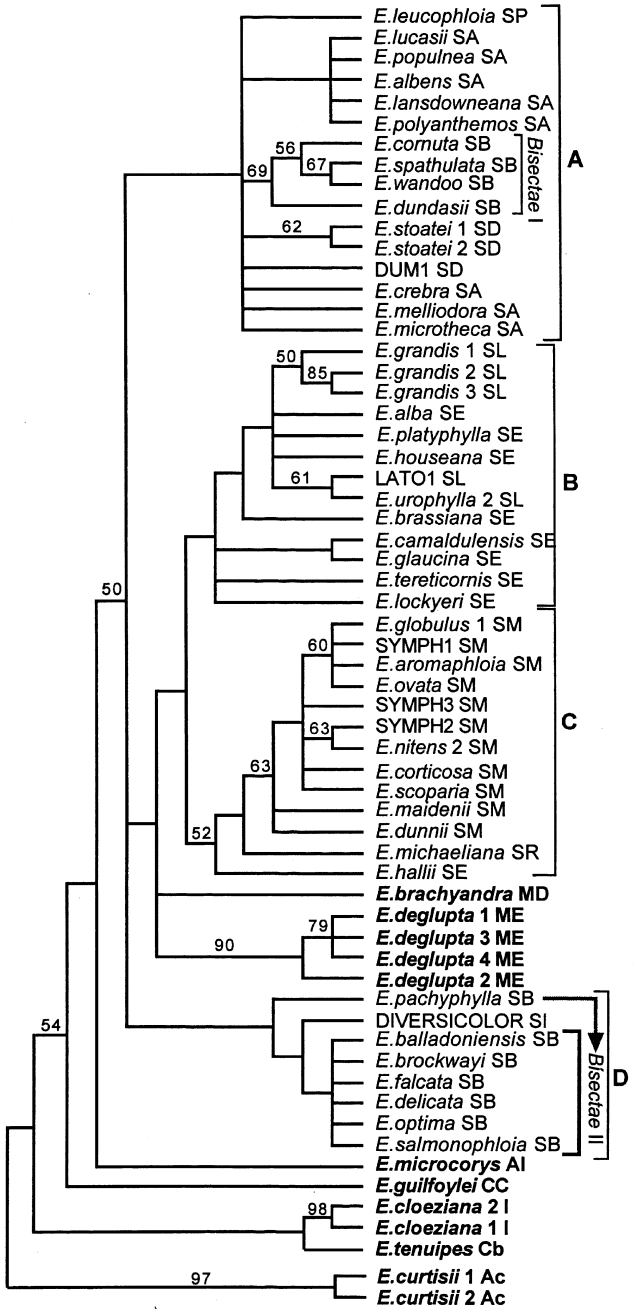

Fig. 5. Strict consensus of 25 180 equally parsimonious cladograms (l_a = 250; CI = 0.449; RI = 0.796) from the SSDS analysis, indicating the stable Clades, A–D. Non-*Symphyomyrtus* taxa are shown in bold type. Bootstrap percentages are shown above branches. *Bisectae* I and *Bisectae* II are shown. Taxonomic codes following species names are detailed in Table 1. DUM1—*E. woodwardii*, *E. torquata*, *E. obtusiflora*; LATO1—*E. urophylla* 1, *E. urophylla* 3, *E. pellita*, *E. wetarensis*; SYMPH1—*E. gunnii* 1, *E. gunnii* 2, *E. perenniana* 1, *E. perenniana* 2, *E. dalrympleana*; SYMPH2—*E. nitens* 1, *E. nitens* 3, *E. pseudoglobulus*, *E. globulus* 2; SYMPH3—*E. globulus* 3, *E. bicostata*; DIVERSICOLOR—*E. diversicolor* 1, 2, 3. Letters following species names represent taxon codes (see Table 1).

Unlike *Bisectae* I, *Bisectae* II is not monophyletic; it is rendered paraphyletic by the exclusion of *E. diversicolor* (sect. *Inclusae*).

The monophyly of subgenus *Minutifructus* is not supported (taxon codes starting with 'M'; Figs 1, 5). However, it appears that *E. brachyandra* and *E. deglupta* are excluded from Clades B + C.

Discussion

The new classification of the eucalypts by Brooker (2000) has added fuel to debate about whether *Angophora* and *Corymbia* should be included in *Eucalyptus s.l.* and whether *Corymbia* is monophyletic. Questions have also been raised about the monophyly of some of the subgenera of *Eucalyptus* (e.g. subgenera *Eucalyptus*, *Symphyomyrtus*, *Eudesmia*, *Minutifructus*), as well as some sections within *Symphyomyrtus*. Expanding our earlier analysis (Steane *et al.* 1999) with 68 new species has helped to resolve some of the outstanding issues of eucalypt systematics.

The AFOG analysis was rooted on *Arillastrum* on the basis of the results of the FDS analysis. However, phylogenetic studies of eucalypts on the basis of chloroplast DNA (Udovicic and Ladiges 2000; S. Whittock, University of Tasmania, unpubl. data) suggest that the clade comprising *Allosyncarpia*, *Eucalyptopsis* and '*Stockwellia*' is sister group to the rest of the eucalypt genera, including *Arillastrum*. Rerooting the AFOG strict consensus (Fig. 1) on this clade had little effect on the topology of the cladogram, except that *Arillastrum* emerges as sister group to *Eucalyptus s.s.*

Our ITS results have fortified the argument against the lumping of *Angophora* and *Corymbia* into the genus *Eucalyptus* and the demotion of each of these to subgeneric level, as advocated by Brooker (2000), without due consideration being given to the relationships of *Allosyncarpia*, *Arillastrum*, *Eucalyptopsis* and '*Stockwellia*' to *Eucalyptus*. As expounded by Ladiges and Udovicic (2000), Brooker's amalgamation of the three genera is 'puzzling', to say the least. Our data, and recent work by Udovicic and Ladiges (2000), emphasise the intricate relationships between the four small genera, '*Stockwellia*' ('Myrtaceae sp.' in Udovicic and Ladiges 2000), *Allosyncarpia*, *Arillastrum*, *Eucalyptopsis* and the larger taxa, *Angophora*, *Corymbia* and *Eucalyptus*. Clearly, if one is to include *Angophora* and *Corymbia* in *Eucalyptus*, then *Allosyncarpia*, *Eucalyptopsis*, '*Stockwellia*' and probably *Arillastrum* (Fig. 2) should also be included. However, Fig. 2 highlights the highly divergent nature of these genera. Long branch lengths distinguish *Angophora* + *Corymbia*, *Allosyncarpia*, *Eucalyptopsis*, '*Stockwellia*' and *Allosyncarpia* from *Eucalyptus s.s.* and from each other. Within *Eucalyptus s.s.*, branch lengths are relatively short, even between the major subgenera, *Symphyomyrtus* and *Eucalyptus* (Fig. 4). Thus, a substantial case could be made

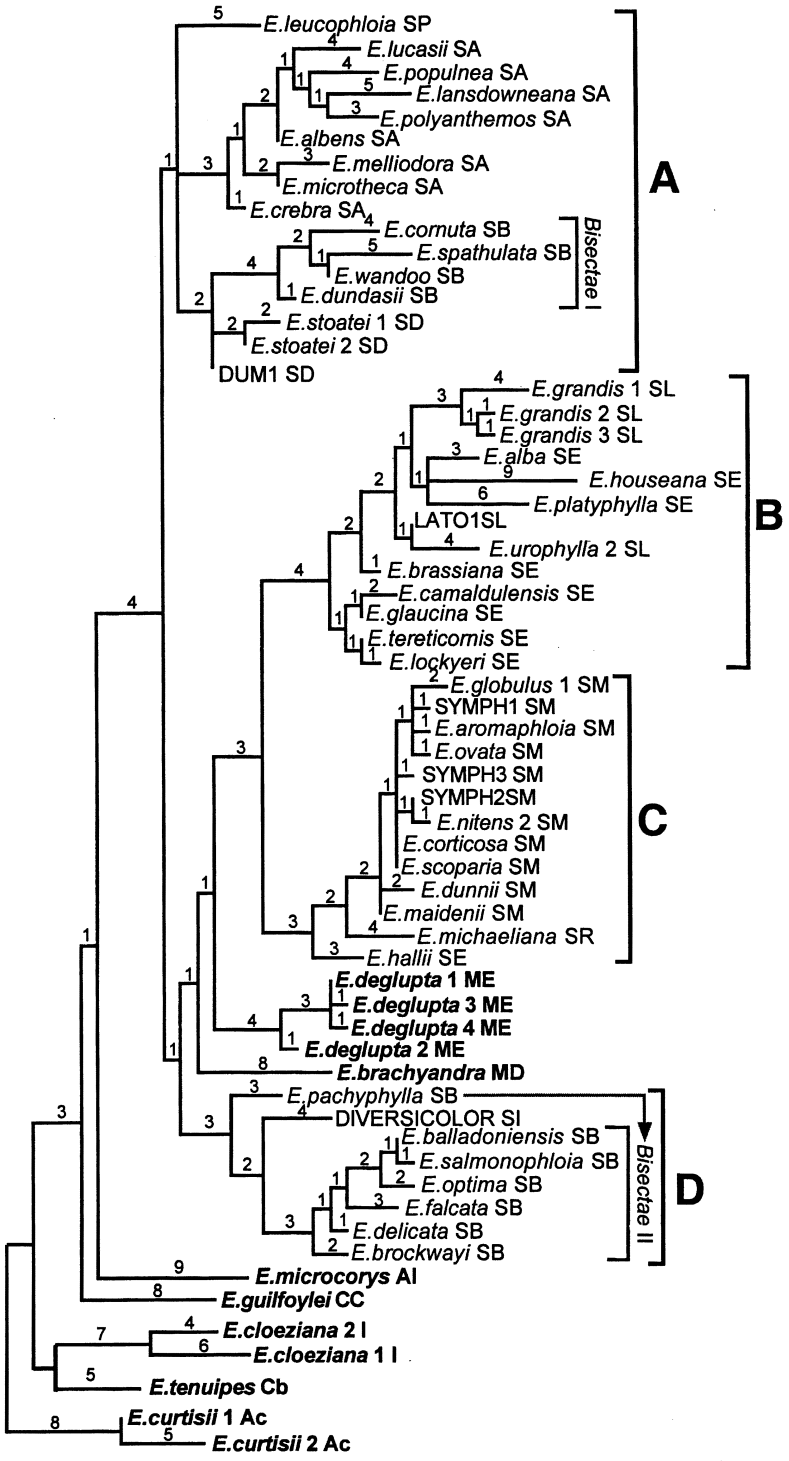

Fig. 6. Phylogram of one of the 25 180 most parsimonious trees obtained from analysis of the SSDS. Clades A–D are indicated. *Bisectae* I and *Bisectae* II are shown. Non-*Symphyomyrtus* taxa are in bold type. Branch lengths are shown above branches. See legend to Fig. 5 for explanation of capitalised terminal taxa. Letters following species names represent taxon codes (Table 1).

for maintaining *Allosyncarpia*, *Arillastrum*, *Angophora* + *Corymbia*, *Eucalyptopsis*, '*Stockwellia*' and *Eucalyptus s.s.* as separate genera.

Angophora and *Corymbia* together form a clade that is well supported by molecular (Figs 1, 2; Udovicic *et al.* 1995; Wilson *et al.* 1999; Udovicic and Ladiges 2000) and morphological (Hill and Johnson 1995) data. Within this alliance are the following three groups: *Angophora* and two groups of *Corymbia*. Following the classification of Hill and Johnson (1995), '*Corymbia* A' comprises the yellow bloodwoods (sect. *Ochraria*), the paper-fruited bloodwoods or ghost gums (sect. *Blakearia*) and the spotted gums (sect. *Politaria*); '*Corymbia* B' comprises the red bloodwoods (sect. *Rufaria*) and the brown bloodwood (*C. trachyphloia*, sect. *Apteria*). These two groups within *Corymbia* are well supported by morphological data (Hill and Johnson 1995), as well as chloroplast-DNA restriction-site data (Sale *et al.* 1993; 1996) and 5S nuclear ribosomal-DNA data (Udovicic *et al.* 1995). The three clades within the *Angophora–Corymbia* alliance have not yet been recognised in a formal taxonomic treatment of the eucalypts. Additional work involving wider sampling among the seven sections (Hill and Johnson 1995) of *Corymbia* is required to further circumscribe these groups.

Increased sampling of *Corymbia* and *Angophora* did not resolve conclusively whether or not *Angophora* is derived from within *Corymbia* (as indicated by Steane *et al.* 1999). As before (Steane *et al.* 1999), the ITS results show that *Angophora* is sister group to *Corymbia* A. Despite low bootstrap and branch support in this analysis, the same relationship has been suggested in analyses of cpDNA data (Udovicic and Ladiges 2000; Whittock 2000). However, the relationship between *Angophora* and the two groups of *Corymbia* is not consistent among data sets (Fig. 1; Udovicic *et al.* 1995; Udovicic and Ladiges 2000) and should be treated as uncertain.

Subgenus *Eudesmia* is a diverse and somewhat heterogeneous assemblage, generally acknowledged to contain some of the most primitive of the non-bloodwood eucalypts (see Ladiges 1997) on the basis of both morphological and molecular data. Our ITS data identified the eudesmids to be a monophyletic group (Fig. 3), albeit with little bootstrap or character support. The eudesmid clade emerged at the base of a clade comprising subgenera *Cuboidea*, *Idiogenes*, *Primitiva* and *Eucalyptus*. The association of eudesmids with subgenera *Idiogenes* and *Eucalyptus* parallels results of Udovicic and Ladiges (2000) in analyses of their 5S rDNA, ITS and *psb*A–*trn*H data sets.

Subgenera *Eucalyptus* (the 'monocalypts'), *Idiogenes* and *Primitiva* together form a monophyletic group. In our analysis, the two representatives of *E. cloeziana* (the only species in subgenus *Idiogenes*) had different DNA sequences (pairwise similarity = 0.92). *Eucalyptus cloeziana* has quite a wide distribution in Queensland and is known to hybridise with *E. acmenoides* (Stokoe *et al.* 2001). It is possible that the sequence divergence observed here is due either to the effects of population subdivision or to interspecific hybridisation. In our phylogenetic analyses, *E. cloeziana* 2 groups with the Western Australian monocalypts. It has a relatively long terminal branch (with no autapomorphies), suggesting that this grouping is probably an artefact of long-branch attraction (Hendy and Penny 1989). The position of the other sample of *E. cloeziana* (1) is basal in the monocalypt clade, but not resolved from the Western Australian monocalypts.

Eucalyptus rubiginosa (subgenus *Primitiva*) is generally thought to be primitive among the monocalypts (Ladiges 1997). In our analysis, *E. rubiginosa* appears to be more-closely related to the eastern monocalypts than the Western Australian monocalypts (e.g. *E. megacarpa*, *E. brevistylis*, *E. diversifolia*, *E. jacksonii*, *E. marginata* and *E. staeri*). The majority of the Western Australian monocalypts (*E. brevistylis*, *E*, *jacksonii*, *E. marginata*, *E. staeri*) are basal to the eastern Australian monocalypts. The position of *E. megacarpa* remains unresolved: the parsimony analyses places it at the base of the monocalypt clade with the remaining western taxa, the eastern taxa and subg. *Idiogenes*. Interestingly, *E. diversifolia*, the one species of subg. *Eucalyptus* that extends from western Victoria into eastern Western Australia (Brooker and Kleinig 1990), is the sister to all eastern species of subg. *Eucalyptus* and subgenus *Primitiva* and appears to bridge the geographic and phylogenetic gap between west and east.

The tropical boxes belonging to subg. *Minutifructus* (formerly subg. *Telocalyptus*) appear, from ITS data, to be nested within subgenus *Symphyomyrtus*. Similar results were obtained by Sale *et al.* (1993, 1996) and Udovicic and Ladiges (2000) also reported a close relationship between the two taxa. Such data indicate that *Minutifructus* should not be maintained as a subgenus. Furthermore, the monophyly of this taxon has yet to be confirmed. No molecular studies (Sale *et al.* 1993, 1996; this study) have supported a monophyletic relationship among the species of the subgenus. The grouping may, in fact, represent a polyphyletic assemblage of highly divergent mono-specific lineages (e.g. see Fig. 6). However, because the sampling of the subgenus in molecular studies has been limited (with two species being the maximum in any one study), greater sampling is essential to resolve this issue.

Symphyomyrtus is the largest subgenus of *Eucalyptus*, comprising several hundred species that occur in all states of Australia as well as in New Guinea, Timor and associated islands. Most of these species fall into the sections *Latoangulatae*, *Bisectae*, *Dumaria*, *Exsertaria*, *Maidenaria* and *Adnataria*. Of these, *Bisectae* is the largest and is predominantly distributed in south-western Australia with several representatives in northern and eastern Australia. It is also probably the most taxonomically interesting group in our

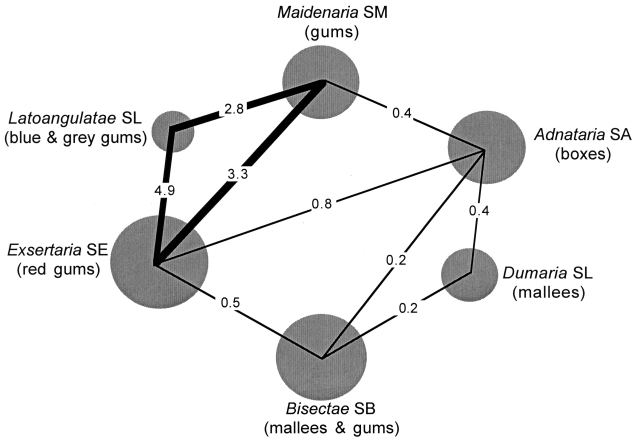

Fig. 7. Occurrence of natural inter-sectional hybrids within *Symphyomyrtus* (data from Griffin *et al.* 1988). Only 40 natural inter-sectional hybrids were reported by Griffin *et al.* (1988) in *Symphyomyrtus*. The figure shows the relative frequency of natural inter-sectional hybrids as a percentage of the number of inter-sectional combinations possible amongst proximal species (with 10 × 10-min area). The area of the circle indicates the number of species in each section.

analysis, because, on the basis of ITS sequence data, it is divided into two groups that appear to be quite unrelated. The first group, '*Bisectae* I', includes *E. cornuta*, *E. spathulata*, *E. dundasii* and *E. wandoo* and appears to be related to sections *Dumaria* and *Adnataria* and the small sect. *Platysperma* (e.g. *E. leucophloia*). The ITS data suggest further that '*Bisectae* II' (*E. pachyphylla*, *E. balladoniensis*, *E. brockwayi*, *E. falcata*, *E. delicata*, *E. optima* and *E. salmonophloia*) is a non-monophyletic group closely associated with *E. diversicolor* (sect. *Inclusae*). This subdivision of *Bisectae* into two groups is correlated with several distinguishing morphological features associated with peduncles, fruit, pith glands and stamen arrangement (Table 2). In addition, most members of *Bisectae* I are endemic to Western Australia, while *Bisectae* II is a more widespread group with fewer Western Australian endemic taxa.

Eucalyptus diversicolor (Karri, restricted to a small region of south-western Western Australia) was placed by Pryor and Johnson (1971) and Brooker and Kleinig (1990) in sect. *Transversaria* (now sect. *Latoangulatae*), as the only Western Australian species of this otherwise eastern section. Recently, however, Brooker (2000) placed *E. diversicolor* in a separate, but closely related, section, *Inclusae*. We included multiple samples of *E. diversicolor* specifically to resolve the affinities of this taxon. All of the ITS sequences were identical. Our data indicate that *E. diversicolor* is not closely related to sect. *Latoangulatae*, but is monophyletic with *Bisectae* II. Although there is reasonably good branch-length support for this relationship (Fig. 6) bootstrap support is low (<50%).

The phylogenetic relationships among the sections of *Symphyomyrtus* that have been revealed by ITS-sequence data correlate well with records of naturally occurring inter-sectional hybrids. Most naturally occurring inter-sectional crosses that have been recorded have been between sections *Latoangulatae*, *Exsertaria* and *Maidenaria* (Fig. 7; D. Nicolle, unpubl. data), with the highest frequency occurring between *Latoangulatae* and *Exsertaria*. This corresponds to the grouping of these two sections together (Clade B), with sect. *Maidenaria* in the sister group (Clade C). The division of section *Bisectae* into two distinct groups is also supported by hybridisation data. In the crossing experiments of Ellis *et al.* (1991), the percentage of ovule penetration by interspecific pollen was much greater in crosses involving species that belong to the same intra-sectional group of *Bisectae* (i.e. within *Bisectae* I or within *Bisectae* II; species were assigned to *Bisectae* I or II on morphological characters, Table 2) than in crosses across those two groups. Furthermore, two surveys of records of natural and cultivated interspecific hybridisations (Griffin *et al.* 1988; D. Nicolle, unpubl. data) involving species of sect. *Bisectae* are congruent with this pattern, recording a total of 76 *Bisectae* I hybrids, 58 *Bisectae* II hybrids and only two *Bisectae* I × *Bisectae* II (natural) combinations. Clearly, it appears that *Bisectae* I and *Bisectae* II have some sort of reproductive barrier operating between them. In addition, the studies of Ellis *et al.* (1991) suggested that ovule penetration by interspecific pollen was greater in crosses between *Bisectae* I and representatives of section *Adnataria*, than between *Bisectae* II and *Adnataria*. This observation supports the hypothesis that *Bisectae* I is phylogenetically closer to *Adnataria* (Clade A) than it is to *Bisectae* II (Figs 5, 6).

Eucalyptus michaeliana (monotypic sect. *Racemus*) is a taxonomically isolated species, restricted to south-eastern Queensland and New South Wales, with no obvious close relatives (Brooker and Kleinig 1994), consistent with the

Table 2. Distinguishing features of two groups of section *Bisectae*
'Bisectae I' includes *E. cornuta*, *E. spathulata*, *E. wandoo*, *E. dundasii*; 'Bisectae II' includes *E. pachyphylla*, *E. balladoniensis*, *E. brockwayi*, *E. falcata*, *E. delicata*, *E. optima* and *E. salmonophloia*

	Bisectae I	*Bisectae* II
Penduncles	Often distally broadened and flattened	Terete
Fruit	Usually longer than wide	As wide as, or wider than, long
Pith glands	Usually present	Always absent
Stamen orientation in bud	Usually erect	Usually inflexed or variously flexed

relatively long branch observed in our analysis (Fig. 6). Brooker (2000) placed *E. michaeliana* close to *E. diversicolor* (sect. *Inclusae*), but our results consistently place it as sister taxon to section *Maidenaria*.

Eucalyptus microcorys was treated by Pryor and Johnson (1971) as a member of subg. *Symphyomyrtus* (sect. *Sebaria*, series *Microcorythes*). Subsequently, Brooker (2000) placed *E. microcorys* in the monotypic subg. *Alveolata*, between subg. *Minutifructus* (previously subg. *Telocalyptus*) and subg. *Cuboidea* (*E. tenuipes*). *Eucalyptus microcorys* has no close relatives (Brooker and Kleinig 1994). It has an unusual morphology, with bud morphology akin to that of scribbly gums, *E. brevistylis* and silvertop ashes (all in the subg. *Eucalyptus*) and the clustered stamen morphology observed in subg. *Eudesmia* (Brooker and Kleinig 1994). The results of our molecular data are consistent with Sale *et al.* (1993, 1996) and Ladiges *et al.* (1995) and confirm that *E. microcorys* is not part of the monocalypt clade but is more-closely related to subgenera *Symphyomyrtus* and *Minutifructus*. With any of *E. curtisii*, *E. clöeziana*, *E. tenuipes* or *E. guilfoylei* as outgroup in the SSDS, *E. microcorys* always emerged as the sister taxon to subg. *Symphyomyrtus*. However, support for this relationship was low (bootstrap percentage >50%; Fig. 5) and the AFOG (Fig. 3) analysis placed it in a hard polytomy (i.e. one that arises due to a lack of characters to resolve it, as opposed to a soft polytomy that arises due to conflicting resolutions) at the base of the *Symphyomyrtus* clade.

Eucalyptus guilfoylei (subgenus *Cruciformes*), the yellow tingle of south-western Australia, is hypothesised to be a remnant of an ancient lineage, confined to remnant patches of wet forest that also support elements of Gondwanan flora and fauna (Wardell-Johnson and Coates 1996). Such a hypothesis is supported by our data, where *E. guilfoylei* appears as a well-differentiated monotypic lineage closely related to subg. *Symphyomyrtus* (Fig. 6).

Conclusion

ITS-sequence data have provided insight into the phylogenetic relationships between sections and subgenera of *Eucalyptus*, as well as between *Eucalyptus* and related genera. Fortifying the results of ITS-sequence data with more-slowly evolving genes (e.g. *rbc*L, *mat*k) and combining molecular and morphological data, will further assist in the effort to resolve the higher taxonomic relationships among the eucalypts. We also hope that by combining morphological data with molecular data from a number of different regions of the genome, we will be able to confirm and clarify some of the areas of uncertainty at the lower taxonomic levels in our cladograms (e.g. the phylogenetic status of subgenus *Eudesmia*; the relative relationships of Clades A–D within subg. *Symphyomyrtus*; the relative positions of subgenera *Idiogenes*, *Primitiva* and *Eucalyptus*; and the relative positions of *Angophora*, *Corymbia* A and *Corymbia* B). The large number of taxa in this survey, relative to the number of informative characters, resulted in large numbers of character-state changes and thus high levels of homoplasy, low CIs and low bootstrap values in the terminal regions of the cladograms. Clearly, there are too few informative characters in the ITS-sequence data to separate species at lower taxonomic levels with great confidence. However, it may be difficult to find molecular data that will be able to resolve accurately the phylogenetic relationships at these lower taxonomic levels. Closely related species often tend to hybridise, which not only confounds nuclear sequence data but also makes species-level phylogenies on the basis of chloroplast DNA unreliable (Steane *et al.* 1998; McKinnon *et al.* 1999).

Acknowledgments

This research was funded by the Australian Research Council and the Cooperative Research Centre for Sustainable Production Forestry. The authors are indebted to the numerous persons who provided eucalypt samples for this study, in particular Hamish Jackson, Paul Toon (QFRI, Gympie, Qld), Mervyn Shepherd (CRC-SPF, Lismore, NSW), Clyde Dunlop (Northern Territory Herbarium) Andrew Ford (CSIRO, Atherton), as well as the Australian Tree Seed Centre (ACT), the Royal Botanic Gardens Sydney, the Royal Tasmanian Botanical Gardens, Waite Arboretum and the Australian National Botanic Gardens. We also thank Simon Whittock for technical assistance.

References

Brooker MIH (2000) A new classification of the genus *Eucalyptus* L'Hér. (Myrtaceae). *Australian Systematic Botany* **13**, 79–148.

Brooker MIH, Kleinig DA (1990) 'Field guide to eucalypts.' Vol. 2. (Inkata Press: Australia)

Brooker MIH, Kleinig DA (1994) 'Field guide to eucalypts.' Vol. 3. (Inkata Press: Australia)

Catalán P, Kellogg EA, Olmstead R G (1997) Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndh*F gene sequences. *Molecular Phylogenetics and Evolution* **8**, 150–166.

Ellis MF, Sedgley M, Gardner, JA (1991) Interspecific pollen–pistil interaction in *Eucalyptus* L'Her. (Myrtaceae): the effect of taxonomic distance. *Annals of Botany* **68**, 185–194.

Griffin AR, Burgess IP, Wolf L (1988) Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Hérit.—a review. *Australian Journal of Botany* **38**, 383–394.

Hasegawa M, Kishino H, Yano T (1985) Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **9**, 366–369.

Hendy MD, Penny D (1989) A framework for the quantitative study of evolutionary trees. *Systematic Zoology* **38**, 297–309.

Hill KD, and Johnson LAS (1995) Systematic studies in the eucalypts. 7. A revision of the bloodwoods, genus *Corymbia* (Myrtaceae). *Telopea* **6**, 185–504.

Ladiges PY (1997) Phylogenetic history and classification of eucalypts. In 'Eucalypt ecology: individuals to ecosystems'. (Eds JE Williams, JCZ Woinarski) pp. 16–29. (Cambridge University Press: Cambridge)

Ladiges PY, Udovicic F (2000) Comment on a new classification of the eucalypts. *Australian Systematic Botany* **13**, 149–152.

Ladiges PY, Udovicic F, Drinnan AN (1995) Eucalypt phylogeny—molecules and morphology. *Australian Systematic Botany* **8**, 483–497.

McKinnon GE, Steane DA, Potts BM, Vaillancourt RE (1999) Incongruence between chloroplast and species phylogenies in *Eucalyptus* subgenus *Monocalyptus* (Myrtaceae). *American Journal of Botany* **86**, 1038–1046.

Mort ME, Soltis PS, Soltis DE, Mabry ML (2000) Comparison of three methods for estimating internal support on phylogenetic trees. *Systematic Biology* **49**, 160–171.

Pryor LD, Johnson LAS (1971) 'A classification of the eucalypts.' (Australian National University Press: Canberra)

Sale MM, Potts BM, West AK, Reid, JB (1993) Relationships within *Eucalyptus* using chloroplast DNA. *Australian Systematic Botany* **6**, 127–138.

Sale MM, Potts BM, West AK, Reid JB (1996) Relationships within *Eucalyptus* (Myrtaceae) using PCR-amplification and southern hybridisation of chloroplast DNA. *Australian Systematic Botany* **9**, 273–282.

Steane DA, Byrne M, Vaillancourt R, Potts, B (1998) Chloroplast DNA polymorphism signals complex interspecific interactions in *Eucalyptus* (Myrtaceae). *Australian Systematic Botany* **11**, 25–40.

Steane DA, McKinnon GE, Vaillancourt RE, and Potts BM (1999) ITS sequence data resolve higher level relationships among the eucalypts. *Molecular Phylogenetics and Evolution* **12**, 215–223.

Stokoe RL, Shepherd M, Lee DJ, Nikles DG, and Henry RJ (2001) Natural inter-subgeneric hybridisation between *Eucalyptus acmenoides* Schauer and *Eucalyptus cloeziana* F.Muell. (Myrtaceae) in southeast Queensland. *Annals of Botany* **88**, 563–570.

Swofford DL (1999) 'PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4.' (Sinauer: Sunderland, MA)

Udovicic F, Ladiges PY (2000) Informativeness of nuclear and chloroplast DNA regions and the phylogeny of the eucalypts and related genera (Myrtaceae). *Kew Bulletin* **55**, 633–645.

Udovicic F, McFadden G, Ladiges PY (1995) Phylogeny of *Eucalyptus* and *Angophora* based on 5S rDNA spacer sequence data. *Molecular Phylogenetics and Evolution* **4**, 247–256.

Wardell-Johnson G, Coates D (1996) Links to the past: local endemism in four species of forest eucalypts in southwestern Australia. In 'Gondwanan heritage: past, present and future of the Western Australian biota'. (Eds SD Hopper, JA Chapill, MS Harvey, AS George) pp. 137–154. (Surrey Beatty and Sons: Sydney)

Whittock S (2000) Chloroplast DNA phylogenetic reconstruction of *Eucalyptus* L'Hérit. (Myrtaceae). BSc Honours Thesis, University of Tasmania, Australia.

Wilson PG, O'Brien MM, Quinn CJ (1999) Molecular phylogeny of the Myrtaceae and the root of the eucalypts. In 'Molecular genetics of *Eucalyptus*. Program and abstracts of CRCSPF symposium'. (Eds RE Vaillancourt, BM Potts) p. 2. (CRC for Sustainable Production Forestry: Hobart)

Manuscript received 20 November 2000, accepted 5 September 2001