SYSTEMATICS OF THE AUSTRALIAN POLYGALACEAE AND XANTHOPHYLLACEAE

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I, Anna Magdalena Monro, certify that this thesis is my own original work, except where otherwise specified in the text, and that it has not been submitted in a previous application for a higher degree.

A.M.Monro

15 September 2003

Comesperma volubile Labill.,

illustrated in *Paxton's Magazine of Botany* 5: 145 (1838), as *C. gracilis*

> "We have been simply seeking to show which tendencies are manifested in a natural group, and to research the causes or at least the links between them. That is to say that the work is very limited and although we have examined all the organs from quite different points of view, we do not flatter ourselves that we have elucidated all the questions which are attached. The 'why' generally escapes our investigations and the causes that influence the formation of groups are so many and often hidden that the researcher hardly raises a corner of the veil."

Chodat & Hochreutiner (1893), Contribution a l'étude du genre *Comesperma* (p. 358)

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Abstract

The Polygalaceae is a large, sub-cosmopolitan flowering plant family. It is represented in Australia by five genera—*Comesperma*, *Muraltia*, *Polygala*, *Salomonia* and *Xanthophyllum*—containing 51 species. Phylogenetic analyses of the family in Australia are presented, using data from morphological characters and two DNA markers. The first molecular dataset contains sequences from the internal transcribed spacer (ITS) regions of nuclear DNA, while the second is taken from the chloroplast *trnL*/*F* region.

All datasets and analysis methods indicate that *Comesperma*, the only endemic Australian genus, is likely to be monophyletic. Furthermore, the molecular data indicate that it is not congeneric with the predominantly South American *Bredemeyera* as previously suggested based on morphological similarity. *Bredemeyera papuana* from New Guinea remains problematic, although the *trnL*/*F* data suggest that it is more closely related to *Comesperma* than to the South American taxa. The utility of the existing sub-generic classification for *Comesperma* is examined in light of the results from the phylogenetic analyses and is found to be largely congruent with the patterns obtained.

Other findings include the likely sister relationship of both *Salomonia* and *Epirixanthes*, rather than their being a single genus as previously suggested. The predominantly Australian species from *Polygala* series *Chloropterae* appear to form a monophyletic group, which confirms their divergent morphology. *Xanthophyllum* is confirmed as the sister group to the remainder of the family and it can thus be considered either a tribe with the Polygalaceae or as the monogeneric family Xanthophyllaceae with equal justification.

The Polygalaceae in Australia have not been revised as a whole since 1863, so a species-level treatment is also provided. Each of 49 taxa from *Comesperma*, *Muraltia*, *Polygala* and *Salomonia* is briefly described, the names used in Australian literature accounted for and maps of the taxon distributions supplied.

Systematics of the Australian Polygalaceae and Xanthophyllaceae

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Introduction and Aims

INTRODUCTION

Polygalaceae

The Polygalaceae Hoffmanns. & Link is a large, sub-cosmopolitan flowering plant family. Some members of this "milkwort" family were well known to medieval herbalists and they believed that consumption of the plants would stimulate milk production, both in livestock and humans (Coombes 1994). This belief is reflected in the family's name, which comes from the Greek *polys*, much or many, and *galos*, milk. Although there is no scientific evidence to indicate that compounds in the Polygalaceae stimulate lactation and the family is not usually considered economically important, many members of the family are known to secrete essential oils and saponins. *Polygala senega* L. (Snakeroot) is traditionally used by some Native American tribes as a treatment for snakebite (Kindscher 1992) and saponins extracted from the roots are today included in bronchitis medications as an expectorant (Grieve 1967). Many species, including those in *Comesperma* Labill., an Australian genus, are known to store methyl salicylate ("oil of wintergreen") in their roots (e.g., Jayasekara *et al.* 2002).

Polygalaceae in the broad sense (including Xanthophyllaceae (Chodat) Gagnep. ex Reveal & Hoogland) currently contains about 1000 species in 17 to 23 genera, depending on circumscription. The bulk of the diversity in the family is contained within the genus *Polygala* L., with about 500 to 750 species, that are distributed mainly in tropical regions but with a few species reaching into temperate areas. With its distributional limits largely being defined by those of *Polygala*, the family as a whole is almost cosmopolitan, being naturally absent only from the polar areas and the Pacific. However, some species have secondarily colonised New Zealand and Pacific islands as introduced weeds.

The pseudo-papilionaceous flowers found in many members of the Polygalaceae have long been considered an indication of convergent evolution between this family and the pea-flowered legumes of Fabaceae subfamily Faboideae. Both groups are primarily pollinated by insects, particularly bees, and are believed to have developed keel flowers (as defined by Westerkamp 1997) as part of a secondary pollination "syndrome".

Secondary pollination is a term used when a floral structure other than the anthers is used to deposit pollen on a potential pollinator. Both pea-flowered legumes and pseudo-papilionaceous members of the Polygalaceae use the style to "present" pollen and in both the asymmetry of the flower results in pollen being deposited on relatively inaccessible parts of a pollinator's body (Yeo 1993). This presumably increases the likelihood of it being carried to other flowers. The functional similarities of keel flowers in both groups have been reviewed in detail by Westerkamp and Weber (1999). The selective pressures on floral morphology in both the Faboideae and the Polygalaceae are assumed to be similar, and, until recently, it was thought that the similarities in their flowers were thus a result of convergence. Strictly speaking, this is correct as the similarities are not anatomically homologous—the enlarged and often petaloid "wings" in a Polygalaceae keel flower are lateral sepals rather than petals and the polygalaceous "keel" is a single folded petal rather than two fused petals as in the papilionaceous flower. However, with molecular evidence showing that the Fabaceae and Polygalaceae are closely related, this is an interesting example of convergence occurring in groups that also share a recent evolutionary history.

According to these recent molecular phylogenies, Polygalaceae forms part of a strongly-supported order Fabales, but the relationship between it and the other three families—Fabaceae, Quillajaceae, Surianaceae—is still somewhat unclear. Several initial studies indicated a sister group relationship between the Polygalaceae and Fabaceae (Chase *et al.* 1993; Fernando *et al.* 1993; Morgan & Soltis 1993; Morgan *et al.* 1994). However, as further sequence data have become available, almost every possible permutation of the four families has been suggested (e.g., Doyle *et al.* 1997; Soltis *et al.* 2000; Savolainen *et al.* 2000). Evidence from two chloroplast regions (*rbcL* and the *trnL* intron; Forest *et al.*, unpublished) indicates that the Surianaceae is sister to the Fabaceae, with Polygalaceae most closely related to these two and with the Quillajaceae then sister to the remainder of the order. However, the support for these relationships was relatively weak. This is also a different pattern from that found by Persson (2001) in his analysis of *trnL/F* sequences, which indicated that Quillajaceae was the sister group to the legumes, with Surianaceae most closely related to these two families and Polygalaceae thus sister to the remainder of the order. Again, the support for these interfamilial relationships was weak. It should be noted that the study by Forest *et al.* was aimed at identifying the sister group to the legumes, while Persson was focusing on the relationships within the Polygalaceae, so their sampling breadths were not equivalent. Nonetheless, it is clear that further data will be required to resolve the question of relationships within the Fabales.

Brief Taxonomic History of Polygalaceae

Linnaeus (1753) placed plants that are now classified as belonging to the Polygalaceae into the class Diadelphia and orders Octandria and Decandria, containing plants with eight or ten partially-fused stamens in two groups. Within this grouping, he described only two genera—*Polygala* L., with 22 species from Europe, Africa, Asia and America, and *Securidaca* L. with one species from America. By the time of Robert Chodat's (1891–1893) *Monographia Polygalacearum* and his treatment for Engler and Prantl's *Die Natürlichen Planzenfamilien* (Chodat 1896), three tribes and ten genera were recognised. *Polygala* was already estimated to contain 400–450 species, with around 230 species across the other genera. These rather massive and very detailed monographs by Chodat remain extremely influential in the current classification of Polygalaceae at all taxonomic levels. For example, the only significant change to the tribal system outlined by Chodat is the addition of the Carpolobieae by Eriksen (1993b), as a result of her morphological cladistic analysis of the family indicating that *Carpolobia* G.Don and *Atroxima* Stapf formed a strongly supported sister group to the Polygaleae. Nonetheless, there have also been indications that Chodat's system is imperfect—the monophyly of both the tribes Polygaleae and Moutabeae has been questioned by various authors on the basis of incongruence between vegetative and reproductive characters (e.g., Styer 1977; Verkerke 1984, 1985; Meijden 1988). Relatively new evidence and analyses (Eriksen 1993b; Persson 2001; Forest *et al.*, unpublished) are enabling testing of many of the tenets of traditional classification in the family.

Polygalaceae in Australia

As circumscribed by Cronquist (1981), the Polygalaceae in Australia is represented by five genera—*Comesperma* Labill., *Polygala* L., *Salomonia* Lour., *Muraltia* DC., and *Emblingia* F.Muell. Cronquist placed *Xanthophyllum* Roxb. into a monogeneric family, a separation which was deemed "a pity" and based on "suspect" differences by Ruud van der Meijden (1982) in his comprehensive revision of the genus. Given that only two species of *Xanthophyllum* occur in Australia and the similarities between them and other members of the Polygalaceae, all six genera were included in this study as Polygalaceae *sens. lat.*

The bulk of the nearly 50 Polygalaceae species in Australia are contained within *Comesperma* and *Polygala*, with the remaining four genera being represented by only five species. There is a general north-south distributional pattern in the two large genera in Australia, with *Polygala* distributed primarily across the north (Figure 1.1) while *Comesperma* is more prevalent in the south (Figure 1.2). Although dramatically characterised by Chodat and Hochreutiner (1893) as "the genus *Polygala* [coming] to die on this shore", this lack of species diversity is probably simply a reflection of the generally tropical distribution of *Polygala* itself, with northern Australia being the southern extreme of the range of many south-east Asian species. Only a few native *Polygala* species occur in sub-tropical Australia, with the other southern taxa being introduced weeds. Within *Comesperma* there are two areas of high species diversity, centred on south-west Western Australia and along the east coast, with only a few species inhabiting the interior or tropical regions.

Joseph Banks collected specimens of the Polygalaceae in eastern Australia in 1770 during Cook's voyage (Banks 1900–1905), although names for the new species were only published by other botanists in the 1800s. However, Robert Brown (1810) did not mention the family in his first account of the Australian flora. Meanwhile, Jacques Julien Houtton de Labillardière (1806) had erected a new Australian genus in the family, *Comesperma*, and described five species based on his own collections from Western Australia and Tasmania. He felt that the form of the capsule and the hairs on the seed coat differed strongly from those in other genera in the family and therefore these new taxa warranted generic rank. In a later work on the flora examined during Matthew Flinders' voyage to Australia, Brown (1814) estimated that 30 species in the Polygalaceae from *Comesperma*, *Polygala* and *Salomonia* were present in Australia. Two of Banks' original collections were then placed into *Comesperma* by A.P. de Candolle in his *Prodromus* (1824), where he listed a total of nine species—five from Labillardiere's original publication, two collected by Banks and two other species collected from Western Australia by Lechenault.

The next major work on *Comesperma* was Ernst Gottlieb von Steudel's (1845) treatment contained in Lehmann's *Plantae Preissianae*. Steudel listed seventeen species

FIGURE 1.1: Distribution map of the genus *Polygala* in Australia, based on herbarium specimen records for those taxa considered endemic or naturalised

FIGURE 1.2: Distribution map of the endemic genus *Comesperma* in Australia, based on herbarium specimen records

from the Western Australian Preiss collections, which included only one name that had been previously published, *C. volubile* Labill. (ironically misapplied to another published species). After some criticism of Steudel's treatments of this and other families, a revision of the entire genus *Comesperma* by Joachim Steetz (1847, 1848) was written just a few years later for publication in the same work. Although this placed many of Steudel's names into synonymy, it also increased the number of species in the genus to twenty-four.

The last revision of the Polygalaceae as a whole in Australia is by George Bentham (1863) and lists only three genera—*Comesperma*, *Polygala* and *Salomonia*. He recognised twenty-one species in *Comesperma*, sinking yet more of Steudel's Western Australian names, but accepting new names published by others (including Mueller and Lindley) in the interim. The treatment also listed only seven species of *Polygala*. Four endemic species are described as new, with the remaining three previously published and extending throughout Asia. The single species listed in *Salomonia* was *S. oblongifolia* DC.—now *S. ciliata* (L.) DC.—which is also widespread through southeast Asia.

As previously mentioned, Chodat's (1896) treatment of the family for *Die Natürlichen Planzenfamilien* has been very influential in Polygalaceae systematics. In this work, Chodat erected a series *Chloropterae* within his *Polygala* section (*Ortho*)*Polygala* for those species with acute, greenish lateral sepals in contrast to the more usual obtuse and petaloid form found in other series within section *Polygala*. All the endemic Australian species belong within sect. *Chloropterae*, along with two species which extend into Malesia and south-east Asia.

Since Bentham's revision of the Polygalaceae, it has received only minor taxonomic attention at family level in Australia. Flora treatments exist for several of the States and Territories (e.g., Stove 1986; Murray 1993; Walsh 1999), there is a thorough revision of *Comesperma* for Queensland (Pedley 1984) and Keighery (2002) has recently published some long-held manuscript names. However, detailed descriptions of many of the species have not yet been written.

Comesperma versus *Bredemeyera*

Willdenow (1801) erected a new genus *Bredemeyera*, based on one species—*B. floribunda* Willd.—from Venezuela. The characters used to diagnose *Bredemeyera*, which Willdenow considered to be most closely allied to *Polygala* and the largely tropical genus *Securidaca* L., included a three-lobed calyx, two-lobed "standard" petal and an unusual bilocular fruit. Although not mentioned in the original description, one of the defining characters for the genus is usually considered to be the long tufts of hair that spring from the hilum of the seed. This is in contrast to the Australian *Comesperma*, which also generally have long hairs on the seed but these emerge from all over the testa. However, a handful of species—*C. aphyllum*, *C. sphaerocarpum* and *C. scoparium*—do not possess this coma. The original description of *C. spinosum* by Mueller (1859) also indicated that this species lacked a coma. This observation was then repeated by Bentham (1863) and is included in more recent treatments (e.g., Grieve 1998), but examination of fruiting specimens shows it to be incorrect.

The similarity in seed characters between *Comesperma* and *Bredemeyera* have led some workers (Steenis 1968) to hypothesise that the two genera are closely related and many overseas treatments have placed *Comesperma* within *Bredemeyera*. Their similarity was first indirectly noted by Saint-Hilaire (1829) in a review of Brazilian species which contained several new names in *Comesperma* and did not mention *Bredemeyera* at all. Later authors (e.g., Thompson 1978) have interpreted this as Saint-Hilaire synonymising the two genera, but this is not conclusively indicated by the original publication. Also, as *Bredemeyera* was published first, the name has priority over *Comesperma* and must be used if the two genera were to be merged. Chodat (1896) provided a detailed treatment of the family, which reduced *Comesperma* to one of three sections of *Bredemeyera*. He further divided Section *Comesperma* into three subsections, one with four series. However, he did not formally make the new combinations required to transfer the Australian species names to *Bredemeyera*.

Steenis (1968), in a paper resulting from work towards a treatment of the Polygalaceae for the *Flora Malesiana*, proposed four sections within *Bredemeyera*. These were:

- Section *Melchiora*—consisting of one newly-described species, *Bredemeyera papuana* from New Guinea;

- Section *Hualania* (previously the genus *Hualania*)—containing spiny, leafless or few-leaved species from Argentina and Chile;
- Section *Bredemeyera*—the "true" *Bredemeyera* from the Caribbean and tropical South America, and;
- Section *Comesperma*—containing all the species from Australia.

In contrast to Chodat's (1896) treatment, no subdivisions were made within Section *Comesperma*. This publication also made the formal combinations required to transfer the Australian species into *Bredemeyera*. Australian workers by contrast seem to favour the retention of *Comesperma* at generic level, with the exception of Ewart & Davies (1917) and Ewart (1930). Steenis (1968) considered this preference "a matter of convenience, not of scientific study" (p. , although his own reasons for preferring *Bredemeyera* are not really any more "scientific" than many of those used by other authors to justify the retention of *Comesperma*. He believed that there was a high level of heterogeneity in *Comesperma*—variation in fruit shape, presence vs. absence of the coma, differences in growth habit and floral construction—and that it lacked sufficiently unambiguous diagnostic characters to merit generic status. Because of the agreement in "the essential characters of flower structure and fruit" (p. 379), Steenis (1968) felt it necessary to merge *Comesperma* with *Bredemeyera*. Furthermore, he described a new species from West New Guinea—*Bredemeyera papuana* Steenis, a lowland rainforest climber. He postulated that this species was a phytogeographic link between the two lineages within the "*Bredemeyera-Comesperma* complex", with true *Bredemeyera* representing "an old tropical rain-forest stock" (p. 380) that had been able to colonise drier sub-tropical areas by adopting a leafless or microphyllous habit in Argentina, Chile and Australia. Interestingly, the Polygalaceae treatment eventually published in the *Flora Malesiana*, *B. papuana* (and thus the entire Section *Melchiora*) was placed into *Polygala* by Meijden (1988). This was due to some rather convoluted reasoning:

(1) Since a small number of *Comesperma* species have no coma and their fruit and seeds somewhat resemble those of *Polygala*, it is not certain that it is a "good" genus and it should probably be merged into *Polygala*.

(2) As the only truly unambiguous character that separates *Comesperma* from *Bredemeyera* is the insertion of the long seed hairs, *Bredemeyera* should perhaps also be placed under *Polygala*, albeit in a separate section from *Comesperma*.

(3) *Bredemeyera papuana* is thus best placed in *Polygala*, within its own Section *Melchiora*.

Perhaps fortunately, Meijden only made the formal transfer of *Bredemeyera papuana* Steenis to *Polygala papuana* (Steenis) Meijden and did not make the necessary combinations to move all of *Comesperma* and *Bredemeyera* into *Polygala*. These ideas have been rejected for the most part by Australian systematists in recent revisions and floras (e.g., Pedley 1984; Walsh 1999), which all uphold *Comesperma* as a separate genus. Steenis (1968) may have regarded this as simply a preference for maintaining the status quo, but a recent analysis using morphological data (Eriksen 1993b) indicates that the two genera are distinct lineages. Moreover, preliminary molecular evidence suggests that the two genera are not sister taxa. A definitive answer to this question is obviously still required for the purposes of this project.

Salomonia* versus *Epirixanthes

A similar situation to the *Comesperma-Bredemeyera* question exists with the largely southeast Asian genera, *Salomonia* and *Epirixanthes* Blume. Both genera are comprised of extremely small and often ephemeral herbs with greatly reduced floral and fruiting structures held in spikes. *Salomonia* is apparently photosynthetic, but *Epirixanthes* are all saprophytes, obtaining their nutrients from decaying plant matter rather than via photosynthesis. Species of *Epirixanthes* thus lack chlorophyll and share a very "reduced" morphology, with the minute flowers and fruit forming on few leafless stems. Bentham & Hooker (1862) and Chodat (1896) suggest that *Epirixanthes* can thus easily be accommodated within *Salomonia*, although both again proposed each genus forms separate sections within the larger *Salomonia*. As only one species of *Salomonia* occurs across northern Australia and the genus has been recently revised by Koyama (1995), this is of marginal importance to an Australian review of the family. However, data from both genera will be included in the cladistic analyses in an attempt to resolve the issue of generic delimitation. Despite the lack of chlorophyll, it is hoped that a remnant chloroplast genome will still be present in *Epirixanthes* as shown in *Epifagus* Nutt., another saprophytic genus (Palmer *et al.* 1990; Depamphilis & Palmer 1990; Feierabend 1992).

Xanthophyllum

Although originally described as a genus within the Polygalaceae by Roxburgh (1820), this large southeast Asian group has been placed at many taxonomic levels within the family, including as a tribe (Baillon 1874; Chodat 1896) or a subfamily (Takhtajan 1980, 1987). By contrast, it has also been considered by some authors to be distinct enough to merit familial status (e.g., Gagnepain 1908; Cronquist 1981; Reveal & Hoogland 1990). The system followed by the *Flora of Australia* (based on Cronquist 1981) recognises *Xanthophyllum* as a distinct family. However, the thorough revision by Meijden (1982) is the most in-depth treatment of the group and strongly suggests that *Xanthophyllum* should be considered as part of the Polygalaceae. Other detailed studies of morphology and anatomy support this (e.g., Dickison 1973; Verkerke 1984; Eriksen 1993a, b). Since two species of *Xanthophyllum* are endemic to northern Queensland, they will be included in the morphological and molecular analyses to determine whether the Australian species at least fall within the rest of the Polygalaceae.

Emblingia

Emblingia calceoliflora F.Muell. is the single species in a genus that is endemic to Western Australia. Its exact distribution within the state is unclear, because it only appears after fire and is succeeded fairly rapidly by other flora. Mueller (1860) originally described it as part of the Capparaceae (mustard family), but it has since been shifted between a number of other families, including the Polygalaceae. Some authors (e.g., Airy Shaw 1965; Dahlgren 1980; Takhtajan 1980; Angiosperm Phylogeny Group 1998) have also postulated that the single species is distinct enough to merit its own family, the Emblingiaceae. This uncertainty is probably due to the unusual morphology of the species, which shares a number of characters with each of the families. Recent molecular evidence from *rbcL* (Chandler & Bayer 2000) indicates that Mueller may have been correct in his original placement, as it seems *Emblingia* falls within the mustard order, Brassicales. Fresh material of *Emblingia* is difficult to obtain because it is a post-fire ephemeral, but it was initially included in the cladistic analyses of the Polygalaceae in Australia, if only to confirm that it did not belong within the family.

AIMS

- To produce a phylogeny for the Polygalaceae within Australia using both molecular and morphological data, with a focus on *Comesperma* as the only likely endemic group of taxa. Some examination of species complexes within *Comesperma* may be required to achieve this aim.

- To determine whether *Comesperma* and *Salomonia* merit separate generic rank or should be placed within other pre-existing genera.

- To determine whether *Xanthophyllum* should be placed within the Polygalaceae rather than as a segregate family.

- To produce a comprehensive revision of the family in Australia, including a treatment for the *Flora of Australia* series.

GENERAL APPROACH

Cladistic Analyses

Cladistic analysis is currently the most commonly used method for inferring the evolutionary history (or phylogeny) of organisms. Attempting to reconstruct the phylogeny of a group of organisms allows us to place observed variation among the taxa of interest into an evolutionary context. Cladistics is based on the identification of monophyletic groups or clades of taxa, defined as those groups that share "derived" character states. Taxa are scored for a list of characters and are grouped together if they exhibit the "derived" or more recently evolved state of any one character. These character states are distinguished through the use of outgroup analysis, where taxa which are outside the group of interest (for example, a family or genus) are scored for the characters. Outgroups provide a polarity to the analysis, as they identify which "primitive" characters are likely to have been shared by the common ancestor of the ingroup and the outgroup/s. The results of a cladistic analysis are usually presented as a tree diagram or cladogram, which represents a hypothesised branching pattern of evolutionary history.

Character data for cladistic analyses may be drawn from a number of sources, including morphology, chemistry, and DNA sequences. Each source of data presents a combination of common and unique issues for such analyses, including how to score characters, weighting the importance of each character, building trees and interpreting results.

Morphological Data

Morphological data were used to construct a phylogeny of the Australian Polygalaceae including all species. This was based primarily on examination of herbarium specimens from the Australian National Herbarium (CANB) and from other Australian and international herbaria. Some morphological characters informative at the generic level were identified by Eriksen (1993a, b) and proved useful in building the character list for this study. Field observation also provided some characters not observable on herbarium specimens. The results of the analyses of morphological data for the Australian Polygalaceae are presented in Chapter 2.

Molecular Data

Use of molecular data for phylogenetic analyses has become increasingly popular in the last decade, as new techniques have become available and direct sequencing in a relatively short time is now feasible. It is possible to target specific regions of the genome that are of interest. This is especially useful in the chloroplast region where regions evolving at different rates have been identified. It is thus possible to choose a region that will be informative at the taxonomic level required, as a faster evolving region would be more useful at the level of species and genera, while a region with relatively low levels of variation is required to elucidate family-level relationships. One necessary consideration with molecular data is the issue of incongruence between data sets from different regions, making it important to obtain sequences for more than one region and to ensure that these data sets are not linked. This will make it more likely that the gene trees obtained by performing cladistic analyses on the sequences reflect the true evolutionary history of the organisms (species trees).

Two DNA regions were examined in the Australian Polygalaceae—one nuclear and one from the chloroplast, as these represented two independent genomes. Choice of these regions was aimed at resolving the problems of generic circumscription, although it was also hoped to clarify the groupings and relationships within *Comesperma*. The results of the analyses of molecular data for the Australian Polygalaceae are presented in Chapter 3.

Reconciling Results from Cladistic Analysis

As the required technology becomes available and more affordable, it is increasingly common for phylogenetic studies to use data gleaned from more than one source. This presents the problem of how or whether to combine the data obtained. The evolutionary constraints acting on a given set of traits may not apply to data collected from a different source, and there is also evidence that traits from a single source may not be governed by a single evolutionary model. The issue of whether to combine data and at what stage this should be done has thus received significant attention in the phylogenetic literature. The results of the phylogenetic analyses of the Australian Polygalaceae using both the morphological and molecular data are compared and a synthesis presented in Chapter 4.

Taxonomic Revision

While a classification scheme does not necessarily have to reflect phylogeny in order to effectively categorise living things, natural classification is one of the goals of phylogenetic systematics. A putative evolutionary history for a group of organisms can be used as a framework for their classification. Monophyletic groups of taxa and the relationships between them are identified via cladistic analysis, using objective and repeatable methods rather than subjective decisions on relatedness. A classification based on groups which share an evolutionary history can then be constructed, although the decision about at which rank a group is recognised remains largely arbitrary. A taxonomic treatment of the Polygalaceae taxa recognised as occurring in Australia and based on the results of the phylogenetic analyses is contained in Appendix 2.

Chapter Two:

Classification and Morphological Phylogeny

INTRODUCTION

Traditional Classification of the Polygalaceae

Defining the family

The Polygalaceae are a morphologically diverse family with numerous growth habits and adaptations to a broad range of habitats. The plants may be trees, climbers, shrubs or small herbs (some of which are hemi-parasitic). Despite its diversity, the family is well-defined by a suite of morphological characters and is thus usually presumed to be monophyletic. Defining characters include:

- leaves usually simple, exstipulate or with stipules reduced to glands;
- flowers bisexual, irregular, subtended by a bract and two bracteoles;
- calyx usually of five sepals (but sometimes four to seven), variously modified—commonly either with the lower two sepals united or with the two inner lateral sepals enlarged and often petaloid;
- corolla usually reduced to three petals (from five), often with the lower petal modified into a keel and sometimes bearing a fringed crest;
- stamens usually eight (reduced from ten), fused to the base of the corolla, often united by the filaments for a varying proportion of their length;
- pollen polycolporate with an excentric disc;
- ovary superior, usually of two united carpels, usually one anatropous ovule per locule with axile placentation;
- style simple and often curved, stigma often bilobed;
- fruit usually a loculicidal capsule, seeds usually arillate and sometimes hairy.

Affinities to other families

Previous classifications based on morphological characters have placed the Polygalaceae as closely related to a number of other families. The similarity of the flowers to those of the papilionoid ("pea-flowered") legumes is well-recognised but has usually been discounted as a superficial resemblance rather than an indicator of a close evolutionary relationship. Takhtajan (1980) considered the Order Polygalales to be closely related to the Geraniales and to be made up of six families—Polygalaceae (including Xanthophyllaceae), Malpighiaceae, Trigoniaceae, Vochysiaceae, Krameriaceae and Tremandraceae. The characters that defined the Polygalales were not explicitly stated. A revision of this classification (Takhtajan 1997) maintained the relationship with the Geraniales but segregated only the Polygalaceae, Xanthophyllaceae, and Emblingiaceae as the Order Polygalales. The other five families were placed into a new sister Order Vochysiales. The Polygalales as circumscribed by Cronquist (1981) included the same taxa as Takhtajan (1980), but he considered them as seven families with Xanthophyllaceae distinct from Polygalaceae. He suggested that the Polygalales were closely related to the Linales and that both were "offshoots" from the Rosales. The families within Cronquist's Polygalales were grouped by common features including simple leaves, hypogynous or rarely perigynous flowers which are often strongly zygomorphic, distinct (or only basally connate) often clawed petals, often poricidal anthers, and a compound, usually plurilocular ovary with mostly only one or two ovules per locule. The families within the order were then distinguished from each other by differences in stipule presence, floral symmetry, anther dehiscence and arrangement, pollen morphology, carpel number and placentation. Thorne (1981) constructed a classification primarily using phytochemical characters and considered this group of families to be a suborder Polygalinae of the Geraniales, with the exception of Tremandraceae, which he placed in the relatively unrelated Pittosporales. In a revision of this classification (Thorne 2000), the Polygalales were raised to ordinal level sister to the Geraniales with Tremandraceae moved into the latter order. However, Vochysiaceae was removed from the Polygalales and Euphroniaceae added. Mabberley (1997) noted that the flowers of members of the Polygalaceae were superficially similar to those of papilionoid legumes, but agreed that the family was "probably derived from the Malpighiaceae-Vochysiaceae complex". Thus, most of the recent classifications based on morphological, anatomical and chemical characters appear to have reached a broad consensus as to the families that are closely related to the Polygalaceae. Nonetheless, recent evidence from molecular systematics (e.g. Angiosperm Phylogeny Group 1998; Savolainen *et al.* 2000; Persson 2001) has called these well-established relationships into question and ironically indicates that the connection between the Polygalaceae and Fabaceae is much closer than previously believed. Since the most obvious morphological connection between the two families is due to convergence, many morphological classifications had dismissed suggestions of a close evolutionary relationship between the two.

Tribal Classification

The situation is less settled within the Polygalaceae than at ordinal level. The number of species—approximately 1000 in 23 genera, when considered in a broad sense—and their great variation in morphological characters makes a robust tribal classification potentially useful. A total of four tribes have been proposed by Chodat (1896) and Eriksen (1993b):

- Polygaleae—Three unequal larger petals and two minute lateral petals, lower petal forming a keel; four or six to eight anthers; two carpels with one ovule per carpel. Fifteen genera (*Acanthocladus*, *Ancyclotropis*, *Badiera*, *Bredemeyera*, *Comesperma*, *Epirhixanthes*, *Monnina*, *Mundtia*, *Muraltia*, *Nylandtia*, *Phlebotaenia*, *Polygala*, *Pteromonnina*, *Salomonia*, *Securidaca*).
- Moutabeae—Calyx and corolla usually fused into a tube; five petals ranging from equal to unequal, lower petal not forming a keel; rarely six or usually eight to ten anthers; three to eight carpels with one ovule per carpel. Five genera (*Balgoya*, *Barnhartia*, *Diclidanthera*, *Eriandra*, *Moutabea*).
- Xanthophylleae—Five equal or more commonly unequal petals, lower petal forming a keel; nearly always eight anthers; two carpels with two or more ovules per carpel. One genus (*Xanthophyllum*).
- Carpolobieae—Five petals of more or less equal length, lower petal forming a keel; five anthers; three carpels with one ovule per carpel. Two genera (*Atroxima*, *Carpolobia*).

The monophyly of these tribes and their relationships to each other remain uncertain. Results from Persson's (2001) analysis of *trnL/F* data indicate that all tribes other than Moutabeae are monophyletic (although Carpolobieae is weakly supported), but that there are serious issues of polyphyly of some of the genera. These findings may make significant taxonomic rearrangements necessary in the future if the classification is to reflect our understanding of the phylogenetic history of the family.

Australian Taxa

The Australian members of the Polygalaceae were the focus of this project because the family as a whole had not been revised here since Bentham (1863) and a new treatment was required for the *Flora of Australia*. A brief outline of the taxonomic history of the Polygalaceae in Australia (previously described in detail in Chapter 1) is shown in Table 2.1. The vast majority of the native and naturalised Polygalaceae taxa occurring in Australia belong within the tribe Polygaleae, with only two endemic species of *Xanthophyllum* from another tribe (Xanthophylleae). The family is present in all states and territories of Australia and the species exhibit a broad range of morphological variation. Cladistic analysis of these morphological characters had not previously been attempted and this approach would enable examination of the sub-generic groupings within *Comesperma*, in particular, and of several previously mooted questions about generic delimitations.

Polygala

The sectional classification for the Australian species of *Polygala* is still largely based on Chodat (1896), who constructed an extremely detailed subdivision of the genus and recognised ten sections. According to this classification, all Australian *Polygala* species belong to section *Polygala* (=section *Orthopolygala* Chodat, *nom. illeg.*). These taxa share two defining characters—an appendage (usually a branched crest) on the back of the keel petal and persistent outer sepals that enclose the mature fruit. Within this grouping, comprised of about 350 species at the time, Chodat recognised fourteen subsections. The species currently found in Australia fall into nine subsections and a number of series, due to the mixture of exotic and endemic species. The endemic species fall within subsection *Deltoideae* and series *Chloropterae*, although Chodat did not explicitly list them all. These taxa all have short axillary inflorescences and flowers with greenish, acute wing sepals.

Comesperma

Steetz (1847) fully revised *Comesperma* and divided it into four sections. However, Chodat (1896) considered *Comesperma* to only warrant sectional rank within *Bredemeyera*. He divided this genus into three sections—*(Eu)Bredemeyera*, *Hualania* and *Comesperma*—on the basis of growth habit, floral morphology and sepal

TABLE 2.1: Brief taxonomic history of Australian members of the Polygalaceae.

Year	Author/s	Publication	Major points
1806	Labillardière	*Novae Hollandiae Plantarum Specimen*	Erected *Comesperma* on the basis of the tuft of hair on the seed coat (coma), included five species.
1845	Steudel	*Plantae Preissianae*	Description of 17 species of *Comesperma*.
1847	Steetz	*Revisio Generis Comesperma* (reprinted in *Plantae Preissianae*; Steetz 1848)	Reworked Steudel's treatment and placed many of his species in synonymy, but also named several new species. Listed 24 species of *Comesperma* in four sections.
1862	Mueller	*Plants Indigenous to the Colony of Victoria*	Erected section *Prosthemosperma* within *Comesperma* to accommodate two species that lacked comose seeds.
1863	Bentham	*Flora Australiensis*	Last Australia-wide revision of the Polygalaceae. Recognised 29 species in three genera—one *Salomonia*, seven *Polygala* and 21 *Comesperma* spp.
1893	Chodat & Hochreutiner	*Bulletin de l'Herbier Boissier*	First part of "Contribution a l'étude du genre *Comesperma*", with a detailed discussion and subdivision of the genus. Further parts were not published.
1896	Chodat	*Die Natürlichen Pflanzenfamilien*	Worldwide treatment of the Polygalaceae, reducing *Comesperma* to a section of *Bredemeyera*. *Bredemeyera* section *Comesperma* contained about 30 species in three sub-sections (based mainly on the sectional classification in Steetz (1847) but using quite different defining characters).
1968	Steenis	*Acta Botanica Neerlandica*	Description of *Bredemeyera papuana* from New Guinea and agreement with Chodat's sinking of *Comesperma*, with formal transfer of most of the Australian species names to *Bredemeyera*.
1969	Adema	*Proceedings of the Royal Society of Queensland*	Listing of thirteen herbaceous species of *Polygala* represented in the Queensland Herbarium with brief comments on each.
1982	Meijden	*Leiden Botanical Series*	Complete revision of *Xanthophyllum*, including the two Australian species.
1984	Pedley	*Austrobaileya*	Revision of *Comesperma* in Queensland, total of fourteen species with six endemic. Rejection of *Comesperma* being synonymised with *Bredemeyera*.
1990	Hnatiuk	*Census of Australian Vascular Plants*	Listing of all 65 Australian taxa in Polygalaceae and Xanthophyllaceae. 32 *Comesperma*, 1 *Emblingia*, 1 *Muraltia*, 27 *Polygala*, 2 *Salomonia* and 2 *Xanthophyllum*.

persistence. These three sections had all previously been regarded as distinct genera. Steetz's (1847) and Chodat's (1896) differing subdivisions of *Comesperma* are shown in Table 2.2. The diagnostic characters used by these two authors are very different and the same sub-generic names do not in all cases refer to the same taxa. Chodat and Hochreutiner (1893) stated that Steetz had not "taken into account the internal organs" and thus "could not seize on the real affinities of the sections of *Comesperma*". They agreed that Steetz's characters "can still be useful" and that "we will employ his methods to a certain extent" but their promise to discuss the issue further was not fulfilled, as the subsequent part or parts of their article on *Comesperma* were apparently never published. Chodat (1896) uses Steetz's sectional names for the most part and lists many of the same taxa under the same names, but apparently prefers to use the stigma characters to diagnose groups over those relating to sepals. Steetz (1847) categorised every species of *Comesperma* known to him but several more were described and named after his treatment was published. Chodat (1896) published his classification after the bulk of the species in *Comesperma* had been described, but listed only a few species he considered "notable" for each subsection and series. Chodat's scheme also separates the most "unusual" taxa and leaves a large portion of the genus in the relatively undivided subsection *Eucomesperma*. Since neither classification is complete, Chodat's (1896) was chosen when categorising taxa for this study, as it extended to the entire Polygalaceae rather than a single genus.

It should be noted that while Chodat (1896) indicated that his sections in Bredemeyera were not considered to be distinct enough to warrant generic status, all three were maintained as separate subgeneric groups, so this is really an issue of rank. Section *Comesperma* was defined on the basis of the following characters: short subshrubs or herbs with small elongate leaves; flowers in racemes; wing sepals petaloid and coloured; ovary glabrous; calyx falling away before mature fruit are formed. By contrast, section (*Eu*)*Bredemeyera* consisted of taxa which shared the following characters: mostly twining shrubs with leathery ovate or long-ovate leaves; flowers mostly in panicles; calyx lobes always free, mostly fleshy and hairy, wing sepals many-nerved, upper petals mostly spathulate and obtuse; stamens free above; stigma little-developed and inconspicuous; fruit a robust capsule; entire calyx falling away by maturity. Finally, section *Hualania* was defined by possession of the following characters: stems and branches glabrous but strongly armed and with very reduced

TABLE 2.2: Comparison of sub-divisions within *Comesperma* as proposed by Steetz (1847) and Chodat (1896), with illustrations of stigmatic characters from Chodat & Hochreutiner (1893).

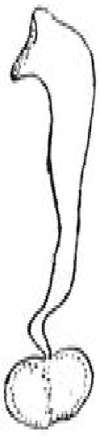
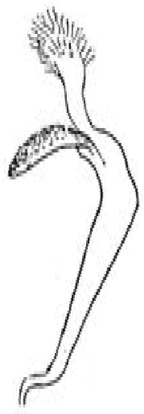
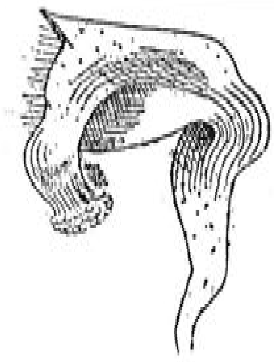
Steetz (1847)—*Comesperma*	**Chodat (1896)—*Bredemeyera* section *Comesperma***
Section *Eucomesperma*: Three external subequal sepals, two anterior and one posterior, 3–4 times shorter than the wings. Series *Erecta*: Stems erect, somewhat woody, ±branched, leafy; wings obovate, apex obtuse, base attenuate; filaments monadelphous at the base, diadelphous above the middle with 4 subsessile anthers in each group; style curved towards the apex. Steetz included *Comesperma retusum*, *C. ericinum*, *C. confertum*, *C. acerosum*, *C. flavum*, *C. secundum*, *C. drummondii*. Series *Volubilia*: Stems twining, usually subherbaceous, more or less branched, sparingly leafy. Flowers in lax racemes, wings broad ovate and clawed at the base; filaments monadelphous, briefly diadelphous towards the middle, free above the middle; style moderately geniculate, apex shortly bilobed. Steetz included *Comesperma volubile*, *C. integerrimum* and *C. ciliatum*.	 Subsection *Eucomesperma*: Stigma not hairy. Wings always obtuse with anastomosing venation and never fused to the petals. Series *Scopariae*: Leafless, virgate, small subshrubs or perennial plants. Capsule similar to *Polygala*. Seeds not comose. Chodat included *Comesperma scoparium* and *C. aphyllum*. Series *Volubiles*: Shoots twining, nearly leafless. Style bent above the middle. Chodat included *Comesperma volubile* and *C. integerrimum*. Series *Confertae*: Mostly heath-like subshrubs, most with many-flowered racemes. Stems erect. Leaves needle-shaped, linear or wider, mostly keeled. Chodat included *Comesperma flavum* and *C. ericinum*. Series *Disepalae*: Similar in habit to *Confertae*. The two lower calyx lobes ±fused. Keel often with a horn-shaped appendage on the back; anthers in fused stamen bundles. Chodat included *Comesperma virgatum* and *C. polygaloides*.
Section *Isocalyx*: Three external subequal sepals, two anterior and one posterior, only slightly shorter than the wings. Wings clawed at the base. Filaments monadelphous at the base, towards the middle diadelphous, above the middle free. Series *Subherbacea*: Stems subherbaceous, unbranched or sparingly branched; lower leaves crowded, upper leaves smaller and remote; style apex curved, "two-legged". Steetz included *Comesperma calymega* and *C. nudiusculum*. Series *Suffruticosum*: Stems somewhat woody, much branched; leaves very small and remote; style apex curved and briefly bilobed. Steetz included *Comesperma scoparium*.	 Subsection *Isocalyx*: Upper stigma tongue-shaped, the apex and lower half similarly hairy. Most small slender herbs; calyx lobes free, the outer lobes barely shorter than the wings, lanceolate; wings without anastomosing venation. Stamens free above. Chodat included *Comesperma lanceolatum*.

TABLE 2.2 (continued)

Steetz (1847)—*Comesperma*	**Chodat (1896)—*Bredemeyera* section *Comesperma***
Section *Disepalum*: Two external unequal sepals, anterior broad with bilobed apex, posterior narrower with entire apex, each 3–4 times shorter than the wings. Steetz included *Comesperma virgatum*.	Not recognised at this level, merged into subsection *Eucomesperma* as series *Disepalae*.
Section *Comespermastrum*: Two external unequal sepals, anterior broad with bilobed apex, posterior narrower with entire apex, each about half the length of the wings. Filaments monadelphous at the base, towards the middle briefly diadelphous, above the middle free. Style curved towards the apex, briefly "two-legged". None of the species which Steetz included are considered to be current.	 Subsection *Comespermastrum*: Upper stigma acutely tooth-shaped; the part between the two stigmas hairy. The lower two calyx lobes fused; venation like *Isocalyx*. Wings shortly stalked, with free nerves and fused with the corolla at the base; anthers ±stalked. Chodat included *Comesperma nudiusculum* and *C. rhadinocarpum*.

leaves; flowers in short pseudo-umbels; calyx retained at maturity; stigma capitate and weakly bilobed.

Troublesome genera: *Emblingia* and *Xanthophyllum*

In Australia, *Emblingia* and *Xanthophyllum* differ significantly from the usual characters displayed by the family. The placement of *Emblingia* in the Polygalaceae has never been particularly stable, as the single species in the genus possesses morphological characters that could indicate affinities to a number of other disparate families. When Mueller (1860) originally described the species, he placed it in the Capparaceae because it possesses an androgynophore (a stalk bearing both androecium and gynoecium above the perianth in a flower), in common with other members of that family. However, later authors have assigned it to different families based on other morphological characters. These families include Polygalaceae (Cronquist 1981), Goodeniaceae (Erdtman *et al.* 1969), Sapindaceae (Thorne 1992) and even a monotypic Emblingiaceae (Airy Shaw 1965). The most recent evidence using the *rbc*L region of chloroplast DNA (Angiosperm Phylogeny Group 1998; Chandler & Bayer 2000) strongly indicates that *Emblingia* is distantly related to the Polygalaceae and cannot be reasonably accommodated within the family without broadening the scope of the Polygalaceae beyond utility. The issue of its familial placement will be discussed again briefly in Chapter Three.

Xanthophyllum, by contrast, has long been closely associated with the Polygalaceae *sens. strict.* The major issue with this group has been whether it forms a tribe nested within the Polygalaceae or whether unique aspects of the morphology justify it being given separate familial rank. Chodat (1896) maintained *Xanthophyllum* as a monogeneric tribe of the Polygalaceae, defining it as those taxa with ±free stamens and many ovules per locule. Cronquist (1981) however, stated that the morphological differences in *Xanthophyllum* were "as significant as those that separate other families in the order" and raised it to familial rank. Meijden (1982), in the most recent revision of the entire genus, considered Cronquist's recognition of the Xanthophyllaceae "a pity" and that the differences between the Polygalaceae and Xanthophyllaceae shown in Cronquist's key were "suspect, even on paper". Only two *Xanthophyllum* species occur in Australia and Meijden placed each in its own monospecific subgenus, indicating that they are extremes in the range of diversity in the genus as a whole. For present purposes, *Xanthophyllum* will be considered a member of the Polygalaceae *sens. lat.*

AIMS

The primary aim of this section of the project was to construct a putative phylogeny of the Australian Polygalaceae via cladistic analysis of morphological data. The robustness of the phylogeny was tested and the characters supporting groups of interest were explored. The phylogeny constructed was also compared to existing classifications in order to evaluate the likelihood of monophyly for the traditionally defined groups. The monophyly of *Comesperma*, the utility of its subgeneric classification by Chodat (1896), and the relationship between it and *Bredemeyera* could thus be addressed.

METHODS

End Taxa

End (or terminal) taxa in a phylogenetic analysis are the taxonomic units whose relationships are being studied. They are represented by the names at the tips of the branches of any tree produced and can be drawn from any taxonomic level. Potential end taxa for these species-level analyses of morphological data were initially identified using the Census of Australian Vascular Plants (Hnatiuk 1990). A number of unpublished "manuscript" names were subsequently added to this list and any names considered synonymous with a taxon already represented were removed. This resulted in a list of 57 Australian taxa—33 *Comesperma* species, one *Emblingia* species, one *Muraltia* species, 19 *Polygala* species, one *Salomonia* species and two *Xanthophyllum* species. Three extra-Australian *Bredemeyera* species were also added, with one representative each from section *Bredemeyera*, section *Hualania* and section *Melchiora*. Under the classification proposed by Chodat (1896), the end taxa can be categorised as shown in Table 2.3.

Characters and states

A list of morphological characters was constructed after detailed examination of a number of herbarium specimens from CANB and the other Australian State herbaria (AD, BRI, DNA, HO, NSW, PERTH) to gauge the level of variation across the family. Some characters, particularly those varying at the level of a genus, were examined and coded after reference to Chodat (1896) and Eriksen (1993b). The list of 56 cladistically informative morphological characters shown in Table 2.4 was thus assembled and a number of specimens of each taxon examined and scored in order to accurately code the

TABLE 2.3: Australian Polygalaceae taxa organised according to current classifications, based on Chodat (1896) and Steenis (1968) for Polygaleae and Meijden (1982) for Xanthophylleae. Blank cells indicate that no name was proposed at this taxonomic level by these authors.

Tribe	Genus	Section	Subsection	Series	Species
Polygaleae	*Bredemeyera*	*(Eu)Bredemeyera*			*Bredemeyera floribunda* Willd.
		Comesperma	*Eucomesperma*	*Scopariae*	*Comesperma aphyllum* Benth. *Comesperma scoparium* J.Drumm. ex Steetz *Comesperma sphaerocarpum* Steetz[1] *Comesperma spinosum* F.Muell.[1]
				Volubiles	*Comesperma ciliatum* Steetz[1] *Comesperma integerrimum* Endl. *Comesperma volubile* Labill.
				Confertae	*Comesperma acerosum* Steetz[1] *Comesperma breviflorum* Pedley[1] *Comesperma confertum* Labill.[1] *Comesperma drummondii* Steez[1] *Comesperma ericinum* DC. *Comesperma esulifolium* Gandoger[1] *Comesperma flavum* DC. *Comesperma hispidulum* Pedley[1] *Comesperma oblongatum* (Benth.) Pedley[1] *Comesperma pallidum* Pedley[1] *Comesperma patentifolium* F.Muell.[1] *Comesperma praecelsum* F.Muell.[1] *Comesperma retusum* Labill.[1] *Comesperma secundum* Banks ex DC.[1] *Comesperma sylvestre* Lindl.[1] *Comesperma viscidulum* F.Muell.[1]
				Disepalae	*Comesperma calcicola* Keighery[1] *Comesperma polygaloides* F.Muell. *Comesperma* sp. A Kimberley[1] *Comesperma virgatum* Labill.
				Incertae sedis	*Comesperma calymega* Labill.[2] *Comesperma defoliatum* F.Muell.[2]

TABLE 2.3 (continued)

Tribe	Genus	Section	Subsection	Series	Species
Polygaleae (continued)	_Bredemeyera_ (continued)	_Comesperma_ (continued)	_Isocalyx_		_Comesperma lanceolatum_ R.Br. ex Benth.
			Comespermastrum		_Comesperma griffiniii_ Keighery[1] _Comesperma nudiusculum_ DC. _Comesperma rhadinocarpum_ F.Muell.
		Hualania			_Bredemeyera microphylla_ (Griseb.) Hieron
		Melchiora			_Bredemeyera papuana_ Steenis
	Muraltia	_(Eu)Muraltia_	_Gymnocarpae_		*_Muraltia heisteria_ (L.) DC.
	Polygala	_(Ortho)Polygala_	_Apterocarpae_	_Tenues_	*_Polygala paniculata_ L.
			Hemipterocarpae		*_Polygala duarteana_ A.St-Hil.
			Migratores	_Persicariaefoliae_	*_Polygala persicariifolia_ DC.
			Fortificatae		_Polygala japonica_ Houtt.
			Leptaleae		_Polygala longifolia_ Poir. _Polygala wightiana_ Wight & Arn.
			Deltoideae	_Chloropterae_	_Polygala chinensis_ L. _Polygala eriocephala_ Benth. _Polygala exsquarrosa_ Adema[1] _Polygala isingii_ Pedley[1] _Polygala linariifolia_ Willd.[1] _Polygala macrobotrya_ Domin[1] _Polygala orbicularis_ Benth.[1] _Polygala rhinanthoides_ Sol. ex Benth.[1] _Polygala tepperi_ F.Muell.[1]
			Virgatae		*_Polygala virgata_ Thunb.
			Formosae		*_Polygala myrtifolia_ L.
			Europeae	_Vulgares_	*_Polygala monspeliaca_ L.[1] *_Polygala vulgaris_ L.
	Salomonia	_(Eu)Salomonia_			_Salomonia ciliata_ (L.) DC.
Xanthophylleae	_Xanthophyllum_	(Subg.) _Grandiflorum_			_Xanthophyllum fragrans_ C.T.White
		(Subg.) _Macintyria_			_Xanthophyllum octandrum_ (F.Muell.) Domin

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[1] Taxon not listed in Chodat (1896), placed in most appropriate subgeneric group based on characters used to define them.

[2] Taxon not listed by Chodat (1896) and unable to be confidently placed into the existing subgeneric classification.

* Taxon secondarily introduced to Australia. These taxa are also marked with an asterisk in some of the figures in this chapter.

TABLE 2.4: List of morphological characters and states used in cladistic analysis of Australian Polygalaceae.

Number	Character with list of possible states
1.	*Plant habit*: **(0)** twining climber; **(1)** erect leafy shrub ("candelabra" type); **(2)** erect virgate or divaricate generally leafless shrub; **(3)** wiry, +/- leafless subshrub; **(4)** prostrate straggling shrub; **(5)** herb; **(6)** tree.
2.	*Plant stem colour*: **(0)** reddish; **(1)** brown/green.
3.	*Stem surface*: **(0)** regularly ribbed; **(1)** irregularly ribbed or smooth.
4.	*Stem cross-section*: **(0)** angular; **(1)** rounded.
5.	*Indumentum/hairs of main branches*: **(0)** glabrous; **(1)** sparsely pubescent; **(2)** moderately pubescent; **(3)** densely hairy.
6.	*Stem hair type*: **(0)** single hair type (crisped); **(1)** two hair types (crisped/erect).
7.	*Glaucousness*: **(0)** leaves and stems glaucous; **(1)** leaves and stems not glaucous .
8.	*Leaf shape (when present)*: **(0)** reduced almost entirely to scales (some true leaves may persist at the base); **(1)** linear/simple; **(2)** elliptic/obovate; **(3)** oblong; **(4)** orbiculate/circular; **(5)** ovate.
9.	*Leaf placement*: **(0)** appressed to stem; **(1)** spreading (held at ~45° to stem); **(2)** patent (held at ~90° to stem).
10.	*Petiole presence*: **(0)** leaves sessile; **(1)** leaves petiolate .
11.	*Leaf base decurrence*: **(0)** present; **(1)** absent.
12.	*Leaf margin deflection*: **(0)** margins strongly recurved; **(1)** margins slightly/moderately recurved; **(2)** margins not recurved.
13.	*Leaf tip mucro*: **(0)** present; **(1)** absent .
14.	*Leaf keel*: **(0)** Leaves strongly keeled below; **(1)** Leaves not or scarcely keeled .
15.	*Leaf surface similarity*: **(0)** concolorous; **(1)** discolourous.
16.	*Inflorescence type*: **(0)** terminal racemes borne on upper branches; **(1)** loose racemes borne on short axillary shoots to the main axis; **(2)** racemes reduced to single scattered flowers on stems; **(3)** spike; **(4)** panicle; **(5)** solitary flowers in the leaf axils.
17.	*Inflorescence shape* : **(0)** corymb-like; **(1)** pyramidal/conical; **(2)** loose; **(3)** solitary and scattered; **(4)** oblong.
18.	*Bract/bracteole shape*: **(0)** all alike, cup-shaped; **(1)** middle bract leaf-like, lateral bracteoles scale-like .
19.	*Ratio of pedicel to flower length*: **(0)** pedicel much longer than flower; **(1)** flower and pedicel of +/- equal length; **(2)** pedicel much shorter than flower/sub-sessile .
20.	*Outer sepal shape*: **(0)** triangular; **(1)** semi-circular; **(2)** ovate; **(3)** oblong; **(4)** elliptic.
21.	*Outer sepal indumentum*: **(0)** glabrous; **(1)** ciliate; **(2)** pubescent.
22.	*Outer sepal fusion*: **(0)** lower pair connate; **(1)** all free; **(2)** all fused into a toothed calyx.
23.	*Lateral sepal modification*: **(0)** enlarged and petaloid; **(1)** enlarged but not petaloid; **(2)** unmodified.
24.	*Relative length of sepals* : **(0)** "wings" much longer than outer sepals; **(1)** "wings" slightly longer or sub-equal to outer sepals .

TABLE 2.4 (continued)

Number	Character with list of possible states
25.	*Flower "wing" colour*: **(0)** blue/mauve; **(1)** pink/magenta; **(2)** white; **(3)** yellow/cream; **(4)** green.
26.	*"Wing" sepal claw*: **(0)** wings distinctly clawed; **(1)** wings not or scarcely clawed.
27.	*Venation of lateral sepals*: **(0)** anastomosing; **(1)** not anastomosing.
28.	*Lateral sepal shape*: **(0)** orbiculate/circular; **(1)** obovate; **(2)** spathulate; **(3)** oblong; **(4)** elliptic; **(5)** hemispherical/falcate; **(6)** ovate.
29.	*Lateral sepal margin curvature*: **(0)** incurved all around; **(1)** partially incurved (esp. near tip); **(2)** flat; **(3)** crisped.
30.	*Apex of lateral sepals*: **(0)** acute/mucronate; **(1)** blunt .
31.	*"Wing"/petal fusion*: **(0)** wings fused to petals at base; **(1)** wings free of petals.
32.	*Upper petals relative to keel*: **(0)** upper petals exceeding keel; **(1)** Upper petals +/- equal to keel; **(2)** Upper petals shorter than keel.
33.	*Corolla tube indumentum*: **(0)** glabrous; **(1)** tuberculate or papillose; **(2)** pubescent.
34.	*Lateral appendages on keel*: **(0)** present; **(1)** absent.
35.	*Keel appendage*: **(0)** absent; **(1)** present, hooked and membranous; **(2)** present, few-branched crest; **(3)** present, finely divided "true" crest.
36.	*Fertile stamen number*: **(0)** four; **(1)** seven; **(2)** eight.
37.	*Anther attachment*: **(0)** sessile; **(1)** stalked.
38.	*Stamen grouping*: **(0)** diadelphous; **(1)** monadelphous; **(2)** three groups.
39.	*Anther dehiscence*: **(0)** short apical slits; **(1)** longitudinal slits.
40.	*Style bending*: **(0)** style straight for most of length; **(1)** style gently curved; **(2)** style distinctly hooked or horse-shoe shaped; **(3)** stigma sessile, i.e. style absent.
41.	*Stigma type*: **(0)** "horse-head" shaped, glabrous; **(1)** upper stigma replaced by a tuft of hairs; **(2)** upper stigma membranous, area between stigmas hairy; **(3)** simple brush; **(4)** capitate, bilobed; **(5)** distant bilobed, glabrous.
42.	*Retention of lateral sepals after pollination (ie mature fruit enclosed by "wings")*: **(0)** sepals retained; **(1)** sepals lost .
43.	*Nectary presence and position*: **(0)** annular nectary below ovary; **(1)** nectary borne on stamens; **(2)** nectary absent.
44.	*Ovary/fruit indumentum*: **(0)** glabrous; **(1)** pubescent; **(2)** ciliate.
45.	*Ovary apex*: **(0)** elongate constriction at apex; **(1)** short "beak" at apex; **(2)** no noticeable extension.
46.	*Ovules per carpel*: **(0)** one; **(1)** more than one.
47.	*Fruit type*: **(0)** capsule; **(1)** fruit fleshy; **(2)** dry indehiscent fruit; **(3)** leathery globose fruit.
48.	*Fruit overall shape*: **(0)** club-shaped; **(1)** circular; **(2)** heart-shaped; **(3)** reniform; **(4)** oblong/ovate.
49.	*Fruit projections (must observe on mature fruit)*: **(0)** horn-like; **(1)** short, emarginate; **(2)** absent; **(3)** stiff spiky "teeth".

TABLE 2.4 (continued)

Number	**Character with list of possible states**
50.	*Fruit wing*: **(0)** absent; **(1)** narrow, both margins; **(2)** broad, both margins; **(3)** narrow, one margin.
51.	*Fruit dehiscence*: **(0)** loculicidally dehiscent; **(1)** septicidally dehiscent; **(2)** irregularly dehiscent; **(3)** indehiscent.
52.	*Coma presence*: **(0)** present, arising all over seed coat; **(1)** present, arising mostly from margins/base of seed; **(2)** present, arising only from hilar area; **(3)** absent.
53.	*Seed shape*: **(0)** ovoid; **(1)** oblong; **(2)** ellipsoidal; **(3)** reniform; **(4)** globular.
54.	*Seed coat surface*: **(0)** smooth; **(1)** tuberculate; **(2)** "engraved".
55.	*Seed appendage type*: **(0)** expanded raphe or strophiole; **(1)** aril; **(2)** none.
56.	*Seed appendage length*: **(0)** short (touching top of seed only); **(1)** moderate - running ~1/2 length of seed; **(2)** long (reaching bottom of seed); **(3)** tailed (reaching bottom of seed and forming tail).

taxon for each character. At least five specimens were examined for each taxon, except in cases where fewer specimens were available. A dissecting microscope was used to score the majority of characters, although scanning electron microscopy (SEM) was necessary in order to examine some of the seed characters and to determine the homologies of these between genera. The matrix of taxa by characters subjected to cladistic analysis is contained in Appendix 1.

Some morphological characters which had been in doubt for *Comesperma* were confirmed using electron microscopy. Thompson (1978) reported that the genus had one-celled anthers opening by a single pore, but anther dissection showed two anther locules (Figure 2.1) and SEM clearly showed dehiscence by short apical slits in all species examined (Figure 2.2). SEM also proved useful in determining the homology of the seed appendages in *Comesperma* and *Polygala*, as it indicated that the appendages of both genera were formed from the same material and were usually three-lobed. However, in *Comesperma*, the lobes are elongated and two of them fused together (Figure 2.3).

Cladistic Analyses

Outgroup Selection

The choice of outgroup for cladistic analyses was primarily based on published molecular results which place *Xanthophyllum* sister to the remainder of the family (Savolainen *et al.* 2000; Persson 2001). A prior cladistic analysis of the family using morphological data and the Trigoniaceae as an outgroup (Eriksen 1993b) had also suggested that the Polygaleae and Carpolobieae were sister taxa, with the Xanthophylleae and Moutabeae sister to these. Since the family is represented in Australia only by the tribes Xanthophylleae and Polygaleae, it seemed reasonable to use *Xanthophyllum* as a means to root the trees. Coding other members of the Fabales, such as legumes, was likely to entail difficult decisions about the homology of various characters and this may well have introduced homoplasy/"noise" into the dataset. (This problem may already have occurred to some extent with the coding of *Emblingia*, given that it is likely to be even more distantly related to the Polygalaceae.) Thus the two Australian species of *Xanthophyllum* were defined as an outgroup for the purpose of the following analyses.

FIGURE 2.1: Transverse section of young anther from *Comesperma ericinum* DC. at 20× magnification. The two locules containing developing pollen can be clearly seen.

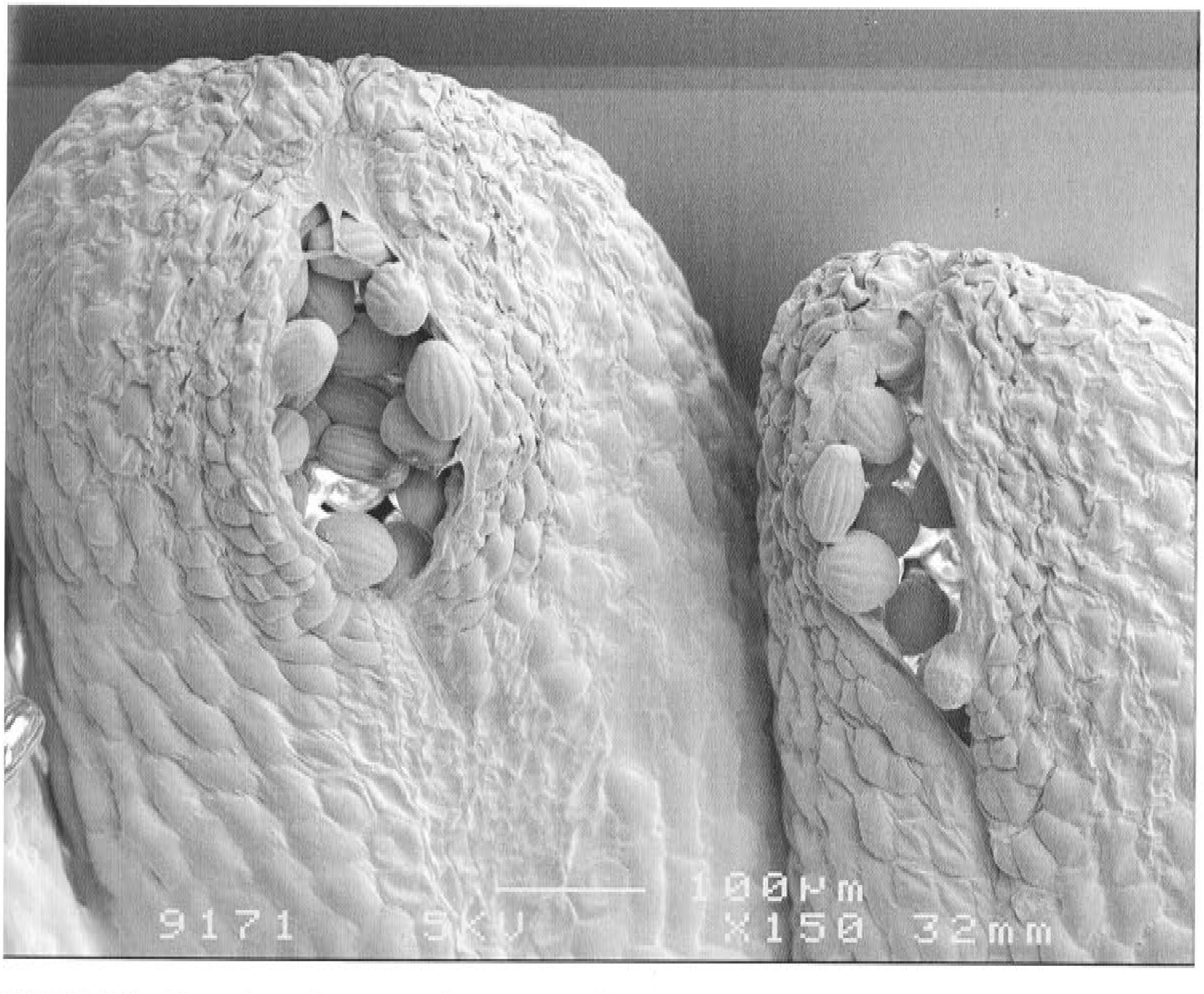

FIGURE 2.2: Scanning electron micrograph of two anthers from *Comesperma ericinum* DC., showing dehiscence by a short apical slit and characteristic polycolporate pollen emerging. The slit tends to widen and the flaps on either side open out as dehiscence progresses. This has led some authors to mistake it for dehiscence via an apical pore.

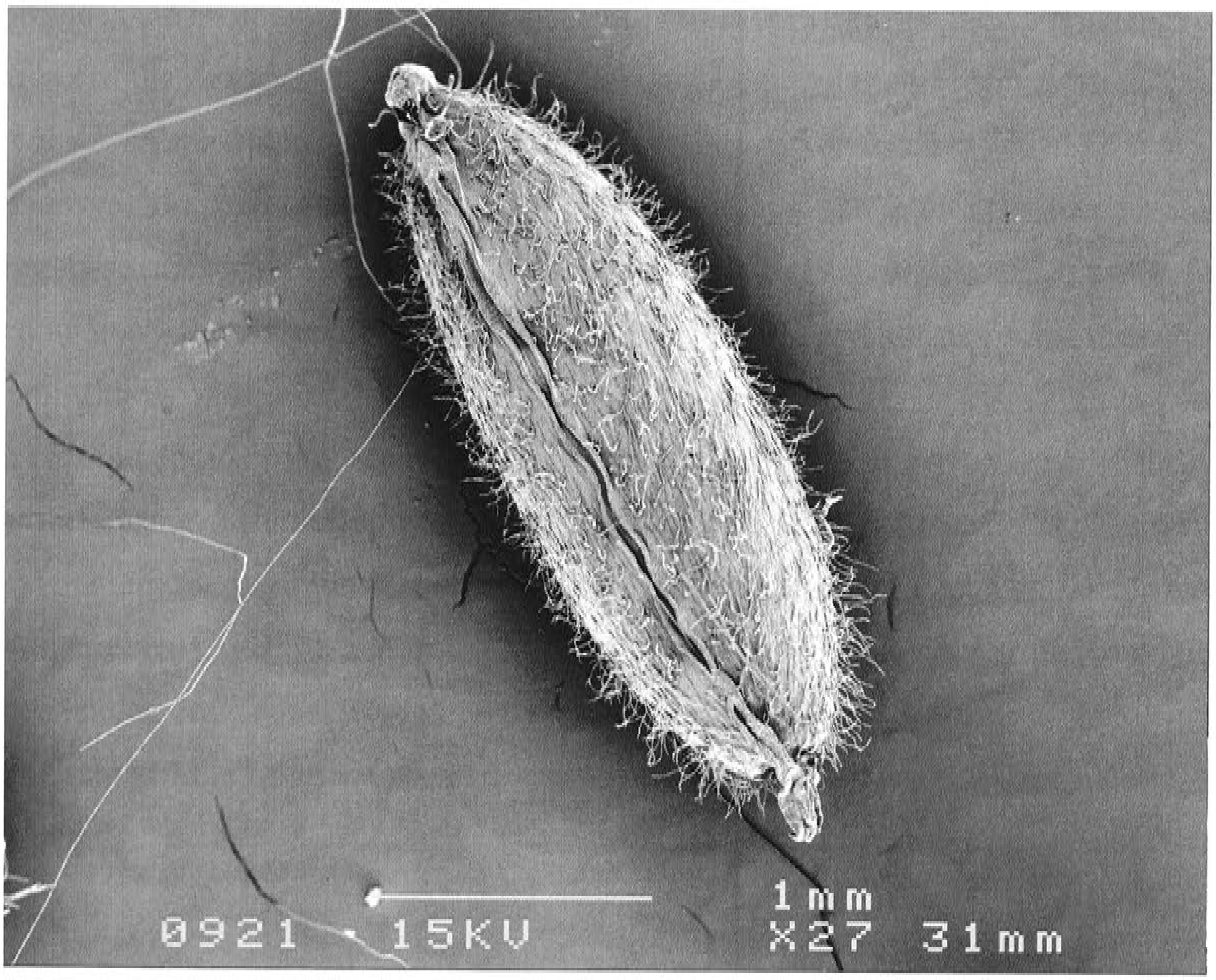

FIGURE 2.3: Scanning electron micrograph of a seed from *Comesperma virgatum* Labill., with coma removed for clarity. The two elongate adaxial lobes of the raphe are visible running the length of the seed coat. The type and size of these seed appendages were coded as characters for the cladistic analysis of morphological data.

Maximum Parsimony

Initial cladistic analyses were undertaken using PAUP* (Phylogenetic Analysis Using Parsimony and Other Methods) versions 4.0b8–10 (Swofford 2002) on a Macintosh G4 computer under maximum parsimony (MP) criteria. Since the dataset is too large to search completely, heuristic searching was employed. A thousand random addition sequence replicates were run and 100 trees saved at each step. Tree-bisection-reconnection (TBR) branch swapping was used throughout to search the "tree space", as it provides the most extensive search available and is thus likely to find a greater number of the shortest-length trees than other methods. The trees obtained using this initial search were then used as starting trees for a second analysis in which all trees found were swapped to completion. A strict consensus of all the shortest trees obtained using these methods was constructed. Bootstrap analysis (Felsenstein 1985) was then used to assess the effect of re-sampling on the dataset and to thus give some measure of the robustness of the clades obtained. One hundred bootstrap replicates were run with 20 random addition sequence replicates within each and saving 100 trees per replicate. TreeRot version 2 (Sorensen 1999) was used to calculate the Bremer (1988, 1994) decay indices for all of the nodes present on the strict consensus tree. This value shows how many steps longer a tree would have to be before the clade in question would not appear and is often considered to give some level of support for each node. The significance of the Bremer values was assessed using T-PTP tests (outlined below).

Permutation Testing

The dataset was subjected to permutation tail probability (PTP) testing (Archie 1989; Faith & Cranston 1991) in order to assess whether the data produce a signal that is significantly different from random. One hundred replicates with ten random addition sequence replicates within each were run in order to assess the significance at the 1% probability level. Topology-dependent PTP tests (T-PTP; Faith 1991) with 100 replicates and five random addition sequence replicates each were also undertaken on clades of interest from the analysis of the unweighted data. Tests were undertaken both on clades that appeared in the strict consensus and also some clades that did not appear. Testing clades that do not appear in the strict consensus shows whether the increase in tree length needed to include this clade is improbable given the data. To some degree, T-PTP tests can provide a measure of the significance of the Bremer decay value on a node by calculating the difference in length between trees that contain the clade of interest and those that do not after permuting the original data. This is equivalent to a null distribution of Bremer values for the dataset.

Reverse Successive Weighting

Due to the poor resolution achieved by the use of maximum parsimony (as discussed below), reverse successive weighting (Trueman 1998) was utilised in order to test for the presence of multiple conflicting signals in the dataset. RSW1.1 (Trueman 2002) uses PAUP* to build a tree using the complete dataset and then eliminates all those characters that are consistent with that tree. The inconsistent characters remaining are then analysed for an alternative signal. RSW builds a second tree from these characters and if this differs significantly from the first tree, a secondary signal in the data is indicated. Additionally, any characters identified as conflicting with the primary signal were then excluded from the dataset and the original cladistic analysis repeated using this "pruned" matrix.

Successive Weighting

Since reverse successive weighting did not identify a strong secondary signal in the morphological data, successive weighting (Farris 1969; Carpenter 1988) was then used. This process minimises the effects of presumably random "noise" in the dataset by building an initial set of trees from all the characters and then re-weighting each character by some measure of how well it "fits" those trees. This is an attempt to reduce the effect of homoplasy in the dataset by giving more weight to those characters that are fully congruent within the current tree/s. The weighting process is repeated until a single tree is obtained or until the trees obtained cannot be further resolved by additional weighting. The successive weighting process was undertaken using the mean consistency index (CI; Kluge & Farris 1969), the mean retention index (RI; Farris 1989), and the mean rescaled consistency index (RC; Farris 1989) for each character.

RESULTS

Maximum Parsimony (MP)

The cladistic analysis indicated that the data were composed of several "islands" of equally parsimonious trees. However, by conducting the second analysis to swap on trees from all the islands found, a reasonable survey of the "tree space" was made and further searching was unlikely to add trees that would significantly change the overall topology. In the initial search, 530 trees of length 447 steps were found. However, in the second stage, most of these trees became redundant after being randomly dichotomised for searching and only 144 trees were retained after swapping. The strict consensus tree of these 144 trees is shown in Figure 2.4 and the characters which change unambiguously on all 144 trees are shown in diagrammatic form in Figure 2.5. The strict consensus (Fig. 2.4) is not completely resolved, but it does indicate some general groupings of taxa. *Comesperma* (node A in Fig. 2.4) is apparently monophyletic, although the relationships within the genus are not clear. There are no characters on this branch that unambiguously support the monophyly of *Comesperma* (i.e. synapomorphies for the genus). Three major groupings appear within a polytomy in *Comesperma*. Firstly, at node B in Fig. 2.4, the three climbing species (series *Volubiles*, *sensu* Chodat (1896)) form a clade that is sister to a group of leafless taxa (series *Scoparieae*, *sensu* Chodat (1896)). One unexpected exception to the "leafless" group is *C. defoliatum*, which falls as sister to the entire climber-leafless grouping. The second major grouping within *Comesperma* is an assemblage containing *Comesperma ericinum* in a polytomy with four species that had previously been split from it at varietal level. However, *Comesperma breviflorum* is also a segregate from *C. ericinum* and it is unresolved in relation to this group. Third, most of the species with fused lower sepals (series *Disepalae*, *sensu* Chodat (1896)) are also clustered together, with the exception of *Comesperma nudiusculum*.

Outside the *Comesperma* clade, *Polygala* forms a monophyletic group sister to *Comesperma*, but only when the three species of *Bredemeyera* in the dataset are included within it. This latter result is unexpected, given that traditional classifications have postulated a close relationship between *Comesperma* and *Bredemeyera* based on morphological similarities. In this phylogeny, however, nine nodes separate the two genera with *Bredemeyera* nested within a clade of *Polygala* species. The characters supporting this grouping (node C in Fig. 2.4, see also Fig. 2.5) all change unambiguously on the shortest trees—#13 (leaf tip mucro absent), #28 (lateral sepal

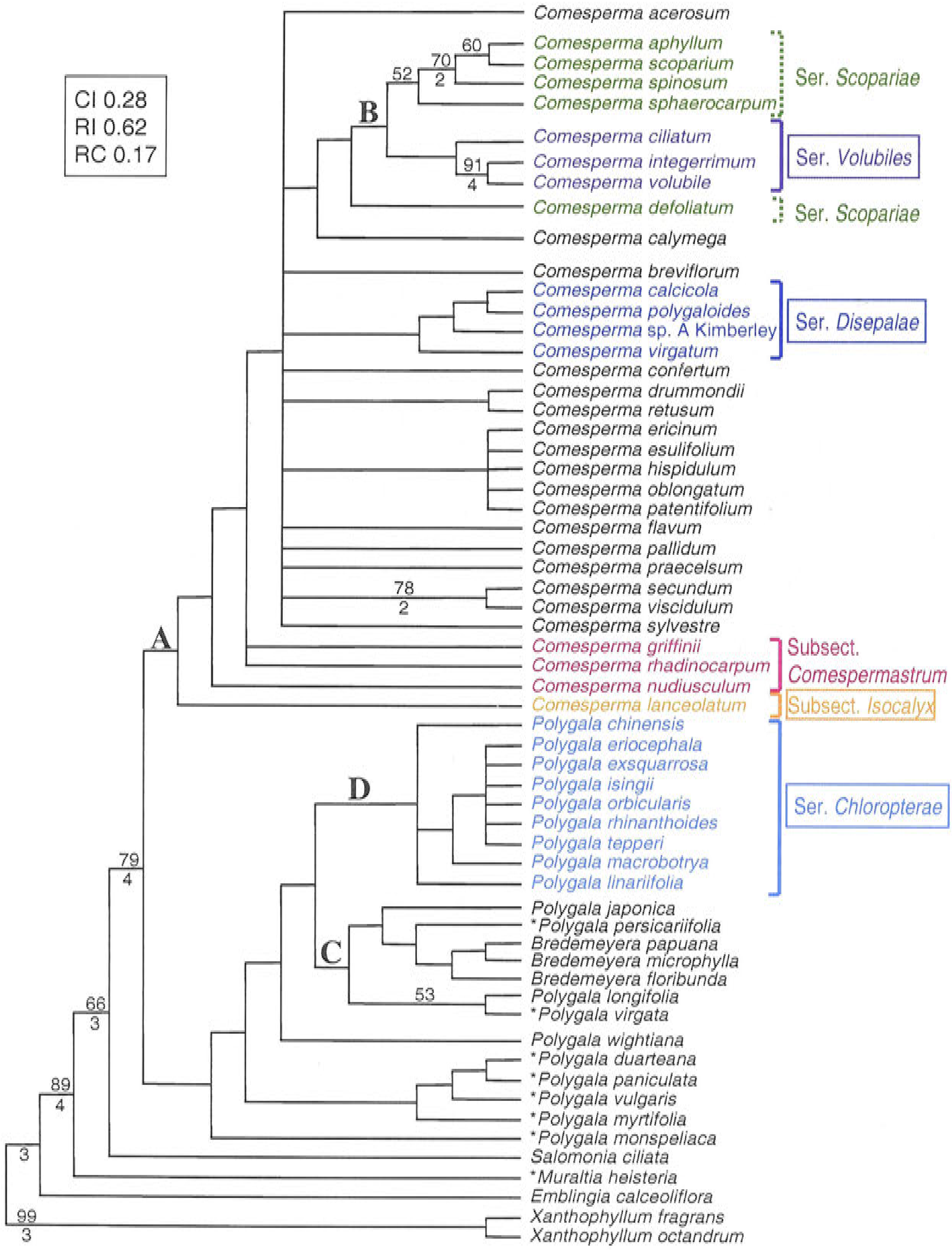

FIGURE 2.4: Strict consensus of 144 shortest trees of length 447 steps from unweighted maximum parsimony analysis of morphological data. Major nodes discussed in the text are marked A–D and some of the groups identified in Chodat (1896) are labelled. Those which are monophyletic are indicated by solid brackets and a box outline around their name, while non-monophyletic groups are enclosed in a dashed bracket and their names are not boxed. Any bootstrap values greater than 50% are also shown above the nodes, while Bremer decay indices over one are given below.

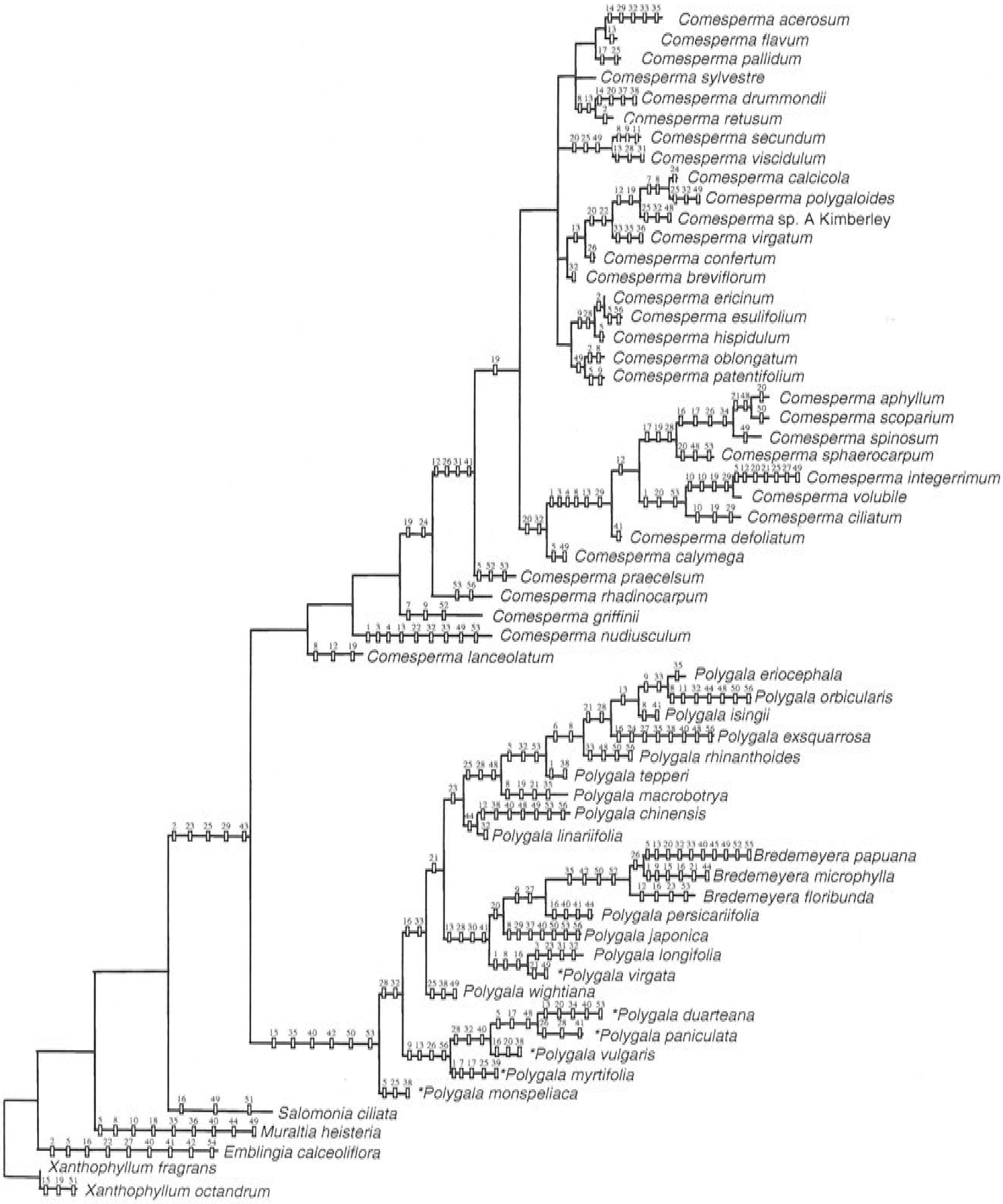

FIGURE 2.5: Tree diagram showing the mapping of the 56 morphological characters on the first of the 144 shortest trees from unweighted maximum parsimony analysis. Characters with unambiguous state changes in all trees are shown as bars with a number corresponding to the character list (Table 4) along the branch where the changes occur. Branches involved in soft polytomies or those with character state changes which differ over the shortest trees are not marked, as their changes cannot be unambiguously defined.

shape circular), #30 (apex of lateral sepals blunt) and #41 (stigma capitate and bilobed). However, *Bredemeyera* in this analysis is defined by unambiguous and non-reversed changes in four characters (shown in Fig. 2.5)—#35 (keel appendage absent), #42 (sepals lost after pollination), #50 (fruit wing absent) and #52 (coma present, arising from hilar area). Sister to the grouping of *Bredemeyera* within a clade of *Polygala* species is a largely unresolved clade of Australian and south-east Asian species (node D in Fig. 2.4, including all the members of series *Chloropterae* which were included in the analysis). *Polygala wightiana* is then sister to this pairing of *Chloropterae*+(other *Polygala* spp.+*Bredemeyera*) and a clade of weedy species from South America, Europe and southern Africa sister to the entire group. Finally, *Polygala monspeliaca* from Europe is placed as sister species to the remainder of the sample of that genus. *Salomonia*, *Muraltia* and *Emblingia* are then progressively distantly related to the (*Comesperma*(*Polygala*+*Bredemeyera*)) grouping.

Any bootstrap values over 50% are shown above the branches in the strict consensus tree in Figure 2.4, while any Bremer decay index values over one are given below the branches. It is immediately obvious that there is limited support for any of the resolution displayed in this tree. However, the values shown indicate that the "backbone" of the tree is reasonably well-supported, while the resolution at internal nodes is not. Thus, the grouping of *Comesperma*, *Polygala* and *Bredemeyera* together is strongly supported, as is their differentiation from *Salomonia*, *Muraltia* and *Emblingia*. In contrast, the clades within the former three genera are almost all unsupported. Three species pairs—*Polygala longifolia* and *P. virgata*, *Comesperma secundum* and *C. viscidulum*, and *C. integerrimum* and *C. volubile*—have bootstrap values over 50% and the latter two pairs also have decay index values of two or more.

Permutation Testing

Despite the limited resolution obtained using maximum parsimony, the PTP test indicated that the trees of length 447 steps obtained were significantly shorter than those obtained by randomising and reanalysing the dataset (P=0.01). The shortest trees produced from the randomised data were 654 steps long. The result means that trees of length 447 steps are unlikely to be obtained by chance alone. Results and implications of the T-PTP tests will be discussed in the Discussion section of this chapter.

Exploration of Characters

The consistency index (CI), retention index (RI) and rescaled consistency index (RC) scores for all characters over the 144 trees found in the unweighted maximum parsimony analysis are shown in Figure 2.6. The consistency index of all characters over the trees is only 0.28, which indicates that 72% of the change observed in the characters can be attributed to homoplasy. Only three characters showed complete consistency with the trees—#46 (ovules per locule), #47 (fruit type) and #51 (fruit dehiscence). A fourth character, #6 (stem hair type), was fully consistent with many of the trees, with an average CI of 0.88.

The retention index is higher at 0.62 over the 144 trees. This index removes the bias due to autapomorphies inherent in the consistency index—these have a CI of one but an RI of zero. It thus measures "the fraction of apparent synapomorphy in the character that is retained as synapomorphy on the tree" (Farris 1989, p. 418). It also corrects the CI for the effect of characters with many state changes, as it takes into account the maximum number of possible changes in that character. As the number of steps in a tree increases, RI decreases to zero in a linear manner while the CI decreases exponentially. For trees longer than the minimum possible number of steps for each character (no homoplasy, CI and RI both equal to one), RI is greater than CI and decreases more slowly to reach zero while CI approaches an asymptote above zero. This pattern is shown in Figure 2.6(a) and (b), with RI higher than CI in all characters except two. These two are #14 (leaf keel) and #36 (fertile stamen number), for which the RI is zero as both exhibit the maximum number of steps possible on all trees. The CI is thus quite misleading for these characters, with values of 0.25 and 0.67 respectively.

The rescaled consistency index for all characters over the 144 trees is very low at 0.17. This value is considered a better measure of the fit of characters on a tree than the CI, because characters with widely different fits to a tree can score the same CI just by virtue of different amounts of possible homoplasy in each character. The rescaled consistency index rescales the CI to a linear function so that when a character fits the tree as poorly as possible for that character, RC equals zero. This value was thus preferred for purposes of successive weighting, as discussed below.

As shown in Fig. 2.6a–c, only the three characters mentioned above (#46, #47, #51) have a CI, RI and RC of one. In other words, these characters map onto all the shortest trees without homoplasy and are not autapomorphies. Figure 2.6c in particular shows

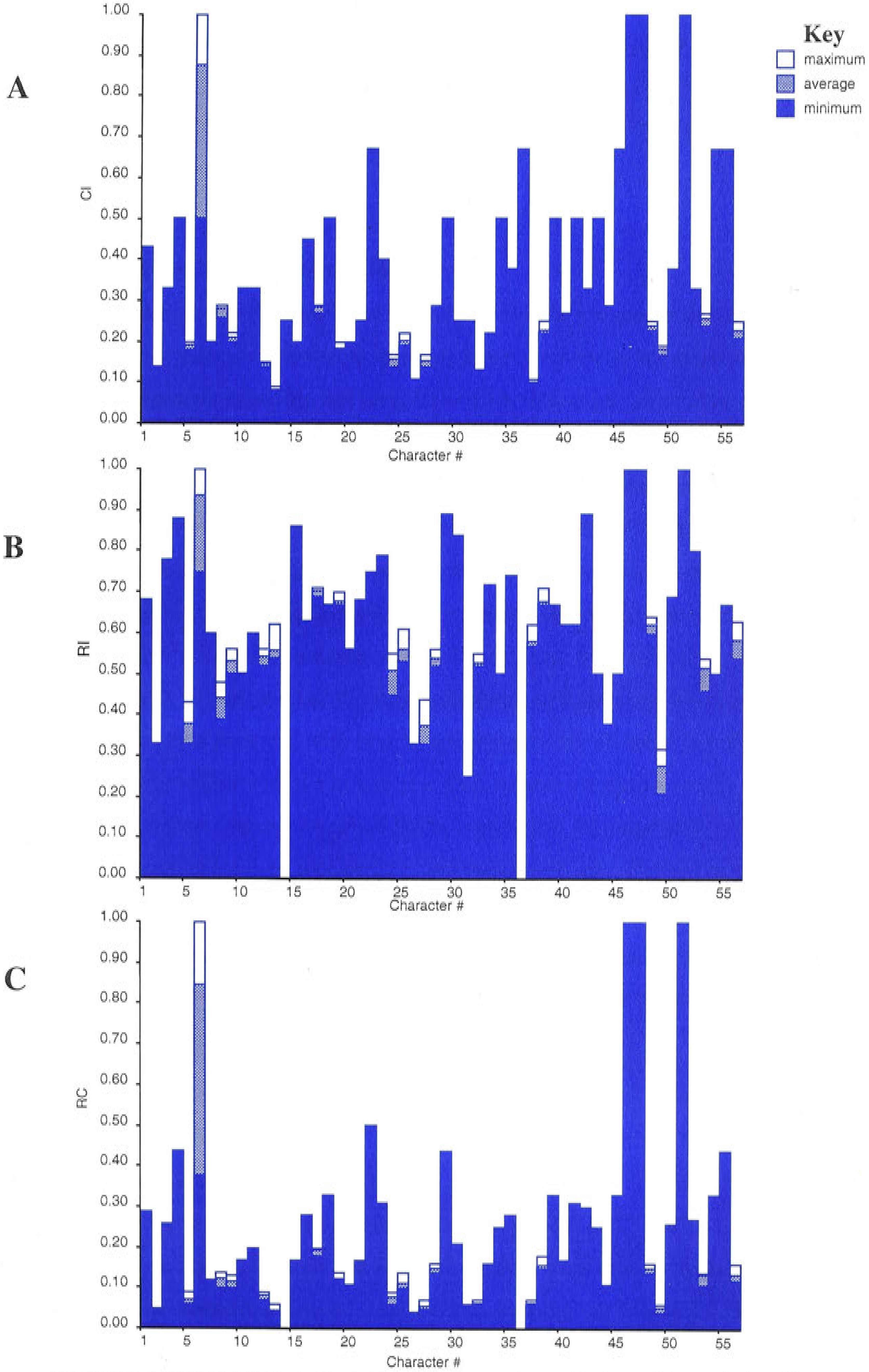

FIGURE 2.6: Maximum, average and minimum values of (a) consistency index (b) retention index and (c) rescaled consistency index over the 144 shortest trees obtained by unweighted maximum parsimony analysis of the 56 morphological characters scored.

the poor fit of the data to the trees, as only the three characters previously mentioned and #6 (stem hair type) have even an average RC of more then 0.5. The 0.5 level represents 50% homoplasy. Thus a high level of homoplasy is inherent in the morphological dataset.

Reverse Successive Weighting (RSW)

Reverse successive weighting did not identify a secondary signal in the morphological data for the Polygalaceae. However, this may have been due to the relatively low total number of characters available and their lack of resolution on the original tree. RSW was unable to build a second resolved tree, as only two characters were identified as being in significant conflict with the remainder of the dataset, and thus the analysis terminated at this point. The two conflicting characters were #19 (ratio of pedicel to flower length) and #32 (upper petals relative to keel). Removing these two characters and re-running the cladistic analysis did not significantly change the relationships identified in the initial strict consensus tree. Ninety-three trees of length 418 steps were found in the second analysis and the strict consensus of these was less resolved than that produced in the first analysis, with several species groups collapsed to polytomies rather than exhibiting conflict with the initial tree. One notable exception to this general pattern was the *Comesperma ericinum* clade, which was fully resolved in the second analysis, rather than forming a polytomy, and included *C. breviflorum* as sister to the remaining five species. The *ericinum* group is defined on this tree by a single unambiguous character—#13 (leaf tip mucro changing from absent to present)—and the grouping does not receive significant bootstrap support, so this difference in topology is unlikely to be significant.

Successive Weighting

Rescaled Consistency Index (RC)

Successive weighting using the rescaled consistency index (RC) settled immediately on four shortest trees of 75.76 steps and further iterations were unable to resolve the trees any further. The strict consensus of these trees is presented in Figure 2.7. This tree includes some of the same groupings found in the original strict consensus tree from unweighted parsimony, but, as expected, much greater resolution was achieved using this method. The strict consensus has only two polytomies and indicates that

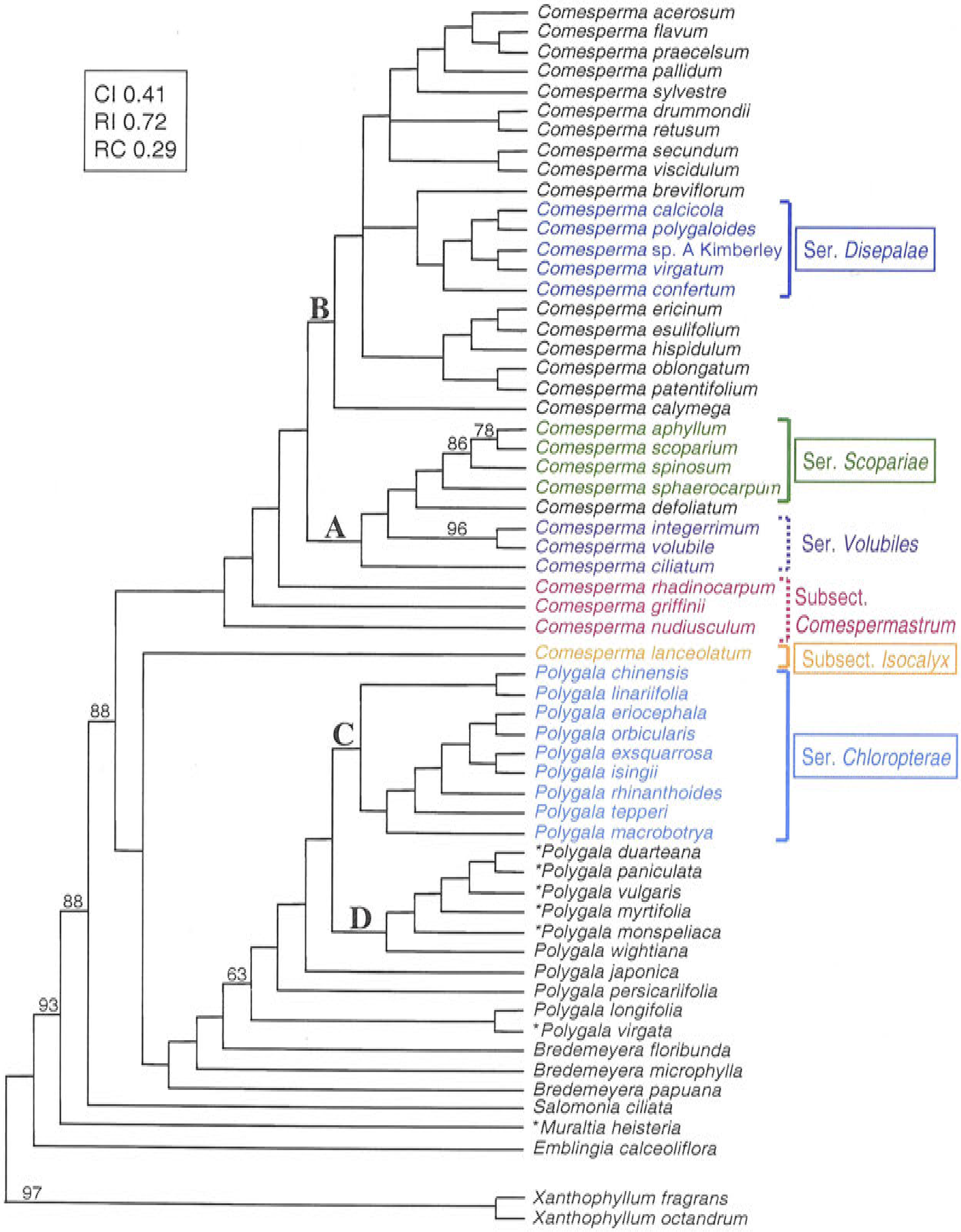

FIGURE 2.7: Strict consensus of four shortest trees of length 75.76 steps from maximum parsimony analysis of morphological data with successive weighting by mean rescaled consistency index (RC) values. Major nodes discussed in the text are marked A–D and clades matching the subsections and series of Chodat (1896) are bracketed and labelled. Those which are monophyletic are indicated by solid brackets and a box outline around their name, while non-monophyletic groups are enclosed in dashed brackets and their names are not boxed. Any bootstrap values greater than 50% are also shown above the nodes.

Comesperma is monophyletic only with the exception of *C. lanceolatum*. This species instead falls sister to a grouping of *Polygala* and *Bredemeyera* species. In this phylogeny, *Polygala* is a monophyletic group progressively sister to the three *Bredemeyera* species included in the analysis. *Bredemeyera* itself is thus not a monophyletic group and is not most closely related to *Comesperma*. The broader grouping of *Comesperma*, *Bredemeyera* and *Polygala* is then progressively more distantly related to *Salomonia*, *Muraltia* and *Emblingia*.

Within *Comesperma* (minus *C. lanceolatum*), several groupings are shown. Similarly to the unweighted MP strict consensus, the climbing and leafless species are closely related to each other (node A in Fig. 2.7). However, in this tree they do not form monophyletic sister groups. Instead the leafless species are a monophyletic group that is sister to a clade consisting of *Comesperma volubile* and *C. integerrimum*. *Comesperma ciliatum* then falls as sister to this entire grouping. There is a large group sister to the climber/leafless clade that includes most of the remaining taxa in the genus (node B in Fig. 2.7). Much greater resolution is obtained within this second group of species than is shown in the unweighted MP tree, although the relationships between the major groups in this clade are still unresolved. There are three monophyletic groups represented. The *Comesperma ericinum* group emerges as a fully resolved clade. Surprisingly *Comesperma breviflorum*, another segregate from *C. ericinum sens. lat.*, is not most closely related to this group and instead falls as sister to a group of species with fused lower sepals (series *Disepalae, sensu* Chodat (1896)). A third grouping consists of several shrubby species, which would all be classified in Chodat's (1896) series *Confertae*. *Comesperma calymega* is then sister to this entire group. *Comesperma rhadinocarpum*, *C. griffinii* and *C. nudiusculum* are then progressively most closely related to the rest of the genus.

Outside the *Comesperma* clade many of the same species groups shown in the unweighted analysis appear, although the relationships between the groups differ widely. *Polygala+Bredemeyera* are a monophyletic group in this analysis with *Comesperma lanceolatum* as sister. However, the only character unambiguously supporting this grouping is #19 (ratio of pedicel to flower length), one of the characters identified as being in conflict with the primary signal in the dataset via reverse successive weighting. Within *Polygala sens. strict.*, the Australian/south-east Asian species from series *Chloropterae* which were a polytomy in the unweighted analysis form a fully resolved clade here (node C in Fig. 2.7). A second grouping of mainly weedy species (node D in Fig. 2.7) forms the sister group to this series. *Polygala japonica*, *P. persicariifolia*, and a pairing of *P. longifolia* with *P. virgata* are then progressively more distantly related to the two major clades. Finally, the three *Bredemeyera* species are each progressively distantly related *to Polygala sens. strict.*, with *B. papuana* from New Guinea being the sister group to all the remaining taxa in this clade.

Weighting by Consistency and Retention Indices

Weighting the data via the consistency and retention indices also resulted in four trees after one search and these could not be further resolved. However, the strict consensus of the trees from unweighted parsimony and the three weighting schemes differed significantly. The results of weighting with CI and RI will only be briefly summarised here, with an emphasis on the groups that conflict with the previous analyses.

The relationships within the main species groups are similar, but the placement of *Bredemeyera* and *Comesperma lanceolatum* varies in all the results. Reweighting characters with their CI values results in a strict consensus tree which shows *Comesperma* as monophyletic only with the inclusion of a clade of the three *Bredemeyera* species in the analysis. The *Bredemeyera* species form a monophyletic sister group to *Comesperma sens. strict.*, with *C. lanceolatum* then sister to this entire group. *Polygala* forms a monophyletic group in this analysis and the clades are almost identically arranged to those from the unweighted analysis (Fig. 2.4), albeit with greater resolution and obviously with the exclusion of *Bredemeyera*. The only other noticeable conflict between this and the unweighted analysis is the movement of *Polygala wightiana* to a position sister to the *Chloropterae* clade in this analysis.

In contrast, reweighting by RI gives a monophyletic but rather internally unresolved *Comesperma*, with *C. lanceolatum* sister to the remainder of the genus. *Polygala* is not monophyletic unless *Bredemeyera* is included and the three species are nested within *Polygala* in the same position as the unweighted analysis.

DISCUSSION

Given the low levels of bootstrap and Bremer decay index support throughout the trees obtained from unweighted, weighted and profile parsimony analyses based on these morphological data, it is difficult to make statements about the relationships within the Australian Polygalaceae with any confidence. Some of the generic delimitations are supported, but *Comesperma*, *Polygala* and *Bredemeyera* form an unresolved grouping and their separation from *Salomonia*, *Muraltia* and *Emblingia* may be at least partially the result of the low numbers of taxa in these latter genera found in Australia. Since only one representative of each of these three genera are coded in this analysis, the level of homoplasy displayed in the characters supporting these nodes may be lower than it would be with a larger sample of the diversity in the genus (although this is of course not possible for the monotypic *Emblingia*). All three of the fully consistent characters change only on nodes leading up to the *Comesperma*, *Polygala* and *Bredemeyera* grouping. This explains the strong bootstrap and decay index support on the "backbone" of the tree.

The three fully consistent characters are all based on the fruit and these characters have traditionally been used to distinguish genera. The remainder of the tree is simply based on very homoplasious characters that do not provide a clear hierarchical signal, rather than being a strong tree with a few troublesome taxa that may be placed in several disparate positions and thus collapse the strict consensus.

Lack of hierarchical signal in a dataset may be the result of several confounding factors. One of the assumptions of cladistic parsimony is that the evolutionary history of a group of organisms has a dichotomous branching structure. This assumption is violated if the taxa have a history of hybridisation. Another problem is convergent evolution, where unrelated taxa develop a similarity in morphology that is not a result of a close historical relationship. Adaptations to a certain pollinator or niche, for example, can cause two relatively unrelated taxa to independently gain the same morphological traits. By contrast, groups of relatively unrelated taxa may also both independently lose a feature. When scoring morphological character states, any or all of these factors can confound the assessments of homology made. Another problem with these data is that the total number of characters scored is rather low. In general, sampling a greater number of cladistically informative characters provides more information which can be used to resolve a tree built from the data. Studies have conclusively shown that scoring a sufficient number of characters is vital to accurate phylogenetic estimation, with accuracy increasing with larger numbers of characters up to a certain point (Hillis *et al.* 1994; Hillis 1998). In the analyses of morphological data described above, the number of end taxa exceeds the number of characters and the vast majority of the characters are binary. Although scoring more characters may have provided greater resolution, a thorough survey of a large number of specimens was made and no further informative characters became obvious.

Weighting the characters by various measures of their fit on the initial trees (CI, RI and RC) produces more resolved phylogenies for the most part, but these trees still receive very little support from measures such as the bootstrap and decay index. The very high level of homoplasy in the characters means that reweighting merely maintains the backbone of the tree and does not produce any robust resolution in other areas. Also if the data set is biased in some way initially, such weighting will merely serve to reinforce potentially incorrect branching patterns. To use such weighting schemes, it is necessary to accept an assumption that the character states are true reflections of the phylogenetic history of the taxa and that the homoplasy or "noise" in the dataset is of a relatively low level and distributed in a random fashion. Given that only a handful of characters in the dataset have high RC values, the fit of the data to the initial trees is quite poor and thus the assumptions inherent in successive weighting techniques may not be met for this dataset. It is also not clear which measure is the best to use for such weighting, as the calculation of each statistic emphasises different aspects of the characters used and thus upweights a different set of these characters. For example, weighting by the RI gives higher weights to a much greater number of the characters than does the CI or RC. It is thus unsurprising that the different weighting schemes result in quite different topologies. It seemed most reasonable to discuss the results of weighting with RC in more detail than those trees obtained via the other weighting schemes, as this measure takes into account the fit of the character as well as its relative contribution to producing a given topology.

Despite the acknowledged limitations of the data available, there are several interesting patterns observed in the cladistic analyses and these can be favourably compared to the existing sub-generic classification in several areas.

Overall Patterns

Unweighted parsimony indicates that *Comesperma* is a monophyletic group. The majority of the genus is also monophyletic in the weighted analyses also, differing only in the inclusion or exclusion of *Comesperma lanceolatum* and/or *Bredemeyera*. Within *Polygala*, series *Chloropterae* appears in all analyses, although the internal relationships differ slightly depending on the weighting scheme used. *Bredemeyera* is a monophyletic group in most of the analyses, except when weighted by RC.

The monophyly of *Comesperma* and the placement of *C. lanceolatum*

The majority of the analyses place *Comesperma lanceolatum* as sister to the remainder of *Comesperma*. The characters that distinguish *C. lanceolatum* in all the shortest trees from unweighted parsimony (Fig. 2.5) are #8 (leaf shape), #12 (leaf margin deflection) and #19 (ratio of pedicel length to flower). Interestingly, none of these characters were used by Chodat (1896) when defining subsection *Isocalyx*, of which *Comesperma lanceolatum* is the only member. The characters that were used (including stigma type) change along this branch in only some of the shortest trees. This indicates that although Chodat correctly identified *Comesperma lanceolatum* as differing markedly from the remainder of the genus, his defining characters are not primarily responsible for the topologies observed.

All analyses show the climbing and leafless species as sister groups, although some differ in the inclusion of *Comesperma defoliatum*. Both groups also appear monophyletic and a T-PTP test of *Volubiles* and *Scopariae* (including *C. defoliatum*) as monophyletic sister groups could not reject this relationship (P=1.00). Characters supporting the *Volubiles* group on all the shortest unweighted trees (Fig. 2.5) are: #1 plant habit (twining climber), #20 outer sepal shape (triangular) and #53 seed shape (oblong). Characters supporting the *Scopariae* grouping on all trees (Fig. 2.5) are: #17 inflorescence shape (solitary or scattered flowers), #19 ratio of pedicel to flower length (pedicel much shorter than flower/sub-sessile), #28 lateral sepal shape (orbiculate/circular). The *Volubiles* group may thus be defined by a greater number of characters than simply plant habit, while *Scopariae* species are not defined by the traditional characters of a *Polygala*-like capsule and lack of a coma on the seed. *Comesperma spinosum* is clearly a member of this group on every other character, but it possesses both an elongated fruit and comose seeds. The sister group relationship between these two groups is perhaps unsurprising, given the shared characters of ribbed stems and being almost or completely leafless, but it has not previously been suggested.

The series *Disepalae* also appears in all analyses as monophyletic, supported unsurprisingly (Fig. 2.5) by characters #20 (outer sepals ovate) and #22 (lower pair of outer sepals connate). Thus the defining character for this series is synapomorphic for the group in these analyses. A T-PTP test also indicates support for this clade (P=0.02).

Finally, the series *Confertae* and section *Comespermastrum* proposed by Chodat (1896) are not supported as monophyletic groups by these analyses. Members of the *Confertae* appear in a number of different clades and some are actually sister to members of the *Disepalae*. Subsection *Comespermastrum* appears as a monophyletic group only in the analysis with characters weighted by CI. In other analyses, the relationship of the species to each other is not resolved, but they fall sister to the majority of the genus.

Comesperma appears as a monophyletic group in all analyses, albeit sometimes with the exception of *C. lanceolatum* or the inclusion of *Bredemeyera*. Also, if *Comesperma lanceolatum* is excluded, *Bredemeyera* forms a monophyletic sister group to *Comesperma* rather than rendering it polyphyletic.

***Polygala* and the *Chloropterae* Clade**

The *Polygala* clade is supported on all the shortest trees (Fig. 2.5) by six characters—#15 (leaf surface similarity changing from concolorous to discolorous), #35 (keel appendage changing from absent to a finely divided crest), #40 (style bending changing from gently curved to distinctly hooked), #42 (retention of lateral sepals after pollination changing from absent to present), #50 (fruit wing changing from absent to narrow and present on both margins) and #53 (seed shape changing from ovoid to oblong). Many of these characters have previously been used to define the tribe Polygaleae, particularly the crested keel and retention of lateral sepals.

The predominantly Australian "endemic" clade of *Polygala* species is supported by only one character in all shortest trees (Fig. 2.5)—#23 lateral sepal modification (enlarged but not petaloid). This coincides with the defining characters for the series *Chloropterae* as described by Chodat (1896). He also listed axillary racemes, but these are found in other sections in this dataset. It does seem clear that the endemic taxa are a monophyletic group sister to the remainder of the genus in Australia, which are primarily weedy taxa that have been introduced or dispersed.

Position of *Bredemeyera*

T-PTP tests of an arrangement with *Comesperma* (including *C. lanceolatum*) and *Bredemeyera* as monophyletic sister taxa indicate that this relationship cannot be rejected based on these data (P=1.00). However, a T-PTP test of *Polygala* and *Bredemeyera* as monophyletic sister groups shows exactly the same result. It is thus impossible to clarify which genus is the closest to *Comesperma*, but it should be noted that trees which support a monophyletic *Bredemeyera* that does not nest within either of the other genera are not significantly longer than the strict consensus. The unexpected placement of *Bredemeyera* deep within *Polygala*, which has never been suggested by traditional classification, thus cannot be viewed with much confidence. Given that evidence is mounting that both *Bredemeyera* and *Polygala* are polyphyletic assemblages (Persson 2001), the placement may be an artefact of taxon sampling, since the dataset does not include representatives from all the tribes in *Polygala*, being biased towards taxa found in Australia.

CONCLUSIONS

Weak support for a putative phylogeny does not necessarily indicate that the pattern observed is incorrect but it does limit the amount of confidence that can be placed in the relationships between taxa and thus the conclusions that can be drawn from them. Tests of these data seem to confirm that they are equivocal, and the lack of a strong signal does not allow much comment on internal relationships. Nonetheless, while the data do not provide a robust or fully-resolved phylogeny, they do indicate interesting groupings within the family. Although the level of phylogenetic signal in the morphological data appears to be low, the trees produced from these analyses are still useful in examining the evolution of the Australian Polygalaceae. The congruence of these data with the phylogenetic pattern revealed in analyses of molecular data from the family will thus be explored in Chapter 4.

Chapter Three:
Molecular Phylogeny

INTRODUCTION

Use of Molecular Data in Systematics

Molecular systematics refers to the use of macromolecules (DNA, RNA, proteins, isozymes etc.) to infer the phylogeny of a group of organisms. The use of molecular data in systematic studies has become prevalent in the last twenty years with increasing access to and automation of technology such as DNA sequencing. This has proved to be a powerful tool in phylogenetic reconstruction, enabling researchers to gather and analyse large datasets from a wide variety of taxa.

Molecular data were at first thought likely to be a "truer" reflection of evolutionary history than morphological characters, as they have a direct genetic basis and were thus considered to be less subject to problems such as convergence and environmental plasticity (e.g. Lamboy 1994; Hedges & Maxson 1996; Givnish & Sytsma 1997). This led to a debate about the "superiority" of one data source over another, but this is now generally recognised as a spurious argument (Hillis 1987; Benton 1999; Hillis & Wiens 2000). Molecular data are subject to some of the same problems encountered when using morphology and also to some unique considerations. Gathering molecular data is still considerably more costly than scoring morphological characters, so it is usually possible to examine more aspects of morphology than molecular regions in a given time. However, the far greater number of molecular characters available may mean a lower end cost per informative character in an efficiently targeted study. Morphological characters are likely to be a product of a number of different genes while most molecular studies are only able to examine a small number of genes or regions. By contrast, molecular data are often considered to be more objective than those obtained from morphology, because the characters and their states are defined and scored in a straightforward and repeatable fashion. Although some debate continues over the relative merits of "molecules versus morphology", it thus seems most productive to use them in conjunction depending on the question of interest.

Some things to be considered when gathering and using molecular data include the rate of evolutionary change, the independence of regions within a genome and secondary structure constraints. The inherent transition/transversion bias of DNA is well known, but the rate of substitutional change can also vary widely both within and between regions of a DNA sequence. This may depend on whether a region is functionally constrained such that substitutions in the sequence would affect transcription or the function of the transcript, but even regions of a similar class may have quite different substitution rates. For example, Small *et al.* (1998) found in a study of tetraploid *Gossypium* species that the *trnL-trnF* intergenic spacer had a 0.12% divergence (measured by the mean nucleotide difference) between the ingroup and the outgroup. By contrast, the *trnT-trnL* spacer had eightfold greater divergence (0.96%) between the same groups. The wide variation in substitution rates and the subsequent differences in base composition can violate the assumptions of some methods of phylogenetic analysis. (This variation is likely to occur in morphological data also, but it is much more difficult to measure and account for in such cases, as the genetic basis of many morphological characters is poorly understood.) However, it is also an extremely important feature for systematic studies, as it allows researchers to choose a region that has informative variation at the desired taxonomic level. Choice of an appropriate region is thus crucial when undertaking a phylogenetic study using DNA sequences. Pilot studies are useful to assess the level of variation in a given region between the taxa of interest. A region with a mixture of faster- and slower-evolving segments can be particularly useful for phylogenetic reconstruction (e.g. the *ndhF* gene; Kim & Jansen 1995), as the differences in rate within the marker provide informative variation at both lower and higher taxonomic levels.

Independence of data is an important consideration when finding characters for any phylogenetic study, as correlated characters can violate the assumptions of some of the methods used to analyse the data. In molecular studies, it may be easier to predict and therefore to minimise than in studies based on morphology, as linkages between genetic regions are somewhat better understood. As an extreme example, all markers from the chloroplast may be considered correlated as they are all contained on a single small (120–200 kbp in land plants; Soltis & Soltis 1998) circular molecule and inherited as a single linkage group (Doyle 1992). At a smaller scale, correlations between regions may be a result of secondary structure. For example, bases forming part of a stem structure are paired with complementary bases on the other side of the stem. These pairings tend to be highly conserved, so that a change in one base affects the probability of change in another (e.g. Wheeler & Honeycutt 1988). Such features should be taken into account when choosing a method to build phylogenetic trees.

The trees produced from an analysis of molecular data are only gene trees, as they are hypotheses of the relationships among the genes under study. It is important to distinguish between these and a tree representing the evolutionary history of the species involved (species trees), as there may be discordance between the topologies. Three main sources of conflict exist among gene trees and between these and species trees—gene duplication, lineage sorting and horizontal gene transfer (Doyle 1992; Maddison 1997). Due to these processes, it may not be valid to convert a gene tree to a species tree simply by replacing the name of the sequence with the name of the taxon from which it was obtained. Page and Charleston (1997) propose a method to "reconcile" gene trees with organismal phylogenies using the computer program GeneTree (Page 1998), which postulates the minimum number of gene duplications and losses necessary to explain the incongruence between two trees. The method requires confidence that both trees are correct representations of the relationships among species and genes respectively, a requirement that is difficult to meet if the true phylogeny is unknown. In this case, gene tree parsimony methods suggest that if the species tree is unknown, the species tree that minimises the number of gene duplications and losses or other "conflict-causing events" across a set of gene trees is preferred (Slowinski & Page 1999). A second approach is uninode coding, proposed by Simmons *et al.* (2000), where data from both duplicated and unduplicated gene copies are combined in phylogenetic analyses of taxa. The relative merits of both methods are still being debated (Simmons & Freudenstein 2002; Cotton & Page in press), but these approaches are developing rapidly. In practice, many researchers adopt a "corroboration" approach, using sequences from independent genetic loci (e.g. nuclear and chloroplast genomes). A similar topology recovered from two or more independent DNA regions is considered more likely to reflect historical processes and thus lends confidence to an hypothesis of species relationships. Again, more rigorous methods to evaluate and score the level of corroboration among datasets are becoming available (e.g. Chen *et al.* 2003).

Previous Molecular Studies in Polygalaceae

The Polygalaceae has not been a particular focus for molecular systematics. Until recently, the only published sequences were those from *rbcL*, 18S rDNA and *atpB* used by the Angiosperm Phylogeny Group (1998) and in other large-scale studies of the relationships between angiosperm plant families (e.g. Chase *et al.* 1993; Morgan *et al.* 1994; Källersjö *et al.* 1998; Soltis *et al.* 2000). Such studies were the first indication that the Polygalaceae and Fabaceae were quite closely related and possibly even sister families (e.g. Doyle *et al.* 1997), countering the traditional assumption that their morphological similarities are a result of convergent evolution.

More recent studies are divided on the detailed relationships among the families in the Order Fabales, now considered to consist of four families—Fabaceae, Surianaceae, Polygalaceae and Quillajaceae. Persson (2001) used the *trnL/F* chloroplast region in a family-wide study of the Polygalaceae and including several outgroups from within the Eurosids I clade of Soltis et al. (2000). His results indicated that Fabaceae and Quillajaceae are sister families, with Surianaceae next most closely related to these two and with Polygalaceae being sister to the remainder of the order. However, Persson was primarily interested in relationships within the Polygalaceae, so his sampling of the outgroup taxa was necessarily limited. A study designed to identify the sister group to the Fabaceae (F.Forest, *pers. comm.* 2003) used a broader sample of taxa and showed a different pattern. Forest gathered sequences from the *trnL* intron, the protein-coding *rbcL* gene, and the 26S ribosomal RNA gene. The data were combined and analysed in a maximum likelihood framework. The results indicated a sister group relationship between the Fabaceae and a clade comprised of the Surianaceae and Polygalaceae. The single species in the Quillajaceae, *Quillaja saponaria* Molina, was then sister to the remainder of the order. However, this topology received only weak support. Thus, while the relationships within the order Fabales based on molecular data have not been satisfactorily resolved to date, it is well-supported as a monophyletic group as currently defined, as is the Polygalaceae *sens. lat.* The Fabales has also been fairly conclusively placed in the eurosids with related orders including Fagales, Rosales, Cucurbitales and Malpighiales (Soltis *et al.* 2000).

The study by Persson (2001) mentioned above is the first large-scale molecular phylogeny undertaken on the Polygalaceae. He sampled widely across the family, including taxa from 64 species in all currently recognised genera except *Eriandra* and *Epirixanthes* as these were not successfully sequenced. This represents less than 10% of the species, but sampling was targeted to include all the previously published subgeneric groups. All tribes and a majority of the subgenera and sections were represented. An equally weighted maximum parsimony analysis was carried out and five thousand of the shortest trees found were saved. The strict consensus of these trees shows that the Polygalaceae *sens. lat.* (including Xanthophyllaceae) is strongly monophyletic, with the node appearing in 100% of the 10000 bootstrap replicates.

Resolution within the family was limited in the strict consensus tree obtained by Persson (2001), but it does contain several interesting groupings. *Xanthophyllum* has a well-supported sister group relationship with the remainder of the family, so its recognition as either a separate tribe or segregate family may be justified. Within the Polygalaceae *sens. strict.* (bootstrap score 92%), the tribes Moutabeae and Carpolobieae form an unresolved group sister to the tribe Polygaleae. The monophyly of and relationships between the Moutabeae and Carpolobieae cannot be confirmed or discounted from these results, although the Polygaleae does have reasonable support for monophyly (77% bootstrap value). Within the Polygaleae, two major well-supported clades appear. Persson (2001)designated these as Polygaleae I (99% bootstrap value) and Polygaleae II (97% bootstrap value). The Polygaleae I clade is fully resolved and consists of *Polygala* sections *Acanthocladus* and *Hebecarpa* and *Bredemeyera* section *Bredemeyera* (*sensu* Chodat 1896), all as monophyletic groups. It also includes a single species from *Bredemeyera* section *Hualania* (*Bredemeyera microphylla*), grouping with *Polygala* section *Hebecarpa* rather than with other members of *Bredemeyera*. Polygaleae II is largely unresolved, but indicates many of the genera included are monophyletic, with the exception of both *Polygala* and *Bredemeyera*. Notably, the three species of *Comesperma* group together and two of the species (*C. hispidulum* and *C. secundum*) share a 188 base-pair inversion relative to the other sequences. However, since *Comesperma* is included within the large Polygaleae II polytomy, no inferences can be drawn about the genus to which it is most closely related. Both *Bredemeyera* and *Polygala* are polyphyletic in Persson's analysis, appearing in both the major Polygaleae clades. However, on the basis of these results, *Comesperma* could be closely related to *Bredemeyera colletioides*, but is unlikely to be sister to the remainder of the genus, as all other members of *Bredemeyera* sampled fall within the Polygaleae I clade. Given the polyphyly of *Bredemeyera sens. lat.*, in contrast to the strong support for a monophyletic *Comesperma*, the most conservative option based on these results would be to treat *Comesperma* as a separate genus until further data are available.

AIMS

The primary aim of this section of the project was to construct putative phylogenies of the Australian Polygalaceae using two DNA regions, one nuclear and one from the chloroplast genome. The results from analysis of these data using both maximum parsimony and Bayesian inference could then be compared to investigate common patterns.

METHODS

Choice of regions for this study

Since it is desirable to choose regions from at least two different genomes in order to compare the topologies obtained from each, one nuclear and one chloroplast marker were selected. The nuclear marker used consists of two internal transcribed spacers (ITS1 and ITS2) and the included 5.8S gene that encodes part of the large ribosomal subunit (structure and priming sites shown in Figure 3.1a). As the name implies, the spacer regions are transcribed but do not form a functional part of the mature ribosomes. Instead they appear to play a role in the maturation and processing of the adjacent rRNAs—deletions or mutations in the spacers have been shown to decrease or inhibit production of rRNAs for both small and large ribosomal subunits (reviewed in Baldwin *et al.* 1995). This hypothetical role seems to be borne out by the similarities in size and base composition observed in the spacer regions across distantly related angiosperm taxa, which may indicate that they are under some evolutionary constraint. As a result, this marker provided a combination of highly conserved genic sequence (5.8S) and some conserved and extremely variable regions within the two spacers. It was hoped these would provide useful information at the taxonomic level of interest. The high copy number of rDNA (Rogers & Bendich 1987) usually makes it easy to amplify and primers from within the conserved flanking ribosomal genes (18S and 26S) were readily available. However, the G+C richness of the ITS regions (50–60% guanine and cytosine in some genera within the Fabaceae: Schiebel & Hemleben 1989, Yokota *et al.* 1989, Wojciechowski *et al.* 1993; but only 48% in the Australian genus *Daviesia* Sm.: Crisp & Cook (2003)) and their inherent secondary structure (Mai & Coleman 1997) can cause difficulties in amplification and sequencing. Another issue is the possible presence of divergent paralogous copies of the ITS region within an individual, which means that in some lineages sampling may pick up sequences with different evolutionary histories. However, nuclear ribosomal DNA is arranged in tandem repeats and usually undergoes rapid concerted evolution (Arnheim 1983; Hillis *et al.* 1991), meaning that divergent paralogues are usually homogenised over time. Direct sequencing can still aid in the detection of taxa with non-homogenised paralogues, as the superpositioning of two or more sequence types is usually visible on a

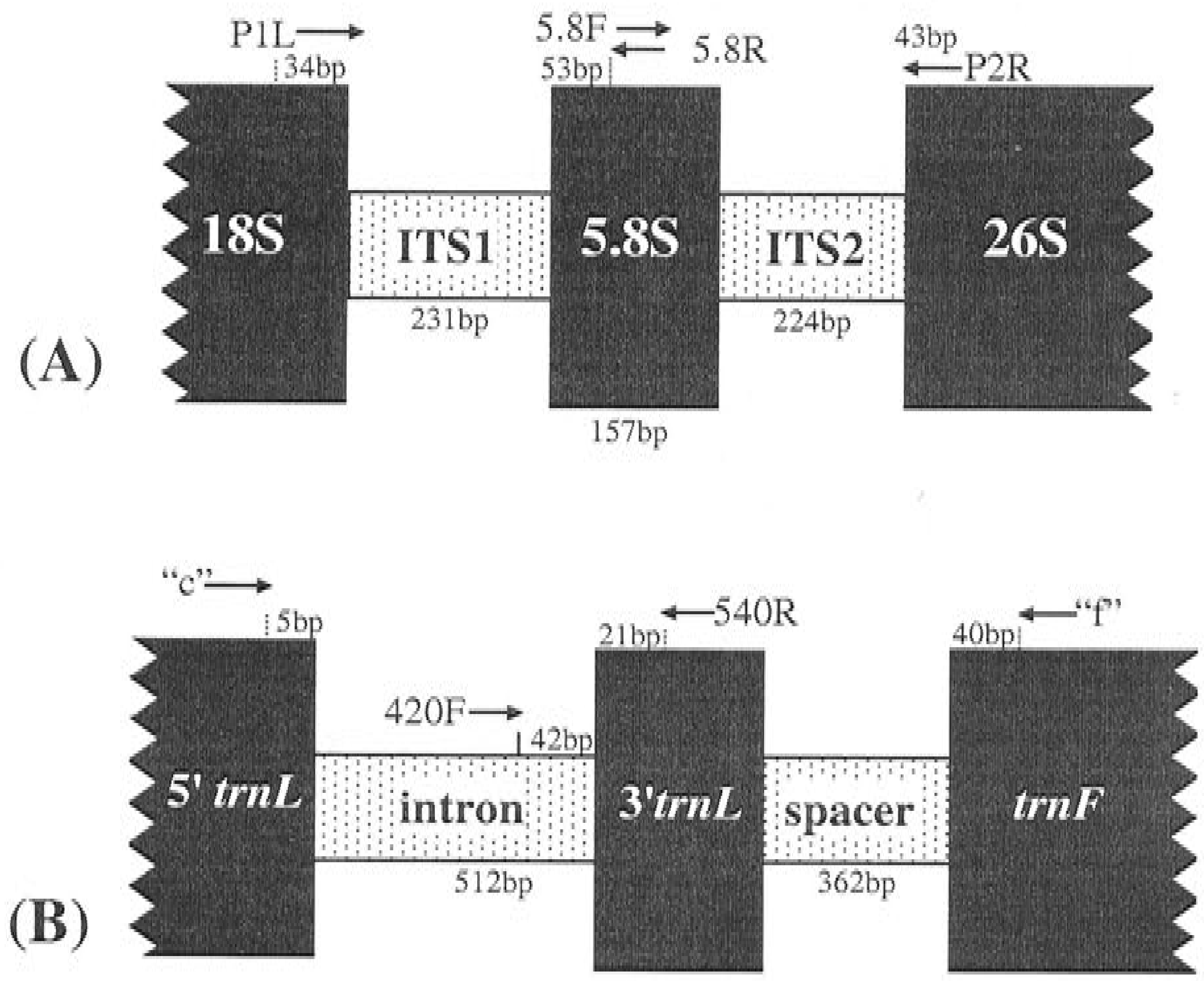

FIGURE 3.1: Structure of (a) the nuclear ribosomal ITS region and (b) the chloroplast *trnL/F* region used as markers in this study (not to scale). Arrows show position and direction of the primers used.

electropherogram. In such taxa, cloning and sequencing of each individual sequence type is required (Ritland *et al.* 1993; Sanderson & Doyle 1992).

The chloroplast marker targeted is part of the *trnL-trnF* region (Taberlet *et al.* 1991), and includes an intron in the *trnL* (transfer RNA) gene, the 3' *trnL* exon and the *trnL/F* intergenic spacer (structure and priming sites shown in Figure 3.1b). Like ITS, this marker is largely non-coding DNA, albeit with a combination of conserved and variable regions. Both the spacer and intron have been successfully used to infer phylogeny at the level of family and below in plants (e.g. within a genus in gentians by Gielly and Taberlet 1994, 1996 within an orchid tribe by Whitten *et al.* 2000; between two legume tribes Mirbelieae and Bossiaeeae by Crisp & Cook (in press); between tribes within the Asteraceae by Bayer & Starr 1998; within the three families now considered to comprise the Apocynaceae *sens. lat.* by Potgieter & Albert 2001). The *trnL/F* region is also subject to several insertion-deletion mutations (indels) and these have proved useful as additional characters in phylogenetic analyses and are often found to delimit clades (e.g. Hauk *et al.* 1997). They need to be interpreted and coded appropriately when constructing an alignment in order to ensure that the sequences have the correct positional homology, as they may cause significant length variation between sequences. The *trnL/F* region is usually easy to amplify due to the proximity of highly conserved genes in which primers can be sited. For this study, it had the added advantage of having been used in a family-wide phylogeny by Persson (2001) and the sequences were available in Genbank. They could thus be used to supplement the sequences gathered for the Australian Polygalaceae.

Extraction, Amplification and Sequencing

For the most part, DNA was extracted from leaf and bud material that had been preserved in a hexadecyltrimethylammonium bromide (CTAB)-saline solution, following Rogstad (1992). In cases where no other material was available, extractions were made from dried herbarium specimens. These specimens were chosen on the basis of collection date, appearance, and amount of available leaf material in order to maximise the possibility of extracting useful DNA while preserving the vouchers.

The extraction methods used are a modification of those given in Doyle & Doyle (1987), scaled to fit into 1.5mL Eppendorf tubes. Leaves of taxa that proved difficult to amplify were ground in liquid nitrogen before incubation, but the vast majority were simply ground directly in the CTAB buffer. After drying, the DNA pellet was resuspended in 30 μL of sterile water and 2 μL of this solution was run out on a 1% agarose gel to check the concentration of the DNA against a standard bacterial size marker (*Spp*1-*Eco*RI). Extracted DNA was generally used undiluted in polymerase chain reactions. Rarely the extracted DNA appeared much brighter than the marker and was thus diluted with sterile water, with dilutions ranging from 1/10 to 1/100.

The total PCR volume was 25 μL per tube, consisting of: 3 μL of 25 mM $MgCl_2$, 2.5 μL 10× Perkin-Elmer reaction buffer, 2 μL of 2 mM dNTPs, 0.5 μL of of each primer at 10 μM, 1 μL (0.2U) of Perkin-Elmer AmpliTaq DNA polymerase (which had been diluted 1 in 5 with storage buffer), 2 μL of template DNA and 13.5 μL sterile water. Reactions were run on a Corbett Research PC-960C cooled thermal cycler, using a "touch-down" procedure (Table 3.1). The temperature was progressively reduced to 45°C and the majority of the cycles carried out at this temperature. Three microlitres of the products were mixed with a loading dye and run out on a 1% agarose gel with the *Spp*1-*Eco*RI marker to determine the size and clarity of the fragment obtained.

Cleaning of PCR products was dependent on the clarity of the band obtained after running out on the gel. Products with bands that were distinct were cleaned using a simple ammonium acetate precipitation. Products with less clear bands or double bands were cleaned using a BRESAclean DNA gel purification kit from GeneWorks Pty Ltd (catalogue number BT-3000, batch number V026) and following manufacturer's instructions. Two microlitres of the purified products were run on a 1% agarose gel with the *Spp*1-*Eco*RI marker and the results used to determine the volume of DNA to be used in the sequencing reactions.

Sequencing reactions for the vast majority of taxa were undertaken using ABI Big Dye® Version 2 terminators, with two batches using Amersham DYEnamic™ ET terminators. The standard reactions included 2 μL Big Dye, 1 μL of the primer, and from 0.5 μL to 3 μL of DNA template depending on the strength of the cleaned PCR products on the gel. In general, cleaned PCR products with bands of equivalent brightness to those of the marker were used at 1 μL, while the volumes for those with brighter or fainter bands were decreased or increased accordingly. Sterile water was used to make the reaction volume up to 10 μL. The sequencing reactions were run on a Corbett Research PC-960C cooled thermal cycler, with 30 cycles of 96°C for 30 seconds, 50°C for 15 seconds and 60°C for four minutes. Tubes were then held at 4°C until they could be removed. Sequencing products were cleaned using a simple sodium acetate precipitation and sequencing was undertaken on an ABI-377 automated sequencer according to manufacturer's directions.

TABLE 3.1: PCR programs used for amplification of Polygalaceae DNA.

Internal transcribed spacer (ITS)

Denaturation	Primer Annealing	Extension Phase	Final extension	Cycles
94°C, 3 min	65°C, 30 sec	72°C, 45 sec		1
94°C, 30 sec	60°C, 20 sec	72°C, 45 sec		2
94°C, 45 sec	55°C, 20 sec	72°C, 30 sec		2
94°C, 45 sec	50°C, 20 sec	72°C, 30 sec		3
94°C, 45 sec	45°C, 20 sec	72°C, 30 sec		2
94°C, 45 sec	45°C, 20 sec	72°C, 30 sec		30
			72°C, 3 min (then held at 4°C)	1

trnL intron, 3' *trnL* exon, *trnL/F* spacer

Denaturation	Primer Annealing	Extension	Final extension	Cycles
94°C, 2 min	65°C, 30 sec	72°C, 1 min 30 sec		1
94°C, 45 sec	60°C, 30 sec	72°C, 1 min 30 sec		2
94°C, 45 sec	55°C, 30 sec	72°C, 1 min 30 sec		2
94°C, 45 sec	50°C, 30 sec	72°C, 1 min 30 sec		2
94°C, 45 sec	45°C, 30 sec	72°C, 1 min 30 sec		37
			72°C, 5 min (then held at 4°C)	1

Primers

The primers used to amplify the ITS region are those listed in Crisp *et al.* (1999). These have been used successfully in Fabaceae subfam. Faboideae and were thus thought likely to be suitable for Polygalaceae. The ITS regions were amplified in two fragments: the first using ITS1 (forward) and 5.8R (reverse), and the second using 5.8F (forward) and ITS2 (reverse). The internal primers were routinely used in both PCR and sequencing.

The primers used to amplify the *trnL/F* region are given in Taberlet *et al.* (1991) and Crisp *et al.* (1999). The *trnL/F* region was amplified in two fragments: the first using "C" (forward) and 540R (reverse),the second using 420F (forward) and "F" (reverse). The internal primers were routinely used in both PCR and sequencing.

Sequence Editing and Alignment

Sequences files were exported to Sequencher 3.0 (GeneCodes Corporation) for assembly and editing. Sequences were aligned by eye for the most part, usually in Se-Al version 2.0 (Rambaut 1996). The ingroup taxa sequenced for cladistic analysis are listed with voucher details in Table 3.2. Due to extreme difficulty in determining the homology of large sections of the ITS sequences, two alignments were subjected to cladistic analysis in order to compare the results. These competing alignments are outlined below. The *trnL/F* alignment was considerably less problematic, as pre-aligned sequences from Persson (2001) could be used as a guide. However, the interpretation and coding of insertion/deletions was modified slightly with inclusion of the new sequences. All alignments used are attached in Appendix 1.

Cladistic Analyses

Outgroups

The outgroups specified in the analysis of the *trnL/F* dataset are the same as those listed in Persson (2001), comprising representatives of the three other families in the Order Fabales (Quillajaceae, Surianaceae and Fabaceae) and three families from within the sister clade to the Fabales according to the Savolainen *et al.* (2000) phylogeny (Fagaceae, Rhamnaceae and Rosaceae). A sequence from another representative of the Fabaceae, *Callistachys lanceolata* Vent. (Genbank accession AY015072) was added to increase the taxon overlap common to the two molecular datasets. The relationships between these families is relatively well-supported by recent molecular evidence, as described above, and it was thus considered reasonable to use them as progressively distantly related outgroups for these analyses. The same reasoning and the availability of Fabaceae sequences in Genbank made it convenient to use those as outgroups for the analysis of the internal transcribed spacer data. Thus, sequences from the ITS region of three legume taxa were used—*Medicago lupulina* L. (Genbank accessions MLZ99216 and MLZ99232), *Callistachys lanceolata* Vent. (Genbank accession AY015189) and *Bauhinia pyrrhoclada* Drake (Genbank accession AF286359).

TABLE 3.2: Details of taxa sequenced for cladistic analyses

Marker/region	Taxon name	Voucher Specimen and Herbarium	Abbreviation
internal transcribed spacer (ITS)	*Bredemeyera colletioides* (Phil.) Chodat	L.J.Marquez 241 (SI)	LM241
	Comesperma acerosum Steetz	A.M.Monro 51 (CANB)	AM51
	Comesperma aphyllum Benth.	J.A.Risler 625 (DNA)	JAR625
	Comesperma calymega Labill.	B.E.Pfeil 305 (CANB)	BP305
	Comesperma ciliatum Steetz	A.M.Monro 50 (CANB)	AM50
	Comesperma drummondii Steetz	A.M.Monro 41 (CANB)	AM41
	Comesperma ericinum DC.	A.M.Monro 102 (CANB)	AM102
	Comesperma esulifolium Gandoger	A.M.Monro 62 (CANB)	ANBG
	Comesperma flavum DC.	S.Donaldson 2066 (CANB)	SD2066
	Comesperma integerrimum Endl.	B.J.Lepschi 4480 (CANB)	BJL4480
	Comesperma rhadinocarpum F.Muell.	E.A.Griffin 8343 (PERTH)	EAG8343
	Comesperma scoparium J.Drumm. ex Steetz	M.D.Crisp 9172 (CANB)	MDC9172
	Comesperma spinosum F.Muell.	G.T.Chandler 810 (CANB)	GTC810
	Comesperma volubile Labill.	A.M.Monro 15 (CANB) A.M.Monro 88 (CANB) A.M.Monro 91 (CANB) M.D.Crisp 9173 (CANB)	AM15 AM88 AM91 MDC9173
	Emblingia calceoliflora F.Muell.[1]	S.D.Hopper 3303 (PERTH)	SDH3303
	Epirixanthes cylindrica Blume	A.D.Poulsen 304 (AAU)	ADP304
	Muraltia heisteria (L.) DC.	No herbarium voucher (Norton Summit, S. Australia)	AD
	Polygala eriocephala Benth.	J.A.Risler 360 (DNA)	JAR360
	Polygala exsquarrosa Adema	R.K.Harwood 804 (CANB)	RKH804
	Polygala japonica Houtt.	L.M.Copeland 2903 (NE, CANB)	LMC2903
	Polygala linariifolia Willd.	L.M.Copeland 2855 (CANB)	LMC2855
	Polygala myrtifolia var. *grandiflora* Hook.	A.M.Monro 67 (CANB)	AM67
	Polygala virgata Thunb.	A.M.Monro 71 (CANB)	AM71

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[1] The sequences obtained for *Emblingia* were not included in the phylogenetic analyses due to problems with making homology assessments for a taxon that was clearly distantly related to the remainder of the sequences. A BLAST search on Genbank indicated that the most closely matching sequences in the database were those from members of the Capparales, in particular the genus *Cleome* L.

TABLE 3.2 (continued)

Marker/region	**Taxon name**	**Voucher Specimen and Herbarium**	**Abbreviation**
internal transcribed spacer (ITS) (continued)	*Salomonia ciliata* (L.) DC.	R.K.Harwood 1023 (CANB)	RKH1023
	Salomonia cantoniensis	K.Larsen 46193 (AAU)	KL46193
	Xanthophyllum fragrans C.T.White	B.Gray 7836 (CANB)	BG7836
	Xanthophyllum octandrum (F.Muell.) Domin	B.Gray 7834 (CANB)	BG7834
trnL/F	*Bredemeyera colletioides* (Phil.) Chodat	L.J.Marquez 241 (SI)	LM241
	Bredemeyera papuana Steenis	E.E.Henty *et al.* NGF33220 (CANB)	NGF33220
	Comesperma ericinum DC.	A.M.Monro 102 (CANB)	AM102
	Comesperma rhadinocarpum F.Muell.	E.A.Griffin 8343 (PERTH)	EAG8343
	Comesperma scoparium J.Drumm. ex Steetz	M.D.Crisp 9172 (CANB)	MDC9172
	Comesperma volubile Labill.	A.M.Monro 88 (CANB)	AM88
	Emblingia calceoliflora F.Muell.[1]	S.D.Hopper 3303 (PERTH)	SDH3303
	Polygala exsquarrosa Adema	R.K.Harwood 804 (CANB)	RKH804
	Polygala japonica Houtt.	L.M.Copeland 2903 (CANB)	LMC2903
	Salomonia ciliata (L.) DC.	R.K.Harwood 1023 (CANB)	RKH1023
	Xanthophyllum fragrans C.T.White	B.Gray 7836 (CANB)	BG7836
	Xanthophyllum octandrum (F.Muell.) Domin	B.Gray 7834 (CANB)	BG7834

Maximum Parsimony

ITS

The alignment of the ITS sequences across the family proved extremely difficult—although making homology assessments within "sequence groups" of presumably closely-related taxa was straightforward, aligning sequences between these groups was often problematic. Given the relatively small length of sequence available, it was considered undesirable to excise areas of ambiguous alignment if they could provide phylogenetic information within the species groups mentioned. Thus, in several areas the sequences were "offset" from each other—in other words, areas of obvious homology in two or more sequences were aligned with each other but not with the remainder of the sequences. Partial Order Analysis (POA) alignment (Lee *et al.* 2002), which balances the need to optimise alignment of each new sequence added to a multiple sequence alignment with the need to produce the best alignment over all the sequences, was then used as an independent method of aligning the ITS sequences. Most of the available algorithms for multiple sequence alignment, such as CLUSTAL (Higgins & Sharp 1988), proceed by a series of pairwise sequence alignments to build an alignment for all sequences. CLUSTAL then builds a tree of the relationships between sequences using measures of their divergences and aligns all sequences in order from "most recently" until all sequences have been included. One problem with this approach is the issue of "local optima"—the alignment may not be optimal for all sequences if an error is made early in the alignment process or if the distance tree is incorrect (Thompson *et al.* 1994). POA, by contrast, uses pairwise dynamic programming rather than reducing an alignment to a linear profile. This guarantees that the optimal alignment of each new sequence against all the sequences already contained in a multiple sequence alignment. POA also runs quickly and was thus able to produce an objective alignment of the ITS sequences, which could be compared to the alignment constructed by hand.

Both alignments were analysed using the following maximum parsimony settings. A heuristic search of 1000 random addition sequence replicates was run, holding ten trees at each step, using tree bisection and reconnection (TBR) branch swapping and the MULTREES option on. A bootstrap analysis was then carried out, using 1000 bootstrap replicates with 10 random addition sequence replicates within each and holding 10 trees at each step. A Bremer decay analysis was also run using TreeRot version 2 (Sorenson 1999), with twenty addition sequence replicates and MAXTREES set to 20000.

trnL/F

The initial parsimony analysis settings used for the *trnL/F* dataset matched those described by Persson (2001), in order to directly compare the published results to those obtained from the expanded dataset. This entailed a two-stage process. An initial heuristic search was run with 500 addition sequence replicates, TBR branch swapping and the MULTREES option off. The second search used the trees found in the initial search as starting trees and used the same search settings but with the MULTREES option on and MAXTREES set to 5000, meaning that multiple shortest trees from each replicate were saved but only the first 5000 of these were retained and swapped to completion. In addition, the second stage of the search was re-run with MAXTREES set to 10000, to ascertain whether this made a significant difference to the topology of the strict consensus of the trees. A bootstrap analysis as described by Persson (2001) was carried out, using 10000 replicates and five random addition sequence replicates within each. Nearest-neighbour interchange (NNI) swapping was used and the MULTREES option switched off. A Bremer decay analysis was also run using TreeRot version 2 (Sorenson 1999), with twenty addition sequence replicates and MAXTREES set to 20000.

Bayesian Analyses

After using ModelTest 3.06 (Posada & Crandall 1998) on both datasets, the general time-reversible model plus invariant sites plus gamma (GTR+I+Γ) was chosen as that which best fit the data. ModelTest evaluates 56 evolutionary models and variations to find the one which best fits the data without adding further parameters. GTR+I+Γ is the most parameter-rich model and allows unequal base frequencies, a substitution rate differing between each pair of nucleotides (but being equal in either direction of change), a proportion of invariant sites, and nucleotide substitution rates between sites following a gamma distribution. Indels were removed from the *trnL/F* alignment and MrBayes version 3.0 (Huelsenbeck & Ronquist 2001) was used to conduct Bayesian analyses of both datasets.

Bayesian analysis approximates the posterior probability distribution of a set of trees given the molecular dataset by using a method called Markov Chain Monte Carlo (MCMC) to sample the treespace and parameter space. It gives some of the advantages of using a more evolutionarily realistic models-based approach to phylogenetic estimation, but generally is much faster to run than true maximum likelihood analyses.

Four Markov chains were run for 500000 generations, sampling a tree every 10 generations. The trees retained during the "burn-in" period before the chains reached apparent stationarity were discarded and a 50% majority rule consensus tree constructed from the remaining trees. The values at each node of the majority-rule tree represent the percentage of the time that the clade occurs among the sampled trees (their posterior probabilities).

A second mixed model Bayesian analysis was run on the *trnL/F* data with indels included. This enabled the two data partitions (nucleotide and indel) to be unlinked and their evolutionary history to be approximated under different models. The GTR+I+Γ model was again used for the nucleotide data, but the indels were subjected to analysis as "standard" characters under a Jukes-Cantor plus gamma (JC+ Γ) model, where all changes between states are equally likely and site-to-site rate variation follows a gamma distribution. In this second analysis, four Markov chains were run for 1000000 generations, sampling a tree every 100 generations. The trees retained during the "burn-in" period before the chains reached apparent stationarity were discarded and a 50% majority rule consensus tree constructed from the remaining trees. This analysis was run five times and the consensus trees from each run compared to ensure the runs all converged on the same tree.

RESULTS

The sequences from the internal transcribed spacer regions are relatively G+C rich, with mean base compositions of 19.1% adenine, 29.3% cytosine, 31.1% guanine and 20.5% thymine. This 60% G+C content is similar to that found in at least some members of the Fabaceae (e.g. 57–59% in *Cercis*, Davis *et al.* 2002) and other angiosperms (e.g. ~53% in the mistletoe genus *Korthalsella*, Molvray *et al.* 1999; 56.46% in *Alyssum*, Mengoni *et al.* 2003). By contrast, the *trnL/F* sequences are markedly A+T rich, with mean base compositions of 35.2% adenine, 17.5% cytosine, 16.9% guanine and 30.4% thymine. This 66% A+T richness is close to the average for several angiosperm groups examined by Bakker *et al.* (2000), which ranged between 64.5% and 67.1%. This A+T richness is evident from the variable-length poly-A and poly-T tracts within this marker. These tracts are responsible for a number of the indels coded for the *trnL/F* dataset, whereas the ITS sequences do not appear to contain indels which can be meaningfully scored.

Maximum Parsimony

ITS Manual Alignment

Using this alignment gave a total sequence length of 1384 characters, with 640 being constant and 327 parsimony-uninformative. This left a total of 417 informative characters in the analysis.

Two shortest trees of 2073 steps were found via the maximum parsimony analysis of the ITS dataset and the results are largely consistent with traditional classification. The strict consensus of the trees obtained is shown in Figure 3.2 and a phylogram representation of one of the shortest trees is shown in Figure 3.3. Relatively equal branches in the phylogram may indicate a relatively constant rate of base substitution along each branch. All the genera included appear as monophyletic groups, albeit with variable levels of support as indicated by the bootstrap statistic and with no supported resolution of inter-generic relationships. *Comesperma* is weakly monophyletic, appearing in only 59% of the bootstrap replicates and the Bremer decay index indicates that only two further steps would be required to render it non-monophyletic. *Polygala* appears as the sister group to *Comesperma* in the strict consensus, although this node does not appear in the bootstrap consensus. The single representative of *Bredemeyera* appears as sister to the *Comesperma-Polygala* pairing, although this again is not supported by the bootstrap. The final clade within the Polygalaceae *sens. str.* comprises *Salomonia* as a strongly monophyletic group as the sister taxon to the single representative of *Epirixanthes* sequenced, with *Muraltia heisteria* falling sister to these two genera. This group breaks down under bootstrap analysis, with the position of *Muraltia* being unresolved and the *Salomonia-Epirixanthes* pairing being only moderately supported. The representatives of *Xanthophyllum* included in this analysis are strongly supported as a monophyletic group and appear as the sister taxon to the remainder of the family. However, this node is also not supported on the bootstrap tree. Thus, although the traditional generic delimitations appear to be supported in this analysis, the relationships between the genera are not resolved. Also, the analysis only includes Australian species for the most part, so it is not possible to comment on the monophyly of the extra-Australian genera.

Within *Comesperma* three main groups are evident in the strict consensus tree (Fig. 3.2). These groupings all fall within subsection *Eucomesperma* and for the most part correspond with the morphological series described by Chodat (1896). The representatives from series *Confertae* form a monophyletic group supported by a

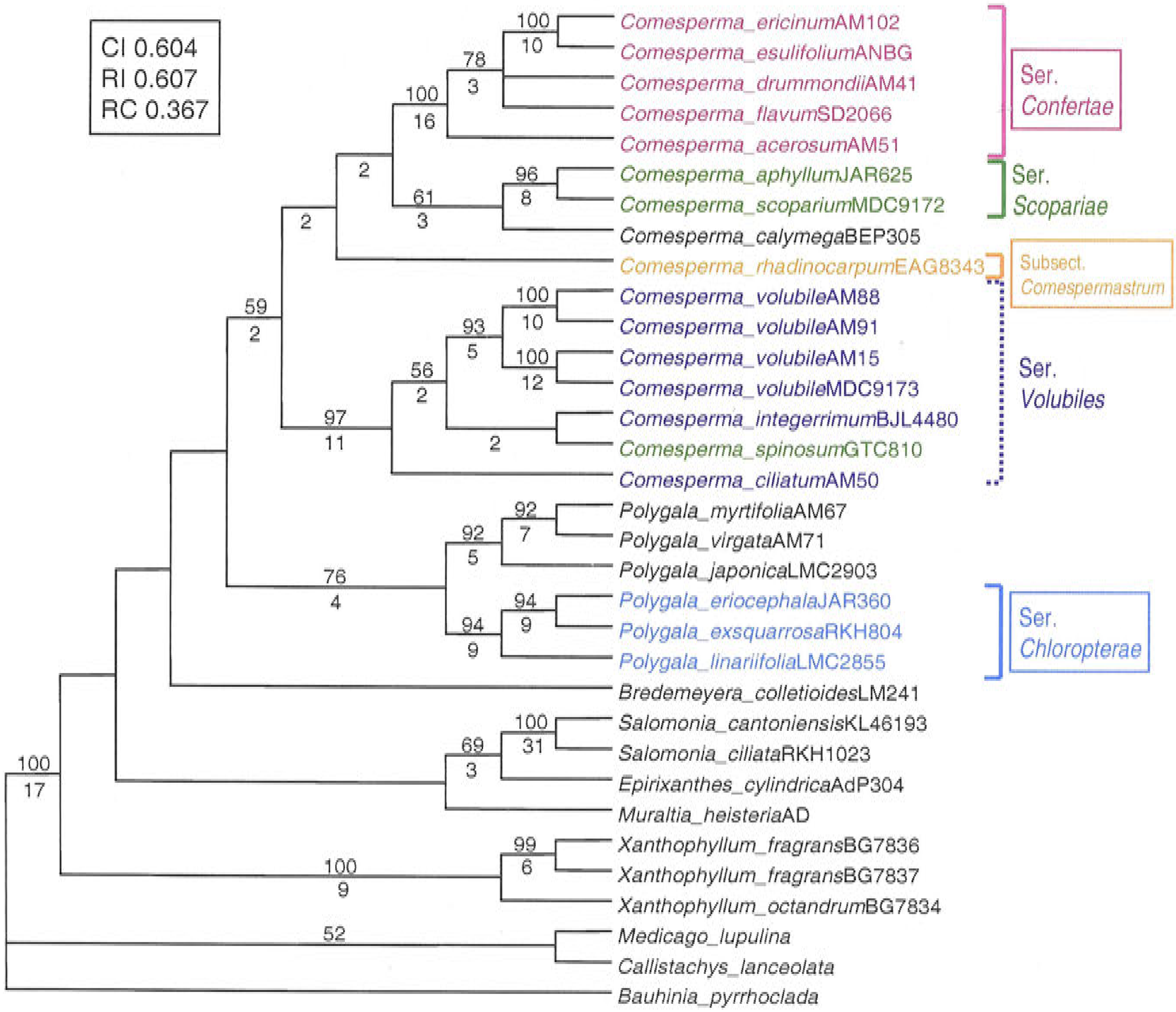

FIGURE 3.2: Strict consensus of two shortest trees obtained from analysis of ITS sequence data aligned by hand under unweighted maximum parsimony criteria. Bootstrap values greater than 50% are shown above the branches, Bremer decay indices greater than one are given below, and groups corresponding to traditional sub-generic classification are labelled. Monophyletic groups are indicated by solid brackets and a box outline around their name, while non-monophyletic groups are enclosed in a dashed bracket and their names are not boxed.

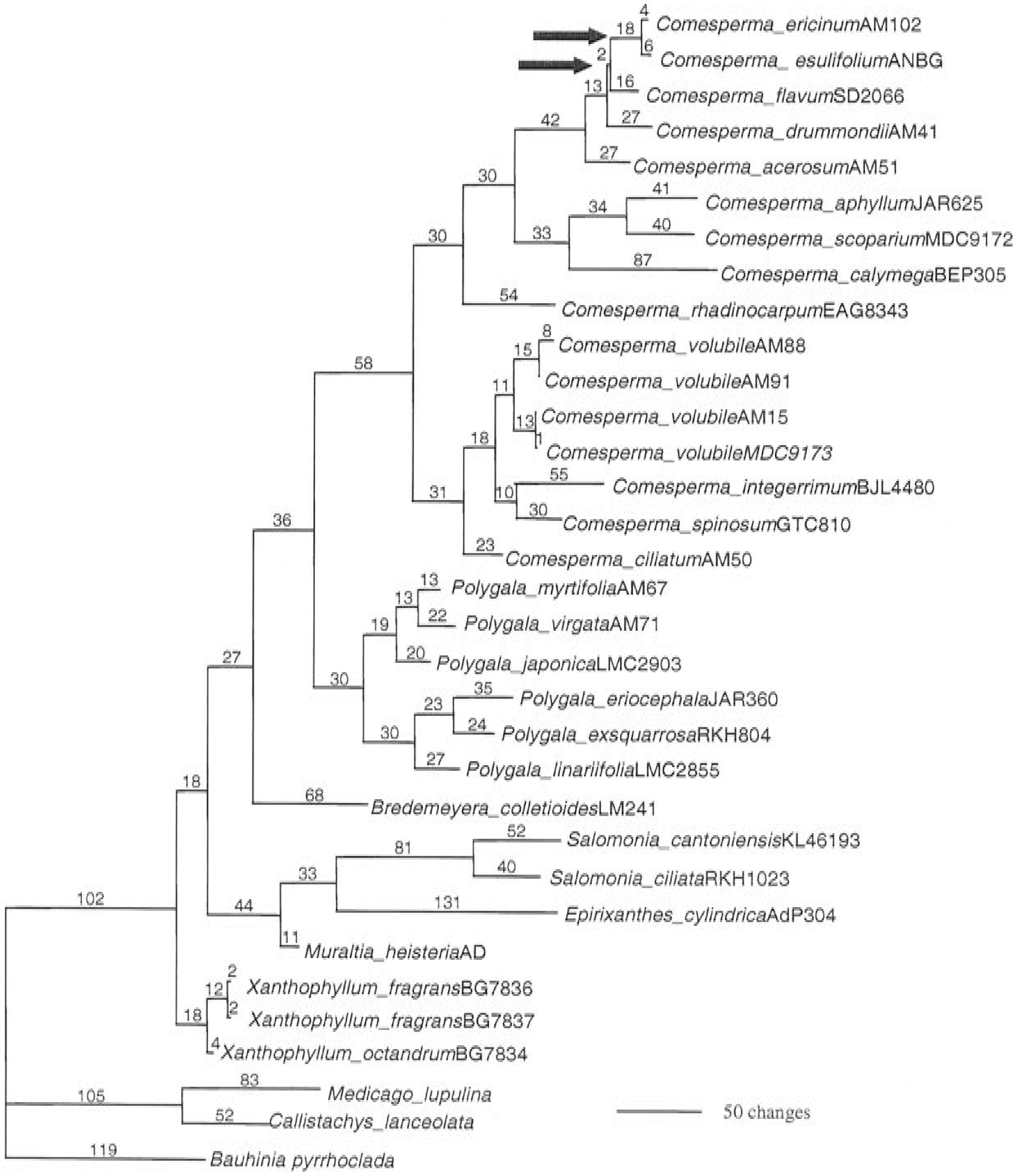

FIGURE 3.3: Phylogram representation of one of the two most parsimonious trees resulting from unweighted maximum parsimony analysis of hand-aligned ITS sequence data for Polygalaceae. The number of steps is given above each branch. Arrows indicate the branches that collapse in a strict consensus of the shortest trees.

bootstrap value of 100% and Bremer decay index of 16. The sister group to this is a clade formed by two members of series *Scopariae* and *Comesperma calymega*. Although this does not match Chodat's classification, it does correspond with that of Steetz (1847), who placed these two species within his rather different concept of section *Isocalyx* (as outlined in Chapter 2). *Comesperma rhadinocarpum*, the only representative of subsect. *Comespermastrum* included in the analysis, is the sister species to this *Confertae*/*Scopariae* pairing. Finally, series *Volubiles* forms a strongly-supported sister clade to the remainder of the genus. One anomaly in this *Volubiles* clade of twining climbers is the presence of *Comesperma spinosum*, a leafless divaricate subshrub and usually classified on that basis as a member of series *Scopariae*. Possible reasons for this placement will be discussed below. It should also be noted that most of the resolution among the groups in *Comesperma* is not well-supported by the bootstrap values—while *Volubiles+C. spinosum*, *Confertae* and *Scopariae* clades appear strongly monophyletic in the bootstrap tree, the three groups simply form a polytomy.

While the representatives of *Polygala* included in this analysis are only moderately supported (76%) as a monophyletic group, there are two strongly supported subclades. The first consists of all the members of series *Chloropterae* included in the analysis. The other subclade contains the other three species sampled, although these were classified into three different series by Chodat (1896). This may be a reflection of a lack of sampling from the other series within *Polygala*, but the groups are very distinct morphologically—*Chloropterae* is partly defined by small flowers with acute, greenish "wing" sepals, while all the other species sampled have larger flowers with obtuse, petaloid "wing" sepals.

ITS POA Alignment

Using this alignment gave a total sequence length of 1408 characters (slightly longer than the alignment obtained by hand), with 844 characters being constant and 132 parsimony-uninformative. This was 204 more constant characters and 195 fewer uninformative characters than the alignment constructed by hand, probably reflecting the difference between a global optimum achieved by POA and the offset method chosen for the manual alignment. Nonetheless, POA alignment left a total of 432 informative characters in the analysis, only slightly more than the 417 informative characters remaining in the analysis of the hand-aligned sequences.

Three shortest trees of length 2208 steps (significantly longer than those produced using the hand alignment, despite the similarity in informative character levels) were found via maximum parsimony analysis of the alignment generated by POA. The strict consensus of these trees is shown in Figure 3.4. It differs from the results obtained by hand alignment in its indication that *Comesperma* is non-monophyletic—the POA alignment results in *Polygala*, *Salomonia*, *Epirixanthes*, *Bredemeyera* and *Muraltia* being nested within *Comesperma*. The relationships within *Comesperma* are also changed, with *Volubiles* and *Scopariae* forming sister groups rather than *Confertae* and Scopariae. However, the species groups obtained from the MP analysis of the hand-aligned sequences are also all recovered from analysis of the POA alignment. It is the relationships among these groups (the "backbone" of the tree) that are changed. Importantly, however, none of these changes in the relationships between groups are well-supported by the bootstrap analysis and the slightly different topologies of the two bootstrap trees are a result of very minor changes in the values obtained. Since bootstrap values will vary slightly when the same number of replicates are run on identical datasets, it is not surprising that the bootstrap trees for the two different alignments are not quite identical. However, the clades that are strongly supported in the 50% bootstrap tree for the hand-aligned sequences are also strongly supported in that for the POA alignment. *Polygala* ser. *Chloropterae*, *Comesperma* ser. *Volubiles* (including *C. spinosum*), *C.* ser. *Scopariae*, *C.* ser. *Confertae* and *Xanthophyllum* all receive high bootstrap values (>90%) in the analysis of the POA-aligned sequences. These results echo those found from the analysis of sequences aligned by hand and offset—despite using two quite different alignment methods, for the most part the same groups are recovered in each case.

trnL/F

One hundred trees of length 1838 steps were found in the initial stage of the maximum parsimony search and used as starting trees for the second stage. The strict consensus of the 5000 trees retained from the second stage of the search is shown in Figure 3.5, as are the consistency index (CI), retention index (RI) and rescaled consistency index (RC) over all of the shortest trees retained. The strict consensus of 10000 trees retained from a second search had an identical topology to that shown in Fig. 3.5, so it is not presented separately. A phylogram representation of one of the shortest trees chosen at random is shown in Figure 3.6. The number of character states changes on each branch within the

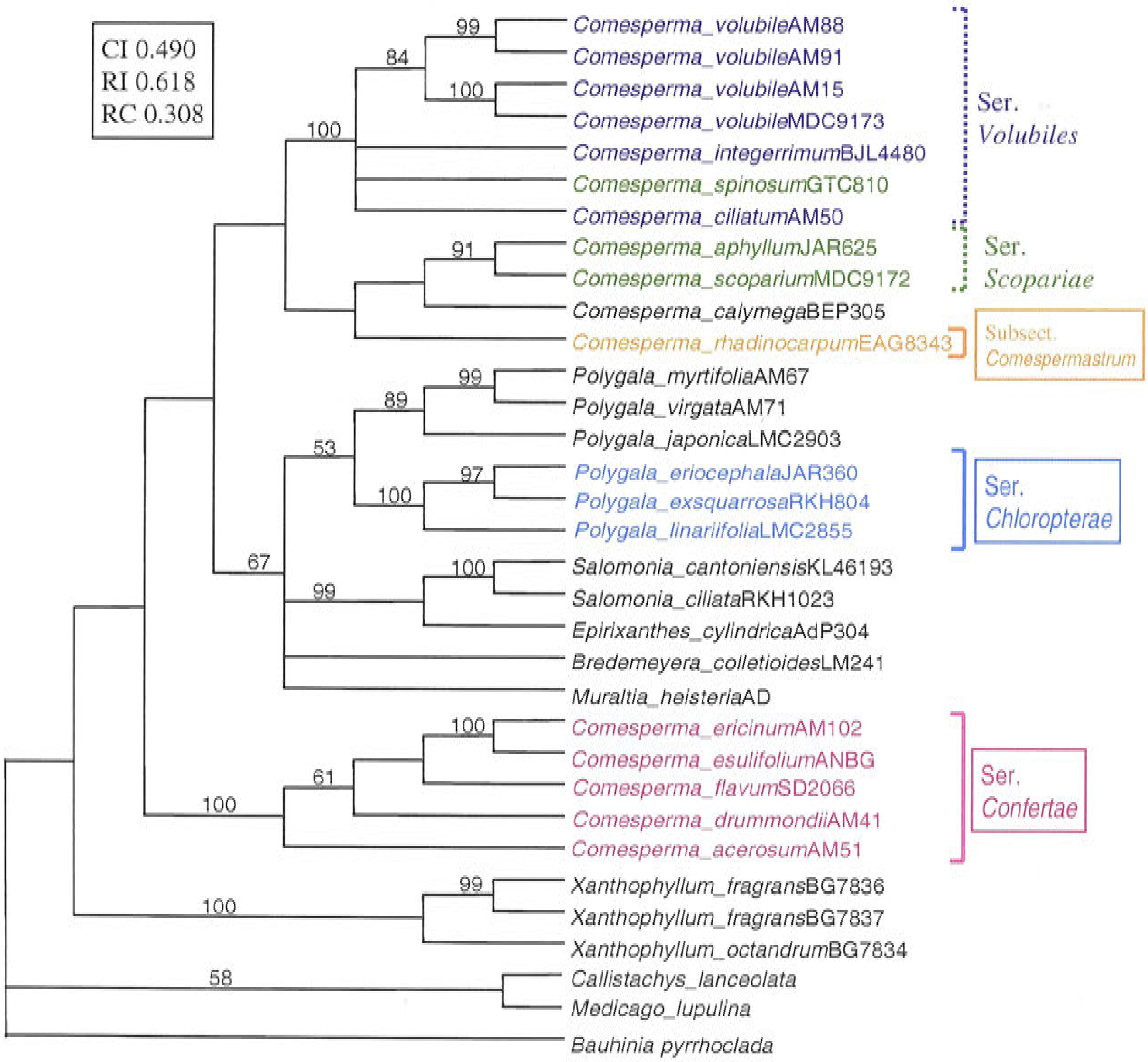

FIGURE 3.4: Strict consensus of three shortest trees obtained from analysis of ITS sequence data aligned using POA under unweighted maximum parsimony criteria. Bootstrap values greater than 50% are shown above the branches and groups corresponding to traditional sub-generic classification are labelled. Those that are monophyletic are indicated by solid brackets and a box outline around their name, while non-monophyletic groups are enclosed in a dashed bracket and their names are not boxed.

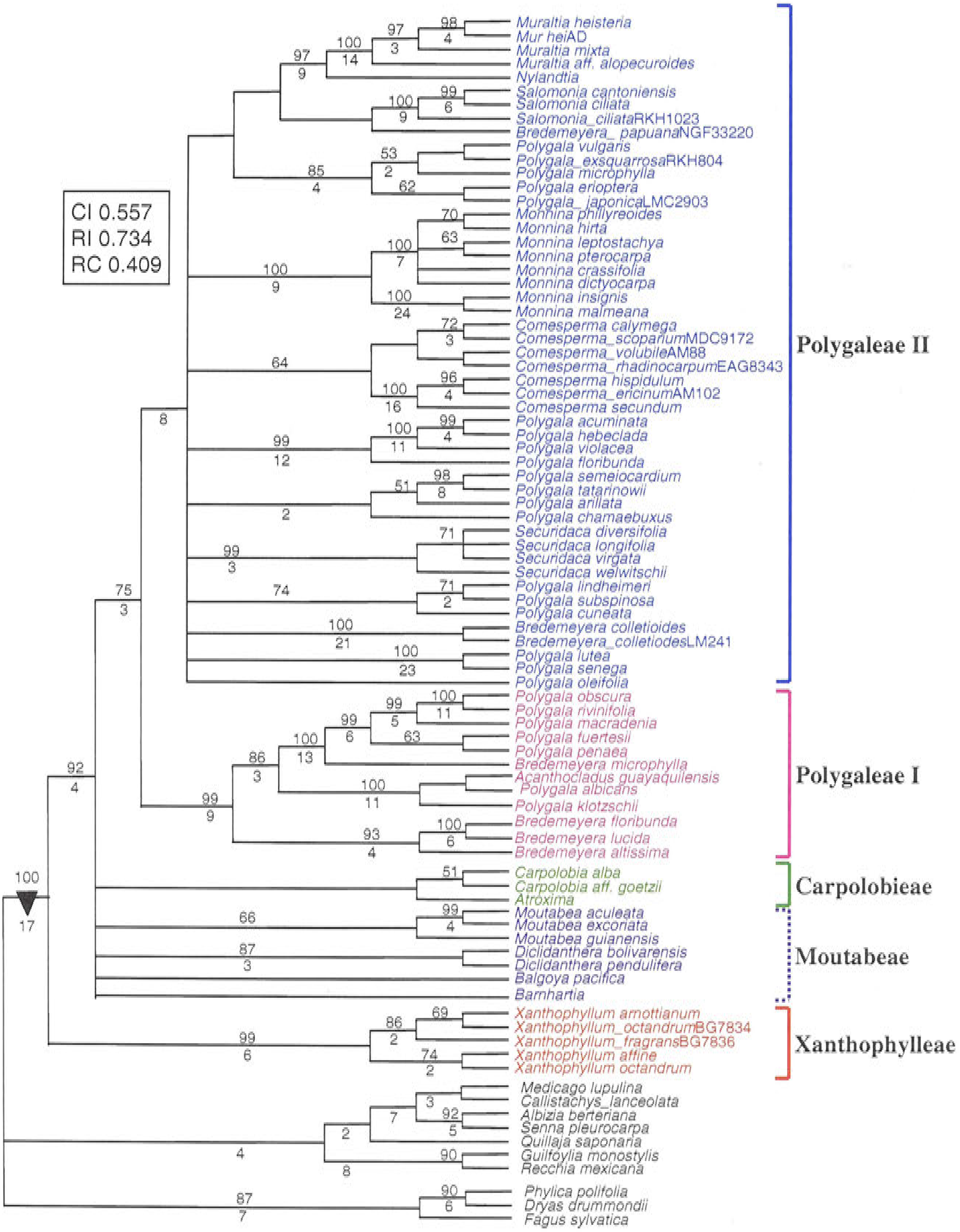

FIGURE 3.5: Strict consensus of 5000 shortest trees obtained from unweighted maximum parsimony analysis of *trn*L/F sequence data taken for the most part from Persson (2001), with the sequences added for this study identified using the block letter abbreviation codes shown in Table Two. Bootstrap values greater than 50% are shown above the branches and Bremer decay indices greater than one are shown below. The existing tribes are labelled, with those that are monophyletic indicated by solid brackets and those that are non-monophyletic indicated by dashed brackets. The Polygalaceae *sens. lat.* is indicated by a solid triangle on the branch leading to the family.

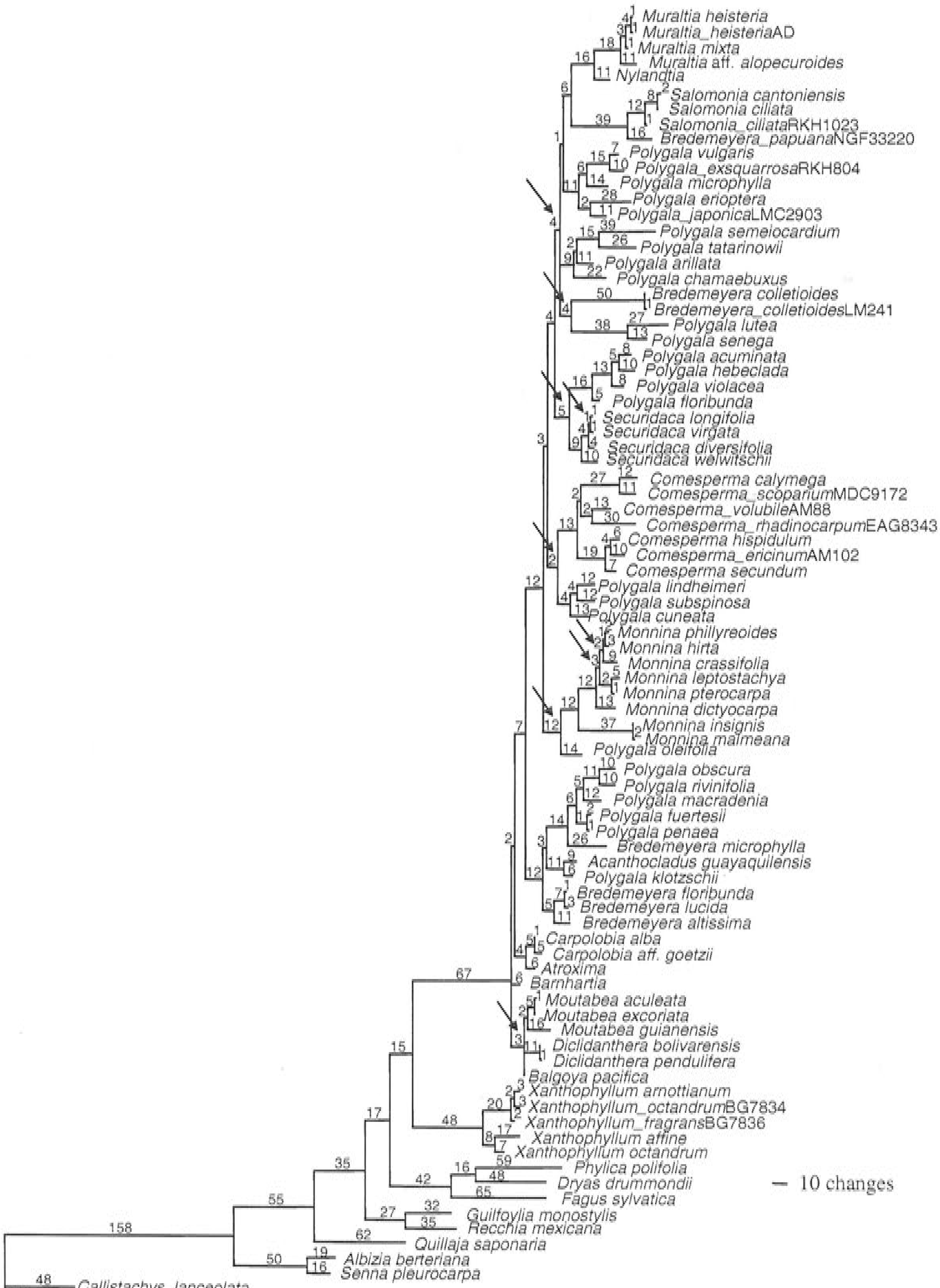

FIGURE 3.6: Phylogram representation of tree #4747 of the 5000 most parsimonious trees resulting from unweighted maximum parsimony analysis of *trnL/F* sequence data for Polygalaceae. The number of steps is given above each branch. Arrows indicate the branches that collapse in a strict consensus of the shortest trees.

Polygalaceae *sens. strict.* is relatively low and this predominance of short branches may indicate relatively recent adaptive radiation in the family relative to the well-differentiated outgroups. This may also mean that a faster-evolving DNA region would be required to elucidate the lower-level relationships among and within genera in the tribe Polygaleae.

The strict consensus tree (Fig. 3.5) largely agrees with that published by Persson (2001), with the additional taxa simply "slotting" into their genera. There are a few anomalies however, with the new sequences forming new clades and with sequences from identical taxa not forming sister groups on the cladogram. *Salomonia ciliata* (RKH1023) is sister to *S. ciliata* and *S. cantoniensis*. Likewise, *Xanthophyllum octandrum* (BG7834) is not most closely related to Persson's sequence for this taxon, as each falls within a different clade within *Xanthophyllum* in this analysis. It is only a small number of characters (mostly indels) that differ between these sequences and it is not clear whether these differences are likely to be real or an artefact of sequencing error or of misidentification of specimens. However, the newly generated sequences have been checked for errors in alignment and the identities of the voucher specimens also reassessed. As only two species of *Xanthophyllum* and one of *Salomonia* are known to occur in Australia and botanists with specialist knowledge of their local flora collected the samples analysed, misidentification seems unlikely.

With a small number of anomalous results aside, the results of the *trnL/F* analysis are largely as expected. The new sequences from *Xanthophyllum* fall within a monophyletic Xanthophyllaceae, which still forms a sister group to the remainder of the family. The Polygaleae remains split into two major groups, the fully-resolved Polygaleae I and unresolved Polygaleae II of Persson (2001).

Within Polygaleae II, *Comesperma* remains a monophyletic group, despite the addition of further taxa, although the bootstrap value of 64% and Bremer decay index of less than two indicate only weak support for the genus. The relationship between the *Comesperma* and the species of *Bredemeyera* included in this analysis is unclear, as both *Comesperma* and *B. colletioides* from *Bredemeyera* sect. *Hualania* fall within the unresolved Polygaleae II clade and thus may or may not be sister taxa. However, since all the members of *Bredemeyera* sect. *Bredemeyera* form a monophyletic group in the Polygaleae I clade and since the only other member of *B.* sect. *Hualania* (*B. microphylla*) also forms part of Polygaleae I clade (albeit as the sister taxon to a group of *Polygala* species), it can be definitely stated that *Comesperma* and the South American members of *Bredemeyera* are not sister genera.

The Australian species of *Polygala* fall within a mixed group of *Polygala* species that includes *P. vulgaris*, the type species for the genus. Since only two Australian *Polygala* species were sequenced for the *trnL/F* region, it is not possible to comment on the monophyly or otherwise of the genus within Australia based on these results. While the sister group relationship between the widespread south-east Asian and Australian species *Polygala japonica* and another south-east Asian species *P. erioptera* seems plausible, it is surprising that the northern Australian/eastern Malesian *P. exsquarrosa* is grouped so closely with the European *P. vulgaris* (found in Australia only as a sparingly naturalised weed). This may however be due to under-representation of the large section *Polygala* in this study and the groupings are not strongly supported by the bootstrap values. However, it does seem reasonable to believe that the previous placement of the Australian species in this section within *Polygala* is correct.

Finally, the placement of *Bredemeyera papuana* Steenis (=*Polygala papuana* (Steenis) Meijden) remains problematic. Although it forms the sister group to *Salomonia* in this analysis, the placement is not supported by the bootstrap values and its position in the bootstrap tree is thus unresolved within the Polygaleae II clade. The difficulty in unambiguous placement may be due to the fact that only a partial sequence of *Bredemeyera papuana* could be obtained from the available herbarium material and the sequence could not replicated despite DNA being extracted from a range of specimens. It is thus difficult to know whether this sequence may actually be contamination from another member of the family. Re-collection of this poorly known species will almost certainly be required to elucidate its relationships.

Bayesian Inference

ITS

The Markov chains reached apparent stationarity after approximately 10000 generations, so the first 1000 trees retained were discarded before constructing a consensus tree. The 50% majority-rule consensus tree is shown in Figure 3.7. Many of the same groups obtained via maximum parsimony analysis are also recovered in the Bayesian analysis and show high posterior probabilities under the chosen model. A monophyletic *Comesperma* was recovered in 100% of the trees retained and the same

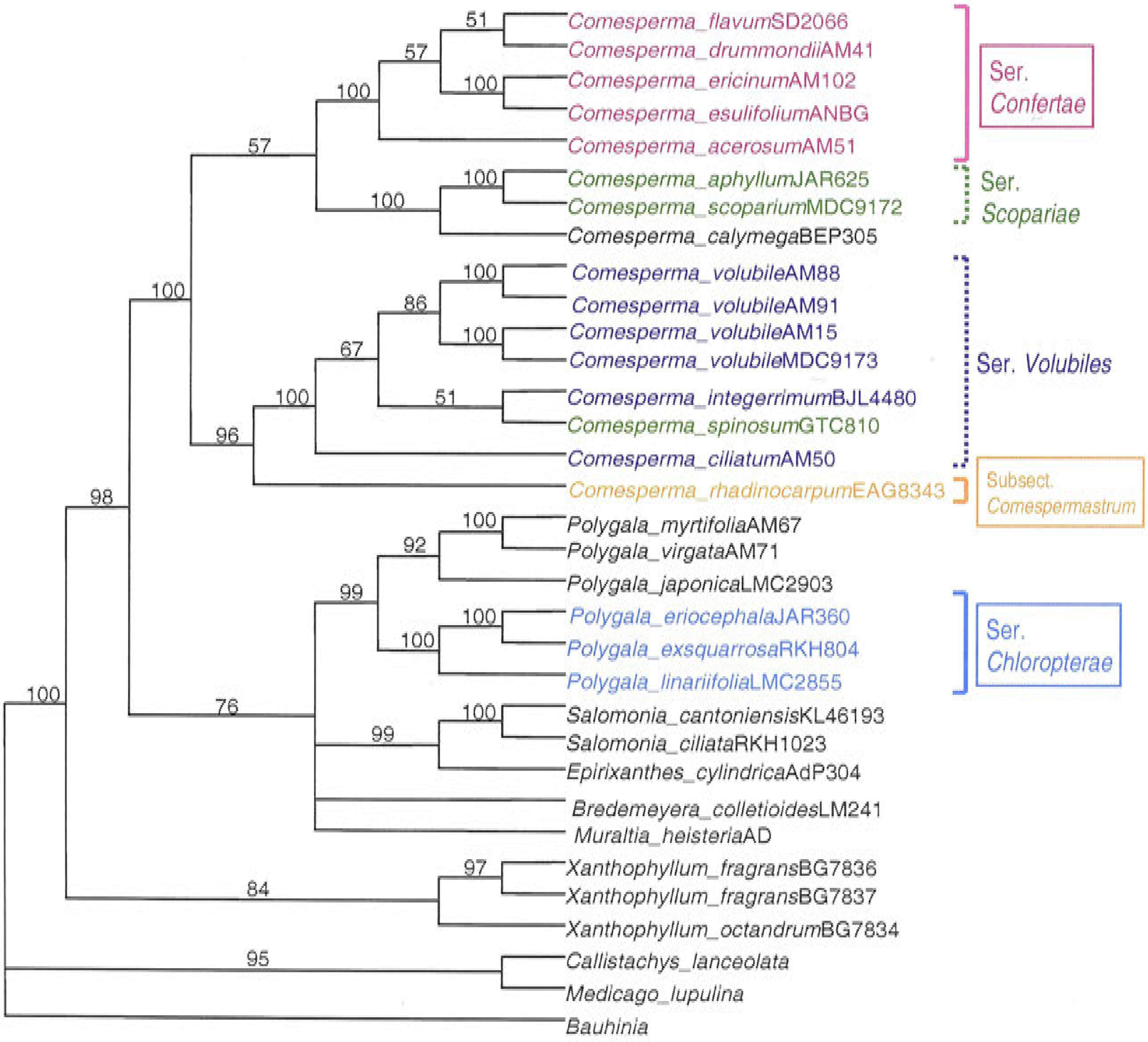

FIGURE 3.7: Fifty percent majority-rule consensus of 49000 trees retained from Bayesian analysis of ITS sequence data using the GTR+I+Γ model, with posterior probability values shown above the branches. Groups discussed in the text are labelled.

three subgeneric groups found in the maximum parsimony analysis (*Confertae*, *Scopariae*, and *Volubiles+C. spinosum*) were also present in all the trees. While the MP analysis of hand-aligned ITS sequences indicates a sister group relationship between the series *Confertae* and *Scopariae* and then places *C. rhadinocarpum* sister to this pairing, the Bayesian results place *C. rhadinocarpum* sister to the third sub-generic group, Ser. *Volubiles* (including *Comesperma spinosum*). The position of *Comesperma rhadinocarpum* differs in the results for the two methods of alignment under MP criteria and in the Bayesian results, and it is thus difficult to conclusively place the species. This was recognised by Chodat (1896) in his placement of it in a different subsection from the vast majority of other species in the genus. However, the Bayesian results placing *Comesperma rhadinocarpum* sister to the (*Volubiles+C. spinosum*) clade are the only ones where its placement receives some stability, having a posterior probability of 96% in this analysis.

Outside *Comesperma*, the results are also similar to those obtained under maximum parsimony. The sister group to *Comesperma* again consists of a grouping of *Polygala*, *Salomonia/Epirrhixanthes*, *Bredemeyera* and *Muraltia*. While this grouping of genera receives an 85% posterior probability value, the relationships within it are again unclear and the genera form a polytomy. *Polygala* ser. *Chloropterae* receives a posterior probability value of 100%, while all the representatives of *Polygala* included in the analysis are grouped together with 99% posterior probability. The grouping of *Salomonia* with *Epirrhixanthes* is recovered in 98% of the Bayesian trees.

trnL/F

The Markov chains in the first analysis reached apparent stationarity after approximately 32000 generations, so the first 3200 trees retained were discarded before constructing a consensus tree. The 50% majority-rule consensus tree is shown in Figure 3.8. Again, the groups obtained via Bayesian analysis are very similar to those appearing in the MP consensus tree. One notable difference is the greater resolution within the Polygaleae II Clade, but this for the most part is not strongly supported. The same small species groups appear with high posterior probabilities in the "twigs" of the 50% majority-rule tree, but the large polytomy is broken up slightly more into subgroups with low posterior probabilities. For example, *Comesperma*, *Monnina* and three species of *Polygala* form a polytomy within the larger Polygaleae II clade but this grouping only receives a posterior probability of 61%. *Comesperma sens. strict.* again

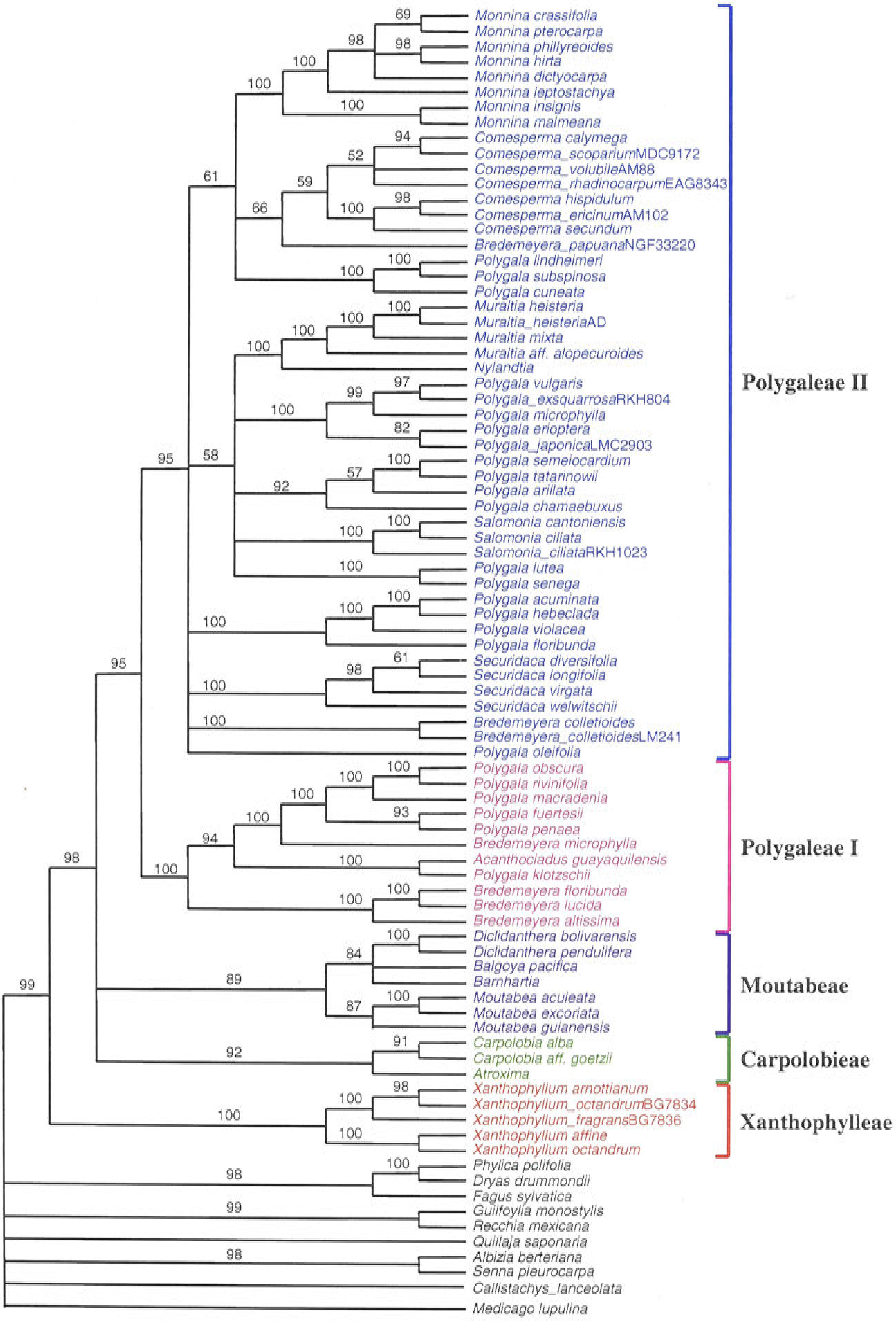

FIGURE 3.8: Fifty percent majority-rule consensus of 46800 trees retained from Bayesian analysis of *trn*L/F sequence data, taken for the most part from Persson (2001). The sequences added to the Persson dataset are identified using the block letter abbreviation codes shown in Table 2. Posterior probability values are shown above the branches and the existing tribes are labelled, with those that are monophyletic indicated by solid brackets.

appears as a weakly-supported group, with only 59% of the trees retained containing the group. Interestingly, *Bredemeyera papuana* (which has previously been linked with the Australian *Comesperma* species) falls as sister taxon to *Comesperma*, but only in 66% of the Bayesian trees. Within *Comesperma*, the results are identical to those obtained from maximum parsimony. The limited sampling does not allow direct comparison to results obtained from the ITS dataset, but the same groups recur in this analysis. *Comesperma scoparium* is closely related to *C. calymega* and these species form a sister group to *C. volubile* and *C. rhadinocarpum*. The sister group to this entire grouping are members of the series *Confertae*.

For the second analysis, which included indels, apparent stationarity was reached after approximately 20000 generations, so the first 2000 trees retained were discarded before constructing the consensus tree for each of the five replicates. The trees obtained from an analysis including indels were nearly identical to those obtained from the analysis where indels were excluded, although *Bredemeyera papuana* once again falls sister to the *Salomonia* clade as it did in the maximum parsimony results. Since the 50% majority-rule consensus trees from the mixed models analysis are otherwise identical to that shown in Figure 3.8, albeit with slightly higher posterior probabilities on many branches, they are not presented separately. It seems likely from these results that the phylogenetic signals contained in both the *trnL/F* nucleotide and indel data are congruent.

DISCUSSION AND CONCLUSIONS

The degree of taxon overlap between the two molecular datasets limits comparison of results to a fairly general level. Nonetheless, some groups and relationships recur across datasets and modes of analysis.

The molecular datasets indicate some support for the traditional classification within the Polygalaceae. For example, *Comesperma* appears as a monophyletic group in all the analyses with the exception of that of the POA-aligned ITS sequences. While the levels of support or credibility that can be placed in this clade vary widely according to the dataset and model of phylogenetic inference, it does seem likely that the *Comesperma* sequences share a common evolutionary history. Also, the Persson (2001) *trnL/F* dataset shows that "section" *Comesperma* is not the sister genus to the remainder of the species in *Bredemeyera sensu* Chodat (1896) and adding more taxa in *Comesperma* did not change this situation. Since *Bredemeyera sens. lat.* is clearly polyphyletic, the most conservative course is to maintain *Comesperma* as a distinct genus in the absence of further evidence to contradict this decision. Neither dataset gives any conclusive indication of which group may be sister to *Comesperma*, as the relationships among the genera are far from clear. Bayesian analysis of the ITS dataset places *Comesperma* as the sister group to all other representatives of the family (excluding *Xanthophyllum*) within Australia. By contrast, maximum parsimony analysis of the same data places *Comesperma* as the sister group to *Polygala*, with all other genera being progressively distantly related to that clade. *trnL/F* sequences leave the position of *Comesperma* within the Polygaleae II clade largely unresolved, despite the Bayesian analysis indicating a weakly-supported grouping of *Monnina*, *Comesperma* and three *Polygala* species from the Americas.

Within *Comesperma*, Chodat's (1896) sub-generic classification is supported by the ITS data. The series *Confertae*, *Scopariae*, and *Volubiles* (albeit with the inclusion of *C. spinosum*) are recovered using this dataset regardless of alignment method or the evolutionary model used. This unexpected placement of *Comesperma spinosum* may be explained by the possibility (previously alluded to in Chapter 2) that it has been misplaced in series *Scopariae*. The species definitely possesses elongate mature fruit and seeds with a coma, while the *Scopariae* are defined by "*Polygala*-like" (i.e. round or cordate) fruit and seeds lacking a coma. Herbarium specimens with mature fruit are rare, however, and the overall habit of the plant is very similar to that of other members of the *Scopariae* (leafless and divaricate shrubs) so it is not difficult to understand this error. In addition to the three series just mentioned, the only representative of subsection *Comespermastrum* included in the analysis, *Comesperma rhadinocarpum*, is weakly supported as the sister group to the *Volubiles* by both datasets. Further representatives of *Comespermastrum* would need to be sampled to test its monophyly, but the placement does indicate that the subsection *Eucomesperma* to which the series listed above belong may be non-monophyletic. The sampling within *Comesperma* for the *trnL/F* data is not sufficient to clarify the sub-generic relationships, but the representatives that are included broadly confirm the monophyly of and divergence between the series listed.

The gross polyphyly of *Polygala* overall and of the type section *Polygala* shown within the *trnL/F* data indicates that significant changes in classification will be required, although this is not advised until further data becomes available to support the results of Persson's (2001) study. He found that the groups could be largely classified geographically, suggesting that the previous characters used to define subgeneric groupings have been artificial. The Australian species included in the analysis of ITS sequences were grouped into two subclades within *Polygala*, with the series *Chloropterae* forming a strong monophyletic group and the other species forming a sister clade of "showy-flowered" species. Obviously this does not cover the great diversity within even section *Polygala*, but does lend support to the morphological grouping of the small-flowered Australian and Malesian species that lack petaloid inner sepals.

Perhaps the most frustrating aspect of both molecular datasets is the lack of resolution between relatively strongly-supported species groups, or the weak "backbone" of the trees obtained. This hampers an understanding of the relationships both within and between genera. While many of the species groups (or sections) indicated in the trees are strongly supported by bootstrap values in the parsimony analyses or receive high posterior probabilities in the Bayesian reconstructions, the links between these groups usually collapse in a bootstrap tree or receive very low probability scores. Sampling also contributes to this problem—for example, the two species of *Salomonia* sampled for ITS form a monophyletic group with *Epirrhixanthes cylindrica* as their sister taxon, but it is not possible to categorically state from this that *Epirrhixanthes* and *Salomonia* should be united as a single genus. Some of the lack of backbone resolution with the ITS sequences may be due to the problem of saturation confounding the alignment. It was easy to align within "groups" of species, but became difficult to align across more distantly-related taxa. Since the internal transcribed spacer regions are relatively fast-evolving, they may have accumulated numerous substitutions in the variable regions and become saturated. It is thus not possible to detect the evolutionary signal in these regions for higher taxonomic levels. ITS then may have been a poor choice of region for a study focusing on a family-level problem. While the Persson (2001) *trnL/F* data casts light on the tribal relationships in the family and indicates a number of strongly-supported generic and sectional groups, further data are required to resolve the Polygaleae II polytomy. Data from other regions of the chloroplast may well be able to fill this gap.

The levels of homoplasy in the molecular datasets, as measured by the consistency and retention indices, are significantly lower than those obtained in the analysis of morphological data. Nonetheless, the two different sources of data have several points of agreement and an attempt to reconcile the topologies obtained will be made in the next chapter.

Chapter Four:
Congruence, Conflict and Conclusions

INTRODUCTION

Using Data from Multiple Sources

It has become increasingly common in recent years for phylogenetic analyses to include data obtained from more than one source. As molecular data have become more readily available, it is common for studies to include sequence data from one or more genomic regions and often also morphological characters. It has thus become necessary to devise methods to deal with data from multiple sources and this is a fairly controversial issue in the systematics literature, with a variety of different approaches being proposed and criticised (reviewed in Huelsenbeck *et al.* 1996).

When using more then one source of data for phylogenetic analyses, congruence of the results obtained is often considered to lend support to hypotheses of relationships and thus to taxonomic decisions based on the results. This is cited as an extension of the scientific principle that hypotheses supported by multiple independent sources of evidence are preferred to those supported by a single source (Miyamoto & Fitch 1995). Phylogenetic trees produced from each source of data are compared and groupings that appear in more than one or all of the supported topologies identified. A consensus topology from a single dataset may be rather poorly resolved, but this approach summarises the consensus of all datasets—the groups on which all agree. One pitfall of this method is that a single taxon that is placed in either of two positions can collapse a consensus tree and thus show less information than is actually present. The agreement in topologies obtained from different data sets is termed taxonomic congruence (Mickevich 1978; Kluge 1989) and separate analyses are sometimes characterised as the "never combine" approach.

Another approach to the issue of synthesising information obtained from more than one source is to "always combine" data, also known as character congruence or total evidence. This method combines the raw data into a single matrix that is then analysed as a whole. Some of the arguments for combining all the available data include maximising the explanatory power of the available information and the possible presence of "hidden support" (Gatesy *et al.* 1999). Groupings that do not appear in the topologies obtained from separate analyses may be found if the data are combined or the support for a given grouping may be greater in a combined analysis than from any of the separate analyses. This is taken to indicate that the presumably random "noise" created by homoplasy is overcome by data combination and the underlying signal in the data is thus highlighted. Combined analysis may also provide positive interaction (Hillis 1987) among the data and resolution of a phylogeny at varying taxonomic levels. For example, a fast-evolving gene can provide information about the relationships within species groups with a more slowly evolving region resolving the relationships between these groups. However, if two datasets both have well-supported but strongly conflicting signals, combination of data is likely only to result in a poorly resolved and inaccurate estimate of the phylogeny. This incongruence has been shown to occur in nuclear and chloroplast genomes from the same group of plants, which are separated within each cell and inherited independently In angiosperms, the haploid mitochondrial and chloroplast genomes are generally inherited maternally (Birky 1995) while the diploid nuclear genome is inherited biparentally. Thus, two of the major reasons cited for using the total evidence approach—the artificial nature of data partitions and the congruence of characters from different sources in organisms which have a common evolutionary history—may not always apply and in these cases a combined analysis is not appropriate.

A third approach to the issue of multiple datasets is conditional combination ("sometimes combine"; Bull *et al.* 1993, Rodrigo *et al.* 1993, de Queiroz 1993). This can be considered a special case of the taxonomic congruence approach, as it also compares the results of separate analyses to check for congruence between them. However, in this method the data are initially analysed separately to ascertain whether there is significant conflict between the topologies obtained from each source and are only combined for a final analysis if they are not strongly incongruent. Several statistical tests exist to evaluate the degree of congruence between multiple data based on maximum parsimony criteria, including the Templeton test (Templeton 1983), incongruence-length difference test (ILD; Farris *et al.* 1994) and the topological incongruence test (Rodrigo *et al.* 1993). The first two tests are character-based and the third topology-based. The conditional combination approach is appealing because it allows exploration of the individual datasets in the initial separate analyses, but does not preclude the advantages of a combined analysis if the results are congruent.

Much of the controversy surrounding separate versus combined analyses was irrelevant to data obtained for the Polygalaceae, as there was unfortunately very little taxon overlap between the datasets. Pruning the datasets to include only those taxa common to all three would result in a very small subset of taxa and a phylogeny of these would be uninformative in regard to the questions initially posed in this study. However, the issue of creating phylogenetic trees from multiple datasets in which not all taxa are represented has been receiving attention in recent years, largely as a result of the "Tree of Life" project. Any attempt to build a phylogenetic tree for all the described taxa on Earth will inevitably have to deal with groups for which no phylogeny exists and with groups that have only partial phylogenies from differing sources. Where more than one putative phylogeny exists for a group, the taxa included seldom correspond exactly. It also may not be possible to combine the datasets used to generate the phylogenies into a "supermatrix", due to difficulties on homology assessment and in the wide variety of data types used. Even in cases where combining the taxon/character matrices would be valid, large amounts of data would be missing for the taxa which are not shared between datasets. In these cases, an approach that combines the existing partial phylogenies into a "supertree" is extremely useful.

Supertree methods have been reviewed by Sanderson *et al.* (1998). They define a supertree as a tree containing all the taxa combined from two or more source topologies. Such supertrees can be constructed using a variety of methods and using either a direct or indirect approach. The direct approach is to construct strict consensus supertrees from the source trees, but this method can only be used if the source trees are compatible—that is, they agree on the relationships between the taxa which they share. Strict supertrees are a conservative method of combining trees from multiple sources, as they illustrate the relationships on which all of the source trees agree. The strict consensus supertree can also be constructed in a relatively short amount of computational time. Some attempts have been made to develop "semi-strict" methods which can construct consensus supertrees from incompatible source trees (e.g. Gower & Wilkinson 1996), but these methods have not yet been implemented in freely-available software. Currently when there is conflict between the source trees, indirect supertree methods must be used.

Perhaps the most widely used indirect approach to supertree construction is matrix representation with parsimony (MRP; Baum 1992, Ragan 1992). This technique converts each source topology into a data matrix where each clade on a source tree becomes a "character" in the matrix, with each taxon scored for its presence (1) or absence (0) in that clade. The matrices from the individual source trees are then combined, with taxa not present in a given source tree scored as missing data (?). A dummy MRP outgroup taxon, which has all the pseudocharacter states scored as zero, is added to root the trees. A parsimony analysis can then be conducted on the whole matrix to find the shortest possible supertrees, which represent the optimal solutions to the combination of the source trees. It is also possible to apply different coding schemes and weights for characters in MRP analyses, depending on the nature of the data being used. In a MRP analysis, it is possible to weight clades from the source trees ("characters") using the relative support value for that clade and thus to give differential importance to the contribution of each data source based on the confidence placed in it. Weighting clades by some index of their overall support in a dataset (e.g. the bootstrap values for each clade) should overcome some of the conflict between datasets when building supertrees, as more value is given to groupings which are robust. Modelling studies (e.g. Bininda-Emonds & Sanderson 2001) have found that weighted MRP analysis provides a better estimate of the true phylogeny than unweighted analyses. However, studies using weighted MRP for real data are still relatively uncommon, probably due to unavailability of software packages which can easily convert bootstrap or other trees with a measure of support into a MRP dataset with weightings.

METHODS

Character Combination

The morphology, ITS and *trnL/F* datasets were pruned to include only those taxa common to all three. This resulted in a very small subset of taxa and ILD testing (Farris *et al.* 1994) indicated incongruence between the three data partitions. Due to the skeletal nature of the taxon sample common to all three, a combined analysis was unlikely to produce a meaningful phylogeny with information bearing on the aims of the project. Character combination was thus not explored further.

Topology Comparisons

Although the three datasets used for the Polygalaceae contain very few overlapping taxa, it was possible to compare the topologies generated from these datasets in general terms. Any similarities cannot be considered true taxonomic congruence due to the missing taxa, but comparisons may still be useful to identify areas of broad agreement or conflict between molecular and morphological data. This will simply be accomplished by comparing the five topologies from the three available datasets (morphology analysed under maximum parsimony, ITS MP and Bayesian, *trnL/F* MP and Bayesian) and identifying any groups which occur in more than one topology.

Topology Combination

Supertree Construction

Unweighted MRP

Five source topologies from three datasets were used to construct supertrees. The source topologies were constructed using morphological data (Fig. 2.1), ITS data (Figs 3.2 & 3.7) and the *trnL/F* dataset largely sourced from Persson (2001; Figs 3.5 & 3.8). Three of the topologies were the strict consensus of all shortest trees found in maximum parsimony analyses and the remaining two were the 50% majority-rule consensus from Bayesian analyses. Taxa which were represented more than once in a dataset (e.g. from the multiple ITS sequences of *Comesperma volubile*) were "pruned" from each topology so that each dataset contributed a single end taxon for each species. The topologies were converted into a matrix for parsimony analysis with RADCON 1.1.5 (Thorley & Page 2000), using the "components coding" option.

Parsimony analysis of the data matrix produced by RADCON was conducted in two stages in PAUP*4.0b10 (Swofford 2002). Two initial heuristic searches of 10000 random addition sequence replicates and saving two trees from each replicate were used to get some idea of the shortest tree length. A series of ten further heuristic searches was then run, ensuring that the minimal tree length found in the initial searches was reached in each search and then saving 20000 trees of that length. Both strict and Adams consensus trees (Adams 1986) from each of the ten searches were then constructed and compared to ensure that the trees saved from each replicate were representative of the analysis as a whole. Adams consensus trees were used because they show the groups which occur in all the optimal trees and collapse taxa that occur in more than one place within a clade to a basal polytomy. It is thus able to show the structure common to all the optimal trees in a replicate without being collapsed by a small number of difficult taxa as a strict consensus can be. However, caution must be used when interpreting an Adams consensus tree (Nelson & Platnick 1980; Wilkinson 1994) as the polytomies within it can be ambiguous. The internal branches in an Adams consensus tree correspond to nestings rather than to the usual dichotomous split and a polytomy may thus be resolved in several more different ways than a polytomy in a strict consensus tree. Since "rogue" taxa are collapsed to the nearest node common to all trees, they may leave behind groups which appear monophyletic but did not appear in any of the underlying trees. Adams consensus trees can be useful for identifying problematic taxa in a dataset—those which are placed in more than one position within the shortest trees—but cannot be interpreted in the same way as a strict consensus. The resolved clades generally indicate relatively close relationships, but the resolved relationships may not be strictly congruent with any of the fundamental phylogenies. The apparent increased resolution offered by an Adams consensus over a strict consensus in a supertree analysis may thus in fact be spurious and misleading.

Weighted MRP

A second supertree analysis was undertaken to examine the effect of using bootstrap "support" values for the clades as a method of weighting. Since bootstrap values were only available for three of the topologies used in the unweighted supertree (the strict consensus trees from the maximum parsimony analyses of the morphology, ITS and *trnL/F* datasets), a new MRP matrix was constructed using only these source trees. One thousand fast bootstrap replicates were run on each of the three datasets and the resulting bootstrap values saved for use as a weights set. Since it was desirable to give more weight to high bootstrap values and relatively less weight to lower values, rather than using a linear weighting scheme, the bootstrap proportions were squared. These "square boot" values thus ranged from 0.25 (50% bootstrap) to 1 (100% bootstrap) and were multiplied by 100 and rounded off to give integer weights as required by PAUP*. Nodes from the three source trees which did not appear in the 50% majority-rule bootstrap trees were given a weight of 20. A weighted maximum parsimony analysis was then conducted in two stages. Initially two sets of 10000 random addition sequence replicates were run, saving only two shortest trees per replicate. The two sets of shortest trees saved were combined and used as starting trees in the second stage of the analysis, with MAXTREES set to 50000 and these were swapped to completion.

RESULTS

Topology Comparisons

The strict consensus of all shortest trees obtained from cladistic analysis of the morphological dataset (Fig. 2.1) indicates that *Comesperma* is a monophyletic group, containing three monophyletic series *sensu* Chodat (1896)—*Volubiles*, *Disepalae* and *Isocalyx*. Series *Scopariae* appears non-monophyletic in this analysis and the majority of the remainder of the species in *Comesperma* (Series "*Eucomesperma*") form a polytomy of a few species groupings. In this analysis, much of Series *Scopariae* forms the sister group to Series *Volubiles*. The sister group to *Comesperma* as a whole in this analysis is *Polygala*, but the latter genus has *Bredemeyera* nested within it. The species from *Polygala* Series *Chloropterae* form a monophyletic group. *Salomonia*, *Muraltia* and *Emblingia* are progressively sister to the *Comesperma+Polygala* (including *Bredemeyera*) clade, with *Xanthophyllum* being used as an outgroup to root the tree.

Broadly speaking, the results from analysis of the ITS dataset using both maximum parsimony and Bayesian analysis are not in conflict with those based on morphology. However, the ITS strict consensus trees (Figs 3.2 & 3.7) are significantly more resolved than that from MP analysis of the morphological data. The ITS dataset also indicates that Series *Scopariae* is non-monophyletic, although the taxon falling outside the series (*Comesperma spinosum*) differs to that in the morphological results (*C. defoliatum*). The ITS consensus trees shows a sister group relationship between Series *Confertae* and Ser. *Scopariae* (minus *C. spinosum*). Series *Volubiles* is the sister group to this pairing, but the position of *C. rhadinocarpum* (*Subsect. Comespermastrum*) differs between MP and Bayesian trees. The two analysis methods also differ in defining the sister group to *Comesperma*, with MP placing *Polygala* as sister to *Comesperma* (as did the morphological analysis, albeit with the inclusion of *Bredemeyera*) and the Bayesian results indicating that a largely unresolved grouping of *Polygala+Salomonia+Epirixanthes+Bredemeyera+Muraltia* is the sister group. Both methods agree on two strong groupings within *Polygala*, one consisting of species with petaloid lateral sepals and the second including members of Series *Chloropterae* with herbaceous lateral sepals. The two analyses also place *Xanthophyllum* as the sister group to the remainder of the family within Australia.

It is difficult to compare these findings to those from analysis of the *trnL/F* sequence data, as only a very small subset of the Australian taxa were present in that dataset. However, in broad terms the groups which are in common show a similar pattern of relatedness to that from morphological and ITS datasets. The representatives of *Comesperma* included form a monophyletic group and include a strongly-supported Series *Confertae*. The representatives of Series *Scopariae* and *Volubiles* again appear closely related and show a similar pattern to the ITS data by grouping with *Comesperma* *calymega* and *C. rhadinocarpum*. The position of *Bredemeyera papuana* is equivocal, appearing as the sister group to *Comesperma* in the Bayesian analysis, but falling rather unexpectedly into a clade with *Salomonia* in the MP consensus. It is not possible to say much about the position of the two Australian *Polygala* species included in the *trnL/F* dataset, other than they fall within the same clade regardless of the analysis method and that they appear to be correctly placed within Section *Polygala*, as the type species for the genus (*P. vulgaris*) is also a member of this grouping. Finally, the addition of two Australian *Xanthophyllum* sequences did not affect the sister-group relationship between this genus and the remainder of the Polygalaceae.

These overall results of the topology comparisons can be summarised as follows:

Agreement among all datasets

- *Comesperma* is a monophyletic group, not a sister genus to *Bredemeyera* as a whole.

Agreement between two datasets

- *Xanthophyllum* is a monophyletic sister group to the remainder of family (thus used as outgroup in morphology dataset).
- Members of *Polygala* Series *Chloropterae* (species with herbaceous rather than petaloid lateral sepals, including the majority of the Australian native or endemic taxa) form a monophyletic group.
- Several of Chodat's (1896) subgeneric groups within *Comesperma*—*Volubiles*, *Scopariae* (minus *C. spinosum* or *C. defoliatum*), *Confertae*—are recovered in morphological and either *trnL/F* or ITS datasets. Since the traditional classification was based on morphology, it is unsurprising that these appear in an analysis of morphological data. However, there is also weak corroboration of these groups from some of the molecular data.

Unresolved or uncorroborated findings (single dataset only)

- *Bredemeyera papuana* groups with South American species using morphology, but its placement in the *trnL/F* consensus trees is ambiguous and changes with the evolutionary model applied.
- A species of *Epirixanthes* forms the sister group to the species of *Salomonia* included in the ITS dataset.

Matrix Representation with Parsimony

Unweighted MRP

The data matrix constructed by RADCON using the five unweighted source topologies from the Polygalaceae data contained 126 taxa (including the MRP outgroup) and 217 "characters". The shortest trees obtained by the initial analyses of the RADCON data matrix were 251 steps long. The strict consensus of the 20000 shortest trees are not identical across replicates and neither are the Adams consensus trees, but each consensus type from all replicates differs only in the position of a few taxa. The least resolved and thus most "conservative" strict consensus tree was also the most common (found in nine of the eleven replicates) and is shown in Figure 4.1. The most common Adams consensus tree (found in seven of the eleven replicates) is shown in Figure 4.2. Measures of the fit (consistency and retention indices) of the three datasets to the strict consensus tree and to one of 20000 shortest trees chosen from a replicate at random are shown in Table 4.1.

TABLE 4.1: Goodness of fit measures for the source tree data to the strict consensus and one tree chosen at random from a replicate which saved 20000 shortest trees

Source of Data	Number of characters in MRP analysis	Strict Consensus		Random MP Tree	
		CI	RI	CI	RI
All trees	217	0.409	0.673	0.865	0.965
Morphology MP	38	0.495	0.534	0.803	0.850
ITS Bayesian	23	0.613	0.555	0.960	0.935
ITS MP	26	0.636	0.623	0.960	0.968
trnL/F Bayesian	67	0.785	0.741	0.955	0.982
trnL/F MP	63	0.814	0.764	0.944	0.968

The most common Adams consensus (Fig. 4.2) indicates that *Comesperma* is a potentially monophyletic group in this analysis, although it completely collapses into a polytomy containing the entire remainder of the tribe Polygaleae in the strict consensus tree. This lack of confirmed monophyly is puzzling initially, as all the source trees used agree on *Comesperma* as a monophyletic group. One might expect this node to appear in the supertrees also, but this is likely to be due to the degradation of the effectiveness

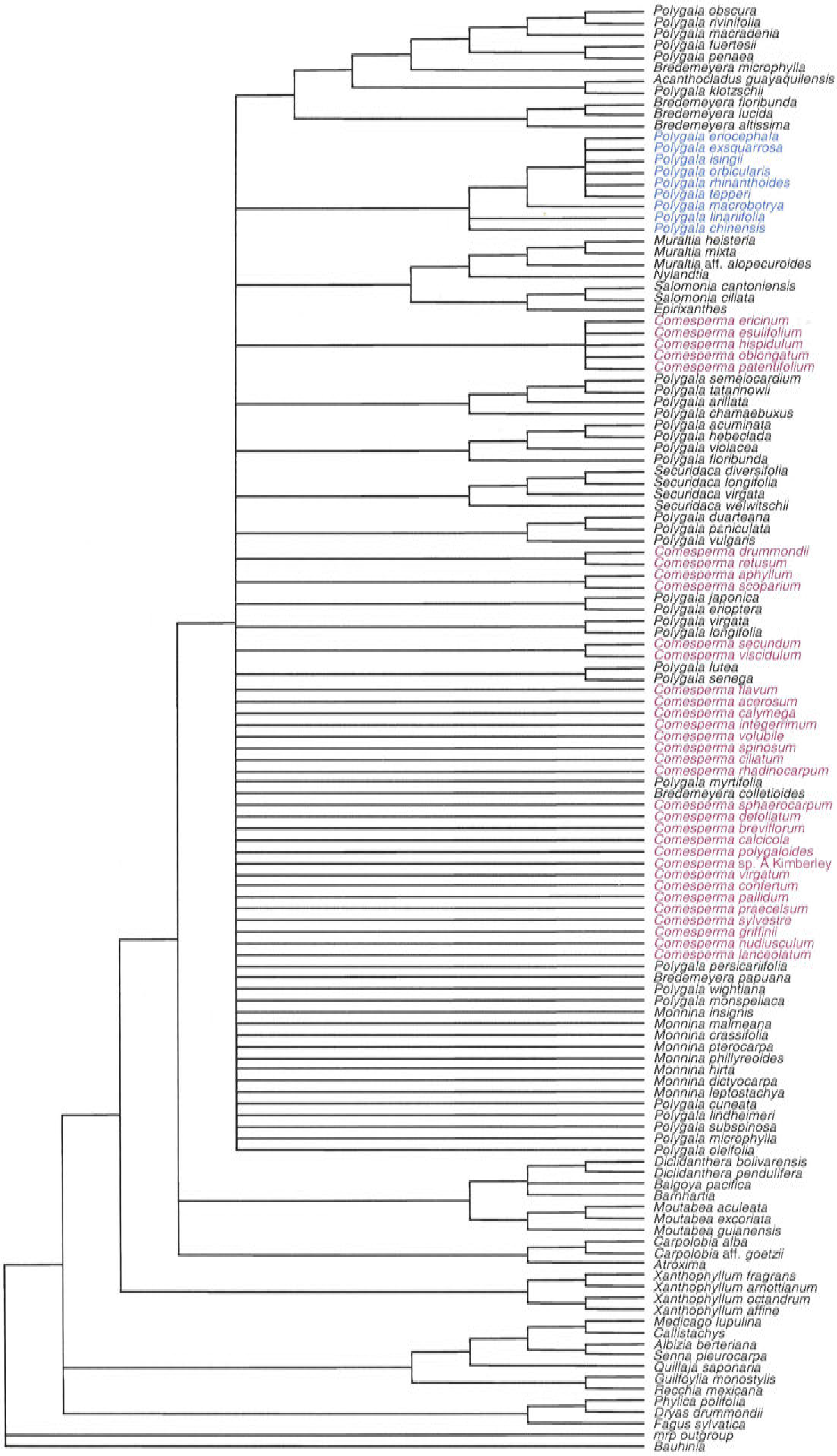

FIGURE 4.1: Strict consensus MRP supertree of 20000 shortest trees obtained from maximum parsimony analysis of five source topologies from three Polygalaceae data sets. Groupings are those on which all five topologies are unequivocal or those which are unique to a single dataset. Names of taxa from *Comesperma* are shown in pink, while those from *Polygala* series *Chloropterae* are shown in blue.

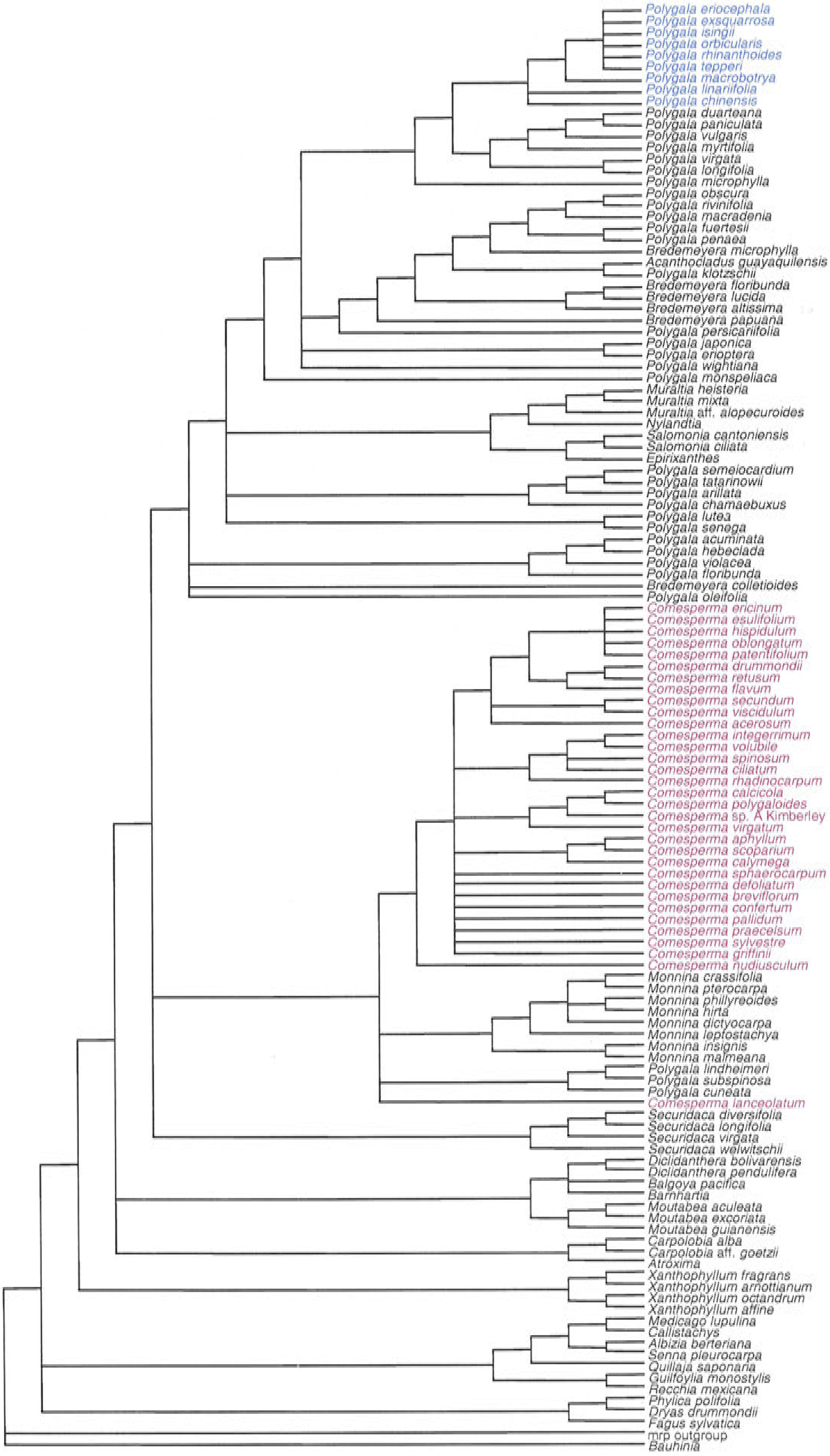

FIGURE 4.2: Adams consensus MRP supertree of 20000 shortest trees obtained from maximum parsimony analysis of five source topologies from three Polygalaceae data sets. Names of taxa from *Comesperma* are shown in pink, while those from *Polygala* series *Chloropterae* are shown in blue.

of all methods of supertree and supermatrix analysis reported by Bininda-Emonds and Sanderson (2001) when taxon overlap between datasets is low. They found that the beneficial effects of using more source trees were negated when taxon overlap between the trees was low and that the most accurate results were obtained when the degree of taxon overlap was greater then 75%. This is an intractable problem for the current study without further data being available.

In the Adams consensus, one species, *Comesperma lanceolatum*, is placed in a polytomy at the node below that leading to the remainder of the genus, but this does not preclude the possibility of a sister relationship and thus monophyly in that tree. *Comesperma lanceolatum* is unique morphologically in the genus, with the upper stigma lobe being membranous and bearing a tuft of hairs, and it is only represented in the morphological dataset, so it is not surprising that the species cannot be conclusively placed in the supertree.

Within *Comesperma*, many of the subgeneric groups described by Chodat (1896) appear, again strongly influenced by the morphological dataset where all taxa are represented. However, the molecular datasets do not conflict with these groupings where the taxa do overlap—the conflict is at the level of the relationship **among** the groups, as represented by the large polytomy containing Series *Volubiles*, *Confertae*, *Disepalae* and a pairing of *Comesperma aphyllum* and *C. scoparium* (Series *Scopariae*).

One somewhat surprising grouping in the Adams consensus supertree is the inclusion of *Comesperma* within a clade including *Monnina* and some *Polygala* species from sections *Chamaebuxus* and *Phlebotaenia*. These taxa are only included in the *trnL/F* dataset, where they all form part of the Polygaleae II clade of Persson (2001). This separation represents a disruption of the two Polygaleae clades indicated by *trnL/F* due to the influence of the morphological and ITS datasets. Since the entire Polygaleae grouping collapses in the strict consensus, it would be premature to postulate a close relationship between *Comesperma* and these other groups without further evidence, but it does lend one further indication that *Comesperma* is not the sister genus to *Bredemeyera sens. strict.*

Finally, the supertrees include *Polygala* series *Chloropterae* as a monophyletic group and the Adams consensus places it as the sister group to the majority of the other *Polygala* species in Australia. This is of course an artefact of their sister relationship in the morphological and ITS analyses. There are likely to be many taxa missing "between" these two groups, as series *Chloropterae* occurs naturally in Australia, while the second clade consists of an "artificial" group of mainly introduced taxa.

In summary, the supertrees constructed by analysis of the MRP dataset are a useful way to visualise the relationships across the Polygalaceae and to examine those on which the three datasets all agree. Much of the structure is derived from *trnL/F*, the largest dataset which includes many unique taxa. The possibility of the larger datasets dominating supertree analysis has been recognised, but Kennedy & Page (2002) found that this problem was minimised when a larger proportion of the taxa in the large analysis are shared by other datasets. They also indicated that the domination of signal from large datasets was more of a problem for their supermatrix analysis of the same data. The tree statistics shown in Table 4.1 indicate that the *trnL/F* dataset is most consistent with the supertree topology, while morphology is the least consistent. However, none of the source trees used is significantly at odds with the supertree topology, as indicated by the difference between the CI and RI for any given source tree in comparison to the CI and RI for the overall supertree. In the Polygalaceae supertrees, both ITS and morphology datasets are useful in providing a check on the relationships indicated by *trnL/F* and the "veto" power of the smaller datasets will collapse any clades on which the source trees conflict. However, this veto power may give undue weight to the relationships supported by the smaller datasets in this analysis or at least downweight the relatively well-supported hypotheses contained in the *trnL/F* topology. The subsequent lack of resolution in the supertrees may thus be a somewhat spurious result. It is for these reasons that a weighting scheme for the MRP pseudocharacters is desirable, as it can upweight those clades which are well-supported in their source tree over those where the support is limited.

Weighted MRP

The weighted analysis contained 126 taxa and 124 "characters" (i.e. nodes from the three source trees). The two sets of trees saved from the two initial replicates included 5312 and 6715 trees respectively, all of length 6715 steps. After using these as starting trees, 50000 trees were saved and strict and Adams consensus trees constructed.

The strict and Adams consensus trees from the weighted analysis are actually less-resolved to those presented for the unweighted replicates (Figs 4.1 & 4.2). They do not conflict with the results from unweighted analyses, but many more species are placed in polytomies. Since they contain less resolution and there are no novel placements of taxa, they are not presented separately.

The weighted analysis again cannot confirm the monophyly of *Comesperma*, and the strict consensus is identical for both unweighted and weighted analysis in regard to this genus. These results indicate that weighting by a measure of support for a clade cannot overcome the problems of low taxon overlap in supertree analysis.

CONCLUSIONS AND FURTHER WORK

This study has examined the evolutionary relationships of the Polygalaceae in Australia and a revision of the family has been completed in light of the phylogenetic analyses (see Appendix 1). One morphological and two molecular datasets have been gathered and analysed under maximum parsimony. The molecular datasets were also subjected to Bayesian analyses. The putative phylogenies are presented separately and have also been combined in the form of MRP supertrees.

At least partial answers have been found for the five specific questions posed at the beginning of this project.

- Is *Comesperma* a "good" genus or is it congeneric with *Bredemeyera*?

All three datasets indicate that *Comesperma* is a monophyletic group and that it is not nested with the representatives of *Bredemeyera* included in the analyses. Although the defining characters for each genus are relatively few, it is clear they should not be combined. Persson's (2001) analysis of *trnL/F* also shows that *Bredemeyera* itself is polyphyletic and splits into sectional groups in his trees. The generic status of *Bredemeyera* should probably be narrowed to exclude *Hualania* also, although further data to confirm these results would strengthen such a decision.

- Where should *Bredemeyera papuana* be placed?

This species remains problematic for several reasons—it has not been re-collected since the late 1960s and it was extremely difficult to extract useful DNA from the few specimens which are available. A half sequence was generated for the *trnL/F* dataset, but its position within the phylogenetic trees differed with analysis method. Bayesian analysis does indicate a sister group relationship to the remainder of *Comesperma* and there are morphological features in common, but without re-extraction and re-sequencing, the position of this species must be considered ambiguous. Attempts to sequence the existing DNA sample for other regions or to resequence the *trnL/F* region failed due to contamination issues and the low levels of whole DNA for the species. Re-collection from the wild and/or improved sequencing techniques may provide a solution for this problem.

- Is *Epirixanthes* a "good" genus or is it congeneric with *Salomonia*?

This question was not conclusively answered in this study. An ITS sequence for *Epirixanthes cylindrica* appeared as the sister species to two species of *Salomonia*, but further sampling from both genera would be required to confirm this result and ascertain that these are sister genera rather than one nesting within the other. My own sequencing and that reported by Persson (2001) failed to recover a *trnL/F* sequence from *Epirixanthes*, which may well indicate that it has a reduced chloroplast genome lacking this region. (Wolfe *et al.* (1992) found that the entire *trnL* gene was either absent or transformed into a pseudogene in *Epifagus virginiana*, a non-photosynthetic parasitic flowering plant.) By contrast, representatives of *Salomonia* were readily sequenced for both chloroplast and nuclear markers. The "negative" result for *Epirixanthes* may thus be further evidence of its separation from *Salomonia*.

- Should the Xanthophyllaceae be maintained as a separate family or sunk into the Polygalaceae?

The two molecular datasets were analysed using taxa from the Fabaceae and other related families as indicated by recent large phylogenies for angiosperm families to provide a root for the trees produced. Both ITS and *trnL/F* strongly suggest that the representatives of *Xanthophyllum* sequenced are monophyletic, forming the sister to all other genera in the analyses. *Xanthophyllum* could thus be maintained as a tribe (Xanthophylleae) of the Polygalaceae or a distinct family (Xanthophyllaceae). Either status would be justified by the current scientific evidence and, since only a small number of species were included in these phylogenetic analyses, it is simplest to maintain the *status quo* unless future phylogenetic analysis of *Xanthophyllum* indicates that it is non-monophyletic.

- Does *Emblingia* belong in the Polygalaceae?

This question was largely answered prior to the commencement of the project, with *rbc*L data (Angiosperm Phylogeny Group 1998; Chandler & Bayer 2000) indicating that *Emblingia* is closely related to the Capparales. A specimen was sequenced for ITS and *trnL/F* and a BLAST search of Genbank indicated that the sequences were closest to those for *Cleome* (Capparaceae). Since this family is only distantly related to Polygalaceae and its inclusion might cause problems with homology assessment, it was omitted from any further analysis.

Future work on the phylogeny of Polygalaceae in Australia needs to be refocused as a result of these findings. The existing phylogenies are largely unresolved in regard to the relationships between species groups—the divisions of the genera are reasonably well supported and so are several smaller groups within these genera, but the relationships between these groups are highly speculative at present. A molecular region intermediate in evolutionary rate between those used in this study may be able to resolve this question. The subgeneric relationships within *Comesperma* outlined by Chodat (1896) are largely supported by the current molecular data, which is a valuable finding. Relating these groups to each other and exploring further groupings would also be useful. Chodat's classification for the most part separated the "oddball" taxa within *Comesperma*, leaving the vast majority of the genus in the polymorphic series *Confertae*. Lower-level studies of this large group of shrubby taxa may provide a better understanding and classification of its diversity, which could then be utilised in a monographic treatment. In a similar vein, the south-east Asian and Australian taxa within *Polygala* series *Chloropterae* need closer examination and perhaps phylogenetic analysis. While the monophyly of this series is strongly indicated, the taxonomy of the Australian taxa in particular is unsatisfactory. There may be several new or previously unrecorded species in northern and central Australia and any study would need to include these as end-taxa.

REFERENCES

Adams, E.N. (1986). *N*-trees as nestings: complexity, similarity and consensus. *J. Classif.* **3**: 299–317.

Adema, F. (1969). Identities of the herbaceous Australian species of *Polygala* represented in the Brisbane Herbarium. *Proc. Roy. Soc. Queensland* **80**(9): 125–130.

Airy Shaw, H.K. (1965). Diagnoses of new families, new names, etc., for the seventh edition of Willis's Dictionary. *Kew Bull.* **18**: 249–273.

Angiosperm Phylogeny Group (1998). An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **88**: 531–553.

Archie, J.V. (1989). A randomization test for phylogenetic information in systematic data. *Syst. Zool.* **38**: 239–252.

Arnheim, N. (1983). Concerted evolution in multigene families, pp. 38–61 *in* M.Nei & R.Koehn (eds), Evolution of Genes and Proteins. Sinauer Associates, Sunderland, Massachusetts.

Baillon, H.E. (1874). Polygalacées, *Hist. Pl.* 5: 71–92.

Bakker, F.T., A.Culham, R.Gomez-Martinez, J.Carvalho, J.Compton, R.Dawtrey & M.Gibby (2000). Patterns of nucleotide substitution in angiosperm cpDNA *trnL* (UAA)–*trnF* (GAA) regions. *Mol. Biol. Evol.* **17**: 1146–1155.

Baldwin, B.G., M.J.Sanderson, J.M.Porter, M.F.Wojciechowski, C.S.Campbell & M.J.Donoghue (1995). The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.* **82**: 247–277.

Banks, J. (1900–1905). Illustrations of Australian plants collected in 1770 during Captain Cook's voyage round the world in H.M.S. *Endeavour*, by the Right Hon. Sir Joseph Banks and Dr Daniel Solander with determinations by James Britten. Trustees of the British Museum, London.

Baum, B.R. (1992). Combining trees as a way of combining data sets for phylogenetic inference, and the desirability of combining gene trees. *Taxon* **41**: 3–10.

Bayer, R.J. & J.R. Starr (1998). Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trnL* intron and *trnL*/*trnF* intergenic spacer. *Ann. Missouri Bot. Gard.* **85**: 242–256.

Bentham, G. (1863). Polygaleae, *Flora Australiensis* 1: 137–149. Lovell Reeve & Co., London.

Bentham, G. & J.D.Hooker (1862). Polygaleae, *Gen. Pl.* 1: 134–140. Lovell Reeve & Co., London.

Benton, M.J. (1999). Early origins of modern birds and mammals: molecules vs. morphology. *BioEssays* **21**: 1043–1051.

Bininda-Emonds, O.R.P & M.J.Sanderson (2001). Assessment of the accuracy of matrix representation with parsimony analysis supertree construction. *Syst. Biol.* **50**: 565–579.

Birky, W.C. (1995). Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proc. Natl Acad. Sci. U.S.A.* **92**: 11331–11338.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10**: 295–304.

Brown, R. (1810). *Prodromus Florae Novae Hollandiae*. Englemann, J.Cramer & Weldon &Wesley Ltd, Weinheim.

Brown, R. (1814). Polygalaeæ *in* M.Flinders, *Voy. Terra. Austral.* 2: 542–543. G. & W.Nicol, London.

Bull, J.J., J.P.Huelsenbeck, C.W.Cunningham, D.L.Swofford, and P.J.Waddell (1993). Partitioning and combining data in phylogenetic analysis. *Syst. Biol.* **42**: 384–397.

Candolle, A.P. de (1824) Polygaleæ. *Prodr.* 1: 338–340. Treuttel & Würtz, Paris.

Carpenter, J.M. (1988). Choosing among multiple equally parsimonious cladograms. *Cladistics* **4**: 291–296.

Chandler, G.T. & R.J.Bayer (2000). Phylogenetic placement of the enigmatic Western Australian genus *Emblingia* based on *rbcL* sequences. *Pl. Sp. Biol.* **15**(1): 67–72.

Chase, M.W., D.E.Soltis, R.G.Olmstead, D.Morgan, D.H.Les, B.D.Mishler, M.R.Duvall, R.A.Price, H.G.Hills, Y.-L.Qiu, K.A.Kron, J.H.Rettig, E.Conti, J.D.Palmer, J.R.Manhart, K.J.Sytsma, H.J.Michaels, W.J.Kress, K.G.Karol, W.D.Clark, M.Hedrén, B.S.Gaut, R.K.Jansen, K.-J.Kim, C.F.Wimpee, J.F.Smith, G.R.Furnier, S.H.Strauss, Q.-Y.Xiang, G.M.Plunkett, P.S.Soltis, S.M.Swensen, S.E.Williams, P.A.Gadek, C.J.Quinn, L.E.Eguiarte, E.Golenberg, G.H.Learn Jr, S.W.Graham, S.C.H.Barrett, S.Dayanandan & V.A.Albert (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Ann. Missouri Bot. Gard.* **80**: 528–580.

Chen, W.J., C.Bonillo, G.Lecointre (2003). Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. *Mol. Phylogenet. Evol.* 26(2): 262–288.

Chodat, R. (1891–1893). Monographia Polygalacearum. *Mém. Soc. Phys. Genève*, Suppl. 1890(7): 1–143, t. 1–12 (1891); *op. cit.* 31(2, 2): i–xii, 1–500, t. 13–35 (1893).

Chodat, R. (1896). Polygalaceae, *in* A.Engler & K.Prantl (eds.), *Natürlich. Pflanzenfam.* III, 4: 323–345. W.Engelmann, Leipzig.

Chodat, R. & G.Hochreutiner (1893). *Contribution a l'etude du genre* Comesperma. *Bull. Herb. Boiss.* **1**: 358–369.

Coombes, A.J. (1994). Dictionary of plant names. Timber Press, Portland, Oregon.

Cotton, J.A. & R.D.M.Page (in press). Gene tree parsimony vs. uninode coding for phylogenetic reconstruction. *Mol. Phylogenet. Evol.*

Crisp, M.D., S.R.Gilmore & P.H.Weston (1999). The phylogenetic relationships of two anomalous species of *Pultenaea* (Fabaceae: Mirbelieae), and description of a new genus. *Taxon* 48: 701–704.

Crisp, M.D. & L.G.Cook (2003). Phylogeny and evolution of anomalous roots in *Daviesia* (Fabaceae: Mirbelieae). *Int. J. Plant Sci.* **164**(4): 603–612.

Crisp, M.D. & L.G.Cook (in press). Phylogeny and embryo sac evolution in the endemic Australasian papilionoid tribes Mirbelieae and Bossiaeeae, *in* B.B.Klitgaard & A.Bruneau (eds), Advances in Legume Systematics 10. Royal Botanic Gardens: Kew.

Cronquist, A. (1981). An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.

Dahlgren, R.M.T. (1980). A revised system of classification of the angiosperms. *Bot J. Linn. Soc.* **80**: 91–124.

Davis, C.C., P.W.Fritsch, J.Li & M.J.Donoghue (2002). Phylogeny and biogeography of *Cercis* (Fabaceae): evidence from nuclear ribosomal ITS and chloroplast *ndhF* sequence data. *Syst. Bot.* **27**(2): 289–302.

Depamphilis, C.W. & J.D.Palmer (1990). Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. *Nature* **348**: 337–339.

De Queiroz, A. (1993). For consensus (sometimes). *Syst. Biol.* **42**(3): 368–372.

Dickison, W.C. (1973). Nodal and leaf anatomy of *Xanthophyllum* (Polygalaceae). *J. Linn. Soc. Bot.* **67**: 103–115.

Doyle, J.J. (1992). Gene trees and species trees: molecular systematics as one-character taxonomy. *Syst. Bot.* **17**: 144–163.

Doyle, J.J. & J.L.Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**: 11–15.

Doyle, J.J., J.L.Doyle, J.A.Ballenger, E.E.Dickson, T.Kajita & H.Ohashi (1997). A phylogeny of the chloroplast gene *rbcL* in the Leguminosae: taxonomic correlations and insights into the evolution of nodulation. *Am. J. Bot.* **84**: 541–554.

Erdtman, G., P.Leins, R.Melville & C.R.Metcalfe (1969). On the relationships of *Emblingia*. *Bot. J. Linn. Soc.* **62**: 169–186.

Eriksen, B. (1993a). Floral anatomy and morphology in the Polygalaceae. *Pl. Syst. Evol.* **186**: 17–32.

Eriksen, B. (1993b). Phylogeny of the Polygalaceae and its taxonomic implications. *Pl. Syst. Evol.* **186**: 33–55.

Ewart, A.J. (1930). Polygalaceae, *Fl. Victoria* 713–717. Government Printers, Melbourne.

Ewart, A.J. & O.B.Davies (1917). Polygalaceae, *Fl. N. Terr.* 160–161. McCarron, Bird & Co, Melbourne.

Faith, D.P. (1991). Cladistic permutation tests for monophyly and nonmonophyly. *Syst. Zool.* **40**: 366–375.

Faith, D.P. & P.S.Cranston (1991). Could a cladogram this short have arisen by chance alone?: On permutation tests for cladistic structure. *Cladistics* **7**: 1–28.

Farris, J.S. (1969). A successive approximations approach to character weighting. *Syst. Zool.* **18**: 374–385.

Farris, J.S. (1989). The retention index and homoplasy excess. *Syst. Zool.* **38**: 406–407.

Farris, J.S., M.Källersjö, A.G.Kluge & C.Bult (1994). Testing significance of incongruence. *Cladistics* **10**: 315–319.

Feierabend, J. (1992). Conservation and structural divergence of organellar DNA and gene expression in non-photosynthetic plastids during ontogenetic differentiation and phylogenetic adaptation. *Bot. Acta* **105**: 227–231.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fernando, E.S., P.A.Gadek, D.M.Crayn & C.J.Quinn (1993). Rosid affinities of Surianaceae: molecular evidence. *Mol. Phylogenet. Evol.* **2**: 344–350.

Gagnepain, F. (1908). Contribution à la connaisance des *Xanthophyllum*. *J. Bot. (Morot)* sér. 2, **1**: 241–253.

Gatesy, J., P.O'Grady & R.H.Baker (1999). Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. *Cladistics* **15**: 271–313.

Gielly, L. & P.L.Taberlet (1994). The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbcL* sequences. *Mol. Biol. Evol.* **11**: 769–777.

Gielly, L. & P.Taberlet (1996). A phylogeny of the European gentians inferred from chloroplast *trnL* (UAA) intron sequences. *Bot. J. Linn. Soc.* **120**(1): 57–75.

Givnish, T.J. & K.J.Sytsma (1997). Homoplasy in molecular vs. morphological data: the likelihood of correct phylogenetic inference, pp. 55–101 *in* T.J.Givnish & K.J.Sytsma (eds), Molecular Evolution and Adaptive Radiation. Cambridge University Press, New York.

Gower, D.J. & M.Wilkinson (1996). Is there any consensus on basal archosaur phylogeny? *Proc. Roy. Soc. London Ser. B* **263**: 1399–1406.

Grieve, M. 1967. A Modern Herbal, vol. II, pp. 733–734. Hafner Publishing Co., New York and London.

Grieve, B.J. (1998). *How to Know Western Australian Wildflowers*: Part II. Restructured and revised 2nd edn. UWA Press, Nedlands.

Hauk, W.D., L.G.Lohmann & S.S.Renner (1997). Indels of the *trnL-F* intergenic spacer as generic markers in woody tropical lianas (Bignoniaceae tribe Bignonieae). *Am. J. Bot.* **84**, suppl.: 201.

Hedges, S.B. & L.R.Maxson (1996). Molecules and morphology in amniote phylogeny. *Mol. Phylogenet. Evol.* **6**: 312–314.

Higgins, D.G. & P.M.Sharp (1988). CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* **73**(1): 237–44.

Hillis, D.M. (1987). Molecular versus morphological approaches to systematics. *Ann. Rev. Ecol. Syst.* **18**: 23–42.

Hillis, D.M. (1998). Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Syst. Bot.* **47**: 3–8.

Hillis, D.M., C.Moritz, C.A.Porter & R.J.Baker (1991). Evidence for biased gene conversion in concerted evolution of ribosomal DNA. *Science* **251**: 308–310.

Hillis, D.M., J.P.Huelsenbeck & C.W.Cunningham (1994). Application and accuracy of molecular phylogenies. *Science* **264**: 671–677.

Hillis, D.M. & J.J.Wiens (2000). Molecules versus morphology in systematics: conflicts, artifacts, and misconceptions, pp. 1–19 *in* J.J.Wiens (ed.), Phylogenetic Analysis of Morphological Data. Smithsonian Institution Press, Washington D.C.

Hnatiuk, R.J. (1990). Census of Australian Vascular Plants. Australian Flora and Fauna Series Number 11, Australian Government Publishing Service.

Huelsenbeck, J.P., J.J.Bull & C.W.Cunningham (1996). Combining data in phylogenetic analysis. *TREE* **11**:152–158.

Huelsenbeck, J.P. & F.Ronquist (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Jayasekara, T.K., P.C.Stevenson, S.R.Belmain, D.I.Farman & D.R.Hall (2002). Identification of methyl salicylate as the principal volatile component in the methanol extract of root bark of *Securidaca longepedunculata* Fers. *J. Mass Spectrom.* **37**(6): 577–80.

Källersjö, M., J.S.Farris, M.W.Chase, B.Bremer, M.F.Fay, C.J.Humphries, G.Petersen, O.Seberg & K.Bremer (1998). Simultaneous parsimony jacknife analysis of 2538 *rbcL* DNA sequences reveals support for major clades of green plants, land plants, seed plants and flowering plants. *Plant Syst. Evol.* **213**: 259–287.

Keighery, G.J. (2002). Two new species of *Comesperma* (Polygalaceae) from Western Australia. *Nuytsia* **15**: 53–57.

Kennedy, M. & R.D.M.Page (2002). Seabird supertrees: combining partial estimates of procellariiform phylogeny. *Auk* **119**: 88–108.

Kim, K.J. & R.K.Jansen (1995). *ndhF* sequence evolution and the major clades in the sunflower family. *Proc. Natl Acad. Sci. U.S.A.* **92**(22): 10379–10383.

Kindscher, K. (1992). Medicinal Wild Plants of the Prairie (pp. 164–168). Univ. Kansas Press, Lawrence.

Kluge, A.G. (1989). A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* **38**: 7–25.

Kluge, A.G. & J.S.Farris (1969). Quantitative phyletics and the evolution of the anurans. *Syst. Zool.* **18**: 1–32.

Koyama, H. (1995). A revision of the genus *Salomonia* (Polygalaceae). *Bull. Natl Sci. Mus. Tokyo, Ser. B.* **21**: 1–12.

Labillardière, J.J.H. de (1806). Diadelphia: Octandria, *Nov. Holl. Pl.* 2: 21–24, t. 159–163.

Lamboy, W.F. (1994). The accuracy of the maximum parsimony method for phylogeny reconstruction with morphological characters. *Syst. Bot.* **19**: 489–505.

Lee, C., C.Grasso & M.Sharlow (2002). Multiple sequence alignment using partial order graphs. *Bioinformatics* **18**: 452–464.

Linnaeus, C. (1753). Diadelphia: Octandria and Decandria. *Sp. Pl.* 1: 701–707. Impensis Laurentii Salvii, Stockholm.

Mabberley, D.J. (1997). The Plant Book (2nd edition). Cambridge University Press, Cambridge.

Maddison, W. (1997). Gene trees in species trees. *Syst. Biol.* **46**: 523–536.

Mai, J.C. & A.W.Coleman (1997). The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *J. Mol. Evol.* **44**: 258–271.

Meijden, R. van der (1982). Systematics and evolution of *Xanthophyllum* (Polygalaceae). *Leiden Bot. Ser.* 7: 1–157.

Meijden, R. van der (1988). Polygalaceae, *in* C.G.G.J van Steenis (ed.), *Fl. Males.* ser. 1, 10(3): 455–539.

Mengoni, A., A.J.M.Baker, M.Bazzicalupo, R.D.Reeves, N.Adigüze, E.Chianni, F.Galardi, R.Gabbrielli & C.Gonnelli (2003). Evolutionary dynamics of nickel hyperaccumulation in *Alyssum* revealed by its nrDNA analysis. *New Phytol.* **159**(3): 691–699.

Mickevich, M.F. (1978). Taxonomic congruence. *Syst. Zool.* **27**:143–158.

Miyamoto, M.M. & W.M.Fitch (1995). Testing species phylogenies and phylogenetic methods with congruence. *Syst. Biol.* **44**: 64–76.

Molvray, M., P.J.Koresa & M.W.Chase (1999). Phylogenetic relationships within *Korthalsella* (Viscaceae) based on nuclear ITS and plastid *trnL-F* sequence data. *Am. J. Bot.* **86**: 249–260.

Morgan, D.R. & D.E.Soltis (1993). Phylogenetic relationships among members of the Saxifragaceae *sensu lato* based on *rbcL* sequence data. *Ann. Missouri Bot. Gard.* **80**: 631–660.

Morgan, D.R., D.E.Soltis & K.R.Robertson (1994). *rbcL* sequence variation in Rosaceae. *Am. J. Bot.* **81**: 890–903.

Mueller, F.J.H. von (1859). Fragmenta Phytographiae Australiae 1: 144–145. John Ferres, Melbourne.

Mueller, F.J.H. von (1860). Fragmenta Phytographiae Australiae 2: 2–4, t. XI. John Ferres, Melbourne.

Murray, L. (1993). Polygalaceae, pp. 3–7 *in* G.J.Harden (ed.), *Flora of New South Wales* vol. 3. N.S.W. University Press, Sydney.

Nelson, G. & N.I.Platnick (1980). Multiple branching in cladograms: two interpretations. *Syst. Zool.* **29**: 86–91.

Page, R.D.M. & M.A.Charleston (1997). From gene to organismal phylogeny: reconciled trees and the gene tree/species tree problem. *Mol. Phylogenet. Evol.* **7**: 231–240.

Page, R.D.M. (1998). GeneTree: comparing gene and species phylogenies using reconciled trees. *Bioinformatics* 14: 819–820.

Palmer, J.D., P.J.Calie, C.W.Depamphilis, J.M.Logsdon, D.S.Katz-Downie & S.R.Downie (1990). An evolutionary genetic approach to understanding plastid gene function: lessons from photosynthetic and nonphotosynthetic plants. *Current Res. Photosyn.* **3**: 475–482.

Pedley, L. (1984). A revision of *Comesperma* (Polygalaceae) in Queensland. *Austrobaileya* **2**: 7–14.

Persson, C. (2001). Phylogenetic relationships in Polygalaceae based on plastid DNA sequences from the *trnL-F* region. *Taxon* **50**(3): 763–779.

Posada, D. & K.A.Crandall (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**(9): 817–818.

Potgieter, K. & V.A.Albert (2001). Phylogenetic relationships within Apocynaceae *s.l.* based on *trnL* intron and *trnL-F* spacer sequences and propagule characters. *Ann. Missouri Bot. Gard.* **88**: 523–549.

Ragan, M.A. (1992). Phylogenetic inference based on matrix representation of trees. *Mol. Phylogenet. Evol.* **1**: 53–58.

Rambaut, A. (1996). Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk/.

Reveal, J.L. & R.D.Hoogland (1990). Validation of five family names in the Magnoliophyta. *Bull. Mus. Natl. Hist. Nat., B, Adansonia* **12**: 205–208.

Ritland, C., K.Ritland & N.Straus (1993). Polymorphism for the ribosomal internal transcribed spacers (ITS1 and ITS2) among eight taxa of the *Mimulus guttatus* species complex. *Mol. Biol. Evol.* **10**: 1273–1288.

Rodrigo, A.G., M.Kelly-Borges, P.R.Bergquist & P.L.Bergquist (1993). A randomisation test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. *New Zealand J. Bot.* **31**: 257–268.

Rogers, S.O. & A.J.Bendich (1987). Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. *Pl. Mol. Biol.* **9**: 509–520.

Rogstad, S.H. (1992). Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analysis. *Taxon* **41**: 701–708

Roxburgh, W. (1820). *Pl. Coromandel* 3(4): 81.

Saint-Hilaire, A.F.C.P. de (1829). Polygaleae, *in* Flora Brasiliae Meridionalis 2: 1–275, t. 81–159. A.Belin, Paris.

Sanderson, M.J. & J.J.Doyle (1992). Reconstruction of organismal and gene phylogenies from data on multigene families: concerted evolution, homoplasy, and confidence. *Syst. Biol.* **41**: 4–17.

Sanderson, M.J., A.Purvis & C.Henze (1998). Phylogenetic supertrees: assembling the trees of life. *TREE* **13**: 105–109.

Savolainen, V., M.W.Chase, S.B.Hoot, C.M.Morton, D.E.Soltis, C.Bayer, M.F.Fay, A.Y.De Bruijn, S.Sullivan & Y.L.Qiu (2000). Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. *Syst. Biol.* **49**: 306–362.

Schiebel, K. & V.Hemleben (1989). Nucleotide sequence of the 18S-25S spacer region from rDNA of mung bean. *Nucl. Acids Res.* **17**: 2852.

Simmons, M.P., C.D.Bailey & K.C.Nixon (2000). Phylogeny reconstruction using duplicate genes. *Mol. Biol. Evol.* **17**: 469–473.

Simmons, M.P. & J.V.Freudenstein (2002). Uninode coding vs gene tree parsimony for phylogenetic reconstruction using duplicate genes. *Mol. Phylogenet. Evol.* **23**(3): 481–498.

Slowinski, J.B. & R.D.M.Page (1999). How should phylogenies be inferred from sequence data? *Syst. Biol.* **48**(4): 814–825.

Small, R.L, J.A.Ryburn, R.C.Cronn, T.Seelanan & J.F.Wendel (1998). The tortoise and the hare: choosing between noncoding plastome and nuclear *Adh* sequences for phylogeny reconstruction in a recently diverged plant group. *Am. J. Bot.* **85**: 1301–1315.

Soltis, D.E. & P.S.Soltis (1998). Choosing an approach and appropriate gene for phylogenetic analysis, pp. 1–42 *in* D.E.Soltis, P.S.Soltis & J.J.Doyle (eds), Molecular Systematics of Plants II: DNA Sequencing. Kluwer Academic Publications, Norwell, Massachusetts.

Soltis, D.E., P.S.Soltis, M.W.Chase, M.E.Mort, D.C.Albach, M.Zanis, V.Savolainen, W.H.Hahn, S.B.Hoot, M.F.Fay, M.Axtell, S.M.Swensen, L.M.Price, W.J.Kress, K.C.Nixon & J.S.Farris (2000). Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Bot. J. Linn. Soc.* **133**: 381–461.

Sorenson, M.D. (1999). TreeRot, version 2. Boston University, Boston, Massachusetts.

Steenis, C.G.G.J. van (1968). Notes on *Bredemeyera* (*Comesperma*) with a new Papuan species and the Australian species listed (Polygalaceae). *Acta Bot. Neerl.* **17**(5): 377–384.

Steetz, J. (1847). Polygaleae, *Revis. Gen.* Comesperma 1–25.

Steetz, J. (1848). Polygaleae, *in* J.G.C.Lehmann (ed.), *Plantae Preissianae* 1: 291–315.

Steudel, E.G. (1845). Polygaleae, *in* J.G.C.Lehmann (ed.), *Plantae Preissianae* 1: 206–211.

Stove, K. (1986). Polygalaceae, *in* J.P.Jessop & H.R.Toelken, *Fl. S. Australia* 4th edn, 2: 788–792. South Australian Government Printing Division.

Styer, C.H. (1977). Comparative anatomy and systematics of Moutabeae (Polygalaceae). *J. Arnold Arbor.* **58**: 109–145.

Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., L.Gielly, G.Pautou & J.Bouvet (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Mol. Biol.* **17**: 1105–1109.

Takhtajan, A. (1980). Outline of the classification of flowering plants (Magnoliophyta). *Bot. Rev.* **46**: 225–359.

Takhtajan, A. (1987). Sistema Magnoliofitov (Systema Magnoliophytorum) 192. Acad. Sciences, USSR, Nauka, Leningrad.

Takhtajan, A. (1997). Diversity and Classification of Flowering Plants. Columbia University Press, New York.

Templeton, A.R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**: 221–244.

Thompson, J. (1978). *Fl. N.S.W.* 112: Polygalaceae. Government Printer, New South Wales.

Thompson, J.D., D.G.Higgins & T.J.Gibson (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**(22): 4673–80.

Thorley, J.L. & R.D.M.Page (2000). RadCon: Phylogenetic tree comparison and consensus. *Bioinformatics* **16**: 486–487.

Thorne, R.F. (1981). Phytochemistry and angiosperm phylogeny: a summary statement, pp. 233–295 *in* D.A.Young & D.S.Siegler (eds), Phytochemistry and Angiosperm Phylogeny. Praeger Publishers, New York.

Thorne, R.F. (1992). Classification and geography of flowering plants. *Bot. Rev.* **58**: 225–348.

Thorne, R.F. (2000). The classification and geography of the flowering plants: dicotyledons of the class Angiospermae (Subclasses Magnoliidae, Ranunculidae, Caryophyllidae, Dilleniidae, Rosidae, Asteridae, and Lamiidae). *Bot. Rev.* **66**: 441–647.

Trueman, J.W.H. (1998). Reverse successive weighting. *Syst. Biol.* **47**: 733–737.

Trueman, J.W.H. (2002). RSW, version 1.1. Available at http://www.anu.edu.au/BoZo/trueman/labsite/software.htm.

Verkerke, W. (1984). Ovule and seed of *Xanthophyllum* (Polygalaceae). *Blumea* **29**: 409–421.

Verkerke, W. (1985). Ovules and seeds of the Polygalaceae. *J. Arnold Arbor.* **66**: 353–394.

Walsh, N.G. (1999). Polygalaceae, pp. 130–137 *in* N.G.Walsh & T.J.Entwisle, *Fl. Victoria* vol. 4. Inkata Press, Melbourne.

Westerkamp, C. (1997). Keel blossoms: bee flowers with adaptations against bees. *Flora* **192**: 125–132.

Westerkamp C. & A.Weber (1999). Keel flowers of the Polygalaceae and Fabaceae: a functional comparison. *Bot. J. Linn. Soc.* **129**(3): 207–221.

Wheeler, W.C. & R.J.Honeycutt (1988). Paired sequence difference in ribosomal RNAs: evolutionary and phylogenetic implications. *Mol. Biol. Evol.* **5**: 90–96.

Whitten, W.M., N.H.Williams & M.W.Chase (2000). Subtribal and generic relationships of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. *Am. J. Bot.* **87**: 1842–1856.

Wilkinson, M. (1994). Common cladistic information and its consensus representation: reduced Adams and reduced cladistic consensus trees and profiles. *Syst. Biol.* **43**: 343–368.

Willdenow, C.L. (1801). Drei neue pflanzen-gattungen. *Ges. Naturf. Freunde Berlin Neue Schriften* **3**: 403–412, t. VI.

Wojciechowski, M.F., M.J.Sanderson, B.G.Baldwin & M.J.Donoghue (1993). Monophyly of aneuploid *Astragalus* (Fabaceae): evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Am. J. Bot.* **80**: 711–722.

Wolfe, K.H., C.W.Morden & J.D.Palmer (1992). Function and evolution of a minimal plastid genome from a non-photosynthetic parasitic plant. *Proc. Natl Acad. Sci. U.S.A.* **89**: 10648–10652.

Yeo, P.F. (1993). Secondary pollen presentation. *Pl. Syst. Evol.*, Suppl. 6. Springer-Verlag, Berlin.

Yokota,Y., T.Kawata, Y.Iida, A.Kato & S.Tanifuji (1989). Nucleotide sequence of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. *J. Mol. Evol.* **29**: 294–301.

Appendix One:
Data Matrices used in Phylogenetic Analyses

	CHARACTERS																																																						
TAXA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Comesperma acerosum	1	1	1	1	0	-	0	1	0	1	0	2	0	0	0	0	0	1	1	?	?	1	0	0	1	1	0	1	1	1	1	0	1	1	1	2	1	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	1	0	0	2
Comesperma aphyllum	2	1	0	0	0	-	1	0	0	0	0	2	1	1	0	2	3	0	2	2	1	1	0	0	0	0	0	0	1	1	1	0	2	1	0	2	1	1	0	0	0	1	0	0	2	0	0	2	2	0	0	3	0	0	0	3
Comesperma breviflorum	1	1	1	1	0	-	1	1	0	1	0	1	0	1	0	0	1	1	1	?	?	1	0	0	1	1	0	1	0	1	1	2	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	?
Comesperma calcicola ms.	1	1	1	1	0	-	0	2	0	1	0	2	1	1	0	0	1	1	2	2	0	0	0	1	1	1	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	?
Comesperma calymega	1	1	1	1	1	0	1	2	0	1	0	1	0	1	0	0	1	1	1	3	0	1	0	0	0	1	0	1	0	1	1	0	0	1	0	2	1	1	0	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	2
Comesperma ciliatum	0	1	0	0	0	-	1	0	0	0	0	2	1	1	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	1	1	0	2	1	0	2	1	1	0	0	0	1	0	0	1	0	0	0	2	0	0	0	1	2	0	2
Comesperma confertum	1	1	1	1	?	-	1	1	0	1	0	1	1	0	0	0	1	1	1	0	0	1	0	0	1	0	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	2
Comesperma defoliatum	3	1	0	0	?	-	1	0	0	1	0	1	1	1	0	0	1	1	1	3	0	1	0	0	0	1	0	2	1	1	1	0	0	1	0	2	1	1	0	0	1	1	0	0	2	0	0	0	2	0	0	0	0	0	0	2
Comesperma drummondii	1	1	1	1	0	-	0	3	0	1	0	1	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	1	0	1	1	1	0	1	0	2	1	1	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	2
Comesperma ericinum	1	0	1	1	0	-	1	2	1	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	3
Comesperma esulifolium	1	0	1	1	1	0	1	2	1	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	2
Comesperma flavum	1	1	1	1	0	-	1	1	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	0	3	1	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	1	0	0	2
Comesperma griffinii ms.	5	1	1	1	0	-	0	2	1	1	0	2	0	1	0	0	1	1	2	0	0	1	0	1	?	0	0	1	0	1	0	1	0	1	0	2	1	1	0	0	2	1	0	0	2	0	0	0	2	0	0	1	0	0	0	2
Comesperma hispidulum	1	1	1	1	2	0	1	2	1	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	3
Comesperma integerrimum	0	1	0	0	3	0	1	1	0	1	0	1	1	1	0	1	2	1	1	1	1	1	0	0	3	0	1	2	1	1	1	0	2	0	0	2	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	3
Comesperma lanceolatum	1	1	1	1	0	-	1	1	0	1	0	0	0	1	0	0	0	1	1	3	0	1	0	1	0	1	1	4	1	0	1	1	0	1	0	2	1	1	0	1	1	1	0	0	2	0	0	0	2	0	0	0	0	0	0	?
Comesperma nudiusculum	3	1	0	0	0	-	1	2	0	1	0	2	1	1	0	0	0	1	2	2	0	0	0	1	0	0	1	4	0	0	0	2	1	1	0	2	1	1	0	1	2	1	0	0	2	0	0	0	1	0	0	0	4	0	0	2
Comesperma oblongatum	1	0	1	1	0	-	1	3	0	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	?
Comesperma pallidum	1	1	1	1	0	-	0	2	0	1	0	2	0	1	0	0	1	1	1	0	0	1	0	0	2	1	0	1	0	1	1	2	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	1	0	0	2
Comesperma patentifolium	1	1	1	1	2	0	1	2	2	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	3
Comesperma polygaloides	1	1	1	1	0	-	0	2	0	1	0	2	1	1	0	0	1	1	2	2	0	0	0	0	0	1	0	1	0	1	1	0	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	2
Comesperma praecelsum	1	1	1	1	2	0	1	1	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	3	1	0	1	0	1	1	2	0	1	0	2	1	1	0	0	0	1	0	0	2	0	0	0	1	0	0	1	2	0	0	?
Comesperma retusum	1	0	1	1	0	-	0	3	0	1	0	2	1	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	0	0	0	0	2
Comesperma rhadinocarpum	1	1	1	1	0	-	1	2	0	1	0	2	0	1	0	0	1	1	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	1	0	2	1	1	0	0	2	1	0	0	2	0	0	0	2	0	0	0	1	0	0	3
Comesperma scoparium	2	1	0	0	0	-	1	0	0	0	0	2	1	1	0	2	3	0	2	3	1	1	0	1	0	1	0	0	1	1	1	0	2	1	0	2	1	1	0	0	0	1	0	0	2	0	0	2	2	1	0	3	0	0	0	3
Comesperma secundum	1	1	1	1	?	0	0	4	1	1	1	1	0	1	1	0	0	1	1	2	0	1	0	0	3	1	?	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	2
Comesperma sp. A Kimberley	1	1	1	1	0	-	1	1	0	1	0	2	1	1	0	0	1	1	2	2	0	0	0	0	2	1	0	1	0	1	1	2	0	1	0	2	?	?	0	0	0	1	0	0	2	0	0	2	2	0	0	1	0	0	0	?
Comesperma sphaerocarpum	3	1	0	0	0	-	1	0	0	1	0	2	1	1	0	0	3	1	2	2	0	1	0	0	0	1	0	0	1	1	1	0	0	1	0	2	1	1	0	0	0	1	0	0	2	0	0	1	2	0	0	3	2	0	0	3
Comesperma spinosum	2	1	0	0	0	-	1	0	0	0	0	2	1	1	0	2	3	0	2	3	0	1	0	1	0	0	0	0	1	1	1	0	2	1	0	2	1	1	0	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	2
Comesperma sylvestre	1	1	1	1	0	-	0	2	0	1	0	2	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	?
Comesperma virgatum	1	1	1	1	0	-	1	1	0	1	0	1	1	0	0	0	1	1	1	2	0	0	0	0	1	1	0	1	0	1	1	1	1	1	1	2	0	0	0	0	0	1	0	0	2	0	0	0	2	0	0	1	0	0	0	3
Comesperma viscidulum	1	1	1	1	0	-	0	2	0	1	0	1	1	1	1	0	0	1	1	2	0	1	0	0	3	1	0	0	0	1	0	1	0	1	0	2	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	2
Comesperma volubile	0	1	0	0	0	-	1	2	0	1	0	2	1	1	0	1	2	1	1	0	0	1	0	0	0	0	0	2	1	1	1	0	?	0	0	2	1	1	0	0	0	1	0	0	0	0	0	0	2	0	0	0	1	1	0	3
Emblingia calceoliflora	4	1	1	1	2	0	1	2	1	1	1	2	1	1	0	5	3	?	2	0	2	2	2	1	4	1	1	4	2	0	1	-	2	1	0	0	1	1	?	3	3	0	2	0	2	0	2	1	2	0	3	3	3	1	1	0
Muraltia heisteria	1	0	1	1	3	0	1	1	0	0	1	2	0	1	0	0	4	0	2	4	0	1	1	1	4	1	0	1	2	0	1	0	2	1	2	1	0	1	1	0	4	1	2	1	2	0	0	1	0	0	0	3	0	0	1	0
Polygala chinensis	5	1	1	1	1	0	1	2	0	1	0	2	0	1	1	1	4	1	2	2	1	1	1	0	0	1	0	5	1	0	1	2	2	1	3	2	1	2	0	1	0	0	0	2	2	0	0	1	3	1	0	3	0	0	0	1
*Polygala duarteana	5	1	1	1	1	?	1	2	1	1	0	1	0	1	1	0	1	1	2	0	0	1	0	0	3	0	0	1	1	1	1	0	0	0	3	2	0	1	0	1	0	0	0	0	2	0	0	4	2	3	0	3	0	0	0	?
Polygala eriocephala	5	1	1	1	3	1	1	3	1	1	0	1	1	1	1	1	4	1	2	4	2	1	1	0	4	1	0	5	1	0	1	1	2	1	2	2	1	1	0	2	0	0	0	1	2	0	0	4	2	1	0	3	1	0	0	0
Polygala exsquarrosa	5	1	1	1	2	1	1	3	0	1	0	1	0	1	1	0	4	1	2	4	2	1	1	1	0	1	1	5	1	0	1	1	2	1	2	2	1	0	0	0	0	0	0	1	2	0	0	2	2	1	0	3	0	0	0	1
Polygala isingii	5	1	1	1	3	1	1	2	0	1	0	2	1	1	1	1	4	1	2	4	2	1	1	0	0	1	0	5	1	0	1	1	2	1	3	2	1	1	0	2	4	0	0	1	2	0	0	4	2	1	0	3	0	0	0	0
Polygala japonica	5	0	1	1	1	0	1	5	0	1	0	1	1	1	1	1	4	1	2	3	1	1	0	0	0	1	0	6	3	1	1	2	2	1	3	2	0	1	0	0	4	0	0	0	2	0	0	2	2	2	0	3	0	0	0	1
Polygala linariifolia	5	1	1	1	1	?	1	2	0	1	0	1	0	1	1	1	4	1	2	2	1	1	1	0	0	1	0	5	1	0	1	1	2	1	3	2	1	1	0	2	0	0	0	2	2	0	0	2	2	1	0	3	1	0	0	0
Polygala longifolia	1	1	0	1	0	-	1	3	0	1	0	1	1	1	1	0	4	1	2	4	1	1	1	0	2	1	0	6	1	1	0	0	2	1	3	2	1	1	0	2	4	0	0	0	2	0	0	2	2	1	0	3	1	0	0	0
Polygala macrobotrya	5	1	1	1	1	?	1	1	0	1	0	1	0	1	1	1	4	1	1	2	2	1	1	0	4	1	0	4	1	0	1	2	2	1	2	2	1	1	0	2	0	0	0	0	2	0	0	4	1	1	0	3	1	0	0	?
*Polygala monspeliaca	5	1	1	1	1	0	1	2	0	1	0	1	0	1	1	0	4	1	2	4	0	1	0	0	2	1	0	4	1	0	1	1	0	1	3	2	0	0	0	2	0	0	0	0	2	0	0	2	2	1	0	3	1	0	0	0
*Polygala myrtifolia	1	1	1	1	0	0	0	2	1	1	0	1	1	1	1	0	0	1	2	4	0	1	0	0	1	0	0	5	1	0	1	2	0	1	3	2	1	1	1	2	0	0	0	0	2	0	0	2	2	1	0	3	1	0	0	1
Polygala orbicularis	5	1	1	1	2	1	1	4	1	1	1	2	1	1	1	1	4	1	2	4	2	1	1	0	4	1	0	5	1	0	1	2	2	1	3	2	1	1	0	2	0	0	0	2	2	0	0	2	2	2	0	3	1	0	0	1
*Polygala paniculata	5	1	1	1	1	0	1	2	1	1	0	1	1	1	1	0	1	1	2	4	0	1	0	0	2	1	0	4	1	0	1	0	0	1	3	2	1	1	0	0	1	0	0	0	2	0	0	4	2	0	0	3	1	0	0	1
Polygala persicariifolia	5	0	1	1	2	0	1	2	1	1	0	1	1	1	1	0	4	1	1	3	1	1	0	0	0	1	1	0	1	1	1	2	2	1	3	2	1	1	0	1	5	0	0	2	2	0	0	1	2	1	0	3	1	0	0	0
Polygala rhinanthoides	5	1	1	1	2	1	1	3	0	1	0	1	0	1	1	1	4	1	2	4	1	1	1	0	4	1	0	4	1	0	1	1	0	1	3	2	1	1	0	2	0	0	0	0	2	0	0	1	2	2	0	3	0	0	0	2
Polygala tepperi	1	1	1	1	2	0	1	2	0	1	0	1	0	1	1	1	4	1	2	0	1	1	1	0	4	1	0	4	1	0	1	1	2	1	3	2	1	2	0	2	0	0	0	1	2	0	0	4	1	1	0	3	0	0	0	0
*Polygala virgata	1	1	1	1	0	0	1	3	0	1	0	1	1	1	1	0	4	1	2	4	0	1	0	0	1	1	0	0	1	1	1	2	2	1	3	2	1	1	0	2	4	0	0	0	2	0	0	2	3	1	0	3	1	0	0	?
*Polygala vulgaris	5	1	1	1	0	0	1	2	1	1	0	1	1	1	1	1	4	1	2	3	0	1	0	0	0	0	0	1	1	1	1	0	0	1	3	2	0	0	0	0	0	0	0	0	2	0	0	2	2	1	0	3	1	0	0	?
Polygala wightiana	5	1	1	1	0	-	1	2	0	1	0	1	0	1	1	1	4	1	2	4	0	1	0	0	3	1	0	5	1	0	1	2	2	1	3	2	1	2	0	2	0	0	0	0	2	0	0	2	3	1	0	3	1	0	0	0
Salomonia ciliata	5	0	1	1	0	-	1	2	0	1	0	2	0	1	0	3	4	1	2	4	0	1	1	1	4	1	0	4	2	0	1	1	0	1	0	0	0	1	0	1	0	1	2	0	2	0	0	2	3	0	1	3	0	0	0	?
Xanthophyllum fragrans	6	0	1	1	0	0	1	2	1	1	1	2	1	1	0	0	0	1	2	2	2	1	2	1	4	1	0	1	2	0	1	-	2	-	0	2	1	1	1	1	4	1	0	0	2	1	3	1	2	0	3	3	4	0	2	-
Xanthophyllum octandrum	6	0	1	1	0	0	1	2	1	1	1	2	1	1	1	0	1	1	1	2	2	1	2	1	4	1	0	1	2	0	1	-	2	-	0	2	1	1	1	1	4	1	0	0	2	1	3	1	2	0	2	3	4	0	2	-
Bredemeyera papuana	0	1	1	1	0	-	1	2	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	0	2	0	1	1	1	1	1	1	0	1	0	2	1	1	?	1	4	1	0	0	1	0	0	0	1	0	0	0	1	0	2	-
Bredemeyera floribunda	0	1	1	1	2	0	1	2	1	1	0	2	1	1	1	4	1	1	2	3	1	1	1	0	3	1	1	1	1	1	1	2	2	1	0	2	1	1	0	2	4	1	0	0	2	0	0	0	2	0	0	2	2	0	0	0
Bredemeyera microphylla	1	1	1	1	2	0	1	2	0	1	0	1	1	1	0	2	3	1	1	3	2	1	0	0	2	0	1	1	1	1	1	2	2	1	0	2	1	1	0	2	4	1	0	1	2	0	0	0	2	0	0	2	1	0	0	?

Taxa by character matrix for Polygalaceae morphology dataset

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          1          11         21         31         41         51         61         71         81         91         101        111
          |          |          |          |          |          |          |          |          |          |          |          |
Bauhinia  ---------- ---------- ---------- ---------- ---------- ---------- --------TC GAAGCCCCAA ACAGCACGAC CCGTGAATTC GTATGCGATA TTGG-GCGGC
Medicago  .......... .......... .......... .......... .......... .......... .TCGATGCCT T.CATG..GT C..A....TG AATCAGT..G AATACAT..G GGTTG..TTG
Callista  .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- ---------- ----.----.
BG7836    .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- ---------- ----.-----
BG7837    .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- ---------- ----.-----
Xan_octB  .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- ---------- ----.-----
Com_drum  .......... .......... .......... .......... ........AA CGACCCGCGG ACACGTATA. ---AGA.G.. CG..GGAAGG GA.G.G-GCA TGCGCT.CGC CCTCTCG.C.
Com_aceA  .......... .......... .......... .......... ........AA CGACCCGCGG ACCCGTAT-. CT--GA.G.C T.G.GGGCGG GA.G.G-GCA TGCGCT.CGC CCTTTCGTC.
Com_eriA  .......... .......... .......... .......... ........AA CGACCCGCGG ACACGTAT-. CT-AAA.G.. TA..GGACGG GA.G.G-GCA TGCGCT.CAC CCTCCCG.C.
Com_esuA  .......... .......... .......... .......... .........A CGACCCGCGG ACACGTAT-. CT-AAAAG.. TA..GGACGG GA.G.G-GCA TGCGCT.CGC CCTCCCG.C.
Com_flaJ  .......... .......... .......... .......... ........AA CGACCCGCGG ACACGTATA. ---AAA.G.. TG..GTACGG GA.G..-GCA TGCGCT.CGC CCTCTCG.C.
Com_intB  .......... .......... .......... .......... .......... .GACATGTGA ACCCGTATCT C.T----G.T TGT.GG.TGG AT.G.G-GCA AGCGCT..GC CCTCTC..C.
Com_vol1  .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- -----...GC CCCTTC-.C.
Com_spiG  .......... .......... .......... .......... ......GAAT GACTTGC.GA ACCCGTACCT CGT----G.T TGC.GGGTGG AT.G.G-GCA TGCGCT..GC CCATCC.CC-
Com_vol2  .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- -----T..GC CCCTTC..C-
Com_volA  .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- ---------- ----TC-.C-
Com_volM  .......... .......... .......... .......... ........AA TGACTCGTGA ACCCGTATCT CGT----G.T TGT.GGGCGG A..G.G-GCA AGCGCT..GC CCCTTC..C-
Com_cili  .......... .......... .......... .......... .......GAA TGACCCGTGA ACCCGTATCT CGT----G.T TGC.GGGCGG AT.G.G-GCA TGCGCT.CGC CC.TTC..C-
Com_rhaE  .......... .......... .......... .......... ........AA CGACCCGCGG ACCTGTATCT TGT----G.T TGT.GGT.GG TA.G.G-GCA TGCGCT.CGC CCTTTCG.AG
Com_calB  .......... .......... .......... .......... .......... ...ATTGTCG AAACCTAA.. C------G.. GAGCG..CCG GT.GAT-GAG TGTCT..TG- ----.-----
Com_aphJ  .......... .......... .......... .......... .......... CGACCTGTGG ACCAGTATA. ACC.TGA... G.G.GCGAGG G..G.T-GCA TGCGTT.CGC CCTTCC..C.
Com_scoM  .......... .......... .......... .......... .......GAA CGACCCGTGG ACCAATTAAT TGCCGTGAC. G.G.GCGAGG GG.GTT-GCA TGCGAT.CGC CCTTCC.---
Pol_japL  .......... .......... .......... .......... ....AAAGAG CGACCGTTGG ACATGTATAT CTT-----.C GGC.GG-TGG AT.G.T-GCA TGCGTT.CGC CCTTCC.CC-
Pol_linL  .......... .......... .......... .......... .......GAG NNANTNTTGC ACATGTATA. CTT-----.C .GC.GG-TGG A..G.T-GCA TGTGCT.TGC CC.TCCACC-
Pol_myrA  .......... .......... .......... .......... .......GAG CGACCGTTGG ACACGTACAT CTT-----.C GGC.GG-CGG A..G.C-GCA TGCGCT.CGC CC.TCT.CCG
Pol_spJR  .......... .......... .......... .......... .......GAG TGACCGTTGG ATATGTATA. CGT-----.T TGC.GG-TGG AT.G.T-GCA TGTGCT.TGC C.ATCCACC-
Pol_virA  .......... .......... .......... .......... .......GAG CGACCGTTGG ACATGTATAT CTT-----.C GGC.GG-TGG AT.G.C-GCA TGCGCT.CGC .C.TCC.CCG
Pol_exsR  .......... .......... .......... .......... .......GAG TGACCGTTGG ACATGTATA. CTT-----.T TGT.GG-TGG A..G.T-GCA TGTGAT.TGC C..TCCACC-
Bre_col   TCTGTAGGTG AACCTGCGGA AGGATCATTG TCGAAACCT. GCCGGAAGGG AGACCGTCGG ATGCGTTCAT CTCTTTA.GC G.G.GG-CGG AT.G.GCGCG TGC.C..TGC CCTTCT.CC-
EpirAdC3  .CTGTAGGTG AACCTGCGGA AGGATCATTG TCAAATCTTA CTCAAAAGGT TGACTGTCGG ACTTGTCTAT CTT------.T GGT.GGA--- -------ACA TGT..AC..T A.TTTCT---
Sal_cant  .......... .......GGA AGGATCATTA TCGAAGTCT. CTCGTAAGAT GGACCGTCGA ACCCGTGACA TG.------.T GTCTGT..GG AGAGCTTG.T TGCCT..GCG CGACAT.TCT
Sal_cilR  .......... .......... .......... .......... .......GAT GGACCGTCGA ACTTGTGACT TG.----A.T T.T..GG.G- --AGAGCACG T.CGCT.CCT .GC.TCGTCT
Mur_heiA  .......... .......... .......... .......... .......... .......... ........-- ---------- ---------- ---------- ---------- ----.-----
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         121        131        141        151        161        171        181        191        201        211        221        231
         |          |          |          |          |          |          |          |          |          |          |          |
Bauhinia GTAGGGTTCT GATT--GCAC CCACCC---- -----ACCCC ATCCTACGTG AGACACACGG GG-------- --TCC---TT CTT------- GTGTGCGCTC ATTTAGGTA- AAGAACAAAA
Medicago AGGT.T.--- CCACACC--- TTGG.TTACC TCTGGTT.AG .GGAAGACGA CA.AGTG..T CC........ ...T.TTTGC GCC....... ---------- ---------. ..A.CTC...
Callista C---..GGGC AGGCTC.GGG GTG.T.CGCA C....CT.AG .C.TCC.CC. T.C.GGGA.A C.C....... ..---...-- ---......T C.CG.GCGC. ..CCTA.CG. .CA.CAC..T
BG7836   ---------- ----.----- ------.... ....------ ---------- ---------- --........ ..---...-- ---....... ---------- ---------. ----------
BG7837   ---------- ----.----- ------.... ....------ ---------- ---------- --........ ..---...-- ---....... ---------- ---------. ----------
Xan_octB ---------- ----.----- ------.... ....------ ---------- ---------- --........ ..---...-- ---....... ---------- ---------. ----------
Com_drum C----.CG.- .GGCTCTGCG .GG..GTGGG GG...----- ---------- ---------- --........ .........C .CC....TGG CC....CGGG CACGT.CG.. .CA.C.....
Com_aceA C---T.CA.- .GGCTC.GTG .GGT.TTGGG GG...----- ---------- ---------- --........ ..-......C .CA....CGG CC....CGGG CACGTATG.. .CA.C.....
Com_eriA C-----.G.- .GGCTC.GTG .GGT.GCGGG GG...----- ---------- ---------- --........ ..-......C .CC....TGG CC....CGGT CACGTACG.. .CA.......
Com_esuA C-----.G.- .GGCTC.GTG .GGT.GCGGG GG...----- ---------- ---------- --........ ..-......C .CC....TGG TC....CGGT CA.GTACG.. .CA.......
Com_flaJ C----.CGT- .GGCTC.GCG .GGT.GTTGG GG...----- ---------- ---------- --........ ..-..TCC.C .CC....TGG CC....CGGG CACGT.CG.. .CA.C.....
Com_intB AC.T.CG--- .GC.GC.T.- TGGTTGTGGG G.....G-.. C.GGCGNCGC G.TGCTG... .-........ ....-....C .CC....CAA C.....TTGG CCC.CTCA.. .CA.C.....
Com_vol1 .C.T.TG--- .G.CGA.TT- .GGTTGGGGG G.....G-T. C.GGC.TCGC G.TGC.G... .-........ ....-....C .CC....TCC CC....CTGG CCC.TACA.. .CA.C.....
Com_spiG CGCAT..GTG .G.CGC.TG- .GGTTTTGGG G.....G-.. G.GG.G.CGC G.TGCT.... .-........ ....-....C .CC....TAT CC....NTGG CCCGTACA.. .CA.C.....
Com_vol2 AGCAC..G-- .G.CGC.T.- .GGTTTTGGG G.....G-T. C.GGC.TCGC G.TGC.G... .-........ ....-....C .CCC...TCT CC....CTGG CCC.TACA.. .CA.C.....
Com_volA CGCAT..G-- .G.CGA.TT- .GGTTGGGGG G.....G-T. C.GGC.TCGC G.TGC.G... .-........ ....-....C .CC....TCC CC....CTGG CCC.TACA.. .CA.C.....
Com_volM AGCAC..G-- .G..GC.T.- .GGTTTTGGG G.....G-T. C.GGC.TCGC G.TGC.G... .-........ ....-....C .CCC...TCT CC....CTGG CCC.TACA.. .CA.C.....
Com_cili CGC.T.CG-- .G.CGC.T.- .GGTTGGGGG GGCAA.G-.. C.GGC.TCGC G.TGCTG... .-........ ....TCC..C ..CC...TAT CC....T.GG CCCAT.CA.. .CA.CA....
Com_rhaE CCCAC..G-- .GCCGG...- GGG..GGGGG ......G-.. C.GGCTTAAC G.TGT..... C-........ ..G.-...-C .CCG...TCT CC....T.GG CCCGCACG.. .T..C.....
Com_calB ---------- ----.----- ------.... ......G-.. G.GGG...G. T.CGG.CT.. .AGCAT.... ..G......C GCC....TCC CC....C.GT C.CAT.CG.T ..A.C.....
Com_aphJ T----..GTG .GGCGT.-TG .GG.TGGGGG GGT...GGAA CCGGCTTCAC CATTCT-... .-........ .....TCC.C .CC....TCC C.....CTG. CCCACACG.. .CA.C.....
Com_scoM ACCCCC.GTG .GGCGT.-TG .GG..GGGGG G.....GG.A CCGG.TTC-- ------C... .-........ ..C......C .CC....TCC CC.C..TTG. CC.GCACG.. .CA.C.....
Pol_japL -CCTCAAGTC .GGCTG.G.- .GGTTGGGTG GTGC.----- -.GGCCTCGC T.CGG.GT.. .-........ .....CC..C .CC......T CC....T.GG C.CGTAC... .CA.C....-
Pol_linL CCCTC.A.T. .GG.GA.G.- TGT.GGGGCG GTGC.----- -.GGCCTCAC T.CAGTG... C-........ .....C...C .CC......T C...C.T.GG CCCGT.C... .CA.C.....
Pol_myrA CCCTCAAGTC .GGCGA.G.- TGGTGGGGCA GG...-TTAA ..GGCCTCGC T.CGG.G... C-........ .....C...C .CC......T CC....T.GG CCCGT.C... .CA.C....-
Pol_spJR CCCTT...TC .TG.GA.G.- TGT.TGGGCG GTGA.----- -.GGCCTCGC T..TG.G... C-........ .....C...C .CC....... ---.T.TT.G CCCGT.CG.. .CA.CT....
Pol_virA CCCTC.AGTC .GGCGA.G.- TGGTTGGGCG GTTC.----- -.GGCCTCGC T.TGG.G... C-........ .....C...C .CC......T CC....T.GA CCCG.AC... .CA.CT...-
Pol_exsR CCCTC...T. .GG.CA.G.- TGT.TGGGTG ......T-G. C.GGCCTCGC T.CTGTG... CC........ .....C...C .CC......T A...C.TTGG CCCGT.CG.. .CA.C.....
Bre_col  C.C.CAAGT. .GGCGA.G.- TGGGGAGAGG GGAG.---G. GAGGCCTCAC N.CGGTG... CCG....... ....TC...G .CC..TCCCT CC..C.T.GG CCCGTACG.. ..A.C.....
EpirAdC3 CCCTCAAGT. ..GGGA.A.T GTTGGGATGG GTG..----- -.GG.G.AGT T..TGTG.CA --........ ...TTCC... T.CTT..... AGA.T.T.CA C.CAT.CGG. -TA.C.T...
Sal_cant CCC------- --CGAGATCG AGTTGG...A CGTGG.AT.G G..GAGTCGC GCT.GTCGC. CC........ .....TC.CC T..C...ACA AC..T...GG C.CGT--G.. TTC.TTCG..
Sal_cilR C.CCT----- --CGAGATCG AGT.GGG..T CGTGG.ATTG G..GAGAAGC GCC.GTCGC. CC........ .....TC..C T..TC..ACG .A....ATGG C.CGT--G.. TTA.CTCG..
Mur_heiA ---------- ----.----- ------.... ....------ ---------- ---------- --........ ..---...-- ---....... ---------- ---------. ----------
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ITS manually-aligned sequences for Polygalaceae (continued)

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          241        251        261        271        281        291        301        311        321        331        341        351
          |          |          |          |          |          |          |          |          |          |          |
Bauhinia  CCCCGGCGCT AGTTGCGCCA AGGAA----- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Medicago  ........T. GAA....T.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Callista  .........G GAA....... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
BG7836    ---------- ---------- -----..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
BG7837    ---------- ---------- -----..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xan_octB  ---------- ---------- -----..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_drum  ..A.....TG C.AA...... ......TCCT ATGGTATGAC CCTGTGCTTG CACCCC.ACC .......... .......... .......... GGCCC.GGAG ACGGAGCTCC GGTGTGT...
Com_aceA  ..A.....TG C.AA...... ......TCCT ATGGTATGAC CCTGTGCTNG CACCCCCACC .......... .......... .......... GGCCC.GGAG ACGGAGCTCC GGTGCGT...
Com_eriA  ..A.....TG C.AA...... ......TCCT ATGGTATGAC CCTGTGCTTG CACC...ACC .......... .......... .......... GGCCC.GGAG ACGGAGTTCC GGTGTGT...
Com_esuA  ..A.....TG C.AA...... ......TCCT ATGGTATGAC CCTGTGCTTG CACA...ACC .......... .......... .......... GGCCC.GGAG ACGGAGTTCC GGTGTGT...
Com_flaJ  ..A.....TG C.AA...... ......TCCT ATGGTATGAA CCTGTGCTTG CACCCCCACC .......... .......... .......... GGCCC.GGAG ACGGAGTTCC GGTGTGTGGC
Com_intB  ..A.....TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_vol1  ........TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_spiG  ..A.....TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_vol2  ..A.....TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_volA  ........TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_volM  ..A.....TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_cili  ..A.....TG ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_rhaE  ..A......G ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_calB  ..AT.....G ...G...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_aphJ  ..A......G .CAA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Com_scoM  T........G ..AA...... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Pol_japL  .T.......G ..AA...... .......... .......... .......... .......... ATTGTACCGA CCGCTCGTGC CCCCTTT... GGCCCCGGAG ACGGTG.... ..........
Pol_linL  .T.......G ..AA...... .......... .......... .......... .......... GATGTACTGA CCGCGCGCGC CCATTTT... GGCCCCGGAG ACGGCG.... ..........
Pol_myrA  .T.......G ..AA...... .......... .......... .......... .......... ATCGTACCGA CCGCG..TGC CC.CTCC... GGCCCCGGAG ACGGTG.... ..........
Pol_spJR  .T..A....G ..A....... .......... .......... .......... .......... GTTATACCGA ATGCA..TTC CCACTTC... GGCCCTGGAG ACAGTG.... ..........
Pol_virA  .T.......G ..AA...... .......... .......... .......... .......... ATCGTACTGA CCGCG..TGC CC.CTTC... GGCCCCGGAG ACGGTG.... ..........
Pol_exsR  .TT.....TG ..AA...... .......... .......... .......... .......... GTCGTATCGA CCGCA..TTC CCACTAC... GGCCCTGGAG ACAGTG.... ..........
Bre_col   -T.......G ..AA...... ...G...... .......... .......... .......... ACTGGACAGA CCGTGCGCGT GCGCCCTA.. GGCCTCCGAG ACGGGG.... ..........
EpirAdC3  .AT.ATT..G ...A.T..G. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Sal_cant  -.G......G ..AA...... ....T..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Sal_cilR  -TG......G ..AA...... ....T..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mur_heiA  ---------- ---------- -----..... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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ITS manually-aligned sequences for Polygalaceae (continued)

```
           361           371           381           391           401           411           421           431           441           451           461           471
           |             |             |             |             |             |             |             |             |             |             |             |
Bauhinia   ----------    ----------    ----------    ----------    ----------    ----------    ----------    ----------    ----------    ----------    ----------    ----------
Medicago   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Callista   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
BG7836     ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
BG7837     ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Xan_octB   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Com_drum   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Com_aceA   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Com_eriA   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Com_esuA   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Com_flaJ   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Com_intB   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGAAAAGAT    TGTGTGCTCG    ACCCTCTCTC    GTGCCGCCAG
Com_vol1   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGAAT.GAT    TGTGCTC..G    ACCCCCTCCT    ..GCCGCCAG
Com_spiG   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGATG.GAT    TGTGCTC..G    ACCCTCTTGT    ..GCCGCTAG
Com_vol2   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGAAG.GAT    TGTGCTTTTG    ACCCCCTCCT    ..GCCGCCAG
Com_volA   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGAAT.GAT    TGTGCTC..G    ACCCCCTCCT    ..GCCGCCAG
Com_volM   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGAAG.GAT    TGTGCTTTTG    ACCCCCTCCT    ..GCCGCCAG
Com_cili   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ........AA    TGGAAG.GAT    TGTGATGTG.    .CCCTCCCCT    ..GCCGCCAG
Com_rhaE   ..........    ..AATGGAC.    TGACTGTGCT    TT.GACCCCC    TCCT.G.CCG    GTACTAAG.C    C.GGCATGCC    CGAGGGGG..    ..........    ..........    ..........    ..........
Com_calB   ..........    ..AGGGGAAC    G.ATTGTGCC    AGCGCTCCCG    CCATAGGCCG    GGAGGGCT..    ..........    ..........    ..........    ..........    ..........    ..........
Com_aphJ   ..........    ..GAGG.AAC    ACATTGCGCT    TT.GCCCAAC    CCGT.GG.CG    GGTGG.....    ..........    ..........    ..........    ..........    ..........    ..........
Com_scoM   ..........    ..GAGGGAAC    AC.TTGTGCT    TA.GCCCCAC    CCGT.GG.CA    GGCGGAAGGC    CTGGCGGGGG    CGGG......    ..........    ..........    ..........    ..........
Pol_japL   TGCCGATTGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Pol_linL   TGTTGACCGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Pol_myrA   TGCCGATCGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Pol_spJR   TGTCGATCGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Pol_virA   TGCTGACCGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Pol_exsR   TGTCGATCGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Bre_col    ...CGACAGG    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
EpirAdC3   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Sal_cant   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Sal_cilR   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
Mur_heiA   ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........    ..........
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ITS manually-aligned sequences for Polygalaceae (continued)

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          481        491        501        511        521        531        541        551        561        571        581        591
          |          |          |          |          |          |          |          |          |          |          |
Bauhinia  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----CCCTT ACAATACAAC ATCCC-----
Medicago  .......... .......... .......... .......... .......... .......... .......... .....TTTAA ATTTTGCTCT GAGCA.A... G..TGG..C. GGAGACGGTT
Callista  .......... .......... .......... .......... .......... .......... .......... .....CTCGA ATTT.GTTAA GCGTG.T.CC G.--GGACC. GGAGACGGTG
BG7836    .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
BG7837    .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Xan_octB  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_drum  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_aceA  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_eriA  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_esuA  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_flaJ  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_intB  TCGGCGAGCA AGGAGGG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_vol1  TGGGCGAGCT GGAGGAG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_spiG  GATGCGAGCT GGAGGGG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_vol2  CCGGCGATCC GGAGGAG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_volA  TGGGCGAGCT GGAGGAG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_volM  CCGGCGATCC GGAGGAG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_cili  TCGGCGAGCT GGAGGAG... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_rhaE  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_calB  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_aphJ  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Com_scoM  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Pol_japL  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Pol_linL  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Pol_myrA  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Pol_spJR  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Pol_virA  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Pol_exsR  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
Bre_col   .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
EpirAdC3  .......... .........A TTAATTTGAG CTATATGTGT AGCCTCTGAC ATCTTTAAAT GAG.TGTCAT CTT....... .......... .....----- ---------- -----.....
Sal_cant  .......... ........TA TGTACCTGAC C.ACGCGTGC GGCCTCCGGG CTCTG..... .......... .......... .......... .....----- ---------- -----.....
Sal_cilR  .......... ........CA TGGACTTGAC C.ACGCGGGC CGCCTTCGGA ATTCGAAGAT GAGATGCAAT CTCT...... .......... .....----- ---------- -----.....
Mur_heiA  .......... .......... .......... .......... .......... .......... .......... .......... .......... .....----- ---------- -----.....
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ITS manually-aligned sequences for Polygalaceae (continued)

```
          601        611        621        631        641        651        661        671        681        691        701        711
          |          |          |          |          |          |          |          |          |          |          |          
Bauhinia  ---------- ---------- --CGTGCGGG GTTTGTTG-- CAACGTGTTT ATCC------ --AAAATGAC TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
Medicago  TTCGTGCGGG .......... ..-------- --------.. TTGT..T..G .CA.ATGATA T????????? ?????????? ?????????? ?????????? ?????????? ??????????
Callista  CTCTTG.... .......... ....G.G.C. TCGC.A.-.. GC.T.AAAA. ..--...... .......... .......... .......... .....T.... .......... ..........
BG7836    .......... .......... ..-------- --------.. ---------- ----...... ..-------- ---------- ---------- ---------- --........ ..........
BG7837    .......... .......... ..-------- --------.. ---------- ----...... ..-------- ---------- ---------- ------.... .......... ..........
Xan_octB  .......... .......... ..-------- --------.. ---------- ----...... ..-------- ---------- ---------- ---------- ---------- ----------
Com_drum  .......... .......... ..G....... CC.-.AA-.. TT.GTC..G. .CA....... ..G...C... .......... .......... .......... .......... ..........
Com_aceA  .......... .......... ..G.G..... CC.-.AA-.. T.TGTC..G. .CA.AC.... ..G...C... ......A... .T........ .......... .......... ..........
Com_eriA  .......... .......... ..G.C..... CCA-.AA-.. TTTGTC..G. .CA....... ..G...C... .......... .......... .......... .......... ..........
Com_esuA  .......... .......... ..G.C..... CCA-.AA-.. TTTGTC..G. .CA....... ..G...C... .......... .......... .......... .......... ..........
Com_flaJ  .......... .......... ..G.C..... CC.-.AA-.. TTTGTC..G. .CA....... ..G...C... .......... .......... .......... .......... ..........
Com_intB  .......... .......... ..G.C..AC. C..-.AC-.. TTTGTCT.G. .CA....... ..TG...... .......... ........T. .......... .......... ..........
Com_vol1  .......... .......... ..G.C..AC. C..-.AC-.. TTTGTC..G. .CA....... ..TG..C... .......... .......... .......... .......... ..........
Com_spiG  .......... .......... ..G.C...C. C..-.AC-.. TTTGTCT.G. .CA....... ..CG..C... .......... .......... .......... .......... ..........
Com_vol2  .......... .......... ..G.C..AC. C..-.AC-.. TTTGTC..G. .CA....... ..TG..C... .......... .......... .......... .......... ..........
Com_volA  .......... .......... ..G.C..AC. C..-.AC-.. TTTGTC..G. .CA....... ..TG..C... .......... .......... .......... .......... ..........
Com_volM  .......... .......... ..G.C..AC. C..-.AC-.. TTTGTC..G. .CA....... ..TG..C... .......... .......... .......... .......... ..........
Com_cili  .......... .......... ..G.C..AC. C..-.AC-.. TTTGTCA.G. .CA....... ..TG...... .......... .......... .......... .......... ..........
Com_rhaE  .......... .......... ....A..AC. C....AC-.. TTTGTC..G. .CA....... ..TG..C... .......T.. .......... .......... .......... ..........
Com_calB  .......... .......... ..G.CA.AC. C..-.AC-.. TTTGTC..G. ..GTTT.... ..TG..C... .......... ........T. .......... .......... ..........
Com_aphJ  .......... .......... ..G.CA.AC. C..-.AC-.. TTTGTC..G. .AAAAT.... ..TG..C... .......... .......... .......... .......... ..........
Com_scoM  .......... .......... ..G.C..AC. C..-.AA-.. TTTGTC..G. .AAAT..... ..TG..C... .......... .......... .......... .......... ..........
Pol_japL  .......... .......... ..-.....C. T.G-.ACATT TTTGTC..G. .AA....... ..TT..C... .......... .......... .T........ .......... ..........
Pol_linL  .......... .......... ..-.C...T. CCG-.ACAAT ATTGTC..G. .AA....... ..TT..C... .......... .......... .......... .......... ..........
Pol_myrA  .......... .......... ..-.C...C. C..-.ACATT TTTGTC..G. .AA....... ..TT..C... .......... .......... .......... .......... ..........
Pol_spJR  .......... .......... ..-...T.C. CCG-.ACAAT ATTGTC..G. .AA....... ..TT..C... .......... .......... .......... .......... ..........
Pol_virA  .......... .......... ..-.C...C. C..-.ACA.T ATTGTC..G. .AA....... ..TT..C... .......... .......... .......... .......... ..........
Pol_exsR  .......... .......... ..-...T.C. CCG-.ACAAT ATTGTC..G. .AA....... ..TT..C... .......... .......... .......... .......... ..........
Bre_col   .......... .......... TGG.CA..C. CGG-.AGA.T TTT....GCG .CAT.GCCAA ...T...... .......... .......... .......... .......... .........G
EpirAdC3  .......... .......... ..G....ACT T.G.T.A-.. TT.GT...G. ..A....... ......CA.. .....A...T ........T. .....T.... .......... ..........
Sal_cant  .......... .......... ..-.....CA ..G-.ACA.. TTCGTC.... .CA....... ..G...C... .......... .......... .......... .......... ..........
Sal_cilR  .......... .......... ..-.C...C. ..G-.ACA.. TTTGTC.... .CA....... ..G...C... .......... ........T. .......... ....------ ..........
Mur_heiA  .......... .......... ..-------- --------.. ---------- ----...... ..-------- ---------- ---------- ---------- ---------- -----.....
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ITS manually-aligned sequences for Polygalaceae (continued)

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          721        731        741        751        761        771        781        791        801        811        821        831
          |          |          |          |          |          |          |          |          |          |          |
Bauhinia  TGCGATACT- TGGTGTGAAT TGC--AGAAT CCCGTGAACC ATCGAGTCTT TGAACGCAAG TTGCGCCCGA AGCCATCA-G GCTGAGGGCA CGTCTGCCTG GGCGTCAAAC ----GTCGCT
Medicago  ?????????? ?????????? ????.????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ????A...AA
Callista  .......... .......... .......... .......... .......T.. .......... ........A. ......T... ..C....... ..C....... ..T....C.. ....A...T.
BG7836    .......... .......... .......... .......... .......... .......... .......... ....T.TG.. ..C....... .......... ..T....CG. .....CA.TC
BG7837    .......... .......... C......... .......... .......... .......... .......... ....T.TG.. ..C....... .......... ..T....CG. .....CA.TC
Xan_octB  ---------. ---------- ---....... .......... .......... .......... .......... ....T..G.. ..C....... .......... ..T....CG. ....ACA.TC
Com_drum  .......A.. ....T..T.. .......... .......... ..T....... ........C. .........C C...T.TT.T ..C....... ..C....... .......CG. ....AC..TC
Com_aceA  ........GA .......... .......... .......... .......... .......... .......... C...T.TT.. ..C....... ..C....... .......CG. ....AC..TC
Com_eriA  .......... .......... .......... .......... .......... .......... .......... C...T.TT.. ..C....... ..C....... .......CG. ....AC..TC
Com_esuA  .......... .......... .......... .......... .......... .......... .......... C...T.TT.. ..C....... ..C....... .......CG. ....AC..TC
Com_flaJ  .......... .......... .......... .......... .......... .......... ........A. C...T.TT.. ..C....... ..C....... .......CG. ....AC..TC
Com_intB  .......... .......... .......... .......... .......... .......... .......... T......... ..C....... .......... ..T....CG. ....AC.ATC
Com_vol1  .......... .......... .......... .......... .......... .......... .......... T...T..T.. ..C....... .......... ..T....CG. ....A..ATC
Com_spiG  .......... .......... .......... .......... .......... .......... .......... T...T..T.. ..C....... .......... ..T....CG. ....A..ATC
Com_vol2  .......... .......... .......... .......... .......... .......... .......... T...T..T.. ..C....... .......... ..T....CG. ....A..ATC
Com_volA  .......... .......... .......... .......... .......... .......... .......... T...T..T.. ..C....... .......... ..T....CG. ....A..ATC
Com_volM  .......... .......... .......... .......... .......... .......... .......... T...T..T.. ..C....... .......... ..T....CG. ....A..ATC
Com_cili  .......... .......... .......... .......... .......... .......... .......... T...T.TTT. ..C....... .......... .......CG. ....A...TC
Com_rhaE  .......... .......... .......... .......... .......... .......... ......T... C...T..T.. ..C....... .......... ..T....CG. ....AC.A.C
Com_calB  .......... .......... .......... .......... .......... .......... .......... T...T..T.. .......... ......T... ..T....CT. ....A.G..C
Com_aphJ  .......... .......... .......... .......... .......... .......... .......... T...T..G.. .......... .......... ..T....CT. ....TCA.TC
Com_scoM  .......... .......... .......... .......... .......... .......... ........-. T....CTT.. .......... .......... ..T....CT. ....CCG.TC
Pol_japL  .......... .......... .......... .......... .......... .......... .......... C...T..T.. ..C....... .......... ..T....CG. ....A...TC
Pol_linL  .......... .......... .......... .......... .......... .......... .......... T...T.TT.. ..C....... .......... ..T....CG. ....AA..TC
Pol_myrA  .......... .......... .......... .......... .......... .......... ........A. C...T..T.. ..C....... .......... ..T....CG. ....A...TC
Pol_spJR  .......... .......... .......... .......... .......... .......... .......T.. C...T.TT.. .......... .......T.. ..T....CG. ....A.T.TC
Pol_virA  .......... .......... .......... .......... .......... .......... ........A. C...T.-T.. ..C....... .......... ..T....CG. ....A...TC
Pol_exsR  .......... .......... .......... .......... .......... .......... .......T.. C...T.TT.. ..C....... .......... ..T....CG. ....A...TC
Bre_col   ........A. .......... .......... .......... .......... .......... .......... ....TC.T.. ..C....... .......... .......CG. ....ACT.TC
EpirAdC3  .......... .......... .......... .......... ..T....... .......... .......T.. .......T.. .TCA...... .......T.. ..A....TG. ....AAT.TC
Sal_cant  .......... .......... .......... .......... .......... .......... .......T.. ...T...T.. ..C....... T......T.. ..T....CGT ....AGT.TC
Sal_cilR  .......... .......... .......... .......... .......... .......... .......T.. .......T.. ..C....... .......T.. ..T....CGT ....A.T.TC
Mur_heiA  .......... .......... -..CG..... .......... .......... .......... .......... C...T..T.. ..C....... T......... .......CG. ....A...TC
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ITS manually-aligned sequences for Polygalaceae (continued)

```
         841       851       861       871       881       891       901       911       921       931       941       951
         |         |         |         |         |         |         |         |         |         |         |         |
Bauhinia GCCCAAA--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Medicago ....CTT... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Callista ....C..... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
BG7836   ....CC-... .GCCGCACCG TCTCCCCGGT GATGGCTTGG GGGC...... .......... .......... .......... .......... .......... .......... ..........
BG7837   ....CC-... .GCCGCACCG TCTCCCCGGT GATGGCTTGG GGGC...... .......... .......... .......... .......... .......... .......... ..........
Xan_octB ....CC-... .GCCGCACCG TCTCCCCGGT GAAGGCTCAG GGGC...... .......... .......... .......... .......... .......... .......... ..........
Com_drum ....CCC... .......... .......... .......... .....CGTGC CTCCTCCCCC TCCTCT.... ....GGGGGC TT........ .......... .......... ..........
Com_aceA ....CCC... .......... .......... .......... ......TTGC CTCCGTCCCC TTCCTT.... ....GGGGGC GT........ .......... .......... ..........
Com_eriA ....CCC... .......... .......... .......... .....CATGC CTCCGTCCCC TCCGTT.... ....GGGGGC TT........ .......... .......... ..........
Com_esuA ....CCC... .......... .......... .......... .....CATGC CTCCGTCCCC TCCGTT.... ....GGGGGC TT........ .......... .......... ..........
Com_flaJ ....CCC... .......... .......... .......... ......GTGC CTCCCTCCCC TCCGTC.... ....AGGGGC TT........ .......... .......... ..........
Com_intB ....CCTCCC .......... .......... .......... .......... .......... .......... .......... ...ATTGCCT CCTTCACCCC CTCTGGGGAA AAAGA.....
Com_vol1 ....CC-... .......... .......... .......... .......... .......... .......... .......... ...TATGGCT CCT.CGCCTC TTCTGGGGA. ...GG.....
Com_spiG ....CCC... .......... .......... .......... .......... .......... .......... .......... ...CATGGCT CCTTCACCTC TTATGGGGA. ...GA.....
Com_vol2 ....CCC... .......... .......... .......... .......... .......... .......... .......... ...TATGGCT CCT.CACCTG TTCTGGGAA. ...GG.....
Com_volA ....CC-... .......... .......... .......... .......... .......... .......... .......... ...TATGGCT CCT.CGCCTC TTCTNGGGA. ...GG.....
Com_volM ....CCC... .......... .......... .......... .......... .......... .......... .......... ...TATGGCT CCT.CACCTG TTCTGGGAA. ...GG.....
Com_cili ....CCC... .......... .......... .......... .......... .......... .......... .......... ...CGTGTCC CCTTCACCCC TTGCGGGGA. ...GG.....
Com_rhaE ....CCC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .....TTTCC
Com_calB ..T.CCC... .......... .......... .......... .......... .......... .......... .......... ...ACGCCTC CTGCCCGCCG .CCCTGTGGG GCGGGGCCT.
Com_aphJ ..T.CC-... .......... .......... .......... .......... .......... .......... .......... ...ACGCCCA TGT...TCCG CCCCGGCGGG GCGGG.....
Com_scoM ....CCCG.. .......... .......... .......... .......... .......... .......... .......... ...CCCCACT CCTCTGTCCG CCCCGATGGG GAGGG.....
Pol_japL ....-TC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CC
Pol_linL ....CTC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CC
Pol_myrA ....-TC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CA
Pol_spJR ....CTC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CC
Pol_virA ....-TC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CC
Pol_exsR ....CTC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CC
Bre_col  ....-TC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ........CG
EpirAdC3 AT..-TC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Sal_cant .A..TTC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Sal_cilR .A..TTC... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mur_heiA ....CTCC.. .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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           961        971        981        991        1001       1011       1021       1031       1041       1051       1061       1071
           |          |          |          |          |          |          |          |          |          |          |          |
Bauhinia   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ACACATTGTT -CTTGCACTG GACTTGT---
Medicago   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... C.CA...TCC TA..TA.TA. .TA....GTG
Callista   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... CGC..A..CC .A..T...G. .CA.C.A...
BG7836     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
BG7837     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Xan_octB   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_drum   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_aceA   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_eriA   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_esuA   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_flaJ   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_intB   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_vol1   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_spiG   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_vol2   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_volA   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_volM   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_cili   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_rhaE   TCCTCTCGCC GTGTTCGCGT GA........ .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_calB   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_aphJ   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Com_scoM   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Pol_japL   CGCC.TTTGC CT.CATCTTT TGGGGC.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Pol_linL   CTAA.TC.GC CTCGGTCTCC TGGG.C.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Pol_myrA   CGCCCTC.GC CT.CATCTTT GGGG.C.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Pol_spJR   CCCCCATTGC CTTCTTCTCG GAGG.C.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Pol_virA   CGCCCTC.GC CTCATTTAT. GGGT.C.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Pol_exsR   CCC..ATTGT CTTGGTCTCC AGGG.C.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
Bre_col    CGCC.TCCGC CTCATGATAT GGCGGC.... .......... .......... .......... .......... .......... .......... .......... ---------- .--------- -------...
EpirAdC3   .......... .......... .......... .......... .........G TCTTCTTGGC CTCATTGCTA TGGCNTGGTG ..ATGGAGG. ---------- .--------- -------...
Sal_cant   .......... .......... .......... .......... .........A CTCGCAGGCC ATCTTT.CGA CGGCGTGCAT AAATGGAGG. ---------- .--------- -------...
Sal_cilR   .......... .......... .......... .......... .........C CTTGCAGGTC ATCTTT.CGA TGGCGTGCAT GGGCGTAGG. ---------- .--------- -------...
Mur_heiA   .......... .......... .......ACT CCCTTGCCTC AAATTGTGGG A......... .......... .......... .......... .......... ---------- .--------- -------...
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ITS manually-aligned sequences for Polygalaceae (continued)

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          1081       1091       1101       1111       1121       1131       1141       1151       1161       1171       1181       1191
          |          |          |          |          |          |          |          |          |          |          |          |
Bauhinia  -GGGGG---- ---------- ------TGTA TGTT-GGCCT CCC-GTGAGC ------ACGG CTGGCGGTTG GCC-TAAA-T ----G-CGGG CCTGTGGTGG CGAG-CAC-C ATGACATAC-
Medicago  CA...TG... .......... ......AT.. .......... .......... .....TCT.T ..CA...... .TT.G...A. ....T.GA.A ...TG.TA.. GTGT.GC..- ....T.G.T.
Callista  ..C...G... .......... ......C.A. ........T. .......... .....TC..T ..C......- AGT.G...A. ....-...A. ..C....... ACGA...... GC..TTA.TT
BG7836    .-----.... .......... ........G. ....T..T.. ...C.C.... .....C...A ..C....A.. ..TTA..CA. ....C.G.A. T.C....C.C .A.A...... GC.T.GC.T.
BG7837    .-----.... .......... ........G. .......T.. .....C.... .....C...A ..C....A.. ..T.....A. ....C.G.A. T.C....C.C .A.A...... GC.T.GC.T.
Xan_octB  .-----.... .......... ......C.G. .......T.. .....C.... .....CG..A ..C....A.. ..T.....A. ....CGG.A. T.C...AC.C ...A.A.... GC.T.GC.T.
Com_drum  .-----..GG GGACCCGGGG GG....C.G. ..A....... .......C.. .....AG..C .G.....A.. ..T.....TG ....CTG.A. GTCCC..C.C .CTA..G... GA.TGGA.T.
Com_aceA  .-----..GA GGACCCGGGG G.....C.G. ..A....... .......C.. .....AG..T .GT....A.. ..T.G...AG ....ATG.A. ..CAC..C.C .CTA..GN.. GC.T..A.T.
Com_eriA  .-----..GA GGACCCGGGG GG....C.G. ..A....... .......C.. .....AG..C .GC....A.. ..T.G...AG ....CGGAA. .TCCC..C.C .CTA..G... GC.T.GA.T.
Com_esuA  .-----..GA GGACCGGGGG G.....C.G. ..A....... .......C.. .....AG..C .GC....A.. ..T.G...AG ....CGGAA. .TCCC..C.C .CTA..G... GC.T.GA.T.
Com_flaJ  .-----..GA GGACCCGGGG G.....C.G. ..A....... .......C.. .....AGT.C .GC....A.. ..T.G...AG ....CTG.A. .TCCC..C.C .CTA..G... GC.T.GA.T.
Com_intB  .-----..GA GGATCTGGGG G.....C... .......... .......T.. .....CG..C .GC....A.. ..T.G...A. ......G.A. .TCAC.A..C .TTAG.G... GC.T.GC.T.
Com_vol1  .-----..GA GTAACTGGTG G.....C... .......... .......T.. .....CG..C .GT....A.. ..T.G...A. ....T.G.A. .TCAC.A..C .TTA.TG... GC.T.GC.T.
Com_spiG  .-----..GA GGATCTTGGG G.....C... C......... .......T.. .....CG..C .GT....A.. ..T.G...A. ....T.G.A. .TCAC.A..C .TTA.TG... GC.T.GC.T.
Com_vol2  .-----..GA GGATATGGGG G.....C... ..C....... .......T.. .....CG..C .GT....A.. ..T.G...A. ....T.G.A. .TCAC.A..C GTTC.TG... GC.T.GC.T.
Com_volA  .-----..GA GTAACTGGTG G.....C... ..C....... .......T.. .....CG..C .GT....A.. ..T.G...A. ....T.G.A. .TCAC.A..C .TTA.TG... GC.T.GC.T.
Com_volM  .-----..GA GGATATGGGG G.....C... N.C....... .......T.. .....CG..C .GT....A.. ..T.G...A. ....T.G.A. .TCAC.A..C GTTC.TG... GC.T.GC.T.
Com_cili  .-----..GA GGATCTGGGG GG....C... .......... .....CTC.. .....CG..C .CC....A.. ..T.G...A. ....T.G.A. .TCAC.AC.C .TTA..G... GC.T.GC.T.
Com_rhaE  .-----..GA GGATCCCGGG G.....C... .......... .....C.C.. ......CT.. TGT....A.. ..A.G...A. ....A.G.A. .TCAC..C.C .TTT.AGT.T GC.T.GC.T.
Com_calB  .-----..GA AGGTTTGTAG GGGGGGC... C.C....... ......TC.. .....CG... .GA....A.. ..T.G....G ....TCG.A. ..CT.....T .TCA..G... GC.C..C.T.
Com_aphJ  .-----..AA GGAAGAGTGG GTG...CC.. A......... ......TC.. .....CTT.C .GC....A.. ..T.G...A. ....C.G.A. .TCAC....T GTTT.TG... GC.T.GC.T.
Com_scoM  .-----..AA GAAAGCTGGG GGGC..C... ..C....... ......TC.. .....AT... .GA....A.. ..T.G...A. ....C.G.A. .TCAC..C.A .TTT..G... GC.T.GC.T.
Pol_japL  .-----..GA GGAGCTGGGG GGA...C.G. .......T.. .......T.. .....CT... .AT....C.. ..T.GC..A. ....CA.A.. A.CAC....C AC.A..G... GC.T.GC.T.
Pol_linL  .-----..AA GGAGTTGGGG GG....C.G. .......T.. .......T.. GCCTACTG.. .ACA...C.. ....G...A. ....CG.A.. A.CAC....C .A.A..G... GC.T.GC.A.
Pol_myrA  .-----..GA GGAGCTGGGG G.....C.G. ..C....T.. .......T.. .....CT... .AT....C.. ..TTA...G. ....CA.A.- A.CAC....C A..A..G... GC.T.GC.T.
Pol_spJR  .-----..GG GGAGTCGGGG ........G. .......T.. .......T.. ACCTACTG.T .AT....C.. ..T.G...A. ....CG.A.. A.CAA..C.T ...A..G... GC.T.GC.T.
Pol_virA  .-----..GA GGAGCCGAGG TG....C.G. ..C....T.. .......T.. .....CGA.T .AT....C.. ..TTA...A. ....CA.A.. A.CAC....C A..A..G... GC.T.GC.T.
Pol_exsR  .-----..GG GGAGCCAGGG G.......G. .......T.. .......T.. ACCTATTG.. .AT....C.. ....G...A. ....TG.A.. A.CAC....C .A.A..G... GC.T.GC.T.
Bre_col   .-----..GA GGTGGCGGGG GGG...C.G. ..C....... .......T.. .....CGG.A .AT....A.. ....G...A. ....CGGA.. ..CAC....T ...A..G... GC.T.CA.T.
EpirAdC3  .-----.... .......... .......AA. .......T.. ....A.AT.T ...TGAGATA .ATTT..A.. A.T.A...T. ....TGT... A..ACAAGTT TAG..TG... ...T.G..T.
Sal_cant  .-----.... .......... ......C.G. C......T.. .....C.T.T ....CCC.TT .AC....A.. ..T.G...AA ...TTGTC.. A.CTC..C.C GCGAGTG.GT GC.TGTC...
Sal_cilR  .-----.... .......... ......C.G. C.C....T.. .....C.T.T ....CTT..T .AC....A.. A.T.G...AA ...TTGTC.T AACTC..C.C AAGAGTG.GT GCATGTC...
Mur_heiA  .-----..GA GGTGTTGGGG A.....C.GT ..A....T.. .......T.. .....TTA.. .AT.T..A.. ..T.G...A. ....CA.A.. A.CAC....A T..A..G... GC.T.GC.T.
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ITS manually-aligned sequences for Polygalaceae (continued)

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         1201       1211       1221       1231       1241       1251       1261       1271       1281       1291       1301       1311
         |          |          |          |          |          |          |          |          |          |          |          |
Bauhinia GGTGGCTGAG TAA------T GCTC--GAAG CC-AGTCTTG CGTGCCTC-- GTCTCGCGCG ATGGCTCTTC TGACCC-TCA TGCATCGTGA ACACGATGCT CTCAACGC-- ---GA-----
Medicago .....A..T. .G.C.....- C.A.....GA ....AA-.CA T....TG.TC TAT.GAATGT ----GGAC.. .TTT--.--- AC.C------ ...T.CGTT. TGT.....TC GTG???????
Callista .....A.... .GTA....TA T.......GA ....A..GC. T...---.CC -....C..T. ..-------- -----.GGG.T .CGCGAC--- C..T.CGTGC .GTC.A.G.. ...C.CCC..
BG7836   .....A.... G..A.....- T.......GA ...C.C.GC. ....TTGTC. ACGGTCTCTC ---T.GACC. --TGT-CG.G CTGC.G---- GT.GCGC-.. T..G...... .....CCCCA
BG7837   .....A.... G..A.....- T.......GA ...C.C.GC. ....TTGTC. ACGGTCTCTC ---T.GACC. --TGT-CG.G CTGC.G---- GT.GCGC-.. T..G...... .....CCCCA
Xan_octB .....A.... G..A.....- T.......GA ...C.C.GC. ....T.GTC. ACGGTCTCGC ---A.GACC. C---GTCG.G CTGCCG.CCG GT.GCGC-.. T..G...... .....CCCCA
Com_drum .....AAAGA GT.A....GA T.....A.GA ...G.A.GC. A.C.TGG.C. C.GG.CTC.T .G-A.C.CC. ----GACGTG CTGCGAT--- G..GCGC-.C .A.G...... .....CCCCA
Com_aceA .....AA-GA GT.AAAAAGA T.......GA ...G.A.GC. T.C.TGG.C. C.GG.CTA.T .G-A.C.CC. ----GACGTG CTGCGAT--- G..GCGC-.C .A.G...... .....CCCCA
Com_eriA .....AAAGA GT.A....GA T.......GA ...C.ATGC. T.C.TGG.C. A.GG.CTT.T .G-A.C.CA- ----GACGTG CTGCGAT--- G..GTGC-.. .A.G...... .....CCCCA
Com_esuA .....AAAGA GT.A....GA T.......GA ...G.ATAC. T.C.TGG.C. A.GG.CTT.T .G-A.C.CC- ----GACGTG CTGCGAT--- G..GTGC-.. .A.G...... .....CCCCA
Com_flaJ .....AAAGA GT.AA...GA T.......GA ...T.A.GC. T.C.TGG.C. C.GG.CTC.T .G-A.C.CC. C---GACGTG CAGCGAT--- G..TCGC-.C .ATG...... .....CCCCA
Com_intB .....A...C GCGA.....- T...T.-GG. .....A.GC. T.C....TC. ..GG.CTC.. ---ATGACC. ---ATATG.G CTGCGAT--- G..GC..-GC TCGG.T.... .....CC...
Com_vol1 .....A.... GCGA.....- T...T.-GGA ...G.A.GC. T.C.TT.T.. ..GGACTC.- ---------- ------.--- ---------- ---------- --------.. ...--.....
Com_spiG .....A.... GCGA.....- T...T.-GGA ...G.A.GC. T.C.TT..T. ..GG.CTC.. ---A.GACC. ---ATATGTG CTGCGAT--- G..GTGC-.C TCAG...... .....CCCCA
Com_vol2 .....A.... GCGA.....- T...T.-GGA ...G.A.GC. T.C.TT.T.. ..GG.CTC-- ---------- ------.--- ---------- ---------- --------.. ...--.....
Com_volA .....A.... GCGA.....- T...T.-GGA ...G.A.GC. T.C.TT.T.. ..GGACTC.. ---ATGACC. ---AAATGTG CTGCAAT--- G..GTGC-GC T-------.. ...--.....
Com_volM .....A.... GCGA.....- T...T.-GGA ...G.A.GC. T.C.TT.T.. ..GG.CTC.T ---A.GACC. ---AAATGTG CTGCGAT--- G..GTGC-GC TCGG...... .....C....
Com_cili .....AC... .TGA.....- T...T.TGGA ...G.A.GC. T.C.TG.TT. ..GG.CTC.. .C.A.GACC. ---ATATGTG CTGCGAT--- G..GTGC-GC TCGG...... .....CCCCA
Com_rhaE .....A.... GCGA.....- T...T.A.GA .....ATGCT T....T..C. ..GG.CTC.C ---A.GACC. ---A.GTGTG CTGTGAT--- G..GCGC-.C T..G...... .....CCCCA
Com_calB .....A..G. GTCA.....- T...T.-.GA ...G.A.GC. ....TTG.C. C.GG.C.C.T ---TGGACC. C--G.ACGTG CTG.GTT--- G..GCGC-.C T..T...... .....CCCCA
Com_aphJ .....A...T .CGA.....- T.C.T.-.GA ..CG.A.GC. T...TTG.C. CG.GGCTC.C TGTA.C.CCT ---A..C.TG CTGCGAC--- G..GCGC-.C ...G...... .....CCCCA
Com_scoM .....A.... .CGA.....- T...T.-.GA ..CG.A.GC. T...TTG.C. CGGGGCTC.C ---AGGACC. C-TG..CGTG CTGTGAT--- G..GCGC-.C ...G...... ....-.....
Pol_japL .....AC... GT.A.....- T...T.A.GA ...G.A.GC. T.C.T.G.C. ..TG.CT.AC ---AGGACC. ----A.TG.G CTGCGAT--- G..GTGC-AC TCGG...... .....CCCCA
Pol_linL .....A.... GT.A.....- T...C.T.GA ...G.A.G.. ..CTT.G.C. ..TG.CTAGA C--AGGACC. ---AAA.G.G CTGCGTT--- G..GTGCCAC TCTG.T.... .....CCCCA
Pol_myrA .....AC... GT.A.....- T...T.A.GA ...G.A.GC. ..CTT.G.C. ..TG.CTATC ---AGGACC. ----A.CG.G CTGC.GT--- G..GTGC-AC TC.G...... .....CCCCA
Pol_spJR .....A.... GT.A.....- T...C.A.GA A..G.A.GC. ..C.T.G.C. ..TG.CTAGA C--ATGACC. ----AATG.G CTGCATT--- G..GTGCCAC TCTG.T..C. .....CCCCA
Pol_virA .....AC... GT.A.....- T...T.A.GA ...G.A.GC. T.CTT.G.C. ..TG.CTATC ---AGGACC. ----A.CG.G CTGC.GT--- G.TGTGC-AC TC.G...... .....CCCCA
Pol_exsR .....A.... GT.A.....- T...C.A.GA ...G.A.GC. ..CA..A.C. ..TG.CTATA G.CATGACC. ----AATG.G CTGCGTT--- GT.GTGCCAC T.GG...... .....CCCCA
Bre_col  .....A..G. GCGA....CC T...CGA.GA ...G.A.GC. T.C.A.G.C. .CGG.CTCGC ---A.GGCC. C.---ATG.G CTGC...--- G..GTG.-.C TC.G.T.... .....CCCCA
EpirAdC3 .T...A..GA A..A.....- T...T.ACGA TT.G.ATG.. A.C.TT--.. ..TGTCTT.A ----TGA.CT .---GATGTG CTGCATT--- G..GTTC-.. ..TC...... .....CCTCA
Sal_cant .T...AC.GA CG.AA....- CG..TAA.GA T..TAAGGCA TCGCG.A... ...G.CGA.. C--.TCACCT C---GATCTG CCGC.AT.-- G.GGCGCC.C A..G...... .....CCCCA
Sal_cilR .....AC.GA G..AA....- CG..T.-GGA G..TAAGGCA TCGCG.A... ...G.CGA.. C--TTGACCT C---GATCTG CCGCATC.-- G.GGCGCC.C A..G...... .....CCCCA
Mur_heiA .....A.... G..AA....- -...T.A.G. ...G.A.G.. T.C.T.G.C. ..GG.CTTGC ---A.GACCT C---GATG.G CTGC.AT--- G..GTG.-.C TC.G...... .....CCCCA
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ITS manually-aligned sequences for Polygalaceae (continued)

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         1321       1331       1341       1351       1361       1371       1381
         |          |          |          |          |          |          |
Bauhinia ---------- ---------- ---------- ------???? ?????????? ?????????? ????
Medicago ?????????? ?????????? ?.???????? ??????.... .......... .......... ....
Callista .......... .......... .......... ......---- ---------- ---------- ----
BG7836   GGTCAGGCGG GA.CTACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
BG7837   GGTCAGTCGG GA.CTACCC. G.CTGAGTTT AAGC.ATATC AAT------- ---------- ----
Xan_octB GGTCAGGCGG GA.CTACCC. G.TTGAGTTT AAGC.ATATC AATAAG-CGG AGG------- ----
Com_drum GGTCAGGCGG GA.CCACCC. G.ATGAGTTT AAGC.ATATC AATAAG-CGG AGGAAA---- ----
Com_aceA G.TCAGGCGC .A.CCACCC. GACTGAGTTC ATACTATATC ACTCGGACGC AGGAAAAG-- ----
Com_eriA GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAA--G-- ---------- ----
Com_esuA GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAGGG ----
Com_flaJ GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAAAGCGG AGGAAAAGAA ----
Com_intB .......... .......... .......... ......---- ---------- ---------- ----
Com_vol1 .......... .......... .......... ......---- ---------- ---------- ----
Com_spiG GGTCAGGCGG GA.ACACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AG-------- ----
Com_vol2 .......... .......... .......... ......---- ---------- ---------- ----
Com_volA .......... .......... .......... ......---- ---------- ---------- ----
Com_volM .......... .......... .......... ......---- ---------- ---------- ----
Com_cili GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
Com_rhaE GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGG------- ----
Com_calB GGTCAGGCGG GA.CCACCC. G.GTGAAGTT TAA...---- ---------- ---------- ----
Com_aphJ GGTCAGGCGG GA.GCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
Com_scoM .......... .......... .......... ......---- ---------- ---------- ----
Pol_japL GGTCAGGCGG GA.TCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAGGC GGGG
Pol_linL GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AG-------- ----
Pol_myrA GGTCAGGCGG GA.TCACCC. .......... ......---- ---------- ---------- ----
Pol_spJR GGTCAGGTGG TA.CCACCCC G.CTGAGTTA ......---- ---------- ---------- ----
Pol_virA GGTCAGGCGG GA.TCACCC. G.CTGAGTTT AAGC.ATATC A--------- ---------- ----
Pol_exsR GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AA....---- ---------- ---------- ----
Bre_col  GGTCAGGCGG GA.CCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGG------- ----
EpirAdC3 GGTCAGGCGA GA.TCACCT. G.CTGAGT.T AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
Sal_cant AGTCAGGCGG GA.TCACCC. G.CTGAGTNT AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
Sal_cilR AGTCAGGCGG GA.TCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
Mur_heiA GGTCAGGTGG GA.TCACCC. G.CTGAGTTT AAGC.ATATC AATAAG-CGG AGGAAAAG-- ----
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ITS manually-aligned sequences for Polygalaceae (end)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    5         15         25         35         45         55         65         75         85         95        105        115
BAUHINIA        T--------- CG-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------AAG CC-CCAAACA GC-ACGACCC
MEDICAGO        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
CALLISTA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7836          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7837          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM        -AACGACCCG CG-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------GAC AC-GTATACA GA-CGAACGA
COM ACEA        -AACGACCCG CG-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------GAC CC-GTATCCT GA-CGACTCG
COM ERIA        -AACGACCCG CG-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------GAC AC-GTATCCT AAACGAATAA
COM ESUA        --ACGACCCG CG-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------GAC AC-GTATCCT AAAAGAATAA
COM FLAJ        -AACGACCCG CG-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------GAC AC-GTATACA AA-CGAATGA
COM INTB        ---------- ---------- ---------- ---------- ---------- ---------- ----GACATG TGAACCCGT- AT-CT-C-AT GATT-GTGGG CT-GGATGGG GG-CAAGCGC
COM VOL1        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SPIG        ---------- ---------- ---------- ---------- ---------- ---------- GAATGACTTG CGAACCCGT- AC-CT-C-GT GATT-GCGGG GT-GGATGGG GG-CATGCGC
COM VOL2        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLM        ---------- ---------- ---------- ---------- ---------- ---------- -AATGACTCG TGAACCCGT- AT-CT-C-GT GATT-GTGGG GC-GGACGGG GG-CAAGCGC
COM CILI        ---------- ---------- ---------- ---------- ---------- ---------- GAATGACCCG TGAACCCGT- AT-CT-C-GT GATT-GCGGG GC-GGATGGG GG-CATGCGC
COM RHAE        ---------- ---------- ---------- ---------- ---------- ---------- -AACGACCCG CGGACCTGT- AT-CT-T-GT GATT-GTGGG TG-GGTAGGG GG-CATGCGC
COM CALB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------ATT GT-CGAAACC
COM APHJ        ---------- ---------- ---------- ---------- ---------- ---------- ---CGACCTG TGGACCAGT- ATACA-CCGT GACAAGCGGG CGAGGGCGGG TG-CATGCGT
COM SCOM        ---------- ---------- ---------- ---------- ---------- ---------- GAACGACCCG TGGACCAAT- TAATTGCCGT GACA-GCGGG CGAGGGGGGT TG-CATGCGA
POL JAPL        ---------- --A------- ---------- ---------- ---------- --------AA GAGCGACCGT TGGACATGT- AT-AT-C-TT CACG-GCGGG TG-G-ATGGG TG-CATGCGT
POL LINL        ---------- ---------- ---------- ---------- ---------- ---------- GAGNNANTNT TGCACATGT- AT-AC-C-TT CACA-GCGGG TG-G-ACGGG TG-CATGTGC
POL MYRA        ---------- ---------- ---------- ---------- ---------- ---------- GAGCGACCGT TGGACACGT- AC-AT-C-TT CACG-GCGGG CG-G-ACGGG CG-CATGCGC
POL SPJR        ---------- ---------- ---------- ---------- ---------- ---------- GAGTGACCGT TGGATATGT- AT-AC-C-GT CATT-GCGGG TG-G-ATGGG TG-CATGTGC
POL VIRA        ---------- ---------- ---------- ---------- ---------- ---------- GAGCGACCGT TGGACATGT- AT-AT-C-TT CACG-GCGGG TG-G-ATGGG CG-CATGCGC
POL EXSR        ---------- ---------- ---------- ---------- ---------- ---------- GAGTGACCGT TGGACATGT- AT-AC-C-TT CATT-GTGGG TG-G-ACGGG TG-CATGTGA
BRE COL         ---------- ---TCTGTAG GTGAACCTGC GGAAGGATCA TTGTCGAAAC CTGC-CGGAA GGGAGACCGT CGGATGCGT- TCATCTC-TT TACGCGCGGG GC-GGATGGG GCGCGTGCTC
EPIRADC3        ---------- ----CTGTAG GTGAACCTGC GGAAGGATCA TTGTCAAATC TTACTCAAAA GGTTGACTGT CGGACTTGT- CT-AT-C-TT -ATG-GTGGG AA-C-ATGTT GA-CAT-TAT
SAL CANT        ---------- ---------- ---------- GGAAGGATCA TTATCGAAGT CTCT-CGTAA GATGGACCGT CGAACCCGTG AC-ATGA-AT GTCT-GTCGG GA-G-AGCTT GT-TTGCCTC
SAL CILR        ---------- ---------- ---------- ---------- ---------- ---------- GATGGACCGT CGAACTTGTG AC-TTGA-AA TTCT-GCGGG GA-G-AGCAC GT-TCGCTGC
MUR HEIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                  125        135        145        155        165        175        185        195        205        215        225        235
BAUHINIA       G-TGAATT-C GTATGCGATA TT-----G-- GGCG-GCGTA GGGTTC-T-G ATT----GCA CC--CA--CC CACCCCATCC TA--C--GTG AGA-C-ACAC GGGGTCCTTC -TT-GTGT--
MEDICAGO       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
CALLISTA       ---------- ---------- --CCGG-G-- GGCA-GGCTC GGGGGT-G-C TCC----GCA CC-TCAGACC TCCCCCGTGC CG--G--GAG ACG-C-TCTC GGGCGCCATC -CTAGCGA--
BG7836         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7837         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM       G-GGAAGG-G AGGGGGCATG CG-----C-- TGCG-CCCTC TCGGCC-C-G CGC----GGG CT--CT--GC GCGGCCGTGG GG--G--TCC TCC-CCTGGC CGTGCCGGGC ACGTGCGA-A
COM ACEA       G-GGGCGG-G AGGGGGCATG CG-----C-- TGCG-CCCTT TCGTCCCT-G CAC----GGG CT--CG--GT GCGGTCTTGG GG--G---CC TCC-CACGGC CGTGCCGGGC ACGTATGA-A
COM ERIA       G-GGACGG-G AGGGGGCATG CG-----C-- TGCA-CCCTC CCGGCC-C-G TGC----GGG CT--CG--GT GCGGTCGCGG GG--G---CC TCC-CCTGGC CGTGCCGGTC ACGTACGA-A
COM ESUA       G-GGACGG-G AGGGGGCATG CG-----C-- TGCG-CCCTC CCGGCC-C-G TGC----GGG CT--CG--GT GCGGTCGCGG GG--G---CC TCC-CCTGGT CGTGCCGGTC ATGTACGA-A
COM FLAJ       G-GTACGG-G AGGGAGCATG CG-----C-- TGCG-CCCTC TCGGCC-C-G CGT----GGG CT--CG--GC GCGGTCGTTG GG--GCCTCC TCC-CCTGGC CGTGCCGGGC ACGTGCGA-A
COM INTB       T-GAGCCC-T CTCCGCCACA TG----CG-- GGCT-GCGTA TGGTTG-T-G GGG----AGC CC-TGGCGNC GCGGTGCTGC GG--G---TC TCC-CCCAAC TGTGCTTGGC CCTCTCAA-A
COM VOL1       -CGAGCCC-- CTTCGCCGCA TG----TG-- GGTC-GAGTT CGGTTG-G-G GGG----AGT CC-TGGCATC GCGGTGCCGC GG--G---TC TCC-CCTCCC CGTGCCTGGC CCTTACAA-A
COM SPIG       T-GAGCCC-A TCCCCCCGCA TG----TGTG GGTC-GCGTG CGGTTT-T-G GGG----AGC CG-TGGTGCC GCGGTGCTAC GG--G---TC TCC-CCTATC CGTGCNTGGC CCGTACAA-A
COM VOL2       T-GAGCCC-C TTCCGCAGCA CG----TG-- GGTC-GCGTA CGGTTT-T-G GGG----AGT CC-TGGCATC GCGGTGCCGC GG--G--TCT CCC-CCTCTC CGTGCCTGGC CCTTACAA-A
COM VOLA       ---------- --TCGCCGCA TG----TG-- GGTC-GAGTT CGGTTG-G-G GGG----AGT CC-TGGCATC GCGGTGCCGC GG--G--TCT CCC-C-TCCC CGTGCCTGGC CCTTACAA-A
COM VOLM       T-GAGCCC-C TTCCGCAGCA CG----TG-- GGTT-GCGTA CGGTTT-T-G GGG----AGT CC-TGGCATC GCGGTGCCGC GG--G--TCT CCC-CCTCTC CGTGCCTGGC CCTTACAA-A
COM CILI       T-GCGCCC-G TTCCGCCGCG TG----CG-- GGTC-GCGTA CGGTTG-G-G GGGGCAAAGC CC-TGGCATC GCGGTGCTGC GG--GTCTCC TCCTCCTATC CGTGCTCGGC CCATGCAA-A
COM RHAE       T-GCGCCC-- TTTCGGAGCC CA----CGTG GGCC-GGGCA GGG-CC-G-G GGG----AGC CC-TGGCTTA ACGGTGTCAC GG--C--GCC CCC-G-TCTC CGTGCTCGGC CCGCACGA-A
COM CALB       T-AATCCG-A AGAGCGACCC GG----TG-- GATGAGTGTC TCGTGA-G-C CGT----GGG AC--GG--GT GCGGCCTGGG AG--CATGCC TCG-CCTCCC CGTGCCCGTC TCATGCGATA
COM APHJ       T-GCGCCC-- TTCCCGCCTG TG----TG-- GGGC-GTGTG CGG-CT-G-G GGGGGTAGGA AC-CGGCTTC ACCATTCTCG GG--TCCTCC TCC-CCTCCC TGTGCCTGCC CCACACGA-A
COM SCOM       T-GCGCCC-- TTCCC-ACCC CC----TGTG GGGC-GTGTG CGG-CC-G-G GG-----GGA GG--CA--CC G-GTTTCCCG GG--C--CCT CCC-C-TCCC CGCGCTTGCC CTGCACGA-A
POL JAPL       T-GCGCCC-- TTCCCCCCCT CA----AGTC GGGC-TGGGA CGGTTG-G-G TG-----GTG C--TGGCCTC GCTGCGGCGT GG--G---TC CCCTCCCCTC CGTGCTCGGC TCGTACTA-A
POL LINL       T-GTGCCC-G TCCACCCCCT CG----ATTT GGGT-GAGGA TGTCGG-G-G CG-----GTG C--TGGCCTC ACTGCAGTGC GG--C---TC CCT-CCCCTC TGTCCTCGGC CCGTGCTA-A
POL MYRA       T-GCGCCCGT CTCCCGCCCT CA----AGTC GGGC-GAGGA TGGTGG-G-G CAG----GTT AAATGGCCTC GCTGCGGCGC GG--C---TC CCT-CCCCTC CGTGCTCGGC CCGTGCTA-A
POL SPJR       T-GTGCCT-A TCCACCCCCT TG----TTTC GTGT-GAGGA TGTCTG-G-G CG-----GTG A--TGGCCTC GCTGATGCGC GG--C---TC CCT-CCCCT- --TCTTTGCC -CGTGCGA-A
POL VIRA       T-GCGCTCGT CCCCCGCCCT CG----AGTC GGGC-GAGGA TGGTTG-G-G CG-----GTT C--TGGCCTC GCTGTGGCGC GG--C---TC CCT-CCCCTC CGTGCTCGAC CCGAACTA-A
POL EXSR       T-GTGCCT-G TCCACCCCCT CG----TTTT GGGT-CAGGA TGTCTG-G-G TG-----ATG CC-TGGCCTC GCTGCTGTGC GG--C---CT CCCTCCCCTA TGTCCTTGGC CCGTGCGA-A
BRE COL        C-GTGCCC-T TCTCCCCTCG CA----AGTT GGGC-GAGGA TGGGGA-GAG GGG----AGG CG-AGGCCTC ACNGCGGTGC GGCCGTCTCT GCCCTCCCTC CGTCCTCGGC CCGTACGA-A
EPIRADC3       T-TTCTCC-- CTCA--AG-T TG----AG-- GGAGAATGTT GGGATG-G-G TGT----GGT GC--AG--TT GATGTGCCAT TTCCT---TT TCT-T-AGA- --TTCTCCAC TCATGCGG--
SAL CANT       G-GCGCGA-- CATCTCTCCC CG----AGAT CGAG-TTGGA CGTGGA-A-T CG-----GT- CG--AG--TC GCGCTCGTCG CGCCTCCTCC CTTTC-ACAA CGTTCGCGGC -TC-GTGA-T
SAL CILR       C-TTGCGT-- CGTCTCTCCT CG----AGAT CGAGTCGGGT CGTGGA-A-T TG-----GT- CG--AG--AA GCGCCCGTCG CGCCTCCTCT CTTTTCACGG AGTGCATGGC -TC-GTGA-T
MUR HEIA       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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POA-aligned ITS sequences (continued)

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              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                 245        255        265        275        285        295        305        315        325        335        345        355
BAUHINIA      GCGCTCATTT A--GGTAAAG AA-----CAA AACC-CC--- GGCGC-TAGT -TGCGCCA-A GGAACC---C TTACAATACA ACA-TCCCC- -GTGCGGGGT T-----TGTT GC---AACG-
MEDICAGO      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
CALLISTA      CAACACAATC CCCGGCGCGG AATGCGCCAA GGAA-CT--- CGAAT-TTGT -TAAGCGT-G CTCCCG---C GGACCCGGAG ACGGTGCTC- -TTGCGGGGG C------GTC GC---GATGC
BG7836        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7837        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM      CAACCAAAAC CACGGCGTGC GAAGCGCCAA GGAATCCTAT GGTAT-GACC CTGTGCTT-G CACCCC--AC CGGCCCGGAG ACGGAGCTCC GGTGTGTGGT G-----CGGG CCT-GAATTA
COM ACEA      CAACCAAAAC CACGGCGTGC GAAGCGCCAA GGAATCCTAT GGTAT-GACC CTGTGCTN-G CACCCC-CAC CGGCCCGGAG ACGGAGCTCC GGTGCGTGGG G-----CGGG CCT-GAATAT
COM ERIA      CAAACAAAAC CACGGCGTGC GAAGCGCCAA GGAATCCTAT GGTAT-GACC CTGTGCTT-G CACCAC---C GG-CCCGGAG ACGGAGTTCC GGTGTGTGGC G-----CGGG CCA-GAATTT
COM ESUA      CAAACAAAAC CACGGCGTGC GAAGCGCCAA GGAATCCTAT GGTAT-GACC CTGTGCTT-G CACAAC---C GG-CCCGGAG ACGGAGTTCC GGTGTGTGGC G-----CGGG CCA-GAATTT
COM FLAJ      CAACCAAAAC CACGGCGTGC GAAGCGCCAA GGAATCCTAT GGTAT-GAAC CTGTGCTT-G CACCCC-CAC CGGCCCGGAG ACGGAGTTCC GGTGTGTGGC GGCG--CGGG CCT-GAATTT
COM INTB      CAACCAAAAC CACGGCGTGA GAAGCGCCAA GGAAAAT--- GGAAAAGATT GTGTGCTC-G ACCCTCTCTC GTGCCGCCAG TCGGCGAGCA AGGAGGGGGC G-----CACG CTT-GACTTT
COM VOL1      CAACCAAAAC CCCGGCGTGA GAAGCGCCAA GGAAAAT--- GGAAT-GA-T -TGTGCTC-G ACCCCC--TC CTGCCGCCAG TGGGCGAGCT GGAGGAGGGC G-----CACG CTT-GACTTT
COM SPIG      CAACCAAAAC CACGGCGTGA GAAGCGCCAA GGAAAAT--- GGATG-GA-T -TGTGCTC-G ACCCTC--TT GTGCCGCTAG GATGCGAGCT GGAGGGGGGC G-----CGCG CTT-GACTTT
COM VOL2      CAACCAAAAC CACGGCGTGA GAAGCGCCAA GGAAAAT--- GGAAG-GATT GTGCTTTT-G ACCCCC--TC CTGCCGCCAG CCGGCGATCC GGAGGAGGGC G-----CACG CTT-GACTTT
COM VOLA      CAACCAAAAC CCCGGCGTGA GAAGCGCCAA GGAAAAT--- GGAAT-GA-T -TGTGCTC-G ACCCCC--TC CTGCCGCCAG TGGGCGAGCT GGAGGAGGGC G-----CACG CTT-GACTTT
COM VOLM      CAACCAAAAC CACGGCGTGA GAAGCGCCAA GGAAAAT--- GGAAG-GATT GTGCTTTT-G ACCCCC--TC CTGCCGCCAG CCGGCGATCC GGAGGAGGGC G-----CACG CTT-GACTTT
COM CILI      CAACAAAAAC CACGGCGTGA GAAGCGCCAA GGAAAAT--- GGAAG-GA-T -TGTGATG-T GCCCTC--CC CTGCCGCCAG TCGGCGAGCT GGAGGAGGGC G-----CACG CTT-GACTTT
COM RHAE      TGACCAAAAC CACGGCGCGA GAAGCGCCAA GGAAAAT--- GGACT-GA-C -TGTGCTT-T GACCCC-CTC CTGCCGGTAC TAAGCCGGCA TGCCCGAGGG GGCGAGCACG CTTTGACTTT
COM CALB      AAACCAAAAC CATGGCGCGA GTGGCGCCAA GGAAAGG--- GGAAC-GA-T -TGTGCCA-- -G--CG---C TC-CCGCCAT A-GGCCGGG- -AGGGCTGGC A-----CACG CTT-GACTTT
COM APHJ      CAACCAAAAC CACGGCGCGA CAAGCGCCAA GGAA-GA--- GGAACACA-T -TGCGCTT-- -T--GC---C CAACCCG--- ----TG-GC- -GGGTGGGGC A-----CACG CTT-GACTTT
COM SCOM      CAACCAAAAT CCCGGCGCGA GAAGCGCCAA GGAA-GA--- GGGAA-CACT -TGTGCTTAG CCCCAC--CC GTGGCAGGCG GAAGGCCTGG CGGGGGCGGG GGCG--CACG CTT-GAATTT
POL JAPL      CAACCAAACT CC-GGCGCGA GAAGCGCCAA GGAAATT--- GTACC-GA-C -CGCTCGT-G CCCCCT--TT GGCCCCGGAG ACGGTGTGCC GATTGGGTGC G-----CGTT GGA-CATTTT
POL LINL      CAACCAAAAC TCCGGCGCGA GAAGCGCCAA GGAAGAT--- GTACT-GA-C -CGCGCGC-G CCCATT--TT GGCCCCGGAG ACGGCGTGTT GACCGGGCGC G-----TGCC GGA-CAATAT
POL MYRA      CAACCAAACT CC-GGCGCGA GAAGCGCCAA GGAAATC--- GTACC-GA-C -CGCG--T-G CCCCTC---C GGCCCCGGAG ACGGTGTGCC GATCGGGCGC G-----CGCT TGA-CATTTT
POL SPJR      CAACTAAAAC TCCAGCGCGA GATGCGCCAA GGAAGTT--- ATACC-GA-A -TGCA--T-T CCCACT--TC GGCCCTGGAG ACAGTGTGTC GATCGGGTGT G-----CGCC GGA-CAATAT
POL VIRA      CAACTAAACT CC-GGCGCGA GAAGCGCCAA GGAAATC--- GTACT-GA-C -CGCG--T-G CCCCTT---C GGCCCCGGAG ACGGTGTGCT GACCGGGCGC G-----CGCT TGA-CATATT
POL EXSR      CAACCAAAAC TTCGGCGTGA GAAGCGCCAA GGAAGTC--- GTATC-GA-C -CGCA--T-T CCCACT--AC GGCCCTGGAG ACAGTGTGTC GATCGGGTGT G-----CGCC GGA-CAATAT
BRE COL       AAACCAAAAT CC-GGCGCGA GAAGCGCCAA GGGAACT--- GGACA-GACC GTGCGCGT-G CGCCCT--AG GC-CTCCGAG ACGGGG-CG- -ACAGGTGGG CACG--CGCG GGA-GATTTT
EPIRADC3      TAACCTAAAC ATCATTGCGA GTAGTGCGAA GGAAATT--- AATTT-GAGC -TATA--T-G TGTAGC-CTC TGACATCTTT AAA-TGAGT- -GTCATCTTG G-----TGCA CTTTGTTTAT
SAL CANT      TCATTCGAAC GC-GGCGCGA GAAGCGCCAA GGATTAT--- GTACCTGA-C -CACGCGT-G CGGCCT---C CGGGCTC--- ----TG-GT- -GCGCAGTGG A-----CATT -------CG-
SAL CILR      TAACTCGAAT GC-GGCGCGA GAAGCGCCAA GGATCAT--- GGACTTGA-C -CACGCGG-G CCGCCT--TC GGAATTCGAA GATGAGATGC AATCTCTGCG C------GCG GTG-GACATT
MUR HEIA      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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POA-aligned ITS sequences (continued)

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               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   365        375        385        395        405        415        425        435        445        455        465        475
BAUHINIA       ---T---GTT TATCCA--AA ATGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
MEDICAGO       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
CALLISTA       --AT---GAA AATATA--AA ATGACTCTCG GCAACGGATA TCTCGGCTCT TGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
BG7836         ---------- ---------- ---------- ---------- ---------- -------ATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
BG7837         ---------- ---------- ---------- ---------- ---------- -GCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATCGC-A GAATCCCGTG AACCATCGAG
XAN OCTB       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------A GAATCCCGTG AACCATCGAG
COM DRUM       --GT--CGTG TACACG--AA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TAAT-TGGTT TGTATTGC-A GAATCCCGTG AACCATTGAG
COM ACEA       --GT--CGTG TACACACGAA ACGACTCTCG GAAACGTATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACGATGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM ERIA       --GT--CGTG TACACG--AA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM ESUA       --GT--CGTG TACACG--AA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM FLAJ       --GT--CGTG TACACG--AA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM INTB       --GT--CTTG TACACT--GA ATGACTCTCG GCAACGGATA TCTTGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM VOL1       --GT--CGTG TACACT--GA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM SPIG       --GT--CTTG TACACC--GA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM VOL2       --GT--CGTG TACACT--GA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM VOLA       --GT--CGTG TACACT--GA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM VOLM       --GT--CGTG TACACT--GA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM CILI       --GT--CATG TACACT--GA ATGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM RHAE       --GT--CGTG TACACT--GA ACGACTCTCG GCTACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM CALB       --GT--CGTG TATGTTTTGA ACGACTCTCG GCAACGGATA TCTTGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM APHJ       --GT--CGTG TAAAAATTGA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
COM SCOM       --GT--CGTG TAAAA-TTGA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
POL JAPL       T-GT--CGTG TAAACT--TA ACGACTCTCG GCAACGGATA TCTCGGTTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
POL LINL       T-GT--CGTG TAAACT--TA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
POL MYRA       T-GT--CGTG TAAACT--TA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
POL SPJR       T-GT--CGTG TAAACT--TA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
POL VIRA       --GT--CGTG TAAACT--TA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
POL EXSR       T-GT--CGTG TAAACT--TA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
BRE COL        C-GTGGCGAC ATGCCAAATA ATGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAGTGCGA TACA-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
EPIRADC3       TAGT--TGTG TATACA--AA ACAACTCTCG ACAATGGATA TCTTGGCTCT TGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATTGAG
SAL CANT       ---T--CGTT TACACG--AA ACGACTCTCG GCAACGGATA TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
SAL CILR       T-GT--CGTT TACACG--AA ACGACTCTCG GCAACGGATA TCTTGGCTCT CGCATCGAT- -----CGTAG CGAAATGCGA TACT-TGGTG TGAATTGC-A GAATCCCGTG AACCATCGAG
MUR HEIA       ---------- ---------- ---------- ---------- ---------- ---------- ---------- CGAAATGCGA TACT-TGGTG TGAATGCCGA GAATCCCGTG AACCATCGAG
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POA-aligned ITS sequences (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      485        495        505        515        525        535        545        555        565        575        585        595
BAUHINIA        TCTTTGAACG CAAGTTGCGC CCGAAGCCAT CAGG-CTGAG GGCACGTCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
MEDICAGO        ---------- ---------- ---------- ---------- ---------- --------TC GATGCCTTAC ATGCCGTCCA ACACGTGAAT CAGTTTGAAT ACATATGGGT TGGCTTGAGG
CALLISTA        TTTTTGAACG CAAGTTGCGC CCAAAGCCAT TAGG-CCGAG GGCACGCCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
BG7836          TCTTTGAACG CAAGTTGCGC CCGAAGCCTT TGGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
BG7837          TCTTTGAACG CAAGTTGCGC CCGAAGCCTT TGGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB        TCTTTGAACG CAAGTTGCGC CCGAAGCCTT CGGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM        TCTTTGAACG CACGTTGCGC CCGCCGCCTT TTTG-CCGAG GGCACGCCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
COM ACEA        TCTTTGAACG CAAGTTGCGC CCGACGCCTT TTGG-CCGAG GGCACGCCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
COM ERIA        TCTTTGAACG CAAGTTGCGC CCGACGCCTT TTGG-CCGAG GGCACGCCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
COM ESUA        TCTTTGAACG CAAGTTGCGC CCGACGCCTT TTGG-CCGAG GGCACGCCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
COM FLAJ        TCTTTGAACG CAAGTTGCGC CCAACGCCTT TTGG-CCGAG GGCACGCCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
COM INTB        TCTTTGAACG CAAGTTGCGC CCGATGCCAT CAGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL1        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM SPIG        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL2        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLA        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLM        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM CILI        TCTTTGAACG CAAGTTGCGC CCGATGCCTT TTTGGCCGAG GGCACGTCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
COM RHAE        TCTTTGAACG CAAGTTGCGC TCGACGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM CALB        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CTGG-CTGAG GGCACGTCTG TCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM APHJ        TCTTTGAACG CAAGTTGCGC CCGATGCCTT CGGG-CTGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
COM SCOM        TCTTTGAACG CAAGTTGCGC C-GATGCCAC TTGG-CTGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
POL JAPL        TCTTTGAACG CAAGTTGCGC CCGACGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
POL LINL        TCTTTGAACG CAAGTTGCGC CCGATGCCTT TTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
POL MYRA        TCTTTGAACG CAAGTTGCGC CCAACGCCTT CTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
POL SPJR        TCTTTGAACG CAAGTTGCGC CTGACGCCTT TTGG-CTGAG GGCACGTCTG CTTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
POL VIRA        TCTTTGAACG CAAGTTGCGC CCAACGCCTT TGG--CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
POL EXSR        TCTTTGAACG CAAGTTGCGC CTGACGCCTT TTGG-CCGAG GGCACGTCTG CCTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
BRE COL         TCTTTGAACG CAAGTTGCGC CCGAAGCCTC CTGG-CCGAG GGCACGTCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
EPIRADC3        TCTTTGAACG CAAGTTGCGC CTGAAGCCAT CTGG-TCAAG GGCACGTCTG CTTGGGAG-- ---------- ---------- ---------- ---------- ---------- ----------
SAL CANT        TCTTTGAACG CAAGTTGCGC CTGAAGCTAT CTGG-CCGAG GGCATGTCTG CTTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
SAL CILR        TCTTTGAACG CAAGTTGCGC CTGAAGCCAT CTGG-CCGAG GGCACGTCTG CTTGGGTG-- ---------- ---------- ---------- ---------- ---------- ----------
MUR HEIA        TCTTTGAACG CAAGTTGCGC CCGACGCCTT CTGG-CCGAG GGCATGTCTG CCTGGGCG-- ---------- ---------- ---------- ---------- ---------- ----------
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POA-aligned ITS sequences (continued)

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             605        615        625        635        645        655        665        675        685        695        705        715
BAUHINIA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
MEDICAGO  TGTTCCACAC CTTGGCTTAC CTCTGGTTCA GAGGAAGACG ACAAAGTGCG TCCTTCTTTG CGCCAAAACT CAAACCCCGG CGTTGAATGC GTCAAGGAAT TTAAATTTTG CTCTGAGCAC
CALLISTA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7836    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7837    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ACEA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ERIA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ESUA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM FLAJ  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM INTB  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL1  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SPIG  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL2  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLM  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM CILI  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM RHAE  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM CALB  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM APHJ  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SCOM  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL JAPL  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL LINL  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL MYRA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL SPJR  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL VIRA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL EXSR  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BRE COL   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
EPIRADC3  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SAL CANT  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SAL CILR  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
MUR HEIA  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   725        735        745        755        765        775        785        795        805        815        825        835
BAUHINIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
MEDICAGO        ACTTGCATGG CACCGGAGAC GGTTTTCGTG CGGGTTGTGT TTTGACACAT GATAT????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
CALLISTA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7836          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7837          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ACEA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ERIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ESUA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM FLAJ        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM INTB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL1        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SPIG        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL2        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLM        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM CILI        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM RHAE        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM CALB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM APHJ        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SCOM        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL JAPL        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL LINL        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL MYRA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL SPJR        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL VIRA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL EXSR        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BRE COL         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
EPIRADC3        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SAL CANT        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SAL CILR        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
MUR HEIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   845        855        865        875        885        895        905        915        925        935        945        955
BAUHINIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC AAACGT---C
MEDICAGO        ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ???????ATC GAA-GC---C
CALLISTA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACACAT---C
BG7836          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCGC---A
BG7837          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCGC---A
XAN OCTB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---A
COM DRUM        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM ACEA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM ERIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM ESUA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM FLAJ        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM INTB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM VOL1        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
COM SPIG        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
COM VOL2        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
COM VOLA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
COM VOLM        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
COM CILI        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
COM RHAE        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---C
COM CALB        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACTCATGGCC
COM APHJ        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACTCTC---A
COM SCOM        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACTCCCGGTC
POL JAPL        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
POL LINL        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAA---C
POL MYRA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
POL SPJR        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCATTGTC
POL VIRA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
POL EXSR        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
BRE COL         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAC---T
EPIRADC3        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ATGCAA---T
SAL CANT        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGTAG---T
SAL CILR        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGTAT---T
MUR HEIA        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------TC ACGCAT---C
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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    965        975        985        995       1005       1015       1025       1035       1045       1055       1065       1075
BAUHINIA        GCTGCCCAA- -AAC--ACAT TGTTCTTG-- CACT-GGACT T----GTG-G G--------G GTGTATGTT- GGCCTCCC-- GTGAGCAC-- -GG-CTGGCG GTTGGCCTAA A-TGCGG--G
MEDICAGO        CCTTCCCAA- -TTT--CCTA TTTAATAG-- GTAT-TGTGT G----CAG-G G--------T GATTATGTT- GGCCTCCC-- GTGAGCTC-- -TGTCTCACG GTTGGTTGAA A-ATTGA--G
CALLISTA        GTTGCCCCA- -ACG--CCAA TGCCATTT-- CACG-GGCAT C----GAGCG G--------G GCGAATGTT- GGCTTCCC-- GTGAGCTC-- -CGTCTCGCG GTTAGT-GAA A-ATCGA--G
BG7836          GTCGCCCCC- -GCC--GCAC CGTCTCCC-- CGGT-GATGG C----TTGGG G--------G CTGGATGTTT GGTCTCCCC- GCGAGCCA-- -CGACTCGCG GATGGCTTAA ACATCGG-AG
BG7837          GTCGCCCCC- -GCC--GCAC CGTCTCCC-- CGGT-GATGG C----TTGGG G--------G CTGGATGTT- GGTCTCCC-- GCGAGCCA-- -CGACTCGCG GATGGCTTAA A-ATCGG-AG
XAN OCTB        GTCGCCCCC- -GCC--GCAC CGTCTCCC-- CGGT-GAAGG C----TCAGG G--------G CCGGATGTT- GGTCTCCC-- GCGAGCCG-- -CGACTCGCG GATGGCTTAA A-ATCGGGAG
COM DRUM        GTCGCCCCC- -CCGT-GCCT CCTCCCCCTC CTCT-GGGGG C----TTGGG GACCCGGGGG GCGGATGAT- GGCCTCCC-- GTGCGCAG-- -CGCCGGGCG GATGGCTTAA A-TGCTGGAG
COM ACEA        GTCGCCCCC- -CTT--GCCT CCGTCCCCTT CCTT-GGGGG C----GTGAG GACCCGGGGG C-GGATGAT- GGCCTCCC-- GTGCGCAG-- -CGTCGTGCG GATGGCTGAA A-AGATGGAG
COM ERIA        GTCGCCCCC- -CCAT-GCCT CCGTCCCCTC CGTT-GGGGG C----TTGAG GACCCGGGGG GCGGATGAT- GGCCTCCC-- GTGCGCAG-- -CGCCGCGCG GATGGCTGAA A-AGCGGAAG
COM ESUA        GTCGCCCCC- -CCAT-GCCT CCGTCCCCTC CGTT-GGGGG C----TTGAG GACCGGGGGG C-GGATGAT- GGCCTCCC-- GTGCGCAG-- -CGCCGCGCG GATGGCTGAA A-AGCGGAAG
COM FLAJ        GTCGCCCCC- -CGT--GCCT CCCTCCCCTC CGTC-AGGGG C----TTGAG GACCCGGGGG C-GGATGAT- GGCCTCCC-- GTGCGCAG-- -TGCCGCGCG GATGGCTGAA A-AGCTGGAG
COM INTB        ATCGCCCCCT CCCATTGCCT CCTTCACCCC CTCT-GGGGA A-AAAGAGAG GATCTGGGGG C-GTATGTT- GGCCTCCC-- GTGTGCCG-- -CGCCGCGCG GATGGCTGAA A-ATGGG-AG
COM VOL1        ATCGCCCCC- -TAT--GGCT CCTCGCCT-- CTTC-TGGGG A----GGGAG TAACTGGTGG C-GTATGTT- GGCCTCCC-- GTGTGCCG-- -CGCCGTGCG GATGGCTGAA A-ATTGG-AG
COM SPIG        ATCGCCCCC- -CCAT-GGCT CCTTCACCTC -TTA-TGGGG A----GAGAG GATCTTGGGG C-GTACGTT- GGCCTCCC-- GTGTGCCG-- -CGCCGTGCG GATGGCTGAA A-ATTGG-AG
COM VOL2        ATCGCCCCC- -CTAT-GGCT CCTCACCT-- GTTC-TGGGA A----GGGAG GATATGGGGG C-GTATGCT- GGCCTCCC-- GTGTGCCG-- -CGCCGTGCG GATGGCTGAA A-ATTGG-AG
COM VOLA        ATCGCCCCC- -TAT--GGCT CCTCGCCT-- CTTC-TNGGG A----GGGAG TAACTGGTGG C-GTATGCT- GGCCTCCC-- GTGTGCCG-- -CGCCGTGCG GATGGCTGAA A-ATTGG-AG
COM VOLM        ATCGCCCCC- -CTAT-GGCT CCTCACCT-- GTTC-TGGGA A----GGGAG GATATGGGGG C-GTANGCT- GGCCTCCC-- GTGTGCCG-- -CGCCGTGCG GATGGCTGAA A-ATTGG-AG
COM CILI        GTCGCCCCC- -CCGT-GTCC CCTTCACCCC -TTG-CGGGG A----GGGAG GATCTGGGGG GCGTATGTT- GGCCTCCC-- GCTCGCCG-- -CGCCCCGCG GATGGCTGAA A-ATTGG-AG
COM RHAE        ACCGCCCCC- -CTT--TCCT CCTCTCGCCG -TGT-TCGCG T----GAGAG GATCCCGGGG C-GTATGTT- GGCCTCCC-- GCGCGCCT-- -GG-TGTGCG GATGGCAGAA A-ATAGG-AG
COM CALB        GCTCCCCACG CCTCCTGCCC GCCGCCCTGT GGGGCGGGGC C-TGAAGGTT TGTAGGGGGG GCGTACGCT- GGCCTCCC-- GTTCGCCG-- -CGGCGAGCG GATGGCTGAA A-GTCGG-AG
COM APHJ        GTCGCTCCC- -ACG--CCCA TGTTCCGCCC CGGC-GGGGC G-GGAAGGAA GAGTGGGTGC C--TAAGTT- GGCCTCCC-- GTTCGCCT-- -TGCCGCGCG GATGGCTGAA A-ATCGG-AG
COM SCOM        GCCCCCCGCC CCAC--TCCT CTGTCCGCCC CGAT-GGGGA G-GGAAGAAA GCTGGGGGGC C-GTATGCT- GGCCTCCC-- GTTCGCAT-- -CGGCGAGCG GATGGCTGAA A-ATCGG-AG
POL JAPL        GTCGCCCTC- -CCC--GCCT TTGCCTCATC -TTT-TGGGG C----GAGGA GCTGGGGGGA C-GGATGTT- GGTCTCCC-- GTGTGCCT-- -CGGCATGCG GCTGGCTGCA A-ATCACAGG
POL LINL        GTCGCCCCT- -CCC--CTAA TCGCCTCG-- GTCT-CCTGG G----CAA-G GAGTTGGGGG GCGGATGTT- GGTCTCCCGT GTGCGCCTAC TGGGCACACG GCTGGCCGAA A-ATCGCAGG
POL MYRA        GTCGCCCTC- -CAC--GCCC TCGCCTCATC -TTT-GGGGC GAGGAGCTGG G--------G GCGGATGCT- GGTCTCCC-- GTGTGCCT-- -CGGCATGCG GCTGGCTTAA AAGTCACAG-
POL SPJR        GCCCCTCCC- -CCC---CCA TTGCCTTCTT CTCG-GAGGC GGGGAGTC-G G--------G GTGGATGTT- GGTCTCCCGT GTGCACCTAC TGGTCATGCG GCTGGCTGAA A-ATCGCAGG
POL VIRA        GTCGCCCTC- -CCC--GCCC TCGCCTCA-- TTTA-TGGGT C----GAGGA GCCGAGGTGC G--GATGCT- GGTCTCCC-- GTGTGCCG-- -AGTCATGCG GCTGGCTTAA AAATCACAGG
POL EXSR        GTCGCCCCTC CCCC---CAT TGTCTTGGTC -TCC-AGGGC GGGGAGCCAG G--------G GTGGATGTT- GGTCTCCCGT GTGCACCTAT TGGGCATGCG GCTGGCCGAA A-ATTGCAGG
BRE COL         GTCGCCCTC- -CGC--GCCT CCGCCTCATG ATAT-GGCGG C----GAG-G TGGCGGGGGG GCGGATGCT- GGCCTCCC-- GTGTGCCG-- -GGACATGCG GATGGCCGAA A-ATCGGAGG
EPIRADC3        GTCATCCTC- -GTC--TTCT TGGCCTCA-- TTGC-TATGG C----NTG-G TG-ATGGAGG T-AAATGTT- GGTCTCCCAT ATGTTGAG-- -ATACATTTG GATGACTAAA A-TTTGTGGG
SAL CANT        GTCGACCTT- -CAC--TCGC AGGCCATC-- TTTC-GACGG C----GTGCA TAAATGGAGG C-GGACGTT- GGTCTCCCGC GTGTCCCC-- -TT-CACGCG GATGGCTGAA AAATTGTCGG
SAL CILR        GTCGACCTT- -CCC--TTGC AGGTCATC-- TTTC-GATGG C----GTGCA TGGGCGTAGG C-GGACGCT- GGTCTCCCGC GTGTCTTC-- -GT-CACGCG GATGACTGAA AAATTGTCGT
MUR HEIA        GTCGCCCCT- -CCAC-TCCC TTGCCTCA-- AATT-GTGGG A----GAG-G TG-TTGGGGA C-GGTTGAT- GGTCTCCC-- GTGTGCTT-- -AGGCATGTG GATGGCTGAA A-ATCACAGG
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                   1085       1095       1105       1115       1125       1135       1145       1155       1165       1175       1185       1195
BAUHINIA         CCTGTGGTGG CG-AGCACC- ATGACATAC- GGTGGCT--G AGTA---ATG -CTCG--AAG CCAG--TCTT GCGTGCCTCG TCTCGCGCGA TGGCTCTTCT GAC---CCTC A-TGCA-T-C
MEDICAGO         ACCTTGGTAG GG-TGTGCC- ATGATAGAT- GGTGGAT--G TGTG---ACC -CACG--AGA CCAAA-TCAT GTGCTGCTC- TATTGAATGT GGACTCTTTT -AC---CCAC A-TGCG-T-T
CALLISTA         CCCGTGGTGG AC-GACACC- GCGATTAATT GGTGGAT--G AGTGT--ATA TCTCG--AGA CCAA--TCGC GTGTGCC-C- TCTCCCGTGA TCGGGCTTCG -CG---ACCC A-TGCG-T-G
BG7836           TCCGTGGCGC CA-AACACC- GCGTCGCAT- GGTGGAT--G AGGA---AAT -CTCG--AGA CCCG--CCGC GCGTGTT-G- TCACG-GT-- ---CTCTCTC GAC---CCTG T-CGCGCT-G
BG7837           TCCGTGGCGC CA-AACACC- GCGTCGCAT- GGTGGAT--G AGGA---AAT -CTCG--AGA CCCG--CCGC GCGTGTT-G- TCACG-GT-- ---CTCTCTC GAC---CCTG T-CGCGCT-G
XAN OCTB         TCCGTGACGC CG-AAAACC- GCGTCGCAT- GGTGGAT--G AGGA---AAT -CTCG--AGA CCCG--CCGC GCGTGTC-G- TCACG-GT-- ---CTCGCAC GAC---CCCG T-CGCGCT-G
COM DRUM         GTCCCGGCGC CC-TACGCC- GAGTGGAAT- GGTGGAA-AG AGTA---AGA TCTCA--AGA CCGG--ACGC GAGCGTG-G- CCCTG-GC-- ---CTC-CTA GAC---CCCC C-GACG-T-G
COM ACEA         CCCACGGCGC CC-TACGNC- GCGTCAAAT- GGTGGAAGAG TAAA-AAAGA TCTCG--AGA CCGG--ACGC GTGCGTG-G- CCCTG-GC-- ---CTA-CTA GAC---CCCC C-GACG-T-G
COM ERIA         CTCCCGGCGC CC-TACGCC- GCGTCGAAT- GGTGGAA-AG AGTA---AGA TCTCG--AGA CCCG--ATGC GTGCGTG-G- CCATG-GC-- ---CTT-CTA GAC---CCCA --GACG-T-G
COM ESUA         CTCCCGGCGC CC-TACGCC- GCGTCGAAT- GGTGGAA-AG AGTA---AGA TCTCG--AGA CCGG--ATAC GTGCGTG-G- CCATG-GC-- ---CTT-CTA GAC---CCCC --GACG-T-G
COM FLAJ         CTCCCGGCGC CC-TACGCC- GCGTCGAAT- GGTGGAA-AG AGTA-AAGAT -CTCG--AGA CCTG--ACGC GTGCGTG-G- CCCTG-GC-- ---CTC-CTA GAC---CCCC CCGACG-T-G
COM INTB         CTCACGATGC CTTAGCGCC- GCGTCGCAT- GGTGGAT--G ACGC---GAT -CTCT--GGG CCAG--ACGC GTGCGCC-T- TCGTG-GC-- ---CTCCGAT GAC---CCAT A-TGCGCT-G
COM VOL1         CTCACGATGC CTTA-TGCC- GCGTCGCAT- GGTGGAT--G AGGC---GAT -CTCT--GGA CCGG--ACGC GTGCGTT-T- T-GTG-GA-- ---CTCC--- ---------- ----------
COM SPIG         CTCACGATGC CTTA-TGCC- GCGTCGCAT- GGTGGAT--G AGGC---GAT -CTCT--GGA CCGG--ACGC GTGCGTT-T- CTGTG-GC-- ---CTCCGAC GAC---CCAT A-TGTGCT-G
COM VOL2         CTCACGATGC GTTC-TGCC- GCGTCGCAT- GGTGGAT--G AGGC---GAT -CTCT--GGA CCGG--ACGC GTGCGTT-T- T-GTG-GC-- ---CTC---- ---------- ----------
COM VOLA         CTCACGATGC CTTA-TGCC- GCGTCGCAT- GGTGGAT--G AGGC---GAT -CTCT--GGA CCGG--ACGC GTGCGTT-T- T-GTG-GA-- ---CTCCGAT GAC---CCAA A-TGTGCT-G
COM VOLM         CTCACGATGC GTTC-TGCC- GCGTCGCAT- GGTGGAT--G AGGC---GAT -CTCT--GGA CCGG--ACGC GTGCGTT-T- T-GTG-GC-- ---CTCCTAC GAC---CCAA A-TGTGCT-G
COM CILI         CTCACGACGC CTTA-CGCC- GCGTCGCAT- GGTGGAC--G AGTT---GAT -CTCT-TGGA CCGG--ACGC GTGCGTG-T- TTGTG-GC-- ---CTCCGAC GACGACCCAT A-TGTGCT-G
COM RHAE         CTCACGGCGC CTTT-AGTT- GCGTCGCAT- GGTGGAT--G AGGC---GAT -CTCT-AAGA CCAG--ATGC TTGTGCT-T- CCGTG-GC-- ---CTCCCAC GAC---CCAC ---GTG-T-G
COM CALB         CCCTTGGTGT CTCA-CGCC- GCGCCACAT- GGTGGAT--G GGGT---CAT -CTCT--AGA CCGG--ACGC GCGTGTT-G- CCCTG-GC-- ---CCC-CTT GGA---CCCC G-CACG-T-G
COM APHJ         CTCACGGTGT GTTT-TGCC- GCGTCGCAT- GGTGGAT--G ATTC---GAT -CCCT--AGA CCCG-GACGC GTGTGTT-G- CC-CGCGG-- ---CTC-CCT GTA---CCCC C-TACCCTTG
COM SCOM         CTCACGGCGA CTTT-CGCC- GCGTCGCAT- GGTGGAT--G AGTC---GAT -CTCT--AGA CCCG-GACGC GTGTGTT-G- CC-CGGGG-- ---CTCCCAG GAC---CCCT G-CCCG-T-G
POL JAPL         ACCACGGTGC AC-AACGCC- GCGTCGCAT- GGTGGAC--G AGGT---AAT -CTCT-AAGA CCGG--ACGC GTGCGTC-G- CCGTT-GC-- ---CTGACAG GAC---CCAC --TGCGCT-G
POL LINL         ACCACGGTGC CA-AACGCC- GCGTCGCAA- GGTGGAT--G AGGT---AAT -CTCC-TAGA CCGG--ACGT GCGCTTC-G- CCGTT-GC-- ---CTAGACA GGA---CCCA --AA-G-C-G
POL MYRA         ACCACGGTGC AG-AACGCC- GCGTCGCAT- GGTGGAC--G AGGT---AAT -CTCT-AAGA CCGG--ACGC GCGCTTC-G- CCGTT-GC-- ---CTATCAG GAC---CCAC --CGCGCT-G
POL SPJR         ACCAAGGCGT CG-AACGCC- GCGTCGCAT- GGTGGAT--G AGGT---AAT -CTCC-AAGA ACGG--ACGC GCGCGTC-G- CCGTT-GC-- ---CTAGACA TGA---CCCA --AT-G-C-G
POL VIRA         ACCACGGTGC AG-AACGCC- GCGTCGCAT- GGTGGAC--G AGGT---AAT -CTCT-AAGA CCGG--ACGC GTGCTTC-G- CCGTT-GC-- ---CTATCAG GAC---CCAC --CGCGCT-G
POL EXSR         ACCACGGTGC CA-AACGCC- GCGTCGCAT- GGTGGAT--G AGGT---AAT -CTCC-AAGA CCGG--ACGC GCGCACCAC- CGTTGCCT-- ---ATAGTCA TGA---CCCA --AT-G-C-G
BRE COL          CCCACGGTGT CG-AACGCC- GCGTCCAAT- GGTGGAT--G GGGC-GACCT -CTCCGAAGA CCGG--ACGC GTGCGAC-G- CCGCG-GC-- ---CTCGCAC GGC---CCCG A-TGCGCT-G
EPIRADC3         ACTACAAGTT TA-GGTGCC- ATGTCGTAT- GTTGGAT--G GAAA---AAT -CTCT-ACGA TTGG--ATGT GAGCGTT-G- T---T-GT-- ---CTT-CAT GAT---CTTG ---ATG-T-G
SAL CANT         ACCTCGGCGC GCGAGTGCGT GCGTGTCAC- GTTGGAC-GG ACGA-AACGT -CTAA--AGA TCTA--AGGC ATCGCGCACG TC-GCCGA-- ---CGCGTC- -AC---CTCG ---ATC-T-G
SAL CILR         AACTCGGCGC AAGAGTGCGT GCATGTCAC- GGTGGAC-GG AGAA---AAC -GTCT--GGA GCTA--AGGC ATCGCGCACG TC-GCCGA-- ---CGC--TT GAC---CTCG ---ATC-T-G
MUR HEIA         ACCACGGTGA TG-AACGCC- GCGTCGCAT- GGTGGAT--G AGGA---AAA -CTCT-AAGG CCGG--ACGT GTGCGTC-G- CCGTG-GC-- ---CTTGCAC GAC---CTCG A-TGCGCT-G
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            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
              1205       1215       1225       1235       1245       1255       1265       1275       1285       1295       1305       1315
BAUHINIA    GTGA------ ACACGATGCT CTCA-ACGC- GA???????? ?????????? ?????????? ---------- ---------- ---------- ---------- ---------- ----------
MEDICAGO    TTGT------ A-ACGCT--- ---------- ---------- ---------- ---------- CGTG?????? ?????????? ?????????? ?????????? ?????????? ??????????
CALLISTA    CCGT------ CAAGGCA--- ---------- ---------- ---------- ---------- C--------- ---------- ---------- ---------- ---------- ----------
BG7836      CTGG------ TAGCGC--CT TTCG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BG7837      CTGG------ TAGCGC--CT TTCG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
XAN OCTB    CCGG-CC-GG TAGCGC--CT TTCG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM DRUM    CTGC-GA-TG CAGCGC--CC CACG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ACEA    CTGC-GA-TG CAGCGC--CC CACG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ERIA    CTGC-GA-TG CAGTGC--CT CACG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM ESUA    CTGC-GA-TG CAGTGC--CT CACG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM FLAJ    CAGC-GA-TG CATCGC--CC CATG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM INTB    CGAT------ GCAGCATGCT CG-G-ATGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL1    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SPIG    CGAT------ GCAGTG--CC CTCAGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOL2    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLA    CAAT------ GCAGTG--CG CT-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM VOLM    CGAT------ GCAGTG--CG CTCGGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM CILI    CGAT------ GCAGTG--CG CTCGGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM RHAE    CTGT-GA-TG CAGCGC--CC TTCG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM CALB    CTGA-GT-TG CAGCGC--CC TTCT-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM APHJ    CTGC-GA-CG CAGCGC--CC CTCG-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
COM SCOM    CTGT-GA-TG CAGCGC--CC CTCG-ACGC- G--------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL JAPL    CGAT------ GCAGTG--CA CTCGGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL LINL    CTGC-GT-TG CAGTGC--CA CTCTGATGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL MYRA    CTGT------ GCAGTG--CA CTCCGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL SPJR    CTGC-AT-TG CAGTGC--CA CTCTGATGCC GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL VIRA    CTGT------ GCTGTG--CA CTCCGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
POL EXSR    CTGC-GT-TG TAGTGC--CA CTTGGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
BRE COL     CTCG------ GCAGTG--TC CTCCGATGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
EPIRADC3    CTGC-AT-TG CAGTTC--CT CTTC-ACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SAL CANT    CCGCTAT-TG CGGCGC--CC CATCGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SAL CILR    CCGC-ATCTG CGGCGC--CC CATCGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
MUR HEIA    CTAT------ GCAGTG--TC CTCCGACGC- GA-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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POA-aligned ITS sequences (continued)

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             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|...
                  1325       1335       1345       1355       1365       1375       1385       1395       1405
BAUHINIA     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------
MEDICAGO     ?????????? ????------ ---------- ---------- ---------- ---------- ---------- ---------- --------
CALLISTA     ---------- ----CC---- ---------- ---------- ---------- ---------- ---------- ---------- --------
BG7836       ---------- ------CCCC AGGTCAGGCG GGACTACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
BG7837       ---------- ------CCCC AGGTCAGTCG GGACTACCCG -CTGA-GTTT AAGC-ATATC AAT------- ---------- --------
XAN OCTB     ---------- ------CCCC AGGTCAGGCG GGACTACCCG -TTGA-GTTT AAGC-ATATC AATAAG-CGG AGG------- --------
COM DRUM     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -ATGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAA---- --------
COM ACEA     ---------- ------CCCC AG-TCAGGCG C-ACCACCCG ACTGA-GTTC ATACTATATC ACTCGGACGC AGGAAAAG-- --------
COM ERIA     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAG---- ---------- --------
COM ESUA     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAGGG --------
COM FLAJ     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAAGCGG AGGAAAAG-- AA------
COM INTB     ---------- ------CC-- ---------- ---------- ---------- ---------- ---------- ---------- --------
COM VOL1     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------
COM SPIG     ---------- ------CCCC AGGTCAGGCG GGAACACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AG-------- --------
COM VOL2     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------
COM VOLA     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------
COM VOLM     ---------- ------C--- ---------- ---------- ---------- ---------- ---------- ---------- --------
COM CILI     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
COM RHAE     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGG------- --------
COM CALB     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -GTGAAGTTT AA-------- ---------- ---------- --------
COM APHJ     ---------- ------CCCC AGGTCAGGCG GGAGCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
COM SCOM     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------
POL JAPL     ---------- ------CCCC AGGTCAGGCG GGATCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAGG- --CGGGG-
POL LINL     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AG-------- --------
POL MYRA     ---------- ------CCCC AGGTCAGGCG GGATCACCC- ---------- ---------- ---------- ---------- --------
POL SPJR     ---------- ------CCCC AGGTCAGGTG GTACCACCCC GCTGA-GTT- ---------- ---------- ---------- -------A
POL VIRA     ---------- ------CCCC AGGTCAGGCG GGATCACCCG -CTGA-GTTT AAGC-ATATC A--------- ---------- --------
POL EXSR     ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AA-------- ---------- ---------- --------
BRE COL      ---------- ------CCCC AGGTCAGGCG GGACCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGG------- --------
EPIRADC3     ---------- ------CCTC AGGTCAGGCG AGATCACCTG -CTGA-GTT- AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
SAL CANT     ---------- ------CCCC AAGTCAGGCG GGATCACCCG -CTGA-GTNT AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
SAL CILR     ---------- ------CCCC AAGTCAGGCG GGATCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
MUR HEIA     ---------- ------CCCC AGGTCAGGTG GGATCACCCG -CTGA-GTTT AAGC-ATATC AATAAG-CGG AGGAAAAG-- --------
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[                              1         11        21        31        41        51        61        71        81        91        101       111          ]
[                              |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine            AATTGGATTGAGCCTTGG-TACGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Xanthophyllum_arnottianum      AATTGGATTGAGCCTTGG-TACGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Xanthophyllum_octandrum        --------------------------------------------------------------------------------?--------------------------------?-?----
Xan_octBG7834                  AATTGGATTGAGCCTTGG-TACGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGGTTTCCGAAAA1C1CAAA
Xan_fraBG7836                  AATTGGATTGAGCCTTGG-TACGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Medicago_lupulina              AATTGGATTGAGCCTTGG-TATGGAAACTTACCAA-GTGAAAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGG0-CAATCCTGAGCCAAATCCTTCTTTCCGAAAA1C1TAAA
Guilfoylia_monostylis          AA?CGTATTGAGCCTTGG-TACGGAARCCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCGAAAA0-1CAAA
Phylica_polifolia              --------CGAGC-TTGG-TATGGAAAC-TA-?AA--TGATAACTTTCAAATTCAGAGAAA-CCTGGAATTACAAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCTGAAAA0-1CAAA
Albizia_berteriana             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCTGTTTTACGAAAA0-1CCAA
Senna_pleurocarpa              --TTGGATTGAGCCTTGGGTATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCGAAAA0-1CCAA
Callistachys_lanc              ---------------------TGGAAACTTACCAA-GTGAAAACTTTCAAATTCAGAGAAACCCCGGAATTCACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA0-1CAAA
Dryas_drummondii               ---------------TGG-TATGG--CCGTACCAGTGTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGG0-CAATCCTGAGCCAAATCCTGTTTTATGAAAA0-1CAAA
Quillaja_saponaria             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCGATTTTCCGAAAA0-1CAAA
Recchia_mexicana               AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGCACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCAAAAA0-1CAAA
Fagus_sylvatica                AATTGGAT-GAGC-TTGG-CATGGAAACTTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGG0-CAATCCTGAGCCAAATCCTATTTTCCGAAAA0-1CAAA
Monnina_insignis               AAWTGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGATAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCAAAAA1A0----
Monnina_malmeana               AA?TGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGATAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCAAAAA1A0----
Atroxima                       -ATCGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Balgoya_pacifica               --------------------------------------------------------------------------------?--------------------------------?-?----
Barnhartia                     --------------------------------------------------------------------------------?--------------------------------?-?----
Bredemeyera_altissima          -------TTGAGCCTTGG-TATGGAAACCTATCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1AAAA
Bredemeyera_colletioides       AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGCTTTCCGAAAG1C1CAAA
Bre_collLM241                  AATTGGAT-GAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCGTGAGCCAAATCCCGCTTTCCGAAAG1C1CAAA
Bredemeyera_floribunda         AATTGGATTGAGCCTTGG-TATGGAAACCTATCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Bredemeyera_lucida             AATTGGATTGAGCCTTGG-TATGGAAACCTATCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Bredemeyera_microphylla        AATTAGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCAAAAA1A1CAAA
Bre_papNGF33220                AATTGGATTGAGCCTTGG-TATGGAAACCTACCGAGTGAGTAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Carpolobia_alba                ATTCGGATTGAGCCTTGG-C?TGGAAACCTCCCAA-GTGAGAACTTTCAAMTTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
'Carpolobia_aff._goetzii'      --------------------------------------------------------------------------------?--------------------------------?-?----
Comesperma_calymega            --------------------------------------------------------------------------------?--------------------------------?-?----
Comesperma_hispidulum          AGTTAGATTGAGCCTTGG-TATGGAAACTTACCGA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCGAAAA1C1CAAA
Comesperma_secundum            AGTTAGATTGAGCCTTGG-TATGGAAACTTACCGA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCGAAAA1C1CAAA
Com_eriAM102                   AGTTAGATTGAGCCTTGG-TATGGAAACTTACCGA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCTTTTTTACGAAAA1C1CAAA
Com_scoMDC9172                 AATTAGATTGAGCCTTGG-TATGGAAACCTACCGA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCTAAAA1A1AAAA
Com_volAM88                    ---------------------------------------G--ACTTT-AAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Com_rhaEAG8343                 -------TTG-GCCTTGG-T-TGGAAACCTACCGA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCAGTTTTCCGAAAA1C1CAAA
Diclidanthera_bolivarensis     --------------------------------------------------------------------------------?--------------------------------?-?----
Diclidanthera_pendulifera      AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A1CAAA
Monnina_crassifolia            AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCG?AAA1A0----
Monnina_phillyreoides          AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A0----
Monnina_hirta                  TATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A0----
Moutabea_aculeata              ----------------------------------------GAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Moutabea_excoriata             --TTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Moutabea_guianensis            ----TCCTTGAGC?TTGG-TATGGAAACCTACCAA-CTGAAAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1AAAA
'Muraltia_aff._alopecuroides'  AATTGGATTGAGCTTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCAAAAA1A1TAAA
Muraltia_heisteria             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A1TAAA
Muraltia_mixta                 AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A1TAAA
Mur_heiAD                      AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A1TAAA
Nylandtia                      AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Acanthocladus_guayaquilensis   AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAATAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAG
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trnL/F alignment, including indels

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[                               1         11        21        31        41        51        61        71        81        91        101       111       ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Polygala_acuminata              AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAGCAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCGAAAA1C1CAAA
Polygala_arillata               AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCATTTTCCGAAAA1C1CAAA
Polygala_chamaebuxus            AATTGAATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCACTTTCCGAAAA1A1TAAA
Polygala_cuneata                AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_erioptera              AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATGCCGTTTTCCGAAAA1C1AAAA
Polygala_floribunda             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_fuertesii              AATTGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_hebeclada              AATTGGATTGAGCATTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAGCAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCCGAAAA1C1CAAA
Polygala_klotzschii             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAG
Polygala_lutea                  AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTTCTAAAA1T1CAAA
Polygala_macradenia             AATTGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_microphylla            AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCTTTTTCCGAAAA1C1CAAA
Polygala_obscura                AATTGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCCAAAA1T1CAAA
Polygala_oleifolia              AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_lindheimeri            AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_penaea                 AATTGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_rivinifolia            AATTGGATTGAGCCTTGG-TATGGAAACCTACTAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG1GCAATCCTGAGCCAAATCCCGTTTTCCGAAAA1T1CAAA
Polygala_semeiocardium          ----GTATTGCCCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCATTTTCCGAAAA1C1CAAA
Polygala_senega                 AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGGTTTCCTAAAA1T1CAAA
Polygala_subspinosa             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Polygala_tatarinowii            AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCTCATTTTTTGAAAA1C1CAAA
Polygala_violacea               AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAGCAATGGG0-CAATCCTGAGCCAAATCCTGTTTTCTGAAAA1C1CAAA
Polygala_vulgaris               --------------------------------------------------------------------------------?--------------------------------?-?----
Pol_exsRKH804                   AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Pol_japLMC2903                  AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGGTTTCCGAAAA1C1CAAG
Monnina_dictyocarpa             AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A0----
Monnina_leptostachya            AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A0----
Monnina_pterocarpa              AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCCGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1A0----
Salomonia_cantoniensis          TATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGCACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCATTTTCCGAAAA1C1CAAA
Salomonia_ciliata               AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGCACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCATTTTCCGAAAA1C1CAAA
Sal_cilRKH1023                  AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGCACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCATTTTCCGAAAA1C1CAAA
Securidaca_diversifolia         AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATGCCGTTTTCCGAAAA1C1CAAA
Securidaca_longifolia           --------------------------------------------------------------------------------?--------------------------------?-?----
Securidaca_virgata              AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA
Securidaca_welwitschii          AATTGGATTGAGCCTTGG-TATGGAAACCTACCAA-GTGAGAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGG0-CAATCCTGAGCCAAATCCCGTTTTCCGAAAA1C1CAAA

[                               121       131       141       151       161       171       181       191       201       211       221       231       ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine             GAAG1AG0-TTCAGAAAGCGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AACGGCATT-------0GCATT-------AG------
Xanthophyllum_arnottianum       GAAG1AG0-TTCAGAAAACGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AACGGCATT-------0GCATT-------AG------
Xanthophyllum_octandrum         ----?--?-------------------------------------------------------------------------------------------?--------------------
Xan_octBG7834                   GAAG1AG0-TTCAGAAAACGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AACGGCATT-------0GCATT-------AG------
Xan_fraBG7836                   GAAG1AG0-TTCAGAAAACGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AACGGCATT-------0GCATT-------AG------
Medicago_lupulina               AAAA1AG0-TTCAGAAAGTGAGGATAGGTGCAGAGACTCGATGGAAGCTGTTCTAACAAA----------TGGAGTT------GACAACATT-------0--------------------
Guilfoylia_monostylis           GAAG1AG0-TTCAGAAAGCGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------GACGGCATT-------0GCGTT-------AG------
Phylica_polifolia               TAAG1GA0-TTCAGAAAGTGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------GGCTGCGAT-------0GCGTT-------AG------
Albizia_berteriana              GAAG1AG0-TTCAGAAAGGGAGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAACAAA----------TGGAGTT------GACGACATTTCGTTTC0TCGTT-------AG------
Senna_pleurocarpa               GAAG1AG0-TTCAGAAAGGGAGGATAGGTGCAGAGACTCAACGGAAGTTGTTCTAACAAA----------TGGAGTT------GACGACATT-------0TCGTTTCGTT--AG------
Callistachys_lanc               GAAA1AG0-CTCAGAAAGCGATGATAGGTGCAGAGACTCAATGGAAGCTGTTCAAACAAA----------TGGAGTT------GACAACATTTCGTTTT0CTACT-------AGGA----
Dryas_drummondii                CAAG1GG1TTTCAGAAAGCGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------GGCTGCATT-------0GTGTT-------AG------
Quillaja_saponaria              GAAG1AG0-TTCAGAAAGCGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------GACGGCATT-------0GCGTT-------AG------
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trnL/F alignment (continued)

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[                             121       131       141       151       161       171       181       191       201       211       221       231       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Recchia_mexicana              GAAG1AG0-TTCAGAAAGCGAGGATAGGTGCAGAGACTCAATGGAAGATGTTCTAAAGAA----------TGGAGTT------GACGGCATT-------0GCGTT-------AG------
Fagus_sylvatica               TAAG1GG0-TTGAGAAGAAAGGGTTTGGTGCAGAGACTCAATGGAAGATGTTCAAACAAATGGGG-----TGGACTT----CGTACGTTATT-------0AAATT-----CAAG------
Monnina_insignis              ----?--?-------------GGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGACATT-------0GCATT-------AG------
Monnina_malmeana              ----?--?-------------GGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGACATT-------0GCATT-------AG------
Atroxima                      GAAG1AG0-TTCAGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Balgoya_pacifica              ----?--?-------------------------------------------------------------------------------------------?--------------------
Barnhartia                    ----?--?-------------------------------------------------------------------------------------------?--------------------
Bredemeyera_altissima         GAAG1AG0-TTCGGCAAGAGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATAGCACT-------0GCATT-------AG------
Bredemeyera_colletioides      GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Bre_collLM241                 GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Bredemeyera_floribunda        GAAG1AG0-TTCGGCAAGAGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCATT-------AG------
Bredemeyera_lucida            GAAG1AG0-TTCGGCAAGAGAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCATT-------AG------
Bredemeyera_microphylla       GAAG1AG0-TTCGGCAAAAGGGGATAGGTGCAGAGACTCAACGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCGCG-------0GCATT-------AG------
Bre_papNGF33220               GAAG1GG0-TTCGGAAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCGAACAAA----------TGGAGTG------AATGGAATT-------0GCATT-------AG------
Carpolobia_alba               GAAG1AG0-TTCAGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCATT-------0GCATT-------AG--ATTA
'Carpolobia_aff._goetzii'     ----?--?-------------------------------------------------------------------------------------------?--------------------
Comesperma_calymega           ----?--?-------------------------------------------------------------------------------------------?--------------------
Comesperma_hispidulum         GAAG1GG0-TTCGACAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGG-----------1--------------------
Comesperma_secundum           GAAG1GG0-TTCGACAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGG-----------1--------------------
Com_eriAM102                  GAAG1GG0-TTCGACAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGG-----------1--------------------
Com_scoMDC9172                GAAG0-G0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCGTT-------0GCATT-------AG------
Com_volAM88                   GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Com_rhaEAG8343                GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Diclidanthera_bolivarensis    ----?--?-------------------------------------------------------------------------------------------?--------------------
Diclidanthera_pendulifera     GAAT1AG0-TTCAGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCATT-------0GCATT-------AG------
Monnina_crassifolia           ----?--?------------AGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Monnina_phillyreoides         ----?--?------------AGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Monnina_hirta                 ----?--?------------AGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Moutabea_aculeata             GAAG1AG0-TTCAGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCATT-------0GCATT-------AG------
Moutabea_excoriata            GAAG1AG0-TTCAGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCATT-------0GCATT-------AG------
Moutabea_guianensis           GGAG1GG0-TTTAGCCAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCATT-------0GCATT-------AG------
'Muraltia_aff._alopecuroides' GAAG0-G0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGTATT-------0GCATT-------AG------
Muraltia_heisteria            GAAG0-G0-TTCGGCAATAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGTATT-------0GCATT-------AG------
Muraltia_mixta                GAAG0-G0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGTATT-------0GCATT-------AG------
Mur_heiAD                     GAAG0-G0-TTCGGCAATAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGTATT-------0GCATT-------AG------
Nylandtia                     GAAG0-G0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------ACTGGCATT-------0GCATT-------AG------
Acanthocladus_guayaquilensis  GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCATT-------AG------
Polygala_acuminata            GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCTTT-------0GCGTT-------AG------
Polygala_arillata             GACG1GG0-TTCGGAAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AACGGCATT-------0GCATT-------AG------
Polygala_chamaebuxus          GACG1GG0-TTCGGCACGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGAATT-------0GCATT-------AG------
Polygala_cuneata              GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAATAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Polygala_erioptera            GAAG1GG0-TTTGGGAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GTATT-------AG------
Polygala_floribunda           GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCTTT-------0GCGTT-------AG------
Polygala_fuertesii            GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCGTT-------AG------
Polygala_hebeclada            GAAG1GG0-TTCGGAAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCTTT-------0GCGTT-------AG------
Polygala_klotzschii           GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCATT-------AG------
Polygala_lutea                TTAA1GG1ATTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Polygala_macradenia           GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTATTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCGTT-------AG------
Polygala_microphylla          GAAG1GG0-GTCGGGAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Polygala_obscura              GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCGTT-------AG------
Polygala_oleifolia            GAAG1GG0-TTCGGCAAGAAGGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TTGAGTG------AATGGCATT-------0GCATT-------AG------
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trnL/F alignment (continued)

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[                               121       131       141       151       161       171       181       191       201       211       221       231       ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Polygala_lindheimeri            GAAA1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAATAAA-----CGGAATGGAGTG------AATGGCATT-------0GCATT-------AG------
Polygala_penaea                 GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCGTT-------AG------
Polygala_rivinifolia            GAAG1AG0-TTCGGCAAGAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTT------AATGGCACT-------0GCGTT-------AG------
Polygala_semeiocardium          GACG1GG0-TTCGACAATTAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Polygala_senega                 TAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGACATT-------0GCATT-------AG------
Polygala_subspinosa             GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAATAAA----------TGGAGTGAGTG--AATGGCATT-------0GCATT-------AG------
Polygala_tatarinowii            GACG1GG0-TTCGGCAAGAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Polygala_violacea               GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAATG------AATGGCTTT-------0GCGTT-------AG------
Polygala_vulgaris               ----?--?-------------------------------------------------------------------------------------------?--------------------
Pol_exsRKH804                   GAAG1GG0-TTCGGTAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Pol_japLMC2903                  GAAG1GG0-TTCGGGAAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Monnina_dictyocarpa             ----?--?------------AGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Monnina_leptostachya            ----?--?------------AGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Monnina_pterocarpa              ----?--?------------AGGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Salomonia_cantoniensis          GAAG1GG0-TTCCGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCGAACAAA----------TGGAGTG------AATGGAATT-------0GCATT-------AG------
Salomonia_ciliata               GAAG1GG0-TTCCGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCGAACAAA----------TGGAGTG------AATGGAATT-------0GCATT-------AG------
Sal_cilRKH1023                  GAAG1GG0-TTCCGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCGAACAAA----------TGGAGTG------AATGGAATT-------0GCATT-------AG------
Securidaca_diversifolia         GAAG1GG0-TTCGGTAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Securidaca_longifolia           ----?--?-------------------------------------------------------------------------------------------?--------------------
Securidaca_virgata              GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------
Securidaca_welwitschii          GAAG1GG0-TTCGGCAAGAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAA----------TGGAGTG------AATGGCATT-------0GCATT-------AG------

[                               241       251       261       271       281       291       301       311       321       331       341       351       ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine             -TAAAAG------1AATCCTTCCATCG0-AAACT-----CCC0-----AAAAAGATCAAGGATGAACG0------------1TATATA1CGTG---------------------------
Xanthophyllum_arnottianum       -TAAAAG------1AATCCTTCCATCG0-AAAGT-----CCC0-----AAAAAG-TCAAGGATGAACG1TATAGATGAACG1TATATA1CGTG---------------------------
Xanthophyllum_octandrum         -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Xan_octBG7834                   -TAAAAG------1AATCCTTCCATCG0-AAAGT-----CCC0-----AAAAAG-TCAAGGATGAACG1TATAGATGAACG1TATATA1CGTG---------------------------
Xan_fraBG7836                   -TGAAAG------1AATCCTTCCATCG0-AAACT-----CCC0-----AAAAAGATCAAGGATGAACG1TATAGATGAACG1TATATA1CGTG---------------------------
Medicago_lupulina               -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Guilfoylia_monostylis           -TAAGGG------1AATCCTTCCATCG0-AAACT-----CCG0-----GAAAAGA--AAGGATCAACC0------------1TATATA1CATA---------------------------
Phylica_polifolia               -TAAAGG------1AATCCTTCCATCG0-AAATT-----CCA0-----GAAAGGAGGAAGGATAAACG0------------1TATATA0--TA---------------------------
Albizia_berteriana              -TAAGGA------1GATCCTTCCATCG0-AAACT-----CCA1GAAAAGAAAGGATCAAGGATGAG--?------------?------1CATA---------------------------
Senna_pleurocarpa               -TAAAGG------1AATCCTTCCATCG0-AAACT-----CCA1GAAAAGAAAGGATCAAGGATGAACA0------------1TATATA0--TA---------------------------
Callistachys_lanc               -AAGAGG------1AATCCTTCCATAA0-AAACT-----TCA0-----GAAAGAATCAAGGAGAAACA0------------1TAGATA1TATATCTATACATATGTCTAGTA--------
Dryas_drummondii                -TAAAGG------1AATCCTTACATCG0-AAACT-----TCC0-----GAAAGGATGAAGGATAAACG0------------1TATATA1CATA---------------------------
Quillaja_saponaria              -TAAAGG------1AATCCTTCCATCG0-AAACT-----CCA1AAAAAGAAAGGATCAAGGATGAACC0------------1TATATA1CATA-------------------TGATA---
Recchia_mexicana                -TAAAGG------1AATCCTTCCATCA0-AAACT-----CCA1GAAAAGAAAGGATCAA-------CT0------------1TATATA1CATA---------------------------
Fagus_sylvatica                 -T-----------1AATCCTTCTATCA0-AAACT-----ACA0-----GAAAGCAT-AAGGATAAACC0------------1TATAAA1CATA---------------------------
Monnina_insignis                -TAAAAG------0-----TTCCATCT0-AAACT-----CCC0-----GAAAAAATCGAGGATGAATG0------------1TATATA1TATA---------------------------
Monnina_malmeana                -TAAAAG------0-----TTCCATCT0-AAACT-----CCC0-----GAAAAAATCGAGGATGAATG0------------1TATATA1TATA---------------------------
Atroxima                        -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------1TATATA1CATA---------------------------
Balgoya_pacifica                -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Barnhartia                      -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Bredemeyera_altissima           -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Bredemeyera_colletioides        -TAAAAG------0-----TTCCATGG0-AAACT-----CTC0-----GAAGAGATCGAAGATGGACG0------------1TCTATA1CATA---------------------------
Bre_collLM241                   -TAAAAG------0-----TTCCATGG0-AAACT-----CTC0-----GAAGAGATCGAAGATGGACG0------------1TCTATA1CATA---------------------------
Bredemeyera_floribunda          -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Bredemeyera_lucida              -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Bredemeyera_microphylla         -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1GATA---------------------------
Bre_papNGF33220                 -TAAAAG------0-----TTCCATCG0-AAATT-----CCC0-----TAAAAGATCGAGGATGAACG0------------1TATATA1CATA---------------------------
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[                             241       251       261       271       281       291       301       311       321       331       341       351       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Carpolobia_alba               GTAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------1TATATA1CATA---------------------------
'Carpolobia_aff._goetzii'     -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Comesperma_calymega           -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Comesperma_hispidulum         -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Comesperma_secundum           -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Com_eriAM102                  -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Com_scoMDC9172                -GAAAAG------0-----TTCCCTCG0-AAACT-----CCT0-----AAAAAGATCGAGGATGAACG0------------1CATATG1CATA---------------------------
Com_volAM88                   -TAAAAG------0-----TTACATCG0-AAATT-----CCC0-----GAAAAGATCGAGGATGAACA0------------1TATATA1CATA---------------------------
Com_rhaEAG8343                -TAAGAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCGAGGATGAACA0------------1TATATA1CATA---------------------------
Diclidanthera_bolivarensis    -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Diclidanthera_pendulifera     -TAAGAG------0-----TTCCACCG0-AAACT-----CCC0-----GAAAAGATCAAGGATGACCA0------------1TATATA1CATA---------------------------
Monnina_crassifolia           -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAAATCGAGGWTGAACG0------------1TATATA1CATA---------------------------
Monnina_phillyreoides         -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAAATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Monnina_hirta                 -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----TAAAAAATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Moutabea_aculeata             -TAAAAG------0-----TTC-ATCG0-AAACT-----CCC0-----AAAAAGATCAAGGATGACCG0------------1TATATA1CATA---------------------------
Moutabea_excoriata            -TAAAAG------0-----TTC-ATCG0-AAACT-----CCC0-----AAAAAGATCAAGGATGACCG0------------1TATATA1CATA---------------------------
Moutabea_guianensis           -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCAAGGATGACCG0------------1TATATA1CATA---------------------------
'Muraltia_aff._alopecuroides' -TAAAAG------0-----TTACATCG1GAAACT-----CTC0-----GAAAAGATCGAAGATGAACG0------------1TATATA1CATA------------------------CAT
Muraltia_heisteria            -TAAAAG------0-----TTACATCG1GAAACT-----CCC0-----GAAAAGATCGAAGATGAACG0------------1TATATA1CATA---------------------------
Muraltia_mixta                -TAAAAG------0-----TTACATCG1GAAACT-----CCC0-----GAAAAGATCGAAGATGAACG0------------1TATATA1CATA---------------------------
Mur_heiAD                     -TAAAAG------0-----TTACATCG1GAAACT-----CCC0-----GAAAAGATCGAAGATGAACG0------------1TATATA1CATA---------------------------
Nylandtia                     -TCAAAG------0-----TTACATCG1GAAACT-----CCC0-----GAAAAGATCGAAGATGAACG0------------1TATATA1CATA---------------------------
Acanthocladus_guayaquilensis  -TAAAAG------0-----TTCTATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Polygala_acuminata            -TAAAAG------0-----TTCCATCG0-AAATT-----CCC0-----AAAAACATCGAAGATAAACA0------------1TATATA1CATA---------------------------
Polygala_arillata             -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Polygala_chamaebuxus          -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Polygala_cuneata              -TAAAAATAAAAA0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Polygala_erioptera            -TCAAAG------0-----TTCCATCG0-AAATT-----TCC0-----GAAAAGATCGAGGATGAACG0------------1TATATA1T?TA---------------------------
Polygala_floribunda           -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCGAAGATGAACG0------------1TATATA1CATA---------------------------
Polygala_fuertesii            -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Polygala_hebeclada            -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAACATCGAAGATAAACA0------------1TATCTA1CATA---------------------------
Polygala_klotzschii           -TAAAAG------0-----TTCTATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Polygala_lutea                -TCAAAG------0-----TTCGATCT0-AAACT-----ACT0-----AAAAAGATCGAGTATGAACG0------------1TATATA1CATA---------------------------
Polygala_macradenia           -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGCACG0------------0----TA1CATA---------------------------
Polygala_microphylla          -TAAAAG------0-----TTCGATCG0-AAACT-----CCC0-----GAAAAAATCGAGGATGAACT0------------1TATATA1CATA---------------------------
Polygala_obscura              -TAAAAG------0-----TTCCGTCA0-AAACT-----CCT0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Polygala_oleifolia            -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGCTCGAGGATGAACG0------------1TATATA1CATA---------------------------
Polygala_lindheimeri          -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Polygala_penaea               -TAAAAG------0-----TTCCATCA0-AAACT-----CCC0-----GAAAAGATCAAGGATGAACG0------------0----TA1CATA---------------------------
Polygala_rivinifolia          -TAAAAG------0-----TTCCATCA0-AAACT-----CCT0-----GAAAAGATCAAGGATGAATG0------------0----TA1CATA---------------------------
Polygala_semeiocardium        -TAAAAG------0-----TTCCATCG1GAAACT-----CCT0-----GAAAAGATCGAGGATAAACG0------------1TATATA1CATA---------------------------
Polygala_senega               -TCAAAG------0-----TTCGATCT0-AAACT-----CCT0-----AAAAAGATCGAGTATGAACG0------------1TATATA1CATA---------------------------
Polygala_subspinosa           -TAAAAG------0-----TTCCATCG0-AAACTAAACTCCC0-----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Polygala_tatarinowii          -TAAAAG------0-----TTCCATTG0-ATCCT-----CCC0-----TAAAAGATTGAGGATAAACG0------------1TATATA1CATA---------------------------
Polygala_violacea             -TAAAAG------0-----TTCCATCG0-AAACT-----CCT0-----GAAAAGATCGAAGATAAACA0------------1TATATA1CATA---------------------------
Polygala_vulgaris             -------------?-------------?--------------?-------------------------?------------?------?-------------------------------
Pol_exsRKH804                 -TAAAAG------0-----TTCTATTG0-AAACT-----CCA0-----GAAAAGATCGAGGATGAACC0------------1TATATA1CATA---------------------------
Pol_japLMC2903                -TAAAAG------0-----TTACATCG0-AAACT-----CCC0-----GAAAAGATCGAGGATGAACT0------------1TATATA1CATA---------------------------
Monnina_dictyocarpa           -TAAAAGTAAAAG0-----TTCCATCG0-AAACT-----CCC0-----GAAAAAATCGAGGATGAACG0------------1TATATA1CATA---------------------------
Monnina_leptostachya          -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAAATCGAGGATGAACG0------------1TATATA1CACA---------------------------
Monnina_pterocarpa            -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0-----GAAAAAATCGAGGATGAACG0------------1TATATA1CATA---------------------------
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trnL/F alignment (continued)

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[                               241       251       261       271       281       291       301       311       321       331       341       351       ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Salomonia_cantoniensis          -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0----GAAAAGATCGAGGATGAACG0------------1TATGTA1CATA----------------------------
Salomonia_ciliata               -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0----GAAAAGATCGAGGATGAACG0------------1TATGTA1CATA----------------------------
Sal_cilRKH1023                  -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0----GAAAAGATCGAGGATGAACG0------------1TATGTA1CATA----------------------------
Securidaca_diversifolia         -TCAAAG------0-----TTACATCG0-AAACT-----CCC0----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA----------------------------
Securidaca_longifolia           -------------?-------------?--------------?------------------------?------------?------?--------------------------------
Securidaca_virgata              -TCAAAG------0-----TTCCATCG0-AAACT-----CCC0----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA----------------------------
Securidaca_welwitschii          -TAAAAG------0-----TTCCATCG0-AAACT-----CCC0----GAAAAGATCGAGGATGAACG0------------1TATATA1CATA----------------------------

[                               361       371       381       391       401       411       421       431       441       451       461       471       ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine             ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA1CTCAAATGATTAA0------------TGATGG----TG0--CGAATC---
Xanthophyllum_arnottianum       ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA1CTCAAATGATTAA0------------TGATGG----CA0--CGAATC---
Xanthophyllum_octandrum         ------------------------------??-------------------------------------?-------------?------------------------?-----------
Xan_octBG7834                   ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA1CTCAAATGATTAA0------------TGATGG----CG0--CGAATC---
Xan_fraBG7836                   ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA1CTCAAATGATTAA0------------TGATGG----CG0--CGAATC---
Medicago_lupulina               ------------------------------??----------------TACTCTTTC------AATAGA0-------------?------------------------?-----------
Guilfoylia_monostylis           ------------------------------11CGTATATGTACTGAAATACTTTCTC------AAATGA0---------TTAA0------------TGACGA----C?0--AAAATA---
Phylica_polifolia               ------------------------------11CGTATACGTACTGAAATACTGTCTCCAAACA-AAATGA0---------TTAA0------------TGACGA----CC0--CGAATCTTT
Albizia_berteriana              ------------------------------11CGTATACGTACTGAAATACTATTTC------AATTGA0---------TTAG1ACC---------AGATAG----AC1CCCAAATA---
Senna_pleurocarpa               ------------------------------11CGTATACGTACTGAAATACTATTTC------AATTGA0---------TTAG1ACCAGAC-----AGACAG----AC1CCCAAATC---
Callistachys_lanc               ------------------------------00--CATATGTACTAAAATACTATTAT------ATTTGT?-------------?------------------------?-----------
Dryas_drummondii                ------------------------------11CGTATACGTACTGAAATACTATCTC-----CAAATGA0---------TTAA0------------TGACGA----CC0--CGAATC---
Quillaja_saponaria              ------------------------------11CGTATACATACTGAAATATTATCTC------AAATGC0---------TTAA0------------TGACGA----TC0--AAAATC---
Recchia_mexicana                ------------------------------11CGTATACGTACTGAAATACTTTCTC------AAATGA0---------TTAA0------------TGACGA----CC0--CAAATC---
Fagus_sylvatica                 ------------------------------11CGTATATGTACTGAAATCCTATCTC------AAATGA0---------TAAA0------------TGACGA----CC0--GGAATC---
Monnina_insignis                ------------------------------11CGTATTCTTACTGAAATACTCTCTC------AAATGA0---------TTAA0------------TGGTGG----CG0--CTAATC---
Monnina_malmeana                ------------------------------11CGTATTCTTACTGAAATACTCTCTC------AAATGA0---------TTAA0------------TGGTGG----CG0--CTAATC---
Atroxima                        ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0--CGAATC---
Balgoya_pacifica                ------------------------------??-------------------------------------?-------------?------------------------?-----------
Barnhartia                      ------------------------------??-------------------------------------?-------------?------------------------?-----------
Bredemeyera_altissima           ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0--CGAATC---
Bredemeyera_colletioides        ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TGAA0-------ATTCATGATGG----TG0--CGAATC---
Bre_collLM241                   ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TGAA0-------ATTCATGATGG----TG0--CGAATC---
Bredemeyera_floribunda          ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTTA0------------TGATGG----CG0--CGAATC---
Bredemeyera_lucida              ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTTA0------------TGATGG----CG0--CGAATC---
Bredemeyera_microphylla         ------------------------------11CGTATTCGTACTCAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0-----------
Bre_papNGF33220                 ------------------------------11TGTATTCGTACTGAAATACTATCTT------GATTGA0-------------0------------TGATGGATGGCG0--CGCATC---
Carpolobia_alba                 ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0--CGAATC---
'Carpolobia_aff._goetzii'       ------------------------------??-------------------------------------?-------------?------------------------?-----------
Comesperma_calymega             ------------------------------??-------------------------------------?-------------?------------------------?-----------
Comesperma_hispidulum           ------------------------------??-------------------------------------?-------------?------------------------?-----------
Comesperma_secundum             ------------------------------??-------------------------------------?-------------?------------------------?-----------
Com_eriAM102                    ------------------------------??-------------------------------------?-------------?------------------------?-----------
Com_scoMDC9172                  ------------------------------11CGTATTTGTACTGAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0--TGAATT---
Com_volAM88                     ------------------------------11CGTATTCGTACTTAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0--CTAATC---
Com_rhaEAG8343                  ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CG0--CGAATC---
Diclidanthera_bolivarensis      ------------------------------??-------------------------------------?-------------?------------------------?-----------
Diclidanthera_pendulifera       ------------------------------11CGTATTCGTACTGAAATACTATCTC------AAATGA0---------TTAA0------------TGATGG----CA0--CGAATC---
Monnina_crassifolia             ------------------------------11CGTATTCGTACTGAAATACTCTCTC------AACTGA0---------TTAA0------------TGATGA----CG0--CGAATC---
Monnina_phillyreoides           ------------------------------11CGTATTCGTACTGAAATACTCTCTC------AACTGA0---------TTAA0------------TGATGA----CG0--CGAATC---
Monnina_hirta                   ------------------------------11CGTATTCGTACTGAAATACTCTCTC------AACTGA0---------TTAA0------------TGATGA----CG0--CGAATC---
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trnL/F alignment (continued)

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[                             361       371       381       391       401       411       421       431       441       451       461       471       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Moutabea_aculeata             ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CA0--CGAATC---
Moutabea_excoriata            ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CA0--CGAATC---
Moutabea_guianensis           ----------------------------11CGTATTCGCACTGAAATACTATCTT-------AAATGA0----------TTAA0------------TGATGG----CA0--CGAATC---
'Muraltia_aff._alopecuroides' A---------------------------11CGTATTCGTACTTAAATACTATCTC-------AAATGA0----------TTAA0------------AGATGG----CG0--CGAATC---
Muraltia_heisteria            ----------------------------11CGTATTCGTATTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGT----CG0--CGAATC---
Muraltia_mixta                ----------------------------11CGTATTCGTATTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Mur_heiAD                     ----------------------------11CGTATTCGTATTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGT----CG0--CGAATC---
Nylandtia                     ----------------------------11TGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Acanthocladus_guayaquilensis  ----------------------------11CGTATTCGTACTGAGATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_acuminata            ----------------------------0?--------------------------------------?--------------?------------------------?-----------
Polygala_arillata             ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_chamaebuxus          ----------------------------11CGTATTCGTACTGAAAGACTATCTA-------AAATTC0----------TTAA0------------TGATGT----CG0--CGAATC---
Polygala_cuneata              ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CA0--CGAATC---
Polygala_erioptera            -?ACGTATTCATATACGTATTC--ATATA11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATT---
Polygala_floribunda           ----------------------------0?--------------------------------------?--------------?------------------------?-----------
Polygala_fuertesii            ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAAGG---
Polygala_hebeclada            ----------------------------0?--------------------------------------?--------------?------------------------?-----------
Polygala_klotzschii           ----------------------------11CGTATTCGTACTGAGATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_lutea                ----------------------------11CGTATTCGTACTTAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CA0--CGAATC---
Polygala_macradenia           ----------------------------11CGTATTCGTACTGAAATACTATCTA-------AAATGA0----------TTAA0------------TGATGG----TG0--CGAATC---
Polygala_microphylla          ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_obscura              ----------------------------11CGTATTC-TACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_oleifolia            ----------------------------11CGTATTCGTACTGAAATATTATCTC-------AAATTA0----------TTAA0------------TGATGG----CG0--CAAATC---
Polygala_lindheimeri          ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_penaea               ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_rivinifolia          ----------------------------11TGGATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_semeiocardium        ----------------------------11CGTATTCGTACTGAAATACTATCTA-------AAATGA0----------TTAA0------------TGATCG----CG0--CGAATC---
Polygala_senega               ----------------------------11CGTATTCGTACTTAAATACTATATC-------AAATGA0----------TTAA0------------TGATGG----CA0--CGAATC---
Polygala_subspinosa           ----------------------------11CGTATTCGTACTTAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_tatarinowii          ----------------------------11CGTATTCGTA------------CTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Polygala_violacea             ----------------------------0?--------------------------------------?--------------?------------------------?-----------
Polygala_vulgaris             ----------------------------??--------------------------------------?--------------?------------------------?-----------
Pol_exsRKH804                 ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------CGATGG----CG0--CAAATC---
Pol_japLMC2903                ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Monnina_dictyocarpa           ----------------------------11CGTATTCGTACTGAAATACTCTCTC-------AACTGA0----------TTAA0------------TGATAA----CG0--CGAATC---
Monnina_leptostachya          ----------------------------11CGTATTCGTACTGAAATACTCTCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Monnina_pterocarpa            ----------------------------11CGTATTCGTACTGAAATACTCTCTC-------AACTGA0----------TTAA0------------TGATGA----CG0--CGAATC---
Salomonia_cantoniensis        ----------------------------?0----TTCGTACTAAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CAAATT---
Salomonia_ciliata             ----------------------------?0----TTCGTACTAAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CAAATT---
Sal_cilRKH1023                ----------------------------?0----TTCGTACTAAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CAAATT---
Securidaca_diversifolia       ----------------------------11CGTATTCGTATTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Securidaca_longifolia         ----------------------------??--------------------------------------?--------------?------------------------?-----------
Securidaca_virgata            ----------------------------11CGTATTCGTATTGAAATACTATCTC-------AAATGA0----------TTAA0------------TGATGG----CG0--CGAATC---
Securidaca_welwitschii        ----------------------------11CGTATTCGTACTGAAATACTATCTC-------AAACGA0----------TTAA0------------TGATGG----CG0--CGAATC---

[                             481       491       501       511       521       531       541       551       561       571       581       591       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine           ---------1TCATCT0-----?-------------------------------1CTATTTA------------------------------TA-TG-----------1ACAAA0---TG
Xanthophyllum_arnottianum     ---------1TCATCT0-----?-------------------------------1CTATTTA------------------------------TT-TG-----------1ACAAA0---TG
Xanthophyllum_octandrum       ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Xan_octBG7834                 ---------1TCATCT0-----?-------------------------------1CTATTTA------------------------------TT-TG-----------1ACAAA0---TG
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trnL/F alignment (continued)

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[                              481       491       501       511       521       531       541       551       561       571       581       591      ]
[                              |         |         |         |         |         |         |         |         |         |         |         |        ]
Xan_fraBG7836                  ---------1TCATCT0-----?-------------------------------1CTATTTA------------------------------TT-TG-----------1ACAAA0---TG
Medicago_lupulina              ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Guilfoylia_monostylis          ---------?--ATCT1TAT?T0---------------------------TTTT1ATATTTA------------------------------TA-TG-----------1ACAAA0---TA
Phylica_polifolia              TTT------1TTTTTT1TATTT0-AT------------------------ATGT1TTATATA------------------------------TA-TGTTAATAT----1GAAAA0---TG
Albizia_berteriana             ---------0-----T1TATTT0---TTTTTA------------------TTTT1ATATTTA------------------------------TA-TG-----------1ACAAA0---TA
Senna_pleurocarpa              ---------0-----C1TATTT0---------------------------TTTA1ATATTTA------------------------------TA-TG-----------1ACAAA0---TA
Callistachys_lanc              ---------?------------------------------------------GA1ATATCTC------------------------------TA-TC-----------1ACAAA0---CG
Dryas_drummondii               ---------0-----T1TCT--0---------------------------TTTT1TTATATT------------------------------TA-TA-----------1TGAAA1AA-TA
Quillaja_saponaria             ---------0-----T1TATTT0---------------------------TTTT0---TTTA------------------------------TA--G-----------1ACAAA0---TG
Recchia_mexicana               ---------0-----T1TATCT0---------------------------TTTT1ATATCTC------------------------------TA-TG-----------1GCAAA0--TTG
Fagus_sylvatica                --------T1TCAT-T1TATTT0-------------------------------1ATATTTC------------------------------TA-TA-----------1AATAA?AC-AC
Monnina_insignis               ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------0-----?-----
Monnina_malmeana               ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------0-----?-----
Atroxima                       ---------1TCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1ACAAA0---TG
Balgoya_pacifica               ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Barnhartia                     ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Bredemeyera_altissima          ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Bredemeyera_colletioides       ---------1CCATCT1TGCTT1G--------------------------TTTT1ATATTTC------------------------------TA-TG-----------1AGAAA0---TC
Bre_collLM241                  ---------1CCATCT1TGCTT1G--------------------------TTTT1ATATTTC------------------------------TA-TG-----------1AGAAA0---TC
Bredemeyera_floribunda         ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1ACAAA0---TG
Bredemeyera_lucida             ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1AAAAA1AA-TG
Bredemeyera_microphylla        ---------?--ATCT1GATTT1T--------T-----------------TTTT1CTATTTC------------------------------TAATG-----------1ACAAA0---TA
Bre_papNGF33220                ---------1CCATCT1TA---0---------------------------TTTT1CTCTATA------------------------------TA-TG-------TATG1AGAAA0---TC
Carpolobia_alba                ---------1TCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1ACAAA0---TG
'Carpolobia_aff._goetzii'      ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Comesperma_calymega            ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Comesperma_hispidulum          ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Comesperma_secundum            ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Com_eriAM102                   ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Com_scoMDC9172                 ---------1CCATCT1CATT-0---------------------------TTTT1CTATTTC------------------------------TA--------------1AAAGA0-----
Com_volAM88                    ---------1CCATCT1TATT-0---------------------------TTTT0-TTTTTC------------------------------TA-TG-----------1AGAAA0---TA
Com_rhaEAG8343                 ---------1CCATCT1TATTT1T---------TT---------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0-----
Diclidanthera_bolivarensis     ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Diclidanthera_pendulifera      ---GAATC-1TCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1GCAAA0---TG
Monnina_crassifolia            ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1AGAAA0---AA
Monnina_phillyreoides          ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1AGAAA0---TC
Monnina_hirta                  ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1ATAAA0---TC
Moutabea_aculeata              ---------1T?ATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1GCAAA0---TG
Moutabea_excoriata             ---------1TCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1GCAAA0---TG
Moutabea_guianensis            ---------1TCATCT1TATTT1G--------------------------TTTT1CTATTTA------------------------------TA-TG-----------1GCAAA0---TG
'Muraltia_aff._alopecuroides'  ---------1CCATCT1TATTT1T-----------TTT------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Muraltia_heisteria             ---------1CCATCT1TATTT0---------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Muraltia_mixta                 ---------1CCATCT1TATTT0---------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Mur_heiAD                      ---------1CCATCT1TATTT0---------------------------TTTT1CTATTTC----------------------------------G-----------1AGAAA0---TA
Nylandtia                      ---------1CCATCT1TATTT0---------------------------TTTT1CTATTTC----------------TATTTC--------TA-TG-----------1AGAAA0---TA
Acanthocladus_guayaquilensis   ---------1CCATCT1GATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Polygala_acuminata             ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Polygala_arillata              ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TG
Polygala_chamaebuxus           ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Polygala_cuneata               ---------1CCATCT1TTTTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---GA
Polygala_erioptera             ---------1CCATCT1TATTT1G--------------------------TTTT1ATATTTC------------------------------TG-AT-----------1AGAAA0---TA
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trnL/F alignment (continued)

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[                               481       491       501       511       521       531       541       551       561       571       581       591      ]
[                               |         |         |         |         |         |         |         |         |         |         |         |        ]
Polygala_floribunda             ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Polygala_fuertesii              ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Polygala_hebeclada              ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Polygala_klotzschii             ---------1CCATCT1GATTT1G--------------------------TTTT1ATATTTC------------------------------CA-TG-----------1ACAAA0---TG
Polygala_lutea                  ---------1CGATCT1TATTT1A--------------------------TTGT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TA
Polygala_macradenia             ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Polygala_microphylla            ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC-------TTTGTTTTCTATTTC--------TA-TG-----------1ATAAA0---TA
Polygala_obscura                ---------1CCATCT1TATTT1G--------------TCTATTTG----TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Polygala_oleifolia              ---------1CCATCT1TATTT1G--------------------------TTTT1CAATTTC------------------------------TA-TG-----------1AGAAA0---TA
Polygala_lindheimeri            ---------1CCATCG1TATTT1T--------------------------TTTT1TTATTTC------------------------------TC-AT-----------1AGAAA0---TA
Polygala_penaea                 ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Polygala_rivinifolia            ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1ACAAA0---TG
Polygala_semeiocardium          ---------1CTATTT1GATTT1T----------------------TTTTTTTT1TTATTTC----------------------TAAA-TTCCA-TG-----------1AGAAA0---TA
Polygala_senega                 ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1ACAAA0---TA
Polygala_subspinosa             ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Polygala_tatarinowii            ---------1CTATTT1TATTT1A--------------------------TTTT0---TTTC------------------------------TA-TG-----------1ATAAA0---AA
Polygala_violacea               ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Polygala_vulgaris               ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Pol_exsRKH804                   ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Pol_japLMC2903                  ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Monnina_dictyocarpa             ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1AGAAA0---TC
Monnina_leptostachya            ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1AGAAA0---TC
Monnina_pterocarpa              ---------1CAATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TA-----------1AGAAA0---TC
Salomonia_cantoniensis          ---------1CCAGCT1TATTT1T--------------------------CTTT1CTCTATT------------------------------TA-TG-----------1AGAAA0---TA
Salomonia_ciliata               ---------1CCAGCT1TATTT1T--------------------------CTTT1CTCTATT------------------------------TA-TG-----------1AGAAA0---TA
Sal_cilRKH1023                  ---------1CCAGCT1TATTT1T--------------------------ATTT1CTCTATT------------------------------TA-TG-----------1AGAAA0---TA
Securidaca_diversifolia         ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Securidaca_longifolia           ---------?------?-----?-------------------------------?-----------------------------------------------------?-----?-----
Securidaca_virgata              ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA
Securidaca_welwitschii          ---------1CCATCT1TATTT1G--------------------------TTTT1CTATTTC------------------------------TA-TG-----------1AGAAA0---TA

[                               601       611       621       631       641       651       661       671       681       691       701       711      ]
[                               |         |         |         |         |         |         |         |         |         |         |         |        ]
Xanthopyllum_affine             --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA1GAAA1GAAAGGAATCGAATATTCATTGGTCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Xanthophyllum_arnottianum       --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA1GAAA1GAAAGGAATCGAATATTCATTGGTCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Xanthophyllum_octandrum         --------?------------------------------------?----?---------------------------------------------------------------------
Xan_octBG7834                   --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA1GAAA1GAAAGGAATCGAATATTCATTGGTCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Xan_fraBG7836                   --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA1GAAA1GAAAGGAATCGAATATTCATTGGTCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Medicago_lupulina               --------?------------------------------------?----?-------ATC--ATATTTATTGATCAAATCAGTCAC------TCCACCAT-AGTCTGA-----TGGATC
Guilfoylia_monostylis           --AAAGAA0----------TTATTGTGAATCGAT?CCAAGCTGAA1GAAA0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCATCAT-AGTCTGA-----TAGATC
Phylica_polifolia               --AAAA--4----------TTGTTGTGAATCGATTCCAAGTAAAA0-A--0-----AAATGGAATATTCGTTGATCAAATCATTTAC------TCCATCGT-AATCTGA-----TATATC
Albizia_berteriana              --AAAGA-5--------------TGTGAATCGATTCCAAGTTGAA1GAAA0-----GAATCAAATATTAATTGATCAAA---TTCAC------TCCATGAT-AGTCTGA-----TAGATT
Senna_pleurocarpa               --AAAGA-5--------------TGTGAATCGATTCCAAGTTGAA1GAAA0-----GAATCGAATATTCATTGAGAAAATCATTCAC------TCCATCAT-AGTCTGA-----TAGATC
Callistachys_lanc               --AAAGT-5--------------TGTGAATCAATTCAAGGTTGAA1GAAA0------AATGGAATATTCATTCATTAAATCATTCAC------TCCATCCT-AGTCTGA-----TAGATC
Dryas_drummondii                --AAAGAA0----------TTGTTGTGAATCGATTCTAAATTGAA1AAAA0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACCAT-AGTCTGA-----TAGATC
Quillaja_saponaria              --AAAGAA0----------TTTTGGAGAATTGATTCCAAGTTGAA1GAAA0-----GAATCGAATATTCAGTGATCAAATCATTCAC------TCCATCAT-AGTCTGA-----TAGATC
Recchia_mexicana                --AAAGAA0----------TTATTGCGAATCGATTCCAAGCTGAA1GAAA0-----GAATCGAATTTTCATTGATCAAATCATTCAC------TCCATCAT-AGTCTGA-----TAGATC
Fagus_sylvatica                 --CGAAAG6AG--------TTGTTGTGAATCGAT-CCAAATGGAA1GAAA0-----GAATCGAATATTTATTAATTCAATTATTTAC------TCCATCAT-AGTCTGA-----TAGATC
Monnina_insignis                --AAAAAA1---------------GTGAATCGATTCCAAGTTGAA0----0-----GAATCAAATATTCATTGATCAAACCATTCAC------TCCACTAG-AATTCGA-----TAATTC
Monnina_malmeana                --AAAAAA1---------------GTGAATCGATTCCAAGTTGAA0----0-----GAATCAAATATTCATTGATCAAACCATTCAC------TCCACTAG-AATTCGA-----TAATTC
Atroxima                        --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACTCT-AGTTTGA-----TAAATC
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trnL/F alignment (continued)

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[                              601       611       621       631       641       651       661       671       681       691       701       711       ]
[                              |         |         |         |         |         |         |         |         |         |         |         |         ]
Balgoya_pacifica               --------?-----------------------------------------?---------------------------------------------------------------------
Barnhartia                     --------?-----------------------------------------?---------------------------------------------------------------------
Bredemeyera_altissima          --AAAAAA0----------TCTTTGTGAATCGATTCCAAATTGAA0----0-----GAATCGAATATTCATTGATCAAAGCATTCAC------TCCACTTT-AGTTCGA-----TAAATC
Bredemeyera_colletioides       --AAAAAA0----------TATTTGTGAATCGACTCTGAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAT------TCCACTCT-AGTTCAA-----TAAATC
Bre_collLM241                  --AAAAAA0----------TATTTGTGAATCGACTCTGAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAT------TCCACTCT-AGTTCAA-----TAAATC
Bredemeyera_floribunda         --CAAAAA0----------TCTTTGTGAATCGATTCCAAATTGAA0----0-----GAATCGAATATTCATTGATCAAAGCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Bredemeyera_lucida             --AAAAAA0----------TCTTTGTGAATCGATTCCAAATTGAA0----0-----GAATCGAATATTCATTGATCAAAGCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Bredemeyera_microphylla        --GAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Bre_papNGF33220                --AA----2----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGARTATTCATTGATCAAACCATTCAC------TCCACTMT-AATTCGA-----TAAATC
Carpolobia_alba                --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACTAT-AGTTTGA-----TAAATC
'Carpolobia_aff._goetzii'      --------?------------------------------------?----?---------------------------------------------------------------------
Comesperma_calymega            --------?------------------------------------?----?---------------------------------------------------------------------
Comesperma_hispidulum          --------?------------------------------------?----?----------------------------CCATTCAC------TTCACTAT-AATTCGA-----TAAATA
Comesperma_secundum            --------?------------------------------------?----?----------------------------CCATTCAC------TTCACTAT-AATTCGA-----TAAATC
Com_eriAM102                   --------?------------------------------------?----?----------------------------CCATTCAC------TTCACTAT-AATTCGA-----TAAATC
Com_scoMDC9172                 --AAGAAA4----------TCTTTGTGAATCGATTCCAAGTGGAA0----0-----GAATCGAATATTTATTGATCAAACCATTCAC------TACACTAT-AATTCGA-----TAAATC
Com_volAM88                    --AA----2----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTTATTGATCAAACCATTCAC------TCCACTCT-AATTCGA-----TAAATC
Com_rhaEAG8343                 --AAA---3----------TATTTGTGAATCGACTCCAAGTTGAA0----0-----GAATCGAATATTTATTGATCAAACCATTCAC------TCCACTCT-AATTCGA-----TAAATC
Diclidanthera_bolivarensis     --------?------------------------------------?----?---------------------------------------------------------------------
Diclidanthera_pendulifera      --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAACC
Monnina_crassifolia            --AAA---3----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTAG-ACTTCGA-----TAATTC
Monnina_phillyreoides          --AAA---3----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTAG-ACTTCGA-----TAATTC
Monnina_hirta                  --AAA---3----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTAG-ACTTCGA-----TAATTC
Moutabea_aculeata              --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Moutabea_excoriata             --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Moutabea_guianensis            --AAAAAA0----------TCTTTGTAAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAATCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
'Muraltia_aff._alopecuroides'  --AAGAAA0----------TCTTTGTGAATTGATTCCAAGTTGAA0----0-----GAATCAAGTATTCATTGATCAAACCATTCAC------TCTACTAT-ATTTCGA-----TAAATC
Muraltia_heisteria             --AAGAAA0----------TCTTTGTGAATTGATACTAAGTTGAA0----0-----GAATCAAGTATTCATTGATCAAACCATTCAC------TCTACTAG-ATTTCGA-----TAAATC
Muraltia_mixta                 --AAGAAA0----------TCTTTGTGAATTGATTCTAAGTTGAA0----0-----GAATCAAGTATTCATTGATCAAACCATTCAC------TCTACTAT-ATTTCGA-----TAAATC
Mur_heiAD                      --AAGAAA0----------TCTTTGTGAATTGATACTAAGTTGAA0----0-----GAATCAAGTATTCATTGATCAAACCATTCAC------TCTACTCG-ATTTCGA-----TAAATC
Nylandtia                      --AATAAA0----------TATTTGTGAATCGATTCCAAATTGAA0----0-----GAATCAAGTATTCATTGATCAAACCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Acanthocladus_guayaquilensis   --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTAATCAAACCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_acuminata             --------?------------------------------------?----?---------------------------------------------------------------------
Polygala_arillata              --AAAAAA0----------TCTTTGTGAATCGATTTCAAGTTGAA0----0-----GAATCAAGTATTCATTGATCAAACCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Polygala_chamaebuxus           --AATAAA0----------TCTTTGTGAATTGATTCCGAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTAT-AGTTCGA-----TAAA-C
Polygala_cuneata               --AA----2----------TCTTTGATCATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCACATTCACTCCACTAG-AATTCGA-----TAAATC
Polygala_erioptera             TAAATAAA0--TAAATAAATCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCAGTGATCAAATCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_floribunda            --------?------------------------------------?----?---------------------------------------------------------------------
Polygala_fuertesii             --GAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCAAATATTCATTGATCAAACCGTTCAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_hebeclada             --------?------------------------------------?----?---------------------------------------------------------------------
Polygala_klotzschii            --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TACACTCT-AGTTCGA-----TAAATC
Polygala_lutea                 --AATAAA0----------TCTTTGTGAATCAATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTAATCAAACCATTCAC------TCCACTAT-AGTTTCA-----TAAATC
Polygala_macradenia            --GAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAAACAAATATTCATTGATCAAACCGTTTAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_microphylla           --AAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACCAT-AGTTCGA-----TAAATC
Polygala_obscura               --GAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCAAATATTCATTAATCAAACCGTTTAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_oleifolia             --AAAAAA0----------TATTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TACACTAT-AGTTCGA-----TAAATC
Polygala_lindheimeri           --AA----2----------TCTTTGTGAATCCATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTAT-AATTCGA-----TAAATC
Polygala_penaea                --GAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCAAATATTCATTGATCAAACCGTTCAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_rivinifolia           --GAAAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCAAATATTCATTGATCAAACCGTTTAC------TCCACTCT-AGTTCGA-----TAAATC
Polygala_semeiocardium         --AATAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TTCACTAT-AGTTCGA-----TAAATC
Polygala_senega                --AATAAA0----------TCTTTGTGAATCGATTCCAAATTGAA0----0-----GAATCGAGTATTCATTAATCAAACCATTCAC------TCCACTAT-AGTTTAA-----TAAATC
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trnL/F alignment (continued)

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[                             601       611       621       631       641       651       661       671       681       691       701       711       ]
[                             |         |         |         |         |         |         |         |         |         |         |         ]
Polygala_subspinosa           --AA----2----------TCTTTGTGAATCCATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCACTCAC------TCCACTCTAAATTCGA-----AAAATC
Polygala_tatarinowii          --AATAAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTAT-AGTTCGATAAAATAAATC
Polygala_violacea             --------?------------------------------------?----?---------------------------------------------------------------------
Polygala_vulgaris             --------?------------------------------------?----?---------------------------------------------------------------------
Pol_exsRKH804                 --AATAAA0----------TCTTTGTTAATCCATTTCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Pol_japLMC2903                --AATAAA0--TAAA----TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Monnina_dictyocarpa           --AAA---3----------TCTTTTTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTAG-ACTTCGA-----TAATTC
Monnina_leptostachya          --AAA---3----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACTATTCAC------TCCACTAG-ACTTCGA-----TAATTC
Monnina_pterocarpa            --AAA---3----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAACCATTCAC------TCCACTAG-ACTTCGA-----TAATTC
Salomonia_cantoniensis        --AATCAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Salomonia_ciliata             --AATCAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Sal_cilRKH1023                --AATCAA0----------TCTTTGTGAATCGATTCCAAGTTGAA0----0-----GAATCGAGTATTCATTGATCAAACCATTCAC------TCCACTCT-AGTTCGA-----TAAATC
Securidaca_diversifolia       --AATAAA0----------TCTTTGTGAATCGATTCTAAGTTGAA0----0-----CAATCGAATATTCATTGATCAAAACATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Securidaca_longifolia         --------?------------------------------------?----?---------------------------------------------------------------------
Securidaca_virgata            --AATAAA0----------TCTTTGTGAATCGATTCTAATTTGAA0----0-----GAATCGAATATTCATTGATCAAAACATTCAC------TCCACTAT-AGTTCGA-----TAAATC
Securidaca_welwitschii        --AATAAA0----------TCTTTGTGAATCGATTCTAAGTTGAA0----0-----GAATCGAATATTCATTGATCAAAACATTCAC------TCCACTAT-AGTTCGA-----TAAATC

[                             721       731       741       751       761       771       781       791       801       811       821       831       ]
[                             |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine           TTTTG-----------------AAAAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Xanthophyllum_arnottianum     TTTTG-----------------AAGAACTGATTAATCGGACGAGAAAAAAGAATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Xanthophyllum_octandrum       ------------------------------------------------------------------------------------------------------------------------
Xan_octBG7834                 TTTTG-----------------AAGAACTGATTAATCGGACGAGAAAAAAGAATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Xan_fraBG7836                 TTTTG-----------------AAGAACTGATTAATCGGACGAGAAAAAAGAATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Medicago_lupulina             TTTTG-----------------AATAACTGATTAATCAGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACATCAATGAAATTTTTAGTAAGAGGAAAATCC
Guilfoylia_monostylis         TTTTG-----------------AAGAACTGATGAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACGAAAATGAAATTTATAGTAAGAGGAAAATCC
Phylica_polifolia             TTTTG-----------------AAAAATTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATATCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Albizia_berteriana            TTTTG-----------------AAGAACTGATTAATCAGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Senna_pleurocarpa             TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Callistachys_lanc             TTTTG-----------------AAGAACTTATGAATCAGACGAGA-------ATAAAGATAGAGTCCCATCCTACATGTCAATACCGACAACAATGAAATTTCTAGTAAGAGGAAAATCC
Dryas_drummondii              TTTTT-----------------AAGAATTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATATTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Quillaja_saponaria            TTTTG-----------------AAGAACTGATTAATCGGATGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Recchia_mexicana              TTTTG-----------------AACAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACCTGTCAATACCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Fagus_sylvatica               TTTTG-----------------AAGAACTAATTTATCGTACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAAGAATGAAATTTATAGTAAGAGGAAAATCC
Monnina_insignis              TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAAAGGAAAATCC
Monnina_malmeana              TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAAAGGAAAATCC
Atroxima                      TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCGTTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Balgoya_pacifica              ------------------------------------------------------------------------------------------------------------------------
Barnhartia                    ------------------------------------------------------------------------------------------------------------------------
Bredemeyera_altissima         TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Bredemeyera_colletioides      TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCAACAACAATGAAATTTATAGTAAGAGGAAAATCC
Bre_collLM241                 TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCAACAACAATGAAATTTATAGTAAGAGGAAAATCC
Bredemeyera_floribunda        TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Bredemeyera_lucida            TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Bredemeyera_microphylla       TTTTG-----------------AAGAACTGATTAATAGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Bre_papNGF33220               TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACMACAATGRAATTTAGAGTAAGAG--------
Carpolobia_alba               TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCGTTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
'Carpolobia_aff._goetzii'     ------------------------------------------------------------------------------------------------------------------------
Comesperma_calymega           ------------------------------------------------------------------------------------------------------------------------
Comesperma_hispidulum         TTTTG-----------------AAGAAATGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Comesperma_secundum           TTTTG-----------------AAGAAATGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
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[                             721       731       741       751       761       771       781       791       801       811       821       831       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Com_eriAM102                  TTTTG-----------------AAGAAATGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Com_scoMDC9172                TTTTGAAAAACTTATTATTTTGAAGAACTTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATRCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Com_volAM88                   TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Com_rhaEAG8343                TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Diclidanthera_bolivarensis    ------------------------------------------------------------------------------------------------------------------------
Diclidanthera_pendulifera     TTTTG-----------CTTTTGAAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Monnina_crassifolia           TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTTTAGTAAAAGGAAAATCC
Monnina_phillyreoides         TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAAAGGAAAATCC
Monnina_hirta                 TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAAAGGAAAATCC
Moutabea_aculeata             TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTATACATGTCAAT?????????????????????????????????????
Moutabea_excoriata            TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Moutabea_guianensis           TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGARGAAAATCC
'Muraltia_aff._alopecuroides' TTTTT-----------------AAGAACTTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTAAATGTCAATTCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Muraltia_heisteria            TTTTT-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATTCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Muraltia_mixta                TTTTT-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATTCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Mur_heiAD                     TTTTT-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATWCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Nylandtia                     TTTTT-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATTACGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Acanthocladus_guayaquilensis  TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAATTCC
Polygala_acuminata            -------------------------------TTAATTGAACGKGA-------ATAAAGATAGAGTCYCATTCTACATGTCAATGCCGACARCAATGAAAATTAGAGTAAGAGGAAAATCC
Polygala_arillata             TTTTG-----------------AAGAACTTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTAGAGTAAGAGGAAAATCC
Polygala_chamaebuxus          TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_cuneata              TTTTG-----------------AAGAGCTTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_erioptera            TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_floribunda           -------------------------------TTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_fuertesii            TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_hebeclada            -------------------------------TTAATTGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAAATTAGAGTAAGAGGAAAATCC
Polygala_klotzschii           TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_lutea                TTTTG-----------------AATAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACACGTCAATGCTGACAATAATGAAATTTATAATAAGAGGAAAATCC
Polygala_macradenia           TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_microphylla          TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTTATTGTAAGAGGAAAATCC
Polygala_obscura              TTTTC-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTAGAGTAAGAGGAAAATCC
Polygala_oleifolia            TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCCACATGTCAATGCCGACAACAATGCAATTTATAGTAGTAGGAAAATCC
Polygala_lindheimeri          TTTTG-----------------AAGAGCTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_penaea               TTTTG-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_rivinifolia          TTTTC-----------------AAGAACTGATTAATCGGACGAGA-------ATAAAGATAGAGTCCCATTTTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_semeiocardium        TTTTG-----------------AAGAACTGATTAATCGAATGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGTCGACAACAATGAAATTTATCGWAGGAGGAAAATCC
Polygala_senega               TTTTG-----------------AATAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACACGTCAATACTGACAATAATGAAATTTGTAGTAAGAGGAAAATCC
Polygala_subspinosa           TTTTG-----------------AAGAGATTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_tatarinowii          TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGTCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Polygala_violacea             -------------------------------TTAATTGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAAATTAGAGTAAGAGGAAAATCC
Polygala_vulgaris             ------------------------------------------------------------------------------------------------------------------------
Pol_exsRKH804                 TTTTG-----------------AAGAAATGATTAATCGAATGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATRCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Pol_japLMC2903                TTTTG-----------------AAGAACTTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Monnina_dictyocarpa           TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTATACATGTCAATGCCGACAACAATGAAATTTATAGTAAAAGGAAAATCC
Monnina_leptostachya          TTTTG-----------------AAGAACTTATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAAAGGAAAATCC
Monnina_pterocarpa            TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTTTAGTAAAAGGAAAATCC
Salomonia_cantoniensis        TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTAGAGTAAGAGGAAAATCC
Salomonia_ciliata             TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTAGAGTAAGAGGAAAATCC
Sal_cilRKH1023                TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTAGAGTAAGAGGAAAATCC
Securidaca_diversifolia       TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Securidaca_longifolia         ------------------------------------------------------------------------------------------------------------------------
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trnL/F alignment (continued)

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[                             721       731       741       751       761       771       781       791       801       811       821       831       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Securidaca_virgata            TTTTG-----------------AAGAACTGATTAATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATGAAATTTATAGTAAGAGGAAAATCC
Securidaca_welwitschii        TTTTG-----------------AAGAACTGATTTATCGAACGAGA-------ATAAAGATAGAGTCCCATTCTACATGTCAATGCCGACAACAATTAAATTTATAGTAAGAGGAAAATCC

[                             841       851       861       871       881       891       901       911       921       931       941       951       ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine           GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTCCC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Xanthophyllum_arnottianum     GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTCCC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Xanthophyllum_octandrum       --------------------------AA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTCCC-TAATTT--1TTTAA1CCTATATT1C------CCTTT
Xan_octBG7834                 GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTCCC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Xan_fraBG7836                 GTCGACTTTATAAATCGYGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTCCC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Medicago_lupulina             GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCC--------------------------------------?-----?--------?------------
Guilfoylia_monostylis         GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGCTT--------AACTTCC-TAATTA--1TTTAT1CCTCTATC1C------TCTTT
Phylica_polifolia             GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCA-------CCCATTT--------GATTCCC-TAATTT--1TTTAT1CCTATACT1C------TCATT
Albizia_berteriana            GTCGACTTTAGAAATCGTGAGGG-TCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTCTC-TAATTA--1TTTAT1CCTATATC1C------TCTTT
Senna_pleurocarpa             GTCGACTTTAGAAATCGTGAGGGTTCAAAGTCC-----------CTCTATCCCCAAAA-GGCACGTTT--------AACTCTC-TAATTA--1TTTAT1CC------?-------TCTTT
Callistachys_lanc             GTCGACTTTCGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-TGCCCGTTC--------CACTCTC-TAATTA--0----T1CCGATATC?-------TTTTT
Dryas_drummondii              GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAAAGACCTGC----------GACTCAT-TAATTA--1TTTAT?------TTT1C------TCATT
Quillaja_saponaria            GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTCCC-TAATTA--1TTTAG1ACTATATC1C------TCTTT
Recchia_mexicana              GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCCCTCTATCCCACTCTATCCCCAAAA-GGCCCGCTT--------AACTTCC-TAATTA--1TTTAT1CCTATATC1C------TCTTC
Fagus_sylvatica               GTCGACTTTAGAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAAAGGCCCGTTT--------GACTCCC-TAATTA--1TTTAT1CCGATCTT1C------TCTTT
Monnina_insignis              GTCGAATTTTAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GTCCTGTTT--------AATTACA-TAATCT--1TTGAA1CCTATATT1T------TCTTT
Monnina_malmeana              GTCGAATTTTAAAATCGTGAGGKTTCAA-GTCC-----------CTCTATCCCCAAAA-GTCCGGTTT--------AATTACA-TAATCT--1TTGAA1CCTATATA1T------TCTTT
Atroxima                      GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Balgoya_pacifica              --------------------------------------------------------------------------------------------?-----?--------?------------
Barnhartia                    -----------------------GT?AAGGTCC-----------??CTA-CACCACRW-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Bredemeyera_altissima         GTCGACTTTCTAAACC????????TCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Bredemeyera_colletioides      GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTACC-TAATTT--1TTTAA1CCTCTATT1C------TCCTT
Bre_collLM241                 GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTACC-TAATTT--1TTTAA1CCTCTATT1C------TCCTT
Bredemeyera_floribunda        GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TGTAA1CCTATATT1A------TCTTT
Bredemeyera_lucida            GTCGACTTTATAAATCSTGAGGGTTAAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TGTAA1CCTATATT1A------TCTTT
Bredemeyera_microphylla       GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAG-GGCCCGTTT--------AACTACC-TAATCC--1TTTAA1CCCCTATT1C------TCTTT
Bre_papNGF33220               --------------------------------------------------------------------------------------------?-----?--------?------------
Carpolobia_alba               GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
'Carpolobia_aff._goetzii'     --------------------------AA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Comesperma_calymega           ------------------------TCAA-GTCC-----------CTCTATCCCCAAAA-GGCCAGTTTAACTACTTAACTACC-TAATTT--1TTTAA1CCTATATT1A------TCTTT
Comesperma_hispidulum         GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCT?TCC?????GTGGCACGTTT--------AACTACC-TCATTT--1TTTAA1CCTATATT1C------TTTTT
Comesperma_secundum           GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCT?TCC???????????CGTTT--------AACTACC-TCATTT--1TTTAA1CCTATATT1C------TTTTT
Com_eriAM102                  GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GACCCGTTT--------AACTACC-TCATTT--1TTGAA1CCTATATT1C------TTTTT
Com_scoMDC9172                GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCC-TT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Com_volAM88                   GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTTAACTACTTAACTACC-CAATTT--1TTTAA1CCCATATT1C------TCTTT
Com_rhaEAG8343                GTCGACTTTAAAAATCTTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTTAACTACTTAATTACC-TCAAT---1TTTAA1CCTATATT1C------TCTTT
Diclidanthera_bolivarensis    --------------------------AA-GTCC-----------CTCTATCCCCAAAA-GTCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Diclidanthera_pendulifera     GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GTCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Monnina_crassifolia           GTCGACTTTAAAAATCCTGA????TCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AATTACC-TAATTT--1TGGAA1CCTATATT1C------TCTTT
Monnina_phillyreoides         GTCGACTTTTAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AATTACC-TAATTT--1TGGAA1CCTATATT1C------TCTTT
Monnina_hirta                 GTCGACTTTAAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AATTACC-TAATTT--1TGGAA1CCTATATT1C------TCTTT
Moutabea_aculeata             ???????????????????????KTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Moutabea_excoriata            GTCGACTTTAAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Moutabea_guianensis           CTCGACTTTAAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
'Muraltia_aff._alopecuroides' GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------C?????????????-GGCCCATTT--------AACTACC-TAATTT--0----A1CCTATATT1C------TCCTT
Muraltia_heisteria            GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCA???-GGCC-ATTT--------CACTACC-TAATTT--0----A1CCTATATT1C------TCCTT
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[                              841       851       861       871       881       891       901       911       921       931       941       951      ]
[                              |         |         |         |         |         |         |         |         |         |         |         |        ]
Muraltia_mixta                 GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCATTT--------AACTACC-TAATTT--0----A1CCTATATT1C------TCCTT
Mur_heiAD                      GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCATTT--------AACTACC-TAATTT--0----A1CCTATATT1C------TCCTT
Nylandtia                      GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTACC-TAATTT--0----A1CCTAT---?------------
Acanthocladus_guayaquilensis   GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGTCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT0----------TT
Polygala_acuminata             GTCGACTTTCTAAATCGTGAGGGTTCAA-G???-----------???TATCCCCAAAA-GGCCTGTTT--------AACTGAC-CCATTT--1TTTTA1CCTATATT1A------TCCTT
Polygala_arillata              GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCCTT
Polygala_chamaebuxus           GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTWTYCCCAAAA-GGCCCGTTT--------AACTACC-CAATTT--1TTTAA1CCTATATT1AATATTATCCTT
Polygala_cuneata               GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTACC-TAATTT--1TTTAA1TCTATATT1C------TCTTT
Polygala_erioptera             GTCGAATTTCTAAATCGTGA??GTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCTTGTTT--------AACTACC-TAATTT--1TTGAA1CTTATATT1A------TCCTT
Polygala_floribunda            GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCC?????-?GCCTGTTT--------AACTGAC--CATTT--1TTTAA1CCTATATT1C------TCCTT
Polygala_fuertesii             GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAG-GGCCCGTTT--------AACTACC-TAATCT--1TTTAA1CCTCTATT1C------TCTTT
Polygala_hebeclada             GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTGAC-CCATTT--1TTGAA1CCTATATT1A------TCCTT
Polygala_klotzschii            GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT0----------TT
Polygala_lutea                 GTCGRATTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-AGACCGTTT--------ACCTACC--AATTT--1TTTAA1CTTACATT1C------TCCTT
Polygala_macradenia            GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAG-GGCCCATTT--------AACTACC-TAATTT--1TTTAA1CCTCTATT1C------TCTTT
Polygala_microphylla           GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCT??????????-GGCACGTTT--------CACTACC-TCATTT--1ATGAA1CCTCTATT1C------TCCTT
Polygala_obscura               GTCGACTTTATAAATCATGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAG-GGCCC?KTT--------AACTACC-TAATCT--1TTTAA1CCTCTATT1C------TCTTT
Polygala_oleifolia             GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTC---------------------------------------------?-----?--------?------------
Polygala_lindheimeri           GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCCGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Polygala_penaea                GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAG-GGCCCGTTT--------AACTACC-TAATCT--1TTTAA1CCTCTATT1C------TCTTT
Polygala_rivinifolia           GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAG-GGACCGTTT--------AACTACC-TAATCT--1TTTAA1CCTCTATT1C------TCTTT
Polygala_semeiocardium         GTCGACTTTAAAAATCGTG?GGGTTCAA-GTCC-----------CTCTATCCCC?AAA-G????GTTT--------AACTACC-TAATCT--1TTTAA1CCCGTATT1C------TTCTT
Polygala_senega                GTCGAATTTTTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGACCGTTT--------AACTACC-TAATTT--1TTTAA1CTTACATT1C------TCCTT
Polygala_subspinosa            GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAAGGGCCCGTTT--------AACTACC-TAATTT--1TTTAA1CCTATATT1C------TCTTT
Polygala_tatarinowii           GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCTCGTTT--------AACTACC-TAATCT--1TTGAA1CCTATATT1C------TACTT
Polygala_violacea              GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AACTGAC-CAATTT--1TTTAA1CCTATATT1C------TCCTT
Polygala_vulgaris              -------------------------------------------------------------CC?GTTT--------AACT?CC-TAATCT--1TTGAA1CCTATATT1C------TCCTT
Pol_exsRKH804                  GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCTCGTTT--------AACTACC-TAATTT--1TGGAA1CCTATATT1C------TCCTT
Pol_japLMC2903                 GTCGACTTTCTAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCTTGTTT--------AACTACC-TAATTT--1TTGAA1CCTATATT1C------TCCTT
Monnina_dictyocarpa            GTCGACTTTAAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCCTGTTT--------AATTACC-TAATTT--1TCGAA1CCTATATT1C------TCTTT
Monnina_leptostachya           GTCGACTTTAAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCTTGTTT--------AATTACC-TAATTT--1TGGAA1CCTATATT1C------TCTTT
Monnina_pterocarpa             GTCGACTTTAAAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGCTTGTTT--------AATTACC-TAATTT--1TGGAA1CCTATATT1C------TCTTT
Salomonia_cantoniensis         GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGACCGTTT--------AACTACC-TCATTT--1TTGAT0-CTAGATT1A------TCCTT
Salomonia_ciliata              GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GTACCGTTT--------AACTACC-TCATTT--1TTGAT0-CTAGATT1A------TCCTT
Sal_cilRKH1023                 GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GTCCCGTTT--------AACTACC-TCATTT--1TTGAT0-CTATATT1A------TCCTT
Securidaca_diversifolia        GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGTCCGTTT--------AACTACC-CAATTT--1TTGAA1CCCATATT1C------TCTTT
Securidaca_longifolia          ------------------------TCAA-GTCC-----------CTCTATCCCCAAAA-GGTCCGTTT--------AACTACC-CAATTT--1TTGAA1CCCATATT1C------TCTTT
Securidaca_virgata             GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGTCCGTTT--------AACTACC-CAATTT--1TTGAA1CCCATATT1C------TCTTT
Securidaca_welwitschii         GTCGACTTTATAAATCGTGAGGGTTCAA-GTCC-----------CTCTATCCCCAAAA-GGACCGTTT--------AACTACC-CAATTT--1TTTAA1CCCATATT1C------TCTTT

[                              961       971       981       991       1001      1011      1021      1031      1041      1051      1061      1071     ]
[                              |         |         |         |         |         |         |         |         |         |         |         |        ]
Xanthopyllum_affine            T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATCTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Xanthophyllum_arnottianum      T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTAGA
Xanthophyllum_octandrum        T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCCTTATCTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Xan_octBG7834                  T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Xan_fraBG7836                  T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Medicago_lupulina              -?--------------------------------------------------------------?-------------------------------------------------------
Guilfoylia_monostylis          T0----------CATT-----AGCGGTTCCAAA------TTCGTTATCTTTA-----CCATT--1CATT----------CTATT-----CTTTCACAAACACAAACGGAT-CTGAGCGGA
Phylica_polifolia              T0----------CGTT-----AGCGGTTCAAAA------TTCGTTATGTTTC-----TCATT--1CATT----------AATTTTTTGATTTTCACAAG------------CCTTGTGA-
Albizia_berteriana             T0---------TTTTT-----AGTGGTTCCAAA------TTCGTTATGTTTC-----TTATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CTGAGTGGA
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trnL/F alignment (continued)

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[                             961       971       981       991       1001      1011      1021      1031      1041      1051      1061      1071      ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Senna_pleurocarpa             T0---------TTTTT-----AGTGGTTCAAAA------TTCGTTATGTTTCC----TTATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CTGAGTGGA
Callistachys_lanc             T0----------TTTT------------------------------------------------?-------------------------------------------------------
Dryas_drummondii              T0-----TGTTTTGTT-----AGCGATTCCAAA------TTCGTTATGTTTA-TCAGTCATTAT1CATT----------CTACT-----CTTTCACAAG------CGGAT-CTGAGCGTA
Quillaja_saponaria            T0----------CGGT-----AGTGGTTCAAAA------TTCGTTATCTTTC-----TCATT--1CATT----TTGATTCTATT-----CTTTCACAAA------CGGAT-CTGAGCGAA
Recchia_mexicana              T0----------CATT-----AGCGGTTCCAAA------TTCGGTATGTTTC-----TCATT--1CAGG----------CTCTT-----TTTTCACAAA------CGGAT-CTGAGCGAA
Fagus_sylvatica               T0----------CGTTTTGTAAGCGTTTTCAAATTGGAATTCGTTATGTTTT-----TCAAT--1CATT----------CTACT-----CTTTTACAAA------TGGAT-CTGATTGTA
Monnina_insignis              T0----------CGTT-----ATTAGTTACAAA------TTCGTTATGTTTC-----CTATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCATGTGGA
Monnina_malmeana              T0----------CGTT-----ATTAGTTACAAA------TTCGTTATGTTTC-----CTATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCATGTGGA
Atroxima                      T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATTTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Balgoya_pacifica              T?----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCA?T--1CATT----------CTATT-----CTTTCACAAA------CAGATCCCGTGTGGA
Barnhartia                    T1CTTTT-----CGTT-----AGYAGTTCYAAA------TTCGTTAYGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAS-CCGTGTGGA
Bredemeyera_altissima         T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Bredemeyera_colletioides      T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------TTATT-----CTTTCACAAA------CGGAT-CCGTATGGA
Bre_collLM241                 T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------TTATT-----CTTTCACAAA------CGGAT-CCGTATGGA
Bredemeyera_floribunda        T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Bredemeyera_lucida            T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Bredemeyera_microphylla       T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATATTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CAGAT-CCGTGTAGG
Bre_papNGF33220               -?--------------------------------------------------------------?-------------------------------------------------------
Carpolobia_alba               T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATTTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
'Carpolobia_aff._goetzii'     T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATTTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Comesperma_calymega           T0----------CATT-----AGTAGTTCCTAA------TTCGTTATCTTTC-----TCATT--1CATT----------CTCTT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Comesperma_hispidulum         T0----------CATT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATG----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Comesperma_secundum           T0----------CATT-----AGTAGTTCCAAA------TTTCTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Com_eriAM102                  T0----------CATT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATC----------ACAAA------CGGAT-CCGTGTGGA
Com_scoMDC9172                T0----------CATT-----AGTAGTTCCTAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCTTGTGGA
Com_volAM88                   T0----------CATT-----AGTAGTTCCTAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Com_rhaEAG8343                T0----------CATT-----AGTAGTTCCTAA------TTCGTTATGTTTC-----TCATT--1CCTT----------TTATT-----CTTTTACAAA------AGGAT-CCGTGTGAA
Diclidanthera_bolivarensis    T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Diclidanthera_pendulifera     T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------TGGAT-CCGTGTGGA
Monnina_crassifolia           T0----------CGTT-----ATTAGTTCCAAA------TTAGCTGTCTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Monnina_phillyreoides         T0----------CGTT-----ATTAGTTCCAAA------TTCGTTGTGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Monnina_hirta                 T0----------CGTT-----ATTAGTTCCAAA------TTCGTTGTGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Moutabea_aculeata             T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Moutabea_excoriata            T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Moutabea_guianensis           T1CTTTT-----CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAG------CAGAT-CCGTGTGGA
'Muraltia_aff._alopecuroides' G0----------AATT-----AGGAGTTCAAAA------TTAATTTTGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGG
Muraltia_heisteria            G0----------AATT-----AGGAGTTCAAAA------TTCATTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGG
Muraltia_mixta                G0----------AATT-----AGGAGTTCAAAA------TTCATTATGTTTC-----TTATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGG
Mur_heiAD                     G0----------AATT-----AGGAGTTCAAAA------TTCATTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGG
Nylandtia                     -?-------------------AGGAATTACAAA------TTCATTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Acanthocladus_guayaquilensis  T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTA-----TCATT--0--------------CTATT-----CTTTCACAAA------CGGAT-CCATGTGGA
Polygala_acuminata            T0----------CATT-----AGGAGTTCAAAA------TTCGTTATGTTTC-----TAATT--1CATT----------CTATT-----TTTTTACAAA------CGGAT-TCGTGTGGG
Polygala_arillata             T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-TCGTGTAGA
Polygala_chamaebuxus          T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----TTTTCACAAA------CGGAT-CCG------
Polygala_cuneata              T0----------CATT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------AGGAT-CCGTGTGGA
Polygala_erioptera            T0----------CATT-----AGGAGTTCCAAA------TTTGTTATGTTTC-----TCATT--0---------------TATT-----CTATCACAWA------AGGAT-TCGTGTGGA
Polygala_floribunda           T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TAATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGG
Polygala_fuertesii            T0----------CGTT-----AGTAGTTCAAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CAGAT-CCGTGTAGG
Polygala_hebeclada            T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TAATT--1TATT----------CTATT-----TTTTCACAAA------CGGAT-TCGTGTGGG
Polygala_klotzschii           T0----------CGTT-----AGTAGTTCCAAA------TTTGTTATGTTTA-----TCATT--0--------------CTATT-----CTTTCACAAA------CGGAT-CCATGTGGA
Polygala_lutea                T0----------CATT-----AGGAGTTCCAAA------TTCGTTATCTTTC-----TCATT--1CATT----------CCATT-----CTTTCACAAA------GGGAT-CCGCATGGA
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trnL/F alignment (continued)

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[                             961       971       981       991       1001      1011      1021      1031      1041      1051      1061      1071      ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Polygala_macradenia           T0----------CGTT-----AGTAGTTGCAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CGTTCACAAA------CAGAT-CCGTGTAGG
Polygala_microphylla          T0----------CATT-----AGGAGTTCCAAA------TTTGTTATGTTTC-----TCATT--1TATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Polygala_obscura              T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CAGAT-CCGTGTAGT
Polygala_oleifolia            -?--------------------------------------------------------------?-------------------------------------------------------
Polygala_lindheimeri          T0----------CATT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CGTTCACAAA------CGGAT-CCGTGTGGA
Polygala_penaea               T0----------CGTT-----AGTAGTTCAAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CAGAT-CCGTGTAGG
Polygala_rivinifolia          T0----------CGTT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--0--------------CTATT-----CTTTCACAAA------CAGAT-CCGTGTAGG
Polygala_semeiocardium        T0----------CATT-----AGGAGTTTCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-TCGTGTGGA
Polygala_senega               T0----------CATT-----AGGAGTTCCAAA------TTCGTTATCTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------GGGAT-CCGCATGGA
Polygala_subspinosa           T0----------CATT-----AGTAGTTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CCTTCACMAA------CGGAT-CCGTGTGGA
Polygala_tatarinowii          T0----------CATT-----AGGAATTCCAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Polygala_violacea             T0----------CATT-----AGGAGTTCCAAA------TTCGTTATGTTTC-----TAATT--1CATT----------CTATT-----TTTTCACAAA------CTGAT-TCGTGTGGG
Polygala_vulgaris             T0----------CATT-----AGGAGTTCAAAA------TGGGTTATGTTTC-----TCATT--1TATTTTTATT----CTATT-----CTTTCACAAA------TGGAT-CCGTGTGGA
Pol_exsRKH804                 T0----------CATT-----TGGAGTTCCAAA------TTGGTTATGTTTC-----TCATT--1TATT----------CTATT-----CTTTCACAAA------CAGAT-CCGTGTGGA
Pol_japLMC2903                T0----------CATT-----AGGAGTTCCAAA------TTTGTTATGTTTT-----TAATT--1TATT----------CTATT-----CTTTCACAAA------CACAA-CCGTGTGGA
Monnina_dictyocarpa           T0----------CGTT-----ATTAGTTATAAA------TTCGTTGTCTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------GGGAT-CCGTGTGAA
Monnina_leptostachya          T0----------CGTT-----ATTAGTTCCAAA------TTCGTTGTGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Monnina_pterocarpa            T0----------CGTT-----ATTAGTTCCAAA------TTCGTTGTGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Salomonia_cantoniensis        T0----------CATT-----AGGAGAAATAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CGTGCACAAA------CCGAT-CCGTGTGGA
Salomonia_ciliata             T0----------CATT-----AGGAGAAATAAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CGTGCACAAA------CCGAT-CCGTGTGGA
Sal_cilRKH1023                T0----------CATT-----AGGAGAAATCAA------TTCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CGTGCACAAA------CCGAT-CCGTGTGGA
Securidaca_diversifolia       T0----------CATT-----AGTAGTTCCAAA------TCCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Securidaca_longifolia         T0----------CATT-----AGTAGTTCCAAA------TCCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Securidaca_virgata            T0----------CATT-----AGTAGTTCCAAA------TCCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------CGGAT-CCGTGTGGA
Securidaca_welwitschii        T0----------CATT-----AGTGGTTCCAAA------TCCGTTATGTTTC-----TCATT--1CATT----------CTATT-----CTTTCACAAA------TGGAT-CCGTGTGGA

[                             1081      1091      1101      1111      1121      1131      1141      1151      1161      1171      1181      1191      ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine           ATTT0-TTC1TTTTTTC--TTTTC1ACAAACCTG-GAATATGTTT--------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Xanthophyllum_arnottianum     ATTT0-TTC0---------TTTTC1ACAAACCTG-GAATATGTTT--------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Xanthophyllum_octandrum       ATTT0-TTC0---------TTTTC1ACAAACCTG-GAATATGTTT--------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Xan_octBG7834                 ATTT0-TTC0---------TTTTC1ACAAACCTG-GAATATGTTT--------------------GGGATATA-----0------AATAT0-----------------11AGAA1A0--A
Xan_fraBG7836                 ATTT0-TTC0---------TTTTC1ACAAACCTG-GAATATGTTT--------------------GGAATATA-----0------AATAT0-AAATAT----------11AGAA1A0--A
Medicago_lupulina             ----?----?--------------?-----------------------------------------------------?-----------?-----------------??----?-?---
Guilfoylia_monostylis         ATTT0-TTC1TTTTTTTT-TTATC1ACAAGTCTTAGAATATGTTT--------------------GAAATTTG-----0-----TAATAT0-----------------0?--AT1A0--T
Phylica_polifolia             ----?----?--------------?-----------------------------------------------------?-----------?-----------------??----?-?---
Albizia_berteriana            ATTT0-TTC1TTTTT-C--TTATC1A??????-------?????T-------------------TGGAATATG-----1TAATGTAATAT0-----------------0?--AT1A0--T
Senna_pleurocarpa             ATTT0-TTC1TTTTT-C--TT---?--??------?????????T-------------------TGGAATATG-----1TAATGTAATAT0-----------------0?--AT1A0--T
Callistachys_lanc             ----?----?--------------?-----------------------------------------------------?-----------?-----------------??----?-?---
Dryas_drummondii              AATT0-TA-?-TTTT-C--TTATC?A???-???????----------------------------GTGTGATA-----0-----------?-----------------??----?-?---
Quillaja_saponaria            ATTT0-CTC1TTTTT-C--TTATC?A??????-----------?T--------------------GGAATATG-----0------TA-AT0-----------------11AGA-?-?---
Recchia_mexicana              ATTT0-TTC1TTTTT-C--TTATC?A??????-------?????T-------------------TGGAATATG-----0------TTTGA0------------A----11ATAT1G0--T
Fagus_sylvatica               AAT-?----?--------------?-----------------------------------------------------?-----------?-----------------??----?-?---
Monnina_insignis              CTTT0-TTC0---------ATTTC1ACAAACACC-GAATATGTTT--------------------GGAATAGA-----0------AATAT0-----------------11AGAA1A0--A
Monnina_malmeana              CTTT0-TTC0---------ATTTC1ACAAACACC-GAATATGTTT--------------------GGAATAGA-----0------AATAT0-----------------11AGAA1A0--A
Atroxima                      ATTT0-TTC0---------TTTTC1ACAAACCCC-GAATATGTTT--------------------TGAATATA-----0------AATAT0-----------------11AGAA1A0--G
Balgoya_pacifica              ATT?0-TTC1?T?TT-C--TTTTC1ACAAACCCC-GAATATGTTTGG?ATATAAAT?ATATGTTTGGAATMTA-----0------AATAT0-----------------11AGAA1A0--A
Barnhartia                    ATTT0-TAC1TTTTT-C--TTTTC1ACAAACCCC-GAATATGTTT--------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Bredemeyera_altissima         ATCT0-TTC0---------TTTTC1ACAAAACCC-GAATATGCTT--------------------GGAATTTA-----0------AATAG0-----------------11AGAA1A0--A
Bredemeyera_colletioides      CTTT1TTTC2T--------TTTTC0-CAAACCCC-GAATATGTTT--------------------GAAATATC-----0------AATAA0-----------------11AGCC1A0---
Bre_collLM241                 CTTT1TTTC2T--------TTTTC0-CAAACCCC-GAATATGTTT--------------------GAAATATC-----0------AATAA0-----------------11AGCC1A0---
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trnL/F alignment (continued)

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[                             1081      1091      1101      1111      1121      1131      1141      1151      1161      1171      1181      1191      ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Bredemeyera_floribunda        ATCT0-TTC0----------TTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATTTA-----0------AATAG0-----------------11AGAA1A0--A
Bredemeyera_lucida            ATCT0-TTC0----------TTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATTTA-----0------AATAG0-----------------11AGAA1A0--A
Bredemeyera_microphylla       ATTT0-TTC0----------TTTTC1AGAAACCCC-GAATATGTTC-------------------GGGATTTA-----0------AATAT0-----------------11AGAA1A0--A
Bre_papNGF33220               ----?----?---------------?----------------------------------------------------?-----------?-----------------??----?-?---
Carpolobia_alba               ATTT0-TTC1TTTTTT-C--TTTTC1ACAAACCCC-AAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
'Carpolobia_aff._goetzii'     ATTT0-TTC1TTTTTT-C--TTTTC1ACAAACCCC-GAATCTGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Comesperma_calymega           CTTT0-TTC0----------TTTTT1ACAAACCCC-GAATATCTTT-------------------AGAATATA-----0------AATAT0-----------------11AGAA1A0--G
Comesperma_hispidulum         CTTT0-TTC2T---------TTTTC0-CAAACACC-CAATCTGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Comesperma_secundum           C---?--TC0----------TTTTG0-CAAACCCC-AAATCTGTTT-------------------GCAATATAATATA0------AATAT0-----------------11AAAA1A0--A
Com_eriAM102                  CTTT0-TTC2T---------TTTTC0-CAAACACC-AAATCTGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Com_scoMDC9172                CTTT0-TTC0----------TTTTT1ACAAACCCC-GAATATGTTT-------------------CGAATATA-----0------AATAT0-----------------11GGAA1A0--G
Com_volAM88                   CTTT0-TTA0----------TTTTT1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Com_rhaEAG8343                CTTT0-TTC0----------TTTTT1ACAAATCCC-GAATATCTTT-------------------GGAATCTA-----0------AATAT0-----------------11AGAA1A0--A
Diclidanthera_bolivarensis    ATTT0-TTC1TTTTTT-C--TTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Diclidanthera_pendulifera     ATTT0-TTC1TTTTTT-C--TTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Monnina_crassifolia           CTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GGAATAGA-----0------AATAT1T----------------11AGAA1A0--A
Monnina_phillyreoides         CTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GGAATAGA-----0------AATAT1T----------------11AGAA1A0--A
Monnina_hirta                 CTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GGAATAGA-----0------AATAT1T----------------11AGAA1A0--A
Moutabea_aculeata             ATTC0-TTC1TTTTTT-C--TTTTC1ACAAACTCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Moutabea_excoriata            ATTC0-TTC1TTTTTT-C--TTTTC1ACAAACTCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Moutabea_guianensis           ATTT0-TTC1TTTTTT-C--TTTTC1ACAAACCCC-GAATATGTTTGGAATAT-----TATGTTTGGAATATA-----0------AATAT0-----------------11AGAA1A0--A
'Muraltia_aff._alopecuroides' TTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GAAATATA-----0------AATAT0-----------------11AGAA1A0--A
Muraltia_heisteria            TTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GAAATATA-----0------AATAT0-----------------11AGAA1A0--A
Muraltia_mixta                TTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GAAATATA-----0------AATAT0-----------------11AGAA1A0--A
Mur_heiAD                     TTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATGTTT-------------------GAAATATA-----0------AATAT0-----------------11AGAA1A0--A
Nylandtia                     CTTT0-TTC0----------TTTTC1GCAAACCCT-GAATATTTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Acanthocladus_guayaquilensis  ATTT0-CTC1TTTTTT-C--TTTTC1ACAAACCCC-GAATATGCTT-------------------GGAATTTA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_acuminata            CTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATATTT-------------------GGGATATA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_arillata             CTTT0-TTC0----------TTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11ATAA1A0--A
Polygala_chamaebuxus          ----?----?---------------?----------------------------------------------------?-----------?-----------------??----?-?---
Polygala_cuneata              CTTT0-TTC0----------TTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_erioptera            CTTT0-TTC0----------TTTTY1ACAAAMTCC-GRAHATATTT-------------------GGRATWTA-----0------AATAT0-----------------11AGAA0-?---
Polygala_floribunda           CTTT0-TTC0----------TTTTT1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11ATAA1A0--A
Polygala_fuertesii            ATTT0-TTC0----------TTTTC1ACAAACCCC-GAATATGCTT-------------------GGGATTTA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_hebeclada            CTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATATTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_klotzschii           ATTT0-CTC1TTTTTT-C--TTTTC1ACAAACCCC-GAATATGCTT-------------------AGAATTTA-----0------AATAT0-------AGAAAT----11AGAA1A0--A
Polygala_lutea                CTTT1GTTC0----------TTTTC1ACAAACCCC-CA----TTTT-------------------GGAATAAA-----0------AATGT0-----------------11AGAA0-?---
Polygala_macradenia           ATTT0-TTC0----------TTTTC1ACAAACCTC-GAATATGCTT-------------------GGGATTTA-----0------AATAT0-----------------11ATAA1A0--A
Polygala_microphylla          CTTT0-TTC0----------TTTTC1ACAAACCCC-GAATCTATTT-------------------GGAATATA-----0------AATAT0-----------------?0----?-?---
Polygala_obscura              ATTT0-TTC0----------TTTTC1ACAAACTTC-AAATATGCTA-------------------TTTATTTA-----0------AATAT0-----------------11ATAA1A0--A
Polygala_oleifolia            ----?----?---------------?----------------------------------------------------?-----------?-----------------??----?-?---
Polygala_lindheimeri          CTTT0-TTC1TTTT--CTCTTTTC1ACAAACCCC-GAATATGTTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_penaea               ATTT0-TTC0----------TTTTC1ACAAACCCC-GAATATGCTT-------------------GGGATTTA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_rivinifolia          ATTT0-TTC0----------TTTTC1ACAAACTTC-AAATATGCTA-------------------TTTTTTTA-----0------AATAT0-----------------11ATAA1A0--A
Polygala_semeiocardium        CTTT0-TTC0----------TTTTC1ACAAACCCC-GAGTTTGGAA-------------------TATAAATA-----0------TATAA0-----------------??----?A?--A
Polygala_senega               CTTT1GTTC0----------TTTTA1ACAAACCCC-TAATATGTTT-------------------GGAATAAA-----0------AATGT0-----------------11AGAA1A0--A
Polygala_subspinosa           CTTT0-TTC0----------TTTTT1ACAAACCCC-G?ATATGTTT-------------------GGAATGTA-----0------AATAT0-----------------11GGAA1A0--A
Polygala_tatarinowii          CTTT0-TTC0----------TTTTC1ACAAACCCC-GAATCCGTTT-------------------GGAATAGA-----0------AATAT0-----------------11ATAA1A0--G
Polygala_violacea             CTTT0-TTC0----------TTTTC1ACAAACCCT-GAATATATTT-------------------GGAATATA-----0------AATAT0-----------------11AGAA1A0--A
Polygala_vulgaris             CTTT0-TTA0----------TTTTC1ACAAACCCC-GAATCTATTT-------------------GGAATAGA-----0------AATAT0-----------------?0----?-?---
Pol_exsRKH804                 CTTT0-TTC0----------TTTTC1ACAAATCCC-GAATCTATTT-------------------GGAATAGA-----0------TATAT0-------------AT--11AGAA0-?---
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trnL/F alignment (continued)

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[                               1081      1091      1101      1111      1121      1131      1141      1151      1161      1171      1181      1191      ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Pol_japLMC2903                  CTTT0-TTC0---------TTTTC1ACAAACCCC-GAATATATTT--------------------GGAATATA-----0------AATAT0-AAATAT----------11AGAA0-?---
Monnina_dictyocarpa             CTTT0-TTC0---------TTTTT1ACAAACCCC-TAATATGTTT--------------------GGAATAGA-----0------AATAT1T----------------11AGAA1A0--A
Monnina_leptostachya            CTTT0-TTC0---------TTTTC1ACAAACCCT-GAATATGTTT--------------------GGAATAGA-----0------AATAT1T----------------11AGAA1A0--A
Monnina_pterocarpa              CTTT0-TTC0---------TTTTC1ACAAACCCT-GAATATGTTT--------------------GGAATAGA-----0------AATAT1T----------------11AGAA1A0--A
Salomonia_cantoniensis          CTTT0-TTT0---------TTTTT1ACAAACCCC-GAATATGGTT--------------------GTAATAGA-----0------AATCT0-----------------11ATAT1A1AAA
Salomonia_ciliata               CTTT0-TTT0---------TTTTT1ACAAACCCC-GAATATGGTT--------------------GTAATAGA-----0------AATCT0-----------------11ATAT1A1AAA
Sal_cilRKH1023                  CTTT0-TTT0---------TTTT-1ACAAACCCC-GAATATGTTT--------------------GTAATAGA-----0------AATCT0---------------AT11ATAA1A0--A
Securidaca_diversifolia         CTTT0-TTA0---------TTTTC1ACAAACCCC-GAATATGTTT--------------------GTAATATA-----0------AATAT0-----------------11AGAA1A0--A
Securidaca_longifolia           CTTT0-TTT0---------TTTTC1ACAAACCCC-GAATATGTTT--------------------GTAATATA-----0------AATAT0-----------------11AGAA1A0--A
Securidaca_virgata              TTTT0-TTA0---------TTTTC1ACAAACCCC-GAATATGTTT--------------------GTAATATA-----0------AATAT0-----------------11AGAA1A0--A
Securidaca_welwitschii          CTTT0-TTA0---------TTTTC1ACAAACCCC-GAATATGTTT--------------------GTAATATA-----0------AATAT0-----------------11AGAA1A0--A

[                               1201      1211      1221      1231      1241      1251      1261      1271      1281      1291      1301      1311      ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine             -------GATA0--------------------TATGATA0-----CATGTAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAG0------TAATT
Xanthophyllum_arnottianum       -------AATA0--------------------TATGATA0-----CATGTAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAG0------TAATT
Xanthophyllum_octandrum         -------AATA0--------------------TATGATA0-----CATGTAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAG0------TAATT
Xan_octBG7834                   -------AATA0--------------------TATGATA0-----CATGTAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAG0------TAATT
Xan_fraBG7836                   -------AATA0--------------------TATGATA0-----CATGTAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAG0------TAATT
Medicago_lupulina               -----------?---------------------------?------------?--------?------------------------?--?------------------?-----------
Guilfoylia_monostylis           -------GATA0---------------------------?-----CACGTAC1AAATAAAA0-----ATATTTGAGCAAAGAATCC1CC0-----------ATATGAA0------TAATT
Phylica_polifolia               -------TATA0--------------------TATGATA0-----CACGTAC1AAATGAAC0-----ATCATTTAGCAAGTAACCC0-C0-----------GATTG--?------TAAAT
Albizia_berteriana              -------GATA1GACGTAAAATTAGGTTTCTATATGATA0-----AATGTAC1AAATGAGC1A-CTTATCTTTGAGCAAGGAATCC1TC0-----------ATTTGAC0------TGATT
Senna_pleurocarpa               -------GATA1GACGTAAGATTTTGTTTCTATATGATA0-----AACGTAC1AAATGAAC1ATCTTATCTTTGAGCAAGGAATCC1TC0-----------ATTTGAA0------TGATT
Callistachys_lanc               -----------?---------------------------?------------?--------?------------------------?--?------------------?-----------
Dryas_drummondii                -------TATA0--------------------TATGATA0-----TGCGTAT1AAATGAAC0-----ATCTTTGAGTAAGGAATCC1CC------------ATTTAAA1TTTGAATAATT
Quillaja_saponaria              -----------?--------------------TATGATA0-----TACGTAC1AAATGAGC0-----ATCTTTGCGCGAGGAATCC1TC0-----------ATTTAAA0------TGATT
Recchia_mexicana                -------AATA0------------------TATATGATA0-----CACATAC1AAATAAAC0-----ATATTTGAGCAAAGAATCC1CC0-----------ATATGAA0------TGATT
Fagus_sylvatica                 -----------?---------------------------?------------?--------?------------------------?--?------------------?-----------
Monnina_insignis                -------GATA0--------------------TATAATA0-----CGCGGAC1AAATGGAC0-----AGCTTTGATAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Monnina_malmeana                -------GATA0--------------------TATAATA0-----CGCGGAC1AAATGGAC0-----AGCTTTGATAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Atroxima                        -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Balgoya_pacifica                -------GATA0--------------------TATGATA0-----CATGGMC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Barnhartia                      -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Bredemeyera_altissima           -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----A----TGAGAAAGGAATCC0-G0-----------ATTTGAA0------TAATT
Bredemeyera_colletioides        ----TATAATA0--------------------TATG-TT0-----CAAATGA1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTGAATAATT
Bre_collLM241                   ----TATAATA0--------------------TATG-TT0-----CAAATGA1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTGAATAATT
Bredemeyera_floribunda          GATATATGATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Bredemeyera_lucida              -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Bredemeyera_microphylla         -------GATA0--------------------TATGATA0-----CATGGAC1AAACGAGC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Bre_papNGF33220                 -----------?---------------------------?------------?--------?------------------------?--?------------------?-----------
Carpolobia_alba                 -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
'Carpolobia_aff._goetzii'       -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Comesperma_calymega             -------CCTA0--------------------TTTAATA0-----CACGTAC1AAATGCAT0-----ATCTTTGAGAAAGGAATTC0-C0------TATTGAATTGAA0------TAATT
Comesperma_hispidulum           -------GATA0--------------------TTTAATA0-----CACAGAC1AAGTAGAT0-----ATCTTTGAGAAAGG-----0--?-------------CTGAA0------TAATT
Comesperma_secundum             -------GAGA0--------------------TTTAATA0-----CACAGAC1AAGTAGAT0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATCTGAA0------TAATT
Com_eriAM102                    -------GATA0--------------------TTTAATA0-----CACAGAC1AAGTAGAT0-----ATCTTTGAGAAGGGAATCC0-C0-----------ATCTGAA0------TAATT
Com_scoMDC9172                  -------GATA0--------------------TTTAATA0-----CACGTAC1AAATAGAT0-----ATCTTTGAGAAAGGAATTC0-C0-----------ATTTGAA0------TAATT
Com_volAM88                     -------GATA0--------------------TTTAATA0-----CACGGA-1AAATGGAT0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Com_rhaEAG8343                  -------GATA0--------------------TTTAATA0-----CACAGAC1AAATGGAT0-----ATCTTTGAGAAAGGGATCC0-C0-----------ATTTGAA0------TAATT
Diclidanthera_bolivarensis      -------GATA0--------------------TATGATA0-----CATGGGC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
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trnL/F alignment (continued)

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[                               1201      1211      1221      1231      1241      1251      1261      1271      1281      1291      1301      1311      ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Diclidanthera_pendulifera       -------GATA0--------------------TATGATA0-----CATGGGC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Monnina_crassifolia             -------GATA0--------------------TATAATA0-----CGCGGAC1AAATGGAC0-----AGCTTTGAGAAAGGAATCC0-C0-----------GTTTGAA0------TAATT
Monnina_phillyreoides           -------GATA0--------------------TATAATA0-----CGCGGAC1AAATGGAC0-----AGCTTTGAGAAAGGAATCC0-C0-----------GTTTGAA0------TAATT
Monnina_hirta                   -------GATA0--------------------TATAATA0-----CGCGGAC1AAATGGAC0-----AGCTTTGAGAAAGGAATCC0-C0-----------GTTTGAA0------TAATT
Moutabea_aculeata               -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Moutabea_excoriata              -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Moutabea_guianensis             -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
'Muraltia_aff._alopecuroides'   -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAC0-----ATCTTTGAGAAAAGAATCC0-C1ATTTTT-----ATTTGAA0------TAATT
Muraltia_heisteria              -------GATA0--------------------TATAATA0-----CATGTAC1AAATGGAC0-----ATCTTTGAGAAAAGAATCC0-C1ATTTTT-----ATTTGAA0------TAATT
Muraltia_mixta                  -------GATA0--------------------TATAATA0-----CATGTAC1AAATGGAC0-----ATCTTTGAGAAAAGAATCC0-C1ATTTTT-----ATTTGAA0------TAATT
Mur_heiAD                       -------GATA0--------------------TATAATA0-----CATGTAC1AAATGGAC0-----ATCTTTGAGAAAAGAATCC0-C1ATTTTT-----ATTTGAA0------TAATT
Nylandtia                       -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAC0-----ATACTTGAGAAAGGAATCC0-C1ATTTTG-----ATTTGAA0------TAATT
Acanthocladus_guayaquilensis    -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_acuminata              -------GATA0--------------------TRTAATA0-----CATGGAC1AAATGGAC0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_arillata               -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTTAATAATT
Polygala_chamaebuxus            -----------?---------------------------?------------?--------?------------------------?--?-----------TTTTGAA0------TAATT
Polygala_cuneata                -------GATA0--------------------TATAATA0-----CACGGAC1AAATGGAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_erioptera              ---------TA0--------------------TATAATA0-----CATGGAM0--------?-----ATCTTTGVGAAAGGAATCC0-C0-----------ATTTGAA1TTTCAATAATT
Polygala_floribunda             -------GATA0--------------------TATAATA0-----CATGGAC1AAATTAAT0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_fuertesii              -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TGATT
Polygala_hebeclada              -------GATA0--------------------TATAATA0-----CATGAAC1AAATGTAC0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_klotzschii             -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_lutea                  ---------TA0--------------------TATAATA0-----CATGTAC1AAATAGAT0-----ATCTTTCACAAAGTAATTC0-T0-----------ATTTGAA1TATGAATTATT
Polygala_macradenia             -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAGC0-----ATCTTTGATAAAGGAATCC0-C0-----------ATTTGAA0------TGATT
Polygala_microphylla            -----------?---------------------ATAATA0-----CATGGAC0--------?-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTGAATAATT
Polygala_obscura                -------GATA0--------------------TATGATA0-----CATGGAC1AAATTAAT0-----ATCTTTGATAAAGGAATCC0-C0-----------ATTTGAA0------TGATT
Polygala_oleifolia              -----------?---------------------------?------------?--------?------------------------?--?------------------?-----------
Polygala_lindheimeri            -------GGTA0--------------------TATAATA0-----CACGGAC1AAATGGAC0----------TGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_penaea                 -------GATA0--------------------TATGATA0-----CATGTAC1AAATGAGC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TGATT
Polygala_rivinifolia            -------GATA0--------------------TATGATA0-----CATGGAC1AAATGAAT0-----ATCTTTTATAAAGAAATCC0-C0-----------ATTTGAA0------TGATT
Polygala_semeiocardium          -------GATA0--------------------TATAATA0-----TCTGGAC1AAATGGAC0-----ATCTTTGAGAAAGTAATCC0--?-----------ATTTGAA1TTTGAATAATT
Polygala_senega                 -------GATA0--------------------TATAATA0-----CATGTAC1AAATGGAC0-----ATCTTTGAGAAAGGAATTC0-G0-----------ATTTGAA1TATGAATTATT
Polygala_subspinosa             -------GGTA0--------------------TATAATA0-----CACGGAC1AAATGGAC0-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_tatarinowii            -------GATA0--------------------TATAATA0-----CATGTAC1AAATGTAC0-----ATCTTTGAGAAAGTAATCC0-C0-----------ATTTTAA1TTTTAATAATT
Polygala_violacea               -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAC0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Polygala_vulgaris               -----------?---------------------ATAATA0-----CATGGAC0--------?-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTGAATAATT
Pol_exsRKH804                   ---------TA0--------------------TATAATA0-----CATGGAC0--------?-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTGAATAATT
Pol_japLMC2903                  ---------TA0--------------------TATAATA0-----CATGGAC0--------?-----ATCTTTGAGAAAGGAATCC0-C0-----------ATTTGAA1TTTGAATAATT
Monnina_dictyocarpa             -------GATA0--------------------TATAATA1TAATACCCGGAC1AAATGGAC0-----AGCTTTGAGAAAGGAATCC0-C0-----------GTTTGAA0------TAATT
Monnina_leptostachya            -------GATA0--------------------TATAATA1TAATACGCGGAC1AAATGGAC0-----AGCTTTGAGAAAGGAATCC0-C0-----------GTTTGAA0------TAATT
Monnina_pterocarpa              -------GATA0--------------------TATAATA1TAATACGCGGAC1AAATGGAC0-----AGCTTTGAGAAAGGAATCC0-C0-----------GTTTGAA0------TAATT
Salomonia_cantoniensis          -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAT0-----ATCGTTGATAAATGAATCC0-G1ATTTTC-----ATTTGAA1TTTGCATAATT
Salomonia_ciliata               -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAT0-----ATCGTTGATAAATGAATCC0-G1ATTTTC-----ATTTGAA1TTTGCATAATT
Sal_cilRKH1023                  -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAT0-----ATCGTTGATAAAGGAATCC0-G1ATTTTC-----ATTTGAA1TTTGAATAATT
Securidaca_diversifolia         -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAT0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Securidaca_longifolia           -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAT0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Securidaca_virgata              -------GATA0--------------------TATAATA0-----CATGGAC1AAATGGAT0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTGAA0------TAATT
Securidaca_welwitschii          -------GATA0--------------------TAGAATA0-----CATGGAC1AAATGGAT0-----ATCCTTGAGAAAGGAATCC0-C0-----------ATTTTAA0------TAATT
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[                             1321      1331      1341      1351      1361      1371      1381      1391      1401      1411      1421      1431      ]
[                             |         |         |         |         |         |         |         |         |         |         |         |         ]
Xanthopyllum_affine           ACCAAC--CCATATCATTAATC1GTACT0-----GAAACTC-0GAAGATCCAA--AAGATTACG-----0-----------AGACTTTGTAATACTGT----AATGGAA-CGTCTTTTT-
Xanthophyllum_arnottianum     ACCAAC--ACATATCATTACTC1GTACT0-----GAAACTC-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGTAATACTGT----AATGGAA-CGTCTTTTTA
Xanthophyllum_octandrum       ACCAAC--CCATATCATTAATC1GTACT0-----GAAACTC-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGTAATACTGT----AATGGAA-CGTCTTTTT-
Xan_octBG7834                 ACCGAC--ACATATCATTACTC1GTACT0-----GAAACTC-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGTAATACTGT----AATGGAA-CGTCTTTTTA
Xan_fraBG7836                 ACCAAC--ACATATCATTACTC1GTACT0-----GAAACTC-0GAAGATCCAA--AAAATTGCG-----0-----------AGACTTTGTAATACTGT----AATGGAA-CGTCTTTTTT
Medicago_lupulina             ----------------------?-----?-------------?--------------------------?--------------------------------------------------
Guilfoylia_monostylis         AAGAAT--ACATATCATTACTC1GTACT0-----GAAACTC-0TAAGATCCAA--GAAATTCCA-----1GAACCTGGATAAGAATTTGTAATA????----??------CGTCTTTTT-
Phylica_polifolia             TGGAATGGTCATATTATCGCTC1GTACT1GTATTGAAACTT-0GAAGATCTAA--GAAATTCCACCA--1AGGCCTGGATAAGACTTTGTAAT-----------------CGTCTTTTT-
Albizia_berteriana            AACAAT--ACATATCATTACTC1CGACT0-----GAAACATG0GAAGATCCAA--GAAATTCCA-----1GGGCTTGGATGAAACTTTATAAT-----------------CGTCTTTTT-
Senna_pleurocarpa             AACAAT--ACATATCATTACTC1CGACT0-----GAAACTT-0GAAGATCCGA--GAAATTCCA-----1GGGCTTGGATAAGACTTTGTAATA----------------CGTCTTTTTA
Callistachys_lanc             ----------------------?-----?-------------?--------------------------?----------------------------------------CGTGTTTTTT
Dryas_drummondii              AACAAT--ACATACCATTACTT1GTACT1GTACTGAAACTT-0GAAGATCCAA--TAAATTCTA---A-1GGGTCTGGATAATACTTTGTAATACTTT----AA------CGTTTTTTT-
Quillaja_saponaria            AACAAT--ACATATCATTGCTC1GTACT0-----GAAACTT-0GAAGATCCAAAAGAAATTCCA-----1GGGTCCGGATAAACCTTTGTAATACTTT----AA------CGTCTTTTT-
Recchia_mexicana              AAAAAT--ACATATCATTACTC1GTACT0-----GAAACTT-0GAAGATCCAA--GAAATTCCA-----1GGGCCTGGATAAGACTTTGTAATACTGT----AA------CGTCTCTTT-
Fagus_sylvatica               ----------------------?-----?-------------?--------------------------?----------------------------------------CTTTTTTTCT
Monnina_insignis              ACCAAT--ACATATAATTACTC1GTACT0-----GAAACGA-0TAAGATACAA--AAAATTAAG-----0-----------ATACTTTTTTTTACTAT----AATAGAA-AGTCTTTTT-
Monnina_malmeana              ACCAAT--ACATATAATTACTC1GTACT0-----GAAACGA-0TAAGATACAA--AAAATTAAG-----0-----------ATACTTTTTTTTACTAT----AATAGAA-AGTCTTTTT-
Atroxima                      ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATGACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Balgoya_pacifica              ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTT-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Barnhartia                    ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTT-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATGCTGT----AATGGAA-CGTCTTTTT-
Bredemeyera_altissima         ACCATA--ACATATCATCACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Bredemeyera_colletioides      ACCAAT----ATACCATTACCC1GTACT0-----GAAACTA-0TAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTTA
Bre_collLM241                 ACCAAT----ATACCATTACCC1GTACT0-----GAAACTA-0TAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTTA
Bredemeyera_floribunda        ACCAAT--CCATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAGATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Bredemeyera_lucida            ACCAAT--CCATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAGATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Bredemeyera_microphylla       ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AGAATTACT-----0-----------AGACTCTGGAATACTGTATGGAATGGAA-CTTCTTTTT-
Bre_papNGF33220               ----------------------?-----?-------------?--------------------------?--------------------------------------------------
Carpolobia_alba               ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATGCTGT----AATGGAA-CGTCTTTTT-
'Carpolobia_aff._goetzii'     ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GACGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATGCGGT----AATGGAA-CGTCTTTTT-
Comesperma_calymega           ACCAAT--ACACATCATTACTC1GTACT0-----GAAAAAA-0GAAGATCCAA--AAAATTACA-----0-----------AGACTTTGGAATACTCT----AACGGAA-CGTCTTTTT-
Comesperma_hispidulum         ACCAAT--ATACATCATTACTC1GTACT0-----GAAAAAA-0GAAGAYCCAA--AAAATTMSR-----0-----------RGACTTTGGAAWACYYY----AWTGGAATCGTCTTTTT-
Comesperma_secundum           ACCAAT--ATACATCATTACTC1GTACT0-----GAAAAAA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACCCT----AATGGAATCGTCTTTTT-
Com_eriAM102                  ACCAAT--ATACATCATTACTC1GTACT0-----GAAAAAA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTTT----AATGGAA-CTTTTTTTT-
Com_scoMDC9172                ACCAAT--ACACACCATTACTC1GTAAT0-----GAAAAAT-0GAAGATCCAA--AAAATTCCA-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Com_volAM88                   ACCAAT--ACACATCATTACTC1GTACT0-----GAAAAAA-0TAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTATTTTT-
Com_rhaEAG8343                ACCAAT--ACACACGATTACTC1GTACT0-----GAAAAAA-0GAAGATCCAA--AAAATGACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Diclidanthera_bolivarensis    ACCAAT--CCATATCATTACTC1GTACT0-----GAAACTT-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Diclidanthera_pendulifera     ACCAAT--CCATATCATTACTC1GTACT0-----GAAACTT-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Monnina_crassifolia           ACCAAT--ACATATCATTACTC1GTACT0-----GAAACGA-0GAAGATACAA--AAAATTATG-----0-----------AGACTTTGGAATATTCT----AATGGAA-CGTCTTTTT-
Monnina_phillyreoides         ACCAAT--ACATATCATTACTC1GTACT0-----GAAACGA-0GAAGATACAA--CAAATTACG-----0-----------AGACTTTGGAATATTCT----AATGGAA-CGTCTTTTT-
Monnina_hirta                 ACCAAT--ACATATCATTACTC1GTACT0-----GAAACGA-0GAAGATACAA--AAAATTACG-----0-----------AGACTTTGGAATATTCT----AATGGAA-CGTCTTTTT-
Moutabea_aculeata             ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAGGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Moutabea_excoriata            ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAGGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Moutabea_guianensis           ACCAAT--ACAGATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
'Muraltia_aff._alopecuroides' ACCAAT--ACATATCATTACCC1GTACT0-----GAAACTA-1---AATCCAA--AAAATTACG-----0-----------AGACTTTGGWAWACTCT----AATGGGA-CATCTTTTT-
Muraltia_heisteria            ACCAAT--ACATATCATTACCC1GTACT0-----GAAACTA-1---WWTYYAA--WMMAWWAMR-----0-----------WKACKWKRSTWTRSTMT----AMTSKAA-YWTYWKTTK-
Muraltia_mixta                ACCAAT--ACATATCATTACCC1GTACT0-----GAAACTA-1---AATCCAA--AAAATTACG-----0-----------AGACTTTGGTATACTCT----AATGGAA-CATCTTTTT-
Mur_heiAD                     ACCAAT--ACATATCATTACCC1GTACT0-----GAAACTA-1---AATCCAA--AAAATTACG-----0-----------AGACTTTGGTATACTCT----AATGGAA-CATCTTTTT-
Nylandtia                     ACCAAT--ACATATCATTACCC1GTACT0-----GAAACTA-0TAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGTATACTCT----AATGGAA-CGTCTTTTT-
Acanthocladus_guayaquilensis  ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAATATCGAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Polygala_acuminata            ACCMAT--ACATATCATTACTC1GT---?-------------?---------A--AAAATTACS----A0-----------AGACTTTGGAATACTCA----AATGGAA-CGTCTTTTT-
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trnL/F alignment (continued)

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[                               1321      1331      1341      1351      1361      1371      1381      1391      1401      1411      1421      1431      ]
[                               |         |         |         |         |         |         |         |         |         |         |         |         ]
Polygala_arillata               ACCAAT--ACATATCATTACTC0-TACT0-----GAAACTC-?--------------------------?---------------------------------ATGGAA-CGTCTTTTT-
Polygala_chamaebuxus            ACCAAT--ACATATCATTACCC0-TATT0-----GAAACTA-?--------------------------?-------------------------TCT----TATGGAA-CGTCTTTTT-
Polygala_cuneata                ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTC-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGAAATACTCC----AATGGAA-CGTCTTTTT-
Polygala_erioptera              ACCAAT--A?ATATCATTACCC1CTACT0-----GAAACTA-0GAAGATCTAA--AAAATTACG-----0-----------AGACTTTGGAATACTCG----AATGGAA-TKTCTTTTT-
Polygala_floribunda             ACCAAT--ACATATCATTACTC1GT---?-------------?---------A--AAAATTACG-----0-----------AGACTTCGGAATACTCA----AATGGAA-CGTCTTTTT-
Polygala_fuertesii              ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AGAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CTTCTTTTT-
Polygala_hebeclada              ACCAAT--ACATATAATTTTTC1GT---?-------------?---------A--AAAATTACG-----0-----------AGACTTTGGAATACTCA----AATGGAA-CGTCTTTTT-
Polygala_klotzschii             ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CGTCTTTTT-
Polygala_lutea                  ACCAAT--ACATATTATTATCC1GTACT0-----CAAACTA-0GAAGATACAA--AAAATTACG-----0-----------AGACTTTGCAATACTCT----AATGGAA-CATCTTTTT-
Polygala_macradenia             ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AGAATTACG-----0-----------CGACTTTGGAATACTGT----AATGCAA-CTTCTTTTT-
Polygala_microphylla            ACCAAT--A-ATATCATTACCC1CTACT0-----GAAACTA-0GAAGATCTAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-TGTCTTTTT-
Polygala_obscura                ATCAAT--ACATATCATTACTC1GTACT0-----GAAAATA-0GAAGATCCAA--AGAATTATG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CTTCTTTTT-
Polygala_oleifolia              ----------------------?-----?-------------?--------------------------?--------------------------------------------------
Polygala_lindheimeri            ACCAAT--ATAGATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Polygala_penaea                 ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AGAATTACG-----0-----------AGACTTTGGAATACTGT----AATGGAA-CTTCTTTTT-
Polygala_rivinifolia            ATCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--ATAATTACG-----0-----------AGACTTT???----TGT----AATGGAA-CTTCTTTTT-
Polygala_semeiocardium          ACCAAT--ACATACCATTACCC0-TACT0-----GAAACT--0GAAGATCAAA--AAAATTATG-----0-----------AGTATTTGTAATAGTCT----AATGGAA-CGTCTTTTT-
Polygala_senega                 ACCAAT--ACATATTATTATCC1TTACT0-----CAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------ATACTTTGCAATACTCT----AATGGAA-CGTCTTTTT-
Polygala_subspinosa             ACCAAT--ATATATAATTACTC1GTACT0-----GAAACTA-0GAAGATCCCA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Polygala_tatarinowii            ACCAAT--ACATACCATTACCC0-TACT0-----GAAACTC-0TAAGATCAAA--AAAATTACG-----0-----------AGTATTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Polygala_violacea               ACCAAT--ACATATCATTACTC1GT---?-------------?---------A--AAAATTACG-----0-----------ATACTTTGGAATACTCC----AATGGAA-CGTCTTTTT-
Polygala_vulgaris               ACCAAT--ACATATCATTACCC1CTACT0-----GAAACTA-0GAAGATCTAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-GGTCTTTTT-
Pol_exsRKH804                   ACCAAT--ACATATCATTACCC1CTACT0-----GAAACTA-0GAAGATCTAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Pol_japLMC2903                  ACCAAT--ACATATCATTACCC1CTACT0-----GAAACTA-0GAAGATCTAA--AAAATTACG-----0-----------AGACATTGGAATACTCT----AATGGAA-CGTCTTTTT-
Monnina_dictyocarpa             ACCAAT--ACATATCATTACTC1GTACT0-----GAAACGA-0GAAGATACAA--AAAATTACG-----0-----------AGACTTTAGAATACTCT----AATGGAA-TGTCTTTTT-
Monnina_leptostachya            ACCAAT--ACATATCATTACTC1GTACT0-----GAAACGA-0GAAGATACAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Monnina_pterocarpa              ACCAAT--ACATATCATTACTC1GTACT0-----GAAACGA-0GAAGATACAA--AAAATTACG-----0-----------AGACTTTGGAATACTCT----AATGGAA-CGTCTTTTT-
Salomonia_cantoniensis          ACCAAT--ACATATCATTATCT1GTACT0-----GAAACG--0GAAAATCCAC--AAAATGACG-----0-----------AGACTTTGGAATACTCT----AATAGAA-CGTCTTTTT-
Salomonia_ciliata               ACCAAT--ACATATCATTATCT1GTACT0-----GAAACG--0GAAAATCCAC--AAAATGACG-----0-----------AGACTTTGGAATACTCT----AATAGAA-CGTCTTTTT-
Sal_cilRKH1023                  ACCAAT--ACATATCATTATCT1GTACT0-----GAAACGA-0GAAAATCCAC--AAAATGACG-----0-----------AGACTTTGGAATACTCT----AATAGAA-CGTCTTTTT-
Securidaca_diversifolia         ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATATTCT----AATGGAA-CGTCTTTTT-
Securidaca_longifolia           ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATATTCT----AATGGAA-CGTCTTTTT-
Securidaca_virgata              ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTACG-----0-----------AGACTTTGGAATATTCT----AATGGAA-CGTCTTTTT-
Securidaca_welwitschii          ACCAAT--ACATATCATTACTC1GTACT0-----GAAACTA-0GAAGATCCAA--AAAATTAAG-----0-----------AGACTTTGGAATATTAT----AATGGAA-CGTCTTTTT-

[                               1441      1451      1461      1471      1481      1491      1501      1511      1521    ]
[                               |         |         |         |         |         |         |         |         |       ]
Xanthopyllum_affine             -----AGTTGACATATATCCAAGTAATCACTTAAAA1TGAAAC0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Xanthophyllum_arnottianum       TTTTTAGTTGACATATATCCAAGTAATCTCTTAAAA1TGAAAC0---------TGAA1GATGATACATC-AGGAATGG-TCGGG
Xanthophyllum_octandrum         -----AGTTGACATATATCCAAGTAATCACTTAAAA1TGAAAC0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Xan_octBG7834                   TTTTTAGTTGACATATATCCAAGTAATCTCTTAAAA1TGAAAC0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Xan_fraBG7836                   TTTTTAGTTGACATATATCCAAGTAATCTCTTAAAA1TGAAAC0---------TGAA1GATGATGCWTC-AGGAATGG-TCGGG
Medicago_lupulina               -----AGTTGACATAGACTCAAGTAATTTCCTAAAA0------?---------TTAG1GGTGGTTTGTC-AAGAATGG-TCGGG
Guilfoylia_monostylis           -----AGTTGACATAGACCCAAGTAATCCCTTAAAA1TTAAAA0---------TGAG1GATGATGCATC-AGGAATGGGTCGGG
Phylica_polifolia               -----AATTGACATAGACCCAAGTCATCTATTAAAA0------?---------TGAG1GATGATGCGTC-GTGACTGG-TCGGG
Albizia_berteriana              TTTTTAGTTGACATATACTCAAGTAATTTCTTAAAA0------?---------TGAG1GATGATGCGTC-ACGAAAGG-TCGGG
Senna_pleurocarpa               TTT--AATTGACATATACTCAAGTAATCTCTTAAAA0------?---------TGAG1GATGATGCGTC-ACGAATGG-TCGGG
Callistachys_lanc               T----CGTTGACATAGATTAAAGTAATCTAATAAAA0------?---------TGAG1TAGGATGCGTC-AAGAATGG-TCGGG
Dryas_drummondii                -----AATTGACATAGACCTAAGTCCTATATTAAAA1TAAAA-0---------TGAG1GCTGATGCGTC-GTGAATGG-TCGGG
Quillaja_saponaria              -----AGTTGACATACATCCAAGTAATTAATTAAAA1TGAAAA0---------TGAG1GATGCTGCGCC-AAGAATGG-TCGGG
Recchia_mexicana                TT---AGTTGACATACATCCCAGTAATCTCATAAAA0------?---------TGAG1GATGACGCATCCAGGAATGG-TCGGG
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trnL/F alignment (continued)

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[                             1441      1451      1461      1471      1481      1491      1501      1511      1521    ]
[                             |         |         |         |         |         |         |         |         |       ]
Fagus_sylvatica               TATT-AATTGACATAAACTCAAGTTATCTATTAAAA0------?---------TAAG1GATGATGCACC-GGTAATGG-TCGGG
Monnina_insignis              -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGGG1GATGATGCATC-CGGAATGG-TCGGG
Monnina_malmeana              -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGGG1GATGATGCATC-CGGAATGG-TCGGG
Atroxima                      -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Balgoya_pacifica              -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Barnhartia                    -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Bredemeyera_altissima         -----AGTTGACATATATCGAAGTAATCTCATAAAA1TAAAAA0---------TCGA1GATAATGCATC-AGGAATGG-TCGGG
Bredemeyera_colletioides      TTTT-AGTTGACATATATCCAAGTAATCTCCTAAAA1TAAGAA0---------TTAG1GATGATACATC-GGTAATGG-TCGGG
Bre_collLM241                 TTTT-AGTTGACATATATCCAAGTAATCTCCTAAAA1TAAGAA0---------TTAG1GATGATACATC-GGTAATGG-TCGGG
Bredemeyera_floribunda        -----AGTTGACATATATCGAAGTAATCTCATAAAA1TAAAAA0---------TCGA1GATGATGCATC-AGGAATGG-TCGGG
Bredemeyera_lucida            -----AGTTGACATATATCGAAGTAATCTCATAAAA1TAAAAA0---------TCGA1GATGATGCATC-AGGAATGG-TCGGG
Bredemeyera_microphylla       -----AGTTGACATATATCGAAGTAATCTCATAAAA1TAAAGA0---------CCGA1GATGATCCATC-AAGAATGG-TCGGG
Bre_papNGF33220               ------------------------------------?------?-------------?--------------------------
Carpolobia_alba               -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
'Carpolobia_aff._goetzii'     -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GAAG-TCCATC-AGG----------
Comesperma_calymega           -----AGTTGACATATATCCAAGTAATCTCATAAAA1TCAAAA0---------TAAG1GATGATTAATC-AGGAATGG-TCGGG
Comesperma_hispidulum         -----AATTGACATATATCCAAGTTATCTCATAAAA1TCAAAA0---------TGAA1GATGATGAATC-AGGAATGG-TCGGG
Comesperma_secundum           -----AGTTGACATATATCCAAGTAATCTCATAAAA1TCAAAA0---------TGAA1GATGATGAATC-AGGAATGG-TCGGG
Com_eriAM102                  -----AGTTGACATATATCCAAGTTATCTAATAAAA1TCAAAA0---------TGAA1GATGATGAATC-GGGAATGG-TCGGG
Com_scoMDC9172                -----AGTTGACATATATCCAAGTAATCTCATAAAA1TCAAAA0---------TGAA1GATGATGAATC-GGGAATGG-TCGGG
Com_volAM88                   -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGAATC-AGGAATGG-TCGGG
Com_rhaEAG8343                -----AGTTGACATATACCCAAGTAATCTGATAAAA1TCAAAA0---------TCAG1GATGATGACCC-GGGAATGG-TCGGG
Diclidanthera_bolivarensis    -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Diclidanthera_pendulifera     -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Monnina_crassifolia           -----AGTTGACATATATCCAACTAATCTCATAAAA1TAAAAA0---------TGGG0---GATGCATC-AGGAATGG-TCGGG
Monnina_phillyreoides         -----AGTTGACATATATCTAACTAATCTCATAAAA1TAAAAA0---------TGGG0---GATGCATC-AGGAATGG-TCGGG
Monnina_hirta                 -----AGTTGACATATATCTAACTAATCTCATAAAA1TAAAAA0---------TGGG0---GATGCATC-AGGAATGG-TCGGG
Moutabea_aculeata             -----AGTTGACATATATCCAAGTAATCCCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Moutabea_excoriata            -----AGTTGACATATATCCAAGTAATCCCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
Moutabea_guianensis           -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGCATC-AGGAATGG-TCGGG
'Muraltia_aff._alopecuroides' -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Muraltia_heisteria            -----ASWTRWCATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Muraltia_mixta                -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Mur_heiAD                     -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Nylandtia                     -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Acanthocladus_guayaquilensis  -----AGTTGACATAGGTCGAAGTAATCTCATAAAA1TGAAAA0---------TCGA1GATGATGCATC-AGGAATGG-TCGGG
Polygala_acuminata            -----AGTTGACATATATACAAGTAATTTCGTAAAA1TAAAAA0---------CGAA1GCTGATGCATC-AGGAATAG-TCGGG
Polygala_arillata             -----AGTTGACATATATTCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-GGGAATGG-TCGGG
Polygala_chamaebuxus          -----AGTTGACATATATTCAACTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATA-AGGAATGG-TCGGG
Polygala_cuneata              -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Polygala_erioptera            -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TTAT1GATGATGCATC-AGGAATGG-TCGGG
Polygala_floribunda           -----AGTTGACATATATCCAAATAATTTCGTAAAA1TAAAAA0---------CGAG1GATGATGCATC-AGGAATAG-TCGGG
Polygala_fuertesii            -----AGTTGACATATATCGAAGTAATCTCATAAAA1TAAAGA0---------CCAA1GATGATGCATC-AGGAATGG-TCGGG
Polygala_hebeclada            -----AGTTGACATATATCCAAGTAATTTCGTAAAA1TAAAAA0---------CGAA1GCTGATGCATC-AGGAATAG-TCGGG
Polygala_klotzschii           -----AGTTGACATAGGTCGAAGTAATCTCATAAAA1TGAAAA0---------TAGA1GATGATGCATC-AGGAATGG-TCGGG
Polygala_lutea                -----AGTTAACATATATCCAAGTAATCTCAGAAAA1TCAAAA0---------TGAG1GATGATGCATC-AGGAACGG-TCGGG
Polygala_macradenia           -----AGTTGACATATATCGAAATAATCTCATAAAA1TAAAGA0---------CCAA1GATGATGCATC-AGGAATGG-TCGGG
Polygala_microphylla          -----AGTTGACATATATCCACGTAATCTCATAAAA1TCAAAA0---------TGAT1GATGATGCATC-AGGAATGG-TCGGG
Polygala_obscura              -----AGTTGACATATATCGAAATAATCTCATAAAA1TAAAGA0---------CCAA1GATGATGCATC-AGGAATGG-TCGGG
Polygala_oleifolia            ------------------------------------?------?-------------?--------------------------
Polygala_lindheimeri          -----AGTTGACATATATCCAACTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
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trnL/F alignment (continued)

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[                              1441      1451      1461      1471      1481      1491      1501      1511      1521    ]
[                              |         |         |         |         |         |         |         |         |       ]
Polygala_penaea                -----AGTTGACATATATCGAAGTAATCTCATAAAA1TAAAGA0---------CCAA1GATGATGCATC-AGGAATGG-TCGGG
Polygala_rivinifolia           -----AGTTGACATATATCGAAATAATCTCATAAAA1TAAAGA0---------CCAA1GATGATGCATC-AGGAATAG-TCGGG
Polygala_semeiocardium         -----AGTTGACATATATTCAAGCAATTTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Polygala_senega                -----AATTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAACGG-TCGGG
Polygala_subspinosa            -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATGG-TCGGG
Polygala_tatarinowii           -----AGTTGACATATATTCAAGCATTTTTATAAAA1TAAAAA0---------TGAG1GATGATGCATC-AGGAATAG-TCGGG
Polygala_violacea              -----AGTTGACATATATCCAAAAAATTTCGTAAAA1TAAAAA0---------CGAA1GATGATGCATC-AGGAATAG-TCGGG
Polygala_vulgaris              -----AGTTGACATATATCCAAGTAATCTCATAAAA1TCAAAA0---------TGAT1GATGATGCATC-AGGAATGG-TCGGG
Pol_exsRKH804                  -----AGTTGACATATATCCAAGTAATCTCATAAAA1TTAAAA0---------TGAT1GATAATGCATC-AGAAATCG-TCGGG
Pol_japLMC2903                 -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TTAT1GATGATGCATC-AGGAATGG-TCGGG
Monnina_dictyocarpa            -----AGTTGACATATATCCAACTAATCTCATAAAA1TAAAAA0---------TGGG0---GATGCATC-GGGAATGG-TCGGG
Monnina_leptostachya           -----AGTTGACATATATCCAACTAATCTCATAAAA1TAAAAA1TAATAAAAATGGG0---GATGCATC-AGGAATGG-TCGGG
Monnina_pterocarpa             -----AGTTGACATATATCCAACTAATCTCATAAAA1TAAAAA1TAATAAAAATGGG0---GATGCATC-AGGAATGG-TCGGG
Salomonia_cantoniensis         -----AGTTGACACATATCCAAGTAATCTCATAAAA1TTAAAA0---------TGAG1GATGATGCATC-AGTAATGG-TCGGG
Salomonia_ciliata              -----AGTTGACACATATCCAAGTAATCTCATAAAA1TTAAAA0---------TGAG1GATGATGCATC-AGTAATGG-TCGGG
Sal_cilRKH1023                 -----AGTTGACACATATCCAAGTAATCTCATAAAA1TTAAAA0---------TGAG1GATGATGCATC-AGTAATGG-TCGGG
Securidaca_diversifolia        -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGTATC-AGGAATGG-TCGGG
Securidaca_longifolia          -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGTATC-AGGAATGG-TCGGG
Securidaca_virgata             -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAG1GATGATGTATC-AGGAATGG-TCGGG
Securidaca_welwitschii         -----AGTTGACATATATCCAAGTAATCTCATAAAA1TAAAAA0---------TGAA1GATGATGTATC-AGGAATGG-TCGGG
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Appendix Two:
Polygalaceae Treatment for the *Flora of Australia*

POLYGALACEAE

A.M.Monro

Trees (not in Australia), shrubs, climbers or herbs. Leaves simple, entire, sometimes reduced to scales; stipules absent or replaced by glands. Inflorescences terminal or axillary racemes, spikes or panicles. Flowers usually zygomorphic, often superficially papilionaceous. Calyx of 5 sepals, free or rarely connate, the two inner "wing" sepals often enlarged and petaloid. Corolla of 5 petals but often reduced to 3, variously fused and/or adnate to the stamens, lower petal often modified to form a keel. Stamens usually 8, but sometimes 10 or 3–7, monadelphous or diadelphous and often fused together by the filaments for at least part of their length; anthers basifixed, often 2-locular, dehiscent by pores or slits. Ovary superior or half-inferior, usually 2-carpellate but sometimes with up to 5 or 7–8 carpels; ovules 1 per locule, anatropous to hemitropous; style often curved or hooked; stigma usually bilobed with the lower lobe fertile or sometimes capitate. Fruit usually a loculicidal capsule but sometimes a samara, drupe or nut. Seeds often hairy and bearing a distinct aril-like appendage.

A sub-cosmopolitan family of about 20 genera and 750–1000 species which is naturally absent only from New Zealand, Polynesia and the polar regions, with much of the diversity being concentrated in the tropics. The distribution of the family is largely matched by that of the large genus *Polygala*. In Australia, there are four genera and 49 species. As circumscribed by Cronquist (1981), Polygalaceae also includes the monotypic Western Australian genus *Emblingia*. This placement is not followed here due to several sources of molecular evidence that indicate *Emblingia* is more closely related to the Order Capparales. Any morphological similarities between *Emblingia* and members of the Polygalaceae are thus likely to be a result of convergence and its inclusion in this family would serve only to confuse rather than clarify the evolutionary relationships.

G.Bentham, Polygaleae, *Fl. Austral.* 1: 137–149 (1863); R.H.Chodat, *Monogr. Polygal.*, *Mém. Soc. Phys. Genève*, Suppl. 1890 (7): 1–168, 12 tables (1891), 31(2, 2): I–VII, 1–500, 23 tables (1893); R.H.Chodat, Polygalaceae, *in* H.G.A.Engler & K.A.E.Prantl (eds), *Nat. Pflanzenfam.* 3(4): 323–345, 10 tables (1896); A.Cronquist, Polygalaceae, *An Integrated System of Classification of Flowering Plants* 775–778 (1981); R. van der Meijden, Polygalaceae, *in* C.G.G.J. van Steenis (ed.), *Fl. Males.* 10(3): 455–539 (1988); B.Eriksen, Floral anatomy and morphology in the Polygalaceae, *Pl. Syst. Evol.* 186: 17–32 (1993); B.Eriksen, Phylogeny of the Polygalaceae and its taxonomic implications, *Pl. Syst. Evol.* 186: 33–55 (1993); C.Persson, Phylogenetic relationships in Polygalaceae based on plastid DNA sequences from the *trn*L-F region, *Taxon* 50(3): 763–779 (2001).

KEY TO GENERA OF POLYGALACEAE IN AUSTRALIA

1 Two lateral sepals slightly larger than or subequal to the outer three; stamens 4–5 or 7

  2 Erect annual herb 10–30 cm; flowers in densely compressed spike; stamens 4–6; fruit with marginal spikes **1. SALOMONIA**

  2: Erect or spreading perennial shrub to around 1.5 m; flowers solitary or paired in leaf axils; stamens 7; fruit margin entire but often with apical horns **2. MURALTIA**

1: Two lateral sepals greatly exceeding the outer three, wing-like and often petaloid, stamens 8

3 Keel petal with a divided appendage below the tip; fruit usually circular or ovate; seeds with a distinct knob-, hood- or cap-like appendage at the apex and lacking a coma **3. POLYGALA**

3: Keel petal usually unadorned (rarely with a membranous hook below the tip); fruit mostly cuneate or clavate; seeds usually with rather obscure linear appendages and nearly always with a coma of long fine hairs arising from the seed coat **4. COMESPERMA**

1. SALOMONIA

Salomonia Lour., *Fl. Cochin.* 1: 14 (1790), *nom. cons.*; named for the Biblical King Solomon, "the first general botanist".

Type: *S. cantoniensis* Lour.

Erect annual herbs. Leaves subsessile or reduced to scales. Inflorescence a terminal raceme or spike, often densely crowded but elongating in fruit. Flowers zygomorphic but not pseudo-papilionaceous. Sepals not petaloid, subequal with the inner pair slightly larger, the outer three retained in fruit. Corolla of three petals adnate to the base of the stamens, keel petal unadorned. Stamens 4–6, monadelphous, sessile; anthers 2-locular and dehiscent by a short slit. Ovary superior, bilocular; style strongly curved; stigma bilobed. Fruit a laterally compressed septicidal capsule with distinct marginal spines. Seeds glabrous, compressed with flattened elongate appendages on the edges.

A genus of about six species (treated here as excluding *Epirrhixanthes* Blume), distributed in tropical areas from India through south-east Asia, Japan, Malesia and Australia; one species native in northern Australia.

G.Bentham, *Salomonia*, *Fl. Austral.* 1: 138 (1863); H.Koyama, A revision of the genus *Salomonia*, *Bull. Natl Sci. Mus., Tokyo ser. B (Bot.)* 21(1): 1–12 (1995); K.F.Kenneally, *Salomonia ciliata* (Polygalaceae), A new generic plant record from the Kimberley, Western Australia, *W. Austral. Nat.* 20(1): 29–31 (1995).

1. **Salomonia ciliata** (L.) DC., *Prodr.* 1: 334 (1824)

Polygala ciliata L., *Sp. Pl.* 2: 705 (1753). T: 'Habitat in India.'; lecto: Herb. Hermann 2: 61, no. 268, BM, *n.v.*, *fide* D.B.Sumithra'arachchi *in* M.D.Dassayanake & F.R.Fosberg (eds), *Rev. Handb. Fl. Ceylon* 6: 316 (1987); photo: BM website.

Salomonia oblongifolia DC., *Prodr.* 1: 334 (1824). T: 'In Nepaulia. Wallich.'; holo: G-DC, *n.v.*; fiche: NSW.

Illustrations: H.Koyama, *Bull. Natl Sci. Mus., Tokyo ser. B (Bot.)* 21(1): 7, fig. 3 (1995); K.F.Kenneally, *W. Austral. Nat.* 20(1): 30, fig. 1 (1995).

Herb to 30 cm high, glabrous. Leaves spherical to ovate, acute, mucronate, sometimes ciliate near apex, up to 1 cm long by 6 mm wide. Inflorescence a dense spike with flowers held perpendicular to the main axis. Sepals lanceolate, around 0.5 mm long, 0.1 mm wide; lateral sepals enlarged to about 1 mm long. Keel petal pale pink with purple tip, about 1 mm long; upper petals whiteish/cream, ¾ mm long. Stamens 4. Style inserted into the notch at the top of the ovary, kinked. Stigma basically oblong, glabrous. Capsule irregularly cordate/reniform, marginal spines 0.5–1 mm long. Seeds globose to ovate, appendage reduced to a linear ridge near the apex.

Found in northern Australia from the Kimberley in W.A. to a few scattered collections in coastal areas of SE Qld. Grows ephemerally on sandy coastal soils or on peaty or clay soils in seasonally wet areas and along river banks. Map 1.

W.A.: McDonald Creek on road to Kalumburu, *K.F.Kenneally 11487* (PERTH). N.T.: Gunn Point road, ca 25 km south of Darwin, *R.K.Harwood 986* (CANB, DNA); Kakadu National Park, ca 4km S of El Sharana, *A.V.Slee 2628 & L.A.Craven* (CANB). Qld: Donkey Spring Ck, Bulleringa NP, 80 km NW Mt Surprise, *P.I.Forster PIF22468* (BRI); Moa Island, c. 1 km NW of Kubin, near airstrip, *D.L.Jones 3624* (BRI, CANB, NSW).

2. MURALTIA

Muraltia DC., *Prodr.* 1: 335 (1824), *nom. cons.*; named for Johann von Muralt (1645–1733), Swiss botanist, physician and professor of physics.

Type: *M. heisteria* (L.) DC., *typ. cons.*

Erect perennial shrubs. Leaves usually fasciculate on short axillary shoots, often stiff and pungent. Flowers zygomorphic but not pseudo-papilionaceous, single or paired in the leaf axils in a reduced raceme. Sepals not petaloid, unequal or subequal with the inner pair larger. Corolla of three petals adnate to the stamens, keel petal with two large petaloid appendages below the tip. Stamens 7, monadelphous and connate by their filaments for part of their length, adnate to the petals; anthers 2-locular and dehiscent by longitudinal slits. Ovary superior, bilocular; style curved at the apex; stigma bilobed. Fruit a compressed capsule often with four horn- or antler-like appendages at the apex. Seeds pubescent, shortly appendaged.

A genus of about 115 species from southern Africa, where it is an important constituent of the fynbos vegetation; 1 species sparingly naturalised in Australia.

M.R.Levyns, The genus *Muraltia*, *J. S. African Bot.* Suppl. 2: 1–247 (1954).

1. ***Muraltia heisteria** (L.) DC., *Prodr.* 1: 335 (1824)

Polygala heisteria L., *Sp. Pl.* 2: 704 (1753). T: 'Habitat in Æthiopia'; lecto: Herb. Clifford 352, *Heisteria* 1, BM, *n.v.*, *fide* D.O.Wijnands *in* C.E.Jarvis *et al.*, *Regnum Veg.* 127: 52 (1993); photo: BM website; photocopy: CANB.

Illustrations: M.R.Levyns, *J. S. African Bot.* Suppl. 2: 213, fig. 86; 215, fig. 87 (1954).

Stiff shrub to about 1 m high, stems pubescent. Leaves sessile, clustered on short shoots, narrowly lanceolate to almost linear, sometimes ciliate and sometimes pubescent on the keel, mucronate, 1–3 mm wide by 4–12 mm long. Flowers solitary or paired. Sepals lanceolate, ciliate, 4–6 mm long; lateral sepals slightly larger than outer 3, sometimes purple towards the tip. Petals pink to mauve or rarely white, glabrous; keel 3–6 mm long, appendage with orbicular lobes; upper petals connate for at least half their length, spathulate to elliptic above. Style broadening towards apex; stigma bilobed with anterior lobe much reduced. Capsules ovate, shortly pubescent, 5–7 mm long including the horn-like appendages. Seeds black, globose, sparsely pubescent, appendage with 2 short membranous lobes.

A garden escape that has become naturalised in small populations around Sydney, Melbourne and Adelaide. Material has not been recently collected from some of these areas and long-term persistence as a weed thus seems unlikely. Map 2.

S.A.: between Ashton and Norton Summit, *D.E.Symon 15213* (AD, NSW); c. 2 miles E of Mount Torrens, *D.J.E.Whibley 510* (AD). N.S.W.: behind Bronte Beach, *R.G.Coveny 7768* (CANB, NSW). Vic.: Kyneton, *D.Martin s.n.*, Nov. 1923 (NSW).

3. POLYGALA

Polygala L., *Sp. Pl.* 2: 701 (1753); *Gen Pl.* 5th edn 315 (1754); from the Greek *polys* (much, many) and *gala* (milk), from a traditional belief that consumption of some species increased milk secretion in humans and livestock.

Type: *P. vulgaris* L.

Herbs, shrubs or small trees (not in Australia). Leaves usually alternate, rarely opposite or whorled. Inflorescence a terminal, lateral or axillary raceme or spike or sometimes a terminal panicle. Flowers zygomorphic. Calyx of 3 unmodified outer sepals with the inner 2 "wing" sepals enlarged and often petaloid. Corolla of 3 petals fused together at the base and variously joined to the stamens, lower petal a boat-shaped "keel" which may be unadorned (not in Australia) or crested. Stamens 8, fused at the base into a staminal tube, monadelphous; anthers 2-celled and dehiscent via a short apical slit. Fruit usually a compressed loculidical capsule, usually obovate or orbiculate, with a broad or narrow marginal wing. Seeds hairy, often with a cap- or hood-like appendage.

A genus of about 500 species with a sub-cosmopolitan distribution but with its main diversity focused in tropical areas; 18 species in Australia, of which 12 are endemic or native. The natural diversity of *Polygala* is highest in the tropics of northern Australia, with most of the taxa in southern regions having been introduced. It can be difficult to judge the peak flowering periods for the species in Australia, as they retain their lateral sepals until the fruit is mature.

G.Bentham, *Polygala*, *Fl. Austral.* 1: 138–141 (1863); F.Adema, A review of the herbaceous species of *Polygala* in Malesia (Polygalaceae), *Blumea* 14(2): 253–276 (1967); F.Adema, Identities of the herbaceous Australia species of *Polygala* represented in the Brisbane Herbarium, *Proc. Roy. Soc. Qld* 80(9): 125–130 (1969); L.Pedley, Two new species of Polygalaceae from Central Australia, *J. Adelaide Bot. Gard.* 3(1): 127–129 (1981).

1 Lateral sepals enlarged, petaloid and usually obtuse, symmetrical

2 Robust erect branching perennial shrubs, usually 1–2 m high

3 Leaves obovate to oblong; inflorescences short and few-flowered among the upper leaves, outer sepals glabrous **1. P. myrtifolia**

3: Leaves narrow-obovate to almost linear (sometimes almost all absent); inflorescences pyramidal and several-flowered exceeding the upper leaves; outer sepals ciliate **2. P. virgata**

2: Slender spreading, ascending or erect herbs, usually less than 60 cm high

4 Main colour of lateral sepals blue to mauve

5: Plant erect, branching in upper areas of main stem **3. P. persicariifolia**

5 Plant lax and rather diffuse, stems branching from near the base and often trailing through surrounding vegetation

6 Inflorescences axillary; fruit with a rather broad crisped marginal wing **4. P. japonica**

6: Inflorescences terminal; fruit narrowly winged **5. P. vulgaris**

4: Main colour of lateral sepals white or cream

7 Seed appendage apparently 2-lobed, with the broad flattened lobes extending some way down the seed

8 Outer sepals white; fruit very narrowly winged on both margins; seed appendage lobes less than half the length of the seed **6. P. paniculata**
8: Outer sepals cream; fruit wing developed on only one margin; seed appendage lobes running almost the length of the seed **7. P. duarteana**

7: Seed appendage 3-lobed and short, confined to top of seed

9 Plant glabrous; outer sepals 3–5 mm long; keel crest few-branched; style distinctly hooked; fruit narrowly winged **8. P. longifolia**
9: Plant puberulous; outer sepals 7–10 mm long; keel crest finely divided; style short and straight; fruit broadly winged **9. P. monspeliaca**

1: Lateral sepals larger than outer sepals, greenish and often acute, asymmetric

10 At least two of the seed appendage lobes running some part of the length of the seed

11 Capsule broadly winged, often broader than the retained lateral sepals; three of the seed appendage lobes elongate **10. P. rhinanthoides**
11: Capsule very narrowly winged, not noticeably exceeding the retained lateral sepals; two of the seed appendage lobes elongate

12 Plant densely villous with long straight hairs; corolla blue to mauve, keel crest relatively simple and undivided **11. P. eriocephala**
12: Plant pubescent with crisped hairs; corolla white to cream, keel crest much-branched **12. P. chinensis**

10: All seed appendages short, reaching only to the top of the seed

13 Leaves oblong, obovate or orbiculate

14 Plant glabrous or sparely haired, leaves orbiculate or very broad obovate **13. P. orbicularis**

14: Plant pubescent to hispidulous, leaves oblong to obovate
15 Plant prostrate or ascending; inflorescence few-flowered; lateral sepals narrow-falcate **14. P. isingii**
15: Plant ascending to erect; inflorescence rather dense; lateral sepals narrow-ovate **15. P. linariifolia**

13: Leaves linear, elliptic or lanceolate

16 Corolla white to cream; stamens arranged in three groups; capsules glabrous or with a few short hairs around the apex **16. P. wightiana**
16: Corolla mauve to blue; stamens monadelphous; capsules distinctly ciliate

17 Keel crest little-incised; capsules obtuse, emarginate; seed appendage rather flattened with three short lobes **17. P. exsquarrosa**
17: Keel crest finely divided; capsules acute, strongly notched; seed appendage knob-like and with a distinct crested ridge **18. P. tepperi**

1. ***Polygala myrtifolia** L., *Sp. Pl.* 2: 703 (1753)

var. myrtifolia

T: Habitat in Aethiopia; lecto: not designated.

Erect shrub 1–2(—4) m high, usually glabrous. Leaves obovate to oblong, obtuse, 8–25 mm wide by 10–40 mm long. Inflorescence a short terminal raceme. Outer sepals ovate, glabrous, 4–6 mm long; wings magenta fading to white, broad-ovate, clawed, 6–12 mm wide by 10–20 mm long. Keel white with pink streaks and deep red apex, bearing a much-branched crest; upper petals white with pink-flecked tips, rounded-oblong; corolla tube glabrous. Stamens free above, monadelphous. Ovary orbiculate. Style curving; lower stigma fertile. Capsules orbiculate, narrowly winged, emarginate, 7–12 mm wide by 7–12 mm long. Seeds black, smooth, pubescent, ovoid; seed appendage 3-lobed. *Myrtle-leaved Milkwort, Sweet Pea Bush, Parrot Bush.*
This taxon, native to southern Africa, is a presumed garden escape in areas surrounding most Australian capital cities. It grows mostly in coastal heath, but has become established in around inland lakes in Victoria. Flowers and fruit can usually be found throughout the year, with a peak in Sept.–Oct. Map 3.

W.A.: Goonaring Springs, gazetted reserve, 18 km SW of Toodyay towards Perth, *G.J.Keighery 6979* (PERTH). S.A.: Coffin Bay township, along the Oyster Walk along the seafront, *D.E.Symon 15204* (AD). N.S.W.: Ulladulla, slope above Ulladulla Harbour, *I.R.Telford 10748* (CANB, NSW). Vic.: c. 500 m E of Cape Otway Lighthouse, *A.M.Lyne 1232 & J.Lyne* (CANB, MEL). Tas.: George Town, She-Oak Point, growing at base of lighthouse between Low Head and George Town, *R.J.Bayer et al. TAS-00003* (CANB, HO).

Polygala myrtifolia var. *myrtifolia* is a serious environmental weed on the Mornington Peninsula in Victoria. By contrast, *Polygala myrtifolia* subsp. *grandiflora* Hook. and horticultural hybrids derived from it are readily commercially available and apparently non-invasive.

2. ***Polygala virgata** Thunb., *Prodr. Pl. Capensis* 120 (1794)

T: not cited; lecto: 'e Cap. b. Spei', *Thunberg*, UPS-THUNB 16262 *n.v.*, fiche: AD.

Virgate shrub 1–2 m high, glabrous. Leaves readily deciduous, narrow-obovate to almost linear, acute, 3–6 mm wide by 20–45 mm long. Inflorescence a loosely pyramidal terminal raceme. Outer sepals ovate, ciliate, 3–6 mm long; wings pinkish-mauve, obovate to nearly orbiculate, sessile, 8–12 mm wide by 10–15 mm long. Keel deep pink to magenta, bearing a finely-divided crest; upper petals pink with deep magenta tips, obovate; corolla tube pubescent. Stamens free above, monadelphous. Ovary obovate. Style distinctly hooked; lower stigma fertile. Capsules obovate, narrowly winged, emarginate, 5–8 mm wide by 6–10 mm long. Seeds black, smooth, pubescent, obloid; seed appendage 3-lobed. *Broom Milkwort, Purple Broom.*
A garden escape originally from southern Africa, naturalised in small populations around most capital cities in southern Australia. Growing mostly along roadsides, railway lines and around rubbish tips (presumably from garden waste). Flowers and fruits present for much of the year, with a peak in July–Dec. Map 4.

W.A.: Mount Shadforth Drive, Denmark, *G.J.Keighery 9381* (PERTH). S.A.: Carlton Farm roadside near Marcollat, *D.E.Murfet 1887 & R.L.Taplin* (AD). Qld: 1.5 km S of Blackbutt , *A.R.Bean 13190* (BRI). N.S.W.: 2.3 km from Dora Creek railway station towards Morisset on the Morisset–Toronto road, *R.G.Coveny 16518, T.Tame & Z.Donabauer* (NSW). Vic.: Mt Eccles National Park, c. 800 m NW of Mt Eccles, *K.Robinson s.n.*, 13 June 1991 (MEL).

3. Polygala persicariifolia DC., *Prodr.* 1: 236 (1824)

T: 'In Nepaulia. Wallich.'; holo: G-DC *n.v.*; fiche: NSW.

Erect herb to about 40 cm, pubescent with crisped hairs. Leaves broad-elliptic, mucronate, 5–12 mm wide by 20–55 mm long. Inflorescence an axillary raceme. Outer sepals linear to elliptic, ciliate, 3–4 mm long; wings pale mauve, orbiculate to broad-ovate, sessile, 3–4 mm wide by 3–5 mm long. Keel bright pink at the tip and paler below, bearing a much-branched crest with rounded ends; upper petals mauve, rounded-cuneate; corolla tube ciliate. Stamens free above, monadelphous. Ovary obovate. Style distinctly hooked; lower stigma fertile. Capsules broad-obovate, narrowly winged and ciliate, emarginate, 3–4 mm wide by 4–5 mm long. Seeds black, smooth, pubescent, obloid; seed appendage hood-shaped with three short lobes.

Extending through Africa, southeast Asia and Malesia, *Polygala persicariifolia* is found in Australia only in a small area around Mareeba in northern Queensland. It grows in open eucalypt woodland in soils derived from granite, often close to creeks. Flowering and fruiting mainly Oct–May. Map 5.

Qld: The Jump-Up ca 20 km S of Mareeba on road to Atherton, *R.J.Henderson H2466* (BRI); Nobby Ck S of Cooktown, *S.T.Blake 21856* (BRI); Mt Molloy, *H.S.McKee 9110* (BRI, CANB); Mareeba near junction of Granite and Spring Creeks, *D.Goodall s.n.*, 19 Mar. 1960 (BRI); N of Adeline Creek, Windsor Tableland, *B.Wannan et al. 1257* (CANB).

4. Polygala japonica Houtt., *Handl. Plant Kruidk.* 10: 89, t. 62, fig. 1 (1779)

T: not cited.

Polygala veronicea F.Muell., *Definitions of Rare or Hitherto Undescribed Australian Plants* 38 (1855); *Polygala veronicifolia* Chodat, *Monogr. Polygal.* 2: 355 (1893), *nom. illeg.* T: In grassy or gravelly places from the King River to the Goulburn River; syn: MEL, K; photos: CANB.

Lax diffuse herb to about 30 cm, tomentose. Leaves elliptic to ovate, acute, 3–6 mm wide by 4–16 mm long. Inflorescence a short lateral raceme. Outer sepals elliptic, 2–3 mm long; wings mauve to blue, obovate, tapering at the base, sessile, 1.5–2.5 mm wide by 4–6 mm long. Keel deep blue with paler tip, bearing bifid much-divided crest; upper petals cream, rounded-oblong; corolla tube pubescent. Stamens monadelphous and sessile. Ovary oblong. Style slightly curved; stigmas distant. Capsules orbiculate with broad crisped wing, emarginate, 3–5 mm wide by 3–5 mm long. Seeds black, smooth, puberulous, ovoid; seed appendage three-lobed.

This species is widespread along the east coast of Australia, from southeastern Queensland to near Melbourne. It grows primarily in open grassy eucalypt woodlands in clay or loam soils. Flowers for much of the year with a peak in Oct.–Dec. Map 6.

Qld: Kangaroo Mtn summit, Little Liverpool Range, *P.Grimshaw PG2741* (BRI). N.S.W.: Landsdown Reserve, Henry Lawson Drive, Georges Hall at Bass Hill, *R.Millar 3 & C.Gibson* (NSW). A.C.T.: Canberra Nature Park, Mulligans Flat, E of 'East View', on boundary, *I.Crawford 2783a* (CANB). Vic.: ca. 100 metres E of Marriage Lane (N side of rail-line), on Melbourne -Bairnsdale rail-line, *I.D.Lunt 90/33* (MEL).

5. *Polygala vulgaris L., *Sp. Pl.* 2: 702 (1753)

T: Habitat in Europae pratis, & pascuis siccis; lecto: Herb. Linn. 882.6, LINN *n.v.*, *fide* G.R.Heubl, *Mitt. Bot. Staatssamml. München* 20: 348 (1984); photocopy: CANB.

Lax sparsely pubescent herb to about 40 cm high. Leaves elliptic, acute, 2–5 mm wide by 10–30 mm long. Inflorescence a terminal raceme. Outer sepals ovate, 2–3 mm long; wings mauve to deep blue, obovate, sessile, 5–6 mm wide by 6–8 mm long. Keel

purple, bearing a finely-divided crest, upper petals purple to blue, rounded-oblong; corolla tube ciliate. Stamens sessile, monadelphous. Ovary obovate. Style straight; lower stigma fertile. Capsules obovate, glabrous, narrowly winged, emarginate, 5–7 mm wide by 6–9 mm long. Seeds black, smooth, pubescent, obloid; seed appendage with 3 membranous lobes about one-third the length of the seed. *Common Milkwort.*
Native to Western Europe and considered endangered in some parts of its natural range, the type species for *Polygala* is sparingly naturalised as a weed in Australia. It is localised in small areas of the Dandenong Ranges outside Melbourne and in north-western Tasmania. It grows mainly on rich loam soils in grassy areas. Flowers Sept.–Feb. Map 7.

Vic.: Dandenong Ranges, Olinda State Forest, E of Olinda Golf Course, track between Mathias Road and Lookout Rock in R.Hamer Forest Arboretum, *J.C.Kissane 704* (MEL); Eastern Highlands, c. 1.5 km ESE of Gembrook on road to Tynong, *I.C.Clarke 2459* (CANB, MEL). Tas.: rocky knoll just south of Rebecca Creek crossing, east side of road, *D.L.Jones et al. 16162* (CANB); Tiger Creek, south of Arthur River, *A.M.Buchanan 15617* (HO).

See B.Jonsell & C.E.Jarvis, *Nordic J. Bot.* 22(1): 80 (2002) for a review of the problems with the current lectotypification for this species.

6. ***Polygala paniculata** L., *Syst. Nat.* 10th edn, 1154 (1759)

T: 'Brown. jam. 287. n. 1'; lecto: *Patrick Browne*, LINN 882.9 *n.v.*, *fide* F.Fawcett & A.B.Rendle, *Fl. Jamaica* 4: 242 (1920); photo: LINN.
Illustration: F.Adema, *Blumea* 14: 267, fig. 12 (1966).

Erect many-branched annual herb, stems hispidulous. Leaves narrowly elliptic, 2–5 mm wide by 6–20 mm long, discolorous. Inflorescence an open terminal raceme. Outer sepals elliptic, 1–2 mm long; wings white with pink flush, elliptic, not clawed, 1–2 mm wide by 2–4 mm long. Keel white, bearing a relatively undivided crest at the tip; upper petals white, narrow-oblong to elliptic; corolla tube glabrous. Stamens with very shortly free filaments, monadelphous. Ovary orbiculate. Style straight; stigmatic surface expanded, membranous, upper stigma replaced by a tuft of hair. Capsules obovate, emarginate, very narrowly winged, 1–3 mm wide by 2–4 mm long. Seeds black, smooth, sparsely hairy, obloid; seed appendage with two lobes which run about half the length of the seed.
A native of tropical America from Mexico to Brazil, *Polygala paniculata* is now naturalised as a weed in Africa, southeast Asia, Malesia, Australasia and the Pacific. In Australia, it is found on the east coast from northern N.S.W. to Bundaberg in Qld, with a disjunction to the Wet Tropics in N Qld. It grows along roadsides and in disturbed sites on a variety of soils. Flowering mainly Sept.–Feb. Map 8.

Qld: Nairn Rd, Morayfield, c 35 km N of Brisbane, *A.R.Bean 16112* (BRI); 16 km ENE of Tully, ca 1 km W of junction of S Mission Beach Rd, Tam O'Shanter Forest, *D.Halford Q317* (BRI). N.S.W.: opposite junction of Empire Vale Road and River Drive, 0.38 km from Empire Vale Post Office, alongside Richmond River, *J.R.Hosking 1593* (CANB); 4 km along South Chowan Road from Manns Road, Nullum State Forest, *K.L.Wilson 8525* (NSW).

7. ***Polygala duarteana** A.St.-Hil., *in* A.F.C.P. de Saint-Hilaire, A. de Jussieu & J.Cambessedes, *Fl. Bras. Merid.* 2: 34 (1829)

T: 'In honorem dixi amicissimi D. Antonio Noguiera Duarte vici Contendas Parochi (in parte desertâ occidentalique provinciae Minas Geraës vulgò Certaô) qui me hospitio excepit mecumque benignè plurimas plantas communicavit'; lecto: ?P *n.v.*

Erect herb to about 50 cm, sparsely pubescent with crisped hairs. Leaves lanceolate to linear, mucronate, 2–6 mm wide by 8–35 mm long. Inflorescence a dense terminal raceme. Outer sepals ovate, ciliate, 1–2 mm long; wings cream to yellowish, obovate to almost orbiculate, sessile, 1–2 mm wide by 2–3 mm long. Keel cream, bearing a few-branched crest of clavarioid appendages; upper petals cream to yellow, deltoid; corolla tube glabrous. Stamens shortly free above, monadelphous. Ovary obovate. Style curved; upper stigma with tuft of hairs, lower stigma fertile. Capsules obovate to oblong, glabrous, narrowly winged on only one side, obtuse, 1–2 mm wide by 3–4 mm long. Seeds black, smooth, densely pubescent, obloid; seed appendage apparently two-lobed with broad flattened lobes running the length of the seed.

Naturally occurring in Brazil and Uruguay; in Australia, *Polygala duarteana* is naturalised only in a small area near Beaudesert in far south-eastern Qld. Flowering recorded July–Dec. Map 9.

Qld: Beaudesert, *M.Everett s.n.*, Dec. 1973 (BRI); Beaudesert, *S.Dunn s.n.*, 22 July 1953 (BRI); near Kerry, *S.L.Everist s.n.*, Sep. 1955 (BRI).

Although somewhat similar to *Polygala paniculata* in habit, *P. duarteana* can easily be distinguished by the presence of a marginal wing on only one side of the fruit—this character is not displayed in any of the other *Polygala* species in Australia. Recent collections of *P. duarteana* have not been made to confirm its persistence.

8. **Polygala longifolia** Poir., *in* J.B.A.P.M. de Lamarck, *Encycl.* 5: 501 (1804)

T: 'Cette plante a été rapportée par Commerson de l'île de Java (v.s. in herb. Lamarck)'; holo: P-LA *n.v.*, fiche: AD; iso: FI *n.v.*, *fide* F.Adema, *Blumea* 14: 266 (1966).
Polygala leptalea DC., *Prodr.* 1: 325 (1824). T: 'in Napauliâ. Wallich'; holo: G-DC *n.v.*; fiche: NSW.
Polygala leptalea var. *australiensis* Domin, *Biblioth. Bot.* 89: 301 (1927). T: 'Nord-Australien: Port Darwin, F.SCHULTZ. No. 276'; holo: ?PR *n.v.*
Polygala abyssinica var. *intercedens* Domin, *Biblioth. Bot.* 89: 301 (1927). T: 'Nord-Australien: Port Essington, ARMSTRONG. No. 365; Upper Victoria River, F. v. MUELLER'; holo: ?PR *n.v.*
[*Polygala abyssinica auct. non* Fresen.: K.Domin, *Biblioth. Bot.* 89: 301 (1927)]
Illustration: F.Adema, *Blumea* 14: 266, fig. 11 (1966).

Erect slender annual 30–120 cm high, glabrous. Leaves linear to narrow-elliptic, acute, 1–4 mm wide by 6–50 mm long. Inflorescence an elongate terminal raceme. Outer sepals elliptic, ciliate, 1–2 mm long; wings cream to white with purple flush, broad-elliptic, sessile, 2–3 mm wide by 3–5 mm long. Keel whiteish with mauve tip, bearing a few-branched crest; upper petals white, obovate to almost quadrangular; corolla tube glabrous. Stamens free above, monadelphous. Ovary orbiculate. Style distinctly hooked; lower stigma fertile. Capsules orbiculate to slightly obovate, narrowly winged, emarginate, 1–2 mm wide by 2–3 mm long. Seeds black, smooth, pubescent, obloid; seed appendage 3-lobed.

This species is distributed throughout tropical south-east Asia and Malesia; in Australia it is scattered across the tropics from the Kimberley in W.A through to the N.T. and Qld. It grows mainly in open grassy eucalypt woodlands in rather rocky areas, often near drainage lines. Flowers and fruits April–Aug. Map 10.

W.A.: Mitchell Plateau mining camp, between camp and airstrip, *P.A.Fryxell & L.A. Craven 4014* (PERTH, CANB). N.T.: South of Maningrida, Arnhem Land, *R.K.Harwood 801* (CANB, DNA); Charles Darwin NP, *P.S.Short 4760* (DNA, MEL). Qld: 62 km SE of Torrens Ck, *E.J.Thompson & D.J.Baumgartner BUC3* (BRI).

9. ***Polygala monspeliaca** L., *Sp. Pl.* 2: 703 (1753)

T: 'Habitat Monspelii in collibus sterilibus'; lecto: LINN (probably 882.7) *n.v., fide* J.A.R.Paiva, *Fontqueria* 50: 286 (1998); photocopy: CANB.

Erect puberulous herb to about 30 cm high. Leaves elliptic, acute, 1–5 mm wide by 10–40 mm long. Inflorescence a terminal raceme. Outer sepals narrow-oblong, 2–3 mm long; wings cream to white, elliptic to narrow-ovate, sessile, 2–4 mm wide by 7–10 mm long. Keel pale pink to white, bearing a finely-divided crest; upper petals white, oblong; corolla tube ciliate. Stamens shortly free above, monadelphous. Ovary obovate. Style short and straight; lower stigma fertile. Capsules obovate, glabrous, broadly winged, emarginate, 6–8 mm wide by 5–8 mm long. Seeds 2–3 mm wide by 3–5 mm long. Seeds black, smooth, flattened-obloid; seed appendage small and hood-shaped. *Annual Milkwort.*

An introduced weed native to the Mediterranean, naturalised in a small area of southern-eastern S.A. and western Vic. Growing in drainage ditches and dry lake beds. Flowering Oct.–Dec. Map 11.

S.A.: 33 km from Robe along road to Millicent, Lucindale turn-off, *M.D.Crisp 3710* (AD, CANB); Big Heath National Park, H[undre]d Spence, S end of the E boundary near drain, *C.R.Alcock 3124* (AD). Vic.: 6 km W of Lillimur South, 17 km WSW of Kaniva P.O., *A.C.Beauglehole 66802* (MEL); Morea Wildlife Reserve, *A.C.Beauglehole 87127* (AD).

10. **Polygala rhinanthoides** Sol. ex Benth., *Fl. Austral.* 1: 140 (1863)

T: 'N. Australia. Upper Victoria river. F.Mueller. Queensland. Endeavour river, R.Brown.'; syn: BM (2); isosyn: NSW (2).
Illustration: R. van der Meijden, *Fl. Males.* 10: 480, fig. 14 (1988).

Straggling puberulous herb to about 40 cm high. Leaves oblong to broad-elliptic, mucronate, 3–8 mm wide by 25–75 mm long. Inflorescence an axillary raceme. Outer sepals elliptic, 1–2 mm long; wings green and herbaceous, falcate, sessile, 2–4 mm wide by 4–6 mm long. Keel cream below with purple tip, bearing a finely-divided crest; upper petals mauve to blue, obdeltoid; corolla tube ciliate. Stamens free above, monadelphous. Ovary orbiculate. Style distinctly hooked; stigma inside curve. Capsules orbiculate, ciliate, broadly winged, emarginate, 5–6 mm wide by 5–7 mm long. Seeds black, smooth, pubescent, flattened-obloid; seed appendage 3-lobed with lobes about half the length of the seed.

Native to tropical regions of Australia, from the Kimberley in W.A. through the N.T. and into the Cape York Peninsula region of Qld; also present in lowland New Guinea. Grows in open grassy woodlands, often on clay soils. Flowering and fruiting recorded Apr.–June. Map 12.

W.A.: near Kelly Creek, Ord River Station, East Kimberley, *A.S.George 14442* (PERTH); along roadside 400 metres N of Piccanniny Creek tee road, Bungle Bungle National Park, *I.Solomon 709* (PERTH). N.T.: 4 km ESE of Jabiru, E of Ranger Plant, *I.R.Telford 7552* (CANB). Qld: Pannikan Springs area 29km W of Mungana, *A.R.Bean 5628* (BRI).

11. **Polygala eriocephala** Benth., *Fl. Austral.* 1: 139 (1863)

T: N. Australia. Upper Victoria river, F.Mueller.; holo: K, photo: CANB.

Erect villous herb to about 50 cm high. Leaves linear to narrow-oblong, acute, 2–6 mm wide by 15–65 mm long. Inflorescence a dense oblong lateral raceme. Outer sepals narrowly triangular, 4–6 mm long; wings green and herbaceous, falcate, sessile, 3–4

mm wide by 6–8 mm long. Keel mauve to mid-blue, bearing a simple bilobed crest; upper petals pale blue, oblong; corolla tube pubescent. Stamens free above, monadelphous. Ovary orbiculate. Style curved; lower stigma fertile. Capsules orbiculate, strongly ciliate, very narrowly winged, emarginate, 3–4 mm wide by 3–5 mm long. Seeds black, smooth, pubescent mainly in lower half, obloid; seed appendage 3-lobed with 2 narrow lobes running the length of the seed.
Apparently endemic to Australia, *Polygala eriocephala* occurs in tropical regions from the Kimberley in W.A. and into the Top End of the N.T. Grows in sedgeland or herbfields on damp clay or silty soils. Flowering and fruiting March–July. Map 13.

W.A.: Kununurra–Timber Creek road, 1.5 km W of Lake Argyle turnoff, *G.W.Carr 3080 & A.C.Beauglehole 46839* (PERTH); ca 6 km S of Gibb River Crossing, Kimberleys, *D.E.Symon 7147A* (ADW, PERTH). N.T.: Auvergne station, *R.K.Harwood 491* (DNA); Yambarran Range; 19km NE Mt Millikmonmir, *G.J.Leach 4481* (DNA, MEL).

12. **Polygala chinensis** L., *Sp. Pl.* 2: 704 (1753)

T: 'Habitat in India'; lecto: Herb. Linn. 882.26, LINN *n.v.*, fide F.Fawcett & A.B.Rendle, *Fl. Jamaica*, Dicot. pt. 2, 4: 244 (1920); photocopy: CANB.
Polygala arvensis Willd., *Sp. Pl.* 4th edn 3(2): 876 (1802). T: 'In India orientali'; holo: B *n.v.*; fiche: CANB.
Polygala chinensis var. *dissitiflora* Domin, *Biblioth. Bot.* 89: 303 (1927). T: Queensland: Sandsteinhügel der Dividing Range bei Pentland (DOMIN II. 1910)'; holo: PR; photo: BRI.
Illustration: F.Adema, *Blumea* 14: 270, fig. 15 (1966).

Ascending branched herb to about 60 cm high, moderately pubescent with crisped hairs. Leaves oblong to obovate, mucronate, 5–16 mm wide by 8–40 mm long. Inflorescence a short axillary raceme. Outer sepals lanceolate, ciliate, 1–2 mm long; wings greenish, asymmetric-falcate, sessile, 2–3 mm wide by 3–5 mm long. Keel cream to white, bearing a much-branched crest; upper petals white, spathulate; corolla tube pubescent. Stamens free above, monadelphous. Ovary orbiculate. Style hooked; fertile lower stigma inside the curve. Capsules broad-obovate to orbiculate, ciliate, narrowly winged, emarginate, 3–4 mm wide by 3–5 mm long. Seeds black, smooth, pubescent, ovoid; seed appendage three-lobed with two of the flattened lobes running about one-third the length of the seed.
Distributed from India through southeast Asia, Malesia and along the northern coast of Qld. Grows in grassland on sandy or rocky soils. Flowering and fruits recorded Oct.–Mar. Map 14.

Qld: Unigan Nature Reserve, Weipa, *P.I.Forster & M.R.O'Reilly PIF6484* (BRI); Preston, *Rev. N.Michael 940* (BRI).

13. **Polygala orbicularis** Benth., *Fl. Austral.* 1: 140 (1863)

Polygala chinensis var. *orbicularis* (Benth.) Domin, *Biblioth. Bot.* 89:302 (1927). T: N. Australia. South Goulburn Island, A. Cunningham; Melville Island, Fraser; n. Coast, Armstrong; syn: K (2); photos: CANB.
Polygala arvensis var. *obovata* Benth., *Fl. Austral.* 1: 141 (1863); *Polygala chinensis* subvar. *obovata* (Benth.) Domin, *Biblioth. Bot.* 29: 303 (1927). T: Cavern Island, Carpentaria, R.Brown; holo: ?BM, *n.v.*

Prostrate to ascending glabrous or sparsely pubescent herb to about 40 cm high. Leaves orbiculate to broad-obovate, mucronate, 8–20 mm wide by 10–40 mm long, discolorous. Inflorescence a short lateral raceme. Outer sepals elliptic, ciliate, 2–3 mm

long; wings green and herbaceous, ovate, acute, sessile, 2–3 mm wide by 3–5 mm long. Keel blue, bearing a bilobed finely-divided crest; upper petals cream with blue tips, broad-obovate; corolla tube ciliate. Stamens free above, monadelphous. Ovary orbiculate, ciliate. Style distinctly hooked; lower stigma fertile. Capsules somewhat asymmetric, orbiculate, narrowly winged, emarginate, 3–4 mm wide by 3–4 mm long. Seeds black, smooth, almost glabrous, ovoid; seed appendage 3-lobed.
Occurs through tropical Australia from the Kimberley region in W.A., through the N.T. to Cape York Peninsula in Qld. Grows in savannah woodlands. Flowering mainly Feb.–May. Map 15.

W.A.: near Willie Rieds camp, King Anna, S end Vansittart Bay, *A.A.Mitchell 2902* (PERTH); 2 km N of Beverley Springs Station Homestead, *R.J.Cranfield 6683* (PERTH). N.T.: Cape Hotham Reserve, Escape Cliff, *I.D.Cowie 3309* (DNA, MEL); Douglas Daly Research Farm, *C.R.Michell 529 & K.J.Nicholl* (DNA). Qld: c 15 km W of Mt Molloy, *J.R.Clarkson 7898 & R.J.F.Henderson* (BRI).

14. **Polygala isingii** Pedley, *J. Adel. Bot. Gardens* 3: 128 (1981)

T: Northern Territory: 5 miles S. of Mt Barkley, "Barkley" Station, 21°40'S, 132°30'E, iii. 1971, Latz 1224; holo: BRI, iso: NT.
Illustration: L.Pedley, *J. Adel. Bot. Gardens* 3: 128, fig. 1A (1981).

Prostrate to ascending hispidulous herb to about 40 cm high. Leaves oblong to broad-obovate, truncate to obtuse, 4–8 mm wide by 5–20 mm long. Inflorescence a short axillary raceme. Outer sepals elliptic, 1–2 mm long; wings green and herbaceous, falcate, sessile, 1–2 mm wide by 3–5 mm long. Keel pale purple with yellowish tip, bearing a many-branched crest; upper petals purple, obovate; corolla tube pubescent. Stamens free above, monadelphous. Ovary orbiculate. Style distinctly hooked; lower stigma fertile. Capsules oblong, ciliate, narrowly winged, emarginate, 3–4 mm wide by 4–6 mm long. Seeds black, smooth, hispid, ovoid; appendage shortly 3-lobed.
This species is restricted to Central Australia and occurs in arid areas of W.A., N.T., S.A. and Qld. It grows mainly in gravelly areas or sand in hummock grassland. Flowering mainly March–Oct. Map 16.

W.A.: 4 miles [6.4 km] W of Dovers Hills, N Gibson Desert, *A.S.George 9008* (PERTH); c. 20 km NNW of Newman, *A.A.Mitchell PRP657* (PERTH, PRH). N.T.: Tarlton Downs Station, Mt Guide, *C.R.Dunlop 2608* (BRI, DNA); Lander River, *N.M.Henry 648* (BRI, DNA). S.A.: Marqualpie Paddock, Innamincka Regional Reserve, *J.Gilen & P.Canty 112* (AD, BRI). Qld: 100 km NW of Mt Isa, 1.5 km E of Inca Ck, *P.L.Harris 680* (BRI).

15. **Polygala linariifolia** Willd., *Sp. Pl.* 3: 877 (1803), as *Linarifolia*

T: 'Habitat in insula Mindanao'; lecto: *Willdenow 12958*, B *n.v.*, *fide* F.Adema, *Blumea* 14: 275 (1966); fiche: CANB.
Polygala rhinanthoides var. *minor* Benth., *Fl. Austral.* 1: 140 (1863). T: 'Upper Victoria river, F.Mueller.'; *n.v.*
Polygala stenoclada var. *stenosepala* Benth., *Fl. Austral.* 1: 141 (1863). T: 'Carpentaria Point and Arnhem Bays, R.Brown (Hb. R.Br.)'; holo: *R.Brown 4971*, K *n.v.*; photo: CANB.
Polygala chinensis subvar. *linariifolia* Domin, *Biblioth. Bot.* 89: 303 (1927), as *linearifolia*. T: 'Queensland: Sandsteinhügel der Dividing Range bei Pentland (DOMIN II. 1910)'; holo: PR; photo: BRI.
Polygala gabrielae Domin, *Biblioth. Bot.* 89: 304 (1927). T: 'Nord-Queensland: auf halbfreien Stellen in den Savannenwäldern bei Chillagoe in Gesellschaft zahlreicher Annuellen (DOMIN. II. 1910)'; holo: PR; photo: BRI.

Illustration: F.Adema, *Blumea* 14: 275, fig. 19 (1966), as *linarifolia*.

Ascending to erect herb to about 30 cm high, pubescent with crisped hairs. Leaves highly variable, usually oblong to obovate, mucronate, 2–8 mm wide by 15–35 mm long. Inflorescence a rather dense axillary raceme. Outer sepals ovate, ciliate, 1.5–2.5 mm long; wings green, asymmetric-ovate, shortly clawed, 3–4 mm wide by 5–7 mm long. Keel blue to mauve, bearing a much-divided crest; upper petals mauve, spathulate; corolla tube ciliate. Stamens free above, monadelphous. Style strongly recurved at the apex, fertile stigma inside the curve. Capsules slightly asymmetric, broad-elliptic to ovate, glabrous or minutely ciliate, very narrowly winged, emarginate, 2–3 mm wide by 3–5 mm long. Seeds black, smooth, densely hairy, obloid; appendage hood-like with three short appendages reaching only to the top of the seed.
Occurs from China throughout southeast Asia and Malesia to Australia, where it is widespread from the Kimberley in W.A., across the N.T. into Qld and extending to northern N.S.W. It grows on a variety of soils in grassland or open forest. Flowering Sept.–Feb. Map 17.

W.A.: Gibb River road, 72.1 km by road W of Wyndham to Kununurra road, *T.E.H.Aplin et al. 627* (PERTH). N.T.: 27 km from Stuart Highway, along Gunn Point road, *J.D.Briggs 751* (CANB). Qld: ca 50 km WNW Of Charters Towers, *L.Pedley 4806* (BRI, CANB). N.S.W.: Munro State Forest (c. 15 km WSW of Copeton Dam), 1 km NNW of Macphersons Gap, 200 m SW of Gap Road, *L.M.Copeland 2855* (NE).

16. Polygala wightiana Wight & Arn., *Prodr. Fl. Penin. Ind. Orient.* 1: 38 (1834)

T: 'India. Peninsula Ind. or.'; syn: *Wallich 4190*, CGE, E, L, NY (2), all *n.v.*; photos: L, NY websites.
Polygala stenoclada Benth., *Fl. Austral.* 1: 141 (1863). T: 'Upper Victoria river, F.Mueller'; holo: K *n.v.*, photo: CANB.
Polygala stenoclada var. *queenslandica* Domin, *Biblioth. Bot.* 89: 305 (1927). T: 'Queensland: Savannenwälder bei Chillagoe, Mareeba, Pentland und auf dem Castle Hill bei Townsville (DOMIN II.–III. 1910); ohne nähere Standortsangabe, BOWMAN'; syn: K *n.v.*, photos: BRI, CANB.
Illustration: F.Adema, *Blumea* 14: 272, fig. 17 (1966).

Erect or ascending herb to around 50 cm high, glabrous. Leaves linear-lanceolate, mucronate, 1–2 mm wide by 5–20 mm long. Inflorescence a relatively long lateral raceme. Outer sepals lanceolate, ciliate apically, 1–2 mm long; wings green, asymmetric, shortly clawed, 1–2 mm wide by 3–4 mm long. Keel cream-yellowish, bearing a much-branched crest; upper petals cream, spathulate; corolla tube ciliate. Stamens free above, in three groups. Ovary elliptic to obovate. Style strongly recurved at the apex, fertile stigma inside the curve. Capsules oblong to obovate, with very few short hairs around the apex, narrowly winged, notched, 2–2.5 mm wide by 3–4 mm long. Seeds black, smooth, densely pubescent, obloid; appendage hood-like with three short appendages reaching only to the top of the seed.
Occurs in India, southeast Asia, Malesia and Australia; in Australia, it occurs in the Kimberley in W.A., the Top End of the N.T. and on Cape York Peninsula in Qld. Apparently grows in silty loams in open woodland. Flowering recorded for May. Map 18.

W.A.: Lennard River, 10 miles [16 km] above the junction of Barker River, *W.V.Fitzgerald 576* (PERTH). N.T.: Flora River, *C.Michell 756 & S.Callis* (DNA). Qld: Herald Is NW of Townsville, *R.J.Cumming 16996* (BRI); ca 89 km N of Hughenden, *E.J.Thompson HUG526* (BRI).

17. **Polygala exsquarrosa** Adema, *Blumea* 14: 268, fig. 13 (1967)

Polygala arvensis var. *squarrosa* Benth., *Fl. Austral.* 1: 141 (1863); *Polygala chinensis* var. *squarrosa* (Benth.) Domin, *Biblioth. Bot.* 89: 303 (1927). T: Endeavour River, R.Brown; Upper Victoria river, F.Mueller; syn: BM (2); isosyn: BRI.
Illustration: F.Adema, *Blumea* 14: 268, fig. 13 (1966).

Erect puberulous herb to about 30 cm high. Leaves linear to narrow-elliptic, mucronate, 1–5 mm wide by 5–17 mm long. Inflorescence an axillary raceme. Outer sepals elliptic, 2–3 mm long; wings green and herbaceous, falcate, mucronate, sessile, 1–2 mm wide by 5–8 mm long. Keel pale blue with deep blue tip, bearing a bilobed membranous crest; upper petals pale blue, obovate; corolla tube pubescent. Stamens free above, diadelphous. Ovary irregular-obovate. Style curving; lower stigma fertile. Capsules oblong to ovate, ciliate, asymmetric, narrowly winged, emarginate, 2–3 mm wide by 3–4 mm long. Seeds black, smooth, ovoid; appendage rather flattened with three membranous appendages.
Distributed in tropical northern Australia, from the Kimberley in W.A. through the N.T. and N Qld and also into adjacent Malesian islands, including New Guinea. Grows on sandy or gravelly soils in open woodlands, often in damp areas. Flowering and fruiting Jan.–Aug. Map 19.

W.A.: Long Creek, 15 km NNW of Beverley Springs Station, W Kimberley, *K.F.Kenneally 11554* (DNA, PERTH); King Edward River, ca 50 km NE of Mitchell River Homestead, *A.C.Beauglehole & E.G.Errey ACB 58855* (PERTH). N.T.: near Berry Springs, c. 20 miles SE of Darwin, *L.G.Adams 1713* (CANB, DNA, K, L). Qld: 15 km W of Bluewater NW of Townsville, *A.R.Bean 4391* (BRI); Cobra Ck between Tinaroo Falls and Malone Rd turnoff, *L.J.Webb 5896* (BRI).

18. **Polygala tepperi** F.Muell., *Vict. Nat.* 7: 38 (1890)

T: 'Roebuck Bay; W.Tepper'; holo: K *n.v.*; photo: CANB.
Polygala arvensis var. *stenosepala* Benth., *Fl. Austral.* 1: 141 (1863); *Polygala chinensis* var. *stenosepala* (Benth.) Domin, *Biblioth. Bot.* 89: 303 (1927). T: 'Victoria River, F.Mueller'; holo: K *n.v.*; photo: CANB.
Illustration: J.R.Wheeler, *in* J.R.Wheeler *et al.*, *Fl. Kimberley Region* 641, fig. 198C, 642, fig. 199F (1990).

Erect herb to around 60 cm high, pubescent with crisped hairs. Leaves narrow-elliptic to linear, mucronate, 1–3 mm wide by 10–30 mm long. Inflorescence a greatly-reduced axillary raceme. Outer sepals narrow-ovate, pubescent, 1.5–2.5 mm long; wings green, slightly asymmetric, sessile, 1.5–2 mm wide by 4–6 mm long. Keel mauve to blue, bearing a much-branched crest; upper petals pink to mauve, spathulate; corolla tube pubescent. Stamens free above, monadelphous. Ovary oblong to obovate. Style strongly recurved at the apex, fertile stigma inside the curve. Capsules oblong to obovate, pubescent, not winged, strongly notched into two pointed lobes, 2–3 mm wide by 4–5 mm long. Seeds black, smooth, densely hairy especially towards base, obloid; appendage knob-like with a ridged crest above, three short appendages reaching only to top of seed.
Endemic to Australia, *Polygala tepperi* occurs in the Western Kimberley region of W.A. and extends across the N.T. to the Gulf of Carpentaria. It grows in sandy soils in grassy eucalypt or mixed woodlands. Flowers and fruits March–May. Map 20.

W.A.: 10 km N of Alistairs Bore at gate near main road, *A.A.Mitchell 1989* (DNA, PERTH); One Arm Point, N Dampier Peninsula, Kimberley coast, *B.J.Carter 360* (PERTH). N.T.: Bungle

Bungle N.P., near Bull Creek, *K.A.Menkhorst 904 & I.Cowie* (DNA); CSIRO Kununurra, new Cockatoo Sand site, *M.H.Andrews 108* (DNA).

4. COMESPERMA

Comesperma Labill., *Nov. Holl. Pl.* 2: 21 (1806); from the Greek *komé* (hair of the head) and *sperma* (seed), for the long tufts of hair on the seeds of most species.

Type: *C. virgatum* Labill.

Shrubs, herbs or twining climbers. Leaves readily deciduous, sometimes reduced to scales. Inflorescence a pyramidal raceme or sometimes corymb-like or rarely reduced to single flowers. Calyx of 3 outer unmodified sepals with lower two sometimes connate and 2 inner enlarged petaloid "wing" sepals. Corolla of 3 petals with the lower one a boat-shaped "keel", fused together at the base and variously joined to the stamens. Stamens 8, fused at the base into a staminal tube and then either all free above or diadelphous and sessile; anthers 2-celled and dehiscent via a short apical slit. Fruit a laterally compressed bilocular capsule, usually clavate and attenuate or rarely spherical. Seeds almost always with readily deciduous coma, arising all over the seed coat or concentrated into particular areas, often with membranous appendages arising from the raphe.

A genus of 29 species endemic to Australia and represented in all States and Territories. The diversity is focused in the south-west of W.A. and along the east coast. A number of species store methyl salicylate ("oil of wintergreen") in their roots.

J.J.H.Labillardière, *Comesperma*, *Nov. Holl. Pl.* 2: 21–24, t. 159–163 (1806); J.Steetz, Polygaleae, *Revis. Gen. Comesperma* 1–25 (1847); R.H.Chodat & G.Hochreutiner, *Contribution a l'étude du genre Comesperma*, *Bull. Herb. Boiss.* 1: 358–369 (1893); C.G.G.J. van Steenis, Notes on *Bredemeyera* (*Comesperma*) with a new Papuan species and the Australian species listed (Polygalaceae), *Acta Bot. Neerl.* 17(5): 377–384 (1968); L.Pedley, Two new species of Polygalaceae from Central Australia, *J. Adelaide Bot. Gard.* 3(1): 127–129 (1981); L.Pedley, A revision of *Comesperma* (Polygalaceae) in Queensland, *Austrobaileya* 2: 7–14 (1984); G.J.Keighery, Two new species of *Comesperma* (Polygalaceae) from Western Australia, *Nuytsia* 15(1): 53–57 (2002).

1 Plant a twining climber
  2 Lateral sepals brownish-green to cream; pedicels pubescent **1. C. integerrimum**
  2: Lateral sepals pale mauve to deep blue or rarely white; pedicels glabrous
    3 Leaves reduced to scales with ciliate margins; inflorescence a terminal raceme; lateral sepals deep blue or purple; mature fruit with a short apical beak and a marginal wing **2. C. ciliatum**
    3: True leaves present, although often caducous; inflorescence a lax raceme on short lateral shoots; lateral sepals pale blue through mauve or rarely white; mature fruit not winged or beaked **3. C. volubile**
1: Plant a shrub or subshrub or herb, stems sometimes trailing but not twining
  4 Plant usually ±leafless or with leaves reduced to scales, stems ribbed; fruit circular or shortly obovate; seeds lacking a coma

5 Plant a few-stemmed lax trailing subshrub; true leaves absent or with a few persisting only at the base; inflorescence a sparse terminal raceme **4. C. sphaerocarpum**

5: Plants usually many-stemmed and upright; leaves reduced to sessile scales; inflorescence reduced to solitary flowers scattered along stems

6 Plant a tangled shrub with rather slender stems; flowers distinctly pedicellate; lateral sepals pink **5. C. aphyllum**

6: Plant a robust, virgate, broom-like shrub; flowers apparently sessile as pedicels hidden by bracts; lateral sepals blue to purple **6. C. scoparium**

4: Plants sometimes ±leafless or with scale leaves, stems sometimes ribbed; fruit cuneate or clavate with base elongated into a stipe; seeds with a coma

7 Stems regularly ribbed; leaves absent over much of the plant or if present reduced to scales

8: Plant a robust, divaricate shrub with spinescent branchlets; inflorescence reduced to solitary flowers scattered along stems; lateral sepals pink to mauve **7. C. spinosum**

8 Plant a slender, erect few-stemmed rather sedge-like subshrub; inflorescence several-flowered and terminal; lateral sepals mauve or blue

9 Inflorescence a few-flowered pyramidal raceme; outer sepals all free, wing sepals fused to corolla at base; seeds tailed and with coma densest on margins **8. C. defoliatum**

9: Inflorescence a dense-corymb-like raceme; lower pair of outer sepals connate for much of their length, wing sepals free of corolla; seeds lacking tail and with coma densest at base **9. C. nudiusculum**

7: Stems not regularly ribbed (although often with decurrent ridges from leaf base); true leaves present over much of the plant

10 Lower pair of outer sepals connate for much of their length

11 Plant a soft sub-shrub; leaves rather thick and leathery, ovate **10. C. calcicola**

11: Plant an erect shrub; leaves not thick or leathery, linear to elliptic

12 Leaves keeled below by prominent midrib; membranous hooked appendage present near tip of keel petal; seeds with short membranous tail **11. C. virgatum**

12: Leaves flat; keel petal unadorned; seeds without tail **12. C. polygaloides**

10: Lower pair of outer sepals free

13 Plant a slender subshrub or herb

14 Plant a rosette herb; leaves oblong or obovate; inflorescence often hidden by or just exceeding upper leaves; lateral sepals white **13. C. griffinii**

14: Plant a slender subshrub; leaves usually elliptic or linear; inflorescence fully exceeding upper leaves; lateral sepals blue

15 Lateral sepals much longer than outer sepals; fruit narrow and fusiform **14. C. rhadinocarpum**

15: Lateral sepals only slightly longer than outer sepals; fruit clavate

16 Leaves linear to elliptic, thin; inflorescence a compressed, corymb-like raceme **15. C. lanceolatum**

16: Leaves variable, usually elliptic to oblong, rather thick and leathery; inflorescence an elongate raceme **16. C. calymega**

13: Plant an erect robust branching shrub

17 Lateral sepals yellow to cream or white

18 Stems viscid; leaves appressed with recurved apices **17. C. viscidulum**

18: Stems not viscid; leaves ± spreading and lacking recurved apices

19 Leaves rather thick and leathery, rounded or cordate at the base; inflorescence often appearing secund **18. C. secundum**

19: Leaves not leathery, tapering at the base; inflorescence not apparently secund

20 Growing usually in rather wetter, swampy habitats; leaves linear to very narrow-elliptic; inflorescence very dense, rather corymb-like and distinct from upper leaves **19. C. flavum**

20: Growing in drier sandy or gravelly areas; leaves oblong to obovate; inflorescence rather short and few-flowered and held among or slightly exceeding upper leaves **20. C. pallidum**

17: Lateral sepals pink to purple

21 Leaves usually oblong or obovate, rather thick and leathery

22 Plant a rather short bushy shrub; inflorescence a dense pyramidal raceme elongating in fruit **21. C. oblongatum**

22: Plant an erect leggy shrub; inflorescence relatively few-flowered and not greatly exceeding the upper leaves

23 Leaves and stems silvery-glaucous, pale green beneath; flowers a pale mauve or pink **22. C. drummondii**

23: Leaves and stems somewhat glaucous but stems often reddish beneath; flowers a deep pink to magenta

24 Leaves oblong to somewhat obovate, acute; seeds with linear appendages on both margins and forming a short tail **23. C. sylvestre**

24: Leaves oblong, truncate; seeds with linear appendage on inner margin, not tailed **24. C. retusum**

21: Leaves elliptic or linear, thin and not leathery

25 Leaves stiff, pungent and strongly keeled below; keel petal with horn-shaped appendage below tip **25. C. acerosum**

25: Leaves not stiff, mucronate and not strongly keeled below; keel petal unadorned

26 Stamens free above; coma concentrated around base of seed **26. C. confertum**

26: Stamens diadelphous and sessile; coma evenly distributed over seed

27 Leaves usually >2 mm wide, flowers medium, with inner sepals and corolla tube 5–8 mm long **27. C. ericinum**

27: Leaves narrow (<2 mm wide), flowers small, with inner sepals and corolla tube ≤4 mm long

28 Plant glabrous; inflorescence elongate (particularly in fruit) and distinct from upper leaves; pedicels 2–3 mm long **28. C. breviflorum**

28: Plant hispidulous; inflorescence short and barely exceeding upper leaves; pedicels 4–6 mm long **29. C. praecelsum**

1. Comesperma integerrimum Endl., *in* S.L.Endlicher *et al.*, *Enum. Pl. Huegel* 7 (1837), as *integerrima*

Bredemeyera integerrima (Endl.) Steenis, *Acta Bot. Neerl.* 17: 393 (1968). T: 'King Georges Sound. (Hügel)'; holo: W.
Comesperma scandens Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 1: 211 (1845). T: 'In arenosi districtus Sussex, 25. Dec. 1839, Herb. Preiss. No. 2376'; holo: LD.

Pubescent twining climber, stems ribbed. Leaves linear to narrow-oblong, apex rounded or truncate, 2–3 mm wide by 15–30 mm long. Inflorescence a loose raceme on short lateral shoots, few to many-flowered. Outer sepals ovate, ciliate, 1.5–2 mm long; wings cream to dull yellow, orbicular to broad-ovate, base slightly auriculate, shortly clawed, 4–6 mm wide by 6–8 mm long. Keel purple at the tip and pinkish-cream below; upper petals cream with reddish flecks, rounded-oblong; base of corolla tube ciliate. Stamens free above. Ovary pyriform. Capsules sharply cuneate, beaked, 3–5 mm wide by 30–40 mm long. Seeds brownish, wrinkled, hemi-ellipsoidal with an elongate pointed tail, 15–20 mm long, coma densest on margins.
Occurs throughout south-western W.A. and in a small area of western N.S.W. between Griffith and Cobar. It grows in a variety of soils, often in mallee in W.A. or *Callitris*-dominated woodland in N.S.W. Flowering mainly Aug.–Nov. Map 21.

W.A.: on northern side of track running east off track around mine ridge, c. 2.4 km W of Landing Ground, c. 13 km NE of Barcooting Hill, Ennuin Station, *N.Gibson 3710 & M.Lyons* (CANB, PERTH); 1.3 km SW of Paynes Find toward Wubin on Great Northern Highway, *S.Donaldson 1387 & G.Flowers* (CANB, PERTH); Tank #8, NW Coastal Highway, 20 km N of "Eurardy" turn-off, *M.D.Crisp 9255 & L.G.Cook* (CANB, PERTH). N.S.W.: McPhersons Range 1 km directly north-west of Pioneer Park, Griffith, *D.J.Mallinson 622* (CANB); Yathong Nature Reserve, via Mt Hope; on the north boundary of the reserve, by the track along the north fence of old Glenlea Station, *A.V.Slee 647 & M.D.Holgate* (CANB).

Although similar to *Comesperma volubile*, particularly in Western Australia, this species is readily distinguished by its generally more pubescent stems and floral parts, by the colour of the flowers and by the larger and acute fruit and seeds.

2. **Comesperma ciliatum** Steetz, *Revis. Gen.* Comesperma 14 (1847)

Bredemeyera ciliata (Steetz) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: Hab. in Novae Hollandiae ora australi-occidentali, in Swan River Colonia, Drummond! Collect. 2. No. 485! (ex parte, cum Comespermate tortuoso commixtum.)'; iso: K, MEL, W.

Twining climber, leaves reduced to scales, stems ribbed. Scale leaves elliptic, 0.5–0.7 mm wide by 1.5–2 mm long, hirsute on back and margins. Inflorescence a dense raceme on long shoots, usually many-flowered. Outer sepals narrow-ovate, ciliate, 2–3 mm long; wings pale to deep blue, ovate, shortly clawed, 3–4 mm wide by 5–6 mm long. Keel deep purple with a yellow tip; upper petals cream with purple tips, rounded-oblong. Stamens free above. Ovary ovate with a short beak. Capsules clavate, 3–5 mm wide by 10–15 mm long, Seeds greenish, with regular marginal channels, hemi-ellipsoidal, without marginal appendages, coma densest on margins.
Occurs mostly in near-coastal regions of south-western W.A. Grows usually in gravelly loams or sand in open eucalypt forest. Flowering mainly Aug.–Nov. Map 22.

W.A.: Cape Le Grand National Park, E of Esperance, *R.D.Royce 9858* (PERTH); Kululinup Nature Reserve, *G.J.Keighery & N.Gibson 2117* (PERTH); lower slopes of Mount Magog, 1 km from picnic area and along gully at picnic area 18 km on Stirling Range Drive on from Chester Pass Road, *R.S.Cowan A-537* (CANB).

Although superficially similar to *Comesperma volubile*, this species can be distinguished by its ciliate scale leaves, and usually deeper blue flowers with longer pedicels in denser racemes. The fruit also have a more truncate apex with a short beak in contrast to those of *C. volubile* which are acute.

3. **Comesperma volubile** Labill., *Nov. Holl. Pl.* 2: 24, t. 163 (1806), as *volubilis*

Bredemeyera volubilis (Labill.) Chodat ex Anon., *Census. Pl. Victoria* 40 (1923). T: in capite Van-Diemen; holo: ?FI *n.v.*, iso: BM.
Comesperma gracile Paxton, *Paxton's Mag. Bot.* 5: 93, 145, pl. (1838), as *gracilis*. T: not cited.
Comesperma tortuosum Steetz, *Revis. Gen.* Comesperma 13–14 (1847). T: 'Hab. In Novae Hollandiae ora australi-occidentali. In Swan-River Colonia legerunt cll. Preiss et Drummond (Herb. Preiss. No. 1015. Herb. Drummond. Collect. 2. No. 485 (ex parte, cum sequente commixtum)'; syn: LD, MEL (*Preiss*), W (*Drummond*).
Comesperma volubile var. *micranthum* Steetz, *Revis. Gen.* Comesperma 13 (1847), as *micrantha*. T: 'Hab. in insula van Diemen, Gunn! Schayer! (v.s.)'; holo: *n.v.*
Comesperma volubile var. *alba* F.M.Bailey, *Queensland Fl.* 1: 80 (1899). T: not cited.
Illustrations: J.J.H. de Labillardiere, *Nov. Holl. Pl.* 2: 24, t. 163 (1806).

Twining climber, often ±leafless, stems ribbed. Leaves narrowly oblong to elliptic, apex acute, 1–3(—5) mm wide by 10–15(—30) mm long. Inflorescence a loose raceme on

short lateral shoots, few- to many-flowered. Outer sepals triangular, 1.5–2 mm long; wings pale blue to mauve, orbiculate to broad-ovate, base slightly cordate, shortly clawed, 5–7 mm wide by 5–8 mm long. Keel deep purple at the tip and cream below, with oblong lateral lobes; upper petals cream with purple-flecked tips, spathulate; corolla tube sometimes pubescent. Stamens free above. Ovary pyriform. Capsules clavate, 3–5 mm wide by 10–20 mm long. Seeds brown, wrinkled, hemi-ellipsoidal, linear appendages on both margins and forming a short tail; coma densest on margins. Flowering mainly Sept.–Nov. *Blue Love Creeper*.
An extremely widespread species, occurring on the coastline in southern Australia from south-western W.A. to southeast Qld. Grows in a wide variety of soils and vegetation types from heath to wet sclerophyll forest. Flowering mainly July–Dec. Map 23.

W.A.: Parker Range, about 2.1 km S of Mt. Caudan, Nth Parker Range, N of track, on ridge, N.Gibson & M.Lyons 2074 (PERTH). S.A.: Tungketta Station, Flinders Highway (West Coast) some 20 km SE of Elliston, *C.R. Alcock 1228* (AD). Qld: Ferntree NP ca 5km N of Nambour, *P.R.Sharpe 4823* (BRI). N.S.W.: cliff top 250 m S of Kew Trig Point, Kew State Forest, *P.Richards PR812* (NSW). A.C.T.: 5 km W of Condor Creek crossing along Brindabella Rd, *G.Stewart 214* (CANB). Vic.: Brisbane Ranges National Park, Macleans Highway near junction of Switch Road, *D.B.Foreman 1578* (CANB, MEL). Tas.: Great Northern Creek, North East Dundas Tramway, *A.E.Orchard 5639* (CANB, HO, NSW).

This species is highly variable throughout its range, often adopting a more robust and "shrubby" habit in Western Australia and South Australia, usually with few large flowers per inflorescence. In general, specimens from eastern Australia are more slender, leafy and floriferous.

4. **Comesperma sphaerocarpum** Steetz, *Revis. Gen.* Comesperma 24 (1847)

Bredemeyera sphaerocarpa (Steetz) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: 'Hab. in Nova Hollandia. Ferd. Bauer! (V.s. in herbario palatino Vindobonensi, cum Comespermate nudiusculo commixtum!)'; holo: W.

Straggling wiry shrub to about 30 cm long, leaves reduced to scales, stems ribbed. Scale leaves elliptic, 0.5–0.75 mm wide by 1–2 mm long, ciliate. Inflorescence a sparse raceme on upper branches. Outer sepals oblong, acute, 3–4 mm long; wings mauve to purple, orbiculate to broad-obovate, base tapering, distinctly clawed, 5–6 mm wide by 6–7 mm long. Keel cream with pink-purple tip; upper petals white, oblong, acute, pubescent; corolla tube pubescent. Stamens free above. Ovary orbiculate. Capsules orbiculate to obovate, 3–5 mm wide by 4–5 long. Seeds black, smooth, obloid, linear appendages on both margins and forming a short tail; coma absent.
Distributed along the east coast of Australia from far southern N.S.W. to around Rockhampton in central Qld and with a disjunction to the Wet Tropics in northern Qld. Grows in wetter areas of open eucalypt forests or heaths, usually on sandy soil. Flowering mainly Oct.–Feb. Map 24.

Qld: Blackdown Tbld Mimosa Ck 0.4km E of crossing, *R.J.Henderson H1379* (BRI); SF 132 10km ESE of Brovinia S of Mundubbera, *A.R.Bean 12583* (BRI). N.S.W.: Yarran Road, 2.1km from eastern junction with Turpentine Road, D.L.Jones 18217 (CANB); 9.9 km S of Boonoo Boonoo River, Mount Lindesay Highway, *P.G.Wilson 1320 & R.Rowe* (NSW).

This species can be difficult to distinguish from *Comesperma defoliatum* in flower, but it has a much laxer habit than *C. defoliatum* and the almost circular non-stipitate capsules are diagnostic.

5. **Comesperma aphyllum** R.Br. ex Benth., *Fl. Austral.* 1: 143 (1863)

Bredemeyera aphylla (R.Br. ex Benth.) Ewart and O.Davies, *Fl. N. Terr.* 160 (1917). T: 'Islands of the N. Coast, R. Brown' (NORTHERN TERRITORY, Cotton's Island (island Y1), near Arnhem Bay, Feb. 18th 1803, *R.Brown 4989*); iso: BM (2), CANB, K, MEL (2).

Tangled spreading shrub to about 50 cm tall, leaves reduced to scales, stems ribbed. Scale leaves triangular, ciliate, 0.25–0.5 mm wide by 1–2 mm long. Inflorescence reduced to solitary flowers scattered along the stems. Outer sepals oblong, 2–3 mm long; wings pink, obovate, base tapering to a short claw, 2–3 mm wide by 4–5 mm long. Keel deep pink with yellow tip; upper petals cream, oblong with somewhat acute tips; corolla tube glabrous. Stamens free above. Ovary circular. Capsules orbicular to obovate, 3–5 mm wide by 3–5 mm long. Seeds black, smooth, obloid, linear appendages on both margins and forming a short membranous tail; coma absent.
Restricted to a small area in Arnhem Land, N.T. Growing on sandstone in eucalypt woodland. Flowers mainly Sept–Mar. Map 25.

N.T.: Kakadu National Park: opposite Mary River Ranger Station, *A.V.Slee 2991* (CANB, DNA);Kakadu National Park, 2 km from Koongarra Saddle along track towards Jim Jim road, 19 km SSW of Jabiru, *I.R.Telford 7876 & J.W.Wrigley* (CANB); Elcho Island, *J.R.Maconochie 2197* (CANB, DNA).

6. **Comesperma scoparium** J.Drumm. ex Steetz, *Revis. Gen.* Comesperma 19 (1847)

Bredemeyera scoparia (J.Drumm. ex Steetz) Chodat ex Anon., *Census Pl. Victoria* 40 (1923). T: 'Hab. in Novae Hollandiae ora australi-occidentali. In Swan-River Colonia leg. cl. Drummond! Collect. 2. No. 487 et 488! (v.s. in herb. palatino Vindobonensi!)'; syn: MEL (4), W (2).

Erect virgate shrub, leaves reduced to scales, stems ribbed. Scale leaves subulate to triangular, 0.5–0.8 mm wide by 1–2 mm long, pubescent in the axils. Inflorescence reduced to solitary flowers scattered along the stems. Outer sepals oblong, 3–5 mm long; wings blue to mauve, orbicular to broad-obovate, base rounded, shortly clawed, 3–4 mm wide by 5–7 mm long. Keel deep purple with yellow tip; upper petals white with purple tips, rounded-oblong; corolla tube ciliate. Stamens free above. Ovary cordate. Capsules obovate with thickened margins, obtuse, 3–5 mm wide by 5–8 mm long. Seeds black, smooth, obloid, linear appendages on both margins and forming a distinct tail; coma absent. *Broom Milkwort, Swan River Broom.*
Very common throughout south-western W.A., with disjunct populations on the Eyre and Yorke Peninsulas, S.A. and in the far north-west of Vic. Recorded by Bentham (*Fl. Austral.* 1: 163 (1863)) as occurring in western N.S.W. based on collections by Mueller, but no specimens have been seen. Grows mainly on sandy soils in mallee woodlands or heath. Flowering mainly July–Oct. Map 26.

W.A.: east -west track S of central southern boundary of Cooloomia Nature Reserve, 2.77 km E of cross-roads (E track to Nerren Nerren), *G.J.Keighery & N.Gibson 1875* (PERTH); c. 3 km SE on Dongolocking Road from Harrismith Road, E. Narrogin area, *L.W.Sage LWS 858 & J.P.Pigott* (PERTH). S.A.: Alligator Gorge. Kingfisher Flat towards Hidden Gorge, *D.J.E.Whibley 8023* (AD). Vic.: Sunset Country, 5km S of Pheenys Track on Underbool Track, *J.H.Browne s.n.*, 27 Sept. 1988 (MEL).

Drummond's description of this species included discussion of the use and sale of the plant as a broom by Aboriginal people in the Perth area. See R.K.Brummitt, *Taxon* 51: 171–174 (2002) for a discussion on the validity of Drummond's name for this species and of "*nomina subnuda*" in general.

7. **Comesperma spinosum** F.Muell., *Fragm.* 1: 144 (1859)

Bredemeyera spinosa (F.Muell.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: In locis arenosis ad montem West Mount Barren sicut in tractu Fitzgerald Range. Mxw; holo: MEL; iso: K.

Divaricate glabrous spiny shrub to 60 cm tall, leaves reduced to scales, stems ribbed. Scale leaves triangular, 0.5–0.8 mm wide by 1.5–2 mm long. Inflorescence reduced to solitary flowers scattered along branchlets. Outer sepals oblong, 2–3 mm long; wings pink to mauve, orbicular to broad-deltoid, base truncate, shortly clawed, 4–8 mm wide by 5–9 mm long. Keel deep pink to purple with yellow tip; upper petals white, rounded-oblong; corolla tube ciliate. Stamens free above. Ovary pyriform. Capsules clavate, beaked, 2–5 mm wide by 10–15 mm long. Seeds black, wrinkled, hemi-ellipsoidal, reduced linear appendage on inner side; coma evenly distributed.
Grows chiefly along the southern coast of W.A. from the Stirling Ranges to about Cape Arid, but extends inland into the wheatbelt. Found mainly on sandy soils in mallee woodlands. Flowering Sept.–Jan. Map 27.

W.A.: along Number 2 Rabbit Proof Fence, ca 35 km SSE of Jerramungup–Ravensthorpe road, ca 30 km N of Bremer Bay, *P.G.Wilson 4381* (PERTH); Fitzgerald River National Park, by Hammersley Drive, 1-2 km from N border of park (WNW of Hopetoun), *A.Strid 21008* (PERTH); 10.7 km ENE of Scaddan, 2.4 km W of Liebecks Street on Norwoods Road, M.A.Burgman MAB 2569 & S.McNee (PERTH).

This species is sometimes described as possessing cordate fruit and as lacking a coma on the seeds. This information would seem to be based on examination of immature material.

8. **Comesperma defoliatum** F.Muell., *Pl. Indig. Victoria* 1: 189 (1862)

Based on *Comesperma nudiusculum sensu* Steetz, *Revis. Gen.* Comesperma 18 (1847), *non* DC. (1824); *Bredemeyera defoliata* (F.Muell.) Chodat ex Anon., *Census Pl. Victoria* 40 (1923). T: Scattered over sandy-heathy ridges from Port Phillip to the Broadribb River. In Tasmania found near Southport; in New South Wales at Illawarra by Mr. Shepherd, at the Clarence River by Dr. Beckler; syn: MEL; isosyn: K.

Erect, virgate subshrub to about 60 cm, often leafless, stems ribbed. Leaves elliptic, 0.25–0.5 mm wide by 3–5 mm long, glabrous. Inflorescence a few-flowered pyramidal raceme. Outer sepals oblong, 2–4 mm long; wings mauve to blue, orbicular to broad-obovate, base gradually tapering to a short claw, 2–3 mm wide by 3–4 mm long. Keel deep purple with yellow tip; upper petals cream, oblong and somewhat acute; corolla tube margins tuberculate. Stamens free above. Ovary orbicular, pubescent. Capsules clavate, apically sparsely pubescent, 2–4 mm wide by 8–12 mm long. Seeds black, smooth, ovoid, narrow appendages on both margins and forming a short tail; coma densest on margins. *Leafless Milkwort.*
Occurs in coastal areas in south-eastern Australia, from Vic. and Tas. through N.S.W. to around Rockhampton in Qld. Grows in wetter areas such as swamp margins and near creeks, often on peaty soils and in wet heath vegetation. Flowering mainly Nov.–Feb. Map 28.

Qld: about 1 km SW of Cape Moreton, NE end of Moreton Island, *R.J.Henderson H2371* (AD, BISH, BRI, CANB, DNA, K, L, LAE, MEL, MO, NSW, PERTH). N.S.W.: 10 km WNW of Milton, *L.G.Adams 3748 & K.Paijmans* (CANB). Vic.: Far SW Lower Glenelg River area, Kentbruck Heath, N. of Mt Kincaid, *A.C.Beauglehole 19513* (MEL). Tas: SW National Park, Middle Giblin River Basin, 8 km NW of Mt Gaffney, 13 km N of Mulcahy Bay, *J.R.Croft 10147 & M.M.Richardson* (CANB, HO, NSW).

9. **Comesperma nudiusculum** DC., *Prodr.* 1: 334 (1824), as *nudiuscula*

Bredemeyera nudiuscula (DC.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: 'in Novâ-Hollandiâ ad portum regis Georgii. Lechenault (v.s.)'; holo: G-DC; fiche: NSW.
Comesperma megapterygum Steud., *Pl. Preiss.* 1: 207 (1845) T: 'In arenosis districtus Sussex, Dec. 1839. Herb. Preiss. No. 2370'; holo: LD.
Comesperma ramosissimum Steud., in J.G.C.Lehmann, *Pl. Preiss.* 1: 209 (1845), as *ramosissima*. T: 'In turfosis arenosis prope oppidulum Albany, 4. Dec. 1840. Herb. Preiss. No. 2369'; iso :LD, MEL (2), W.

Ascending to erect open subshrub, 30–60 cm tall, often almost leafless, stems ribbed. Leaves linear, 1–2 mm wide by 3–6 mm long, acute, glabrous. Inflorescence a many-flowered corymb-like raceme. Outer sepals ovate, acute, 3–4 mm long, lower pair connate and emarginate; wings mauve to deep blue, ovate, base tapering to a short claw and fusing with corolla, 3–4 mm wide by 4–6 mm long. Keel blue with yellow tip; upper petals white, elliptic, acute; corolla tube margins papillose. Stamens free above. Ovary pyriform. Capsules clavate with a short apical beak, sparsely pubescent, 1–2 mm wide by 4–6 mm long. Seeds black, smooth, spherical, reduced linear appendages on both margins; coma densest at base.
Endemic to W.A., *Comesperma nudiusculum* is found only in the far south-west of the State. It grows in seasonally-wet black sand flats. Flowering mainly Dec.–Mar. Map 29.

W.A.: N side of Lake William Road, near Dunskys Road junction, West Cape Howe National Park, *N.Gibson & M.Lyons 1055* (PERTH); along the western boundary of Two Peoples Bay Nature Reserve, Section 1, *G.T.Smith & L.A. Moore s.n.*, 6 May 1976 (PERTH); N side of Scott Road, 250 metres E of Lake Smith, 1.8 km E of Lake Jasper Road, intersection, D'Entrecasteaux National Park, *N.Gibson & M.Lyons 1128* (PERTH); Poole Swamp, Blythe Road, Yelverton Forest, NNW Margaret River, *G.J.Keighery 12691* (PERTH); Black Point Road, *R.Cranfield 11045* (PERTH).

This species is similar in habit to *Comesperma defoliatum* but has a crowded and corymb-like inflorescence rather than an open pyramidal raceme.

10. **Comesperma calcicola** Keighery, *Nuytsia* 15(1): 54 (2002)

T: southern margin of Truslove Nature Reserve, Western Australia, 20 October 2000, *G.J.Keighery & N.G.Gibson 3127*; holo: PERTH, iso: CANB, K, MEL.
Illustrations: G.J.Keighery, *Nuytsia* 15(1): 55, fig. 1A–B

Erect glaucous clonal subshrub to about 40 cm tall, glabrous. Leaves leathery, obovate, acute, 2–4 mm wide by 3–9 mm long. Inflorescence a many-flowered pyramidal raceme held among upper leaves. Outer sepals ovate, 1–2 mm long, lower pair fused with an apical notch; wings apparently cream to pink with reddish tips, narrowly obovate, base tapered, sessile, 1–1.5 mm wide by 2–3 mm long. Keel cream with maroon flushing at tip; upper petals cream, oblong, acute; corolla tube glabrous. Stamens diadelphous and sessile. Ovary circular. Capsules obovate to shortly clavate, 2–3 mm wide by 4–6 mm long. Seeds black, smooth, ovoid, linear appendages on both margins and forming a short tail; coma evenly distributed.
Endemic to W.A. and collected only a few times, *Comesperma calcicola* is distributed near the south coast. It grows on calcareous or somewhat saline soils in mallee or shrublands. Flowering mainly Oct.–Jan. Map 30.

W.A.: 0.5 km SE of Kau Rock, 65 km NE of Esperance, *W.Archer 1301901* (PERTH); 4 km S of Forrestania crossroads towards Digger Rocks/Hatter Hill, *B.J.Lepschi 2185* (PERTH); 21 km NNW of Mount Ragged, 23 km ESE of Mount Buraminya, *B.Archer s.n.*, 17 Jan. 1989.

Listed as Priority 3 (several known populations and not currently threatened) in the CALM Conservation Codes for Western Australian Flora.

11. Comesperma virgatum Labill., *Nov. Holl. Pl.* 2: 21, t. 159 (1806), as *virgata*

Bredemeyera virgata (Labill.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: 'in terrâ Van - Leuwin'; holo: ?FI *n.v.*; iso: BM.

Comesperma simplex Endl., *Enum. Pl. Huegel* 7 (1837); *Comesperma virgatum* var. *simplex* (Endl.) Steetz, *Revis. Gen.* Comesperma 22 (1847). T: Freemantle ad Swan-River (Hügel.); holo: W.

Comesperma corniculatum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 206 (1845), as *corniculata*; *Comesperma virgatum* var. *corniculatum* (Steud.) Domin, *Mem. Soc. Roy. Sci. Boheme* 1921–2: 2 : 58 (1923). T: In glariosis sylvatica circa Muljenup ditionis Plantagenet, 14 Oct. 1840. Herb. Preiss. No. 2360; lecto: LD, isolecto: MEL (2), W.

Comesperma longebracteatum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 207 (1845), as *longibracteata*; *Comesperma virgatum* var. *longebracteatum* (Steud.) Steetz, *Revis. Gen.* Comesperma 22 (1847), as *longebracteata*. T: In turfosis fruticosis districtus Princess Royal Harbour, 27. Sept. 1840. Herb. Preiss. No. 2361; iso: LD, MEL (2).

Comesperma roseum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 207 (1845), as *rosea*. T: 'In humoso-arenosis depressis umbrosis prope Monger'slake, 4. Febr. 1839. Herb. Preiss. No. 2363'; iso: LD [marked 2263], MEL (2)/LD.

Comesperma aemulum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 208 (1845), as *aemula*. T: In arenosis districtus Sussex, 19. Dec. 1839. Herb. Preiss. No. 2371; holo: LD, iso: MEL (2).

Comesperma contractum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 208 (1845), as *contracta*; *Comesperma virgatum* var. *contractum* (Steud.) Steetz, *Revis. Gen.* Comesperma 22 (1847), as *contracta*. T: In turfosis inter arundines ad marginem lacus prope villam Dr. Helms, districtus Perth, 24. May 1839. Herb. Preiss. No. 2373; holo: LD, iso: MEL.

Comesperma laxiusculum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 210 (1845), as *laxiuscula*. T: In lapidosis districtus York, 21. Mart. 1839. Herb. Preiss. No. 2372; holo: LD.

Erect leggy shrub, to about 1.5 m tall, glabrous. Leaves keeled below, linear to narrow-elliptic, acute, 1–3 mm wide by 4–20 mm long. Inflorescence a many-flowered pyramidal raceme. Outer sepals ovate, 1.5–2 mm long, lower pair connate and emarginate; wings pale pink to magenta, obovate, base tapering, sessile, 3–5 mm wide by 6–8 mm long. Keel deep pink with yellow tip and hooked dorsal appendage; upper petals cream, rounded-oblong; corolla tube papillose on the margins. Anthers diadelphous and sessile. Ovary obovate. Capsules clavate, shortly beaked, 2–3 mm wide by 5–8 mm long. Seeds black, smooth, obloid, linear appendages on both margins (reduced on outer side) and forming a short membranous tail; coma concentrated on base.

Endemic to W.A. and found in near-coastal regions in the south-west. It grows in sandy soils often in jarrah/karri forest. Flowering mainly Sept.–Feb. Map 31.

W.A.: 11 miles [17.5 km] from Denmark toward Manjimup, *M.E.Phillips s.n.*, 12 Oct. 1962 (CANB); Driver Rd, off Old Coast Road. Australind, *L.J.Nunn 414* (CANB, PERTH); Albany, *W.E.Blackall s.n.*, Jan. 1938 (PERTH); Crampton Nature Reserve, 26 km N of Bunbury, *G.J.Keighery 13320* (PERTH); Yalgorup National Park, *A.Strid 21356* (PERTH).

This species has distinct similarities to *Comesperma acerosum*, including the keeled leaves, crowded inflorescences and the hooked appendage on the keel. However, it is easily distinguished by the connation of the lower pair of outer sepals.

12. Comesperma polygaloides F.Muell., *Trans. Philos. Soc. Victoria* 1: 7 (1854)

Bredemeyera polygaloides Chodat ex Anon., *Census Pl. Victoria* 40 (1923). T: In barren plains at the Avoca, Guichen Bay and Encounter Bay; syn: MEL (3), K.

Erect glaucous shrub, 30–60 cm tall. Leaves usually crowded, keeled below, elliptic to narrowly obovate, acute, 1–3 mm wide by 6–15 mm long. Inflorescence a many-flowered pyramidal raceme. Outer sepals ovate, 2–3 mm long, lower pair fused with an apical notch; wings pale pink to mauve often with a green stripe, oblong to obovate, base tapered, sessile, 2–3 mm wide by 4–6 mm long. Keel deep pink with a yellow tip; upper petals pale cream to white, rounded-oblong; corolla tube glabrous. Stamens diadelphous and sessile. Ovary circular. Capsules clavate, 2–3 mm wide by 6–9 mm long. Seeds black, smooth, ovoid, linear appendages on both margins, not tailed; coma evenly distributed. *Small Milkwort.*

This species is distributed in southern regions of W.A., with a disjunction across the Nullarbor Plain and is then present from southeastern S.A. into southwestern Vic. and as far as Melbourne. It grows on rather swampy clay soils in grassy woodlands. Flowering mainly Nov.–Feb. Map 32.

W.A.: Arthur River flats, 22.8 km WNW of Albany Highway on road to Moodiarrup, *T.R.Lally 874 & B.J.Lepschi* (CANB, PERTH). S.A.: Newland Head Cons[ervation] Park, *D.E.Murfet 1715 & R.L.Taplin* (AD); Sec[tion] 262, H[undre]d of Warrow, *S.Ronson s.n.*, 10 Feb. 1993 (AD). Vic.: Eastern Little Desert NP, *A.C.Beauglehole 87713* (MEL).

13. Comesperma griffiniii Keighery, *Nutysia* 15(1): 56 (2002)

T: Indarra Nature Reserve, on Ardingly South Rd, 20 km south-west of Mullewa, Western Australia, 25 October 1988, *G.J.Keighery s.n.*; holo: PERTH.
Illustration: G.J.Keighery, *Nuytsia* 15(1): 55, fig. 1C–D (2002).

Glaucous rosette herb to about 15 cm high, glabrous. Leaves broad obovate, leathery, 5–10 mm wide by 10–30 mm long, mucronate. Inflorescence a few-flowered pyramidal raceme held among upper leaves. Outer sepals ovate, acute, 1–1.5 mm long; wings apparently pale pink, obovate, base tapering, shortly clawed, fused to keel at the base, 1–2 mm wide by 2–3 mm long. Keel deep pink with yellow tip; upper petals cream, obovate, acute; corolla tube margins glabrous. Stamens free above. Ovary orbiculate. Capsules clavate, 1–2 mm wide by 4–5 mm long. Seeds smooth, black, ovoid, linear appendage only on inner margin, forming a short tail; coma concentrated around base.

Endemic to W.A., this species has only been collected a few times in scattered regions of the southwest. It is recorded as growing in sand in dry heath vegetaion. Flowering mainly Sept.–Oct. Map 33.

W.A.: c. 60 km direct NE of Wubin, c. 19 km direct SW of Mt Gibson, 1.5 km E of Great Northern Highway along road to Mt Gibson gold mine, *A.M.Lyne et al. 864* (AD, CANB, MEL, PERTH); verge on W side of road, 51 km S of Marvel Loch along Southern Cross road towards Lake King, *A.M.Monro 38* (CANB); Allied Eneabba Leases, 15 km S of Eneabba, *E.A.Griffin 1405* (PERTH); Petrudor Rock Reserve, SE of Dallwallinu, *M.Hislop 1883* (PERTH).

Listed as Priority 2 (few known populations but not currently threatened) in the CALM Conservation Codes for Western Australian Flora.

14. Comesperma rhadinocarpum F.Muell., *Fragm.* 11: 1 (1845)

Bredemeyera rhadinocarpa (F.Muell.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: 'In virgultis ad fluvios Greenough's, Arrowsmith's et Irwin's River; F.M.'; holo: MEL; iso: K.

Ascending to erect shrub, 30–50 cm tall. Leaves elliptic, leathery, acute, 2–4 mm wide by 6–25 mm long. Inflorescence an open slender raceme. Outer sepals ovate, acute, 1–1.5 mm long; wings deep blue, orbiculate to asymmetric-ovate, base truncate, shortly clawed, 2–2.5 mm wide by 2–3 mm long. Keel cream with deep blue tip, upper petals white, blunt-oblong; corolla tube glabrous. Stamens free above. Ovary pyriform. Capsules fusiform with a short apical beak, 1–3 mm wide by 9–15 mm long. Seeds brown, wrinkled, hemi-ellipsoidal with an elongate-pointed tail, 6–8 mm long; coma densest on margins. *Slender-fruited Comesperma.*

Comesperma rhadinocarpum has a restricted distribution around the Eneabba area, W.A. It grows on sand in coastal heath vegetation. Flowering mainly Sept.–Dec. Map 34.

W.A.: Bibby road, W of Badgingarra National Park, W of Badgingarra, *E.A.Griffin 8343* (PERTH); ca 8 km S of Eneabba, *R.J.Hnatiuk 771489* (PERTH); Kenwick, Nature Reserve owned by Botany Department Western Australian University, *K.H.Rechinger 59475* (PERTH, W).

Comesperma rhadinocarpum superficially resembles *C. calymega* but is easily distinguished from all other species by the spindle-shaped capsules. Listed as Priority 2 (few known populations but not currently threatened) in the CALM Conservation Codes for Western Australian Flora.

15. **Comesperma lanceolatum** R.Br. ex Benth., *Fl. Austral.* 1: 48 (1863)

Bredemeyera lanceolata (R.Br. ex Benth.) Chodat ex Anon., *Census. Pl. Victoria* 40 (1923). T: 'W. Australia. S.Coast, east of King George's Sound, R.Brown (Hb. R.Br.).' (Bay 1, South Coast, *R.Brown 4980*); holo: BM/K.

Ascending shrub to about 20 cm high, glabrous. Leaves linear to elliptic, acute, 1–2 mm wide by 3–10 mm long. Inflorescence a corymb-like raceme. Outer sepals elliptic, 3–5 mm long; wings blue, obovate, base tapered, very shortly clawed, 2–3 mm wide by 4–6 mm long. Keel cream below with a purple and yellow tip; upper petals cream with blue-flecked tips, oblong and somewhat acute; corolla tube papillose. Stamens free above. Ovary ovate, shortly beaked. Capsules clavate to somewhat elliptic, beaked, 2–3 mm wide by 4–5 mm long. Mature seeds not seen.

This species has a restricted distribution on the southern coast of W.A. It grows on white sand in open shrubland. Map 35.

W.A.: 21 km NW of Cape Riche, *K.R.Newbey 4630* (PERTH); 11 km NE of Woolbernup Hill (Fitzgerald River National Park), *K.R.Newbey 11071* (PERTH); No. 2 Rabbit Proof Fence, ca 29 miles S of Ongerup–Ravensthorpe Road, *A.S.George 1849* (CANB).

Listed as Priority 2 (few known populations but not currently threatened) in the CALM Conservation Codes for Western Australian Flora.

16. **Comesperma calymega** Labill., *Nov. Holl. Pl.* 2: 23, t. 162 (1806)

Bredemeyera calymega (Labill.) Chodat ex Anon., *Census Pl. Victoria* 40 (1923). T: 'In Capite van-Diemen'; holo: ?FI *n.v.*

Comesperma strictum Endl., *in* S.L.Endlicher *et al.*, *Enum. Pl. Huegel* 7 (1837), as *stricta*; *Comesperma calymega* var. *strictum* (Endl.) Domin, *Mem. Soc. Roy. Sci. Boheme* 1921–2, 2: 57 (1923). T: 'King George's Sound. (Hügel)'; holo: W.

Comesperma tenue Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 208 (1845), as *tenuis*. T: In subumbrosis ad Aron [Avon] Dale, districtus York, 4. Febr. 1839. Herb. Preiss. No. 2367; iso: LD, MEL.

Comesperma varians Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 210 (1845). T: In subturfosis arenosis inter frutices prope Strawberryhill. 24. Nov. 1840. Herb. Preiss. No. 2365; iso: LD, MEL.
Comesperma herbaceum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 211 (1845), as *herbacea*; *Comesperma subherbacea* Steetz, *Revis. Gen.* Comesperma 17 (1847), *nom. illeg.* T: In Australasia occid. Herb. Preiss. No. 2366.; holo: LD.
Comesperma parviflorum Steud., *Pl. Preiss.* 1: 210 (1845). T: 'In calculosis montium continuorum Darling'srange districtus Perth, 15. Oct. 1839. Herb. Preiss. No. 2374'; iso: LD, W.

Comesperma strictum var. *latifolium* Steetz, *Revis. Gen.* Comesperma 18 (1847), as *latifolia*. T: 'Hab. in Novae Hollandiae ora australi-occidentali, in Swan River Colonia. (herb. Drummond. Collect. I No. 429!) V.s. in herbario palatino Vindobonensi!'; holo: W.

Comesperma calymega var. *latifolium* Benth., *Fl. Austral.* 1: 148 (1863). T: Swan River, Drummond; King George's Sound, R.Brown; syn: K (Drummond).

Erect to ascending shrub, 30–60 cm tall, glabrous. Leaves leathery, narrow- to very broad-elliptic, acute, 3–5(—15) mm wide by 8–20(–35) mm long. Inflorescence a many-flowered pyramidal raceme. Outer sepals oblong and somewhat acute, 4–5 mm long; wings deep purple to blue, orbiculate, base tapering to a short claw, 4–5 mm wide by 5–6 mm long. Keel cream below with purple and yellow tip; upper petals cream with blue tips, oblong and somewhat acute; corolla tube glabrous. Stamens free above. Ovary circular and shortly beaked. Capsules asymmetrically clavate and emarginate, 2–3 mm wide by 6–9 mm long. Seeds black, smooth, ovoid, no obvious appendages; coma arising mainly from the base. *Blue Spike Milkwort.*
This species is widespread in southern Australia. It grows through southwestern W.A. with a disjunction across the Nullarbor Plain and is then distributed in near-coastal areas from Yorke Peninsula in S.A. to about Bairnsdale in Vic. and into Tas. It usually grows in sandy soils in heaths or open woodlands. Flowering mainly Sept.–Jan. Map 36.

W.A.: Yallingup–Margaret River road opposite Miamup swamp, *T.A.Halliday 227* (AK, CANB). S.A.: road between Sec[tion]s 90 and 97, H[undre]d of Wanilla, *C.R.Alcock 2564* (ADW, CANB). Vic.: Dunkeld Rifle Range, c. 4.5 km N of Dunkeld off the Halls Gap road, S end of range around target bunker, *I.C.Clarke 2230* (CANB, MEL). Tas.: Freycinet National Park, Hazards Beach track, middle section, *A.E.Orchard 5473* (HO).

17. Comesperma viscidulum F.Muell., *Fragm.* 10: 4 (1876)

Bredemeyera viscidula (F.Muell.) Steenis, *Acta Bot. Neerl.* 17:383 (1968). T: Ad fontes eremi Victoria's Springs, Young; iso: K.

Viscid glaucous erect shrub to about 1.5 m high, glabrous. Leaves appressed, crowded, oblong to obovate, mucronate and recurved at the apex, 2–3 mm wide by 4–7 mm long. Inflorescence a short pyramidal raceme held among upper leaves but elongating in fruit. Outer sepals ovate, 1–2 mm long; wings yellow, obovate, base rounded, sessile, 3–4 mm wide by 5–6 mm long. Keel cream below with a yellow tip; upper petals cream, rounded-oblong; corolla tube glabrous. Stamens diadelphous and sessile. Ovary deltoid. Capsules clavate with two apical horns, 3–4 mm wide by 8–12 mm long. Seeds black, smooth, ovoid, linear appendage on inner margin; coma concentrated on outer margin and base. *Viscid Milkwort.*
This species has a scattered distribution in central Australia, from eastern W.A. into southern parts of the N.T. and with a disjunction to collections from the Eyre Peninsula, S.A. It grows mainly on red sand in inland dune systems. Flowers mainly Sept.–Dec. Map 37.

W.A.: Little Sandy Desert, 17.8 km WNW of Lake Sunshine, 18.7 km SSW of Yanneri Lake, 22.5 km ENE of Kulonoski East Well on Beyondie Station, 38.9 km SE of Canning Well on Ilgarari Creek, *S. van Leeuwen 5001* (CANB, PERTH, PRH). N.T.: 30 km WSW of Mount Olga, *P.K.Latz 5726* (AD, CANB, DNA, PERTH, K); Reedy Rockhole, 5 km SE, *P.K.Latz 8995* (CANB, DNA). S.A.: sand dunes 3 km SE of Pine Lodge out station, *R.Bates 6891* (AD).

18. **Comesperma secundum** Banks ex DC., *Prodr.* 1: 334 (1824), as *secunda*

Bredemeyera secunda (Banks ex DC.) Chodat ex Ewart & O.Davies, *Fl. N. Terr.* 160 (1917). T: 'In Nov. Holl. prope flumen Endeavour (v.s. in h. Banks)'; holo: G-DC *n.v.*, fiche: NSW.

Erect shrub 60–100 cm tall, glabrous or hispidulous. Leaves leathery, elliptic to ovate, rounded or cordate, 6–10 mm wide by 7–13 mm long. Inflorescence a corymb-like raceme with a superficially secund appearance. Outer sepals ovate, 1–2 mm long; wings cream, obovate, base tapered, sessile, 3–4 mm wide by 4–5 mm long. Keel cream below with a yellow tip; upper petals yellowish-cream, rounded-oblong; corolla tube glabrous. Stamens diadelphous and sessile. Ovary circular. Capsules clavate with two apical horns, 2–4 mm wide by 10–16 mm long. Seeds black, smooth, ovoid, linear appendages on both margins; coma concentrated around base.

Comesperma secundum is one of the few species in the genus distributed across northern Australia, scattered from the Kimberley region in W.A. across the Top End of the N.T. and into Cape York Peninsula, Qld. It grows in sandy or rocky soils in open woodlands. Flowering mainly Mar.–June. Map 38.

W.A.: Koolan Island: near Acacia Ore Body in central part of island, *P.A.Fryxell 4609 et al.* (CANB, PERTH); Eva Creek and slope leading to Bold Bluff, *R.J.Cranfield 6611* (CANB). N.T.: Edith Falls lookout area, *R.W.Purdie 3398* (CANB, DNA); Arnhem Land, ca. 18 km ESE of Ramingining, *I.D.Cowie 9407* (BRI, CANB, DNA, MEL). Qld: head of Hann Ck, *P.I.Forster PIF4539* (BRI).

As noted by J.R.Wheeler , *in* J.R.Wheeler *et al.*, *Fl. Kimberley Region* 639 (1990), specimens from W.A. are glabrous while the Type specimen and some collections from other States are hispidulous. However, this character varies through the range of the species and it does not seem necessary to split the taxon based on such variation.

19. **Comesperma flavum** DC., *Prodr.* 1: 334 (1824), as *flava*

Bredemeyera flava (DC.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: 'in Nova Hollandia orâ orientali Lechenault'; holo: G-DC, fiche: NSW.

Comesperma xanthocarpum Steud., *Pl. Preiss.* 1: 209 (1845), as *xanthocarpa*; *Comesperma anthocarpum* Walp., *Repert. Bot. Syst.* 5: 66 (1845), *nom. illeg.* T: 'In subturfosis arenosis prope oppidulum Perth, 18 Dec. 1838. Herb. Preiss. No. 2368'; iso: LD, MEL.

Erect glaucous slender shrub, 0.3–1 m tall. Leaves linear to narrow elliptic, acute, 1–2 mm wide by 10–25 mm long. Inflorescence a many-flowered corymb-like raceme. Outer sepals ovate, 1–2 mm long; wings pale yellow, obovate, base rounded, sessile, 3–4 mm wide by 4–6 mm long. Keel deep yellow; upper petals cream, very broad oblong; corolla tube margins tuberculate. Stamens diadelphous and sessile. Ovary obovate. Capsules narrow-clavate, shortly beaked, 2–3 mm wide by 6–9 mm long. Seeds brown, smooth, hemi-ellipsoidal, linear appendages reduced to narrow lines on both margins, not tailed.

Endemic to W.A., *Comesperma flavum* is distributed in coastal areas in the southwest corner of the State, with a few disjunct collections from the coast north of Geraldton. It seems to favour winter-wet swampy habitats, growing mainly in sand over clay. Flowering mainly Oct.–Jan. Map 39.

W.A.: 17 km ESE of Walpole, 4 km N of Point Irwin., *B.Barnsley 789* (CANB); Gazetted Reserve 32926, Anstey Road, Forrestdale, *G.J.Keighery 11811* (CANB, PERTH); Pinticup Nature Reserve, *G.J.Keighery & N.Gibson 2510* (CANB, PERTH); Yerina Springs Road W side of near dam c. 3.5 km S of Ogilvie Road, *S.Patrick SP2230* (PERTH); Walpole-Nornalup National Park, Nut Road, 0.3 km N of junction with Ficifolia Road, *J.R.Wheeler & S.J.Patrick JRW3834* (PERTH).

20. **Comesperma pallidum** Pedley, *J. Adelaide. Bot. Garden* 3: 127 (1981)

T: 'Mitchell District: Torrens Creek, Mar 1933, *White* 8703'; holo: BRI, iso: BRI, K *n.v.*
Illustration: L.Pedley, *J. Adelaide. Bot. Garden* 3: 128. fig. 1B (1981).

Erect glaucous shrub, 1.5–2 m tall, glabrous. Leaves thick, elliptic to obovate, mucronate, 2–6 mm wide by 10–25 mm long. Inflorescence a many-flowered pyramidal raceme. Outer sepals ovate, 1–2 mm long; wings cream, obovate, base tapering, sessile, 3–4 mm wide by 4–5 mm long. Keel purplish at the tip; upper petals cream, oblong and somewhat acute; corolla tube glabrous. Anthers diadelphous and subsessile. Ovary circular. Capsules clavate, emarginate, 3–4 mm wide by 10–16 mm long. Seeds black, smooth, obloid; linear appendages unequal, inner forming a short tail; coma densest on base and margins.
Comesperma pallidum has a rather disjunct distribution in central Australia, from eastern desert areas of W.A. with a few collections in the nearby southern N.T. and then a large gap to central Qld. This disjunction may be an artefact of being poorly collected. *C. pallidum* grows on sandy or gravelly soils in open eucalypt woodlands. Flowering mainly Sept.–Feb. Map 40.

W.A.: 90 km E of Calvert Range, *G.J.Morse 213* (CANB, PERTH); Great Sandy Desert, Canning Stock Route, at Well 45, *P.Wicksteed 8* (CANB). N.T.: 18 km WNW of Lake Surprise, *P.K.Latz 11048* (DNA). Qld: Burra Range, 4 km from Great Dividing Range crest towards Pentland along Flinders Highway, *I.R.Telford 11433* (BRI, BISH, CANB, NSW, MEL); south side of Flinders Highway, 144 km west of Charters Towers (Charters Towers=1476 km north-west of Brisbane), *P.Jobson 462* (BRI, CANB).

This species is similar to *Comesperma sylvestre* in general habit, but the plant is generally much paler and the flowers are cream to yellow instead of deep pink. *C. sylvestre* also favours generally moister sites than *C. pallidum*, which is one of the few species in the genus to inhabit central Australia. Early records of *C. sylvestre* from W.A. and the N.T. are misidentifications of *C. pallidum*. *C. pallidum* is listed as Priority 3 (several known populations and not currently threatened) in the CALM Conservation Codes for Western Australian Flora.

21. **Comesperma oblongatum** (Benth.) Pedley, *Austrobaileya* 2: 13 (1984)

Comesperma ericinum var. *oblongatum* Benth., *Fl. Austral.* 1: 147 (1863) T: 'East Coast, R.Brown' (QUEENSLAND: Port Clinton (Port 2), Aug. 22nd. 1802, *R.Brown 4986*); iso: BM (2), K.

Rounded shrub to about 50 cm tall, glabrous. Leaves oblong, thick, mucronate, 2–5 mm wide by 10–25 mm long. Inflorescence a dense pyramidal raceme elongating in fruit. Outer sepals ovate, 1–2 mm long; wings pink to magenta, obovate, base tapered, sessile, 2–3 mm wide by 4–6 mm long. Keel pink below with a yellow tip; upper petals pale pink to cream, rounded-oblong; corolla tube glabrous. Stamens diadelphous and sessile.

Ovary circular. Capsules clavate with a slight apical notch, 3–4 mm wide by 8–11 mm long. Seeds black, smooth, linear appendages on both margins and forming a short tail; coma evenly distributed over seed. *Byfield Matchstick.*
Restricted to a small headland area on the coast of Qld northeast of Rockhampton, mostly contained within an Australian Army training area. It grows in shallow rocky soil in a wind-pruned shrub community. Flowers mainly Aug.–Dec. Map 41.

Qld: Byfield-Five Rocks area, 19 Aug. 1982, *L.Lindsay s.n.* (BRI); roadside, headland above 3 Rivers, Shoalwater Bay Military Training Area, 16 Aug. 1993, *S.Barry s.n.* (BRI); headland above falls at Switzerland Beach, Shoalwater Bay Military Training Area, 17 Aug. 1993, *S.Barry s.n.* (BRI); Stockyard Pt, Byfield, *M.Melzer RM232* (BRI); Stockyard Point, a very exposed rocky headland ca 15.5km ENE of Byfield, *J.R.Clarkson 964 & T.D.Stanley* (BRI, CANB).

22. **Comesperma drummondii** Steetz, *Revis. Gen.* Comesperma 11–12 (1847)

Bredemeyera drummondii (Steetz) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: Hab. in Novae Hollandiae ora australi-occidentali, in Swan-River Colonia, Drummond Collect. 2 No. 491; iso: BM, K, MEL, W.

Erect silvery-glaucous shrub, around 30–75 cm tall. Leaves thick, usually oblong or broad-elliptic, truncate, 1–5 mm wide by 5–12 mm long. Inflorescence a few-flowered corymb-like raceme. Outer sepals ovate, 1–2 mm long; wings pale pink, orbicular to obovate, base rounded, not clawed, 4–5 mm wide by 4–6 mm long. Keel deep pink to purple with yellow tip; upper petals cream to yellow, oblong and somewhat acute; corolla tube glabrous. Stamens diadelphous and sessile. Ovary circular. Capsules cuneate, 3–5 mm wide by 7–10 mm long. Seeds brown, smooth, hemi-ellipsoidal, narrow linear appendages on both margins; coma densest on margins. *Drummond's Milkwort.*
This species is distributed across southwestern W.A. It is usually found on white sand in mixed heathland. Flowering mainly Aug.–Dec. Map 42.

W.A.: 3 miles [5 km] E. of Lake Grace, *M.E.Phillips s.n.*, 30 Oct. 1962 (CANB); between Ravensthorpe & Lake King, 311–312 mp [i.e. 499 km from Perth], to W of Lake Chidnup, *E.M.Canning WA/68 7351* (CANB); Mount Ragged, *S.Barrett 486* (PERTH); 90 mile Tank, ca 80 km W of Kumarl, *P.G.Wilson 5721* (CANB, PERTH); Tompkins Rd, 10.2 km W from Natta Road. 40 km NNW of Eneabba., *N.Hoyle 155* (CANB, PERTH).

This species has sweetly scented flowers.

23. **Comesperma sylvestre** Lindl., *in* T.L.Mitchell, *J. Exped. Trop. Australia* 342 (1848), as *sylvestris*

Bredemeyera sylvestris (Lindl.) Chodat ex Ewart & O.Davies, *Fl. N. Terr.* 160 (1917). T: not cited; ?iso: *Mitchell 396*, K; probable iso: *Mitchell 391*, NSW.

Erect glaucous shrub, 1–2 m tall, stems reddish. Leaves thick, oblong to narrow-obovate, mucronate, 2–6 mm wide by 10–20 mm long. Inflorescence a relatively few-flowered pyramidal raceme. Outer sepals ovate, 2–3 mm long; wings deep pink to magenta, obovate, base rounded, sessile, 4–5 mm wide by 6–8 mm long. Keel deep pink with a yellow tip; upper petals cream to white, rounded-oblong; corolla tube glabrous. Anthers diadelphous and sessile. Ovary circular. Capsules clavate, 2–4 mm wide by 9–15 mm long. Seeds black, smooth, hemi-ellipsoidal, linear appendages on both margins and forming a short tail; coma densest on base and margins.

This species is scattered on tablelands from southern central Qld into northeastern N.S.W. It grows on a variety of substrates, including sandstone, in open sclerophyll forest. Flowering mainly Oct.–Jan. Map 43.

Qld: 6 km N of Marlong Gate, Mt Moffatt NP, *A.R.Bean 12853* (BRI); Palmgrove NP, Bigge Ra, NW Taroom, Bat Cave Gorge, *P.I.Forster & R.Booth PIF24706* (BRI). N.S.W.: Lairds Lookout near Euglah Spring, 36 km E of Narrabri, Mount Kaputar National Park, *R.G.Coveny 8833 & S.K.Roy* (NSW); E side Sth Boonoo Boonoo NP, 35 km NE Tenterfield, *J.Wilkes 23* (NE).

24. **Comesperma retusum** Labill., *Nov. Holl. Pl.* 2: 22, t. 160 (1806), as *retusa*

Bredemeyera retusa (Labill.) Chodat ex Anon., *Census Pl. Victoria* 40 (1923), as *retusum*. T: 'in capite Van-Diemen'; holo: ?FI *n.v.*; iso:BM.
Comesperma compactum Sieber ex Steud., *Nomencl. Bot.* 2nd edn, 400 (1840), *nom illeg.* T: not cited.

Erect slender glaucous shrub, 1–1.5 m tall, stems reddish. Leaves thick, oblong, truncate, 3–5 mm wide by 6–15 mm long. Inflorescence a few-flowered corymb-like raceme. Outer sepals ovate, 2–2.5 mm long; wings deep pink to magenta, obovate, base rounded, scarcely clawed, 2–3 mm wide by 4–7 mm long. Keel deep pink to purple and yellow-green at the tip; upper petals white, rounded-oblong; corolla tube glabrous. Anthers diadelphous and sessile. Ovary cordate. Capsules clavate, emarginate, 2–4 mm wide by 10–15 mm long. Seeds brownish-black, smooth, ovoid, linear appendage on inner margin; coma densest on margins and base. *Mountain Milkwort.*
Comesperma retusum is distributed along the east coast of Australia from south-eastern Qld through N.S.W and the A.C.T. into Vic. and Tas. It grows mostly in peaty soils in wet areas of sub-alpine and alpine regions, but is occasionally also found on sand in swampy coastal heaths. Flowering mainly Dec.–Jan. Map 44.

Qld: Russell Island, *E.N.Parker s.n.*, Sept. 1917 (BRI). N.S.W.: Barrington Tops ca. 40 miles N of Singleton, *R.Pullen 3793* (A, AD, CANB, K, L, MEL, NSW). A.C.T.: Mt Bimberi, *M.Gray 5028* (CANB). Vic.: Mt Buffalo National Park, c. 700 m S of Reservoir along creek, *P.S.Short 1375* (CANB, MEL). Tas.: Sanctuary Bay–Spero Bay, *A.Moscal 5832* (HO).

25. **Comesperma acerosum** Steetz, *Revis. Gen.* Comesperma 9 (1847)

Bredemeyera acerosa (Steetz) Steenis, *Acta Bot. Neerl.* 17: 382 (1968). T: 'Hab. in Novae Hollandiae ora australi-occidentali, in Swan-River Colonia, Drummond (Herbar. Drummond. Collect. 1. No. 431 Collect. 2. No. 492)'; syn:. *Drummond 1: 431* W, *Drummond 2: 492* BM, K, MEL, W.

Glaucous erect leggy shrub 1–1.5 m tall, glabrous. Leaves stiff, elliptic, strongly keeled below, 1–3 mm wide by 6–17 mm long. Inflorescence a dense pyramidal raceme. Outer sepals semi-circular, 1–2 mm long; wings pale pink to magenta, obovate, base tapered, sessile, 3–5 mm wide by 5–7 mm long. Keel deep pink to purple with a yellow tip and horn shaped appendage below; upper petals pinkish cream, square; corolla tube papillose. Stamens diadelphous and sessile. Ovary circular. Mature capsules apparently clavate. Seeds not seen.
Comesperma acerosum is restricted to a small coastal area near Eneabba, W.A. It grows on sandy soils in low open heaths. Flowering mainly Oct.–Dec. Map 45.

W.A.: in large block of remnant vegetation on private farmland (Breakaway, J. & J.Browne) off Green Head–Coorow Road, c. 3 km W of Brand Highway, *M.Hislop 220* (PERTH); track heading north 7.0 km east on Eneabba-Coolimba Rd from intersection with Coastal Rd, *E.D.Kabay 664* (PERTH); 19 km E of Green Head on Green Head-Jurien road, foothills of Gairdner Range, *T.A.Halliday 143* (AK, CANB); Cockleshell Gully, NE of Jurien, *E.A.Griffin 2426* (CANB, PERTH).

Listed as Priority 3 (several known populations and not currently threatened) in the CALM Conservation Codes for Western Australian Flora.

26. **Comesperma confertum** Labill., *Nov. Holl. Pl.* 2: 23, t. 161 (1806), as *conferta*

Bredemeyera conferta (Labill.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: in terrâ van-Leuwin; holo: ?FI *n.v.*; iso: BM, MEL.
Comesperma longifolium Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 206 (1845), as *longifolia*. T: In arenosis sylvae prope Limekiln districtus Perth, 29. Nov. 1839. Herb. Preiss. 2359; lecto: LD, MEL (2), W.
Comesperma hirtulum Steud., *in* J.G.C.Lehmann, *Pl. Preiss.* 2: 209 (1845), as *hirtula*; *Comesperma confertum* var. *hirtulum* (Steud.) Steetz, *Revis. Gen.* Comesperma 9 (1847). T: In turfosis humidis umbrosis, Dec. 1838. Herb. Preiss. 2362 et 2364; syn: LD (2), MEL (4), W.
Comesperma confertum var. *bracteosum* Domin, *Mem. Soc. Roy. Sci. Boheme* 1921–2, 2: 125 (1923). T: not cited.

Erect leggy shrub to about 1.5 m tall, glabrous or hispidulous. Leaves linear, crowded, 1–3 mm wide by 10–25 mm long, acute. Inflorescence an elongate dense pyramidal raceme. Outer sepals ovate, acute, 2–3 mm long; wings deep pink to magenta, obovate, base tapering, sessile, 2–3 mm wide by 4–6 mm long. Keel pink to purple with yellow tip; upper petals white, rounded-oblong; corolla tube margins papillose. Stamens free above. Ovary orbiculate. Capsules clavate, 2–3 mm wide by 5–7 mm long. Seeds black, obloid, linear appendages on both margins with inner side enlarged, forming a short tail; coma concentrated around base.
Comesperma confertum is mainly distributed in near-coastal areas of southwestern W.A. from around to Perth to Esperance. It grows in a variety of soils, including sand and clay loams, in heaths or rather open eucalypt forest. Flowering mainly Oct.–Dec. Map 46.

W.A.: 17.5km along Redmond West Road from Redmond, c. 20km direct SW of Narrikup. *A.M.Monro 57* (CANB); Witchcliffe, *J.Scott 47* (PERTH); Thomas River, Cape Arid National Park, E of Esperance, *R.D.Royce 9929* (PERTH); Bold Park, Floreat Park, 8 km W of Perth, *G.J.Keighery 9536* (PERTH).

This species is similar to *Comesperma acerosum* and herbarium specimens are often misidentified as that species. *C. confertum* can be distinguished by the less stiff and unkeeled leaves and by the lack of an appendage on the keel petal.

27. **Comesperma ericinum** DC., *Prodr.* 1: 334 (1824), as *ericina*

Bredemeyera ericina (DC.) Chodat, *Nat. Pflanzenfam.* 3(4): 338, fig. 117Q (1896), as *ericine*. T: in Novae-Hollandiae orâ orientali; holo:G-DC *n.v.*, fiche: NSW.
Comesperma cordifolium A.Cunn., *in* B.Field, *Geog. Mem. N. S. W.* 337 (1825). T: 'Brushes on the Blue Mountains'; holo: K.
Comesperma latifolium Steetz, *Revis. Gen.* Comesperma 5–6 (1847); *Comesperma ericinum* var. *latifolium* (Steetz) J.D.Hook., *Fl. Tasman.* 1: 32 (1855). T: 'Hab. in insula van Diemen. Ferd. Bauer (Vidi specimina in herb. palatino Vindobonensi)'; holo: W.
Comesperma acutifolium Steetz, *Revis. Gen.* Comesperma 6 (1847); *Comesperma ericinum* var. *acutifolium* (Steetz) J.D.Hook., *Fl. Tasman.* 1: 32 (1855), as *acutifolia*. T: Sieber herb. Nov. Holl. No. 534: lecto: LD, isolecto: MEL, W (2).

Comesperma linariifolium A.Cunn. ex Steetz, *Revis. Gen.* Comesperma 7 (1847), as *linariaefolium*. T: 'Hab. in Novae Hollandiae ora australi-orientali. Cunningham! Hügel! Ferd. Bauer! (v.s.)'; lecto: *n.v.*

Comesperma patentifolium F.Muell., *Fragm.* 1: 48 (1858); *Comesperma ericinum* var. *patentifolium* (F.Muell.) Benth., *Fl. Austral.* 1: 147 (1863). T: Raro provenit in montibus rupestribus dumosis ad flumen Burnett; holo: MEL; iso: K.

Comesperma planifolium Hassk., *Ann. Mus. Bot. Lugduno-Batavum* 1: 185 (1864). T: 'Herb. SIEBER, n. 364 e Nova Hollandia, sub nomine C. ericini DC.'; iso: K, LD, MEL.

Comesperma esulifolium Gandoger, *Bull. Soc. Bot. France* 60: 456 (1913). T: N.S. Wales, ad cacumen montis Warning (Forsyth); holo: LY; iso: NSW.

Comesperma uncinatum Gandoger, *Bull. Soc. Bot. France* 60: 456 (1913). T: Australia, N. S. Wales (R.T.Baker); holo: LY.

Comesperma tasmanicum Gandoger, *Bull. Soc. Bot. France* 60: 456 (1913). T: 'Tasmania (Spicer)'; holo: LY.

Comesperma aristulosum Gandoger, *Bull. Soc. Bot. France* 60: 456 (1913). T: 'Australia, Queensland (Walter); in alpibus Victoriae (Walter, Reader, Mac Lean etc.)'; syn: LY.

Comesperma ericifolia Sulman, *Austral. Wildflowers* ser. 2: t. 40 (1913), *nom. illeg.* T: not cited.

Comesperma hispidulum Pedley, *Austrobaileya* 2: 10 (1984). T: Moreton District: Plunkett [ca 40 km S of Brisbane], Aug. 1930, Hubbard 3785; holo: BRI, iso: K.

Erect slender shrub, 1–2 m tall, stems often reddish, glabrous, puberulous or hispidulous. Leaves discolorous, linear to elliptic to narrow-obovate, mucronate, 2–4 mm wide by 10–30 mm long. Inflorescence a pyramidal raceme, many-flowered. Outer sepals ovate, 1–2 mm long; wings magenta-pink, obovate, not clawed, 3–4 mm wide by 5–8 mm long. Keel deep pink with a white tip; upper petals white to cream, rounded-oblong; corolla tube glabrous. Stamens diadelphous and sessile. Ovary circular. Capsules spathulate, emarginate, 3–4 mm wide by 6–8 mm long. Seeds black, smooth, ellipsoidal, linear appendages on both margins and forming a short tail; coma evenly distributed over seed. *Heath Milkwort*, *Match Heads*.

This is an extremely widespread species, occurring along the east coast of Australia from central Qld through N.S.W, Vic. and Tas. It grows on a variety of soils, including sandstone and granite, and is often found in coastal heath or open eucalypt forests. Flowers mainly Oct.–Dec. Map 47.

Qld: Lower Bellbird Circuit Track, Binna Burra Section, Lamington NP, *P.Grimshaw G853* (BRI). N.S.W.: North Rocks Rd, Nightcap Range, Whian Whian State Forest, *L.Haegi 1506* (BRI, NSW); Tuncurry, N of town, *M.E.Phillips s.n.*, 12 Oct. 1969 (CANB, NE). Vic.: Burrowa Pine Mountain NP, *P.G.Smith s.n.*, Nov. 1978 (MEL). Tas.: Furneaux Group, Clarkes Island, near a steep-sided eastern tributary of Maclaines Creek, *J.S.Whinray 5092* (CANB, HO, MEL).

A species with highly variable leaf morphology, *Comesperma ericinum* has previously been split into a number of varieties. Since the leaf characters appear to vary continuously throughout the range of the species and other characters remain fairly constant, a broad concept is used here.

28. Comesperma breviflorum Pedley, *Austrobaileya* 2: 11 (1984)

T: Mt Greville, Oct 1934, Michael 2072; holo: BRI.

Erect slender shrub to around 1 m high, stems greenish-grey, glabrous. Leaves concolorous, linear to narrow-oblong, acute, 0.8–1.2 mm wide by 8–16 mm long. Inflorescence an elongate pyramidal raceme, many-flowered. Outer sepals ovate, 1–1.5mm long; wings pink, obovate, not clawed, 3–4 mm wide by 3–5 mm long. Keel deeper pink with yellowish tip; upper petals white to cream, spathulate; corolla tube glabrous. Stamens diadelphous and sessile. Ovary rounded-deltoid with a short apical projection. Capsules clavate, emarginate with a narrow marginal

wing, 2–3 mm wide by 4–5 mm long. Seeds black, smooth, hemi-ellipsoidal, linear appendages on both margins and forming a short tail; coma evenly distributed over seed.
Comesperma breviflorum is restricted to a few mountain peaks in the McPherson Range on the Qld/N.S.W. border. It grows on volcanic rock flows in a heathland community. Map 48.

Qld: E peak, Mt Maroon, *G.Leiper 20* (BRI); Mt Barney south ridge, *P.I.Forster PIF11872* (BRI); Knapps Peak, *P.I.Forster PIF11962* (BRI); Mt Ernest, *P.I.Forster PIF12374* (BRI, MEL). N.S.W.: 15 km W of Woodenbong near Queensland border in the Bald Knob State Forest, *A.Bedwell s.n.*, 14 Oct. 1998 (NSW).

29. **Comesperma praecelsum** F.Muell., *Fragm.* 11: 2 (1878)

Bredemeyera praecelsa (F.Muell.) Steenis, *Acta Bot. Neerl.* 17: 383 (1968). T: In montibus orariis ad Rockingham's Bay; Dallachy; iso: :K.

Erect shrub 1–3m tall, puberulous. Leaves linear to narrow-elliptic, mucronate, 1–3 mm wide by 8–30 mm long. Inflorescence a short pyramidal raceme held among the upper leaves. Outer sepals ovate, 0.5–1.5 mm long; wings pink, obovate, 2–3mm wide by 3–5 mm long. Keel deep pink with a white and yellow tip; upper petals cream, oblong; corolla tube glabrous. Stamens free above. Ovary circular. Capsules clavate, 2–4 mm wide by 10–15 mm long. Seeds black, smooth, hemi-ellipsoidal, linear appendages on both margins; coma evenly distributed.
Comesperma praecelsum is restricted to Hinchinbrook Island and adjacent parts of the mainland in northern Qld. It grows in wetter areas near creeks on sandy or rocky substrates. Flowering mainly Oct.–Jan.(—May). Map 49.

Qld: Hinchinbrook Is., Little Ramsay Bay, N end, *P.Sharpe 1662* (BRI); SF 461, Five Mile Ck, 6.5km S of Cardwell, *A.Thorsborne 475, M.Thorsborne & W.Travers* (BRI, CANB); between Sweet Water & Zoe Ck, Hinchinbrook Is., *D.G.Fell DF751* (BRI);Zoe Ck, Hinchinbrook Is., *D.G.Fell DF1032* (BRI); Zoe Ck, Hinchinbrook Is., *D.G.Fell DF1035* (BRI).

Doubtful Names

Comesperma nudum Backh., *in* J.Ross, *Hobart Town Almanack* 78 (1835), as *nuda*.

T: not designated.

It is not possible to conclusively identify this taxon from the description given—"Naked Seeded Comesperma. A small plant with indigo coloured flowers, found near George-town and at Port Arthur, differing from the two preceding species in the seeds being destitute of the hair-like appendages from which the genus is named...". Since none of the taxa which truly lack a coma on the seeds are currently present in Tasmania and the coma of most species is readily deciduous, it is difficult to know what is meant by this observation. By a process of elimination, this taxon seems likely to be referrable to either *Comesperma calymega* Labill. or *C. defoliatum* F.Muell., but there is not enough information to choose between them.

Comesperma paucifolium Turcz., *Bull. Soc. Imp. Naturalistes Moscou* 27(2): 352 (1855).

T: 'Nova Hollandia occidentalis. Gilbert coll. n. 86'; holo: ?KW *n.v.*

The Type for this species was requested from KW, but never received. It is thus not possible to determine the status of this name.

Comesperma selaginoides Turcz., *Bull. Soc. Imp. Naturalistes Moscou* 27(2): 352–353 (1855).

T: 'Nova Hollandia occidentalis. Drum. coll. III n. 215'; holo: ?KW *n.v.*

The Type for this species was requested from KW, but never received. It is thus not possible to determine the status of this name, although from the description it appears likely to be a synonym of *Comesperma virgatum* Labill. The only marked difference mentioned is the collection notes indicating the plant had white flowers and as this mutation is not uncommon in *Comesperma*, it is unlikely to be significant.

Comesperma spathulatum Turcz., *Bull. Soc. Imp. Naturalistes Moscou* 27(2): 352 (1855).

T: 'cum priore. Gilbert n. 88' [i.e. *Comesperma paucifolium, Gilbert 86*]; holo: ?KW *n.v.*

The Type for this species was requested from KW, but never received. It is thus not possible to determine the status of this name.

1. *Salomonia ciliata* (L.) DC.

2. **Muraltia heisteria* (L.) DC.

3. **Polygala myrtifolia* L. var. *myrtifolia*

4. **Polygala virgata* Thunb.

5. *Polygala persicariifolia* DC.

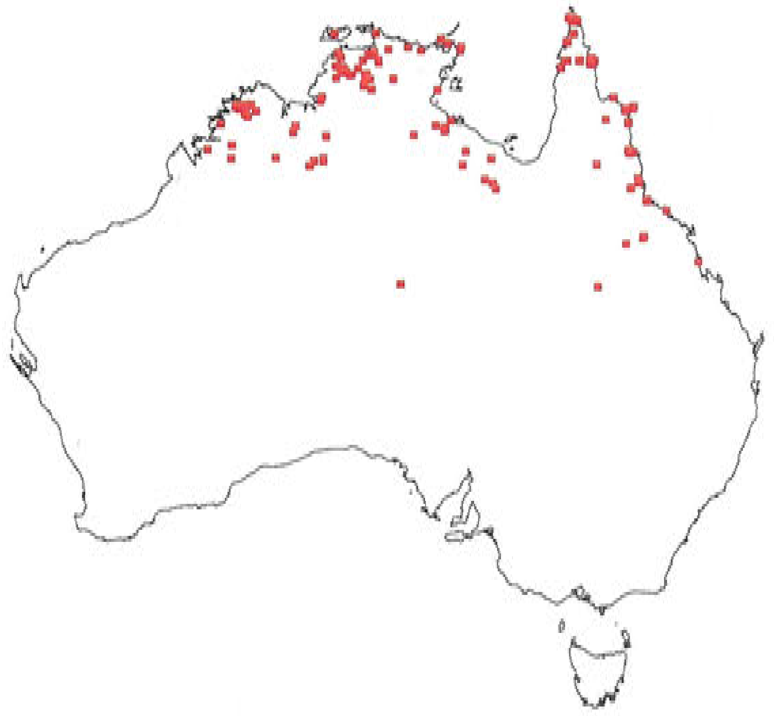
6. *Polygala japonica* Houtt.

MAPS 1–6: Distribution of Polygalaceae in Australia, based on herbarium specimen records

7. **Polygala vulgaris* L.

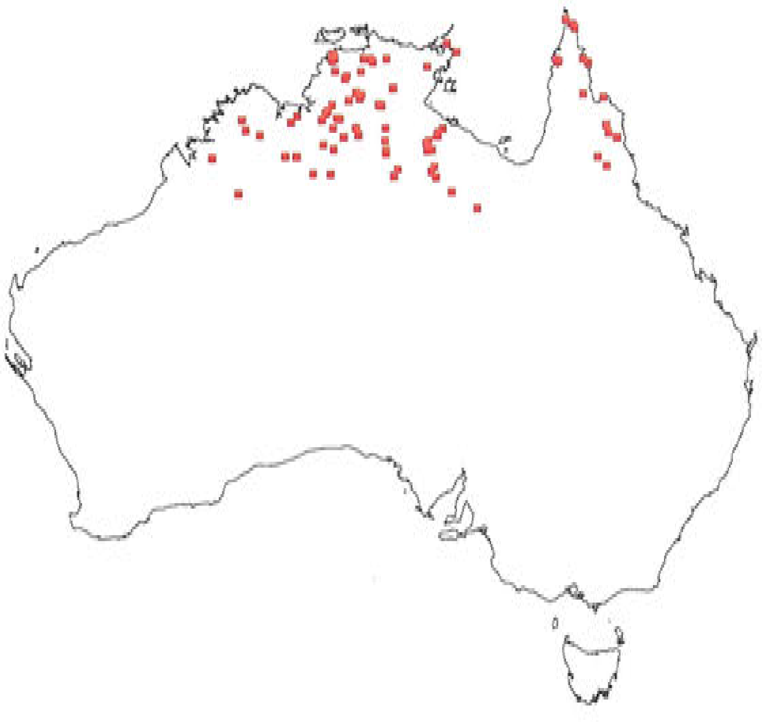
8. **Polygala paniculata* L.

9. **Polygala duarteana* A.St.-Hil.



10. *Polygala longifolia* Poir.

11. **Polygala monspeliaca* L.



12. *Polygala rhinanthoides* Benth.

MAPS 7–12: Distribution of Polygalaceae in Australia, based on herbarium specimen records

13. *Polygala eriocephala* Benth.

14. *Polygala chinensis* L.

15. *Polygala orbicularis* Benth.

16. *Polygala* isingii Pedley

17. *Polygala linariifolia* Willd.

18. *Polygala wightiana* Wight & Arn.

MAPS 13–18: Distribution of Polygalaceae in Australia, based on herbarium specimen records

19. *Polygala exsquarrosa* Adema

20. *Polygala tepperi* F.Muell.

21. *Comesperma integerrimum* Endl.

22. *Comesperma ciliatum* Steetz

23. *Comesperma volubile* Labill.

24. *Comesperma sphaerocarpum* Steetz

MAPS 19–24: Distribution of Polygalaceae in Australia, based on herbarium specimen records

25. *Comesperma aphyllum* Benth.

26. *Comesperma scoparium* J.Drumm. ex Steetz

27. *Comesperma spinosum* F.Muell.

28. *Comesperma defoliatum* F.Muell.

29. *Comesperma nudiusculum* DC.

30. *Comesperma calcicola* Keighery

MAPS 25–30: Distribution of Polygalaceae in Australia, based on herbarium specimen records

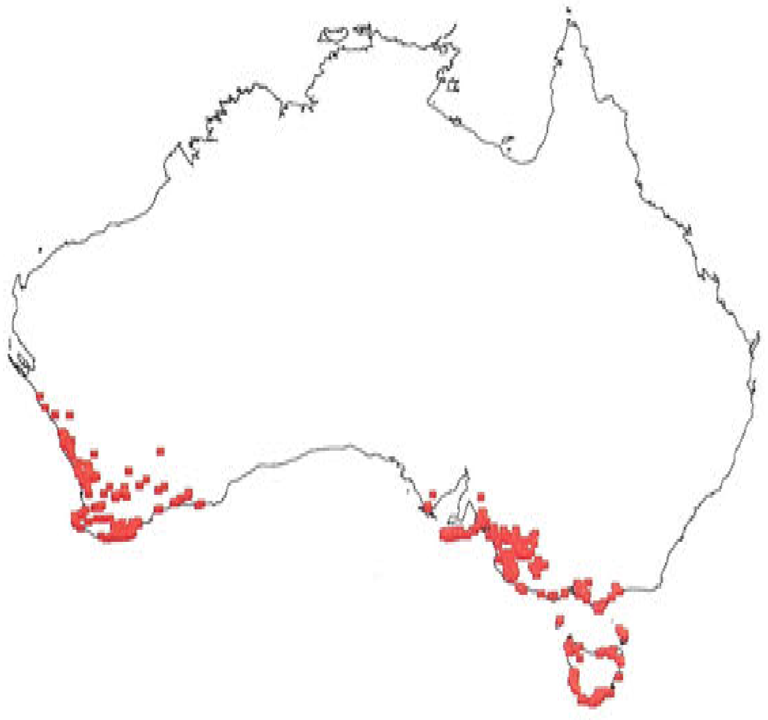
31. *Comesperma virgatum* Labill.

32. *Comesperma polygaloides* F.Muell.

33. *Comesperma griffinii* Keighery

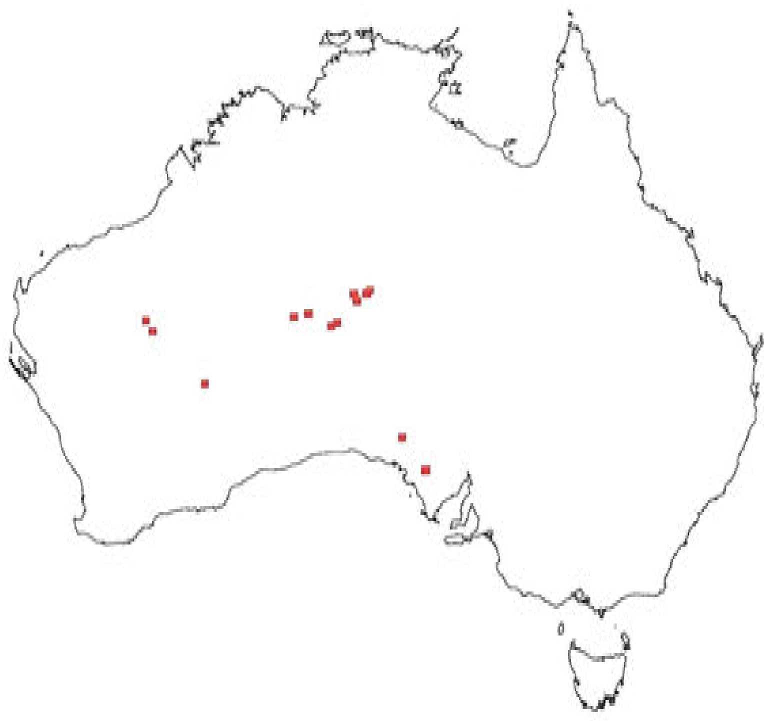
34. *Comesperma rhadinocarpum* F.Muell.

35. *Comesperma lanceolatum* R.Br. ex Benth.



36. *Comesperma calymega* Labill.

MAPS 31–36: Distribution of Polygalaceae in Australia, based on herbarium specimen records



37. *Comesperma viscidulum* F.Muell.

38. *Comesperma secundum* Banks ex DC.

39. *Comesperma flavum* DC.

40. *Comesperma pallidum* Pedley

41. *Comesperma oblongatum* (Benth.) Pedley

42. *Comesperma drummondii* Steetz

MAPS 37–42: Distribution of Polygalaceae in Australia, based on herbarium specimen records

43. *Comesperma sylvestre* Lindl.

44. *Comesperma retusum* Labill.

45. *Comesperma acerosum* Steetz

46. *Comesperma confertum* Labill.

47. *Comesperma ericinum* DC.

48. *Comesperma breviflorum* Pedley

MAPS 43–48: Distribution of Polygalaceae in Australia, based on herbarium specimen records

49. *Comesperma praecelsum* F.Muell.

MAP 49: Distribution of Polygalaceae in Australia, based on herbarium specimen records

INDEX OF ACCEPTED TAXON NAMES