

## CLADES, CLOCKS, AND CONTINENTS: HISTORICAL AND BIOGEOGRAPHICAL ANALYSIS OF MYRTACEAE, VOCHYSIACEAE, AND RELATIVES IN THE SOUTHERN HEMISPHERE

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Some of the most interesting but still most contentious disjunct biogeographical distributions involve Southern Hemisphere tropical and warm temperate families. The PHMV clade of Myrtales includes four families (Psiloxylaceae, Heteropyxidaceae, Myrtaceae, and Vochysiaceae) that exhibit a number of these biogeographical patterns. The related Psiloxylaceae and Heteropyxidaceae are small families restricted in distribution to the recent volcanic Mascarene Islands to the east of Madagascar and to southeast Africa, respectively. Myrtaceae are found on three major Gondwanan regions (South America, Australasia, and Africa). Because the New World taxa are almost exclusively fleshy fruited, it is unclear whether the family distribution is a classic Gondwanan vicariance pattern or results from one or more long-distance dispersal events over ocean barriers. The Vochysiaceae represent one of a handful of families with ampho-Atlantic distributions vigorously argued to support both long-distance dispersal over the Atlantic and vicariance of western Gondwanan biota by Atlantic seafloor spreading. Molecular phylogenetic relationships, fossil dating of nodes, and penalized likelihood rate smoothing of maximum likelihood trees were employed for a Myrtales-wide analysis using *rbcl* and *ndhF* and an analysis of the PHMV analysis using *ndhF* and *matK*. The results indicate that the PHMV differentiated during the late Cretaceous. The African lineage of Vochysiaceae is nested within a South American clade and probably arose via long-distance dispersal in the Oligocene at a time when the Atlantic had already rifted 80 m.yr. at the equatorial region. The African/Mascarene Island families, most closely related to Myrtaceae, differentiated during the late Eocene, with subsequent but recent long-distance dispersal from Africa to the Mascarenes. Myrtaceae show a rapid differentiation of a basal, paraphyletic subf. Leptospermoideae in Australasia. Fleshy-fruited taxa (subf. Myrtoideae) are not monophyletic. Vicariance of a widespread warm temperate Southern Hemisphere distribution is likely in explaining the South American–Australasian disjunction, with subsequent dispersal events between the two and to Africa and the Mediterranean basin.

**Keywords:** biogeography, dispersal, Heteropyxidaceae, *matK*, molecular clock, Myrtaceae, *ndhF*, phylogenetics, Psiloxylaceae, *rbcl*, vicariance, Vochysiaceae.

### Introduction

The distribution patterns and implied biogeographical events of southern hemisphere organisms have been vigorously studied and debated from the late 1960s since the incorporation of continental drift ideas into biogeography. The breakup of Gondwana starting in the early Cretaceous has provided a strong vicariance focal point (Cracraft 1988) for explaining present-day disjunct patterns in early mammals (Murphy et al. 2001), passerine birds (Edwards and Boles 2002), flightless ratites (Haddrath and Baker 2001), *Araucaria* beetles (Sequeira and Farrell 2001), and a number of angiosperm clades (Raven and Axelrod 1974; Manos 1997;

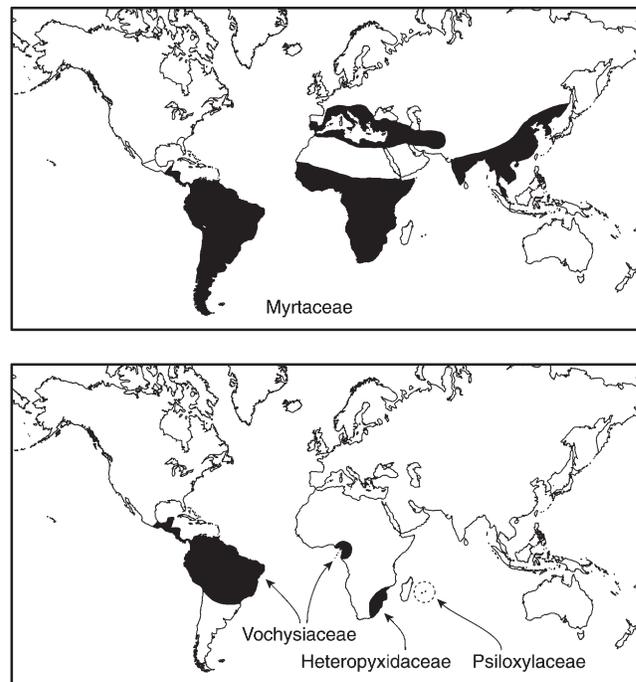
Renner et al. 2000; Swenson et al. 2001; Vinnersten and Bremer 2001; Bremer 2002). In many of these examples, however, evidence for more recent intercontinental dispersal in at least some subclades of these southern hemisphere groups has been presented (Haddrath and Baker 2001; Vinnersten and Bremer 2001; Bremer 2002). Further complicating the study of southern continent biogeography are the distinct separation times for southern temperate compared with tropical biota because of such factors as the counterclockwise rotation of Africa while separating from South America (Pittman et al. 1993), bridging of temperate flora of South America and Australia via Antarctica until ca. 50 Ma (Berry et al. 1990, 2004; Sytsma et al. 1991; Hallam 1994), the early separation (ca. 80 Ma) of New Zealand with apparently repeated dispersal events from Australia (Winkworth et al. 2002), and the long isolation of India (Conti et al. 2002).

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Vicariance as the sole explanation for disjunct patterns in southern hemisphere groups not only has been tempered, as evident in some of the examples already cited, but has been ruled out in others. These include Tertiary migration and long-distance dispersal for the pantropical Melastomataceae (Renner et al. 2001), the Laurasian origin and subsequent southern hemisphere dispersal of Lauraceae (Chanderbali et al. 2001), and the recent long-distance dispersal in the amphi-Atlantic distributed Bromeliaceae and Rapateaceae (Givnish et al. 2000, 2004). The latter families are only two examples of the distinctive disjunction between South America and Africa involving ca. 12 families (including Cactaceae, Humiriaceae, Mayacaceae, and Vochysiaceae). The relationships among these families of tropical South America and west Africa have been the subject of much systematic and biogeographic discussion before any recent sequence-based biogeographical analyses (Thorne 1972, 1973; Brenan 1978; Goldblatt 1978, 1993; Renner 2004). The vicariance versus dispersalist stances in explaining these amphi-Atlantic disjunctions arose decades ago and have been largely untested since. Axelrod (1970, p. 293) suggested that the shared, disjunct African–South American families indicate “the splitting apart of a more homogeneous flora by fragmentation of Gondwana.” In contrast, most others (Thorne 1972, 1973; Smith 1973; Barthlott 1983) strongly argued for more recent over-water dispersal to account for many, if not all, of these disjunctions.

The issues involving vicariance of widespread taxa with subsequent erection of barriers or dispersal of taxa across preexisting barriers, however, cannot be satisfactorily addressed unless three items are known: robust estimates of phylogenetic relationships, ages of relevant clade formation, and a geological time sequence of barrier formation. Here we have attempted with DNA sequence-based phylogenetic analyses, fossil dating of nodes, and molecular clock calibrations to unravel the biogeographic history of a clade of rosoid angiosperms in Myrtales that exhibits many of these southern hemisphere disjunct patterns (fig. 1). This clade (hereafter referred to as the PHMV clade) comprises two small families, Psiloxylaceae and Heteropyxidaceae, restricted to the Mascarene Islands and southeast Africa, respectively, the diverse and largely South American and Australian Myrtaceae, and the moderate-sized, amphi-Atlantic disjunct Vochysiaceae.

The Myrtaceae represent one of the most striking ecological and taxic radiations of the Gondwanan flora. Myrtaceae comprise ca. 130–140 genera and nearly 4000 species and, with their not-too-distant relative the Melastomataceae, represent the majority of species in the order Myrtales. The family is widespread in the southern hemisphere, with concentrations in the Neotropics and Australia, is somewhat rarer in the Australasian tropics, and is very rare in the African tropics. A single genus (*Myrtus*) occurs in the Mediterranean region. Table 1 shows the latest division of the family based on Johnson and Briggs (1984). The traditional two-subfamily classification, the capsular-fruited Leptospermoideae and the indehiscent-fruited Myrtoideae, is maintained here, although recent cladistic analyses of morphological and molecular data have demonstrated paraphyly of the Leptospermoideae (Wilson et al. 2001). Informally recognized “alliances” and “suballiances” are used, although these also



**Fig. 1** Current distribution of Myrtaceae, Vochysiaceae, Heteropyxidaceae, and Psiloxylaceae (modified after Heywood 1993). The occurrences of Myrtaceae and Vochysiaceae in Africa are due to only three and two genera, respectively.

have been reordered as new information has accumulated (Wilson et al. 2001). Of the two roughly equal subfamilies, the indehiscent-fruited Myrtoideae show a number of geographical connections between the Old and New Worlds but are almost entirely unrepresented on very nutrient-deficient sites in Australia and southern Africa. Two genera, *Eugenia* and *Syzygium*, occur in tropical Africa and vicinity. Within the Myrtoideae, all genera are endemic to their respective geographical areas, with the exception of *Eugenia* and *Syzygium*. The dry-fruited Leptospermoideae are represented in the New World (*Tepaulia*) and Africa (“*Metrosideros*” *angustifolia*) by only one endemic genus each, their diversification being largely Australasian.

The remainder of the PHMV clade consists of Heteropyxidaceae (three dioecious species) from southeast Africa possessing dry fruits; the monotypic, dioecious, but fleshy-fruited Psiloxylaceae from the Mascarenes, a volcanic archipelago 800 km east of Madagascar that is up to 8 m.yr. old and is linked floristically to Africa and Madagascar; and the largely Neotropical Vochysiaceae but with two genera endemic to tropical west Africa (fig. 1). Vochysiaceae is of considerable interest not only because of its biogeographical distributional pattern that it shares with 11 other tropical families restricted to the Neotropics and the African tropics but also because the flowers are strikingly different from its closely related families. The flowers are zygomorphic with a single fertile stamen and with reduction seen in petal number from five down to three or even one. The tribe Vochysieae have trilocular ovaries forming loculicidal capsules, and

**Table 1**  
**Biogeography of Myrtaceae Using an Informal Two-Subfamily Classification**

	Total Number of Genera : Number of Endemic Genera				Total
	Central and South America	Australasia	Africa	Mediterranean	
Leptospermoideae:					
<i>Metrosideros</i> alliance	1 : 1	23 : 23	1 : 1		25
<i>Backhousia</i> alliance		2 : 2			2
<i>Arillastrum</i> alliance		3 : 3			3
<i>Eucalyptus</i> alliance		9 : 9			9
<i>Leptospermum</i> alliance		14 : 14			14
<i>Chamelaucium</i> alliance		18 : 18			18
Myrtoideae:					
<i>Myrcia</i> alliance	7 : 7				7
<i>Myrtus</i> alliance	15 : 15	13 : 13		1 : 1	29
<i>Cryptorbiza</i> alliance	6 : 6	1 : 1			7
<i>Osbornia</i> alliance	1 : 1			1	
<i>Acmena</i> alliance	8 : 7	1 : 0	1 : 0		8
<i>Eugenia</i> alliance	18 : 17	3 : 2	1 : 0		20

Sources. Modified after Briggs and Johnson (1979) and Johnson and Briggs (1984).

Note. These "alliances" are now superseded by informal "groups" based on molecular results given in Wilson et al. (2001), used in figure 5.

the tribe Erismeeae, including one Neotropical genus and both African genera, have unilocular ovaries forming indehiscent, samara-like fruits (Litt 1999; Litt and Cheek 2002).

Earlier molecular evidence based solely on *rbcl* placed these four families in a well-supported group (Conti et al. 1996, 1997), although relationships of the four, except for the strong support for the pair of African/Mascarene Island families (Heteropyxidaceae and Psiloxylaceae), were weak. This Myrtaceae group is sister to a clade of Melastomataceae in the broad sense and a biogeographically disjunct lineage of five small families referred to as the CAROP (Crypteroniaceae, Alzateaceae, Rhynchoalcycaee, Oliniaceae, Penaeaceae) lineage (Conti et al. 2002).

The PHMV clade thus poses a number of interesting biogeographic, evolutionary, and ecological issues. For the purpose of this article, the issues are distilled to three interrelated problems: (1) What are the phylogenetic relationships within the PHMV clade (relationships of the four families found on three southern continents, relationships of the New vs. Old World Myrtaceae, relationships of African vs. American members of Vochysiaceae)? (2) What are the ages of the various splits in the group (separation of African Heteropyxidaceae and Mascarene endemic Psiloxylaceae, separation of American vs. Old World Myrtaceae, timing of the American-African disjunction within Vochysiaceae)? and (3) Are these major separations the result of vicariance of southern continents and/or more recent over-water dispersal?

We employ here a nested, two-stage survey of phylogenetic relationships and biogeographical history within the PHMV clade using maximum likelihood (ML) and penalized likelihood (PL) to examine these questions. The first is a broad survey of Myrtales using *rbcl* and *ndhF* sequences to examine the placement of the PHMV clade in the Myrtales; the second survey focuses on the PHMV clade using *ndhF* and *matK* sequences, both considerably faster-evolving than *rbcl*. Likelihood ratio tests of sequence evolution, penalized likeli-

hood analyses, and clock calibration with fossils of Myrtaceae and other Myrtales were subsequently done to address the issue of date calibration of the resulting tree nodes and inference of biogeographical events, whether vicariance or dispersal. Presented elsewhere will be a full phylogenetic analysis of the Myrtaceae using additionally maximum (MP) and Bayesian analyses and resulting conclusions on systematics, evolution of morphological characters, and ecological specializations (K. J. Sytsma, M. L. Zjhra, J. C. Pires, M. Nepokroeff, J. Walker, C. J. Quinn, and P. G. Wilson, unpublished manuscript).

## Material and Methods

### Sampling Design

The survey of all Myrtales using *rbcl* + *ndhF* included 79 ingroup plus four outgroup species (appendix). As this analysis was intended to place the PHMV clade within the order Myrtales and to provide a ML framework under clock assumptions in order to date fossils outside the PHMV clade, sampling was more intensive in other families: 13 from the PHMV clade, 34 from Melastomataceae s.l., five from the CAROP clade, 17 from Onagraceae, six from Lythraceae, and four from Combretaceae. Most larger-scale phylogenetic studies of rosids place Myrtales either isolated in Rosids (Savolainen et al. 2000; Soltis et al. 2000) or most closely related to Malvales, Sapindales, and Brassicales (Conti et al. 1996, 1997). The four outgroups selected came from Malvales and Brassicales. The majority of the *rbcl* sequences came from previous studies (Conti et al. 1996; Clausing and Renner 2001). The *ndhF* sequences are new except for the majority of Melastomataceae (Clausing and Renner 2001; Renner and Meyer 2001; Renner et al. 2001).

The *ndhF* + *matK* analysis of the PHMV clade included 65 species of Myrtaceae representing all alliances and

suballiances of Johnson and Briggs (1984), one species each of Heteropyxidaceae and Psiloxylaceae, and seven species of Vochysiaceae, representing all American and African genera except for the recently discovered *Korupodendron* from west Africa (Litt and Cheek 2002). The sister group to the PHMV is clearly Melastomataceae s.l. plus the CAROP clade (Conti et al. 1996). Because of long branches (and resulting problems in clock tests) seen generally in Melastomataceae s.l. relative to either the CAROP or PHMV clades based on the first survey and previous studies (Renner et al. 2001), *Rhynchocalyx* of the CAROP group was used as outgroup. All but three *ndhF* sequences are new; 26 Myrtaceae and all Vochysiaceae *matK* sequences are new. Different species were sequenced for *rbcL* and *ndhF* in two genera, *Mouriri* (Memecylaceae) and *Tococa* (Melastomataceae), and for *ndhF* and *matK* in four genera, *Psidium* and *Xanthomyrtus* (Myrtaceae) and *Callisthene* and *Qualea* (Vochysiaceae), but each pair was treated as one taxon when combining data sets. The lack of evidence for nonmonophyly of these genera and the congruence of placements of each genera with phylogenetic analysis of either gene indicate that this approach is not providing misleading evidence.

#### DNA Extraction, Amplification, and Sequencing

DNA was extracted using a modified 6% CTAB extraction (Doyle and Doyle 1987; protocol D of Smith et al. 1991) or with DNeasy Plant Mini Kit (QIAGEN, Valencia, Calif.). In the former method, DNA was precipitated with ethanol and either sodium chloride or ammonium acetate because these cause fewer polysaccharides to coprecipitate with the DNA than is the case with isopropanol or with ethanol in the presence of sodium acetate (Bult et al. 1992). Particularly in the case of some Myrtaceae and Melastomataceae, subsequent salt washes (Sytsma 1994) were required in order to consistently amplify the DNA. PCR amplification and cycle-sequencing followed the methods described elsewhere (Conti et al. 1993, 1996; Givnish et al. 2000; Hall et al. 2002). All PCR products were cleaned using QIAquick PCR purification kits (QIAGEN). Sequences were generated on an ABI Prism 377 DNA sequencer. Contiguous alignments were edited using Sequencher version 3.0 (Gene Codes, Ann Arbor, Mich.).

Overlapping sequence fragments of *rbcL* were obtained from both strands using up to 10 primers (Conti et al. 1997 for details). Overlapping sequence fragments of the 3' end of *ndhF* were obtained from both strands using primers 5–14 (Olmstead et al. 1993; Sytsma et al. 2002). Amplification and sequencing of both strands for *matK* (Vochysiaceae only) used primers described in Johnson and Soltis (1994). Myrtaceae *matK* primers and protocols have been published elsewhere (Wilson et al. 2001). Sequences of *ndhF* and *matK*, for which indels existed, were easily aligned visually in Se-AL version 2.0a6 (Rambaut 2001).

#### Phylogenetic Analyses

As the goal of this study was to explore the utility of sequence data to provide a framework for clock calibration, only ML methodologies were employed here. However, the

main phylogenetic relationships important for the biogeographical analyses are robust with either MP or Bayesian analyses (K. J. Sytsma, M. L. Zjhra, J. C. Pires, M. Nepokroeff, J. Walker, C. J. Quinn, and P. G. Wilson, unpublished manuscript). ML analyses of the data sets of *rbcL/ndhF* for Myrtales and *ndhF/matK* for the PHMV clade were performed using PAUP\* version 4.0b10 (Swofford 2002) on a Macintosh G4. Model test version 3.06 (Posada and Crandall 1998) was used to explore 56 ML models of DNA substitution in a hierarchical testing framework for each data set with a starting MP tree (obtained in PAUP\*) instead of the default neighbor-joining tree. A heuristic ML search using default settings with TBR branch-swapping was then conducted using parameters determined for the best model of sequence evolution and using a MP topology as a starting tree. ML bootstrap values (Felsenstein 1985) were obtained using the procedure outlined in Nepokroeff et al. (2003) for large data sets. This method used a constraint tree of clades supported 100% in MP bootstrap analyses, ML model of DNA substitution estimated previously, Addition Sequence = random, and SEARCH = FASTSTEP.

#### Fossils and Clock Calibration

As sequences from multiple species have accumulated over the last decades, it has become apparent that a molecular clock is not fully supported in general (Britten 1986; Gillespie 1986; Li 1997; Wray 2001; Smith and Peterson 2002) or specifically in angiosperms (Wilson et al. 1990; Bosquet et al. 1992; Gaut et al. 1992; Clegg et al. 1995; Sytsma and Baum 1996; Sanderson 1998; Sanderson and Doyle 2001). To evaluate whether the chloroplast DNA sequences in each data set evolved in a clocklike fashion, a likelihood ratio test (Felsenstein 1988) was performed by comparing the scores of ML trees with and without the clock. Following rejection of the clock assumption, the ML trees (with branch lengths) for each of the two data sets were rate smoothed with the penalized likelihood (PL) method of Sanderson (2002) as implemented in r8s version 1.60 (Sanderson 2003) using the truncated Newton algorithm. PL averages local differences in the rate of DNA evolution on different branches and assigns a penalty for rate changes among branches that are too rapid or frequent based on a smoothness parameter. Cross-verification in r8s was used to find the optimal value of the smoothness parameter by varying the latter from  $10^0$  to  $10^3$  in steps of 0.25 in the exponent.

Five fossil dates were used in PL to constrain specific nodes to minimum, maximum, or fixed ages. Issues with these approaches are known (Doyle and Donoghue 1993; Smith and Peterson 2002), but the use of multiple independent fossil calibration points can provide increased confidence in resulting dates. Two fossils of the Melastomataceae, utilized by Renner et al. (2001), were used with the *rbcL + ndhF* data set. The first leaf fossil (Wehr and Hopkins 1994; fossil 1, fig. 3) fixes the node representing the most recent common ancestor (MRCA) of *Pternandra* and all other Melastomataceae at 53 Ma. The second seed fossil (fossil 2, fig. 3) fixes the node representing the MRCA of *Monochaetum* and *Rhexia* at 23 Ma. We did not use the putative combretaceous fossils described as *Esgueiria* (Campanian-Maastrichtian of

Portugal [Friis et al. 1991] and early Santonian of Japan [Takahashi et al. 1999]). Although Magallón et al. (1999) and Wikström et al. (2001) used *Esgueiria* to constrain the Combretaceae node at 84 Ma in their angiosperm-wide analyses, its flowers differ significantly in stylar branches from extant Combretaceae, and placement in the family is not at all certain or represents a member of an early stem lineage (Friis et al. 1991; E. M. Friis, personal communication; fig. 3). We also did not use *Diporites* (Berry et al. 1990), representing Old World *Fuchsia* (Onagraceae) pollen (fig. 3) because our sampling with only one *Fuchsia* species precludes proper placement within the genus (Berry et al. 2004).

The fossil status for the PHMV group is sketchy. No fossils have been reported that unambiguously belong to Heteropyxidaceae, Psiloxylaceae, or the Vochysiaceae. The earliest fossil record for the PHMV group is *Myrtaceidites* (= *Syncolporites*) pollen from Gabon during the Santonian or 86 Ma (Herngreen 1975; Muller 1981). This pollen is also seen later in the Cretaceous from Colombia and from Borneo (van der Hammen 1954; Muller 1968). Although *Myrtaceidites* is attributed to the Myrtaceae, the pollen of Myrtaceae, Heteropyxidaceae, and Psiloxylaceae are difficult to distinguish (Schmid 1980; Patel et al. 1984). Thus we gave a maximum age of 86 Ma to node C (figs. 3, 5), representing the MRCA of the three families in both analyses. However, we also explored fixing this node at 86 Ma, or placing a maximum age of 86 Ma at the crown node of Myrtaceae (node D; fig. 3), or simply removing the fossil date from the PL analyses. The fourth fossil dates from the middle Eocene, is the earliest of eucalyptoid fruits and leaves from central Australia (Greenwood 1991), and provides a fixed age of ca. 48 Ma for the MRCA of *Eucalyptus* and *Angophora* in the *ndbF* + *matK* analysis (fossil 4, fig. 5). Since this node is not present in the *rbcL* + *ndbF* analysis, we constrained a minimum age of 48 Ma (i.e., it will likely be older) on the node representing the MRCA of *Angophora* and *Acmena* (fossil 4, fig. 3). The fifth set of fossils is *Paleomyrtinaea* from the latest Paleocene of North Dakota and early Eocene of British Columbia (Crane et al. 1990; Pigg et al. 1993; Manchester 1999). *Paleomyrtinaea* material comprises distinctive fruits and seeds seen only in the largely American subtribe Myrtinae (Pigg et al. 1993). Seed coat features of *Paleomyrtinaea* are similar to extant genera (Lantern and Sharp 1989). In addition, the unornamented C-shaped embryo in *Paleomyrtinaea* is similar to that of extant Myrtinae (McVaugh 1968; Lantern and Stevenson 1986). Thus, *Paleomyrtinaea* can be used to date the node (56 Ma; Paleocene/Eocene border) from which Myrtinae first diverge within the subf. Myrtoideae. As the Myrtinae are broadly paraphyletic in our analyses (fig. 4), this node is essentially the crown radiation of the tribe Myrteae (the Myrtoideae in the strict sense). We did not use the still unplaced fossil *Syzygioides* (Manchester et al. 1998; Manchester 1999). In addition, we constrained with a maximum age of 120 Ma the root of Myrtales, Sapindales, Brassicales, and Malvales in the *rbcL* + *ndbF* analysis (fig. 3) because this age is consistent with eudicot fossil records (125 Ma in Barremian-Aptian for early eudicots; Magallón et al. 1999). The node representing the crown group of PHMV (node B, fig. 5) was fixed at 93 Ma in the *ndbF* + *matK* analysis based on the results from the larger-scale analysis.

The ML trees with PL-smoothed branch lengths were then visualized and saved in TreeEdit version 1.0a10 (Rambaut and Charleston 2001) as chronograms for subsequent analysis. Geographical area was mapped onto resulting trees using MacClade version 4.05 (Maddison and Maddison 2002). For the purposes of the broad biogeographical questions posed here, areas of endemism were restricted to continental regions (South America, Africa, Australasia, Mediterranean). A more detailed examination of Myrtaceae (K. J. Sytsma, M. L. Zjhra, J. C. Pires, M. Nepokroeff, J. Walker, C. J. Quinn, and P. G. Wilson, unpublished manuscript) defines more exclusive areas of endemism in Australia, Asia, and the Neotropics. For dates of geological periods, epochs, and ages, we used the Obradovich (1993) Cretaceous time scale as modified by Everhart (2003) and the Harland et al. (1990) Tertiary time scale as modified by Poling (1997).

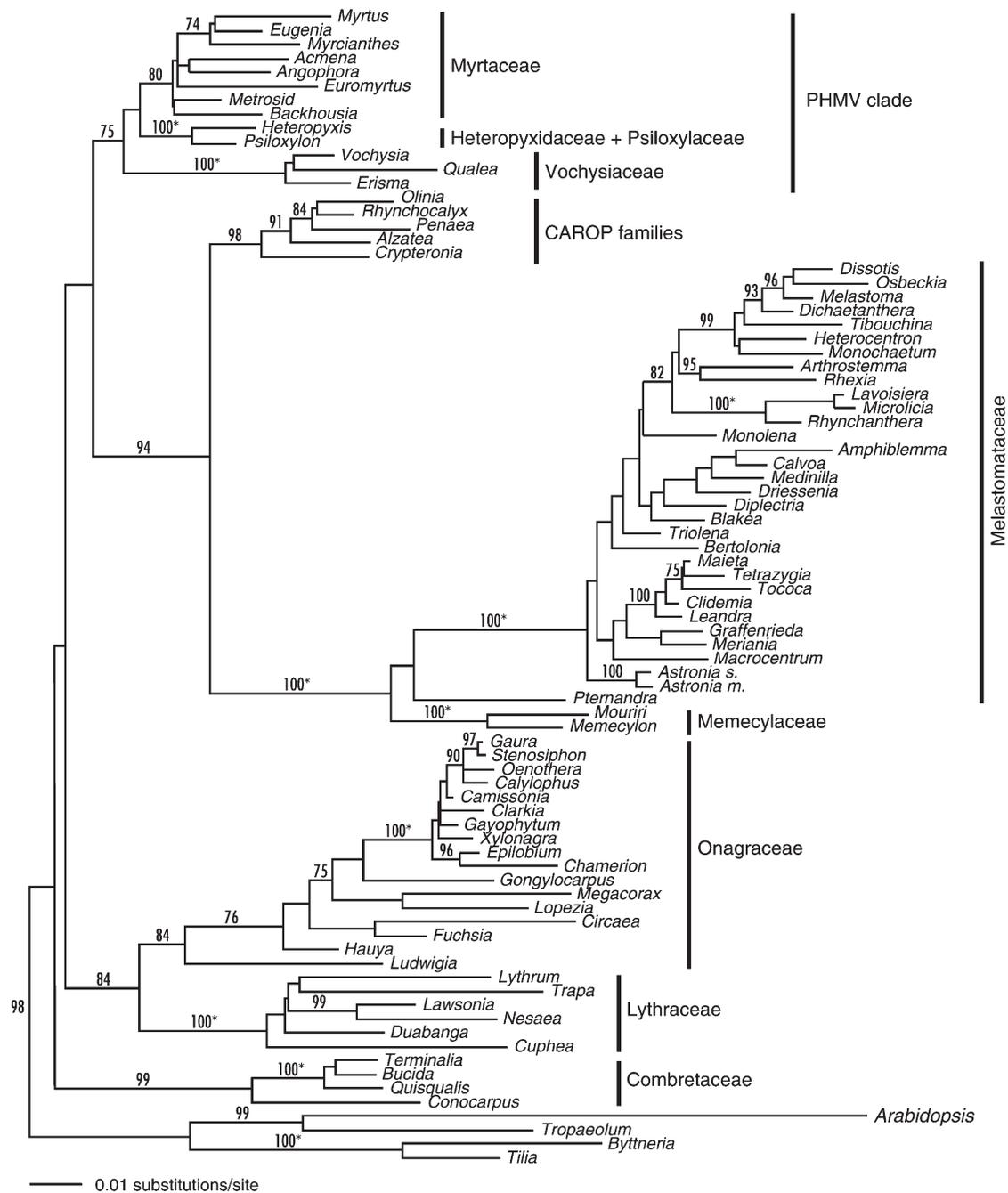
## Results

### Placement of PHMV Clade in Myrtales

The ML tree (fig. 2) for *rbcL* + *ndbF* sequences using GTR + I + G likelihood settings obtained from Modeltest highlights four important phylogenetic results in the Myrtales-wide survey: (1) Combretaceae is identified as the sister clade to the rest of the order, although support is not strong. (2) The PHMV clade is fairly well supported and is sister to the clade comprising Melastomataceae + CAROP. (3) The African families Heteropyxidaceae and Psiloxylaceae are strongly monophyletic and are sister to Myrtaceae, although not with strong ML bootstrap support. and (4) Vochysiaceae is sister to all other members of the PHMV clade.

### Clock Calibration and Lineage Dating in Myrtales-Wide Analysis

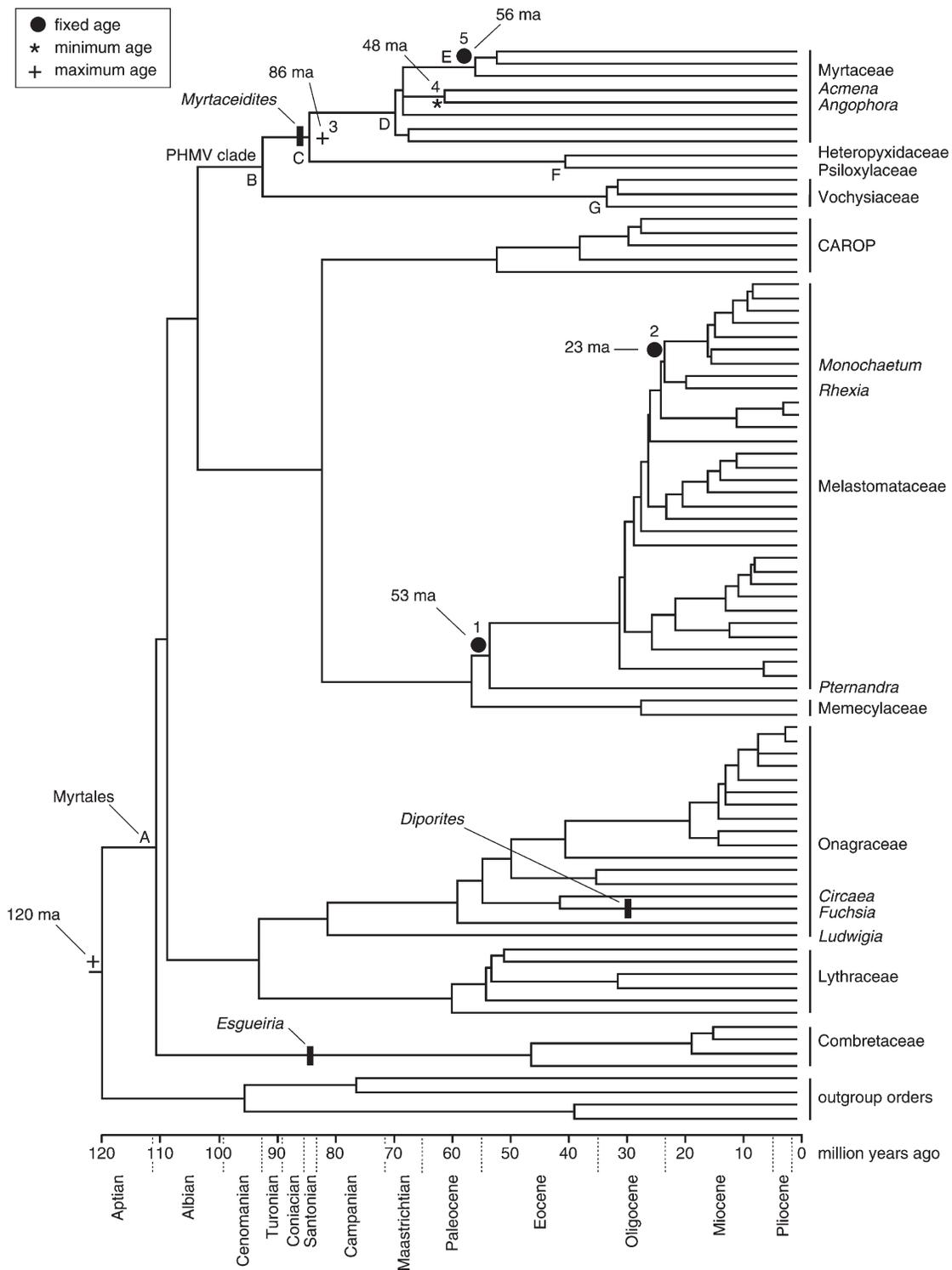
As is evident from a cursory glance of the ML tree (fig. 2), rates of sequence evolution are not clocklike in the Myrtales as a whole, based on the likelihood ratio test. The woody Myrtaceae lineage has a slow rate of sequence change, whereas the Melastomataceae have an accelerated rate—approaching that of *Arabidopsis* in the outgroup. Removal of Melastomataceae and/or *Arabidopsis* still did not permit passing of the likelihood ratio test. Cross verification of the *rbcL* + *ndbF* data set with PL indicated that the optimum smoothing rate was 100.00. Figure 3 represents the PL chronogram using information from all five fossils and placing a maximum age of 120 Ma on the root. This chronogram is based on the assumption that *Myrtaceidites* is a representative of the stem lineage leading to Myrtaceae, Heteropyxidaceae, and Psiloxylaceae (i.e., node C, fig. 3, has a maximum age of 86 Ma). This placement of *Myrtaceidites* is supported by almost an identical chronogram obtained using only the four other fossils and with *Myrtaceidites* removed from the analysis (table 2). The chronogram obtained by fixing node C at 86 Ma (instead of maximum 86 Ma constraint) is similar to that in figure 3 but gives slightly older dates within the Myrtaceae/Heteropyxidaceae/Psiloxylaceae clade (table 2). Assuming that *Myrtaceidites* belongs to the Myrtaceae lineage only (i.e., node D, fig. 3, has a maximum age of 86 Ma) provides considerably older dates within the Myrtaceae clade



**Fig. 2** Maximum likelihood tree depicting phylogenetic relationships within the order Myrtales based on *rbcL* and *ndhF* sequences. ML bootstrap values 70% and greater are above branches (those constrained in ML bootstrap runs are asterisked).

that are not consistent with the dates obtained from the four other fossils alone (table 2). The PL chronogram (fig. 3) thus places the 86 Ma *Myrtacoidites* nearly at the crown group radiation of Myrtaceae/Heteropyxidaceae/Psiloxylaceae that occurred at 84 Ma. *Esgueiria* occurs farther back in time from the crown group radiation of Combretaceae sampled here (fig. 3). As expected, *Diporites* is placed on the *Fuchsia* lineage after the 41 Ma split of *Fuchsia* and *Circaea* in the Onagraceae (fig. 3).

Based on the preferred PL chronogram, the crown group of Myrtales (node A, fig. 3) diverged 111 Ma in the earliest Albian. By the end of the Albian, the four major lineages within Myrtales are already evident: Combretaceae, Onagraceae + Lythraceae, Melastomataceae + CAROP, and PHMV. The first divergence within the PHMV clade (node B, fig. 3) involving the separation into the stem lineages for Vochysiaceae and for the other three families occurred at 93 Ma. The Cretaceous dates for both the origin of Myrtales



**Fig. 3** Penalized likelihood chronogram of Myrtales based on *rbcL* + *ndbF* sequences. Information from five fossils (indicated by numbers) was used; see text for details of implementation. Two fossils from the Melastomataceae (1, 2) were used to fix dates at two nodes. The date of *Myrtaceidites* (86 Ma) was used as a maximum age constraint of node C (alternative analyses fixed the age of node C at 86 Ma or constrained node D at 86 Ma; chronograms not shown). The date of the earliest eucalyptoid fossil (4) was used as a minimum age constraint on the most recent node leading to the sole eucalyptoid genus (*Angophora*). The date of *Paleomyrtinaea* (fossil 5; 56 Ma) was used to fix the age of node E leading to the Myrtoide lineage. The rosid root of the tree was maximally constrained at 120 Ma. Probable placements of two fossils not used in the analyses (*Diporites*, *Esgueiria*) are shown. Nodes identified by letter are discussed in the text.

**Table 2**  
**Divergence Times for Clades within Myrtales and the PHMV Lineage Based on Penalized Likelihood Analyses of Two Data Sets**

	Myrtales ( <i>rbcL</i> + <i>ndhF</i> ) <sup>a</sup>				PHMV ( <i>ndhF</i> + <i>matK</i> )
	4 fossils + Myrtaceidites set as Myrtaceae max	4 fossils + Myrtaceidites set as PHM fix	4 fossils + Myrtaceidites set as PHMmax	4 fossils + (no Myrtaceidites)	2 fossils + Myrtaceidites set as PHMmax
PHMV	100	94	93	93	93 (fixed)
PHM	95	86	84	84	86
Myrtaceae <sup>b</sup>	(86)	(70)	(70)	(70)	80 (78)
PH	45	41	40	40	38
Vochysiaceae	35	33	33	33	36
African Vochysiaceae	...	...	...	...	28

Note. In the Myrtales analysis, four fossil dates were used with or without *Myrtaceidites*. The latter's placement was allowed to vary (maximum constraint for Myrtaceae or as either a maximum constraint or a fixed aged for the PHM clade). Two fossils plus *Myrtaceidites* were used as a maximum age constraint in the PHMV analysis.

<sup>a</sup> Clade age based on differential placement of 86 Ma *Myrtaceidites*.

<sup>b</sup> Because Myrtaceae was sampled more extensively in the PHMV analysis, the Myrtaceae crown nodes for the two analyses are not exactly the same. Values in parentheses indicate ages for the Myrtaceae node seen in the less-sampled Myrtales analysis and for the comparable node in the PHMV analysis.

and of the PHMV clade are clearly in the range indicating Gondwanan vicariance as probably important in their biogeographical history. However, the crown group of the extant amphi-Atlantic Vochysiaceae (node G, fig. 3) dates back only to 33 Ma from the Oligocene of the Tertiary, a time by which ca. two-thirds of the Atlantic seafloor rifting had already occurred. The separation of southeast African Heteropyxidaceae and Psiloxylaceae from the Mascarenes (node F, fig. 3) is dated at ca. 40 Ma in the Eocene, well before the age of present-day Mascarenes (ca. 8 Ma).

#### Phylogenetic Relationships within the PHMV Clade

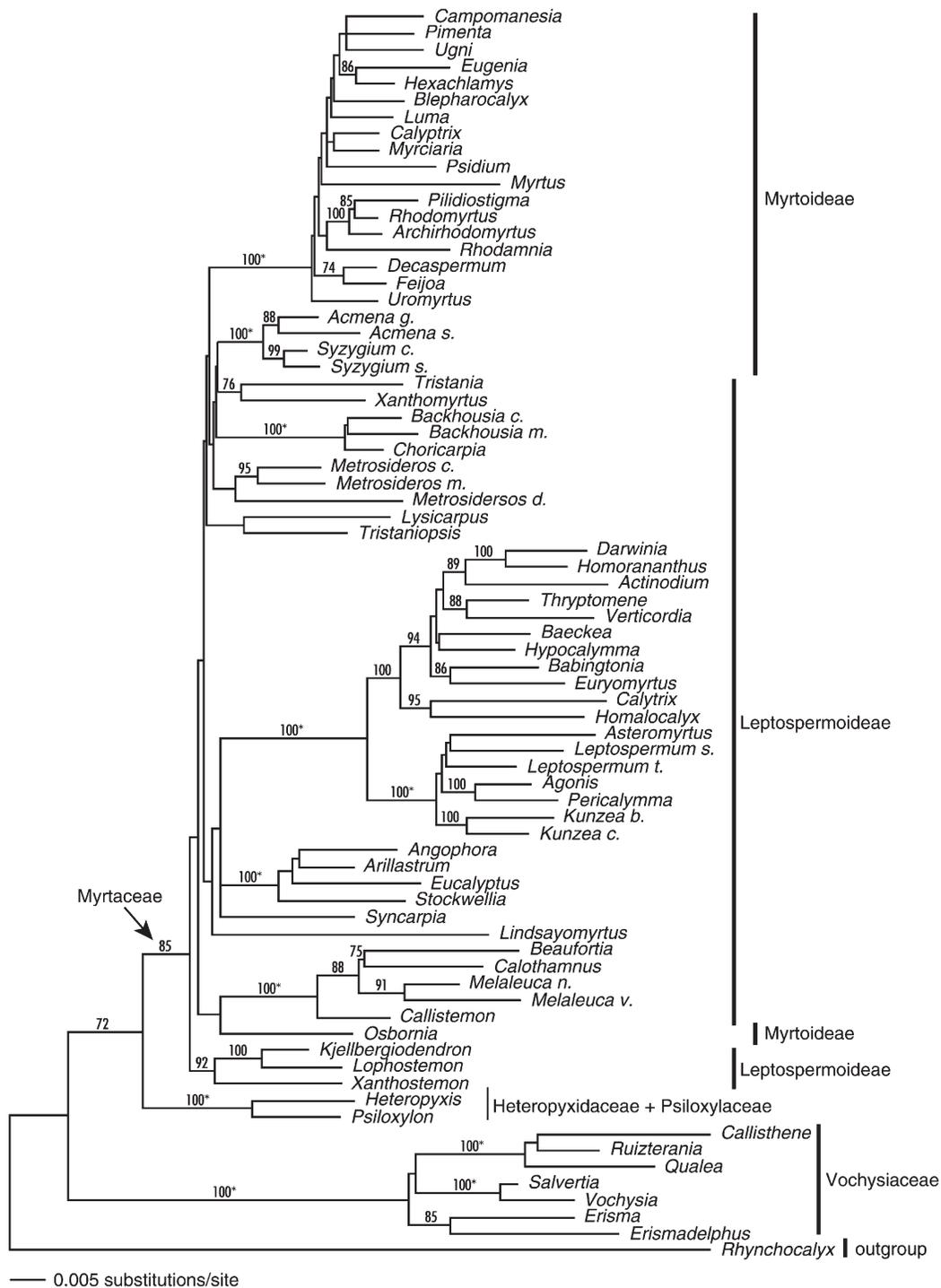
The ML analysis of Myrtaceae, Vochysiaceae, and relatives in the PHMV clade based on *ndhF* + *matK* sequences using GTR + G settings shows clear relationships among the four families in the PHMV clade but does not strongly resolve the basal lineages within Myrtaceae (fig. 4). The inclusion of Melastomataceae as outgroups (in addition to Rhynchochalcaceae of the CAROP clade) does not affect relationships within the PHMV clade (tree not shown). As in the previous Myrtales data set, the molecular clock is rejected in this focused survey of the PHMV clade using *ndhF* and *matK*. The ML tree reveals five important points about relationships within the PHMV clade: (1) The relationships of the four families are exactly the same as indicated by the broad Myrtales survey using *rbcL* and *ndhF*. Heteropyxidaceae and Psiloxylaceae are monophyletic and are sister to Myrtaceae and, with Vochysiaceae, are sister to these three families. (2) Within Vochysiaceae, the tribe Erismeeae, represented here with the South American *Erisma* and the tropical west African *Erismadelphus*, is clearly monophyletic. This clade in turn is sister to all other Vochysiaceae. (3) Although the backbone of the Myrtaceae is not strongly supported, the first diverging lineages are consistently obtained with *ndhF* and *matK* singly or in combination: the "Lophostemon" group diverges first, followed by the mangrove *Osbornia* and/or "Calothamnus-Melaleuca" group (fig. 5). (4) The

fleshy-fruited Myrtoid group is strongly supported as monophyletic and well derived within the family, making the subfamily Leptospermoideae paraphyletic. and (5) Fleshy, indehiscent fruits are derived at least three times, once in the Myrtoid group, once in the "Acmena" group, and once for *Osbornia*, making the subfamily Myrtoideae polyphyletic.

When geographical area is mapped onto the ML tree (fig. 5), three important results are seen: (1) The base of the PHMV clade is ambiguous for area with three major Gondwanan regions (South America, Africa, and Australasia) occurring in the basal lineages. This basal ambiguity in geographical area is mirrored in the outgroup CAROP clade (fig. 3), a result obtained whether the Melastomataceae are included or not. (2) The biogeographical shift within the Vochysiaceae between South America and Africa, whether due to dispersal or vicariance, occurs after the crown group node in Vochysiaceae has already diversified in South America. and (3) Despite the apparent rapid radiation of the major lineages within Myrtaceae, which are often strongly supported, there is a clear signal for the origin of the extant Myrtaceae within Australasia. Biogeographical shifts to South America (and to the Mediterranean and Africa) are more recent.

#### Clock Calibration and Lineage Dating in PHMV Clade

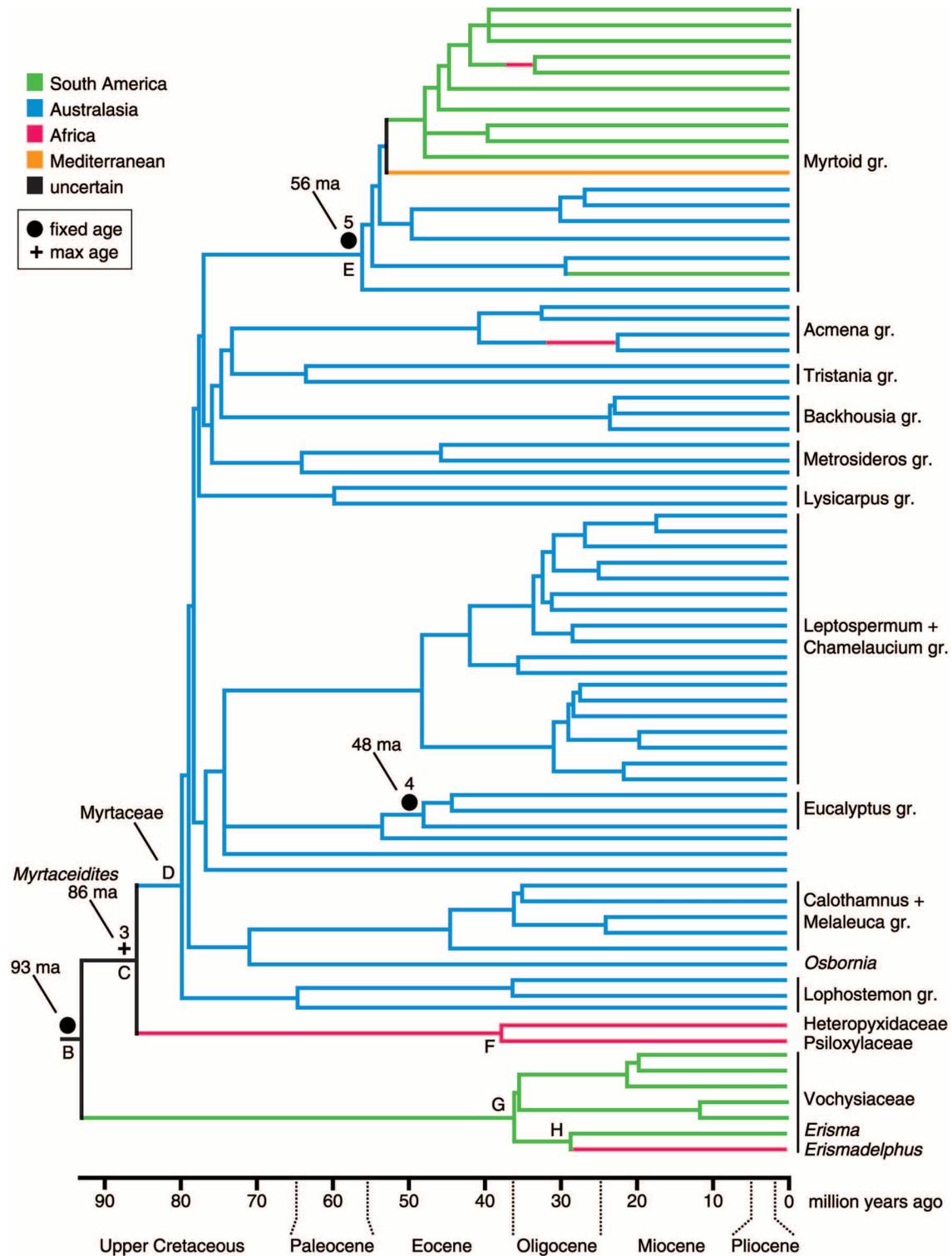
The PL chronogram for *ndhF* + *matK* (fig. 5) used a fixed 48 Ma date for the eucalyptoid fossil node; a fixed 56 Ma date for the base of Myrteae; a constrained maximum 86 Ma date for the MRCA of Myrtaceae, Heteropyxidaceae, and Psiloxylaceae (node C, fig. 5); and a fixed date of 93 Ma for the PHMV clade node based on the first *rbcL* + *ndhF* PL analysis. Dates of most nodes in the *ndhF* + *matK* chronogram are quite similar to those seen in the *rbcL* + *ndhF* chronogram except for the age of Myrtaceae, 80 versus 70 Ma, respectively (table 2). However, the *ndhF* + *matK* analysis has considerably greater sampling of Myrtaceae, and thus nodes D (defining the Myrtaceae crown group) in figures 3 and



**Fig. 4** Maximum likelihood tree depicting phylogenetic relationships within the PHMV clade based on *ndhF* and *matK* sequences. Subfamily designation for Myrtaceae based on Briggs and Johnson (1979) and Johnson and Briggs (1984). ML bootstrap values 70% or greater are above branches (those constrained in ML bootstrap runs are asterisked).

5 are not exactly the same. Both data sets provide similar dates for the separation of Heteropyxidaceae and Psiloxylaceae (node *F*; 40–38 Ma) in the late Eocene (table 2). The Vochysiaceae crown group divergence dates back to the Eocene-Oligocene boundary (node *G*; 36–33 Ma). The Afri-

can *Erismadelphus* and its American sister *Erisma* (both of tribe Erismeae) show a separation at 28 Ma (node *H*, fig. 5) near the end of the Oligocene, long after complete separation of tropical South America and west Africa (ca. 80 Ma; see below). The Myrtaceae crown group diverged at 70–80 Ma



**Fig. 5** Penalized likelihood chronogram of the PHMV clade based on *ndhF* and *matK* sequences. Information from three fossils (indicated by numbers) also used in the Myrtales-wide survey (fig. 3) was used; see text for details of implementation. The date of *Myrtaceidites* (86 Ma) was used as a maximum age constraint of node C. The date of the earliest eucalyptoid fossil (4) was used to fix the age of the node representing the most recent common ancestor of all eucalyptoid genera. The date of *Paleomyrtinaea* (56 Ma) was used to fix the age of node E representing the Myrtoids. The node representing the crown group of the PHMV clade was fixed at 93 Ma based on the Myrtales-wide analysis (fig. 3). Nodes identified by letter are discussed in the text. Areas of endemism at the continental scale for each clade are provided. The branches leading to *Eugenia* and *Syzygium* are indicated as polymorphic since both additionally contain African species (these not sampled). Informal “groups” within Myrtaceae follow the designations of Wilson et al. (2001).

(node *D*, figs. 3, 5; table 2). The Myrtoidean stem lineage is old (77 Ma), but the crown group diversified at 56 Ma (table 2) at the Paleocene-Eocene boundary. Separation of the Neotropical elements of Myrtaceae (excluding capsular-fruited *Tepaulia*, not sampled here) from the basal grade of Australasian Myrtoidean elements is less clear but occurred shortly thereafter (fig. 5).

### Discussion

This two-tiered approach at estimating phylogenetic placement and relationships within the biogeographically disjunct PHMV clade comprising Myrtaceae, Vochysiaceae, Heteropyxidaceae, and Psiloxylaceae provides four important conclusions on the phylogenetic relationships and biogeographic history of the clade: (1) The PHMV clade separated from its sister clade of Melastomataceae and relatives in the Albian of the Lower Cretaceous and the PHMV crown group diversified 12 m.yr. later around the Cenomanian-Turonian boundary of the Upper Cretaceous. (2) Vochysiaceae are moderately supported as the sister clade to the other three families in the PHMV clade, but extant genera of Vochysiaceae did not diversify until ca. 60 m.yr. later in the early Oligocene of the Tertiary, and thus the South American-African disjunction (28 Ma) must be from long-distance dispersal rather than vicariance. (3) The African/Mascarene endemic families, Heteropyxidaceae and Psiloxylaceae, are closely related, and they diverged from each other in the late Eocene. (4) The widespread Myrtaceae date back to the Campanian of the late Cretaceous, extant members clearly are Australasian in origin (basal lineages of the paraphyletic subfamily Leptospermoideae), and a more recent shift to America (including most members of the polyphyletic subfamily Myrtoideae) occurred in the early Eocene, perhaps via the Antarctic temperate land bridge.

#### *Biogeographical History of Myrtales and Origin of the PHMV Clade*

Phylogenetic relationships presented here using *rbcL* and *ndbF* expand on and clarify the emerging picture of familial relationships within and biogeography of Myrtales based only on *rbcL* (Conti et al. 1996, 1997, 2002). Probably an outcome of its ancient age, Myrtales have not been strongly placed within the Rosids and typically are placed outside both Eurosids I and II (APG 1998, 2003; Magallón et al. 1999; Savolainen et al. 2000; Soltis et al. 2000). Based on these analyses, the crown group of Myrtales (node *A*, fig. 3) began differentiation at 111 Ma during the early Albian of the Lower Cretaceous. This date is similar to the 107 Ma in the Albian estimated by Wikström et al. (2001) based on *rbcL* alone.

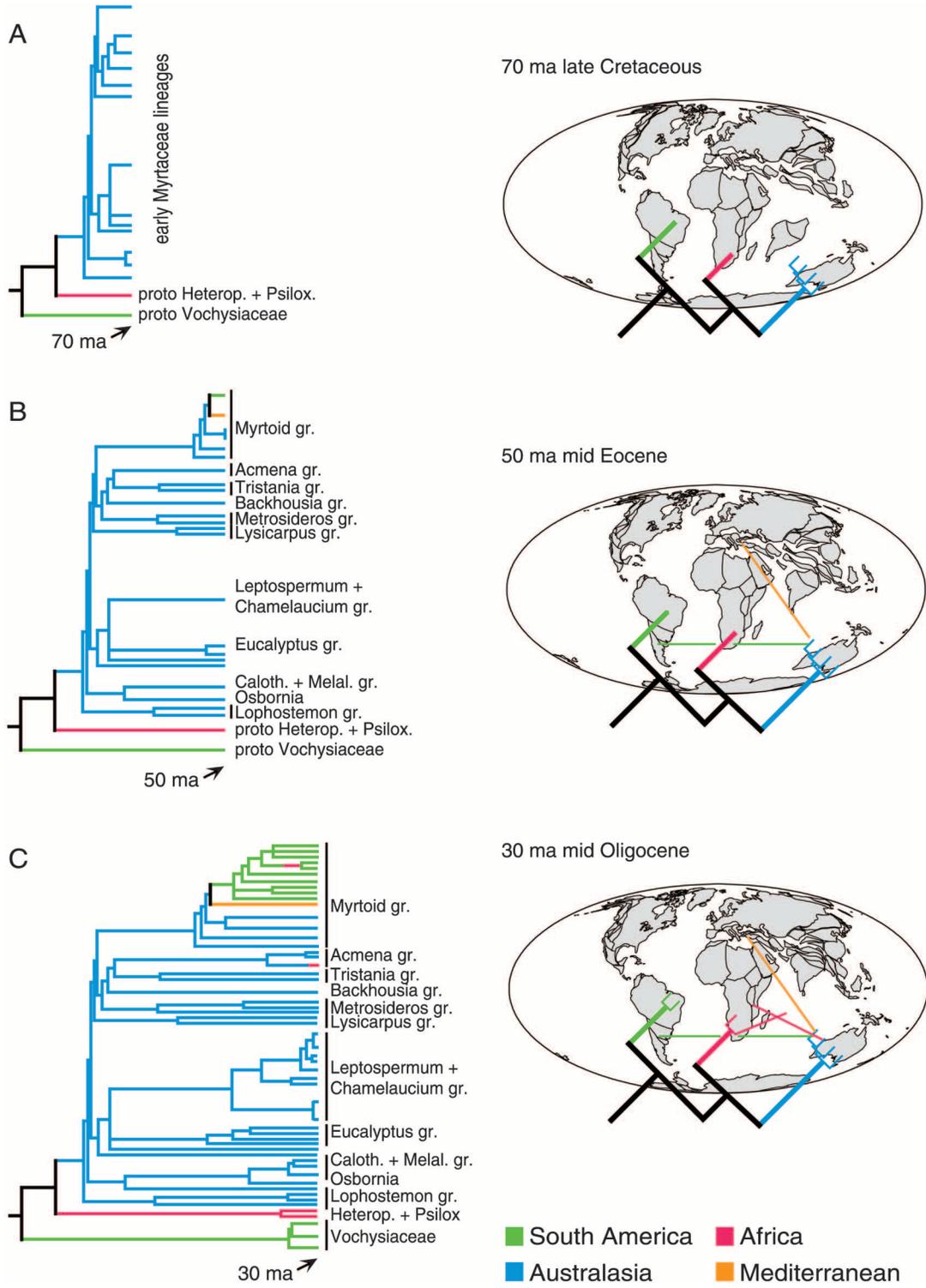
Combretaceae are shown now to represent the sister family to all other Myrtales with the ML analysis (figs. 2, 3, although not strongly supported), whereas in previous MP *rbcL* analyses the family was weakly linked as sister to the Lythraceae-Onagraceae clade. The combretaceous fossil *Esgueiria* is placed considerably earlier than the node defining the crown radiation of extant Combretaceae (fig. 3). This earlier occurrence is consistent with the issues (see above) of

placing *Esgueiria* in extant Combretaceae. Additionally, Combretaceae is the one family in Myrtales that has not been adequately sampled yet (e.g., *Strephonema* of subf. Strephonematoideae; Tan et al. 2002; Stace, in press), so the crown group radiation might actually be older and thus more consistent with *Esgueiria* dates. Of the remaining Myrtales sister to Combretaceae, two large clades diverged shortly thereafter (109 Ma): (1) PHMV + Melastomataceae s.l. + CAROP and (2) Lythraceae + Onagraceae. The separation of the PHMV clade from Melastomataceae and the CAROP clade occurred in the late Albian at 105 Ma. Onagraceae and Lythraceae are strongly supported as sister families; they diverged at 93 Ma near the Cenomanian-Turonian border.

Although the breakup of Gondwana into a western portion (South America, Africa) and an eastern portion (Antarctica, Australia, India, Madagascar) occurred prior to these early splits within Myrtales (180–150 Ma; Scotese et al. 1988; Scotese and McKerrow 1990; Scotese 1997; McLoughlin 2001; Briggs 2003), subsequent continental movements brought South America and Antarctica into contact and permitted direct west-east floral exchange well into the Tertiary (Hallam 1994). Evidence was previously presented for the role of the breakup of Gondwana in the diversification of the CAROP clade and subsequent rafting and dispersal out of the Indian subcontinent for Crypteroniaceae (Conti et al. 2002; Rutschmann et al. 2004). Renner et al. (2001) suggested with *ndbF* data that the diversification of Melastomataceae s.s. probably occurred during the Tertiary (Paleocene/Eocene) and in the north of the Tethys Sea (not Gondwanan), and their results imply a Melastomataceae s.s.–Memecylaceae split of 53 Ma and an earlier separation with the CAROP clade at ca. 73 Ma. These latter dates are consistent with our *rbcL* + *ndbF* results (56 and 82 Ma, respectively) and point toward Gondwanan separation events in the early formation of the PHMV clade and its sister lineage (fig. 6A).

#### *Vochysiaceae: Vicariance or Dispersal?*

The molecular phylogenetic relationships and clock calibration presented here (fig. 5) provide unambiguous resolution of the controversy involving the vicariance (Axelrod 1970) versus dispersalist (Thorne 1972, 1973; Smith 1973; Barthlott 1983) interpretations of the amphi-Atlantic disjunction for Vochysiaceae (fig. 1). Although Vochysiaceae represent the sister lineage to Myrtaceae, Heteropyxidaceae, and Psiloxylaceae, and this separation dates back to ca. 93 Ma in the Turonian of the Upper Cretaceous, the crown group of extant American and African genera did not diversify until ca. 60 m.yr. later in the early Oligocene of the Tertiary (36–33 Ma, based on PHMV or Myrtales data set calibrations, respectively). Inclusion of the African *Erismadelphus* in the second PHMV analysis (figs. 4, 5) provides a separation time of 28 Ma (late Oligocene) of this African genus from its sister *Erisma* in South America. The topology of the ML trees argues that South America is the plesiomorphic geographic area for Vochysiaceae, a finding maintained in *matK* analyses of many more taxa (Litt 1999; A. Litt, unpublished data, 2001). Although the second African genus *Korupodendron* was not available for DNA analysis, it is clearly more closely related to *Erismadelphus* in Africa than to other members of



**Fig. 6** Sequence of lineage diversification in the PHMV clade. *A*, Late Cretaceous; *B*, mid-Eocene; *C*, mid-Oligocene. Chronograms derived from penalized likelihood analyses (fig. 5). Stem lineages for extant families indicated by *proto*. Informal “groups” within Myrtaceae follow the designations of Wilson et al. (2001). Paleomaps showing relative placements of continents are based on Scotese (1997). Note that African Vochysiaceae have not differentiated yet by the mid-Oligocene (*C*) and that the lineage leading to the Mascarene endemic Psiloxylaceae is shown in paleomap *C* although these volcanic islands in their present form are considerably younger. Additional intercontinental movements not shown involve *Eugenia* (South America to Africa) and capsular *Tepaulia* (Australasia to South America).

Erismeeae in South America (Litt and Cheek 2002). Synapomorphies the African genera share with *Erisma* include petal number greater than one, five-winged fruit (vs. three-winged), and simple (vs. stellate) hairs. Thus, the nesting of African taxa within the South American Vochysiaceae rather than sister to all South American taxa is strong with both molecular and morphological data. Additionally, as the American-African lineage separation is on the order of ca. 28 Ma, the disjunct distribution must be attributed to dispersal over a considerable Atlantic water barrier rather than due to vicariance caused by disruption of a once more continuous American-African area of endemism (fig. 6C).

Raven and Axelrod (1974) argued that it appears likely that upwards of 80 large, fairly primitive to moderately specialized angiosperm families were widely distributed in tropical regions before the Gondwanan continents separated. According to Pittman et al. (1993), the beginning of the separation of Africa from South America occurred just before 135 Ma in the early Cretaceous (Valanginian). The early phase of rifting lasted from 135 Ma to ca. 106 Ma (middle Albian) but, due to the rotation of Africa away from South America, tropical west Africa was still in contact with northeastern South America. Complete separation of the two continents was achieved sometime between 106 and 84 Ma. Pittman et al. (1993) suggested that by between 100 and 95 Ma (Cenomanian), Africa and South America were effectively isolated from each other except to a few easily dispersed terrestrial organisms (Goldblatt 1993). The distance from the eastern bulge of Brazil to the nearest point in west Africa is 2900 km. Assuming an initial continental separation of tropical South America and Africa at 106 Ma and a steady rate of separation (27.4 km/m.yr.), the movement of Vochysiaceae to Africa would have entailed a long-distance dispersal event over 2100 km of water during the late Oligocene (fig. 6C).

Of the 12 or so families of flowering plants exhibiting the striking amphi-Atlantic disjunct pattern involving only the Americas and Africa, eight are essentially American families (e.g., Bromeliaceae, Cactaceae, Caricaceae, Humiriaceae, Loasaceae, Mayacaceae, Rapateaceae, Vochysiaceae) with ca. 3500 species in the Americas compared with just over 10 in Africa (Thorne 1973). Previously, Givnish et al. (2000, 2004) conclusively demonstrated with a molecular phylogenetic analysis of Rapateaceae that the single African genus *Maschocephalus* is of recent origin (late Miocene) via long-distance dispersal. Arguments have been made for similar young ages of African taxa in Bromeliaceae and Mayacaceae (Givnish et al. 1999, 2000, 2004) and perhaps the remainder of these eight families. Many of the families possess seed or fruit dispersal mechanisms likely to account for long-distance dispersal over water: fleshy fruits in *Rhizophora* (Cactaceae) for internal bird dispersal; sticky seeds in Caricaceae for external bird dispersal; small seeds in muddy or wet areas in Bromeliaceae, Mayacaceae, Rapateaceae for external bird dispersal; and drift fruits in Humiriaceae and Vochysiaceae (one species only) for water dispersal. The fruits of Vochysiaceae are either loculicidal capsules with few winged seeds (tribe Vochysieae) or samaroid with a single seed encased by enlarged sepals (tribe Erismeeae) (Kawasaki 1992). Of the latter, the fruit of one species of inundated Amazonian forests, *Eris-*

*meae calcaratum*, is further modified into a wingless, nutlike, and corky structure that is dispersed by water. Despite the lack of information on specific relationships of the African species to those in *Erisma* and on fruit type in the lineage leading to the African clade, the potential for long-distance dispersal exists in the tribe Erismeeae in which occurred the amphi-Atlantic dispersal event.

#### Historical Biogeography of Heteropyxidaceae and Psiloxylaceae

The strong phylogenetic affinity of Heteropyxidaceae and Psiloxylaceae seen in these analyses is consistent with previous MP results based solely on *rbcL* (Conti et al. 1996, 1997) or *matK* (Gadek et al. 1996; Wilson et al. 2001). The present ML results using combinations of *rbcL*, *ndhF*, and *matK* (figs. 2–5) strongly support these two families as sister to Myrtaceae, a relationship strongly supported (95% bootstrap) by *matK* alone (Wilson et al. 2001) but not *rbcL* alone (Conti et al. 1996, 1997); the latter could not resolve with high bootstrap support the polytomy of Vochysiaceae + (Heteropyxidaceae + Psiloxylaceae) + Myrtaceae. Cladistic analysis of morphological characters (Wilson et al. 2001) links Heteropyxidaceae and Psiloxylaceae to Myrtaceae by the presence of glands in the anther connective and of secretory cavities in leaves (followed by loss of essential oils in *Psiloxylon*). The phylogenetic results support the recognition of *Heteropyxis* and *Psiloxylon* as a clade within a more broadly defined Myrtaceae and sister to the Myrtaceae s.s. (Gadek et al. 1996). The APG (2003) continues to recognize all three families, although a single family is logically possible. The argument, however, could be made that if these two small families are merged into Myrtaceae, then a similar merging of the five small CAROP families should also occur, and perhaps also with Melastomataceae s.l.

The separation of *Heteropyxis* and *Psiloxylon* from the Myrtaceae lineage is old (86–84 Ma; fig. 6A). The restriction of *Heteropyxis* to southeast Africa (West Gondwana) versus the Australian or East Gondwanan nature of basal lineages in Myrtaceae (see below) indicates that this early separation might be Gondwanan. However, these dates are younger than typically are indicated for Gondwanan vicariance and might indicate dispersal over at least moderate ocean barriers. *Psiloxylon* is restricted to Mauritius and Réunion, part of the hot spot basalt volcanic Mascarene Island chain in the western Indian Ocean formed after the final breakup of India from Madagascar and Seychelles at ca. 65 Ma (Storey et al. 1995; Briggs 2003). The Mascarene Islands are situated in a line along a submerged ridge, the Seychelles-Mauritius Plateau, located 640–800 km east of Madagascar in the western Indian Ocean, with the oldest, Mauritius, probably no older than 8 m.yr. (Bonneville et al. 1988; Hantke and Scheidegger 1998). Since the divergence between *Heteropyxis* and *Psiloxylon* occurred between 40 and 38 Ma in the late Eocene (figs. 3, 5, 6C), the lineage leading to *Psiloxylon* must have occupied other areas for most of the Tertiary. There is the possibility that this lineage was situated on earlier islands that are now submersed, as has been argued for the ancestor of *Fuchsia* in Tahiti (Sytsma et al. 1991; Berry et al. 2004) and Hawaiian lobelioids (Givnish et al. 1995). The flora of the Mascarenes is highly endemic but exhibits ties mainly to

Africa and Madagascar (Renvoize 1979; D. Lorence, personal communication), although connections also are seen with Australasia and Polynesia (Carlquist 1974) and even the Neotropics (Graham 2002). Thus, the precise biogeographical history of these two monogeneric families remains obscure. The most likely scenario involves an early Gondwanan origin in Africa for the small clade, a dispersal event of unknown age (but no earlier than 40 Ma) of the *Psiloxylon* lineage to the volcanic islands forming along the Seychelles-Mauritius Plateau after the India-Madagascar-Seychelles separation of the early Tertiary and subsequent island hopping and older island extinction events (fig. 6C).

#### *Biogeographical History of the Amphi-Tropical Myrtaceae*

The molecular phylogenetic tree (figs. 4, 5) depicts a rapid radiation at the base of the family Myrtaceae with only the more recent lineages well supported and the paraphyly of subf. Leptospermoideae. The detailed phylogenetic results of the combined *ndbF* + *matK* data sets using MP and Bayesian analyses, along with morphological data, will be presented elsewhere, but they largely agree with that seen here with ML and with the MP analysis of only *matK* (Wilson et al. 2001). Despite the lack of strong support for basal relationships, the historical biogeographical results seen here (fig. 5) are strongly compelling for an origin of extant members of Myrtaceae in Australasia and more recent shifts to the Americas, Africa, the Mediterranean, with perhaps subsequent dispersals back to Australasia (fig. 6). The “Lophostemon” group (*Lophostemon*, *Xanthostemon*, *Kjellbergiodendron*), centered in northeast Australia, New Caledonia, New Guinea, and out to Sulawesi, is sister to the remainder of the family. The Australasian-distributed mangrove, *Osbornia*, and the largely Australian “*Calothamnus*-*Melaleuca*” group (e.g., *Calothamnus*, *Eremaea*, *Beaufortia*, *Melaleuca*, and *Callistemon*) are also early diverging lineages in Myrtaceae. Thus the largely capsular-fruited subf. Leptospermoideae is a paraphyletic grade at the base of the family (figs. 4, 5). In addition, as suggested by Johnson and Briggs (1984) based on a cladistic morphological analysis and Wilson et al. (2001) using only *matK*, the fleshy-fruited subf. Myrtoideae is polyphyletic with the Acmena group (*Acmena*, *Syzygium*, and related genera) unrelated to the Myrtoid group (figs. 4, 5).

Whether or not the base of the stem lineage for Myrtaceae is also Australasian is unclear. The closest relatives of Myrtaceae in the PHMV clade are African (or African derived), and thus an origin in either west or east Gondwana is possible (Wilson et al. 2001). This ambiguity is mirrored in the fossil record since the first fossil of myrtaceous type (including Heteropyxidaceae and Psiloxylaceae) is the Upper Cretaceous *Myrtacidites* from Africa (Gabon) but shortly thereafter also from South America (Colombia) and Asia (Borneo). This early pantropical distribution of the Myrtaceae/Heteropyxidaceae/Psiloxylaceae clade must have been followed by extinction in South America. Much of the diversification of the early diverging Australian lineages (including New Caledonia and New Guinea) of the Myrtaceae took place between 80 and 30 Ma (fig. 6A, 6B). This was a time

period of temperate connection with Antarctica (and thus to South America), followed by separation of the Australian plate from Antarctica, increasing aridification, and the rise of the distinctive scleromorphic/xeromorphic vegetation characterizing the late Miocene to the Recent of Australia (Christophel and Greenwood 1989; Hill 1994; White 1994).

Myrtaceae now occur in three areas outside the plesiomorphic area of Australasia: the Americas, Africa, and the Mediterranean (table 1; figs. 1, 6C). The Mediterranean endemic *Myrtus* appears to be a Tertiary dispersal event out of the Myrtoid group (fig. 5), discussed below. The African taxa comprise three unrelated groups: *Syzygium* of the Acmena group; *Eugenia* of the Myrtoid group; and “*Metrosideros*” *angustifolia* of uncertain placement. Thus, the occurrence of all extant Myrtaceae in Africa (except perhaps for the capsular-fruited “*Metrosideros*”) may all represent more recent dispersal events by fleshy-fruited taxa followed by species radiations. Recent long-distance dispersal via air currents of the small seeds of *Metrosideros* from Australasia to the Pacific islands has been argued with molecular data (Wright et al. 2000, 2001).

All Neotropical genera belong to the Myrtoid group (except for the capsular-fruited *Tepaulia* not sampled here), the largest fleshy-fruited lineage of the subf. Myrtoideae (*Myrcia*, *Myrtus*, *Cryptorhiza*, and *Eugenia* alliances; table 1). The stem lineage for the Myrtoid group spans ca. 20 m.yr. (77–56 Ma; fig. 5), a time when the temperate Australia/Antarctica/South American connection was still in contact (fig. 6B, 6C; see below). This portion of the Tertiary also experienced the height of the Cenozoic warm period and saw the incursion of a vast, climate-moderating epicontinental sea in North America and thus the extension of tropical and subtropical climates to high northern latitudes (50°N) (Graham 1993). The Myrtoid group radiated quickly ca. 56 Ma (figs. 4, 5) in both South America and Australasia, and relationships among the Neotropical and Australasian genera are not strongly supported (only *Eugenia* occurs in both areas). With the *ndbF* + *matK* data there is not enough resolution to address biogeographical relationships. Additional molecular markers are needed to verify the geographical pattern suggested by *ndbF* + *matK* data (figs. 5, 6C)—a paraphyletic Australasian Myrtoid group from which the American taxa diverged.

The Australasian-American disjunct pattern is consistent with a vicariance model based on geologically and fossil calibrated phylogenetic trees with some subsequent dispersal events occurring between Australasia and South America and Africa (*Tepaulia*, “*Metrosideros*” *angustifolia*, *Eugenia*, and *Syzygium*) (fig. 6). The stem lineage of the Myrtoid group diverged from Australasian lineages in the Campanian of the Upper Cretaceous (77 Ma) followed 20 m.yr. later by the rapid crown group radiation in both Australasia and the Americas (fig. 6B, 6C). Although tropical areas of Gondwana had already been separated for at least 20 m.yr. when the Myrtoid stem lineage is seen, warm temperate connections existed between South America and west Antarctica and between east Antarctica and Australia well into the Tertiary (fig. 6C; Hallam 1994; Scotese 1997; McLoughlin 2001). It is clear that neither the Drake Passage between Antarctica and South America nor the passage between Australia and

Antarctica was open at 40 Ma, but both passageways were open to middle and deep water circulation by the early Oligocene (32 Ma) (Lawver and Gahagan 1998; McLoughlin 2001). A widespread warm temperate Myrtoid stem lineage across these southern continents would have experienced vicariance at about the time the crown group quickly radiated in the earliest Eocene (56 Ma) or when the first American lineages are seen (48 Ma) (figs. 5, 6C). A similar warm temperate Antarctic link between South America and Australia/New Zealand explains the phylogenetic pattern, fossil (*Diporites*) dates, and distribution for *Fuchsia* (Onagraceae) (Berry et al. 1990, 2004; Sytsma et al. 1991).

#### Phylogenetic-Biogeographic Summary for the PHMV Clade

To summarize these phylogenetic and biogeographic events within the PHMV clade of four families, paleogeographic reconstructions of the continents are examined at three time intervals: late Cretaceous, 70 Ma (fig. 6A); mid-Eocene, 50 Ma (fig. 6B); and Oligocene, 30 Ma (fig. 6C). The relevant subclades (stem lineages and extant lineages) that have evolved at each time interval are presented using a consensus clock-calibrated phylogenetic tree for the PHMV clade. Prior to the Cretaceous/Tertiary boundary, 70 Ma (fig. 6A), Myrtales had already been diverging into at least four main clades for more than 40 m.yr. in concert with the separation of Gondwana. Of these, the PHMV clade had also been diverging for almost 20 m.yr. into the three lineages leading to Myrtaceae, to the African/Mascarene Island families, and to Vochysiaceae. At the Cretaceous/Tertiary boundary (fig. 6A), stem lineages are still present only for Vochysiaceae and the African/Mascarene Island families. However, the Myrtaceae are now diversifying into several Australasian groups, perhaps partly in response to the movement, beginning isolation, and aridification of Australia. The Myrtoid stem lineage has just separated from other early basal lineages.

During the mid-Eocene of the Tertiary at 50 Ma (fig. 6B), tropical regions are now quite in isolation from each other,

but a temperate connection between South America and Australia via the Antarctic temperate land bridge will be present for at least another 10 m.yr. The crown groups of extant Vochysiaceae and of Heteropyxidaceae and Psiloxylaceae still are not evident. Most of the major Australasian lineages of Leptospermoideae are now present and are actively diversifying. Two fleshy-fruited stem lineages in Myrtaceae are now seen—the *Acmena* and the Myrtoid groups. The Myrtoid crown group is differentiating in Australia, and the American and European lineages now appear. This may have involved a single overland migration of fleshy fruits to the Americas. Additional dispersal events to the Mediterranean (Myrtoid group) and Africa (*Acmena* group) occur from the Eocene on.

Finally, a majority of the genera sampled (at least their stem lineages) are present by the mid-Oligocene at 30 Ma (fig. 6C). The Myrtoid lineages of both the Neotropics and Australasia are almost fully differentiated. Heteropyxidaceae and Psiloxylaceae have just differentiated, presumably in Africa since the Mascarenes will not be available for colonization by Psiloxylaceae for some time. Only American lineages of Vochysiaceae are now evident, with the African lineage not to diverge for another 2 m.yr., well after the formation of a considerable Atlantic water barrier.

#### Acknowledgments

We are grateful to Chris Quinn, Marcelle O'Brien, and Margaret Heslewood for making available unpublished Myrtaceae *matK* sequences and to the Molecular Systematics Laboratory, Royal Botanic Gardens, Kew, for initial help in extraction of Vochysiaceae DNAs. Graphical support was provided by Kandis Elliot. This research was supported by NSF grants DEB 9407270 and 9981587 to K. J. Sytsma and by Australian Research Council grant A19602649 to P. G. Wilson and C. J. Quinn.

#### Appendix

Table A1

Species Names, Sources, and Genbank Accession Numbers of the DNA Sequences Used in the Analyses

Taxon	GenBank accession numbers			Voucher/source
	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	
Myrtaceae:				
<i>Acmena graveolens</i> (F.M. Bailey) L.S. Sm.		AY498759*	AF368194	Gadek s.n., UNSW (9)
<i>Acmena smithii</i> (Poir.) Merrill & Perry	U26315	AY498760*	AY525127*	Conti 104, WIS (3)
<i>Actinodium cunninghamii</i> Scahuer		AY498761*	AF489394	UNSW22989B (10)
<i>Agonis flexuosa</i> (Willd.) Sweet		AY498762*	AF184711	UNSW23029 (5)
<i>Angophora hispida</i> (Sm.) Blaxell	U26317	AY498763*	AF368196	Conti 109, WIS (3, 9)
<i>Archirhodomyrtus beckleri</i> (F. Muell.) A.J. Scott		AY498764*	AF368197	UNSW23517 (9)
<i>Arillastrum gummiferum</i> Panch ex. Baillon		AY498765*	AF368198	Weston 1635, NSW (9)
<i>Asteromyrtus lysicephala</i> (F. Muell. & F.M. Bail.) Craven		AY498766*	AF184718	UNSW23530 (5)
<i>Babingtonia tozerensis</i> A.R. Bean		AY498767*	AF368199	Wilson 1338, NSW (9)
<i>Backhousia citriodora</i> F. Muell.	U26318	AY498768*	AY525129*	Conti 110, WIS (3)
<i>Backhousia myrtifolia</i> Hook.		AY498769*	AF368200	UNSW22391 (4, 9)
<i>Baeckea frutescens</i> L.		AY498770*	AF489365	UNSW23529 (10)

Table A1

(Continued)

Taxon	GenBank accession numbers			Voucher/source
	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	
<i>Beaufortia orbifolia</i> F. Muell.		AY498771*	AY521530*	Conti s.n., WIS
<i>Blepharocalyx tweediei</i> O. Berg		AY498772*	AY521531*	Katinas 200, LP
<i>Callistemon polandii</i> F.M. Bail.		AY498773*	AF184705	Jacobs 5362, NSW (5)
<i>Calothamnus validus</i> S. Moore		AY498774*	AF184704	Wilson 1331, NSW (5)
<i>Calyptranthes pallens</i> Griseb.		AY498775*	AF368201	Sytsma s.n., WIS (9)
<i>Calytrix tetragona</i> Labill.		AY498776*	AF489396	UNSW21772 (4, 10)
<i>Campomanesia guazumifolia</i> (Chambess.) Berg		AY498777*	AY521532*	UNSW22385 (4)
<i>Choricarpia subargentea</i> (C.T. White) L.A.S. Johnson		AY498778*	AF368202	UNSW22896 (9)
<i>Darwinia fascicularis</i> Rudge		AY498779*	AF368204	Conti s.n., WIS (9)
<i>Decaspermum humile</i> (G. Don) A.J. Scott		AY498780*	AY521534	Conti s.n., WIS
<i>Eucalyptus curtisii</i> Blakely & C.T. White		AY498781*	AF368206	Conti s.n., WIS (9)
<i>Eugenia uniflora</i> L.	AF294255	AF15592	AF368207	(6, 9)
<i>Euryomyrtus ramosissima</i> (A. Cunn.) Trudgen	U26319	AY498782*	AF489376	Conti 103, WIS (3, 10)
<i>Feijoa sellowiana</i> Berg [Acca sellowiana (Berg) Burret]		AY498783*	AY525128*	Conti s.n., WIS
<i>Hexachlamys edulis</i> (Berg) Kausel & D. Legrand		AY498784*	AY525131*	Katinas 201, LP
<i>Homalocalyx aurea</i> (C.A. Gardner) Craven		AY498785*	AF489398	UNSW22947 (10)
<i>Homoranthus darwinioides</i> Cheel		AY498786*	AF489399	UNSW23267 (10)
<i>Hypocalymma linifolium</i> Turcz.		AY498787*	AF489379	UNSW22915 (10)
<i>Kjellbergiodendron celebicum</i> (Koord.) Merrill		AY498788*	AF368209	Zich s.n., NSW (9)
<i>Kunzea baxteri</i> (Klotzsch) Schauer		AY498789*	AF184722	Hardie 11, NSW (5)
<i>Kunzea capitata</i> (Smith) Heynh.		AY498790*	AF184723	Bruhl 1653, NSW (5)
<i>Leptospermum spectabile</i> J. Thompson		AY498791*	AY521539*	Conti s.n., WIS
<i>Leptospermum trinervium</i> (Smith) J. Thompson		AY498792*	AF184735	UNSW23515 (5)
<i>Lindsayomyrtus racemoides</i> (Greves) Craven		AY498793*	AF184706	Hill 2039, NSW (4, 5)
<i>Lophostemon confertus</i> (R. Br.) P.G. Wilson & J.T. Waterh.		AY498794*	AF184707	UNSW23606 (5)
<i>Luma apiculata</i> (DC.) Burret		AY498795*	AY521540*	Conti s.n., WIS
<i>Lysicarpus angustifolius</i> (Hook.) Druce		AY498796*	AF368210	Conti s.n., WIS (9)
<i>Melaleuca</i> aff. <i>nesophila</i> F. Muell.		AY498797*	AY525135*	Crayn 13, UNSW
<i>Melaleuca viridiflora</i> Gaertner		AY498798*	AF184708	Hind 616, NSW (5)
<i>Metrosideros carminea</i> W.R.B. Oliv.		AY498799*	AY521541*	UNSW23266
<i>Metrosideros diffusa</i> (G. Forst.) Sm.		AY498800*		Conti s.n., WIS
<i>Metrosideros diffusa</i> (G. Forst.) Sm.			AY521542*	Wright s.n., AK232791
<i>Metrosideros macropus</i> Hook. & Arn.		AY498801*	AF368212	Sytsma s.n., WIS (9)
<i>Metrosideros nervulosa</i> C. Moore & F. Muell.	AJ235785	AY498802*		Conti s.n., WIS
<i>Myrcianthes fragrans</i> (Sw.) McVaugh	U26328	AY498803*		Conti 108, WIS (3)
<i>Myrciaria vexator</i> McVaugh		AY498804*	AY521544*	Conti s.n., WIS
<i>Myrtus communis</i> L.	AF294254	AF215593	AY525136*	Sytsma 7205, WIS
<i>Osbornia octodonta</i> F. Muell.		AY498805*	AF368213	UNSW23593 (9)
<i>Pericalymma ellipticum</i> (Endl.) Schauer		AY498806*	AF184740	UNSW23002 (5)
<i>Pilidistigma papuanum</i> (Lauterb.) A.J. Scott		AY498807*	AF368214	Hill 2061, NSW (4, 9)
<i>Pimenta racemosa</i> (Mill.) J.W. Moore		AY498808*	AY521545*	Conti s.n., WIS
<i>Psidium guineense</i> Sw.		AY498809*		UNSW22388 (4)
<i>Psidium longipes</i> (Berg) McVaugh			AY521546*	Conti s.n., WIS
<i>Rhodammia argentea</i> Benth.		AY498810*	AF368217	UNSW22389 (4, 9)
<i>Rhodomyrtus macrocarpa</i> Benth.		AY498811*	AY525137*	Conti s.n., WIS
<i>Stockwellia quadrifida</i> D.J. Carr, S.G.M. Carr & B. Hyland		AY498812*	AY525138*	Wilson 1351, NSW
<i>Syncarpia glomulifera</i> (Sm.) Nied.		AY498813*	AF368220	UNSW23246 (9)
<i>Syzygium cumini</i> Skeels		AY498814*	AY525140*	Hahn 5897, WIS
<i>Syzygium samarangense</i> (Bl.) Merrill & Perry		AY498815*	AY525141*	Conti s.n., WIS
<i>Thryptomene saxicola</i> (Hook.) Schauer		AY498816*	AF184709	UNSW23856 (5)
<i>Tristania nerifolia</i> (Sims) R. Br.		AY498817*	AF368224	UNSW23243 (9)
<i>Tristaniopsis laurina</i> (Smith) P.G. Wilson & J.T. Waterh.		AY498818*	AF184710	UNSW22390 (5)
<i>Ugni molinae</i> Turcz.		AY498819*	AY525142*	Conti s.n., WIS
<i>Uromyrtus australis</i> A.J. Scott		AY498820*	AY527230*	Conti s.n., WIS
<i>Verticordia pennigera</i> Endl.		AY498821*	AF489402	Sytsma s.n., WIS (10)

Table A1

(Continued)

Taxon	GenBank accession numbers			Voucher/source
	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	
<i>Xanthomyrtus biengbenensis</i> Guillaumin		AY498822*		UNSW22387
<i>Xanthomyrtus papuana</i> Merrill & Perry			AF368226	(9)
<i>Xanthostemon chrysanthus</i> (F. Muell.) Benth.		AY498823*	AF368227	Weston 524, NSW (9)
Heteropyxidaceae:				
<i>Heteropyxis natalensis</i> Harv.	U26326	AY498824*	AF368208	Conti 1002, WIS (3, 9)
Psiloxylaceae:				
<i>Psiloxylon mauritianum</i> Baill.	U26333	AY498825*	AF368215	Conti 101, WIS (3, 9)
Vochysiaceae:				
<i>Callisthene major</i> Mart.		AY498826*		Litt 10, NY
<i>Callisthene fasciculata</i> Mart.			AY572436/7*	Litt s.n., NY
<i>Erisma floribunda</i> Rudge	U26324	AY498827*	AY572438/9*	Mori 22847, NYBG (3)
<i>Erismadelphus sessilis</i> Keay & Stafleu		AY498828*	AY572440/1*	Litt 19, NY
<i>Qualea grandiflora</i> Mart.			AF368216	(9)
<i>Qualea</i> sp.	U02730	AY498829*		(1)
<i>Ruizterania cassiquiarensis</i> (Spruce ex Warm.) Marc.-Berti		AY498830*	AY572442/3*	Rib 1473, NY
<i>Salvertia convallariaeodora</i> A.St.Hil.		AY498831*	AY572444/5*	Litt 15, NY
<i>Vochysia hondurensis</i> Sprague	U26340	AY498832*	AY572446*	Iltis s.n., WIS (3)
CAROP:				
<i>Alzatea verticillata</i> Ruiz & Pav.	U26316	AF215591		Conti 107, WIS (3, 6, 8)
<i>Crypteronia paniculata</i> Blume	AY078153	AY498833*		Chase 1235, K (11, 13)
<i>Olinia ventosa</i> (L.) Cuf.	AF215525	AF215594		(6, 8)
<i>Penaea mucronata</i> L.	AY078155	AF270756		(6, 8, 11)
<i>Rhynchochalyx lawsonioides</i> Oliver	U26336	AF270757	AF368218	(3, 6, 9)
Melastomataceae:				
<i>Amphiblemma cymoum</i> (Schr. & Wendl.) Naudin	AF215543	AF215588		(6, 8)
<i>Arthrostemma ciliatum</i> Pavón ex D. Don	AF215522	AF215562		(6)
<i>Astronia macrophylla</i> Bl.	AF215510	AF215548		(6, 8)
<i>Astronia smilacifolia</i> Triana ex C.B. Clarke	AF215511	AF215549		(6, 8)
<i>Bertolonia maculata</i> DC.	AF215512	AF215550		(6, 8)
<i>Blakea trinervis</i> L.	AF215516	AF215555		(6, 8)
<i>Calvoa orientalis</i> Taub.	AF215544	AF215589		(6, 8)
<i>Clidemia rubra</i> (Aubl.) Mart.	AF215535	AF215579		(6, 8)
<i>Dichaetanthera asperrima</i> Cogn.	AF215523	AF215564		(6, 8)
<i>Diplectria divaricata</i> (Willd.) O. Ktze.	AF270746	AF215556		(6, 8)
<i>Dissotis rotundifolia</i> (Sm.) Triana	U26323	AF215565		Conti s.n., WIS (3, 6, 8)
<i>Driessenia glanduligera</i> Stapf.	AF270749	AF215586		(6, 8)
<i>Graffenrieda rotundifolia</i> (Bonpl.) DC.	AF215532	AF215576		(6, 8)
<i>Heterocentron subtriplinerviium</i> (Link & Otto) A.	AF270747	AF215566		(6, 8)
Brown & Bouché				
<i>Lavoisiera cordata</i> Cogn.	AF215540	AF215582		(6, 8)
<i>Leandra mexicana</i> (Naud.) Cogn.	AF215536	AF215580		(6, 8)
<i>Macrocentrum repens</i> (Gleason) Wurdack	AF215513	AF324498		(6, 8)
<i>Maieta guianensis</i> Aubl.	AF215537	AF215581		(6, 8)
<i>Medinilla humbertiana</i> H. Perrier	AF215517	AF215557		(6, 8)
<i>Melastoma malabathricum</i> L.	AF270748	AF272810		(6, 7)
<i>Meriania nobilis</i> Triana	AF215533	AF215577		(6, 8)
<i>Microlicia fasciculata</i> Cogn.	AF215541	AF215583		(6, 8)
<i>Monochaetum calcaratum</i> (DC.) Triana	AF215524	AF215568		(6, 8)
<i>Monolena primuliflora</i> J.D. Hooker	AF215514	AF215553		(6, 8)
<i>Osbeckia stellata</i> Wall.	U26330	AF272818		Conti 1004, WIS (3, 7, 8)
<i>Pternandra caeruleascens</i> Jack	AF215518	AF215558		(6, 8)
<i>Rhexia virginica</i> L.	U26334	AF215587		Post s.n., WIS; (3, 6, 8)
<i>Rhynchanthera grandiflora</i> (Aubl.) DC.	AF215542	AF215584		(6, 8)
<i>Tetrazygia urbanii</i> Cogn.	AF215538	AF270753		(6, 8)
<i>Tibouchina urvilleana</i> (DC.) Cogn.	U26339	AF272820		Goldblatt s.n., WIS (3, 8)
<i>Tococa guianensis</i> Aubl.		AY498834*		Almeda 7494; CAS
<i>Tococa rotundifolia</i> (Triana) Wurdack	AF215539			(6)
<i>Triolena obliqua</i> (Triana) Wurdack	AF215518	AF215558		(6, 8)

Table A1

(Continued)

Taxon	GenBank accession numbers			Voucher/source
	<i>rbcL</i>	<i>ndbF</i>	<i>matK</i>	
Memecylaceae:				
<i>Memecylon edule</i> Roxb.	AF215515	AF215574		(6, 8)
<i>Mouriri cyphocarpa</i> Standl.	U26327			Nepokroeff & Hammel 764, WIS (3)
<i>Mouriri guianensis</i> Aubl.		AF215575		(6)
Onagraceae:				
<i>Calylophus hartwegii</i> (Benth.) P.H. Raven	AF495767	AF495790		(12)
<i>Camissonia boothii</i> (Douglas) P.H. Raven	AF495766	AF495789		(12)
<i>Chamerion angustifolium</i> (L.) Holub	L10217	AF495784		(2, 12)
<i>Circaea alpina</i> L.	L10216	AF495780		(2, 12)
<i>Clarkia xantiana</i> A. Gray	L10225	AF495787		(2, 12)
<i>Epilobium rigidum</i> Hausskn.	AF495763	AF495785		(12)
<i>Fuchsia cyrtandroides</i> J.W. Moore	L10220	AF495779		(2, 12)
<i>Gaura mutabilis</i> Cav.	AF495769	AF495792		(12)
<i>Gayophytum heterozygum</i> F.H. Lewis & Szweyk.	AF495765	AF495788		(12)
<i>Gongylocarpus fruticulosus</i> (Benth.) Brandegees	AF495762	AF495783		(12)
<i>Hauya elegans</i> DC.	L10227	AF495778		(2, 12)
<i>Lopezia riesenbachia</i> Plitmann, P.H. Raven &	L10219	AF495781		(2, 12)
Breedlove				
<i>Ludwigia peruviana</i> (L.) H. Hara	L10221	AF495777		Zardini & Gentry 2206, MO (2, 12)
<i>Megacorax graciellana</i> Gonzalez & Wagner	AF495774	AF495797		(12)
<i>Oenothera elata</i> Kunth	NC002693	NC002693		(2, 12)
<i>Stenosiphon linifolius</i> (Nutt.) Heynh.	AF495768	AF495791		(12)
<i>Xylonagra arborea</i> (Kellogg) Donn. Sm. & Rose	AF495764	AF495786		(12)
Lythraceae:				
<i>Duabanga moluccana</i> Blume	AY496862*	AY498835*		Chai s.n.
<i>Cuphea llavea</i> Lex.	AF495773	AF495796		(12)
<i>Lawsonia inermis</i> L.	AY496863*	AY498836*		Correll 45915, TEX
<i>Lythrum salicaria</i> L.	AF495760	AF495775		(12)
<i>Nesaea aspera</i> (Guill. & Perr.) Koehne	AY496864*	AY498837*		Drummond 11446, KE
<i>Trapa natans</i> L.	L10226	AY498838*		Graham 1102, KE (2)
Combretaceae:				
<i>Bucida macrostachya</i> Standl.	U26321	AY498839*		Conti 111, WIS (3)
<i>Conocarpus erectus</i> L.	AF281477	AY498840*		Conti s.n., WIS
<i>Quisqualis indica</i> L.	L01948	AY498841*		Anderson s.n., MICH (1)
<i>Terminalia catappa</i> L.	U26338	AY498842*		Conti 103, WIS (3)
Brassicaceae:				
<i>Arabidopsis thaliana</i> (L.) Heynh.	NC000932	NC000932		
Tropaeolaceae:				
<i>Tropaeolum majus</i> L.	L14706	AJ236281		
Malvaceae:				
<i>Byttneria aculeata</i> (Jacq.) Jacq.	AF022123	AF111775		
<i>Tilia americana</i> L.	AF022127	AF111760		

Source. 1 = Chase et al. 1993; 2 = Conti et al. 1993; 3 = Conti et al. 1996; 4 = Gadek et al. 1996; 5 = O'Brien et al. 2000; 6 = Clausen and Renner 2001; 7 = Renner and Meyer 2001; 8 = Renner et al. 2001; 9 = Wilson et al. 2001; 10 = Lam et al. 2002; 11 = Conti et al. 2002; 12 = Levin et al. 2003; 13 = Rutschmann et al. 2004.

Note. Newly generated sequences are indicated by asterisks.

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