



Phylogeny of the Linnaea clade: Are *Abelia* and *Zabelia* closely related?

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ABSTRACT

Phylogenetic investigations based on molecular and morphological data have improved our understanding of Dipsacales phylogeny dramatically over the past 20 years. The *Linnaea* clade, however, has mostly been neglected and *Zabelia* has rarely been included in previous studies. We present the results of a molecular investigation including nine *Abelia* and five *Zabelia* species based on nuclear (ITS) and plastid (*trnK*, *matK*, *atpB-rbcL*, *trnL-F*) sequence data using maximum parsimony, Bayesian inference, and maximum likelihood. Our results indicate that *Abelia* is paraphyletic and possibly polyphyletic. The genus falls apart into a Mexican clade, corresponding to *Abelia* section *Vesalea*, and an Asian clade (excluding *A. spatulata*), corresponding to *Abelia* section *Abelia*. A close relationship between *Zabelia* and other members of the traditional *Linnaea* clade is not recovered by our analyses. Instead, *Zabelia* is associated with either the *Morina* or the *Valeriana* clade. Support for a monophyletic *Linnaea* clade without *Zabelia* is strong.

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1. Introduction

With 1100 species in 41 genera and two families (Adoxaceae and Caprifoliaceae), Dipsacales is a fairly small order in the campanulid clade (asterids). Despite its size, morphological diversity is surprisingly high, including annual and perennial herbs, vines, shrubs, and large trees. The order originated in the late Cretaceous in eastern Asia and each major lineage still has roots in Asia today (e.g., Backlund, 1996; Wikstrom et al., 2001; Bell and Donoghue, 2003, 2005a,b; Winkworth and Donoghue, 2005; Moore and Donoghue, 2007; Smith, 2009; Smith and Donoghue, 2010). Dipsacales is, together with the small, Asian-Oceanic order Paracryphiales, sister to Apiales (Winkworth et al., 2008a; APG III, 2009).

Over the past 20 years, considerable effort has been made to improve our understanding of Dipsacales evolution (e.g., Zhang et al., 2003; Donoghue et al., 2003; Winkworth et al., 2008a,b). Although the relationships between the major Dipsacales lineages are well understood, several questions have proven difficult to answer, such as (1) the systematic position of *Heptacodium miconioides* Rehder, (2) the intergeneric relationships of the *Lonicera* clade, and (3) the intergeneric relationships of the *Linnaea* clade s.l. (*Linnaeaceae* sensu Backlund and Pyck, 1998; *Linnaeae* sensu Donoghue et al., 2001). This study addresses the latter question. While previous investigations have tried to resolve the intergeneric

relationships of the *Linnaea* clade s.l. (e.g., Verlaque, 1983; Kim et al., 1999; Pyck, 2001), the phylogeny of the *Linnaea* clade s.l. and the relationship between *Abelia* and *Zabelia* have remained equivocal.

Due to our increased knowledge of Dipsacales phylogeny, a number of new names and classifications have been proposed (e.g., Backlund and Pyck, 1998; Donoghue et al., 2001). Because this has led to a fair amount of nomenclatural confusion, we have chosen to assign informal names to the major lineages of Caprifoliaceae (Fig. 1). In what follows, the *Linnaea* clade s.l. corresponds to the traditional circumscription of the *Linnaea* clade (*Linnaeaceae* sensu Backlund and Pyck, 1998; *Linnaeae* sensu Donoghue et al., 2001) comprising *Abelia* R.Br. (Fig. 2A), *Dipelta* Maxim., *Kolkwitzia* Graebn., *Linnaea* L., and *Zabelia* (Rehder) Makino (Fig. 2B), whereas the *Linnaea* clade s.s. omits *Zabelia* (Fig. 1).

The traditional *Linnaea* clade s.l. has been recovered as the first diverging lineage of the *Linnaea* clade by a number of recent studies (e.g., Pyck, 2001; Donoghue et al., 2001; Bell et al., 2001; Winkworth et al., 2008b; Fig. 1). In the *Linnaea* clade, the woody *Linnaea* clade s.l. is often considered as an early diverged, ancestral lineage in comparison with the highly derived and predominantly herbaceous *Morina*, *Dipsacus*, and *Valeriana* clades. Although the monophyly of the *Linnaea* clade s.l. received strong support in the aforementioned studies, morphological synapomorphies supporting this hypothesis are scarce. Fukuoka (1968, 1969, 1972) described the members of the *Linnaea* clade s.l. as plants with cymose inflorescences, slightly zygomorphic flowers with one or three

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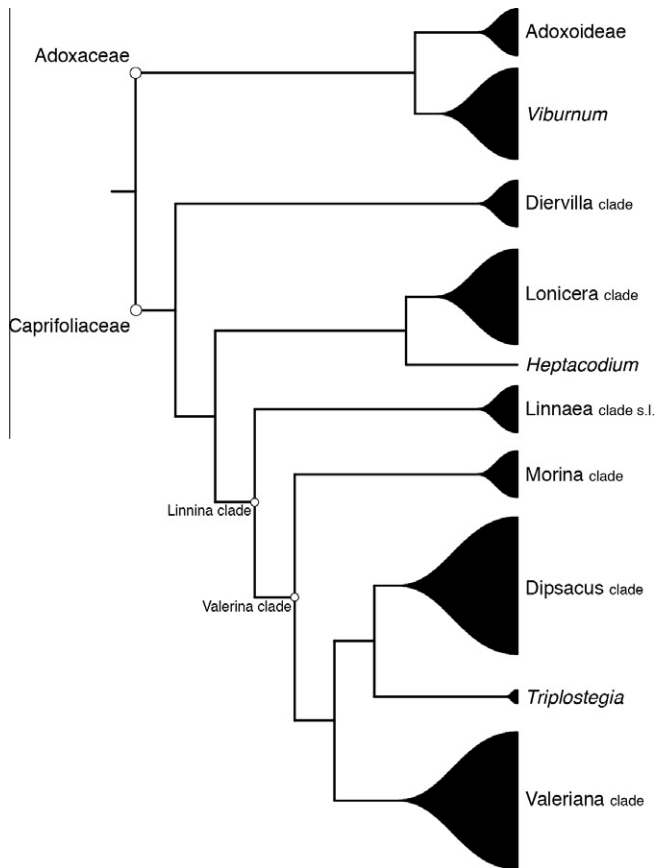


Fig. 1. Summary of Dipsacales phylogeny (based on Winkworth et al., 2008a, Fig. 3A).

(*Abelia* section *Vesalea*) nectaries, four didynamous stamens (sometimes five in *Zabelia*), and achenes as fruits. Free supernumerary bracts subtending each flower are only found in the Linnaea clade s.l., *Heptacodium*, and some members of the Valeriana clade.

With about 30 species of shrubs and subshrubs in five genera, the traditional Linnaea clade s.l. is one of the smaller lineages in Caprifoliaceae. Despite its size, the morphological diversity is remarkably high. *Abelia* (Fig. 2A) and *Zabelia* (Fig. 2B) are the most species rich genera with, according to Hara (1983), 15 and 10 species, respectively. *Dipelta* consists of three species, while *Kolkwitzia* and *Linnaea* are both monospecific. With the rest of the Linnina clade, the Linnaea clade s.l. shares a tricarpellate, inferior ovary with one fertile and two sterile carpels. The fertile carpel holds one, pendulous, anatropous ovule (although additional sterile ovules may be present), whereas the sterile carpels each contain several sterile ovules. After fertilization, the ovary matures into a dry, single-seeded, indehiscent achene (Wilkinson, 1948; Fukuoka, 1968; Hara, 1983). *Dipelta* is unique in the Linnina clade by having tetralocular ovaries with two fertile and two sterile locules, maturing into two-seeded, winged achenes (e.g., Fukuoka, 1968; Hara, 1983).

Kolkwitzia amabilis Graebn. (beauty bush) has received little attention from systematists and, as a result, we know fairly little about this shrub. It naturally occurs in Central and North China (Fukuoka, 1969; Hara, 1983). Its flowers are pentamerous and highly similar to the flowers of *Dipelta* (Hara, 1983). The inferior ovary has a long, slender neck, and a persistent, pentamerous calyx. The broad ovary base is densely covered with numerous, stiff trichomes that might aid in fruit dispersal. Several supernumerary bracts sclerify during fruit development and form a thick protective layer partially enclosing the fruit.



Fig. 2. (A) The flowers of *Abelia engleriana* Rehder (section *Abelia*) have a wide, infundibuliform corolla tube much reminiscent of those of *Dipelta* Maxim. (and *Kolkwitzia amabilis* Graebn.). (B) Flowering specimen of *Zabelia mosanensis* (Chung ex Nakai) Hisauti & Hara (series *Zabelia*) with much narrower and slightly curved corolla tubes.

Dipelta comprises three species distributed in Central and West China (Hara, 1983). In the Linnaea clade s.l., *Dipelta* is morphologically unique in several ways. After fertilization, several supernumerary bracts develop into large, papery appendages enclosing the fruit and promoting wind dispersal (Wilkinson, 1948; Fukuoka, 1968, 1969, 1972; Hara, 1983). As mentioned earlier, tetralocular ovaries and two-seeded achenes are only found in *Dipelta*. Like most members of the Linnina clade, the sterile locules contain several sterile ovules whereas the fertile locules each contain one fertile ovule (Jacobs B., unpublished data). While extant species of *Dipelta* are only distributed in eastern Asia, the fossil record shows that in the late Eocene/early Oligocene the genus was distributed as far as southern England (Manchester and Donoghue, 1995). Also, in North America, fossils (Late Eocene, Oligocene, and Miocene) were found of an extinct close relative of *Dipelta*, *Diplodipelta* (Manchester and Donoghue, 1995). Although the fruits of *Diplodipelta* are strikingly similar to those of *Dipelta* by having well-developed papery wings, it seems the wings of *Dipelta* and *Diplodipelta* are not homologous (Manchester and Donoghue, 1995).

Despite declining numbers of *Linnaea borealis* L. (twinflower), the small, evergreen subshrub remains widespread in boreal regions (Wilcock and Jennings, 1999). *Linnaea borealis* is characterized by long stolons sprouting both vegetative and flowering shoots (Fukuoka, 1969; Hara, 1983; Niva, 2003). The flowering shoots generally bear two flowers (hence its common name) with a pentamerous, deciduous calyx and an infundibuliform, slightly zygomorphic corolla (Wilkinson, 1948; Hara, 1983). Achenes hold one (sometimes

two) seed and are enclosed by two, fleshy bracteoles (supernumerary bracts) covered with numerous large, glandular hairs (Wilkinson, 1948). The seed holds a small embryo and copious endosperm (Hara, 1983).

Fukuoka (1968) segregated *Abelia* (Fig. 2A) into two sections, *Abelia* and *Vesalea*, in part based on the disjunct distribution of *Abelia*. Members of section *Abelia* are distributed in East and Central Asia, whereas section *Vesalea* is endemic to Mexico. *Zabelia* (Fig. 2B) is predominantly found in parts of the Middle East (Afghanistan, Turkistan) and North and East Asia (Hara, 1983). Originally, *Zabelia* was included in *Abelia* (or *Linnaea*) with the latter divided into two sections, *Euabelia* and *Zabelia* (Rehder, 1911). It was Makino (1948) who raised section *Zabelia* to the generic rank. Several subsequent studies provided additional morphological support for the separation of *Abelia* and *Zabelia* (e.g., Erdtman, 1952; Ikuse and Kurosawa, 1954; Fukuoka, 1968, 1969). Although both genera have tricolporate pollen grains, pollen of *Abelia* are echinate, whereas those of *Zabelia* are psilate (Erdtman, 1952; Verlaque, 1983). Furthermore, *Zabelia* has petioles with a dilated, connate base, a feature absent in *Abelia* (Hara, 1983). Ogata (1991) emphasized that wood anatomy of *Abelia* and *Zabelia* differs considerably. The wood of *Zabelia* is characterized by aggregate rays, visible as six regular, broad lines in cross section and corresponding to the distinct grooves on its branches and twigs (Ogata, 1991). Ogata (1991) further described the perforation plates of the wood of *Abelia* as scalariform, while *Zabelia* has vessels with simple (rarely scalariform) perforation plates.

Studies addressing the intergeneric relationships of the Linnaea clade s.l. and the systematic position of *Zabelia* are scarce. The phylogenetic study of Kim et al. (1999), based on *matK* sequence data, hinted at the paraphyly of the Linnaeae clade s.l. In this study, the genus *Linnaea* appeared to be more closely related to the Dipsacus and Valeriana clades than to *Zabelia*. Pyck (2001) carried out a phylogenetic investigation based on *ndhF* sequence data, including both *Abelia* and *Zabelia*, and reported a possible affinity between *Zabelia* and the Morina clade, confirming an earlier palynological study done by Verlaque (1983). However, support for the relationship between *Zabelia* and the Morina clade was low. Other investigations dealing with Dipsacales phylogeny have only included a single species of either *Abelia* or *Zabelia*. Two recent AFLP analyses (Zhou and Qian, 2003; Zhou et al., 2004) concentrated on the interspecific relationships of *Abelia*. Taxon sampling of these studies, however, was limited and only Asian *Abelia* species and two *Zabelia* species (*Abelia* section *Zabelia* in their study) were included. In the study of Zhou et al. (2004), *Kolkwitzia amabilis* appeared to be nested inside a clade of *Zabelia* species, whereas Zhou and Qian (2003) reported a sister relationship between *Abelia* and *Zabelia*. Despite considerable effort, molecular research has not been able to resolve the intergeneric relationships of the Linnaea clade s.l., (e.g., Pyck, 2001; Bell et al., 2001; Donoghue et al., 2001; Winkworth et al., 2008b). A recent, in-depth study of Dipsacales phylogeny stressed once more the problematic nature of the Linnaea clade s.l. (Winkworth et al., 2008b). Concurrent with previous studies, the analyses recovered *Linnaea borealis* as the basalmost taxon of the Linnaea clade s.l. However, *Zabelia* was not sampled and only one *Abelia* species (section *Abelia*) was included. In addition, intergeneric relationships changed with data sets, data partitioning, and evolutionary models. The lack of resolution in the Linnaea clade s.l. might be due to ancient, rapid radiation (Bell and Donoghue, 2005a; Winkworth et al., 2008b). Both clades originated in the Eocene and have radiated in a relatively short time-frame (Bell and Donoghue, 2005a).

With this study, we aim to further resolve the phylogeny of the Linnaea clade s.l. by including a broad sampling of the clade in addition to all the major Dipsacales lineages. The aims of the study are fivefold: (1) assessing the monophyly of *Abelia* and *Zabelia* as well as the Linnaea clade s.l.; (2) investigating the phylogenetic

relationship between *Abelia* and *Zabelia*; and (3) improving our understanding of the intergeneric relationships of the Linnaea clade s.l. By addressing these questions, we hope to further unravel the evolution of the order Dipsacales.

2. Materials and methods

2.1. Taxon sampling

Silica dried leaf material of 51 Dipsacales was collected in the field and botanic gardens, or acquired through collaboration with herbaria (Appendix A). The sampling includes 22 species of the Linnaea clade s.l. with nine *Abelia* and five *Zabelia* species. The sampling of *Abelia* included three Mexican species (section *Vesalea*; *A. floribunda*, *A. mexicana*, *A. occidentalis*) and six Asian species (section *Abelia*; *A. biflora*, *A. chinensis*, *A. engleriana*, *A. graebneriana*, *A. schumanii*, *A. spathulata*). Additionally, 26 species of all major caprifolioid lineages were added to the sampling and three members of Adoxaceae were included as an outgroup (Appendix A).

2.2. DNA isolation, amplification, and sequencing

A modified CTAB protocol was used for DNA isolation (Tel-Zur et al., 1999). Secondary metabolites were removed by washing ground leaf material with extraction buffer (100 mM Tris-HCl pH 8, 5 mM EDTA pH 8, 0.35 M sorbitol). After the addition of 700 μ l CTAB lysis buffer (as described in Chase and Hills (1991) with addition of 3% PVP-40) and 30 μ l Sarkosyl, the samples were incubated for 1 h (60 °C). Chloroform-isoamylalcohol (24/1 v/v) extraction was done twice, followed by an ethanol-salt precipitation (absolute ethanol, sodium acetate 3 M). After centrifugation, the pellet was washed twice (70% ethanol), air-dried, and dissolved in 100 μ l TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8).

Amplification of all five regions was done using standard PCR (25 μ l). Reactions initiated with a 3 min heating at 95 °C followed by 30–35 cycles consisting of 95 °C for 30–90s, 50–56 °C for 30–90s, and 72 °C for 30–90s. Reactions ended with a 3 min incubation at 72 °C.

Amplification and sequencing primers for ITS were adopted from White et al. (1990). The primers described by Young et al. (1999) were used for amplification and sequencing of the *trnK* and *matK* regions. Amplification and sequencing of *trnL-F* and *atpB-rbcL* was done using the primers of Taberlet et al. (1991) and Manen et al. (1994), respectively.

Purified PCR products were sequenced by the MacroGen sequencing facilities (MacroGen, Seoul, South Korea). Sequence editing and assembly was done in Geneious Pro v4.7 (Drummond et al., 2009). MUSCLE v4.0 (Edgar, 2004) was used for initial sequence alignment with a maximum of 25 iterations. In Geneious Pro v4.7 (Drummond et al., 2009), we scrutinized and manually optimized the alignment. Indels of plastid regions were coded as binary characters following simple indel coding as described by Simmons and Ochoterena (2000). Matrices and trees were submitted to TreeBASE (<http://www.treebase.org>, S2618) and newly obtained sequences to GenBank (Appendix A).

2.3. Phylogenetic analyses

Maximum parsimony (MP), Bayesian inference (BI), and maximum likelihood (ML) were used to analyze three data sets: (1) ITS sequence data; (2) plastid sequence data (*trnK*, *matK*, *atpB-rbcL*, *trnL-F*); and (3) combined sequence data (Table 1). The outgroup consisted of *Adoxa moschatellina* L., *Sambucus ebulus* L., and *Viburnum acerifolium* L.

Table 1
Statistics for the three data sets and the MP- and ML-analyses.

	Nuclear data set	Plastid data set	Combined data set (nuclear and plastid)
<i>Data sets</i>			
Number of taxa	51	51	51
Number of sites	795	3908	4703
Number of variable sites	368	1448	1816
Number of parsimony informative sites	246	852	1098
<i>MP</i>			
Number of shortest trees	6616	120	180
Tree length	1025	2260	3299
CI/RI	0.60/0.74	0.77/0.85	0.71/0.81
<i>ML</i>			
Likelihood value	6071	17120	23448

MP analyses were carried out using the command line version of PAUP* v4.0b10 (Swofford, 2002). Heuristic searches were conducted on 1000 random addition replicates with five trees held at each step, tree-bisection reconnection branch swapping in effect, and all characters unordered and equally weighted. Bootstrap analyses with 100 pseudoreplicates and 100 repetitions were carried out to assess branch support. Apart from the number of repetitions, settings of the bootstrap analyses were identical to those of the original analyses. For each data set, three subsets were created and analyzed: (1) all data; (2) indels excluded; and (3) *Zabelia* excluded. Indels were excluded from the second subset to allow for a more accurate comparison with results of the ML analyses in which indels were not included. *Zabelia* was excluded from the third subset to scrutinize the impact of *Zabelia* on the resulting phylogenetic hypotheses.

BI analyses were carried out using MrBayes v3.1 (Ronquist and Huelsenbeck, 2003) with each marker placed in a separate partition and partitions unlinked. MrModeltest v2.2 (Nylander, 2004) selected a GTR+I+G model for the nuclear data and a GTR+G model for each plastid region. Indels were assigned to a separate partition and analyzed using an F81-based model, as suggested by Ronquist and Huelsenbeck (2003), with an ascertainment bias set to variable. Four chains, one cold and three heated initiated from a random starting tree, were run for two million generations with sample frequency and burn-in set to 100 and 2500, respectively. Analyses were terminated when the log likelihood values of the cold chain became stationary and the average standard deviation of split frequencies dropped below 0.01. The latter, however, was not always the case, not even after significantly increasing the number of generations. For each data set, we created and analyzed three subsets, identical to those used in the MP analyses.

ML analyses were conducted using RAxML-VI-HPC v2.2.3 (Stamatakis, 2006) with indel data excluded from all data sets. The argument for indel exclusion is that RAxML-VI-HPC v2.2.3 does implement a model for analyzing binary data. Each analysis included 1000 inferences on the original alignment with a random initial starting tree for each inference and GTRMIX set as the nucle-

otide substitution model. After 1000 inferences, the topology with the highest likelihood was chosen. Non-parametric bootstrapping was done on 2000 replicates with GTRMIX set as the nucleotide substitution model. The results were plotted onto the previously chosen topology with the best likelihood.

2.4. Comparing data sets and topologies

Using PAUP* v.4.0b10 (Swofford, 2002), a series of incongruence length difference tests (ILD; Farris et al., 1995) were carried out to search for incongruencies between the five markers (Table 2). To investigate the impact of (a) the instability of the Lonicera clade and (b) *Zabelia* on possible incongruencies, ILD tests were rerun with the Lonicera clade and/or *Zabelia* excluded (Table 2). Topologies were compared visually and by means of the approximately unbiased (AU, Shimodaira, 2002) and Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa, 1999) tests. The ML topologies based on the three initial data sets were compared with the MP, BI, and ML hypotheses from our analyses. Additionally, four constrained ML hypotheses (based on our combined data set) were added to the AU and SH tests. The applied constraints included (1) *Abelia* and *Zabelia* forming a clade, (2) *Abelia* being monophyletic, (3) *Abelia* section *Abelia* forming a clade with *Zabelia*, and (4) *Abelia* section *Vesalea* and *Zabelia* being monophyletic. Similar to the ILD tests, AU and SH tests were performed with and without *Zabelia*. Site-wise log-likelihoods were calculated with PAUP* v.4.0b10 (Swofford, 2002) and used as input for multiscale bootstrap resampling using Consel v0.1j (Shimodaira and Hasegawa, 2001). The multiscale bootstrap resampling was conducted with ten sets of 10000 replicates.

3. Results

Our results are congruent with previous investigations, that is, strong support for the monophyly of Adoxaceae and Caprifoliaceae, a well-supported basalmost position of the Diervilla clade in Caprifoliaceae, and a moderate to strong sister relationship of *Heptacodium miconioides* and the Lonicera clade. The sister relationship of the Linnina clade and the clade comprising *Heptacodium* and the Lonicera clade is also moderately to strongly supported. The instability of the Lonicera clade as shown by previous investigations (e.g., Theis et al., 2008; Winkworth et al., 2008b; Smith, 2009) is also apparent in our analyses. Support for the intergeneric relationships in the Lonicera clade ranged from weak to strong and differed with data sets and optimality criteria.

The following paragraphs focus on the relationships in the Linnina clade s.l. and the relationships between the major lineages of the Linnina clade. However, first, we briefly describe the intergeneric relationships in the Morina, Dipsacus, and Valeriana clades since they are consistent throughout our analyses (Figs. 3–5). In the Morina clade, *Acanthocalyx* is consistently recovered as the basalmost taxon, sister to *Morina* and *Cryptothladia*. As regards the Dipsacus clade, *Scabiosa* is sister to *Succisa* and *Succisella*. The sister relationship between *Triplostegia glandulifera* and the Dipsacus clade is recovered in all analyses and mostly with high support.

Table 2

Incongruence length difference test results. Tests are based on our complete sampling (1) as well as three subsets of this sampling: (2) *Zabelia* excluded; (3) outgroup limited to *Lonicera dioica* to assess the impact of the instability of the Lonicera clade; and (4) both *Zabelia* excluded and outgroup limited to *Lonicera dioica*.

	ITS/trnK	ITS/matK	ITS/atpB-rbcL	ITS/trnL-F
1. Complete sampling	0.80	<0.05	<0.05	0.55
2. <i>Zabelia</i> excluded	0.46	<0.05	<0.05	0.23
3. Outgroup limited to <i>Lonicera dioica</i>	0.67	<0.05	<0.05	0.44
4. <i>Zabelia</i> excluded and outgroup limited to <i>L. dioica</i>	0.28	<0.05	<0.05	0.17

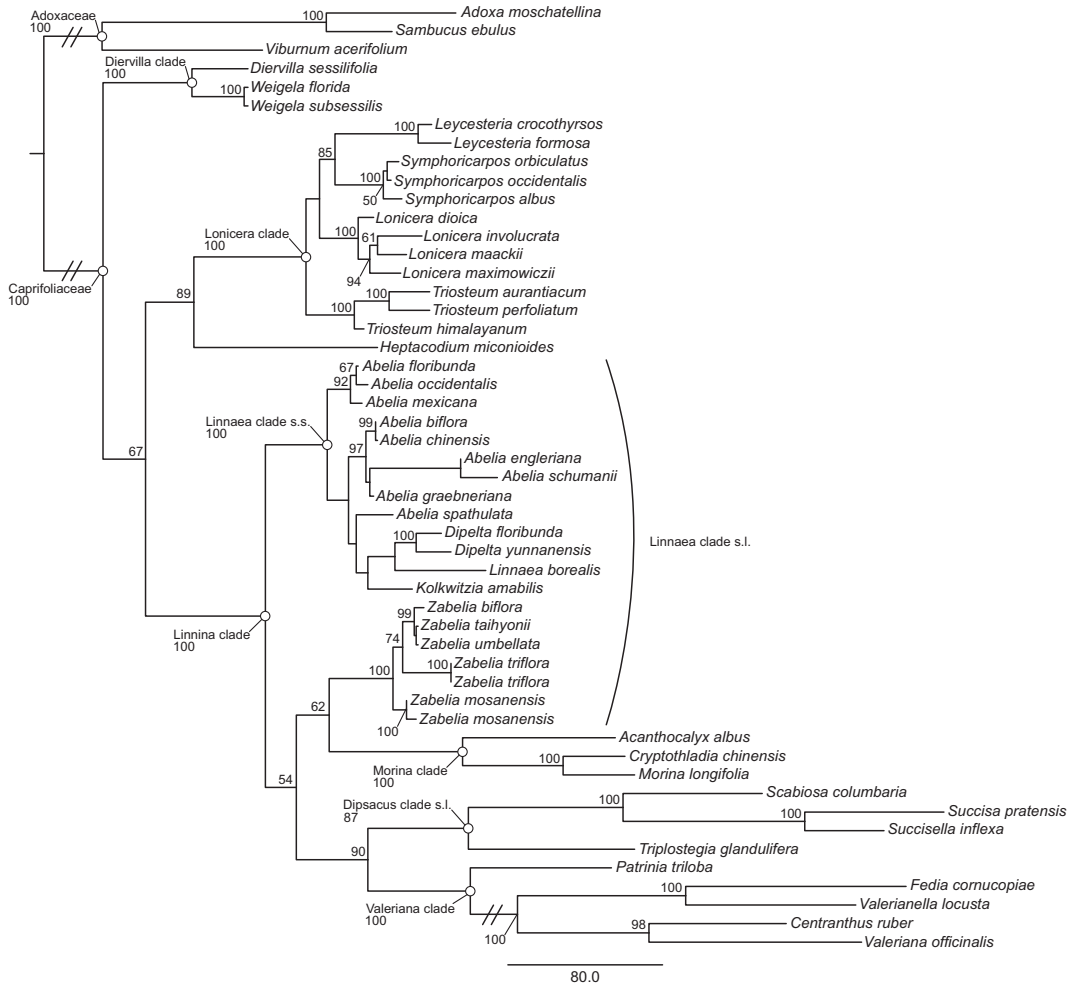


Fig. 3. Maximum parsimony hypothesis of Dipsacales phylogeny based on nuclear and chloroplast sequence data of 51 taxa. Branch support is indicated by bootstrap values.

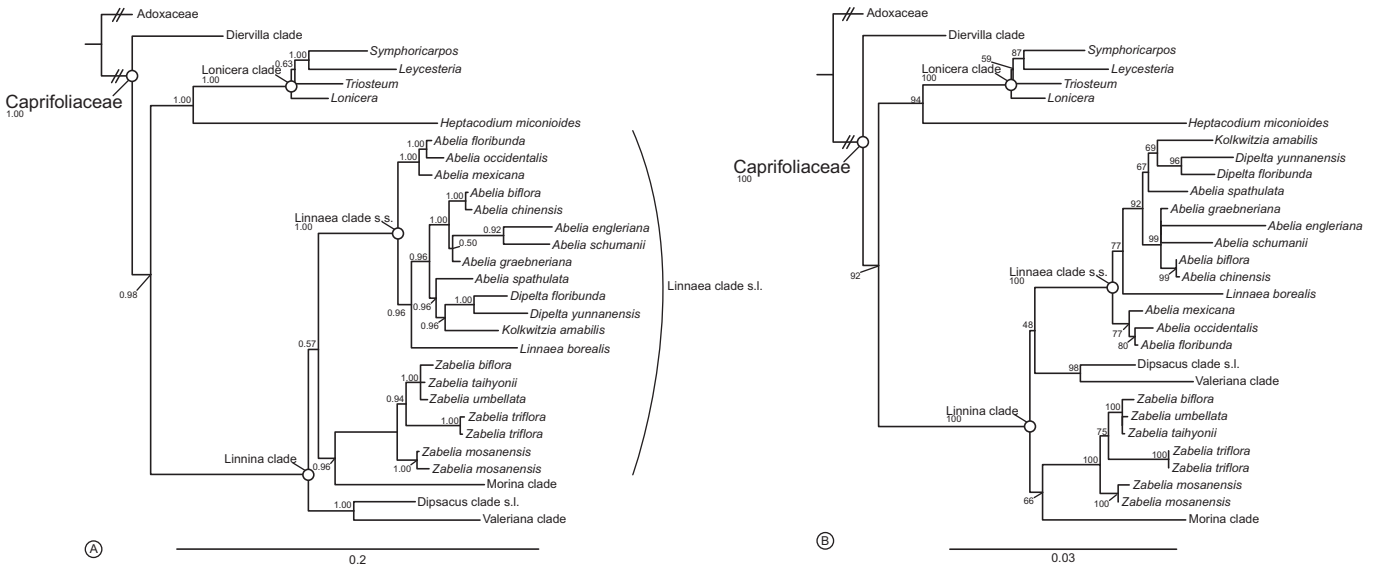


Fig. 4. (A) Bayesian inference hypothesis of Dipsacales phylogeny based on nuclear and chloroplast sequence data of 51 taxa. Branch support is indicated by posterior probability values. (B) Maximum likelihood hypothesis of Dipsacales phylogeny based on nuclear and chloroplast sequence data of 51 taxa. Branch support is indicated by bootstrap values.

For simplicity reasons, we refer to the clade comprising *T. glandulifera* and the Dipsacus clade as the Dipsacus clade s.l. Support for

Patrinia being sister to the core valerians (*Nardostachys* is not sampled) gains high support in our analyses. Inside the core valerians,

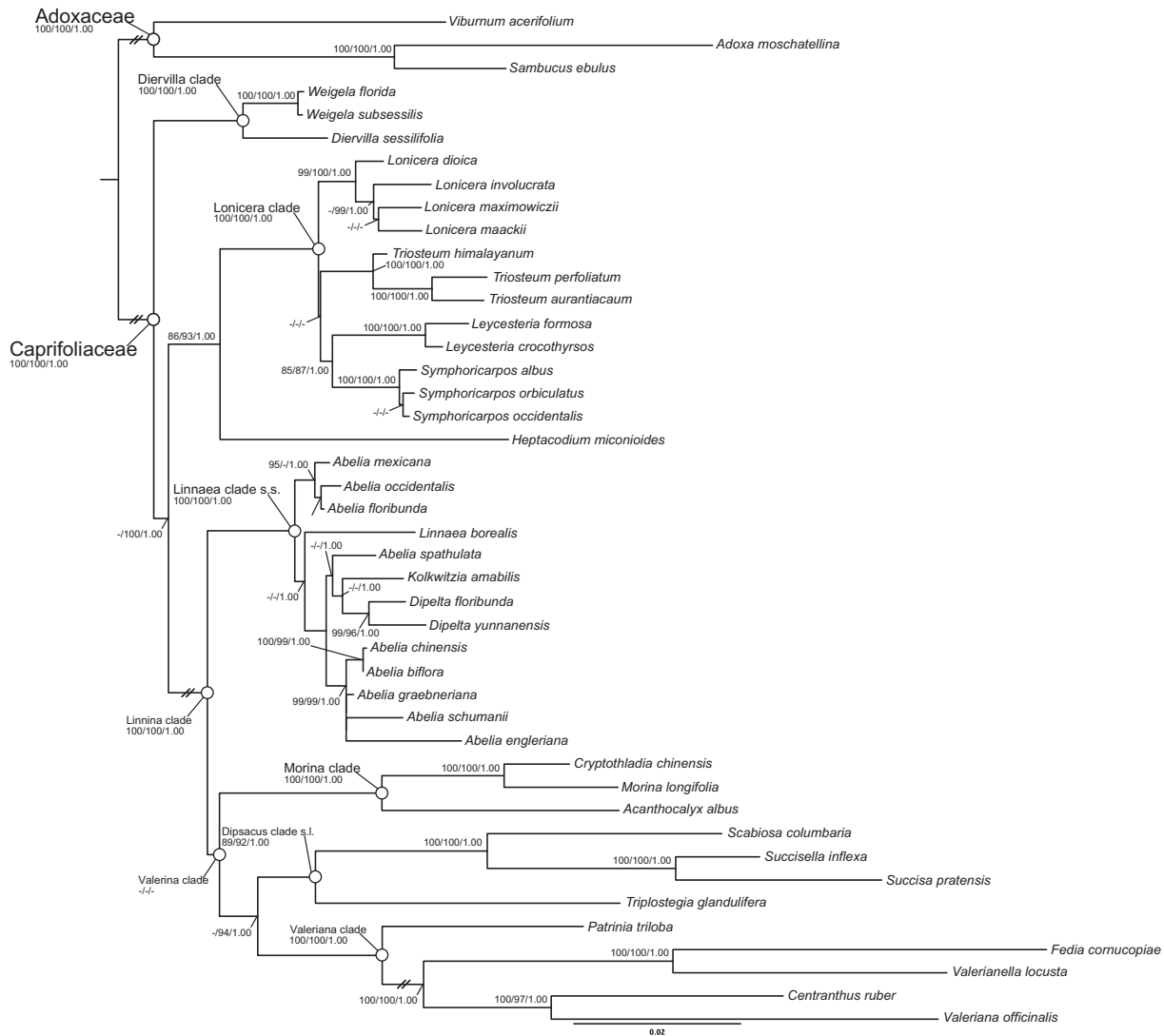


Fig. 5. Maximum likelihood hypothesis of Dipsacales phylogeny based on nuclear and chloroplast sequence data with *Zabelia* excluded. MP (first) and ML (middle) bootstrap branch support and Bayesian posterior probabilities (last) are indicated above a cut off value of 85 and 0.95, respectively.

Fedia and *Valerianella* are consistently recovered as sister to *Centranthus* and *Valeriana*.

3.1. Comparing data sets and topologies

ILD testing (Table 2) shows significant incongruencies between ITS and two of the plastid markers, *matK* and *atpB-rbcL*. Excluding *Zabelia* from the ILD tests and/or restricting the sampling of the *Lonicera* clade to *Lonicera dioica* does not resolve these incongruencies. Rerunning the MP-, BI-, and ML-analyses with the restricted *Lonicera* clade does not alter the relationships in the Linnina clade. These results indicate that the underlying cause of the incongruencies between the ITS and plastid sequence data is not restricted to the *Lonicera* clade or the inclusion of *Zabelia*.

AU and SH testing (Tables 3 and 4) confirms that topological incongruencies are caused by three factors: (1) incongruencies between markers (see ILD testing); (2) in- or exclusion of *Zabelia*; and (3) the optimality criterion. The MP topologies in particular differ significantly from the BI and ML topologies, which is confirmed by the AU and SH tests (Tables 3 and 4). Adding the four constrained ML hypotheses to the AU and SH tests shows that we can safely reject the hypothesis that *Abelia* (or any of its sections) and *Zabelia* form a clade. Although the SH tests do not reject the

hypothesis that *Abelia* section *Vesalea* and *Zabelia* form a clade, the likelihood value differs substantially from the likelihood values of the original ML topologies (Table 3). Not all tests reject the monophyly of *Abelia*. Although the AU tests are mostly unequivocal, the SH tests are not, which could be ascribed to the fact that SH tests are heavily biased (Shimodaira, 2002). The AU and SH tests illustrate this well as several alternative hypotheses that are rejected by the AU tests, are not by the SH tests.

3.2. Nuclear data set

The nuclear data set consists of 795 characters of which 368 are variable and 246 parsimony informative. Our MP analysis results in 6616 shortest trees with a tree length of 1025 (CI = 0.60, RI = 0.74). Although the MP consensus tree is not fully resolved, several major lineages are apparent. In Caprifoliaceae a basal polytomy includes the Diervilla and *Lonicera* clades, *Heptacodium miconioides*, and the Linnina clade. In the Linnina clade, another polytomy contains the Linnina clade s.s. (<85% BS), a monophyletic *Zabelia* (100% BS), and the Morina (91% BS), Dipsacus s.l. (60% BS), and Valeriana (74% BS) clades. Several clades are recovered in the Linnina clade s.s.: the Mexican *Abelia* species (98% BS); the Asian *Abelia* species; and *Dipelta*. Relationships between these lineages, however, are

Table 3

Comparing topologies using likelihoods as well as approximately unbiased (AU) and Shimodaira-Hasegawa (SH) tests.

Topology		ML nuclear			ML plastid			ML combined		
		Δ ln L	AU	SH	Δ ln L	AU	SH	Δ ln L	AU	SH
MP	Nuclear data set	154	<0.01	<0.01	1031	<0.01	<0.01	1107	<0.01	<0.01
	Plastid data set	55	<0.01	0.03	28	0.06	0.57	47	<0.01	0.40
	Plastid data set no indel data	77	<0.01	<0.01	20	0.01	0.62	66	<0.01	0.31
	Combined data set (nuclear and plastid)	35	0.01	0.13	44	<0.01	0.40	46	0.015	0.41
	Combined data set no indel data	34	0.03	0.13	15	0.01	0.72	15	0.109	0.79
BI	Nuclear data set	2	0.45	0.87	136	<0.01	0.10	107	<0.01	0.16
	Plastid data set	65	<0.01	<0.01	4	0.40	0.85	39	0.013	0.47
	Plastid data set no indel data	48	<0.01	0.04	0	0.71	1.00	17	0.034	0.74
	Combined data set (nuclear and plastid)	29	<0.01	0.20	0	0.71	0.99	1	0.665	0.98
	Combined data set no indel data	29	<0.01	0.20	2	0.11	0.98	2	0.133	0.97
ML	Nuclear data set	0	0.69	0.89	150	<0.01	0.07	117	<0.01	0.14
	Plastid data set	50	<0.01	0.03	0	0.61	0.97	16	0.053	0.76
	Combined data set (nuclear and plastid)	27	0.04	0.23	1	0.64	0.98	0	0.817	0.99
<i>Constrained hypotheses</i>										
	<i>Abelia</i> and <i>Zabelia</i> monophyletic (ML)	11	<0.01	0.12	161	<0.01	<0.01	165	<0.01	<0.01
	<i>Abelia</i> monophyletic (ML)	6	0.07	0.41	52	<0.01	0.36	39	<0.01	0.52
	sect. <i>Abelia</i> and <i>Zabelia</i> monophyletic (ML)	9	<0.01	0.13	162	<0.01	<0.01	164	<0.01	<0.01
	sect. <i>Vesalea</i> and <i>Zabelia</i> monophyletic (ML)	12	<0.01	0.10	107	<0.01	0.15	110	<0.01	0.13

Table 4Comparing topologies using likelihoods as well as approximately unbiased (AU) and Shimodaira-Hasegawa (SH) tests with *Zabelia* excluded from the sampling.

Topology		ML nuclear			ML plastid			ML combined		
		Δ ln L	AU	SH	Δ ln L	AU	SH	Δ ln L	AU	SH
MP	Nuclear data set	78	<0.01	<0.01	650	<0.01	<0.01	672	<0.01	<0.01
	Plastid data set	49	<0.01	0.03	25	0.09	0.52	43	0.02	0.35
	Combined data set	29	0.07	0.14	42	<0.01	0.33	43	0.02	0.35
BI	Nuclear data set	12	0.01	0.57	242	<0.01	<0.01	223	<0.01	<0.01
	Plastid data set	59	<0.01	<0.01	4	0.37	0.79	38	0.02	0.41
	Combined data set	22	0.11	0.27	1	0.29	0.89	0	0.09	0.92
ML	Nuclear data set	0	0.97	0.99	261	<0.01	<0.01	242	<0.01	<0.01
	Plastid data set	42	<0.01	0.04	0	0.82	0.98	15	0.04	0.69
	Combined data set	22	0.11	0.27	1	0.29	0.89	0	0.99	1.00

unresolved. In *Zabelia*, *Z. mosanensis* is sister to the rest of the genus (100% BS) and *Z. triflora* is sister to a trichotomy of *Z. biflora*, *Z. taihyonii*, and *Z. umbellata* (73% BS). Resolution of the BI topology is considerably higher than that of the MP consensus topology. BI recovers two major clades in the Linnaea clade: (1) *Zabelia* sister to the Valeriana clade (0.84 PP); and (2) the Linnaea clade s.s. (0.98 PP) sister to a clade holding the Morina and Dipsacus s.l. clades. In the Linnaea clade s.s., the Mexican *Abelia* species are sister to a trichotomy comprising (1) the Asian *Abelia* species, (2) *Linnaea borealis*, and (3) a clade holding *Dipelta* sister to *Kolkwitzia amabilis*. Interspecific relationships of *Zabelia* are identical to those recovered by MP. The ML topology with the highest likelihood is congruent with the BI topology. In the Linnaea clade s.s., however, the basalmost clade contains *K. amabilis* and *Dipelta*, followed by *L. borealis* being sister to a monophyletic *Abelia*. In the latter, the Mexican species are sister to the Asian species. Apart from the monophyly of the Mexican *Abelia* species (92% BS) and the sister relationship of *A. biflora* and *A. chinensis* (93% BS), bootstrap support is low for the relationships in the Linnaea clade s.s.

Excluding *Zabelia* from the analyses results in few changes. The MP consensus topology shows a Morina clade being sister to the Dipsacus s.l. clade. Based on BI, the relationships in the Linnaea clade s.s. alter with *Abelia* being monophyletic and divided into a Mexican and an Asian clade. Relationships between the genera are unresolved. Additionally, a clade holding the Morina and Dipsacus s.l. clades is recovered (0.99 PP) and appears to be sister to the Valeriana clade. In the ML topology (*Zabelia* excluded), the Mexican *Abelia* species are the basalmost clade in the Linnaea clade s.s. followed by *Dipelta*. *Linnaea borealis* is sister to a clade containing

Kolkwitzia amabilis and the Asian *Abelia* species. Among the Asian *Abelia* species, *A. spathulata* is hypothesized to be the basalmost taxon. However, support for the relationships within the Linnaea clade s.s. is low.

3.3. Chloroplast data set

The plastid data set consists of 3908 characters of which 1448 are variable and 852 parsimony informative. Indel data accounts for 158 parsimony informative characters. Our MP analysis results in 120 shortest trees with a tree length of 2260 (CI = 0.77, RI = 0.85). The resolution of the MP consensus tree is considerably higher than the nuclear based MP consensus topology. The Linnaea clade falls apart into a basal trichotomy: (1) the Linnaea clade s.s.; (2) *Zabelia* and the Morina clade; and (3) the Dipsacus s.l. and Valeriana clades. Interspecific relationships are unresolved in *Zabelia*. In the Linnaea clade s.s., the Mexican *Abelia* species are sister to the rest of the clade (100% BS). A clade comprising all Asian *Abelia* species but *A. spathulata* appears to be sister to a clade holding *A. spathulata*, a monophyletic *Dipelta*, *K. amabilis*, and *L. borealis*. In the latter clade, *A. spathulata* is sister to the rest of the clade with *K. amabilis* being sister to *Dipelta* and *L. borealis*. Based on BI and ML, the Linnaea clade is segregated into two clades: (1) the Dipsacus s.l. and Valeriana clades (1.00 PP; 80% BS); and (2) a clade holding *Zabelia* and the Morina clade (1.00 PP; 92% BS) sister to the Linnaea clade s.s. (1.00 PP; 100% BS). In the Linnaea clade s.s., the sole difference between BI and MP is the position of *Linnaea borealis*. Instead of being sister to *Dipelta* in the MP consensus topology, in the BI topology, *L. borealis* is sister to the Linnaea clade s.s. without the Mexican *Abelia* species.

Based on BI, the Mexican *Abelia* clade is the basalmost lineage in the Linnaea clade s.s. In the ML topology, the Mexican *Abelia* species and *L. borealis* switch positions so that *L. borealis* is the basalmost lineage of the Linnaea clade s.s. (100% BS).

Excluding *Zabelia* renders no changes in the MP and BI topologies apart from the Morina and Linnaea s.s. clades becoming sister clades. In the ML topology, the relationships in the Linnaea clade s.s. become identical to those of the BI topology after excluding *Zabelia* from the analysis.

Omitting indel data from the MP analysis leads to a lower resolved Linnaea clade s.s. with a basal polytomy containing *L. borealis*, the Mexican *Abelia* species, and a clade in which the Asian *Abelia* species (except for *A. spathulata*) are sister to a polytomy of *A. spathulata*, *Dipelta*, and *K. amabilis*. The BI topology remains unchanged.

3.4. Combined data set

Combining nuclear and plastid data results in 4703 characters of which 1816 are variable and 1098 parsimony informative. Indel data accounts for 158 parsimony informative characters. Our MP analysis results in 180 shortest trees with a tree length of 3299 (CI = 0.71, RI = 0.81). In the Linnina clade, the relationships between the major lineages change with the optimality criterion. Based on MP (Fig. 3), the Linnaea clade s.s. is sister to the rest of the Linnina clade (Fig. 3). *Zabelia* and the Morina clade are sister to the Dipsacus s.l. and Valeriana clades, but for this relationship support is low (Fig. 3). The BI topology (Fig. 4A) resolves the Linnaea clade s.s. as sister to *Zabelia* and the Morina clade, whereas the ML topology (Fig. 4B) resolves the Linnaea clade s.s. as sister to the Dipsacus s.l. and Valeriana clades. MP, BI, and ML agree on the basalmost position of the Mexican *Abelia* species in the Linnaea clade s.s. (Figs. 3 and 4). In the BI (Fig. 4A) and ML (Fig. 4B) topologies, *Linnaea borealis* is sister to a clade in which the Asian *Abelia* species (apart from *A. spathulata*) are sister to a clade with *A. spathulata*, *K. amabilis*, and a monophyletic *Dipelta*. In the latter clade, *A. spathulata* is sister to *K. amabilis* and *Dipelta* (Fig. 4). Apart from a strongly supported Asian *Abelia* clade (apart from *A. spathulata*) and a monophyletic *Dipelta*, relationships in the rest of the Linnaea clade s.s. are unresolved or poorly supported in the MP topology (Fig. 3). The close relationship of *A. chinensis* and *A. biflora* gains strong support in all analyses (99% BS, 1.00 PP; Figs. 3 and 4).

Topological changes after the exclusion of *Zabelia* are limited to the relationships between the major lineages of the Linnina clade (Fig. 5). In all analyses, the Linnaea clade s.s. is recovered as sister to the Valeriana clade, in which the Morina clade is resolved as sister to the Dipsacus s.l. and Valeriana clades. Changes within the major caprifolean lineages are not observed.

Excluding indel data from the MP and BI analyses results in a basal trichotomy in the Linnina clade: (1) the Linnaea clade s.s.; (2) *Zabelia* and the Morina clade (60% BS; <0.95 PP); and (3) the Dipsacus s.l. and Valeriana clades (91% BS; 1.00 PP). Additionally, the relationships in the Linnaea clade s.s. are also affected in the MP analyses: (1) a basal trichotomy composed of *Linnaea borealis*, the Mexican *Abelia* species, and the rest of the Linnaea clade s.s.; and (2) a sister relationship between the Asian *Abelia* species (without *A. spathulata*) and a clade with *A. spathulata* being sister to *Dipelta* and *K. amabilis*.

4. Discussion

4.1. Taxon sampling, incongruencies, and rapid radiation

In the past two decades, a significant number of phylogenetic studies have focused on Dipsacales (e.g., Pyck, 2001; Donoghue et al., 2001; Bell et al., 2001; Zhang et al., 2003; Bell and Donoghue, 2005a; Winkworth et al., 2008b; Smith and Donoghue, 2008) and

its major lineages (e.g., Kim and Kim, 1999; Pyck et al., 1999, 2002; Gould and Donoghue, 2000; Bell, 2004, 2007, 2010; Bell and Donoghue, 2003, 2005b; Theis et al., 2008; Carlson et al., 2009; Avino et al., 2009; Smith, 2009; Smith and Donoghue, 2010), which has resulted in a substantial improvement in our comprehension of Dipsacales evolution. Although the studies aimed at resolving the phylogeny of Dipsacales have all sampled the Linnaea clade s.l., sampling has often been limited (only one of two sections of *Abelia*) or incomplete (absence of *Zabelia*). Poor taxon sampling may significantly influence phylogenetic inference (e.g., Wortley et al., 2005; Jian et al., 2008), which is apparent in our analyses. The absence of *Zabelia* from most previous studies has created the false impression that the Linnaea clade s.l. is monophyletic. However, our study is not the first to report the possible paraphyly of the Linnaea clade s.l. Based on palynological data, Verlaque (1983) indicated the close relationship between *Zabelia* and the Morina clade. Also, two more recent molecular investigations (Kim et al., 1999; Pyck, 2001) have hinted at the paraphyly of the traditional Linnaea clade s.l. Employing *matK* sequence data, Kim et al. (1999) hypothesized that *Linnaea* is more closely related to the Dipsacus and Valeriana clades than to *Zabelia*. However, the authors did not sample other genera of the Linnaea clade s.l. Pyck (2001) came to similar conclusions by using *ndhF* sequence data and sampling all genera of the Linnaea clade s.l. She also confirmed Verlaque's (1983) hypothesis that *Zabelia* is more closely related to the Morina clade than to any other member of the Linnaea clade s.l. Support for this relationship, however, was weak. To date, no molecular study has thoroughly dealt with the phylogeny of the Linnaea clade s.l. by broadly sampling its members as well as both sections of *Abelia*, and this has led to the assumption that the genus *Abelia* is monophyletic. In addition, some authors continue to treat *Abelia* and *Zabelia* as a single genus (e.g., Zhou and Qian, 2003; Zhou et al., 2004).

Phylogenetic analysis and statistical testing have shown that incongruencies between nuclear and plastid sequence data are predominantly located in the Linnaea clade s.l. The incongruencies, for example, persist after restricting the outgroup (to *Lonicera dioica*) and excluding *Zabelia*. Furthermore, the intergeneric relationships are consistent in the Morina, Dipsacus s.l., and Valeriana clades independent of data set or optimality criterion. Two factors significantly contribute to these incongruencies and the lack of a clear, consistent phylogenetic signal in the Linnaea clade s.s. (1) Fast evolving molecular markers (such as ITS) may suffer from nucleotide substitutional saturation and this can result in an increase of homoplasious characters and a decrease of synapomorphies. In the end, this may lead to incongruencies as well as a weak and even incorrect phylogenetic signal (Wortley et al., 2005). It is important to note that nucleotide substitutional saturation is not the result of bad alignment, although a poor alignment may exacerbate incongruencies between data sets and conceal the phylogenetic signal. (2) Several recent investigations have shown evidence of rapid, ancient radiations in two problematic Dipsacales lineages, the Linnaea clade s.l. and the *Lonicera* clade (Bell and Donoghue, 2005a; Winkworth et al., 2008b; Smith, 2009; Smith and Donoghue, 2008, 2010). So far, the *Lonicera* clade is the only clade that has been examined in greater detail, although with limited success (Pyck et al., 1999; Theis et al., 2008; Smith, 2009; Smith and Donoghue, 2010). The short branches in our BI (Fig. 4A) and ML (Fig. 4B) topologies are the result of these rapid radiations and are a direct reflection of the limited number of synapomorphies supporting the recovered relationships in these clades. The radiations in the Linnaea clade s.l. have taken place in a timeframe no longer than 6–9 Mya (Bell and Donoghue, 2005a; Smith, 2009; Smith and Donoghue, 2008, 2010). The herbaceous lineages of the Linnina clade have also radiated explosively, however, these radiations coincided with a significantly increased rate of molecular evolution, which is visible in the longer branches in our BI (Fig. 4A) and ML (Fig. 4B) topologies. Ancient, rapid radiations, such as the radiation of the Linnaea clade s.l., are known to hinder phylogenetic inference due to a weak phylogenetic signal

caused by a lack of synapomorphies, increased homoplasy as well as extinction (Jian et al., 2008).

As mentioned earlier, the branch lengths of the BI and ML topologies also show the significant differences in rates of molecular evolution between the woody Caprifoliaceae (*Heptacodium*, and *Diervilla*, *Lonicera*, and *Linnaea* s.l. clades) and the herbaceous lineages (*Morina*, *Dipsacus* s.l., and *Valeriana* clades). Smith and Donoghue (2008) argue that the generally shorter life histories of herbaceous lineages result in an increased rate of molecular evolution. Lineage dating has shown that the majority of Dipsacales diversity emerged only recently (since 10 Mya) as a result of this explosive radiation of the herbaceous *Dipsacus* and *Valeriana* clades.

4.2. *Abelia*

Based on the disjunct distribution of *Abelia*, inflorescence structure, and flower morphology, Fukuoka (1968) segregated the genus into two sections: (1) section *Abelia*, comprising the Asian species; and (2) section *Vesalea*, including the Mexican species. Section *Abelia* was further divided into three series, series *Abelia*, which Fukuoka denoted as “most primitive”, series *Uniflorae*, and series *Serratae*. Several plesiomorphic features characterize section *Vesalea* and these made Fukuoka (1968) assume that the section is one of the first diverging lineages of the *Linnaea* clade s.l. Our results confirm the latter hypothesis as the Mexican *Abelia* species are recovered (with strong support) as the basalmost lineage of the *Linnaea* clade s.s. in most of our analyses. The monophyly of the Mexican *Abelia* species and their basalmost position in the *Linnaea* clade s.s. consistently received strong support. Fukuoka’s section *Abelia* is paraphyletic in our analyses, except for the BI- and ML-analyses based on nuclear data (poorly supported). Support for a monophyletic Asian clade without *Abelia spathulata*, however, gains moderate to strong support.

Although Fukuoka’s individual sections are in part supported by our analyses, the monophyly of the genus is not. A sister relationship between both sections is only recovered by the ML analysis based on nuclear data. This relationship, however, receives low support. Based on our results, we can conclude that *Abelia* is paraphyletic and possibly polyphyletic as *A. spathulata* seems more closely related to *Kolkwitzia amabilis* and *Dipelta* than to any other Asian *Abelia*.

A number of morphological differences separate the Mexican and Asian *Abelia* species. The former have flowers with three narrow, adnate nectaries, a narrow, subregular corolla tube without a protuberance at their base, and a pentamerous calyx, whereas the Asian *Abelia* species have one nectary, a wide, zygomorphic corolla tube with a small protuberance at their base (in which the nectary resides), and a di- to pentamerous calyx (Rehder, 1911; Fukuoka, 1968, 1969; Hara, 1983; Villareal, 1997; Villareal and de La Rosa, 2000).

4.3. *Zabelia*

Although analysis of the nuclear data set suggests a close relationship with the *Valeriana* clade, support for this relationship is low. Analysis of the plastid and combined data sets, however, hypothesize a close relationship with the *Morina* clade and corroborates with the findings of Verlaque (1983) and Pyck (2001). Either way, the *Linnaea* clade s.l. is paraphyletic as *Zabelia* appears to share no close relationship with any member of the *Linnaea* clade s.s. Palynological data support the results of Pyck (2001) and this study. *Zabelia* and the *Morina* clade both have psilate pollen characterized by an endocingulum, two features absent in other Dipsacales lineage (Verlaque, 1983; Jacobs et al., in press).

The monophyly of *Zabelia* is strongly supported regardless of the data set or optimality criterion. A number of morphological characters support this monophyly. Wood anatomy, in particular, appears to provide strong support for the monophyly of *Zabelia*. Ogata (1991)

emphasized that the presence of aggregate rays in the wood of *Zabelia* is one of its most distinguishing features and unique in Dipsacales. The aggregate rays are visible as six regular, broad lines in cross section and correspond to the six distinct grooves on branches and twigs (Ogata, 1991). Several other wood characters separate *Abelia* and *Zabelia*. In *Abelia* perforation plates of the vessels are scalariform with many bars, whereas *Zabelia* has simple or rarely scalariform perforation plates with few bars (Ogata, 1991; Jacobs et al., in press). Furthermore, the wood of *Zabelia* is ring-porous instead of diffuse-porous as observed in *Abelia* (Ogata, 1991; Jacobs et al., in press). Despite the fact that our results do not hypothesize a relationship with the *Lonicera* clade, Ogata (1991) indicated that *Lonicera* and *Symphoricarpos* share simple perforation plates and ring-porous wood with *Zabelia*. Finally, pollen of *Zabelia* is psilate, whereas all members of the *Linnaea* clade s.s. have echinate pollen grains (Erdtman, 1952; Jacobs et al., in press). The development of curved achenes and flowers with an hypocrateriform corolla tube are sometimes considered characteristic of *Zabelia* (e.g., Hara, 1983). However, curved achenes are also found in *Heptacodium* and flowers with a narrow corolla tube (the base of the corolla tube, in particular) also characterize the Mexican *Abelia* species (Villareal, 1997; Villareal and de La Rosa, 2000).

Based on inflorescence structure and flower morphology, Hara (1983) segregated *Zabelia* into two series, series *Zabelia* and series *Biflorae*. Ogata (1991), however, saw no wood anatomical differences between both series. Series *Zabelia* and *Biflorae* were both included in our sampling and our results do not support Hara’s serial classification. *Zabelia mosanensis* (series *Zabelia*) is recovered as sister to a clade in which *Z. triflora* (series *Zabelia*) is sister to a trichotomy of *Z. biflora* (series *Biflorae*), *Z. taihyonii* (series *Zabelia*), and *Z. umbellata* (series *Biflorae*). Additional evidence is needed before considering abandoning Hara’s serial classification.

Two recent AFLP analyses (Zhou and Qian, 2003; Zhou et al., 2004) have provided evidence for a paraphyletic *Zabelia* (*Abelia* section *Zabelia* in their study) as *Kolkwitzia amabilis* was resolved nested inside *Zabelia*. The authors also reported a close affinity between *Abelia chinensis* and *A. spathulata*, while our results strongly link *A. chinensis* with *A. biflora*, and *A. spathulata* with *K. amabilis* and *Dipelta*. However, taxon sampling in these AFLP investigations was restricted to *Abelia* section *Abelia*, *K. amabilis*, and two species of *Zabelia* (*Abelia* section *Zabelia* in their study). *Dipelta* nor *Linnaea borealis* were included in their analyses.

4.4. Phylogenetic implications

Our results strongly indicate that the *Linnaea* clade s.l. is paraphyletic and possibly polyphyletic. A close relationship between *Zabelia* and any of the other members of the traditional *Linnaea* clade s.l. is not recovered in our analyses. Instead, *Zabelia* appears to be either sister to the *Valeriana* (low support) or the *Morina* clade. However, support for a monophyletic *Linnaea* clade s.s. is strong.

The ecology and reproductive biology of *Linnaea borealis* has been studied extensively (e.g., Wilcock and Jennings, 1999; Niva, 2003), whereas its systematic position remains an enigma. Despite the fact that the affinity between *Linnaea borealis* and other members of the *Linnaea* clade s.s. has been known for quite some time (e.g., Fukuoka, 1968; Hara, 1983), its exact placement inside the *Linnaea* clade s.s. has remained unclear. Morphologically and biogeographically, *Linnaea borealis* is the most peculiar genus of the *Linnaea* clade s.s. Our results are not entirely clear about the placement of *Linnaea borealis*, but a basal position, that is, being the first or second diverging clade, within the *Linnaea* clade s.s., gains strong support in our BI- and ML-analyses and is congruent with earlier hypotheses (e.g., Pyck, 2001; Bell et al., 2001; Donoghue et al., 2001; Zhang et al., 2003; Winkworth et al., 2008b). MP suggests a sister relationship with *Dipelta*, but with low support.

In the Linnina clade, the morphology of *Dipelta* is unique in several ways (e.g., ovary and fruit morphology) and it is therefore not surprising that our results strongly support its monophyly. A close relationship with *Kolkwitzia amabilis* is recovered in most of our analyses and corroborates with the results of previous studies (e.g., Pyck, 2001; Bell et al., 2001; Donoghue et al., 2001; Zhang et al., 2003; Winkworth et al., 2008b). Alternative phylogenetic hypotheses are poorly supported. One character supporting the relationship between *Dipelta* and *K. amabilis* is the persistence of sterile locules in mature achenes (Jacobs B., unpublished data). In *Abelia*, *Zabelia*, and *Linnaea borealis*, the fertile locule expands after fertilization and compresses the sterile locules. As a result, traces of the sterile locules are mostly absent at maturity (Jacobs et al., in press).

With our current knowledge, we can hypothesize that the ancestor giving rise to the Linnina clade had flowers and fruits much like those of *Abelia* and *Zabelia*, that is, zygomorphic flowers with a well-developed, 5-lobed corolla tube subtended by free supernumerary bracts, a trilobular ovary with two sterile locules containing many ovules and one fertile locule with one fertile ovule, and a dry, cylindrical, narrow achene bearing a persistent calyx and containing one spindle-shaped seed.

4.5. Conclusions

Based on our results, the following conclusions can be drawn: (1) the traditional *Linnaea* clade s.l. (Linnaeaceae sensu Backlund and Pyck, 1998; Linnaeaeae sensu Donoghue et al., 2001) is paraphyletic; (2) the *Linnaea* clade s.s. is monophyletic; (3) *Abelia* is paraphyletic and possibly polyphyletic; (4) the Mexican *Abelia* species (*Abelia* section *Vesalea*) form a monophyletic group and are most likely the first diverging lineage of the *Linnaea* clade s.s. (5) the Asian *Abelia* species (*Abelia* section *Abelia*) are paraphyletic; (6) *Zabelia* is monophyletic but not closely related to *Abelia*; and (7) *Kolkwitzia amabilis* and *Dipelta* are sister groups.

4.6. Future prospects

It is clear that more work needs to be done to further improve our understanding of the phylogeny of the *Linnaea* clade s.l. and the evolution of Dipsacales in general. Future phylogenetic studies should combine a broad taxon sampling with an increased amount of data, both morphological and molecular, followed by careful analysis. In addition, thorough morphological studies are much needed as they provide the key to understand character evolution.

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Appendix Appendix. 1

Source, voucher information, and GenBank Accession details of sampled taxa. Accessions in bold are newly acquired sequences for this study, whereas missing sequences are indicated by a dash. (spe-

cies, source, voucher, *trnK*, *matK*, *atpB-rbcL*, *trnL-F*, ITS). *Abelia biflora* Turcz. (sect. *Abelia*), Botanic Garden Esveld, The Netherlands, Jacobs 2009-12, -, **GU168639**, **GU168598**, **GU168691**, **GU168618**; *Abelia chinensis* R.Br. (sect. *Abelia*), Sir Harold Hillier Gardens and Arboretum, United Kingdom, Pyck 1989-2220, -, AY310461, -, AF366913, **GU168619**; *Abelia engleriana* Rehder (sect. *Abelia*), Botanic Garden Esveld, The Netherlands, Jacobs 2009-14, **GU168666**, **GU168640**, **GU168599**, **GU168692**, **GU168620**; *Abelia floribunda* Decne. (sect. *Vesalea*), Botanic Garden Esveld, The Netherlands, Jacobs 2009-11, -, **GU168641**, **GU168600**, **GU168693**, **GU168621**; *Abelia graebneriana* Rehder (sect. *Abelia*), Sir Harold Hillier Gardens and Arboretum, United Kingdom, Pyck 1977-0008, **GU168667**, **GU168642**, **GU168601**, **GU168694**, **GU168622**; *Abelia mexicana* Villareal (sect. *Vesalea*), National Herbarium Nederland (Leiden University Branch), The Netherlands, Calzada 21100, **GU168668**, **GU168643**, **GU168602**, **GU168695**, **GU168623**; *Abelia occidentalis* Villareal (sect. *Vesalea*), Nationaal Herbarium Nederland (Leiden University Branch), The Netherlands, Garcia 2406, **GU168669**, **GU168644**, **GU168603**, -, **GU168624**; *Abelia schumanii* Rehder (sect. *Abelia*), National Botanic Garden Belgium, Belgium, Pyck 85-0252, **GU168670**, **GU168645**, **GU168604**, **GU168696**, **GU168625**; *Abelia spathulata* Siebold & Zucc. (sect. *Abelia*), National Botanic Garden Belgium, Belgium, Pyck 92-2291-43, **GU168671**, **GU168646**, **GU168605**, **GU168697**, **GU168626**; *Acanthocalyx albus* (Hand.-Mazz.) M.J. Cannon, -, -, AY290027, AF446913, AF447003, AF446973, AY236183; *Adoxa moschatellina* L., -, -, EF490235, EF490235, AF446990, AF366927, U88194; *Centranthus ruber* (L.) DC., National Botanic Garden Belgium, Belgium, Pyck 001, **GU168672**, AF446926, AF447016, **GU168698**, **GU168627**; *Cryptothladia chinensis* (Pai) M.J. Cannon, -, -, AY290026, AF446914, AF447004, AF446974, AY236184; *Diervilla sessilifolia* Buckley, National Botanic Garden Belgium, Belgium, Pyck 82-6494, FJ745402, AF446907, AF446997, **GU168699**, AY236177; *Dipelta floribunda* Maxim., Sir Harold Hillier Gardens and Arboretum, United Kingdom, Pyck 1978-4099, -, **GU168647**, **GU168606**, **GU168700**, **GU168628**; *Dipelta yunnanensis* Franch., National Botanic Garden Belgium, Belgium, Pyck 93-1274-05, AY290042, AF446910, AF447000, **GU168701**, AY236180; *Fedia cornucopiae* (L.) Gaertn., National Botanic Garden Belgium, Belgium, Pyck 97-1125-86, **GU168673**, **GU168648**, AF447013, AF446983, AY236193; *Heptacodium miconioides* Rehder, National Botanic Garden Belgium, Belgium, Pyck 92-0130-16, FJ745412, AF446906, AF446996, **GU168702**, AY236176; *Kolkwitzia amabilis* Graebn., National Botanic Garden Belgium, Belgium, Roels DDM/88/0215, **GU168674**, AF446912, AF447002, **GU168703**, AY236182; *Leycesteria crocothyrsos* Airy Shaw, National Botanic Garden Belgium, Belgium, Pyck 1992-1691, **GU168675**, FJ745393, EU265520, **GU168704**, AF265277; *Leycesteria formosa* Wall., National Botanic Garden Belgium, Belgium, Pyck 82-6395, FJ745405, AF446902, AF446992, **GU168705**, AF265276; *Linnaea borealis* L., Linnaeus Garden Uppsala University, Sweden, Hansson HL20080001, AY290040, AF446911, AF447001, **GU168706**, AY236181; *Lonicera dioica* L., National Botanic Garden Belgium, Belgium, Pyck 51-3590, -, **GU168649**, EU265571, -, EU240713; *Lonicera involucrata* (Richardson) Banks ex Spreng., National Botanic Garden Belgium, Belgium, Pyck 53-6481, **GU168676**, **GU168650**, EU265550, **GU168707**, **GU168629**; *Lonicera maackii* (Rupr.) Maxim., National Botanic Garden Belgium, Belgium, Pyck 88-1731, **GU168677**, **GU168651**, EU265553, **GU168708**, FJ217883; *Lonicera maximowiczii* (Rupr.) Regel, National Botanic Garden Belgium, Belgium, Pyck 81-1860, **GU168678**, **GU168652**, EU265554, **GU168709**, **GU168630**; *Morina longifolia* Wall., Royal Botanic Gardens Edinburgh, United Kingdom, RBGE 1969-5386, AY290020, AF446915, AF447005, **GU168710**, AY236185; *Patrinia triloba* Miq., -, -, AY794316, AF446921, AF447011, AF446981, AY236191; *Sambucus ebulus* L., -, -, EF490239, EF490239, -, DQ679833, DQ521256; *Scabiosa columbaria* L., -, -, AY290032, AF446918, AF447008, AF446978, AY236188; *Succ-*

isa pratensis Moench, National Botanic Garden Belgium, Belgium, Pyck 19752365, AY290033, FJ745401, **GU168607**, AY290007, AY290018; *Succisella inflexa* (Kluk) Beck, National Botanic Garden Belgium, Belgium, Pyck 19761319, **GU168679**, **GU168653**, **GU168608**, **GU168711**, **GU168631**; *Symphoricarpos albus* (L.) S.F. Blake, Kasteelpark Arenberg Katholieke Universiteit Leuven, Belgium, Roels 004, FJ745410, AY310459, –, **GU168712**, AF265282; *Symphoricarpos occidentalis* Hook., National Botanic Garden Belgium, Belgium, Pyck 90-1416, **GU168680**, –, EU265523, **GU168713**, EU240668; *Symphoricarpos orbiculatus* Moench, National Botanic Garden Belgium, Belgium, Pyck 80-0921, **GU168681**, AF446904, AF446994, **GU168714**, AF265281; *Triosteum aurantiacum* E.P. Bicknell, Linnaeus Garden Uppsala University, Sweden, Pyck 1978-2045, **GU168682**, **GU168655**, **GU168609**, GU168715, AF265290; *Triosteum himalayanicum* Wall., Linnaeus Garden Uppsala University, Sweden, Pyck 1963-1032, **GU168683**, **GU168656**, –, **GU168716**, AF265286; *Triosteum perfoliatum* L., Linnaeus Garden Uppsala University, Sweden, Pyck 1963-1028, FJ745409, AF446905, AF446995, **GU168717**, AY236175; *Triplostegia glandulifera* Wall. ex DC., –, –, AY290034, AF446919, AF447009, AF446979, AY236189; *Valeriana officinalis* L., –, –, AY794362, AY310467, –, AY360120, DQ180745; *Valerianella locusta* (L.) Betcke, National Botanic Garden Belgium, Belgium, Pyck 92-2077-23, AY794398, AF446922, AF447014, **GU168718**, DQ354168; *Viburnum acerifolium* L., –, –, AY265160, AF446897, AF446987, AF446957, AY265114; *Weigela florida* (Bunge) DC., National Botanic Garden Belgium, Belgium, Pyck 51-0632, FJ745404, **GU168657**, **GU168611**, **GU168719**, AF078711; *Weigela subsessilis* L.H. Bailey, National Botanic Garden Belgium, Belgium, Pyck 93-1547-84, FJ745403, **GU168658**, **GU168612**, **GU168720**, AF078706; *Zabelia biflora* (Turcz.) Makino (ser. *Biflorae*), Arnold Arboretum, U.S.A., AA#94-23 B, **GU168684**, **GU168659**, **GU168613**, **GU168721**, **GU168632**; *Zabelia mosanensis* (Chung ex Nakai) Hisauti & Hara (ser. *Zabelia*), Botanic Garden Esveld, The Netherlands, Jacobs 2009-13, **GU168685**, **GU168660**, **GU168614**, **GU168722**, **GU168633**; *Zabelia mosanensis* (Chung ex Nakai) Hisauti & Hara (ser. *Zabelia*), Botanic Garden Utrecht University, U-2009RD701, **GU168686**, **GU168661**, **GU168615**, **GU168723**, **GU168634**; *Zabelia taihyonii* (Nakai) Hisauti & Hara (ser. *Zabelia*), Holden Arboretum, USA, Tubising 89-583, **GU168687**, **GU168662**, **GU168616**, **GU168724**, **GU168635**; *Zabelia triflora* (R.Br.) Makino (ser. *Zabelia*), National Botanic Garden Belgium, Belgium, Pyck 82-6561, **GU168688**, **GU168663**, **GU168617**, **GU168725**, **GU168636**; *Zabelia triflora* (R.Br.) Makino (ser. *Zabelia*), Private Garden Leuven, Belgium, Pyck 2008-0001, **GU168689**, **GU168664**, –, **GU168726**, **GU168637**; *Zabelia umbellata* (Graebn. & Buchw.) Makino (ser. *Biflorae*), Sir Harold Hillier Gardens and Arboretum, United Kingdom, Pyck 1977-3240, **GU168690**, **GU168665**, –, **GU168727**, **GU168638**.

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