



**Phylogenetic Relationships in Maloideae (Rosaceae): Evidence from Sequences of the Internal Transcribed Spacers of Nuclear Ribosomal DNA and Its Congruence with Morphology**

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# PHYLOGENETIC RELATIONSHIPS IN MALOIDEAE (ROSACEAE): EVIDENCE FROM SEQUENCES OF THE INTERNAL TRANSCRIBED SPACERS OF NUCLEAR RIBOSOMAL DNA AND ITS CONGRUENCE WITH MORPHOLOGY<sup>1</sup>

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We used sequences from both internal transcribed spacers (ITS) and a small portion of the 5.8S gene of nuclear ribosomal DNA (nrDNA) for phylogenetic reconstruction of 19 genera of Maloideae and four potential outgroups from the Rosaceae. Parsimony analyses indicate that Maloideae are not monophyletic; *Vauquelinia*, which is traditionally placed in Spiraeoideae, and two genera of the Maloideae, *Eriobotrya* and *Rhaphiolepis*, form a well-supported clade that is the sister to the remainder of the subfamily. Although our ITS phylogenetic hypothesis is highly resolved, there is considerable homoplasy, and support, as indicated by bootstrap values and decay indices, is relatively weak for all groups except four: *Eriobotrya-Rhaphiolepis-Vauquelinia*, *Crataegus-Mespilus*, *Amelanchier-Peraphyllum-Malacomeles*, and *Cydonia-Pseudocydonia*. Our DNA sequence data do not support a broad interpretation of *Sorbus*. Intergeneric hybridization, which is prevalent in Maloideae, occurs between genera that are far removed from one another on our most-parsimonious trees. We infer an overall phylogeny from separate analyses of ITS DNA sequences and recently published morphological and wood anatomical studies of Maloideae and from analyses after pooling these data sets. The four most strongly supported clades of the ITS phylogeny appear in the phylogeny based on pooled data.

The Maloideae contain  $\approx 28$  genera and 940 species of mostly north temperate trees and shrubs, including economically and ecologically important groups, such as apples, pears, cotoneasters, mountain ashes, hawthorns, and shadbushes (Robertson et al., 1991). This subfamily is remarkable for its hypothesized allopolyploid origin, extensive intergeneric hybridization, intergeneric grafting compatibility, and high incidence of polyploidy and apomixis (reviewed in Campbell, Green, and Dickinson, 1991; Phipps et al., 1991; Robertson et al., 1991). The perceived history of hybridization has strongly influenced taxonomic concepts of the subfamily and its genera.

The allopolyploid origin of the Maloideae from Amygdaloideae and Spiraeoideae ancestors has many proponents (Sax, 1932; Stebbins, 1950; Challice and Kovanda, 1981; Phipps et al., 1991). Primary evidence for this origin

is unique fruit type (pome) and base chromosome number ( $x = 17$ ; amygdaloids are  $x = 8$  and spiraeoids mostly  $x = 9$ ). When fruit type and base chromosome number are emphasized, the subfamily is asserted to be monophyletic (Phipps et al., 1991). Wood anatomy, even in conjunction with these two potential synapomorphies, however, does not establish monophyly of the Maloideae (Zhang, 1992), and groups lacking these potential synapomorphies vie for inclusion. For example, *Kageneckia*, *Lindleya*, and *Vauquelinia*, with dry fruits and  $n = 15$  or  $17$ , are traditionally assigned to the Spiraeoideae, but morphological, anatomical, cytological, and DNA sequence data point to affinities with Maloideae (Stebbins, 1958; Sterling, 1966; Goldblatt, 1976; Savile, 1979; Morgan, Soltis, and Robertson, 1994).

Delimitation of genera of Maloideae has varied with the importance attached to intergeneric hybridization. *Pyrus* and *Sorbus*, in particular, and the genera they resemble morphologically and/or with which they hybridize (e.g., *Aria*, *Aronia*, *Chamaemespilus*, *Cornus*, *Malus*, *Torminalis*) have been treated as either one genus or up to eight genera. Robertson (1974) united all five segregates of *Sorbus* s.l. plus *Malus* and *Aronia* into *Pyrus*, and Sax (1931) broached the extreme position of lumping all Maloideae under one genus. In contrast, Robertson et al. (1991) discounted the taxonomic importance of intergeneric hybridization and recognized *Malus*, *Pyrus*, and five genera in place of a broad interpretation of *Sorbus*.

Relationships of other genera of Maloideae are also poorly understood. Two formal subdivisions of the sub-

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family have been proposed. One divides the genera into those wherein the ovary wall hardens and each carpel develops into a separate pyrene (Crataegeae) and those with a membranous to cartilaginous ovary wall and united carpels (Sorbeae; Koehne, 1891). The other subdivision defines the Maleae as having two (rarely one) ovules per carpel as opposed to several in the *Cydonia* group (Kalkman, 1988). Recent studies of the Maloideae based on morphology (Phipps et al., 1991; Rohrer, Robertson, and Phipps, 1991, 1994) and wood anatomy (Zhang, 1992) do not support either of these divisions. Phenotypic similarities, in some cases supplemented by crossability and grafting compatibility, tie some taxa into groups of two (e.g., *Eriobotrya* and *Raphiolepis*; *Crataegus* and *Mespilus*; *Pyrus* and *Cydonia*; and *Cotoneaster* and *Pyracantha*) or three (e.g., *Amelanchier*, *Malacomeles*, and *Peraphyllum*). Beyond this there is little agreement about suprageneric groupings.

We used DNA sequences to augment morphological and other data on phylogenetic relationships among 19 of the larger genera of the Maloideae. We chose nuclear DNA sequencing to avoid problems of uniparentally inherited genomes, such as chloroplast DNA, in groups characterized by extensive hybridization (Rieseberg and Soltis, 1991; Rieseberg and Brunsfeld, 1992). The 18S–26S nrDNA gene family has been widely used at all taxonomic levels in plant phylogenetic and evolutionary studies (e.g., Hillis and Dixon, 1991; Schaal, O’Kane, and Rogstad, 1991; Baldwin, 1992, 1993; Hamby and Zimmer, 1992; Suh et al., 1993; Wojciechowski et al., 1993; Kim and Jansen, 1994; Baldwin et al., in press; Wendel, Schnabel, and Seelanan, in press). Systematic versatility of nrDNA comes from varying rates of evolution among different regions, ease of polymerase-chain-reaction (PCR) amplification because of multiple copies in this midrepetitive gene family (hundreds to thousands of repeats on one or more chromosomes), and intraspecific repeat homogeneity through concerted evolution. The generally high levels of concerted evolution in nrDNA raise the probability that parsimony analysis of nrDNA sequences will find the correct phylogeny (Sanderson and Doyle, 1992).

DNA sequencing of ITS 1 and ITS 2 has been phylogenetically insightful within and among genera of angiosperms (e.g., Baldwin, 1992, 1993; Suh et al., 1993; Wojciechowski et al., 1993; Baldwin et al., in press, and references therein). These regions separate the 18S and 5.8S genes and 5.8S and 26S genes, respectively, and appear to be instrumental in processing the mature RNA into the three gene products (van der Sande et al., 1992). ITS 1 ranges from 187 to 298 base pairs (bp) and ITS 2 from 187 to 252 bp in flowering plants (Baldwin et al., in press).

The recent advent of phylogenetic hypotheses based on molecular data poses questions of how and when to integrate these with phylogenetic information from morphology (Kluge, 1989; Barrett, Donoghue, and Sober, 1991; Swofford, 1991; Donoghue and Sanderson, 1992; Doyle, 1992; Bull et al., 1993; de Queiroz, 1993; Chippendale and Wiens, 1994). To integrate independent data sets, one may combine the data themselves prior to phylogenetic analysis or evaluate the consensus of separate phylogenetic analyses. According to de Queiroz (1993), the choice

of method depends upon independence of characters within data sets and congruence among data sets. The combined approach is likely to produce better phylogenetic estimates when characters are independent within data sets and separate analyses do not strongly conflict with one another. With nonindependence within one or more data sets and strongly divergent phylogenetic signals (e.g., as judged by bootstrap analyses), de Queiroz favors consensus, and both approaches are encouraged when there is internal nonindependence but not strong conflict. Congruence among separate data sets may be assessed by the extent of agreement between taxonomic patterns (taxonomic congruence) or between data sets (character congruence). The former approach suffers from the fact that taxonomic pattern is removed from underlying character evidence (Miyamoto, 1985; Kluge, 1989).

We explored congruence of our ITS sequence data with phylogenetic data from recent studies of Maloideae morphology (Phipps et al., 1991) and wood anatomy (Zhang, 1992). Phipps et al.’s work is the most relevant to our ITS study because it included 36 phylogenetically informative characters for 18 of the genera for which we have DNA sequences; Zhang’s work involved only seven phylogenetically informative characters and 15 genera in common with our study. ITS DNA sequences are likely independent of morphology and wood anatomy, but the latter two data sets may not be for some characters (e.g., those associated with adaptation to xeric conditions). Because of the potential for nonindependence within each of the individual data sets and the apparent lack of strong conflict among data sets, we analyzed both separate and pooled data sets.

## MATERIALS AND METHODS

**Genera of Maloideae**—We sampled single representatives of 19 genera of Maloideae (Table 1). This includes all major genera of the subfamily as defined by Robertson et al. (1991). Together they account for  $\approx$  900 species or 96% of the species recognized in the subfamily; the other nine genera contain a total of 32 species, and the only genera with more than four species are the South and Central American *Hesperomeles* (11 species; we did not have access to fresh material of this genus) and Asian *Pyracantha* (nine species; we were unable to obtain unambiguous DNA sequences for all of ITS 1 and 2 of this genus). For outgroups we included representatives of the other three commonly recognized subfamilies—Amygdaloideae (*Prunus*), Rosoideae (*Rosa*), and Spiraeoideae (*Spiraea* and *Vauquelinia*, the latter a genus of three species of southwestern North America [Hess and Henrikson, 1987]).

**Plant samples**—Total genomic DNAs were extracted from either field-collected (stem cuttings maintained in cold water for up to 5 d, then stored at  $-80^{\circ}\text{C}$ ) or greenhouse-maintained plants (Table 1) using the  $2\times$  CTAB procedure of Doyle and Doyle (1987). DNAs were further purified by centrifugation to equilibrium in cesium chloride-ethidium bromide gradients (Sambrook, Fritsch, and Maniatis, 1989).

TABLE 1. Taxa of Maloideae and other Rosaceae used in phylogenetic analyses.

Taxon	Origin <sup>a</sup>	Accession
<b>Maloideae</b>		
<i>Amelanchier bartramiana</i> (Tausch) Roemer	Maine	CSC <sup>b</sup> , B3
<i>Aria alnifolia</i> (Sieb. & Zucc.) Decaisne <sup>c</sup>	South Korea	AA <sup>d</sup> , 17261
<i>Chaenomeles cathayensis</i> (Hemsl.) Schneider	China	CSC <sup>b</sup> , 92-6
<i>Cornus domestica</i> Spach	Denmark	AA <sup>d</sup> , 11671
<i>Cotoneaster lacteus</i> W. W. Smith	China	UA campus <sup>e</sup>
<i>Crataegus mollis</i> (T. & G.) Scheele	North America	CSC <sup>b</sup> , 94-1
<i>Cydonia oblonga</i> Miller	Asia	UA campus <sup>e</sup>
<i>Eriobotrya japonica</i> Lindley	China	UA campus <sup>e</sup>
<i>Heteromeles arbutifolia</i> M. Roemer	California	BTSA <sup>f</sup>
<i>Malacomeles denticulata</i> (Kunth) Engler	Mexico	T17M-96s <sup>g</sup>
<i>Malus domestica</i> Borkh.	Europe, Russia	CSC <sup>b</sup> , 92-2
<i>Mespilus germanica</i> L.	Europe	AA <sup>d</sup> , 1026-84
<i>Osteomeles schwerinae</i> Schneider	Hawaii	BTSA <sup>f</sup>
<i>Peraphyllum ramosissimum</i> Nutt.	Colorado	CSC <sup>b</sup> , 91-49
<i>Photinia pyrifolia</i> (Lamarck) Robertson and Phipps <sup>h</sup>	Maine	CSC <sup>b</sup> , 92-4
<i>Pseudocydonia sinensis</i> (Thouin) Schneider	South Korea	AA <sup>d</sup> , 534-83
<i>Pyrus calleryana</i> Decne.	China	CSC <sup>b</sup> , 94-10
<i>Raphiolepis indica</i> (L.) Lindley	China	UA campus <sup>e</sup>
<i>Sorbus aucuparia</i> L.	Europe	CSC <sup>b</sup> , 92-1
<b>Other Rosaceae</b>		
<i>Vauquelinia californica</i> (Torr.) Sarg.	North America	UA campus <sup>e</sup>
<i>Prunus cerasifera</i> Ehrh.	Asia	CSC <sup>b</sup> , 92-7
<i>Rosa</i> sp.	Unknown	UA campus <sup>e</sup>
<i>Spiraea</i> × <i>vanhouttei</i> (Briot.) Zab.	Asia	UA campus <sup>e</sup>

<sup>a</sup> Country, continent, or, if in the United States, state.

<sup>b</sup> Christopher S. Campbell, collection numbers; vouchers at the University of Maine Herbarium.

<sup>c</sup> Included in *Sorbus* by many authors (see Robertson et al., 1991).

<sup>d</sup> Arnold Arboretum, Jamaica Plain, MA; the number is the arboretum's accession number.

<sup>e</sup> Plantings on the University of Arizona campus; vouchers in the University of Arizona Herbarium (genus—collector, collection number or date): *Cotoneaster*—G. Starr, C29; *Cydonia*—G. Starr, C277; *Eriobotrya*—G. Starr, C169; *Raphiolepis*—K. L. Gibson, 2 April 1975; *Vauquelinia*—K. L. Gibson, 19 June 1975; *Spiraea*—R. B. Streets, 2 May 1946.

<sup>f</sup> Boyce Thompson Southwestern Arboretum, Superior, AZ.

<sup>g</sup> Yucca Do Nursery, Waller, TX.

<sup>h</sup> Commonly known as *Aronia arbutifolia* (L.) Elliott (see Robertson et al., 1991).

**PCR and DNA sequencing**—PCR amplification and sequencing of ITS and adjacent regions of nrDNA from the taxa in Table 1 followed procedures outlined by Baldwin (1992; see his Fig. 1). Single-stranded DNAs of ITS 1 and ITS 2 were amplified directly by 40 cycles of asymmetric PCR (20:1 molar ratio) using the “ITS” primers of White et al. (1990). PCR amplifications were performed in 25  $\mu$ l reactions containing 10–100 ng genomic DNA, 200  $\mu$ M deoxynucleotide triphosphates (equimolar), 0.5 units *AmpliTag* DNA polymerase (Perkin Elmer Cetus, Norwalk, CT), 1  $\mu$ l glycerol, and oligonucleotide primers at 25–500 nmol. PCR cycle conditions consisted of 1 min at 97 C for denaturation, 1 min at 48 C for primer annealing, and 45 sec at 72 C for primer extension. Primer extension times were increased by 4 sec with each cycle, followed by a final extension of 7 min. Single- and double-stranded DNA PCR products were analyzed together by electrophoresis in 1.5% agarose gels in 1 × TBE buffer (pH 8.3) and purified by differential filtration in Millipore Ultrafree-MC tubes (Millipore UFC3 THK00). Purified DNAs were sequenced according to reaction conditions specified by the manufacturers by the dideoxy-chain-termination technique using *Taq* DNA Polymerase (TA-Quence<sup>®</sup>, U.S. Biochemical Co., Cleveland, OH), [ $\alpha$ -<sup>35</sup>S]dATP (Amersham) and 7-deaza-dGTP, substituted for dGTP to prevent base compressions. Samples were

electrophoresed in 5% acrylamide–8M urea gels. Gels were fixed in 5% methanol/5% acetic acid for at least 30 min, vacuum dried at 80 C for 1 hr, and exposed to autoradiographic film for at least 12 hr.

Single-stranded DNA from ITS repeats was amplified using “ITS” primers (see Baldwin [1992] for base composition of primers) obtained from Operon Technologies, Inc. (Alameda, CA). “ITS5” and “ITS2” (in molar excess) were used to amplify the 3′-18S DNA-ITS 1-5.8S nrDNA-5′ region. This region was sequenced using primer “ITS5.” Single-stranded DNA of 3′-5.8S nrDNA-ITS 2–25S nrDNA-5′ sequences were amplified using primers “ITS3” and “ITS4” (in molar excess) and sequenced using primer “ITS3.” For almost all taxa both ITS 1 and ITS 2 were also amplified and sequenced in the reverse direction by reversing molar ratios of primers in the amplification and using the limiting primer for sequencing reactions. The sequences reported in this study are available in the GenBank Libraries (accession number U151591 for *Amelanchier* and numbers U16185–U16206 for the remaining taxa in Table 1).

DNA sequences were aligned by the PILEUP option of the computer program GCG (Genetics Computer Group) with slight modifications by eye. Alignment required incorporation of gaps scattered over ITS 1 and 2 regions. Indels were coded as missing data. Regions where align-

	10	20	30	40	50		110	120	130	140	150	
AMELANCHIER	TCGAACCTG	---CACA	GCAGAACGACCCGAGA	ACCAGTTT	---CAA	CG	AMELANCHIER	-----CCT	---GTCCCGGGAGCYCGCT	CCCCGG	---GCGC	
ARIA	.....	.....	.....	T.....	.....	.....	ARIA	-----T	---A.....	C.....	GC.TAT	
CHAENOMELES	.....	G.....	.....	G.....	.....	.....	CHAENOMELES	-----T	---A.....	C.....	.....T	
CORMUS	.....	---G.....	.....	G.....	.....	.....	CORMUS	-----TT	---A.....	C.....	GC.TA.	
COTONEASTER	.....	.....	C.....	G.....	CAA.....	.....	COTONEASTER	-----T	---A.....	C.....	---T	
CRATAEGUS	.....	---G.....	.....	.....	.....	.....	CRATAEGUS	-----YCGC	.....	C.....	.....	
CYDONIA	.....	A.....	.....	G.....	.....	.....	CYDONIA	-----T	---A.....	C.....	.....T	
ERIOBOTRYA	.....	---G.....	.....	A.....	T.G.....	.....	ERIOBOTRYA	-----T	---A.....	A.....	CT...T.....T	
HETEROMELES	C.....	---G.....	.....	.....	.....	.....	HETEROMELES	-----C	.....	C.....	.....T	
MALACOMELES	.....	.....	.....	.....	.....	.....	MALACOMELES	-----T	---A.....	TC.....	---T	
MALUS	.....	---G.....	.....	C.....	.....	.....	MALUS	-----T	---CGT..C..	GAGC.CGCT..	C.....T	
MESPILUS	.....	.....	.....	.....	.....	C.....	MESPILUS	-----T	GC.....	C.....	Y...GC...	
OSTEOMELES	.....	---G.....	.....	G.....	---A.....	.....	OSTEOMELES	-----T	---A.....	C.....	---T	
PERAPHYLLUM	.....	.....	.....	.....	Y.....	.....	PERAPHYLLUM	-----T	---A.....	TC.....	---T	
PHOTINIA	.....	G.....	G.....	.....	C.....	.....	PHOTINIA	-----T	---A.....	C.....	---T	
PSEUDOCYDONIA	.....	---T.....	.....	G.....	---A.....	.....	PSEUDOCYDONIA	-----T	---A.....	C.....	---T	
PYRUS	.....	---C.....	.....	C.....	C---G.....	.....	PYRUS	-----YT	---A.....	Y.....	---T	
RHAPHIOLEPIS	.....	---R.G.....	---R.....	A.....	Y.G.....	.....	RHAPHIOLEPIS	-----T	---A.....	A.....	AY...T.....T	
SORBUS	.....	.....	.....	.....	.....	.....	SORBUS	-----T	---A.....	C.....	---T	
VAUQUELINIA	.....	---R.....	---S.....	A.....	Y.G.....	.....	VAUQUELINIA	-----T	---A.....	A.....	AY...T.....T	
PRUNUS	GC..A.CT	---GC.TA	.....	T.....	---AGC	.....	PRUNUS	TC	TCGGGGGGGTTGCGTTG	GC-TCG	GCA.CCGG.C.CTT.C.A...T	
ROSA	....A.CT	---GC.TA	.....	AT.....	.....	.....	ROSA	AGTGTCTT	GCGYGTTCATT	CG..G	TTYCGCTTGAYCGA.CCTCC.AG	
SPIRAEA	....A.CTGCC	....A.....	.....	GA.....	CACA.G...	.....	SPIRAEA	CGTCTCCTTT	CGGGACTCG..GC	CG	GCTCC.TC.T.CT...CGG...T	
	60	70	80	90	100			160	170	180	190	200
AMELANCHIER	YCGGGGGTCGGCGG	GCCTTCGGGCT	CGG-CGTCCC	-----	-----	AMELANCHIER	AC-AAACAAACACCGCGCGTGTTCGCGCAAGGAACCCGAAACGAAAGA	---				
ARIA	T.....	.....	.....	.....	-----	ARIA	..T...G.....	C.....	A.....	C.....	---	---
CHAENOMELES	T.....	C.....	.....	.....	-----	CHAENOMELES	..T...G.....	C.....	C.....	T.....	---	---
CORMUS	T.....	C.....	.....	.....	-----	CORMUS	..T...G.....	C.....	C.....	T.....	---	---
COTONEASTER	T.....	C.....	.....	A.....	M.....	COTONEASTER	..T...G.....	C.....	C.....	T.....	---	---
CRATAEGUS	T.....	A.G.C.GC.G	.....	.....	-----	CRATAEGUS	..T...G.....	C.....	C.....	T.....	---	---
CYDONIA	T.....	C.....	.....	C.....	A.....	CYDONIA	..T...G.....	C.....	C.....	Y.....	---	---
ERIOBOTRYA	T.....	.....	G.....	C.....	---	ERIOBOTRYA	..T...G.....	A.....	C.....	T.....	---	---
HETEROMELES	T.....	AC.....	.....	CC.....	---	HETEROMELES	G.....	C.....	C.....	Y.....	---	---
MALACOMELES	T.....	.....	Y.....	.....	---	MALACOMELES	..T...G.....	C.....	C.....	T.....	---	---
MALUS	T.....	.....	C.....	T.....	C.....	MALUS	..T...TT.....	.....	C.....	T.....	---	---
MESPILUS	T.....	TC.GC.G	.....	C.....	---	MESPILUS	..T...G.....	C.....	C.....	T.....	---	GC
OSTEOMELES	T.....	.....	.....	T.....	---	OSTEOMELES	..T...G.....	A.....	C.....	T.....	---	---
PERAPHYLLUM	T.....	K.....	.....	.....	---	PERAPHYLLUM	..T...G.....	C.....	C.....	T.....	---	W.....
PHOTINIA	T.....	C.....	.....	C.....	A.....	PHOTINIA	..T...G.....	C.....	C.....	T.....	---	---
PSEUDOCYDONIA	T.....	C.....	.....	C.....	A.....	PSEUDOCYDONIA	..T...G.....	C.....	C.....	T.....	---	---
PYRUS	T.....	C.....	T.....	C.....	Y.....	PYRUS	..T...G.....	C.....	C.....	T.....	---	---
RHAPHIOLEPIS	T.....	.....	G.....	C.....	---	RHAPHIOLEPIS	..T...G.....	A.....	A.....	C.....	T.....	---
SORBUS	T.....	.....	.....	.....	---	SORBUS	..T...G.....	A.....	A.....	C.....	T.....	---
VAUQUELINIA	T.....	.....	G.....	C.....	Y.....	VAUQUELINIA	..T...G.....	C.....	C.....	T.....	---	---
PRUNUS	GG...ACGA..G.CC	T..CG.CCGG-T	CT.C..GTCCT	-----TTA	---	PRUNUS	..T...G.....	AA.....	TT.....	G.....	---	---
ROSA	CTT...G..KA..T..RC	.....T.CGCC..YATCCTAGGAGGCA	---	---	---	ROSA	G.GT.CTG.....	T.AA.....	TT.....	T.....	---	---
SPIRAEA	T.CCTT.AATC.C.C	GGG...C..C..CG..CGTT	-----CGCT	---	---	SPIRAEA	..T...G.....	A.....	AACC...A.G.ATC..T.....	T.....	---	---

Fig. 1. DNA sequences for the taxa of Table 1. Columns are nucleotide sites, and rows are individual DNA sequences. Sites are numbered (5' to 3') from 1 at the 5' end of ITS 1, to the 3' end of ITS 1 (site 268), through 35 sites at the 3' end of the 5.8S gene (site 303), to the end of the ITS 2 (site 547). Sequence symbols: A, C, G, T = dATP, dCTP, dGTP, dTTP; K = G or T; M = A or C; R = A or G; S = C or G; W = A or T; Y = C or T; periods = the same nucleotide as the first taxon (*Amelanchier*); hyphens = gaps; question marks = nucleotides of unknown identity.

ment was ambiguous (sites 352–355, 382–386, and 533–538, Fig. 1) were eliminated from analysis. Boundaries of the ITS regions and coding sequences were determined by comparison to those of *Arabidopsis thaliana*, *Cucumis*, *Daucus carota*, *Nicotiana rustica*, and *Vicia faba* (Kavanagh and Timmis, 1988; Yokota et al., 1989; Unfried and Grunder, 1990; Venkateswarlu and Nazar, 1991).

**Phylogenetic analyses**—Phylogenies were reconstructed using Fitch parsimony as implemented in PAUP 3.1.1 (Swofford, 1993). Owing to the large number of taxa, heuristic searches were conducted and included two sets of addition sequences and branch swapping: CLOSEST addition and TBR (tree bisection-reconnection) swapping and 10–50 replications of RANDOM addition and TBR to search for multiple islands of most-parsimonious trees (Maddison, 1991). Character state changes were equally weighted in all analyses except for one set of heuristic

searches with character-state-weighted parsimony; transversions were weighted over transitions by a factor of 5:1 using PAUP's step matrix option.

Sets of equally parsimonious trees were summarized using strict consensus. Bootstrapping, taken as an index of support for individual clades (Felsenstein, 1985; Hillis and Bull, 1993), was implemented in PAUP using 100 replicates of heuristic searches with CLOSEST addition sequence and TBR. We used decay indices as another measure of the robustness of individual clades (Bremer, 1988; Donoghue et al., 1992). These were computed by heuristic searches, with ten replications of random-addition sequence, for trees one or more steps longer than the most-parsimonious trees, with each set of trees equal to a certain length summarized by semistrict consensus (Hillis, 1987; Bremer, 1990).

Separate phylogenetic analyses (heuristic searches with 10 RANDOM addition sequences) were performed for

	210	220	230	240	250
AMELANCHIER	--GCGCGCTCCGCG--CGCCCCGGAACGGTGC	CGCGCGCGGGT--GCGTCGT			
ARIA	--A.....T.....			GC--A..	
CHAENOMELES	.....				
CORMUS	.....A.....A.....			C.....	
COTONEASTER	--T.....T.....T.....			CGCGTCKTA	
CRATAEGUS	--T.....T.....A.....			CGCGTC..	
CYDONIA	--T.....TCY.....			G.....C.....	
ERIOBOTRYA	--A.....T.....				
HETEROMELES	.....				
MALACOMELES	.....				
MALUS	.....M.....W.....				
MESPILUS	GC.....			G.T.CGT.....	
OSTEOMELES	.....T.....			A.....	
PERAPHYLLUM	.....Y.....			R.....	
PHOTINIA	.....T.....			AG.....T.....	
PSEUDOCYDONIA	--T.....T.....			CT.GC.GA.....	
PRUNUS	--A.....			CG.....	
RHAPHIOLEPIS	.....T.....			R.....	
SORBUS	.....A.....				
VAUQUELINIA	.....T.....			R.....	
PRUNUS	.....GT.C..AT.G..CCGG.AACG.T.T..			CG.....	
ROSA	.....T.T.C..G..GT.C.G.GAC..T.T..			G.TT.....	
SPIRAEA	AG.....GA.....GA.....CT.....C				

	260	270	280	290	300
AMELANCHIER	CGTCTTC--AA-TATGTCTTTAGGCCGAGGGC	CACGCTGCCTGGGCGTCA			
ARIA	.....-C.CA..G.....				
CHAENOMELES	.....T..C.....YG.....				
CORMUS	.....G.....G.C.....				
COTONEASTER	.....G.....G.C.....				
CRATAEGUS	.SK.....G.....A.....				
CYDONIA	.....G.....C.....G.C.-CG.....			G.....	
ERIOBOTRYA	.....AGT--C.....G.....				
HETEROMELES	.....-A.C.....G.C..CG.....			G.....	
MALACOMELES	.....				
MALUS	.....G.....A.....A.Y.....				
MESPILUS	.T..-GA--T.....AC.....				
OSTEOMELES	.....G.....A.....G.....				
PERAPHYLLUM	.....A.....			Y.....	
PHOTINIA	.....A.....				
PSEUDOCYDONIA	.....G.....C.....G.C.....				
PYRUS	.....TGT..A.A.....				
RHAPHIOLEPIS	.....AGC-A.C.....G.C.....				
SORBUS	.....A.....				
VAUQUELINIA	.....AGC-A.....G.C..CG.....				
PRUNUS	.A.....A.....A.....				
ROSA	.....A.....A.....T.....				
SPIRAEA	..AA.....ATT.C.....G.C..CG.....				

Fig. 1. Continued.

	310	320	330	340	350
AMELANCHIER	CACGCCGTTGTCCCCC--GCGC-----	CTCCCTCGGGAGCGTC--			
ARIA	.....CT.....				
CHAENOMELES	.....C.....CAA..A.....			G.....	
CORMUS	.....C...T..GAC.....			C..A.....	
COTONEASTER	.....C.....YY--				
CRATAEGUS	.G.....C.....T.....				
CYDONIA	.....C.C.....				
ERIOBOTRYA	.....C.C.AT..C--K.....			G--	
HETEROMELES	.....C.C.....			C.....	
MALACOMELES	.....C.....			A.....	
MALUS	.....C.C.....			A.....	
MESPILUS	.....C.....T.....				
OSTEOMELES	.....C.....			A.....	
PERAPHYLLUM	.....C.....			A.....Y.....	
PHOTINIA	.....CT.....C.C..A.....			A.....GGT	
PSEUDOCYDONIA	.....C.....C--GA.....			T.....GGG	
PYRUS	.....C.....				
RHAPHIOLEPIS	.....C.C.T.....C--			C..G--	
SORBUS	.....T.....C.....				
VAUQUELINIA	.....C.C.T.....C--			C..G--	
PRUNUS	.....T.....C.....G-AT-AA-----			T.....TT-----	
ROSA	.....T.....C.....AA-----			C..TYG..AGTT-----	
SPIRAEA	.....C.....GCGC.C.GCTCCCT..TTT.G..AGCGAA.TCG				

	360	370	380	390	400
AMELANCHIER	G-GGGGGCGGAGGATGGCCTCCCGTCCGACA--	CCCCGCGCGGTTGGCA-			
ARIA	-G.....C.....	TRC...T.G--A.....C.....			
CHAENOMELES	.....CT.....	T.....			
CORMUS	.....C.....	T.....			
COTONEASTER	.G...A...C.....				
CRATAEGUS	.....C.....C.....C.....			T.....	
CYDONIA	.....A.....C.....T.C--T.....			C.....	
ERIOBOTRYA	.G.A.....C.....CC--T.....			C.....	
HETEROMELES	.....A.....C.....			G.....	
MALACOMELES	.....C.....			CC--G.....	
MALUS	.....C.....			T.....	
MESPILUS	.....C.....C.....			T.....C.....	
OSTEOMELES	.....A.....C.....			TGCAG.....G	
PERAPHYLLUM	.....A.....				
PHOTINIA	.....C.....			T.....	
PSEUDOCYDONIA	.A.....AT.....A.T.....			T.....	
PYRUS	.....C.....			T.....C.....	
RHAPHIOLEPIS	.A.....C.....			T.G--C.....	
SORBUS	.....T.....C.....			T.....	
VAUQUELINIA	.A.....C.....CGTCAG.....			C.....	
PRUNUS	CG.....T.....R.C.T--GT.....				
ROSA	.AT...A...T.....CTM--AGT.A.....				
SPIRAEA	.G.C...T..T.G.....CTCCG..G..TG.T.G.C--				

Fig. 1. Continued.

ITS 1 plus the 35 sites at the 3' end of the 5.8S region and for ITS 2 to compare their phylogenetic signals and to determine the level of character congruence between them.

To examine robustness of various clades and the impact of particular taxa on tree topology, we performed jackknifing. We deleted *Aria*, *Cormus*, *Chaenomeles*, *Heteromeles*, *Malus*, *Osteomeles*, *Photinia*, *Pyrus*, *Prunus*, *Rosa*, and *Spiraea*, individually and in some combinations prior to bootstrapping with 100 replicates of heuristic searches with CLOSEST addition sequence and TBR.

We also tested for structure or phylogenetic signal in our data (as opposed to random noise), as indicated by significantly skewed distribution of tree lengths (Hillis, 1991; Hillis and Huelsenbeck, 1992; Porter, 1993). Using the random-trees option in PAUP, we compared *gl* values for the distribution of tree lengths of sets of 10,000 random trees with critical values ( $P = 0.05$ ) of *gl* from Hillis and Huelsenbeck (1992) for 500 four-state characters. We made these comparisons for our entire data set and after sequentially removing the most strongly supported clades—

*Crataegus-Mespilus-Amelanchier-Peraphyllum-Malacomeles-Eriobotrya-Rhaphiolepis-Vauquelinia*, and *Cydonia-Pseudocydonia*. We also removed *Prunus*, *Rosa*, and *Spiraea*, performed an exhaustive search of this data set of ten taxa, and compared the *gl* value of the distribution of tree lengths of the 2,027,025 trees with Hillis and Huelsenbeck's (1992) critical value.

For comparison of ITS-based phylogenies with those from morphology and wood anatomy, we reanalyzed portions of the data of Phipps et al. (1991) and Zhang (1992). We extracted from Phipps et al. (1991) a 36-character-by-18-genera data matrix. For 11 of the genera, Phipps et al. scored the same plant species as the one we sequenced (see our Table 1 and their Table 2). For the other seven genera we arbitrarily used the first species listed for each genus in their Table 2. We took 18 wood anatomical characters from Zhang's (1992) data matrix (Table 4) for *Prunus*, *Rosa*, *Spiraea*, *Vauquelinia*, and the 15 genera of Maloideae in common with our study. Zhang did not differentiate *Aria* and *Cormus* from *Sorbus* and did not include *Malacomeles* and *Pseudocydonia*. With all char-

	410	420	430	440	450		510	520	530	540
AMELANCHIER	CAAATGCCGCGTCCCGCGG	GCGAACGCCACGA	CAATCGGTGGTTGTC			AMELANCHIER	-GCTCGCGACGATCGTCTGCTC	-T-GCTTCGGCCGAGCTTTCAAC	-G	
ARIA	.....T.....A.....	.....	.....	.....	.....	ARIA	---G.TGCGACGCTCGCTG	.CC.GC.....G.G.....	---	
CHAENOMELES	.....T.....A.....	.....	.....	.....	.....	CHAENOMELES	-C.CGCGACGTCA.C.RCT	---C.....G.....	---	
CORMUS	.....T.....A.....	.....	.....	.....	.....	CORMUS	A..GY.....C.....	.CGGC.AAC.-CTG	.....R..	
COTONEASTER	T.....T.....T.....	A.....	.....	.....	.....	COTONEASTER	----C.CG.....A..AAACGCG	.C..C..G.....	---	
CRATAEGUS	.....A.....A.....	.....	.....	.....	.....	CRATAEGUS	.....CCCATTAAGGCCCT	.....G.....	---	
CYDONIA	.....A.....T.....	A.....	.....	.....	.....	CYDONIA	-CT.....C..C.....	CT.T.....G.....	---	
ERIOBOTRYA	.....A.....A.....	T.....GA.....	A.GG.....	.....	.....	ERIOBOTRYA	---T..CGACGGT.G.TG.TCGCA	.CGACGGAGC.....	---	
HETEROMELES	.....A.....T.....	A.....	.....	.....	.....	HETEROMELES	-C.CGCGACA.C.....	---C.....A.G.....	G..A..	
MALACOMELES	.....A.....A.....	.....	.....	.....	.....	MALACOMELES	.....T.....T.....	---A.....	???	
MALUS	.....T.....T.....	A.....	.....	.....	.....	MALUS	-C.CGCGACG.CC.....	---C.....G.....	---	
MESPILUS	.....A.....A.....	.....	.....	.....	.....	MESPILUS	.....ACC---	CGCGCTCT.....	G..C.....	
OSTEOELES	.....T.....T.....	A.....	.....	.....	.....	OSTEOELES	ACG.....CA..	CTGCGCTCT.....	RR.....C.....	
PERAPHYLLUM	.....A.....A.....	.....	.....	.....	.....	PERAPHYLLUM	.....AC.....	.....	RR.....A..	
PHOTINIA	T.....A.....T.....	A.....	.....	.....	.....	PHOTINIA	C.TGCGACG-C.....	---T.....	---	
PSEUDOCYDONIA	.....T.....T.....	A.....	.....	.....	.....	PSEUDOCYDONIA	---C..CGACC.CTC	.CGCTCT.....	C..G.....C.....	
PYRUS	.....T.....T.....	A.....	.....	.....	.....	PYRUS	-C.G..ACG.TCC.....	C.....	.....G.....	
RHAPHIOLEPIS	.....A.....T.....	A.....	.....	.....	.....	RHAPHIOLEPIS	---T..CGACGGTGCA.G.TCCGC	A..T.G.....	???????????	
SORBUS	.....A.....T.....	A.....	.....	.....	.....	SORBUS	..YG..ATG.TCG.TGCT..T---	.....G.....	---	
VAUQUELINIA	.....A.....T.....	A.....	.....	.....	.....	VAUQUELINIA	---T..CRCGGTCGATG.TCCCT	.CAT.T.G.....	V.....	
PRUNUS	T.....AA.....T.....	A..C.....	.....	.....	CG	PRUNUS	--GAG.GCT..AAAAATTG	..TGG...C.K..TCG	.....	
ROSA	T.....A..AA.....T.....	A..C.....	.....	.....		ROSA	--G.GCTTT.TTAA.AATGYGTGTCGA	..T.GAT.....	---	
SPIRAEA	.....A.....A.....	A..TG.....	.....	.....	AG	SPIRAEA	C.GG.T..CAT.G.....	CT.GGCGCACAGT	..G.....C.....	

Fig. 1. Continued.

	460	470	480	490	500
AMELANCHIER	AA-ACCT-CGGTTGCC-TGTTG	TGCGCTTTCGCCGCGCT	-CCGGGCG-		
ARIA	C.....C.....	.....T.....	CA.GA....	---	
CHAENOMELES	GT.....C.....	.....T.....	CR.GA.CG..	---	
CORMUS	CG.....C.....	CG..C.....	T.....A.GAACG	---	
COTONEASTER	.....C.....C.....	C.....T.....	C.GA.CG..	---	
CRATAEGUS	..G.....C.....	.....C.....	C.G..CG..	---	
CYDONIA	G..G.....C.....	.....A.....	T.....CG.GA.CG..	---	
ERIOBOTRYA	.....C.....	.....AG..	T.....C.GA.CGT	---	
HETEROMELES	.....C.....	.....T.....	C.GA.CG..	---	
MALACOMELES	.....C.....	.....	.....	---	
MALUS	G..GG.....A.....	.....T.....	C.GAACG	---	
MESPILUS	..G.....C.....	.....C.G..CG..	---		
OSTEOELES	..G.....T.....	.....T.....	C.GA.AG.CG	---	
PERAPHYLLUM	.....C.....	.....	.....	---	
PHOTINIA	G.....AC.....	.....T.....	G.GA..G..	---	
PSEUDOCYDONIA	..G.....Y.....	.....C.....	CG.GA.CG..	---	
PYRUS	G.....C.....	.....T.....	C.GATCG..	---	
RHAPHIOLEPIS	G.....C.....	.....T.....	T.T.GA.CG..	---	
SORBUS	.....C.....	.....C.Y.Y.....	C.GA.CGC	---	
VAUQUELINIA	G.....C.....	.....T.....	T.T.GA.CG..	---	
PRUNUS	.....C.....	CAG..T..GG..	TT..AT-	---	
ROSA	.....T.C.....C.....	CGCT.TT..AT.GA	-----		
SPIRAEA	G.....CCT.TCGCTAG	..CC.....CC..	-----GT.GC.T.ACG		

Fig. 1. Continued.

acters ordered (except for four that Zhang [1992] did not order), we analyzed these morphological and wood anatomical data sets separately, after pooling them, after pooling each separately with the ITS data, and after pooling both with the ITS sequences. We performed analyses of all three data sets for those taxa for which there is complete data (all taxa of Table 1 except *Aria*, *Cormus*, *Malacomeles*, *Pseudocydonia*, *Vauquelinia*, *Prunus*, *Rosa*, and *Spiraea*). In addition, we followed the "pseudofossil" method (Weins and Reeder, in press) and analyzed all taxa of Table 1, assigning "?" for missing data. We explored these data sets with MacClade (version 3.04; Maddison and Maddison, 1992) to trace anatomical and morphological character state changes on trees based on ITS DNA sequence data to search for individual character congruence between data sets.

We assessed taxonomic congruence between DNA-sequence and morphological topologies with strict consensus techniques. We measured character congruence among anatomical, DNA-sequence, and morphological data sets and between ITS 1 plus the 35 sites at the 3' end of the

5.8S region and ITS 2. We used two indices of character incongruence:  $I_{MF}$ , the Mickevich-Farris index (Mickevich and Farris, 1981), and  $I_M$ , an index attributed to Miyamoto (Kluge, 1989; Swofford, 1991). These indices are both based on the ratio of among-data-set homoplasy to total homoplasy, where among-data-set homoplasy = total homoplasy - within-data-set homoplasy. For  $I_M$ , total homoplasy is the sum of the extra steps in reciprocal fittings of data sets onto each other's most-parsimonious trees; that is, total homoplasy =  $\sum_{ij} (S_{i \rightarrow j} - M_i)$ , where  $S_{i \rightarrow j}$  is the length of the most-parsimonious tree (s) when data set  $i$  is fitted onto the most-parsimonious tree (s) of data set  $j$ , and  $M_i$  is the minimum possible length of data set  $i$ . Within-data-set homoplasy is the sum of the extra steps for  $n$  data sets ( $S_1 - M_1 + S_2 - M_2 + \dots S_n - M_n$ ). For  $I_{MF}$ , total homoplasy is the number of extra steps in parsimony analysis of pooled data sets (observed tree length of pooled data sets [ $S_p$ ] - minimum tree length of pooled data sets [ $M_p$ ]). For two data sets, then,

$$I_M = \frac{S_{1 \rightarrow 2} - M_1 + S_{2 \rightarrow 1} - M_2 - (S_1 - M_1 + S_2 - M_2)}{S_{1 \rightarrow 2} - M_1 + S_{2 \rightarrow 1} - M_2};$$

$$I_{MF} = \frac{S_p - M_p - (S_1 - M_1 + S_2 - M_2)}{S_p - M_p}.$$

These indices range from 0 (no incongruence) to 1 (complete incongruence). They confound total homoplasy and among-data-set homoplasy and thereby generally under-value conflict among data sets (Campbell, unpublished data). We therefore also used Farris's (1989) retention index to measure character congruence:

$$RI = \frac{G_T - S}{G_T - M_T},$$

where  $G_T$  is maximum total character evolution or number of steps for the data sets of interest;  $M_T$  is minimum total character evolution or number of steps for the data sets; and  $S$  is the length of the most-parsimonious tree(s) either from parsimony analysis of pooled data sets ( $S_p$ )

or from the sum of the lengths of the most-parsimonious tree(s) of the data sets fitted onto the topology of the most-parsimonious tree(s) of the other data sets ( $S_F$ ). This index may therefore be measured with  $S_{F_3}$  and then is  $RI_{F_3}$  or with  $S_P$  and then is  $RI_P$ . The former expression of RI is preferred because parsimony minimizes conflict among pooled data sets and thereby undervalues conflict (Campbell, unpublished data). RI ranges from 0 and no congruence (when  $G_T = S$ ) to 1 and complete congruence (when  $S = M_T$ ).

## RESULTS

**ITS length variation, alignment, divergence, and G + C content**—Within Maloideae lengths of ITS 1 and 2 are nearly identical and vary little among the genera sampled (Fig. 1, Table 2). Sequence alignment required 44 insertions, all of which were less than four bases in length except for one four-base gap to align an insertion in *Mespilus* at sites 199–202; a four-base deletion in the same species at sites 513–516; gaps at two to seven bases (*Cotoneaster*) for several species at sites 499–505; and a six-base gap in *Pyrus* at sites 520–525. Eighteen of these indels are autapomorphic. There are four autapomorphic gaps in the 35 sites at the 3' end of the 5.8S subunit (sites 269–303, Fig. 1). Only a few indels, such as the two-base insertion at sites 122–123 for *Crataegus* and *Mespilus*, are congruent with the relationships of the most-parsimonious tree (Fig. 2). Indels account for  $\approx 9\%$  of all sites within the Maloideae. Lengths of ITS 1 and 2 of *Vauquelinia* fall within the range of the Maloideae (Table 2), whereas lengths of *Prunus*, *Rosa*, and *Spiraea* lie outside the range of Maloideae. Alignment of sequences of Maloideae to these three outgroup genera requires major gaps in Maloideae sequences—seven- and 18-base gaps for *Prunus*; an 18-base gap for *Rosa*; and seven-, nine-, and 18-base gaps for *Spiraea*. Gaps introduced into Maloideae sequences to align these outgroup genera account for 8% of the total length of ITS 1 and 2 and the 35 sites at the 3' end of the 5.8S subunit. Overall, alignment of sequences was relatively unambiguous; alignment uncertainty required exclusion of 15 sites (352–355, 382–386, and 533–538 of Fig. 1).

Sequence divergence within the Maloideae plus *Vauquelinia* ranges from 2.7% (*Malacomeles* and *Peraphyllum*) to 16.1% (*Crataegus* and *Eriobotrya*). The three outgroup rosaceous genera, *Prunus*, *Rosa*, and *Spiraea*, diverge from all Maloideae plus *Vauquelinia* by 25.4–39.3%. Sequence divergence is greater in ITS 2 than ITS 1 (Table 2).

In the Maloideae G + C content ranges from 65% to 72% (mean  $67 \pm 1.7\%$ ) for ITS 1 and from 67% to 72% (mean  $69 \pm 1.5\%$ ) for ITS 2 (Table 2). Overall G + C content for both spacers averages 68% ( $\pm 1.5$ ), which is slightly more than the level of  $65 \pm 3\%$  for outgroup genera. These values are toward the high end of the range for plants (31%–73% for ITS 1 and 30%–77% for ITS 2; Baldwin et al., in press).

**Phylogenetic analyses of ITS 1 and 2 pooled**—The full data set contains 354 variable and 191 potentially informative sites (Table 2). There is a total of 228 variable

TABLE 2. Characteristics of ITS 1 and ITS 2 in the Maloideae and outgroups.

	ITS 1	ITS 2
Length		
Maloideae	208–213–221 <sup>a</sup>	211–216–224 <sup>a</sup>
<i>Vauquelinia</i>	212	220
<i>Prunus</i>	242	209
<i>Rosa</i>	249	207
<i>Spiraea</i>	251	230
Variable sites		
Maloideae <sup>b</sup>	108	120
Rosaceae <sup>c</sup>	196	158
Potentially informative sites		
Maloideae <sup>b</sup>	45	72
Rosaceae <sup>c</sup>	90	101
% G + C content		
Maloideae <sup>b</sup>	$67 \pm 1.7^d$	$69 \pm 1.2^d$
Outgroup genera	$64 \pm 1.3^d$	$66 \pm 4.0^d$
% Sequence divergence		
Maloideae <sup>b</sup>	$8.9 \pm 3.1^d$	$14.8 \pm 3.7^d$
Rosaceae <sup>c</sup>	$15.1 \pm 11.4^d$	$19.3 \pm 8.3^d$

<sup>a</sup> Lowest length–mean–longest length.

<sup>b</sup> Includes *Vauquelinia*.

<sup>c</sup> Includes all taxa of Table 1.

<sup>d</sup> Mean and SD.

sites and 117 potentially informative sites within Maloideae and *Vauquelinia*, with a majority of these sites in ITS 2. The 35 sites at the 3' end of the 5.8S subunit (sites 269–303, Fig. 1) contain eight variable and five potentially informative sites in the Maloideae plus *Vauquelinia* and nine variable and six potentially informative sites when *Prunus*, *Rosa*, and *Spiraea* are included.

Heuristic searches, with both CLOSEST and RANDOM addition sequences of taxa, produce one maximally parsimonious tree of 820 steps (autapomorphies included; Fig. 2). Character state changes were equally weighted in these analyses; we obtained the same results when transversions were weighted over transitions by a factor of 5:1. Relationships at the base of the Rosaceae are unclear, and it is not obvious how to root the Rosaceae. We have some confidence that the root does not lie within the apparently derived Maloideae (Morgan, Soltis, and Robertson, 1994). We therefore included representatives of the three other subfamilies, including *Vauquelinia* of the Spiraeoideae because of the possibility that it might be close to the Maloideae. Our approach was to carry out a simultaneous parsimony analysis (Farris, 1982; Maddison, Donoghue, and Maddison, 1984) with these taxa included and then root the resulting network along the branch connecting the outgroups to the ingroup. However, it emerged in the analysis that all of the potential outgroups did not attach to the Maloideae along a single branch, i.e., the four outgroups are not convex on the network. We therefore chose to root the tree along the branch connecting representatives of all three subfamilies, which then implies that *Vauquelinia* is actually nested within Maloideae.

Interior branch lengths within the Maloideae are short (Fig. 2) showing significantly less character state change than terminal branches ( $t$ -test,  $P < 0.001$ ; SAS Institute,



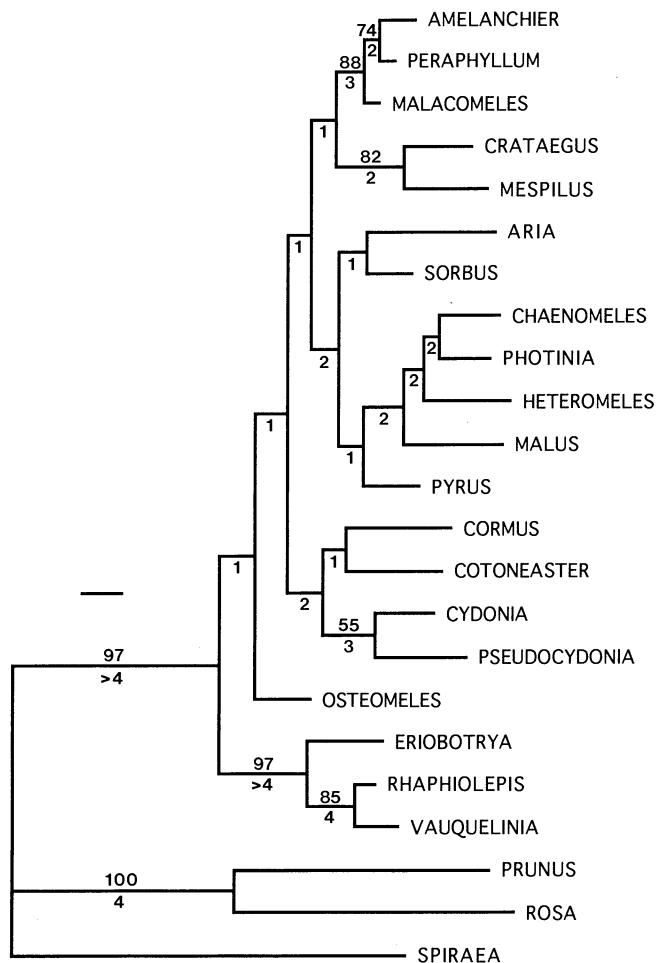


Fig. 2. Phylogram of the single most-parsimonious tree of 820 steps from parsimony analysis of ITS 1, 35 sites at the 3' end of the 5.8S gene, and ITS 2 DNA sequences (sites 352–355, 382–386, and 533–538 from Fig. 1 excluded) for all taxa in Table 1. The tree was generated from 50 replications of RANDOM addition sequence with TBR and is rooted between *Prunus*, *Rosa*, and *Spiraea* and the 19 Maloideae genera plus *Vauquelinia*. Branch lengths are based on accelerated transformation (ACCTRAN) of characters. The separate bar indicates the branch length equivalent to ten substitutions. Numbers above branches indicate bootstrap % values for clades found in both this tree and bootstrap majority rule tree. Numbers below branches are decay index values. See Table 3, single data set 5. a. and text for fuller explanation.

Inc., 1989). These short interior branches, coupled with considerable homoplasy, reduce confidence in our phylogenetic hypotheses (Donoghue and Sanderson, 1992).

Within the Maloideae-plus-*Vauquelinia* lineage, the first branch is the well-supported (bootstrap value of 97%, decay index greater than 4) group of *Eriobotrya*, *Rhaphiolepis*, and *Vauquelinia* (Fig. 2). The latter two genera are strongly linked (bootstrap value of 85%, decay index of 4).

*Osteomeles* is the sister of the remaining group of Maloideae (Fig. 2). Two clades within this core Maloideae—*Crataegus* plus *Mespilus*, *Amelanchier* plus *Peraphyllum* and *Malacomeles*—consistently appeared in all jackknifing experiments. The *Cydonia* plus *Pseudocydonia* clade

is not supported above the 50% level when *Aria*, *Cotoneaster*, *Malus*, *Photinia*, *Prunus*, or *Rosa* are deleted prior to bootstrap analyses in PAUP.

Relationships of the ten genera of Maloideae outside the four most strongly supported clades—*Eriobotrya*-*Rhaphiolepis*-*Vauquelinia*, *Crataegus* plus *Mespilus*; *Amelanchier* plus *Peraphyllum* and *Malacomeles*, and *Cydonia* plus *Pseudocydonia*—are fully resolved (Fig. 2). Nevertheless low bootstrap and decay index values indicate weak support for the relationships of these other genera. Overall, ITS regions do not appear to be strongly informative about phylogenetic relationships of our sample of these other ten genera.

Distributions of tree lengths are significantly skewed and therefore show phylogenetic signal for the entire data set ( $g1 = -1.14$ ; for 25 taxa the critical value at the  $P = 0.05$  level of  $g1 = -0.07$  and more negative values indicate significant structure [Hillis and Huelsenbeck, 1992]). Values of  $g1$  are significant even after removal of the most strongly supported clades;  $g1 = -1.36$  when the *Crataegus*-*Mespilus*-*Amelanchier*-*Peraphyllum*-*Malacomeles* clade is removed;  $g1 = -1.77$  when the *Eriobotrya*-*Rhaphiolepis*-*Vauquelinia* clade is also removed; and  $g1 = -1.75$  when the *Cydonia*-*Pseudocydonia* clade is also removed. When *Prunus*, *Rosa*, and *Spiraea* are removed  $g1 = -0.37$  for the distribution of lengths of the 2,027,025 trees produced by an exhaustive search; this value is significant at the  $P = 0.05$  (critical value of  $g1 = -0.16$ ) and at the  $P = 0.01$  (critical value of  $g1 = -0.27$ ) levels.

**Phylogenetic analyses of ITS 1 and 2 separately**—Phylogenetic analysis of ITS 1 plus the 35 sites at the 3' end of the 5.8S region results in 61 most-parsimonious trees (Table 3, single data set 5. b), whereas ITS 2-based phylogenies are fully resolved (Table 3, single data set 5. c). The most-parsimonious trees from both analyses (not shown) include the *Prunus*-*Rosa* and *Eriobotrya*-*Rhaphiolepis*-*Vauquelinia* clades, although the latter is nested within the other Maloideae and not sister to the remaining species of the subfamily. ITS-1 trees include the *Cydonia*-*Pseudocydonia* clade but not the *Crataegus*-*Mespilus*-*Amelanchier*-*Peraphyllum*-*Malacomeles* clade. The ITS-2 tree includes the latter but not the former clade.

**Congruence among data sets**—Phylogenies based on equivalent sets of genera for ITS DNA sequence, morphological, and wood anatomical data sets show a wide range of values for character congruence (Table 4).  $I_M$  ranges from 0.309 to 0.335 and is considerably higher than  $I_{MF}$ , which ranges from 0.054 to 0.086. RI values range from 0.199 to 0.473 and indicate lower levels of congruence than those recorded by  $I_{MF}$  and  $I_M$ . To illustrate calculation of these indices using values from Table 3, consider congruence between ITS DNA sequences (D) and morphology (M):

$$I_M = \frac{S_{D \rightarrow M} - M_D + S_{M \rightarrow D} - M_M - (S_D - M_D + S_M - M_M)}{S_{D \rightarrow M} - M_D + S_{M \rightarrow D} - M_M} \\ = \frac{535 - 269 + 384 - 111 - (440 - 269 + 303 - 111)}{535 - 269 + 384 - 111} \\ = 0.326.$$

TABLE 3. Summary of information from Maloideae character congruence analysis.

Character set	Taxa <sup>a</sup>	Number of trees <sup>b</sup>	Tree length			CI <sup>c</sup>	RI <sup>d</sup>
			Observed <sup>b</sup>	Minimum <sup>b</sup>	Maximum <sup>b</sup>		
Single data set							
1. Anatomy (A)	A	994	23	13	39	0.545	0.615
2. A	B	288	55	36	99	0.604	0.698
3. ITS1–ITS2 (D <sup>e</sup> )	B	8	725	486	945	0.554	0.488
4. D <sup>e</sup>	C	2	440	269	571	0.499	0.434
5. a. D <sup>e</sup>	D	1	820	510	1,086	0.507	0.462
b. ITS1 + 5.8S	D	61	392	282	517	0.580	0.532
c. ITS2	D	1	410	228	569	0.556	0.466
6. Morphology (M)	A	1	258	111	389	0.430	0.471
7. M	C	1	303	111	482	0.366	0.482
Pooled data sets							
1. A + D <sup>e</sup>	B	3	777	522	1,044	0.542	0.473
2. A + M	A	1	290	124	428	0.426	0.454
3. D <sup>e</sup> + M	C	1	777	380	1,053	0.414	0.410
4. A + D <sup>e</sup> + M	A	2	676	367	903	0.463	0.424
5. A + D <sup>e</sup> + M	D	1	1,228	662	1,666	0.450	0.436
One data set fitted onto (→) the most parsimonious trees from another							
1. A → D <sup>e</sup>	B	N/A <sup>f</sup>	72–75 <sup>g</sup>	36	99	0.446	0.429
2. D <sup>e</sup> → A	B	N/A <sup>f</sup>	838–858 <sup>g</sup>	486	945	0.457	0.233
3. A → M	A	N/A <sup>f</sup>	33 <sup>g</sup>	13	39	0.375	0.231
4. M → A	A	N/A <sup>f</sup>	318–355 <sup>g</sup>	111	389	0.349	0.255
5. D <sup>e</sup> → M	C	N/A <sup>f</sup>	535 <sup>g</sup>	269	571	0.390	0.119
6. M → D <sup>e</sup>	C	N/A <sup>f</sup>	384–386 <sup>g</sup>	111	482	0.288	0.289
7. ITS1 + 5.8S → ITS2	D	N/A <sup>f</sup>	419	517	282	0.526	0.417
8. ITS2 → ITS1 + 5.8S	D	N/A <sup>f</sup>	452–461 <sup>g</sup>	228	569	0.427	0.343

<sup>a</sup> Set A includes all Maloideae genera except *Aria*, *Cormus*, *Malacomeles*, and *Pseudocyonidia*; set B includes *Prunus*, *Rosa*, *Spiraea*, *Vauquelinia*, and all Maloideae genera except *Aria*, *Cormus*, *Malacomeles*, and *Pseudocyonidia*; set C includes all Maloideae genera except *Pseudocyonidia*; set D includes all taxa of Table 1.

<sup>b</sup> From heuristic searches with ten replicates of random addition sequence. The minimum and maximum are the shortest (no homoplasy) and longest (maximum homoplasy) tree lengths.

<sup>c</sup> Consistency index, excluding uninformative characters.

<sup>d</sup> Retention index.

<sup>e</sup> Includes all of ITS 1, 35 sites at the 3' end of the 5.8S region, and all of ITS 2 except sites 352–355, 382–386, and 533–538 (see Fig. 1).

<sup>f</sup> Not applicable.

<sup>g</sup> The range of tree lengths from mapping one data set onto the most-parsimonious trees of another; CI and RI values are for the shortest tree lengths.

$$I_{MF} = [S_P - M_T - (S_D - M_D + S_M - M_M)] / (S_P - M_T) \\ = [777 - 380 - (440 - 269 + 303 - 111)] / (777 - 380) \\ = 0.086.$$

$$RI_F = \frac{G_T - (S_{D \rightarrow M} + S_{M \rightarrow D})}{G_T - M_T} = \frac{1053 - (535 + 384)}{1053 - 380} \\ = 0.199.$$

$$RI_P = \frac{G_T - S_P}{G_T - M_T} = \frac{1053 - 777}{1053 - 380} = 0.410.$$

These values are generally similar to those between ITS 1 plus the 35 bases of 5.8S and ITS 2:  $I_M = 0.191$ ,  $I_{MF} = 0.058$ ,  $RI_F = 0.462$ , and  $RI_P = 0.373$ .

**Phylogenetic analysis of ITS data for a reduced taxon sample**—Parsimony analysis of the ITS data for the 18 genera in common with the morphological data of Phipps et al. (1991) produces two most-parsimonious, 440-step trees (Table 3, single data set 4; the strict consensus is shown in Fig. 3). The data set of 36 morphological char-

acters for the 18 genera of Maloideae in common with our ITS study yielded one shortest, 303-step tree (Table 4, single data set 7; the cladogram is shown in Fig. 4). Strict consensus of the three trees from these two analyses is completely unresolved. Inspection of the separate analyses (Figs. 3, 4), however, shows that the two data sets do share some relationships. Both data sets include *Crataegus*, *Mespilus*, *Amelanchier*, and *Peraphyllum* in a small clade. *Cotoneaster*, *Cydonia*, and *Heteromeles* are members of the same clade, albeit with very different taxonomic composition in the two data sets. The pooled data set of ITS DNA sequences and morphology yields one most-parsimonious tree of 777 steps (Table 4, pooled data set 3; the cladogram is shown in Fig. 5). Two major clades are recognized. The first includes the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade and a group of the three maloid genera with compound leaves *Cormus*, *Osteomeles*, and *Sorbus*. The remaining eight genera form the other clade.

**Phylogenetic analysis of pooled data sets**—When all taxa for which there is complete data for ITS DNA sequences, morphology, and wood anatomy are analyzed, there are

TABLE 4. Character incongruence and congruence between wood anatomical, ITS DNA sequence, and morphological data sets for the Maloideae and outgroups.<sup>a</sup>

Anatomy	DNA	Morphology	Anatomy	DNA	Morphology
	0.062 <sup>b</sup>	0.054 <sup>c</sup>	Anatomy	0.473 <sup>b</sup>	0.454 <sup>c</sup>
0.335 <sup>b</sup>		0.086 <sup>d</sup>	DNA	0.257 <sup>b</sup>	0.410 <sup>d</sup>
0.309 <sup>c</sup>	0.326 <sup>d</sup>		Morphology	0.253 <sup>c</sup>	0.199 <sup>d</sup>

<sup>a</sup> For the matrix on the left values above the diagonal are character incongruence index,  $I_{MF}$ , and values below the diagonal are character incongruence index,  $I_M$ ; for the matrix on the right, values above the diagonal are the retention index for character congruence, measured with the data sets pooled ( $RI_P$ ), and values below the diagonal are the retention index for character congruence, measured with  $S$  determined by fitting data sets onto each other's most-parsimonious trees ( $RI_F$ ); see text for an explanation of these indices and Table 3 for values from which they were computed.

<sup>b</sup> Based on *Prunus*, *Rosa*, *Spiraea*, *Vauquelinia*, and all Maloideae genera except *Aria*, *Cormus*, *Malacomeles*, and *Pseudocydonia*; wood anatomical data from Zhang (1991).

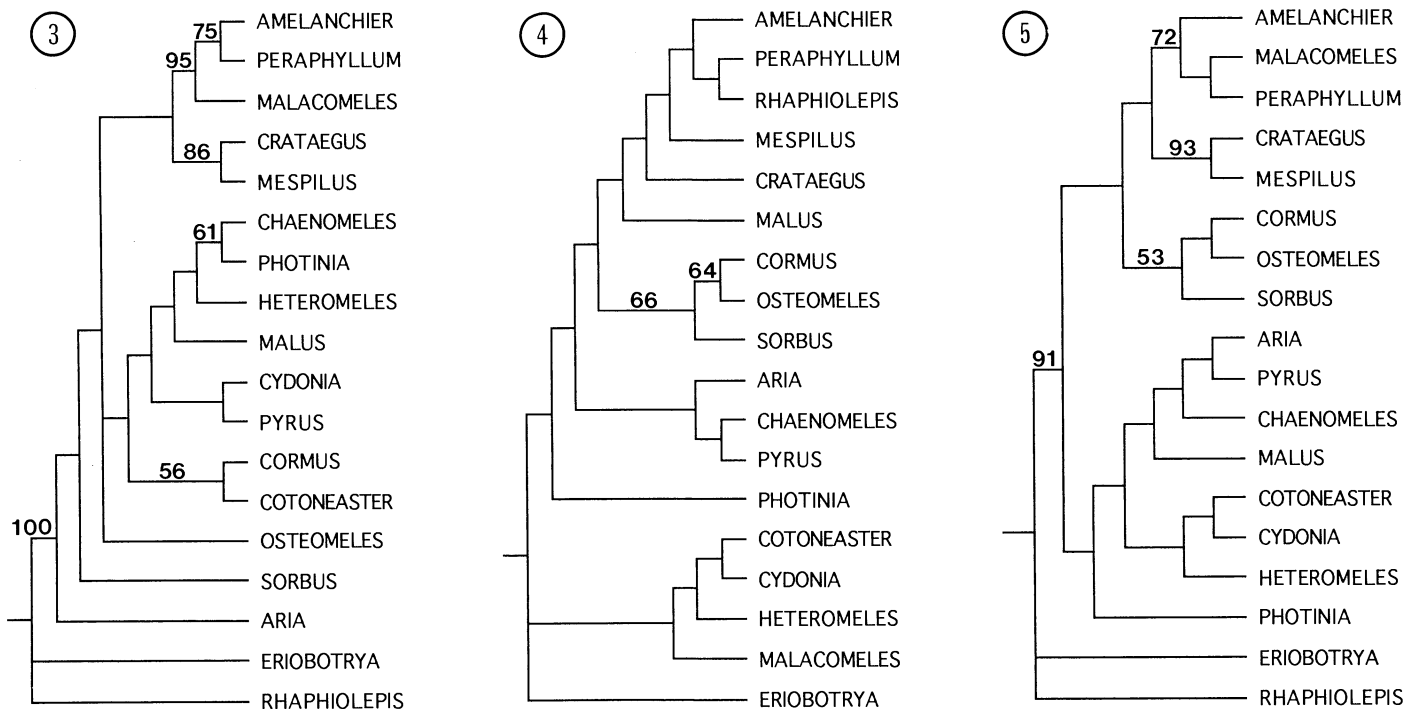
<sup>c</sup> Based on all Maloideae genera except *Aria*, *Cormus*, *Malacomeles*, and *Pseudocydonia*; wood anatomical data from Zhang (1991) and morphological data from Phipps et al. (1991).

<sup>d</sup> Based on all Maloideae genera except *Pseudocydonia* and *Vauquelinia*; morphological data from Phipps et al. (1991).

two most-parsimonious trees of 676 steps (Table 3, pooled data set 4; the strict consensus is shown in Fig. 6). These trees were rooted between *Eriobotrya* plus *Rhaphiolepis* and the remainder of the Maloideae. Both trees recognize

the *Amelanchier-Peraphyllum* and *Crataegus-Mespilus* clades, and group *Chaenomeles*, *Malus*, and *Pyrus* together and *Cotoneaster* and *Cydonia* together, as in the ITS phylogeny (Fig. 2). The *Amelanchier-Peraphyllum* and *Crataegus-Mespilus* clades are the most strongly supported clades within the core Maloideae, and they are members of the same monophyletic group. The position of *Photinia*, the sister to the group of *Chaenomeles*, *Cotoneaster*, *Cydonia*, *Heteromeles*, *Malus*, and *Pyrus*, differs from being nested within a clade of four of these genera in the ITS phylogeny (Fig. 2). The two trees for the pooled ITS DNA sequence, morphological, and wood anatomical data differ in the attachment of the clade of *Osteomeles* and *Sorbus*; in one tree this clade is sister to the remaining Maloideae (except for *Eriobotrya* and *Rhaphiolepis*), and in the other it is the sister of the *Amelanchier-Peraphyllum-Crataegus-Mespilus* group.

Analysis of the pooled data set of ITS DNA sequences, morphology, and wood anatomy for all the taxa of Table 1 generates one shortest, 1,228-step tree (Table 3, pooled data set 5; the phylogram is shown in Fig. 7). This reconstruction identifies the *Eriobotrya-Rhaphiolepis-Vauquelinia*, *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus-Sorbus*, and *Cydonia-Pseudocydonia* clades. *Osteomeles* groups with the two other genera with compound foliage (*Cormus* and *Sorbus*). There is some decay-index support for *Aria-Chaenomeles-Malus-Pyrus* and *Cotoneaster-Cydonia-Heteromeles-Pseudocydonia*



Figs. 3–5. Parsimony analyses of DNA sequences (ITS 1, 35 sites at the 3' end of the 5.8S gene, and ITS 2 (sites 352–355, 382–386, and 533–538 from Fig. 1 excluded) and morphology (from Phipps et al., 1991) for 18 genera of Maloideae (i.e., excluding *Pseudocydonia*). 3. Strict consensus of the two most-parsimonious, 440-step trees for ITS DNA sequences. Numbers above branches indicate bootstrap % for clades found in both the strict consensus and bootstrap majority rule trees. See Table 3, single data set 4 and text for fuller explanation. 4. The single most-parsimonious 303-step tree for morphology. Numbers above branches indicate bootstrap % for clades found in both the most-parsimonious and bootstrap majority rule trees. See Table 3, single data set 7 and text for fuller explanation. 5. The single most-parsimonious 777-step tree for pooled ITS DNA sequences and morphology. Numbers above branches indicate bootstrap % for clades found in both the most-parsimonious and bootstrap majority rule trees. See Table 3, pooled data set 3 and text for fuller explanation.

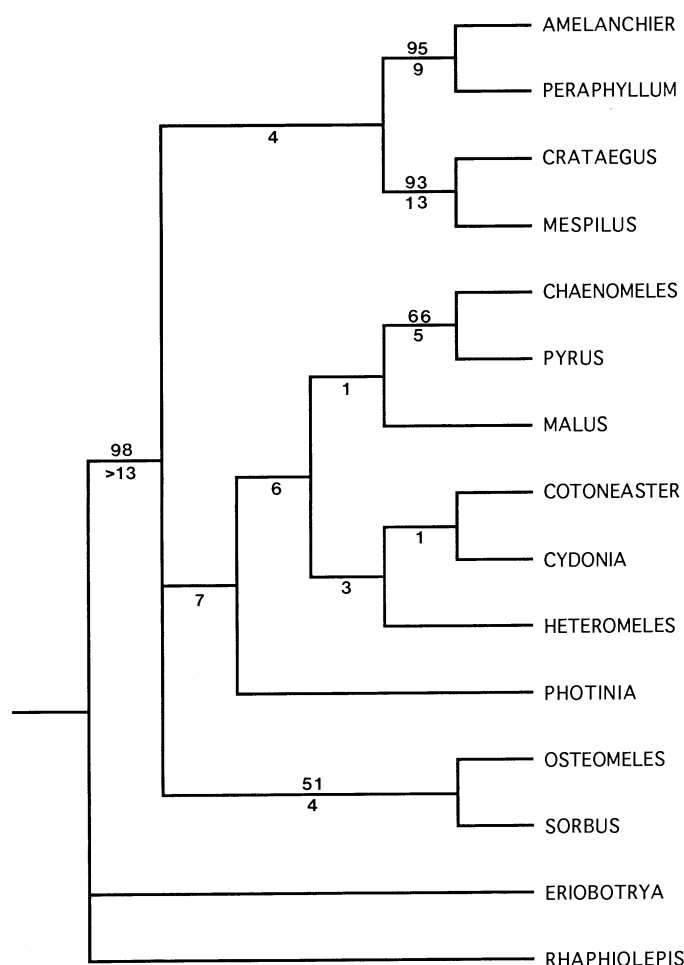


Fig. 6. The strict consensus of the two most-parsimonious trees of 676 steps based on wood anatomy (from Zhang, 1992), DNA sequences (ITS 1, 35 sites at the 3' end of the 5.8S gene, and ITS 2 (sites 352–355, 382–386, and 533–538 from Fig. 1 excluded), and morphology (from Phipps et al., 1991) for the 15 Maloideae genera for which the three data sets are complete (namely all genera except *Aria*, *Cormus*, *Malacomeles*, and *Pseudocydonia*). Numbers above branches indicate bootstrap % values for clades found in both the strict consensus and bootstrap majority rule trees, and numbers below branches are decay index values. See Table 3, pooled data set 4, and text for fuller explanation.

clades, and the sister-group relationship of *Photinia* to these two clades.

## DISCUSSION

**Sister group relationships and the origin of the Maloideae**—Phipps et al. (1991, p. 317) noted “broadly equivalent similarity of maloids to spiraeoids and amygdaloids.” Wood anatomy (Zhang, 1992) and Kalkman’s (1988) four hand-generated cladograms of the Rosaceae also do not settle the question of sister-group relationships of the Maloideae. The two lineages of Maloideae recognized by Kalkman (Maleae and the *Cydonia* group) are sister groups in a clade that includes the amygdaloid Osmonarieae (all four trees presented), Pruneae (two trees), Kerrieae of the Rosoideae or Spiraeoideae (two trees), and the trio of Quillajeae genera, *Exochorda*, *Lindleya*, and

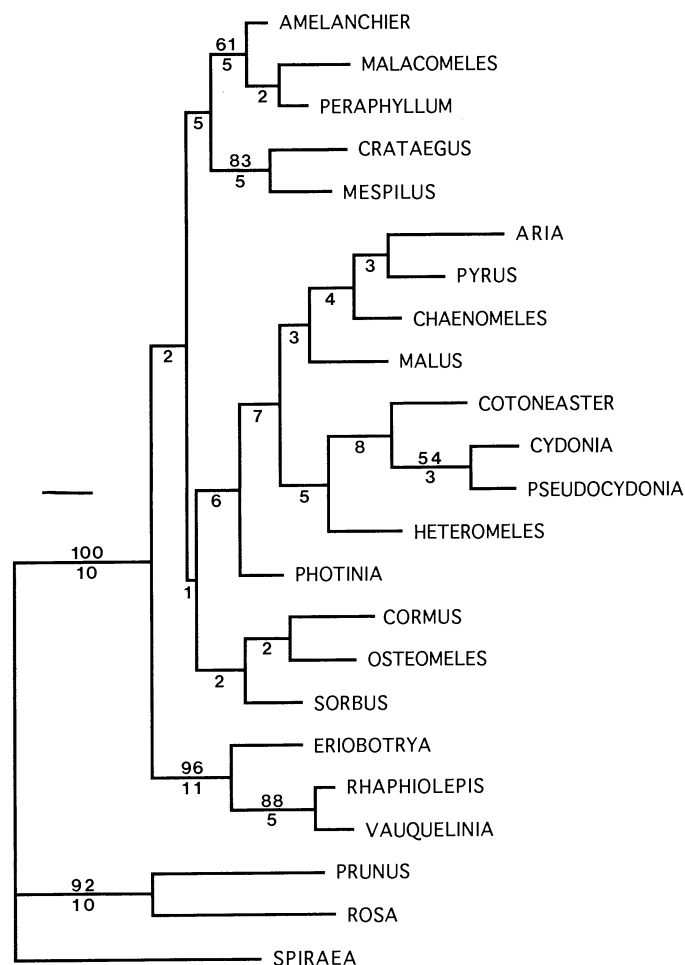


Fig. 7. The single most-parsimonious tree for all taxa of Table 1 based on the pooled data set of wood anatomy (from Zhang, 1992), DNA sequences (ITS 1, 35 sites at the 3' end of the 5.8S gene, and ITS 2 (sites 352–355, 382–386, and 533–538 from Fig. 1 excluded), and morphology (from Phipps et al., 1991). Missing-data values (“?”) were assigned for morphological characters of *Prunus*, *Rosa*, *Spiraea*, *Vauquelinia*, and *Pseudocydonia* and for anatomical characters of *Aria*, *Cormus*, *Malacomeles*, and *Pseudocydonia*. The separate bar indicates the branch length equivalent of ten substitutions. Numbers above branches indicate bootstrap % for clades found in both this tree and bootstrap majority rule trees. Numbers below branches are decay index values. See Table 3, pooled data set 5, and text for fuller explanation.

*Vauquelinia* (one tree). Because of uncertainty about the root of the Rosaceae (other than that it is unlikely to be within Maloideae), we cannot determine the sister-group relationships of the Maloideae with the ITS DNA sequence data set presented here. We did experiment with ITS sequences of a more distant outgroup (*Celtis*, Ulmaceae). In these analyses the Rosaceae are rooted along the branch leading to *Prunus* plus *Rosa*, leading to the conclusion that *Spiraea* is most closely related to the Maloideae. This finding is tentative and requires additional study.

Phipps et al. (1991, p. 319) found “extremely strong support for . . . Sax-Stebbins allopolyploid theory.” If, however, *Vauquelinia* is nested within the Maloideae, as ITS sequences suggest, then either base chromosome number

and fruit type are not synapomorphic for the subfamily or the character states of *Vauquelinia* and possibly related groups were derived subsequent to the origination of the Maloideae. Further, the Maloideae as usually defined may not be exclusively  $x = 17$ . There have been reports of  $n = 16$  in six genera of Maloideae (Campbell, Greene, and Dickinson, 1991), including *Eriobotrya*, which ITS DNA sequences indicate is close to *Vauquelinia* ( $n = 15$ ). Zhang (1992) noted that if Maloideae arose through hybridization, then wood anatomy rules out amygdaloids as one of the parents. One could attempt to rescue the allopolyploid hypothesis by arguing that *Vauquelinia* evolved  $n = 15$  through aneuploid reduction and its capsular (Hess and Henrikson, 1987) or follicular (Kalkman, 1988) fruit from a pome.

The status of *Vauquelinia* has been unsettled. The common view (see Phipps et al., 1991) places this genus and other genera of tribe Quillajeae (*Exochorda*, *Kageneckia*, *Lindleya*, *Lyonothamnus*, and *Quillaja*) in the Spiraeoideae. Zhang (1992) considered the Quillajeae to be Amygdaloideae or a separate subfamily between Amygdaloideae and Maloideae. Morgan, Soltis, and Robertson (1994) concluded that *Quillaja* should be removed from the Rosaceae. Sterling (1966) noted similarities between *Vauquelinia* and Maloideae in the configuration of the ovary, fusion of carpels along the inner margins, basal ovules, and floral vascularization. Goldblatt (1976) and Morgan, Soltis, and Robertson (1994) recommended moving *Lindleya* ( $n = 17$ ) and *Vauquelinia* into the Maloideae. Morgan, Soltis, and Robertson (1994) also see *Kageneckia* ( $n = 17$ ) as a member of the Maloideae.

**ITS phylogeny of Maloideae**—The most strongly supported ITS clade in this data set is the *Eriobotrya-Rhaphiolepis-Vauquelinia* group (Fig. 2). These genera are evergreen, diploid (i.e.,  $n = 15, 16$ , or  $17$ ) shrubs of relatively low latitudes (Goldblatt, 1976; Hess and Henrikson, 1987; Robertson et al., 1991). Perhaps these genera, along with other groups of Quillajeae (except perhaps *Quillaja*), belong to an early radiation distinct from the core of the subfamily. Analysis of other members of the Quillajeae might uncover other phylogenetic relationships among the *Eriobotrya-Rhaphiolepis-Vauquelinia* group.

*Eriobotrya* and *Rhaphiolepis* are considered closely related on the basis of coreless fruits with usually one large seed and thin exocarp (Robertson et al., 1991). The capsular fruits of *Vauquelinia*, with ten seeds (two in each of the five carpels; Hess and Henrikson, 1987), are quite different. ITS sequence data suggest that either the coreless, few-seeded fruits of *Eriobotrya* and *Rhaphiolepis* arose in parallel or such fruits were ancestral to the dry, many-seeded fruits of *Vauquelinia*. In addition to its nonpome fruit, *Vauquelinia* differs from Maloideae in wood ray anatomy (Zhang, 1992). The similarity in ITS sequences of *Rhaphiolepis* and *Vauquelinia*—they differ at only 3.6% of the sites in Fig. 1—is astonishing given their traditional placement in separate subfamilies and their different fruits. Fruit type, however, may not be as phylogenetically informative as chromosome number in the Rosaceae (Morgan, Soltis, and Robertson, 1994). ITS DNA sequences support the conclusion that *Vauquelinia* is a dry-fruited member of the Maloideae (Goldblatt, 1976; Morgan, Soltis, and Robertson, 1994). Our finding represents a case

of appreciable phenotypic evolution with little nrDNA sequence divergence.

Within the remaining Maloideae, *Osteomeles* is sister to the other genera of Maloideae in the full data set (Fig. 2), but not in reduced data set (Fig. 3). This genus of about three species of eastern Asia and Hawaii is the only member of the subfamily with pinnately compound leaves besides *Cormus* and *Sorbus* s. str. Unlike *Sorbus* it produces pyrenes and was therefore placed in the Crataegaeae, along with *Crataegus*, *Cotoneaster*, *Hesperomeles*, *Mespilus*, and *Pyracantha*. This tribe is not considered tenable (Robertson et al., 1991; Rohrer, Robertson, and Phipps, 1991), and dispersion of its four members represented in the ITS data set to three parts of the shortest tree (Fig. 2) corroborates doubt concerning its monophyly. Phipps et al.'s (1991) phylogeny (fig. 11) includes all the genera of Crataegaeae, except *Cotoneaster* and *Pyracantha*, as a paraphyletic group referred to as the *Crataegus* group, and Rohrer, Robertson and Phipps (1991) clustered *Crataegus*, *Hesperomeles*, *Mespilus*, and *Osteomeles* together in their fruit group 3, defined by hard pyrenes separated by a thin fleshy layer. *Hesperomeles* (not included in the present study) shares with *Osteomeles* the state of single ovules per carpel (Rohrer, Robertson, and Phipps, 1991). This state is unique in the Maloideae, and these two genera are apparently closely related (Robertson et al., 1991). In Zhang's (1992) phylogeny, *Osteomeles* is part of a multichotomy with several other genera of Maloideae, not including *Hesperomeles*.

The largest single ITS clade within the Maloideae that we consistently recovered contains *Crataegus* plus its sister *Mespilus*, and *Amelanchier*, *Malacomeles*, and *Peraphyllum*. There is considerably less bootstrap and decay-index support for this group of five genera than there is for two separate clades, *Crataegus-Mespilus* and *Amelanchier-Malacomeles-Peraphyllum* (Fig. 2). *Crataegus* and *Mespilus* hybridize (Robertson et al., 1991) and are believed closely related (Phipps, 1990) because they both bear superposed ovules (in contrast to collateral ovules for all other Maloideae except some species of *Sorbus*), thorny stems, and pyrenes separated by a thin fleshy layer in the fruits (Robertson et al., 1991). *Amelanchier*, *Malacomeles*, and *Peraphyllum* share a potential synapomorphy in their pseudoberries with false septa (Jones, 1946; Robertson et al., 1991). Fully connate carpels and the deciduous habit distinguish *Amelanchier* and *Peraphyllum* from *Malacomeles*.

Traditional emphasis on fruit characters in the Maloideae would not favor grouping *Crataegus* and *Mespilus*, with hard pyrenes, with *Amelanchier*, *Peraphyllum*, and *Malacomeles*, with berrylike fruits. On the other hand, calyx lobe morphology and ovary connation and adnation unite *Amelanchier* and *Crataegus* (Rohrer, Robertson, and Phipps, 1991). Furthermore, these five genera are distributed predominantly in the Western Hemisphere. *Malacomeles* and *Peraphyllum* are endemic to southwestern North America;  $\approx 62\%$  of the species of *Crataegus* and  $83\%$  of the species of *Amelanchier* are also from the Western Hemisphere; and *Mespilus*, long thought to include only one European species, now contains a recently discovered North American species as well (Phipps, 1990). Neither this lineage of five genera nor close groupings of any of its genera appear in Zhang's (1992) trees.

*Aria* and *Sorbus* are large genera with intertwined histories; they have traditionally been united because some of their species frequently hybridize in the wild (Robertson et al., 1991). *Aria* contains 97 species ranging through Eurasia, and *Sorbus* is a north-temperate genus of 92 species. ITS sequence data unite them weakly (Fig. 2). Robertson et al. (1991) concluded that *Aria* is probably more closely related to *Malus* and *Pyrus* than to *Sorbus*, a view not strongly incongruent with the relationships of these genera based on ITS sequences (Fig. 2).

Most broadly circumscribed genera, such as *Pyrus* (Robertson, 1974) and *Sorbus* (McAllister, 1986), have not been favored by recent students of the Maloideae (Phipps et al., 1991; Robertson et al., 1991). Recent usages of *Sorbus* s. l. vary widely in their inclusiveness, from recognition of five segregate genera (*Aria*, *Chamaemespilus*, *Cormus*, *Sorbus* s. str., and *Torminalis*; Robertson et al., 1991) to inclusion of all these taxa within *Sorbus* (Robertson, 1974; McAllister, 1986; Zhang (1992) lumped the first three genera into *Sorbus* but did not sample *Torminalis*). Robertson et al. (1991) published a few new combinations for species of segregate genera of *Sorbus*, whereas Phipps et al. (1991) and Rohrer, Robertson, and Phipps (1991, 1994) continue to use *Sorbus* in the broad sense while arguing for its dismemberment. The scattered placement of *Cormus* relative to *Aria* and *Sorbus* on our shortest trees dictates a narrow definition of *Sorbus*. *Chamaemespilus* (not sampled), with one species of southern Europe, is considered closely related to *Aria* (Robertson et al., 1991), and *Torminalis* (not sampled), with two species of Europe to Asia Minor and North Africa, hybridizes with *Aria* but differs from this genus in leaf morphology (Robertson et al., 1991). Members of *Sorbus* s. str. hybridize with members of five other genera of Maloideae (*Amelanchier*, *Aria*, *Cotoneaster*, *Crataegus*, and *Photinia*), more than any other genus in the subfamily (see Fig. 1 in Robertson et al., 1991). Additional studies of *Aria* and *Sorbus* are needed to clarify their relationship.

The remaining nine genera of Maloideae in our study form two weakly supported ITS lineages that are not recovered in the majority of bootstrap searches and that decay in trees that are one or two steps longer than the shortest (Fig. 2). One lineage contains three large, primarily Eurasian genera (*Photinia*, *Malus*, and *Pyrus*), *Chaenomeles* (four species of eastern Asia), and *Heteromeles* (one species of western North America). *Photinia*, for which we follow Robertson et al.'s (1991) interpretation as including the small North American genus *Aronia*, is superficially similar to but distinct from *Heteromeles* (Phipps, 1992). Phipps et al.'s (1991) phylogeny places *Photinia serrulata* near *Heteromeles* and quite removed from other species of *Photinia*. Rohrer, Robertson, and Phipps (1994) considered *Pyrus* and *Cydonia* to be sister groups because they share the unusual feature of styles passing through a pit in the floral cup. They also hybridize. ITS DNA sequences support the sister-group relationship of these two genera when *Pseudocydonia* (which Phipps et al.'s study [1991] did not include) is deleted from analysis (Fig. 3). Detailed studies of reproductive structures (Rohrer, Robertson, and Phipps, 1991, 1994) and wood anatomy (Zhang, 1992) do not indicate that *Malus* and *Pyrus* are sister taxa, and floral and fruit evidence fail to identify the relationships of these two

important genera. ITS DNA sequences and morphology do agree that these two genera belong to the same clade, although the composition of this clade varies greatly (Figs. 2–5).

Robertson et al. (1991) indicated that *Chaenomeles* is related to *Malus*, while Kalkman (1988) united *Chaenomeles*, *Cydonia*, *Pseudocydonia*, and *Docynia* (a south-east Asian genus of two species not included in the present study) because of their multiovulate carpels. *Chaenomeles* either joins *Docynia* and *Malus* (p.p.) if one considers fruits alone (Rohrer, Robertson, and Phipps, 1991), or its fruit is phenetically distinct and it is isolated from other genera (Rohrer, Robertson, and Phipps, 1994). *Chaenomeles* resembles *Pseudocydonia* in flowers and gland-tipped teeth of the foliage (Robertson, Phipps, and Rohrer, 1992). Robertson et al. (1991) and Rohrer, Robertson, and Phipps (1991) noted the similarity of the fruits of *Heteromeles* and *Cotoneaster*.

The second, weakly supported group contains *Cormus*, *Cotoneaster*, *Cydonia*, and *Pseudocydonia* (Fig. 2). ITS data give relatively strong support to the sister group relationship of *Cydonia* and *Pseudocydonia*. Weber (1964) noted the similarity of these genera, but Rohrer, Robertson, and Phipps (1994) found that *Pseudocydonia* sometimes links with *Cydonia* and other times with *Chaenomeles*. Our data indicate that *Cotoneaster* (plus *Cormus*) is the sister group of *Cydonia* plus *Pseudocydonia*, but there do not appear to be any morphological indicators of this relationship. *Cormus* joins this group only in the shortest trees (Fig. 2). Kalkman's clade of multiovulate genera, his *Cydonia* group—*Chaenomeles*, *Cydonia*, *Pseudocydonia*, and *Docynia*—does not correspond to any clade uncovered by ITS data. Floral and fruit morphology have tied *Cotoneaster* to *Pyracantha*, which was not included in our study (Robertson et al., 1991).

**Hybridization and maloid phylogeny**—Hybridization has been hypothesized in the genesis of the Maloideae and is currently prevalent among its genera (see Robertson et al., 1991, fig. 1). McAllister (1986) even discussed a trigenic hybrid (*Aria*, *Sorbus*, and *Torminalis*). It is important, then, to consider the potential impact of hybridization on phylogenetic estimation. Hybridization, especially if it is ancient, can be difficult to detect from ITS sequence data because concerted evolution rapidly homogenizes nrDNA repeats (Hillis and Dixon, 1991). Multiple hybridizations between the same taxa, followed by concerted evolution and lineage sorting (Neigel and Avise, 1986; reviewed by Doyle, 1992) of the ITS region, could lead to discordance between phylogenies based on ITS and morphology. Hybrid derivatives could eventually be of two types, one with the nrDNA repeat of one parent and the other with the nrDNA repeat of the other parent. Wendel, Schnabel, and Seelanan (in press) demonstrated fixation of Old World and New World nrDNA repeats in different cotton allopolyploids and consequent discordance between organismal and gene phylogenies.

Hybridization could also be substantially misleading if current genera are hybrid derivatives of phylogenetically distant parents (McDade, 1992). Hybridization between more or less distantly related parents is certainly possible in the Maloideae, as crosses have been reported between genera that are not close to one another in the most-

parsimonious reconstructions based on pooled ITS DNA sequences, morphology, and wood anatomy (Fig. 7). For example, all five genera with which *Sorbus* crosses (*Ame-lanchier*, *Aria*, *Cotoneaster*, *Crataegus*, and *Photinia*) attach to different parts of the tree. Clearly the ability to interbreed is not a reliable indicator of relationships in the Maloideae but is instead a long-retained, ancestral character state.

Genera of Maloideae are either uniformly diploid ( $x = 17$ ) or include both diploid and polyploid species (Campbell, Greene, and Dickinson, 1991); there are no genera that are exclusively polyploid and therefore potentially allopolyploid in origin. It may be that some of the larger genera are not monophyletic, a possibility consistent with morphological data (see below). Some actual monophyletic units then may be hybrid derivatives. Homoploid reticulate evolution is possible in the Maloideae, although it has rarely been well documented in flowering plants (Rieseberg, 1991).

**Integrating ITS DNA sequence, morphological, and wood anatomical data for an overall estimate of phylogeny of Maloideae**—Recent studies of Maloideae phylogeny (Phipps et al., 1991; Rohrer, Robertson, and Phipps, 1991; Zhang, 1992) involve more extensive taxonomic sampling than the present study. Phipps et al.'s (1991) data include 36 vegetative, floral, and fruit characters for 96 Maloideae species and 21 genera. They did not include outgroups due to uncertainty in their selection and the problem of homology assessment of fruiting characters. Homoplasy was extensive, as indicated by the consistency index of 0.12. It is not possible to say whether or not this level is excessive because Sanderson and Donoghue's (1989) regression of consistency index on number of taxa does not extend beyond  $\approx 60$  taxa. Phipps et al. (1991) attributed this homoplasy in part to "the idea that several clades of Maloideae originated independently in a highly reticulate system existing shortly after the original allotetraploid cross" (p. 303; see also Robertson, Phipps, and Rohrer, 1992). Even though they published the network (fig. 11) that "matches phenetic genera best," it is noteworthy that no genus with more than 40 species (i.e., *Aria*, *Cotoneaster*, *Crataegus*, *Malus*, *Photinia*, *Pyrus*, and *Sorbus* s. str.) is monophyletic.

Zhang (1992) used 18 wood anatomical characters, chromosome number, and fruit type for 64 genera of Rosaceae. Using a "Bailey transformation series of wood anatomy" (p. 98) to polarize all but four characters, Zhang obtained over 100 minimal-length trees with a consistency index of 0.29. The 21 Maloideae genera in Zhang's study, although homogeneous in terms of wood anatomy, form a paraphyletic assemblage (within which the rosoid *Cercocarpus* is nested) in the one published tree for the Rosaceae. *Sorbus* is treated as including *Aria*, *Cormus*, and *Chamaemespilus* (*Torminalis* was not included in the study) but not *Malus*, *Micromeles*, and *Pyrus*. Zhang also analyzed genera of Maloideae separately using one of the genera (*Polylepis*) in the presumed sister clade as an outgroup and again recovered over 100 minimal-length trees. The tree chosen for publication has little in common with the results for all Rosaceae.

Finally Rohrer, Robertson, and Phipps (1991) used 18

quantitative fruit characters for a Manhattan-distance/UPGMA phenetic analysis of 173 Maloideae species from all the major genera. Species clustered with congeners for the most part, and, while noting that taxonomic structure above the rank of genus is difficult to recognize in the Maloideae, they identified nine "fairly consistent groupings of genera" (p. 1627).

Morphological (Phipps et al., 1991) and wood anatomical (Zhang, 1992) studies show similar values of the consistency index or retention index for analyses of genera in common with our ITS sample (Table 3, single data sets 2 vs. 3 and 4 vs. 7). These results support Donoghue and Sanderson's (1992) observation that morphological data are not necessarily more homoplastic than molecular data.

Levels of congruence among anatomical, ITS DNA sequence, and morphological data sets differ depending upon the type of measurement (taxonomic or character congruence) and character congruence index. That the strict-consensus of ITS-DNA-sequence trees (Fig. 3) and the morphological tree (Fig. 4) is unresolved is due to the very different placement of many of the taxa by these two data sets. The character state of compound leaves, for example, ties together *Osteomeles*, *Cormus*, and *Sorbus* in the morphological consensus (Fig. 4), whereas ITS DNA sequences do not record a close relationship among these three genera (Fig. 3). Strict consensus may be "too strict" (Swofford, 1991, p. 298), as it misses the common signal coming from pooled analysis of ITS DNA sequences and morphology (Fig. 5).

Recent comparisons of molecular and morphological phylogenies uncover high taxonomic congruence, although the number of taxa is lower than the 18 genera of the present study. Kluge (1989) studied 11 species of snakes; DeSalle and Grimaldi (1991) nine taxa of Drosophilidae; Bousquet, Strauss, and Li (1992) six species in the birch family, and Omlund (1994) nine species of ducks. Not surprisingly, levels of character incongruence are low in all these studies except that of DeSalle and Grimaldi (1991), who only considered taxonomic congruence.

Values of incongruence among anatomical, ITS DNA sequence, and morphological data sets, as measured by  $I_M$ ,  $I_{MF}$ ,  $RI_F$ , and  $RI_P$ , span a wide range of values. For ITS DNA sequences and morphology, for example,  $I_{MF}$  is 0.086 (only 8.6% of the total homoplasy is between the data sets), whereas the  $RI_F$  value of 0.199 shows a low level of congruence between the data sets. Some of great range in these four indices probably comes from the undervaluing of incongruence by  $I_M$ ,  $I_{MF}$ , and  $RI_P$  (Campbell, unpublished data).

Levels of character congruence among anatomical, ITS DNA sequence, and morphological data sets (Table 4) are similar to those between ITS 1 plus the 3' end of the 5.8S region and ITS 2. We assume then that the three data sets do not conflict strongly and that pooling them is appropriate (de Queiroz, 1993). We obtain similar phylogenetic relationships among genera whether we pool only those genera for which the data are complete in all three data sets (Table 3, combined data set 4; Fig. 6) or use the pseudofossil approach (Weins and Reeder, in press) and pool the full set of genera of Table 1 (Table 3, pooled data set 5; Fig. 7). *Eriobotrya*, *Raphiolepis*, and (for the full



analysis, Fig. 7) *Vauquelinia* form a clade that is sister to the remainder of (or core) Maloideae. The data support recognition of the *Crataegus-Mespilus-Amelanchier-Peraphyllum* (and *Malacomeles* for the full data set) clade and the small clade of *Chaenomeles*, *Malus*, and *Pyrus* belong to a small clade that also includes *Aria* for the full data set (Fig. 7). *Cotoneaster*, *Cydonia*, and *Heteromeles* also form a small clade, which *Pseudocydonia* joins in the full data set (Fig. 7).

Additional data are required to bring into better focus overall relationships within the Maloideae, Quillajaceae, and especially within labile genera of Maloideae, such as *Aria*, *Cotoneaster*, *Heteromeles*, *Osteomeles*, and *Photinia*. Combined analysis of diverse data will hopefully amplify phylogenetic signal, dampen random noise, and clarify relationships within this evolutionarily complex group.

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