Systematic studies in Neotropical Myrtaceae with an emphasis on *Myrcia s.l.*: The evolution and biogeography of a large South American clade

Thesis

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E. J. Lucas

Doctor of Philosophy

Open University

Royal Botanic Gardens, Kew

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Abstract

Low morphological variation at all taxonomic levels give Neotropical Myrtaceae a reputation as a 'difficult' family to identify even to genus, resulting in a lack of taxonomic data on every front. The subtribal classification of the predominantly Neotropical and exclusively fleshy-fruited tribe Myrteae (49 genera and c. 2500 species) is unstable, as are generic boundaries within it. Neotropical Myrtaceae are particularly species rich in some of South America's most threatened habitats. The second largest Neotropical genus, *Myrcia s.l.*, comprises >700 taxonomically 'difficult' species with species diversity reaching its peak in the Brazilian Atlantic rainforest and *cerrado*, habitats in urgent need of inventories of their plant species before conservation initiatives can be undertaken.

Phylogenetic hypotheses are provided for evolutionary relationships within Myrteae and *Myrcia s.l.* based on nuclear ITS and ETS ribosomal DNA and plastid *psbA-trnH* and *matK* DNA sequences, using parsimony and Bayesian inference. Four morphological characters of Myrteae are optimized on the resulting trees and nineteen are used in a cladistic analysis of *Myrcia s.l.* Myrteae appear monophyletic, comprising seven clades plus two isolated taxa. Of the four previously accepted genera of subtribe Myrciinae *sensu* DC., two are polyphyletic and all emerge in a single clade treated here as *Myrcia s.l.* Morphological characters exhibit homoplasy at both ranks, although in combination are useful for clade diagnosis.

Biogeographical analysis is inconclusive regarding tribal ancestral areas, but South American colonization before northern radiation via the Andes appears likely. The largest genera, *Eugenia* and *Myrcia s.l.*, have western and southeastern South American origins, respectively. Nonmetric multidimensional scaling and ordination techniques are employed to divide the distribution of *Myrcia s.l.* into discrete areas of endemism and historical biogeographical scenarios are discussed. Finally, modern, natural, subtribal and infrageneric classifications are proposed and concluding inferences are drawn regarding drivers of large genera using Myrtaceae and *Myrcia s.l.* as case studies.

"The 500 supposed species of Myrcia may probably be reduced to 300; but this is still a very large number, rendering the task of grouping exceedingly difficult when there is so very little in their characters of absolute difference definable in words."

Bentham (1869, p.148)

"The classification of the South-American species of Campomanesia, Psidium, Myrtus, Myrcia, Marlierea, Calyptranthes, and Eugenia is a labour to be entirely recommenced when a botanist shall be found courageous enough to undertake so tedious a task".

Bentham (1869, p.148)

"The divisions [Berg] proposes are not natural enough to enable us to sort the specimens approximatively without examination; and at the same time, if taken as artificial sections, their characters (beyond the embryo, which is so rarely to be met with) are too vague and undefined to serve for practical purposes."

Bentham (1869, p.148)

"The species of American Myrtaceae are distressingly alike in aspect and in most individual characters, making identification and classification of both genera and species a correspondingly difficult and tedious matter."

McVaugh (1968, p.359)

"It is difficult to determine fertile, let alone sterile material from the wet forests of eastern Brazil north of Santa Catarina. Because of the importance of Myrtaceae, a complete ecological description of these forests is contingent upon a taxonomic revision of the family. Unfortunately such a revision soon may not be possible because eastern Brazilian wet forests are disappearing at an unprecedented rate."

Mori et al. (1983, p.70)

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Chapter 1 - Introduction

Background

Myrtaceae are generally considered a well-delimited family (Conti et al. 1996) of c. 140 genera and between 3800 and 5600 species (Wilson et al. 2001; Govaerts et al. 2006.) with centres of diversity in Australasia and South America. There are approximately 2300 species of Myrtaceae in the Neotropics, making up 10 to 15 percent of total tree species in the moist forests and *cerrados* of Brazil - more than any other family (Oliveira-Filho & Fontes 2000). Given that these and other Myrtaceae-rich areas are under severe environmental threat, an understanding of the evolutionary history of Myrteae, the richest tribe in terms of numbers of genera, is necessary for prioritization of conservation initiatives.

Delimitation of taxa at all ranks has been unclear since the family first was described (Adanson 1763); today, there is still considerable disagreement among workers regarding circumscription of genera and species (Legrand & Klein 1967-1977; Landrum & Kawasaki 1997; Sobral 2003). The first taxonomic accounts of the family date from the 18th and 19th Centuries; these were treatments that delimited genera using few and often polymorphic characters such as number of calyx-lobes, number of locules or seeds (Landrum 1981a).

The earliest concept of the family (De Candolle 1826) encompassed five tribes (Schlechtendahl 1827): Leptospermeae; Myrteae; Chamaelaucieae; Barringtonieae and Lecythideae. This concept of Myrtaceae was narrowed by Niedenzu (1893) into two subfamilies divided by fruit morphology; a mostly Neotropical fleshy-fruited Myrtoideae, comprising tribe Myrteae, and an almost exclusively Paleotropical, dry, capsular-fruited Leptospermoideae, consisting of tribes Leptospermeae and Chamaelaucieae. Niedenzu also transferred tribes Barringtonieae and Lecythideae to Lecythidaceae, a family now known to be only distantly related to Myrtaceae (in Ericales; Morton et al. 1996; APG, 2003)

In recent years, key molecular and/or morphology-based studies have been carried out on the Australian and other Paleotropical groups (e.g. Gadek et al. 1996; Ladiges et al. 1999;

Udovicic and Ladiges 2000; Brown et al. 2001; Wilson et al. 2001; Harrington & Gadek 2004; Wilson et al. 2004). Consequently, the phylogenetics and systematics of these groups are becoming better known. Although some genera of Neotropical Myrtaceae have been the subject of recent studies and revisions, (*Myrceugenia* (Landrum 1981a), *Campomanesia, Pimenta, Blepharocalyx, Legrandia, Acca, Myrrhinium* and *Luma* (Landrum 1986), *Siphoneugena* (Proença 1990), *Myrcianthes* (Grifo 1992), *Gomidesia* (Nic Lughadha 1997) and *Mosiera* (Salywon et al. 2002), systematics of the Neotropical Myrtaceae remain poorly studied phylogenetically compared to those occurring in the old world.

The two largest genera of Neotropical Myrtaceae are *Eugenia* and *Myrcia* (comprising more than 550 and 250-500 species, respectively; Mabberley 1997; Govaerts et al 2006); species diversity reaches its peak in the Brazilian Atlantic rainforests, a habitat disappearing rapidly under human pressure. Despite the importance of these taxa in this threatened environment, there is an acute lack of taxonomic, morphological, evolutionary, biogeographical and ecological understanding of these genera. Neotropical Myrtaceae, in particular the most species-rich genera, are considered a taxonomic nightmare; it is common for one species to be described several times so that ultimately an accepted species name may have many synonyms. Examples of this can easily be found for common species such as *Myrcia splendens* DC., *Myrcia guianensis* (Aubl.) DC., *Blepharocalyx salicifolia* (Kunth) O.Berg and *Eugenia punicifolia* (Kunth) DC. with 108, 85, 65 and 58 synonyms (not including infra-specific names) respectively (Govaerts et al. 2006). The resulting situation is one of extreme taxonomic instability; it is often difficult to know where to start when making identifications as even confidently assigning a specimen to genus can be a frustrating and tedious task.

Ecology

The rainforests that once covered the Brazilian Atlantic coast are termed *Mata Atlântica* (Azevedo 1950). Despite a rapid decline in the area covered by this forest, what remains today, situated between the eastern Brazilian coast and 55° longitude and between 14° 00' and 26° 30'S latitude, still makes up the second largest tropical moist forest area of South America (Oliveira-Filho & Fontes 2000). The Atlantic rainforest is an environment extremely rich in angiosperm species diversity (estimated at 20,000 species; Conservation International, 2007). Similarly, high species diversity is found in other taxa, such as mammals (Fonseca et al. 1999) and birds (Harris et al. 2005). Mori et al. (1983) demonstrated that the *Mata Atlântica* is different from other Neotropical lowland wet forests, i.e. Amazonian, and that ecologically the most important family within it is Myrtaceae. More recently, Oliveira-Filho & Fontes (2000) compared tree diversity in Atlantic forest, Amazonian rainforest, semiarid *caatinga* of northeastern Brazil and the *cerrado* of south-central Brazil (Table 1.1). Myrtaceae are the most speciose family in both *Mata Atlântica* and *cerrado,* making up 10 to 15 percent of total tree species found in these areas. Furthermore, the most species-rich tree genus in the Atlantic rain and semi-deciduous forests was *Eugenia,* with *Myrcia* ranked third most diverse. If the four probably paraphyletic (McVaugh 1968; Lucas et al. 2005) genera of *Myrcia sensu lato* (*Calyptranthes, Gomidesia, Marlierea, Myrcia*) are combined, 109 species of this group occur in the Atlantic rain forest sites sampled, one more than *Eugenia*; *Myrcia sensu stricto* is also identified as the most species-rich tree (dbh >10 cm) genus in the *cerrado;* were woody shrub species also taken into account, this proportion would be likely to rise still further.

Table 1.1. Percentage of total tree flora comprising Myrtaceae in *Mata Atlantica, cerrado* (Oliveira-Filho & Fontes 2000) and Amazonian rainforest vegetation (Prance 1973).

	Mata Atlantica	Atlantic semi-deciduous forest	Amazonian rainforest	Cerrado
Number of areas surveyed	48	77	22	98
Total number of tree species	2012	1533	1530	528
Total number of Myrtaceae tree species	308	187	40	51
Position of Myrtaceae when ranked against tree species number in all other families	1	1	11	1
% Myrtaceae makes up of tree flora	15	12	3	10
Total number of *Eugenia* species	108	59	18	12
% Eugenia makes up of tree flora	5	4	1	2
Position of *Eugenia* when ranked against tree species number in all other families	1	1	11	4
Total number of *Myrcia* species	57	46	not listed	24
% *Myrcia* makes up of tree flora	3	3	-	5
Position of *Myrcia* when ranked against tree species number in all other families	3	3	-	1

Having originally extended between 6 and 30°S, along more than 3300 km of Brazil's Atlantic coastline, covering some 1.1 million km^2 and making up 12 % of Brazil's surface (SOS Mata Atlântica & INPE 1993), this biologically rich and diverse vegetation is now under immediate threat from pressures associated with the urban expansion of some of the most populated areas of Brazil. In 1981, Mori et al. estimated between 65.8% and 93% of the original Atlantic forests had been destroyed, whereas less than 0.1% in their southern Bahia study area had been protected as biological reserves or national parks. By 1998, the *Mata Atlântica* forest was reduced to less than 5% of its original range, most of this made up of patches of secondary forest and areas on slopes unsuitable for agricultural or other

development (SOS Mata Atlântica 1998). The *Mata Atlântica* and associated ecosystems, such as the Atlantic semi-deciduous forest and *cerrado,* are in critical need of conservation (Murray-Smith et al. submitted). Departing from the focus on the *Mata Atlântica* and *cerrado*, Myrtaceae are the eleventh most important family in terms of number of tree species in the Amazonian forest (Table 1.1), a further threatened area where effective conservation strategies are also required.

Given human requirements for farming and population expansion, it seems unlikely that it will be possible to conserve everything that remains of today's global flora. By documenting the botanical resources in a given area, the richest areas can be identified and prioritised for conservation (Murray-Smith et al. in prep.). The botanical and taxonomic complexity of Myrtaceae makes identification of individuals extremely difficult, and their use for ecological analysis is limited. Herbaria generally contain large numbers of unidentified or poorly identified Myrtaceae specimens, impeding identification of new collections, resulting in specimens collected during ecological surveys being routinely identified as 'morpho-species', e.g. 'Myrtaceae indet. sp. 1' (Guedes & Orge 1998; Zappi et al. 2003). Furthermore, numbers of Myrtaceae species are regularly underestimated, and the species diversity of an area therefore appears poorer. Common in Myrtaceae literature are calls for more studies to be carried out on the more complex genera or in more poorly studied areas (e.g. McVaugh 1956, 1968; Mori et al. 1983; Holst 2003).

Monographic revisions of these large and frequently occurring Myrtaceae genera over their entire range would be extremely useful for conservation of areas such as the *Mata Atlântica.* With systematic and taxonomic understanding in Myrtaceae as poor as it is today, however, this is still a long way off.

Taxonomy

The last attempt at a family level monograph was by Otto Berg in Von Martius's *Flora Brasiliensis* in the 1850's. Berg (1855–56, 1857–1859) treated 1726 Myrtaceae species in this work, many of which were known from few or incomplete collections. Berg had a tendency to 'split' taxa, publishing over 1000 new species and 30 new genera, many of which have since been relegated to synonymy. Since this detailed but now out-of-date work, smaller, more easily defined groups have been revised, but the bulk of species, particularly those from the largest genera or generic complexes, such as *Eugenia, Myrcia* and *Psidium* have yet to be treated. Regional treatments exist, such as McVaugh's treatments for Peru (1958b), Guatemala, (1963b) and the Guianas (1969), Legrand & Klein's (1967-1977a) treatment for Santa Catarina, Sobral's (2003) treatment for Rio Grande do Sul and Holst et al's (2003) treatment for Venezuelan Guayana. On a still smaller scale, national parks or similar sized remnants of natural vegetation have been subject to floristic study (e.g. Kawasaki 1989; Duarte unpubl.), although often, results of these surveys remain unpublished.

The study of large genera

Willis (1949) suggested that for a genus to qualify as large, it should contain a minimum of 500 species. Dating back to pre-Linnaean times, the genus has been defined as 'the smallest "kind" recognizable without expert study' (Frodin 2004). After the introduction of Linnaeus's binomial system (1735, 1737), the concept of the genus quickly became the means to associate species epithets and has since maintained a fundamental and relatively stable place in systematic botany (although not necessarily its application or delimitation). Linnaeus advocated that for practicality, a genus should not exceed 100 species and the ideal size of genera has been a subject of debate among naturalists ever since (Brown 1883; Willis 1949; Mabberley 1997; Frodin 2004). Articulation or consolidation (splitting and lumping) of genera have alternated as the preferred approach; to split a genus into several smaller, similar

sized ones was favoured by those seeking to memorise and apply classification; to lump species into natural groups of unlimited size was preferable to botanists such as De Candolle (1824-1896) who worked towards 'synthesis' of understanding. In an attempt to do both, De Candolle introduced the concept of infrageneric units, which facilitated the memorization and use of the classification while contributing to the growing acceptance of large genera (Frodin 2004).

Once authors were free to enlarge genera without the constraint of an ideal size, it became apparent that some genera were naturally much larger than others; by the end of the 19th century some patterns were emerging. Brown (1883) published the first list of large genera, in this case those of 300 species or more, 28 genera were listed including two Myrtaceae; *Syzygium* (including *Eugenia*; 500-700 species) and *Myrcia* (300-500 species), ranked 7th and 23rd, respectively. Further large genera lists were subsequently compiled, typically settling at a threshold closer to 500 species (Willis 1949; Mabberley 1997; Frodin 2004). *Eugenia* and *Syzygium* feature in each of these lists; *Myrcia* was listed by Willis (1949), but has not been again since Mabberley's (1997) list with a lower limit of 250 species. Frodin's (2004) most recent estimates are that 57 genera contain more than 500 species, totalling over 53 000 species and accounting for about 15% of all extant species.

Willis (1922) demonstrated the pattern that genus size follows a 'hollow curve' distribution, i.e. small numbers of large genera and large numbers of small genera; seemingly describing the same pattern, Frodin (2004) noted that a large genus is often accompanied by several, often small, 'satellite' taxa. Scotland & Sanderson (2004) noted differences in slope and length of tail between real hollow curve datasets (shallower with shorter tails) and those generated by modeling. These authors conclude that this lack of fit is due to taxonomic as opposed to evolutionary factors. Citing a lack of objective criteria for recognizing higher taxa, Scotland & Sanderson (2004) suggest this causes taxonomists to avoid circumscribing genera so large they are cumbersome to work with and often transpire to be nonmonophyletic,

and to refrain from describing monotypic genera as they contain no information about relationships.

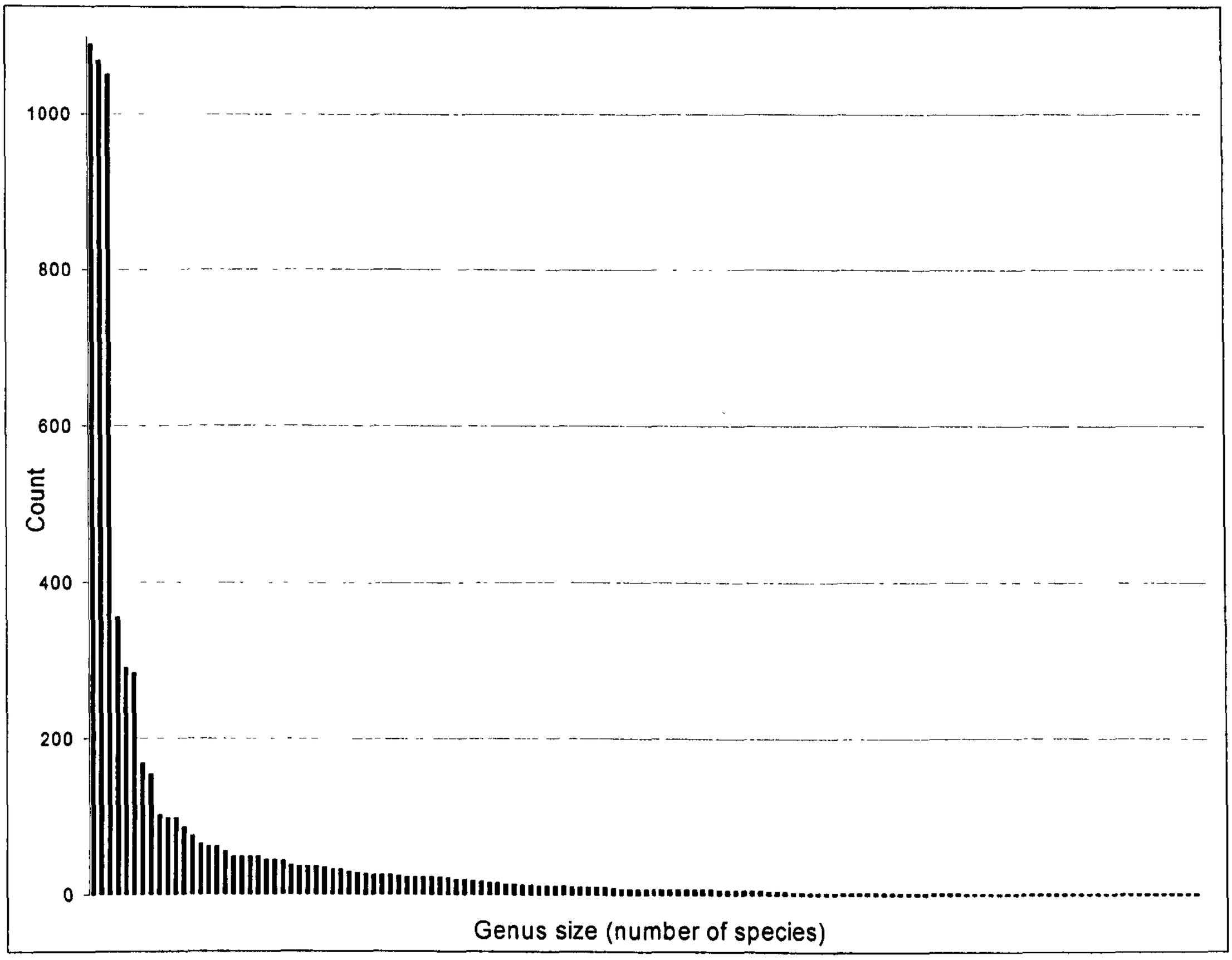


Figure 1.1. The hollow curve of Myrtaceae.

The hollow curve for Myrtaceae (Figure 1.1) demonstrates an exceedingly steep slope and long tail, suggesting that in this family, taxonomists have had no such qualms and the resulting taxonomy is one that well fits the predicted natural pattern of the hollow curve, i.e. the association of very many small genera with a few very large ones.

Historically, divisions within and between angiosperm genera were based on few characters, particularly reproductive ones. From the 1960s onwards, characters were added from other data sources such as micromorphology, anatomy, palynology and karyology. These studies supported unity of some large groups such as *Salvia* (El-Gazzar et al. 1968) while breaking down other previously recognised large groups such as *Eupatorium* (King & Robinson 1970).

The last decades of the 20th century saw growth in popularity of both phylogenetic techniques (Hennig 1966) and taxonomy based on comparative DNA sequence data, often but not always in conjunction with morphology. Today these studies are producing support for large genera such as *Solanum* (Bohs & Olmstead, 1997) and *Astragalus* (Wojciechowski et al. 1999), whereas genera such as *Acacia* (Miller & Bayer 2001) and *Euphorbia* (Steinmann & Porter 2002) have been shown to be poly- or paraphyletic and recircumscribed accordingly. *Eupatorium* has since been confirmed as paraphyletic (Schmidt & Schilling 2000), as has *Salvia* (Walker et al. 2004).

Although molecular phylogenetic techniques have advanced our understanding, the art of taxonomic revisions and monographs is in decline, particularly for large genera (Stuessy 1993; Landrum 2001; Frodin 2004). A monograph is a comprehensive account of all taxonomic data relating to a group, stabilizing the taxonomy and understanding of the taxon, the information compiled in it serving as a base-line for other analytical techniques and stimulating additional work on the group (Stace 1989). As genera became larger, they became harder to manage (Frodin 2004); with more material now lodged in more disparate herbaria, the time and expense required to process specimens for monographic work has increased. Monographing relatively small genera is possible in a timeframe and at a cost deemed reasonable and practical by the scientific community; genera of 30-40 species can be expected to be tackled over three years (Stuessy 1993), the supposed time of a doctoral degree; it is extremely rare for a PhD study to attempt to tackle more than 50–100 species. sulting treatments are then transferable to, or written expressly for scientific journals, or as fascicles for floristic accounts (e.g. Landrum 1981a, 1986; Prance & Mori 1979, Mori & Prance 1990). Larger works do exist and may be published as flora fascicles or in journals but are more often published in book form, the result of a lifetime's work by a single researcher (e.g. *Utricularia;* Taylor 1989, *Inga;* Pennington 1997).

It has been suggested that large genera are not real entities, that instead they are artefacts of incomplete study, "nothing but monuments to the ignorance and timidity of past taxonomists"

(Robinson 1987: p64). Frodin (2004) argued that large genera do exist and can be defined, using molecular and morphological techniques, as monophyletic groups united by synapomorphy. Frodin (2004) debated whether or not large genera have any 'worth' in terms of understanding patterns of evolution and concluded that modern, multi-disciplinary studies are allowing greater understanding of the lineages of large genera and this is now allowing detailed resolution of internal structure, immediate relationships and evolution. Studies such as those of Sanderson & Wojciechowski (1996) on *Astragalus* and Berry et al. (2005) on *Croton* have done just this, and for these groups, conclusions have been drawn regarding changes in evolutionary diversification rates, adaptive radiations, key innovations, and chromosomal rearrangements; these genera are concluded to very definitely exist as robust taxonomic groups both in terms of morphological and molecular criteria.

This study uses the principles and approaches of these previous works to make similar judgements of existence and 'worth' of the large genus *Myrcia* and its relation to subtribe Myrciinae. Previous classifications are the starting point for re-organization of these taxa and their intended division into natural sub-groups of manageable size. As Frodin (2004) suggested, this group will be tackled first by a rapid review of work to date, including assessments of classifications and/or evolutionary hypotheses available, followed by "fuller studies using traditional and contemporary methods of reasonably extensive, well-selected and properly named ranges of species in an effort to clarify phylogenies as well as historical biogeography" (p763). The resulting framework will allow the eventual resolution of the whole group to be undertaken systematically, at a reasonable cost and within a realistic time frame. To attempt a monographic revision of such a large group, without a suitable framework with which to break it down to manageable groups would be overwhelming. Production of a robust estimate of the phylogeny of genera such as these will provide the framework for much needed, detailed, future monographic revisions. Patterns of evolution that have shaped the large genera of Myrtaceae will be discussed in the concluding part of this work, as will the question of their existence as real genera or as artefacts of incomplete study.

Aims

The aims of this thesis and the chapters in which they are addressed follow:

Chapter 2: To summarise taxonomic history and current systematic knowledge of the fleshy-fruited Myrtaceae, comparing the most significant classifications of the past and illustrating the most enduring groups, with a particular emphasis on subtribe Myrciinae.

Chapter 3: To construct a phylogenetic estimate of tribe Myrteae (sensu Wilson et al. 2004) based on DNA sequence data, with a view to evaluating monophyly of the subtribal classifications of tribe Myrteae. To investigate relationships of Neotropical Myrteae genera and to interpret these in the light of morphology.

Chapter 4: To construct a phylogenetic estimate of Myrciinae using DNA sequence and morphological data, which will allow simultaneous interpretation of this phylogenetic hypothesis and the morphology of this group.

Chapter 5: To hypothesise major biogeographical events in Myrteae and Myrciinae history and to correlate the latter results with the evolutionary history of the *Mata Atlântica*.

Chapter 6: To produce a modern, natural, infrageneric classification of Myrciineae.

Chapter 2 – Review of fleshy-fruited Myrteae

Until the works of Briggs & Johnson (1979), Johnson & Briggs (1984) and Wilson et al. (2001, 2004), most authors recognised the two groups suggested by Niedenzu as subfamilies although not always at this rank, and some authors, such as Schmid (1980), reinstated *Chamaelaucium* as tribe Chamaelaucoideae (Table 2.1).

Briggs & Johnson (1979) and Johnson & Briggs (1984) used extensive morphological data to evaluate the subfamilial arrangement in Myrtaceae. These studies were based on a variety of characters including those of the inflorescence, fruit and embryo, trichomes, germination and distribution. In the 1979 analysis, Briggs & Johnson maintained the dry-fleshy fruit division as subfamilies Leptospermoideae and Myrtoideae (Table 2.1). Thirteen alliances and 13 suballiances with no absolute definition were proposed and described as roughly equivalent to tribes and subtribes. The 1984 paper presented a cladistic analysis of this large data set and resulted in the authors modifying some of the 1979 alliance/suballiance framework, in particular concluding that the mostly Asian *Acmena* group, containing *Syzygium* (previously Johnson & Briggs (1984) and considered to be allied with *Eugenia*), had arisen independently from the predominantly Neotropical Myrtoideae *sensu stricto*. This supported the work of Schmid (1972), who had demonstrated that the floral anatomy of *Eugenia sensu stricto* is distinct from that of *Syzygium*, in particular in the ovule vascular supply system that is transeptal in *Eugenia* and axile in *Syzygium*. Johnson & Briggs (1984) rejected the traditional subfamily classification for the first time, the authors stating (p:752) that "continued reference to them is misleading in setting phylogenetic context and is phytogeographically irrelevant". Johnson & Briggs (1984) concluded their paper by recommending "a formal system of tribes and subtribes, more or less equivalent to our informal scheme of alliances and suballiances" (p.752). Ultimately, they never followed-up their initial work, and undertaking a phylogenetic analysis of Myrtaceae and its classification fell to others.

Wilson et al. (1994) began this task by scoring a wide range of genera and reviewing the morphological characters used by Johnson & Briggs (1984); they found that extensive homoplasy, already noted in Myrtaceae by Landrum (1981b), resulted in a poorly resolved cladogram. Wilson et al. (2001) combined *matK* sequence data with additional morphological characters "used in defining relationships of genera and tribes (alliances) in Myrtaceae" (p. 2014). The molecular analysis of Wilson et al. (2001) indicated the '*Acmena*' (fleshy-fruited) group emerged in a clade with a '*Tristania*' (capsular-fruited) group and the fleshy-fruited *Xanthomyrtus,* and that these were sister to the capsular '*Backhousia*' and '*Metrosideros*' groups. The clade formed by all of these clades is sister to a clade containing various capsular-fruited taxa, and the group consisting of the majority of other fleshy-fruited genera previously referred to tribe Myrteae (predominantly Neotropical, fleshy-fruited genera). Based on combined analyses of morphological and molecular data sets in Wilson et al. (2001), the authors concluded that the dry fruit-type is the plesiomorphic state and that fleshy fruits have arisen on four independent occasions. Discounting genera with leathery fruits such as *Osbornia* and *Darwinia*, there are three lineages that include fleshy-fruited genera. In one of these, including the '*Acmena*' and 'myrtoid' groups as well as *Xanthomyrtus*, fleshy fruits appear to have arisen on two separate occasions. In Wilson et al. (2001), the authors critically review the use of a thoroughly investigated suite of morphological characters for determining groups within Myrtaceae. The only character of use for distiniguishing the 'myrtoid' group was one of pubescence, with "standard" unicellular hairs (as opposed to no hairs) appearing plesiomorphic, lost on several occasions but present in the 'myrtoid' group and absent in the *Acmena* group.

By 2005, Wilson et al. had compiled sufficient *matK* plus 5' spacer region sequence data to propose and publish a new subfamilial classification of Myrtaceae that comprised two subfamilies, Psiloxyloideae and Myrtoideae, the former comprising two genera, and the latter including the rest of the family. Within Myrtoideae, fifteen monophyletic groups were identified and formally published as tribes (Table 2.1). According to this classification,

Myrteae, roughly equating to Briggs & Johnson's (1979) Myrtoideae *sensu stricto*, contain 29 Neotropical and 18 Paleotropical fleshy-fruited genera. In the resulting tree (Wilson et al. 2005), Neotropical genera form a clade, including the Australian genus *Lenwebbia,* sister to the remaining Paleotropical Myrteae genera.

Table 2.1. Subfamilial groupings of Myrtaceae according to different authors. Taxa associated with subfamilies Myrtoideae, Leptospermoideae and Psiloxyloideae are indicated by light, intermediate and dark shading respectively.

De Candolle ex Schlechtendahl (1827)	Niedenzu (1893)	Kausel (1956)	Johnson & Briggs (1984)	Schmid (1980)	Wilson et al. (2005)
Tribe Leptospermeae Tribe Chamaelaucieae	Subfamily Leptospermoideae	Family Leptospermaceae	Subfamily Leptospermoideae	Subfamily Leptospermoideae Subfamily Chamaelaucoideae	
Tribe Myrteae	Subfamily Myrtoideae	Family Myrtaceae	Subfamily Myrtoideae*	Subfamily Myrtoideae	Subfamily Myrtoideae**
Tribe Barringtonieae Tribe Lecythideae	Family Lecythidaceae (excluded from Myrtaceae)				
				Subfamily Psiloxyloideae	Subfamily Psiloxyloideae

*Including 3 groups:
Osbornia
'Myrtoideae sensu stricto'
The *Acmena* alliance

**Including 15 tribes:
Eucalypteae, Kanieae, Leptospermeae, Lindsayomyrteae, Lophostemoneae,
Melaleuceae, Metrosidereae, Osbornieae, Syncarpieae, Tristanieae,
Myrteae, Syzygieae

Neotropical Myrteae

A review of the taxonomic history and various classifications of tribe Myrteae in the Neotropics follows. Although not explicitly defined in his work, Linnaeus (1753) divided Myrtaceae according to numbers of calyx lobes, locules and seeds (Table 2.2). He recognised three fleshy-fruited genera: *Myrtus* with a pentamerous calyx and a trilocular, one-seeded berry; *Psidium* with a pentamerous calyx and a unilocular, many-seeded berry; and *Eugenia* with a tetramerous, unilocular, one-seeded drupe. Willdenow (1800) described two further genera: *Calyptranthes* with a calyptrate calyx and a unilocular, 1-4-seeded berry and *Plinia* with a 4-5-merous calyx and a sulcate drupe. As new specimens arrived from the Neotropics, these distinctions no longer served to delimit generic boundaries neatly; subsequent authors, such as Kunth (1823) and Sprengel (1825), with no robust characters with which to distinguish genera, opted to lump *Eugenia* into an inclusive *Myrtus*. Along with the subfamilial classification that divided fleshy-fruited Myrtaceae from dry-fruited ones, De Candolle (1828) further subdivided the fleshy-fruited genera of subfamily Myrtoideae into three 'principal groups' depending on the nature of the embryo within the seed; he never formally published these groups.

Berg (1855-1856, 1857-59) published De Candolle's three groups of Myrteae genera as subtribes Myrcioideae, Eugenioideae and Pimentoideae. He recognised two further monogeneric subtribes, Myrrhinioideae (*Myrrhinium*) and Orthostemonoideae (*Acca;* Table 2.2). *Myrrhinium* and *Acca* have since been associated with Myrtinae (McVaugh 1968; Landrum 1986) and current myrtologists are accustomend to using the former three subtribes only (Table 2.2), characterised as follows (see Landrum 1981a; 1986; Fig. 2.1): **Myrciinae,** embryo with thin, membranous cotyledons folded into a bundle and a long, horseshoe-shaped hypocotyl curled around the cotyledons; **Eugeniinae,** embryo with thick, fleshy cotyledons (similar to those of a bean) sometimes fused together in a homogenous mass, the hypocotyls evident or not; **Myrtinae,** embryo consisting of a long, thin, horseshoe- or hook-shaped

(Landrum 1981a) or coiled (Snow et al. 2003) hypocotyl curved towards two, reduced, thin cotyledons or surrounding them.

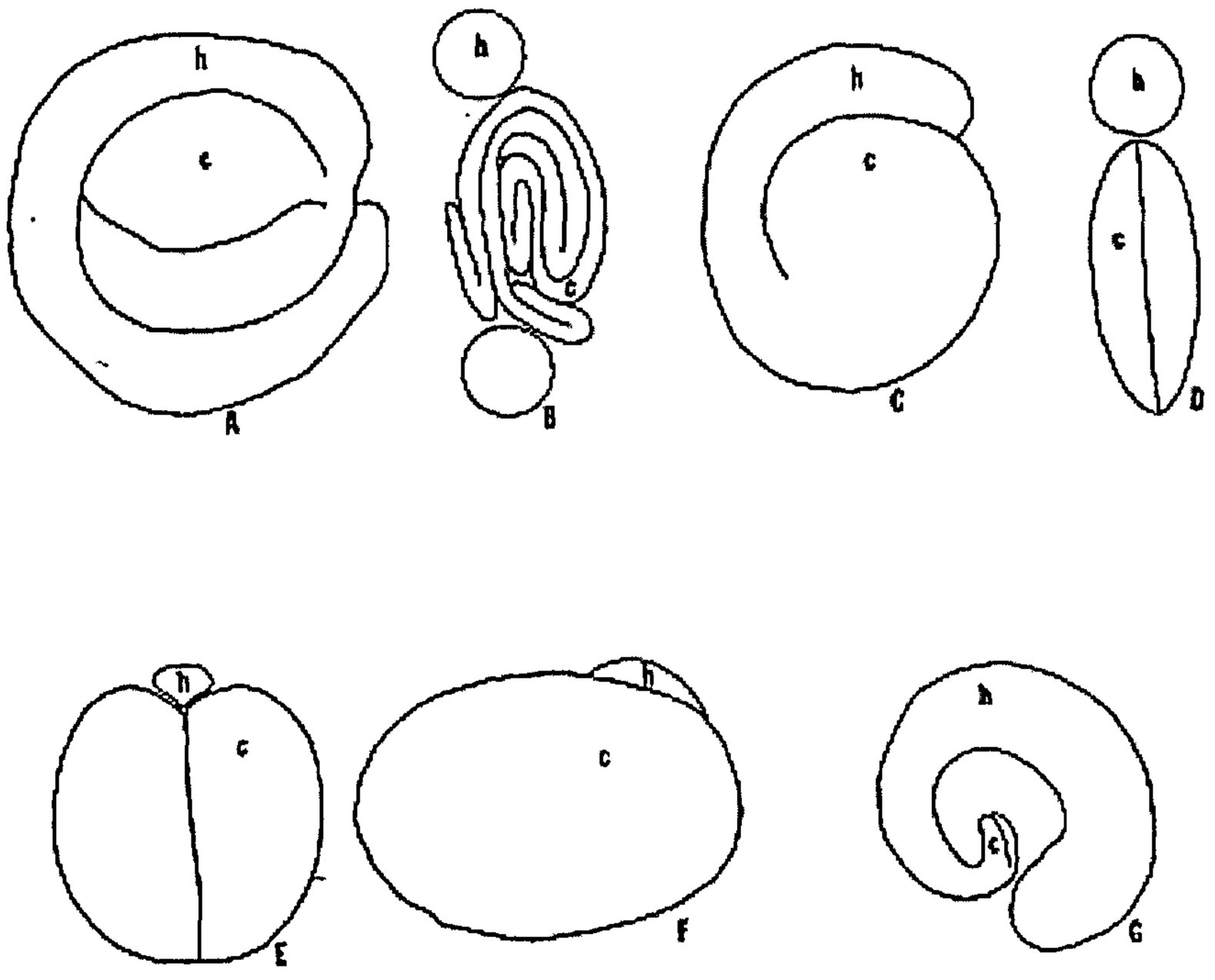


Figure 2.1. Myrteae embryos. Embryo of Myrciinae: A, side view; B, cross section. Embryo of Luma, suggested intermediate between the eugenioid and myrcioid embryos: C, side view; D, cross section. Embryo of Eugeniinae: E, cross section, F, side view. Embryo of Myrtinae: G, side view. (h – hypocotyl; c – cotyledon). From Landrum (1981a).

Paleotropical genera were presented in Berg's subtribal scheme for the first time by Bentham (1869); Snow recently (2000) provided a conspectus of Australasian Myrtinae; this arrangement enhanced by other Paleotropical studies on Eugeniinae genera (Briggs & Johnson 1979; Johnson & Briggs, 1984; Wilson et al. 2001) is presented in Table 2.3.

The subtribal arrangement of Berg, based on characters from the embryo, was criticized as artificial (Bentham 1869; Kausel 1947, 1956, 1966). Kausel (1956, 1966) reclassified Myrteae and Leptospermeae as Myrtaceae and Leptospermaceae; he then divided Myrtaceae (Myrteae *sensu* Wilson et al. 2005) into five subfamilies divided into two groups based on patterns of seed germination: those with hypogeal germination, Eugenioideae (*Eugenia, Stenocalyx, Calycorectes, Myrciaria, Pseudomyrcianthes*), Plinioideae (*Myrcianthes,*

Acreugenia, Siphoneugena, Paramyrciaria) and Cryptorhizoideae (*Campomanesia, Britoa, Blepharocalyx*) versus those with epigeal germination, Myrtoideae (*Amomyrtus, Amomyrtella, Myrrhinium, Psidium, Ugni, Myrteola*) and Myrcioideae (*Aulomyrcia, Calyptranthes, Marlierea, Myrceugenia, Myrcia, Myrceugenella, Feijoa*). Plinioideae have separate, swollen cotyledons, Cryptorhizoideae were erroneously thought by Kausel to have large cotyledons surrounding the hypocotyl, whereas Eugenioideae have undivided cotyledons indistinguishable from the hypocotyl. Kausel was later shown to be wrong in the case of *Myrcianthes,* which has hypogeal germination (Landrum pers. comm.).

As a result of the artificiality of the available groupings, McVaugh (1968) moved away from attempts to designate "subtribes [or other ranked groups] representing natural units" (p.362). After a study based on characters of the cotyledons, embryo, seed coat, ovary, calyx and inflorescence structure, McVaugh (1968) concluded that the 30-35 genera and approximately 1200 species in the traditional three Neotropical subtribes had evolved along six main evolutionary lines that he called 'informal groups' (Table 2.2). Of these, groups 4, 5 and 6 contain the genera of Myrtinae, groups 2 and 3 contain the genera of Eugeniinae and group 1 contains the genera that make up Berg's Myrciinae.

Linnaeus (1753)	Sprengel (1825)	De Candolle (1826)	Berg (1855-56, 1857-59)	Kausel (1956, 1966)	McVaugh (1968)	Landrum (1986)
	Calyptranthes	Principal group of genera associated with *Myrcia*	Subtribe Myrcioideae (later Myrciinae)	Subfamily Myrcioideae	Group 1. The 'Myrcioid' genera	
	Plinia	Principal group of genera associated with *Eugenia*	Subtribe Eugenioideae (later Eugeniinae)	Subfamily Plinioideae	Group 2. The 'Eugenioid' genera	
Eugenia	*Myrtus*			Subfamily Eugenioideae		
					Group 3. *Myrcianthes* and related genera	
Myrtus		Principal group of genera associated with *Myrtus*	Subtribe Pimentoideae (later Myrtinae)	Subfamily Myrtoideae	Group 5. *Psidium* and related genera	
Psidium	*Psidium*				Group 6. *Pseudocaryophyllus* and *Pimenta*	*Campomanesia* complex
	Campomanesia			Subfamily Cryptorhizoideae	Group 4. *Campomanesia* and related genera	
					Imperfectly known *Cryptorhiza*	
			Subtribe Myrrhinioideae (later Myrrhinieae)		Unplaced genera	
			Subtribe Orthostemonoideae (later Feijoinae)			

Table 2.2. Groupings of Neotropical Myrteae *sensu* Wilson et al. (2005), according to different authors. Taxa associated with Berg's subtribes Myrciinae, Eugeniinae and Myrtinae are indicated by light, intermediate and dark shading respectively.

Subtribe	Genera recognised by De Candolle (1828)	Genera recognised or described by Berg (1855-56, 1857-59)	Genera of the *Campomanesia* complex *sensu* Landrum and Stevenson (1986)	Paleotropical Myrtinae genera (Snow 2000, Snow et al. 2003)
Myrciinae	*Myrcia* DC. *Calyptranthes* Sw.	*Myrcia* DC., *Calyptranthes* Sw. *Marlierea* Cambess. *Aulomyrcia, Calycampe,* *Calyptromyrcia, Cerqueiria* *Eugeniopsis, Gomidesia,* *Myrceugenia, Rubachia* (all described by O.Berg).		
Eugeniinae	*Eugenia* L., *Syzygium* Gaertn., *Acmena* DC., *Caryophyllus* L., *Jambosa* DC.	*Eugenia* L., *Syzygium* Gaertn., *Acmena* DC., *Caryophyllus* L., *Jambosa* Adans. *Phyllocalyx, Stenocalyx,* *Myrcianthes, Mitranthes,* *Calycorectes, Schizocalyx,* *Myrciaria, Siphoneugena,* *Hexachlamys, Aulacocarpus* (all described by O.Berg).		
Myrtinae	*Campomanesia* Ruiz & Pav., *Psidium* L., *Myrtus* L.	*Campomanesia* Ruiz & Pav., *Myrtus* L., *Pimenta* Lindley, *Psidium* L., *Ugni* Turcz. *Abbevillea, Acranda,Amomis,* *Blepharocalyx, Britoa, Calycolpus,* *Calyptropsidium,* *Lacerdaea, Myrteola, Paivaea,* *Pseudocaryophyllus, Psidiopsis* (all described by O.Berg). *Myrrhinium* (subsequently described by Schott).	*Campomanesia* Ruiz & Pav. [incl. *Abbevillea, Acranda, Britoa, Lacerdaea, Paivaea* (all described by Berg)]. *Pimenta* Lindley [(incl. *Amomis* O.Berg, *Cryptorhiza, Krokia, Myrtekmania* (all described by Urban)], *Mentodendron* Lundell, *Blepharocalyx* (incl. *Marlieriopsis* Kiaersk., *Temu* O.Berg), *Legrandia* Kausel and *Pilidiostigma* Burret.	*Archirhodomyrtus* (Nied.) Burret, *Austromyrtus* (Nied.) Burret, *Decaspermum* J.R.Forst. & G.Forst., *Gossia* N.Snow & Guymer, *Lenwebbia* N.Snow & Guymer, *Lithomyrtus* F. Muell., *Lophomyrtus* Burret, *Myrtella* F. Muell., *Myrtastrum* Burret, *Neomyrtus* Burret, *Octamyrtus* Diels, *Pilidiostigma* Burret, *Rhodamnia* Jack, *Rhodomyrtus* (DC.) Rchb., *Uromyrtus* Burret

Table 2.3. Genera assigned by various authors to the three previously recognized subtribes of Myrteae *sensu* Wilson et al. (2005).

Although McVaugh (1968) assigned nearly all accepted genera to one of these groups, eight genera did not fit satisfactorily into any group. McVaugh (1968) regarded these as evolutionary lines that had arisen from the same proto-myrtaceous ancestors as the others but had not been as successful and consequently remained evolutionarily isolated but affiliated to these ancestral groups. Of these anomalous genera, *Nothomyrcia* is now considered a synonym of *Myrceugenia* (Landrum 1981a), affiliated with group 1 (the 'myrcioid genera'). *Legrandia* was affiliated with group 2 (the 'eugenioid genera'), *Luma* was affiliated with group 3 (*Myrcianthes* and related genera) and *Amomyrtus* and *Amomyrtella* were affiliated with group 5 (*Psidium* and related genera). Two remaining genera, *Myrrhinium* and *Acca* were listed as '*genera incertae sedis*' and correspond to Berg's subtribes Myrrhinieae and Feijoinae, respectively. McVaugh (1968) doubted the validity of *Cryptorhiza* and therefore Cryptorhizoideae by suggesting the (destroyed) type of the species was *Myrcianthes fragrans* (Sw.) McVaugh.

Briggs & Johnson (1979) divided Myrtoideae into six informal groups, the Neotropical genera falling into four of these, the *Myrcia, Myrtus, Eugenia* and *Cryptorhiza* alliances. Later, no longer recognizing Myrtoideae, Johnson & Briggs (1984) erected three fleshy-fruited groups (Table 2.1), having reduced those four containing Neotropical taxa into a single alliance, 'Myrtoideae sensu stricto' (in their sense: *Austromyrtus, Eugenia, Fenzlia, Meteoromyrtus, Myrcia, Myrtella, Myrtus, Pilothecium, Psidium, Sterocaryum, Uromyrtus* and *Xanthomyrtus*).

As a precursor to revisionary work, Landrum & Stevenson (1986) studied embryo variability in Myrtinae. They proposed a '*Campomanesia* complex' (Table 2.3) in which the seed coat is usually soft and the hypocotyl swollen (usually much wider than the cotyledons), often containing a visible vascular core that in the past had been mistaken for the radicle and the surrounding cortex interpreted as fused cotyledons in several genera, such as *Cryptorhiza* (Urban 1921; Kausel 1956, 1966). Landrum (1988) conceptualized a further group, linking

Myrteola, *Ugni*, *Myrtastrum*, *Neomyrtus*, *Myrtus*, *Lophomyrtus*, and those *Austromyrtus* species that Snow et al. (2003) later distinguished as *Austromyrtus* sensu stricto (2003). This association is based on the shared characteristics of C-shaped embryos with cotyledons roughly the same length as the hypocotyl and not folded back over it, smooth, hard seed coats opening by means of opercula and uniflorous peduncles in the leaf axils. Berg (1855-56, 1857-59) had also noted the division of Myrtinae genera between those with bony and soft testae, although he did not create any formal taxonomy for these groups.

The first molecular study focused on Myrteae was that of Salywon et al. (2002) and Salywon (2003). This study sampled mainly tropical American genera, especially Myrtinae and *Mosiera*. The study strongly supported a monophyletic *Mosiera* but suggested that *Psidium* may be paraphyletic, with *Mosiera* and other genera of Myrtinae nested within it. Most Paleotropical Myrtinae grouped together except *Lophomyrtus*, *Gossia* and *Lenwebbia*. The study further indicated that some Myrtinae may be sister to Myrciinae and Eugeniinae and that Eugeniinae are probably polyphyletic. Salywon (pers. comm.) further reported problems with low levels of variability and low bootstrap support.

Wilson et al.'s (2005) analysis contained 19 Myrteae genera, seven Paleotropical genera and eleven Neotropical. Results suggested that the Neotropical genera plus the Australian genus *Lenwebbia* constitute the group sister to the Mediterranean *Myrtus*, which in turn is sister to the rest of the Paleotropical genera (Fig. 2.2). Berg's subtribe Myrtinae appear paraphyletic, the two Eugeniinae genera included (*Eugenia* and *Hexachlamys*) emerge in a monophyletic group, whereas *Calyptranthes* is the only Myrciinae genus represented, placed in an ambiguous polytomy (Fig. 2.2). Jackknife support is low, and few conclusions can be drawn regarding relationships of the Neotropical genera or Berg's subtribes. Sytsma et al. (2004) presented analyses of *ndhF* and *matK* sequences for a similar set of genera but excluded *Lenwebbia* and *Calyptranthes*. This study recovered a similar arrangement with *Calytrix* (tribe Chamelaucieae *sensu* Wilson) nested in the Neotropical genera and *Acca* (syn. *Feijoa*)

emerging with the Paleotropical taxa (Fig. 2.3). Again support is low, and few firm taxonomic conclusions can be drawn.

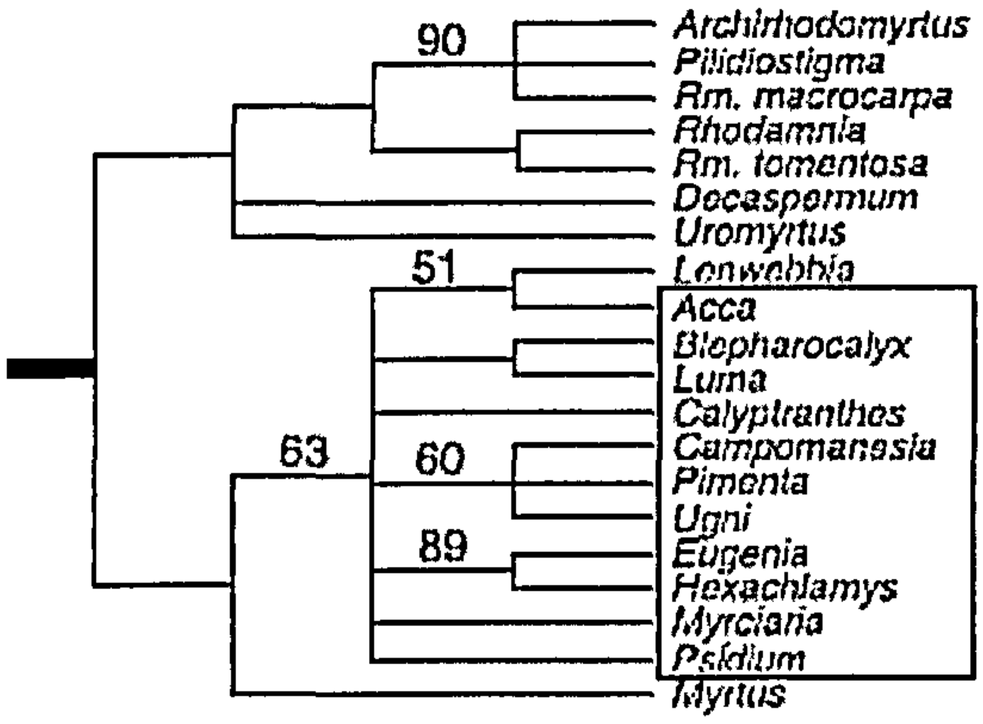


Figure 2.2. Strict consensus from heuristic search of *matK* sequences. Thick branches indicate at least 95% jackknife support; other values >50% shown above branches. Box indicates Neotropical taxa. *Rm.* = *Rhodomyrtus*. Modified from Wilson et al. (2005).

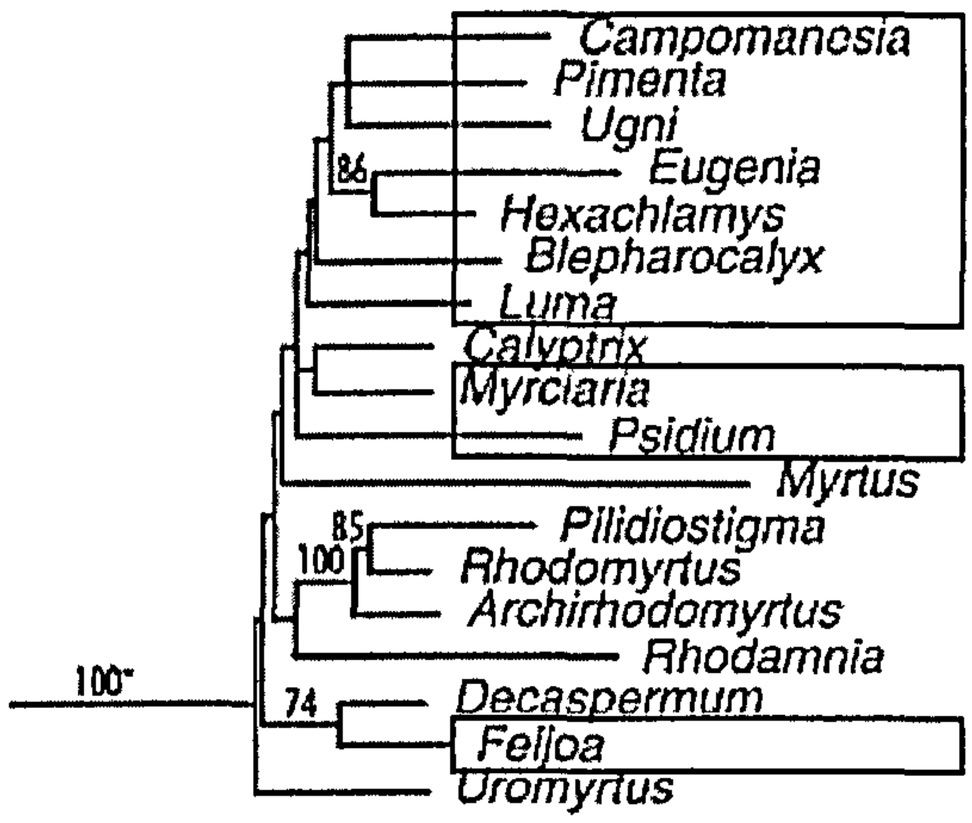


Figure 2.3. Strict consensus from maximum likelihood analysis of *ndhF* and *matK* sequences. ML bootstrap percentages >70 shown above branches. Boxes indicate Neotropical taxa. (*Calyptrix*: miss-spelling of *Calytrix* Labill.). Modified from Sytsma et al. (2004).

Therefore, despite several attempts to group the genera of Myrteae, there is still no framework for understanding relationships in the tribe. Molecular studies confirm that tribe Myrteae is monophyletic without *Syzygium* and allies and suggest a New World-Old World split, rendering Myrtinae *sensu* Berg paraphyletic (Systsma et al. 2004; Wilson et al. 2005). There is neither a morphological understanding of this division of Myrtinae nor a well-supported hypothesis of relationships within and between the remaining two subtribes, in particular

Myrciinae, the focus of this study. Also lacking are reliable phylogenetic hypotheses of relationships between the genera of the Neotropics *vs*. Paleotropics.

Myrciinae

A taxonomic review of previous classifications of the genera of this subtribe follows. The number of genera assigned to Myrciinae has fluctuated widely over taxonomic history (Table 2.4). The major characters on which these genera have been based are summarised in Table 2.5. Grisebach (1864) treated Myrtaceae for the British West Indies following Berg's classification, except in synonymising *Aulomyrcia* as a section of *Myrcia*. Bentham (1869) reduced Berg's 11 genera to four, discussing in depth the imperceptible intervals between character states ascribed to the calyx and anthers. The subsequent arrangement by Niedenzu (1893) differed only in maintaining *Gomidesia* (including *Cerqueiria*). A few months after Niedenzu, Kiaerskou (1893) independently reduced Myrciinae to *Myrcia*, *Marlierea* and *Calyptranthes* only. Kausel (1947, 1956, 1966) published two additional genera, *Myrceugenella* and *Nothomyrcia*, reinstated *Gomidesia*, *Aulomyrcia* and *Myrceugenia*, and associated *Feijoa* with Myrciinae for the first time, since the cotyledons are larger than in most Myrtinae, twisted and reflexed across the hypocotyl.

Gray (1854) described *Luma*, a taxon unknown to Berg; *Luma* has characters that suggest intermediacy between Myrciinae and Eugeniinae. The embryo has two broad, thin cotyledons and a short but distinct hypocotyl, whereas the tetramerous flowers are arranged either in inflorescences that can be interpreted as derivations of dichasial branching inflorescences, such as three-flowered dichasial cymes, or as solitary flowers in the leaf-axils. McVaugh (1968) realised that Kausel's *Myrceugenella* was a superfluous name for *Luma*. He did not place *Luma* within any of his main groups but related it to '*Myrcianthes* and associated genera', suggesting an association with Eugeniinae. In the Flora of Surinam, Amshoff (1951) reinstated *Aulomyrcia* as a distinct genus and reduced *Calycampe* to *Myrcia*.

McVaugh (1958a, 1968, 1969) agreed with the majority of nomenclatural reduction preceding him. Berg (1855-1856, 1857-1859) and Kausel (1942) assigned *Myrceugenia* and *Nothomyrcia* to Myrciinae since they have the same foliaceous embryo type as the other genera. McVaugh (1968) considered *Nothomyrcia* an aberrant species of *Myrceugenia* but did not merge them. McVaugh (1968) did not include these genera directly in his 'myrcioid' group, but after consideration associated them on the basis of shared embryo structure.

Characters that separate *Myrceugenia* and *Nothomyrcia* from core Myrciinae include: the inflorescence (flowers in axillary racemes, solitary, in three-flowered cymes or made up of multiple such units and superposed if there is more than one inflorescence per axil, never a Myrciinae-type panicle); in *Myrceugenia* and *Nothomyrcia* the number of ovules in each locule (usually several rather than the usual two in Myrciinae); the number of locules in the ovary (two to three in *Nothomyrcia* and usually three in *Myrceugenia* rather than the usual two); the number of calyx lobes (mostly four instead of the usual five); and bracteoles (persistent until anthesis in *Myrceugenia*, deciduous in Myrciinae and *Nothomyrcia*).

Berg (1855-1856, 1857-1859) described the multilocular, calyptrate *Mitranthes* without fruit and placed it in Eugeniinae; he was followed by Legrand (1961), who included a new combination from *Calyptranthes*. Burret (1941) transferred several of Berg's species to the new genus, *Mitropsidium,* on account of Myrtinae-type placentation and embryo. McVaugh (1968) concurred for species with Myrtinae affinities but noted that some species, such as *Mitranthes obscura* (DC.) Legr., and in particular some multilocular, calyptrate species with a Myrciinae embryo, are more allied to Myrciinae. McVaugh (1968) finally placed *Mitranthes* among 'imperfectly known genera', concluding (p. 411) that this is 'a relatively unspecialised member of the myrcioid alliance'. McVaugh (1968) included *Krugia* in *Marlierea,* and *Aguava, Cumetea* and *Mozartia* in *Myrcia.* McVaugh (1968; 409) also noted that the cotyledons of *Feijoa* 'bear little resemblance to the broad, crumpled cotyledons of Myrcia' supporting its sinking into the older name *Acca* by Burret (1941) and disassociating it from the 'myrcioid group'.

After long and eloquent discussion on the usefulness of characters of the calyx and hypanthium in the 'myrcioid' genera and detailed descriptions of the opening calyx, McVaugh (1968) concluded that there was no consistent way to separate *Myrcia* and *Marlierea* or the latter from *Calyptranthes*. He advocated maintaining the genera on account of his understanding of them as "natural units; assemblages of species in which correlations of evolutionary tendencies can be demonstrated" (p. 375) and of it being practically desirable. *Marlierea* is then distinguished from *Myrcia* in having deciduous calyx lobes that often continue to tear into the disc of the flower and tetramerous flowers often with an explanate disc and dibrachiate hairs. *Calyptranthes* is distinguished from *Marlierea* in having calyx lobes fused into a calyptra, the disc not tearing or becoming explanate at anthesis. It must, however, be emphasised that intermediate stages can often be found for every combination of characters.

Landrum (1981a) revised *Myrceugenia*, sinking *Nothomyrcia* into it, and placing it in Myrciinae but noting its different characters. He suggested that its anomalies may be characteristic of extant taxa similar to ancestors of the three subtribes of Myrteae. *Myrceugenia* (Myrciinae), *Blepharocalyx* (Myrtinae), *Myrcianthes* (Eugeniinae) and *Luma* (subtribe unknown) share some or all of the characters considered by Landrum (1981a) to be inconsistent with other genera of their respective subtribes and therefore potentially plesiomorphic. These characters include: no fusion of calyx-lobes beyond the top of the ovary; no fusion of the embryonic cotyledons; an indefinite number of ovules in each locule, usually 5-20; 2-4 locules per ovary, usually 2; most or all species with tetramerous flowers; seeds with membranous testa; and a strongly developed dichasium in at least some species. In this account, Landrum hypothesised that the characters of *Luma* are anomalous to the extent that this genus may be similar to an ancestor of all Myrteae. Landrum (1984) described two new calyptrate *Myrceugenia* and concurred with McVaugh (1968) in the placement of *Mitranthes* in Myrciinae. Later, in his revision of *Luma*, Landrum (1986) further discussed the anomalous characters of *Luma* and concluded that if *Luma* is to be

assigned to a subtribe it should be placed in Myrciinae as it appears most closely allied with *Myrceugenia*.

Despite McVaugh's (1968; p. 373) opinion that the 'myrcioid' group is '*the most readily comprehensible of the groups of Myrteae*', difficulty in demarcating genera within the group means that modern Myrtaceae researchers asked to list the Myrciinae genera would struggle to reach a consensus. They would likely begin, '*Myrcia* (including *Aulomyrcia*, *Calycampe* and *Calyptromyrcia*), *Calyptranthes*, *Marlierea* (including *Rubachia* and *Eugeniopsis*), perhaps *Mitranthes*', although they would explain that the division between *Myrcia* and *Marlierea* is not a good one and neither is that between *Calyptranthes* and *Marlierea*. Some authors (McVaugh 1968; Landrum 1981a; NicLughadha 1997) have favoured separating *Gomidesia* (including *Cerqueira*), whereas others such as Landrum & Kawasaki (1997); Sobral (2003) and Holst (2003), have treated *Gomidesia* as a synonym of *Myrcia*. Landrum & Kawasaki (1997) additionally included *Marlierea* in *Myrcia* but retained *Calyptranthes*. At this point in the taxonomic history of the subtribe, this survey finds hesitation (McVaugh 1968; Landrum 1981a) to place unreservedly *Myrceugenia* (including *Nothomyrcia*) and *Luma* in a subtribe or group, with *Luma* more quickly disassociated from the other myrcioid genera than *Myrceugenia*.

Whether or not *Calyptranthes*,*Gomidesia, Marlierea* and *Myrcia* are paraphyletic and combined into one vast genus, as suggested by McVaugh (1968), revision of *Myrcia*, the largest component of this quartet, will facilitate understanding and ultimately classification of the others.

Table 2.4. Taxonomy of Myrciinae genera. Authors accepted names in bold, grey box signifies genera not placed directly in the 'myrcioid' group but associated with it. Modified from Nic Lughadha (1997).

Berg (1855-56, 1857-59)	Bentham (1869)	Niedenzu, (1893)	Kiaerskou (1893)	Kausel (1942, 1956, 1966)	McVaugh (1968)	Landrum (1981a)
Subtribe Myrcioideae	Myrciinae	Myrciinae	Myrcioideae	Myrcioideae	'myrcioid' genera	Myrciinae
Cerqueiria	*= Myrcia*	*= Gomidesia*			*= Gomidesia*	
Gomidesia	*= Myrcia*	***Gomidesia***	*= Myrcia*	***Gomidesia***	***Gomidesia***	***Gomidesia***
Rubachia	*= Marlierea*	*= Marlierea*	*= Marlierea*		*= Marlierea*	
Marlierea	***Marlierea***	***Marlierea***	***Marlierea***		***Marlierea***	***Marlierea***
Calyptranthes	***Calyptranthes***	***Calyptranthes***	***Calyptranthes***	***Calyptranthes***	***Calyptranthes***	***Calyptranthes***
Calpytromyrciaa	*= Myrcia*	*= Myrcia*			*= Myrcia*	
Aulomyrcia	*= Myrcia*	*= Myrcia*	*= Myrcia*	***Aulomyrcia***	*= Myrcia*	
Eugeniopsis	*= Marlierea*	*= Marlierea*	*= Marlierea*		*= Marlierea*	
Myrcia	***Myrcia***	***Myrcia***	***Myrcia***	***Myrcia***	***Myrcia***	***Myrcia***
Myrceugenia	***Myrceugenia***	***Myrceugenia***	*= Myrcia*	***Myrceugenia***	*Myrceugenia*	***Myrceugenia***
Calycampe	*= Myrcia*	*= Myrcia*				
				Nothomyrcia	*Nothomyrcia*	*= Myrceugenia*
				Myrceugenella		***Luma***
					Mitranthes	***Mitranthes***
					Feijoa	

Table 2.5. Genera of Myrciinae, date of publication and diagnostic characters. All have the Myrciinae-type embryo, unless specified.

Genus	Date	Characters on which originally circumscribed.
Calyptranthes Sw.	1788	Calyx lobes fused into a calyptra.
Myrcia DC.	1826	Locules bi-ovular, calyx lobes free, hypanthium barely extended above ovary, base of calyx constricted. Calyx lobes not separating from hypanthium.
Marlierea Cambess.	1833	Locules bi-ovular, calyx lobes fused in bud, sometimes with an apical pore, tearing open unevenly at anthesis.
Aguava Raf.	1838	Protologue unavailable.
Cumetea Raf.	1838	Protologue unavailable.
Mitranthes O.Berg	1855–56	Calyx and inflorescence of *Calyptranthes*. Ovary multilocular and multiovulate.
Luma A.Gray	1854	Inflorescence uniflorous or dichasial. Flowers tetramerous, ovary bilocular, multiovulate. Intermediate embryo with extended hypocotyl and cotyledons somewhat swollen.
Cerqueiria O.Berg	1855–56	Locules bi-ovular, calyx lobes free, hypanthium barely extended above ovary, base of calyx constricted, anthers quadrilocular, dehiscing by means of four apical pores.
Gomidesia O.Berg	1855–56	Locules bi-ovular, calyx lobes free, hypanthium barely extended above ovary, base of calyx constricted, anthers sub-quadrilocular, dehiscing laterally by means of an s-shaped fissure.
Rubachia O.Berg	1855–56	Locules bi-ovular, calyx lobes not completely fused in bud, 3–5 short sepals eventually dividing all the way to the summit of the ovary.
Calyptromyrcia O.Berg	1855–56	Locules bi-ovular, calyx lobes free, reflexed, always deeply pentamerous.
Aulomyrcia O.Berg	1855–56	Locules bi-ovular, calyx lobes free, hypanthium strongly extended above ovary, base of calyx never constricted.
Eugeniopsis O.Berg	1855–56	Locules bi-ovular, calyx lobes semi-closed in bud, shortly four-merous.
Myrceugenia O.Berg	1855–56	Bi or trilocular ovary. Locules with roughly six ovules per locule, tetramerous flowers in axillary racemes.
Calycampe O.Berg	1855–56	Locules bi-ovular, calyx lobes free, hypanthium barely extended above ovary, base of calyx constricted. Calyx lobes separating to some degree from hypanthium.
Krugia Urb.	1893	Petals adhering to a calyptriform segment of the calyx, breaking free from the flower together.
Mozartia Urb.	1923	Calyx and hypanthium as *Aulomyrcia*. Ovary imperfectly bilocular or unilocular, ovules solitary or sometimes two when ovary unilocular.
Myrceugenella Kausel	1942	= *Luma* Gray (Name illegitimate and superfluous)
Nothomyrcia Kausel	1947	Bilocular ovary with roughly six ovules per locule, tetramerous flowers in axillary racemes.

Myrcia

Exact number of species of *Myrcia* has never been determined, and estimates have varied from 300(–500) (Brown, 1883) to 500 (Willis, 1949), more recently dropping to 250 (Mabberley 1997), with c. 350 species currently accepted in the World Checklist of Myrtaceae (Govaerts et al. 2006) and 749 names on IPNI (2005). McVaugh (1969) estimated c. 300 species of *Myrcia*, listing approximately 35 species known from Peru, 50 from the Guayana region, 'very few' from the West Indies and North America, with the remainder of species from Brazil, particularly in the south and east. This section will deal with historical subdivisions of *Myrcia sensu* McVaugh (1968), the sense in which it is accepted today in the majority of local and international herbaria.

De Candolle (1828) divided *Myrcia* into two sections: Sphaerocarpae with round fruits from the Caribbean, the Guianas and Brazil, and Oocarpae with ovate or oblong fruits from Brazil and Colombia. He further subdivided Sphaerocarpeae into those with truly round fruits and consistently two locules in the ovary and those, exclusively Brazilian species, with subglobular fruits and one to three locules per ovary. The division of species according to fruit shape was similar to Berg's division of *Myrcia* from *Aulomyrcia*; *Myrcia* have a tendency towards cylindrical, elongate fruits, whereas *Aulomyrcia* fruits are more rounded.

In *Flora Brasiliensis*, Berg (1857-59) described 240 species of *Aulomyrcia* in 18 unranked infrageneric groups (Table 2.6) and 184 species of *Myrcia* in 11 unranked infrageneric groups (Table 2.7). Apart from using locularity to associate the sections of *Aulomyrcia*, no attempt was made to classify them.

Myrcia was specifically divided into sections for the first time by Grisebach (1864); these were sections *Eumyrcia* and *Aulomyrcia*, and corresponded to Berg's genera *Myrcia* and *Aulomyrcia*. Niedenzu (1893) followed this arrangement, ranking the two groups as subgenera (untergattung) and expanding *Myrcia* to include *Calyptromyrcia* by creating subgenus *Aulomyrcia* section *Calyptromyrcia*. Although no species descriptions or discussion

were provided, many of Niedenzu's groupings corresponded to Berg's infrageneric groups (Table 2.8). Niedenzu (1893) attempted to group some species, dividing subgenus *Myrcia* into sections *Bracteatae* and *Debracteatae* depending on the presence or absence of persistent bracts subtending flowers.

Table 2.6. Divisions of *Aulomyrcia* (Berg 1857-59). (Hypanthium strongly extended above ovary, base of calyx never constricted).

Section	Characters (from Berg, 1857-59)
Ovary bilocular	
Pauciflorae	Peduncle mostly 1-3 flowered, rarely 9-12 flowered, very rarely 20 flowered.
Cymosae	Inflorescence cymose.
Paniculatae	Leaves rigid, opaquely punctate. Panicles pyramidal, axillary and subterminal.
Perforatae	Leaves mostly membranaceous, sometimes chartaceous, often pellucid puncate. Panicles axillary and subterminal.
Lateriflorae	Indumentum more or less grey villous. Young leaves nearly always membranaceous or at least rigid, often opaquely punctate. Panicles arise from young branchlets; smaller branchlets without supporting bracts, larger branchlets often with axillary bracts. Flower buds greyish white, cylidrical or at least clavate.
Grandifoliae	Leaves usually large, mostly chartaceous. Panicles strong and robust, multiflorous, axillary and subterminal.
Cordatae	Leaves cordate or subcordate, ovary bi- or rarely trilocular.
Ovary tri- or tetralocular	
Cordatae II	Leaves cordate or subcordate.
Amethystina	Branchlets strongly thickened, bark thick, with a corky covering. Branchlets, leaves and peduncles more or less covered with violet. Leaves not cordate. Ovary trilocular, with 6 ovules.
Rugosae	Leaves rigid, rugose or raised reticulate. Ovary tri- or tetra- or rarely bilocular.
Paniculatae II	Indumentum not at all rufo-hirsute. Leaves mostly membranaceous or chartaceous, sparsely or obscurely pellucid-punctate. Panicles axillary and subterminal. Ovary trilocular.
Thyrsiflorae	Panicles terminal and solitary. Ovary tri- or tetralocular.
Cymosae II	Mature leaves always coriaceous, mostly obscurely or opaquely punctate. Inflorescence always cymose. Ovary bi-, tri- or tetralocular.
Coriaceae	Leaves thick coriaceous, thick, suborbicular, obovate, ovate or rarely ovate-oblong, opaque-punctate. Panicles axillary and subterminal, rarely aborting terminally. Ovary trilocular, rarely bilocular.
Tomentosae	Branchlets, underside of leaves, panicles and flower buds, tomentose. Leaves often alternate or subverticillate, coriaceous and opaque-punctate. Panicles axillary and subterminal. Bi-, tri- or tetralocular.
Rufipedes	Indumentum of reddish bristles or velutinous. Leaves rigid, chartaceous or coriaceous, occasionally alternate or verticillate, mature leaves often opaque-punctate, not tomentose below. Panicles axillary and subterminal. Ovary trilocular.
Exsuccae	Puberulous indumentum soon disappearing. Leaves linear, ovate-oblong, sparsely glandular, opposite or verticillate, chartaceous or coriaceous, dried looking, veins raised on both surfaces or reticulate, glands impressed or elevated. Inflorescence racemose, thyrsoid, paniculate or corymbose. Ovary bi-, tri- or tetralocular.
PaucifloraeII	1-3 Peduncles, rarely 5-7-9

Table 2.7. Divisions of *Myrcia* (Berg 1857-59). (Hypanthium barely extended above ovary, base of calyx constricted).

Section	Ovary bilocular	Characters
Bracteatae		Indumentum wooly, hairs patent, reddish or brown. Leaves occasionally coriaceous, acuminate. Inflorescence simple racemes or panicles, flowers glomerulous, bracts and bracteoles membranaceous, equal or surpassing the flower buds. Sepals erect in buds, surpassing the globe of the petals.
Costatae		Leaves often rigid, acuminate, midrib generally evident below or clearly pinnately nerved. Panicles axillary and subterminal, multiflowered, bracts and bracteoles caducous. Flower bud sepals globose, petals short obtuse.
Bullatae		Indumentum silky, velutinous or villose, not exactly tomentose. Leaves often chartaceous, mid-vein impressed and often bullate. Peduncles rarely 3-5 flowered, often paniculate and multi-flowered, inflorescence axillary and subterminal, bracts and bracteoles caducous. Sepals obtuse, rarely acute, mostly shorter than the globe of petals.
Acuminatae		Indumentum silky or minutely tomentose. Leaves mostly rigid coriaceous, obtuse–acuminate. Panicles axillary and subterminal, bracts and bracteoles caducous. Sepals obtuse.
Abrupte acuminatae		Indumentum silky or puberulent. Leaves chartaecous or coriaceous, abruptly acuminate. Panicles axillary and subterminal, bracts and bracteoles caducous. Sepals obutse, rarely acute.
Perforatae		Indumentum pubescent or subvillose. Leaves membranaceous, becoming chartaceous, acuminate, pellucid puctate dots large and close together. Panicles axillary and subterminal. Sepals obtuse or acute.
Rostratae		Indumenteum silky or velutinous. Leaves membranaceous becoming chartaceous, rarely coriacous, rostrate–acuminate, minutely pellucid-punctate, venation narrow reticulate. Panicles axillary and subterminal.
Obtusifoliae		Leaf undersurfaces glabrous, leaf apex narrow-obutse, obtuse or rounded, often obovate. Flowers close to the apex of the stem often in axillary and subterminal, congested panicles. Sepals obtuse.
Cupreae		Indumentum copper coloured, silky or velvety, later becoming pale. Leaves stiff coriaceous, opaque-punctate or minutely glandular. Panicles axillary and subterminal; bracteoles mostly caducous. Sepals obtuse or acute.
Tomentosae		Indumentum clearly tomentose (covered in dense, rigid short hairs, perceptible to the touch.)
Cordifoliae		Leaves cordate or subcordate, sometimes subrotund.

Table 2.8. Subgeneric division of *Myrcia* (Niedenzu 1893).

Subgeneric groupings of Niedenzu (1893)				**Correspondence with Berg's classification (1957-59)**
Subgenus *Eumyrcia*	Section 1 *Debracteatae*	'group' A	'subgroup' a	*Myrcia* section *Acuminatae*
				Myrcia section *Abrupte acuminatae*
				Myrcia section *Perforatae*
			'subgroup' b	*Myrcia* section *Rostratae*
			'subgroup' c	*Myrcia* section *Obtusifoliae*
			'subgroup' d	*Myrcia* section *Cordifoliae*
		'group' B	'subgroup' a	*Myrcia* section *Costatae*
				Myrcia section *Bullatae*
			'subgroup' b	*Myrcia* section *Cupreae*
				Myrcia section *Tomentosae*
	Section 2 *Bracteatae*	'groups' A and B		*Myrcia* section *Bracteatae*
Subgenus *Aulomyrcia*	Section 1 *Eu-Aulomyrcia*			*Aulomyrcia*
	Section 2 *Calyptromyrcia*			*Calyptromyrcia*

Kiaerskou (1893) returned to Grisebach's (1864) ranking of *Eumyrcia* and *Aulomyrcia* as sections, providing detailed descriptions of species treated. These diagnoses are arranged in groups by dividing contrasting statements similar to a key. Within *Myrcia* section *Myrcia*, Kiearskou's groups followed Berg's infrageneric classification. In section *Aulomyrcia*, species were presented in 30 unnamed groups. If these groups are interpreted in the same way and considered as Kiaerskou's taxonomic concepts, it becomes clear that Kiaerskou's idea of species groupings in section *Aulomyrcia* bears little similarity to Berg's. This is particularly evident in Kiaerskou's use of vegetative branching pattern as the primary character for grouping species, at no point using ovary locule number. Some groups of species recognised by Kiaerskou correspond (1893) well to those of Berg, but others comprise species assigned by Berg to widely differing groups.

Legrand (1961) documented Myrtaceae in Santa Catarina, ultimately producing genus-by-genus accounts for the state (Legrand and Klein 1967-1977). Legrand (1961) divided *Myrcia* into *Eumyrcia* and *Aulomyrcia*, ranking them as subgenera. Subgenus *Eumyrcia;* (6 species) was not subdivided; subgenus *Aulomyrcia* was divided into two unnamed groups of undescribed sections based on ovary locule number (Table 2.9).

In discussing subgeneric classification, McVaugh (1968, 1969) described in detail the nature of the hypanthium and calyx and mode of opening of the flower. He concluded that such characters may be useful for distinguishing species but restated their unsuitability for naturally grouping species. Despite these conclusions and with reservations, McVaugh (1968) subdivided *Myrcia* (Table 2.10), citing the same practical necessity with which he justified maintaining the 'myrcioid' genera and his belief that real taxonomic entities exist.

McVaugh (1968) replaced subgenus *Eumyrcia* with section *Myrcia,* the name being automatically created at this rank when *Aulomyrcia* was assigned sectional status.

McVaugh's concept of section *Myrcia* is entirely that of Berg's *Myrcia* (1855-56, 1857-59). *Myrcia* section *Aulomyrcia* is essentially that of *Aulomyrcia sensu* Berg excluding a mixed group of species that McVaugh (1968) referred to as *Myrcia* section *Armeriela,* created to house a "small group of species that are evidently closely interrelated and that seem to bridge the gap between *Myrcia* and *Marlierea*" (p379).

Table 2.9. Division of *Myrcia* subgenera *Eumyrcia* and *Aulomyrcia* (Legrand 1961).

Subgenus *Myrcia (as Eumyrcia)*		
	Ovary locule division	**species**
		M. anomala Camb., *M. arborescens* O.Berg, *M. acuminatissima* O.Berg, *M. rostrata* DC., *M. rupicola* Legr., *M .incisa* Legr.
Subgenus *Aulomyrcia*		
Section		**species**
	Ovary bi-locular	
Ramulosae		*M. smithi* Legr. and Kausel, *M. myrtillifoliae* DC., *M. ramulosa* DC., *M. lajeana* Legr., *M. laruotteana* Camb., *M. multiflora* (Spreng.) DC., *M. glaucescens* (O.Berg) Kiaersk.
Crassae		*M. pubipetala* Miq., *M. hatschbachii* Legr., *M. breviramis* (O.Berg) Legr., *M. calumbaensis* Kiaersk., *M. dichrophylla* Legr.
Chartaceae		*M. bombycina* (O.Berg) Kiaersk., *M.tenuivenosa* Kiaersk., *M. racemosa* (O.Berg) Kiaersk.
Microsiphonatae		*M. microsiphonata* Legr.
	Ovary tri- or tetralocular	
Reticulatae		*M. kauseliana* Legr., *M. richardiana* (O.Berg) Kiaersk., *M. castrensis* (O.Berg) Legr.
Obovatae		*M. citrifolia* (Aubl.) Legr., *M. obtecta* (O.Berg) Kiaersk., *M. alternifolia* Miq., *M. glabra* (O.Berg) Legr., *M. bicarinata* (O.Berg) Legr., *M. crassicaulis* Camb.
Ovatae		*M. heringii* Legr., *M. debilis* Camb.

Table 2.10. Subgeneric groupings of *Myrcia* (McVaugh 1968, 1969)

Section	Defining characteristics
Myrcia	Summit of the ovary and inner hypanthium pubescent, scarcely prolonged beyond the ovary, the centre of the flower more or less flat; flowers pentamerous; ovary bilocular; fruit usually oblong-ellipsoid; outer side of the hypanthium appressed-hairy.
Aulomyrcia	Hypanthium usually glabrous within and prolonged above the summit of the ovary; flowers penta- or tetramerous; disc not generally explanate at anthesis, calyx lobes generally persistant; ovary bi- or tri- (or tetra-) locular; fruit globose or subglobose; flowers often glabrous without; dibrachiate hairs often present.
Armeriela	Hypanthium usually glabrous within and prolonged above the summit of the ovary; flowers 4 or 5 merous; tendency for disc to be explanate at anthesis, calyx lobes generally deciduous; ovary bi- or tri- (or tetra-) locular; fruit globose or subglobose; flowers often glabrous without; dibrachiate hairs not usually present.

Marlierea and *Calyptranthes*

This section summarises previous schemes dividing these genera at a subgeneric level. The last account of *Marlierea* was that for the Guayanan highlands (McVaugh 1969); the genus was originally described by Berg (1855-56, 1857-58), who additionally described *Rubachia* and *Eugeniopsis* (Table 2.11). Berg distinguished *Marlierea* by its completely or nearly completely closed bud whereas *Rubachia* and *Eugeniopsis* have short sepals in bud, 3–5 sepals in the case of *Rubachia* that eventually tear to the summit of the ovary, and 4 sepals in *Eugeniopsis* that tear more or less regularly into the hypanthium without reaching the ovary. Kiaerskou (1893) included the last two genera in a larger *Marlierea* comprising three subgenera: *Eumarlierea*, *Rubachia* and *Eugeniopsis* (Table 2.11). Legrand (1962a) recognised only *Marlierea* section *Marlierea* (with two subsections; subsect. *Clausae*; bud closed, tearing into 4-5 lobes, and subsect. *Apertae*; bud open, tearing into (3) 4-5 lobes, with *Rubachia* in synonymy of the latter subsection) and section *Eugeniopsis*, eventually describing a third section, *Pseudocalyptra* (Legrand 1975) to house *Marlierea eugeniopsoides* after its transfer from *Calyptranthes* (Table 2.11). *Calyptranthes* has never been subdivided into formal taxonomic sections although Legrand (1962b) divided the southern Brazilian species into two unequally sized groups based on vegetative branch ramification (monopodial, 4 species, vs sympodial, 24 species); the sympodial group, was further subdivided based on characters of the peduncles and inflorescences, with occasional reference to bract shape and leaf size (Table 2.12).

Table 2.11. Three classifications of *Marlierea* (McVaugh 1969)

Species recognised Berg (1857-59)	**Additional species recognised Kiaerskou (1893)**	**Additional species recognised Legrand (1962a)**
Marlierea spathulata O.Berg, *M. tomentosa* Camb., *M. strigipes* O.Berg, *M. suaveolens* Camb. *M. capitata* O.Berg, *M. parvifolia* O.Berg, *M. sessiliflora* O.Berg, *M. spruceana* O.Berg, *M. estrellensis* O. Berg, *M. umbraticola* (Kunth.) O.Berg, *M. excoriata* Mart. *M. obscura* O.Berg, *M. schottiana* O.Berg, *M. grandifolia* O.Berg, *M. glabra* Camb. *M. resupinata* (Vell.) O.Berg, *M. rufa* (Vell.) O.Berg	<u>*Marlierea* subgenus *Eumarlierea*</u> *Marlierea regeliana* O.Berg, *M. warmingiana* Kiaersk., *M. langsdorffii* O.Berg	<u>*Marlierea* section *Marlierea* subsect. *Clausae*</u> *Marlierea verticillaris* O.Berg, *M. dimorpha* O.Berg, *M. uaupensis* O.Berg, *M. regeliana* O.Berg, **Extra-Brazilian:** *M. glomerata* O.Berg, *M. multiglomerata* Amsh., *M. macrophylla* Amsh., *M. subulata* McVaugh, *M. biptera* Amsh., *M. imperfecta* McVaugh, *M. caudata* McVaugh, *M.schomburgkiana* O.Berg, *M. montana* (Aubl.) Amsh., *M. salticola* Amsh.
Rubachia obumbrans O.Berg, *R. involucrata* O.Berg, *R. antonia* O.Berg, *R. neuwiedeana* O.Berg, *R. spiciflora* (Nees & Mart.) O.Berg, *R. lateriflora* (DC.) O.Berg, *R. glomerata* O.Berg	<u>*Marlierea* subgenus *Rubachia*</u> *M. antrocola* Kiaersk., *M. brachymischa* Kiaersk., *M. choriophylla* Kiaersk., *M. glaziouviana* Kiaersk.	<u>*Marlierea* section *Marlierea* subsect. *Apertae*</u> *Marlierea macedoi* Legrand, *M. edulis* Nied., *M. occhionii* Legrand, *M. obversa* Legrand, *M. scytophylla* Diels, *M. umbraticola* (Kunth) O. Berg, *M. bipennis* (O. Berg) McVaugh, *M. velutina* McVaugh, *M. antonia* (O. Berg) Legrand **Extra-Brazilian:** *M. areolata* McVaugh, *M. squarrosa* McVaugh, *M. cuprea* Amsh.
Eugeniopsis acuminatissima O.Berg, *E. affinis* O.Berg, *E. angustifolia* O.Berg, *E. cannifolia* O.Berg, *E. clausseniana* O.Berg, *E. gardneriana* O.Berg, *E. gaudichaudiana,* O.Berg, *E. grandifolia* O.Berg, *E. laevigata* O.Berg, *E. luschnathiana* O.Berg, *E. ovata* O.Berg, *E. paniculata* O.Berg, *E. polygama* O.Berg, *E. richardiana* O.Berg, *E. riedeliana* O.Berg, *E. rubiginosa* O.Berg, *E. schottiana* O.Berg, *E. sylvatica* O.Berg, *E. teuscheriana* O.Berg	<u>*Marlierea* subgenus *Eugeniopsis*</u> *M. subacuminata* Kiaersk., *M. racemosa* (Vell.) Kiaersk., *M. silvatica* (Gardn.) Kiaersk., *M. laevigata* (DC.) Kiaersk.	<u>*Marlierea* section *Eugeniopsis*</u> *Marlierea bergiana* Legrand, *M. reitzii* Legrand, *M. eugeniodes* (Camb.) Legrand, *M. schottii* Legrand **Extra-Brazilian:** *M.guyanensis* Legrand

Table 2.12. An informal classification of southern Brazilian *Calyptranthes* (Legrand 1962b)

Primary division	Secondary division	Species
Monopodial ramification		*C. angustifolia; C. caudata; C. grammica; C. tetraptera*
Sympodial ramification	Peduncles (inflorescence bearing appendages) 1-3 flowered	*C. tricona*
	Peduncles winged; rachis short to absent	*C. pteropoda*
	Bracteate inflorescence with subfiliform rachis	*C. langsdorffii*
	Inflorescence with rachis not strictly opposite, axes of the same arrangement or more flexible.	*C. obovata; C. pulchella; C. hatschbachii; C. lucida; C. polyantha*
	Inflorescence with several pairs of strictly opposite rachis, often further subdivided (inflorescence paniculate).	*C. loranthifolia; C. glazioviana; C. widgreniana; C. grandifolia; C. rufa; C. clusiaefolia*
	Inflorescence typically with one pair of strictly opposite rachis, rarely accompanied by another shorter pair, rarely more (rachis trifurcate, more rarely paniculate). Ultimate flowers sessile, generally in apical groups, rarely these rachis abort.	*C. concinna; C. klienii; C. reitziana; C. variabilis; C. strigipes; C. eriopoda*
	Inflorescence with a sole pair or less frequently two pairs of strictly opposite, terminal rachis with a sole flower or with a pedicelate or subpedicelate dichasium	*C. eugeniopsoides; C. pileata*
	Large panicle with wide, spicate, opposite rachis. Large leaves.	*C. aromatica*
	Inflorescence with opposite rachis, with large basal bracts.	*C. lanceolata*

Chapter 3 – Tribe Myrteae

Introduction

Myrteae DC. (sensu Wilson et al. 2005; Myrtaceae) are trees or occasionally shrubs with a Pantropical distribution. They comprise 49 genera and c. 2500 species (Govaets et al. 2006), including the large genera *Eugenia* and *Myrcia* (estimated at 1023 and 351 species respectively; Govaerts et al. 2006). The tribe is distinguished from the 14 other tribes in subfamily Myrtoideae (Wilson, et al. 2005) by their indehiscent fleshy fruits, transeptal vascular system, and, commonly, presence of uni- or multi-cellular hairs (Schmid 1972; Wilson et al. 2001). Previous classifications of Myrtaceae (De Candolle 1828; Berg 1855-56, 1857-59) used subfamily Myrtoideae and tribe Myrteae interchangeably; however, following reclassification by Wilson et al. (2005), the two ranks have different circumscriptions and are no longer considered synonymous. The most enduring subfamilial and subtribal classifications of Myrtaceae and Myrteae are reviewed in Lucas et al. (2005), with additional interpretations discussed in chapter 2 (Tables 2 and 3). The highest species diversity in Myrteae occurs in South America, particularly along the eastern coast of Brazil, the Guayana Highlands, and the Caribbean (McVaugh, 1968); genera occurring in the Paleotropics are listed in Table 2.3.

Myrteae

The most commonly recognized previous classifications of Myrteae are the three, embryology-based subtribes of Berg (1855-56, 1857-59) and the six 'informal groupings' of McVaugh (1968; Chapter 2, Tables 3 and 4; Fig. 1.1), a detailed taxonomic review is given in Chapter 2.

This chapter has three main goals: (1) to clarify sister relationships between the (included) genera of Myrteae as currently defined and to determine their monophyly; (2) to assess in light of these results morphological characters as potential synapomorphies with which groups of genera may be delimited; and (3) to evaluate previously proposed supra-generic classifications of Myrteae.

Methodology

Sampling

Eighty eight Myrtaceae accessions were sequenced for the ETS, ITS, and *psbA-trnH* studies (Appendix 1). This sampling represents 31 Myrteae genera, with species selected to encompass as much morphological diversity and geographical variation as possible; ten outgroup genera (13 species) were selected from seven other tribes of Myrtoideae. To improve bootstrap support, the phylogenetic hypothesis produced by the three-region analysis was used to select a subset of 46 taxa, comprising representatives from each clade, for which a portion of *matK* was also sequenced (Appendix 1). This section of *matK* is approximately 750 base pairs (bp) long, extending from bp 1075 to 1822 relative to the tobacco *trnK* map (Sugita et al., 1985).

DNA extraction, sequencing and alignment

Total genomic DNA was extracted from c. 0.3 g of fresh, silica gel- or air-dried leaf material using the CTAB extraction protocol of Doyle & Doyle (1987) but purified by equilibrium centrifugation in CsCl-ethidium bromide gradients (1.55 g ml^{-1}) after precipitation in -18°C 100% ethanol. Ethidium bromide was removed with butanol extraction, followed by dialysis to remove the caesium chloride. Target regions were amplified in an Applied Biosystems Inc. (ABI) Gene Amp 9700 PCD system; amplification conditions and primers used are listed in Table 3.1. A new forward primer was designed for ETS and designated ETS MyrtF (5'-CTCCGTGCTGGTGCATCGAACTGC-3'). PCR products were purified using QIAGEN® QIAquick™ Spin Columns, according to the manufacturer's protocol. Sequencing reactions were carried out with the BigDye Terminator v3.1 Cycle Sequencing Kit™ (Applied Biosystems, Inc). Sequences were read on an ABI 3100 Genetic Analyzer. Electropherograms were assembled and edited using Sequencher™ version 4.5 (Gene Codes Corporation). Sequences were aligned by eye following the guidelines of Kelchner (2000).

Table 3.1. PCR primers, reagents and conditions.

	ITS	**ETS**	***psbA-trnH***	***matK***
Primers	17SE (F); 26SE (R) Sun et al. (1994)	Myrt (F) see text; 18S (R) Wright et al. (2001)	psbA (F); trnH (R) Hamilton (1999)	390 (F); 1326 (R) Johnson & Soltis (1994)
PCR conditions	2 min at 94° C followed by 30 cycles of 1 min at 94° C, 1 min at 52° C, 1 min at 72° C	4 min at 94° C followed by 30 cycles of 1 min at 94° C, 1 min at 50° C, 1 min at 72° C	4 min at 94° C followed by 30 cycles of 1 min at 94° C, 1 min at 48° C, 2 min 30 seconds at 72° C	2 min at 94° C followed by 30 cycles of 1 min at 94° C, 30 seconds at 50° C, 1 min at 72° C

Phylogenetic analyses

All character transformations are treated as equally likely and unordered (Fitch, 1971), and parsimony analysis was carried out using PAUP* 4.0b10 (Swofford, 2002). Thirteen potentially informative, non-overlapping indels (Table 3.2) were scored as separate, binary characters at the end of the data matrix, according to the simple method of Simmonds & Ochoterena (2000), with absence of a gap coded as 0 and presence as 1. Overlapping indels were not included as they occurred only in sequence regions where it was impossible to obtain an unambiguous alignment of all taxa; all such regions were excluded from the phylogenetic analyses. Alignments are provided in Appendix 2.

Table 3.2. Informative indels scored.

Indel	**Positions affected**
1	ITS 327
2	ITS 340–342
3	*psbA-trnH* 215–220
4	*psbA-trnH* 359–369
5	*psbA-trnH* 783–829
6	*psbA-trnH* 842–857
7	*psbA-trnH* 858–883
8	*psbA-trnH* 892–902
9	*psbA-trnH* 936–958
10	*psbA-trnH* 977–988
11	ETS 58–59
12	ETS 495–505
13	*matK* 267–275

Heuristic searches consisted of 1000 replicates using random taxon entry order, tree bisection reconnection (TBR) and branch-swapping with MulTrees (saving multiple trees) on but only saving 10 trees per replicate to reduce time searching suboptimal islands of trees (Maddison, 1991). After completing the replicates, the resulting trees were used as starting trees for a further search using TBR and branch-swapping with MulTrees on, this time saving no more than 10,000 trees. Successive approximations weighting was used (SW; Farris, 1969); characters were reweighted according to the rescaled consistency indices of the best trees, and after each round of reweighting a heuristic search of ten random replicates was performed. Optimal trees were again collected and used as starting trees to collect all shortest trees; once tree length remained the same in two successive rounds, these were the successive approximation weighted (SW) trees. The bootstrap (Felsenstein, 1985) was used to assess support for individual clades without weights from the re-weighting scheme. The heuristic search settings were: 1000 bootstrap replicates with taxa added by simple stepwise addition, TBR swapping with MulTrees on and saving no more than 10 trees per replicate. Bootstrap support percentages (BP) of 50–74 are considered weakly supported, 75–84 moderately supported and 85–100 strongly supported (Chase et al., 2000). Plastid regions were analyzed together as both exhibit low levels of sequence divergence and incongruence was not expected from the uniparentally inherited non-recombining plastid genome. The combined plastid regions, ITS, and ETS data sets were initially analysed separately before the three datasets were analyzed together. Congruence among pair-wise combinations of the three data sets and for all data sets combined was evaluated by visual inspection of resulting trees, with incongruence recognized by the presence of inconsistent groupings of taxa with moderate to high bootstrap support (>75 BP) in the component matrices when analyzed alone.

Bayesian analysis was performed on the combined data set in MrBayes v3.0b4 (Huelsenbeck & Ronquist 2001; Ronquist et al. 2005). Models of nucleotide substitution were selected for the sequence partitions using Modeltest 3.06 (Posada & Crandall, 1998). The following models were recommended under the Akaike information criterion, for ITS, ETS, *psbA-trnH* and *matK,* respectively; GTR+I+G, TVM+G, TVN+G, TVM+G. All models require six substitution types

(NST=6) and a gamma model of among-site rate heterogeneity (rates=gamma). Data sets were analyzed in combination with these model parameters fit independently to the separate data partitions. Four such analyses were conducted with 500,000 generations of Monte Carlo Markov chains and a sampling frequency of 10 to ensure the analysis did not stall on suboptimal tree islands. Trees generated were plotted against likelihoods in Microsoft Excel to determine the post 'burn in' set of trees; this set of 7,500 trees was deleted. The remaining trees were imported into PAUP to produced majority rule consensus tree displaying the posterior probabilities (PP) of all nodes.

Biffin et al. (2006) use Myrtaceae RNA secondary structure predictions to assess the use of structural partitioning in ITS. Their study concluded that bias in the mutational dynamics of stems and loops and non-independence amongst nucleotides in stem-pairing sites is such that partitioning significantly improves the likelihood of more weakly supported (i.e. PP≤95%) relationships in the resulting topology whereas well-supported clades [nodes] were generally found to be consistent with those inferred from other DNA regions regardless of the evolutionary model or partitioning used. The study presented here concurs: phylogenetic signal in Myrtaceae ITS is insufficient to resolve relationships among short internal branches, regardless if partitioned or not, and conclusions in this study are therefore only considered reliable for those well-supported relationships (i.e. PP ≥ 95). For these reasons and also due to the relatively small effect that similar weighting schemes of loops versus stems for 18S rDNA data had on analysis of that region (Soltis & Soltis, 1998), the partitioning of the ITS data matrix recommended by Biffin et al. (2006) was not implemented here.

Morphological optimization

Morphological data in Myrtaceae are notoriously homoplasious, possibly due to rapid and recent speciation in the family (Landrum 1981a; Wilson et al. 2001). Particular attention was therefore paid to Stevens' (2000) recommendation that before embarking on a morphological phylogenetic exercise all aspects of methodology, character selection, distribution of characters and variation

patterns within the group should be carefully considered. Cladistic analysis of morphological characters was inappropriate at this scale due to low numbers of putative homologous characters available and the limitations of using place-holders for genera. The approach applied was to select key characters for optimization onto the molecular tree without including them in the tree-building process. After consideration and rapid rejection of a wide range of characters, these were narrowed down to twelve characters for serious consideration, from the gynoecium, androecium, embryo, perianth, wood anatomy, seeds and inflorescence. The majority proved impossible to score sensibly throughout the tribe, invariant, or inapplicable to all taxa and ultimately only four suitable characters were selected. Character coding was developed here based on ranges of states and characters taken from the generic descriptions of Bentham (1869); Grifo (1992); Holst (2003); Landrum (1981b, 1986, 1988); Landrum & Kawasaki (1997); McVaugh (1963a, 1968, 1969); Proença (1990); Snow et al. (2003); Landrum & Salywon (2004), and results of a survey of the wood anatomy of Myrteae by Telford et al. (unpubl.). If additional information was required, characters were scored directly from herbarium material. Optimization of morphological characters was performed with MacClade 4.0 (Maddison & Maddison 2000). The four potentially useful characters were optimized onto a single most parsimonious tree (MPT) resulting from the successively weighted parsimony analyses. Outgroup genera were pruned from the MPT, and monophyletic clades of individual genera reduced to single terminals. Results presented in this study and in Lucas et al. (2005) suggest that the four core genera of subtribe Myrciinae form a poorly resolved monophyletic group with low internal support in which *Myrcia* and *Marlierea* are paraphyletic with *Gomidesia* and *Calyptranthes* nested within them; in light of this, *Myrcia* is here used in a broad sense, including these other genera.

Characters

1. *Embryo – (0) hypocotyl wrapped around well-developed, crumpled cotyledons (Fig. 3.1A); (1) hypocotyl much reduced, cotyledons swollen, homogenous or fused (Fig. 3.1B); (2) hypocotyl swollen, usually wider than the cotyledons, sometimes spiralled (Fig. 3.1C, G, H); (3) cotyledons inconspicuous, hypocotyl linear or C-shaped (Fig. 3.1D); (4) short hypocotyl enclosed by entire,*

plano-convex cotyledons (Fig. 3.1E); (5) short hypocotyl enclosed by lobed and thickened cotyledons (Fig. 3.1F). The embryo is the most frequently used feature for grouping genera in previous Myrteae taxonomy (see Lucas et al., 2005, Chapter 2 Fig. 1.1 for historical use of embryo types). There has recently been a move away from such heavy dependence on embryo type (e.g. McVaugh, 1968), with Snow et al. (2003) rejecting the three traditional embryo types on the basis that they constitute several independent characters, favouring partitioning the three conditions into several characters more likely to be homologous. Snow & Guymer (1999) successfully developed such a system for *Myrtella* and *Lithomyrtus* but suggested it might 'be of limited use in the Myrtaceae as a whole' (p. 206). The assertion of Snow et al. (2003) is accepted, that intrageneric variation in Myrteae embryos is such that ultimately the complete abandonment of traditional embryonic archetypes may be necessary. However, the intention here is to observe the fit of the original tripartite system, modified according to subsequent authors, to the DNA tree to provide a starting point for future subdivision of embryo characters based on phylogenetic relationships. To that aim, in addition to those states (0, 1 and 3) that correlate with the original three embryo types, states 2, 4 and 5 correspond to those of the *Campomanesia* complex sensu Landrum and Stevenson (1986), *Luma* and *Algrizea,* respectively (see Proença et al. (2006) for discussion of the *Luma* and *Algrizea* embryos). If embryo type is inconsistent within a genus, the condition of the outgroup is scored; for example, *Gossia* can have typical 'myrtoid' embryos (character state 3) or spiralled embryos (character state 2). *Austromyrtus*, the sister group to *Gossia*, and all other members of their clade have the 'myrtoid' condition leading us to assume that character state 3 is plesiomorphic in *Gossia* and to assign it as such.

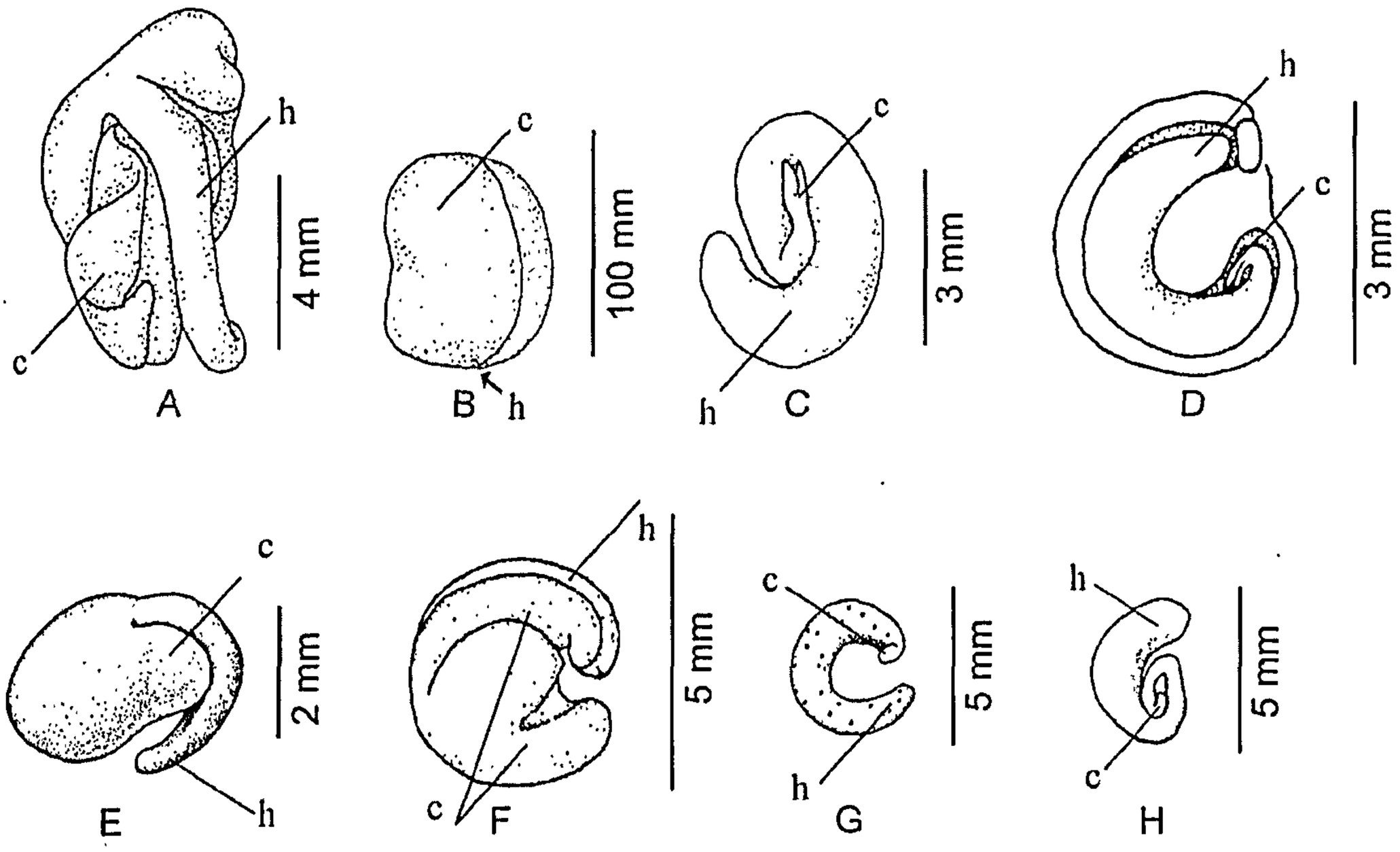


Figure 3.1. Embryo variation in tribe Myrteae. A. Myrcioid embryo *sensu* Berg (*Myrcia*); B. Eugenioid embryo sensu Berg (*Myrcianthes*); C. Embryo of the *Campomanesia* complex *sensu* Landrum (*Campomanesia*); D. Myrtoid embryo *sensu* Berg (*Psidium*); E. *Luma*; F. *Algrizea*; G. *Blepharocalyx cruckshanksii*; H. *B. salicifolius*. Labels: c, cotyledon; h, hypocotyl. Reprinted with permission; A–D from Landrum & Kawasaki (1997); E. G. H. from Landrum (1986); F from Proença et al. (2006).

2. *Mean number of ovules per ovary – (0) <70; (1) >120* (Fig. 2.2). Numbers of locules and ovules are characters that have been, and still are, used independently in distinguishing between genera of Myrteae, with trends in both characters associated with specific groups. Berg (1855-56, 1857-59) found the ovaries of Myrciinae and Eugeniinae to be constant, placing little emphasis on them while providing a detailed discussion on ovary variability in Myrtinae. Bentham (1869, p. 119) believed 'modifications of the ovules and their placentation afford the best generic characters'. Kausel (1956) suggested that taxonomic decisions might be made based on a combination of ovule number, position of the placenta, and structure of the latter. McVaugh (1968) concluded that number of ovules per locule is probably taxonomically significant although to an unknown extent. This study found that infrageneric variation in locule and ovule number is such that neither character can be divided into discrete states. Seeking a new approach that would

allow these characters to be optimised on a molecular tree, it is here hypothesized that the developmental trend in Myrteae has been a decrease in number of ovules per ovary and that numbers of either locules or ovules are of secondary taxonomic importance. This hypothesis is suggested with some reservation as it is recognised that evolutionary pressures are more likely to have individually influenced the component characters, however, in agreement with previous authors (Bentham 1869; Kausel 1956; McVaugh 1968) and partially influenced by Kausel (1956), it is here believed that there is systematic information to be teased from ovary and ovule characters and that observation of these characters in combination may uncover previously unnoticed patterns for use in the clarification of the Myrteae phylogeny.

3. Minimum and maximum locule and ovule per locule numbers were recorded for each genus, and mean number of ovules per ovary calculated. Genera were ranked according to this value (Fig. 3.2; minimum and maximum values indicate range), and the character was coded according to the single break observed in the data (indicated by a dashed line).

Figure 3.2. Ranked means of ovules per ovary, including mean maximum and minimum values. Dashed line represents delimitation of character states

4. *Placentation – (0) ovules mostly arising at a single point on septum (Fig. 3.3A, B); (1) ovules in one or more series along a non-peltate placenta (Fig. 3.3D, E); (2) ovules in one or more series around a protruding placenta (Fig. 3.3C).* Berg (1855-56, 1857-59) discussed placental diversity when defining *Myrtus* and *Psidium* whereas Kausel (1947, 1956) emphasized position and shape of the placenta in his definition of Argentinian genera. McVaugh (1968; p.360) doubted that 'valid conclusions as to generic limits can yet be drawn on the basis of [placentation]'. Unilocular ovaries with parietal placentation (Fig. 2.3F) are found in *Rhodamnia* and *Neomyrtus*; incomplete septal division is reported in *Myrteola* (Landrum, 1988) and *Myrtus* (Bentham, 1869). McVaugh (1963a) considered relationships within a group of *Psidium* now accepted as *Chamguava* (Landrum, 1991), one of which posseses a unilocular ovary. He concluded that 'generic distinction based on [ovary characters] among species that are otherwise so strikingly alike, would seem to be specious'. A similar view is taken here; it is assumed that the axile condition is not significantly different from the parietal one and that it is a homoplasious condition, with the incomplete septum and unilocular conditions arising from incomplete or 'non-development of the placenta-bearing dissepiments' (Bentham 1869, p. 119); those genera with incomplete septal development demonstrate a common but unrelated process. Within the unilocular ovary of *Rhodamnia* and *Neomyrtus,* placentation is in a single series on a non-peltate placenta; therefore, character state 2 has been assigned. Placentation in *Psidium* varies with a non-peltate condition (character state 1) somewhat less common than the peltate (character state 2). Despite the sister group to *Psidium* having non-peltate placentation, the majority of further outgroup genera have peltate placentas leading us to assume that character state 2 is plesiomorphic in *Psidium* and to describe it as such.

5. *Vessel elements – (0) simple perforation plates (Fig. 3.4C, K); (1) scalariform perforation plates (Fig. 3.4G, J, L); (2) mixed simple, reticulate or scalariform perforation plates (Fig. 3.4A, B, D--F, H, I).* Wood anatomical surveys of Myrtaceae by Ingle & Dadswell (1953), Meylan & Butterfield (1975, 1978), Ragonese (1976), Schmid and Baas (1984), and Patel (1995) have

reported helical thickenings of vessel elements, scalariform plates and tracheids in the wood of Myrteae. Additional wood anatomy data (Telford et al., unpubl.) suggest that, of these, the most and possibly only taxonomically useful character of the wood for distinguishing Myrteae genera is occurrence of scalariform perforation plates.

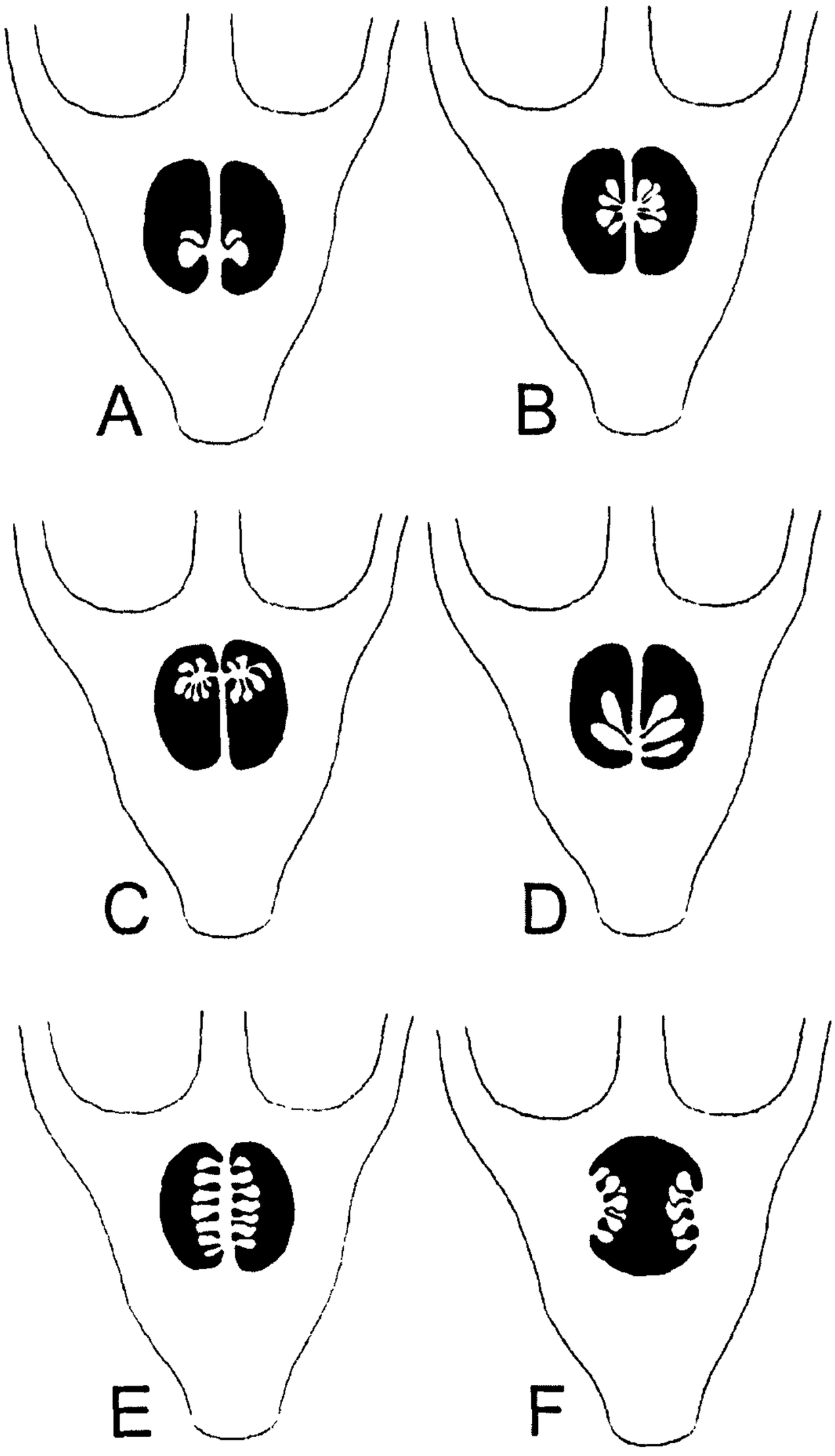


Figure 3.3. Placental variation in tribe Myrteae. Predominant placentation in A. *Myrcia*, *Plinia*, *Myrciaria*; B. *Eugenia*; C. *Amomyrtus*, *Legrandia*, *Lophomyrtus*, *Pimenta*, *Myrcianthes*, *Myrteola*, *Ugni*; D. *Siphoneugena*, *Neomitranthes*; E. *Acca*, *Campomanesia*, *Decaspermum*, *Octamyrtus*, *Psidium*; and F. *Rhodamnia*, *Neomyrtus*.

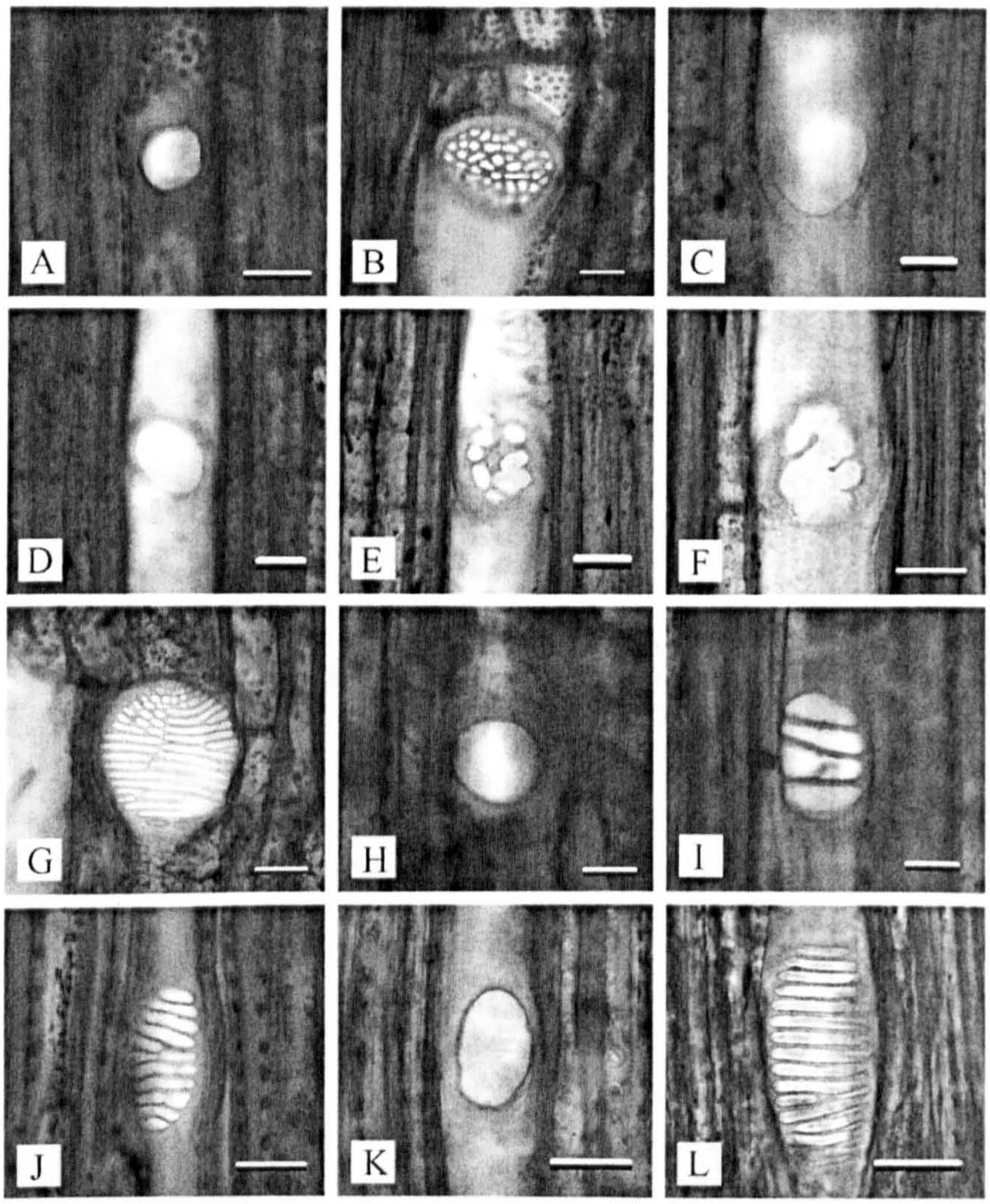


Figure 3.4. Vessel element perforation plates in tribe Myrteae, from radial sections. A. B. *Blepharocalyx cruckshanksii*, simple in A, reticulate in B (RBGE 1996-1223); C. *B. salicifolius*, simple (Lucas 78); D. E. F. *Lophomyrtus obcordata*, simple in D, reticulate in E & F (Hort., Kew '51); G. *Luma apiculata*, scalariform, becoming reticulate towards top (RBGK 1958-50308); H. I. *Myrceugenia alpigena*, simple in H, scalariform with only 3 bars in I (de Lima 2560); J. *Myrteola nummularia*, scalariform, with one bifurcating bar (RBGE 1996-1096); K. *Myrtus communis*, simple (RBGK 2000-3513); L. *Ugni molinae*, scalariform, with one bifurcating bar (RBGE 1992-3445). Scale bars; A. B. D--G. J--L. = 20µm; C. H. I. = 10µm.

Results

Sequence data

Statistics from the parsimony analyses are summarized in Table 3.3; the plastid analysis comprises 503 and 806 aligned bp from *psbA-trnH* and *matK*, respectively. The results from the SW analyses of individual data partitions (not shown) demonstrate low bootstrap support along the spine of the trees and low resolution in the in-group. Five genera (*Amomyrtus, Gossia, Luma, Siphoneugena* and *Rhodamnia*) receive >50% bootstrap support (BP) in all the three topologies. At the suprageneric level, three further clades consistently receive >50 BP; these are a clade containing all Myrteae genera, *Siphoneugena+Neomitranthes* and *Neomyrtus+Lophomyrtus*. The ITS and ETS topologies each receive >50 BP for *Gomidesia, Myrceugenia* and *Myrcianthes,* whereas *Eugenia* receives >50 BP in the plastid and ETS topologies. *Calyptranthes, Campomanesia, Myrceugenia* and *Myrcianthes* receive >50 BP in the ETS topology alone as do *Pimenta* and *Psidium* in the plastid topology. A clade comprising the core Myrciinae genera plus *Neomitranthes, Siphoneugena, Plinia, Myrciaria*, and *Algrizea* also receives >50 BP in the plastid analysis as do clades comprising *Eugenia+Myrcianthes* and *Austromyrtus+Gossia* in the ETS analysis. The only incongruity between groupings with >50 BP in the three topologies (i.e., hard incongruities) were present between *Decaspermum, Octamyrtus, Rhodamnia* and *Austromyrtus* which emerged with > 50 BP at each of the following nodes: ETS ((*Decaspermum, Rhodomyrtus*) *Octamyrtus*), unrelated to *Austromyrtus*; ITS ((*Rhodomyrtus, Octamyrtus*) *Decaspermum*), unrelated to *Austromyrtus*; plastid ((*Octamyrtus, Rhodamnia, Austromyrtus) Decaspermum).

	ITS	ETS	plastid	combined
Characters	686	448	1309	2443
No. variable characters (%)	305 (44.)	292 (65.2)	403 (30.8)	1000 (41)
No. potentially parsimony informative characters (%)	195 (28.4)	215 (48)	190 (14.5)	600 (24.6)
Trees	>10000	>10000	>10000	360
Trees (SW)	>10000	1610	>10000	>10000
Length (L)	1117	1128	674	3013
L (SW) (Fitch length of the SW result)	265 (1125)	310 (1134)	396 (675)	467.8 (1286)
Consistency Index (CI)	0.40	0.43	0.71	0.46
CI (SW)	0.40	0.42	0.70	0.50
Retention Index (RI)	0.52	0.59	0.77	0.58
RI (SW)	0.52	0.59	0.77	0.60

Table 3.3. Data set and parsimony tree characteristics for ITS, ETS, plastid and combined analyses plus indels, before and after successive weighting (SW).

Phylogenetic analysis

The 'soft incongruence' of the partitioned analyses is attributed to lack of phylogenetic signal (sampling error due to too few polymorphic sites), rather than evidence of hybridization or horizontal transfer, and these data are considered suitable for combined analysis (Sheahan & Chase, 2000). A single MPT with branch lengths (DELTRAN optimization; Fig. 3.5) from the combined analysis demonstrates that the length of the branch separating *Myrtus communis* from the rest of Myrteae is one of the longest in the analysis, whereas other branch lengths within Myrteae are relatively short. The strict consensus tree from the combined analysis is shown in Fig. 3.6 and comprises seven clades, including all but three isolated taxa; informal subtribal groupings are provided in inverted commas (e.g. the '*Myrcia* group'). The combined analysis provides better resolution and higher bootstrap support for clades than any of the three separate analyses with every group receiving >50% BP in any of the individual analysis receiving increased support in the combined analysis. Despite this increase in support, the combined topology still provides little bootstrap support along the spine of the tree, with the exception of the well-supported (BP 100) tribe Myrteae (Clade H, Fig. 3.6) and the sister relationship of

Myrtus communis with the rest of the tribe (BP 61). Other suprageneric relationships receiving bootstrap support in the combined topology are: the '*Plinia* group' (BP 95, Clade A, Fig. 3.6) comprising *Neomitranthes*, *Siphoneugena*, *Plinia* and *Myrciaria*, the '*Myrcia* group' (BP 64, Clade B, Fig. 7) comprising the four core genera of the Myrciinae, *Calyptranthes, Gomidesia, Marlierea*, and *Myrcia*; the relationship (BP 55) between *Blepharocalyx cruckshanksii* and *Luma;* the relationship of *Gossia* and *Austromyrtus* (BP 89); and an association (BP 66) of *Gossia+Austromyrtus* with the remaining genera of the Australasian clade (Clade G, Fig. 3.6).

A 50% majority rule consensus of the trees produced by the Bayesian analysis was almost entirely congruent with the SW tree with an apparent increase in support. Nodes supported by >95 posterior probability (PP) are indicated by bold branches in Fig. 3.6, whereas the four nodes incongruent with the SW analysis are indicated by arrows. The four incongruent arrangements in the Bayesian topology are: 1) *Myrtus communis* sister to the Australasian clade (PP 71); 2) *Rhodomyrtus* and *Decaspermum* with a sister relationship (PP 96); 3) the '*Eugenia* group' emerging as sister to the '*Myrteola* group' (PP 39); and 4) *E. punicifolia* as sister to the *E. stictosepala–E. latifolia* clade (PP 91).

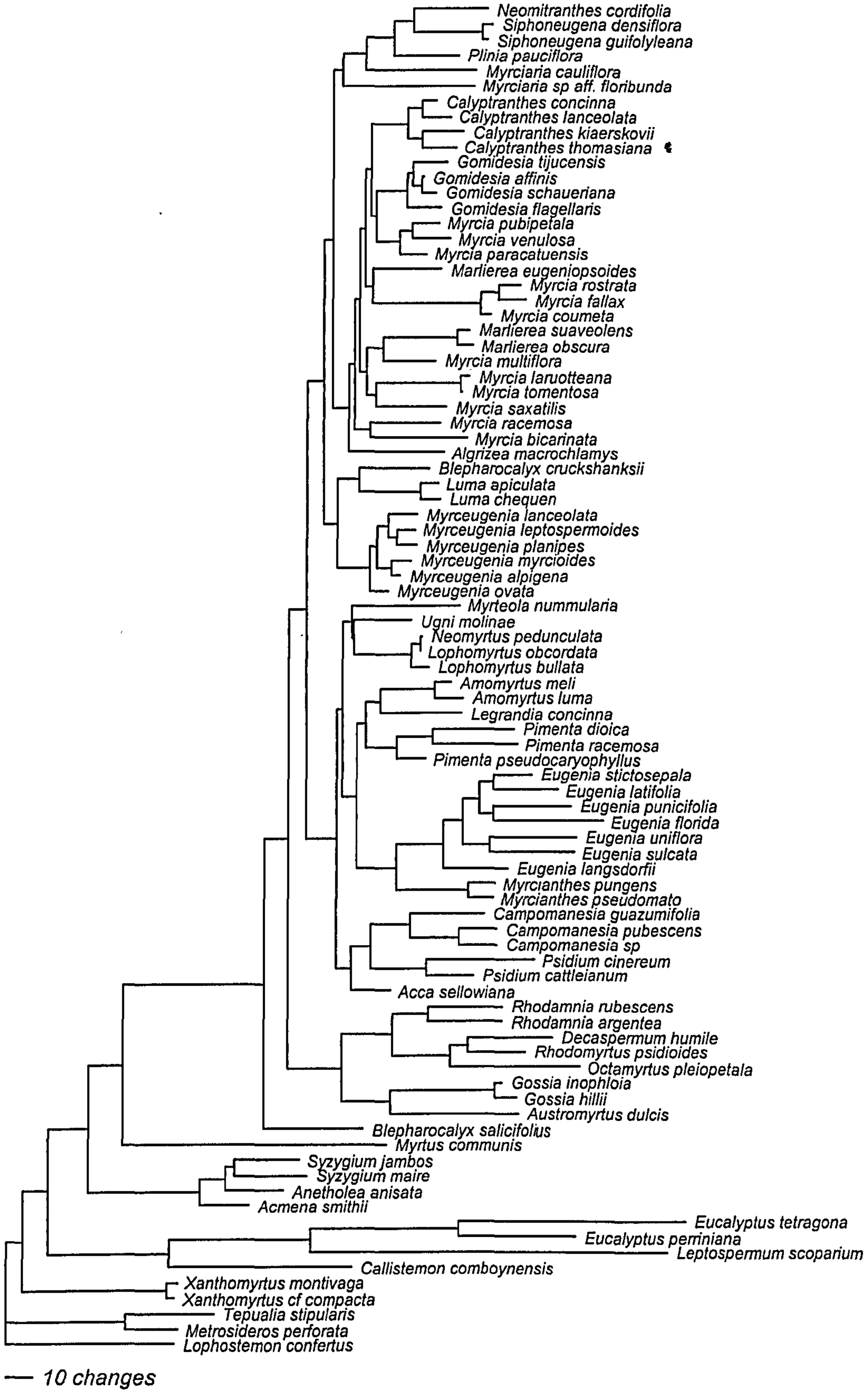


Figure 3.5. A single tree of 1427 minimum length trees generated by the heuristic search of combined ITS, ETS, *psbA-trnH*, and *matK* data.

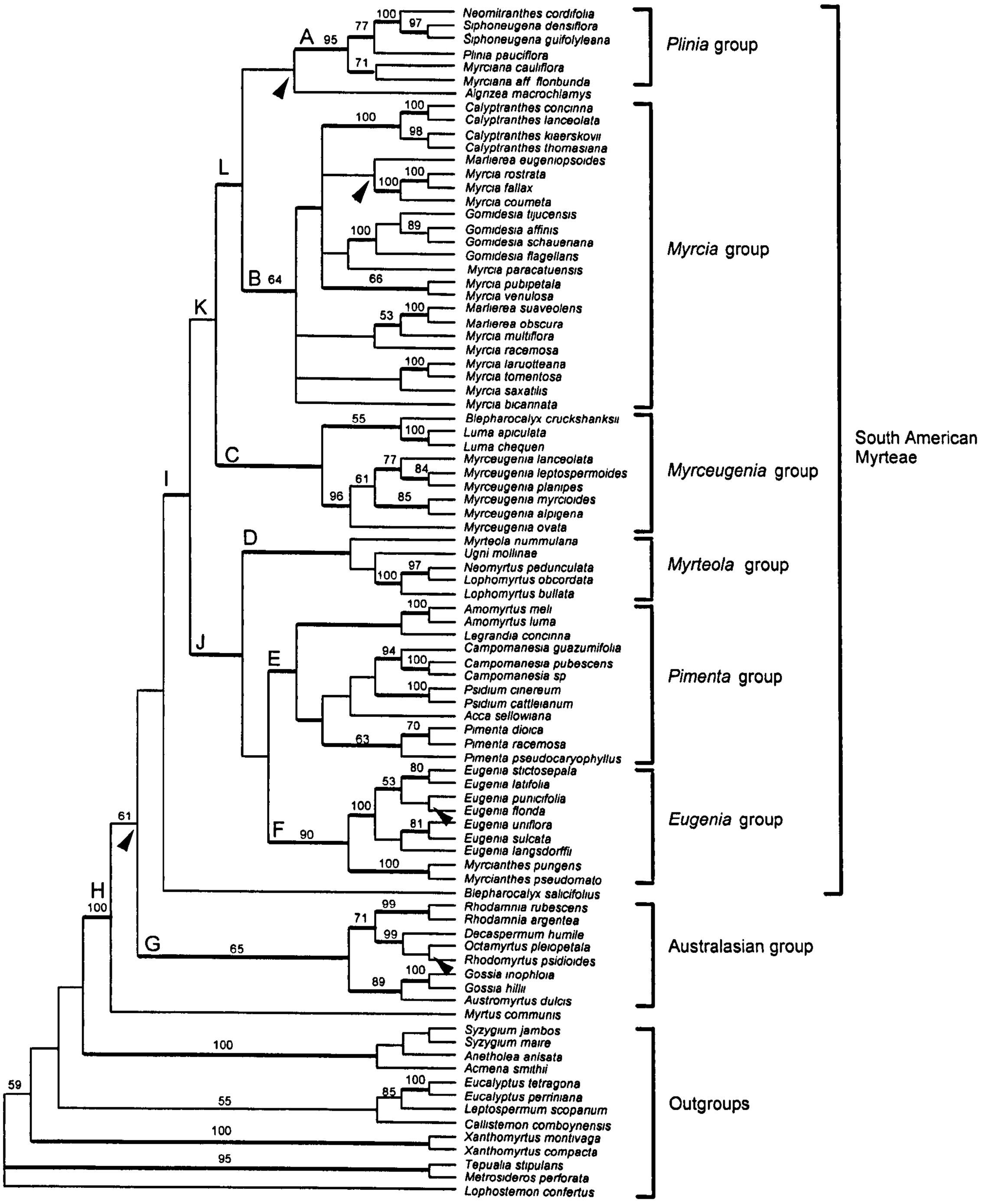


Figure 3.6. Strict consensus tree resulting from parsimony analysis with successive weighting and Bayesian analysis of the ITS, ETS, *psbA-trnH* and *matK* data. Bootstrap percentages greater than 50 are shown above branches; clades that receive Bayesian probabilities greater than 0.95 are marked in bold; clades not recovered in the Bayesian analysis are marked with solid arrowheads.

Morphological optimization

Of the morphological characters under study (Fig. 3.7), the nature of the perforation plates of the vessel elements and the mean number of ovules per ovary provide unambiguous support for clades. Characters based on embryo and placentation correlate less well, each displaying some level of homoplasy when optimized over the entire Myrteae tree. Despite this, combinations of characters may often be used to diagnose clades, albeit with exceptional taxa in several cases (see discussion).

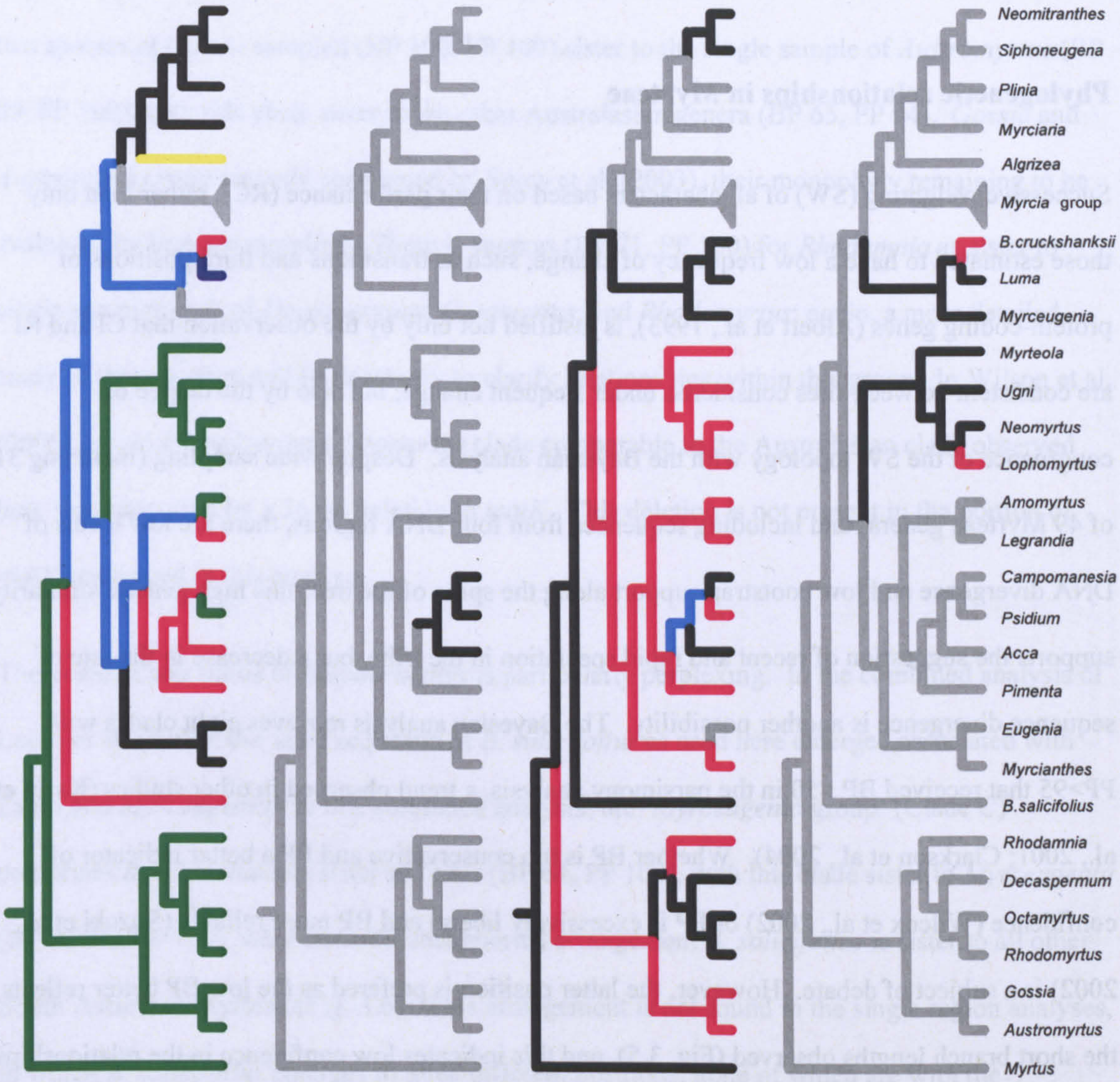


Figure 3.7. Character optimization over a single, randomly selected tree from the combined analysis. A. Embryo. B. Mean number of ovules per ovary. C. Placentation. D. Vessel elements.

Discussion

Phylogenetic relationships in Myrteae

Successive weighting (SW) of all characters based on their performance (RC), rather than only those estimated to have a low frequency of change, such as transitions and third positions of protein-coding genes (Albert et al., 1993), is justified not only by the observation that CI and RI are consistent between sites considered under frequent change, but also by the degree of congruence of the SW topology with the Bayesian analysis. Despite wide sampling (including 31 of 49 Myrteae genera) and including sequences from four DNA regions, there are low levels of DNA divergence and low bootstrap support along the spine of the tree; this high genetic similarity supports the suggestion of recent and rapid speciation in the tribe, but a decrease in the rate of sequence divergence is another possibility. The Bayesian analysis retrieves eight clades with PP>95 that received BP <50 in the parsimony analysis, a trend observed in other studies (Karol et al., 2001; Clarkson et al., 2004). Whether BP is too conservative and PP a better indicator of confidence (Wilcox et al., 2002) or PP is excessively liberal and BP more reliable (Suzuki et al., 2002) is a subject of debate. However, the latter position is prefered as the low BP better reflects the short branch lengths observed (Fig. 3.5), and this indicates low confidence in the relationships obtained.

The SW and Bayesian analyses presented here strongly support (BP 100, PP 100) monophyly of Myrteae (Clade H, Fig. 3.6). Within the tribe, Sytsma et al. (2004) placed Mediterranean *Myrtus* sister to the Neotropical genera, whereas its position in Wilson et al. (2005) varied, emerging as in Sytsma et al. (2004) in a *matK* analysis or sister to all other Myrteae when data from the spacer between *matK* and the 5' exon of *trnK* was included, but with low BP in both cases. The strongest evidence for the position of *Myrtus* in the present analysis is from the parsimony analyses (Fig. 3.6), in which it emerges as sister to the rest of Myrteae in the ITS, plastid, and combined analyses and in an unresolved position within the Myrteae clade in the ETS analysis.

Low bootstrap support, however, and with a different position in the Bayesian topology mean that its position is still unclear. The 'Australasian group' (Clade G) is split into two clades with the two species of *Gossia* sampled (BP 100, PP 100) sister to the single sample of *Austromyrtus* (BP 89, PP 100), with this clade sister to the other Australasian genera (BP 65, PP 94). *Gossia* and *Austromyrtus* were recently segregated by Snow et al. (2003), their monophyly remaining to be evaluated by broader sampling. There is support (BP 71, PP 100) for *Rhodamnia* as sister to single samples each of *Decaspermum, Octamyrtus*, and *Rhodomyrtus*; again, a more detailed study of these groups will be necessary to clarify relationships within this group. In Wilson et al. (2005), an *Archirhodomyrtus-Uromyrtus* clade comparable to the Australasian clade observed here was supported by a 36-bp deletion in *matK*. This deletion is not present in the portion of *matK* sequenced in this analysis.

The position and status of *Blepharocalyx* is particularly perplexing. In the combined analysis of Lucas et al. (2005), the same sequence of *B. salicifolius* as used here emerged associated with *Luma* and *Myrceugenia*. In this combined analysis, the '*Myrceugenia* group' (Clade C) comprises *B. cruckshanksii* sister to *Luma* (BP 61, PP 100), with this clade sister to *Myrceugenia* (BP <50%, PP 100), whereas in an unsupported arrangement *B. salicifolius* is sister to all other South American Myrteae (Fig. 3.6). This arrangement is not found in the single region analyses, in which *B. salicifolius* emerges in three different positions, none of which are with the '*Myrceugenia* group'; the species was resequenced for each region and using a second sample, the same result was always obtained. Performing the SW search on the taxonomic sample used here, using only the ITS and *psbA-trnH* sequence data as in Lucas et al. (2005), *B. salicifolius* retains the position it has in this analysis (BP <50), suggesting that the position of *B. salicifolius* in this analysis is a result of increased sampling. *Blepharocalyx salicifolius* is an extremely variable and wide ranging species; nevertheless, these mixed results suggest a complex evolutionary history may be discovered in futher analyses.

In Landrum's (1986) account of *Blepharocalyx*, the genus is considered to contain three species, each considered a separate genus by previous authors; although characters such as placentation

unite these three species, other characters vary. *Blepharocalyx salicifolius* is the type species with dichasial inflorescences, an open calyx with lobes that fall at about anthesis and a spiralled hypocotyl. *Blepharocalyx eggersii* has a paniculate inflorescence, a closed calyx splitting into four lobes that fall at anthesis and a large, homogenous embryo with little distinction between the cotyledons; it was described as *Marlieriopsis* Kiaersk. *Blepharocalyx cruckshanksii* has dichasial inflorescences, an open calyx with four persistent lobes and a C-shaped embryo; it was originally described as *Temu* O.Berg. Should the results presented here stand up to greater taxonomic sampling and addition of characters, *Temu* will need to be reinstated. *Blepharocalyx eggersii* was not sampled here, and only its inclusion in a future analysis will indicate if it is related to the type species or if *Marlieriopsis* will require resurrection.

Again referring to Figure 3.6, South American Myrteae, excluding *B. salicifolius,* fall into two groups (Clades J and K). Clade J (BP <50, PP 100) comprises the South American Myrtinae *sensu* DC plus *Eugenia* and *Myrcianthes*. The '*Myrteola* group' (BP <50, PP 99, Clade D) comprises *Myrteola*, *Ugni*, and the two New Zealand genera, *Lophomyrtus* and *Neomyrtus,* with strong support for the monophyly of the New Zealand genera (BP 100, PP 100) but no support for their sister relationship to *Ugni* or the sister relationship of *Myrteola* to the rest. In a weakly supported sister relationship to the '*Myrteola* group' is a clade (BP <50, PP 98) composed of two subclades, one of which, the '*Pimenta* group' (Clade E), comprises *Amomyrtus* (BP 100, PP 100) sister to *Legrandia* (BP <50, PP 100), with this last clade sister (BP <50, PP 98) to a clade of *Campomanesia, Psidium, Pimenta*, and *Acca*, all of which appear monophyletic. The '*Eugenia* group' (Clade F) comprises two traditional Eugeniinae genera, apparently monophyletic *Eugenia* (BP 100, PP 100) in a well-supported (BP 90, PP 100) sister relationship to (BP 100, PP 100) *Myrcianthes*.

Poorly supported Clade K suggests a sister relationship of the '*Plinia* group' plus *Algrizea* and the '*Myrcia* group' (Clade L) to the '*Myrceugenia* group' (Clade C). This contrasts with previous results (Proença et al. 2006) and the Bayesian analysis (PP 85) presented here that support a sister relationship between *Algrizea* and the '*Myrcia* group'. Whereas the association of *Algrizea* with

the other genera of Clade L is clear, its fluctuating position between analyses and lack of support renders this another taxon for which exact phylogenetic position requires further investigation. The sister relationship (BP <50, PP 100, Clade L) between the '*Plinia* group' (Clade A) of genera previously ascribed to Eugeniinae, *Algrizea* and the '*Myrcia* group' (Clade B) supports previous analyses (e.g. Salywon et al. 2004; Sytsma et al. 2004; Wilson et al. 2005) indicating paraphyly of Eugeniinae *sensu* O. Berg. The '*Myrcia* group' appears monophyletic (BP 64, PP 100), including the four core genera of Berg's subtribe Myrciinae; *Calyptranthes, Gomidesia, Marlierea*, and *Myrcia*. The '*Myrcia* group' will be discussed in greater depth in a future paper devoted entirely to it; however, key differences from Lucas et al. (2005) are an increase in support for monophyletic *Calyptranthes* and *Gomidesia* (both BP, PP 100), with some support (BP 67, PP 100) for a sister grouping between *Myrcia pubipetala* and *M. venulosa.*

Morphological optimizations

Embryo – Landrum & Stevenson (1986) suggested the ancestral Myrteae embryo was small, thin, and unfolded, similar to that of *Ugni* (Myrtinae), and that the swollen or folded embryos of Eugeniinae and Myrciinae evolved independently to increase the capacity of the embryo to store food. This study supports that idea, with genera of the 'Australasian group', *Myrtus communis*, and the '*Myrteola* group' sharing the myrtoid embryo and these genera successively emerging from the deepest nodes of Myrteae (Fig. 3.7A). With hindsight, it appears that a further division of the myrtoid embryo state might support the '*Myrteola* group' as distinct from the remainder of Clade J, specifically those genera of Clade E. In *Acca, Accara, Myrtus, Myrrhinium, Ugni, Lophomyrtus, Myrtastrum, Myrteola* and *Neomyrtus*, the embryo has cotyledons that roughly equate in length or that are slightly shorter than the hypocotyl and not reflexed (Landrum pers. com; see Fig. 2F, Landrum 1986). Clade D, members of the '*Myrteola* group' exclusively possess this embryo type, while the majority of the remainder of Myrtinae *sensu* Berg have embryos as depicted in Fig 3.1D, with cotyledons much shorter that the hypocotyl and generally reflexed on it. The embryo condition found in the '*Myrteola* group' however is also found in

Myrtus and *Acca*, also in unsampled, unplaced *Accara, Myrrhinium* and *Myrtastrum.* The outcome of future studies to clarify arrangements along the Myrteae backbone and in particular within clade J will enable a full assessment of this character's subdivision to be made. For now however, the arrangement suggests that the oldest Myrteae lineages (according to Sytsma et al. 2005) have linear or slightly incumbent embryos with small, undeveloped cotyledons and that the condition depicted in Fig. 3.1D may be a derived state, with the primitive condition persisting in *Acca* and possibly *Accara* and *Myrrhinium* should they also emerge in the '*Pimenta* group'. Of the other potentially derived conditions of the Myrteae embryo, enlarged, homogenous cotyledons are also found in genera of tribe Syzygieae; spiralled embryos or crumpled cotyledons however, are not found anywhere else in the family. The spiralled embryo of *B. salicifolius*, therefore, and the position of this taxon nested between otherwise myrtoid clades suggests that this species has long been isolated from the rest of the South American Myrteae, developing the spiralled embryo type independently from the other genera of the *Campomanesia* complex (Landrum & Stevenson, 1986), a group now confirmed as artificial with its genera spread widely through the tribe.

Results presented here indicate that the apparently homogenous eugenioid embryo has arisen independently in the '*Plinia'* and '*Eugenia*' groups. Cotyledons are less likely to be fully fused in *Plinia* and associated genera (Kausel, 1956; McVaugh, 1968), whereas they are almost always entirely so in *Eugenia* although in *Myrcianthes* the cotyledons are nearly always free and plano-convex (McVaugh, 1963a). The foliaceous myrcioid embryo occurs throughout the '*Myrcia* group' and also in *Myrceugenia*, suggesting either its repeated loss in the '*Plinia* group', *Algrizea*, *Luma*, and *Blepharocalyx cruckshanksii* or its independent evolution in the '*Myrcia* group' and *Myrceugenia.* Developmental studies will be necessary before such reversals or parallelisms can be confirmed. Optimization of a modified tripartite embryo arrangement confirms that states relied on by previous authors are not suitable as the sole characteristics for the grouping of Myrteae genera, each having arisen more than once. The modified tripartite embryo characterisation can, however, be used to assess the phylogenetic hypothesis presented here. Within some subtribal clades (the '*Myrcia'*, *'Plinia'*, *'Myrteola'*, and '*Eugenia'* groups), embryo type is largely consistent, and in these cases, in conjunction with other morphological characters,

optimization of the form of the embryo provides potential synapomorphies for these groups. Future ontogenetic studies seeking developmental differences between seemingly identical states with independent origins (e.g. swollen cotyledons in the *'Plinia'* and *'Eugenia'* groups) would be helpful, as would similar studies in clades in which more than one embryo type is present (e.g. the '*Myrceugenia* group'). Ultimately, the gradual accumulation of such information should allow an unambiguous partitioning of the embryo into several, homologous characters, a much-improved situation (McVaugh, 1968; Landrum, 1986; Snow et al., 2003) likely to be of significance in the natural classification of Myrteae genera.

Number of ovules per ovary — Locular complement in Myrteae varies from unilocular *Rhodamnia* to up to 20-locular *Campomanesia,* whereas number of ovules per locule varies from two in the '*Myrcia* group' and some genera of the '*Plinia* group' to just under fifty in *Acca.* Optimization of mean numbers of ovules per ovary (Fig. 3.7B) indicates that ancestral Myrteae had between 12 and 70 ovules, with the average number subsequently increasing in *Campomanesia*, *Psidium*, and *Acca.* The *Campomanesia-Pimenta-Acca* association is supported by overall morphology; Bentham (1869, p.150) described finding 'nothing in habit or character to separate *Acca* (at that time in Orthostemonoideae) from *Psidium'* other than the presence of endosperm in the seed of *Acca* (later found to be erroneous) and by *Psidium* and *Campomanesia* forming a group distinct from those other Myrtinae genera in which 'the ovary [is] never more than tricelled', a generalization that holds true in Neotropical Myrtinae *sensu* DC. except for *Ugni*, in which the ovary is occasionally tetralocular. Optimization of ovule number per ovary may support the paraphyly of Eugeniinae since *Eugenia* and *Myrcianthes* of the '*Eugenia* group' contain an average of 22 and 50 ovules per ovary, respectively, numbers closer to the range of the majority of genera in the '*Pimenta'* and '*Myrteola*' groups, to which the '*Eugenia* group' appears more closely allied, and higher than the range in the '*Plinia* group'. The average number of ovules per ovary in the '*Plinia* group' is 4-9 (Fig. 3.2), an arrangement more similar to that of the '*Myrcia* group' (with a mean of 4 to 5 ovules per ovary) than to the '*Eugenia* group' with which it was previously associated. Despite this trend, the interval between the highest number of the

'*Plinia* group' and the lowest numbers of the next ranked taxon is not discrete, preventing the delimitation of an additional state within this character. In addition, there is some overlap with *Rhodomyrtus* and *Pimenta* of the 'Australasian' and '*Pimenta*' groups, respectively, both averaging 10 ovules per ovary, only one more than *Siphoneugena*, whereas *Algrizea*, nested between the '*Plinia*' and '*Myrcia*' groups, averages 11 ovules per locule. The hypothesis that an increased number of ovules per ovary has more taxonomic significance than number of locules or ovules is not supported except in the case of the *Campomanesia-Psidium-Acca* association, the character apparently of limited use as currently employed despite going some way in supporting the '*Plinia* group'+*Algrizea*+'*Myrcia* group' association. It remains for a further study to interpret the characters of the ovary and ovules in a phylogenetic sense; while this study does not advocate the continued use of the characters combined as they are here, a methodology employing them independently does not at this stage appear appropriate either.

Placentation. — Results indicate that placentation in ancestral Myrteae was bi- or multi-seriate on flat or parietal placentas covering at least part of the septum (Fig. 3.7C), with arrangements of ovules on peltate or otherwise extruded placentas having arisen independently in the 'Australasian group' and the '*Myrteola*' and '*Pimenta*' groups and *Myrcianthes*. It appears that the subsequent transition to the arrangement in which ovules arise at a single point on the septum occurred independently in *Eugenia* and the '*Plinia* group'+*Algrizea*+'*Myrcia* group' association, with an at least partial reversal to the plesiomorphic state apparent in *Neomitranthes* and *Siphoneugena*. It is tempting to hypothesize a link between placentation in the '*Myrcia* group', *Algrizea* and those genera of the '*Plinia* group' in which the ovules arise at a single point, particularly as in each case the ovules emerge from below the midpoint of the septum. The link is unclear, however, when the genera of the '*Plinia* group' with ovules arranged in rows along at least part of the septum are considered. Should placentation at a single point be shown to be synapomorphic for Clade L, it might be distinguished from the ovules of the '*Eugenia* group' by its position below the midpoint of the septum, whereas in the latter group placentation of ovules usually occurs at the middle of the septum or above. This fine splitting of characters, however,

after more striking differences (such as unilocular vs bi– or multilocular ovaries) have been disregarded, is tenuous. Nature of placentation has limited use as a character for defining groups within Myrteae; it provides potential synapomorphies only if a high and unlikely number of reversals and parallelisms are assumed to have taken place, and further use of this character will require developmental investigation of the gynoecium similar to that undertaken by Bohte & Drinnan (2005).

Vessel elements. — South American Myrteae with scalariform perforation plates are consistently from (sub)montane Andean localities. In the tropical taxa in which scalariform perforation plates do occur, they are probably retained as an adaptation to cold and are therefore more prevalent at high elevations (Jansen et al., 2004). The function of scalariform plates may be to trap air bubbles during the thawing of ice, or they may be a relictual state in taxa in warmer environments that have lost scalariform plates to provide a more efficient hydraulic system during the wet season (Schmid & Baas, 1984; Carlquist, 1988; Jansen et al., 2004). Scalariform plates are usually considered to be associated with more ancient taxa (Bailey & Tupper, 1918; Jansen et al., 2004), a trend not supported here as the earliest branching Myrteae lineages, *Myrtus* and the 'Australasian group', have simple perforation plates. The presence of scalariform perforation plates in Andean *Tepualia* of tribe Metrosidereae (Jansen et al., 2004) supports a scenario whereby taxa in Myrteae and Myrtaceae speciated in the Andes and lost their scalariform perforation plates as they spread into the tropical lowlands. The optimization of scalariform plates (Fig. 3.7D) suggests that they developed twice in the history of Myrteae, in the '*Myrteola* group' and the '*Myrceugenia* group', but an alternative, although less parsimonious explanation could be that they are relictual in these two groups and there have been multiple losses in the other genera. Arguments along these lines can be found in Baas & Wheeler (1996). The presence of scalariform perforation plates in *Neomyrtus* and *Lophomyrtus* supports the suggestion of the phylogenetic topology that these genera arrived in New Zealand after dispersal from western South America. Mixed scalariform and simple plates are also found in *Lenwebbia* (Australia) and *Myrtastrum* (New Caledonia), suggesting that these genera result from similar dispersal events by

(an) ancestor(s) of the '*Myrteola* group' and supports the placement of *Lenwebbia* outside of the 'Australasian group' (Wilson et al. 2005). The perforation plate character is the most robust of those evaluated here, clearly defining two clades and associating the apparently less closely related '*Myrceugenia* group' with the '*Myrteola* group', challenging the weakly supported, molecular-based grouping of the former with the '*Plinia* group'+*Algrizea* +'*Myrcia* group' association. The division of the character into mixed scalariform vs. entirely scalariform is unnecessary, as the '*Myrceugenia* and '*Myrteola* groups' would be equally supported if the character had been divided into simple vs. non-simple perforation plate states.

Assessment of previous supra-generic arrangements

The following section attempts to evaluate previous classifications of tribe Myrteae in light of the new results presented in this work.

De Candolle and Berg

De Candolle (1828) and Berg's (1855-56, 1857-59) division of Neotropical Myrtaceae into three subtribes based on embryo morphology is artificial (Landrum 1986, McVaugh 1968, Wilson et al. 2001, 2005). Myrtinae *sensu* O. Berg are paraphyletic with genera previously ascribed to Eugeniinae *sensu* O. Berg nested within it, whereas the latter subtribe and subtribe Myrciinae *sensu* O. Berg (including *Myrceugenia*) are polyphyletic.

Kausel

The subfamilial groupings of Kausel (1956, 1966) are to date the only arrangement that did not accept a direct relationship between *Plinia* and *Eugenia*; as Kausel noted, *Plinia* cotyledons are discrete and plano-convex, not homogeneous or fused as they are in *Eugenia*. Kausel transferred those *Myrciaria* species (including *M. cauliflora*) with free cotyledons to *Plinia* (Plinioideae), an inclusion questioned by subsequent authors (e.g. Sobral, 1993; Barrie, 2004) and here challenged by the grouping of two species of *Myrciaria* (including *M. cauliflora*) separately from the only

included species of *Plinia*; Kausel included what remained of *Myrciaria* in Eugenioideae. *Pseudomyrcianthes* and *Acreugenia* (both now treated in *Myrcianthes*) were placed separately in Eugenioideae and Plinioideae. These last genera are represented here by *Myrcianthes pseudomato* and *M. fragrans,* respectively, their emergence together here supports the monophyly of an inclusive *Myrcianthes*, although this could change when the type species of *Pseudomyrcianthes*, *P. pyriformis* (Cambess.) Kausel is included, as Legrand considered it a *Eugenia* (McVaugh, 1968). Cryptorhizoideae *sensu* Kausel are artificial for reasons already discussed regarding the *Campomanesia* complex of Landrum and Stevenson (1986). Myrtoideae *sensu* Kausel are also artificial, including some but not all members of the '*Myrteola*' and '*Pimenta*' groups. Within Myrcioideae *sensu* Kausel, *Myrceugenia*, *Luma* (as *Myrceugenella*) and *Acca* (as *Feijoa*) do not appear directly related to the '*Myrcia* group', contradicting Kausel's association of these genera based on his belief that the folded embryos of *Myrceugenia* and the '*Myrcia* group' and the large, involute, reflexed cotyledons of *Acca,* had a common origin.

McVaugh

In the first realignment attempting to depart from the three subtribe, embryo-based viewpoint, McVaugh (1968) recognized six informal groups of American Myrteae with a further eight unaligned genera, six of which were nevertheless allied to a group. McVaugh's group 1 comprised the four core Myrciinae genera with *Myrceugenia* and *Nothomyrcia* (a synonym of *Myrceugenia*) allied, based mainly on embryo morphology. The analysis presented here indicates McVaugh's group 1 to be monophyletic, excluding *Myrceugenia*.

McVaugh's group 2 comprised *Eugenia, Calycorectes, Myrciaria, Plinia* and *Siphoneugena* with *Legrandia* allied, united by possession of fused cotyledons. The analysis presented here indicates that this group is polyphyletic, with *Myrciaria, Plinia*, and *Siphoneugena* emerging in the '*Plinia* group', *Eugenia* in its own group, and *Legrandia* in the '*Pimenta* group'. A phylogenetic study of the '*Eugenia* group' by Mazine et al., (2006) indicates a paraphyletic *Eugenia* including *Calycorectes*. McVaugh's group 3 united *Myrcianthes, Reichea*, and *Pseudanamomis,* with *Luma*

allied, based on the dichasial inflorescence, swollen cotyledons, and bilocular ovary. Grifo (1992) recognized that *Pseudanamomis* is closely related to *Myrcianthes* (including *Reichea*), McVaugh appearing to have correctly associated these genera. A relationship between *Myrcianthes* and *Luma* is refuted here; instead *Myrcianthes* is associated with *Eugenia*. McVaugh's group 4 unites *Campomanesia, Paivaea, Britoa, Blepharocalyx*, and *Temu* based on possession of soft testa and enlarged, spiralled embryos, a suite of characters all demonstrated to be homoplastic.

McVaugh's group 5 united *Calycolpus, Myrtus, Psidium, Ugni*, and *Myrteola,* with *Amomyrtus* and *Amomyrtella* allied, based on possession of a 'myrtoid' embryo, solitary or three flowered peduncles, bony seeds and ovaries mostly tri-locular or less. Results indicate that *Psidium*, anomalous in McVaugh's group 5 due to a multi-locular ovary, is not directly related to *Ugni*, *Myrteola*, or *Myrtus*. McVaugh (1968) did not explain his assumption of an affinity of *Amomyrtus* and *Amomyrtella* with the core group, but it is assumed to be based on shared possession of a myrtoid embryo. These last genera possess solitary (*Amomyrtus*, *Amomyrtella*) or racemose (*Amomyrtus*) inflorescences and subapical protruding placentas; these are characters that support their association in this study with *Legrandia* and the '*Pimenta* group'. Biochemical similarities suggested that *Amomyrtella* is closely related to *Amomyrtus* (Weyerstahl et al., 1992); the former has yet to be sequenced, and its position remains unconfirmed. Results within the '*Myrteola* group' support developmental floral morphology-based observations by Belsham (2003), of a possible common origin for *Myrteola nummularia* and *Lophomyrtus obcordata*, whereas floral morphology and ontogeny in *Ugni* differ from the New Zealand species. Snow and Guymer (2001) have shown similarities between floral morphology in *Ugni* and the Australian genus *Uromyrtus*; it is clear that further research on these groups is required. However, the shared possession of scalariform perforation plates supports the inclusion of *Ugni* in the '*Myrteola* group'. Unpublished data from Wilson et al., (pers. comm.) suggest that in *matK*-based analyses *Calycolpus* is sister to *Myrtus*, and this congruence with the analysis presented here suggests that their inclusion in McVaugh's fifth group is erroneous.

McVaugh's group 6 unites *Pseudocaryophyllus* and *Pimenta,* a grouping noted by Landrum's (1986) subsequent synonymization of the former with the latter and supported by this analysis. In addition to these six groups, McVaugh left *Acca* and *Myrrhinium* with no placement in his scheme, tentatively ascribing their affinities to the 'myrtoid' genera. *Acca* emerges with *Psidium* in the '*Pimenta* group', and Landrum (1986) suggested that *Myrrhinium* shares significant morphological similarities with *Acca*; however, *Myrrhinium* was not sampled in this analysis, and its affinities remain unconfirmed.

Ultimately, despite McVaugh's (1968) determinations otherwise, his subtribal classification did not place less importance on embryo characters than previous authors as many of his groups were based primarily on this character. Of his six groups, only three (groups 1, 3, and 6), taken in their strict sense, agree with the results presented here. The six genera not satisfactorily placed in any of McVaugh's groups, but tentatively allied with them, are without exception erroneously associated with the majority of genera in whichever group they were associated. McVaugh's hypothesis that the unplaced genera plus *Acca* and *Myrhinnium* were evolutionary lines from less successful proto-myrtaceous ancestors that today remain morphologically similar to ancestral groups is refuted by their emergence here nested in terminal clades. Despite the shortcomings of the systems of Kausel (1956, 1966) and McVaugh (1968), they remain two of the most frequently cited and accurate classifications of Myrteae genera to date, identifying many subtribal groups that follow outlines of groups identified here.

Landrum

Landrum (1981b) suggested that anomalous characters preventing the straightforward placement of *Myrceugenia* in a subtribal classification may be plesiomorphic and shared by *Blepharocalyx*, *Myrcianthes, Myrceugenia,* and *Luma* and that the three former genera are similar to ancestors of the three previously accepted subtribes of tribe Myrteae, whereas *Luma* is an early offshoot of the tribe. This study suggests that the characters suggested as plesiomorphic by Landrum are in fact synapomorphic for *Blepharocalyx cruckshanksii*, *Myrceugenia*, and *Luma*, contradicting the idea

that these genera are similar to precursors of independent groups. The weakly supported placement of the '*Myrceugenia* group' in the overall topology prevents conclusions from being drawn as to whether it is an early branching lineage of Myrteae or not. If the position of *Blepharocalyx salicifolius* is correct, then, based on this analysis, it is this species, rather than *Luma,* that is the South American taxon most distantly related to the remainder of tribe Myrteae; if, however, *Calycolpus* is sister to *Myrtus,* then these two genera will share this position.

Landrum (1988) recognised a group associated with *Myrteola*, including genera with myrtoid embryos, bony testae, and uniflorous peduncles; this correlates with the '*Myrteola* group'. Genera in the '*Myrteola* group' have scalariform perforation plates and subapical, protruding placentation as do two extra-South American genera, *Myrtastrum* (New Caledonia) and *Lenwebbia* (Australia), which on this basis are predicted to fall within it. Although *Myrtus* and *Austromyrtus* possess embryo, testa and inflorescence characters leading to their inclusion in Landrum's group, their placentation and lack of scalariform perforation plates supports their exclusion.

Summary

In summary, seven subtribal groupings within Myrteae *s.l.* are identified. Relationships between these groups do not have high support, and of all suggested relationships those considered most likely to change in future analyses are the positions of *Myrtus communis, Blepharocalyx salicifolius*, and the '*Myrceugenia* group', with several morphological characters suggesting that this last group in particular might have closer associations with the subtribal groups of Clade J.

The seven subtribal groups distinguished by the molecular analysis can, to a certain extent, be distinguished by combinations of morphological characters (Table 3.4). Increased taxonomic sampling representing all Myrteae genera is desirable, and further sequence data are required before the tribe can be formally reclassified along these lines (although see Chapter 6 for taxonomic implications so far). The molecular results do not conflict with modern concepts of genera, with the exception of *Blepharocalyx*, which appears polyphyletic. Of the Neotropical

genera sampled, six are represented by one accession only, eight by two, and a further eight represented by three or more accessions. Efforts to ensure that these samples were from widely separated ends of the taxonomic spectrum add weight to suggestions of their monophyly, but when sampling is low and, particularly in the case of the Australasian genera, the majority are represented by one sample only, further data are required to establish monophyly. Comparison of the molecular results with morphology of the tribe supports McVaugh's opinion (1968) that the most obvious characters with which to classify Myrteae genera, such as merosity, degree of prolongation of the calyx or hypanthium, and nature of the calyx lobes are of use at the specific level, but not at the level of genus or subtribe.

Informal group	Character combinations
'*Plinia* group'	Plano-convex fleshy cotyledons not completely fused, testa soft; a mean of nine ovules per ovary; ovules at a single point on the septum, usually below the mid-point or inserted along its lower part; scalariform plates absent.
'*Myrcia* group'	Foliaceous cotyledons, testa soft; a mean of five ovules per ovary; ovules arising at a single point on the septum, usually below the mid-point; scalariform plates absent.
'*Myrceugenia* group'	Foliaceous or reduced cotyledons, testa soft; a mean of between 20 and 70 ovules per ovary; ovules inserted along the length of the septum; scalariform plates present.
'*Myrteola* group'	C-shaped embryo, testa bony; a mean of between 20 and 70 ovules per ovary; placenta subapical and protruding; scalariform plates present.
'*Pimenta* group'	C-shaped embryo or sometimes spiralled, testa bony or soft; a mean of 20 to 120 ovules per ovary (except *Pimenta*); placentation protruding or along the length of the septum; scalariform plates absent.
'*Eugenia* group'	Plano-convex or fully fused cotyledons, testa soft; a mean of 20 to 70 ovules per ovary; ovules at a single point on the septum; scalariform plates absent; inflorescence racemose (sometimes reduced and appearing fasciculate).
'Australasian group'	C-shaped embryo, testa bony (except some species of *Gossia*); a mean of 20 to 70 ovules per ovary (except *Rhodomyrtus*); scalariform plates absent; inflorescence racemose (except some species of *Gossia* and *Decaspermum*).

Table 3.4. Informal subtribal groupings of tribe Myrteae, based on clades generated by the combined molecular analyses (Fig. 3.6) and combinations of morphological characters useful for their circumscription.

Chapter 4 – *Myrcia sensu lato*

Introduction

Obvious morphological synapomorphies (shared, homologous, derived characters) are too few and rare across the tribe Myrteae to justify their inclusion in the tree-building process. This approach, however, may be more appropriate for a phylogenetic study of *Myrcia s.l.* (*Calyptranthes*, *Gomidesia*, *Marlierea* and *Myrcia*), as circumscribed by DNA analysis (Chapter 3); particularly, species are more closely related than in the Myrteae analysis and sampling is sufficiently dense to allow the detection of homoplasy instead of it being mistaking for true phylogenetic signal. Despite recent doubts over the usefulness of morphological characters for phylogenetic analysis (Scotland et al. 2003), such an analysis was desirable to establish synapomorphic macroscopic characters for definition of *Myrcia s.l.* sub-groups. It also allows for more rigourous evaluation of the taxonomic usefulness of characters considered by previous authors and some considered here for the first time. A well-executed morphological cladistic analysis provides an evaluation of congruence between morphological and molecular data, a 'reality check' as described by Wiens (2004); if data sets are congruent, overall phylogenetic hypotheses can benefit from increased statistical support (Wiens 2004). Bearing in mind that "rewarding morphological characters may be recognised better after circles of reciprocal illumination between phylogenetic and evolutionary studies" (Endress 2003, p.148), it was concluded that a cladistic analysis of morphological characters, in light of the molecular phylogenetic hypotheses within *Myrcia s.l.* generated in this chapter, would be appropriate, allowing sampling strategy and outgroup analysis to be based on the most up-to-date phylogenetic hypothesis available.

In this chapter, the most distinctive morphological characteristics of *Myrcia s.l.* are reviewed, and rationale and steps behind cladistic character selection are discussed. Morphological characters of *Myrcia s.l.* genera are described and compared to those of the other groups of tribe Myrteae.

Aims

The specific aims of this chapter were therefore: 1) to review strengths and weaknesses of previous morphological cladistic analyses in Myrteae; 2) to select appropriate morphological character selection criteria; 3) to review morphology in *Myrcia s.l.* and assess suitability of characters for species delimitation and species-level cladistic analysis; 4) to perform separate morphological and molecular analyses to identify areas where the two data sets are most incongruent; 5) to perform a combined analysis; 6) to determine and discuss any resulting incongruence. In this way morphological data can identify potential problems in the molecular results and act as a rigorous independent phylogenetic evaluation of them.

Review of morphological phylogenetic analyses in Myrtaceae

Johnson & Briggs (1984) compiled a dataset of 64 morphological characters for the first phylogenetic analysis in Myrtaceae; characters were weighted according to the degree of transformation they had undergone and the phylogenetic algorithm CLAX was used to systematically join taxa with the highest number of apomorphies and obtain a tree. A wide range of characters was used, with little discussion of why or how they were chosen. As this was primarily a study of capsular-fruited Myrtaceae genera, Myrteae were represented entirely as a single operational taxonomic unit, the 'Myrtoideae *s.s*' and characters were not therefore chosen on the basis of their use in subdividing Myrteae, but they were sufficient for the authors to be able to propose the taxonomy detailed in Chapter 2 (Table 2.1). Despite the lack of propriety for assessing Myrteae phylogeny, Johnson & Briggs's characters (Table 4.1) provide a useful starting point for character choice within the family. The study of Johnson and Briggs (1984, p.752) was first to highlight the shortcomings of morphological data in Myrtaceae, stating that "parallelisms and convergence must have been rife at all levels in the history of the [family]".

Landrum (1981b), in his study of *Myrceugenia*, performed the first Myrtaceae cladistic morphological analysis at generic level. Until this work, characters used to define higher order groups were those of the fruit, seed, embryo and wood anatomy, since replaced by a preference for using characters based on pubescence type and floral or vegetative measurements and their ratios. The matrix was analysed using the phylogenetic program 'Wagner 78' (Farris 1970); characters states were assessed by outgroup comparison, but as a result of low confidence in these polarity judgements the final phylogram was unrooted. In conjunction with a phenetic exercise (from which the cladistic results differed little) including geological and ecological data, Landrum (1981b) was able to reorganise the genus and hypothesise a phylogenetic and biogeographic history for it that corresponds with the limited molecular sample analysed here. In conclusion, Landrum (1981b) pointed out that there is a lack of characters available for morphological analysis in the Myrtaceae and that those already tested are generally poor indicators of evolutionary history due to homoplasy.

Landrum (1986) presented a tentative phylogenetic analysis of the genus *Pimenta* in which he used half as many (binary, floral and indumentum) characters as taxa. The phylogenetic method of character compatibility was used to construct groups of species, and the tree was arranged using *Blepharocalyx* as an outgroup. Despite the low number of characters used per taxon in this analysis, the phylogenetic estimate produced appeared to correlate with the geography of the species, a result that Landrum found 'encouraging'. He went on to emphasise that the study was based on little data and that he was unaware of characters linking these geographical groups. There have been no subsequent studies in *Pimenta, and* therefore the phylogeographic hypothesis generated by Landrum (1986) and the classification based on it has never been further evaluated.

Table 4.1. Numbers and nature of morphological characters used in previous cladistic analyses of Myrtaceae.

	Johnson & Briggs (1984) – Myrtaceae	Landrum (1981b) – *Myrceugenia*	Landrum (1986) – *Pimenta*	Grifo (1992) – *Myrcianthes*	Wilson et al. (2001) – Myrtaceae
Wood anatomy	15	-	-	-	11
Hairs	6	8	2	-	2
Buds, stipules, leaves	7	5	-	1	4
Bracts/bracteoles	-	3	-	2	-
Inflorescence	3	5	1	3	4
Non-sexual flower parts	4	12	1	4	1
Androecium	3	1	-	-	5
Pollen	2	-	-	-	1
Gynoecium	15	2	3	2	7
Fruit	3	-	-	1	4
Seeds	3	-	-	1	2
Embryo	3	-	-	4	3
Karyotype	-	-	-	-	1
Flowering period	-	1	-	-	-
Number of taxa	**16**	**45**	**15**	**18**	**44**
Number of characters	**64**	**38**	**7**	**18**	**45**
Number of states	**142**	**117**	**14**	**43**	**117**
Characters/taxon	**4**	**1.18**	**0.47**	**1**	**1.02**
States/character	**2.22**	**3.08**	**2**	**2.39**	**2.6**

Grifo (1992) used the program Hennig86 (Farris 1988) and characters based on the leaves, fruits, ovary, stigma, sexual system, embryo and seeds to carry out three morphological cladistic analyses on Myrtaceae, Myrteae and Eugeniinae. Subsequent analyses considering morphological and molecular evidence (Wilson et al. 2001; this work, Chapter 3) have since demonstrated the results of these analyses to be highly incongruent with current understanding. Grifo (1992) performed two analyses investigating tribal and subtribal arrangements based on unfeasibly limited datasets of seven and six characters, respectively, and a more detailed analysis (Table 4.1) of relationships in Eugeniinae. Sampling in these analyses included taxa since shown to be distantly related (Lucas et al. 2005; Wilson et al. 2001, 2005); to compare taxa such as *Syzygium, Myrceugenia, Luma, Eugenia* and *Plinia* is attempting comparison at a greater scale even than that used in the present study for Myrteae (Chapter 3). Based on the conclusions of Landrum (1981b, 1986) it seems to have been somewhat naïve in 1992 to compare distantly

related taxa using characters highly susceptible to homoplasy such as the pedicellate nature of the flowers or presence of a distinct marginal vein.

Wilson et al. (2001) added four new characters to the family-level dataset of Johnson and Briggs (1984) and removed nine autapomorphic characters and the character weighting previously employed. Broadening of the sampling increased the number of scored taxa to 44 and included five Myrteae genera, including two from South America. This allowed the first inferences to be made on phylogenetically useful characters for this tribe. Indications from this study are that characters of the indumentum, anther arrangement and essential oil biochemistry may be useful for exploring subtribal relationships. Several clades were supported by both molecular and some morphological characters, but Wilson et al. (2001) observed that resolution was limited and emphasised the 'marked homoplasy in many of the non-molecular characters', stating that 'almost every morphological character displays some degree of homoplasy on the molecular and combined estimates of phylogeny' (p. 2021). This was the last attempt at morphological cladistics in Myrtaceae.

In summary, this review demonstrates that morphological phylogenetic analyses in Myrtaceae are difficult procedures, due to high levels of homoplasy. To extract information from a morphological dataset for maximally accurate phylogenetic analysis, major issues that must be addressed are: i) selection of characters to minimise homoplasy (given that a morphological dataset will likely be small and homoplasy will not become 'background noise' as it may in DNA comparisons) and to reflect variation across the sampled taxa, and ii) a broad but careful taxonomic sampling of the group in question based on current taxonomic opinion, to ensure that a maximum amount of variation in the group is represented.

Morphological Methodology

Character selection criteria

Character conceptualisation, homology assessment and character independence

Morphological cladistic analysis involves the analysis of phenotypic variation in a group of organisms represented in a data matrix, for which columns and rows represent characters and taxa, respectively, and intersecting cells contain the appropriate character state. Hawkins (2000) distinguished characters as 'comparable categories', whereas character states are 'hypotheses of groupings'. Authors such as Hawkins (2000) and Poe and Wiens (2000) emphasised the need to describe explicitly the criteria on which characters are selected or rejected and how states are assigned. Characters should be homologous features, i.e. they are similar due to common descent and therefore potentially synapomorphic. It is accepted (Hawkins 2000; Williams & Humphries 2003) that there are two phases to the assessment of homology and thereby character recognition: these are the initial observation that two features are similar followed by the subjection of the proposed character to tests to confirm its homology; Patterson (1982) proposed three tests of homology; similarity, congruence and conjunction. The conjunction test rules hypotheses of homology are refuted if both structures are found in one organism, because presence of both structures in one organism indicates their independence. Rieppel (1988) and De Pinna (1991) asserted that conjunction is a weak test of homoplasy, and Hawkins (2000) noted that conjunction is frequently conducive to the coding of independent states as a single character. All three authors ultimately accepted only two tests of primary homology, those of congruence and similarity. Rieppel (1988) and De Pinna (1991) categorised the two phases of homology assessment as "topographical correspondence and homology" and "primary and secondary homology". In the opinion of these authors, using the terminology of De Pinna (1991), primary homology assessment involves the observation of similar features, this constituting the first test, that of similarity. Secondary homology assessment is applied during cladistic analysis after character states have been assigned, thus constituting the test of congruence. This sequence is adhered to in

the study presented here, with the assumption that characters in a cladistic data matrix must conform to the criteria of similarity and congruence but not of conjunction.

To provide independent evidence of relationships and avoid the over-significance and influence of a single biological event by the use of dependent characters, characters in this study were selected as far as possible to be independent of each other. Aspects of some organs and structures, such as the flower or inflorescence vary more and therefore offer more characters than others, and in a group as morphologically difficult as tribe Myrteae it is necessary to exploit fully these relatively few potential synapomorphies. This has resulted here in the use of three inflorescence and seven floral characters. The majority of characters used here, however, are from parts of the flower of separate origin and under independent genetic control. In the case of those characters of the inflorescence (characters 6 – 9), character 6 is concerned with the position of the inflorescence, considered here to be independent of characters 7, 8 and 9, which are concerned with inflorescence shape.

Coding quantitative and qualitative data

Characters used in this study are dealt with in different ways, depending on their nature, in particular, on where gaps were found between character states. Characters that appear qualitative (with clearly demarcated, discrete states) are often found to be quantitative (continuous data where states form a continuum) when explored in detail. An example of this is when a character divisible into apparently discrete states proves divisible only because material available under-represents natural variation. The use of continuous characters in cladistic analysis was initially discouraged (Pimental & Riggins 1987; Kraus 1988;) on the grounds that such data are too noisy to have any recoverable phylogenetic signal, with Cranston & Humphries (1988) believing they should be removed from such analyses altogether. More recent analyses, however, indicate morphological variation described by continuous characters to be important in distinguishing species and that it increases resolution in resulting cladograms (e.g. Roalson et al 2002). A variety of proposed methods for grouping or dividing continuous variation into character states

are available (see Forey & Kitching 2000 for an overview); however, in this analysis all characters are discontinuous and qualitative.

Polymorphic characters

To be useful for taxonomic grouping, character states must be largely invariant within taxa, and strong objections exist to the use of characters polymorphic within taxa in cladistics (e.g. Crother 1990, Siddall & Kluge 1997). Species that exhibit no intraspecific variation whatsoever are rare (Wiens 1995), and their careful inclusion encouraged by Wiens (2000). In this study, polymorphism was present in a single species; there are two forms of *Gomidesia anacardiifolia* , from Santa Catarina and from Rio de Janeiro, with tri and bi-locular ovaries, respectively. The species sampled in the molecular analysis is the form from Rio de Janeiro, and the species is scored as having a bi-locular ovary. As this analysis aims to define subgeneric groupings rather than to determine precise species level relationships it is felt that the scoring of this character one way or the other will not influence the subgeneric group in which it emerges.

Missing or inapplicable data

Empty cells due to missing data occur either when the character is inapplicable to the taxon (e.g. character 4 in which two states exist, hairs simple or hairs dibrachiate and species with no hairs are recorded as 'missing') or when the material available is insufficient to record the character (e.g. is available only in fruit, not flower). Recent studies suggested that, providing the sampling of a group is thorough and sufficient characters are utilized, incomplete taxa can be accurately placed in trees and furthermore that the addition of such incomplete taxa can dramatically improve results in dividing misleading long branches (Wiens 2006). Less than 2% (30 cells) of the data matrix presented here are scored as missing data.

Character exclusion

As in Chapter 3, a wide range of characters were considered and rejected; in the search for the best characters, they were rejected if they were quantitative due to required arbitrary circumscription of states or if there was doubt as to whether or not they reflect homology. To be disqualified for the analysis presented here, a character was rejected if it was applicable to less than 80% of the species sampled or if it exhibited variation too extreme to be accounted for under any polymorphism criterion (Wiens 2000). Additional details of characters are listed with comments on state scoring and weighting on a case-by-case basis.

Characters

The following description of '*Myrcia* group' characters uses *Myrcia* sections *Myrcia* and *Myrcia Aulomyrcia and Marlierea*, *Calyptranthes* and *Gomidesia* sensu Niedenzu (1893; Table 2.8). All photographed vouchers are lodged in the Kew herbarium.

Wood anatomy

Dias-Leme et al. (1995) surveyed wood anatomy of the four core genera of *Myrcia s.l.* and reported the wood to be diffuse porous, the vessels typically solitary with simple perforations and alternate, vestured inter-vessel pits. Fibres in core *Myrcia s.l.* wood are mostly thick-walled with bordered, occasionally vestured pits. Parenchyma is scanty paratracheal, diffuse or diffuse-in-aggregates forming complete or interrupted bands and strands of 4-8 (3-10) cells. Rays are 1-3 (-4) seriate with body ray cells procumbent with over four rows of marginal cells. Disjunctive ray parenchyma cell walls are present. The possession of scanty paratracheal parenchyma, diffuse-in-aggregates and in continuous tangential bands up to three cells wide united the seven *Calyptranthes* species analysed with five of the six *Marlierea* (excluding *M. umbraticola* (Kunth) O. Berg). The four species of *Gomidesia* analysed were united by parenchyma type that was found to be scanty paratracheal and diffuse-in-aggregates but was rarely banded and only diffuse in *G. affinis* (Cambess.) D.Legrand. *Myrcia* was found to have variable parenchyma. A review

of Myrteae wood anatomy (Telford et al. in prep.) found little variation between the four '*Myrcia* group' genera, and therefore no attempt has been made here to use wood anatomy characters for cladistic analysis.

Vegetative branching

The most common vegetative branching system in mature individuals of *Myrcia s.l.* is monopodial branching with a single or pair of weaker side shoots emerging from the main rachis resulting in a somewhat irregular pattern. This is the case in *Myrcia, Gomidesia*, in some *Marlierea* and in few *Calyptranthes.* In some species of *Marlierea* and *Myrcia* and in many *Calyptranthes* the tendency is rather for a sympodial system of dichotomous substitution, in which axillary branches arise in a continually repeated pattern from two or three sides of a suppressed central axis resulting in a characteristic repeating forked or trifurcating pattern of branchlets by which sterile material may often be recognised.

Character #1: Vegetative branching: 0 = monopodial; 1 = sympodial.

Leaves

Myrtaceae leaves are characteristically opposite and decussate with pinnate venation. In *Myrcia s.l.* this is almost exclusively the case with a few exceptional species demonstrating a distinctive whorled arrangement, even in vegetative bud, at the apex of the branch. Primary and secondary venation varies widely in prominence and density both between, and to some degree, within '*Myrcia* group' species. Two main types of venation can be distinguished throughout the family: brochidodromous venation in which the primary lateral veins curve towards the margin of the lamina and eventually curve distally and fuse, running parallel to the edge of the leaf, or less commonly, or not found in *Myrcia s.l.* and; eucamptodromous in which the secondary veins do not terminate at the margins but gradually diminish inside the margin and are connected to the secondary veins by a series of cross-veins without forming prominent marginal loops. Attempts to distinguish homologous types of marginal venation (Lucas 1999) were unsuccessful, indicating

the high homoplasticity of the character. A venation character with more potential for taxonomic use may be found in the midvein. The midvein is almost without exception conspicuous on the abaxial leaf surface and flattened or somewhat impressed adaxially. More rarely the midvein is raised or in some species it is adaxially raised as much or more than its width.

Character #2: Leaf arrangement: 0 = entirely opposite; 1 = entirely whorled or consistently whorled at branch apices.

Character #3: Adaxial midvein: 0 = impressed for more than half of the leaf's length; 1 = flat for more than half of the leaf's length; 2 = raised for more than half of the leaf's length.

Indumentum

Pubescence varies greatly in position, shape, density, colour and length throughout Myrtaceae. Most pubescent '*Myrcia* group' species have simple, erect or appressed, basifixed hairs; however, in some species, particularly in *Calyptranthes* and *Marlierea*, they are dibrachiate (McVaugh 1958a, 1968). An individual can bear a mixture of both simple and dibrachiate hairs that are usually appressed to the surface, often attached near one end so that the two-branched condition is not always obvious. McVaugh (1958a) associated dibrachiate hairs mainly with *Marlierea* and *Calyptranthes,* but McVaugh (1969) stated that they 'are of a more frequent occurrence' p.60. Nic Lughadha (1997) noted the presence of indumentum and describes its variation amongst the species of *Gomidesia*.

Character #4: 0 = simple hairs absent; 1 = simple hairs present

Character #5: 0 = dibrachiate hairs absent; 1 = dibrachiate hairs present

Inflorescence

A detailed overview of the Myrtaceae inflorescence can be found in Briggs and Johnson (1979). This system has been reviewed in particular for genera of Myrteae by Landrum (1981a), Nic

Lughadha (1997) and Lucas (1999), the general conclusion being that despite their use of an extensive, new and at times over-complex descriptive terminology, the resulting system is helpful in the analysis of the myrtaceous inflorescence. McVaugh (1956) was first to focus on inflorescence variation within the Neotropical genera, noting that subtribe Eugeniinae was characterised by racemes (sometimes extensively reduced giving the impression of fascicles), subtribe Myrciinae was characterised by a paniculate inflorescence, whereas subtribe Myrtinae was characterised by truly solitary or dichasial inflorescences.

The standard 'myrcioid panicle' (McVaugh 1956) can be in terminal or axillary; the apex of the rachis usually bears sessile flowers, either solitary or aggregated in threes, with the primary branchlets arranged oppositely on the central rachis, the lower ones usually longer than the rest and compound (Fig. 4.1a). Branchlets may partially lose their opposite arrangement (Fig. 4.1b) after the abortion of one or both buds at a given node, a process that can result in distortion or a zig-zagged appearance of the remaining axis (McVaugh 1956); this reduction can also be abrupt with the ultimate part of the main rachis resembling a spike (Fig. 4.1c). In practice, these arrangements are more common in *Myrcia*, *Gomidesia* and some *Marlierea*, whereas *Calyptranthes*, other species of *Marlierea* and a few *Myrcia* have characteristically different panicles in which the terminal bud aborts at the first node from where two branchlets emerge, the terminal bud of each branchlet then aborts and generates two branchlets of its own; this system gives the inflorescence a dibrachial appearance with multiple paired spikes or panicles arising from the same axil (McVaugh 1956; Fig. 4.1d) and is often, though not always, correlated with dibrachiate vegetative branching. There is a further inflorescence type in some species of *Myrcia* and *Marlierea* in which all primary axes emerge in a whorl from a single, terminal point that represents a compression of all inflorescence nodes and internodes. In this cymose system, each branchlet is roughly the same length with little secondary branching at the base and a rapid transition to sessile flowers; this sort of inflorescence is commonly markedly broader than long. In some *Calyptranthes* and *Myrcia/Marlierea* species, the axes of the inflorescence may be winged and the orientation of these wings used as a character to distinguish between sterile

specimens. Wings in the former genus run in the same plane as the leaves whereas in the other genera they are at right angles. This character is present in a minority of species in these genera and is therefore not used here for cladistic analysis.

Outgroup taxa in this study do not have paniculate inflorescences; *Neomitranthes, Plinia* and *Siphoneugena* either have racemose inflorescences, often reduced and appearing fasciculate, or fascicles with no evidence of a raceme; the *Algrizea* inflorescence is dichasial.

Character #6: 0 = inflorescences regularly spaced in more than two terminal or subterminal nodes, usually in several; 1 = inflorescences congested in terminal node, occasionally in ultimate two nodes.

Character #7: 0 = inflorescence a panicle; 1 = inflorescence a raceme, often reduced and/or appearing fasciculate; 2 = inflorescence a dichasium.

Character #8: 0 = ultimate panicle branchlets decrease steadily in length resulting in a broadly triangular panicle; 1 = ultimate panicle branchlets decrease abruptly in length resulting in a narrowly triangular or spiciform panicle.

Character #9: 0 = more than 70% of secondary rachises of panicles opposite; 1 = more than 70% of secondary rachises of panicles alternate.

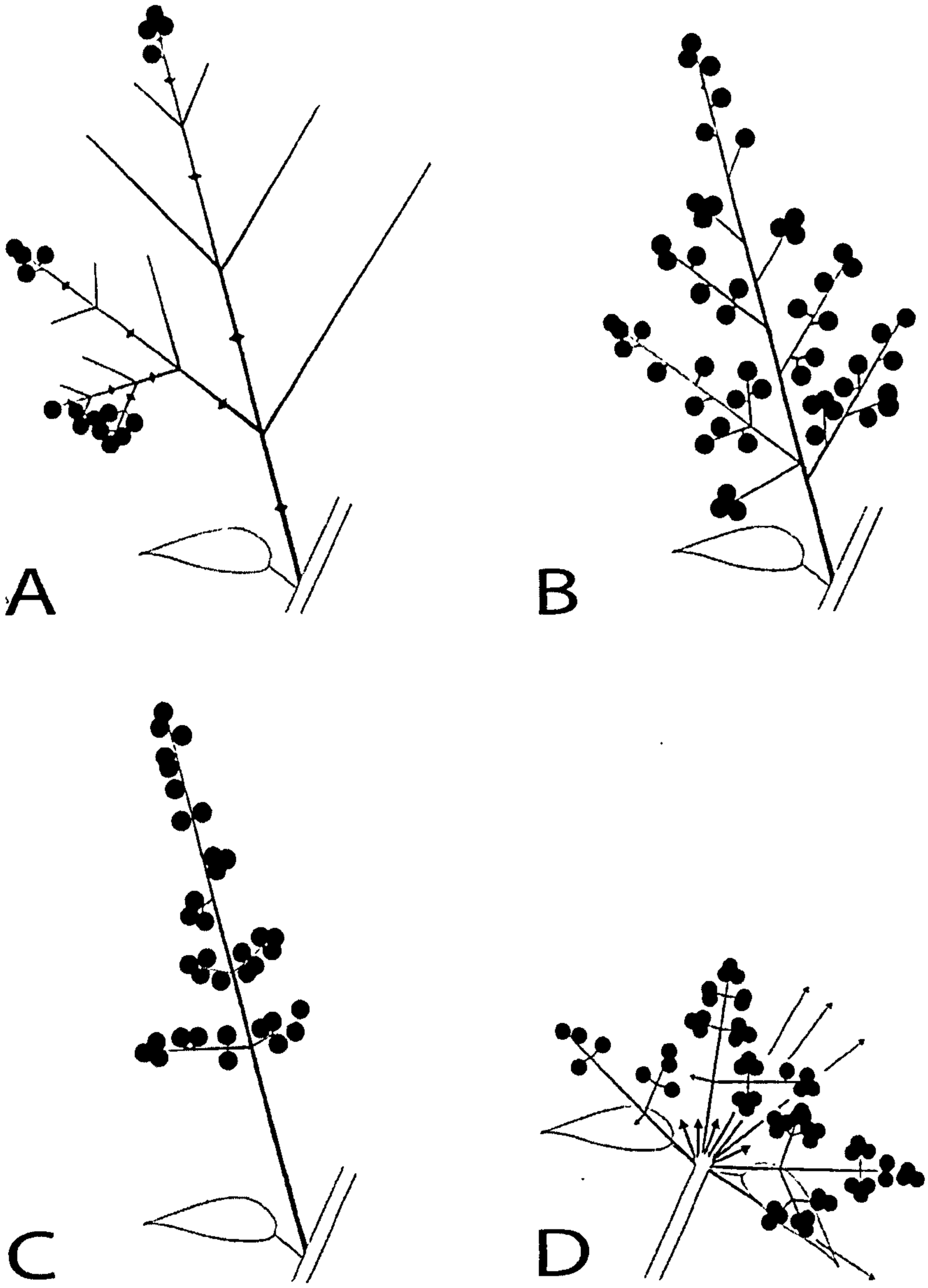


Figure 4.1. Panicles of *Myrcia s.l.* Position of flowers and branches indicated to the extent necessary to make clear the branching pattern repeated in each part of the panicle. Omitted branches are indicated by triangles. Adapted from McVaugh (1956).

Floral bracts and bracteoles

Each flower and each pair of branchlets in the 'myrcioid panicle' is subtended by a pair of bracts. Following a decrease in primary rachis, internode length in the inflorescence, these bracts can persist in a group at the point of insertion of the resulting whorled primary branchlets (Fig. 4.2a). Although this character is dependant to a degree on that of inflorescence type, not all species with whorled inflorescences bear such a group of bracts (Fig. 4.2b). It is common for these persistent bracts to be somewhat enlarged and evident. In some, particularly more northerly species of *Myrcia* and *Marlierea*, bracts subtending flowers are characteristically pointed and persistent up to the point where the fruits fall (Fig. 4.2c).

Character #10: 0 = whorl of bracts absent below inflorescences/new growth; 1 = whorl of bracts present below inflorescences/new growth.

Character #11: 0 = pair of bracts below flowers deciduous after flowers/fruit fall; 1 = pair of bracts below flowers persistent after flowers/fruit fall.

Calyx and hypanthium

Historical divisions between and within *Myrcia s.l.* genera have been closely linked to the nature of the calyx. Drawing attention to the grading of these characters, McVaugh (1968) suggested that the manner of the opening of the calyx is not correlated with any other characters and that "there is hardly any real distinction between *Calyptranthes* and *Marlierea*" and "*Myrcia* is no more distinct from *Marlierea* than the latter is from *Calyptranthes*" (p.374). McVaugh (1968) also noted that in some *Myrcia* species the number of calyx lobes varies between four and five, concluding that number of lobes, along with the nature of opening of the calyx, may be characters useful at the specific but not the generic level. Following extensive study of herbarium and wild-growing material and based on the experience of using calyx and hypanthium morphology to identify *Myrcia s.l.* taxa to genus/section/species, it is here suggested that, correctly interpreted, this character can reflect the evolutionary history of *Myrcia s.l.*

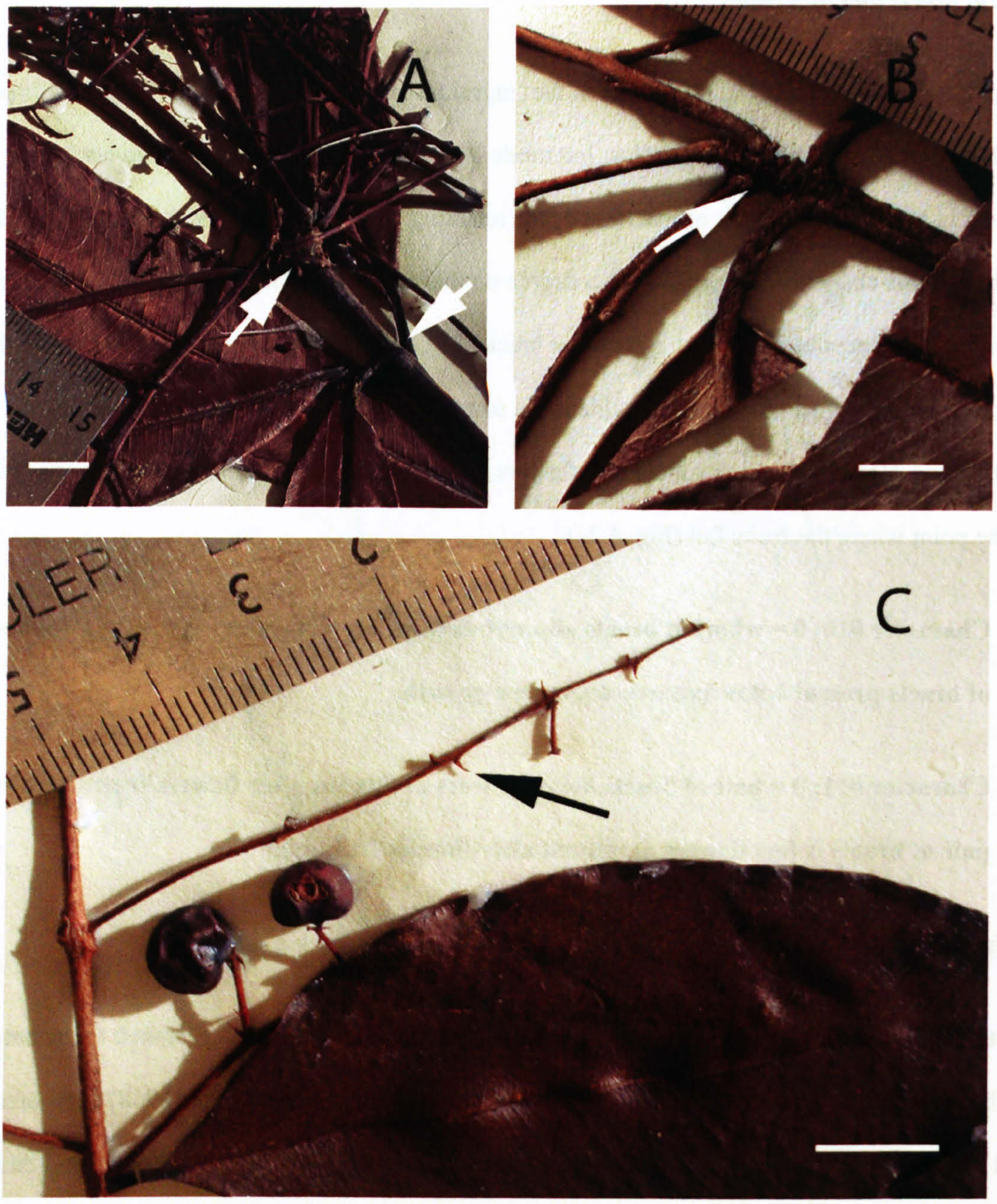


Figure 4.2. Floral bracts and bracteoles. A. RH arrow indicates whorled leaves, LH arrow indicates bracts subtending the whorled inflorescence; *Myrcia insularis, Hatschbach 13139.* B. Arrow indicates whorled inflorescence without bracts; *Marlierea tomentosa, Lucas 188.* C. Arrow indicates pointed, persistent, sub-floral bracts *Myrcia saxatilis*. Scale bars = 1 cm.

Species of *Myrcia* section *Myrcia* have five, free, imbricate calyx lobes approximately the length of the hypanthium; the apex of the ovary is flat, the disc is flat and glabrous, and there is no hypanthial tube (Fig. 4.3Aii); at anthesis the calyx lobes are attached to the disc at the apex of the ovary and open regularly without tearing (Fig. 4.3Aiii). Species of *Myrcia* section *Aulomyrcia* have more than one arrangement at anthesis. The majority of species have four or five short, calyx lobes on top of an extended hypanthium that is usually thin and prolonged into a glabrous tube above the ovary, with a reduced, usually concave disc (Fig. 4.3Bii, Cii, Dii).

Some species of *Myrcia* section *Aulomyrcia* and *Marlierea* with particularly elongate, turbinate hypanthia (Fig. 4.3Ciii) open as the hemispherical calyx lobes that are too small to reflex to the degree necessary for the flower to open and partially detach at either side of the lobe and tear away from the rim of the hypanthium (Fig. 4.3Cii). These tears mostly run horizontally and parallel to the rim of the hypanthium, leaving irregular, often apparently angular, well-spaced portions of calyx upon the hypanthial rim. A similar elongate, tubinate bud is normal also in *Calyptranthes,* (Fig. 4.3Fiii) in which the fused calyptra detaches entirely from the rim of the hypanthium at anthesis. It is unusual for these systems that begin with an elongate, tubular bud to result in flowers in which the tearing reaches the disc, and thus the flowers are explanate.

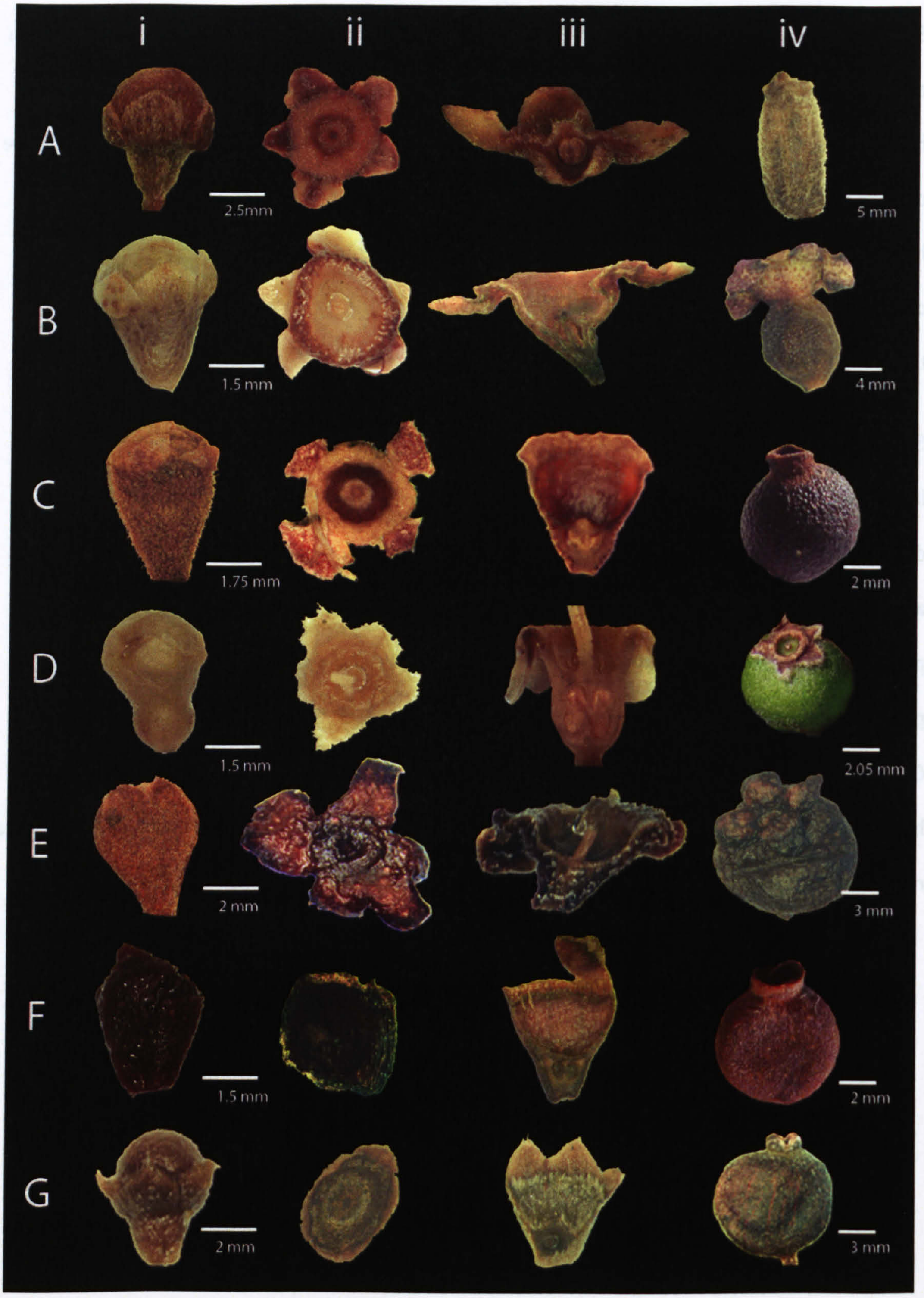


Figure 4.3. Calyx, disc, hypanthium, fruit. A. *Myrcia formosiana, Lucas 165*; B. *M. paracatuensis, Mello-Silva 1713*; C. *M. pulchra, Lucas 138.*; D. *M. tomentosa, Soares-Silva 752*; E. *Marlierea suaveolens*, bud: *Matsumoto 830*, fl & frt: *Marques 129.*; F. *Calyptranthes concinna, Lucas 183.*, G. *Gomidesia fenzliana, Lucas 256.* RH scale bars: columns i, ii, iii; LH scale bars: column iv.

A further group, including species of *Marlierea* and a minority of *Myrcia* section *Aulomyrcia,* have calyx lobes wholly or partially closed in the bud with a pore-like or no opening (Fig. 4.3Ei). In these species the distinction between the disc and the hypanthium is poorly marked, the flower opening by deep tearing during which the hypanthium tube may flare outwards; they thus, become strongly revolute and flattened, giving the impression that the lobes are larger or more uneven than they actually are (Fig. 4.3Eii). In *Marlierea*, the remnants of the torn calyx are more irregular and more likely to divide into three or four, rather than five, segments although there is an unexpected regularity to such apparently random tearing and open flowers can appear star shaped. In these, the tears in the hypanthium are more likely to extend into the staminal ring, leaving an extremely small piece of entire, flattened tissue around the style.

A further group of *Myrcia* section *Aulomyrcia* have five, free, calyx lobes abruptly reflexed away from a flat disc with a short hypanthium and often a slightly convex disc (Fig. 4.3Dii), a condition not found elsewhere. *Gomidesia* species have five free calyx lobes arranged in a similar fashion to species of *Myrcia* section *Myrcia*, a usually prolonged hypanthium with a flattened or shallowly domed and pubescent disc (Fig. 4.3Gii).

Even within this framework the distribution of characters is not completely uniform. At anthesis, even within a single species, the hypanthium can vary in degree of reflection and tearing and in the number of calyx lobes. The outgroup taxa of the '*Plinia* group' complicate assignation of states further; *Plinia* species open by vertical tearing between calyx lobes and through the hypanthium, the flowers of *Myrciaria, Neomitranthes* and *Siphoneugena* have free or fused calyx lobes which fall by means of a transverse tear as a single unit with or without the hypanthium. Despite all character possibilities in the calyx and hypanthium, to score characters across both the in- and out-groups, only two characters were finally employed.

Character #12: 0 = no part of flower tearing at anthesis; 1 = flower opening by tearing vertically through hypanthium wall; 2 = flower opening by tearing parallel to hypanthium ring

Character #13: 0 = calyptra absent; 1 = calyptra present

Flower buds

Species of *Myrcia* section *Myrcia* have more or less obovate buds (Fig. 4.3Ai), whereas those of *Myrcia* section *Aulomyrcia* have flower buds that are variously obovate (Fig. 4.3Bii), semi-globose (Fig. 4.3Eii) or elongate, turbinate (Fig. 4.3Ci). Species of *Marlierea* can have globose, semi-globose (Fig. 4.3Eii), or elongate-turbinate (Fig.4.3Ci) buds, whereas species of *Calyptranthes* mostly have these latter elongate-turbinate buds (Fig.4.3Fi). *Gomidesia* species have buds of a similar shape to those of *Myrcia* (Fig. 4.3Ai). When distinguishing *Aulomyrcia* from *Myrcia,* one of the primary characters used by Berg (1857-59) was the degree of constriction of the base of the calyx; never constricted in *Aulomyrcia* but constricted in *Myrcia.* As a result of McVaugh's (1968) general dismissal of characters of the calyx and hypanthium for subgeneric Myrteae classification, these traditional characters have come to be seen as variable, homoplastic and unreliable. Closer examination however, has indicated that the constriction or not of the bud between the ovary and calyx may indeed prove useful for species grouping within *Myrcia s.l.*. The buds of species such as *Myrcia tomentosa* (Rich.) DC. and *M. laruotteana* Cambess. have a distinct constriction in this position (Fig. 4.3Di) that is indistinct in pubescent species, in these cases only visible once the hairs are removed. Peculiarly, Berg mostly placed these species with this constriction in *Aulomyrcia* in his unranked group *Lateriflorae*, despite declaring the constriction to be a character of *Myrcia.* It is not clear if the constriction of the bud emphasised here is in fact the character used by Berg because after extensive examination, no buds or flowers of the species of Berg's *Myrcia* were found to have a constriction at the base of the calyx and Berg's sense of this character remains unclear (Berg, 1855-56, 1857-59).

Character #14: 0 = buds without constriction above the ovary; 1 = buds with constriction above the ovary.

Disc and staminal ring

McVaugh (1956, 1958b, 1968) believed that a reduction series existed for the number of stamens present in the flowers of most groups of Myrtaceae. However, within his 'myrcioid group' McVaugh believed that smaller stamen number was associated simply with a smaller disc. The use of stamen number as a taxonomic character was investigated by Nic Lughadha (1997), who concluded that *Myrcia s.l* (including *Myrceugenia*) stamen number is correlated with disc diameter and style length and consequently with flower size, whereas Myrtinae and Eugeniinae stamen numbers correlate only with flower size. Within *Myrcia s.l.*, Nic Lughadha (1997)

demonstrated a pattern in stamen number at generic level, reporting *Calyptranthes*, *Marlierea*, *Myrcia* and *Gomidesia* with median stamen numbers of 100 or fewer, 140 or fewer, 200 or fewer and 125 (rarely exceeding 175), respectively. Measurement of the disc in species of Myrtaceae may be unreliable, however, as the age of the flower and the degree of tearing of the hypanthium can distort the organ. Stamens in *Myrcia s.l.* species are borne on a ring of tissue surrounding the disc, from here on termed as the staminal ring. In species of *Myrcia* section *Myrcia,* the staminal ring is multi-seriate, broad, laterally flattened, hairy and clearly differentiated from the top of the hypanthium tube (Fig. 4.3Aii, iii). Other '*Myrcia* group' species have much narrower and essentially glabrous staminal rings with fewer rows of stamens, the rows relatively undifferentiated from the hypanthium wall (Fig. 4.3B–Giii). In such species, there is an unclear distinction between disc, hypanthium and staminal ring that may begin anywhere from the middle to the apex of the hypanthium. In species in which stamens are inserted in the apical portion of the hypanthium, staminal scars can appear to be situated at apices of the calyx lobes. Herbarium dissections suggest that number of stamens is correlated with staminal ring width, with this character used here to detect generic level trends in the former.

Character #15: 0 = staminal ring at anthesis comprising 60 percent or more of disc width; 1 = staminal ring at anthesis comprising less than 40 percent of disc width.

Character #16: 0 = disc (excluding staminal ring) glabrous; 1 = disc pubescent.

Anthers

Most *Myrcia s.l.* have bi-locular, longitudinally dehiscent anthers, whereas *Gomidesia* have incompletely or completely tetra-locular anthers, with the interior sac of each theca distally displaced and apparently opening extrorsely at the tip whilst the exterior sac appears to open introrsely at the base. The degree of displacement varies greatly, with some species having clearly four locular anthers, whereas some barely differ from the regular arrangement of *Myrcia*

s.l. Legrand (1959), McVaugh (1968) and Nic Lughadha (1997) maintained *Gomidesia,* in the case of the last author on the grounds that 'the combination of specialised anthers, prolonged hypanthium which is densely pubescent internally and calyx lobes erect or connivent in fruit (rather than spreading) renders *Gomidesia* at least as distinct from *Myrcia* as *Marlierea* is from *Calyptranthes* (or indeed from *Myrcia*)' (p.45). However, some authors did not recognise the group. Most recently, Sobral (2003) in his treatment of Myrtaceae from Rio Grande do Sul, has included all *Gomidesia* species within *Myrcia* on the grounds that in anthers of some species of *Gomidesia*, the degree of thecal displacement is so slight that it is impossible to tell them from those of other '*Myrcia* group' species. With this in mind, this study looks at a further character of *Myrcia s.l.* anther, degree of reflexion of the thecal halves. It is proposed in Nic Lughadha (1997, p.19) that in most *Myrcia s.l.*, 'the thecal arcs generally lose or reverse their curvature at dehiscence, exposing the interior of the thecal halves, sometimes diverging to the extent that finally they are back to back with the dorsal thecal arcs of the opposite theca' (Fig. 4.4A). This is in contrast with the anthers of *Gomidesia* species in which the 'thecal halves retain more or less their original curvature or are, at most somewhat flattened after dehiscence' (Fig. 4.4B). This character is usually evident and more consistent than the degree of thecal displacement, and for this reason it is selected for use here.

Character #17: 0 = thecal arcs reflexed after dehiscence; 1 = thecal arcs maintaining their original curvature or flattened after dehiscence.

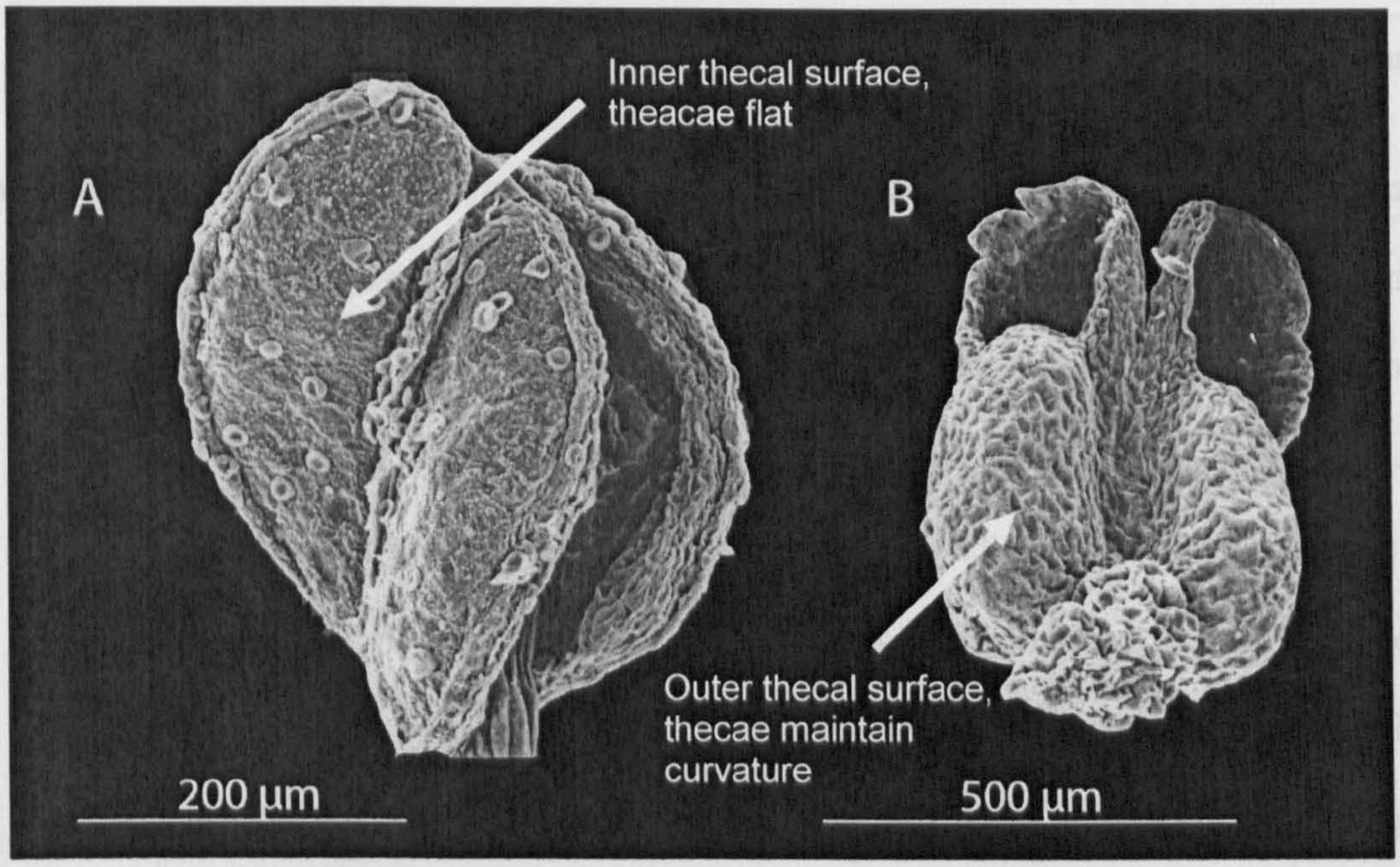


Figure 4.4. Anthers of *Myrcia s.l.* A. *Myrcia variabilis Mello-Silva 1699*; B. *Gomidesia tijucensis Nic Lughadha 195.*

Pollen

Pike (1956) surveyed Myrtaceae pollen from the southwestern Pacific genera, finding the majority of Myrtaceae taxa to possess syn- or parasyncolpate grains with an extremely faintly patterned or unpatterned exine or brevi- or brevissimicolpate grains with a smooth exine. Genera of Myrteae were found to almost exclusively possess longicolpate pollen grains with a faintly patterned exine. Within Myrteae, exceptional genera were *Octamyrtus*, which has pollen c. six times larger than any other and *Pimenta* in which grains are smaller and more rounded than average in the tribe and have no extensions to the colpi. Only *Calyptranthes* was examined from *Myrcia s.l.*, and its pollen was found to be similar to the rest of the tribe. Given the similarity of pollen within Myrteae genera it seems unlikely that many differences would be found within sections of *Myrcia s.l.*; however, a future study of Myrteae and *Myrcia s.l.* pollen would be

worthwhile, particularly with increased sampling of *Myrcia s.l*, to confirm pollen conservation within the group.

Locularity

'*Myrcia* group' species (except *Mitranthes*, see Chapter 1) have two ovules per locule. Locularity varies between bi- or tri- or occasionally tetralocular, with the majority of species having the first condition and a minority, mostly associated with *Aulomyrcia* and *Gomidesia,* the second; tetralocular ovaries are rare and exclusive to *Gomidesia*. De Candolle (1828) used number of locules per ovary in part to describe groups in *Myrcia*, although these groups were heavily and erroneously influenced by the distributions of species. Berg (1855-56, 1857-59) used the ovary locularity similarly but independent of geography to distinguish groups within *Aulomyrcia*, defining seven unranked, infrageneric groupings in part upon this character (Table 2.6). This character is unambiguous to code and has potential to give a clear taxonomic signal. None of the species used in this analysis had tetralocular ovaries.

Character #18: 0 = ovary bilocular; 1 = ovary trilocular; 2 = ovary tetralocular.

Fruit

De Candolle (1828) named his primary divisions of *Myrcia,* sections *Sphaerocarpae* and *Oocarpae,* according to the shape of the fruit, further dividing section *Sphaerocarpeae* into those with truly round fruits *vs* those with subglobular fruits (see Chapter 1), correlating these subdivisions with locularity and geographical distribution. Subsequent workers (Berg 1855-56; Niedenzu 1893; Legrand, 1961; McVaugh 1968,) have noted the tendency for *Myrcia* section *Myrcia* to have cylindrical, elongated fruits (Fig. 4.3Aiv), whereas *Myrcia* section *Aulomyrcia* fruits are more rounded (Fig. 4.3B–Giv). None of these subsequent authors however have used fruit shape as a defining character, possibly due to its plasticity; it is possible (although rare) to find species of one or other section apparently displaying the 'wrong' fruit shape. Despite this, when mean fruit heights and widths of those species included here were plotted against each

other, a break in the data corresponded to this described difference in shape (Fig. 4.5). As a result, the use of this fruit shape character for phylogenetic reconstruction will be attempted here.

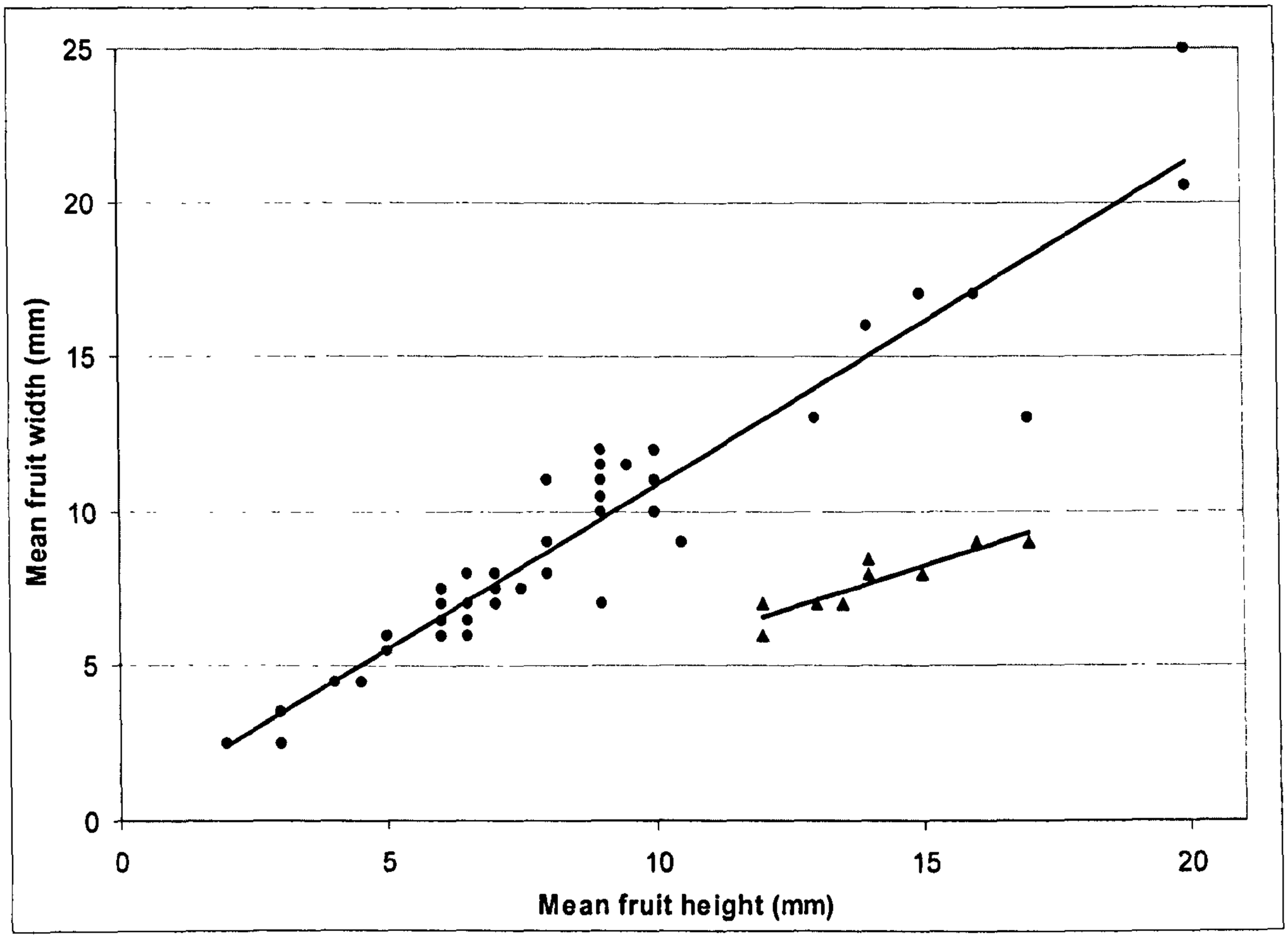


Figure 4.5. Fruit height vs. width in *Myrcia s.l.* Triangular and circular points correspond to character states identified (triangles = 0, circles = 1).

As a result of variation in the calyx and hypanthium and opening of the flower, there is considerable variation in posture and arrangement of the remains of the calyx upon the fruit. Species of *Myrcia* section *Myrcia* have erect, rounded calyx lobes in fruit, held c. 90° from the top of ovary (Fig. 4.3Aiv), and *Gomidesia* species have a similar arrangement except the lobes are usually truncate (Fig. 4.3Giv), although some can be acute. Species of those *Myrcia* section *Aulomyrcia* and *Marlierea* with an elongated hypanthium and horizontal tearing of the calyx tend to have cylindrical remains of the hypanthium with the calyx lobes having cleanly fallen away or some small fragments remaining (Fig. 4.3Civ); those with vertical calyx tearing tend to have the torn calyx lobes remaining splayed upon the disc (Fig. 4.3Eiv) or falling. *Calyptranthes* species have a similar arrangement with the calyptra falling or attached by a small piece of tissue (Fig.

4.3Fiv). Those species of *Myrcia* section *Aulomyrcia* that open without tearing tend to have the calyx lobes and hypanthium persistent and flared and held at a c. 45° angle from top of ovary (Fig. 4.3Div), whereas those with a shorter tube and reflexed calyces have lobes entirely reflexed back upon upper portion of fruit. Desptite trends in calyx posture on the fruit, variation and exception to these arrangements was such that it was impossible to transform patterns into discrete character states.

Character #19: 0 = fruit cylindrical (more than one and a half times as tall as broad); 1 = fruit globose (less than one and a half times as tall as broad).

Biochemistry

Porter et al. (2000) conducted a biochemical survey of Myrtaceae including 67 species in 33 Myrteae genera, 20 of these of *Myrcia s.l.* That study did not identify subtribal or subgeneric trends other than to support the separation of *Eugenia* from Syzygieae (*sensu* Wilson et al. 2005) and tentatively segregate *Luma* from *Myrcia s.l.* A further survey of *Myrcia s.l.* biochemistry is currently underway (Matsumoto et al. in prep.) and will build on the foundations laid by Porter et al. (2000) to detect biochemical patterns present within this group.

Data collection

Nineteen characters were investigated and scored for this analysis (Table 4.2). Seven characters are associated with flowers and one with fruits whereas four are concerned with the inflorescence and seven characterise vegetative aspects. Morphological characters were each coded from an ideal minimum of three herbarium specimens (or as many as were available; Appendix 3), including those used for the molecular part of this study (Appendix 4) available at Kew, and also from field observations in Brazil and French Guiana and additional field observations by Marcos Sobral, Kazue Matsumoto and Eimear Nic Lughadha (pers. comm.). The matrix was compiled for 80 species, representing the core genera of *Myrcia s.l.* as well as *Rubachia*, *Eugeniopsis* and 18 out of 29 of Berg's infrageneric groups of *Myrcia* and *Aulomyrcia*. Seven outgroup species

were also included, six from the *'Plinia group'* plus *Algrizea macrochlamys*. Phylogenetic analysis was performed using PAUP * 4.0b2 (Swofford 2002) using the same settings as described in Chapter 3. Optimization of morphological characters was performed with MacClade 4.0 (Maddison & Maddison 2000), and characters were optimized onto a randomly selected single most parsimonious tree (MPT) resulting from parsimony analyses.

Table 4.2. Morphological matrix. Inapplicable character -, state unknown ?, sequenced by K. Matsumoto *.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Neomitranthes cordifolia	0	0	2	1	0	0	1	-	-	1	0	0	0	0	1	0	0	0	1
Plinia pauciflora	0	0	0	1	0	0	1	-	-	0	0	1	0	0	1	0	0	0	1
Siphoneugena densiflora	0	0	2	1	0	0	1	-	-	0	0	2	0	1	1	0	0	0	1
Siphoneugena guifolyleana	0	0	2	1	0	0	1	-	-	1	0	2	0	1	1	0	0	0	1
Myrciaria cauliflora	0	0	2	1	0	0	1	-	-	1	0	1	0	0	1	0	0	0	1
Calyptranthes concinna	1	0	0	1	0	0	0	0	0	0	0	-	1	0	1	0	0	0	1
Calyptranthes lanceolata	1	0	0	0	0	1	0	0	0	0	0	-	1	0	1	0	0	0	1
Calyptranthes kiaerskovii	1	0	0	0	0	0	0	-	0	0	0	-	1	0	1	0	0	0	1
Calyptranthes thomasiana	1	0	0	0	1	0	0	-	0	0	0	-	1	0	1	0	0	0	1
Calyptranthes grandifolia	1	0	0	0	0	1	0	0	0	0	0	-	1	0	1	0	0	0	1
Calyptranthes clusiifolia	1	0	0	1	0	1	0	0	0	0	0	-	1	0	1	0	0	0	1
Marlierea eugeniopsoides	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1
Marlierea suaveolens	1	0	0	0	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1
Marlierea obscura	0	0	0	1	0	0	0	0	1	0	1	1	0	0	1	1	0	0	1
Marlierea pilodes	1	0	0	0	1	1	0	0	0	0	0	2	0	0	1	0	0	0	1
Myrcia clavija	0	1	2	1	0	1	0	1	1	1	1	1	0	0	1	0	?	0	1
Marlierea angustifolia	0	0	0	1	0	0	0	-	0	0	0	2	0	0	1	0	0	0	1
Marlierea subacuminata	1	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	1
*Marlierea obversa**	0	0	2	1	0	1	0	1	1	1	1	1	0	0	1	0	0	0	1
Myrcia eumecephylla	0	1	2	1	0	1	0	0	1	1	?	1	0	?	1	0	?	?	1
*Marlierea regeliana**	1	0	0	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1
*Marlierea warmingiana**	0	0	2	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1
*Marlierea sucrei**	0	0	1	1	0	1	0	1	1	1	0	1	0	0	1	0	0	0	1
*Marlierea parviflora**	0	0	0	0	0	1	0	0	1	0	1	1	0	1	1	0	0	0	1
*Marlierea tomentosa**	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0	0	1
*Marlierea clausseniana**	1	0	0	1	0	0	0	0	0	0	0	2	0	0	1	0	0	0	1
*Marlierea glazioviana**	1	0	0	0	0	1	0	0	0	0	0	2	0	0	1	0	0	0	?
Gomidesia tijucensis	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
Gomidesia pubescens	0	0	0	1	0	0	0	-	0	0	0	0	0	0	1	1	1	0	1
Gomidesia affinis	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
Gomidesia schaueriana	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
Gomidesia spectabilis	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
Gomidesia flagellaris	0	0	0	1	0	0	0	-	0	0	0	0	0	0	1	1	1	0	1
Gomidesia martiana	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
Gomidesia anacardiifolia	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
Gomidesia sellowiana	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1
Myrcia tenuivenosa	1	0	0	1	0	0	0	0	0	0	?	0	0	0	1	0	0	0	?
Myrcia pubipetala	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia reticulosa	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia inaequiloba	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	1
Myrcia laruotteana	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1	0	0	0	1
Myrcia tomentosa	0	0	2	1	0	0	0	1	1	1	1	0	0	1	1	0	0	0	1
Myrcia multiflora	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Myrcia torta	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
Myrcia paracatuensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia guianeensis	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia rorida	0	0	2	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
Myrcia grandiflora	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

continued...

Table 4.2. continued

Myrcia saxatilis	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1
Myrcia racemosa	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1
Myrcia bicarinata	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	?	
Myrcia rostrata	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia coumeta	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia venulosa	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	
Myrcia bracteata	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia velutina	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia suffruticosa	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia detergens SP	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	1
Myrcia linguaeformis	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia selloi	0	1	0	0	0	0	0	1	1	1	1	0	0	1	1	0	0	0	1
Myrcia amazonica PR	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	1
Myrcia obtecta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia pulchra	1	0	0	0	1	0	0	0	0	0	0	2	0	0	1	0	0	0	1
Myrcia formosiana	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia detergens PR	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	1
Myrcia insularis	0	1	2	0	0	1	0	1	1	1	1	0	0	0	1	0	0	0	1
Myrcia citrifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia amazonica	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	1
Myrcia decorticans	0	0	0	0	0	0	0	1	1	0	1	1	0	0	1	0	0	0	1
Myrcia arborescens	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia anceps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia subverticillaris	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1
Myrcia eriopus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrcia aff. selloi RJ	0	0	0	0	0	0	0	0	0	1	0	0	0	?	1	0	0	0	1
Myrcia variabilis	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1
Myrcia rufipes	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Myrcia mischophylla	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
Myrcia micropetala	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1
Algrizea macrochlamys	0	0	0	1	0	-	2	-	0	0	0	0	0	0	1	1	0	2	1

Molecular methodology

Fifty-one further '*Myrcia* group' accessions were sequenced for ETS, ITS, and *psbA-trnH* and 18 for *matK* (Appendix 4), following the same DNA extraction, sequencing and alignment protocols described in Chapter 3. Eight of these, marked with an asterisk (*) in Table 4.2 were collected and sequenced for ITS and *psbA-trnH* by Kazue Matsumoto as part of a separate study underway at the Universidade de Campinas, SP, Brazil; these data are used here with permission.

Again, the sampling represents as much morphological diversity and geographical variation as possible with species included from as many of the geographical distribution centres of the clade

and from as many of Berg's (1855-56, 1857-59) infrageneric groups as possible. The inclusion of genera distantly related from *Myrcia s.l.* in the morphological analysis would make it even harder to devise a representative and homologous set of characters for phylogenetic analysis. To reduce the number of genera and also to accelerate search times, the molecular sample was first analysed independently using all non-'*Myrcia* group' taxa included in the Myrteae analysis of Chapter 3; then, beginning with the clade most distant to *Myrcia s.l.*, clades were removed one by one, with the analysis re-performed after each removal and the effect within the in-group observed. Support and resolution in the in-group remained stable throughout this process, until *Algrizea macrochlamys* and the '*Plinia* group' were removed, when resolution and support within the resulting phylogeny reduced dramatically; the two latter taxa were therefore selected as outgroups.

Alignment and phylogenetic analysis were performed using PAUP * 4.0b10 (Swofford, 2002) using the same settings as described in Chapter 3. Successive weighting was not used in this case as the resolution obtained from the parsimony analyses was sufficient for phylogenetic results and taxonomic comment. Alignments are provided in Appendix 5 and indels are listed in Table 4.3.

Table 4.3. Informative indels scored.

Indel	Positions affected
1	ITS 327
2	ITS 340–342
3	*psbA-trnH* 215–219
4	*psbA-trnH* 783–829
5	*psbA-trnH* 892–902
6	*psbA-trnH* 917–965
7	ETS 495–505

Combined analysis

To assess congruence among pairwise combinations of the morphological and molecular datasets, 100 replicates of the partition homogeneity test (Farris et al. 1994) were applied to the data, with a full heuristic search and random taxon addition. This was followed by analysis of the combined dataset using parsimony analysis in PAUP * 4.0b10 (Swofford 2002) and Mr Bayes v3.0b4 (Huelsenbeck & Ronquist 2001) using the same settings as described in Chapter 3.

Results

Sequence data

Statistics from the parsimony analyses are summarised in Table 4.4; the plastid analysis comprises 495 and 805 base pairs from *psbA-trnH* and *matkK*, respectively. Topologies are characterised by lack of bootstrap support along the spine of the trees and low resolution in the ingroup, as in Chapter 3. The *Myrcia s.l.* clade plus *Algrizea* is maintained as distinct from the five '*Plinia* group' species in the ITS, ETS and plastid analyses with 59, 99 and 60 bootstrap percentages (BP), respectively. Ten clades [*Calyptranthes concinna, C. lanceolata, C. grandifolia, C. clusiifolia*], [*Marlierea subacuminata, M. clausseniana*], [*Marlierea suaveolens, M. obscura, M. regeliana, M. warmingiana, M. sucrei, M. parviflora, M. tomentosa*], [*Marlierea pilodes, M. angustifolia, M. glazioviana, Myrcia bicarinata, Myrcia pulchra*], [*Gomidesia spectabilis, G. martiana*], [*Myrcia reticulosa, M. venulosa*], [*Myrcia laruotteana, M. tomentosa, M. selloi, M. cf. selloi*], [*M. multiflora, M. torta, M. rorida*] [*Myrcia rostrata, M. fallax, M. velutina, M. bracteata, M. formosiana, M. eriopus, M. anceps, M. coumeta* (excluding this last species in the plastid tree)] and [*Myrcia detergens* SP, *M. detergens* PR, *M. amazonica* PR] receive >50 BP in all the three topologies. The ITS and ETS topologies each receive >50 BP for the additional clades; [*Marlierea eugeniopsoides, M. subacuminata, M. clausseniana*], [*Marlierea obversa, Myrcia eumecyphylla*], [*Gomidesia tijucensis, G. pubescens, G. schaueriana, G.*

flagellaris, G. anacardiifolia, G. sellowiana , Myrcia mischophylla], and [*Myrcia rostrata, M. fallax, M. velutina, M. bracteata, M. formosiana, M. eriopus, M. anceps, M. coumeta, M. grandiflora, M. suffruticosa, M. arborescens*]. The ETS and plastid topologies each receive >50 BP for the clades [*Myrcia obtecta, M. subverticillaris*] and [*M. micropetala, M. racemosa*]. The ITS tree alone receives >50 BP for the clade [*Myrcia clavija, Marlierea obversa, Myrcia eumecephylla, Myrcia insularis*]. The ETS tree alone receives >50 BP for the clade [*Myrcia paracatuensis, M. linguaeformis, M. rufipes, M. variabilis, M. guianensis, M. obtecta, M. subverticillaris, M. citrifolia*]. The plastid tree alone receives >50 BP for the clades [*Marlierea suaveolens, M. obscura, M. regeliana, M. warmingiana, M. sucrei, M. parviflora, M. tomentosa, M. clavija, Marlierea obversa, M. eumecephylla, M. multiflora, M. detergens* SP, *M. detergens* PR, *M. amazonica* PR, *M. insularis, M. amazonica*], [*M. pubipetala, Myrcia reticulosa, M. venulosa*] and [*Myrcia torta, M. rorida, M. micropetala, M. racemosa*]. There was no hard incongruence between any groupings (i.e., >50 BP) in the three analyses.

Table 4.4. Data set and parsimony-based tree characteristics.

	ITS	ETS	plastid	Combined molecular	Morphology	Combined molecular and morphology
Characters	686	447	1304	2437	19	2456
No. variable characters (%)	181 (26)	213 (47.7)	168 (12.9)	562 (23.1)	19 (100)	607 (24.7)
No. potentially parsimony informative characters (%)	112 (16.3)	133 (29.8)	72 (5.5)	317 (12.7)	19 (100)	357 (14.5)
Trees	>10,000	>10,000	>10,000	>10,000	>10,000	2411
Length (L)	395	490	213	1143	72	1242
Consistency Index (CI)	0.58	0.57	0.83	0.6	0.32	0.57
Retention Index (RI)	0.73	0.79	0.86	0.76	0.8	0.75

Combined sequence data

Statistics from the combined sequence data analysis are summarised in Table 4.4. The tree resulting from Bayesian analysis of the combined molecular datasets is shown in Fig. 4.6, with bootstrap percentages from parsimony analysis of the same data indicated. This topology provides a resolved tree upon which parts of the following discussion are based; support, however, is low along the spine of this tree, and at several nodes there is neither bootstrap (BP >50) nor Bayesian support (PP >95).

Morphological data

Statistics from the analysis of the morphological data are summarised in Table 4.4; the combined consensus tree from the morphological analysis is shown in Fig. 4.7. The data failed to provide resolution between many species, with the following clades identified in what is otherwise a polytomy of all sampled species: [*Siphoneugena densiflora, Siphoneugena guifolyleana*; BP 55], [*Myrcia clavija, M. eumecephylla, M. insularis*; BP <50], [*Marlierea obscura* [[[*Gomidesia tijucensis, G. spectabilis, G. martiana, G. sellowiana* BP <50] *G. pubescens, G. affinis, G .schaueriana, G. flagellaris, G. anacardiifolia* BP <50], *Myrcia mischophylla, Algrizea macrochlamys*; BP <50] BP <50], [*Myrcia ramulosa, M. micropetala*; BP 59] and [*Myrcia grandiflora, M. rostrata, M. coumeta,* [*M. fallax, M. anceps;* BP 52] *M. bracteata, M. velutina, M. suffruticosa, M. formosiana, M. arborescens, M. eriopus*; BP 66]. Selected morphological optimisations are presented in Figs. 4.13–4.20.

Combined data

Statistics from the analysis of the combined molecular and morphological data are summarised in Table 4.4; the combined consensus tree this analysis is shown in Fig. 4.8. The molecular and morphological data sets provide different degrees of resolution and although little resolution is provided by the morphological analysis alone; those clades that are resolved in Fig. 4.7 are

congruent with molecular results. Despite this, the partition homogeneity test indicated that there is significantly different phylogenetic signal between the two data sets ($P = 0.01$).

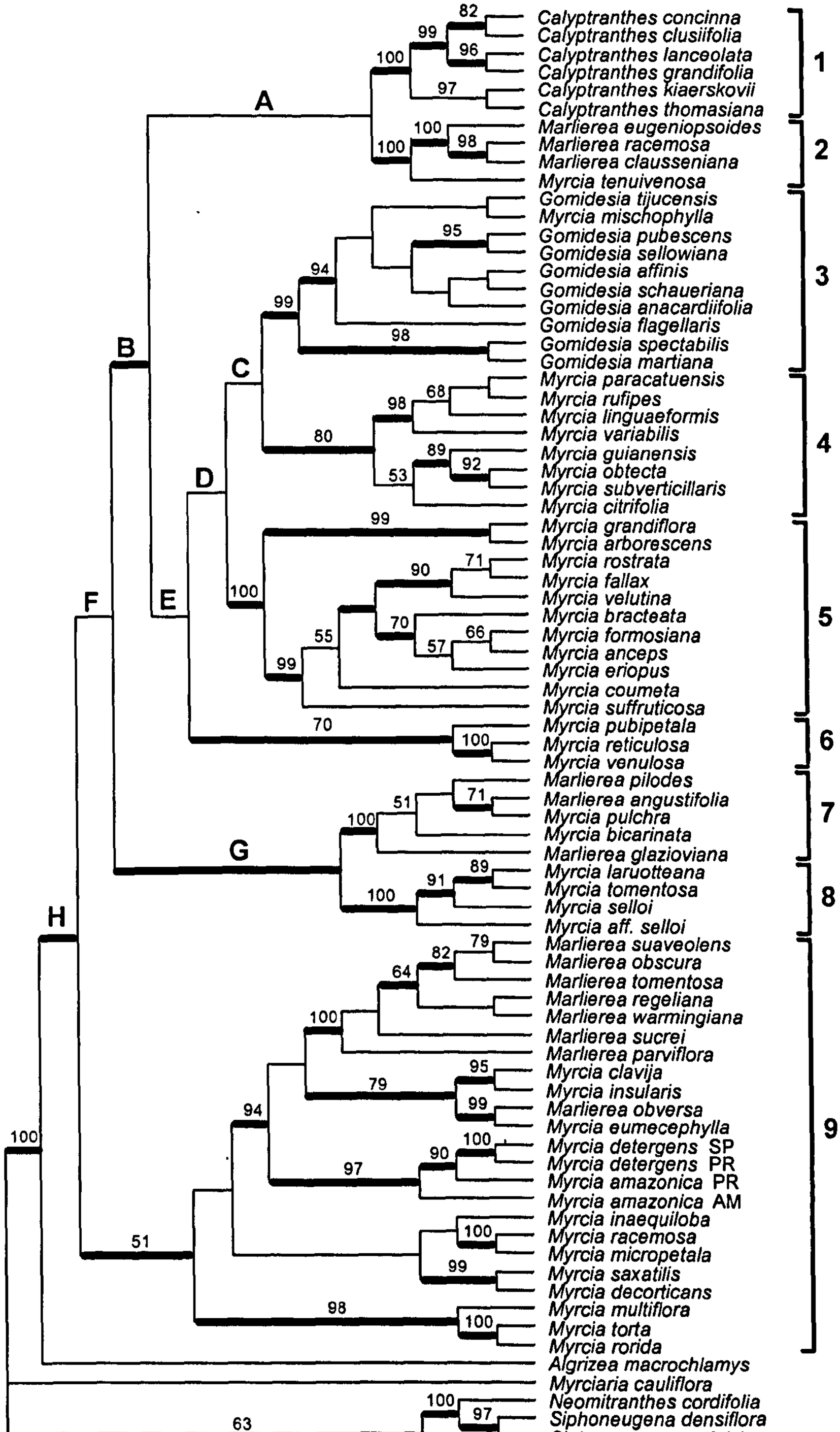


Figure 4.6. Consensus tree resulting from Bayesian analysis of the ITS, ETS, *psbA-trnH* and *matK* data. Bootstrap percentages (from parsimony analysis) greater than 50 are shown above branches; clades that receive Bayesian probabilities greater than 0.95 are marked in bold.

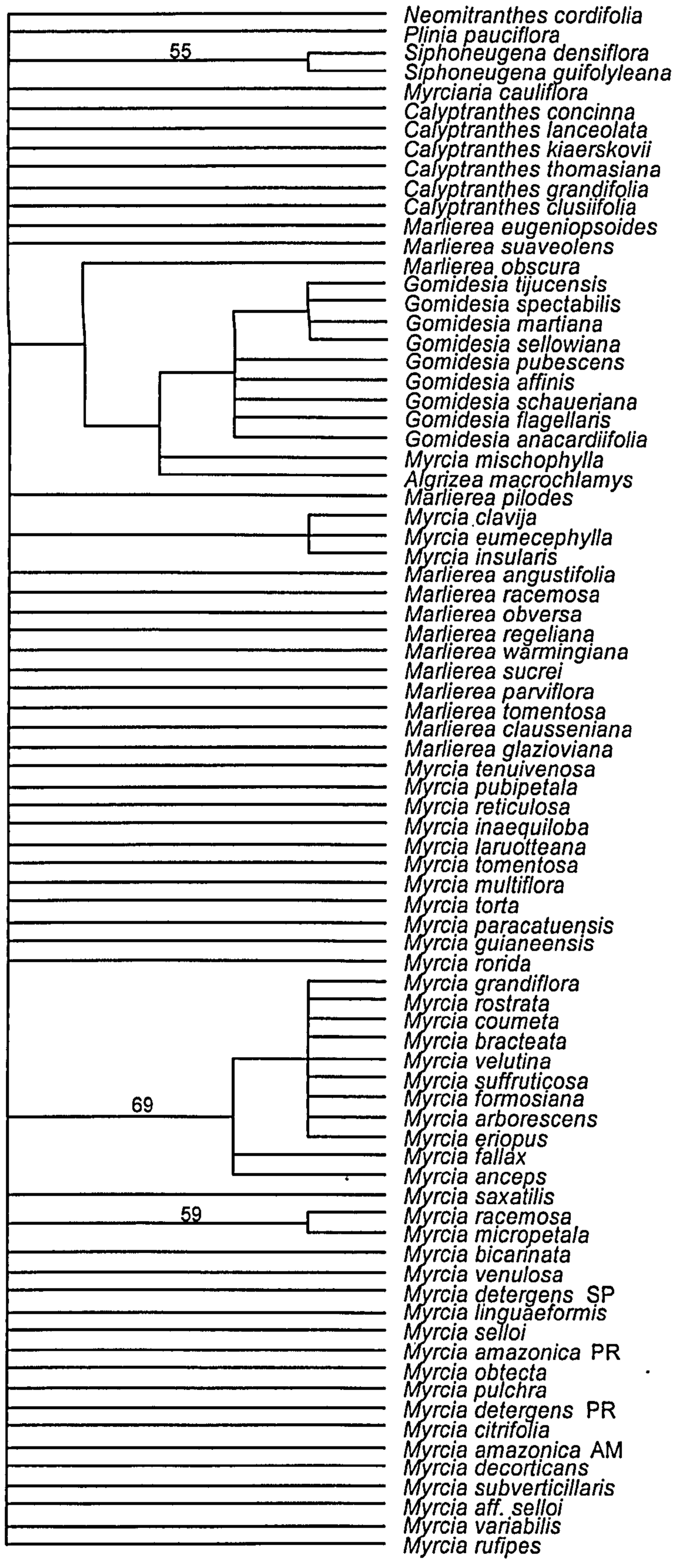


Figure 4.7. Strict consensus tree resulting from parsimony analysis of the morphological dataset. Bootstrap percentages greater than 50 are shown above branches.

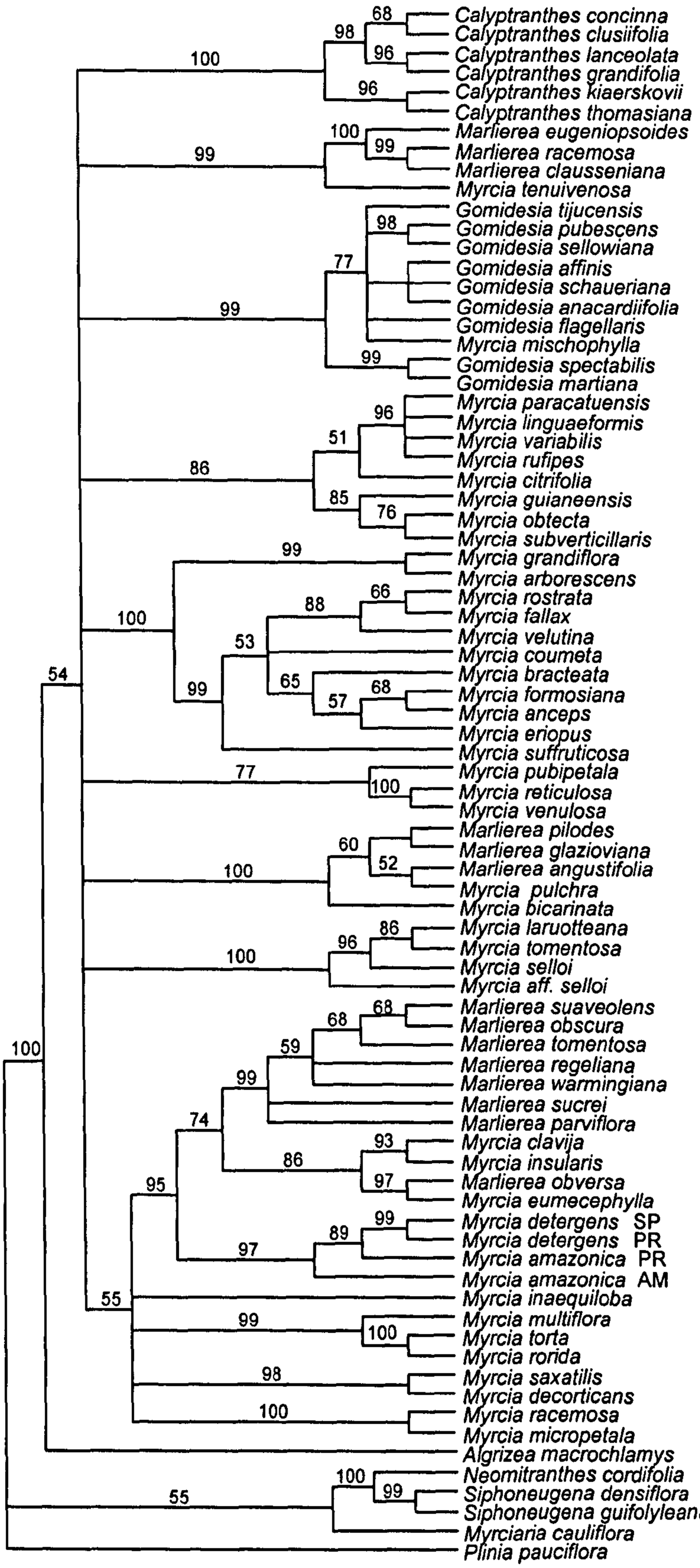


Figure 4.8. Strict consensus tree resulting from parsimony analysis of the ITS, ETS, *psbA-trnH*, *matK* and morphological data. Bootstrap percentages greater than 50 are shown above branches.

Discussion

Molecular analyses of *Myrcia s.l.*

Both Bayesian and parsimony analyses of the molecular data produce trees in which the '*Plinia* group' outgroup emerges sister to *Myrcia s.l.* and *Algrizea macrochlamys* (BP 100, PP 100). In the parsimony analysis, the clade of *Algrizea* and *Myrcia s.l.* is a polytomy of nine clades (each BP > 50 and numbered in Fig. 4.8), whereas in the Bayesian analysis *Algrizea* alone is sister to *Myrcia s.l.* (PP 97). This position of *Algrizea,* associated firmly with *Myrcia s.l.*, brings some clarity to the position of *Algrizea* after the tribal analysis of Chapter 3 in which it emerged, with little support, as sister to the '*Plinia* group' in parsimony analysis but sister to the '*Myrcia* group' in the Bayesian analysis. High support (BP 100 PP 100) for this arrangement clarifies this more local analysis and suggests that it is the addition of outgroups that reduces support and resolution in the more general analysis. The remainder of Fig. 4.6 will be discussed in terms of the nine numbered clades comprising the *Myrcia s.l.* polytomy that receive BP > 50 and PP > 95 in the strict consensus of the parsimony analysis. No other clades suggested in the Bayesian topology presented in Fig. 4.6 receive BP > 50 and only two, those marked B and F, receive PP > 95; these less well supported clades marked A–H will then be discussed.

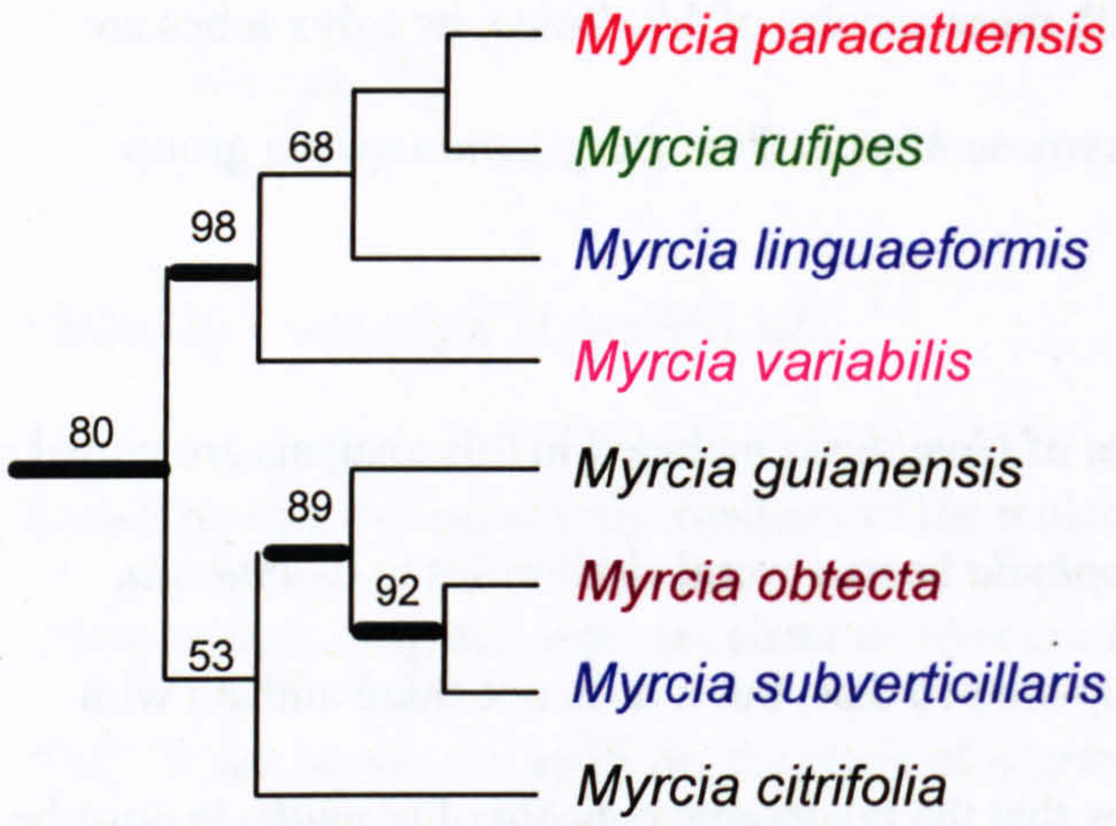
In Clade 1 (BP 100, PP 100) *Calyptranthes* is strongly supported as monophyletic, uniting the Caribbean species *C. kiaerskovii* and *C. thomasiana* (BP 97, PP 95) and linking them as sister to the Brazilian species sampled (BP 100, PP 100).

In Clade 2 *M. clausseniana* and *M. subacuminata* (both *Marlierea* sect. *Eugeniopsis sensu* Legrand 1962a; BP 98, PP 99) are united with *M. eugeniopsioides* (*Marlierea* sect. *Pseudocalyptra sensu* Legrand 1962a; BP 100, PP 99) and *Myrcia tenuifolia* (*Aulomyrcia* group *Cymosae sensu* O.Berg 1855-56; BP 100, PP 100). Although it is unsurprising that *M. tenuifolia* with sympodial branching and other characters reminiscent of cymose *Marlierea*

and *Calyptranthes* should be associated with these species of *Marlierea*, its calyx lobes are free and this species also resembles other cymose *Myrcia* from Berg's *Aulomyrcia* group *Cymosae* that fall in Clade 7.

In Clade 3 (BP 99, PP 100) the nine species of *Gomidesia* included in this analysis are united with *Myrcia mischophylla*. *Myrcia mischophylla* bears several similarities to *Gomidesia*, particularly in the possession of a hairy, cup-shaped disc, but it does not share anthers with displaced or reflexed thecae. This indicates that the taxonomic concept of *Gomidesia* must be broadened to include some species that do not share every morphological character, and as in so many cases in *Myrcia s.l,* the clade must be recognised based on a combination of morphological characters. The remainder of the clade groups *G. spectabilis* and *G. martiana* as sister (BP 98) to the rest of the clade; these species differ from the remainder in their possession of a hairy hypocotyl (Nic Lughadha 1997). Their sister relationship to the rest of the clade is unsurprising.

Clade 4 is also strongly supported (BP 80, PP 100) and contains eight species of *Myrcia* with regular, free calyx lobes, tri-locular ovaries, hypanthia prolonged into a tube above the ovary and symmetrical, well-developed panicles distributed in two to several of the higher branch axils with a lack of persistent bracts. This clade includes species distributed by Berg in five of his eleven tri- or tetralocular groups of *Aulomyrcia* (Fig 4.9); only *M. guianensis* and *M. citrifolia* did not feature in Berg's treatment. These two species are the only Amazonian representatives of a clade otherwise containing only species from southeastern Brazil, they were placed by McVaugh (1969) in his Guyanan *Myrcia* sects. *Armeriela* and *Aulomyrcia*, respectively. *Myrcia guianensis* is widespread, found throughout the range of *Myrcia s.l.,* and the sampled specimen of *Myrcia citrifolia* is from the British Virgin Islands although the species is also recorded from the Amazon.



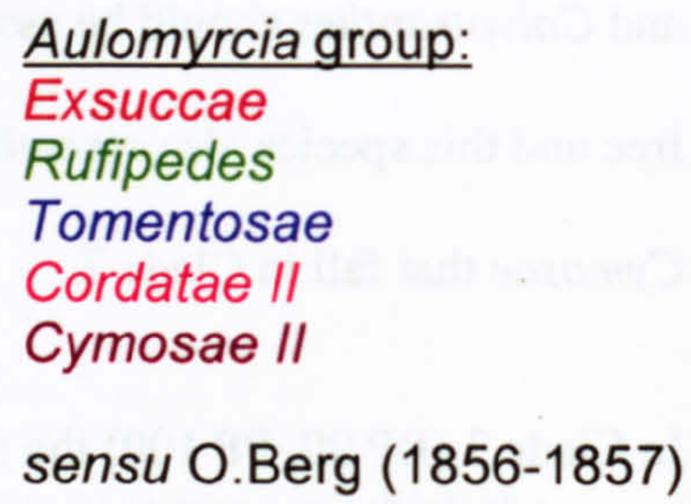


Figure 4.9. Detail of Clade 4. Colours of names correspond to unranked infrageneric groups of *Aulomyrcia sensu* O. Berg (1857-59). Names in black were not assigned to infrageneric groups by Berg.

The results for Clade 5 strongly suggests (BP 100, PP 100) that Berg's genus *Myrcia* (with a few exceptions, discussed later), which is equivalent to Niedenzu's subgenus *Eumyrcia* and McVaugh's *Myrcia* sect. *Myrcia)* is monophyletic and composed of bi-locular species with hypanthia clearly not extended above the ovary, a pubescent staminal ring more than twice as wide as the flat disc and symmetrical and well-developed panicles distributed in two to several of the higher branch axils with a lack of persistent bracts. Of the eleven samples in this clade, eight were distributed by Berg in five infrageneric groups (Fig. 4.10); the clade is comprised of two principal groups, with *M. grandiflora* and *M. arborescens* emerging sister to the rest. Again, the majority of species sampled are from southeastern and central Brazil; however, Amazonian representatives of widespread species such as *M. splendens* and species with distributions limited to the Amazon such as *M. coumeta* are nested within the clade.

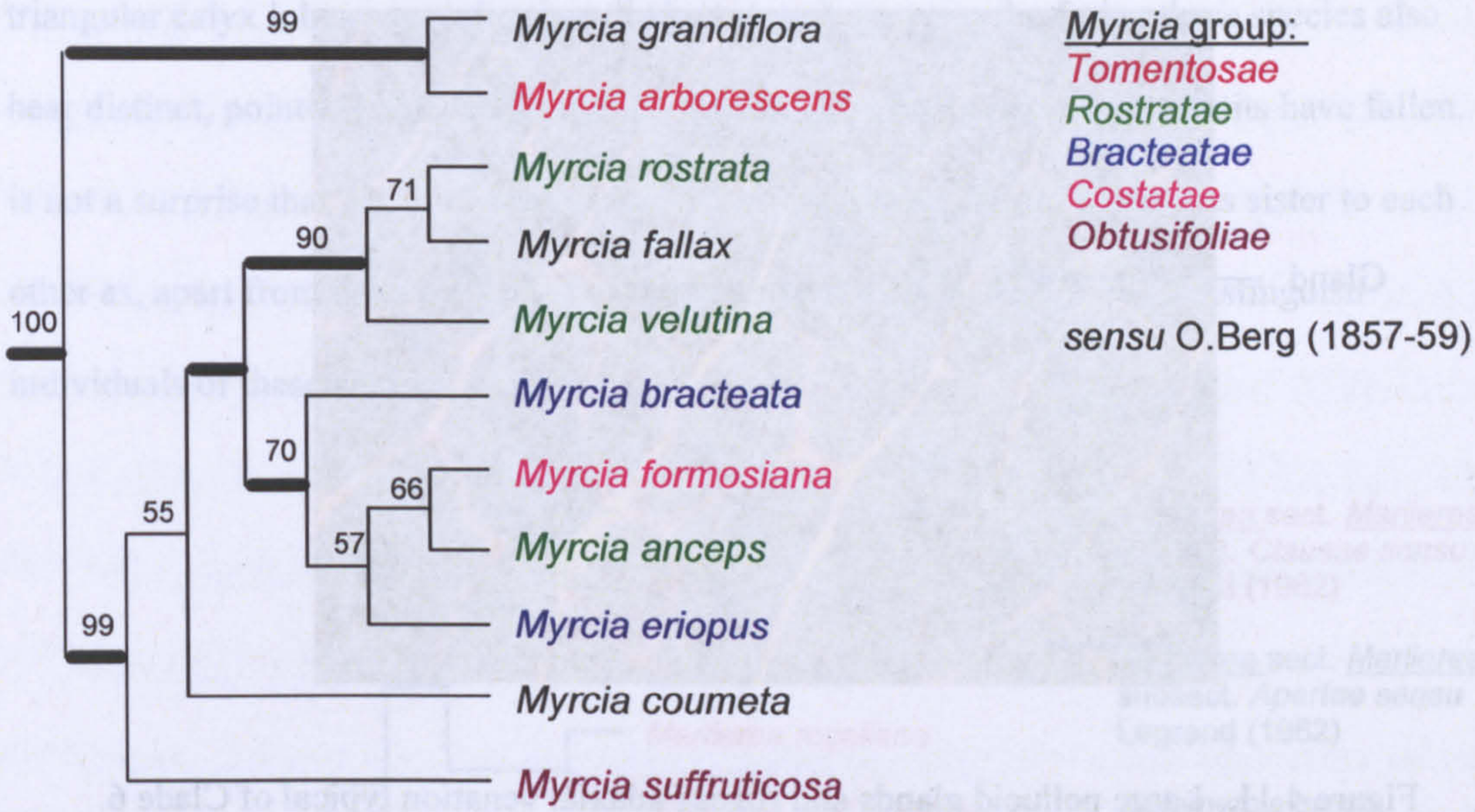


Figure 4.10. Detail of Clade 5. Colours of names correspond to unranked infrageneric groups of *Myrcia sensu* O. Berg (1857-59). Names in black were not assigned to groups by Berg.

Clade 6 (BP 70, PP 100) comprises three species, of which *Myrcia pubipetala* was recorded by Berg (1857–59) and Legrand (1961) to be bilocular (it is in fact trilocular) and was classified by Berg in *Myrcia* sect. *Myrcia* and by Legrand as *Myrcia* subgen. *Aulomyrcia* series *Crassae.* The remaining two species in this clade, *M. reticulosa* and *M. venulosa* (both trilocular) were assigned by Berg to *Aulomyrcia*, infrageneric groups *Cordatae II* and *Rugosae*, respectively. These three species share similar morphology to the *Aulomyrcia* species in Clade 4 but differ in having particularly coriaceous leaves with distinctly raised venation on the abaxial, and particulary distinct on adaxial surfaces, and often large pellucid glands, distributed one or few per cell of the network of veins (Fig. 4.11); it is predicted that species such as the relatively common *M. richardiana* or the rarer *M. heringii*, which both display these foliar characters, will fall into this clade.

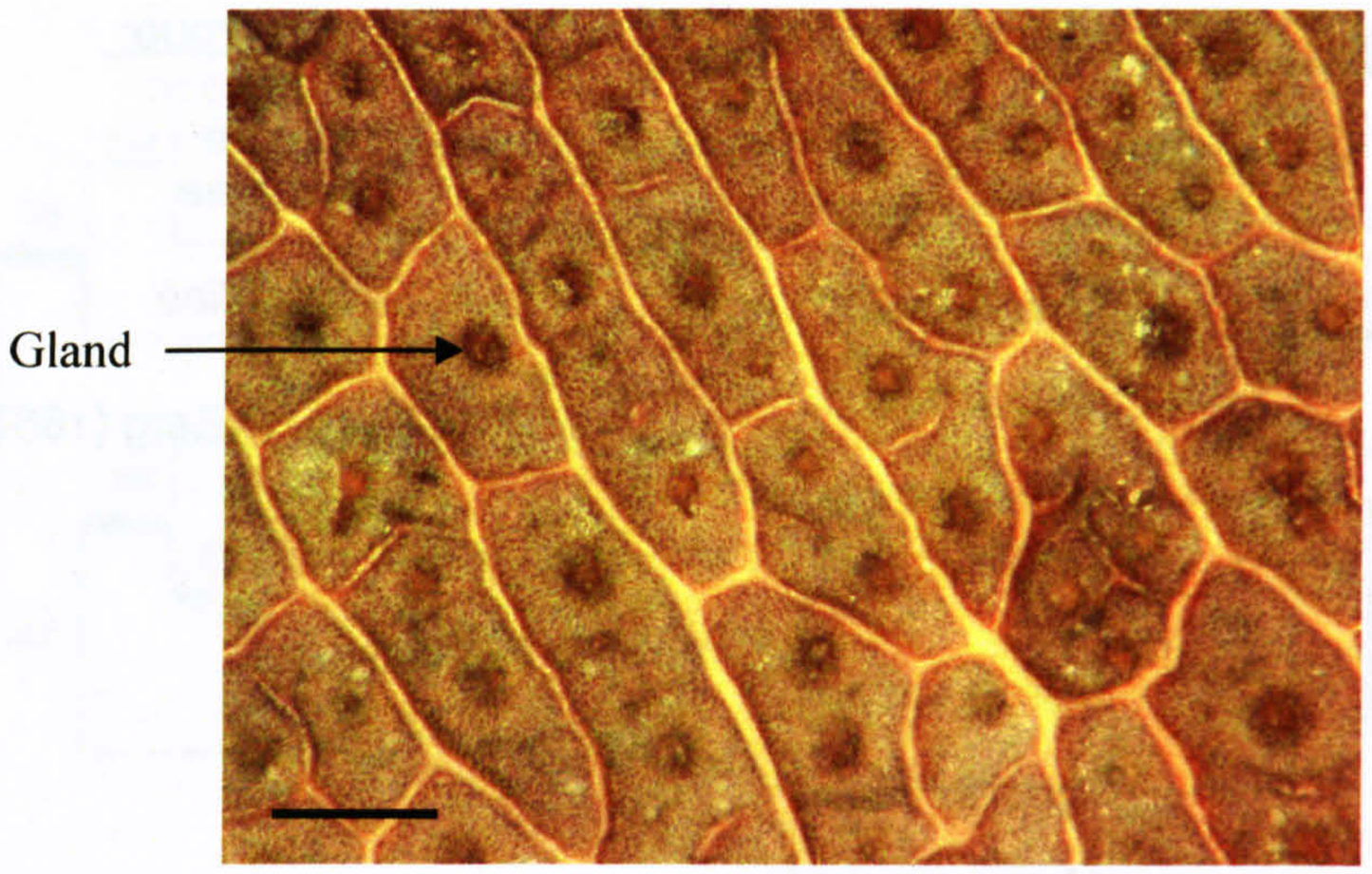


Figure 4.11. Large pellucid glands and rugose adaxial venation typical of Clade 6. *Myrcia reticulosa* Harley 50309. Scale bar = 1.5 mm.

In Clade 7 there is strong support (BP 100, PP 100) for a clade comprising three species of *Marlierea*; *M. angustifolia* (*Marlierea* sect. *Eugeniopsis sensu* Legrand), *M. pilodes* (*Marlierea* sect. *Marlierea* subsect. *Apertae sensu* Legrand) and two species of *Myrcia*, *Myrcia pulchra* (*Aulomyrcia* group *Cymosae sensu* Berg) and *M. bicarinata* (*Aulomyrcia* group *Cymosae II sensu* Berg). All these species demonstrate sympodial vegetative branching and cymose panicles; *Marlierea pilodes* was initially described in *Myrcia* by Kiaerskou and placed in his first *Aulomyrcia* group with other dichotomous branching species including *Myrcia pulchra*; *M. pilodes* was transferred to *Marlierea* by Kawasaki (1989). The morphological similarities of this group explain the grouping of the species within it; however, unification of *Marlierea* species from two of the sections of *Marlierea sensu* Legrand suggest that these sections based entirely on characters of the calyx are artificial.

Clade 8 strongly unites (BP 100, PP 100) *Myrcia laruotteana, M. tomentosa, M. selloi* and an unidentified species with similarities to, but distinct from, *M. selloi*. These southeastern Brazilian species are united by a characteristic morphology, such as buds with a distinct constriction below the ovary, inflorescences narrowing abruptly into narrow triangular panicles with asymmetrical branching and open flowers with acutely reflexed pointed,

triangular calyx lobes remaining in a distinct star-shape upon the fruits; these species also bear distinct, pointed bracts beneath each flower that usually persist after fruits have fallen. It is not a surprise that *Myrcia laruotteana* and *M. tomentosa* should emerge as sister to each other as, apart from the pubescence of the latter species it is often hard to distinguish individuals of these species.

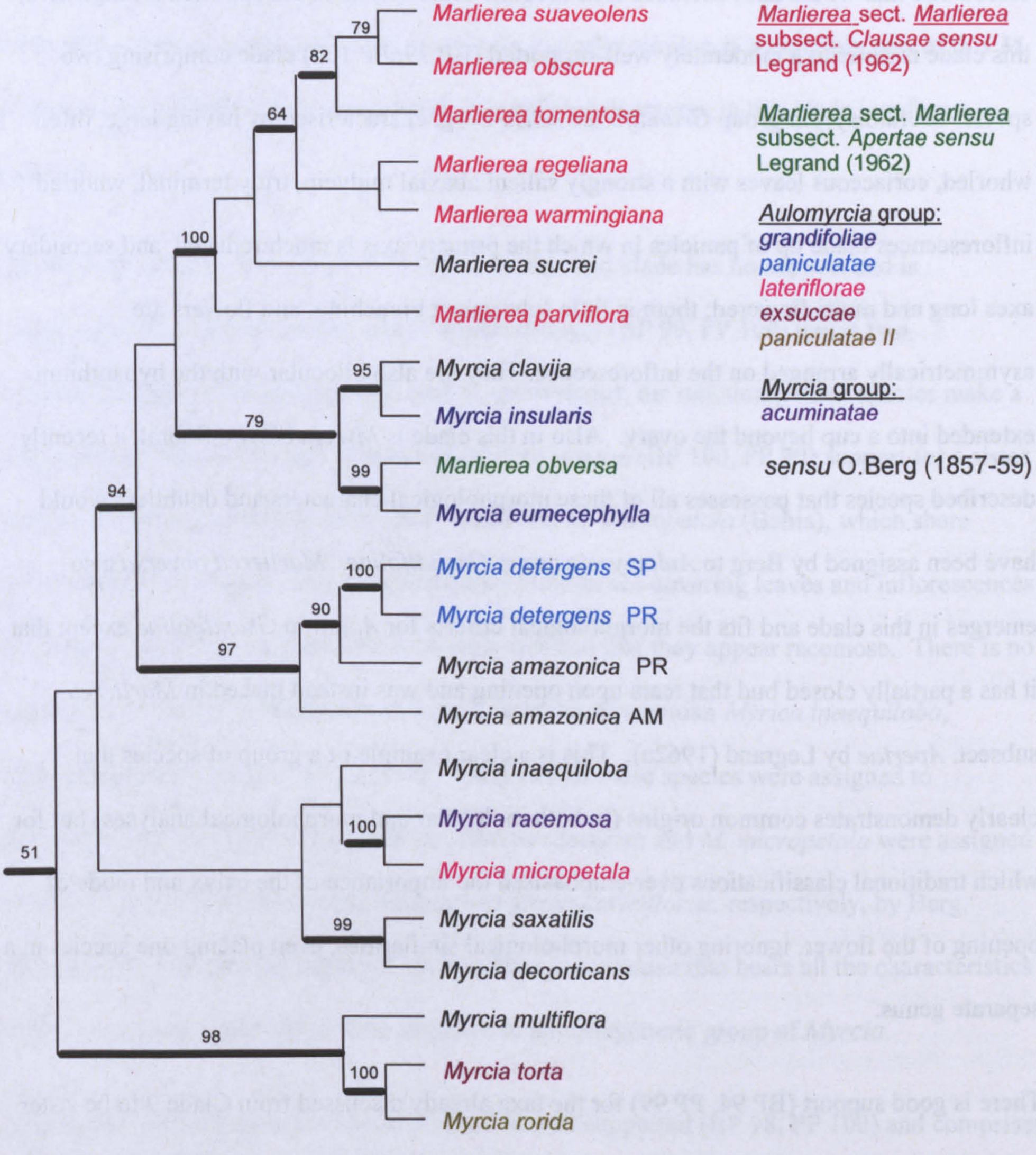


Figure 4.12. Detail of Clade 9. Colours of names correspond to sections of *Marlierea sensu* Legrand (1962) and infrageneric groups of *Aulomyrcia* or *Myrcia sensu* O. Berg (1857-59); names in black were not assigned to groups by these authors.

Containing 23 species, Clade 9 is the largest of those recognised in the *Myrcia s.l.* polytomy; it is poorly supported in the parsimony analysis (BP 51), but well supported in the Bayesian analysis (PP 96). A detailed look at this clade (Fig. 4.12) indicates it to contain a well-supported (BP 100, PP 100) clade of six species of *Marlierea* sect. *Marlierea* subsect. *Clausae sensu* Legrand (1962a) plus *M. sucrei*, described after Legrand's work; it has a closed bud that would have included it in subsect. *Clausae*. In an unsupported arrangement, this clade is sister to a moderately well-supported (BP 79, PP 100) clade comprising two species of *Aulomyrcia* group *Grandifoliae sensu* Berg, characterised by having large, often whorled, coriaceous leaves with a strongly salient abaxial midvein, truly terminal, whorled inflorescences made up of panicles in which the primary axis is much reduced, and secondary axes long and multi-flowered; there is little subsequent branching, and flowers are asymmetrically arranged on the inflorescence. They are also bilocular with the hypanthium extended into a cup beyond the ovary. Also in this clade is *Myrcia clavija* Sobral, a recently described species that possesses all of these morphological characters and doubtless would have been assigned by Berg to *Aulomyrcia* group *Grandifoliae*. *Marlierea obversa* also emerges in this clade and fits the morphological criteria for *A.* group *Grandifoliae* except that it has a partially closed bud that tears upon opening and was instead placed in *Marlierea* subsect. *Apertae* by Legrand (1962a). This is a clear example of a group of species that clearly demonstrates common origins (in both molecular and morphological analyses) but for which traditional classifications over-emphasised the importance of the calyx and mode of opening of the flower, ignoring other morphological similarities, even placing one species in a separate genus.

There is good support (BP 94, PP 99) for the taxa already discussed from Clade 9 to be sister to *Myrcia detergens* and *M. amazonica*. It was previously thought that the former species occurred in southeastern Brazil, whereas the latter was from the Amazon, extending to the more northerly states of Matto Grosso and Bahia and that the two species distributions do not overlap. The collection of *M. amazonica* made in the southern Brazilian state of Paraná

remained unidentified for a long time until Marcos Sobral (pers. comm.) was able to identify it as *M. amazonica,* and after subsequent examination of more samples of each it appears that they are in fact the same species. To evaluate this, two samples of each species were included; the emergence of the Paraná *M. amazonica* sister to the two species of *M. detergens* and the Amazonian representative of *M. amazonica* emerging sister to those three, supports the idea that this clade comprises a single species. During the study, it was further noted that the Caribbean *Myrcia leptoclada* DC. is morphologically similar; it is certainly part of this *M. detergens/amazonica* complex and would be predicted to emerge in this clade in a future analysis.

Continuing the analysis of Clade 9 in Fig. 4.12, the next clade has no support and is comprised of two further clades, one of which strongly (BP 99, PP 100) unites two Amazonian species, *Myrcia saxatilis* and *M. decorticans*; the remaining three species make a single, also unsupported clade within which there is good (BP 100, PP 99) support for a sister relationship between *M. ramulosa* (São Paulo) and *M. micropetala* (Bahia), which share morphological characters such as a dense grey pubescence covering leaves and inflorescences in axillary, asymmetrical panicles so severely reduced that they appear racemose. There is no support for the sister arrangement of this clade to the Amazonian *Myrica inaequiloba,* morphologically similar to *M. saxatilis*. Only two of these species were assigned to infrageneric groups by previous authors; *Myrcia ramulosa* and *M. micropetala* were assigned to *Myrcia* group *Acuminatae* and *Aulomyrcia* group *Lateriflorae,* respectively, by Berg, although it is unclear why a species such as *Myrcia ramulosa* that bears all the characteristics of an *Aulomyrcia* should have been assigned to an infrageneric group of *Myrcia*.

Finally, the clade sister to the rest of Clade 9 is well supported (BP 98, PP 100) and comprises *Myrcia multiflora, M. torta* and *M. rorida,* the last two species well supported as sisters (BP 100 PP, 100). Although the rest of Clade 9 have somewhat asymmetrical, whorled or bunched inflorescences, persistent subfloral bracts and somewhat to extremely unevenly sized calyx lobes held irregularly or reflexed on the fruit, these three species are different,

resembling more the trilocular, more regularly flowered, bractless *Aulomyrcia* species of Clade 4 with calyx lobes flared or urn-shaped upon the fruit. *Myrcia multiflora* is recorded as bilocular (Legrand 1961; McVaugh 1968), and although this name is not included in Berg's treatments (1855-56; 1857-59), the clearly synonymous *Myrcia sphaerocarpa* DC. (Govaerts et al. 2006) is assigned to bilocular *Aulomyrcia* group *Perforatae*. *Myrcia torta* and *M. rorida* were assigned to quadri-/trilocular groups of *Aulomyrcia* by Berg (sects. *Exsuccae* and *Paniculatae II,* respectively) although dissections revealed *M. torta* to be bi- or trilocular (the sample included in the DNA analysis was bilocular, and an isotype at Kew, *Pohl 1026,* was trilocular), whereas *M. rorida* was found to be consistently bilocular (including the sample used in the DNA analysis plus isotypes at Kew *Riedel 2491,* labelled as *Aulomyrcia rorida* O.Berg, and *Glaziou 21164,* labelled as *M. rorida* (O.Berg) Kiaersk. var. *microphylla* Kiaersk.).

Of the lettered clades (A–H) that make up the nine clades of the *Myrcia s.l.* polytomy in the Bayesian analysis, Clade A contains the *Calyptranthes* clade (Clade 1) and Clade 2 and is unsupported in both the parsimony and Bayesian analyses. It is difficult to see what morphological characters these groups might share, although *Marlierea eugeniopsoides* was originally described in *Calyptranthes* by Legrand and Kausel (Legrand 1962a) on account of its extremely unequal tearing of the calyx resulting in a single lobe appearing to resemble a 'pseudocalyptra' (Legrand 1975). Clade B has < 50 BP but 100 PP; this clade contains Clades 1–6, separating them from the cymose and asymmetrically flowered species in Clades 7–9. Clades C–E form successive nodes within Clade B, and all are weakly supported in both the parsimony and Bayesian analyses. The topology within Clade C suggests that the sister group to the *Gomidesia* Clade 3 is the trilocular, non-roughly veined *Aulomyrcia* Clade 4; the relationships in Clade D suggests a sister grouping of this latter clade to *Myrcia sensu* Berg (Clade 5), whereas the results for Clade E suggests that the trilocular, roughly veined *Aulomyrcia* (Clade 6) are sister to Clade D. Clade F maintains Clades 7–8 as sister to Clade B and again, is unsupported in both the parsimony and Bayesian analyses; morphological

optimisations demonstrate several characters to suggest that Clades 7–8 might share a common origin with Clade 9 but again, the lack of support at this node makes speculation unnecessary. Clades G and H have 97 and 100 PP, respectively, but < 50 BP. Despite a lack of clear morphological synapomorphy, Clade G links Clades 7–8; as mentioned, the topology in both of these clades suggest that there should be similarities to Clade 9, but, other than DNA characters, the morphological evidence for their association is unclear. In Clade H *Algrizea macrochlamys* is distinct from *Myrcia s.l.*, in this case however, morphological differentiation between separating these taxa is clear (see Chapter 3).

Morphology and morphological optimizations

Homoplasy in many morphological characters is striking; nearly every morphological character displays some degree of homoplasy on the molecular and combined estimates of relationships (Figs 4.6 and 4.8). In some cases this homoplasy is extreme; for example, characters 3 (adaxial midvein impressed/flat/raised) and 4 (presence/absence of simple hairs) require 16 and 18 steps, respectively, on Fig. 4.6. There are however, some characters that correlate strongly with clades of the molecular tree that receive good support. Clade 3 is supported as distinct from the rest of *Myrcia s.l.* by its possession of a pubescent disc (characters 16/state 1; Fig. 4.13), although this character has developed in parallel in *Algrizea*. Clade 3 (with the exception of *Myrcia mischophylla*) is also distinguished by having anthers in which the thecal arcs are reflexed after dehiscence as opposed to maintaining their original curvature or being held parallel to each other, not reflexed (17/0, Fig. 4.14). Clade 5 is supported as distinct from the rest of *Myrcia s.l.* by the staminal ring at anthesis comprising 60 percent or more of the total width of the disc rather than significantly less (15/0, Fig. 4.15) and by typically cylindrical as opposed to globose fruits (19/0, Fig. 4.16). In this sample, trilocular ovaries (18/1, Fig. 4.17) are found throughout Clades 4, and Clade 6, and in *Gomidesia schaueriana*. Nic Lughadha (1997) recorded that *Gomidesia* can have three or four locules, but as discussed, this clade possesses morphological synapomorphies that

distinguish it from the others, whereas apart from the venation character described above (Fig. 4.11), Clades 4 and 6 are morphologically similar, sharing regular, free calyx lobes, trilocular ovaries, hypanthia prolonged into a tube above the ovary, symmetrical, and well-developed panicles distributed in two to several of the higher branch axils with a lack of persistent bracts. This optimization suggests that the basic condition in *Myrcia s.l.* is bilocular and that the evolution of the trilocular condition in these three clades arose independently. Lack of support for Clades C, D and E prevent drawing of clear conclusions regarding the arrangement of Clades 3, 4, 5 and 6 to each other and the possibility that the two trilocular Clades 3 and 6 share a single origin cannot be discounted.

Character 1 indicates sympodial vegetative branching to occur, as McVaugh (1958a) noted, in species of *Calyptranthes* and *Marlierea*. All *Calyptranthes* (Clade 1) studied had sympodial branching, and although this was a common characteristic of Clade 2, *Marlierea eugeniopsoides* has monopodial branching. If this topology is correct, the homoplasious nature of this character can be noted from the additional sympodial branching by all members of Clade 7 except *M. angustifolia* and by two members of Clade 9, *M. suaveolens* and *M. regeliana*. Low support, however, at critical nodes (B–F) prevents being certain that Clades 1, 2 and 7 are not more closely related. Two of the sympodially branching species of Clade 7 are species of *Myrcia, M. pulchra* and *M. bicarinata*, species that resemble in particular species of *Calyptranthes* in having turbinate buds and globose fruits with a cylindrical calyx tube (Fig. 4.3Ci, iv) remaining after the calyx tubes have fallen, similar to the fruit of *Calytpranthes* (Fig. 4.3Fiv).

Despite whorled leaves with a raised midvein providing some support for the clade within Clade 9 comprising *Myrcia clavija, M. insularis, M. eumecephylla* and *Marlierea obversa*, characters 2 and 3 are homoplastic, with the former occurring also in some species of Clades 4 and 8 and the latter additionally in Clades 3 and 9.

Characters 4 and 5 concern the presence/absence of simple or dibrachiate hairs, respectively; character 4 is one of the most homoplasious of the data set, with simple hairs equally likely to be present or absent in each of the nine numbered clades. Outgroup species exclusively have simple hairs and for this reason, their presence appears to be the ancestral state. Character 5/state 1 (dibrachiate hairs present) occurs in a single *Calyptranthes* species (Clade 1) and in two species of *Marlierea* (Clade 7). The low support for nodes B and F ensure that the precise association of these two clades remains uncertain; however, in a study of this scale, the character is of little taxonomic use, and it remains for more detailed studies of, or better sampling in, these two genera, to indicate the taxonomic use or otherwise of the dibrachiate hair character.

Of the four characters of the inflorescence used, character 6/state 1 (inflorescences congested in terminal node, occasionally in ultimate two nodes as opposed to 0, inflorescences regularly spaced in more than two terminal or subterminal nodes, usually in several) is present in but not exclusive to Clades 1, 7 and 9. It is noted that this character state is distinctive for the (unsupported) sub-clade beginning with *Marlierea suaveolens* and ending with *Myrcia eumecephylla* (excluding *Marlierea obscura*), but homoplasy across the topology suggests that congested terminal inflorescences have arisen more than once in *Myrcia s.l.* Character 7/state 1 is exclusive to the '*Plinia* group' out-groups, and state 2 is exclusive to *Algrizea*. This character provides no resolution to the in-group but serves to suggest that the ancestral inflorescence may have been a raceme and that the dichasial and paniculate inflorescences are derived from this, contradicting Briggs and Johnson's (1979) suggestion that the panicle was the ancestral character from which all extant Myrtaceae inflorescences was derived by a process of reduction.

Characters 8, 9 and 11 provide some support for an association between Clades 8 and 9, with a similar pattern recovered for each (Figs 4.18–4.20). In each case, the majority of species in each of these clades is in possession of character state 1; in the case of character 8, the

ultimate panicle branchlets decrease abruptly in length resulting in a narrowly triangular or spiciform panicle as opposed to steadily decreasing and resulting in a broadly triangular panicle. In character 9, the majority of secondary rachises of panicles are alternate as opposed to opposite whereas in character 11, the majority of species have a pair of pointed bracts below the flowers that persist until after the flowers/fruit fall as opposed to these bracts being deciduous. This combination of characters combines to form similar but distinctive panicle types for these clades; the alternate branching and often persistent, pointed bracts often produce and emphasise 'zig-zag' shaped branchlets and panicles. In addition, panicles in Clade 8 species have a whorled arrangement with persistent bracts at the base of these whorls (10/1) shared with a minority of Clade 9 species: *Marlierea sucrei* plus the Clade comprising *Myrcia clavija, M. insularis, M. eumecephylla* and *Marlierea obversa*. Despite the grouping that characters 8, 9 and 11 provide for Clades 8 and 9, none of these characters is synapomorphic for every species. The species that do not share state 1 for any of these characters varies; for characters 8 and 11, however, the three species, *Myrcia multiflora, M. torta* and *M. rorida,* share state 0 (ultimate panicle branchlets decrease steadily in length resulting in a broadly triangular panicle), whereas for character 9 this state (more than 70% of secondary rachises of panicles opposite) is present in *M. multiflora*. As discussed, these three species differ morphologically from the rest of Clade 9. Once again, low levels of bootstrap support along the backbone of this topology prevent firm conclusions from being drawn, but morphological similarities between Clades 8 and 9 might suggest that a future topological change bringing them closer together would not be a surprise, nor would a change that removed *Myrcia multiflora, M. torta* and *M. rorida* from Clade 9.

Characters 12 and 13 attempt to interpret the *Myrcia s.l.* calyx with a minimum of homoplasy; results, however, indicate that this character has more than one origin within the *Myrcia s.l.* clade. Character 13/state 1 (calyptra present as opposed to absent) is synapomorphic for the *Calyptranthes* clade; this is unsurprising because the *Calyptranthes* species sampled all had clear calyptra - none possessed intermediate states such as an irregular calyptra with remnants

of tissue on the hypanthium rim resembling calyx lobes; equally no *Marlierea* sampled had uneven calyx lobes with one resembling a calyptra, arrangements often found in *Myrcia s.l.* Character 12/state 0 (calyx not tearing at anthesis) is present in the majority of taxa and appears to be the ancestral state for *Myrcia s.l.* It is unfortunate that no species with these intermediate states were analysed as sampling was limited to those species available for DNA analysis; future studies are planned that will include such taxa. State 1 (calyx tearing vertically at anthesis) is present in the outgroup species, *Myrciaria cauliflora* and *Plinia pauciflora;* it has also been assigned to *Marlierea eugeniopsoides*, as despite its reported pseudocalyptra, this organ could not be interpreted in specimens in the Kew herbarium in which instead a closed bud with a vertically tearing calyptra was observed. As such, it appears that this character state evolved three times within *Myrcia s.l.* because it is also present in *Myrcia decorticans* and each species of Clade 9, from *Marlierea suaveolens* to *Myrcia eumecephylla*, excluding *Myrcia insularis*. This result supports a common origin for the species of this latter clade and a tendency for vertical tearing of the calyptra in Clade 9. Character state 2 (calyx tearing parallel to hypanthium ring at anthesis) is present in two species of Clade 2, which is sister to the *Calyptranthes* clade, and suggests a possible mechanism in which the calyx began to transform into a laterally tearing calyptra. This horizontally tearing calyx is also present in the majority of species of Clade 7; the lack of any internal support for node F and only Bayesian support for node G raise the possibility that Clade 7 could be more closely related to Clades 1 and/or 2 than is suggested by Fig. 4.6., an arrangement supported by character 1 (discussed above).

Character 14 concerns bud shape and does not contribute much to the dataset, except possibly the possession of state 1 (buds constricted below the ovary) supporting a closer relationship between Clades 8 and 9 than reflected by Fig. 4.6., as also suggested by characters 8, 9 and 11 (discussed above).

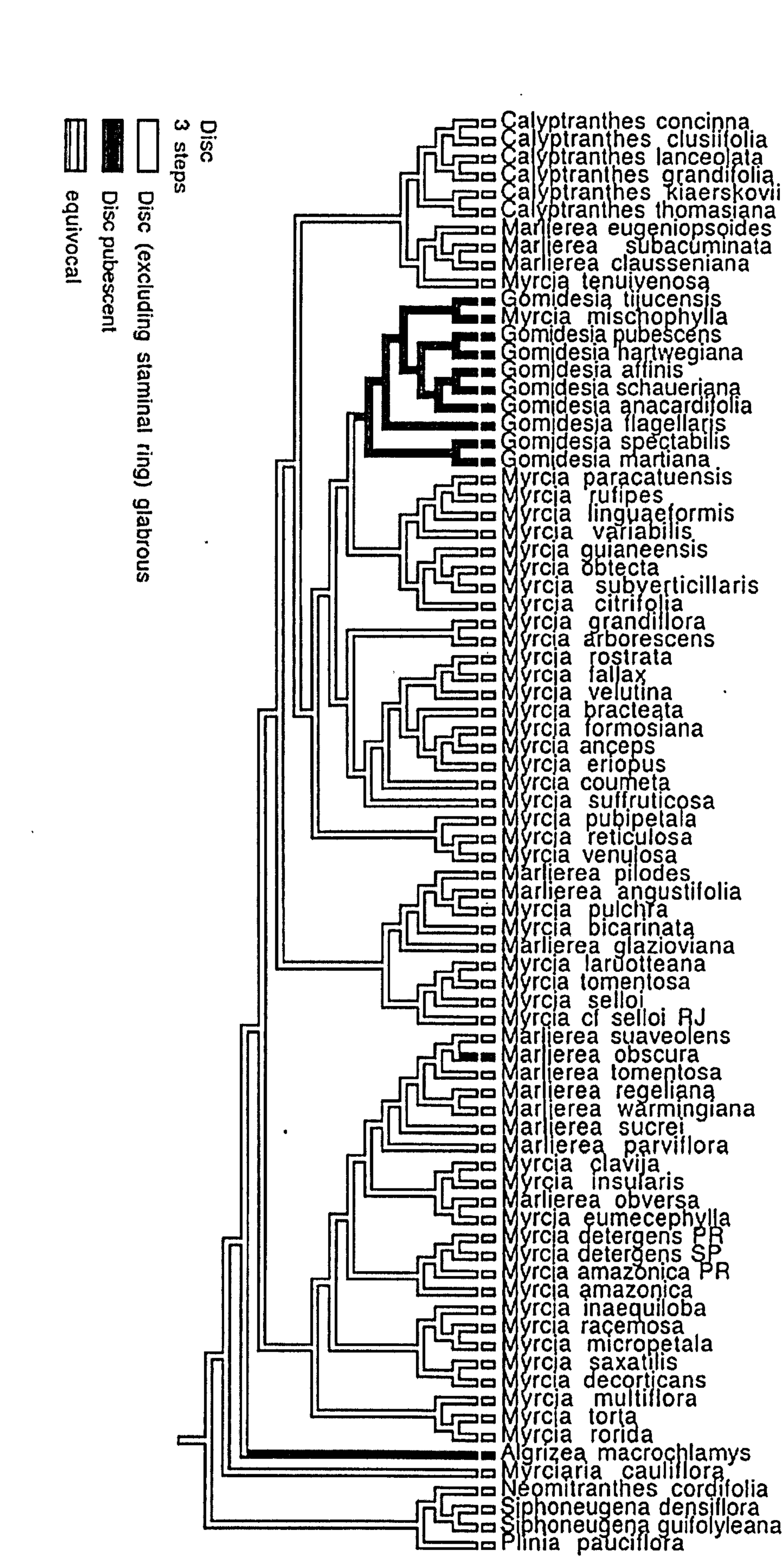


Figure 4.13. Optimisation of character 16: disc.

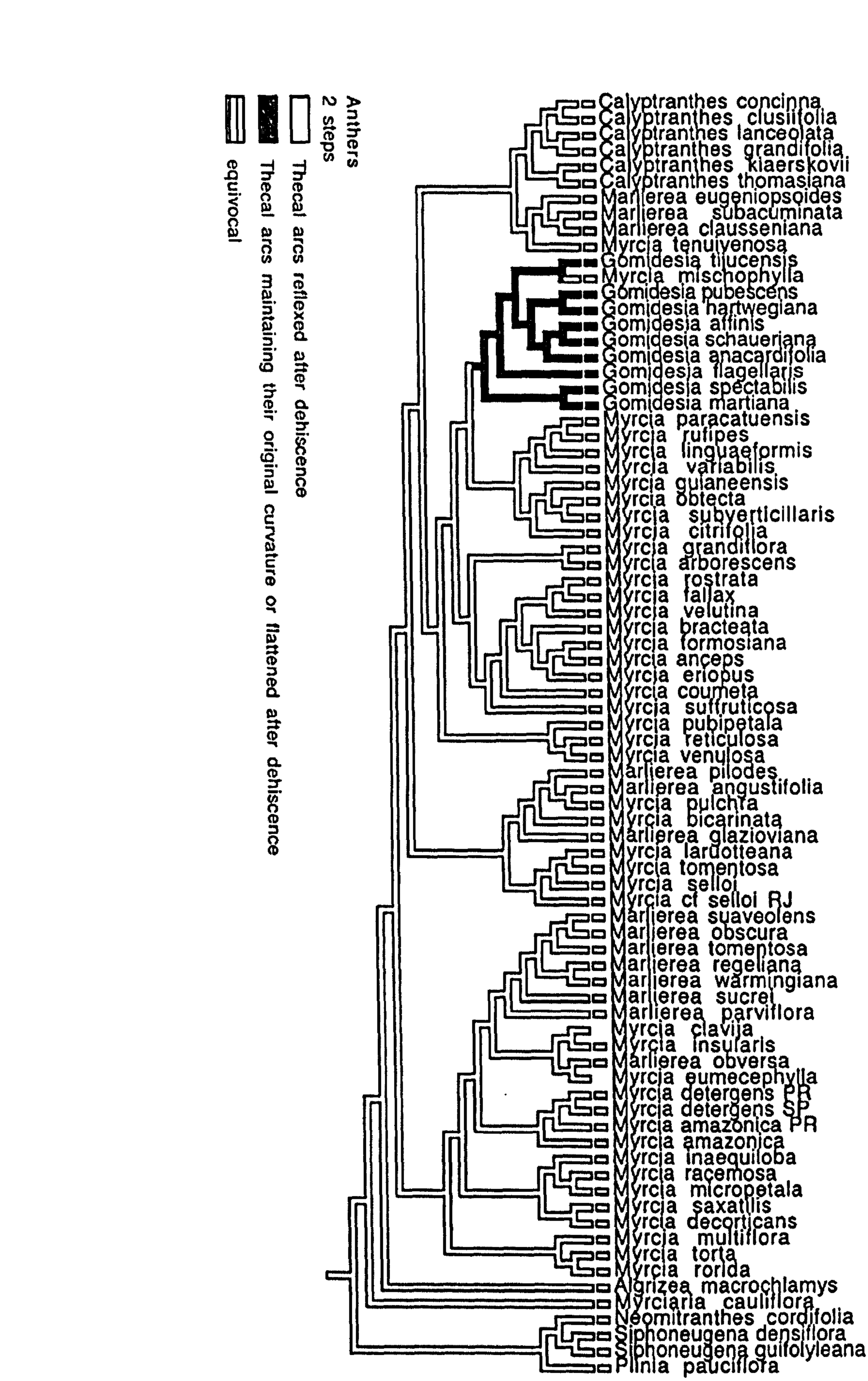


Figure 4.14. Optimisation of character 17: anthers.

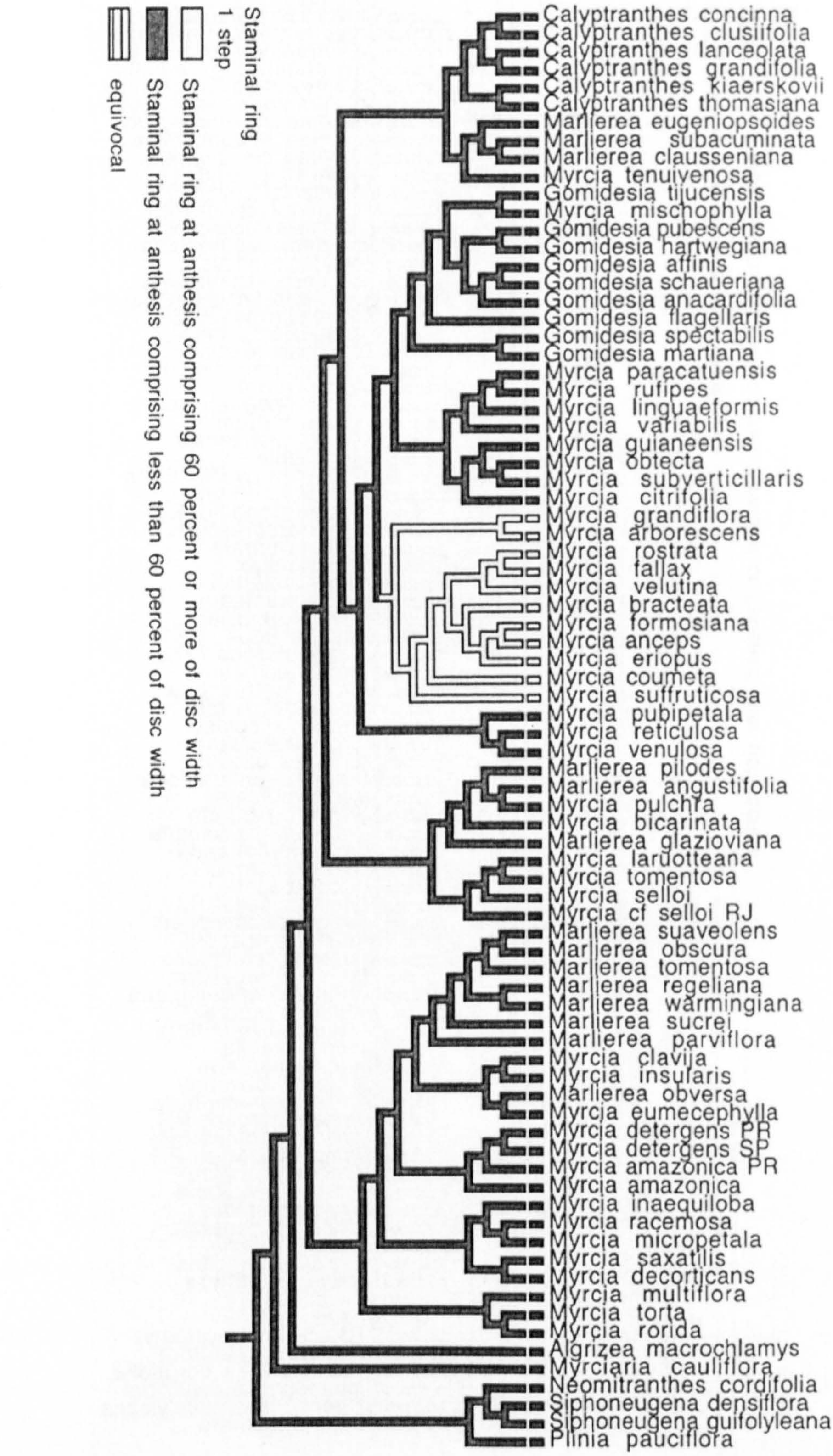


Figure 4.15. Optimisation of character 15: staminal ring.

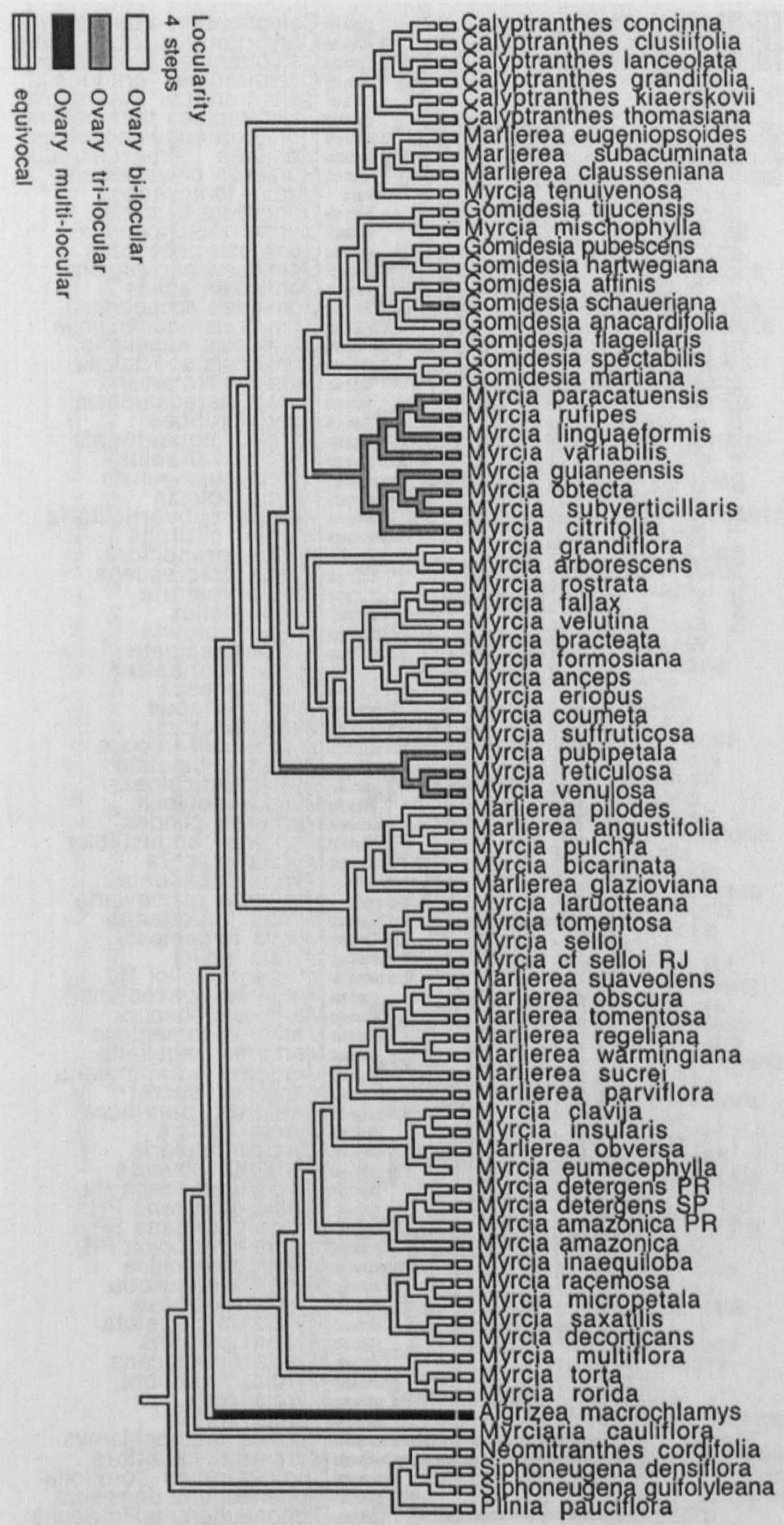


Figure 4.16. Optimisation of character 18: locularity.

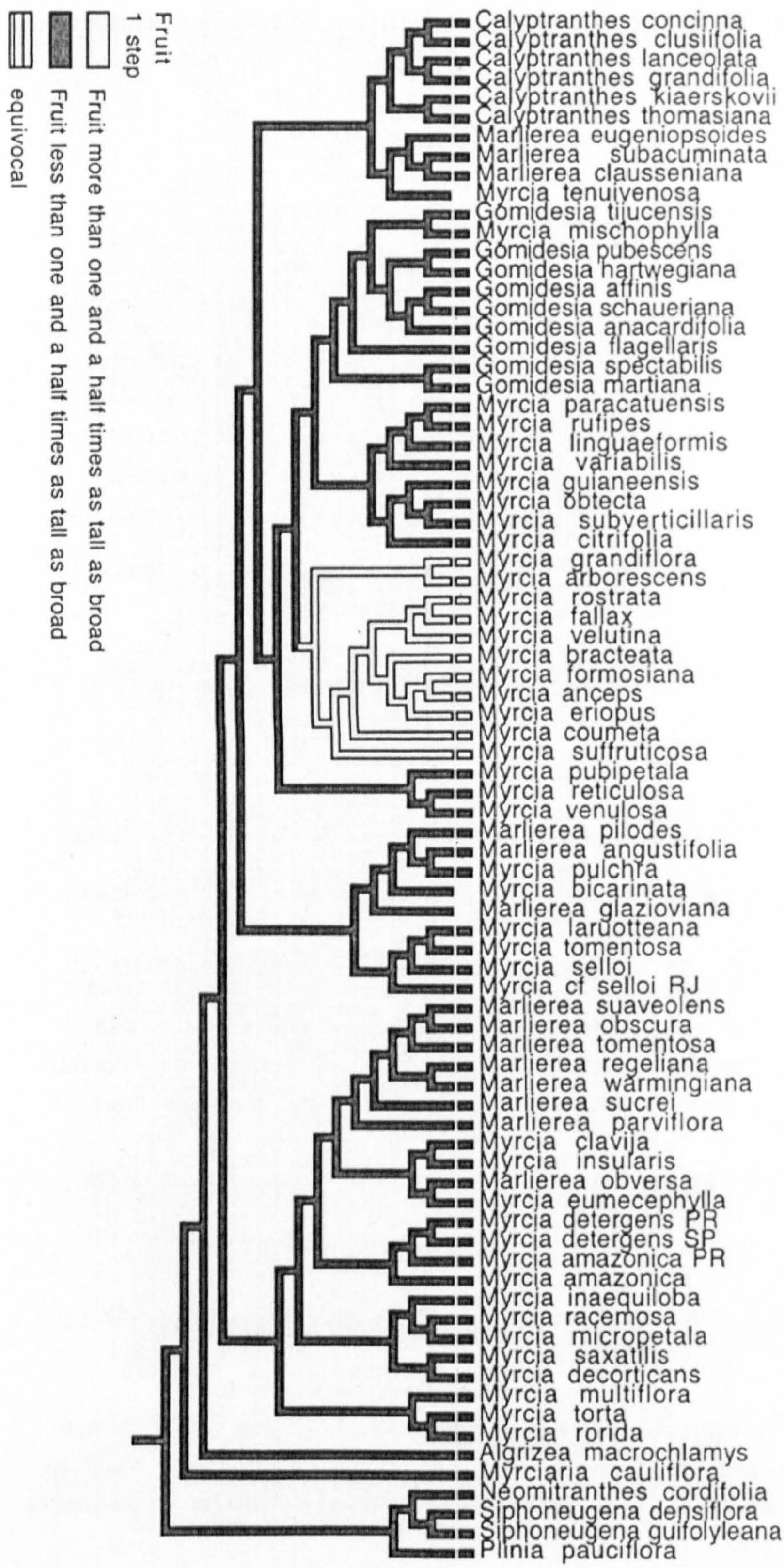


Figure 4.17. Optimisation of character 19: fruit shape.

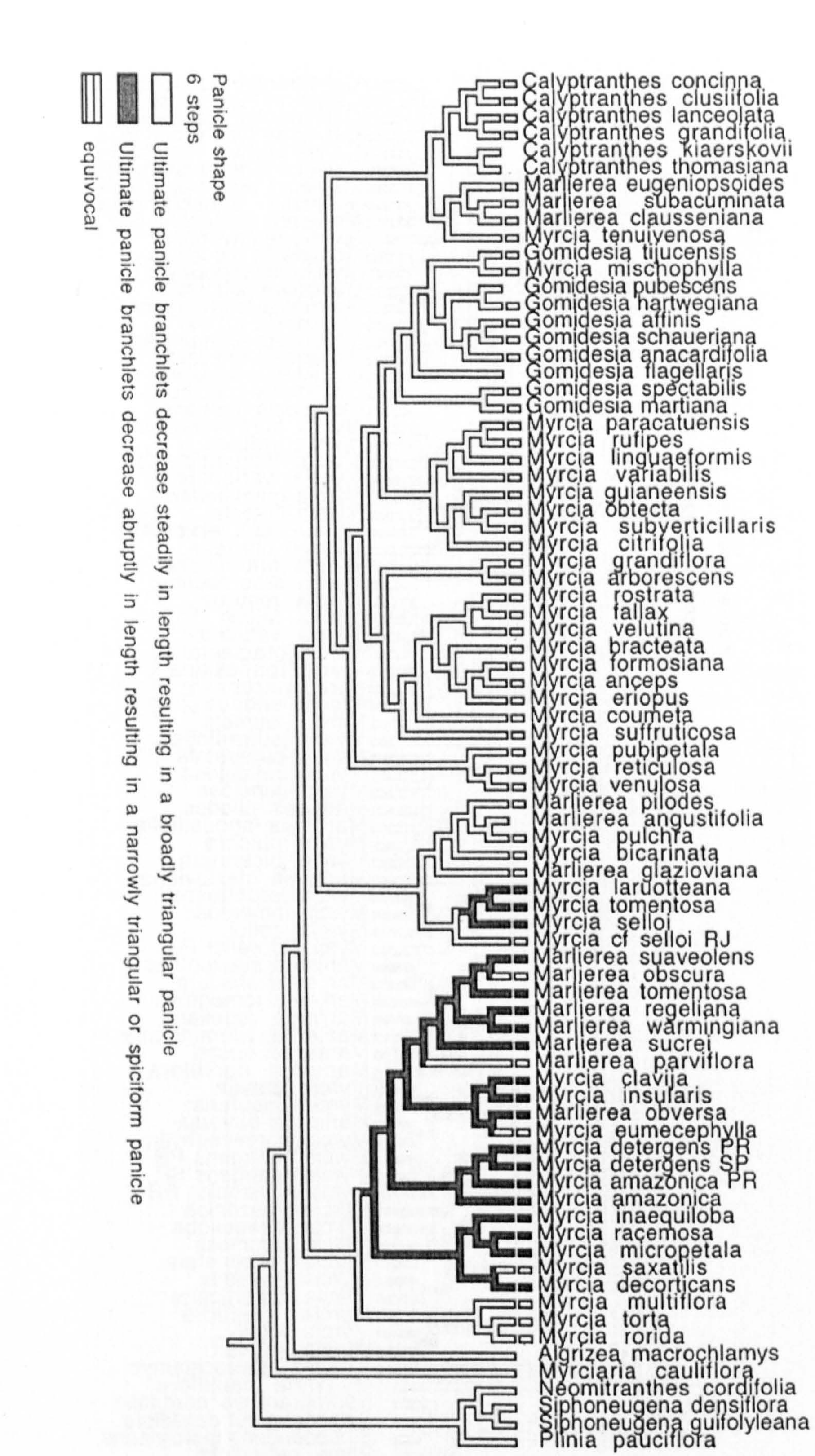


Figure 4.18. Optimisation of character 8: panicle shape.

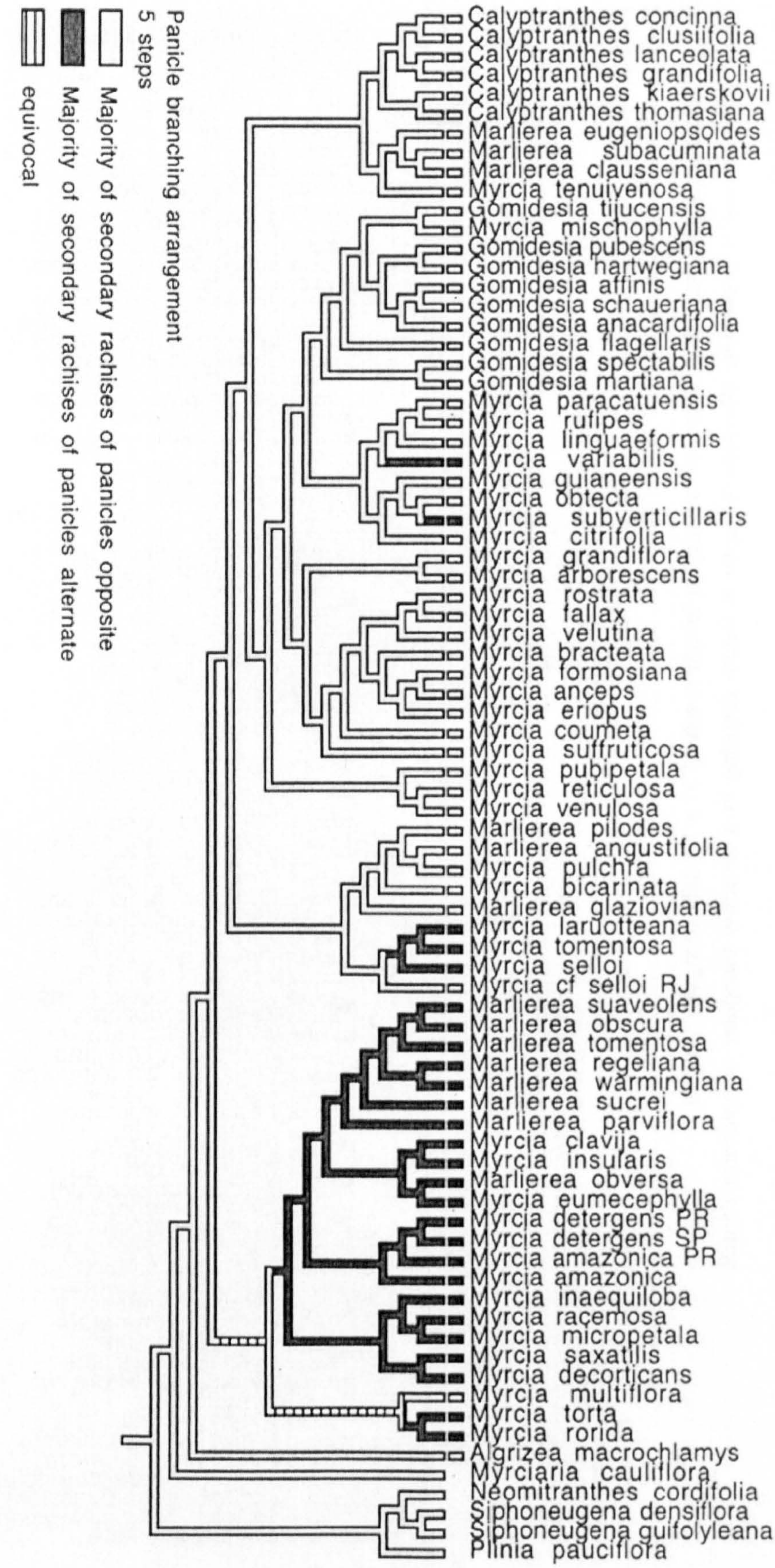


Figure 4.19. Optimisation of character 9: panicle branching arrangement.

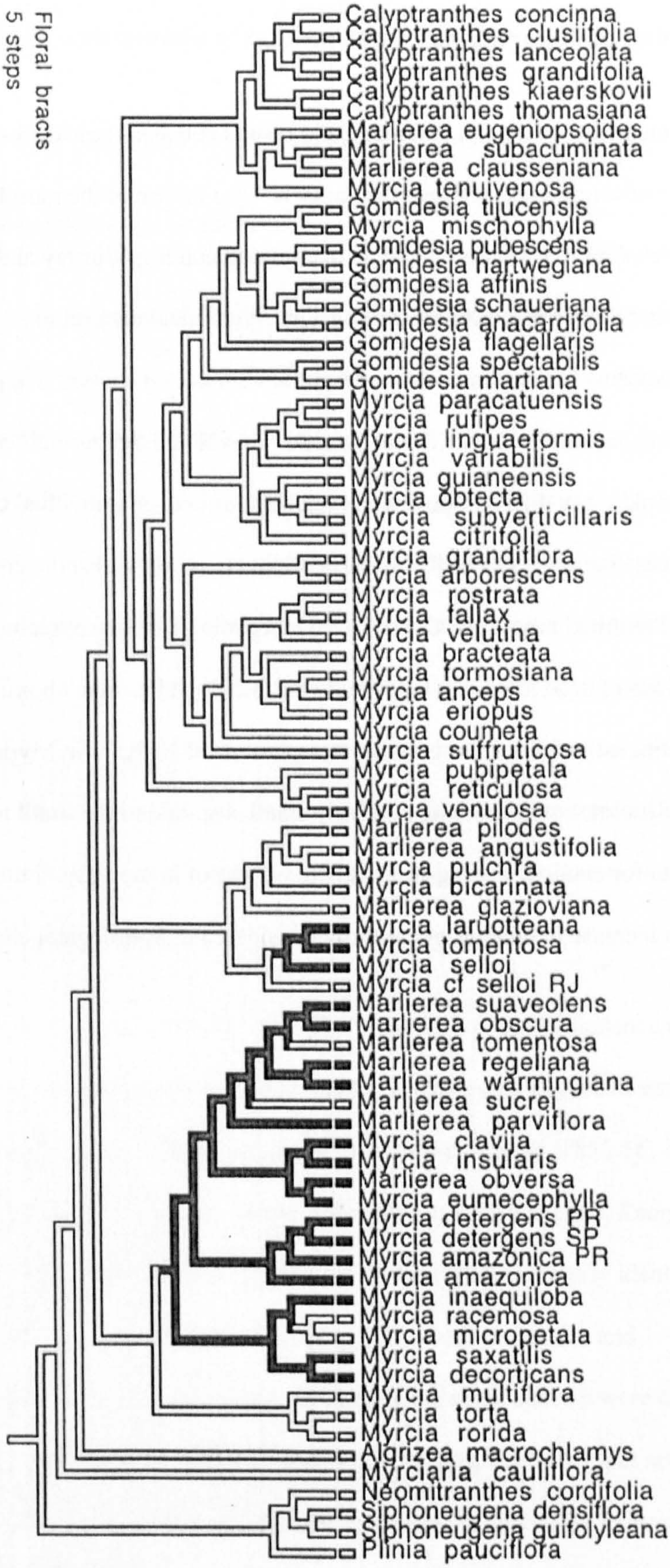


Figure 4.20. Optimisation of character 11; floral bracts.

Combining data partitions

It is assumed that the overall congruence in results between these different data sources is indicative of congruence between the datasets. The failure of the morphological dataset to increase resolution or support is not an uncommon pattern; Wortley and Scotland (2006) reported that although in general, adding a morphological data set to an existing molecular one will have a significant impact on resolution but not on support, support may increase or decrease at some nodes in individual studies. They noted that a small change in resolution or support could mask different positive or negative effects on individual clades; this is assumed to be the case here, with overall congruence in pattern but with some conflict evident at individual terminal branches. Overall, this morphological dataset seems useful for addressing the questions of *Myrcia s.l.* phylogeny and not beset with as much homoplasy as might have been anticipated judging from previous morphological analyses in Myrtaceae. A problem with this data set, however, is its relatively small size, due to the small numbers of characters appropriate for cladistic coding and likely to be free of homoplasy. Future analyses will require an increase in number of carefully considered morphological characters.

Assessment of previous sub-generic arrangements

Myrcia

De Candolle, Berg, Grisebach and Niedenzu

Despite low support along the spine of Fig. 4.6, it is clear that three of the four core Myrciinae genera of De Candolle (1828) and Berg (1855-56, 1857-59) do not stand up to phylogenetic analysis, and none emerge as monophyletic. For now, *Calyptranthes* appears a monophyletic group embedded in a paraphyletic *Myrcia*; the sample size of the genus, however, may not reflect diversity throughout the group (c. 260 species; Govaerts et al. 2006), with some as yet unsampled species, particularly from eastern South America being distinct morphologically, casting doubt on their future inclusion in this *Calyptranthes* clade. The emergence of *Myrcia mischophylla* within the *Gomidesia* clade provides strong support for the paraphyly of *Gomidesia*. There are other *Myrcia* species (e.g. *M. aliena*) that do not share the *Gomidesia* anther characters but for which overall aspect strongly suggests *Gomidesia* and which are likely to emerge in this clade in future.

The divisions of *Myrcia* by Berg (1855-56, 1857-59), Grisebach (1864), Niedenzu (1893), Kiaerskou (1893), Legrand (1961) and McVaugh (1968) are based on degree of extension of the hypanthium above the ovary. The group identified as *Myrcia* (Berg, 1855-56, 1857-59), sect. *Eumyrcia* (Grisebach, 1864), subgen. *Myrcia* (Niedenzu, 1893), subgen. *Eumyrcia* (Kiaerskou, 1893; Legrand, 1961) or sect. *Myrcia* McVaugh (1968) is clearly identified in Clade 5 and is one of the best supported clades in Fig 4.6 in both molecular and morphological terms (as discussed). In earlier classifications, some species were erroneously included in this group (e.g. *M. pubipetala*, *M. tomentosa*, *M. mischophylla*), but no species is known to have been mistakenly placed outside of it. Attempts to subdivide *Myrcia s.s.* do not correlate with results presented here, with no evidence of groupings of species assigned to the various infrageneric groups of Berg. Grisebach and Niedenzu's subdivision of this group

closely followed Berg's classification whereas Kiaerskou's subdivisions differ somewhat; in both cases, the initial subdivision was into two groups based on the possession or otherwise of bracts; these groups are not evident in this analysis, with bracteate species *M. bracteata* and *M. eriopus* emerging in the same clade but with non-bracteate species; there is no evidence for either of the further subdivisions of these authors.

The group identified as *Aulomyrcia* (Berg, 1855-56, 1857-59), sect. *Aulomyrcia* (Grisebach, 1864), subgen. *Aulomyrcia* (Niedenzu 1893; Kiaerskou 1893; Legrand 1961) or sect. *Aulomyrcia* (McVaugh, 1968) is dispersed throughout Fig. 4.6 (Clades 2, 4, 6–9). All of these classifications make an initial division of species into those with two versus three locules, a demarcation that fits the topology well (as discussed), with Clades 4 and 6 exclusively and uniquely bearing trilocular ovaries and containing species from Berg's trilocular infrageneric groups. Occasionally, Berg would place a bilocular species in a tri- or tetralocular group, e.g. *Aulomyrcia bicarinata* O.Berg, placed in group Cymosae II; said to be 2–4 locular and thereby polyphyletic. Alternatively, a trilocular species was placed in a group said to be bilocular, e.g. *Myrcia pubipetala*, placed in exlclusivey bilocular *Myrcia* group Abrupte-acuminatae. An image of the type of this species lodged in the Geneva herbarium matches the commonly accepted species concept, making it hard to understand Berg's apparent error. Niedenzu made only one further division in *Aulomyrcia*, creating sect. *Eu-Aulomyrcia* for the bulk of species and monospecific sect. *Calyptromyrcia* containing *Myrcia cymosa*; unfortunately, this last species could not be included in this analysis, preventing discussion of Niedenzu's classification. Kiaerskou's *Aulomyrcia* classification differed from those of Berg and Niedenzu, its basic structure is comparable to the results presented here.

Kiaerskou

Kiaerskou (1893) avoided locularity completely as a means of dividing these groups, initially dividing the group into three according to vegetative branching patterns; group A with dichotomous or sympodial branching is comparable to Clade 7 (Fig. 4.6), including *Myrcia*

pulchra and *M. pilodes*, later transferred to *Marlierea*; group B with two further partially dichotomous species, *M. andromedoides*, a synonym of trilocular *M. venulosa* (Clade 6) and *M. atropunctata*, a species not encountered at Kew or in any of the major herbaria and not sampled here, and group C with monopodial branching, containing the remainder of species and further divided into three groups based on inflorescence type. Of these three, groups II (inflorescences racemiform with terminal flower, secondary inflorescence usually in uniflorous dichasia) and III (inflorescences in 1–3 flowered dichasia) contain five and one confirmed or likely tri-locular species, respectively. Group I (inflorescence paniculate) contains the 67 remaining species divided into four more or less equally sized groups depending on the shape of the panicle. Under this system, the first two groups, a and b (lower case), contain species in which the penultimate inflorescences are usually more or less spike shaped or racemiform; these include *Myrcia laruotteana,* several synonyms of *M. tomentosa, M. detergens, M. torta* and *M. rorida.* The remaining two groups, c and d (lower case), contain species in which the penultimate inflorescences are usually in three-flowered pseudodichasia or three to seven flowered true dichasia, including *Myrcia multiflora, M. tenuivenosa, M. subverticillaris, M. obtecta, M. variabilis, M. rufipes, M. pubipetala* and *M. venulosa.*

With some exceptions, these groups link type and shape of inflorescence to the pattern of locularity already noted by Berg (1855-56, 1857-59) and demonstrated by results presented here. Those species in groups a and b are bilocular and comparable to Clades 7–9 (Fig. 4.6), including *Myrcia torta* and *M. rorida*, despite discussed doubts regarding their inclusion in Clade 9. The majority of species in groups c and d are trilocular and correspond to Clades 4 and 6 (Fig. 4.6); exceptions include *Myrcia multiflora* (and several its synonyms) that emerges with *M. torta* and *M. rorida* in Clade 9 in Fig. 4.6. and *M. tenuivenosa*, listed close to *M. multiflora* on page 84 of Kiaerskou's (1893) treatment, which may have been placed here simply as a result of its perceived proximity to this last species. As discussed, morphologically *M. multiflora* shares many similarities with Clades 4 and 6, notably in its

well-developed, symmetrical inflorescence, and non-tearing calyx held upright and flared in fruit; it is understandable that Kiaerskou should have placed this species here. Of all of the previous classifications of *Myrcia s.s* and *Aulomyrcia*, Kiaerskou's appears to be the closest to a natural system, remarkable for such homoplasious and morphologically complex groups.

Legrand

Legrand (1961) reverted to a less novel system, dividing *Myrcia* into subgenera *Myrcia* (as *Eumyrcia)*, which he did not attempt to further divide, and *Aulomyrcia*, divided into two groups of four and three sections based on locularity. The seven infrageneric groups of subgen. *Aulomyrcia* were based variously on inflorescence shape (sect. Ramulosae), leaf texture (sects. Crassae, Chartaceae and Reticulatae) and shape (sects. Obovatae and Ovatae) and on single characters of the flowers (sect. Microsiphonatae). Of the bilocular sections, series Ramulosae includes a majority of species with asymmetrical, spike-like inflorescences that correspond to Clade 8 (Fig. 4.6), but also, apparently erroneously included *Myrcia multiflora*, which can vegetatively or in bud be mis-identified as *M. laruotteana.* Series *Crassae* and *Chartaceae* do not correspond to results presented here, but series *Crassae* is notable in containing *M. pubipetala*, once again classifying this species as being bilocular.

Series *Microsiphonatae* contained the single species *M. microsiphonata*, later synonymised with *M. ramulosa* (Legrand & Klein 1967-77) and likely to be associated with series *Ramulosae* or Clade 8 (Fig. 4.6).

Of the trilocular series, ser. *Reticulatae* contains *Myrcia richardiana* and *M. kauseliana*, a likely synonym of the first species, plus *M. castrensis,* a likely synonym of *M. venulosa.* *Myrcia richardiana* possesses the large pellucid glands and reticulate venation shared by Clade 6 (Fig. 4.6) that includes *M. venulosa,* this series may therefore correlate with Clade 6. Ser. *Obovatae* contains *M. citrifolia, M. obtecta;* the remaining species also morphologically resemble those of Clade 4 (Fig. 4.6) except for *M. bicarinata*, a species with affinities to *Marlierea* and here included in Clade 7. Ser. *Ovatae* contains *M. heringii,* a species with

reticulate venation and extremely large glands that could be affiliated with ser. *Reticulatae* or Clade 6 (Fig. 4.6) and *M. debilis*, a poorly known species of uncertain affinities. Legrand's (1961) classification is concerned with too few species to allow its accuracy to be rigourously judged; it does, however, include at least some natural groupings.

McVaugh

McVaugh's (1969) classification of *Myrcia s.s.* based on chiefly Guayanan species (Fig. 4.21) accurately defined *Myrcia* sect. *Myrcia* ,whereas his sects. *Aulomyrcia* and *Armeriela* now appear non-monophyletic. These results reflect once again the confusion at the *Myrcia* and *Marlierea* interface and the futility of trying to force paraphyletic genera into meaningful groups.

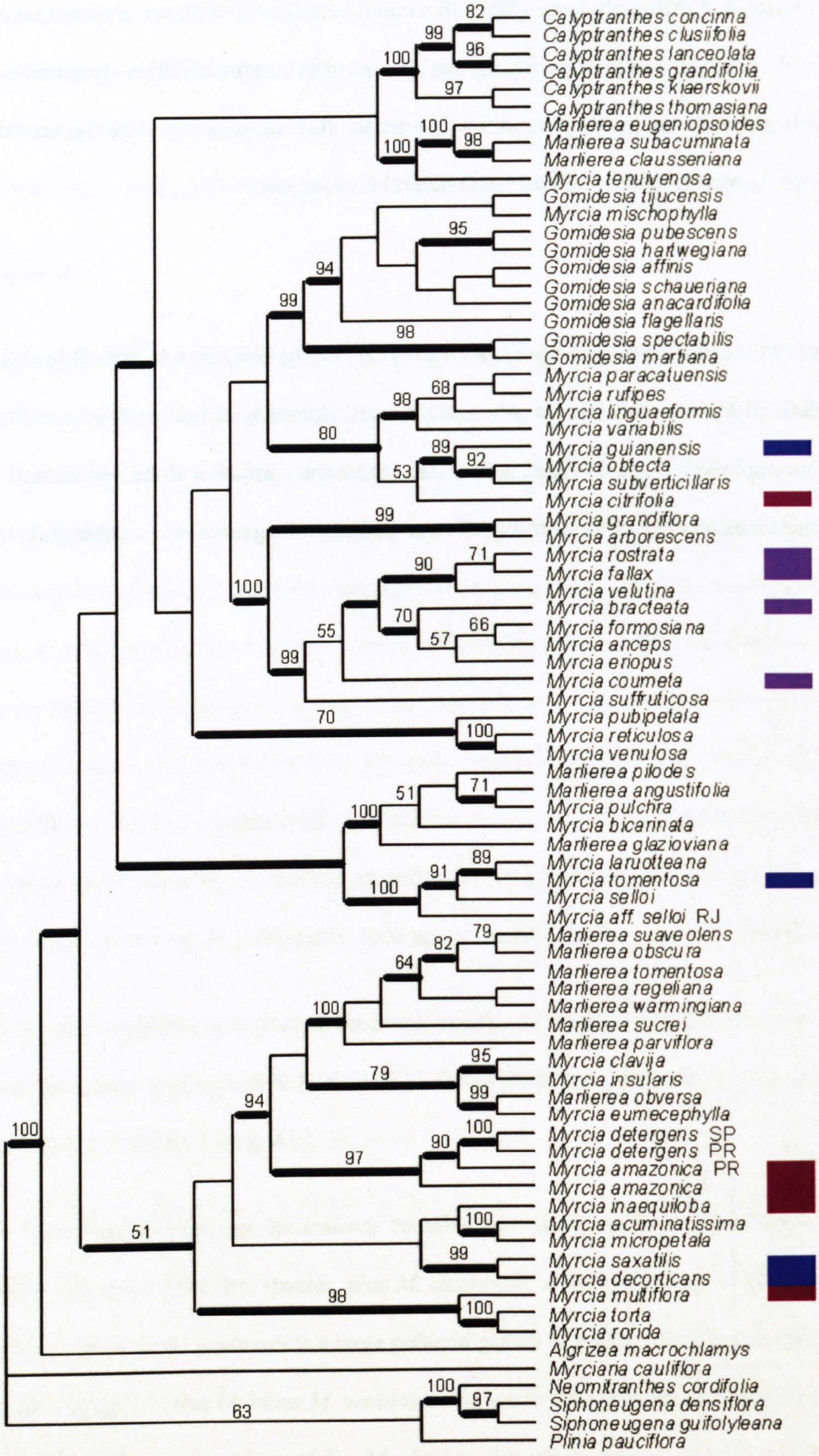


Figure 4.21. McVaugh's (1969) classification of Guianan *Myrcia s.l.* Blue squares: sect. *Aulomyrcia*; maroon squares: sect. *Armeriela*; lilac squares: sect. *Myrcia*.

Marlierea

Clear evidence for the polyphyly of *Marlierea* is its emergence in three of the nine clades of Fig. 4.6. with BS >50. There is some evidence of the existence of groups that reflect the three subgeneric groups recognised at various ranks by Berg (1855-56, 1857-59), Kiaerskou (1893) and Legrand (1962a); two of the three species in Clade 2 represent sect. *Eugeniopsis sensu* Legrand (the remainder represent sect. *Pseudocalyptra;* Legrand, 1975), and all but one species in Clade 9 represent sect. *Marlierea* subsect. *Clausae sensu* Legrand, whereas the additional species in Clade 9 represent sect. *Marlierea* subsect. *Apertae sensu* Legrand. Of the two species emerging in Clade 7 and included in Legrand's classification, one each is from sect. *Eugeniopsis* and sect. *Marlierea* subsect. *Apertae.* It is tempting to conclude that the *Marlierea* syndrome of completely closed buds that tear vertically into star-shaped open flowers is more likely to have originated once, whereas the syndrome of a bud with short, unevenly tearing calyx lobes, especially tearing parallel to the rim of the hypanthium, is more likely to have several origins. It is clear that the tearing hypanthium syndrome is common in *Myrcia s.l.* and associated in this sample with three groups; wider sampling and/or increased support may indicate that it has arisen in further groups and may confirm the indication here that in some cases it is associated with the development of a calyptra (Clades 1 and 2), whereas in others, it is not (Clades 7 and 9).

Calyptranthes

Present results indicate a geographical division with the two Caribbean species emerging as sister group to the Brazilian species. The small sample of *Calyptranthes* in this analysis prevents assessment of Legrand's (1962b) informal subgeneric divisions; the four Brazilian species emerge in three separate groups, with *C. grandifolia* and *C. clusiifolia* emerging in one of Legrand's groups but separated in this analysis. It is not possible at this stage to comment on the morphological significance of the arrangement of these four Brazilian species and it should be noted that no Central American, Amazonian or northeastern *Calyptranthes* (94, 19, 12 species respectively; Govaerts et al. 2006) were sampled. The high

bootstrap percentages in Clade 1 (*Calyptranthes*) suggest that this group would produce a robust phylogeny at species level if a broader sample were analysed using similar protocols.

Combined analysis

Although some (Farris et al. 1994) would say that results of the partition homogeneity test indicate significantly different phylogenetic signal between the molecular and morphological data sets ($P = 0.01$) and should therefore not be combined, others (Dowton & Austin 2002; Barker & Lutzoni 2002) doubt that the partition homogeneity test is an effective measure of congruence, particularly when two datasets differ as widely in size as they do here. The datasets were therefore combined, but results have been interpreted in the light that incongruence may be affecting final topologies. Bootstrap support fluctuates slightly as a result of combining the data sets; slightly less than 50% of clades receive increased BP whereas a slim majority have BP decrease; the overall effect on the trees however, is negligible, with hard incongruence (BP >50) visible at only two points. In Clade 4, morphological data provide weak support (BP 51) for *Myrcia citrifolia* as sister to *M. paracatuensis*, *M. rufipes*, *M. lingueaformis* and *M. variabilis*, whereas the molecular data place *M. citrifolia* sister to the other subclade of Clade 4 (again with weak support; BP 53). In Clade 7 weak support (BP 51) for a sister relationship between *Marlierea glaziouviana* and the rest of the clade is increased slightly to provide BP 60 for this taxon to emerge in the main subclade of four taxa in an unsupported sister relationship with *Marlierea pilodes*. Other increases in BS are notable in Clade 9, which benefits from a slight overall increase and node H, which is supported only by Bayesian probability in the molecular analysis but receives BP 54 in the combined molecular and morphological analysis.

Chapter 5 – Biogeography

Introduction

Myrtaceae

The mostly tropical, disjunct distributions of Myrtaceae and tribe Myrteae (Figure 5.1) have generated debate over their historical biogeography (Berry, 1915; Raven & Axelrod, 1974; Johnson & Briggs 1984; Pigg et al. 1993; Wilson et al. 2001; Sytsma et al. 2004). Following putative Myrteae fossil finds in North American Tertiary deposits, initial hypotheses (Berry 1914, 1915) were of a fleshy-fruited plesiomorphic state and North American origin. However, Raven & Axelrod (1974) suggested that differentiation between then recognized subfamilies Leptospermoideae and Myrtoideae took place from ancestral stock that occupied west Gondwana (Myrtoideae) and Australasia (Leptospermoideae) and modern distributions were mainly the result of early Cretaceous Gondwanan vicariance, with long-distance dispersal resulting in disjunct distributions. Phylogenetic analyses of morphological (Briggs & Johnson 1979; Johnson & Briggs 1984) and later DNA (Wilson et al. 2001, 2005; Sytsma et al. 2004) data suggested a Gondwanan origin of Myrtaceae, with Myrteae originating and diversifying in Australasia between 77–56 mya, when Australia was connected to South America via warm-temperate Antarctic land bridges. Following at least two separate dispersal events, South American *Eugenia* occupied Africa (van der Merwe et al. 2005), *Syzygium* and *Metrosideros* migrated from Australasia to Africa, *Tepualia* from Australasia to South America, and c. 50 mya (Sytsma et al. 2004), *Myrtus* migrated from an unknown locality to the Mediterranean.

Myrteae

As discussed above, a variety of authors have considered and hypothesised events that have shaped Myrtaceae distribution over time; there has however, been little speculation specifically regarding the colonization of South America by tribe Myrteae. Based on generic morphology and modern distributions, McVaugh (1968) concluded that Myrteae were initially established in southern South America and subsequently spread throughout the continent, this area of origin is unconfirmed, however, as are the number of colonization events.

With probably five locules, C-shaped embryos and parietal placentation, the fossil genus *Paleomyrtinaea* from the late Palaeocene of North Dakota and early Eocene of British Columbia (Pigg et al. 1993) is known only from fruit. This genus is thought by some to be most closely related to *Psidium* or *Mosiera* (Pigg et al. 1993), whereas others remain unconvinced of its affinity to Myrtaceae (Landrum pers. comm.). If Pigg et al. (1993) are followed, the presence of this fossil indicates a previously much wider distribution of Myrteae. This wider distribution suggests that some species may have arrived in North America via Africa/Asia, perhaps via North Atlantic land connections during periods of Arctic warming that began c. 60 mya. (Davis et al. 2002), or alternatively, that at some period in the history of Myrteae, its distribution over the North and South American continents was continuous.

Myrcia s.l.

The *Myrcia s.l.* clade is endemic to the Neotropics, ranging from southern Florida, through Central America and the Caribbean, with the most southerly species reaching northern Argentina and Paraguay (Fig. 5.2). Centres of species diversity for the four traditional 'core' genera of Myrciinae *sensu* O.Berg, have been suggested as follows: *Calyptranthes* in the Caribbean, *Myrcia* in southern or central Brazil, *Gomidesia* in southern Brazil and *Marlierea* in the Guayana highlands and/or extra-Amazonian Brazil (McVaugh, 1968). Ranges of the

Myrcia s.l. sub-clades described in Chapter 4 vary considerably (Table 5.1), and almost nothing is known of the origin of these newly recognised infrageneric groups, or of their evolutionary history.

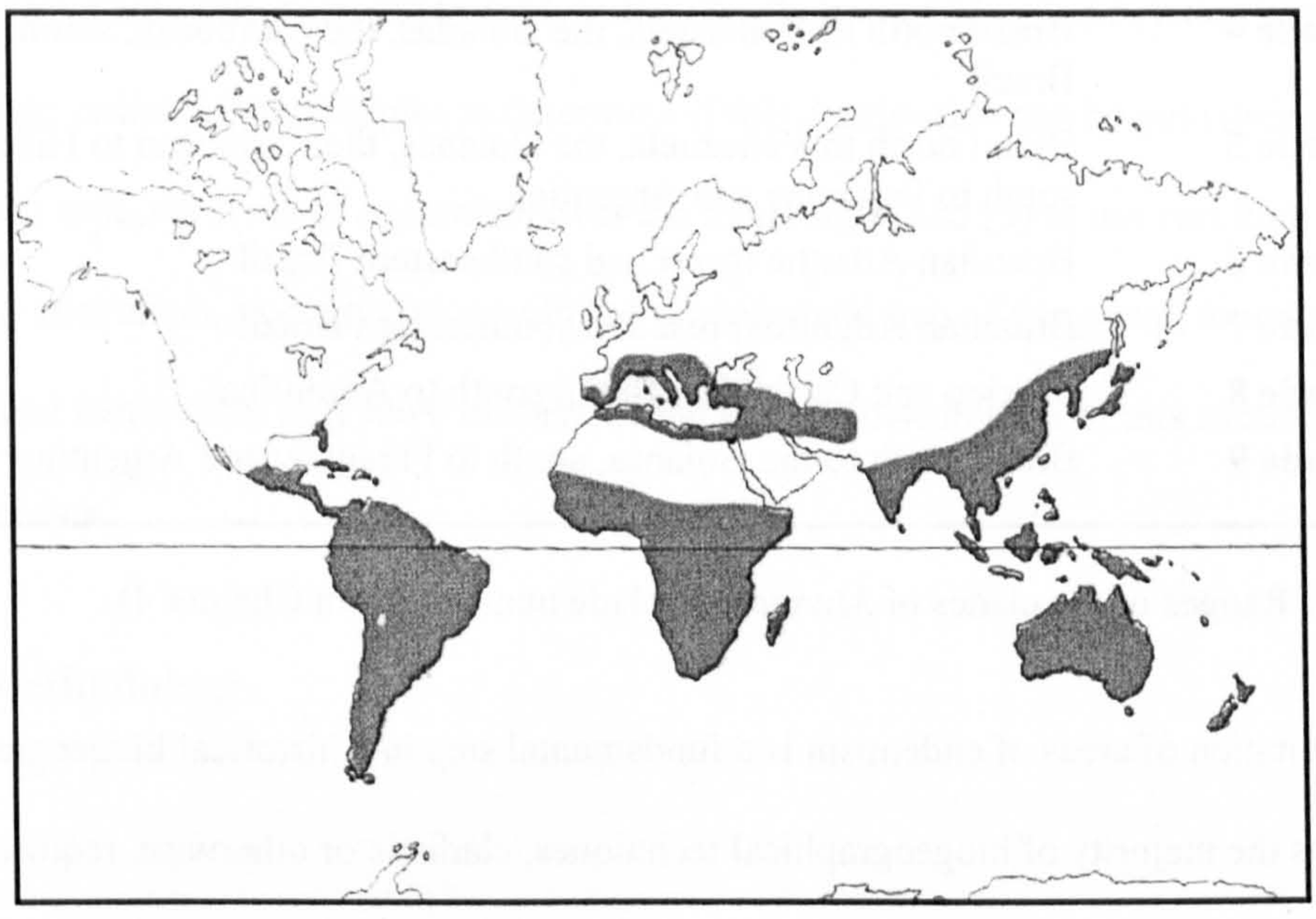


Figure 5.1. Myrtaceae and Myrteae distributions. From Heywood (1993).

Figure 5.2. *Myrcia s.l.* distribution. Points represent herbarium specimen collection localities. From Myrtaceae database, Royal Botanic Gardens, Kew.

Section	Range
Clade 1	Florida, Mexico and Caribbean, Mexico south to N. Argentina
Clade 2	Southeastern Brazil
Clade 3	Brazil north to Venezuela, the Guianas, the Caribbean to Hispaniola, south to Paraguay and Argentina
Clade 4	Brazil north to Venezuela, the Guianas, the Caribbean, southeastern Brazil
Clade 5	Brazil north to Venezuela, the Guianas, the Caribbean to Hispaniola, south to Paraguay and Argentina
Clade 6	Brazilian Atlantic forest and southeastern Brazil
Clade 7	Brazilian Atlantic forest and southeastern Brazil
Clade 8	Mexico and Caribbean, Brazil south to Argentina
Clade 9	Brazil north to the Guianas, south to Paraguay and Argentina

Table 5.1. Ranges of the clades of *Myrcia s.l.* (clade numbers from Chapter 4).

The delimitation of areas of endemism is a fundamental step in a historical biogeographic analysis as the majority of biogeographical techniques, cladistic or otherwise, require *a priori,* a hypothesis of clearly delimited biogeographical areas (Crisci et al. 2003). That these areas should be 'areas of endemism' was emphasised by Heads (1999), who described how the non-integration of local vicariance and/or the use of polyphyletic areas of endemism in an analysis would inevitably result in cladograms incongruent with any geological split sequence.

Due to limitations of the phylogenetic results of Chapter 3, discussed in greater detail below, the biogeographical focus of the study presented here regarding *Myrcia s.l.* biogeography shifted from an attempt to discover the biogeographical history of the group, to a study of its areas of endemism using statistical rather than phylogenetic techniques. It is hoped that a study of this nature will provide basic areas of study for future, phylogeny-based biogeographical studies. In this chapter however, the resulting areas are simply discussed in light of the phylogeny.

Aims

This Chapter has three main goals: (1) to investigate the biogeographical relationships of the major clades of tribe Myrteae; (2) to analyse the distribution of *Myrcia s.l.*, using non-phylogenetic ordination techniques to determine if this distribution can be convincingly divided into separate areas of endemism over the total range; and (3) to use resulting areas of *Myrcia s.l.* endemism, in conjunction with the phylogenetic tree of the group, to understand the historical factors that may have influenced the modern distribution of this subtribe in South America.

Methodology

Myrteae

Dispersal vicariance analysis (DIVA 1.1; Ronquist, 1996, 1997) was used to calculate likely distributions of the now extinct ancestors represented by the internal nodes of the Myrteae tree and examine biogeographical relationships of major Myrteae clades. DIVA employs an event-based method to reconstruct ancestral distributions of taxa, taking into account historical processes of vicariance, dispersal, or extinction, and applying a set of optimization rules and costs to them. The method maximizes the probability of vicariance events (no cost) and minimizes the probability of dispersal and extinction (cost of 1), allowing the existence of reticulate relations among the areas, not only hierarchical ones.

DIVA requires delimitation of Myrteae distribution into phytogeographically meaningful, yet phylogeny-independent divisions. Myrteae distribution was divided into 14 such regions (Fig. 5.3); North/Central America (NCA), western South America (WSA), northern South America (NSA), north/central Brazil (NCB), eastern South America (ESA), southern South America (SSA), North Africa/Mediterranean (NAM), Indochina (INC), Malesia (MAL), Papuasia (PAP), Australia (AUS), Pacific (PAC), New Zealand (NZ), and sub-Saharan Africa

(AFR). South American regions (Table 5.2) are amalgamations of the phytogeographically distinct divisions of Cabrera and Willink (1980), sometimes divided in consideration of Myrteae distribution, separating areas with most species diversity and endemism, such as the Atlantic forest and *cerrado* of Brazil (Oliveira-Filho & Fontes, 2000), the Amazon basin (Prance et al., 1976), the Guianas (McVaugh, 1969) and the central to southern Andes (Landrum, 1981b, 1986). The two remaining areas (North/Central America, western South America) are areas of similar size to the others, without remarkable Myrtaceae species diversity but with distinct phytogeographical compositions (Cabrera & Willink, 1980). Myrteae distribution outside the Americas was divided according to the phytogeographically distinct areas proposed by Brummitt et al. (2001). The MPT on which the biogeographical analyses were based was the same as for the morphological optimizations, with *Syzygium* as the outgroup due to its position as sister to tribe Myrteae in Chapter 3. Distributional data were collected from Kew herbarium collections (Brummitt & Brummitt 2001) and available literature. Genera were scored as present or absent in each area with the distribution of a genus taken as the sum of areas that it currently inhabits (Appendix 6).

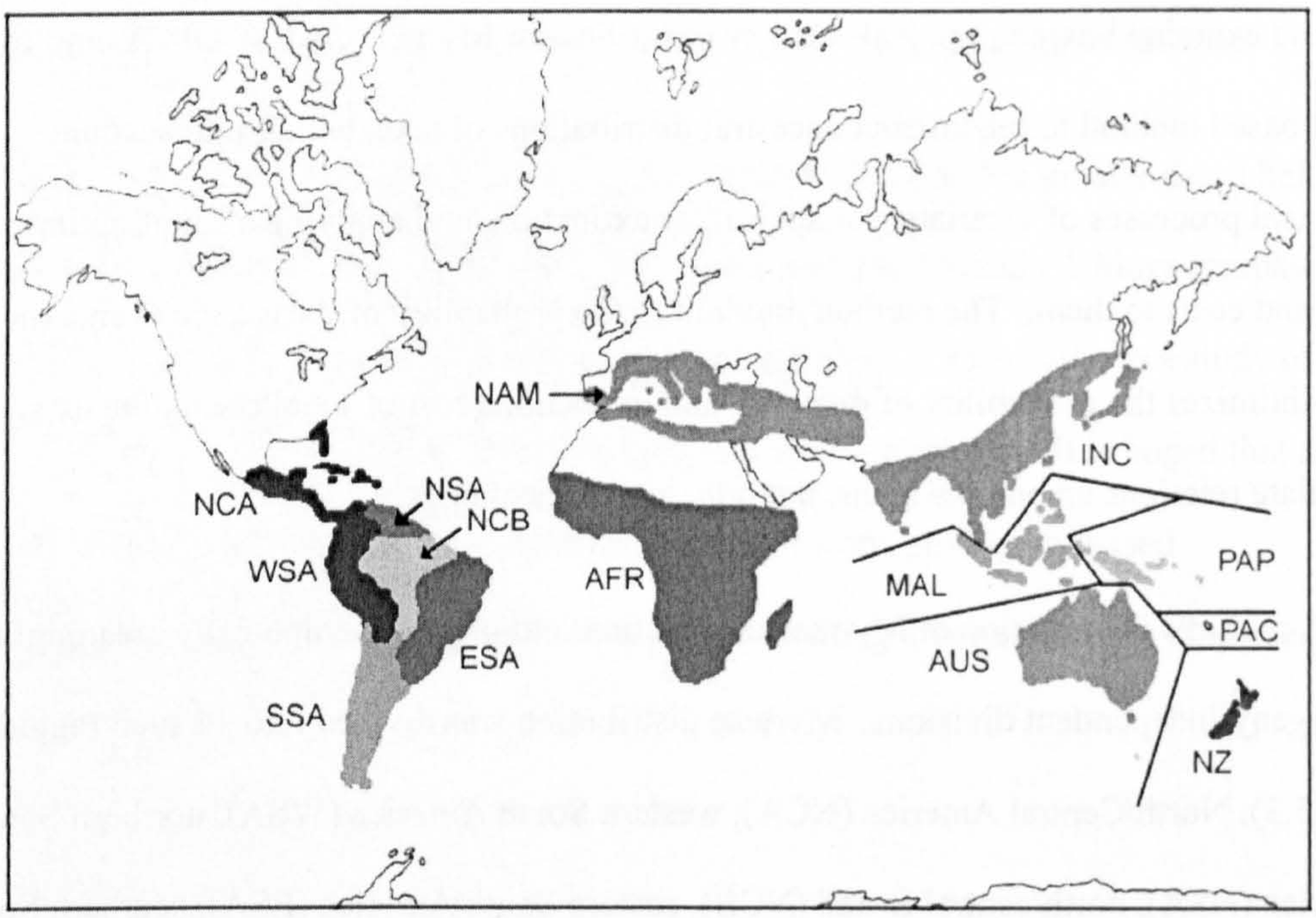


Figure 5.3. Myrteae distribution with biogeographical divisions used for dispersal-vicariance (DIVA) analysis. Adapted with permission from Heywood (1993).

Area	Definition	Phytogeographical provinces of Cabrera & Willink (1980)
North/Central America (NCA)	USA (Florida), Mexico, Central America to Panama, Greater Antilles.	Mesoamericana, Mexicana, Caribe (*pro parte*), Guajira, las Galápagos.
Western South America (WSA)	Colombia, Ecuador, Peru and Bolivia.	Amazónica (*pro parte*), Pacífica, las Yungas, Venezolana, la Sabana, Páramo, Monte (*pro parte*), Altoandina (*pro parte*), Puneña, Oceánico Peruana-Chileno (*pro parte*).
Northern South America (NSA)	Venezuela, the Guianas, Brazil north of 1°N, Lesser Antilles.	Caribe (*pro parte*), Amazónica (*pro parte*), Guayana.
North/central Brazil (NCB)	Brazil south of 1°N, including the states of Acre, Amapá, Amazonas, Brasília, Goiás, Mato Grosso, Mato Grosso do Sul, Maranhão, Pará, Rondônia, Roraima, Sergipe, Tocantins.	Amazónica (*pro parte*), Cerrado.
Eastern South America (ESA)	Uruguay, Argentina and Paraguay east of the Paraná and Paraguai rivers, Brazilian states of Alagoas, Bahia, Ceará, Espírito Santo, Minas Gerais, Paraíba, Paraná, Rio de Janeiro, Rio Grande do Sul, Rio Grande do Norte, Santa Catarina, São Paulo, Sergipe.	Paranense, Atlantica, Caatinga, Chaqueña, Espinal (*pro parte*), Monte (*pro parte*), Pampeana, Oceánico tropical.
Southern South America (SSA)	Chile, Argentina and Paraguay west of the Paraná and Paraguai rivers.	Prepueña, Altoandina (*pro parte*), Desierto, Chilena Central, Patagónica, Subantártica, Insular, Juan Fernández, Antártica, Oceánico Magallánico, Oceánico Peruana-Chileno (*pro parte*), Oceánico Antártica.

Table 5.2. Definitions of Neotropical areas based on established phytogeographic centres (Cabrera & Willink 1980).

Myrcia s.l.

Areas of endemism

The availability to this study of a comprehensive, georeferenced database of c. 12220 *Myrcia s.l.* specimens meant that species ranges were understood more precisely and the biogeography of the group and its areas of endemism could be investigated at a more accurate scale than was possible for tribe Myrteae. This database comprised all specimen records held at the herbaria of the Royal Botanic Gardens, Kew, the New York Botanical Garden, the National Herbarium of French Guiana, Botanical Gardens of Rio de Janeiro and the National Herbarium Nederland [Utrecht]. For species endemic to the *Mata Atlântica,* additional records were obtained from the online herbarium resources of the Field Museum; the Smithsonian Institute; TROPICOS (Missouri Botanic Gardens) and original literature sources. For taxonomic uniformity, species names were standardised according to the World Checklist of Myrtaceae (Govaerts et al. 2006) and Nic Lughadha's (1997) account of *Gomidesia*. Each record of the database represents a herbarium sheet and the ecological information from its collection label.

The database was queried to remove duplicate records, those with locality data insufficient for georeferencing and those not or unreliably named; 5801 records remained. This reduced dataset was mapped onto an outline of South America using ArcGis 9.1 (ESRI, www.esri.com; Fig. 5.2) and sequentially overlaid with twelve grids generated using the GIS extension 'Repeating Shapes' (Jenness, 2006). Maps and grids were projected using an Albers Equal Area Conic projection for South America included in the ArcGis 9.1 package that accounted for the curvature of the earth and ensured uniformity of grid-cell size. A grid-cell size was sought that would provide a compromise in size, small enough to resolve biogeographical patterns between cells yet sufficiently large to represent internal species composition, allowing comparisons of the cells to be made. A grid-cell size of 300 km^2 was selected and overlaid on the *Myrcia s.l.* distribution map, initially with the horizontal grid bars

parallel to the equator; the grid was then replaced three times with the horizontal bars randomly angled according to the equator. Success of the 300 km^2 grid cell in meeting the criteria discussed above and providing clear results, was assessed by repeating this process using smaller (150 km^2) and larger (400 km^2) grids.

The twelve resulting grids were overlaid one at a time, onto a hypothetical biome map (Fig. 5.4) covering the extent of *Myrcia s.l.* distribution; this was based on the IBGE (2004) biome map for Brazil and extended using satellite imagery available from Global Land Cover 2000 project (www.gvm.jrc.it/glc2000/Products) and the Perry-Castañeda Library Map Collection used courtesy of the University of Texas Libraries, The University of Texas at Austin (www.lib.utexas.edu/maps). The six IBGE biomes (Amazon, Atlantic Forest, *caatinga*, *cerrado*, Pampas, Pantanal) were extended to cover similar vegetation beyond the Brazilian border, and a further three categories were added, Central America, the Caribbean and Amazonian Savannah (Fig. 5.4).

ArcGIS 9.1 was used to query the maps to assign each grid cell to a biome; cells bisected by two or more biomes were manually assigned to whichever occupied most of the cell. Maps were then queried for the species composition of each grid square with results reported as a presence/absence matrix for each species in each square. The Beals Smoothing data transformation was applied to the matrix, replacing each cell with a probability of the species occurring there, based on joint occurrences of the target species with species actually in the sample (McCune & Mefford 1999). The 'slow and thorough' autopilot mode of Nonmetric multidimensional scaling (NMS; Kruskal 1964) in PC-ORD (McCune & Mefford 1999) was then applied, so grouping the grid cells in multidimensional space by similarity of species composition. NMS is an iterative search for the best positions of a given number of entities on the optimal number of axes that minimises the stress of the configuration (McCune & Grace 2002). PC-ORD uses a distance matrix to measure distance between the rows (entities) and columns (attributes) of a data matrix. Arrangements are attempted using one to six axes, and "stress" is measured in relation to the number of axes. The 'slow and thorough' setting

used Sørensen distances to express community resemblances, performing 40 runs with the real data along with 50 runs with randomized data for a Monte Carlo test of significance; the proportion of randomised runs with stress less than or equal to the observed stress is reported as p and the arrangement with the lowest stress and lowest p is selected as the 'best' solution.

NMS results were plotted on the appropriate number of axis (two), and the data series corresponding to the cells assigned to each of the nine hypothetical biomes were coloured to assess the grouping of points (cells).

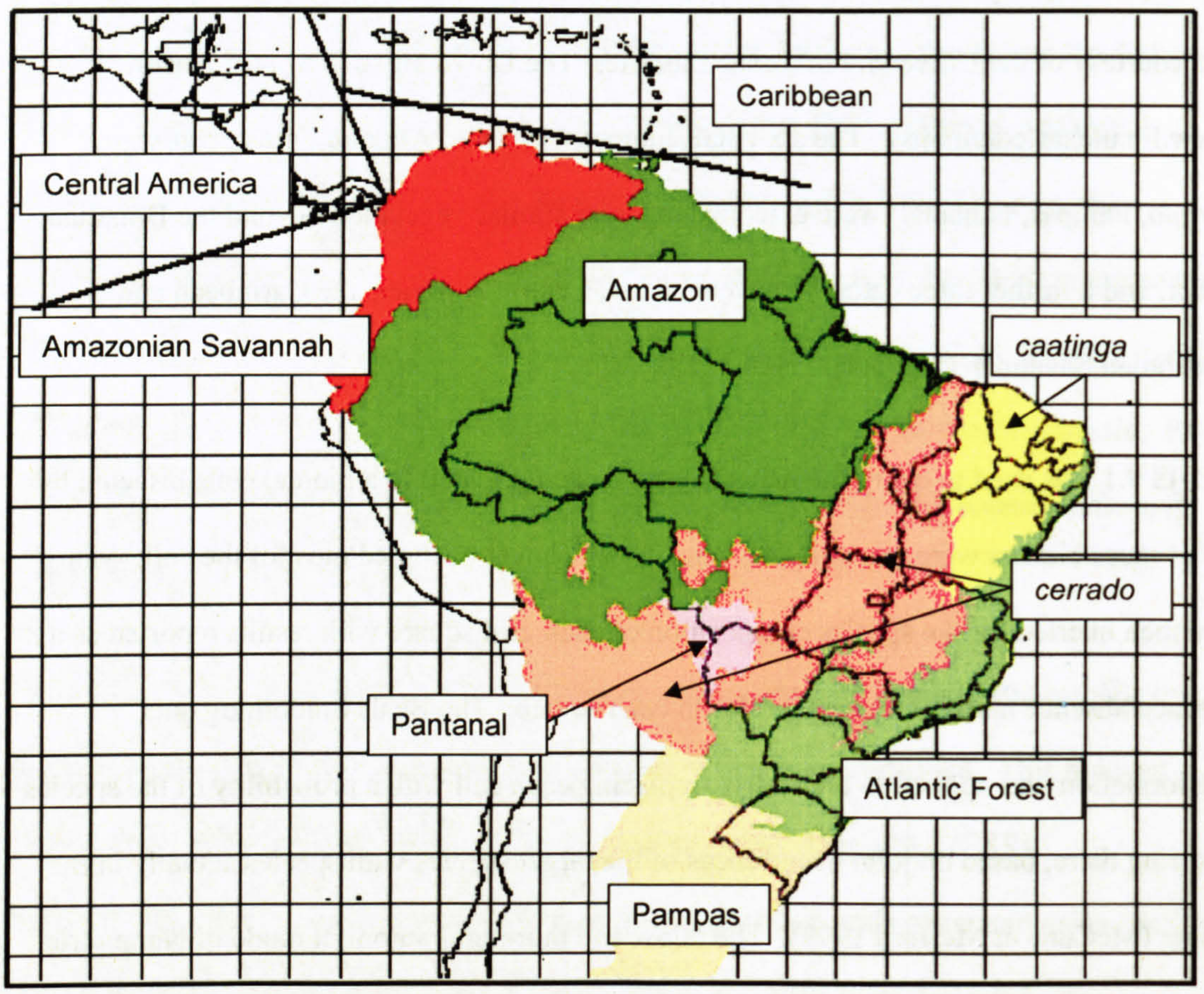


Figure 5.4. Hypothetical biome map of South America.

Historical biogeography

A historical biogeography methodology was then sought with which to analyse the resulting objectively identified areas of *Myrcia s.l.* species endemism in conjunction with the

phylogenetic tree produced in Chapter 4. The main objective was to identify factors that have influenced the modern distribution of this group in South America.

Model-based methods such as DIVA and methods which optimize distribution areas onto a phylogeny were considered for this dataset but were discounted. Model-based methods are concerned with the terminal taxonomic units of a phylogenetic tree – either species or, as is the case here and in the Myrteae analysis, terminal clades. If a species-level analysis is to be subjected to DIVA, complete or near-complete sampling is required to ensure phylogenetic and geographical completeness. If clades are the focus of the study, again there must be a level of confidence that these clades adequately cover the phylogenetic and geographical spectrum of a taxon and, once again, that the distributions of the individual clades are fully known. In the case of *Myrcia s.l.*, despite confidence that the tree produced in Chapter 4 provided good representation of phylogenetic variation within the taxon, it was impossible to predict the phylogenetic positions of the several hundred unsampled species and to assign accurate distributions to the nine identified sub-clades. The same problem applied to the use of optimization methods, with additional problems associated with the choice of parsimony algorithm to use. Instead, the phylogenetic tree (Fig. 4.6) was simply examined in light of the presence/absence of members of the main clades in the areas of endemism here identified.

Results

Myrteae

The DIVA programme provides decreasing certainty regarding the area of origin towards the root node of a tree (Ronquist, 1996); as a result, the most conservative suggestion for the area of origin of tribe Myrteae involves eight of the fourteen areas used in the analysis and is not further discussed.

The 'Australasian group' (Fig. 3.6, Clade G), shares its distribution with the majority of Myrtaceae genera with capsular-fruits; this fruit type is believed to be plesiomorphic and it is assumed that the oldest Myrtaceae genera are found in the Australasian region also (Wilson et al. 2001). DIVA results (Table 5.3) therefore suggest that Clade G evolved *in situ* in Indochina, Malesia, Australia, Papuasia, and the Pacific.

The South American Myrteae clade and Clade I (South American Myrteae, excluding *Blepharocalyx salcifolius*) receive identical DIVA results (Table 5.3), indicating the origin of each in any or all of the South American areas suggested here, compatible with the hypothesised movement of one or more ancestral taxa from present-day Australasia to South America. DIVA suggests that of the two main clades into which the ancestor of Clade I diverged (J and K), the origin of Clade J was in either western or southern South America, suggesting an Andean origin for the '*Myrteola*', '*Pimenta*' and '*Eugenia*' groups, a scenario supported by the suggestion of the same areas of origin for all three of these last clades (D, E, and F, respectively) with the additional inclusion of eastern South America in the case of the '*Pimenta* group'. This suggests that some large genera with centres of diversity in eastern or northeastern South America, such as *Eugenia* and *Psidium,* may have migrated from southwestern to north or northeastern South America and speciated there, perhaps reaching the east via temperate to subtropical forests covering what is now Chile and southern Argentina in the Oligocene (Landrum, 1981a).

Node	Area of ancestral distribution of key Myrteae clades inferred from DIVA
'*Plinia* group' (Clade A)	NCB; ESA; NCB-ESA; NCA-NCB-ESA; NSA-NCB-ESA; NCA-NSA-NCB-ESA; WSA-NCB-ESA; NCA-WSA-NCB-ESA; NSA-WSA-NCB-ESA; NCA-NSA-WSA-NCB-ESA; NCB-ESA-SSA; NCA-NCB-ESA-SSA; NSA-NCB-ESA-SSA; NCA-NSA-NCB-ESA-SSA; WSA-NCB-ESA-SSA; NCA-WSA-NCB-ESA-SSA; NSA-WSA-NCB-ESA-SSA; NSA-NCA-WSA-NCB-ESA-SSA.
'*Plinia* group' + *Algrizea*	NCB; NCB-ESA.
'*Myrceugenia* group' (Clade C)	SSA.
'*Myrteola* group' (Clade D)	WSA; SSA.
'*Pimenta* group' (Clade E)	WSA-SSA; ESA-SSA.
'*Eugenia* group' (Clade F)	WSA; SSA.
'*Australasian* group' (Clade G)	INC-MAL-AUS-PAP-PAC.
Clade I	NCA-NSA-WSA-NCB-ESA-SSA.
Clade J	WSA; SSA.
Clade K	NCA-NSA-NCB-ESA-SSA; NCA-NSA-WSA-NCB-ESA-SSA.
Clade L	NCA-NSA-NCB-ESA; NCA-NSA-WSA-NCB-ES; NCA-NSA-NCB-ESA-SSA; NCA-NSA-WSA-NCB-ESA-SSA.
South American Myrteae	NCA-NSA-WSA-NCB-ESA-SSA.

Table 5.3. Areas of ancestral distributions of key Myrteae clades as inferred from DIVA. North/Central America (NCA), western South America (WSA), northern South America (NSA), north/central Brazil (NCB), eastern South America (ESA), southern South America (SSA), North Africa/Mediterranean (NAM), Indochina (INC), Malesia (MAL), Papuasia (PAP), Australia (AUS), Pacific (PAC), and New Zealand (NZ). Clades identified by letters refer to Fig. 3.6.

Any or all South American divisions are suggested as areas of origin for Clades A, B, K, and L, allowing no precise conclusions to be drawn regarding the area of origin of the ancestor of the '*Plinia* group'+*Algrizea* +'*Myrcia* group' association and the '*Myrceugenia* group'. The same lack of certainty applies to the areas of origin of the '*Plinia*' and '*Myrcia*' groups and *Algrizea*; however, the area of origin of the '*Myrceugenia* group' is exclusively suggested as southern South America, supporting Landrum's conclusions (1981a).

Myrcia s.l.

Areas of endemism

In PC-Ord, the Monte Carlo test result was $p = 0.196$ for all grid cell analyses, regardless of cell size or orientation; in each case mean stress was lowest for the 2-D solution (Table 5.4). Rules of thumb for stress levels used here follow Clarke (1993) who suggested that stress of 10-20 can correspond to a usable picture although with potential to mislead and with unreliable details; all of the plots in this study fall into this category.

Despite differences in resolution amongst data plots resulting from analyses of the three different grid cell size, overall patterns are reassuringly similar; in each case one example is selected and presented (Figs 5.5–5.7). Overall patterns at all grid cell sizes are affected by spurious cells containing single records, particularly if these are records of species with only one occurrence in the database; these cells appear in the plots at the extremities of the axes in an arc pattern. This occurs more often at the smaller 150 km^2 grid cell size and may explain why there is slightly more stress in the data in the four analyses at this cell size than at 300 km^2. Spurious outlier cells are considered to contain little significant information and are not considered when interpreting results. In terms of areas of endemism, results from all analyses indicate a similar pattern; there is a significant amount of overlap between several hypothetical biomes, but species compositions in the Central American and Caribbean biomes are clearly very different from the rest, with some similarity to each other detectable. It is

difficult to distinguish the Amazonian savannah biome in any analysis, with cells so assigned falling well within the spread of the Amazonian cells or as spurious outliers indicating their inclusion of single species only and suggesting high levels of endemism and/or serious under collection in this region. Herbarium collections studied during this project and analysis of the *Myrcia s.l.* database suggest that despite the presence of endemic species in the Amazonian savannah, the area is so poorly collected and represented in the world's herbaria that under-collection is more likely to be the cause of these ambiguous results. Atlantic forest cells are shown to group independently from the large majority of Amazon cells; the *caatinga* and *cerrado* biomes appearing to grade gradually from the Amazon to the Atlantic forests, with much overlap between them.

Grid cell size - run	Number of squares occupied by *Myrcia s.l.*	Mean stress in real data	Mean stress in randomised data
150 km^2: Run 1	439	15.283	41.919
150 km^2: Run 2	451	15.733	41.923
150 km^2: Run 3	450	17.661	34.780
150 km^2: Run 4	447	15.920	41.923
150 km^2: Mean	446.8	16.149	40.136
300 km^2: Run 1	184	13.814	33.805
300 km^2: Run 2	182	16.725	34.932
300 km^2: Run 3	187	15.006	33.626
300 km^2: Run 4	180	16.954	33.958
300 km^2: Mean	183.3	15.625	34.080
400 km^2: Run 1	120	20.901	34.013
400 km^2: Run 2	123	15.332	41.371
400 km^2: Run 3	122	16.178	41.375
400 km^2: Run 4	119	15.021	33.859
400 km^2: Mean	121	16.858	37.656

Table 5.4. Grid cell size and mean stress values for the 2-D solution for real and random data for each of twelve grid cell analyses.

The majority of *cerrado* cells group tightly, overlapping and linking the *caatinga* and Atlantic forest groups; some *cerrado* cells fall outside of this group and cluster with the Amazon group in the 150 km^2 grid cell analyses and closer examination indicates these to be cells

adjacent to cells assigned to this latter biome. The Pampas and Pantanal cells fall within the overlapping *cerrado* and Atlantic forest groups.

Based on these results, the *Myrcia s.l.* areas of endemism hypothesis suggested by the biome map (Figure 5.4) can be altered to better fit the observed situation, with other groups identified that suggest additional areas of species endemism. In Figure 5.5, dashed lines indicate divisions in the points representing the Amazon biome. On closer inspection it appears that these divisions, apparent in all analyses at 150 and 300 km^2 (with some exceptional points) represent natural data partitions; those Amazonian points labelled A represent grid cells falling in a type I peri-Amazonian arc of De Granville (1992). Grid cells of the hypothetical Amazonian savannah of this analysis also fall within this peri-Amazonian group, as do three Caribbean points which represent the Windward Islands of the Lesser Antilles, where it appears that *Myrcia s.l.* species composition is more similar to that of the Amazon than to the rest of the Caribbean (Fig. 5.8).

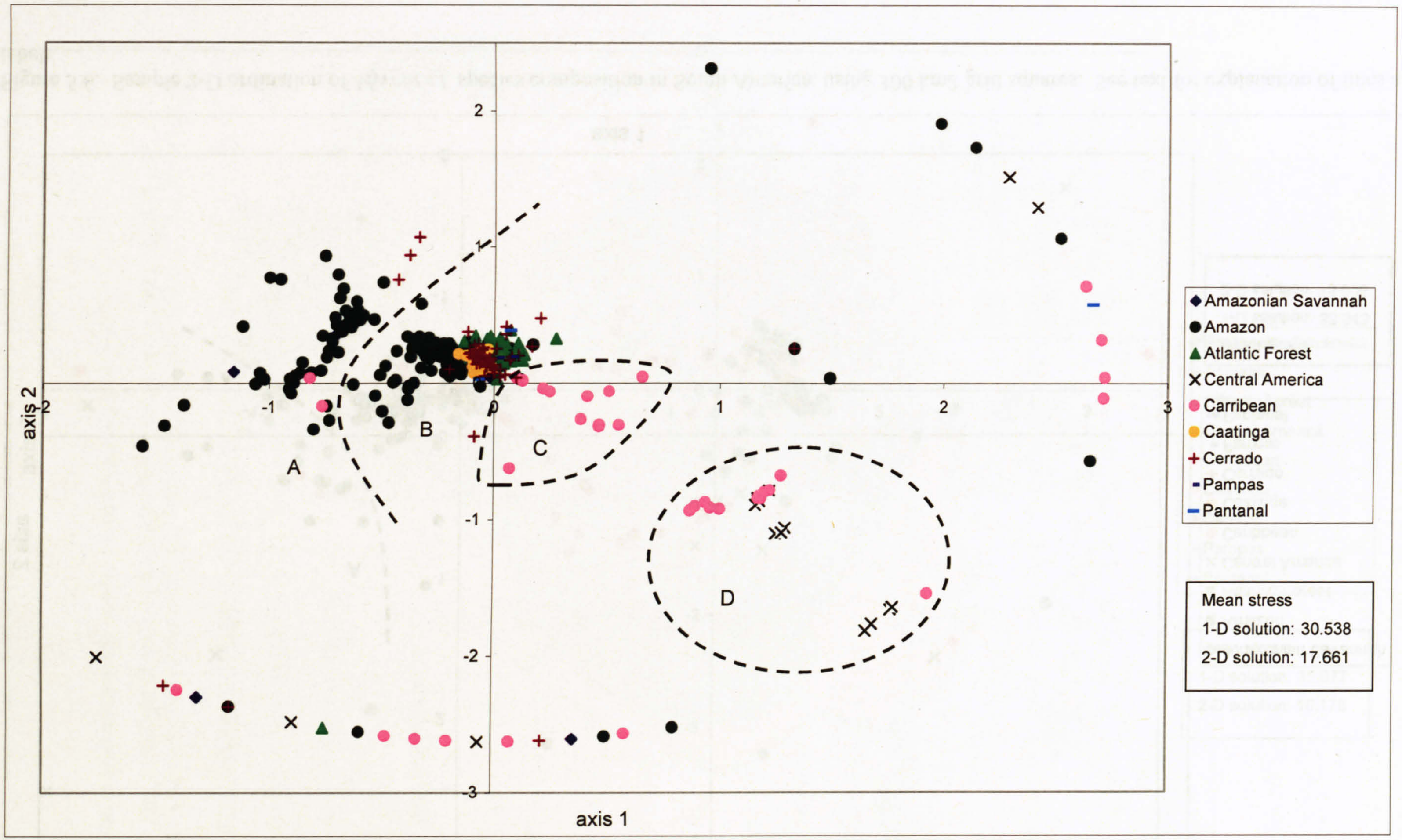


Figure 5.5. Sample 2-D ordination of *Myrcia s.l.* species composition in South America, using 150 km2 grid squares. See text for explanation of lines and labels.

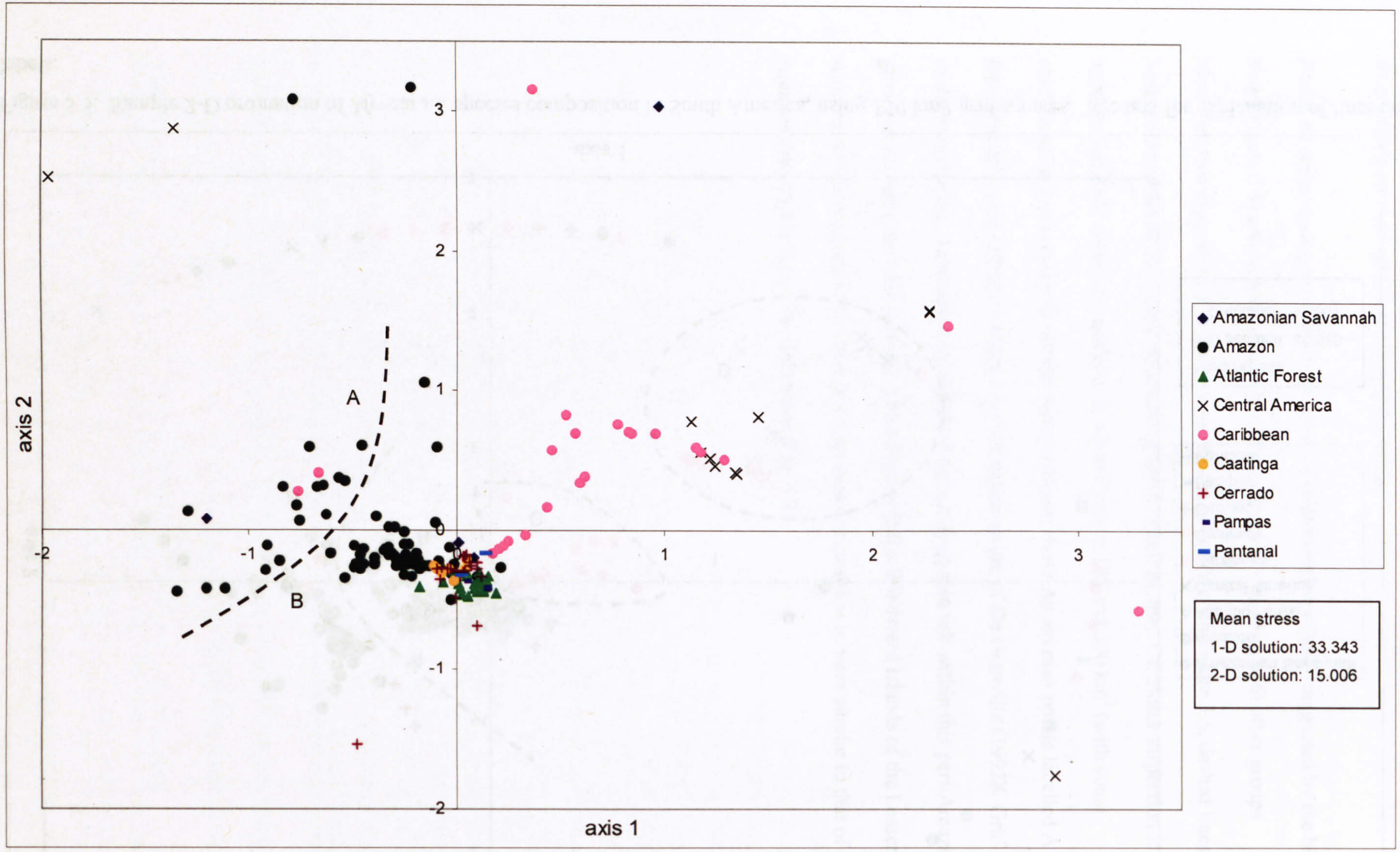


Figure 5.6. Sample 2-D ordination of *Myrcia s.l.* species composition in South America, using 300 km2 grid squares. See text for explanation of lines and labels.

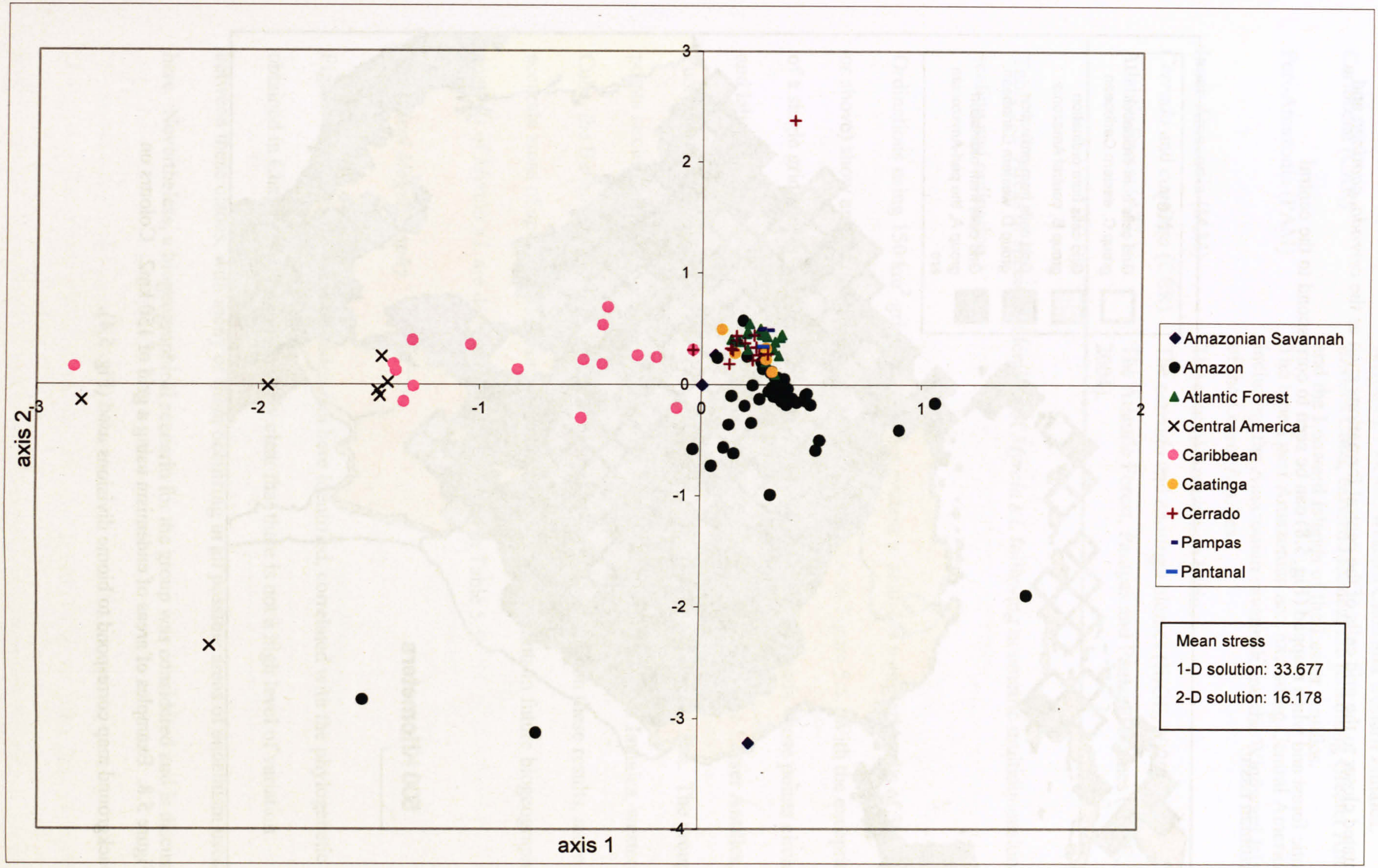


Figure 5.7. Sample 2-D ordination of *Myrcia s.l.* species composition in South America, using 400 km2 grid squares.

The remaining group of Amazonian cells are labelled B in Fig. 5.5, these are spatially orientated closer to the grid cells of the central formations such as the *cerrado*, *caatinga* and Atlantic forest and when mapped (Fig. 5.8) can be seen to correspond to the central Amazonian basin.

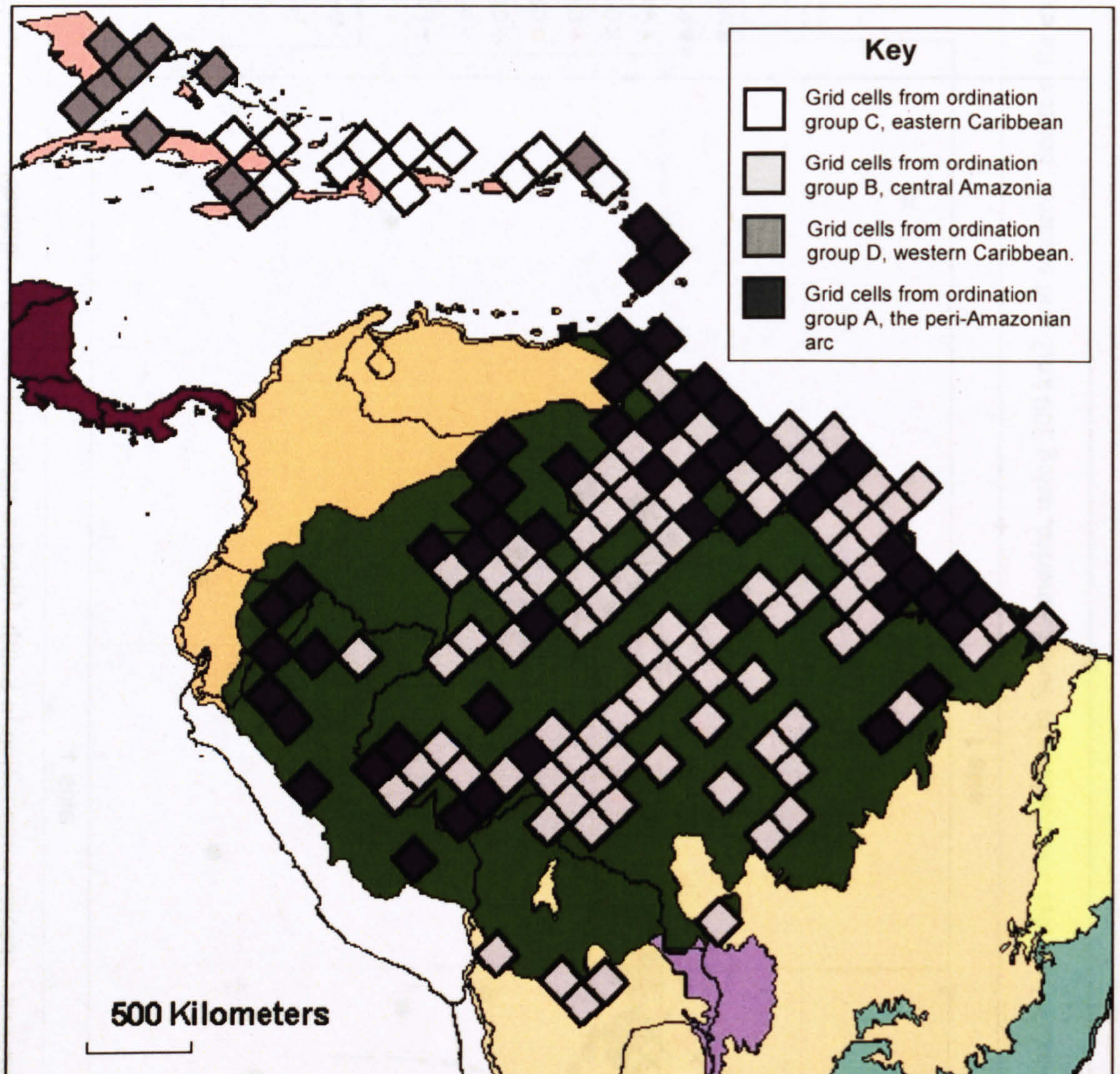


Figure 5.8. Examples of areas of endemism using a grid of 150 km2. Colours on background map correspond to biome divisions used (Fig. 5.4).

Area	Definition
Central America (CAM)	Including Florida, western Cuba and western Jamaica.
Caribbean (CAR)	Eastern Cuba, eastern Jamaica, the remaining Greater Antilles and the Leeward Islands of the Lesser Antilles.
Peri-Amazonia (PAM)	The type I peri-Amazonian arc, excluding Central America but including the Amazonian savannah and the Windward Islands of the Lesser Antilles.
Inner Amazonia (IAM)	The central Amazonian basin.
Cerrado and *caatinga* (CER)	The *cerrado* and *caatinga* biomes (IBGE, 2004).
Atlantic Forest (ATL)	The Atlantic Forest, Pampas and Pantanal biomes (IBGE, 2004).

Table 5.5. Areas of species endemism of *Myrcia s.l.* following nonmetric multidimensional scaling and ordination.

Ordinations using 150 km^2 grid cells (but not evident in analyses using grid cells of 300 km^2 or above) show an additional division indicated by circles in Figure 5.5. With the exception of a single grid square at the approximate position of the Virgin Islands, those points circled and labelled C represent cells corresponding to the Leeward Islands of the Lesser Antilles, Puerto Rico, the Dominican Republic, Haiti, eastern Jamaica and eastern Cuba. The group of points circled and labelled D correspond to grid squares covering western Jamaica, western Cuba, the Bahamas, Central America and Florida (Fig. 5.8). Based on these results, more accurate areas of species endemism for use as biogeographical units in future biogeographical analysis of *Myrcia s.l.* are defined and are presented in Table 5.5.

Historical biogeography

Figure 5.9 depicts the areas of endemism here identified, correlated with the phylogenetic tree obtained in Chapter 4. It is immediately clear that there is not a high level of variation between these clades, with many of them occurring in all possible areas of endemism used here. Nevertheless, a biogeographical scenario for the group was considered and is discussed.

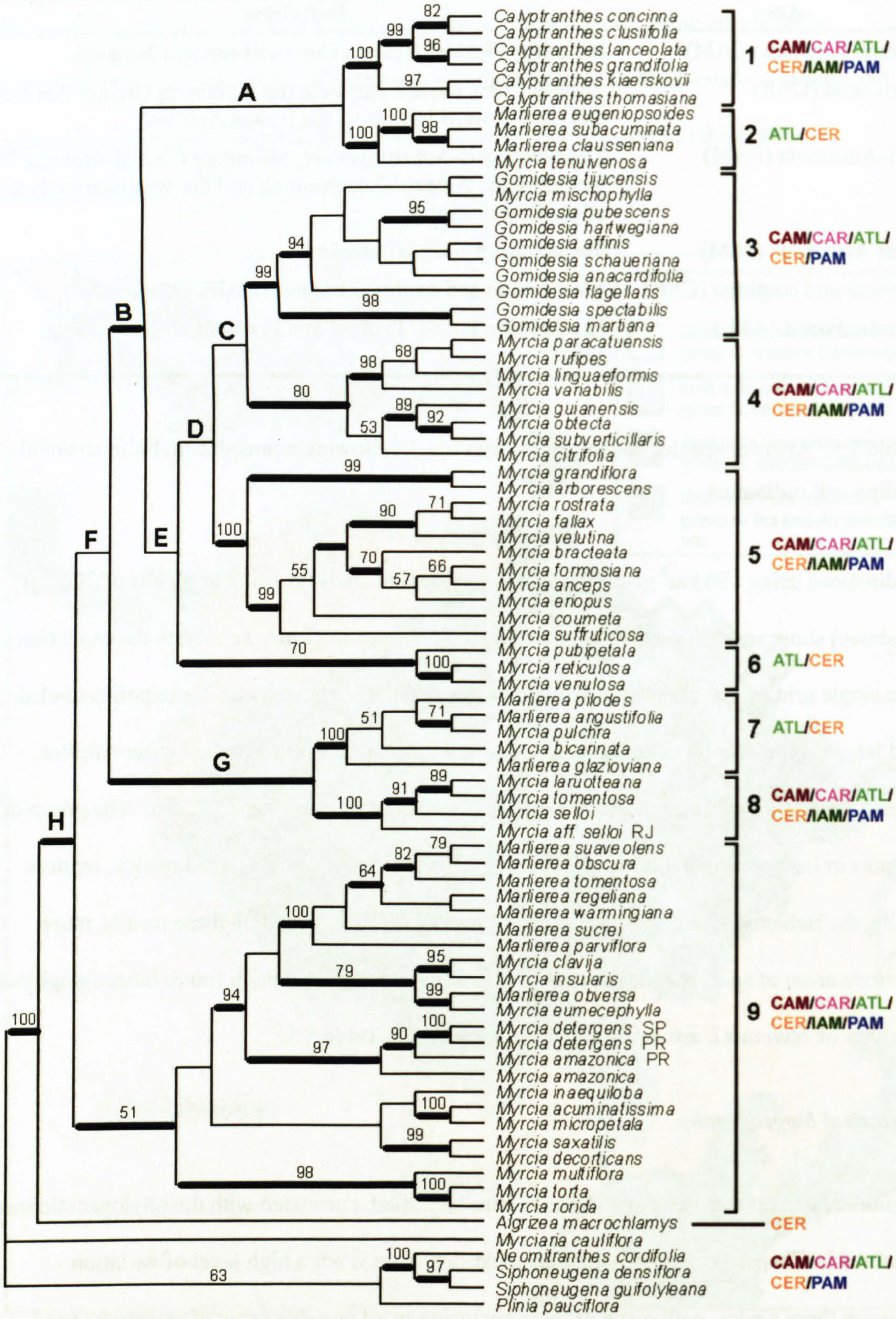


Figure 5.9. Occurrence of the main *Myrcia s.l.* clades in identified areas of endemism (see Table 5.4 for explanation of abbreviations used). Shading serves to visually unite areas on this figure only.

Discussion

Myrteae

The biogeographical scenario resulting from DIVA supports the hypothesis that Myrteae originated in Gondwana and migrated to South America, with ancestors of several, currently widespread genera originating in the south of the continent and moving northwards. There is no evidence of a Laurasian colonization of the Americas; the explanation suggested by these results for the presence of Paleocene *Paleomyrtinaea* fossils in North America is more likely due to range extension of the tribe from the south during warmer periods in the Paleocene and Eocene. Ancestors of *Eugenia* and the New Zealand genera, *Lophomyrtus* and *Neomyrtus*, appear to have originated in southern South America before dispersing, in the case of the first genus to South East Asia, Africa and Madagascar, and in the last two genera, to New Zealand. Based on information from Wilson (pers. comm.) of a likely relationship between *Myrtus* and *Calycolpus*, DIVA analysis was performed with *Calycolpus* as sister to *Myrtus*. This addition did not significantly change the resulting biogeographical scenario, usually increasing or sometimes decreasing the number of combinations of potential ancestral areas at a given node but without introducing any additional regions into the results.

The important role of dispersal in the history of Southern Hemisphere biogeography is currently undergoing a period of re-acceptance (Sanmartín & Ronquist, 2004). This pattern is supported here, with long-distance dispersals more likely to explain at least some of the intercontinental movements of Myrteae species than vicariance. The higher cost of these dispersal events in DIVA, however, results in an unknown number of optimal ancestral area combinations being missed. DIVA also assumes that ancestral species distributions occupied only one unit area, and although efforts were made to use phytogeographically distinct areas, it is impossible to determine accurately the optimal size of an area in which a taxon might have arisen. The results of DIVA may be further influenced by the geographic scale of this analysis and the large, somewhat coarse and debatable areas into which the distribution of

Myrteae has been divided. Debate regarding these divisions has in particular questioned the Southern South American (SSA) area as used here, noting that the phytogeographical provinces of Chilena Central, Sabantártica, Insular and Juan Fernández (Cabrera & Willink 1980) encompass the ranges of a significant number of endemic genera, namely *Luma, Temu, Legrandia* and *Amomyrtus*. This concentration of endemic taxa suggest that a further subdivision of SSA, introducing a southern-most region accounting for those four phytogeographical provinces listed above might provide improved division of the range of Myrteae. The use of such a scheme in future DIVA analyses might produce results that more accurately reflect the historical biogeography of the group and that minimise the incongruence of using areas of endemism that do not correspond to the evolution of the group under study – as may have been done here.

It should also be noted that DIVA results presented here are based on a topology in which some nodes are weakly supported and thus may be subject to future change. The consequence of the limitations of this analysis and those resulting from the assumptions of the DIVA program are that the biogeographical scenarios presented here can be considered only as starting points upon which future, more refined investigations can be based and from which the probabilities of taxa moving by dispersal *vs*. vicariance may be assessed using molecular dating techniques.

Myrcia s.l.

Areas of endemism

The areas of endemism resulting from the ordination study presented here are crude divisions, somewhat 'blurred' at the edges, but they are a first attempt at rigorous, repeatable analysis of distribution patterns in South American Myrtaceae. Results divide the distribution area of *Myrcia s.l.* into useful areas with which to consider speciation and biogeographical history of the group; future work refining the ordination protocols developed here, re-inforcing the

Myrcia s.l. tree and accumulating a more detailed knowledge of the species membership of the clades and thereby their distributions will allow the areas of endemism to be used in future model-based or cladistic biogeography analyses of the biogeography of the group.

Non-metric multidimensional scaling is considered the most generally effective ordination method for ecological data (McCune & Grace 2002) and has performed well here except in the case of rare species and those cells containing single or few species occurrences. In these exceptional cases, results are unreliable and cells are unassignable to groups. Future analyses will be honed to remove cells including single species and to include more complete distributions of rare species that may increase definition of areas. Further analysis of the link between data resolution and scale will also be investigated; the smallest grid-cell-size to which this type of analysis can be successfully applied is currently unknown, but future studies will investigate the possibilities of using still smaller units with an aim to determine the optimal size.

Historical biogeography

The phylogenetic hypothesis presented in Chapter 3 suggests that *Myrcia s.l*, *Algrizea* and the '*Plinia* group' may have arisen in any of the South American areas employed for DIVA analysis. The two main criteria used for determining the centre of origin of a group in the majority of classic biogeographical methods such as phylogenetic biogeography (Hennig 1966) and ancestral areas (Bremer 1992) are: i) that areas present on deep branches in a cladogram are more likely parts of the ancestral area than areas positionally more apomorphic; and ii) that areas represented on numerous branches of the cladogram are more likely parts of the ancestral areas than are areas represented on few branches. The lack of species of *Myrcia s.l*, *Algrizea* or the '*Plinia* group' in Chile or further south than northeastern Argentina therefore suggests the centre of origin of the clade comprising them to not be in these areas. Instead, the majority of species are found in the overlapping *cerrado*, *caatinga*

and Atlantic forest biomes (Figs 5.5–5.7), and it is here assumed that these are the ancestral areas of *Myrcia s.l.*

After initial colonisation of the southernmost parts of South America by Myrteae ancestors (Chapter 3), *Myrcia s.l.* ancestors may have arrived in the north-east of the continent via humid subtropical forests that migrated south during the late Paleocene and Eocene, reaching their maximum advance to c. 40° S by the middle Eocene (c. 50 Ma.). Here they mixed with the still temperate elements of the southernmost parts of the continent, including ancestors of Myrteae genera such as *Myrceugenia* (Landrum 1981a). Meanwhile, evidence suggests that the flora of the sub-Antarctic sub-province, including these ancestral Myrteae probably began to migrate and disperse northwards, eventually covering the continent and reaching the most northern parts of northern America (e.g. British Columbia, where fossil *Paleomyrtinae* has been found), most likely by long distance dispersal. It is here speculated that it was during this time of limited distributional boundaries that the Myrteae lineage in South America began to divide into ancestors of the main extant clades in Chapter 3, some of which, notably the '*Eugenia* group', appear to have been particularly successful and are now found in most of South America.

This expansive South American Myrteae distribution may then have undergone disjunction as a result of climate and habitat changes resulting from the arrival and retreat of the Atlantic Salamancan sea transgression that covered parts of Patagonia, Bolivia and Peru in the Eocene to Oligocene (55–24 Ma.). This was followed by new oceanic transgressions in Patagonia (the Patagoniana Sea) and in the Amazon (the Amazonian sea) in the late Oligocene to mid-Miocene (26–11 Ma.) (Ortiz-Jaureguizar & Cladera 2006), further cleaving the distribution of the group. Divided in this way, it is here suggested that the ancestors of current extant genera such as *Myrcia s.l.* may have begun to diverge in the relatively large areas in which they remained; one of the most important of these areas may have been the moist eastern forests – the current centre of diversity of Neotropical Myrtaceae.

The uplift of the Andes in the late Miocene and early Pliocene (c. 11–3 Ma.) formed a barrier to moisture laden South Pacific winds, decreasing rainfall and causing the development of dry, grasslands (*cerrado*) and deserts (*caatinga*) in the centre and east of the continent (Gentry 1993). This induced marked habitat diversification and opportunities for speciation, and it appears that *Myrcia s.l* may have diverged into its oldest lineages such as *Algrizea* at this point. This was followed by the formation of the Isthmus of Panama c. 5–3 Ma., allowing the migration of more northerly species to Central America and subsequently, the Caribbean. The onset of the last period of glaciation in the Pleistocene (c. 1 Ma.) would have led to the extinction of any Myrtaceae populations in all but the southernmost parts of North America and has been hypothesised to have caused the formation of habitat-island forest refugia within certain habitats in which extremely rapid allopatric multiplication of species took place (Haffer 1969; Prance 1973, 1982). It is suggested that the majority of *Myrcia s.l.* species arose at this time. The following section discusses possible biogeographical events in each of the areas of species endemism identified above.

Atlantic forest: The Atlantic forests of southeastern Brazil are botanically diverse, with an estimated 13,000 angiosperm species, accounting for c. 16% of the Neotropical flora (Gentry 1982). Overall endemism is very high in the Atlantic forest, with an estimated 73% of angiosperm species endemic (Gentry 1992). Prior to the uplift of the Andes, forests were well established on the Atlantic coast of South America that extended uninterrupted from the rich soils of the Brazilian Shield, over what is now *cerrado* and *caatinga* and the igneous, sandy, impoverished soils of the Guayana Shield, to the Amazon (Mori et al. 1981; Melo Santos et al. 2007). The Atlantic forests were isolated several times from the Amazon, initially by the formation of the *caatinga* and *cerrado* regions as a result of the uplift of the Andes, and again during the Pleistocene glaciation. The result of these periods of connection and isolation is that the biota of the region comprises not only old elements that differentiated during the Tertiary but also elements that colonized the region more recently during the Quaternary (Melo Santos et al. 2007). In the case of *Myrcia s.l.*, it is here proposed that the rapid

speciation during the late Miocene and Pliocene, followed by accelerated or explosive speciation during the Pleistocene discussed above (habitat-island forest refugia and allopatric multiplication) occurred primarily within the Atlantic forest, *cerrados* and *caatinga* and that it was here that the majority of extant species arose.

Independent centres of endemism for *Myrcia s.l.* have been identified within the Atlantic forests (Murray-Smith et al. submitted); these are in the Serra do Mar mountain range between São Paulo and Rio de Janeiro (SP–RJ) and between the north of the state of Espirito Santo and the south of Bahia (ES–BA). Separation of the these areas of endemism occurs in the region of the 'cold cape' or Cabo Frio, just north of the Paraíba River in the state of Rio de Janeiro close to 23° S, where there is a strong upwelling of cold water from the ocean floor that reduces rainfall rates along the coast. This hydrodynamic coastal area is a biogeographical boundary between distributions of both marine and terrestrial organisms, such as shrimp (Maggioni et al. 2003) and birds (da Silva et al. 2004). Species of angiosperm genera in families such as Meliaceae (Pennington 1981) and Rubiaceae (Zappi pers. comm.) also follow this pattern. Field visits to these areas and studies of herbarium collections confirm that whereas angiosperm species compositions in these regions are more similar to each other than either is to the Amazon, *Myrcia s.l.* species composition in the northern ES–BA area displays several similarities of species composition to that of the Amazon region. Meanwhile, the SP–RJ area has more species composition links with the southeastern Brazilian states and northern Argentina.

It is here proposed that these divisions of *Myrcia s.l.* diversity within the Atlantic forest are due to a combination of factors. The geology of the northern ES–BA area is a Guayana Shield derived quartzite sand more similar to the substrate of the upper Amazon that should promote the success of similar species. This area is also in closer proximity to the Amazon and to forest relicts in the caatinga containing Amazonian species and linking the areas. The coldwater upwelling between the two regions provides an additional, effective geographical boundary preventing significant mixing of these floras. If it is assumed that the ancestors of

Myrcia s.l. developed in the north-east of the continent, it then appears more likely that south-eastern endemics are the result of arrivals from the Amazon influenced north-eastern forests.

***Cerrado* and *caatinga*:** As discussed, these areas were formed as a result of severely reduced rainfall after the uplift of the Andes in the Miocene and early Pliocene (c. 11–3 Ma.) and may have been the site of accelerated speciation in *Myrcia s.l.* since the Pleistocene. It has been suggested (Oliveira-Filho & Ratter 1995; Melo Santos et al. 2007) that these semi-arid biomes dominated by scrub vegetation contain remnants of the tropical rain forests that used to link the Atlantic forests to the Amazon. In the *cerrado*, these remnants are known as gallery forest enclaves (Oliveira-Filho & Ratter 1995), wheras in the *caatinga*, they are two main subregions; the Pernambuco Centre between the sea and the Borborema Plateau and the collection of patches of tropical rain forest enclaves that cover the slopes of some isolated plateaus within the large *caatinga* depression known as the Brejos Nordestinos (Melo Santos et al. 2007).

A further indicator that the ancestral area of *Myrcia s.l.* may have been in the northeastern part of South America and that this group is a relatively recent one comes from the phylogenetic position and distribution of *Algrizea*. It has been noted that species-poor clades are often sister to species-rich lineages (Davies et al. 2004), suggesting that the former are experimental lineages, devoid of the adaptations that allowed the rapid speciation of their sister group (Davies et al. 2004, Warren & Hawkins 2006). If this were true of *Algrizea* it would suggest that it is an early, rather unsuccessful proto-*Myrcia s.l.* lineage that developed in the *cerrado* and never escaped. The presence of three *Myrcia s.l.* clades (2, 6 and 7) in the *cerrado* and Atlantic forest only also contributes to the theory that early lineages of this taxon originated in this region.

Inner Amazonia: Theories explaining the dearth of peri-Amazonian species in the central Amazon basin are presented by de Granville (1992). It is suggested that these species may be absent due to intolerance of high levels of heat or humidity in the Amazonian basin, or to a

lack of inventories documenting their presence. Further explanations include the retreat of species to peri-Amazonian refugia during periods of extreme drought during the Pleistocene glaciations, the presence of the Amazonian sea oceanic transgression in the late Oligocene to mid-Miocene and the presence of a large, shallow lake that covered the Amazonian basin in the post-glacial Holocene (c. 8–7,000 years ago). De Granville (1992) discounted the hypotheses that the pattern is due to lack of inventories and also to the theory of peri-Amazonian refugia. He concluded that the lack of peri-Amazonian species in this area is due to recent environmental factors such as high temperature and humidity combined with the repeated historical flooding of the area. He believed that the Amazonian basin has been recolonized more recently by humid forests; in terms of *Myrcia s.l.*, this may explain the relative lack of species and endemics in this area and the frequent presence of widespread species such as *Myrcia guianensis*, *M. fallax, M. tomentosa* and *M. amazonica.* Exceptions to this pattern are found in the sandy, black-water, nutrient-poor and frequently inundated Igapó and white-sand soil habitats that make up less than 2% of the Amazon basin (Gentry 1993) and in which *Myrcia s.l.* diversity and endemism are significantly higher than in the remaining low-lying Amazon.

Peri-Amazonia: The type I peri-Amazonian ring of De Granville (1992) is illustrated in Figure 5.10; its putative origins are discussed above. It matches the distribution (Fig. 5.8) of grid cells used in the ordination analysis and labelled A in Figure 5.5 with some exceptions, particularly in the French Guianan lowlands and adjacent parts of Brazil, which in the case of *Myrcia s.l.* appear to have a species composition more similar to those of the central Amazon basin, implying that these parts of modern French Guiana were subject to the same limiting factors (in terms of species richness) described above for inner Amazonia.

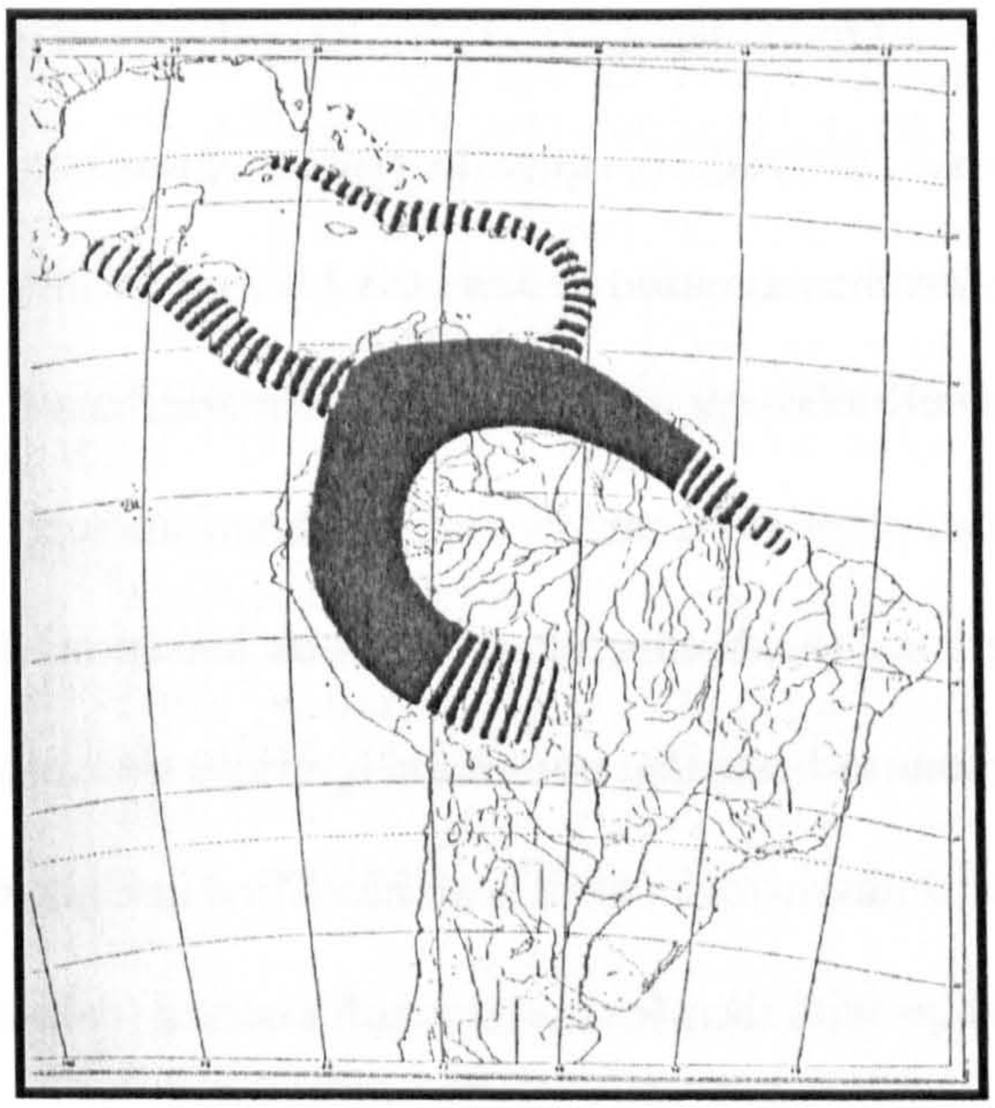


Figure 5.10. Extent of the type I peri-Amazonian ring. From De Granville (1992).

At the generic level, McVaugh (1968, 1969) suggested relationships between the Guayana Highland and the uplands of southern Brazil, although these are not borne out by the results of the ordination analysis. Clade 9 (Fig. 4.6), however, contains a particularly great number of species from this peri-Amazonian region and is well represented also in *restingas* of the Atlantic forests, particularly in the northern BA-ES area of endemism. This may be further floristic evidence for ancient links between these the Amazon and Atlantic forests; *Marlierea* and *Siphoneugena* were cited by McVaugh (1968) as examples of this shared distribution.

Central America: The Atlantic plain of North America, including Florida and the Bahamas, was submerged until the Oligocene (Adams 1997); the Isthmus of Panama region that now links the two American continents is younger (c. 3–5 Ma.) and has acted as a bridge between them since the Pliocene. As a result of this two-directional migration, the Meso-American region contains a high estimated number of species (15,000–17,000; Gentry 1978). The region underwent periods of strong tectonic activity during the meeting of the two American plates, resulting in the volcanic mountain ranges, frequent earthquakes and landslides that form a dominant feature of the heterogeneous region today. Despite this heterogeneity, total species endemism is low, estimated at 14% (Gentry 1982). Although some Myrtaceae

genera, such as *Eugenia* or *Plinia*, demonstrate significant levels of diversity and endemism in Central America (Barrie 2004, 2005), equivalent levels of diversity are not encountered in *Myrcia s.l.*, suggesting that the migration of *Myrcia s.l.* was a recent event. An explanation for the grouping of grid cells corresponding to Central America and the western Greater Antilles in the ordination analysis is found by comparison of the representation of endemic with non-endemic genera and the distribution of the latter in Central America and the Greater Antilles. Such comparisons indicate that northern migrations took place from the former to the latter and in addition, Jurassic rocks have been identified in Cuba that suggest continental and geological relationships with the Mexican state of Yucatán (Adams 1997). Figure 5.9 suggests that *Myrcia s.l.* species arrived in the Central American region on at least six occasions, with Clades 1, 3, 4, 5, 8 and 9 containing species that occur there. A single event might explain the presence of all of the clades in Clades B or D in the Central American region, however, in many cases the species representing the numbered clades are extremely widespread (e.g., *Myrcia guianensis* in Clade 4, *M. fallax* in Clade 5) suggesting that these species, benefiting from whichever adaptations allowed them to be so successful elsewhere, were also able to reach these areas independently and thrive there.

Caribbean: As described above, it appears that the indigenous Antillean floras were recruited from pre-existing continental taxa with northern migrations from Central America to the Greater Antilles occurring independently of movements from South America to the more recent volcanic islands of the Lesser Antilles (Adams 1997). This makes it relatively easy to explain the results of the ordination analysis in which the Lesser Antilles are indicated to share their closest *Myrcia s.l.* composition links with the northern Amazonian forests. It is less straightforward to explain the extension of this Lesser Antillean species composition through Puerto Rico and Hispaniola to the western parts of Cuba and Jamaica despite the shared geological history of the Greater Antilles. Evidence from biological distribution patterns suggests a land-mass may have occupied the Caribbean Sea in the Cretaceous, allowing direct links between northern South America and the Greater Antilles avoiding

Central America (Stearn 1971); this might explain the species composition patterns observed for the Caribbean in this analysis but suggests ancient migrations of *Myrcia s.l.* that are not borne out by branch lengths in the DNA studies or geological evidence that suggests that Jamaica and Hispaniola have been undergoing whole or partial submersion since the mid Eocene (Buskirk 1985). In the case of *Myrcia s.l.,* it appears that since the speculated explosive speciation of *Myrcia s.l.* in or around the Miocene, species colonising the Lesser Antilles via northern Venezuala were able to move north-westwards by a gradual process of dispersal or migration along the Antillean island chain, while a separate group of species that had reached Central America via the Andes and northern Colombia, crossed land bridges to present day Cuba and colonised western Jamaica. Why this colonisation seems to have been limited to the west of these islands remains unknown.

Figure 5.9 suggests that *Myrcia s.l.* species arrived in the Caribbean region on at least six occasions, with the same clades containing the species occurring there as in the Central American region and the same arguments suggesting one event was not responsible for the presence of any or all of these clades. In contrast to the results of the ordination analysis, the similarity in composition of clades present in Central America and the Caribbean suggest similarities in biogeographical history, again this can be explained by the presence in several of the clades of widespread species with a propensity for successful colonisation of any suitable area. Clade 1 (*Calyptranthes*) is particularly well represented in the Caribbean region, suggesting that it may have undergone a significant amount of *in situ* speciation in this region after the arrival of the genus from South America.

These biogeographical scenarios are highly speculative and proposed as starting points from which to consider the evolutionary history of *Myrcia s.l.* It is hoped that future phylogenetic studies will produce trees with greater resolution and support, to which molecular dating can be applied, to support or reject hypotheses proposed here.

Chapter 6 - Conclusions

Taxonomic implications

Myrteae

As discussed in Chapter 2, circumscription of tribe Myrteae (De Candolle 1826) has remained essentially the same through several centuries except for the relatively recent resolution of the *Eugenia/Syzygium* controversy (Schmid 1972) and the demonstration that *Syzygium* and its allies form a relatively distantly related, independent tribe Syzygieae (Wilson et al. 2005). Since the monumental works of the late 19th century authors Berg (1855-1856, 1857-59), Niedenzu (1893) and Kiaerskou (1893), subtribal classification has also remained relatively stable, with the three subtribes of Berg (1855-1856, 1857-59) remaining the most common terms of reference for placement of Myrteae genera, despite the modified classifications of Kausel (1956) and McVaugh (1968). Despite their intentions to move away from an embryo-based system, these recent workers nevertheless could not erase its influence from their thinking.

The work on phylogenetic relationships in Myrteae presented in Chapter 3 supports the tribal circumscription of Myrteae (De Candolle 1826) minus Syzygieae (Wilson et al. 2001). It nevertheless challenges previous subtribal classifications and indicates that a new Myrteae classification is required to stabilize subtribal nomenclature for future phylogenetic work and to reflect the current improved understanding of the composition of monophyletic groups of genera. Despite the low levels of support for the deep nodes of the Myrteae tree, there is sufficient evidence presented in Chapter 3 for a new sub-tribal classification proposing nine subtribes. It is anticipated that formal publication of the following classification will follow establishment of clearer estimates of relationship and increased sampling of both taxa and loci. These descriptions are therefore not formally published here, and, to prevent them from being mistaken as valid publications, Latin diagnoses are deliberately ommitted.

The genus *Temu* O.Berg is reinstated, removing *B. cruckshanksii* from *Blepharocalyx*, and the core genera of the Myrciinae *sensu* O.Berg are treated as a single large genus *Myrcia s.l.* (discussed below). Apart from these cases, generic delimitation in tribe Myrteae is not challenged, and nomenclatural changes to genera are not discussed here. The only doubtful placement is of *Myrrhinium*; this genus is not sampled here and is morphologically distinct from any other genus, distinguished by the presence of setose appendages at the base of the petals (Landrum, 1986). Landrum (1986) speculated that *Myrrhinium* is closely related to *Acca*; the genus is unfamiliar to me and, in the interests of taxonomic stability, for now, *Myrrhinium* is treated with *Acca* in subtribe Psidinae. *Pilidiostigma* Burret is also included in the otherwise exclusively South American subtribe Psidinae on the basis of morphological similarities to other genera of this group described by Landrum & Stevenson (1986); this inclusion suggests a further long-distance dispersal event from South America to Australasia.

Classification

MYRTEAE DC. in Schltdl., Linnaea 2: 504. Jul 1827. Type genus: *Myrtus* L.

Trees, sometimes shrubs; hairs simple, occasionally dibrachiate. Leaves opposite. Inflorescences axillary, sometimes paniculate; perianth free or calyx calyptrate; stamens free, numerous; anthers versatile, dehiscing by longitudinal slits. Ovary inferior, mostly 2- or 3-locular; placentas axile, ovules radiating, sometimes pendulous; vascular supply to ovary trans-septal. Fruit indehiscent, fleshy; seeds usually numerous; embryo variable with cotyledons small and straight, elongate and curved, well developed and leafy or homogenous (from Wilson et al. 2005).

1. **Pliniinae E.Lucas** subtrib. nov. Type genus: *Plinia* L.

Small trees or shrubs; hairs simple; inflorescences usually a glomerule or a short bracteate shoot; bracteoles caducous; perianth 4-tetramerous, parts free or calyx calyptrate and opening by longitudinal tearing, perianth sometimes cicumscissile and falling with the calyx at anthesis, hypanthium occasionally extending into a tube beyond the summit of ovary; ovary bi-locular with 2–many ovules per ovary; ovules at a single point on septum, usually below the mid-point or inserted along its lower part; embryo with plano-convex, free cotyledons, hypocotyl reduced; testa soft; scalariform plates absent.

Included genera: *Myrciaria, Neomitranthes, Plinia, Siphoneugena.*

2. **Myrciinae O. Berg,** Linnaea 27: 4. 1855 (as 'Myrcioideae'). Type genus: *Myrcia* DC.

Trees, shrubs or sub-shrubs; hairs simple, sometimes dibrachiate; inflorescences usually a regularly or cymosely branching panicle or dichasium, terminal flowers often in groups of three; bracteoles caducous or persistent beyond fruit fall; perianth (4–)5-merous, parts free or calyx partially fused to calyptrate, opening by longitudinal or transverse tearing or circumscissile and falling as a calyptra at anthesis, hypanthium often extending into a

tube beyond the ovary; ovary bi- to tri-locular with 2–3(–8) ovules per locule; ovules arising at a single point on septum, usually below the mid-point; cotyledons foliaceous and folded, surrounded by a long hypocotyl; testa soft; scalariform plates absent.

Included genera: *Algrizea, Myrcia s.l.*

3. **Myrceugeniinae E.Lucas** subtrib. nov. Type genus: *Myrceugenia* O.Berg

Trees or shrubs; hairs dibrachiate (in *Temu* and many *Myrceugenia*) or simple; inflorescences usually uniflorous, less often a dichasium or bracteate shoot, peduncles of *Myrceugenia* often superimposed in a row in the axils of leaves or bracts; bracteoles usually persistent beyond fruit fall; perianth 4-merous, párts free or calyx occasionally calyptrate, cicumscissile and falling as a calyptra at anthesis, hypanthium never extending beyond the ovary; ovary bi- to quadri-locular with many ovules per ovary; ovules inserted along length of septum; cotyledons foliaceous and folded and surrounded by long hypocotyl, reduced with c-shaped hypocotyl or plano-convex surrounded by a short hypocotyl; testa mostly soft; scalariform plates usually present.

Included genera: *Luma, Myrceugenia, Temu.*

4. **Myrteoliinae E.Lucas** subtrib. nov. Type genus: *Myrteola* O.Berg

Small trees, shrubs or sub-shrubs; hairs simple; inflorescences usually solitary and uniflorous or occasionally triflorous; bracteoles persistent or caduceus at anthesis; perianth 4–5 merous, parts free, hypanthium never extending beyond the ovary; ovary bi- to tri-locular with a many ovules per ovary; ovules inserted along length of septum; cotyledons not reflexed, equal to or slightly shorter than the hypocotyl; embryo c-shaped; testa bony; scalariform plates present.

Included genera: *Lenwebbia, Lophomyrtus, Myrtastrum, Myrteola, Neomyrtus, Ugni.*

5. **Psidinae E.Lucas** subtrib. nov. Type genus: *Psidium* L.

Trees, shrubs or sub-shrubs; hairs simple; inflorescences usually solitary and uniflorous or racemose bracteate shoots or occasionally panicles; bracteoles usually caducous; perianth 4–5 merous, parts free or calyx sometimes fused and opening by irregular tearing, hypanthium seldom extending beyond the ovary; ovary bi- to tri-locular with many ovules per ovary; placentation apical and protruding or protruding along length of septum; cotyledons usually much shorter than hypocotyl and reflexed; embryo c-shaped, sometimes spiralled (or some genera with embryo as in Myrteoliinae); testa bony or soft; scalariform plates absent.

Included genera: *Acca, Accara, Amomyrtella, Amomyrtus, Campomanesia, Chamguava, Mosiera, Myrrhinium, Pilidiostigma, Pimenta, Psidium.*

6. **Eugeniinae O. Berg,** Linnaea 27: 4. 1855 (as 'Eugenioideae'). Type genus designated here: *Eugenia* L.

Trees, shrubs or sub-shrubs; hairs simple; inflorescences usually solitary and uniflorous, in fascicles or racemose bracteate shoots or sometimes dichasia; bracteoles usually persistent beyond fruit fall; perianth 4(–5) merous, parts free or calyx fused and opening by lateral tearing; hypanthium never extending beyond the ovary; ovary bi-locular with 2–many ovules per ovary; ovules attached at a single point on septum; cotyledons plano-convex or fully fused, hypocotyl reduced; testa soft; scalariform plates absent.

Included genera: *Eugenia, Calycorectes, Hexachlamys, Myrcianthes.*

7. **Blepharocalinae E.Lucas** subtrib. nov. Type genus: *Blepharocalyx* O.Berg

Small trees; hairs simple; inflorescences in complex, branching dichasia of up to 35 flowers; bracteoles caducous; perianth 4-merous, parts free, hypanthium never extending beyond the ovary; ovary bi-locular with 14–30 ovules per ovary; ovules inserted along length of septum; cotyledons reduced, testa soft; scalariform plates absent.

Sole genus: *Blepharocalyx*.

8. **Rhodamniinae E.Lucas** subtrib. nov. Type genus: *Rhodamnia* Jack

Trees or shrubs or sub-shrubs; hairs simple; inflorescences solitary, bracteate shoots or paniculate dichasia; bracteoles usually caducous; perianth 4–5-merous, parts free, sometimes markedly unequal in size, hypanthium never extending beyond the ovary; ovary 1- to multi-locular (13 locules are reported in *Decaspermum*) with many ovules per ovary; placentation apical and protruding or along length of septum; cotyledons usually reduced and hypocotyl c-shaped, sometimes spiralled; testa bony or soft; scalariform plates absent.

Included genera: *Archirhodomyrtus, Austromyrtus, Decaspermum, Gossia, Legrandia, Lithomyrtus, Meteoromyrtus, Myrtella, Octamyrtus, Rhodamnia, Rhodomyrtus, Sterocaryum, Uromyrtus.*

9. **Myrtinae O. Berg,** Linnaea 27: 4. 1855 (as 'Pimentoideae'). Type genus: *Myrtus* L.

Trees or shrubs; hairs simple or dibrachiate; inflorescences solitary or very short racemose bracteate shoots; bracteoles usually caducous; perianth 5-merous, parts free, calyx occasionally with an apical leaf appendage; hypanthium never extending beyond the ovary; ovary bi- to multi-locular with many ovules per ovary; ovules inserted along the length of the septum, nearly peltate in *Calycolpus*; cotyledons usually reduced and hypocotyl c-shaped; testa bony; scalariform plates absent.

Included genera: *Calycolpus, Myrtus.*

Genera incertae sedis

Marlieriopsis Kiaersk., *Mitranthes* O.Berg

Myrcia s.l.

For similar reasons as described above for Myrteae, a new sub-generic system is also tentatively proposed for *Myrcia s.l.* (from here on referred to simply as *Myrcia*). This classification is both liable and likely to change following future work on *Myrcia* phylogeny, it is currently of interest, however, as it provides a much-needed framework with which to divide the genus into groups suitable for monography or other study. Application of names to these sections will also serve as 'handles', allowing them to be debated, discussed and visualised with greater ease than leaving them as 'Clade 1', 'Clade 2' etc.

As anticipated by recent authors (Landrum & Kawasaki 1997; Sobral 2003), results of this study and the following new classification merge the four currently accepted core genera of Myrciinae. Prior to making the necessary taxonomic modifications, consideration was given to the alternative scenario of 'splitting' the nine main clades of the subtribe. Were this divisory approach applied, the four genera could be maintained as follows: Clade 1: *Calyptranthes*, Clade 3: *Gomidesia*, Clade 5: *Myrcia*, Clade 9: *Marlierea*. Five further clades would require additional generic names, necessitating an estimated 150 new combinations (roughly based on figures from Govaerts et al. 2006), inflating considerably the numbers of names in an already taxonomically fraught subtribe. In the interests of taxonomic stability, the decision was therefore made to 'lump' as described.

This 'lumping' unfortunately, produces the predictable (McVaugh 1968) problem that *Calyptranthes*, the oldest genus name in the subtribe takes priority over *Myrcia,* and all names in other genera would require new combinations in the former genus. To avoid this undesirable situation and for the following reasons, it is suggested that the name *Myrcia* should be conserved: following intense recent taxonomic activity and synonymisation in Brazilian *Myrcia* and *Marlierea* (Govaerts et al. 2006), there are currently 1.3 times as many accepted names in *Myrcia* (351; Govaerts et al. 2006) than *Calyptranthes* (263; Govaerts et al. 2006). Additional taxonomic deflation is anticipated in Caribbean *Calyptranthes*

(McVaugh 1968) that would further emphasise this imbalance. In addition, *Gomidesia* has, on at least one occasion, been treated in *Myrcia,* and, as a result, of 60 names currently accepted in *Gomidesia*, 46 have combinations available in *Myrcia* (Govaerts et al. 2006); a small number of *Marlierea* and *Calyptranthes* (three and four respectively) also have existing combinations in *Myrcia.* The existence of these names further reduces the number of new combinations required were the name *Myrcia* conserved over *Calyptranthes* compared to the acceptance of *Calyptranthes* over *Myrcia*, with current estimates suggesting 368 and 509 such combinations respectively (Govaerts et al. 2006). The classification assumes that this most necessary step is taken in the near future; as with the Myrteae classification, Latin diagnoses have been omitted to prevent the assumption that this represents formal publication.

Preceding the classification is a key to sections of *Myrcia s.l* proposed within it. Identification keys define groups based on few characters concentrated in succinct couplets, in this case the key relies on inflorescence, calyx and hypanthial characters. As described in Chapter 4 however, these characters alone cannot provide perfect distinction between groups, the sections identified below are in fact distinguished by more subtle combinations of characters and occasional exceptions will not fit into this key; the following sectional descriptions provide a more comprehensive explanation of morphological differences between groups.

Key to sections of Myrcia s.l.

1. Inflorescence a cymosely branching panicle with an abortive, congested terminal primary axis, buds turbinate, hypanthium extended beyond ovary in a turbinate tube.....2

   Inflorescence a symmetrical or asymmetrical, regularly or cymosely branching triangular panicle, buds globose, hypanthium not extended beyond ovary or extended in a flared tube 4

2. Calyx fused and calyptrate, circumscissile and falling as a calyptra at anthesis, often remaining attached by a small piece of tissue at one side of the hypanthium .... **1. Sect.** ***Calyptranthes***

   Calyx fused, with very short or free tips of the calyx lobes, tearing at anthesis or opening regularly.....3

3. Calyx tearing vertically through the calyx and hypanthial tissue, tearing irregular resulting in calyx lobes of markedly different sizes ..... **2. Sect.** ***Pseudocalyptra***

   Calyx tearing parallel to rim of hypanthium, tearing relatively regularly resulting in somewhat angular calyx lobes of similar sizes..... **7. Sect.** ***Pulchra***

4. Staminal ring and/or floral disc pubescent.....5

   Staminal ring glabrous or only sparsely pubescent, floral disc glabrous 6

5. Internal sac of each pair of anther thecae clearly or otherwise overtopping the external sac, open thecae retaining or losing curvature on dehiscence but never reversing, floral disc pubescent; staminal ring thin, comprising less than 40% of total disc width ....**3. Sect.** ***Gomidesia***

   Internal sac of each pair of anther thecae reversing curvature on dehiscence, floral disc hard to distinguish from broad, densely sericeous staminal ring comprising 60% or more of disc width .....**5. Sect.** ***Myrcia***

6. Inflorescence usually a symmetrical, regularly or cymosely branching triangular panicle, ovary tri-locular 7

Inflorescence usually an asymmetrical, irregularly branching panicle giving a zig-zagged appearance, occasionally appearing spike-like, ovary bi-locular 8

7. Venation not rugose, glands less obvious, often several per reticulation, veins lightly raised abaxially, occasionally also adaxially .....**4. Sect. *Guianensis***

Venation distinctly rugose, often with one or few large and distinct glands per reticulation, veins strongly raised both abaxially and adaxially.**6. Sect. *Reticulosa***

8. Calyx lobes free, triangular, acute and adaxially pubescent, strongly reflexed and appressed to fruit .....**8. Sect. *Tomentosa***

Calyx lobes free to partially or completely fused, irregularly tearing open vertically through the calyx and hypanthial tissue leaving calyx lobes of markedly different sizes or of regular triangles, where tears are deep, staminal scars appear to be at the tips of calyx lobes.....**9. Sect. *Aulomyrcia***

Classification

Myrcia DC. in Dictionnaire Classique d'Histoire Naturelle 11: 378 [Jan 1827]. Type species: *M. bracteolaris* (Poir.) DC.

Aguava Raf. Sylva Tellur. 107. 1838. Type species: *A. guianensis* (Aubl.) Raf. (basionym: *Eugenia guianensis* Aubl. Hist. Pl. Guiane 1: 506. 1775).

Aulomyrcia O.Berg Linnaea 27: 35. 1855. Type species: *A. multiflora* (Lam.) O.Berg (basionym: *Eugenia multiflora* Lam. Encycl. 3: 202. 1789).

Calycampe O.Berg Linnaea 27: 129. 1856. Type species: *C. latifolia* O.Berg

Calyptranthes Sw. Nova Genera & Species Plantarum seu Prodromus (Swartz) 5 (79). 1788. Type species: *C. chytraculia* (L.) Sw. (basionym: *Myrtus chytraculia* L. Syst. Nat., ed. 10. 1056. 1759).

Calyptromyrcia O.Berg Linnaea 27: 34. 1855. Type species: *C. cymosa* O.Berg

Cerqueiria O.Berg Linnaea 27: 5. 1855. Type species: *C. sellowiana* O.Berg.

Cumetea Raf. Sylva Tellur. 106. 1838. Type species: *C. alba* Raf.

Marlierea Cambess. in A. St. Hil. Fl. Bras. Mer. ii. 373. t. 156. 1829. Type species: *M. suaveolens* Cambess.

Eugeniopsis O.Berg Linnaea 27: 80. 1855. Type species: *E. laevigata* (DC.) O.Berg

Gomidesia O.Berg Linnaea 27: 6. 1855. Type species: *G. spectabilis* (DC.) O.Berg.

Krugia Urb. Ber. Deutsch. Bot. Ges. 11: 375. 1893. Type species: *K. elliptica* (Griseb.) Urb.

Mozartia Urb. Symb. Antill. (Urban). 9: 87. 1923. Type species: *Mozartia gundlachii* (Krug & Urb.) Urb.

Rubachia O.Berg Linnaea 27: 11. 1855. Type species: *R. spiciflora* O.Berg

Trees, shrubs or sub-shrubs; hairs simple, sometimes dibrachiate; inflorescence usually a regularly or cymosely branching panicle, terminal flowers often in groups of three or in a sub-opposite arrangement upon the rachis; bracteoles rounded or acute, caducous or persistent beyond fruit fall; perianth (0–)4–5-merous, parts free or calyx partially fused to calyptrate, opening by longitudinal or transverse tearing or circumscissile and falling as a calyptra at anthesis; disc flat and hairy or glabrous with hypanthium extending into a tube beyond the ovary; ovary bi- to tri-locular with 2–3(–8) ovules per locule; ovules arising at a single point on the septum, usually below the mid-point; cotyledons

foliaceous and folded, surrounded by a long hypocotyl; testa soft; scalariform plates absent.

1. **Sect. *Calyptranthes* (Sw.) E.Lucas** stat. nov. Type species: *C. chytraculia* (L.) Sw. Nova Genera & Species Plantarum seu Prodr. 5 (79). 1788. (basionym: *Myrtus chytraculia* L. Syst. Nat., ed. 10. 1056. 1759).

Trees or shrubs; hairs simple or often dibrachiate; branchlets often bearing lenticels; branching sympodial, frequent in vegetative and fertile branches; branchlets often two-winged, with distal ends of wings between the leaf-bases at opposite sides of a node; bracteoles rounded or triangular and acute, usually caducous; inflorescence usually a cymosely branching panicle with an abortive, congested terminal primary axis and terminal flowers in groups of three; buds turbinate and apiculate at summit; petals 0–5, small, calyx fused and calyptrate, circumscissile and falling as a calyptra at anthesis, often remaining attached by a small piece of tissue at one side of the hypanthium; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium glabrous internally, extending into a turbinate tube beyond the ovary; ovary bi-locular with 2 ovules per locule; fruits globose with persistent apical hypanthium tube, calyptra generally falling or occasionally still attached at one side.

Included species: *C. concinna, C. clusiifolia, C. lanceolata, C. grandifolia, C. kiaerskovii, C. thomasiana.*

Section also presumed to include all other species of perfectly calyptrante *Calyptranthes.*

2. **Sect. *Pseudocalyptra* (D.Legrand) E.Lucas** comb. nov. Type species: *Marlierea eugeniopsoides* (Kausel & D.Legrand) D.Legrand Bradea 2: 7. 1975.

Trees or shrubs; hairs simple or occasionally t-shaped; branchlets terete, often bearing lenticels; branching usually sympodial; bracteoles rounded or acute, usually caducous; inflorescence usually a cymosely branching panicle with an abortive, congested terminal primary axis and terminal flowers in groups of three; buds clavate; petals (4–)5, calyx partially to completely fused, tearing open vertically through the calyx and hypanthial tissue, tearing irregular and calyx lobes can be of markedly different sizes; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium extending into an abruptly flared tube beyond the ovary; ovary bi-locular with 2 ovules per locule; fruits globose with persistent, apical hypanthium tube, calyx remains generally falling.

Included species: *Marlierea eugeniopsoides, M. subacuminata, M. clausseniana, Myrcia tenuivenosa.*

3. **Sect. *Gomidesia* (O.Berg) E.Lucas** stat. nov. Type species: *Gomidesia spectabilis* (DC.) O.Berg. Fl. Bras. 14(1): 12. 1857.

Trees or shrubs; often covered in a brownish pubescence, hairs simple or unevenly dibrachiate; branchlets terete; branching usually monopodial; bracteoles rounded or acute, usually caducous; inflorescence formed from conflorescences of 2–6(–8) generally symmetrical uniflorescences; buds globose; perianth usually 5-merous, petals and sepals distinct and imbricate, abaxially pubescent, calyx lobes generally truncate; anthers tetra-locular with the internal sac of each pair clearly or otherwise overtopping the external sac, open thecae retaining or losing curvature on dehiscence but never reversing and exposing interior of sacs as a convex surface; floral disc pubescent; staminal ring thin, comprising less than 40% of total disc width; hypanthium internally glabrous or pubescent, extending into a short tube beyond the ovary; ovary 2–3 (–5)-locular with 2 ovules per locule; fruits globose, often pubescent, with persistent calyx lobes erect at apex.

Included species: *Gomidesia affinis, G. anacardiifolia, G. flagellaris, G. martiana, G. pubescens, G. schaueriana, G. sellowiana, G. spectabilis, G. tijucensis, Myrcia mischophylla.*

Section also presumed to include all *Gomidesia* treated by Nic Lughadha (1997), also *Myrcia aliena* McVaugh, *M. luschnathiana* O.Berg, *Myrcia neesiana* DC., *M. nobilis* O.Berg.

4. **Sect. *Guianensis* E.Lucas** sect. nov. Type species: *Myrcia guianensis* (Aubl.) DC. Prodr. 3: 245. 1828. (basionym: *Eugenia guianensis* Aubl. Hist. Pl. Guiane 1: 506. 1775).

Trees, shrubs or woody sub-shrubs; hairs simple; branchlets terete; branching usually monopodial; bracteoles rounded or acute, usually caducous; inflorescences usually a symmetrical, regularly or cymosely branching triangular panicle; buds globose; perianth 5-merous, petals and sepals distinct and imbricate, sepals may be abaxially and/or adaxially pubescent; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium usually internally glabrous extending into a flared tube beyond the ovary; ovary tri-locular with 2 ovules per locule; fruits globose, often with persistent calyx lobes and/or flared hypanthium tube at apex.

Included species: *Myrcia citrifolia* (Aubl.) Urb., *M. guianensis* (Aubl.) DC., *M. linguaeformis* (O.Berg) Sib., *M. obtecta* (O.Berg.) Kiaersk., *M. paracatuensis* Kiaersk., *M. subverticillaris* (O.Berg) Kiaersk., *M. rufipes* DC., *M.variabilis* DC.

Section also presumed to include: *Myrcia aethusa* N.Silveira, *M. amethystina* (O.Berg.) Kiaersk., *M. angustifolia* Nied., *M. blanchetiana* (O.Berg) Mattos, *M. calumbaensis* Kiaersk., *M. chapadensis* S. Moore, *M. crassifolia* (Miq.) Kiaersk., *M. cymosa* Nied., *M. desertorum* (O.Berg) N.Silveira, *M. diaphanosticta* Kiaersk., *M. dictyophleba* (O.Berg) D.Legrand, *M. dictyophylla* (O.Berg) Mattos & D.Legrand, *M. intermedia* (O.Berg.)

Kiaersk., *M. linearifolia* Cambess., *M. lingua* (O.Berg) Mattos & D.Legrand, *M. littoralis* DC., *M. morroqueimadensis* Kiaersk., *M. oblongata* DC., *M. obovata* Nied., *M. pachyclada* (O.Berg) N.Silveira, *M. pistrinalis* McVaugh, *M. stewartiana* O.Berg, *M. tortuosa* (O.Berg) N.Silveira, *M. variabilis* DC., *M. vestita* DC.

5. **Sect. *Myrcia*** Type species: *M. bracteolaris* (Poir.) DC. Prodr. 3: 245. 1828 [=*Myrcia splendens* DC.].

Myrcia Sect. *Sphaerocarpae* DC. Type species: *M. bracteolaris* (Poir.) DC.

Myrcia Sect. *Oocarpae* DC. Lectotypification case in preparation, candidate species: *M. rostrata* DC. Prodr. 3: 255. 1828.

Myrcia Sect. *Eumyrcia* Griseb. *nom. inval.* (Art. 21.3 ICBN 2006).

Myrcia Sect. *Debracteatae* Nied. Lectotypification case in preparation, candidate species: *M. splendens* DC. Prodr. 3: 244. 1828.

Myrcia Sect. *Bracteatae* O.Berg ex Nied. Type species: *M. bracteata* (Rich.) DC. Prodr. 3: 245. 1828.

Trees, shrubs or woody sub-shrubs; hairs simple; branchlets terete; branching usually monopodial; venation often closed with little distinction between primary and secondary veins; bracteoles rounded or acute, usually caducous; inflorescences usually a symmetrical, regularly branching triangular panicle; buds globose; perianth 5-merous, petals and sepals distinct, imbricate and acute, abaxially and/or adaxially pubescent, adaxial hairs frequently silver, silky and appressed; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc flat, hard to distinguish from broad, densely sericeous staminal ring comprising 60% or more of disc width; hypanthium short, glabrous, scarcely extending into a tube beyond the ovary, outer surface appressed, silky-hairy to copiously lanate; ovary bi-locular with 2 ovules per locule; fruits cylindrical, with persistent calyx lobes held separated and erect at apex.

Included species: *Myrcia anceps* O.Berg, *M. arborescens* Legr., *M. bracteata* (Rich.) DC., *M. coumeta* (Aubl.) DC., *M. eriopus* DC., *M. fallax* (Rich.) DC., *M. formosiana* DC., *M. grandiflora* Nied., *M. rostrata* DC., *M. suffruticosa* O.Berg, *M.velutina* O.Berg.

Section also presumed to include: *M. anomala* Cambess., *M. bella* Cambess., *M. bergiana* O.Berg, *M. capitata* O.Berg, *M. costa-ricensis* O.Berg, *M. diamantinensis* Glaziou, *M. fasciata McVaugh, M. fascicularis* O.Berg, *M. fenestrata* DC., *M. ferruginea* DC., *M. friburgensis* O.Berg, *M. glabra* (O.Berg) D.Legrand, *M. guajavaefolia* O.Berg, *M. hirsuta* O.Berg, *M. impressa* O.Berg, *M. klotzschiana* O.Berg, *M. lacunosa* (O.Berg) N.Silveira, *M. langsdorffii* O.Berg, *M. lasiantha* DC., *M. mollis* DC., *M. nitida* Cambess., *M. nivea* Cambess., *M. ovata* Cambess., *M. paivae* O.Berg, *M. recurvata* O.Berg, *M. riparia* O.Berg, *M. rufidula* Schlecht., *M. salzmanni* O.Berg, *M. schaueriana* O.Berg, *M. schuechiana* O.Berg, *M. sellowiana* O.Berg, *M. splendens* (Sw.) DC., *M. sporadosticta* Kiaersk., *M. sylvatica* (Meyer) DC., *M. undulata* O.Berg, *M. vauthiereana* O.Berg, *M. virgata* Cambess., *M. ypanemensis* O.Berg.

6. **Sect. *Reticulosa* E.Lucas** sect. nov. Type species: *Myrcia reticulosa* Miq. Linnaea 22: 794. 1850.

Trees or shrubs; often covered in a grey or reddish-brown felt, hairs simple; branchlets terete; branching usually monopodial; venation distinctly rugose, often with one or few large and distinct glands, veins raised both abaxially and adaxially, bracteoles rounded or acute, usually caducous; inflorescence usually a symmetrical, regularly or cymosely branching triangular panicle; buds globose; perianth 5-merous, petals and sepals distinct and imbricate, sepals may be abaxially and/or adaxially pubescent, often acute and ciliate; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium usually internally glabrous extending into a

somewhat flared tube beyond the ovary; ovary tri-locular with 2 ovules per locule; fruits globose, often with persistent calyx lobes and/or flared hypanthium tube at apex.

Included species: *Myrcia pubipetala* Miq., *M. reticulosa* Miq., *M. venulosa* DC.

Section also presumed to include: *Aulomyrcia klotzschiana* O.Berg, *Aulomyrcia langsdorffii* O.Berg, *Aulomyrcia riedeliana* O.Berg; *M. almasensis E. NicLughadha, M. castrensis* (O.Berg) D.Legrand, *M. daphnoides* DC., *M. dilucida* Barroso, *M. grandiglandulosa* Kiaersk., *M. heringii* D.Legrand, *M. jaguariaivensis* Mattos & D.Legrand, *M. labordeana Glaziou, M. laureola* (O.Berg) Kiaersk., *M. melanosepala* Kiaersk., *M. oligantha* O.Berg, *M. oreioeca* Kiaersk., *M. rhabdoides* Kiaersk, *M. richardiana* (O.Berg) Kiaersk, *M. rugosa* (O.Berg) Kiaersk., *M. shirleyana* Mattos, *M. sonderiana* (O.Berg) Mattos, *M. subrugosa* Kiaersk.

7. **Sect. *Pulchra* E.Lucas** sect. nov. Type species: *Myrcia pulchra* (O.Berg) Kiaersk. Enum. Myrt. Bras. 65. 1893.

Trees or shrubs; hairs mostly simple; branching frequently sympodial in vegetative and fertile branches; branchlets terete or winged with distal ends of wings terminating between leaf bases; bracteoles generally rounded, usually caducous; inflorescence usually a cymosely branching panicle with an abortive, congested terminal primary axis and terminal flowers in groups of three; buds apiculate; perianth (4–)5-merous, calyx often fused in the bud or calyx lobes small, tearing relatively regularly, parallel to rim of hypanthium upon opening; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium internally glabrous, extending into a turbinate tube beyond the ovary; ovary bi-locular with 2 ovules per locule; fruits globose with persistent apical hypanthium tube, calyx lobes usually falling or remnants occasionally still attached.

Included species: *Marlierea angustifolia* (O.Berg) Mattos, *M. pilodes* (Kiaersk.) M.L.Kawas., *M. glazioviana* Kiaersk.; *Myrcia bicarinta* (O.Berg) D.Legrand, *M. pulchra* (O.Berg) Kiaersk.

Section also presumed to include: *Marlierea rubiginosa* (Cambess.) D.Legrand; *Myrcia bicolor* Kiaersk., *M. bombycina* (O.Berg) Kiaersk., *M. pulchra* (O.Berg) D.Legrand, *M. calyptranthoides* (O.Berg) Mattos, *M. coelosepala* Kiaersk., *M. jacobinensis* Mattos, *M. lineata* (O.Berg) Nied., *M. mutabilis* (O.Berg) N.Silveira, *M. pilotantha* Kiaersk., *M. platyclada* DC., *M. plusiantha* Kiaersk.

8. **Sect. *Tomentosa* E.Lucas** sect. nov. Type species: *Myrcia tomentosa* (Aubl.) DC. Prodr. 3: 245. 1828. (basionym: *Eugenia tomentosa* Aubl. Hist. Pl. Guiane 1: 504 1775).

Trees or shrubs; hairs simple; branchlets terete; branching usually monopodial; bracteoles usually triangular and acute, usually persistent after fruit fall; inflorescence usually an asymmetrical, irregularly branching panicle giving a zig-zagged appearance and occasionally appearing spike-like; buds ovate with constriction beneath ovary; perianth 5-merous, petals and sepals distinct, triangular, acute, imbricate and adaxially pubescent; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium usually internally glabrous extending into a short tube beyond the ovary; ovary bi-locular with 2 ovules per locule; fruits globose with triangular calyx lobes strongly reflexed and appressed to fruit in a characteristic star shape.

Included species: *Myrcia laruotteana* Cambess., *M. ramulosa* DC., *M. selloi* (Spreng.) N.Silveira, *M. tomentosa* (Aubl.) DC.

Section also presumed to include: *Myrcia acutata* O.Berg, *M. alloiota* (O.Berg) Kiaersk., *M. caracasana Klotzsch ex* O.Berg, *M. curatellaefolia* DC., *M. puberula* Cambess., *M.*

rhodeosepala Kiaersk., *M. rosulans* (O.Berg) Kiaersk., *M. valenzuelana* Griseb.

9. **Sect. *Aulomyrcia* (O.Berg) Griseb.** Type species: *Myrcia multiflora* (Lam.) DC. Prodr. 3: 244. 1828. (basionym: *Eugenia multiflora* Lam. Encycl. 3: 202. 1789).

Marlierea Cambess. Type species: *Marlierea suaveolens* Cambess. A. St. Hil. Fl. Bras. Mer. ii. 374.

Myrcia Sect. *Eu-Aulomyrcia* Nied. Lectotypification case in preparation, candidate species: *Myrcia rufipes* DC. Prodr. 3: 247. 1828.

Myrcia Sect. *Armeriela* McVaugh Type species: *Myrcia inaequiloba* (DC.) Lemée Fl. Guyane Franç. 3: 150 (1954). (basionym: *Eugenia inaequiloba* DC. Prodr. 3: 282 (1828).

Trees or shrubs; hairs mostly simple; branchlets terete; branching usually monopodial; bracteoles usually triangular and acute, usually persistent after fruit fall; inflorescence usually of paniculate axes emerging from a single terminal whorl representing a compression of all primary inflorescence nodes, primary axes appear asymmetrical and irregularly branched, often with a zig-zagged appearance and occasionally appearing spike-like; buds clavate or ovate; perianth 4–5-merous, calyx lobes free to partially or completely fused, irregularly tearing open vertically through the calyx and hypanthial tissue, leaving calyx lobes of markedly different sizes or of regular triangles in a 'star' shape, where tears are deep, staminal scars appear to be at the tips of calyx lobes; anthers tetra-locular, thecae symmetrical, reversing curvature on dehiscence, exposing interior of sacs as a convex surface; floral disc glabrous; staminal ring thin, comprising less than 40% of total disc width; hypanthium extended in a flared tube beyond the ovary but inconspicuous after deep tearing; ovary bi-locular with 2 ovules per locule; fruits globose, calyx remains generally caducous.

Included species: *Marlierea obscura* O.Berg, *M. obversa* D.Legrand, *M. parviflora* O.Berg, *M. regeliana* O.Berg, *M. suaveolens* Cambess., *M. sucrei* G.M.Barroso & Peixoto, *M. tomentosa* Cambess., *Marlierea umbraticola* (Kunth) O.Berg, *M. warmingiana* Kiaersk.;

Myrcia amazonica DC., *M. clavija* Sobral, *M. decorticans* DC., *M. detergens* Miq., *M. eumecephylla* Nied., *M. inaequiloba* (DC.) McVaugh, *M. insularis* Gardn., *M. micropetala* Nied., *M. multiflora* (Lam.) DC., *M. rorida* Kiaersk., *M. ramulosa* DC., *M. saxatilis* (Amshoff) McVaugh, *M. torta* DC.

Section also presumed to include: *Aulomyrcia grandifolia* O.Berg; *M. cuprea* (O.Berg) Kiaersk., *M. hexasticha* Kiaersk., *M. leptoclada* DC., *M. lucida* McVaugh, *M. magna* D.Legrand, *M. pyrifolia* (Desv. ex Hamil.) Nied., *M. rupta* M.L.Kawasaki & Holst, *M. subobliqua* (Benth.) Nied.

Myrcia in the context of large genera

Based on the definition of large genera presented in Chapter 2, *Myrcia* qualifies without doubt as large, particularly under its new and more inclusive circumscription, including an estimated 1500 names and 800 species (Govaerts et al. 2006). *Myrcia* is one of several large genera in the Neotropics, other examples under recent study include *Inga* (Leguminosae; Richardson et al. 2001), *Costus* (Costaceae; Kay et al. 2005), *Lupinus* (Leguminosae; Hughes & Eastwood 2006) and *Eugenia* (Myrtaceae; Mazine 2006). Faced with large genera, frequent questions posed by various authors concern their evolutionary history; when did these genera become so large and why (Richardson et al. 2001; Klak et al. 2003; Kay et al. 2005; Hughes & Eastwood 2006)? As these questions are answered for an ever-increasing number of taxa, the picture of tropical historical biogeography and evolution becomes clearer, allowing the implementation of increasingly precise conservation initiatives in order to conserve those areas containing not only most species diversity but also genetic diversity.

Timing hypotheses

It has been suggested that speciation and diversification may occur at a faster rate in the tropics (Gentry 1989; Schemske 2002). This is likely due to a combination of factors such as high levels of potential biotic interactions between highly diverse species from all kingdoms (Schemske 2002), high levels of energy from sunlight, constant precipitation and low seasonality (Givnish 1999), suggesting that the Neotropics are so species-rich today as a result of recent biotic and environmental factors and that the diversity is relatively new. An alternative theory is that Neotropical plant lineages are much older (Dick et al. 2003) as a result of the relatively stable tropical climate during the Teritary period that allowed species to accumulate, with few catastrophic events resulting in mass extinctions during this time. Proponents of this theory (Dick et al. 2003) doubt the effects of more recent perturbations in tropical climates, such as through allopatric differentiation of populations in separate Pleistocene refugia, suggested by others to be responsible for rapid and recent speciation in

the tropics, particularly in South America (Haffer 1969; Prance 1973). Empirical evidence exists for recent (Richardson et al. 2001; Klak et al. 2004; Kay et al. 2005; Hughes & Eastwood 2006) and ancient (Dick et al., 2003) tropical plant species differentiation, and it has lately been suggested that the tropics are in fact both a cradle and a museum of biodiversity with the majority of taxa originating in the tropics and subsequently expanding into higher latitudes. This has been supported by data suggesting that the tropics contain both old and young taxa and that higher latitudes are progressively populated by younger taxa (Jablonski et al. 2006).

The case studies discussed above measure numbers of substitutions on branches of phylogenetic trees and use these in conjunction with molecular clock techniques to produce estimates of divergence times among the species of the respective genera. A molecular clock approach was beyond of the scope of this project; however, both Myrteae and *Myrcia* trees are characterised by small numbers of substitutions, low bootstrap support and unresolved nodes, all consistent with a hypothesis of recent speciation.

Drivers for large genera

To address the question of 'why is *Myrcia* so large', three main hypothetical drivers of speciation in taxa that have undergone recent speciation are considered. They are i) shifts in diversification rates due to the evolution of key morphological or physiological innovations, ii) fine niche partitioning and founder effects in rapidly changing landscapes and iii) changes in genome structure.

Key innovations

The first of these processes is described in a study of Aizoaceae in the South African Karoo (Klak et al. 2004) in which climatic and ecological factors are discounted as major reasons for the reported imbalance in net speciation rates between two sister clades under study. Instead, three morphological characters are proposed as key innovations facilitating a major radiation

of one of these clades. These adaptations are wide-band tracheids preventing collapse of primary cell walls, cylindrical or trigonous leaves that provide reduction in leaf surface area and water loss and specialized hygrochastic capsules that open and release seeds only when moistened in rain, all of which provided individuals with a higher tolerance to extreme aridity, whereas intermittent seed dispersal reduces gene-flow distances and promotes population isolation.

Such innovative adaptations may also affect biotic interactions with other organisms as described in a study of speciation and pollination systems in the northern Andes/Central American-centered *Costus* subgenus *Costus* (Costaceae; Kay et al. 2005). This study identifies hummingbird pollination as an innovative change from orchid-bee pollinated older clades that have evolved independently seven or more times in the Neotropics, allowing *Costus* populations to exploit higher, cooler Andean elevations where orchid bees are less active but hummingbirds are common. As a result, hummingbird-pollinated *Costus* flowers underwent rapid adaptive divergence in floral morphology and colour and subsequent effective reproductive isolation from their bee-pollinated relatives.

These characters illustrate advantages to survival and dispersal strategies quickly transformed into reproductive isolation and sympatric or allopatric speciation. In the case of Costaceae, molecular dating suggests that the diversification of this Neotropical clade has coincided with recent, extreme geological change such as the uplift of the Andes and the closing of the Isthmus of Panama, providing new geographical terrain for colonisation by opportunistic species with the potential for developing new adaptations; this niche partitioning is the second driver of speciation to be discussed here.

Niche partitioning and founder effects

A study of South American *Lupinus* (Hughes & Eastwood 2006) found no morphological or physiological traits that were obviously responsible for rapid and spectacular diversification in the Andean clade. Instead, the study (p. 10337) suggested that *Lupinus* "diversification

was driven by ecological opportunities similar to those on islands created by the emergence of largely unoccupied habitats after Andean uplift and subsequent Pleistocene glaciation". Large-scale geological change creates environments with less competition for often abundant resources as newly created or exposed areas benefit from increased sunlight and freshly exposed substrates. This frequently results in habitat fragmentation and geographical isolation of species in which newly arriving individuals or 'founders' and their offspring thrive. Often these new areas will have somewhat different environmental conditions than surrounding areas, the cool, dry, oxygen-poor Andes for example, compared to surrounding warm, moist, lowland forests; this provides new survival challenges to newly arrived organisms. If these 'founders' undergo genetic mutation resulting in a chance morphological or ecological advantage to meet these environmental challenges, the result will likely be reproductive success in those areas and often an inability to return to exploit the environments from which they came – effective reproductive isolation and allopatric speciation.

If these new or newly exposed areas are islands, the effects of low competition and new resources are exacerbated and result in some of the fastest reported plant radiations, such as the Hawaiian silver-swords (Robichaux et al. 1990). Formations such as the peaks of the Andes behave as islands in terms of species divergence rates with many examples of rapid, even explosive species radiation found here (see Hughes & Eastwood 2006 for more details). The fastest plant speciation rate recorded to date is for Andean *Lupinus*, with a conservative estimated of 1.93–2.78 species per million years. Continental taxa can also speciate rapidly, although the fastest reported, at 0.77–1.75 species per million years for Aizoaceae in the Karoo, does not outpace the 'island dwelling' *Lupinus*. The reason for this difference is thought to be the relative lack of ecological opportunity on continents compared to islands, suggesting that it is opportunity, in the form of extrinsic circumstances and events rather than evolutionary innovation that plays a dominant role in driving episodes of great species diversification (Hughes & Eastwood 2006).

Changes in genome structure

It has been suggested that polyploid clades may be larger than diploid clades and that polyploidy may allow species to radiate into unoccupied niches (Tate & Simpson 2003) and/or outcompete their diploid counterparts (Soltis et al. 1995), with greatest species richness being found in genera characterized by having 50–75% polyploid species (Otto & Whitton 2000). Polyploidy in its most basic form results in sympatric speciation as polyploids are genetically isolated from their diploid parents in just a few generations. This process becomes more complex and speciation more pronounced when it occurs in conjunction with large-scale geological change and reduction in competition and availability of new resources as described above. A study of ploidy and phylogeny within Rosaceae sought to deduce the main mechanisms behind the correlation between polyploidy and species richness and concluded that polyploid clades are not more evolutionarily successful than diploid clades and do not experience increased rates of speciation or less extinction; instead, it appears that reproductive isolation from the parental clade(s) following polyploidy is responsible for increased species richness (Vamosi & Dickinson 2006).

Large genera and Myrtaceae

Using the criteria discussed in Chapter 2, Myrtaceae contains four large genera (of over 500 species), *Eugenia, Syzygium, Eucalyptus* and *Myrcia s.l.* (1023, 1043, 820 and 772 species, respectively; Govaerts et al. 2006). The only other families thought to include four or more large genera are Orchidaceae, Leguminosae and Compositae, with (7, 5 and 5 such genera respectively; Frodin 2004), much larger families in terms of species and genera. The following section discusses potential causal factors for the unusual occurrence of so many large genera in Myrtaceae.

The basic Myrtaceae chromosome number is $2n = 22$, with little variation distributed through the tribes and few occurrences of polyploidy reported in the dry-fruited genera (Rye 1979). Polyploid series and apomixis have however, been found in several genera, particularly in

tribe Syzygieae with the highest recorded chromosome number being 2n=110 in *Syzygium samarangense* (Blume) Merr. & L.M.Perry (Roy & Jha 1962). Small studies by Costa & Forni-Martins (2006a, b, in press) on Myrteae genera demonstrate that polyploidy is present in c. 39% of studied *Eugenia* (31 species studied, 12 polyploid or mixed), *Myrciaria* (4 species/0 polyploid) and *Plinia* (2 species/0 polyploid), c. 50% of studied *Psidium* (9 species/8 polyploid) and *Campomanesia* (5 species/0 polyploid), and c. 17% of core Myrciinae (*Gomidesia*: 4 species/1 polyploid; *Marlierea*: 3 species/0 polyploid; *Myrcia*: 11 species/2 polyploid) . Although never common, this discrepancy between dry and tropical taxa, suggests that polyploidy in Myrtaceae is linked to evolution in tropical, moist environments and that whilst polyploidy has had an effect on species differentiation in some Myrtaceae genera, notably *Psidium* and *Syzygium*, it probably did not play a significant part in the period of rapid speciation that produced the largest Myrteae genera, *Eugenia* and *Myrcia.*

Myrteae genera have remarkably uniform morphology, with most species bearing small, white, short-lived, bee-pollinated flowers and fleshy fruits adapted to dispersal chiefly by birds and small mammals (Nic Lughadha & Proença 1996). More morphological diversity is evident in characters linked to the embryo, seed and seed dispersal; however, Myrteae genera are notorious for their morphological homogeneity, and it would be difficult to realistically suggest that the phenomenal success of the tribe or any of its genera is chiefly due to any one morphological or ecological innovation or to a combination of these. Outside of Myrteae, *Syzygium* and *Eucalyptus* also show little intra-generic diversity in flower morphology, and in the case of *Eucalyptus* even embryo and seed characters are relatively invariable.

Instead it seems more likely, as in the case of *Lupinus* (Hughes & Eastwood 2006), that the main driver of speciation in Myrtaceae genera was habitat fragmentation, and success of founder species in combination with a few, key, advantageous morphological characters. The following section seeks to discuss where possible, key episodes of habitat fragmentation and

morphological characters for each of the four large Myrtaceae genera: *Eugenia*, *Myrcia*, *Syzygium* and *Eucalyptus*.

Myrteae

The biogeographical scenario presented in Chapter 5 provides ideal conditions for allopatric speciation and adaptive radiation on a large scale. In summary, this suggests that Myrteae genera were isolated in a few large and disjunct areas that were severely fragmented by Pleistocene/Holocene geological and particularly, climatic changes, preserving the ancestors of currently extant species in forest refugia protected from effects of glaciation where they differentiated and were able to dominate newly exposed areas as the post-glacial environment warmed. Morphological adaptations that allowed genera such as *Eugenia* and *Myrcia* to dominate in these new environments may represent a suite of characters associated with rapid speciation and large woody genera in the Neotropics.

Despite the relative homogeneity of Myrteae flower types, flower size varies, as does flowering strategy, from mass-flowering in just a few days, to pulsed or steady flowering lasting up to 90 days. Nic Lughadha & Proença (1996) noted that with these differences in flower size and strategy, Myrteae genera take full advantage of all bee taxa that visit them and exploit any differences in bee behaviour that they might display such as trap-lining and buzzing at frequencies suitable for buzz-pollination. This flexibility in bee-pollination may have provided Myrteae taxa with reproductive advantages when exploiting new areas, but additionally, Nic Lughadha & Proença (1996, p. 499) suggested that the 'rather narrow pollination spectrum is reminiscent of other large groups with 'faithful bee pollination' such as Malpighiaceae, Melastomataceae and Solanaceae, implying that a relatively constant pollination strategy with some flexibility in detail may be a shared source of success for these taxa.

Givnish (1999) suggested that areas of high rainfall and low seasonality promote evolution of endozoochory and consequentially promote speciation in understorey trees by promoting a

relationship between forest-interior birds (and presumably also small mammals) for seed dispersal. The same study suggests that the resulting limited dispersal of these seeds may create a larger species pool in a given area and may be responsible for some of the largest genera of angiosperms (e.g. *Piper, Psychotria, Chamaedorea, Geonoma, Solanum*) being forest understorey groups with fleshy fruits.

Landrum (1981a) hypothesised that morphological divergences in seeds from the small, c-shaped seeds with hard coats of some subtribes (e.g. Myrteolinae and Rhodamniinae) may also contribute to the success of the large Myrteae genera *Eugenia* and *Myrcia.* This hypothesis suggests significant evolutionary advantage in the possession of either green, well-developed cotyledons and hypocotyl, ready to photosynthesize (e.g. *Myrcia*), or homogenous cotyledons and hypocotyl, ready to provide plentiful food stored as starch (e.g. *Eugenia*).

Syzygium

South American *Eugenia* and south-eastern Asian *Syzygium* are morphologically similar, and for most of the 19th and 20th centuries were sporadically synonymized into each other. The two genera share similarities in habit, both dominating lowland, moist forests, and have similar morphology, bearing solitary flowers, spiciform inflorescences or more rarely panicles and homogenous seeds with soft testae. There is additional evidence that they share a close symbiosis with forest mycorrhizae required for successful germination and root development (Ashton pers. comm.), a phenomenon that requires further investigation. Biffin et al (2006) identified pachychalazal seeds (where all or part of the seed coat, possibly derived from the chalaza, intrudes beneath the cotyledons within the seed) in *Syzygium s.l.* and drew attention to their presence also in several southern African species of *Eugenia s.l.* (Van Wyk & Botha, 1984). Pachychalazal seeds may provide a mechanism for the rapid transfer of "nutrients" to the developing embryo (Von Teichman & Van Wyk 1996), with the same authors (Von Teichman & Van Wyk 1991) making an association between this condition and rainforest taxa with large seeds. Biffin (pers. comm.) suggests that this pachychalazal condition may be

more common in South American *Eugenia* and perhaps *Myrcia* than has been previously recognised, a hypothesis requiring further investigation. If this is the case, then it would be tempting to suggest this as an additional character providing evolutionary advantage to these large, rainforest Myrtaceae genera.

Apomixis in the form of polyembryony appears relatively common in *Plinia* and *Syzygium* (Nic Lughadha & Proença 1996). Apomixis may drive speciation in these genera by the production of exceptionally successful and numerous individuals of a single genotype that subsequently becomes recognized as single species. Although *Syzygium* is a large genus, *Plinia* is not, and this condition has not been reported in *Eucalyptus, Eugenia* or *Myrcia,* therefore if apomixis played a role in the massive speciation of *Syzygium*, it remains a phenomenon unique to this genus and not a common driver of Myrtaceae speciation. Further studies are necessary to assess the extent of this condition in Myrtaceae genera and its impact on species numbers.

Eucalyptus

The radiation of *Eucalyptus* has been hypothesised to be older than those of Myrteae and Syzygieae (Wilson et al. 2001; Sytsma et al. 2004; Basinger et al. 2007). Ladiges et al. (2003) suggested that the main period of diversification in this genus took place from the late Paleocene to the Cenozoic (70-65 mya) at a relatively slow pace compared to Myrteae. This diversification appears to have coincided with the rifting of Australia from Gondwana and subsequent desertification of southern and central Australia. As a result it is likely that the key morphological innovations that allowed *Eucalyptus* to exploit new niches provided by these environmental changes were adaptations to desiccation and fire. As in Myrteae and Syzygieae, *Eucalyptus* flowers are mostly small and white and produced in profusion, either simultaneously or in a pulsed manner, generating a large display for effective pollinator attraction (Beardsell et al. 1993). Stamens act as the main visual attractants, and pollination is mostly by birds such as honey eaters seeking nectar as a reward, although bees also play a

significant role in *Eucalyptus* pollination (as in Myrteae), as do bats and small marsupials (Beardsell et al. 1993). Despite increased reliance on bird rather than bee pollination in *Eucalyptus*, this 'big-bang' flowering strategy appears equally effective. The fruits of *Eucalyptus* are woody, protecting seeds enclosed by the woody exocarp, which are indehiscent in the canopy or soil until conditions are suitably moist for germination; then, seeds are rapidly shed from slits at the capsule summit (Beardsell et al. 1993). These characteristics are common, however, in many genera of Australian Myrtaceae (e.g. *Melaleuca*, *Callistemon*), which though successful, have not reached the species numbers of *Eucalyptus,* countering the suggestion that these characters alone are responsible for the extraordinary number of *Eucalyptus* species. Instead, it is here suggested that in combination with development of these drought resistance characters, the character exclusive to *Eucalyptus* that may have been key to its reproductive success is its capability to regenerate prolifically from seed, with extremely rapid subsequent growth. This phenomenon allows *Eucalyptus* seedlings to quickly outcompete surrounding plants and dominate an area. It is unclear which morphological characteristics brought about reproductive isolation of *Eucalyptus* populations and subsequent speciation.

Summary

The characters consistent throughout the four largest Myrtaceae genera, which appear, as a result of their shared nature, to contribute to the phenomenon that 'Myrtaceae genera are so large', are flower morphology and flowering strategy. In addition, in the tropics, fleshy fruits in co-evolution with mammals and particularly birds appear to have given genera of Myrteae a competetive advantage, grouping them with other successful taxa with similar flowering strategy and fruits. The development of the embryo also appears to have played an advantageous role in Myrcia, Eugenia and Syzygium, while the rapid growth of Eucalyptus seedlings is an advantage unique to Eucalyptus. The answer to the question 'why are Myrtaceae genera so large' is therefore answered as a combination of niche partitioning and

founder effects combined with a few key morphological innovations, some general to the four largest genera and some unique to one or a few.

Conclusions

Contributions of the study

The findings presented here have fully met the aims set out in Chapter 1. Set against a consolidation of taxonomic history of tribe Myrteae and subtribe Myrciinae, the phylogenetic analyses presented in Chapters 3 and 4 have provided a framework upon which a variety of subsequent projects can be based; studies currently underway and referring to the new arrangements presented here range from chromosome and karyotype studies (Costa et al. in prep.) and further phylogenetic studies, to large-scale monographs of Myrteae genera (Lucas et al. in prep) and sections of *Myrcia* (e.g. Rosario et al. in prep.; Lucas et al. in prep.). It is hoped that availability of these frameworks will stimulate further research on these groups. In addition, this study provides both evolutionary and biogeographical hypotheses for Myrteae and Myrciinae. Suggestions such as these can now be evaluated in molecular clock studies and incorporated into larger-scale studies of angiosperm biogeography and evolution in Gondwana and the Neotropics.

The molecular phylogenetic analyses presented here provide means by which hypotheses regarding the evolution of key morphological characters within genera can be devised and tested. In the case of Myrteae, for example, it is now clear that the character of the embryo, on which so much historical taxonomic emphasis has been placed, is far more complex than the original tri-part divisions made by De Candolle (1828) and Berg (1855-56, 1857-59), and these 'myrtoid', 'eugenioid' and 'myrcioide' conditions have arisen on more than one occasion. In the case of *Myrcia*, there is no longer any doubt that the mode of opening of the calyx and degree of prolongation of the hypanthium tube are morphological forms that have arisen repeatedly within the genus; these characters provide taxonomic signal only when combined with other morphological data.

Distribution data compiled for the areas of species endemism analysis presented in Chapter 5 have been employed in a study that used Myrciinae diversity as a surrogate for angiosperm

diversity in the Atlantic rainforests of Brazil (Murray-Smith et al. submitted). As a result, plant diversity sub-hotspots were identified within the already established Atlantic rainforest hotspot and were assessed for effective conservation of maximum biodiversity in this biome. The areas of species endemism circumscribed in Chapter 5 will further serve as the basis for future model-based biogeographical studies requiring division of the *Myrcia s.l.* distribution into phytogeographically meaningful areas, always controversial and often arbitrary.

Future studies

Future research stemming from the studies presented here will commence with the monography of a single section of *Myrcia.* This will complement parallel studies seeking to strengthen the Myrteae molecular phylogenetic hypothesis by the addition of further DNA regions and the *Myrcia* phylogenetic hypothesis, by first including further, already collected samples and then by adding more DNA regions. Once robust phylogenetic hypotheses are available, it is intended that further dating and biogeographical studies will be undertaken at both ranks, that the name *Myrcia* will be conserved over *Calyptranthes* as discussed in this Chapter, and that the classifications proposed here will be formally published.

The next decade of Myrteae and *Myrcia* research should witness steady development of reliable alpha-taxonomic systems that will feed into ongoing parallel studies on the diversification of these lineages. These more narrowly focused studies will be of interest in their own right, but ultimately will promote our understanding of the remarkable species richness of the Neotropical flora and contribute to its conservation. An over-arching study is highly desirable, it should combine phylogenetic analyses of these taxa, biogeography and timing of diversification, along with examination of the evolution and development of ecological features, such as pollination systems, ploidy, biochemistry and morphological adaptations that have contributed to their spectacular speciation.

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Appendix 1. Voucher details of specimens used in molecular analysis of tribe Myrteae.

* sequenced for *matK*.

Species	Origin	Voucher details	Herbarium
Acca sellowiana (O. Berg) Burret *	Cult. RBG Kew	Lucas, E.J. 205	K
Acmena smithii (Poir.) Merr. & L.M.Perry *	Cult. Dunedin Botanic Gardens	Belsham, S. M37	OTA
Algrizea macrochlamys Proença & Nic Lughadha *	Brazil	Giulietti & Harley 1648	K
Amomyrtus luma (Molina) D.Legrand & Kausel	Cult. RBG Edinburgh	RBGE 1996-1065	E
Amomyrtus meli (Phil.) D.Legrand & Kausel *	Cult. RBG Edinburgh	RBGE 1996-1083	E
Anetholea anisata (Vickery) Peter G.Wilson	Cult. RBG Sydney	Belsham, S. M85	OTA
Austromyrtus dulcis (C.T.White) L.S.Sm. *	Cult. RBG Melbourne	Belsham, S. M77	OTA
Blepharocalyx cruckshanksii (Hook. & Arn.) Nied. *	Cult. RBG Edinburgh	RBGE 1998-073D	E
Blepharocalyx salicifolia O.Berg *	Brazil	Lucas, E.J. 78	K
Callistemon comboynensis Cheel *	Cult. RBG Kew	Lucas, E.J. 206	K
Calyptranthes concinna DC. *	Brasil	Lucas, E.J. 74	K
Calyptranthes kiaerskovii Krug. & Urb. *	British Virgin Islands	Pollard, B.J. 1194	K
Calyptranthes lanceolata O.Berg	Brazil	Lucas, E.J. 84	K
Calyptranthes thomasiana O.Berg	British Virgin Islands	Pollard, B.J. 1195	K
Campomanesia pubescens O.Berg	Brazil	Farinaccio & Costa 1669	K
Campomanesia sp *	Brazil	Lucas, E.J. 201	K
Campomanesia guazumifolia (Cambess.) O.Berg *	Cult. RBG Sydney	Belsham, S. M86	K
Decaspermum humile (G.Don) A.J.Scott	Cult. RBG Melbourne	Belsham, S. M82	OTA
Eucalyptus perriniana F.Muell. ex Rodway *	Cult. RBG Kew	Lucas, E.J. 283	K
Eucalyptus tetragona F. Muell. *	Victoria, Australia	Udovicic, F. 177	MELU
Eugenia florida DC.	Fench Guiana	Lucas, E.J. 106	K
Eugenia langsdorffii O.Berg	Brazil	Da Silva & Farias 4528	K
Eugenia latifolia Aubl.	French Guiana	Prevost, M.F. 4707	K
Eugenia punicifolia (Kunth) DC.	French Guiana	Prevost, M.F. 4724	K
Eugenia stictosepala Kiaersk.	Brazil	Zappi, D.C. 406	K
Eugenia sulcata Spring ex Mart. *	Brazil	Lucas, E.J. 68	K
Eugenia uniflora L. *	Cult. RBG Kew	Lucas, E.J. 207	K
Gomidesia affinis (Cambess.) D.Legrand	Brasil	Lucas, E.J. 64	K

continued...

...Appendix 1 continued

Species	Origin	Voucher details	Herbarium
Gomidesia flagellaris D.Legrand *	Brazil	Lucas, E.J. 83	K
Gomidesia schaueriana O.Berg *	Brazil	Lucas, E.J. 62	K
Gomidesia tijucensis (Kiaersk.) D.Legrand	Brazil	Zappi, D.C. 305	K
Gossia inophloia (J.F.Bailey & C.T.White) Snow & Guymer	Cult. RBG Melbourne	Belsham, S. M79	OTA
Gossia hillii (Benth.) Snow & Guymer	Cult. RBG Melbourne	Belsham, S. M78	OTA
Legrandia concinna (Phil.) Kausel *	Cult. RBG Edinburgh	RBGE 1999-0656	E
Leptospermum scoparium J.R.Forst. & G.Forst. *	Cult. RBG Kew	Lucas, E.J. 284	K
Lophomyrtus bullata (Soland. ex A.Cunn.) Burret *	Cult. Dunedin Botanic Gardens	Belsham, S. M31	OTA
Lophomyrtus obcordata (Raoul) Burret *	Cult. Dunedin Botanic Gardens	Belsham, S. M41	OTA
Lophostemon confertus (R.Br.) Peter G.Wilson & J.T.Waterh. *	Victoria, Australia	FU 337	MELU
Luma apiculata (DC.) Burret *	Cult. RBG Kew	Lucas, E.J. 208	K
Luma chequen (DC.) Burret	Cult. RBG Edinburgh	RBGE 1998-0725G	E
Marlierea eugeniopsoides (D.Legrand & Kausel) D.Legrand *	Brasil	Lucas, E.J. 61	K
Marlierea obscura O.Berg *	Brazil	Lucas, E.J. 88	K
Marlierea suaveolens Cambess.	Brazil	Lucas, E.J. 85	K
Metrosideros perforata (J.R.& G.Forst.) A.Rich. *	Cult. RBG Kew	Lucas, E.J. 209	K
Myrceugenia alpigena (DC.) Landrum	Brazil	Lucas, E.J. 167	K
Myrceugenia lanceolata (Juss. ex J.St.-Hil.) Kausel	Cult. RBG Edinburgh	RBGE 1998-0662	E
Myrceugenia leptospermoides (DC.) Kausel *	Cult. RBG Edinburgh	RBGE 1989-1714C	E
Myrceugenia myrcioides O.Berg *	Brazil	Lucas, E.J. 82	K
Myrceugenia ovata O. Berg	Cult. RBG Edinburgh	RBGE 1998-2353C	E
Myrceugenia planipes O. Berg	Cult. RBG Edinburgh	RBGE 1998-1561B	E
Myrcia bicarinata (O.Berg) D.Legrand	Brasil	Lucas, E.J. 71	K
Myrcia coumeta (Aubl.) DC.	French Guiana	Lucas, E.J. 107	K
Myrcia fallax (Rich.) DC. *	French Guiana	Prevost, M.F. 4716	K
Myrcia laruotteana Cambess. *	Brasil	Mello-Silva, R. 1705	K
Myrcia multiflora (Lam.) DC. *	Brasil	Lucas, E.J. 65	K
Myrcia paracatuensis Kiaersk.	Brasil	Mello-Silva, R. 1713	K
Myrcia pubipetala Miq. *	Brasil	Lucas, E.J. 86	K
Myrcia racemosa (O.Berg) Kiaersk. *	Brasil	Lucas, E.J. 63	K

continued...

...Appendix 1 continued

Species	Origin	Voucher details	Herbarium
Myrcia rostrata DC.	Brazil	Lucas, E.J. 73	K
Myrcia saxatilis (Amsh.) McVaugh *	French Guiana	Lucas, E.J. 98	K
Myrcia subverticillaris (O.Berg) Kiaersk. *	Brasil	Lucas, E.J. 251	K
Myrcia tomentosa (Aubl.) DC.	Brazil	Soares-Silva, L.H. 752	K
Myrcianthes pseudomato (D.Legrand) McVaugh	Bolivia	Beck 9667	K
Myrcianthes pungens (O.Berg) D.Legrand	Argentina	Tressens et al 5481	K
Myrciaria aff. floribunda (H.West ex Willd.) O.Berg	Brazil	Mazine, F.F. 796	ESA
Myrciaria cauliflora O.Berg *	Cult. RBG Kew	Lucas, E.J. 210	K
Myrteola nummularia (Lam.) O.Berg *	Cult. RBG Edinburgh	RBGE 1996-1096	E
Myrtus communis L. *	Cult. RBG Kew	Lucas, E.J. 211	K
Neomitranthes cordifolia (D.Legrand) D.Legrand	Brazil	Forster, W.F. 1011	ESA
Neomyrtus pedunculata (Hook.f.) Burret*	Cult. Dunedin Botanic Gardens	Belsham, S. M42	OTA
Octamyrtus pleiopetala Diels	Irian Jaya	Johns, R.J. s.n.	K
Pimenta dioica (L.) Merr. *	Cult. RBG Kew	Lucas, E.J. 212	K
Pimenta pseudocaryophyllus (Gomes) Landrum *	Brazil	Lucas, E.J. 161	K
Pimenta racemosa (Mill.) J.W.Moore*	Cult.	Holst, B.H. 8866	K
Plinia pauciflora M.L.Kawas. & B.Holst	Brazil	Mazine, F.F. 957	ESA
Psidium cattleianum Sabine *	Cult. RBG Kew	Lucas, E.J. 213	K
Psidium cinereum Mart. ex DC.	Brazil	Da Silva & Farias, 4535	K
Rhodamnia argentea Benth.	Cult. RBG Melbourne	Belsham, S. M81	OTA
Rhodamnia rubescens (Benth.) Miq. *	Cult. RBG Melbourne	Belsham, S. M83	OTA
Rhodomyrtus psidioides (G.Don) Benth.	Cult. Dunedin Botanic Gardens	Belsham, S. M72	OTA
Siphoneugena densiflora O.Berg	Brazil	Mazine, F.F. 1050	ESA
Siphoneugena guilfoyleana C.Proença*	Brazil	Lucas, E.J. 70	K
Syzygium jambos (L.) Alston *	Cult. RBG Kew	Lucas, E.J. 214	K
Syzygium maire (A.Cunn.) W.R.Sykes & P.J.Garnock-Jones	Cult. Dunedin Botanic Gardens	Belsham, S. M84	OTA
Tepualia stipularis (Hook. & Arn.) Griseb.	Cult. RBG Edinburgh	RBGE 1995-2370A	E
Ugni mollinae Turcz. *	Cult. Dunedin Botanic Gardens	Belsham, S. M69	OTA
Xanthomyrtus compacta Diels	Irian Jaya	Edwards, P. 4213A	K
Xanthomyrtus montivaga A.J.Scott	Irian Jaya	Lucas, E.J. 16	K

NO CD/DVD

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Appendix 2. Molecular matrix: Myrteae analysis. See CD in back cover.

Appendix 3. Voucher details of specimens used in morphological analysis of *Myrcia s.l.*

Not including specimens used in the molecular analysis and available at K, see Appendix 4.

Species	Origin	Voucher details	Herbarium
Algrizea macrochlamys Proença & Nic Lughadha	Brazil	Ganev, W. 2699	K
Algrizea macrochlamys Proença & Nic Lughadha	Brazil	Harley, R.M. 26906	K
Calyptranthes clusiifolia (*Miq.*) O.Berg	Brazil	Heringer, E.P. 5998	K
Calyptranthes clusiifolia (Miq.) O.Berg	Brazil	Mori, S.A. 11437	K
Calyptranthes clusiifolia (Miq.) O.Berg	Brazil	Riedel, L. 2380	K
Calyptranthes clusiifolia (Miq.) O.Berg	Brazil	Lucas, E.J. 253	K
Calyptranthes concinna DC.	Brazil	Lucas, E.J. 74	K
Calyptranthes concinna DC.	Brazil	Lucas, E.J. 183	K
Calyptranthes grandifolia O.Berg	Brazil	Lucas, E.J. 122	K
Calyptranthes grandifolia O.Berg	Brazil	Kollmann, L. 247	K
Calyptranthes grandifolia O.Berg	Brazil	Matsumoto, K. 804	K
Calyptranthes grandifolia O.Berg	Brazil	Hatschbach, G. 20899	K
Calyptranthes Kiaersk*ovii* Krug & Urb.	British Virgin Islands	Pollard, B.J. 1194	K
Calyptranthes Kiaersk*ovii* Krug & Urb.	British Virgin Islands	Acevedo-Rodríguez, P. 10928	K
Calyptranthes lanceolata O.Berg	Brazil	Sucre, D. 8496	K
Calyptranthes lanceolata O.Berg	Brazil	Glaziou, A.F.M. 6541	K
Calyptranthes thomasiana O.Berg	British Virgin Islands	Pollard, B.J. 1195	K
Calyptranthes thomasiana O.Berg	Virgin Islands (U.S.)	Acevedo-Rodríguez, P. 5103	K
Gomidesia affinis (Cambess.) D.Legrand	Brazil	Santos, D.P. s.n.	K
Gomidesia affinis (Cambess.) D.Legrand	Brazil	Rossi, L. 431	K
Gomidesia affinis (Cambess.) D.Legrand	Brazil	Santos, D.P. s.n	K
Gomidesia anacardiifolia (Gardner) O.Berg	Brazil	Lucas, E.J. 120	K
Gomidesia anacardiifolia (Gardner) O.Berg	Brazil	Pereira, T.S. 12	K
Gomidesia flagellaris D.Legrand	Brazil	Hatschbach, G. 13552	K
Gomidesia flagellaris D.Legrand	Brazil	Hatschbach, G. 26266	K
Gomidesia martiana O.Berg	Brazil	Thomas, W.W. 9044	K

continued...

Species	Origin	Voucher details	Herbarium
Gomidesia martiana O.Berg	Brazil	Harley, R.M. 17947	K
Gomidesia pubescens (DC.) D.Legrand	Bolivia	Williams, R.S. 17	K
Gomidesia pubescens (DC.) D.Legrand	Brazil	Glaziou, A.F.M. 10792	K
Gomidesia pubescens (DC.) D.Legrand	Brazil	Filgueiras, T.S. 1555	K
Gomidesia schaueriana O.Berg	Brazil	Martins, H.F. 145	K
Gomidesia schaueriana O.Berg	Brazil	Reitz, P.R. 2083	K
Gomidesia schaueriana O.Berg	Brazil	Glaziou, A.F.M. 657	K
Gomidesia schaueriana O.Berg	Brazil	Lucas, E.J. 127	K
Gomidesia sellowiana O.Berg	Brazil	Lucas, E.J. 129	K
Gomidesia sellowiana O.Berg	Brazil	Lindeman, J.C. 4706	K
Gomidesia sellowiana O.Berg	Brazil	Mello-Silva, R. 62	K
Gomidesia sellowiana O.Berg	Brazil	Lucas, E.J. 129	K
Gomidesia spectabilis (DC.) O.Berg	Brazil	Custodio Filho, A. 1541	K
Gomidesia spectabilis (DC.) O.Berg	Brazil	Morawetz, W. 16875	K
Gomidesia spectabilis (DC.) O.Berg	Brazil	Toledo, C.B. 540	K
Gomidesia tijucensis (Kiaersk.) D.Legrand	Brazil	Hatschbach, G. 13573	K
Gomidesia tijucensis (Kiaersk.) D.Legrand	Brazil	Sucre, D. 7964	K
Marlierea angustifolia (O.Berg) Mattos	Brazil	Kawasaki, M.L. 1019	K
Marlierea angustifolia (O.Berg) Mattos	Brazil	Matsumoto, K. 793	K
Marlierea clausseniana (O.Berg) Kiaersk.	Brazil	Claussen, P. 0	K
Marlierea clausseniana (O.Berg) Kiaersk.	Brazil	Costa, I.R. 451	K
Marlierea eugeniopsoides (D.Legrand & Kausel) D.Legrand	Brazil	Zappi, D.C., D. 311	K
Marlierea eugeniopsoides (D.Legrand & Kausel) D.Legrand	Brazil	Urbanetz, C. 324	K
Marlierea eugeniopsoides (D.Legrand & Kausel) D.Legrand	Brazil	Matsumoto, K. 829	K
Marlierea eugeniopsoides (D.Legrand & Kausel) D.Legrand	Brazil	Barbosa, E. 38	K

continued...

Species	Origin	Voucher details	Herbarium
Marlierea glazioviana Kiaersk.	Brazil	Glaziou, A.F.M. 11998	K
Marlierea obscura O.Berg.	Brazil	Sugiyama 1201	F
Marlierea obscura O.Berg	Brazil	Hatschbach, G. 14455	F
Marlierea obscura O.Berg	Brazil	Lucas, E.J. 88	K
Marlierea obversa D.Legrand	Brazil	Amorim, A.M. 449	K
Marlierea obversa D.Legrand	Brazil	Mori, S.A. 14129	K
Marlierea obversa D.Legrand	Brazil	Amorim, A.M. 449	K
Marlierea obversa D.Legrand	Brazil	Matsumoto, K. s.n.	K
Marlierea parviflora O.Berg	Brazil	Lucas, E.J. 151	K
Marlierea parviflora O.Berg	Brazil	Martinelli 10980	K
Marlierea parviflora O.Berg	Brazil	Reitz & Klein 7336	K
Marlierea parviflora O.Berg	Brazil	Kollmann, L. 279	K
Marlierea pilodes Kiaersk.	Brazil	Peron 629	K
Marlierea pilodes Kiaersk.	Brazil	Peron 537	K
Marlierea pilodes Kiaersk.	Brazil	Ganev, W. 1271	K
Marlierea pilodes Kiaersk.	Brazil	Glaziou A.F.M. 16976	K
Marlierea racemosa (Vell.) Kiaersk	Brazil	Riedel 348	K
Marlierea racemosa (Vell.) Kiaersk	Brazil	Marquete 1822	K
Marlierea racemosa (Vell.) Kiaersk	Brazil	Glaziou, A.F.M. 11996	K
Marlierea regeliana O.Berg	Brazil	Glaziou, A.F.M. 10804	K
Marlierea regeliana O.Berg	Brazil	Pereira, E. 4224	K
Marlierea regeliana O.Berg	Brazil	Jacques, J.L. 90	K
Marlierea suaveolens Cambess.	Brazil	Giordano, L.C. 1695	K
Marlierea suaveolens Cambess.	Brazil	Marques, M.C. 129	K
Marlierea sucrei G.M.Barroso & Peixoto	Brazil	Mori, S.A. 11723	K
Marlierea sucrei G.M.Barroso & Peixoto	Brazil	Mori, S.A. 13030	K
Marlierea sucrei G.M.Barroso & Peixoto	Brazil	Mori, S.A. 9846	K
Marlierea tomentosa Cambess.	Brazil	Lucas, E.J. 188	K
Marlierea tomentosa Cambess.	Brazil	Dusén, P.K.H. 8136	K

continued...

Species	Origin	Voucher details	Herbarium
Marlierea tomentosa Cambess.	Brazil	Hage, J.L. 2222	K
Myrcia amazonica DC.	Brazil	Mori, S.A. 17645	K
Myrcia amazonica DC.	Brazil	Cid Ferreira, C.A. 9627	K
Myrcia amazonica DC.	Brazil	Kawasaki, M.L. 160	K
Myrcia anceps (Spreng.) O.Berg	Brazil	Glaziou, A.F.M. 2875	K
Myrcia anceps (Spreng.) O.Berg	Brazil	Guimaraes, E.F. 1417	K
Myrcia anceps O.Berg	Brazil	Guimaraes, 1417	K
Myrcia anceps O.Berg	Brazil	Duarte, 8444	K
Myrcia bicarinata (O.Berg) D.Legrand	Brazil	Sellow, F. s.n.	K
Myrcia bracteata (Rich.) DC.	Brazil	Souza, M.A.D. de 3	K
Myrcia bracteata (Rich.) DC.	Brazil	Souza, M.A.D. de 151	K
Myrcia bracteata (Rich.) DC.	Brazil	Assuncao, P.A.C.L. 282	K
Myrcia bracteata (Rich.) DC.	Brazil	Prance, G.T. 3920	K
Myrcia citrifolia (Aubl.) *Urb.*	Antigua	Nicholson, 75	K
Myrcia citrifolia (Aubl.) *Urb.*	British Virgin Islands	Fishlock, N.C. 462	K
Myrcia citrifolia (Aubl.) *Urb.*	Brazil	Irwin, H.S. 9307	K
Myrcia decorticans DC.	Guyana	Maas, P.J.M. 3879	K
Myrcia decorticans DC.	Guyana	Mutchnick, P. 192	K
Myrcia decorticans DC.	French Guiana	Prevost, M.F. 3833	K
Myrcia detergens Miq.	Brazil	Mexia, Y. 5466	K
Myrcia detergens Miq.	Brazil	Lewis, G.P. 7472	K
Myrcia detergens Miq.	Brazil	Peron, M.V. 729	K
Myrcia detergens Miq.	Brazil	Pirani, J.R. 7465	K
Myrcia detergens Miq.	Brazil	Mori, S.A. 11285	K
Myrcia detergens Miq. (SP)	Brazil	Lucas, E.J. 59	K
Myrcia eriopus DC.	Brazil	Sucre, D. 5848	K
Myrcia eriopus DC.	Brazil	Schott, 1004	K
Myrcia eumecephylla (O.Berg) Nied.	Brazil	Matsumoto, K. 803	K
Myrcia fallax (Rich.) DC.	Brazil	Prance, G.T. 15180	K

continued...

Species	Origin	Voucher details	Herbarium
Myrcia fallax (*Rich.*) DC.	Brazil	Prance, G.T. 8837	K
Myrcia fallax (*Rich.*) DC.	Brazil	Mori, S.A. 9442	K
Myrcia fallax (*Rich.*) DC.	Brazil	Cid Ferreira, C.A. 9393	K
Myrcia formosiana DC.	Brazil	Hatschbach, G. 29770	K
Myrcia formosiana DC.	Brazil	Hatschbach, G. 24676	K
Myrcia guajavaefolia O.Berg	Brazil	Peron, M.V. 895	K
Myrcia guianensis (Aubl.) DC.	Brazil	Proenca, C. 1842	K
Myrcia guianensis (Aubl.) DC.	Brazil	Stannard, B. 6649	K
Myrcia guianensis (Aubl.) DC.	Suriname	Oldenburger, F.H.F. 332	K
Myrcia guianensis (Aubl.) DC.	Brazil	Maas, P.J.M. 406	K
Myrcia guianensis (Aubl.) DC.	Brazil	Carvalho, A.M.V. de 2436	K
Myrcia inaequiloba (DC.) Lemée	Suriname	Irwin, H.S. 57568	K
Myrcia inaequiloba (DC.) Lemée	Guyana	Henkel, T.W. 4585	K
Myrcia inaequiloba (DC.) Lemée	French Guiana	Sagot, P.A. 891	K
Myrcia inaequiloba (DC.) Lemée	Suriname	Irwin, H.S. 57531	K
Myrcia insularis Gardner	Brazil	Hatschbach, G. 13139	K
Myrcia insularis Gardner	Brazil	Hatschbach 25824	K
Myrcia insularis Gardner	Brazil	Hatschbach, G. 13139	K
Myrcia insularis Gardner	Brazil	Hatschbach, G. 25824	K
Myrcia insularis Gardner	Brazil	Lanna Sobrinho, J.P. 1591	K
Myrcia laruotteana Cambess.	Brazil	Pereira, B.A.S. 1418	K
Myrcia laruotteana Cambess.	Brazil	Peron, M.V. 366	K
Myrcia leptoclada DC.	St. Vincent	Resil, G. 181	K
Myrcia linguaeformis (O.Berg) N.Silveira	Brazil	Hatschbach, G. 24550	K
Myrcia linguaeformis (O.Berg) N.Silveira	Brazil	Pohl 1065	K
Myrcia mischophylla Kiaersk.	Brazil	Guedes, M.L. 1388	K
Myrcia mischophylla Kiaersk.	Brazil	Ganev, W. 3097	K
Myrcia mischophylla Kiaersk.	Brazil	Giulietti, A.M. 1312	K
Myrcia multiflora (Lam.) DC.	Brazil	Simon, M.F. 219	K

continued...

Species	Origin	Voucher details	Herbarium
Myrcia multiflora (Lam.) DC.	French Guiana	Lescure, J.P. 619	K
Myrcia multiflora (Lam.) DC.	Brazil	Simon, 9945	K
Myrcia multiflora (Lam.) DC.	Brazil	Handro, O. 9	K
Myrcia multiflora (Lam.) DC.	Brazil	Costa, I.R. 479	K
Myrcia multiflora (Lam.) DC.	Brazil	Berg, C.C. 496	K
Myrcia obtecta (O.Berg) Kiaersk.	Brazil	Sucre, D. 4278	K
Myrcia obtecta (O.Berg) Kiaersk.	Brazil	Lucas, E.J. 135	K
Myrcia obtecta Kiaersk.	Brazil	Lucas, E.J. 116	K
Myrcia obtecta Kiaersk.	Brazil	Lucas, E.J. 136	K
Myrcia paracatuensis Kiaersk.	Brazil	Glaziou, A.F.M. 21132	K
Myrcia pubipetala Miq.	Brazil	Burchell, W.J. 4371	K
Myrcia pubipetala Miq.	Brazil	Klein, R.M. 1912	K
Myrcia pubipetala Miq.	Brazil	Peron, M.V. 774	K
Myrcia pubipetala Miq.	Brazil	Reitz, P.R 9484	K
Myrcia pubipetala Miq.	Brazil	Reitz, P.R 7313	K
Myrcia pubipetala Miq.	Brazil	Hatschbach, G. 56366	K
Myrcia pulchra Kiaersk.	Brazil	Hatschbach, G. 12095	K
Myrcia pulchra Kiaersk.	Brazil	Hatschbach, G. 12095	K
Myrcia pulchra Kiaersk.	Brazil	Harley, R.M. 4530	K
Myrcia pulchra Kiaersk.	Brazil	Hatschbach 17686	K
Myrcia pulchra Kiaersk.	Brazil	Hatschbach, G. 17686	K
Myrcia racemosa (O.Berg) Kiaersk.	Brazil	Sucre, D. 8194	K
Myrcia racemosa (O.Berg) Kiaersk	Brazil	Mori, S.A. 11706	K
Myrcia racemosa (O.Berg) Kiaersk.	Brazil	Sucre, D. 9171	K
Myrcia ramulosa DC.	Paraguay	Hassler, E. 1551	K
Myrcia ramulosa DC.	Brazil	Carvalho 25	K
Myrcia reticulosa Miq.	Brazil	Blanchet, J.S. 3728	K
Myrcia rorida (O.Berg) Kiaersk.	Brazil	Glaziou, A.F.M. 21164	K

continued...

Species	Origin	Voucher details	Herbarium
Myrcia rorida (O.Berg) Kiaersk.	Brazil	Harley, R.M. 10856	K
Myrcia rostrata DC.	Brazil	Tatlo, L. 80769	K
Myrcia rostrata DC.	Brazil	Lima, H.C. de 2642	K
Myrcia rostrata DC.	Brazil	Dusén, P.K.H. 17410	K
Myrcia rufipes DC.	Brazil	Peron, M.V. 415	K
Myrcia rufipes DC.	Brazil	Esteves, G.L. 5993	K
Myrcia saxatilis (Amsh.) McVaugh	French Guiana	Granville, J.J. de 13565	K
Myrcia saxatilis (Amsh.) McVaugh	Brazil	Prance, G.T. 28838	K
Myrcia subverticillaris Kiaersk.	Brazil	Claussen sn 1840	K
Myrcia subverticillaris Kiaersk.	Brazil	Burchell 5135	K
Myrcia tenuivenosa Kiaersk.	Brazil	Glaziou, A.F.M. 3004	K
Myrcia tenuivenosa Kiaersk.	Brazil	Hatschbach, G. 22470	K
Myrcia tomentosa (Aubl.) DC.	Brazil	Eiten, G.E. 9423	K
Myrcia tomentosa (Aubl.) DC.	Brazil	Ratter, J.A. 3628	K
Myrcia torta DC.	Brazil	Hensold, N. 7723	K
Myrcia torta DC.	Brazil	Heringer, E.P. 5340	K
Myrcia torta DC.	Brazil	Costa, I.R. 455	K
Myrcia variabilis DC.	Brazil	Irwin, H.S. 7265	K
Myrcia variabilis DC.	Brazil	Hunt, D.R. 5710	K
Myrcia venulosa DC.	Brazil	Mori, S.A. 13522	K
Myrcia venulosa DC.	Brazil	Harley, R.M. 24551	K

Appendix 4. Voucher details of specimens used in molecular analysis of *Myrcia s.l.*

In addition to those listed in Appendix 1. * sequenced for *matK*.

Species	Origin	Voucher details	Herbarium
Calyptranthes clusiifolia O.Berg	Brazil	Lucas, E.J. 253	K
Calyptranthes grandifolia O.Berg *	Brazil	Lucas, E.J. 122	K
Gomidesia anacardiifolia O.Berg	Brazil	Nadruz, M. 999	K
Gomidesia martiana O.Berg	Brazil	Zappi, D.C. 455	K
Gomidesia pubescens (DC.) D.Legrand	Brazil	IBGE s.n.	K
Gomidesia sellowiana O.Berg	Brazil	Lucas, E.J. 155	K
Gomidesia spectabilis O.Berg	Brazil	Lucas, E.J. 75	K
Marlierea angustifolia (O.Berg) Mattos *	Brazil	Lucas, E.J. E.J. 263	K
Marlierea clausseniana (O.Berg) Kiaersk.	Brazil	Matsumoto, K. 752	K, UNICAMP
Marlierea glazioviana Kiaersk.	Brazil	Matsumoto, K. 799	K, UNICAMP
Marlierea obscura O.Berg	Brazil	Matsumoto, K. 836	K, UNICAMP
Marlierea obversa D.Legrand	Brazil	Matsumoto, K. 820	K, UNICAMP
Marlierea parviflora O.Berg	Brazil	Matsumoto, K. 825	K, UNICAMP
Marlierea pilodes (Kiaersk.) M.L.Kawasaki *	Brazil	Mazine, F.F. 1052	K
Marlierea racemosa (Vell.) Kiaersk. *	Brazil	Lucas, E.J. 225	K
Marlierea regeliana O.Berg	Brazil	Matsumoto, K. 814	K, UNICAMP
Marlierea sucrei G.M.Barroso & Peixoto	Brazil	Matsumoto, K. 824	K, UNICAMP
Marlierea tomentosa Cambess.	Brazil	Matsumoto, K. 798	K, UNICAMP
Myrcia amazonica DC. (AM) *	French Guiana	Prevost, M.F. 4751	K
Myrcia amazonica DC. (PR)	Brazil	Lucas, E.J. 130	K
Myrcia anceps (Spreng.) O.Berg	Brazil	Lucas, E.J. 236	K
Myrcia bracteata (Rich.) DC.	French Guiana	Prevost, M.F. 4712	K
Myrcia pulchra Kiaersk. *	Brazil	Lucas, E.J. 138	K
Myrcia citrifolia (Aubl.) Urb.	British Virgin Islands	Pollard, B.J. 1193	K

continued...

Species	Origin	Voucher details	Herbarium
Myrcia clavija Sobral *	Brazil	Lucas, E.J. 244	K
Myrcia decorticans DC. *	French Guiana	Prevost, M.F. 4749	K
Myrcia detergens Miq. (PR) *	Brazil	Lucas, E.J. 189	K
Myrcia detergens Miq. (SP) *	Brazil	Lucas, E.J. 59	K
Myrcia eriopus DC.	Brazil	Lucas, E.J. 258	K
Myrcia eumecyphylla Nied.	Brazil	Matsumoto, K. 803	K, UNICAMP
Myrcia formosiana DC.	Brazil	Lucas, E.J. 165	K
Myrcia grandiflora (O.Berg) Nied. *	Brazil	Lucas, E.J. 60	K
Myrcia guianensis (Aubl.) DC.	Brazil	Harley, R.M. 50307	K
Myrcia inaequiloba (DC.) Lemée *	French Guiana	Lucas, E.J. 105	K
Myrcia insularis Gardner *	Brazil	Lucas, E.J. 194	K
Myrcia linguaeformis (O.Berg) N.Silveira	Brazil	Lucas, E.J. 93	K
Myrcia micropetala Nied.	Brazil	Paixao, 289	K
Myrcia mischophylla Kiaersk.	Brazil	Sobral, M. s.n	K, BH
Myrcia obtecta Kiaersk.	Brazil	Lucas, E.J. 141	K
Myrcia reticulosa Miq.	Brazil	Harley, R.M. 50309	K
Myrcia retorta Cambess.	Brazil	Lucas, E.J. 179	K
Myrcia rorida (O.Berg) Kiaersk.	Brazil	Bridgewater, S. 1076	K
Myrcia rufipes DC.	Brazil	Lucas, E.J. 280	K
Myrcia aff. selloi (Spreng.) N.Silveira	Brazil	Lucas, E.J. 274	K
Myrcia selloi (Spreng.) N.Silveira *	Brazil	Lucas, E.J. 110	K
Myrcia suffruticosa O.Berg	Brazil	Mello Silva 1690	K
Myrcia tenuivenosa Kiaersk. *	Brazil	Lucas, E.J. 87	K
Myrcia torta DC. *	Brazil	Soares-Silva, L.H. 751	K
Myrcia variabilis DC. *	Brazil	Lucas, E.J. 277	K
Myrcia velutina O.Berg	Bolivia	Wood, J. 15435	K
Myrcia venulosa DC.	Brazil	Cruz, J.M. 195	K

NO CD/DVD

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Appendix 5. Molecular matrix: *Myrcia s.l.* analysis. See CD in back cover.

Appendix 6. Presence/absence matrix of Myrteae genera in phytogeographic regions: DIVA analysis.

Region	NAF	INC	MAL	AUS	NZ	PAP	PAC	NCA	NSA	WSA	NCB	ESA	SSA
Acca	0	0	0	0	0	0	0	0	0	1	0	1	0
Amomyrtus	0	0	0	0	0	0	0	0	0	0	0	0	1
Austromyrtus	0	0	0	1	0	0	0	0	0	0	0	0	0
Blepharocalyx	0	0	0	0	0	0	0	0	0	1	0	1	0
Campomanesia	0	0	0	0	0	0	0	1	1	1	1	1	0
Decaspermum	0	1	1	1	0	1	1	0	0	0	0	0	0
Eugenia	0	0	0	0	0	0	0	1	1	1	1	1	1
Gossia	0	0	0	1	0	1	1	0	0	0	0	0	0
Algrizea	0	0	0	0	0	0	0	0	0	0	1	0	0
Legrandia	0	0	0	0	0	0	0	0	0	0	0	0	1
Lophomyrtus	0	0	0	0	1	0	0	0	0	0	0	0	0
Luma	0	0	0	0	0	0	0	0	0	0	0	0	1
Myrceugenia	0	0	0	0	0	0	0	0	0	0	0	1	1
Myrcia	0	0	0	0	0	0	0	1	1	1	1	1	0
Myrcianthes	0	0	0	0	0	0	0	1	1	1	1	1	1
Myrciaria	0	0	0	0	0	0	0	1	1	1	1	1	0
Myrteola	0	0	0	0	0	0	0	0	0	1	0	0	1
Myrtus	1	0	0	0	0	0	0	0	0	0	0	0	0
Neomitranthes	0	0	0	0	0	0	0	0	0	0	0	1	0
Neomyrtus	0	0	0	0	1	0	0	0	0	0	0	0	0
Octamyrtus	0	0	1	0	0	1	0	0	0	0	0	0	0
Pimenta	0	0	0	0	0	0	0	1	0	1	0	1	0
Plinia	0	0	0	0	0	0	0	1	1	1	1	1	0
Psidium	0	0	0	0	0	0	0	1	1	1	1	1	1
Rhodamnia	0	1	1	1	0	1	1	0	0	0	0	0	0
Rhodomyrtus	0	1	1	1	0	0	1	0	0	0	0	0	0
Siphoneugena	0	0	0	0	0	0	0	1	0	0	1	1	0
Temu	0	0	0	0	0	0	0	0	0	0	0	0	1
Ugni	0	0	0	0	0	0	0	1	1	1	0	0	1