Molecular Phylogenetics and Evolution 87 (2015) 50–64

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeny of *Rosa* sections *Chinenses* and *Synstylae* (Rosaceae) based on chloroplast and nuclear markers

Zhang-Ming Zhu [a,b], Xin-Fen Gao [a,*], Marie Fougère-Danezan [a,c]

[a] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan 610041, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China

ARTICLE INFO

Article history:
Received 31 October 2014
Revised 4 March 2015
Accepted 14 March 2015
Available online 23 March 2015

Keywords:
Rosa
Section *Chinenses*
Section *Synstylae*
Phylogeny
Incongruence
Hybridization

ABSTRACT

Rosa sections *Chinenses* and *Synstylae* contain approximately 39 wild species mainly distributed in East Asia and are closely related according to previous studies. But the specific relationships within these two sections were still obscure due to limited sampling, low genetic variation of molecular markers, and complex evolutionary histories. In this study, we used four chloroplast (*ndhC-trnV*, *ndhF-rpl32*, *ndhJ-trnF* and *psbJ-petA*) and two nuclear (ribosomal *ITS* and *GAPDH*) markers with an extensive geographic and taxonomic sampling to explore their evolutionary history. Our phylogenetic analyses suggested that *Rosa* sections *Chinenses* and *Synstylae* defined in traditional taxonomic system are not monophyletic and close to sections *Caninae* and *Gallicanae*. Additionally, our results showed incongruence between chloroplast and nuclear markers, and the patterns of incongruence might be due to ancient hybridization (genetic introgression). One putative hybrid species and three samples identified as interspecific hybrids are further discussed in terms of topological incongruence, biological characters and distribution patterns.

1. Introduction

Hybridization has long been recognized as an important driving force in plant evolution (Abbott, 1992; Mallet, 2007; Paun et al., 2009; Rieseberg, 1995, 1997), which can lead to high intraspecific genetic diversity, creation of new species or ecotypes, and reticulate evolution (Anderson, 1948; Linder and Rieseberg, 2004; Whitham et al., 1994). As a result, it is particularly challenging to reconstruct species relationships for groups including hybrids. In the genus *Rosa* L. (Rosaceae), interspecific hybrids are easily formed, which have contributed substantially to the diversity in this genus (Atienza et al., 2005; Rehder, 1940; Wissemann, 2003). Many hybridization events have been reported in wild roses (Atienza et al., 2005; Joly et al., 2006; Matthews, 1920; Schanzer and Kutlunina, 2010). The whole section *Caninae* seems to be of hybrid origin (Fougère-Danezan et al., 2015; Ritz et al., 2005; Wissemann, 1999). Some species of *Rosa* sects. *Rosa* and *Synstylae* were also shown to have undergone hybridization during their evolutionary histories (Joly et al., 2006; Ohba et al., 2000). Indeed, hybridization has been recognized as one of the major difficulties in the taxonomy of roses even since the early times (Crépin, 1889; Linnaeus, 1753).

The genus *Rosa* comprises about 150–200 species widely distributed in the temperate and subtropical regions of the northern hemisphere (Ku and Robertson, 2003; Yü et al., 1985). The widely adopted taxonomic system built by Rehder (1940) divided *Rosa* species into four subgenera, i.e., *Hesperhodos* Cockerell, *Hulthemia* (Dumort.) Focke, *Platyrhodon* (Hurst) Rehder and *Rosa*. The first three subgenera contain only one or two species, while species from the subgenus *Rosa* are further divided into ten sections, i.e., *Banksiae* Lindl., *Bracteatae* Thory, *Caninae* (DC.) Ser., *Carolinae* Crép., *Cinnamomeae* (DC.) Ser., *Gallicanae* (DC.) Ser., *Indicae* Thory, *Laevigatae* Thory, *Pimpinellifoliae* (DC.) Ser., and *Synstylae* DC. We use *R.* sect. *Chinenses* (DC.) Ser. in this study instead of *R.* sect. *Indicae* since the former is the oldest valid name while the latter is a synonym (Seringe, 1818; Thory, 1820). Because *R.* sect. *Cinnamomeae* includes the type species (*R. cinnamomea* L.) of the genus (Barrie, 2006; Jarvis, 1992), the section name *Cinnamomeae* is invalid and should be replaced by the autonym (*R.* sect. *Rosa*; Art. 22.1, McNeill et al., 2012). So we use *R.* sect. *Rosa* instead of *R.* sect. *Cinnamomeae* in this study.

Many attempts were made to reconstruct the phylogeny of this genus, most of which suggested that the divisions of most subgenera and sections based on morphology were artificial (Bruneau

* Corresponding author.
E-mail address: xfgao@cib.ac.cn (X.-F. Gao).

http://dx.doi.org/10.1016/j.ympev.2015.03.014
1055-7903/

et al., 2007; Koopman et al., 2008; Jan et al., 1999; Matsumoto et al., 1998, 2000; Millan et al., 1996; Wu et al., 2000, 2001; Wissemann and Ritz, 2005). And *Rosa* sects. *Chinenses* and *Synstylae* were resolved as closely related. For example, both Matsumoto et al. (1998) and Wu et al. (2000) found that *R.* sect. *Chinenses* was nested within *R.* sect. *Synstylae*, which is similar to a recent phylogenetic study of 39 Chinese wild roses (Qiu et al., 2012). Both nuclear and chloroplast DNA data from Wissemann and Ritz (2005) and Bruneau et al. (2007) suggested that sections *Chinenses* and *Synstylae* formed a clade with other sections, which may have been due to sparse taxon sampling, garden-grown samples that could be introgressed, and low variation in genetic markers. In an analysis of amplified fragment length polymorphism (AFLP) by Koopman et al. (2008), two East Asian species (*R. wichurana* and *R. multiflora* Thunb.) from sect. *Synstylae* formed a clade, and two other species (*R. arvensis* Huds. and *R. moschata* Mill.) from Europe were grouped with sections *Rosa* and *Caninae*. However, in these studies, both the sampling and the molecular markers used were limited. Further research with more extensive taxon sampling and molecular data from both nuclear and chloroplast genomes is needed to solve the phylogenetic relationships within sections *Chinenses* and *Synstylae*.

Rosa sects. *Chinenses* and *Synstylae* are mainly distributed in East Asia. And species from these two sections include most of the wild ancestors of modern cultivated roses, such as *R. multiflora*, *R. luciae* Franch. & Rochebr. in *R.* sect. *Synstylae* and *R. chinensis* var. *spontanea* (Rehder & E.H. Wilson) T.T. Yu & T.C. Ku, *R. odorata* var. *gigantea* (Crép.) Rehder & E.H. Wilson in *R.* sect. *Chinenses* (Ku and Robertson, 2003; Rehder, 1940; Yü et al., 1985). Species of *R.* sect. *Synstylae* can be distinguished by their exserted and columnated styles. This section contains approximately 36 wild species with about 31 distributed in East Asia (mainly in China), one in North America, three in Europe, and one in East Africa and South Arabian Peninsula (Ku and Robertson, 2003; Wissemann, 2003). In Flora of China (Yü et al., 1985), *R.* section *Synstylae* has been divided into two series (ser. *Brunonianae* Yu and Ku and *Multiflorae* Yu and Ku). Species of ser. *Multiflorae* have pectinate or serrate stipules while those in ser. *Brunonianae* have entire ones. *Rosa* sect. *Chinenses* has similarly exserted styles, but the free styles and much bigger hips distinguish this section from sect. *Synstylae*. *R.* section *Chinenses* contains three species that are all endemic to China (Ku and Robertson, 2003). The ploidy of approximately a third wild species of these two sections have been reported in previous studies, and nearly all of them are diploid (Cole and Melton, 1986; Roberts et al., 2009; Ueda and Akimoto, 2001; Yokoya et al., 2000). Thus, unlike *R.* sects. *Caninae* and *Rosa* (Joly et al., 2006; Ritz et al., 2005), polyploidization does not seem to be an important factor in the evolution of *R.* sects. *Chinenses* and *Synstylae*. However, hybridization seems to be at work in these two sections as suggested by previous studies (Ohba et al., 2000; Meng et al., 2011) and numerous intermediate morphological characters from specimens and field observations.

In this study, we used four chloroplast and two nuclear genetic markers to reconstruct the phylogeny of *R.* sects. *Chinenses* and *Synstylae*. Our sampling covered the whole distribution area and most wild species of these two sections. Species belonging to other sections and subgenus were also sampled. The objectives of this study are: (1) to reconstruct a more comprehensive and better-resolved phylogeny of *Rosa* sections *Chinenses* and *Synstylae*, and (2) to test the significance of species hybridization in these two sections.

2. Materials and methods

2.1. Taxon sampling

Samples for this study were obtained from various sources. Most of our samples were collected in the field and the voucher specimens were deposited in the Herbarium of Chengdu Institute of Biology (CDBI) after identified with Flora of China (Ku and Robertson, 2003; Yü et al., 1985) and Flora Europaea (Tutin et al., 1978). Others were obtained from cultivated material or herbarium specimens at the Royal Botanic Gardens Kew and Edinburgh, Wageningen University Botanical Garden, and Botanic Garden Meise in Belgium. In total, we sampled 126 individuals (including three outgroups) of which 85 (representing 34 species) belonging to sect. *Synstylae*, ten (representing three species) belonging to sect. *Chinenses*, and three putative hybrids related to these two sections. According to previous studies (Eriksson et al., 2003; Potter et al., 2007, 2002), *Potentilla parvifolia* Fisch. and *Rubus idaeopsis* Focke were chosen as outgroups. All samples with detailed information are listed in Supplementary Table S1.

2.2. Lab protocols

Total genomic DNA was extracted from silica-gel dried or fresh leaves using the Tiangen plant genome DNA extraction Kit (Tiangen Biotech., Beijing, China).

Table 1
Primers used in this study.

Region	Name	Primer sequence (5′–3′)	Source
ndhC-trnV	ndhCretF	AAGTTTCTCCGGTCCTTTGC	Timme et al. (2007)
	trnVretR	TCTACGGTTCGAGTCCGTATAG	Timme et al. (2007)
	ndhC-1F	GAATGAAAATGCCAAAATAG	This study
	trnV-1R	TTTCGAGTCCGTATAGCC	This study
ndhF-rpl32	ndhF	GAAAGGTATKATCCAYGMATATT	Shaw et al. (2007)
	rpl32R	CCAATATCCCTTYYTTTTCCAA	Shaw et al. (2007)
	ndhF-1F	ATTGTTTCTGATTCACCAGTT	This study
	rpl32-2R	CGGTTCATCGGTTGGATG	This study
ndhJ-trnF	ndhJ	ATGCCYGAAAGTTGGATAGG	Shaw et al. (2007)
	tabE	GGTTCAAGTCCCTCTATCCC	Taberlet et al. (1991)
psbJ-petA	psbJ	ATAGGTACTGTARCYGGTATT	Shaw et al. (2007)
	petA	AACARTTYGARAAGGTTCAATT	Shaw et al. (2007)
nrITS	ITS-4	TCCTCCGCTTATTGATATGC	White et al. (1990)
	ITS-A	GGAAGGAGAAGTCGTAACAAGG	White et al. (1990)
GAPDH	GPDX7F	GATAGATTTGGAATTGTTGAGG	Strand et al. (1997)
	GPDX11R	GACATTGAATGAGATAAACC	Joly et al. (2006)
	GAPDH_ZF	CTCCATCACTGGTGAGTT	This study
	GAPDH_ZR	TGTCACCAATGAAGTCGG	This study

Four chloroplast regions (*ndhC-trnV*, *ndhF-rpl32*, *ndhJ-trnV*, and *psbJ-petA*), nuclear ribosomal internal spacers (*nrITS*) and a single copy nuclear gene coding for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were used in this study. Primers used in this study are listed in Table 1. Polymerase chain reaction (PCR) amplification were performed in 25 μL volumes containing *Taq* DNA polymerase buffer (Tiangen, Beijing) with 2 mM $MgCl_2$, 20 μM of dNTP, 0.2 μM of each primer, 5 U of *Taq* DNA polymerase and 0.5 μL of genomic DNA (20–100 ng). Conditions for amplification consisted of an initial denaturation at 95 °C for 4 min, followed by 34 cycles at 95 °C for 45 s, then at 53 °C (49 °C for *GAPDH*) for 45 s and at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplifications were conducted using a PTC-0200 thermocycler (Bio-rad, Beijing).

Amplification products were purified using the Universal DNA Purification Kit (Tiangen, Beijing, China) after gel-separation. Purified products were directly sequenced at Invitrogen Biotech. (Shanghai, China).

Heterozygous (double) peaks were observed in chromatograms of most nuclear (*ITS* and *GAPDH*) sequences, which might be caused by different alleles. A strategy similar to Joly et al. (2006) was used to sample all the alleles. Sequences with reliable chromatograms were used directly. When heterozygous peak occurred at only one site, cloning was not considered necessary since the alleles could be easily distinguished. If sequences had more than one heterozygous peak or could not be directly sequenced, cloning was performed. Amplification and purification were performed as previously described except that the PCR volume was 50 μL. The purified PCR products were cloned with pEASY-T1 Cloning Kit (Transgen, Beijing, China) following the manual provided by the manufacturer. Finally, five to twenty positive clones were sequenced.

2.3. Sequence treatment, alignment and gap coding

Sequences were edited and assembled using Sequencher v4.1.4 (GeneCodes Inc., Ann Arbor, MI, USA). For nuclear sequences, the samples that had only one heterozygous peak were separated into two sequences labeled as S1 and S2. Cloned sequences were treated following the strategy of Yang et al. (2013). First, PCR errors were corrected by comparing the cloned sequences with the original sequences from direct sequencing. Second, if more than two cloned sequences of one sample shared identical bases, they were treated as one consensus sequence. Third, PCR-mediated recombination and pseudogene sequences were excluded.

Sequences were aligned using ClustalW (Thompson et al., 1994) in BioEdit v7.0.9.0 (Hall, 1999) and manually adjusted when necessary. Single base repeats as well as ambiguously aligned indels were excluded. Other substitutions and indels were considered as variations. Indels in the regions of unambiguous alignment were coded following the modified complex indel coding (Müller, 2006; Simmons and Ochoterena, 2000) for maximum parsimony (MP) analyses and simple coding (Simmons and Ochoterena, 2000) for Bayesian Inference (BI) using SeqState v1.4.1 (Müller, 2005).

2.4. Phylogenetic analysis

The chloroplast genome are maternally inherited, and our preliminary analyses did not show obvious incongruence among the four chloroplast DNA (cpDNA) regions, so we combined them to get better resolution of the phylogeny. All the concatenated chloroplast dataset and the nuclear datasets were analyzed using MP, maximum likelihood (ML) and BI. MP analyses were conducted with PAUP* v4.0 b10 (Swofford, 2002). For each dataset we performed a heuristic tree search using a set of 1000 random addition sequence. MP bootstrap support was summarized from 1000 bootstrap trees.

Model selection was performed in jModelTest v2.1.1 (Darriba et al., 2012) with the default setting to select the best-fit DNA substitution model for each dataset. The best-fit models and parameter values determined by Akaike Information Criterion (AIC) (Akaike, 1974) are presented in Supplementary Table S2.

ML analyses were performed using RAxML-HPC2 on XSEDE v8.0.24 (Stamatakis, 2014) in The Cipres Science Gateway (Miller et al., 2010). All parameters were set according to the best-fit models or the most similar ones available in Cipres. ML bootstrap support was summarized from 5000 bootstrap trees.

BI analyses were conducted using MrBayes v3.2.1 (Ronquist et al., 2012). All parameters were set according to the best-fit or the most similar model available in MrBayes v3.2.1. The Markov chain Monte Carlo (MCMC) algorithm was run for 30,000,000 (50,000,000 for *GAPDH*) generations with one cold and three heated chains, starting with a random tree and sampling one tree every 1000 generations. The first 7,500,000 (12,500,000 for *GAPDH*) generations were treated as burn-in. An adequate burn-in value for each analysis was assessed using the software Tracer 1.5 (Rambaut and Drummond, 2009) and a 50% majority-rule consensus tree was then computed.

Phylogenetic networks were constructed for the nuclear datasets using SplitsTree v4.13.1 (Huson and Bryant, 2006). Phylogenetic network analyses were performed using the NeighborNet algorithm with Kimura 2-parameter (K2P) distances and Ordinary Least Square Method.

2.5. Molecular dating analysis

Since topologies obtained from the nuclear dataset were weakly supported, we selected the concatenated chloroplast dataset to perform molecular clock analyses in BEAST v 1.7.5 (Drummond et al., 2012) to estimate divergence times. We used the GTR+I+G model that is similar to the best-fit model to execute the analysis with an uncorrelated lognormal relaxed clock model and a Yule process of speciation. Two independent Markov chain Monte Carlo (MCMC) runs were set to 80,000,000 generations with parameters sampled every 1000 generations and 25% burn-in. Samples were combined and convergence of chains was checked in Tracer v 1.5 (Rambaut and Drummond, 2009). Although many fossils of *Rosa* were found, most of them were hard to assign to an extant species. Thus, we chose the oldest fossil *R. germerensis* (55.8–48.6 Ma; Idaho, USA) (Edelman, 1975) as the only calibration point. We used this age to constrain the root node (separation of *Rosa* from outgroups) with a prior normal distribution centered on 50.0 Ma and a 97.5% confidence interval between 51.0 and 49.0 Ma.

3. Results

3.1. Phylogeny of concatenated chloroplast DNA regions

After removing ambiguous sites (e.g. single base repeats), our aligned chloroplast *ndhC-trnV*, *ndhF-rpl32*, *ndhJ-trnF*, and *psbJ-petA* DNA regions contained 763 base pairs (bp), 1275 bp, 1319 bp, and 1051 bp, respectively. The final concatenated cpDNA matrix was composed of 126 taxa and 4408 bp, of which 755 (17.1%) were variable and 396 (9.0%) were potentially parsimony informative.

The results of the MP, ML and BI analyses were congruent on major lineages. MP bootstrap supports (MPBS), ML bootstrap supports (MLBS) and BI posterior probabilities (PP) were shown on the 50% majority-rule consensus tree from BI analysis (Fig. 1). The monophyly of *Rosa* was well supported by all analyses (100% MPBS, 100% MLBS, 1.00 PP). In addition, two well-supported clades

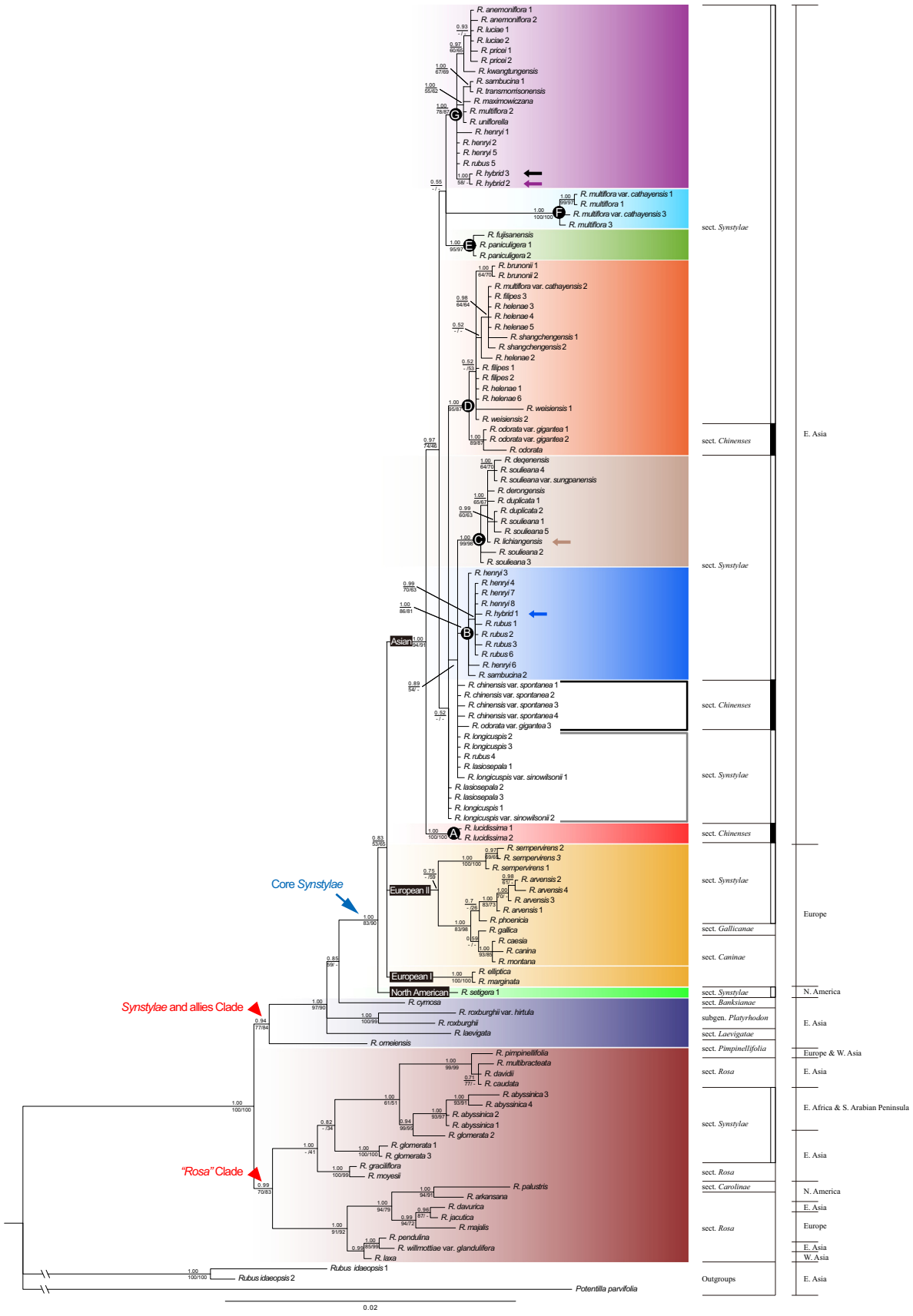

Fig. 1. Phylogram resulting from Bayesian Inference of concatenated chloroplast dataset. Double slashes on branches indicate branch lengths not in proportion. Numbers above branches are posterior probability values; numbers below branches are bootstrap values from MP/ML analyses. Clades, subclades or lineages are indicated by labels on branches and gradual colors of boxes covered on taxa names.

("*Rosa*" Clade, and *Synstylae* and allies Clade) were recovered within *Rosa*.

"*Rosa*" Clade included sections *Carolinae*, *Rosa*, and one species from section *Pimpinellifoliae*, as well as two species from section *Synstylae* (Fig. 1). All accessions of *Rosa glomerata* Rehder & E. H. Wilson and *Rosa abyssinica* R. Br. (Sect. *Synstylae*) were nested in "*Rosa*" Clade. The monophyly of *R. abyssinica* is strongly supported by all analyses and sister to *R. glomerata* 2 from Ebian (Sichuan, China). The other two samples of *R. glomerata* from Luding (*R. glomerata* 1) and Yingjing (*R. glomerata* 3) counties (Sichuan, China) formed another subclade with high support values.

Synstylae and allies Clade included most samples from section *Synstylae*, all samples from section *Chinenses*, as well as samples from subgenus *Platyrhodon* and sections *Banksianae*, *Caninae*, *Gallicanae*, *Laevigatae* and *Pimpinellifoliae* (Fig. 1). Both sections *Chinenses* and *Synstylae* were not recovered as monophyletic. Asian species of sections *Chinenses* and *Synstylae* formed a well-supported subclade (Asian) in which seven well-supported lineages (A-G) were resolved. The only *Synstylae* species (*R. setigera* Michx.) distributed in North America formed another subclade (North American). European *Synstylae* species clustered with *Caninae* species and formed the subclade European II while the remaining *Caninae* species clustered in a subclade we named European I. These four subclades formed a well-supported (83% MPBS, 90% MLBS, 1.00 PP) clade named Core *Synstylae* in this study.

3.2. Phylogeny of ITS region

The final *ITS* dataset contained 224 accessions and 622 aligned nucleotides, of which 168 (27.0%) were variable and 103 (16.6%) were parsimony informative.

The phylogenetic analyses of the *ITS* dataset using MP, ML, and BI methods showed similar topologies. The backbone of the phylogenetic tree had weak support at many nodes (Fig. 2). The genus *Rosa* was resolved as monophyletic and well supported. But neither section *Synstylae* nor section *Chinenses* was resolved as monophyletic (Fig. 2). Unlike cpDNA results, *R. cymosa* from sect. *Banksianae* was sister to the remaining *Rosa* species. Additionally, none of the clades identified by cpDNA was supported here except *R. setigera* samples, the only *Synstylae* species endemic to North America. Most accessions of "*Rosa*" Clade in the chloroplast tree formed a clade. Moreover, *R. glomerata* and *R. abyssinica* showed a closer relationship to species from Core *Synstylae* clade.

3.3. Phylogeny of GAPDH region

PCR amplification of *GAPDH* region failed for five samples, and one sequence (DQ091174, *R. setigera*) was obtained from GenBank (Joly et al., 2006). Finally, the *GAPDH* dataset included 272 accessions and 915 aligned nucleotides that contained 418 (45.7%) variable sites and 270 (29.5%) potential parsimony informative sites.

Phylogenetic analyses of *GAPDH* dataset using three methods (MP, ML and BI) did not show obvious incongruence except support values (Fig. 3). The monophyly of the genus *Rosa* was well-supported, while the phylogenetic relationships within *Rosa* were very different from the chloroplast and *ITS* trees. Two early-diverging clades (One included species from subgenus *Platyrhodon*, sections *Banksianae* and *Laevigatae*, and another included species from section *Pimpinellifoliae*.) were sister to the remaining species forming a polytomy. The positions for species of section *Synstylae* from Europe and North America, and species from sections *Caninae*, *Carolinae*, *Rosa*, and *Gallicanae* were unresolved. Other species (mainly Asian) from sections *Chinenses* and *Synstylae* showed a little more resolution. *R. glomerata* formed a clade sister to species of *Rosa soulieana* group. Seven accessions of *R. abyssinica* formed a clade and another one clustered with species of *R. soulieana* group.

3.4. Phylogenetic networks

Phylogenetic networks of nuclear *ITS* and *GAPDH* regions (Figs. 4 and 5) were similar to the corresponding phylogenetic trees (Figs. 2 and 3) and showed clearer backbones of genus *Rosa*. Species from sections *Chinenses*, *Gallicanae*, and some sequences from section *Caninae* were nested in *Synstylae*. Section *Chinense* was split into different groups in both networks.

3.5. Divergence times

The maximum clade credibility (MCC) tree (Supplementary Fig. S1) generated from divergence time analyses was congruent with the cpDNA phylogenetic tree (Fig. 1) in major clades. The age of the first divergence event in *Rosa*, which is the divergence time between "*Rosa*" Clade and *Synstylae* and allies Clade, was estimated to be 40.9 Ma (95% highest posterior density (HPD): 30.0–50.2 Ma). The crown age of Core *Synstylae* was estimated to be 24.2 Ma (95% HPD: 15.4–33.7 Ma), and the crown age of subclade Asian was 18.4 Ma (95% HPD: 11.2–26.4 Ma).

4. Discussion

4.1. Low resolution and phylogenetic incongruence

Our results provided some significant information for the evolutionary history of genus *Rosa*, while resolution was limited between closely related species. Low resolution in phylogeny can be due to causes such as insufficient data, noisy sequences, rapid diversification, polyploidization, and reticulate evolution (Baurain et al., 2007; Calvino et al., 2008; Campbell et al., 2007; Lo et al., 2009; Xu et al., 2012). In this study, we sampled most species of sections *Chinenses* and *Synstylae* covering the whole distribution area. The concatenated chloroplast dataset had much lower parsimony informative (PI) rate than nuclear markers (Table 2), while a better resolved phylogeny was obtained. It seems that insufficient variation is not the key reason in this case. Many reticulate evolution events were indicated by the nuclear networks (Figs. 4 and 5). Hybridization is easy for *Rosa* species because of overlapping distribution areas and flowering times, common generalist pollinators, and fertile crossbreeds (Jesse et al., 2006; Kevan et al., 1990; Matthews, 1920). Many studies have reported that hybridization has occurred in this genus not only between closely related species but also between species from different sections (Matthews, 1920; Mercure and Bruneau, 2008; Ritz et al., 2005; Schanzer and Kutlunina, 2010; Uggla and Carlson-Nilsson, 2005; Wissemann and Hellwig, 1997). Most species from sections *Chinenses* and *Synstylae* are diploid, but polyploidy is very common in other sections especially *Caninae* and *Rosa*. A lot of studies have documented polyploidization events and evaluated its significance for the evolution of the genus *Rosa* (Erlanson, 1938; Joly et al., 2006; Wissemann, 2002). Therefore, we attribute the low resolution of nuclear phylogenies to the complex evolutionary history of this genus rather than insufficient data.

Although the resolution was low in nuclear phylogenies, some moderately to strongly supported clades were recovered. Numerous inconsistencies (*R. glomerata*, *R. abyssinica* and *R. rubus* group) were revealed between the chloroplast and nuclear phylogenies (Figs. 1–3). Either abiotic (stochastic errors and systematic errors) or biological factors (convergent evolution, genetic introgression following hybridization, incomplete lineage sorting,

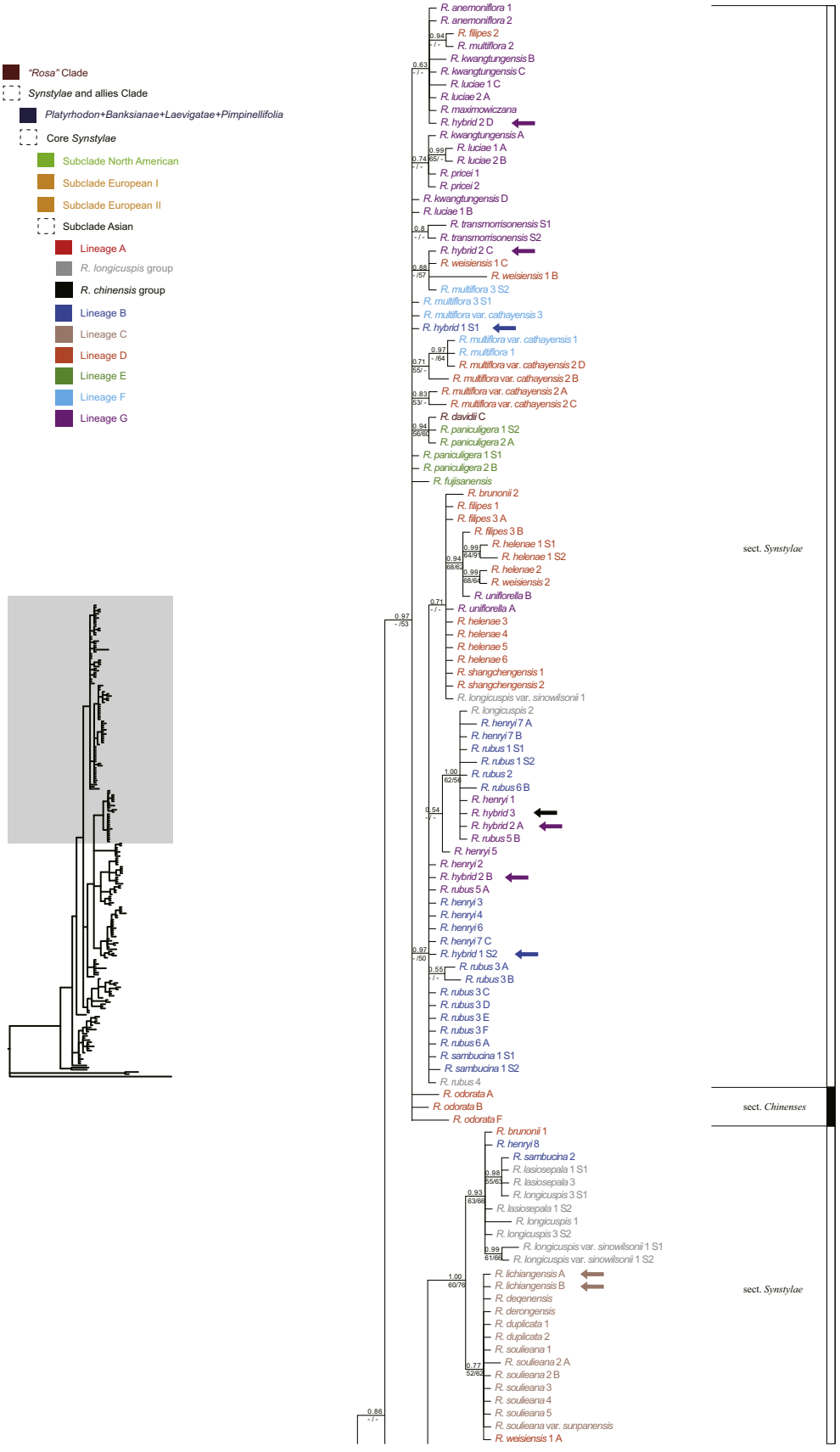

Fig. 2. Phylogram resulting from Bayesian Inference of *nrITS* dataset. Numbers above branches are posterior probability values; numbers below branches are bootstrap values from MP/ML analyses. Taxa colors corresponding to Fig. 1 indicate their position in chloroplast phylogeny.

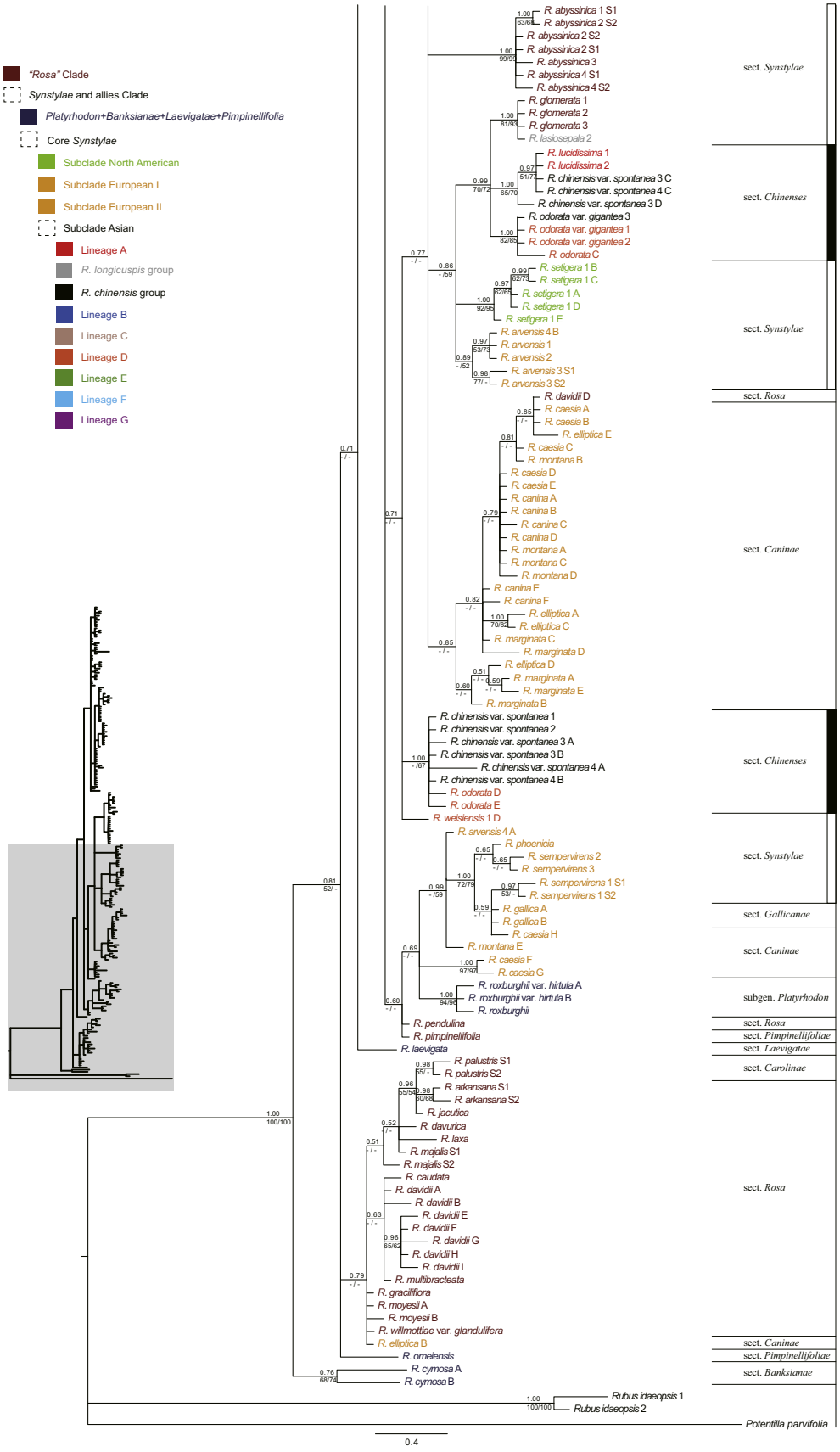

Fig. 2 (*continued*)

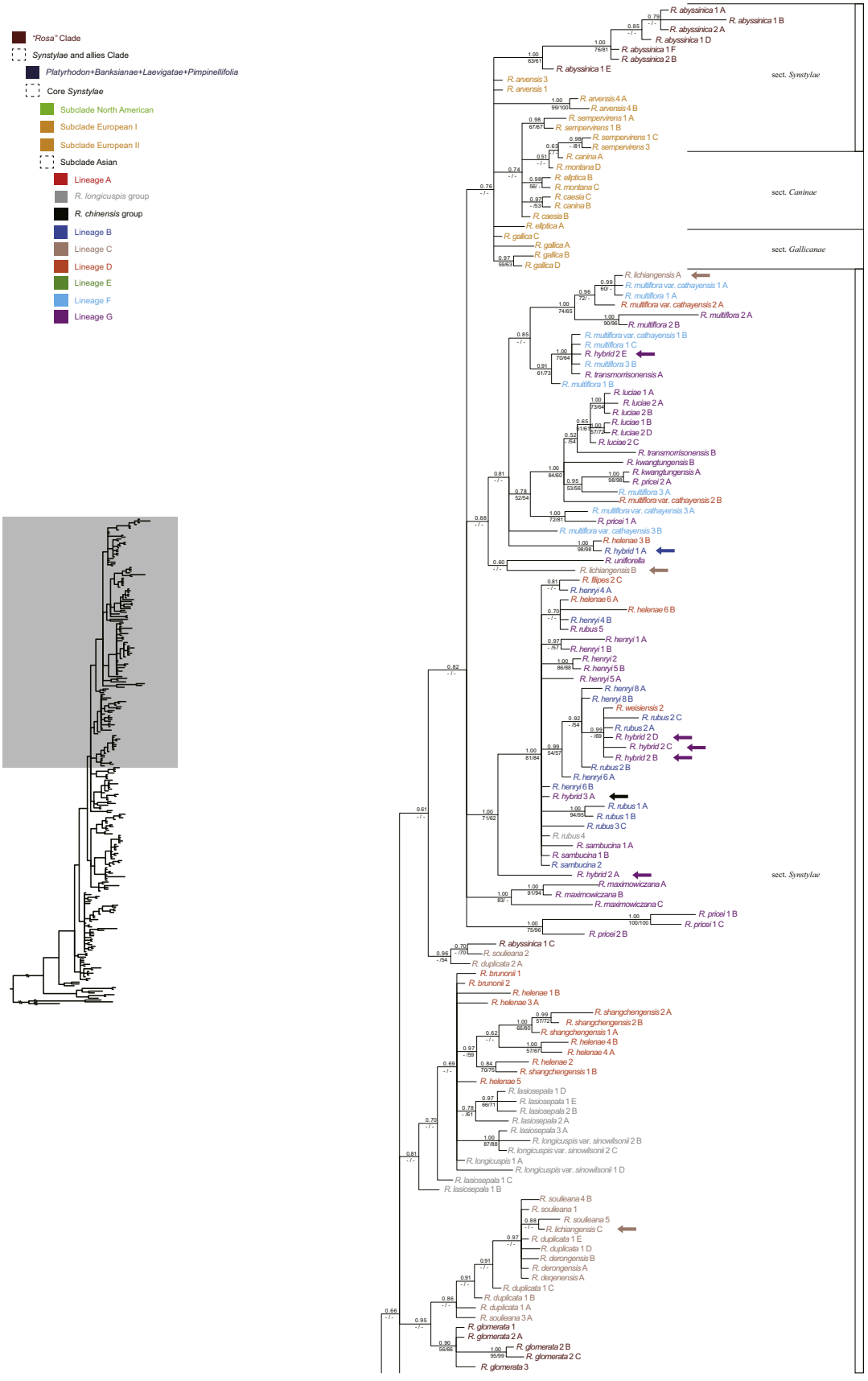

Fig. 3. Phylogram resulting from Bayesian Inference of *GAPDH* dataset. Double slashes on branches indicate branch lengths not in proportion. Numbers above branches are posterior probability values; numbers below branches are bootstrap values from MP/ML analyses. Taxa colors corresponding to Fig. 1 indicate their position in chloroplast phylogeny.

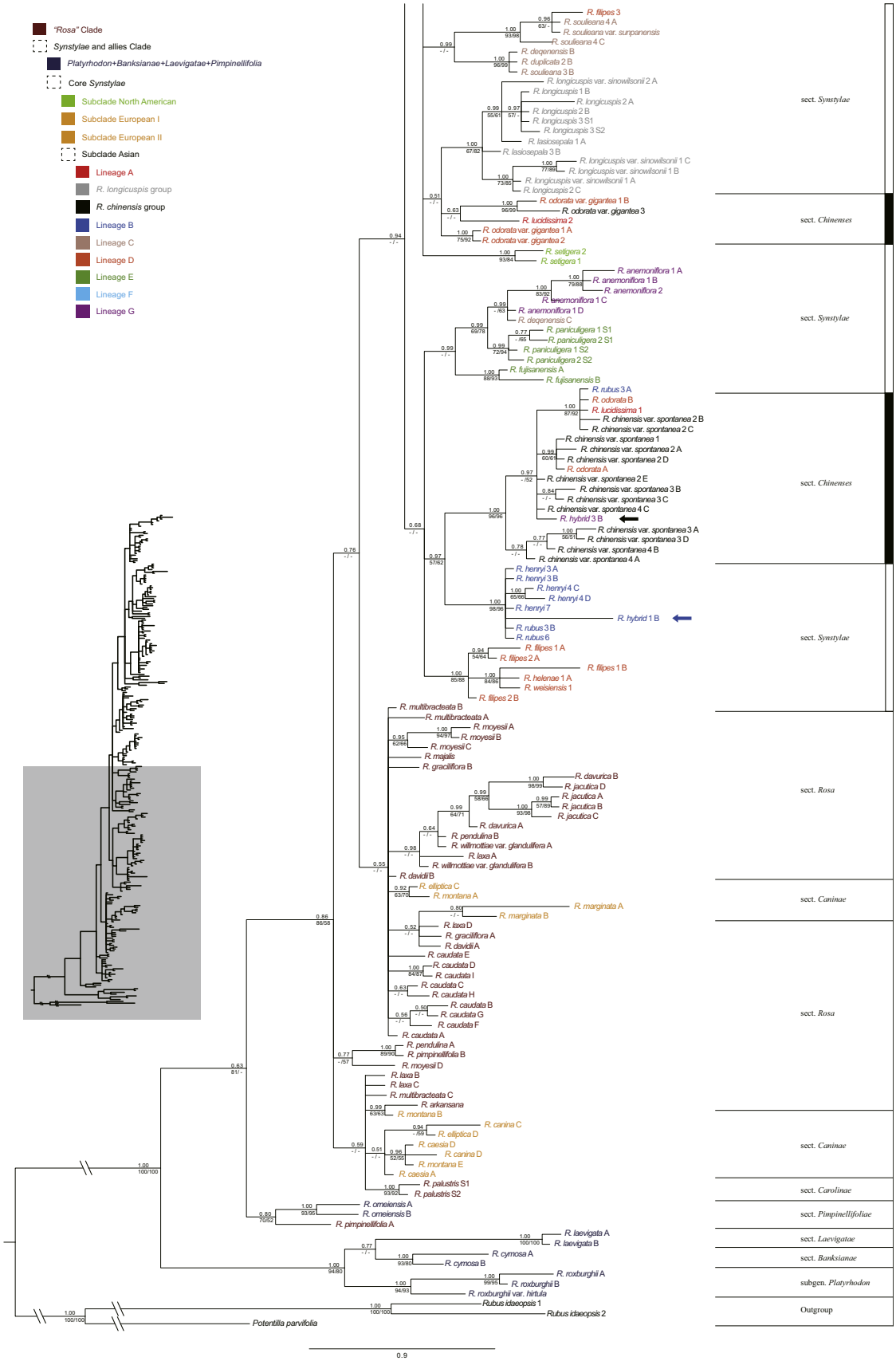

Fig. 3 (*continued*)

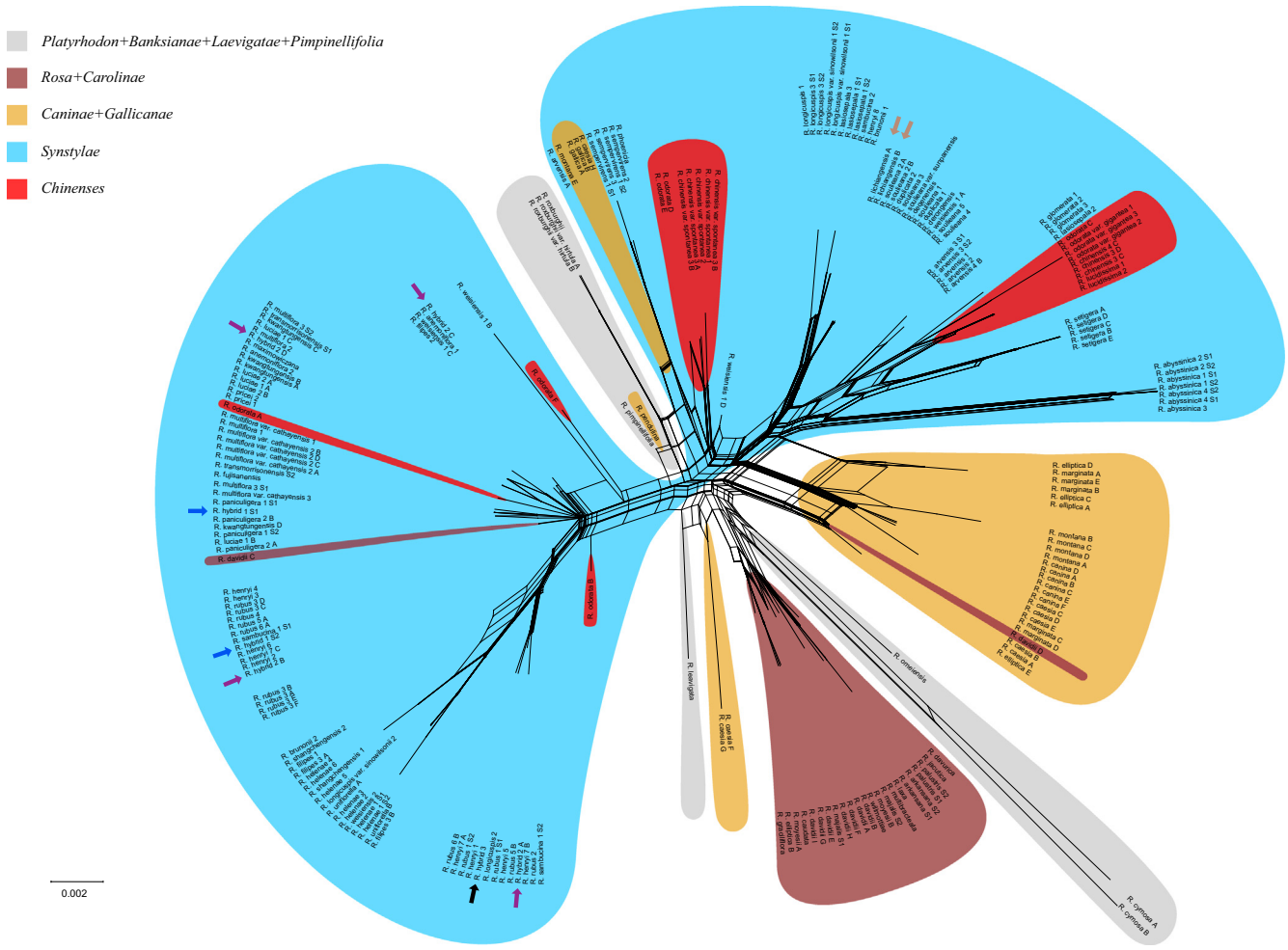

Fig. 4. Phylogenetic network from NeighorNet analysis of *nrITS* dataset. Different colors indicate subgenus or sections in traditional taxonomy.

and horizontal gene transfer) can cause these phylogenetic inconsistencies (Cronn and Wendel, 2004; Linder and Rieseberg, 2004; Maddison, 1997; Rieseberg and Soltis, 1991; Wendel and Doyle, 1998; Zou and Ge, 2008). Among these causes, introgression and incomplete lineage sorting have been frequently discussed as the prominent factors of phylogenetic incongruence at lower taxonomic levels (Cronn and Wendel, 2004; Joly et al., 2009; Maddison and Knowles, 2006). Hybridization/introgression plays a significant role in the evolutionary history of most plant taxa (Abbott, 1992; Barton, 2001; Hewitt, 1988; Whitney et al., 2010). As mentioned above, many species in genus *Rosa* were proved to have undergone hybridization events. Incongruent phylogenetic relationships among our gene trees could be caused by introgression/hybridization. Incomplete lineage sorting does not seem a reasonable explanation in these cases. Incomplete lineage sorting often corresponds to recent divergence events with insufficient time to fully sort gene polymorphism from the ancestors (Knowles and Carstens, 2007). In our study, the divergence time of those species (Fig. S1) seemed to be long enough for a full lineage sorting. Therefore, we rather attribute the discordance to genetic introgression than incomplete lineage sorting in this case.

4.2. Phylogeny of Rosa sections Chinenses and Synstylae

Our analyses all gave strong support to the monophyly of genus *Rosa*. This is consistent with previous molecular phylogenetic studies (Bruneau et al., 2007; Eriksson et al., 2003; Potter et al., 2007; Wissemann and Ritz, 2005). Though this study focused on sections *Chinenses* and *Synstylae*, our extensive sampling did help understanding relationships within the whole genus. As previous studies found (Bruneau et al., 2007; Wissemann and Ritz, 2005; Wu et al., 2000), none of our three phylogenies (Figs. 1–3) supported traditional taxonomy of subgenera and sections (Rehder, 1940; Wissemann, 2003). Our phylogenetic analyses suggested that the sections *Chinenses* and *Synstylae* are not monophyletic since sections *Caninae*, *Chinenses*, *Gallicanae* and *Synstylae* showed very close relationships in our phylogenies. Our nuclear phylogenetic networks (Figs. 4 and 5) also indicated significant interrelations of these four sections in their evolutionary histories. These results were also consistent with a new worldwide study of the whole genus based on several markers (Fougère-Danezan et al., 2015).

Species of sections *Chinenses* and *Synstylae* are mainly distributed in China (Ku and Robertson, 2003). The Chinese species of section *Synstylae* have been divided into two series (ser. *Multiflorae* and *Brunonianae*) in Flora of China (Yü et al., 1985) according to the character of stipules. If the series definition is applied to the whole section, Japanese species (e.g., *R. fujisanensis* Makino and *R. paniculigera* Makino ex Momiy.) should be assigned to ser. *Multiflorae* and others to ser. *Brunonianae*, but these series have not been strictly recovered in our comprehensive molecular phylogenies (Figs. 1–3). The phylogenetic relationships within these two sections were not consistent with traditional taxonomy in many clades either, but a lot of information on their relationships was still provided by our molecular phylogenetic analyses.

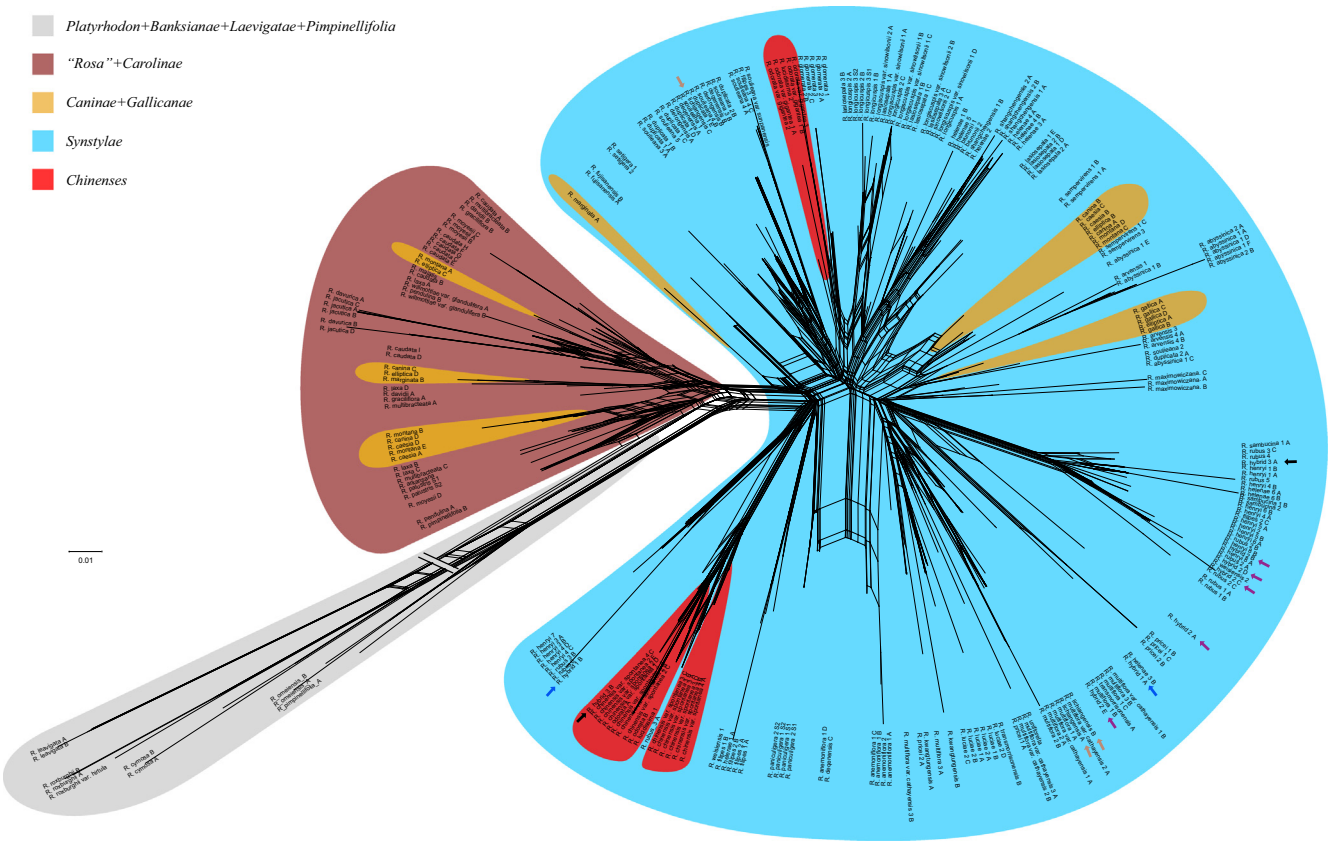

Fig. 5. Phylogenetic network from NeighorNet analysis of *GAPDH* dataset. Different colors indicate subgenus or sections in traditional taxonomy.

Table 2
Statistics of data partitions used in this study.

Region	Number of accessions		Aligned nucleotide length (bp)		Variable sites (%)		Parsimony informative sites (%)	
	R+O[a]	R[b]	R+O[a]	R[b]	R+O[a]	R[b]	R+O[a]	R[b]
ndhC-trnV	126	123	763	703	139 (18.22)	61 (8.68)	68 (8.91)	34 (4.84)
ndhF-rpl32	126	123	1275	1227	243 (19.06)	129 (10.51)	132 (10.35)	76 (6.19)
ndhJ-trnF	124	121	1319	1269	206 (1319)	75 (5.91)	92 (6.97)	38 (2.99)
psbJ-petA	124	121	1051	999	167 (15.89)	57 (5.71)	104 (9.90)	28 (2.80)
Concatenated chloroplast data	126	123	4408	4198	755 (17.13)	322 (7.67)	396 (8.98)	176 (4.19)
nrITS	224	221	622	616	168 (27.01)	108 (17.53)	103 (16.56)	67 (10.88)
GAPDH	272	269	915	908	418 (45.68)	318 (35.02)	270 (29.51)	210 (23.13)

[a] *Rosa* plus outgroups.
[b] *Rosa*.

4.2.1. Rosa section Chinenses

Rosa sect. *Chinenses* includes three wild taxa with coriaceous leaves and exserted free styles. *Rosa chinensis* var. *spontanea* and *R. lucidissima* H. Lev. have three to five leaflets per leaf while *R. odorata* var. *gigantea* has five to nine. In addition, *Rosa lucidissima* has a unique character consisting of congested bristles on branchlets, pedicels and receptacles. Because this section includes important garden plants or wild ancestors of cultivated roses, research on this section has been prolific (see Meng et al., 2011; Scalliet et al., 2008; Scariot et al., 2006), but most studies focused on the garden roses or double-petaled varieties. In this study, we sampled all three wild taxa and double-petaled *R. odorata* (Andrews) Sweet var. *odorata*. All samples were nested within section *Synstylae* (Figs. 4 and 5). This relationship was also recovered in previous studies (Bruneau et al., 2007; Fougère-Danezan et al., 2015; Meng et al., 2011; Qiu et al., 2012; Wissemann and Ritz, 2005). Our phylogenies showed that these species together are not monophyletic, and chloroplast phylogeny resolved *R. lucidissima* as the earliest divergent species in the Asian subclade.

4.2.2. Rosa abyssinica

Rosa abyssinica is the only *Synstylae* species distributed in East Africa (Ethiopia) and the South Arabian Peninsula (Yemen and Saudi Arabia). There was some discordance regarding the position of this species between chloroplast and nuclear phylogenies. Its exserted and columnated styles provide strong evidence for its inclusion in section *Synstylae*, while in our chloroplast tree, the four samples formed a well-supported lineage nested in "*Rosa*" Clade (Fig. 1). We attribute the abnormal position of *R. abyssinica* in chloroplast phylogeny to hybridization/introgression instead of incomplete lineage sorting because the divergence time (40.9 Ma, Fig. S1) seems to be long enough for a full lineage sorting as discussed before. Most previous studies of *Rosa* did not include *R. abyssinica* except the recent analyses of Fougère-Danezan et al.

(2015) in which the results were consistent with ours and suggested that *R. abyssinica* could have a hybrid origin.

4.2.3. North American Synstylae

Only one species (*Rosa setigera*) from section *Synstylae* is distributed in North America. It has pink flowers and three leaflets per leaf, which is relatively rare in sect. *Synstylae*. This species is also the only rose that is dioecious (Focke, 1897; Kevan et al., 1990). *R. setigera* was recovered as monophyletic with a good support in our phylogenetic trees (Figs. 1–3). And its particular distribution, morphological characters and sexual system match its position in the chloroplast tree as sister to all the other species of Core *Synstylae*.

4.2.4. European Synstylae

Eight samples representing three European species (*Rosa arvensis*, *R. sempervirens* L., and *R. phoenicia* Boiss) were investigated in this study. They showed close relationships to the European species from sections *Caninae* and *Gallicanae* rather than other species of sect. *Synstylae*. Previous studies (De Riek et al., 2013; Graham and Primavesi, 1993) have reported frequent hybridization between sections *Synstylae* and *Caninae*, which is an important reason for the complexity of section *Caninae*. In our nuclear results (Figs. 2–5), some copies from these species clustered with *Caninae* species suggesting an interrelation in their evolutionary history. However, those three European species separately formed good monophyletic lineages with obvious divergence in their chloroplast genomes (Fig. 1).

4.2.5. Rosa glomerata

Rosa glomerata is widely distributed in Southwest China with typical exserted and columnated styles as well as white flowers. But its leathery leaflets with abaxially dense pubescence and prominent veins make it very different from other *Synstylae* species. Nuclear and chloroplast results for this species were inconsistent as mentioned above. *R. glomerata* has an overlapping distribution area with "*Rosa*" species (*R. moyesii* Hemsley & E.H. Wilson, *R. gracilliflora* Rehder & E.H. Wilson and *R. multibracteata* Hemsley & E.H. Wilson) and showed close relationships in chloroplast tree (Fig. 1). Considering the early divergence (40.9 Ma, Fig. S1) of "*Rosa*" Clade and *Synstylae* and allies Clade, together with the sympatric distribution with some "*Rosa*" species we suggest that *R. glomerata* has undergone some ancient hybridization events.

4.2.6. Rosa rubus group

This group mainly corresponded to Lineage B in the chloroplast tree and included three species (*R. rubus*, *R. henryi* Boulenger, and *R. sambucina* Koidz.) (Fig. 1). These species are highly similar with long climbing or repent branches, five leaflets per leaf, similar size and shape of leaflets, and white flowers in corymbs. But *R. rubus* is densely pubescent or glandular on the abaxial side of leaflets while leaflets of *R. henryi* and *R. sambucina* are glabrous on both sides. *R. rubus* and *R. henryi* share a large distribution area in central and south China while *R. sambucina* is distributed in Japan and Taiwan (China). In our phylogenetic analyses, the boundaries between *R. rubus*, *R. henryi*, and *R. sambucina* were very obscure. Additionally, in the chloroplast tree one sample of *R. rubus* and three of *R. henryi* were recovered as closely related to sympatric species of *Rosa multiflora* group in Lineage G (Fig. 1). Rechecking of specimens excluded misidentifying of these species. The non-monophyletic positions could be due to incomplete lineage sorting or genetic introgression as mentioned above. Here, the incongruences are more likely the result of genetic introgression/hybridization than incomplete lineage sorting, because lineage B and G diverged about 15 Mya (Fig. S1) which is long enough for a full lineage sorting.

4.2.7. Rosa soulieana group

The *Rosa soulieana* group is distributed in Hengduan Mountains (H-D Mountains) and eastern edge of Qinghai-Tibet Plateau (Q-T Plateau) with about five species and varieties. *Rosa soulieana* Crép. is the first described species of this group and occupies the whole distribution area. The other four species and varieties (*R. deqenensis* T.C. Ku, *R. derongensis* T.C. Ku, *R. duplicata* T.T. Yu & T.C. Ku, and *R. soulieana* var. *sungpanensis* Rehder) are morphologically similar, narrowly distributed, and sympatric with *R. soulieana*. They were documented according to their different characters of leaflets or flowers (Ku, 1990; Rehder, 1930; Yü et al., 1980). All samples of this group clustered together in our chloroplast (Lineage C; Fig. 1) and *ITS* analyses (Fig. 2). Our molecular phylogenies did not show a deep divergence in contrast with the morphological diversity exhibited by those species, and despite the fact that H-D Mountains and Q-T Plateau have been suggested as the divergence center of many plant taxa because of their rich topography and complex history (Gao et al., 2013; Sun et al., 2014; Wang et al., 2009).

4.2.8. Rosa helenae group

The *Rosa helenae* group included five species (*R. brunonii* Lindl., *R. filipes* Rehder & E. H. Wilson, *R. helenae* Rehder & E. H. Wilson, *R. shangchengensis* T. C. Ku, and *R. weisiensis* T. T. Yu & T. C. Ku). They generally have seven (*R. weisiensis* has five to seven) leaflets per leaf and corymb or compound corymb inflorescences. In the chloroplast tree (Fig. 1) these species clustered as a subclade of Lineage D that was sister to *R. odorata* and *R. odorata* var. *gigantea* of sect. *Chinenses* (not recovered in nuclear phylogenies). Either introgression or incomplete lineage sorting, or both of them could be the cause for this relationship.

4.2.9. Rosa multiflora group

The *Rosa multiflora* group corresponds to the series *Multiflorae* including about 12 species and varieties in our study. *Rosa multiflora* is naturally distributed in China and Japan but now considered as an invasive species in other parts of the world (e.g. Australia, Pakistan and USA). Other species and varieties occur at coastal regions of China and Japan. They formed a clade that further divided into three highly supported lineages (Lineage E, Lineage F and Lineage G) in the chloroplast tree (Fig. 1). Morphological characters are not consistent with our molecular results. For example, the white flowered *R. multiflora* and its pink flowered variety *R. multiflora* var. *cathayensis* did not segregate in two separate clades. *Rosa anemoniflora* has light pink flowers and three leaflets per leaf which is rare in sect. *Synstylae*. However, despite this peculiarity, this species was not monophyletic in our molecular phylogenies.

4.2.10. Rosa longicuspis group

This group included two species (*R. longicuspis* and *R. lasiosepala*) and a variety (*R. longicuspis* var. *sinowilsonii* (Hemsley) T.T. Yu & T.C. Ku) distributed in South China. *Rosa longicuspis* and *R. longicuspis* var. *sinowilsonii* are distributed in Yunnan, Guizhou and Sichuan provinces. *Rosa lasiosepala* is endemic to Guangxi and Yunnan provinces. They have shiny and coriaceous leaves with prominent veins that are particular in sect. *Synstylae*. Despite these species have different morphological characteristics, they did not exhibit obvious genetic divergences in our analyses.

4.3. Putative hybrids in Rosa sections Chinenses and Synstylae

As mentioned above, hybridization occurred frequently among wild roses and driven the diversification of this genus (Joly et al., 2006; Matthews, 1920; Schanzer and Vagina, 2007; Uggla and Carlson-Nilsson, 2005). Indeed, it generated a lot novel characters (e.g. intermediate characters) and promoted speciation in this genus.

Rosa lichiangensis T.T. Yu & T.C. Ku was first documented in 1981 (Tsue-Chih, 1981). It was supposed to be hybrid-origin from our field and specimen observations. And its hybrid origin was confirmed by both molecular and morphological evidences in another study (Zhu and Gao, unpublished). The results of cpDNA and *GAPDH* in this study also displayed the hybrid scenario of *R. lichiangensis* (Figs. 1 and 3). When documenting hybridization events in plant using *ITS* sequences, it is necessary to consider concerted evolution. Some studies suggested that homogenization of *ITS* copies can eliminate one of the parents copies in few generations (Hillis et al., 1991; Wendel et al., 1995; Aguilar et al., 1999). Our *ITS* results support the same scenario in *R. lichiangensis* (Fig. 2). The homogenization of parental alleles in the *ITS* sequences resulted in the display of maternal (*R. soulieana*) genetic information only and the *ITS* gene could not reveal the hybridization event in this case. However, although ten clones were sequenced, it is not possible to completely rule out the possibility that all the *ITS* types were not completely collected.

Rosa rubus and *R. multiflora* have a wide distribution with a large overlapping area. A few putative hybrids between these two species were observed in the field. Two samples (*R. hybrid* 1 and *R. hybrid* 2) respectively located in Enshi (Hubei, China) and Guiyang (Guizhou, China) were included in this study. In both localities, *Rosa rubus* and *R. multiflora* grow together and have the same flowering time from May to July. In morphology, the putative hybrids possessed lobed stipules that were intermediate between those of *R. rubus* and *R. multiflora*. They had five to seven leaflets per leaf and the size of leaflet was intermediate between the probable parents. In our chloroplast phylogeny (Fig. 1), the putative hybrids were close to *R. rubus* in Clade B or G. Our nuclear phylogenies (Figs. 2–5) were consistent with the hybrid hypothesis for these two individuals. Their nuclear accessions clustered into two different lineages of which one contained *R. rubus* and its close relatives and the other contained *R. multiflora* and its close relatives. All these results suggested that *R. rubus* and *R. multiflora* were probably the parents of the hybrids.

Another putative hybrid (*R. hybrid* 3) that seemed to be morphologically related to sections *Synstylae* (*R. rubus*) and *Chinenses* (*R. lucidissima*) was also sampled in this study. Several characters seemed to be intermediate between the two possible parents. *Rosa lucidissima* has bright coriaceous leaves and dark red flowers. *Rosa rubus* has pubescent herbaceous leaves and white flowers. This putative hybrid had slightly coriaceous leaves and light red flowers. Moreover, *Rosa lucidissima* has long (app. 2 mm) glandular bristles on pedicels and branchlets while *R. rubus* only has glands adhering to pedicels. The putative hybrid had short (app. 0.5–1 mm) glandular bristles on pedicels and branchlets. In addition, this individual was located in the overlapping area of *R. lucidissima* and *R. rubus*. Although *R. lucidissima* flowers much earlier (April to May) than *R. rubus* (late April to July), there is an overlap in their flowering time from late April to early May. We obtained two *GAPDH* accessions for this putative hybrid with one close to sect. *Chinenses* (*R. lucidissima*) and the other close to *R. rubus* group (Fig. 3). The *GAPDH* phylogeny confirmed our inference based on morphology. The chloroplast tree (Fig. 1) suggested that *R. rubus* might play the female parent, and *R. lucidissima* might be the male parent. However, the *ITS* sequences of this sample did not show different copies (Fig. 2). This pattern in the *ITS* phylogeny is similar to the situation of *R. lichiangensis* mentioned above. As before, we attributed this pattern to homogenization of parental genetic information obscuring hybridization events.

5. Conclusions and perspectives

This study presents phylogenies of *Rosa* sections *Chinenses* and *Synstylae* based on three different kinds of genetic markers (chloroplast DNA, nuclear ribosome DNA, and single copy nuclear DNA) with extensive taxon sampling. The two sections were not resolved as monophyletic in any of our analyses. A lot of discordance was observed between chloroplast and nuclear trees. And we conclude that hybridization, rather than incomplete lineage sorting has been the main driving force of the incongruence. The lack of resolution in phylogenies (especially nuclear phylogenies) definitely hindered the acquisition of accurate relationships among species. However, we confirmed the hybrid origin of the species *R. lichiangensis* and other putative hybrids.

Chloroplast markers provided a better backbone for phylogenies, but ignored hybridization because of their maternal inheritance. Insufficient variation among close species was also a problem. The *ITS* region has been widely used as a nuclear marker in former studies of *Rosa* since it is of biparental inheritance and provides more variation than chloroplast markers. However, concerted evolution and homogenization of parental polymorphism, as discussed above, definitely hampered our understanding of evolution history. Single copy nuclear gene *GAPDH* provided more variation than chloroplast and *ITS* genes. In addition, the *GAPDH* tree provided the most comprehensive and accurate knowledge of the reticulate evolution of *Rosa* although the resolution was low for some nodes. Further studies including more low copy nuclear genes are necessary to better understand the complex evolutionary history of roses (e.g. hybridization and polyploidization). Additionally, population level studies seem more effective for tracking evolutionary history and circumscribing close species (Joly et al., 2006; Liu et al., 2012; Sun et al., 2014). Thus, studies focusing on population level could be critical to the further understanding of sections *Chinenses* and *Synstylae* and even the whole genus since a lot of close species (for example *R. soulieana* group, *R. rubus* group, *R. multiflora* group) have undergone a fast diversification, incomplete lineage sorting, or reticulate evolution.

Acknowledgments

We are grateful to Dr. Liang Zhang, Dr. Yu-Lan Peng, Dr. Bing-Qiang Xu, Wen-Bin Ju, Yu Zhang, Xue-Li Zhao, Xia Zheng, and Prof. Mark P. Simmons for their help in collecting samples; to Prof. Li-Bing Zhang, Dr. Yun-Dong Gao and Dr. Bo Xu for their suggestion and comment on data analysing and manuscript preparing. We thank the Royal Botanic Gardens Kew and Edinburgh, Wageningen University Botanical Garden, and Botanic Garden Meise in Belgium for providing samples, and Ya-Ling Cao, Yong-Hua He, and Jian Gu for collecting and cultivating roses for Maoxian Ecological Station. We are also grateful to Dr. Zhen-Xiang Xi and Prof. Kyle Tomlinson for some useful suggestion and English editing of the manuscript. Additionally, we would like to thank the curators and staff of the herbaria A/GH, BM, CDBI, E, HNWP, IBSC, K, KATH, KUN, PE, TUCH, and WUK for their help when Xin-Fen Gao visited their herbarium. Financial support was provided by The National Natural Science Foundation of China (NSFC Grand Nos. 31070173, 31110103911), Knowledge Innovation Program of the Chinese Academy of Sciences (Grand Nos. KSCX2-EW-J-22, KSCX2-EW-Z-1), the Ministry of Science and Technology (Grand

No. 2007FY110100), and Project of Platform Construction for Plant Resources of Sichuan Province to Xin-Fen Gao.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.03.014.

References

Aguilar, J.F., Rossello, J.A., Feliner, G.N., 1999. Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). Mol. Ecol. 8, 1341–1346.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723.

Anderson, E., 1948. Hybridization of the habitat. Evolution 2, 1–9.

Atienza, S.G., Torres, A.M., Millan, T., Cubero, J.I., 2005. Genetic diversity in *Rosa* as revealed by RAPDs. Agric. Conspec. Sci. 70, 75–85.

Barrie, F.R., 2006. Report of the general committee: 9. Taxon 55, 795–800.

Barton, N.H., 2001. The role of hybridization in evolution. Mol. Ecol. 10, 551–568.

Baurain, D., Brinkmann, H., Philippe, H., 2007. Lack of resolution in the animal phylogeny: closely spaced cladogeneses or undetected systematic errors? Mol. Biol. Evol. 24, 6–9.

Bruneau, A., Starr, J.R., Joly, S., 2007. Phylogenetic relationships in the genus *Rosa*: new evidence from chloroplast DNA sequences and an appraisal of current knowledge. Syst. Bot. 32, 366–378.

Calvino, C.I., Martinez, S.G., Downie, S.R., 2008. The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): rapid radiations, long distance dispersals, and hybridizations. Mol. Phylogenet. Evol. 46, 1129–1150.

Campbell, C.S., Evans, R.C., Morgan, D.R., Dickinson, T.A., Arsenault, M.P., 2007. Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): limited resolution of a complex evolutionary history. Plant Syst. Evol. 266, 119–145.

Cole, P., Melton, B., 1986. Self-and cross-compatibility relationships among genotypes and between ploidy of the rose. J. Am. Soc. Hortic. Sci. 111, 122–125.

Crépin, F., 1889. Sketch of a new Classification of Roses. Spottiswoode & Company, Printers.

Cronn, R., Wendel, J.F., 2004. Cryptic trysts, genomic mergers, and plant speciation. New Phytol. 161, 133–142.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

De Riek, J., De Cock, K., Smulders, M.J.M., Nybom, H., 2013. AFLP-based population structure analysis as a means to validate the complex taxonomy of dogroses (*Rosa* section *Caninae*). Mol. Phylogenet. Evol. 67, 547–559.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Edelman, D.W., 1975. The Eocene Germer Basin Flora of South-Central Idaho [M.S. Thesis], Moscow, University of Idaho, 142pp.

Eriksson, T., Hibbs, M.S., Yoder, A.D., Delwiche, C.F., Donoghue, M.J., 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (*ITS*) of nuclear ribosomal DNA and the trnL/F region of chloroplast DNA. Int. J. Plant Sci. 164, 197–211.

Erlanson, E.W., 1938. Phylogeny and polyploidy in *Rosa*. New Phytol. 37, 72–81.

Focke, W.O., 1897. Neue Beobachtungen über Artenkreuzung und Selbsterilitat. Abh. Natwiss. Ver. Brem. 14, 297–304.

Fougère-Danezan, M., Joly, S., Bruneau, A., Gao, X.F., Zhang, L.B., 2015. Phylogeny and biogeography of wild roses with specific attention to polyploids. Ann. Bot. 115, 275–291. http://dx.doi.org/10.1093/aob/mcu245.

Gao, Y.D., Harris, A.J., Zhou, S.D., He, X.J., 2013. Evolutionary events in *Lilium* (including *Nomocharis*, Liliaceae) are temporally correlated with orogenies of the Q-T plateau and the Hengduan Mountains. Mol. Phylogenet. Evol. 68, 443–460.

Graham, G.G., Primavesi, A.L., 1993. Roses of Great Britain and Ireland. BSBI Handbook, No. 7 Botanical Society of the British Islas, London, p. 208.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hewitt, G.M., 1988. Hybrid zones-natural laboratories for evolutionary studies. Trends Ecol. Evol. 3, 158–167.

Hillis, D.M., Moritz, C., Porter, C.A., Baker, R.J., 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. Science 251, 308–310.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Jan, C.H., Byrne, D.H., Manhart, J., Wilson, H., 1999. Rose germplasm analysis with RAPD markers. HortScience 34, 341–345.

Jarvis, C., 1992. Seventy-two proposals for the conservation of types of selected Linnaean generic names, the report of Subcommittee 3C on the lectotypification of Linnaean generic names. Taxon 41, 552–583.

Jesse, L.C., Moloney, K.A., Obrycki, J.J., 2006. Insect pollinators of the invasive plant, *Rosa multiflora* (Rosaceae), in Iowa, USA. Weed Biol. Manage. 6, 235–240.

Joly, S., Starr, J.R., Lewis, W.H., Bruneau, A., 2006. Polyploid and hybrid evolution in roses east of the Rocky Mountains. Am. J. Bot. 93, 412–425.

Joly, S., McLenachan, P.A., Lockhart, P.J., 2009. A statistical approach for distinguishing hybridization and incomplete lineage sorting. Am. Nat. 174, E54–E70.

Kevan, P.G., Eisikowitch, D., Ambrose, J.D., Kemp, J.R., 1990. Cryptic dioecy and insect pollination in *Rosa setigera* Michx (Rosaceae), a rare plant of Carolinian Canada. Biol. J. Linn. Soc. 40, 229–243.

Knowles, L.L., Carstens, B.C., 2007. Delimiting species without monophyletic gene trees. Syst. Biol. 56, 887–895.

Koopman, W.J.M., Wissemann, V., De Cock, K., Van Huylenbroeck, J., De Riek, J., Sabatlno, G.J.H., Visser, D., Vosman, B., Ritz, C.M., Maes, B., Werlemark, G., Nybom, H., Debener, T., Linde, M., Smulders, M.J.M., 2008. AFLP markers as a tool to reconstruct complex relationships: a case study in *Rosa* (Rosaceae). Am. J. Bot. 95, 353–366.

Ku, T.C., 1990. Taxa nova *Rosacearum Sinicarum* (VI). Bull. Bot. Res. 10, 1–15.

Ku, T.C., Robertson, K.R., 2003. *Rosa* Linnaeus. In: Wu, C.Y., Raven, P.H. (Eds.), Flora of China, vol. 9. Science Press/Missouri Botanical Garden Press, Beijing/St, Louis, pp. 339–381.

Linder, C.R., Rieseberg, L.H., 2004. Reconstructing patterns of reticulate evolution in plants. Am. J. Bot. 91, 1700–1708.

Linnaeus, C.V., 1753. Species Plantarum, vols. 2. Salvius, Stockholm.

Liu, J.Q., Sun, Y.S., Ge, X.J., Gao, L.M., Qiu, Y.X., 2012. Phylogeographic studies of plants in China: advances in the past and directions in the future. J. Syst. Evol. 50, 267–275.

Lo, E.Y.Y., Stefanović, S., Christensen, K.I., Dickinson, T.A., 2009. Evidence for genetic association between East Asian and western North American *Crataegus* L. (Rosaceae) and rapid divergence of the eastern North American lineages based on multiple DNA sequences. Mol. Phylogenet. Evol. 51, 157–168.

Maddison, W.P., 1997. Gene trees in species trees. Syst. Biol. 46, 523–536.

Maddison, W.P., Knowles, L.L., 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. 55, 21–30.

Mallet, J., 2007. Hybrid speciation. Nature 446, 279–283.

Matsumoto, S., Kouchi, M., Yabuki, J., Kusunoki, M., Ueda, Y., Fukui, H., 1998. Phylogenetic analyses of the genus *Rosa* using the matK sequence: molecular evidence for the narrow genetic background of modern roses. Sci. Hortic. (Amst.) 77, 73–82.

Matsumoto, S., Kouchi, M., Fukui, H., Ueda, Y., 2000. Phylogenetic analyses of the subgenus *Eurosa* using the *ITS* nrDNA sequence. Acta Hort. 521, 193–202.

Matthews, J.R., 1920. Hybridism and classification in the genus *Rosa*. New Phytol. 19, 153–171.

McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, D.L., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Proud'Homme van Reine, W.F., Smith, J.F. Wiersema, J.H. (Eds.), 2012. International Code of Nomenclature for algae, fungi and plants (Melbourne Code): Adopted by the Eighteenth International Botanical Congress, Melbourne, Australia, July 2011. Regnum Vegetable, vol. 154, pp. 1–274.

Meng, J., Fougere-Danezan, M., Zhang, L.B., Li, D.Z., Yi, T.S., 2011. Untangling the hybrid origin of the Chinese tea roses: evidence from DNA sequences of single-copy nuclear and chloroplast genes. Plant Syst. Evol. 297, 157–170.

Mercure, M., Bruneau, A., 2008. Hybridization between the escaped *Rosa rugosa* (Rosaceae) and native *R. blanda* in eastern North America. Am. J. Bot. 95, 597–607.

Millan, T., Osuna, F., Cobos, S., Torres, A.M., Cubero, J.I., 1996. Using RAPDs to study phylogenetic relationships in *Rosa*. Theor. Appl. Genet. 92, 273–277.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), 2010. IEEE, pp. 1–8.

Müller, K., 2005. SeqState. Appl. Bioinformatics 4, 65–69.

Müller, K., 2006. Incorporating information from length-mutational events into phylogenetic analysis. Mol. Phylogenet. Evol. 38, 667–676.

Abbott, R.J., 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. Trends Ecol. Evol. 7, 401–405.

Ohba, H., Akiyama, S., Mikanagi, Y., 2000. Interspecific hybrids between the Japanese species in *Rosa* section *Synstylae* (Rosaceae). J. Jpn. Bot. 82, 45–53.

Paun, O., Forest, F., Fay, M.F., Chase, M.W., 2009. Hybrid speciation in angiosperms: parental divergence drives ploidy. New Phytol. 182, 507–518.

Potter, D., Gao, F., Bortiri, P.E., Oh, S.H., Baggett, S., 2002. Phylogenetic relationships in Rosaceae inferred from chloroplast matK and trnL-trnF nucleotide sequence data. Plant Syst. Evol. 231, 77–89.

Potter, D., Eriksson, T., Evans, R.C., Oh, S., Smedmark, J.E.E., Morgan, D.R., Kerr, M., Robertson, K.R., Arsenault, M., Dickinson, T.A., Campbell, C.S., 2007. Phylogeny and classification of Rosaceae. Plant Syst. Evol. 266, 5–43.

Qiu, X.Q., Zhang, H., Wang, Q.G., Jian, H.Y., Yan, H.J., Zhang, T., Wang, J.H., Tang, K.X., 2012. Phylogenetic relationships of wild roses in China based on nrDNA and matK data. Sci. Hortic. (Amst.) 140, 45–51.

Rambaut, A., Drummond, A.J., 2009. Tracer v1.5. <http://tree.bio.ed.ac.uk/software/tracer/>.

Rehder, A., 1930. New species, varieties and combinations from the herbarium and collections of the Arnold arboretum. J. Arnold Arbor. XI, 161.

Rehder, A., 1940. Manual of Cultivated Trees and Shrubs Hardy in North America, second ed. Macmillan, New York.

Rieseberg, L.H., 1995. The role of hybridization in evolution – old wine in new skins. Am. J. Bot. 82, 944–953.

Rieseberg, L.H., 1997. Hybrid origins of plant species. Annu. Rev. Ecol. Syst. 28, 359–389.

Rieseberg, L.H., Soltis, D.E., 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trend. Plant 5, 65–84.

Ritz, C.M., Schmuths, H., Wissemann, V., 2005. Evolution by reticulation: European dogroses originated by multiple hybridization across the genus rosa. J. Hered. 96, 4–14.

Roberts, A.V., Gladis, T., Brumme, H., 2009. DNA amounts of roses (*Rosa* L.) and their use in attributing ploidy levels. Plant Cell Rep. 28, 61–71.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Scalliet, G., Piola, F., Douady, C.J., Rety, S., Raymond, O., Baudino, S., Bordji, K., Bendahmane, M., Dumas, C., Cock, J.M., Hugueney, P., 2008. Scent evolution in Chinese roses. Proc. Natl. Acad. Sci. USA 105, 5927–5932.
Scariot, V., Akkak, A., Botta, R., 2006. Characterization and genetic relationships of wild species and old garden roses based on microsatellite analysis. J. Am. Soc. Sci. 131, 66–73.
Schanzer, I.A., Kutlunina, N.A., 2010. Interspecific hybridization in wild roses (*Rosa* L. sect. *Caninae* DC.). Biol. Bull. 37, 480–488.
Schanzer, I.A., Vagina, A.V., 2007. ISSR (Inter Simple Sequence Repeat) markers reveal natural intersectional hybridization in wild roses [*Rosa* L., sect. *Caninae* (DC.) Ser. and sect. *Cinnamomeae* (DC.) Ser.]. Wulfenia 14, 1–14.
Seringe, N.C., 1818. Observations générales sur les Roses. In: Musée helvétique d'histoire naturelle, (part Botanique). tome I. Genève: Chez l'auteur, Berne, p. 2.
Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am. J. Bot. 94, 275–288.
Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Strand, A.E., Leebens-Mack, J., Milligan, B.G., 1997. Nuclear DNA-based markers for plant evolutionary biology. Mol. Ecol. 6, 113–118.
Sun, Y.S., Abbott, R.J., Li, L.L., Li, L., Zou, J.B., Liu, J.Q., 2014. Evolutionary history of Purple cone spruce (*Picea purpurea*) in the Qinghai-Tibet Plateau: homoploid hybrid origin and Pleistocene expansion. Mol. Ecol. 23, 343–359.
Swofford, D., 2002. PAUP 4.0 b10: Phylogenetic Analysis Using Parsimony. Sinauer Associates, Sunderland, MA, USA.
Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Thory, C.A., 1820. Prodrome de la monographie des espèces et variétés connues du genre rosier, divisées selon leur ordre naturel. P. Dufart, Paris, p. 128.
Timme, R.E., Kuehl, J.V., Boore, J.L., Jansen, R.K., 2007. A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: identification of divergent regions and categorization of shared repeats. Am. J. Bot. 94, 302–312.
Tsue-Chih, K., 1981. Taxa nova *Rosacearum Sinicarum* (III). Bull. Bot. Res. 1, 1–33.
Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A., 1978. Roseaceae to Umbelliferae. Flora Europaea, vol. 2. Cambridge University Press, Cambridge, pp. 1–455.
Ueda, Y., Akimoto, S., 2001. Cross-and self-compatibility in various species of the genus *Rosa*. J. Hortic. Sci. Biotechnol. 76, 392–395.
Uggla, M., Carlson-Nilsson, B.U., 2005. Screening of fungal diseases in offspring from crosses between *Rosa* sections *Caninae* and *Cinnamomeae*. Sci. Hortic. (Amst.) 104, 493–504.
Wang, Y.J., Susanna, A., Von Raab-Straube, E., Milne, R., Liu, J.Q., 2009. Island-like radiation of *Saussurea* (Asteraceae: Cardueae) triggered by uplifts of the Qinghai-Tibetan Plateau. Biol. J. Linn. Soc. 97, 893–903.
Wendel, J.F., Doyle, J.J., 1998. Phylogenetic incongruence: window into genome history and molecular evolution. Molecular Systematics of Plants, vol. II. Springer, pp. 265–296.
Wendel, J.F., Schnabel, A., Seelanan, T., 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. USA 92, 280–284.
White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gefland, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press Inc., San Diego, pp. 315–322.
Whitham, T.G., Morrow, P.A., Potts, B.M., 1994. Plant hybrid zones as centers of biodiversity: the herbivore community of two endemic *Tasmanian eucalypts*. Oecologia 97, 481–490.
Whitney, K.D., Ahern, J.R., Campbell, L.G., Albert, L.P., King, M.S., 2010. Patterns of hybridization in plants. Perspect. Plant Ecol. Evol. Syst. 12, 175–182.
Wissemann, V., 1999. Genetic constitution of *Rosa* sect. *Caninae* (*R. canina*, *R. jundzillii*) and sect. *Gallicanae* (*R. gallica*). Angew. Bot. 73, 191–196.
Wissemann, V., 2002. Molecular evidence for allopolyploid orign of the *Rosa canina* – complex (Rosaceae, Rosoideae). J. Appl. Bot. 76, 176–178.
Wissemann, V., 2003. Classification. In: Roberts, A., Debener, T., Gudin, S. (Eds.), Encyclopedia of Rose Science. Elsevier, London, pp. 111–135.
Wissemann, V., Hellwig, F.H., 1997. Reproduction and Hybridisation in the Genus *Rosa*, Section *Caninae* (Ser.) Rehd. Rehd. Bot. Acta 110, 251–256.
Wissemann, V., Ritz, C.M., 2005. The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of *nrITS*-1 and atpB-rbcL intergenic spacer (IGS) versus conventional taxonomy. Bot. J. Linn. Soc. 147, 275–290.
Wu, S.Q., Ueda, Y., He, H.Y., Nishihara, S., Matsumoto, S., 2000. Phylogenetic analysis of Japanese *Rosa* species using matK sequences. Breed. Sci. 50, 275–281.
Wu, S., Ueda, Y., Nishihara, S., Matsumoto, S., 2001. Phylogenetic analysis of Japanese *Rosa* species using DNA sequences of nuclear ribosomal internal transcribed spacers (*ITS*). J. Hortic. Sci. Biotech. 76, 127–132.
Xu, B., Wu, N., Gao, X.F., Zhang, L.B., 2012. Analysis of DNA sequences of six chloroplast and nuclear genes suggests incongruence, introgression, and incomplete lineage sorting in the evolution of *Lespedeza* (Fabaceae). Mol. Phylogenet. Evol. 62, 346–358.
Yang, H.M., Zhang, Y.X., Yang, J.B., Li, D.Z., 2013. The monophyly of Chimonocalamus and conflicting gene trees in Arundinarieae (Poaceae: Bambusoideae) inferred from four plastid and two nuclear markers. Mol. Phylogenet. Evol. 68, 340–356.
Yokoya, K., Roberts, A.V., Mottley, J., Lewis, R., Brandham, P.E., 2000. Nuclear DNA amounts in roses. Ann. Bot. 85, 557–561.
Yü, T.T., Lu, L.T., Ku, T.C., 1980. Taxa nova Rosacearum Xizangensium. Acta Phytotax. Sin. 18, 490–503.
Yü, T.T., Lu, L.T., Ku, T.C., Kuan, K.C., Li, C.L., 1985. Rosaceae (2). Flora Reipublicae Popularis Sinicae, vol. 37. Science Press, Beijing, pp. 1–516.
Zou, X.H., Ge, S., 2008. Conflicting gene trees and phylogenomics. J. Syst. Evol. 46, 795–807.