Abelia and relatives: phylogenetics of Linnaeeae (Dipsacales–Caprifoliaceae *s.l.*) and a new interpretation of their inflorescence morphology

SVEN LANDREIN*, GERHARD PRENNER, MARK W. CHASE and JAMES J. CLARKSON

Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK

We present a phylogenetic analysis with sampling from all taxonomic groups in the tribe Linnaeeae (Caprifoliaceae) and interpret the results in relation to inflorescence morphology. Characters from inflorescences are shown to be highly informative for the classification of Linnaeeae; however, they have been neglected in recent studies, possibly as a result of confusion between descriptive and typological views. The phylogenetic relationships of 27 taxa are investigated using the nuclear ribosomal internal transcribed spacer (ITS) region and multiple plastid regions (*rbcL*, *ndhF*, *matK*, *trnL* intron and *trnL-F* spacer). Two additional plastid regions, *atpB-rbcL* and the *trnK* intron, are also investigated for a subset of taxa. Results indicate that several taxonomic groups, including sections and genera, are not monophyletic. Several groups are supported, e.g. *Zabelia*, *Vesalea* and *Abelia* section *Abelia*, but *Abelia* series *Serratae* is not supported. A new hypothesis for inflorescence evolution in Dipsacales is proposed, and the distinctiveness of *Abelia* series *Serratae* is reinforced. The position of tribe Zabeliinae remains unclear within Caprifoliaceae. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, **169**, 692–713.

ADDITIONAL KEYWORDS: *Abelia* series *Serratae* – *Diabelia* – thyrse – Zabeliinae.

INTRODUCTION

In recent phylogenetic studies (Bell *et al*., 2001; Donoghue *et al*., 2001; Zhang *et al*., 2002a; Jacobs, Pyck & Smets, 2010), Linnaeeae forms a distinct clade more closely related to Morinaceae, Dipsacaceae and Valerianaceae than to Caprifolieae and Diervilleae. In previous phylogenetic studies, Caprifoliaceae had been redefined to include Caprifolieae, Diervilleae, Linnaeeae, Morinaceae, Dipsacaceae and Valerianaceae (Bell *et al*., 2001). The name Linnina for the clade including Linnaeeae and Valerina (Morinaceae, Dipsacaceae and Valerianaceae) was also introduced by Donoghue *et al*. (2001). Backlund & Pyck (1998)

preferred to raise Linnaeeae and Diervilleae to family level, but this system was not followed by Donoghue *et al*. (2001) because it does not retain most of the names currently in use.

Caprifoliaceae *s.l.* (APG III, 2009) is used here because of the problematic position of *Heptacodium* Rehder, which has morphological characters that are a mixture of those in Linnaeeae and Caprifolieae. A genus *incertae sedis* within a family is acceptable, but a genus without a clear family placement is problematic; therefore, we favour Caprifoliaceae *sensu* APG III (2009). As in Linnaeeae, ovaries of *Heptacodium* are trilocular with a single fertile locule, and the achenes have a persistent accrescent calyx. However, flowers have five stamens, and the inflorescence is reminiscent of *Lonicera* L. subgenus *Periclymenum* Mill. (Caprifolieae). The phylogenetic study of Pyck &

*Corresponding author. E-mail: s.landrein@kew.org

Smets (2000) placed *Heptacodium* as an unresolved lineage in Caprifoliaceae *s.l.* A karyomorphological study (Zhang, Zhou & Gu, 2002b) suggested that *Heptacodium* perhaps resulted from hybridization and polyploidization between two ancestral species, possibly with a base chromosome number of $x = 8$ or 9 and having characters of Caprifolieae and Linnaeeae (dislocation hypothesis; Stebbins, 1971). Another reason not to raise Linnaeeae to family level was presented in a phylogenetic study by Jacobs *et al.* (2010), in which the relationship between *Abelia* R.Br. and *Zabelia* (Rehder) Makino was questioned, and a weakly supported position of *Zabelia* sister to the *Morina* clade rather than Linnaeeae was shown.

A classification of Linnaeeae was first proposed by Vatke (1872), which was followed by that of Zabel (1893). Graebner (1900) published the first complete study of Linnaeeae and produced an artificial classification. Rehder (1911) and Fukuoka (1968) produced more recent classifications based solely on morphology. An unpublished thesis using molecular data (nuclear ribosomal DNA sequences) by Kim (1998) produced more insight into the classification of Linnaeeae; he included one new subtribe, Zabeliinae, but this remains unpublished. Kim's (1998) generic delimitation is based on a number of traditionally used morphological characters (mostly inflorescences) and molecular data. He recognized six genera (Table 1).

The centre of diversity for Linnaeeae is China. *Linnaea* L. is circumboreal, and other taxa also occur in Japan, Korea, easternmost Russia, Central Asia, the Himalayas and Mexico. The total number of species recognized is 17–39, depending on the author. Members of Linnaeeae are important in horticulture, and clarification of the generic limits in this clade is necessary. With this in mind, we initiated this molecular study of Linnaeeae based on five plastid regions (*rbcL*, *ndhF*, *trnL* intron, *trnL-F* spacer and *matK*) and the nuclear ribosomal internal transcribed spacer (nrITS) region; two more plastid regions (*atpB-rbcL* spacer and *trnK* intron) were sampled for a smaller dataset. The resulting phylogenetic trees were used to re-evaluate the morphology and evolution of the inflorescence in this group.

INFLORESCENCE

The DNA analyses performed here provide us with a framework to reinterpret inflorescence architecture; the molecular data create a backbone against which hypotheses can be tested. Most taxonomic studies of Linnaeeae have mentioned the inflorescence as the most important morphological character to examine, but inflorescence studies suffer from confusion between descriptive and typological views (idealized

Table 1. Linnaeeae classification by Kim (1998)

Tribe	Subtribe	Genus	Section/Series	Species number	Species names
Linnaeeae Dumortier (1829)	Zabeliinae T.Kim & B.Sun, ined.	Zabelia Makino cf. Ikuse & Kurosawa (1954)	Zabelia	3	Z. corymbosa, triflora and tyaihyoni
Linnaeeae	Zabeliinae	Zabelia	Biflorae (Zabel) Fukuoka (1968)	3	Z. biflora, dielsii and integrifolia
Linnaeeae	Linnaeinae	Linnaea L. (1753)	–	1	L. borealis
Linnaeeae	Linnaeinae	Vesalea Martens & Galeotti (1843)	–	2	V. coriacea and floribunda
Linnaeeae	Linnaeeae	Abelia Brown (1818)	Abelia	3	A. chinensis, forrestii and uniflora
Linnaeeae	Linnaeeae	Abelia	Serratae Graebn.	3	A. serrata, spathulata and tetrasepala
Linnaeeae	Linnaeinae	Kolkwitzia Graebn. (1901)	–	1	K. amabilis.
Linnaeeae	Linnaeinae	Dipelta Maxim. (1878)	–	3	D. elegans, floribunda and yunnanensis

morphology; Endress, 2010). In order to establish relationships between taxa, it is nevertheless not possible to use only a descriptive approach, and a theory of idealized morphological characters needs to be developed.

Inflorescence descriptions can sometimes be difficult, and it is often more suitable to focus on a smaller group of closely related taxa, which may be easier to interpret (Endress, 2010). Prenner, Vergara-Silva & Rudall (2009) proposed four main inflorescence types: cymes, panicles, racemes and thyrses. More recently, Endress (2010) suggested two main inflorescence types: racemes and cymes with intermediates between the two. The inflorescence of Caprifoliaceae *s.l.* is a thyrse, which is a raceme of cymes. Members of Linnaeeae also follow this branching pattern, but have fewer flowers within each cyme and shorter internodes between prophylls, resulting in a distinct epicalyx.

The terminology of inflorescence morphology is currently under debate (Prenner *et al.*, 2009; Endress, 2010). In a three-flowered cyme, the primary flower is subtended by a pair of bracts that become supernumerary bracts for secondary flowers. Secondary flowers are preceded by two bracteoles and subtended by one bract, which is also a bracteole of the primary flower. Endress (2010) used the term 'pherophylls' for the phyllome subtending a new branch, which is therefore equivalent to the bracts and supernumerary bracts; for bracteoles, he used the term 'prophylls', described as the two first phyllomes.

CHOICE OF TAXA AND DNA REGIONS TO BE SEQUENCED

The overall aim was to elucidate generic limits in Linnaeeae and to evaluate infrageneric groups within *Abelia* and related genera. Because there are only a few species in each group, we used nearly every available species; *Vesalea coriacea* (Hemsl.) T.Kim & B.Sun ex Landrein, *Diabelia tetrasepala* (Koidz.) Landrein, *A. forestii* (Diels) J.Q.Hu and *Dipelta elegans* Batalin were not available. We used a narrow species concept and included 19 species (Kim, 1998). Three recently published species from Mexico (Villareal, 1997, 2000) were not sampled because of the lack of available material.

The nrITS region (Baldwin, 1992) and plastid *rbcL* (Bell *et al.*, 2001; Donoghue *et al.*, 2001), *ndhF* (Pyck & Smets, 2000; Bell *et al.*, 2001; Zhang *et al.*, 2002a), *trnL* intron and trn*L-F* spacer (Taberlet *et al.*, 1991), *matK* (Bell *et al.*, 2001) and *atpB* (Bell *et al.*, 2001) regions were used in previous studies and showed insufficient taxon sampling of Linnaeeae, with the exception of Kim (1998), who only employed nrITS, and this analysis lacked resolution. Recently, Jacobs *et al.* (2010) used the *atpB-rbcL* spacer and *trnK* intron regions in a study of the group and we were able to analyse these sequences with our dataset. Based on previous analyses, *Heptacodium miconioides* Rehder was included as an outgroup (Pyck & Smets, 2000; Donoghue *et al.*, 2001; Zhang *et al.*, 2002b).

MATERIAL AND METHODS

DNA EXTRACTION AND AMPLIFICATION

Most material of Linnaeeae was cultivated at the Royal Botanic Gardens, Kew, and the Royal Horticultural Society garden at Wisley (UK) and extracted directly from fresh tissue. Some taxa were available only from herbarium specimens or silica gel material dried in the field. Collections made in the field were dried and stored in silica gel (Chase & Hills, 1991). Accessions are vouchered at RBG Kew (K), Makino (MBK) or Harvard (GH) (Table 2).

Total DNA was extracted from 0.3 g of silica gel-dried leaf tissue, 0.1 g of herbarium material or 1 g of fresh tissue using the $2 \times$ cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987) with additional purification on a caesium chloride/ethidium bromide gradient (1.55 g mL^{-1}), followed by dialysis and removal of the ethidium bromide.

Target regions were amplified in a Gene Amp 9700 PCR system (ABI, Applied Biosystems, Warrington, Cheshire, UK) using ReddyMix PCR Mastermix at 2.5 mM MgCl$_2$ concentration (ABgene, Epsom, Surrey, UK) with the addition of 1 μL of bovine serum albumin (BSA, 0.4%), 0.25–0.50 μL of each primer (Table 3) and 0.5–1.0 μL of template in a final reaction volume of 25 μL. Regions were amplified using the following programme: 4 min of initial denaturation at 94 °C, followed by 28 cycles of 1 min of denaturation at 94 °C, 1 min of annealing at 48 °C and 1 min of extension at 72 °C, and a final extension of 7 min at 72 °C.

PCR products were cleaned using miniprep columns (Life Technologies, Paisley, Strathclyde, UK) following the manufacturer's protocols. Templates were sequenced on a 48-capillary 3730 DNA Analyser (ABI) using Big Dye terminator v3.1 chemistry, following the manufacturer's protocols (ABI). For cleaning of cycle sequencing products, precipitation in ethanol was used.

Characters were divided into five matrices to take into account the potential for incongruence between nuclear and plastid regions and also to incorporate recently published data (Jacobs *et al.*, 2010) and an unpublished thesis (Kim, 1998). A single large combined dataset would have been less reliable because of

Table 2. List of taxa used in this study, their Royal Botanic Gardens (RBG) Kew DNA Bank accession numbers and GenBank accession numbers. Accessions in bold are new sequences published in this study, missing sequences are indicated by a dash. Herbarium acronyms follow http://sciweb.nybg.org/science2/IndexHerbariorum.asp

Taxa	Voucher and provenance data	DNA bank accession	rbcL	matK	trnL intron and trnL_F spacer	ndhF	ITS	atpB-rbcL	trnK
Abelia chinensis R. Br.	(K) RBG Kew Living Collection 1978-2338 donated by Coblands nurseries	25461	**HQ680737**	**HQ693935**	**HQ693980**	**HQ693958**	Kim (1998)	GU168616	–
Abelia uniflora R.Br. ex Wall.	(K) RBG Kew Living Collection 1985-4595. Provenance: China, Guizhou, near Guaxi, alt. 400 m, 28.x.1985. Collected by *Simmons et al* #288	24054	**HQ680734**	**HQ693932,**	**HQ693977**	**HQ693955**	–	–	–
Abelia uniflora R.Br. ex Wall.	(K) RBG Kew Living Collection 2002-410, Provenance: China, Sichuan, Bazhong pref., Ji Gong Shan, alt. 1670 m, N32°40′18″, E107°03′21″, 30.ix.1999. Collected by *T. Kirkham, Cole, Flanagan & McNamara* #SICH2029	24028	**HQ680736**	**HQ693934**	**HQ693979**	**HQ693957**	–	GU168604	GU168670
Abelia uniflora R.Br. ex Wall.	(K) RBG Kew Living Collection 1958-80619 donated by A.Vogt	24026	**HQ680735**	**HQ693933**	**HQ693978**	**HQ693956**	Kim (1998)	–	–
Abelia ×grandiflora 'Francis Mason'	(K) RBG Kew Living Collection 1984-1355 donated by Waterers Nurseries	35309	**HQ680738**	**HQ693936**	**HQ693981**	**HQ693959**	–	–	–
Acanthocalyx albus (Hand.-Mazz.) M.J.Cannon	GenBank	GenBank	AF446943	AF446913	AF446973	AY496556	AY236183	AF447003	AY290027
Cryptothladia chinensis (Pai) M.J.Cannon	GenBank	GenBank	AF446944	AF446914	AF366925	AF447034	AY236184	AF447004	AY290026
Diabelia serrata (Siebold et Zucc.) Landrein	(K) RBG Kew Living Collection 1998-1337 Provenance: Japan, Shikoku, Mt Yokohura, x.1993. Collected by *Yuji Kurashiga* #0665	23922	**HQ680744,**	**HQ693942**	**HQ693987**		Kim (1998)	–	–
Diabelia serrata (Siebold et Zucc.) Landrein	(MBK) *Gale, S.* #2007005 Provenance: Japan, Kochi pref., Tengu-Kogen, N33°28′46″, E133°00′43″, alt. 1484.9 m, 10.vi.2007	30478	**HQ680745**	**HQ693943**	**HQ693988**	**HQ693965**	–	–	–
Diabelia serrata (Siebold et Zucc.) Landrein	(MBK) *Gale, S.* #2007009 Provenance: Japan, Ehime pref., Shigiyama, Mikame-cho, N33°24′45″, E133°25′17″, alt. 250 m, 23.vi.2007	30479	**HQ680746**	**HQ693944**	**HQ693989,**	**HQ693966**	–	–	–
Diabelia spathulata (Siebold et Zucc.) Landrein	(K) RBG Kew Living Collection 1996-4676 Provenance: Japan, Honshu, Kanagawa pref., Hakone, alt. 970 m, 01.xi.1996. Collected by *Townsend & Bridger* #80	23918	**HQ680743**	**HQ693941**	**HQ693986**	**HQ693964**	Kim (1998)	GU168605	GU168671
Dipelta floribunda Maxim.	(A; K) *D.E. Boufford, Q.Tian & Z.Y.Zhang* #37425 Provenance: China, Gansu, Wen Xian: Shilong Gou, N32°41′38″, E105°16′35″, alt. 900–1150 m, 06.v.2007	30914	**HQ680740**	**HQ693938**	**HQ693983**	**HQ693961**	–	–	–
Dipelta floribunda Maxim.	(K) Cultivated at Cambridge Botanical Gardens, UK	35317	**HQ680741**	**HQ693939**	**HQ693984**	**HQ693962**	Kim (1998)	GU168606	–
Dipelta yunnanensis Franch.	(A, K) *Boufford* #37836, Provenance: China, Gansu, Wen Xian: W of the Qiujiaba field station, N32°53′48″, E104°19′42″, alt. 2170–2425 m, 21.v.2007	30915	**HQ680742**	**HQ693940**	**HQ693985**	**HQ693963**	Kim (1998)	AF447000	AY290042
Fedia cornucopiae (L.) Gaertn.	GenBank	GenBank	AF446953	AF446923	AF446983	AF447043	AY236193	AF447013	GU16873

Table 2. *Continued*

Taxa	Voucher and provenance data	DNA bank accession	rbcL	matK	trnL intron and trnL_F spacer	ndhF	ITS	atpB-rbcL	trnK
Heptacodium miconioides Rehder	GenBank	GenBank	AF446936	AF446906	AF446966	AF447026	Kim (1998)	AF446996	FJ745412
Kolkwitzia amabilis Graebn.	(K) RBG Kew Living Collection 1996-1609, Provenance: China, Hubei, Near Yan Chi He, alt. 400 m. Collected by the *North American China Plants exploration consortium s.n.* 9.xii.1994	35318	HQ680739	HQ693937	HQ693982	HQ693960	Kim (1998)	AF447002	GU168674
Linnaea borealis L.	(K) Cultivated from White Cottage Nurseries	25460	HQ680732	HQ693930	HQ693975	HQ693953	Kim (1998)	AF447001	AY290040
Morina longifolia Wall. ex DC.	GenBank	GenBank	AF446945	AF446915	AF44975	AF447035	Kim (1998)	AF447005	AY290020
Succisa pratensis Moench	GenBank	GenBank	FJ640593	FJ45401	AY290007	AF130491	AY290018	GU168607	AY290033
Triplostegia glandulifera Wall. ex DC.	GenBank	GenBank	AF446949	AF446949, AF446919	AF446979	AF447039	AF446919	AF447009	AY794323
Valeriana officinalis L.	GenBank	GenBank	L13934	AY362532	AF366917	AY362469	DQ180745	AF448583	AY94362
Vesalea floribunda M. Martens et Galeotti	(K) Cultivated from Royal Horticultural Society at Wisley	25410	HQ680733	HQ693931	HQ693976	HQ693954	Kim (1998)	GU168600	GU168668
Zabelia biflora (Turcz.) Makino	(K) RBG Kew Living Collection 1993-372. Provenance: South Korea, Chungchong Pukto, Tanyang Gun, 10.vi.1989. Collected by the *U.S. National Arboretum: USNA no: 61752*	23923	HQ680728	HQ693926	HQ693971	HQ693949	–	–	–
Zabelia biflora (Turcz.) Makino	(K) RBG Kew Living Collection 1995-1638. Provenance: China, Sichuan, Above Kanding on Paoma Shan, alt. 2580 m, 20.ix.1991. Collected by *Simmons et al. #SICH 525*	23917	HQ680729	HQ693927	HQ693972	HQ693950	Kim (1998)	GU168598	GU168684
Zabelia biflora (Turcz.) Makino	(K) RBG Kew Living Collection 1999-4173. Provenance: Japan, Shikoku, Kochi pref., bank of Makiyama river, alt. 560 m, 13.x.1999. Collected by *Townsend and Bridger #SOKU 112*	23921	HQ680730	HQ693928	HQ693973	HQ693951	–	–	–
Zabelia biflora (Turcz.) Makino	(MBK) *Gale, S. #2007003* Provenance: Japan, Kochi pref, Tengu-Kogen N33°28'46", E133°00'43", alt. 1484.9 m, 10.vi.2007	30480	HQ680731	HQ693929	HQ693974	HQ693952	–	–	–
Zabelia corymbosa (Regel et Schmalh.) Makino	(K) *Landrein, S. #121.* Provenance: Kyrgyzstan, Talas Oblast, Talas Rayon, Chychkan Valley, N42°09'49", E72°54'25", alt. 2062 m, 05.vii.2008	35319	HQ680726	HQ693924	HQ693969	HQ693947	–	–	–
Zabelia corymbosa (Regel et Schmalh.) Makino	(K) *Landrein, S. #152.* Provenance: Kyrgyzstan, Jalal-Abad Oblast, Ala-Buka Rayon, 6 km west of Terek-Say, N41°28'51", E71°05'41", alt. 1581 m, 08.vii.2008	35321	HQ680727	HQ693925	HQ693970	HQ693948	–	–	–
Zabelia triflora (R. Br. ex Wall.) Makino	(K) RBG Kew Living Collection 1999-3330. Provenance: Pakistan, Palas, Bush to Barser and onto Pharlogah, alt. 1970 m, 09.ix.1995. Collected by *Sinnott, Burnell & Jackson #182*	24053	HQ680725	HQ693923	HQ693968	HQ693946	Kim (1998)	GU168617	GU168689
Zabelia tyaihyoni (Nakai) Hisauchi et H. Hara	(K) Cultivated from Royal Horticultural Society at Wisley	25411	HQ680724	HQ693922	HQ693967	HQ693945	Kim (1998)	GU168616	GU168687.

Table 3. Sources of primers used in this study

Primer region name	Primer sequences published	Primers used in this study
trnL intron and *trnL-F* intergenic spacer	Taberlet *et al.* (1991)	c (forward), f (reverse)
ndhF	Olmstead & Palmer (1994)	972F (forward)-2110R (reverse)
matK	Sun, McLewin & Fay (2001)	390F (forward)-1326R (reverse)
rbcL	Olmstead *et al.* (1992)	1F (forward)-724R (reverse), 636F (forward)-1460R (reverse)

Table 4. Statistics of the eight regions

	rbcL	*trnL-F**	*matK*	*ndhF*	*atpB-rbcL*	*trnK*	ITS	Combined
No. taxa	31	31	31	30	18	19	22	19
Total aligned	1382	958	826	1061	658	752	766	6403
Constant	1223	704	613	770	431	467	452	4660
Variable	80	155	100	160	153	187	131	966
Potentially parsimony informative	58	77	91	110	52	76	111	575
No. trees	5667	553	4	1751	251	2763	18	1
No. steps	177	280	245	351	263	329	522	2167
Consistency index	0.83	0.92	0.88	0.87	0.90	0.89	0.64	0.83
Retention index	0.82	0.91	0.88	0.89	0.80	0.73	0.70	0.72
Evolution model	HKY + I	GTR + G	GTR + I	GTR + I	GTR + G	GTR + G	GTR + G	

*For the *trnL* intron and *trnL-F* spacer combined.

the different species sampling. The five matrices were:

1. A large plastid matrix with new data collected for this paper (*rbcL*, *ndhF*, *matK*, *trnL* intron and *trnL-F* spacer).
2. The nrITS matrix from Kim (1998) re-entered by hand from the unpublished thesis.
3. A large plastid and nuclear ITS dataset with new data collected for this paper and nrITS data from Kim (1998).
4. The extended combined plastid dataset: new data collected for this paper plus *atpB-rbcL* spacer and *trnK* region (Jacobs *et al.*, 2010); with some taxa missing.
5. All available data combined (with some taxa missing data).

The sequences were assembled and edited using Sequencher version 4.5 (Gene Codes, Ann Arbor, MI, USA). They were aligned by eye in PAUP* 4.0b10 (Swofford, 2002). Gaps were coded as missing data. Newly obtained sequences were submitted to GenBank and matrices to Tree Base (http://www.treebase.org, S12323) (Table 2).

ANALYSIS OF SEQUENCE DATA

PAUP* 4.0b10 (Swofford, 2002) was used for parsimony analyses. Heuristic searches were performed using tree bisection–reconnection (TBR) branch swapping and 1000 replicates of random taxon addition with 10 trees held at each step to reduce time searching suboptimal 'islands' of trees. All character transformations were treated as equally likely and unordered (Fitch, 1971). To assess internal support, 1000 bootstrap replicates (Felsenstein, 1985) were performed with equal weights using TBR branch swapping with 10 trees held at each step and simple taxon addition. Individual and combined matrix statistics are presented in Table 4; one sequence [*ndhF* for *Diabelia serrata* (Siebold & Zucc.) *Landrein 23922*] was missing because of technical difficulties.

Bayesian analyses were performed using MrBayes v3.1 (Huelsenbeck & Ronquist, 2001) at the freely available Bioportal server (http://www.bioportal.uio.no). Two runs were performed and each marker was placed in unlinked separate partitions. jModelTest v0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) selected a GTR + G model for the nuclear data, GTR + G model for *trnL* intron, *trnK* and *atpB-rbcL*

and a GTR + I model for *matK* and *ndhF*, and HKY + I for *rbcL*. The analyses were performed with 20 million generations of Monte Carlo Markov chains with equal rates and a sampling frequency of 1000. Tracer v1.5 (Rambaut & Drummond, 2011) was used to plot the generation number against ln *L* to find the 'burn in'. Trees of low posterior probability (PP) were deleted, and all remaining trees were used. A majority rule consensus tree was produced showing the frequencies (i.e. PP) of all observed partitions.

RESULTS

Matrix 1 (four plastid regions): for 31 taxa, the number of characters was 4227, 495 (11.7%) of which were variable and 336 (7.9%) of which were potentially parsimony informative. The number of characters contributed by each individual region was 1382 from *rbcL*, 513 from the *trnL* intron and 445 from the *trnL-F* spacer, 826 from *matK* and 1061 from *ndhF*. Analysis produced 12 equally most-parsimonious trees [length = 1076 steps, consistency index (CI) = 0.86 and retention index (RI) = 0.86]. Details for each region are provided in Table 4. In the Bayesian analysis (Fig. 1), two million trees of low likelihood were eliminated, and the tree shown is a consensus of 18 000 individual trees; frequencies of node occurrence are recorded as PPs, which are shown next to each node. Bootstrap percentages (BPs) consistent with the strict consensus tree are shown above each branch.

Zabeliinae (BP 100, PP 1.0) and Linnaeinae (BP 100, PP 1.0) form well-supported clades, but Zabeliinae has an unresolved position in both analyses and forms a poorly supported clade. *Abelia* (BP 100, PP 1.0), *Diabelia* (BP 83, PP 0.99), *Dipelta* Maxim. (BP 100, PP 1.0) and *Zabelia* section *Biflorae* (Zabel) Fukuoka (BP 82, PP 1.0) form well-supported groups. *Dipelta* and *Kolkwitzia* Graebn. are sister to *Diabelia* (Siebold & Zucc.) Landrein (BP 59, PP 0.99), and *Vesalea* M.Martens & Galeotti is sister to the rest of Linnaeinae, except *Linnaea* (BP 75, PP 0.91). *Diabelia*, *Dipelta* and *Kolkwitzia* form a moderately supported clade (BP 59, PP 0.99). Finally, *Zabelia* section *Zabelia* is not monophyletic; *Z. tyaihyoni* (Nakai) Hisauti & Hara is sister to the rest of *Zabelia* (BP 100, PP 1.0).

Matrix 2 (nrITS only) (Kim, 1998): for 21 taxa, the number of characters was 766, 131 (17.1%) of which were variable and 111 (14.5%) of which were potentially parsimony informative. Analysis produced 18 equally most-parsimonious trees (length = 522 steps, CI = 0.64 and RI = 0.70). Details are provided in Table 4. The Bayesian analysis is shown in Figure 2 with PPs given next to each node. BPs consistent with the strict consensus tree are shown above each branch.

Zabeliinae (BP 100, PP 1.0) and Linnaeinae (BP 94, PP 0.99) form well-supported clades. Zabeliinae are sister to a clade containing *Dipsacus* L., *Morina* L. and *Valeriana* L. (BP 76, PP 0.97). Clades in Linnaeinae are not well resolved, but *Abelia* and *Diabelia* form a weakly supported clade (BP 67, PP 0.52). Clades within Zabeliinae are also not well resolved.

Matrix 3 (four plastid regions and nrITS data): for 30 taxa, the number of characters was 4993, 622 (12.4%) of which were variable and 499 (10.0%) of which were potentially parsimony informative. Analysis produced eight equally most-parsimonious trees (length = 1612 steps, CI = 0.81 and RI = 0.81). Details are provided in Table 4. The Bayesian analysis is shown in Figure 3 with PPs given next to each node. BPs consistent with the strict consensus tree are shown above each branch.

Zabeliinae (BP 100, PP 1.0) and Linnaeinae (BP 100, PP 1.0) form well-supported clades, but Zabeliinae have an unresolved position in both analyses. *Abelia* (BP 100, PP 1.0), *Diabelia* (BP 100, PP 1.0), *Dipelta* (BP 100, PP 1.0) and *Zabelia* section *Biflorae* (BP 67, PP 0.99) form well-supported groups. *Diabelia*, *Dipelta* and *Kolkwitzia* form a moderately supported clade (BP 53, PP 0.98). *Vesalea* is sister to the rest of Linnaeinae, except *Linnaea* (BP 91, PP 0.96). Finally, *Zabelia* section *Zabelia* is not monophyletic; *Z. tyaihyoni* is sister to the rest of *Zabelia* (BP 100, PP 1.0).

Matrix 4 (six plastid regions including *atpB-rbcL* spacer and *trnK* intron; Jacobs *et al*., 2010): for 19 taxa, the number of characters was 5637, 835 (14.8%) of which were variable and 464 (8.2%) of which were potentially parsimony informative. The numbers of characters contributed by each individual region were 1382 from *rbcL*, 513 from the *trnL* intron and 445 from the *trnL-F* spacer, 826 from *matK*, 1061 from *ndhF*, 658 from *atpB-rbcL* and 752 from the *trnK* intron. Analysis produced six equally most-parsimonious tree (length = 1665 steps, CI = 0.86 and RI = 0.74). Details for each region are provided in Table 4. The Bayesian analysis is shown in Figure 4 with PPs given next to each node. BPs consistent with the strict consensus tree are shown above each branch.

Zabeliinae (BP 100, PP 1.0) and Linnaeinae (BP 100, PP 1.0) form well-supported clades, but Zabeliinae have an unresolved position in both analyses. The Morina clade is well supported (BP 100, PP 1.0). *Abelia* (BP 91, PP1.0) and *Dipelta* (BP 100, PP 1.0) form well-supported groups. *Diabelia*, *Dipelta* and *Kolkwitzia* form a well-supported clade (BP 82 PP 1.0). *Vesalea* is sister to the rest of Linnaeinae, except *Linnaea* (BP 58, PP 0.91). Finally, *Zabelia* section *Zabelia* is not monophyletic; *Z. tyaihyoni* is sister to the rest of *Zabelia* (BP 100, PP 1.0).
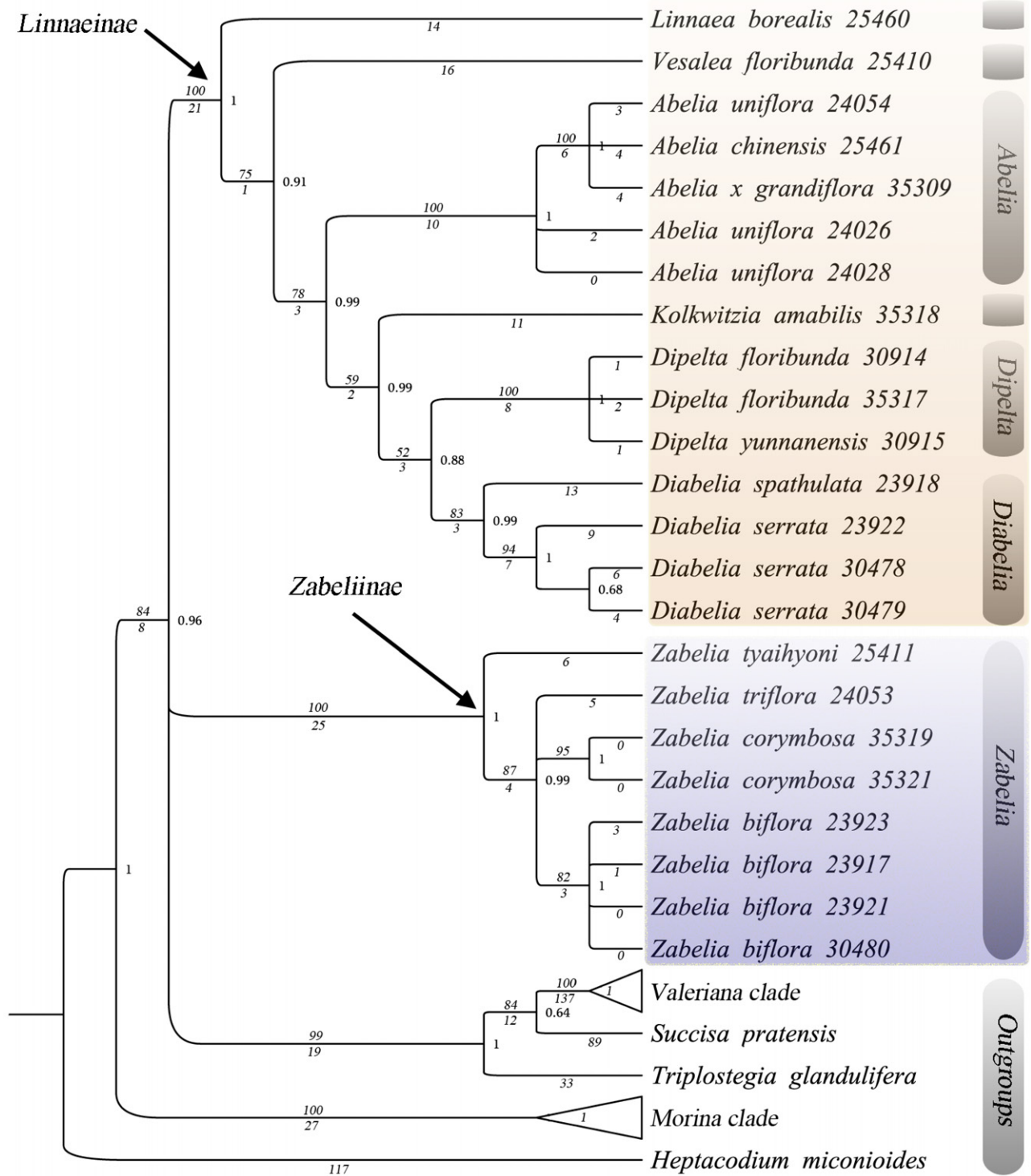
Figure 1. Bayesian consensus of 18 000 trees from a 31-taxon analysis of plastid data (*rbcL*, *trnL* intron, *trnL-F* spacer, *matK*, *ndhF*). Posterior probabilities (PPs) are shown next to the nodes. Parsimony analysis branch lengths (DELTRAN optimization) are shown below the branches. Bootstrap percentages (BPs) > 50 and consistent with the strict consensus tree are shown above the branches.
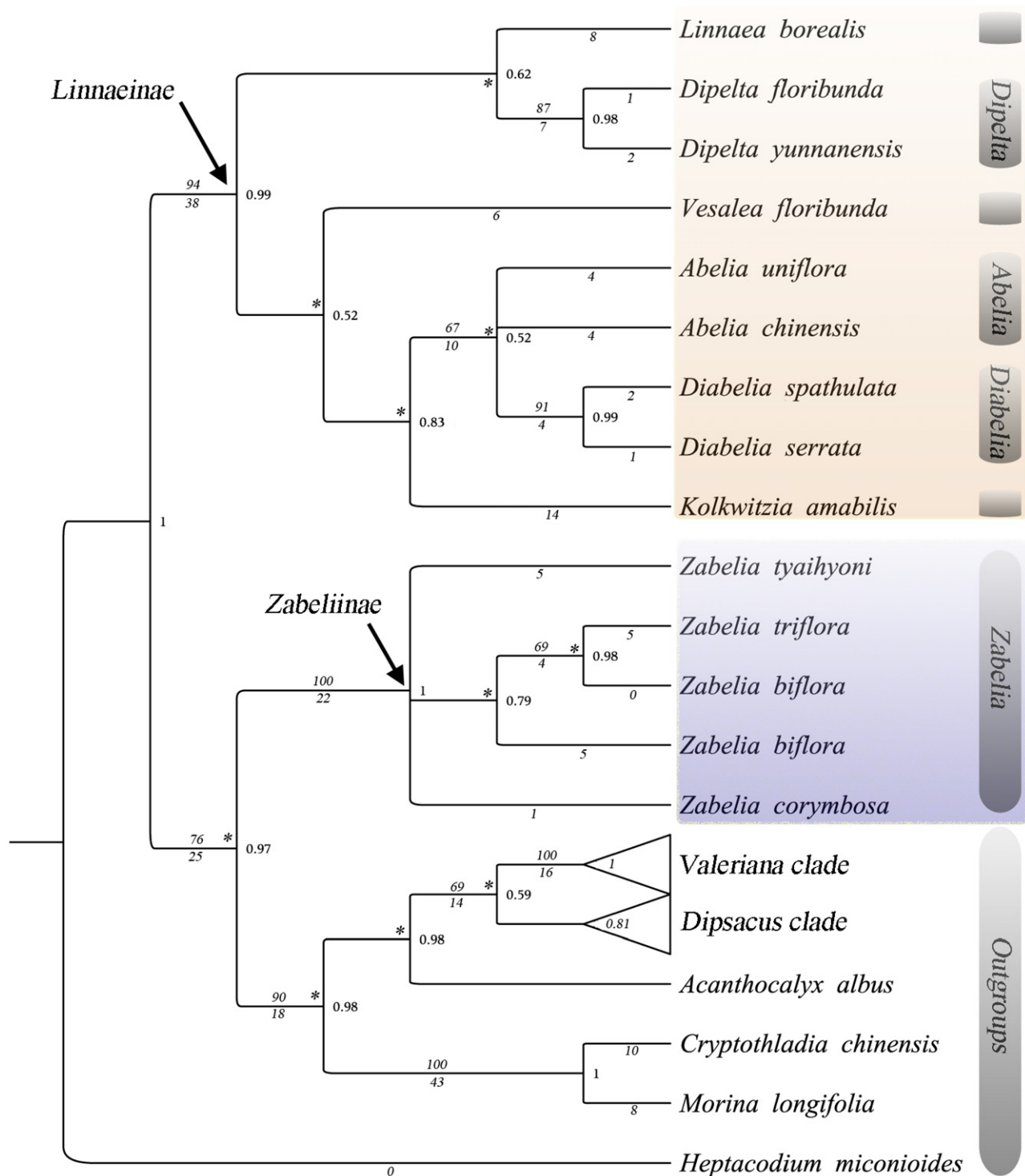
Figure 2. Bayesian consensus of 18 000 trees from a 22-taxon analysis of nuclear data (nrITS). Posterior probabilities (PPs) are shown next to the nodes. Parsimony analysis branch lengths (DELTRAN optimization) are shown below the branches. Bootstrap percentages (BPs) > 50 and consistent with the strict consensus tree are shown above the branches. Stars show the node incongruences when compared with all the other figures.
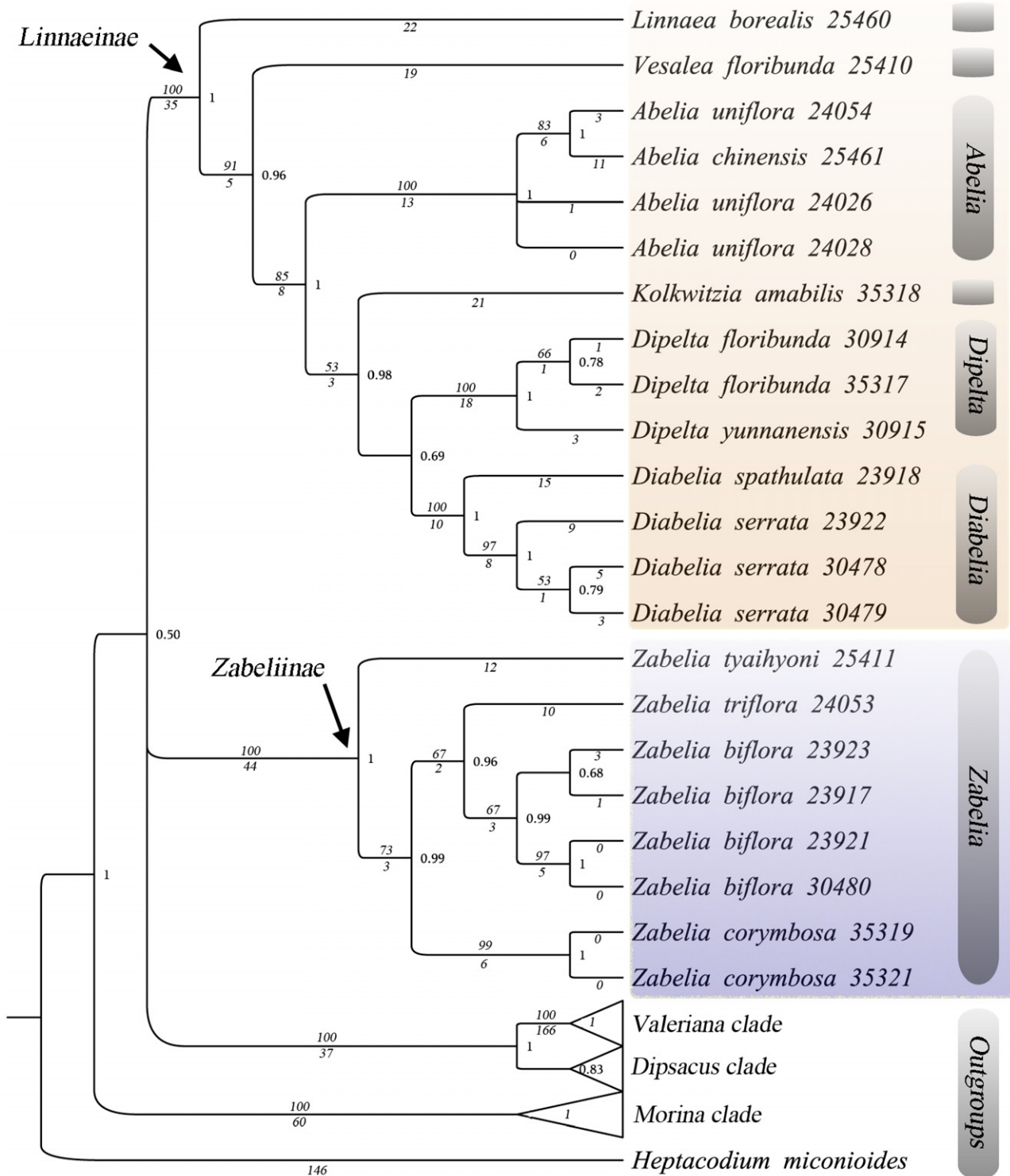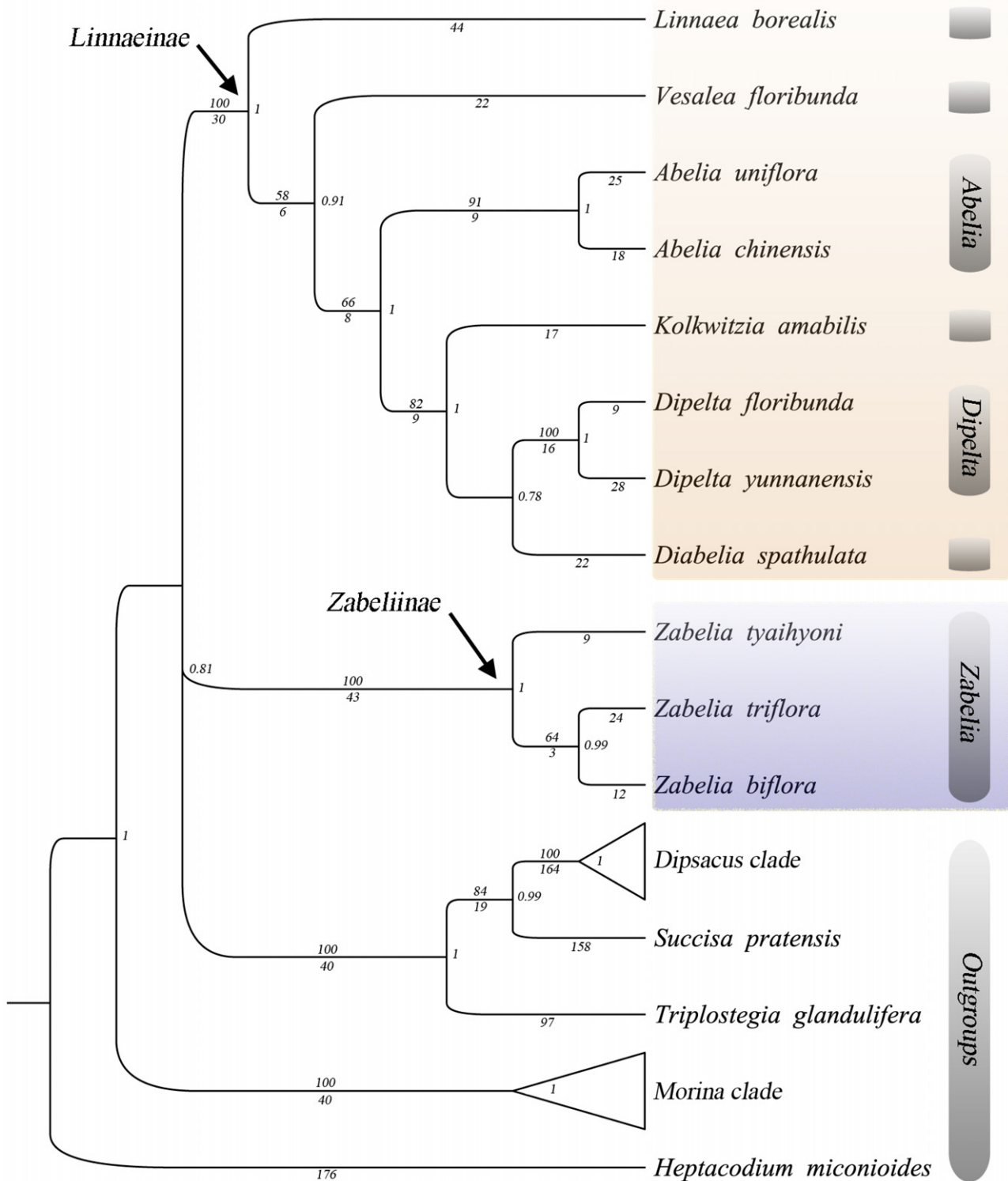
Figure 3. Bayesian consensus of 18 000 trees from a 30-taxon analysis of nuclear and plastid data (*rbcL*, *trnL* intron, *trnL-F* spacer*, matK*, *ndhF* and nrITS). Posterior probabilities (PPs) are shown next to the nodes. Parsimony analysis branch lengths (DELTRAN optimization) are shown below the branches. Bootstrap percentages (BPs) > 50 and consistent with the strict consensus tree are shown above the branches.

Figure 4. Bayesian consensus of 18 000 trees from a 19-taxon analysis of plastid data (*rbcL*, *trnL* intron, *trnL-F* spacer, *matK*, *ndhF*, *atpB-rbcL* and *trnK* intron). Posterior probabilities (PPs) are shown next to the nodes. Parsimony analysis branch lengths (DELTRAN optimization) are shown below the branches. Bootstrap percentages (BPs) > 50 and consistent with the strict consensus tree are shown above the branches.

Matrix 5 (all data, six plastid regions and nrITS data): for 19 taxa, the number of characters was 6403, 966 (15.1%) of which were variable and 575 (9.0%) of which were potentially parsimony informative. This analysis produced one most-parsimonious tree (length = 2167 steps, CI = 0.83 and RI = 0.72). Details for each region are provided in Table 4. The Bayesian analysis is shown in Figure 5 with PPs given next to each node. BPs consistent with the strict consensus tree are shown above each branch.

Zabeliinae (BP 100, PP 1.0) and Linnaeinae (BP 100, PP 1.0) form well-supported clades, but Zabeliinae form a moderately supported clade with the clade of *Valeriana*, *Dipsacus* and *Morina* (BP 79, PP 0.89). *Abelia* (BP 98, PP 1.0) and *Dipelta* (BP 100, PP 1.0) form well-supported groups. *Diabelia*, *Dipelta* and *Kolkwitzia* form a moderately supported clade (BP 79, PP 1.0), and *Vesalea* is sister to the rest of Linnaeinae, except *Linnaea* (BP 84, PP 0.95). Finally, *Zabelia* section *Zabelia* is not monophyletic; *Z. tyaihyoni* is sister to the rest of *Zabelia* (BP 100, PP 1.0).

INFLORESCENCE DATA

To avoid confusion in the terminology for inflorescences, we decided to use the terms 'prophylls-1' for bracteoles of secondary flowers, 'prophylls-2' for bracts of secondary flowers and bracteoles of primary flowers and 'prophylls-3' for bracts of primary flowers and supernumerary bracts of secondary flowers (Fig. 6A). All prophylls are located at the base of ovaries and form a more or less distinct epicalyx.

Zabelia section *Zabelia* inflorescences are terminal compact thyrses with one- to three-flowered cymes. They appear on short shoots developing from winter buds surrounded by several pairs of scales (Fig. 6B). Inflorescences of *Zabelia* section *Biflorae* are terminal and composed of one pair of flowers opening simultaneously. Inflorescences appear on short shoots developing from winter buds surrounded by several pairs of scales (Fig. 6C). *Vesalea* inflorescences are compact racemes; they are grouped at ends of branches. Racemes appear on short shoots developing from winter buds surrounded by several pairs of scales (Fig. 6D). *Linnaea* inflorescences are similar to those of *Vesalea*, but each inflorescence is reduced to a pair of opposed flowers. Paired nodding flowers develop at ends of single short erect shoots along a woody and creeping stem, and the opposed bud does not develop (Fig. 6E). *Kolkwitzia* inflorescences are terminal thyrses formed at the end of short shoots; flowers are paired or single, when paired they open consecutively. Short shoots develop from winter buds surrounded by several pairs of scales (Fig. 6F). *Dipelta* inflorescences are terminal thyrses formed at ends of short shoots.

Short shoots develop from winter buds surrounded by several pairs of scales (Fig. 6G). *Diabelia* inflorescences are terminal and composed of one pair of flowers opening simultaneously. Inflorescences appear on short shoots developing from winter buds surrounded by several pairs of scales (Fig. 6C). *Abelia* inflorescences are axillary cymes or racemes; flowers can be solitary. Inflorescences appear at the ends of long arching shoots. *Abelia chinensis* R.Br. and *A. uniflora* Wall. are closely related, but their inflorescences differ; flowers are single in *A. uniflora* (Fig. 6H) but are paired in *A. chinensis* and open consecutively (Fig. 6I). Detailed results, including ontogenetic data, will be published elsewhere (S. Landrein, unpubl. data).

DISCUSSION

TAXON SAMPLING AND INCONGRUENCES

Recently, some major phylogenetic studies have focused on Dipsacales and have improved our understanding of the classification of the group (Bell *et al*., 2001; Donoghue *et al*., 2001; Zhang *et al*., 2002a). The sampling of the tribe Linnaeeae has nevertheless always been incomplete. Jacobs *et al*. (2010, 2011) provided the first good sampling of the tribe, and we have improved this sampling to include all taxonomic groups. We have also added more markers, allowing for better resolution and support (Wortley *et al*., 2005).

Phylogenetic analyses using four different matrices and six plastid regions were all congruent, despite the differences in sampling and regions sequenced. The nuclear ribosomal dataset nevertheless showed many incongruences when compared with the plastid data. It is well documented that, unlike the plastid genome, ITS is not always maternally inherited (e.g. Chase *et al*., 2003). This difference in the inheritance pattern of the two datasets could explain the differences in topologies, and thus result in gene trees rather than species trees.

LINNAEEAE

The placement of Zabeliinae is not fully resolved in any of the results of our five matrices. In all plastid analysis trees (Figs 1, 4), Zabeliinae are unresolved. When using the plastid and nuclear data, we find moderate support (Fig. 5; BP 79, PP 0.89) for Zabeliinae sister to the clade of *Dipsacus*, *Morina* and *Valeriana*.

The inclusion of nrITS data results in Zabeliinae being sister to a clade containing *Dipsacus*, *Morina* and *Valeriana* using both parsimony and Bayesian methods. This sister relationship receives moderate support (BP 76 and PP 0.97) from nrITS alone
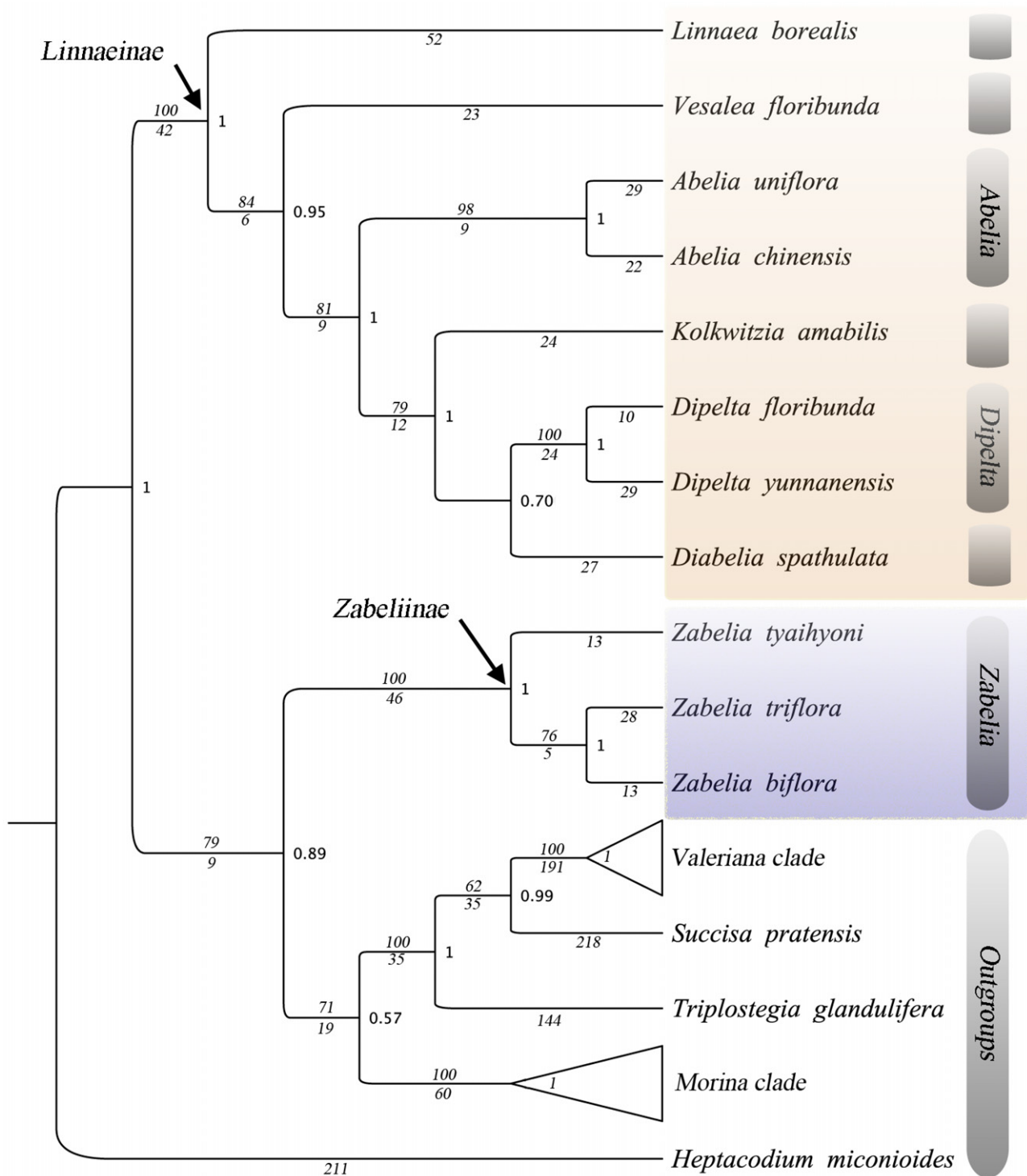
Figure 5. Bayesian consensus of 18 000 trees from a 19-taxon analysis of nuclear and plastid data (*rbcL*, *trnL* intron, *trnL-F* spacer, *matK*, *ndhF*, *atpB-rbcL*, *trnK* intron and nrITS). Posterior probabilities (PPs) are shown next to the nodes. Parsimony analysis branch lengths (DELTRAN optimization) are shown below the branches. Bootstrap percentages (BPs) > 50 and consistent with the strict consensus tree are shown above the branches.
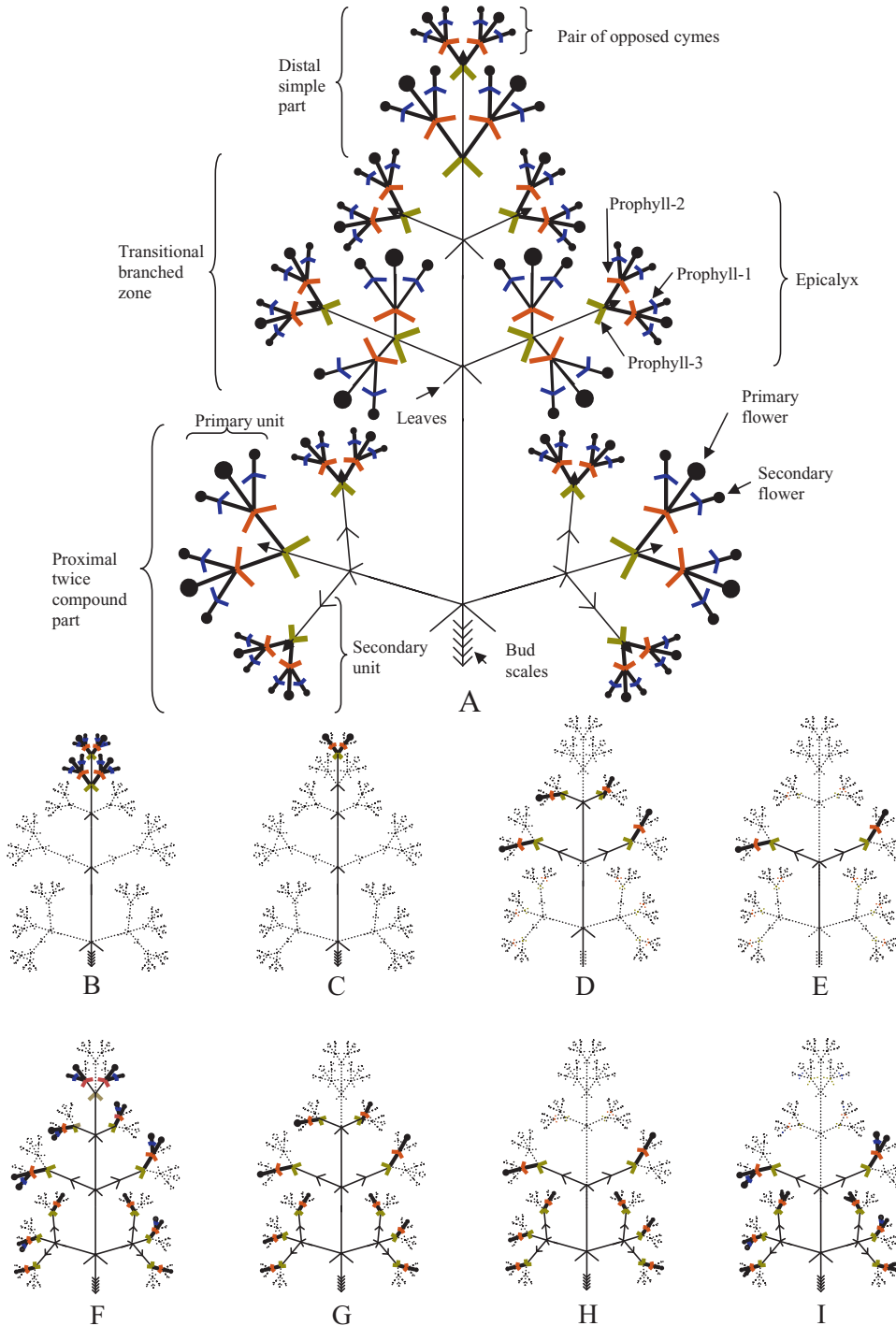
Figure 6. The inflorescence of Linnaeeae: A, terminology; B, *Zabelia* section *Zabelia*; C, *Zabelia* sections *Biflorae* and *Diabelia*; D, *Vesalea*; E, *Linnaea*; F, *Kolkwitzia*; G, *Dipelta*; H, *Abelia uniflora*; I, *Abelia chinensis* (dotted lines represent aborted flowers/branches).

(Fig. 2). It is also seen in the combined trees (Fig. 5) with similar support levels (BP 79 and PP 0.89). Therefore, the current evidence points towards Zabeliinae being sister to *Dipsacus*, *Morina* and *Valeriana* (as in Jacobs *et al.*, 2010).

A general trend in the reduction of sepal number from five to two is confirmed here. This character transition has occurred in several clades: *Zabelia*, *Abelia* and *Diabelia*. There is also a tendency for groups with a specialized epicalyx to have a reduced

or deciduous calyx, e.g. *Linnaea*, *Kolkwitzia* and *Dipelta* (Table 5, Fig. 7).

ZABELIINAE

As revealed in previous studies, Zabeliinae and Linnaeinae are well supported by both molecular and morphological data (Ikuse & Kurosawa, 1954; Fukuoka, 1968; Kim, 1998; Jacobs *et al*., 2010). The monophyly of *Zabelia* section *Zabelia* is not supported by the molecular results (Figs 1–5, 7); although morphologically similar to *Zabelia* section *Zabelia*, *Z. corymbosa* (Regel & Schmalh.) Makino has tetramerous flowers like *Zabelia* section *Biflorae*. Karyology, wood anatomy, pollen and petiole bases are all clear characters for *Zabelia*. Its distribution in the Himalayas, Tian-Shan and mountains of Korea to China and Japan shows an extension from the centre of diversity of Linnaeeae, but its thyrse, with only a simple distal part with three-flowered cymes (often reduced to one flower) and subregular flowers, is probably a plesiomorphic character (Table 5, Fig. 7).

LINNAEINAE

As seen in a previous analysis (Kim, 1998), phylogenetic relationships in Linnaeinae are less resolved than in other groups of Caprifoliaceae (Figs 1–5). Nevertheless, we are able to draw some conclusions. *Linnaea* and *Vesalea* appear to be distinct and successively sister to the rest of Linnaeinae. They both have thyrses reduced to the proximal part and one-flowered basic units [sometimes two-flowered in *Vesalea coriacea* (Hemsl.) T.Kim & B.Sun ex Landrein] with tubular-campanulate flowers in which the nectary does not form a distinct bulge at the base of the corolla tube. *Linnaea* and *Vesalea* have disjunct distributions; *Vesalea* occurs in Mexico, and *Linnaea* is circumboreal, but the latter also occurs in the centre of diversity for Linnaeeae, China. *Linnaea* differs in its habit and structure of the two prophylls-3 covered with long glandular and sticky hairs, which can be explained as a fruit dispersal mechanism (Giger, 1912).

It is clear from the phylogenetic results that *Abelia* is not monophyletic and that *Kolkwitzia*, *Dipelta* and *Diabelia* form a clade (BP 82, PP 1.0 in the combined plastid analysis, Fig. 4). *Abelia* differs from the rest in its unspecialized epicalyx, but mainly in its synflorescence of thyrses that appears on long arching shoots rather than short shoots as in the rest of Linnaeeae. The bilabiate and colourful flowers are strikingly similar to those of *Kolkwitzia*, *Dipelta* and *Diabelia*, but *Abelia chinensis* has an infundibuliform corolla with exserted stamens and style. One synapomorphy for Linnaeinae is a cyme

with single or paired flowers (primary and secondary flowers). *Kolkwitzia*, *Dipelta* and *Diabelia* have a characteristic inflorescence on short shoots. They also show a trend towards a specialization of the epicalyx; *Dipelta* and *Kolkwitzia* both have specialized bracts forming the epicalyx, which enhances fruit dispersal. *Dipelta* has broadly peltate prophyll-2 bracts, and *Kolkwitzia* has prophylls that are covered with stiff hairs that become woody; these hairs also cover achenes in the fruit. Molecular data give weak support to *Dipelta* as sister to *Diabelia* (BP 52, PP 0.88, matrix 1, Fig. 1); the morphology of the achenes and inflorescences indicates that *Kolkwitzia* and *Dipelta* are closely related.

The most puzzling group is *Diabelia*, which has always been considered part of *Abelia*, but the molecular results place it with *Dipelta* and *Kolkwitzia* (BP 82, PP 1.0; Fig. 4). A re-examination of its morphology also supports this; *Abelia* spp. are summer-flowering shrubs with synflorescences of thyrses reduced to the proximal twice-compound portion that appears on long arching shoots. Species of *Diabelia* are spring-flowering shrubs with thyrses on short shoots that are reduced to the simple distal part. It is noticeable that the terminal thyrses of *Diabelia* are reduced to the terminal pair of opposed cymes, each being one-flowered and therefore similar to *Zabelia* section *Biflorae*, which is presumably a reversion. This group and *Zabelia* section *Biflorae* are also the only ones with a reduction in the number of sepals from five to four (Table 5, Fig. 7). It is now clear that *Diabelia* is distinct from *Abelia* and more closely related to *Dipelta* and *Kolkwitzia*. Landrein (2010) proposed the name *Diabelia* for *Abelia* series *Serratae*. A list of currently accepted genera is presented in Table 6.

INFLORESCENCE MORPHOLOGY

A broad interpretation that takes into account the genetic controls of the inflorescence, the repetitive (modular) nature of plant structure (Prenner *et al*., 2009; Endress, 2010) and the phylogenetic results is proposed here: we consider a pair of three-flowered opposed cymes as the basic unit that forms the thyrse. A thyrse can be divided into three regions: (1) a distal simple part; (2) a transitional branched zone; and (3) a proximal part that is twice compound. Within the proximal part, each secondary unit is subtended by two further bracts that are not part of the epicalyx (Fig. 6A).

Each unit is initially composed of pairs of opposed three-flowered cymes (Fig. 6B) that later become reduced in three steps:

1. Units with a single pair of opposed cymes with two primary flowers from each cyme; paired flowers

Table 5. Morphological characters of Linnaeeae (bold text shows putatively primitive character states). Personal observations (SL), except when stated

Subtribe	Groups	Petioles/character state	Life form	Wood (Ogata, 1988)	Distribution (Kim, 1998)	Karyology	Inflorescence
Linnaeinae Fukuoka	**Abelia** R.Br.	**Base not dilated**	Erect shrub	Diffuse-porous, scalariform perforation	China, Japan	$x = 8$ (Kim, 1998)	Synflorescence of thyrses reduced to proximal twice compound part
Linnaeinae Fukuoka	**Diabelia** Landrein	**Base not dilated**	**Erect shrub**	Diffuse-porous, scalariform perforation	Japan, China (one locality)	$x = 8$ (Kim, 1998)	Thyrse reduced to distal simple part with two flowers
Linnaeinae Fukuoka	**Dipelta** Maxim.	**Base not dilated**	Erect shrub	Diffuse-porous, scalariform perforation	**China**	$x = 8$ (Kim, 1998)	Thyrses with or without distal simple part
Linnaeinae Fukuoka	**Kolkwitzia** Graebn.	**Base not dilated**	Erect shrub	Diffuse-porous, scalariform perforation	**China**	$x = 8$ (Li & Shang, 1989)	Thyrses with or without distal simple part
Linnaeinae Fukuoka	**Linnaea** L.	**Base not dilated**	Creeping subshrub	Diffuse-porous, scalariform perforation	Circumboreal	$x = 8$ (Arohonka, 1982)	Thyrses reduced to proximal twice compound part with only two flowers
Linnaeinae Fukuoka	**Vesalea** Martens & Galeotti	**Base not dilated**	**Erect shrub**	Diffuse-porous, scalariform perforation	Mexico	?	Thyrses with or without distal simple part
Zabeliinae T.Kim & B.Sun, ined	**Zabelia** section **Biflorae** (Zabel) Fukuoka	Base dilated	**Erect shrub**	**Ring-porous wood, simple perforations. Branches with six longitudinal grooves**	Russia Far East, Japan, Korea, China	$x = 9$ (Kim, 1998)	Thyrse reduced to distal simple part with two flowers
Zabeliinae T.Kim & B.Sun, ined	**Zabelia** section **Zabelia**	Base dilated	**Erect shrub**	**Ring-porous wood, simple perforations. Branches with six longitudinal grooves**	Himalaya, Korea, Tian-Shan.	**$x = 9$ (Bedi, Bir & Gill, 1980)**	Thyrse reduced to distal simple part

Table 5. *Continued*

| Subtribe	Epicalyx	Corolla	Calyx	Ovary	Nectaria trichomalia	Pollen
Linnaeinae Fukuoka	Four (single flowers) or six (paired flowers); nonaccrescent	Infundibuliform to bilabiate	Two–five-lobed accrescent	**Trilocular, one fertile ovule**	Bulging	Echinulate without endocingulum
Linnaeinae Fukuoka	Six nonaccrescent	Bilabiate	Two–five-lobed accrescent	**Trilocular, one fertile ovule**	Bulging, clavate and intruding inside tube	Echinulate without endocingulum
Linnaeinae Fukuoka	Four (two accrescent and wing-like in fruit)	Bilabiate	Five-lobed nonaccrescent	Tetralocular, two fertile ovules	Bulging	Echinulate without endocingulum
Linnaeinae Fukuoka	Four (single flowers) or six (paired flowers); accrescent becoming woody, spongy	Bilabiate	Five-lobed nonaccrescent	Tri/tetralocular, one/two fertile ovules	Bulging	Echinulate without endocingulum
Linnaeinae Fukuoka	Four, two covered with stalked large glandular hair and accrescent	Infundibuliform	Five-lobed deciduous	**Trilocular, one fertile ovule**	**Four lines of compact glandular hairs**	Echinulate without endocingulum
Linnaeinae Fukuoka	Four nonaccrescent	Long tubular to campanulate	**Five-lobed accrescent**	**Trilocular, one fertile ovule**	**Four lines of compact glandular**	Echinulate without endocingulum
Zabeliinae T.Kim & B.Sun, ined	Six nonaccrescent	**Hypocrateriform**	Four-lobed accrescent	Tri/tetralocular, one/two fertile ovules	**Four lines of compact glandular hairs**	**Smooth with endocingulum**
Zabeliinae T.Kim & B.Sun, ined	**Fourteen, leaf-like and nonaccrescent**	**Hypocrateriform**	Four/five-lobed accrescent	**Trilocular, one fertile ovule**	**Four lines of compact glandular**	**Smooth with endocingulum**
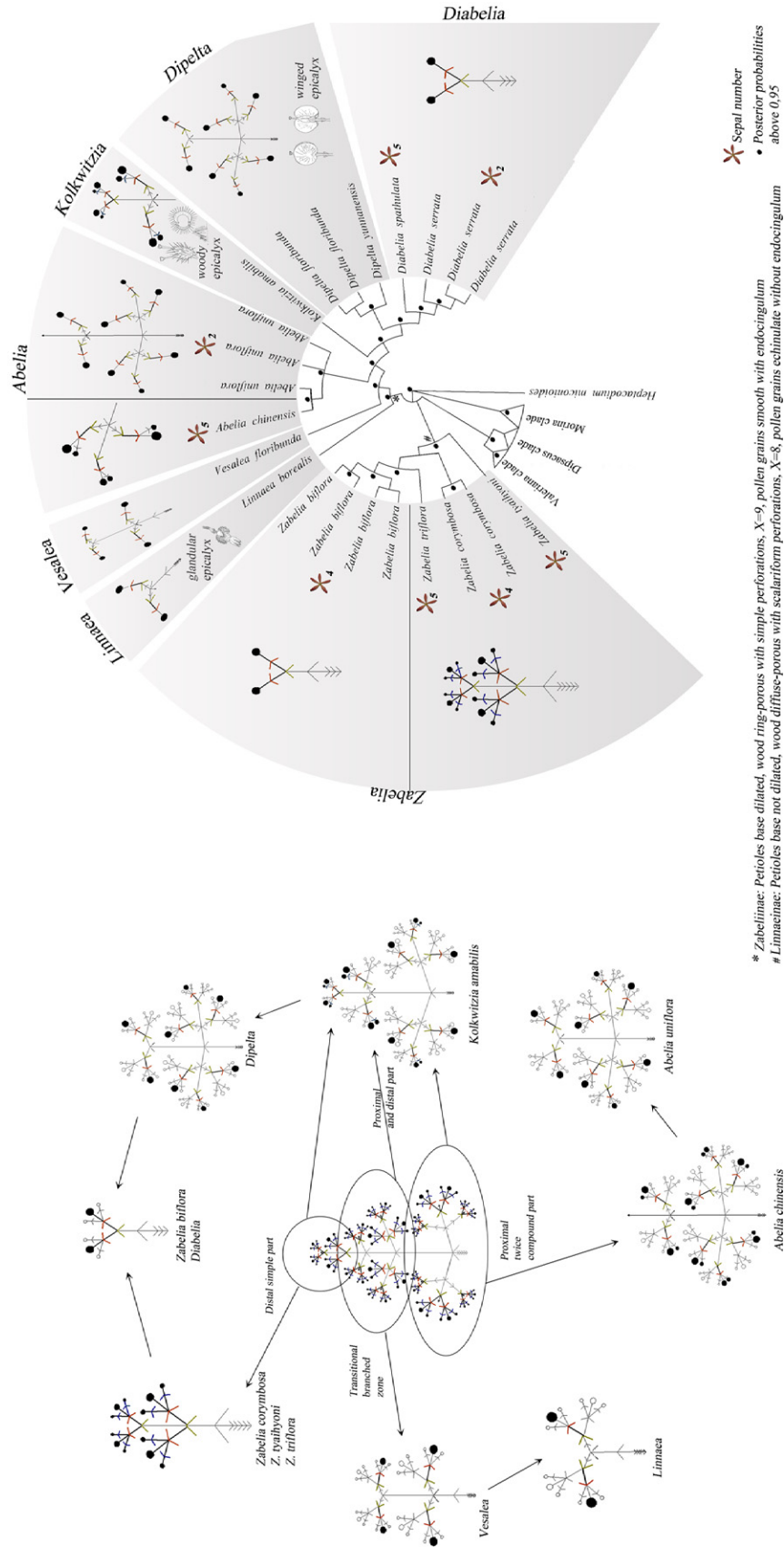
Figure 7. Summary tree of Linnaeeae phylogenetics displaying the theory of inflorescence evolution (left) and a circular phylogenetic tree from a Bayesian consensus of 18 000 trees from a 30-taxon analysis of nuclear and plastid data (*rbcL*, *trnL* intron, *trnL-F* spacer, *matK*, *ndhF* and nrITS) (Figure 3) (right). Also displayed on the tree are the most informative morphological characters, including the epicalyx and the sepal number.

Table 6. Classification used in this article

Clade	Tribe	Subtribe	Genus	Species number	Species names
Linnina	Linnaeeae	Zabeliinae	*Zabelia*	4	*Z. biflora, corymbosa, triflora* and *tyaihyoni*
Linnina	Linnaeeae	Linnaeinae	*Linnaea*	1	*L. borealis*
Linnina	Linnaeeae	Linnaeinae	*Vesalea*	2 (5)	*V. coriacea* and *floribunda* (three newly published species by Villareal, 1997, 2000)
Linnina	Linnaeeae	Linnaeinae	*Abelia*	3	*A. chinensis, forrestii* and *uniflora*
Linnina	Linnaeeae	Linnaeinae	*Kolkwitzia*	1	*K. amabilis*
Linnina	Linnaeeae	Linnaeinae	*Dipelta*	3	*D. elegans, floribunda* and *yunnanensis*
Linnina	Linnaeeae	Linnaeinae	*Diabelia*	3	*D. serrata, spathulata* and *tetrasepala*

open simultaneously and face their adaxial side. The epicalyx is formed of six prophylls from two reduced internodes: a pair of opposed cymes and two cymes (Fig. 6C).

2. Units with a single pair of opposed cymes with one primary and one secondary flower from a single cyme; paired flowers open consecutively and are oriented at an angle of around 90°. The epicalyx is formed by six prophylls from three reduced internodes: a pair of opposed cymes, the remaining cyme and a secondary flower (Fig. 6F, I).

3. Units with a single pair of opposed cymes with only one primary flower from a single cyme. The epicalyx is formed of four prophylls from two reduced internodes: a pair of opposed cymes and the remaining cyme (Fig. 6D, E, G, H).

This interpretation allows us to hypothesize that two parts of the same inflorescence, a distal simple part and a twice-compound proximal part, can coexist in the same plant. This also shows that Linnaeeae is a good case in which the transition between an open inflorescence (thyrse) to a closed inflorescence (thyrsoid) has occurred by reduction of a lateral cyme within a pair of opposed cymes. Thus, the tribe fits well between Caprifoliae with open thyrses (which can be mistaken for a racemose inflorescence when the primary flowers of a pair of opposed cymes do not develop, as in *Leycesteria* Wall. or *Symphoricarpos* Duhamel) and the rest of Linnina with purely cymose inflorescences.

INFLORESCENCE EVOLUTION

An extended discussion of the relationships between inflorescence types in Caprifoliaceae was provided by Troll & Weberling (1966), and this was further discussed by Fukuoka (1969). Roels & Smets (1996) and Donoghue, Bell & Winkworth (2003) also discussed inflorescences in Caprifoliaceae in general, but did not focus on Linnaeeae.

Fukuoka's (1969: 162) interpretation was based on the work of Troll & Weberling (1966): 'Troll and others consider that the polytelic type is derived from the monotelic type by the extinction of [the] terminal flower on each axis of inflorescence. However, two forms of this polytelic type may be considered in our tribe. One is the inflorescence that has never borne any terminal flower from first, as the raceme seen in *Symphoricarpos*. The other is that derived from the cymose type by the reduction of [a] terminal flower, as in the other members of our tribe (transitional type). Thus, the evolutionary trend of the inflorescence may be considered as follows: the racemose type is more primitive; the cymose type is more advanced; the polytelic cymose type, which is the cymose type without terminal flower, is most advanced'.

Unfortunately, as shown by Endress (2010: 226), the two basic types, 'monotelic' and 'polytelic', are problematic. 'A difficulty in Troll's inflorescence concept is his distinction between a "descriptive" and a "typological" (i.e. idealistic) classification. Unfortunately, the "typological" classification with two basic types, monotelic and polytelic inflorescences, appears to be of limited use, as seen from current evolutionary knowledge on inflorescences.'

Nevertheless, some parallels with Troll & Weberling (1966) and Fukuoka (1969) can be drawn. The transition between the 'polytelic' and 'monotelic' types can be explained by abortion of a single cyme in a pair of opposed cymes. Therefore, the 'monotelic' type seems also to be derived from the 'polytelic' type if we follow the terminology of Troll & Weberling (1966). Fukuoka (1969) considered the cymose type as derived from a 'racemose' type. 'Racemose' can be interpreted as a thyrse in which each three-flowered cyme is reduced to just the primary flower. Therefore, in our interpretation, the 'racemose' type is derived from the thyrse (cymose inflorescence) if we follow the terminology of Fukuoka (1969).
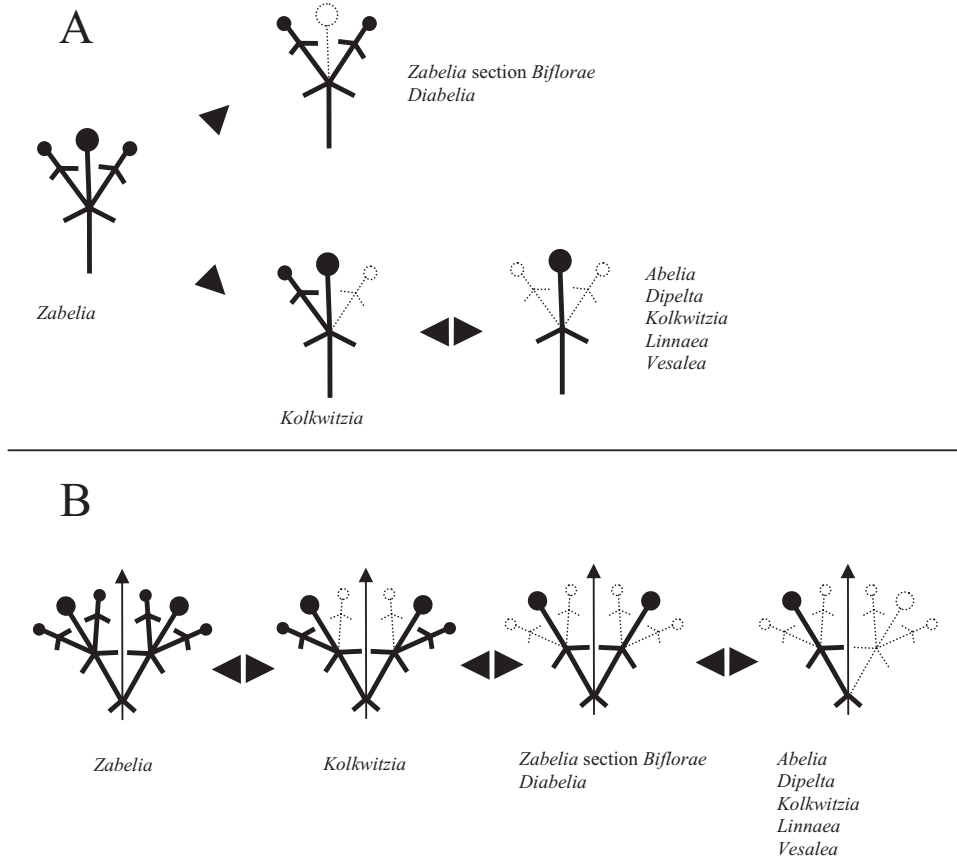
Figure 8. Simplified schematic representation of the evolution of cymes by: A, Troll and Weberling (1966) and Fukuoka (1969); B, according to this article.

One of the points in which our interpretation differs significantly from that of previous authors is for the two-flowered inflorescences found in *Diabelia* and *Zabelia* section *Biflorae*. Troll & Weberling (1966) and Fukuoka (1969) interpreted the paired flowers as two secondary flowers of a single cyme or, as in *Abelia chinensis*, the terminal and secondary flower of one cyme. In our interpretation, the two-flowered inflorescences seen in *Diabelia* and *Zabelia* section *Biflorae* are the primary flowers of a pair of opposed cymes (Fig. 8). When viewing our interpretation against the framework of the molecular results, a clear correlation with the new interpretation is evident. Only one evolutionary pathway is necessary from the less derived or ancestral type of *Zabelia* to the more derived type of *Diabelia*, *Dipelta*, *Kolkwitzia*, *Linnaea* and *Vesalea*. This hypothesis also accommodates the position of *Diabelia* as an intermediate type; this could be the result of a reversion from the most derived type. Troll's and Fukuoka's interpretations involved the existence of two evolutionary pathways, which would not involve a parsimonious interpretation for cases of reversion, as in *Diabelia*.

CONCLUSIONS

Abelia contains numerous shrubs of horticultural interest and, like many recently investigated genera, appears not to be monophyletic. This result encouraged us to re-examine the morphological characters and to search for synapomorphies. Reversions and convergence may be present in Linnaeeae and could explain the difficulty in classifying this small group of genera/species. Thanks to the molecular study, we were able to identify *Abelia* series *Serratae* as a distinct group, now renamed *Diabelia*; thyrses with a distal simple part, large accrescent calyx and small epicalyx are thus likely to be independent reversions and not indicative of a shared history. The position of Zabeliinae in Linnaeeae and in relation to the *Dipsacus*, *Morina* and *Valeriana* clades is more problematic, and our study did not manage to completely resolve these relationships. We feel that Linnaeeae inflorescences provide great insight into the evolution of Caprifoliaceae *s.l.*, and thyrses with a twice compound portion seem to have evolved within Linnaeeae; they are absent in Caprifolieae (except in

Heptacodium), but present in Valerina. More work is still needed to resolve the relationships within species and the position of Zabeliinae, and this could eventually lead to a better understanding of the phylogeny and evolution of this group.

ACKNOWLEDGEMENTS

REFERENCES

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

Arohonka PA. 1982. Chromosome counts of vascular plants of the island Scili in Anuva, SW Finland. *Turu Yliopistou Biologian-Laitoksen Jalkaisuja* **3:** 1–12.

Backlund A, Pyck N. 1998. Diervillaceae and Linnaeaceae, two new families of caprifolioids. *Taxon* **47:** 657–661.

Baldwin BG. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* **1:** 3–16.

Bedi YS, Bir SS, Gill BS. 1980. Chromosome number reports LXVII. *Taxon* **29:** 353–355.

Bell CD, Edwards EJ, Kim A-T, Donoghue M. 2001. Dipsacales phylogeny based on chloroplast DNA sequences. *Harvard Papers in Botany* **6:** 481–499.

Brown R. 1818. Abelia. In: Clarke A, ed. *Narrative of a journey in the interior of China and of a voyage to and from that country in the years 1816 and 1817.* London: Printed for Longman, Hurst, Rees, Orme, and Brown, 376–378.

Chase MW, Hills HG. 1991. Silica gel: an ideal desiccant for preserving field-collected leaves for use in molecular studies. *Taxon* **41:** 215–220.

Chase MW, Knapp S, Cox AV, Clarkson JJ, Butsko Y, Joseph J, Savolainen V, Parokonny AS. 2003. Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). *Annals of Botany* **92:** 107–127.

Donoghue MJ, Bell CD, Winkworth RC. 2003. The evolution of reproductive characters in Dispsacales. *International Journal of Plant Sciences* **164:** 453–464.

Donoghue MJ, Eriksson T, Reeves PA, Olmstead RG. 2001. Phylogeny and phylogenetic taxonomy of Dipsacales, with special reference to *Sinadoxa* and *Tetradoxa* (Adoxaceae). *Harvard Papers in Botany* **6:** 459–479.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin, Botanical Society of America* **19:** 11–15.

Dumortier B-C. 1829. *Caprifoliaceae. Analyse des familles des plantes.* Tournay: Imprimerie de J. Casterman, Aine: I. 33.

Endress PK. 2010. Disentangling confusions in inflorescence morphology: patterns and diversity of reproductive shoot ramification in angiosperms. *Journal of Systematics and Evolution* **48:** 225–239.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20:** 406–416.

Fukuoka N. 1968. Phylogeny of the tribe Linnaeeae. *Acta Phytotaxonomica et Geobotanica* **23:** 82–94.

Fukuoka N. 1969. Inflorescence of Linnaeeae (Caprifoliaceae). *Acta Phytotaxonomica et Geobotanica* **23:** 153–162.

Giger E. 1912. *Linnaea borealis* L., eine monographische Studie. *Beihefte zum Botanischen Centralblatt* **30:** 1–78.

Graebner P. 1900. Die Gattung *Linnaea* (einschliefslich *Abelia*). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **29:** 120–145.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Ikuse M, Kurosawa S. 1954. Notes on sect. *Zabelia* Rehder of the genus *Abelia*. *The Journal of Japanese Botany* **29:** 107–110.

Jacobs B, Geuten K, Pyck N, Huysmans S, Jansen S, Smets E. 2011. Unraveling the phylogeny of *Heptacodium* and *Zabelia* (Caprifoliaceae): an interdisciplinary approach. *Systematic Botany* **36:** 231–252.

Jacobs B, Pyck N, Smets E. 2010. Phylogeny of the Linnaea clade: are *Abelia* and *Zabelia* closely related? *Molecular Phylogenetics and Evolution* **57:** 741–752.

Kim T. 1998. Phylogenetic studies of tribe Linnaeeae (Caprifoliaceae). DPhil Thesis, Chonbuk National University.

Landrein S. 2010. *Diabelia*, a new genus of tribe Linnaeeae subtribe Linnaeinae (Caprifoliaceae). *Phytotaxa* **3:** 34–38.

Li RJ, Shang ZY. 1989. The chromosome observation on 5 species of rare plants of China. *Journal of Wuhan Botanical Research* **7:** 217–220.

Linnaeus. 1753. *Species plantarum.* Stockholm: Salvius.

Martens M, Galeotti H. 1843. Enumeratione synoptica plantarum phanerogamicarum ab Henrico Galeotti in regionibus Mexicanus collectarum. Loniceræ. *Bulletin de l'Academie Royale de Bruxelles* **10:** 241–242.

Maximowicz CJ. 1878. Diagnoses plantarum novarum asiaticarum. *Bulletin de l'Academie Imperiale des Sciences de St-Petersbourg* **24:** 50–51.

Ogata K. 1988. Wood anatomy of the Caprifoliaceae of Japan. *IAWA Bulletin* **9:** 299–316.

Olmstead RG, Michaels HJ, Scott KM, Palmer JD. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcL*. *Annals of the Missouri Botanical Garden* **79:** 249–265.

Olmstead RG, Palmer JD. 1994. Chloroplast DNA system-

atics: a review of methods and data analysis. *American Journal of Botany* **81:** 1205–1224.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Prenner G, Vergara-Silva F, Rudall PJ. 2009. The key role of morphology in modelling inflorescence architecture. *Trends in Plant Science* **14:** 302–309.

Pyck N, Smets E. 2000. A search for the phylogenetic position of the seven-son flower (*Heptacodium*, Dipsacales): combining molecular and morphological evidence. *Plant Systematics and Evolution* **225:** 185–199.

Rambaut A, Drummond AJ. 2011. Tracer v1.5. Available at: http://tree.bio.ed.ac.uk/software/tracer/. Accessed 02/2012.

Rehder A. 1911. Wilson expedition to China. In: Sargent CS, ed. *Plantae wilsonianae*. Cambridge: The University Press, 118–128.

Roels S, Smets E. 1996. A floral ontogenetic study in the Dipsacales. *International Journal of Plant Sciences* **157:** 203–218.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold.

Sun H, McLewin W, Fay M. 2001. Molecular phylogeny of *Helleborus* (Ranunculaceae), with an emphasis on the East Asia–Mediterranean disjunction. *Taxon* **50:** 1001–1018.

Swofford DL. 2002. *PAUP* phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sunderland, MA: Sinauer Associates.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17:** 1105–1109.

Troll W, Weberling F. 1966. Die Infloreszenzen der Caprifoliaceen und ihre systematische Bedeutung. *Abhandlungen der Mathematisch-Naturwissenschaftlichen Klasse. Akademie der Wissenschaften und der Literatur in Mainz* **4:** 455–605.

Vatke W. 1872. Üeber die Gattung *Abelia* R.Br. *Öesterreichische Botanische Zeitschrift* **32:** 290–291.

Villareal JA. 1997. A new species of *Abelia* (Caprifoliaceae) from western Mexico. *Brittonia* **52:** 172–176.

Villareal JA. 2000. Two new species of *Abelia* (Caprifoliaceae) from Mexico. *Brittonia* **52:** 172–176.

Wortley AH, Rudall PJ, Harris DJ, Scotland RW. 2005. How much data are needed to resolve a difficult phylogeny? Case study in Lamiales. *Systematic Biology* **54:** 697–709.

Zabel H. 1893. Über die Gattung *Abelia*. *Mitteilungen der Deutschen Dendrologischen Gesellschaft* **2:** 29–31.

Zhang WH, Chen ZD, Li JH, Chen HB, Tang YC. 2002a. Phylogeny of the Dipsacales *s.l.* based on chloroplast *trnL-F* and *ndhF* sequences. *Molecular Phylogenetics and Evolution* **26:** 176–189.

Zhang ZY, Zhou ZK, Gu ZJ. 2002b. Karyomorphology of *Heptacodium* (Caprifoliaceae *s.str.*) and its phylogenetic implications. *Taxon* **51:** 499–505.