

PHYLOGENY AND HISTORICAL BIOGEOGRAPHY
OF THE AUSTRALIAN CAMPHOROSMEAE
(CHENOPODIACEAE)

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1 INTRODUCTION

1.1 Systematics of Camphorosmeae (Salsoloideae, Chenopodiaceae)

Chenopodiaceae are a family of herbs, shrubs or subshrubs particularly successful in dry, saline or disturbed habitats of temperate and subtropical climates in the northern and southern hemispheres. In Australia, the family characterizes the vast arid and saline desert and semi-desert regions as well as coastal and inland salt marshes. It is thus considered a key component of the continent's modern flora (Diels, 1906; Christophel, 1989). Chenopodiaceae are a prominent element of the South Australian vegetation in the so called chenopod shrublands (Figure 1: AUSLIG, 1990). These regions are dominated mostly by species of *Atriplex* and *Maireana*, which, together with other genera of Chenopodiaceae, also show isolated dominance in certain habitats of the remaining floristic provinces of the continent. The Australian flora comprises 32 genera with 302 species of Chenopodiaceae, of which 28 genera and 279 species are endemic (Wilson, 1984). The native Chenopodiaceae occurring in the Australian continent belong to five different tribes/subfamilies (Kadereit et al., 2005), i.e., Camphorosmeae (147 species, 12 genera), Salicornieae (36 species, six genera), Suaedeae (two species, one genus), Salsoleae (one species), and Chenopodioideae (99 species; six genera).

Camphorosmeae are the most species-rich tribe of Chenopodiaceae in Australia, with 147 of the 149 currently recognized species in 14 genera being endemic to the continent. Of these, *Sclerolaena* (64 spp.) and *Maireana* (57 spp.), together with *Atriplex* (57 native species; Chenopodioideae), are the most well-represented genera of Chenopodiaceae in Australia. The degree of endemism in the Australian Camphorosmeae is nearly 100% compared to 80% average for Australian plant species in general (Crisp et al., 1999). The tribe Camphorosmeae, though essentially adapted to the arid and semi-arid conditions of Central and West Australia, is present in all floristic regions of the continent, occurring as well in tropical North Australia and temperate and Mediterranean South Australia mainly in the so called interzones (Burbidge, 1960). Several species are salt or gypsum tolerant and their occurrence is linked to inland salt lakes (e.g. *Didymanthus roei*, *Maireana amoena* and *Sclerolaena fimbriolata*, Wilson, 1984). Some species extend into agricultural lands and coastal habitats where they are able to tolerate slightly saline soils;

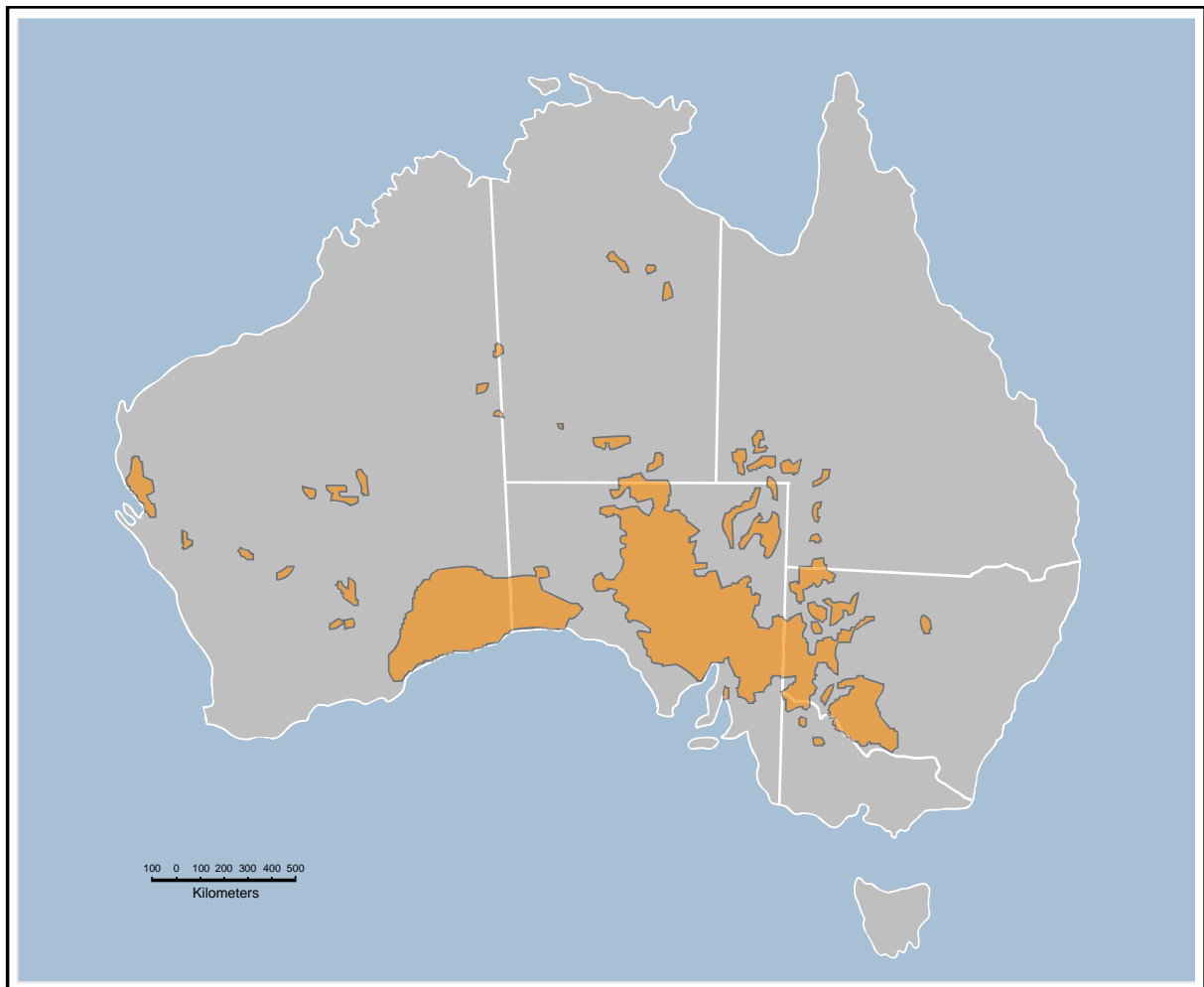


Figure 1. Chenopod Shrublands in Australia (shaded orange). Areas where shrubs of Chenopodiaceae are prominent in the natural vegetation (modified from AUSLIG, 1990).

nevertheless, some taxa prefer non-saline conditions (e.g., *Eriochiton sclerolaenoides* and *Maireana convexa*, Wilson, 1984). Furthermore, the distribution patterns are very variable with species being present only in few localities, such as *Roycea spinescens*, to widespread, such as *Enchylaena tomentosa* and *Maireana georgei*. *Enchylaena tomentosa* is also present in New Caledonia, and the coastal species *Threlkeldia diffusa* also occurs along the northern coast of Tasmania.

Camphorosmeae belong to subfamily Salsoloideae (Kadereit et al., 2003), and comprise ca. 30 to 40 extra-Australian and 149 Australian species. Outside Australia, Camphorosmeae are centered in Europe, and West and Central Asia. A few species also occur in northern and South Africa, as well as in North America.

The taxonomic history of Camphorosmeae during the last 75 years has been characterized by a number of rearrangements. Ulbrich (1934) classified this tribe under Cyclolobeae uniting all tribes of Chenopodiaceae that have a more or less annular embryo, whereas Scott (1978a) elevated the group to subfamily rank and divided it into three different tribes (Table 1). His tribes were distinguished from each other on the basis of the general plant habit, and the characters of the fruiting perianth. Camphorosmeae *sensu* Scott contained genera that are essentially Eurasian in distribution and frequently annual with thin perianths and tepaline appendages. The tribes Sclerolaeneae and Maireaneae contained all the Australian genera. These two were distinguished from each other by the position of the wings, spines or lobes on the fruiting perianth. Kühn and colleagues (1993) subsumed Camphorosmioideae under Chenopodioideae and reorganized Scott's tribes placing all Australian genera into Sclerolaeneae.

Recent molecular phylogenetic studies in Chenopodiaceae resolved Camphorosmeae as monophyletic within Salsoloideae (Kadereit et al., 2003; 2005). The tribe as a whole can be distinguished from other Salsoloideae by the absence of bracteoles, a horseshoe- or ring-like embryo (versus a spirally twisted embryo in other Salsoloideae), and by leaf anatomical characters. Molecular data (Kadereit et al., 2003) suggest that the Australian lineage of Camphorosmeae (= Sclerolaeneae *sensu* Kühn et al., 1993) is monophyletic and is derived from within the Holarctic Camphorosmeae, being resolved sister to a species-poor clade of Central Asian taxa (Kadereit et al., 2005).

Table 1. Classification of Camphorosmioideae (Scott, 1978a).

Tribe	General Characteristics
Camphorosmeae	Annuals with herbaceous stems; inflorescences spicate or paniculate; fruits unmodified and with tepaline appendages
Sclerolaeneae	Perennials with usually solitary flowers; fruits with intertepaline spines, lobes or vertical wings
Maireaneae	Perennials with usually solitary flowers; fruits usually with tepaline wings, spines or lobes

The genera of the Holarctic and Australian Camphorosmeae show great morphological similarity and are difficult to delimitate (Wilson, 1975; Scott, 1978a). All endemic Australian Camphorosmeae exhibit non-Kranz type leaf anatomy typical of the C₃ pathway of photosynthesis, whereas most Eurasian taxa are C₄ plants (Carolin et al., 1975; Kadereit et al., 2003). The Australian species have at various times been included in *Bassia* (or *Echinopsilon*), *Kochia*, and *Chenolea*. Nevertheless, Scott (1978a) and Wilson (1975) pointed out three main morphological differences between Australian and extra-Australian Camphorosmeae: (1) the Australian species are mainly perennials with a woody stem while the extra-Australian species are mainly annuals with an herbaceous stem. (2) The flowers of the Australian species are solitary or paired while those of the extra-Australian species are aggregated in condensed cymes. (3) The fruiting perianth of the Australian Camphorosmeae is typically modified opposite the radicle (radicular anomaly) while the perianth of extra-Australian Camphorosmeae remains chartaceous and is not modified opposite the radicle. However, none of these characters are unique to either the Australian or the Holarctic lineages of Camphorosmeae (Kadereit et al., 2005) and the only consistent differences between the two groups are found in the fruiting perianth.

Wilson (1984) pointed out that generic delimitation within the Australian Camphorosmeae is likely to be artificial. The different genera show strong affinities with each other based on both vegetative and reproductive structures, so that a clear delimitation of taxa remains problematic. Members of Camphorosmeae in Australia are distinguished from one another mainly on the basis of fruiting perianth characters (Figure 2). The perianth in most cases becomes hardened and develops appendages in the fruiting stage.



Figure 2. Representative species of the Australian Camphorosmeae showing the different fruiting perianth types and the variety of fruiting perianth appendages.

A – *Dissocarpus paradoxus* showing a woody and woolly aggregate fruit possessing spines; B – *Sclerolaena obliquicuspis* with closely woolly fruits possessing 2 obliquely spreading spines; C – *S. anisacanthoides* exhibiting a glabrous and cylindrical fruiting perianth bearing 6 erect spines; D – *Threlkeldia diffusa* with a succulent fruiting perianth bearing a cup-shaped outgrowth; E – *Enchylaena tomentosa* showing a succulent fruiting perianth with a vertical slit opposite the radicle; F – *Maireana pentatropis* bearing a horizontal wing characteristic of the genus (Photo credits: A-E, S. Jacobs; F, R. Saffrey).

Although these appendages exhibit a range of morphological plasticity, they are indispensable in identifying species (Wilson 1984).

Wilson (1984) while recognizing the complexity in the taxonomy of the group avoided mass rearrangements of species because additional evidences, such as molecular data, were lacking.

Recent molecular phylogenetic studies on the Australian Chenopodiaceae indicate that Camphorosmeae entered Australia during the late Miocene or early Pliocene probably originating from Central Asian ancestors (Kadereit et al., 2005). In this study which is based on *rbcL* and ITS sequence data, the clade of the Australian Camphorosmeae remained largely unresolved. Nevertheless, a polyphyletic *Maireana* appears to be supported within the tribe. The molecular markers used in the abovementioned study do not have a sufficient amount of nucleotide variation to give a clear phylogenetic signal for the Australian clade. This lack of variation was interpreted by Kadereit et al. (2005) as a fast radiation of the ancestors of the tribe shortly after their arrival in Australia which did not allow for the accumulation of numerous mutations. Another possibility is that these markers are not suitable in dealing with phylogenetic problems at the generic level in Camphorosmeae.

1.2 Australian aridification

The arid or Eremaean zone in Australia is generally defined in terms of the 250 mm isohyet. So defined, 65 to 70 percent of the Australian continental land area at present time is considered arid or semi-arid (Barlow, 1981; AUSLIG, 1990). The development of aridification in Australia, whether regional or continental, has been the subject of numerous studies. These studies based mainly on stratigraphy, clay mineralogy, palaeobotany, and palaeogeography agree that the Australian environment has been influenced by the geological evolution of the continent as a result of its changing position on the globe, the evolution of its landforms, and wide swings in the global climate (Frakes, 1999). Furthermore, almost all of these hypotheses suggest that the onset of aridification in Australia was sometime during the Tertiary, but admittedly with discrepancies (Burbidge, 1960; Beard, 1977; Bowler, 1976, 1982; Quilty, 1982; Stein & Robert, 1986; Markgraf et al., 1995; Martin, 1978, 1998; Zheng et al., 1998; Frakes, 1999).

The climatic history of Australia from the mid-Tertiary onward has largely been one of increasing aridity. Several studies suggest that most of Australia underwent extensive aridification beginning approximately 10–15 million years ago (Beard, 1977; Stein & Robert, 1986; Markgraf *et al.*, 1995; Martin, 1998). Other studies propose that aridification commenced near the end of the Miocene (ca. 5–6 Myr) based on findings that the prehistoric eastern rainforests of the continent contracted and fragmented at this time (Kemp 1981; Singh & Geissler, 1985). Evidence for rainforest contraction comes from both palynology (Martin, 1978, 1998) and comparative phylogeography (Schneider *et al.*, 1998). However, it has been established that the arid zone has experienced increasingly intense cyclical climatic fluctuations all throughout the Quaternary (Crocker & Wood, 1947; Burbidge, 1960; Bowler 1982; Schodde, 1989; Martin, 1998). The following section presents several hypotheses on the Tertiary aridification of Australia.

Quilty (1982) hypothesized the inception of aridity in the Eocene some 50 million years ago based on evidences from sedimentation off the western margin of the continent. Laterite formation or terrigenous sedimentation requires decomposition of underlying rocks in warm and humid climates. Carbonate sedimentation on the other hand is the formation of sedimentary rock by the precipitation of organic or inorganic carbon from aqueous solutions of carbonates. The change from terrigenous to carbonate sedimentation infers cessation of efficient drainage from the land, and thus the drying up of river systems or lacustrine bodies. According to Quilty (1982), an episode of such a shift was recorded in the Eocene, indicating the start of aridification in Australia.

Beard (1977) put forward a hypothesis based on continental drift stating that aridity started in the northwest of the continent in the mid Eocene, some 45 million years ago. Australia has drifted 15° of latitude northwards since breaking away from Antarctica, reaching the dry anticyclone belt, and thus gradually drifting into aridity. By the Oligocene, desert conditions were established in the north and northwest, with the first Mediterranean climatic zone appearing, also in northwest Australia. By the Miocene, deserts extended to almost their present limits, and the Mediterranean zone had moved southward to become more extensive than at present. Based on his findings, the age of extensive aridity on Australia would be at least 15 Myr, and it would have impinged on the Australian continent from the north.

Relationships between different types of clay minerals may be used as palaeoclimatic indicators (Singer, 1980). Two such clay minerals used in studying climatic history are smectite and illite. Smectite is a type of soil formed normally in humid to semi-arid climates. Illite, on the other hand, is formed from predominantly physical weathering. The smectite/illite ratio over a period of geological time can serve as an indicator of palaeoclimate (Stein & Robert, 1986). Low ratios indicate aridity, while high ratios suggest otherwise. In the mid-Miocene (ca. 14-15 Myr), the S/I ratio has been reported to decline, suggesting an increased aridification in the northern to central parts of the continent (Stein & Robert, 1986). Southern Australia at the same time may have been dominated by alternating humid and semi-arid climatic conditions. It was not until the Late Miocene that decreased S/I ratios in the south were observed. This Miocene southwards extension of aridity coincided with the late Miocene low sea level and the palaeobotanical evidence for a drier climate. This hypothesis concurs with Beard's theory based on continental drift.

Based on palaeobotanical data but mainly on pollen fossil evidence, the trend to a drier climate started in the Miocene and continued throughout the Pliocene (Martin, 1998). From the Upper Eocene to the Early Miocene southern Australia may be generalized to have been dominated by a complex closed forest system, experiencing warm temperate to tropical climate (Christophel, 1989). During the Oligocene-Miocene boundary, recovered palynological records still contained rainforest species, a little *Nothofagus* and abundant swamp taxa. However, the mid-Miocene was a time of profound change, when there was a drastic turnover from rainforests to myrtaceous and sclerophyllous forests. By the end of the Tertiary, ca. 2-3 million years ago, arid conditions approaching those of today had already been reached, but aridity has intensified especially in the last half million years (Martin, 1998). According to plant fossil assemblages, occurrence of arid conditions would have started from the Northeast and progressed to the Southeast. This deduction was based mainly on palaeobotanical data obtained from southern Australia, and the onset of aridity could have originated in the Northwest as well as in the Northeast. The scarcity of fossil data from the northern parts of the continent prevents the verification or rejection of this hypothesis.

Still another hypothesis suggesting the onset of aridity in Australia is that of Bowler (1982). Based on his studies on the sediments of lakes in southeastern Australia,

aridification was dated to have commenced 5-6 million years ago. During this time, there was a drastic change when the lakes dried out and the sediments became deeply weathered. High lake levels returned briefly at 2.5 million years ago and from this time on, the lake levels oscillated between lake-full and lake-dry conditions. This pattern persisted to recent times, with intensifications during the last 700,000 years. Bowlers' hypothesis advocates that aridification started in the south and expanded northwards.

1.3 Statement of research

This PhD project aims at investigating the systematics and biogeographical history of the Australian Camphorosmeae based on molecular and morphological data.

To date, there is no comprehensive phylogeny of the Australian Camphorosmeae, although familial treatments with a small sampling of the group have already been published (Kadereit et al., 2003). With the use of multiple molecular markers from the nuclear and plastid genomes, it is the aim of this work to provide a broadly-sampled phylogeny of the tribe, and to determine intergeneric relationships within this morphologically difficult group. The phylogeny will be further used to establish morphologically-supported clades. The characters of the fruiting perianth will be evaluated for their systematic utility, for the reason that these characters have been traditionally used to delimitate species and genera.

This study also attempts to reconstruct historical landscape evolution based on the phylogeny of Camphorosmeae. It has been previously proposed that Camphorosmeae diversification in Australia occurred during the Middle to Late Miocene (Kadereit et al., 2005). This age coincides with several known hypotheses for the onset of aridification of the continent. It is therefore of interest to clarify the role of aridity development for the phylogenetic history of the group. Historical biogeographic study of the Australian Camphorosmeae could in turn provide support for existing theories on aridification in the continent. The suitability of this group for the study of the aridity events in Australia is strengthened by its widespread distribution in all arid and semi-arid regions of the continent.

2 MATERIALS AND METHODS

2.1 Taxon sampling and DNA extraction

A total of 73 species, representing the 12 currently recognized endemic genera of the Australian Camphorosmeae, and four representatives of the extra-Australian Camphorosmeae (*Kochia americana*, *Chenolea diffusa* and the Central Asian *Kochia melanoptera* and *Bassia dasyphylla*) as outgroup were included in this study. The designation of the outgroup followed the results of recent family-wide phylogenetic studies on Chenopodiaceae in which the Australian Camphorosmeae were resolved as sister to the Central Asian members of this tribe (Kadereit et al., 2005). Plant materials used for this study, together with collection and other pertinent information, are enumerated in Appendix 1. Dr. Surrey W.L. Jacobs of the Royal Botanic Gardens Sydney, Australia, collected most of the plant materials used for this study. Several specimens were acquired during an institute excursion in southwest Australia. Voucher specimens are kept either in the Herbarium of the Johannes Gutenberg-Universität (MJG) or in the Royal Botanic Gardens Herbarium in Sydney (NSW). Additional materials used for the morphological investigation of the group were provided by the following herbaria: Western Australian Herbarium (PERTH), the Herbarium of the Australian National Botanic Gardens (CBG), and the Herbarium of the Royal Botanic Gardens in Sydney (NSW).

The taxonomic integrity of plant materials used for the succeeding molecular work was verified by checking published descriptions of the species. In addition, voucher materials were compared to other herbarium samples and to the type specimens, whenever the type was available, to avoid possible misidentifications due primarily to the plasticity of vegetative and reproductive characters.

Tissue samples were kept either in CTAB solution or as dried specimen prior to extraction. Total genomic DNA was extracted mostly from leaf material using a Macherey & Nagel Nucleospin Extrakt Kit (Düren, Germany) following the manufacturers protocol. DNA was extracted from herbarium materials only when the age of the specimen did not exceed 25 years. Extraction from samples older than 25 years yielded DNA of poor quality, and produced non-usable sequences when amplified.

2.2 Selection and evaluation of molecular markers

There are numerous markers, both nuclear and plastid, being used to address phylogenetic problems on the intrafamilial level. Two of the most popular nuclear sequences used in developing phylogenetic inferences in plants are the internal transcribed spacer (ITS) region and the 3' external transcribed region of the 18S–5.8S–26S nuclear ribosomal DNA (Figure 3). The suitability of these non-coding regions of the nuclear rDNA cistron has been proven in resolving different levels of phylogenetic problems (Baldwin & Markos, 1998; Clevinger & Panero, 2000; Linder et al., 2000; Markos & Baldwin, 2002; Roberts & Urbatsch, 2003; Starr et al., 2003; Vander Stappen et al., 2003; Álvarez & Wendel, 2003 and references therein).

The use of noncoding chloroplast DNA (cpDNA) has continually increased to augment characters from either nuclear DNA markers or from coding genes of the chloroplast. The utility of these markers for phylogenetics vary depending mostly on the study group (Bayer & Starr, 1998; McDade & Moody 1999; Bell & Patterson, 2000; Cuénoud et al., 2000; Hardig et al., 2000; Xu et al., 2000; Bellstedt et al., 2001; Ge et al., 2002; Goldblatt et al., 2002; Klak et al., 2003; Muellner et al., 2003; Samuel et al., 2003; Xu & Ban, 2004; Shaw et al., 2005, 2007; Andersson et al., 2006; Biffin et al., 2006; Flannery et al., 2006; Hedenas, 2006; Jiang et al., 2006; Moore & Jansen, 2006; Nie et al., 2006; Whittall et al., 2006; Mort et al., 2007).

In this study, two nuclear and five plastid markers were tested for variability and suitability for use in resolving phylogenetic and biogeographic problems in the Australian Camphorosmeae. The internal transcribed spacer (ITS) region and the 3' external transcribed region (ETS) of the nuclear ribosomal DNA, the *trnP-psaJ* IGS, the *trnS-trnG* IGS, the *trnL-trnF* spacer, the *rpS16* intron and the *rpL16* intron have been used for this study. The plastid markers are all located on the large single copy region (LSC) of the chloroplast genome (Figure 4).

2.3 DNA amplification and sequencing

Amplification and sequencing of the 3' ETS were conducted using primers **ETS-992 for** (5'-ATG AGC GTA AYG AGG TGA GG-3') and **18S-II rev** (5'-CTC TAA CTG ATT TAA TGA GCC ATT CGC A-3'; Ochsmann, 2000), and the primer **ETS-442 rev** (5'-

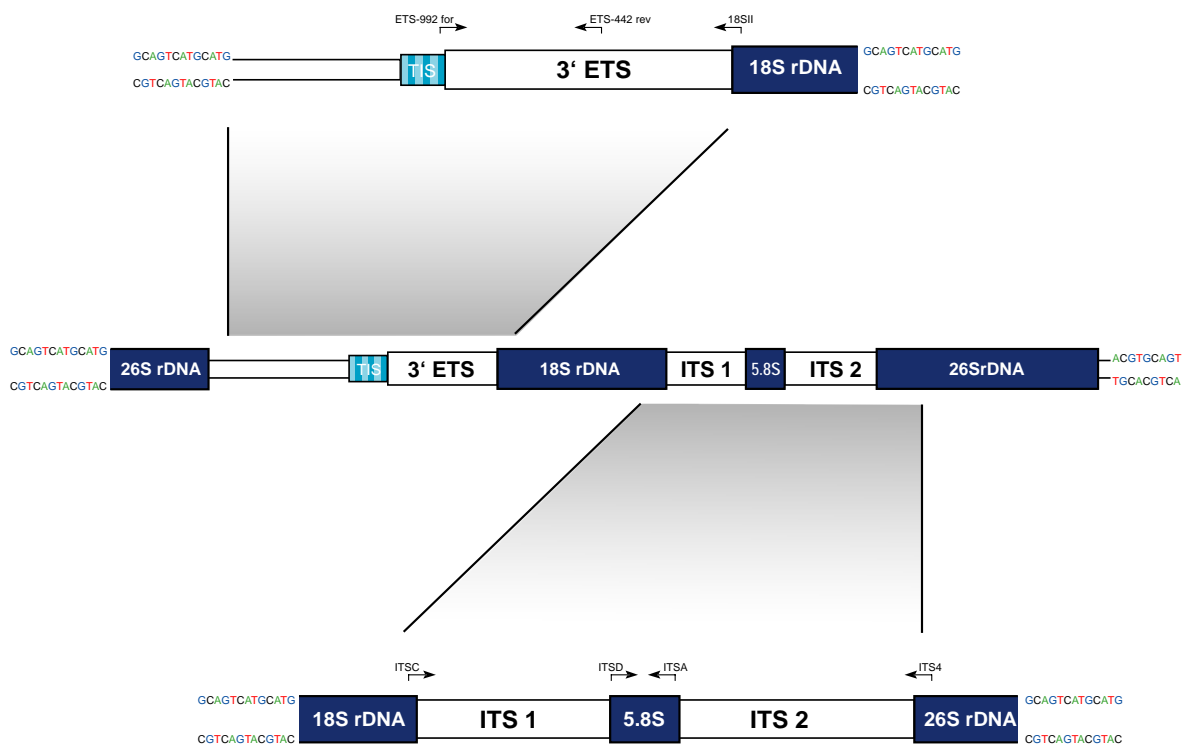


Figure 3. The nuclear ribosomal DNA cistron. Amplification and sequencing primer names are shown above each segment with directional arrows. The schematic diagrams are not drawn to scale (modified from Linder et al., 2000).

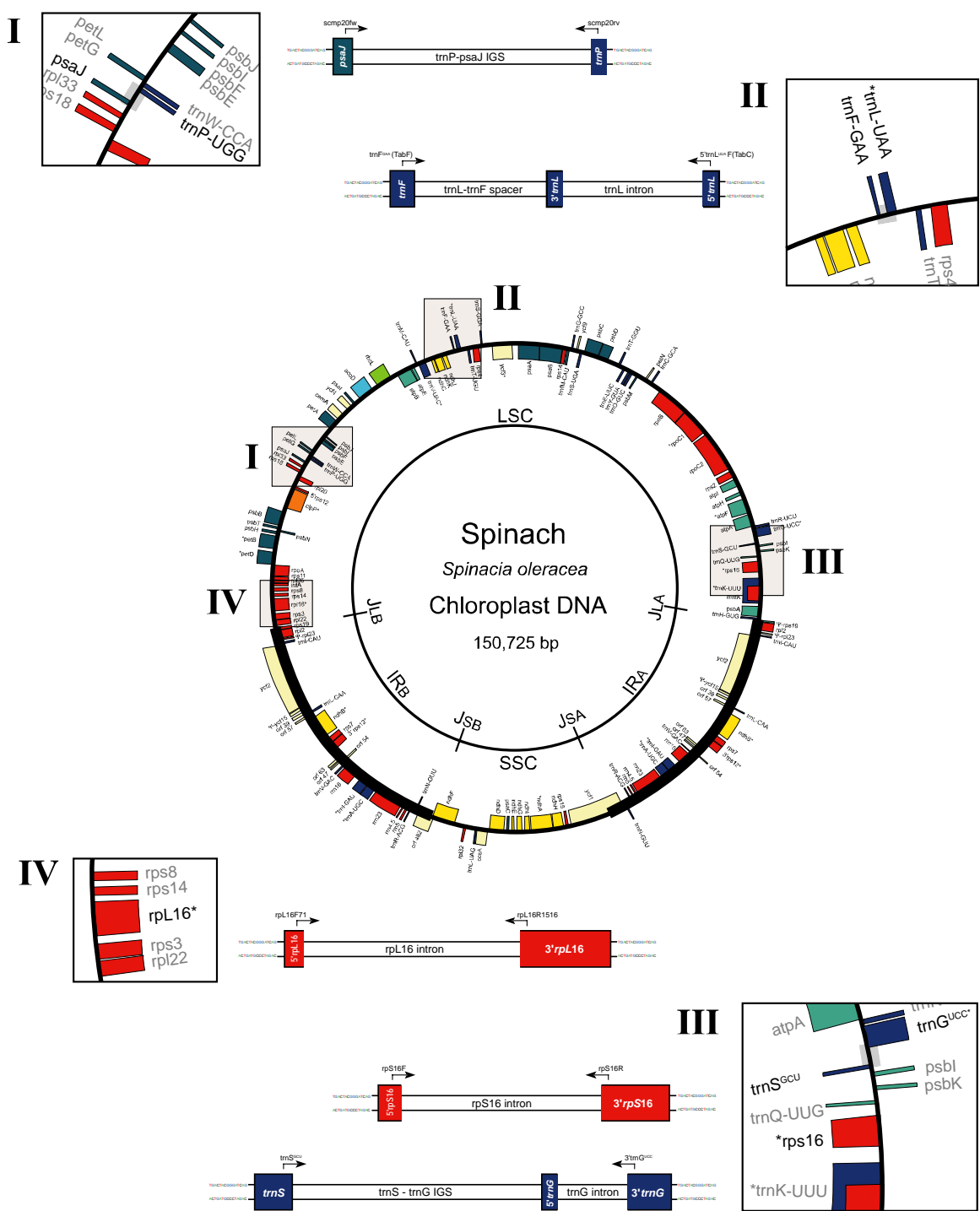


Figure 4. The orientation and relative positions of the molecular markers along the large single copy (LSC) region of the chloroplast genome. Amplification and sequencing primer names are shown above each segment with directional arrows. I: *trnP-psaJ* spacer; II: *trnL-trnF* spacer; III: *rpS16* intron, *trnS-trnG* spacer; IV: *rpl16* intron. The schematic diagrams are not drawn to scale (modified with permission from Schmitz-Linneweber et al., 2001).

ACC AAA TAC CAC TCA TAC GCT-3') was used as an additional internal sequencing primer. A PTC-100 Cycler (MJ Research Inc., USA) was used according to the following protocol: 95°C for 3 min; 30 cycles of 95°C for 30 s, 50.5°C for 45 s, 72°C for 2 min; 72°C for 8 min.

Amplification of the ITS was performed using primers **ITS-A** (5'-GGA AGG AGA AGT CGT AAC AAG G-5'; Blattner, 1999), **ITS-C** (5'-GCA ATT CAC ACC AAG TAT CGC-5'; Blattner, 1999), **ITS-D** (5'-CTC TCG GCA ACG GAT ATC TCG-5'; Blattner, 1999), and **ITS-4** (5'-TCC TCC GCT TAT TGA TAT GC-5'; White et al., 1990) with the parameters 94°C for 3 min; 35 cycles of 94°C for 18 s, 55°C for 30 s, 72°C for 1 min; 55°C for 1 min 18 s, 72°C for 7 min .

trnS^{GCU}-trnG^{UUC} spacer. For this region, the following protocol using the primers **trnS^{GCU}** (AGA TAG GGA TTC GAA CCC TCG GT; Shaw et al., 2005) and **3'trnG^{UUC}** (GTA GCG GGA ATC GAA CCC GCA TC; Shaw et al., 2005) was implemented: primer annealing and chain extension occurred at the same temperature, using the parameters 80°C, 5 min; 30x (95°C, 1 min; 66°C, 4 min); 66°C, 10 min (Shaw et al., 2005).

trnP-psaJ IGS. The amplification of this region was done using the primers **scmp20 fw** (AAA CAA ACG CGC TAC CAA G; Möller, 2005) and **scmp20 rv** (GTC AAC GCA TCT GGG AAA AA; Möller, 2005) with the same PCR parameters as that used for the amplification of ITS.

trnL5'-trnF was amplified using primers **trnL5'^{UAA}F (TabC)** (CGA AAT CGG TAGACG CTA CG; Taberlet et al., 1991) and **trnF^{GAA} (TabF)** (ATT TGA ACTGGT GAC ACG AG; Taberlet et al., 1991) with the parameters 80°C, 5 min; 35 x (94°C, 1 min; 50°C, 1 min; 72°C, 2 min); 72°C, 5 min (Shaw et al., 2005).

rpS16. This region was amplified using the parameters 80°C, 5 min; 35 x (94°C, 30 s; 50–55°C, 30 s; 72°C, 1 min); 72°C, 5 min (Shaw et al., 2005), with primers **rpS16F** (AAA CGA TGT GGT ARA AAG CAA C) and **rpS16R** (AAC ATC WATTGC AAS GAT TCG ATA), which were modified from Oxelman et al., (1997).

rpL16. This region was amplified and sequenced using primers **rpL16F71** (GCT ATG CTT AGT GTGTGA CTC GTT G; Shaw et al., 2005) and **rpL16R1516** (CCC TTC ATT CTT CCT CTA TGT TG; Small et al., 1998). Amplification parameters were 80°C, 5 min; 35 x (95°C, 1 min; 50°C, 1 min with a ramp of 0.3°C/s; 65°C, 5 min); 65°C, 4min

(Shaw et al., 2005).

All amplification reactions were prepared in 25 μ L aliquots containing 10X Buffer (Peqlab TM, Erlangen, DE), 2.25 mM MgCl₂ (Peqlab TM), 100 pmol forward and reverse primers, 0.6 U Taq polymerase (Abgene House, Surrey, UK), 4% DMSO, and 4-10% template DNA. Amplifications were subsequently visualized on a 0.8% agarose gel, and then purified using a PCR extraction kit (QiaGen GmbH, Hilden, DE). DNA sequencing of all markers was performed using ABI's BigDye Terminator v.3 Kit following the manufacturers Protocol on an ABI capillary sequencer.

Alignment of the sequences was straightforward and unambiguous. The repeating units upstream the 3' ETS, where the purported transcription initiation site (TIS; Volkov et al., 1996; Borisjuk et al., 1997; Baldwin & Markos, 1998) of the rDNA is located, were excluded in succeeding analyses.

2.4 Tests of incongruence

To compare the pattern of phylogenetic signal present in the different DNA regions, an incongruence length difference test (ILD test, Farris et al., 1995) implemented in PAUP* 4.0b10 (Swofford, 2001) as the partition homogeneity test (with heuristic search, parsimony optimality criterion and 100 addition replicates) was performed. This test was done to ensure the suitability of the different markers for combined phylogenetic analyses.

2.5 Phylogenetic analyses

2.5.1 Model selection

Hierarchical phylogenetic relationships among sampled taxa were estimated using Bayesian and maximum likelihood optimality criteria. Modeltest (Posada and Crandall, 1998) was used to select the model of nucleotide substitution that best fit the sequence data. The TVM+I+G (transversional model including invariable sites and rate variation among sites) model of sequence evolution was chosen for the ETS sequence data by using the Akaike information criterion as suggested by Modeltest V3.06 (Posada & Crandall, 1998). The following settings representing the best-fit evolutionary model for ETS were used: rate (Φ) matrix set to: $\Phi_{A-C} = 1.0310$; $\Phi_{A-G} = 4.5798$; $\Phi_{A-T} = 2.9469$; $\Phi_{C-G} = 0.4782$; $\Phi_{C-T} = 4.5798$; $\Phi_{G-T} = 1$; base frequencies (Π) set to: $\Pi_A = 0.3233$; $\Pi_C = 0.2718$; $\Pi_G =$

0.1799; $\Pi_T = 0.2250$; proportions of invariable sites (I) set to: $I = 0.2576$; variable sites with gamma-distribution shape (Γ) set to: 0.6725.

The best-fit model of sequence evolution chosen by Modeltest V3.06 (Posada & Crandall, 1998) using the Akaike information criterion for the ITS sequence data was the GTR+I+G (general time reversible model including invariable sites and rate variation among sites). The following parameter settings were implemented: rate (Φ) matrix set to: $\Phi_{A-C} = 1.0355$; $\Phi_{A-G} = 2.5008$; $\Phi_{A-T} = 0.9863$; $\Phi_{C-G} = 0.3019$; $\Phi_{C-T} = 6.1398$; $\Phi_{G-T} = 1$; base frequencies (Π) set to: $\Pi_A = 0.2120$; $\Pi_C = 0.2731$; $\Pi_G = 0.2867$; $\Pi_T = 0.2282$; proportions of invariable sites (I) set to: $I = 0.5707$; variable sites with gamma-distribution shape (Γ) set to: 0.6739.

For the *trnS-trnG* intergenic spacer the GTR+I+G (general time reversible model including invariable sites and rate variation among sites) model of sequence evolution was chosen using the Akaike information criterion implemented in Modeltest V3.06 (Posada & Crandall, 1998). The following parameter settings were applied for all succeeding model-based analyses using this molecular marker: rate (Φ) matrix set to: $\Phi_{A-C} = 0.5770$; $\Phi_{A-G} = 1.1108$; $\Phi_{A-T} = 0.1428$; $\Phi_{C-G} = 0.4568$; $\Phi_{C-T} = 0.4273$; $\Phi_{G-T} = 1$; base frequencies (Π) set to: $\Pi_A = 0.3857$; $\Pi_C = 0.1506$; $\Pi_G = 0.1245$; $\Pi_T = 0.3392$; proportions of invariable sites (I) set to: $I = 0.5138$; variable sites with gamma-distribution shape (Γ) set to: 0.6384.

The K81uf+I (general time reversible model including invariable sites) model of sequence evolution was chosen for the *trnP-psaJ* spacer sequence data by using the Akaike information criterion as suggested by Modeltest V3.06 (Posada & Crandall, 1998). The following settings representing the best-fit evolutionary model for the spacer were used: rate (Φ) matrix set to: $\Phi_{A-C} = 1.0000$; $\Phi_{A-G} = 1.3399$; $\Phi_{A-T} = 0.2491$; $\Phi_{C-G} = 0.7089$; $\Phi_{C-T} = 1.3399$; $\Phi_{G-T} = 1.0000$; base frequencies (Π) set to: $\Pi_A = 0.3154$; $\Pi_C = 0.1476$; $\Pi_G = 0.1371$; $\Pi_T = 0.3999$; proportions of invariable sites (I) set to: $I = 0.7089$.

The concatenated matrix of the ETS and ITS sequence data follows the GTR+I+G (general time reversible model including invariable sites and rate variation among sites) model of sequence evolution, with the following parameter settings: rate (Φ) matrix set to: $\Phi_{A-C} = 1.0236$; $\Phi_{A-G} = 4.1936$; $\Phi_{A-T} = 2.4229$; $\Phi_{C-G} = 0.3981$; $\Phi_{C-T} = 5.5088$; $\Phi_{G-T} = 1.00$; base frequencies (Π) set to: $\Pi_A = 0.2762$; $\Pi_C = 0.2693$; $\Pi_G = 0.2247$; $\Pi_T = 0.2298$;

proportions of invariable sites (I) set to: $I = 0.4480$; variable sites with gamma-distribution shape (Γ) set to: 0.625.

2.5.2 Bayesian statistics implementing the Markov-Chain-Monte-Carlo algorithm

Bayesian phylogenetic analyses were then implemented in MrBayes 3.0B4 (Huelsenbeck & Ronquist, 2001) using a corresponding model of sequence evolution. Four incrementally heated Markov chains were run for 10×10^6 generations, sampling every 1000 generations for a total of 10000 samples. To ensure that the Markov chains reached a stable equilibrium, \ln -likelihood values for sampling points were plotted against generation time. Only 5000 of the generated samples were used to compute a 50% majority-rule consensus tree, where the percentage of samples that recover a particular node represents the posterior probability of that clade. To verify that analyses were not trapped on local optima, five replicate searches were conducted. The independent analyses were considered to have converged on the optimal joint posterior distribution if similar \ln -likelihood scores and parameter estimates were achieved (Huelsenbeck & Bollback, 2001).

2.5.3 Maximum likelihood analysis and parsimony bootstrap

Maximum likelihood (ML) analyses were conducted on all data sets as implemented in PAUP* 4.0b10 (Swofford, 2001). Using the best-fit model determined by Modeltest for all sequence data, maximum likelihood searches were performed with a heuristic search, each with 10 random sequence addition replicates.

To provide support for the resulting maximum likelihood trees, bootstrap support values were calculated from 100 replicates of heuristic searches using 10 random additions of sequences with TBR swapping.

2.6 Molecular clock test and divergence time calculation

Only the ML tree generated from the 3' ETS data was used to estimate the divergence times within the Australian Camphorosmeae. The ETS matrix contained 71 species, and with it the best-sampled among the datasets. A likelihood ratio test as implemented in Modeltest was conducted to determine if a strict molecular clock can be enforced on the ETS sequence for the group. In this case, the log-likelihood scores of the

ML tree with and without enforced molecular clocks varied significantly, and a smoothing method was needed to determine the age of Camphorosmeae. The ML tree obtained in the absence of a molecular clock was subjected to a rate smoothing applying penalized likelihood (PL) approach using the computer software r8s v1.70 (Sanderson, 2002, 2004). An optimal smoothing factor was chosen based on a data-driven cross-validation procedure implemented in r8s (Sanderson, 2004).

In the absence of a suitable fossil to calibrate the molecular phylogeny of the Australian Camphorosmeae, an estimated age of split between the Eurasian and Australian taxa inferred from *ndhF* and *rbcL* sequence data was used. Both crown and stem nodes of the split were calibrated using the following ages: 8.1 – 3.6 myr based on *rbcL*, and 12.91 – 14.33 myr based on *ndhF* (Kadereit et al., in prep).

2.7 Morphological characterization

Characters of the fruiting perianth and the pubescence of the entire plant body have been investigated for their systematic relevance. Although the characters of the fruiting perianth are extremely plastic, these characters are valuable in discriminating among species (Wilson, 1984). Pubescence has also been suggested to be of taxonomic importance (Carolin, 1983; Kadereit, 2003). Several studies showed that the indumentum varies among and within groups in the family (Ulbrich, 1934; Carolin et al., 1975; Scott, 1978b; Carolin, 1983; Wilson, 1984; Simon, 1996; Klopper & van Wyk, 2001; Mosyakin & Clemants, 2002; Kadereit, 2003). Carolin (1983) reported three major trichome types occurring within Camphorosmeae, and members of the tribe in Australian exhibit two of these three (Wilson, 1984). It is therefore of interest to know, whether or not these characters would support the phylogeny of the tribe derived from molecular sequence data.

Patterns of phenotypic evolution were assessed by mapping fruiting perianth and vegetative characters onto the maximum likelihood tree derived from ETS sequence data. The program Mesquite version 1.11 (Maddison & Maddison, 2006) was used to reconstruct character state changes by implementing the “Reconstruct Ancestral States” function of the program employing multiple parsimony algorithms.

Table 2. Characters and characters states scored for Camphorosmeae

No.	Character	Character States
1	Phyllotaxy	0 – alternate 1 – opposite 2 – rosette
2	Infructescence type	0 – solitary 1 – paired 2 – aggregate
3	Distribution of sexes	0 – bisexual 1 – unisexual
4	Fruit type/appendage	0 – berry-like fruiting perianth with no obvious appendage 1 – hardened fruiting perianth with no obvious appendage 2 – hardened fruiting perianth with vertical wings 3 – hardened fruiting perianth with horizontal wings 4 – hardened fruiting perianth with spines 5 – non-woody fruiting perianth, with cylindrical or spinescent appendage
5	Fruit detachability	0 – detachable 1 – not detachable
6	Seed orientation	0 – horizontal 1 – vertical to oblique
7	Fruit pubescence	0 – glabrous 1 – only on perianth tube 2 – entire perianth
8	Hair type (fruit)	0 – not applicable 1 – simple 2 – simple with tubercles/short extensions 3 – dendritic
9	Hair cellularity (fruit)	0 – not applicable 1 – unicellular 2 – multicellular
10	Leaf pubescence	0 – glabrous 1 – pubescent
11	Hair type (leaf)	0 – not applicable 1 – simple 2 – simple with tubercles/short extensions 3 – dendritic
12	Hair cellularity (leaf)	0 – not applicable 1 – unicellular 2 – multicellular

13	Stem pubescence	0 – glabrous 1 – pubescent
14	Hair type (stem)	0 – not applicable 1 – simple 2 – simple with tubercles/short extensions 3 – dendritic
15	Hair cellularity (stem)	0 – not applicable 1 – unicellular 2 – multicellular

All the characters were scored equally and therefore no reference to ancestry was considered. Heterogeneous character states, i.e. a taxon exhibiting 2 or more character states, were recorded equivocally.

2.8 Ancestral area reconstruction

Continental Australia has been divided into 15 different regions for the biogeographic analyses of Camphorosmeae. These areas were defined based on previously recognized biogeographic regions in Australia (Figure 5; Cracraft, 1991; Crisp et al., 1995; Crisp, 1999). The regional descriptions presented below were taken from Cracraft (1991), unless otherwise noted.

- A. Kimberley – The Kimberley area of endemism is an elevated region of woodland and low woodland isolated to the southwest by the Fitzroy river drainage and the Great Sandy Desert, to the south by the northern interior desert, and to the east from Arnhem Land by lowlands of the Victoria and Daly valleys.
- B. Arnhem – The woodland and low woodland of Arnhem Land are isolated from the Kimberley Plateau to the west by the Victoria and Daly River valleys, to the south by the lowlands of the northern interior desert, and to the southeast by drier, more open habitats around the Gulf of Carpentaria.
- C. Cape York – This area is characterized by medium closed forest (rainforest), open forest, and woodland. The area is bounded to the southeast by the uplands of the Atherton Plateau and to the south and southwest by drier, more open habitats.
- D. Atherton – The Atherton Tableland is generally characterized by moist closed forest (rainforest) and surrounded by the lowlands to the north, by drier, more open

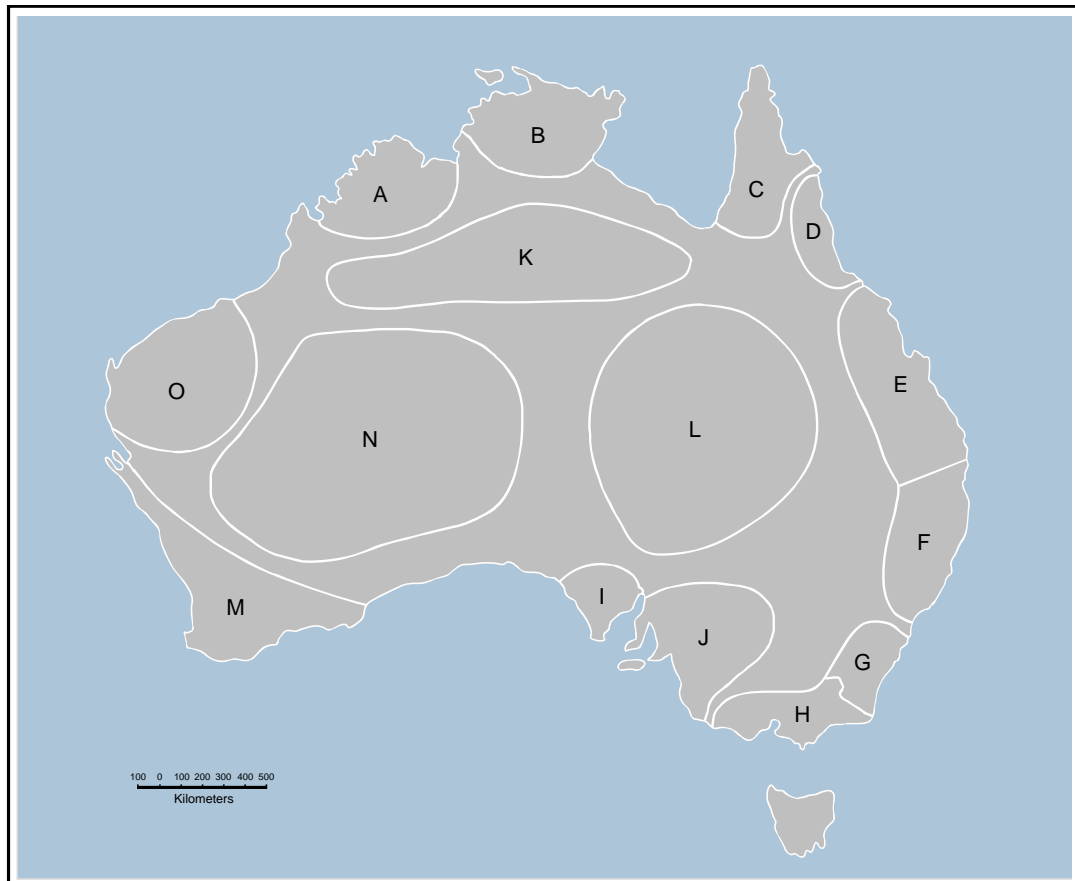


Figure 5. Floristic regions of continental Australia. A = Kimberley; B = Arnhem; C = Cape York; D = Atherton; E = Eastern Queensland; F = McPherson-Macleay; G = Southeastern New South Wales; H = Victoria; I = Eyre; J = Adelaide; K = Northern Desert; L = Eastern Desert; M = Southwest; N = Western Desert; O = Pilbara (modified from Crisp et al., 1995).

- habitats to the west, and by open woodland in the region of the Burdekin River valley.
- E. Eastern Queensland – This area is characterized by wet forest habitats which have their northern limits at the Burdekin River Valley, limited westward by drier, more open woodland habitats east of the Great Dividing Range, and by the Glasshouse Mountains in the south (Crisp, 1995).
- F. McPherson-MacLeay – This region is limited to the north by the Glasshouse Mts., to the west by the Great Dividing Range, and extends to the Hunter River valley in the south (Crisp, 1995).
- G. Southwestern New South Wales – This area is bounded to the north by the Hunter River valley, to the southwest by the Victoria-New South Wales border, to the northwest (inland) by the Great Dividing Range or drier, more open habitats, and at its western boundary by lowlands vegetated by mallee (Crisp, 1995).
- H. Victoria – This area is bounded to the northeast by the Victoria-New South Wales border and to the north by mallee-vegetated lowlands, which are on the southern end of the Great Dividing Range next to the Darling River valley (Crisp, 1995).
- I. Eyre – This region is limited to the north and east by the more arid Eyrean barrier and the Flinder Ranges and to the west by the drier area of the Nullarbor Plain.
- J. Adelaide – This region includes the mallee vegetation east of the Flinders and Mount Lofty Ranges, south of the Darling River valley, west of the Great Dividing Range.
- K. Northern Desert – The Northern Desert area of endemism consists mostly of low open woodland and lies between the southern edges of the Kimberley Plateau and Arnhem Land areas of endemism and the northern boundaries of the Eastern and Western desert areas. The area extends eastward to the Gulf of Carpentaria.
- L. Eastern Desert – This area of endemism lies to the east of the Central Highlands and the drier regions of the Simpson Desert and extends to the uplands of the Great Dividing Range, and is limited to the south by the Darling River or areas of mallee vegetation.
- M. Southwest – This region is characterized by open forests and woodlands at the south-western corner of Australia. The area is limited to the east by the low

shrubland of the Nullarbor Plain and to the north by drier habitats including open scrub and tall shrubland.

- N. Western Desert – This region extends to the south and west of the Pilbara east to the Simpson Desert. It roughly conforms to the distribution of the mulga vegetation.
- O. Pilbara – The uplands of the Pilbara (Hammersley Plateau) are vegetated primarily with tall shrubland and tall open shrubland. This area is bounded to the north by the lowlands of the Great Sandy Desert, to the west by the Gibson Desert, and to the south by the lowlands.

Tasmania was excluded in all biogeographic analysis due to the fact that only one species of Camphorosmeae (*Threlkeldia diffusa*) occurs on this island. The presence of this taxon could be considered as a recent dispersal, and is therefore uninformative for further historical biogeographic analyses.

In order to obtain historical scenarios on the biogeography of the Australian Camphorosmeae, dispersal-vicariance analysis (DIVA; Ronquist, 1996), primary Brooks parsimony analysis (1° BPA: Brooks, 1990), cladistic analysis of distributions and endemism (CADE: Porzecanski & Cracraft, 2005; also parsimony analysis of endemism *sensu* Cracraft, 1991), ancestral area analysis (AAA: Bremer, 1992), and weighted ancestral area analysis (WAAA: Hausdorf, 1998) were conducted using the ETS data.

2.8.1 Dispersal-vicariance analysis

To estimate the number of dispersal events that explains the current biogeographical distribution of Camphorosmeae in Australia, dispersal-vicariance analysis was performed using the computer software DIVA 1.1a (Ronquist, 1996, 1997). DIVA uses reversible parsimony and a three-dimensional cost matrix for dispersal and extinction events (the cost of a vicariance event is 0) to find a combination of ancestral areas that requires the least number of dispersal/extinction events to explain the distributions of the descendant taxa. Since vicariance is the default mode of speciation recognized by the program, optimization was therefore conducted with the maximum number of ancestral areas set to a maximum of two to favour dispersal rather than vicariance (Ronquist, 1996, 1997; Zink et al., 2000; Donoghue et al., 2001). This mode of analysis also helps identify

the possible geographical distribution of the ancestors of the group (Zink et al., 2000). Because the ML tree contained multiple zero-length branches, including the three most basal clades, separate maximum likelihood analyses on sub-sets of the ETS matrix were performed to produce a completely bifurcating tree required by the program.

One-hundred semi-random sub-samples of 28 to 44 taxa were used for the determination of the basal clades and the resolution of several polytomies found in the ML tree. The best model of sequence evolution for each of the smaller matrices was selected using the Akaike information criterion derived by Modeltest (Posada & Crandall, 1998). Five taxa were always included in the analysis: one outgroup species and a taxon from each of the four most basal clades in the ML tree, i.e. *Maireana erioclada* – *M. pentatropis* clade, *Roycea* clade, *M. brevifolia* clade, and *M. pyramidata* – *Eremophea* clade.

2.8.2 Primary Brooks parsimony analysis

Area cladograms were reconstructed using 1° BPA (see Brooks, 1990, 2001; van Veller & Brooks, 2001; Green et al., 2002) and CADE (Cracraft, 1991; Porzecanski & Cracraft, 2005). Primary BPA requires a single area-taxon matrix for all clades to be analysed. The areas are entered by row and the taxa in the columns. If a taxon is present in an area it is coded with a 1; absence is coded with a 0; unavailable information for a taxon or when all species of a clade are absent may be coded with a question mark (?). BPA assumes vicariance as the primary explanation for observed patterns, because this is a general process. Therefore, the distributions of hypothetical ancestral taxa (the internal nodes in taxon cladograms) are also entered as the sum of distributions of their daughter species. Other mechanisms affecting distribution patterns, such as dispersal, extinction etc. are not denied. However, with the assumption that the ancestral taxa of the study group was absent in Australia, and reached the continent via long-distance dispersal, invocation of vicariance as default mode would be inappropriate. In this case, an all-zero outgroup has been added. An all-one and an all-unknown outgroup were also used to check whether the choice of character state affects the resulting area cladogram. For the 1° BPA, a single area cladogram was reconstructed using a 1000-replicate bootstrap parsimony analysis, with TBR and 10 random addition sequence replicates performed in PAUP*4.0b10 (Swofford, 2001).

2.8.3 Cladistic analysis of distributions and endemism

Cladistic analysis of distributions and endemism (CADE: Cracraft, 1991; Porzecanski & Cracraft, 2005) is a direct derivation of Rosen's (1988) static parsimony analysis of endemism (PAE). CADE, like PAE, is a biogeographical method that uses a parsimony algorithm to obtain an area cladogram, based on taxa inhabiting the study areas (Rosen 1988). However, in contrast to static PAE, CADE is historically-based (Porzecanski & Cracraft, 2005; Nihei, 2006) and is therefore more suitable for the study of historical biogeography than the former. Furthermore, CADE uses predefined areas of endemism based on the distributional congruence among the studied taxa, and it incorporates cladistic information with distributions limited to inclusive taxonomic levels (i.e. for a genus and its species, or a family/tribe and the genera belonging to it). In addition, Cracraft (1991) suggests that using a single taxonomic level (e.g. species) would generally be insufficient to resolve area relationships, but if the hierarchical information implied by accepted classification schemes, or better still, by phylogenetic relationships, were used as a framework to code distributions, historical signal about area relationships would be increased.

Just like 1° BPA, CADE requires an area-taxon matrix, in which areas are treated as "taxa", and the distribution of taxa as characters. Two analyses implementing CADE were conducted: (1) using all biogeographical information from all members of the tribe as listed in the Flora of Australia (Wilson, 1984), (2) and using only taxa represented in the phylogenetic tree. Both analyses were performed using the following settings: 100 bootstrap replicates, TBR and 10 random addition sequence replicates. Primary BPA and CADE were performed in PAUP*4.0b10 (Swofford, 2001).

2.8.4 Ancestral area analysis

Bremer's (1992) ancestral area analysis allows one to identify the ancestral area of a group based on the topological information of a suitable cladogram, in most cases a phylogenetic tree. In AAA, each area is treated as a two-state character (present/absent), which is then optimized on the cladogram while searching for the most-parsimonious solution. By comparing the numbers of gains and losses, it is possible to estimate areas most likely to have been part of the ancestral areas. The probability of an area having been part of an ancestral range (probability index, or PI), is estimated as a ratio of area

appearances on the cladogram (with the assumption that the area in question was not part of the ancestral range) over the number of area losses from the cladogram (with the assumption that the area was part of the ancestral range) calculated using Camin-Sokal parsimony.

Table 3. Distribution of the native Australian Camphorosmeae genera in Australia (A = Kimberley; B = Arnhem; C = Cape York; D = Atherton; E = Eastern Queensland; F = McPherson-Macleay; G = Southeastern New South Wales; H = Victoria; I = Eyre; J = Adelaide; K = Northern Desert; L = Eastern Desert; M = Southwest; N = Western Desert; O = Pilbara)

Genera	Distribution
<i>Didymanthus</i>	M, N, O
<i>Dissocarpus</i>	I, J, L, M, N, O
<i>Enchylaena</i>	E, F, G, H, I, J, K, L, M, N, O
<i>Eremophea</i>	M, N, O
<i>Eriochiton</i>	I, J, L, M, N, O
<i>Maireana</i>	A, B, E, F, G, H, I, J, K, L, M, N, O
<i>Malacocera</i>	I, J, L, N
<i>Neobassia</i>	J, K, L, M, N, O
<i>Osteocarpum</i>	I, J, L, N, O
<i>Roycea</i>	M, N
<i>Sclerolaena</i>	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O
<i>Threlkeldia</i>	H, I, J, K, M, O, Tasmania

2.8.5 Weighted ancestral area analysis

Like AAA, WAAA treats each area as a two-state character (present/absent), but similar to DIVA, it permits reversible change between presence and absence of an area on a cladogram. It implements a weighting scheme, which weights steps in positionally plesiomorphic branches more highly than steps in positionally apomorphic branches. This

means that each appearance or loss of an area on a cladogram (gain step) is weighted (divided) by the number of nodes separating this appearance or loss from the common ancestor of a clade (Hausdorf, 1998). With the assumption that the speciation of Camphorosmeae in Australia resulted from a series of dispersals, extinctions and recolonizations, all WAAA optimizations for the weighted gain steps (GSW) were done with the DELTRAN (delayed transformation) resolving option, and the weighted loss steps (LSW) with the ACCTRAN (accelerated transformation) resolving option (Hausdorf, 1998). However, the choice of resolving option for both gain and loss steps has had no significant effect on the results, usually altering the values only slightly.

Area optimizations for AAA and WAAA were performed using MacClade 4.0 (Maddison & Maddison, 2000).

Biogeographic information for all genera of Camphorosmeae native to Australia were taken from Wilson (1984). All descriptive distributions and distribution maps were converted to the corresponding floristic regions before they could be used for the biogeographic analyses (Table 3; Appendix 2).

3 RESULTS

3.1 DNA sampling

A total of 69 Australian Camphorosmeae and 4 outgroup species representing members of the tribe occurring outside the continent have been used for this study (Table 4). The sampling covered all recognized genera of the endemic Camphorosmeae in the continent. Attempts to use only DNA sequences from the Eurasian taxa *Bassia dasyphylla* and *Kochia melanoptera* as outgroup were not always successful for the plastid markers. Therefore, two other members of the extra-Australian Camphorosmeae were used, namely *Chenolea diffusa* and *Kochia americana*, which amplified using the available primers.

Table 4. Total number of Camphorosmeae used in this study

Genera	Total No. of Species	No. of sampled taxa			
		ETS	ITS	<i>trnS-trnG</i>	<i>trnP-psaJ</i>
Ingroup					
<i>Didymanthus</i>	1	1	1	1	1
<i>Dissocarpus</i>	4	2	2	2	2
<i>Enchylaena</i>	2	2	2	1	1
<i>Eremophea</i>	2	2	2	0	0
<i>Eriochiton</i>	1	1	1	1	1
<i>Maireana</i>	57	23	12	12	6
<i>Malacocera</i>	4	2	1	1	1
<i>Neobassia</i>	2	2	1	1	1
<i>Osteocarpum</i>	5	2	1	1	2
<i>Roycea</i>	3	2	2	2	2
<i>Sclerolaena</i>	64	29	12	15	9
<i>Threlkeldia</i>	2	1	1	1	1
Total	147	69	38	38	27
Outgroup					
<i>Bassia dasyphylla</i>		✓	✓		
<i>Chenolea diffusa</i>					✓
<i>Kochia americana</i>				✓	
<i>Kochia melanoptera</i>		✓	✓	✓	

3.2 Characteristics of the molecular markers

The aligned sequence characteristics of the molecular markers in *Camphorosmeae* used in this study are summarized in Table 5.

Table 5. Characteristics and variation of the molecular markers in *Camphorosmeae*

Sequence Characteristics	3'ETS	ITS	ETS+ITS	<i>trnS-trnG</i> IGS	<i>trnP-psaJ</i> IGS
<i>Within Australian Camphorosmeae</i>					
Total length (bp)	653	663	1316	915	582
Constant sites (bp %)	403 (61.7%)	537 (81.0%)	1011 (76.8%)	775 (84.7%)	559 (96.0%)
Variable sites (bp)	250 (38.3%)	126 (19.0%)	305 (23.2%)	140 (15.3%)	23 (4.0%)
Informative sites (bp)	142 (21.7%)	63 (9.5%)	163 (12.4%)	53 (5.8%)	3 (0.5%)
GC content (mean %)	45.17	55.94	51.79	36.89	28.09
Transition/transversion	2.05	3.42	2.32	0.62	0.52
<i>Australian Camphorosmeae with outgroup</i>					
Total length (bp)	664	701	1365	915	583
Constant sites (bp)	385 (58.0%)	552 (78.7%)	988 (72.4%)	747 (81.6%)	548 (94.0%)
Variable sites (bp)	279 (42.0%)	149 (21.3%)	377 (27.6%)	168 (18.4%)	35 (6.0%)
Informative sites (bp)	157 (23.6%)	75 (10.7%)	192 (14.1%)	62 (6.8%)	8 (1.4%)
GC content (mean %)	40.83	55.97	51.84	37.51	28.47
Transition/transversion	2.02	3.26	2.23	0.62	0.63
<i>Pairwise sequence divergence</i>					
<i>Within Australian Camphorosmeae</i>					
Range (%)	0.0-8.7	0.0-6.09	0.27-7.08	0.0-5.89	0.0-2.21
Mean (%)	4.01	2.69	3.41	1.79	0.36
<i>Australian Camphorosmeae with outgroup</i>					
Range (%)	0.0-11.83	0.0-7.47	0.27-8.83	0.0-6.70	0.0-4.91
Mean (%)	4.31	3.01	3.81	2.03	0.67

Preliminary amplification and sequencing of three plastid markers, i.e. the *rpS16* intron, *rpL16* intron and *trnL-trnF* spacer produced unsatisfactory results. In several species these DNA loci did not amplify and when they did, variation is relatively less than the other markers used in this study indicating the unsuitability of these markers for resolving intrafamilial phylogenetic problems in *Camphorosmeae*. Comparison of

potentially informative characters (PIC, Shaw et al., 2005) between *Roycea divaricata* and *Sclerolaena eurotioides* showed the lowest values for the abovementioned plastid markers (Table 6). Therefore, all succeeding analyses were performed using sequence data from the remaining molecular markers, which appear to possess more potentially informative characters.

Table 6. Comparison of potentially informative characters (PIC) between *Roycea divaricata* and *Sclerolaena eurotioides*

	ETS	ITS	<i>trnS-trnG</i>	<i>trnP-psaJ</i>	<i>rpS16</i>	<i>rpL16</i>	<i>trnL-trnF</i>
Substitutions	36	-	5	-	8	5	2
Indels	5		12		-	1	2
PIC	6.7 %		2.2%		1.2%	0.7%	0.7%

The partition-homogeneity test indicated no significant heterogeneity between the 3' ETS and the ITS sequence data at the 95% confidence level (Table 7). However, there existed significant sequence heterogeneity among the two spacer regions of the nuclear rDNA and the plastid marker *trnS-trnG* IGS, and therefore these data sets were not analysed in combination.

Table 7. *P*-values for the partition-homogeneity tests

Partitions	<i>P</i> value
3' ETS – ITS	0.09
3' ETS – ITS – <i>trnS-trnG</i> IGS	0.01*

*Significant heterogeneity at the 95% confidence level

3.3 Phylogeny of Camphorosmeae

3.3.1 Phylogeny based on ETS sequence data

The molecular phylogeny of the Australian Camphorosmeae based on ETS sequence data from 71 accessions analyzed using maximum likelihood is illustrated in Figure 6. These accessions included sequences from all species of *Didymanthus*, *Enchylaena*, *Eremophea*, *Eriochiton*, and *Neobassia*, and available species of the

remaining Australian genera. Statistical support in the form of maximum parsimony bootstrap (BS) and Bayesian posterior probabilities (PP) are shown on the tree.

Major phylogenetic relationships resolved using maximum likelihood analysis of the ETS sequence data are the following:

- a. The two most speciose genera of the Australian Camphorosmeae, *Sclerolaena* and *Maireana*, were polyphyletic.
- b. Most *Sclerolaena* species occurred in two clades (*Sclerolaena* clade I & II). However, these clades were supported by neither bootstrap nor Bayesian posterior probability. *Sclerolaena stelligera* was resolved in a clade of 3 *Maireana* species. This relationship received low bootstrap support (59%), but 100% posterior probability. *Sclerolaena fimbriolata* was placed in a statistically unsupported polytomy with the clade containing *S. stelligera* and a clade containing *Maireana astrotricha* and *Enchylaena lanata* (BS=77; PP=100).
- c. *Threlkeldia* originated from within the *Sclerolaena* clade I.
- d. Six clades each containing at least 2 species of *Maireana* were resolved. These clades received varying degrees of statistical support. The clade containing *M. erioclada* and *M. pentatropis* received high bootstrap (96%) and high posterior probability (100%), whereas the relationship of *M. microphylla* to *M. planifolia* and *M. villosa* was statistically unsupported. In addition, several species, such as *M. coronata*, *M. astrotricha*, *M. georgei* and *M. platycarpa*, were placed sister to other Camphorosmeae genera.
- e. *Roycea* was resolved monophyletic and placed in a basal polytomy on the spine of the tree. The monophyly of the two species, *R. spinescens* and *R. divaricata*, was highly supported by both bootstrap and Bayesian posterior probability.
- f. *Eremophea* was resolved paraphyletic with respect to *Neobassia astrocarpa*. This relationship was supported by 100% bootstrap and 100% posterior probability (*Eremophea* clade).
- g. *Neobassia* was polyphyletic. *Neobassia astrocarpa* was sister to *Eremophea* with moderate bootstrap and 99% posterior probability. *Neobassia proceriflora* was placed sister to the two *Sclerolaena* clades and the *Osteocarpum* clade, although this was not supported by either bootstrap or posterior probability.



- h. The monophyly of *Osteocarpum* was supported by both bootstrap and Bayesian posterior probabilities, 99% and 100% respectively. This genus was resolved as sister to *Maireana platycarpa*.
- i. *Malacocera* was resolved monophyletic with 99% bootstrap and 100% posterior probability. This clade was placed sister to a clade containing *Sclerolaena fimbriolata*, *Sclerolaena stelligera*, *Enchylaena lanata* and several *Maireana* species.
- j. The monotypic genera *Eriochiton* and *Didymanthus* were placed in a clade together with *Dissocarpus* (*Dissocarpus* clade). The relationships among these genera were weakly supported by bootstrap (51%), but showed high Bayesian posterior probabilities (99%). *Dissocarpus* was resolved paraphyletic in this clade. *Dissocarpus paradoxus* formed a sister relationship to *Didymanthus roei*, receiving weak bootstrap support (64%), but high posterior probability (95%). *Dissocarpus biflorus* was placed sister to *Eriochiton sclerolaenoides*, although this relationship was supported by neither bootstrap nor Bayesian probability.
- k. *Enchylaena* was polyphyletic. However, both sampled species appeared sister to species of the genus *Maireana* occurring at different positions on the tree with moderate to strong bootstrap support and high Bayesian posterior probabilities.

3.3.2 Phylogeny based on ITS sequence data

Forty accessions from all 12 currently recognized genera of Australian Camphorosmeae were sampled for ITS sequence data (Table 4). The maximum likelihood analysis of the ITS resulted in a single tree (Figure 7). The monophyly of the Australian Camphorosmeae was highly supported by both maximum parsimony bootstrap and Bayesian posterior probability. Although the tree topology showed relatively few unresolved branches, only few of the resolved relationships received maximum parsimony bootstrap support or Bayesian posterior probabilities.

Major phylogenetic relationships resolved through the maximum likelihood analysis of the ITS sequence data are as follows:

- a. Like the ETS-derived phylogeny, the species-rich genera *Maireana* and *Sclerolaena* were not monophyletic.
- b. Most *Sclerolaena* species formed a paraphyletic clade with respect to *Threlkeldia*

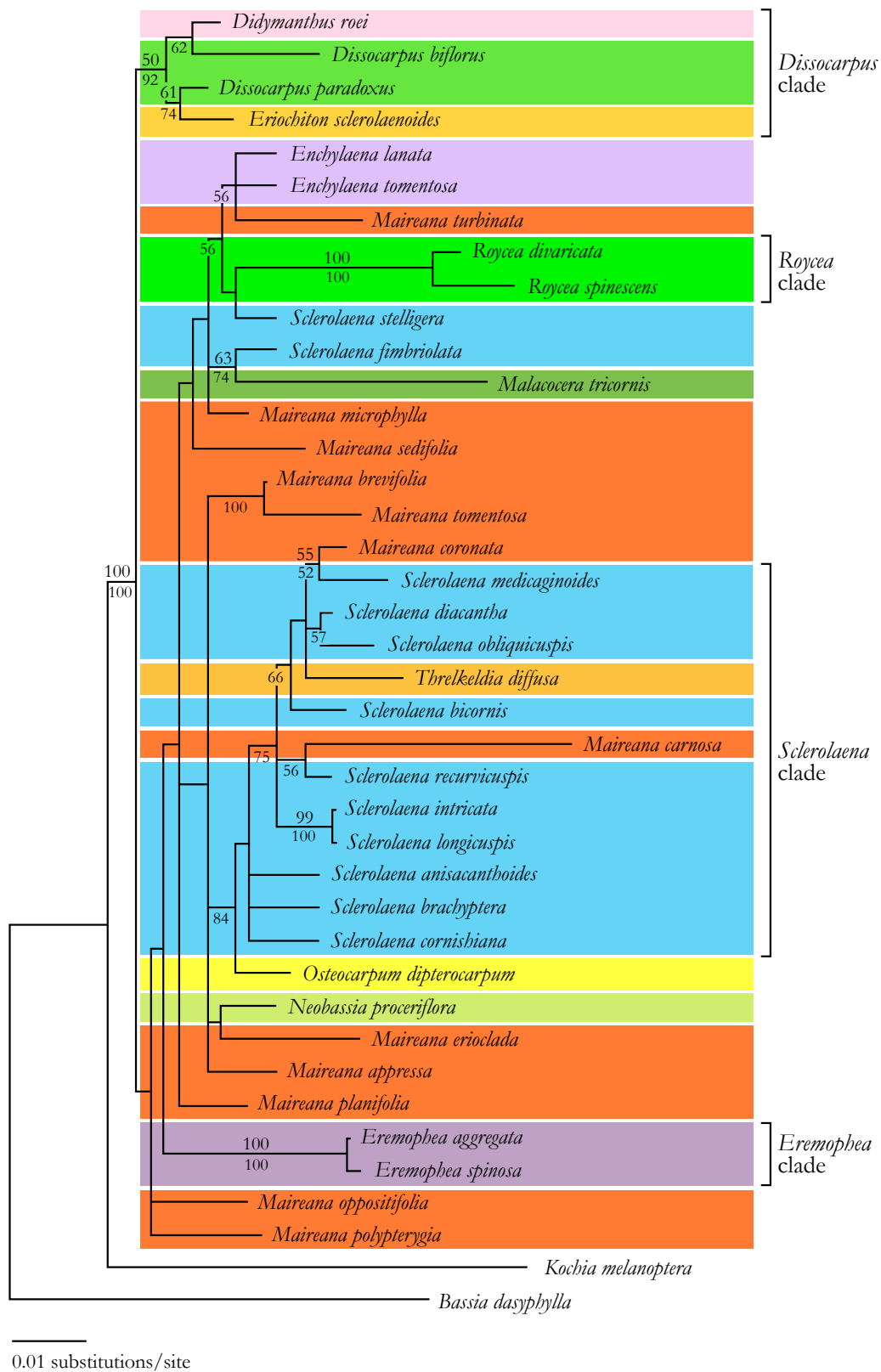


Figure 7. ML tree derived from ITS sequence data. MP bootstrap support are listed above branches, Bayesian posterior probabilities below (CI=0.605, RI=0.57).

diffusa and *Maireana coronata* (*Sclerolaena* clade). This clade did not receive statistical support. *Sclerolaena fimbriolata* was sister to *Malacocera tricornis*, receiving low bootstrap support (63%) and low posterior probability (74%). *Sclerolaena stelligera* was resolved sister to *Roycea*, although receiving neither maximum parsimony bootstrap nor Bayesian posterior probabilities.

- c. *Eremophea* was monophyletic and was strongly supported by both parsimony bootstrap (100%) and Bayesian posterior probability (100%).
- d. The monophyly of *Roycea* received 100% bootstrap support as well as 100% Bayesian posterior probability.
- e. Like in the ETS-derived phylogeny, the monotypic genera *Eriochiton* and *Didymanthus* were placed in a clade together with *Dissocarpus*. The relationships among these genera were weakly supported by bootstrap. *Dissocarpus* was resolved paraphyletic in this clade. *Dissocarpus paradoxus* formed a sister relationship to *Eriochiton sclerolaenoides*, receiving weak bootstrap support (61%) and weak posterior probability (74%). *Dissocarpus biflorus* was placed sister to *Didymanthus roei*, receiving no bootstrap support, but weak Bayesian probability (62%).
- f. *Osteocarpum dipterocarpum* was resolved sister to the *Sclerolaena* clade. This relationship was supported by moderate Bayesian posterior probability (84%).
- g. *Enchylaena* formed a clade with *Maireana turbinata*. However, this relationship was not supported by parsimony bootstrap, and received only weak Bayesian posterior probability (84%).

Although ITS sampling was smaller in comparison with ETS, the resulting phylogenetic hypotheses from both nuclear markers showed very similar topologies. The major differences were the resolution of the two *Enchylaena* species as belonging to a single clade, the relationships within the *Dissocarpus* clade and the somewhat derived position of the *Roycea* clade in the ITS phylogeny. Nevertheless, these relationships received weak or no statistical support.

3.3.3 Phylogeny based on the concatenated ETS-ITS sequence data

The concatenated matrix of ETS and ITS sequence data consisted of 40 accessions from representative taxa of the 12 recognized native genera of the Australian

Camphorosmeae and the two Eurasian outgroup species. Maximum likelihood analysis of this data set showed a monophyletic Australian Camphorosmeae, which was highly supported by both parsimony bootstrap and Bayesian posterior probability (Figure 8). Most phylogenetic relationships resolved using the combined data matrix were congruent with those derived from either ETS or ITS sequences. There was only one case where a terminal relationship from the combined analysis has been reduced to a polytomy (i.e. the sister relationship of *Dissocarpus paradoxus* and *Eriochiton sclerolaenoides*). However, this relationship was not resolved in the analysis of the ETS sequences. Furthermore, it was poorly supported by bootstrap or Bayesian statistics in the analysis of the ITS sequence data.

3.3.4 Phylogeny based on *trnS-trnG* IGS sequence data

Sequences of the *trnS-trnG* IGS from 40 accessions were used to reconstruct the phylogeny of the Australian Camphorosmeae. All native genera of the tribe in Australia were sampled except for *Eremophea*, which did not amplify using the available PCR primers. In addition, *Bassia dasyhylla* could not be amplified with the same primer and therefore an alternative outgroup, *Kochia americana*, was used. The phylogeny resulting from the maximum likelihood analysis of the *trnS-trnG* spacer sequences is shown in Figure 9.

The phylogenetic hypothesis derived from this plastid marker differed considerably from the phylogenies based on either ETS or ITS sequence data. The monophyly of the Australian Camphorosmeae was neither supported by maximum parsimony bootstrap nor by Bayesian posterior probability. Furthermore, phylogenetic resolution among the sampled species of *Maireana* and *Sclerolaena* was not improved using the *trnS-trnG* IGS. Instead, the two consisted of still smaller, mostly unsupported clades.

Roycea was polyphyletic, although both sampled species belonged to a polytomy containing *Enchylaena*, *Eriochiton*, *Malacocera*, *Threlkeldia*, and several species of *Maireana* and *Sclerolaena*.

Neobassia, *Osteocarpum* and *Threlkeldia* were resolved sister to different species of *Sclerolaena*.

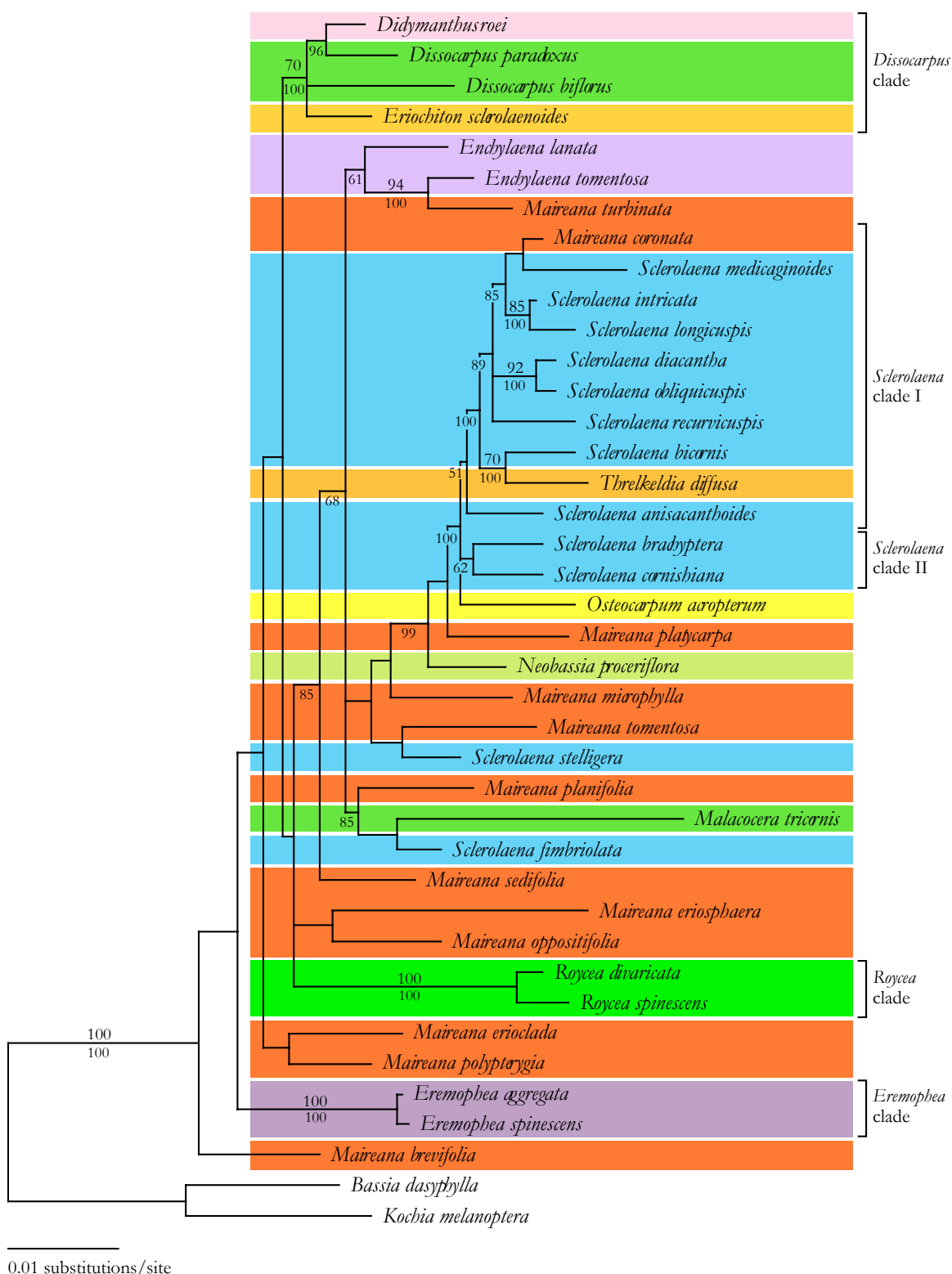


Figure 8. Maximum likelihood tree derived from the analysis of the concatenated matrix of ETS and ITS sequence data. Parsimony bootstrap support are listed above branches, Bayesian posterior probabilities below.



Figure 9. ML tree derived from *trnS-trnG* IGS sequence data. Parsimony bootstrap support are listed above branches, Bayesian posterior probabilities below (CI=0.687, RI=0.470).

The monospecific *Didymanthus* retained its sister relationship to *Dissocarpus biflorus*, like in the phylogenies derived from the non-coding regions of the nuclear rDNA. However, this relationship received no statistical support.

3.3.5 Phylogeny based on *trnP-psaJ* spacer sequence data

Twenty-nine *trnP-psaJ* spacer sequences were used to generate a phylogeny of Camphorosmeae. Accessions represent all native genera of the tribe in Australia with the exception of *Eremophea*, which could not be amplified using the available PCR primers. *Chenolea diffusa*, a South African Camphorosmeae taxon, was used as outgroup, since the preferred Eurasian taxa did not amplify using the PCR primers. The maximum likelihood tree is given in Figure 10. The monophyly of the Australian Camphorosmeae received weak support from both parsimony bootstrap and Bayesian statistics. Furthermore, all relationships within the Australian Camphorosmeae, with the exception of a clade composed of *Maireana aphylla* and *Sclerolaena stelligera*, were supported neither by parsimony bootstrap nor by Bayesian probabilities.

3.4 Age of the Australian Camphorosmeae

The cross-validation procedure implemented in the program r8s (Sanderson, 2004) using the penalized likelihood algorithm resulted in a smoothing value of 100000. The penalized likelihood chronograms (i.e. the “smoothed” ML tree) showing the relative minimum ages of the Australian Camphorosmeae clades using two different age estimates for the split between the Australian Camphorosmeae and its sister group in Central Asia are shown in Figures 11a and 11b. The different ages of the calibration point were based on *rbcL* and *ndhF* divergence age estimations from an ongoing study of Camphorosmeae (Kadereit et al., in prep).

Estimated ages of the calibration point differed from each other by about 4.81-9.01 myr. The age of split between the Australian clade from the Central Asian clade was dated to 12.91-14.33 using *ndhF* sequence data. Age estimates using *rbcL* for the same event was found to be 8.1-3.6 myr. Ages determined from the calibration of the ETS ML tree using the *ndhF*-derived estimate implementing either the non-parametric rate smoothing (NPRS) algorithm or the penalized likelihood (PL) algorithm were in all cases relatively older than

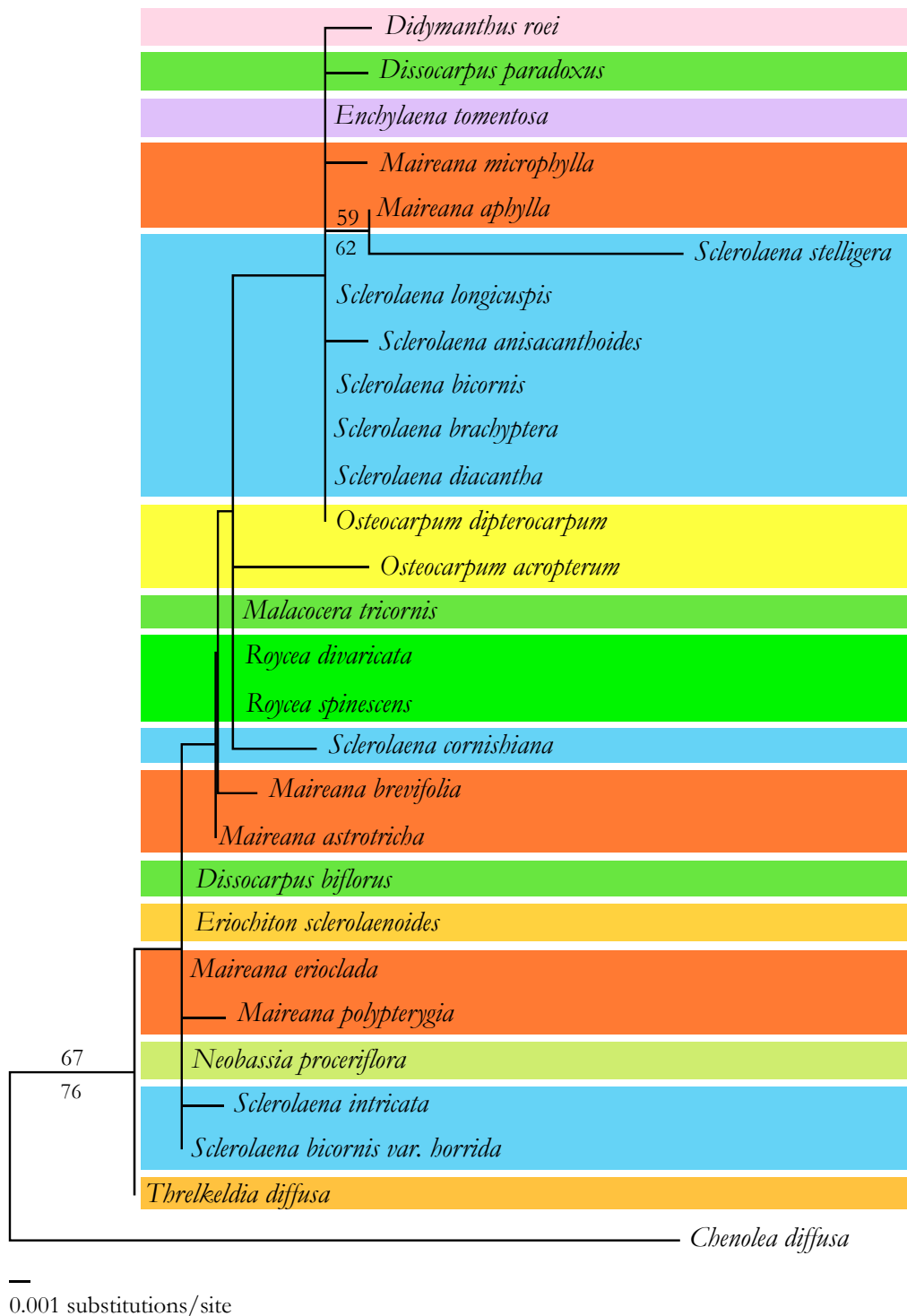


Figure 10. ML tree derived from *trnP-psaJ* spacer sequence data. Parsimony bootstrap support are listed above branches, Bayesian posterior probabilities below (CI=0.957, RI=0.889).

that using the *rbcL*-derived age, especially for nodes near the calibration point (see Appendix 3).

The results of both NPRS and PL analyses using a single calibration point did not substantially differ from each other (Appendix 3). In all cases except in two terminal nodes, age estimates using the former algorithm were older than that of the latter (see nodes 27 and 100 in Appendix 3). Age estimates for the same nodes using an *ndhF* calibration implementing the two algorithms differed from each other by about 0.03 to about 3.60 million years. In the case of the *rbcL*-calibrated tree, age differences for the same nodes determined using the two methods ranged from 0.04 to 2.07 myr. NPRS chronograms and a table showing all age-estimates derived from the different methods and calibrations are given in Appendix 3.

Based on the *ndhF* calibration implementing PL, predecessors of the tribe reached Australia during the Late Miocene (ca. 7.38 myr) and eventually diversified during the Late Tertiary beginning ca. 7.09 myr and continued to diversify all throughout the Quaternary (Figure 11a). However, PL estimates using *rbcL* calibration suggested that the same event did not happen before the Early Pliocene ca. 4.18 myr (Figure 11b), and speciation was gradual throughout the rest of the Tertiary and all the way through the Quaternary.

3.5 Morphological characterization

The character state matrix is shown in Appendix 4. Cladograms showing parsimoniously optimized analyses of morphological characters against the ML phylogenetic tree for Camphorosmeae are given in Figure 12 and Appendix 5a-5n.

Of 15 characters of the fruiting perianth and the general pubescence of Camphorosmeae, no character provided a clear phylogenetic pattern, except for the type of fruiting appendages.

The fruiting perianth in the Australian Camphorosmeae shows a degree of variation significant enough to suggest a potential systematic contribution (Figure 13). The fruiting perianth of Camphorosmeae has been categorized into six different types according to the following criteria:

- a. Berry-like fruiting perianth with no obvious appendage – the perianth in the fruiting

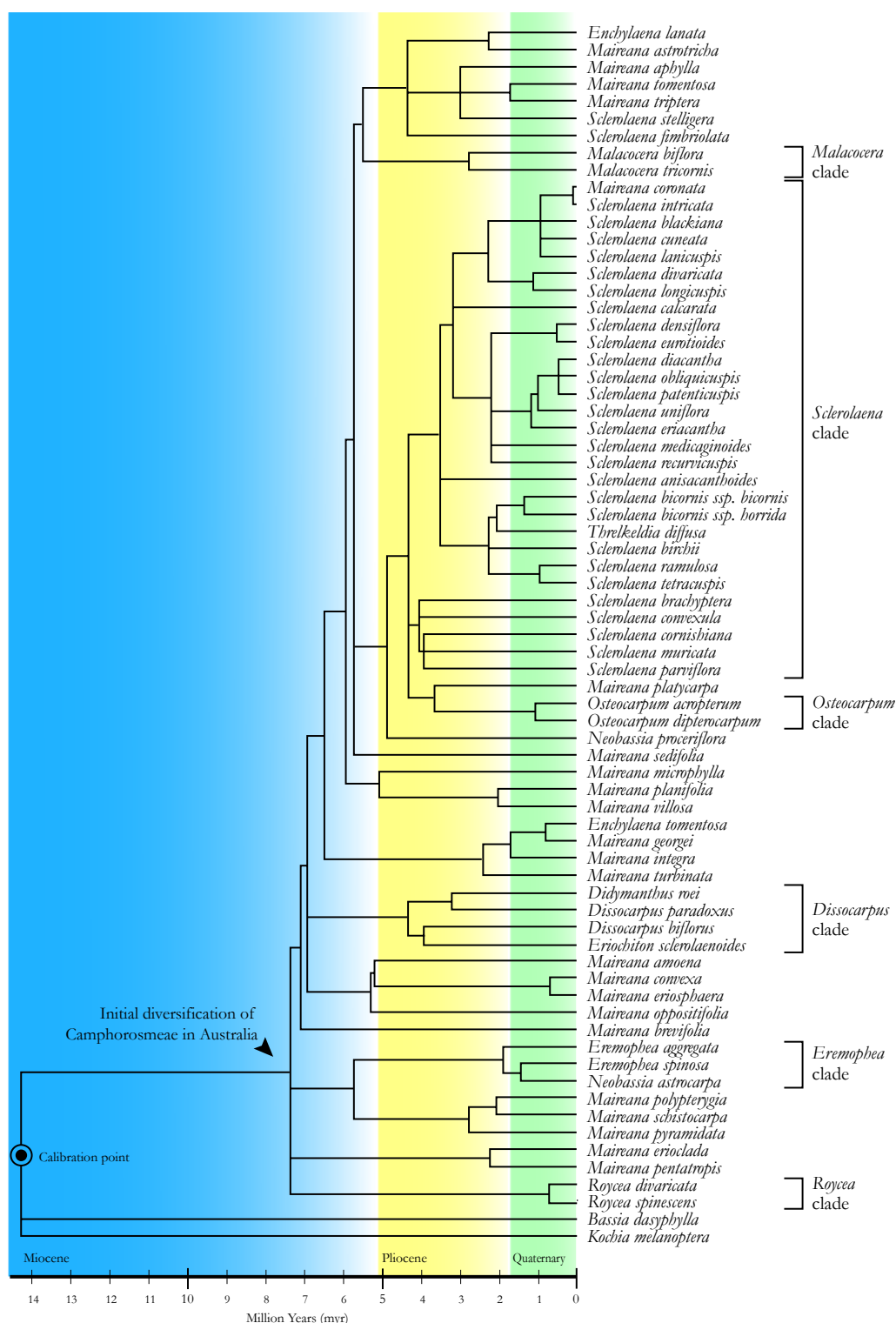


Figure 11a. Relaxed maximum likelihood tree of the Australian Camphorosmeae with estimated ages derived from divergence time analysis of the ETS sequence data using a relaxed clock method implementing penalized likelihood. Calibration used age estimates of the split between the Australian and the Central Asian clades of the tribe inferred from *ndhF* sequence data.

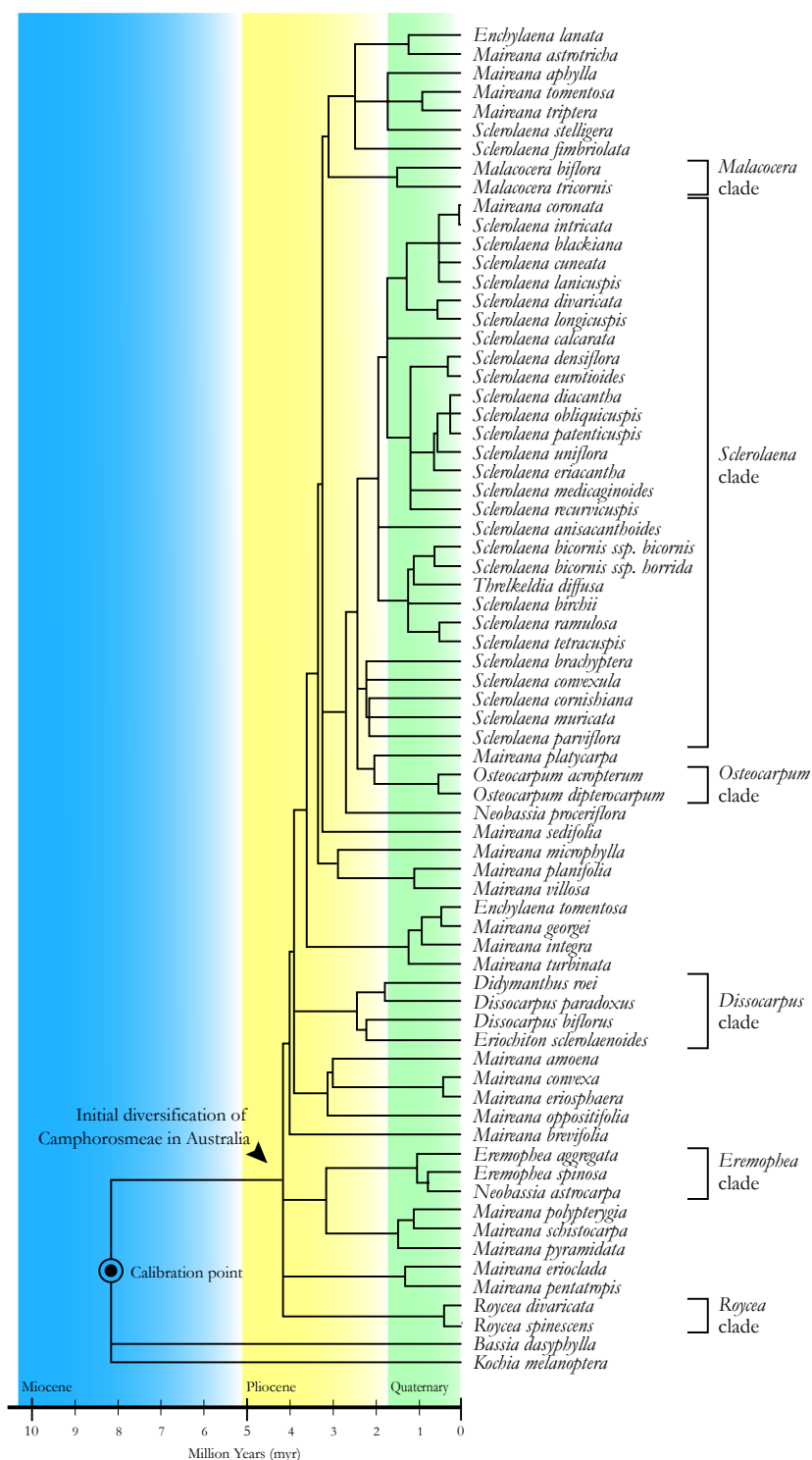


Figure 11b. Relaxed maximum likelihood tree of the Australian Camphorosmeae with estimated ages derived from divergence time analysis of the ETS sequence data using a relaxed clock method implementing penalized likelihood. Calibration used age estimates of the split between the Australian and the Central Asian clades of the tribe inferred from *rbcL* sequence data.

phase persists and becomes succulent with a cartilaginous to woody inner layer. It develops no obvious appendages but forms a cup-shaped extension (Figure 13a.E & 13a.I). The pericarp is thin and crustaceous above, and membranous below. This type of fruiting perianth characterizes *Enchylaena* and *Threlkeldia* differing mainly on the thickness of the succulent layer and the general shape of the fruiting perianth. *Enchylaena* has a globular to subglobular fruiting perianth with a relatively thicker fleshy covering than *Threlkeldia* which possesses a tubular to urceolate fruiting perianth.

- b. Hardened fruiting perianth with no obvious appendage – the perianth does not become modified in the fruiting phase, but persists and surrounds the fruit at its base. The pericarp is thin and crustaceous, and does not develop any appendage. *Roycea* is distinguished from all other members of Camphorosmeae by this fruit type (see Figure 37, p. 217 in Wilson, 1984).
- c. Hardened fruiting perianth with vertical wings – the perianth possess a prominent radicular tubercle, either bare or bearing an erect wing, and 0-4 intertepaline tubercles being modified into vertical wings in the fruiting phase. This fruiting perianth type characterizes *Osteocarpum* (Figure 13a.C).
- d. Hardened fruiting perianth with horizontal wings – these fruiting perianths develop a horizontal papery or rarely woody wing, or five separate wings which grow from the base of the fruit lobes. In some cases accessory wings form at the base or the side of the perianth tube, resembling vertical wings. This fruiting perianth type is observed mainly in *Maireana* (Figures 13a.J, K and L) and *Didymanthus* (see Figure 43, p. 237 in Wilson, 1984).
- e. Hardened fruiting perianth with spines – crustaceous to woody fruiting perianths typically with 2-6 spines, or occasionally nine, and often with a contiguous pair opposite the radicle. Fruiting perianths of *Sclerolaena* (Figure 13b), *Eremophea* (Figure 13a.A and B), *Dissocarpus* (see Figure 40, p. 227 in Wilson, 1984) and *Neobassia* (Figure 13a.F) possess spines, which differ mainly from each other based on their position on the fruiting perianth and their growth form. Spines on the fruit of *Sclerolaena* arise between the perianth lobes, whereas, fruits of the other three genera develop spines from the base or opposite the perianth lobes.

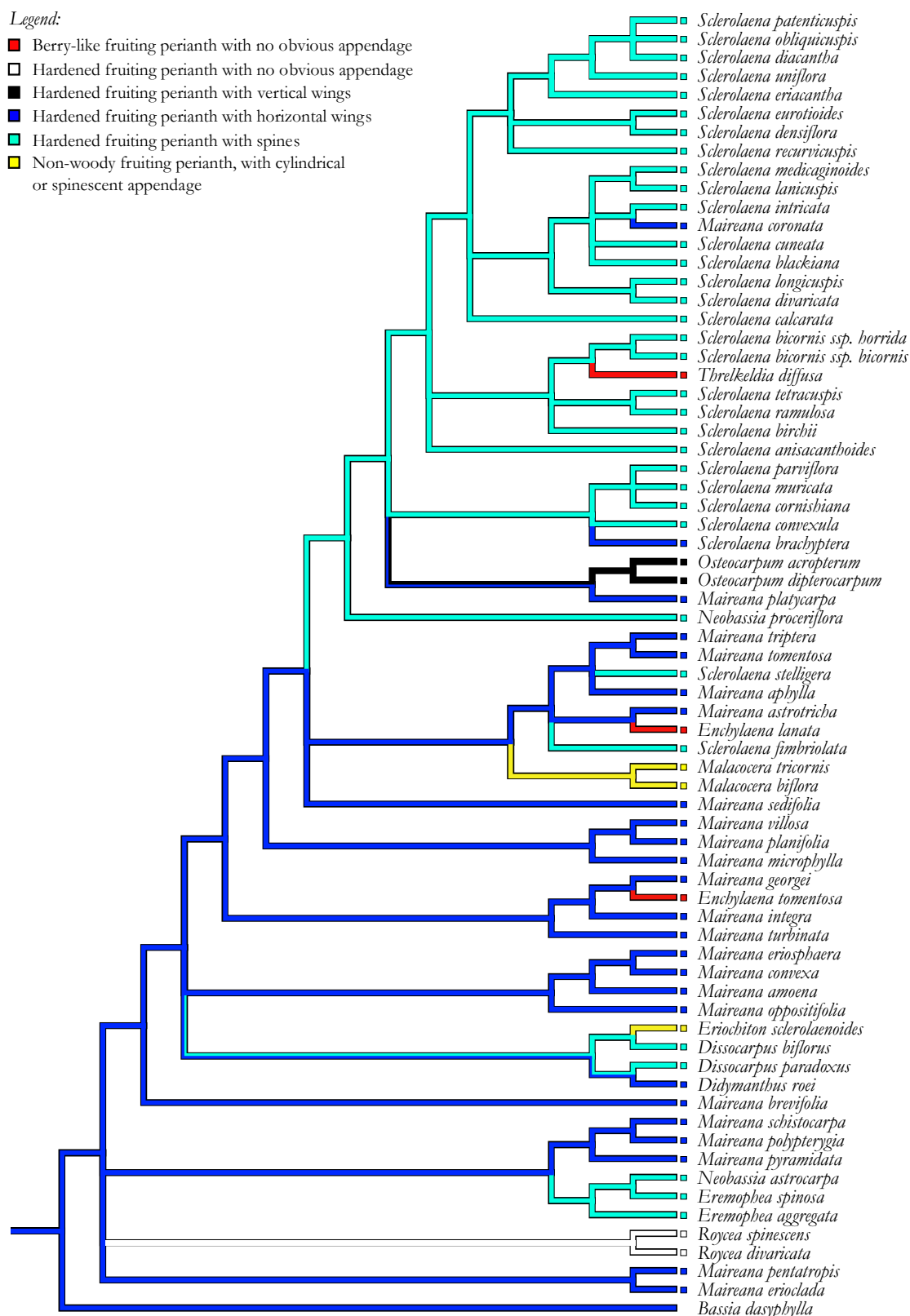


Figure 12. Parsimoniously optimized analysis of the fruiting perianth appendages of the Australian Camphorosmeae on the ML tree derived from ETS sequence data.

- f. Non-woody fruiting perianth, with cylindrical or spinescent appendage – the fruiting perianth possesses flattened or sub-cylindrical processes, attached either along the length of the perianth tube or at its base. *Malacocera* (Figure 13a.G and H) and *Eriochiton* have fruiting perianth developing such appendages, differing mainly from each other based on their positions on the perianth tube, and their shapes.

Parsimony optimization of the fruiting perianth appendage types on the ETS-derived ML tree is shown in Figure 12. Among the six analyzed character states only two were resolved monophyletic, namely, the hardened fruiting perianth with no obvious appendage and the hardened fruiting perianth with vertical wings. The non-appendaged character state was placed on the basal polytomy of the tree, whereas the vertical-winged fruiting perianth was resolved on a derived position. The horizontally-winged fruiting perianth was determined as the plesiomorphic state, also being observed in the Eurasian members of Camphorosmeae. Five clades of not less than two taxa distinguished by this character state were monophyletic. Clades characterized by the spiny fruiting perianth were placed in derived positions in the distance tree. The berry-like fruiting perianth arose three times, whereas the hard, cylindrical or spinescent appendage arose twice.

Alternate phyllotaxy was observed in 64 (92.75%) species of the investigated Australian Camphorosmeae (Appendix 5a). The opposite and whorled leaf arrangements only occur in few phylogenetically unrelated taxa. Of the 71 accessions, only two species showed an opposite leaf arrangement, i.e. *Maireana platycarpa* and *Didymanthus roei*. *Sclerolaena fimbriolata*, on the other hand, exhibited a whorled phyllotaxy.

In Camphorosmeae, flowers/fruits are solitary, paired or aggregate (Appendix 5b). The solitary fruit form was most common in species of the tribe in Australia and was represented in the analysis by 56 taxa (81.16%). Several other species, such as *Didymanthus roei* and *Malacocera biflora*, exhibit paired fruits that are fused along the modified perianth tube. Still other species form an aggregate of unilocular fruits which fuse along the perianth tube as the perianth matures and is modified. This fruit form has been observed in *Eremophea aggregata* and *Dissocarpus paradoxus*.

Plasticity of fruit form has also been recorded for seven taxa (10%) included in this study. For example, *Maireana planifolia*, *M. villosa*, *M. sedifolia* and *Sclerolaena bicornis*

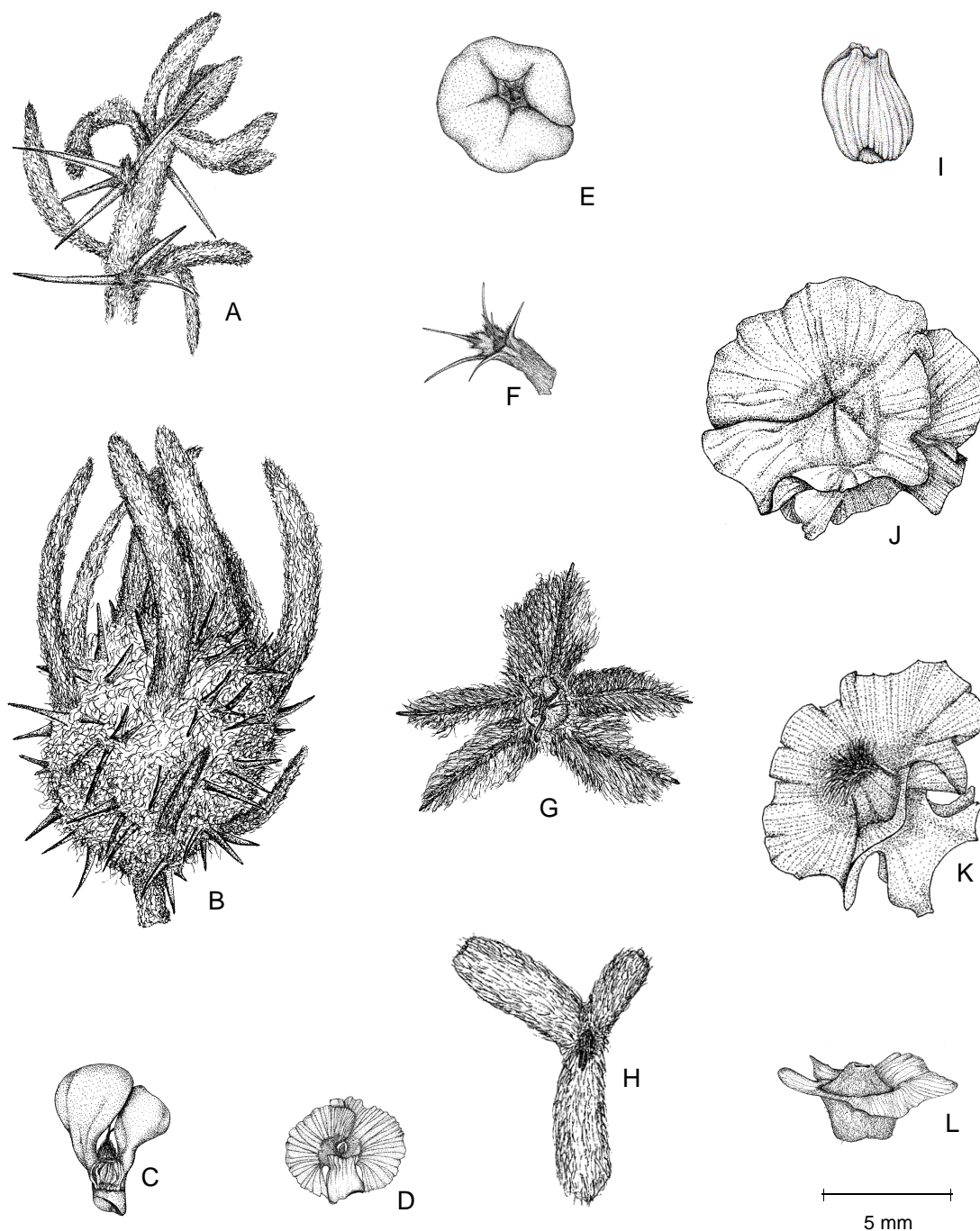


Figure 13a. Fruiting perianth in Australian Camphorosmeae. Berry-like fruiting perianth: E. *Enchylaena tomentosa* and I. *Threlkeldia diffusa*; Hard, cylindrical or spinescent fruiting perianth: G. *Malacocera gracilis* and H. *M. tricornis*; Woody fruiting perianth with spines: A. *Eremophea spinosa*, B. *E. aggregata* and F. *Neobassia proceriflora*; Woody fruiting perianth with vertical wings: C. *Osteocarpum acropterum* and D. *O. dipterocarpum*; Woody fruiting perianth with horizontal wings: J. *Maireana erioclada*, K. *M. pyramidata* and, L. *M. convexa* (drawn by H. Mohr).

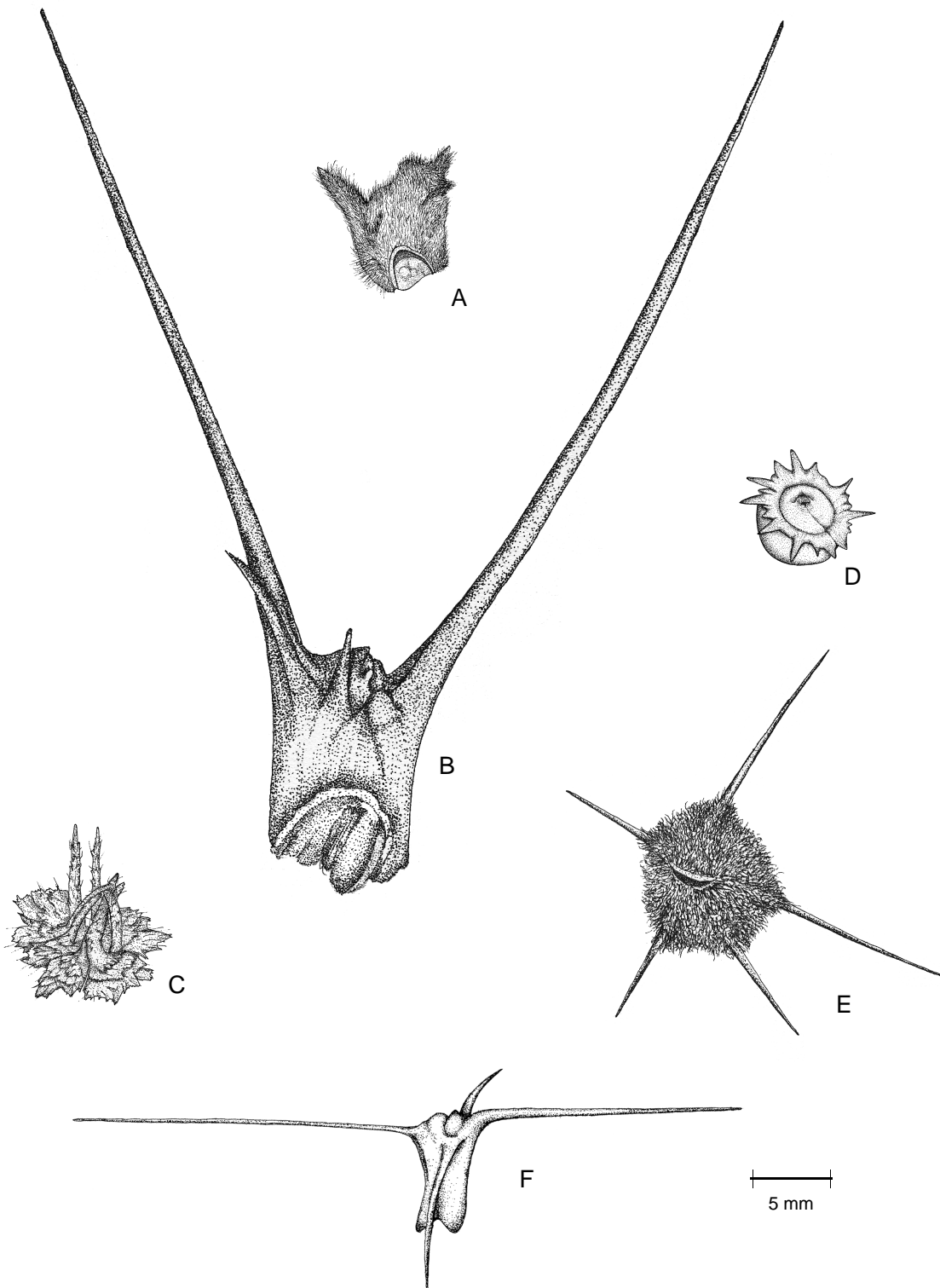


Figure 13b. Variation of the spiny and woody fruiting perianth in *Sclerolaena*. A. *S. uniflora*; B. *S. longicuspis*; C. *S. fimbriolata*; D. *S. stelligera*; E. *S. cornishiana*; and, F. *S. divaricata* (drawn by H. Mohr).

var. *bicornis* may possess both solitary and paired fruits, either on an individual or between individuals of the same species. On the other hand, *Eremophea spinosa* and *Sclerolaena densiflora* may have either solitary or aggregate fruits.

Sixty-one accessions (88.41%) were found to be bisexual (Appendix 5c), and eight (11.59%) of the studied samples were unisexual. The unisexual taxa were found in different positions and appeared not to be phylogenetically related, with the exception of *Maireana polypterygia* and *M. pyramidata*, whose relationship was supported by both parsimony bootstrap and Bayesian posterior probability (Figure 6).

The ability of the fruits to detach from the plant has also been assessed (Appendix 5d). In 64 (92.75%) accessions, the dried fruits detached easily from the plant. However, there were five species (7.25%) observed whose fruits are fused with the stem axis, and do not detach from the plant without external mechanical assistance.

Parsimony optimization of the pubescence of the fruiting perianth on the cladogram resulted in no apparent groupings (Appendix 5e). Pubescent fruiting perianth was observed in 38 (55.07%) of the sampled ingroup accessions, 30.43% (21 species) have wholly pubescent fruits, and 17 taxa (24.64%) were partially hairy, mostly with hairs localized on the perianth tube.

Among taxa possessing pubescent fruits, hair types and hair cellularity optimizations also resulted in ambiguous divisions. The three different hair types did not form distinct groupings. Taxa having dendritic hairs occurred in two different positions on the cladogram (Appendix 5f). Sixteen (23.19%) accessions have simple, unbranched hairs while 18 (26.09%) taxa have hairs possessing tubercles or short extensions. Both of these characters also arose in multiple positions.

From the taxa sampled possessing pubescent fruits, all accessions except *Sclerolaena parviflora* exhibited multicellular hairs (Appendix 5g). Hair length and hair cellularity have been observed to be correlated. Taxa having long hairs tend to be multicellular, whereas short hairs are normally unicellular, such as in the case of *Sclerolaena parviflora*.

Seeds of the Camphorosmeae were in 52 (75.36%) accessions horizontally positioned within the fruiting perianth (Appendix 5h). Seventeen or 24.64% of the sampled

species have seeds that are vertically or obliquely oriented. These two states formed equivocal groupings.

Mapping pubescence characters of the leaves and stems of the sampled accessions also showed vague distributions along the cladograms (Appendix 5i-5n). However, most taxa have pubescent leaves (46 accessions or 66.67%) and stems (51 sampled species or 73.91%). Dendritic hairs were present only in *Eremophea aggregata*, *E. spinosa* and *Maireana astrotricha*.

3.6 Historical biogeography of Camphorosmeae

A summary of the results from all biogeographic analyses is presented in Table 8.

Table 8. Summary of most probable ancestral areas for the Australian Camphorosmeae determined using multiple tests of biogeography

Methods	Ancestral Area
Dispersal-Vicariance Analysis (DIVA)	Southwest, Western Desert, Pilbara
1° Brooks Parsimony Analysis (1° BPA)	Western Desert
Cladistic Analysis of Distributions and Endemisms (CADE; sampled taxa)	Western Desert
CADE (all taxa)	Eastern Desert
Ancestral Area Analysis (AAA)	Southwest
Weighted Ancestral Area Analysis (WAAA)	Eyre

3.6.1. Dispersal-vicariance analysis

Dispersal-vicariance analysis required a completely bifurcating tree. Since the maximum likelihood tree was not completely resolved, a modified likelihood tree derived from multiple ML analyses of sub-samples of the ETS matrix was used for this test. The robustness of the basal clades and relationships among taxa in polytomies was tested by redundant sub-sampling and re-sampling of the accessions. The clade containing *Maireana erioclada* was resolved as the most basal clade in the Australian Camphorosmeae in 70%

of the replicates. The *Maireana brevifolia* clade was resolved most basal only 16% of the time, and 14% of the total replicates showed a basal polytomy.

Dispersal-vicariance analysis of Camphorosmeae resulted in an exact solution when the maxareas option was set to two (Appendix 6). This result suggested three alternative ancestral distributions for the root of the Australian Camphorosmeae (Figure 14): (1) the Southwest floristic region, (2) the Southwest and the Western Desert regions combined, and (3) the Southwest and the Pilbara floristic provinces.

From the Southwest, DIVA implied an initial eastward range expansion into the Eastern Desert region and eventually occupying the central southern coast of the continent into the Eyre and Adelaide floristic zones (Figure 15A). Further dispersal into the southeastern regions of Australia, i.e. into Eastern Queensland, McPherson-Macleay, Southeastern New South Wales and Victoria, was suggested by DIVA before a northward expansion took place.

The northern tropical regions of Australia did not belong to the possible ancestral areas of Camphorosmeae, suggesting that the presence of the plant group in these areas is a result of recent dispersal/expansion.

3.6.2 Primary Brooks parsimony analysis

Primary BPA of all 15 ingroup clades (see Appendix 7) produced two equally parsimonious cladograms, differing only in the position of the Western Desert Region. However, these cladograms have a consistency index (CI) of only 50.8%, indicating almost 50% homoplasy. Although weakly supported, parsimony bootstrap analysis of the taxon-area matrix resolved the Western Desert Region as the earliest split from all other regions in the 1° BPA (Figure 16A). The remaining relationships were moderately to strongly supported by parsimony bootstrap. From the Western Desert, 1° BPA suggested an initial range expansion into the East – Southeast, particularly into the Eastern Desert, Eyre and Adelaide (Figure 15B). Succeeding dispersal events included a westward expansion into the Southwest and Pilbara regions, and northwards into the Northern Desert. A secondary eastward range expansion took place shortly after colonization of the Northern Desert into Southeastern NSW, Eastern Queensland and McPherson-Macleay. Finally, the interzones of northern tropical Australia were most recently colonized by Camphorosmeae.

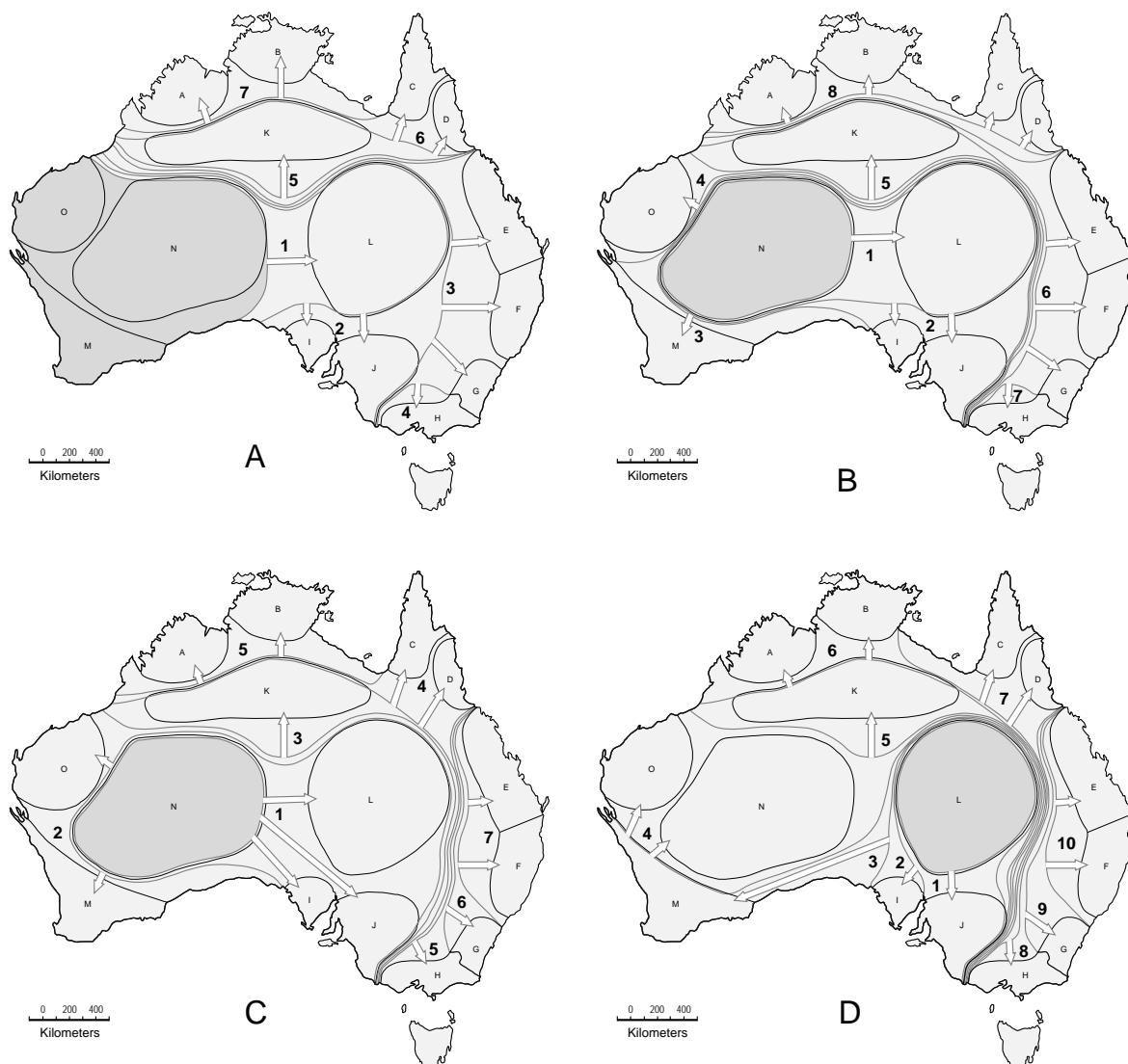


Figure 15. Dispersal routes of *Camphorosmeae* in Australia inferred from multiple biogeographical tests. A. Dispersal vicariance analysis; B. Brooks parsimony analysis; C. Cladistic analysis of distributions and endemism (sampled taxa); and, D. Cladistic analysis of distributions and endemism (all taxa). Dark-shaded areas represent putative ancestral regions; numbers and arrows represent sequences and directions of dispersal events.

Figure 14. The exact biogeographic solution using dispersal-vicariance analysis. Possible ancestral centers of endemism are shown on the nodes of the cladogram. Physically non-adjacent solutions are written in italicized lowercase.



E = Eastern Queensland; F = McPherson-Macleay; G = Southeastern New South Wales; H = Victoria; I = Eyre; J = Adelaide; K = Northern Desert; L = Eastern Desert; M = Southwest; N = Western Desert; O = Pilbara.

3.6.3 Cladistic analysis of distributions and endemism

Like 1° BPA, cladistic analysis of distributions and endemism of all sampled taxa resolved the earliest split of Camphorosmeae in Australia between the Western Desert region and the rest of the continent (73% parsimony BS; Figure 16B). Most other relationships resolved by CADE were already found out using 1° BPA. The main difference between the two methods was that more polytomies were suggested by CADE than by 1° BPA. In addition, CADE suggested an earlier expansion event into the northern interzones of tropical Australia than into the east coast, i.e. into Eastern Queensland, Southeastern NSW and into MacPherson-Mcleay (Figure 15C).

Complete CADE of the Australian Camphorosmeae produced a somewhat different scenario of area relationships (Figure 17). Inclusion of all taxa in the CADE of all floristic regions of the continent, suggested an initial split of the Eastern Desert Region from the rest of Australia (Figure 15D). This was followed by a southward range expansion into Adelaide and Eyre, and eventually westwards into the Western Desert, Southwest, and Pilbara floristic zones. A northward dispersal event commenced thereafter, initially reaching the Northern Desert Region and subsequently the interzones of tropical Northern Australia. The last expansion events were suggested to occur eastwards into Eastern Queensland, Southeastern New South Wales and MacPherson-Mcleay.

3.6.4 Ancestral area analysis

The AAA suggested that the ancestor of Camphorosmeae initially inhabited the Southwest region and from there has dispersed eastwards into the Western Desert region and Eyre (Table 9). The succeeding expansion event was a long-distance dispersal into MacPherson-Mcleay, which bypassed the Eastern Desert of the Ereman region. Following this event, the predecessors of the present-day Camphorosmeae dispersed along the eastern coast of Australia, into Eastern Queensland and Southeastern New South Wales. The Eastern Desert and the Adelaide regions were shown to have been colonized most recently, after the expansion into the northern tropical interzones.

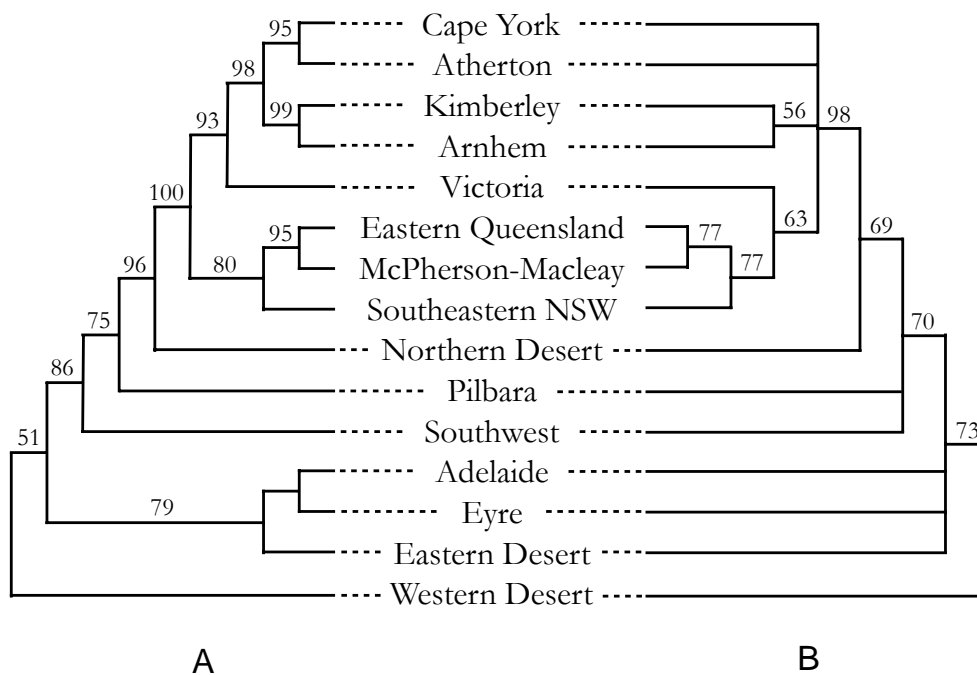


Figure 16. Area relationships derived from Primary Brooks parsimony analysis (A) and cladistic analysis of distributions and endemism (B) of the Australian Camphorosmeae. Values above branches are parsimony bootstrap support.

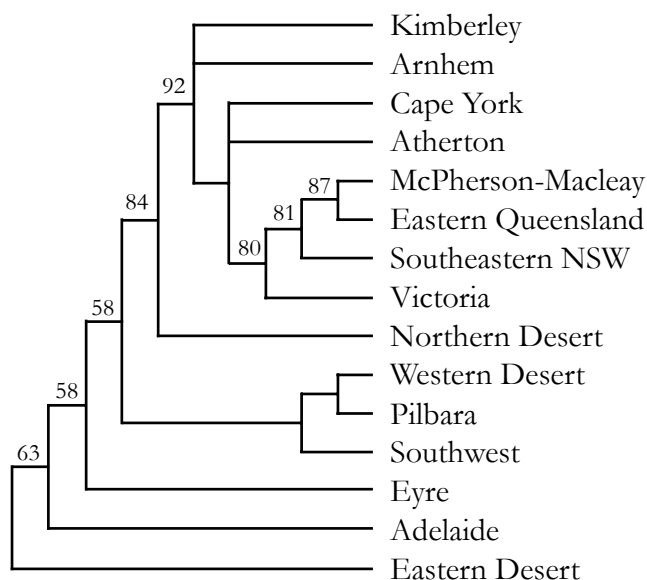


Figure 17. An area cladogram resulting from the cladistic analysis of distributions and endemism of the floristic regions of Australia based on the native Camphorosmeae taxa on the continent. Values above branches are parsimony bootstrap support.

3.6.5 Weighted ancestral area analysis

According to WAAA, ancestors of the Australian Camphorosmeae entered the continent through the Eyre floristic region (Table 9). From this region, possible multiple expansion routes were taken by the ancestral Camphorosmeae taxa. A gradual northward migration into the Central Australian Deserts was suggested by WAAA. Simultaneously other ancestral taxa dispersed westwards into the direction of the Southwest region and eastwards initially to Adelaide, then reached the eastern coast of the continent. The interzones of tropical northern Australia were colonized the latest.

Table 9. Probability indices (PI) for all possible ancestral ranges inferred from ancestral area analysis (AAA) and weighted ancestral area analysis (WAAA). (GSW – weighted gain step; LSW – weighted loss step)

Floristic regions	AAA			WAAA		
	Gains	Losses	PI	GSW	LSW	PI
Kimberley	2	2	1.00	0.22	2.00	0.11
Arnhem	2	2	1.00	0.22	2.00	0.11
Cape York	1	2	0.50	0.09	2.00	0.05
Atherton	2	2	1.00	0.15	2.00	0.07
Eastern Queensland	10	3	3.33	1.12	2.07	0.54
McPherson-Macleay	11	2	5.50	1.11	2.00	0.56
Southeastern NSW	7	2	3.50	0.81	2.00	0.41
Victoria	5	2	2.50	0.47	2.07	0.23
Eyre	20	3	6.67	3.49	0.67	5.17
Adelaide	3	20	0.15	2.06	2.15	0.96
Northern Desert	8	12	0.67	2.47	1.60	1.54
Eastern Desert	3	19	0.16	1.07	2.81	0.38
Southwest	18	2	9.00	1.85	2.00	0.92
Western Desert	12	2	6.00	0.99	2.00	0.49
Pilbara	7	9	0.78	1.33	2.56	0.52

4 DISCUSSION

4.1 The phylogeny of the Australian Camphorosmeae

4.1.1 Suitability of molecular markers in phylogenetic studies of *Camphorosmeae*

DNA sequences have become an indispensable source of data for studies on the phylogeny, biogeography and systematics of plants. Almost all contemporary phylogenetic studies make use of sequences from either protein-coding or non-coding DNA fragments of the different genomes. For lower level taxonomic studies or for questions regarding purportedly recently-evolved taxa, non-coding regions are more suitable under the assumption that these regions should be under less functional constraint than protein-coding regions and should provide greater levels of variation for phylogenetic analyses (Gielly & Taberlet, 1994; Xu et al., 2000; Shaw et al., 2005, 2007; Mort et al., 2007). Several of the most variable non-coding DNA segments used in numerous plant systematic work from the nuclear and chloroplast genomes have been tested for the phylogeny and biogeography of the Australian Camphorosmeae, with varying degrees of utility.

Of seven molecular markers, i.e. the external transcribed spacer (ETS) and the internal transcribed spacer (ITS) of the nuclear rDNA, *trnL-trnF* spacer, *rpS16* intron, *rpL16* intron, *trnS-trnG* IGS and *trnP-psaJ* IGS, only four were used to generate phylogenetic hypotheses for the Australian Camphorosmeae, two regions from the nuclear genome and two from the plastid genome. However, in terms of phylogenetic utility, the ETS was the most useful, having a high percentage of parsimony informative (PI) characters and a moderate retention index, suggesting a relatively low level of homoplasy (Table 5 & Figure 6). Although the internal transcribed spacer of the nuclear rDNA (ITS) belongs to the same transcriptional unit as the ETS, it is not as rapidly evolving and thus has fewer PI characters than ETS.

In the Australian Camphorosmeae, the degree of variation in ETS based on pairwise base difference ranged from 0 to 12%, with a mean of 4%. This is relatively low compared to other groups, which ETS may show a pairwise distance of up to 40% (e.g. Asteraceae, Bayer et al., 2002). Furthermore, sequenced ETS in several plant groups although shorter than that in Camphorosmeae provided more parsimony informative characters (*Ourisia*, Plantaginaceae 475 bp (29%), Meudt & Simpson, 2006; Tribe

Neillieae (Rosaceae) 427 bp (16%), Oh & Potter, 2005; Tribe Gnaphalieae (Asteraceae) 553 bp (55%), Bayer et al., 2002; *Ericameria* (Asteraceae) 505 bp (14%), Roberts & Urbatsch, 2003). Nevertheless, ETS resolved several statistically supported clades and is the most variable among the markers used in this study. It is important to note that in some taxa multiple sequences from different vouchers of the same species were obtained (e.g. *Maireana brevifolia*, *Roycea divaricata*, and *Sclerolaena diacantha*). These sequences varied from each other by several base positions which could be explained by several evolutionary and speciation events. Such intraspecific variation could be due to incomplete lineage sorting as supported by the relatively young age of the group (see 4.1.3 for a detailed discussion). Other possibilities are that these copies represent results of recent hybridization or that the samples from which the sequences were obtained represent different unrecognized subspecies or maybe even different species. To ensure that the last option was not the reason for this intraspecific variation of the ETS, vouchers were controlled for taxonomic integrity. However, not all vouchers were available and in such cases only sequences with available vouchers were included in the following phylogenetic analyses. It may be of interest to mention that when these sequences were analyzed simultaneously, they form monophyletic clades, suggesting the correctness of taxonomy, the homogenization of the ETS copies still being driven by concerted evolution, or the positive selection of one of the differing copy sequences.

The ITS is probably the most sequenced DNA segment in molecular plant phylogenetics. The ITS has been used extensively for plant phylogenetic reconstruction because it evolves rapidly, is subject to concerted evolution (Zimmer et al., 1980; Hillis et al., 1991), and is easily amplified using universal primers in the flanking genes (White et al., 1990; Baldwin, 1992). Numerous studies have used this region to resolve phylogenetic problems at different taxonomic levels (reviewed in Álvarez & Wendel, 2003). It has been previously reported that pairwise divergence values ranged from 0 to 39% in pairwise comparisons between related taxa, with 5–59% of these being potentially phylogenetically informative (Baldwin et al., 1995). In Camphorosmeae, the degree of variation in ITS ranged only from 0 to 6%, and is thus lower than the variation of the ETS.

In most studies involving both the ETS and the ITS, it has been shown that the ETS region evolves more rapidly than the ITS (Baldwin & Markos, 1998; Bena et al., 1998;

Wright et al., 2001). The same is true for the Australian Camphorosmeae. Although both regions occur within the same transcriptional unit, it appears that the ITS experiences more functional constraint than the ETS.

The combinability of ETS and ITS data sets have been proven in numerous phylogenetic studies of plant groups (e.g. *Calycadenia*, Asteraceae, Baldwin & Markos, 1998; *Silphium*, Asteraceae, Clevinger & Panero, 2000; *Machaeranthera*, Asteraceae, Morgan, 2003; *Ericameria*, Asteraceae, Roberts & Urbatsch, 2003; *Uncinia*, Cyperaceae, Starr et al., 2003; *Gaura*, Onagraceae, Hoggard et al., 2004; *Montanoa*, Asteraceae, Plovanich & Panero, 2004; Tribe Neillieae, Rosaceae, Oh & Potter, 2005; *Ourisia*, Plantaginaceae, Meudt & Simpson, 2006). Combining these two regions improved phylogenetic resolution and has yielded more robust phylogenies in many other groups compared to phylogenies based on either ITS or ETS alone (Baldwin & Markos, 1998; Clevinger & Panero, 2000; Markos & Baldwin, 2001; Morgan, 2003; Wright et al., 2003; Meudt & Simpson, 2006). Although this may seem to be the case in Camphorosmeae, the resulting phylogeny was poorly supported by either maximum parsimony bootstrap or Bayesian posterior probability. This lack of statistical support may be the result of the low number of parsimony informative characters.

The use of non-coding chloroplast DNA regions has continually increased and is now routinely employed for studies of phylogeny at intergeneric and interspecific levels (Small et al., 1998; Shaw et al., 2005, 2007; Mort et al., 2007). In most cases, these markers are able to resolve phylogenetic relationships at different taxonomic levels. In the Australian Camphorosmeae, relationships among species suggested by the cpDNA markers differed considerably between each other and to those suggested by the nuclear markers. Although several studies assert that these plastid markers can be useful in resolving phylogenetic relationships at different levels (*trnL-trnF* spacer: Gielly & Taberlet, 1994; Sang et al., 1997; Bayer & Starr, 1998; Small et al., 1998; McDade & Moody, 1999; Bremer et al., 2002; Cronn et al., 2002; Goldblatt et al., 2002; Hartmann et al., 2002; Mast and Givnish, 2002; Mort et al., 2002; Borsch et al., 2003; Fukuda et al., 2003; Jobson et al., 2003; Miller et al., 2003; Salazar et al., 2003; Simpson et al., 2003; Stech et al., 2003; Shaw & Small, 2004; Xu & Ban, 2004; Barfuss et al., 2005; Flannery et al., 2006; Nie et al., 2006; Jiang et al., 2006; *rpL16*: Biffin et al., 2006; Hedenas, 2006; Whittall et al.,

2006; *rpS16*: Goldblatt et al., 2002; Jobson et al., 2003; Popp & Oxelman, 2004; Shaw & Small, 2004; Barfuss et al., 2005; Meudt & Simpson, 2006; Moore & Jansen, 2006; Nie et al., 2006; *trnS-trnG* IGS: Sakai et al., 2003; Kita & Kato, 2004; Shaw & Small, 2004; Levin et al., 2005), the results of this study suggest otherwise. Variation among sequences of the plastid markers is so low, that relationships among the Australian Camphorosmeae could not be resolved.

The plastid *trnS-trnG* IGS and the *trnP-psaJ* IGS showed varying degrees of phylogenetic utility, but as is typical of other chloroplast regions they are not as rapidly evolving as their nuclear counterparts (Levin et al., 2005) and thus have fewer PI characters than either ETS or ITS (Table 5). Furthermore, the utility of the *trnS-trnG* IGS is dubious. The resulting phylogenetic tree is characterized by a low retention index indicating a high degree of homoplasy (Figure 9). The *trnP-psaJ* IGS showed a very high retention index suggesting a low level of homoplasy (Figure 10) even though its PI characters are the lowest among all sequenced markers (Table 5).

Preliminary sequencing of the *trnL-trnF* spacer, *rpL16* intron and *rpS16* intron revealed that they are unsuitable for resolving phylogenetic relationships in the Australian Camphorosmeae. They possess insufficient variation required to resolve interspecific relationships within the tribe. Pair-wise base differences among sequenced taxa showed a divergence of only up to 0.8%. This suggests that these spacer regions are under strong selection, possibly related to the selective pressure on the flanking genes. The lack of informative characters from these markers could also be the result of recent speciation events or hybridization among members of the tribe, and the sequencing of a shared plastid genome.

The ETS provided the most parsimony informative characters compared to the allegedly rapidly evolving plastid loci examined here. While the ITS did not provide as much variation as the ETS, these two markers have the same phylogenetic signal because of concerted evolution. Although several reports suggest that the chloroplast markers included in this study are among the most variable in several plant groups (see Shaw et al., 2005, 2007; Mort et al., 2007), sequence variation in these regions were not enough to resolve relationships in the Australian Camphorosmeae. This finding proves that the utility of the non-coding plastid markers is not universal, and that each has to be tested for

phylogenetic studies of different plant groups. Attempts to find a plastid marker suitable for elucidating evolutionary relationships within the Australian Camphorosmeae have failed. And however important it is to have a phylogeny based on both nuclear and chloroplast markers, this study could only provide hypotheses from the former.

4.1.2 Phylogenetic relationships among Australian Camphorosmeae

Australian Camphorosmeae

A comprehensive phylogeny of the Australian Camphorosmeae has not yet been proposed until this study. Nevertheless, previous phylogenetic studies in Chenopodiaceae based on two molecular markers suggested that the Australian Camphorosmeae is monophyletic and sister to the Central Asian members of the tribe (Kadereit et al., 2003, 2005). The affinity of the Australian and Central Asian Camphorosmeae is rather unexpected since these two groups are not particularly morphologically similar (Scott, 1978a; Wilson, 1975, 1984). The Central Asian taxa *Kochia melanoptera*, *K. krylovii* and *Bassia dasyphylla* are all annual and have herbaceous stems (Zhu et al., 2003), whereas members of the tribe in Australia are perennials, sometimes shrubby, and possess woody stems. With respect to growth form, several Eurasian taxa such as *Camphorosma monspeliaca* and *Kochia prostrata* are more similar to the Australian group than are the Central Asian taxa (Zhu et al., 2003). Nevertheless, the close relationship of Australian Camphorosmeae to the Central Asian species is supported by obvious similarities in leaf structure. In both groups the leaves are succulent and have a C₃ sclerolaenoid anatomy, whereas in the Eurasian group all species are C₄ except *Bassia sedoides*, *B. hirsuta* and *Kochia saxicola* (reviewed in Jacobs, 2001; Kadereit et al., 2005; Kadereit, pers. comm.).

Relationships within the Australian Camphorosmeae

Results of the phylogenetic analyses based on the ETS and ITS markers support the monophyly of the Australian Camphorosmeae with high maximum parsimony bootstrap and high Bayesian posterior probabilities (Figures 6 & 7). The monophyly of the group based on the plastid markers did not receive any bootstrap support and only moderately high Bayesian posterior probability for the *trnP-psaJ* IGS-derived phylogeny (Figures 9 &

10). This result is rather surprising since apparently less variable plastid markers such as the *ndhF*, *rbcL* and the *atpB-rbcL* spacer clearly support the monophyly of the Australian Camphorosmeae (Kadereit et al., 2003, 2005, in prep). Furthermore, hypotheses of phylogenetic relationships within the Australian Camphorosmeae were partly consistent only between the nuclear markers, and between maximum likelihood and Bayesian methods. Support for relationships among species was not at all times obtained, nevertheless, no strongly supported clades from either marker or either method were found in conflict between each other. A similar interspecific relationship was recovered when both partitions were combined (Figure 8). Although the phylogeny resulting from the concatenated nuclear sequence data is almost completely resolved, deep-level relationships did not receive statistical support in the form of bootstrap or posterior probability.

There is no agreement among markers as to which taxa are most basal in the Australian Camphorosmeae (see Figures 6-10). In addition, all results of the phylogenetic analyses suggest that the spine of the trees are unresolved, supported by neither maximum parsimony bootstrap nor Bayesian posterior probability. The base of the ETS phylogeny is a polytomy of clades representing lineages consisting of the monophyletic genus *Roycea*, and *Maireana* together with *Eremophea* and *Neobassia astrocarpa*. Relationships within the three most basal clades are highly supported statistically (Figure 6). The ITS phylogeny suggested that the *Dissocarpus* clade is the earliest evolved clade, and sister to the rest of Camphorosmeae (Figure 7). The results of the phylogenetic analysis of the concatenated matrix of the two nuclear markers unexpectedly showed that the earliest split within the Australian clade is between *Maireana brevifolia* and the rest of the tribe. This disagreement and the irresolution of the basal taxa could be the result of a simultaneous and rapid diversification as arid conditions developed on the continent (discussed further in 4.1.3).

There is also very little agreement among markers with respect to other interspecific relationships within the Australian Camphorosmeae. However, it could be said that in all cases *Sclerolaena*, *Osteocarpum*, *Malacocera* and *Enchylaena* occupied derived positions and thus most likely are not part of the basal stock. *Threlkeldia* also assumed a derived position using both nuclear markers and the *trnS-trnG* IGS. Its apparent basal position in the *trnP-psaJ*-based phylogeny could be the combined effect of outgroup

selection and the extent of sampling. All three markers included *Kochia melanoptera* together with another taxon as outgroup, whereas in the case of *trnP-psaJ* only *Chenolea diffusa* was used. Amplification and sequencing of the *trnP-psaJ* IGS for *K. melanoptera* proved difficult and the use of another outgroup was necessary.

Roycea

This genus, represented by two of three species in the nuclear-based phylogenetic trees, was resolved monophyletic (Figures 6-8). The basal position inferred by the ETS phylogeny was not recovered by all other markers used. Affinity to other Camphorosmeae genera remains inconclusive, although leaves, indumentum and flowers of *Roycea* correspond closely to those found in *Maireana oppositifolia* (Wilson, 1975, 1984), to which it was resolved sister based on the *trnS-trnG* spacer. However, the fruiting perianth is quite different and is unique among all other genera of the tribe in Australia. *Roycea* possesses relatively small, crustaceous fruits surrounded by a persistent perianth at the base. The perianth does not become modified, and the fruit does not develop any extensions or appendages. The unsampled taxon *R. pycnophylloides* shares similar morphological characters with the species included in this study; the major difference among these three is found in the growth form. *Roycea pycnophylloides* is characterized by a dense mat-forming herbaceous habit, whereas the remaining two taxa are erect shrubs with woody branches (Wilson, 1984).

Eremophea

Eremophea is monophyletic (including *Neobassia astrocarpa*, see *Neobassia* below). Both currently recognized species of the genus were sampled in this study. This genus was placed in a relatively basal position in the phylogenetic trees based on the nuclear markers (Figures 6-8). *Eremophea* shows close morphological affinity to *Sclerolaena* owing to the characters of the fruiting perianth. Species of the genus have woody or hardened fruiting perianths which possess five spines (Wilson, 1984).

Neobassia

Neobassia is polyphyletic. But like *Eremophea*, *Neobassia* shows close affinity to *Sclerolaena* by virtue of the fruiting perianth characters. The fruiting perianth of the genus is hardened and possesses five spines (Wilson, 1984). *Neobassia proceriflora*, was not resolved sister to *N. astrocarpa*, but was placed sister to the *Sclerolaena* clades. The non-sister relationship of the two species of *Neobassia* was not surprising considering their taxonomic history. These two species were previously classified as belonging to *Bassia* (most Australian species now transferred to *Sclerolaena* by Wilson (1984)) and *Threlkeldia*, respectively (Ulbrich, 1934). Wilson (1975) observed that both species show similarity in flower structure and habit, and suggested that they be segregated as a distinct genus, which Scott (1978a) in his revision of the Camphorosmioideae did. However, the characters used by Scott to elevate the species into a separate genus are not unique (i.e. vertical seeds and tepaline spines). The same characters are also found in *Eremophea* and several species of *Sclerolaena* (e.g. *S. stelligera*).

Maireana

Maireana is polyphyletic. The genus occupies both basal and derived positions in the phylogenetic tree of the Australian Camphorosmeae (see Figures 6-10). Twenty-three of the 57 species of *Maireana* were sampled in this study. Several *Maireana* species show statistically-supported close relationships with members of *Sclerolaena* (e.g. *M. coronata* and *Sclerolaena intricata* in Figure 6), and to other species belonging to the relatively smaller genera (e.g. *M. astrotricha* and *Enchylaena lanata*, Figure 6). Wilson (1975, 1984) attempted to bring order to the taxonomy of *Maireana*, and suggested morphologically recognizable interspecific relationships, e.g. *M. tomentosa* and *M. integra*, *M. sedifolia* and *M. astrotricha*, *M. pentatropis* and *M. erioclada*, etc. Unfortunately, the results of the molecular sequence-based phylogeny did not always provide statistical and robust support for these relationships. Nevertheless, the ETS phylogeny resolved several statistically-supported clades corresponding to Wilson's observations. For example, the clade composed of *Maireana erioclada* and *M. pentatropis* (96% bootstrap and 100% Bayesian posterior probability, Figure 6). Both species possess semicircular wings that extend along the perianth tube (Wilson, 1984), are often confused to be a single taxon. They can be

distinguished from each other by the raised woolly upper perianth in *M. pentatropis*. Another example is the clade containing *M. villosa* and *M. planifolia* (97% bootstrap and 100 % posterior probability, Figure 6), in which both species have solitary or paired flowers and simple horizontal wings with a single radial slit. In both cases, the characters shared between members of these two clades are not unique to them. Several other *Maireana* species not belonging to these clades exhibit the same characters (e.g. extension of the wings along the perianth tube in *M. polypterygia* and *M. triptera*).

Malacocera

Malacocera is monophyletic. Although only two of the four currently recognized species were sampled for this study, it could be assumed with some of certainty that the remaining two taxa, *M. albolanata* and *M. gracilis*, would form a monophyletic clade with *M. biflora* and *M. tricornis*. *Malacocera biflora* shares more morphological similarities with *M. albolanata* than it does with *M. tricornis*, which is more similar morphologically to *M. gracilis* (Wilson, 1984). The relationship of *M. biflora* to *M. tricornis* inferred from ETS sequence data received 99% BS support and 100% Bayesian posterior probability (Figure 6).

Enchylaena

Enchylaena is polyphyletic. Both recognized species of *Enchylaena* were used in this study. Based on ETS sequence data, *E. lanata* and *E. tomentosa* are not closely related (Figure 6). On the other hand, the ITS-derived phylogeny placed both of them in the same clade together with *M. turbinata* (Figure 7). This relationship however did not receive bootstrap support and only a weak Bayesian posterior probability (56%). Affinity of the genus with species of *Maireana*, with which they have been reported to hybridize in nature, has been previously suggested (Wilson, 1975, 1984). *Enchylaena* differs mainly from *Maireana* based on the succulence of the fruiting perianth. The fruiting perianth of *E. lanata* also possesses wings typical of *Maireana*. In *E. tomentosa*, wings are replaced by a ring-like succulent outgrowth over the perianth lobes (Wilson, 1975). Wilson (1975) observed hybridization between *Enchylaena tomentosa* and *Maireana georgei* wherever the two species are growing together. The phylogenetic relationship of these two species

derived from ETS sequence data (Figure 6) is highly supported by bootstrap (96%) and Bayesian posterior probability (100%). *Maireana turbinata* has also been reported to hybridize with *E. tomentosa* to produce a shrub with fleshy fruits that have a short erect wing (Wilson, 1975, 1984).

Osteocarpum

Osteocarpum is monophyletic. Two of the five recognized species of the genus were included in this study, and their monophyly based on ETS sequence data is highly supported by both bootstrap (99%) and Bayesian posterior probability (100%). The two sampled taxa, *O. acropterum* and *O. dipterocarpum*, have less morphological affinity to each other than they do to the remaining species of *Osteocarpum* (Wilson, 1984), thus, it is expected that the inclusion of the remaining taxa would still result in a monophyletic group.

Threlkeldia

Only one of the two species of *Threlkeldia* was sampled for this study. Whether or not *T. inchoata* would form a sister relationship to *T. diffusa* is difficult to determine in the absence of molecular data. The two recognized species do not appear to be particularly closely related in terms of morphological characters, although both have close affinities with species of *Sclerolaena* (Wilson, 1984). Except for the fact that both species have fruiting perianths devoid of spines, which excludes them from *Sclerolaena*, the two *Threlkeldia* species have relatively dissimilar fruiting perianths (e.g. shape, succulence, seed position, etc.).

Didymanthus

The monospecific genus *Didymanthus* (*D. roei*) was always resolved sister to either *Dissocarpus paradoxus* (ETS & *trnP-psaJ* spacer, Figures 6 & 10) or *Dissocarpus biflorus* (ITS & *trnS-trnG* spacer, Figures 7 & 9). This finding is unexpected since these two genera exhibit significant morphological variation. *Didymanthus* exhibits morphological affinity to *Maireana*, differing principally in the position of the embryo (Wilson, 1975) and the paired fruiting perianth (Wilson, 1984).

Dissocarpus

Dissocarpus is monophyletic (including *Didymanthus roei* and *Eriochiton sclerolaenoides*). Two of the four recognized species of *Dissocarpus* were used in this study, *D. biflorus* and *D. paradoxus*. Although these two species always formed a statistically supported clade with *Didymanthus* and *Eriochiton* (Figures 6-10) they do not appear to be particularly closely related to each other. The remaining two unsampled species of *Dissocarpus*, *D. latifolius* and *D. fontinalis*, share high morphological similarities with *D. paradoxus* but not with *D. biflorus*. The only difference between *D. latifolius* and *D. paradoxus* is the leaf shape. *Dissocarpus latifolius* has narrowly obovate and flat leaves, whereas *D. paradoxus* has linear-terete leaves. Wilson's (1984) decision to recognize *D. latifolius* as a separate species from *D. paradoxus* on this basis is debatable since leaf plasticity is observed in the latter. It should be subsumed as a variety in *D. paradoxus* as previously suggested by Black (1922) and Ulbrich (1934).

Eriochiton

The monotypic genus *Eriochiton* (*E. sclerolaenoides*) is sister to *Dissocarpus*. *Eriochiton* has two rows of spinescent appendages comparable to the spines found in both *Sclerolaena* and *Dissocarpus*. The apparent close relationship of this genus to *Maireana* (Wilson, 1975) is not supported by molecular data, although the entire *Dissocarpus* clade occurs along a grade of basal *Maireana* clades (see Figure 6).

Sclerolaena

Sclerolaena is polyphyletic. Two clades (*Sclerolaena* clades I and II) occur in a polytomy with the *Osteocarpum* clade, and two species, *S. fimbriolata* and *S. stelligera*, were placed in a clade composed mainly of *Maireana* species (Figure 6). Very few interspecific relationships within Clades I and II received statistical support. Furthermore, the bootstrap- and posterior-probability-supported relationships do not appear to be closely related based on characters of the fruiting perianth (e.g. *S. densiflora* and *S. eurotioides*, *S. longicuspis* and *S. divaricata*; Wilson, 1984).

Sclerolaena stelligera and *Sclerolaena brachyptera* have been proven problematic because of the presence of both spines and "wings" on the fruiting perianth (Scott, 1975;

Wilson, 1984; Jacobs, 1988). For this reason, they were considered two different monotypic genera by Scott (1978a), i.e. *Stelligera endecaspinis* and *Sclerochlamys brachyptera*, and maintained in the Flora of Australia by Wilson (1984), which Jacobs (1988) recently subsumed into *Sclerolaena*. Scott (1978a) admitted that *Stelligera* and *Sclerochlamys* are related to *Sclerolaena*. *Stelligera* differs from *Sclerolaena* in possessing both tepaline and intertepaline spines as opposed to the characteristic intertepaline spines of the latter. On the other hand, *Sclerochlamys* differentiates itself from *Sclerolaena* by the presence of a wing along the top plane of the perianth tube with intertepaline ribs extending from it to form short spines. Jacobs (1988) found little justification from Scott's reasoning of maintaining the two taxa as distinct, and therefore placed both in *Sclerolaena*. According to him, the characters of the fruiting perianth represent extreme forms in *Sclerolaena*, and such forms can be observed elsewhere in the genus (e.g. the fimbriate spines of *S. fimbriolata* and *S. symoniana*, and the hollow base and reduction in number of spines in the *S. uniflora* group). The phylogenetic results do not support the generic status in both cases. *Sclerolaena brachyptera* was placed within *Sclerolaena* clade II supporting its inclusion in the genus as suggested by Jacobs. On the other hand, *S. stelligera* was resolved in a polytomy dominated by *Maireana*, and thus neither justifies its subsumption in *Sclerolaena* nor the resurrection of a separate genus.

4.1.3 Age of the Australian Camphorosmeae

In this study, two methods that relax the assumption of a strict molecular clock have been implemented. These smoothing methods (Sanderson, 1997, 2002) reduce dimensionality of a molecular clock by imposing an autocorrelation among the parameters. They allow one rate for every branch but then restrict how much difference in rate is allowed between branches. Nonparametric rate smoothing (NPRS; Sanderson, 1997) and penalized likelihood (Sanderson, 2002) both penalize the parameter estimates of rates by comparing them to their phylogenetically immediate neighbours.

Initial diversification of the Australian lineage of Camphorosmeae using these smoothing methods was suggested to have occurred from the Late Miocene to the Early Pliocene, after its arrival during the Middle Miocene (Figures 11a & 11b, Appendix 3c & 3d). This age coincides with several hypotheses on the onset of aridity in Australia (Beard,

1977; Bowler, 1982; Stein & Robert, 1986; Markgraf et al., 1995; Martin, 1998; see 4.5.2 for a detailed discussion). At this time the Australian continent has already been separated from the rest of the southern landmasses (Veevers, 1986, 2000; Stagg & Willcox, 1992; McLoughlin, 2001) suggesting that ancestors of the group must have arrived there via long-distance dispersal and therefore are not of Gondwanan origin. However, several workers on Australian biogeography suggest that progenitors of the arid flora of Australia, including Chenopodiaceae, were already present on the continent even before the onset of aridification (Burbidge, 1960; Beadle, 1981; Christophel, 1989; Schodde, 1989). They have initially occupied coastal habitats which enabled them to adapt to the novel environment which resulted from the drying up of the Australian landmass.

It is parsimonious to assume that the “ancestral stock” giving rise to the present day Camphorosmeae were already adapted to arid conditions before they arrived in Australia. Other members of the tribe in Eurasia and elsewhere are especially adapted to such conditions, and this character must have developed once in the evolutionary history of the group. This is supported further by hypotheses on the onset of aridification of continental Asia, which was estimated to have taken place at the beginning of the Miocene ca. 22 mya (Guo et al., 2002). The apparent meagerness of lineages shortly after the supposed entry of the basal stock in Australia (Figures 11a & 11b, Appendix 3c & 3d) would suggest that the necessary environmental conditions upon arrival of the ancestral Camphorosmeae taxa that would have encouraged diversification in the group did not exist. Then as the arid habitat developed in Australia during the Late Miocene to the Early Pliocene, Camphorosmeae also gradually diversified into the newly opening niches. Most stocks of the present day Australian Camphorosmeae have already existed during the Pliocene (at least 72% based on *ndhF* calibration and 45% based on *rbcL*). This process continued all throughout the Quaternary until the group assumed its current diversity.

It must be emphasized that the ages of the calibration point used for the molecular dating analyses in this study is secondary in nature (i.e. they are not independent fossil-based dates) and represent dates based on two molecular markers calibrated using multiple fossil records (Kadereit et al., 2005, in prep.). Nevertheless, the general divergence ages for Camphorosmeae in Australia determined by the molecular dating analyses are considerably similar, falling within the range of the Late Tertiary. Furthermore, these

results make sense in light of the climatic history of the continent (discussed further in Chapter 4.5).

Roycea together with *Maireana* appeared to be old genera which diverged from the ancestral stock during the initial diversification phase of the group on the continent. The clade containing *Dissocarpus* differentiated from the rest of the tribe during the Mid- to Late Pliocene ca. 3-4 mya (Figures 11a & 11b, Appendix 3c & 3d). From this stock also came the genus *Didymanthus*, although of fairly recent origin. This species separated from the former ca. 3 to 1.8 mya during the Pliocene-Quaternary boundary. Ancestral clades that gave rise to several other genera could be traced back to the Late Tertiary and the Quaternary. *Osteocarpum*, *Threlkeldia*, and *Eremophea* were the result of recent diversifications in Australia.

Neobassia and *Enchylaena* were both polyphyletic, stocks of which diverged from the rest of the tribe once during the Pliocene and again in the Quaternary. The genus *Maireana* has a rather complex history, arising multiple times during the evolution of the group. The oldest *Maireana*-containing clades appeared during the Late Miocene. *Sclerolaena*, on the other hand, first arose during the Mid-Pliocene, and showed several diversification events throughout the Late Pliocene and all the way through the Quaternary (see Appendix 3 for a detailed list of the divergence age estimates).

The age estimates provided here represent only possible minimum ages for the Australian Camphorosmeae. The scantiness of palaeontological evidences makes it difficult to confirm whether or not these ages come close to the “real ages” of the group. Nevertheless, divergence age estimates using the two relaxed clock methods did not produce ages that conflict with the earliest fossil record for Chenopodiaceae in Australia, which was dated from the Oligocene-Miocene boundary (Martin, 1981; Christophel, 1989). Furthermore, it could be said with some degree of certainty, that the diversification in the Australian Camphorosmeae would have been influenced greatly by environmental evolution, and thus by ecological opportunity and most-probably by niche pre-emption.

4.2 Morphological mapping

Of the 15 investigated morphological characters, only the type of the fruiting appendages (or its absence) showed a pattern comparable to the ETS-based phylogeny.

This is surprising since characters of the fruiting perianths, particularly their appendages, are believed to be homoplasious and that similarities between taxa were the result of parallel evolution (Wilson, 1984). But for the lack of substantial homologous and taxonomically significant characters, Wilson (1984) used structures of the fruiting perianth in discriminating species, and based the most recent taxonomic key to the tribe in the Flora of Australia on these characters.

Analyses of the remaining characters resulted to no clear taxonomic signals. This is also surprising, since some of these characters, particularly the indumentum, has been studied considerably in other groups of Chenopodiaceae, and in some cases was shown to be of systematic significance (Ulbrich, 1934; Carolin et al., 1975; Scott, 1978b; Carolin, 1983; Wilson, 1984; Simon, 1996; Klopper & van Wyk, 2001; Mosyakin & Clemants, 2002; Kadereit, 2003). In the Australian Camphorosmeae, no defined groupings were obtained from the analyses of the different indumentum characters indicating convergent evolution, or loss of characters in different, distantly related species (see Appendix 5).

Cladistic mapping of the fruiting perianth appendages on the phylogenetic tree appears to establish two major groups: a basal grade of taxa possessing wings, from which another group of species whose fruiting perianth have spines arose. However, there are some instances showing that the spiny fruit appendages resulted from convergent evolution. The winged fruiting perianth also did not form a monophyletic clade; instead, it belongs to multiple basal clades and it appeared thrice in derived positions in the maximum likelihood tree (Figure 12). It could be assumed that the winged-fruit is the plesiomorphic character and the presence of this character state in terminal positions could be interpreted as a reversal to the ancestral state. The same conclusion was given by Scott (1978a) in his revision of Camphorosmioideae. According to him, the ancestor of the entire subfamily (= Camphorosmeae) may have been most similar to some species of *Maireana*, that is a shrub with opposite branches, probably dioecious flowers arranged in groups of 3 in the leaf axils, and with a thin, accrescent perianth with an annular, membranous wing. He also suggested that the annual, herbaceous habit eventually developed in the Eurasian genera, including the Central Asian taxa. Furthermore, the intertepaline spines of *Sclerolaena* and the tepaline spines of *Neobassia* and *Dissocarpus* are derived characters, which developed in different lines of evolution.

4.2.1 Winged fruiting perianth

The wing-like structures observed on the fruiting perianth of the Australian Camphorosmeae (see Figures 13J, 13K, 13L) are also present in members of the tribe occurring in Eurasia and North America, as well as in species of the Central Asian sister group (e.g. in *Pandertia pilosa*, Scott, 1978a; *Bassia dasyphylla* and *Kochia melanoptera*, Zhu et al., 2003). Many species of the tribe Salsoleae with which Camphorosmeae shares a sister relationship based on molecular data (Kadereit et al., 2003) also have winged perianth, such as *Salsola*, *Girgensohnia* and *Sympegma* (Zhu et al., 2003). According to Scott, the wings in the fruiting perianth of *Pandertia* developed from the winged ancestor by reduction, and thus are homologous to those found in *Kochia*, *Bassia*, and *Maireana*. The Eurasian taxa have been suggested to be older than the Australian members of the group (ca. 21.6-14.5 mya based on *rbcL* sequence data, Kadereit et al., 2003), and probably gave rise to the latter more derived clade (Kadereit et al., 2005). Therefore, the idea that winged fruiting perianths are the plesiomorphic character state is further supported. The development of wings on fruits in general could be seen in the evolutionary point of view as an adaptation to wind dispersal (Howe & Smallwood, 1982; Jurado et al., 1991).

The vertical wings of *Osteocarpum* are not homologous to the horizontal wings of *Maireana*, but are more similar to the spines of *Sclerolaena* (Wilson, 1984).

4.2.2 Other forms of the fruiting perianth

Spiny fruiting perianth

The spiny appendage of the fruiting perianth is a derived character state which arose five times in the Australian Camphorosmeae. Again this is a clear case of convergent evolution possibly driven by adaptation to zoochory, particularly to exozoochory. Spines on fruits or, in this case, on the fruiting perianth allow dispersals through animals in which they become attached to hairs, feathers, fur or hooves (Howe & Smallwood, 1982). However, there are reports that some species of *Sclerolaena* (e.g. *S. bicornis* and *S. divaricata*) are dispersed through endozoochory by emus (Rogers et al., 1993). Spines as adaptive response against herbivory may be disclosed, since herbivores in the arid regions of Australia are practically non-existent, and were recent introductions by European settlers (Anderson, 1982), and thus did not play a significant role in the morphological

evolution of Camphorosmeae. *Sclerolaena*, the most speciose genus of Camphorosmeae in Australia with currently 64 recognized species, has crustaceous to woody fruiting perianth which possess such an appendage (Figure 13b). It arose at least thrice in the genus: once in *S. fimbriolata*, again in *S. stelligera*, and lastly in the clade containing the rest of the *Sclerolaena* species (Figure 12).

Two other instances of independent evolution of the spiny fruiting perianth are found in the *Eremophea* + *Neobassia* clade and in the *Dissocarpus* clade. The presence of *Didymanthus* within the *Dissocarpus* clade could be interpreted as a reversal to the plesiomorphic character state. However, spines in the two sampled *Dissocarpus* species are probably not homologous as they differ in their position on the fruiting perianth. The spines in *D. biflorus*, when present, develop from the perianth lobe, whereas those of *D. paradoxus* arise from the base of the perianth lobe. This ontogenetic difference argues for two independent origins of spines in *Dissocarpus*. The remaining *Dissocarpus* species, *D. latifolius* and *D. fontinalis*, possess spines similar in morphology to those of *D. paradoxus*, although spines of the former are often flattened (Wilson, 1984).

Unappendaged fruiting perianth

The reduction in size and the loss of appendages occurred multiple times in the development of the Australian Camphorosmeae (Figure 12). In *Roycea*, the loss of appendages could be seen as an ecological adaptation. Once the taxa became highly specialized in particular regions of Australia as indicated by their present-day, relatively limited distribution, the necessity for specialized dispersal structures became superfluous. Members of this genus became edaphic specialists in heavy saline soils of the Southwestern floristic province. However, if the distribution of *Roycea* is the result of edaphic specialization or of the inability of the fruits to be widely dispersed remains to be determined. Furthermore, if the loss of appendage occurred shortly after the split from the winged basal stock or during the evolutionary development of the genus could only be resolved by paleontological data. Unfortunately, such information does not exist, and this question could at this point not be conclusively evaluated.

The berry-like fruiting perianth is also a result of convergent evolution, arising three times independently in two different genera. Again, this could be interpreted as an

ecological adaptation since such fruits could be more widely dispersed through animal/bird dispersers. Fruits of *Enchylaena* are dispersed by birds (Rogers, 1993) and those of *Threlkeldia* may be assumed to be bird-dispersed because of their succulence which is believed to be attracting birds and encouraging them to consume the fruits (Howe & Smallwood, 1982; Gosper et al., 2005). *Enchylaena* is one of the most widespread taxa of the Australian Camphorosmeae. This is due mainly to its fruiting perianth which is eaten by birds (Rogers, 1993), and its non-preference to specialized edaphic conditions (Wilson, 1984). The limited distribution of *Threlkeldia* along coastal regions of continental Australia may be indicative of specialization to littoral conditions. Inland localities where the species occur are also limited to the vicinity of inland salt lakes. Furthermore, the fruiting perianth of *Threlkeldia* is less succulent and visually duller than those of *Enchylaena*, which would then be preferred for consumption by avian dispersers (Howe & Smallwood, 1982; Gosper et al., 2005).

Fruiting perianth with spinescent or wing-like appendages

Malacocera and *Eriochiton* have varying fruiting perianth appendages resembling tubercles or elongated perianth lobes, which cannot be strictly classified into either wings or spines. This form of fruiting perianth appendage arose at least twice in the evolution of the tribe. However, the exact relationship between these two genera and their fruiting perianth is not clear. A considerable morphological variation exists between the two, and an evolutionary sister-relationship based on molecular data was also not resolved. *Malacocera* was resolved sister to a clade composed of several *Maireana* species, *Enchylaena lanata*, *Sclerolaena fimbriolata* and *S. stelligera*. *Eriochiton*, on the other hand, has always been resolved to belong to the *Dissocarpus* clade. The homology of the characters is therefore doubtful, and they may represent two different appendage types and thus could not be considered as a case of parallel evolution. The sub-cylindrical and woolly tepaline processes characteristic of *Malacocera* arose once from the plesiomorphic character state. These processes are distinguished from that of members of *Sclerolaena* by their position and their softness (Chinnock, 1980). The monotypic genus *Eriochiton* with its spinescent appendages was derived from a clade characterized by a spiny fruiting

perianth. These appendages fit traits for fruits or fruiting perianth that are dispersed by either wind or animals (Howe & Smallwood, 1982).

4.2.3 Homology vs. convergent evolution

A major consideration when dealing with morphological data applied in phylogenetic problems is the question of homology. In the case of Camphorosmeae, optimization of characters on the phylogenetic tree established several cases of convergent evolution. The possession of similar character states by different species was derived not from common ancestry but was driven by ecological adaptation. Furthermore, the ETS-inferred phylogeny (Figure 6) showed that the degree of homoplasy in the appendages of the Australian Camphorosmeae is considerably high, especially involving taxa with either wings or spines.

The development of multiple types of appendages and fruiting perianth types in Camphorosmeae is seen as an adaptation to the then forming arid environments. Several genera have specialized appendages that are adapted to zoochory, and others to wind-dispersal. These differences would have improved the chances of dispersal and novel niche occupation. Another group of arid-adapted plants which occupies a similar distribution as the Camphorosmeae in Australia is *Atriplex*. This genus has fruits that possess bracteoles believed to be an adaptation to arid conditions (Anderson, 1982). However, such adaptation has little to do with dispersal rather with germination (reviewed in Anderson, 1982). Nevertheless, bracteoles of *Atriplex* may possess winged appendages, which were suggested to be of evolutionary significance (Parr-Smith, 1982).

4.3 Phylogeny and generic concepts: implications to the taxonomy of Camphorosmeae

The Australian Camphorosmeae is a relatively young group of plants, which members are still undergoing the process of speciation. These “incomplete species” are morphologically very similar, and complicate the taxonomy of the tribe. The phylogeny of the Australian Camphorosmeae based on molecular sequence data also did not improve the delimitation of taxa. Both nuclear and plastid markers provided insufficient degree of variation unsuitable for a complete phylogenetic reconstruction in the Australian Camphorosmeae. Although the nuclear markers resolved several statistically supported

clades, several decisive relationships remain unresolved. However, the problem of resolution is based considerably on the young age of the group, and consequently on incomplete lineage sorting resulting to high degree of DNA sequence similarity.

As another consequence of the age of the group, different taxa often hybridize among each other; strengthening support that differentiation is not complete or is fairly recent to allow reproduction between different taxa. Furthermore, present-day hybridization has been observed to occur between several species (Wilson, 1975, 1984). *Enchylaena tomentosa* hybridizes with *Maireana georgei* in West Australia, and *M. turbinata* in South Australia and Victoria. The species *Sclerolaena stylosa* and *S. napiformis* are purported hybrids resulting from a cross involving *S. forrestiana* and *S. parviflora*, respectively. Several other examples of hybridization occur between the following taxa: possibly *Osteocarpum salsuginosum* with *Sclerolaena urceolata*; *O. acropterum* either with *S. calcarata* or *S. anisacanthoides* (= *O. scleropterum*); *S. obliquicuspis* with both *S. brevifolia* and *S. patenticuspis*; *S. uniflora* and *S. diacantha*; *S. constricta* with either *S. parallelicuspis* or *S. holtiana*; *S. articulata* and *S. cuneata*; *Maireana lanosa* with *M. lobiflora* in north-western West Australia; *M. integra* with *M. villosa*; *M. planifolia* with *M. villosa* (Wilson, 1984). Unfortunately, only a few taxa reported to hybridize were available for use in this study. Nevertheless, those taxa that were included occurred in almost always the same clade as the species to which they hybridize (e.g. *Enchylaena tomentosa* and *M. georgei*, *M. planifolia* and *M. villosa*, *S. obliquicuspis* and *S. patenticuspis*, etc. in Figure 6). Although hybridization explains several relationships resulting from the phylogenetic analysis of the tribe, the lack of resolution and dubious interspecific affinities are most likely the result of incomplete lineage sorting.

Workers of the group agree that the taxonomy of Camphorosmeae and Chenopodiaceae in general is complex. Morphological characters used by various authors for distinguishing the individual genera have not been satisfactory. In most cases, these characters represent plesiomorphic characters (i.e. the fruiting perianth wings of *Maireana*) and therefore unsuitable for use in delimiting genera. Furthermore, results of the morphological mapping of fruiting perianth characters and characters of the indumentum on the phylogenetic tree suggest that several character states resulted from parallel

evolution, and thus are not homologous.

The biggest problem lies with the taxonomy of the two most speciose genera of Australian Camphorosmeae, *Sclerolaena* and *Maireana*. These genera have been the subject of numerous nomenclatural and taxonomic rearrangements (reviewed in Anderson, 1923 and Wilson, 1975), and their exact delimitation is still a matter of the authors' opinion. In addition, these two genera in the past also included all other genera of Camphorosmeae on the continent. Anderson (1923) in his revision of *Bassia* (now *Sclerolaena*) and Wilson in his revision of *Maireana* (1975) and in the Flora of Australia (1984), although recognizing the need for a "thorough study" of the group, desisted from mass-rearrangements, even considering it "unwise" to do so, due to the lack of key characters required to circumscribe genera.

Based on the results of this study, several genera do not merit the generic status and should be demoted to species of the remaining genera (Table 10).

The generic and the species concept in the Australian Camphorosmeae must be further reevaluated. Members of this tribe show high degree of phenetic similarity, which does not allow for sharp interspecific or even intergeneric delimitations. Admittedly, the meagerness of morphological characters makes such a task more difficult, but one should consider the possibility that less well-defined genera is better than more highly ambiguously-defined taxa.

4.4 The biogeography of Camphorosmeae and Australian Tertiary aridification

4.4.1 Biogeographic history of the Australian Camphorosmeae

There was a fairly good agreement that the stock from which the Australian Chenopodiaceae have evolved must have been present in the continent during the Tertiary and prior to aridification (Burbidge, 1960; Beadle, 1981; Christophel, 1989). According to these studies, progenitors of the family were most likely coastal taxa, which were adapted to salt-influenced and arid conditions. However, a previous study of Chenopodiaceae based on molecular data suggested that the divergence time of the Australian lineages ranged from late Pliocene as the youngest to late Eocene/early Oligocene as the oldest, with a clear accumulation of arrivals during the late Miocene and the entire Pliocene (Kadereit et al., 2005). The authors suggested that a Gondwanan stock from which the present-day

Table 10. Historical classification of genera in the Australian Camphorosmeae

Ulbrich (1934)	Scott (1978a)	Wilson (1984)	Kühm et al. (1993)	This study
<i>Austrobassia</i>	<i>Stelligera</i>	<i>Stelligera</i>	(<i>Sclerolaena</i>)	(<i>Sclerolaena</i>)
	<i>Eriochiton</i>	<i>Eriochiton</i>	(<i>Maireana</i>)	<i>Eriochiton</i>
<i>Babbagia</i>	<i>Babbagia</i>	<i>Osteocarpum</i>	<i>Babbagia</i>	(<i>Sclerolaena</i>)
<i>Coilocarpus</i>	(<i>Threlkeldia</i>)			
<i>Didymanthus</i>	<i>Didymanthus</i>	<i>Didymanthus</i>	<i>Didymanthus</i>	<i>Didymanthus</i>
<i>Dissocarpus</i>	<i>Dissocarpus</i>	<i>Dissocarpus</i>	<i>Dissocarpus</i>	<i>Dissocarpus</i>
<i>Duriala</i>	(<i>Maireana</i>)			
<i>Enchylaena</i>	<i>Enchylaena</i>	<i>Enchylaena</i>	<i>Enchylaena</i>	(<i>Maireana</i>)
	-	<i>Eremophea</i>	<i>Eremophea</i>	<i>Eremophea</i>
<i>Kochia</i>	<i>Maireana</i>	<i>Maireana</i>	<i>Maireana</i>	<i>Maireana</i>
	<i>Sclerochlamys</i>	<i>Sclerochlamys</i>	(<i>Maireana</i>)	(<i>Sclerolaena</i>)
<i>Malacocera</i>	<i>Malacocera</i>	<i>Malacocera</i>	<i>Malacocera</i>	<i>Malacocera</i>
-	<i>Neobassia</i>	<i>Neobassia</i>	<i>Neobassia</i>	(<i>Eremophea</i> & <i>Sclerolaena</i>)
	<i>Roycea</i>	<i>Roycea</i>	<i>Roycea</i>	<i>Roycea</i>
<i>Sclerobassia</i>				
<i>Sclerolaena</i>	<i>Sclerolaena</i>	<i>Sclerolaena</i>	<i>Sclerolaena</i>	<i>Sclerolaena</i>
<i>Threlkeldia</i>	<i>Threlkeldia</i>	<i>Threlkeldia</i>	<i>Threlkeldia</i>	(<i>Sclerolaena</i>)

Chenopodiaceae were derived did not exist; rather, the presence of the family in Australia has been the result of multiple arrivals at different times. For example *Atriplex*, with its ca. 61 species in Australia, is believed to have colonized the continent at least three times during the Pliocene and the Quaternary (Kadereit, pers. comm.). According to this study, Camphorosmeae diverged from its Central Asian sister group ca. 6.5–2.9 mya based on *rbcL* data, and ca 8.1–3.6 mya according to the ITS data. Furthermore, it was suggested that the colonizers arrived via long-distance dispersal from the temperate semi-arid to arid parts of continental Eurasia. The divergence age estimation implementing a relaxed molecular clock using ETS sequence data and the results of the biogeographic analyses in Camphorosmeae support the Miocene to Pliocene dispersal hypotheses proposed by Kadereit and colleagues (2005).

All biogeographic tests, phylogenetically- or distribution-based, showed that the most probable ancestral area of endemism of Camphorosmeae in Australia was or laid in close proximity to the southern coast of the continent. Barlow's (1981) hypothesis that Chenopodiaceae may actually have radiated from within the arid zone, i.e. Central Australia, is not corroborated by the biogeography of Camphorosmeae presented here. From the South, there was a multidirectional radiation into the neighboring regions until it reached the temperate east coast and the interzones of tropical north Australia. These events were most likely driven by the formation of arid regions as Australia dried up starting during the Late Tertiary.

Dispersal vicariance analysis resulted to three biogeographic zones as the most probable ancestral areas of Camphorosmeae: the Southwest, Western Desert and Pilbara floristic regions. The apparent absence of a distinct distribution pattern from the determined ancestral centers of origin could indicate recent multiple dispersals into the present distribution areas of the group. Such biogeographic relationships would have been the result of contractions and expansions of areas of endemisms (i.e. into the arid regions), and consequently extinction, migration, colonization and recolonization of these regions. Although a general biogeographic pattern could not initially be deduced from DIVA, superimposing the DIVA tree into the calibrated phylogeny of the tribe resulted to the general area cladogram presented in Figure 18 (see also Figure 15a).

One major drawback of DIVA is that it underestimates dispersal because sister taxa

having the same areas of distribution are assumed to be derived from a common ancestor that speciated through vicariance simultaneously in all areas (Voelker, 1999). Similar to the problem of morphological or molecular homology, the case of parallel dispersals could as well account for similarities in distribution areas and not common ancestry. Another problem lies with the reconstruction of ancestral areas when different descendants occupy different areas (Drovetski, 2003). DIVA favours a widespread ancestor that includes all areas occupied by its descendant species forcing an unrealistic assumption that an ancestor had a much wider distribution than any of its descendants (Ronquist, 1997). It is generally believed that the ancestral distribution of *Camphorosmeae* in Australia was not widespread, and thus vicariance could not account for the current distribution of the group. In this case, DIVA was set to resolve a maximum of two ancestral areas per node to favour dispersal. This led to the identification of sequential migration events (as opposed to sequential vicariance events) for the ancestors of the tribe. Migration would have favoured dispersal scenarios between adjacent areas. Kimberley, Arnhem, Cape York and Atherton were colonized most recently since these are non-arid regions, and the presence of *Camphorosmeae* here is limited to the interzones (Wilson, 1984).

Biogeographic patterns obtained from 1°BPA and CADE (sampled taxa) are more congruent to each other than to that obtained by DIVA. Ancestors of *Camphorosmeae* occupied the Western Desert region and gradually radiated in similar multidirectional patterns. But not unlike DIVA, the interzones of northern tropical Australia and the temperate east coast were colonized the latest.

Cladistic analysis of distributions and endemism using distribution data from all *Camphorosmeae* species differed mainly from the other analyses in that the Eastern Desert region was resolved to be the center of origin of the tribe. The sequence of radiation was similar to those suggested by either 1°BPA or CADE (sampled taxa). There was an initial expansion from the Eastern Desert to the west into Eyre and the Southwest, eventually migrating northwards into Pilbara and the Northern Desert region, and finally into the interzones of northern tropical Australia and the temperate east coast.

Although the results are similar, the incongruence between the two CADE analyses demonstrates the importance of sampling when addressing problems in phylogeny and biogeography. It is of course ideal and optimal for such studies that a complete or nearly

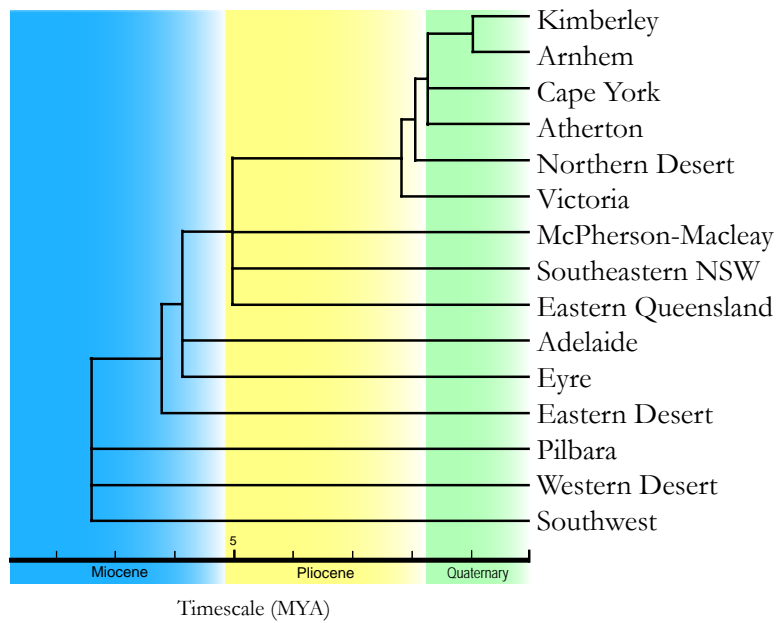
complete sampling be utilized. Unfortunately, it is a logistical reality that specimen for studies are not always readily available.

4.4.2 The role of aridity in the evolutionary history of Camphorosmeae in Australia

There are no significant geological features that are present in Australia that should prevent continuous gene flow within the continent (Barker & Greenslade, 1982; Byrne & Hines, 2004). One would therefore assume that discrepancies in the distribution of plants in the central arid region of the continent have been influenced mainly by climatic changes, competition and micro-environmental factors.

Deeper-level diversifications within the Australian Camphorosmeae are most likely to have been gradual and the Tertiary aridification of the continent appears to have played a key role in the historical biogeography of tribe. The molecular dating analyses imply that initial divergence within the group occurred during the Late Miocene ca. 7.38 mya (based on *ndhF* calibration). By the end of the Pliocene ca. 1.8 mya, there was a 2.5-fold increase in the diversification rate (based on a pure-birth model) of the Camphorosmeae. If correctly dated, the period between the initial diversification and the end of the Pliocene would coincide with the onset of Australian aridification based on Bowler (1982). He suggested that 6-2.5 mya was a period of sustained aridity. Based on weathering profiles of lake sediments, he proposed that this time was associated with the initiation and intensification of the high pressure system. This resulted in a change from a climate where rainfall was evenly distributed to one of summer rainfall similar to that experienced in northern Australia today (Kershaw et al., 1994). Continued atmospheric intensification and movement north of the high pressure system at a greater rate than Australia's drift eventually brought southern Australia under the influence of the westerly wind belt. This reflects the onset of seasonal aridity with wet summers continuing to about 2.5 mya. Thereafter the continental interior would dry while the seasonally intense stresses on the southern margin were relaxed by the incursion of cold and humid, winter air masses coming from the Southern Ocean.

Although widespread arid habitat was probably nonexistent prior to the Late Miocene, the presence of the ancestral stock of Camphorosmeae in Australia ca. 13 mya indicates that suitable habitat must have existed. Burbidge (1960) and Schodde (1989)



A



B

Figure 18.A. General area relationships derived from dispersal vicariance analysis showing possible ages of colonization of the current distribution areas. B. Floristic regions of continental Australia. A = Kimberley; B = Arnhem; C = Cape York; D = Atherton; E = Eastern Queensland; F = McPherson-Macleay; G = Southeastern New South Wales; H = Victoria; I = Eyre; J = Adelaide; K = Northern Desert; L = Eastern Desert; M = Southwest; N = Western Desert; O = Pilbara (modified from Crisp et al., 1995).

suggested that the arid regions of Central Australia were colonized by speciation of taxa occurring along the coasts of the continent from the Late Tertiary until the present. This would mean that progenitors of the family were mostly coastal taxa, which were adapted to salt-influenced conditions (Burbidge, 1960; Beadle, 1981; Christophel, 1989). Burbidge (1960) considered that the adaptation of Chenopodiaceae to salinity pre-adapted them to an arid climate. A similar suggestion has been made with respect to the entry of *Lepidium* (Brassicaceae) into the Australian environment (Mummenhoff et al., 2004). Both drought and salinity-tolerance require mechanisms for managing osmotic stress, and although the mechanisms can be different (Kefu et al., 2003), tolerance of both stresses is widespread in the chenopods (Crisp et al., 2004).

The theory that the ancestors of Camphorosmeae in Australia must come from Eurasia is supported not just by previous biogeographic studies of the tribe and Chenopodiaceae (Kadereit, et al., 2003, 2005), but also by the age of aridification of both the Asian and the Australian continents. The onset of desertification in the Asian interior inferred from loess deposits was dated to be 22 million years ago (Guo et al., 2002). This predates the commencement of aridity in Australia by several million years, which implies an earlier possibility of progenitors of the present-day Camphorosmeae to adapt to arid conditions in Asia.

Vicariance did not play a major role in the historical biogeography of the Australian Camphorosmeae. Previous studies suggested that the ancestors of Camphorosmeae had a narrower distribution than its current distribution. They were localized along the coasts of the continent before aridity took over. The absence of a suitable environment upon the entry of Camphorosmeae in Australia would have prevented the group to occupy a wider distribution range, and thus precluding a possible vicariant scenario.

Long-distance colonization would likely to have played a role in the distribution pattern of the Australian Camphorosmeae, and the current distribution could be explained by gradual and subsequent contractions and expansion of both population and species range. Camphorosmeae radiated concurrently with the development of the arid regions, and it has also reduced its distribution to residual areas as the arid zones contracted. This climatic oscillation pattern has not only influenced the biogeography of the group, but also played a pivotal role in the amount of diversity in Camphorosmeae.

4.4.3 Comparison of biogeographic patterns

Identifying hierarchical patterns of area relationships within continents is difficult (Crisp et al., 1999). The main problem of identifying such patterns is the absence of clear geographical barriers that would delimitate regions into distinct areas of endemism (Crisp et al., 1995). In contrast to continents and islands which are bounded by coastlines, a continuous land mass seldom has sharp internal boundaries, such as big bodies of water or high mountain ranges that provide natural borders. One should therefore exercise caution when determining natural areas of endemism when addressing biogeographic problems within continents. The Australian landmass has no topographical features of major significance that divides the continent into distinct regions, although the Great Dividing Range in eastern Australia forms what appears to be a definite migration route (Burbidge, 1960). Previous workers of Australian biogeography have therefore relied considerably on existing climatic regimes (Burbidge, 1960) or distributional limits of species of vertebrates (Cracraft, 1991) and plants (Crisp et al., 1995) as apparent boundaries, for the lack of a better solution.

Another problem resulting from the absence of apparent geographical barriers is that multiple taxa could differentiate within the same areas at the same time or at different periods. Contemporaneous diversification of multiple taxa with the same areas of distribution need not show similar hierarchical patterns of area relationships. The fitness of the organism, interactions between taxa, climatic situation, micro-environmental conditions and the dynamics between these factors would determine such a pattern. Consequently, this would lead to different area relationships for the same areas of endemism (e.g. in Cracraft, 1982, 1986, 1991: Australian birds, mammals, snakes, lizards and frogs; in Crisp et al., 1995: angiosperm families). For example, black soil plains would be a barrier to both the plant genus *Persoonia* and the bird genus *Platycercus*, whereas calcareous plains supporting forest would be a barrier only to *Persoonia* (Crisp et al., 1995). Therefore, the inherent dispersal ability or the ecological tolerance of *Platycercus* would result in a different dispersal scenario as to that of *Persoonia*, although initially both taxa occupied the same region. Another example, consider two hypothetical plant species, Taxon A and Taxon B, which occupy a coastal region bounded on three sides by three areas of different soil types, e.g. lateritic soil, calcareous sand and clayey loam. In this

scenario, there are no mountains or rivers that would prevent the colonization of the bounding regions by either taxon, in short, no topographical barrier. For some innate biological or genetic (or both) reason, Taxon A is predisposed to disperse only to the region characterized by lateritic soils, whereas Taxon B is an edaphic generalist, and thus is capable of dispersing in all three areas. After several thousand of years, Taxon A developed the capabilities of an edaphic generalist and was able to occupy the remaining two regions, which were already invaded by Taxon B. In the absence of palaeontological or temporal evidence of origin of either taxon, one might assume that both taxa shared the same biogeographic histories by virtue of their current distributions and by their ability to occupy variable soil types, although this ability developed later in Taxon A.

Very few cladistic studies have been done on hierarchical patterns of area relationships within Australia, particularly over the whole continent. Two studies which included a complete representation of all biogeographic regions of Australia were those done by Cracraft (1991) and Crisp et al. (1995). Recent studies are methodically different to the two abovementioned studies disclosing a sensible comparison or are restricted to specific regions of Australia owing to the relatively narrow distribution of the study groups (e.g. Byrne & Hines, 2004; Chapple et al., 2004; Crisp et al., 2004; Hunter, 2005; Ladiges et al., 2005; Strasburg & Kearney, 2005; Crayn et al., 2006).

Cracraft's (1991) general area cladogram for all vertebrates (Figure 19a) shows three major clusters of areas (excluding Tasmania). The first includes a basal divergence between regions linking the Southwest to Adelaide, and the rest of the continent. The second cluster includes the four interior desert region (Pilbara, Western desert, Eastern desert and Northern Desert), and the final cluster includes the areas of the east coast and tropics. The wet tropics (Atherton) are closely related to the Southeast regions and to the adjacent monsoonal areas (Cape York, Arnhem Land and the Kimberley). On this basis, Cracraft hypothesized an ancient vicariance event running east-west along the Nullarbor Plain, followed by another event separating eastern and northern areas from the arid central region. However, his hypothesis was based on the parsimony analysis of endemism (PAE; Rosen, 1988) of vertebrates with widespread distributions, and did not use phylogenetic data from these groups, and thus lacks historical information (reviewed in Crisp et al., 1999; Nihei, 2006). The proposed area relationships therefore cannot explain the history of

the areas of endemism, although he attempted to do so by reconciling different area cladograms, which unfortunately are all non-historical. His study, although presenting one of earliest attempts to provide a hierarchical pattern of area relationships in Australia using cladistic methods, received numerous criticisms for using only distribution data from widespread taxa (Nelson & Ladiges, 1991, 1996; Crisp & Weston, 1995; Crisp et al., 1995; Stafford Smith et al., 1995).

In contrast to Cracraft, Crisp and colleagues (1995) provided a general area cladogram based on phylogenies of eleven taxa from different angiosperm families. The preferred area cladogram from Crisp et al. (Figure 19b) differs from Cracraft's mainly in having an early split between the northern tropical regions (Kimberley, Arnhem, Cape York, New Guinea and Northern desert) and the rest of Australia (except Tasmania, which is basal). Also, the Southwest is most closely related to the Western Desert rather than to the south coastal areas, and the wet tropics are related to eastern and southern coastal areas (Eyre, Adelaide, Victoria, SE New South Wales, McPherson-Macleay, Eastern Queensland), rather than to the monsoonal tropics (Kimberley, Arnhem, Cape York). Although they recognized more areas of endemism based on possible barriers of migration (e.g. the Glasshouse Mountains dividing Eastern Queensland into a northern Eastern Queensland *sensu stricto* and a southern McPherson-Macleay area), they failed to incorporate in their general area cladogram three regions which appear to have played a great role in the diversification of the arid flora of Australia. Furthermore, the basic assumption of vicariance in Crisp's and colleagues, and Cracraft's studies (and in most other biogeographic studies) lends not only support to the incomparability of results obtained from this study to those from both studies, but also question the possibility of vicariance in a closed system practically devoid of significant topographical or geographical barrier. The assumption of vicariance biogeographers that Australia at one time in its relatively long history must have experienced a homogeneous climatic regime or environmental condition that has supported a widespread group of organisms seems to be a farfetched theory. Australia in its current orientation experiences a rather defined climatic system, ranging from tropical climates in the north, to the arid regions of Central Australia and the temperate zones of the Southwest and the Southeast. It is therefore improbable that such a condition existed after its separation from the rest of the southern landmasses (see

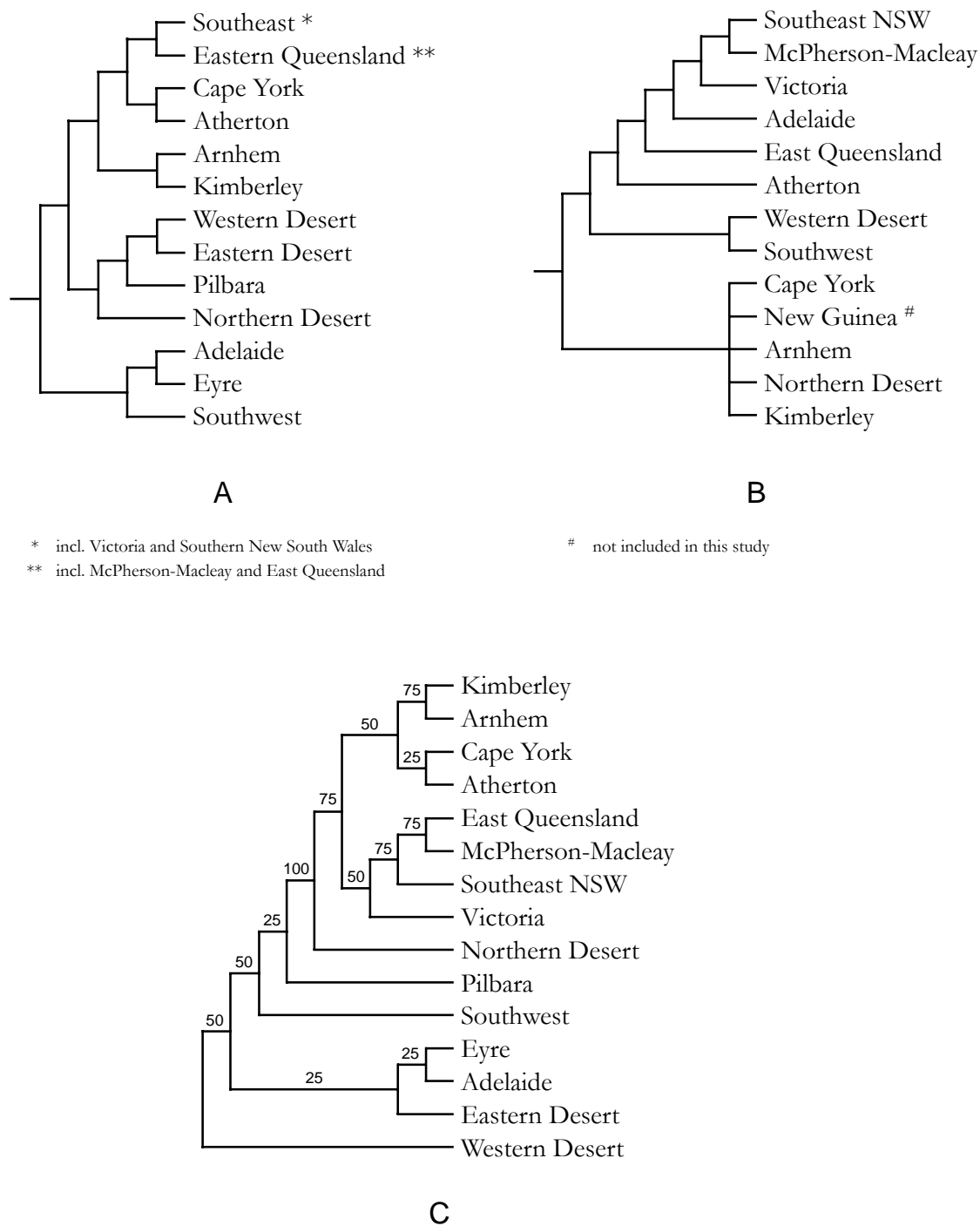


Figure 19. Hypothesized general area cladograms for Australia derived from hierarchical pattern in plants and animal distributions. A. Derived from the combined distributions of birds, mammals, snakes, lizards and frogs (Cracraft, 1991). B. Derived from the phylogenies of 11 taxa in Proteaceae, Myataceae and Fabaceae (Crisp et al., 1995). C. Nelson consensus tree derived from four biogeographic analyses of Camphorosmeae distribution data. Values above branches represent consensus indices.

Frakes, 1999 for a chronology of the evolution of Australian environments).

Ancestral taxa giving rise to the current *Camphorosmeae* arrived in Australia during the Miocene via long-distance dispersal from continental Eurasia. Accordingly, they were not as widespread on the continent as the present-day members of the group. Nevertheless, several area relationships retrieved in both studies are congruent to those found using cladistic and parsimony analyses of distribution data and phylogeny of the Australian *Camphorosmeae* (Figure 19c); for example, the close relationships of regions in the monsoonal tropics proposed by Cracraft (1991) and the eastern coastal areas and the southern coastal regions suggested by both. However, this study provides a more detailed temporal view of the environmental evolution in Australia (Figure 18) which the other two failed to present. The area relationships derived from the historical biogeography of *Camphorosmeae* represents possible responses of these areas of endemism to the then developing arid conditions in Australia. And again, it should be stressed that dispersal and not vicariance was the basis of the resolved hierarchical patterns of area relationships. Furthermore, the area cladograms (Figure 16, 17, 18a & 19c) based on the distribution of *Camphorosmeae* give a complete representation of all areas of endemism in continental Australia, as opposed to the non-representation of Pilbara, Eyre and Eastern desert in Crisp's and colleagues' general area cladogram.

The history of environmental evolution of Australia is obviously complicated, and no single study has yet satisfied the question of the hierarchical pattern of area relationships within the continent. Furthermore, the absence of geographical barriers that might have driven speciation questions the effectiveness of vicariance as the mechanism of the observed patterns presented by previous workers of Australian biogeography. Dispersal and range expansion are gaining a greater role in providing evidences for the evolution of environmental conditions in Australia.

5 SUMMARY

Molecular and morphological data were used to reconstruct the phylogeny and biogeographic history of the arid adapted plant group Camphorosmeae in Australia. Molecular phylogenies were constructed using Bayesian statistics and maximum likelihood. Nonparametric rate smoothing and penalized likelihood were employed to estimate divergence times within the Australian lineage. Morphological characters were parsimoniously mapped on the molecular-based phylogeny of Camphorosmeae. Primary Brooks parsimony analysis, cladistic analysis of distributions and endemism, dispersal-vicariance analysis, ancestral area analysis and weighted ancestral area analysis were performed to infer sequence and directionality of biogeographic pathways.

From seven molecular markers tested, only the nuclear ETS and ITS provided enough variation for the successive analyses of the group; the plastid markers *trnL-trnF* spacer, *trnP-psaJ* spacer, *rpS16* intron, *rpL16* intron and the *trnS-trnG* spacer showed degrees of variation unsuitable for phylogenetic studies in the Australian Camphorosmeae. Phylogenetic hypotheses inferred using the nuclear markers do not completely support the current taxonomy of the group. *Neobassia*, *Threlkeldia*, *Osteocarpum* and *Enchylaena* should be subsumed to the speciose genera *Sclerolaena* or *Maireana*. The results of the cladistic analyses of the fruiting perianth appendage characters support the taxonomic implications of the DNA-based phylogeny. However, indumentum character, which was reported to be of taxonomic significance in several groups in Chenopodiaceae, did not provide support for the phylogeny of Camphorosmeae in Australia.

Ancestors of the present-day Camphorosmeae arrived in Australia during the Miocene (ca. 8-14 mya) via long-distance dispersal probably from continental Asia. Initial diversification of the group occurred during the Late Miocene until the Early Pliocene (ca. 4-7 mya), and by the end of the Pliocene 45% - 72% of the extant lineages were already present, indicating rapid diversification. This age coincides with previous hypotheses on the onset of aridification of the continent, suggesting that environmental evolution played a significant role in speciation in the group. Ancestors of the group appeared to have occupied the southern coastal regions of the continent prior to the aridity of the continent. They then “migrated” in multiple directions as aridity developed during the Late Tertiary and throughout the Quaternary. The success of the group in the then newly forming environment was determined by the adaptation of the ancestral lineages to arid conditions.

The apparent absence of clear phylogenetic and generic boundaries among taxa in the Australian Camphorosmeae is believed to be the result of the young age of the group and its rapid diversification, therefore not allowing the accumulation of mutations and clear morphological distinctions.

6 ZUSAMMENFASSUNG

Diese Studie befasst sich mit der Phylogenie und Biogeographie der australischen Camphorosmeae, die ein wichtiges Element der Flora arider Gebiete Australiens sind. Die molekularen Phylogenien wurden mit Hilfe Bayes'scher Statistik und „maximum likelihood“ berechnet. Um das Alter der Gruppe und interner Linien abzuschätzen, wurden die Methoden „Nonparametric rate smoothing“ und „penalized likelihood“ benutzt. Morphologische Merkmale wurden nach Kriterien der Parsimonie auf den molekularen Baum aufgetragen. „Brooks parsimony analysis“, „cladistic analysis of distributions and endemism“, „dispersal-vicariance analysis“, „ancestral area analysis“ und „weighted ancestral area analysis“ wurden angewandt, um Abfolge und Richtungen der Ausbreitung der Gruppe in Australien zu analysieren.

Von sieben getesteten Markern hatten nur die nukleären ETS und ITS genügend Variation für die phylogenetische Analyse der Camphorosmeae. Die plastidären Marker *trnL-trnF* spacer, *trnP-psaJ* spacer, *rpS16* intron, *rpL16* intron und *trnS-trnG* spacer zeigten kein ausreichendes phylogenetisches Signal. Die gefundenen phylogenetischen Hypothesen widersprechen der jetzigen Taxonomie der Gruppe. *Neobassia*, *Threlkeldia*, *Osteocarpum* und *Enchylaena* sollten den Gattungen *Sclerolaena* bzw. *Maireana* zugeordnet werden. Die kladistische Analyse der Fruchthängsel unterstützt die taxonomischen Ergebnisse der auf DNA basierenden Phylogenie. Allerdings hat die Behaarung, die bei anderen Gruppen der Chenopodiaceae als wichtiges taxonomisches Merkmal herangezogen wird, die Phylogenie nicht unterstützt.

Vorfahren der heutigen Camphorosmeen sind im Miozän, vor ca. 8-14 Millionen Jahren, durch Fernausbreitung vermutlich aus Asien in Australien eingewandert. Anfängliche Diversifizierung fand während des späten Miozäns bis in das frühe Pliozän vor ca. 4-7 Millionen Jahren statt. Am Ende des Pliozäns existierten schon 45% - 72% der Abstammungslinien der jetzigen Camphorosmeen. Dies weist auf eine schnelle Ausbreitung hin. Das Alter stimmt mit dem Einsetzen der Aridisierung Australiens überein, und deutet darauf hin, dass die Ausbreitung der ariden Gebiete eine große Rolle bei der Diversifizierung der Gruppe spielte. Die Vorfahren der australischen Camphorosmeae scheinen die Südküste Australiens zuerst besiedeln zu haben. Dies geschah vor dem Einsetzen der Aridisierung des Kontinents. Die anschließende Ausbreitung erfolgte in verschiedene Richtungen und folgte der fortschreitenden Austrocknung im späten Tertiär und im ganzen Quartär. Durch ihre Anpassung an Trockenheit ist der Erfolg der Camphorosmeae in den ariden Gebieten zu erklären.

Die Abwesenheit von klaren phylogenetischen und artspezifischen Signalen zwischen Arten der australischen Camphorosmeae ist auf das junge Alter und die schnelle Diversifizierung der Gruppe zurückzuführen, welche die Häufung von Mutationen und eine starke morphologische Differenzierung nicht zugelassen haben.

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Appendix 1. Taxa sampled, vouchers, GenBank accession numbers, and references for sequences that are already in GenBank. Herbarium acronyms are according to the Index Herbariorum.

Taxon	DNA Source / Voucher	Genbank Accession Numbers			
		ITS	ETS	<i>trnS-trnG</i> IGS	<i>trnP-psaI</i> IGS
<i>Bassia dasyphylla</i>	Kadereit et al. 2005	AY489195	EF607140	-	-
<i>Chenolea diffusa</i>	L. Mucina 6914/1 (MJG); South Africa, Western Cape	-	-	-	EF613653
<i>Kochia americana</i>	Kadereit et al. 2005	-	-	EF613659	-
<i>Kochia melanoptera</i>	Kadereit et al. 2005	AY489215	EF607149	EF613660	-
<i>Didymanthus roei</i>	S. Jacobs 9132 (NSW); Australia; W Australia, Avon, Mortlock River	EF613597	EF607141	EF613654	EF613626
<i>Dissocarpus biflorus</i>	Kadereit et al. 2005	AY489205	EF607142	EF613655	EF613627
<i>Dissocarpus paradoxus</i>	Kadereit et al. 2005	AY489206	EF607143	EF613656	EF613628
<i>Enchylaena lanata</i>	F.H. Vachell 12/03 (NSW); Australia; W Australia, Kellerberrin	-	EF607144	-	-
<i>Enchylaena tomentosa</i>	Kadereit et al. 2005	AY489207	EF607145	EF613657	EF613629
<i>Eremophea aggregata</i>	Hopper 3262 (KEW, ex PERTH); Australia; W Australia, S of Wooramei	EF613598	EF607146	-	-
<i>Eremophea spinosa</i>	A.C. Beauglehole ACB 23480 (NSW); Australia; C Australia, George Gill Range, Bagot Creek	EF613599	EF607147	-	-
<i>Eriochiton sclerolaenoides</i>	Kadereit et al. 2005	AY489208	EF607148	EF613658	EF613630

<i>Maireana amoena</i>	S. Jacobs 9198 (NSW); Australia; W Australia, Coolgardie	-	EF607150	-	-
<i>Maireana aphylla</i>	S. Jacobs 9106 (NSW); Australia; New South Wales, South Far Western Plains, S of Broken Hill	-	EF607151	-	EF613631
<i>Maireana astrotricha</i>	S. Jacobs 9097 (NSW); Australia; New South Wales, North Far Western Plains, Scopes Range	-	EF607152	-	EF613632
<i>Maireana brevifolia</i>	S. Jacobs 9098 (NSW); Australia; New South Wales, Broken Hill	EF613600	EF607153	EF613661	EF613633
<i>Maireana carnosa</i>	S. Jacobs 9186 (NSW); Australia; W Australia, Austin, Willuna Rd.	-	-	-	-
<i>Maireana convexa</i>	S. Jacobs 9184 (NSW); Australia; W Australia, Austin, Willuna Rd.	-	EF607154	-	-
<i>Maireana coronata</i>	S. Jacobs 8722 (NSW); Australia; New South Wales, Tibooburra Rd. N of Fowlers Gap	EF613601	EF607155	EF613662	-
<i>Maireana erioclada</i>	Kadereit et al. 2005; S. Jacobs 8699 (NSW); Australia; New South Wales, Euston Rd. W of Balranald	AY489222	EF607156	EF613663	EF613634
<i>Maireana eriosphaera</i>	S. Jacobs 9174 (NSW); Australia; W Australia, Austin, Willuna Rd.	EF613602	EF607157	EF613664	-
<i>Maireana georgei</i>	S. Jacobs 9195 (NSW); Australia; W Australia, Austin, Leonora Rd.	-	EF607158	-	-
<i>Maireana integra</i>	S. Jacobs 9102 (NSW); Australia; New South Wales, North Far Western Plains, Mundi Mundi Lookout	-	EF607159	-	-
<i>Maireana microphylla</i>	S. Jacobs 9076 (NSW); Australia; Queensland, Toowoomba Rd. E of Pittsworth	EF613603	EF607160	EF613665	EF613635
<i>Maireana oppositifolia</i>	S. Jacobs 9155 (NSW); Australia; W Australia, Austin, Yalgoo Rd.	EF613604	EF607161	EF613666	-
<i>Maireana pentatropis</i>	S. Jacobs 9091 (NSW); Australia; New South Wales, North Western Plains, Wilcannia Rd.	-	EF607162	-	-
<i>Maireana planifolia</i>	S. Jacobs 9194 (NSW); Australia; W Australia, Leonora Rd. S of Leinster	EF613605	EF607163	EF613667	-
<i>Maireana platycarpa</i>	S. Jacobs 9142 (NSW); Australia; W Australia, Avon, Cowcowing Lakes	EF613606	EF607164	EF613668	-
<i>Maireana polypterygia</i>	S. Jacobs 9178 (NSW); Australia; W Australia, Carnarvon	EF613607	EF607165	EF613669	EF613636

<i>Maireana pyramidata</i>	S. Jacobs 9093 (NSW); Australia; New South Wales, North Far Western Plains, Wilcannia Rd.	-	EF607166	-	-
<i>Maireana schistocarpa</i>	S. Jacobs 9110 (NSW); Australia; New South Wales, North Far Western Plains, Tibooburra Rd.	-	EF607167	-	-
<i>Maireana sedifolia</i>	S. Jacobs 9096 (NSW); Australia; New South Wales, Scopes Range, E of Wilcannia	EF613608	EF607168	EF613670	-
<i>Maireana tomentosa</i>	S. Jacobs 9171 (NSW); Australia; West Australia, Carnarvon	EF613609	EF607169	EF613671	-
<i>Maireana triptera</i>	S. Jacobs 9090 (NSW); Australia; New South Wales, North Western Plains, Wilcannia Rd.	-	EF607170	-	-
<i>Maireana turbinata</i>	S. Jacobs 8709 (NSW); Australia; New South Wales, Poongcarrie Rd N of Wentworth	EF613610	EF607171	EF613672	-
<i>Maireana villosa</i>	S. Jacobs 9193 (NSW); Australia; West Australia, Austin, Leonora Rd.	-	EF607172	-	-
<i>Malacocera biflora</i>	Ising 75234 (NSW); Australia; South Australia, Evelyn Downs Station, Oodnadatta	-	EF607173	-	-
<i>Malacocera tricornis</i>	S. Jacobs 8706 (NSW); Australia; New South Wales, Poongcarrie Rd N of Wentworth	EF613611	EF607174	EF613673	EF613637
<i>Neobassia astrocarpa</i>	S. Jacobs 4191 (NSW); Australia, West Australia, Broome	-	EF607175	-	-
<i>Neobassia proceriflora</i>	S. Jacobs 9088 (NSW); Australia; New South Wales, North Western Plains, Bourke Rd.	EF613612	EF607176	EF613674	EF613638
<i>Osteocarpum acropterum</i>	S. Jacobs 8717 (NSW); Australia; New South Wales, Tibooburra Rd. N of Broken Hill	-	EF607177	EF613675	EF613639
<i>Osteocarpum dipterocarpum</i>	Kadereit et al. 2005; S. Jacobs 8731 (NSW); Australia: New South Wales, North Far Western Plains, near Tibooburra	AY489226	EF607178	-	EF613640
<i>Roycea divaricata</i>	S. Jacobs 9147 (NSW); Australia; W Australia, Avon, N of Koorda	EF613613	EF607179	EF613676	EF613641
<i>Roycea spinescens</i>	S. Jacobs 9138 (NSW); Australia; W Australia, Avon, Mortlock River, E of Meckering	EF613614	EF607180	EF613677	EF613642
<i>Sclerolaena anisacanthoides</i>	S. Jacobs 9082 (NSW); Australia; Queensland, Darling Downs, Goondiwindi Rd.	EF613615	EF607181	EF613678	EF613643
<i>Sclerolaena bicornis</i>	S. Jacobs 8723 (NSW); Australia; New South Wales, Tibooburra Rd. N of Fowlers Gap	EF613616	EF607182	EF613679	EF613644

<i>S. bicornis</i> ssp. <i>horrida</i>	S. Jacobs 9087 (NSW); Australia; New South Wales, North Western Plains	-	EF607183	-	EF613645
<i>Sclerolaena birchii</i>	S. Jacobs 9079 (NSW); Australia; Queensland, Darling Downs, Toowoomba Rd.	-	EF607184	-	-
<i>Sclerolaena blackiana</i>	R.G.Coveny 13616, B. Wiecek & M.Savio (NSW); Australia; New South Wales, Sturt National Park, N Far W Plains	-	EF607185	-	-
<i>Sclerolaena brachyptera</i>	S. Jacobs 8708 (NSW); Australia; New South Wales, Pooncarrie Rd N of Wentworth	EF613617	EF607186	EF613680	EF613646
<i>Sclerolaena calcarata</i>	S. Jacobs 9086 (NSW); Australia; New South Wales, North Western Plains	-	EF607187	-	-
<i>Sclerolaena convexula</i>	W. Greuter 20757 (NSW); Australia, New South Wales, Buckeroo Mtn. c. 12 km NE of Coolabah	-	EF607188	-	-
<i>Sclerolaena cornishiana</i>	N. Schmalz 227 (MJG); Australia; North Territory, Kings Canyon Resort	EF613618	EF607189	EF613681	EF613647
<i>Sclerolaena cuneata</i>	S. Jacobs 9199 (NSW); Australia; W Australia, Coolgardie, Norseman Rd.	-	EF607190	EF613682	-
<i>Sclerolaena densiflora</i>	S. Jacobs 9179 (NSW); Australia; W Australia, E of Middalya	-	EF607191	-	-
<i>Sclerolaena diacantha</i>	Kadereit et al. 2005	AY489231	EF607192	EF613683	EF613648
<i>Sclerolaena divaricata</i>	S. Jacobs 9084 (NSW); Australia; Queensland, Darling Downs, Toowoomba Rd.	-	EF607193	-	-
<i>Sclerolaena eriacantha</i>	S. Jacobs 9160 (NSW); Australia; West Australia, Austin, Yalgoo Rd.	-	EF607194	-	-
<i>Sclerolaena eurotioides</i>	S. Jacobs 9143 (NSW); Australia; West Australia, Avon, Koorda Rd.	-	EF607195	EF613684	-
<i>Sclerolaena fimbriolata</i>	S. Jacobs 9188 (NSW); Australia; West Australia, Austin, Leinster Rd.	EF613619	EF607196	EF613685	-
<i>Sclerolaena intricata</i>	S. Jacobs 8707 (NSW); Australia; New South Wales, Pooncarrie Rd N of Wentworth	EF613620	EF607197	EF613686	EF613649
<i>Sclerolaena lanicuspis</i>	S. Jacobs 9104 (NSW); Australia; New South Wales, South Far Western Plains, Wentworth Rd.	-	EF607198	-	-
<i>Sclerolaena longicuspis</i>	Kadereit et al. 2005; S. Jacobs 8727 (NSW); Australia; New South Wales, Sturt National Park	AY489232	EF607199	EF613687	EF613650

<i>Sclerolaena medicaginooides</i>	S. Jacobs 9180 (NSW); Australia; W Australia, Ashburton	EF613621	EF607200	EF613688	-
<i>Sclerolaena muricata</i>	S. Jacobs 9081 (NSW); Australia; Queensland, Darling Downs, Goondiwindi Rd.	-	EF607201	-	-
<i>Sclerolaena obliquicuspis</i>	S. Jacobs 9092 (NSW); Australia; New South Wales, North Western Plains, Wilcannia Rd.	EF613622	EF607202	EF613689	-
<i>Sclerolaena parviflora</i>	P.G. Kadela 552, G.Chapple, R.G.Coveny & H.McPherson (NSW); Australia; Arumpo Station, New South Wales, S Far W Plains	-	EF607203	-	-
<i>Sclerolaena patenticuspis</i>	S. Jacobs 9094 (NSW); Australia; New South Wales, North Far Western Plains, Wilcannia Rd.	-	EF607204	-	-
<i>Sclerolaena ramulosa</i>	S. Jacobs 9272 (NSW); Australia, Queensland, St Lawrence	-	EF607205	EF613690	-
<i>Sclerolaena recurvicuspis</i>	S. Jacobs 9165 (NSW); Australia; W Australia, Carnarvon	EF613623	EF607206	EF613691	-
<i>Sclerolaena stelligera</i>	S. Jacobs 8733 (NSW); Australia; New South Wales, Bourke Rd. SE of Fords Bridge	EF613624	EF607207	EF613692	EF613651
<i>Sclerolaena tetracuspis</i>	S. Jacobs 9080 (NSW); Australia; Queensland, Darling Downs, Goondiwindi Rd.	-	EF607208	-	-
<i>Sclerolaena uniflora</i>	S. Jacobs 9170 (NSW); Australia; W Australia, Carnarvon	-	EF607209	-	-
<i>Threlkeldia diffusa</i>	S. Jacobs 9205 (NSW); Australia; S Australia, Eyre Peninsula, Ceduna	EF613625	EF607210	EF613693	EF613652

Appendix 2. Distribution of the sampled, native Camphorosmeae in Australia (A = Kimberley; B = Arnhem; C = Cape York; D = Atherton; E = Eastern Queensland; F = McPherson-Macleay; G = Southeastern New South Wales; H = Victoria; I = Eyre; J = Adelaide; K = Northern Desert; L = Eastern Desert; M = Southwest; N = Western Desert; O = Pilbara).

Taxa	Distribution (Floristic Region)
<i>Didymanthus roei</i>	In the drier inland south-western portion of Western Australia (M, N, O)
<i>Dissocarpus biflorus</i>	Widespread in arid and semi-arid regions of Northern Territory, South Australia, Queensland, New South Wales and Victoria (I, J, L, N)
<i>Dissocarpus paradoxus</i>	Widespread from north-western Victoria, western New South Wales, and south-western Queensland, westwards to Shark Bay region of Western Australia (I, J, L, M, N, O)
<i>Enchylaena lanata</i>	Inland south-western Australia (M, N)
<i>Enchylaena tomentosa</i>	Widespread in all mainland states (E, F, G, H, I, J, K, L, M, N, O)
<i>Eremophea aggregata</i>	In Western Australia between Geraldton and Carnarvon (M)
<i>Eremophea spinosa</i>	In north-western South Australia and southern Northern Territory westwards to the North West Cape area in Western Australia (N, O)
<i>Eriochiton sclerolaenoides</i>	Temperate Western Australia eastwards to western New South Wales and north-western Victoria (I, J, L, M, N, O)
<i>Maireana amoena</i>	Widespread in Western Australia from the latitude of Carnarvon S to Norseman (N)
<i>Maireana aphylla</i>	West of the Great Dividing Range in New South Wales and southern Queensland, also in north-western Victoria, eastern South Australia, southern Northern Territory, and in the Carvarvon-Wiluna area of Western Australia (F, G, I, J, K, L, N, O)
<i>Maireana astrotricha</i>	Western New South Wales, central South Australia and the southern portion of Northern Territory (I, J, L)
<i>Maireana brevifolia</i>	In all mainland states, mostly S of 26°S latitude (E, F, G, H, I, J, M, N)
<i>Maireana convexa</i>	Western Australia between latitudes 25°S and 30°S (M, N, O)
<i>Maireana coronata</i>	Central and eastern Australia from the vicinity of Alice Springs E to the Great Dividing Range and as far as Dubbo, New South Wales (L, N)
<i>Maireana erioclada</i>	Southern WA eastwards to western New South Wales and Victoria (I, J, M)
<i>Maireana eriosphaera</i>	In arid areas of Western Australia from the Rudall River S to Norseman (N)

<i>Maireana georgei</i>	Widespread in Australia but absent from the wetter southern and northern regions and east of the Great Dividing Range (A, B, I, J, K, L, M, N, O)
<i>Maireana integra</i>	Western New South Wales westwards through South Australia and southern Northern Territory into south-central Western Australia (I, J, L, N)
<i>Maireana microphylla</i>	South-eastern Queensland and eastern New South Wales (E, F, G)
<i>Maireana oppositifolia</i>	Along the south coast of Western Australia and South Australia (I, J, M, N)
<i>Maireana pentatropis</i>	Central and south-eastern Western Australia, southern Northern Territory, South Australia and western New South Wales and Victoria (I, J, L, M, N)
<i>Maireana planifolia</i>	Western Australia between 22° and 31°S latitude, in southern Northern Territory and northern South Australia (L, M, N, O)
<i>Maireana platycarpa</i>	West Australia from Carnarvon south-east to Norseman (M, N, O)
<i>Maireana polypterygia</i>	North-western Western Australia between Exmouth Gulf and Shark Bay (M, O)
<i>Maireana pyramidata</i>	Inland Western Australia and eastwards through South Australia to western New South Wales and north-western Victoria (G, I, J, L, N, O)
<i>Maireana schistocarpa</i>	Central Australia in south-western Queensland, north-western New South Wales, south-eastern Northern Territory and north-eastern South Australia (L)
<i>Maireana sedifolia</i>	South-western New South Wales and north-western Victoria, South Australia and southern Northern Territory and south-eastern Western Australia (I, J, L, N)
<i>Maireana tomentosa</i> var. <i>tomentosa</i>	Western Australia and southern Northern Territory (M, N, O)
<i>Maireana triptera</i>	Widespread in the drier areas of Western Australia south of 20°S latitude and eastwards to western districts of Queensland, New South Wales, and Victoria (J, K, L, M, N, O)
<i>Maireana turbinata</i>	Central Queensland, western New South Wales, north-western Victoria and W to south-eastern Western Australia (I, J, L, M)
<i>Maireana villosa</i>	Widespread in arid areas of all mainland states except Victoria (E, K, L, M, N, O)
<i>Malacocera biflora</i>	In the Lake Eyre Basin region of South Australia and southern Northern Territory (L, N)
<i>Malacocera tricornis</i>	Inland southern Western Australia, southern Northern Territory, central and eastern South Australia, southern Queensland, western New South Wales and far north-western Victoria (I, J, L, N)

<i>Neobassia astrocarpa</i>	In the W coast of Western Australia (Shark Bay to Broome) eastwards to western Northern Territory (K, M, N, O)
<i>Neobassia proceriflora</i>	Southern Northern Territory and northern South Australia to eastern New South Wales and Queensland (J, L)
<i>Osteocarpum acropterum</i>	North-western Western Australia through southern Northern Territory and north-western South Australia to south-western Queensland and western New South Wales (J, L, O)
<i>Osteocarpum dipterocarpum</i>	Southern Northern Territory to western New South Wales (I, J, L, N)
<i>Roycea divaricata</i>	In southern central Western Australia from Yuna east to Laverthon and south to Koorda and Southern Cross (M, N)
<i>Roycea spinescens</i>	Morawa south to Merredin, Western Australia (M)
<i>Sclerolaena anisacanthoides</i>	In north-western and central New South Wales, and Queensland (E, F, G, L)
<i>Sclerolaena bicornis</i> var. <i>bicornis</i>	North-western Western Australia, eastwards through southern Northern Territory and South Australia to Queensland and central and western New South Wales (A, B, I, J, K, L, N, O)
<i>Sclerolaena bicornis</i> var. <i>horrida</i>	Northern New South Wales and in central and southern Queensland (E, F, J, L)
<i>Sclerolaena birchii</i>	South-eastern Queensland, central New South Wales, and occasionally in southern Northern Territory and south-eastern South Australia (E, F, G, H, J, L, N)
<i>Sclerolaena blackiana</i>	Northern South Australia, south-western Queensland and western New South Wales (J, L)
<i>Sclerolaena brachyptera</i>	South-western Queensland, central and western New South Wales, western Victoria, South Australia, southern Northern Territory and north-central Western Australia (F, H, I, J, L, N)
<i>Sclerolaena calcarata</i>	Central and eastern Australia excluding Victoria (D, E, J, L)
<i>Sclerolaena convexula</i>	In Central Australia and eastwards to southern Queensland and northern New South Wales (E, J, L, N, O)
<i>Sclerolaena cornishiana</i>	Northern Central Australia and western Queensland (C, K, L, N, O)
<i>Sclerolaena cuneata</i>	Inland Australia, except Victoria (I, J, K, L, M, N, O)
<i>Sclerolaena densiflora</i>	Central and northern desert areas of West Australia (N, O)
<i>Sclerolaena diacantha</i>	Widespread in subtropical and temperate Australia (I, J, K, L, M, N, O)
<i>Sclerolaena divaricata</i>	Western and central New South Wales and eastern South Australia (I, J, L)

<i>Sclerolaena eriacantha</i>	Inland Australia, except Victoria (I, J, K, L, M, N, O)
<i>Sclerolaena eurotioides</i>	In the drier areas of subtropical Western Australia (M, N, O)
<i>Sclerolaena fimbriolata</i>	In the desert of central Western Australia (N)
<i>Sclerolaena intricata</i>	Southern Northern Territory, south-western Queensland, western New South Wales and north-eastern South Australia (J, L, N)
<i>Sclerolaena lanicuspis</i>	Central and northern Western Australia, Northern Territory, South Australia, central and western Queensland, western New South Wales, and north-western Victoria (I, J, K, L, N, O)
<i>Sclerolaena longicuspis</i>	Southern Northern Territory, northern and eastern South Australia, south-western Queensland and north-western New South Wales (L)
<i>Sclerolaena medicaginoides</i>	Western Australia c. 300 km NE of Carnarvon (M)
<i>Sclerolaena muricata</i>	In eastern Australia from central Queensland south to northern Victoria and eastern South Australia (D, E, F, J, L)
<i>Sclerolaena obliquicuspis</i>	South-central and eastern Western Australia, south-western Northern Territory, western New South Wales, and far north-western Victoria (I, J, L, M, N)
<i>Sclerolaena parviflora</i>	In the drier central and southern portions of Australia (I, J, M, N)
<i>Sclerolaena patenticuspis</i>	Central New South Wales and north-western Victoria, extending to central and south-eastern Western Australia (I, J, L, N)
<i>Sclerolaena ramulosa</i>	South-central and central-eastern Queensland (E, L)
<i>Sclerolaena recurvicuspis</i>	North-western Western Australia (M, O)
<i>Sclerolaena stelligera</i>	Southern Queensland, central and western New South Wales, eastern South Australia, and north-western Victoria (E, F, J, L)
<i>Sclerolaena tetracuspis</i>	South-eastern Queensland and north-eastern New South Wales (E, F)
<i>Sclerolaena uniflora</i>	In coastal South Australia, and in Western Australia as far north as Port Hedland (I, M, N, O)
<i>Threlkeldia diffusa</i>	Near Broome in Western Australia, south around the coast to Victoria and Tasmania, also at scattered inland localities (H, I, J, K, M, O)

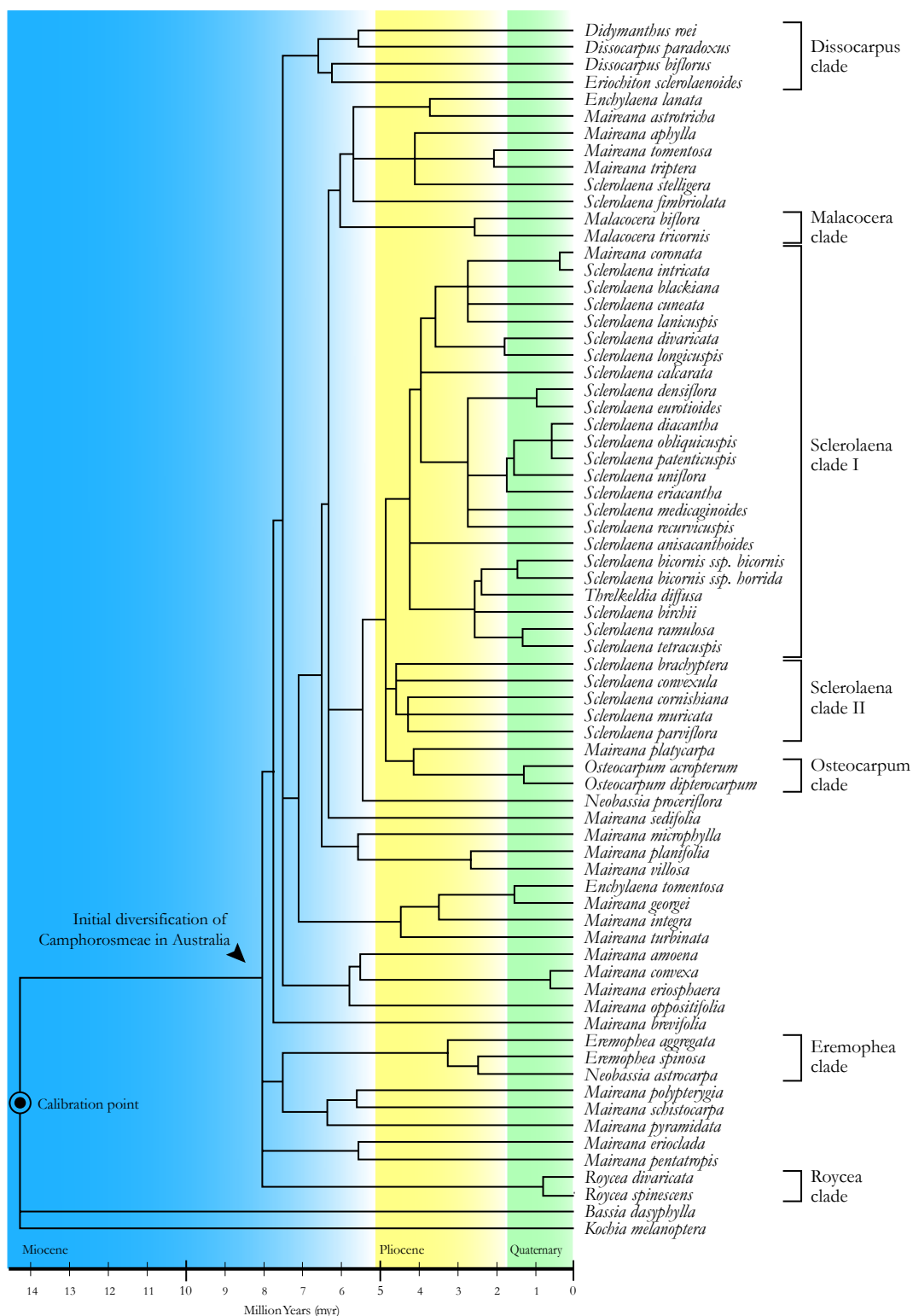
Appendix 3a. Results of the maximum likelihood tree dating using two rate smoothing algorithms as implemented in the program r8s. The ML tree was calibrated using an age constraint of 12.91 – 14.33 mya based on *ndhF* and 8.1 – 3.6 based on *rbcL* for the split between the Australian and the Central Asian members of Camphorosmeae. All values are in million years.

Node	Non-parametric Rate Smoothing			Penalized Likelihood			<i>ndhF</i>	<i>rbcL</i>
	<i>ndhF</i>	<i>rbcL</i>	<i>ndhF-rbcL</i>	<i>ndhF</i>	<i>rbcL</i>	<i>ndhF-rbcL</i>	NPRS-PL	NPRS-PL
AU	8,04	4,58	3,46	7,38	4,18	3,20	0,66	0,40
3	7,75	4,41	3,34	7,09	4,02	3,07	0,66	0,39
4	7,57	4,31	3,26	6,92	3,92	3,00	0,65	0,39
5	6,64	3,77	2,87	4,32	2,45	1,87	2,32	1,32
6	5,65	3,20	2,45	3,17	1,81	1,36	2,48	1,39
9	6,22	3,52	2,70	3,96	2,23	1,73	2,26	1,29
12	7,10	4,04	3,06	6,49	3,68	2,81	0,61	0,36
13	6,47	3,68	2,79	5,95	3,36	2,59	0,52	0,32
14	6,27	3,56	2,71	5,78	3,27	2,51	0,49	0,29
15	6,06	3,44	2,62	5,57	3,15	2,42	0,49	0,29
16	5,70	3,23	2,47	4,36	2,5	1,86	1,34	0,73
17	3,71	2,10	1,61	2,29	1,32	0,97	1,42	0,78
20	4,10	2,31	1,79	3,03	1,72	1,31	1,07	0,59
22	2,10	1,14	0,96	1,73	0,95	0,78	0,37	0,19
27	2,65	1,47	1,18	2,88	1,61	1,27	-0,23*	-0,14*
30	5,42	3,07	2,35	4,84	2,72	2,12	0,58	0,35
31	4,86	2,76	2,10	4,34	2,44	1,90	0,52	0,32
32	4,24	2,40	1,84	3,53	1,96	1,57	0,71	0,44
33	3,98	2,25	1,73	3,17	1,77	1,40	0,81	0,48
34	3,58	2,02	1,56	2,25	1,28	0,97	1,33	0,74
35	2,78	1,57	1,21	0,93	0,54	0,39	1,85	1,03
36	0,38	0,21	0,17	0,01	0	0,01	0,37	0,21
42	1,81	1,02	0,79	1,15	0,66	0,49	0,66	0,36
46	2,77	1,56	1,21	2,20	1,21	0,99	0,57	0,35
47	0,98	0,74	0,24	0,53	0,33	0,20	0,45	0,41
50	1,79	1,01	0,78	1,13	0,63	0,50	0,66	0,38
51	1,57	0,88	0,69	1,00	0,56	0,44	0,57	0,32
52	0,60	0,34	0,26	0,40	0,23	0,17	0,20	0,11
61	2,69	1,61	1,08	2,25	1,28	0,97	0,44	0,33
62	2,42	1,44	0,98	2,07	1,17	0,90	0,35	0,27
63	1,44	0,80	0,64	1,35	0,7	0,65	0,09	0,10
68	1,35	0,81	0,54	0,97	0,56	0,41	0,38	0,25
71	4,16	2,36	1,80	3,72	2,09	1,63	0,44	0,27
73	1,26	0,71	0,55	1,07	0,54	0,53	0,19	0,17

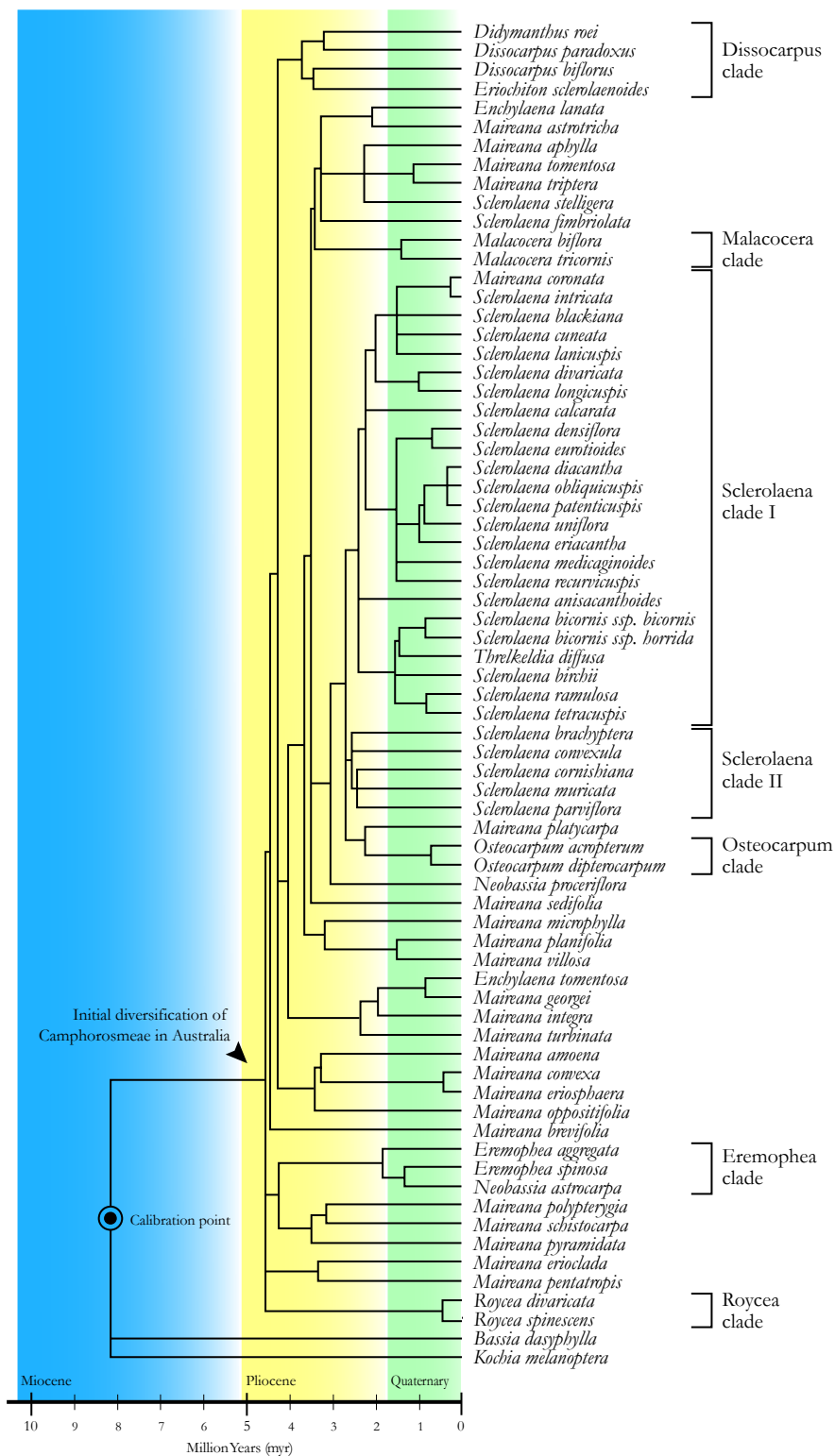
76	4,57	2,59	1,98	4,09	2,3	1,79	0,48	0,29
79	4,31	2,44	1,87	3,94	2,21	1,73	0,37	0,23
85	5,62	3,18	2,44	5,11	2,88	2,23	0,51	0,30
87	2,72	1,53	1,19	2,03	1,16	0,87	0,69	0,37
90	4,42	2,49	1,93	2,45	1,38	1,07	1,97	1,11
91	3,50	1,96	1,54	1,75	0,97	0,78	1,75	0,99
92	1,66	0,88	0,78	0,83	0,43	0,40	0,83	0,45
97	5,78	3,41	2,37	5,34	3,12	2,22	0,44	0,29
98	5,52	3,26	2,26	5,14	3,01	2,13	0,38	0,25
100	0,68	0,40	0,28	0,77	0,45	0,32	-0,09*	-0,05*
105	7,49	4,26	3,23	5,75	3,22	2,53	1,74	1,04
106	3,30	1,88	1,42	1,90	1,07	0,83	1,40	0,81
108	2,42	1,35	1,07	1,44	0,79	0,65	0,98	0,56
111	6,37	3,60	2,77	2,78	1,55	1,23	3,59	2,05
112	5,69	3,19	2,50	2,09	1,12	0,97	3,60	2,07
116	5,65	3,35	2,30	2,18	1,3	0,88	3,47	2,05
119	0,81	0,42	0,39	0,78	0,38	0,40	0,03	0,04



Appendix 3b. The maximum likelihood tree of the Australian Camphorosmeae showing node numbers.



Appendix 3c. Relaxed maximum likelihood tree of the Australian Camphorosmeae with estimated ages derived from divergence time analysis of the ETS sequence data using a relaxed clock method implementing non-parametric rate smoothing. Calibration used age estimates of the split between the Australian and the Central Asian clades of the tribe inferred from *ndhF* sequence data.



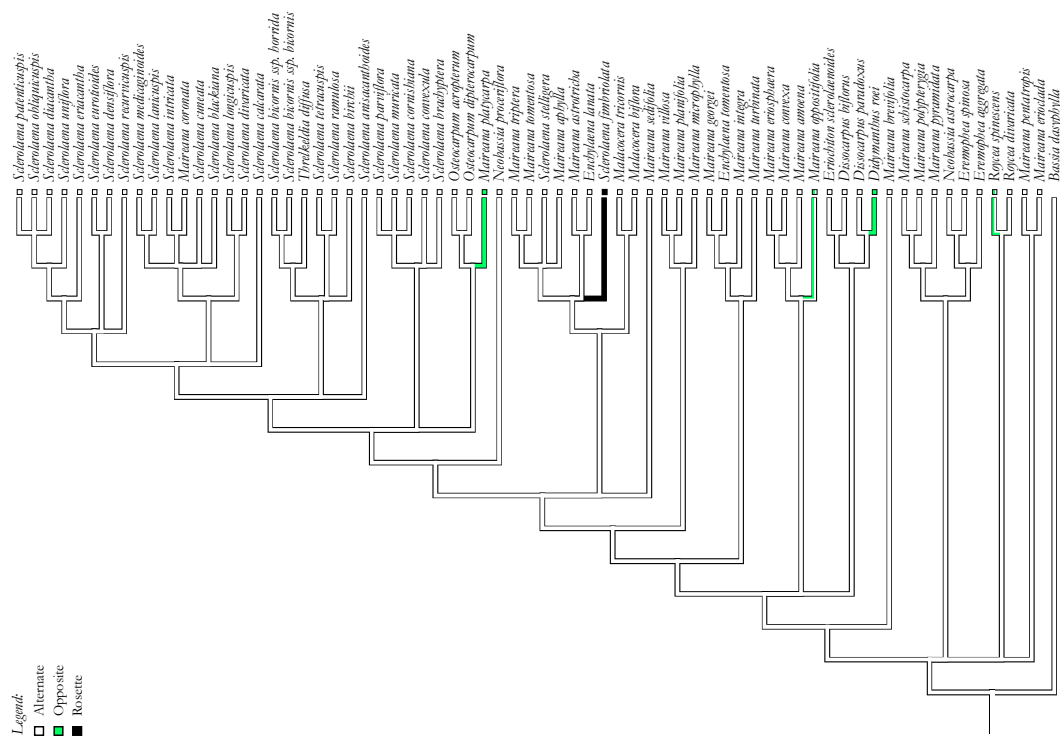
Appendix 3d. Relaxed maximum likelihood tree of the Australian Camphorosmeae with estimated ages derived from divergence time analysis of the ETS sequence data using a relaxed clock method implementing non-parametric rate smoothing. Calibration used age estimates of the split between the Australian and the Central Asian clades of the tribe inferred from *rbcL* sequence data.

Appendix 4. Morphological matrix showing the different character states scored for the Australian Camphorosmeae

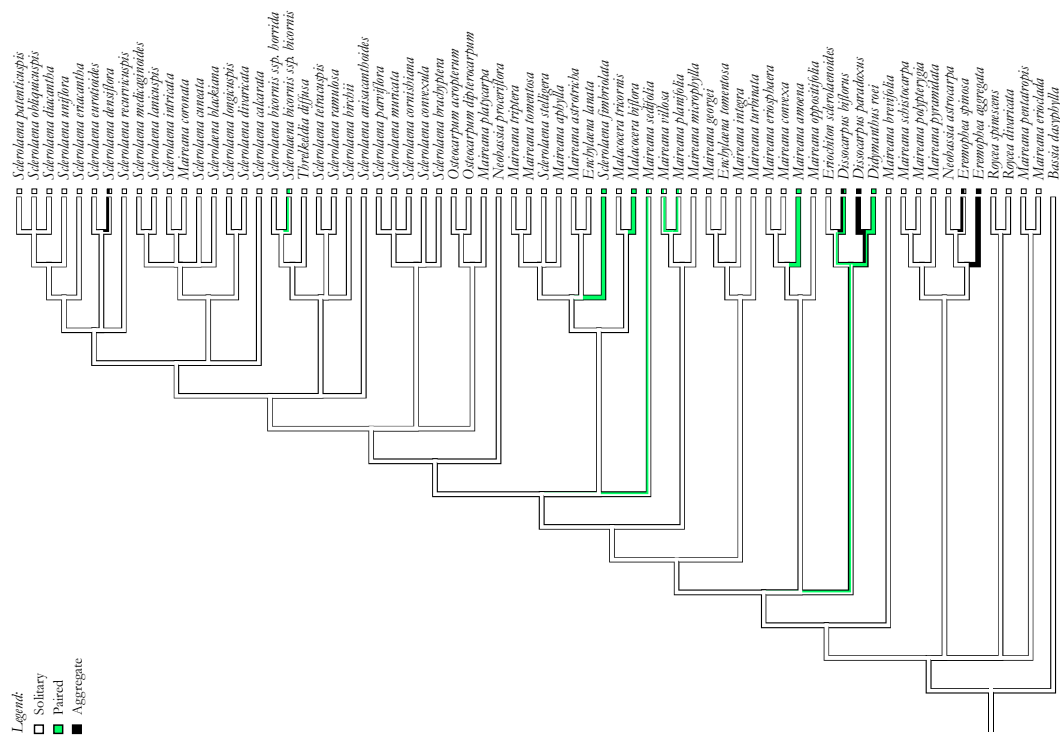
Taxa	Phyllotaxy	Infructescence type	Distribution of sexes	Fruit type/ appendage	Fruit detachability	Seed orientation	Fruit pubescence	Hair type (fruit)	Hair cellularity (fruit)	Leaf pubescence	Hair type (leaf)	Hair cellularity (leaf)	Stem pubescence	Hair type (stem)	Hair cellularity (stem)
<i>Didymanthus roei</i>	1	1	0	1	0	1	1	1	2	1	2	2	1	1	2
<i>Dissocarpus biflorus</i>	0	2	0	2	0	0	2	2	2	1	2	2	1	2	2
<i>Dissocarpus paradoxus</i>	0	1&2	0	2	0	0	2	2	2	1	2	2	1	2	2
<i>Enchylaena lanata</i>	0	0	0	4	0	0	2	2	2	1	2	2	1	2	2
<i>Enchylaena tomentosa</i>	0	0	0	4	0	0	0	0	0	1	2	2	1	2	2
<i>Eremophea aggregata</i>	0	2	0	2	1	1	1	3	2	1	3	2	1	3	2
<i>Eremophea spinosa</i>	0	0&2	0	2	1	1	2	3	2	1	3	2	1	3	2
<i>Eriochiton sclerolaenoides</i>	0	0	0	1	0	0	2	2	2	1	2	2	1	2	2
<i>Maireana amoena</i>	0	1	0	1	0	0	2	2	2	1	2	2	1	1	2
<i>Maireana aphylla</i>	0	0	1	1	0	0	0	0	0	1	2	2	1	0	0
<i>Maireana astrotricha</i>	0	0	1	1	0	0	1	3	2	1	3	2	1	3	2
<i>Maireana brevifolia</i>	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2
<i>Maireana convexa</i>	0	0	0	1	0	0	1	1	2	1	2	2	1	2	2
<i>Maireana coronata</i>	0	0	0	1	0	0	2	1	2	1	1	2	1	1	2
<i>Maireana erioclada</i>	0	0	0	3	0	0	0	0	0	0	0	0	1	1	2
<i>Maireana eriosphaera</i>	0	0	0	1	0	0	2	1	2	1	1	2	1	1	2
<i>Maireana georgei</i>	0	0	0	1	0	0	0	0	0	1	2	2	1	2	2
<i>Maireana integra</i>	0	0	0	1	0	0	0	0	0	1	2	2	1	2	2
<i>Maireana microphylla</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Maireana oppositifolia</i>	0&1	0	1	1	0	0	0	0	0	1	1	2	1	1	2
<i>Maireana pentatropis</i>	0	0	0	3	0	0	0	0	0	0	0	0	1	1	2
<i>Maireana planifolia</i>	0	0&1	0	1	0	0	1	2	2	1	2	2	1	2	2
<i>Maireana platycarpa</i>	1	0	0	1	0	0	1	2	2	1	2	2	1	2	2
<i>Maireana polypterygia</i>	0	0	1	3	0	0	1	1	2	1	2	1	1	2	1
<i>Maireana pyramidata</i>	0	0	1	1	0	0	1	2	2	1	2	1	1	2	2
<i>Maireana schistocarpa</i>	0	0	0	3	0	0	1	1	2	1	2	2	1	2	2

<i>Maireana sedifolia</i>	0	0&1	1	1	0	0	1	1	2	1	2	2	1	2	2
<i>Maireana tomentosa</i>	0	0	0	1	0	0	0	0	0	1	2	2	1	2	2
<i>Maireana triptera</i>	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
<i>Maireana turbinata</i>	0	0	0	1	0	0	0	0	0	1	2	2	1	2	2
<i>Maireana villosa</i>	0	0&1	0	1	0	0	0	0	0	1	1	2	1	2	2
<i>Malacocera biflora</i>	0	1	0	1	0	0	2	1	2	1	2	2	1	2	2
<i>Malacocera tricornis</i>	0	0	0	1	0	0	2	1	2	1	2	2	1	1	2
<i>Neobassia astrocarpa</i>	0	0	0	2	0	1	2	2	2	1	2	2	1	2	1
<i>Neobassia proceriflora</i>	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0
<i>Osteocarpum acropterum</i>	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
<i>Osteocarpum dipterocarpum</i>	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
<i>Roycea divaricata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Roycea spinescens</i>	0&1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sclerolaena anisacanthoides</i>	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Sclerolaena bicornis</i> v. <i>bicornis</i>	0	0&1	0	2	0	0	1	2	2	0	0	0	1	2	2
<i>Sclerolaena bicornis</i> v. <i>horrida</i>	0	0	0	2	0	0	1	2	2	1	2	2	1	2	2
<i>Sclerolaena birchii</i>	0	0	0	2	1	1	1	2	2	1	2	2	1	2	2
<i>Sclerolaena blackiana</i>	0	0	0	2	0	0	2	1	2	0	1	2	1	1	2
<i>Sclerolaena brachyptera</i>	0	0	0	2	0	0	0	0	0	1	2	2	1	0	0
<i>Sclerolaena calcarata</i>	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
<i>Sclerolaena convexula</i>	0	0	0	2	0	0	1	2	2	1	2	2	1	2	2
<i>Sclerolaena cornishiana</i>	0	0	0	2	0	0	1	2	2	1	2	2	1	2	2
<i>Sclerolaena cuneata</i>	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
<i>Sclerolaena densiflora</i>	0	0&2	0	2	0	1	2	1	2	1	1	2	1	1	2
<i>Sclerolaena diacantha</i>	0	0	0	2	0	0	2	2	2	1	2	2	1	2	1
<i>Sclerolaena divaricata</i>	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
<i>Sclerolaena eriacantha</i>	0	0	0	2	0	0	2	1	2	1	1	2	1	1	2
<i>Sclerolaena eurotioides</i>	0	0	0	2	0	0	2	1	2	1	1	2	1	2	2
<i>Sclerolaena fimbriolata</i>	2	1	0	2	0	0	2	1	2	1	2	2	1	1	2
<i>Sclerolaena intricata</i>	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
<i>Sclerolaena lanicuspis</i>	0	0	0	2	0	1	2	1	2	1	1	2	1	1	2
<i>Sclerolaena longicuspis</i>	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
<i>Sclerolaena medicaginooides</i>	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0

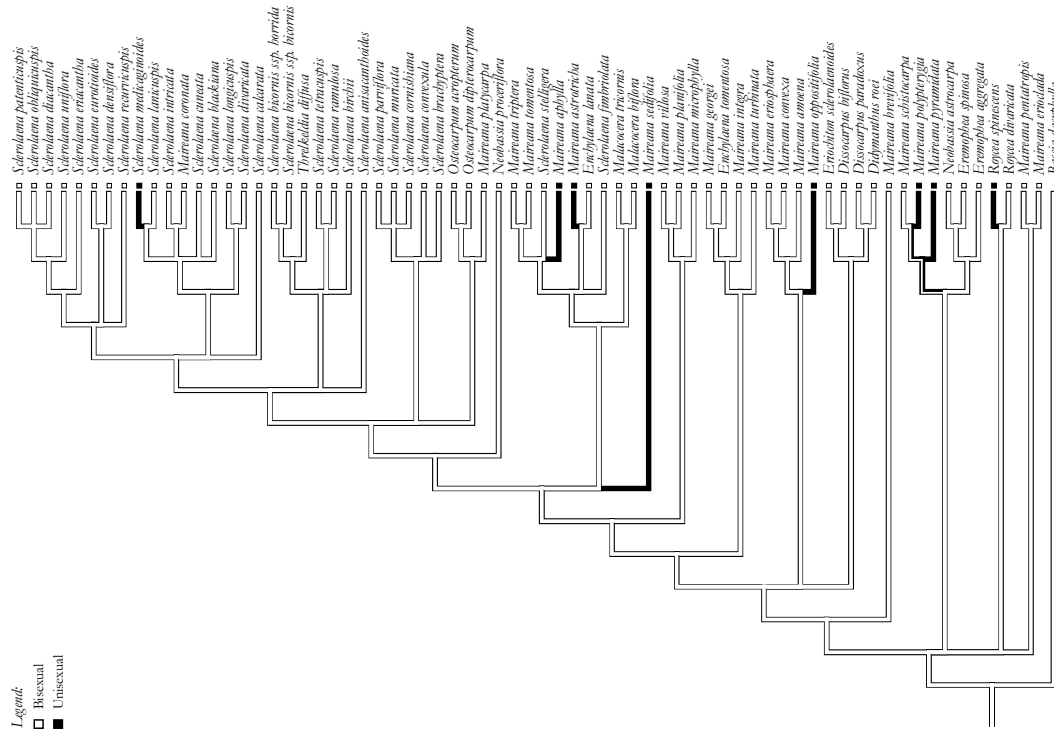
<i>Sclerolaena muricata</i>	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Sclerolaena obliquicuspis</i>	0	0	0	2	0	1	1	2	2	1	2	2	1	2	2
<i>Sclerolaena parviflora</i>	0	0	0	2	0	0	2	2	1	1	2	1	1	2	1
<i>Sclerolaena patenticuspis</i>	0	0	0	2	0	1	1	2	2	1	2	2	1	2	2
<i>Sclerolaena ramulosa</i>	0	0	0	2	0	0	2	?	?	1	?	?	1	?	?
<i>Sclerolaena recurvicuspis</i>	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
<i>Sclerolaena stelligera</i>	0	0	0	2	0	0	0	0	0	1	2	2	1	2	2
<i>Sclerolaena tetracuspis</i>	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0
<i>Sclerolaena uniflora</i>	0	0	0	2	0	0	2	1	2	1	2	2	1	2	2
<i>Threlkeldia diffusa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



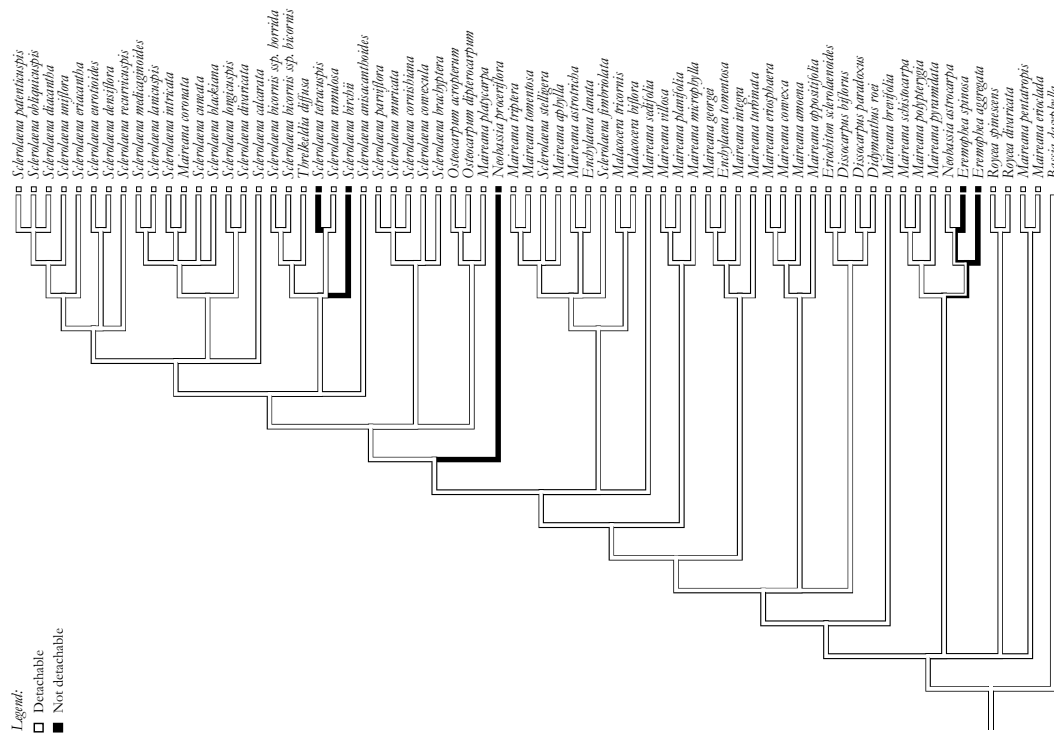
Appendix 5a. A cladogram showing parsimoniously optimized analysis of phyllotaxy on the ML tree derived from ETS sequence data.



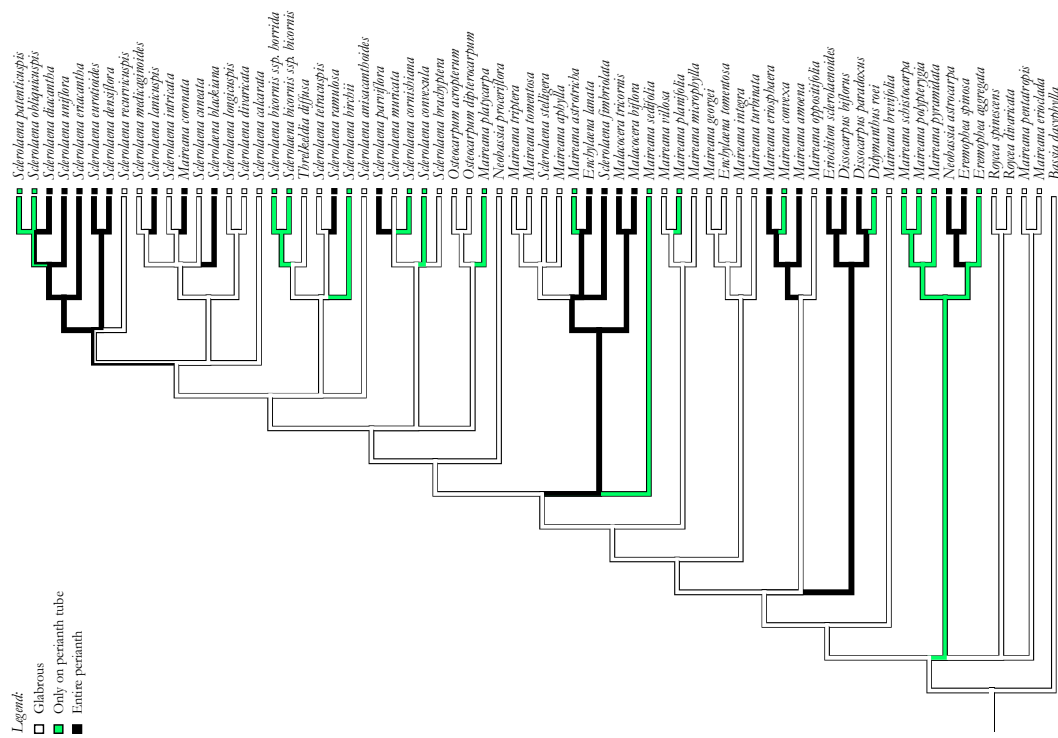
Appendix 5b. A cladogram showing parsimoniously optimized analysis of infructescence types against the ML tree derived from ETS sequence data.



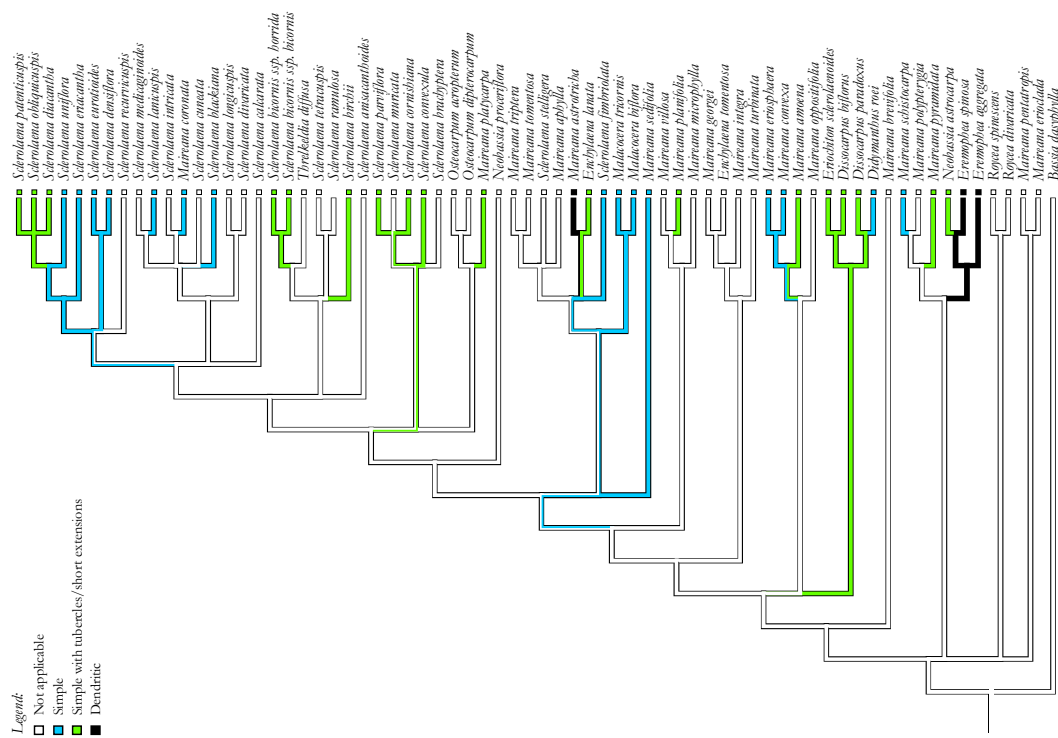
Appendix 5c. A cladogram showing parsimoniously optimized analysis of distribution of sexes against the ML tree derived from ETS sequence data.



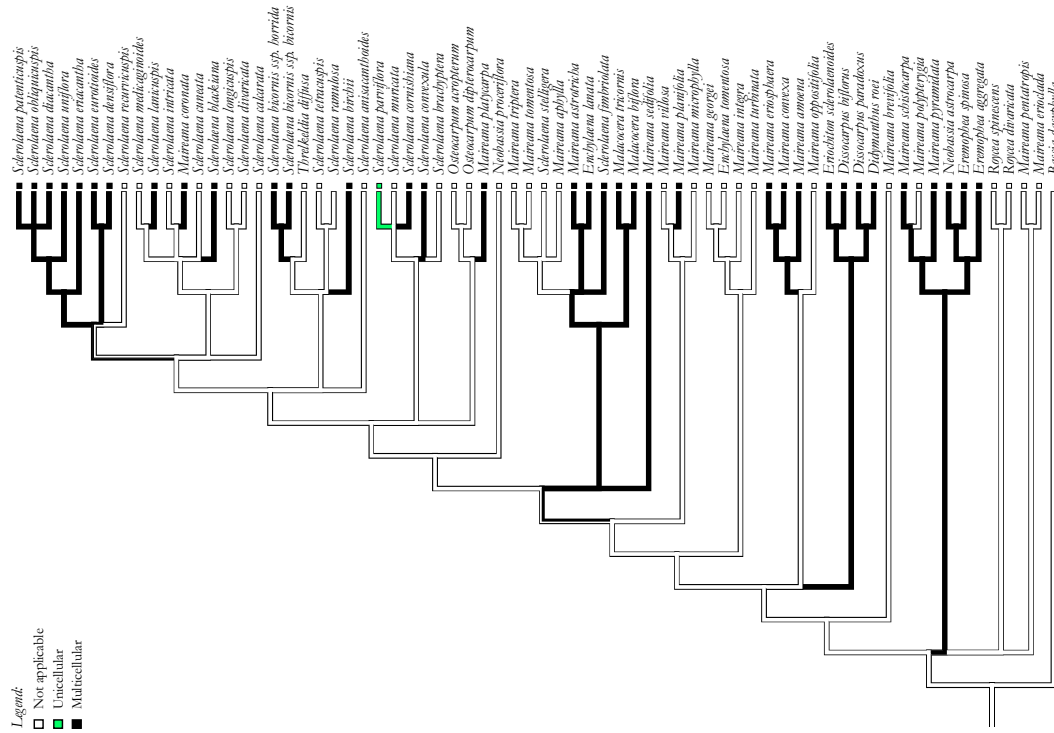
Appendix 5d. A cladogram showing parsimoniously optimized analysis of fruit detachability against the ML tree derived from ETS sequence data.



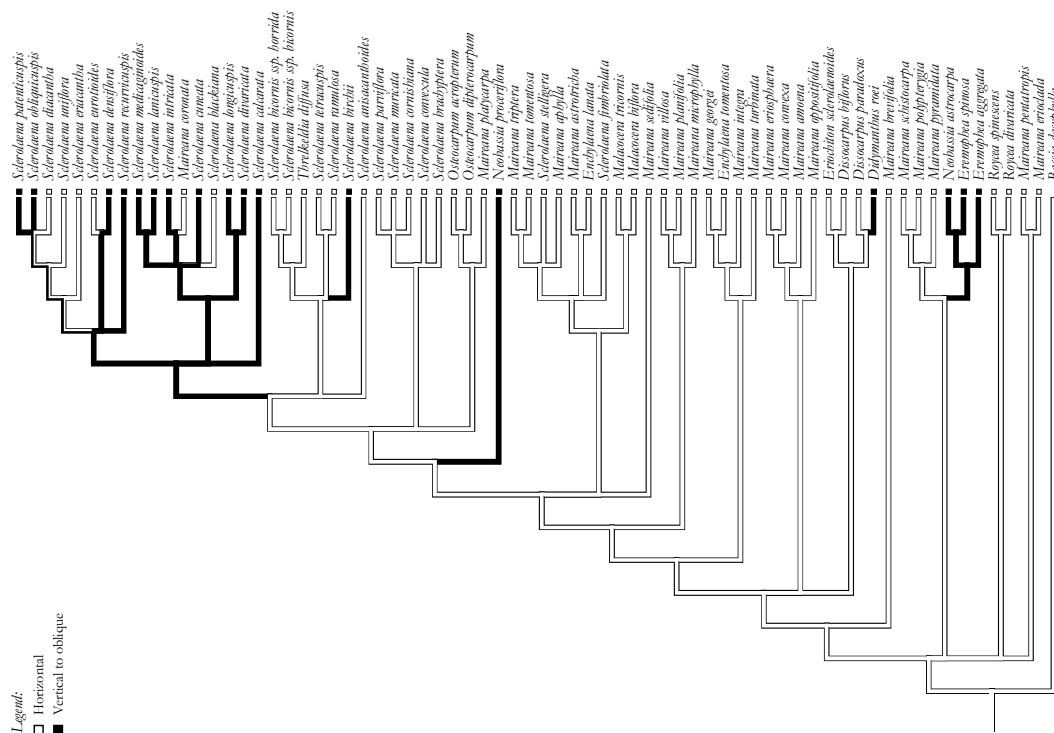
Appendix 5e. A cladogram showing parsimoniously optimized analysis of perianth pubescence against the ML tree derived from ETS sequence data.



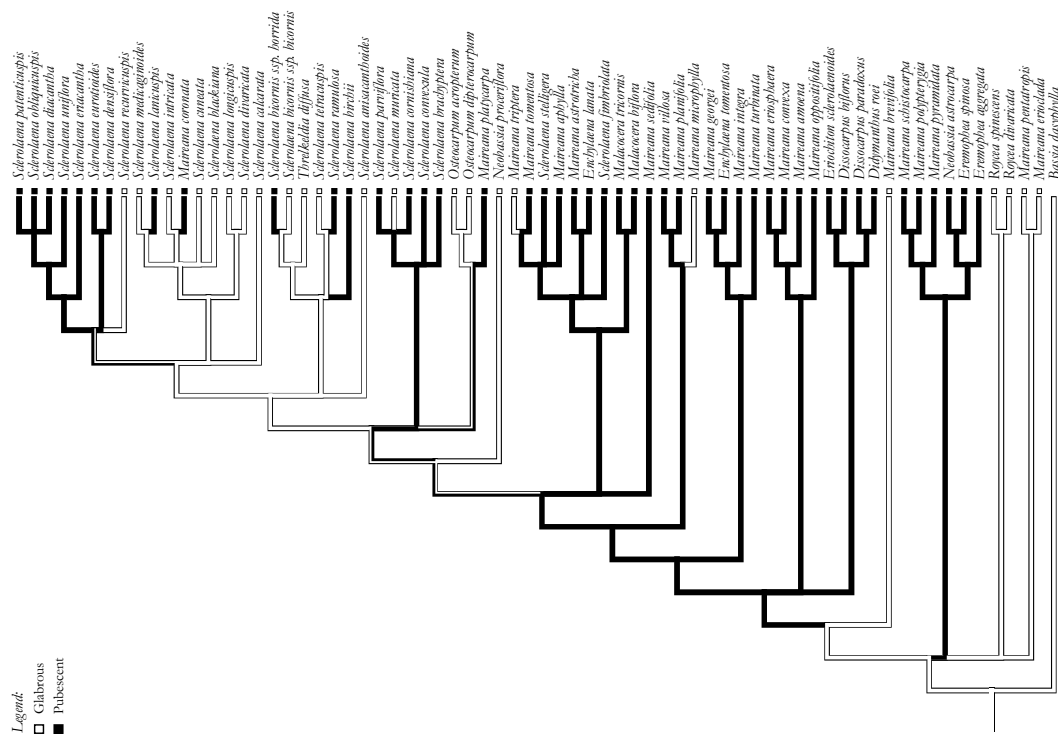
Appendix 5f. A cladogram showing parsimoniously optimized analysis of perianth hair types against the ML tree derived from ETS sequence data.



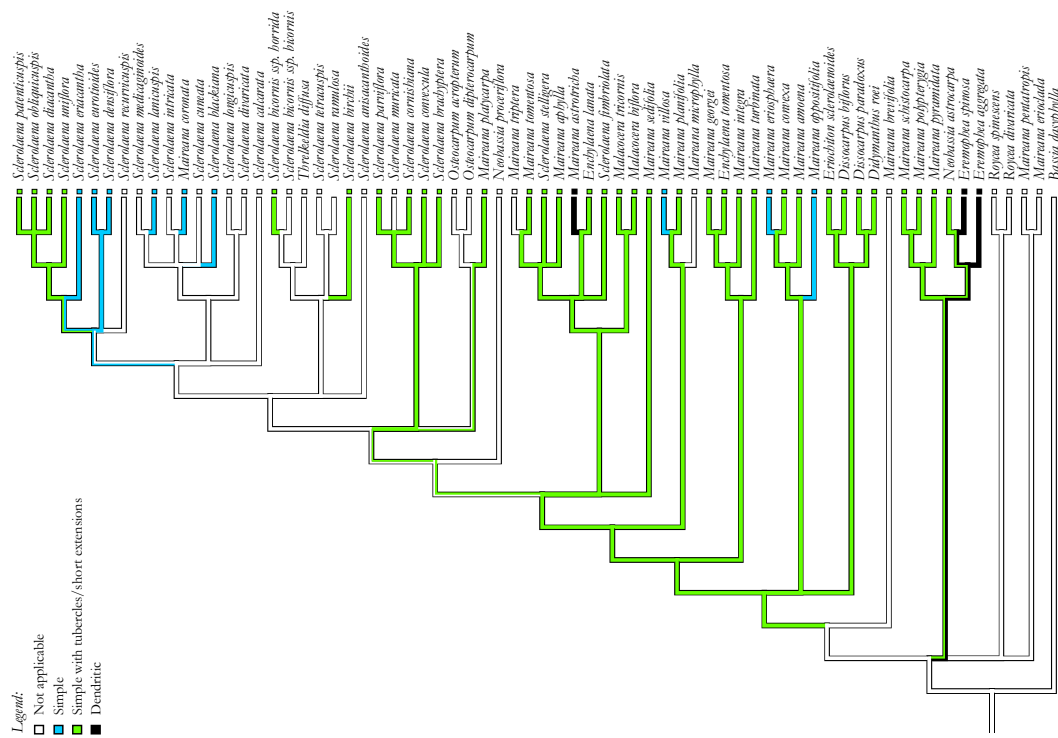
Appendix 5g. A cladogram showing parsimoniously optimized analysis of perianth hair cellularity against the ML tree derived from ETS sequence data.



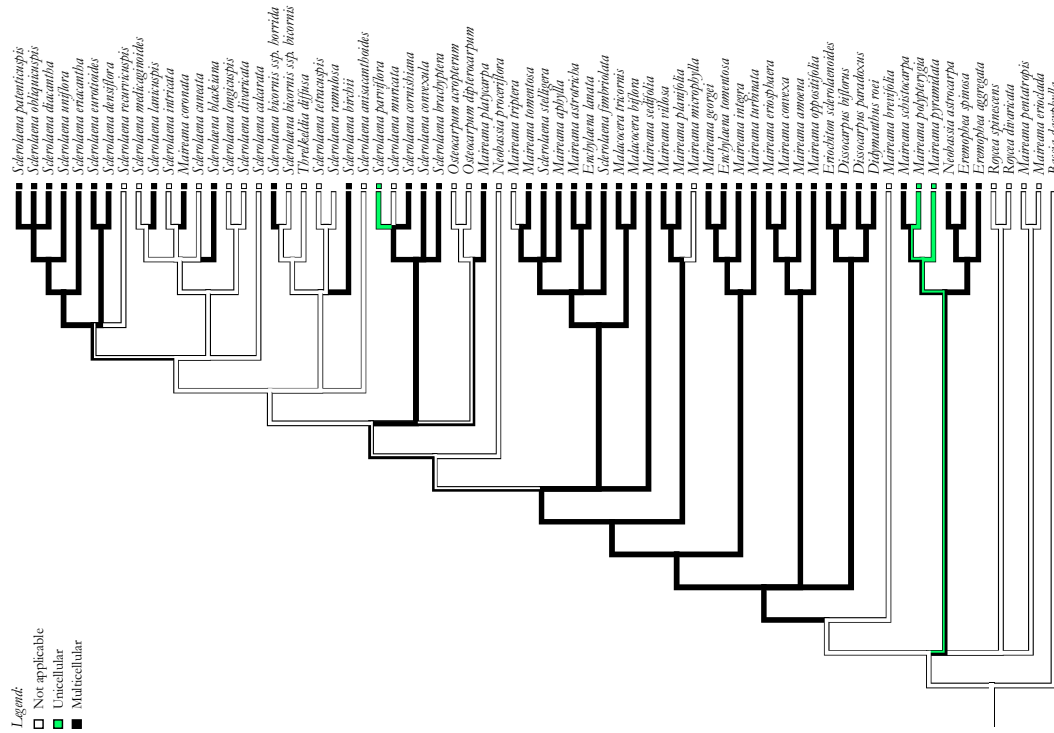
Appendix 5h. A cladogram showing parsimoniously optimized analysis of seed position against the ML tree derived from ETS sequence data.



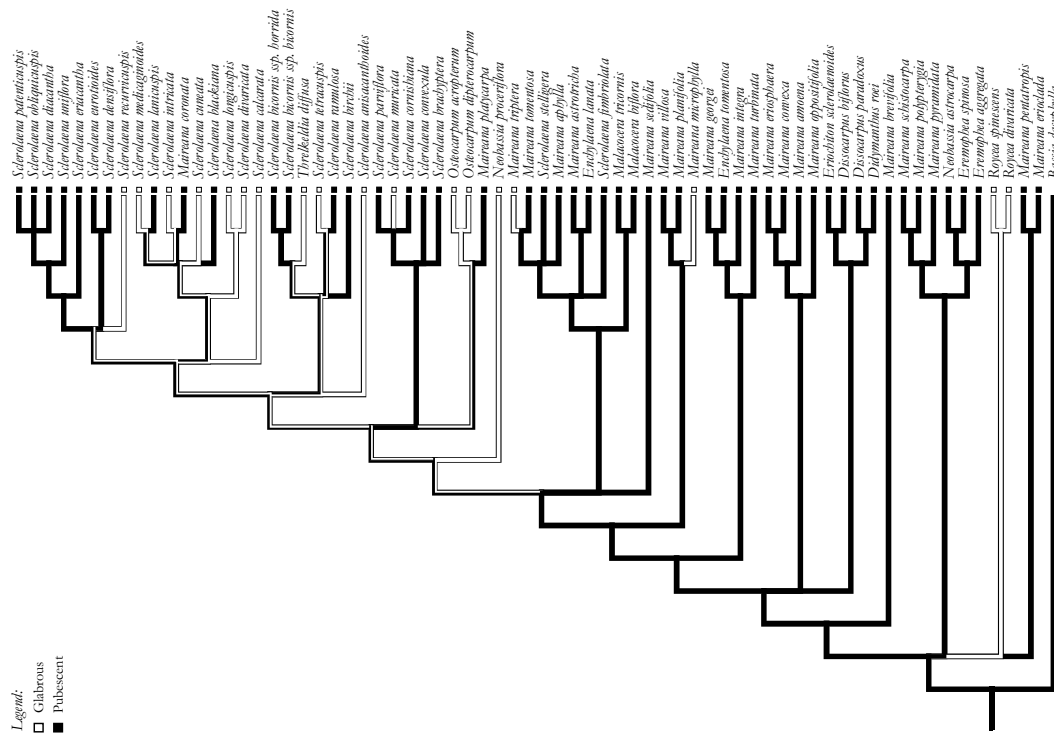
Appendix 5i. A cladogram showing parsimoniously optimized analysis of leaf pubescence against the ML tree derived from ETS sequence data.



Appendix 5j. A cladogram showing parsimoniously optimized analysis of leaf hair types against the ML tree derived from ETS sequence data.



Appendix 5k. A cladogram showing parsimoniously optimized analysis of leaf hair cellularity against the ML tree derived from ETS sequence data.



Appendix 5l. A cladogram showing parsimoniously optimized analysis of stem pubescence against the ML tree derived from ETS sequence data.

Appendix 6. DIVA optimization.

optimization successful - exact solution

settings: maxareas=2, bound=250, hold=1000, weight=1.000, age=1.000

optimal reconstruction requires 219 dispersals

optimal distributions at each node:

node 71 (anc. of terminals 1-3): N
 node 72 (anc. of terminals 1-2): N
 node 73 (anc. of terminals 1-8): N
 node 74 (anc. of terminals 7-34): N
 node 75 (anc. of terminals 6-34): MN
 node 76 (anc. of terminals 6-12): N
 node 77 (anc. of terminals 24-25): LM MN O LO NO
 node 78 (anc. of terminals 24-26): L LN LO
 node 79 (anc. of terminals 6-26): N LN NO
 node 80 (anc. of terminals 1-26): N
 node 81 (anc. of terminals 38-39): M MN
 node 82 (anc. of terminals 1-39): N MN
 node 83 (anc. of terminals 9-20): N
 node 84 (anc. of terminals 1-20): N
 node 85 (anc. of terminals 4-11): IN JN LN
 node 86 (anc. of terminals 4-54): N
 node 87 (anc. of terminals 28-29): N
 node 88 (anc. of terminals 10-29): N
 node 89 (anc. of terminals 10-65): EN FN JN LN
 node 90 (anc. of terminals 4-65): N
 node 91 (anc. of terminals 32-33): N
 node 92 (anc. of terminals 27-33): N
 node 93 (anc. of terminals 4-33): N
 node 94 (anc. of terminals 14-55): L
 node 95 (anc. of terminals 14-48): L
 node 96 (anc. of terminals 14-56): L
 node 97 (anc. of terminals 51-58): L
 node 98 (anc. of terminals 14-58): L
 node 99 (anc. of terminals 40-43): L
 node 100 (anc. of terminals 14-43): L
 node 101 (anc. of terminals 14-45): L
 node 102 (anc. of terminals 49-53): N MN O MO
 node 103 (anc. of terminals 49-66): M MN O MO
 node 104 (anc. of terminals 52-68): M O
 node 105 (anc. of terminals 52-59): M MO
 node 106 (anc. of terminals 49-59): M O MO
 node 107 (anc. of terminals 50-63): L
 node 108 (anc. of terminals 50-61): L
 node 109 (anc. of terminals 49-61): LM LO
 node 110 (anc. of terminals 14-61): L
 node 111 (anc. of terminals 41-57): L
 node 112 (anc. of terminals 42-64): L
 node 113 (anc. of terminals 42-69): HL IL JL LM LO
 node 114 (anc. of terminals 41-69): L
 node 115 (anc. of terminals 14-69): L

node 116 (anc. of terminals 35-44): J L
node 117 (anc. of terminals 62-67): EI FI EJ FJ EM FM EN FN
node 118 (anc. of terminals 60-67): E F J EL FL IL JL LM LN
node 119 (anc. of terminals 46-67): E J L EL FL JL
node 120 (anc. of terminals 35-67): J L EL JL
node 121 (anc. of terminals 35-47): L JL
node 122 (anc. of terminals 14-47): L
node 123 (anc. of terminals 36-37): L
node 124 (anc. of terminals 14-37): L
node 125 (anc. of terminals 14-23): LN
node 126 (anc. of terminals 4-23): N
node 127 (anc. of terminals 22-31): N
node 128 (anc. of terminals 4-31): N
node 129 (anc. of terminals 1-31): N
node 130 (anc. of terminals 5-17): I J L N
node 131 (anc. of terminals 5-18): I J L N
node 132 (anc. of terminals 5-30): I J L IN JN LN MN
node 133 (anc. of terminals 1-30): N IN JN LN
node 134 (anc. of terminals 13-16): N
node 135 (anc. of terminals 13-19): EN FN GN
node 136 (anc. of terminals 1-19): EI FI GI EJ FJ GJ N IN JN
node 137 (anc. of terminals 1-21): I J N IN JN MN
node 138 (anc. of terminals 1-15): I J M IN JN MN
node 139 (anc. of terminals 1-70): M IM JM MN

Appendix 7. Primary Brooks parsimony analysis matrix.

#NEXUS

begin data;

dimensions ntax=16 nchar=136;

format datatype=standard;

Matrix

	1	2	4	6	7	8	9	10	11	12	14	16	22	23	29	30	32	35	43	45	48	49	50	51
Kimberley	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Arnhem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Cape York	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atherton	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eastern Qld	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
McPherson	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
SW NSW	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
Victoria	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Eyre	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	0	1	1	0	1	1	0	0	0
Adelaide	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	0	1	1	0	1	1	0	0	0
N Desert	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
E Desert	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	0	1	1	0	0	1	1	0	0
Southwest	1	0	1	1	1	1	0	1	0	0	0	1	1	0	1	0	1	0	0	1	1	1	1	1
W Desert	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	0
Pilbara	1	0	1	0	1	0	1	1	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1	1

	53	56	58	63	65	66	67	69	71	72	73	74	75	79	81	82	84	86	88	89	93	96	97	100
Kimberley	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Arnhem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Cape York	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Atherton	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Eastern Qld	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0
McPherson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0
SW NSW	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Victoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Eyre	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	0	0	0	0	1
Adelaide	1	0	1	0	1	1	0	0	1	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1
N Desert	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1
E Desert	1	1	1	0	1	1	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1
Southwest	0	0	0	1	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1
W Desert	1	0	1	1	1	0	1	1	1	1	0	0	1	1	0	1	0	1	1	0	0	1	1	1
Pilbara	1	0	0	1	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1

	102	104	105	107	108	110	116	118	120	121	125	127	128	130	131	132	136	141	145	146	t1	t2	
Kimberley	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arnhem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape York	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atherton	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Eastern Qld	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0
McPherson	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0
SW NSW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Victoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Eyre	0	1	1	1	0	0	0	1	0	0	0	1	1	1	0	0	0	1	0	1	0	1	0
Adelaide	0	1	1	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	1	1	1	1	1
N Desert	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
E Desert	0	1	1	1	0	0	1	1	1	0	1	1	0	1	1	0	0	0	1	0	1	1	1
Southwest	0	1	0	1	1	0	0	0	0	1	0	1	1	0	0	1	0	1	0	1	0	1	0
W Desert	1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1
Pilbara	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	1

	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16	t17	t18	t19	t20	t21	t22	t23	t24
Kimberley	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
Arnhem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
Cape York	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atherton	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Eastern Qld	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
McPherson	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
SW NSW	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0
Victoria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0
Eyre	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
Adelaide	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N Desert	1	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0
E Desert	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Southwest	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1
W Desert	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pilbara	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0

	t25	t26	t27	t28	t29	t30	t31	t32	t33	t34	t35	t36	t37	t38	t39	t40	t41	t42	t43	t44	t45	t46
Kimberley	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Arnhem	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Cape York	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Atherton	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Eastern Qld	1	0	1	1	1	0	1	1	0	0	1	0	0	1	0	0	1	1	1	1	0	0
McPherson	1	1	1	1	1	0	1	1	0	1	1	0	0	1	0	0	1	1	0	1	0	0
SW NSW	0	0	0	0	1	0	1	1	0	1	1	0	0	1	0	0	1	1	0	1	0	0
Victoria	0	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Eyre	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	0
Adelaide	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
N Desert	0	0	0	1	1	0	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1
E Desert	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
Southwest	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1
W Desert	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pilbara	1	0	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1

	t47	t48	t49	t50	t51	t52	t53	t54	t55	t56	t57	t58	t59	t60	t61	t62	t63	t64	t65	t66	t67	t68
Kimberley	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1
Arnhem	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1
Cape York	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1
Atherton	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1
Eastern Qld	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1
McPherson	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1
SW NSW	1	1	1	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1
Victoria	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1
Eyre	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1
Adelaide	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1
N Desert	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1
E Desert	0	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1
Southwest	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
W Desert	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pilbara	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1

;
End;

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