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Phylogenetic Relationships in the Genus *Rosa*: New Evidence from Chloroplast DNA Sequences and an Appraisal of Current Knowledge

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ABSTRACT. The genus *Rosa* (roses) comprises approximately 190 shrub species distributed widely throughout the temperate and subtropical habitats of the northern hemisphere. Despite numerous recent studies examining phylogenetic relationships in the genus, relationships remain obscure due to problems such as poor identification of garden specimens, hybridization in nature and in the garden, and low levels of chloroplast and nuclear genome variation. Phylogenetic analyses of non-coding chloroplast sequences from the *trnL-F* region and *psbA-trnH* intergenic spacer for 70 taxa show slightly more variation than previous analyses of the genus. Bayesian and parsimony analyses suggest that subg. *Rosa* can be divided into two large clades, each with low internal resolution. One comprises species from sections *Carolinae*, *Cinnamomeae* and *Pimpinellifoliae* p.p., whilst the other consists of all of the remaining sections of subg. *Rosa* (*Banksianae* p.p., *Bracteatae*, *Caninae*, *Indicae*, *Laevigatae*, *Rosa, Synstylae* and *Pimpinellifoliae* p.p.). A fairly complete sampling of field-collected North American taxa has been incorporated in this analysis. Analyses indicate that migration into North America occurred at least twice within this primarily Old World genus. Most North American taxa, except *R. setigera* and *R. minutifolia*, fall into a single clade that includes Asian and European taxa. Analyses also are consistent with the notion that cultivated commercial roses have a relatively narrow genetic background. Six of the seven primary taxa believed to be involved in the creation of domesticated roses are found within the same large clade that mostly includes Asian and European taxa.

KEYWORDS: chloroplast DNA phylogeny, classification, origin of garden roses, *Rosa*.

The genus *Rosa* (roses) comprises approximately 190 shrub species distributed widely throughout the temperate and subtropical habitats of the northern hemisphere (Rehder 1940; Matthews 1995). Roses are of worldwide economic importance as the centre of a large ornamental shrub and cut flower industry. They also are economically important as a source of essential oils for perfumes and scents (i.e., attar; Krüssman 1981), and pharmacological research has identified significant radioprotective (Akhmadieva et al. 1993) and anti-inflammatory (Winther et al. 1999) properties in rose extracts. Moreover, traits such as small nuclear genomes (Dickson et al. 1992; Yokoya et al. 2000), extensive cross-species fertility (Erlanson 1934), and advanced industrial horticultural and micro-propagation techniques (e.g., Gudin 2000; Crespel et al. 2002; Dugo et al. 2005; Squirrell et al. 2005) suggest that roses could provide an ideal model for exploring woody plant genomes. This is particularly significant considering that most of the economically important temperate bush (e.g., raspberries, genus *Rubus*) and tree fruits (e.g., apples, genus *Malus*; cherries, peaches, plums, genus *Prunus*) are members of the rose family (Rowley 1978).

Modern molecular techniques are powerful tools for deciphering the inheritance and molecular basis of characters. Recently, linkage maps have been created for *Rosa* (Debener and Mattiesch 1999; Dugo et al. 2005), and the first MADS-box genes involved in floral development have been sequenced (Kitahara and Matsumoto 2000; Kitahara et al. 2001; Hibino et al. 2006). However, modern molecular biology and crop improvement programs are still selecting taxa and making biological comparisons within the context of a classification that is at least sixty years old (Rehder 1940), and heavily dependant on late 19th century arrangements (Crépin 1889, 1891). A thorough and robust phylogenetic hypothesis is needed to provide the genus-wide perspective necessary to determine the origins of natural and horticultural roses and to properly orient the molecular revolution that is under development in our understanding of floral evolution, trait genomics, and rose breeding.

Until now, relationships in *Rosa* have remained obscure due to the difficulty of circumscribing species and a taxonomy that is notoriously complex (Matthews 1920; Rowley 1959, 1978; Clarke 1980; Matthews 1995; Cairns et al. 2000; Wissemann 2003; Wissemann and Ritz 2005). The

ability of rose species to cross relatively easily, even between seemingly divergent groups, has certainly been a boon to horticulture, but from the earliest of times it has also been recognized as the source of difficulty in the taxonomy of the genus (Linnaeus 1753; Crépin 1893). Despite numerous taxonomic studies of this well know genus (e.g., Crépin 1889, 1891, 1896; Baker 1905; Willmott 1910–1914; Herring 1925; Boulenger 1934, 1936; Erlanson 1938; Rehder 1940; Lewis 1957; Wissemann 2003), species relationships within *Rosa* remain problematic. Species boundaries have been notoriously difficult to define because of intraspecific variability, polyploidy, and interspecific hybridization (Crépin 1893; Erlanson 1929; Erlanson-MacFarlane 1966; Melville 1967; Wissemann 2003). *Rosa* taxonomy is further complicated by the publication of numerous names given to morphological variants and hybrids. Depending on the author, between 14 and 4,000 species of *Rosa* are accepted (e.g., Linnaeus 1753; Gandoger 1881). Despite these problems at the species level, the subgeneric and sectional classification system of Rehder (1940) is often used (four subgenera, ten sections). Rehder's (1940) adaptation of Crépin's (1891) classification is widely adopted because of the ease with which sections and subgenera may be identified and by an apparent correlation between his taxonomy and chromosome numbers.

The first phylogenetic analyses of the genus, based on isozyme (Kim and Byrne 1994, 1996) and RAPD data (e.g., Debbener et al. 1996; Millan et al. 1996; Jan et al. 1999), suggested that Rehder's classification was largely natural. Similar conclusions were obtained from microsatellite analyses of small samples of wild and cultivated roses (Scariot et al. 2006). However, phylogenetic analyses of sequences from the internal transcribed spacer of 18–26S nuclear ribosomal genes (ITS; Iwata et al. 2000; Matsumoto et al. 2000, 2001; Wu et al. 2001; Wissemann and Ritz 2005), the chloroplast *matK* gene (Matsumoto et al. 1998, 2001; Wu et al. 2000) and the *atpB-rbcL* intergenic spacer (Wissemann and Ritz 2005) generally do not support the monophyly of Rehder's sections, nor is there agreement among these analyses as to the relative phylogenetic position of the subgenera and sections. In all of these previous studies, phylogenetic resolution is poor, and where clades are resolved, support is often low. This is explained partly by the extremely low levels of sequence divergence observed across the genus (e.g., Matsumoto et al. 1998; Wissemann and Ritz 2005). A further concern is the clustering in different clades of conspecific samples. These conflicts suggest either problems with the identification of plant material or that hybrids may have been sequenced. Likewise, in their recent analyses of ITS and *atpB-rbcL* data for a much larger taxonomic sampling than previous studies, Wissemann and Ritz (2005) noted several contradictions between the chloroplast and nuclear gene phylogenies suggesting that hybridization is frequent and that it complicates phylogeny reconstruction in roses. Thus, despite substantial recent efforts, the phylogeny and classification of *Rosa* remain ambiguous.

The objectives of this paper are to circumscribe the monophyletic groups in *Rosa* and to examine the phylogenetic origins of cultivated roses using variable chloroplast DNA sequences. We examine phylogenetic relationships in the genus *Rosa* using the chloroplast *trnL* intron, and the *trnL-F* and *psbA-trnH* intergenic spacer sequences, which preliminary analyses had found to be relatively variable. A fairly complete sampling of field-collected North American taxa, supplemented with garden-grown Asian and European species has been incorporated in this analysis. Finally, we draw conclusions on our current knowledge of *Rosa* phylogeny by comparing the similarities and differences between this and previous phylogenetic studies of the genus.

Materials and Methods

Taxon Sampling. A total of 79 specimens representative of all four subgenera and 10 sections of the large subgenus *Rosa* were included in analyses. Most of the North American species were obtained from field collected specimens, whilst European and Asian taxa mostly were represented by garden-grown material (Appendix 1). The identification of garden-grown specimens was confirmed using the keys of Rehder (1940), Clarke (1980), Klastersky (1968), or Gu and Robertson (2003). Because of the potential for misidentification and hybridization of garden-grown specimens, a second accession from either a different garden or from wild collected material was sequenced whenever possible.

Three species (*Potentilla fruticosa* L., *P. nivea* L., *Alchemilla glomerulans* Buser) were initially sequenced as outgroup taxa based on their position within the sister group to *Rosa* in the molecular analyses of Rosoideae by Eriksson et al. (2003). However, because two of these potential outgroups displayed considerable size differences in their the *trnL-F* and *psbA-trnH* regions as compared to roses, final analyses included only *Potentilla nivea* as an outgroup (see below).

DNA Extraction, PCR and Sequencing. DNA was extracted from 20–30 mg of leaf tissue of single individuals dried in silica gel or removed from herbarium specimens using a CTAB method (Doyle and Doyle 1987) with modifications as in Joly et al. (2006).

Eight non-coding chloroplast regions were evaluated for their variability on a small, but taxonomically diverse sample of individuals: *trnK* intron (both 3′ and 5′ of *matK*), *psbA – trnH* spacer, *trnT – trnE* spacer, *trnE – trnD* spacer, *trnF –* ORF spacer, *trnL* intron, and *trnL – trnF* spacer. Of these eight regions, four were deemed variable enough for phylogenetic analyses (*psbA – trnH*, *trnL* intron, *trnL – trnF*, *trnT – trnD*), although the *trnT – trnD* region was subsequently abandoned due to difficulties with amplification and sequencing.

The *trnL* intron and *trnL-F* spacer were either amplified as a complete unit using primers ''c'' and ''f'' (Taberlet et al. 1991), or when necessary as separate amplicons using the ''c'' and ''d'' and ''e'' and ''f'' primer pairs (Taberlet et al. 1991). The *psbA-trnH* intergenic spacer was amplified using the primers of Sang et al. (1997). Reaction mixtures contained 5 µL of 10 × PCR reaction buffer (Roche Diagnostics, Laval, Québec, Canada; contains 1.5 mM $MgCl_2$), 200 µM of each dNTP, 0.5 µM of each primer, 10–50 ng template DNA and 2–2.5 units *Taq* DNA polymerase, adjusted to an end volume of 50 µL with de-ionized water. PCR products were produced on an ABI 9700 thermal cycler (Applied Biosystems, Foster City, California) via 35 cycles of DNA denaturation at 95°C for 30 sec, primer annealing at 48–55°C for 30 sec, and DNA strand extension at 72°C for 1 min 20 sec. The PCR was terminated by a final extension step of 72°C for 7 min. Double-stranded PCR products were then purified using QiaQuick columns (QiaGen, Mississauga, Ontario, Canada) following the manufacturer's protocol.

Purified PCR products were cycle sequenced using the ABI Prism™ BigDye™ terminator Kit (Applied Biosystems). Termination products for all regions were produced on an ABI 9700 thermal cycler using PCR primers and the following cycling conditions: 95°C for 3 min (pretreatment), then 25 cycles of 96°C for 30 sec, 50°C for 15 sec, and 60°C for 4 min. Products were run on an ABI 3100-*avant* capillary sequencer according to the protocols of the manufacturer.

Sequence Analysis. Intron and spacer boundaries for the *trnL-F* and *psbA-trnH* regions were determined by comparison to sequences for *Rosa* (Iwata et al. 2000; Potter et al. 2002). Complete sequences for the *psbA-trnH* spacer were obtained for all taxa; however, 33 bp and 26 bp from the 5′ ends of the *trnL* intron and the *trnL-F* spacer, respectively, were excluded from analyses because they could not be obtained for most taxa. Sequences were assembled and edited in Sequencher™ 4.1 (Gene Codes Corporation, Inc., Ann Arbor, Michigan), and multiple alignments were performed in ClustalX (Thompson et al. 1997) with manual adjustments to the alignment assessed by parsimony as described in Starr et al. (2004). Primary sequence characteristics, pair-wise sequence divergence and parsimony character statistics were calculated in PAUP* 4.0b10 (Swofford 2002) using the BASEFREQ and SHOWDIST commands.

Phylogenetic Analyses. Tree searches under parsimony were conducted using PAUP* and a combined data set of *trnL-F* and *psbA-trnH* sequences. Searches were performed with both the inclusion and exclusion of indels as coded by GapCoder (Young and Healy 2003), a program that scores gaps according to the simple gap-coding method of Simmons and Ochoterena (2000). Two hundred and twenty-six characters (positions 445–453, *trnL-F*; 1073–1250, 1279–1287, 1396–1425, *psbA-trnH*) were excluded from all analyses due to alignment ambiguity. Initial searches excluding indels used a heuristic search strategy with tree-bisection-reconnection (TBR) branch swapping and a random addition of taxa (RAT) for 20,000 replicates. Heuristic searches that included indels (TBR branch swapping, RAT, 163 replicates) were forced to limit the number of trees saved and swapped per replicate to 10,000 as a single replicate could not otherwise be completed in a reasonable amount of time. Both analyses (with and without indels) were also repeated with the outgroup taxon excluded in order to determine whether the outgroup had an effect on ingroup topology. Clade support was assessed by bootstrap values (BS; Felsenstein 1985) calculated from 10,000 replicates of a heuristic search strategy with TBR branch swapping and the MULTREES option ''off''. Such searches are computationally fast, but simulations (DeBry and Olmstead 2000) suggest that they provide bootstrap values that are essentially identical to the values of a typical bootstrap analysis where the MULTREES option is ''on''.

Bayesian analyses (indels excluded) were performed with MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). The posterior tree distribution was estimated via a Metropolis-coupled Markov Chain Monte Carlo (MC^3) run of 10,000,000 generations with tree sampling conducted every 5,000 generations from one (the ''cold'' chain) of four simultaneously run Markov chains. A general-time-reversible (GTR) model (default settings) incorporating a correction for rate heterogeneity across sites (i.e., a gamma distribution, Γ; Yang 1993) was enforced during the running of the chain. This model was chosen using MrModeltest 1.1b (J. A. A. Nylander, Uppsala University), a simplified version of Modeltest (Posada and Crandall 1998) that performs hierarchical likelihood ratio tests only for those DNA substitution models that are common to both PAUP* and MrBayes. Plots of run parameters versus generation number were used to determine the point where the chain leveled off and began to fluctuate around a stable value (i.e., the stationary phase). The first million generations were thus excluded from analyses. In order to assess whether enough generations had been run to reach convergence and to determine whether sufficient mixing of the chain had occurred to provide reliable parameter estimates, a second independent analysis using the same initial parameters as above was conducted. Convergence and mixing were assessed by a comparison of likelihood values, the mean and variance of model parameters, and the topologies of majority rule consensus trees derived from the first and second analyses. Posterior probabilities of trees, clades and parameter estimates were determined from the trees obtained in both analyses once the burnin sample was removed.

RESULTS

Sequence Analysis. Summary sequence statistics for the combined *trnL-F* and *psbA-trnH* regions are presented in Table 1. The range of sequence divergence among taxa was low regardless of whether the outgroup was included (0.0–5.9%) or excluded (0.0–2.3%). The level of divergence was higher in the *psbA-trnH* spacer (0.0–8.4%) than in the *trnL-F* region (0.0–5.5%). However, over 43% of *psbA-trnH* aligned sequences had to be excluded because of two highly repetitive regions that could not be reliably aligned (positions 1073–1250, 1396–1425). In addition, a microsatellite (9–18 T's) at positions 1279–1287 in the *psbA-trnH* region and another (8–13 T's) at positions 445–453 in the *trnL-F* region were excluded from analyses. Greater than 47 % of all pairwise sequences compared (indels included) differed by less than 1% (<12 absolute differences), and no difference between sequences was seen in 58 pairwise comparisons (~2%). The *trnL-F/psbA-trnH* matrix alignment (including indels) is available in TreeBASE (study number S1730).

Phylogenetic Analyses. Tree topologies of analyses that excluded the outgroup taxon were entirely compatible with those that included it. *Potentilla nivea* was therefore deemed an appropri-

TABLE 1. Sequence statistics for separate and combined *trnL-F* and *psbA-trnH* datasets used in phylogenetic analyses.

	trnL-F	*psbA-trnH*	Both regions combined
Length range (bp)			
- ingroup	884–900	256–402	1147–1294
- outgroup included	856–900	256–402	1147–1294
Length mean (bp)			
- ingroup	892.5	318.6	1211.0
- outgroup included	892.0	319.1	1211.1
Aligned length (bp)			
- outgroup included	934	503	1437
Sequence divergence (%)			
- ingroup	0.0–1.9	0.0–4.0	0.0–2.3
- outgroup included	0.0–5.5	0.0–8.4	0.0–5.9
Number of indels			
- ingroup	20	9	29
- outgroup included	28	10	38
Excluded characters			
- outgroup included	9 (1.0%)	217 (43.1%)	226 (15.7%)
Potentially informative indels			
- ingroup	12	3	15
- outgroup included	12	3	15
Number of variable characters			
- ingroup	84 (9.0%)	43 (8.5%)	127 (8.8%)
- outgroup included	124 (13.3%)	56 (11.1%)	180 (12.5%)
Potentially informative characters			
- ingroup	40 (4.3%)	22 (4.4%)	62 (4.3%)
- outgroup included	40 (4.3%)	24 (4.8%)	64 (4.5%)

ate outgroup for rooting trees in all subsequent analyses.

Heuristic searches of combined *trnL-F/psbA-trnH* data including and excluding indels produced 180 and 152 variable characters of which 64 and 49 were potentially informative. Heuristic searches including indels produced 710,000 trees, 235 steps in length (CI = 0.79; RI = 0.91), whereas searches excluding indels found only 13 trees, 171 steps in length (CI = 0.87; RI = 0.95). The strict consensus tree produced from the indel analysis is presented in Fig. 1. Topological differences between this analysis and analyses lacking indels are marked on Fig. 1 by arrows (clades not present in analyses excluding indels) and ellipses (clades present in analyses excluding indels).

The Bayesian 50% majority rule consensus of trees sampled in the stationary phase of the two analyses is presented in Fig. 2. Although results from Bayesian analyses are most similar to those parsimony analyses that excluded indels, both parsimony and Bayesian analyses are similar in terms of the clades recovered (see open circles on Fig. 1). Both analyses suggest that the genus *Rosa* may be divided into two principal clades. The first clade (Clade I) consists of sections *Cinnamomeae* and *Carolinae* and a part of the Eurasian section *Pimpinellifoliae*. If we ignore the unresolved relationships of *R. foetida* and *R. primula* (sect. *Pimpinellifoliae*), this clade is well supported in Bayesian trees (Bayesian posterior probability [BPP] of 92%) and poorly supported in parsimony trees (BS < 50%). The second clade (Clade II) comprises the remaining sections of subg. *Rosa* (*Banksianae* p.p., *Bracteatae*, *Caninae*, *Indicae*, *Laevigatae*, *Pimpinellifoliae* p.p., *Rosa, Synstylae*). Defined as such, Clade II is well supported in the Bayesian analysis (BPP = 96%). A similar poorly supported clade is resolved in the parsimony analysis, but it includes two subg. *Rosa* species (*R. wichurana* and *R. arvensis*) that are excluded from Clade II in the Bayesian analysis. In both analyses, *R. banksiae* (subg. *Rosa* sect. *Banksianae*) and *R. roxburghii* (subg. *Platyrhodon*) are either placed as successive sisters to Clade II (BPP = 91%, BS < 50%) or in a trichotomy with Clade II (BPP = 91%). *Rosa persica* (subg. *Hulthemia*) is sister to this *R. banksiae*/*R. roxburghii*/Clade II group (BPP = 92%, BS < 50%). In the parsimony analysis, subg. *Hesperhodos* is sister to all other *Rosa* species (BS < 50%), whereas its position is unresolved along with *R. wichurana* and *R. arvensis* in the Bayesian analysis.

Some taxa for which more than one sample was sequenced (noted in bold in Figs. 1, 2) occurred at different positions in the phylogeny. This was seen in three polyploid species, *R. acicularis*, *R. nutkana* and *R. californica* (specimens sampled from different field collected populations), as well as for *R. wichurana* (specimens sampled from different botanical gardens).

DISCUSSION

Sequence Analysis. The very low level of sequence divergence in *Rosa* detected in this chloroplast analysis confirms the conclusion of Matsumoto et al. (1998), based on a more limited data set, that the genus has a very narrow plastid genetic background. Studies based on nuclear, single-copy genes (Joly et al. 2006; Joly and Bruneau 2006) and ribosomal spacers (Wissemann and Ritz 2005; Ritz et al. 2005) indicate that this low-level of molecular divergence amongst rose species is not peculiar to the chloroplast, suggesting that most extant rose species have a very recent origin (Wissemann and Ritz 2005). For example, within North American taxa of sect. *Cinnamomeae*, no rose species differed in its sequence by more than 1.4% (15 absolute differences, 26 taxa). Thus it seems that the difficulty in distinguishing North American roses by morphology alone (e.g., Erlan-

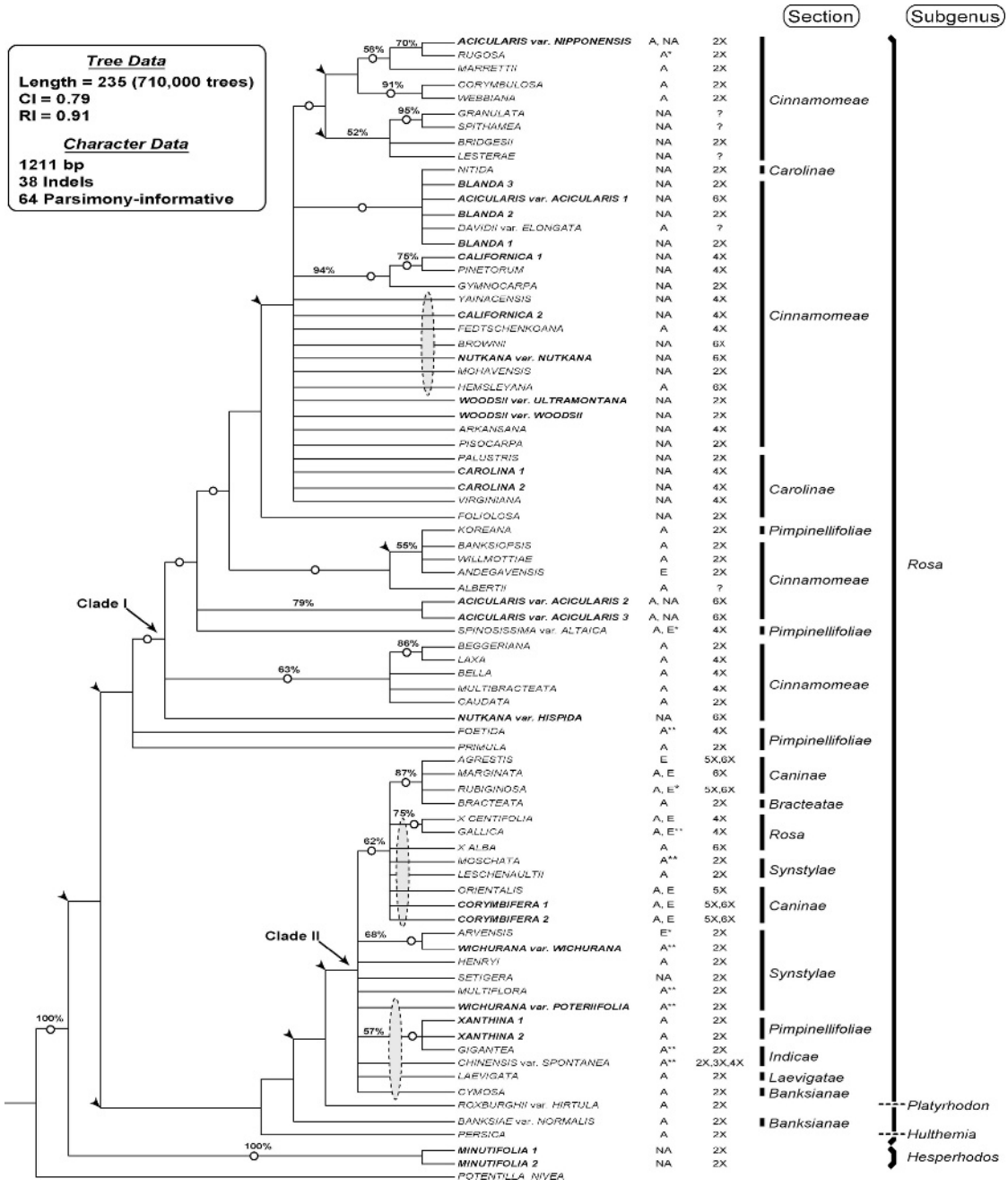

FIG. 1. Strict consensus of 710,000 trees resulting from the combined parsimony analysis of *trnL-F* and *psbA-trnH* noncoding sequences and their indels. Tree and character statistics are given at the top left. The typical ploidy level for each taxon is given after specific epithets following Erlanson (1929), Lewis (1959; 1966), Krüssman (1981) and Cairns et al. (2000). Geographic information (NA = North America; A = Asia; E = Europe) is taken from Rehder (1940) and Krüssman (1981). Asterisks after distribution indicate whether the taxon is believed to have made either a major (**) or minor (*) genetic contribution to domestic garden roses. Multiple specimens from the same species are highlighted in bold. Bars to the right of specific epithets indicate the sections and subgenera of *Rosa* as circumscribed by Rehder (1940). Numbers above branches represent bootstrap values for clades with support >50%. Arrows indicate branches that were not present in analyses where indels were excluded. Ellipses across branches link species that formed clades in analyses without indels (Bayesian and parsimony; cf. Fig. 2). Clades common to both Bayesian and parsimony analyses (with and without indels) are marked by empty circles on branches.

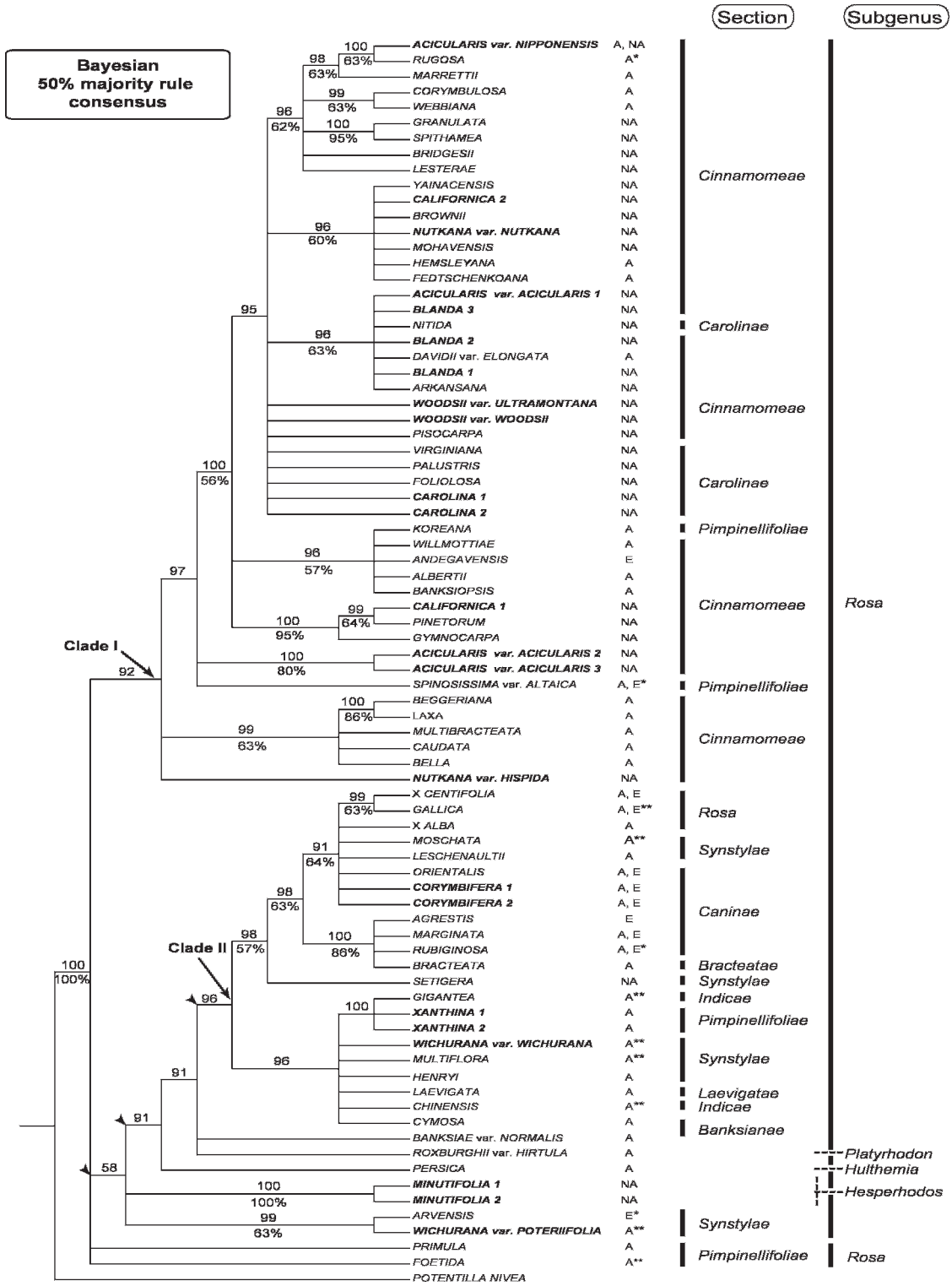

FIG. 2. Bayesian 50% majority rule consensus of trees sampled in the stationary phase of two analyses. Values above branches are the posterior probabilities for each clade (%), whereas values below branches are bootstrap values (>50%) for the same clades found during parsimony searches that excluded indels. Arrows indicate branches that were not recovered in the strict consensus of parsimony analyses that excluded indels. Additional information as in Fig. 1.

son 1934; Lewis 1957; Erlanson-Macfarlane 1966; Joly 2006), where features represent more of a continuum than discrete characteristics, is mirrored by the paucity of differentiation between rose species at the molecular level. This low level of sequence divergence explains the difficulty encountered when reconstructing the phylogeny of this taxonomically difficult genus, whether based on non-coding chloroplast sequences or on nuclear ribosomal spacers. In part for this reason, but also because of potential problems with hybridization or introgression in garden material, inferences of phylogenetic relationships must at this time be based on a careful comparison of clades that are similarly resolved in this and all previous phylogenetic analyses.

***Phylogenetic Relationships in* Rosa.** The present phylogenetic analysis of chloroplast DNA sequences does not fully support the classification of the genus *Rosa* as proposed by Rehder (1940). Rehder (1940) divided *Rosa* into the small subgenera *Hulthemia* (Dumort.) Focke, *Platyrhodon* (Hurst) Rehder and *Hesperhodos* Cockrell (each with 1 or 2 species), and the large subgenus *Rosa* (= *Eurosa* Focke), which is divided into ten sections: *Pimpinellifoliae* (DC.) Ser., *Rosa* [= *Gallicanae* (DC.) Ser.], *Caninae* (DC.) Ser., *Carolinae* Crép., *Cinnamomeae* (DC.) Ser., *Synstylae* (DC.), *Indicae* Thory, *Banksianae* Lindl., *Laevigatae* Thory, *Bracteatae* Thory. Although not fully resolved, the relationships observed indicate the presence of two main clades of subg. *Rosa* species (sects. *Carolinae/Cinnamomeae/Pimpinellifoliae* p.p. [Clade I] vs. all remaining subg. *Rosa* sections, except sect. *Banksianae* p.p. [Clade II]), with *R. banksiae* (sect. *Banksianae*), *R. roxburghii* (subg. *Platyrhodon*) and *R. persica* (subg. *Hulthemia*) as sister to Clade II (BPP = 92%, BS < 50%). Our parsimony analyses also suggest that *R. minutifolia* (subg. *Hesperhodos*) is sister to the entire genus, but the support for this relationship is weak. This pattern would contradict the analyses of the chloroplast *atpB-rbcL* sequences (Wissemann and Ritz 2005) and those of nuclear ITS sequences (Wu et al. 2001), which suggest that *R. persica* is sister to the rest of the genus. The other small subgenera are either unresolved in a basal polytomy, which also includes subg. *Hesperhodos* (*mat*K; Matsumoto et al. 1998), or they occur in poorly supported contradictory positions within subg. *Rosa* (e.g., Wu et al. 2001; Wissemann and Ritz 2005). Regardless, neither the present analysis nor any of the previous molecular analyses would support distinct subgeneric status for these taxa, and when resolution is available, all data seem to indicate a closer relationship to Clade II than to Clade I.

The presence of the same two main subg. *Rosa* clades is generally reflected in the analyses of RAPD data (Millan et al. 1996; Jan et al. 1999) and it is partially supported in the *matK* analysis of Matsumoto et al. (1998) and the *atp*B-*rbc*L intergenic spacer study of Wissemann and Ritz (2005). However, in the study by Wissemann and Ritz (2005), sect. *Bracteatae* (*R. bracteata*) occurs in the equivalent of our Clade I in the ITS analysis, and in their *atpB-rbcL* analysis, this section and sect. *Banksianae* (*R. banksiae*) occur in Clade I (with low support), rather than in (or associated with) Clade II, as in our analyses.

Few of the available molecular analyses support as monophyletic the sections proposed by Rehder (1940). However, in the analyses by Wissemann and Ritz (2005), with a strong concentration of *Rosa* sect. *Caninae* species, and in those of Wu et al. (2001), with a large number of native Japanese sect. *Synstylae* species, these sections tend to be supported as monophyletic. Similarly, in our study, with relatively better sampling of North American species for sects. *Cinnamomeae* and *Carolinae*, the analyses suggest that together these two sections may form a monophyletic group. Section *Cinnamomeae* is the largest rose section comprising over 40% of the species in the genus. Rehder (1940) separated sects. *Carolinae* and *Cinnamomeae* by sepals spreading and deciduous (*Carolinae*) versus upright and usually persistent (*Cinnamomeae*), and by achenes inserted at the base of the receptacle (*Carolinae*) versus achenes inserted on the wall and base of the receptacle (*Cinnamomeae*). However, subsequent authors have suggested that the two sections should be merged because of morphological similarity and the lack of consistency in the characters that are supposed to separate them (Lewis 1957; Robertson 1974). The distinction of these two sections is not substantiated by the molecular analyses presented here, nor by a recent analysis of a low-copy number nuclear gene for North American species that illustrated that the allopolyploid origin of the sect. *Carolinae* polyploid taxa renders the section polyphyletic (Joly et al. 2006). A biochemical study also argues against recognising section *Carolinae* (Grossi et al. 1998).

Rosa gymnocarpa, a western North American species, and several Asiatic species in our analysis such as *R. beggeriana*, *R. albertii*, and *R. willmottiae* have sometimes been segregated from section *Cinnamomeae* as section *Gymnocarpae* Crép., a group based on an unusual character where the entire calyx detaches at an articulation near the summit of the receptacle when the hip approaches maturity (Crépin 1896; Clarke 1980). Based on morphological and biogeographic evidence and a presumed

relationship to *R. gymnocarpa*, Ertter (2001) tentatively placed three further short-growing, rhizomatous species, *R. bridgesii*, *R. spithamea*, and *R. pinetorum*, restricted to California and Oregon, in sect. *Gymnocarpae* despite their lack of a deciduous calyx. Although the present analysis does not provide clear resolution as to the position of all these taxa, there is an indication that sect. *Gymnocarpae* as circumscribed by deciduous calices or sensu Ertter is polyphyletic.

In our analyses and previous chloroplast DNA studies (e.g., Matsumoto et al. 1998, 2001), sect. *Pimpinellifoliae* is resolved as polyphyletic with certain species clearly belonging to Clade I, others to Clade II, and still others with unresolved positions at the base of the chloroplast DNA phylogeny. The position of *R. koreana* (sect. *Pimpinellifoliae*) with sect. *Cinnamomeae* is fully expected given the phenetic analysis of sect. *Pimpinellifoliae* by Roberts (1977). Roberts transferred this species and *R. farreri* Stapf to sect. *Cinnamomeae* on the basis that both species were more closely related to *R. forrestiana* Boulenger, a species like many in sect. *Cinnamomeae* (e.g., *R. multibracteata*, *R. bella*; Clarke 1980) that resembles the *Pimpinellifoliae*. It is therefore not too surprising that two species of sect. *Pimpinellifoliae*, *R. koreana* and *R. spinosissima*, would appear to be embedded within section *Cinnamomeae*. Matsumoto et al. (1998) found a similar position for their sample of *R. spinosissima*. Two further species of sect. *Pimpinellifoliae*, *R. foetida* and *R. primula*, may be found either in a trichotomy with Clade I or in a polytomy at the base of the genus. Again, the results for *R. foetida* are supported by the *matK* analyses of Matsumoto et al. (1998, 2001). The tetraploid *R. foetida* is known to have low fertility (Roberts 1977) and may represent an ancient hybrid of unknown parentage (Wylie 1954).

Within Clade I it is interesting to note the varied phylogenetic positions of polyploid taxa for which more than one individual was sequenced. In particular, the position of the diploid *R. acicularis* var. *nipponensis* as sister to *R. rugosa* in an entirely Asian clade, and separate from polyploid *R. acicularis* may support Lewis's (1959) contention that this taxon should be treated at the species level. The relatively well-supported grouping of *R. rugosa*, *R. marrettii* and *R. acicularis* var. *nipponensis* also is resolved in the ITS analyses of Wu et al. (2001). The remaining samples of *R. acicularis* occur in different positions in Clade I. *Rosa acicularis* has both hexaploid and octoploid populations, and for this species, the incongruence also may suggest that *R. acicularis* has multiple independent origins from different maternal parents as has been seen in many other polyploid species (e.g., Doyle et al. 1990; Soltis et al. 1995). A similar explanation is possible for two polyploid western North American species, *R. nutkana* (where two varieties were sampled) and *R. californica*. For other taxa, the incongruence may be the consequence of the non purity (hybridization, introgression) of the garden samples included in the analysis. For example, this may explain the conflicting positions of the two *R. wichurana* specimens as either nested within Clade II or as sister to it, where we used two garden-collected specimens, but from different gardens. This also may explain contradictory positions for the same species among the different chloroplast DNA analyses that have been published to date (present study; Matsumoto et al. 1998, 2001; Wissemann and Ritz 2005). For example, we suspect that contaminated botanical garden samples explains the position of *R. californica* within ''Clade II'' in the *matK* analyses of Matsumoto et al. (1998, 2001) and of *R. palustris* (sect. *Carolinae*) and *R. rugosa* (sect. *Cinnamomeae*) at the base of ''Clade II'' in the *atpB-rbcL* analyses of Wissemann and Ritz (2005). Likewise the position of *R. bracteata* (sect. *Bracteatae*) in our analyses as nested within Clade II, rather than as sister to *R. cymosa* (sect. *Banksianae*), contradicts the analyses of Matsumoto et al. (1998, 2001) and of Wissemann and Ritz (2005), and suggests that our sample of *R. bracteata* may represent a botanical garden contaminant.

Although poor clade support makes it difficult to draw strong conclusions, some interesting biogeographic patterns are apparent in our analyses. The presence of rose species in North America appears to be the consequence of multiple introductions in a genus that mostly is concentrated in the Old World (Europe and Asia). If we consider *R. minutifolia* as sister to all other *Rosa* species, then there would be a split at the base of the phylogeny between North American and Old World taxa. Based on these data it is therefore not possible to determine whether the genus *Rosa* has an Old or New World origin. An obvious introduction of *Rosa* in North America occurred in Clade II with *R. setigera*, the only species of this clade that is native to North America. Within Clade I, with the exception of *R. nutkana* var. *hispida* (a probable polyploid) and the polyploid *R. acicularis* (parsimony analysis), all of the first branching taxa are Asian in origin, whereas the later branching taxa are a mix of both North American and Asian species. It also is noteworthy that all diploid North American roses in Clade I are contained within a single subclade, although they are intermixed with Asian taxa. This suggests a single New World introduction for all diploid North American

species of subg. *Rosa*, but because of the low resolution we cannot firmly conclude whether the Old World species in the mostly North American clade represent reintroductions in the Old World or an ancestral presence.

Origin of Garden Roses. Most authors believe that only seven species, *R. chinensis*, *R. foetida*, *R. gallica*, *R. gigantea*, *R. moschata*, *R. multiflora*, and *R. wichurana*, have made major contributions to the creation of the modern commercial rose, with a further seven species providing only ''minor'' contributions (Wylie 1954). By repeated crosses and backcrosses over time, rose breeders have used these putative parents to incorporate such desirable traits as brilliant yellow (*R. foetida*), winter hardiness (*R. wichurana*), perpetual flowering (*R. chinensis*, *R. gigantea*), and a sweet bouquet (*R. gallica*) into garden cultivars. This has led some authors to suggest that the genetic background of the modern domesticated rose is narrow (e.g., Matsumoto et al. 1998), although this is disputed (Debener et al. 1996). Of the species listed by Wylie (1954) as having made major contributions to the origin of cultivated roses, six of the seven sampled are found within Clade II. The seventh, *Rosa foetida*, is unresolved between the two subg. *Rosa* clades or is weakly supported as sister to Clade I. Although we have not sampled all the species considered to have made a minor contribution to the development of garden roses, it is likely that of the seven listed by Wylie (1954), only three (*R. spinosissima, R. cinnamomea* and *R. rugosa*) occur in Clade I. Thus the concentration of the limited number of both major and minor contributors to the domestic rose within a single clade largely supports the conclusion of Matsumoto et al. (1998) that commercial roses have a narrow genetic background.

Assuming maternal chloroplast inheritance in Rosaceae (e.g., Corriveau and Coleman 1988; Raspé 2001; Brettin et al. 2000; Panda et al. 2003) and in *Rosa* (Corriveau and Coleman 1988), our phylogeny would suggest that the maternal parent of *R.* × *centifolia* var. *mucosa*, a complex hybrid believed to have been formed by the crossing of multiple species from sects. *Rosa* (*R. gallica*, *R.* × *damascena*), *Synstylae* (*R. moschata*) and *Caninae* (*R. canina*) (Matthews 1995), is *Rosa gallica*. The chloroplast genome of *R. gallica* and *R.* × *centifolia* for the markers examined is identical. Likewise, the position of *R.* × *alba* (sect. *Rosa*), the White Rose of York, would suggest that its disputed origin, whether from a cross between *R. gallica* and *R. arvensis* or *R. corymbifera*, or even a cross between *R. canina* and *R.* × *damescena*, can at least eliminate *R. gallica* as the maternal species of the cross. In this analysis, *R.* × *alba* shared identical chloroplast genomes to *R. corymbifera*, *R. leschenaultii*, *R. moschata*, and *R. orientalis*.

In conclusion, although none of the presently available phylogenetic analyses has by itself recovered strongly supported groups within the genus *Rosa*, the occurrence of some of the same clades in multiple analyses increases support for their monophyly. For example, most studies have recovered two major clades within the genus, one consisting of sections *Cinnamomeae* (including *Carolinae*) and *Pimpinellifoliae* and the other of sections *Banksianae*, *Bracteatae*, *Caninae*, *Rosa*, *Indicae*, *Laevigatae*, *Pimpinellifoliae*, and *Synstylae*. One cautionary note concerning this and previous phylogenies is that most of the material used in this study had its origins in gardens. Even though this is a concern for any study that uses garden material it is especially troublesome when studying groups that are known to hybridize. For logistical and political reasons it is not always possible to collect wild material. Although this does not entirely negate the results of this and previous studies it should be noted that the possibility that garden material may be the result of artificial crosses, whether by human or by open pollination in a garden, cannot be excluded. Future studies should attempt to confirm the sequences presented here for each taxon with sequences derived from wild collected material. Moreover, the little genetic variation of the chloroplast genome found here and in previous studies demonstrates that in order to recover well supported clades future phylogenetic studies of the genus will need to use non-coding regions of low-copy nuclear genes such as those used by Joly and Bruneau (2006).

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APPENDIX 1. Classification and voucher data for taxa used in combined analysis of *trnL-F* and *psbA-trnH* sequences in *Rosa*. Ingroup taxa are arranged in alphabetical order according to section, with the outgroup taxon placed last. Subgeneric and sectional delimitation follows Rehder (1940), with nomenclatural modifications noted in Wissemann (2003). Multiple specimens from the same species are numbered 1, 2 or 3. Asterisks indicate whether a taxon is considered to have made a minor (*) or major (**) genetic contribution to rose cultivars according to Wylie (1954).

Abbreviations for garden material are as follows: JBM (Montreal Botanical Garden), AA (Arnold Arboretum), RBGE (Royal Botanic Gardens, Edinburgh), WAG (Wageningen University Botanical Garden), RASABG (Rancho Santa Ana Botanical Garden), UCB (University of California, Berkeley). The first accession number is for the *trnL* region, the second for the *psbA-trnH* spacer.

Subg. *Hesperhodos* Cockerell: *R. minutifolia* Engelm. – 1, UCB 86.0999 – MEXICO: Baja California del Norte, Voucher deposited at UC, DQ778865, DQ778786; *R. minutifolia* Engelm. – 2, MEXICO: Baja California (garden-grown specimen) *Ertter G-422* (UC), DQ778866, DQ778787.

Subg. *Hulthemia* (Dumort.) Focke.: *R. persica* Michx., RBGE 19802360B – UZBEKISTAN: between Tashkent and Chimigan, *Matthews 1108* (E), DQ778878, DQ778799.

Subg. *Platyrhodon* (Hurst.) Rehder: *R. roxburghii* Tratt. var. *hirtula* (Reg.) Rehd. & Wils., JBM 1294-2000 – UNITED KINGDOM: Royal Botanic Gardens Kew, *Dickson 1165* (MT), DQ778883, DQ778804.

Subg. *Rosa*: Sect. *Banksianae* Lindl.: *R. banksiae* Ait. var. *normalis* Reg.: WBG 96BG52202 – SPAIN: Real Jardin Botanico, Madrid, no voucher, DQ778828, DQ778749; *R. cymosa* Tratt. RBGE 19911805A – CHINA: Yunnan, *Howick & McNamara 1567* (E) DQ778846, DQ778767; **Sect. *Bracteatae* Thory:** *R. bracteata* Wendl., AA 670-81 – CHINA: Shanghai Bot. Garden (wild collected in China, Anhuei Province, Huanshan Mt), *Thornton, Reynolds & Harrison 231* (A)*; Chapin 77* (A)*; Gamble, Wunderle & Thornton 131* (A), DQ778833, DQ778754; **Sect. *Caninae* DC.:** *R. agrestis* Savi.. JBM 1623-75 – LATVIA: Academia Scientiarum, Salaspils, *Bruneau 1192* (MT), *Gervais 92* (MT), DQ778823, DQ778744; *R. corymbifera* Borkh. – 1, JBM 1102-76 – UZBEKISTAN: Acad. Scient. Taschkent., *Gervais 116* (MT); DQ778838, DQ778759; *R. corymbifera* Borkh – 2, WBG BG21920 – NETHERLANDS: Frederiksoord, MtuS, *Gervais 142* (MT), DQ778844, DQ778765; R. *rubiginosa* L.*, JBM 1936-76 – NORWAY: Universitas Bergensis, Bergen, *Bruneau 1191* (MT), *Gervais 103* (MT), DQ778848, DQ778769; *R. marginata* Wallr., JBM 890-77 – GERMANY: Botanischer Garten, Dortmund, *Dickson 1155* (MT), *Gervais 101* (MT), DQ778864, DQ778785; *R. orientalis* Dupont ex Seringe, WBG BG23960 – RUSSIA: Mescherkoje, *Gervais 152* (MT), DQ778876, DQ778797; **Sect. *Carolinae* Crép.:** *R. carolina* L. – 1, USA: Virginiana, Augusta Co., *Joly 528 & Starr* (MT), DQ778839, DQ778760; *R. carolina* L. – 2: USA: West Virginia, Randolph Co., *Joly 535 & Starr* (MT), DQ778840, DQ778761; *R. foliolosa* Nutt., USA: Oklahoma, Okmulgee Co., *Lewis 15846-1* (MO), DQ778851, DQ778772; *R. nitida* Willd., CANADA: New Brunswick, Albert Co., *Joly 942, Starr & Thibeault* (MT), DQ778873, DQ778794; *R. palustris* Marsh., USA: Pennsylvania, Erie Co., *Joly 560 & Starr* (MT), DQ778877, DQ778798; *R. virginiana* Miller, USA: Maryland, Worcester Co., *Joly 517 & Starr* (MT), DQ778888, DQ778809; **Sect. *Cinnamomeae*:** *R. acicularis* Lindl. var. *acicularis* Lindl. – 1, CANADA: Saskatchewan, Sakatoon, *Charest & Brouillet 2* (MT), DQ778820, DQ778741; *R. acicularis* Lindl. var. *acicularis* Lindl. – 2, CANADA: Alberta, Redwood Meadows, *Dickson 1175* (MT), DQ778821, DQ778742; *R. acicularis* Lindl. var. *acicularis* Lindl. – 3, CANADA: Manitoba, Winnipeg, *Joly 709 & Starr* (MT), DQ778822, DQ778743; *R. acicularis* Lindl. var. *nipponensis* (Crép.) Koehne, JAPAN: Honshu Shizuoka Pref., Mt. Fuji, *Togashi s.n.* (WIS), DQ778872, DQ778793; *R. albertii* Regel, AA 837-90 – RUSSIA: Acad. Science, Tallinn (wild collected in Turkestan), *Straate & Uyterhoeven 326-02* (A), DQ778825, DQ778746; *R. andegavensis* Bast. (=*R. nanothamnus* Boulenger var. *litvinovii* Boulenger), RBGE 19612577A – unknown provenance, collected in 1961, voucher deposited at (E), DQ778871, DQ778792; *R. arkansana* Porter, CANADA: Manitoba, *Joly 730 & Starr* (MT), DQ778826, DQ778747; *R. banksiopsis* Baker, JBM 1097-76 – UZBEKISTAN: Academiae Scientarum, Taschkent, *Dickson 1147* (MT), *Gervais 111* (MT), DQ778829, DQ778750; *R. beggeriana* Schrenk, AA 1048-83 – BRITAIN: Royal Botanic Gardens Edinburgh, *Brown, Kelley & Thornton 153* (A), DQ778830, DQ778751; *R. bella* Reher & Wilson, AA 455-83A – SWEDEN: Uppsala University Botanical Garden (wild collected in China), *Chapin 352* (A), DQ778831, DQ778752; *R. blanda* Ait. – 1, CANADA: New Brunswick, York Co., *Joly 409 & Starr* (MT), DQ778832, DQ778753; *R. blanda* Ait. (=*R. johannensis* Fern.) – 2, CANADA: Québec, Parc du Bic, *Bruneau 1240* (MT), DQ778858, DQ778779; *R. blanda* Ait. (=*R. rousseauiorum* Boivin) – 3, CANADA: Québec, Saint-Fabien, *Bruneau 1250* (MT), DQ778882, DQ778803; *R. bridgesii* Crép. ex Rydb., USA: California, Madera Co., Willow Creek (garden grown specimen), *Ertter 12207* (UC), DQ778834, DQ778755; *R. brownii* Rydb., USA: California, Shasta Co., *Ertter 17967* (UC), DQ786403, DQ786402; *R. californica* Cham. & Schlechtend – 1, USA: California, Alameda Co., *Ertter 17954* (UC), DQ778836, DQ778757; *R. californica* Cham. & Schlechtend – 2, USA: California, Tehama Co., *Ertter 17975* (UC), DQ778837, DQ778758; *R. caudata* Baker, JBM 1106-76 – NORWAY: Universitas Bergensis, Bergen, *Dickson 1145* (MT), *Gervais 109* (MT), DQ778841, DQ778762; *R. corymbulosa* Rolfe., JBM 1108-76 – NORWAY: Universitas Bergensis, Bergen, *Dickson 1144* (MT), *Gervais 108* (MT), DQ778845, DQ778766; *R. davidii* Crép. var. *elongata* Rehder & Wilson, AA 1073-83 – BELGIUM: J. Massart Experimental Garden, *Brown, Kelley & Thornton 108* (A), DQ778847, DQ778768; *R. fedtschenkoana* Regl., AA 622-78 – USA: Cary Arboretum, Millbrook, NY (wild collected in Kyrgyzstan), *Gamble, Wunderle & Thornton 126* (A), DQ778849, DQ778770; *R. granulata* Greene, USA: California, San Luis Obispo Co., *Ertter 14881* (UC), DQ778854, DQ778775; *R. gymnocarpa* Nutt., USA: Idaho, Nez Perce, above Waha Lake, *Ertter 18001* (UC), DQ778855, DQ778776; *R. hemsleyana* Tackholm, JBM 1564-78 – POLAND: Dendrologiae Institutum, Kornik, *Dickson 1146* (MT), *Gervais 110* (MT), DQ778856, DQ778777; *R. laxa* Retzius cultivar 'Retzius', JBM 3128-93 – CANADA: Paul Olsen, Roseberry Gardens, Thunder Bay, Ontario, *Dickson 1140* (MT), *Gervais 127* (MT), DQ778861, DQ778782; *R. lesterae* Eastwood, USA: California, Yuba Co., *Ertter 17983* (UC), DQ778835, DQ778756; *R. marretii* H. Lev., WBG BG22228 – CANADA: University of Guelph Arboretum, Ontario, no voucher, DQ778863, DQ778784; *R. mohavensis* Parish, USA: Nevada, Clark Co., *Ertter 17525* (UC), DQ778867, DQ778788; *R. multibracteata* Hemsl. & Wils., WBG BG21940 – NETHERLANDS: Den Haag, S.G.A. Doorenbos, *Gervais 155* (MT), DQ778869, DQ778790; *R. nutkana* Presl var. *nutkana* Presl, USA: California, Mendocino Co., *Ertter 18013* (UC), DQ778874, DQ778795; *R. nutkana* Presl var. *hispida* Fern., USA: Idaho, Latah Co., *Ertter 18011* (UC), DQ778875, DQ778796; *R. pinetorum* Heller, USA: California, Monterey Co. SFB Morse Botanical Reserve, *Ertter 11888* (UC), DQ778879, DQ778800; *R. pisocarpa* A. Gray, JBM 1126-76 – NORWAY: University of Bergen Botanical Garden, *Bruneau, Joly & Gauthier 1270* (MT), DQ778880, DQ778801; *R. rugosa* Thunb.*, JBM 1427-70 – USA: Smith College Botanical Garden, Northampton, Massachusetts, *Gervais 104* (MT); *Dickson 1142* (MT), DQ778884, DQ778805; *R. spithamea* S. Watson, USA: California, San Luis Obispo Co., *Ertter 14880* (UC), DQ778887, DQ778808; *R. webbiana* Royle, JBM 896-77 – GERMANY: Botanischer Garten, Dortmund, *Dickson 1138* (MT), DQ778889, DQ778810; *R. willmottiae* Hemsl., JBM 0047-96 – FRANCE: Les Rosiers Du Loire, Les Brettes, Nantes, *Bruneau, Joly & Gauthier 1271* (MT), DQ778892, DQ778813; *R. woodsii* Lindl. var. *woodsii* Lindl., CANADA: Saskatchewan, Antler River Valley, *Joly 750 & Starr* (MT), DQ778893, DQ778814; *R. woodsii* Lindl. var. *ultramontana* (S. Wats.)

Heller, USA: Idaho, Nez Perce Co., *Ertter 18002* (UC), DQ778894, DQ778815; *R. yainacensis* Greene, WBG 89BG05002 – GERMANY: Dortmund, B.G. Rombergpark, no voucher, DQ778897, DQ778818; **Sect. *Indicae* Thory:** *R. chinensis* Jacq. var. *spontanea* (Rehder & E.H.Wilson) T.T.Yu & T.C.Ku**, RBGE 19890840 – CHINA: Sichuan, C: Wenjiang Pref., Xinjin Co., *Sichuan Expedition (1988) 237* (E), DQ778843, DQ778764; *R. gigantea* Collett ex. Crép.**, JBM 2562-93 – CHINA: Inst. Botanici Kunminensis, Junming, Yunnan, no voucher, DQ778853, DQ778774; **Sect. *Laevigatae* Michx.:** *R. laevigata* Michx., RBGE 19599848 – cultivated origin, no voucher, DQ778860, DQ778781; **Sect. *Pimpinellifoliae* DC.:** *R. foetida* Herrm. var. *bicolor* (Jacq.) Willm.**, JBM 2019-92 – CANADA: Michel A. Otis, Montréal, *Dickson 1143* (MT), *Gervais 93* (MT), DQ778850, DQ778771; *R. koreana* Komarov, WBG BG22246 – RUSSIA: Moscow, H.B. Principals, no voucher, DQ778859, DQ778780; *R. primula* Boulenger, JBM 2403-78 – CHINA: Peking, Beijing, *Gervais 98* (MT), DQ778881, DQ778802; *R. spinosissima* L. var. *altaica* (Willd.) Rehd.*, JBM 1650-75 – LATVIA: Acad Scient, Salaspils, Latvia, *Gervais 118* (MT), *Dickson 1149* (MT), DQ778886, DQ778807; *R. xanthina* Lindl. (=*R. hugonis* Hemsl.) – 1, JBM 2898-90 – CANADA: Parc Floral, 9/07/1990, *Gervais 120* (MT), DQ778895, DQ778816; *R. xanthina* Lindl. (=*R. hugonis* Hemsl.) – 2, WBG 96BG09802 – BRITAIN: University Botanic Garden, Bristol, *Gervais 150* (MT); DQ778896, DQ778817; **Sect. *Rosa*:** *R.* X *alba* L., JBM 2284-77 – RUSSIA: Academiae Scientiarum, Moscow, *Bruneau 1194* (MT), *Gervais 82* (MT), DQ778824, DQ778745; *R.* X *centifolia* L. var. *muscosa* (Mill.) Ser., AA 266-94 – cultivated origin, voucher deposited at A, DQ778842, DQ778763; *R. gallica* L.**, JBM 1715-91 – GERMANY: Forstbotanischer Garten und Arboretum der Universität Göttingen, *Bruneau 1185* (MT), DQ778852, DQ778773; **Sect. *Synstylae* DC.:** *R. arvensis* Huds.*, JBM 1626-75 – LATVIA: Academia Scientiarum, Salaspils, *Bruneau 1182* (MT), Gervais 83 (MT), DQ778827, DQ778748; *R. henryi* Bouleng., WBG 86BG645914 – CHINA: Zhejiang Prov., Chang-Hua pref., *Wieringa 3460* (WAG), DQ778857, DQ778778; *R. leschenaultiana* Wight & Arnott, WBG 81BGN2102 – Roseraie de L'Hay, no voucher, DQ778862, DQ778783; *R. moschata* Herrm.**, JBM 2639-93 – SPAIN: Real Jardin Botanico, Madrid, *Dickson 1162* (MT), DQ778868, DQ778789; *R. multiflora* Thunb.**, USA: Massachusetts, Essex Co., *Joly 456 & Starr* (MT), DQ778870, DQ778791; *R. setigera* Michx., USA: Pennsylvania, Erie Co., *Joly 553 & Starr* (MT), DQ778885, DQ778806; *R. wichurana* Crép. var. *wichurana* Crép. **, JBM 1132-76 – UZBEKISTAN: Academiae Scientarum, Taschkent, *Bruneau 1195* (MT), *Gervais 84* (MT), DQ778890, DQ778811; *R. wichurana* Crép. var. *poteriifolia* Koidz.**, AA 1556-83 – JAPAN: Shikoku region, Murotozaki, Kochi-ken (wild collected by J. Creech), *Harrison & Brown 80* (A), DQ778891, DQ778812.

Outgroup taxon: *Potentilla nivea* L., JBM 2480-96 – ITALY: Gran Paradiso National Park, Alpine Botanical Garden ''Paradisia'', Cogne, Valnontey, no voucher, DQ778898, DQ778819.