

# PHYLOGENY OF DIPSACALES BASED ON CHLOROPLAST DNA SEQUENCES

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Donoghue et al., 1992

Judd et al., 1994

Backlund and Donoghue, 1996

Pyck et al., 1999

Donoghue et al., in prep.

In recent years there have been several studies of the phylogeny of the Dipsacales based on both morphological and molecular, as well as combined evidence (e.g., Donoghue, 1983; Donoghue et al., 1992; Judd et al., 1994; Backlund and Donoghue, 1996; Backlund and Bremer, 1997; Pyck et al., 1999; Donoghue et al., in prep.). All of these studies have concluded that *Viburnum* is most closely related to *Sambucus* and *Adoxa*, and that the remainder of the Caprifoliaceae (in the traditional sense) are related to Morinaceae, Valerianaceae, and Dipsacaceae. Within the later clade previous studies have supported the monophyly of Caprifoliaceae, Diervillieae, and Linnaeae (all of traditional Caprifoliaceae), with Linnaeae being the sister group to a clade containing Morinaceae, Valerianaceae, and Dipsacaceae. Support for these clades varies considerably among analyses and the exact placement of several key taxa (e.g., *Heptacodium*, *Triplostegia*) remains unresolved. Here we report the results of combined analyses using chloroplast DNA sequences from four regions: *ndhF*, *rbcL*, *matK*, and *trnL*.

## METHODS AND MATERIALS

Taxon sample--Sequence data from 30 taxa, representing all major lineages within Dipsacales were obtained from herbarium specimens, material stored in silica gel, or from GenBank. DNA extraction and amplification--Total DNAs were extracted using a standard CTAB method and further purified with the Prep-A-Gene(r) DNA Purification Kit (Bio-Rad). DNA sequences were amplified for *ndhF*, *rbcL*, *matK* and *trnL* regions of the chloroplast genome using previously published primers. Sequencing--All sequencing was done via dye terminator cycle sequencing using the protocol specified by the ABI PRISM(r) Dye Primer Cycle Sequencing Ready Reaction Kit (Revision B, August 1995, Perkin-Elmer) and visualized on a ABI 377 automated DNA sequencer. Alignment--Sequence fragments were assembled using Sequencher (Gene Codes Corp., 1994) and aligned visually. MacClade version 3.06 (Maddison and Maddison, 1992) was used to translate DNA sequences to protein sequences to help with the alignment. Phylogenetic analyses--Maximum parsimony and maximum likelihood searches were conducted using heuristic search methods with TBR branch swapping, collapse of zero-length branches, and weighting all characters equally. The analyses were repeated 100 times with the RANDOM ADDITION option (Maddison, 1991). Equally parsimonious trees were summarized using strict consensus. Bootstrap tests (Felsenstein, 1985) were performed, for both parsimony and likelihood searches, using 300 replicates. All analyses were done using PAUP\* 4.0b4a (Swofford, 2000). For likelihood analyses, a series of likelihood ratio tests (LRT) were performed to determine which model of sequence evolution best fit the data using a program written in Python (available from CDB), for individual and combined data sets, and searches were carried with this model.

*Adoxa* *Sinadixa* *Viburnum* *Weigela* *Lycorhiza* *Tristemon* *Dipelta* *Acanthocyphus* *Scabiosa* *Triplostegia* *Patrinia* *Aurastachys*

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Strict Consensus of 2 MPR from combined data

Donoghue et al., in prep.

## RESULTS AND DISCUSSION

Our aligned matrix contained 2,199 *ndhF*, 1,428 *rbcL*, 1,808 *matK*, and 1,196 *trnL* sites, for a total of 6,631 characters. Results of separate and combined analyses are summarized in Table 1. The strict consensus of the two most parsimonious trees of 3954 steps (CI=0.6585) from the combined analysis is shown above. In general, our separate and combined analyses yield trees that are consistent with previously published results, though with stronger support (most clades with bootstrap values of 100%). Most importantly, (1) *Viburnum* and *Sambucus* are strongly linked with *Adoxa* and relatives, (2) *Sinadixa* is the sister group of *Adoxa*, *Tetradoxa* (3) Linnaeae are strongly united with a Morinaceae-Dipsacaceae-Valerianaceae clade. We find reasonable support for (1) placing *Heptacodium* sister to Caprifoliaceae, (2) the placement of *Diervilla* and *Weigela* sister to the rest of the Caprifoliaceae (sensu Judd et al., 1994; i.e., Caprifoliaceae, Linnaeae, and Valerina: see Figure above), and (3) uniting *Triplostegia* with the Dipsacaceae and not the Valerianaceae, as in previous studies (e.g., Backlund and Donoghue, 1996). These results are consistent with some previous hypotheses concerning morphological evolution. For example, they support the view (e.g., Manchester and Donoghue, 1995; Roels and Smets, 1996) that the epicalyx characteristic of Morinaceae, *Triplostegia* and Dipsacaceae is homologous with the supernumerary bracts in Linnaeae. In contrast, they do not clearly support the view (e.g., Hara, 1983; Liang, H. 1997) that the split stamens and open inflorescence of *Tetradoxa* are ancestral in the *Adoxa-Tetradoxa-Sinadixa* clade (Adoxina in Fig. A).