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A PHYLOGENETIC ANALYSIS OF RHAMNACEAE USING *RBCL* AND *TRNL-F* PLASTID DNA SEQUENCES[1]

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Previous tribal classifications of Rhamnaceae have been based on fruit characters, resulting in the delimitation of large and otherwise heterogeneous groups. We evaluated the most recent classification with DNA sequences of two regions of the plastid genome, *rbcL* and *trnL-F,* from 42 genera of Rhamnaceae and representatives of the related families Elaeagnaceae, Barbeyaceae, Dirachmaceae, Urticaceae, Ulmaceae, Moraceae, and Rosaceae. The *trnL-F* trees have higher consistency and retention indices than the *rbcL* trees, and patterns of change in *rbcL* and *trnL-F* are compared. The closest relatives of Rhamnaceae are Dirachmaceae and Barbeyaceae, followed by the urticalean families. The plastid trees support the monophyly of the family and provide the basis for a new tribal classification. Three strongly supported clades are identified, but morphological characters could not be found to underpin a formal taxonomic description of these three clades as subfamilies. We therefore only recognize groups that are also defined by morphological characters. The biogeography of Rhamnaceae is discussed with reference to the molecular trees.

Key words: molecular phylogeny; *rbcL*; Rhamnaceae; *trnL-F*.

Rhamnaceae are a cosmopolitan family of trees, shrubs, climbers, and one herb consisting of ~50 genera and ~900 species. They are characterized by flowers with petal-opposed stamens (obhaplostemony) and a tendency towards xeromorphism. Obhaplostemony is a relatively rare feature in angiosperms, and this has resulted in Rhamnaceae being associated with other families such as Vitaceae and Cornaceae exhibiting this arrangement. The xeromorphic adaptations exhibited by some members of the family include reduced or absent leaves, crowding of leaves, shortening of branch axes, presence of thorns or spines, and a low, shrubby habit. There are few plants of economic value in Rhamnaceae, the most notable being the jujube (*Ziziphus jujuba*), a fruit tree, and the ornamental shrubs *Ceanothus* and *Colletia.*

The history of the taxonomic relationships of Rhamnaceae is presented in Table 1. Two patterns have generally been followed: either Rhamnaceae have been placed with groups such as Vitaceae on the basis of shared floral features (Takhtajan, 1980; Cronquist, 1988) or with Elaeagnaceae on the basis of shared vegetative characteristics (Thorne, 1992; Takhtajan, 1997).

Previous suprageneric classifications of Rhamnaceae are summarized in a tribal revision by Richardson et al. (2000). The most recent revision of the entire family by Suessenguth (1953) recognized Hooker's (1862) five tribes and included a total of 58 genera and 984 species. A summary of Suessenguth's system is presented in Table 2. Prior to this study Rhamnaceae comprised 49 genera (a few genera have been described subsequent to Suessenguth's treatment and others are now treated as congeneric; see Richardson et al., 2000). The suprageneric or tribal classification of Rhamnaceae had been based largely on fruit characters, which in Suessenguth's system resulted in the circumscription of two large and otherwise heterogeneous tribes, Rhamneae Hook. f. and Zizipheae Brongn. (= Paliureae Reiss. ex Endl.).

An example of this heterogeneity can be found when comparing the genera *Ziziphus* and *Berchemia,* which were placed in the tribe Zizipheae because they both have drupaceous fruits. These two genera differ in a number of other characters, such as ovary position and leaf venation, which indicate relationships to genera in other tribes. Some of the tribes recognized by Suessenguth, Colletieae Reiss. ex Endl., Gouanieae Reiss. ex Endl. and Ventilagineae Hook. f., appear to be morphologically homogeneous and thus more likely to be monophyletic.

An analysis of sequences of the plastid gene *rbcL* for 499 species of angiosperms (Chase et al., 1993) showed that Rhamnaceae are part of a weakly supported group also containing Rosaceae, Urticales, and Fagales. Further studies using *rbcL* (Soltis et al., 1995) indicated a close relationship between Elaeagnaceae and Rhamnaceae. Studies using 18S nuclear ribosomal DNA, *atpB* and *rbcL* sequence data (Soltis et al., 1997; Savolainen et al., 2000) have supported the link between Rhamnaceae and Elaeagnaceae. Sequence data for a plastid noncoding region have placed Barbeyaceae and Dirachmaceae in association with Rhamnaceae (Thulin et al., 1998). The occurrence of nitrogen-fixing symbioses in some Rhamnaceae, Elaeagnaceae, Ulmaceae, and Rosaceae offers further support for a close relationship among these families (Soltis et al., 1995; Swensen, 1996).

Taxa from the above families were included in this analysis

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TABLE 1. A suprafamilial taxonomic history of Rhamnaceae.

Author	Order	Families
Hutchinson (1959)	Rhamnales	Rhamnaceae, Heteropyxidaceae, Elaeagnaceae, Vitaceae
	Urticales	Barbeyaceae
	Tiliales	Dirachmaceae
Takhtajan (1980)	Rhamnales	Rhamnaceae, Vitaceae, Leeaceae
	Elaeagnales	Elaeagnaceae
	Barbeyales	Barbeyaceae close to Hammamelidales
	Geraniales	Dirachmoideae, a subfamily of Geraniaceae
Cronquist (1988)	Rhamnales	Rhamnaceae, Vitaceae, Leeaceae
	Proteales	Elaeagnaceae, Proteaceae
	Urticales	Urticaceae, Ulmaceae, Cannabaceae, Moraceae, Cecropiaceae, Barbeyaceae
	Geraniales	Dirachmaceae
Thorne (1992)	Rhamnales	Rhamnaceae, Elaeagnaceae
	Geraniales	Dirachmaceae—as Dirachmoideae, a subfamily of Geraniaceae
	Incertae sedis	Barbeyaceae
Takhtajan (1997)	Barbeyales as superorder Barbeyanae	Barbeyaceae
	Malvales	Dirachmaceae
	Rhamnales in superorder Rhamnanae	Rhamnaceae
	Elaeagnales in superorder Rhamnanae	Elaeagnaceae

in an attempt to refine ideas about relationships among them as well as among genera within Rhamnaceae. Sequences were obtained from two regions of the plastid genome for 66 taxa in Rhamnaceae and related families. Sequence data from *rbcL* have been widely applied at the intrafamilial level, for example in Dipsacales (Donoghue, 1992), Geraniaceae (Price and Palmer, 1993), Cornaceae (Xiang et al., 1993), Saxifragaceae sensu stricto (Soltis et al., 1993), Rosaceae (Morgan, Soltis, and Robertson, 1994), Droseraceae (Williams, Albert, and Chase, 1994), Zygophyllaceae (Sheahan and Chase, 1996), Themidaceae (Fay and Chase, 1996), Lecythidaceae (Morton et al., 1997), and Plumbaginaceae (Lledó et al., 1998). We have extended the sampling for the *trnL* (UAA) 5′ intron and the intergenic spacer between the *trnL* (UAA) 3′ exon and *trnF* (GAA; Taberlet et al., 1991) from that of Thulin et al. (1998). This region, subsequently referred to as *trnL-F,* has been used in suprageneric phylogenetic analysis of Asparagales (Fay et al., 2000) and Haemodoraceae (Hopper et al., 1999). The results of the combined analysis of these data were used in part to redefine the suprageneric classification of Rhamnaceae (Richardson et al., 2000). A discussion of the comparative utility in phylogenetic analysis and aspects of the molecular evolution of *rbcL* and *trnL-F* data are also presented.

TABLE 2. Summary of Suessenguth's (1953) classification of Rhamnaceae.

Tribe	Genera
Colletieae	*Adolphia, Colletia, Discaria, Kentrothamnus, Retanilla, Talguenea, Trevoa*
Gouanieae	*Crumenaria, Gouania, Helinus, Pleuranthodes, Reissekia*
Rhamneae	*Ampelozizphus, Alphitonia, Ceanothus, Colubrina, Cormonema, Cryptandra, Emmenosperma, Hovenia, Hybosperma, Lasiodiscus, Macrorhamnus, Nesiota, Noltea, Oreorhamnus, Phylica, Pomaderris, Rhamnus, Sageretia, Schistocarpaea, Scutia, Siegfriedia, Spyridium, Trymalium, Tzellemtinia*
Ventilagineae	*Smythea, Ventilago*
Zizipheae	*Auerodendron, Berchemia, Berchemiella, Chaydaia, Condalia, Condaliopsis, Dallachya, Doerpfeldia, Lamellisepalum, Microrhamnus, Karwinskia, Krugiodendron, Maesopsis, Paliurus, Phyllogeiton, Reynosia, Rhamnella, Rhamnidium, Sarcomphalus, Ziziphus*

MATERIALS AND METHODS

Materials for molecular analysis—Sources of plant material and vouchers used in this analysis are listed in Table 3. Forty-two genera of Rhamnaceae were sampled, including at least one representative of each of Suessenguth's five tribes. All genera of Elaeagnaceae, Barbeyaceae, and Dirachmaceae and nine genera from Urticales and Rosaceae were also included. Rosaceae were chosen as the ultimate outgroup because earlier molecular analyses (Chase et al., 1993; Soltis et al., 1995; Thulin et al., 1998) had shown this family to be the sister of the rest.

DNA extraction—DNA was extracted from ~1.0 g fresh, 0.2–0.25 g silica gel dried leaves or 0.1–0.2 g of material from herbarium sheets using a 2X CTAB (hexadecyltrimethylammonium bromide) method modified from Doyle and Doyle (1987); DNA was precipitated using isopropanol instead of ethanol because it was found to be more reliable for Rhamnaceae, and DNA extracted from herbarium material was left to precipitate for at least 3 wk at −20°C (Fay et al., 1998) because this has been shown to give better yields. The reasons for this are unclear, but it could be due to the presence of altered secondary compounds that form as a result of the degradation associated with drying (making the DNA more difficult to precipitate) or simply because the DNA from herbarium specimens is degraded and therefore takes longer to precipitate. DNA was extracted from herbarium specimens for 21 of the 66 taxa. All DNA samples were purified on cesium chloride/ethidium bromide gradients (1.55 g/mL).

Gene amplification and purification—For most taxa, amplification of the *rbcL* exon was carried out in two overlapping halves using forward primers beginning at positions 1 and 636 and reverse primers beginning at position 724 and at a downstream ribosomal control site (Fay et al., 1998). DNA from some herbarium specimens had to be amplified in shorter pieces using forward primers beginning at positions 636 and 895 and reverse primers beginning at position 1024 and the downstream site. Amplification of the *trnL-F* region (Taberlet et al., 1991) was carried out using the forward primer c and the reverse primer f, and some taxa had to be amplified in shorter pieces using the primer pairs c and d and e and f. The d and e primers are exact complements so these sequences have a 20 base-pair (bp) gap where the primer site

is located. Amplification products were purified using Magic mini-columns (Promega, Southampton, Hampshire, UK) or QIAquick columns (Qiagen, Crawley, West Sussex, UK), following protocols provided by the manufacturers.

DNA sequencing—Standard dideoxy methods using S^{35} (for 12 *rbcL* sequences) or modified dideoxy cycle sequencing with dye terminators run on an ABI 373A or 377 automated sequencer (according to the manufacturer's protocols; Applied Biosystems, Inc., Warrington, Cheshire, UK) were used to sequence the amplification products directly. Automated sequence output files were edited and assembled using Sequence Navigator and Autoassembler (Applied Biosystems Inc.). All sequences have been submitted to GenBank (for accession numbers, see Table 3).

Sequence alignment—Alignment of *rbcL* sequences was easily performed manually because of the absence of insertions or deletions. An initial alignment was performed for five *trnL-F* sequences using Clustal version 1.61 (Higgins, Bleasby and Fuchs, 1992). Subsequent sequences were aligned manually (aligned matrices available at http://www.botany.org/bsa/ajbsupp/v86/s01-01.html and from the first and last authors j.richardson@rbge.org.uk/m.chase@rbgkew.org.uk).

After alignment of the *trnL-F* matrix, a matrix of insertion/deletion characters was prepared (characters were coded as present or absent). These characters were given weight equal to that of all single characters in the matrix. A large deletion can mask other smaller deletions, and taxa that have these larger deletions are coded as unknown for deletions that occur entirely within them. For example, there is a deletion between positions 891 and 941 for some taxa, whereas in other taxa there are smaller deletions between these positions, which we have coded as missing for taxa with the larger deletion.

We used 1399 *rbcL* characters and 1258 *trnL-F* characters. The ends were clipped from the sequences to remove primer sites (i.e., 20 bp from beginning of *rbcL,* 24 bp from the beginning and 28 bp from the end of *trnL-F*). Two regions of 59 and 16 bp of the *trnL-F* matrix were too ambiguous to be confidently aligned and so were excluded from all analyses.

Phylogenetic analysis—Data were analyzed using the parsimony algorithm of the software package PAUP version 3.1.1 for Macintosh (Swofford, 1993). Searches were conducted on the separate *rbcL* and *trnL-F* data sets (which included the matrix of 16 *trnL-F* indel characters) and on both data sets combined. Tree searches were conducted under the equal and unordered weights criterion (Fitch parsimony; Fitch, 1971) with 1000 random sequence additions and TBR (tree bisection-reconnection) swapping, but permitting only five trees to be held at each step. The limit on the number of trees held at each step was implemented to cut down the time spent searching on suboptimal trees. All shortest trees collected in the 1000 replicates were then used as starting trees for another round of heuristic search, and all these trees were swapped on to completion or swapped on until 6000 trees were produced, at which point we limited the number of trees and swapped on the 6000 trees collected. This search strategy should find islands of equally parsimonious trees (Maddison, 1991). To investigate the existence of islands, we then used one of the shortest trees as a starting tree and if all 6000 trees found produced the same strict consensus tree, then we concluded that only one island of trees was present (all shortest trees appeared to be from one island, so this topic will not be discussed further).

Successive approximations weighting (SW; Farris, 1969) was then carried out on these trees with a limit of ten trees per replicate and ten replicates per round. The trees collected in the ten replicates were then swapped to completion (or until we reached 6000 trees, as above) before the next round of weighting was implemented. Further rounds of SW continued until tree lengths were the same in two consecutive rounds. Characters were reweighted according to their rescaled consistency indices (RC), with a base weight of 1000. This procedure was designed to downweight or eliminate characters that were highly homoplasious.

One thousand replicates of the bootstrap (Felsenstein, 1985) were then carried out with the successive weights applied. We applied the following scheme of support: bootstrap values of 50–74% represent weak support, 75–84% moderate support, and 85–100% strong support. Unless otherwise noted, we use the word "support" to mean internal support, in this case estimated by the bootstrap.

MacClade version 3.05 (Maddison and Maddison, 1992) was used to calculate the number of steps and consistency index (CI) and retention index (RI) for each codon position in the *rbcL* analysis (Table 4), and CI and RI values of indel characters from the *trnL-F* matrix (Table 5). MacClade was also used to plot the number of unambiguous steps per character optimized on the most parsimonious SW tree from the combined analysis and the number of characters per number of steps on both the *trnL-F* and *rbcL* trees. The Fitch CI and RI were each calculated for transitions and transversions on the SW tree of the combined analysis using a step matrix in which transitions were weighted to zero, thus calculating the CI and RIs of transversions on the combined tree, and from these we calculated the number and CI/RI of transitions (Table 6). Tree lengths for all matrices are given in Table 7.

RESULTS

Analysis of rbcL data—The *rbcL* data matrix had 474 variable characters and 270 potentially informative characters out of a total of 1399 characters used, i.e., 19% of characters were variable in two or more taxa. The heuristic search under the Fitch criterion produced >6000 equally parsimonious trees with a length of 1174 steps. The CI for these trees was 0.52 (0.39 excluding autapomorphies) and the RI was 0.66. With SW, there were seven trees with a length of 423 378 steps, CI was 0.84 (0.63 excluding autapomorphies), and RI was 0.86. The Fitch length for these trees was also 1174 steps, i.e., the weighted trees were a subset of the Fitch trees from the same island. Figure 1 shows one of the SW trees with its Fitch branch lengths (ACCTRAN optimization) above the branches and SW bootstrap percentages below; branches that collapse in the strict consensus tree of the Fitch analysis are marked with a solid arrow, and those not present in the strict consensus of the weighted trees are marked with an open arrow.

The trees indicate that Rhamnaceae are not a monophyletic group because Elaeagnaceae, Barbeyaceae, and Dirachmaceae are all nested within them (Fig. 1). The sister group to this clade includes members of the families Moraceae, Ulmaceae, and Cannabaceae. However, there is little morphological evidence to indicate that Elaeagnaceae, Dirachmaceae, and Barbeyaceae should be included within Rhamnaceae, and bootstrap support for this grouping is low. Tribes Rhamneae and Zizipheae Brongn. are paraphyletic, but Colletieae are potentially a strongly supported monophyletic group (the other genera in this tribe need to be sampled to confirm monophyly of this group), and Gouanieae, with the exception of *Pleuranthodes,* are a strongly supported monophyletic group. Using MacClade to recalculate tree length, it requires only three additional steps to move *Pleuranthodes* from its unresolved position and place it within Gouanieae.

Within Rhamnaceae, strongly bootstrap-supported major groups are identified: a ziziphoid group that has Elaeagnaceae as a sister group; an ampeloziziphoid group that contains the genera *Ampeloziziphus, Doerpfeldia,* and *Bathiorhamnus* (the inclusion of *Ventilago* in this group does not occur in all Fitch trees and recieves <50% SW bootstrap support); and a rhamnoid group that has the ampeloziziphoid group as its sister.

Other strongly supported groups within these larger groups include: (1) in the ziziphoid group: (a) Pomaderreae Reiss. ex Endl., a group of Australian taxa (*Pomaderris, Siegfriedia, Spyridium,* and *Trymalium*), (b) *Ceanothus,* (c) Phyliceae Reiss. ex Endl., a group of taxa with a southern African center of distribution (*Phylica, Nesiota,* and *Noltea*), (d) *Colubrina,*

TABLE 3. Taxon accession data. (K) = Royal Botanic Gardens, Kew, (MICH) = University of Michigan. Numbers in parentheses after species names represent the number of the species indicated in Figs. 1-3 (e.g., *Spyridium* 2 in these figures is *S. complicatum*). In some instances different species were sequenced for *trnL-F* and *rbcL* but treated as single species in the analysis, and in such cases the region sequenced for each accession is indicated in parentheses. Tribes indicated are those of Richardson et al. 2000.

Species	Source	Voucher/citation	Material type/age	GenBank accessions *rbcL/trnL-F* [a]
Rhamnaceae				
Ampeloziziphеae				
Ampeloziziphus amazonicus Ducke	Brazil	*Vilhena & Taylor 1004* (K)	herbarium; 1983	GBAN-AJ390037/GBAN-AJ390341
Bathiorhamneae				
Bathiorhamnus cryptophorus Capuron	Madagascar	*Labar & DuPuy 2044* (K)	herbarium; 1990	GBAN-AJ390036/GBAN-AJ390340
Colletieae				
Adolphia infesta (H.B.K.) Meisn.	Mexico	*McVaugh 7506* (K)	herbarium; 1945	GBAN-AJ390055
Colletia ulicina Gill. & Hook.	Chile	Swensen et al., 1996	fresh	GBAN-U59819/GBAN-AJ390364
Discaria chacaye (G. Don) Tortosa	Chile	Swensen et al., 1996	fresh	GBAN-U59826/GBAN-AJ225797
Trevoa trinervis Miers	Chile	*Wall & Sparre 2430* (K)	herbarium; 1947	GBAN-AJ390056
Doerpfeldieae				
Doerpfeldia cubensis Urban	Cuba	*Howard et al. 246* (K)	herbarium; 1950	GBAN-AJ390038/GBAN-AJ390342
Gouanieae				
Crumenaria erecta Reiss.	Brazil	*Ratter & Rocha R5015* (K)	herbarium; 1984	GBAN-AJ390042/GBAN-AJ390346
Gouania mauritiana Lam.	Mauritius	*Chase 904* (K)	silica	GBAN-AJ390040/GBAN-AJ390344
Helinus integrifolius Kuntze	South Africa	*Thulin & Warfa 5865* (K)	herbarium; 1986	GBAN-AJ390043/GBAN-AJ390347
Pleuranthodes hillebrandii (Oliver) Weberb.	Hawaii	*Hutchinson 2776* (K)	herbarium; 1967	GBAN-AJ390045/GBAN-AJ390348
Reissekia smilacina Endl.	Brazil	*Arbo et al. 4921* (K)	herbarium; 1991	GBAN-AJ390041/GBAN-AJ390345
Maesopsideae				
Maesopsis eminii Engl.	Australia	*Chase 1338* (K)	silica	GBAN-AJ390034/GBAN-AJ390336
Paliureae				
Hovenia dulcis Thunb.	South Korea	*Chase 968* (K)	fresh	GBAN-AJ390039/GBAN-AJ390343
Paliurus spina-christi Mill.	Bulgaria	*Chase 969* (K)	fresh	GBAN-AJ390051/GBAN-AJ390354
Ziziphus ornat Miq. (2)	Sumatra	*Chase 2117* (K)	silica	GBAN-AJ390052/GBAN-AJ390355
Ziziphus glabrata Heyne ex. Roth. (1)	Saudi Arabia	Thulin et al., 1998	silica	GBAN-U60313/GBAN-AJ225799
Phyliceae				
Nesiota elliptica (Roxb.) Hook. f.	St Helena	Thulin et al., 1998	fresh	GBAN-AJ225783/GBAN-AJ225803
Noltea africana (L.) Reichb.	South Africa	*Bayliss BS6824 49* (K)	herbarium; 1974	GBAN-AJ390054/GBAN-AJ390357
Phylica nitida Lam. (1)	Mauritius	*Soorer 64-5* (MICH)	herbarium; 1964	GBAN-AJ390053/GBAN-AJ390356
Phylica polifolia (Vahl) Pillans (*rbcL*) (2)	St Helena	*Chase 1751* (K)	fresh	GBAN-AJ225784
Phylica polifolia (Vahl) Pillans (*trnL-F*) (2)	St Helena	*Chase 2269* (K)	fresh	GBAN-AJ390373
Phylica pubescens Ait. (3)	South Africa	Thulin et al., 1998	fresh	GBAN-Y16769 & GBAN-Y16770/Y16771
Pomaderreae				
Cryptandra cf. *spyridioides* F. Muell.	W Australia	*Chase 2180* (K)	silica	GBAN-AJ390060/GBAN-AJ390360
Pomaderris rugosa Cheeseman	W Australia	*Chase 857* (K)	fresh	GBAN-AJ390063/GBAN-AJ390363
Siegfriedia darwinioides C.A. Gardner	W Australia	*Chase 2181* (K)	silica	GBAN-AJ390064/GBAN-AJ390375
Spyridium globulosum (Labill.) Benth. (3)	W Australia	*Chase 2021* (K)	silica	GBAN-AJ390058/GBAN-AJ390358
Spyridium complicatum F. Muell. (2)	W Australia	*Chase 2182* (K)	silica	GBAN-AJ390059/GBAN-AJ390359
Spyridium cf. *forrestianum* F. Muell. (1)	W Australia	*Chase 2183* (K)	silica	GBAN-AJ390057/GBAN-AJ251690
Trymalium ledifolium Fenzl (1)	W Australia	*Chase 2184* (K)	silica	GBAN-AJ390061/GBAN-AJ390361
Trymalium floribundum Steudel (2)	W Australia	*Chase 2185* (K)	silica	GBAN-AJ390062/GBAN-AJ390362

TABLE 3. Continued.

Species	Source	Voucher/citation	Material type/age	GenBank accessions *rbcL/trnL-F* [a]
Rhamneae				
Berchemia discolor (Klotch) Hemsley	Saudi Arabia	Thulin et al., 1998	silica	GBAN-AJ225786/GBAN-AJ225793
Condalia microphylla Cav.	Argentina	*Kiesling et al. 5967* (K)	herbarium; 1986	GBAN-AJ390032/GBAN-AJ390334
Frangula alnus L.	Switzerland	*Chase 1745* (K)	silica	GBAN-AJ390026/GBAN-AJ251691
Karwinskia humboldtiana (Roem. & Schult) Zucc.	Mexico	*Brennan 14483* (K)	herbarium; 1977	GBAN-AJ390031/GBAN-AJ390333
Krugiodendron ferreum (Vahl) Urban	West Indies	*Lundell 17449* (K)	herbarium; 1963	GBAN-AJ390028/GBAN-AJ390331
Reynosia uncinata Urban	Cuba	*Chase 363* (K)	silica	GBAN-AJ390029/GBAN-AJ390339
Rhamnella franguloides (Maxim.) Weberb.	South Korea	*Chase 912* (K)	silica	GBAN-AJ390027/GBAN-AJ390330
Rhamnidium elaeocarpum Reiss.	Brazil	*Santos et al. 693* (K)	herbarium; 1983	GBAN-AJ390030/GBAN-AJ390332
Rhamnus cathartica L. (2)	USA	Chase et al., 1993	fresh	GBAN-L13189
Rhamnus lycioides L. (1)	Spain	*Chase 1884* (K)	silica	GBAN-AJ390070/GBAN-AJ390374
Sageretia thea (Osbeck) M.C. Johnston	Saudi Arabia	Thulin et al., 1998	silica	GBAN-AJ225785/GBAN-AJ225792
Scutia buxifolia Reiss.	Argentina	*Chase 858* (K)	fresh	GBAN-AJ390033/GBAN-AJ390335
Ventilagineae				
Ventilago leiocarpa Benth. (2)	Hong Kong	*Hu 11890* (K)	herbarium; 1972	GBAN-AJ390338
Ventilago viminalis Hook. (1)	W Australia	*Kenneally 9507* (K)	herbarium; 1985	GBAN-AJ390035/GBAN-AJ390337
incertae sedis				
Alphitonia excelsa Reiss.	Australia	*Chase 2179* (K)	silica	GBAN-AJ390049/GBAN-AJ390352
Ceanothus coeruleus Lag. (*trnL-F*) (1)	USA	Thulin et al., 1998	fresh	GBAN-AJ225798
Ceanothus sanguineus Nutt. (*rbcL*) (1)	USA	Soltis et al., 1993	—	GBAN-U06795
Ceanothus thyrsiflorus Esch. (2)	USA	Swensen et al., 1996	fresh	GBAN-U59827
Colubrina asiatica Brongn. (1)	Sumatra	*Chase 905* (K)	silica	GBAN-AJ390047/GBAN-AJ390350
Colubrina reclinata (L'Hér.) Brongn. (2)	West Indies	*Chase 2115* (K)	silica	GBAN-AJ390065/GBAN-AJ390370
Emmenosperma alphitonioides F. Muell.	Australia	*Clarkson 8826* (K)	herbarium; 1990	GBAN-AJ390048/GBAN-AJ390351
Lasiodiscus mildbraedii Engl.	Sao Tomé	*Figueiredo et al. 29* (K)	herbarium; 1993	GBAN-AJ390050/GBAN-AJ390353
Schistocarpaea johnsonii F. Muell.	Australia	*Gray 1247* (K)	herbarium; 1979	GBAN-AJ390046/GBAN-AJ390349
Barbeyaceae				
Barbeya oleoides Schweinf.	Saudi Arabia	Thulin et al., 1998	silica	GBAN-AJ224820/GBAN-AJ225795
Cannabaceae				
Cannabis sativa L.	ex. cult.	*Chase 2992* (K)	fresh	GBAN-AJ390068/GBAN-AJ390367
Dirachmaceae				
Dirachma socotrana Schweinf.	Socotra	Thulin et al., 1998	silica	GBAN-AJ225789/GBAN-AJ225796
Elaeagnaceae				
Elaeagnus angustifolia Blanco (*rbcL*)		Soltis et al., 1995	fresh	GBAN-U17038
Elaeagnus sp. (*trnL-F*)	China	Thulin et al., 1998	fresh	GBAN-AJ225800
Hippophae salicifolia D. Don	Nepal	Swensen et al., 1996	fresh	GBAN-U59821/GBAN-AJ225801
Shepherdia canadensis (Pursh.) Nutt. (*rbcL*)	USA	Soltis et al., 1995	—	GBAN-U17039
Shepherdia argentea Schlecht. (*trnL-F*)		*Chase 3176* (K)	fresh	GBAN-AJ390372
Moraceae				
Dorstenia psilurus Welw.	South Africa	*Chase 2416* (K)	fresh	GBAN-AJ390066/GBAN-AJ390365
Artocarpus heterophylla Lam.	India	*Chase 2415* (K)	fresh	GBAN-AJ390376
Ficus pretoriae Burtt-Davy	South Africa	*Chase 2412* (K)	fresh	GBAN-AJ390067/GBAN-AJ390366

TABLE 3. Continued.

Species	Source	Voucher/citation	Material type/age	GenBank accessions *rbcL/trnL-F* [a]
Rosaceae				
Dryas drummondii Richards.	Canada	Swensen et al., 1996	—	GBAN-U59818/GBAN-AJ225802
Spiraea × *vanhouttei* Zabel (*rbcL*)		Morgan et al., 1994	—	GBAN-L11206
Spiraea betulifolia Pall. (*trnL-F*)	Russia	*Chase 2503* (K)	fresh	GBAN-AJ390368
Pyrus serotina Rehder	China	*Chase 1018* (K)	silica	GBAN-AJ390369
Ulmaceae				
Gironniera subaequalis Planch.	Java	Thulin et al., 1998	silica	GBAN-Y16772
Urticaceae				
Boehmeria biloba Miq.	Java	*Chase 2532* (K)	silica	GBAN-AJ390069/GBAN-AJ390371

[a] The prefix GBAN-has been added to link the online version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

(e) Paliureae, including *Ziziphus, Paliurus,* and *Hovenia,* and (f) Gouanieae (excluding *Pleuranthodes*), and (2) in the rhamnoid group: Rhamneae, a clade composed of *Karwinskia, Condalia, Krugiodendron, Reynosia, Rhamnella, Rhamnidium, Berchemia, Sageretia, Rhamnus, Frangula,* and *Scutia* with the monotypic tribe Maesopsideae being sister to Rhamneae.

Analysis of trnL-F data—The aligned *trnL-F* data matrix had 595 variable characters and 353 potentially informative characters out of a total of 1258 characters used (i.e., 28%). The heuristic search produced >6000 equally parsimonious Fitch trees with 1339 steps, CI = 0.67 (0.57 excluding autapomorphies), and RI = 0.75. Application of SW produced >6000 trees with a length of 652 105 steps, CI = 0.87 (0.76 excluding autapomorphies), and RI = 0.91. The Fitch length of the SW trees was 1339, i.e., the weighted trees were a subset of the Fitch trees from the same island. Figure 2 shows one of the weighted trees with Fitch branch lengths (ACCTRAN optimization) and SW bootstrap percentages; branches that collapse in the strict consensus tree of the Fitch analysis are marked with a solid arrow, and those not present in the strict consensus of the weighted tree are marked with an open arrow. The performance of the indel characters is shown in Table 5. The average CI was 0.84, and the average RI was 0.90.

Rhamnaceae are a strongly supported monophyletic group with a clade containing Dirachmaceae and Barbeyaceae as sister. In some trees, Elaeagnaceae form a sister group to a clade containing Rhamnaceae, Barbeyaceae, Dirachmaceae, and Urticales. Therefore the main differences between trees produced by the separate *rbcL* and *trnL-F* matrices were that the *rbcL* trees placed Elaeagnaceae, Dirachmaceae, and Barbeyaceae within Rhamnaceae but with low bootstrap support, whereas the *trnL-F* trees placed these families outside Rhamnaceae with high bootstrap support (94%) for the monophyly of Rhamnaceae. Also, *Pleuranthodes*' position within Gouanieae is strongly supported by the bootstrap (94%) in the *trnL-F* analysis.

TABLE 4. Performance of each codon position in the *rbcL* analysis.

Codon position	Number of steps	CI	RI
1	277	0.47	0.50
2	167	0.57	0.44
3	747	0.51	0.70

Within Rhamnaceae, the strongly supported major groups identified in the *rbcL* analysis (i.e., the ziziphoid, rhamnoid, and ampeloziziphoid groups) receive further support. The inclusion of *Ventilago* in the rhamnoid group and not the ampeloziziphoid group (as in the *rbcL* tree) is strongly supported. Generally speaking, the generic relationships and the larger clades identified are highly congruent with the *rbcL* results. We did not use any congruence metrics because these are too coarse to be useful (Wiens, 1998) and instead preferred node by node inspection to determine if evidence of strongly bootstrap-supported incongruence was present (i.e., less resolution and lower bootstrap percentages in the combined trees).

Analysis of combined rbcL and trnL-F data—The combined matrix produced 324 Fitch trees with a length of 2559 steps, a CI = 0.59 (0.48 excluding autapomorphies) and RI = 0.70. With SW there was only one tree with two trichotomies, tree length of 1 068 277 steps, CI = 0.85 (0.71 excluding autapomorphies), and RI = 0.88. Figure 3 shows this single tree with its Fitch branch lengths (ACCTRAN optimization) and SW bootstrap values; branches that collapse in the strict consensus tree of the Fitch analysis are marked with a solid arrow. The Fitch length of this tree was 2559 steps (i.e., it was one of the Fitch trees from the same island).

The combined trees show a greater similarity to the *trnL-F* tree than to the *rbcL* tree. Rhamnaceae are monophyletic with a clade consisting of Dirachmaceae and Barbeyaceae forming their sister group. Elaeagnaceae fall on a long branch nearest the outgroup. The ziziphoid, rhamnoid, and ampeloziziphoid groups are again strongly bootstrap-supported as are the groups within these clades that were strongly supported in the separate analyses. *Pleuranthodes* is strongly supported as a member of Gouanieae as in the *trnL-F* analysis. No group was less resolved than in either of the individual trees, demonstrating that the differences in topology were instances of "soft" incongruence (Seelanen, Schnabel, and Wendel 1997)/sampling error (Huelsenbeck, Bull, and Cunningham, 1996).

Molecular evolution—Figure 4 shows a plot of the number of steps per character optimized on the single most parsimonious SW tree from the combined analysis. The *trnL-F* region has a more even distribution of substitutions along its length

TABLE 5. Performance of *trnL-F* indel characters (numbered 1–16).

	Indel character															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CI	1	1	1	1	1	1	1	1	0.25	0.50	1	1	1	1	0.50	0.25
RI	1	1	1	1	1	1	1	1	0.50	0.67	1	1	1	1	0.80	0.40

than *rbcL*. Figure 5 shows the number of steps per character on both the *trnL-F* and *rbcL* trees, indicating that some *rbcL* characters change up to 16 times, whereas the *trnL-F* positions change up to nine times only. We consider the existence of such highly homoplasious positions to justify the use of SW, which downweights only those characters that change frequently based on the best RC from any tree.

Table 4 shows that in the *rbcL* analysis the third codon positions have by far the greatest number of steps, followed by the first and then second positions. The CI value is highest for the second position followed by the first and the third position. However, the RI value is highest for the third position, followed by the first, and then the second position. Table 5 shows that most of the *trnL-F* indel characters have higher CI and RI values than base positions. On average, variable *rbcL* positions change 2.48 times (tree length divided by number of variable positions), whereas each variable *trnL-F* position changes 2.25 times. There are more, variable *trnL-F* positions (595 vs. 474), but they change fewer times on average.

The transition/transversion ratio for *rbcL* calculated on the single combined SW tree was 1.17. The *trnL-F* transition/transversion ratios based on the combined SW tree were calculated separately for the intron, exon, intergenic spacer, and they were (258/282) 0.91, (6/1) 6.0, and (340/342) 0.99, respectively. Thus, for *rbcL* there is a bias for transitions, whereas noncoding regions have no bias. Transitions have higher CIs and RIs (Table 6) than tranversions for both *rbcL* and *trnL-F*.

Table 7 shows the tree lengths when analyzed alone for *rbcL* and *trnL-F* as well as the number of steps for *rbcL* and *trnL-F* data sets optimized on the single combined SW tree. Both of the separate analyses underestimate the number of substitutions relative to the combined tree. The *trnL-F* region had a 1339/1347 difference, which is a 0.6% underestimate of change in the *trnL-F* tree compared to the combined tree. The *rbcL* gene had a 1174/1194 difference, which is a 1.7% underestimate of change in the *rbcL* tree compared to the combined tree. Thus the *rbcL* tree was less accurate than the *trnL-F* tree. We consider the combined tree more accurate because overall bootstrap percentages are higher than either *rbcL* or *trnL-F* analyzed separately.

TABLE 6. Tree scores for transitions (TS) and transversions (TV) on the single successively weighted tree from the combined *rbcL/trnL-F* analysis.

	rbcL			*trnL-F*		
	TS	TV	Ratio	TS	TV	Ratio
Number of steps	646	548	1.17	677	664	1.02
CI	0.553	0.465		0.694	0.620	
RI	0.721	0.567		0.786	0.690	

DISCUSSION

Molecular evolution—In the *rbcL* trees Rhamnaceae are paraphyletic with Barbeyaceae, Dirachmaceae, and Elaeagnaceae nested within a weakly supported clade, whereas the *trnL-F* analysis indicates that Rhamnaceae are a strongly supported monophyletic group. There are two possible explanations for this result: either the two data sets are really incongruent, or the nesting of Barbeyaceae, Dirachmaceae, and Elaeagnaceae in the *rbcL* tree is an artifact of sampling error. We assume that there is a common phylogenetic signal present in all sequence matrices, but overlying this there may be other patterns. Functional constraints exist in protein-coding genes such as *rbcL* (Albert et al., 1994), and third positions in codons are expected to be more variable than first or second positions, as is the case with this *rbcL* data set (Table 4). Direct combination of matrices should enhance the common although perhaps partially to wholly overshadowed patterns, and this could result in a slightly different but more strongly supported pattern than either matrix analyzed separately (as is the case here).

Because of the degenerate nature of the genetic code, first and second positions in a codon are under higher levels of direct selection, and therefore fewer of these can change than third positions. In noncoding regions such as *trnL-F* there is probably less functional constraint than there is in *rbcL* (constraints in noncoding regions could involve ribosome control sites and other structural features). Given similar constraints among noncoding characters, rates of change among noncoding characters should be more similar, and this is what we found, i.e., *trnL-F* has a more even pattern of change than *rbcL* (Fig. 4). Also, on average each *rbcL* position changes more often than in *trnL-F,* and *rbcL* has more hypervariable positions than *trnL-F* (Fig. 5). This uneven pattern of variation in *rbcL* makes it harder to detect all changes (i.e., all instances of homoplasy) in such positions and is therefore more likely to produce misrepresentations of relationships (i.e., underestimates in the actual amount of change). As compared to the combined tree, which is better supported by the bootstrap, the *rbcL* tree misses more homoplasious changes and has a less accurate overall topology (*rbcL* is a 1.7% underestimate of the combined tree, whereas *trnL-F* is only a 0.6% underestimate).

As discussed above, combining matrices should strengthen only shared signal, which is likely to be the phylogenetic one (see also Chase and Cox, 1998). In general, similar weakly supported patterns of separate data sets would be expected to

TABLE 7. Comparison of number of steps for the separate analyses vs the combined trees.

Tree	*rbcL* tree length	*trnL-F* tree length	Length on combined tree	Difference	% difference
rbcL	1174	–	1194	+20	1.7
trnL-F	–	1339	1347	+8	0.6

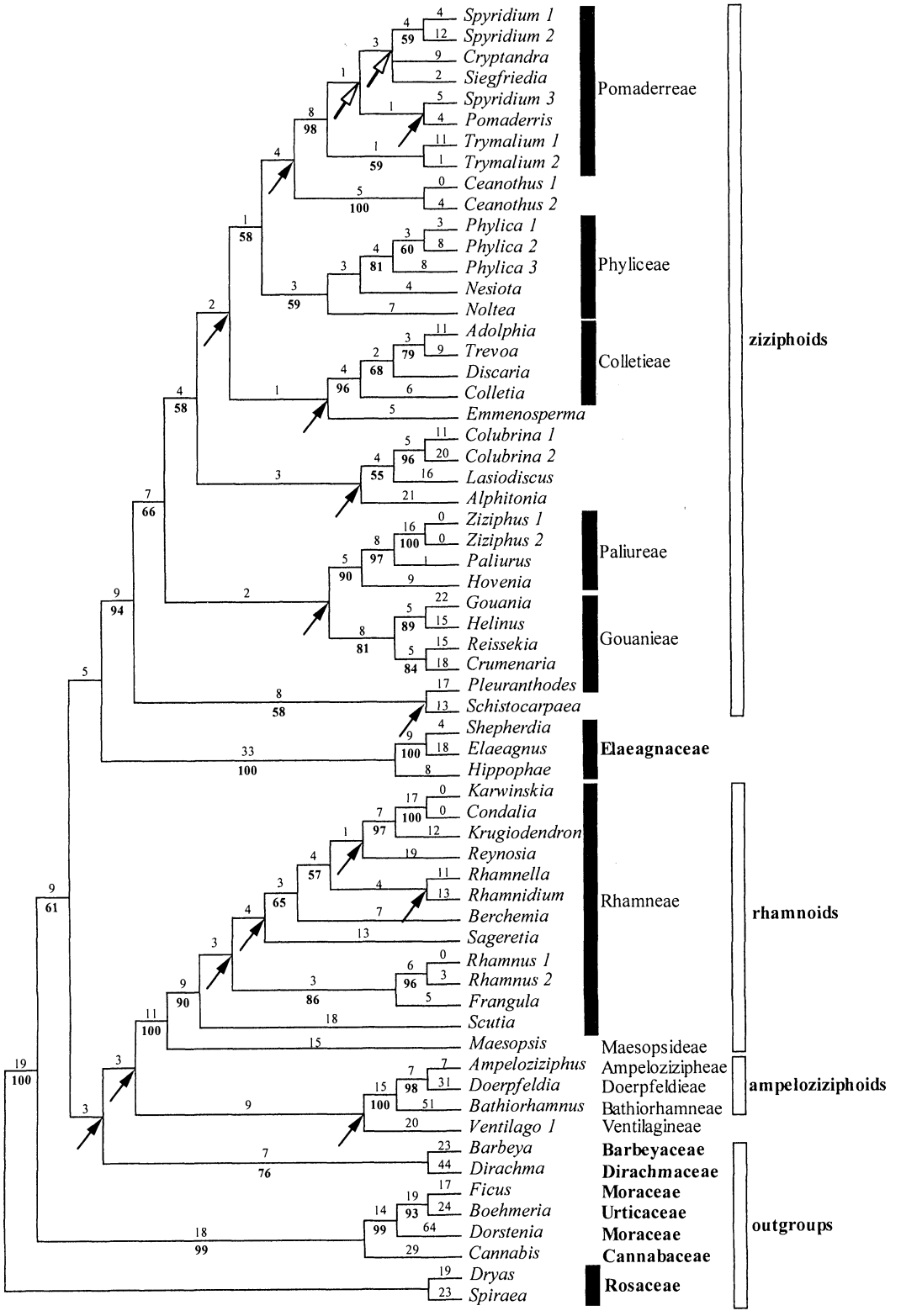

Fig. 1. One optimal SW tree from the *rbcL* analysis with its Fitch lengths (above branches; ACCTRAN optimization) and SW bootstrap values (below). Branches not present in the Fitch strict consensus tree are indicated by a solid arrow, and those not present in the SW strict consensus tree are indicated by an open arrow. Heuristic search under the Fitch criterion produced >6000 equally parsimonious trees with a length of 1174 steps. The CI for these trees was 0.52 and the RI was 0.66. There were only seven SW trees with a length of 423 378 steps, CI = 0.84, and RI = 0.86 (Fitch length, 1174 steps). This figure shows the paraphyly of Rhamnaceae and indicates the three major "cryptic" clades within Rhamnaceae sensu stricto.

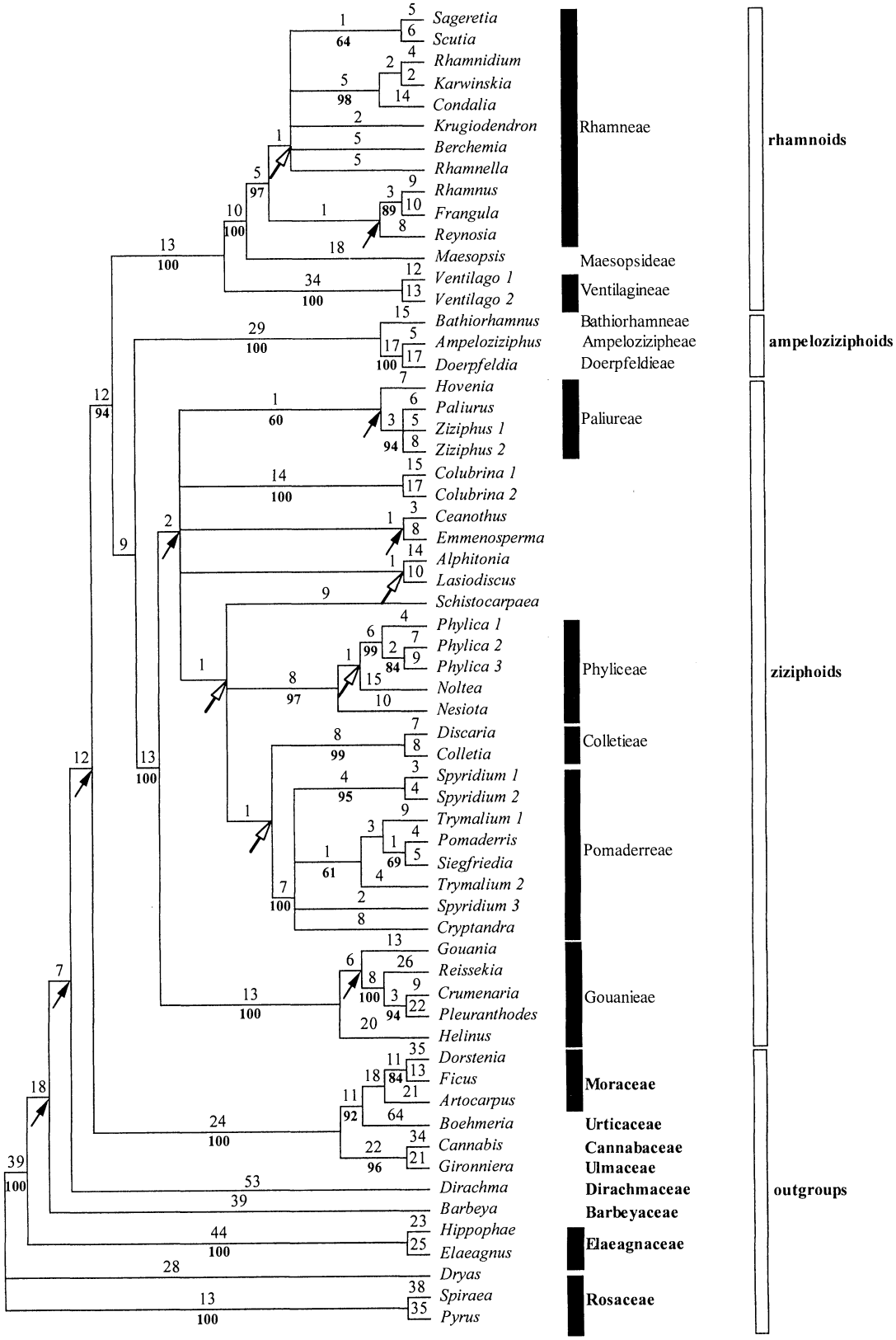

Fig. 2. One optimal SW tree from the *trnL-F* analysis with its Fitch lengths (above branches; ACCTRAN optimization) and SW bootstrap values (below). Branches not present in the Fitch strict consensus tree are indicated by a solid arrow, and those not present in the SW strict consensus tree are indicated by an open arrow. Heuristic search under the Fitch criterion produced >6000 equally parsimonious trees with a length of 1339 steps, CI = 0.67, and RI = 0.75. SW produced >6000 trees and a length of 652 105 steps, CI = 0.87, and RI = 0.91 (Fitch length, 1339 steps). This figure shows the monophyly of Rhamnaceae and indicates the three major "cryptic" clades within the family.

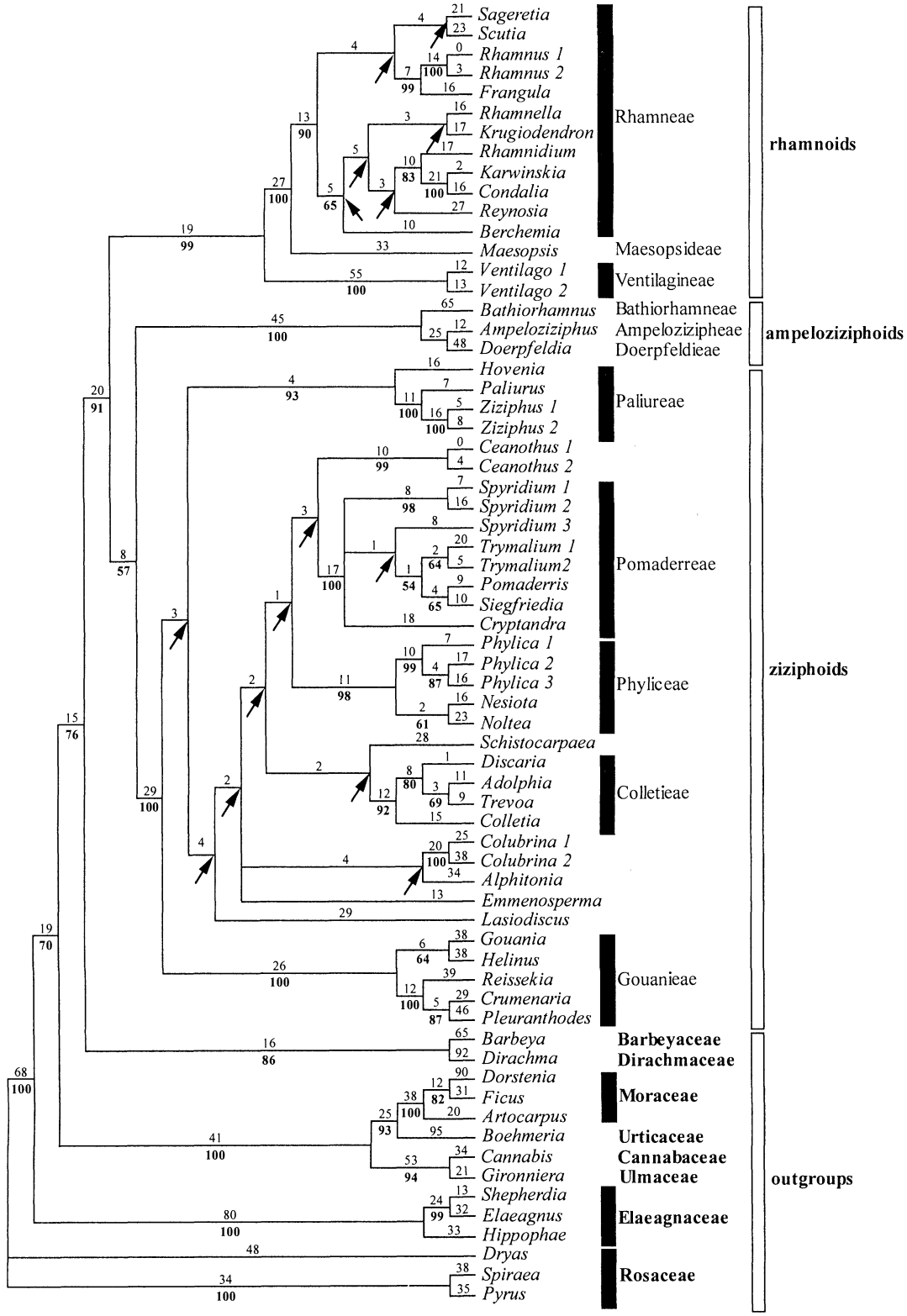

Fig. 3. The single optimal SW tree from the combined *rbcL/trnL-F* analysis with its Fitch lengths (above branches; ACCTRAN optimization) and SW bootstrap values (below). Branches not present in the Fitch strict consensus tree are indicated by a solid arrow. Heuristic search under the Fitch criterion produced 324 Fitch trees with a length of 2559 steps, CI = 0.59, and RI = 0.70. SW produced one tree with two trichotomies and a tree length of 1 068 277 steps, CI = 0.85, and RI = 0.88 (Fitch length, 2559 steps). This figure shows the monophyly of Rhamnaceae and indicates the tribes as circumscribed by Richardson et al. (2000). Genera for which a tribe is not indicated are unplaced (incertae sedis).

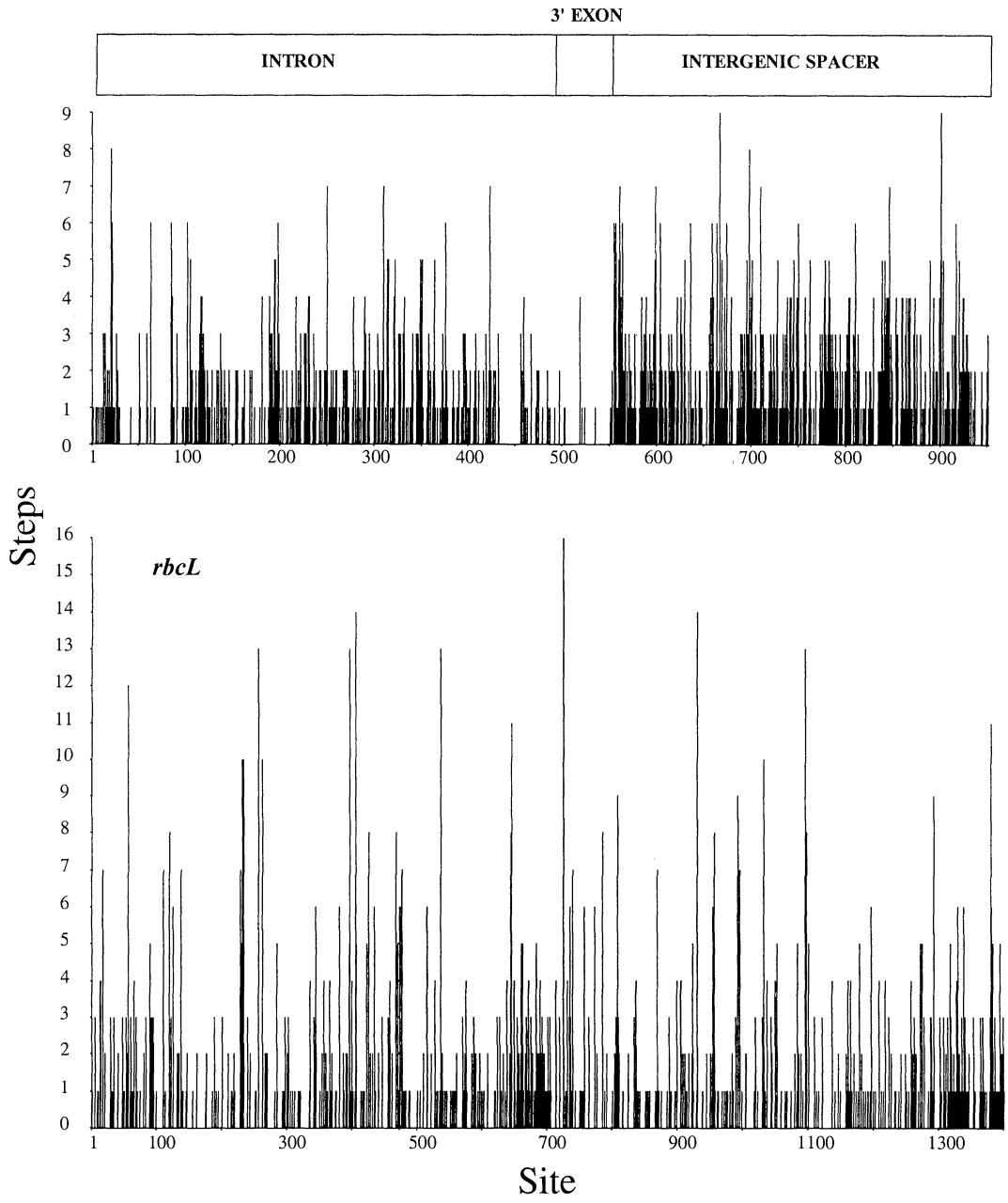

Fig. 4. Number of changes per character based on the single SW tree from the combined *rbcL/trnL-F* analysis.

be more strongly supported when combined. Finally, combining data sets detects evidence for additional substitutions not found in the individual matrices, thus permitting more accurate overall character reconstruction and estimates of relationships. As a result, combined trees might be expected to be longer than any of the individual matrix trees because combined matrices should recover more of the unobserved substitutions, which is the case in this study (Table 7). The greater underestimate in change for *rbcL* compared to *trnL-F* may have resulted in the spurious nesting of Elaeagnaceae, Barbeyaceae, and Dirachmaceae within Rhamnaceae in the *rbcL* analysis.

A further *rbcL* analysis was run in which the monophyly of Rhamnaceae was constrained. This analysis produced a Fitch tree of 1175 steps, i.e., only one step longer than the nonconstrained analysis. Such underestimates of change on single gene matrices highlight the limitations of too little data in which patterns are too weak for accurate reconstruction (i.e. sampling error sensu Huelsenbeck et al., 1997), not the unre-

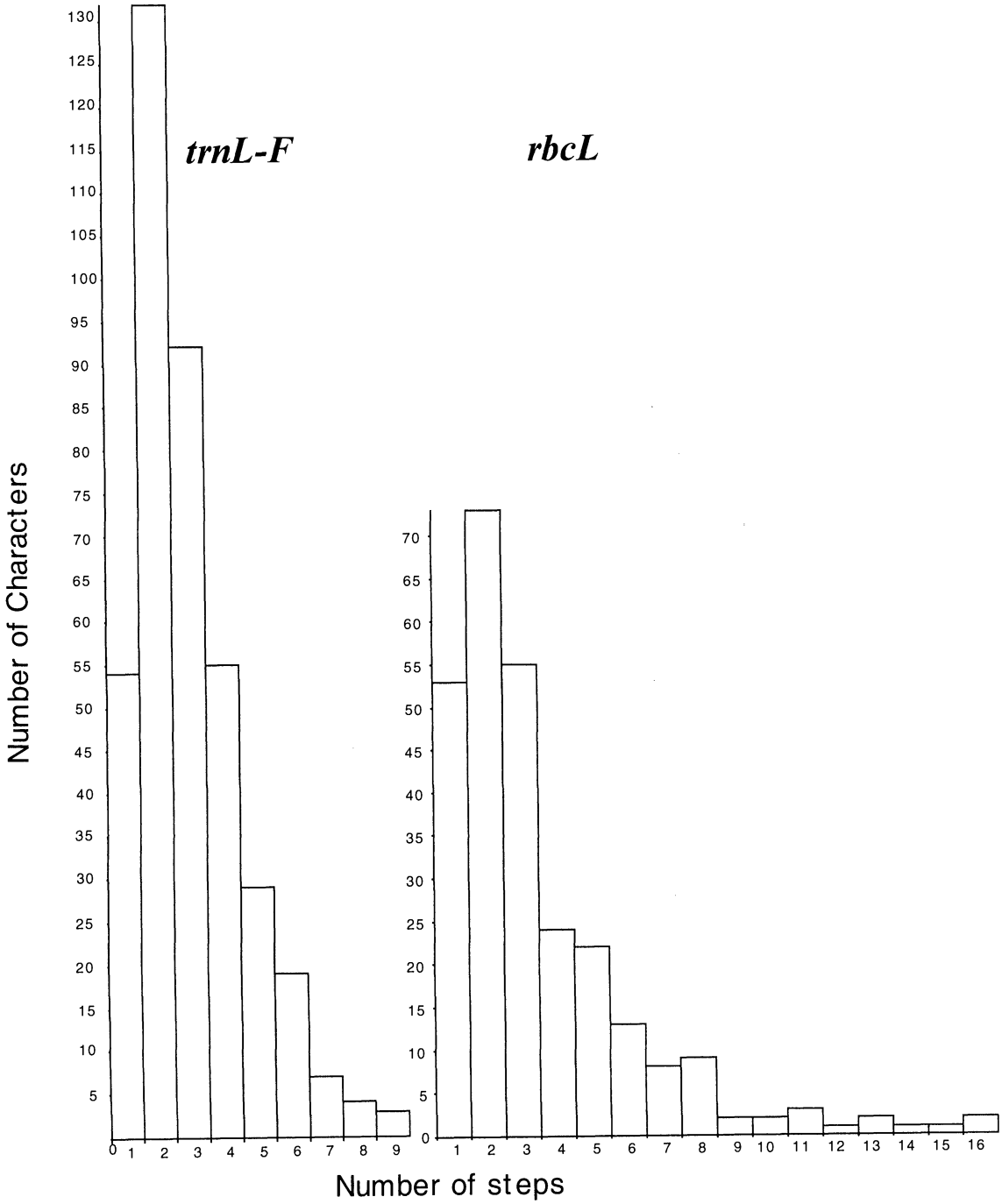

Fig. 5. Number of steps each of the variable sites produced on the single SW tree from the combined *rbcL/trnL-F* analysis.

liability of parsimony as an optimality criterion. The following sections of the discussion will focus mainly on the combined tree, which we view as the most accurate, for the reasons explained above.

Thirteen of the 16 indel characters from the *trnL-F* data set were nonhomoplasious synapomorphies. Therefore, in this analysis, indel characters appear to be good phylogenetic markers. Six indels appear to be unique in sequence, and the other ten are copies or near copies of adjacent regions.

In this data set, coding regions have a transition bias, whereas introns or nontranscribed spacers have no apparent bias. The lack of bias in noncoding *trnL-F* (Table 6) is consistent with the findings of Morton and Clegg (1995) who demonstrated that substitutions in noncoding regions of the plastid genome were affected by the two immediately flanking bases. When both the 5′ and 3′ flanking nucleotides are G or C, Morton and Clegg found that only 25% of the observed substitutions were transversions, whereas if the flanking nucleotides were both A or T 57% of the substitutions were transversions. Because noncoding regions of the plastid genome are more A/T rich, the relative proportion of transversions increases, resulting in a more even transition/transversion ratio.

Differential rates of change (codon positions or ts/tv) have been used to justify relative weighting schemes in phylogenetic analyses (e.g., Fu, 1998; Smith, 1998; Zink and Blackwell, 1998). The enhanced performance of transitions (Table 6) and third codon positions in *rbcL* as indicated by CI and RI values (Table 4) shows that differential weighting of whole classes of characters is not justified.

Relationships of Rhamnaceae—The *Dirachma/Barbeya* alliance is strongly supported by the bootstrap analysis. This clade is the sister group of Rhamnaceae in the combined tree with moderate support. Thulin et al. (1998) suggested that the families Barbeyaceae and Dirachmaceae should be retained because they differ so significantly in morphology, and the results here also indicate that this would be the best circumscription for these families given the large number of morphological and molecular differences between them and the other families. Greater sampling from within the urticalean families and Rosaceae may result in a better placement of Barbeyaceae and Dirachmaceae, but their combination of traits otherwise restricted to either Rhamnaceae or the urticalean families (Thulin et al., 1998) appears to indicate either a position as obtained here or as sister to the urticalean families is appropriate.

Relationships within Rhamnaceae—Classification based solely on DNA sequence data should be treated with caution unless corroborated by evidence from other sources, but the *rbcL/trnL-F* data have indicated patterns that were not apparent from previous non-phylogenetic studies of morphology and anatomy. The single SW tree from the combined analysis shows that Rhamnaceae are a well-supported monophyletic group and also provides support for some of Suessenguth's tribes. However, these data show a division of Rhamnaceae into three clades supported by bootstrap values of 99 or 100, but for which there are no obvious morphological apomorphies (no formal morphological phylogenetic analysis is presented here, but see Richardson et al., 2000). Such groups were described as "cryptic clades" (Wojciechowski et al., 1993) in a study that identified a strongly supported clade of dysploid North American *Astragalus,* which was found to be supported by three different lines of genotypic evidence (chromosomal, nuclear rDNA, and plastid DNA) but for which there were no morphological apomorphies. The authors suggested that the group should be given an informal name, and we have likewise chosen to adopt informal names for the three cryptic clades identified here. The only morphological evidence for these groups comes from studies of gynoecium ontogenesis (Medan, 1988). A molecular phylogenetic study of Saxifragaceae (Soltis, Soltis, and Thompson, 1992) indicated that inferior or superior ovaries, which had been used in the classification of *Lithophragma,* were homoplasious. However, a study of gynoecium ontogenesis revealed that patterns in the initial development of the ovary were consistent with the molecular phylogeny. Monophyletic groups within *Lithophragma* could be defined on the basis of whether the floral apex in the initial stages of development is more or less flat or with a circular depression. Subsequent ontogenetic development leads to superior or inferior ovaries from both meristem conditions, and thus ovary conditions at maturity are not always homologous. Without ontogenetic investigation this would seem to represent a case of parallel evolution. A similar phenomenon could be occurring in Rhamnaceae; Medan (1988) studied the shape of the floral apex and the degree of intercalary growth at carpellary bases in 17 genera of Rhamnaceae. In some taxa the floral apex is more or less flat at the time of primordia differentiation, e.g., *Condalia* and *Rhamnus,* and these taxa usually go on to form superior ovaries. In other taxa the floral apex shows a circular depression at the time of primordia differentiation, e.g., *Colletia, Noltea, Phylica,* and *Pomaderris,* and these taxa usually go on to form inferior or semi-inferior ovaries. We could therefore have a situation in Rhamnaceae similar to that of *Lithophragma* in which the latter stages of ontogenetic development of floral apices may obscure the initial patterns. The limited sampling in the Medan (1988) study could be expanded and provide morphological character support for the cryptic clades defined by the molecular data.

Group 1: rhamnoid clade—This clade is divided into three strongly supported subgroups. The first of these comprises Rhamneae with genera such as *Rhamnus* and *Berchemia* that have drupaceous fruits, superior ovaries and a nectariferous disc either partly or totally adnate to the calyx tube. The interrelationships of the genera within this group are not particularly well supported. The second subgroup, the monotypic tribe Maesopsideae, consists of the monotypic genus *Maesopsis,* which is a sister to Rhamneae. It is so morphologically divergent that its inclusion in Rhamneae seems unwarranted. Ventilagineae are the third distinct subgroup with strong support as sister to the Maesopsideae–Rhamneae alliance. All members of this third tribe are climbers with apically winged fruits and semi-inferior ovaries. We had no success with herbarium DNA for *Smythea,* which is the only other genus previously placed in this tribe. However, this genus is similar in morphology to *Ventilago* and should therefore be tentatively included within Ventilagineae.

Group 2: ampeloziziphoid clade—This group consists of three highly divergent genera that have palmately veined leaves and drupaceous fruits: *Ampeloziziphus,* a monotypic Brazilian climber with semi-inferior ovaries and a thick nectariferous disc; *Doerpfeldia,* a monotypic tree from Cuba with small leaves and a superior ovary thinly covered by the nectariferous disc; and *Bathiorhamnus,* a genus of two Madagascan tree species with a superior ovary and thick nectariferous disc. There are, however, no exclusive morphological similarities linking these genera. The high levels of molecular divergence between these genera and their highly disjunct distribution indicates that they are only distantly related, and it is likely that they are remnants of groups that were formerly more diverse and widespread. These three genera have each been placed in a separate tribe because of their highly divergent nature (Richardson et al., 2000).

Group 3: ziziphoid clade—The third major clade comprises genera that usually have semi-inferior to inferior ovaries and capsular fruits. There are, however, exceptions to this, e.g., *Ziziphus* has drupaceous fruits. In addition some genera of the tribe Colletieae have superior ovaries or drupaceous fruits. This ziziphoid group may be further split into a number of subgroups. Suessenguth's more derived tribes Colletieae and Gouanieae are strongly supported monophyletic groups. There are some differences between *rbcL* and *trnL-F* regarding the position of *Pleuranthodes,* but moving this taxon from its unresolved position in the *rbcL* tree to within Gouanieae using

TABLE 8. Summary of revised tribal classification of Rhamnaceae (Richardson et al., 2000).

Tribe	Genera included	Distribution
Paliureae Reiss. ex Endl.	*Paliurus, Ziziphus, Hovenia*	tropics and warm temperate regions
Colletieae Reiss. ex Endl.	*Adolphia, Colletia, Discaria, Kentrothamnus, Retanilla, Trevoa*	South America, New Zealand, Australia
Phyliceae Reiss. ex Endl.	*Nesiota, Noltea, Phylica*	southern Africa, Atlantic and Indian Ocean islands
Gouanieae Reiss. ex Endl.	*Alvimiantha, Crumenaria, Gouania, Helinus, Pleuranthodes, Reissekia*	tropical and warm Americas, Africa, Madagascar, NW India, Indian Ocean Islands
Pomaderreae Reiss. ex Endl.	*Blackallia, Cryptandra, Pomaderris, Siegfriedia, Spyridium, Trymalium*	Australia, New Zealand
Rhamneae Hook. f.	*Auerodendron, Berchemia, Berchemiella, Condalia, Dallachya, Karwinskia, Krugiodendron, Reynosia, Rhamnella, Rhamnidium, Rhamnus, Sageretia, Scutia*	tropics to northern temperate regions
Maesopsideae Weberb.	*Maesopsis*	tropical Africa
Ventilagineae Hook. f.	*Smythea, Ventilago*	Old World tropics
Ampeloziziphеae J. F. Richardson	*Ampeloziziphus*	Brazil
Doerpfeldieae J. F. Richardson	*Doerpfeldia*	Cuba
Bathiorhamneae J. F. Richardson	*Bathiorhamnus*	Madagascar
Genera incertae sedis	*Ceanothus*	USA
	Emmenosperma	Australia, New Guinea, New Caledonia, Fiji
	Schistocarpaea	Australia
	Alphitonia	Malaysia, Australia, Polynesia, Hawaii
	Colubrina	tropical and warm areas in the Americas, Africa and southeast Asia
	Lasiodiscus	Africa, Madagascar

MacClade does not significantly increase tree length. With *trnL-F, Pleuranthodes* is strongly supported in an embedded position in Gouanieae. We view this shift as merely an indication that *rbcL* has no clear signal for *Pleuranthodes,* which is indicated by strong support at several nodes as a member of Gouanieae in the combined tree. Gouanieae are climbers with tendrils and longitudinally winged fruits; Colletieae are a group of strongly armed trees or shrubs. Australian Pomaderreae are characterized by the presence of stellate hairs.

Ziziphus, Paliurus, and *Hovenia* make up another strongly supported tribe in the combined analysis, Paliureae. *Hovenia* appears to have a close relationship with *Ziziphus* and *Paliurus* in that they both have cymose inflorescences, a base chromosome number of $x = 12$, and a similar pollen exine structure, and on the basis of this evidence *Hovenia* is placed in Paliureae. The strongly supported, predominantly South African Phyliceae, consisting of *Phylica, Nesiota,* and *Noltea,* also appear distinct and are generally characterized by having an ericoid shrubby habit, inferior ovaries, and leaves with revolute margins and tomentose undersurfaces.

Colubrina, which includes trees or shrubs with the nectariferous disc filling the receptacle and surrounding the ovary, and the similar *Lasiodiscus* were always thought to be closely related (Johnston, 1971; Figueiredo, 1995), but only the *rbcL* matrix produced trees in which *Colubrina* and *Lasiodiscus* form a clade. Further sampling of *Lasiodiscus* and studies of other sequences might be necessary to lend more molecular support for a *Colubrina/Lasiodiscus* grouping. The two morphologically similar genera may eventually be treated as a distinct tribe, but at this time there is insufficient evidence to recognize this group.

The affinities of a number of other genera are unclear. The arborescent genus *Alphitonia* from Malaysia, Australia, and the western Pacific has fruit exocarps that are thick, spongy, and friable at maturity, and *Emmenosperma* is similar in having red arillate seeds persisting on the receptacle after dehiscence. Again further evidence is needed to place these two genera together in a separate tribe. According to the *trnL-F* and combined analyses, *Schistocarpaea* appears to be closely related to Colletieae, in spite of having few supporting morphological characters.

The North American genus *Ceanothus* is characterized by having receptacles and nectariferous discs persisting on the pedicel, and its relationship with the other clades is unresolved. *Ceanothus,* and a number of genera in Colletieae (*Colletia, Discaria, Kentrothamnus, Retanilla,* and *Trevoa*) engage in root nodular fixation of nitrogen in a symbiotic association with the actinomycete bacterium *Frankia* (Baker and Schwintzer, 1990). Soltis et al. (1995) showed that root-nodulating angiosperms fall within one clade and may share a genetic predisposition to nodulation, even though most of the members of this clade do not nodulate. Root-nodulating members of Rhamnaceae also fall within one clade, the ziziphoids, in which most of the members do not nodulate, but relationships between groups within this clade are not clearly resolved. It could be that with additional data *Ceanothus* and Colletieae could end up as sister taxa, in which case root nodulation has arisen only once within the family (with a subsequent loss in *Adolphia*).

The molecular trees from the combined analysis have been used in conjunction with a phylogenetic analysis of morphological data to recircumscribe the tribal classification of Rhamnaceae (Richardson et al., 2000). Tribes were delimited on the basis of strong bootstrap support in the molecular results with additional support from morphology (summarized in Table 8).

Biogeography of Rhamnaceae—Raven and Axelrod (1974) stated that "Rhamnaceae are so well represented both in tropical and temperate regions that it is difficult to trace the history of the family." The Rose Creek flower was ascribed to Rosaceae by Basinger and Dilcher (1984), but it is clearly a member of Rhamnaceae with obhaplostemonous flowers and

"rhamnaceous" pollen. This plant has been dated to 94–96 million years old, and thus it establishes this as the minimum age for the family. Given such an age, it is likely that Rhamnaceae had already become cosmopolitan before the continents became widely separated, and the following discussion is based upon this assumption.

Two general patterns in the distribution of the three major groups within Rhamnaceae can be observed. The ampeloziziphoid group illustrates a pattern of disjunction also found in other groups between northern South America and Madagascar (e.g., Fay et al., 1998). In this case there are long branch lengths and a lack of morphological similarities, indicating that this group is ancient and probably had a much wider distribution subsequently reduced by extinction, particularly in Africa. The other major groups likely had a similarly wide distribution not reduced by extinction to the same extent as the ampeloziziphoid group. Overlaid on this pattern, is another, presumably post-Gondwanan, in which groups are more or less restricted to individual plates or later separating plates (e.g., Colletieae in South America, Australia, and New Zealand, Phyliceae in Africa, and Pomaderreae in Australia).

The ziziphoid group is cosmopolitan with a predominantly southern hemisphere distribution and could be of Gondwanan origin with the exception of *Ceanothus,* which has a North American distribution. This indicates that either this whole southern group was widespread throughout Gondwanaland and parts of Laurasia (in what is now North America) and subsequently contracted, largely to the southern hemisphere, or that *Ceanothus* arrived at its present location by long-distance dispersal. California has many examples of relictual taxa from lineages that are otherwise restricted to the Old World or the southern hemisphere; these include species of *Paeonia* (Paeoniaceae), *Odontostomum* (Tecophilaeaceae; Brummitt et al., 1998) and *Fremontodendron* (Malvaceae; Bayer et al., 1999). *Ceanothus* is sister to other clades within the ziziphoid group and morphologically highly autapomorphous (Richardson et al., 2000); therefore we do not consider it to be a recent derivative of one of these clades and thus the most likely explanation for its present distribution is that it is relictual and reasonably old (~65 million years).

Gouanieae have a similar distribution to the ampeloziziphoid group, with some genera of the group also being found in Africa. *Colubrina* is predominantly found in northern South America, although species are also found in Asia, Hawaii, Madagascar, and South Africa. *Lasiodiscus* is found in Africa and Madagascar, and this distribution may represent the remnants of previously more widespread groups that are now only found on Madagascar or in rain and coastal forests in the tropical parts of sub-Saharan Africa and east Africa. *Alphitonia,* Pomaderreae, and *Schistocarpaea* are Australasian taxa, representing isolated clades. Colletieae are a mostly South American group, but two species of *Discaria* are found in Australia and New Zealand. This is a southern hemisphere disjunction, which is also found in other groups such as *Orthrosanthus, Libertia, Berberidopsis* and *Eucryphia,* and these are probably relicts of formerly more widespread groups that were present throughout southern South America, east Antarctica, Tasmania, New Zealand, and eastern Australia (Africa and Madagascar having split earlier from Gondwana).

Within Rhamneae, relationships are not clearly resolved by *trnL-F* and *rbcL* sequence data. A more in-depth molecular study using a more variable region and additional taxon sampling is needed to clarify relationships before any biogeographical conclusions can be drawn. However, they do form a strongly supported monophyletic unit that has a wide distribution throughout the tropics and into northern temperate regions. Ventilagineae, found in the Old World tropics but with a center of diversity in India, could have had a Gondwanan origin and subsequently spread into Asia when India collided with Asia. More species in each genus throughout the family need to be analyzed to make a fine-scale biogeographic assessment.

General conclusions—Rhamnaceae are an old monophyletic family with Barbeyaceae and Dirachmaceae forming their sister. Further data from other fields such as anatomy or chemistry are necessary to provide more evidence for the three "cryptic clades" resolved by the molecular data. Although there is strong molecular support for these major divisions in Rhamnaceae, we have been unable to identify morphological characters that could adequately describe these groups (see also, Richardson et al., 2000).

What is clear from these results is that the tribes Rhamneae and Zizipheae as circumscribed by Suessenguth are unnatural, and a reclassification of some tribes in Rhamnaceae is necessary. All of the tribes proposed by Richardson et al. (2000) are strongly supported by the bootstrap in the combined analysis. The molecular data indicate that many morphological character states have evolved in parallel (e.g., leaf venation patterns, fruit type, and pollen exine architecture), but it is not a simple matter of morphology vs. molecules. Classifications based on one particular morphological character (such as Suessenguth's reliance on fruit characters) often do not compare well with those based on many morphological characters. A classification based largely on molecular data with the support of some morphological characters seems a better solution.

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