Phylogenetic Relationships in the Genus *Rosa* Revisited Based on *rpl16*, *trnL-F*, and *atpB-rbcL* Sequences

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Abstract. Three chloroplast DNA (cpDNA) sequences [the *rpl16* intron, the *trnL-F*, and *atpB-rbcL* intergenic spacer (IGS)] were employed to study phylogenetic relationships in the genus *Rosa*. Phylogenetic analyses using these three concatenated sequences were performed using maximum parsimony (MP) and Bayesian inference (BI) methods. Both analyses results suggest that the molecular phylogeny conforms closely to the conventional classification of botanical sections. Morphological similarities between *R. sects. Synstylae* and *Chinenses*, and *R. sects. Rosa* and *Carolinae* are corroborated on the molecular level in our analyses. Four taxa from *R. sect. Pimpinellifoliae* are further divided into two small clades, which reflect the morphological characters for these species on a molecular level. Whereas three accessions of *R. foetida* from *R. sect. Pimpinellifoliae* form a separate clade. *R. ×fortuniana* forms a clade with *R. laevigata* based on its maternal inheritance of cpDNA. *R. ×cooperii* is hypothesized to be a hybrid with seed parent from *R. sects. Synstylae* and *Chinenses*. And *R. roxburghii* should be classified as a section within the *R. subgen. Rosa*, rather than being treated as its own subgenus, based on molecular analyses.

The genus *Rosa* L. comprises ≈200 species distributed widely throughout the temperate and subtropical regions of the northern hemisphere (Ku and Robertson, 2003; Rehder, 1940; Wissemann, 2003). However, less than 10 species have likely contributed to modern cultivated roses (Wylie, 1954). The genetic background of cultivated roses is,

therefore, very narrow compared with the abundant germplasm resources available in this genus (Bruneau et al., 2007; Matsumoto et al., 1998). Genetic relationship studies within the genus based on morphology are difficult owing to intraspecific variability, polyploidy, and interspecific hybridization (Bruneau et al., 2007; Wissemann, 2003).

Conventional taxonomy (Rehder, 1940; Wissemann, 2003) divides the genus into four subgenera: *R. subgen. Hulthemia* (Dumort.) Focke, *R. subgen. Platyrrhodon* (Hurst) Rehder, *R. subgen. Hesperhodos* Cockerell, and *R. subgen. Rosa*. The first three subgenera are monotypic or contain two species, whereas the *R. subgen. Rosa* contains all the remaining species and is divided into 10 sections: [*R. sect. Pimpinellifoliae* (DC.) Ser., *R. sect. Cinnamomeae* (DC.) Ser., *R. sect. Synstylae* DC., *R. sect. Indicae* Thory, *R. sect. Banksianae* Lindl., *R. sect. Laevigatae* Thory, *R. sect. Bracteatae* Thory, *R. sect. Gallicanae* (DC.) Ser., *R. sect. Carolinae* Crép., and *R. sect. Caninae* (DC.) Ser.]. However, species within this genus are only divided into *R. subgen. Hulthemia* and *R. subgen. Rosa* in *Flora of China* (Ku and Robertson, 2003), where the *R. subgen. Platyrrhodon* is treated as *R. sect. Microphyllae* Crép. in *R. subgen. Rosa* (Crép., 1889; Ku and Robertson, 2003), and the *R. subgen. Hesperhodos*, *R. sects. Carolinae* and *Caninae* are not included in this classification system at all.

Phylogenetic relationships on a molecular level within this genus have previously been investigated based on restriction/amplified fragment length polymorphism analyses (Koopman et al., 2008; Matsumoto et al., 1997), random amplified polymorphic DNA analyses (Debener et al., 1996; Jan et al., 1999; Millan et al., 1996), and microsatellite analyses (Scariot et al., 2006; Tang et al., 2008). Furthermore, nucleotide sequence data have increasingly been used in phylogenetic studies of this genus, for instance, the internal transcribed spacer (*ITS*) of nuclear ribosomal DNA (nrDNA) (Matsumoto et al., 2000; Wissemann and Ritz, 2005; Wu et al., 2001), the cpDNA *matK* gene (Matsumoto et al., 1998; Wu et al., 2000), the cpDNA *atpB-rbcL* IGS (Wissemann and Ritz, 2005), the combined cpDNA *trnL-F* and *psbA-trnH* IGS (Bruneau et al., 2007), and the combined nrDNA *ITS* and cpDNA *matK* gene (Qiu et al., 2012). Although *Rosa* species phylogeny has been researched using these different markers, when comparing with this notoriously complex genus, its resolution remains poor. Additionally, contradictory results exist between these previous investigations.

In this study, we analyzed 48 genotypes including several mutations and variations within this genus by using the cpDNA *rpl16* intron, the *trnL-F*, and *atpB-rbcL* IGS sequences, which initial analyses have shown to be reasonably variable. Several species used in the present study were not included in previous analyses. Moreover, there are few reports regarding phylogenetic relationships in *Rosa* that combine three or more different DNA sequence regions (Fougère-Danezan et al., 2015; Leus et al., 2004; Zhu et al., 2015). The objectives of this study are to construct a robust, well-resolved phylogeny for *Rosa* accessions derived from multiple molecular marker systems, and to provide suggestions regarding the taxonomic classification by comparing the present findings

with results from previous phylogenetic studies of this genus. For the purpose of this research, *R. roxburghii* Tratt. is classified in *R. sect. Microphyllae* following Crépin (1889) and Ku and Robertson (2003), thus, eleven sections are represented by the taxa in our investigation. Besides, in this study *R. sect. Chinenses* (DC.) Ser. was used instead of *R. sect. Indicae* since the former is the oldest valid name while the latter is a synonym (Seringe, 1818; Thory, 1820). And following the proposal that the type species of this genus is *R. cinnamomea* L. (Jarvis, 1992), *R. sect. Rosa* was used instead of *R. sect. Cinnamomeae* in this study.

Materials and Methods

Plant materials. The list of wild roses used in this study is provided in Table 1. Sample numbers 1–5, 8–11, 13–24, and 29–46 were grown in the field at the Rose Trial Station of the Shenzhen Park Service, Guangdong, China. Sample numbers 6, 7, 14, 15, 26, and 27 were grown in the field at the Flower Research Institute nursery, Yunnan Academy of Agricultural Sciences, Yunnan, China. Sample numbers 12, 28, 47, and 48 were grown in the field at the Shanghai Chenshan Botanical Garden, Shanghai, China. Morphological traits and literature descriptions were used to confirm the identity of specimen (Ku and Robertson, 2003; Rehder, 1940; Wissemann, 2003). Four species from the related genera *Rubus* L. and *Spiraea* L. (*Rubus lambertianus* Ser., *Rubus hirsutus* Thunb., *Rubus corchorifolius* L., and *Spiraea trilobata* L.) were chosen as the out-group and samples were collected from Zijin Mountain, Jiangsu, China. Young leaves of all accessions were collected and froze at -80°C for storage until use. All the voucher samples were preserved and archived in the Herbarium of Nanjing Forestry University.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing. Seven DNA regions from three genomes were examined for their variability among the accessions: the ITS of nrDNA, the *rpl16* intron, the *trnL-F* and *atpB-rbcL* IGS of cpDNA, and the *ccb256*, *cox2*, and *nad1* genes of mitochondrial DNA (mtDNA). Among these seven regions, the ITS region was abandoned owing to extensive

sequence polymorphisms within a single genotype (Matsumoto et al., 2000; Wissemann and Ritz, 2005; Zhu et al., 2015), and the mtDNA *ccb256*, *cox2*, and *nad1* genes were abandoned because of a lack of informative sites in the data matrix (0.4%, 0.3%, and 0.6%, respectively, excluding the out-group, unpublished results). The three cpDNA regions were comparatively variable and were used for phylogenetic analyses.

Total DNA from the leaves of individual plants was isolated using a Genomic DNA Extraction Kit (DP305; TianGen Co., Ltd, Beijing, China). The three cpDNA regions were amplified by PCR in a reaction mixture of 25- μL total volume, containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl_2 , 200 μM deoxynucleotide triphosphates, 2.5 U Taq-DNA polymerase, 50 ng template DNA, and 1.0 μM forward and reverse primers of *rpl16*, *trnL-F*, and *atpB-rbcL*, accordingly (Table 2). The amplification conditions for each region were as follows: for *rpl16*, an initial 4 min at 95°C , followed by 32 cycles of 1 min at 94°C , 1 min at 52°C , 1 min at 72°C , and 10 min at 72°C for the final extension; for *trnL-F*, an initial 3 min at 95°C , followed by 35 cycles of 30 s at 95°C , 30 s at 50°C , 1 min 20 s at 72°C , and 7 min at 72°C for the final extension (Bruneau et al., 2007); and for *atpB-rbcL*, an initial 3 min at 95°C , followed by 30 cycles of 30 s at 95°C , 45 s at 48°C , 1 min at 72°C , and 5 min at 72°C for the final extension (Wissemann and Ritz, 2005). Amplifications were performed in an Eppendorf thermocycler (Mastercycler Nexus X1). The amplified products were sequenced by Invitrogen (Shanghai, China). Sequencing primers were identical to those used for amplification.

Phylogenetic analyses. The sequences were aligned using Clustal X (Thompson et al., 1997) and checked manually in BioEdit (Hall, 1999). Our three cpDNA sequence alignments were used to construct separate (not shown in this paper) and combined data matrixes. Modeltest (Posada and Crandall, 1998) was used to perform hierarchical likelihood ratio tests to estimate an optimal nucleotide substitution model for use with MrBayes (Ronquist and Huelsenbeck, 2003). The MP method in PAUP* 4.0b10 (Swofford, 2002) was used with its heuristic search option to find the most parsimonious trees. Insertions and/or deletions (indels) were included in the matrixes, and the resulting gaps were treated as missing data. Character state changes were all weighted equally. Searches were performed using 1000 replicates for bootstrap analysis (Felsenstein, 1985).

BI using Monte Carlo Markov chain (MCMC) was performed with MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). Four incrementally heated simultaneous MCMCs were run over 2,000,000 generations, using the general time reversible nucleotide substitution model with gamma distributed substitution rates, random starting trees, and default nucleotide substitution model starting values. Trees were sampled every 100 generations resulting in an overall sampling of 20,001 trees. The first 1000 “burn-in” trees

were excluded from each analysis. A 50% majority rule consensus tree was computed to obtain estimates of a posteriori probabilities. Two independent analyses were conducted using these initial parameters.

Results

Sequence analysis. Summary sequence statistics for the three cpDNA regions were presented in Table 3. Among these three regions, the proportion of informative characters was highest in the *rpl16* region (7.6% and 4.6%, depending on inclusion or exclusion of the out-group, respectively). The percentage of informative characters in the *trnL-F* and *atpB-rbcL* were 3.5% and 2.4%, within the in-group data matrixes, respectively. These three regions were all relatively easy to align; however, two microsatellites (9–12 A's and 8–15 A's) at positions 342–353 and 418–432 in the *rpl16* region, and one microsatellite (9–12 T's) at positions 427–438 in the *trnL-F* spacer were excluded from analyses data because these regions could not be reliably aligned (Bruneau et al., 2007).

Phylogenetic analyses. A total of 2610 nucleotides were used for phylogenetic analyses, heuristic searches of combined data matrix including indels produced 285 variable characters and 207 informative characters, between in-group and out-group in the data matrix (Table 3). The heuristic search resulted in 2000 trees, with a tree length of 607, a consistency index 0.873, and a retention index of 0.902. The Bayesian 50% majority rule consensus of 19,001 trees in stationary phase from the two analyses is presented in Fig. 1. Results from the Bayesian analyses were very similar to those using parsimony.

Both methods suggested that the 48 taxa used in our study should be divided into two major groups. The first group was composed of taxa from *R. sects. Pimpinellifoliae*, *Rosa*, and *Carolinae*. Within this group, taxa from *R. sects. Rosa* and *Carolinae* formed an inner clade, two other clades were set apart and were constituted by taxa both from *R. sect. Pimpinellifoliae*. The second group included all the remaining taxa and was further divided into five clades: the first clade consisted of taxa from *R. sects. Synstylae*, *Chinenses*, *Gallicanae*, and *Caninae* as well as *R. xcooperii* (sample no. 43), the molecular data placed taxa from *R. sect. Caninae*, and *R. arvensis* (sample no. 28) of *R. sect. Synstylae* in a sister relationship to the reminder accessions. The second clade consisted of taxa from *R. sect. Banksianae*, except for *R. xfortuniana* (sample no. 37), which formed the third clade together with *R. laevigata* (sample no. 42) of *R. sect. Laevigatae*. The fourth clade and the fifth clade contained accessions from *R. sects. Microphyllae* and *Bracteatae*, respectively.

Discussion

The 48 taxa phylogeny presented (Fig. 1) based on our three concatenated sequence regions conforms closely to the conventional

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Table 1. Taxa sampled for *rpl16*, *trnL-F* and *atpB-rbcL* sequence analyses.

Sample no.	Species	Source	Year	Color	Petals	GenBank accession no.		
						<i>rpl16</i>	<i>trnL-F</i>	<i>atpB-rbcL</i>
Sect. 1. <i>Pimpinellifoliae</i>								
1	<i>R. foetida</i> Herrm.	London, UK	2010	Deep-yellow	5	KT359376	KT359428	KT359480
2	<i>R. foetida</i> var. <i>bicolor</i> Willmott	London, UK	2010	Orange-red	5	KT359377	KT359429	KT359481
3	<i>R. foetida</i> f. <i>persiana</i> (Lem.) Rehd.	London, UK	2010	Golden-yellow	30–45	KT359378	KT359430	KT359482
4	<i>R. primula</i> Bouleng.	Chengde, CHN	2009	Light yellow	5	KT359379	KT359431	KT359483
5	<i>R. xanthina</i> Lindl.	Chengde, CHN	2009	Yellow	5	KT359380	KT359432	KT359484
6	<i>R. omeiensis</i> Rolfe	Dali, CHN	2011	White blend	4	KT359381	KT359433	KT359485
7	<i>R. sericea</i> Lindl.	Dali, CHN	2011	White blend	4	KT359382	KT359434	KT359486
Sect. 2. <i>Rosa</i>								
8	<i>R. forrestiana</i> Bouleng.	Lijiang, CHN	2007	Deep pink	5	KT359383	KT359435	KT359487
9	<i>R. sweginzowii</i> Koehne	Pingwu, CHN	2012	Pink	5	KT359384	KT359436	KT359488
10	<i>R. davurica</i> Pall.	Datong, CHN	2008	Deep pink	5	KT359385	KT359437	KT359489
11	<i>R. rugosa</i> Thunb.	Kunming, CHN	2011	Red	5	KT359386	KT359438	KT359490
12	<i>R. rugosa</i> var. <i>albo-plena</i> Rehd.	Kunming, CHN	2011	White blend	17–25	KT359387	KT359439	KT359491
13	<i>R. majalis</i> Herrm.	Akesu, CHN	2010	Carmine-pink	5	KT359388	KT359440	KT359492
14	<i>R. murielae</i> Rehd. et Wils.	Lijiang, CHN	2011	White blend	5	KT359389	KT359441	KT359493
15	<i>R. macrophylla</i> Lindl.	Zhongdian, CHN	2010	Deep pink	5	KT359390	KT359442	KT359494
Sect. 3. <i>Synstylae</i>								
16	<i>R. multiflora</i> Thunb.	Nanjing, CHN	2009	White	5	KT359391	KT359443	KT359495
17	<i>R. multiflora inermis</i>	Chiba, JPN	2008	White blend	5	KT359392	KT359444	KT359496
18	<i>R. multiflora</i> var. <i>cathayensis</i> Rehd. et Wils.	Nanjing, CHN	2009	Light pink	5	KT359393	KT359445	KT359497
19	<i>R. multiflora</i> f. <i>watsoniana</i> Matsumura	Chiba, JPN	2008	Light pink to white	5	KT359394	KT359446	KT359498
20	<i>R. brunonii</i> Lindl.	Kunming, CHN	2011	White blend	5	KT359395	KT359447	KT359499
21	<i>R. helenae</i> Rehd. et Wils.	Kunming, CHN	2011	White	5	KT359396	KT359448	KT359500
22	<i>R. lasiosepala</i> Metc.	Yulin, CHN	2012	White	5	KT359397	KT359449	KT359501
23	<i>R. soulieana</i> Crép.	Deqin, CHN	2010	White	5	KT359398	KT359450	KT359502
24	<i>R. longicuspis</i> Bertol.	Mile, CHN	2011	White	5	KT359399	KT359451	KT359503
25	<i>R. taiwanensis</i> Nakai	Changhua, CHN	2007	White blend	5	KT359400	KT359452	KT359504
26	<i>R. lichiangensis</i> Yu et Ku	Lijiang, CHN	2010	Pink	5	KT359401	KT359453	KT359505
27	<i>R. weisiensis</i> Yu et Ku	Weixi, CHN	2012	White	5	KT359402	KT359454	KT359506
28	<i>R. arvensis</i> Huds.	Sangerhausen, DE	2007	White	5	KT359403	KT359455	KT359507
Sect. 4. <i>Chineses</i>								
29	<i>R. chinensis</i> Jacq. f. <i>mutabilis</i> Rehd.	Lyons, FR	2008	Yellow to pink, ages to crimson	5	KT359404	KT359456	KT359508
30	<i>R. chinensis</i> Jacq. var. <i>minima</i> (Sims) Voss	Lyons, FR	2008	Carmine-pink, ages to light pink	35–50	KT359405	KT359457	KT359509
31	<i>R. odorata</i> (Andr.) Sweet	Kunming, CHN	2011	White to pink, reverse marked with purple	25–45	KT359406	KT359458	KT359510
32	<i>R. odorata</i> var. <i>erubescens</i> (Focke) Yu et Ku	Lijiang, CHN	2010	Pink to light pink	17–25	KT359407	KT359459	KT359511
33	<i>R. odorata</i> var. <i>gigantea</i> (Crép.) Rehd. et Wils.	Chuxiong, CHN	2010	Butter-yellow to white	5	KT359408	KT359460	KT359512
Sect. 5. <i>Banksianae</i>								
34	<i>R. banksiae</i> Ait.	Dali, CHN	2012	White blend	25–45	KT359409	KT359461	KT359513
35	<i>R. banksiae</i> var. <i>normalis</i> Regel	Dali, CHN	2012	White	5	KT359410	KT359462	KT359514
36	<i>R. banksiae</i> f. <i>lutea</i> (Lindl.) Rehd.	Kunming, CHN	2012	Light yellow	35–50	KT359411	KT359463	KT359515
37	<i>R. cymosa</i> Tratt.	Yixing, CHN	2011	White	5	KT359412	KT359464	KT359516
38	<i>R. cymosa</i> var. <i>puberula</i> Yu et Ku	Wenshan, CHN	2010	White	5	KT359413	KT359465	KT359517
39	<i>R. × fortuniana</i> Lindl.	Kunming, CHN	2011	White	35–45	KT359414	KT359466	KT359518
Sect. 6. <i>Microphyllae</i>								
40	<i>R. roxburghii</i> Tratt.	Jiangyou, CHN	2012	Lilac pink	35–65	KT359415	KT359467	KT359519
41	<i>R. roxburghii</i> f. <i>normalis</i> Rehd. et Wils.	Guangyuan, CHN	2008	Rose-pink to blush	5	KT359416	KT359468	KT359520
Sect. 7. <i>Laevigatae</i>								
42	<i>R. laevigata</i> Michx.	Ji'an, CHN	2007	White	5	KT359417	KT359469	KT359521
43	<i>R.× cooperii</i>	Lyons, FR	2008	White	5	KT359418	KT359470	KT359522
Sect. 8. <i>Bracteatae</i>								
44	<i>R. bracteata</i> Wendl.	Fuzhou, CHN	2010	White	5	KT359419	KT359471	KT359523
Sect. 9. <i>Caninae</i>								
45	<i>R. canina</i> L.	Lyons, FR	2008	Light pink to white	5	KT359420	KT359472	KT359524
46	<i>R. rubrifolia</i> Vill.	Lyons, FR	2008	Pink blend, white center	5	KT359421	KT359473	KT359525
Sect. 10. <i>Gallicanae</i>								
47	<i>R. × damascena</i> Mill.	Lyons, FR	2008	Pink	25–35	KT359422	KT359474	KT359526
Sect. 11. <i>Carolinae</i>								
48	<i>R. palustris</i> f. <i>plena</i> W. H. Lewis	Vancouver, CA	2011	Pink	17–25	KT359423	KT359475	KT359527
Outgroup taxa								
49	<i>Rubus lambertianus</i> Ser.	Nanjing, CHN	2013	White	5	KT359424	KT359476	KT359528
50	<i>Rubus hirsutus</i> Thunb.	Nanjing, CHN	2013	White	5	KT359425	KT359477	KT359529
51	<i>Rubus corchorifolius</i> L.	Nanjing, CHN	2013	White	5	KT359426	KT359478	KT359530
52	<i>Spiraea trilobata</i> L.	Nanjing, CHN	2013	White	5	KT359427	KT359479	KT359531

Table 2. Primers for PCR amplification and sequencing.

Region	Name	Sequences (5'-3')	References
<i>rpl16</i>	rpl16F	CCCTTTCATTCTTCCCCTATGTTG	Small et al., 1998
	rpl16R	GCTATGCTTAGTGTGCGACTCGTTG	
<i>trnL-F</i>	trnL	CGAAATCGGTAGACGCTACG	Taberlet et al., 1991
	trnF	ATTTGAACTGGTGACACGAG	
<i>atpB-rbcL</i>	2	GAAGTAGTAGGATTGATTCT	Savolainen et al., 1994
	10	CATCATTATTGTATACTCTTTC	

Table 3. Sequence and parsimony analysis statistics for separate and combined chloroplast DNA data sets used in phylogenetic analyses.

Character	<i>rpl16</i>	<i>trnL-F</i>	<i>atpB-rbcL</i>	Combined data
Length range (bp)				
In-group	891–911	959–968	539–591	2,397–2,463
Out-group included	875–911	903–968	539–591	2,317–2,463
Aligned length (bp)				
Out-group included	996	993	621	2,610
Number of indels				
In-group	4	2	4	10
Out-group included	6	9	4	19
Variable characters				
In-group	41 (4.1%)	11 (1.1%)	13 (2.1%)	65 (3.0%)
Out-group included	110 (11.0%)	117 (11.8%)	58 (9.3%)	285 (11.0%)
Informative characters				
In-group	46 (4.6%)	35 (3.5%)	15 (2.4%)	96 (3.7%)
Out-group included	76 (7.6%)	78 (7.8%)	34 (5.5%)	188 (7.2%)
Number of trees				
Out-group/indels included	1,998	2	1,998	2,000
Tree length				
Out-group/indels included	262	235	107	607
Consistency index				
Out-group/indels included	0.813	0.932	0.916	0.873
Retention index				
Out-group/indels included	0.861	0.948	0.928	0.902

bp = base pair.

classification of *Rosa* botanical sections (Matsumoto et al., 1998; Qiu et al., 2012). For example, five species of the traditional *R. sect. Banksianae* formed a clade in our phylogeny with high posteriori probability and bootstrap support (100% and 86%, respectively). The phylogenetic results are also consistent with other studies in which taxa from genus *Rosa* are generally divided into two main clades, named *Cinnamomeae* (*Rosa*) clade or Clade I, and *Synstylae* clade or Clade II (Bruneau et al., 2007; Fougère-Danezan et al., 2015). However, notable exceptions exist.

Three accessions (sample no. 1–3) of *R. foetida* were separated from the other taxa (sample no. 4–7) of *R. sect. Pimpinellifoliae*. This result has also been found in previous studies (Bruneau et al., 2007; Fougère-Danezan et al., 2015; Matsumoto et al., 1998). The fertility of tetraploid *R. foetida* is strikingly low (Roberts, 1977; Rowley, 1967), and it may represent an ancient hybrid of unknown parentage (Wylie, 1954). Our clustering results resolved the other four taxa (sample no. 4–7) of *R. sect. Pimpinellifoliae* as a sister relationship to the taxa from *R. sects. Rosa* and *Carolinae*. While in a more extensive taxon sampling of *R. sect. Pimpinellifoliae* made by Fougère-Danezan et al. (2015), several species (*R. spinosissima* L., *R. tsinglingensis* Pax & K. Hoffm., *R. farreri* Stapf ex Cox, and *R. koreana* Kom.) of this section are embedded in the *Rosa*-*Carolinae* clade. Moreover, the above four taxa are further divided into two small clades based

on our molecular analyses (Fig. 1). In *Flora of China* (Ku and Robertson, 2003), *R. primula* (sample no. 4) and *R. xanthina* (sample no. 5) belong to series *Spinosissimae* with five petals, whereas *R. omeiensis* (sample no. 6) and *R. sericea* (sample no. 7) belong to series *Sericeae* with four petals. Our molecular results seem to well reflect the morphological peculiarities of these species in *R. sect. Pimpinellifoliae*. Similar results have also been obtained in previous molecular phylogenetic studies in this genus (Fougère-Danezan et al., 2015; Matsumoto et al., 1998; Qiu et al., 2012).

The *Carolinae* section should be merged with the *Rosa* section according to several authors (Bruneau et al., 2007; Fougère-Danezan et al., 2015; Koopman et al., 2008; Matsumoto et al., 1998; Scariot et al., 2006; Takeuchi et al., 2000). This result was confirmed in our analysis. Morphologically *R. sects. Rosa* and *Carolinae* can be distinguished by week differences: the sepals are usually upright and persistent in *R. sect. Rosa* vs. reflected and deciduous in *R. sect. Carolinae* (Rehder, 1940; Wissemann, 2003). However, the distinction of this character is not substantiated on molecular level in these two sections. *R. palustris* f. *plena* (sample no. 48), traditionally placed in the *Carolinae* section (Table 1), formed a group with all the accessions (samples no. 8–15) of *R. sect. Rosa* in our study with 100% posteriori probability and 91% bootstrap support (Fig. 1).

Taxa from *R. sect. Synstylae* were clustered with accessions of *R. sect. Chinenses* in

the phylogeny (Fig. 1). Several authors have already pointed out the close relationship between *R. sects. Synstylae* and *Chinenses* (Bruneau et al., 2007; Fougère-Danezan et al., 2015; Jan et al., 1999; Matsumoto et al., 1998, 2000; Millan et al., 1996; Qiu et al., 2012; Wissemann and Ritz, 2005; Wu et al., 2000; Zhu et al., 2015). The morphological similarity of these two sections also concurs with our molecular study, for both sections have adnate and persistent stipules, and exserted styles, taxa of *R. sect. Chinenses* only lack the connected styles (Ku and Robertson, 2003; Rehder, 1940). It appears these two sections are not monophyletic in the phylogeny in our study (Fig. 1). Similar results have been reported in several previous studies (Bruneau et al., 2007; Fougère-Danezan et al., 2015; Matsumoto et al., 1998; Qiu et al., 2012; Wissemann and Ritz, 2005; Wu et al., 2000; Zhu et al., 2015), but there are also contrary studies suggested that these two sections are independently monophyletic (Jan et al., 1999; Matsumoto et al., 2000; Millan et al., 1996). Moreover, taxa from *R. sect. Synstylae* can be further divided into series *Multiflorae* and *Brunonianae*, respectively, depending on the stipules are pectinate or entire according to *Flora of China* (Ku and Robertson, 2003). However, this morphological character was not discovered in our study, nor in a recent report by Zhu et al. (2015). Besides, three samples of *R. odorata* (*R. sect. Chinenses*, sample no. 31–33) formed an inner clade with *R. brunonii* (sample no. 20), *R. helenae* (sample no. 21), and *R. weisiensis* (sample no. 27) of *R. sect. Synstylae*, is similar to two recent studies (Fougère-Danezan et al., 2015; Zhu et al., 2015). *R. chinensis* var. *mutabilis* (sample no. 29) was first reported in our study, and it presented in the *Synstylae*-*Chinenses* clade with an unresolved position (Fig. 1), which may be due to its remarkable variation in the flower color during blooming (Table 1). Nevertheless, more comprehensive molecular studies are still needed to ascertain the position of this peculiar species.

Within the *Synstylae*-*Chinenses* clade, *R. xdamascena* (sample no. 47) from *R. sect. Gallicanae* was merged with *R. sects. Synstylae* and *Chinenses*, while two taxa of *R. sect. Caninae*, together with *R. arvensis* (sample no. 28), formed a separate small clade (Fig. 1). The results show that *R. sect. Gallicanae*, rather than *R. sect. Caninae*, is a closer relative to *R. sects. Synstylae* and *Chinenses*, and that *R. sect. Caninae* appears to be sister to the *Synstylae*-*Chinenses* clade. Several previous studies obtained similar results based on the research on *R. gallica* L. of *R. sect. Gallicanae* (Koopman et al.,

Fig. 1. Bayesian 50% majority rule consensus of trees sampled in the stationary phase of two analyses. Values above the branches are the posterior probabilities for each clade, below the branches are bootstrap values (>50%) for the same clades found during parsimony searches.

2008; Millan et al., 1996; Scariot et al., 2006; Wissemann and Ritz, 2005). However, in recent studies, *R. gallica* formed a clade with taxa from *R. sect. Caninae*, and both of these two sections appear sister relationship to the *Synstylae-Chinenses* clade (Fougère-Danezan et al., 2015; Zhu et al., 2015). *R.*

xdamascena is thought to be a hybrid with three parents of wild species, and *R. moschata* Herrm. of *R. sect. Synstylae* is considered the female parent (Iwata et al., 2000; Millan et al., 1996). This viewpoint is supported by Takeuchi et al. (2000) whose study showed that *R. odorata* var. *gigantea*,

R. moschata, and *R. xdamascena* were grouped into one clade apart from *R. sect. Gallicanae*.

It is interesting that the only European species of *R. sect. Synstylae*, *R. arvensis*, together with *R. canina* (sample no. 45) of *R. sect. Caninae* formed a small clade in our

analysis (Fig. 1); this result is consistent with previous studies (Wissemann and Ritz, 2005; Zhu et al., 2015), and it also helps to explain why the origin of *R. ×alba* L. is ambiguous, because they are both hypothetical parents of *R. ×alba* (Klastersky, 1968; Millan et al., 1996). Furthermore, the origin of *R. ×cooparii* (sample no. 43) is still in dispute. *R. ×cooparii* is thought to be a seedling of *R. laevigata* (Beales, 1985), or a natural hybrid between a seed parent, *R. odorata* var. *gigantea*, and a pollen parent, *R. laevigata* (Young and Schorr, 2007). However, on the basis of our cpDNA molecular data, it formed a clade with *R. odorata* var. *gigantea* (*Synstylae-Chinenses* clade), rather than being clustered with *R. laevigata* (Fig. 1). This result suggests that the seed parent of *R. ×cooparii* might not be *R. laevigata* considering the maternal inheritance of cpDNA in *Rosa* (Matsumoto et al., 1998). Meanwhile, since this species was also not clustered with *R. odorata* var. *gigantea* in the *Synstylae-Chinenses* clade, it could not be confirmed whether the seed parent of *R. ×cooparii* is *R. odorata* var. *gigantea*, but is possibly a parent from *R. sects. Synstylae* and *Chinenses*.

Our clustering results resolved five samples (sample no. 34–38) of *R. sect. Banksianae* as monophyletic and as sisters to the *Synstylae-Chinenses* clade, without considering the position of *R. ×fortuniana* (sample no. 39). Similar results were obtained in previous studies (Fougère-Danezan et al., 2015; Qiu et al., 2012; Wu et al., 2001). *R. ×fortuniana*, which is traditionally placed in the *R. sect. Banksianae*, formed a clade with *R. laevigata* with 100% posteriori probability and 100% bootstrap confidence in our plastid sequence analyses (Fig. 1). *R. ×fortuniana* is thought to be a hybrid between a *R. laevigata* seed parent and a *R. banksiae* pollen parent, and its cpDNA *matK* sequence matches that of *R. laevigata*, not *R. banksiae* (Matsumoto et al., 2001). This *matK* sequence result is consistent with our molecular data. *R. sects. Laevigatae* and *Bracteatae* were also resolved as sister relationship to the *Synstylae-Chinenses* clade in the second group in our study. Similar results were indicated in other studies for *R. sect. Laevigatae* (Jan et al., 1999; Zhu et al., 2015), for *R. sect. Bracteatae* (Wissemann and Ritz, 2005), or for the both sections (Fougère-Danezan et al., 2015; Qiu et al., 2012). Furthermore, *R. sects. Banksianae*, *Laevigatae*, and *Bracteatae* are morphologically united in a large clade according to the free and deciduous stipules (Rehder, 1940). However, *R. sect. Banksianae* discriminates from *R. sect. Bracteatae* (which is readily featured by its woolly or tomentose young receptacle and branchlet) by the nonpubescent receptacle and branchlet, and discriminates from *R. sect. Laevigatae* by the reflexed and deciduous sepals. In addition, the stipules of *R. sect. Bracteatae* are deeply incised, which obviously differs from the other two sections. Several previous analyses also indicate that these three sections are distantly related to each other (Fougère-Danezan et al., 2015; Jan et al., 1999; Qiu et al., 2012). So it seems that the morphological characterization of free and

deciduous stipules in these three sections indicates not synapomorphy but convergence or plesiomorphy (Wissemann and Ritz, 2005). Besides, *R. roxburghii* (sample no. 40) and *R. roxburghii* f. *normalis* (sample no. 41) formed a clade, and in parallel with *R. sects. Banksianae*, *Laevigatae*, and *Bracteatae* (Fig. 1). This agrees with previous suggestions that *R. roxburghii* should be placed on a sectional level (Crépin, 1889; Fougère-Danezan et al., 2015; Jan et al., 1999; Koopman et al., 2008; Ku and Robertson, 2003; Wissemann and Ritz, 2005), rather than being classified as a subgenus (Rehder, 1940; Wissemann, 2003).

In summary, our phylogenetic reconstruction (Fig. 1) derived from the combined three cpDNA data for 48 genotypes shows substantial consistency with the conventional classification of infrageneric sections (Table 1). However, the results are mostly not consistent with the conventional taxonomy in a better sampling of both taxa and molecular markers (Fougère-Danezan et al., 2015; Zhu et al., 2015). The differences might be caused by the hybridization (genetic introgression) and/or polyploidization existing among the taxa of this genus. For instance, *R. lichiangensis* (sample no. 26) has been confirmed to be a hybrid species (Zhu et al., 2015), and the decaploid *R. praelucens* Byhouwer is also regarded as an allopolyploid resulting from hybridization (Fougère-Danezan et al., 2015; Jan et al., 2010). Moreover, chloroplast markers ignore hybridization (because of their maternal inheritance), and concerted evolution and homogenization of parental polymorphism exist in the *ITS* region of nrDNA. Therefore, low/single copy nuclear genes, such as those used by Zhu et al. (2015), should be employed in future studies to better understand this complex genus.

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