

DIPSACALES PHYLOGENY BASED ON CHLOROPLAST DNA SEQUENCES

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Abstract. Eight new *rbcL* DNA sequences and 15 new sequences from the 5' end of the chloroplast *ndhF* gene were obtained from representative Dipsacales and outgroup taxa. These were analyzed in combination with previously published sequences for both regions. In addition, sequence data from the entire *ndhF* gene, the *trnL-F* intergenic spacer region, the *trnL* intron, the *matK* region, and the *rbcL-atpB* intergenic spacer region were collected for 30 taxa within Dipsacales. Phylogenetic relationships were inferred using maximum parsimony and maximum likelihood methods. Inferred tree topologies are in strong agreement with previous results from separate and combined analyses of *rbcL* and morphology, and confidence in most major clades is now very high. Concerning controversial issues, we conclude that Dipsacales in the traditional sense is a monophyletic group and that *Triplostegia* is more closely related to Dipsacaceae than it is to Valerianaceae. *Heptacodium* is only weakly supported as the sister group of the Caprifoliaceae (within which relationships remain largely unresolved), and the exact position of Diervilleae is uncertain. Within Morinaceae, *Acanthocalyx* is the sister group of *Morina* plus *Cryptothladia*. Dipsacales now provides excellent opportunities for comparative studies, but it will be important to check the congruence of chloroplast results with those based on data from other genomes.

Keywords: Dipsacales, Adoxaceae, Caprifoliaceae, Morinaceae, Dipsacaceae, Valerianaceae, phylogeny, chloroplast DNA.

The Dipsacales has traditionally included the Caprifoliaceae (*sensu lato*, i.e., including *Viburnum* and *Sambucus*), Adoxaceae, Dipsacaceae, and Valerianaceae (e.g., Cronquist, 1988), and sometimes segregates such as Morinaceae and Triplostegiaceae. In contrast, the classification of the Angiosperm Phylogeny Group (APG, 1998) included the bulk of the Caprifoliaceae in Dipsacales, along with Dipsacaceae, Valerianaceae, and Morinaceae, but excluded an expanded Adoxaceae (including *Viburnum*, *Sambucus*, and *Adoxa*), which they left unassigned to any order within Asteridae. The molecular phylogenetic studies reported here concern Dipsacales in the traditional sense.

The circumscription of the Caprifoliaceae has also become confusing, and to avoid misunderstandings we need to clarify our usage of the name. Caprifoliaceae in the traditional sense includes *Sambucus*, *Viburnum*, Caprifoliaceae (*Leycesteria*, *Lonicera*, *Symphoricarpos*, and *Triosteum*, and possibly *Heptacodium*), Diervilleae (*Diervilla* and *Weigela*), and Linnaeae (*Abelia*, *Dipelta*, *Kolkwitzia*, and

Linnaea). It excludes *Adoxa* and its relatives, as well as Dipsacaceae, Morinaceae, and Valerianaceae. We reject this traditional circumscription of Caprifoliaceae on the grounds of non-monophyly. According to all recent phylogenetic studies (e.g., Donoghue, 1983, 1985; Donoghue et al., 1992; Judd et al., 1994; Backlund and Donoghue, 1996; Backlund and Bremer, 1997; Kim et al., 1999; Olmstead et al., 2000; Donoghue et al., 2001), *Sambucus* and *Viburnum* are more closely related to *Adoxa* and its relatives than they are to Caprifoliaceae, Diervilleae, and Linnaeae, which are instead more closely related to Morinaceae, Dipsacaceae, and Valerianaceae.

A more restricted usage of Caprifoliaceae excludes *Sambucus* and *Viburnum*, on the grounds that these are probably more closely related to *Adoxa* and its relatives, but retains Caprifoliaceae, Diervilleae, and Linnaeae. Again, we reject the use of Caprifoliaceae in this restricted sense because it is not monophyletic. Specifically, all recent analyses (references above) indicate that the Linnaeae is

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more closely related to Morinaceae, Dipsacaceae, and Valerianaceae, than to the Caprifoliaceae or Diervilleae.

In view of the phylogenetic results, and wishing to retain monophyly, Backlund and Bremer (1998) suggested another strategy, which Backlund and Pyck (1998) followed with the naming of two new families. They restricted the name Caprifoliaceae to the former Caprifoliaceae, and then elevated Diervilleae to Diervillaceae and Linnaeae to Linnaeaceae. This usage was adopted in the Angiosperm Phylogeny Group ordinal classification (APG, 1998). We reject this strategy on the grounds that it causes instability and confusion to substitute the names Caprifoliaceae, Diervillaceae, and Linnaeaceae for the already well established names Caprifoliaceae, Diervilleae, and Linnaeae solely for the sake of adjusting taxonomic ranks. These names refer to the same entities, and rank assignments are arbitrary (APG, 1998).

Our usage of both the names Adoxaceae and Caprifoliaceae follows the initial suggestion of Judd et al. (1994) and the node-based phylogenetic definitions of Donoghue et al. (2001). The mapping of these names onto a phylogenetic hypothesis is shown in Fig. 1, adapted from Donoghue et al. (2001). Adoxaceae here refers to the clade that includes *Viburnum*, *Sambucus*, and *Adoxa* and its relatives. Caprifoliaceae includes Caprifoliaceae, Diervilleae, Linnaeae, Morinaceae, Dipsacaceae, and Valerianaceae. The unusual and most obviously beneficial feature of this nomenclature is that all of the traditional names are retained (so long as these refer to clades). The potentially confusing aspect of this treatment is that, in addition to including Caprifoliaceae, Diervilleae, and Linnaeae, the Caprifoliaceae also includes Morinaceae, Dipsacaceae, and Valerianaceae. We believe that the benefits of this solution far exceed any potential confusion. Donoghue et al. (2001) provide additional discussion of this approach.

Previous analyses of Dipsacales phylogeny have been based on morphological characters, molecular data, and a combination of the two (e.g., Donoghue, 1983; Donoghue et al., 1992; Judd et al., 1994; Backlund and Donoghue, 1996; Backlund and Bremer, 1997; Pyck et al., 1999; Pyck and Smets, 2000; Donoghue et al., 2001). As mentioned above, these studies have clearly established that traditional Caprifoliaceae do not form a monophyletic group. *Viburnum* is closely related to a *Sambucus*-

Adoxa clade, and the remainder of the traditional Caprifoliaceae are paraphyletic. Specifically, the Linnaeae appears to be more closely related to Morinaceae, Valerianaceae, and Dipsacaceae than it is to either Caprifoliaceae or Diervilleae. However, support for these major clades varies considerably among analyses, and the exact placement of several key taxa (e.g., *Heptacodium*, *Triplostegia*) remains unresolved. Moreover, there is disagreement concerning the placement of several other taxa, especially *Linnaea* and the Diervilleae, as well as relationships within the Caprifoliaceae. Finally, several lineages have received insufficient attention. For instance, we still know little about relationships among *Morina*, *Acanthocalyx*, and *Cryptothladia* of the Morinaceae.

Here we present broad phylogenetic analyses including 8 new *rbcL* sequences and 15 new DNA sequences from the 5' end of the *ndhF* gene. For more detailed studies within Dipsacales we have added sequences of several other chloroplast coding and non-coding regions: *matK*, the *trnL* intron, and the intergenic spacer (IGS) regions of *trnL-F* and *rbcL-atpB*. Our hope was that separate and combined analyses of this much-expanded chloroplast dataset would further clarify Dipsacales phylogeny.

MATERIALS AND METHODS

Samples

We carried out analyses on two different datasets. Our 46-taxon dataset included *rbcL* and *ndhF* sequences from 32 representatives of major lineages within Dipsacales and 14 asterid outgroup taxa. Our 30-taxon dataset consisted of Dipsacales taxa alone scored for all six chloroplast gene regions. Information on the source of plant materials and on GenBank accession numbers is provided in Tables 1 and 2.

DNA Extraction and PCR Protocols

Total DNAs were extracted using the CTAB method of Doyle and Doyle (1987). The DNA extracts were further purified with the Prep-A-Gene DNA Purification Kit (Bio-Rad). Double-stranded copies of all regions were amplified using standard Polymerase Chain Reaction (PCR) in 25- to 50- μ L reactions. All reactions were heated at 94 C for 3 min. The reactions entailed 35 cycles consisting of 94 C for 1.5 min, 48–56 C for 2 min, and 72 C for 3 min. Dye terminator cycle sequencing followed the protocol specified by the ABI PRISM Dye

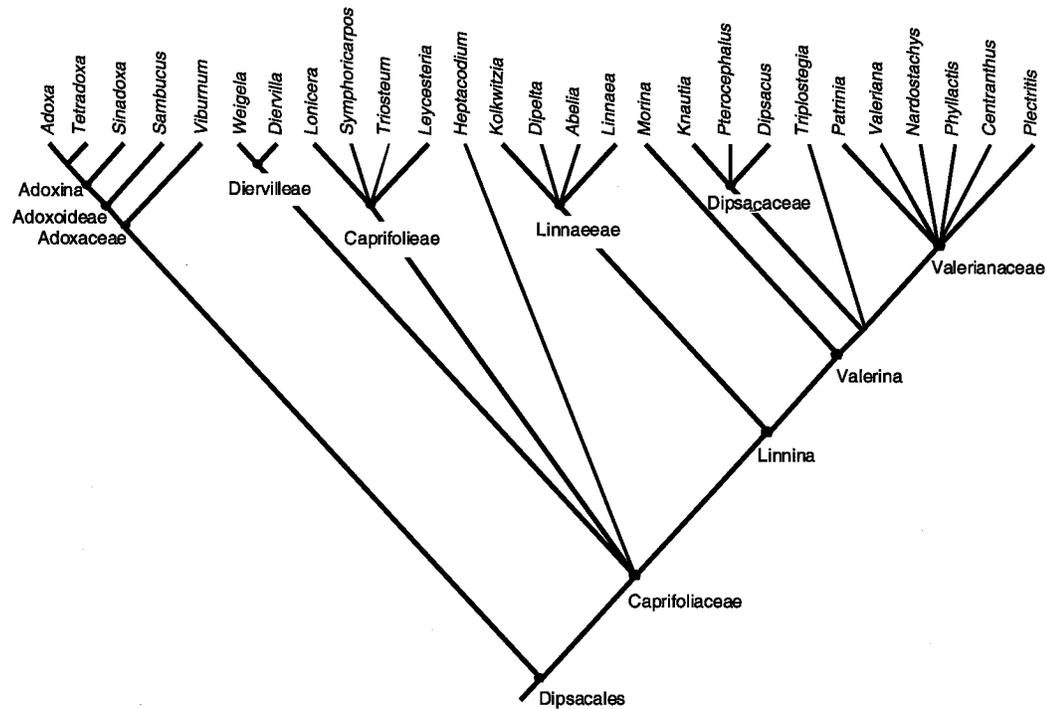


FIGURE 1. Application of names according to the phylogenetic taxonomy of Donoghue et al. (2001).

Primer Cycle Sequencing Ready Reaction Kit (Revision B, August 1995, Perkin-Elmer) and was visualized using an ABI 377 automated DNA sequencer.

rbcL. *rbcL* was amplified and sequenced using standard primers (see, e.g., Olmstead et al., 1992, 1993).

ndhF. A portion of the 5' end of the chloroplast gene *ndhF* was amplified with primers *ndhF*-1 and *ndhF*-1318R (Olmstead and Sweere, 1994). Specifically, we sequenced the first 1320 base pairs of the coding region, which corresponds to the region studied by Pyck et al. (1999). Amplification primers, along with *ndhF*-274, *ndhF*-274R, *ndhF*-803, and *ndhF*-803R (Olmstead and Sweere, 1994) were then used to sequence each corresponding region. The entire *ndhF* molecule was also sequenced for these and additional taxa (see Tables 1 and 2).

matK. The *matK* region, as well as a portion of the flanking region at the 3' end, was amplified and sequenced with the primers used by Young et al. (1999). To obtain the entire coding region, a variety of primer pairs were employed to amplify small fragments of the molecule, which were sequenced using appropriate inter-

nal primers (see Young et al., 1999). The complete coding region was sequenced from 29 taxa. From *Valerianellalocosta* we were able to sequence only the first 507 base pairs. This partial sequence was included in our phylogenetic analyses, with undetermined nucleotide positions scored as missing data ("?") in PAUP* (Swofford, 2001).

trnL-F IGS and *trnL* intron. The *trnL*-F IGS region and *trnL* intron were amplified and sequenced using the universal primers *trnL*-c, *trnL*-d, *trnL*-e, and *trnL*-f of Taberlet et al. (1991).

rbcL-atpB IGS. The *rbcL-atpB* IGS region was amplified with the primers *rbcL-atpBR* and *atpB-F* of Manen et al. (1994). Sequencing was accomplished using the amplification primers along with two additional primers we designed specifically for Dipsacales (*atpB*-sqK1: 5'-CATATMNTATGGCGCAAACC-3'; *atpB*-sqK1R: 5'-GGTTGCGCCATAKATATG-3').

Sequence Alignment

Contiguous sequences were assembled using Sequencher 2.1 (Gene Codes Corp., Madison, Wis.). MacClade (Maddison and Maddison, 1992) was used to translate DNA sequences into protein sequences to aid in the alignment

of *rbcL*, *ndhF*, and *matK* sequences. All sequences were readily aligned by eye, with few ambiguities. The resulting data matrices are available in TreeBASE (www.herbaria.harvard.edu/treebase) or upon request from the first author.

Datasets

The sequence data were partitioned into various datasets differing in the number of taxa and characters. One set of analyses focused on a broader dataset, including representative Dipsacales and potentially related groups of Asteridae. This consisted of 46 taxa with data from *rbcL* and the first 1320 base pairs of the *ndhF* gene. A second set of separate and combined analyses focused on an expanded sample of Dipsacales and consisted of 30 taxa scored for *rbcL*, the entire *ndhF* gene, *trnL*-IGS, *trnL* intron, *matK*, and *atpB*-IGS. On the basis of the broader analyses, and on previous molecular and morphological studies, the resulting Dipsacales trees were rooted along the branch connecting Adoxaceae to Caprifoliaceae (see below).

Phylogenetic Analyses

All analyses were conducted using PAUP* (Swofford, 2001). Maximum parsimony searches were conducted using heuristic search methods with tree bisection reconnection (TBR) branch swapping, collapse of zero-length branches, and equal weighting of all characters. The analyses were repeated 100 times with the "random addition" option to minimize problems of multiple islands of most parsimonious trees. Sets of equally most parsimonious trees were summarized by a strict consensus tree. To assess confidence in clades, bootstrap tests (Felsenstein, 1985) were performed using 300 replicates with heuristic search settings identical to those of the original search.

A series of likelihood ratio tests was performed (on a variety of tree topologies) to determine which model of sequence evolution best fit the data using the program PORN* (Bell, 2001). A variety of "best fitting" models were found, depending on the taxa and data partition being examined (see Table 3). Maximum likelihood searches were carried out in PAUP* using the appropriate model. Parameters for each search were simultaneously estimated via maximum likelihood for all

datasets. Heuristic search methods were used with TBR branch swapping and collapse of zero-length branches. Analyses were repeated 100 times with the "random addition" option. Bootstrap tests were performed using 1000 replicates with nearest neighbor interchange (NNI) branch swapping. Parameters for bootstrap tests were fixed to values estimated from the maximum likelihood tree.

RESULTS

46-Taxon *rbcL* dataset

This data matrix consisted of 1428 aligned base pairs, of which 434 were variable and 250 parsimony informative. Maximum parsimony analyses resulted in 102 trees of 1040 steps (CI= 0.538, 0.425 excluding invariant characters; RI= 0.619). The strict consensus of these trees is presented in Fig. 2, which marks those branches with bootstrap support of 50% or higher. Our maximum likelihood search resulted in a single tree with a $-\ln L$ value of 7846.4885 (1041 steps under parsimony).

The traditional Dipsacales form a clade in all trees; however, bootstrap support for this conclusion is rather low (60%). Even less certain are conclusions about possible close relatives of Dipsacales. Within Dipsacales there is strong support for a basal split between Adoxaceae and everything else. Adoxaceae here includes *Viburnum*, *Sambucus*, and a strongly supported clade consisting of *Sinadoxa*, *Tetradoxa*, and *Adoxa*. *Tetradoxa* and *Adoxa* are more closely related to one another than either is to *Sinadoxa*.

The remaining Dipsacales constitute the Caprifoliaceae *sensu* Judd et al. (1994) and Donoghue et al. (2001), including traditional Caprifoliaceae, Diervilleae, Linnaeae, Morinaceae, Valerianaceae, and Dipsacaceae. Within this well-supported clade, basal relationships are poorly resolved, with only weak support for the idea that Caprifoliaceae plus *Heptacodium* form an early branch (Pyck and Smets, 2000). As shown in Fig. 2, relationships within Caprifoliaceae (i.e., among *Leycesteria*, *Lonicera*, *Symphoricarpos*, and *Triosteum*) are highly uncertain.

Within the remainder of the Caprifoliaceae there are a few well supported clades, including the traditional Morinaceae, Valerianaceae, and Dipsacaceae, but all other relationships are uncertain in this dataset. As noted previously by Donoghue et al. (2001), a particular anomaly in *rbcL* analyses is the separation of

Linnaea from the other Linnaeae. In addition, the remaining Linnaeae appear in *rbcL* trees to be paraphyletic, with *Abelia* as the sister group of the Diervilleae. However, bootstrap support for these groupings is uniformly low (usually <50%).

Our maximum likelihood result also supports the monophyly of Dipsacales, which is linked to a clade consisting of *Columellia* and *Desfontainia*. However, support for these relationships is weak (bootstrap value <50%). Bootstrap support for clades within Dipsacales is generally low, with the exception of Caprifoliaceae (93%), Morinaceae (100%), and Dipsacaceae (100%).

The maximum likelihood and parsimony trees are consistent with one another. Furthermore, there is general agreement with previous analyses based on *rbcL* sequences for Dipsacales (Donoghue et al., 1992; Backlund and Donoghue, 1996; Backlund and Bremer, 1997) and for Asteridae (e.g., Olmstead et al., 1992), as well as on morphological characters (Donoghue, 1983; Judd et al., 1994; Backlund and Donoghue, 1996).

46-Taxon *ndhF* dataset

This data matrix consisted of 1239 aligned base pairs, of which 506 were variable and 306 parsimony informative. Maximum parsimony analyses resulted in six most parsimonious trees of 1313 steps (CI = 0.565, 0.469 excluding invariant characters; RI = 0.705). The strict consensus is shown in Fig. 2, again with branches marked that are supported at 50% or more. Our maximum likelihood search resulted in a single topology with a -lnL value of 8700.22647 (1318 steps under parsimony).

As with *rbcL*, maximum parsimony searches recover a monophyletic Dipsacales, which in this case is weakly linked with an Apiales clade. Again, *Viburnum* and *Sambucus* are united with *Adoxa* and its relatives in a clade that is sister to the Caprifoliaceae. Within the Caprifoliaceae, members of the Caprifoliaceae are strongly united, but support for relationships within this clade is mostly poor. The link between Caprifoliaceae and *Heptacodium* is weak, as is the connection to Diervilleae.

In contrast to *rbcL* analyses, the *ndhF* data strongly support the monophyly of Linnaeae in the traditional sense, with *Linnaea* firmly positioned at the base of this clade. Sister to the Linnaeae is a clade consisting of Morinaceae,

Valerianaceae, and Dipsacaceae. Within this group we see some support for uniting *Triplostegia* with Dipsacaceae, rather than with Valerianaceae as in prior analyses (e.g., Backlund and Donoghue, 1996; Backlund and Bremer, 1997).

Our *ndhF* maximum likelihood tree is in strong agreement with those recovered using parsimony, except for the placement of Diervilleae. In parsimony trees (Fig. 2) the Diervilleae is united with Caprifoliaceae plus *Heptacodium*, whereas in the maximum likelihood tree it is the sister group of all other Caprifoliaceae. In general, however, our *ndhF* results match previously published trees based on *ndhF* sequences (Pyck et al., 1999; Pyck and Smets, 2000) and on the combination of *rbcL* and morphology (Backlund and Donoghue, 1996; Donoghue et al., 2001).

46-Taxon Combined dataset

Our combined *rbcL* and *ndhF* data matrix consisted of 2667 aligned base pairs, of which 940 were variable and 556 parsimony informative. Maximum parsimony analyses resulted in 48 most parsimonious trees of 2382 steps (CI = 0.5460, 0.4432 excluding invariant characters; RI = 0.6612). Our maximum likelihood search resulted in a single tree with a -lnL value of 16846.62023 (2383 steps under parsimony). This tree is presented in Fig. 3, which shows branches with bootstrap values of 80% or more.

Our parsimony results for the combined dataset are similar to those found using *ndhF* alone. We see improved support for Dipsacales as a clade (70%), but again weak support (54%) for a link between Dipsacales and Apiales. Adoxaceae splits from Caprifoliaceae at the base of the Dipsacales. Within Adoxaceae, most relationships are now supported at near 100%, whereas relationships at the base of the Caprifoliaceae and within the major clades (e.g., Caprifoliaceae, Linnaeae), continue to be poorly supported. Interestingly, *Heptacodium* and the Caprifoliaceae together form a basal clade, and Diervilleae is united with the rest of the Caprifoliaceae with a bootstrap value of 53%. In agreement with the *ndhF* sequences alone, *Triplostegia* is united with Dipsacaceae rather than with Valerianaceae.

Results from the maximum likelihood analyses (Fig. 3) agree with those from parsimony. Perhaps most importantly, these results even more strongly unite the Dipsacales (88% bootstrap

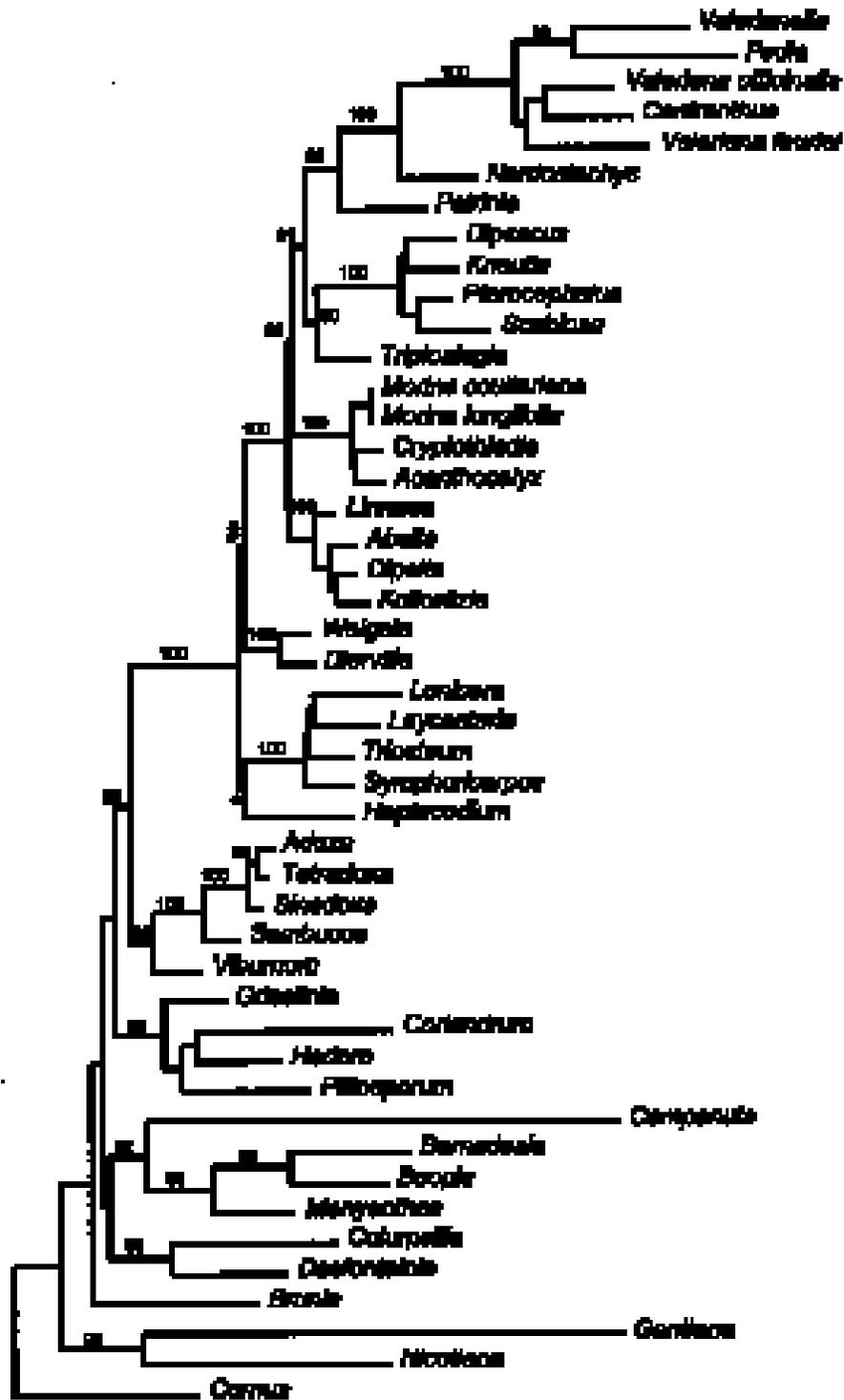


FIGURE 3. Maximum likelihood tree from the 46-taxon combined analysis of *rbcL* and *ndhF* (see text). Numbers above the branches are bootstrap values greater than 80% (support for the clade indicated by * was 66%; support for the clade indicated by # was 46%).

support) and fortify the placement of *Linnaea* with the rest of the Linnaeae (100%) and of *Triplostegia* with the Dipsacaceae (80%).

30-Taxon datasets

Summary statistics for our parsimony and maximum likelihood analyses of separate and combined datasets are summarized in Table 3. Trees obtained from separate searches using each of the additional chloroplast markers (not shown) were generally in strong agreement with one another as well as with those from analyses of the combined data. The Dipsacales tree recovered from our maximum likelihood analysis of the combined dataset is presented in Fig. 4. On the basis of our broader analyses of *rbcL* and *ndhF* (see above), this is rooted along the branch connecting Adoxaceae and Caprifoliaceae. For each 30-taxon analysis, bootstrap values are given in Table 4 for the major clades defined by Donoghue et al. (2001), which are indicated on the tree in Fig. 1.

Focusing on the results from our combined analyses, and specifically on the maximum likelihood tree in Fig. 4, it is especially noteworthy that bootstrap support for most clades (16 of 27) is now at 100% and that four other branches are supported above 95%. These well-supported relationships include several formerly controversial links, most notably the connection between *Triplostegia* and Dipsacaceae. Within the Caprifoliaceae, Diervilleae is identified as the sister group of the rest, which form a clade supported at 84%. The remaining problems, with bootstrap values between 60% and 70%, are the relationships (1) between *Heptacodium* and the Caprifoliaceae, (2) among the major branches within Caprifoliaceae, (3) among Linnaeae, Morinaceae, and Valerianaceae plus Dipsacaceae, and (4) within the core Valerianaceae.

DISCUSSION AND CONCLUSIONS

Our separate and combined analyses of Dipsacales chloroplast DNA sequences yield trees that are broadly consistent with previously published results. And, in every case, the phylogenetic classification proposed by Donoghue et al. (2001) is upheld (Fig. 1). The present study is exceptional, however, in providing far greater confidence in the major clades within the Dipsacales; our combined analyses support most of the major clades with bootstrap values of 100% (Fig. 3–4).

Because Dipsacales monophyly was called into question by some previous studies (e.g.,

Backlund and Donoghue, 1996; Backlund and Bremer, 1997), the APG (1998) classification did not assign Adoxaceae to their more restricted Dipsacales. However, our analyses support the monophyly of the Dipsacales as traditionally circumscribed (see Fig. 2–3). Trees recovered when *ndhF* and *rbcL* are analyzed separately unite Adoxaceae with Caprifoliaceae (Fig. 2), though with weak bootstrap support. Support for this conclusion is, however, reasonably strong (70% for parsimony, 88% for maximum likelihood) in combined *rbcL/ndhF* analyses (Fig. 3). Importantly, our results do not support the inclusion of *Columellia* and *Desfontainia* within Dipsacales, as suggested previously (Backlund and Donoghue, 1996; Backlund and Bremer, 1997).

The basal split within Dipsacales separates the Adoxaceae from the Caprifoliaceae (*sensu* Judd et al., 1994; Donoghue et al., 2001). As in previous analyses, *Viburnum* is strongly linked with the compound-leaved Adoxoideae (*Sambucus* and *Adoxa* and its relatives). Within Adoxoideae, our analyses strongly support the herbaceous Adoxina clade of Donoghue et al. (2001), with *Sinadoxa* as the sister group of *Tetradoxa* plus *Adoxa* (contra Liang, 1997, who united *Sinadoxa* and *Adoxa*).

Relationships at the base of the Caprifoliaceae remain somewhat uncertain. Our combined results place Diervilleae (*Diervilla* plus *Weigela*) as the sister group of the remaining taxa, but support for this position is weak, and the alternative that Caprifoliaceae is sister to the rest of the Caprifoliaceae cannot be rejected with confidence. Unfortunately, this uncertainty limits our ability to infer the basal condition for several key morphological features, especially carpel number (2, 3, or 5) and fruit type (capsule or berry).

Traditionally, the Caprifoliaceae has included *Leycesteria*, *Lonicera*, *Symphoricarpos*, and *Triosteum*. The monophyly of this group is very strongly supported, but relationships among the major lineages remain obscure. The position of *Heptacodium* also remains uncertain. Although our combined analyses support its placement as the sister group of the Caprifoliaceae (as in Pyck and Smets, 2000), confidence in this arrangement is limited in the individual and combined analyses. Understanding the evolution of several characters, especially inflorescence architecture, depends on the resolution of this issue.

Our combined analyses strongly support the monophyly of Linnaeae, despite the separation of *Linnaea* in *rbcL* analyses. Furthermore,

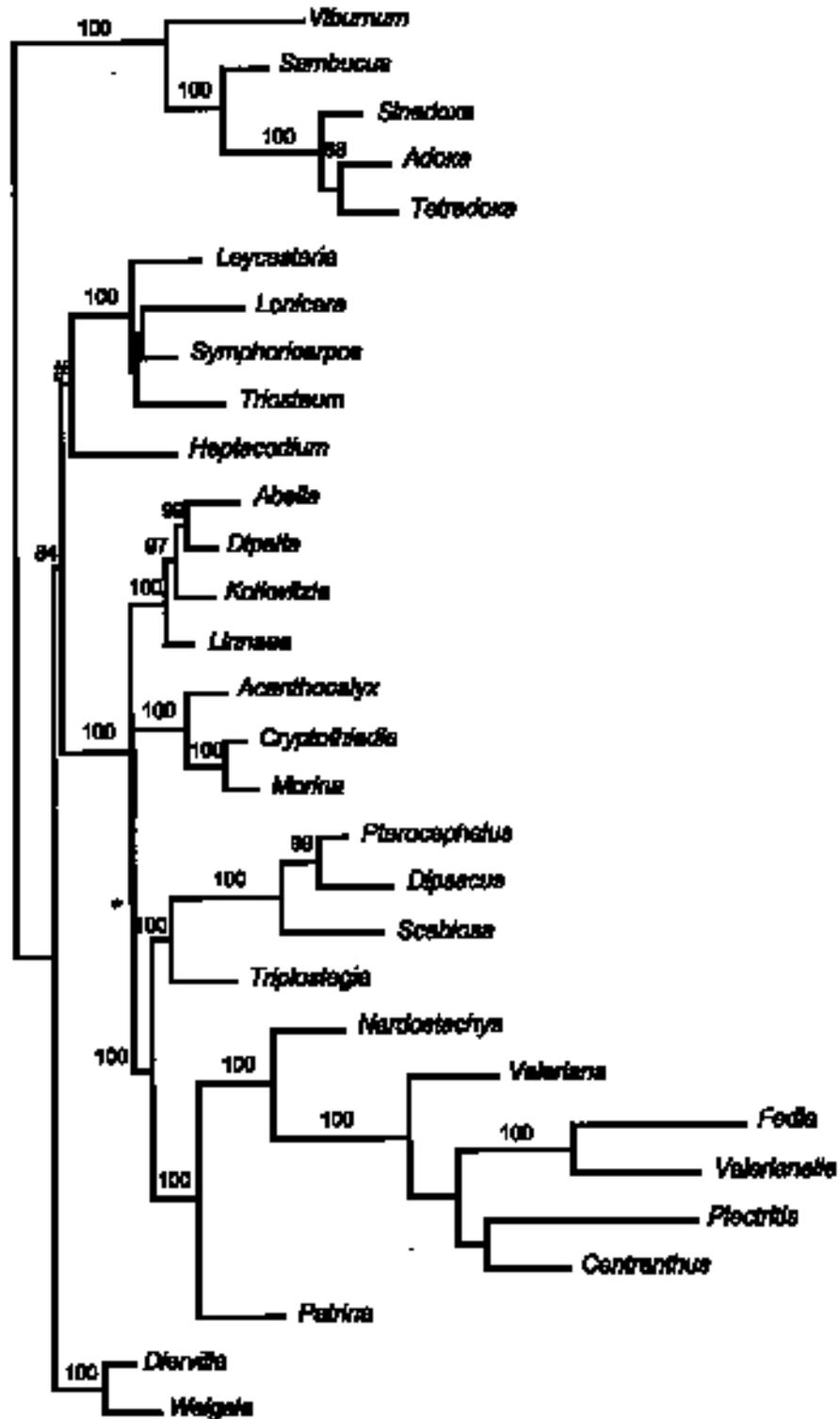


FIGURE 4. Maximum likelihood tree from the combined chloroplast DNA dataset for 30 taxa (Table 3). Numbers above the branches are bootstrap values greater than 80% (support for the clade indicated by * was 60%; support for the clade indicated by # was 67%).

relationships within Linnaeaceae now seem well established, with *Linnaea* being the sister group of the rest, and *Abelia* and *Dipelta* more closely related to one another than either is to *Kolkwitzia*. These findings, along with the description of the fossil *Diplodipelta* (Manchester and Donoghue, 1995), provide a context for the more-detailed developmental studies that are needed to establish homologies within the inflorescence.

As suggested previously (e.g., Judd et al., 1994; Backlund and Donoghue, 1996), the Linnaeaceae is strongly united in our analyses with the Valerina clade of Donoghue et al. (2001), which includes Morinaceae, Dipsacaceae, and Valerianaceae. This supports the view that the characteristic abortion of two carpels (Wilkinson, 1949) and the origin of achene fruits (Fukuoka, 1972) occurred in the common ancestor of Linnaeaceae and Valerina. Valerina is monophyletic in all of our analyses except for the separate analysis of *matK* sequences, though bootstrap support remains weak. This clade is marked by a number of morphological changes, including a shift to herbaceous habit.

Within Valerina, Morinaceae is the sister group of a clade including the Dipsacaceae, *Triplostegia*, and Valerianaceae. Support for Morinaceae is very strong, and within this clade *Morina* and *Cryptothladia* are more closely related to one another than either is to *Acanthocalyx*. These results are supported by a variety of morphological characters (Cannon and Cannon 1984; Caputo and Cozzolino, 1994). Specifically, *Morina* and *Cryptothladia* are united by possession of whorled leaves, a two-lipped calyx, reduction from four to two functional stamens, lobed floral nectaries, and pollen with extraordinary equatorial protrusions.

One of the most interesting results of the present analysis is the strong support obtained for the linkage of *Triplostegia* with the Dipsacaceae, as opposed to with the Valerianaceae (e.g., Backlund and Donoghue, 1996; Donoghue et al. 2001), or with Morinaceae plus Dipsacaceae (Peng et al., 1995). This result is consistent with some previous hypotheses concerning morphological evolution. For example, it supports the view (e.g., Hofmann and Gottmann, 1990; Manchester and Donoghue, 1995; Roels and Smets, 1996) that the epicalyx in Morinaceae, *Triplostegia*, and Dipsacaceae was derived by fusion of the supernumerary bracts seen in Linnaeaceae. It is possible that the 12-ribbed epi-

calyx of Morinaceae originated independently of the 8-ribbed condition found in Dipsacaceae and the inner epicalyx of *Triplostegia*. Alternatively, the epicalyx may have originated in the ancestor of the Valerina clade and then been lost in the Valerianaceae. The winged fruits of *Patrinia*, situated at the base of the Valerianaceae, may represent an independent fusion of bracts or a stage in the loss of the epicalyx; additional developmental studies are needed to evaluate these possibilities.

Relationships remain uncertain within Dipsacaceae, in part owing to the limited sampling of taxa. Our 46-taxon *ndhF* analysis and our 30-taxon analyses indicate that *Dipsacus* and *Pterocephalis* are more closely related to one another than either is to *Scabiosa*. In the combined 30-taxon analysis, the bootstrap support for *Pterocephalis* plus *Dipsacus* reaches 99%. However, this is contradicted by our 46-taxon *rbcL* analysis and (weakly) by our combined *rbcL* and *ndhF* analysis, as well as by the morphological analysis of Caputo and Cozzolino (1994).

Finally, within Valerianaceae the conclusion that *Patrinia* and *Nardostachys* are basal lineages is now very well established. This suggests that the group initially diversified within Asia, probably within the eastern Himalayas. Relationships within the core Valerianaceae are much less clear, aside from the strong connection between *Fedia* and *Valerianella*. We are encouraged by the weak union of *Centranthus* with *Plectritis*, which both have characteristic nectar spurs, but further resolution will require additional taxonomic sampling and molecular markers. Of special interest is the origin and spectacular diversification of the South American species, which are not represented in this dataset.

Having now achieved a very high level of confidence in the backbone phylogeny of Dipsacales, the stage is now set for continued resolution of relationships within major clades, especially *Viburnum*, *Lonicera*, Dipsacaceae, and core Valerianaceae. Dipsacales also now provide an excellent system for studies of character evolution, diversification rate, and historical biogeography. We caution, however, that our results are based solely on chloroplast DNA data. Although chloroplast trees are consistent with those based on morphology, we look forward to the addition of nuclear and/or mitochondrial sequences to solidify our understanding of Dipsacales phylogeny.

TABLE 1. Voucher information and GenBank accession numbers for the 46-taxon *rbcl* and *ndhF* analyses.

TAXON	<i>rbcl</i> VOUCHER OR REFERENCE	GENBANK No.	<i>ndhF</i> VOUCHER OR REFERENCE	GENBANK No.
<i>Abelia x grandiflora</i> Rehder	Donoghue et al., 2001	AJ420875	Cult. Wake Forest Univ., NC., 1999; Donoghue, voucher lacking	AF447029
<i>Acanthocalyx alba</i> (Hand.-Mazz.) M. Cannon	<i>Boufford et al. 28401</i> (A)	AF44693	<i>Boufford et al. 28401</i> (A)	AF447033
<i>Adoxa moschatellina</i> L.	Donoghue et al., 1992	L01884	Pyck et al., 1999	AF060156
<i>Barnadesia caryophylla</i> (Vell.) S. F. Blake	Olmstead et al., 1992	L01887	Kim and Jansen, 1995	L39394
<i>Boopis anthenoides</i> Juss.	Olmstead et al., 1993	L13860	Kim and Jansen, 1995	L39384
<i>Brunia albiflora</i> Phillips	Backlund and Bremer, 1997	Y10674	Roels et al., unpubl.	AF060159
<i>Campanula ramulosa</i> Wall.	Olmstead et al., 1992	L13861	Kim and Jansen, 1995	L39387
<i>Centranthus ruber</i> (L.) DC.	Donoghue et al., 2001	AJ420879	Pyck et al., 1999	AF161297
<i>Columellia oblonga</i> Ruiz & Pav.	Backlund and Bremer, 1997	Y10675	Roels et al., unpubl.	AF060160
<i>Coriandrum sativum</i> L.	Olmstead et al., 1992	L11676	Olmstead et al., 2000	AF130199
<i>Cornus florida</i> L.	Xiang et al., 1993	L11215	Olmstead et al., 2000	AF130220
<i>Cryptothladia chinensis</i> (Pai) M. Cannon	<i>Boufford et al. 27846</i> (A)	AF446944	<i>Boufford et al. 27846</i> (A)	AF447034
<i>Desfontainia spinosa</i> Ruiz & Pav.	Bremer et al., 1994	Z29670	Pyck et al., 1999	AF060163
<i>Diervilla sessilifolia</i> Buckley	Bremer et al., 1994	Z29672	Pyck et al., 1999	AF060164
<i>Dipelta ventricosa</i> Hemsli.	N/A	N/A	Pyck et al., 1999	AF161295
<i>Dipelta floribunda</i> Maxim.	Donoghue et al., 2001	AJ420876	N/A	N/A
<i>Dipsacus sativus</i> (L.) Honck.	Olmstead et al., 1992	L13824	Olmstead et al., 2000	AF130190
<i>Fedia cornicopiae</i> (L.) Gaertner	Cult. Bergius Bot. Gard., Sweden; <i>Eriksson s.n.</i> , 2 Nov. 1999 (SBT)	AF446953	Cult. Bergius Bot. Gard., Sweden; <i>Eriksson s.n.</i> , 2 Nov. 1999 (SBT)	AF447043

TABLE 1. (CONT.)

TAXON	<i>rbcL</i> VOUCHER OR REFERENCE	GENBANK No.	<i>ndhF</i> VOUCHER OR REFERENCE	GENBANK No.
<i>Gentiana procera</i> Holm	Olmstead et al., 1993	L14398	Reeves and Olmstead, 1995	L36400
<i>Griselinia lucida</i> G. Forst.	Xiang et al., 1993	L11225	Olmstead et al., 2000	AF130205
<i>Hedera helix</i> L.	Olmstead et al., 1992	L01924	Olmstead et al., 2000	AF130203
<i>Heptacodium miconioides</i> Rehder	Donoghue et al., 2001	AJ420873	Cult. Arnold Arboretum, 1549-80; <i>Koller s.n.</i> , 12 Oct. 1984 (A)	AF447026
<i>Knautia arvensis</i> (L.) Coult.	N/A	N/A	Pyck et al., 1999	AF161298
<i>Knautia intermedia</i> Parnh. & Wettst.	Backlund and Bremer, 1997	Y10698	N/A	N/A
<i>Kolkwitzia amabilis</i> Graebn.	Donoghue et al., 2001	AJ420877	Pyck et al., 1999	AF161294
<i>Leycesteria formosa</i> Wall.	Donoghue et al., 2001	AJ420872	Pyck et al., 1999	AF161290
<i>Limnaea borealis</i> L.	Donoghue et al., 2001	AJ420878	Pyck et al., 1999	AF060166
<i>Lonicera orientalis</i> Lam.	Gustafsson et al., 1996	X87389	Oxelman et al., unpubl.	AF027274
<i>Menyanthes trifoliata</i> L.	Olmstead et al., 1993	L14006	Kim and Jansen, 1995	L39388
<i>Morina longifolia</i> Wallich ex DC.	Cult. Bergius Bot. Gard., Sweden; <i>Eriksson s.n.</i> , 2 Nov. 1999 (SBI)	AF446945	Cult. Bergius Bot. Gard., Sweden; <i>Eriksson s.n.</i> , 2 Nov. 1999 (SBI)	AF447035
<i>Morina coulteriana</i> Royle	Backlund and Bremer, 1997	Y10706	<i>Grady et al.</i> 6553 (A)	AF447047
<i>Nardostachys jatamansii</i> (D. Don) DC.	Backlund and Bremer, 1997	Y10705	<i>Boifford et al.</i> 28099 (A)	AF447040
<i>Nicotiana tabacum</i> L.	Lin et al., 1985	M16896	Olmstead et al., 1993	L14953
<i>Patrinia rupestris</i> (Pall.) DuRoi.	Backlund and Bremer, 1997	Y10704	N/A	N/A
<i>Patrinia triloba</i> Miq.	N/A	N/A	Pyck et al., 1999	AF161296
<i>Pittosporum japonicum</i> Hort. ex C. Presl.	Morgan and Soltis, 1993	L11202	Olmstead et al., 2000	AF130201

TABLE 1. (CONT.)

TAXON	<i>rbcl</i> VOUCHER OR REFERENCE	GENBANK No.	<i>ndhF</i> VOUCHER OR REFERENCE	GENBANK No.
<i>Pterocephalus hookeri</i> (C. B. Clarke) V. Meyer & Ehrendorfer	<i>Boufford et al.</i> 28691 (A)	AF446946	<i>Boufford et al.</i> 28691 (A)	AF447036
<i>Pterocephalus lasiospermus</i> Link ex Buch	Backlund and Bremer, 1997	Y10702	N/A	N/A
<i>Sambucus cerulea</i> Raf.	Donoghue et al., 2001	AJ420867	N/A	N/A
<i>Sambucus racemosa</i> L.	N/A	N/A	<i>Boufford et al.</i> 27670 (A)	AF447018
<i>Scabiosa columbaria</i> DC.	<i>Bell</i> 199 (Yale)	AF446948	<i>Bell</i> 199 (Yale)	AF447038
<i>Sinadoxa corydalifolia</i> C. Y. Wu, Z. L. Wu & R. F. Huang	Donoghue et al., 2001	AJ420866	<i>Boufford et al.</i> 26555 (A)	AF447019
<i>Symphoricarpos albus</i> (L.) S. F. Blake	Olmstead et al., 1992	L11682	Pyck et al., 1999	AF161291
<i>Tetradoxa omeiensis</i> (H. Hara) C. Y. Wu	Donoghue et al., 2001	AJ420865	<i>Donoghue et al.</i> 4000 (A)	AF447021
<i>Triossteum perfoliatum</i> L.	Donoghue et al., 2001	AJ420871	Pyck et al., 1999	AF161293
<i>Triplostegia glandulifera</i> Wall. ex DC.	Backlund and Bremer, 1997	Y10700	<i>Boufford et al.</i> 28440 (A)	AF447039
<i>Valeriana fauriei</i> Briquet	<i>Yokokura</i> 96175 (A)	AF447047	Olmstead et al., 2000	AF130192
<i>Valeriana officinalis</i> L.	Olmstead et al., 1992	L13934	<i>Boufford et al.</i> 28729 (A)	AF447048
<i>Valerianella locusta</i> (L.) Laterrade	<i>Patterson, 2001</i> (SFSU)	AF446954	<i>Patterson, 2001</i> (SFSU)	AF447044
<i>Viburnum acerifolium</i> L.	Olmstead et al., 1992	L01959	<i>Davis</i> CCD32 (A)	AF446927
<i>Weigela hortensis</i> (Sieb. & Zuck.) C. A. Mey.	Donoghue et al., 2001	AJ420874	Pyck et al., 1999	AF161293

TABLE 2. Voucher information and GenBank accession numbers for the 30-taxon chloroplast DNA analyses.

TAXON	VOUCHER OR REFERENCE	GENBANK NO.				
		<i>rbcL</i>	<i>ndhF</i>	<i>matK1</i>	<i>trnL2</i>	<i>atpB3</i>
<i>Abelia x grandiflora</i> Rehder	Cult. Wake Forest Univ., NC., 1999; Donoghue, voucher lacking	AF446939	AF447029	AF446909	AF446969	AF446998
<i>Acanthocalyx alba</i> (Hand.-Mazz.) M. Cannon	<i>Boufford et al.</i> 28401 (A)	AF446943	AF447033	AF446913	AF446973	AF447003
<i>Adoxa moschatellina</i> L.	<i>Boufford et al.</i> 28906 (A)	AF446930	AF447020	AF446900	AF446960	AF446990
<i>Centranthus ruber</i> (L.) DC.	<i>Bell.</i> 203 (Yale)	AF446956	AF447046	AF446926	AF446986	AF447016
<i>Cryptothladia chinensis</i> (Pai) M. Cannon	<i>Boufford et al.</i> 27846 (A)	AF446944	AF447034	AF446914	AF446974	AF447004
<i>Diervilla sessilifolia</i> Buckley	Cult. Arnold Arboretum 960-79; <i>Elsik and Zimman</i> 3286 (A)	AF446937	AF447027	AF446907	AF446967	AF446997
<i>Dipelta yunnanensis</i> Franchet	<i>Boufford et al.</i> 29340 (A)	AF446940	AF447030	AF446910	AF446970	AF447000
<i>Dipsacus mitis</i> D. Don	<i>Boufford et al.</i> 27724 (A)	AF446947	AF447037	AF446917	AF446977	AF447007
<i>Fedia cornicopiae</i> (L.) Gaertner	Cult. Bergius Bot. Gard., Sweden; <i>Eriksson s.n.</i> , 2 Nov. 1999 (SBI)	AF446953	AF447043	AF446923	AF446983	AF447013
<i>Heptacodium miconioides</i> Rehder	Cult. Arnold Arboretum, 1549-80; <i>Koller s.n.</i> , 12 Oct. 1984 (A)	AF446936	AF447026	AF446906	AF446966	AF446996
<i>Kolkwitzia amabilis</i> Graebn.	Cult. Arnold Arboretum, 18090; <i>Elsik and Siegel</i> 1558 (A)	AF446942	AF447032	AF446912	AF446972	AF447002
<i>Leycesteria formosa</i> Wall.	Cult. Kew Botanic Gardens, UK; Donoghue, voucher lacking	AF446932	AF447022	AF446902	AF446962	AF446992
<i>Linnaea borealis</i> L.	Door County, WI; Donoghue, 1990, voucher lacking	AF446941	AF447031	AF446911	AF446971	AF447001
<i>Lonicera nervosa</i> (D. Don) DC.	<i>Boufford et al.</i> 29295 (A)	AF446933	AF447023	AF446903	AF446963	AF446993
<i>Morina longifolia</i> Wallich ex DC.	Cult. Bergius Bot. Gard., Sweden; <i>Eriksson s.n.</i> , 2 Nov. 1999 (SBI)	AF446945	AF447035	AF446915	AF446975	AF447005

TABLE 2. (CONT.)

TAXON	VOUCHER OR REFERENCE	GENBANK NO.				
		<i>rbcL</i>	<i>ndhF</i>	<i>matK1</i>	<i>trnL-2</i>	<i>atpB3</i>
<i>Nardostyachys jatamansii</i> (D. Don) DC.	Boufford et al. 28099 (A)	AF446950	AF447040	AF446920	AF446980	AF447010
<i>Patrina triloba</i> Miq.	Cult. Bergius Bot. Gard., Sweden; Eriksson 807 (SBT)	AF446951	AF447041	AF446921	AF446981	AF447011
<i>Plectritis macroceras</i> Torr. & Gray	Patterson, 2000 (SFSU)	AF446955	AF447045	AF446925	AF446985	AF447015
<i>Pteroccephalus hookeri</i> (C. B. Clarke) V. Meyer & Ehrendorfer	Boufford et al. 28691 (A)	AF446946	AF447036	AF446916	AF446976	AF447006
<i>Sambucus racemosa</i> L.	Boufford et al. 27670 (A)	AF446928	AF447018	AF446898	AF446958	AF446988
<i>Sinadixa corydalifolia</i> C. Y. Wu, Z. L. Wu & R. F. Huang	Boufford et al. 26555 (A)	AF446929	AF447019	AF446899	AF446959	AF446989
<i>Scabiosa columbaria</i> L.	Bell, 199 (Yale)	AF446948	AF447038	AF446918	AF446978	AF447008
<i>Symphoricarpos oreophilus</i> A. Gray	vic. Tucson, AZ, 1993; Donoghue, voucher lacking	AF446934	AF447024	AF446904	AF446964	AF446994
<i>Tetradoxa omeiensis</i> (H. Hara) C. Y. Wu	Donoghue et al. 4000 (A)	AF446931	AF447021	AF446901	AF446961	AF446991
<i>Triosteum perfoliatum</i> L.	vic. Madison, WI, 1990; Donoghue, voucher lacking	AF446935	AF447025	AF446905	AF446965	AF446995
<i>Triplostegia glandulifera</i> Wallich ex DC.	Boufford et al. 28440 (A)	AF446949	AF447039	AF446919	AF446979	AF447009
<i>Valeriana minutiflora</i> Hand.-Mazz.	Boufford et al. 27928 (A)	AF446952	AF447042	AF446924	AF446982	AF447012
<i>Valerianella locosta</i> (L.) Latarrade	Patterson, 2001 (SFSU)	AF446954	AF447044	AF446922	AF446984	AF447014
<i>Viburnum acerifolium</i> L.	Davis CCD32 (A)	AF446927	AF447017	AF446897	AF446957	AF446987
<i>Weigela hortensis</i> (Seib. & Zucc.) C. A. Mey.	Cult. Arnold Arboretum 1897-77-A; Kelly and Buckalind 28 (A)	AF446938	AF447028	AF446908	AF446968	AF446998

¹ *matK* coding region and *matK* intron.² *trnL-F* intergenic spacer region and *trnL* intron.³ *rbcL-atpB* intergenic spacer region.

TABLE 3. Summary of maximum parsimony and maximum likelihood analyses of 30-taxon datasets.

	<i>rbcl</i>	<i>ndhF</i>	<i>matK</i>	<i>trnL</i>	<i>atpB</i>	COMBINED
Sequence length	1428	2199*	1808	1180	978	7593
No. of variable characters	291	787	863	470	511	2916
No. of parsimony-informative characters	145	488	512	276	334	1819
No. of MP trees	4	1	2	12	24	1
Length of MP trees	468	1478	1592	751	965	5378
CI (all characters)	0.718	0.701	0.726	0.842	0.754	0.717
CI (excluding invariant characters)	0.574	0.612	0.634	0.802	0.688	0.632
RI	0.755	0.771	0.775	0.826	0.839	0.785
-lnL	4961.537	11516.643	11353.176	5823.373	6121.587	41192.291
Model of sequence evolution	GTR+I+G	GTR+I+G	GTR+G	GTR+G	GTR+G	GTR+I+G
No. of steps on ML tree	470	1484	1606	754	966	5379

NOTE: MP = most parsimonious, CI = consistency index, RI = retention index, GTR = general time reversible, I = invariable sites, G = among site rate variation modeled to fit a discrete gamma distribution, ML = maximum likelihood.
 * Includes 8 indels of 3-9 base pairs in length.

TABLE 4. Bootstrap support values (maximum parsimony/maximum likelihood) for taxa recognized by Donoghue et al. (2001).

CLADE	<i>rbcL</i>	<i>ndhF</i>	<i>matK₁</i>	<i>trnL₂</i>	<i>atpB₃</i>	COMBINED
Adoxaceae	100/100	100/100	100/100	100/100	100/100	100/100
Adoxoideae	93/83	100/100	100/100	74/<50	99/98	100/100
Adoxina	100/100	100/100	100/100	100/100	99/95	100/100
Caprifoliaceae	100/100	100/100	100/100	100/100	100/100	100/100
Diervilleae	89/88	100/100	100/100	99/100	97/98	100/100
Caprifoliaceae	72/80	100/100	100/100	100/99	100/74	100/100
Linnina	51/<50	100/100	100/100	97/98	99/93	100/100
Linnaeaceae	<50/<50	100/100	99/87	100/100	100/100	100/100
Valerina	<50/<50	89/86	<50/<50	<50/<50	100/100	60/61
Dipsacaceae	99/100	100/100	100/100	100/100	71/<50	100/100
Valerianaceae	86/75	100/100	100/76	100/99	100/100	100/100

NOTE: See Fig. 1 for the connection between names and clades.

¹ *matK* coding region and *matK* intron.

² *trnL-F* intergenic spacer region and *trnL* intron.

³ *rbcL-atpB* intergenic spacer region.

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