Nuclear and plastid phylogenomic analyses provide insights into the biogeography and reticulate evolution of the Sino-Japanese disjunctive *Diabelia* (Caprifoliaceae)

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Abstract: Understanding biological diversity and the mechanisms of Sino-Japanese disjunctions are major challenges in eastern Asian biogeography. The Sino-Japanese flora has been broadly studied as an ideal model, however, few studies have focused on phylogeography and diversification at the genus level utilizing both nuclear and plastid data. *Diabelia* (Caprifoliaceae) is an East Asian genus, with a disjunctive distribution across the Sino-Japanese flora. However, relationships within *Diabelia* remain elusive, with none of the previous studies having inferred a species phylogeny using nuclear loci. In this study, we reconstructed the phylogenetic relationships among *Diabelia* based on nuclear and plastid sequence data by using target enrichment and genome skimming approaches, respectively. We found that the main clades within *Diabelia* were discordant between nuclear and plastid trees. Both nuclear and plastid phylogenetic analyses supported five main clades: *D. serrata*, *D. tetrasepala*, *D. spathulata* var. *sanguinea*, *D. spathulata* var. *stenophylla* and *D. spathulata* var. *spathulat*a. *Diabelia tetrasepala* was inferred to be the result of a hybridization event from the species network analyses. Divergence time estimation and ancestral area reconstructions showed that *Diabelia* originated in Japan during the early Miocene, with subsequent gene flow between China, Japan and Korea. Overall, our results support the division of *Diabelia* into five main clades and this research provides new insights in the speciation process and taxonomy of *Diabelia*.

Keywords: *Diabelia;* Phylogenomic; Incongruence; Evolution; Hybridization

1 Introduction

Understanding biological diversity and how different factors shaped the current biodiversity setting are major challenges for evolutionary biology (Jiang et al., 2016; Casebolt & Kowalewski, 2018; Wang et al., 2018; Martínez-Espinosa, 2021; Maguilla et al., 2021). The Sino-Japanese flora (SJFR) of East Asia was an important glacial sanctuary during the quaternary glacial period with the largest diversity of temperate plant species in the world (Qiu et a1., 2009; 2011; Zhao et al., 2019) and currently serves as a model system to study the genetic effects of geographic isolation on speciation. The SJFR extends widely over an area from Southwest China to the northernmost point of Japan, with complex topography and multiple climates (Qiu et a1., 2011; Lu et al., 2020). The eastern edge of the SJFR experienced dramatic plate changes during the Miocene and Pliocene, which may have played key roles in isolating ancient Japanese plant species from continental species (Ota, 1988). Studies investigating the mechanisms of disjunctive distributions have focused on vicariance and long-distance dispersal (Doyle et al., 2004; Baenfer et al., 2006; Bobo-Pinilla et al., 2018; Torres-Cambas et al., 2019; Wang et al., 2020; Nge et al., 2021).

Diabelia Landrein (Caprifoliaceae) is an East Asian endemic genus with a disjunctive distribution in China, Korea and Japan (Hara, 1983). *Diabelia* belongs to the subfamily Linnaeoideae (Landrein, 2010; Wang et al., 2015; Landrein and Farjon, 2020; Wang et al., 2021) and includes three species: *Diabelia serrata* with two sepals, *D. tetrasepala* with four big and one smaller sepal, and *D. spathulata* with five sepals (Fig. 1; Hara, 1983). The number of sepals in *Diabelia* range from two to five, with the intermediate sepal form observed in *D. tetrasepala* distributed in the sympatric areas of *D. serrata* and *D. spathulata* (Zhao et al., 2019; Wang et al., 2020). This intermediate form may be the result of hybridization. There have been several previous studies on the disjunctive distributions between China and Japan (e.g., Lu et al., 2020; Haq et al., 2020; Takano et al., 2020), with most studies focusing on species that are widely distributed in Japan. The disjunctive distribution of *Diabelia* dates back to the middle Oligocene, covering the long geological history of the formation of flora in China and

Japan (Yang et al., 2011; Shin et al., 2012; Zhao et al., 2019; Wang et al., 2020). Overall, exploring the internal phylogenetic relationship and biogeographic diversification of *Diabelia* is conducive for further inference of the disjunctive distribution mechanisms of SJFR in East Asia.

There have been several previous molecular phylogenetic studies involving *Diabelia* (Zhou et al., 2004; Landrein et al., 2012; Wang et al., 2015). However, relationships within *Diabelia* remain controversial due to limited markers and sampling. Zhou et al. (2004) conducted an Amplified Fragment Length Polymorphism (AFLP) analysis including six *Diabelia* samples and found that *D. serrata* from China was closely related to accessions of the same species from Japan. Using the nuclear internal transcribed spacer (ITS) and plastid fragments (*rbcL, ndhF, matK, trnL* intron and *trnL-F* intergenic spacer), Landrein et al. (2012) found a close relationship between *D. serrata* and *D. spathulata*, with *Dipelta* Maxim being sister to *Diabelia*. Wang et al. (2015) confirmed the same results of *Dipelta* sister to *Diabelia* based on nine plastid markers.

Subsequent molecular phylogenetic studies have enriched sampling across the SJFR to further investigate the phylogenetic relationship of *Diabelia*. Based on plastid fragments from 37 *Diabelia* samples, Zhao et al. (2019) found the non-monophyly of *Diabelia* species. Increasing the number of informative sites by using complete plastomes, Wang et al. (2020) successfully recovered the monophyly of *D. serrata* while the relationships of the other species remained unresolved. Overall, sufficient informative molecular data and a large taxonomic sampling are essential for the comprehensive reconstruction of phylogenetic relationships within *Diabelia*.

Organelle genes often exhibit phylogenetic patterns significantly different from nuclear markers (Toews and Brelsford, 2012; Leducq et al., 2017; Ji et al., 2019; Yao et al., 2019; Wang et al., 2020). Currently, target enrichment is emerging as the method of choice to obtain target sequence for numerous nuclear orthologs of many complex taxa (Wanke et al., 2017; Buys et al., 2019; Schneider et al., 2020; Granados Mendoza et al., 2020; Wang et al., 2021). Many studies have applied this method to obtain data sets for analyzing cyto-nuclear discordance, speciation, hybridization and polyploidy (e.g.,

Bogarin et al., 2018; Morales-Briones et al., 2018; 2021). Given that the species-level phylogeny of *Diabelia* was previously unknown, we obtained data through target enrichment and genome skimming of broadly sampled *Diabelia* across the SJFR to (1) explore robust intrageneric phylogenetic relationships and compare them against previous phylogenies, and (2) reconstruct the biogeography and evolution of the genus to offer a comprehensive phylogenetic framework for future studies.

2 Materials and Methods

2.1 Sampling

A data set of 47 accessions was analyzed including 42 *Diabelia* samples (including all three species of *Diabelia* and three varieties of *D. spathulata*) and five outgroup samples representing Linnaeoideae (*Abelia macrotera* var. *parvifolia*, *Dipelta floribunda*, *Kolkwitzia amabilis*, *Vesalea floribunda*, *Linnaea borealis*) (Landrein et al., 2012; Wang et al., 2015; Zhao et al. 2019; Wang et al., 2020). Voucher specimens have been deposited in the herbarium of the Institute of Tropical Agriculture and Forestry of Hainan University (HUTB), Haikou, China. Detailed information on the geographical distribution and voucher details for the 42 samples and the localities of the populations sampled in this study are shown in Table S1.

2.2 DNA extraction and sequencing

We used a modified CTAB method (Doyle & Doyle, 1987) to extract total genomic DNA from silica gel-dried tissue or herbarium accessions. The concentration of each extraction was checked with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). A total of 400 ng of DNA was sonicated with a Covaris S2 (Covaris, Woburn, MA) to produce fragments ~150-350 bp in length prior to library preparation. Libraries of genomic DNA were made following Weitemier et al. (2014). To ensure that genomic DNA was sheared approximately to the selected fragment size, all samples were evaluated on a 1.2% (w/v) agarose gel.

To obtain nuclear data we used a target enrichment approach (Weitemier et al.,

2014) and genome skimming for plastid data (Wang et al., 2020). Baits designed across Dipsacales (Wang et al., 2021) were used to target 428 putatively single-copy genes. Hybridization, enrichment and sequencing followed Wang et al. (2021). Plastid sequencing followed Wang et al. (2020).

2.3 Read processing and assembly

Trimmomatic v.0.36 (Bolger et al., 2014) was used to remove adaptor sequences and low-quality bases from the raw reads (ILLUMINACLIP: TruSeq_ADAPTER: 2:30:10 SLIDINGWINDOW: 4:5 LEADING: 5 TRAILING: 5 MINLEN: 25). HybPiper v.1.3.1 (Johnson et al., 2016) was employed to assemble nuclear loci. Exons were assembled individually to avoid chimeric sequences in multi-exon genes produced by potential paralogy (Morales-Briones et al., 2018), in which exons longer than 150 bp were set as a reference. Paralog detection was undertaken for all exons using the 'paralog_investigator' option in HybPiper. To obtain 'monophyletic outgroup'(MO) orthologs (Yang and Smith, 2014), all assembled loci (with and without paralogs detected) were processed following Morales-Briones et al. (2021).

For the plastome assemblies, clean reads were extracted from raw sequencing reads by using SOAPfilter_v2.2 to remove adapter sequences and low-quality reads. The resulting reads were then used as the input to assemble plastomes using MITObim v1.8 (Hahn et al. 2013) following Wang et al. (2020).

2.4 Phylogenetic analyses

Nuclear data sets. We used concatenation and coalescent-based methods to analyze the nuclear data. For concatenation analyses, sequence alignments of individual nuclear exons were performed with MAFFT v.7.407 (Katoh & Standley, 2013) and aligned columns with more than 90% missing data were removed using Phyutility (Smith and Dunn, 2008). A maximum likelihood tree from the concatenated matrix was inferred with RAxML v.8.2.20 (Stamatakis, 2014) using a partition-by-locus scheme and the GTRGAMMA substitution model for all partitions.

We assessed clade support with 100 rapid bootstrap replicates. In addition, Quartet Sampling (QS; Pease et al., 2018) was used to distinguish strong conflict from weakly supported branches with 1000 replicates. For coalescent species tree estimation, ASTRAL-III v.5.7.1 (Zhang et al., 2018) was used to estimate a species tree based on the individual exon trees constructed using RAxML with a GTRGAMMA model. We used local posterior probabilities (LPP) to calculate branch support (Sayyari and Mirarab, 2016).

Plastome data set. Complete plastomes were aligned using MAFFT v.7.407 (Katoh and Standley, 2013). A ML tree was inferred with IQ-TREE v.1.6.1 (Nguyen et al., 2015) under the extended model and 200 non-parametric bootstrap replicates for branch support. Additionally, branch support was evaluated using QS with 1000 replicates.

2.5 Species network analysis

We used PhyloNet (Wen et al., 2018) to infer a maximum likelihood species network with the InferNetwork_MPL command (Yu and Nakhleh, 2015). Due to computational restrictions and given that we were primarily concerned with the underlying network between different species, we reduced the 47-taxon data set to one outgroup and 14 ingroup taxa to represent all major clades within *Diabelia*. Network searches were undertaken using only nodes in the gene trees that had at least 50 percent bootstrap support, permitting up to five hybridization events, optimizing the branch lengths and genetic probability of the returned species networks under the full likelihood. The command 'CalGTProb' in PHYLONET was used to infer the maximum likelihood of the concatenated RAxML, ASTRAL, and plastid trees, given the individual gene trees to estimate the optimal number of hybridization events and inspect whether the species network represented a better model than a purely bifurcating tree. The bias-corrected Akaike information criterion (AICc; Sugiura, 1978), Akaike information criterion (AIC; Akaike, 1973), and the Bayesian information criterion (BIC; Schwarz, 1978) were used for model selection, with the best-fit-species

network having the lowest AICc, AIC and BIC scores.

2.6 Divergence time estimation

Divergence times were estimated with BEAST v.2.4.0 (Bouckaert et al., 2014). Following Wang et al. (2021), we used nuclear loci for estimating node ages owing to potential problems of recombination and incomplete concerted evolution. The root age was set to 50 Ma (lognormal prior distribution 32.66 - 57.81 Ma) following Wang et al. (2021). We selected the fruit fossil of *Diplodipelta* Manchester & Donoghue (offset 36 Ma, lognormal prior distribution 34.07 - 37.20 Ma, Bell & Donoghue, 2005) that could confidently be placed in our tree as an internal calibration point. Dating analyses were carried out using an uncorrelated lognormal relaxed clock under the GTR + G model for each marker partitioned separately and a Yule tree prior. The MCMC chains were run for 500,000,000 generations and sampling every 5000 generations. Tracer v.1.7 (Drummond et al., 2012) was used to check convergence with the first 10% of trees removed as burn-in and to assess that all effective sample size (ESS) values were ≥ 200. The produced tree files were combined using LogCombiner v1.8.2 and the maximum clade credibility tree was generated in TreeAnnotator v1.8.4 (Drummond et al., 2012) and visualized using FigTree 1.4.2.

2.7 Ancestral area reconstruction

The ancestral area of the genus was reconstructed using Statistical Dispersal-Vicariance Analysis (S-DIVA) in RASP version 4.2 (Yu et al., 2015) based on Bayesian Binary Method (BBM) with the nuclear concatenated data set. To prevent biased inferences towards wide or unlikely distributions for the crown node of the ingroup (only *Diabelia* species) due to the uncertainty in the root area of the outgroup, we pruned the five outgroups for our ancestral state reconstructions. Three areas were defined to cover the present distribution range of *Diabelia*: (A) Japan, (B) Korea and (C) China. Distribution areas of all populations in this study were defined according to

field observations (Table S1). The BBM analyses ran for 500,000,000 generations generations using 10 MCMC chains.

2.8 Analysis of character evolution

Morphological character states of *Diabelia* were coded from previous studies, specifically from Hara (1983) and Landrein et al. (2020). In this study, four traits (the number of sepals, the number of flowers, corolla colour and corolla shape) were scored to infer the evolution of morphological characters. The number of sepals were scored with the following: (0) Two sepals; (1) Five unequal sepals; (2) Five subequal sepals. The number of flowers were scored with the following: (0) Single flower; (1) Flowers paired; (2) Clusters of four-seven flowers. Corolla colour was scored with the following: (0) Yellow; (1) Pale yellow; (2) Red; (3) White. Corolla shape was scored with the following: (1) Tubular; (2) Bilabiate; (3) Infundibuliform. Ancestral character state reconstructions were carried out using the maximum-likelihood RAxML trees (nuclear and plastid data) in Mesquite v.3.51 (Maddison and Maddison, 2018). We selected the one-parameter Markov k-state evolution model for the analysis of discrete unordered characters.

2.9 Data accessibility

Raw Illumina data from target capture is available at the Sequence Read Archive (SRA) under accession SUB9796722(see Table S1 for individual sample SRA accession numbers). DNA alignments, phylogenetic trees and results from all analyses and data sets can be found in the Dryad data repository.

3 Results

3.1 Exon assembly

The number of assembled exons per species (with > 75% of the target length) (except the outgroups) ranged from 587 (*D. serrata* E314) to 989 (*D. spathulata* var. *sanguinea* E301), with an average of 889 exons per sample (Table S2; Fig. S1). The

number of exons with paralog warnings (except the outgroups) ranged from 34 in *D. serrata* E314 to 619 in *D. spathulata* var. *sanguinea* E301 (Table S2). The concatenated alignment had a length of 392,600 bp, including 18,057 parsimony-informative sites, and a matrix occupancy of 86.7 % (Table 1). The plastid alignment resulted in a matrix of 159,365 bp with 4,693 parsimony-informative sites and a matrix occupancy of 98.2 % (Table 1).

3.2 Phylogenetic reconstruction

We performed phylogenetic analyses based on nuclear genes and plastomes (Figs. 2, 3, S2 and S3). All nuclear phylogenetic analyses recovered five main clades: *D. serrata*, *D. tetrasepala*, *D. spathulata* var. *sanguinea*, *D. spathulata* var. *stenophylla* and *D. spathulata* var. *spathulata*. However, the relationships of the five main groups within *Diabelia* varied in the different analyses.

With respect to the nuclear concatenated ML tree, the phylogeny was recovered with high support values (BS > 70; except for the placement of *D. spathulata* var. *stenophylla*, BS = 56) along the five main branches (Fig. 2). *Diabelia serrata* forms a clade with *D. serrata* var. *buchwalddi*; *D. tetrasepala* was monophyletic and sister to all *D. serrata* accessions; *D. spathulata* var. *sanguinea* (including one sample of *D. spathulata*), *D. spathulata* var. *stenophylla* and *D. spathulata* var. *spathulata* formed respective clades. *Diabelia spathulata* var. *spathulata* was sister to the remainder of *Diabelia* (Fig. 2). Within the QS analyses based on the nuclear ML tree, *Diabelia* was shown to have strong QS support (1/ – /1; Fig. S2).

The coalescent species tree (Fig. 3) presented a largely congruent topology compared to the nuclear ML tree with all five major groupings recovered, each being respectively monophyletic. The main clades were maximally supported (LPP ≥ 0.93) except for the placement of *D. spathulata* var. *sanguinea* (LPP = 0.44). The conflict analyses revealed full QS support within *Diabelia* (1/ – /1; Fig. 3). The clade of *D. serrata* had strong QS support with signal for a possible alternative topology (0.92/0.38/1). The sister relationship of the clade of *D. spathulata* var. *sanguinea* and *D. serrata* had counter QS support with signal for a possible alternative topology

(-0.22/0.21/0.99). *Diabelia tetrasepala* was sister to the clade of *D. serrata* + *D. spathulata* var. *sanguinea*. The clade of *D. spathulata* var. *stenophylla* between China and Japan had strong QS support with signal for a possible alternative topology (0.9/0.2/1)

The phylogenetic relationships in the plastid ML tree of *Diabelia* (Fig. 2 and S3) were also confirmed with strong QS support (1/ – /1). The clade of *D. spathulata* var. *sanguinea* + *D. spathulata* var. *stenophylla* (Japan) was sister to the *D. serrata* + *D. tetrasepala* clade, and the combined clade had strong BS support (98) and moderate QS support (0.14/0.57/0.98). There were some observed differences between the nuclear and plastid trees: *D. tetrasepala* was nested in *D. serrata* with strong BS support (98); *D. spathulata* var. *sanguinea* was not monophyletic; *D. spathulata* var. *stenophylla* from Zhejiang was sister to the remainder of *Diabelia*, rather than *D. spathulata* var. *spathulata*.

3.3 Species network analysis

The species network analyses (15-taxa data set) recovered topologies with one to five reticulation events, which appear to be a better model than a strictly bifurcating tree (Table 2; Fig. S4). Two reticulation events were inferred for the best network (Fig. 4). The best network showed that *D. tetrasepala* was the product of a hybridization event involving *D. serrata* and *D. spathulata* var. *spathulata*. Another reticulation event was observed indicating the clade of *D. spathulata* var. *spathulata* + *D. spathulata* var. *stenophylla* had genetic contributions from the lineage leading to *D. spathulata* var. *sanguinea*.

3.4 Divergence time estimation

Our dating estimates based on the nuclear data suggested an early Miocene crown age for *Diabelia* of 22.53 Ma (95% Highest Posterior Density (HPD): 17.89 - 27.73 Ma, node 1 in Fig. 5). The sister relationships of *D. serrata* and *D. tetrasepala* diverged during the early Miocene 18.17 Ma (95% HPD: 14.07 - 22.41 Ma, node 4 in Fig. 5). The crown age of *D. serrata* was dated to 14.88 Ma (95% HPD: 11.10 - 18.91

Ma, node 5 in Fig. 5). The split between *D. spathulata* and *D. spathulata* var. *sanguinea* occurred between 3.90 and 9.41 Ma (node 7 in Fig. 5). The diversification of *D. spathulata* var. *spathulata* between Korea and Japan was estimated as 4.78 Ma (95% HPD: 3.02 - 6.84 Ma, node 8 in Fig. 5). The divergence time of *D. spathulata* var. *stenophylla* between China and Japan was 6.30 Ma (95% HPD: 3.90 - 9.41 Ma, node 6 in Fig. 5).

3.5 Ancestral area reconstruction

The reconstruction results of the ancestral distribution of *Diabelia* were presented in Fig. 6. There were three dispersal events and three vicariance events identified within the three defined biogeographic areas (Fig. 6). Our analyses revealed that the ancestor of *Diabelia* was present throughout Japan (region A) in the early Miocene (Fig. 6), followed by dispersal or vicariance events across Japan, Korea and China. Migration events occurred primarily during the Neogene. *Diabelia spathulata* var. *spathulata* from Korea was also suggested to have originated in Northeast Japan. However, one vicariance and one dispersal event was detected for the ancestral nodes of *D. spathulat*a var. *stenophylla* from China, indicating gene flow from Japan.

3.6 Character evolution

Character evolution with likelihood inference based on the inferred nuclear tree indicated that all four selected morphological characters showed some level of homoplasy in *Diabelia* (Figs. 7 - 8). Sepal number showed little homoplasy with our results suggesting that the ancestral character state was most likely five subequal sepals, with two independent shifts to five unequal sepals (four big and one smaller sepal) in *D. tetrasepala* and to two sepals in *D. serrata* (Fig. 7). Flower number was similar to sepal number by having little homoplasy, with the inferred ancestral character of paired flowers (Fig. 7). The ancestral corolla colour of *Diabelia* was most likely white (Fig. 8). Corolla shape exhibited a high level of homoplasy, with the state “infundibuliform” inferred to have originated twice, once in *D. spathulata* and the other in the clade of *D. tetrasepala* (Fig. 8).

The results of character evolution based on the plastid tree are shown in Figs. S5 and S6. The inferred ancestral character of sepal number, flower number and corolla colour from plastid tree are similar to those from the nuclear gene tree, but the ancestral corolla shape was most likely tubular (Figs. S5 - S6). The main difference of character state evolution between plastid tree and nuclear gene tree is the position of *D. tetrasepala* with its corresponding characters.

4 Discussion

4.1 Phylogenetic incongruence and hybridization

This study reconstructed well-supported phylogenetic relationships of the three species of *Diabelia* (*D. serrata, D. tetrasepala, D. spathulata:* including *D. spathulata* var. *sanguinea, D. spathulata* var. *stenophylla* and *D. spathulata* var. *spathulata*) based on both nuclear and plastid data. Our phylogenetic analyses indicate that the backbone relationships of *Diabelia* in the ML nuclear tree are congruent with these of the ASTRAL tree, but the relationships among these clades are incongruent between nuclear and plastid data (Figs. 2 and 3). For example, *D. serrata* and *D. tetrasepala* were recovered as monophyletic in ML nuclear tree, while *D. tetrasepala* was nested in *D. serrata* in the ML plastid tree (Fig. 2).

Comparing the tree topologies between the nuclear and plastid data, we find that the topology formed by the nuclear gene data was more stable. The nuclear data recovered five strongly supported clades (Figs. 2 and 3), with *D. serrata* forming a stable monophyletic group that was consistent with previous studies supporting the monophyletic nature of *D. serrata* (Wang et al., 2020). *Diabelia spathulata* var. *stenophylla* and *D. spathulata* var. *spathulata* were also recovered as monophyletic with the nuclear data. While in the plastid data *D. spathulata* var. *sanguinea* does not form a monophyletic group (Fig. 2). We hypothesize that plastid capture (Liu et al., 2017; Renoult et al., 2009) resulted in the non-monophyly of *D. spathulata* var. *sanguinea* observed in the plastome data. Previous phylogenetic relationship of *Diabelia* based on plastid data have also been unstable (Zhao et al.,2019; Wang et al., 2020). Therefore, the nuclear data provides a more robust estimate of the species tree

compared to plastid data. However, the analyses only include one *D. spathulata* sample, and the internal relationships of the clade encompassing *D. spathulata* need to be further resolved. Hence, we need more samples to obtain a more accurate and robust inference of relationships for future studies.

In the present study, we found extensive strong conflict between plastome and nuclear data (Fig. 2). Several processes can lead to gene tree inconsistencies between closely related groups, including hybridization, incomplete lineage sorting (ILS), horizontal gene transfer, and gene duplication and loss (Morales-Briones et al., 2018; Bogarin et al., 2018; Carter et al., 2019; Stull et al., 2020). Ancestral polymorphisms may lead to incomplete genealogical classification, therefore phylogenetic relationships between organelle markers may fail to capture the true process of population differentiation, or inconsistencies in gene trees may reflect interspecies hybridization and cytoplasmic infiltration (Lee-Yaw et al., 2019; Wielstra & Arntzen, 2020; Tkach et al., 2020). Therefore, the causes of inconsistencies in gene trees can explain the relative influences of drift, gene flow and selection on the maintenance of organelle variation within and between groups, which is helpful in revealing the evolutionary process of inter-group relationships (Mao et al., 2020; Dufresnes et al., 2020).

A previous study (Zhao et al., 2019) reported gene introgression events between *Diabelia* species and hybridization was hypothesized as the main driver based on sample observations, specifically that *D. tetrasepala* is a hybrid species. In this study, we conducted network analyses for *Diabelia*, with *D. tetrasepala* being inferred to result from a hybridization event between *D. serrata* and *D. spathulata* var. *spathulata*. These results support our hypothesis: *D. tetrasepala* (four big and one small sepals) is a hybrid of *D. serrata* (two sepals) and *D. spathulata* (five sepals). Samples of *D. tetrasepala* are distributed in the sympatric areas of *D. serrata* and *D. spathulata* (Table S1; Zhao et al., 2019; Wang et al., 2020; Landrein and Farjon, 2020). Therefore, we confirm that these geographical conditions provide the possibility of interbreeding between *D. serrata* and *D. spathulata*.

4.2 Molecular dating and demographic analyses

Based on our nuclear chronogram (Fig. 5), all *Diabelia* lineages differentiated 22.53-18.17 Ma (Fig. 5). Notably, this point estimate broadly coincides with the isolation of the Japanese island (24-22 Ma) (Hotta, 1974; Iijima and Tada, 1990; Li et al., 1996; Maekawa, 1998) and the estimated dates are earlier than previous estimates based on chloroplast data (Wang et al., 2020). The East China Sea (ESC) land bridge likely acted as a barrier or corridor to the dispersal of plant species during the LGM and earlier cold periods, despite its repeated exposure during the Miocene (7.0-5.0 Ma) and Quaternary (2.0-1.3 Ma, 0.2-0.015 Ma) (Harrison, 2001). Even though the Japanese archipelago was not covered by a major ice sheet during the last glacial period (Ono, 1984), the mean annual temperature was 5℃–9℃ cooler and the precipitation was less than at present (Yasuda and Narita, 1981; Tsukada, 1988). In addition, as a result of the lower sea levels (ca. 100 m below present), Shikoku and Kyushu were continuous with Honshu, and the continental shelf, ca. 20–30 km from the present coastline, emerged around the archipelago (Ohta and Yonekura, 1987). The distribution data of *D. serrata* suggests that the group is widely distributed in Japan except for Hokkaido. As the climate warmed, the species recovered and expanded northward or towards higher altitudes (Tsukada, 1988; Takahara et al., 2000), therefore, forming the current isolated distribution.

Diabelia species are relatively tolerant to cold and arid climates, which might have facilitated gene exchange across the glacially exposed ECS land bridge up until its latest submergence. All *Diabelia* species have fruits forming samaras, which increase long-distance dispersal abilities (Landrein, 2010). Our molecular dating indicates that *Diabelia* originated in the Japanese archipelago (Fig. 5) and the onset of diversification of *D. spathulata* var. *stenophylla* from Japan and *D. spathulata* var. *stenophylla* from China occurred during the early Pliocene. Our results suggest a vicariance event and a dispersal event associated with *D. spathulata* var. *stenophylla* from China (Fig. 5). Under the hypothesis of long-distance dispersal, a predicted lineage in one region should nest within a lineage from a separate disjunctive region.

In contrast, under vicariance, lineages from different geographic regions would be monophyletic, with relatively comparable levels of genetic diversity in each region of the distribution (Yoder & Nowak, 2006; Liao et al., 2016; Thomas et al., 2017). The disjunction between *D. spathulata* var. *stenophylla* in Japan and *D. spathulata* var. *stenophylla* from China may have resulted from migration across this land bridge followed by vicariance. However, given that *Diabelia* fruits are winged (Landrein, 2010) the hypothesis of direct long-distance dispersal between Japan and China cannot be ruled out.

Our results show that *D. spathulata* var. *spathulata* is comprised of two phylogroups with distinct geographic distributions, one in Japan and the other in Korea. Furthermore, our results also confirm that populations of *D. spathulata* var. *spathulata* in Korea are likely due to more recent episodes of long-distance dispersal and ancient vicariant events (Fig. 6). Our divergence time estimates suggest that species differentiation in *D. spathulata* var. *spathulata* occurred during the late Miocene to the early Pliocene (4.78 Ma, 95% HPD: 3.02–6.84 Ma), suggesting that extant populations likely differentiated well before the LGM. The cooling and drying climate in the late Miocene drove the formation of some species. The changing climate in the late Pliocene and Pleistocene are related to lineage differentiation, genetic diversity formation, and population contraction and expansion such as observed in *Cercdiphyllum japonicum* (Qi et al., 2012) and *Euptelea* (Cao et al., 2016). This study adds another example of this well-documented pattern.

4.3 Evolution of morphological characters

Character state reconstructions performed on the ML tree (Figs. 7 - 8 and S5 - S6) have the potential to exhibit hemiplasy and xenoplasy (Avise & Robinson, 2008; Robinson et al., 2008; Copetti et al., 2017; Wang et al., 2020). Based on nuclear data there are five distinct clades in both the ASTRAL and concatenation trees (Figs. 1 - 2), but distinguishing taxonomic rank remains difficult. These relative lineages have constrained biogeographic histories, which may also explain the high level of morphological variation observed in some clades.

Sepal number, flower number and corolla colour have been regularly used to recognize *Diabelia* (Hara, 1983; Zhao et al., 2019; Wang et al., 2020; Landrein and Farjon, 2020). The evolution pattern of sepal number reveals that five subequal sepals is the likely ancestral state of *Diabelia*, while the five unequal sepals (four big and one smaller sepal) observed in *D. tetrasepala* (Fig. 1 C) and two sepals in *D. serrata* (Fig. 1 A) are derived characters that evolved independently. Robayo et al. (2020) pointed out that the calyx of Loranthaceae degenerated in the evolutionary history of the family. In addition, Nakagawa et al. (2020) demonstrated that the dorsal-to-ventral inhibitions and the floral stem cell region (meristem) determine the number and position of the sepals. Based on our previous hybridization analysis, we hypothesize that hybridization events may cause sepals to decrease in number throughout the process of evolution.

Paired flowers are an important character in the classification within *Diabelia*. Landrein (2010) noted that the paired flowers appearing at the end of short shoots of *Diabelia* differ from those of *Abelia*. Based on this important characteristic, *Diabelia* was separated from *Abelia*. Our ancestral state reconstruction revealed paired flowers as the inferred ancestral character of *Diabelia*, with the number of flowers increasing to clusters of four to seven in *D. serrata*.

The evolution of corolla color within *Diabelia* is complicated, with a white corolla appearing to be the ancestral condition. Based on our ancestral state reconstructions, there does not appear to be any clear evolutionary patterns (Figs. 8 and S6). There are some overlapping colour characters within *Diabelia*, for instance the corolla of *D. serrata* is usually yellow (sometimes pale yellow, Fig. 1 A); *D. spathulata* is usually white (Fig. 1 E) but sometimes pale yellow (Fig. 1 D); *D. tetrasepala* is usually pale yellow but sometimes light pink. Selection driven by biotic agents can generate variation in floral characters. Abiotic selection, however, also drives the diversity of floral features, especially with respect to patterns of pigmentation (Koski & Ashman, 2016; Hong et al., 2019; Meng et al., 2021).

Overall, five subequal sepals, paired flowers and white corolla are inferred as the likely ancestral states of this genus. The clarification of the evolution patterns of these

characters is significant for the taxonomy of *Diabelia*, which contributes towards a sound taxonomic treatment of the genus.

5 Conclusions

The robust phylogeny of *Diabelia* was reconstructed with nuclear and plastid data based on target enrichment and genome skimming approaches. The inferred phylogenies from both nuclear and plastome data indicate that *Diabelia* can be divided into five main clades, which are further supported by morphological traits such as sepal number. These results suggest that *Diabelia* can be divided into five species or sub species. The PhyloNet results further confirmed the existence of reticulation and natural hybridization events in *Diabelia*, confirming *D. tetrasepala* is a natural hybrid of *D. serrata* and *D. spathulata* var. *spathulata*. The divergence time and biogeography analyses further support the differentiation and propagation of *Diabelia* with multiple vicariances from the perspectives of time and space, further supporting the complex natural hybridization and evolutionary network of the disjunctive flora of Japan and China. Cladistic evolution and reticulate evolution should be considered when reconstructing phylogenetic relationships among closely related species. The appropriate choice of data to construct phylogenetic trees is important in the era of genomics data. More studies are needed to further clarify the origin, dispersal and evolution of Sino-Japanese disjunct species with nuclear and plastid data at the population level.

6 TAXONOMIC TREATMENT

Landrein (2010) separated *Diabelia* from *Abelia* by the paired inflorescences that appear at the ends of short branches. Sepal number is commonly regarded to distinguish the apparent characteristics of *Diabelia* species. Hara (1983) divided the genus into three species, based on the properties of number and size of the sepals: *D. spathulata* has five unequal sepals, *D. serrata* has 2-3 sepals, and *D. tetrasepala* has four sepals and a variation of shorten sepals. Landrein (2010) and Landrein et al. (2020) raised four species within *Diabelia* based on the number of sepals, position of nectar

pad and colour of corolla. In the light of the phylogenetic results and morphological evidence in this study, we identified and accepted three species of *Diabelia* Landrein, with *D. spathulata* containing three varieties.

6.1 *Diabelia serrata*（Siebold& Zucc.）Landrein

Basionym: *Abelia spathulata* Siebold & Zucc. (Siebold & Zuccarini 1838: 34); *Linnaea spathulata* (Siebold &Zucc.) Graebn. (Graebner 1900: 142). Type: Japan, Buerger, Herb. Siebold. 899.68-6 (lectotype L!).

Abelia spathulata var. *elliptica* Miq. (Miquel 1866: 269). Type: Shizuoka Pref., Simoda, 15 May 1855, C. Wrights.n. (K!).

Abelia spathulata f. pilosa Nakai (1921). Type: Japan, Honshu, Osaka Pref., Kawachi, Aug. 1899, T. Tadas.n. (lectotype TI!).

Abelia spathulata var. *micrantha* Nakai (in Nakai & Koidzumi 1927: 504). Type: Japan, Shinano, H. Koidzumi s.n. (TI!).

Abelia serrata var. *integerrima* Nakai (in Nakai & Koidzumi 1927: 625); Abelia integerrima (Nakai) Sugim. (Sugimoto 1936: 361). Type not located.

Description: sepals 2 often dissected into 2 - 4 lobes, flowers paired or more often in clusters of 4 - 7 flowers, corolla 9 - 22 mm long, yellow, pale yellow or less frequently pale pink or white. Corolla tubula rinfundibulform with or without an inflated mouth. Nectary cushion fused to the corolla tube, 1 - 3 mm in diameter.

Distribution: Zhejiang (China); Kagoshima; Shimane; Ehime; Shiga; Fukui; Tokushima; Gifu; Okayama; Oita; Kochi; Hyogo; Nagasaki; Yamanashi; Yamaguchi

6.2 *Diabelia tetrasepala* (Koidz.) Landrein

Basionym: *Diabelia stenophylla* (Honda) Landrein var. *tetrasepala* (Koidz.) Landrein comb. nov. KEW BULLETIN 74: 70 (Landrein & Farjion 2019);

Abelia spathulata Siebold & Zucc. var. *tetrasepala* Koidz., Bot. Mag. (Tokyo) 29 (348): 311(Koidzumi 1915);

Abelia tetrasepala (Koidz.) H. Hara & S. Kuros. (in Kurosawa & Hara 1955: 296); Diabelia tetrasepala (Koidz.) Landrein (2010: 37). Type: Japan, Honshu, Saitama Pref. [Musashi, Chichibu], 23 April 1878, J. Matsumaras.n. (holotype TI!).

Abelia spathulata Siebold & Zucc. var. *subtetrasepala* Makino (1917: 18). Type: Japan, Honshu, Kochi Pref., Nanokawa-mura, 22 April 1888, K. Watanabe s.n. (holotype TI!).

Abelia spathulata Siebold & Zucc. var. *subtetrasepala* Makino f. flavescens Honda (1935: 569). Type: Japan, Gunma Pref., Ikaho, 27 May 1934, T. Sakai 152 (holotype TI!).

Abelia spathulata Siebold & Zucc. var. *subtetrasepala* Makino f. rubescens Sugim. (Sugimoto 1961: 456). Type and description not located.

Description: Sepals 4 plus a reduced adaxial sepal lobe, corolla often larger, 30 - 35 mm long, paired flowers, ovary subappressed pubescent.

Distribution: Tokushima; Shiga; Mie; Shiga; Fukushima; Ibaraki.

6.3 Diabelia spathulata (Siebold & Zucc.) Landrein

Description: Sepals 5, all of similar size, corolla pink, white or yellowish-white, campanulate in the upper part, 16–28 mm long, sepals acute at apex, nectary cushion clavate and apically free from the corolla tube.

Distribution: Kochi; Niigata; Toyama; Shizuoka; Ehime; Kanagawa; Ibaraki; Fukui; Aichi; Nara; Tokushima; Gifu; Fukushima; Yamanashi; Shiga; Gyeongsangnam-do (Korea)

var. *sanguinea* Corolla red, campanulate in the upper part, 11–22 mm long, sepals obtuse at apex. Nectary cushion flat and fused to the corolla tube or shortly clavate and apically free from the corolla tube.

Basionym: *Diabelia sanguinea* (Makino) Landrein comb. et stat. nov. KEW BULLETIN 74: 70 (Landrein & Farjion 2019);

Abelia spathulata Siebold & Zucc. var. *sanguinea* Makino, Bot. Mag. (Tokyo) 18: 106 (1904); *Linnaea spathulata* (Siebold & Zucc.) Graebn. var. sanguinea (Makino) Matsumura (1912: 598);

Abelia sanguinea (Makino) Makino (1917: 14). Type: Japan, Honshu, Tochigi, Hosoomura, 11 June 1878, S. Okubo s.n. (lectotype TI!).

Abelia curviflora Nakai (in Nakai & Koidzumi 1927: 503). Type: Japan, Nagano,

Shinno, Asage mura, Shimoinagun, 18 July 1959, H. Koidzumi 8308 (holotype TI!).

Abelia sanguinea (Makino) Makino var. *purpurascens* Honda (1930: 409). Type: Japan, Tochigi, Nasu, 8 June 1930, M. Honda s.n. (holotype TI!).

var. *stenophylla* Corolla yellowish white, rarely pinkish, 25-45 mm long; leaves often larger, smooth; young branches, petioles, midrib of leaves beneath, and ovary often with long (up to 1mm long) spreading hairs; bracts linear-lanceolate, 2-6mm long.

Basionym: Diabelia stenophylla (Honda) Landrein comb. et stat. nov. KEW BULLETIN 74: 70 (Landrein & Farjion 2019);

Abelia spathulata Siebold & Zucc. var. stenophylla Honda, Bot. Mag. (Tokyo) 50: 436 (1936). Type: Japan, Honshu, Akita, Higashidatemura, Kitaakita-gun, June 1931, M. Matsuda 14b (holotype TI!).

Abelia spathulata Siebold & Zucc. var. macrophylla Honda (1941: 202). Type: Japan, Honshu, Akita, Shimoshinzan, Oga., 25 Aug. 1929, T. Hida s.n. (holotype TI!).

Abelia ionostachya Nakai (1953: 23). Type: Honshu, Niigata, Kirinzan, Tsugawa-machi, 26 May 1950, T. Nakai & N. Maruyama s.n. (holotype TNS).

var. *spathulata* Nectary-gland inside the base of the corolla-tube, short clavate or globular, free or partly adnate; corolla 1.5-3cm long, white, yellowish, rarely rose-red; leaves generally with impressed veins above, pilose generally with short hairs.

Basionym: *Abelia spathulata* Siebold & Zucc. (Siebold & Zuccarini 1838: 34); Linnaea spathulata (Siebold & Zucc.) Graebn. (Graebner 1900: 142). Type: Japan, Buerger, Herb. Siebold. 899.68-6 (lectotype L!).

Abelia spathulata var. elliptica Miq. (Miquel 1866: 269). Type: Shizuoka Pref., Simoda, 15 May 1855, C. Wrights.n. (K!).

Abelia spathulata f. pilosa Nakai (1921). Type: Japan, Honshu, Osaka Pref., Kawachi, Aug. 1899, T. Tada s.n. (lectotype TI!).

Abelia spathulata var. micrantha Nakai (in Nakai & Koidzumi 1927: 504). Type: Japan, Shinano, H. Koidzumi s.n. (TI!).

Abelia serrata var. integerrima Nakai (in Nakai & Koidzumi 1927: 625); Abelia integerrima (Nakai) Sugim. (Sugimoto 1936: 361). Type not located.

Abelia spathulata f. lucida Makino (1933: 255). Type: Japan, Honshu, Chiba Pref., Futtsu, Mt Nokogiri, 28 April 1952, M. Mizushima 1721 (A!, TI)

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Author contributions

H.F.W. and J.W. conceived the study. H.F.W. and D.F.M-B. performed the research and analyzed the data. X.R.K., H.F.W., D.F.M-B., J.W. HXW, Q.H.S and J.B.L wrote and revised the manuscript.

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Figure Legends

Figure 1. Floral diversity of *Diabelia*. (A) *Diabelia serrata*; (B) *D. serrata var. buchwaldii*; (C) *D. tetrasepala*; (D)- (E) *D. spathulata*; (F) *D. spathulata var. sanguinea*.

Figure 2. Tanglegram of the nuclear concatenated (left) and plastid (right phylogenies. Dotted lines connect taxa between the two phylogenies. Maximum likelihood bootstrap support values are shown above branches. The asterisks indicate maximum likelihood bootstrap support of 100%. Major taxonomic groups or main clades in the family as currently recognized are indicated by branch colors as a visual reference to relationships.

Figure 3. ASTRAL-III species tree; Clade support is depicted as: LPP/QC/QD/QI (local posterior probability/quartet concordance/quartet differential/quartet informativeness).

Figure 4. Best supported species networks inferred with Phylonet for the 15-taxa. Numbers next to the inferred hybrid branches indicate inheritance probabilities. Blue curved lines represent major hybrid edges. Red curved lines represent minor hybrid edges (edges with an inheritance contribution < add space 0.50).

Figure 5. BEAST analysis of divergence times based on the nuclear data set. Calibration points are indicated by A, B. Numbers 1-8 represent major divergence events in *Diabelia*; mean divergence times and 95% highest posterior densities (HDP) are provided for each nodes of interests. Blue bars represent 95% HDP.

Figure 6. Ancestral area reconstruction for *Daibelia*. Areas of endemism are as follows: (A) Japan, (B) Korea, (C) China. The numbered nodes represent crown nodes of important colonization events, as discussed in the text.

Figure 7. Likelihood inference of character evolution in *Diabelia* using Mesquite v.3.51 based on nuclear phylogeny. Left, The number of sepals; Right, The number of flowers.

Figure 8. Likelihood inference of character evolution in *Diabelia* using Mesquite v.3.51 based on nuclear phylogeny. Left, Corolla colour; Right, Corolla shape.

Table. 1 Data set statistics, including the number of taxa, number of characters, number

of PI characters, missing data.

Table. 2 Model selection of the different species networks and bifurcating trees.

Fig. S1 Heatmaps showing gene recovery efficiency for the nuclear genes in 45 species of *Diabelia*. Columns represent genes, and each row is one sample. Shading indicates the percentage of the reference locus length coverage.

Fig. S2 nuclear RAxML tree; Clade support is depicted as: LPP/QC/QD/QI (local posterior probability/quartet concordance/quartet differential/quartet informativeness).

Fig. S3 plastid RAxML tree; Clade support is depicted as: LPP/QC/QD/QI (local posterior probability/quartet concordance/quartet differential/quartet informativeness).

Fig. S4 Best species networks of the selective nuclear data set estimated with PhyloNet for the 15-taxa. A: One hybridization event; B: Two hybridization events; C: Three hybridization events; D: Four hybridization events; F: Five hybridization events. Blue branches connect the hybrid nodes. Numbers next to blue branches indicate inheritance probabilities.

Figure S5. Likelihood inference of character evolution in *Diabelia* using Mesquite v.3.51 based on plastid phylogeny. Left, The number of sepals; Right, The number of flowers.

Figure S6. Likelihood inference of character evolution in *Diabelia* using Mesquite v.3.51 based on plastid phylogeny. Left, Corolla colour; Right, Corolla shape.

Table. S1 List of species and vouchers used in this study.

Table. S2 HybPiper assembly statistics.

Table 1. Data set statistics, including the number of taxa, number of characters, number of PI characters, missing data.

Alignment	No. of taxa	No. sites	Missing data (%)	No. of variable/Parsimony informative sites
Nuclear	47	392,600	13.2	43,250/18,057
plastid	47	159,365	1.8	8,243/4,693

Table 2. Model selection of the different species networks and bifurcating trees.

					Information criteria		
Topology	lnL	Parameters	Loci	Number of hybridizations	AIC	AICc	BIC
15 taxa							
nuclear ASTRAL	-6134.788576	27	241	N/A	12323.577153	12330.675744	12417.666670
nuclear RAxML	-6137.811629	27	241	N/A	12329.623259	12336.721850	12423.712776
plastid	-6427.555081	27	241	N/A	12909.110161	12916.208753	13003.199679
Network 1	-6079.839883	29	241	1	12217.679766	12225.926212	12318.738877
Network 2	**-6006.511111**	**31**	**241**	**2**	**12075.022221**	**12084.515044**	**12183.050926**
Network 3	-6079.836581	33	241	1	12225.673162	12236.513742	12340.671461
Network 4	-6038.368637	35	241	4	12146.737274	12159.029957	12268.705166
Network 5	-6088.526401	37	241	5	12251.052802	12264.905019	12379.990288

Order	Ingroup/ Outgroup	Taxon	Coding	Locality	Voucher specimen number	SRA accession
1	Ingroup	*Diabelia serrata*	A	Ogawara plateau,Tokushima , Japan	HUTB, E314	
2	Ingroup	*Diabelia serrata* var. *buchwaddi*	A	Takanose-kyo gorge, Kito, Naka-cho, Kochi, Japan	HUTB, E324	
3	Ingroup	*Diabelia serrata*	A	Mt. Shiraga, Motoyama-cho, Kochi, Japan	HUTB, E137	
4	Ingroup	*Diabelia serrata*	A	Kochi, Japan	HUTB, E140	
5	Ingroup	*Diabelia serrata*	A	Kakiki-mura, Kanoashi-gun, Shimane, Japan	HUTB, E122	
6	Ingroup	*Diabelia serrata*	A	Anan, Tokushima, Japan	HUTB, E123	SRR13705758
7	Ingroup	*Diabelia serrata*	A	Mt. Shiraga, Motoyama-cho, Kochi, Japan	HUTB, E138	
8	Ingroup	*Diabelia serrata*	A	Mt. Shiraga, Motoyama-cho, Kochi, Japan	HUTB, E139	
9	Ingroup	*Diabelia serrata*	A	Takanose-kyo gorge, Kito, Naka-cho, Kochi, Japan	HUTB, E128	
10	Ingroup	*Diabelia serrata*	A	Takakuma, Kagoshima, Japan	HUTB, E321	SRR13890747
11	Ingroup	*Diabelia serrata*	A	Mt. Shozanji, Kamiyama, Tokushima, Japan	HUTB, E125	
12	Ingroup	*Diabelia serrata*	A	Ogawara plateau,Tokushima , Japan	HUTB, E126	
13	Ingroup	*Diabelia serrata*	A	Takaga, Seki city, Gifu, Japan	HUTB, E124	
14	Ingroup	*Diabelia tetrasepala*	A	Mt. Takamaru, Kamikatsu, Tokushima, Japan	HUTB, E142	
15	Ingroup	*Diabelia tetrasepala*	A	Mt. Takamaru, Kamikatsu, Tokushima, Japan	HUTB, E143	
16	Ingroup	*Diabelia tetrasepala*	A	Mt. Takamaru, Kamikatsu, Tokushima, Japan	HUTB, E185	
17	Ingroup	*Diabelia tetrasepala*	A	Mt. Takamaru, Kamikatsu, Tokushima, Japan	HUTB, E312	
18	Ingroup	*Diabelia tetrasepala*	A	Mt. Takamaru, Kamikatsu, Tokushima, Japan	HUTB, E311	
19	Ingroup	*Diabelia spathulata*	A	Tochigi, Japan	HUTB, E271	
20	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Yumoto, Nasu-cho, Nasu-gun, Tochigi, Japan	HUTB, E130	
21	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Mt. Takamaru, Kamikatsu, Tokushima, Japan	HUTB, E294	
22	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Zike, Toyama city, Toyama, Japan	HUTB, E131	
23	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Sendai, Miyagi, Japan	HUTB, E301	SRR13705755

24	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Mt. Kinkan, Numadzu city, Shizuoka, Japan	HUTB, E303	SRR13890746
25	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Tanyama, Nagano, Japan	HUTB, E308	
26	Ingroup	*Diabelia spathulata* var. *sanguinea*	A	Tanyama, Nagano, Japan	HUTB, E306	SRR13705756
27	Ingroup	*Diabelia spathulata* var. *spathulata*	B	Naewon temple forests,Yangsan-si, Gyeongsangnam-do, Korea	HUTB, E190	
28	Ingroup	*Diabelia spathulata* var. *spathulata*		Kowaki-cho, Higashiomi city, Shiga, Japan	HUTB, E200	
29	Ingroup	*Diabelia spathulata* var. *spathulata*	B	Naewon temple forests,Yangsan-si, Gyeongsangnam-do, Korea	HUTB, E141	
30	Ingroup	*Diabelia spathulata* var. *spathulata*	A	Kowaki-cho, Higashiomi city, Shiga, Japan	HUTB, E198	SRR13705757
31	Ingroup	*Diabelia spathulata* var. *spathulata*	A	Hatasawa, Obanasawa city, Yamagata, Japan	HUTB, E129	
32	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E204	SRR13705754
33	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E203	
34	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E201	
35	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E202	
36	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E205	
37	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E170	
38	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E172	
39	Ingroup	*Diabelia spathulata* var. *stenophylla*	C	Wenzhou, Zhejiang, China	HUTB, E171	
40	Ingroup	*Diabelia spathulata* var. *stenophylla*	A	Saitama, Japan	HUTB, E240	
41	Ingroup	*Diabelia spathulata* var. *stenophylla*	A	Tabitomachi, Iwaki-shi, Fukushima Pref, Japan	HUTB, E295	
42	Ingroup	*Diabelia spathulata* var. *stenophylla*	A	Kami-sanbonmatsu, Honzyuku-cho, Okazaki city, Aichi, Japan	HUTB, E132	
43	Outgroup	*Abelia macrotera* var. *parvifolia*		Yichang, Hubei, China	HUTB, E45	SRR13890750
44	Outgroup	*Dipelta floribunda*		Cambridge, UK.	HUTB, E55	SRR13705794
45	Outgroup	*Kolkwitzia amabilis*		Weinan, Shanxi, China	HUTB, E9	SRR13705797
46	Outgroup	*Vesalea floribunda*		Oaxaca, Mexico	HUTB, E112	

47	Outgroup	*Linnaea borealis*	Yili, Xinjiang, China	HUTB, E59	SRR13705788

Table S2. HybPiper assembly statistics

Species	Number of reads	Number of reads on target	Percent reads on target	Number of exons with reads	Number of exons with contigs	Number of exons with sequences	Number of exons with sequences > 25% of the target length	Number of exons with sequences > 50% of the target length	Number of exons with sequences > 75% of the target length	Number of exons with sequences > 150% of the target length	Number of exons with paralog warnings
D. spathulata var. *spathulata* E129	16390784	4958995	0.303	1114	1025	994	992	982	934	1	101
D. spathulata var. *sanguinea* E306	9313351	1888267	0.203	1119	1007	977	974	960	889	0	79
D. spathulata var. *sanguinea* E308	5571741	780542	0.14	1097	981	960	956	936	875	0	69
D. tetrasepala E142	14145605	1023090	0.072	1089	987	963	962	952	899	1	79

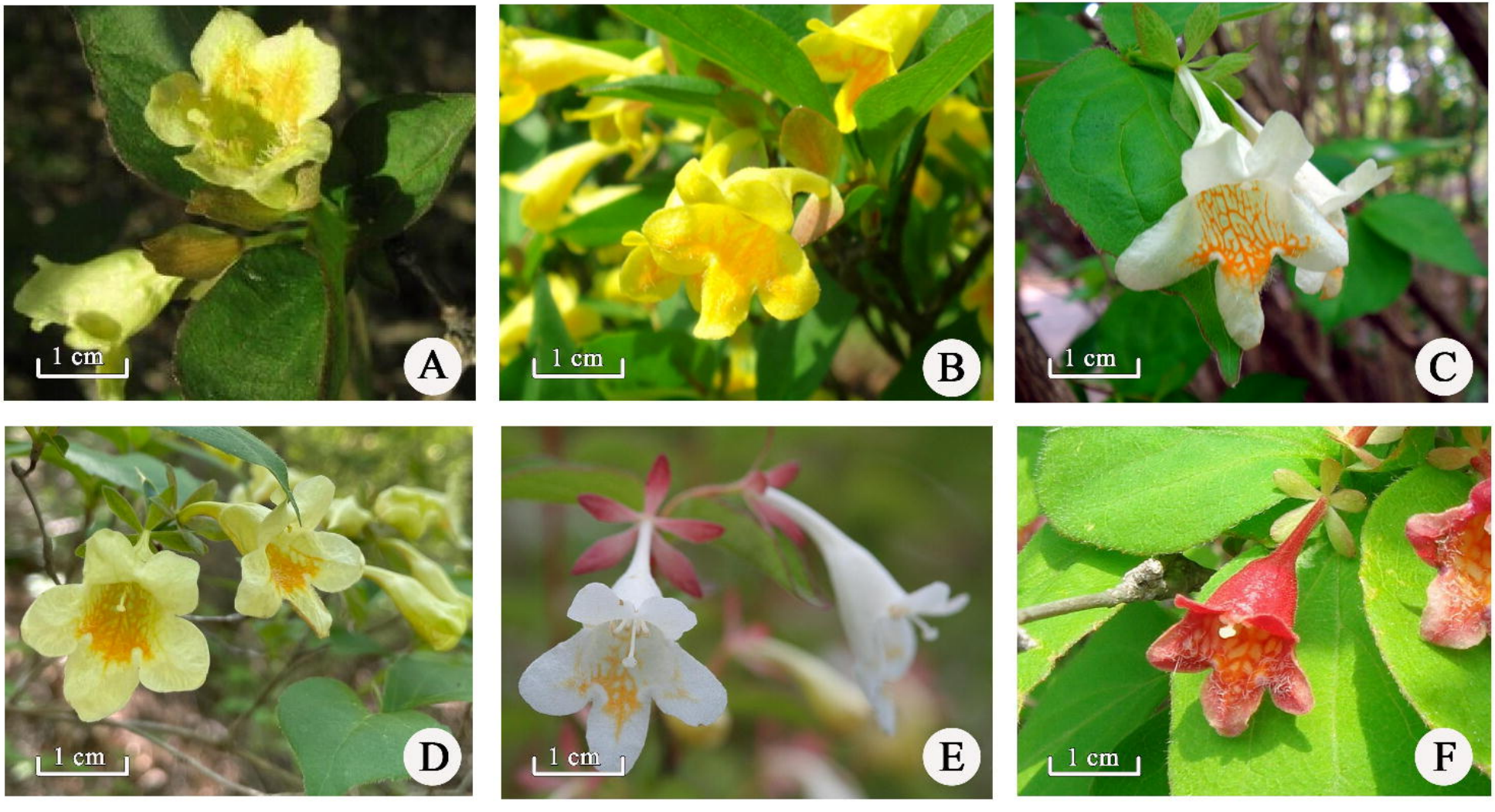
D. tetrasepala E143	4395804	228392	0.052	1050	900	878	878	851	783	0	54
D. tetrasepala E185	5533465	1430420	0.259	1114	1008	978	977	959	898	0	90
D. spathulata var. *sanguinea* E294	1071353	238126	0.222	1021	876	862	861	841	779	0	56
D. spathulata var. *stenophylla* E295	9507905	2627497	0.276	1138	1024	992	992	971	904	0	92
D. tetrasepala E311	12638082	3514670	0.278	1144	1030	995	994	982	937	2	98
D. tetrasepala E312	26768128	7923496	0.296	1130	1022	995	994	984	940	2	102
D. spathulata var. *stenophylla* E170	8333538	2519416	0.302	1127	1017	995	994	979	922	1	90
D. spathulata var. *stenophylla* E171	8838954	2672116	0.302	1108	1017	987	985	972	923	1	96
D. spathulata var. *stenophylla* E172	6849894	2064638	0.301	1103	1008	983	981	962	908	1	92
D. spathulata var. *stenophylla* E201	10281957	3225974	0.314	1112	1015	988	987	973	922	1	86

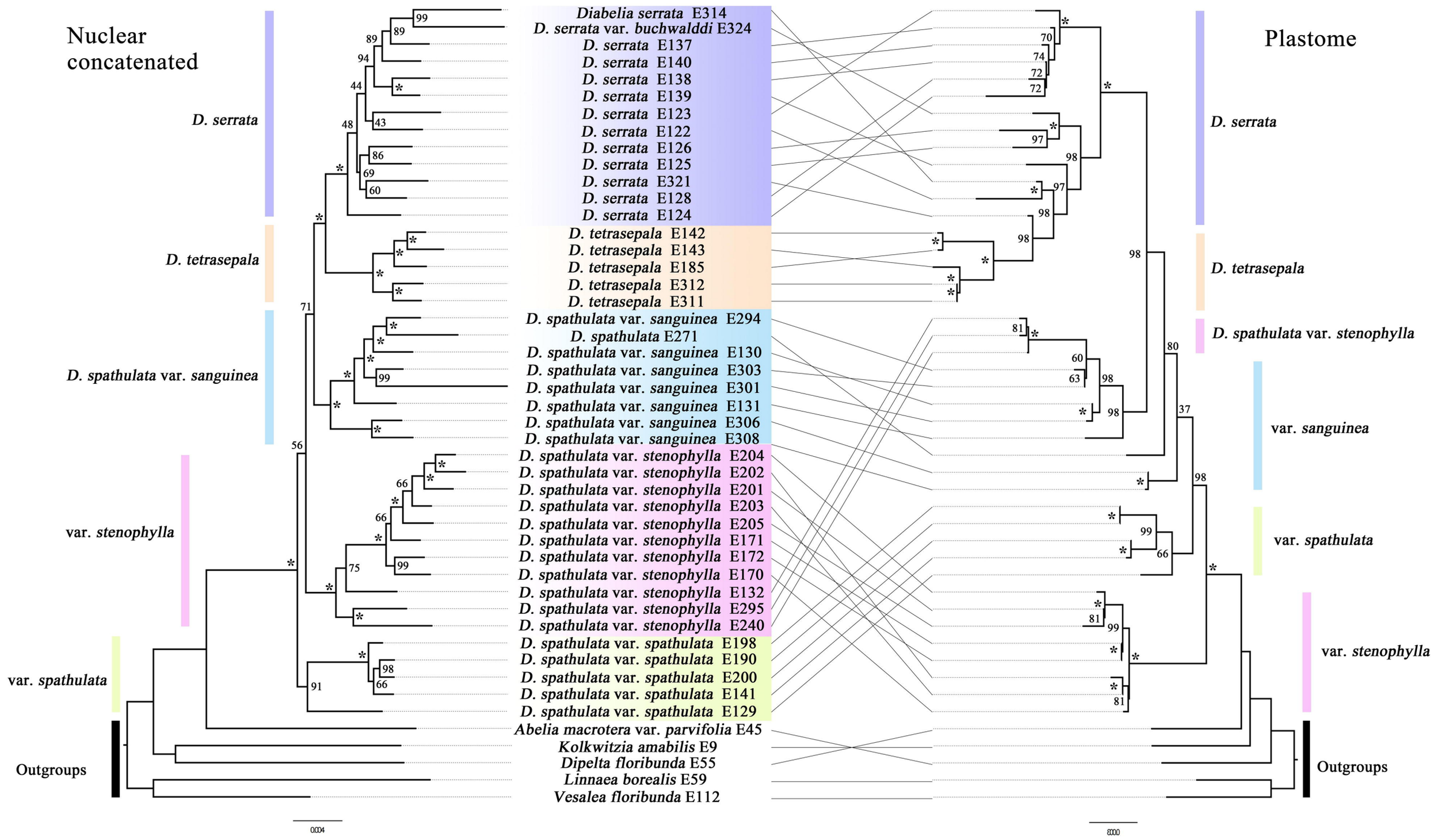
D. spathulata var. *stenophylla* E202	7944302	2326039	0.293	1109	1012	986	985	968	915	1	86
D. spathulata var. *stenophylla* E203	8895646	3097609	0.348	1122	1022	996	995	980	924	1	85
D. spathulata var. *stenophylla* E204	6642696	2077347	0.313	1103	1006	983	982	962	908	1	76
D. spathulata var. *stenophylla* E205	9630997	2810192	0.292	1128	1020	995	993	972	920	1	80
D. spathulata var. *sanguinea* E130	4766791	879875	0.185	1065	981	958	956	942	887	0	79
D. spathulata var. *stenophylla* E240	8121657	922592	0.114	1098	988	963	961	933	838	0	66
D. spathulata var. *sanguinea* E301	12028399	2724711	0.227	1151	1062	1032	1032	1021	989	3	619
D. spathulata var. *sanguinea* E303	10970170	3075238	0.28	1134	1025	991	988	973	914	0	91
D. serrata var. *buchwaddi* E324	6665872	419228	0.063	1093	872	852	849	803	683	0	39
D. serrata E122	5880405	1892938	0.322	1064	999	978	977	962	895	0	85
D. serrata E123	2163968	7331755	0.339	1120	1033	998	996	980	933	1	136

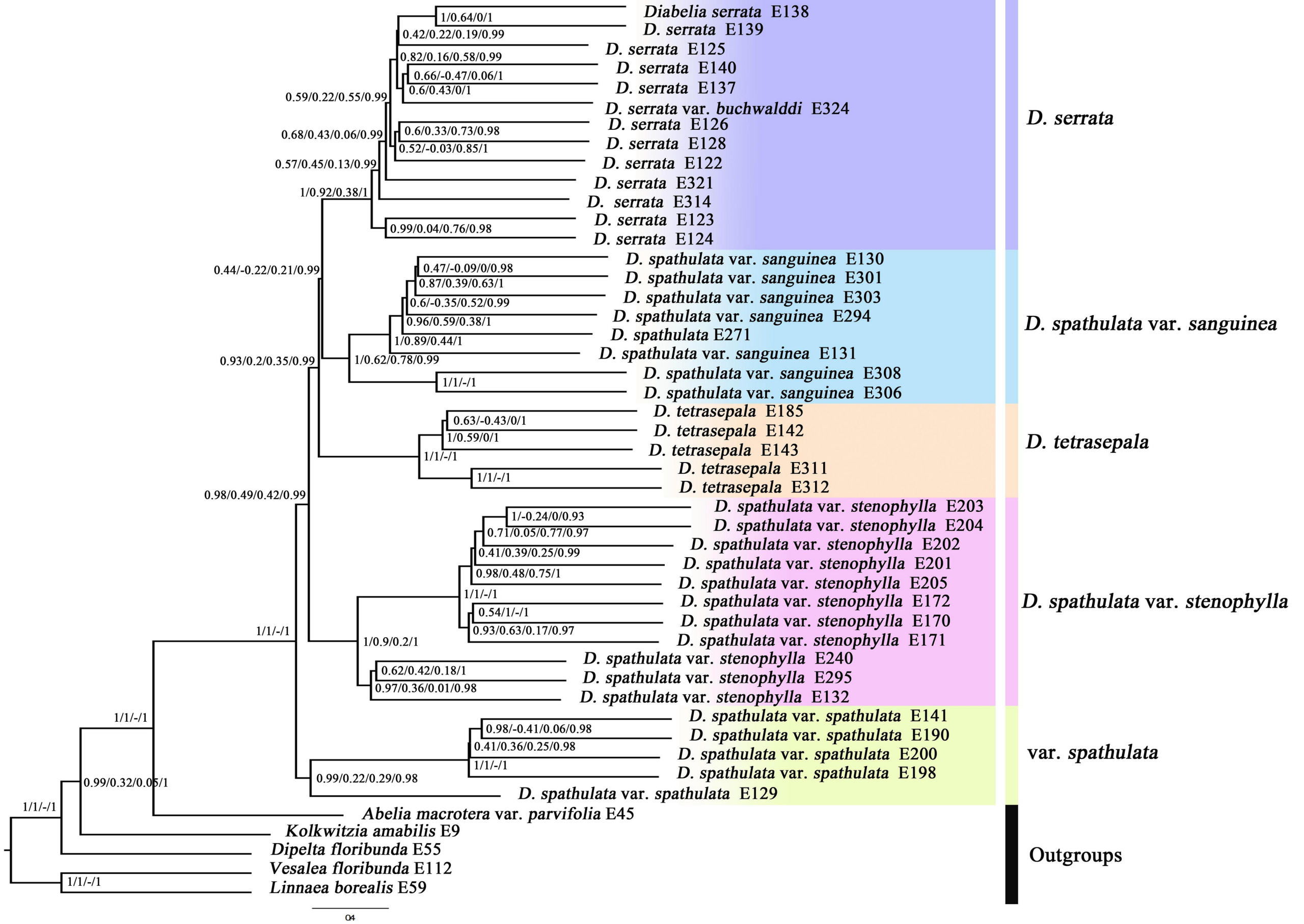
	0										
D. serrata E124	2291434 9	9714647	0.424	1116	1031	1000	999	984	940	2	97
D. serrata E125	1501139 4	6415541	0.427	1105	1034	1003	1002	986	938	0	88
D. serrata E126	3065202 1	9145847	0.298	1124	1028	1002	1000	984	937	0	112
D. serrata E128	1393433 3	2581418	0.185	1091	999	975	974	959	909	1	102
D. serrata E137	4273733	735627	0.172	1060	974	952	950	933	871	0	79
D. serrata E138	1414860 9	3912863	0.277	1100	1020	993	992	979	924	1	83
D. serrata E139	1011296	2465780	0.244	1102	1002	983	982	968	910	0	96

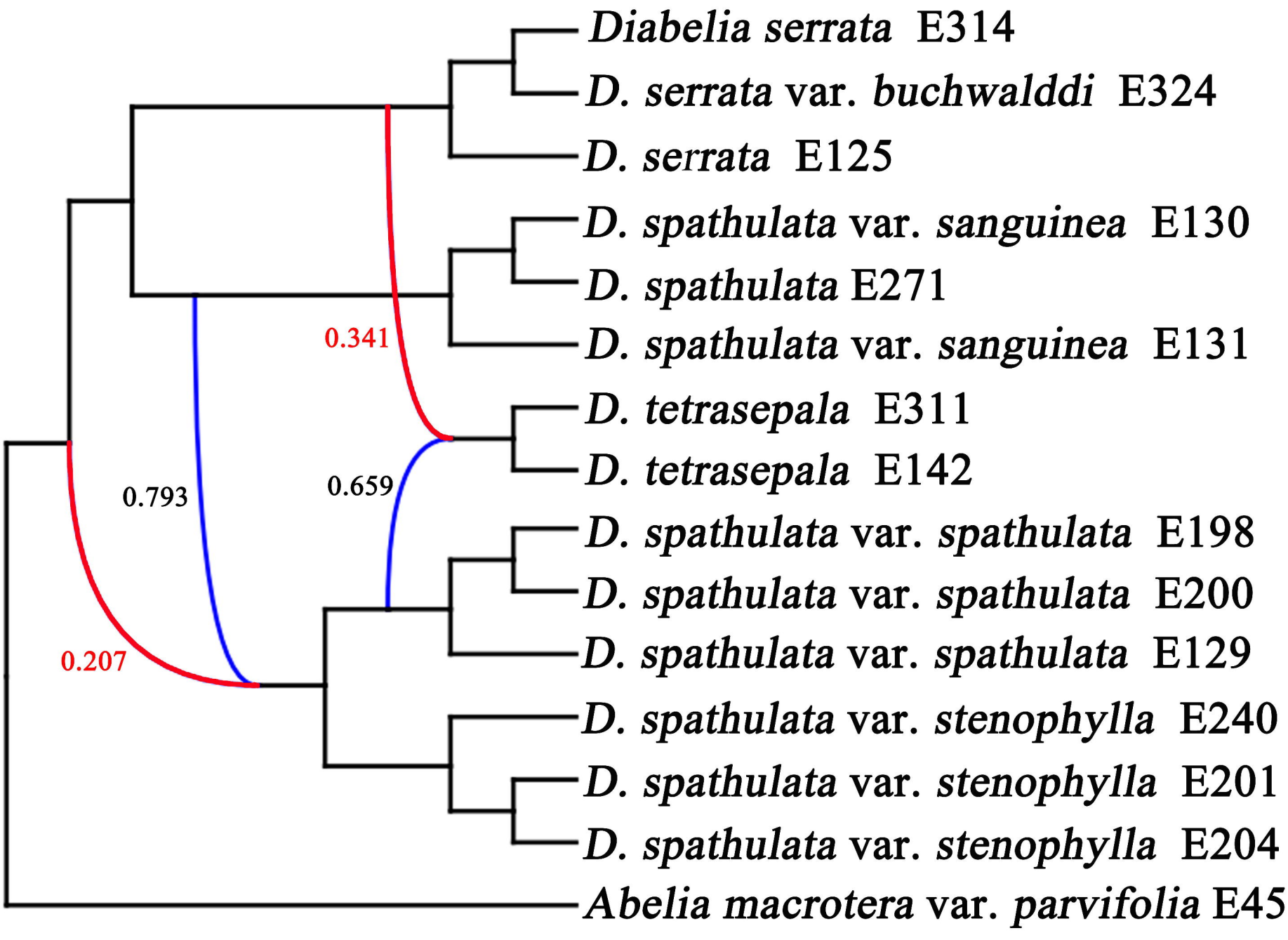
	2										
D. serrata E314	5056940	378381	0.075	1050	877	850	839	746	587	1	36
D. serrata E321	13220295	4190116	0.317	1138	1037	1002	1000	986	934	0	93
D. spathulata E271	3089596	212920	0.069	992	827	809	806	762	661	0	39
D. spathulata var. *sanguinea* E131	13699360	3897086	0.284	1107	1018	991	990	979	930	1	87
D. spathulata var. *stenophylla* E132	7051574	1749371	0.248	1081	1000	976	974	958	905	1	86
D. serrata E140	6481952	1399052	0.216	1081	992	967	966	949	888	0	88
D. spathulata var. *spathulata* E141	6471860	1497704	0.231	1075	992	966	965	952	900	0	87
D. spathulata var. *spathulata* E190	8953298	2806002	0.313	1124	1022	989	986	973	928	2	86
D. spathulata var. *spathulata* E198	9267804	3459777	0.373	1130	1022	993	992	981	928	1	92
D. spathulata var. *spathulata* E200	8307344	2742274	0.33	1118	1018	991	990	980	922	1	86

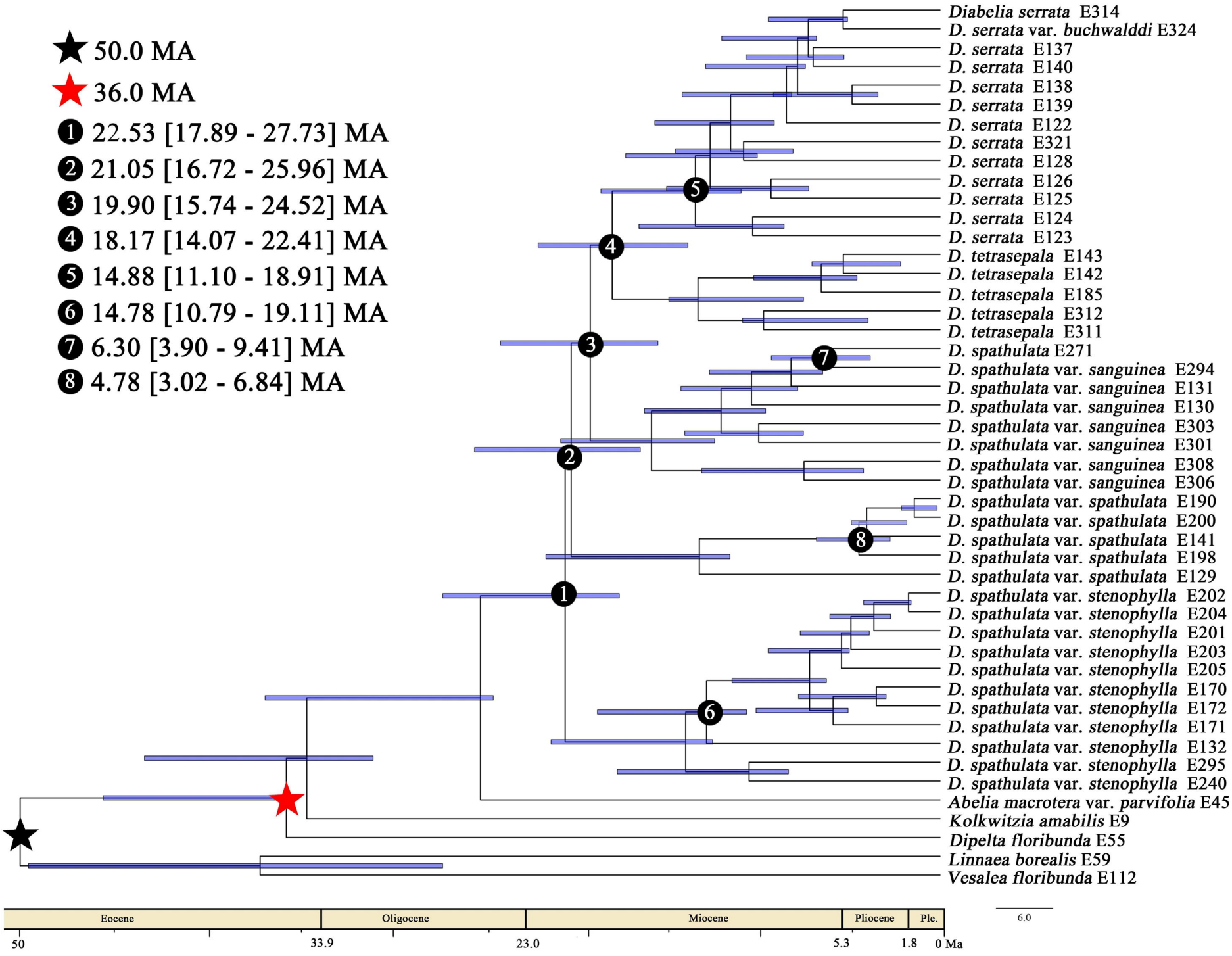
Abelia macrotera var. *parvifolia* E45	1833543	32422	0.018	825	436	424	417	355	253	0	6
Dipelta floribunda E55	4636783	105360	0.023	924	660	642	639	601	507	0	27
Kolkwitzia amabilis E9	9779050	2641147	0.27	1097	1005	984	983	967	916	0	75
Linnaea borealis E59	3831881	51899	0.014	867	536	516	507	443	333	0	11
Vesalea floribunda E112	3834861	805440	0.21	1061	970	947	946	915	856	0	52

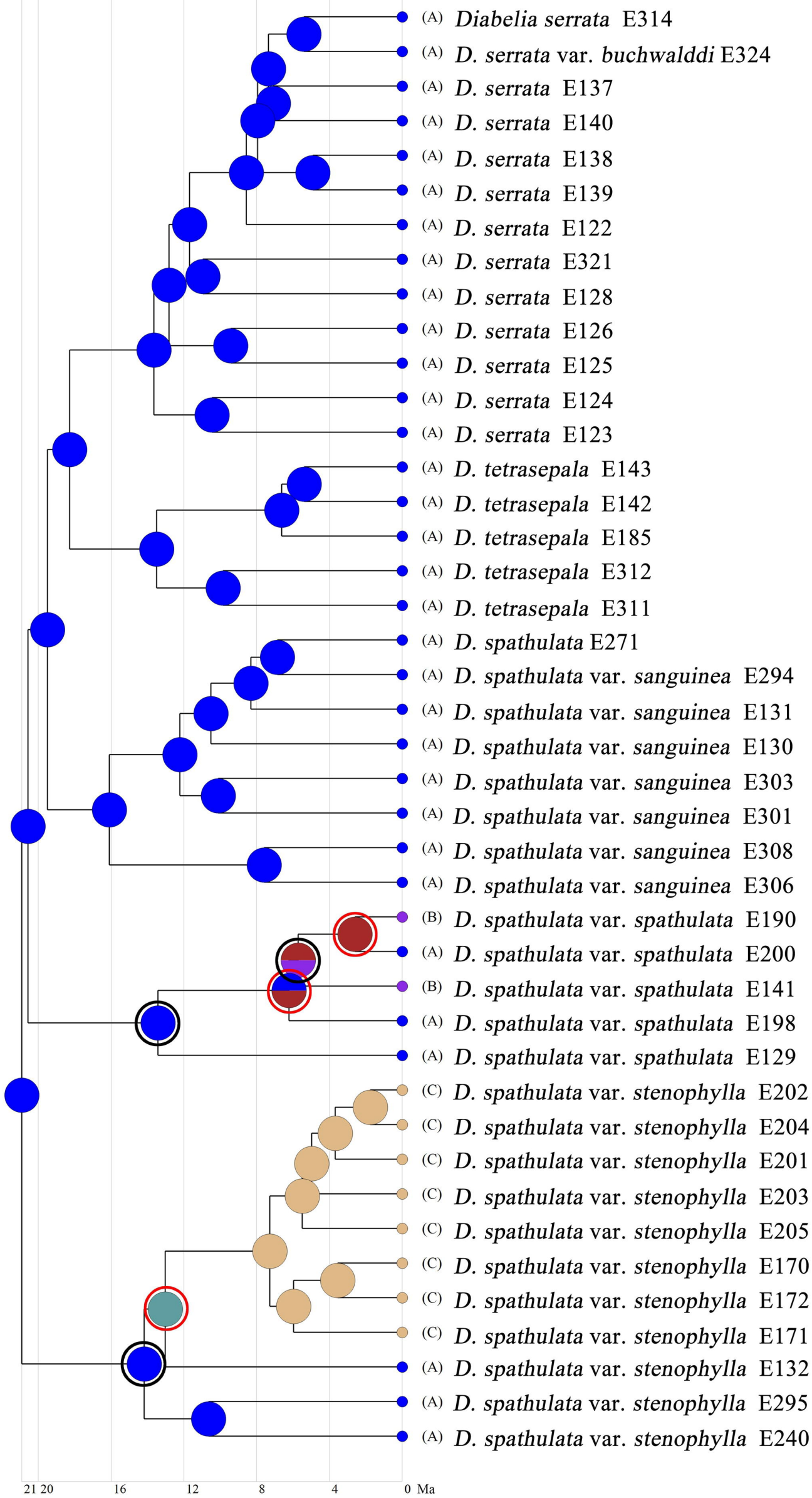

1 cm
A
1 cm
B
1 cm
C
1 cm
D
1 cm
E
1 cm
F

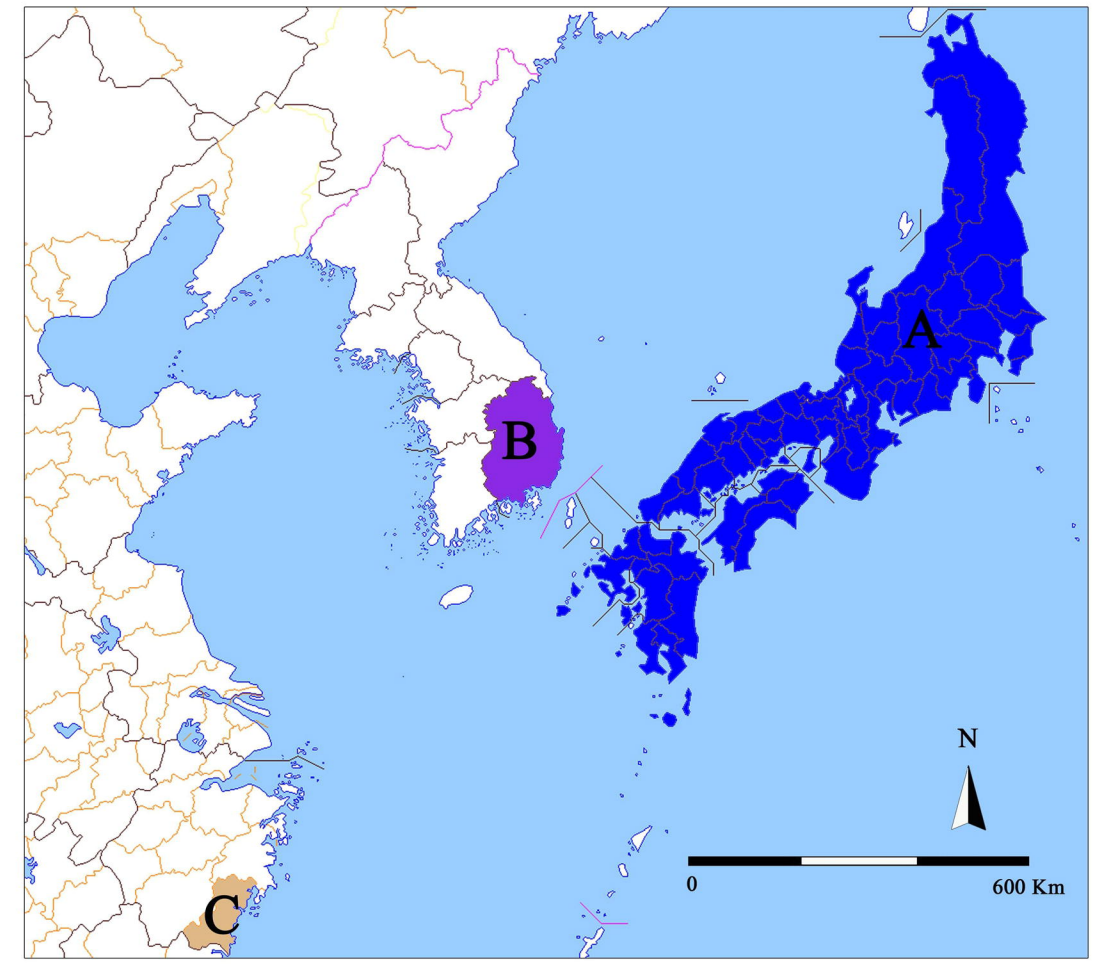

Nuclear concatenated
Plastome
D. serrata
D. tetrasepala
D. spathulata var. sanguinea
var. stenophylla
var. spathulata
Outgroups
Diabelia serrata E314
D. serrata var. buchwalddi E324
D. serrata E137
D. serrata E140
D. serrata E138
D. serrata E139
D. serrata E123
D. serrata E122
D. serrata E126
D. serrata E125
D. serrata E321
D. serrata E128
D. serrata E124
D. tetrasepala E142
D. tetrasepala E143
D. tetrasepala E185
D. tetrasepala E312
D. tetrasepala E311
D. spathulata var. sanguinea E294
D. spathulata E271
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E131
D. spathulata var. sanguinea E306
D. spathulata var. sanguinea E308
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E170
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
D. spathulata var. spathulata E198
D. spathulata var. spathulata E190
D. spathulata var. spathulata E200
D. spathulata var. spathulata E141
D. spathulata var. spathulata E129
Abelia macrotera var. parvifolia E45
Kolkwitzia amabilis E9
Dipelta floribunda E55
Linnaea borealis E59
Vesalea floribunda E112
D. serrata
D. tetrasepala
D. spathulata var. stenophylla
var. sanguinea
var. spathulata
var. stenophylla
Outgroups
0.004
0.008

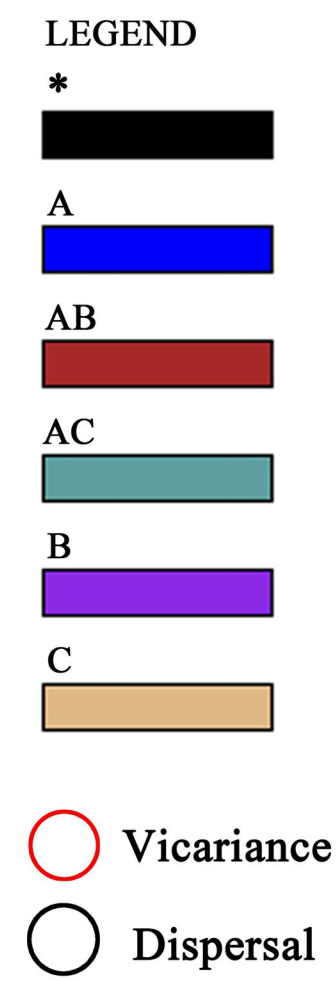

Diabelia serrata E138
D. serrata E139
D. serrata E125
D. serrata E140
D. serrata E137
D. serrata var. buchwalddi E324
D. serrata E126
D. serrata E128
D. serrata E122
D. serrata E321
D. serrata E314
D. serrata E123
D. serrata E124
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E294
D. spathulata E271
D. spathulata var. sanguinea E131
D. spathulata var. sanguinea E308
D. spathulata var. sanguinea E306
D. tetrasepala E185
D. tetrasepala E142
D. tetrasepala E143
D. tetrasepala E311
D. tetrasepala E312
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E170
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E240
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E132
D. spathulata var. spathulata E141
D. spathulata var. spathulata E190
D. spathulata var. spathulata E200
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
Abelia macrotera var. parvifolia E45
Kolkwitzia amabilis E9
Dipelta floribunda E55
Vesalea floribunda E112
Linnaea borealis E59
1/0.64/0/1
0.42/0.22/0.19/0.99
0.82/0.16/0.58/0.99
0.66/-0.47/0.06/1
0.6/0.43/0/1
0.59/0.22/0.55/0.99
0.6/0.33/0.73/0.98
0.52/-0.03/0.85/1
0.68/0.43/0.06/0.99
0.57/0.45/0.13/0.99
1/0.92/0.38/1
0.99/0.04/0.76/0.98
0.47/-0.09/0/0.98
0.87/0.39/0.63/1
0.6/-0.35/0.52/0.99
0.96/0.59/0.38/1
1/0.89/0.44/1
1/0.62/0.78/0.99
0.44/-0.22/0.21/0.99
1/1/-/1
0.93/0.2/0.35/0.99
0.63/-0.43/0/1
1/0.59/0/1
1/1/-/1
1/1/-/1
0.98/0.49/0.42/0.99
1/-0.24/0/0.93
0.71/0.05/0.77/0.97
0.41/0.39/0.25/0.99
0.98/0.48/0.75/1
1/1/-/1
0.54/1/-/1
0.93/0.63/0.17/0.97
1/0.9/0.2/1
1/1/-/1
0.62/0.42/0.18/1
0.97/0.36/0.01/0.98
1/1/-/1
0.98/-0.41/0.06/0.98
0.41/0.36/0.25/0.98
1/1/-/1
0.99/0.22/0.29/0.98
0.99/0.32/0.05/1
1/1/-/1
1/1/-/1
D. serrata
D. spathulata var. sanguinea
D. tetrasepala
D. spathulata var. stenophylla
var. spathulata
Outgroups
04

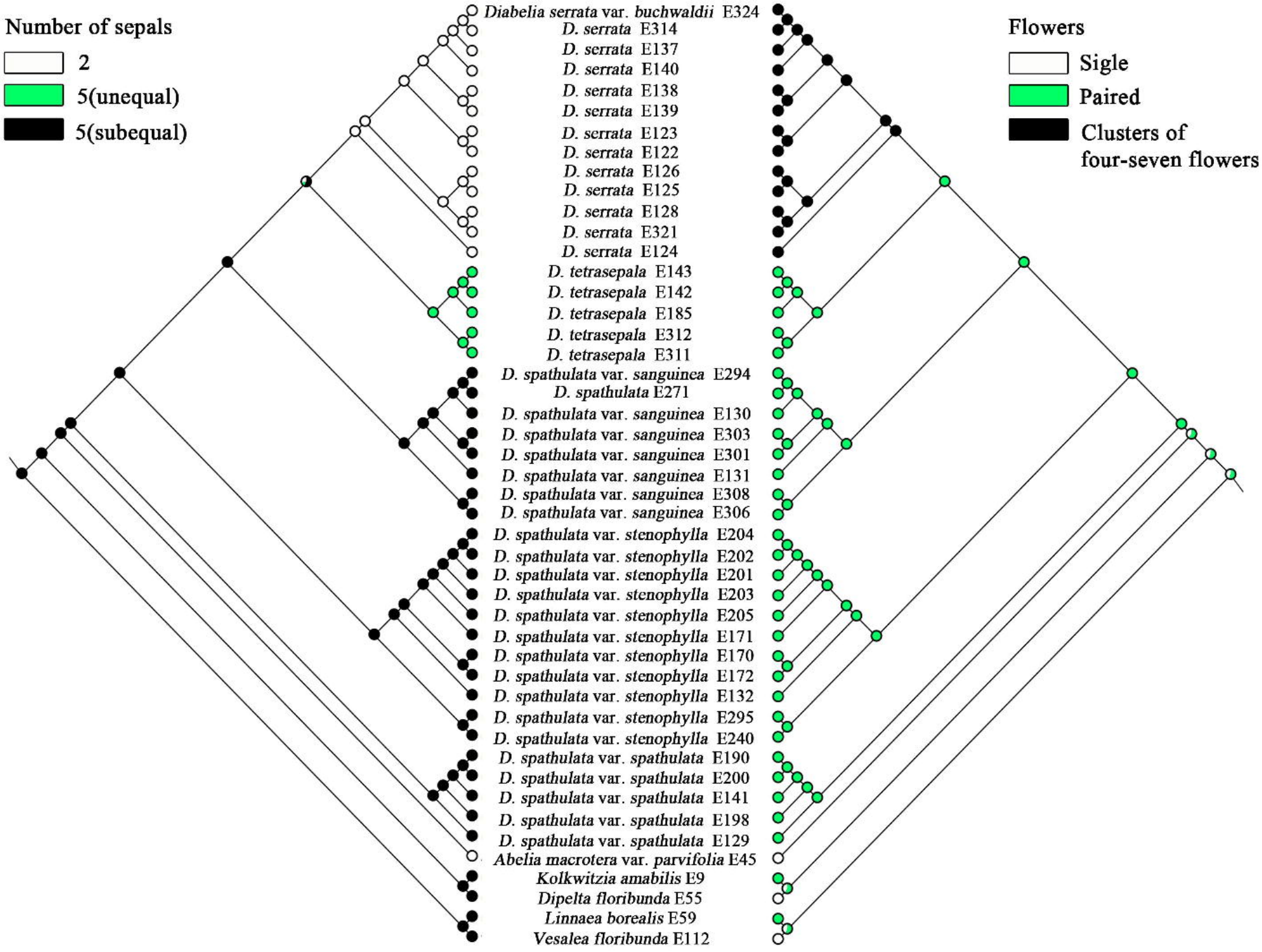

Diabelia serrata E314
D. serrata var. buchwalddi E324
D. serrata E125
D. spathulata var. sanguinea E130
D. spathulata E271
D. spathulata var. sanguinea E131
D. tetrasepala E311
D. tetrasepala E142
D. spathulata var. spathulata E198
D. spathulata var. spathulata E200
D. spathulata var. spathulata E129
D. spathulata var. stenophylla E240
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E204
Abelia macrotera var. parvifolia E45
0.341
0.793
0.659
0.207

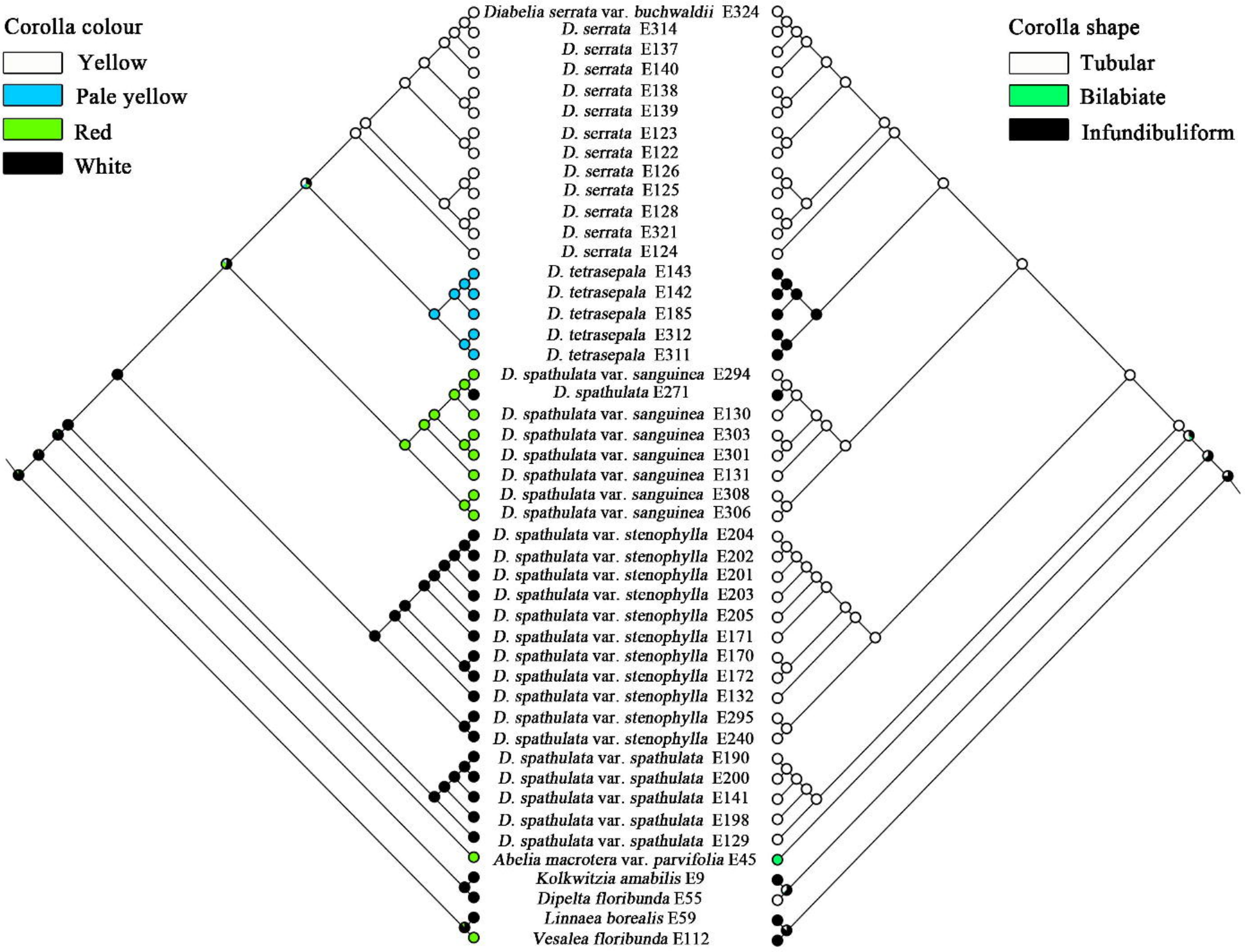

50.0 MA
36.0 MA
1 22.53 [17.89 - 27.73] MA
2 21.05 [16.72 - 25.96] MA
3 19.90 [15.74 - 24.52] MA
4 18.17 [14.07 - 22.41] MA
5 14.88 [11.10 - 18.91] MA
6 14.78 [10.79 - 19.11] MA
7 6.30 [3.90 - 9.41] MA
8 4.78 [3.02 - 6.84] MA
Diabelia serrata E314
D. serrata var. buchwalddi E324
D. serrata E137
D. serrata E140
D. serrata E138
D. serrata E139
D. serrata E122
D. serrata E321
D. serrata E128
D. serrata E126
D. serrata E125
D. serrata E124
D. serrata E123
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. tetrasepala E312
D. tetrasepala E311
D. spathulata E271
D. spathulata var. sanguinea E294
D. spathulata var. sanguinea E131
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E308
D. spathulata var. sanguinea E306
D. spathulata var. spathulata E190
D. spathulata var. spathulata E200
D. spathulata var. spathulata E141
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E170
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
Abelia macrotera var. parvifolia E45
Kolkwitzia amabilis E9
Dipelta floribunda E55
Linnaea borealis E59
Vesalea floribunda E112
Eocene
Oligocene
Miocene
Pliocene
Ple.
50
33.9
23.0
5.3
1.8
0 Ma
6.0

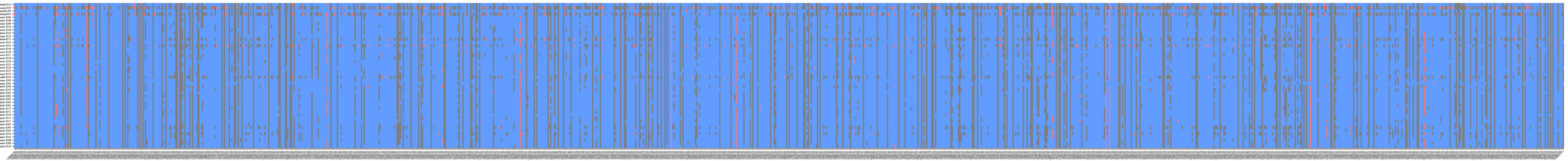

(A) Diabelia serrata E314
(A) D. serrata var. buchwalddi E324
(A) D. serrata E137
(A) D. serrata E140
(A) D. serrata E138
(A) D. serrata E139
(A) D. serrata E122
(A) D. serrata E321
(A) D. serrata E128
(A) D. serrata E126
(A) D. serrata E125
(A) D. serrata E124
(A) D. serrata E123
(A) D. tetrasepala E143
(A) D. tetrasepala E142
(A) D. tetrasepala E185
(A) D. tetrasepala E312
(A) D. tetrasepala E311
(A) D. spathulata E271
(A) D. spathulata var. sanguinea E294
(A) D. spathulata var. sanguinea E131
(A) D. spathulata var. sanguinea E130
(A) D. spathulata var. sanguinea E303
(A) D. spathulata var. sanguinea E301
(A) D. spathulata var. sanguinea E308
(A) D. spathulata var. sanguinea E306
(B) D. spathulata var. spathulata E190
(A) D. spathulata var. spathulata E200
(B) D. spathulata var. spathulata E141
(A) D. spathulata var. spathulata E198
(A) D. spathulata var. spathulata E129
(C) D. spathulata var. stenophylla E202
(C) D. spathulata var. stenophylla E204
(C) D. spathulata var. stenophylla E201
(C) D. spathulata var. stenophylla E203
(C) D. spathulata var. stenophylla E205
(C) D. spathulata var. stenophylla E170
(C) D. spathulata var. stenophylla E172
(C) D. spathulata var. stenophylla E171
(A) D. spathulata var. stenophylla E132
(A) D. spathulata var. stenophylla E295
(A) D. spathulata var. stenophylla E240
21 20 16 12 8 4 0 Ma

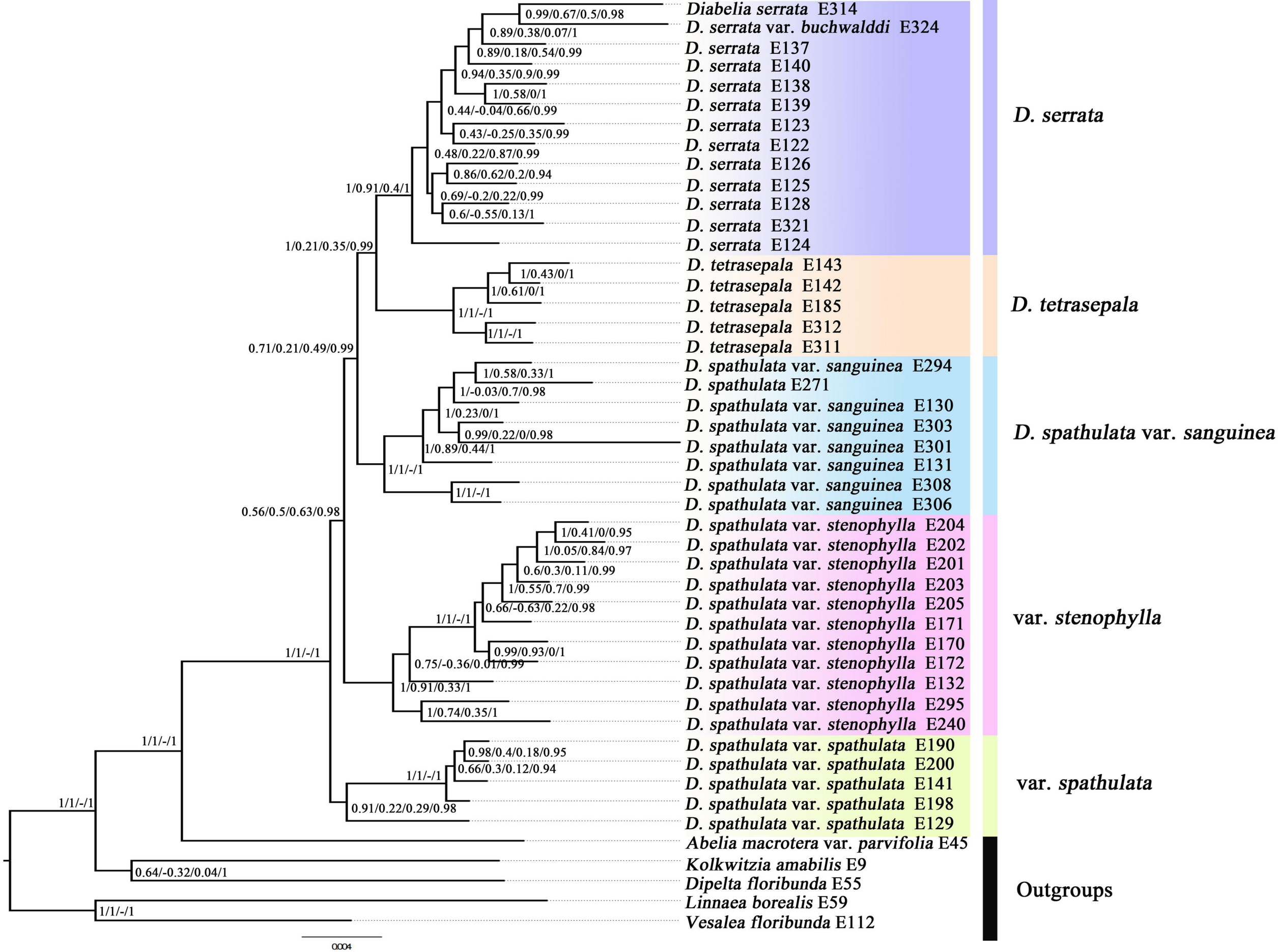

A
B
C
N
0
600 Km

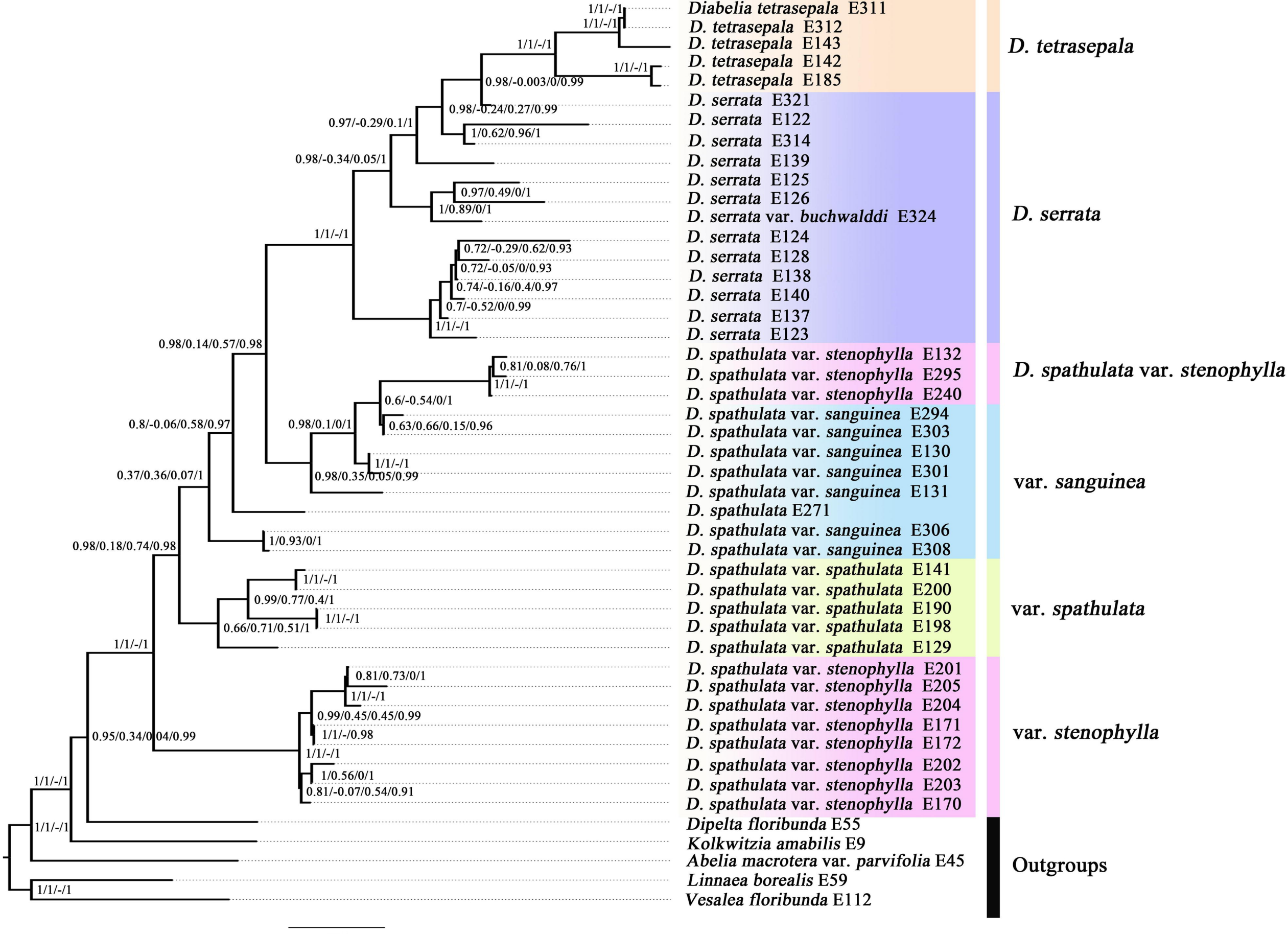

LEGEND
*
A
AB
AC
B
C
Vicariance
Dispersal

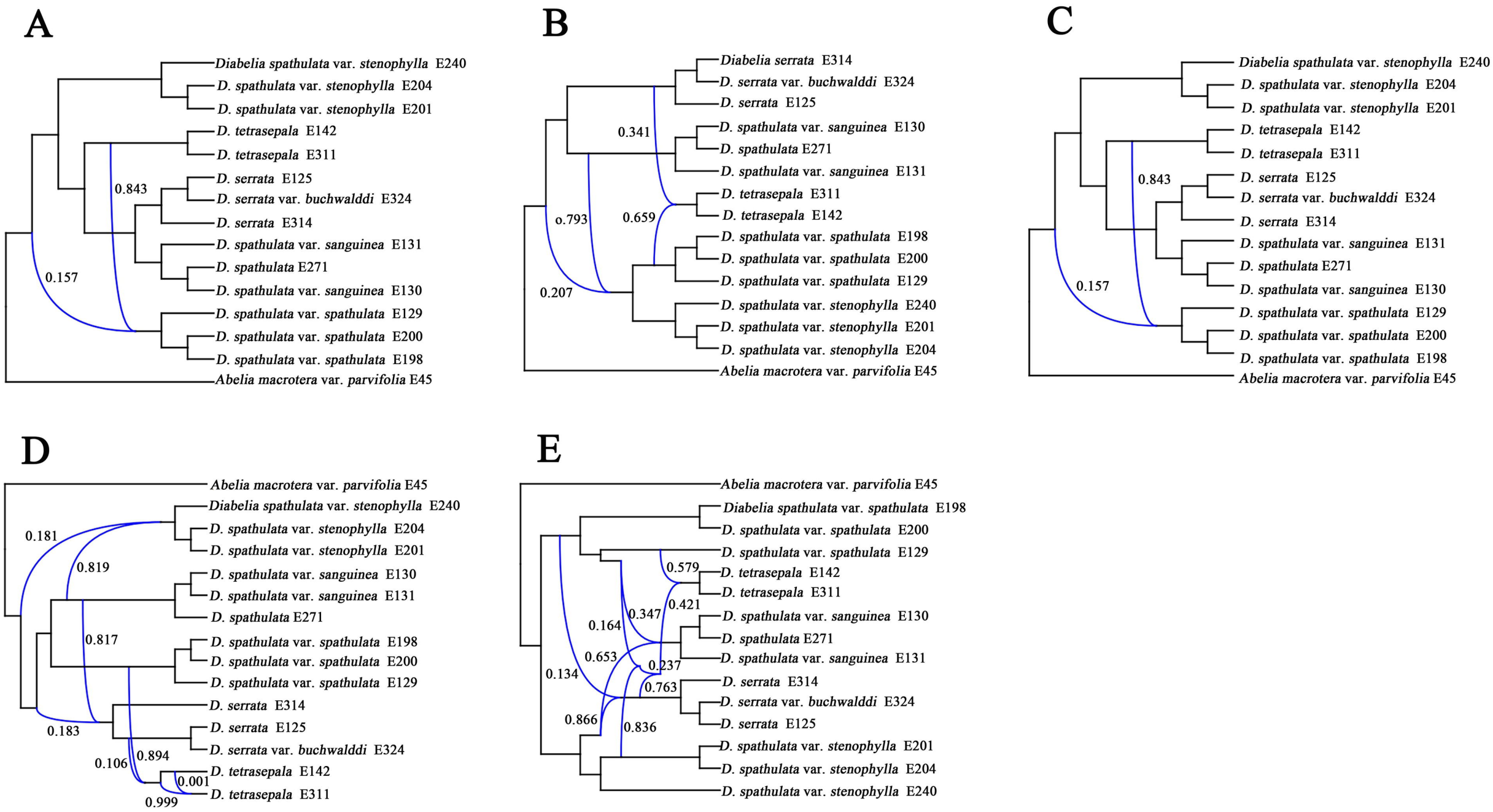

Number of sepals
2
5(unequal)
5(subequal)
Flowers
Sigle
Paired
Clusters of four-seven flowers
Diabelia serrata var. buchwaldii E324
D. serrata E314
D. serrata E137
D. serrata E140
D. serrata E138
D. serrata E139
D. serrata E123
D. serrata E122
D. serrata E126
D. serrata E125
D. serrata E128
D. serrata E321
D. serrata E124
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. tetrasepala E312
D. tetrasepala E311
D. spathulata var. sanguinea E294
D. spathulata E271
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E131
D. spathulata var. sanguinea E308
D. spathulata var. sanguinea E306
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E170
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
D. spathulata var. spathulata E190
D. spathulata var. spathulata E200
D. spathulata var. spathulata E141
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
Abelia macrotera var. parvifolia E45
Kolkwitzia amabilis E9
Dipelta floribunda E55
Linnaea borealis E59
Vesalea floribunda E112

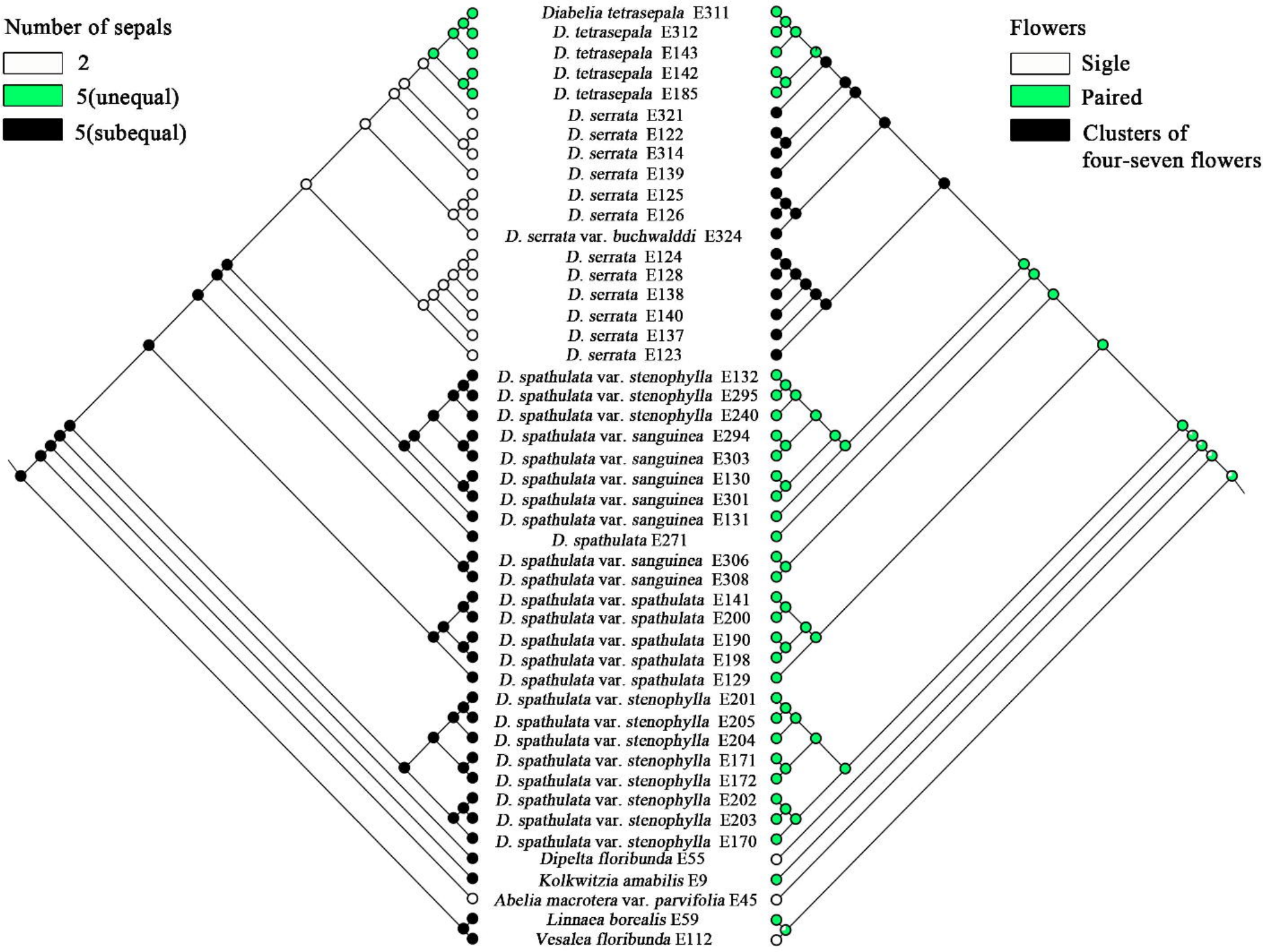

Corolla colour
Yellow
Pale yellow
Red
White
Corolla shape
Tubular
Bilabiate
Infundibuliform
Diabelia serrata var. buchwaldii E324
D. serrata E314
D. serrata E137
D. serrata E140
D. serrata E138
D. serrata E139
D. serrata E123
D. serrata E122
D. serrata E126
D. serrata E125
D. serrata E128
D. serrata E321
D. serrata E124
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. tetrasepala E312
D. tetrasepala E311
D. spathulata var. sanguinea E294
D. spathulata E271
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E131
D. spathulata var. sanguinea E308
D. spathulata var. sanguinea E306
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E170
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
D. spathulata var. spathulata E190
D. spathulata var. spathulata E200
D. spathulata var. spathulata E141
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
Abelia macrotera var. parvifolia E45
Kolkwitzia amabilis E9
Dipelta floribunda E55
Linnaea borealis E59
Vesalea floribunda E112

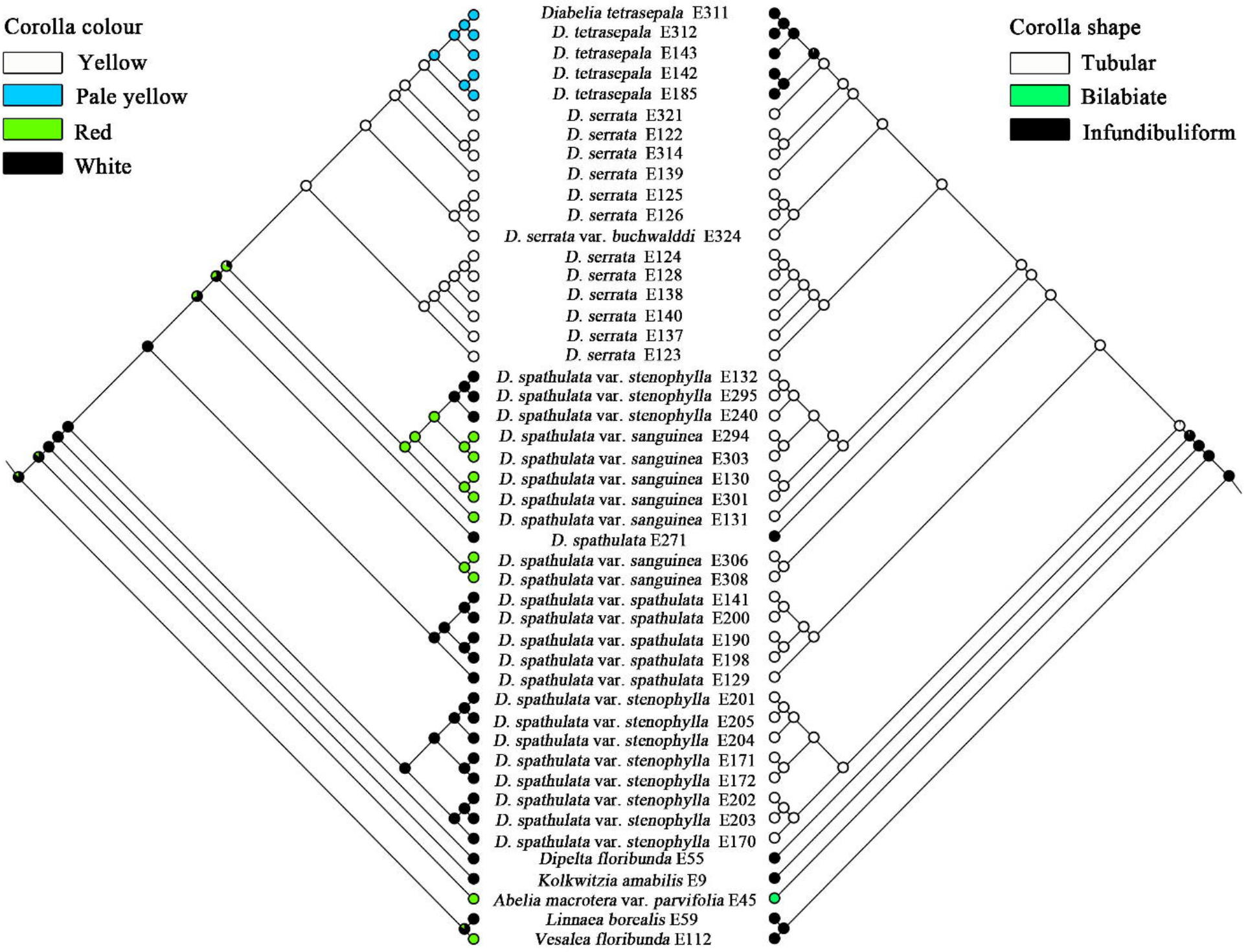

value
1.00
0.75
0.50
0.25
0.00


Diabelia serrata E314
D. serrata var. buchwalddi E324
D. serrata E137
D. serrata E140
D. serrata E138
D. serrata E139
D. serrata E123
D. serrata E122
D. serrata E126
D. serrata E125
D. serrata E128
D. serrata E321
D. serrata E124
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. tetrasepala E312
D. tetrasepala E311
D. spathulata var. sanguinea E294
D. spathulata E271
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E131
D. spathulata var. sanguinea E308
D. spathulata var. sanguinea E306
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E170
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
D. spathulata var. spathulata E190
D. spathulata var. spathulata E200
D. spathulata var. spathulata E141
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
Abelia macrotera var. parvifolia E45
Kolkwitzia amabilis E9
Dipelta floribunda E55
Linnaea borealis E59
Vesalea floribunda E112
D. serrata
D. tetrasepala
D. spathulata var. sanguinea
var. stenophylla
var. spathulata
Outgroups
0.99/0.67/0.5/0.98
0.89/0.38/0.07/1
0.89/0.18/0.54/0.99
0.94/0.35/0.9/0.99
1/0.58/0/1
0.44/-0.04/0.66/0.99
0.43/-0.25/0.35/0.99
0.48/0.22/0.87/0.99
0.86/0.62/0.2/0.94
1/0.91/0.4/1
0.69/-0.2/0.22/0.99
0.6/-0.55/0.13/1
1/0.21/0.35/0.99
1/0.43/0/1
1/0.61/0/1
1/1/-/1
1/1/-/1
0.71/0.21/0.49/0.99
1/0.58/0.33/1
1/-0.03/0.7/0.98
1/0.23/0/1
0.99/0.22/0/0.98
1/0.89/0.44/1
1/1/-/1
1/1/-/1
0.56/0.5/0.63/0.98
1/0.41/0/0.95
1/0.05/0.84/0.97
0.6/0.3/0.11/0.99
1/0.55/0.7/0.99
0.66/-0.63/0.22/0.98
1/1/-/1
1/1/-/1
0.99/0.93/0/1
0.75/-0.36/0.01/0.99
1/0.91/0.33/1
1/0.74/0.35/1
1/1/-/1
0.98/0.4/0.18/0.95
0.66/0.3/0.12/0.94
1/1/-/1
0.91/0.22/0.29/0.98
1/1/-/1
0.64/-0.32/0.04/1
1/1/-/1
0.004


Diabelia tetrasepala E311
D. tetrasepala E312
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. serrata E321
D. serrata E122
D. serrata E314
D. serrata E139
D. serrata E125
D. serrata E126
D. serrata var. buchwalddi E324
D. serrata E124
D. serrata E128
D. serrata E138
D. serrata E140
D. serrata E137
D. serrata E123
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
D. spathulata var. sanguinea E294
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E131
D. spathulata E271
D. spathulata var. sanguinea E306
D. spathulata var. sanguinea E308
D. spathulata var. spathulata E141
D. spathulata var. spathulata E200
D. spathulata var. spathulata E190
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E170
Dipelta floribunda E55
Kolkwitzia amabilis E9
Abelia macrotera var. parvifolia E45
Linnaea borealis E59
Vesalea floribunda E112
D. tetrasepala
D. serrata
D. spathulata var. stenophylla
var. sanguinea
var. spathulata
var. stenophylla
Outgroups
1/1/-/1
1/1/-/1
1/1/-/1
1/1/-/1
0.98/-0.003/0/0.99
0.98/-0.24/0.27/0.99
0.97/-0.29/0.1/1
1/0.62/0.96/1
0.98/-0.34/0.05/1
0.97/0.49/0/1
1/0.89/0/1
1/1/-/1
0.72/-0.29/0.62/0.93
0.72/-0.05/0/0.93
0.74/-0.16/0.4/0.97
0.7/-0.52/0/0.99
1/1/-/1
0.98/0.14/0.57/0.98
0.81/0.08/0.76/1
1/1/-/1
0.6/-0.54/0/1
0.8/-0.06/0.58/0.97
0.98/0.1/0/1
0.63/0.66/0.15/0.96
1/1/-/1
0.37/0.36/0.07/1
0.98/0.35/0.05/0.99
1/0.93/0/1
0.98/0.18/0.74/0.98
1/1/-/1
0.99/0.77/0.4/1
1/1/-/1
0.66/0.71/0.51/1
1/1/-/1
0.81/0.73/0/1
1/1/-/1
0.99/0.45/0.45/0.99
1/1/-/0.98
0.95/0.34/0.04/0.99
1/1/-/1
1/0.56/0/1
0.81/-0.07/0.54/0.91
1/1/-/1
1/1/-/1
1/1/-/1
0.003


A
Diabelia spathulata var. stenophylla E240
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E201
D. tetrasepala E142
D. tetrasepala E311
D. serrata E125
D. serrata var. buchwalddi E324
D. serrata E314
D. spathulata var. sanguinea E131
D. spathulata E271
D. spathulata var. sanguinea E130
D. spathulata var. spathulata E129
D. spathulata var. spathulata E200
D. spathulata var. spathulata E198
Abelia macrotera var. parvifolia E45
0.843
0.157
B
Diabelia serrata E314
D. serrata var. buchwalddi E324
D. serrata E125
D. spathulata var. sanguinea E130
D. spathulata E271
D. spathulata var. sanguinea E131
D. tetrasepala E311
D. tetrasepala E142
D. spathulata var. spathulata E198
D. spathulata var. spathulata E200
D. spathulata var. spathulata E129
D. spathulata var. stenophylla E240
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E204
Abelia macrotera var. parvifolia E45
0.341
o.793
0.659
0.207
C
Diabelia spathulata var. stenophylla E240
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E201
D. tetrasepala E142
D. tetrasepala E311
D. serrata E125
D. serrata var. buchwalddi E324
D. serrata E314
D. spathulata var. sanguinea E131
D. spathulata E271
D. spathulata var. sanguinea E130
D. spathulata var. spathulata E129
D. spathulata var. spathulata E200
D. spathulata var. spathulata E198
Abelia macrotera var. parvifolia E45
0.843
0.157
D
Abelia macrotera var. parvifolia E45
Diabelia spathulata var. stenophylla E240
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E201
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E131
D. spathulata E271
D. spathulata var. spathulata E198
D. spathulata var. spathulata E200
D. spathulata var. spathulata E129
D. serrata E314
D. serrata E125
D. serrata var. buchwalddi E324
D. tetrasepala E142
D. tetrasepala E311
0.181
0.819
0.817
0.183
0.106
0.894
0.001
0.999
E
Abelia macrotera var. parvifolia E45
Diabelia spathulata var. spathulata E198
D. spathulata var. spathulata E200
D. spathulata var. spathulata E129
D. tetrasepala E142
D. tetrasepala E311
D. spathulata var. sanguinea E130
D. spathulata E271
D. spathulata var. sanguinea E131
D. serrata E314
D. serrata var. buchwalddi E324
D. serrata E125
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E240
0.579
0.421
0.347
0.164
0.653
0.237
0.134
0.763
0.866
0.836


Number of sepals
2
5(unequal)
5(subequal)
Flowers
Sigle
Paired
Clusters of four-seven flowers
Diabelia tetrasepala E311
D. tetrasepala E312
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. serrata E321
D. serrata E122
D. serrata E314
D. serrata E139
D. serrata E125
D. serrata E126
D. serrata var. buchwalddi E324
D. serrata E124
D. serrata E128
D. serrata E138
D. serrata E140
D. serrata E137
D. serrata E123
D. spathulata var. stenophylla E132
D. spathulata var. stenophylla E295
D. spathulata var. stenophylla E240
D. spathulata var. sanguinea E294
D. spathulata var. sanguinea E303
D. spathulata var. sanguinea E130
D. spathulata var. sanguinea E301
D. spathulata var. sanguinea E131
D. spathulata E271
D. spathulata var. sanguinea E306
D. spathulata var. sanguinea E308
D. spathulata var. spathulata E141
D. spathulata var. spathulata E200
D. spathulata var. spathulata E190
D. spathulata var. spathulata E198
D. spathulata var. spathulata E129
D. spathulata var. stenophylla E201
D. spathulata var. stenophylla E205
D. spathulata var. stenophylla E204
D. spathulata var. stenophylla E171
D. spathulata var. stenophylla E172
D. spathulata var. stenophylla E202
D. spathulata var. stenophylla E203
D. spathulata var. stenophylla E170
Dipelta floribunda E55
Kolkwitzia amabilis E9
Abelia macrotera var. parvifolia E45
Linnaea borealis E59
Vesalea floribunda E112


Corolla colour
Yellow
Pale yellow
Red
White
Corolla shape
Tubular
Bilabiate
Infundibuliform
Diabelia tetrasepala E311
D. tetrasepala E312
D. tetrasepala E143
D. tetrasepala E142
D. tetrasepala E185
D. serrata E321
D. serrata E122
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D. spathulata var. spathulata E129
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Linnaea borealis E59
Vesalea floribunda E112