Molecular Phylogenetics and Evolution 142 (2020) 106641

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Plastid phylogenomic insights into the evolution of the Caprifoliaceae *s.l.* (Dipsacales)

Hong-Xin Wang[a,1], Huan Liu[b,c,1], Michael J. Moore[d], Sven Landrein[e], Bing Liu[f,g], Zhi-Xin Zhu[a], Hua-Feng Wang[a,*]

[a] Key Laboratory of Tropical Biological Resources of Ministry of Education, School of Life and Pharmaceutical Sciences, Hainan University, Haikou 570228, China
[b] BGI-Shenzhen, Beishan Industrial Zone, Yantian District, Shenzhen 518083, China
[c] State Key Laboratory of Agricultural Genomics, BGI-Shenzhen, Shenzhen 518083, China
[d] Department of Biology, Oberlin College, Oberlin, OH 44074, USA
[e] Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, 666303, China
[f] State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Science, Beijing 100093, China
[g] Sino-African Joint Research Centre, Chinese Academy of Science, Wuhan 430074, China

ARTICLE INFO

Keywords:
Caprifoliaceae *s.l.*
Dipsacales
Plastome
Phylogenetics

ABSTRACT

The family Caprifoliaceae *s.l.* is an asterid angiosperm clade of ca. 960 species, most of which are distributed in temperate regions of the northern hemisphere. Recent studies show that the family comprises seven major clades: Linnaeoideae, *Zabelia*, Morinoideae, Dipsacoideae, Valerianoideae, Caprifolioideae, and Diervilloideae. However, its phylogeny at the subfamily or genus level remains controversial, and the backbone relationships among subfamilies are incompletely resolved. In this study, we utilized complete plastome sequencing to resolve the relationships among the subfamilies of the Caprifoliaceae *s.l.* and clarify several long-standing controversies. We generated and analyzed plastomes of 48 accessions of Caprifoliaceae *s.l.*, representing 44 species, six subfamilies and one genus. Combined with available Caprifoliaceae *s.l.* plastomes on GenBank and 12 outgroups, we analyzed a final dataset of 68 accessions. Genome structure was strongly conserved in general, although the boundaries between the Inverted Repeat were found to have contracted across Caprifoliaceae *s.l.* to exclude *rpl2, rps19,* and *ycf1*, all or parts of which are typically present in the IR of most angiosperms. The *ndhF* gene was found to have been inverted in all plastomes of Adoxaceae. Phylogenomic analyses of 68 complete plastomes yielded a highly supported topology that strongly supported the monophyly of *Zabelia* and its sister relationship to Morinoideae. Moreover, a clade of Valerianoideae + Dipsacoideae was recovered as sister to a clade of Linnaeoideae + *Zabelia* + Morinoideae clade, and *Heptacodium* was sister to remaining Caprifolioideae. The Diervilloideae and Caprifolioideae were successively sister to all other Caprifoliaceae *s.l.* Major lineages of Caprifoliaceae *s.l.* were estimated to have diverged from the Upper Cretaceous to the Eocene (50–100 Ma), whereas within-genus diversification was dated to the Oligocene and later, concomitant with global cooling and drying. Our results demonstrate the power of plastid phylogenomics in improving estimates of phylogeny among genera and subfamilies, and provide new insights into plastome evolution across Caprifoliaceae *s.l.*

1. Introduction

Complete plastome sequences have become a powerful tool for resolving plant phylogenies (e.g., Jansen et al., 2007; Moore et al., 2010; Yang et al., 2016; Lu et al., 2016; Niu et al., 2018; Pinard et al., 2019; Li et al., 2019) and have long been used in population-level analyses as well (e.g., Shaw et al., 2014). The plastome has some advantages over the nuclear genome, such as being haploid, having maternal inheritance, and possessing a more or less canonical structure in vascular plants with minimal gene duplication (Wu et al., 2010; Li et al., 2017; Bi et al., 2018). Specifically, the vast majority of plastomes in vascular plants have a quadripartite structure composed of two copies of a large inverted repeat (IR) separating the Large and Small Single-copy regions (LSC and SSC, respectively) (Saski et al., 2005; Zhu et al., 2016).

The family Caprifoliaceae *s.l.*, in the order Dipsacales, includes

* Corresponding author.
E-mail address: wanghuafeng2012@foxmail.com (H.-F. Wang).
[1] Authors contributed equally to this study.

https://doi.org/10.1016/j.ympev.2019.106641
Received 21 May 2019; Received in revised form 2 October 2019; Accepted 6 October 2019
Available online 09 October 2019

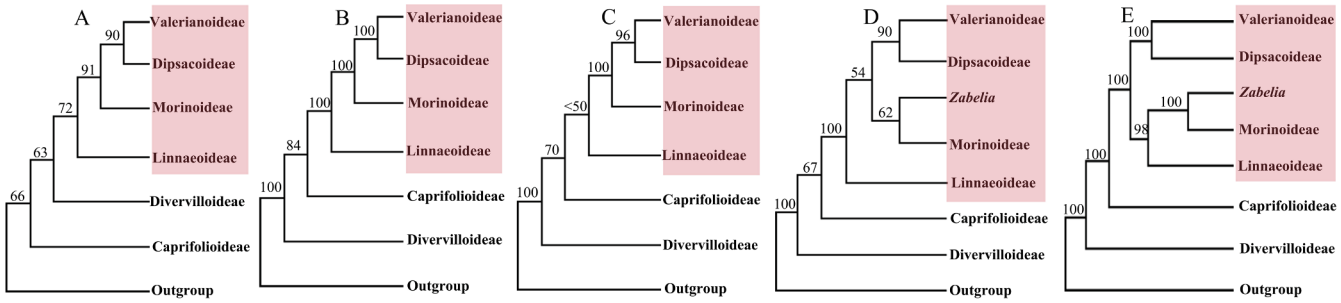

Fig. 1. Alternative relationships for the Caprifoliaceae *s.l.* backbone based on previous analyses. (A) Donoghue et al. (2001); parsimony analyses based on chloroplast *rbcL* sequences and morphological characteristics; (B) Bell et al. (2001); maximum likelihood tree from the combined chloroplast DNA data; (C) Zhang et al. (2003); maximum likelihood tree based on chloroplast *trnL-F* and *ndhF* sequences; (D) Jacobs et al. (2010); maximum parsimony Dipsacales phylogeny based on nuclear and chloroplast sequence data; (E) this study; maximum likelihood tree based on 68 complete plastomes.

about 960 species in 41 genera. The family has a nearly cosmopolitan distribution, with centers of diversity in eastern North America and eastern Asia, but is absent in tropical and southern Africa (Manchester and Donoghue, 1995; Bell, 2004). In recent phylogenetic analyses based on limited numbers of genes, all species in the family have been resolved into seven major clades: Diervilloideae, Caprifolioideae, Linnaeoideae, Morinoideae, *Zabelia*, Dipsacoideae and Valerianoideae (Donoghue et al., 1992; Jacobs et al., 2010; Smith et al., 2010; Landrein et al., 2012; Angiosperm Phylogeny Group (APG), 2016; Stevens, 2019). Although the major groups within Dipsacales are well understood, the relationships among them are less clear. For example, early studies of Dipsacales phylogeny based on limited chloroplast DNA data recovered Linnaeoideae as sister to a clade of Valerianoideae + Dipsacoideae + Morinoideae with weak support and found a sister relationship between Morinoideae and Valerianoideae + Dipsacoideae with lower bootstrap support (Fig. 1A, B, C) (Donoghue et al., 2001; Bell et al., 2001; Zhang et al., 2003). Using nuclear and chloroplast sequence data across Dipsacales, Jacobs et al. (2010) recovered Valerianoideae as sister to Dipsacoideae + Morinoideae with weak support, and Linnaeoideae as sister to Valerianoideae + Dipsacoideae (Fig. 1D). Based on nuclear ribosomal cistron data and more extensive plastid data, Tank and Donoghue (2010) recovered the same position for Linnaeoideae as Jacobs et al. (2010). Based on molecular and pollen data, Xu et al. (2011) recovered Linnaeoideae as sister to a clade of Valerianoideae + Dipsacoideae + Morinoideae. Hence, the relationships among the *Zabelia* + Morinaceae clade, Linnaeoideae, and the Dipsacoideae + Valerianoideae clade are still uncertain.

Likewise, the systematic positions of *Zabelia* and *Heptacodium* have not yet been fully resolved (Landrein et al., 2012; Bittrich and Kadereit, 2016). Originally, *Zabelia* was included in *Abelia* or in *Linnaea* (Rehder, 1911; Fukuoka, 1972; Xu, 1988). Subsequent studies supported the separation of *Abelia* and *Zabelia* based on morphological evidence (Erdtman, 1952; Ikuse and Kurosawa,1954; Fukuoka, 1968, 1969) and molecular evidence (Bell et al., 2001; Donoghue et al., 2001; Winkworth et al., 2008). Based on molecular data, Jacobs et al. (2010) raised sect. *Zabelia* to a genus, and more recent studies have confirmed the distinctiveness of *Zabelia* (Landrein et al., 2012; Wang et al., 2015), often finding it sister to Morinoideae, although with low to moderate support (Donoghue et al., 1992; Jacobs et al., 2010; Tank and Donoghue, 2010; Wang et al., 2015).

The position of *Heptacodium* has also been somewhat uncertain. Similarities in inflorescence morphology have suggested that *Heptacodium* may be related to Caprifolioideae (Fukuoka, 1972), although it has usually been placed in Linnaeoideae (Hara, 1983; Takhtajan, 1987; Xu, 1988). Based on *ndhF* sequence data, *Heptacodium* was recovered as sister to all members of Caprifolioideae with weak support (Pyck and Smets, 2000), a result confirmed in numerous later studies (Bell et al., 2001; Donoghue et al., 2001; Zhang et al., 2003; Tank and Donoghue, 2010).

Despite progress in understanding Dipsacales phylogeny, most advances have been based on relatively limited molecular and/or morphological data (e.g., Donoghue, 1983; Donoghue et al., 1992, 2001; Judd et al., 1994; Jacobs et al., 2010; Landrein et al., 2012; Wang et al., 2015). Only one study has examined Dipsacales phylogeny using plastome-scale data (Fan et al., 2018), but this study employed only 14 taxa and omitted many key lineages of Caprifoliaceae *s.l.* Here, we test the power of complete plastome sequences for resolving the broader phylogeny of Caprifoliaceae *s.l.* by generating and analyzing complete plastomes of 48 accessions across most key lineages of Caprifoliaceae *s.l.* Compared with phylogenetic studies limited to a few complete plastomes or a few plastid loci, plastome phylogenomic studies provide potentially much greater resolution and support (Burke et al., 2012). In addition, based on comparative genomic analyses, mutational hotspots can be identified within plastomes for use as informative regions for phylogenetics or DNA barcoding at lower taxonomic levels (Doorduin et al., 2011; Li et al., 2013, 2014). Our research objectives were as follows: (1) to reconstruct the phylogenetic relationships for the major lineages within Caprioliaceae *s.l.*; (2) to investigate global structural patterns of Caprifoliaceae *s.l.* complete plastomes; and (3) to explore plastome differentiation and divergence times in Dipsacales.

2. Materials and methods

2.1. Taxon sampling

Our sampling strategy involved maximizing the taxonomic and geographical coverage within Caprifoliaceae *s.l.* In total, 56 accessions representing 47 species of Caprifoliaceae *s.l.* were analyzed (Table 1), including representatives of all seven major clades of Caprifoliaceae *s.l.* as described by APG (2016) and Stevens (2019). We added 12 Caprifoliaceae *s.l.* plastomes from GenBank (Table 1), yielding a grand total of 23 Linnaeoideae accessions, eight *Zabelia* accessions, two Morinoideae accessions, five Valerianoideae accessions, five Dipsacoideae accessions, 11 Caprifolioideae accessions, and two Diervilloideae accessions. Also included were 12 outgroup taxa from Adoxaceae (Table 1) whose selection was based on previous genus-level, family-wide analyses (Jacobs et al., 2010; APG, 2016; Fan et al., 2018; Stevens, 2019). Samples were collected in the field (33 accessions), at botanical gardens (nine accessions), and from herbarium material (eight accessions); Table 1 provides locality and voucher information.

2.2. DNA extraction and sequencing

Total genomic DNA was extracted from dried leaf tissue using the cetyltrimethyl ammonium bromide (CTAB) protocol of Doyle and Doyle (1987). Genomic DNA from each sample was analyzed for quality and quantity using an Agilent BioAnalyzer 2100 (UCDAVIS Genome Center,

Table 1
Sampling information and the GenBank accession numbers of sequences.

Order	Subfamily	In/Out group	Taxon	Locality	Voucher specimen number	Accession number in GeneBank
1	Linnaeoideae	Ingroup	*Dipelta floribunda* Maximowicz Bull.	Cambridge, UK.	HUTB, SDM	MN524643
2	Linnaeoideae	Ingroup	*Dipelta floribunda* Maximowicz Bull.	Cambridge, UK.	HUTB, dy3	MN524642
3	Linnaeoideae	Ingroup	*Dipelta floribunda* Maximowicz Bull.	Xi'an, Shanxi, China	NA	NC_037955.1
4	Linnaeoideae	Ingroup	*Dipelta floribunda* Yi F. Wang & Y. S. Lian	Pingliang, Gansu, China	HUTB, dy2	MN524641
5	Linnaeoideae	Ingroup	*Dipelta yunnanensis* Franch	Nujiang, Yunnan, China	HUTB, dy1	MN524644
6	Linnaeoideae	Ingroup	*Diabelia serrata* (Siebold et Zucc.) Landrein	Tokushima, Japan	HUTB, e0310	MN524640
7	Linnaeoideae	Ingroup	*Diabelia ionostachya* var. *tetrasepala* (Siebold & Zucc.) Landrein	Pref, Japan	HUTB, t3209	MK033553
8	Linnaeoideae	Ingroup	*Diabelia sanguinea* (Siebold & Zucc.) Landrein	Miyagi, Japan	HUTB, p2102	MK033544
9	Linnaeoideae	Ingroup	*Diabelia spathulata* var. *spathulata* (H. Hara) Landrein	Shiga, Japan	HUTB, p2801	MK033548
10	Linnaeoideae	Ingroup	*Kolkwitzia amabilis* Graebn.	Weinan, Shanxi, China	NA	NC_029874.1
11	Linnaeoideae	Ingroup	*Kolkwitzia amabilis* Graebn.	Weinan, Shanxi, China	HUTB, ka1	MN524646
12	Linnaeoideae	Ingroup	*Kolkwitzia amabilis* Graebn.	Weinan, Shanxi, China	HUTB, ka2	MN524647
13	Linnaeoideae	Ingroup	*Abelia macrotera* (Graebn. et Buchw.) Rehd.	Nanchuan, Shanxi, China	HUTB, C215	MN524637
14	Linnaeoideae	Ingroup	*Abelia uniflora* R. Brown	Wuyishan, Fujian, China	HUTB, 339	MN524638
15	Linnaeoideae	Ingroup	*Abelia chinensis* R. Brown	Jujiang, Jiangxi, China	HUTB, JJ02	MN384463
16	Linnaeoideae	Ingroup	*Abelia* × *grandiflora* (André) Rehd.	Zhengzhou, Henan, China	HUTB, ag1	MN524635
17	Linnaeoideae	Ingroup	*Abelia forrestii* (Diels) W. W. Smith	Nujiang, Yunnan, China	HUTB, af	MN524636
18	Linnaeoideae	Ingroup	*Vesalea coriacea* (Hemsl.) T.Kim & B.Sun ex Landrein	San Luis Potosi, Mexico	HUTB, vc3	MN524620
19	Linnaeoideae	Ingroup	*Vesalea mexicana* Villarreal	San Luis Potosi, Mexico	HUTB, C184	MN524622
20	Linnaeoideae	Ingroup	*Vesalea floribunda* M.Martens & Galeotti	Oaxaca, Mexico	HUTB, C189	MN524621
21	Linnaeoideae	Ingroup	*Vesalea occidentalis* (Villarreal) Wang, H.F. & Landrein	Durango, Mexico	HUTB, V08	MN524623
22	Linnaeoideae	Ingroup	*Linnaea borealis* Linn.	Yili, Xinjiang, China	HUTB, Ib13	MN524649
23	Linnaeoideae	Ingroup	*Linnaea borealis* Linn.	Yili, Xinjiang, China	HUTB, Ib8	MN524648
24	*Zabelia*	Ingroup	*Zabelia coreana* (Nakai) Hisauti & H.Hara	Sinchon, Korea	HUTB, AB02	MN524629
25	*Zabelia*	Ingroup	*Zabelia biflora* Turcz.	Dushanbe, Tajikistan	HUTB, LDM	MN524627
26	*Zabelia*	Ingroup	*Zabelia integrifolia* Koidz	Kyushu, Japan	HUTB, zi	MN524632
27	*Zabelia*	Ingroup	*Zabelia dielsii* (Graebn.) Makino	Ganzi, Sichuan, China	HUTB, zd1	MN524631
28	*Zabelia*	Ingroup	Zabelia corymbosa (Regel & Schmalh.) Makino	Dushanbe, Tajikistan	HUTB, zc	MN524630
29	*Zabelia*	Ingroup	*Zabelia triflora* R.Br. ex Wall.	Bangalore, India	HUTB, zt	MN524633
30	*Zabelia*	Ingroup	*Zabelia buddleioides* W. W. Smith	Nujiang, Yunnan, China	HUTB, AB09	MN524628
31	*Zabelia*	Ingroup	Zabelia tyaihyoni (Nakai) Hisauti & H. Hara	Sinchon, Korea	HUTB, AB03	MN524634
32	Monrinoideae	Ingroup	*Morina longifolia* Wall. ex DC.	Dushanbe, Tajikistan	HUTB, m1	MN524607
33	Monrinoideae	Ingroup	*Acanthocalyx alba* (Hand.-Mazz.) M. Connon	Nujiang, Yunnan, China	HUTB, m01	MN524639
34	Valerianoideae	Ingroup	*Patrinia scabra* Bunge	Beijing, China	HUTB, B269	MN524610
35	Valerianoideae	Ingroup	*Patrinia heterophylla* Bunge	Beijing, China	HUTB, B268	MN524608
36	Valerianoideae	Ingroup	*Patrinia_scabiosifolia* Fisch. ex Trevir.	Yanqing, Beiijing, China	HUTB, C77	MN524609
37	Valerianoideae	Ingroup	*Patrinia saniculifolia* Hemsl.	Kangwon, Korea	NIBRVP0000642096	NC_036835.1
38	Valerianoideae	Ingroup	*Valeriana officinalis* Linn.	Baoding, Hebei, China	HUTB, B267	MN524619
39	Dipsacoideae	Ingroup	*Scabiosa tschiliensis* Grüning	Chang ping, Beijing, China	HUTB, C72	MN524616
40	Dipsacoideae	Ingroup	*Scabiosa tschiliensis* Grüning	Yanqing, Beijing, China	HUTB, C78	MN524617
41	Dipsacoideae	Ingroup	*Pterocephalus hookeri* (C.B. Clarke) Diels	Basu,Tibet, China	HUTB, C66	MN524611
42	Dipsacoideae	Ingroup	*Dipsacus japonicus* Miq.	Beijing, China	HUTB, B271	MN524645
43	Dipsacoideae	Ingroup	*Triplostegia glandulifera* Wall. ex DC.	Motuo,Tibet, China	HUTB, C64	MN524618
44	Caprifolioideae	Ingroup	*Lonicera stephanocarpa* Franch.	Xi'an, Shanxi, China	NA	NC_037954.1
45	Caprifolioideae	Ingroup	*Lonicera hispida* Pall. ex Schult.	Zhouzhi, Shanxi, China	HUTB, C71	MN524605
46	Caprifolioideae	Ingroup	*Lonicera fragrantissima* var. *lancifolia* (Rehder) Q.E. Yang, Landrein	Ankang, Shanxi, China	NA	MG738669.1
47	Caprifolioideae	Ingroup	*Lonicera elisae* Franch.	Miyun, Beijing, China	HUTB, C70	MN524603
48	Caprifolioideae	Ingroup	*Lonicera japonica* Trunb	Beijing, China	NA	NC_026839.1
49	Caprifolioideae	Ingroup	*Lonicera confusa* D.C.	Haikou, China	HUTB, B272	MN524602
50	Caprifolioideae	Ingroup	*Lonicera tatarinowii* Maxim.	Xinglong, Hebei, China	HUTB, C73	MN524606
51	Caprifolioideae	Ingroup	*Lonicera ferdinandii* Franch.	Haidian, Beijing, China	HUTB, C80	MN524604
52	Caprifolioideae	Ingroup	*Lonicera calcarata* Hemsl.	Wenshan, Yunnan, China	HUTB, C60	MN524650
53	Caprifolioideae	Ingroup	*Triosteum pinnatifidum* Maxim.	Baoji, Shanxi, China	NA	NC_037952.1
54	Caprifolioideae	Ingroup	*Heptacodium miconioides* Rehder	Hangzhou,Zhejiang, China	HUTB, B158	MH712480
55	Divervilloideae	Ingroup	*Weigela florida* (Bunge) A. DC.	Wendeng, Shandong, China	HUTB, C75	MN524626
56	Divervilloideae	Ingroup	*Weigela florida* (Bunge) A. DC.	Kangwon, Korea	NIBRVP0000642096	NC_037950.1
57	Adoxoideae	Outgroup	*Sambucus williamsii* Hance.	Haidian, Beijing, China	HUTB, C91	MN524615
58	Adoxoideae	Outgroup	*Sambucus williamsii* Hance.	Haidian, Beijing, China	HUTB, C90	MN524614
59	Adoxoideae	Outgroup	*Sambucus williamsii* Hance.	Heyuan, Guangdong, China	NA	NC_033878.1
60	Adoxoideae	Outgroup	*Sambucus nigra* Linn.	Beijing, China	HUTB, sn1	MN524612
61	Adoxoideae	Outgroup	*Sambucus nigra* Linn.	Beijing, China	HUTB, sn2	MN524613
62	Adoxoideae	Outgroup	*Tetradoxa omeiensis* (H. Hara) C.Y. Wu	NA	NA	NC_034793.1
63	Adoxoideae	Outgroup	*Adoxa moschatellina* linn.	NA	NA	NC_034792.1
64	Adoxoideae	Outgroup	*Sinadoxa corydalifolia* C.Y. Wu, Z.l. Wu & R.F. Huang	Yushu, Qinghai, China	NA	NC_032040.1
65	Opuloideae	Outgroup	*Viburnum betulifolium* Batalin	Xi'an, Shanxi, China	NA	NC_037951.1
66	Opuloideae	Outgroup	***V****iburnum fordiae* Hancew	Wuyishan, Fujian, China	HUTB, B156	MN524625
67	Opuloideae	Outgroup	*Viburnum brachybotryum* Hemsl.	Ganzhou, Jiangxi, China	HUTB, B160	MN524624
68	Opuloideae	Outgroup	*Viburnum utile* Hemsley J. Linn.	Nanyang, Henan, China	NA	NC_032296.1

Davis, California, USA). Approximately 0.8 μg of DNA was sheared and used to prepare paired-end libraries with 200–400 bp insert size. Samples were sequenced using the BGISEQ-500 platform at BGI Shenzhen (China), yielding about 8 Gb high quality per sample with 100 bp paired-end reads. We trimmed raw reads with SOAPfilter_v2.2 (BGI-Shenzhen, China) using the following criteria: first, reads with > 10% N's; second, reads with > 40% low quality bases (quality score < 10); third, reads contaminated by adaptor sequence and produced by PCR duplication.

2.3. Plastome assembly, annotation, and structural analyses

About 6 Gb of clean data were assembled against the plastomes of *Lonicera japonica* (NC_026839), *Kolkwitzia amabilis* (NC_029874), and *Viburnum utile* (NC_032296) using MITO bim v1.8 (Hahn et al. 2013). All contigs were

aligned to the reference plastomes [*Lonicera japonica* (NC_026839), *Kolkwitzia amabilis* (NC_029874), and *Viburnum utile* (NC_032296)] using BLAST (Li et al., 2017) as implemented in Geneious R11.0.5 (Biomatters Ltd., Auckland, New Zealand). To verify sequencing depth and contig overlap, cleaned reads were mapped to reference plastomes in Geneious R11.0.5. Newly sequenced plastomes were annotated in a variety of ways, including using DOGMA (Dual Organellar GenoMe Annotator; Wyman et al., 2004), with corrections for start/stop codons based on published Caprifoliaceae plastomes. Intron/exon boundaries were further determined using alignments in MAFFT v7 (Katoh and Standley, 2013) against the plastomes of. *Lonicera japonica* (NC_026839) and *Kolkwitzia amabilis* (NC_029874). In addition, tRNAscan-SE1.21 was used to verify all of the tRNA genes (Peter et al., 2005). IR boundaries were confirmed with Unipro UGENE v1.32 (Rose et al., 2018). The annotated plastomes sequences were deposited in GenBank with accession numbers (MN524602-MN524650, MN384463) (Table 1). Genome maps were drawn using OGDRAW (Lohse et al., 2013), with subsequent manual editing. Sequences were aligned using PROGRESSIVEMAUVE v2.4.0 to compare the structure and gene contents among the plastomes (Darling et al., 2010).

2.4. Phylogenetic analysis

Sequences were aligned using MAFFT v7 (Katoh and Standley, 2013) under default parameters and regions with > 80% missing data were excluded from phylogenetic analyses. Unpartitioned, aligned plastid data were analyzed using maximum likelihood (ML) and Bayesian inference (BI) approaches. After selecting the best-fitting model of nucleotide substitution for the entire dataset (GTR + I + G), as determined by the Akaike Information Criterion (AIC) in jModelTest v2.1.7 (Santorum, et al., 2014), the ML and BI analyses were conducted in RAxML-HPC v8.2.20 with GTR + I + G (Stamatakis, 2014; with 1000 bootstrap replicates) and MrBayes Version v.3.2.7a (Ronquist et al, 2012), respectively. For Bayesian inference (burnin = 1,000), two independent chains were run with a random starting tree and default priors for 400,000,000 generations, with trees sampled every 1, 000 generations. The first 25% of calculated trees were discarded as burn-in, and a consensus tree was constructed from the remaining trees to estimate posterior probabilities. Convergence of the MCMC chains was assumed when the average standard deviation of split frequencies reached 0.01 or less. Adoxaceae was designated as the outgroup for rooting based on the APG (2016) classification system. ML analyses were also conducted using the following seven data partitions: (1) complete plastomes; (2) coding regions; (3) non-coding regions; (4) LSC region; (5) IR region; and (6) SSC region; (7) complete plastomes minus one copy of the IR region. All analyses were performed on the CIPRES Science Gateway website (Miller et al., 2010). FigTree v.1.3.1 (Drummond et al., 2012) was used to visualize the resulting phylogenetic trees.

2.5. Genome comparative analysis and molecular marker identification

Plastome comparisons across the 44 Caprifoliaceae *s.l.* species (employing only one accession per species) were performed in Shuffle-LAGAN mode in mVISTA (Frazer et al., 2004) using the plastome of *Vesalea floribunda* as a reference. To explore variability among all protein-coding and noncoding (intergenic spacer and intron) regions for future population genetic and species identification studies, nucleotide diversity (P_i) was evaluated with DnaSP v.5.10 (Librado and Rozas, 2009).

2.6. Divergence time estimation

Bayesian searches for tree topologies and node ages were conducted on the complete data set in BEAST using a GTR + G substitution model selected by MrModelTest (Posada, 2008) and an uncorrelated lognormal relaxed clock (Drummond et al., 2012). A Yule process was specified as tree prior. Three calibration points were used to constrain each node. First, a fruit fossil of the genus *Diplodipelta* from the late Eocene Florissant flora of Colorado (36 Ma; Manchester and Donoghue, 1995) was considered to be the oldest possible date for the age of *Diabelia*. Hence we set the stem of *Dipelta* with a lognormal mean = 0, SD = 1.0 and an offset = 36 Ma. Second, Bell and Donoghue (2005) suggested that the Dipsacales node to be 102–110 Ma, which originated by the mid-Cretaceous. In this study, the Dipsacales node (the root of our tree) was constrained to 103 Ma, with a normal prior, a mean = 103 Ma, and a SD = 5, which is based on a secondary calibration of the Dipsacales node to be 102–110 Ma (Bell and Donoghue 2005). Third, the stem of *Viburnum* was set to a lognormal mean = 0, a SD = 1.0 and an offset = 89.3 Ma based on a leaf fossil of *Viburnum* from the Upper Cretaceous in North America (Bell, 1957). The analyses were run for 900,000,000 generations and the parameters were sampled every 1,000 generations. The effective sample size (> 200) was determined using Tracer v 1.6 (Drummond et al., 2012) and the first 25% of the samples were discarded as burn-in. TreeAnnotator v.1.8.0 (Drummond et al., 2012) was used to summarize the set of post-burn-in trees and their parameters in order to produce a maximum clade credibility chronogram showing the mean divergence time estimates with 95% highest posterior density (HPD) intervals. FigTree v1.3.1 (Drummond et al., 2012) was used to visualize the resulting divergence times.

3. Results

3.1. Plastome features and gene content

The plastomes of Caprifoliaceae *s.l.* species differed little in sequence length, ranging in size from 151,267 bp (*Patrinia scabra*) to 158,313 bp (*Scabiosa tschiliensis*) (Table 2). All plastomes displayed the typical quadripartite structure of nearly all land plants, consisting of a pair of IRs (22,064–26,410 bp) separated by the LSC regions (86,715–90,956 bp) and SSC regions (16,288–22,622 bp). The GC content among these 44 plastomes was very similar (37.7–39.0%) (Table 2). The 44 plastomes encoded 128 genes, including 83 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Within the IR, eight tRNA genes, seven protein-coding genes, and all four rRNA genes were completely duplicated. Among all genes, 14 genes harbored a single intron (*trnK-UUU*, *trnG-GCC*, *trnI-GAU*, *trnL-UAA*, *trnV-UAC*, *trnI-GAU*, *atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpoC1*, *rpl2* and *rps16*) and two genes (*ycf3* and *rps12*) harbored two introns. The *ndhF* gene was found to be inverted in all Adoxaceae (Fig. 3B).

3.2. Boundaries between the IR and SC regions

Expansion and contraction at the borders of IR regions was the main reason for size variation among Caprifoliaceae *s.l.* plastomes (Fig. 2).

Table 2
Summary of major characteristics of Dipsacales plastomes, including aspects of genome size, G-C content, and gene number (per type and location).

Species	Genome size (bp)	LSC Length (bp)	SSC Length (bp)	IR Length (bp)	Total GC %	LSC GC %	SSC GC %	IR GC %	Protein Coding	tRNA LSC	rRNA IR_A
Dipelta florifbunda	157,206	90,426	18,984	23,898	38.4	36.5	33.1	44.1	82[8]	30[7]	4[4]
D. yunnanensis	156,972	90,184	18,966	23,911	38.4	36.5	33.1	44.2	82[8]	30[7]	4[4]
Diabelia serrata	156,755	89,910	18,857	24,051	38.5	36.6	33.1	44.2	82[8]	30[7]	4[4]
Ionostachya var. tetrasepala	156,635	89,947	18,820	23,934	38.5	36.6	33.2	44.1	82[8]	30[7]	4[4]
D. sanguinea	156,842	90,912	18,894	23,923	38.5	36.6	33.2	44.2	82[8]	30[7]	4[4]
D. spathulata var. Spathulata	156,810	90,051	18,863	23,948	38.4	36.5	33.0	44.2	82[8]	30[7]	4[4]
Kolkwitzia amabilis	157,389	90,570	19,057	23,881	38.4	36.4	33.2	44.2	82[8]	30[7]	4[4]
Abelia macrotera	157,186	90,344	18,999	23,921	38.4	36.5	33.1	44.2	82[8]	30[7]	4[4]
A. uniflora	157,038	90,219	18,998	23,910	38.4	36.5	33.1	44.1	82[8]	30[7]	4[4]
A. chinensis	157,310	90,464	19,043	23,902	38.4	36.4	33.0	44.1	82[8]	30[7]	4[4]
A. × grandiflora	157,107	90,360	18,993	23,877	38.4	36.5	33.0	44.2	82[8]	30[7]	4[4]
A. forrestii	157,263	90,463	18,996	23,902	38.4	36.5	33.1	44.1	82[8]	30[7]	4[4]
Vesalea floribunda	157,330	90,345	19,035	23,975	38.4	36.5	33.2	44.1	82[8]	30[7]	4[4]
V. mexicana	157,448	90,424	19,056	23,984	38.4	36.5	33.1	44.1	82[8]	30[7]	4[4]
V. coriacea	157,736	90,638	19,122	23,988	38.4	36.5	33.2	44.1	82[8]	30[7]	4[4]
V. occidentalis	157,399	90,377	19,074	23,974	38.4	36.5	33.1	44.2	82[8]	30[7]	4[4]
Linnaea borealis	157,271	90,402	19,017	23,926	38.5	36.5	33.3	44.2	82[8]	30[7]	4[4]
Zabelia dielsii	157,201	90,449	19,066	23,843	38.4	36.5	33.1	44.2	82[8]	30[7]	4[4]
Z. integrifolia	157,551	90,533	19,014	24,002	38.4	36.5	32.9	44.1	82[8]	30[7]	4[4]
Z. coreana	156,714	90,083	18,807	23,912	38.4	36.6	33.1	44.1	82[8]	30[7]	4[4]
Z. buddleioides	157,823	90,938	19,181	23,852	38.4	36.5	33.4	44.1	82[8]	30[7]	4[4]
Z. triflora	156,827	90,173	18,982	23,836	38.4	36.6	33.0	44.1	82[8]	30[7]	4[4]
Z. tyaihyoni	152,441	87,551	18,634	23,128	38.4	36.5	32.9	44.1	82[8]	30[7]	4[4]
Z. biflora	157,136	86,715	17,734	26,410	38.4	36.5	32.6	43.3	82[8]	30[7]	4[4]
Z. corymbosa	157,325	90,265	18,874	24,093	38.5	36.6	33.2	44.0	82[8]	30[7]	4[4]
Morina longifolia	157,149	90,292	18,679	24,089	38.6	36.7	33.4	44.1	82[8]	30[7]	4[4]
Acanthocalyx alba	157,999	90,750	19,413	23,918	38.4	36.5	33.3	44.0	82[8]	30[7]	4[4]
Patrinia scabra	151,267	87,269	18,020	22,989	38.6	36.8	33.2	44.2	82[8]	30[7]	4[4]
P.scablosifolisa	154,019	89,132	17,297	23,795	38.5	36.6	33.6	43.8	82[8]	30[7]	4[4]
P. heterophylla	151,964	87,380	18,506	23,039	38.6	36.8	33.3	44.3	82[8]	30[7]	4[4]
Valeriana officinalis	151,505	87,619	16,288	23,799	38.4	36.5	32.5	44.0	82[8]	30[7]	4[4]
Dipsacus japonicus	154,709	88,605	18,538	23,783	39.0	37.2	33.6	44.4	82[8]	30[7]	4[4]
Scabiosa tschiliensis	158,313	90,843	21,022	23,224	37.7	36.5	28.7	44.1	82[8]	30[7]	4[4]
Pterocepalus hookeri	158,012	90,794	22,622	22,064	37.7	36.4	29.8	44.3	82[8]	30[7]	4[4]
Triplostegia glandulifera	157,560	90,239	21,099	23,111	37.9	36.5	30.7	44.1	82[8]	30[7]	4[4]
Lonicera confusa	155,346	89,122	18,634	23,795	38.6	37.1	33.5	43.4	82[8]	30[7]	4[4]
L. elisae	156,550	90,466	18,830	23,627	38.2	36.7	32.9	43.4	82[8]	30[7]	4[4]
L. tatarinowii	155,781	89,458	18,637	23,843	38.3	36.8	33.1	43.1	82[8]	30[7]	4[4]
L. hispida	155,857	89,353	18,705	23,815	38.3	36.7	33.1	43.4	82[8]	30[7]	4[4]
L. ferdinandii	155,904	89,532	18,930	23,721	38.4	36.8	33.0	43.4	82[8]	30[7]	4[4]
L. calcarata	158,010	90,956	21,823	22,616	37.9	36.3	33.2	43.5	82[8]	30[7]	4[4]
Heptacodium miconioides	156,313	89,760	18,745	23,904	38.4	36.6	32.9	43.8	82[8]	30[7]	4[4]
Sambucus williamsii	158,375	86,889	18,948	26,269	37.9	36.3	31.7	43.0	84[8]	30[7]	4[4]
Sambucus nigra	158,321	87,075	18,274	26,261	38.0	36.3	31.8	43.0	84[8]	30[7]	4[4]
Viburnum fordiae	157,627	86,544	18,773	26,155	38.1	36.5	32.0	43.1	84[8]	30[7]	4[4]
Viburnum brachbotryum	157,433	86,552	18,615	26,133	38.1	36.4	32.0	43.1	84[8]	30[7]	4[4]

Abbreviations: CDS, protein-coding sequences/genes; LSC, large single-copy region; SSC, small single-copy region; IR, inverted repeat (A or B) regions.
Numbers in brackets mean the number of duplicated genes, e.g. 80[8] means there were 80 unique genes in the plastome, of which 8 were duplicated in the IRs.

Within the Adoxaceae outgroups, all plastomes shared similar IR/SC boundaries within the *rps19* and *ycf1* genes, similar to most other angiosperms (Fig. 2). However, within most Caprifoliaceae *s.l.*, the IR/LSC junction was found to reside within the *rpl23* gene (Fig. 2). In several cases, however, the IR was found to have contracted to exclude *rpl23* entirely, in *Weigela, Scabiosa, Pterocephalus*, *Triplostegia*, and *Lonicera hispida*. In *Patrinia scabiosifolia*, the IR was found to have expanded to include *trnH-GUG* (Fig. 2). Likewise, the IRa/SSC junction was also found to have contracted in Caprifoliaceae *s.l.*, to exclude *ycf1*. The IRb/SSC junction was located in the *ndhF* gene in *Lonicera confusa* (Fig. 2).

3.3. Phylogenetic relationships

ML and BI analyses of 68 Dipsacales plastomes yielded identical tree topologies except for the position of some species of *Lonicera*, which swapped positions between ML and BI trees (Fig. 4). Support was generally very high for most relationships within Caprifoliaceae *s.l.* except for some branches within *Dipelta, Diabelia and Vesalea* (Fig. 4). Within Dipsacales, two major lineages were clearly defined: Adoxaceae and Caprifoliaceae *s.l.* with maximal support (Fig. 4). Caprifoliaceae *s.l.* was resolved into seven major clades with a highly supported topology of (Diervilloideae, (Caprifolioideae, ((Dipsacoideae, Valerianoideae), (Linnaeoideae, (Morinoideae, *Zabelia*))))). and, each with maximal support (Fig. 4). *Heptacodium* was sister to remaining Caprifolioideae with maximal support (Fig. 4). Within Linnaeoideae, all genera (*Dipelta*, *Diabelia*, *Kolkwitzia*, *Abelia*, *Vesalea* and *Linnaea*) were recovered as monophyletic with strong support (Fig. 4). Relationships among these genera were fully resolved with maximal support (Fig. 4).

3.4. Sequence divergence analysis

The mVISTA plot is provided in Fig. 5, and percent variation is provided in Supplementary Table S1. Mean percent sequence variation was 2.80% among the 56 Caprifoliaceae *s.l.* plastomes. Percent variation in coding regions (mean = 1.71%) was lower than that in non-coding regions (mean = 3.89%). Among coding regions, the five genes with the highest nucleotide diversity (P_i) values were *rpl*22, *psbJ*, *rps15*,

Fig. 2. Comparison of the IR/SC junctions among 47 Dipsacales plastomes.

rpl33 and *rpl32*. all of which had values > 4%. The most variable noncoding regions (all with P_i > 7%) were found to be the *rpl2* intron, the *trnV-UAC-trnM-CAU* spacer region, *psbJ-psbL* spacer region, the *ycf3* intron, the *infA-rps8* spacer region, the *rrn5-trnR-ACG* spacer region, the *trnE-UUC-trnT-GGU* spacer region, the *psbI-trnS-GCU* spacer region, the *ycf3-trnS-GGA* spacer region, and the *rpl32-trnL-UAG* spacer region.

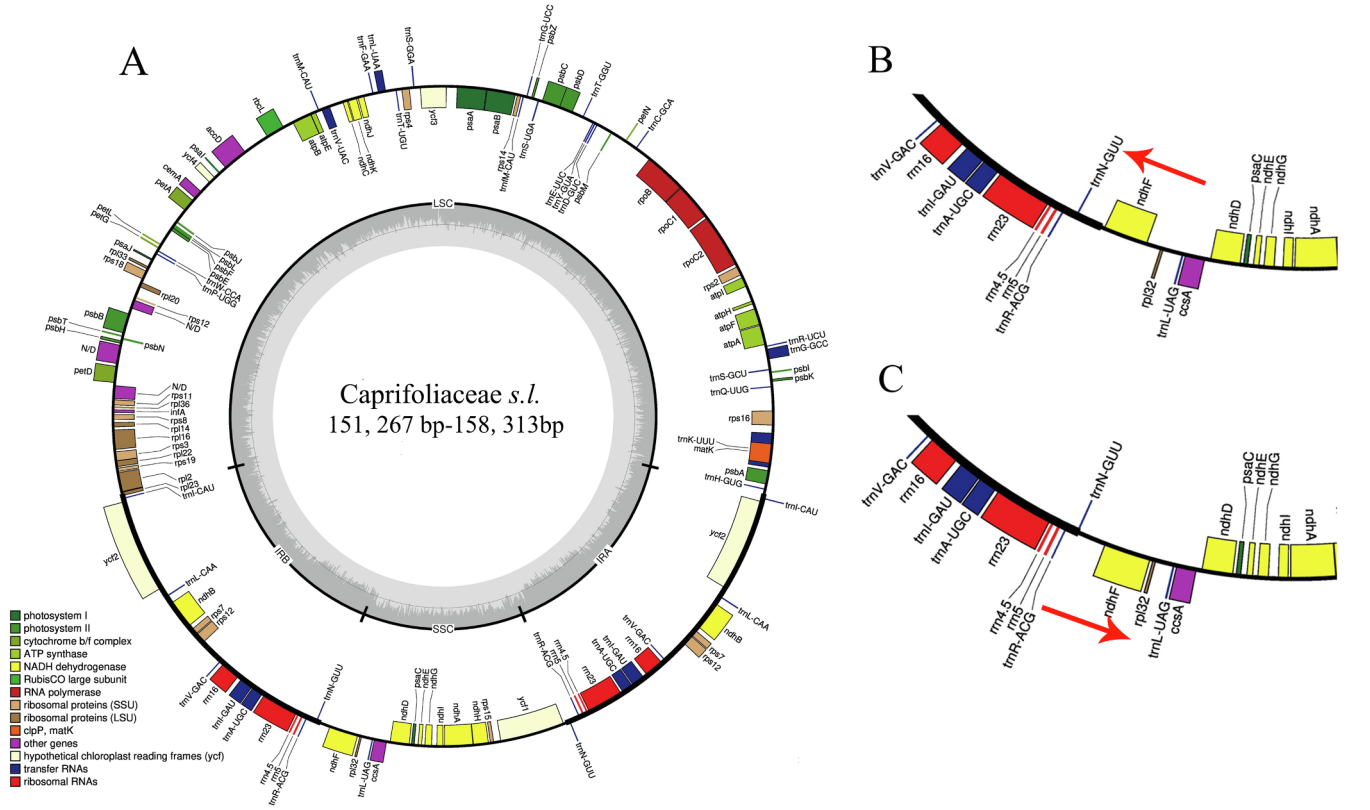

Fig. 3. Generalized maps of Dipsacales complete plastomes. (A) Plastome map of Caprifoliaceae *s.l.*; (B) Inset map of the plastome of *Sambucus nigra,* showing the inverted orientation of *ndhF* in the outgroup; (C) Inset map of the plastome of *Kolkwitzia amabilis*, showing typical orientation of *ndhF.* Genes inside and outside the outer circle are transcribed clockwise and counterclockwise, respectively.

3.5. Divergence times of major lineages

The results of the BEAST analyses are provided in Fig. 6. The earliest split in crown group Caprifoliaceae *s.l.* was dated to 100.49 Ma (95% HPD = 67.37–119.49 Ma), with the following split dated to 93.91 Ma (95% HPD = 76.21–115.56 Ma). The divergence of remaining Caprifoliaceae *s.l.* was dated to 78.88 Ma (95% HPD = 60.03–99.17 Ma). The divergence between Dipsacoideae and Valerianoideae was dated to 70.19 Ma (95% HPD = 51.23–92.43 Ma). The earliest divergence of crown group Linnaeoideae was dated to 52.19 Ma (95% HPD = 40.61–65.26 Ma), and that of crown group *Zabelia* to 48.15 Ma (95% HPD = 24.41–80.61 Ma).

4. Discussion

4.1. Plastome structural evolution

Although the overall gene content and arrangement within the 44 Caprifoliaceae *s.l.* plastomes is highly similar (Table 2), the positions of the IR boundaries vary within *Caprifoliaceae* s.l. and between the ingroup and outgroup (Fig. 2). Variation at the IR boundaries is well known, and often contributes significantly to overall length variation among angiosperm plastomes (e.g., Downie and Jansen, 2015; Yang et al., 2016; Xu et al., 2017; Yan et al., 2018). Given the near uniformity of IR boundaries in Caprifoliaceae *s.l.,* it is clear that these shifts must have occurred in the ancestor of the clade.

Another notable finding of our work is the inversion of *ndhF* in Adoxaceae. Numerous studies have documented plastome inversions in many angiosperm lineages, including Asteraceae (Kim et al., 2005; Walker et al., 2014), Fabaceae (Martin et al., 2014; Schwarz et al., 2015), and Styracaceae (Yan et al., 2018). Because of their relative rarity, easily determined homology, and easily inferred state polarity, plastome inversions are considered highly valuable in phylogenetics (Cosner et al., 1997; Dugas et al., 2015; Schwarz et al., 2015). The cause of inversions is not fully known, but explanations have generally focused on intramolecular recombination between dispersed short inverted/direct repeats and tRNA genes (Cosner et al., 1997; Haberle et al., 2008; Sloan et al., 2014). Given the position of *ndhF* near the IRb/SSC boundary, it is also possible that this inversion is due to an expansion of the IR to include *ndhF*, followed by a contraction of the boundary to exclude it. This would leave *ndhF* in an inverted state. However, it is not possible to sort between this possibility and a simple inversion of the gene.

4.2. Phylogenetic relationships

Our study presents highly resolved phylogenies of Caprifoliaceae *s.l.* based on a comprehensive plastome sampling of major lineages, including species representing all major clades of Caprifoliaceae *s.l.* and representative outgroups. Compared to prior studies based on small numbers of genes (Pyck, 2001; Zhang et al., 2003; Landrein et al., 2010, 2012; Wang et al., 2015; Niu et al., 2018), we focused on the usefulness of complete plastomes to resolve phylogenetic relationships in Caprifoliaceae *s.l.*, exploring more of the plastome to obtain additional informative sites and regions.

4.2.1. Backbone relationships of Caprifoliacee s.l.

Our analyses strongly support the seven major clades found in previous analyses of the family based on fewer genes (Jacobs et al., 2010; APG, 2016): Linnaeoideae, *Zabelia*, Morinoideae, Valerianoideae, Dipsacoideae Caprifolioideae, and Diervilloideae (Fig. 4). Importantly, our phylogenomic analyses provide much stronger support for the relationships among these clades than has been recovered in previous work. This is particularly true within regions of shorter branches, as in

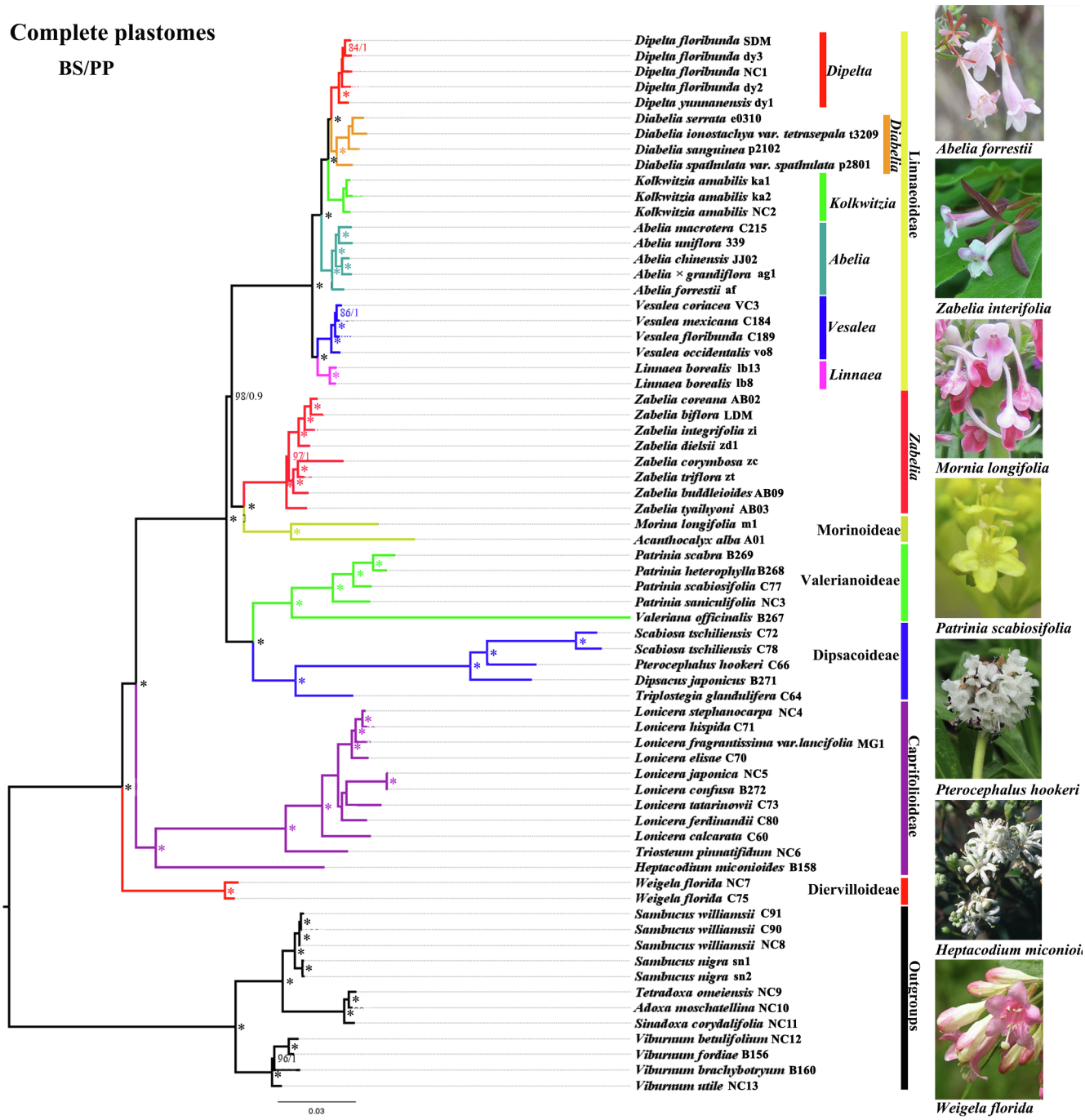

Fig. 4. Phylogenetic relationships of Dipsacales inferred from maximum likelihood (ML) and Bayesian inference (BI) based on 68 complete plastome sequences. Representative images of seven major backbone clades of Caprifoliaceae *s.l.* on the right, respectively. Support values above the branches are maximum likelihood bootstrap support/Bayesian posterior probability; " * " indicates 100%/1.0 support values. Major clades/genera of Caprifoliaceae *s.l.* are indicated by different colors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the earliest diversification of Linnaeoideae, *Zabelia,* Morinoideae, Valerianoideae, and Dipsacoideae (Fig. 4). For example, using *rbcL*, Donoghue et al. (2001) recovered Linnaeoideae as sister to (Valerianoideae + Dipsacoideae) + Morinoideae with weak support (Fig. 1A). Later, using expanded chloroplast gene sampling, Zhang et al. (2003) also recovered weak support for these relationships (Fig. 1C). Based on nuclear and chloroplast sequence data, Jacobs et al. (2012) also found Linnaeoideae as sister to the (Valerianoideae + Dipsacoideae) + (Morinoideae + *Zabelia*) clade, but again with weak support (Fig. 1D). It should not be surprising that our analyses recovered much stronger support, given that our phylogenomic analyses included far greater amounts of data, and employed noncoding regions as well as coding regions.

4.2.2. Relationships within the Linnaeoideae

Our analyses provide excellent support for the monophyly of each of the six genera of Linnaeoideae included in our analyses (*Abelia, Dipelta, Diabelia, Kolkwitzia, Vesalea* and *Linnaea*), as well as their relationships (Fig. 4). Our study confirms that *Abelia*, *Diabelia* and *Vesalea*, all formerly treated as part of *Abelia* based on morphological similarities (Kim et al., 1999; Jacobs et al., 2010), are best treated as distinct genera, as advocated by Landrein et al. (2010) and Wang et al. (2015). Moreover,

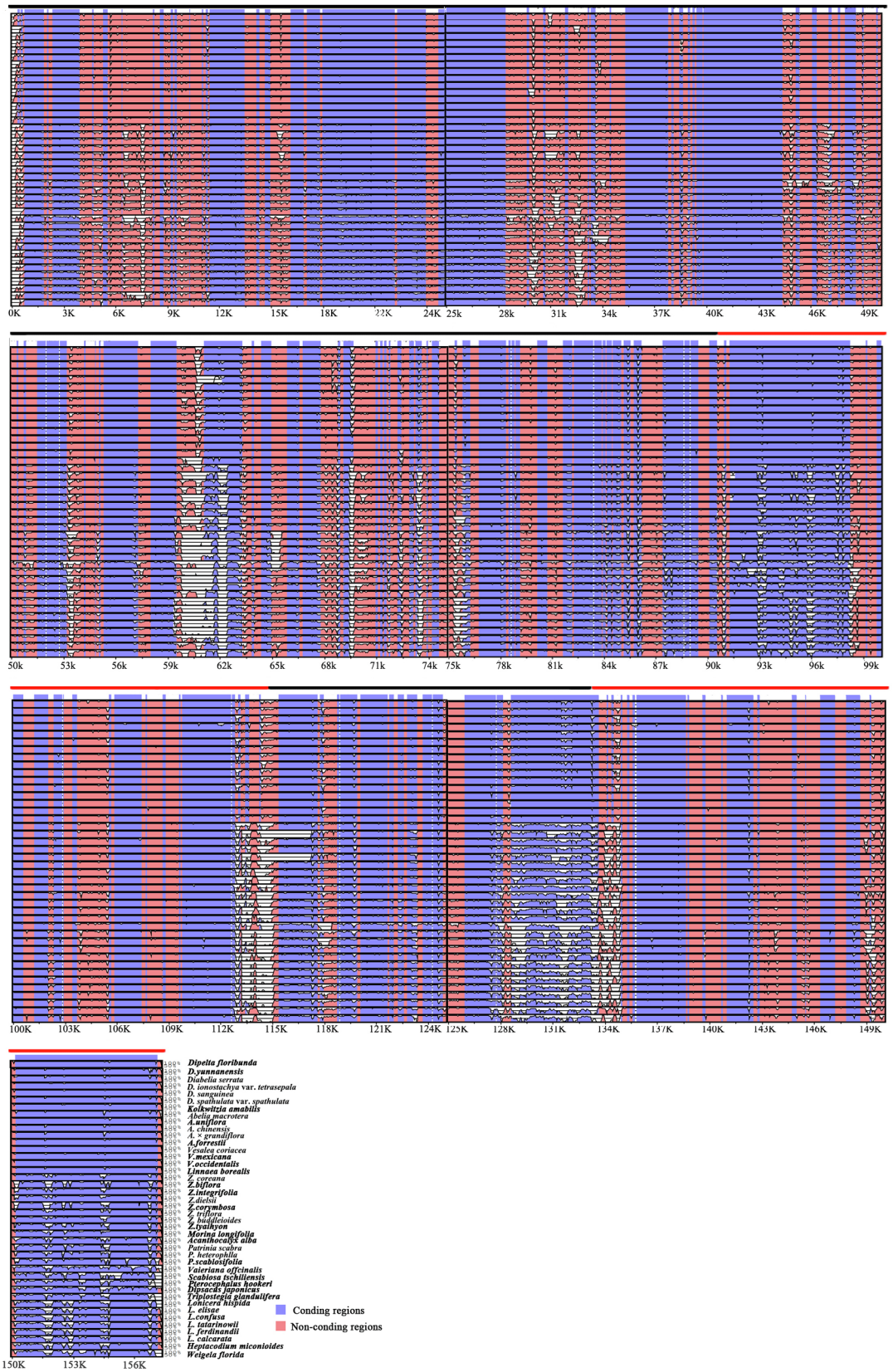

Fig. 5. Alignment of 44 Caprifoliaceae *s.l.* complete plastome sequences. Annotated genes are displayed along the top. Sequence identity is shown as a percentage between 50 and 100% on the y-axis. On the x-axis, *V. floribunda* genes are indicated on the top line, and arrows represent the transcriptional direction. Genome regions are distinguished by color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

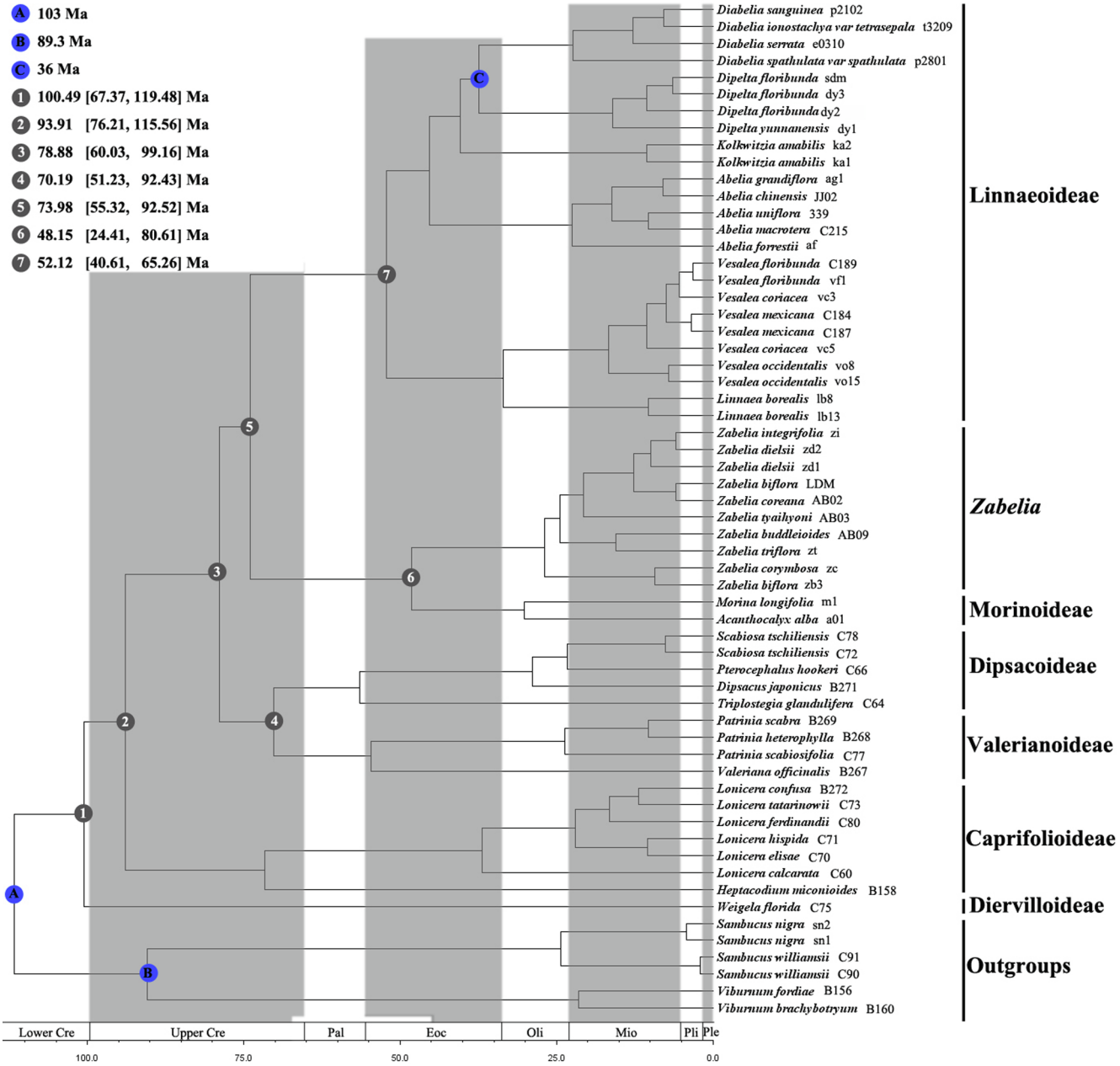

Fig. 6. BEAST analysis of divergence times based on the plastome alignment. Calibration points are indicated by A, B. and C. Numbers 1–7 represent major divergence events in Dipsacales; mean divergence times and 95% highest posterior densities are provided for each.

our results support the sister relationship of *Diabelia* and *Dipelta,* as well as that of *Linnea* and *Vesalea*, as detected in previous studies (Wang et al., 2015). Landrein et al. (2016) suggested that nectary morphology is a shared character between *Linnaea* and *Vesalea.* While the relationships recovered here for Linnaeoideae are promising, further research should sample more species in this clade as well as additional nuclear data. For example, Heckenhauer et al. (2019) indicated that discordance in placement of *Parashorea* between phylogenetic trees based on complete plastome and nuclear single nucleotide polymorphism (SNP) data may be evidence of ancient hybridization.

4.2.3. Relationships within the Caprifolioideae

In previous studies, *Heptacodium* has been placed in different tribes, sometimes in Linnaeeae (Hara, 1983; Takhtajan, 1987; Tang and Li, 1994), and sometimes in Caprifolieae *s.s.* (Fukuoka, 1972; Donoghue, 1983). Using *ndhF,* Pyck and Smets (2000) recovered *Heptacodium* as sister to the clade consisting of *Leycesteria*, *Lonicera*, *Symphoricarpos* and *Triosteum*, but only with weak support. We found strong support for the sister relationship of *Heptacodium* to *Lonicera* + *Triosteum* (Fig. 4). This result is inconsistent with previous morphological studies (Hara, 1983; Takhtajan, 1987; Xu, 1988), but has been recovered consistently in molecular studies (e.g. Bell et al., 2001; Donoghue et al., 2001; Zhang et al., 2003; Pyck and Smets, 2004; Xiang et al., 2019).

4.2.4. Phylogenetic position of Zabelia

The phylogenetic position of *Zabelia* has been a longstanding issue (Bell et al., 2001; Donoghue et al., 2001; Winkworth et al., 2008). Based on nuclear (ITS) and plastid (*trnK*, *matK*, *atpB-rbcL*, *trnL-F*) sequence data, Jacobs et al. (2010) found that *Zabelia* appeared to be either sister to Valerianoideae (low support) or the Morinoideae. Wang et al. (2015) found it to be sister to *Zabelia* and Morinoideae based on sequences of the nuclear ribosomal ITS and nine plastid regions. Our results strongly

support the monophyly of *Zabelia* and its sister relationship to Morinoideae (Fig. 4). The taxonomic status of *Zabelia* at the suprageneric level has been debated recently, with some treating it as a separate family within Dipsacales (Bittrich and Kadereit, 2016), and others subsuming *Zabelia* within Morinoideae (Xu et al., 2011). The phylogenetic and morphological distinctiveness of *Zabelia* seems to warrant its treatment as its own subfamily (Zabelioideae) within Caprifoliaceae *s.l.*, but it would be prudent to wait until additional nuclear phylogenomic data can be gathered to test this hypothesis before formally proposing such a change. In particular, it is important to investigate whether the position of *Zabelia* will differ between nuclear and plastid phylogenomic data, and whether such incongruence may result from hybridization and/or incomplete lineage sorting (Lin et al., 2019; Heckenhauer et al., 2019; Olmstead and Bedoya, 2019).

4.3. Molecular dating

Our estimated divergence times (Fig. 6) are older than those previously reported for the clade, including the plastome studies of Dipsacales by Fan et al. (2018), the Dipsacales study of Bell and Donoghue (2005), and those based on broader studies of angiosperms (Li et al, 2019). For example, we recovered a mid-Cretaceous age for the diversification of crown group Caprifoliaceae *s.l.,* earlier than the Tertiary ages recovered for this clade in Bell and Donoghue (2005), Bell (2010), and Wikström et al. (2015). The older dates recovered here may be a function of the constraint we placed at the base of the tree, but differences in amount of data and taxon sampling can also drive differences in dating estimates because they influence branch length.

We recovered pre-Oligocene ages for divergences among genera of Caprifoliaceae *s.l.* and post-Eocene ages for diversification within genera. Hence it would appear that the diversification of these major herbaceous lineages within Dipsacales may have generally coincided with the global cooling and drying that have characterized much of the Earth's history since the end of the Eocene (Zachos et al., 2001).

5. Conclusions

This work represents a major advance in understanding Dipsacales phylogenetics and plastome evolution. Our results clearly document the power of the plastome to resolve relationships, and they also document strong phylogenetic patterns of plastome structural evolution. Nevertheless, we must emphasize that our results are from plastome sequence alone, and are not taxonomically comprehensive. Differences in tree topologies are often noted between genomic compartments and even among nuclear loci (Heckenhauer et al., 2019; Olmstead and Bedoya, 2019). A number of factors may account for these topological differences, including differences in taxon sampling and biological factors such as hybridization/introgression, incomplete lineage sorting, gene duplication and/or loss, and horizontal gene transfer (Degnan and Rosenberg, 2006; Naciri and Linder, 2015; Nicola et al., 2019; Lin et al., 2019). Although we included species from all major clades of Caprifoliaceae *s.l.*, our taxon sampling was not comprehensive for all genera, and it is possible that the inclusion of additional genera may alter the topology and/or support values. Moreover, we cannot resolve reticulate evolution using our data set because plastome DNA is generally uniparentally inherited. Future studies should investigate patterns of incongruence among nuclear and plastid loci more rigorously as data become available.

Acknowledgements

We sincerely thank the editor and reviewers for their comments and suggestions to our initial submission. This study was funded by National Natural Scientific Foundation of China (31660055 and 31660074) and by start-up funds from Hainan University [kyqd1633 and kydq(zr) 1840], and the China Scholarship Council (Grant No. 201907565012), supporting H-FW's research visit to the Smithsonian Institution, Washington, DC, United States. It was also supported by the grants of Basic Research Program, Shenzhen Municipal Government of China (No. JCYJ20150831201123287, No. JCYJ20160331150739027), and the Guangdong Provincial Key Laboratory of Genome Read and Write (Grant No. 2017B030301011). It was also supported by National Wild Plant Germplasm Resource Center.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.106641.

References

APG IV, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linnean Soc. 181, 1–20.
Bell, W.A., 1957. Flora of the Upper Cretaceous Nanaimo Group of Vancouver Island, British Columbia. Geol. Surv. Canada, Memoir. 293, 1–84.
Bell, C.D., Edwards, E.J., Kim, S.T., Donoghue, M.J., 2001. Dipsacales phylogeny based on chloroplast DNA sequences. Harvard Papers in Botany 6, 481–499.
Bell, C.D., 2004. Preliminary phylogeny of Valerianaceae (Dipsacales) inferred from nuclear and chloroplast DNA sequence data. Mol. Phylogenet. Evol. 31, 340–350.
Bell, C.D., 2010. Towards a species level phylogeny of *Symphoricarpos* (Caprifoliaceae), based on nuclear and chloroplast DNA. Syst. Bot. 35, 442–450.
Bell, C.D., Donoghue, M.J., 2005. Dating the Dipsacales: Comparing models, genes, and evolutionary implications. Am. J. Bot. 92, 284–296.
Bi, Y., Zhang, M.F., Xue, J., Dong, R., Du, Y.P. Zhang X.H., 2018. Chloroplast genomic resources for phylogeny and DNA barcoding: a case study on Fritillaria. Front. Plant Sci., vol. 8, 1184.
Bittrich, V., Kadereit, J.W., 2016. Introduction to the Orders and Families of uncertain placement of this volume. In: Kadereit, J.W., Bittrich, V. (Eds.) The Families and Genera of Vascular Plants vol. xiv: Flowering plants, eudicots, Aquifoliales, Boraginales, Bruniales, Dipsacales, Escllonialeas, Garryales, Paracryphiales, Solanales (except Convollulaceae), Icacinaceae, Metteniusaceae, Vahliaceae. Berlin: Springer, pp. 1–18.
Burke, S.V., Grennan, C.P., Duvall, M.R., 2012. Plastome sequences of two New World bamboos-*Arundinaria gigantea* and *Cryptochloa strictiflora* (Poaceae)-extend phylogenomic understanding of Bambusoideae. Am. J. Bot. 99, 1951–1961.
Cosner, M.E., Jansen, R.K., Palmer, J.D., Downie, S.R., 1997. The highly rearranged chloroplast genome of *Trachelium caeruleum* (Campanulaceae): multiple inversions, inverted repeat expansion and contraction, transposition, insertions/deletions, and several repeat families. Curr. Genet. 31, 419–429.
Darling, A.E., Mau, B., Perna, N.T., 2010. ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5, e11147.
Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. PLoS Genet. 2, 0762.
Donoghue, M.J., 1983. A preliminary analysis of phylogenetic relationships in *Viburnum* (Caprifoliaceae s.l.). Syst. Bot. 8, 45–58.
Donoghue, M.J., Olmstead, R.G., Smith, J.F., Palmer, J.D., 1992. Phylogenetic relationships of Dipsacales based on rbcL sequences. Ann. Missouri Bot. Gard. 79, 333–345.
Donoghue, M.J., Eriksson, T., Reeves, P.A., Olmstead, R.G., 2001. Phylogeny and phylogenetic taxonomy of Dipsacales, with special reference to *Sinadoxa* and *Tetradoxa* (Adoxaceae). Harvard Papers Bot. 6, 459–479.
Doorduin, L., Gravendeel, B., Lammers, Y., Ariyurek, Y., Chin-A-Woeng, T., Vrieling, K., 2011. The complete chloroplast genome of 17 individuals of pest species *Jacobaea vulgaris*: SNPs, microsatellites and barcoding markers for population and phylogenetic studies. DNA Res. 18, 93–105.
Downie, S.R., Jansen, R.K., 2015. A comparative analysis of whole plastid genomes from the Apiales: expansion and contraction of the inverted repeat, mitochondrial to plastid transfer of DNA, and identification of highly divergent noncoding regions. Syst. Bot. 40, 336–351.
Dugas, D.V., Hernandez, D., Koenen, E.J.M., Schwarz, E., Straub, S., Hughes, C.E., Jansen, R.K., Nageswara-Rao, M., Staats, M., Trujillo, J.T., Hajrah, N.H., Alharbi, N.S., AlMalki, A.L., Sabir, J.S.M., Bailey, C.D., 2015. Mimosoid legume plastome evolution: IR expansion, tandem repeat expansions, and accelerated rate of evolution in *clpP*. Sci. Rep. 5, 16958.
Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.
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.
Erdtman, G., 1952. Pollen Morphology and Plant Taxonomy. Almqvist & Wiksell, Stockholm.
Fan, W.B., Wu, Y., Yang, J., Shahzad, K., Li, Z.H., 2018. Comparative chloroplast genomics of Dipsacales species: insights into sequence variation, adaptive evolution, and phylogenetic relationships. Front. Plant Sci. 9, 689.
Frazer, K.A., Pachter, L., Poliakov, A., Rubin, E.M., Dubchak, I., 2004. VISTA: computational tools for comparative genomics. Nucl. Acids Res. 32, 273–279.
Fukuoka, N., 1968. Phylogeny of the tribe Linnaeeae. Acta Phytotax. Geobot. 23, 82–94.
Fukuoka, N., 1969. Inflorescence of Linnaeeae (Caprifoliaceae). Acta Phytotax. Geobot., vol. 23, pp. 153–162.

Fukuoka, N., 1972. Taxonomic study of the Caprifoliaceae. Mem. Fac Sci. Kyoto Univ Ser Biol. 6, 15–58.
Hahn, C., Bachmann, L., Chevreux, B., 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads-a baiting and iterative mapping approach. Nucl. Acids Res. 41, e129.
Haberle, R.C., Fourcade, H.M., Boore, J.L., Jansen, R.K., 2008. Extensive rearrangements in the chloroplast genome of *Trachelium caeruleum* are associated with repeats and tRNA genes. J. Mol. Evol. 66, 350–361.
Hara, H., 1983. A revision of Caprifoliaceae of Japan with reference to allied plants in other districts and the Adoxaceae. Ginkgoana 5, 184.
Heckenhauer, J., Paun, O., Chase, M.W., Ashton, P.S., Kamariah, A.S., Samuel, R., 2019. Molecular phylogenomics of the tribe Shoreeae (Dipterocarpaceae) using whole plastid genomes. Ann. Bot. 123, 857–865.
Ikuse, M., Kurosawa, S., 1954. Notes on section *Zabelia* Rehder of the genus *Abelia*. J. Jap. Bot. 29, 107–110.
Jacobs, B., Pyck, N., Smets, E., 2010. Phylogeny of the *Linnaea* clade: are *Abelia* and *Zabelia* closely related? Mol. Phylogenet. Evol. 57, 741–752.
Jansen, R.K., Cai, Z., Raubeson, L.A., Daniell, H., Depamphilis, C.W., Leebensmack, J., Müller, K.F., Guisinger-Bellian, M., Haberle, R.C., Hansen, A.K., Chumley, T.W., Lee, S.B., Peery, R., McNeal, J.R., Kuehl, J.V., Boore, J.L., 2007. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc. Natl. Acad. Sci. USA 104, 19369–19374.
Judd, W.S., Sanders, R.W., Donoghue, M.J., 1994. Angiosperm family pairs-preliminary phylogenetic analyses. Harvard Pap. Bot. 5, 1–51.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software Version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kim, T.J., Sun, B.Y., Park, C.W., Suh, Y., 1999. Phylogenetic implications of matK sequences in Caprifoliaceae. Am. J. Bot., vol. 86 (Suppl.), 3202 (Abstract 86).
Kim, K.J., Choi, K.S., Jansen, R.K., 2005. Two chloroplast DNA inversions originated simultaneously during the early evolution of the sunflflower family (Asteraceae). Mol. Biol. Evol. 22, 1783–1792.
Landrein, S., 2010. *Diabelia*, a new genus of tribe Linnaeeae subtribe Linnaeinae (Caprifoliaceae). Phytotaxa 3, 34–38.
Landrein, S., Prenner, G., Chase, M.W., Clarkson, J.J., 2012. *Abelia* and relatives: Phylogenetics of Linnaeeae (Dipsacales Caprifoliaceae s.l.) and a new interpretation of their inflorescence morphology. Bot. J. Linn. Soc. 169, 692–713.
Landrein, S., Prenner, G., 2016. Structure, ultrastructure and evolution of floral nectaries in the twinflower tribe Linnaeeae and related taxa (Caprifoliaceae). Bot. J. Linn. Soc. 181, 37–69.
Li, R., Ma, P.F., Wen, J., Yi, T.S., 2013. Complete sequencing of five Araliaceae chloroplast genomes and the phylogenetic implications. PLoS One 8, e78568.
Li, P., Lu, R.S., Xu, W.Q., Ohi-Toma, T., Cai, M.Q., Qiu, Y.X., Cameron, K.M., Fu, C.X., 2017. Comparative Genomics and Phylogenomics of East Asian Tulips (Amana, Liliaceae). Front. Plant Sci. 8, 451.
Li, Q.S., Li, Y., Song, J., Xu, H.B., Xu, J., Zhu, Y.J., Li, X.W., Gao, H.H., Dong, L.L., Qian, J., Sun, C., Chen, S.L., 2014. High-accuracy de novo assembly and SNP detection of chloroplast genomes using a SMRT circular consensus sequencing strategy. New Phytol. 204, 1041–1049.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Li, H.T., Yi, T.S., Gao, L.M., Ma, P.F., Zhang, T., Yang, J.B., Gitzendanner, M.A., et al., 2019. Origin of angiosperms and the puzzle of the Jurassic gap. Nature Plants 5, 461–470.
Lin, H.Y., Hao, Y.J., Li, J.H., Fu, C.X., Pamela, S.S., Douglas, E.S., Zhao, Y.P., 2019. Phylogenomic conflict resulting from ancient introgression following species diversification in *Stewartia s.l.* (Theaceae). Mol. Phylogenet. Evol. 135, 1–11.
Lohse, M., Drechsel, O., Kahlau, S., Bock, R., 2013. Organellar GenomeDRAW-a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucl. Acids Res 41, 575–581.
Lu, R.S., Li, P., Qiu, Y.X., 2016. The Complete Chloroplast Genomes of Three *Cardiocrinum* (Liliaceae) Species: comparative genomic and phylogenetic analyses. Front. Plant Sci. 7, 2054.
Manchester, S.R., Donoghue, M.J., 1995. Winged Fruits of Linnaeeae (Caprifoliaceae) in the Tertiary of Western North America: Diplodipelta gen. nov. Int. J. Plant Sci. 156, 709–722.
Martin, G.E., Rousseau-Gueutin, M., Cordonnier, S., Lima, O., Michon-Coudouel, S., Naquin, D., Carvalho, J.F., Ainouche, M., Salmon, A., Ainouche, A., 2014. The first complete chloroplast genome of the Genistoid legume Lupinus luteus: evidence for a novel major lineage-specifific rearrangement and new insights regarding plastome evolution in the legume family. Ann. Bot., vol. 113, pp. 1197–1210.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for-inference of large phylogenetic trees. Gateway Comput. Environ. Workshop 1–8.
Moore, M.J., Dhingra, A., Soltis, P.S., Shaw, R., Farmerie, W.G., Folta, K.M., Soltis, D.E., 2010. Rapid and accurate pyrosequencing of angiosperm plastid genomes. BMC Plant Biol. 6, 1–13.
Naciri, Y., Linder, P., 2015. Species delimitation and relationships: the dance of the seven veils. Taxon 64, 3–16.
Niu, Y.T., Florian, J., Russell, L., 2018. Combining complete chloroplast genome sequences with target loci data and morphology to resolve species limits in *Triplostegia* (Caprifoliaceae). Mol. Phylogenet. Evol. 129, 15–26.
Nicola, M.V., Johnson, L.A., Pozner, R., 2019. Unraveling patterns and processes of diversification in the South Andean-Patagonian *Nassauvia* subgenus *Strongyloma* (Asteraceae, Nassauvieae). Mol. Phylogenet. Evol. 136, 164–182.
Olmstead, R.G., Bedoya, A.M., 2019. Whole genomes: the holy grail. A commentary on: 'Molecular phylogenomics of the tribe Shoreeae (Dipterocarpaceae) using whole plastid genomes'. Ann. Bot. 123, 4–5.
Peter, S., Angela, N.B., Todd, M.L., 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucl. Acids Res. 33, 686–689.
Pinard, D., Myburg, A.A., Mizrachi, E., 2019. The plastid and mitochondrial genomes of *Eucalyptus grandis*. BMC Genom. 20, 1471–2164.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Pyck, E., Smets, 2000. A search for the phylogenetic position of the seven-son flower (*Heptacodium*, Dipsacales): combining molecular and morphological evidence. Plant Syst. Evol. 225, 185–199.
Pyck, N., 2001. Phylogenetic relationships within Dipsacales: a combined molecular and morphological approach. Ph.D. dissertation. Katholieke University, Leuven.
Pyck, N., Smets, E., 2004. On the systematic position of *Triplostegia* (Dipsacales): a combined molecular and morphological approach. Belg J. Bot. 137, 125–139.
Rehder, A., 1911. Synopsis of the genus Abelia. In: Sargent, C.S. (Ed.), Plantae Wilsonianae. Cambridge Univ. Press, Cambridge, pp. 122–129.
Ronquist, F., Teslenko, M., Paul, V.D.M., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MRBAYES 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. Syst. Biol. 61, 539–542.
Rose, R., Golosova, O., Sukhomlinov, D., Tiunov, A., Prosperi, M., 2018. Flexible design of multiple metagenomics classification pipelines with UGENE. Bioinformatics 35, 1963–1965.
Santorum, J.M., Darriba, D., Taboada, G.L., Posada, D., 2014. jmodeltest.org selection of nucleotide substitution models on the cloud. Bioinformatics 30, 1310–1311.
Saski, C., Lee, S.B., Daniell, H., Wood, T.C., Tomkins, J., Kim, H.G., Jansen, R.K., 2005. Complete chloroplast genome sequence of *Glycine max* and comparative analyses with other legume genomes. Plant Mol. Biol. 59, 309–322.
Schwarz, E.N., Ruhlman, T.A., Sabir, J.S.M., Hajrah, N.H., Alharbi, N.S., Al-Malki, A.L., Bailey, C.D., Jansen, R.K., 2015. Plastid genome sequences of legumes reveal parallel inversions and multiple losses of *rps16* in papilionoids. J. Syst. Evol. 53, 458–468.
Sloan, D.B., Triant, D.A., Forrester, N.J., Bergner, L.M., Wu, M., Taylor, D.R., 2014. Arecurring syndrome of accelerated plastid genome evolution in the angiosperm tribe Sileneae (Caryophyllaceae). Mol. Phylogenet. Evol. 72, 82–89.
Smith, S.A., Beaulieu, J.M., Donoghue, M.J., 2010. An uncorrelated relaxedclock analysis suggests an earlier origin for flowering plants. Proc. Natl. Acad. Sci. USA 107, 5897–5902.
Shaw, J., Shafer, H.L., Leonard, O.R., Kovach, M.J., Schorr, M., Morris, A.B., 2014. Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. Am. J. Bot. 101, 1987–2004.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Stevens, P.F., 2019. Angiosperm Phylogeny Website. Version Apr. 15, 2019 < http://www.mobot.org/MOBOT/research/APweb/ > .
Takhtajan, A.L., 1987. Systema Magnoliphytorum. Nauka, Leingrad.
Tank, D.C., Donoghue, M.J., 2010. Phylogeny and phylogenetic nomenclature of the Campanulidae based on an expanded sample of genes and taxa. Syst. Bot. 35, 425–441.
Tang, Y.C., Li, L.Q., 1994. The phytogeography of Caprifoliaceae s.str. with its implications for understanding eastern asiatic flora. Acta Phytotax. Sin. 32, 197–218.
Walker, J.F., Zanis, M.J., Emery, N.C., 2014. Comparative analysis of complete chloroplast genome sequence and inversion variation in *Lasthenia burkei* (Madieae, Asteraceae). Am. J. Bot. 101, 722–729.
Wang, H.F., Landrein, S., Dong, W.P., Nie, Z.L., Kondo, K., Funamoto, T., Wen, J., Zhou, S.L., 2015. Molecular phylogeny and biogeographic diversification of Linnaeoideae (Carprifoliaceae *s.l.*) disjunctively distributed in Eurasia, North American and Mexico. PLoS One 10, e0116485.
Wu, F.H., Chan, M.T., Liao, D.C., Hsu, C.T., Lee, Y.W., Daniell, H., Duvall, M.R., Lin, C.S., 2010. Complete chloroplast genome of *Oncidium* Gower Ramsey and evaluation of molecular markers for identification and breeding in Oncidiinae. BMC Plant Biol. 10, 68.
Wyman, S.K., Jansen, R.K., Boore, J.L., 2004. Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20, 3252–3255.
Winkworth, R.C., Bell, C.D., Donoghue, M.J., 2008. Mitochondrial sequence data and Dipsacales phylogeny: mixed models, partitioned Bayesian analyses, and model selection. Mol. Phylogenet. Evol. 46, 830–843.
Wikström, N., Kainulainen, K., Razafimandimbison, S.G., Smedmark, J.E.E., Bremer, B., 2015. A revised time tree of the asterids: establishing a temporal framework for evolutionary studies of the coffee family (Rubiaceae). PLoS One 10, e0126690.
Xiang, C.L., Dong, H.J., Landrein, S., Zhao, F., Yu, W.B., Soltis, D.E., Soltis, P.S., Backlund, A., Wang, H.F., Li, D.Z., Peng, H., 2019. Revisiting the phylogeny of Dipsacales: new insights from phylogenomic analyses of complete chloroplast genome sequences. J. Syst. Evol. 00 (0), 1–15. https://doi.org/10.1111/jse.12526.
Xu, B.S., 1988. Caprifoliaceae (Linnaeeae, excluding Heptacodium and Symphoricarpos). In: Hsu Ping-sheng (Ed.), Fl. Reipubl. Popularis Sin., vol. 72, pp. 112–131.
Xu, C., Dong, W.P., Li, W.Q., Lu, Y.Z., Xie, X.M., Jin, X.B., Shi, J.P., He, K.H., Suo, Z.L., 2017. Comparative analysis of six *Lagerstroemia* complete chloroplast genomes. Front. Plant Sci. 8, 15.
Xu, L., Lu, L., Li, D.Z., Wang, H., 2011. Evolution of pollen in the Dipsacales. Plant Divers. Resour. 33, 249–259.
Yan, M.H., Fritsch, P.W., Moore, M.J., Feng, T., Meng, A.P., Yang, J., Deng, T., Zhao, C.X., Yao, X.H., Sun, H., Wang, H.C., 2018. Plastid phylogenomics resolves infrafamilial relationships of the Styracaceae and sheds light on the backbone relationships of the Ericales. Mol. Phylogenet. Evol. 121, 198–211.
Yang, Y., Zhou, T., Duan, D., Yang, J., Feng, L., Zhao, G., 2016. Comparative analysis of the complete chloroplast genomes of five *Quercus* species. Front. Plant Sci. 7, 573–575.

Zachos, J.S., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693.
Zhang, W.H., Chen, Z.D., Li, J.H., Chen, H.B., Tang, Y.C., 2003. Phylogeny of the Dipsacales s.l. based on chloroplast *trnL-F* and *ndhF* sequences. Mol. Phylogenet. Evol. 26, 176–189.
Zhu, A., Guo, W., Gupta, S., Fan, W., Mower, J.P., 2016. Evolutionary dynamics of the plastid inverted repeat: the effects of expansion, contraction, and loss on substitution rates. New Phytol., vol. 209, pp. 1747–1756.