Dissolution of Viburnum Section Megalotinus (Adoxaceae) of Southeast Asia and Its Implications for Morphological Evolution and Biogeography
Author(s): Wendy L. Clement and Michael J. Donoghue
Reviewed work(s):
Source: *International Journal of Plant Sciences*, Vol. 172, No. 4 (May 2011), pp. 559-573
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/658927
Accessed: 20/09/2012 12:19

Int. J. Plant Sci. 172(4):559–573. 2011.

1058-5893/2011/17204-0008$15.00 DOI: 10.1086/658927

DISSOLUTION OF *VIBURNUM* SECTION *MEGALOTINUS* (ADOXACEAE) OF SOUTHEAST ASIA AND ITS IMPLICATIONS FOR MORPHOLOGICAL EVOLUTION AND BIOGEOGRAPHY

Wendy L. Clement[1,]* and Michael J. Donoghue*

*Department of Ecology and Evolutionary Biology, Yale University, P.O. Box 208105, New Haven, Connecticut 06520, U.S.A.

This study marks a significant increase in the number of species and genetic loci used in reconstructing the phylogeny of *Viburnum*. In particular, we expanded sampling of the morphologically heterogeneous section *Megalotinus* of Southeast Asia, which to date has been represented by only one species. Our results provide increased support for the monophyly of most of the previously named clades and for relationships within them. However, the four subsections of *Megalotinus* are placed with confidence in widely separate places in the phylogeny, and their disparate relationships are supported by morphological characters including branching patterns, inflorescence types, and trichomes. These findings, along with the phylogenetic placement of several additional Southeast Asian species, are critical in assessing the ancestral condition of *Viburnum* inflorescence architecture and endocarp shape. Our results also highlight a new biogeographic possibility, namely that *Viburnum* may have originated and initially diversified in montane subtropical forests in Southeast Asia and later moved into northern temperate forests, which most of them are associated with today. This study provides a clear-cut example of the importance of including in phylogenetic studies rare and difficult-to-obtain species from outside the main centers of diversity and of the value of dismantling nonmonophyletic taxonomic groups.

Keywords: *Viburnum*, *Megalotinus*, phylogeny, biogeography, morphological evolution, Southeast Asia.

Online enhancements: appendixes.

Introduction

Considerable attention has been paid to elucidating the phylogeny of *Viburnum* (Adoxaceae, Dipsacales, Campanulidae), a well-supported clade of ~160 woody species distributed mainly in temperate forests around the Northern Hemisphere (Donoghue 1983*a*). The first phylogenetic work was based on morphology (Donoghue 1983*b*), but recent analyses have been based on DNA sequences (Donoghue et al. 2004; Winkworth and Donoghue 2004, 2005). The most recent study (Winkworth and Donoghue 2005) provided robust results in many areas of the tree on the basis of which a new set of clade names was proposed (fig. 1). Their biogeographic analysis of *Viburnum* supported at least five movements from the Old World into the New World through Beringia. These studies also provided the basis for analyses of diversification shifts in *Viburnum* and other Adoxaceae (Moore and Donoghue 2007, 2009) and detailed studies of fruit and seed characters (Jacobs et al. 2008).

While these studies sampled the taxonomic diversity of *Viburnum* as reflected in prior classification systems (Oersted 1861; Rehder 1908; Kern 1951; Hara 1983), one group in particular, section *Megalotinus*, has remained poorly represented. To date, only one species (*V. cylindricum*) has been included from this mostly Southeast Asian group with some 19 described species (table 1). This poor sampling reflects the fact that most of the species of *Megalotinus* are apparently rare and, in any case, have been poorly collected (Kern 1951; LaFrankie 2010). This is clearly insufficient sampling, especially since previous studies have noted the possible importance of Southeast Asia in the early evolution of *Viburnum* (Winkworth and Donoghue 2005). Importantly, it appears that *V. clemensiae* (previously placed in section *Solenotinus*), which is endemic to northern Borneo, represents one of the earliest diverging lineages within crown *Viburnum* (Donoghue et al. 2004; Winkworth and Donoghue 2004, 2005). Furthermore, it has long been appreciated that *Megalotinus* is morphologically diverse; indeed, Kern (1951) formally divided it into four subsections; *Coriacea*, *Lutescentia*, *Punctata*, and *Sambucina* (table 1). In a cladistic analysis of morphological characters, *Megalotinus* subsections *Coriacea* (represented by *V. cylindricum*) and *Punctata* (represented by *V. punctatum*) were not united (Donoghue 1983*b*). Also, the recent study of *Viburnum* fruit and seed characters (Jacobs et al. 2008) highlighted the heterogeneity of *Megalotinus*. In effect, *Megalotinus* is the last great unknown in our understanding of the overall structure of *Viburnum* phylogeny.

Today, there are two major centers of *Viburnum* diversity; in temperate eastern Asia (~82 spp.) and in the mountains of Latin America (~39 spp.). Previous phylogenetic analyses have suggested that *Viburnum* originated in eastern Asia, where the greatest number of species and sections are represented. In contrast, the Latin American diversity is interpreted as a rapid recent radiation of a single *Viburnum* lineage, the *Oreinodontotinus* clade. Overall, *Viburnum* contains

[1] Author for correspondence; e-mail: wendy.clement@yale.edu.

Manuscript received July 2010; revised manuscript received December 2010.

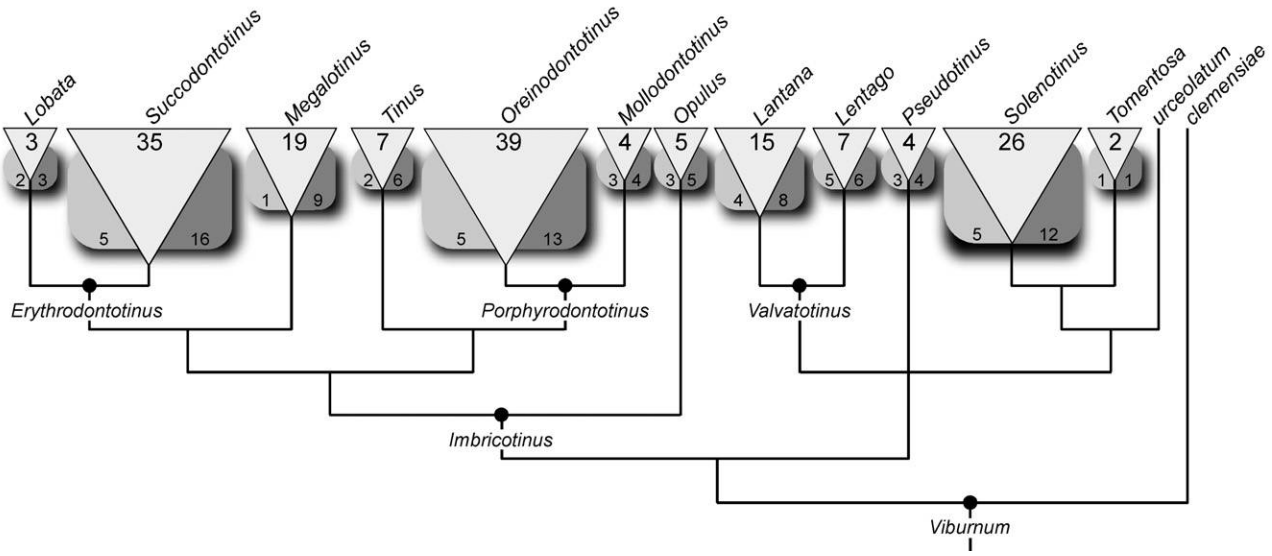

Fig. 1 Phylogenetic hypothesis for the named clades of *Viburnum* according to Winkworth and Donoghue (2005). The number of species assigned to each clade is shown in the corresponding triangle. To the left of each triangle (lighter gray) is the number of species in the clade sampled by Winkworth and Donoghue (2005); to the right (darker gray) is the number of species sampled in this study.

~20 species with their geographic ranges partly or entirely in Southeast Asia, with most of these (14 spp.) belonging to *Megalotinus*. As noted above, the Bornean species *V. clemensiae* is genetically very distinct from the rest of *Viburnum* (Donoghue et al. 2004; Winkworth and Donoghue 2004, 2005) and is clearly very distantly related to members of the *Solenotinus* clade, with which it traditionally has been united on the basis of its paniculate inflorescences. The remaining Southeast Asian species occur in three other clades (fig. 1): *Solenotinus* (3 spp.), *Tinus* (1 sp.), and *Succodontotinus* (1 sp.). Of the 20 Southeast Asian species, only three (*V. clemensiae*, *V. cylindricum*, and *V. odoratissimum*) have been included in previous molecular phylogenetic studies.

In general, the Southeast Asian species of *Viburnum* have been viewed as being southern offshoots of several otherwise temperate clades, with movements into more subtropical climates therefore being derived and accompanied by certain repeated character changes (e.g., shifts from dentate to entire leaf margins; Donoghue and Levin 1986). The alternative view, that *Viburnum* originated in Southeast Asia and spread from there to temperate eastern Asia, has not been proposed and, in any case, has been impossible to evaluate, given the poor representation of the Southeast Asian species in phylogenetic analyses.

Here we present the results of phylogenetic analyses of a greatly increased sample of *Viburnum* species, from 41 species in previous studies to 90 species (fig. 1). This includes additional representatives from all of the major known lineages and species complexes within *Viburnum* but with a special emphasis on the inclusion of *Megalotinus* species and others from Southeast Asia. Within *Megalotinus* we have added two representatives of all four of the previously recognized subsections. Previous studies recovered only weak to moderate support for relationships among major *Viburnum* clades (i.e., the relationships of *Imbricotinus* to the rest of *Viburnum*; Winkworth and Donoghue 2004, 2005). To try to improve the resolution, we also greatly expanded the number of gene regions included. We increased the taxon sampling for the four gene regions used previously: *trn*H-*psb*A (Winkworth and Donoghue 2005), *trn*K (Donoghue et al. 2004), *trn*S-*trn*G (Moore and Donoghue 2007), and the internal transcribed spacer (Donoghue et al. 2004). To these we added six additional chloroplast gene regions (coding regions *mat*K, *ndh*F, and *rbc*L, and chloroplast intron and intergenic spacer regions *pet*B-*pet*D, *rpl*32-*trn*L$^{(UAG)}$, and *trn*C-*ycf*6), yielding a matrix with 9552 bp, as compared to 2017 bp in previous work.

As we develop below, this greatly expanded sampling provides several insights of general importance. First, this study provides an excellent demonstration of the power of including rare species from outside of the main centers of diversity, from the standpoint of understanding the evolution of the characters that distinguish the major clades but also in understanding historical biogeography. More specifically, they provide us with insights into the movement of lineages among subtropical, temperate, and cold environments—transitions that may be critical in understanding global biodiversity patterns and the phylogenetic conservatism/lability of ecological tolerances (Donoghue 2008). Finally, this work highlights both the possibility of past extinction in Southeast Asia and the likelihood of losing rare species that appear to hold the key to our understanding of *Viburnum* evolution.

Material and Methods

Taxon Sampling

We sampled nine chloroplast (CP) gene regions and the nuclear ribosomal internal transcribed spacer region (hereafter termed "ITS") for 90 species of *Viburnum*. This included 40 species from Winkworth and Donoghue (2004; excluding *V.*

Table 1

Classification of *Viburnum* Section *Megalotinus* According to Kern (1951) and Hara (1983)

Subsection, species	Geographic range
Coriacea (Maxim.) Kern:	
V. beccarii Gamble	Malaysia
V. coriaceum Blume[a]	Himalaya, India, Myanmar, Thailand, Indochina, west and central China, Malaysia
V. cornutidens Merrill	Philippines
V. crassifolium Rehder	West China
V. cylindricum Buch-Ham ex D.Don[a]	China, Bhutan, India, Indonesia, Myanmar, Nepal, Pakistan, Thailand, Vietnam
V. glaberrimum Merrill	Philippines
V. hebanthum Wight et Arn.[a]	South India
V. platyphyllum Merrill	Philippines
Lutescentia Kern:[b]	
V. amplifolium Rehder	West China
V. colebrookeanum Wall.[a]	East Himalaya, Indochina, Hainan
V. lutescens Blume[a]	Malaysia, Indochina, south China
V. pyramidatum Rehder	West China
Punctata Kern:	
V. lepidotulum Merrill & Chun[a]	West China
V. punctatum Hamilton Ex D.Don[a]	Himalaya, Deccan, Thailand, Indochina, west China, Sumatra
Sambucina Kern:	
V. hispidulum Kern	Borneo
V. inopinatum Craib[a]	Myanmar, Thailand, Indochina, west China
V. sambucinum Blume	Malaysia, Indochina
V. ternatum Rehder[a]	West China
V. vernicosum Gibbs	Borneo

[a] Species sampled in this study.

[b] Hara (1983) correctly recognized that subsection *Lutescentia* should be referred to as subsection *Megalotinus*. However, for our purposes, so as to avoid confusion between section *Megalotinus* and subsection *Megalotinus*, we chose to use *Lutescentia*. Further, in the spirit of Winkworth and Donoghue (2005), we use *Lutescentia* as the informal clade name for this lineage.

davidii) and 50 species new to the study of *Viburnum* phylogeny. We note that the species labeled *V. cordifolium* in prior studies (Donoghue et al. 2004; Winkworth and Donoghue 2004, 2005; Moore and Donoghue 2007), is here referred to as *V. nervosum*, its correct species name (Hara 1983). Although we increased the sample for all described *Viburnum* clades (fig. 1), we sampled at least twice as many species from *Succodontotinus* (+11 spp.), *Megalotinus* (+8 spp.), *Tinus* (+4 spp.), *Oreinodontotinus* (+8 spp.), *Lantana* (+4 spp.), and *Solenotinus* (+7 spp.). The most important increase was from one species of *Megalotinus* (*V. cylindricum*) in previous studies to nine species, representing each of Kern's (1951) four subsections (table 1). Two Southeast Asian species, *V. amplificatum* and *V. inopinatum* were included in the analysis despite being successfully sequenced for only two and three genes regions, respectively (app. A). *Viburnum amplificatum* is the only species that has been assigned to section *Solenotinus* that has a flat-topped inflorescence rather than the paniculate inflorescence characteristic of that group. Samples of newly included *Viburnum* species were obtained from herbarium specimens in the Harvard University Herbaria (A, GH), the Missouri Botanical Garden (MO), and the New York Botanical Garden (NY) or from silica-dried leaf material accompanying our own voucher specimens in the Yale Herbarium (YU; app. A).

Plant Extractions and Sequencing

Genomic DNA was extracted from silica-dried leaf material or herbarium material using a Qiagen DNeasy plant kit (Valencia, CA) following the manufacturer's protocol. Total genomic DNA was used to amplify and sequence ITS; CP coding regions *mat*K, *ndh*F, and *rbc*L; and CP intron and intergenic spacer regions *pet*B-*pet*D, *rpl*32-*trn*L$^{(UAG)}$, *trn*C-*ycf*6, *trn*H-*psb*A, *trn*K, and *trn*S-*trn*G.

Amplification of *trn*H-*psb*A followed Winkworth and Donoghue (2005), and amplification of ITS and the 5′ portion of the *trn*K intron (hereafter *trn*K) followed Donoghue et al. (2004). Amplification of a 5′ portion of *mat*K followed protocols published by the CBOL Plant Working Group (CBOL et al. 2009). The same reaction conditions were used for the amplification of *rpl*32-*trn*L$^{(UAG)}$, *trn*C-*ycf*6, *trn*S-*trn*G, and *pet*B-*pet*D. Reactions were prepared in a total volume of 25 μL and included ×10 Qiagen PCR buffer, 25 mM $MgCl_2$, 62.5 mM dNTPs (NE Biolabs, Ipswich, MA), 25 μM forward and reverse primers (table B1 in the online edition of the *International Journal of Plant Sciences*), 1 U of Qiagen Taq DNA polymerase, and 10–50 ng of total genomic DNA. Unsuccessful amplifications were repeated with the same conditions but with the addition of 1% bovine serum albumin (NE Biolabs) compensated by a reduction in the volume of water. Thermocycler conditions were as follows: 94°C for 5

min, 30 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min, and a final extension of 72°C for 7 min.

Amplifications of *rbc*L and *ndh*F were prepared in 25-uL reactions including ×10 Qiagen PCR buffer, 25 mM $MgCl_2$, 37.5 mM dNTPs (NE Biolabs), 12.5 μM each of forward and reverse primers (table B1), 1.25 U of Qiagen Taq DNA polymerase, and 10–50 ng of total genomic DNA. Thermocycler conditions were as follows: 94°C for 2 min, 25 cycles of 94°C for 30 s, 48°C for 1 min, and 68°C for 1.5 min, followed by a final extension of 72°C for 7 min.

All amplification reactions were cleaned using either PEG purification or a Qiagen PCR cleanup kit following the manufacturer's protocols. Automated sequencing was performed using ABI Big Dye Technology in 1/8 reactions and analyzed using an Applied Biosystems 3730xl DNA genetic analyzer at either the DNA Analysis Facility on Science Hill or the W. M. Keck Facility, both of which are at Yale University.

Sequence Alignment

Each gene region was aligned using Muscle 3.6 (Edgar 2004) followed by visual inspection. Ambiguous portions of the alignment and apomorphic indels were removed before analysis. Unambiguously aligned indels were not coded as separate characters. All analyzed data sets (and trees published here) are available in TreeBASE (http://www.treebase.org; S10714) and from W. L. Clement.

Phylogenetic Analyses of Individual Gene Regions

Each gene region was analyzed separately using maximum parsimony (MP), maximum likelihood (ML), and Bayesian criteria. MP analyses were performed using PAUP*4b10 (Swofford 2002), with uninformative characters excluded, tree bisection-reconnection branch swapping, and 100,000 replications. If the heuristic search did not adequately search tree space (i.e., sampled a single island), we used the parsimony ratchet (Nixon 1999) as implemented by Pauprat (Sikes and Lewis 2001). Each Pauprat analysis consisted of 10 independent runs, each with 1000 replicates and 10% of the characters weighted. All resulting trees were pooled, and zero branch lengths were collapsed. This set of trees was filtered to keep only the trees of shortest length and summarized using 50% majority rule consensus. Clade support was evaluated using nonparametric bootstrap analysis (Felsenstein 1985) implemented in PAUP*4b10 (Swofford 2002). Each gene region was assessed with 10,000 bootstrap replicates, each with 1000 addition sequence replicates with the maximum number of trees saved per replicate set to 100.

ML analyses were performed using RAxML 7.0.4 (Stamatakis 2006) implementing a GTR + G model of sequence evolution. Each analysis ran 500 rapid bootstrap replicates immediately followed by a likelihood analysis that was replicated three times to ensure that likelihood scores were similar.

Before conducting Bayesian analyses, the best-fitting model for each gene region was selected by MrModeltest 2.3 (Nylander 2004) following the Akaike Information Criterion evaluation criteria. Each Bayesian analysis was run in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001), with six chains for 10–20 million generations, sampling the posterior distribution every 100 generations. Analyses were run until the split frequencies were lower than 0.01 and there was sufficient evidence of chain swapping. We further assessed convergence and determined an appropriate burn-in (no less than 10% of the total number of generations) by viewing plots of likelihood and model parameters in Tracer 1.5 (Rambaut and Drummond 2007).

Phylogenetic Analyses of Combined Data

We compiled two concatenated data matrices for analysis using Phyutility (Smith and Dunn 2008). The first matrix combined all the CP data, and the second combined the CP data with ITS data (hereafter CP + ITS). Criteria for MP analyses were the same as described for the individual gene region analyses. ML analyses were also conducted as described previously but with the addition of a partition file designating an individual partition for each gene region included in the analysis.

Three partitioning schemes were explored for Bayesian analyses of the CP and CP + ITS data sets. First, we analyzed the CP and CP + ITS data sets with a single model of sequence evolution, GTR + I + G. Second, we divided the CP data into two partitions: coding regions (*mat*K, *ndh*F, and *rbc*L) and noncoding regions (*pet*B-*pet*D, *rpl*32-*trn*$L^{(UAG)}$, *trn*C-*ycf*6, *trn*H-*psb*A, *trn*K, and *trn*S-*trn*G). We implemented a GTR + I + G model of sequence evolution for both data partitions. For the CP + ITS data set, we implemented a GTR + I + G model for each of three partitions; CP coding regions, CP noncoding regions, and ITS. Third, we conducted an analysis in which each gene region was assigned its own model of sequence evolution. As a result, the CP data set was divided into nine data partitions, and each partition was assigned the best model of sequence evolution identified for the individual gene region analyses (see above). The CP + ITS data set was analyzed in the same way but with an extra partition for ITS. All Bayesian analyses were run with 12 chains for 30 million generations, sampling the posterior every 1000 generations. Stationarity and burn-in were determined as described above. For analyses of data divided among more than one partition, the model parameters and overall rate of evolution were unlinked.

Rooting the Tree

Previous phylogenetic studies have used species of *Sambucus* to root the *Viburnum* tree (Donoghue et al. 2004; Winkworth and Donoghue 2005). However, the enormous genetic distance between *Sambucus* and *Viburnum* makes it difficult to reliably align many of the fast-evolving noncoding CP gene regions used in this study. We conducted a combined analysis of *Viburnum* and *Sambucus* as described for the CP + ITS data set. We sampled *Sambucus canadensis* and *S. racemosa* for ITS, *mat*K, *ndh*F, *rbc*L (*S. racemosa* only), *rpl*32-*trn*$L^{(UAG)}$, *trn*H-*psb*A (*S. canadensis* only), *trn*K, and *trn*S-*trn*G (*S. racemosa* only). This analysis (results not shown) strongly supported the results of previous studies in placing *V. clemensiae* as sister to the rest of *Viburnum* (Winkworth and Donoghue 2005). Therefore, in order to maximize the amount of unambiguously aligned data, in all other analyses described here we simply rooted the *Viburnum* tree along the *V. clemensiae* branch.

Results

Individual Gene Phylogenies

For the three CP coding regions sampled in this study, we sequenced *mat*K for 86 taxa (750 bp, 35 parsimony informative characters [PICs]), *ndh*F for 62 taxa (2022 bp, 84 PICs), and *rbc*L for 80 taxa (1332 bp, 48 PICs). For three of the noncoding CP regions, we sequenced the *pet*B-*pet*D region for 70 taxa (909 bp, 37 PICs, 5 indels), *rpl*32-*trn*L$^{(UAG)}$ for 85 taxa (875 bp, 66 PICs), and *trn*K for 48 taxa (85 taxa total, 1146 bp, 59 PICs, 3 indels). The indels in these regions were retained for analysis. Of the three remaining noncoding CP regions, we sequenced the *trn*H-*psb*A for 50 taxa (87 taxa total, 416 bp, 49 PICs, 7 indels), *trn*S-*trn*G for 44 taxa (68 taxa total, 63 bp, 48 PICs, 7 indels), and *trn*C-*ycf*6 for 79 taxa (820 bp, 35 PICs, 5 indels). Indels in these regions that could be unambiguously aligned were retained for analysis; those that could not were removed. In total, before analysis we excluded 56 bp from *trn*H-*psb*A, 55 bp from *trn*C-*ycf*6, and 27 bp from *trn*S-*trn*G. Finally, we sequenced ITS for 47 additional taxa (84 taxa total, 642 bp, 127 PICs), and no data were excluded before analysis. Phylogenies reconstructed using MP, ML, and Bayesian criteria for the individual gene trees were topologically congruent (table C1; fig. C1 in the online edition of the *International Journal of Plant Sciences*; individual CP gene trees are not shown).

Combined Analyses

We compared the topologies of the individual CP gene regions to identify conflicts supported by high MP and ML bootstrap proportions ($\geq$70) and significant posterior probabilities (PPs; $\geq$0.95). The topologies were largely congruent with one another, except for relationships within the *Pseudotinus* clade, as described by Winkworth and Donoghue (2004, 2005). For our purposes, we concatenated the CP data for a combined analysis on the grounds that the conflicts were limited to species within *Pseudotinus* and all CP gene regions supported the monophyly of this clade.

We analyzed a combined CP matrix of 90 species and 8910 bp. We found that the analysis with two partitions ($-\ln L = 21,232.21$) yielded a better likelihood score than the analysis with a single partition ($-\ln L = 21,738.10$). After 30 million generations, the analysis with nine partitions failed to converge. Therefore, we present the results from the analysis with two partitions, which divided the data among coding and noncoding regions (table 2; fig. C2). Also, the topologies resulting from the MP, ML, and bipartitioned Bayesian analyses were very similar to one another.

Conflicts confined to relationships within the *Pseudotinus* and *Lentago* clades were recovered when comparing the combined CP tree to the ITS tree. Conflicts within these clades have been discussed by Winkworth and Donoghue (2004, 2005) and do not appear to affect our ability to confidently reconstruct relationships among the major clades. Relationships among *Succodontotinus*, *Lobata*, and *Megalotinus* subsection *Coriacea* are not entirely clear and differ among the two data sets (figs. C1, C2). Specifically, these data call into question the monophyly of *Lobata* and *Succodontotinus* but not the monophyly of *Megalotinus* subsection *Coriacea*. Many *Succodontotinus* species remain to be sampled (fig. 1), and it is likely that both increased taxon sampling and additional data will be needed to fully address this apparent conflict. For the time being, we concatenated the CP and ITS data for further analysis.

The combined CP + ITS data matrix included 90 species and 9552 bp. We compared partitioning schemes for Bayesian analyses and found that a three-partition scheme (CP coding, CP noncoding, and ITS) resulted in the best likelihood scores ($-\ln L$ for 1 partition $= 26,409.95$; $-\ln L$ for 3 partitions $= 25,250.70$). The topologies from the MP, ML, and Bayesian analyses were very similar to one another, and we present the results from the Bayesian search in table 2 and figure 2.

The Bornean species, *V. clemensiae*, with paniculate inflorescences, is well supported as widely separated from *Solenotinus*, which contains all other species with panicles. Strong support was obtained for the monophyly of the informally named clades *Pseudotinus*, *Lantana*, *Solenotinus*, *Tinus*, *Mollodontotinus*, *Oreinodontotinus*, *Opulus*, and *Succodontotinus* as well as for the more inclusive clades *Porphyrodontotinus* and *Imbricotinus* (figs. 1, 2). *Erythrodontotinus* (including *Lobata* and *Succodontotinus*) and *Valvatotinus* (including *Lantana* and *Lentago*) are not monophyletic, due to the addition of *Megalotinus* sections *Coriacea* and *Sambucina* into *Erythrodontotinus* and the addition of *Megalotinus* section *Punctata* into *Valvatotinus* (figs. 1, 2).

With many newly added species and an expanded molecular data set, we have identified strongly supported clades within the major named clades (fig. 2). As noted above, two clades

Table 2

Results of Bayesian Analyses for Combined Analyses of the Concatenated Chloroplast (CP) and CP + Internal Transcribed Spacer (ITS) Data Sets

			Base pair frequencies				Rate matrix							
Gene region, partition	−lnL	TL	A	C	G	T	AC	AG	AT	CG	CT	GT	G	I
CP	21,232.21	17.4906												
Coding			.2792	.1787	.1985	.3436	.2291	.2370	.0579	.1355	.1933	.1473	.0510	.8523
Noncoding			.3317	.1631	.1661	.3391	.2581	.1648	.0868	.0704	.1699	.2500	1.0970	.7175
CP + ITS	25,250.70	17.4444												
Coding			.2786	.1792	.1987	.3435	.2279	.2408	.0582	.1392	.1919	.1420	.0507	.8529
Noncoding			.3313	.1630	.1658	.3399	.2578	.1642	.0875	.0704	.1694	.2506	1.0888	.7195
ITS			.1888	.3285	.2921	.1906	.0697	.2035	.0404	.0294	.6166	.0404	.7656	.4587

Note. −lnL = likelihood score; TL = tree length. Parameter estimates for base pair frequencies, rate matrix, gamma (G), and proportion of invariable sites (I) are provided for each analysis.

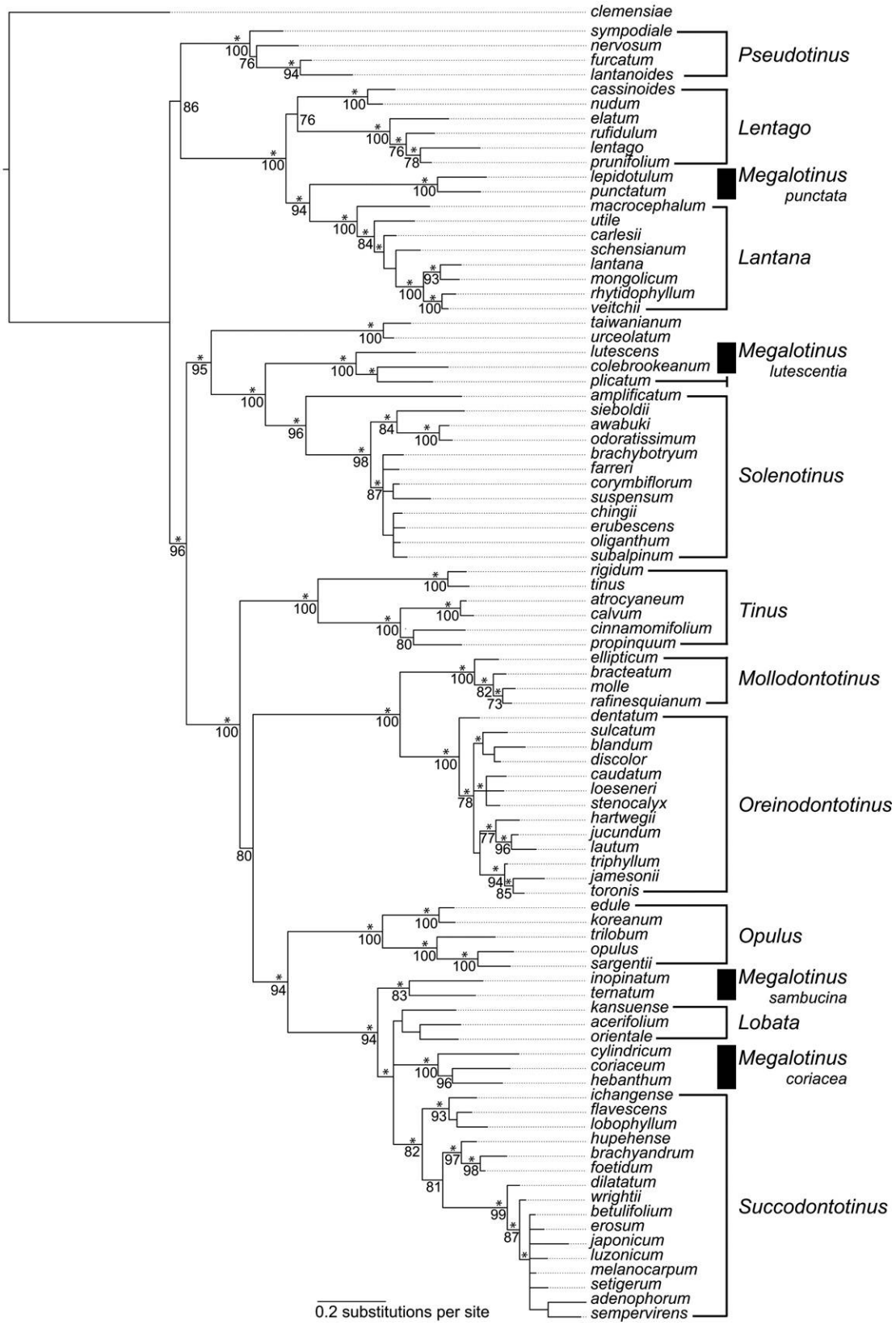

Fig. 2 Bayesian majority rule consensus tree resulting from the analysis of the concatenated chloroplast + internal transcribed spacer data matrix. Branches marked with an asterisk are supported by posterior probabilities greater than or equal to 0.95. Maximum likelihood bootstrap proportion values greater than 70 are indicated below the branches. Clade names proposed by Winkworth and Donoghue (2005) are shown to the right.

were recognized within *Lentago*, one containing *V. cassinoides* and *V. nudum*, with stalked inflorescences, and the other containing *V. elatum*, *V. lentago*, *V. prunifolium*, and *V. rufidulum*, with derived sessile inflorescences. Within *Lantana*, we found strong support for a clade containing *V. lantana*, *V. mongolicum*, *V. rhytidophyllum*, and *V. veitchii*. *Viburnum macrocephalum*, which produces enlarged sterile flowers around the margin of its inflorescences, appears as the sister to all other *Lantana* species, which lack such flowers. Within *Opulus*, sterile marginal flowers are lacking in the clade containing *V. edule* and *V. koreanum* but present in the *V. opulus*–*V. sargentii*–*V. trilobum* clade. The basal split within *Tinus* makes geographic sense; one clade contains *V. rigidum* and *V. tinus* from Europe, and the other contains *V. atrocyaneum*, *V. calvum*, *V. cinnamomifolium*, and *V. propinquum* from Asia. Likewise, within *Mollodontotinus*, the western North American *V. ellipticum* is sister to the other species, which extend from the eastern United States to northeastern Mexico.

In *Oreinodontotinus*, *V. dentatum* from the eastern United States was sister to a large clade of Latin American species. Within this clade several subgroups correspond well with geography, including *V. caudatum*, *V. loeseneri*, and *V. stenocalyx* from eastern and central Mexico, and *V. jamesonii*, *V. toronis*, and *V. triphyllum* from South America. Finally, we recovered some structure within clades with many closely related species. Within *Solenotinus*, *V. awabuki*, *V. odoratissimum*, and *V. sieboldii* form a well-supported clade. Within *Succodontotinus*, several well-supported clades are evident, such as one containing *V. brachyandrum*, *V. foetidum*, and *V. hupehense*.

Polyphyly of Megalotinus

Our analysis with an expanded sample of section *Megalotinus* clearly demonstrates that this group is not monophyletic (fig. 2). Instead, we recovered four separate clades of *Megalotinus* species.

Viburnum lepidotulum and *V. punctatum* form a strongly supported clade (ML bootstrap = 100; PP = 1.0) that is only distantly related to the other *Megalotinus* species. Its position within *Viburnum* is strongly supported as part of *Valvatotinus* (ML bootstrap = 100; PP = 1.0) and, within this clade, as sister to *Lantana* (ML bootstrap = 94; PP = 1.0).

Viburnum lutescens and *V. colebrookeanum* form a strongly supported clade together with *V. plicatum* of *Tomentosa* (ML bootstrap = 100; PP = 1.0). In turn, this clade is strongly supported as sister to *Solenotinus* (ML bootstrap = 100; PP = 1.0).

Viburnum cylindricum was the only member of *Megalotinus* included in prior phylogenetic analyses of *Viburnum*, where it was consistently linked with *Lobata* and *Succodontotinus*. In our analyses, *V. cylindricum* is joined by *V. coriaceum* and *V. hebanthum* (ML bootstrap = 100; PP = 1.0). This group remains strongly connected with *Erythrodontotinus* (PP = 1.0), although relationships among these groups remain uncertain.

Also closely associated with *Erythrodontotinus*, we recovered a clade containing *V. inopinatum* and *V. ternatum* (ML bootstrap = 83; PP = 1.0). Our results show that this clade is sister to the clade containing *Lobata*, *Megalotinus* subsection *Coriacea,* and *Succodontotinus* (ML bootstrap = 94; PP = 1.0).

Discussion

Viburnum *Phylogeny and the Dissolution of* Megalotinus

Apart from the disposition of the newly added *Megalotinus* species (discussed below), our results support the clades recovered and named by Winkworth and Donoghue (2005). However, our trees do not strongly support a monophyletic *Lobata* (fig. 2). Also, while Winkworth and Donoghue (2005) found weak support for a large clade containing *Pseudotinus*, *Solenotinus*, *Tomentosa*, *Lantana*, *Lentago*, and *V. urceolatum*, we find that *Pseudotinus* is instead weakly linked with *Valvatotinus* (*Lantana* + *Lentago*; ML bootstrap = 86; fig. 2), and we recover a clade containing *V. urceolatum* (and its newly added relative *V. taiwanianum*), *Tomentosa*, and *Solenotinus*, which is more closely related to *Imbricotinus* (ML bootstrap = 96, PP = 1; fig. 2).

Like Winkworth and Donoghue (2005), we also found conflicting signals when comparing cpDNA and ITS (in addition to conflicts among CP gene trees), especially within *Pseudotinus* and *Lentago* (for detailed discussion, see Winkworth and Donoghue 2005). This could be explained by several instances of homoploid hybrid speciation, as previously suggested (Winkworth and Donoghue 2005). However, we also note the possibility of biparental rather than strictly maternal inheritance of CPs, which has recently been demonstrated in Caprifoliaceae (Hu et al. 2008).

Comparing our results to the trees obtained from the nuclear granule–bound starch synthase gene region GBSSI, or "waxy" (Winkworth and Donoghue 2004), there are several differences within the *Imbricotinus* clade. Both analyses support a clade containing *Oreinodontotinus* and *Mollodontotinus*, but they differ with respect to the placement of *Tinus* and *Opulus*. Our data moderately support *Tinus* as sister to the rest of *Imbricotinus* (ML bootstrap = 80; PP = 0.93). In contrast, waxy trees suggested that *Tinus* is more closely related to *Porphyrodontotinus* (ML bootstrap = 92) and that *Opulus* is sister to the rest of *Imbricotinus* (ML bootstrap = 94).

The main difference between our study and all previous molecular analyses concerns the placement of the *Megalotinus* species. Previous studies included only *V. cylindricum*, of subsection *Coriacea*, which was confidently linked with *Lobata* and *Succodontotinus* within the larger *Imbricotinus* clade. To this we have added eight other *Megalotinus* species—two representatives of each of the four subsections recognized by Kern (1951; table 1). Our results unequivocally demonstrate the nonmonophyly of section *Megalotinus* and recover four clades of *Megalotinus* that correspond to the four subsections described by Kern (1951; table 1; fig. 1): *Coriacea*, *Lutescentia*, *Punctata*, and *Sambucina*. The species assigned to each subsection do appear to be closely related to one another, but the subsections themselves are only distantly related, falling into several widely separated clades within *Viburnum*. *Viburnum lepidotulum* and *V. punctatum*, of subsection *Punctata*, are strongly supported as belonging to the *Valvatotinus* clade (which includes the *Lantana* and *Lentago* groups). *Viburnum lutescens* and *V. colebrookeanum*, of subsection *Lutescentia*, are strongly connected to *V. plicatum* of the small *Tomentosa* clade and in turn to the *Solenotinus*

clade. *Viburnum hebanthum* and *V. coriaceum*, of subsection *Coriacea*, connect as expected with *V. cylindricum*, which generally maintains its previously determined position in relation to *Lobata* and *Succodontotinus*. Finally, *V. inopinatum* and *V. ternatum*, of subsection *Sambucina*, form a separate lineage sister to the *Succodontotinus-Lobata-Coriacea* clade (fig. 2).

Based on these results it is clear that section *Megalotinus* must be abandoned. To accommodate these discoveries, we recommend that the circumscription of Winkworth and Donoghue's (2005) *Valvatotinus* (fig. 1) be expanded to include the newly recognized *Punctata* lineage (which includes *V. lutescens* and *V. punctata*). We suggest that the name *Tomentosa* should continue to apply to *V. plicatum* and *V. hanceanum*. A new name will eventually be needed for the clade that includes *Tomentosa* and the *Lutescentia* species. However, this should await the sampling of additional species assigned to this group (see below). The *Coriacea* and *Sambucina* clades should both be recognized, but additional nomenclatural recommendations to reflect their placements (e.g., possible expansion of *Erythrodontotinus*) will require more confident resolution in this portion of the *Imbricotinus* clade.

Morphological Support and Character Evolution

The findings reviewed above have a number of important implications for our understanding of morphological evolution, which we explore clade by clade in this section. Additionally, we discovered morphological features a posteriori that complement the significant molecular support for the placement of these clades in various parts of the *Viburnum* phylogeny.

Punctata. We were able to sample both species of *Megalotinus* subsection *Punctata* (table 1). Our finding that these are placed in the *Valvatotinus* clade is consistent with the distribution of several morphological characters. First, the pollen grains of *Punctata* species share a derived character state with members of the *Lantana* and *Lentago* groups, namely a semitectate exine with regularly scabrate muri (class b; Donoghue 1985), as opposed to the more common and ancestral condition of a semitectate exine with psilate muri (class a; Donoghue 1985). Further modifications of the exine occur within a subclade of *Lentago* (*V. elatum–V. rufidulum–V. lentago–V. prunifolium*), which exhibits the derived condition of an intectate, regularly retipilate exine (class c; Donoghue 1985). Second, the endocarps of *Punctata* species are strongly compressed but undulate in cross section (Jacobs et al. 2008). Very similar flattened, undulate endocarps characterize members of the *Lantana* clade, but this is presumably the ancestral condition within the *Valvatotinus* clade. The derived condition is found in the *Lentago* clade, where the endocarps become less undulate to the point of being nearly completely flattened (Jacobs et al. 2008). Finally, we note that seeds of *V. lepidotulum* in particular, and to a lesser extent those of *V. punctatum*, exhibit what Jacobs et al. (2008) referred to as type 3 rumination, in which the seed coat is multilayered and locally proliferating into the endosperm. The placement of the *Punctata* group in our trees supports a separate origin of this type of rumination, which also occurs in *Solenotinus* and *Pseudotinus* species. However, contrary to Jacobs et al. (2008), closer inspection of a wider array of *Lentago* and *Lantana* species reveals the presence of type 3 rumination in some of these species as well. For example, we see evidence of this condition in *V. macrocephalum* of the *Lantana* group and *V. obovatum* of the *Lentago* group. As these are both early branching species within their respective clades, it is possible that type 3 rumination is the ancestral condition for the entire *Valvatotinus* clade, which was subsequently lost within the *Lentago* and *Lantana* groups.

In addition to these morphological similarities at the level of *Valvatotinus*, *Punctata* species appear to share several additional derived features with members of the *Lentago* group, which explains the direct connection between these two groups recovered in an earlier morphological analysis (Donoghue 1983*b*). *Punctata* plants have entire leaves with minute, reddish, peltate trichomes on the abaxial leaf surfaces (fig. 3*A*). Members of the *Lentago* clade are the only other viburnums to produce similar peltate trichomes (Donoghue 1983*b*; fig. 3*B*). Variously stalked stellate hairs are also present in *Valvatotinus*, particularly in the *Lantana* species, which are typically densely pubescent (fig. 3*C*). The fruits of the two *Punctata* species are distinctly large at maturity (Kern 1951; Hara 1983) and are approached in size only by some members of the *Lentago* clade. In view of these observations, our results are somewhat surprising in linking *Punctata* directly with *Lantana* as opposed to with *Lentago* within *Valvatotinus*. If the current results hold and *Punctata* remains sister to *Lantana*, peltate trichomes and large fruits may have evolved independently in *Lentago* and in *Punctata* or were perhaps further modified in the *Lantana* group.

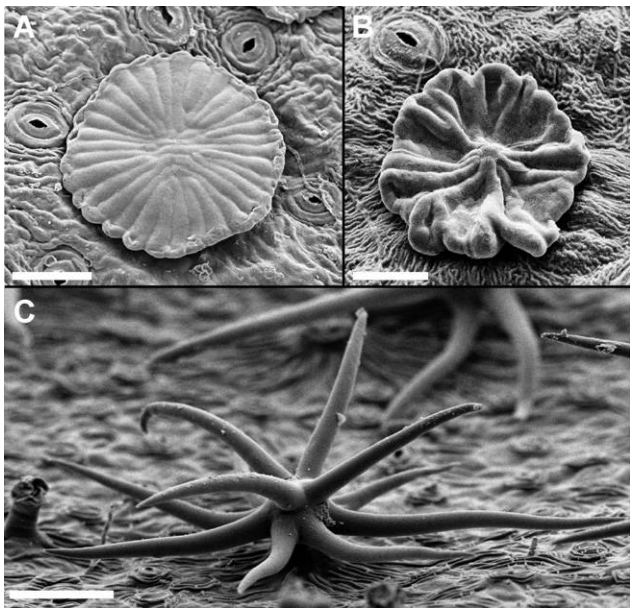

Fig. 3 SEM of trichome types in *Valvatotinus*. *A*, Peltate trichome of *V. punctatum* (scale bar = 44 μm). *B*, Peltate trichome of *V. nudum* (scale bar = 30 μm). *C*, Stellate trichome of *V. carlesii* (scale bar = 64 μm).

Lutescentia. Species of *Megalotinus* subsection *Lutescentia* form a well-supported clade together with *V. plicatum* of *Tomentosa*, which also includes *V. hanceanum* (not included in our analyses). *Viburnum plicatum* and *V. hanceanum* have serrate leaf margins, and their inflorescences terminate short lateral shoots occurring along both sides of the monopodial plagiotropic branches (Donoghue 1981, 1982). This branching pattern yields the distinctive appearance of two rows of inflorescences along the elongate axes of *V. plicatum*; hence its common name, the "double-file" viburnum (fig. 4*A*). Consistent with our phylogenetic findings, it appears that *Lutescentia* species share these peculiar features with the *Tomentosa* species. Both *V. colebrookeanum* and *V. lutescens* have serrate leaves and appear to produce many of their inflorescences on short shoots along monopodial branches (fig. 4*A*). *Viburnum colebrookeanum*, which appears as sister to *V. plicatum* in our analyses, consistently exhibits this characteristic branching pattern, while this is more variable in *V. lutescens*. *Tomentosa* species are instantly distinguished from their *Lutescentia* relatives by the production of greatly enlarged, asymmetrical, sterile flowers around the margins of the inflorescences.

In our trees, the *Lutescentia-Tomentosa* clade is sister to *Solenotinus* (fig. 2), whose members are distinguished by the production of pyramid-shaped paniculate (as opposed to the usual flat-topped corymbose) inflorescences (fig. 4*C*). Two other species of the *Lutescentia* group, *V. amplifolium* and *V. pyramidatum*, remain to be sampled. *Viburnum amplifolium* from western China bears its inflorescences on short lateral shoots (Rehder 1908), which suggests that it will fall within the *Lutescentia-Tomentosa* clade. *Viburnum pyramidatum*, on the other hand, is highly unusual in having terminal pyramid-shaped inflorescences, which appear to be intermediate between standard flat-topped inflorescences and panicles. They produce an umbel-like whorl of rays at the apex of the primary stalk of the inflorescence, but the central ray appears to elongate, accounting for the pyramid shape (Rehder 1908). This morphology suggests the possibility that *V. pyramidatum* may be directly linked with *Solenotinus*, perhaps providing a clue to the origin of the fully paniculate inflorescences of that group (see discussion of *V. amplificatum* below). However, we also note that *V. lutescens* is surprisingly variable in inflorescence morphology, and can produce both umbel-like and panicle-like inflorescences on the same plant (M. Donoghue, personal observation). Taken together, these observations suggest that there may have been several shifts in inflorescence morphology within the entire *Tomentosa-Lutescentia-Solenotinus* clade.

In this lineage, *Viburnum lutescens* seeds exhibit well-developed type 3 rumination. There are some signs of this form of rumination in *V. plicatum* (although this appears to be variable), and as noted by Jacobs et al. (2008), this condition is well developed in the nearby *Solenotinus* clade. In fact, our further inspection of *Solenotinus* species indicates that this condition is almost universally present in *Solenotinus* (with the possible exception of *V. erubescens*). Again, this raises the possibility that type 3 rumination evolved in the ancestor of the entire *Tomentosa-Lutescentia-Solenotinus* clade.

Coriacea. *Viburnum cylindricum*, of subsection *Coriacea*, was the only *Megalotinus* species included in prior phylogenetic studies, where it was found to be related to *Erythrodontotinus* (*Succodontotinus* plus *Lobata*; figs. 1, 2). In our analysis, *V. cylindricum* maintains this phylogenetic position but is joined by the two other *Coriacea* species in-

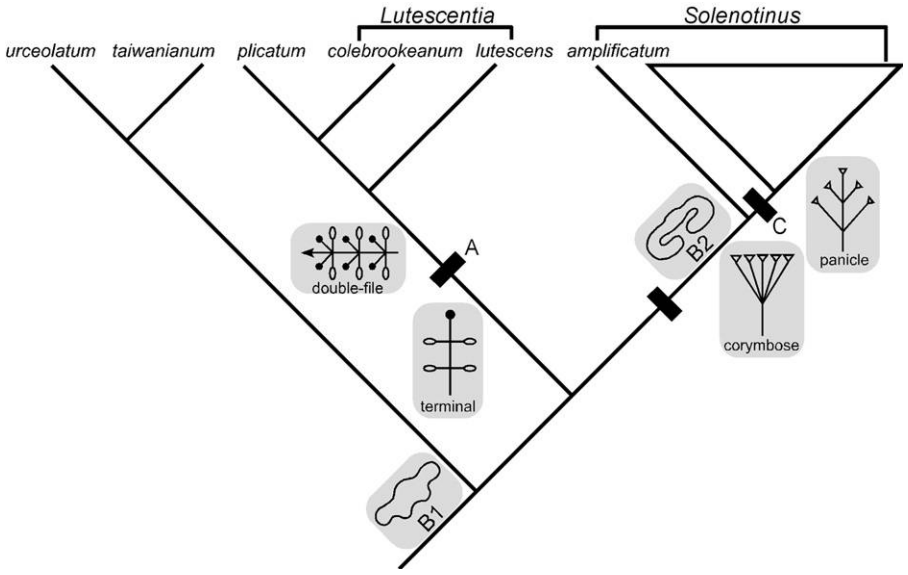

Fig. 4 Hypotheses of morphological evolution in the clade including *Solenotinus*, *Tomentosa*, and species assigned to *Megalotinus* subsection *Lutescentia*. *A*, Proposed shift from producing inflorescences (black circles) at the tips of orthotropic branches to producing inflorescences on axillary short shoots along monopodial plagiotropic axes (Donoghue 1981, 1983). *B*, Proposed shifts in endocarp shape: *B1*, compressed undulate endocarp; *B2*, compressed undulate endocarp with a "bilobed" central intrusion. *C*, Proposed shift in inflorescence type from a flat-topped corymbose form to a pyramid-shaped paniculate form.

cluded here, *V. coriaceum* and *V. hebanthum*. *Erythrodontotinus* species are marked by inconspicuous nectar-producing glands embedded in the abaxial leaf lamina on both sides of the leaf base (M. Weber, personal communication). The *Coriacea* species included here, as well as the four remaining species assigned to this group (*V. beccarii*, *V. cornutidens*, *V. glaberrimum*, and *V. platyphyllum*), also possess these laminar glands.

Sambucina. The final *Megalotinus* subsection, *Sambucina,* is represented in our trees by *V. inopinatum* and *V. ternatum*. These are also strongly linked with *Erythrodontotinus* and may be the sister group of a clade that includes *Succodontotinus*, *Lobata*, and *Coriacea*. These species also generally produce laminar nectary glands (Kern 1951); the unsampled members of this group, *V. hispidulum*, *V. sambucinum*, and *V. vernicosum* require more careful study.

It is noteworthy that the placement of both the *Coriacea* and *Sambucina* clades with *Succodontotinus* and *Lobata* is also consistent with the distribution of endocarp size and shape traits (Jacobs et al. 2008). Here we note a subtle but consistent distinction among the phylogenetically widely distributed endocarps that were described by Jacobs et al. (2008) as being undulate in cross section. In general, such endocarps in the *Valvatotinus* clade (here expanded to include the *Punctata* group) are larger and thicker, and the two margins generally appear rounded when the endocarp is viewed in cross section. In contrast, the undulate endocarps of members of the *Sambucina-Coriacea-Succodontotinus-Lobata* clade are typically smaller and flatter, and the margins appear to narrow to a point in cross section.

The distribution of fruit colors in the *Sambucina-Coriacea-Succodontotinus-Lobata* clade warrants further study. Members of *Succodontotinus* are characterized by their bright red fruits at maturity, with the exception of an evident reversal to dark purple in the aptly named *V. melanocarpum*. Of the species assigned to *Lobata*, *V. kansuense* of the eastern Himalayas and *V. orientale* of the Caucasus region produce red fruits at maturity, whereas the North American *V. acerifolium* sets dark purple fruits. Thus, *V. acerifolium* may represent another reversal from red to purple at maturity. Members of the *Sambucina* and *Coriacea* clades are described as being dark purple at maturity, with the exception of *V. inopinatum*, which is described as red. Depending in part on the ultimate disposition of *V. kansuense* (whether it is linked or not to *V. orientale* and *V. acerifolium*) and of the red-fruited *Opulus* clade (whether sister to the entire *Imbricotinus* clade or just to the *Sambucina-Coriacea-Succodontotinus-Lobata* clade), red fruits could be ancestral in the entire *Sambucina-Coriacea-Succodontotinus-Lobata* clade, with several reversions to dark purple.

Other Southeast Asian *Viburnum.* Although it has not been assigned to *Megalotinus*, the position of the Southeast Asian species *V. amplificatum* is worthy of special attention in view of its significance for understanding morphological evolution and biogeography. This rare Bornean endemic was first described by Kern in 1951 (Kern 1951; Haron 1996), who assigned it to what was then section *Thyrsosma* (now *Solenotinus*; Hara 1983) on the basis of its endocarp shape, which he referred to as having a "bilobate furrow" in cross section (fig. 4*B2*). Indeed, this shape is quite similar to that seen in many *Solenotinus* species, though there are further modifications within that group, especially the much rounder endocarps, with more pronounced central intrusions, seen in the *V. sieboldii–V. odoratissimum–V. awabuki* clade (Jacobs et al. 2008). Kern's assignment was a bold one in view of the fact that *V. amplificatum* produces standard flat-topped umbel-like inflorescences, as opposed to the pyramid-shaped panicles characteristic of the *Solenotinus* group. In our analyses, *V. amplificatum* appears as sister of the rest of *Solenotinus*. This is consistent with Kern's assignment, but it also supports a single origin of the panicle inflorescence within this clade, without needing to invoke a reversal to the umbel-like inflorescence (fig. 4*C*). However, as noted above, a full analysis of inflorescence evolution will need to take into consideration the variation observed in *V. pyramidatum* and *V. lutescens*.

The placement of *V. amplificatum* is also critical from the standpoint of the broader issue of whether the panicle is ancestral or derived within *Viburnum*. The wide separation of *V. clemensiae*, which also produces panicles, from *Solenotinus* and its possible placement as sister to the rest of *Viburnum* (Winkworth and Donoghue 2005) has left open as a possibility the traditional interpretation (e.g., Wilkinson 1948; Egolf 1962; Hara 1983), namely, that the panicle is ancestral in *Viburnum* and that the umbel-like corymbs found in most species are derived. Our placement of *V. amplificatum* argues against this view, rendering it even more unparsimonious to suppose that the panicles of *Solenotinus* were retained from the ancestor of *Viburnum*. Instead, taken at face value, the panicle inflorescence seen in *Solenotinus* was clearly derived from an umbel-like inflorescence, and it is not homologous to the similar condition in *V. clemensiae*.

Implications for Historical Biogeography

The phylogenetic and biogeographic analyses of Winkworth and Donoghue (2005) supported the view that *Viburnum* originated somewhere in Asia, diversified primarily in temperate eastern Asia, and then moved both into Europe (at least three times) and into North America (at least five times). Based on the inferred timing of these events, they argued that movements into the New World most likely took place through Beringia. Subsequently, one clade (*Oreinodontotinus*) moved down through the mountains of Mexico and Central America and radiated recently in the Andes of South America. This scenario left open the question of the origin of the Southeast Asian species, though it has generally been supposed that these represent southern outliers of clades that are now centered in eastern Asia, and that their characteristics (e.g., entire leaves) were derived adaptations to newly inhabited subtropical regimes. This interpretation was consistent with the trees produced by Winkworth and Donoghue (2005), in which the three included Southeast Asian species were placed in three widely separated clades. In their analyses, *V. clemensiae* appeared as the sister of the rest of *Viburnum*, and *V. odoratissimum* and *V. cylindricum* were nested well within distantly related clades.

In addition to nine species of *Megalotinus*, which are mainly centered in Southeast Asia, we added three species from this region that do not belong to *Megalotinus*: *V. amplificatum*, *V. luzonicum*, and *V. propinquum*. The phylogenetic placement of all of these Southeast Asian species suggests,

for the first time, a very different biogeographic interpretation (fig. 5). As detailed above, the four subsections of *Megalotinus* are widely dispersed in our trees, and their distribution adds Southeast Asian species as early diverging branches in three major clades that previously contained only northern temperate species (fig. 5). In addition, our placement of *V. amplificatum* adds a Southeast Asian species as sister to the mainly temperate *Solenotinus* clade and, along with *V. odoratissimum*, opens the possibility of a Southeast Asian origin of that clade. Finally, we have added a Southeast Asian element, *V. propinquum*, within the *Tinus* clade. Taken together, these placements render it nearly equally parsimonious to imagine a northern temperate origin of *Viburnum* followed by multiple (~9) entries into southeast Asia or a southern subtropical montane origin followed by multiple (~11) entries into temperate Asia. Although this issue cannot be confidently resolved until additional Southeast Asian species are included, it is noteworthy that, either way, there appear to have been multiple shifts within *Viburnum* between temperate and subtropical regions in Asia. Thus, despite the fact that most viburnums live in temperate forests, and they have largely conserved their temperate-forest "niche" as they have moved around the Northern Hemisphere and down into the mountains of Latin America, this group is likely to prove useful in dissecting the key physiological transitions that may underpin many global biodiversity and biogeographic patterns (Donoghue 2008).

Although crown Adoxaceae may have originated in the late Cretaceous (~75–65 mya; Bell and Donoghue 2005; J. M. Beaulieu, D. C. Tank, and M. J. Donoghue, unpublished manuscript), and the *Viburnum* stem lineage may therefore have been in existence since that time, crown-clade *Viburnum* may go back only ~40–25 mya (Moore and Donoghue 2007, 2009). A more precise assessment must await the inclusion of additional fossil calibrations within *Viburnum*. In any case, it is likely that although much of the diversification of crown *Viburnum* has taken place during a period of expansion of temperate and cold climates, the early evolution of the group (along the stem and within the crown) may have occurred at a time when tropical and subtropical climates were more extensive. Together with the relatively early divergence of a number of the Southeast Asian *Viburnum* lineages reported here, this leaves open the possibility that at least some of the extant Southeast Asian species, which occupy more subtropical montane forest habitats, are remnants of the early phase of evolution of the group. It is noteworthy that many of these species, which could hold the key to understanding *Viburnum* evolution, are apparently rare in nature and are certainly poorly collected. Several are known from just one or two localities and from only a handful of woefully incomplete specimens (e.g., *V. amplificatum*, *V. cornutidens*, *V. glaberrimum*, *V. junghuhnii*, and *V. platyphyllum*). Considering their importance for understanding morphological evolution and biogeography, every effort should be made to obtain additional material of these species and to protect their rapidly vanishing habitats.

Conclusions

Overall, this study provides an example of how the discovery of the nonmonophyly of a long-recognized taxonomic group can dramatically improve our understanding of character evolution and biogeography. It also highlights the importance of including rare and difficult-to-obtain species

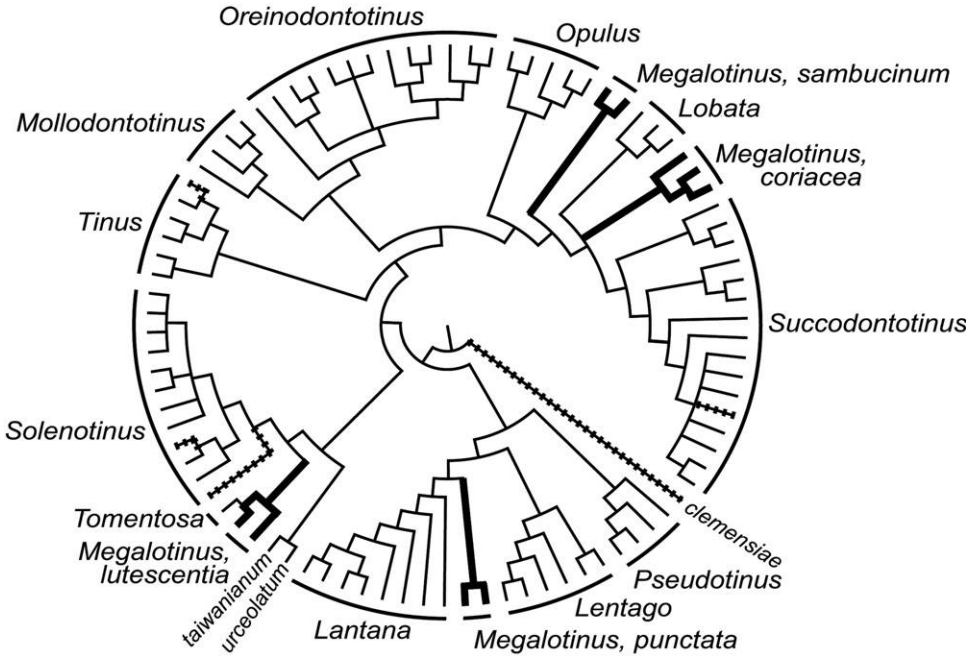

Fig. 5 Phylogenetic position of the Southeast Asian *Viburnum* species included in this analysis. Southeast Asian *Megalotinus* are marked with thick black branches, and other Southeast Asian *Viburnum* species are marked with dashed branches (*V. amplificatum* and *V. odoratissimum* within *Solenotinus*; *V. propinquum* within *Tinus*; *V. luzonicum* within *Succodontotinus*; *V. clemensiae*).

from areas outside of the main centers of diversity of a group. Our confident phylogenetic placement of the four subgroups of section *Megalotinus* as early diverging lineages within distantly related *Viburnum* clades has helped us to trace the origin of several distinctive morphological characters, such the "double-file" arrangement of inflorescences in *Tomentosa* and panicle inflorescences in *Solenotinus*. Moreover, it has highlighted a new biogeographic scenario for the group, namely, the possible origin and initial radiation of *Viburnum* in subtropical montane forests in Southeast Asia. Although this issue can only be settled by comprehensively sampling the rare Southeast Asian species, our analyses already make it clear that there have been numerous transitions between subtropical and temperate habitats within *Viburnum*.

Acknowledgments

We would like to thank R. C. Winkworth and K. L. Heckman for *Viburnum* sequences, M. Weber for kindly sharing her work on *Viburnum* leaf glands, and K. Schmandt for *Viburnum* collections. We would also like to thank the members of the Donoghue and Near labs of Yale University and the Edwards lab of Brown University for many helpful discussions regarding this work. Our sincere thanks to the Arnold Arboretum, Harvard Herbaria, the Field Museum, the Missouri Botanical Garden, and the New York Botanical Garden and their curators for permission to work with their specimens. Support for this work was provided by the National Science Foundation (IOS-0842800).

Appendix A

Voucher Information and GenBank Accession Data

Viburnum species examined in this study. Species names are organized by the clade names of Winkworth and Donoghue (2005). Voucher information for each species is organized as follows: species name and author; collector and collector number; and herbarium abbreviated as follows: A = Arnold Arboretum, Harvard University Herbaria, K = Kew Royal Botanic Gardens; MO = Missouri Botanical Garden, NY = New York Botanical Garden, WTU = University of Washington Herbarium; and YU = Yale University Herbarium. GenBank accession numbers immediately follow voucher information for each species in the following order: *trn*H-*psb*A, *rpl*32$^{(UAG)}$-*trn*L, ITS, *trn*K, *mat*K, *rbc*L, *ndh*F, *trn*C-*ycf*6, *trn*S-*trn*G, *pet*B-*pet*D. Sequences that we were unable to obtain are indicated by "-." Species names and GenBank numbers in bold are new to the study of *Viburnum*.

Lantana: *Viburnum carlesii* Hemsl. ex Forb. & Hemsl.; M.J. Donoghue & R.C. Winkworth 24; YU; AY627385, **HQ591873**, AY265115, AY265161, **HQ591566**, **HQ591710**, **HQ591645**, **HQ592117**, **HQ591823**, **HQ591996**. *Viburnum lantana* L.; M.J. Donoghue & R.C. Winkworth 26; YU; AY627404, **HQ591902**, AY265134, AY265180, **HQ591595**, **HQ591736**, **HQ591667**, **HQ592145**, EF490278, **HQ592019**. ***Viburnum macrocephalum*** Fortune; M.J. Donoghue 101; YU; **HQ592086**, **HQ591911**, EF462984, EF490247, **HQ591604**, **HQ591745**, **HQ591673**, **HQ592153**, **HQ591842**, **HQ592027**. ***Viburnum mongolicum*** Rehder; M.J. Donoghue s.n.; YU; **HQ592087**, **HQ591914**, EF462985, EF490248, **HQ591607**, **HQ591748**, **HQ591676**, **HQ592155**, **HQ591844**, **HQ592029**. ***Viburnum rhytidophyllum*** Hemsl. ex Forb. & Hemsl.; M.J. Donoghue & R.C. Winkworth 8; YU; **HQ592092**, **HQ591925**, AY265146, AY265192, **HQ591618**, **HQ591759**, **HQ591685**, **HQ592166**, **HQ591850**, **HQ592036**. ***Viburnum schensianum*** Maxim.; Boufford et al. 26082; A; **HQ592094**, **HQ591929**, **HQ591975**, **HQ591808**, **HQ591622**, **HQ591763**, **HQ591689**, **HQ592169**, **HQ591851**, **HQ592040**. *Viburnum utile* Hemsl.; Egolf 2336-E; cultivated plant; AY627424, **HQ591945**, AY265156, AY265202, **HQ591638**, **HQ591778**, **HQ591698**, **HQ592184**, EF490291, **HQ592054**. ***Viburnum veitchii*** C.H. Wright; Boufford et al. 27597; A; **HQ592106**, **HQ591946**, **HQ591985**, **HQ591817**, **HQ591639**, **HQ591779**, **HQ591699**, -, **HQ591861**, **HQ592055**.

Lentago: ***Viburnum cassinoides*** L.; Arnold Arboretum 874–85A, 0182773; A; **HQ592067**, **HQ591874**, **HQ591956**, **HQ591789**, **HQ591567**, **HQ591711**, **HQ591646**, **HQ592118**, **HQ591824**, **HQ591997**. *Viburnum elatum* Benth.; M.J. Donoghue 472; YU; AY627394, **HQ591887**, AY265124, AY265170, **HQ591578**, **HQ591721**, -, -, EF490272, **HQ592003**. *Viburnum lentago* L.; M.J. Donoghue & R.C. Winkworth 21; YU; AY627406, **HQ591905**, AY265136, AY265182, **HQ591598**, **HQ591739**, **HQ591670**, **HQ592148**, EF490280, **HQ592022**. *Viburnum nudum* L.; M.J. Donoghue NVI; AY627410, **HQ591915**, AY265140, AY265186, **HQ591608**, **HQ591749**, **HQ591677**, **HQ592156**, EF490282, **HQ592030**. *Viburnum prunifolium* L.; M.J. Donoghue & R.C. Winkworth 13; YU; AY627413, **HQ591922**, AY265144, AY265190, **HQ591615**, **HQ591756**, **HQ591683**, **HQ592163**, EF490286, **HQ592033**. *Viburnum rufidulum* Raf.; M.J. Donoghue & R.C. Winkworth 14; YU; AY627415, **HQ591927**, AY265147, AY265193, **HQ591620**, **HQ591761**, **HQ591687**, **HQ592167**, EF490287, **HQ592038**.

Lobata: *Viburnum acerifolium* L.; M.J. Donoghue & R.C. Winkworth 27; YU; AY627384, **HQ591863**, AY265114, AY265160, **HQ591557**, **HQ591701**, **HQ591641**, **HQ592108**, **HQ591819**, **HQ591987**. *Viburnum kansuense* Batalin; Boufford et al. 27416; A; AY627403, **HQ591901**, AY265133, AY265179, **HQ591594**, **HQ591735**, **HQ591666**, **HQ592144**, EF490276, **HQ592018**. ***Viburnum orientale*** Pall.; Merello et al. 2291; MO; **HQ592089**, **HQ591919**, EF462986, EF490249, **HQ591612**, **HQ591753**, **HQ591680**, **HQ592160**, EF490284, **HQ592031**.

Megalotinus: ***Viburnum colebrookeanum*** Wall.; Parker 3220; A; **HQ592070**, **HQ591879**, **HQ591959**, **HQ591791**, **HQ591570**, **HQ591715**, -, **HQ592123**, -, **HQ592000**. ***Viburnum coriaceum*** Blume; L. Averyanov et al. VH3300; MO; **HQ592071**, **HQ591881**, **HQ591960**, **HQ591792**, **HQ591572**, **HQ591717**, **HQ591650**, **HQ592125**, -, **HQ592001**. *Viburnum cylindricum* Buch.-Ham ex D.Don; Boufford et al. 29342; A; AY627389, **HQ591883**, AY265119, AY265165, -, -, -, **HQ592127**, EF490269, -. ***Viburnum hebanthum*** Wight & Arn.; J. Klackenberg 32; NY; **HQ592076**, **HQ591895**, -,

HQ591795, **HQ591587**, **HQ591729**, **HQ591660**, **HQ592138**, **HQ591833**, **HQ592012**. ***Viburnum inopinatum*** Craib; Rock 1603; A; **HQ592079**, -, -, -, **HQ591590**, -, -, **HQ592141**, -, -. ***Viburnum lepidotulum*** Merr. & Chun; Lau 27991; A; **HQ592083**, **HQ591906**, -, **HQ591800**, **HQ591599**, **HQ591740**, -, -, -, -. ***Viburnum lutescens*** Blume; Wu et al. WP531; A; -, **HQ591909**, **HQ591969**, **HQ591802**, **HQ591602**, **HQ591743**, **HQ591672**, **HQ592151**, **HQ591841**, **HQ592025**. ***Viburnum punctatum*** Buch.-Ham. ex D. Don; Sino-British Expedition 133; A; **HQ592091**, **HQ591923**, **HQ591973**, **HQ591806**, **HQ591616**, **HQ591757**, -, **HQ592164**, **HQ591848**, **HQ592034**. ***Viburnum ternatum*** Rehder; Bartholomew et al. 2268; A; **HQ592102**, **HQ591939**, **HQ591981**, **HQ591813**, **HQ591632**, **HQ591772**, -, **HQ592179**, **HQ591856**, **HQ592048**.

Mollodontotinus: ***Viburnum bracteatum*** Rehder; Arnold Arboretum 1067–87A, 0227564; A; **HQ592065**, **HQ591871**, -, -, **HQ591564**, **HQ591708**, **HQ591643**, **HQ592115**, **HQ591822**, **HQ591994**. *Viburnum ellipticum* Hook.; M.J. Donoghue NVI; AY627395, -, AY265125, AY265171, **HQ591579**, **HQ591722**, **HQ591653**, **HQ592131**, **HQ591830**, **HQ592004**. *Viburnum molle* Michx.; M.J. Donoghue & R.C. Winkworth 5; YU; AY627409, **HQ591913**, AY265139, AY265185, **HQ591606**, **HQ591747**, **HQ591675**, **HQ592154**, EF490281, -. *Viburnum rafinesquianum* Schult.; M.J. Donoghue & R.C. Winkworth 4; YU; AY627414, **HQ591924**, AY265145, AY265191, **HQ591617**, **HQ591758**, **HQ591684**, **HQ592165**, **HQ591849**, **HQ592035**.

Opulus: *Viburnum edule* (Michx.) Raf.; NVI ; AY627393, -, AY265123, AY265169, **HQ591577**, **HQ591720**, -, -, EF490271, -. *Viburnum koreanum* Nakai; H. Yamaji 5170; MO; **HQ592081**, -, EF462983, EF490246, -, -, -, -, EF490277, -. *Viburnum opulus* L.; M.J. Donoghue & R.C. Winkworth 32; YU; -, **HQ591918**, **HQ591972**, **HQ591805**, **HQ591611**, **HQ591752**, **HQ591679**, **HQ592159**, **HQ591847**, -. *Viburnum sargentii* Koehne; M.J. Donoghue & R.C. Winkworth 17; YU; AY627416, **HQ591928**, AY265148, AY265194, **HQ591621**, **HQ591762**, **HQ591688**, **HQ592168**, EF490288, **HQ592039**. ***Viburnum trilobum*** Marshall; Arnold Arboretum 22900A, 0174487; A; **HQ592104**, **HQ591942**, **HQ591983**, **HQ591815**, **HQ591635**, **HQ591775**, **HQ591695**, **HQ592182**, EF490290, **HQ592051**.

Oreinodontotinus: ***Viburnum blandum*** C.V. Morton; M.J. Donoghue 464; YU; **HQ592062**, **HQ591869**, **HQ591952**, **HQ591785**, **HQ591562**, **HQ591706**, -, **HQ592113**, -, **HQ591992**. ***Viburnum caudatum*** Greenm.; M.J. Donoghue 64; YU; **HQ592068**, **HQ591875**, **HQ591957**, **HQ591790**, -, -, -, **HQ592119**, **HQ591825**, -. *Viburnum dentatum* L.; M.J. Donoghue & R.C. Winkworth 33; YU; AY627391, **HQ591884**, AY265121, AY265167, **HQ591574**, **HQ591718**, **HQ591651**, **HQ592128**, **HQ591827**, **HQ592002**. ***Viburnum discolor*** Benth.; M.J. Donoghue 507; YU; **HQ592073**, **HQ591886**, -, **HQ591793**, **HQ591576**, -, -, **HQ592130**, **HQ591829**, -. *Viburnum hartwegii* Benth.; M.J. Donoghue 486; YU; AY627400, **HQ591894**, AY265130, AY265176, **HQ591586**, -, **HQ591659**, **HQ592137**, **HQ591832**, **HQ592011**. ***Viburnum jamesonii*** (Oersted) Killip & A.C.Sm.; P.W. Sweeney 1636; YU; **HQ592080**, **HQ591898**, **HQ591966**, **HQ591798**, **HQ591591**, **HQ591732**, **HQ591663**, **HQ592142**, **HQ591836**, **HQ592015**. *Viburnum jucundum* C.V. Morton; M.J. Donoghue 244; YU; AY627402, **HQ591900**, AY265132, AY265178, **HQ591593**, **HQ591734**, **HQ591665**, -, **HQ591838**, **HQ592017**. ***Viburnum lautum*** C.V. Morton; M.J. Donoghue 72; YU; **HQ592082**, **HQ591904**, **HQ591967**, **HQ591799**, **HQ591597**, **HQ591738**, **HQ591669**, **HQ592147**, **HQ591839**, **HQ592021**. ***Viburnum loeseneri*** Graebn.; M.J. Donoghue 2547; YU; **HQ592084**, **HQ591908**, **HQ591968**, **HQ591801**, **HQ591601**, **HQ591742**, -, **HQ592150**, -, **HQ592024**. *Viburnum stenocalyx* Hemsl.; M.J. Donoghue 60; YU; **HQ592097**, **HQ591933**, **HQ591978**, **HQ591810**, **HQ591626**, **HQ591767**, -, **HQ592173**, -, **HQ592043**. ***Viburnum sulcatum*** Hemsl.; M.J. Donoghue 207; YU; **HQ592099**, **HQ591935**, **HQ591980**, **HQ591812**, **HQ591628**, -, -, **HQ592175**, -, -. ***Viburnum toronis*** Killip & A.C. Smith; P.W. Sweeney 1799; YU; **HQ592103**, **HQ591941**, **HQ591982**, **HQ591814**, **HQ591634**, **HQ591774**, **HQ591694**, **HQ592181**, **HQ591858**, **HQ592050**. *Viburnum triphyllum* Benth.; P.W. Sweeney 1783; YU; **HQ592105**, **HQ591943**, **HQ591984**, **HQ591816**, **HQ591636**, **HQ591776**, **HQ591696**, **HQ592183**, **HQ591859**, **HQ592052**.

Pseudotinus: *Viburnum furcatum* Blume ex Hook.f. & Thomson; Tsugaru & Takashi 19958; MO; AY627399, **HQ591893**, AY265129, AY265175, **HQ591585**, **HQ591728**, **HQ591658**, **HQ592136**, EF490275, **HQ592010**. *Viburnum lantanoides* Michx.; M.J. Donoghue & R.C. Winkworth 2; YU; AY627405, **HQ591903**, AY265135, AY265181, **HQ591596**, **HQ591737**, **HQ591668**, **HQ592146**, EF490279, **HQ592020**. *Viburnum nervosum* D. Don; Boufford et al. 27388; A; AY627388, **HQ591880**, AY265118, AY265164, **HQ591571**, **HQ591716**, **HQ591649**, **HQ592124**, EF490268, -. ***Viburnum sympodiale*** Graebn.; Lai & Shan 4529; MO; **HQ592100**, **HQ591937**, EF462988, EF490252, **HQ591630**, **HQ591770**, -, **HQ592177**, EF490289, **HQ592046**.

Solenotinus: ***Viburnum amplificatum*** J. Kern; Pereira et al. 677; A; **HQ592058**, **HQ591865**, **HQ591949**, -, -, -, -, -, -, -. ***Viburnum awabuki*** Hort.Berol. ex K. Koch; Liu 141; A; **HQ592060**, **HQ591867**, **HQ591951**, **HQ591783**, **HQ591560**, **HQ591704**, -, **HQ592111**, -, **HQ591990**. ***Viburnum brachybotryum*** Hemsl. ex Forb. & Hemsl.; NVI ; **HQ592064**, -, **HQ591954**, **HQ591787**, -, -, -, -, -, -. ***Viburnum chingii*** P.S. Hsu; Bartholomew et al. 973; A; **HQ592069**, **HQ591876**, **HQ591958**, -, -, **HQ591712**, -, **HQ592120**, -, -. ***Viburnum corymbiflorum*** P.S. Hsu & S.C. Hsu; Gao 1706; A; **HQ592072**, **HQ591882**, **HQ591961**, -, **HQ591573**, -, -, **HQ592126**, -, -. *Viburnum erubescens* Wall.; Boufford et al. 27190; A; AY627397, **HQ591889**, AY265127, AY265173, **HQ591581**, **HQ591724**, **HQ591655**, **HQ592133**, **HQ591831**, **HQ592006**. *Viburnum farreri* Stearn; M.J. Donoghue & R.C. Winkworth 18; YU; AY627398, **HQ591890**, AY265128, AY265174, **HQ591582**, **HQ591725**, **HQ591656**, **HQ592134**, EF490274, **HQ502007**. *Viburnum odoratissimum* Ker-Gawl.; R. Olmstead 118; WTU; AY627411, **HQ591916**, AY265141, AY265187, **HQ591609**, **HQ591750**, **HQ591678**, **HQ592157**, **HQ591845**, -. ***Viburnum oliganthum*** Batalin; Boufford et al. 27175; A; **HQ592088**, **HQ591917**, **HQ591971**, **HQ591804**, **HQ591610**, **HQ591751**, -, **HQ592158**, **HQ591846**, -. *Viburnum sieboldii* Miq.; M.J. Donoghue & R.C. Winkworth 3; YU; AY627417, **HQ591932**, AY265149, AY265195, **HQ591625**, **HQ591766**, **HQ591691**, **HQ592172**, **HQ591853**, **HQ592042**. ***Viburnum***

subalpinum Hand.-Mazz.; Heng 11878; A; **HQ592098**, **HQ591934**, **HQ591979**, **HQ591811**, **HQ591627**, **HQ591768**, -, **HQ592174**, -, **HQ592044**. *Viburnum suspensum* Lindl.; M.J. Donoghue & R.C. Winkworth 36; YU; AY627419, **HQ591936**, AY265151, AY265197, **HQ591629**, **HQ591769**, **HQ591692**, **HQ592176**, **HQ591854**, **HQ592045**.

Succodontotinus: ***Viburnum adenophorum*** W.W. Sm.; Boufford & Bartholomew 24402; A; **HQ592057**, **HQ591864**, **HQ591948**, **HQ591781**, **HQ591558**, **HQ591702**, -, **HQ592109**, -, **HQ591988**. ***Viburnum betulifolium*** Batalin; Boufford et al. 29335; A; **HQ592061**, **HQ591868**, -, **HQ591784**, **HQ591561**, **HQ591705**, -, **HQ592112**, -, **HQ591991**. ***Viburnum brachyandrum*** Nakai; Mizushima 568; A; **HQ592063**, **HQ591870**, **HQ591953**, **HQ591786**, **HQ591563**, **HQ591707**, -, **HQ592114**, **HQ591821**, **HQ591993**. *Viburnum dilatatum* Thunb.; M.J. Donoghue & R.C. Winkworth 19; YU; AY627392, **HQ591885**, AY265122, AY265168, **HQ591575**, **HQ591719**, **HQ591652**, **HQ592129**, **HQ591828**, -. *Viburnum erosum* Thunb.; M.J. Donoghue & R.C. Winkworth 16; YU; AY627396, **HQ591888**, AY265126, AY165172, **HQ591580**, **HQ591723**, **HQ591654**, **HQ592132**, EF490273, **HQ592005**. ***Viburnum flavescens*** W.W. Sm.; Boufford et al. 32758; A; **HQ592074**, **HQ591891**, **HQ591962**, **HQ591794**, **HQ591583**, **HQ591726**, **HQ591657**, -, -, **HQ592008**. ***Viburnum foetidum*** Wall.; C.-H. Lin 563; MO; **HQ592075**, **HQ591892**, **HQ591963**, EF490245, **HQ591584**, **HQ591727**, -, **HQ592135**, -, **HQ592009**. ***Viburnum hupehense*** Rehder; Bartholomew et al. 1286; A; **HQ592077**, **HQ591896**, **HQ591964**, **HQ591796**, **HQ591588**, **HQ591730**, **HQ591661**, **HQ592139**, **HQ591834**, **HQ592013**. ***Viburnum ichangense*** Rehder; Bartholomew et al. 1889; A; **HQ592078**, **HQ591897**, **HQ591965**, **HQ591797**, **HQ591589**, **HQ591731**, **HQ591662**, **HQ592140**, **HQ591835**, **HQ592014**. *Viburnum japonicum* Spreng; NVI ; YU; AY627401, **HQ591899**, AY265131, AY265177, **HQ591592**, **HQ591733**, **HQ591664**, **HQ592143**, **HQ591837**, **HQ592016**. *Viburnum lobophyllum* Graebn.; M.J. Donoghue & R.C. Winkworth 25; YU; AY627407, **HQ591907**, AY265137, AY265183, **HQ591600**, **HQ591741**, **HQ591671**, **HQ592149**, **HQ591840**, **HQ592023**. ***Viburnum luzonicum*** Rolfe; Shen 673; A; **HQ592085**, **HQ591910**, **HQ591970**, **HQ591803**, **HQ591603**, **HQ591744**, -, **HQ592152**, -, **HQ592026**. *Viburnum melanocarpum* P.S. Hsu; M.J. Donoghue & R.C. Winkworth 12; YU; AY627408, **HQ591912**, AY265138, AY265184, **HQ591605**, **HQ591746**, **HQ591674**, -, **HQ591843**, **HQ502028**. *Viburnum sempervirens* K. Koch; Hu & But 21891; A; **HQ592095**, **HQ591930**, **HQ591976**, **HQ591809**, **HQ591623**, **HQ591764**, -, **HQ592170**, -, -. ***Viburnum setigerum*** Hance; M.J. Donoghue 102; YU; **HQ592096**, **HQ591931**, **HQ591977**, EF490251, **HQ591624**, **HQ591765**, **HQ591690**, **HQ592171**, **HQ591852**, **HQ592041**. ***Viburnum wrightii*** Miquel; Yonekura 1362; A; **HQ592107**, **HQ591947**, **HQ591986**, **HQ591818**, **HQ591640**, **HQ591780**, **HQ591700**, **HQ592185**, **HQ591862**, **HQ592056**.

Tinus: ***Viburnum atrocyaneum*** C.B. Clarke; Boufford et al. 34956; A; **HQ592059**, **HQ591866**, **HQ591950**, **HQ591782**, **HQ591559**, **HQ591703**, **HQ591642**, **HQ592110**, **HQ591820**, **HQ591989**. ***Viburnum calvum*** Rehder; Li & Soukup 934; A; **HQ592066**, **HQ591872**, **HQ591955**, **HQ591788**, **HQ591565**, **HQ591709**, **HQ591644**, **HQ592116**, -, **HQ591995**. *Viburnum cinnamomifolium* Rehder; Olmstead 120; WTU; AY627386, **HQ591877**, AY265116, AY265162, **HQ591568**, **HQ591713**, **HQ591647**, **HQ592121**, **HQ591826**, **HQ591998**. ***Viburnum propinquum*** Hemsl.; M.J. Donoghue 100; YU; **HQ592090**, **HQ591921**, EF462987, EF490250, **HQ591614**, **HQ591755**, **HQ591682**, **HQ592162**, -, -. ***Viburnum rigidum*** Ventenat; Stearn 1116; A; **HQ592093**, **HQ591926**, **HQ591974**, **HQ591807**, **HQ591619**, **HQ591760**, **HQ591686**, -, -, **HQ592037**. *Viburnum tinus* L.; M.J. Donoghue & R.C. Winkworth 35; YU; AY627420, **HQ591940**, AY265152, AY265198, **HQ591633**, **HQ591773**, **HQ591693**, **HQ592180**, **HQ591857**, **HQ592049**.

Tomentosa: *Viburnum plicatum* Thunberg; M.J. Donoghue & R.C. Winkworth 10; YU; AY627412, **HQ591920**, AY265143, AY265189, **HQ591613**, **HQ591754**, **HQ591681**, **HQ592161**, EF490285, **HQ592032**.

Unassigned: *Viburnum clemensiae* Kern; J. Beaman 11781; K; AY627387, **HQ591878**, AY265117, AY265163, **HQ591569**, **HQ591714**, **HQ591648**, **HQ592122**, EF490267, **HQ591999**. ***Viburnum taiwanianum*** Hayata; W.-H. Hu et al. 2186; MO; **HQ592101**, **HQ591938**, EF462989, EF490253, **HQ591631**, **HQ591771**, -, **HQ592178**, **HQ591855**, **HQ592047**. *Viburnum urceolatum* S. & Z.; M.J. Donoghue NVI; AY627423, **HQ591944**, AY265155, AY265201, **HQ591637**, **HQ591777**, **HQ591697**, -, **HQ591860**, **HQ592053**.

Literature Cited

Bell CD, MJ Donoghue 2005 Dating the diversification of Dipsacales: comparing models, genes, and evolutionary implications. Am J Bot 92:284–296.

CBOL (Consortium for the Barcode of Life) Plant Working Group 2009 A DNA barcode for land plants. Proc Natl Acad Sci USA 106:12794–12797.

Donoghue MJ 1981 Growth patterns in woody plants with examples from the genus *Viburnum*. Arnoldia 41:2–23.

——— 1982 Systematic studies in the genus *Viburnum*. PhD diss. Harvard University, Cambridge, MA.

——— 1983*a* The phylogenetic relationships of *Viburnum*. Columbia University Press, New York.

——— 1983*b* A preliminary analysis of phylogenetic relationships in *Viburnum* (Caprifoliaceae s.l.). Syst Bot 8:45–58.

——— 1985 Pollen diversity and exine evolution in *Viburnum* and the Caprifoliaceae sensu lato. Arnoldia 66:421–469.

——— 2008 A phylogenetic perspective on the distribution of plant diversity. Proc Natl Acad Sci USA 105:11549–11555.

Donoghue MJ, BG Baldwin, J Li, RC Winkworth 2004 *Viburnum* phylogeny based on chloroplast trnK intron and nuclear ribosomal ITS DNA sequences. Syst Bot 29:188–198.

Donoghue MJ, GA Levin 1986 Leaf morphology and evolution in the genus *Viburnum*. Am J Bot 73:760.

Edgar RC 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.

Egolf DR 1962 A cytological study of the genus *Viburnum*. J Arnold Arbor Harv Univ 43:132–172.

Felsenstein J 1985 Confidence limits on phylogenies: an approach using the bootstrap. Evolution 38:783–791.

Hamilton MB 1999 Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol Ecol 8: 521–523.

Hara H 1983 A revision of Caprifoliaceae of Japan with reference to allied plants in other districts and the Adoxaceae. Academia, Tokyo.

Haron NW 1996 Caprifoliaceae. Ampang, Kuala Lumpur.

Hu Y, Q Zhang, G Rao, Sodmergen 2008 Occurrence of plastids in the sperm cells of Caprifoliaceae: biparental plastid inheritance in angiosperms is unilaterally derived from maternal inheritance. Plant Cell Physiol 49:958–968.

Huelsenbeck JP, FR Ronquist 2001 MrBayes: Bayesian inference of phylogeny. Biometrics 17:754–755.

Jacobs B, MJ Donoghue, F Bouman, S Huysmans, E Smets 2008 Evolution and phylogenetic importance of endocarp and seed characters in *Viburnum* (Adoxaceae). Int J Plant Sci 169:409–431.

Kern JH 1951 The genus *Viburnum* (Caprifoliaceae) in Malaysia. Reinwardtia 1:107–170.

LaFrankie JV 2010 Trees of tropical Asia: an illustrated guide to diversity. Black Tree, Philippines.

Löhne C, T Borsch 2005 Molecular evolution and phylogenetic utility of the *pet*D group II intron: a case study in basal angiosperms. Mol Biol Evol 22:317–332.

Moore BR, MJ Donoghue 2007 Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. Am Nat 170(suppl):S28–S55.

——— 2009 A Bayesian approach for evaluating the impact of historical events on rates of diversification. Proc Natl Acad Sci USA 106:4307–4312.

Nixon KC 1999 The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15:407–414.

Nylander JAA 2004 MrModeltest. Version 2. Evolutionary Biology Centre, Uppsala University. http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html.

Oersted AS 1861 Til belysning af slaegten *Viburnum*. Vidensk Medd Dan Naturhist Foren 13:267–305.

Olmstead RG, HJ Michaels, KM Scott, JD Palmer 1992 Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of rbcL. Ann Mo Bot Gard 79:249–265.

Olmstead RG, JA Sweere 1994 Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Syst Biol 43:467–481.

Rambaut A, AJ Drummond 2007 Tracer. Version 1.4. http://beast.bio.ed.ac.uk/Tracer.

Rehder A 1908 The viburnums of eastern Asia. Pages 105–116 *in* CS Sargent, ed. Trees and shrubs. Vol II, pt II. Houghton Mifflin, Boston.

Sang T, DU Crawford, TF Stuessy 1997 Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Am J Bot 84:1120–1136.

Shaw J, EB Lickey, JT Beck, ST Farmer, W Liu, J Miller, KC Siripun, CT Winder, EE Schilling, RL Small 2005 The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am J Bot 92: 142–166.

Shaw J, EB Lickey, EE Schilling, RL Small 2007 Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94:275–288.

Sikes DS, PO Lewis 2001 Beta software. Version 1. PAUPRat: PAUP* implementation of the parsimony ratchet. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs. http://users.iab.uaf.edu/~derek_sikes/software2.htm

Smith SA, CW Dunn 2008 Phyutility: a phyloinformatics tool for trees, alignments, and molecular data. Bioinformatics 24:715–716.

Stamatakis A 2006 RAxML-Vl-HPC: maximum likelihood–based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Swofford DL 2002 PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, MA.

White TJ, T Bruns, S Lee, J Taylor 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 *in* MA Innis, DH Gelfand, JJ Sninsky, TJ White, eds. PCR protocols. Academic Press, San Diego, CA.

Wilkinson AM 1948 Floral anatomy and morphology of some species of the genus *Viburnum* of the Caprifoliaceae. Am J Bot 35:455–465.

Winkworth RC, MJ Donoghue 2004 *Viburnum* phylogeny: evidence from the duplicated nuclear gene GBSSI. Mol Phylogenet Evol 33: 109–126.

——— 2005 *Viburnum* phylogeny based on combined molecular data: implications for taxonomy and biogeography. Am J Bot 92: 653–666.

Young ND, KE Steiner, CW dePamphilis 1999 The evolution of parasitism in Scrophulariaceae/Orobanchaceae: plastid gene sequences refute an evolutionary transition series. Ann Mo Bot Gard 86:876–893.