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Large indels obscure phylogeny in analysis of chloroplast DNA (*trn*L-F) sequence data: Pomaderreae (Rhamnaceae) revisited

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Abstract

Phylogenetic analysis of 69 ingroup-taxa of Pomaderreae using *trn*L-F sequences confirm the monophyly of the tribe. The analysis was impeded by a paucity of informative characters and the presence of apparently homoplasious indel characters and base changes within the P8 region of the *trn*L intron: the strict consensus tree of the *trn*L-F analysis is less resolved and had fewer supported clades than in a previous ITS analysis (Kellermann et al. 2005). The backbone of the cladogram is not supported and relationships between genera/clades are somewhat uncertain. The genera *Cryptandra*, *Stenanthemum* and *Polianthion* are well supported. *Pomaderris* groups with *Siegfriedia* and *Trymalium*, but only individual clades within these genera receive support. *Blackallia biloba* is related to two atypical species of *Stenanthemum* and *B. connata* to *Cryptandra*, but this grouping depends on the exclusion of homoplasious indel characters. Species of *Spyridium* only group in one clade when these indels are excluded, otherwise they are located in a polytomy at the base of the cladogram. The results mostly agree with earlier findings using ITS sequence data. Two new genera containing atypical species of *Stenanthemum* are suggested. A synopsis of the Australian genera of Rhamnaceae is provided.

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Introduction

Australia has a very rich Rhamnaceae flora with about one quarter of the world's species (c. 250 out of 950) occurring in the country. The majority of species (over 90%) belong to the tribe Pomaderreae, which is almost endemic to Australia. The remaining 10% of species are mostly from genera that are also widespread in the Malesian or Pacific region, and some species occur in southern Australia. A synopsis of the Australian genera of Rhamnaceae is presented in Table 1.

Species of Pomaderreae are found mainly in the temperate to semi-arid southern regions of Australia, but some occur in the tropical north, and arid centre of the continent, and eight taxa are found in New Zealand. There are about 230 species, which are currently classified in eight genera (Table 1). The complex taxonomic history of the major genera in the tribe is reviewed in Kellermann et al. (2005) and Kellermann (2007).

The tribe has been the focus of recent and on-going research in the Australian Rhamnaceae. Walsh revised *Pomaderris* and published an infrageneric classification of the genus (e.g., Walsh 1988, 1990; Walsh & Coates 1997). Rye (1995, 2001) re-instated the genus *Stenanthemum* and revised species from Western Australia (e.g., Rye 1996b).

Table 1. Currently accepted genera of Australian Rhamnaceae.
Tribal classification follows Medan & Schirarend (2004) and Richardson et al. (2000b). Six genera of Rhamnaceae are not assigned to a tribe; five of these occur in Australia.

Tribe	Genus	Species in Australia
Paliureae Reissek ex Endl.	*Hovenia* Thunb.	1 (introduced)
	Ziziphus Mill.	4
Colletieae Reissek ex Endl.	*Discaria* Hook.	2
Phyliceae Reissek ex Endl.	*Noltea* Rchb.	1 (introduced)
Gouanieae Reissek ex Endl.	*Gouania* Jacq.	2
Pomaderreae Reissek ex Endl.	*Blackallia* C.A.Gardner	2
	Cryptandra Sm.	c. 55
	Polianthion K.R.Thiele	4
	Pomaderris Labill.	c. 70
	Siegfriedia C.A.Gardner	1
	Spyridium Fenzl	40–45
	Stenanthemum Reissek	c. 30
	Trymalium Fenzl	13
Rhamneae Hook.f.	*Dallachya* F.Muell.	1
	Rhamnus L.	2 (1 native, 1 introduced)
	Sageretia Brongn.	1
Ventilagineae Hook.f.	*Ventilago* Gaertn.	3
Genera *incertae sedis*	*Alphitonia* Reissek ex Endl.	5
	Colubrina Rich. ex Brongn.	1
	Emmenosperma F.Muell.	2
	Granitites Rye	1
	Schistocarpaea F.Muell.	1

An atypical species of *Pomaderris* was excluded from the tribe and segregated into its own genus, *Granitites* (Rye 1996a). Thiele & West (2004) and Thiele (2007) elucidated the delimitations of the genera *Cryptandra*, *Spyridium* and *Stenanthemum*. Bean (2004) published new species of *Cryptandra* and *Stenanthemum* for Queensland. Kellermann (2006b, 2007) clarified the position of several *Spyridium* taxa that were misplaced in other genera. The revision of the south-eastern species of *Cryptandra* has resulted so far in three publications (Kellermann 2006a, 2006c; Kellermann & Udovicic 2007). Kellermann et al. (2005) published a molecular phylogeny using ITS sequence data, as a result of which a new genus, *Polianthion* K.R.Thiele, was established (Kellermann et al. 2006).

The ITS phylogeny confirmed the monophyly of Pomaderreae, corroborating earlier results by Richardson et al. (2000a) and Fay et al. (2001). The clades found in the strict consensus tree were mostly consistent with the currently accepted genera in the tribe. Some species were clearly misplaced, but re-examination of the morphology of these species confirmed their placement in the molecular phylogeny. The major genera/clades, except *Stenanthemum* and *Blackallia*, received moderate to strong bootstrap and jackknife support. *Stenanthemum* was split into two well-supported clades with the atypical *St. gracilipes* inserted in between the two clades. *Blackallia biloba* and *St. grandiflorum* were sister taxa, and not allied to any of the remaining genera; *B. connata* was placed in *Cryptandra*.

This study was initiated to clarify questions that could not be resolved in the analysis of ITS data (Kellermann et al. 2005) and to augment the molecular data-set available for Pomaderreae with sequences from the *trn*L-F region of cpDNA. In this paper, the resulting phylogenies of the *trn*L-F analysis are presented and we report on the presence of unforeseen problems relating to the structure of the *trn*L-F region, which hampered and complicated the cladistic analysis of the data. The results add to the base of knowledge needed for the completion of the *Flora of Australia* treatment of Rhamnaceae (K.R. Thiele, F. Udovicic, N.G. Walsh & J. Kellermann, in prep.).

Materials & Methods

Sixty-nine ingroup taxa were sequenced from all genera of Pomaderreae. The outgroup consisted of five species from related tribes of Rhamnaceae. Voucher and collection details are listed in Appendix 1. Manuscript names of taxa are used as they are listed in FloraBase (http://florabase.calm.wa.gov.au) at the time of writing (Mar. 2007). In this paper, the abbreviations used for the genera *Pomaderris*, *Polianthion*, *Siegfriedia*, *Spyridium* and *Stenanthemum* are '*P.*', '*Pol.*', '*Si.*', '*Sp.*' and '*St.*'.

Choice of DNA region

The *trn*L-F region consists of the complete *trn*L intron, *trn*L 3' exon, and the intergenic spacer (IGS) between the *trn*L and the *trn*F genes of the chloroplast genome. These genes encode the chloroplast's transfer RNA for Leucine and Phenylalanine, respectively. Both the *trn*L intron and the *trn*L-F IGS are non-coding regions. The *trn*L intron is the only group I intron in the chloroplast genome and has a conserved secondary structure (Simon et al. 2004).

The *trn*L-F region was first used in phylogenetic analyses of *Gentiana* L. (Gielly & Taberlet 1994) and Crassulaceae (Ham et al. 1994). Currently, it is applied in studies at all taxonomic levels. Borsch et al. (2003) used the *trn*T-*trn*F region, which includes the *trn*L-F region, to infer a phylogeny of basal angiosperms. Most frequently, however, *trn*L-F is used for infrafamilial studies, e.g., in Araliaceae (Plunkett et al. 2004), Gentianaceae (Yuan et al. 2003), *Oxylobium* Andrews and related genera (Crisp & Cook 2003), or *Acacia* Mill. (Murphy et al. 2000).

The region has already been employed to examine the relationships of Rhamnaceae with other families (Sytsma et al. 2002; Thulin et al. 1998), to resolve the tribal limits of the family (Fay et al. 2001; Richardson et al. 2000a, b), and in studies on the genera *Ceanothus* Mill. (Islam & Simmons 2006), *Phylica* L. (Richardson et al. 2001) and *Rhamnus* L. s. lat. (Bolmgren & Oxelman 2004).

DNA isolation and sequencing

Genomic DNA was isolated using the method described in Kellermann et al. (2005). A few samples of the *trn*L-F region had to be purified using the QIAquick Gel Extraction Kit (QIAGEN). The *trn*L-F region was amplified using the primers designed by Taberlet et al. (1991). For most species the whole region was amplified with primers C and F with one hold at 95°C for 15 min preceding 30 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and followed by one hold at 72°C for 5 min. In other species, the *trn*L intron and the *trn*L-F IGS had to be amplified separately using primer pairs C/D and E/F, respectively. While the *trn*L-F IGS amplified readily, the annealing temperature frequently had to be lowered to 55°C or 52°C when amplifying the *trn*L intron. Some species with a low yield of genomic DNA, in particular from herbarium specimens, had to be amplified with a semi-nested PCR protocol (Udovicic & Murphy 2002) using products from a previous amplification with primers C and F as template for a second round of PCR. In this second round the *trn*L intron and the *trn*L-F IGS were amplified using the primer pairs C/D and E/F, respectively, and a lower annealing temperature of 55°C. Amplification with primers C and F in the second round of PCR was unsuccessful, a fact already noted by Richardson et al. (2001) for other species of Rhamnaceae.

Phylogenetic analysis

Sequences were aligned as outlined in Kellermann et al. (2005) and analysed using the computer program PAUP*, version 4.0b10 (Swofford 2002). Individual base positions were coded as unordered multistates and gaps were treated as missing data. Insertion/deletion (indel) characters were coded as single binary characters. Uninformative characters were excluded from the data matrix.

A two step search was employed, since the computer ran out of memory when using a more straightforward search strategy (e.g., Kellermann et al. 2005). In the first round, a heuristic search was performed with 1000 replicates using random stepwise addition of taxa and TBR branch swapping. Only five trees were held in each replicate. All shortest trees collected in the 1000 replicates were then used as starting trees for a second round of heuristic search. All trees were swapped to completion, or until a maximum number of 10,000 trees was produced, at which point the search was limited and the 10,000 trees saved were swapped. Strict consensus and majority-rule consensus trees were calculated for the 10,000 equally parsimonious trees. Trees were rooted using the outgroup taxa (Maddison et al. 1984).

To test the support for nodes in the tree, both bootstrap (Felsenstein 1985) and jackknife (Farris et al. 1996) values were calculated in PAUP*. Bootstrap analysis was carried out with 1,000 replicates, TBR branch swapping and a limit of 1,000 trees per replicate. To calculate jackknife values, the 'Jac' emulation as implemented in PAUP* was performed with 100,000 replicates and 37% deletion, using the fast heuristic search option.

Results

Sequences

Sequences were obtained for 69 species of Pomaderreae and five outgroup species from related tribes. Two accessions were obtained for each of six taxa to test infraspecific variation: *Cryptandra amara*, *C. mutila*, *Siegfriedia darwinioides*, *Spyridium globulosum*, *Sp. parvifolium* and *Trymalium ledifolium*. The sequence variation between two sequences of the same species was ≤1.6% in all cases and in some cases, sequences were identical. Because of the low sequence variation, only a single sequence of each species, the first listed in Appendix 1, was used in the analysis of the *trn*L-F sequence data.

Large indels

In the alignment of the *trn*L-F sequences, several large indels were identified. In particular, one deletion of approximately 125 base pairs (indel no. 9) seemed to have occurred in unrelated species, a result revealed in the first analysis (A). Subsequently two more analyses were undertaken to explore the effect of indel no. 9 on the resulting topology of the tree. The following analyses of the *trn*L-F data-set were carried out:

Analysis A included all species and characters;

Analysis B excluded two of the three sequences with indel no. 9, namely those of *Pomaderris rotundifolia* and *Cryptandra triplex*, but included all characters;

Analysis C included all species, but excluded the DNA region in which indel no. 9 occurred, and all potential characters therein (following Quandt et al. 2004; see below for discussion).

Characteristics of sequences & phylogeny

The alignment of the *trn*L-F data set had 1145 base positions. Four regions in the alignment were ambiguous and unalignable and therefore excluded from the analysis. This reduced the data-set by 46 characters to 1099 base positions. Twenty-three indels were identified in the alignment and coded separately using the simple indel coding method of Simmons and Ochoterena (2000).

When all species and all characters were included in analysis A, the alignment provided 90 parsimony-informative characters (8.2%) and 21 out of 23 indel characters were potentially informative characters. In analysis B, the number of parsimony-informative characters in the alignment was reduced due to the exclusion of two species: 87 base characters (7.9%) and 20 of 23 the indels were potentially informative. Analysis C excluded a stretch of 261 bases from the alignment and reduced the number of

characters to 838 base positions; this also eliminated 8 indel characters from the data-set. Analysis C included 67 potentially informative base characters (8.0 %), and 13 parsimony-informative indels.

In all three parsimony analyses, the maximum number of 10,000 trees was reached when using the two step search strategy. The trees of analysis A had a CI=0.566 and RI=0.789. The CI and RI for analysis B were 0.564 and 0.786, respectively. The trees in analysis C had a CI=0.572 and a RI=0.805. The strict consensus tree of analysis A (Fig. 1) showed 30 nodes common to all most parsimonious trees (27 nodes common to the ingroup); 23 nodes had bootstrap support (BS) and 22 nodes had jackknife support (JS) ≥50%. The strict consensus of analysis B (excluding the sequences containing indel no. 9; Fig. 2), had 28 nodes (25 nodes in the ingroup), of which 21 had bootstrap support and 20 nodes had jackknife support above 50%. Analysis C had 24 nodes present in the strict consensus tree (22 nodes in the ingroup; Fig. 3), statistical support ≥50% in the bootstrap and jackknife analyses was obtained for 19 nodes.

Cladogram topology

The strict consensus trees for analyses A and B are shown in Figures 1 and 2. Tree topology is the same in both cladograms, except that in analysis A the species containing indel no. 9 are grouped in one clade within the genus *Trymalium*. This clade is indicated in bold in Figure 1. Bootstrap (BS) and jackknife (JS) values differ only slightly between the analyses. The strict consensus in analysis C (Fig. 3) has a similar topology to the previous two trees, but is less resolved. However, the genus *Spyridium* was resolved in one clade (at node 3) in analysis C, and *Blackallia connata* grouped with *Cryptandra* (node 24) and not with *Stenanthemum gracilipes*, *St. grandiflorum* ms and *B. biloba* (clade at node 22). Only the tree in Figure 2 (analysis B) is discussed in the following sections and Figure 1 and 3 are only referred to when there are differences between the analyses.

Monophyly of the tribe Pomaderreae is very strongly supported with 100% bootstrap and jackknife support. Sister to Pomaderreae is either *Schistocarpaea johnsonii* (not placed in any tribe by Richardson et al. 2000b), *Adolphia californica* (tribe Colletieae), or a weakly supported clade (BS: 57%; JS: <50%) containing *Alphitonia* aff. *incana* (unplaced genus), *Ceanothus coeruleus* (unplaced genus) and *Phylica buxifolia* (tribe Phyliceae).

The backbone of the cladogram lacks bootstrap or jackknife support above 50% and thus the relationships among the main clades (genera) are unresolved. Of the currently accepted genera, only *Cryptandra* and two clades of *Stenanthemum* have bootstrap/jackknife support. The species of *Spyridium* do not group in a clade in the strict consensus tree in analyses A and B. However, they form a clade in 94% of trees in a majority rule consensus tree (majority rule tree not shown). In analysis C the species of *Spyridium* are united in a clade, albeit without bootstrap or jackknife support above 50%.

Within *Spyridium*, three species from New South Wales (*Sp. scortechinii*, *Sp. buxifolium* and *Sp. burragorang*) form a weakly supported clade at node 4. The two Tasmanian species included, *Sp. ulicinum* and *Sp. gunnii*, are sister taxa (node 6; BS: 61%, JS: 58%). *Spyridium mucronatum* and *Sp. cordatum* are strongly supported as sister taxa (node 7), but their relationship with the third Western Australian species included, *Sp. globulosum*, is unresolved. *Spyridium daltonii* and *S.* ×*ramosissimum* from the

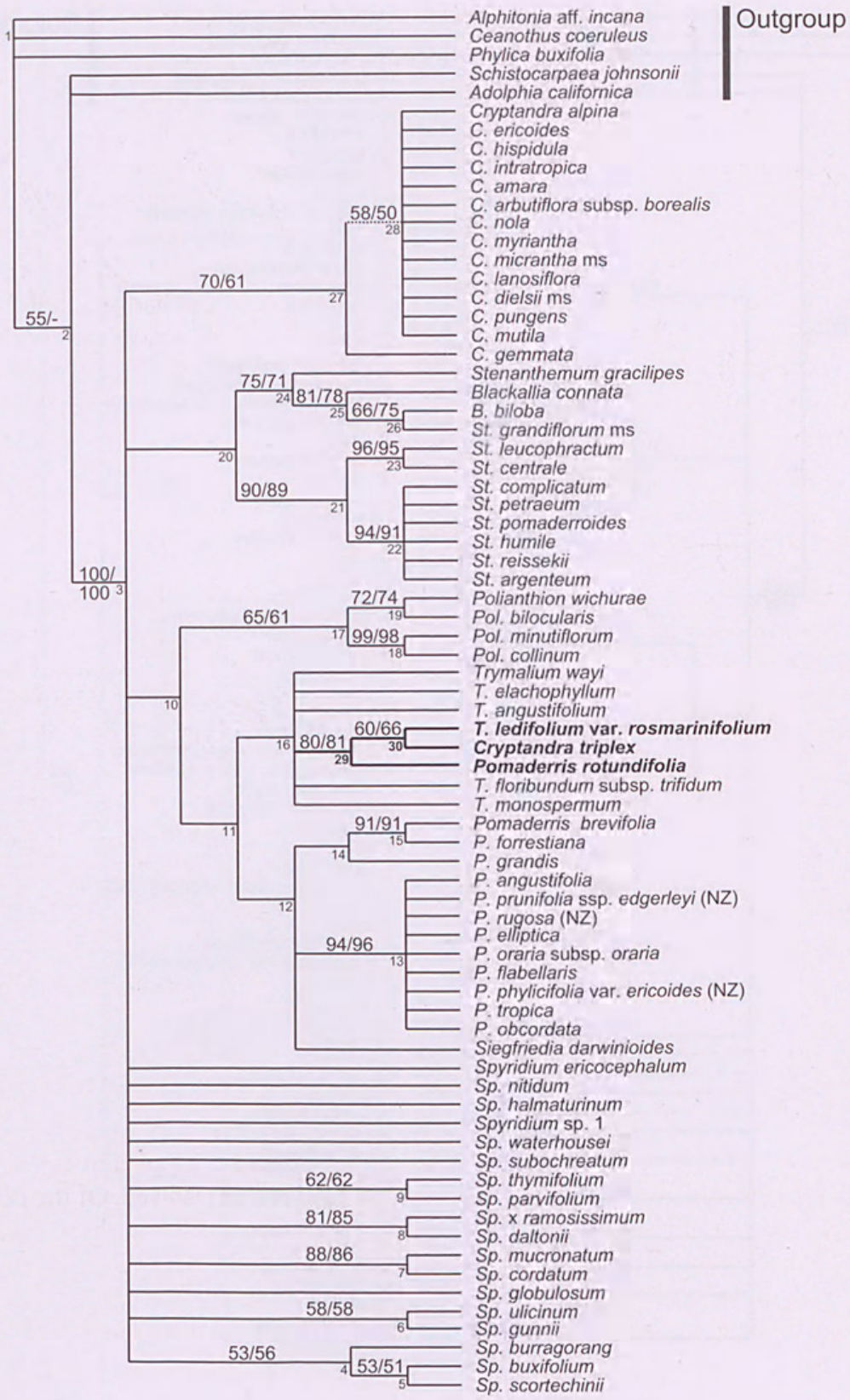

Fig. 1. Strict consensus tree of analysis A of the *trn*L-F data-set (10,000 equally parsimonious trees of 226 steps each, CI=0.57, RI=0.79), i.e., analysis of the full data-set. Bootstrap/jackknife values are indicated on branches. Node numbers are indicated in smaller type. The clade highlighted in bold contains taxa that share indel no. 9. The branch denoted by a dotted line is only present in bootstrap and jackknife analyses. Species from New Zealand are indicated (NZ).

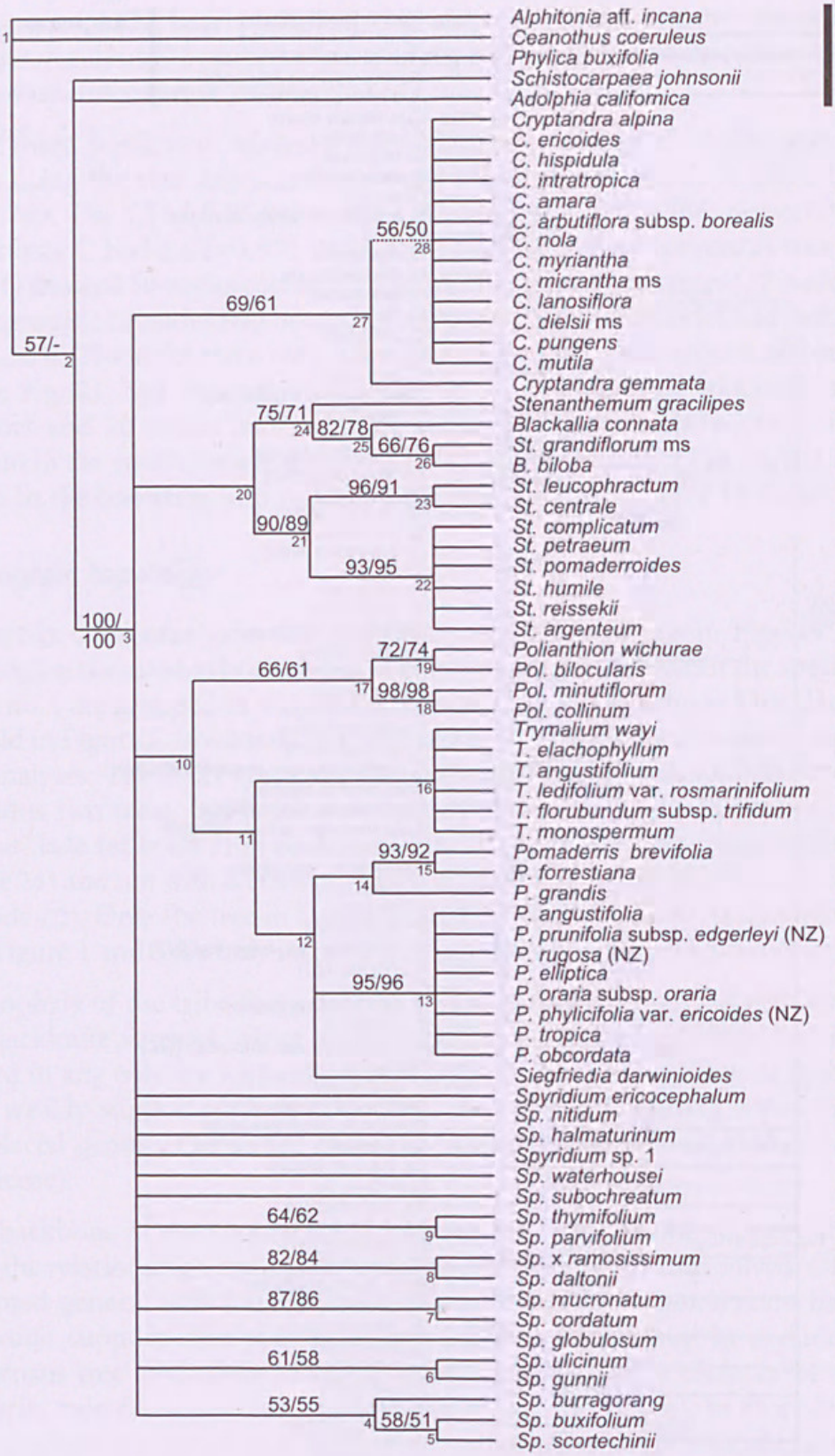

Fig. 2. Strict consensus tree of analysis B of the *trn*L-F data-set (10,000 equally parsimonious trees of 220 steps each, CI=0.56, RI=0.79), i.e., parsimony analysis excluding sequences from two taxa with indel no. 9 (*Pomaderris rotundifolia*, *Cryptandra triplex*). Bootstrap/jackknife values are indicated on branches. Node numbers are indicated in smaller type. The branch denoted by a dotted line is only present in bootstrap and jackknife analyses. Species from New Zealand are indicated (NZ).

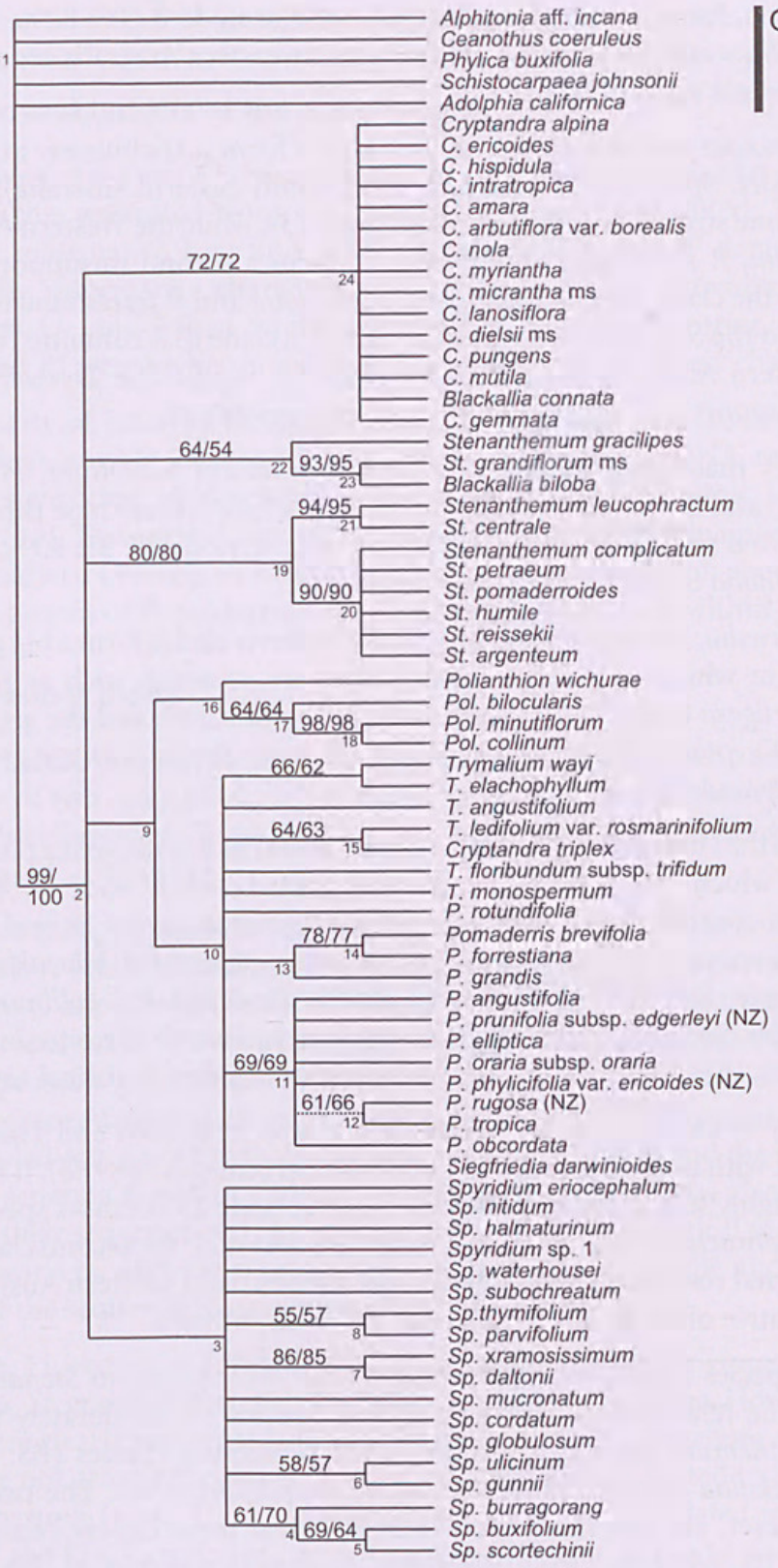

Fig. 3. Strict consensus tree of analysis C of the *trn*L-F data-set (10,000 equally parsimonious trees of 259 steps each, CI=0.57, RI=0.81), i.e., parsimony analysis excluding the DNA region containing indel no. 9. Bootstrap/jackknife values are indicated on branches. Node numbers are indicated in smaller type. The branch denoted by a dotted line is only present in bootstrap and jackknife analyses. Species from New Zealand are indicated (NZ).

Grampians, Victoria, form a strongly supported clade at node 8 (BS: 82%, JS: 84%). *Spyridium parvifolium* and *Sp. thymifolium* from south-eastern Australia are sister taxa (node 9 with moderate support).

Siegfriedia darwinioides and two clades of *Pomaderris* form a trichotomy at node 12, which lacks support. Species of *Pomaderris* from south-eastern Australia and New Zealand group in one strongly supported clade (node 13), while the Western Australian species, *P. brevifolia*, *P. forrestiana* and *P. grandis*, form a second unsupported clade (node 14). Within the clade the relationship of *P. brevifolia* and *P. forrestiana* is strongly supported. Sister to the *Siegfriedia-Pomaderris* clade is a clade that contains *Trymalium* species from Western Australia and South Australia (*T. wayi*). The *Trymalium* clade does not receive support >50% and shows no internal resolution.

If the two species that share indel no. 9 with *Trymalium ledifolium*, *Pomaderris rotundifolia* (W.A.) and *Cryptandra triplex* (N.T.), are included, then these three species form a well supported sub-clade within *Trymalium* (Fig. 1, node 29; BS: 80%, JS 81%). *Pomaderris rotundifolia* is sister to the other two species.

In analysis C, *Siegfriedia*, *Trymalium* and the two *Pomaderris* clades form a big polytomy (Fig. 3, node 10) in which only a few relationships are resolved, such as the sister-relationship of *P. rugosa* from New Zealand with *P. tropica* (Qld), and the grouping of *T. ledifolium* with *Cryptandra triplex*. In analysis C, *P. rotundifolia* does not group with either of the two *Pomaderris* clades.

The sister-group to the *Pomaderris-Siegfriedia-Trymalium* clade is the genus *Polianthion*, the four species of which group in a moderately supported clade at node 17 (Fig. 2; BS: 66%, JS: 61%) that consists of two well supported sub-clades (nodes 18 and 19). Two species from south-western Western Australia, *Pol. wichurae* and *Pol. bilocularis*, group in the first sub-clade; *Pol. minutifolium* from Queensland and *Pol. collinum* (W.A.) form the second sub-clade. In analysis C, *Pol. wichurae* is sister to the remaining three species (Fig. 3, node 16); this topology, however does not receive statistical support.

The genus *Stenanthemum* sensu stricto (according to Rye 1995, 2001 and Thiele 2007) is found at node 21 with high bootstrap and jackknife percentages (≥89 %). It is divided further into two highly supported groups. One clade at node 23 contains species from southern (*St. leucophractum*) and central Australia (*St. centrale*); the second clade (node 22) shows no internal resolution and contains five species from Western Australia and the only representative of the genus in Queensland, *St. argenteum*.

A group of four species from Western Australia is the sister group to *Stenanthemum* s. str. (node 24); the relationships between all four species are moderately to highly supported. *Stenanthemum gracilipes* is sister to the remaining species (BS: 75%, JS: 71%), namely *Blackallia connata*, *B. biloba* and *St. grandiflorum* ms. The two species of *Blackallia*, however, are not sister taxa, with *B. biloba* most closely related to *St. grandiflorum* ms (BS: 66%, JS: 76%). *Blackallia connata* is not part of this clade in analysis C, but is part of the *Cryptandra* clade (Fig. 3, node 24).

Fourteen species of *Cryptandra* form a large polytomy in the strict consensus tree (Fig. 2, node 27). This clade receives moderate support with bootstrap and jackknife values ≥61%. In analyses A and B, *C. gemmata* from Arnhem Land (N.T.) is sister to all remaining species, but this relationship is only resolved in the bootstrap and jackknife trees, not in the strict consensus tree (BS: 56%; JS: 50%). As stated previously, *Blackallia connata* is placed in *Cryptandra* in analysis C.

Discussion

Overall characteristics of the *trn*L-F region

The analysis of the *trn*L-F sequence data resulted in a less resolved tree, when compared with the tree generated from ITS data (Kellermann et al. 2005). This is mainly due to fewer informative base and indel characters. The *trn*L-F data-set contained 110 potentially informative characters, with the ITS region providing 270 informative characters, i.e., more than double the number in a shorter region of DNA. However, the CI and RI were higher in the *trn*L-F analyses, indicating less character conflict.

This paucity of informative characters reflects the fact that chloroplast DNA evolves slower than nuclear DNA and that "even non-coding cpDNA regions often fail to provide significant phylogenetic information at low taxonomic levels" (Small et al. 2004, p. 147). The cpDNA region *trn*L-F provides resolution mainly at the generic level in this analysis. This region neither provides much information about the relationships between genera of Pomaderreae nor resolves the clades well within genera.

Homoplasious indels

A conspicuous feature of the *trn*L-F region during alignment and analysis was the presence of two large indels in the *trn*L intron, namely a deletion between bases 380–576 in the alignment (indel no. 9; c. 125 bp) and a deletion between bases 443–487 (indel no. 11, c. 45 bp).

Indel no. 9 groups *Trymalium ledifolium*, *Pomaderris rotundifolia* and *Cryptandra triplex* in analysis A (clade at node 29). This relationship is also supported by a base change from A to G at position 660 in the alignment, which is unique to these species. Results from the analysis of ITS sequences do not support this clade, since they place *C. triplex* into *Cryptandra* and *P. rotundifolia* with its congeners into a clade of *Pomaderris* species from Western Australia. An error during lab-work or cross-contamination of samples can be ruled out, since the DNA extraction of these species and the PCR reactions were done at separate times. In addition, Richardson et al. (2000a) also reported this long indel in their sequence of *T. ledifolium*; our sequence is identical to that of Richardson and co-workers, with the exception of two deletions of a single nucleotide towards the 3' end of the sequence (base positions 1194 and 1198).

Indel no. 11 occurs in *Spyridium buxifolium*, *Siegfriedia darwinioides*, *Stenanthemum gracilipes*, *St. grandiflorum* ms, *Blackallia connata* and *B. biloba*. However, some of these species are clearly not related, and in the resulting trees of analyses A and B (Fig. 1 & 2) some are not grouped together: *Spyridium buxifolium* is related to *Sp. burragorang* and *Sp. scortechinii* (node 4), *Si. darwinioides* is most closely related to *Pomaderris* (node 12) and the four-taxon clade at node 24 is the sister group to *Stenanthemum* (node 21). In analysis C, *B. connata* is part of the *Cryptandra* clade (Fig. 3, node 24). It is obvious from these results, that indel no. 11 is a homoplasious character and cannot be relied upon for the correct delimitation of relationships. This is corroborated by the placement of these species in the ITS analysis (Kellermann et al. 2005).

The *trn*L intron, in which both long indels occur, is part of the Group I Intron family and its RNA has a conserved secondary structure (Borsch et al. 2003; Cech 1988; Quandt et al. 2004). This complex structure consists of several stem-loop regions and paired

sequence elements. Borsch et al. (2003, p. 565) have shown that in angiosperms "the P6 and P8 stem-loop regions account for most of the sequence length variation in the [*trn*L] intron" and can contain highly variable regions. Although a detailed modelling of the secondary structure of the *trn*L intron has not been attempted here, it is possible to map most of the conserved regions of Group I Introns by simple sequence comparison. Using the secondary structure of *Nymphaea odorata* Ait. (Borsch et al. 2003, Fig. 2) as a model, it can be deduced that indels no. 9 and no. 11 are indeed located within the P8 stem-loop region. Indel no. 9 nearly encompasses the whole P8 region. It is therefore most likely that these indels are the result of a loss of an arm, stem or loop from the P8 stem-loop region. Homoplasious indels in analyses of non-coding regions of cpDNA have been reported by Morton & Clegg (1993), Mes et al. (2000) and others, who concluded that homplasious indels can be associated with hairpin structures.

In a phylogeny of land plants, Quandt et al. (2004) excluded the P8 region from the analysis, since it was not alignable across the species in the data-set and was assumed to be not even homologous across all lineages. These authors recommend the exclusion of P8, and possibly P6, in studies of higher level phylogenies. This advice was followed in analysis C, where the P8 region was not included.

The homoplasious indels in Pomaderreae could have been created several times independently, for example in the particularly labile P8 region, which could easily lose a hairpin or stem-loop region. Alternatively, if these homoplasious indels originated only once, they must have been transferred to these species through the introgression of a chloroplast genome containing the indels from one species to another (Rieseberg & Brunsfeld 1992).

The first possibility seems likely in the case of indel no. 11, which appears independently in at least three lineages: *Siegfriedia darwinioides*, *Spyridium buxifolium*, the clade at node 24, and possibly *Blackallia connata*, if it is part of *Cryptandra* as suggested by analysis C and the ITS sequence analysis (Kellermann et al. 2005).

The case is equivocal for indel no. 9. The presence of additional supporting characters for clade 29, namely a base change from A to G at position 660 in the alignment, a common base change from C to A (position 1132) in *Trymalium ledifolium* and *Cryptandra triplex*, and the fact that the three species in that clade share an insertion with *T. elachophyllum* and *T. angustifolium* (indel no. 8, positions 355–362 in the alignment), suggest a possible single origin of indel no. 9. On the other hand, the sequence of *Pomaderris rotundifolia* shares a base change from C to G (position 836) with all species of *Pomaderris* and *Siegfriedia*, as well as a base change from A to C (position 806) with *P. forrestiana*, which is the sister taxon of *P. rotundifolia* in the ITS sequence analysis (Kellermann et al. 2005). The latter two base changes clearly show a relationship of *P. rotundifolia* with *Pomaderris* and not with *Trymalium* and would indicate that indel no. 9 is homoplasious. Flower morphology also supports a relationship of *P. rotundifolia* with *Pomaderris* (Walsh & Coates 1997) and of *C. triplex* with *Cryptandra* (Kellermann 2006a).

Since the region in which indels no. 9 and no. 11 occur is part of the highly variable P8 region, which is prone to homoplasious indels, the possibility of more homplasious characters cannot be ruled out, in particular in this region. As such, a phylogenetic analysis C, excluding the region, might be the best representation of the relationships in Pomaderreae, a fact that is corroborated in some degree by slightly higher CI and RI values for analysis C.

Pomaderreae

The tribe Pomaderreae is monophyletic and supported with very high bootstrap and jackknife percentages. This confirms the results of the ITS data-set (Kellermann et al. 2005) and of previous analyses, using combinations of *trn*L-F and *rbc*L sequence data (Fay et al. 2001; Richardson et al. 2000a) and *trn*L-F/ITS sequences (Richardson et al. 2001; Islam & Simmons 2006). The sister group to Pomaderreae, however, is unclear from the results of the *trn*L-F data-set. In previous analyses, either *Ceanothus* or the tribe Colletieae (Richardson et al. 2000a), or the genera *Alphitonia* and *Granitites* (Fay et al. 2001) were the closest relatives to Pomaderreae. Islam & Simmons (2006) also reported *Alphitonia* as the weakly to moderately supported sister group to Pomaderreae when analysing combined molecular and morphological data-sets. The results of Kellermann et al. (2005) indicated with weak support that Colletieae (represented by *Adolphia*) might be the sister taxon to Pomaderreae.

Spyridium

Species of *Spyridium* do not form a clade in the strict consensus trees in analyses A and B. However, they group together in 94% of most parsimonious trees in these analyses (majority rule tree not shown) and in analysis C. Within *Spyridium*, several species form small clades that are moderately to well supported. A clade of closely related species from New South Wales, *Sp. scortechinii* and relatives (node 4), and a clade of Tasmanian species (*Sp. ulicinum* and *Sp. gunnii*, node 6) were found also in the ITS analysis. This corroborates the unique position of *Sp. scortechinii* and relatives in the genus; they are the only species of *Spyridium* in New South Wales with a very long hypanthium tube (Thiele & West 2004), which was the reason these species were not included in *Spyridium* for a long time. This feature also occurs in *Sp. waterhousei* (S.A.), a species that was recently transferred back into *Spyridium* (Kellermann 2007). *Spyridium mucronatum* and *Sp. cordatum* from Western Australia form a well supported clade (node 7), but the position of the third western species, *Sp. globulosum*, was unresolved. The ITS data-set groups *Sp. mucronatum* and *Sp. globulosum* species into one clade (*Sp. cordatum* was not included in the ITS analysis). The remaining species from south-eastern Australia do not group together, as they do in the ITS results (Kellermann et al. 2005), but form a polytomy. The chloroplast data-set also confirms the close relationship of *Sp. daltonii* and *Sp.* ×*ramosissimum* (node 8) and corroborates previous findings that these species were misplaced in *Trymalium* (Kellermann 2006b).

The poor result for *Spyridium* is caused by both a conflict of characters and lack of informative characters for the group. There is only one synapomorphy for the genus as a whole, a base change from A to G (position 869). In analysis A and B this phylogenetic signal is in conflict with other base changes that occur in the region of indel no. 9. Once this DNA region is excluded (analysis C), species of *Spyridium* group in one clade. Overall, the results for *Spyridium* are not in conflict with the ITS analysis

Pomaderris and *Siegfriedia*

Pomaderris is divided into two geographically separated clades that form a trichotomy (node 12) with *Siegfriedia darwinioides*. This highlights the close relationship of *Siegfriedia* and *Pomaderris*, which is corroborated by ITS data (Kellermann et al. 2003) and morphology: both genera have a basal valve in each fruitlet of their schizocarpic fruits (Rye 1996b).

The two *Pomaderris* clades contain species from Western Australia (node 14) and south-eastern Australia (including New Zealand; node 13), respectively. The clade at node 14 is not supported, only the relationship between *P. forrestiana* and *P. brevifolia* is highly supported. All Western Australian species share a deletion of five bases (indel no. 5, bp 161–164 in the alignment). They have been perceived as distinct from the remaining species of *Pomaderris* before (N.G. Walsh, pers. comm.), because of their umbellate (or contracted) inflorescences and flowers with a very conspicuous annular disc (Rye 1996b). Most south-eastern Australian species (node 13) have a disc that is inconspicuous or absent. *Pomaderris obcordata* from mallee scrubland in South Australia and Victoria is part of this clade, and not, as in the ITS analysis, at the base of *Pomaderris*. It is, however, anomalous in the genus, because of the absence of a clearly defined valve in the fruitlets. A few other species from *Pomaderris* sect. *Apetalae* N.G.Walsh, sect. *Flabellares* N.G.Walsh and sect. *Psilogyne* N.G.Walsh may not have a clearly defined valve as well (Walsh & Coates 1997). The south-eastern Australian clade receives high support, but relationships within it are not resolved.

Trymalium

The sister group to the *Siegfriedia-Pomaderris* clade is a clade that contains species of *Trymalium* from Western Australia and South Australia (node 16). This relationship, however, does not receive statistical support ≥50%; its synapomorphy is one unique base change at position 878 in the alignment (C to G). When *Pomaderris rotundifolia* and *Cryptandra triplex* are included (analysis A) they form a clade with *Trymalium ledifolium* (Fig. 1, node 29), as discussed above. Morphologically, *P. rotundifolia* displays characters typical for *Pomaderris*, such as the basal valve in each fruitlet, a deeply divided style, and a densely hairy ovary summit (Rye 1996b). It differs from other species of *Pomaderris* in its unique, compact, head-like inflorescences and flowers with more strongly hooded petals. Morphologically, *C. triplex* seems to be very similar to two species of *Cryptandra* from northern Australia (Kellermann 2006a): *C. filiformis* A.R.Bean (not included in this analysis) and *C. intratropica* W.Fitzg. The three species share dense indumentum on all parts of the plant. *Cryptandra intratropica* and *C. triplex* were included in the ITS analysis and are nested deep within *Cryptandra* in a clade of northern species (node 52 in Kellermann et al. 2005, Fig. 1). The presence of stipules that are fused around the base of the petiole and a ring of bracts at the base of the flower are important morphological characters (Thiele & West 2004) that indicate a relationship of *C. triplex* with *Cryptandra*. The cpDNA characters that place these two species into *Trymalium* seem to be homoplasious indels and base changes.

Polianthion

The clade at node 17 unites four species that were labelled as the 'Bilocular Clade' by Kellermann et al. (2005) and have since been described as the new genus *Polianthion* (Kellermann et al. 2006). It is well supported and consists of two species pairs: *Pol. minutiflorum* groups with *Pol. collinum* (node 18; JS/BS: 98%), and *Pol. bilocularis* with *Pol. wichurae* (node 19; JS/BS: ≥72%). The same relationships were reported by Kellermann et al. (2005), and are corroborated with this cpDNA data-set. The four species share a biloculate ovary and a conspicuous dense indumentum on all surfaces of the plant. The clade is here sister to *Pomaderris*, *Siegfriedia* and *Trymalium*. All

species in these genera share a 1 bp deletion (indel no. 20, position 1088), but the relationship does not receive bootstrap or jackknife support above 50%. Kellermann et al. (2005) report an association of *Polianthion* with *Cryptandra*, *Blackallia biloba* and *Stenanthemum grandiflorum* ms, but this too did not receive statistical support.

Stenanthemum

Three species currently included in *Stenanthemum*, namely *St. gracilipes*, *St. grandiflorum* ms and *St. intropubens* Rye ms (the last species not included in this analysis) are in conflict with the definition of the genus (Rye 1995, 2001; Thiele 2007), since they do not share typical morphological characters, such as a disc that is lining the hypanthium tube. Two strongly supported clades containing species of *Stenanthemum* s. str. (i.e., according to Rye and Thiele) from south-eastern and central Australia (SE-central clade, node 23) and Western Australia and Queensland (WA-Qld clade, node 22) are sister-taxa in the *trn*L-F analysis (node 21; BS/JS: ≥89%). This confirms the monophyly of *Stenanthemum* s. str. These two clades were also found in the ITS results. The atypical species included in the analysis, *St. grandiflorum* ms and *St. gracilipes*, appear in the clade at node 24 (see below). This is in contrast to the results of Kellermann et al. (2005), which placed *St. gracilipes* as sister taxon to the WA-Qld clade with low support (node 35 in Kellermann et al. 2005: BS: 53%, JS: 57%).

Species associated with *Stenanthemum*

The clade at node 24 that is shown as sister to *Stenanthemum*, contains four species, which are very different in appearance and habit: *St. gracilipes*, *Blackallia connata*, *St. grandiflorum* ms and *B. biloba*.

This clade contradicts the results from the ITS data (Kellermann et al. 2005), in which *Blackallia connata* is placed into *Cryptandra* and *St. gracilipes* into *Stenanthemum*. However, in analysis C, *B. connata* falls within a well supported *Cryptandra*, thus agreeing with the ITS results. The homoplasious nature of some indels in the P8 stem-loop region of the *trn*L intron has been discussed above. All characters that unite *B. connata* with these other three species are located within the P8 region, and this grouping might therefore be an artefact of an incorrect phylogenetic signal caused by homoplasious indels (see also Quandt et al. 2004). Once the P8 region is excluded from the analysis, the results also agree with morphology. *Blackallia connata* has single, sessile flowers that are surrounded by rows of bracts and contain a pubescent disc that surrounds the ovary; the stipules are connate below the attachment point of the petiole. These and other features of the species are typical for *Cryptandra* (Thiele & West 2004, Thiele 2007).

The three remaining species in the clade have glabrous discs surrounding the ovary, and a simple cymose inflorescence; stipules are free from one another or connate between petiole and stem. In *Stenanthemum gracilipes* and *Blackallia biloba* the flowers have long pedicels. These characters are at odds with the placement of *St. gracilipes* in the *Stenanthemum* clade in Kellermann et al. (2005), since putative synapomorphies for *Stenanthemum* s. str. are a disc that is adnate to the hypanthium tube and sometimes confluent with the filament bases, and dense cymose heads with sessile flowers (Rye 1995, 2001; Thiele 2007).

Stenanthemum grandiflorum ms is resolved as the sister taxon to *Blackallia biloba* in both the *trn*L-F and ITS analyses. An unnamed species, *St. intropubens* ms (not included in the analysis), is similar to *St. grandiflorum*. Although *B. biloba* shares long-pedicellate flowers with *St. gracilipes*, its flower morphology and habit are distinct from *St. gracilipes*, *St. grandiflorum* ms and *St. intropubens* ms.

Stenanthemum gracilipes has long been seen as a unique species with no apparent close relatives and these results strongly suggest the recognition of a monotypic genus for this species. A new genus containing *St. grandiflorum* ms and *St. intropubens* ms is also supported by our results and was already recommended by Kellermann et al. (2005) as 'New genus A'. The description of these new genera will be published in the near future, in addition to a new circumscription of *Blackallia* (Kellermann et al., in press).

Cryptandra

All remaining species of *Cryptandra* fall into one clade. However, there is no resolution within that clade, with the exception of *C. gemmata*, which is sister to the remaining species in the jackknife and bootstrap trees, but not in the strict consensus tree. Synapomorphies for the genus *Cryptandra* were mentioned above and comprise stipules that are connate at the base of the petiole, single flowers, surrounded by row(s) of bracts, a pubescent annular disc around the base of the ovary and schizocarpic fruits that release dehiscent fruitlets (Thiele & West 2004, Thiele 2007). *Cryptandra gemmata* is unique in the genus since it is apparently the only species with truly terminal inflorescences (Bean 2004); it is also one of very few species of *Cryptandra* to occur in the tropical north of Australia (Kellermann 2006a). Some of these tropical species fall into one clade in the analysis of Kellermann et al. (2005, clade at node 52).

Conclusions

The analysis of DNA sequence data from the chloroplast *trn*L-F region confirmed most findings of the ITS data-set (Kellermann et al. 2005). However, it was hampered by the lack of informative characters and the presence of apparently homoplasious indel characters and base changes within the P8 region of the *trn*L intron. As such, the strict consensus tree of the *trn*L-F analysis was less resolved and had fewer supported clades than in the ITS analysis.

The genera *Cryptandra*, *Stenanthemum* and *Polianthion* were well supported. Species of *Trymalium* from Western Australia and South Australia formed one clade, but when two taxa with a large homoplasious indel (no. 9), *P. rotundifolia* and *C. triplex*, were included in the analysis they appeared in the *Trymalium* clade. This was also in contrast to the ITS results. *Pomaderris* was divided into a Western Australian clade and a clade containing south-eastern Australian species; these two clades formed an unresolved trichotomy with the monotypic *Siegfriedia*. *Stenanthemum grandiflorum* ms and *Blackallia biloba* are confirmed as closely related species. Their sister taxon is *B. connata*, with *St. gracilipes* next in the phylogenetic sequence, when the P8 region is included in the analysis. When the region is excluded, *B. connata* changes its position and moves into the *Cryptandra* clade, a result that is supported both by morphology and ITS sequence data. All species of *Spyridium* do not group in a clade, but are resolved in a polytomy at the base of Pomaderreae with the clades described above. If the P8 region is excluded, they form a clade, albeit without statistical support.

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Appendix 1. Sources of plant material used for sequencing of *trn*L-F DNA regions. All samples were collected in Australia, except where indicated. GenBank accessions in bold indicate sequences obtained for this project. ANBG: Australian National Botanic Gardens, Canberra; cult.: plant collected from cultivation; NSW: New South Wales; N.P.: National Park; NT: Northern Territory; Qld: Queensland; RBG: Royal Botanic Gardens, Melbourne; SA: South Australia; Tas.: Tasmania; Vic.: Victoria; WA: Western Australia. Standard herbarium abbreviations according to Holmgren et al. (1990).

Species	Locality	Voucher/citation	Material type /date of collection	GenBank accession No.
OUTGROUP				
Adolphia infesta Meisn.	USA: California	Aagesen et al. (2005)		AY460408
				AY642142
Alphitonia aff. *incana* (Roxb.) Kurz	Australia	Richardson et al. (2000a) (as *Alphitonia excelsa* Reissek)		AJ390352
Ceanothus coeruleus Lag.	USA: California	Thulin et al. (1998)		AJ225798
Phylica buxifolia L.	South Africa	Richardson et al. (2001)		AF327614
Schistocarpaea johnsonii F.Muell.	Australia	Richardson et al. (2000a)		AJ390349
INGROUP				
Blackallia biloba C.A.Gardner	WA: Northampton	*J. Kellermann 257* (MEL)	fresh; 2001	**EF528505**
B. connata (C.A.Gardner) C.A.Gardner	WA: Laverton Downs Station	*H. Pringle 2494* (CANB)	herb.; 1989	**EF528503**
Cryptandra alpina Hook.f.	ANBG B8903607 Sect. 231	*R. Burns 98* (CBG at CANB)	silica; 2003	**EF528488**
C. amara Sm. **1**	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528489**
C. amara Sm. **2**	NSW: Nimmitabel	Rosetto et al. (2001)		AF300321
C. arbutiflora var. *borealis* Rye	WA: N of Geraldton	*J. Kellermann 224* (MEL)	fresh; 2001	**EF528491**
C. dielsii C.A.Gardner ms	WA: Trayning-Kellerberrin Rd	*J. Kellermann 292* (MEL)	fresh; 2001	**EF528500**
C. ericioides Sm.	NSW: Saltwater Creek	*D.E. Albrecht* 3989 (MEL)	herb.; 1990	**EF528487**
C. hispidula Reissek & F.Muell. ex Reissek	SA: Kangaroo Island	*G. Jackson 3187* (MEL)	herb.; 1994	**EF528492**
C. intratropica W.Fitzg.	WA: King Edward River	*L.A. Craven & C.L. Brubaker 9163* (CANB)	herb; 1983	**EF528495**
C. lanosiflora F.Muell.	ANBG 617457 Sect. 99F	*S. Fethers 8* et al. (CANB)	silica; 2003	**EF528490**
C. micrantha Rye ms	WA: Wongan Hills	*J. Kellermann 287* (MEL)	fresh; 2001	**EF528493**
C. myriantha Diels	WA	Richardson et al. (2000a) (as *Cryptandra* cf. *spyridioides* F.Muell.)		AJ390360

C. mutila Nees ex Reissek **1**	WA: Singleton	*B. Keighery s.n.* (PERTH)	herb.; 1999	**EF528499**	
C. mutila Nees ex Reissek **2**	WA	*M. Hislop 1068* (PERTH)	herb.; 1998	**EF528498**	
C. nola Rye	WA: Pinjar	*B.L. Rye 239044* (PERTH)	herb.; 2003	**EF528496**	
C. pungens Steud.	WA: Ravensthorpe	*J. Kellermann 375* (MEL)	fresh; 2001	**EF528497**	
C. triplex K.R.Thiele ex Kellermann	NT: Jabiru East	*L.A. Craven 6546* (MEL)	herb.; 1981	**EF528549**	
C. gemmata A. R. Bean	NT: SE of Oenpelli	*C. Dunlop 4919* (MEL)	herb.; 1978	**EF528494**	
Polianthion bilocularis (A.S.George) Kellermann	WA: Dongolocking	*S. Patrick 394* (PERTH)	herb.; 1986	**EF528502**	
Polianthion collinum Rye	WA: Yalgoo	*A. Chant 9* (PERTH)	herb.; 2000	**EF528511**	
Pol. minutiflorum (E.M.Ross) K.R.Thiele	Qld: Coominglah State Forest	*A.R. Bean 9107 & G. Turpin* (CANB)	herb.; 1995	**EF528510**	
Pol. wichurae (Nees ex Reissek) K.R.Thiele	WA: Hi Vallee Farm, Badgingarra	*J. Kellermann 183* (MEL)	fresh; 2001	**EF528501**	
Pomaderris angustifolia N.A.Wakef.	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528518**	
P. brevifolia N.G.Walsh	WA: Ravensthorpe	*J. Kellermann 388* (MEL)	silica; 2001	**EF528513**	
P. elliptica Labill.	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528519**	
P. forrestiana F.Muell.	WA: Mt Jimberiana	*B. Archer 2271* (MEL)	herb.; 2002	**EF528514**	
P. grandis F.Muell.	WA: Mount Manypeaks	*N.G. Walsh 2776* (MEL)	herb.; 1989	**EF528512**	
P. obcordata Fenzl	SA: Eyre Peninsula	*N.G. Walsh 3999* (MEL)	herb.; 1995	**EF528516**	
P. oraria F.Muell. ex Reissek subsp. *oraria*	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528515**	
P. prunifolia subsp. *edgerleyi* (Hook.f.) L.Moore	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528521**	
P. phylicifolia var. *ericioides* Maiden & Betche	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528520**	
P. rotundifolia (F.Muell.) Rye	WA: Ravensthorpe-Esperance area	*J. Kellermann 379* (MEL)	fresh; 2001	**EF528550**	
P. rugosa Cheeseman	New Zealand	Richardson et al. (2000a)		AJ390363	
P. tropica N.A.Wakef.	Qld: Walsh's Pyramid	*I.R. Telford 12045* (CBG at CANB)	herb.; 1994	**EF528517**	
Siegfriedia darwinioides C.A.Gardner **1**	N.G.Walsh (cult.)	*N.G. Walsh s.n.* (MEL)	fresh; 2000	**EF528507**	
Si. darwinioides C.A.Gardner **2**	WA	Richardson et al. (2000a)		AJ390375	
Spyridium burragorang K.R.Thiele	ANBG c606176 Sect. 31	*S. Donaldson 903* (CBG at CANB)	silica; 2001	**EF528536**	
Sp. buxifolium (Fenzl) K.R.Thiele	NSW: E of 'Boonara'	*J.R. Hosking 1848* (MEL)	herb.; 2000	**EF528508**	
Sp. cordatum (Turcz.) Benth.	WA: Ravensthorpe	*J. Kellermann 370* (MEL)	fresh; 2001	**EF528530**	
Sp. daltonii (F.Muell.) Kellermann	Vic.: Grampians	*J. Read s.n.* (MEL)	fresh; 2000	**EF528534**	
Sp. eriocephalum Fenzl var. *eriocephalum*	ANBG 9106516 Sect. 100	*A.M. Lyne 675* (CBG at CANB)	silica; 2003	**EF528522**	
Sp. globulosum (Labill.) Benth. **1**	WA: Dempster Head	*B. Archer 2255* (MEL)	silica; 2002	**EF528529**	
Sp. globulosum (Labill.) Benth. **2**	WA	Richardson et al. (2000a)		AJ390358	

Sp. gunii (Hook.f.) Benth.	ANBG 9902964 Sect. 99	*R. Burns 74* (CBG at CANB)	silica; 2003	**EF528524**
Sp. halmaturinum (F.Muell.) F.Muell.	ANBG 631019 Nursery	*J. Nightingale 143* (CANB)	silica; 2003	**EF528527**
Sp. mucronatum Rye	WA: Lake King	*J. Kellermann 367* (MEL)	silica; 2001	**EF528528**
Sp. nitidum N.A.Wakef.	SA: Kangaroo Island	*I. Jackson 3253* (MEL)	herb.; 1997	**EF528531**
Sp. parvifolium (Hook.) F.Muell. **1**	Melbourne University (cult.)	*J. Kellermann 112* (MEL)	fresh; 2001	**EF528526**
Sp. parvifolium (Hook.) F.Muell. **2**	Australia	Rossetto et al. (2001)		AF300322
Sp. ×ramosissimum (Audas) Kellermann	Vic.: Grampians	*J. Kellermann 122* (MEL)	silica; 2001	**EF528535**
Sp. scortechinii (F.Muell.) K.R.Thiele	Melbourne University (cult.)	*J. Kellermann 409* (MEL)	fresh; 2002	**EF528537**
Sp. subochreatum (F.Muell.) Reissek	ANBG 636419c Nursery	*J. McAuliffe 250* (CANB)	silica; 2003	**EF528532**
Sp. thymifolium Reissek	ANBG 631015 Nursery	*J. Nightingale 139* (CANB)	silica; 2003	**EF528533**
Sp. ulicinum (Hook.) Benth.	Tas.: Fehlbergs Road	*A.M. Buchanan 15952* (MEL)	fresh; 2002	**EF528523**
Sp. waterhousei F.Muell.	ANBG 9700078	*I. Jackson 13* (CBG at CANB)	silica; 2003	**EF528538**
Sp. sp. 1 *sensu* N.G.Walsh *in* Fl. Victoria	RBG Melbourne	*J. Kellermann 113* (MEL)	fresh; 2000	**EF528525**
Stenanthemum argenteum A.R.Bean	Qld: Mt Mulligan	*J.R. Clarkson 8895* (CANB)	herb.; 1990	**EF528542**
St. centrale K.R.Thiele	NT: Palm Valley	*D.V. Matthews s.n.* (MEL)	silica; 2002	**EF528544**
St. complicatum (F.Muell) Rye	WA: Kalbarri N.P.	*J. Kellermann 239* (MEL)	fresh; 2001	**EF528539**
St. gracilipes Diels	WA: Northampton	*J. Kellermann 262* (MEL)	fresh; 2001	**EF528506**
St. grandiflorum (C.A.Gardner) Rye ms	WA: Peterwangi Hill	*J. Kellermann 274* (MEL)	fresh; 2001	**EF528504**
St. humile Benth.	WA: Hi Vallee Farm, Badgingarra	*J. Kellermann 194* (MEL)	silica; 2001	**EF528540**
St. leucophractum (Schldl.) Reissek	Vic.: Little Desert	*J. Kellermann 136* (MEL)	silica; 2001	**EF528545**
St. petraeum Rye	NT: Watarrka N. P.	*T.L. Collins s.n.* (MEL)	fresh; 2002	**EF528541**
St. pomaderroides (Reissek) Reissek	WA	Richardson et al. (2000a) (as *Spyridium* cf. *forrestianum* F.Muell.)		AJ251690
St. reissekii Rye	WA: Hi Vallee Farm, Badgingarra	*J. Kellermann 197* (MEL)	silica; 2001	**EF528543**
Trymalium angustifolium Reissek	WA: Talbot State Forest, York	*J. Kellermann 302* (MEL)	fresh; 2001	**EF528548**
T. elachophyllum Rye	WA: Hopetoun	*J. Kellermann 384* (MEL)	fresh; 2001	**EF528547**
T. floribundum Steud.	WA	Richardson et al. (2000a)		AJ390362
T. ledifolium var. *rosmarinifolium* (Steud.) Benth **1**	WA: Flynn State Forest, York	*J. Kellermann 294* (MEL)	fresh; 2001	**EF528551**
T. ledifolium Fenzl **2**	WA	Richardson et al. (2000a)		AJ390361
T. monospermum Rye	WA: Narrogin	*L.W. Sage 1540* (MEL)	herb.; 1999	**EF528546**
T. wayi F.Muell. & Tate	SA: E of Crystal Brook	*D.N. Kraehenbuehl 5197* (CBG at CANB)	herb.; 1989	**EF528509**