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PHYLOGENETIC RECONSTRUCTION BETWEEN THE OLD AND NEW WORLD SPIROIDES INFERRED FROM PLASTID TRNL-F AND NRDNA ITS SEQUENCES

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Abstract

Distribution of similar taxa between the East Asia (old world) and new worlds fascinated the Botanists and Bio-geographers since the Linnaean time. The current study represents phylogenetic investigation of 39 *Spiraea* L., species covering their entire distribution in the new world (America) to the old world (Tibet PR China). In this study we used both plastidial marker (*trn*L-F) and nuclear internal transcribed spacer (nrDNA ITS). Phylogeny among the species implemented in the program PAUP* while divergence time was calibrated in BEAST 1.7.5. The analysis revealed that all 39 species of the genus are monophyletic. The study further suggested that ancestors of *Spiraea* were in the new world but their center of diversification is in East Asia. The estimated divergence time showed that *S. virginiana* endemic to North America is the oldest species with divergence time 21.1 MY. The divergence time of *Spiraea* species with disjunct distribution in the new world, Europe and East Asia e.g., *S. hypericifolia*, *S. chamaedryfolia*, *S. douglasii*, *S. salicifolia* and *S. latifolia* ranged from 7.44-1.83 MY with most during Pliocene (within the last 5 MY). The results concluded that isolation of *Spiraea* species occurred during the severe eco-climatic fluctuations period throughout the late Tertiary and Quaternary.

Key words: Radiation, Disjunction, Vicariance, Dispersal, Migration, Phylogeny.

Introduction

The disjunct distributions of similar plant taxa between eastern Asia and eastern North America have fascinated plant biologists since the Linnaean era Phylogenetic, molecular, geological, and Paleontological data all favor the hypothesis that the eastern Asian and eastern North American disjunct distributions are relicts in the northern hemisphere during the Tertiary. In many genera of flowering plants, molecular and fossil data suggest that the disjunct patterns were established during the Miocene (Wen, 1999). The Tertiary relicts include survivors from plant communities that were distributed throughout a large part of the Northern Hemisphere during the Tertiary (i.e., 65–15 MY). Molecular Phylogenetics showed that within East Asia the relict flora is best divided into two clear refugial groups, found on the Japan/Korea/northeast China and southeast China/Himalaya regions, which lead towards the understanding of the origins and evolution of Tertiary relict floras in East Asia. The studies also indicate two putative clusters of divergence times for East Asian-North American Tertiary relict disjunct occurring at 5 MY and 10 MY while the divergence times for transatlantic Tertiary relict disjuncts generally fall between 10 MY and 40 MY. This might result from large-scale allopatric speciation, together with stabilizing selection (Richard *et al.,* 2002). Even though East Asia is an important harboring platform for tertiary relicts still most of the studies concern biogeography and evolutionary histories conducted about Europe and North America rather than in East Asia (Liu *et al.,* 2012). East Asia was mostly ice-free contrary to Europe and North America during the last three million years (Hewitt, 2000 & 2004; Shi, 2002; Abbott & Brochmann, 2003).

Family Rosaceae has great morphological diversity, taxonomically difficult to explain, and classified in different ways by different systematists (Schulze-Menz, 1964; Hutchinson, 1964; Takhtadzhian, 1997; Morgan *et al.,* 1994). Phylogenetic study based on rbcL sequence indicated that the family is monophyletic (Morgan *et al.,* 1994), with some modification, in Rosoideae, Amygdaloideae and Maloideae, but Spiraeoideae were paraphyletic (Schulze-Menz, 1964). According to Morgan *et al.* (1994) the tribe Spiraeeae includes eight genera, developed into two groups, i.e., evergreen and deciduous ones, where *Spiraea* is considered as the most primitive genus in deciduous group (Lu, 1996). East-Asia is considered the center of distribution and differentiations of the species belong to the tribe (Donoghue & Smith, 2004). In tribe Spiraeeae, *Spiraea* is a rich genus represented by 70-80 species distributed throughout the north temperate regions from East Asia to North America. The PR China include 48 endemic species of this genus, the remaining species are with disjunct distribution in Korea, Japan, Russia, Mongolia, Sikkim, North India, and North Myanmar in south Asia (Flora of China). Different taxonomists divided and subdivided the genus into subgenera and sections in different ways, based on their inflorescence. Batta (1977) has divided the genus into three sections: Chamaedryon, Calospira, and Spireae. Spireas are important ornamental Shrubs-Dirr (1990) lists 13 species used as ornamentals. Most of the important ornamentals species have been introduced from China and Japan (Dirr, 1990).

Molecular phylogeny is an insight to understand the land plants origin and to elucidate relations among them (Kranz *et al.,* 1995; Kaellersjoe *et al.,* 1998; Chase *et al.,* 1993; Qiu *et al.,* 1999; Soltis *et al.,* 1997) and have provided a new vision in the reconstruction of phylogenetic history. Phylogenetic reconstruction gives significant insights in biology and comparison of different taxa (Avise, 1994). The plastid *mat*K, *rbc*L and *trn*L-*trn*F sequences analyses resolved most of these clades but different in details among them, though most of these relationships were not well supported by any of the analyses (Potter *et al.,* 2002). The common ancestor of tribe Spiraeeae occurred in western North America, with independent migrations to the Old World occurring in *Aruncus*, *Sibiraea*, and *Spiraea* (Potter *et al.,* 2007). According to Potter *et al.,* 2007, the ancestral area for *Spiraea* could not be reconstructed explicitly based on our results, and suggested a complex biogeographic history of the genus. The complex biogeographic history involved multiple dispersal and/or vicariant events between the Old and New Worlds through several independent migrations between Europe, western/ central Asia, and eastern Asia. Based on the above literature and distribution of *Spiraea* we aimed to address the following objectives: (1) to find out the relationship among *Spiraea* species found in both the old and new world covering most of their distribution; and (2) to find the origin of the genus *Spiraea*.

Materials and Methods

Data taxon sampling: Two internal transcribed regions including one nuclear and one chloroplast DNA sequences were analyzed in the current study. All the sequences used in this study were obtained from available database NCBI (National Center for Biotechnology Information). Thirty nine species from *Spiraea* were included in the study. The species included has three species exclusively found in the new world, two species to both the new and old world, while the remaining species belong to the old world. Outgroup taxa comprised five taxa which are closed to the genus from the same family (Fig. 1; Table 1). These species representing all the three sections of tribe Spiraeeae by Rehder (1940).

Sequence alignment and phylogenetic analyses: All the sequences were checked for genetic polymorphism through MEGA5 (Tamura *et al*., 2011). The CLUSTAL X (with default alignment option) was used to align the sequences (Thompson *et al.,* 1997) by following the similarity criterion (Simmons, 2004). Phylogenetic analyses were carried out in three different data matrices. The first data matrix comprised the cpDNA *trn*L-F including 39 species with five out group taxa with 44 terminals. The second one consisted of nrDNA ITS sequences, involved 38 sequences and five outgroup taxa resulted of 43 terminals. While the third data matrix included the concatenated sequences of *trn*L-F and ITS matrices, involved 38 sequences and five outgroup taxa. The phylogenetic relationships were constructed for both the individual's data matrices and concatenated data matrix by means of maximum parsimony (MP) and neighbor joining (NJ) methods. Both the MP and NJ trees were implemented in the program PAUP* ver. 4.0b10 (Swofford, 2003). All the characters and transitions/ transversion were equally weighted and gaps were coded as missing data to avoid weighting characters. Hundreds replicates of heuristic searches were performed with random taxon-addition sequences to ensure that all islands of shortest trees were found. To obtain estimates of bootstrap (BS) support (Felsenstein, 1985) for each individual lineage, 1000 replicates were conducted in the bootstrap analysis with random taxon-addition sequences, tree-bisection- reconnection (TBR) branch swapping, options Multrees and Steepest Descent in operation, with saving up to 10 trees per replicate.

Bayesian divergence time estimations: Estimation of divergence times among the main lineages of *Spiraea* species was performed across the ITS sequences by employing Bayesian Markov Chain Monte Carlo using the program BEAST ver. 1.7.5 (Drummond *et al.,* 2012). By using the General Time reversible (GTR+G+I) substitution model and four rate categories, a Yule tree model was implemented with a Log normal relaxed molecular clock model. A substitution rate of 5.69×10^{-9} substitutions per site per year was used (Mes *et al.,* 1996). *Sorbus koehneana* and *Sorbaria kirilowii* were selected as reference points for calibration, using their Oligocene and Eocene fossil records from the QTP in China (Tao & Xiang, 1986). Posterior distributions of parameters were approximated by using two independent 10 million generation runs, sampled after every 1,000 generations, discarding 25 % of generations as burn-in. The effective sample size >200 of all parameters was obtained through TRACER ver. 1.5. The software TREE-ANNOTATOR ver. 1.7.5 was used to summarize the output results. The tree with ages for each node and their 95% highest posterior density displayed in FIGTREE ver. 1.3.1 (Drummond & Rambaut, 2007).

Results

Phylogenetic analyses: Relevant sequence characteristics on the length and composition of each gene region as well as tree statistics from separate and combined analyses of the two regions are listed in Table 2. The alignment of the *trn*L-F dataset for 39 taxa of *Spiraea* and five outgroup taxa produced a matrix of 976 nucleotide sites, of which 202 sites were variable sites and 89 sites were parsimony informative. Both the trees were topologically almost the same, with resolved branches, showed monophyly of all the species. The tree divided all the species into three groups or clades. The clade I clustered 12 species, the clade II with five species, while the clade III grouped the remaining species (Fig. 2). The second analyses based on the nrDNA ITS data matrix for 38 species and five outgroup taxa constitute of 615 align base pairs included 263 variables sites and 177 parsimony informative sites. The MP and NJ trees based on nrDNA were with same topology, much resolved branches, and highly supported bootstraps compare with that of cpDNA matrix. The tree again showed monophyly of all the species with three distinct clades. But here the clade I constitute four species i.e. *S. sericea*, *S. chamaedryfolia*, *S. latea* and *S. rosthornii*, clade II with ten species and clade III with 23 species. In this case the clade I was very similar to the clade II with the tree of cpDNA matrix, while clade II with Clade I of cpDNA matrix (Fig. 3). The combined data matrix was 1592 nucleotide sites in length, of which 482 and 273 sites were variable and parsimony informative sites respectively. The concatenated data matrix produced MP and NJ trees with same topology with highly supported three clades with monophyly of all the species (Fig. 4).

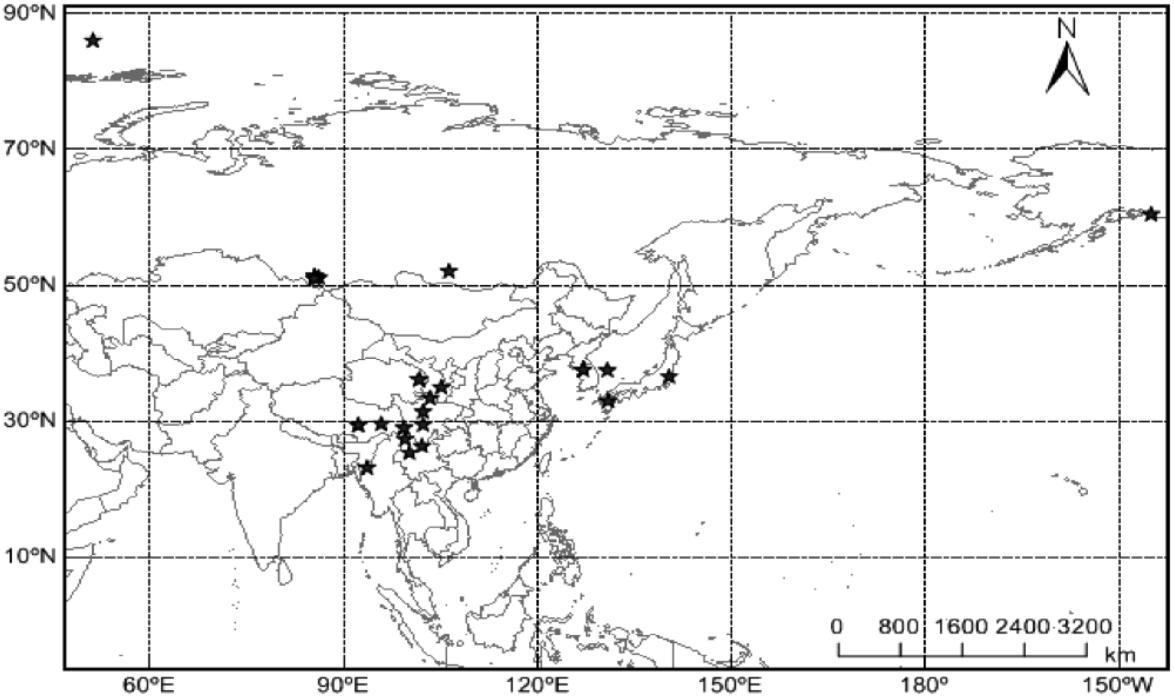

Fig. 1. Map showing geographical distribution of the species used in the study. Details of the species are given in Table 1.

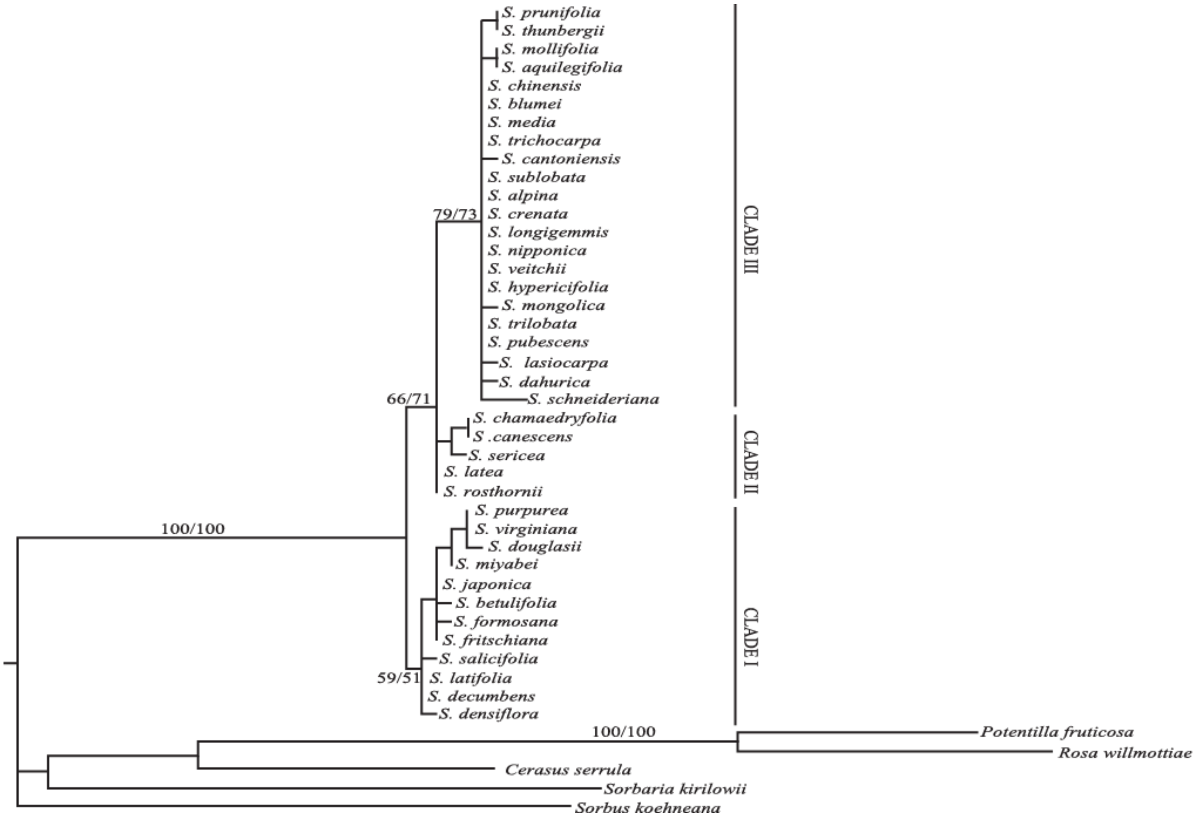

Fig. 2. Maximum Parsimony (MP) and neighbor joining (NJ) Trees based on the cpDNA marker as implemented in PAUP* 4.0 (Swofford, 2003), with Sorbaria kirilowii and Sorbus koehneana as outgroup taxa. All the characters were equally weighted and treated as unordered. A heuristic search parameters were used with the random addition sequence (1000 replicates), tree-bisection-reconnection (TBR) branch swapping and the MULTREES options selected. The number above the branches indicates bootstraps support the first one for MP followed By NJ.

Table 1. Origin of samples, GenBank accession numbers, distribution, coordinates (from published data) and the markers [ITS (nrDNA) and *trn*L-F (cpDNA)].

No.	Species	Source	Distribution	Longitude	Altitude	GenBank accessions	
						ITS	*trnL-F*
	In group taxa						
1.	*S. trichocarpa*	Gene bank	China/Korea	127.01	37.59	JQ041779	JQ041815
2.	*S. salicifolia*	Gene bank	China/ Japan/Korea/Mongolica	127.01	37.59	JQ041778	JQ041814
3.	*S. miyabei*	Gene bank	China/Japan/Korea	127.01	37.59	JQ041776	JQ041812
4.	*S. media*	Gene bank	Central Asia/China/Europe/Japan/Korea/Russia	127.01	37.59	JQ041775	JQ041811
5.	*S. chinensis*	Gene bank	China	127.01	37.59	JQ041774	JQ041810
6.	*S. blumei*	Gene bank	China/Japan/Korea	127.01	37.59	JQ041773	JQ041809
7.	*S. prunifolia*	Gene bank	China/Japan/Korea	127.01	37.59	JQ041769	JQ041805
8.	*S. latifolia*	Gene bank	Canada/France/USA	-145	58.5	DQ897619	DQ897588
9.	*S. japonica*	Gene bank	China/Japan/Korea	102.18	29.51	KF983149	KF983124
10.	*S. chamaedryfolia*	Gene bank	China/Europe/Japan/Korea/Mongolia/Russia	130.86	37.5	GU217797	GU217793
11.	*S. virginiana*	Gene bank	USA	140.43	36.59	DQ897632	DQ897601
12.	*S. veitchii*	Gene bank	China	105	35	DQ897629	DQ897598
13.	*S. trilobata*	Gene bank	China/Korea/Russia	-	-	DQ897628	DQ897597
14.	*S. thunbergii*	Gene bank	China/Japan	-	-	DQ897626	DQ897595
15.	*S. pubescens*	Gene bank	China/Korea/Mongolia/Russia	-	-	DQ897624	DQ897593
16.	*S. nipponica*	Gene bank	USA	-	-	DQ897622	DQ897591
17.	*S. longigemmis*	Gene bank	China	-	-	DQ897620	DQ897589
18.	*S. lasiocarpa*	Gene bank	China	-	-	DQ897618	DQ897587
19.	*S. hypericifolia*	Gene bank	China/Canada/Southwest Asia/Southeast Europe/Mongolia/Russia	-	-	DQ897615	DQ897584
20.	*S. fritschiana*	Gene bank	China	-	-	DQ897614	DQ897583
21.	*S. formosana*	Gene bank	Taiwan	-	-	DQ897613	DQ897582
22.	*S. douglasii*	Gene bank	Canada/USA	-	-	DQ897612	DQ897581
23.	*S. decumbens*	Gene bank	Europe	-	-	DQ897611	DQ897580
24.	*S. crenata*	Gene bank	Europe	-	-	DQ897610	DQ897579
25.	*S. cantoniensis*	Gene bank	China/Japan/Korea	85.36	51.03	DQ897609	DQ897578
26.	*S .canescens*	Gene bank	China/Bhutan/North India/Nepal/ Sikkim	-	-	DQ897608	DQ897577
27.	*S. densiflora*	Gene bank	Canada	-	-	DQ886362	AF348571
28.	*S. betulifolia*	Gene bank	USA	-	-	-	AJ390368
29.	*S. alpina*	Gene bank	China/Mongolia/Russia/Sikkim	101.58	36.17	KF983137	KF983115
30.	*S. mongolica*	Gene bank	China	101.58	36.17	KF983138	KF983120
31.	*S. rosthornii*	Gene bank	China	103.27	33.4	KF983148	KF983130
32.	*S. sericea*	Gene bank	China/Japan/Mongolia/Korea	102.02	26.36	KF983147	KF983129
33.	*S. latea*	Gene bank	China	99.4	27.37	KF983150	KF983131
34.	*S. schneideriana*	Gene bank	China	99.24	29.04	KF983135	KF983122
35.	*S. dahurica*	Gene bank	China/Mongolia/Russia	102.18	31.42	KF983136	KF983123
36.	*S. mollifolia*	Gene bank	China	92.12	29.42	KF983132	KF983113
37.	*S. purpurea*	Gene bank	Bhutan/China/India/Nepal/Sikkim	95.72	29.62	KF983142	KF983125
38.	*S. sublobata*	Gene bank	China	93.53	23.16	KF983134	KF983121
39.	*S. aquilegifolia*	Gene bank	China/Mongolia/Russia	92.21	29.48	KF983133	KF983114
	Outgroup taxa						
40.	*Sorbaria kirilowii*	Gene bank	China	103.27	33.4	KF983155	KF983231
41.	*Potentilla fruticosa*	Gene bank	Asia/Europe/North AMERICA	100.13	34.13	KF983151	KF983235
42.	*Rosa willmottiae*	Gene bank	China	97.21	32.53	KF983152	KF983236
43.	*Cerasus serrula*	Gene bank	China	100.49	32.19	KF983153	KF983232
44.	*Sorbus koehneana*	Gene bank	China	103.13	33.36	KF983154	KF983234

Table 2. Sequence characteristics and tree statistics for the three data sets. Models of sequence evolution were chosen by the Akaike information criterion using Modeltest3.7. nr, nuclear ribosomal; cp, chloroplast.

Characteristic	nr *ITS*	cp *trnL-F*	Combine Data
Sequence characteristics			
Aligned length including out group taxa	615	976	1592
Variable sites	263	202	482
Parsimony informative sites	177	89	273
Aligned length excluding out group taxa	607	870	1477
Variable sites	125	23	160
Parsimony informative sites	78	10	89
Model of sequence evolution	GTR+1+G	K81uf+G	GTR+I+G
Tree statistics			
Length	875	247	594
Consistency index (CI)	0.7360	0.9069	0.6734
Retention index (RI)	0.7735	0.9145	0.7605

Fig. 3. Maximum Parsimony (MP) and neighbor joining (NJ) Trees based on the ncDNA Internal transcribed spacer as implemented in PAUP* 4.0 (Swofford, 2003), with *Sorbaria kirilowii* and *Sorbus koehneana* as outgroup taxa. All the characters were equally weighted and treated as unordered. A heuristic search parameters were used with the random addition sequence (1000 replicates), tree-bisection-reconnection (TBR) branch swapping and the MULTREES options selected. The number above the branches indicates bootstraps support the first one for MP followed By NJ.

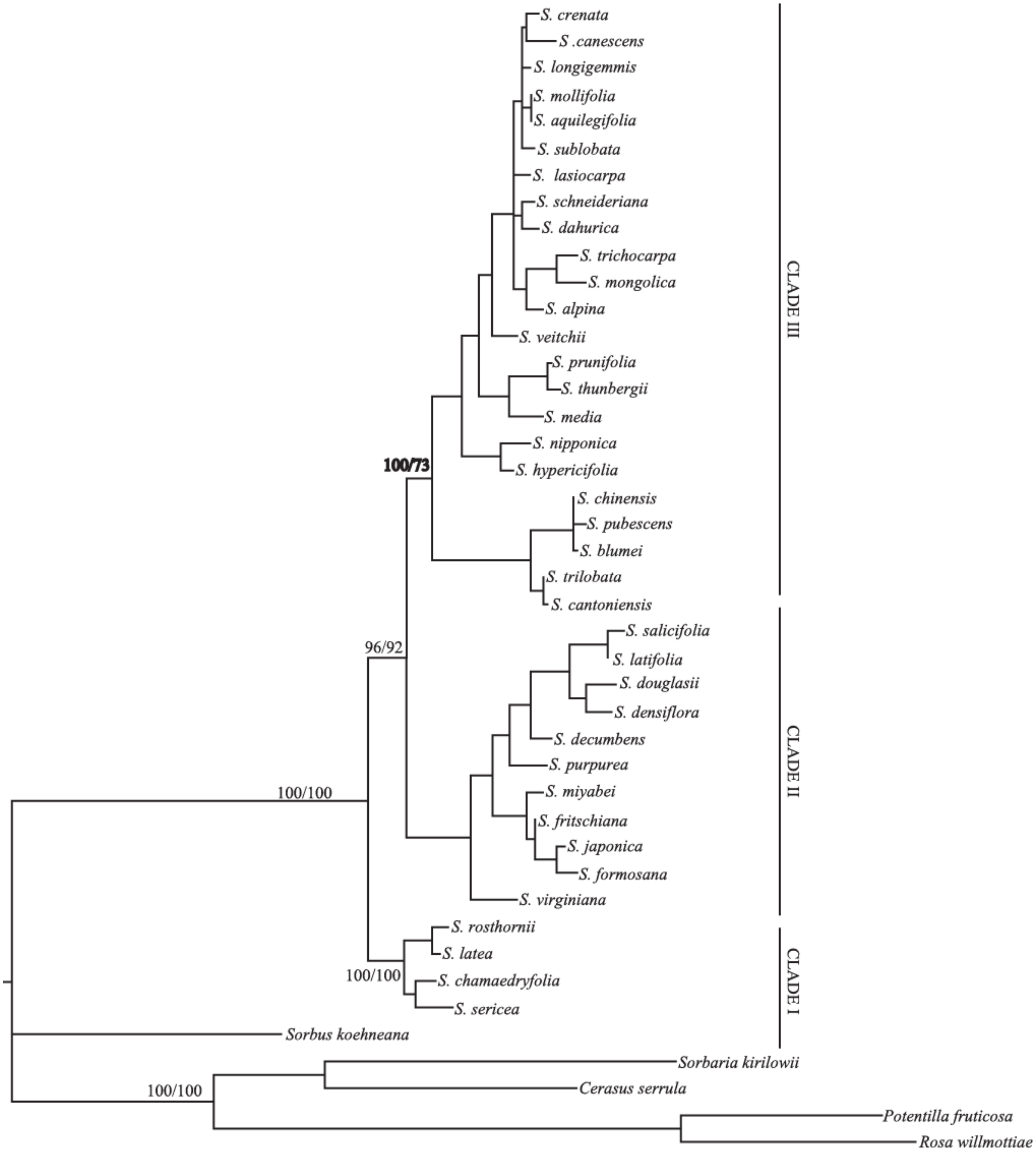

Fig. 4. Maximum Parsimony (MP) and neighbor joining (NJ) Trees based on concatenated cpDNA and nrDNA Internal transcribed spacer as implemented in PAUP* 4.0 (Swofford, 2003), with Sorbaria kirilowii and Sorbus koehneana as outgroup taxa. All the characters were equally weighted and treated as unordered. A heuristic search parameters were used with the random addition sequence (1000 replicates), tree-bisection-reconnection (TBR) branch swapping and the MULTREES options selected. The number above the branches indicates bootstraps support the first one for MP followed By NJ.

Estimation of divergence time: The tree obtained through the BEAST had the same topology with three clades (Fig. 5). The Markov Chain Monte Carlo runs yielded high effective sample sizes > 200 for all relevant parameters like branch lengths, topology and clade posteriors probabilities, indicated adequate sampling of posterior distribution. The divergence time revealed that the main lineage of *Spiraea* diversified 31.12 MY during the tertiary. The tree showed monophyly of all the species, no species or out group taxa showed paraphyly or polyphyly. *Spiraea virginiana* (Endemic to new world) is the oldest species 21.1 MY from the new world, while the youngest species are *S. chinensis* (Endemic to PR China) and *S. pubescens* (Distributed in PR China, Korea, Mongolia, and Russia).

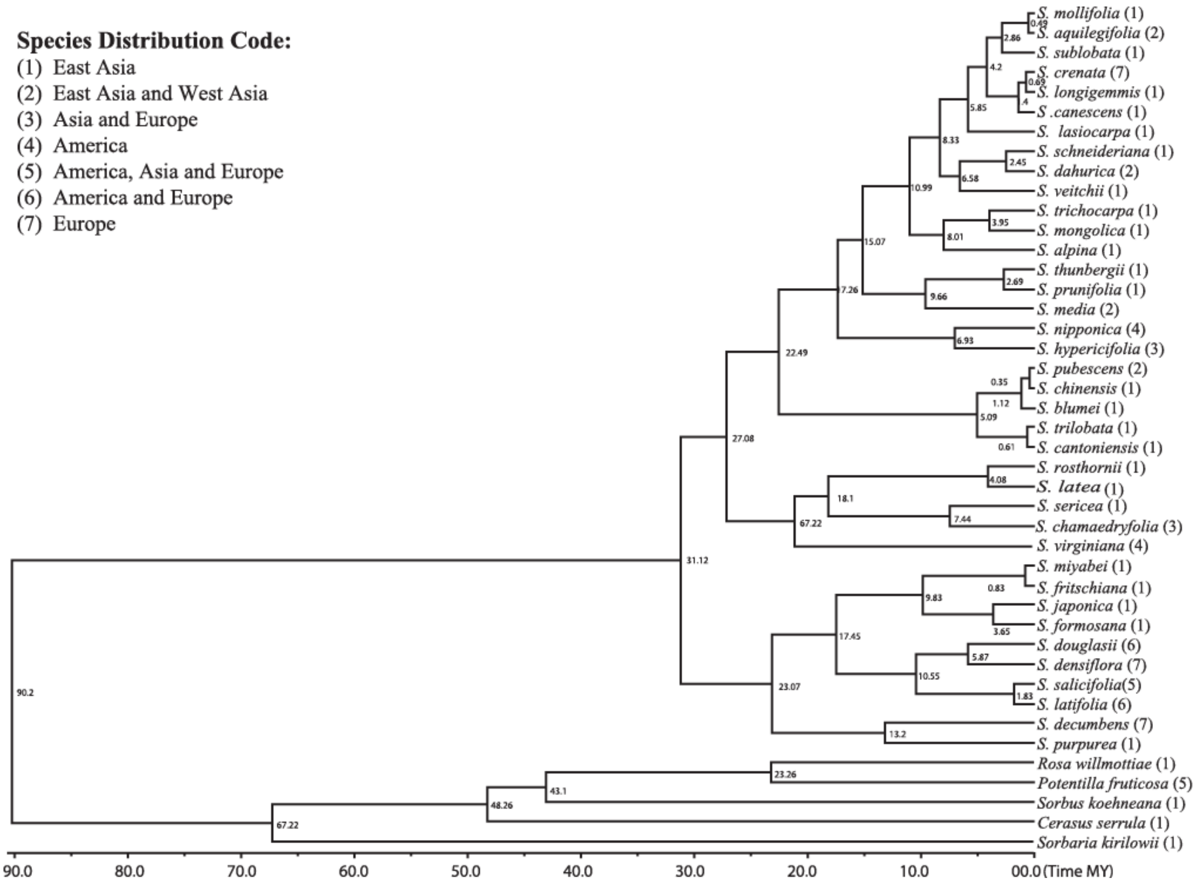

Fig. 5. Bayesian chronogram of genus *Spiraea* based on nuclear internal transcribed spacer, axis scale represents million years ago (Ma); numbers above the branches represent the divergence time (estimated with the substitution rate of 5.69 x 10^{-9} s s^{-1} y^{-1}.) with 95% highest posterior density.

Discussion

The present molecular phylogenetic investigation of spiroides shrubs revealed that all the 39 species of *Spiraea* found in the new and old worlds are monophyletic. The origin of their ancestors was most likely in the new world, while diversification of these spiroides is the East Asian platform. The biogeographic history of the genus involved dispersal and/or vicariant events between the Old and New Worlds proceeded in different direction. The present study is the most inclusive study of *Spiraea* including 39 species from both the old and new worlds. Phylogenetic analyses based on the three data matrices i.e., *trn*L-F (cpDNA), ITS (nrDNA) and concatenated data set (cpDNA and nrDNA) revealed monophyly of the genus. The concatenated data matrix and nrDNA given more clearer and resolved trees than the trees based on individual data matrix of cpDNA. Trees based on the concatenated data matrix divided 38 species into three clear clades. The clade I consisted of four species e.g. *S. chamaedryfolia*, *S. sericea*, *S. rosthornii* and *S. laeta*. The clade II grouped 10 species as *S. foramosana*, *S. japonica*, *S. fritschiana*, *S. miyabei*, *S. decumbens*, *S. densiflora*, *S. douglasii*, *S. virginiana*, *S. latifolia* and *S. salicifolia*. The remaining 24 species clustered in clade III. Our results were similar to Rehder (1940), Potter *et al.* (2007) and Khan *et al.* (2015) who suggested that the genus *Spiraea* is monophyletic. However the composition of each group or clade of *Spiraea* species in the current study was different from that of Rehder (1940) who studied their phylogeny based on morphology. The work of Potter *et al.* (2007) were limited to only 24 species. According to *Potter et al.* (2007) the inclusion of only two outgroups allowed a limited test of the hypotheses to say that the genus is monophyletic. In the current study we used five outgroups to overcome this limitation. Our results suggested that the 39 *Spiraea* species representing all of the three sections of Rehder's (1940) covering their full geographic range (i.e., from new world to Tibetan region in the old world) are monophyletic, because none of the other genera was nested within it. Both the morphological and molecular evidences are suggested to be carried out to give a clear hypothesis about the complete phylogenetics and evolutionary trends of this diverse and rich genus of tribe Spiraeeae (Rosaceae).

The common ancestor of Spiroides shrubs occurred in western North America, with independent migrations to the Old World occurring in *Aruncus*, *Sibiraea*, and *Spiraea* (Potter *et al.,* 2007), but the ancestral area for *Spiraea* could not be reconstructed explicitly. The estimated divergence time showed that the main lineages of *Spiraea* diversified

31.12 MY during the Oligocene. *Spiraea virginiana* (Endemic to new world) is the oldest species from the new world, while the youngest species are *S. chinensis* (Endemic to PR China) and *S. pubescens* (Distributed in PR China, Korea, Mongolia, Russia). The results showed that the ancestors of genus *Spiraea* had originated in the new world but the center of diversification of the genus is the East Asia. The results further showed that diversification of the genus was probably controlled by the quaternary climatic oscillation and orogenic processes. Most of the species showed divergence during the period in which the East Asian platform had been through severe uplifting of Qinghai-Tibetan Plateau (QTP) and eco-environmental process since the last 4 MY. The effects of QTP uplifting and severe climatic oscillation on the genus *Spiraea* species distributed in QTP has been reported (Khan *et al.,* 2014). The divergence time of *Spiraea* species with disjunct distribution in the new world, Europe and East Asia ranges e.g., *S. hypericifolia*, *S. chamaedryfolia*, *S. douglasii*, *S. salicifolia* and *S. latifolia* ranged from 7.44-1.83 MY with most within the last 5 MY (during Pliocene). These results unraveled that the isolation of disjunct species in eastern Asia and eastern North America occurred during the severe eco-climatic fluctuations period throughout the late Tertiary and Quaternary. The results of estimated time is closely correlated with paleontological evidence. The hypothesis which considers the eastern Asian–eastern North American floristic disjunction to be the result of the range restriction of a once more or less continuously distributed mixed mesophytic forest of the Northern Hemisphere that occurred during the late Tertiary and Quaternary is in accordance to our results. Similar results were obtained by Xiang *et al.* (2000). Potter *et al.* (2007) suggested that the complex biogeographically history of the genus *Spiraea*, involved multiple dispersal and/or vicariant events between the Old and New Worlds, and several independent migrations between Europe, western/central Asia, and eastern Asia, with the possibility that the different events may have proceeded in different directions.

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