Phylogeny and Biogeography of the Arbutoideae (Ericaceae): Implications for the Madrean-Tethyan Hypothesis

Lena C. Hileman[1], Michael C. Vasey, and V. Thomas Parker

Department of Biology, San Francisco State University, San Francisco, California 94132
[1] Present address: Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138

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Abstract. Phylogenetic relationships within subfamily Arbutoideae (Ericaceae) were estimated using parsimony and maximum likelihood analyses of sequence data from the ITS region and part of the large subunit of nuclear ribosomal DNA. The data support the monophyly of *Arctostaphylos, Arctous,* and *Comarostaphylis,* but suggest that *Arbutus* is not monophyletic, with Mediterranean Basin species more closely related to the clade containing *Arctostaphylos, Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus* than to the western North American species of *Arbutus.* Calibration of branch lengths with the fossil record suggests that a vicariance event occurred among members of the Arbutoideae between western North America and the Mediterranean Basin at the Paleogene/Neogene boundary, consistent with the Madrean-Tethyan hypothesis.

Vicariance biogeography is of considerable interest to many botanists, and the question of disjunct mediterranean floras has been given substantial attention (see Liston 1997 for a review). Studies from the Pinaceae (Liston et al. 1999), Datiscaceae (Liston et al. 1989a; Liston et al. 1992) and this study suggest that the occurrence of disjunct taxa can be explained by vicariance in the middle Tertiary. Other studies from the Asteraceae (Liston et al. 1989b; Liston and Kadereit 1995) and Styracaceae (Fritsch 1996) instead suggest that mediterranean disjuncts may be the result of more recent dispersal events, or convergent evolution. The phylogenetic and biogeographic relationships of taxa comprising mediterranean floras are proving to be complex, and it is likely that there will be low levels of correlated history among the different groups investigated. However, as more data are gathered from taxa exhibiting North American/Mediterranean Basin disjunctions, a clearer understanding of the flora's historical biogeography will be gained.

Various hypotheses have been suggested for the origin of disjunct taxa found in the mediterranean climates of western North America and the Mediterranean Basin (see Fritsch 1996 for a review). Axelrod (1973, 1975) hypothesized that a continuous, sclerophyllous, dry-adapted flora existed between western North America (''Madrean'') and Eurasia (''Tethyan'') until the end of the Oligocene (ca. 25 MYA). It is generally accepted (McKenna 1975, 1983; Wolfe 1975; Tiffney 1985b; and references therein) that western Europe and North America were in contact until late Eocene time (ca. 40 MYA).

However, Axelrod suggests that even as North America continued moving westward during the Oligocene, drops in sea level and the presence of microcontinents and volcanic islands maintained the potential for gene flow across a still narrow Atlantic Ocean until the Paleogene/Neogene boundary (see also Tiffney 1985b). Alternatively, Raven (1973) and Wolfe (1975) have suggested that the present-day distribution of elements appearing to belong to a Madrean-Tethyan flora could be the result of convergent adaptation to similar dry climates, or the result of long-distance dispersal. For a group to be consistent with the Madrean-Tethyan hypothesis, disjunct taxa should (1) show a sister-group relationship and (2) exhibit an estimated divergence time greater than approximately 25 million years, corresponding to the Paleogene/Neogene boundary (Fritsch 1996).

The Arbutoideae is a distinct and natural group within the Ericaceae based on fruit and flower morphology, as well as anatomy and phytochemistry (Cox 1948; Stevens 1971). Six genera are commonly recognized; *Arbutus, Arctostaphylos, Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus* (Small 1914; Adams 1940; Stevens 1971; Diggs and Breckon 1981). The Arbutoideae are dry-adapted sclerophyllous taxa and most of the diversity in the group is in regions of mediterranean climate in western North America (Table 1). Exceptions to this primary distribution include the circumarctic *Arctous alpina,* circumboreal *Arctostaphylos uva-ursi,* and four species of *Arbutus* that occur in mediterranean regions of Europe, North Africa, and the Middle East.

TABLE 1. Genera comprising the Arbutoideae with species number and general distributions.

Taxon	# of species	Distribution
Arbutus L.	ca. 11	West coast of N. America through Mexico and Central America; western Europe through the Mediterranean region, northern Africa and parts of the Middle East.
Arctostaphylos Adans.	ca. 62	Northern hemisphere distribution that consists mainly of the circumboreal *A. uva-ursi.* The center of diversity is the California floristic province.
Arctous Nied.	2	Circumarctic distribution.
Comarostaphylis Zucc.	9	Southern California to northern Baja California and from the Mexican highlands to Central America.
Ornithostaphylos Small	1	Southern California to northern Baja California.
Xylococcus Nutt.	1	Southern California to northern Baja California.

Arbutus is characterized by baccate fruit, with multiple ovules per locule, and papillose fruit surface (Small 1914; Diggs and Breckon 1981; Sørensen 1995). Three species of *Arbutus* occur in the Mediterranean region from North Africa to the Middle East; *A. unedo, A. andrachne,* and *A.* x *andrachnoides.* The latter is hypothesized to be of hybrid origin between *A. unedo* and *A. andrachne* (Callan 1941). *Arbutus canariensis* is endemic to the Canary Islands. The remaining eight species of *Arbutus* occur in the Western Hemisphere. Of these, *A. xalapensis, A. texana, A. peninsularis, A. tessellata, A. arizonica, A. occidentalis,* and *A. madrensis* have a Neotropical distribution with *A. xalapensis* being the most widespread and variable. *Arbutus menziesii* extends from British Columbia to Baja California along the West Coast of North America. Because of the disjunct distribution between mediterranean climates of western North America and the Mediterranean Basin, Axelrod (1975) cited *Arbutus* as a component of the Madrean-Tethyan flora.

Arctostaphylos has a northern hemispheric distribution that consists mainly of the circumboreal *Arctostaphylos uva-ursi.* The peak of distribution for *Arctostaphylos* is the California Floristic Province where all 62 species occur. *Arctostaphylos* is characterized by a drupaceous fruit with a smooth surface with nutlets that are usually separable, as well as inflorescences that begin development at the end of the growing season, but over-winter to bloom the following season (nascent inflorescences; Wells 1992). The wide range of character states within *Arctostaphylos* may account for the previous inclusion of *Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus.*

Arctous ranges from tundra to alpine and subalpine habitats, and of the two species of *Arctous, A. alpina* makes up most of the circumarctic distribution. Although *Arctous* has, at times, been subsumed within *Arctostaphylos* (Candolle 1839; Klotzsch 1851; Walpers 1852; Hooker 1876; Parry 1884; Gray 1886; Webb 1972; Wilber and Luteyn 1978), distinguishing characters include deciduous foliage, lack of recurved appendages on the anthers and lack of nascent inflorescences (Drude 1891; Small 1914; Eastwood 1934; Adams 1940; Hitchcock 1959; Watson 1965; Stevens 1971).

The nine species of *Comarostaphylis* occur in subtropical chaparral and oak-pine forests of Central America, Mexico and the southwestern United States. *Comarostaphylis,* at times placed within *Arctostaphylos* (Candolle 1839; Parry 1884; Gray 1886; Drude 1891; Watson 1965; Wilber and Luteyn 1978), is distinguished by drupaceous fruit with a papillose surface and lack of nascent inflorescences (Walpers 1852; Small 1914; Eastwood 1934; Adams 1940; Stevens 1971; Diggs and Breckon 1981).

The monotypic *Ornithostaphylos* occurs in chaparral from northern Baja California to southern California. Like *Comarostaphylis, Ornithostaphylos* has, at times, been treated as *Arctostaphylos* (Parry 1884; Watson 1965; Wilber and Luteyn 1978). However, characters such as opposite or whorled leaves, carpels each containing two locules and two seeds, and a unique, much branched inflorescence give support to its generic circumscription (Small 1914; Adams 1940; Stevens 1971).

The monotypic *Xylococcus* has often been included within *Arctostaphylos* (Walpers 1852; Hooker 1876; Parry 1884; Gray 1886; Drude 1891; Watson 1965; Wilber and Luteyn 1978), but can be distinguished by corolla, filament, and fruit morphology (Small 1914; Eastwood 1934; Adams 1940; Stevens 1971). *Xylococcus* has a similar distribution to that of *Ornithostaphylos* but its range extends farther

TABLE 2. Taxa included in phylogenetic analysis based on the ITS region and partial 28S sequence data. P = V. T. Parker, V = M. C. Vasey, M = S. Markos, LH = Lenz and Henrickson, SFSU = Herbarium at San Francisco State University, OSU = Herbarium at Oregon State University, RSA = Herbarium at Rancho Santa Anna, HBG = Huntington Botanic Garden. Taxa indicated with * were included in the maximum likelihood analysis.

Taxon	Specimen	GenBank Acc. ITS	GenBank Acc. LSU
Ingroup:			
*Arbutus andrachne L.	PV 0599 (SFSU)	AF091954	AF091980
Arbutus arizonica (A. Gray) Sarg.	V 0223 (SFSU)	AF091958	AF091984
Arbutus canariensis Duham.	PV 0596 (SFSU)	AF091992/AF091993	AF091981
Arbutus menziesii Pursh	V 0541 (SFSU)	AF086828	AF086829
Arbutus occidentalis McVaugh & Rosatti	LH 295 (RSA)	AF091955	N/A
*Arbutus peninsularis Rose & Goldman	HBG 23722	AF091956	AF091982
Arbutus texana Buckl.	PV 0597 (SFSU)	AF091959/AF091960	AF091985
*Arbutus unedo L.	PV 0601 (SFSU)	AF091952	AF091979
Arbutus unedo L.	Liston 1003 (OSU)	AF091953	AF091978
*Arbutus xalapensis Kunth	PV 0598 (SFSU)	AF091957	AF091983
Arctostaphylos andersonii A. Gray	V 0086 (SFSU)	AF091990/AF091991	AF091976
*Arctostaphylos hookeri G. Don	M 0610 (SFSU)	AF091951	AF091977
*Arctostaphylos nummularia A. Gray	V 0040 (SFSU)	AF091949	AF091974
Arctostaphylos tomentosa (Pursh) Lindl.	V 0243 (SFSU)	AF091988/AF091989	AF091972
*Arctostaphylos uva-ursi (L.) Spreng.	T 0440 (SFSU)	AF091950	AF091975
Arctous alpina (L.) Nied.	P 0861 (SFSU)	N/A /AF091961	AF091986
*Arctous rubra (Rehd. & E. H. Wilson) Nakai	P 0860 (SFSU)	AF091944	AF091968
Comarostaphylis arbutoides Lindl.	PV 0595 (SFSU)	AF091942	AF091966
Comarostaphylis discolor (Hooker) Diggs	MV 0593 (SFSU)	AF091946	AF091970
Comarostaphylis discolor 2	UCBot 68.0109	AF091945	AF091969
*Comarostaphylis discolor 3	UCBot 76.2006	AF091943	AF091967
*Comarostaphlis diversifolia (Parry) Greene	UCBot 85.1235	AF091947	AF091971
Ornithostaphylos oppositifolia (Parry) Small	V 0708 (SFSU)	N/A /AF091962	AF091987
*Xylococcus bicolor Nutt.	V 0737 (SFSU)	AF091948	AF091973
Outgroup:			
Vaccinium ovatum Pursh	YAU 018 (SFSU)	AF091941	AF091965
*Enkianthus campanulatus G. Nicholson	Arnold Arb. 14528C	AF091940	AF091964
Pyrola rotundifolia L.	Kron 1906 (NCU)	AF091939	AF091963

north along the coast of California and farther south into the Baja California peninsula.

In this paper, we estimate phylogenetic relationship among species of the Arbutoideae based on molecular sequence data from the ITS region (ITS1, 5.8S, and ITS2) and part of the 28S region of nuclear ribosomal DNA (rDNA). Testing for a constant rate of molecular evolution across the arbutoid rDNA tree, and calibrating nodes with the fossil record allows us to investigate historical biogeography within the group, and determine the extent to which disjunct taxa in the Arbutoideae fit the Madrean-Tethyan hypothesis.

MATERIALS AND METHODS

Sequencing DNA from Arbutoideae Taxa and Outgroups. Genomic DNA was isolated from dried leaf tissue following a CTAB extraction protocol (Cullings 1992). Representatives from all genera of Arbutoideae were included in our analyses (Table 2). We included three of the four Mediterranean Basin species of *Arbutus* (*A. andrachne, A. canariensis,* and *A. unedo*); *A.* x *andrachnoides,* of putative hybrid origin between *A. andrachne* and *A. unedo,* was not included. In addition, we sampled six of the ca. eight North American species of *Arbutus* (*A. occidentalis, A. peninsularis, A. arizonica, A. xalapensis, A. menziesii,* and *A. texana*). Of the ca. 62 species of *Arctostaphylos,* we included five species representing both subgenera, subgenus *Micrococcus* (*A. nummularia*) and subgenus *Arctostaphylos* (*A. hookeri, A. uva-ursi, A. andersonii,* and *A. tomentosa*; Wells 1992). Molecular phylogenetic analysis of *Arctostaphylos* (Markos et al. 1999) does not support this classifi-

cation, but rather suggests that species of *Arctostaphylos* fall within two main lineages: lineage I (represented by *A. nummularia* and *A. hookeri*) and lineage II (represented by *A. uva-ursi, A. tomentosa,* and *A. andersonii*). We included both species of *Arctous* (*A. rubra* and *A. alpina*) in this analysis, as well as the monospecific genera *Ornithostaphylos* (*O. oppositifolia*) and *Xylococcus* (*X. bicolor*). *Comarostaphylis* consists of nine species and no subgeneric segregation is considered warranted (Diggs 1995). We sampled *C. diversifolia* (considered morphologically distinct from the other species of *Comarostaphylis,* Diggs 1995), *C. arbutoides,* and *C. discolor.*

Taxa of Arbutoideae have been treated at the tribal level (Arbuteae) within the subfamily Vaccinioideae (Stevens 1971). Recent molecular phylogenetic work (Kron and Chase 1993; Kron 1996) suggests that *Enkianthus, Pyrola,* and Arbutoideae (represented by *Arbutus* [Kron 1996], or *Arbutus* and *Arctostaphylos* [Kron and Chase 1993]) form basal lineages within Ericaceae. *Vaccinium* is nested within the major clade of Ericaceae. Based on this phylogenetic hypothesis, we included *Vaccinium ovatum, Enkianthus campanulatus* and *Pyrola rotundifolia* as outgroup taxa.

Three hundred base pairs of the rDNA large subunit (28S) were PCR-amplified following Markos et al. (1999) with the plant specific primer 28KJ (Cullings 1992), and either the universal primer 28C or the universal primer 28B (Cullings 1992). Based on alignment with 28S sequence data from *Arabidopsis thaliana* (GenBank accession X52320), the region of 28S sequenced from Arbutoideae taxa corresponds to base pair 324–623 from the 5′ end of the large subunit. The region of rDNA including ITS1, 5.8S, and ITS2 was amplified using the compliment-28KJ primer (Cullings 1992) and the universal primer its5 (White et al. 1990). PCR products were sequenced manually following Markos et al. (1999) or using an ABI 377 automated sequencing system following ABI dye-terminator suggested protocol. We used primers 28KJ, 28B, and 28C for sequencing the rDNA large subunit, and its1, its2, its4, and its5 (White et al. 1990) for sequencing the region including ITS1, 5.8S, and ITS2. ITS1, 5.8S, and ITS2 sequence data from *Arbutus peninsularis* and *A. occidentalis* were kindly provided by Dr. Aaron Liston.

Sequences were edited and aligned using the sequence analysis program Sequencher (Genecodes Corp.). Final alignments were determined manually. In some regions alternative alignments were possible between genera. In these cases we selected the alignment that minimized the number of potentially informative sites. In some regions of the ITS1 and ITS2, outgroup alignment was random relative to the ingroup, in which case we re-coded the outgroup sequences as missing data. At least one attempt at optimal alignment within these regions provided the same results as when outgroup sequences were coded as missing data.

Phylogenetic Analysis. Phylogenetic analyses were conducted using PAUP* 4.0b1 (Swofford 1998). Parsimony trees were generated with the branch and bound search option, and gaps were treated as missing data. Characters were treated as equally weighted and unordered. Bootstrap percentages (Felsenstein 1985) were estimated with 1000 heuristic search replicates, gaps treated as missing, and the simple stepwise addition and TBR options invoked. The decay index (DI; Bremer 1988; Donoghue et al. 1992) for clades of interest were determined by searching for the shortest tree lacking a given constraint clade with the heuristic algorithm (as described above for bootstrap analysis).

Due to computational limitations, maximum likelihood trees were generated for a subset of 12 taxa (Table 2). We pruned taxa containing regions of missing data while maintaining representation from the major lineages found in the most parsimonious trees; *Enkianthus campanulatus* was used as an outgroup. Three models of molecular evolution were investigated following the approach of Baum et al. (1998). The simplest model (F81) assumes equal rates of change across sites and equal probabilities of transitions and transversions (Felsenstein 1981). The HKY85 model differs from F81 by allowing the transition/transversion bias to be estimated by maximum likelihood and allowing for unequal base frequencies (Hasegawa et al. 1985). The HKY85-Γ is based on the HKY85 model but allows for among site rate heterogeneity using a discrete approximation to a Γ distribution with four rate categories and estimating the shape parameter, α, by maximum likelihood. All maximum likelihood searches were conducted with the heuristic search algorithm as implemented in PAUP* with the as-is stepwise addition and TBR options invoked. The likelihood scores of the trees found under each model were compared using a likelihood ratio test (Felsenstein 1981; Goldman 1993; Yang et al. 1995; Huelsenbeck and Rannala 1997).

To determine if the data support *Arbutus* paraphyly significantly better than *Arbutus* monophyly, we conducted Wilcoxon sign-rank (Templeton 1983; Larson 1994; Mason-Gamer and Kellogg 1996) and Kishino-Hasegawa (Kishino and Hasegawa 1989)

tests as implemented in PAUP*. The Kishino-Hasegawa test was performed with the HKY85-Γ assumptions of molecular evolution and empirical base frequencies. The two topologies compared were one of the six equally most parsimonious trees, and one of 16 equally most parsimonious trees from a branch and bound search with the constraint of *Arbutus* monophyly enforced.

Historical biogeography of Arbutoideae was inferred from the phylogenetic estimate using standard Fitch parsimony character optimization in MacClade (Maddison and Maddison 1992; Fritsch 1999). The data matrix was constructed by coding "area" as a single multistate character (character states: North America, Mediterranean Basin, Asia).

Molecular Clock Analysis. A molecular clock hypothesis requires that the rate of molecular change is relatively constant for a particular gene and group of taxa to be useful in calibrating a linear timeline of divergences (Hillis et al. 1996). We used a likelihood-ratio test to test for rate-constant evolution (Felsenstein 1981, 1993; Huelsenbeck and Rannala 1997; Baum et al. 1998). The likelihood score of the optimal tree generated under the HKY85-Γ model of sequence evolution was compared to the likelihood score of the optimal tree generated under the same model, but with a molecular clock enforced. A likelihood-ratio test was also performed, in the same manner as described above, but with the constraint of *Arbutus* monophyly enforced. If the likelihood ratio, $2[-\ln L1 + \ln L2]$, is significant as determined from a X^2 test with ten degrees of freedom (number of taxa $-$ 2), then the null hypothesis of rate homogeneity across the tree must be rejected. If clock-like behavior cannot be rejected, estimates of optimal tree branch lengths can be considered proportional to time. Standard errors around nodes were obtained using the computer program PAML (Yang 1998) by inputting a user tree under the HKY85-Γ model with a molecular clock enforced.

RESULTS

Aligned sequences of the ITS region spanned 682 bp, and partial sequence of the large subunit spanned 298 bp. The percent of variable characters with all taxa considered (with only ingroup taxa considered) was 46% (28%) for ITS1, 10% (8%) for 5.8S, 46% (33%) for ITS2, and 31% (29%) for the region of 28S sequenced. The percent of phylogenetically informative characters with all taxa considered (with only ingroup taxa considered) was

21% (10%) for ITS1, 5% (3%) for 5.8S, 14% (8%) for ITS2, and 20% (19%) for the region of 28S sequenced. Sequence data were combined into a single matrix. Of the total data matrix, 5.4 percent of sites were coded as missing. This is due in large part to four regions for which sequence data were not collected because of difficulty in amplifying DNA from herbarium material: the ITS1 regions from *Ornithostaphylos oppositifolia* and *Arctous alpina,* the 5.8S region from *Arbutus texana,* and the large subunit region from *Arbutus occidentalis.*

Phylogenetic Analysis. Parsimony analysis resulted in six equally most parsimonious trees of 548 steps that differ only in the relationship among western North American species of *Arbutus* (Fig. 1). These trees support *Arctostaphylos* and *Arctous* as monophyletic (100% bootstrap support for each clade, and DI of 12 and 6, respectively), and that species of *Comarostaphylis* form a monophyletic group but with low bootstrap support (<50%) and a DI of 1. *Arbutus* is not monophyletic in this analysis. Rather, Mediterranean Basin species of *Arbutus* are more closely related to the other five North American genera than to species of western North American *Arbutus* (77% bootstrap support, DI=4). The other inter-generic relationship with moderate support is the clade containing *Arctostaphylos, Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus* (bootstrap of 63%, DI=3).

The maximum likelihood analyses resulted in the same topology corresponding to a pruned version of the parsimony trees (Fig. 1). Based on likelihood ratio tests, we determined that the HKY85-Γ model of evolution fits the data significantly better than either the HKY85 or the F81 models (Table 3). Under the HKY85-Γ model, the α estimate was 0.16, and the Ti/Tv ratio estimate was 3.15.

We considered the apparent paraphyly of *Arbutus* to warrant further analytical investigation. Specifically, we investigated whether there is significant conflict between the most parsimonious topology (*Arbutus* paraphyly) and the most parsimonious trees in which western North American and Mediterranean Basin species of *Arbutus* form a monophyletic group. The Wilcoxon signed-rank test (P = 0.011) suggests that the difference in tree topologies between the most parsimonious (paraphyly) and the alternative (monophyly) hypotheses cannot be explained by random variation within the data. In contrast, the Kishino-Hasegawa test (P = 0.39) suggests that the data do not significantly discriminate significantly between the two trees.

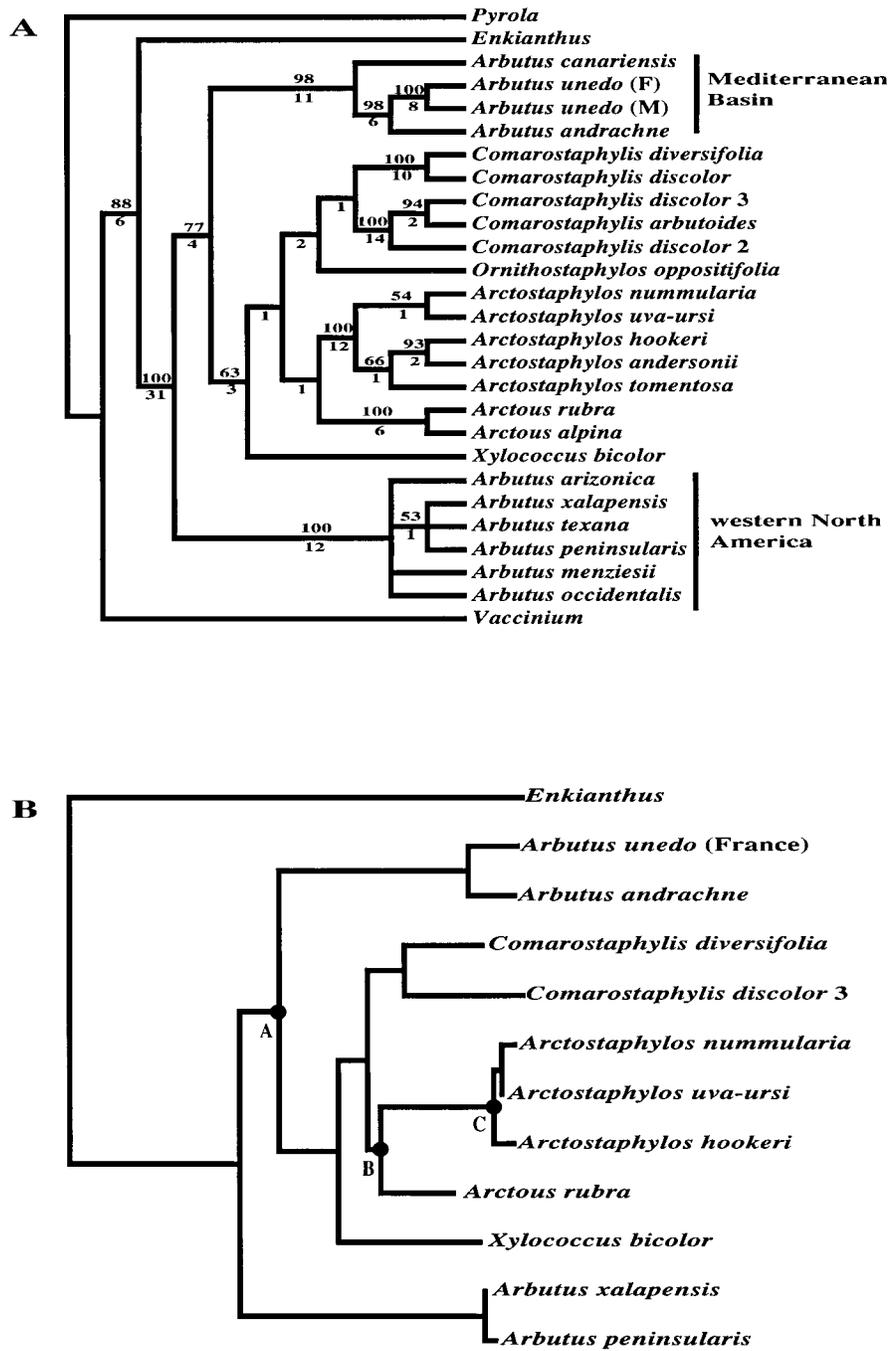*Molecular Clock Analysis.* The maximum like-

FIG. 1. Results of phylogenetic analyses. A) Strict consensus of six equally most parsimonious trees from branch and bound search with gaps treated as missing data, length=548 steps, CI=0.706, RI=0.812, RC=0.573. Numbers above branches are bootstrap values, numbers below branches are decay indices. F or M following *Arbutus unedo* indicates France or Morocco respectively. B) Single tree found in maximum likelihood analyses under three models of molecular evolution; F81, HKY85, and HKY85-Γ.

TABLE 3. Comparison of likelihood scores for different models of evolution. In each comparison, model 1 is the simpler model and represents a special case of model 2. The likelihood-ratio test is based on twice the difference between the log-likelihoods for the two models [2(−lnL1+lnL2)]. The P values are based on a chi-square distribution with either 1 (in the comparison between models) or 10 (in the comparison of likelihood scores with and without a molecular clock) degrees of freedom. [1] Comparison of clock vs. non-clock models with the constraint of *Arbutus* monophyly enforced. ** P < 0.001.

Model 1	Model 2	[−log L1]	[−log L2]	[2(−lnL1+lnL2)]
F81	HKY	3084.61	2988.27	192.68**
HKY	HKY-Γ	2988.27	2892.43	191.68**
HKY-Γ (no clock)	HKY-Γ (clock)	2892.43	2897.86	10.86
[1]HKY-Γ (no clock)	[1]HKY-Γ (clock)	2890.76	2897.06	12.60

lihood tree generated under the HKY85-Γ model of evolution with the enforcement of a molecular clock corresponds to a rooted version of the HKY85-Γ tree without the enforcement of a molecular clock. A likelihood ratio comparison of the clock and non-clock versions of the HKY85-Γ model could not reject the hypothesis of a molecular clock (P>0.25; Table 3). With *Arbutus* monophyly enforced, a likelihood ratio comparison of the clock and nonclock versions of the HKY85-Γ could not reject the hypothesis of a molecular clock (data not shown). Branch lengths and standard errors around nodes of interest are presented in Figure 2.

To test the Madrean-Tethyan hypothesis, we estimated the divergence time between Mediterranean Basin and North American Arbutoideae (node A, Fig. 1). The oldest fossil identified as *Arctostaphylos* (*A. masoni*) is from leaf impressions dated at 15.8 MYA from the Stewart Spring Flora, Nevada (Wolfe 1964; Shorn unpublished data). The combination of smooth, flat leaf margins with margin tertiary venation forming a series of ladder-like loops distinguishes these fossils from other genera of the Arbutoideae. This fossil *Arctostaphylos* can be mapped to the Arbutoideae phylogeny between node B and node C (Fig. 1). Mapping fossil data at node B provides a conservative (recent) estimate for the timing of divergence between Mediterranean Basin species of *Arbutus* and the primarily North American taxa of Arbutoideae; the conservative estimate is 21.2 to 39.2 MYA (Fig. 2). Mapping fossil data at node C results in an estimated divergence of 99.6 to 240.2 MYA, near the origin of Angiosperms (Doyle and Hickey 1976; Sun et al. 1998). The divergence of North American species from Mediterranean Basin species if *Arbutus* is constrained to be monophyletic is estimated to be between 32 and 168 MYA (data not shown).

DISCUSSION

Phylogenetic Relationships. Historically, many arbutoid genera (*Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus*) have been placed in *Arctostaphylos* (reviewed by Diggs and Breckon 1981). Both maximum parsimony and maximum likelihood analyses suggest that each of these genera (including *Arctostaphylos*) form monophyletic groups. Therefore, classifications that recognize the above genera are consistent with this analysis (Adams 1940; Stevens 1971; Diggs and Breckon 1981). However, our phylogenetic estimate for Arbutoideae taxa does not reject alternative classifications such as those of Klotzsch (1851), Walpers (1852), and Webb (1972), which subsume *Arctous* within *Arctostaphylos*. Our analyses also do not reject classifications that subsume *Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus* within *Arctostaphylos* (Hooker 1876; Parry 1884; Gray 1886; Wilber and Luteyn 1978).

Few studies have addressed generic relationships within the Arbutoideae (Diggs and Breckon 1981). In the absence of an explicit phylogenetic analysis based on morphology, the hypothesis that *Arctostaphylos, Arctous, Comarostaphylis, Ornithostaphylos,* and *Xylococcus* form a monophyletic group is supported by putative synapomorphies including drupaceous fruit with one ovule per locule and a stony endocarp. Our analyses always find *Arbutus* to be non-monophyletic; in addition, the Templeton test rejects the alternative hypothesis of *Arbutus* monophyly. The Kishino-Hasegawa test, however, does not reject *Arbutus* monophyly. Because the Kishino-Hasegawa test is parametric, as opposed to the nonparametric Templeton test, the Kishino-Hasegawa test may not perform well if assumptions of the underlying model (all nucleotide sites are independently and identically distributed) are not
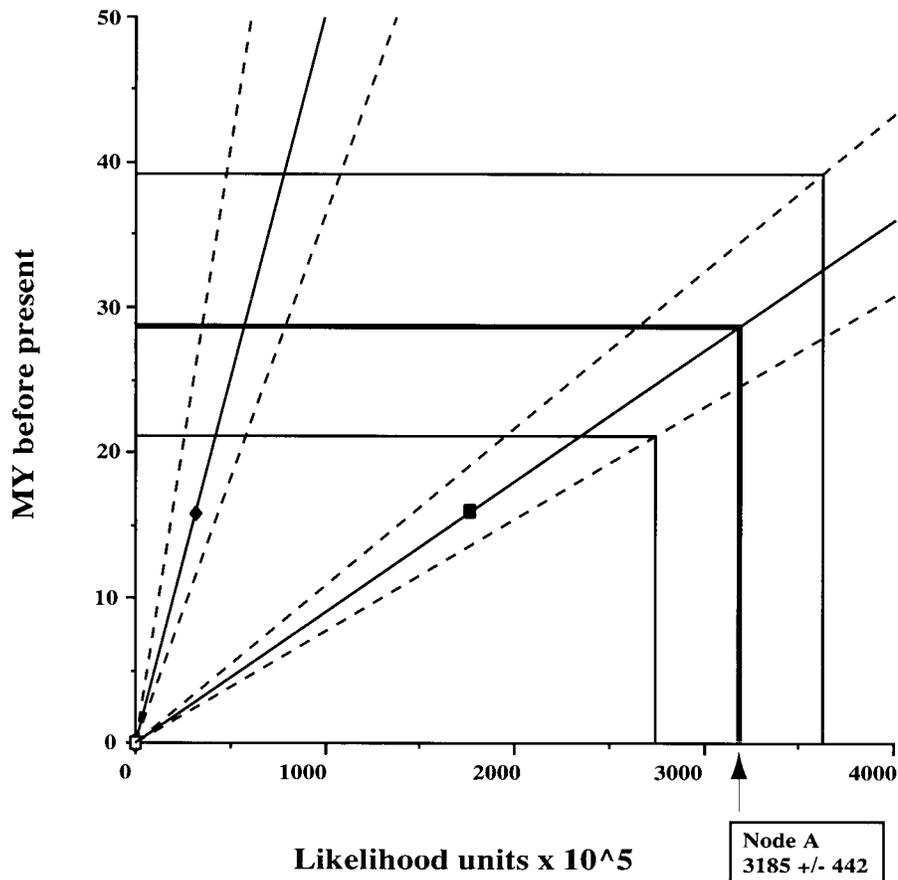
Fig. 2.   Calibration of likelihood branch lengths, including standard error around nodes, against the fossil record. Along the X axis is relative branch length in likelihood units x 10^5; along the Y axis is a time-line in millions of years before present. The regression line through ♦ corresponds to a molecular clock calibration with node C (Fig. 1) fixed at 15.8 MY before present. The regression line through ■ corresponds to a molecular clock calibration with node B (Fig. 1) fixed at 15.8 MY before present. Dashed lines around both regressions indicate standard errors around branch lengths. Relative branch length in likelihood units x 10^5 for nodes A, B, and C (Fig. 1) are 3185 ± 442, 1754 ± 294, and 314 ± 121 respectively.

met (Swofford et al. 1996). This may well be the case for rDNA sequence data from the Arbutoideae. The paraphyletic relationship of *Arbutus* species implies that characters formerly used to diagnose the genus (fleshy fruit with a soft endocarp, numerous ovules per locule and a papillose fruit surface) may be plesiomorphic for the Arbutoideae, or the result of convergent evolution.

Biogeography.   In Axelrod's original formulation (Axelrod 1973, 1975) *Arbutus* was described as a genus conforming to the expectations of the Madrean-Tethyan hypothesis. For *Arbutus* to be consistent with the Madrean-Tethyan hypothesis, we expect *Arbutus* taxa, exhibiting a disjunct distribution,

to form sister clades. Because we do not find species of *Arbutus* to be monophyletic in this analysis, it is possible to view the question of disjunction from within the Arbutoideae. In doing so, the sister group comparison for addressing vicariance between western North America and the Mediterranean Basin are the clade containing Mediterranean Basin species of *Arbutus* and its sister clade containing species of *Arctous, Arctostaphylos, Comarostaphylis, Ornithostaphylos,* and *Xylococcus* (Fig. 1).

The second expectation of the Madrean-Tethyan hypothesis is a divergence time of greater than approximately 25 MY, i.e., the boundary between the Paleogene and Neogene or earlier. The question of

divergence time can be addressed in the context of a molecular clock applied to a phylogenetic hypothesis. When the standard error around branch lengths are considered, this earliest possible date of divergence is 21.2 MYA, just after the Paleogene/Neogene boundary. Based on this analysis, we are, strictly speaking, unable to accept the Madrean-Tethyan hypothesis for vicariance within the Arbutoideae. The fossil species, *A. masoni,* does not correspond to any extant lineage within *Arctostaphylos,* giving us confidence that it should be placed somewhere below node C (Fig. 1). Placement at node B provides us with the most recent estimate of divergence between Mediterranean Basin *Arbutus* species, and the remaining five genera (node A). Using this conservative estimate, we still find that most of the estimated range lies within the time frame prior to 25 MYA, and is therefore consistent with the second expectation of the Madrean-Tethyan hypothesis.

Although there may be many alternatives, we suggest the following historical scenario that is consistent with the phylogenetic estimate of the Arbutoideae based on rDNA sequences. During the late Cretaceous and Early Tertiary, an *Arbutus* complex, including ancestral taxa belonging to lineages I, II and III (Fig. 3) evolved in western North America (Axelrod 1958). During equable climates (low mean annual range of temperature and high mean annual temperature) at high latitudes (Axelrod 1975; Savin 1977; Wolfe 1978; Tiffney 1985a, 1985b), this complex spread across North America and ancestral taxa of lineage III dispersed into Eurasia. Axelrod suggested that *Arbutus* was part of the Madro-Tertiary geoflora, a broadleaved sclerophyllous vegetation that extended across North America and Eurasia during the Paleogene. Recent analyses (Wolfe 1975; Tiffney 1985a, 1985b) present little evidence for such a geoflora due to the absence of a continuous dry belt from the southwestern United States to the Mediterranean region during the Eocene. Wolfe (1975) suggested that a humid to mesic flora (the boreotropical flora), with no clear center of origin, evolved and spread across northern latitudes during the Paleogene. The spread of the boreotropical flora was aided by the Bering and North Atlantic land bridges (Wolfe 1975; McKenna 1983; Tiffney 1985b). Wolfe also stated that "the presence of a genus such as *Arbutus* in both western North America and Asia Minor in all probability is a reflection of a more wide-spread distribution of the genus in humid to mesic broadleaved and coniferous forests of the Paleogene." Axelrod (1966) has,

in fact, recorded *Arbutus* from an Eocene flora, for which he inferred a "microthermal climate with abundant precipitation well distributed throughout the year."

Once this early *Arbutus* complex achieved a North American/Eurasian distribution, it was affected by climatic and geographic events during the early to middle Tertiary (Wolfe 1975; McKenna 1983; Tiffney 1985a, 1985b). Our analysis supports a migration route between eastern North America and Europe through the Oligocene via microcontinents and volcanic island stepping stones (Axelrod 1975). As reviewed by Tiffney (1985b), many investigators agree that a migration route between eastern North America and Eurasia across North Atlantic land bridges persisted only until the late Eocene. A divergence time earlier than 21.2 MYA for North American and mediterranean Arbutoideae does not reject late Eocene vicariance.

Uplift of the Rocky Mountains and deteriorating climates in the Oligocene led to the spread of savannas, followed by grasslands across much of central North America (Tiffney 1985b). These climatic changes likely affected the distribution of Arbutoid taxa, restricting lineages I and II to western North America. The major radiation of Arbutoideae in western North America (primarily within *Arctostaphylos* and *Comarostaphylis*) did not occur until the Pleistocene, following their origin in the Miocene (Raven and Axelrod 1978). The development of diverse edaphic conditions, recurrent climatic fluctuations and diverse topography in the late Tertiary contributed to rapid speciation in this group.

The phylogenetic relationships and biogeographic histories of taxa comprising the Mediterranean Flora are complex, and it is becoming clear that there are low levels of correlated history among the different groups investigated (Liston et al. 1992; Liston and Kadereit 1995; Fritsch 1996; Liston et al. 1989a; Liston et al. 1989b; Liston et al. 1999; Manos et al. 1999). The approach taken in this analysis has provided insight into phylogenetic relationships within the Arbutoideae. In addition, the clock-like behavior of rDNA within the Arbutoideae and the presence of a fossil record has allowed us to rigorously test the Madrean-Tethyan hypothesis. We find that the divergence of North American from Mediterranean basin Arbutoideae supports that Madrean-Tethyan hypothesis; however, earlier divergence times are not rejected by our data. Studies of this kind, that investigate additional taxa belonging to the Mediterranean Flora, will lead to a clear-
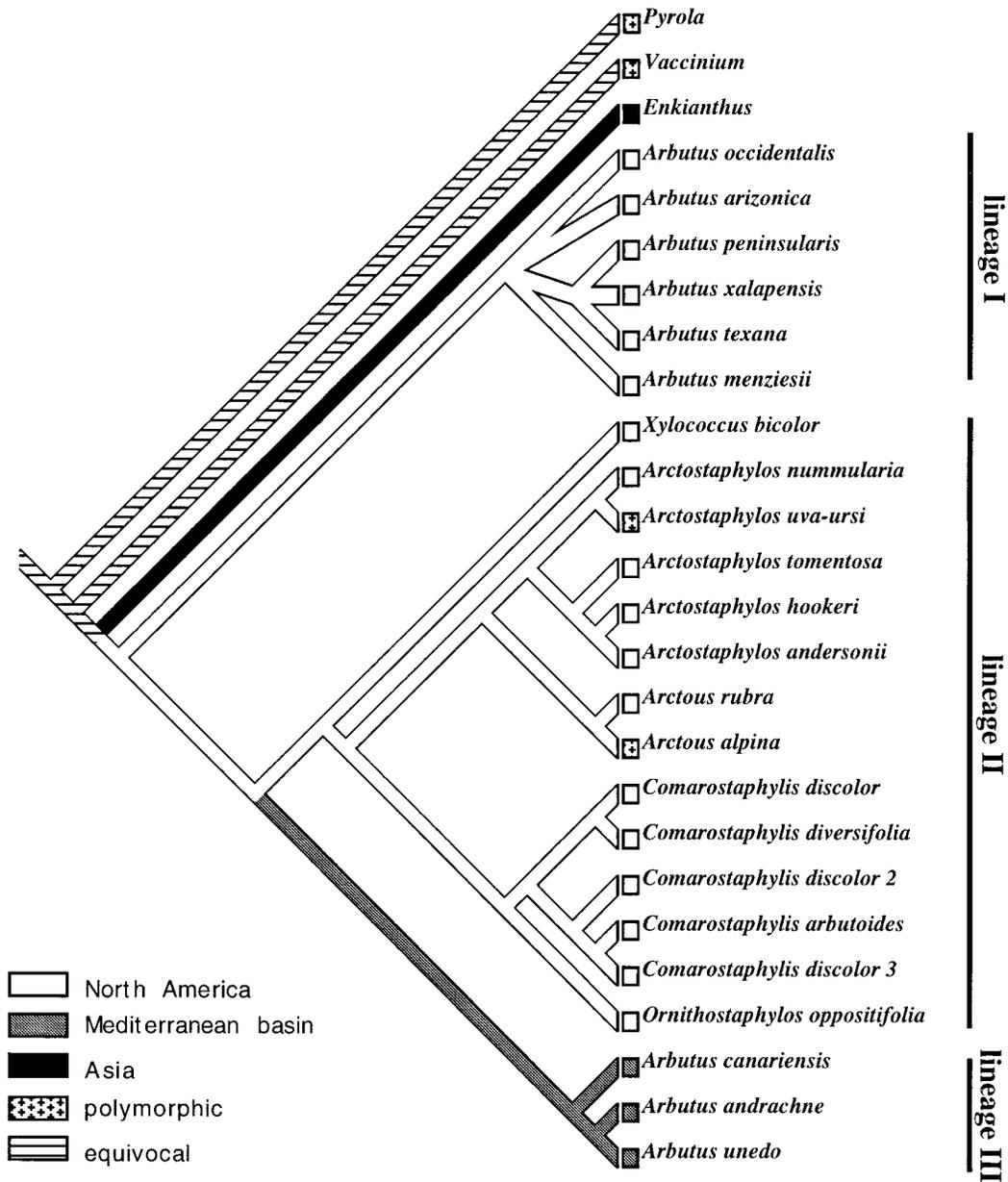
FIG. 3. Optimization of ancestral state area assignments using Fitch parsimony analysis. Lineages I, II and III refer to the ancestral lineages of the Arbutoideae that are thought to have evolved in western North America during the early Tertiary (see discussion).

er understanding of Northern Hemisphere historical biogeography.

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