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Phylogeny of *Rhus* (Anacardiaceae) Based on Sequences of Nuclear *Nia*-i3 Intron and Chloroplast *trnC-trnD*

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ABSTRACT. A phylogenetic analysis of *Rhus* (Anacardiaceae) was conducted using nuclear and chloroplast sequences. The nuclear (*Nia*-i3) and chloroplast (*trnC-trnD*) sequence data generated in this study were compared with previously published phylogenies of *Rhus* based on nuclear ribosomal ITS data and chloroplast *trnL-F* and *ndhF* sequences. The *Nia*-i3 data provided more parsimony-informative characters than ITS; the *trnC-trnD* data provided the most parsimony-informative characters among three chloroplast markers. All data sets support the monophyly of *Rhus*. Within *Rhus*, nuclear data support the monophyly of subgen. *Lobadium* and the monophyly of subgen. *Rhus*. Chloroplast data suggest a paraphyletic subgenus *Lobadium* with *R. microphylla* and *R. rubifolia* of subgen. *Lobadium* placed within subgen. *Rhus*. *Rhus coriaria* and *R. michauxii* of subgen. *Rhus* also have discordant positions in cpDNA and nuclear trees. Each species with discordant positions (*R. coriaria*, *R. microphylla*, *R. michauxii* and *R. rubifolia*) has a single allele or different alleles of the same species forming a monophyletic group in the nuclear ITS and *Nia*-i3 data. Incongruence among nuclear and chloroplast datasets, together with the phylogenetic positions, sympatric distributions, and morphological intermediacy of discordant taxa, suggest possible reticulate evolution among members of *Rhus*.

KEYWORDS: Anacardiaceae, *Nia-i3*, phylogeny, reticulate evolution, *Rhus*, *trnC-trnD*.

Rhus Tourn. ex L., the sumac genus (Anacardiaceae), is one of the most widespread and recognizable genera in the North Temperate Zone. With 35 species, the genus exhibits a disjunct distribution in Eurasia, North America, and Hawaii (Barkley 1937; Miller et al. 2001; Yi et al. 2004). All *Rhus* species studied so far are diploids with $2n = 30$ ($x = 15$; Löve and Connor 1982; Mullingan 1984; Parfitt et al. 1985; Wcislo 1987; Parfitt et al. 1990; Shang et al. 1990; Singhal and Gill 1990). Barkley (1937) recognized two subgenera within *Rhus*: subgen. *Rhus* and subgen. *Lobadium.* Subgenus *Lobadium* was subdivided into five sections: *Lobadium* (Raf.) DC., *Pseudoschmaltzia* Barkley, *Pseudosumac* Barkley, *Rhoeidium* (Greene) Barkley and *Styphonia* (Nutt.) Barkley; Barkley 1937). Young (1978) merged Barkley's (1937) sect. *Rhoeidium* with sect. *Lobadium*, and sect. *Pseudoschmaltzia* with sect. *Styphonia*, thus recognizing only three sections within subgen *Lobadium* (sect. *Lobadium* (Raf.) DC., sect. *Styphonia* (Nutt. in T. & G.) Barkley, and sect. *Terebinthifolia* Young). He also divided sect. *Styphonia* into three subsections (*Compositae* Young, *Intermediae* Young, and *Styphoniae* Young). A classification scheme has not been proposed for subgen. *Rhus*.

Two molecular studies have investigated the evolutionary history of *Rhus* (Miller et al. 2001; Yi et al. 2004). Miller et al. (2001) sampled 13 species of *Rhus* in the broad analysis of the relationships of the *Rhus* complex, which includes *Actinocheita* (DC.) Barkley, *Cotinus* Miller, *Malosma* (Nutt.) Abrams, *Melanococca* Blume, *Metopium* P. Brown, *Searsia* F. A. Barkley, and *Toxicodendron* Miller. With a broader taxon sampling of *Rhus* (25 species), Yi et al. (2004) used chloroplast (*trnL-F* and *ndh*F) and ITS sequences to clarify the intrageneric relationships within the genus. The monophyly of *Rhus* was strongly supported by both molecular analyses; however, the relationships among subgen. *Rhus* and subgen. *Lobadium* were not well resolved. The ITS data in both studies suggested a paraphyletic subgen. *Rhus* with a monophyletic subgen. *Lobadium* nested within it (Miller et al. 2001; Yi et al. 2004). In contrast, analyses of chloroplast sequence data (*ndhF* and *trnL-F*; Yi et al. 2004) resolved a paraphyletic subgen. *Lobadium* with a monophyletic subgen. *Rhus* nested within it. A third scenario was suggested with combined ITS and cpDNA sequences (Yi et al. 2004): species of subgen. *Rhus* formed a monophyletic group and species of subgen. *Lobadium* were monophyletic.

In earlier phylogenetic analyses of *Rhus*, the relative positions of several species were incon-

gruent between the chloroplast and nuclear data (Yi et al. 2004). Incongruence between nuclear and chloroplast datasets can be indicative of reticulate evolution through chloroplast capture and ancient hybridization events, ongoing gene flow, or unrecognized paralogy problems with nrDNA (Doyle 1992; Wendel et al. 1995). Discordant phylogenetic positions of *Rhus* taxa in chloroplast and nuclear datasets were only weakly supported in previous studies, and need to be examined more thoroughly to understand the role of reticulation, if any, in the evolutionary history of the genus. We employed two additional markers to better resolve the relationships within *Rhus*: the third intron of nuclear nitrate reductase (*Nia*-i3) gene, and the chloroplast *trnC-trnD* region (the *trnC-petN* intergenic spacer, the partial *petN* gene, the *petN-psbM* intergenic spacer, the partial *psbM* gene, the *psbM-trnD* intergenic spacer, and the partial *trnD* gene). *Nia*-i3 and the *trnC-trnD* region have been useful in resolving relationships among closely related species (Howarth and Baum 2002; Lee and Wen 2004). In this study, the phylogeny of *Rhus* was estimated using the nuclear *Nia*-i3 and chloroplast *trnC-trnD* sequences. These sequences were then compared, and where appropriate, combined with previously published data sets based on ITS, *ndhF* and *trnL-F* sequences.

The objectives of this study were to: 1) test the utility of *Nia*-i3 and *trnC-trnD* for phylogenetic reconstruction of *Rhus*; 2) clarify evolutionary relationship of subgen. *Lobadium* and subgen. *Rhus*, and detect possible evidence for reticulation between the two subgenera; and 3) examine relationships of species within subgen. *Lobadium* and subgen. *Rhus*.

Materials and Methods

Species Examined. Twenty-two of the estimated 35 *Rhus* species were included in this study. All ten subgen. *Rhus* species recognized by Barkley (1937) and Young (1975, 1978, 1979) were sampled (Appendix 1). Twelve (of 25) species of subgen. *Lobadium* were included in the study, with three species from sect. *Lobadium*, eight species from sect. *Styphonia*, and one species from sect. *Terebinthifolia* (Appendix 1). We sampled both subgenera throughout their distribution and included representatives of subgen. *Lobadium* corresponding to all five sections of Barkley (1937) or all three sections of Young (1978). Representative species of four genera in the *Rhus* complex, *Actinocheita*, *Malosma*, *Searsia*, and *Toxicodendron* were chosen as outgroups because of their close relationships to *Rhus* (Miller et al. 2001; Pell 2004). *Pistacia* and *Schinus*, two genera that have never been considered to be a part of the *Rhus* complex, but are grouped with *Rhus* in tribe Rhoeae within Anacardiaceae, were included as more distant outgroups.

DNA Extraction, PCR Amplification, and Sequencing. Total DNA was extracted from silica-gel dried or fresh leaf material following the CTAB method of Doyle and Doyle (1987). DNA amplifications were performed in 20 µl reactions with approximately 10–50 ng of total DNA, 20 mM Tris buffer (pH 8.3, with 50 mM KCl, 1.5 mM $MgCl_2$, and 0.1% Tween 20), 0.15 mM of each dNTP, 5 µM of each primer, 2 µl of *Taq* polymerase. Amplification of the *Nia*-i3 region employed primers NIA3F and NIA3R (Howarth and Baum, 2002). The *trnC-trnD* region was amplified with three pairs of primers: trnC and petN2R, petN1 and psbM2R, and psbM1 and trnD as in Lee and Wen, 2004. The PCR products were electrophoresed using 1% low-melting-point NuSieve GTG agrose gels (FMC BioProducts, Rockland, Maine), in 1× Tris-acetate buffer (pH 7.8), with one-tenth the EDTA concentration (Sambrook et al., 1989), and containing ethidium bromide. The amplicon was cut from the gel and digested using the GELase™ Agarose Gel-Digesting preparation, using the ''Fast Protocol'' method (Epicentre Technologies, Madison, Wisconsin). The sequencing reaction was performed in a 10 µl final volume using the BigDye Terminator cycle sequencing kit (PE Applied Biosystems, Foster City, California) following the manufacturer's instructions. The same amplification primers were used for sequencing. The sequencing reaction products were viewed with an ABI 3100 automated DNA sequencer (Applied Biosystems). The resulting sequences were aligned and edited using Sequencher version 3.1.1 (GeneCodes Corporation, Ann Arbor, Michigan). Alignments were further adjusted by eye in PAUP*4.0b10 (Swofford, 2003). All sequences have been deposited in GenBank (Appendix 1).

Directly sequencing the *Nia*-i3 PCR products of *R. aromatica*, *R. coriaria*, *R. glabra*, *R. michauxii*, *R. microphylla*, and *R. typhina* produced ambiguous sequences. The PCR products of these samples were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, California). Initially six clones for each sample were selected and sequenced. When the discordant positions of *R. coriaria*, *R. michauxii*, *R. microphylla* and *R. rubifolia* were observed in the chloroplast and nuclear data, we further cloned the nuclear ITS and *Nia* of these species. Ten to 27 clones were sequenced for each marker of these species. Only one sequence was included in the analysis when different clones produced identical sequences; when different sequences were found for a species, all sequences were included.

Phylogenetic Analysis. Phylogenetic analyses were conducted for five data sets: (1) nuclear *Nia*-i3; (2) combined nuclear data (*Nia*-i3 and ITS); (3) chloroplast *trnC-trnD*; (4) combined chloroplast data (*trnC-trnD*, *ndhF*, and *trnL-F*); and (5) combined nuclear and chloroplast data matrices (minus two discordant taxa, see below). Parsimony analyses with PAUP*4.0b10 (Swofford, 2003) were performed with heuristic searches, tree-bisection-reconnection (TBR) branch swapping, MULTREES option, and 100 random taxon addition. Tree topologies did not change with gaps included in analyses; however, support values along some branches were higher. We thus coded each different gap as a separate binary character following the method of Simmons and Ochoterena (2000). Internal branch support was estimated with 1000 bootstrap replicates (Felsenstein 1985), using the same heuristic search strategy as above.

Bayesian analyses were conducted using MrBayes version 3.0 (Huelsenbeck and Ronquist 2001). The MCMC algorithm was run for 2,000,000 generations with 6 incrementally heated chains, starting from random trees and sampling one out of every 100 generations. A majority-rule consensus tree was calculated with PAUP* from the last 18,001 out of the 20,001 trees sampled. The first 2,000 trees (burn-in) were excluded to avoid trees that might have been sampled prior to convergence of the Markov chains. The posterior probability of each topological bipartition was estimated by the frequency of these bipartitions across all 18,001 trees sampled. Clades with posterior probabilities $\geq$ 95% were

considered well supported. All trees presented here were submitted to TreeBASE (study number S1770). Characteristics of the data matrices and the tree statistics for *Nia*-i3 and *trnC-trnD* data are presented in Tables 1 and 2, together with statistics for ITS, *ndh*F, and *trnL-F* data.

Tests of Data Incongruence. Independent Length Difference (ILD; Farris et al. 1995), Templeton's (Templeton 1983), and Shimodaira-Hasegawa (SH) tests (Shimodaira and Hasegawa 1999) are three commonly used methods to evaluate congruence between different data sets. The ILD test was used to detect the difference of tree lengths between combined data partitions and each data partition. This test is sensitive to differences in among-site rate variation between partitions, overall evolutionary rates, levels of noise, and relative size of data partitions, but is still a useful, conservative initial test of congruence between data partitions (reviewed by Hipp et al. 2004). Templeton's test and SH test were used to compare tree topologies from each data partition. The SH test tends to overestimate the confidence interval around the optimal tree, and Templeton's test tends to underestimate the confidence interval around optimal trees (Shimodaira 2002). These three tests were used to evaluate the congruence among the three data sets: (1) *Nia*-i3, (2) ITS, and (3) chloroplast data (*ndh*F, *trnL-F* and *trnC-trnD*). For all ILD tests, 100 replications were performed with PAUP*4.0b10 (Swofford 2003). Parsimony heuristic searches were employed with tree-bisection-reconnection (TBR) branch swapping, ACCTRAN character optimization, and gaps treated as missing data. Topological congruence between the gene trees was evaluated with Templeton's test, which was implemented in PAUP* as the nonparametric pairwise test. The SH test was executed with PAUP* with the sequence substitution model for each data set estimated using Modeltest version 3.6 (Posada and Crandall 1998), RELL optimization, and 1000 bootstrap (BS) replicates to compare the difference between the RELL optimization and the computationally much more intensive full optimization. We compared the optimal trees (unconstrained) from the maximum likelihood analysis of separate data sets with constraint trees.

RESULTS

Nuclear DNA Data. Eight species of *Rhus* (*R. aromatica*, *R. coriaria*, *R. glabra*, *R. lanceolata*, *R. michauxii*, *R. microphylla*, *R. potaninii*, and *R. typhina*) produced two or more sequences for the *Nia*-i3 region. Sequences from six of the eight species each formed a monophyletic group. *Rhus lanceolata*, however, showed a higher level of sequence heterogeneity. Two clonal sequences of *R. potaninii* formed a clade with *R. punjabensis*, and three clonal sequences of *R. lanceolata* formed a monophyletic group with *R. copallina* (Fig. 1).

Alignment of *Nia*-i3 data, including the outgroup taxa, required 67 gaps. Including only *Rhus* species, alignment of *Nia*-i3 data required 24 gaps. The strict consensus tree (Fig. 1) of 59 MPTs was consistent with the 50% majority-rule consensus of 18,001 trees (20,001 trees minus 2,000 burn-in trees) resulting from the Bayesian analysis.

The *Nia*-i3 data strongly supported the monophyly of *Rhus* sensu Barkley (1937). *Pistacia* was most closely related to *Rhus* among the outgroup

TABLE 1. Characteristics of different data sets and tree statistics for *Nia*-i3, ITS, *trnC-trnD*, *ndhF* and *trnL-F* for *Rhus* (excluding outgroup taxa) including number of species (specimens) sampled, number of characters, number of variable characters (VC) and percentage of variable characters (%), number of parsimony-informative characters (PIC), percentage of cells in the data matrix scored as missing or inapplicable (% miss.), pair-wise sequences divergence (PSD), number of most parsimonious trees (MPTs), MPT length, consistency index (CI), and rescaled consistency index (RC).

	Gaps treated as missing data					Each Gap coded as separate character				
	Nia-i3	ITS	*trnC-trnD*	*ndhF*	*trnL-F*	*Nia*-i3	ITS	*trnC-trnD*	*ndhF*	*trnL-F*
species #	22	22	22	22	22	22	22	22	22	22
char. #	687	715	2303	2073	940	712	721	2333	2079	954
VC # (%)	118 (17.18%)	93 (13.01%)	126 (5.47%)	111 (5.34%)	49 (5.21%)	143 (20.08%)	99 (13.73%)	156 (6.69%)	117 (5.63%)	63 (6.60%)
PIC# (%)	51 (7.42%)	64 (8.95%)	68 (2.95%)	65 (3.14%)	24 (2.55%)	69 (9.69%)	69 (9.57%)	89 (3.81%)	68 (3.27%)	33 (3.46%)
% miss.	2.27	0.60	1.79	2.58	0	2.19	0.60	1.78	2.57	0
PSD	0.15%–6.21%	0–6.08%	0–1.81%	0.05%–1.80%	0–1.66%	0.15%–6.81%	0–6.08%	0–1.88%	0.05%–1.80%	0–1.66 %
MPTs #	19	24	845	460	3600	16	24	30	799	1181
MPT length	132	133	140	137	53	159	149	190	152	74
CI	0.96	0.77	0.92	0.85	0.94	0.93	0.73	0.85	0.84	0.88
RI	0.96	0.88	0.91	0.86	0.94	0.94	0.85	0.85	0.85	0.87

TABLE 2. Matrix and tree statistics for *Nia*-i3, ITS, *trnC-trnD*, *ndhF* and *trnL-F* for *Rhus* and outgroup taxa, including number of species (specimens) sampled, number of characters, number of variable characters (VC) and percentage of variable characters (%), number of parsimony-informative characters (PIC), percentage of cells in the data matrix scored as missing or inapplicable (% miss.), pair-wise sequences divergence (PSD; = pair-wise sequence divergence between *Rhus* species and outgroup taxa), number of most parsimonious trees (MPTs), length of MPTs, consistency index (CI), and rescaled consistency index (RC).

	Gaps as missing data					Each gap coded as separate characters				
	Nia-i3	ITS	*trnC-trnD*	*ndhF*	*trnL-F*	*Nia*-i3	ITS	*trnC-trnD*	*ndhF*	*trnL-F*
species #	35	35	35	35	35	35	35	35	35	35
char. #	758	737	2425	2091	994	825	759	2464	2098	1011
VC # (%)	322 (42.48%)	200 (27.13%)	243 (10.02%)	182 (8.70%)	104 (10.46%)	389 (47.15%)	222 (29.25%)	282 (11.44%)	189 (9.01%)	121 (11.96%)
PIC# (%)	150 (19.79%)	142 (19.26%)	114 (4.70%)	101 (4.83%)	42 (4.23%)	189 (22.91%)	153 (20.16%)	140 (5.68%)	106 (5.05%)	52 (5.14%)
% miss.	2.08	1.25	1.64	1.68	0	1.91	1.21	1.62	1.68	0
PSD	5.88%–13.70%	4.96%–12.03%	0.78%–3.32%	0.41%–1.94%	0.44%–2.76%	5.37%–13.84%	4.96%–12.03%	0.78%–3.32%	0.41%–1.94%	0.44%–2.76%
MPTs #	108	24	193	5804	>10,000	54	48	400	>10,000	>10,000
MPT length	468	455	288	243	117	551	501	358	262	146
CI	0.86	0.61	0.89	0.81	0.93	0.85	0.60	0.83	0.79	0.88
RI	0.88	0.75	0.88	0.84	0.93	0.88	0.73	0.84	0.83	0.88

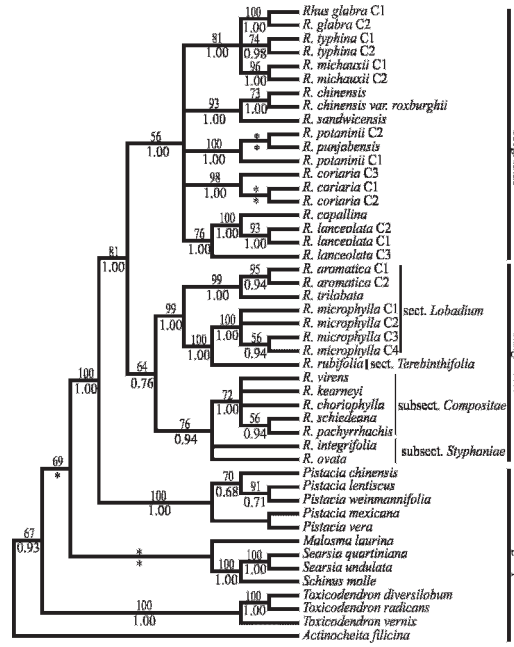

FIG. 1. The strict consensus tree of 59 MPTs of *Nia*-i3 data of *Rhus*, with gaps coded as separate binary characters (CI = 0.82, RI = 0.82). The bootstrap values in 1000 replicates >50% are shown above the branches, and the Bayesian posterior probabilities are indicated below the branches. * indicates bootstrap value less than 50%. C1, C2, C3, and C4 represent clonal sequences when the sample has multiple *Nia*-i3 copies.

taxa included in the study. Species of subgen. *Lobadium* formed a monophyletic group (Posterior Probability (PP) = 0.76, Bootstrap (BS) = 64%). Subgenus *Lobadium* species formed two clades, one corresponded to sect. *Styphonia*, and the other clade included species of sect. *Lobadium* and sect. *Terebinthifolia* plus one clonal sequence of *R. lanceolata* (subgen. *Rhus*). Aside from this clonal sequence of *R. lanceolata*, species of subgen. *Rhus* formed a monophyletic group (PP = 1.00, BS = 56%). Within the subgen. *Rhus* clade, five subclades were recognizable: (1) *R. glabra-R. michauxii-R. typhina* (PP = 1.00; BS = 81%); (2) *Rhus chinensis* var. *chinensis-R. chinensis var roxburghii-R. sandwicensis* (PP = 1.00, BS = 93%); (3) *R. coriaria*; (4) *R. copallina-R. lanceolata* (PP = 1.00, BS = 76%); and (5) *R. potaninii-R. punjabensis* (PP = 1.00, BS = 100%; Fig. 1).

The ILD, Templeton's, and SH tests showed the *Nia*-i3 and ITS data sets were marginally congruent. With the *Nia*-i3 strict consensus tree as the constraint topology, all 25 ITS trees were incongruent to *Nia*-i3 data in the Templeton's test (Table 3; $P < 0.01$), and when using the ITS strict consensus tree as the constraint topology, 54 of 108 MPTs were incongruent to ITS data in the Templeton's test (Table 3). However, when the

TABLE 3. The ILD, Templeton's, and Shimodaira-Hasegawa (SH) tests of *Rhus* and its outgroup taxa. Data were divided into *Nia*-i3, ITS, the combined *Nia*-i3 and ITS, and the combined cpDNA partition for analysis. The data matrices in the first line were used as the constraints.

	Tests	*Nia*-i3	ITS	*Nia*-i3 + ITS	cpDNA
Nia-i3	ILD	—	0.440	—	0.010
	Templeton	—	0.009–0.128	0.082–1.000	<0.001
	SH	—	0.016	0.052	<0.001
ITS	ILD	0.440	—	—	0.010
	Templeton	<0.001–0.003	—	0.136–0.868	<0.001
	SH	0.010	—	0.061	<0.001
Nia-i3 + ITS	ILD	—	—	—	0.010
	Templeton	0.125–1.000	0.219–1.000	—	<0.001
	SH	0.371	0.662	—	<0.001
cpDNA	ILD	0.010	0.010	<0.001	—
	Templeton	<0.001	<0.001	<0.001	—
	SH	<0.001	<0.001	<0.001	—

combined *Nia*-i3 and ITS data were used as the constraint tree, all three tests showed *Nia*-i3 data and ITS data were congruent to the combined tree (Table 3; $P > 0.05$). The most strongly supported clades in the ITS tree (see Fig. 2 of Yi et al. 2004) were also strongly supported in *Nia*-i3 data (Fig. 1): (1) Species of *Rhus* form a monophyletic group; (2) *Rhus lanceolata* and *R. copallina* are sister taxa; (3) *R. chinensis* and *R. sandwicensis* form a clade; (4) *R. punjabensis* and *R. potaninii* form a clade; (5) *R. typhina, R. glabra*, and *R. michauxii* form a clade; (6) species of subgen. *Lobadium* form a monophyletic clade (moderate support in *Nia*-i3 dataset); (7) members of subgen. *Lobadium* sect. *Styphoniae* form a clade (*R. virens, R. kearneyi, R. choriophylla*, *R. schiedeana, R. pachyrrachis, R. integrifolia*, and *R. ovata*); (8) members of subgen. *Lobadium*, sect. *Styphonia*, subsect. *Compositae* form a clade (*R. virens, R. kearneyi, R. choriophylla, R. schiedeana*, and *R. pachyrrachis*); and (9) *R. aromatica* and *R. trilobata* are sister taxa. The primary difference between the *Nia*-i3 and ITS datasets is the status of subgen. *Rhus*. In the ITS data, subgenus *Rhus* is paraphyletic with subgen. *Lobadium* nested within it (see Fig. 2 of Yi et al. 2004); in the *Nia*-i3 data, subgenus *Rhus* was resolved as a monophyletic group (PP = 1.00; BS = 79%).

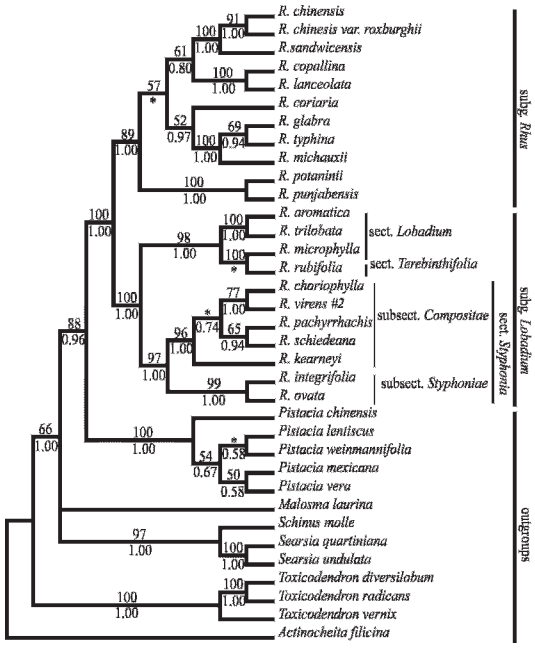

FIG. 2. The strict consensus tree of two MPTs of the combined ITS and *Nia*-i3 data of *Rhus* with gaps coded as separate binary characters (CI = 0.72, RI = 0.80). The bootstrap values in 1000 replicates >50% are shown above the branches, and the Bayesian posterior probabilities are indicated below the branches. * indicates bootstrap value less than 50%.

We combined ITS and *Nia*-i3 data sets based on their support of most clades. In species for which multiple clones were found but the clones formed a monophyletic group, we included only one clonal sequence in the combined analysis (*R. aromatica*, *R. coriaria*, *R. glabra*, *R. michauxii*, *R. microphylla*, *R. typhina*). When different clonal sequences of the same species of *R. lanceolata* and *R. potaninii* do not form a monophyletic group, we arbitrarily chose clonal sequence 1 in combined analyses. Coding each gap as a separate binary character, the combined *Nia*-i3 and ITS dataset has 1583 aligned positions, 608 variable characters, and 342 parsimony-informative characters. The parsimony analysis produced two MPTs of 1059 steps (CI = 0.72; RI = 0.80; and RC = 0.58). The strict consensus tree of the parsimony analysis was consistent with the 50% majority-rule consensus of 18,001 trees (20,001 trees minus 2,000 burn-in trees) that resulted from the Bayesian analysis

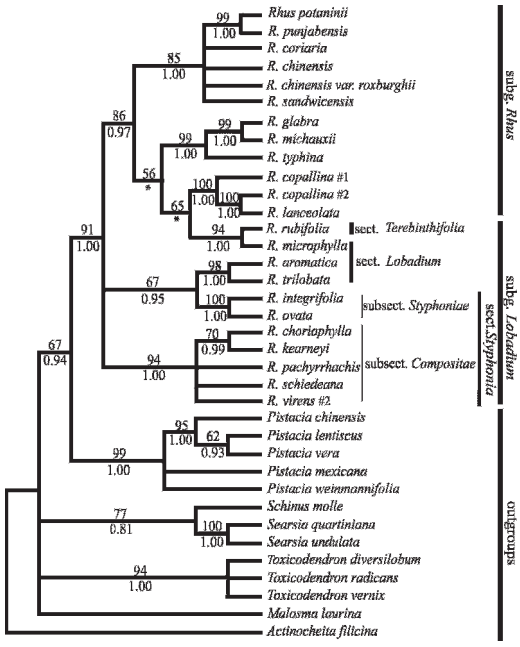

FIG. 3. The strict consensus tree of 400 MPTs of *trnC-trnD* data of *Rhus*, with gaps coded as separate binary characters (CI = 0.83, RI = 0.84). The bootstrap values in 1000 replicates >50% are shown above the branches, and the Bayesian posterior probabilities are indicated below the branches. * indicates bootstrap value less than 50%.

(Fig. 2). The combined ITS and *Nia*-i3 tree was largely congruent with *Nia*-i3 tree. Subgenus *Lobadium* was well supported as a monophyletic group in the combined tree (PP = 1.00; BS = 100%). In addition, subg. *Rhus* was well-supported as a monophyletic group (PP = 1.00, BS = 89%). The combined tree resolved two clades that were not observed in the *Nia*-i3 tree: (1) *R. copallina–R. lanceolata* clade was weakly supported to be sister of *R. chinensis–R. chinensis var. roxburghii–R. sandwicensis* clade (PP = 0.80; BS = 61%); and (2) *R. coriaria* was weakly supported to be sister of *R. glabra–R. michauxii–R. typhina* clade (PP = 0.97; BS = 52%).

Chloroplast DNA Data. The alignment of *trnC-trnD* sequences of *Rhus* and outgroup taxa required 39 gaps. Including only *Rhus* species, the aligned *trnC-trnD* data has 33 gaps. The strict consensus tree of 400 MPTs of *trnC-trnD* is presented in Fig. 3. *Rhus* was strongly supported as monophyletic (PP = 1.00, BS = 91%). *Pistacia* was sister to *Rhus* among included outgroup taxa. With two notable exceptions (*R. microphylla* and *R. rubifolia*), species of subgen. *Lobadium* were resolved into two clades: (1) *Rhus choriophylla–R. kearneyi–R. pachyrrhachis–R. schiedeana–R. virens;* and (2) *R. aromatica–R. trilobata* and *R. integrifolia–R. ovata* (Fig. 3). *Rhus microphylla* and *R. rubifolia*, two taxa that have been considered a part of subgen. *Lobadium* based on morphological and nuclear sequence data (Young 1978, 1979; Miller 2001; Yi et al. 2004) formed a monophyletic clade (PP = 1.00; BS = 94%) that was nested within subgen. *Rhus* as the sister of the *R. copallina–R. lanceolata* clade. The eastern Asian *R. chinensis*, *R. chinensis* var. *roxburghii*, *R. potaninii*, and *R. punjabensis* formed a clade with the southern European and western Asian *R. coriaria* and the Hawaiian *R. sandwicensis* (PP = 1.00, BS = 85%). The North American subgen. *Rhus* species were resolved into two strongly supported clades, the *R. copallina-R. lanceolata* clade (PP = 1.00, BS = 100%) and the *R. glabra-R. michauxii-R. typhina* clade (PP = 1.00, BS = 100%). *Rhus glabra* and *R. michauxii* were strongly supported as sisters (PP = 1.00, BS = 99%), and *R. typhina* was sister to the *R. glabra-R. michauxii* clade (PP = 1.00, BS = 99%).

Because the chloroplast genome behaves as a single recombination unit, we combined the *trnC-trnD*, *ndhF*, and *trnL-F* data in our analysis. When coding gaps as separate data, the aligned data matrix had 5573 total characters with 599 variable and 298 parsimony-informative sites. Maximum parsimony analysis produced 270 MPTs with a length of 772 steps, a CI of 0.82, an RI of 0.84, and an RC of 0.69. The strict consensus tree (Fig. 4.) was congruent with the 50% majority-rule consensus of the 18,001 trees (20,001 trees minus 2000 burn-in trees) resulting from the Bayesian inference. The combined cpDNA tree was largely consistent with the *trnC-trnD* tree, but the combined data showed a higher resolution (cf. Figs. 3, 4).

Data Congruence/Incongruence Between Chloroplast and Nuclear Data. Congruence between the combined nuclear (ITS + *Nia*-i3) and combined chloroplast data sets (*ndhF* + *trnC-trnD* + *trnL-F*) was assessed using *Pistacia chinensis* as the sole outgroup. All previous analyses indicated that *P. chinensis* was the outgroup that was most closely related to *Rhus*; furthermore, the removal of excess outgroups prevented analytical complications resulting from the complex and poorly resolved relationships among the outgroups. The ILD, Templeton's, and SH tests revealed incongruence between the combined nuclear and chloroplast data (Table 3). We examined the trees from the separate analyses and found that the discordant relationships reflected whether or not species of subgen. *Lobadium* formed a monophyletic group, and the phylogenetic positions of the *R. microphylla –R. rubifolia* clade (Figs. 2, 4). Following the conditional combination approach, we excluded these two species and conducted parsimony

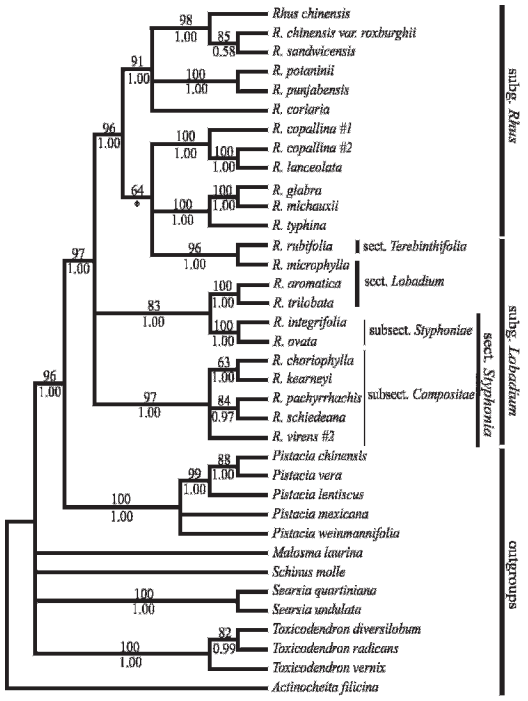

FIG. 4. The strict consensus tree of 270 MPTs of the combined chloroplast DNA data of *Rhus* with gaps coded as separate binary characters (CI = 0.82, RI = 0.84). The bootstrap values in 1000 replicates >50% are shown above the branches, and the Bayesian posterior probabilities are indicated below the branches. * indicates bootstrap value less than 50%.

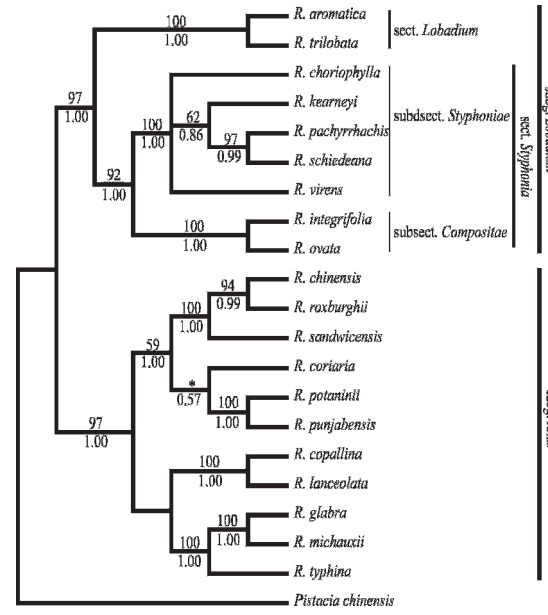

FIG. 5. The strict consensus tree of two MPTs of the combined nuclear and chloroplast DNA data of *Rhus* with gaps coded as separate binary characters (CI = 0.82, RI = 0.81). The bootstrap values in 1000 replicates >50% are shown above the branches, and the Bayesian posterior probabilities are indicated below the branches. * indicates bootstrap value less than 50%.

analysis of three data sets: (1) ITS + *Nia*-i3, (2) chloroplast, and (3) combined nuclear and chloroplast (Bull et al. 1993; Johnson and Soltis 1998). The combined nuclear and chloroplast data produced two MPTs (CI = 0.82; RI = 0.81). Our visual examination found no topological conflicts among the trees from the three data sets, except that the combined chloroplast and nuclear data (Fig. 5) had higher resolution. Interestingly, ILD, Templeton's, and SH tests all suggested incongruence between the chloroplast and nuclear data. When we used the combined chloroplast and nuclear tree as the constraint topology, the SH test suggests incongruence between the nuclear and combined data ($p < 0.01$), and the chloroplast and combined data ($P < 0.01$). Templeton's test suggests the congruence between chloroplast and combined data ($P > 0.01$), but incongruence between nuclear and combined data ($P < 0.01$). When using either the chloroplast trees or the nuclear trees as the constraint topology, congruence between chloroplast and combined data, as well as between nuclear and combined data, is well supported ($p > 0.01$). We therefore presented the single maximally parsimonious tree of the combined nuclear and chloroplast data in Fig. 5. In the combined nuclear and chloroplast tree, subgenus *Rhus* was strongly supported as monophyletic (PP = 1.00; BS = 97%), within which species from Eurasia and Hawaii form a clade (PP = 1.00; BS = 59%) and species from North America form a clade (PP = 0.69; BS < 50%). Species from subgen. *Lobadium* form another clade (PP = 1.00; BS = 97%). Sections *Lobadium* and *Styphonia* were strongly supported and two subsections of sect. *Styphoniae* were also well supported.

Sequence Characteristics of* Nia-*i3 and* trnC-trnD *in comparison with other markers. Within *Rhus*, the *Nia*-i3 region provided more variable characters than ITS (118 vs. 93, 17.18% vs. 13.01%; Table 1). The pair-wise sequence divergence varied from 0.15% to 6.81% in the *Nia*-i3 data, and 0 to 6.08% in the ITS data of *Rhus*. *Nia*-i3 has slightly fewer parsimony-informative characters than ITS (51 vs. 64, 7.42% vs. 8.95%) with gaps treated as missing data (Table 1). Eighteen of the 25 coded gaps are parsimony-informative in *Nia*-i3 data, and five of six coded gaps are parsimony-informative in the ITS data. The *Nia*-i3 (69, 9.69%) and ITS (69, 9.57%) had similar parsimony-informative characters when gaps were coded as separate characters (Table 1). Among *Rhus* and closely related genera, *Nia*-i3 provided more parsimony-informative characters, and had greater sequence divergence than

ITS (Table 3). The pair-wise sequence divergence among *Rhus* species and closely allied genera ranged from 5.37% to 13.84% in *Nia*-i3, and from 4.96% to 12.03% in ITS (Table 2). Thirty-eight of the 67 coded gaps in *Nia*-i3 (vs. 11 of the 22 coded gaps in ITS) are parsimony-informative among *Rhus* and allied genera (Table 2).

Within *Rhus*, the *trnC-trnD* (68, 2.95%) provided slightly more parsimony-informative characters than the ITS region (64, 8.95%) and the *ndhF* gene (65, 3.14%), and many more parsimony-informative characters than *trnL-F* (24, 2.55%; Table 1). The pairwise *trnC-trnD* sequence divergence within *Rhus* varied from 0 to 1.81%, 0.05% to 1.80% in *ndhF*, and 0 to 1.66% in *trnL-F* (Table 1). With gaps coded as separate binary characters, 30 coded gaps provided 21 parsimony-informative characters in *trnC-trnD* (three of six gaps in *ndhF*, and nine of 14 gaps in *trnL-F*, Table 1). Among *Rhus* and its closely allied genera, *trnC-trnD* (114, 4.70%) provided fewer parsimony-informative characters than ITS (142, 19.26%), but more than *ndhF* (101, 4.83%) and *trnL-F* (42, 4.12%; Table 2). The pairwise sequence divergence among *Rhus* and closely related genera varied from 0.78% to 3.32% in *trnC-trnD*, from 0.41% to 1.94% in *ndhF* data, and from 0.44% to 2.76% in *trnL-F* data (Table 2). With gaps coded as separate binary characters among *Rhus* and allied genera, 26 of 36 gaps in *trnC-trnD* (vs. five of seven gaps in *ndhF*, and 10 of 17 gaps in *trnL-F*) are parsimony-informative (Table 2).

DISCUSSION

***Monophyly of* Rhus.** *Rhus* sensu Barkley (1937) is morphologically heterogeneous. The only known morphological synapomorphy of the genus is the presence of red fruits with glandular hairs (Young 1978; Miller et al. 2001; Yi et al. 2004). Despite the heterogeneity of this group, the monophyly of *Rhus* sensu Barkley (1937) was strongly supported in previous analyses (Miller et al. 2001; Yi et al. 2004), as well as by *Nia*-i3, the combined nuclear dataset, *trnC-trnD*, the combined chloroplast dataset, and the combined nuclear and chloroplast data presented here (Figs. 1–5).

Evolutionary Relationship of Subgen.* Rhus *and Subgen.* Lobadium*, and the Discordant Placement of the* R. microphylla-R. rubifolia *Clade. The two subgenera of *Rhus* (subgen. *Lobadium* and subgen. *Rhus*) were distinguished initially based on differences in inflorescence structure, bracts, bracteoles, flowering time, fruit pubescence and flavonoid chemistry (Heimsch 1940; Barkley 1937, 1942, 1963; Brizicky 1962, 1963; Young 1975, 1979; Li et al. 1999). Based on morphological data, Barkley (1937) suggested that subgen. *Rhus* species were primitive relative to the species of subgen. *Lobadium*; however, overlapping variation in the morphological characters used to distinguish the subgenera complicated efforts to understand the evolutionary relationship of subgen. *Lobadium* and subgen. *Rhus*, precluding formal morphological cladistic analyses (A. J. Miller and D. A. Young, unpubl. data). Flavonoid chemistry data supported Barkley's hypothesis of a primitive subgen. *Rhus* (Young 1979), as well as molecular analyses based on nuclear ITS sequence data (Miller 2001; Yi et al. 2004). In contrast, nuclear data presented here based on *Nia*-i3 sequences and *Nia*-i3 + ITS sequences support a monophyletic subgen. *Rhus* and a monophyletic subgen. *Lobadium* (Figs. 1, 2). Chloroplast sequences from the *trnC-trnD*, *ndh*F, and *trnL-F* regions contradict both previously published scenarios, indicating that subgen. *Rhus* is a paraphyletic group with two *Lobadium* species nested within it (*R. microphylla* and *R. rubifolia*). Thus, subgen. *Lobadium* is polyphyletic. Incongruence in the two nuclear and chloroplast trees is the result of the variable placement of two taxa that form a well-supported clade, *R. microphylla* and *R. rubifolia* (discussed below). The removal of these taxa from analyses results in a monophyletic subgen. *Lobadium* and a monophyletic subgen. *Rhus* (Fig. 5).

Various factors may contribute to incongruence among gene trees (Mason-Gamer and Kellogg 1996; Johnson and Soltis 1998; Wendel and Doyle 1998). When a species has discordant systematic positions between the maternally inherited chloroplast and biparentally inherited nuclear gene trees, this species may be a hybrid or an allopolyploid (Soltis and Kuzoff 1995; Mason-Gamer and Kellogg 1996). Hybridization and introgression have been shown to be widespread in plants, and reticulate evolution is likely to be the most common reason for phylogenetic discordance (Rieseberg and Soltis 1991; Rieseberg and Brunsfeld 1992; Soltis and Kuzoff 1995; Wendel and Doyle 1998; Sang and Zhong 2000). A few examples of natural hybridization have been reported in *Rhus* (Barkley 1937; Brizicky 1963; Hardin and Philips 1985; Burke and Hamrick 2002); consequently, hybridization and introgression may be responsible for these taxa showing ''hard incongruence'' (Seelanen et al. 1997).

The varying position of the *R. microphylla-R. rubifolia* clade is critical to the interpretation of the available DNA sequence data with respect to the relative relationships of the two subgenera, and is likely an indication of hybridization between members of subg. *Rhus* and subg. *Lobadium*. Historically, *R. microphylla* and *R. rubifolia* have

been grouped within subgen. *Lobadium* based on morphological and flavonoid chemistry characters (Barkley 1937; Young 1978, 1979). The nuclear data (*Nia*-i3, combined *Nia*-i3+ITS) place the *R. microphylla-R. rubifolia* clade within subgen. *Lobadium*, while in the chloroplast datasets (*trnC-trnD*, combined *trnC-trnD*, *ndhF*, and *trnL-F*) the *R. microphylla-R. rubifolia* clade groups firmly within subgen. *Rhus* (PP = 0.97, BS = 86%, Fig. 3; PP = 1.00, BS = 96%, Fig. 4).

The discordance between the nuclear and chloroplast datasets with regard to the placement of the *R. microphylla-R. rubifolia* clade may be an indication of chloroplast capture. Both geographic and morphological data are consistent with this hypothesis. The geographic distribution *of Rhus microphylla* overlaps with both the species of subg. *Lobadium* and and the species of subg. *Rhus* with which it was allied in nuclear and choroplast datasets, respectively. *Rhus microphylla* is known from northern central Mexico and the southwestern United States (Arizona, New Mexico, and Texas). In the nuclear dataset, the *R. microphylla-R. rubifolia* clade grouped with the *R. aromatica-R. trilobata* clade (subgen. *Lobadium*) that occurs throughout the United States, including the southwestern United States and northcentral Mexico, where it overlaps with *R. microphylla*. In the chloroplast datasets, the *R. microphylla-R. rubifolia* clade groups with the North American members of subgen. *Rhus*, *R. copallina* and *R. lanceolata.* The range of *R. microphylla* overlaps extensively with *R. lanceolata* in Texas and northern Mexico; with *R. glabra* in New Mexico, Arizona, and northern Mexico; and with *R. copallina* in western Texas and northeastern Mexico (Barkley 1937; Global Biodiversity Information Facility; WWW.gbif.org). The present distributions of *R. microphylla, R. aromatica-R. trilobata, R. lanceolata*, and *R. copallina* indicate that the opportunity for hybridization between some or all of these species exists (and likely existed) in the southwestern U.S. and northern Mexico. Although the contemporary geographic distribution of *R. rubifolia* does not overlap with any of the putative parent species, when *R. microphylla* was removed from the analyses, *R. rubifolia* remains nested within subgen. *Rhus*.

In addition to the geographical sympatry of *R. microphylla* with members of subgen. *Lobadium* (*R. trilobata*) and subgen. *Rhus* (*R. copallina, R. lanceolata, R. glabra*), *R. microphylla* displays some morphological characteristics that are intermediate between the two subgenera. Specifically, *R. microphylla* resembles members of subgen. *Lobadium* in its persistent bracts and two bracteoles, sessile flowers that occur in solitary or small clustered spikes and appear before the leaves, flavonoid chemistry, and shrubby growth form (Barkley 1937; Young 1978). *Rhus microphylla* displays some similarity to members of subgen. *Rhus*, such as its imparipinnately compound leaves and winged rachis. The leaves of *R. microphylla* look like highly reduced *R. copallina* leaves.

***Relationships within Subgen.* Lobadium.** Subgenus *Lobadium* consists of approximately 25 species distributed primarily in the southwestern United States, Mexico, and northern Central America. The most recent classification of subgen. *Lobadium* delimited three sections: sect. *Lobadium*, sect. *Styphonia*, and sect. *Terebinthifolia* based on morphological and flavonoid chemistry data (Young 1978, 1979). Section *Styphonia* was divided into three subsections, *Compositae, Intermediae*, and *Styphoniae* (Young 1978, 1979).

Conflict between datasets existed for sampled members of sect. *Lobadium* (*R. aromatica, R. microphylla*, and *R. trilobata*), sect. *Terebinthifolia* (*R. rubifolia*), and sect. *Styphonia* subsect. *Styphoniae* (*R. integrifolia* and *R. ovata*). For example, in the combined nuclear datasets, the sampled representatives of sect. *Lobadium* group with sect. *Terebinthifolia* (PP = 1.00, BS = 100%), and representatives of sect. *Styphonia* subsect. *Styphoniae* (*R. integrifolia* and *R. ovata*) group with species of sect. *Styphonia* subsect. *Compositae* (*R. choriophylla, R. integrifolia, R. kearneyi, R. ovata, R. pachyrrhachis, R. schiedeana*, and *R. virens*). In contrast, in the combined chloroplast datasets, *R. aromatica* and *R. trilobata* (sect. *Lobadium*) form a clade with *R. integrifolia* and *R. ovata* (Sect. *Styphonia* subsect. *Stypohoniae*), while *R. microphylla* (sect. *Lobadium*) and *R. rubifolia* (sect. *Terebinthifolia*) group within subgen. *Rhus* (see discussion above).

The cpDNA results for subgen. *Lobadium* may reflect an ancient chloroplast capture, indicating that reticulate evolution has possibly occurred among some subgen. *Lobadium* species. A similar result was detected in *Heuchera* (Soltis et al. 1991; Soltis and Kuzoff 1995). However, additional sampling of subgen. *Lobadium* (this study includes 12 of the ~25 known species in the subgenus) is required to reconstruct a robust phylogeny for subg. *Lobadium* in order to test sectional classification scheme and detect possibly hybridization events within this sugbenus.

***Relationships within Subgen.* Rhus.** Subgenus *Rhus* consists of approximately 10 species with four in eastern Asia, four in North America, one in Europe, and one in Hawaii. The relationships among subgen. *Rhus* species have proven difficult to disentangle, most likely a reflection of the complex and long biogeographic history of subgen.

Rhus in the North Temperate zone (Yi et al. 2004). In *Nia*-i3 data, the species of subgen. *Rhus* formed five subclades (Fig. 1, see results). In the combined chloroplast tree, species from the eastern Asian, central Asia/Europe, and Hawaii formed a well-supported monophyletic group, and species from North America plus two subgen. *Lobadium* species formed another, albeit weakly supported, monophyletic group (Fig. 4). Two taxa occupied discordant position in the nuclear and chloroplast data (*R. microphylla* and *R. rubifolia*); with these taxa removed, the combined nuclear and cpDNA trees reveal two weakly supported clades within subgen. *Rhus*: an Asian/Hawaiian clade, and a North American clade (Fig. 5).

Within subgen. *Rhus*, two taxa have discordant positions in the nuclear and chloroplast sequence data: *R. coriaria* and *R. michauxii*. *Rhus coriaria* is the only *Rhus* species native to southeastern Europe and western Asia. In the nuclear data, *R. coriaria* was weakly supported as the sister species of the North American *R. glabra–R. michauxii–R. typhina* clade (combined *Nia*-i3 and ITS, PP = 0.97, BS = 52, Fig. 4; ITS data see Yi et al., 2004, PP = 0.92, BS < 50%). The chloroplast data, in contrast, grouped *R. coriaria* in a well-supported clade together with eastern Asian and Hawaiian species (*trnC-trnD* PP = 1.00, BS = 85%, Fig. 3; combined cp data PP = 1.00, BS = 91%, Fig. 4), although the systematic position of *R. coriaria* within this clade was not clear. Similar results in the combined cpDNA and nuclear data, *Rhus coriaria* formed a monophyletic group together with eastern Asia and Hawaiian species (PP = 1.00, BS = 59%). Three *Nia*-i3 sequence types were detected in *R. coriaria* from 15 clonal sequences, and these three types of sequence formed a monophyletic group. Only one type of ITS sequence was obtained from eight clonal sequences. At present, the available molecular and morphological data fail to identify unambiguously the extant *Rhus* species that are most closely related to *R. coriaria*.

Discordant placement between nuclear and chloroplast datasets was also observed for *R. michauxii*, a North American member of subgen. *Rhus*. *Rhus michauxii* was thought to have distinct morphology from other sympatric congeners (*R. glabra, R. typhina, R. copallina, R. aromatica*); initially, its closest relatives were suggested to be the east Asian *R. chinensis* and south European and west Asian *R. coriaria* (Barkely 1937). In all molecular analyses, *R. michauxii* formed a strongly supported clade together with the North American species *R. glabra* and *R. typhina*. Within the *R. michauxii–R. glabra–R. typhina* clade, the relative positions of *R. michauxii, R. glabra*, and *R. typhina* are not congruent among chloroplast and nuclear datasets (Figs. 1–4; Yi et al., Figs. 2, 3). In the combined nuclear tree, *R. michauxii* is the sister taxon to a *R. glabra–R. typhina* clade (Fig. 2). In the combined chloroplast tree, *R. michauxii* forms a clade with *R. glabra* and *R. typhina* is the sister of the *R. michauxii–R. glabra* clade. Natural hybridization has been reported between *R. glabra* and *R. michauxii*, and between *R. michauxii* and *R. typhina*, based on morphological data (Hardin and Philips 1985). In addition, molecular evidence for ongoing gene flow between *R. michauxii* and *R. glabra* was detected using allozymes (Burke and Hamrick 2002). Our data are consistent with previous reports of hybridization between these three taxa.

***Phylogenetic Utility of* Nia-*i3 and* trnC-trnD.** Previous studies suggested the nuclear region *Nia*-i3 as a useful marker for reconstructing relationships among closely related species (Howarth and Baum 2002). In this study, we found more variable characters, and more parsimony informative characters, in *Nia*-i3 than in ITS within *Rhus* or among *Rhus* and closely related genera. Similar results were found in *Scaevola* with the percentage sequence divergence 1.3- to 5.4-fold greater in *Nia*-i3 than in ITS (Howarth and Baum 2002). Aligning *Nia*-i3 required more gaps than that of ITS. The *Rhus* data matrix of *Nia*-i3 has 25 gaps, 18 of which are parsimony-informative, in comparison with five of six informative gaps in ITS. In the data matrix of *Rhus* and its outgroups, 38 of 67 gaps are informative in *Nia*-i3, and 11 of 22 gaps are informative in ITS. Compared with ITS, the *Nia*-i3 dataset has higher CI and RI values, suggesting a lower level of homoplasy of *Nia*-i3.

The *trnC-trnD* region was recently considered to be phylogenetic marker (Lee and Wen 2004). The *trnC-trnD* region has moderate evolutionary rate. Two of three noncoding regions of *trnC-trnD*, *ycf6-psbM* and *psbM-trnD*, were attributed into rank of Tier2, and the third region of *trnC-ycf6* was attributed into rank of Tier3 (Shaw et al. 2005). However, the entire *trnC-trnD* region provided more informative characters in comparison with other noncoding cpDNA regions for its relative greater length (Shaw et al. 2005). This region has recently demonstrated to be useful to resolve intrageneric relationships of several plant groups (Hartmann et al. 2002; Lee and Wen 2004; Fritsch et al., 2006; Ran et al. 2006; Smedmark et al. 2006). Similar results were found within *Rhus*, in which *trnC-trnD* provided slightly more parsimony-informative characters than the ITS region and the *ndhF* gene, and many more parsimony-informative characters than *trnL-F*. The *trnC-trnD* region has a low level of homoplasies in *Panax* (Lee and Wen

2004), *Picea* (Ran et al. 2006), and *Symplocos* (Fritsch et al. 2006). Within *Rhus*, this region has higher CI and RI values than that of *ndhF* and slightly lower CI and RI values than that of *trnL-F*. The *trnC-trnD* region appears to be prone to indels, and have higher resolution and clade support in analyses with gaps coded as new characters (Lee and Wen 2004; Fritch et al 2006). Comparable results were found within *Rhus*.

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APPENDIX 1. Voucher information and GenBank numbers of sequences from *Rhus* and outgroups included in this study. Voucher details are listed in the following sequence: taxon name, collection number and herbarium at which the voucher is deposited (parentheses), collection locality, distribution, GenBank accession numbers (*Nia*-i3, *trnC-trnD*, ITS, *ndhF*, and *trnL-F*). When marker from one sample has more than one sequence, different clonal sequences are included in parentheses. C1, C2, C3 and C4 represent clone 1, clone 2, clone 3 and clone 4, respectively.

***Rhus* subgenus *Rhus*:** *R. chinensis* Mill.; *Wen 6389* (F); Morton Arb., Illinois (cult.); E. Asia to SE Asia; DQ382286, DQ400536, AY641480, AY640435, AY643095. *R. chinensis* Mill. var. *roxburghii* Steud.; *Wen 6526* (F); China, Yunnan; E Asia; DQ382314, DQ400551, AY641482, AY640436, AY633892. *R. copallina* L. #1; *Wen 7134* (F); USA, Illinois; E North America; -, DQ400538, AY641483, AY640437, AY643097. *R. copallina* L. #2; *Wen 7165* (F); USA, Alabama; E North America; DQ382288; DQ400539, AY641484, AY640438, AY643098. *R. coriaria* L.; *Wen 7150* (F); Oak Park, Illinois (cult.); W Asia to S Europe; (C1, DQ382290; C2, DQ382291), DQ400540, AY641485, AY640439, AY643099. *R. glabra* L.; *Wen 7171* (F); USA, Alabama; North America; (C1, DQ382292; C2, DQ382293), DQ400541, AY641486, AY640440, AY643100. *R. lanceolata* Gray ex Engler; *Wen 7277* (F); USA, Texas; E North America; (C1, DQ382296; C2, DQ382297; C3, DQ382298), DQ400544, AY641487, AY640441, AY643101. *R. michauxii* Sargent; *Hardin 13984* (F); USA, North Carolina; E North America; (C1, DQ382306; C2, DQ382307), DQ400545, AY641488, AY640442, AY643102. *R. potaninii* Maxim.; *Wen 7138* (F); Morton Arb., Illinois (cult.); E Asia; (C1, DQ382310; C2, DQ382311), DQ400549, AY641489, AY640443, AY643103. *R. punjabensis* J. L. Stew. ex Brand.; *Wen 7137* (F); Morton Arb., Illinois (cult.); E Asia; DQ382312, DQ400550, AY641490, AY640444, AY643104. *R. sandwicensis* A. Gray; *Wen 7052* (F); Hawaii: Hawaii; Hawaii; DQ282316, DQ400553, AY641491, AY640445, AY643105. *R. typhina* L.; *Wen 7082* (F); USA, Wisconsin; E North America; (C1, DQ382318; C2, DQ382319), DQ400556, AY641492, AY640446, AY643106.

Rhus* subgenus *Lobadium (Raf.) Torrey & Gray: **Sect. *Lobadium*** (Raf.) DC. *R. aromatica* Ait.; *Wen 7086* (F); USA, Illinois; E North America; (C1, DQ382284; C2, DQ382285), DQ400535, AY641493, AY640447, AY643107. *R. microphylla* Engelm. ex Gray; *Wen 7288* (F); USA, Texas; SW America to N Mexico; (C1, DQ382300; C2, DQ382301; C3, DQ382302; C4, DQ382303), DQ400546, AY641495, AY640448, AY643108. *R. trilobata* Nutt. ex Torr. & Gray; *Miller* 21 (CS); USA, Colorado; North America; DQ382317, DQ400555, AY641497, AY640449, AY643109. **Sect. *Styphonia*** (Nutt.) Barkley; *R. choriophylla* Woot. & Standl.; *Miller 27* (CS); USA, Arizona; S Arizona and New Mexico to Sonora of Mexico; DQ382287, DQ400537, AY641498, AY640450, AY643110. *R. integrifolia* (Nutt. ex Torrey & Gray) Benth. & Hook f. ex Rothr.; *Miller 28* (CS); Rancho Santa Ana Bot Gard, CA (cult.); S California to N Lower California; DQ382294, DQ400542, AY641499, AY640451, AY643111. *R. kearneyi* Barkl.; *Ickert-Bond 1298* (F); USA, Arizona (cult.); S Arizona; DQ382295, DQ400543, AY641500, AY640452, AY643112. *R. ovata* Wats.; *Miller 6* (CS); USA, Arizona; C Arizona to S California; DQ382308, DQ400547, AY641501, AY640453, AY643113. *R. pachyrrhachis* Hemsl.; *Steinmann et al. 3724* (F); Mexico, Nuevo León; NE Mexico; DQ382309, DQ400548, AY641503, AY640455, AY643115. *R. schiedeana* Schlecht.; *Steinmann et al. 3696* (F); Mexico, Querétaro; S Mexico to Guatemala; DQ382318,

DQ400554, AY641504, AY640456, AY643116. *R. virens* Lindh. ex Gray #2; *Wen 7282* (F); USA, Texas; SW America to N Mexico; DQ382320, DQ400557, AY641506, AY640458, AY643118. **Sect. *Terebinthifolia*** Young. *R. rubifolia* Turcz.; *Steinmann & Carranza 3146* (F); Mexico, Michoacán; S Mexico; DQ382315, DQ400552, AY641508, AY640459, AY643119.

Outgroups. *Actinocheita filicina* (D.C.) Barkl.; *Panero s.n. 44* (CS); S Mexico; S Mexico; DQ382321, DQ400558, AY641509, AY640460, AY643120. *Malosma laurina* (Nutt.) Nutt. ex Engl.; *Miller 34* (CS); Rancho Santa Ana Bot Gard, CA (cult.); S California and N Lower California; DQ382322, DQ400559, AY641510, AY640461, AY643121. *Pitacia chinensis* Bge. *Wen 7090* (F); E Asia; DQ382323, DQ400560, DQ390466, DQ390462, DQ390470. *Pistacia lentiscus* L.; *Ickert-Bond* 1299 (F); USA, Arisona (cult.); Mediterranean; DQ382324, DQ400561, DQ390467, DQ390463, DQ390471. *Pistacia mexicana* H.B.K.; *Parfitt 27* (F); USA, California (cult.); Mexico to C America; DQ382325, DQ400562, DQ390468, DQ390464, DQ390472. *Pistacia vera* L.; *Golan 1.539* (F); Israel, cultivated; C Asia; DQ382326, DQ400563, AY677201, AY677209, AY677204. *Pistacia weinmannifolia* Poisson; *Ji 0174* (KUN); China, Yunnan; E Asia; DQ382327, DQ400564, DQ390469, DQ390465, DQ390473. *Schinus molle* L.; *Wen 6686* (F); USA, Los Angeles, CA (cult.); California and Texas; DQ382333, DQ400565, AY641512, AY640463, AY643123. *Searsia quartiniana* (A. Rich.) A. J. Miller; *Miller 51* (CS); Phoenix Desert Bot Gard, AZ (acc. # 1980007001); Africa; DQ382331, DQ400566, AY641517, AY640468, AY643128. *Searsia undulata* (A. Rich) T. S. Yi, A. J. Miller & J. Wen; *Miller s.n.* (CS); Phoenix Desert Bot Gard, AZ (acc. # 19800071); Africa; DQ382332, DQ400567, AY541519, AY640470, AY643130. *Toxicodendron diversilobum* (Torrey & Gray) Greene; *Wen 6693* (F); USA, California; W North America; DQ382328, DQ400568, AY677202, AY677208, AY677205. *Toxicodendron radicans* (L.) Kuntze; *Wen 6236* (F); USA, Illinois; North America; DQ382329, DQ400569, AY677203, AY677207, AY677206. *Toxicodendron vernix* (L.) Kuntze; *Wen 7146* (F); Morton Arb., Illinois (cult.); E North America; DQ382330, DQ400670, AY541520, AY640471, AY643131.