

Phylogenomics of the green ash eucalypts (Myrtaceae): a tale of reticulate evolution and misidentification

Susan Rutherford^{A,B,C}, Peter G. Wilson^B, Maurizio Rossetto^B and Stephen P. Bonser^A

^AEvolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney, NSW 2052, Australia.

^BNational Herbarium of NSW, Royal Botanic Garden Sydney, Mrs Macquaries Road, Sydney, NSW 2000, Australia.

^CCorresponding author. Email: susan.rutherford@rbgsyd.nsw.gov.au

Abstract. *Eucalyptus* is a genus that occurs in a range of habitats in Australia, Papua New Guinea, Timor, Sulawesi and the Philippines, with several species being used as sources of timber and fibre. However, despite its ecological and commercial significance, understanding its evolutionary history remains a challenge. The focus of the present study is the green ashes (subgenus *Eucalyptus* section *Eucalyptus*). Although previous studies, based primarily on morphology, suggest that the green ashes form a monophyletic group, there has been disagreement concerning the divergence of taxa. The present study aims to estimate the phylogeny of the green ashes and closely related eucalypts (37 taxa from over 50 locations in south-eastern Australia), using genome-wide analyses based on Diversity Arrays Technology (DArT). Results of analyses were similar in topology and consistent with previous phylogenies based on sequence data. Many of the relationships supported those proposed by earlier workers. However, other relationships, particularly of taxa within the Sydney region and Blue Mountains, were not consistent with previous classifications. These findings raise important questions concerning how we define species and discern relationships in *Eucalyptus* and may have implications for other plant species, particularly those with a complex evolutionary history where hybridisation and recombination have occurred.

Additional keywords: Australia, DArT, Diversity Arrays Technology, *Eucalyptus*, hybridisation, phylogenetics, recombination.

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Introduction

Eucalyptus L'Hér. (Myrtaceae) is a highly diverse genus encompassing more than 700 species distributed across Australia, Papua New Guinea, Timor, Sulawesi and the Philippines (Smith *et al.* 2003; McKinnon *et al.* 2008; Wilson 2011). Over 98% of species within the genus are endemic to Australia where they are the dominant or co-dominant component of many vegetation types (Potts and Wiltshire 1997; Hager and Benson 2010). *Eucalyptus* is also commercially important, with many species (such as *Eucalyptus grandis* W.Hill, *E. globulus* Labill. and *E. tereticornis* Sm.) being grown around the world as sources of timber and fibre (Eldridge *et al.* 1993; Grattapaglia *et al.* 2012). Eucalypts are considered Gondwanan in origin (Crisp *et al.* 2011; Gandolfo *et al.* 2011; Hermsen *et al.* 2012; Thornhill and Macphail 2012), forming a minor part of Tertiary Australian rainforests (Hill 1994; Hager and Benson 2010). Macrofossil evidence suggests that the distribution of eucalypts expanded in response to increasing aridity during the Miocene, and pollen evidence indicates that they became

widespread only in the Pleistocene (5–1.5 million years ago; Pole *et al.* 1993; Rozefelds 1996; Potts and Pederick 2000; Macphail 2007). Many present-day eucalypt species complexes are thought to be the result of recent and ongoing speciation (McKinnon *et al.* 2004; Byrne 2007; Yeoh *et al.* 2013). Morphological differences among species are often narrowly defined (Hill 1991), and clinal variation and morphological convergence between taxa are common (McKinnon *et al.* 2004). Defining species boundaries is further complicated by interspecific hybridisation, often between distantly related taxa (Griffin *et al.* 1988; Rossetto *et al.* 1997; McKinnon *et al.* 2001; Field *et al.* 2011a, 2011b; Steane *et al.* 2011; Pollock *et al.* 2013, 2015). As a result, understanding evolutionary relationships in *Eucalyptus*, particularly between closely related species, remains a major challenge.

The focus of the present study is the green ashes in subgenus *Eucalyptus* section *Eucalyptus*^A (Brooker 2000). The green ashes are characterised by alternate juvenile leaves, adult leaves with moderate to no reticulation, pedicellate buds, reniform anthers

^AAuthors of plant names are given in Table 2 and authors of both species and higher taxonomic ranks are listed in Appendices 1 and 5.

and brown to red–brown seeds (Brooker 2000). They are found in a range of habitats in south-eastern Australia, with some species occurring as trees in tall forests on fertile soils and others as small trees or mallees on shallow soils on sandstone (Ladiges *et al.* 2010). Thirteen species were recognised by Brooker (2000), including *Eucalyptus regnans* (the tallest flowering plant in the

world, up to 100 m tall), the timber species, *E. obliqua* and *E. fastigata*, and the mallee, *E. cunninghamii*, which is often less than 1 m in height (Fig. 1). Of these, nine are rare, restricted or localised (e.g. *E. paliformis* is known from only seven populations in Wadbilliga National Park, Prober *et al.* 1990). Previous studies, based primarily on morphology, suggest that the



Fig. 1. Taxa from the green ash group. A. *Eucalyptus regnans* from Mount Field National Park (Tasmania). B. *E. codonocarpa* from Washpool National Park (New South Wales). C. *E. langleyi* from Nowra (New South Wales). D. *E. cunninghamii* from the Greater Blue Mountains World Heritage Area (New South Wales).

green ashes form a monophyletic group (Ladiges *et al.* 1987, 1989). However, there has been much disagreement concerning the divergence and differentiation of taxa, and, in particular, the number of recognised species (Table 1). A small number of species, namely *E. regnans*, *E. fastigata*, *E. obliqua*, *E. triflora*, *E. obtusiflora*, *E. stricta*, *E. apiculata*, *E. kybeanensis* and *E. approximans*, were placed as part of series *Obliquae* (section *Renantheria*) by Pryor and Johnson (1971). The green ashes were only later referred to as a 'group' (e.g. Ladiges *et al.*

1987, 1989; Moran *et al.* 1990; Prober *et al.* 1990; Hill 1991, 2002). Ladiges *et al.* (1989) recognised more species (e.g. *E. dendromorpha*, *E. rupicola*, *E. paliformis* and *E. burgessiana*), although they considered *E. obtusiflora* (now *E. obstans*) to be a subspecies of *E. stricta*. Hill (1991, 2002) treated several taxa as species, e.g. *E. codonocarpa* (formerly *E. approximans* subsp. *codonocarpa*), *E. spectatrix*, *E. laophila* and *E. microcodon*, and recognised *E. langleyi*, *E. obstans* (formerly *E. obtusiflora*) and *E. cunninghamii* (formerly *E. rupicola*). However, many of the

Table 1. Classifications of the green ashes

Eucalyptus obtusiflora in Pryor and Johnson (1971) is *E. stricta* subsp. *obtusiflora* in Ladiges *et al.* (1989) and *E. obstans* in Hill (1991, 2002). *Eucalyptus obtusiflora* var. *dendromorpha* in Pryor and Johnson (1971) is *E. dendromorpha* in Ladiges *et al.* (1989), Hill (1991, 2002) and Brooker (2000), whereas *E. rupicola* in Ladiges *et al.* (1989) is *E. cunninghamii* in Hill (1991, 2002) and Brooker (2000). *Eucalyptus spectatrix*, *E. obstans*, *E. laophila*, *E. codonocarpa* and *E. microcodon*, which are recognised by Hill (1991, 2002), are not recognised by Brooker (2000). Brooker and Kleinig (2006) and Slee *et al.* (2006) considered *E. spectatrix* to be *E. stricta*, *E. laophila* to be *E. apiculata*, *E. obstans* to be *E. burgessiana* and *E. codonocarpa* and *E. microcodon* included within *E. approximans*

Pryor and Johnson (1971)	Ladiges <i>et al.</i> (1989)	Hill (1991, 2002)	Brooker (2000)
Subgenus <i>Monocalyptus</i>	Subgenus <i>Monocalyptus</i>	Subgenus <i>Monocalyptus</i>	Subgenus <i>Eucalyptus</i>
Section <i>Renantheria</i>	Superseries <i>Eucalyptus</i>		Section <i>Eucalyptus</i>
Series <i>Obliquae</i>	Series <i>Regnaninae</i>	Green-leaved ashes	Series <i>Regnantes</i>
Subseries <i>Obliquinae</i>	<i>E. regnans</i>	<i>E. fastigata</i>	<i>E. regnans</i>
<i>E. obliqua</i>	<i>E. fastigata</i>	<i>E. obliqua</i>	<i>E. fastigata</i>
Subseries <i>Delegatensinae</i>	Series <i>Eucalyptus</i>	<i>E. triflora</i>	Series <i>Eucalyptus</i>
<i>E. delegatensis</i>	<i>E. obliqua</i>	<i>E. dendromorpha</i>	<i>E. obliqua</i>
Subseries <i>Regnantinae</i>	Series <i>Strictinae</i>	<i>E. apiculata</i>	Series <i>Strictae</i>
<i>E. regnans</i>	Subseries <i>Dendromorphitae</i>	<i>E. laophila</i>	Subseries <i>Irregulares</i>
<i>E. fastigata</i>	<i>E. dendromorpha</i>	<i>E. stricta</i>	<i>E. triflora</i>
Subseries <i>Luehmannianinae</i>	Subseries <i>Strictitae</i>	<i>E. spectatrix</i>	<i>E. dendromorpha</i>
<i>E. oreades</i>	<i>E. triflora</i>	<i>E. burgessiana</i>	<i>E. apiculata</i>
<i>E. luehmanniana</i>	<i>E. stricta</i>	<i>E. langleyi</i>	<i>E. stricta</i>
Subseries <i>Considenianinae</i>	subsp. <i>stricta</i>	<i>E. obstans</i>	<i>E. burgessiana</i>
<i>E. consideniana</i>	subsp. <i>obtusiflora</i>	<i>E. cunninghamii</i>	<i>E. langleyi</i>
<i>E. remota</i>	<i>E. burgessiana</i>	<i>E. approximans</i>	Subseries <i>Regulares</i>
<i>E. sieberi</i>	Subseries <i>Approximanitae</i>	<i>E. codonocarpa</i>	<i>E. approximans</i>
<i>E. multicaulis</i>	<i>E. kybeanensis</i>	<i>E. microcodon</i>	<i>E. cunninghamii</i>
Subseries <i>Pauciflorinae</i>	<i>E. paliformis</i>	<i>E. paliformis</i>	<i>E. paliformis</i>
<i>E. pauciflora</i>	<i>E. approximans</i>	<i>E. kybeanensis</i>	Series <i>Contiguae</i>
subsp. <i>pauciflora</i>	subsp. <i>approximans</i>		<i>E. kybeanensis</i>
subsp. <i>niphophila</i>	subsp. <i>codonocarpa</i>		
subsp. <i>debeuzevillei</i>	<i>E. rupicola</i>		
var. <i>nana</i>	<i>E. apiculata</i>		
Subseries <i>Strictinae</i>			
<i>E. fraxinoides</i>			
<i>E. triflora</i>			
<i>E. obtusiflora</i>			
var. <i>dendromorpha</i>			
<i>E. obtusiflora</i>			
<i>E. stricta</i>			
<i>E. apiculata</i>			
<i>E. approximans</i>			
subsp. <i>approximans</i>			
subsp. <i>codonocarpa</i>			
Subseries <i>Kybeanensinae</i>			
<i>E. kybeanensis</i>			
Subseries <i>Mitchellianinae</i>			
<i>E. mitchelliana</i>			
Subseries <i>Stellulatinae</i>			
<i>E. stellulata</i>			
<i>E. moorei</i>			
var. <i>laticula</i>			

species recognised by Hill (1991, 2002) were not recognised by Brooker (2000) (Fig. 2). In the green ashes, Brooker and Kleinig (2006) and Slee *et al.* (2006) considered *E. obstans* to be a coastal variant of *E. burgessiana*, *E. spectatrix* to be a southern outlier of *E. stricta*, *E. laophila* to be a synonym of *E. apiculata*, and include both *E. codonocarpa* and *E. microcodon* within *E. approximans* subsp. *codonocarpa*. Many of these species are very similar morphologically and are difficult to distinguish in the field (Lassak and Southwell 1982; Ladiges *et al.* 1989). Consequently, although much effort has gone into the systematics and classification of this group, the ranking of taxa and the nature of the relationships among species remain uncertain.

Over the past two decades, molecular methods have become increasingly important in resolving questions concerning evolutionary relationships among taxa. The development of sequence datasets has enhanced our understanding of relationships between eucalypt genera and major subgenera (e.g. Udovicic *et al.* 1995; Steane *et al.* 1999, 2002; Udovicic and Ladiges 2000; Whittock *et al.* 2003; Parra-O. *et al.* 2006, 2009; Ochieng *et al.* 2007b). However, although standard DNA markers have successfully been used to resolve relationships at higher taxonomic levels within *Eucalyptus*, these have generally been unsuccessful in resolving relationships among closely related species (McKinnon *et al.* 2008). Most phylogenetic studies use sequence data from only a single or few regions of the genome (e.g. chloroplast DNA) and are not sufficiently variable in closely related species (e.g. Steane *et al.* 1998). There are also problems associated with using sequence data from some regions of nuclear DNA (e.g. ITS and ETS) in eucalypts because of the functional constraints imposed on neutral change of nucleotides during evolution (Bayly and

Ladiges 2007; Ochieng *et al.* 2007a; Bayly *et al.* 2008). Marker systems theoretically representing the whole genome, such as microsatellites and amplified fragment length polymorphisms (AFLPs), have been used to overcome some of these issues (Steane *et al.* 2011). However, although microsatellites have moderate levels of throughput, and are highly polymorphic and transferable across populations, their transferability across species is sometimes poor (Rossetto *et al.* 2000; Semagn *et al.* 2006). The genotyping density obtained even with AFLPs is only hundreds of markers per sample and because it is a gel-based technique, it is comparatively labour intensive (Sansaloni *et al.* 2010).

With the advent of next generation sequencing (NGS), analytical approaches that have wider genome coverage have been developed. Bayly *et al.* (2013) used whole chloroplast genome sequences to construct a phylogeny of 39 eucalypt species, with many branches having 97–100% bootstrap support. Another technique that has recently been used in *Eucalyptus* is Diversity Arrays Technology (DArT) (Hudson *et al.* 2012). DArT is a microarray hybridisation-based technique that simultaneously assays hundreds to thousands of markers across the genome (Jaccoud *et al.* 2001; Sansaloni *et al.* 2010; Kullán *et al.* 2012). Steane *et al.* (2011) used over 8000 DArT markers (primarily nuclear) to construct a phylogeny of *Eucalyptus*, where relationships among higher taxa were generally concordant with traditional taxonomy and ITS-based phylogenies, with high resolution within major clades (including between some closely related species) relative to previous techniques.

Although several green ash taxa (e.g. *Eucalyptus regnans*, *E. obliqua*, *E. triflora*) have been included in molecular

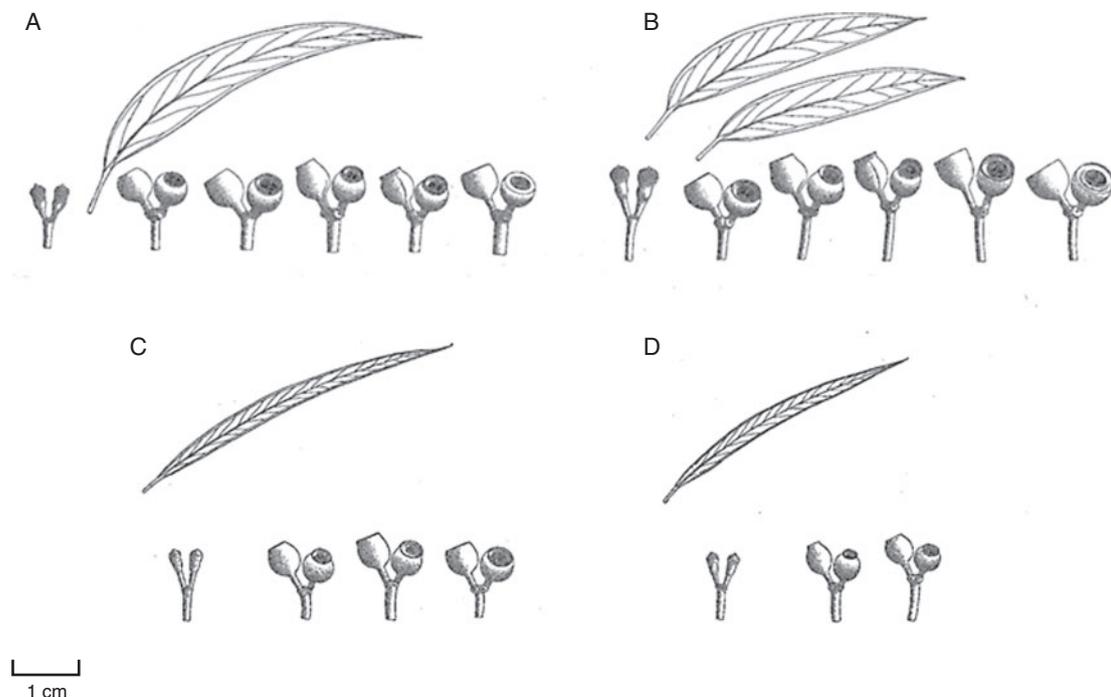


Fig. 2. Morphological characters (leaf, bud and fruits) of four green ash taxa found in the Sydney and Greater Blue Mountains World Heritage Area. A. *Eucalyptus burgessiana*. B. *E. obstans*. C. *E. laophila*. D. *E. apiculata* (Klaphake 2012: 47, 49).

Table 2. Taxa collected showing taxonomy, provenance and codes used in phylogenetic analyses

Columns 2 and 3 follow the classification of Brooker (2000), whereas Column 4 follows the classification of Hill (1991, 2002). Abbreviations: ABG, Australian Botanic Garden, Mount Annan; BMBG, Blue Mountains Botanic Garden, Mount Tomah; CCA, Currency Creek Arboretum; cult., cultivated; GBMWA, Greater Blue Mountains World Heritage Area; NSW, New South Wales; Qld, Queensland; RBG, Royal Botanic Garden, Sydney; SA, South Australia; SW, south-west; Vic., Victoria

Taxon	Subgenus	Section	Group	Provenance	Code
<i>E. apiculata</i> R.T.Baker & H.G.Sm.	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Woodford, GBMWA, NSW Hilltop, NSW	<i>apiculata</i> Wood <i>apiculata</i> Hill
<i>E. approximans</i> Maiden	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Berrima, NSW cult. ABG	<i>apiculata</i> Berr
<i>E. burgesiana</i> L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Barren Mountain, NSW cult. BMBG Warrimoo, GBMWA, NSW Linden, GBMWA, NSW	<i>approximans</i> <i>burgesiana</i> Warri <i>burgessiana</i> Lind
<i>E. codonocarpa</i> Blakely & McKie	<i>Eucalyptus</i>		Green ash	Springwood, GBMWA, NSW Faulconbridge Point, GBMWA, NSW Waratah Trig, NSW Warra National Park, NSW	<i>burgessiana</i> Sprin <i>burgessiana</i> Faul <i>codonocarpa</i> Wara <i>codonocarpa</i> Warr
<i>E. cunninghamii</i> G.Don	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Girraween National Park, Qld Mount Banks, GBMWA, NSW	<i>codonocarpa</i> Girr <i>cunninghamii</i> Bank
<i>E. dendromorpha</i> (Blakely) L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Kings Tableland, GBMWA, NSW Mount Wilson, GBMWA, NSW Mount Banks, GBMWA, NSW Blackheath, GBMWA, NSW	<i>cunninghamii</i> King <i>dendromorpha</i> Wils <i>dendromorpha</i> Bank <i>dendromorpha</i> Blac
<i>E. fastigata</i> H.Deane & Maiden	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Main falls, Wentworth Falls, GBMWA, NSW	<i>dendromorpha</i> Went M
<i>E. kybeanensis</i> Maiden & Cambage	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Princes Rock Track, Wentworth Falls, GBMWA, NSW Fitzroy Falls, NSW Redhills Road, Fitzroy Falls, NSW	<i>dendromorpha</i> Went P <i>dendromorpha</i> Fitz <i>dendromorpha</i> Redh
<i>E. langleyi</i> L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Mount Tomah, GBMWA, NSW Wadbilliga National Park, NSW Kosciusko National Park, NSW Snowy River National Park, Vic. Braidwood Road, Nowra, NSW Parma Creek Fire Road, Nowra, NSW	<i>fastigata</i> <i>kybeanensis</i> Wadb <i>kybeanensis</i> Kosc <i>langleyi</i> Brai (Nowra) <i>langleyi</i> Parm
<i>E. laophila</i> L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>		Green ash	Braidwood Road, 17 km SW Nowra, NSW Wollemi National Park, NSW Garden of Stone National Park, NSW Kings Tableland, GBMWA, NSW Lithgow, NSW	<i>langleyi</i> Brai (17 km SW of Nowra) <i>laophila</i> Woll <i>laophila</i> Gard <i>laophila</i> King <i>laophila</i> Lith
<i>E. microcodon</i> L.A.S.Johnson & K.D.Hill	<i>Eucalyptus</i>		Green ash	Woodendong, NSW	<i>microcodon</i>
<i>E. obliqua</i> L'Hér.	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Mt Murray, NSW	<i>obliqua</i>
<i>E. obstans</i> L.A.S.Johnson & K.D.Hill	<i>Eucalyptus</i>		Green ash	Beacon Hill, Sydney, NSW Royal National Park, NSW Jervis Bay, NSW	<i>obstans</i> Beac <i>obstans</i> Roya <i>obstans</i> Jerv
<i>E. patiformis</i> L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Wadbilliga National Park, NSW	<i>patiformis</i>
<i>E. regnans</i> F.Muell.	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Great Ocean Road, Vic. cult. CCA, SA	<i>regnans</i>
<i>E. spectatrix</i> L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>		Green ash	Doctor George Mountain, NSW Wadbilliga National Park, NSW Newnes Plateau, NSW	<i>spectatrix</i> Geor <i>spectatrix</i> Wadb <i>stricta</i> Newn
<i>E. stricta</i> Sieber ex Spreng.	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash		

<i>E. triflora</i> (Maiden) Blakely	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Green ash	Mount Banks, GBMWH, NSW	<i>stricta</i> Bank
<i>E. copulans</i> L.A.S.Johnson & K.D.Hill	<i>Eucalyptus</i>	<i>Eucalyptus</i>	Black sallies	Blackheath, GBMWH, NSW	<i>stricta</i> Blac
<i>E. moorei</i> Maiden & Cambage	<i>Eucalyptus</i>	<i>Longitudinales</i>	Black sallies	Katoomba, GBMWH, NSW	<i>stricta</i> Kato
<i>E. luehmannaiana</i> F.Muell.	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash A	Little Switzerland track, Kings Tableland, GBMWH, NSW	<i>stricta</i> King L
<i>E. oreades</i> R.T.Baker	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash A	Tableland Road, Kings Tableland, GBMWH, NSW	<i>stricta</i> King T
<i>E. piperita</i> Sm.	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash A	Stanwell Tops, NSW	<i>stricta</i> Stan
<i>E. consideniana</i> Maiden	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Sassafras, NSW	<i>stricta</i> Sassa
<i>E. haemastoma</i> Sm.	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Nerriga, NSW Cult. ABG	<i>triflora</i>
<i>E. multicaulis</i> Blakely	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Jamison Creek, Wentworth Falls, GBMWH, NSW cult. RBG	<i>copulans</i> Went J
<i>E. rossii</i> R.T.Baker & H.G.Sm.	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Darwins track, Wentworth Falls, GBMWH, NSW	<i>copulans</i> Went D
<i>E. sclerophylla</i> (Blakely) L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Darwins track, Wentworth Falls, GBMWH, NSW	<i>moorei</i> Went D
<i>E. sieberi</i> L.A.S.Johnson	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Wentworth Falls Lake, GBMWH, NSW	<i>moorei</i> Went Lake
<i>E. stenostoma</i> L.A.S.Johnson & Blaxell	<i>Eucalyptus</i>	<i>Cineraceae</i>	Blue ash B	Sir Bertram Stevens Drive, Royal National Park, NSW	<i>luehmannaiana</i> Roya S
<i>E. radiata</i> Sieber ex DC.	<i>Eucalyptus</i>	<i>Aromatica</i>	Peppermint	Karlo track, Royal National Park, NSW	<i>luehmannaiana</i> Roya K
<i>E. caliginosa</i> Blakely & McKie	<i>Eucalyptus</i>	<i>Capillulus</i>	Stringybark	Katoomba, GBMWH, NSW	<i>oreades</i>
<i>E. deauensis</i> Boland & Gilmour	<i>Eucalyptus</i>	<i>Capillulus</i>	Stringybark	Kings Tableland, GBMWH, NSW	<i>piperita</i> King
<i>E. oblonga</i> DC.	<i>Eucalyptus</i>	<i>Capillulus</i>	Stringybark	Hilltop, NSW	<i>piperita</i> Hill
<i>E. sparsifolia</i> Blakely	<i>Eucalyptus</i>	<i>Capillulus</i>	Stringybark	Woodford, GBMWH, NSW	<i>consideniana</i> Wood
<i>E. williamsiana</i> L.A.S.Johnson & K.D.Hill	<i>Eucalyptus</i>	<i>Capillulus</i>	Stringybark	Braidwood Road, Nowra, NSW	<i>consideniana</i> Brai (Nowra)
<i>E. cloeziana</i> F.Muell.	<i>Idiogenes</i>	<i>Capillulus</i>	Stringybark	Beacon Hill, Sydney, NSW	<i>haemastoma</i> Beac
				Royal National Park, NSW	<i>haemastoma</i> Roya
				Linden, GBMWH, NSW	<i>multicaulis</i>
				Wollemi National Park, NSW	<i>rossii</i>
				Linden, GBMWH, NSW	<i>sclerophylla</i> Lind
				Braidwood Road, Nowra, NSW	<i>sclerophylla</i> Brai (Nowra)
				Wentworth Falls, GBMWH, NSW	<i>sieberi</i> Went
				Hilltop, NSW	<i>sieberi</i> Hill
				Wadbilliga National Park, NSW	<i>stenostoma</i>
				Katoomba, GBMWH, NSW	<i>radiata</i>
				Cult. RBG	<i>caliginosa</i>
				North-east of Mongamulla Mountain, NSW cult. RBG	<i>deauensis</i>
				Stanwell Tops, NSW	<i>oblonga</i>
				Linden, GBMWH, NSW	<i>sparsifolia</i>
				Queanbeyan, NSW	<i>williamsiana</i>
				Cult. RBG	<i>cloeziana</i>

phylogenies over the past 10 years (Bayly and Ladiges 2007; Steane *et al.* 2011; Bayly *et al.* 2013), there has been no broader study of the green ash eucalypts using these more advanced techniques. Prober *et al.* (1990) used allozyme data to investigate diversity in the green ashes, and this revealed low differentiation among taxa and many relationships that were not consistent with those derived from morphological characters. Although the green ashes are widely distributed in south-eastern Australia, they are particularly diverse in the Sydney region and Blue Mountains (the latter was listed as a World Heritage Area partly because of its eucalypt diversity (Hager and Benson 2010)). Within this area, the green ashes are distributed across a range of environments and occur sympatrically with other closely related eucalypts, such as blue ashes (including scribbly gums), black sallies, stringybarks and peppermints (sections *Cineraceae*, *Longitudinales*, *Capillulus* and *Aromatica* respectively; Brooker 2000). The distribution of taxa in this heterogeneous environment, therefore, provides a unique opportunity for using more recent genomic techniques to address specific evolutionary questions concerning the green ashes and closely related taxa. Our objective was to estimate the phylogeny of the green ashes using DArT markers, so as to resolve relationships within the green ash group and between the green ashes and other taxa in subgenus *Eucalyptus*. Therefore, we aimed to address the following questions: (1) do the green ashes form a monophyletic group, (2) is there evidence of hybridisation among taxa, (3) are phylogenetic relationships of the green ashes and closely related taxa consistent with previous classifications (primarily based on morphological characters), and (4) are phylogenetic relationships correlated with geography and substrate?

Materials and methods

Sampling of taxa

Leaf material was collected from all taxa assigned to the green ash group by the major authorities (Pryor and Johnson 1971; Ladiges *et al.* 1989; Hill 2002; Brooker 2000). Table 2 lists the species sampled, following the species concepts of Hill (2002). For most of these species, more than one individual was sampled from multiple locations. So as to sample across the diversity and geographic range of the group, we collected from 44 locations between southern Queensland and Victoria (Fig. 3). Locations of green ash taxa and habitat details were obtained from the National Herbarium of New South Wales database (Royal Botanic Garden Sydney) and Benson and McDougall (1998) (full accession details are listed in Appendix 1; habitat details, and latitude and longitudes are provided in Appendix 2). During the sampling, several new populations of green ash taxa (e.g. *Eucalyptus stricta*) were discovered and included. In addition, closely related co-occurring taxa in subgenus *Eucalyptus* (sections *Aromatica*, *Capillulus*, *Cineraceae* and *Longitudinales*) were sampled (often from more than one individual per species from different locations). *Eucalyptus cloeziana* (subgenus *Idiogenes*) was included as an out-group to subgenus *Eucalyptus* on the basis of previous studies (Sale *et al.* 1993; Hill and Johnson 1995; Ladiges *et al.* 1995; Steane *et al.* 1999; Udovicic and Ladiges 2000; Steane *et al.* 2011). Most taxa were sampled directly in the field and their geographic

position (including elevation) was recorded (GPS model: Garmin Rino 650, Garmin Australasia, Sydney, NSW, Australia); vouchers of these were lodged in the National Herbarium of New South Wales. Other species (namely *E. approximans*, *E. regnans*, *E. deuaensis*, *E. caliginosa*, *E. cloeziana* and *E. apiculata* from the Berrima population) were sourced from specimens cultivated at the Currency Creek Arboretum (South Australia), the Royal Botanic Garden Sydney, the Australian Botanic Garden (Mount Annan) and the Blue Mountain Botanic Garden (Mount Tomah). All leaf samples were dried in silica gel and stored at -20°C until used for DNA extraction.

DNA isolation

Total genomic DNA was extracted from samples using a CTAB protocol modified from Doyle and Doyle (1990). A total of 1–1.5 g of leaf material per sample was ground under liquid nitrogen and the following modifications were made: (1) 2-mercaptoethanol was replaced by sodium metabisulfite (0.5%); (2) addition of sorbitol (0.35 M), polyvinylpyrrolidone (4%) and sarcosyl (5%) to the CTAB isolation buffer; and (3) DNA was purified using a Zymo-Spin I-96 Plate and the ZR-96 Clean and Concentrator Kit (Zymo Research Corporation, CA, USA). DNA quality of each sample was tested by restriction of 2 μL of DNA with 3 μL of the restriction endonuclease, *RsaI* (New England Biolabs, Irvine, CA, USA), and digests were visualised on a 1.0% agarose gel. DNA concentrations were measured using a Qubit 2.0 Fluorometer (Invitrogen, Melbourne, Vic., Australia) and each sample was made up to between 400 and 1000 ng of DNA (targeting a concentration of 50 ng μL^{-1}). Samples were sent to Diversity Arrays Technology Pty Ltd (Canberra, ACT, Australia) for genotyping, using the microarray platform developed by Sansaloni *et al.* (2010).

Phylogenetic analysis of DArT markers

The DArT microarray genotyping platform produces a binary output showing the marker name, its presence or absence in each sample and statistics regarding the quality and reliability of each marker. The DArT dataset produced for the present study consisted of a total of 2702 presence or absence markers. Phylogenetic trees were constructed using parsimony, Bayesian and distance analyses. To ensure that only the higher-quality markers were used, markers with a call rate below 90% and reproducibility less than 100% were removed from the dataset (leaving 1780 markers). Maximum parsimony (MP) analyses were conducted in PAUP 4.0 b10 (D. L. Swofford, Sinauer, Sunderland, MA, USA). The MP analysis was performed with a heuristic search using 1000 random addition sequences and tree bisection and reconnection (TBR) branch swapping (characters were equally weighted, gaps were treated as missing and character states were unordered). Bootstrapping for the MP analysis (branch lengths shown) comprised heuristic searches and 1000 replicates. Bayesian analyses were conducted in MrBayes 3.2.4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist *et al.* 2012) using a restriction site (binary) model of evolution and default priors. The final analysis was run for 150 million generations sampling every 1000 generations, with two parallel runs each with four chains (three hot and one cold). Convergence was considered reached

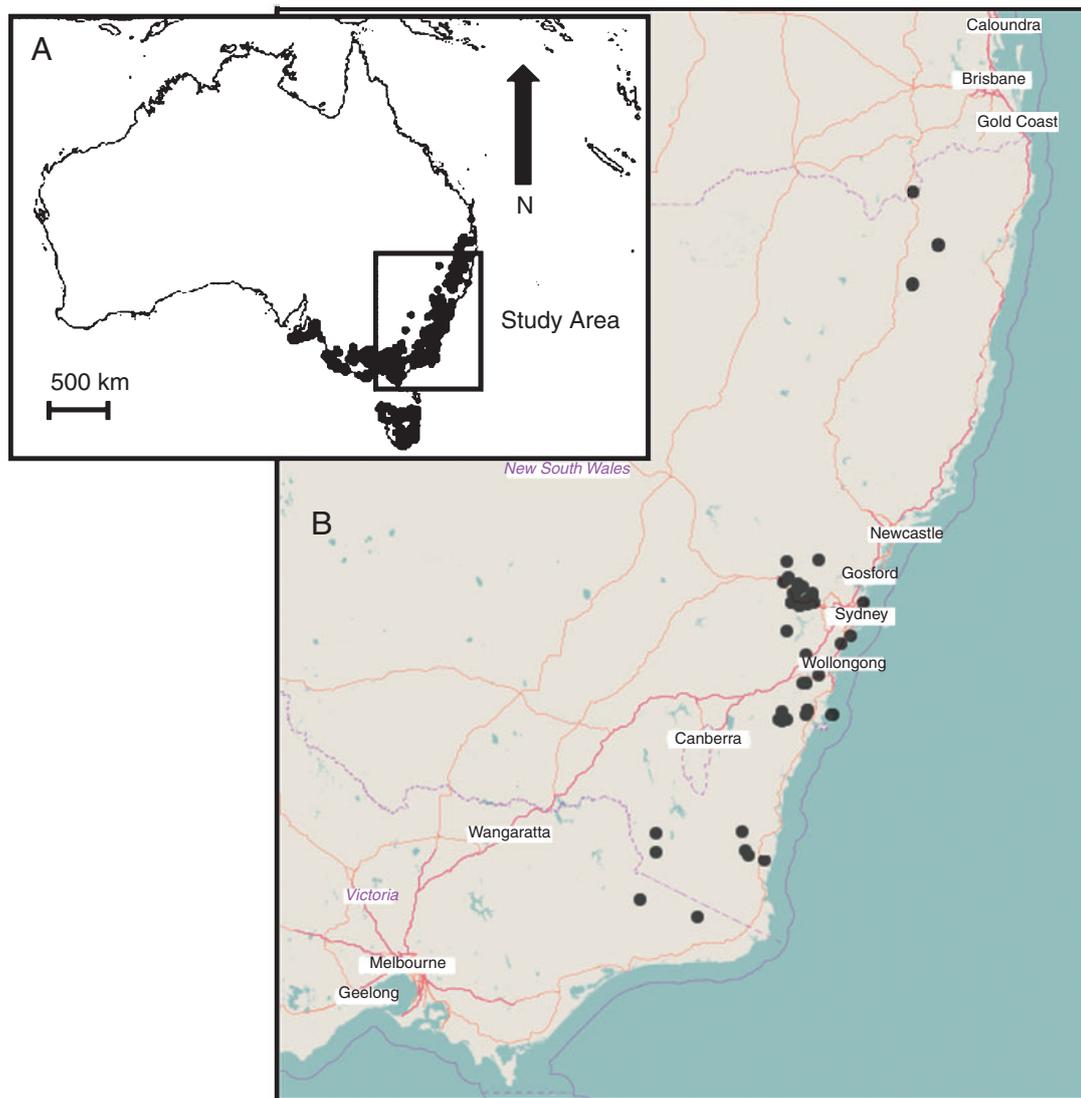


Fig. 3. Study area. A. Distribution of the green ash eucalypts (subgenus *Eucalyptus* section *Eucalyptus*) in south-eastern Australia. B. Region from southern Queensland to Victoria where leaf material of green ashes and co-occurring taxa in subgenus *Eucalyptus* were sourced (see Table 2 for location details). Maps generated using Atlas of Living Australia (2015) and Australia's Virtual Herbarium (2015).

on the basis of the standard deviation of split frequencies (<0.01) and the first 25% of trees were discarded as burn-in.

Diversity Arrays Technology (DArT) datasets are considered to follow a Dollo model of evolution (because it is much easier for a DArT marker to be lost than gained; Woodhams *et al.* 2013). Although Dollo data have been traditionally analysed using parsimony methods (Le Quesne 1974; Farris 1977), it is well known that parsimony does not take into account branch-length information (Woodhams *et al.* 2013). Therefore, a distance-based phylogenetic approach, which implements the Dollo model of evolution, was used in the present study. A distance matrix of the DArT data (Partitioned Additive Dollo Distance, or PADD) was calculated following the method outlined by Woodhams *et al.* (2013) and a tree found by minimum evolution in FastME (Desper and Gascuel 2002; Lefort *et al.* 2015). Branch support

was obtained using a bootstrap analysis in PAUP 4.0 b10 (D. L. Swofford). This comprised a heuristic search and 1000 replicates (under the minimum-evolution criterion). Nexus files containing the raw data and all tree files are available on TreeBase at <http://purl.org/phylo/treebase/phyloids/study/TB2:S18461> (accessed 9 November 2015).

Relationship networks based on the full DArT dataset (2702 markers) were generated in *SplitsTree4* (version 4.13.1) (Huson 1998; Huson and Bryant 2006) using the default settings of the software. Relationship networks are implicit representations of evolutionary history that are used to represent agreement and incompatibilities in the dataset (Huson and Bryant 2006). Therefore, use of the full DArT dataset for these analyses was considered appropriate. In a relationship network, the parallel edges indicate splits in the data and allow samples to be assigned

to groups, with the longer lines suggesting more support for that particular split (Huson and Bryant 2006). Relationship networks are an effective way of depicting the character conflicts of DArT markers and allow the complexity of the datasets to be visualised (Steane *et al.* 2011).

Reconstruction of ancestral states and character evolution

To examine patterns and variation in morphology, ancestral reconstructions were performed on the following diagnostic traits: (1) habit (mallee or tree) and (2) leaf width. These parameters were chosen as they are considered important when identifying species in subgenus *Eucalyptus* in the classifications of Brooker (2000) and Hill (2002). Leaf width was measured at the widest point (following the method of McGowen *et al.* 2001) from five random leaves per voucher specimen to the nearest millimetre, with a digital Vernier calliper (Kincrome, Melbourne, Victoria, Australia). For *Eucalyptus microcodon* and *E. williamsiana*, vouchers from the same population were used for leaf-width measurements (because of the unavailability of leaves from the samples used for DNA analysis). Categories for leaf length and leaf width have not been standardised in eucalypts. However, in the treatment of Hill (2002), *E. stricta* is described as narrow-leaved (with leaves <10 mm wide), whereas *E. burgessiana* is described as broad-leaved (leaf width >15 mm). Therefore, the categories used here for leaf width were based on the descriptions of Hill (2002) and divided into narrow (<10 mm), intermediate (10–15 mm) and broad (>15 mm). The contribution of two environmental variables (altitudinal zone and substrate) to the evolutionary diversification of taxa was also investigated. Altitudinal divisions followed the zones defined by Turak *et al.* (2011) and were classified as follows: coastal and lowland (0–235 m), upland (235–1065 m) and highland (\geq 1065 m). The substrate observed (sandstone, granite, basalt or rhyolite) was recorded per sample at each site at the time of collection of leaf material for DNA analysis. Ancestral reconstructions of each morphological and environmental parameter were traced onto the Bayesian phylogenetic tree by using MP reconstructions in the Mesquite software package v. 3.03 (W. P. Maddison and D. R. Maddison, see <http://mesquiteproject.org>). The character data matrix is presented in Appendix 2.

Results

Phylogenetic analysis of DArT markers

The DArT dataset used to produce phylogenetic trees (comprising 1780 markers) consisted of 76 samples (representing 37 taxa), with the proportion of missing data for most samples being less than 5%. Five samples had 5–10% missing data, whereas *Eucalyptus regnans* had the highest proportion of missing data (22%). The overall topology and groupings of taxa produced from all analyses were similar. The MP analysis recovered two trees, each with a tree length of 15 909, consistency index (CI) of 0.11, and retention index (RI) of 0.34. Of the 1780 markers in the dataset, 1695 were parsimony informative. The strict consensus tree had 74 nodes, 28 of which had bootstrap support (BS) greater than 50% (Fig. 4). *Eucalyptus piperita* (section *Cineraceae*) from two locations (Hilltop and Kings Tableland) was sister to the remainder of the taxa in subgenus

Eucalyptus (although *E. piperita* did not form a monophyletic group). The stringybarks (section *Capillulus*) formed a monophyletic group (84.9% BS), which was sister to a clade comprising the green ash tall trees, *E. regnans*, *E. obliqua* and *E. fastigata*. *Eucalyptus regnans* and *E. obliqua* formed a clade (81.7% BS). The clade comprising the stringybarks and the green ash tall trees was sister to the remainder of the blue ashes (section *Cineraceae*), black sallies (section *Longitudinales*), the peppermint (section *Aromatica*) and the majority of the green ashes. The remainder of the blue ashes (apart from *E. consideniiana* from Nowra) formed a monophyletic group comprising three main clades. The first was of *E. oreades* and *E. luehmanniana* (100% BS), the second included *E. multicaulis*, *E. sieberi* and *E. stenostoma* (99.3% BS), and the third comprised *E. consideniiana* from Woodford and the scribbly gums (*E. haemastoma*, *E. sclerophylla* and *E. rossii*). Whereas the samples of *E. luehmanniana* emerged in a monophyletic group (76.9% BS), the samples of other species (namely *E. sieberi*, *E. haemastoma* and *E. sclerophylla*) did not form a clade.

With the exception of *Eucalyptus apiculata* from Hilltop, the remainder of the green ashes, the black sallies, *E. radiata* and *E. consideniiana* from Nowra formed a clade. Within this group were two main clades. The first comprised *E. codonocarpa*, *E. approximans*, *E. microcodon*, *E. cunninghamii*, *E. kybeanensis*, *E. paliformis*, *E. radiata* and the black sallies. *Eucalyptus codonocarpa* from all locations was monophyletic (89.8% BS), as were *E. cunninghamii* (94.2% BS) and *E. kybeanensis* (98.9% BS). The black sallies formed a monophyletic clade (99.9% BS); however, within this group, *E. copulans* from all locations and *E. moorei* from all locations did not form separate monophyletic clades. The second main clade included *E. spectatrix*, *E. consideniiana* from Nowra and most of the green ash taxa from the Sydney region and Greater Blue Mountains World Heritage Area (GBMWA). *Eucalyptus spectatrix* from both locations was monophyletic (89.3% BS), as was *E. dendromorpha* from Fitzroy Falls and Redhills Road, and *E. burgessiana* from three locations in the GBMWA (Linden, Springwood and Faulconbridge). The three *E. langleyi* samples, *E. dendromorpha* from the Princes Rock track (Wentworth Falls, GBMWA) and *E. consideniiana* formed a clade. With the exception of *E. stricta* from Blackheath and Mount Banks, all other *E. stricta* populations emerged in a clade that also included *E. apiculata* from Woodford, *E. laophila* from Lithgow and Wollemi National Park, and *E. dendromorpha* from Mount Banks. *Eucalyptus apiculata* from Hilltop was separate from the other green ash taxa (being sister to all other taxa with the exception of *E. piperita*).

Bayesian analyses produced a phylogeny with 70 nodes, 49 of which had Bayesian posterior probability (PP) greater than 0.95 (Fig. 5). As in the MP analysis, *E. piperita* from Hilltop was sister to the remainder of taxa in subgenus *Eucalyptus*, and the green ash tall trees (*E. regnans*, *E. obliqua* and *E. fastigata*) formed a clade separate from the other green ashes (PP: 0.99). In contrast to the MP analysis, the remainder of the blue ashes were not monophyletic. However, as with the MP analysis, *E. luehmanniana* and *E. oreades* formed a monophyletic group (PP: 1), as did samples of *E. multicaulis*, *E. sieberi* and *E. stenostoma* (PP: 1). The remainder of the green ash taxa

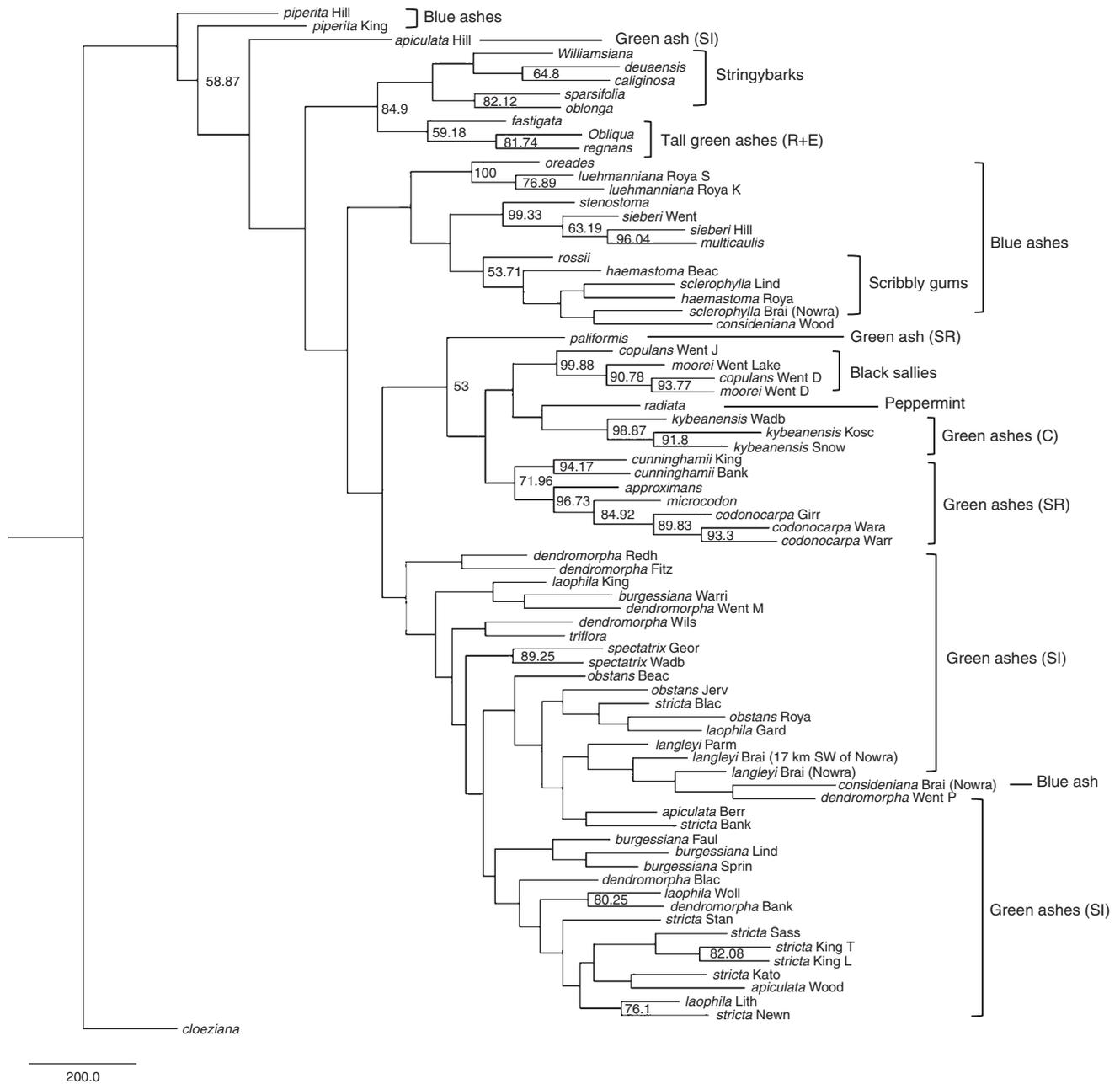


Fig. 4. One of two most parsimonious trees (based on 1780 Diversity Arrays Technology (DArT) markers) of the green ashes (section *Eucalyptus*) and other taxa in subgenus *Eucalyptus*: the black sallies (section *Longitudinales*), blue ashes (including the scribbly gums, section *Cineraceae*), peppermints (section *Aromatica*) and stringybarks (section *Capillus*). *Eucalyptus cloeziana* (subgenus *Idiogenes*) is the outgroup. Sample codes correspond to those in Table 2 (Column 6). Series and subseries (Brooker 2000) within the green ashes are shown: series *Regnantes* (R), *Eucalyptus* (E), *Strictae* subseries *Regulares* (SR), *Strictae* subseries *Irregulares* (SI) and *Contiguae* (C). Node numbers represent bootstrap values greater than 50%.

formed a clade with the black sallies and *E. radiata* (section *Aromatica*). This clade was split into the same two main groups as in the MP analysis. However, in contrast to the MP analysis, *E. apiculata* from Hilltop was grouped with *E. spectatrix* from southern New South Wales and the majority of green ash taxa from the Sydney region and GBMWA (PP: 1). Also, unlike the MP analysis, all the *E. burgessiana* samples formed a monophyletic group (PP: 0.97); the *E. langleyi* samples used

formed a monophyletic group (PP: 0.99); and the blue ash from Nowra, *E. consideniana*, was not grouped with *E. langleyi*, but was in a clade with the rest of the blue ashes.

The minimum evolution tree produced in FastME from the PADD data had 74 nodes (38 of which had BS greater than 50%, Appendix 3). In contrast to the Bayesian and MP analyses, the two *E. piperita* samples were monophyletic (63.9% BS). Also, unlike the Bayesian and MP analyses, the

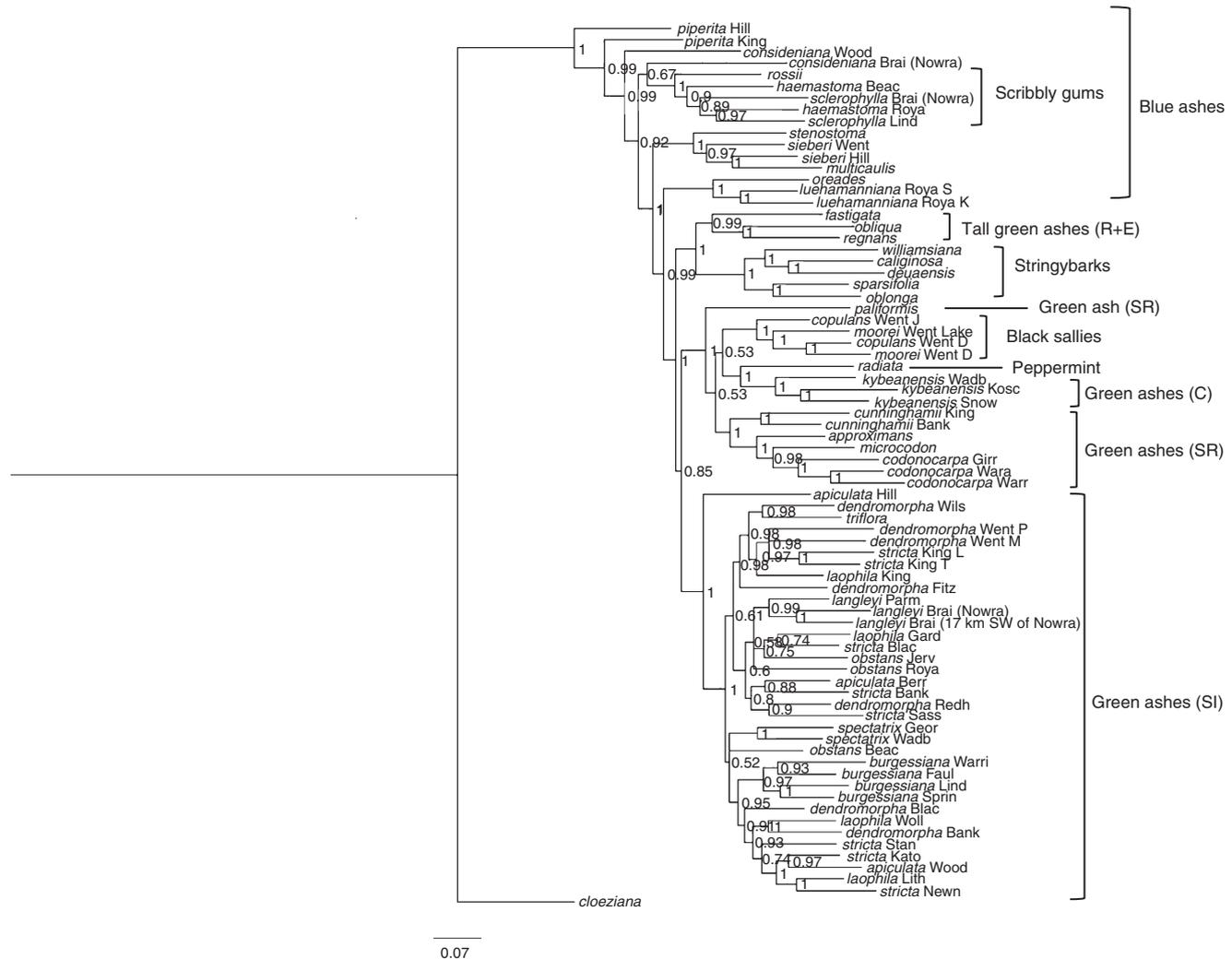


Fig. 5. Bayesian 50% majority consensus tree (based on 1780 Diversity Arrays Technology (DART) markers) of the green ashes (section *Eucalyptus*) and other taxa in subgenus *Eucalyptus*: black sallies (section *Longitudinales*), blue ashes (including the scribbly gums, section *Cineraceae*), peppermints (section *Aromatica*) and stringybarks (section *Capillulus*). *Eucalyptus cloeziana* (subgenus *Idiogenes*) is the outgroup. Sample codes correspond to those in Table 2 (Column 6). Series and subspecies (Brooker 2000) within the green ashes are shown: series *Regnantes* (R), *Eucalyptus* (E), *Strictae* subspecies *Regulares* (SR), *Strictae* subspecies *Irregulares* (SI) and *Contiguae* (C). Node values are Bayesian posterior probability (PP) values.

stringybarks were sister to the remainder of taxa in subgenus *Eucalyptus*. However, the groupings of most taxa in this tree were similar to those in the Bayesian and MP trees. For example, as with the Bayesian and MP analyses, the green ash tall trees (*E. regnans*, *E. fastigata* and *E. obliqua*) were separate from the remainder of the green ashes (which formed a clade comprising the same two major groups). As in the Bayesian tree (but unlike the MP analysis), *E. apiculata* from Hilltop was sister to *E. spectatrix* and the green ashes from the Sydney region and GBMWA. However, in contrast to the Bayesian tree (but like in the MP analysis), *E. consideniana* from Nowra was grouped with *E. langleyi* and *E. dendromorpha* from Princes Rock track (Wentworth Falls). Like in the Bayesian tree and unlike the MP tree, all samples of *E. burgessiana* were monophyletic.

Two relationship networks were generated using *SplitsTree4*, one including all taxa in subgenus *Eucalyptus* and the other comprising the green ash taxa only. In the relationship network comprising all samples, taxa formed the same broad groups as in the MP, Bayesian and PADD analyses (Appendix 4). The relationship network comprising the green ash taxa only (Fig. 6) was also largely in agreement with the MP, Bayesian and PADD analyses, and allowed geographic differentiation among taxa to be visualised (with the clustering of northern New South Wales and southern Queensland taxa and the clustering of southern New South Wales and northern Victorian taxa). The taxa from the Sydney region and GBMWA generally clustered together, although *Eucalyptus spectatrix* from southern New South Wales was nested within this group (as indicated by all phylogenetic analyses). As with

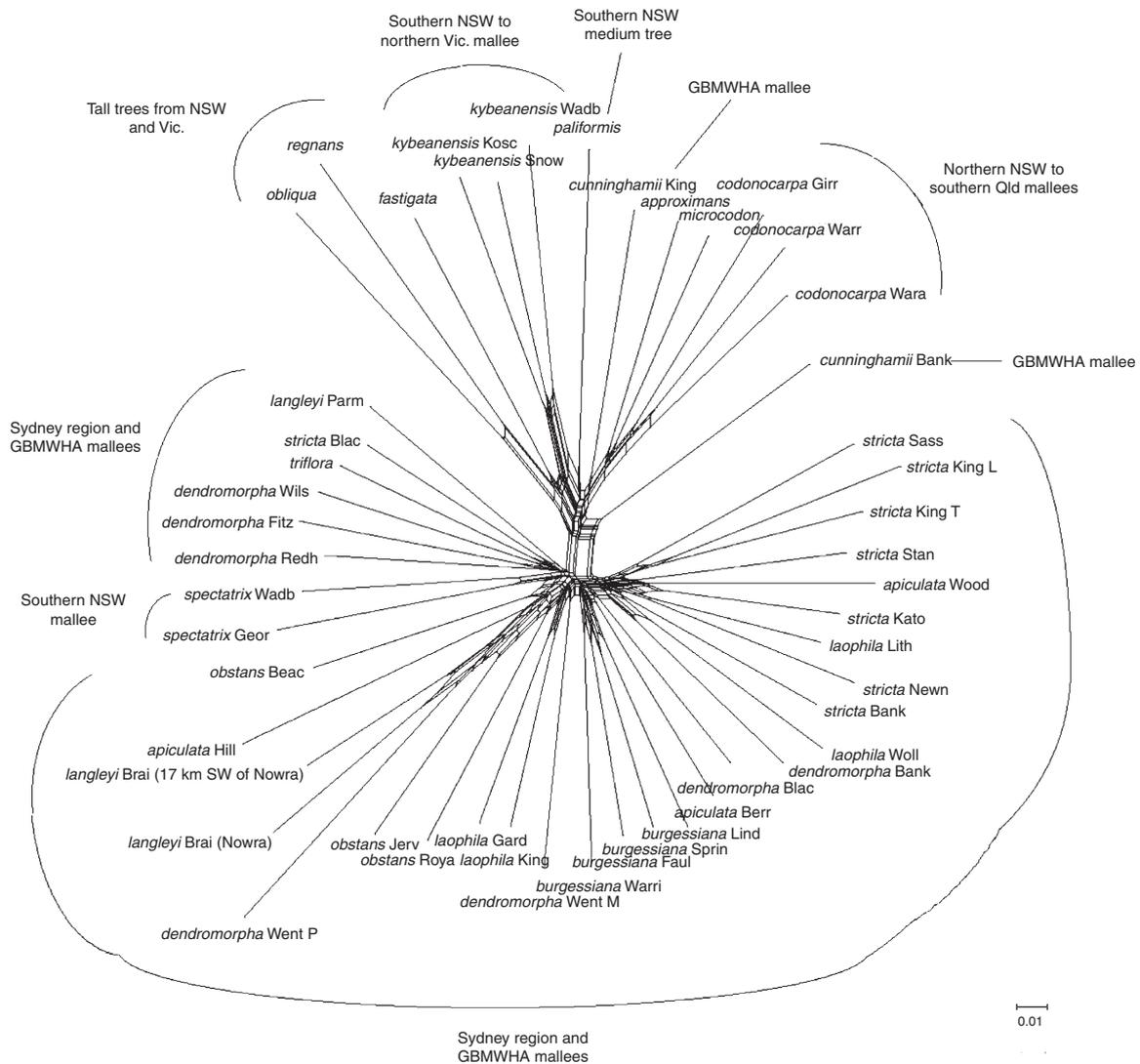


Fig. 6. Network generated by *SplitsTree4* (version 4.13.1) showing relationships among the green ashes (based on 2702 Diversity Arrays Technology (DArT) markers). Sample codes correspond to those in Table 2 (Column 6). Abbreviations: GBMWHA, Greater Blue Mountains World Heritage Area; NSW, New South Wales; Qld, Queensland; Vic., Victoria. Scale bar shows uncorrected *P* genetic distance equivalent to 0.01.

the Bayesian and PADD phylogenies, and in contrast to the MP analysis, *E. apiculata* from Hilltop was grouped with the other green ash taxa from the Sydney region and GBMWHA.

Reconstruction of ancestral states and character evolution

Overall, the Bayesian analysis produced a phylogeny that was most consistent with the current taxonomy of subgenus *Eucalyptus* (Brooker 2000). In the MP tree, *E. apiculata* from Hilltop was separate from the other green ashes and closer to the blue ash, *E. piperita*. Similarly in both the PADD and MP analyses, the blue ash, *E. consideniiana*, from Nowra, was grouped close to the green ash, *E. langleyi*. Therefore, the Bayesian topology was selected for ancestral reconstructions.

The distribution of growth habit showed a marked dichotomy in the Bayesian 50% majority consensus phylogeny (Fig. 7). The deeper diverging clades of the phylogeny (and the ancestral habit of the green ashes) was reconstructed as the tree form. *Eucalyptus luehmanniana* and *E. multicaulis* represented the only change to mallee form in the blue ash group and *E. deuaensis* was the only change to mallee form in the stringybark group. Within the clades comprising the majority of mallees, there were very few reversions to the tree form. For example, *E. triflora* and *E. dendromorpha* (from Fitzroy Falls and Redhills Road) represented the only reversions to tree form in the clade comprising most of the Sydney and GBMWHA taxa. Patterns in leaf width were not as significant on the phylogeny as growth form, although narrow leaves appeared only in the green ash mallee group. Nevertheless, there were

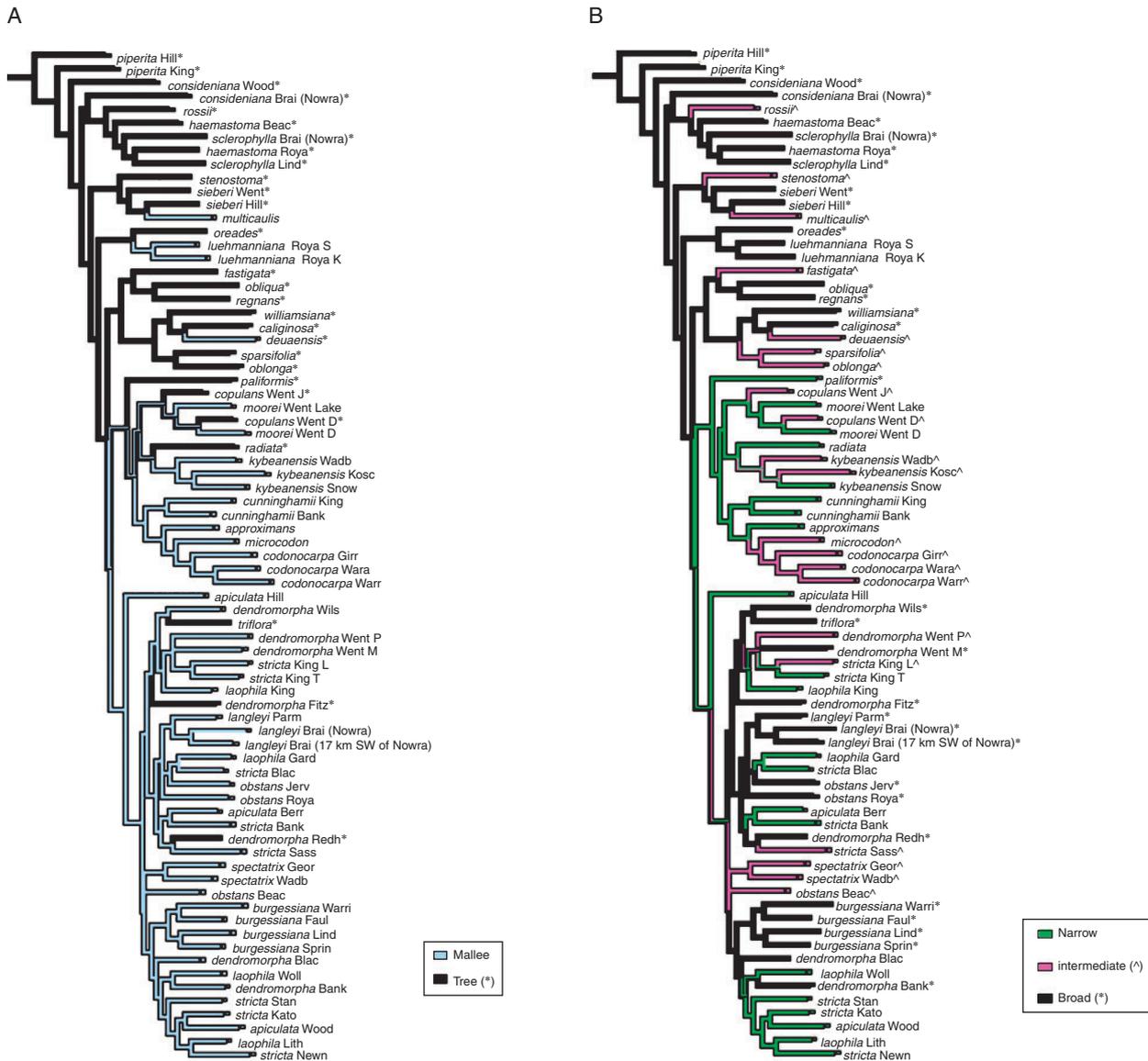


Fig. 7. Ancestral state reconstruction of taxa in subgenus *Eucalyptus*: A. Habit. B. Leaf width. C. Altitudinal zones. D. Substrate. Leaf width was divided into narrow (<10 mm), intermediate (10–15 mm) and broad (>15 mm). Altitudinal zones are defined as coastal and lowland (0–235 m), upland (235–1065 m) and highland (≥ 1065 m). Ancestral states were traced on the Bayesian phylogeny (Fig. 5) using maximum parsimony in Mesquite ver. 3.03. Sample codes correspond to those in Table 2 (Column 6).

many reversions in this group to intermediate and broad leaves. There was some correlation between clades on the Bayesian 50% majority consensus tree and environmental parameters. The majority of taxa studied occurred in upland habitats on sandstone, with some clades radiating into lowland and coastal habitats on sandstone (e.g. *E. langleyi* and *E. luehmanniana*) and other clades radiating into upland or highland habitats on granite (taxa from northern New South Wales, southern Queensland, southern New South Wales and northern Victoria). The green ash tall trees (*E. regnans*, *E. obliqua* and *E. fastigata*) differed from all other groups being a clade on basalt, as did *E. deuaensis*, which was the only taxon to occur on rhyolite.

Discussion

Phylogenetic relationships and the monophyly of the green ashes

Bayesian, MP and PADD analyses produced phylogenies with similar topologies and groupings of taxa. The phylogenies produced here were more resolved than were previous phylogenies of subgenus *Eucalyptus* using traditional one-region sequence data (e.g. Steane *et al.* 1999, 2002; Bayly and Ladiges 2007). These findings demonstrate that phylogenetic analyses based on DArT markers can provide insights into evolutionary relationships among

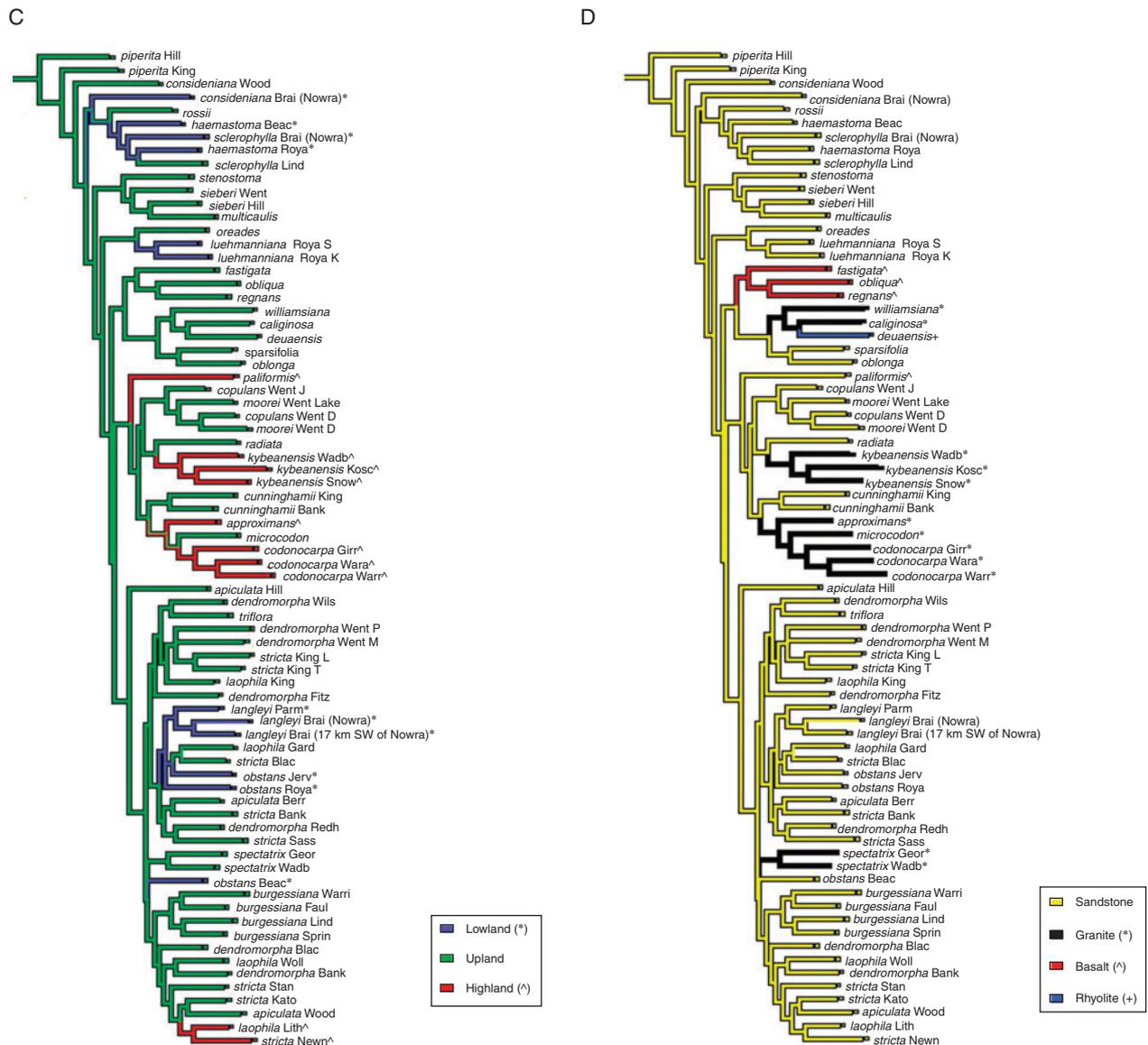


Fig. 7. (continued)

closely related species and groups that are taxonomically challenging. Although only a few taxa from the present study were included in more recent phylogenies, the relationships found here were generally consistent with the findings of Steane *et al.* (1999, 2002, 2011, for the relationship of *Eucalyptus obliqua* and *E. regnans*) and Bayly and Ladiges (2007, for the close relationship of *E. triflora*, *E. spectatrix* and *E. paliformis*). The results from the present study support many of the relationships proposed by Ladiges *et al.* (1989), Hill (2002) and Brooker (2000). The clade comprising *E. williamsiana*, *E. deuaensis* and *E. caliginosa* is consistent with Brooker's (2000) section *Capillulus* and Hill's (2002) stringybarks, whereas the close relationship of *E. regnans* and *E. fastigata*, and the relationship of the *E. approximans*–*codonocarpa*–*microcodon* clade with *E. cunninghamii* and

E. paliformis is congruent with Ladiges *et al.* (1989) and Brooker (2000).

In the present study, the green ashes (subgenus *Eucalyptus* section *Eucalyptus*) as circumscribed by Brooker (2000) did not form a monophyletic group. The separation of the green ash tall trees from the remainder of the green ashes is in contrast to Brooker (2000) and Ladiges *et al.* (1989). The blue ashes (section *Cineraceae*) in the present study were polyphyletic, which disagrees with the classifications of Brooker (2000) and Hill (2002). Similarly, the positions of the black sallies (section *Longitudinales*) and *E. radiata* were unexpected. The monophyly of *E. cunninghamii* from both locations, *E. kybeanensis* from all locations and *E. luehmanniana* from both locations support the species circumscriptions of Brooker (2000). However, many taxa from different locations (e.g.

E. haemastoma and *E. sclerophylla* and the majority of green ashes from the Sydney region and GBMWA do not appear to fit into the species delimitations of Ladiges *et al.* (1989), Hill (2002) or Brooker (2000). Consequently, these results highlighted the need for a potential revision of the infrageneric ranking of the green ashes, blue ashes, black sallies and peppermints. The implications of the findings from the present study to the taxonomy and classification of subgenus *Eucalyptus* are discussed in further detail below.

Gene flow and hybridisation

Some of the relationships found in the present study differed from a chloroplast genome study on eucalypts (Bayly *et al.* 2013), in which *E. obliqua* and *E. radiata* formed a monophyletic group, and *E. sieberi* and *E. elata* also formed a monophyletic group with *E. regnans*, all within the 'Monocalypt' clade (=subgenus *Eucalyptus*). Numerous studies have highlighted the issue of incongruence between phylogenies based on chloroplast and nuclear DNA (e.g. Soltis and Kuzoff 1995; Kim and Donoghue 2008; Wang *et al.* 2011; Yu *et al.* 2013; Govindarajulu *et al.* 2015) and, therefore, differences between the findings of Bayly *et al.* (2013) and the present study (based on DArT markers, which are predominantly nuclear) are not surprising. McKinnon *et al.* (1999) found extensive sharing of chloroplast DNA haplotypes among sympatric species from subgenus *Eucalyptus* in Tasmania, which showed a clear correlation with geographic patterns rather than phylogenetic relationships. Consequently, analyses using uni-parentally inherited markers alone may confound phylogenetic reconstruction in groups that frequently hybridise (McKinnon *et al.* 1999; Bayly and Ladiges 2007). However, McKinnon *et al.* (2010) found that, although *E. globulus* and *E. cordata* maintained strongly differentiated nuclear gene pools, leakage of nuclear DNA did occur between the two species (although cpDNA sharing was much more extensive).

In the present study, some of the relationships found were indicative of hybridisation and introgression between lineages. For example, in the PADD tree, the *E. obstans* sample from Jervis Bay was in a clade with *E. langleyi* (from which it is morphologically distinct but geographically proximate). In the MP and PADD analyses, one sample of the blue ash, *E. considiana*, appeared in the same clade as *E. langleyi* from the same location (although it was grouped with the other blue ashes in the Bayesian phylogeny). Similarly, whereas the sample of *E. apiculata* from Hilltop was grouped with the other green ashes in the Bayesian and PADD trees, in the MP analysis it was separate from the remainder of the green ashes and closer to *E. piperita*. Comparisons made between this specimen and a specimen at the National Herbarium of New South Wales (Chippendale 1002, NSW327081) recorded as a likely hybrid between *E. apiculata* and *E. piperita* (from Berrima, which is geographically close to Hilltop) revealed similarities in leaf colour, shape and size. In addition, the fruit shape from the specimen used in the present study was more spherical, suggesting that it may be the result of hybridisation with *E. piperita*. Patterns of morphological variation and introgression of eucalypt species in Tasmania indicate that reticulate evolution occurred between divergent lineages

during the Quaternary (McKinnon *et al.* 2004), and Hager and Benson (2010) suggested that such processes are likely to have played a major role in the evolutionary history of the green ashes of the GBMWA. Future studies should, therefore, use both chloroplast and nuclear genomes to explore the role that reticulate evolution may have played in the evolution of this group. Ecological and phenological studies (e.g. differences in flowering time) focussing on sympatric populations and taxa may also provide insights into patterns of gene flow and hybridisation.

Classification, morphology and the issue of misidentification

Historically, species within subgenus *Eucalyptus* have been difficult to identify because many of the subgroups have few obvious distinguishing synapomorphic morphological characters (McKinnon *et al.* 1999). The green ashes exemplify this, with the majority of species being distinguished on the basis of characters such as leaf length, leaf width, fruit size and bud size (characters that can be variable across large geographic areas). In the present study, those taxa that have notably distinctive morphological traits or are geographically isolated tend to form well supported clades. For example, *E. cunninghamii* is easily identified on the basis of its small, soft-textured, silvery-green leaves, *E. kyeansensis* is distinguished on the basis of its conical or hemispherical fruits and sessile buds, whereas the *E. approximans* clade (including *E. codonocarpa*, and *E. microcodon*) is geographically disjunct.

However, with the exception of *E. cunninghamii*, the morphological traits used for species identification in the Sydney region and GBMWA (such as leaf length and width) often overlap between taxa (the ancestral reconstructions of leaf width in the present study highlighted such overlaps between taxa). Furthermore, previous studies have demonstrated that such morphological traits can be highly plastic. For example, in *Nothofagus cunninghamii*, it was found that although leaf length and width partially depended on genotype, there was a significant effect of environmental factors on morphology (leaves became smaller and thicker with increasing altitude, Hovenden and Vander Schoor 2004). In the case of the green ashes, many taxa that are difficult to identify on the basis of morphology alone can be assigned to a particular taxon on the basis of geographical location. For example, *E. laophila* and *E. apiculata* from the GBMWA are often distinguished on the basis of the elevation at which they occur (*E. laophila* is considered to occur at higher altitudes than is *E. apiculata*). Species definitions that are in large part based on geographical location have likely led to misidentifications, which is an obvious issue in phylogenetic reconstructions. Another problem with such taxa (which are synonymous in Brooker's (2000) classification) is that there is the possibility that they are the one highly plastic species that has been distinguished on the basis of morphological differences that are not useful in species delimitations. However, although some taxa from the present study (e.g. *E. copulans* and *E. moorei*) would become a monophyletic clade if re-labelled according to the classification of Brooker (2000), re-labelling samples used for other taxa (e.g.

E. apiculata and *E. laophila*) does not make them monophyletic in the MP, PADD or Bayesian phylogenies. Many studies have highlighted the importance of comparing physiology and anatomy with phylogenetic information to better understand evolutionary diversification in both plants and animals (e.g. Ackerly *et al.* 2000; Garland *et al.* 2005; Hodson *et al.* 2005). The relationship between genetic variation and physiological and anatomical traits of seedlings, juvenile and adult plants may, therefore, provide insights into the evolution of green ash taxa in the Sydney region and GBMWA and should be the focus of future studies.

Geography, substrate and evolutionary models

Although the majority of taxa in the present study occupy upland habitats on sandstone, the ancestral reconstructions support the hypothesis of radiation of the green ashes and other taxa in subgenus *Eucalyptus* into a multitude of habitats, such as lowland and coastal habitats on sandstone, upland and highland habitats on granite, and upland habitats on basalt. There was also a correlation between habit and substrate (e.g. the tall green ash trees, *Eucalyptus regnans*, *E. fastigata* and *E. obliqua*, were found on basalt, whereas the smaller trees and mallees were found on sandstone or granite). The relationship networks in the present study indicated geographic structuring of many taxa and indicated that there is likely to be recombination, hybridisation and introgression. Previous studies have discussed the possibility that evolution in many eucalypts may not necessarily have been divergent (Chappill and Ladiges 1996; McKinnon *et al.* 2008) and that speciation in both plants and animals can occur during partial reproductive isolation (Wu 2001; Lexer and Widmer 2008; Mallet 2005). Although more traditional evolutionary models assume a tree, it is well known that more complex evolutionary scenarios (such as rapid radiation and reticulate evolution) are poorly described by these models (Huson and Bryant 2006; Morrison 2014). Phylogenetic networks, which allow horizontal reticulation events as well as vertical processes to be visualised, are increasingly being recognised as providing a more comprehensive picture of evolutionary history (Francis and Steel 2015). In the present study, the relationship networks suggested a complex pattern of evolution in the green ashes and closely related eucalypts. The role of environmental parameters (especially substrate and soil type) in the evolutionary diversification of these groups should be investigated. A detailed population-genomic study targeting taxa in the Sydney region and GBMWA will also be required to better understand the complexity of evolution in the green ashes and to clarify species boundaries.

Consequences for the classification of *Eucalyptus* subgenus *Eucalyptus*

The classification of Brooker (2000) and draft scheme of Nicolle (2015) are largely in agreement with regard to the groupings of species considered in the present study (see Appendix 5 for a direct comparison between the two classifications). The major difference is in the ranking; Brooker (2000) recognised several named sections, whereas Nicolle (2015) included the same species in a single section, section *Eucalyptus*, divided into several series, most of which

correspond with Brooker's groupings. The analyses presented here suggest that some of these groupings should be revised. In the case of *E. deuaensis*, both Brooker and Nicolle placed this taxon in a series separate from series *Pachyphloiae* (Appendix 5), the stringybarks, but the MP, Bayesian and PADD trees clearly placed *E. deuaensis* within the stringybark group as sister to *E. caliginosa*. The series *Psathyroxylon* is supported as monophyletic if the monotypic series, series *Stenostomae*, is included. In all analyses, the sole species in this series, *E. stenostoma*, is consistently strongly associated (>99% BS, PP: 1) with some species of the subseries *Considenianae*. The position of *E. consideniana* itself is problematic, with the different analyses suggesting divergent affinities for the two accessions included, possibly as a result of gene flow from other species in subgenus *Eucalyptus*. The scribbly gums (*E. haemastoma*, *E. sclerophylla* and *E. rossii*), subseries *Haemastomae*, are well supported as monophyletic in the Bayesian analysis (PP: 1), with *E. rossii* indicated as sister to the other species (*E. rossii* is also sister to the other scribbly gums in the MP analysis, although not in the PADD tree).

All analyses indicated that series *Strictae*, as recognised by both Brooker (2000) and Nicolle (2015), is not monophyletic and that the rank of the two included subseries should be revised because only subseries *Irregulares sensu* Brooker is monophyletic, whereas subseries *Regulares* is paraphyletic and not unambiguously sister to subseries *Irregulares*. The placement of *E. cunninghamii* differs between their classifications; both included it within series *Strictae* (Appendix 5), Nicolle included it in subseries *Irregulares* with *E. stricta* and its allies, whereas Brooker placed it in subseries *Regulares* with *E. approximans* and allied species. In the present study, all analyses (>70% BS, PP: 1) agreed with Brooker's placement. A member of Nicolle's subseries *Regulares* is *E. kybeanensis*, which Brooker considered to be a member of the monotypic series *Contiguae*. Here, also, the phylogeny supports Brooker's position; the three accessions of *E. kybeanensis* form a well supported clade sister to the peppermint, *E. radiata*, rather than to other taxa from subseries *Regulares*. A fourth species, *E. paliformis*, is included by both Brooker and Nicolle in series *Strictae* subseries *Regulares*. In this case, the MP and Bayesian analyses suggested that this species is sister to a clade that includes not only other members of subseries *Regulares*, but also the peppermint, *E. radiata*, and the black sallies. A fifth species, *E. spectatrix*, was not recognised by Brooker, but it was included in series *Strictae* subseries *Irregulares* by Nicolle. In all of our analyses, *E. spectatrix* received strong support as a distinct species, even though most other species, with the possible exception of *E. langleyi* (monophyletic in the Bayesian tree, but not in the MP and PADD analyses) and *E. burgesiana* (monophyletic in the Bayesian and PADD trees, but not in the MP analysis), did not appear monophyletic. *Eucalyptus langleyi* and other taxa from the Sydney region and GBMWA are the focus of ongoing research (S. Rutherford, P. G. Wilson, M. Rossetto and S. P. Bonser, unpubl. data).

Conclusions

Phylogenetic analysis of DArT markers recovered trees that were consistent with previous phylogenies of subgenus

Eucalyptus based on sequence data, with many relationships supporting those from previous classifications. However, some relationships, particularly of taxa in the Sydney region and GBMWA, were not consistent with previous classifications, highlighting the need for a revision of the green ashes and other taxa in subgenus *Eucalyptus*. As with many eucalypts, relationships in the green ashes have been defined on the basis of quantitative characters such as leaf length, leaf width, fruit size and bud size, as well as geographic location. However, the results here suggest that some morphological traits may not necessarily be reflective of evolutionary relationships within and among taxa. Defining species boundaries on the basis of geographic location is likely to be equally problematic. A detailed population genomic study focussing on taxa from the Sydney region and GBMWA is required to better understand patterns of gene flow, species boundaries and the evolutionary history of the group.

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References

- Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Randall Linder C, Sandquist DR, Geber MA, Evans AS, Dawson TE, Lechowicz MJ (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* **50**, 979–995. doi:10.1641/0006-3568(2000)050[0979:TEOPET]2.0.CO;2
- Atlas of Living Australia (2015) Spatial portal. Available at http://spatial.ala.org.au/?q=qid%3A1423528839339&qc=data_hub_uid:dh2# [Verified 1 August 2015]
- Australia's Virtual Herbarium (2015) Occurrence records. Available at http://avh.ala.org.au/occurrences/search?q=collector_text%3ARutherford+matched_name_children%3AEucalyptus#tab_mapView [Verified 1 August 2015]
- Bayly MJ, Ladiges PY (2007) Divergent paralogues of ribosomal DNA in eucalypts (Myrtaceae). *Molecular Phylogenetics and Evolution* **44**, 346–356. doi:10.1016/j.ympev.2006.10.027
- Bayly MJ, Udovicic F, Gibbs AK, Parra-O C, Ladiges PY (2008) Ribosomal DNA pseudogenes are widespread in the eucalypt group (Myrtaceae): implications for phylogenetic analysis. *Cladistics* **24**, 131–146. doi:10.1111/j.1096-0031.2007.00175.x
- Bayly MJ, Rigault P, Spokevicius A, Ladiges PY, Ades PK, Anderson C, Bossinger G, Merchant A, Udovicic F, Woodrow IE, Tibbitts J (2013) Chloroplast genome analysis of Australian eucalypts: *Eucalyptus*, *Corymbia*, *Angophora*, *Allosyncarpia* and *Stockwellia* (Myrtaceae). *Molecular Phylogenetics and Evolution* **69**, 704–716. doi:10.1016/j.ympev.2013.07.006
- Benson D, McDougall L (1998) Ecology of Sydney plant species Part 6: dicotyledon family Myrtaceae. *Cunninghamia* **5**, 808–987.
- Brooker MIH (2000) A new classification of the genus *Eucalyptus* L'Hér. (Myrtaceae). *Australian Systematic Botany* **13**, 79–148. doi:10.1071/SB98008
- Brooker MIH, Kleinig DA (2006) 'Field Guide to Eucalypts', 3rd edn. (Bloomings Books: Melbourne)
- Byrne M (2007) Phylogeography provides an evolutionary context for the conservation of a diverse and ancient flora. *Australian Journal of Botany* **55**, 316–325. doi:10.1071/BT06072
- Chappill JA, Ladiges PY (1996) Phylogenetic analysis of *Eucalyptus* informal subgenus *Symphyomyrtus* section *Maidenaria*. *Australian Systematic Botany* **9**, 71–93. doi:10.1071/SB9960071
- Crisp MD, Burrows GE, Cook LG, Thornhill AH, Bowman DMJS (2011) Flammable biomes dominated by eucalypts originated at the Cretaceous–Palaeogene boundary. *Nature Communications* **2**, 193. doi:10.1038/ncomms1191
- Desper R, Gascuel O (2002) Fast and accurate phylogeny reconstruction algorithms based on the minimum-evolution principle. *Journal of Computational Biology* **9**, 687–705. doi:10.1089/106652702761034136
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.
- Eldridge K, Davidson J, Harwood C, Van Wyk G (1993) 'Eucalypt Domestication and Breeding.' (Oxford University Press: New York)
- Farris J (1977) Phylogenetic analysis under Dollo's law. *Systematic Biology* **26**, 77–88. doi:10.1093/sysbio/26.1.77
- Field DL, Ayre DJ, Whelan RJ, Young AG (2011a) Patterns of hybridization and asymmetrical gene flow in hybrid zones of the rare *Eucalyptus aggregata* and common *E. rubida*. *Heredity* **106**, 841–853. doi:10.1038/hdy.2010.127
- Field DL, Ayre DJ, Whelan RJ, Young AG (2011b) The importance of pre-mating barriers and the local demographic context for contemporary mating patterns in hybrid zones of *Eucalyptus aggregata* and *Eucalyptus rubida*. *Molecular Ecology* **20**, 2367–2379. doi:10.1111/j.1365-294X.2011.05054.x
- Francis AR, Steel M (2015) Tree-like reticulation networks: when do tree-like distances also support reticulate evolution? *Mathematical Biosciences* **259**, 12–19. doi:10.1016/j.mbs.2014.10.008
- Gandolfo MA, Hermsen EJ, Zamalao MC, Nixon KC, Gonzalez CC, Wilf P, Cuneo NR, Johnson KR (2011) Oldest known *Eucalyptus* macrofossils are from South America. *PLoS One* **6**, e21084. doi:10.1371/journal.pone.0021084
- Garland T, Bennett AF, Rezende EL (2005) Phylogenetic approaches in comparative physiology. *The Journal of Experimental Biology* **208**, 3015–3035. doi:10.1242/jeb.01745
- Govindarajulu R, Parks M, Tennessen JA, Liston A, Ashman T-L (2015) Comparison of nuclear, plastid, and mitochondrial phylogenies and the origin of wild octoploid strawberry species. *American Journal of Botany* **102**, 544–554. doi:10.3732/ajb.1500026
- Grattapaglia D, Vaillancourt RE, Shepherd M, Thumma BR, Foley W, Külheim C, Potts BM, Myburg AA (2012) Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. *Tree Genetics & Genomes* **8**, 463–508. doi:10.1007/s11295-012-0491-x

- Griffin AR, Burgess IP, Wolf L (1988) Patterns of natural and manipulated hybridization in the genus *Eucalyptus* L'Herit: a review. *Australian Journal of Botany* **36**, 41–66. doi:10.1071/BT9880041
- Hager T, Benson D (2010) The eucalypts of the Greater Blue Mountains World Heritage Area: distribution, classification and habitats of the species of *Eucalyptus*, *Angophora* and *Corymbia* (family Myrtaceae) recorded in its eight conservation reserves. *Cunninghamia* **11**, 425–444.
- Hermesen EJ, Gandolfo MA, del Carmen Zamalao M (2012) The fossil record of *Eucalyptus* in Patagonia. *American Journal of Botany* **99**, 1356–1374. doi:10.3732/ajb.1200025
- Hill KD (1991) Myrtaceae: *Eucalyptus*. In 'Flora of New South Wales. Vol. 2'. (Ed. GJ Harden) pp. 76–142. (New South Wales University Press: Sydney)
- Hill RS (1994) Chapter 16: the history of selected Australian taxa. In 'History of the Australian Vegetation: Cretaceous to Recent'. (Ed. RS Hill) pp. 390–419. (Cambridge University Press: Cambridge, UK).
- Hill KD (2002) Myrtaceae: *Eucalyptus*. In 'Flora of New South Wales. Vol. 2', revised edn. (Ed. GJ Harden) pp. 96–164. (University of New South Wales Press: Sydney)
- Hill KD, Johnson LAS (1995) Systematic studies in the eucalypts. 7. A revision of the bloodwoods, genus *Corymbia* (Myrtaceae). *Telopea* **6**, 185–504. doi:10.7751/telopea19953017
- Hodson MJ, White PJ, Broadley MR (2005) Phylogenetic variation in the silicon composition of plants. *Annals of Botany* **96**, 1027–1046. doi:10.1093/aob/mci255
- Hovenden MJ, Vander Schoor JK (2004) Nature vs nurture in the leaf morphology of Southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *New Phytologist* **161**, 585–594. doi:10.1046/j.1469-8137.2003.00931.x
- Hudson CJ, Freeman JS, Kullán ARK, Petroli CD, Sansaloni CP, Kilian A, Detering F, Grattapaglia D, Potts BM, Myburg AA, Vaillancourt RE (2012) A reference linkage map for eucalypts. *BMC Genomics* **13**, 240. doi:10.1186/1471-2164-13-240
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755. doi:10.1093/bioinformatics/17.8.754
- Huson DH (1998) SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* **14**, 68–73. doi:10.1093/bioinformatics/14.1.68
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23**, 254–267. doi:10.1093/molbev/msj030
- Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research* **29**, 25e. doi:10.1093/nar/29.4.e25
- Kim S-T, Donoghue MJ (2008) Incongruence between cpDNA and nrITS trees indicates extensive hybridisation within *Eupersicaria* (Polygonaceae). *American Journal of Botany* **95**, 1122–1135. doi:10.3732/ajb.0700008
- Klaphake V (2012) 'Eucalypts of the Sydney Region', 3rd edn. (Van Klaphake: Byabarra, NSW, Australia)
- Kullán ARK, van Dyk MM, Jones N, Kanzler A, Bayley A, Myburg AA (2012) High-density genetic linkage maps with over 2400 sequence-anchored DArT markers for genetic dissection in an F2 pseudobackcross of *Eucalyptus grandis* × *E. urophylla*. *Tree Genetics & Genomes* **8**, 163–175. doi:10.1007/s11295-011-0430-2
- Ladiges PY, Humphries CJ, Brooker MIH (1987) Cladistic and biogeographic analysis of Western Australian species of *Eucalyptus* L'Herit., informal subgenus *Monocalyptus* Pryor & Johnson. *Australian Journal of Botany* **35**, 251–281. doi:10.1071/BT9870251
- Ladiges PY, Newnham MR, Humphries CJ (1989) Systematics and biogeography of the Australian 'green ash' eucalypts (*Monocalyptus*). *Cladistics* **5**, 345–364. doi:10.1111/j.1096-0031.1989.tb00568.x
- Ladiges PY, Udovicic F, Drinnan AN (1995) Eucalypt phylogeny: molecules and morphology. *Australian Systematic Botany* **8**, 483–497. doi:10.1071/SB9950483
- Ladiges PY, Bayly MJ, Nelson GJ (2010) East–west continental vicariance in *Eucalyptus* subgenus *Eucalyptus*. In 'Beyond Cladistics: the Branching of a Paradigm'. (Eds DM Williams, S Knapp) pp. 267–301. (University of California Press: Los Angeles, CA)
- Lassak EV, Southwell IA (1982) The stem volatile leaf oils of some species of *Eucalyptus* subseries *Strictinae*. *Phytochemistry* **21**, 2257–2261. doi:10.1016/0031-9422(82)85188-1
- Le Quesne WJ (1974) The uniquely evolved character concept and its cladistic application. *Systematic Biology* **23**, 513–517. doi:10.1093/sysbio/23.4.513
- Lefort V, Desper R, Gascuel O (2015) FastME 2.0: a comprehensive, accurate and fast distance-based phylogeny inference program. *Molecular Biology and Evolution* doi:10.1093/molbev/msv150
- Lexer C, Widmer A (2008) The genic view of plant speciation: recent progress and emerging questions. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **363**, 3023–3036. doi:10.1098/rstb.2008.0078
- Macphail M (2007) Australian Palaeoclimates: Cretaceous to Tertiary: a review of palaeobotanical and related evidence to the year 2000. CRC LEME special volume, open file report 151. CRC LEME, Perth, WA, Australia.
- Mallet J (2005) Hybridization as an invasion of the genome. *Trends in Ecology & Evolution* **20**, 229–237. doi:10.1016/j.tree.2005.02.010
- McGowen MH, Wiltshire RJE, Potts BM, Vaillancourt RE (2001) The origin of *Eucalyptus vernicosa*, a unique shrub eucalypt. *Biological Journal of the Linnean Society. Linnean Society of London* **74**, 397–405. doi:10.1111/j.1095-8312.2001.tb01401.x
- 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. doi:10.2307/2656621
- McKinnon GE, Vaillancourt RE, Jackson HD, Potts BM (2001) Chloroplast sharing in the Tasmanian eucalypts. *Evolution* **55**, 703–711. doi:10.1554/0014-3820(2001)055[0703:CSITTE]2.0.CO;2
- McKinnon GE, Jordan GJ, Vaillancourt RE, Steane DA, Potts BM (2004) Glacial refugia and reticulate evolution: the case of the Tasmanian eucalypts. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **359**, 275–284. doi:10.1098/rstb.2003.1391
- McKinnon GE, Vaillancourt RE, Steane DA, Potts BM (2008) An AFLP marker approach to lower-level systematics in *Eucalyptus* (Myrtaceae). *American Journal of Botany* **95**, 368–380. doi:10.3732/ajb.95.3.368
- McKinnon GE, Smith JJ, Potts BM (2010) Recurrent nuclear DNA introgression accompanies chloroplast DNA exchange between two eucalypt species. *Molecular Ecology* **19**, 1367–1380. doi:10.1111/j.1365-294X.2010.04579.x
- Moran GF, Bell JC, Prober S (1990) The utility of isozymes in the systematics of some Australian tree groups. *Australian Systematic Botany* **3**, 47–57. doi:10.1071/SB9900047
- Morrison DA (2014) Phylogenetic networks: a review of methods to display evolutionary history. *Annual Research & Review in Biology* **4**, 1518–1543. doi:10.9734/ARRB/2014/8230
- Nicolle D (2015) Classification of the eucalypts (*Angophora*, *Corymbia* and *Eucalyptus*) Version 2. Available at <http://www.dn.com.au/Classification-Of-The-Eucalypts.pdf> [Verified 25 August 2015]
- Ochieng JW, Henry RJ, Baverstock PR, Steane DA, Shepherd M (2007a) Nuclear ribosomal pseudogenes resolve a corroborated monophyly of the eucalypt genus *Corymbia* despite misleading hypotheses at functional ITS paralogs. *Molecular Phylogenetics and Evolution* **44**, 752–764. doi:10.1016/j.ympev.2007.04.017

- Ochieng JW, Steane DA, Ladiges PY, Baverstock PR, Henry RJ, Shepherd M (2007b) Microsatellites retain phylogenetic signals across genera in eucalypts (Myrtaceae). *Genetics and Molecular Biology* **30**, 1125–1134. doi:10.1590/S1415-47572007000600016
- Parra-O C, Bayly M, Udovicic F, Ladiges P (2006) ETS sequences support the monophyly of the eucalypt genus *Corymbia* (Myrtaceae). *Taxon* **55**, 653–663. doi:10.2307/25065641
- Parra-O C, Bayly MJ, Drinnan A, Udovicic F, Ladiges P (2009) Phylogeny, major clades and infrageneric classification of *Corymbia* (Myrtaceae), based on nuclear ribosomal DNA and morphology. *Australian Systematic Botany* **22**, 384–399. doi:10.1071/SB09028
- Pole MS, Hill RS, Green N, Macphail MK (1993) The Oligocene Berwick Quarry flora: rainforest in a drying environment. *Australian Systematic Botany* **6**, 399–427. doi:10.1071/SB9930399
- Pollock LJ, Bayly MJ, Nevill PG, Vesk PA (2013) Chloroplast DNA diversity associated with protected slopes and valleys for hybridizing *Eucalyptus* species on isolated ranges in south-eastern Australia. *Journal of Biogeography* **40**, 155–167. doi:10.1111/j.1365-2699.2012.02766.x
- Pollock LJ, Bayly MJ, Vesk PA (2015) The roles of ecological and evolutionary processes in plant community assembly: the environment, hybridization, and introgression influence co-occurrence of *Eucalyptus*. *American Naturalist* **185**, 784–796. doi:10.1086/680983
- Potts BM, Pederick LA (2000) Morphology, phylogeny, origin, distribution and genetic diversity of eucalypts. In 'Diseases and Pathogens of Eucalypts'. (Eds PJ Keane, GA Kile, FD Podger, BN Brown) pp. 11–34. (CSIRO Publishing: Melbourne)
- Potts BM, Wiltshire RJE (1997) Eucalypt genetics and genecology. In 'Eucalypt Ecology'. (Eds JE Williams, JCZ Woinarski) pp. 56–91. (Cambridge University Press: Cambridge, UK)
- Prober S, Bell JC, Moran G (1990) A phylogenetic and allozyme approach to understanding rarity in three 'green ash' eucalypts (Myrtaceae). *Plant Systematics and Evolution* **172**, 99–118. doi:10.1007/BF00937801
- Pryor LD, Johnson LAS (1971) 'A Classification of the Eucalypts.' (The Australian National University: Canberra)
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. doi:10.1093/bioinformatics/btg180
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542. doi:10.1093/sysbio/sys029
- Rossetto M, Lucarotti F, Hopper SD, Dixon KW (1997) DNA fingerprinting of *Eucalyptus graniticola*: a critically endangered relict species or a rare hybrid? *Heredity* **79**, 310–318. doi:10.1038/hdy.1997.159
- Rossetto M, Harriss FCL, Mclauchlan A, Henry RJ, Baverstock PR, Lee LS (2000) Interspecific amplification of tea tree (*Melaleuca alternifolia* – Myrtaceae) microsatellite loci: potential implications for conservation studies. *Australian Journal of Botany* **48**, 367–373. doi:10.1071/BT98084
- Rozefelds AC (1996) *Eucalyptus* phylogeny and history: a brief summary. *Tasforests* **8**, 15–26.
- Sale MM, Potts BM, West AK, Reid JB (1993) Relationships within *Eucalyptus* using chloroplast DNA. *Australian Systematic Botany* **6**, 127–138. doi:10.1071/SB9930127
- Sansaloni CP, Petroli CD, Carling J, Hudson CJ, Steane DA, Myburg AA, Grattapaglia D, Vaillancourt RE, Kilian A (2010) A high-density Diversity Arrays Technology (DArT) microarray for genome-wide genotyping in *Eucalyptus*. *Plant Methods* **6**, 16. doi:10.1186/1746-4811-6-16
- Semagn K, Bjørnstad Å, Ndjioudjop MN (2006) An overview of molecular marker methods for plants. *African Journal of Biotechnology* **5**, 2540–2568.
- Slee AV, Brooker MIH, Duffy SM, West JG (2006) 'EUCLID – Eucalypts of Australia', 3rd edn. (CD-ROM) (CSIRO Publishing: Melbourne)
- Smith S, Hughes J, Wardell-Johnson G (2003) High population differentiation and extensive clonality in a rare mallee eucalypt: *Eucalyptus curtisii*. *Conservation Genetics* **4**, 289–300. doi:10.1023/A:1024077315820
- Soltis DE, Kuzoff RK (1995) Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). *Evolution* **49**, 727–742. doi:10.2307/2410326
- Steane DA, Byrne M, Vaillancourt RE, Potts BM (1998) Chloroplast DNA polymorphism signals complex interspecific interactions in *Eucalyptus* (Myrtaceae). *Australian Systematic Botany* **11**, 25–40. doi:10.1071/SB96028
- Steane DA, McKinnon GE, Vaillancourt RE, Potts BM (1999) ITS sequence data resolve higher level relationships among the eucalypts. *Molecular Phylogenetics and Evolution* **12**, 215–223. doi:10.1006/mpev.1999.0612
- Steane DA, Nicolle D, McKinnon GE, Vaillancourt RE, Potts BM (2002) Higher-level relationships among the eucalypts are resolved by ITS-sequence data. *Australian Systematic Botany* **15**, 49–62. doi:10.1071/SB00039
- Steane DA, Nicolle D, Sansaloni CP, Petroli CD, Carling J, Kilian A, Myburg AA, Grattapaglia D, Vaillancourt RE (2011) Population genetic analysis and phylogeny reconstruction in *Eucalyptus* (Myrtaceae) using high-throughput, genome-wide genotyping. *Molecular Phylogenetics and Evolution* **59**, 206–224. doi:10.1016/j.ympev.2011.02.003
- Thornhill AH, Macphail M (2012) Fossil myrtaceous pollen as evidence for the evolutionary history of Myrtaceae: a review of fossil *Myrtaceidites* species. *Review of Palaeobotany and Palynology* **176–177**, 1–23. doi:10.1016/j.revpalbo.2012.03.003
- Turak E, Marchant R, Barmuta LA, Davis J, Choy S, Metzeling L (2011) River conservation in a changing world: invertebrate diversity and spatial prioritisation in south-eastern coastal Australia. *Marine and Freshwater Research* **62**, 300–311. doi:10.1071/MF09297
- 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. doi:10.2307/4118780
- Udovicic F, McFadden GI, Ladiges PY (1995) Phylogeny of *Eucalyptus* and *Angophora* based on 5S rDNA spacer sequence data. *Molecular Phylogenetics and Evolution* **4**, 247–256. doi:10.1006/mpev.1995.1023
- Wang H, Sun D, Sun G (2011) Molecular phylogeny of diploid *Hordeum* species and incongruence between chloroplast and nuclear datasets. *Genome* **54**, 986–992. doi:10.1139/g11-063
- Whitlock S, Steane DA, Vaillancourt RE, Potts BM (2003) Molecular evidence shows that the tropical boxes (*Eucalyptus* subgenus *Minutifructus*) are over-ranked. *Transactions of the Royal Society of South Australia* **127**, 27–32.
- Wilson PG (2011) Myrtaceae. In 'The Families and Genera of Vascular Plants. Vol. 10. Flowering Plants Eudicots'. (Ed. K Kubitzki) pp. 212–271. (Springer-Verlag: Berlin)
- Woodhams M, Steane DA, Jones RC, Nicolle D, Moulton V, Holland BR (2013) Novel distances for Dollo data. *Systematic Biology* **62**, 62–77. doi:10.1093/sysbio/sys071
- Wu C-I (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology* **14**, 851–865. doi:10.1046/j.1420-9101.2001.00335.x
- Yeoh SH, Ho SYW, Thornhill AH, Foley WJ (2013) Regional population expansion in *Eucalyptus globulus*. *Molecular Phylogenetics and Evolution* **68**, 498–501. doi:10.1016/j.ympev.2013.04.020
- Yu W-B, Huang P-H, Li D-Z, Wang H (2013) Incongruence between nuclear and chloroplast DNA phylogenies in *Pedicularis* section *Cyathophora* (Orobanchaceae). *PLoS One* **8**, e74828. doi:10.1371/journal.pone.0074828

Appendix 1. Collection details of taxa sampled showing collector number and name, location, accession number (if cultivated) and voucher number(s)
 Column 1 follows the taxonomic hierarchy of Brooker (2000), although species names follow those of Hill (1991, 2002). All vouchers were lodged at the National Herbarium of New South Wales unless otherwise stated. Abbreviations: ABG, Australian Botanic Garden (Mount Annan); AD, State Herbarium of South Australia; AO, A. Orme; BMBG, Blue Mountains Botanic Garden (Mount Tomah); CCA, Currency Creek Arboretum; cult., cultivated; DC, D. Ciric; DN, D. Nicolle; GBMWH, Greater Blue Mountains World Heritage Area; JB, J. Benson; NSW, New South Wales; Qld, Queensland; RBG, Royal Botanic Garden Sydney; SA, South Australia; SR, S. Rutherford; SW, south-west; TW, T. Wilson; Vic., Victoria

Taxon	Source
Subgenus <i>Eucalyptus</i>	
Section <i>Aromatica</i> Brooker	
Series <i>Radiatae</i> Chippendale	
<i>E. radiata</i> Sieber ex DC.	SR100, Katoomba (GBMWH, NSW), NSW905807, NSW905808
Section <i>Capillulus</i> Brooker	
Series <i>Pachyphloiae</i> Blakely	
<i>E. caliginosa</i> Blakely & McKie	SR163, cult. (RBG), Accession number 16211, NSW4138578
<i>E. oblonga</i> DC.	SR32, Stanwell Tops (NSW), NSW901027, NSW901028
<i>E. sparsifolia</i> Blakely	SR8, Linden (GBMWH, NSW), NSW900784, NSW900785
<i>E. williamsiana</i> L.A.S.Johnson & K.D.Hill	JB2912, Queanbeyan (NSW), NSW931179, JB2909, Queanbeyan (NSW), NSW888063
Series <i>Limitares</i> Brooker	
<i>E. deuaensis</i> Boland & Gilmour	SR162, North-east of Mongamulla Mountain (NSW), cult. (RBG), Accession number 841864, NSW4112513
Section <i>Cineraceae</i> Brooker	
Series <i>Fraxinales</i> Blakely	
<i>E. luehmanniana</i> F.Muell.	SR27, Sir Bertram Stevens Drive (Royal National Park, NSW), NSW900953, NSW900954, NSW900955; DC s.n. (NSW971562), Karloo track (Royal National Park, NSW)
<i>E. oreades</i> R.T.Baker	SR101, Katoomba (GBMWH, NSW), NSW905809
Series <i>Psathyroxylon</i> Blakely	
Subseries <i>Considenianae</i> Brooker & Slee	
<i>E. consideniana</i> Maiden	SR52, Braidwood Road (Nowra, NSW), NSW901228, NSW901230; SR67, Woodford (GBMWH, NSW), NSW902351, NSW902361
<i>E. multicaulis</i> Blakely	SR3, Linden (GBMWH, NSW), NSW897519, NSW897520
<i>E. sieberi</i> L.A.S.Johnson	SR11, Wentworth Falls (GBMWH, NSW), NSW900793; SR45, Hilltop (NSW), NSW901213, NSW901214
Subseries <i>Haemastomae</i> Brooker	
<i>E. haemastoma</i> Sm.	SR24, Beacon Hill (Sydney, NSW), NSW900884, NSW900885; SR28, Royal National Park (NSW), NSW900956, NSW900957
<i>E. rossii</i> R.T.Baker & H.G.Sm.	SR42, Wollemi National Park (NSW), NSW901061
<i>E. sclerophylla</i> (Blakely) L.A.S.Johnson & Blaxell	SR6, Linden (GBMWH, NSW), NSW897521, NSW897522; SR53, Braidwood Road (Nowra, NSW), NSW901231, NSW901232
Series <i>Stenostomae</i> Brooker	
<i>E. stenostoma</i> L.A.S.Johnson & Blaxell	SR156, Wadbilliga National Park (NSW), NSW971600, NSW971601
Series <i>Piperitales</i> Blakely	
<i>E. piperita</i> Sm.	SR18, Kings Tableland (GBMWH, NSW), NSW900858; SR44, Hilltop (NSW), NSW901211, NSW901212
Section <i>Eucalyptus</i>	
Series <i>Regnantes</i> Chippendale	
<i>E. fastigata</i> H.Deane & Maiden	SR33, Mt Tomah (GBMWH, NSW), NSW901039
<i>E. regnans</i> F.Muell.	DN4316, Great Ocean Road (Vic.), cult. (CCA, SA), AD164457
Series <i>Eucalyptus</i>	
<i>E. obliqua</i> L'Hér.	SR50, Mount Murray (NSW), NSW901221, NSW901222
Series <i>Strictae</i> L.A.S.Johnson ex Brooker	
Subseries <i>Irregulares</i> Brooker	
<i>E. apiculata</i> R.T.Baker & H.G.Sm.	SR46, Hilltop (NSW), NSW901216; SR69, Woodford (GBMWH, NSW), NSW902380; SR129, Berrima (NSW), Cult. (ABG), Accession number 903465, NSW4120313
<i>E. burgessiana</i> L.A.S.Johnson & Blaxell	SR4, Linden (GBMWH, NSW), NSW897458, NSW897465; SR62, Warrimoo (GBMWH, NSW), NSW902178, NSW902180; SR106, Springwood (GBMWH, NSW), NSW905815; SR107, Faulconbridge Point (GBMWH, NSW), NSW905816
<i>E. dendromorpha</i> (Blakely) L.A.S.Johnson & Blaxell	SR12, Princes Rock track (Wentworth Falls, GBMWH, NSW), NSW900795, NSW900797; SR13, Main falls (Wentworth Falls, GBMWH, NSW), NSW900798, NSW900799; SR36, Mt Wilson (GBMWH, NSW), NSW901045; SR47, Redhills Road (Fitzroy Falls, NSW), NSW901217; SR49, Fitzroy Falls (NSW), NSW901220; SR73, Blackheath (GBMWH, NSW), NSW902476, NSW902477; SR121, Mt Banks (GBMWH, NSW), NSW970903

(continued next page)

Appendix 1. (continued)

Taxon	Source
<i>E. langleyi</i> L.A.S.Johnson & Blaxell	SR51, Braidwood Road (Nowra, NSW), NSW901226; SR91, Parma Creek Fire Road (Nowra, NSW), NSW904641; SR94, Braidwood Road (17 km SW of Nowra, NSW), NSW904644
<i>E. laophila</i> L.A.S.Johnson & Blaxell	SR39, Wollemi National Park (NSW), NSW901056, NSW901057; SR83, Kings Tableland (GBMWH, NSW), NSW904368; SR125a, Lithgow (NSW), NSW984456; TW429, Garden of Stone National Park (NSW), NSW906163
<i>E. obstans</i> L.A.S.Johnson & K.D.Hill	SR21, Beacon Hill (Sydney, NSW), NSW900872, NSW900873, NSW900874; SR25, Royal National Park (NSW), NSW900947, NSW900949, NSW900950; SR85a, Jervis Bay (NSW), NSW904635
<i>E. spectatrix</i> L.A.S.Johnson & Blaxell	SR136, Doctor George Mountain (NSW), NSW971575; SR160, Wadbilliga National Park (NSW), NSW971605
<i>E. stricta</i> Sieber ex Spreng.	SR9, Little Switzerland track (Kings Tableland, GBMWH, NSW), NSW900786, NSW900787; SR16, Tableland Road (Kings Tableland, GBMWH, NSW), NSW900847, NSW900853; SR30, Stanwell Tops (NSW), NSW901022; SR37, Newnes Plateau (NSW), NSW901047, NSW901049; SR55, Sassafras (NSW), NSW901235; SR75, Blackheath (GBMWH, NSW), NSW902556; SR98, Katoomba (GBMWH, NSW), NSW905718; SR123a, Mount Banks (GBMWH, NSW), NSW984458
<i>E. triflora</i> (Maiden) Blakely	SR127, Nerriga (NSW), cult. (ABG), Accession number 861018, NSW4124002
Subseries <i>Regulares</i> Brooker	
<i>E. approximans</i> Maiden	SR114, Barren Mountain (NSW), Cult. (BMBG), Accession number 872906, NSW707119
<i>E. codonocarpa</i> Blakely & McKie	SR109, Waratah Trig (NSW), NSW906600, NSW906601; SR112, Warra National Park (NSW), NSW906606; SR131, Girraween National Park (Qld), NSW970972
<i>E. cunninghamii</i> G.Don	SR104, Kings Tableland (GBMWH, NSW), NSW905813; SR 118a, Mount Banks (GBMWH, NSW), NSW984419
<i>E. microcodon</i> L.A.S.Johnson & K.D.Hill	AO1054, Woodendong (NSW), NSW931184; AO1052, Woodendong (NSW), NSW848075
<i>E. paliformis</i> L.A.S.Johnson & Blaxell	SR139, Wadbilliga National Park (NSW), NSW971580
Series <i>Contiguae</i> Brooker & Slee	
<i>E. kybeanensis</i> Maiden & Cabbage	SR143, Wadbilliga National Park (NSW), NSW971584; SR147, Kosciusko National Park (NSW), NSW971591; SR151, Snowy River National Park (Vic.), NSW971595
Section <i>Longitudinales</i> (Blakely) Brooker	
<i>E. copulans</i> L.A.S.Johnson & K.D.Hill	SR164, Jamison Creek, Wentworth Falls (GBMWH, NSW), cult. (RBG), Accession number 961650, NSW4211706; AO1049, Darwins track (Wentworth Falls, GBMWH, NSW), NSW973317
<i>E. moorei</i> Maiden & Cabbage	AO1048, Darwins track (Wentworth Falls, GBMWH, NSW), NSW973316; AO1051, Wentworth Falls Lake (GBMWH, NSW), NSW973329
Subgenus <i>Idiogenes</i> L.D.Pryor & L.A.S.Johnson ex Brooker	
<i>E. cloeziana</i> F.Muell.	SR181, cult. (RBG), Accession number 811164, NSW4138580

Appendix 2. Character matrix used for ancestral reconstructions

Sample codes correspond to those in Table 2 (Column 6). Location details unavailable for *E. cloeziana* and latitude and longitude values unavailable for *E. caliginosa*

Code	Habit	Mean leaf width (mm)	Altitude (m)	Substrate	Latitude (S)	Longitude (E)
<i>apiculata</i> Hill	Mallee	8.50	600	Sandstone	34°19'28.8"	150°28'23.5"
<i>apiculata</i> Wood	Mallee	8.16	589	Sandstone	33°45'19.3"	150°29'8.3"
<i>apiculata</i> Berr	Mallee	5.26	660	Sandstone	34°29'15"	150°15'55"
<i>approximans</i>	Mallee	6.25	1430	Granite	30°24'0"	152°29'45"
<i>burgessiana</i> Lind	Mallee	17.20	584	Sandstone	33°41'44.7"	150°29'27"
<i>burgessiana</i> Warri	Mallee	18.34	295	Sandstone	33°44'23.9"	150°35'2.2"
<i>burgessiana</i> Sprin	Mallee	19.46	433	Sandstone	33°39'20.6"	150°33'25.2"
<i>burgessiana</i> Faul	Mallee	17.93	445	Sandstone	33°37'1.7"	150°33'55.3"
<i>codonocarpa</i> Wara	Mallee	12.00	1180	Granite	29°29'45.2"	152°18'24.9"
<i>codonocarpa</i> Warr	Mallee	13.27	1263	Granite	29°59'3.8"	151°55'30.3"
<i>codonocarpa</i> Girr	Mallee	12.85	1232	Granite	28°51'47.9"	151°57'34.8"
<i>cunninghamii</i> Bank	Mallee	4.87	960	Sandstone	33°35'4.9"	150°22'4.5"
<i>cunninghamii</i> King	Mallee	4.63	794	Sandstone	33°46'15.1"	150°22'33.7"
<i>dendromorpha</i> Went P	Mallee	11.81	833	Sandstone	33°43'33.9"	150°32'13.6"
<i>dendromorpha</i> Went M	Mallee	16.95	846	Sandstone	33°43'39.6"	150°22'29.5"
<i>dendromorpha</i> Wils	Mallee	17.38	1004	Sandstone	33°31'15.7"	150°22'14.8"
<i>dendromorpha</i> Redh	Tree	17.30	635	Sandstone	34°38'47.0"	150°26'10.3"
<i>dendromorpha</i> Fitz	Tree	21.33	658	Sandstone	34°38'54.1"	150°28'47.1"
<i>dendromorpha</i> Blac	Mallee	20.68	960	Sandstone	33°37'40.4"	150°18'42"
<i>dendromorpha</i> Bank	Mallee	17.38	963	Sandstone	33°35'5.8"	150°22'6.1"
<i>fastigata</i>	Tree	11.83	992	Basalt	33°32'57.9"	150°25'26.4"
<i>kybeanensis</i> Wadb	Mallee	10.84	1338	Granite	36°20'31.3"	149°36'7.3"
<i>kybeanensis</i> Kosc	Mallee	11.39	1465	Granite	36°21'23.7"	148°24'35.6"
<i>kybeanensis</i> Snow	Mallee	9.05	1198	Granite	37°6'27.8"	148°11'46.6"
<i>langleyi</i> Brai (Nowra)	Mallee	25.46	224	Sandstone	34°58'25.3"	150°29'40.2"
<i>langleyi</i> Parm	Mallee	24.67	220	Sandstone	34°59'30.6"	150°29'13.3"
<i>langleyi</i> Brai (17 km SW of Nowra)	Mallee	23.79	235	Sandstone	35°0'22.4"	150°28'35.8"
<i>laophila</i> Lith	Mallee	7.83	1114	Sandstone	33°29'55.3"	150°9'59.2"
<i>laophila</i> Woll	Mallee	7.51	939	Sandstone	33°15'23.7"	150°13'7.3"
<i>laophila</i> King	Mallee	6.96	866	Sandstone	33°43'57.8"	150°22'22.3"
<i>laophila</i> Gard	Mallee	8.45	c. 1000	Sandstone	33°16'16"	150°5'58"
<i>microcodon</i>	Mallee	10.56	960–970	Granite	28°22'2"	152°45'47"
<i>obliqua</i>	Tree	24.9	625	Basalt	34°33'33.5"	150°38'22'
<i>obstans</i> Beac	Mallee	10.82	135	Sandstone	33°44'34.8"	151°15'35.6"
<i>obstans</i> Roya	Mallee	19.92	120	Sandstone	34°7'16.4"	151°4'31.7"
<i>obstans</i> Jerv	Mallee	17.79	50	Sandstone	35°0'30.5"	150°49'51.9"
<i>paliformis</i>	Tree	9.8	1305	Sandstone	36°20'30.8"	149°35'47.1"
<i>regnans</i>	Tree	30.99	330–500	Basalt	38°45'32"	143°35'51"
<i>spectatrix</i> Geor	Mallee	12.7	323	Granite	36°39'34.4"	149°54'19.5"
<i>spectatrix</i> Wadb	Mallee	14.31	277	Granite	36°35'17.6"	149°41'21.6"
<i>stricta</i> King L	Mallee	10.36	853	Sandstone	33°44'15.6"	150°22'21.9"
<i>stricta</i> King T	Mallee	9.37	843	Sandstone	33°45'17.6"	150°22'32.7"
<i>stricta</i> Stan	Mallee	9.06	328	Sandstone	34°12'39.7"	150°57'20.4"
<i>stricta</i> Newnes	Mallee	8.44	1183	Sandstone	33°27'10.4"	150°13'53.1"
<i>stricta</i> Sass	Mallee	14.41	738	Sandstone	35°04'22"	150°12'24.2"
<i>stricta</i> Blac	Mallee	9.79	906	Sandstone	33°37'57"	150°18'48"
<i>stricta</i> Kato	Mallee	6.98	965	Sandstone	33°44'3.7"	150°16'56.2"
<i>stricta</i> Bank	Mallee	9.54	940	Sandstone	33°34'59.8"	150°22'1.5"
<i>triflora</i>	Tree	18.04	750	Sandstone	35°5'	150°9'
<i>caliginosa</i>	Tree	18.36	800–950	Probably granite		
<i>consideniana</i> Brai (Nowra)	Tree	16.56	224	Sandstone	34°58'25.7"	150°29'37.2"
<i>consideniana</i> Wood	Tree	17.08	587	Sandstone	33°45'20.3"	150°29'7.4"
<i>copulans</i> Went J	Tree	10.38	850	Sandstone	33°42'32"	150°22'26"
<i>copulans</i> Went D	Tree	12.45	850	Sandstone	33°43'0.2"	150°22'31.5"
<i>cloeziana</i>	Tree	13.38				
<i>deuaensis</i>	Mallee	11.28	660	Rhyolite	35°49'	149°49'
<i>haemastoma</i> Beac	Tree	26.81	139	Sandstone	33°44'35.1"	151°15'35.7"
<i>haemastoma</i> Roya	Tree	30.32	118	Sandstone	34°7'16.3"	151°4'31.5"

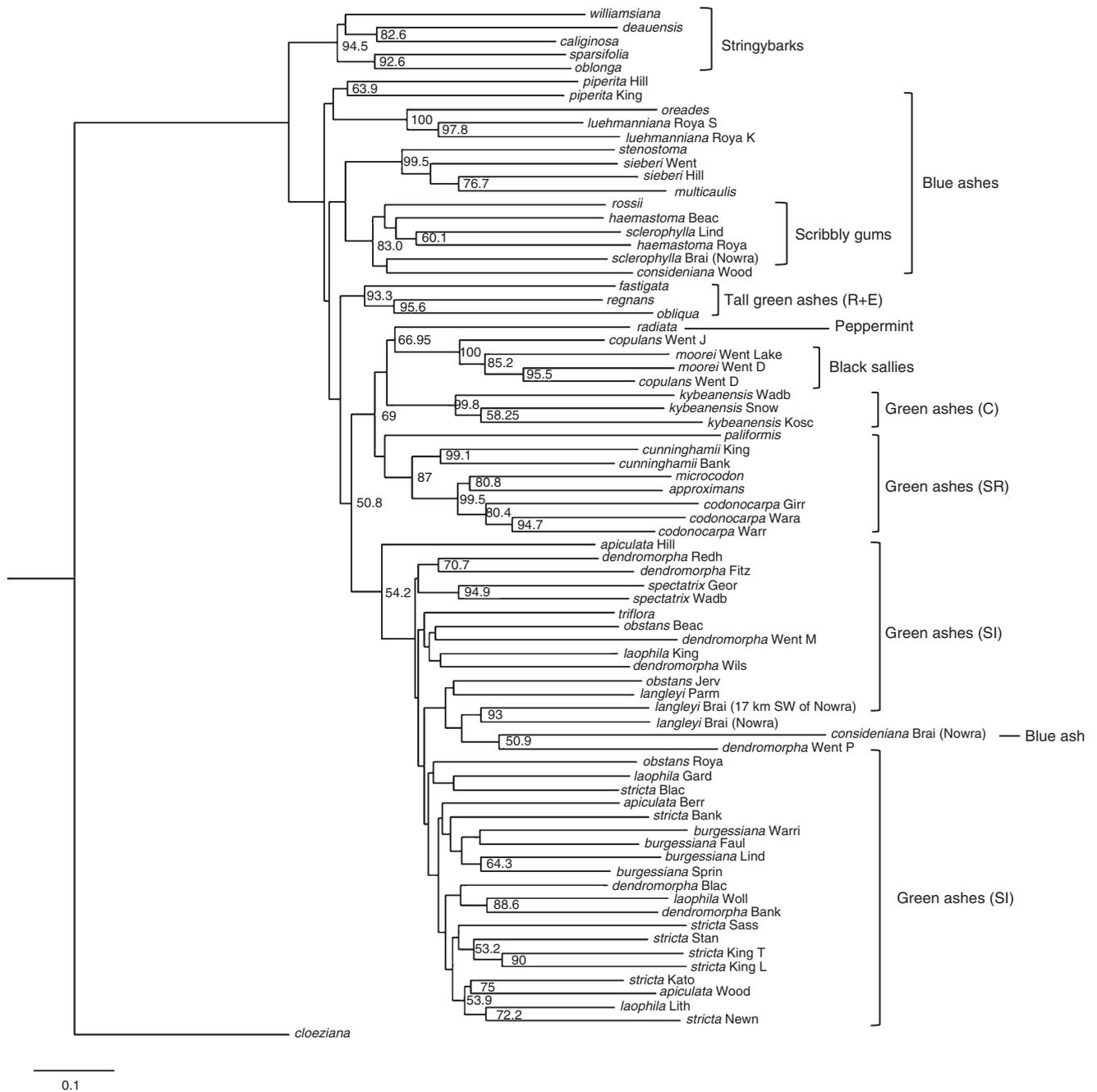
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Appendix 2. (continued)

Code	Habit	Mean leaf width (mm)	Altitude (m)	Substrate	Latitude (S)	Longitude (E)
<i>luehmanniana</i> Roya K	Mallee	25.75	115	Sandstone	34°6'16"	151°2'5"
<i>luehmanniana</i> Roya S	Mallee	22.81	116	Sandstone	34°7'16.6"	151°4'31"
<i>moorei</i> Went D	Mallee	8.71	850	Sandstone	33°42'60"	150°22'31.5"
<i>moorei</i> Went Lake	Mallee	7.46	890	Sandstone	33°42'3.4"	150°22'16.8"
<i>multicaulis</i>	Mallee	14.71	583	Sandstone	33°41'54.1"	150°29'27.6"
<i>oblonga</i>	Tree	14.69	330	Sandstone	34°12'38.9"	150°57'21.6"
<i>oreades</i>	Tree	20.29	969	Sandstone	33°44'2.4"	150°16'58"
<i>piperita</i> King	Tree	19.49	842	Sandstone	33°45'19.2"	150°22'34"
<i>piperita</i> Hill	Tree	21.44	598	Sandstone	34°19'29.4"	150°28'25.7"
<i>radiata</i>	Tree	9.09	967	Sandstone	33°44'2.3"	150°16'58"
<i>rossii</i>	Tree	10.58	930	Sandstone	33°15'22.6"	150°13'6.4"
<i>sclerophylla</i> Lind	Tree	27.15	583	Sandstone	33°41'44.8"	150°29'26.3"
<i>sclerophylla</i> Brai (Nowra)	Tree	24.72	221	Sandstone	34°58'26.2"	150°29'36.4"
<i>sieberi</i> Hill	Tree	17.50	600	Sandstone	34°19'29.7"	150°28'25.6"
<i>sieberi</i> Went	Tree	18.26	850	Sandstone	33°43'33.8"	150°22'19.3"
<i>sparsifolia</i>	Tree	12.46	583	Sandstone	33°41'44.8"	150°29'26.3"
<i>stenostoma</i>	Tree	12.87	712	Sandstone	36°32'12.8"	149°38'57.6"
<i>williamsiana</i>	Tree	40.52	716	Granite	35°25'2"	149°14'51"

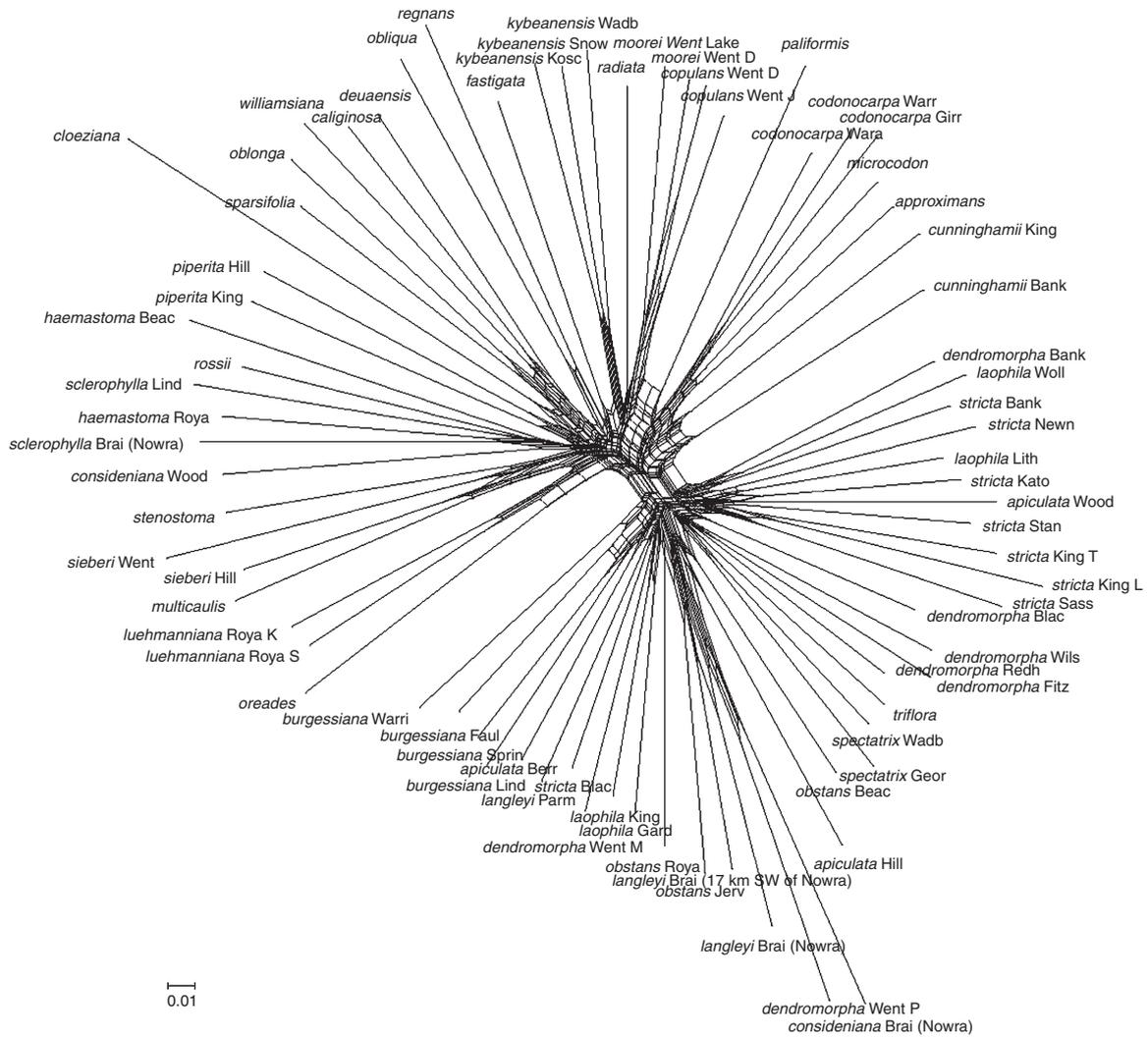
Appendix 3. Phylogeny derived from 1780 Diversity Arrays Technology (DArT) markers analysed with Partitioned Additive Dollo Distance (PADD) and minimum-evolution tree estimation of the green ashes (section *Eucalyptus*) and other taxa in subgenus *Eucalyptus*

Includes the black sallies (section *Longitudinales*), blue ashes (including the scribbly gums, section *Cineraceae*), peppermints (section *Aromatica*) and stringybarks (section *Capillulus*). *Eucalyptus cloeziana* (subgenus *Idiogenes*) is the outgroup. Sample codes correspond to those in Table 2 (Column 6). Series and subseries (Brooker 2000) within the green ashes are shown: *Regnantes* (R), *Eucalyptus* (E), *Strictae* subseries *Irregulares* (SI), *Strictae* subseries *Regulares* (SR) and *Contiguae* (C). Node numbers represent bootstrap values greater than 50%



Appendix 4. Network generated in *SplitsTree4* (version 4.13.1) based on 2702 Diversity Arrays Technology (DArT) markers

Relationships among the green ashes (section *Eucalyptus*) and other taxa in subgenus *Eucalyptus* (sections *Longitudinales*, *Cinereae*, *Aromatica* and *Capillus*). Sample codes correspond to those in Table 2 (Column 6). Scale bar shows uncorrected *P* genetic distance equivalent to 0.01



Appendix 5. Comparison of the published classification of subgenus *Eucalyptus* by Brooker (2000) with the online draft scheme of subgenus *Eucalyptus* section *Eucalyptus* by Nicolle (2015)

Asterisks indicate dubious species; hashes indicate possible or probable hybrid. Authors of plant names are given in Table 2 and authors of both species and higher taxonomic ranks are listed in Appendices 1 and 5

Brooker (2000)	Nicolle (2015)
Subgenus <i>Eucalyptus</i>	Subgenus <i>Eucalyptus</i>
Section <i>Amentum</i> Brooker	Section <i>Eucalyptus</i>
<i>E. acmenoides</i> Schauer	Series <i>White-mahoganies</i> Blakely (white mahoganies)
<i>E. apothalassica</i> L.A.S.Johnson & K.D.Hill	<i>E. acmenoides</i> Schauer
<i>E. psammitica</i> L.A.S.Johnson & K.D.Hill	<i>E. apothalassica</i> L.A.S.Johnson & K.D.Hill
<i>E. carnea</i> R.T.Baker	<i>E. psammitica</i> L.A.S.Johnson & K.D.Hill
<i>E. umbra</i> R.T.Baker	<i>E. carnea</i> R.T.Baker
	<i>E. umbra</i> R.T.Baker
	<i>E. mediocris</i> L.A.S.Johnson & K.D.Hill
	<i>E. irritans</i> L.A.S.Johnson & K.D.Hill
	<i>E. helidonica</i> K.D.Hill
	<i>E. portuensis</i> K.D.Hill
	<i>E. latisinensis</i> K.D.Hill
Section <i>Pseudophloius</i> Brooker	Series <i>Pseudostringybarks</i> Blakely
<i>E. pyrocarpa</i> L.A.S.Johnson & Blaxell	<i>E. pyrocarpa</i> L.A.S.Johnson & Blaxell
<i>E. pilularis</i> Sm.	<i>E. pilularis</i> Sm.
Section <i>Aromatica</i> Brooker	
Series <i>Radiatae</i> Chippendale	Series <i>Radiatae</i> Chippendale (peppermints)
<i>E. elata</i> Dehnh.	<i>E. elata</i> Dehnh.
<i>E. radiata</i> Sieber ex DC.	<i>E. radiata</i> Sieber ex DC.
<i>E. croajingolensis</i> L.A.S.Johnson & K.D.Hill	<i>E. croajingolensis</i> L.A.S.Johnson & K.D.Hill
<i>E. willisii</i> Ladiges, Humphries & Brooker	<i>E. willisii</i> Ladiges, Humphries & Brooker
<i>E. dives</i> Schauer	<i>E. dives</i> Schauer
Series <i>Insulanae</i> Brooker	<i>E. amygdalina</i> Labill.
<i>E. amygdalina</i> Labill.	<i>E. pulchella</i> Desf.
<i>E. pulchella</i> Desf.	<i>E. tenuiramis</i> Miq.
<i>E. tenuiramis</i> Miq.	<i>E. risdonii</i> Hook.f.
<i>E. risdonii</i> Hook.f.	<i>E. nitida</i> Hook.f.
<i>E. nitida</i> Hook.f.	<i>E. coccifera</i> Hook.f.
<i>E. coccifera</i> Hook.f.	<i>E. robertsonii</i> Blakely
	<i>E. nebulosa</i> A.M.Gray
	<i>E. arenicola</i> Rule
	<i>E. falciformis</i> (Newnham, Ladiges & Whiffin) Rule
	<i>E. molyneuxii</i> Rule* (possibly <i>E. falciformis</i> (Newnham, Ladiges & Whiffin) Rule)
Section <i>Capillulus</i> Brooker	
Series <i>Pachyphloiae</i> Blakely	Series <i>Pachyphloiae</i> Blakely (stringybarks)
<i>E. muelleriana</i> A.W.Howitt	<i>E. muelleriana</i> A.W.Howitt
<i>E. laevopinea</i> R.T.Baker	<i>E. laevopinea</i> R.T.Baker
<i>E. macrorrhyncha</i> F.Muell.	<i>E. macrorrhyncha</i> F.Muell.
<i>E. cannonii</i> R.T.Baker	<i>E. expressa</i> S.A.J.Bell & D.Nicolle
<i>E. mackintii</i> Kottek	<i>E. mackintii</i> Kottek
<i>E. williamsiana</i> L.A.S.Johnson & K.D.Hill	<i>E. williamsiana</i> L.A.S.Johnson & K.D.Hill
<i>E. youmanii</i> Blakely & McKie	<i>E. youmanii</i> Blakely & McKie
<i>E. capitellata</i> Sm.	<i>E. capitellata</i> Sm.
<i>E. cameronii</i> Blakely & McKie	<i>E. cameronii</i> Blakely & McKie
<i>E. globoidea</i> Blakely	<i>E. globoidea</i> Blakely
<i>E. caliginosa</i> Blakely & McKie	<i>E. caliginosa</i> Blakely & McKie
<i>E. eugenioides</i> Sieber ex Spreng.	<i>E. eugenioides</i> Sieber ex Spreng.
<i>E. sparsifolia</i> Blakely	<i>E. sparsifolia</i> Blakely
<i>E. tenella</i> L.A.S.Johnson & K.D.Hill	<i>E. tenella</i> L.A.S.Johnson & K.D.Hill
<i>E. mckieana</i> Blakely	<i>E. mckieana</i> Blakely
<i>E. ligustrina</i> DC.	<i>E. ligustrina</i> DC.
<i>E. conglomerata</i> Maiden & Blakely	<i>E. conglomerata</i> Maiden & Blakely
<i>E. tindaliae</i> Blakely	<i>E. tindaliae</i> Blakely
<i>E. agglomerata</i> Maiden	<i>E. agglomerata</i> Maiden
<i>E. blaxlandii</i> Maiden & Cambage	<i>E. blaxlandii</i> Maiden & Cambage

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Appendix 5. (continued)

Brooker (2000)	Nicolle (2015)
<i>E. camfieldii</i> Maiden	<i>E. camfieldii</i> Maiden
<i>E. bensonii</i> L.A.S.Johnson & K.D.Hill	<i>E. bensonii</i> L.A.S.Johnson & K.D.Hill
<i>E. arenacea</i> Marginson & Ladiges	<i>E. arenacea</i> Marginson & Ladiges
<i>E. imitans</i> L.A.S.Johnson & K.D.Hill	<i>E. imitans</i> L.A.S.Johnson & K.D.Hill
<i>E. serraensis</i> Ladiges & Whiffin	<i>E. serraensis</i> Ladiges & Whiffin
<i>E. verrucata</i> Ladiges & Whiffin	<i>E. verrucata</i> Ladiges & Whiffin
<i>E. curta</i> Brooker	<i>E. oblonga</i> DC.* (possibly <i>E. globoidea</i> Blakely)
<i>E. baxteri</i> (Benth.) Maiden & Blakely ex J.M.Black	<i>E. yangoura</i> Blakely* (possibly <i>E. globoidea</i> Blakely)
Series <i>Limitares</i> Brooker	<i>E. erosa</i> A.R.Bean* (possibly <i>E. eugenioides</i> Sieber ex Spreng.)
<i>E. deuaensis</i> Boland & Gilmour	<i>E. aurifodina</i> Rule
	<i>E. alpina</i> Maiden
	<i>E. victoriana</i> Ladiges & Whiffin
	<i>E. reducta</i> L.A.S.Johnson & K.D.Hill* (possibly <i>E. tindaliae</i> Blakely)
	<i>E. prominula</i> L.A.S.Johnson & K.D.Hill* (possibly <i>E. youmanii</i> Blakely & McKie)
Section <i>Nebulosa</i> Brooker	Series <i>Olsenianae</i> Chippendale
<i>E. olsenii</i> L.A.S.Johnson & Blaxell	<i>E. olsenii</i> L.A.S.Johnson & Blaxell
	<i>E. boliviana</i> J.B.Williams & K.D.Hill
	<i>E. deuaensis</i> Boland & Gilmour
Section <i>Eucalyptus</i>	Series <i>Regnantes</i> Chippendale
Series <i>Regnantes</i> Chippendale	<i>E. fastigata</i> H.Deane & Maiden
<i>E. fastigata</i> H.Deane & Maiden	<i>E. regnans</i> F.Muell.
<i>E. regnans</i> F.Muell.	Series <i>Eucalyptus</i>
Series <i>Eucalyptus</i>	<i>E. obliqua</i> L'Hér.
<i>E. obliqua</i> L'Hér.	Series <i>Strictae</i> L.A.S.Johnson ex Brooker (mallee ashes)
Series <i>Strictae</i> L.A.S.Johnson ex Brooker	Subseries <i>Irregulares</i> Brooker
Subseries <i>Irregulares</i> Brooker	<i>E. triflora</i> (Maiden) Blakely
<i>E. triflora</i> (Maiden) Blakely	<i>E. dendromorpha</i> (Blakely) L.A.S.Johnson & Blaxell
<i>E. dendromorpha</i> (Blakely) L.A.S.Johnson & Blaxell	<i>E. apiculata</i> R.T.Baker & H.G.Sm. (includes <i>E. laophila</i> L.A.S.Johnson & Blaxell)
<i>E. apiculata</i> R.T.Baker & H.G.Sm. (includes <i>E. laophila</i> L.A.S.Johnson & Blaxell)	<i>E. stricta</i> Sieber ex Spreng. (includes <i>E. spectatrix</i> L.A.S.Johnson & Blaxell)
<i>E. stricta</i> Sieber ex Spreng. (includes <i>E. spectatrix</i> L.A.S.Johnson & Blaxell)	<i>E. burgessiana</i> L.A.S.Johnson & Blaxell (includes <i>E. obstans</i> L.A.S.Johnson & K.D.Hill)
<i>E. burgessiana</i> L.A.S.Johnson & Blaxell (includes <i>E. obstans</i> L.A.S.Johnson & K.D.Hill)	<i>E. langleyi</i> L.A.S.Johnson & Blaxell
<i>E. langleyi</i> L.A.S.Johnson & Blaxell	<i>E. spectatrix</i> L.A.S.Johnson & Blaxell* (possibly <i>E. stricta</i> Sieber ex Spreng.)
	<i>E. cunninghamii</i> G.Don
Subseries <i>Regulares</i> Brooker	Subseries <i>Regulares</i> Brooker
<i>E. approximans</i> Maiden (includes <i>E. codonocarpa</i> Blakely & McKie and <i>E. microcodon</i> L.A.S.Johnson & K.D.Hill)	<i>E. approximans</i> Maiden
	<i>E. microcodon</i> L.A.S.Johnson & K.D.Hill
<i>E. cunninghamii</i> G.Don	<i>E. codonocarpa</i> Blakely & McKie
<i>E. paliformis</i> L.A.S.Johnson & Blaxell	<i>E. paliformis</i> L.A.S.Johnson & Blaxell
Series <i>Contiguae</i> Brooker & Snee	<i>E. kybeanensis</i> Maiden & Cabbage
<i>E. kybeanensis</i> Maiden & Cabbage	
Section <i>Longitudinales</i> (Blakely) Brooker	Series <i>Longitudinales</i> (Blakely) Brooker (sallees)
<i>E. mitchelliana</i> Cabbage	<i>E. mitchelliana</i> Cabbage
<i>E. stellulata</i> Sieber ex DC.	<i>E. stellulata</i> Sieber ex DC.
<i>E. moorei</i> Maiden & Cabbage	<i>E. moorei</i> Maiden & Cabbage
	<i>E. forresterae</i> Molyneux & Rule * (possibly <i>E. stellulata</i> Sieber ex DC.)
	<i>Eucalyptus</i> × <i>copulans</i> L.A.S.Johnson & K.D.Hill # (<i>E. moorei</i> Maiden & Cabbage subsp. <i>moorei</i> × <i>E. stellulata</i> Sieber ex DC. hybrid)
Section <i>Cineraceae</i> Brooker	Series <i>Fraxinales</i> Blakely
Series <i>Fraxinales</i> Blakely	<i>E. fraxinoides</i> H.Deane & Maiden
<i>E. fraxinoides</i> H.Deane & Maiden	<i>E. luehmanniana</i> F.Muell.
<i>E. luehmanniana</i> F.Muell.	

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Appendix 5. (continued)

Brooker (2000)	Nicolle (2015)
<i>E. oreades</i> R.T.Baker	<i>E. oreades</i> R.T.Baker
<i>E. delegatensis</i> R.T.Baker	<i>E. delegatensis</i> R.T.Baker
Series <i>Sphaerocarpace</i> Brooker	<i>E. sphaerocarpa</i> L.A.S.Johnson & Blaxell
<i>E. sphaerocarpa</i> L.A.S.Johnson & Blaxell	
Series <i>Pauciflorae</i> L.A.S.Johnson ex Brooker & Slee	Series <i>Pauciflorae</i> L.A.S.Johnson ex Brooker & Slee (snow gums)
<i>E. lacrimans</i> L.A.S.Johnson & K.D.Hill	<i>E. lacrimans</i> L.A.S.Johnson & K.D.Hill
<i>E. gregsoniana</i> L.A.S.Johnson & Blaxell	<i>E. gregsoniana</i> L.A.S.Johnson & Blaxell
<i>E. pauciflora</i> Sieber ex Spreng.	<i>E. pauciflora</i> Sieber ex Spreng.
Series <i>Psathyroxylon</i> Blakely	Series <i>Psathyroxyla</i> Blakely
Subseries <i>Considenianae</i> Brooker & Slee	Subseries <i>Considenianae</i> Brooker & Slee
<i>E. sieberi</i> L.A.S.Johnson	<i>E. sieberi</i> L.A.S.Johnson
<i>E. multicaulis</i> Blakely	<i>E. multicaulis</i> Blakely
<i>E. remota</i> Blakely	<i>E. remota</i> Blakely
<i>E. consideniana</i> Maiden	<i>E. consideniana</i> Maiden
<i>E. andrewsii</i> Maiden	<i>E. andrewsii</i> Maiden
<i>E. olida</i> L.A.S.Johnson & K.D.Hill	<i>E. olida</i> L.A.S.Johnson & K.D.Hill
	<i>E. campanulata</i> R.T.Baker & H.G.Sm.
Subseries <i>Haemastomae</i> Brooker	Subseries <i>Haemastomae</i> Brooker (scribbly gums)
<i>E. racemosa</i> Cav.	<i>E. racemose</i> Cav.
<i>E. haemastoma</i> Sm.	<i>E. haemastoma</i> Sm.
<i>E. rossii</i> R.T.Baker & H.G.Sm.	<i>E. rossii</i> R.T.Baker & H.G.Sm.
Series <i>Stenostomae</i> Brooker	Series <i>Stenostomae</i> Brooker
<i>E. stenostoma</i> L.A.S.Johnson & Blaxell	<i>E. stenostoma</i> L.A.S.Johnson & Blaxell
Series <i>Piperitales</i> Blakely	Series <i>Piperitales</i> Blakely
<i>E. piperita</i> Sm.	<i>E. piperita</i> Sm.
Section <i>Insolitae</i> Brooker	Series <i>Planchoniana</i> Chippendale
<i>E. planchoniana</i> F.Muell.	<i>E. planchoniana</i> F.Muell.
Section <i>Pedaria</i> L.A.S.Johnson ex Brooker	
<i>E. brevistylis</i> Brooker	
Section <i>Longistylus</i> Brooker	
Subsection <i>Arboreae</i> Brooker	
Series <i>Jacksoniae</i> Brooker	
<i>E. jacksonii</i> Maiden	
Series <i>Occidentales</i> Blakely	
<i>E. marginata</i> Donn ex Sm.	
<i>E. staeri</i> Kessell & C.A.Gardner	
Series <i>Patentes</i> L.A.S.Johnson ex Brooker	
<i>E. patens</i> Benth.	
Subsection <i>Frutices</i> Brooker	
Series <i>Diversiformae</i> Blakely	
Subseries <i>Neuropterae</i> (Maiden) Brooker	
<i>E. diversifolia</i> Bonpl.	
<i>E. pachyloma</i> Benth.	
<i>E. erectifolia</i> Brooker & Hopper	
<i>E. lateritica</i> Brooker & Hopper	
<i>E. todtiana</i> F.Muell.	
<i>E. johnsoniana</i> Brooker & Blaxell	
Subseries <i>Cochleatae</i> (Maiden) Brooker	
<i>E. buprestium</i> F.Muell.	
Subseries <i>Finales</i> Brooker	
<i>E. dolorosa</i> Brooker & Hopper	
Series <i>Angulares</i> Brooker	
<i>E. angularis</i> Brooker & Hopper	
Series <i>Muricatae</i> Maiden	
<i>E. exilis</i> Brooker	
<i>E. pendens</i> Brooker	
<i>E. sepulcralis</i> F.Muell.	
Series <i>Calcicolae</i> Brooker	
<i>E. calcicola</i> Brooker	

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Appendix 5. (*continued*)

Brooker (2000)

Nicolle (2015)

-
- E. ligulata* Brooker
 - Series *Preissianae* L.D.Pryor & L.A.S.Johnson ex Brooker & Slee
 - Subseries *Glandulares* Blakely
 - E. megacarpa* F.Muell.
 - E. aquilina* Brooker
 - E. coronata* C.A.Gardner
 - Subseries *Pluriloculares* Blakely
 - E. preissiana* Schauer
 - Series *Proximae* Brooker
 - E. acies* Brooker
 - Series *Subereae* Chippendale
 - E. suberea* Brooker & Hopper
 - Subsection *Unicae* Brooker
 - E. insularis* Brooker
-