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Research Article

Revisiting the phylogeny of Dipsacales: New insights from phylogenomic analyses of complete plastomic sequences

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Abstract Phylogenetic relationships in Dipsacales have long been a major challenge. Although considerable progress has been made during the past two decades, questions remain; the uncertain systematic positions of *Heptacodium*, *Triplostegia*, and *Zabelia*, in particular, impede our understanding of Dipsacales evolution. Here we use 75 complete plastomic sequences to reconstruct the phylogeny of Dipsacales, of which 28 were newly generated. Two primary clades were recovered that form the phylogenetic backbone of Dipsacales. Seven of the primary clades correspond to the recognized families Adoxaceae, Caprifoliaceae s. str., Diervillaceae, Dipsacaceae, Linnaeaceae, Morinaceae, and Valerianaceae, and one corresponds to *Zabelia*, which was found to be the closest relative of Morinaceae in all analyses. Additionally, our results, with greatly increased confidence in most branches, show that *Heptacodium* and *Triplostegia* are members of Caprifoliaceae s. str. and Dipsacaceae, respectively. The results of our study indicate that the complete plastomic sequences provide a fully-resolved and well-supported representation of the phylogenetic relationships within Dipsacales.

Key words: Adoxaceae, Caprifoliaceae, *Heptacodium*, Morinaceae, *Triplostegia*, *Zabelia*.

1 Introduction

Dipsacales (Donoghue, 1983) are a clade of asterids (Soltis et al., 2011; Ruhfel et al., 2014; APG IV, 2016) comprising approximately 1100 species (Backlund & Bittrich, 2016; Hofmann & Bittrich, 2016a, 2016b; Mayer, 2016; Weberling & Bittrich, 2016) distributed all over the world. Recognized as an order by APG IV (2016), this clade is generally characterized by opposite and usually simple leaves and generally large inflorescences. Flowers are generally pentamerous, with successive reductions in the number of carpels and stamens. Pollen grains are triaperturate. However, high morphological variations occur across this order and non-molecular synapomorphies for subclades are relatively few.

Over the past two decades, enormous progress has been made in understanding overall phylogenetic relationships and evolution in Dipsacales (Donoghue et al., 1992, 2001, 2003; Eriksson & Donoghue, 1997; Pyck & Smets, 2000; Bell et al., 2001, 2012; Bell & Donoghue, 2003, 2005; Zhang et al., 2003; Bell, 2004; Moore & Donoghue, 2007; Theis et al., 2008; Winkworth et al., 2008a, 2008b; Carlson et al., 2009; Jacobs et al., 2009, 2010a, 2010b, 2011; Landrein et al., 2012; Clement et al., 2014; Wang et al., 2015; Landrein & Prenner, 2016). Although most genera and all families in Dipsacales have been included in molecular studies cited above, no single study has included representatives for each clade by using plastomic sequences. The uncertain systematic positions of *Heptacodium* Rehder, *Triplostegia* Wall. ex DC., and *Zabelia* (Rehder) Makino remain particularly problematic.

The monophyly of Dipsacales has been well documented (e.g., Winkworth et al., 2008a, 2008b), and several previous studies (i.e., Donoghue et al., 2001, 2003; Zhang et al., 2003; Jacobs et al., 2010b) have consistently clarified the composition of Dipsacales and recovered two lineages. One is Adoxaceae s. l. which comprises five genera (*Adoxa* L., *Tetradoxa* C. Y. Wu, *Sinadoxa* C. Y. Wu, Z. L. Wu & R. F. Huang, *Viburnum* L., and *Sambucus* L.) and 175–210 species, and the other is a larger clade (i.e., Caprifoliaceae s. l.) comprising Caprifoliaceae s. str. (or Caprifolieae, 14 genera with approximately 230 species), Morinaceae (2 genera and 12 species), Dipsacaceae (including *Triplostegia*, 15 genera with approximately 300 species), and Valerianaceae (5 genera with approximately 400 species) (see also Bittrich & Kadereit, 2016). Although the main clades of Dipsacales have been ascertained, details of their interrelationships are still unclear (Judd et al., 1994; Backlund & Donoghue, 1996; Bell et al., 2001; Donoghue et al., 2001, 2003; Pyck, 2001; Zhang et al., 2003; Jacobs et al., 2011; Boyden et al., 2012; Landrein et al., 2012; Wang et al., 2015).

Circumscriptions of some families, however, have been controversial, especially for Caprifoliaceae, as its traditional concept is not supported by phylogenetic analyses. The traditionally recognized Caprifoliaceae are not monophyletic, and two different treatments were used in order to recognize monophyletic groups. Backlund & Pyck (1998) retained a narrowly defined Caprifoliaceae and established two new families, Diervillaceae and Linnaeaceae, to refer to Caprifoliaceae subfamilies Diervilloideae and Linnaeoideae, respectively. The narrowly circumscribed concept of the family is also adopted by some other authors (APG, 1998; Yang et al., 2011; Yang & Landrein, 2011). In contrast, Donoghue et al. (2001) proposed a phylogenetic scheme that lumped Morinaceae, Dipsacaceae, Valerianaceae, and Caprifoliaceae s. str. into the expanded family Caprifoliaceae, as suggested by Judd et al. (1994). Accordingly, they named the two main clades of Dipsacales as Valerina clade (including Morinaceae, Dipsacaceae, and Valerianaceae) and Linnina clade (including Diervilleae, Caprifolieae, and Linnaeeae), but the concepts of Morinaceae, Dipsacaceae, and/or Valerianaceae were still used in their subsequent studies (Bell & Donoghue, 2003, 2005; Donoghue et al., 2003; Moore & Donoghue, 2007; Carlson et al., 2009; Boyden et al., 2012).

Assignment of certain genera in Dipsacales has long been controversial, particularly the positions of *Heptacodium* and *Zabelia* (Pyck & Smets, 2000; Jacobs et al., 2009; Landrein et al., 2012; Bittrich & Kadereit, 2016). The position of the monotypic *Heptacodium* has been enigmatic ever since its publication (Rehder, 1916) due to incongruence between molecular and morphological evidence (Pyck & Smets, 2000; Zhang et al., 2003) and its possible hybrid origin (Zhang et al., 2002; Jacobs et al., 2011). Some studies (Pyck & Smets, 2000; Donoghue et al., 2003; Winkworth et al., 2008b; Jacobs et al., 2009, 2010b) have shown that *Heptacodium* is a member of Caprifoliaceae and sister to the *Lonicera* clade, whereas other studies suggest a sister relationship of the *Linnina* clade (e.g., Winkworth et al., 2008a; Jacobs et al., 2009). Based on cytological evidence, Zhang et al. (2002) suggested that this genus possibly has a polyploid origin following hybridization between two ancestral extinct species. To date, the exact phylogenetic position of *Heptacodium* remains to be determined.

Zabelia has long been thought closely related to *Abelia* R. Br. in Caprifoliaceae (Hara, 1983; Tang & Lu, 2005), however, Verlaque (1983) discovered that the genus *Morina* shows some affinities with *Zabelia* in pollen microscopic characters. Later, Ogata (1991) found that the presence of aggregate rays is a unique character to *Zabelia* and suggested that *Abelia* and *Zabelia* are less closely related. Other morphological characters, such as the types of inflorescence (Fukuoka, 1969) and pollen morphology (Hu & He, 1988) also supported the separation of *Zabelia* from *Abelia*. Using molecular datasets, Tank & Donoghue (2010) and Jacobs et al. (2011) found that *Zabelia* is sister to Morinaceae, but a close relationship between *Zabelia* and the Valeriana clade was also recovered by Jacobs et al. (2010b). Based on six molecular loci and inflorescence morphology, Landrein et al. (2012) concluded that the position of *Zabelia*, which is the sole member of the tribe Zabeliinae, remains unclear. This view was adopted by Bittrich & Kadereit (2016) and they treated this genus as *incertae sedis* in Caprifoliaceae.

Likewise, the systematic position of *Triplostegia* has also been enigmatic and it has been variously placed in Valerianaceae, Dipsacaceae, Morinaceae, or Triplostegiaceae. It is now generally thought to have a close relationship with Dipsacaceae (Bell, 2004; Hidalgo et al., 2004; Pyck & Smets, 2004; Soltis et al., 2011). Morphologically, *Triplostegia* was considered to be closely related to Dipsacaceae by sharing the pseudomonomerous ovary and epicalyx (Peng et al., 1995), but other characters (i.e., inflorescence structure, pollen morphology, and phytochemistry) indicate affinities to Valerianaceae (Backlund & Nilsson, 1997; Backlund & Moritz, 1998). For all these reasons, Bittrich & Kadereit (2016) thought it was not easy to resolve the systematic position of *Triplostegia*. The uncertain positions of these problematic genera have hindered a better understanding of evolution in Dipsacales (Jacobs et al., 2011).

Next-generation sequencing has provided a wealth of genome sequence data from an increasingly diverse set of green plants (Givnish et al., 2010; Ruhfel et al., 2014; Brassac & Blattner, 2015), and the use of complete plastomic sequences has successfully resolved difficult phylogenetic problems in flowering plants at different taxonomic levels (e.g., Jansen et al., 2007, 2011; Moore et al., 2007, 2010; Lin et al., 2010; Zhong et al., 2010; Xi et al., 2012; Barrett et al., 2014; Ma et al., 2014; Yang et al., 2014b; Stull et al., 2015; Luo et al., 2016; Williams et al., 2016; Yu et al., 2017; Zhang et al., 2017). Although some plastid genes, mitochondrial genes, and nuclear DNA regions have long been exploited for phylogenetic inference in Dipsacales (e.g., Donoghue et al., 1992, 2001, 2003; Pyck & Smets, 2000; Bell et al., 2001, 2012; Bell & Donoghue, 2003, 2005; Zhang et al., 2003; Moore & Donoghue, 2007; Winkworth et al., 2008a, 2008b; Carlson et al., 2009; Jacobs et al., 2010a, 2010b, 2011; Landrein et al., 2012; Clement et al., 2014; Wang et al., 2015; Landrein & Prenner, 2016), the problems noted above still need dedicated investigation. We therefore used complete plastomic sequences to reconstruct Dipsacales phylogeny.

In this study, we obtained 28 newly sequenced complete plastomes across the major lineages of Dipsacales and combined these with 47 published plastomic data for more comprehensive phylogenetic analyses. The major objectives of this study are to: (i) infer a robust phylogeny of Dipsacales using the complete plastomic sequences; (ii) investigate the phylogenetic placements of *Heptacodium*, *Zabelia*, and *Triplostegia*; and (iii) comment on current taxonomy based on the recovered phylogeny.

2 Material and Methods

2.1 Taxon sampling

Four species from Apiales (*Panax ginseng* C. A. Mey, *Aralia undulata* Hand.-Mazz., *Anthriscus cerefolium* (L.) Hoffm., and *Daucus carota* L.) were chosen as outgroups based on previous studies (Winkworth et al., 2008a; Tank & Donoghue, 2010; Beaulieu & Donoghue, 2013; APG IV, 2016). Ingroup taxon samplings included Dipsacales (70 accessions representing 64 species in 25 genera) and its sister clade Paracryphiales (*Quintinia verdonii* F. Muell.). In total, 28 species were newly sequenced in this study. Voucher specimens for the newly sequenced taxa were deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN; Table 1).

2.2 DNA isolation and sequencing

Young healthy leaves were collected and dried in silica gel. Total genomic DNA was extracted from approximately 3 g of leaf material using the CTAB protocol of Doyle & Doyle (1987). We used two methods for next-generation sequencing. Eight samples (asterisked in Table 1) were sequenced by the long-range polymerase chain reaction method using nine universal primer pairs developed by Yang et al. (2014a). The amplified DNA fragments were pooled together in roughly equal concentrations for subsequent sequencing. For the remaining 20 samples, 0.5 μg genomic DNA was directly used for sequencing. The genomic DNAs or DNA fragments were sheared into fragments of ca. 300 bp in size, then used to construct libraries following the manufacturer's protocols (Illumina, San Diego, CA, USA). Paired-end sequencing of 90 bp (*Abelia forrestii* (Diels) W. W. Sm., *Acanthocalyx nepalensis* (D. Don) M. J. Cannon, and *Viburnum cylindricum* Buch.-Ham. ex D. Don 02) or 150 bp (other species) was undertaken on the Illumina HiSeq 2000 (Illumina) at BGI-Shenzhen (Shenzhen, Guangdong, China).

2.3 Plastome assembly, gene region extraction, and alignment

Raw paired-end reads were assessed using FastQC (Andrews, 2010), and the high quality paired-end reads were assembled using the GetOrganelle toolkit (Jin et al., 2018). To filter the plastid-like reads, we set the published plastome of *Lonicera japonica* Thunb. (NC026839) as seeds/references (He et al., 2017). Those filtered reads were *de novo* assembled into contigs/scaffolds using SPAdes version 3.10 (Bankevich et al., 2012). The circular complete plastomes were visualized and exported using Bandage version 0.8.1 (Wick et al., 2015). The new plastomes were automatically annotated using DOGMA (Wyman et al., 2004; http://dogma.ccbb.utexas.edu/). The positions of start and stop codons and intron/exon boundaries were determined according to the published plastome of *Lonicera japonica*. The coding regions (CR) and non-coding regions (NCR) were extracted from the annotated complete plastomic sequences for phylogenetic analyses. All initial alignments were undertaken using MAFFT version 7.308 (Katoh & Standley, 2013) within Geneious version 11.0.4 (Kearse et al., 2012) before being further refined by eye.

2.4 Phylogenetic analyses

We followed Clement et al. (2014) for selection of CR and NCR. In addition, we also included some extra genes and regions (i.e., *accD, ndhB, ndhK, rps7, ycf5, atpF-atpH, petB-petD*, and *petN-psbM*) that were not used by Clement et al. (2014) for analyses. The removal of problematic alignment regions can lead to better trees (Talavera & Castresana, 2007), so ambiguously aligned positions (e.g., characters of uncertain homology among taxa and single-taxon insertions; see also Chen et al., 2016; Drew et al., 2017; Hu et al., 2018; Xiang et al., 2018) were removed manually in our analyses (Tables S1, S2). The corresponding datasets are indicated by "−M" subscript in Table 2.

Because the plastome is uniparentally inherited in these plants and generally does not undergo recombination, genes can safely be concatenated for phylogenetic analysis, as suggested by Olmstead & Sweere (1994). Thus, we combined all sequences (i.e., the complete plastomic dataset including CR and NCR; dataset CPG) for phylogenetic analyses. Because sequences of five species obtained from the 1KP project (http://onekp.com; *Dipsacus sativus* (L.) Honck., *Lonicera japonica*, *Symphoricarpos sinensis* Rehder, *Valeriana officinalis* L., and *Viburnum odoratissimum* Ker Gawl.) and seven species obtained from published transcriptome data (*Diabelia spathulata* (Siebold & Zucc.) Landrein, *Dipsacus laciniatus* L., *Lonicera alpigena* L. subsp. *glehnii* (Fr. Schm.) H. Hara., *L. caerulea* L., *L. macranthoides* Hand.-Mazz., *Patrinia triloba* (Miq.) Miq. var. *takeuchiana* (Makino) Ohwi, and *Sambucus canadensis* L.) and NCR for these taxa are unavailable. So, all coding regions (dataset CR) for all species were combined for an additional analysis, and PartitionFinder 1.0.1 (Lanfear et al., 2012) was used to evaluate the optimal partitioning strategy. Under the corrected Akaike Information Criterion (AIC), the "greedy" algorithm, and "models = all", PartitionFinder identified 32 partitions (Table S3), which were then used in Bayesian inference (BI) and maximum likelihood (ML) analyses.

The ML analyses of all combined datasets were implemented with RAxML version 8.2.9 (Stamatakis, 2014) using the CIPRES Science Gateway (http://www.phylo.org/). The ML tree was inferred with the combined rapid bootstrap (1000 replicates) and searched for the best-scoring ML tree simultaneously (the "-f q" option). The GTR+G model was used in all analyses (Table 2). In addition, for the dataset of coding regions ($\text{CR}_{-\text{M}}$), a partitioned model (-q) was selected, and 1000 bootstrap iterations (-#/-N) were carried out, with other parameters using the default settings.

Bayesian inference analyses were carried out using MrBayes version 3.2.2 (Ronquist et al., 2012). The optimal substitution models were selected using the "ModelFinder" feature (Kalyaanamoorthy et al., 2017) of IQ-TREE version 1.6.8 (Nguyen et al., 2015) according to the Akaike Information Criterion. Model parameters were estimated directly during the runs (Table S4). Markov chain Monte Carlo analysis was executed for 25 million generations, each starting with a random tree and sampling one tree every 1000 generations. Convergence of runs was accepted when the average standard deviation of split frequencies dropped below 0.01. Tracer version 1.6.0 (Rambaut et al., 2014) was used to assess effective sample size values for estimated parameters. The first 25% of the resulting trees was discarded as burn-in. Statistics for the BI analysis are provided in Table S4. All resulting trees with nodal support values were visualized and edited in FigTree version 1.4.2 (Rambaut, 2014).

Table 1 Newly sampled species in this study for which complete or majority of the chloroplast genome sequences are available

Family/lineage	Species	Voucher specimen
Adoxaceae	*Adoxa moschatellina* L. 01	China, Beijing, Huairou, Labagou, elevation 1200 m, 116°27′10″E, 40°57′25″N, 19 June 2016, *Xiang CL 1278*
	Tetradoxa omeiensis (H. Hara) C. Y. Wu 01*	China, Sichuan, Emeishan City, Mountain Emei, elevation 2300 m, 103°20′38.94″E, 29°32′41.43″N, 20 April 2014, *Li XJ Y004*
	Sambucus williamsii Hance 01	China, Yunnan, Lijiang, Baisha, elevation 2480 m, 100°12′57.36″E, 26°57′27.28″N, 27 June 2016, *Xiang CL 1298*
	Sambucus javanica Blume*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 12 May 2013, *Dong HJ 130411*
	Viburnum cylindricum Buch.-Ham. ex D. Don 02	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 12 May 2013, *Dong HJ 130412*
	Viburnum hupehense Rehder	China, Yunnan, Lijiang, Jade Dragon Snow Mountain, elevation 3200 m, 100°17′8.56″E, 27°12′34.4″N, 26 June 2016, *Xiang CL 1288*
Dipsacaceae	*Dipsacus asper* Wall. ex DC.*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 12 May 2013, *Dong HJ 130415*
	Pterocephalus bretschneideri (Batalin) E. Pritz.	China, Yunnan, Lijiang, on the way from Lijiang to Daju, elevation 3000 m, 100°17′15″E, 27°12′33.05″N, 26 June 2016, *Xiang CL 1286*
	Pterocephalus hookeri (C. B. Clarke) Diels	China, Yunnan, Lijiang, on the way from Lijiang to Daju, elevation 3200 m, 100°17′15.5″E, 27°12′34.44″N, 26 June 2016, *Xiang CL 1284*
	Triplostegia grandiflora Gagnep.*	China, Yunnan, Lijiang, Lijiang Alpine Botanic Garden, elevation 2810 m, 100°11′35.34″E, 27°0′0.19″N, 27 June 2016, *Xiang CL 1295*
Valerianaceae	*Valeriana flaccidissima* Maxim.	China, Yunnan, Lijiang, Lijiang Alpine Botanic Garden, elevation 2810 m, 100°11′35.34″E, 27°0′0.19″N, 27 June 2016, *Xiang CL 1294*
	Valeriana jatamansi Jones*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 12 May 2013, *Dong HJ 130414*
Linnaeaceae	*Abelia chinensis* R. Br.	China, Beijing, cultivated in the Institute of Botany, 11 June 2017, *Li YY s.n.*
	Abelia forrestii (Diels) W. W. Sm.	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 12 May 2013, *Dong HJ 130416*
	Dipelta floribunda Maxim.	China, Yunnan, Lijiang, Jade Dragon Snow Mountain, elevation 3230 m, 100°17′9.22″E, 27°12′28.67″N, 26 June 2016, *Xiang CL 1290*
	Linnaea borealis L.	China, Jilin Antu, from Erdaobaihe town to western slop of Changbai mountain, elevation 799, 128°05′57.78″E, 42°21′49.25″N, 26 May 2016, *Wu Y. D. s.n.*
Morinaceae	*Acanthocalyx delavayi* (Franch.) M. J. Cannon	China, Yunnan, Lijiang, on the way from Lijiang to Daju, elevation 3200 m, 100°17′18.20″E, 27°12′35.18″N, 26 June 2016, *Xiang CL 1282*
	Acanthocalyx nepalensis (D. Don) M. J. Cannon	China, Tibet, Dangxiong County, Yangbajin, elevation 4097 m, 30°08′04.6″E, 97°23′25.2″N, 20 April 2014, *Xiang CL 1422*
	Morina chlorantha Diels	China, Yunnan, Lijiang, Baisha, Lijiang Alpine Botanic Garden, elevation 2732 m, 100°11′57.34″E, 26°59′47.90″N , 27 July 2016, *Liang H. 03*
Zabelia	*Zabelia biflora* (Turcz.) Makino*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 9 April 2013, *Dong HJ 130455*
	Zabelia dielsii (Graebn.) Makino	China, Chongqing, Nanchuan, Jinfoshan, elevation 2040 m, 107°10′34.49″E, 29°1′19.85″N, 7 June 2017, *Xiang CL 1308*
Caprifoliaceae s. str.	*Heptacodium miconioides* Rehder*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 13 June 2013, *Dong HJ 130469*
	Leycesteria formosa Wall.	China, Yunnan, Lijiang, Wenhai, Lijiang Alpine Botanic Garden, elevation 3297 m, 100°10′38.56″E, 26°59′48.71″N, 27 June 2016, *Xiang CL s.n.*
	Lonicera japonica Thunb. 02*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 3 August 2013, *Dong HJ 130494*
	Lonicera tangutica Maxim.	China, Yunnan, Lijiang, on the way from Lijiang to Daju,

Continued

Table 1 Continued

Family/lineage	Species	Voucher specimen
		elevation 3200 m, 100°17′38.56″E, 27°12′34.4″N, 26 June 2016, *Xiang CL 1287*
	Symphoricarpos sinensis	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, 8 June 2017, *Xiang CL s.n.*
	Triosteum himalayanum Wall.	China, Yunnan, Lijiang, Wenhai, Lijiang Alpine Botanic Garden, elevation 3297 m, 100°10′38.56″E, 26°59′48.71″N, 27 June 2016, *Xiang CL 1299*
Diervillaceae	*Weigela florida* (Bunge) A. DC.*	China, Yunnan, Kunming, cultivated in Kunming Botanical Garden, elevation 1900 m, 3 August 2013, *Dong HJ 130493*

*Long-range polymerase chain reaction method. Voucher specimens were deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

3 Results

3.1 Characteristics of datasets

Our sequencing generated 290 012 to 25 888 742 clean reads for the 28 sampled species, with mean coverage of base from 146.344 to 2907.078. Statistics about the assemblies for each newly sequenced species were provided in Table S5. Accession numbers for all DNA sequences longer than 200 bp used in this study are listed in Table S6. Twenty protein-coding genes (from a total of 86 genes) and 32 NCR (from a total of 56 regions) included in our analyses do not have GenBank accession numbers because they are shorter than 200 bp. A list of gene regions and GenBank datasets, however, was submitted to TreeBASE (study ID: S23466). Properties of the different datasets are summarized in Table 2, and the best-fit models that partitioned the CR matrix by gene (32 partitions) are summarized in Table S3.

In our analyses, 86 CR and 56 NCR were used. The combined dataset was 57 278 bp for CR and 23 473 bp for NCR. Ambiguous sites were excluded from analyses and resulted coding and non-coding datasets were 56 450 bp (CR$_{-M}$) and 19 671 bp (NCR$_{-M}$), respectively. Of the total 76 121 characters that we analyzed (CPG$_{-M}$), 56 206 sites were constant (73.84%) and 14 338 sites were parsimony informative (18.84%). The aligned regions and the excluded ambiguous sites of the individual loci are listed in Tables S1 and S2.

3.2 Phylogeny of Dipsacales

For the CR and CPG datasets, the backbone phylogenies obtained from both ML and BI analyses were identical in topology (Figs. 1, 2, S1–S3). In all analyses, the monophyly of Dipsacales was strongly supported (ML bootstrap = 100%, BI posterior probability (PP) = 1.00, hereafter) and *Quintinia verdonii* of Paracryphiales, was sister to Dipsacales.

Three species of *Valeriana* formed a long branch (Figs. 1, 2, S1). Removing the long-branch taxa and the third codon positions could reduce long-branch attraction artifacts (Lyons-Weiler & Hoelzer, 1997; Bergsten, 2005). After excluding faster evolving third codon positions (dataset CR12$_{-M}$), however, the long-branch still existed (Fig. S4). To test the effect of this long-branch on the topology, as practiced by some studies to alleviate the long-branch attraction artifact (Duvall & Ervin, 2004; Hampl et al., 2009), we excluded three long-branched taxa and only one representative (*Patrinia triloba* var. *takeuchiana*) of Valerinaceae was retained. Following the removal of these taxa, the topology and relationships among all remaining clades were not altered (Fig. S2). As a result, we included *Valeriana* species in the final analyses.

Within Dipsacales, two major clades were recovered (Figs. 1, 2, S1–S5). The first one is Adoxaceae s. l., comprising *Viburnum*, *Tetradoxa*, *Adoxa*, *Sinadoxa*, and *Sambucus*. In this clade, two subclades can be recognized, *Viburnum* (100%, 1.00; Figs. 1, 2), and *Tetradoxa*, *Adoxa*, *Sinadoxa*, and *Sambucus* (100%, 1.00; Figs. 1, 2). Many relationships within *Viburnum* are well supported and the topology of the genus is identical to that of Clement et al. (2014) and thus the clade name used here also followed with their study. *Viburnum clemensiae* Kern is sister to the remaining members of the clade (98%, 1.00; Fig. 1). The next branching member is the "*Pseudotinus*" clade plus the "*Valvatotinus*" clade (76%, 1.00; Fig. 1), which together are sister to the remainder of the *Viburnum* clade that were well supported (99%, 1.00; Fig. 1) and divided into two subclades, that is, the *Perplexitinus* clade (100%, 1.00; Fig. 1) and the *Nectarotinus* clade (100%, 1.00; Fig. 1), as suggested by Clement et al. (2014).

The second major clade is Caprifoliaceae s. l. (100%, 1.00; Fig. 1). Within this clade, *Weigela florida* (Bunge) A. DC. (Diervillaceae s. str.) is sister to the remainder of the Caprifoliaceae s. l. (100%. 1.00; Figs. 1, 2). Following this branch, two subclades can be recognized. The first subclade is Caprifoliaceae s. str. (100%, 1.00; Fig. 1) comprising *Heptacodium*, *Symphoricarpos* Duhamel, *Triosteum* L., *Leycesteria* Wall., and *Lonicera* L. The monotypic genus *Heptacodium* is sister to other Caprifoliaceae s. str. (or Caprifolieae) (100%, 1.00; Fig. 1). Two species of *Symphoricarpos* did not group together, one is with *Triosteum himalayanum* Wall., and the other is with *Leycesteria formosa* Wall. The second subclade comprises *Zabelia*, Morinaceae, Linnaeaceae, Valerianaceae, and Dipsacaceae. In all analyses, the sister relationship between *Zabelia* and Morinaceae (100%, 1.00; Fig. 1) and between Dipsacaceae and Valerianaceae (100%, 1.00; Fig. 1) are well supported. The monotypic genus *Triplostegia* is a member of Dipsacaceae and its placement received maximal support in all analyses.

4 Discussion

4.1 Overview of Dipsacales and placement of *Heptacodium*

The present study is based on combined analyses of complete plastomic sequences and represents a greatly increased number of genes from previous work (Backlund & Bremer, 1997; Bell et al., 2001; Donoghue et al., 2001; Zhang

Table 2 Data characteristics with models selected for each dataset used for phylogenetic analysis in this study

Dataset	Number of taxa	Number of sites after excluding ambiguous sites	Missing data (%)	GC (%)	ML	BI	−lnL (ML)	Harmonic mean in log-likelihood (BI)
CR	75	57 278	1.36	39.2	GTR+G	GTR+F +I+G4	−231 401.521922	−238 589.23
CR_{-M}	75	56 450	1.36	39.2	GTR+G	GTR+F +I+G4	−229 818.725842	−238 321.29
NCR	75	23 473	1.84	32.6	GTR+G	TVM+F+G4	−105 483.258072	−105 579.38
NCR_{-M}	62	19 671	1.84	33.4	GTR+G	TVM+F+G4	−93 120.500711	−93 216.04
CPG	75	80 751	1.51	37.8	GTR+G	GTR+F +I+G4	−339 456.237387	−344 387.41
CPG_{-M}	75	76 121	1.51	38.0	GTR+G	GTR+F +I+G4	−324 797.425020	−328 658.44
CR12	75	38 168	1.36	39.2	GTR+G	GTR+F +I+G4	−125 104.955908	−133 072.87
$CR12_{-M}$	75	38 060	1.36	43.1	GTR+G	TVM+F +I+G4	−124 889.181206	−132 883.88

BI, Bayesian inference; CPG, chloroplast genome sequences (CR and NCR); CR, coding regions; CR12, codon positions 1 and 2 (with the exclusion of faster evolving third codon positions); GC, guanine-cytosine content; $_{-M}$, ambiguously aligned positions were removed manually; ML, maximum likelihood; NCR, non-coding chloroplast regions.

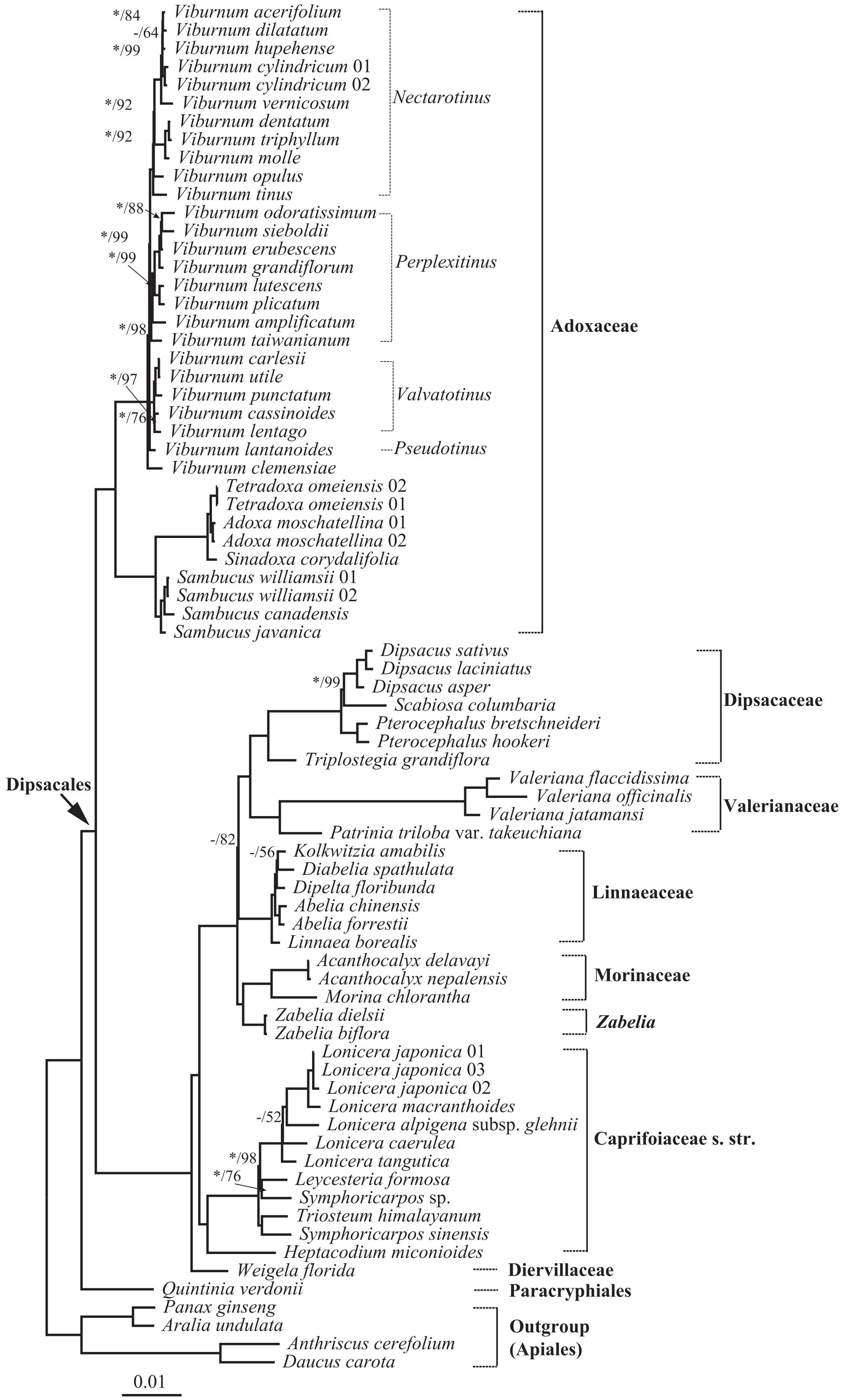

Fig. 1. Bayesian inference (BI) analysis of Dipsacales based on complete plastomic sequences (coding regions and non-coding regions) dataset, ambiguous sites were excluded for analysis. All clades are maximally supported for both BI posterior probability (PP) (1.00) and maximum likelihood bootstrap support (ML BS) (100%) except where noted. *BI PP = 1.00. –, ML BS and BI PP values <50% and PP <0.8.

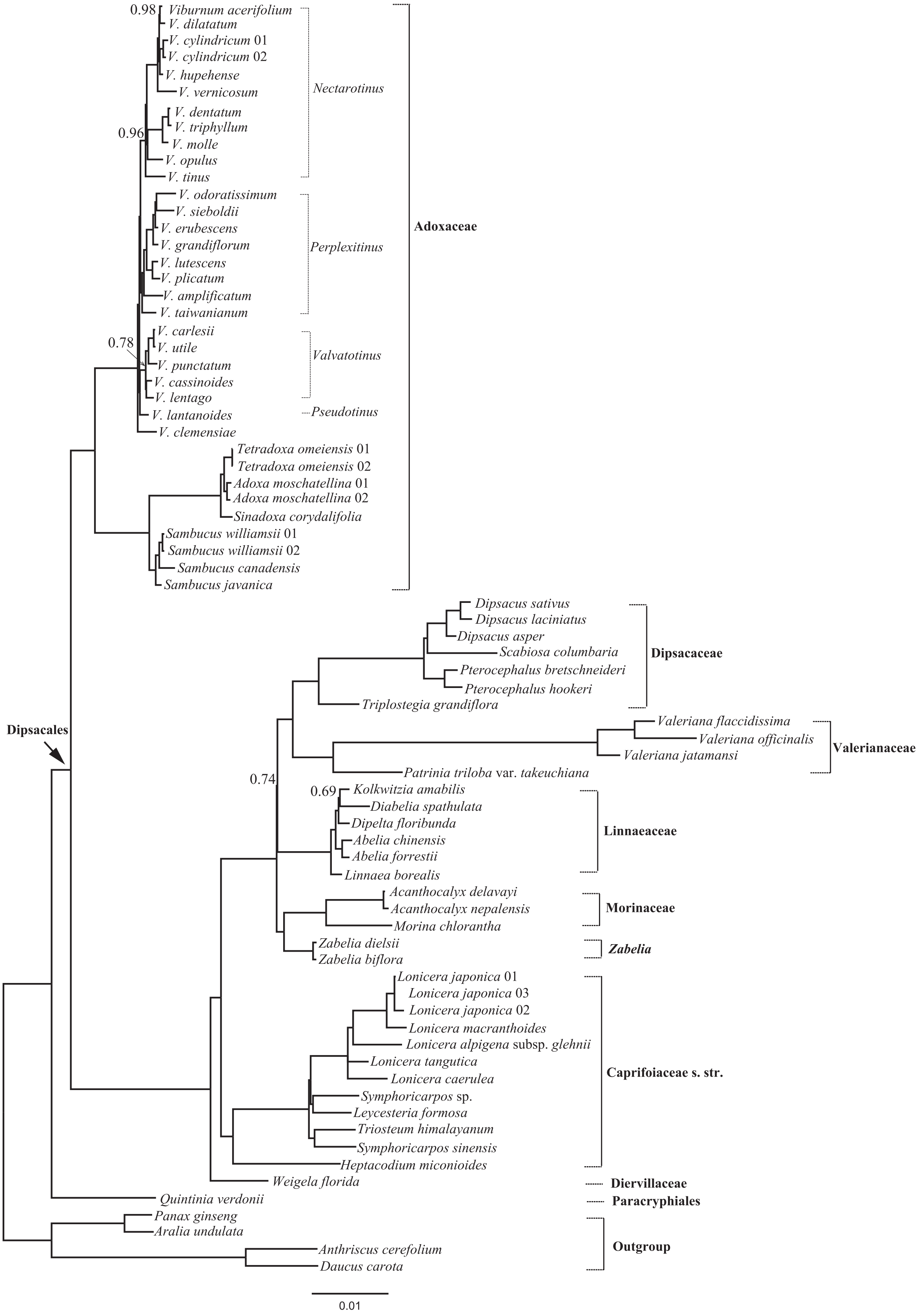

Fig. 2. Bayesian inference phylogeny of the Dipsacales, estimated with a partitioned analysis of dataset CR123$_{-M}$, ambiguous sites were excluded for analyses. All clades are maximally supported (1.00 posterior probability) except where noted.

et al., 2003; Beaulieu & Donoghue, 2013). The phylogenetic tree obtained here is in close agreement with previously published results based on far fewer genes (Bell et al., 2001; Donoghue et al., 2001; Zhang et al., 2003) and also mitochondrial DNA sequences (Winkworth et al., 2008a). We found that internal support values have a general increase here as compared to earlier studies (e.g., Donoghue et al., 1992; Backlund & Bremer, 1997; Bell et al., 2001; Zhang et al., 2003).

Although the genus *Symphoricarpos* is not a focal group for this study, the monophyly of *Symphoricarpos* was not recovered here. An unidentified *Symphoricarpos* species was sequenced by the 1KP project and we used those data here. A quick blast of the *rbcL* amino acid sequence from the 1KP sample shows 100% identity to that of *Symphoricarpos occidentalis* Hook. (Bennett et al., 2013). These results indicate the 1KP sample is indeed *Symphoricarpos* species. Actually, relationships among *Symphoricarpos*, *Triosteum* and *Leycesteria* varied in earlier studies. Some studies showed that *Symphoricarpos* and *Leycesteria* formed a sister group (Pyck & Smets, 2000; Smith, 2009; Jacobs et al., 2010b, 2011), others showed that *Symphoricarpos* grouped with *Triosteum* (Bell, 2010; Beaulieu & Donoghue, 2013; Wang et al., 2015). Thus, perhaps it is not strange that two *Symphoricarpos* species grouped with *Leycesteria* and *Triosteum*, respectively, in the current study. As a future research goal, the evolutionary history and relationships among these genera need further clarification.

The systematic position of *Heptacodium* has been challenging ever since its publication (Rehder, 1916). It has been placed in Linnaeaceae (Hara, 1983; Takhtajan, 1987; Tang & Li, 1994) or Caprifoliaceae (Fukuoka, 1972; Donoghue, 1983), or placed it within its own tribe, Heptacodieae (Golubkova, 1965). Molecular phylogenetic analyses have also provided contradictory results for the systematic position of *Heptacodium*. The systematic position of the genus was investigated for the first time by Pyck & Smets (2000) using molecular sequence data from *ndhF* and *rbcL* genes. As a result, *Heptacodium* was found to be phylogenetically closely related to *Lonicera*, *Triosteum*, *Symphoricarpos*, and *Leycesteria.* Taking into account molecular data and morphological characters, Pyck & Smets (2000) believed that *Heptacodium* is a member of the Caprifoliaceae s. str. Using more plastid DNA markers, Bell et al. (2001) and Zhang et al. (2003) also found *Heptacodium* is the sister group of Caprifoliaceae s. str., but with weakly supported values. Donoghue et al.'s (2003) combined analysis of nuclear ribosomal internal transcribed spacer (nrITS) and chloroplast DNA sequences obtained the same relationship with increased bootstrap support value. Most studies that followed suggest a position of *Heptacodium* as sister to Caprifoliaceae s. str. (Bell & Donoghue, 2005; Theis et al., 2008; Jacobs et al., 2010b; Wang et al., 2015), but in Winkworth et al.'s (2008a) study based on mitochondrial and plastid sequence data, the placement of *Heptacodium* among Caprifoliaceae s. str. and Linnaeaceae varied in different data matrices, and Jacobs et al. (2009) found a sister relationship between *Heptacodium* and the Linnina clade (i.e., Morinaceae–Caprifoliaceae s. str.–Dipsacaceae–Valerianaceae). Here, our analyses agree with previous findings (Pyck & Smets, 2000; Bell et al., 2001; Donoghue et al., 2003; Zhang et al., 2003; Theis et al., 2008; Jacobs et al., 2010b; Wang et al. 2015) that *Heptacodium* occupied a basal position with respect to the remaining members of Caprifoliaceae s. str.

A cytological study (Zhang et al., 2002) showed that *Heptacodium* has an unusual chromosome number ($2n = 28$) and is hypothesized to be the result of hybridization and polyploidization between two ancestral species of Caprifoliaceae s. str. and Linnaeaceae, which might have possessed a base chromosome number of $x = 8$ or 9. This hypothesis can explain why *Heptacodium* has many intermediate morphological characters between Caprifoliaceae s. str. and Linnaeaceae (see Jacobs et al., 2010a). For example, *Heptacodium* shares similar inflorescence structure with Caprifoliaceae s. str. (Airy-Shaw, 1952; Weberling, 1966) and unusual form of ovary and fruit development with Linnaeaceae (Hara, 1983; Tang & Li, 1994). Thus, Jacobs et al. (2010a) considered that the phylogenetic position of *Heptacodium* remains uncertain. In the most recent classification of Dipsacales, Hofmann & Bittrich (2016a) agreed with Jacobs et al.'s (2010a) argument.

Here, analyses based on complete plastomic sequences showed that *Heptacodium* is a member of Caprifoliaceae s. str. and sister to remaining members (Figs. 1, 2, S1–S5), and the cladistics analysis-based palynological data also support the placement of *Heptacodium* within Caprifoliaceae (Xu et al., 2011). The hybrid origin of *Heptacodium* is probably another scenario, but here maternally inherited plastid data cannot provide more explanation to this question. Future studies involving single-copy nuclear genes and increased taxon sampling could provide insights into the complex history of this genus.

4.2 Systematic position of *Triplostegia*

The taxonomic affiliation and the systematic position of the genus *Triplostegia* within Dipsacales have long been disputed and it has been placed in four different families: Valerianaceae (de Candolle, 1830; Verlaque, 1977; Cronquist, 1981), Dipsacaceae (Bentham & Hooker, 1873; Thorne, 1983), Morinaceae (Van Thieghem, 1909) or in a separate monotypic family, Triplostegiaceae (Airy-Shaw, 1973; Dahlgren, 1975; Takhtajan, 1987; Thorne, 1992a, 1992b).

The most controversial views are the placement of *Triplostegia* in Dipsacaceae or Valerianaceae. For a long time, *Triplostegia* has been associated with Valerianaceae, in both traditional classification (de Candolle, 1830; Höck, 1902; Graebner, 1906; Wagenitz, 1964; Verlaque, 1977; Cronquist, 1981) and modern analysis of morphological data (Backlund & Nilsson, 1997) or DNA sequences (Backlund & Bremer, 1997; APG, 1998). Some morphological traits united *Triplostegia* with Valerianaceae. For example, endosperm reduction in *Triplostegia* (Peng et al., 1995) or complete loss in Valerianaceae (Donoghue et al., 2001), apertures of pollen grains have a distinctive "halo" (Backlund & Nilsson, 1997), the presence of three distinct carpels (two of which abort), the presence of valepotriate iridoin compounds (Backlund & Moritz, 1998). Thus, Backlund & Nilsson (1997) recommend to put *Triplostegia* at subfamily level within Valerianaceae and later a subfamily name, Triplostegioideae, was proposed by Reveal (2012). However, other distinctive characters of *Triplostegia* differentiate this genus from members of Valerianaceae, for example, the basic number $x = 9$ found in *Triplostegia* is uncommon, and all genera in Valerianaceae have pollen with a disrupted nexine adjacent to the colpus margin (Eriksen, 1989).

As Donoghue et al. (2001) and Pyck & Smets (2004) indicated, *Triplostegia* is linked with both Valerianaceae and Dipsacaceae on the basis of simple perforation plates (or nearly so), further reduction of the calyx lobes, and the presence of chlorophyllous embryos (Yakovlev & Zhukova, 1980), as well as several pollen characters. Thus some authors place the genus in Dipsacaceae (Höck, 1902; Graebner, 1906; Wagenitz, 1964; Verlaque, 1977; Cronquist, 1981; Thorne, 1983), supported by some molecular phylogenetic studies. Using *trnL-trnF*, Zhang et al. (2001) found *Triplostegia* is sister to Dipsacaceae and suggested its placement in the Dipsacaceae as a subfamily. Later, Bell & Donoghue (2003) found that *Triplostegia* plus Dipsacaceae form a clade that is sister to Valerianaceae. Likewise, in Pyck & Smets's (2004) study, the topology of the combined *ndhF* and *rbcL* sequences showed *Triplostegia* to be sister to Dipsacaceae, but the morphological data placed *Triplostegia* as a sister of Valerianaceae. Later, Hidalgo et al. (2004) and Bell (2004) repeatedly confirmed the sister relationship between *Triplostegia* and Dipsacaceae. Different findings were reported in some studies. For example, *Triplostegia* was found to be sister to a clade including Dipsacaceae and Valerianaceae by Avino et al. (2009) based on four DNA regions (*trnL* intron, *trnL-trnF* intergenic spacer, *psbB-psbH*, and nrITS). Caputo & Cozzolino (1994) and Peng et al. (1995) also suggested that *Triplostegia* is a sister-group to a clade containing the Dipsacaceae and Morinaceae. Thus, in the most recent classification of Dipsacales, Mayer (2016) included it in Dipsacaceae, although its position in Dipsacaceae is unresolved. Very recently, Niu et al. (2018, 2019) put *Triplostegia* in its own subfamily (Triplostegioideae) under Caprifoliaceae.

Some previous analyses using nrITS, chloroplast DNA markers, and the combined data (Zhang et al., 2001; Bell, 2004; Hidalgo et al., 2004; Pyck & Smets, 2004) and our present data showed that *Triplostegia* and Dipsacaceae form a well-supported clade (Figs. 1, 2; 100%, 1.00). It might no longer be appropriate to refer to *Triplostegia* as *incertae sedis* because its phylogenetic position has now been inferred with considerable confidence. However, taxonomic and nomenclatural questions remain: should *Triplostegia* remain outside the family Dipsacaceae as a separate family, Triplostegiaceae, as adopted by some researchers (Airy-Shaw, 1973; Dahlgren, 1975; Thorne, 1992a, 1992b), or should the limits of Dipsacaceae be expanded to include *Triplostegia*? One consideration is the comparative degree of support for the two clades to which name might be applied. In all our analyses (Figs. 1, 2, S3), the support values for Dipsacaceae s. l. (including *Triplostegia*) (1.00, 100%) were identical for Dipsacaceae s. str. (excluding *Triplostegia*). With this in mind, either choice is tenable. Another consideration in deciding where to assign the family name is diagnosability by non-molecular means. Here, we prefer to include *Triplostegia* as a member of Dipsacaceae.

4.3 Systematic position of *Zabelia*

The systematic position of *Zabelia* has long been uncertain and thus impedes our understanding of Dipsacales evolution (Jacobs et al., 2011). *Zabelia* was originally placed in *Abelia* sect. *Zabelia* Rehder (1911) and later Makino (1948) segregated it from *Abelia* as a distinct genus. Subsequent studies added more morphological (Ikuse & Kurosawa, 1954; Fukuoka, 1968, 1969; Hara, 1983; Ogata, 1991), cytological (Kim et al., 2001), and palynological evidence (Erdtman, 1952; Hu & He, 1988; Kim et al., 2001) in support of Makino's concept.

Even though *Zabelia* is morphologically distinct from *Abelia*, it has been assumed that these two genera are closely related, and some molecular studies also reported a sister relationship between them (Zhou & Qian, 2003). Verlaque (1983) first hypothesized the close relationship between *Zabelia* and *Morina* L. based on pollen morphological characters. Using only *ndhF* sequence data, Pyck (2001) then confirmed the affinity between *Zabelia* and *Morina* clade. With broad sampling of Campanulidae as a whole, Tank & Donoghue (2010) came to similar conclusions using more DNA markers. Here, in all analyses, *Zabelia* is consistently resolved as sister to *Morinaceae* with maximal support (1.00, 100%; Figs. 1, 2). Jacobs et al. (2011) hypothesized two relationships regarding *Zabelia*, that is, sister to the *Morina* clade, *Abelia*, or the Valeriana clade, and our analysis confirmed the first hypothesis.

Kim et al. (1999) suggested that *Linnaea* L. is more closely related to the *Dipsacus* and *Valeriana* clades than to *Zabelia*, but species of other genera were not included in their study. Later, to address this controversial question, Jacobs et al. (2010b) used both nuclear and plastid DNA sequences for nine *Abelia* and five *Zabelia* species. As a result, *Zabelia* appeared to be either sister to the *Morina* clade or the *Valeriana* clade. However, support for the latter relationship was weak. Using intensive sampling of tribe Linnaeeae, Landrein et al. (2012) also found *Zabelia* (Zabeliinae) as sister to the *Dipsacus–Morina–Valeriana* clade, and they still considered the placement of *Zabelia* as not fully resolved. One more recent molecular investigation (Wang et al., 2015) found that *Zabelia* is neither sister to the *Morina* clade nor clustered with the *Abelia* or *Valeriana* clade, but sister to the *Morina* and *Linnaea* clade. In our analyses (Figs. 1, 2), sister relationships between *Zabelia*–Morinaceae and Linnaceae–Valerianaceae–Dipsacaceae were not highly supported. In order to resolve relationships among these group, broader sampling and more DNA markers are necessary in future studies.

The systematic position of *Zabelia* implies the paraphyly of Linnaeaceae but supported the monophyly of the subtribe Linnaeinae (Kim, 1998) or redefined and expanded genus *Linnaea* by Christenhusz (2013). Backlund & Pyck (1998) included five genera in Linnaeaceae, that is, *Abelia*, *Dipelta* Maxim., *Linnaea*, *Kolkwitzia* Graebn., and *Zabelia*. Because several molecular analyses have shown that the traditionally defined *Abelia* is polyphyletic (Landrein et al., 2012), to resolve *Abelia* as monophyletic, an old genus, *Vesalea* Martens & Galeotti, was resurrected and composing three Mexican species from *Abelia*. At the same time, a new genus, *Diabelia* Landrein (2010), was separated from *Abelia*, corresponding to three species from *Abelia* sect. *Serratae* (Graebner) T. Kim & B. Sun. *Vesalea* and *Diabelia* were not sampled in the present study, but their systematic affinities were well resolved by other studies (Landrein et al., 2012; Wang et al., 2015). Kim (1998) established a new subtribe, Zabeliinae T. Kim & B. Sun, to accommodate the systematic position of *Zabelia*. Here, our study indicated that *Zabelia* should be excluded from Linnaeaceae.

As to the systematic placement of *Zabelia*, there are four possibilities that can be considered based on the present study. The first strategy is to consider *Zabelia* as a separate family in Dipsacales, as suggested by Bittrich & Kadereit (2016), who thought that "an additional family will be needed for this genus" and recognized each clade as a family. Although *Zabelia* can be readily distinguished from other families within Dipsacales by having six distinct, longitudinal grooves on twigs and branches, and the development of aggregate rays (Ogata, 1991) and the unique nectary hair (Landrein & Prenner, 2016). Currently it is too early to propose a new family based on only a plastid tree, unless more evidence can be provided by subtle morphological studies and phylogenetic studies using nuclear genes in the future.

The second option is to include *Zabelia* in Morinaceae, as suggested by Xu et al. (2011). This treatment is acceptable technically, because the expanded Morinaceae does not violate the principle of monophyly. However, only one morphological character is now known to support the *Zabelia*–Morinaceae clade, that is, the shared presence of psilate pollen grains with an endocingulum (Kim et al., 2001). Thus, a Morinaceae with *Zabelia* included would be a family well-supported by molecular data, but hard to characterize using external morphological characters. Third, we can adopt the expanded definition of Caprifoliaceae and divide it into seven tribes to accommodate each clade. Four new tribe names need to be proposed to accommodate the *Zabelia* clade, Dipsacaceae, Morinaceae, and Valerianaceae, respectively. Otherwise, we can only raise *Zabelia* to subtribe level and still keep Dipsacaceae, Morinaceae, and Valerianaceae in Caprifoliaceae s. l., as treated by Donoghue et al. (2001) and Bell et al. (2001). Finally, we can still keep *Zabelia* as a separate genus. As noted above, we prefer to recognize *Zabelia* as a distinct genus and do not provide any taxonomical or nomenclatural changes in this study, unless future studies provide more convincing evidence.

5 Conclusion

Our study represents the first plastid phylogenomic investigation of the Dipsacales as a whole and offers new and important insights into the deep phylogenetic relationships of Dipsacales. The well-supported molecular phylogeny provides evidence for delineation of families within Dipsacales and seven families (Adoxaceae s. l., Caprifoliaceae s. str., Diervillaceae, Dipsacaceae, Linnaeaceae, Morinaceae, and Valerianaceae) were strongly supported as monophyletic. *Heptacodium* and *Triplostegia* were resolved as members of Caprifoliaceae s. str. and Dipsacaceae, respectively. In all analyses, *Zabelia* is sister to Morinaceae. These relationships were mostly resolved and strongly supported, but relationships among *Zabelia* plus Morinaceae, Linnaeaceae, and Dipsacaceae plus Valerianaceae need to be clarified in further studies. The phylogenetic backbone we provided should establish a framework for future comparative studies on Dipsacales evolution.

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/1.1111/jse.12526/suppinfo:

Fig. S1. Maximum likelihood (ML) phylogram of Dipsacales based on CPG-M dataset, ambiguous sites were excluded for analysis. A, Showing branch lengths, where tips were nor present but in the same order as shown in B. Branch represent the mean number of nucleotide substitutions per site. B, Bootstrap support values (ML-BS ≥ 50%) in maximum likelihood (RAxML) are shown above the branches.

Fig. S2. Bayesian Inference (BI) phylogram of Dipsacales based on CPG-M dataset, with the exclusion of three Valeriana species. A, Showing branch lengths, where tips were nor present but in the same order as shown in B. Branch represent the mean number of nucleotide substitutions per site. B, Bayesian posterior probabilities (PP ≥ 0.90) and bootstrap support values (ML-BS ≥ 50%) in maximum likelihood (RAxML) are shown above and below the branches, respectively.

Fig. S3. Phylogenetic relationship of Dipsacales as inferred from combined data set (CR-M) of 87 genes of coding regions, ambiguous sites were excluded for analysis. A, Bayesian inference phylogram, where tips were nor present but in the same order as shown in B. Branch represent the mean number of nucleotide substitutions per site. B, Maximum likelihood phylogram, bootstrap support values (ML-BS ≥ 50%) in maximum likelihood (RAxML) and Bayesian posterior probabilities (PP ≥ 0.90) are shown above and below the branches, respectively.

Fig. S4. Phylogenetic relationship of Dipsacales as inferred from CR12-M data set (including the first and second codon positions of 87 genes of coding regions), ambiguous sites were excluded for analysis. A, Bayesian inference phylogram, where tips were nor present but in the same order as shown in B. Branch represent the mean number of nucleotide substitutions per site. B, Maximum likelihood phylogram, bootstrap support values (ML-BS ≥ 50%) in maximum likelihood (RAxML) and Bayesian posterior probabilities (PP ≥ 0.90) are shown above and below the branches, respectively.

Fig. S5. The ML tree from the CR-M dataset with partitioned analysis, ambiguous sites were excluded for analysis. A, Showing branch lengths, where tips were not present but in the same order as shown in B. B, Cladogram of the ML tree, bootstrap support values (ML-BS ≥ 50%) in maximum likelihood (RAxML) are shown above or below the branches.

Table S1. Excluded ambiguous sites for 86 genes of coding regions (dataset CR123).

Table S2. Excluded ambiguous sites for 56 locus of non-coding regions (dataset NCR).

Table S3. Thirty-two partitions and models identified by PartitionFinder for dataset CR123.

Table S4. Best-fitting models and parameter values of datasets used for Bayesian inference analysis and resulting tree statistics.

Table S5. De novo assembly statistics summary of 28 new sequenced species in present study.

Table S6. Accession numbers of newly sequenced species.