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Morphological and Molecular Evidence of Polyphyly in *Rhodomyrtus* (Myrtaceae: Myrteae)

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***Abstract*—**The monophyly of the genus *Rhodomyrtus* (Myrtaceae) was tested using data from morphology and the nuclear ribosomal ITS regions (ITS-1, ITS-2) and 5.8S gene. Representative species from baccate genera hypothesized to be closely related to *Rhodomyrtus* were included, such as *Archirhodomyrtus*, *Octamyrtus*, *Kanakomyrtus*, and some genera believed to be more distantly related, including *Rhodamnia*, *Decaspermum*, *Pilidiostigma*, and *Myrtastrum*. Up to four capsular-fruited outgroup species were used to root the trees (*Heteropyxis natalensis*, *Carpolepis tardiflora*, *Lophostemon confertus,* and *Metrosideros rotundifolia*). Morphological data using neighbor joining scattered species of *Rhodomyrtus* across several branches but generally recovered genera other than *Rhodomyrtus*. Using parsimony, the morphological data analysis also rejected the monophyly of *Rhodomyrtus* and resulted in consensus trees with relatively low resolution and bootstrap support. Based on traditionally recognized generic boundaries, results from DNA sequence data (parsimony, Bayesian analysis) rejected the hypothesized monophyly of *Rhodomyrtus* and typically dispersed species of *Rhodomyrtus* irregularly into two relatively large branches designated as Clades A and B. Species other than *Rhodomyrtus* contained in either Clade A or B from the molecular results were some, but not all, members of *Archirhodomyrtus*, *Octamyrtus*, and *Kanakomyrtus*. Partition tests indicated that phylogenies based on morphological characters differed significantly from those based on molecular data so a combined analysis was not conducted. DNA sequence variation ranged from no variation among sequences within a species up to 61 base pair differences plus four 1 or 2 bp gaps between *Rhodomyrtus misimana* and *R. montana*. Although results from morphological and molecular analyses reject the hypothesis that *Rhodomyrtus* is monophyletic, additional data are needed before *Rhodomyrtus* can be split confidently into demonstrably monophyletic genera.

Keywords*—Archirhodomyrtus*, ITS sequence variation, *Kanakomyrtus*, Myrtaceae, Myrteae, *Octamyrtus*, *Rhodomyrtus*, systematics, Syzygieae, *Syzygium*.

The Myrtaceae comprise some 145 genera and 5,500 + species (Mabberley 2008; Govaerts et al. 2008; see also Rye 2009) and are now considered the eighth largest family of angiosperms (Table 1). The estimated level of specific diversity for the Myrtle family increased a remarkable 43% in the two-decade period between the first and latest editions of Mabberley (1987, 2008). Aside from Malvaceae, whose broadened taxonomic boundaries also now include Bombacaceae, Sterculiaceae, and Tiliaceae, no other family registered such a steep rise in estimated specific diversity over that time frame (Table 1). The increased estimates of alpha diversity indicate significant taxonomic progress (e.g. Takeuchi 2002; Parra 2002; Snow et al. 2003; Barrie 2005; Trudgen and Rye 2005; Ashton 2006; Proença et al. 2006; Salywon and Landrum 2007; Rye and Trudgen 2008; Snow 2009; Mazine and Souza 2009; Kawasaki and Holst 2009; Rye 2009; Snow and Craven 2010), but they also reflect our limited knowledge of the family overall.

Briggs and Johnson (1979) established a taxonomic framework for Myrtaceae based on morphological, anatomical, and cytological data that guided many taxonomic studies of the family over the next two decades. By the mid 1990s molecular studies began to provide a newer evolutionary framework for understanding supraspecific relationships in the family (Gadek et al. 1996; Wilson et al. 2001; Sytsma et al. 2004). Wilson et al. (2005) used data from plastid *matK* DNA sequences and morphology to propose a new classification for Myrtaceae at the subfamilial and tribal levels, which is now used as a basis for interpreting evolutionary relationships (e.g. Lucas et al. 2007; Biffin et al. 2010).

An important finding to emerge from molecular studies is that baccate (fleshy) fruits evolved at least three times from capsular-fruited ancestors (Gadek et al. 1996; Sytsma et al. 2004; Wilson et al. 2005; Lucas et al. 2005, 2007; Biffin et al. 2010). Wilson et al. (2005) described the new tribe Syzygieae Peter G. Wilson, which includes *Syzygium* Gaertn. and some related paleotropical, baccate genera that mostly are now merged into *Syzygium* (Craven 2001; Craven et al. 2006; Biffin et al. 2010; Craven and Biffin 2010). In addition to Syzygieae, baccate fruits evolved in the Malesian-Melanesian genus *Xanthomyrtus* Diels (Scott 1979a), the sole baccate genus of tribe Tristanieae Peter G. Wilson. All remaining baccate genera of Myrtaceae are now placed in tribe Myrteae DC. in Schltdl. (sensu Wilson et al. 2005; Biffin et al. 2010).

Another important finding from molecular studies (Wilson et al. 2005; Biffin et al. 2006, 2010; Lucas et al. 2007) was that a globular embryo ("eugenioid" fide Landrum and Kawasaki [1997]) enveloped by a soft (not sclerotic) testa has evolved independently in Syzygieae and some genera of Myrteae, including (as defined by Lucas et al. 2007) the "Plinia group" (*Neomitranthes* D. Legrand, *Siphoneugena* O. Berg, *Plinia* L., *Myrciaria* O. Berg) and "Eugenia group" (*Eugenia* L., *Hexachlamys* O. Berg, and *Myrcianthes* O. Berg). Results from the present study (see below) also demonstrate that a globular embryo, thickened and fused (or partially fused) cotyledons, and a soft testa, evolved independently in the Australian-New Guinean genus *Pilidiostigma* Burret (Snow 2004).

The first published studies of baccate genera of Myrtaceae were those of Lucas et al. (2005, 2007). These authors investigated suprageneric relationships of Myrteae using nuclear ITS and ETS ribosomal DNA and the plastid markers *psbA-trnH* and *matK*, and focused on relationships among Neotropical genera. Lucas et al. (2007) proposed seven

TABLE 1. Changes in the estimated levels of diversity of the ten largest families of flowering plants based on Mabberley (1987 and 2008). Numbers to the left and right of the forward slash reflect the estimates from 1987 and 2008, respectively; the percentage change is indicated in parentheses. Because Malvaceae now include Bombacaceae, Sterculiaceae, and Tiliaceae, and Euphorbiaceae now exclude Phyllanthaceae, Picrodendraceae, and Putranjivaceae, the changes by percentage for these families are less meaningful given that the taxonomic boundaries of the other families are essentially unchanged. Unpublished data (Govaerts, pers. comm. 2009) suggest that Cyperaceae is probably larger than Malvaceae and Melastomataceae (contra Mabberley 2008). * The generic estimate of 131 by Mabberley (2008) for Myrtaceae should be increased by 10–15 genera; current work by Rye (2009) and colleagues is clarifying some of the boundaries.

Family	Estimated genera	Estimated species
Asteraceae	1,341 / 1,590 (+ 18%)	21,000 / 23,600 (+ 12%)
Orchidaceae	796 / 779 (– 3%)	17,500 / 22,500 (+ 28%)
Fabaceae	657 / 720 (+ 10%)	16,400 / 19,500 (+ 19%)
Rubiaceae	630 / 563 (– 11%)	10,400 / 10,900 (+ 5%)
Poaceae	635 / 715 (+ 13%)	9,000 / 10,550 (+ 17%)
Euphorbiaceae	321 / 229 (– 29%)	7,950 / 6,500 (- 19%)
Lamiaceae	221 / 238 (+ 8%)	5,600 / 6,500 (+ 16%)
Myrtaceae	120 / 131* (+ 9%)	3,850 / 5,500 (+ 43%)
Melastomataceae	215 / 179 (– 17%)	4,750 / 5,150 (+ 8%)
Malvaceae	116 / 113 (– 3%)	1,550 / 5,000 (+ 322%)

informal suprageneric groups based on the clades that resulted from combined molecular data sets, and indicated the character combinations that generally apply to each group. Apart from the widespread genus *Eugenia* L., all Australasian taxa fell into their "Australasian Group." Although generic sampling (Lucas et al. 2007) was not complete among genera now included in Myrteae, clade membership of the Australasian group (i.e. *Rhodamnia* Jack, *Decaspermum* J. R. Forst. & G. Forst., *Rhodomyrtus* (DC.) Rchb., *Octamyrtus* Diels, *Gossia* N. Snow & Guymer, and *Austromyrtus* (Nied.) Burret) corroborated the results of previous studies (Salywon et al. 2004; Biffin et al. 2006) that generally placed members of Myrteae (sensu Wilson et al. 2005) into a single clade, with the exception of *Eugenia* (limited sampling), which placed with other Neotropical taxa in Lucas et al. (2007, Fig. 6).

Despite progress in our understanding of phylogenies at the suprageneric level, the use of molecular data to test hypotheses of generic boundaries remains at an early stage for most baccate Myrtaceae. The study of Csizmadi ([now McFadden] 2006) was the first to focus on the generic boundaries in *Rhodomyrtus* and allied genera, using morphological and nuclear sequence data from the ITS-1, 5.8S, and ITS-2 ribosomal regions. As currently understood and traditionally circumscribed, *Rhodomyrtus* consists of 23 species and one possibly undescribed taxon (Snow et al. 2008; Snow and Cantley 2010). In their native ranges, species of *Rhodomyrtus* typically grow in seasonally dry to moist forests from India and Sri Lanka to China and the Philippines, Malesia, and northeastern Australia, and east to New Caledonia and the Solomon Islands (Scott 1978a; Guymer 1991; Snow 2006; Snow et al. 2008; Snow and Cantley 2010). The highest specific diversity of *Rhodomyrtus* (Snow et al. 2008) occurs on New Guinea or adjacent islands (13 species; *R. macrocarpa* and *R. trineura* also occur in Australia [see Appendix 1 for taxonomic authorities at species level]), followed by Australia (seven species), whereas other areas have only one or a few species. The type species of the genus, *Rhodomyrtus tomentosa*, is an ornamental that has become invasive in several areas (Wagner et al. 1999; Meyer 2000; Weber 2003; Staples and Herbst 2005).

Diagnostic morphological characters traditionally used to recognize *Rhodomyrtus* have included 5-merous flowers, axile placentation, baccate fruits, and seeds bearing a sclerotic outer testa (Bentham 1867; Scott 1978a; Guymer 1991; Csizmadi 2006). Some authors have included acrodromous (Hickey 1973) venation as a character for the genus or parts thereof (Bentham 1867; Burret 1941; Scott 1978a), although approximately half of the species have eucamptodromous or brochidodromous venation, or are intermediate between these types (Snow et al. 2008; Snow and Cantley 2010).

Snow (1999, 2000) first discussed the inconsistencies among characters used to diagnose *Rhodomyrtus*, including differences in leaf venation, hypanthium texture in flower, locule number, stigma shape, placentation type, fruit morphology, and the presence or absence of thinly membranous partitions between individual seeds. Given the inability of any combination of characters to consistently diagnose *Rhodomyrtus*, Csizmadi (2006) tested the null hypothesis of monophyly in *Rhodomyrtus* against the alternate hypothesis of polyphyly using morphology and DNA sequences from the nuclear ribosomal DNA regions ITS-1, 5.8S, and ITS-2. This paper expands the results from Csizmadi (2006) using an updated and expanded source of morphological data and modified DNA sequence alignments.

Materials and Methods

***Morphological Data*—**Character data were taken from specimens listed in Appendix 1. For scoring characters we also consulted Schmid (1980), Scott (1978a-b; 1979b), Guymer (1991), Hyland et al. (1999), Craven and Sunarti (2004), Snow (2004, 2006, 2007), and Snow et al. (2008). Approximately 225 specimens housed in fifteen herbaria (see Acknowledgements) were used to score 32 characters, representing 99 character states (Appendices 2, 3). The sampling included most species of *Rhodomyrtus*, *Octamyrtus* (two species, including one possibly undescribed, indicated as "sp. nov.?"), *Archirhodomyrtus* (all four species), and *Kanakomyrtus* (three species), all of which appear to be closely related by virtue of having thin membranous partitions between the seeds. Other taxa sampled among Australasian baccate Myrteae included *Decaspermum humile*, *Myrtastrum rufo-punctatum*, two species of *Pilidiostigma*, and three species of *Rhodamnia*. We also scored four capsular-fruited species because molecular data consistently revealed the capsular state as being pleisiomorphic in the family (e.g. Salywon et al. 2004; Wilson et al. 2005; Lucas et al. 2005, 2007), including *Heteropyxis natalensis*, *Carpolepis tardiflora*, *Metrosideros rotundifolia*, and *Lophostemon confertus*. Wilson et al. (2005) demonstrated that *Heteropyxis natalensis* and *Psiloxylon mauritianum* (Hook. f.) Baill. (the latter not sampled here) are the earliest-arising extant members of Myrtaceae, so the former was designated a priori as the outgroup in molecular analyses. Vegetative measurements were taken from dried and pressed herbarium specimens and from notes taken by the first author while collecting most Australian species in cultivation or in the field. Measurements for flowering and fruiting characters also used dried material, material rehydrated using boiling water, or specimens stored in 70% ethanol. Characters that were unknown or could not be scored with certainty for some taxa were treated as missing data.

***DNA Sequence Data*—**Six species of *Rhodomyrtus* were not included in the ITS sequence data set. One species (*R. trineura*) could not be amplified successfully, and four newly described species were unknown when the molecular data were generated and analyzed (*R. mengenensis*, *R. kaweaensis*, *R. guymeriana*, and *R. takeuchii*). Samples for DNA extractions were obtained from herbarium specimens (with permission from appropriate curators; see Acknowledgments) or from young leaves collected in the field by the first author and preserved in silica gel. Some ITS sequences from previous studies (Salywon et al. 2004) were incorporated into this study.

Genomic DNA was extracted using Qiagen DNA plant mini kits (Valencia, California) or a slightly modified extraction protocol for tropical plants (Scott and Playford 1996). In the latter procedure, 0.4% polyvinylpyrolidone was added to the extraction solution to increase DNA yields, and the 0.1% serum bovine albumin was excluded from the extraction buffer. At least 2 mg of herbarium material or field-collected material dried in silica gel was used in each extraction.

After extraction the DNA was washed with 70% ethanol and resuspended in TE buffer for storage. Total genomic DNA was used as the template for amplification of the nrDNA ITS regions 1–2 using PCR. Primers used for amplification were developed by Beyra-Matos and Lavin (1999): "ITS 18" (5′ - GTCCACTGAACCTTATCATTTAGAGG-3′) and "ITS-26" (5′-GCCGTTACCTAAGGGAATCCTTGTTAG-3′). Amplification conditions included a hot start method beginning at 94°C for 2 min to initiate denaturation. After denaturation the cycle was held at 94°C until after *Taq* polymerase was added to each reaction tube. After addition of *Taq*, 35 repetitions of the thermocycler conditions followed using an additional 30-sec denaturation step at 94°, annealing at 55° for 30 sec, primer extension at 72°C, followed by a holding temperature of 4°C. Each 25 µl reaction contained approximately 10 µl of genomic DNA, 5 µM dNTP mix, 5 µM oligonucleotide primer, 1 × PCR buffer, and 1 unit of Taq polymerase (Qiagen Corp.). Amplification products were purified using a Qiagen PCR cleanup kit.

Sequencing was performed using an ABI 3730xl high-throughput sequencing machine at Polymorphic (Alameda, California). Sequences were edited and contigs were created with the aid of Sequencher 4.1 (GeneCodes, Ann Arbor, Michigan). The raw sequences were imported into PAUP*4.0b10 (Swofford 2002) and aligned visually. Gaps were treated as missing data. A total of 33 base pairs of high variability were excluded from the analyses. Insertion-deletion mutations required gaps of varying length to be added to both ITS-1 and ITS-2 regions during alignment, resulting in an aligned sequence of 645 characters.

Phylogenetic Analyses—All parsimony and maximum likelihood analyses used PAUP* version 4.0b10 (Swofford 2002) on a G4 Power Macintosh computer or a MacBook Pro. The ILD partition test (Bull et al. 1993; Farris et al. 1994) was used to assess whether morphological and molecular data sets produced significantly different topologies ($p < 0.01$). *Metrosideros rotundifolia*, a species included in the molecular studies, was excluded in the morphological data matrix given uncertainties of some character states.

Morphological Data: Phenetic Distance—With the understanding that analyses based on overall similarity cannot distinguish between homologous and homoplastic character states (e.g. Hennig 1966; Patterson 1982) we used neighbor joining to explore phenetic groupings. This was deemed appropriate given that some morphological traits are consistent within genera (apart from *Rhodomyrtus* s. l.; see Discussion) and because of the limited ability of the ITS markers to resolve all branches strongly.

Morphological Data: Parsimony—Analyses of skewness typically revealed g1 values of about –0.29, suggesting greater phylogenetic signal in the data than that expected at random. Data were analyzed using parsimony or jackknife approaches using random addition sequences with unordered and unweighted characters. Characters 14 and 15 represent different ways to score abaxial petal indumentum, which appears to have taxonomic and possible phylogenetic significance, but either character 14 or 15 was deleted from each analysis. Phenetic similarity among the taxa was analyzed with neighbor joining methods using the Tajima-Nei distance measure with ties broken randomly.

Molecular Data—In contrast to the morphological studies, in which *Kanakomyrtus myrtopsidoides* (the type of the genus) was used, we substituted *K. prominens* given the availability of much fresher DNA material. Furthermore, we excluded *K. prominens* from the morphological analyses because of large amounts of missing data due to the lack of known flowering material (Snow 2009). Parsimony analysis of ITS data used heuristic methods implementing stepwise random addition (300 replicates), MAXTREES (10,000), TBR branch-swapping, and swap on best trees only. Gaps were treated as missing data (Simmons and Ochoterena 2000). A total of 100 replicates of bootstrap resampling (Felsenstein 1985) were employed to determine the relative support for each clade using stepwise addition, with 100 replicates held at each step, TBR branch-swapping, and MAXTREES = 1,000. A pruned neighbor-joining tree was used as a starting tree for maximum likelihood analyses. Starting branch lengths were obtained using the Rogers-Swofford approximation method (Rogers and Swofford 1998). One tree was held at each step and a total of 10 random addition sequences were conducted. These data were distributed using a Γ (gamma) distribution with an estimated α shape parameter of 0.5812. Maximum likelihood analyses used Modeltest 3.06 (Posada and Crandall 1998) to determine that the data were most appropriately analyzed using the Trn + Γ model of substitution. Bayesian analyses were conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). For the Bayesian analysis one accession was randomly chosen for inclusion among species having multiple DNA samples. An appropriate model of evolution was selected (F81 + I + Γ) using a hierarchical ratio test as implemented in Modeltest (Posada and Crandall 1998). The analysis consisted of six chains with 5,000,000 generations with samples taken every 10,000 generations. Trees from the first 125 samples were considered the burn-in phase and discarded. The data matrices and the trees here were submitted to TreeBASE (study number S10866).

Results

Morphological Data: Phenetic Similarity—The neighbor joining tree (Fig. 1) supports the alternative hypothesis that *Rhodomyrtus* is polyphyletic, and shows members of *Rhodomyrtus* scattered across numerous branches of the phenogram. Congeneric members of the ingroup placeholder genera *Rhodamnia* and *Pilidiostigma* grouped together (Fig. 1). One of the most prominent differences between the neighbor joining tree (Fig. 1) and sequence-based trees (Figs. 2–4) is the monophyly of *Archirhodomyrtus* and *Octamyrtus* in the former. Unlike the molecular trees (Figs. 2–4), the three species of *Kanakomyrtus* clustered closely together in a branch that also included *Rhodomyrtus macrocarpa*. The pairing of *Rhodomyrtus macrocarpa* with *Kanakomyrtus* was unexpected given a number of differences in the morphology of flowers and fruits. The species of *Rhodomyrtus* with acrodromous venation cluster together in the branch that includes *R. canescens* through *R. sericea*.

Morphological Data: Parsimony—Phylogenetic trees based on parsimony or jackknife analyses had relatively low levels of consensus and support for clades, irrespective of which search options and outgroup combinations were used. Despite the low consensus and bootstrap values, the hypothesis that *Rhodomyrtus* is monophyletic could be rejected in all search permutations. With some exceptions, the analyses recovered monophyletic groups represented by congeneric species in *Rhodamnia*, *Pilidiostigma*, *Archirhodomyrtus*, and *Octamyrtus* (results not shown). The relative topological placements of *Decaspermum humile* and *Myrtastrum rufo-punctatum* varied, but neither was ever nested within either of two main clades of *Rhodomyrtus* that generally were resolved in molecular-based analyses (designated Clades A and B, see Figs. 3–4). Two of three species of *Kanakomyrtus* always grouped together (*K. dawsoniana* + *K. myrtopsidoides*), although *K. longipetiolata* sometimes grouped among species of *Rhodomyrtus*. The only clades recovered in the strict consensus tree based on morphological data were: *Rhodomyrtus elegans* + *R. guymeriana*, *Archirhodomyrtus* (all four species), *Pilidiostigma* (both species), *Octamyrtus* (all accessions), *Rhodamnia* (all three species), and *Kanakomyrtus longipetiolata* + *K. myrtopsidoides*. The bootstrap was not run on the final analysis because of consistently low levels of clade support in previous analyses.

DNA Sequence Data—The final dataset of the nrDNA ITS regions consisted of complete sequences of the ITS-1, the 5.8S gene, and ITS-2 regions from 49 accessions, representing 36 species, with an aligned length of 645 base pairs. A total of 33 bp were excluded due to uncertainties in the alignments, resulting in 613 included characters. The percentage of cells scored as missing data was only ca. 1.0%. Much of the missing data came from long (20–39 bp) regions of ambiguity at the beginning of some sequences (e.g. *Archirhodomyrtus turbinata* [30 bp], *Kanakomyrtus longipetiolata* [28 bp], *Octamyrtus pleiopetala* [30 bp], *Rhodomyrtus elegans* [30 bp], *R. obovata* [30 bp], and *R. surigaoensis* [39 bp]). *Kanakomyrtus longipetiolata* also was notable for its 26 bp region of ambiguity at the end of its sequence. Most of the remaining missing cells occurred at or toward the end of the sequences, and generally involved only two to five contiguous bp. *Heteropyxis natalensis* has a five bp

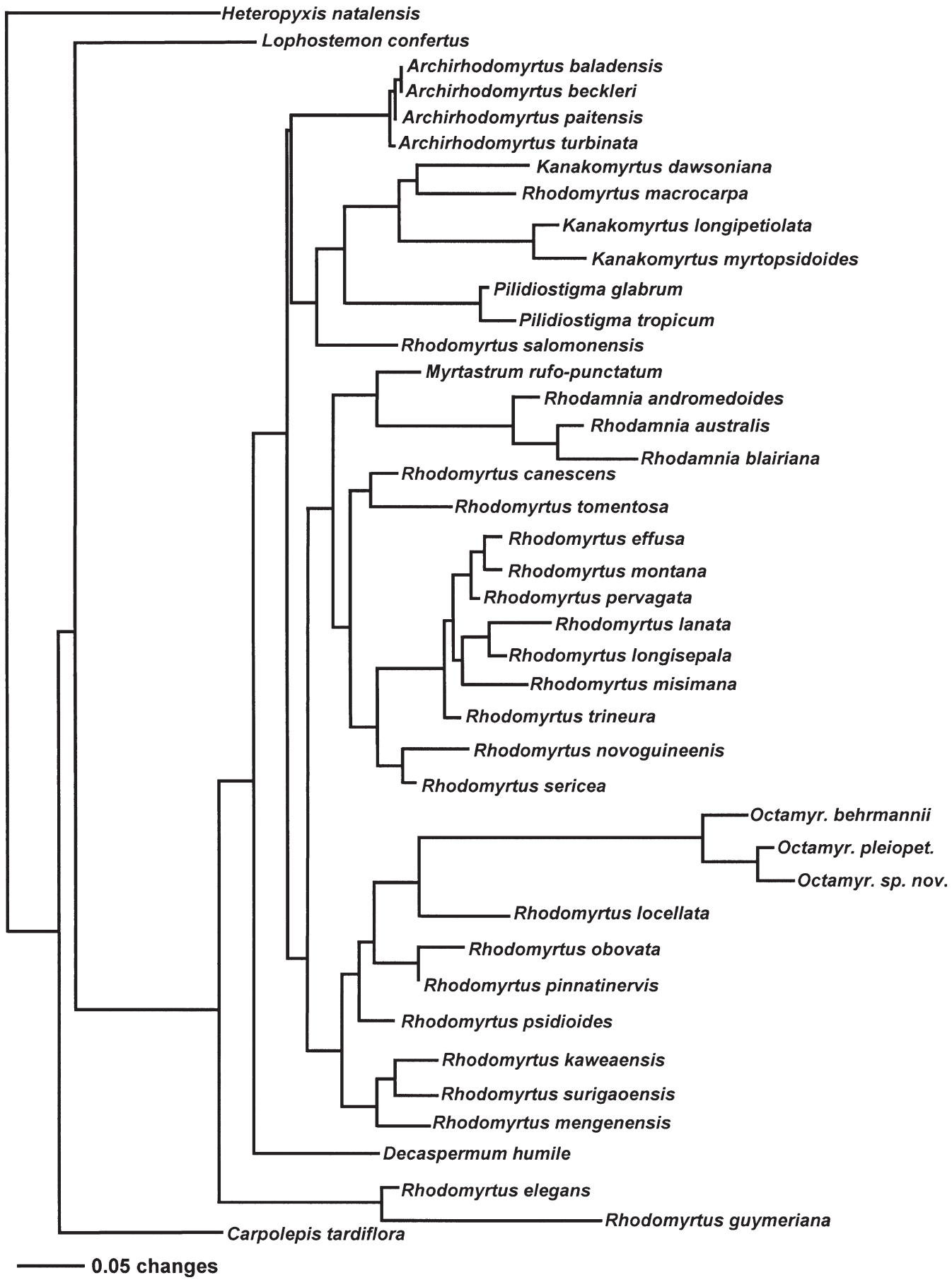

FIG. 1. Phenogram produced by neighbor-joining analysis of *Rhodomyrtus* and putatively related taxa using morphological data.

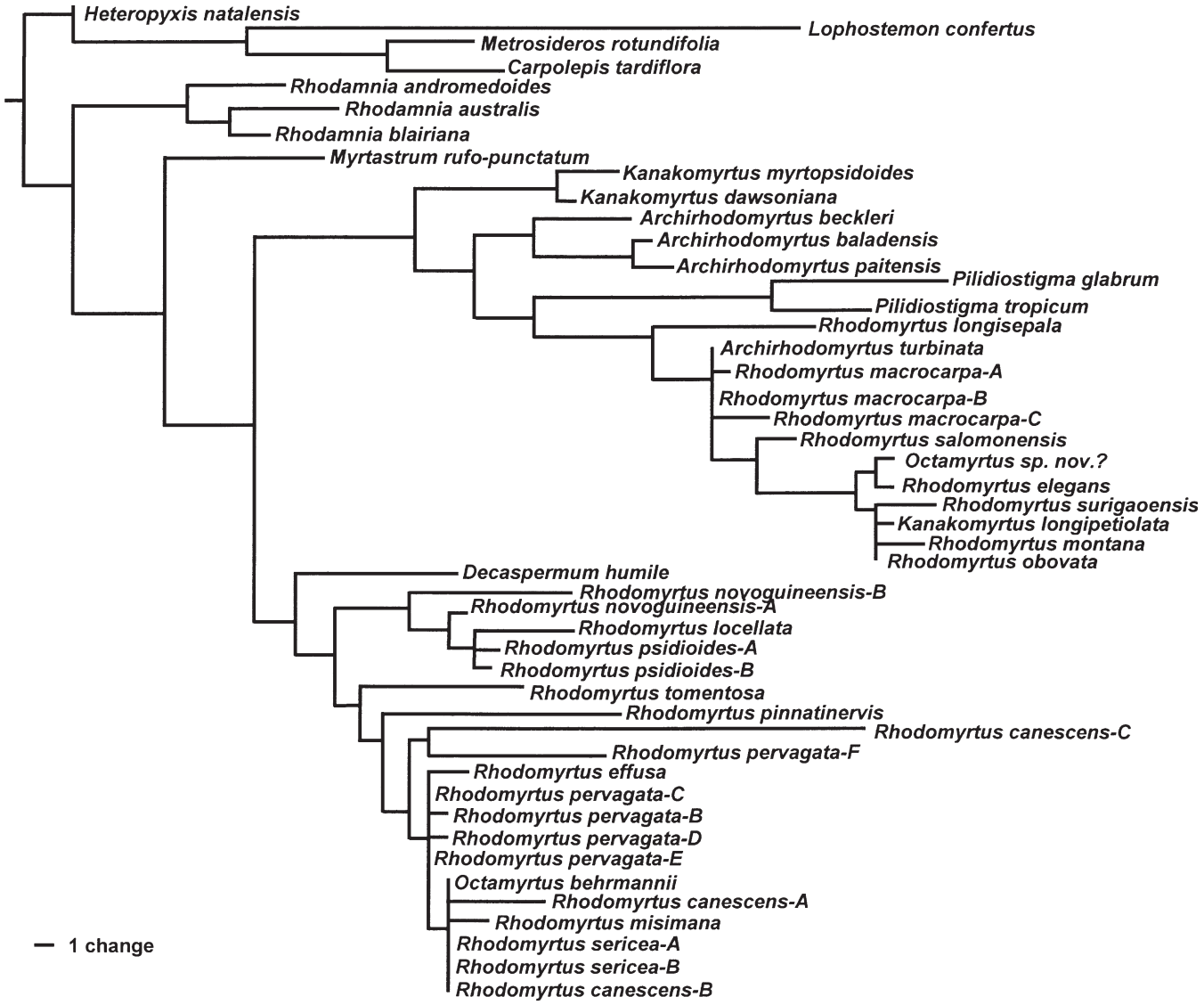

FIG. 2. A single, randomly selected most parsimonious tree of *Rhodomyrtus* s. l. and putatively related genera, selected from 10,000 trees based on sequence data from the ITS-1, 5.8S, and ITS-2 regions. Length = 535 steps; CI = 0.70; RI = 0.83; RC = 0.59.

sequence absent in all other taxa, and given that it is the earliest arising extant lineage in Myrtaceae among those sampled for this study (Wilson et al. 2005; Biffin et al. 2010), the absence of that short sequence from other taxa probably represents an evolutionary deletion among other taxa. A number of taxa had no missing (ambiguous) data (*Archirhodomyrtus baladensis*, *Kanakomyrtus dawsoniana*, *Octamyrtus behrmannii*, *Rhodomyrtus canescens* [all accessions]), *R. effusa*, *R. lanata*, *R. locellata*, *R. macrocarpa* [accessions A and B], *R. montana*, *R. novoguineensis* [both accessions], *R. pervagata* [all accessions], *R. salomonensis*, and *R. sericea* [both accessions]). Among the ambiguities present were a 16 bp sequence in the ITS-1 spacer and a 17 bp sequence in the ITS-2 spacer. No ambiguities occurred in the 5.8S gene. Of the base pairs used in the analyses, 355 (55%) were constant, 151 (23%) were variable but parsimony uninformative, and 140 (22%) were variable and parsimony informative. Overall, the nrDNA ITS region used in phylogenetic inferences had 46% variable nucleotide sites. Most insertions and deletions ranged in size from one to five bp. The highest number of indels (counted as one or more missing base pairs) among species of *Rhodomyrtus* was 25, which occurred in *Rhodomyrtus canescens*-C; the lowest was 18 in *R. locellata*. Among other baccate, place-holding taxa in Myrteae, the highest number of indels was 23 in the New Caledonian *Rhodamnia andromedoides*, which also was notable for having the longest indel (40 bp) among all sequences; the fewest indels was 18 in *Pilidiostigma tropicum*. The number of indels for the outgroup taxa were *Heteropyxis* (16), *Carpolepis* (24), *Lophostemon* (23), and *Metrosideros* (24). The nrDNA 5.8S region was 163 bp long in all taxa examined, suggesting an absence of insertion-deletion events in this location, which was helpful in making alignments. Over 60 bp differences and four indels existed between *R. misimana* and *R. montana* (see Discussion). The nrDNA ITS region had nucleotide frequencies with somewhat higher occurrences of G-C content (A = 0.22, C = 0.28, G = 0.27, T = 0.23). According to Modeltest (Posada and Crandall 1998), the data show a gamma distribution of variance of 0.58 as estimated by the GTR + Γ model (Tavaré 1986).

Parsimony—The complete 49-taxon nrDNA ITS data set reached the preselected limit of 10,000 equally parsimonious trees (L = 535; CI = 0.70; RI = 0.83; RC = 0.59). A randomly

FIG. 3. Strict consensus of 2,740 trees of *Rhodomyrtus* and putatively related genera produced by parsimony analysis of data from the ITS-1, 5.8S, and ITS-2 regions. Bootstrap values equal to or greater than 50% are shown above the branches.

FIG. 4. Inferred phylogeny of *Rhodomyrtus* and allied genera produced by Bayesian analysis of data from the ITS-1, 5.8S, and ITS-2 regions. Numbers above the branches reflect posterior probabilities.

selected most parsimonious tree (Fig. 2) illustrates the relative branch lengths. The ITS data were unable to group species with multiple accessions consistently (i.e. *R. canescens*, *R. macrocarpa*, *R. novoguineensis*, *R. pervagata*, *R. psidioides,* and *R. sericea*), reflecting infraspecific variation in the ITS region. Although many branches are short, the most prominent feature among the ingroup taxa is the long branch leading to *Rhodomyrtus canescens*-C, the specimen with an indel of 25 bp (see above). The voucher specimen for this accession easily fits into the concept of this species as it is presently understood, and the voucher does not differ appreciably from two other conspecific accessions collected nearby on the same day (Snow, pers. obs.). *Rhodomyrtus pervagata* also was collected by the first author at the same site as *R. canescens*-C. Chromosome numbers of these species are unknown, but there remains the possibility that the long branch of *R. canescens*-C may represent a past hybridization event, particularly since *R. sericea*, *R. effusa*, and *R. trineura* subsp. *trineura* also occur in the general region of the collection (Hyland et al. 1999; A. Ford, pers. comm. 2010). As explained more in the Discussion, it is possible also that a pseudogene of ITS may have been amplified in *R. canescens*-C.

The strict consensus tree of the ITS data (Fig. 3) scattered members of *Rhodomyrtus* across two clades, labeled A and B, which allows rejection of the null hypothesis that *Rhodomyrtus* is monophyletic. No known morphological or anatomical character states or combinations thereof can consistently diagnose Clades A and B, or the species of *Rhodomyrtus* included in each clade. The genera sampled in Myrteae for this study included representatives only from Australia, Malesia, and New Caledonia. In light of previous results (Wilson et al. 2005; Salywon et al. 2004; Lucas et al. 2007; Biffin et al. 2010), *Rhodamnia* and *Myrtastrum* (the latter a monotypic but ecologically common genus from New Caledonia) may be among the two earliest branching lineages of baccate genera in tribe Myrteae. The strict consensus tree (Fig. 3) supports the monophyly of *Rhodamnia* and *Pilidiostigma*, but *Archirhodomyrtus turbinata*, *Octamyrtus* sp. nov? and *Kanakomyrtus longipetiolata* are nested within *Rhodomyrtus* Clade A, separate from other members of their respective genera. Aberrant members of Clade A are *Rhodomyrtus montana* and *R. longisepala*, two species with acrodromous venation in a clade that otherwise contains species with brochidodromous or eucamptodromous venation. Clade B is comprised of some species of *Rhodomyrtus* and *Octamyrtus behrmannii* (Fig. 3). Clade B also contains variations of leaf venation, and no morphological character state or combinations thereof can unite members of Clade B. Bootstrap support values of 100% for clades in tribe Myrteae (Fig. 3) included an unnamed branch that includes Clade A and three clades arising prior to Clade A, as well as 100% support for two species each in *Archirhodomyrtus*, *Pilidiostigma,* and *Kanakomyrtus*. Clades in Myrteae with over 95% support include a branch within Clade B that contains accessions of three Australian or Melanesian species of *Rhodomyrtus* (*R. locellata* of New Caledonia, *R. novoguineensis* of New Guinea, and *R. psidioides* of Australia).

***Bayesian Analysis*—**The nrDNA ITS data set for Bayesian analysis was reduced to 36 species, which yielded a log likelihood score of –3,571.16 and also rejected the monophyly of *Rhodomyrtus* (Fig. 4). The Bayesian tree was unable to unambiguously show that the capsular genera (*Heteropyxis*, *Carpolepis*, *Lophostemon*) evolved prior to the genus *Rhodamnia*, which has been supported in part by other studies (e.g. Gadek et al. 1996; Wilson et al. 2005; Lucas et al. 2007; Biffin et al. 2010). The collective membership of Clade A in the Bayesian results (Fig. 4) is nearly identical to the results from parsimony (Fig. 3), although there is greater resolution in the Bayesian tree among the species. In the Bayesian tree *Decaspermum humile* arises before Clade B (Fig. 4), whereas it was unresolved between Clades A and B in the parsimony analysis (Fig. 3). Although the values are not directly comparable, the posterior probability of 0.99 for Clade B in the Bayesian tree (Fig. 4) appears to provide stronger support than the bootstrap for Clade B of 62% in the consensus tree (Fig. 3).

As with the parsimony trees (Figs. 2, 3), species of *Rhodomyrtus* in Clade A (Fig. 4) of the Bayesian tree also include those with acrodromous leaf venation (*R. longisepala*, *R. montana*) and brochidodromous or eucamptodromous venation (all others). Clade B (Fig. 4) also (as in Fig. 3) has a mixture of species with acrodromous venation (*R. canescens*, *R. effusa*, *R. misimana*, *R. pervagata*, *R. novoguineensis*, *R. tomentosa*) and brochidodromous or eucamptodromous venation (*R. locellata*, *R. psidioides*, *R. pinnatinervis*). In a similar manner, Clades A and B (Figs. 3–4) are a mixture of species of *Rhodomyrtus* having smooth (terete) versus longitudinally ribbed/rugose hypanthium species (Snow et al. 2008, Table 1 therein). Thus, a combination of character states cannot be used to diagnose *Rhodomyrtus* s. l. or to diagnose the constituent species of *Rhodomyrtus* in Clades A and B, upholding the initial observations of Snow (1999).

***Combined Analysis*—**Given that partition tests indicated that the topologies from morphological and DNA sequence data were significantly different, the data sets were not combined.

Discussion

Molecular studies have begun to illuminate the evolutionary history of Myrtaceae (Gadek et al. 1996; Wilson et al. 2001, 2005; Salywon et al. 2004; Lucas et al. 2005, 2007; Biffin et al. 2006, 2010) and resolve some of the taxonomic uncertainties highlighted by the comprehensive family analysis of Briggs and Johnson (1979).

The first published molecular studies testing evolutionary hypotheses with relatively broad sampling among the baccate genera of Myrteae nonetheless focused somewhat either on Neotropical genera (Lucas et al. 2005, 2007) or on *Syzygium* and paleotropical (Biffin et al. 2006, 2010). The latter study of Lucas et al. (2007) included one species each from *Rhodomyrtus* and *Octamyrtus* among the eight species (representing six genera) of Australasian Myrteae. In the strict consensus tree of molecular data based on parsimony analysis and successive weighting (Lucas et al. 2007; Fig. 7), their Australasian clade emerged as the earliest-diverging clade of Myrteae apart from *Myrtus communis* L., which was the sole member of that clade. Earlier (Salywon et al. 2004; Wilson et al. 2005) and more recent analyses (Biffin et al. 2010) also suggested that Australasian lineages were among the earliest to arise in tribe Myrteae. This study is the first to test the hypothesis of the monophyly of *Rhodomyrtus* and attempt to infer the relationships of that (polyphyletic) genus and related genera.

***Morphological Data*—**Table 2 summarizes characters traditionally used to separate *Rhodomyrtus* from morphologically similar baccate genera in Australasia, and includes recently generated data. For example, Snow et al. (2008) described circinate embryos and a soft, fibrous testa in some species of

TABLE 2. Character differences generally used to diagnose *Rhodomyrtus* from morphologically similar genera. Data were taken from specimens (Appendix 1), Snow (2007, 2009) and Snow et al. (2008). Abbreviations: AC (acrodromous), EU (eucamptodromous), BR (brochidodromous); SM (smooth), RB (ribbed or rugulose).

Character	*Rhodomyrtus*	*Archirhodomyrtus*	*Octamyrtus*	*Kanakomyrtus*	*Rhodamnia*
leaf venation	AC, BR, EU	BR	BR, EU	BR, EU	EU (rarely BR)
petal number	5 (4 in 1 sp.)	5	6,8,10,12	4,5,6,7,8	4 (5 in 1 sp.)
petal lengths	mostly equal	mostly equal	highly unequal (inner petals much elongated)	mostly equal	mostly equal
hypanthial texture	SM, RB	SM	RB	SM	SM
placentation	axile (1 sp. parietal)	axile	axile	axile	parietal
stigma shape	peltate, capitate	peltate	peltate	2–4 lobed	capitate
stigma length	slightly exserted	slightly exserted	greatly exserted	slightly exserted	slightly exserted
partitions between seeds	absent, present	present	present	absent, present	absent
native range	SE Asia, Malesia, Melanesia, Australia	Australia, New Caledonia	New Guinea, Moluccas	New Caledonia	SE Asia, Malesia, Australia, New Caledonia

Rhodomyrtus, making these characters more common among baccate genera than previously reported (Snow et al. 2003; Lucas et al. 2007). Additional characters were observed, such as the presence of large, fleshy, and dark maroon cells (when fresh) between seeds in the fruit (see Table 1, Snow et al. 2008), which leave pitted depressions on the testa of the seeds of some species (e.g. most prominently in *Rhodomyrtus locellata*). Although we analyzed the morphological data using parsimony, poor levels of resolution and low support indices were obtained (results not shown). However, despite low levels of resolution, parsimony analyses consistently rejected the monophyly of *Rhodomyrtus.* We believe that characters from morphology and anatomy have the potential to illuminate relationships among the ingroup taxa, but that much greater study is required. For example, anatomical studies of *Rhodomyrtus* and related genera that focus on venation patterns and epidermal characters of the leaves, fruits, and floral ontogeny (e.g. Belsham and Orlovich 2002, 2003) likely will provide additional taxonomic markers. It should be noted also that some species circumscriptions in *Rhodomyrtus* need testing with additional sampling and observation in the field, particularly among some taxa on New Guinea. For example, the diagnostic traits that putatively separate *R. effusa*, *R. montana, R. misimana, R. novoguineensis*, *R. pervagata*, and *R. trineura* need additional study.

The phenogram based on morphological characters using neighbor joining (Fig. 1) strongly rejects the hypothesis that *Rhodomyrtus* comprises a single lineage, but it corroborates the taxonomic boundaries of some of the genera as they currently are defined (Fig. 1). For example, members of *Archirhodomyrtus*, *Pilidiostigma*, *Rhodamnia*, and *Octamyrtus* each are restricted to their own phenetic branch. Species of *Kanakomyrtus* occupy the same branch, but one that also includes *Rhodomyrtus macrocarpa,* which is atypical in *Rhodomyrtus* with its elongated fruits, unilocular ovary, and parietal placentation.

The flowers of the sole New Caledonian member of *Rhodomyrtus*, *R. locellata*, are significantly larger than others of the genus. Its placement (Fig. 1) adjacent to the three species of *Octamyrtus* is of potential evolutionary interest. The inner petals, stamens, and style of *Octamyrtus* are significantly elongated compared to other related genera (Scott 1978b; Craven and Sunarti 2004; Craven 2006). Craven (2006) suggested that this modified floral syndrome might reflect a selective shift by avian pollinators. A highly worthwhile field study would investigate what kinds of pollinators visit flowers in *Octamyrtus* and what birds and (presumably) mammals eat the fruits. The relatively large, reddish-pinkish flowers of *R. locellata* and its placement in a branch (Fig. 1) with *Octamyrtus* that includes most of the other eucamptodromous- and brochidodromous-veined species suggests the possibility of a close relationship between *Rhodomyrtus locellata* and *Octamyrtus*. The remaining members of *Rhodomyrtus* that cluster broadly with *R. locellata* and *Octamyrtus* also share a wrinkled to rugose hypanthium. The membranous testa and circinate embryos of *R. elegans* and *R. guymeriana*, which are atypical character states for *Rhodomyrtus* (Snow et al. 2008), account for these species clustering together. Collectively, the phenogram does not support a close overall similarity among species of *Rhodomyrtus* s. l., but it upholds the morphological recognition of the other genera based on overall similarity.

During the present study it became increasingly evident that indumentum patterns in *Octamyrtus* on the abaxial laminar surface, hypanthium, calyx lobes and petals probably merit additional study. In particular, considerable variation has been noted among specimens presently placed into *O. behrmannii* and *O. pleiopetala* (e.g. Craven and Sunarti 2004). Variation observed by the first author (Snow, unpubl.) on the abaxial leaf surfaces leads him to believe that one or more undescribed taxa may exist in *Octamyrtus,* one specimen of which has been treated here as *Octamyrtus pleiopetala*, but which was treated by Csizmadi (2006) as sp. nov. Indumentum patterns have been shown to have diagnostic value in *Rhodamnia* (Snow 2007), and given that some species in both *Kanakomyrtus* and *Octamyrtus* have highly contorted, colored trichomes, and the genera likely are closely related within Myrteae (Snow 2009), additional study of the indumentum of these genera is warranted.

***Molecular Data*—**A number of papers have discussed potential problems with ribosomal DNA and ITS sequences (e.g. Buckler et al. 1997; Mayol and Roselló 2001; Muir et al. 2001; Bailey et al. 2003; Razafimandimbison et al. 2004; Feliner and Roselló 2007). After our molecular data mostly were generated and analyzed, Bayly et al. (2008) demonstrated pseudogenes among ITS sequences in some capsular fruited members of Myrtaceae. These authors noted that a given set of primers and PCR conditions can selectively amplify different rDNA copies among different taxa, and that some species contain both typical and pseudogene ITS sequences. The only taxon shared between their analysis and ours, *Lophostemon confertus*, which we used as an outgroup, was not identified as having a pseudogene in their analysis (Bayly et al. 2008). One potential indicator of pseudogenes in our sequence

data is its level of C-G content, which at 55% falls closer to the pseudogene values reported by Bayly et al. (2008: 137). A second potential indicator of paralogy is the failure of all accessions of *Rhodomyrtus canescens* and *R. pervagata* to group together (Fig. 3). In contrast, several lines of evidence suggest an absence or low incidence of pseudogenes in our data. First, the absence of any substitutions in our data in the 5.8S cistron (see Results) is contrary to expectations of relatively high rates of substitutions (Bayly et al. 2008: 137). Second, our molecular results mostly were lacking in relatively long branches among the ingroup taxa, apart from the long branch of *R. canescens*-C (Fig. 2). It is possible that this long branch reflects amplification of a pseudogene. Third, in much larger analyses of baccate genera, ITS data almost always grouped together congeneric species (Salywon et al. 2004, and unpubl.; Lucas et al. 2007). Fourth, the relationships revealed in studies having broad generic samples (Lucas et al. 2007; Salywon et al. 2004, and unpubl.) based on ITS data generally do not contradict taxonomic insights from morphology and anatomy. On balance, we thus are relatively unconcerned that pseudogenes may have affected the results significantly. But we agree that paralogues can confound phylogenetic analyses (Bayly et al. 2008) and, when properly identified, potentially can provide an additional source of character data for phylogenetic studies.

The ITS sequence data consistently rejected the null hypothesis that *Rhodomyrtus* is monophyletic (Figs. 2–4), irrespective of which outgroups (alone or in combination) or analytical methods were used. Trees from all molecular results placed species of *Rhodomyrtus* in two clades designated as Clades A and B with highly similar (although not always identical) members (Figs. 2–4). Although different results would have been obtained with the inclusion of sequence data from the nonsampled taxa in *Rhodomyrtus* (see Methods), *Octamyrtus*, and *Kanakomyrtus* (which appear to be most closely related morphologically based on aspects of fruit morphology), we consider it unlikely that *Rhodomyrtus* would have been monophyletic based on greater breadth of sampling.

The Bayesian tree (Fig. 4) was insufficiently resolved at the basal nodes to shed light on the relative sequential and geographical origins of the baccate genera sampled herein. In contrast, the parsimony analyses of the ITS data (Figs. 2–3) suggests that *Rhodamnia* arose before the other sampled baccate genera, a result supported by Biffin et al. (2010). This result differs from Lucas et al. (2007), who sampled less densely among the genera studied here but more broadly overall, and whose results are ambiguous with regards to the relative order of branching of *Rhodamnia*, *Rhodomyrtus*, *Octamyrtus*, *Decaspermum*, and two genera not sampled here, *Gossia* N. Snow & Guymer and *Austromyrtus* (Nied.) Burret. *Rhodamnia* generally differs (Snow 2007) from *Rhodomyrtus* and other genera sampled here by its 4-merous flowers, parietal placentation, and lack of membranous partitions between seeds (Table 2). *Rhodamnia* is alone among the sampled genera in having exclusively parietal placentation, although parietal occurs in the unilocular ovary of *Rhodomyrtus macrocarpa*, and axile and parietal placentation occur in *Pilidiostigma* (Snow 2004). Since the Australian and New Guinean/Asian members of *Rhodomyrtus* are mixed in Clades A and B (geographic occurrences indicated in Appendix 1), the molecular phylogenies (Figs. 2–4) offer no immediate clues concerning the geographic origin of *Rhodomyrtus*. However, the Bayesian results (Fig. 4) suggest that Clade B may be more recent in origin, given the placement of *Kanakomyrtus*, *Archirhodomyrtus*, and *Pilidiostigma* relative to the origin of Clade A, but after the most common ancestor shared between Clades A and B.

Infraspecific variation in the ITS region prevented some accessions from grouping together. For example, the strict consensus tree of ITS data (Fig. 3) showed accessions A and F of *Rhodomyrtus pervagata* somewhat apart from other accessions of this species (Fig. 3). Likewise, *Rhodomyrtus canescens*-C was not in the same clade as its two conspecific accessions, the latter of which were also part of a polytomy. Three accessions of *R. macrocarpa* were not resolved as a distinct clade, nor did two accessions each of *R. novoguineensis, R. psidioides,* or *R. sericea* (Fig. 3). Generally, levels of infraspecific variation were low. Some accessions within a species showed no variation (e.g. *Rhodomyrtus pervagata* accessions C-E) or only one bp difference (e.g. *R. sericea*). Excluding ambiguous regions, others had five or fewer bp changes, (*R. pervagata*-F from accessions C-E; *R. psidioides* accessions A and B, *R. novoguineensis* A and B). The reasons underlying the diversity in the nrDNA region merits additional attention in future studies. Such diversity, as discussed above, may reflect paralogy, hybridization, or relatively old ages of species. The lattermost possibility cannot be ruled out given the relative climatic stability of rainforests in eastern Australia, which have shifted in their latitudinal extent through time. Sequence diversity within species may also reflect genetic drift or selection for different environments. The taxa with the most variable ITS sequences (e.g. *Rhodomyrtus canescens*, *R. pervagata*) occur in eastern Australia in the wet tropics, a region that is believed to harbor many ancient lineages of vascular plants.

An interesting finding between species of *Rhodomyrtus* was the high degree of variation in ITS sequence data between *R. montana* and *R. misimana* (Appendix 3), which strongly corroborates the hypothesized distinctness of these two morphologically similar species from New Guinea. As currently understood, they differ by character states of indumentum of the branchlets and abaxial laminar surface, and occupy different elevations at widely disjunct localities (Snow et al. 2008). While it would be ideal to repeat the sequencing to confirm the high degree of ITS variation noted here, the aligned sequences differed by 61 base pairs. In addition, *R. montana* has two indels (gaps) of two bp relative to *R. misimana*, and *R. misimana* has one gap each of one bp and two bp relative to *R. montana*. Of the 65 total differences (61 bp plus four indels), 36 differences occurred in the first 250 bp; the remaining 29 were spread more or less equally between bp 383–641. Snow and Veldkamp (2010) reported a specimen from New Guinea intermediate between the two species concerning the density of the abaxial laminar indumentum, but given the high levels of ITS variation and the initiation of recent collecting activity in New Guinea by Bishop Museum, which may find additional material that helps address the taxonomy of the species, did not chose to make any taxonomic alterations.

Biogeography—The baccate (ingroup) genera differ significantly in their native distributions (Table 2), although none extend beyond Australasia. Among the ingroup genera of greatest interest in this study, *Rhodomyrtus* ranges from India and southern China to eastern Australia and New Caledonia (Scott 1978a; Snow et al. 2008), *Kanakomyrtus* is endemic to New Caledonia (Snow 2009), *Archirhodomyrtus* has three species in New Caledonia and one in eastern Australia, and *Octamyrtus* is nearly restricted to New Guinea (Scott 1978b; Craven and Sunarti 2004), with one species occurring on New

Guinea and the Aru Islands (Indonesia). Among the other ingroup taxa believed to be less closely related to *Rhodomyrtus* s. l., *Decaspermum humile* is Australian (with the genus having an Australasian distribution), *Rhodamnia* ranges from Thailand and southern China to the Solomon Islands (Snow 2007), *Pilidiostigma* is Australian with one species (*P. papuanum* [Lauterb.] A. J. Scott) extending into southwestern Papua New Guinea (Snow 2004), and *Myrtastrum rufo-punctatum* is a monotypic genus from New Caledonia.

Among species of *Rhodomyrtus*, the results from parsimony (Fig. 3) and Bayesian (Fig. 4) analyses did not clearly separate the species geographically. The membership of Clade A in the two trees (Figs. 3–4) is identical, apart from the removal of duplicate accessions in the Bayesian study (Fig. 4). None of its members are exclusively Australian, although *R. macrocarpa* is mostly Australian, with some occurrences in New Guinea. Given that southern New Guinea is part of the Australian craton, it is not surprising that some taxa are shared between southern New Guinea and Australia (e.g. *Rhodomyrtus macrocarpa*, *Pilidiostigma papuanum*). Most other species of *Rhodomyrtus* are Papuasian (*R. elegans*, *R. longisepala*, *R. montana*, *R. obovata*, *R. salomonensis*), whereas one (*R. surigaoensis*) is from the Philippines and another (*R. salamonensis*) is from the Solomon Islands (which geologically is part of the oceanic ridge that includes Bougainville Island (Papua New Guinea)). The members of *Rhodomyrtus* in Clade B collectively have a wider distribution. Some are restricted to Australia (*R. canescens*, *R. pervagata*, *R. psidioides*, *R. sericea*) or New Guinea (*R. misimana*, *R. effusa*, *R. novoguineensis*, *R. pinnatinervis*), one is endemic to New Caledonia (*R. locellata*), and one weedy species (*R. tomentosa*) whose native distribution is widespread across tropical and subtropical Asia. Clade B also lacks any clear biogeographical significance with regards to its members of *Rhodomyrtus*.

Relative Species Richness Among Baccate Genera—The focal genera of this study occur in Myrteae. Biffin et al. (2010) demonstrated elevated diversification rates of tribes Syzygieae and Myrteae relative to others in Myrtaceae based on their parallel acquisition of baccate fruits from lineages with capsular fruits. These authors also discussed other factors that may have been involved with increased speciation rates, and suggested that more complex hypotheses may be necessary apart from the acquisition of baccate fruits.

Members of Syzygieae produce fruits with one (or at most a few) seeds, which have a soft (membranous to leathery) testa surrounding a relatively large and globular embryo. The cotyledons vary from free to fused or mostly fused. In contrast, Myrteae is well known for its variation in the structure of the ovary, placenta, seed, and embryo (Landrum and Stevenson 1986; Landrum and Kawasaki 1997; Lucas et al. 2007). The majority of genera in Myrteae bear fruits with a "myrtoid" character-state syndrome (Landrum and Kawasaki 1997); namely the condition of having numerous small seeds, relatively small embryos with relatively small cotyledons, and seeds that are enclosed by a sclerotic testa. Landrum and Stevenson (1986) and Landrum and Kawasaki (1997) discussed variation in the context of subtribe Myrtinae (see also Lucas et al. 2007 for more recent summary), which Wilson et al. (2005) include within Myrteae. Included among the "myrtoid" character syndrome are all of the focal genera of the present study. However, apart from *Psidium* (ca. 95 species, Govaerts et al. 2008), no other genus in Myrteae with the "myrtoid" character state syndrome exceeds 50 species. As such, genera having the "myrtoid" syndrome, including *Rhodomyrtus*, contribute only minimally to the elevated diversification of Myrteae.

Genera of subtribe Myrciinae (see Landrum and Kawasaki 1997) also are included in Myrteae by Wilson et al. 2005) and have fruits characterized by one or a few green embryos having relatively large, well-formed cotyledons that are enclosed in a soft testa. *Myrcia* DC. ex Guillemin, a polyphyletic genus (ca. 380 species s. s., E. Lucas pers. comm. 2010) and related genera (ca. 370 species, Lucas, pers. comm., 2010; see also Landrum and Kawasaki 1997) also have contributed to the elevated speciation rates of Myrteae noted by Biffin et al. (2010).

Several genera of Myrteae have a eugenioid embryo (figures from Govaerts et al. 2008: *Myrcianthes* O. Berg, ca. 35 species; *Myrciaria* O. Berg, ca. 22 species; *Neomitranthes* D. Legrand, ca. 17 species; *Plinia* Plum. ex L., ca. 67 species; *Siphoneugena* O. Berg, ca. nine species), but *Eugenia* (ca. 1,100 species, including *Calycorectes* O. Berg [ca. 30 species]; Snow, unpubl.) contributes most significantly to the elevated species richness of Myrteae. Remarkably, and perhaps not insignificantly from the point of adaptive radiations, the fruits of *Eugenia* (like *Syzygium*) produce one or a few seeds that contain relatively large, nutrient-rich embryos with large (compared to other baccate genera in the tribe), free to fused cotyledons that are surrounded by a soft (not sclerotic) testa (Landrum and Kawasaki 1997). It is noteworthy in this regard that the baccate genus *Xanthomyrtus* of Tristanieae (small, few to numerous seeds with relatively small embryos and a sclerotic testa) has not undergone a radiation. Given that parallel natural selection has produced nearly identical seed and embryo morphologies in the two genera that have had the largest radiations among baccate Myrtaceae (*Syzygium* and *Eugenia*), the hypothesis that the evolution of a baccate fruit is the key innovation of species radiations in Syzygieae and Myrteae needs to be reconsidered. Specifically, it appears that the evolution of a "eugenioid" seed and embryo character state syndrome, occurring after the acquisition of baccate fruits, may be the key innovation that has been most important in the diversification of *Syzygium* and *Eugenia* and their respective tribes. Given that *Eugenia* has a nearly pantropical distribution and notably elevated species numbers in some insular areas such as Sri Lanka, New Caledonia, and Madagascar (Snow 2008; Snow and Craven 2010), future workers should consider formulating and testing hypotheses of radiations in *Eugenia* not as a singular event, but as a recurring phenomenon that has occurred more than once in space and time (Biffin et al. 2010) and under different ecological conditions.

In summary, while this study has documented that *Rhodomyrtus* is polyphyletic using morphological and molecular data, additional work is needed to resolve the generic boundaries of *Rhodomyrtus* and related genera. When such boundaries are more confidently circumscribed and phylogenetic relationships are better understood, newer and more complex hypotheses can be tested concerning evolutionary processes such as vicariance, dispersal, speciation and community assembly involving Myrtaceae (e.g. Hubbell 2001; Lavin 2006; Dick and Heuertz 2008; Wang et al. 2009).

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Literature Cited

Ashton, P. S. 2006. New *Syzygium* (Myrtaceae) from northern Borneo. *Kew Bulletin* 61: 107–144.

Bailey, C. D., T. G. Carr, S. A. Harris, and C. E. Hughes. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435–455.

Barrie, F. R. 2005. Thirty-five new species of *Eugenia* (Myrtaceae) from Mesoamerica. *Novon* 15: 4–49.

Bayly, M. J., F. Udovicic, A. K. Gibbs, C. Parra-O., and P. Y. Ladiges. 2008. Ribosomal DNA pseudogenes are widespread in the eucalypt group (Myrtaceae): implications for phylogenetic analysis. *Cladistics* 24: 131–146.

Belsham, S. R. and D. A. Orlovich. 2002. Development of the hypanthium and androecium in New Zealand Myrtoideae (Myrtaceae). *New Zealand Journal of Botany* 40: 687–695.

Belsham, S. R. and D. A. Orlovich. 2003. Development of the hypanthium and androecium in South American Myrtoideae (Myrtaceae). *New Zealand Journal of Botany* 41: 161–169.

Bentham, G. 1867. *Rhodomyrtus* DC. Pp. 271–273 in *Flora Australiensis* vol. 3. London: L. Reeve & Co.

Beyra-Matos, A. and M. Lavin. 1999. Monograph of *Pictetia* (Leguminosae-Papilionoideae) and review of the Aeschynomeneae. *Systematic Botany Monographs* 56: 1–93.

Biffin, E., M. G. Harrington, M. D. Crisp, L. A. Craven, and P. A. Gadek. 2006. Structural partitioning, paired-sites models and evolution of the ITS transcript in *Syzygium* and Myrtaceae. *Molecular Phylogenetics and Evolution* 43: 124–139.

Biffin, E., E. J. Lucas, L. A. Craven, I. Ribeiro da Costa, M. G. Harrington, and M. D. Crisp. 2010. Evolution of exceptional species richness among lineages of fleshy-fruited Myrtaceae. *Annals of Botany* 106: 79–93.

Briggs, B. S. and L. A. S. Johnson. 1979. Evolution in the Myrtaceae – Evidence from inflorescence structure. *Proceedings of the Linnean Society of New South Wales* 102: 157–256.

Buckler, E. S., A. Ippolito, and T. P. Holtsford. 1997. The evolution ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.

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

Burret, M. 1941. Myrtaceen-Studien. *Notizblatt Botanische Gartens Berlin-Dahlem* 15: 479–550.

Craven, L. A. 2001. Unraveling knots or plaiting rope: What are the major taxonomic strands in *Syzygium* sens. lat. (Myrtaceae) and what should be done with them? Pp. 75–85 in *Taxonomy: the cornerstone of biodiversity*, eds. L. G. Saw, L. S. L. Chua, and K. C. Khoo. Kuala Lampur: Institute Penyelidikan Perhutanan Malaysia.

Craven, L. A. 2006. Myrtaceae of New Guinea. Pp. 429–434 in *The ecology of Papua*, eds. A. J. Marshall and B. M. Beehler. Singapore: Periplus Editions.

Craven, L. A. and S. Sunarti. 2004. A new species of, and reinstatements in, *Octamyrtus* (Myrtaceae). *Gardens' Bulletin Singapore* 56: 147–152.

Craven, L. A., E. Biffin, and P. S. Ashton. 2006. *Acmena, Amenosperma, Cleistocalyx, Piliocalyx,* and *Waterhousea* formally transferred to *Syzygium* (Myrtaceae). *Blumea* 51: 131–142.

Craven, L. A. and E. Biffin. 2010. An infrageneric classification of *Syzygium* (Myrtaceae). *Blumea* 55: 2010, doi: 10.3767/000651910X499303.

Csizmadi, J. 2006. *An assessment of generic boundaries of* Rhodomyrtus *(Myrtaceae) based on morphology and the nuclear internal transcribed spacer (ITS-1,2) region sequence data*. M. S. thesis. Greeley: University of Northern Colorado.

Dick, C. W. and M. Heuertz. 2008. The complex biogeographic history of a widespread tropical tree species. *Evolution* 62: 2760–2774.

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

Feliner, G. N. and J. A. Roselló. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* 44: 911–919.

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

Gadek, P. A., P. G. Wilson, and C. J. Quinn. 1996. Phylogenetic reconstruction in Myrtaceae using *matK*, with particular reference to the position of *Psiloxylon* and *Heteropyxis*. *Australian Systematic Botany* 9: 283–290.

Govaerts, R., M. Sobral, P. Ashton, F. Barrie, B. K. Holst, L. L. Landrum, K. Matsumoto, F. F. Mazine, E. Nic Lughadha, C. Proença, L. H. Soares-Silva, P. G. Wilson, and E. Lucas. 2008. *World checklist of Myrtaceae*. Kew: Kew Publishing, Royal Botanic Gardens.

Guymer, G. P. 1991. Revision of the *Rhodomyrtus trineura* (F. Muell.) F. Muell. ex Benth. (Myrtaceae) species complex. *Austrobaileya* 3: 377–387.

Hennig, W. 1966. *Phylogenetic systematics*. Urbana: University of Illinois Press.

Hickey, L. J. 1973. Classification of the architecture of dicotyledonous leaves. *American Journal of Botany* 60: 17–33.

Hubbell, S. P. 2001. *The unified neutral theory of biodiversity and biogeography*. Princeton: Princeton University Press.

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

Hyland, B. P. M., T. Whiffin, D. C. Christophel, B. Gray, R. W. Elick, and A. J. Ford. 1999. *Australian tropical rainforest trees and shrubs*. Collingwood: CSIRO Publishing.

Kawasaki, M. L. and B. K. Holst. 2009. Five new species of Myrtaceae from Ecuador. *Journal of the Botanical Research Institute of Texas* 3: 123–132.

Landrum, L. R. and D. Stevenson. 1986. Variability of embryos in subtribe Myrtinae (Myrtaceae). *Systematic Botany* 11: 155–162.

Landrum, L. R. and M. L. Kawasaki. 1997. The genera of Myrtaceae in Brazil: an illustrated synoptic treatment and identification keys. *Brittonia* 49: 508–536.

Lavin, M. 2006. Floristic and geographic stability of discontinuous seasonally dry tropical forests explains patterns of plant phylogeny and endemism. Pp. 433–448 in *Neotropical savannas and seasonally dry forests: plant biodiversity, biogeographic patterns and conservation*, eds. R. T. Pennington, J. A. Ratter, and G. P. Lewis. Boca Raton: CRC Press.

Lucas, E. J., S. R. Belsham, E. M. Nic Lughadha, D. A. Orlovich, C. M. Sakuragui, M. W. Chase, and P. G. Wilson. 2005. Phylogenetic patterns in the fleshy-fruited Myrtaceae – preliminary molecular evidence. *Plant Systematics and Evolution* 251: 35–51.

Lucas, E. J., S. A. Harris, F. F. Mazine, S. R. Belsham, E. M. Nic Lughadha, A. Telford, P. E. Gasson, and M. W. Chase. 2007. Suprageneric phylogenetics of Myrteae, the generically richest tribe in Myrtaceae (Myrtales). *Taxon* 56: 1105–1128.

Mabberley, D. J. 1987. *The plant book. A portable dictionary of the higher plants*. Reprinted version, with corrections, published 1989. Cambridge: Cambridge University Press.

Mabberley, D. J. 2008. *Mabberley's plant book. A portable dictionary of the higher plants*. Ed. 3. Cambridge: Cambridge University Press.

Mayol, M. and J. A. Roselló. 2001. Why nuclear ribosomal spacers (ITS) tell different stories in *Quercus*. *Molecular Phylogenetics and Evolution* 19: 167–176.

Mazine, F. F. and V. C. Souza. 2009. New species of *Eugenia* sect. *Racemosae* (Myrtaceae) from Brazilian Amazon Rainforest. *Kew Bulletin* 64: 147–153.

Meyer, J.-Y. 2000. Preliminary review of the invasive plants in the Pacific islands (SPREP member countries). Pp. 85–114 in: *Invasive species in the Pacific: a technical review and draft regional strategy*, ed. G. Sherley. Samoa: South Pacific Regional Environment Programme.

Muir, G., C. C. Flemming, and C. Schlötterer. 2001. Three divergent rDNA clusters predate the species divergence in *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. *Molecular Biology and Evolution* 18: 112–119.

Parra-O., C. 2002. Dos nuevas especies de Myrtaceae de Colombia. *Caldasia* 24: 95–102.

Patterson, C. 1982. Morphological characters and homology. Pp. 21–74 in: *Systematics Association special volume 21, Problems in phylogenetic reconstruction*. London: Academic Press.

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

Proença, C. E. B., E. M. Nic Lughadha, E. J. Lucas, and E. M. Woodgyer. 2006. *Algrizea* (Myrteae, Myrtaceae): a new genus from the highlands of Brazil. *Systematic Botany* 31: 320–326.

Razafimandimbison, S. G., E. A. Kellogg, and B. Bremer. 2004. Recent origin and phylogenetic utility of divergent ITS putative pseudogenes: a case study from Naucleeae (Rubiaceae). *Systematic Biology* 53: 177–192.

Rogers, J. S. and D. L. Swofford. 1998. A fast method for approximating maximum likelihoods of phylogenetic trees from nucleotide sequences. *Systematic Biology* 47: 77–89.

Ronquist, F. and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 17: 754–755.

Rye, B. L. 2009. An interim key to the Western Australian tribes and genera of Myrtaceae. *Nuytsia* 19: 313–323.

Rye, B. L. and M. E. Trudgen. 2008. *Seorsus*, a new Gondwanan genus of Myrtaceae with a disjunct distribution in Borneo and Australia. *Nuytsia* 18: 235–257.

Salywon, A. M. and L. R. Landrum. 2007. *Curitiba* (Myrtaceae): a new genus from the Planalto of southern Brazil. *Brittonia* 59: 301–307.

Salywon, A., N. Snow, M. F. Wojciechowski, J. Csizmadi, and L. Landrum. 2004. Phylogenetic relationships of Myrtaceae as inferred from nrDNA ITS sequence data. Snowbird, Utah: Abstract presented at Botany 2004.

Schmid, R. 1980. Comparative anatomy and morphology of *Psiloxylon* and *Heteropyxis*, and the subfamilial and tribal classification of Myrtaceae. *Taxon* 29: 559–595.

Scott, A. J. 1978a. A revision of *Rhodomyrtus* (Myrtaceae). *Kew Bulletin* 33: 311–329.

Scott, A. J. 1978b. A revision of *Octamyrtus* (Myrtaceae). *Kew Bulletin* 33: 303–309.

Scott, A. J. 1979a. A revision of *Xanthomyrtus* (Myrtaceae). *Kew Bulletin* 33: 461–484.

Scott, A. J. 1979b. The Austral-Pacific species of *Decaspermum* (Myrtaceae). *Kew Bulletin* 34: 59–67.

Scott, K. D. and J. Playford. 1996. DNA extraction technique for PCR in rain forest plant species. *BioTechniques* 20: 974–978.

Simmons, M. P. and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic studies. *Systematic Biology* 49: 369–381.

Snow, N. 1999. Notes on generic concepts in *Rhodomyrtus, Archirhodomyrtus, Decaspermum*, and *Pilidiostigma* (Myrtaceae). *Australian Systematic Botany Society Newsletter* 99: 5–7.

Snow, N. 2000. Systematic conspectus of Australasian Myrtinae (Myrtaceae). *Kew Bulletin* 55: 647–654.

Snow, N. 2004. Systematics of *Pilidiostigma* (Myrtaceae). *Systematic Botany* 29: 393–406.

Snow, N. 2006. New species of *Gossia* N. Snow & Guymer and *Rhodomyrtus* (DC.) Hassk. (Myrtaceae) from Papua New Guinea. *Austrobaileya* 7: 325–340.

Snow, N. 2007. Systematics of the Australian species of *Rhodamnia* (Myrtaceae). *Systematic Botany Monographs* 82. Ann Arbor: American Society of Plant Taxonomists.

Snow, N. 2008. Studies in Malagasy Eugenia (Myrtaceae) – I: Two new species from the Masoala Peninsula and generic transfers from *Monimiastrum*. *Systematic Botany* 33: 343-348.

Snow, N. 2009. *Kanakomyrtus* (Myrtaceae): A new and endemic genus from New Caledonia with linear stigma lobes and baccate fruits. *Systematic Botany* 34: 330–344.

Snow, N. and J. Cantley. 2010. New species of *Uromyrtus* and *Rhodomyrtus* (Myrtaceae: Myrteae) from Kamiali Wildlife Management Area, Papua New Guinea, and an updated key to *Rhodomyrtus*. *Harvard Papers in Botany* 15: 63–70.

Snow, N. and L. A. Craven. 2010. Five new species of *Syzygium* (Myrtaceae) from New Guinea. *Harvard Papers in Botany* 15: 123–126.

Snow, N. and V. F. Veldkamp. 2010. Miscellaneous taxonomic and nomenclatural notes for Myrtaceae. *Austrobaileya* 8: 177–186.

Snow, N., G. P. Guymer, and G. Sawvel. 2003. Systematics of *Austromyrtus, Lenwebbia*, and the Australian species of *Gossia* (Myrtaceae). *Systematic Botany Monographs 65*: Ann Arbor: American Society of Plant Taxonomists.

Snow, N., J. McFadden, and J. P. Atwood. 2008. Three new species of *Rhodomyrtus* (DC.) Rchb. (Myrtaceae) from Papua New Guinea. *Austrobaileya* 7: 691–706.

Staples, G. W. and D. R. Herbst. 2005. *A tropical garden flora: plants cultivated in the Hawaiian Islands and other tropical places*. Honolulu: Bishop Museum Press.

Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.b10. Sunderland: Sinauer Associates Inc.

Sytsma, K. J., A. Litt, M. L. Zjhra, J. C. Pires, M. Nepokroeff, E. Conti, J. Walker, and P. Wilson. 2004. Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the southern hemisphere. *International Journal of Plant Sciences* 165(S4): S85–S105.

Takeuchi, W. 2002. Notes and new species in Papuasian *Syzygium* (Myrtaceae). *Edinburgh Journal of Botany* 59: 259–272.

Thiers, B. 2010 Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. New York: New York Botanical Garden. http://sweetgum.nybg.org/ih/.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.

Trudgen, M. E. and B. L. Rye. 2005. *Astus*, a new Western Australian genus of Myrtaceae with heterocarpidic fruits. *Nuytsia* 15: 495–512.

Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1999. *Manual of the flowering plants of Hawai'i*. Rev. ed. 2 vols. Honolulu: University of Hawaii Press.

Wang, H., M. J. Moore, P. S. Soltis, C. D. Bell, S. F. Brockington, R. Alexandre, C. C. Davis, M. Latvis, S. R. Manchester, and D. E. Soltis. 2009. Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academy of Sciences USA* 106: 3853–3858.

Weber, E. 2003. *Invasive plant species of the world. A reference guide to environmental weeds*. Cambridge, Massachusetts: CABI Publishing.

Wilson, P. G., M. M. O'Brien, P. A. Gadek, and C. J. Quinn. 2001. Myrtaceae revisited: A reassessment of infrafamilial groups. *American Journal of Botany* 88: 2013–2025.

Wilson, P. G., M. M. O'Brien, M. M. Heslewood, and C. J. Quinn. 2005. Relationships within Myrtaceae sensu lato based on a *matK* phylogeny. *Plant Systematics and Evolution* 251: 3–19.

Appendix 1. Taxa and specimens studied for analyses of morphological data and for DNA sampling. DNA vouchers are indicated in bold; GenBank numbers follow the corresponding vouchers. Morphological data for species of *Rhodamnia* (not listed here) are from Snow (2007) and those of *Pilidiostigma* from Snow (2004). All specimens are housed at BISH unless indicated otherwise in parentheses following standard herbarium acronyms (Thiers 2010). In some cases more than one duplicate of a given collection was measured. Geographic occurrence given in square brackets following author(s): AF = Africa; AU = Australia; NC = New Caledonia; NG = New Guinea; PH = Philippines; widely ranging species are designated as appropriate.

Archirhodomyrtus baladensis (Brongn. & Gris) Burret [NC]: ***T. Jaffré 3285*** (HQ225434), *E. Merrill & L. M. Perry 2178* (NY). ***Archirhodomyrtus beckleri*** (F. Muell.) A. J. Scott [AU]: ***A. Ford 3166*** (HQ225435), *Clemens 247* (NY), *Constable 66820* (NY), *A. Ford 3165 et al.*, *A. Ford 2565 & W. Cooper*, *N. Snow 7763*. ***Archirhodomyrtus paitensis*** (Schltr.) Burret [NC]: ***N. Snow 9268 et al.*** (HQ225436), *N. Snow 9240 et al.* ***Archirhodomyrtus turbinata*** (Schltr.) Burret [NC]: ***N. Snow 9260 et al***. (HQ225437), *Dawson WELTU 16572*. ***Carpolepis tardiflora*** J. W. Dawson [NC]: (AF211495). ***Decaspermum humile*** (G. Don) A. J. Scott [AU]: ***Belsham M82*** (OTA) (AM234128), *A. Snow 7462* & *J. Bradford*, *A. Snow 7467* & *J. Bradford*, *A. Snow 7738*. ***Heteropyxis natalensis*** Harv. [AF] (HM160104/05). ***Kanakomyrtus dawsoniana*** N. Snow [NC]: ***Dumontet et al. s. n.*** (WELTU) (HQ225445). ***Kanakomyrtus longipetiolata*** N. Snow [NC]: ***Litaudon 110*** (NOU) (HQ225443), *H. MacKee 41450* (NOU), *G. McPherson 4759* (NOU), *Schmid 1169* (NOU, WELTU). ***Kanakomyrtus myrtopsidoides*** (Guillaumin) N. Snow [NC]: *G. McPherson 2238* (MO, NOU), *G. McPherson 2242* (MO), *J. Veillon 5610* (P), *J. Veillon 16575* (BISH, WELTU). ***Kanakomyrtus prominens*** N. Snow [NC]: ***N. Snow 9204*** (ASU) (HQ225444), *H. MacKee 26617* (NOU, WELTU), *G. McPherson & P. Lowry 18489*, *J. Munzinger et al. 3181* (NOU). ***Lophostemon confertus*** (R. Br.) Peter G. Wilson & J. T. Waterh. [AU]: (AF390444). ***Metrosideros rotundifolia*** J. W. Dawson [NC]: (AF211507). ***Myrtastrum rufo-punctatum*** (Panch. ex Brongn. & Gris) Burret [NC]: ***N. Snow 9188 et al.*** (HQ225439), *N. Snow 9267 et al.*, *Ziarnik 23*. ***Octamyrtus behrmannii*** Diels [NG]: ***W. Takeuchi 6271*** (HQ225446), *W. Takeuchi 4868*, *W. Takeuchi 6180*, *W. Takeuchi 6271*, Vink *16823*. ***Octamyrtus pleiopetala*** Diels [NG]: *Burley & Ismail 4504*, *Powell 1694*, *R. Schodde 5027 & L. Craven*, *W. Takeuchi 5326*, *P. van Royen 11574*. ***Octamyrtus* sp. nov.?** [NG]: ***W. Takeuchi 4502*** (HQ225447). ***Pilidiostigma glabrum*** Burret [AU]:

N. Snow 7762 (HQ225448), *N. Snow 7762*, *N. Snow 7470 & J. Bradford*, *L. Webb & J. Tracey 8148*. ***Pilidiostigma tropicum*** L. S. Sm. [AU]: ***N. Snow 7760*** (HQ225449), *B. Gray 4596*, *B. Gray 4726*, *B. Gray 8796*, *B. Hyland 13335*, *B. Hyland 13629*, *T. Risley 408*. ***Rhodamnia andromedoides*** Guillaumin [NC]: ***N. Snow 9264*** (HQ225440). ***Rhodamnia australis*** A. J. Scott [AU]: ***A. Ford 2319*** (HQ225441). ***Rhodamnia blairiana*** F. Muell. [AU]: ***A. Ford 3241*** (HQ225442). ***Rhodomyrtus canescens*** C. T. White [AU]: ***N. Snow 9941 & A. Ford*** **(accession-A)** (HQ225450), ***N. Snow & A. Ford 9945*** **(accession-B)** (HQ225468), ***N. Snow 9942 & A. Ford*** **(accession-C)** (HQ225470), *L. Brass 2098* (A, BISH), *A. Ford 2402*, *A. Ford 2425, A. Ford 3254, B. Gray 5328*, *V. Moriarty 1947* (A), *L. Smith 5039* (A), *C. White 4800* (A). ***Rhodomyrtus effusa*** Guymer [AU]: ***A. Ford 2402*** (HQ225451), *A. Ford 3254, L. Smith 11503*. ***Rhodomyrtus elegans*** (Blume) A. J. Scott [NG]: ***C. Ridsdale NGF31720*** (**BISH**, US) (HQ225452), *L. Brass 3600* (A), *C. Carr 11991* (A), *M. Galore & Vandenberg NFG41068* (BRI), *T. Hartley 10636* (A, BRI), *R. Johns 12268* (A), *R. Johns 14418* (A), *R. Pullen 8167* (A), *H. Streimann & A. Kairo NGF17479* (A, BISH, L, US), *P. van Royen & H. Sleumer 6326* (A). ***Rhodomyrtus kaweaensis*** N. Snow [NG]: *Foreman LAE 52304*, *H. Streimann & D. Foreman NGF24432*. ***Rhodomyrtus lanata*** Guymer [NG]: *L. Brass 12053* (L), *L. Brass 29576* (K, NY), *B. Conn et al. 328* (K), *L. Craven & R. Schodde 1311* (A, BRI), *M. Fallen 398*, *L. Gressitt BMF10*, *L. Gressitt BMF331*, *K. Kerenga & D. Wabo LAE74380* (K), *A. Millar NGF12156* (K, L), *J. Regalado & P. Katik 1022* (BISH, NY), *C. Ridsdale 30279* (BISH, BRI), *C. Sayers NGF12609* (BISH, BRI), *C. Sayers NGF19502* (BISH, BRI, K), *C. Sayers NGF19935* (K), *J. Szent-Ivany & R. Straatman BMF10/A, W. Takeuchi 4056*, *B. Verdcourt & R. Johns 5110* (K), *J. Womersley 5375* (BISH, BRI, US), *J. Womersley & R. Thorne NGF12810* (BRI). ***Rhodomyrtus locellata*** (Guillaumin) Burret [NC]: ***G. McPherson 19191*** (MO) (HQ225454), *Catala-Stucki C92* (MO), *G. McPherson 3831* (MO, WELTU), *G. McPherson 5328* (MO), *J. Dawson WELTU 16582* (WELTU), *J. Dawson WELTU 16607* (WELTU), *M. LeRat & M. LeRat 2906* (P), *P. Weston 1600 et al.* (BRI). ***Rhodomyrtus longisepala*** N. Snow & J. McFadden [NG]: ***W. Takeuchi 4550*** (**BISH**, BRI, CANB, NY) (HQ225455). ***Rhodomyrtus macrocarpa*** Benth. [AU, NG]: ***N. Snow 9939*** **(accession-A)** (HQ225456), ***R. Elick 310*** **(accession-B)** (HQ225466), ***A. Ford 3285 & Holmes*** **(accession-C)** (HQ225467), *L. Brass 5989* (NY), *L. Brass 33718* (QRS), *H. Flecker 14245* (NY), *A. Irvine 680* (QRS), *N. Snow 9938*, *L. Webb & J. Tracey 6035*. ***Rhodomyrtus mengenensis*** N. Snow [NG]: *P. Stevens & Y. Lelean LAE58784* (A, BRI, CANB, E, K). ***Rhodomyrtus misimana*** N. Snow [NG]: ***Harrison-Gagné 2155*** (HQ225453), *K. Damas LAE74597*. ***Rhodomyrtus montana*** Guymer [NG]: ***H. Sleumer & Vink BW14152*** (**BISH,** BRI) (HQ225457), *J. Burley & T. Ismail 4500* (A, BISH), *M. Galore & B. Seruta NGF41199* (K), *P. van Royen & H. Sleumer 8205* (K), *P. van Royen & H. Sleumer 8087* (CANB), *P. van Royen & H. Sleumer 8205* (K). ***Rhodomyrtus novoguineensis*** Diels [NG]: ***J. Wiakubu & O. Gideon LAE50641*** (NY; **accession A**) (HQ225458), ***J. Regalado & P. Katik 1022*** (NY; **accession-B**) (HQ225471), *N. Bowers 643* (US), *L. Brass 27450* (US), *L. Brass 27093* (K), *L. Craven & R. Schodde 1108* (A, BRI), *K. Damas 74597* (BISH), *P. Darbyshire & R. Hoogland 8365* (BISH, BRI), *D. Frodin et al. 4372*, *D. Frodin NGF26201* (K, NY), *L. Gressitt 331*, *J. Havel NGF17273*, *F. Hellwig 590* (K), *R. Hoft 2095* (L), *R. Hoogland 4611* (BRI), *R. Hoogland 9158* (K, US), *Y. Lelean & P. Stevens LAE51245* (K), *A. Millar NGF23475*, *R. Schlechter 19223* (A, BISH, NY), *R. Schodde 5372* (L), *F. Schram 14969* (BISH, L), *B. Stone & H. Streimann 10377*, *H. Streimann & A. Katik NGF28988* (BISH, US), *J. Vandenberg & M. Galore NGF42113* (BRI, K). ***Rhodomyrtus obovata*** C. T. White [NG]: ***K. J. White & E. Gray NGF10404*** (**A**, BRI, L) (HQ225459), *L. Brass 6566* (A, BRI), *L. Brass 8678* (A), *Laman et al. TL791* (NY). ***Rhodomyrtus pervagata*** Guymer [AU]: ***A. Ford 3295*** **(accession-A)** (HQ225473), ***A. Ford 2426*** **(accession-B)** (HQ225475), ***N. Snow 9943 & A. Ford*** **(accession-C)** (HQ225474), ***R. Elick 311*** **(accession-D)** (HQ225476), ***N. Snow 9940 & A. Ford*** **(accession-E)** (HQ225477), ***R. Elick 309*** **(accession-F)** (HQ225478), *M. Luckow 3799* (NY), *N. Snow 9944 & A. Ford*. ***Rhodomyrtus pinnatinervis*** C. T. White [NG]: ***J. Regalado & P. Katik 1195*** (**A**, BISH) (HQ225460), *M. Benjamin LAE67967*, *L. Brass 4905* (NY), *L. Brass 22975* (A, US), *L. Brass 25711* (A, US), *L. Brass 29125* (US), *L. Brass 29568* (US), *Clemens 6382* (A), *M. Coode & P. Katik NGF 32851*, *L. Craven & R. Schodde 1139* (US), *T. Hartley 11648* (A), *A. Millar 22800*, *A. Millar & R. Holttum 15731*, *R. Pullen 7820* (A), *C. Ridsdale NGF30180* (A, BISH), *C. Sayers NGF19938* (US), *Smith 1390* (L), *P. Stevens & J. Veldkamp 55590* (US), *H. Streimann & A. Kairo NGF17498* (BISH, US), *J. Womersley 11035* (A), *P. Wood et al. NGF17954*. ***Rhodomyrtus psidioides*** (G. Don) Benth. [AU]: ***N. Snow 9162*** **(accession-A)** (HQ225461), ***N. Snow 7453 & J. Bradford*** **(accession-B)** (HQ225472), *M. Clemens 247* (NY), *N. Snow 7730* (BRI). ***Rhodomyrtus salomonensis*** (C. T. White) A. J. Scott [NG; Solomon Islands]: ***P. Lavarack & C. Ridsdale NGF31292*** (A) (HQ225462), *L. Brass 3257* (A, BISH), *L. Craven 325* & *R. Schodde* (A), *J. Sore BSIP2314* (A), *T. Whitmore 6064* (A). ***Rhodomyrtus sericea*** Burret [AU]: ***R. Elick 308*** **(accession A)** (HQ225463), ***N. Snow 9937*** **(accession B)** (HQ225469), *L. Brass 2098* (A), *L. Brass 20061* (A), *A. Ford 2425*, *P. Forster 27691* (BRI, NY). ***Rhodomyrtus surigaoensis*** Elmer [PH]: ***Ramos & Lonvocar 83788*** (A) (HQ225464), *Catalan s. n.* (US), *Elmer 13709* (US), *Ramos & Pascasio 34579* (US), *Sulit PNH6422* (A). ***Rhodomyrtus tomentosa*** (Aiton) Hassk. [widespread, SE Asia to Malesia]: ***A. Salywon 758*** (ASU) (HQ225465), *L. Abbe & T. Smitinand 9503* (NY), *L. Abbe & T. Smitinand 9593* (NY), *L. Abbe & Tang 10035* (NY), *P. Ashton 2296* (A), *Bartlett 8327* (NY, US), *Beauchamp 897*, *Beck 1135* (NY), *Bembower 150* (NY), *Boeea 5952* (NY, US), *L. Brass 4085* (NY), *Cuming 3630* (NY), *O. Degener 8190* (NY), *F. Fosberg 37709* (BISH, NY, US), *C. Lei 531* (BISH, NY), *E. Merrill 10957* (NY), *S. Sohmer et al. 8428*, *N. Snow 7728, B. Stone 11233*. ***Rhodomyrtus trineura*** (F. Muell.) F. Muell. ex Benth. [AU, NG]: *L. Brass 1948* (A, BISH), *L. Brass 5110* (NY), *L. Brass 12455* (A), *M. Clemens s. n.* (BISH 166423), *A. Ford 3175, A. Ford 3294, A. Ford 4514*, *E. Henty & D. Foreman 52614* (A, BRI), *V. Moriarty 1974* (A), *R. Pullen 1427* (A), *G. Stocker 660* (A, BISH), *W. Takeuchi & Kuland 11390* (A), *C. White 4800* (A), *J. Witono 26*.

Appendix 2. List of characters used in the morphological cladistic analyses. Character 15 is simply character 14 scored in a more general manner, which was helpful in some analyses in Csizmadi (2006).

***Vegetative Characters*. 1. Terminal internode transsectional shape:** elliptic to round (0); bearing one longitudinal groove per side (1); bearing two longitudinal grooves per side (2); quadrangular (3); bearing two wings on each side (4); bearing several to many fine longitudinal striations (5). **2. Leaf position on mature plants:** alternate (0); opposite (1). **3. Leaf venation:** brochidodromous (0); acrodromous (1); eucamptodromous (2). **4. Abaxial indumentum of mature leaves:** glabrous (0); sericeous (1) [this includes the indumentum of *Kanakomyrtus dawsoniana*, which was described as glandular-hoary (Snow 2009)]; tomentose (2); villous-hispid (3) [consistently differentiating between hispid or villose has not been possible among these taxa, but the hyphenated concept is easily distinguishable from other types recognized herein]; tomentose with individual hairs irregularly contorted or bent at sharp angles (4); stellate (5). **5. Abaxial indumentum color on mature leaves:** whitish (0); ferrugineous (1).

***Reproductive Characters*: 6. Inflorescence type (following Briggs and Johnson 1979)**: flower solitary or in fascicles (0); triad (1); botryoid (2); metabotryoid (3). **7. Hypanthium texture in flower (excluding glands and hairs):** smooth (0); ribbed to rugulose (1). **8. Flower sexuality:** hermaphroditic (0); unisexual (1). **9. Floral indumentum color (excluding ciliate hairs of calyx lobes):** white (0); ferrugineous (1); brownish (2); yellowish (3). **10. Calyx lobe number:** four (0); five (1); six (2). **11. Petal number:** three (0); four (1); five (2); six (3); eight (4); ten (5); twelve (6). **12. Petal lengths:** equal or nearly so (0); highly unequal (1). **13. Calyx lobe position in flower:** non-overlapping at bases (0); partially overlapping at bases (1). **14. Abaxial petal surface indumentum (excluding ciliate margins):** glabrous (0); sericeous (1); irregularly stellate-glandular (2); villous (3); tomentose (4); stellate (5). **15. Abaxial petal surface indumentum (excluding ciliate margins):** glabrous or nearly so (0); more or less hairy (1). **16. Petal base shape:** broadly attached (0); somewhat narrowed (1); clawed (2). **17. Style length at anthesis:** about the same length or only slightly longer than longest stamens (0); greatly exserted relative to longest stamens (1). **18. Stigma shape:** terete-capitate (same width or only slightly wider than apex of style) (0); peltate (1); with 2-4 narrow lobes (2). **19. Stamen length relative to petals at anthesis:** slightly exserted (0); greatly exserted (1). **20. Anther sac shape at anthesis:** subglobular to globular (0); subcylindrical (1); cylindrical to linear (2). **21. Anther connective glands (excluding apex of connective):** absent (0); present (1). **22. Anther attachment** (some data from Hyland et al. 1999): basifixed (0); dorsifixed (1). **23. Locule number:** one (0); two (1); three (2); four (3). **24. Placentation type:** parietal (0); axile (1). **25. Fruit type:** capsule (0); berry (1). **26. Mature fruit shape:** subglobular/globular (0); subcylindrical/cylindrical (1); ellipsoidal (2); turbinate (3); fusiform (5). **27**. **Fruits ribbed longitudinally:** no (0), yes (1). **28. Seed arrangement in locules:** irregular (0); stacked in even rows (1); surrounded by a single encapsulating membrane (2). **29. Horizontal membranous partitions between seeds:** absent (0); present [partial to complete] (1). **30. Seed coat texture** (Snow et al. 2008)**:** sclerotic (0), membranous (1); fibrous (2). **31. Seed coat pits:** absent (0), present (1). **32. Embryo shape:** c-shaped (0); circinate (1); globular (2); straight (3).

Appendix 3. Data matrix for morphological cladistic analyses of *Rhodomyrtus* and closely related genera. Taxa represented do not exactly match those of the DNA analyses, due to lack of information for some taxa (e.g., *Metrosideros rotundifolia*). See Appendix 2 for a summary of character descriptions. a = (0/1); b = (0/2); c = (0/1/2/3); d = (2,3); e = (1/2); f = (0/3/4); g = (3/4); h = (4/5/6); i = (0/5); j = (0/1/2); k = (0/3); l = (2/3/4); m (5/6); x = (not applicable); ? = (unknown or not confirmed). Not all characters were used in all analyses (see Discussion).

	00000	00001	11111	11112	22222	22223	33
	12345	67890	12345	67890	12345	67890	12
Archirhodomyrtus baladensis	01000	a0001	20000	00100	00211	00110	00
Archirhodomyrtus beckleri	01000	c0001	20000	00100	00211	00110	00
Archirhodomyrtus paitensis	01000	a0001	20000	00100	00211	a0110	00
Archirhodomyrtus turbinata	a1000	a00x1	20000	00100	00211	a0110	00
Carpolepis tardiflora	d1010	10001	00000	10012	11210	00?01	?3
Decaspermum humile	51000	e0001	20000	00000	01d11	00200	00
Heteropyxis natalensis	b0010	d0101	20000	20011	01100	20?01	?3
Myrtastrum rufo-punctatum	01?10	00101	20000	0000a	00211	00?00	00
Kanakomyrtus dawsoniana	210a1	10120	40100	00?0a	10???	?0???	??
Kanakomyrtus longipetiolata	5100x	e1121	20141	0020e	10511	101a0	00
Kanakomyrtus myrtopsidoides	51021	e1a21	20141	00202	10311	10110	00
Lophostemon confertus	50b0x	0a001	20011	10010	01210	30?01	?3
Octamyrtus behrmannii	112fa	01010	g1121	01112	10311	0011?	00
Octamyrtus pleiopetala	a1201	01010	h1100	01112	10311	0011?	00
Octamyrtus sp. nov?	01221	01010	m1a00	01112	10d11	?????	??
Pilidiostigma glabrum	i100x	b00x1	20000	00101	10211	j0011	02
Pilidiostigma tropicum	i100x	j00x1	20000	00101	10001	j0011	02
Rhodamnia andromedoides	01110	00001	20011	00000	00001	00000	00
Rhodamnia australis	01a10	j0000	10011	00000	00001	00000	00
Rhodamnia blairiana	01151	00010	10051	0000a	00001	a0000	00
Rhodomyrtus canescens	111e0	ja0a1	20011	00001	0a211	00110	00
Rhodomyrtus effusa	11131	00011	20131	00000	0a211	00100	00
Rhodomyrtus elegans	a1030	a1011	20011	00000	00111	e00a2	01
Rhodomyrtus guymeriana	21030	11001	??0??	?????	??111	41001	01
Rhodomyrtus kaweaensis	21210	01001	20000	00000	10211	001??	?0
Rhodomyrtus lanata	21131	00011	20031	00001	00211	10100	10
Rhodomyrtus locellata	51220	j10a1	20a00	00102	10211	00120	10
Rhodomyrtus longisepala	01131	j0011	20031	00001	00211	10100	00
Rhodomyrtus macrocarpa	212k0	e1101	20100	00102	10a01	10121	01
Rhodomyrtus mengenensis	21210	0?001	20000	00?01	0?211	00100	00
Rhodomyrtus misimana	01131	00011	20131	0000?	??211	10110	00
Rhodomyrtus montana	01131	00011	20131	00000	0?211	00100	00
Rhodomyrtus novoguineensis	21130	00011	20131	0000a	10211	10100	00
Rhodomyrtus obovata	21230	01031	20100	00a00	1a211	a01a0	00
Rhodomyrtus pervagata	21131	a0011	20131	0000a	00211	a0100	00
Rhodomyrtus pinnatinervis	21b30	j1001	201ka	0010a	10211	001a0	10
Rhodomyrtus psidioides	512k0	j1001	20a00	00001	10l11	001a0	10
Rhodomyrtus salomonensis	510a0	a0011	20a00	00001	10l11	001a0	10
Rhodomyrtus sericea	21110	000a1	20111	00000	1a211	001a0	00
Rhodomyrtus surigaoensis	21e10	01001	20100	00000	00211	001a0	10
Rhodomyrtus tomentosa	21120	a1001	20131	00000	00d11	001a0	00
Rhodomyrtus trineura	01131	00011	20031	00000	0a211	001a0	00