American Journal of Botany 92(4): 653–666. 2005.

# *VIBURNUM* PHYLOGENY BASED ON COMBINED MOLECULAR DATA: IMPLICATIONS FOR TAXONOMY AND BIOGEOGRAPHY[1]

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We investigated *Viburnum* phylogeny using separate and combined analyses of DNA sequence data from two chloroplast and three nuclear loci. Separate analyses of nuclear and chloroplast data sets resulted in gene trees that were generally congruent with one another and with trees from two previous analyses. Our gene trees do differ in the position of section *Pseudotinus*, as well as in species relationships within sections *Pseudotinus* and *Lentago*. However, tests for incongruence indicate that differences between the nuclear and chloroplast data are not significant. Furthermore, gene trees from combined analyses were highly similar to those found in separate analyses, suggesting that these localized differences do not affect other parts of the tree. Our analyses provide convincing support for numerous relationships, although there is still uncertainty at the base of the tree. To facilitate future study, we propose informal names for 12 well-supported species groups, as well as for several higher-level clades. We also discuss the biogeographic implications of our phylogeny, focusing on repeated, although apparently temporally incongruent, patterns of disjunction between the Old and New Worlds.

**Key words:** biogeography; data combination; phylogeny; taxonomy; *Viburnum*.

*Viburnum* (Adoxaceae, Dipsacales) is a clade of ca. 160 species of shrubs and small trees. Although broadly distributed around the Northern Hemisphere, *Viburnum* has centers of species diversity in eastern Asia and Latin America (Rehder, 1908; Hara, 1983; Donoghue, 1983a), and its range extends into the Southern Hemisphere in the mountains of southeast Asia (Kern, 1951) and South America (Killip and Smith, 1931). The group displays relatively little variability in flower and fruit morphology, but conspicuous diversity in numerous other characters; variation in endocarp shape, fruit color, leaf morphology, bud morphology, and inflorescence form is particularly striking.

Linnaeus (1753) originally erected *Viburnum*. A later revision by Oersted (1861) clearly identified the principal variable characters within the group, although his proposed subdivision into five genera was never widely adopted. Since Oersted's (1861) treatment, few botanists have considered the classification of *Viburnum* as a whole. One exception was Rehder (1940), who, although concentrating on the Asian species, recognized nine sections. More recently, Hara (1983) greatly clarified the subgeneric nomenclature with his review of the section-level classification. This treatment recognized 10 sections (Table 1)—the nine suggested by Rehder (1940) plus section *Oreinotinus* to accommodate the Latin American species, for which Morton (1933) had proposed 10 sections. There have been few section level revisions. The exceptions include a thorough treatment of the eastern North American species of section *Lentago* (Jones, 1983) and a monograph of several species complexes in Mexico and Central America (Donoghue, 1982). In other studies, Hsu (1975) segregated a new section (Platyphylla) from *Megalotinus*, Kern (1951) recognized four subsections within *Megalotinus*, and Hara (1983) proposed subsections within sections *Odontotinus*, *Viburnum*, and *Solenotinus*. Regional treatments have dealt with the Asian (Hara, 1983; Rehder, 1908; Hsu et al., 1988), Malesian (Kern, 1951; Kern and van Steenis, 1951), South American (Killip and Smith, 1929, 1931), Mexican and Central American (Morton, 1933; Donoghue, 1982), and North American species (McAtee, 1956). These have varied in scope, but mostly contain only keys and descriptions; species relationships within or between regions have seldom been considered.

The monophyly and phylogenetic placement of *Viburnum* are now clearly established (e.g., Wilkinson, 1948; Donoghue, 1983b; Pyck et al., 1999; Bell et al., 2001; Donoghue et al., 2001, 2003). In contrast, despite an earlier morphological study (Donoghue, 1983a) and several unpublished molecular data sets (see abstracts by Donoghue and Sytsma, 1993; Donoghue and Baldwin, 1993; see also Baldwin et al., 1995) evolutionary relationships within *Viburnum* have remained largely unresolved. However, two recent molecular phylogenetic studies—Donoghue et al. (2004) using chloroplast *trnK* intron and nuclear ribosomal ITS DNA sequences, and Winkworth and Donoghue (2004) using sequences from the low-copy number nuclear gene *GBSSI*—have improved our understanding of relationships within *Viburnum*. In broad terms, they confirm the monophyly of many section-level taxa. Section *Odontotinus* was the most striking exception, being divided into (1) a purple-fruited North American lineage that also includes the Latin American section *Oreinotinus*, and (2) a mostly red-fruited Asian lineage that also contains the North American purple-fruited species, *V. acerifolium*. Sections *Lentago*, *Solenotinus*, and *Viburnum* were also found to be nonmonophyletic. However, for each of these groups, a core clade contains all but one representative of the section (see below).

Perhaps more importantly, the studies of Donoghue et al. (2004) and Winkworth and Donoghue (2004) provided consistent resolution of relationships among the sectional-level lineages, identifying three well-supported suprasectional clades. The largest suprasectional lineage contains subclades

[1] Manuscript received 5 June 2004; revision accepted 7 January 2005.

The authors thank Bob Cook and the staff of the Arnold Arboretum for allowing us to make extensive collections on the grounds and for their general support. Lab groups at Harvard and Yale provided valuable feedback. We thank Valéry Malécot for input on species numbers. We also thank Richard Olmstead and an anonymous reviewer for helpful comments on an earlier version.

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TABLE 1. Traditionally recognized sections of *Viburnum* (e.g., Hara, 1983).

| Section | Approximate number of species | Generalized geographic range |
|---|---|---|
| *Lentago* | 7 | Eastern North America, except *V. elatum* in Mexico |
| *Megalotinus* | 18 | Southeast Asia, extending west to India and south to Indonesia |
| *Odontotinus* | 37 | Temperate Asia and Eastern North America, except *V. orientale* in the Caucasus Mountains |
| *Opulus* | 5 | Circumboreal |
| *Oreinotinus* | 38 | Mexico, Caribbean, Central and South America |
| *Pseudotinus* | 4 | Asia, except *V. lantanoides* in Eastern North America |
| *Solenotinus* | 26 | Asia, extending west to India and south to Indonesia |
| *Tinus* | 7 | Asia, except *V. tinus* in Europe |
| *Tomentosa* | 2 | China, Japan |
| *Viburnum* | 14 | Asia, except *V. lantana* in Europe |

corresponding to (1) section *Opulus*, (2) section *Tinus*, (3) the New World *Odontotinus* plus *Oreinotinus*, and (4) the Old World *Odontotinus–acerifolium* lineage plus *V. cylindricum* of section *Megalotinus*. Each of the two remaining suprasectional lineages consists of a well-supported pair of section level clades. In one of these, *V. plicatum* (section *Tomentosa*) is sister to the core of section *Solenotinus*; in both studies this grouping is weakly associated with *V. urceolatum* (traditionally of section *Viburnum*). The third suprasectional lineage contains the core species groups of sections *Lentago* and *Viburnum*. This lineage also includes *V. nudum* (traditionally of section *Lentago*), although its exact relationship to these section-level clades remains unclear.

The most striking difference between the gene trees inferred by Donoghue et al. (2004) and those of Winkworth and Donoghue (2004) relates to the duplication of the *GBSSI* paralogs in several *Viburnum* lineages. Specifically, *V. erubescens*, *V. odoratissimum*, and *V sieboldii* of section *Solenotinus* and the *Oreinotinus–dentatum* clade were represented twice on *GBSSI* gene trees. These lineage-specific duplications of the *GBSSI* loci are consistent with karyological evidence for polyploidy in these groups (Winkworth and Donoghue, 2004). Although in these two lineages the placement of *GBSSI* homoeologues tends to favor an allopolyploid origin, such inferences are complicated by (1) loss or failure to identify homoeologues in certain taxa and (2) placement of related diploid species. As noted by Winkworth and Donoghue (2004), data from additional nuclear loci are needed to confirm inferences about the origins of polyploidy in *Viburnum*.

In addition to differences in locus copy number, there were several localized regions of uncertainty or conflict between gene trees recovered in the two studies. In most cases, the alternative arrangements seem to reflect limited phylogenetic signal rather than differences in evolutionary history. However, one possible exception involves relationships within the core of section *Lentago*. Here, differences between the relationships suggested by nuclear and chloroplast markers are consistent with an earlier hypothesis suggesting homoploid hybrid speciation (Donoghue et al., 2004). Perhaps the most critical outstanding issue involves relationships at the base of *Viburnum*. Although the analyses of Donoghue et al. (2004) and Winkworth and Donoghue (2004) have resolved many major clades, relationships near the base remain uncertain. Specifically, (1) the placement of section *Pseudotinus*, (2) relationships among the suprasectional lineages, *V. urceolatum*, and *V. clemensiae*, and (3) the position of the root all remain unresolved.

In the present study, we further investigate phylogenetic relationships within *Viburnum* by combining the data sets of Donoghue et al. (2004) and Winkworth and Donoghue (2004) with sequences from an additional chloroplast marker, the *psbA-trnH* intergenic spacer (IGS). For our analyses, we formed new data partitions. Specifically, a chloroplast data set that included *trnK* intron sequences from Donoghue et al. (2004) and our *psbA-trnH* IGS sequences, and a nuclear partition that consisted of nrITS (Donoghue et al., 2004) and *GBSSI* (Winkworth and Donoghue, 2004) sequences. We used gene trees from analyses of chloroplast, nuclear, and combined data sets to test phylogenetic hypotheses suggested by earlier studies, to investigate differences between the data sets, and to clarify relationships left unresolved in previous analyses. The results provide a firm basis for reclassification of *Viburnum* and for a preliminary biogeographic analysis.

## MATERIALS AND METHODS

***Taxon sampling***—For this study, we included the same set of taxa (i.e., 41 *Viburnum* species plus an outgroup, *Sambucus canadensis*) that were used by Winkworth and Donoghue (2004). This sample includes approximately one quarter of all *Viburnum* species, but more importantly it includes representatives of all traditionally recognized sections and major species complexes. Although we broadly covered *Viburnum* diversity, our sampling within certain clades was limited. In particular, section *Megalotinus* was represented by just one of the 18 recognized species.

***Molecular methods***—In general, molecular methods (i.e., genomic DNA isolation, PCR amplification, fragment purification, and sequencing) followed the protocols described by Winkworth and Donoghue (2004). For PCR, we used the oligonucleotide primers psbAF and trnHR (Sang et al., 1997) and a thermocycling profile that incorporated a 53°C annealing temperature and a 1-min extension time. For some accessions, direct sequencing of PCR fragments consistently failed due to the presence of long adenosine or thymine strings. In such cases, amplification products were cloned, screened, and sequenced as described by Winkworth and Donoghue (2004). Voucher information for the accessions used in this study is as reported in Winkworth and Donoghue (2004); accession details and GenBank numbers for our *psbA-trnH* IGS sequences are available in the Appendix (see Supplemental Data accompanying the online version of this article).

***Sequence alignments and data matrices***—Preliminary multiple sequence alignments for the *psbA-trnH* IGS DNA sequences were performed using ClustalX (Thompson et al., 1994), followed by visual inspection. Prior to phylogenetic analyses, all ambiguous positions were excluded from the data matrix. Furthermore, to remain consistent with the data matrices of Donoghue et al. (2004) and Winkworth and Donoghue (2004), we excluded all gapped positions from our *psbA-trnH* IGS data set. We discuss the compatibility of several parsimony informative gaps with the topology based on nucleotide substitutions in the combined chloroplast data set.

For phylogenetic analyses, we prepared three data matrices, each containing the same taxa as used by Winkworth and Donoghue (2004). A chloroplast data set was compiled by combining the *trnK* intron matrix from Donoghue et al. (2004) and our new *psbA-trnH* IGS sequences. All taxa were represented by both loci in this matrix. A nuclear data set was prepared by combining the nrITS data matrix (Donoghue et al., 2004) with the *GBSSI* matrices from Winkworth and Donoghue (2004). In most species of *Viburnum*, *GBSSI* is present as two paralogues; Winkworth and Donoghue (2004) characterized the region between exons 8 and 12 for both of these loci. In addition to these main copies, a pair of homoeologues were identified from several species. We considered several methods for combining the *GBSSI* matrices with the nrITS data set, in which each species is represented by a single sequence (e.g., uninode coding, Simmons et al., 2000). However, to account for the *GBSSI* homoeologues in our combined data set, either nrITS or *GBSSI* sequences would need to be duplicated, thereby increasing the influence of that partition on the resulting gene tree. We opted instead to include in the nuclear matrix those *GBSSI* homoeologues that were topologically congruent with the nrITS phylogeny of Donoghue et al. (2004). Specifically, we used the Winkworth and Donoghue (2004) *B* homoeologues from the *Oreinotinus–dentatum* clade and the *D* homoeologues from *V. erubescens*, *V. odoratissimum*, and *V. sieboldii*. Winkworth and Donoghue (2004) did not recover *GBSSI* sequences for several taxa—*WAXY1* sequences are missing for *V. odoratissimum*, as are *WAXY2* sequences for *V. dentatum*, *V. edule*, section *Tinus*, and *Sambucus canadensis*. These sequences are represented as missing data in our nuclear matrix. We prepared a combined data set by concatenating our chloroplast and nuclear matrices.

***ILD and Templeton tests***—We tested for incongruence between data partitions within the chloroplast and nuclear data matrices using the incongruence length difference (ILD) test as implemented in PAUP* 4.0b10 (Swofford, 2002). For the chloroplast matrix, this required a single pairwise comparison; for the nuclear data set, we made all pairwise tests and a simultaneous comparison of all three markers.

We also tested for incongruence between the chloroplast and nuclear matrices. Based on previous studies and our preliminary analyses, we identified several potential sources of conflict between these two data sets—specifically, relationships within sections *Lentago* and *Pseudotinus* and the placement of *Pseudotinus*. To test these differences, we conducted a series of ILD tests to compare levels of incongruence in the complete data set with those in matrices containing different subsets of taxa from sections *Lentago* and *Pseudotinus*. We used this approach to evaluate the relative contribution of the different areas of conflict to the overall level of incongruence. We also examined differences between the chloroplast and nuclear data using the significantly less parsimonious test (SLP test; Templeton, 1983). In these tests, we used the 70% majority rule consensus of MP trees as our ''test tree'' and constructed a ''rival tree'' by constraining the node of interest to reflect the topology suggested by the other data set (''test'' and ''rival'' are used as in Mason-Gamer and Kellogg, 1996). For example, the rival topology for evaluating the placement of section *Pseudotinus* suggested by the chloroplast data was identical to the test tree except that *Pseudotinus* was placed sister to the *Solenotinus–Tomentosa* clade. Tests were performed as implemented in PAUP* 4.0b10.

***Phylogenetic analyses***—We inferred phylogenetic relationships using both the maximum parsimony (MP) and maximum likelihood (ML) optimality criteria as implemented in PAUP* 4.0b10. MP analyses used heuristic search methods, tree-bisection-reconnection (TBR) branch swapping, zero-length branches collapsed, and all characters equally weighted. Analyses were repeated 100 times with RANDOM ADDITION. Parsimony bootstrap analyses were performed with 1000 replicates and nearest neighbor interchange (NNI) branch swapping. For ML analyses, the best-fitting evolutionary model was found using a hierarchical series of likelihood ratio tests as implemented in PORN* (Bell, 2001). Subsequent heuristic searches used the best-fit model (model parameters were simultaneously estimated using ML), TBR branch swapping, and collapsed zero-length branches. Analyses were repeated 100 times with RANDOM ADDITION. ML bootstrap analyses used 1000 replicates with NNI branch swapping; parameters values were estimated from the optimal ML tree. Our trees and data matrices are available in TreeBASE (study accession number S1153, matrix accession numbers M1980–1982).

***Biogeographic reconstructions***—We investigated biogeographic disjunctions between the Old World and the New World using dispersal-vicariance analysis (Ronquist, 1997) as implemented in the program DIVA version 1.1 (Ronquist, 1996). This method reconstructs historical biogeography by minimizing the number of vicariance, dispersal, and extinction events required to explain specific distribution patterns. For this analysis, we used the maximum likelihood topology from our analysis of the full combined data set, with one modification. Molecular analyses do not strongly support the placement of the eastern North American species *V. nudum*; based on morphological evidence (Donoghue, 1983a; Jones, 1983), we resolved this taxon as sister to the remainder of section *Lentago*. We used the default cost matrix, which assigns a cost of 1 to dispersal and extinction events, and 0 to vicariance.

## RESULTS

***Aligned psbA-trnH IGS DNA sequences***—We were unable to directly sequence the *psbA-trnH* IGS for three *Viburnum* species, specifically, *V. clemensiae*, *V. furcatum*, and *V. sargentii*. For these, we cloned PCR fragments, obtaining DNA sequences from four or five clones. Preliminary analyses indicated that clones from a given accession formed a clade in the *Viburnum* phylogeny. Therefore, we selected a single representative of each species for inclusion in the final matrix.

Our *psbA-trnH* IGS DNA sequences for 41 *Viburnum* accessions and *Sambucus canadensis* ranged in length from 410 to 444 nucleotides, resulting in an aligned data matrix of 525 nucleotides positions. Although this alignment contained several gaps, the high degree of nucleotide similarity resulted in a matrix with little alignment ambiguity. There were only two regions where alignment proved problematic. In one case, this was due to several overlapping gaps (i.e., alignment positions 351–377), in the other it resulted from the variable length of a string of adenosine residues (i.e., alignment positions 455–461). Following exclusion of all gapped and ambiguous sites, the final data set contained 330 nucleotide positions; this included 62 varied positions, of which 24 were parsimony informative.

***Combined analyses of chloroplast sequences***—The chloroplast data matrix contained a total of 1461 nucleotides positions; 1131 from the *trnK* intron and 330 from the *psbA-trnH* IGS. This included 178 varied positions, of which 68 were parsimony informative. The ILD test indicated no significant conflict between these two partitions ($P = 0.681$). A MP analysis of the combined chloroplast data set recovered 10 optimal trees, each 222 steps long with a consistency index (CI) of 0.892 (without autapomorphies, CI = 0.774) and a retention index (RI) of 0.930. These trees differed only in (1) the monophyly and inferred relationships of section *Tinus*, and (2) the placement of *V. odoratissimum* relative to the remaining core species of section *Solenotinus*. The ML search used a GTR + G substitution model and resulted in a single topology ($-\ln L = 3468.22102$; Fig. 1) that was highly similar to those recovered using parsimony. Bootstrapping indicated moderate (i.e., greater than 65%) to strong (i.e., greater than 80%) support for many of the major clades.

As described by Donoghue et al. (2004), the alignment of *trnK* intron sequences contained a single gap that was parsimony informative with respect to ingroup relationships—this deletion, which marks the *Pseudotinus* clade, is also consistent

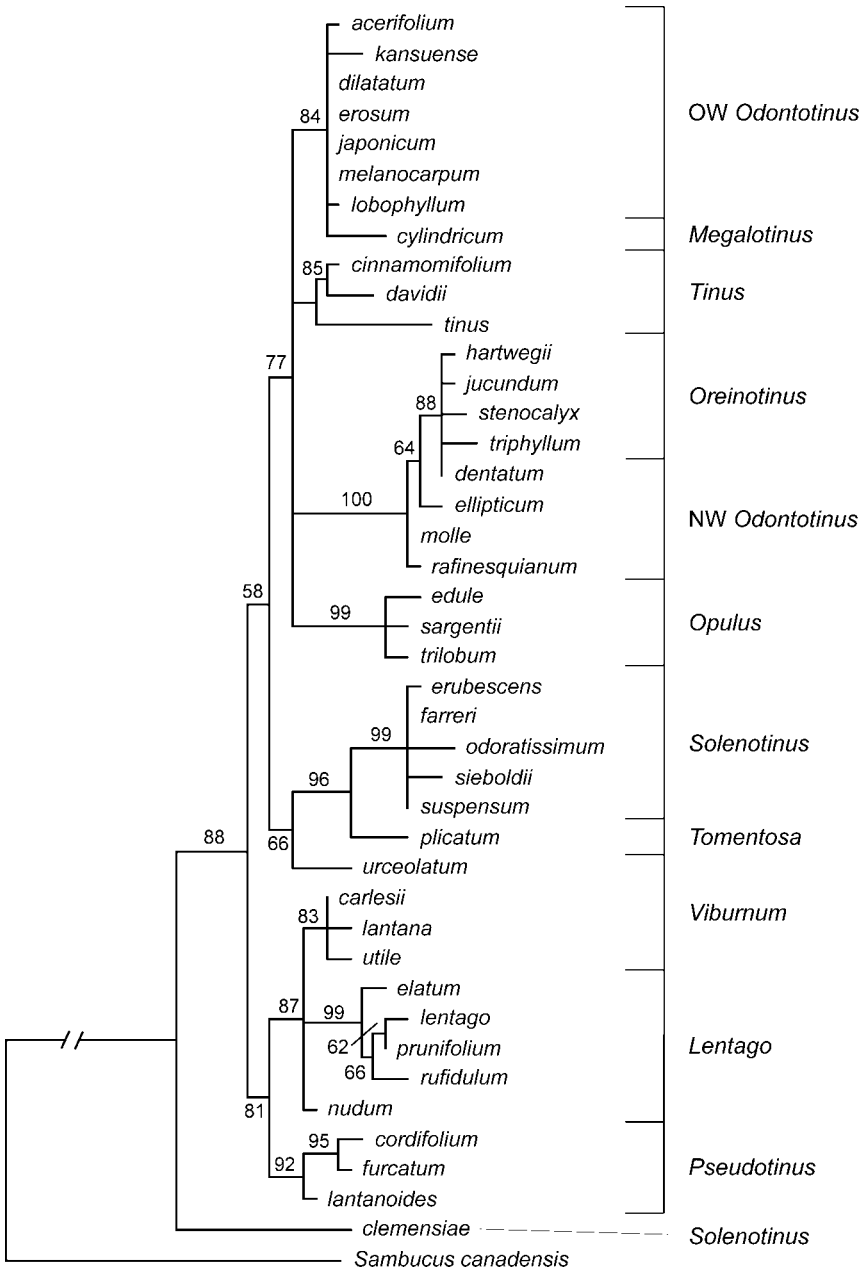


Fig. 1. Single optimal maximum likelihood tree obtained from the analysis of combined *trnK* intron and *psbA-trnH* IGS sequences for *Viburnum*, showing bootstrap values over 50% (1000 replicates). Names of traditionally recognized sections are noted at the right.

with our combined chloroplast analyses based on nucleotide substitutions. As noted earlier, the alignment of *psbA-trnH* IGS sequences contained several gaps. Unique and unambiguous indels support the core species group of section *Solenotinus* (two insertions and a deletion) and the *Oreinotinus–dentatum* clade (one insertion).

***Combined analyses of nuclear sequences***—The nuclear matrix contained 2148 nucleotide positions; 556 from nrITS, 807 from *WAXY1*, and 785 from *WAXY2*. In this matrix, 141 positions were varied, of which 69 were parsimony informative. ILD tests of the nuclear data matrix included only those taxa that were represented in all three data sets. In all pairwise comparisons and in a test that included all three markers, no significant conflict was detected—*P* values ranged from 0.763 for the nrITS/*WAXY2* comparison to 0.067 for the three-marker comparison. All taxa were included for phylogenetic analysis, with absences coded as missing data; missing cells accounted for approximately 6% of the data matrix. The MP search recovered 432 equally parsimonious trees, each 876 steps long with CI = 0.731 (without autapomorphies, CI = 0.568) and RI = 0.812. The optimal MP trees differed in (1) the placement of *Sambucus canadensis* (and hence the root) and *V. nudum*, and (2) species relationships within the core clade of section *Solenotinus*, within the Old World *Odontotinus* clade, and within both the *Oreinotinus–dentatum* and *rafinesquianum* species complexes. For the ML search a GTR + I + G substitution model was used, resulting in a single op-

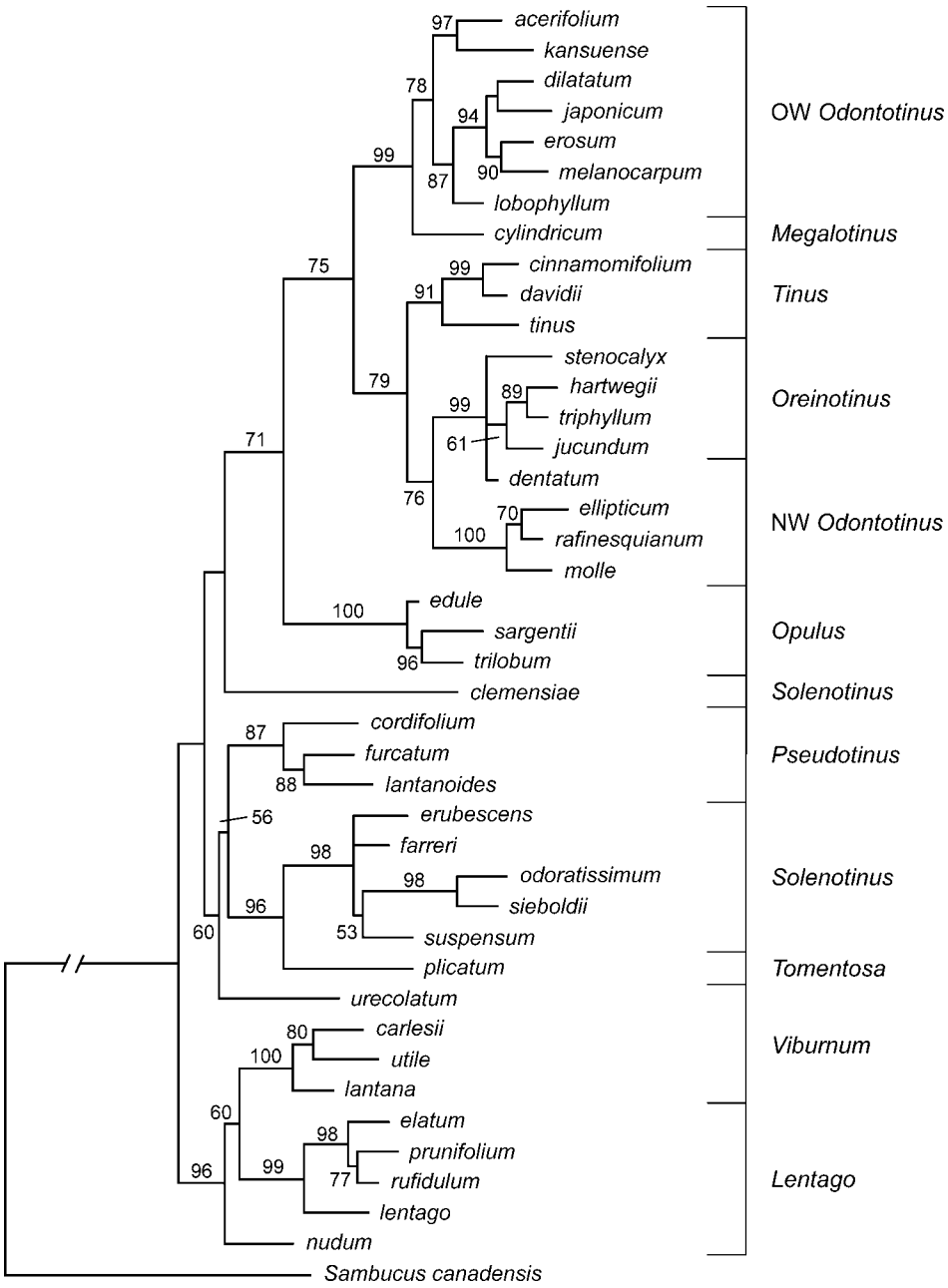


Fig. 2. Single optimal maximum likelihood tree obtained from the analysis of combined nrITS, *WAXY1,* and *WAXY2* sequences for *Viburnum,* showing bootstrap values over 50% (1000 replicates). Names of traditionally recognized sections are noted at the right.

timal topology (−lnL = 8446.05975; Fig. 2). This topology was very similar to those found using parsimony, and many of the inferred relationships were well supported by bootstrap analyses.

Winkworth and Donoghue (2004) identified several gaps in the *GBSSI* alignments that were consistent with clades in their gene trees based on nucleotide substitutions. In all but three cases, the indels reported by these authors were also consistent with clades found in our combined analyses of *GBSSI* and nrITS sequences. Two insertions in *WAXY1*—one uniting *V. dilatatum, V. erosum,* and *V. melanocarpum,* and one shared by *V. edule* and *V. trilobum*—are incompatible with our nuclear trees; similarly an insertion in *WAXY2* that unites *V. cordifolium* and *V. furcatum* is inconsistent with our nuclear analyses.

***Tests for incongruence between the chloroplast and nuclear data sets***—Visual inspection of gene trees from our separate analyses of chloroplast and nuclear data suggest several areas of incongruence between these data sets (Figs. 1 and 2). Specifically, the chloroplast and nuclear trees differ with respect to (1) the position of *Pseudotinus,* (2) species relationships within *Pseudotinus,* and (3) species relationships within *Lentago*. We explored these possible conflicts using the ILD and SLP tests.

TABLE 2. Incongruence length difference test scores from comparisons of chloroplast and nuclear data partitions in a series of *Viburnum* data matrices.

| Data matrix | *N* | *P* |
|---|---|---|
| Full taxa set | 1 | 0.015 |
| Single exemplars of both *Lentago* and *Pseudotinus* | 15 | 0.078–0.271 |
| Single exemplar of *Lentago*/all of *Pseudotinus* | 5 | 0.019–0.044 |
| All of *Lentago*/single exemplar of *Pseudotinus* | 3 | 0.040–0.053 |
| Single exemplar of *Lentago* and all *Pseudotinus* removed | 5 | 0.882–0.968 |
| All *Lentago* removed and single exemplar *Pseudotinus* | 3 | 0.239–0.335 |
| All *Lentago* included and all *Pseudotinus* removed | 1 | 0.556 |
| All *Lentago* removed and all *Pseudotinus* included | 1 | 0.054 |
| Both *Lentago* and *Pseudotinus* removed | 1 | 0.959 |

*Note:* $N$ = number of tests.

*ILD tests*—We tested for incongruence between the nuclear and chloroplast partitions in the full combined data set (i.e., all taxa for which all markers were represented were included) and a series of matrices that contained differing subsets of taxa. We used this approach as a heuristic to examine the effect of taxa or clades on conflict between the chloroplast and nuclear data sets. Results of these tests are summarized in Table 2.

An ILD test of the full combined data set is consistent with our observations of topological differences between chloroplast and nuclear gene trees. That is, the $P$ value ($P = 0.015$) for this test indicates some conflict between these two data partitions. Furthermore, our tests of reduced data matrices suggest that the three areas of topological conflict we have identified do not contribute equally to the overall level of incongruence. Specifically, differences between $P$ values for data sets that exclude *Pseudotinus* and matrices that contain one or more exemplars of this section are greater than between tests that include all representatives of a section and tests that contain single exemplars. This suggests that topological conflict within *Pseudotinus* and *Lentago* contributes less to the overall level of incongruence than do differences in the overall placement of *Pseudotinus*.

*SLP tests*—The SLP test provides a useful method for evaluating incongruence between data matrices because it can be applied at various topological levels (Seelanan et al., 1997). In our tests, we directly compared alternative arrangements for the three incongruent regions noted above. Results of these tests are summarized in Table 3.

Tests on the placement of *Pseudotinus* are not conclusive. Although the chloroplast data were a significantly worse fit to the nuclear rival tree ($P = 0.0253$), the reciprocal test was not statistically significant (i.e., the number of characters that required different numbers of steps on the test and rival tree was not $>5$). Similarly, tests that used the chloroplast data and nuclear rival tree to evaluate within section differences were not significant because of insufficient character differences between the test and rival trees. Within *Lentago*, the chloroplast topology resulted in a significantly worse fit of the nuclear data ($P = 0.0067$) than the test tree. The same test of relationships within *Pseudotinus* could not reject the null hypothesis ($P = 0.1797$).

TABLE 3. Significantly less parsimonious test scores from comparisons of chloroplast and nuclear data matrices for *Viburnum*.

| Test data and tree | Rival constraint | *N* | *P* |
|---|---|---|---|
| Placement of section *Pseudotinus* | | | |
| Chloroplast | Nuclear | 5 | 0.0253 |
| Nuclear | Chloroplast | 4 | 0.0455 |
| Species relationships within section *Pseudotinus* | | | |
| Chloroplast | Nuclear | 3 | 0.0833 |
| Nuclear | Chloroplast | 5 | 0.1797 |
| Species relationships within section *Lentago* | | | |
| Chloroplast | Nuclear | 2 | 0.1573 |
| Nuclear | Chloroplast | 11 | 0.0067 |

*Note:* $N$ = the number of characters requiring a different number of steps to be reconstructed on the test and rival tree.

***Combined analyses of chloroplast and nuclear DNA sequences***—Our separate analyses and statistical tests suggested highly localized incongruence between the chloroplast and nuclear data. In particular, our tests suggest that differences in the placement of *Pseudotinus* make a substantial contribution to the overall level of incongruence. To further explore the effect of this difference, we conducted a pair of combined analyses, one using the full set of taxa and the other with representatives of section *Pseudotinus* removed. In both cases, missing cells accounted for approximately 4% of the total cells in the data matrix.

*Full taxon set*—Parsimony tree searches resulted in 288 trees of 1109 steps, with CI = 0.756 (without autapomorphies, CI = 0.591) and RI = 0.830. Optimal trees in this search differed with respect to (1) the relationships among the three suprasectional lineages, *Pseudotinus*, and *V. clemensiae*, and (2) species relationships within several clades (i.e., *Pseudotinus*, core *Solenotinus*, Old World *Odontotinus*, *Oreinotinus*–*dentatum*, and the *rafinesquianum* species complex). A GTR + I + G substitution model was used in the ML search and resulted in a single optimal topology ($-\ln L = 12163.30184$; Fig. 3). This tree is generally consistent with those from separate analyses of nuclear and chloroplast data sets, combining well-supported clades from each. Bootstrap tests provided moderate to strong support for many relationships within *Viburnum*.

With respect to relationships that differed in separate chloroplast and nuclear analyses, the combined tree generally favored the nuclear resolution. In particular, the relationships inferred within sections *Pseudotinus* and *Lentago* are identical to those recovered in nuclear analyses. The combined tree also favored a direct relationship between *Pseudotinus* and the clade containing *Solenotinus*, *Tomentosa*, and *V. urceolatum*. In this case, however, the exact placement of *Pseudotinus* differs between the nuclear and combined analyses, although

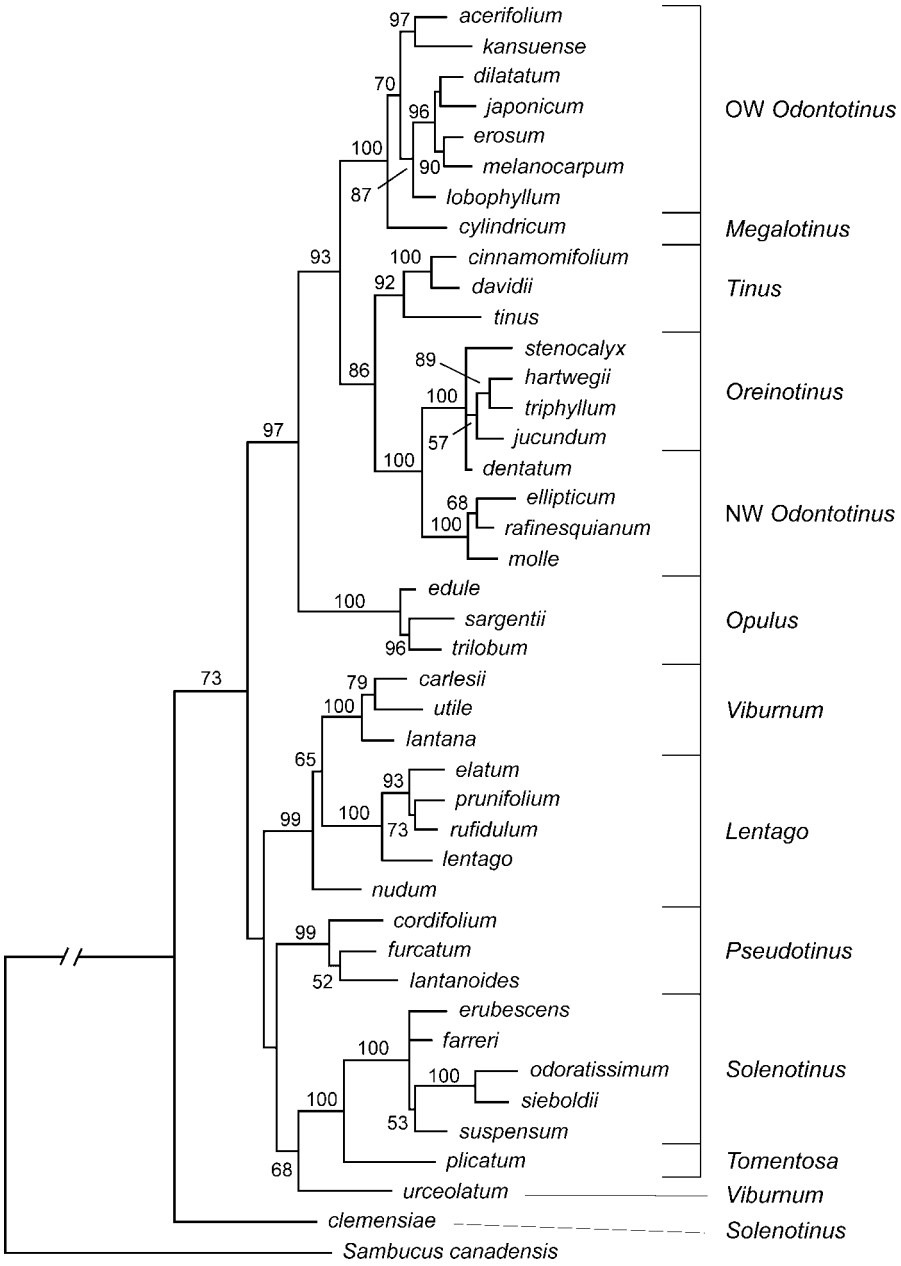


Fig. 3. Single optimal maximum likelihood tree obtained from the combined analysis of chloroplast and nuclear sequences for *Viburnum*, showing bootstrap values over 50% (1000 replicates). Names of traditionally recognized sections are noted at the right.

bootstrap support for these relationships is weak. In any case, the inclusion of *Pseudotinus* in the combined analysis had little effect on the overall structure and support for clades elsewhere in the tree.

*Minus Pseudotinus*—The MP search resulted in 144 trees of 1038 steps, with CI = 0.761 (without autapomorphies, CI = 0.591) and RI = 0.835. These trees differed slightly with respect to the relationships inferred within the core *Solenotinus* clade, the Old World *Odontotinus* clade, the *Oreinotinus–dentatum* clade, and the *rafinesquianum* species complex. ML analyses used a GTR + I + G substitution model and resulted in a single optimal topology ($-\ln L = 11662.62886$). This tree was very similar to those recovered using parsimony; the relationships inferred were well resolved and supported, except in those lineages where maximum parsimony trees differed. The ML tree was also largely consistent with those recovered in separate analyses of chloroplast and nuclear data sets, as well as our full combined data set. In *Lentago*, where separate analyses suggested differing resolutions, the relationships supported by the nuclear data were recovered in the combined analysis.

***Biogeographic reconstructions***—Dispersal-vicariance analysis (Fig. 5b) suggests an initial diversification of *Viburnum* in the Old World, with a number of transitions into the New World. In four cases, the movement is unequivocally reconstructed as from the Old World to the New World. Specifically,

*V. acerifolium*, *V. lantanoides*, *Lentago*, and the clade containing the New World *Odontotinus* and *Oreinotinus* all appear to be derived from Old World ancestors. Transitions between the Old and New Worlds are also inferred within *Opulus*, although in this case the directionality is uncertain. DIVA reconstructions suggest either (1) that the New World taxa *V. edule* and *V. trilobum* are independently derived from Old World ancestors, or (2) there was a transition to the New World at the base of the clade and the Old World species, *V. sargentii*, represents a transition back to the Old World.

## DISCUSSION

When multiple data sets are available, a common question is whether or not to combine the individual sources into a single analysis. The issue of data combination has received considerable attention in the literature and views on the most appropriate approach to this problem have varied from always to never combine (see de Queiroz et al., 1995; Johnson and Soltis, 1998). This lack of consensus reflects the knowledge that different data sets (i.e., different gene loci or data types) may differ in their underlying evolutionary histories and, therefore, provide alternative estimates of the organismal phylogeny. The issue is further confounded because it is clear that there are several possible causes of topological incongruence, at least some of which are artifactual. Consequently, it has become important to assess whether the differences between data sets are of potential biological significance.

In this study, we have used several data sets to investigate phylogenetic relationships within *Viburnum*. Generally, our gene trees are very similar to each other and also to those reported by Donoghue et al. (2004) and Winkworth and Donoghue (2004). However, our inclusion of additional chloroplast sequences, separate and combined analyses of the chloroplast and nuclear partitions, and statistical testing of apparent topological conflicts provide several new insights. In the following sections, we describe the key features of our trees and insights from the statistical tests. We compare our results to trees from earlier studies and discuss implications for our understanding of *Viburnum* taxonomy and biogeography.

***Gene trees***—In this section, we discuss the impact of our gene trees and statistical tests on our understanding of *Viburnum* phylogeny. Given the overall similarity of our combined chloroplast and nuclear analyses, we discuss results from analyses of the full taxa set, unless otherwise noted.

*The monophyly of traditional sections*—Our analyses provide strong support for the monophyly of sections *Opulus*, *Pseudotinus*, and *Tinus*. Bootstrap support for these clades is 87–100%, except in chloroplast analyses where *Tinus* receives less than 50% bootstrap support. We also consider our results to be consistent with the monophyly of section *Oreinotinus* (c.f. Winkworth and Donoghue, 2004), although in this case the result is not well supported.

As in previous molecular studies, four of the traditional sections were found not to be monophyletic, specifically, *Lentago*, *Odontotinus*, *Solenotinus*, and *Viburnum*. In the case of *Odontotinus*, our analyses again suggest two major lineages, a predominantly red-fruited Asian clade and a purple-fruited North American clade that also includes section *Oreinotinus*. Generally, these two lineages are well supported (78–100% bootstrap support); the exception is in the chloroplast analyses where the Old World *Odontotinus* species form a polytomy with *V. cylindricum* (*Megalotinus*). For the three remaining nonmonophyletic sections, our gene trees also support the previous analyses, placing a single species outside of a monophyletic core group. For section *Lentago*, the core clade is strongly supported (99–100% bootstrap support), but the placement of *V. nudum* remained uncertain. In chloroplast analyses, this species formed a polytomy with the core clades of sections *Lentago* and *Viburnum*; in nuclear and combined trees, it was sister to a clade containing these two lineages (60 and 65% bootstrap support, respectively). In the case of section *Solenotinus*, five of the six representatives form a strongly supported clade (98–100% bootstrap support). In all of our analyses, *V. clemensiae* was well separated from *Solenotinus* and all other species groups; consistent with previous studies, our chloroplast and combined analyses suggest that this rare Bornean species is sister to the remainder of *Viburnum* (88 and 73% bootstrap support, respectively). For section *Viburnum*, the Taiwanese–Japanese species, *V. urceolatum*, is well separated from the core clade that is supported by bootstrap values of 83–100%. In all of our optimal ML trees, *V. urceolatum* is consistently placed sister to a clade containing the core *Solenotinus* plus *V. plicatum* of section *Tomentosa*, albeit with limited support (see next section).

Only single representatives of sections *Megalotinus* and *Tomentosa* were included in our analyses. Additional sampling will be required to assess the monophyly of these groups.

*Suprasectional lineages*—Donoghue et al. (2004) and Winkworth and Donoghue (2004) identified three major subclades within *Viburnum*. These groups were also recovered in our separate and combined analyses, generally with improved bootstrap support. Further, within these lineages, relationships among section-level clades were well resolved and supported.

The largest suprasectional clade contains the traditional sections *Megalotinus*, *Odontotinus*, *Opulus*, *Oreinotinus*, and *Tinus* (71–97% bootstrap support). This clade is further divided into four well-supported subclades (92–100% bootstrap support in the combined analysis), corresponding to (1) section *Opulus*, (2) section *Tinus*, (3) the New World *Odontotinus* and section *Oreinotinus*, and (4) an Old World *Odontotinus–acerifolium* lineage plus *V. cylindricum* of *Megalotinus*. Relationships among these lineages were unresolved in chloroplast analyses, but based mainly on resolution provided by *GBSSI*, our nuclear and combined analyses provide moderate to strong support for relationships among these clades. Section *Opulus* appears to be sister to the other groups (75 and 93% bootstrap support in nuclear and combined analyses, respectively). In turn, the Old World *Odontotinus–acerifolium–cylindricum* clade is sister to one containing section *Tinus* and the New World *Odontotinus–Oreinotinus* lineage. In the first clade, our analyses placed *V. cylindricum* at the base (78 and 70% bootstrap support, respectively), with *V. acerifolium* and *V. kansuense* united in a clade (97% bootstrap support in both analyses) that is sister to the remainder of the Old World *Odontotinus* (87% bootstrap support in both analyses). Within the second group, *Tinus* is sister to an exclusively New World lineage (76 and 100% bootstrap support, respectively). This New World group is divided into two clades, one containing *V. ellipticum*, *V. molle*, and *V. rafinesquianum* (100% bootstrap support in both analyses) and the other containing *V. dentatum* of Eastern North America plus the Latin American

section *Oreinotinus* (99 and 100% bootstrap support, respectively).

In our trees, the two remaining suprasectional lineages each contain a pair of section-level lineages. In one, the core clade of section *Viburnum* (i.e., excluding *V. urceolatum*) is directly linked to section *Lentago* (87–99% bootstrap support). This grouping includes *V. nudum*, although its exact position is uncertain. The third suprasectional lineage consists of core *Solenotinus* (i.e., with the exception of *V. clemensiae*) and *V. plicatum* of section *Tomentosa* (96–100% bootstrap support). This group also appears to include the morphologically distinctive *V. urceolatum*. As in previous molecular analyses, our gene trees consistently place this species sister to the *Solenotinus–Tomentosa* clade (60–68% bootstrap support). This relationship is most strongly supported in our combined analyses that excluded section *Pseudotinus*. In this case, the link between *V. urceolatum* and the *Solenotinus–Tomentosa* clade was supported by a bootstrap value of 81%.

Although there is now considerable support for the presence of three main groups within *Viburnum*, the placement of section *Pseudotinus* relative to the two smaller suprasectional clades remains unclear. In the two previous molecular phylogenetic studies, these relationships were unresolved, and it was assumed that resolution of relationships simply required more data. However, our separate analyses of nuclear and chloroplast data matrices suggest conflicting placements of *Pseudotinus*. Specifically, chloroplast analyses favor a link with the *Lentago–Viburnum* clade (81% bootstrap support), whereas the nuclear data suggest a closer relationship with the *Solenotinus–Tomentosa–urceolatum* clade (60% bootstrap support). Our statistical tests are also consistent with conflict concerning the placement of *Pseudotinus*. In particular, ILD tests indicate that differences in the placement of this lineage make a substantial contribution to the overall level of incongruence between data sets. To confidently place this lineage within the phylogeny will require more data. However, our analyses highlight the possibility that hybridization and/or lineage sorting events may have played a role in the early evolution of *Pseudotinus*.

*Relationships among suprasectional lineages and the root position*—Previous studies have not clearly resolved relationships at the base of *Viburnum*. Unfortunately, even with our larger combined data matrices support for basal relationships remains weak. Both chloroplast and combined analyses suggest that the root falls along the branch separating *V. clemensiae* from a clade containing the remainder of *Viburnum* (Figs. 1 and 3). In contrast, analyses of the nuclear data set place the root along the edge separating the *Lentago–Viburnum* clade from everything else. Beyond this, there is uncertainty about the relationships among the major clades. Specifically, it is unclear whether *Viburnum* is divided into two clades as in our combined analysis (Fig. 3) or whether relationships at the base of the tree are a grade relative to a large clade containing the traditional *Megalotinus*, *Odontotinus*, *Opulus*, *Oreinotinus*, and *Tinus* as in our separate analyses (e.g., Figs. 1 and 2).

The great genetic distance to extant relatives and the relatively short branches close to the base of *Viburnum* suggests that understanding basal relationships will be an ongoing challenge. To this end, it may be useful to expand sampling of the *Sambucus–Adoxa* clade, especially if *GBSSI* can be routinely recovered from this group. However, previous results (e.g., Bell et al., 2001; Donoghue et al., 2001) suggest this may not help because including *Adoxa* and its relatives would simply add another exceptionally long branch to the problem.

*Relationships within sections*—Relationships within the section-level clades were generally unresolved in our chloroplast analyses. In contrast, the nuclear and combined data sets provided much greater resolution and support for these relationships. In general, inferred relationships are highly similar to those reported by Winkworth and Donoghue (2004) based on *GBSSI* sequences alone. Here we focus on the two cases where the chloroplast and nuclear data sets suggest different phylogenetic histories—the core of *Lentago* and *Pseudotinus*.

Our separate analyses of nuclear and chloroplast data sets suggest relationships within core *Lentago* that are consistent with those recovered by Donoghue et al. (2004). In chloroplast analyses, *V. lentago*, *V. prunifolium*, and *V. rufidulum* of eastern North America are united to the exclusion of the Mexican species, *V. elatum* (66% bootstrap value); within the eastern North American clade, *V. lentago* is more closely related *V. prunifolium* (62% bootstrap value). In contrast, analyses of nuclear data place *V. lentago* sister (98% bootstrap support) to a clade containing *V. elatum*, *V. prunifolium*, and *V. rufidulum*, within which *V. prunifolium* and *V. rufidulum* are united with 77% bootstrap support. Although not conclusive, these results are consistent with the hypothesis that *V. prunifolium* originated following hybridization between *V. rufidulum* and *V. lentago* (Brumbaugh and Guard, 1956; Rader, 1976; Donoghue et al., 2004). When assessed with the ILD test, these differences do not, in themselves, result in a significant level of incongruence. However, a SLP test showed that the nuclear tree is a significantly better fit to these data than the rival chloroplast topology. In the reciprocal test, there were insufficient sites for a statistically significant result; most likely reflecting the lack of resolution for species level relationships in the chloroplast data set.

Previous molecular analyses left relationships within *Pseudotinus* uncertain. Only *WAXY1* sequences had provided clear resolution, uniting the Japanese *V. furcatum* and North American *V. lantanoides* (99% bootstrap support) to the exclusion of *V. cordifolium* from the Himalayas (Winkworth and Donoghue, 2004). This relationship was also recovered in analyses of our nuclear matrix (88% bootstrap support). In contrast, analyses of the chloroplast data strongly suggest a direct link between *V. cordifolium* and *V. furcatum* (95% bootstrap support). We also note that this relationship is supported by a unique insertion in *WAXY2* (Winkworth and Donoghue, 2004). Use of the ILD test to evaluate this conflict is confounded by differences in the placement of section *Pseudotinus* inferred from chloroplast and nuclear partitions. However, a SLP test using the nuclear matrix indicates that the chloroplast rival tree is not a significantly worse partitioning of these data; there were insufficient sites that differed in length between the topologies for the reciprocal test to have statistical significance.

Clearly final resolution of relationships within both *Lentago* and *Pseudotinus* requires additional sampling and markers.

***Informal clade names***—Molecular phylogenetic analyses (e.g., Donoghue et al., 2004; Winkworth and Donoghue, 2004; this study) have greatly improved our understanding of *Viburnum* phylogeny. Given the general congruence between these studies and the strong support for a number of major clades, it seems appropriate to begin to reclassify *Viburnum*. Although we recognize the importance of providing formal

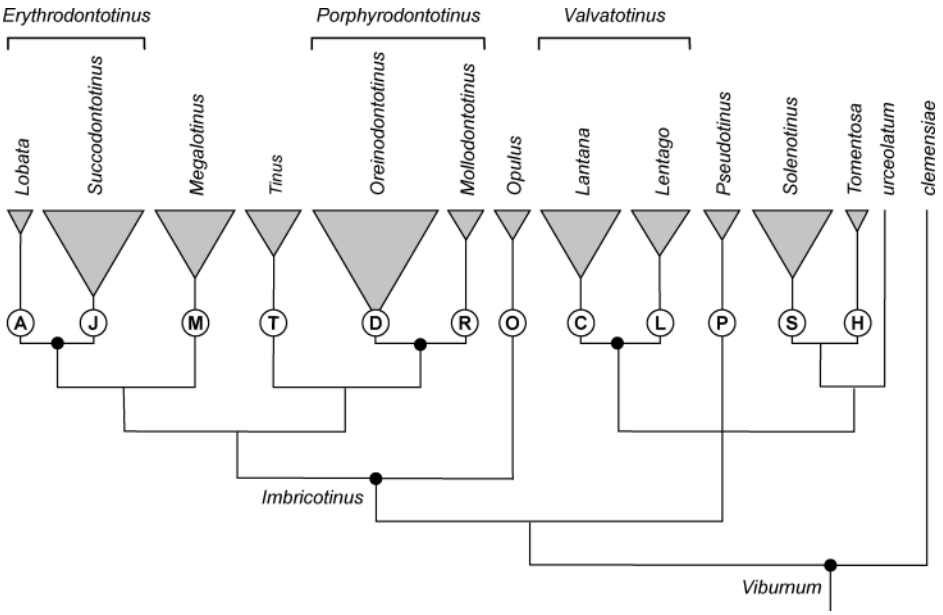


Fig. 4. Schematic tree diagram with informal phylogenetic nomenclature for some key *Viburnum* lineages. Triangles represent species groups and are approximately scaled to species number. Species groups are coded: A, *Lobata*; C, *Lantana*; D, *Oreinodontotinus*; H, *Tomentosa*; L, *Lentago*; J, *Succodontotinus*; M, *Megalotinus*; O, *Opulus*; P, *Pseudotinus*; R, *Mollodontotinus*; S, *Solenotinus*; T, *Tinus*.

phylogenetic definitions for taxonomic names, at present we merely wish to provide a system of informal names to facilitate discussion and future work. We leave the formal naming of clades until we have achieved a better understanding of species-level relationships.

Molecular and morphological studies have provided clear evidence for the monophyly of some, and the nonmonophyly of other, traditionally recognized sections. It would be useful to have names that can be consistently applied to the newly identified lineages. Specifically, we will provide names for the 12 major species groups and several of the more inclusive clades (Fig. 4). In choosing names, we have attempted to minimize potential confusion and to maintain continuity with the taxonomic literature (e.g., Hara, 1983). To this end, we have retained previous section names when these correspond to clades that contain all, or almost all, of the traditionally included species. Specifically, we have maintained *Lentago*, *Opulus*, *Pseudotinus*, *Solenotinus*, and *Tinus*. *Megalotinus* and *Tomentosa* are also retained; these groups have been represented in our studies by single exemplars, but it seems reasonable to use these names until the sampling is expanded. The exception to our strategy of retaining sectional names is the name *Viburnum* itself. Although this group, with the exception of *V. urceolatum*, does correspond to a well-supported clade, the name *Viburnum* is most often applied to the genus as a whole. To minimize confusion, we will refer to this group as the *Lantana* lineage, after the widely cultivated European species, *V. lantana*.

Each of the four remaining species groups (Fig. 4) contains taxa traditionally assigned to section *Odontotinus*, which molecular and morphological analyses have repeatedly shown to be nonmonophyletic. For the clade represented by *V. acerifolium* and *V. kansuense*, we propose the name *Lobata*, in reference to their tri-lobed leaves (also found in *Opulus*). This clade appears to correspond to taxon that Hara (1983) recognized as subsection *Lobata* within section *Odontotinus*. For the three other clades, we have used *Odontotinus* as the basis for our new names, in each case adding a prefix that reflects some feature of the included plants. The sister group of *Lobata* contains the remaining Asian members of the traditional section *Odontotinus*. For this clade, we coin the name *Succodontotinus*, a reference to the juicy fruits produced by most of these species. The two remaining species groups contain New World taxa that were placed by Hara (1983) in *Odontotinus* subsection *Dentata*. For the clade containing *V. ellipticum*, *V. molle*, and *V. rafinesquianum*, we suggest *Mollodontotinus*, which refers to the soft pubescence on the leaves in some members of this group. The final group consists of *V. dentatum* and all representatives of the Latin American section *Oreinotinus*. To highlight the inclusion of the large Latin American clade, we refer to this lineage as *Oreinodontotinus*.

Our phylogenetic analyses have also identified several well-supported higher-level clades. Two of these contain representatives of the traditional section *Odontotinus*. Most members of *Lobata* and *Succodontotinus* produce red fruits (*V. acerifolium* of *Lobata* and *V. melanocarpum* of *Succodontotinus* are exceptions), and we therefore suggest the name *Erythrodontotinus* for the clade that includes these two lineages. Similarly, in reference to their purple fruits, we propose *Porphyrodontotinus* for the clade comprised of *Oreinodontotinus* and *Mollodontotinus*. *Erythrodontotinus* and *Porphyrodontotinus* along with *Opulus* and *Tinus* form the largest of the suprasectional clades identified in our molecular analyses. We refer to this entire clade as *Imbricotinus* because two pairs of imbricate bud scales generally characterize these plants. This contrasts with *Valvatotinus*, the name we apply to the suprasectional clade that contains *Lentago* and *Lantana*. These plants produce a single pair of valvate bud scales in *Lentago* and naked buds with non-overlapping involute vernation in *Lantana*.

***Biogeographic implications***—The wide distribution of *Viburnum* makes it an excellent model for investigating Northern

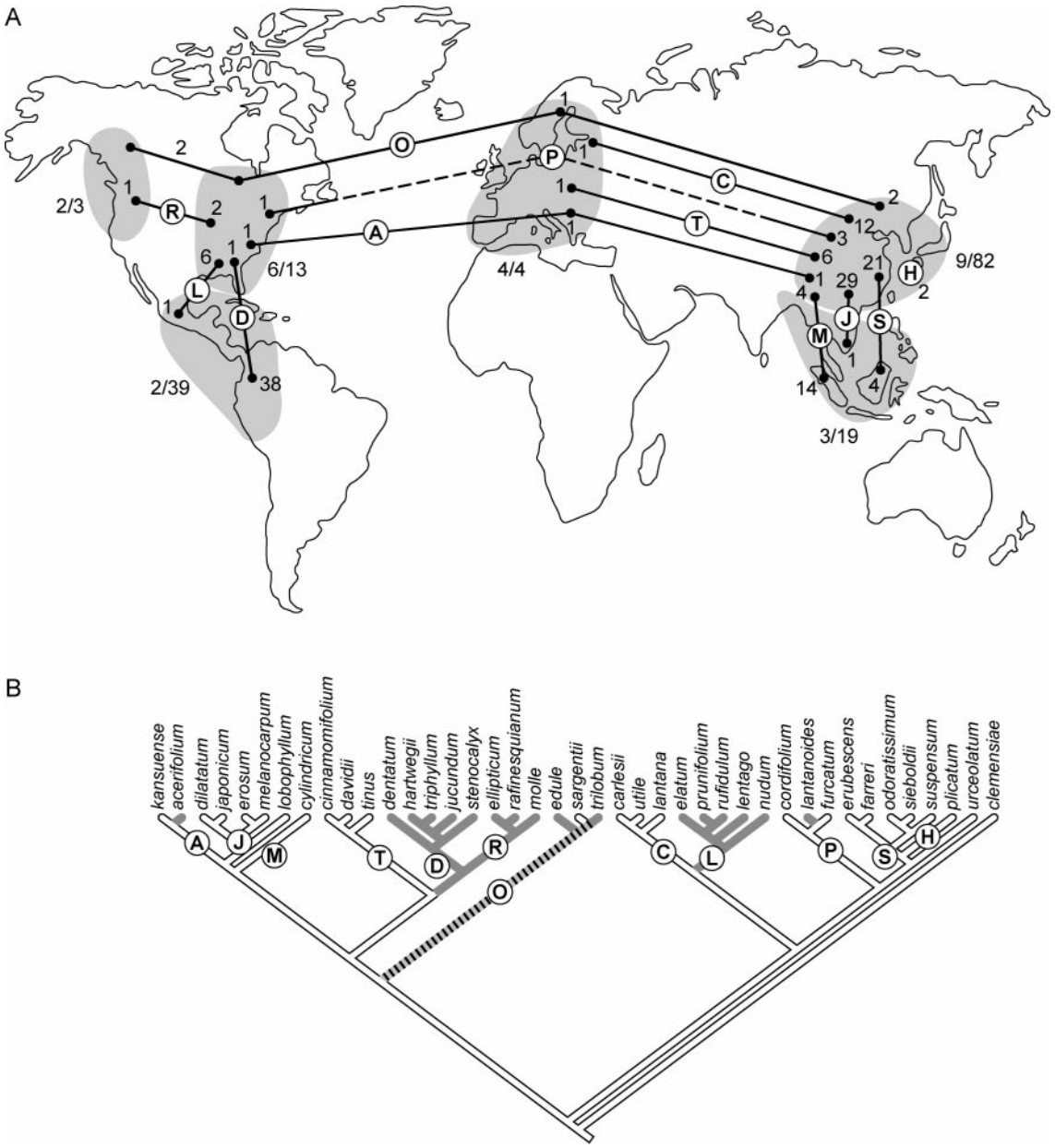


Fig. 5. Biogeographic patterns within *Viburnum*. (A) Map illustrating six broadly defined regions of *Viburnum* diversity and the distribution of our 12 species groups across these areas. For each clade, a line connects the regions in which it is present; the number of species present in each of the regions is also given. Species groups are coded: A, *Lobata*; C, *Lantana*; D, *Oreinodontotinus*; H, *Tomentosa*, L, *Lentago*; J, *Succodontotinus*; M, *Megalotinus*; O, *Opulus*; P, *Pseudotinus*; R, *Mollodontotinus*; S, *Solenotinus*; T, *Tinus*. The partially dotted line for *Pseudotinus* indicates that no species are present in Europe. The formula associated with each region indicates the number of clades. Lineages represented by *V. clemensiae* and *V. urceolatum* in our analyses fall outside the 12 major clades but are included in our estimates of regional species diversity. (B) Ancestral area reconstruction of Old World–New World disjunctions in *Viburnum* from DIVA (Ronquist, 1996). Unshaded = Old World; shaded = New World; striped = equivocal. Species groups denoted as for (A).

Hemisphere biogeography. In particular, it provides an opportunity to investigate classic patterns of disjunction between the Old and New Worlds (e.g., Wen, 1999, 2001; Xiang et al., 2000; Donoghue et al., 2001; Manos and Donoghue, 2001; Sanmartín et al., 2001; Xiang and Soltis, 2001; Donoghue and Moore, 2003; Donoghue and Smith, 2004). For the purposes of providing a biogeographic overview, we have estimated that there are 158 species of *Viburnum* worldwide. This number is based on a list of specific and infraspecific taxa kindly provided by V. Malécot (Institut National d'Horticulture, France; personal communication), a review of the taxonomic and floristic literature, and on M. Donoghue's (unpublished data) assessment of species limits in several clades. With the exception of *V. clemensiae* and *V. urceolatum*, the remaining 156 can be assigned to one of our species groups (Fig. 5a). For most clades, we expect the estimated species numbers to be relatively stable, although considerable taxonomic uncertainty remains in *Megalotinus* and *Oreinotinus*. In general, however, future species-level taxonomic changes are unlikely to alter our general conclusions.

For our preliminary biogeographic analysis, we recognized six broadly defined geographic regions—western North America, eastern North America, and Latin America in the New World, and temperate eastern Asia, southeastern Asia, and Europe in the Old World (Fig. 5a). Using this scheme, temperate eastern Asia, with ca. 82 species, is the most species rich, followed by Latin America (with ca. 39 spp.), southeastern Asia (ca. 19 spp.), eastern North America (ca. 13 spp.), Europe (ca. 4 spp.), and western North America (ca. 3 spp.). At the extremes of regional species richness, there is a clear relationship between the total of species and clade diversity. Specifically, temperate eastern Asia has the greatest diversity of both species and major clades (i.e., 82 species in nine clades), whereas western North America is the least diverse in both species and clades (i.e., three species in two clades). However, this relationship is not straightforward in the remaining regions. Although Latin America ranks second in total species richness, these species represent just two clades. Indeed, all but one of the 39 species in Latin America belong to *Oreinodontotinus*; the exception being the Mexican *V. elatum* of *Lentago*. In stark contrast, each of the four European species represents a different and distantly related clade: *Lantana*, *Lobata*, *Opulus*, and *Tinus*. Species from these two regions represent the extremes of dispersion across the phylogeny (Webb et al., 2002). That is, the distribution of the Latin American species is highly localized in the phylogeny (i.e., their closest relatives are found within the same region), whereas the four European species are hyper-dispersed (i.e., they are distantly related to one another, and each is more closely related to a species outside of the region). The two remaining regions fall between these two endpoints. Six clades occur in Eastern North America. Three of these are represented by a single species (*Lobata*, *Oreinodontotinus*, and *Pseudotinus*), two by a pair of species (*Mollodontotinus* and *Opulus*), and one by six species (*Lentago*). Similarly, the 19 species found in southeastern Asia belong to three clades; one represented by a single species (*Succodontotinus*), one by four species (*Solenotinus*), and the third by 14 species (*Megalotinus*).

Our phylogenetic analyses also highlight patterns of disjunction within *Viburnum*. With the exception of the *Tomentosa* clade, which contains two species confined to temperate eastern Asia, each of the other 11 species groups occupies more than one region (Fig. 5a). Most commonly, these lineages are present in neighboring regions. Three are shared between the two Asian regions (*Megalotinus*, *Solenotinus*, and *Succodontotinus*), two between eastern North America and Latin America (*Lentago* and *Oreinodontotinus*), and one between eastern and western North America (*Mollodontotinus*). The three remaining two-region clades are more widely disjunct. Most species of *Tinus* and *Lantana* occur in temperate eastern Asia, but each of these clades includes a single European species. *Pseudotinus* is disjunct between the Old and New Worlds, with three species in temperate eastern Asia and one in eastern North America. The two remaining species groups, *Lobata* and *Opulus*, are found in three and four regions, respectively. Single representatives of *Lobata* occur in temperate eastern Asia, Europe, and eastern North America (based on preliminary data, we include *V. orientale* of the Caucusus mountains in this clade; R. Winkworth and M. Donoghue, unpublished data). In the circumboreal *Opulus* clade, five species are shared between four areas; a single species in Europe, two in temperate eastern Asia, and two that occur in both eastern and western North America.

In addition to disjunctions within the 12 species groups, our phylogeny indicates two Old World-New World disjunctions between these lineages. Specifically, in *Valvatotinus* the Eurasian *Lantana* clade is sister to *Lentago* of eastern North America and Mexico. Likewise, the Eurasian *Tinus* is sister to the exclusively New World *Porphyrodontotinus* clade.

Our ancestral area reconstructions using DIVA (Fig. 5b), together with recent molecular age estimates for Dipsacales (Bell and Donoghue, 2005), provide a framework for making preliminary inferences about the historical biogeography of *Viburnum*. Despite uncertainty at the base of the tree, these analyses suggest that the *Viburnum* stem lineage most likely arose in Asia during the Late Cretaceous or Early Tertiary (ca. 60–80 mya). It also appears that the initial diversification of the crown clade occurred within Asia. Indeed, several species groups may never have extended beyond Asia (i.e., *Megalotinus*, *Solenotinus*, *Succodontotinus*, and *Tomentosa*). However, our analyses indicate transitions between the Old World and New World in several lineages. In four cases, DIVA analyses unequivocally reconstruct the movement as being from the Old World to the New World (Fig. 5b). For the *Tinus–Porphyrodontotinus* and the *Lantana–Lentago* splits, arrival in the New World appears to have occurred relatively early. Absolute dates are unclear, because we have yet to confidently date the crown clade, but relatively early splits are suggested by substantial morphological and molecular disparity within and between the subclades, as well as by interregional disjunctions within the subclades (i.e., between Asia and Europe in *Lantana* and *Tinus*, and between eastern North America and Latin America in *Lentago* and *Oreinodontotinus*). Uncertainty about the placement of *V. nudum* leaves open the possibility of an additional Old World-New World disjunction within *Valvatotinus*. In contrast to disjunctions between these major species groups, the disjunctions within *Lobata* and *Pseudotinus* appear to have arisen much more recently. This is suggested by limited morphological and molecular differentiation between the Old and New World species, but it is also consistent with the low species diversity in each of these clades. Specifically, in *Pseudotinus* the eastern North American species, *V. lantanoides*, is morphologically very similar to the three Asian species, in particular to *V. furcatum* of Japan and northeastern China. Similarly, the three species of *Lobata* are similar in appearance, especially the eastern North American *V. acerifolium* and the European *V. orientale*.

Our analyses imply at least one additional Old World–New World disjunction within the circumboreal *Opulus* clade. However, both the exact number and the directionality of events in this clade remain uncertain because *V. opulus* and *V. koreanum* are missing from our analyses. Gene trees are consistent with either two independent shifts from the Old World to the New World or, alternatively, an early transition to the New World followed by re-entry into the Old World. Despite this uncertainty, the morphological similarity and high latitude distribution of the five recognized species suggests that the circumboreal distribution of the *Opulus* lineage arose quite recently.

Phylogenetic analyses of *Viburnum* are consistent with similar studies on a broad range of plant taxa and with our current understanding of the historical biogeography of northern temperate forests. This study highlights the finding that patterns of disjunction are reiterated over time (Donoghue and Moore, 2003). Consistent with other molecular studies (e.g., Donoghue et al., 2001), multiple, temporally incongruent disjunctions appear to have arisen within *Viburnum* during the Ter-

tiary; often these involved movements from the Old World to the New World, presumably via Beringia (Donoghue and Moore, 2003; Donoghue and Smith, 2004). That these events appear to have occurred at different times suggests that the contemporary community has been assembled over an extended period and that as a result, interactions between lineages will have existed for differing amounts of time. In the case of *Viburnum*, for example, it would appear that *Lentago* and *Porphyrodontotinus* were present in the New World before the entry of the *Lobata*, *Opulus*, or *Pseudotinus* lineages. A more detailed account of this dynamic history must await more precise estimates for the absolute timing of events and more detailed phylogenetic work within the major clades.

***Future directions***—Molecular systematic studies have provided a clear and consistent picture of *Viburnum* phylogeny, 12 well-supported species groups have been identified and relationships among most of these lineages have been resolved with confidence. However, despite this progress, relationships at the base of the phylogeny remain uncertain. Specifically, there is little support for relationships among the three major lineages, *Pseudotinus*, *V. urceolatum*, and *V. clemensiae*, or for the exact position of the root. The large genetic distance separating *Viburnum* from its closest living relatives and the structure of the phylogeny itself (i.e., short branches close to the base of the tree) suggest that the resolution of this problem will require much more data. Although such uncertainty limits our understanding of some broad scale evolutionary patterns within *Viburnum*, the current results provide an excellent framework for further research on character evolution, rates of diversification, and historical biogeography.

Future studies will need to expand taxon sampling, especially for clades represented by one or a few species in the current analyses. The sample used in our recent analyses has yielded some quite unexpected results. Most importantly, the inclusion of *V. clemensiae* from Borneo revealed the nonmonophyly of section *Solenotinus* and suggested a novel position for the root of *Viburnum*. We expect, however, that adding more species will not greatly change the overall picture. In most cases, we anticipate that additional species will fall within the clades that we have identified, although species assigned to the morphologically diverse *Megalotinus* could provide some important surprises. Another important goal in the future will be to obtain more precise estimates of divergence times within *Viburnum* through the careful study and the inclusion fossils.

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