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Phylogeny of Elms (*Ulmus*, Ulmaceae): Molecular Evidence for a Sectional Classification

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ABSTRACT. The approximately 45 woody species of *Ulmus* (Ulmaceae) have been placed in five to nine sections on the basis of morphological characters. Cladistic analyses of chloroplast DNA restriction site variation were employed to examine phylogenetic relationships among 29 *Ulmus* accessions, including representatives from all proposed sections and subsections, and *Zelkova serrata*. Sufficient variation was detected to construct cladograms with branches both well-resolved and supported. The cpDNA results are largely congruent with those based on nuclear ribosomal DNA. Inclusion of 18 morphological/chemical characters further resolved relationships within the genus. Intrageneric relationships implied by the molecular and combined cladograms differ from previous classifications in a number of respects. Three species, *U. crassifolia*, *U. serotina*, and *U. thomasii*, which have been placed in two or three sections, were found to form a well differentiated monophyletic group (sect. *Trichoptelea*). The maintenance of sections *Anisoptelea* and *Trichocarpus* and the recognition of subsections *Foliaceae* and *Glabrae* within section *Ulmus* are not supported. The inclusion of *U. mexicana*, sometimes treated as the distinct genus *Chaetoptelea*, within *Ulmus* is supported. The molecular evidence supports the distinctiveness of *U. rubra* and the recognition of two subgenera: *Oreoptelea* (sects. *Blepharocarpus*, *Chaetoptelea*, and *Trichoptelea* s. l.) and *Ulmus* (sects. *Lanceifolia*, *Microptelea*, and *Ulmus*).

Ulmus comprises approximately 45 woody species, widely distributed throughout the north temperate regions (excluding western North America), and extending to the subtropics in Central America and southeast Asia (Pennington and Sarukhan 1968; Fu 1980). Although the genus is generally well defined, the delimitation of species and their taxonomic affinities have been controversial. These difficulties have been attributed to the paucity of taxonomic characters in a group characterized by simple, highly reduced flowers and fruits, variable vegetative characters, few barriers to interspecific hybridization, and little phytochemical differentiation (Richens 1983). Additional complications result from human disturbance of natural species distributions and nomenclatural controversies including the type designation of Linnaeus. Taxonomic problems are particularly rampant within sect. *Ulmus* (Heybroek 1976).

The most widely recognized treatment of *Ulmus* was published by Schneider (1916) who divided the genus into five sections (Table 1): *Microptelea*, *Trichoptelea*, *Chaetoptelea*, *Blepharocarpus*, and *Madocarpus* (= sect. *Ulmus*, see following). Grudzinskaya accepted the lectotypification of *U. americana* L. by Hutchinson (1967). This was done at a time when the name *U. campestris* L., which had been designated the type by Britton and Brown (1913), was considered invalid by reason of *nomen ambiguum* [the name had been widely applied to different biological identities (Melville 1938; Grudzinskaya 1971)]. Current nomenclatural practice, however, does not recognize the *nomen ambiguum* argument, and thus *U. campestris* must still be recognized as the type (F. R. Barrie, pers. comm.). Although the species to which the name applies has not been established, two possibilities, now referred to as *U. glabra* Hudson and *U. carpini-*

folia Ruppius ex Suckow (and various other names) are included by Schneider (1916) in sect. *Madocarpus*. We therefore refer to this section, sensu Schneider, as *Ulmus*. Under Grudzinskaya's system, however, *U. glabra* is still placed in sect. *Madocarpus*, but *U. carpinifolia* is transferred to sect. *Foliaceae*. Until one of these is designated a lectotype, there is no means of determining which of Grudzinskaya's sections should be named *Ulmus*, although the use of *Ulmus* as a subgeneric name for the group containing *U. americana* and not "*U. campestris*" is now considered inappropriate.

Sections *Microptelea* (ditypic) and *Trichoptelea* (monotypic) differ from the rest of the genus in having calyx lobes free for more than half their length. Both sections are found in southeastern North America but with the third species in east Asia. Schneider distinguished sect. *Microptelea* from sect. *Trichoptelea* by compressed floral axes and short pedicels in the former versus the distinctly racemose cymes in the latter. The calyces in the other three sections are shallowly lobed. The North American sect. *Chaetoptelea* has elongated floral axes and pedicels, and pubescent and ciliate fruit, whereas sect. *Blepharocarpus*, distributed in eastern North American and Europe, has fasciculate cymes with elongated pedicels, and ciliate but otherwise glabrous samaras. Section *Madocarpus/Ulmus*, basically Eurasian except for *U. rubra* in eastern North America, has compressed floral axes, short pedicels, and samaras ranging from glabrous and eciliate to pubescent and cilate. The position of the seed, in the center or near the apex of the samara, was used to distinguish subsects. *Glabrae* and *Foliaceae*, respectively, within sect. *Madocarpus/Ulmus*.

The classification system of Schneider (1916), although widely accepted (Yarmolenko 1936; Rehder 1940; Elias 1970), has been the subject of a number of revisions as new methods of data analysis, new data (e.g., flavonoid data), and new theories to explain patterns of variation have called into question the species composition of almost every one of the sections (Cheng et al. 1963; Sweitzer 1971; Bate-Smith and Richens 1973; Grudzinskaya 1975, 1980; Heybroek 1976; Fu 1980; Table 1).

There are four main points of contention with Schneider's (1916) classification system. 1) Should *Ulmus mexicana* (Liebm.) Planch. be excluded from *Ulmus* and regarded as a separate monotypic genus, *Chaetoptelea*? 2) Are there sufficient grounds for recognizing two or more subgenera within the genus? 3) Are *U. alata* Michx., *U. crassifolia* Nutt., *U. mexicana*, *U. serotina* Sarg., and *U. thomasii* Sarg. more closely related than is indicated by their placement in three different sections? 4) Does Schneider's sect. *Madocarpus/Ulmus*, in fact, comprise two or more distinct sections?

Cladistic analyses of chloroplast (cp) DNA restriction site variation have proven useful in resolving taxonomic problems in numerous plant taxa at this rank (see reviews in Palmer et al. 1988; Soltis et al. 1992; Sytsma and Hahn 1994). Although cpDNA results can sometimes be misleading due to chloroplast "capture" (Smith and Sytsma 1990; Sytsma 1990; Soltis et al. 1991; Rieseberg and Brunsfeld 1992; Rieseberg and Wendel 1993), these inconsistencies can be readily determined by comparing the chloroplast cladogram to one derived from nuclear DNA. Taxonomic and nomenclatural changes based in part on chloroplast DNA studies are becoming more frequent [e.g., *Heterogaura/Clarkia* (Sytsma and Gottlieb 1986a; Lewis and Raven 1992), *Boisduvalia/Epilobium* (Hoch and Raven 1992; Baum et al. 1994), subfamily Barnadesioideae (Jansen et al. 1992; Bremer et al. 1992), tribe Tarchonanthe (Keeley and Jansen 1991), *Lycopersicon/Solanum* (Spooner et al. 1993), *Papaver* (Kadereit and Sytsma 1992), *Psilactis/Machaeranthera* (Morgan 1993), *Sphenostylis/Nesphostylis* (Potter and Doyle, 1994), and *Rollandia/Cyanea* (Lammers et al. 1993; Givnish et al. 1994)]. In the current study, representatives of all but one of the proposed sections and all subsections of *Ulmus* were examined using cladistic analysis of cpDNA restriction site data. We also explored the ramifications of combining the cpDNA data set and a preliminary morphological/chemical data set. The resulting phylogenies are used to track the evolution of specific morphological and chemical characters, assessed relative to other nuclear DNA data, compared to the various classification systems, and then used to recommend certain revisions in the classfication that more closely reflect the evolutionary history of *Ulmus*.

Materials and Methods

Plant Materials. Twenty-nine accessions of 14 species representing eight of the nine pub-

TABLE 1. Differing taxonomic classifications for the species of *Ulmus* studied. Bate-Smith and Richens' designations are for "ad hoc" groups, to which formal names were not given. Their *U. davidiana* may be synonymous with *U. japonica*. The designation — indicates that this taxon was not included in the classification system or study. § = section.

Species	Schneider (1916)	Sweitzer (1971)	Bate-Smith & Richens (1973)	Grudzinskaya (1975, 1980)	Heybroek (1976)	Fu (1980)
Ulmus americana L.	§ *Blepharocarpus* Dumort.	*Ulmus* L.	C	subg. *Ulmus*, § *Ulmus*, ser. *Ulmus*	§ *Blepharocarpus* Dumort.	§ *Blepharocarpus* Dumort.
U. laevis Pallas	§ *Blepharocarpus* Dumort.	*Ulmus* L.	C	subg. *Ulmus*, § *Ulmus*, ser. *Ulmus*	§ *Blepharocarpus* Dumort.	§ *Blepharocarpus* Dumort.
U. mexicana (Liebm.) Planchon	§ *Chaetoptelea* (Liebm.) C. Schneider	*Chaetoptelea* Liebm.	A	subg. *Ulmus*, § *Chaetoptelea* (Liebm.) C. Schneider	§ *Chaetoptelea* (Liebm.) C. Schneider	§ *Chaetoptelea* (Liebm.) C. Schneider, ser. *Mexicanae* L. K. Fu
U. alata Michx.	§ *Chaetoptelea* (Liebm.) C. Schneider	*Ulmus* L.	A	subg. *Ulmus*, § *Chaetoptelea* (Liebm.) C. Schneider	§ *Chaetoptelea* (Liebm.) C. Schneider	§ *Chaetoptelea* (Liebm.) C. Schneider, ser. *Mexicanae* L. K. Fu
U. thomasii Sarg.	(as *U. racemosa* Thomas) § *Chaetoptelea* (Liebm.) C. Schneider	*Ulmus* L.	B	subg. *Ulmus*, § *Trichoptelea* C. Schneider	§ *Chaetoptelea* (Liebm.) C. Schneider	§ *Chaetoptelea* (Liebm.) C. Schneider, ser. *Thomasianae* L. K. Fu
U. serotina Sarg.	§ *Trichoptelea* C. Schneider	*Ulmus* L.	A	subg. *Ulmus*, § *Trichoptelea* C. Schneider	§ *Trichoptelea* C. Schneider	§ *Trichoptelea* C. Schneider
U. crassifolia Nutt.	§ *Microptelea* (Spach) Benth. & Hook.	*Ulmus* L.	A	subg. *Ulmus*, § *Anisoptelea* Grudz.	—	—
U. parvifolia Jacq.	§ *Microptelea* (Spach) Benth. & Hook.	*Ulmus* L.	E	subg. *Ulmus*, § *Microptelea* (Spach) Benth. & Hook.	§ *Microptelea* (Spach) Benth. & Hook.	§ *Microptelea* (Spach) Benth. & Hook.
U. rubra Muhlenb.	(as *U. fulva* Michx.) § *Madocarpus* Dumort., ssect. *Glabrae* (Moss) C. Schneider, ser. *Fulvae* C. Schneider	*Ulmus* L.	F	subg. *Dryoptelea* (Spach) Planchon, § *Madocarpus* C. Schneider	§ *Ulmus*, ser. *Fulvae* C. Schneider	§ *Ulmus*, ser. *Fulvae* C. Schneider

TABLE 1. Continued.

Species	Schneider (1916)	Sweitzer (1971)	Bate-Smith & Richens (1973)	Grudzinskaya (1975, 1980)	Heybroek (1976)	Fu (1980)
U. glabra Hudson	§ *Madocarpus* Dumort., ssect. *Glabrae* (Moss) C. Schneider, ser. *Euglabrae* C. Schneider	*Ulmus* L.	F	subg. *Dryoptelea* (Spach) Planchon, § *Madocarpus* C. Schneider	§ *Ulmus*, ser. *Euglabrae* C. Schneider	§ *Ulmus*, ser. *Glabrae* Moss
U. carpinifolia Ruppius ex Suckow	(as *U. foliaceae* Gilbert), § *Madocarpus* Dumort., ssect. *Foliaceae* C. Schneider, ser. *Nitentes* Moss	*Ulmus* L.	G	(as *U. campestris*) subg. *Dryoptelea* (Spach) Planchon, § *Foliaceae* (C. Schneider) Grudz., ser. *Nitentes* Moss	§ *Ulmus*, ser. *Nitentes* Moss	—
U. macrocarpa Hance	§ *Madocarpus* Dumort., ssect. *Glabrae* (Moss) C. Schneider, ser. *Wallichiana* C. Schneider	*Ulmus* L.	F	subg. *Dryoptelea* (Spach) Planchon, § *Trichocarpus* Cheng	§ *Trichocarpus* Cheng	§ *Ulmus*, ser. *Glabrae* Moss
U. glaucescens Franchet	§ *Madocarpus* Dumort., ssect. *Foliaceae* C. Schneider, ser. *Pumilae*, C. Schneider	—	—	subg. *Dryoptelea* (Spach) Planchon, § *Trichocarpus* Cheng	§ *Trichocarpus* Cheng	§ *Ulmus*, ser. *Nitentes* Moss
U. japonica (Rehd.) Sarg.	§ *Madocarpus* Dumort., ssect. *Foliaceae* C. Schneider, ser. *Nitentes* Moss	—	G?	subg. *Dryoptelea* (Spach) Planchon, § *Foliaceae* (C. Schneider) Grudz., ser. *Nitentes* Moss	§ *Ulmus*, ser. *Nitentes* Moss	§ *Ulmus*, ser. *Nitentes* Moss

lished sections and both subsections (Schneider 1916; Cheng et al. 1963; Grudzinskaya 1975, 1980) of *Ulmus* were studied. In addition to naturally occurring individuals, specimens of verified taxa from selected arboreta were incorporated whenever possible (Table 2). Only sect. *Madocarpus/Ulmus* with over 30 species was not extensively sampled in this analysis. Preliminary results with a larger set of species (21) and accessions (44) from this section indicate that a more thorough study of the section is needed to clarify relationships within one portion of the section, although monophyly of the section is not questioned (Wiegrefe 1992; Wiegrefe et al., in mss.). The six species selected from sect. *Madocarpus/Ulmus* for this genus wide survey represent the basic clades detected in the more detailed study.

Zelkova serrata Mak. was used as the outgroup (Watrous and Wheeler 1981). *Zelkova* was placed in the Ulmoideae by Grudzinskaya (1967; as Ulmaceae s. str.) and Giannasi (1978), and was found to be closely related to *Ulmus* on the basis of cpDNA restriction site mapping and *rbc*L (large subunit gene for ribulose-1,5-bisphosphate carboxylase/oxygenase) sequencing among genera in the family (Wiegrefe 1992; Wiegrefe et al., in mss.). *Hemiptelea* and *Celtis* (both Ulmaceae s. l.) were used initially along with *Zelkova* for global outgroup analysis (sensu Maddison et al. 1984), but were subsequently omitted when restriction site homology could not be determined relative to *Ulmus* and *Zelkova* without resorting to detailed restriction site mapping using double digestions (Wiegrefe 1992).

Sample Preparation and Data Collection. Total DNA was extracted from leaf tissue using protocol B of Smith et al. (1992), involving a CTAB lysis following an initial organelle isolation. The following modifications and clarifications were necessary: PEG (polyethylene glycol) was added to the grinding buffer to a concentration of 2% w/v; all alcohol precipitations were carried out using two volumes cold ethanol (including step 7); RNAse was not used, nor were the samples purified with phenol and phenol/chloroform (steps 12 through 14); and, following the final alcohol precipitation, the samples were washed twice by covering the samples with cold 70% ethanol in water for a minimum of 20 min before air drying. Multiple extractions were necessary for some accessions, each using 0.9–2.0 g of frozen tissue to acquire sufficient DNA for the restriction enzyme survey.

Aliquots of the DNA's were digested with 25–30 restriction endonucleases according to manufacturers' specifications (Bethesda Research Labs and New England Biolabs) with each enzyme recognizing a different six-basepair sequence. The restriction enzymes used were: *Apa*I, *Apa*LI, *Ava*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Cla*I, *Dra*I, *Eco*NI, *Eco*O1090, *Eco*RI, *Eco*RV, *Hin*dIII, *Mlu*I, *Pst*I, *Pvu*II, *Sca*I, *Sst*I, *Sst*II, *Stu*I, *Sty*I, *Xba*I, and *Xmn*I. Preliminary work showed no intrageneric variation in the restriction sites of *Bst*BI, *Kpn*I, *Sal*I, *Sma*I, or *Xho*I, and thus these enzymes were not utilized on all accessions. Procedures for fragment electrophoresis, bidirectional transfer, and permanent bonding of DNA to nylon membranes (Biodyne) followed Smith et al. (1992). Filters were sequentially probed with 10 heterologous cpDNA fragment clones or clone combinations that cover almost the entire chloroplast genome: S6, S8, P3, P6, P8, P10, P12, P14, P18, and P19 from *Petunia* (Sytsma and Gottlieb 1986b), and *Lactuca* clones 18.8 (L1), *Pst*I/*Sst*I 6.2, *Sst*I 3.5 and *Sst*I 1.8 (Jansen and Palmer 1988). Nick translations, hybridizations, and washes followed the protocol of Sytsma and Schaal (1985). Autoradiography was employed for fragment visualization.

A morphological/chemical data set was constructed for the same taxa examined for cpDNA variation. The data set includes 18 characters (Table 3) taken from leaves (3), wood (1), flowers (6), fruits (6), and flavonoids (2). Difficulties in finding a sufficient number of useful characters, in assessing homology, and in obtaining data for all species precluded finding a morphological/chemical consensus tree with adequate resolution. The morphological/chemical data set was subsequently combined with the cpDNA data to examine relationships based on all available evidence.

Phylogenetic Analyses. The most parsimonious cpDNA trees were identified using PAUP version 3.1.1 (Swofford 1993) with the branch-and-bound or the heuristic options (the latter using simple addition sequence, TBR branch swapping, steepest descent, and collapsing zero-length branches). Both Wagner parsimony (Farris 1970) and weighted parsimony (Albert et al. 1992) were employed. Character state weighting was used to discriminate

against convergent gains using several character-state step matrices. The weighting schemes used values (from 1.05 to 2.0, gains:losses) that bracket the range empirically determined to be appropriate for cpDNA studies within angiosperms (Albert et al. 1992). Support for each branch was estimated using bootstrap analysis (Felsenstein 1985) in PAUP, and by examining trees one through three steps longer using the keep function in PAUP [Bremer 1988; Donoghue et al. 1992 (as "decay" function)]. Strict consensus trees were constructed using the PAUP consensus tree option for all shortest trees and also trees $\leq$ n steps long. The decay values for all branches maintained after relaxing parsimony by three steps were determined by using topological constraints (Swofford 1993). The shortest trees violating each constraint were found by using 10 replications of random addition sequence (shortest trees were often not found with a single run of simple addition sequence) with the heuristic approach (TBR branch swapping and steepest descent). Alternative topologies suggested by previous studies and the number of extra steps they require were examined using MacClade 3.0 (Maddison and Maddison 1992). The combined data set of cpDNA and morphological/chemical characters was analyzed by maintaining all state transformations unordered unless otherwise noted. Character state changes in morphological/chemical characters were placed on the combined data set trees using MacClade and PAUP under various optimization criteria.

Results

Colinearity with the *Petunia* chloroplast genome was established by restriction site mapping of *Ulmus thomasii* using nine restriction enzymes (Wiegrefe 1992; Wiegrefe et al., in mss.). The mode of chloroplast inheritance could not be demonstrated conclusively, but preliminary evidence indicates that it is via the seed parent. Maternal inheritance is supported by epifluorescence microscopy observations (Corriveau and Coleman 1988).

A total of 667 cpDNA restriction sites was analyzed per accession, accounting for 3.0 percent of the 160 kb chloroplast genome in *Ulmus*. One hundred twenty-six mutations were detected within *Ulmus* (122 informative at the accession level) and 22 unpolarized mutations were detected between *Ulmus* and *Zelkova* (Appendix 1). One verified deletion of ca. 200 bp was detected in *Ulmus rubra* Muhl.-23 in the S8 probe region, but was not included in the analyses.

High mucilage content, especially in older leaves, led to difficulties in extracting high purity DNA and obtaining complete digestion with certain restriction enzymes in several accessions. These difficulties resulted in significant amounts of missing data for these few accessions, inclusion of which generated large numbers of trees and collapsed well-supported clades during "decay" analyses. Four accessions (11, 13, 15, 24) were not included in these phylogenetic analyses to circumvent these problems. In all four cases, however, multiple representatives of each species were being examined. Thus, the missing information for the four accessions was not critical, and the four accessions could be placed *a posteriori* onto the resulting cladograms using diagnostic synapomorphies.

The branch-and-bound option of PAUP identified five most parsimonious Wagner trees 168 steps (including autapomorphies) in length using the cpDNA data set. These trees have consistency indices (Kluge and Farris 1969) of 0.881/0.856 (with/without autapomorphies included), retention index of 0.962, and rescaled consistency indices of 0.848/0.824. One of these five trees is depicted in Fig. 1 and includes locations of the mutations (numbered following Appendix 1), the bootstrap confidence level and decay index for each branch, and Schneider's (1916) classification of the taxa. The strict consensus of the five trees is fully resolved with four exceptions: 1) a soft polytomy (two possible resolutions: one trichotomy and one fully dichotomized tree) remains for the three clades (*U. americana* + *U. laevis* Pall.), (*U. alata* + *U. mexicana*), and (*U. crassifolia* + *U. serotina* + *U. thomasii*); 2) two possible resolutions of relationships for *U. thomasii* and the subclade *U. crassifolia* + *U. serotina*; 3) *U. crassifolia* and *U. serotina* are indistinguishable, and 4) a hard trichotomy (i.e., zero-length branches) remains within sect. *Madocarpus*/*Ulmus*) involving five of the six species examined.

The consensus tree from the 91 cpDNA trees of $\leq$ 169 steps (decay at +1 steps) results in six additional polytomies (see Fig. 1): two involve lack of intraspecies resolution (*Ulmus laevis* and *U. parvifolia* Jacq.); one grouping *U. macrocarpa*

TABLE 2. Accessions examined, code numbers used in Figs., material sources, voucher numbers, and origins. Sources codes: CA = Los Angeles State and County Arboretum, Arcadia, California; GA = Kris Medic Thomas, Pine Mountain, Georgia; IL = Morton Arboretum, Lisle, Illinois; MA = Arnold Arboretum of Harvard University, Jamaica Plain, Massachusetts; MN = University of Minnesota Landscape Arboretum, Chanhassan, Minnesota; PA = Morris Arboretum of the University of Pennsylvania, Philadelphia, Pennsylvania; WI = University of Wisconsin, Madison, College of Agriculture and Life Sciences Arlington Research Station, Arlington, Wisconsin.

No. Taxon	Source	Tree	Voucher	Origin
1. *Zelkova serrata* Mak.	PA	81-502	*Wiegrefe & Stonehill 123* (WIS)	wild: *Meyer 143*, S. Korea
2. *Ulmus americana* L.	WI	—	*Wiegrefe 187* (WIS)	cult.: Madison, Wisconsin
3. *U. americana* L.	WI	2166	*Weigrefe 186* (WIS)	cult.: Pineville, Louisiana
4. *U. laevis* Pallas	WI	2051-17	—	cult.: Yining, People's Republic of China
5. *U. laevis* Pallas	IL	559-64	*Wiegrefe 73* (WIS, MOR)	United States Department of Agriculture PI #298952
6. *U. laevis* Pallas	MA	6951	—	—
7. *U. mexicana* (Liebm.) Planchon	WI	—	*Wiegrefe & Castillo 131* (WIS, XAL)	wild: Xalapa, Veracruz, Mexico
8. *U. alata* Nutt.	IL	—	*Wiegrefe & Ware 144* (WIS, MOR)	cult.: Cook Co., Illinois
9. *U. alata* Nutt.	GA	—	—	cult.: Pine Mountain, Georgia
10. *U. thomasii* Sarg.	WI	—	*Wiegrefe 74* (WIS)	cult.: Columbia Co., Wisconsin
11. *U. thomasii* Sarg.	MN	58-0627	*Wiegrefe & Zuzek 140* (WIS)	wild: St. Paul, Minnesota
12. *U. thomasii* Sarg.	IL	178-84	*Altvatter & Bradtke 5805V93* (MOR, WIS)	wild: Manitowoc Co., Wisconsin
13. *U. thomasii* Sarg.	MA	17926-C	—	—
14. *U. serotina* Sarg.	IL	1039-23	*Wiegrefe 83* (WIS, MOR)	cult.: Forest Nursery Co., McMinnville, Tennessee
15. *U. crassifolia* Nutt.	IL	385-68	*Altvatter & Bradtke 5808V93* (MOR, WIS)	wild: Sequin, Texas
16. *U. crassifolia* Nutt.	CA	52-P-288	—	—
17. *U. parvifolia* Jacq.	WI	948-3	*Wiegrefe 175* (WIS)	wild: *Heybroek 157*, Japan
18. *U. parvifolia* Jacq.	IL	410-68	*Gavlak 4236V90* (MOR)	cult.: Taylor Arboretum Chester, Pennsylvania
19. *U. parvifolia* Jacq.	MA	17917-B	—	cult.: Tokyo Government Forestry School, Japan
20. *U. macrocarpa* Hance	MA	17911	*Every et al. 1781* (WIS)	cult.: United States Department of Agriculture
21. *U. macrocarpa* Hance	IL	179-84	*Ware 5712V93* (MOR, WIS)	cult.: Harbin Bot. G., People's Republic of China
22. *U. rubra* Muhl.	WI	—	*Wiegrefe 77* (WIS)	cult.: Madison, Wisconsin
23. *U. rubra* Muhl.	IL	184-66	*Wiegrefe 72* (WIS, MOR)	wild: Clermont, Kentucky

TABLE 2. Continued.

No. Taxon	Source	Tree	Voucher	Origin
24. *U. rubra* Muhl.	MA	21966-C	—	wild: West Virginia
25. *U. glabra* Hudson	WI	901-2	—	wild: Eidsvold, Norway
26. *U. glabra* Hudson	MA	17143	—	—
27. *U. japonica* (Rehd.) Sarg.	WI	906-47	*Wiegrefe 76* (WIS)	wild: *Heybroek 58*, Japan
28. *U. japonica* (Rehd.) Sarg.	MA	4119	—	—
29. *U. glaucescens* Franchet	IL	537-76	*Altvatter & Bradtke 5806V93* (MOR, WIS)	cult.: Beijing Bot. G., Bot. Inst. of the Chinese Acad. Sci., People's Republic of China
30. *U. carpinifolia* Ruppius ex Suckow	IL	1463-24	—	cult.: Arnold Arboretum, Jamaica Plain, Massachusetts

Hance and *U. glaucescens* Fr.; one grouping several clades within sect. *Madocarpus/Ulmus*; one collapsing sect. *Blepharocarpus*; and one collapsing sect. *Madocarpus/Ulmus* with *U. parvifolia*. At ≤171 steps (2,969 trees with decay at +3 steps) eight clades were maintained. Eight clades are maintained even after parsimony is relaxed 5 steps, with one of these finally collapsing at 18 extra steps. Support for branches uniting presumed conspecific accessions were estimated at 90% or more from 100 bootstrap replicates with the following exceptions: *Ulmus americana* 81%, *U. macrocarpa* 56%, and *U. glabra* 86%. Strong support (100% bootstrap level) is found for the division of the genus into two clades (Fig. 1). Strong support for the monophyly of clades formed by *U. crassifolia*, *U. serotina*, and *U. thomasii* (100%), by *U. glabra*, *U. glaucescens*, *U. japonica* Sarg., *U. carpinifolia*, and *U. macrocarpa* (100%), and by *U. glaucescens* and *U. macrocarpa* (100%) was also found. Less support was provided for the clades formed by *U. americana* and *U. laevis* (71%), by *U. alata* and *U. mexicana* (77%), and by *U. glabra* and *U. carpinifolia* (57%).

Discrimination against convergent gains or loss/gains using character state weights of 1.05 to 1.3:1 (gains : losses) resulted in a single shortest cpDNA tree equivalent to one of the five Wagner trees. This tree is depicted in Fig. 1 and represents a likely estimation of relationships in *Ulmus* based on cpDNA. At weights of 1.5 to 2.0, unrealisticly high weights for studies within genera (Albert et al. 1992), one shortest tree was found that is equivalent to one of the 86 Wagner trees with a length of 169 (one step longer than the most parsimonious trees).

The morphological/chemical data alone generated a consensus tree (not shown) with very little resolution (5800+ trees of 68 steps). The combined data (149 molecular + 18 morphological/chemical) analysis produced two trees at 236 steps (consistency index of 0.869/0.723). The consensus of the combined data trees (Fig. 2) resolves two nodes forming trichotomies in the consensus of the trees based solely on cpDNA. Resolution is seen within sect. *Madocarpus/Ulmus* and the "rock elm" clade is identified (*U. mexicana*, *U. alata*, *U. thomasii*, *U. serotina*, and *U. crassifolia*), a group recognized in only two of the five cpDNA trees. Only *U. thomasii* is unresolved, forming either a monophyletic species or a basal and paraphyletic species in its section. Mapping character state changes onto the cpDNA or combined trees (Fig. 2), indicates considerable disagreement of some of the morphological and chemical characters with the cpDNA data. In particular, three apomorphies are shared between *U. parvifolia* and various members of the "rock elm clade," relationships strongly opposed by the cpDNA tree (Figs. 1, 2).

DISCUSSION

The Revival of Subgeneric Designations. The most conspicuous feature of the cpDNA cladograms is the great number of mu-

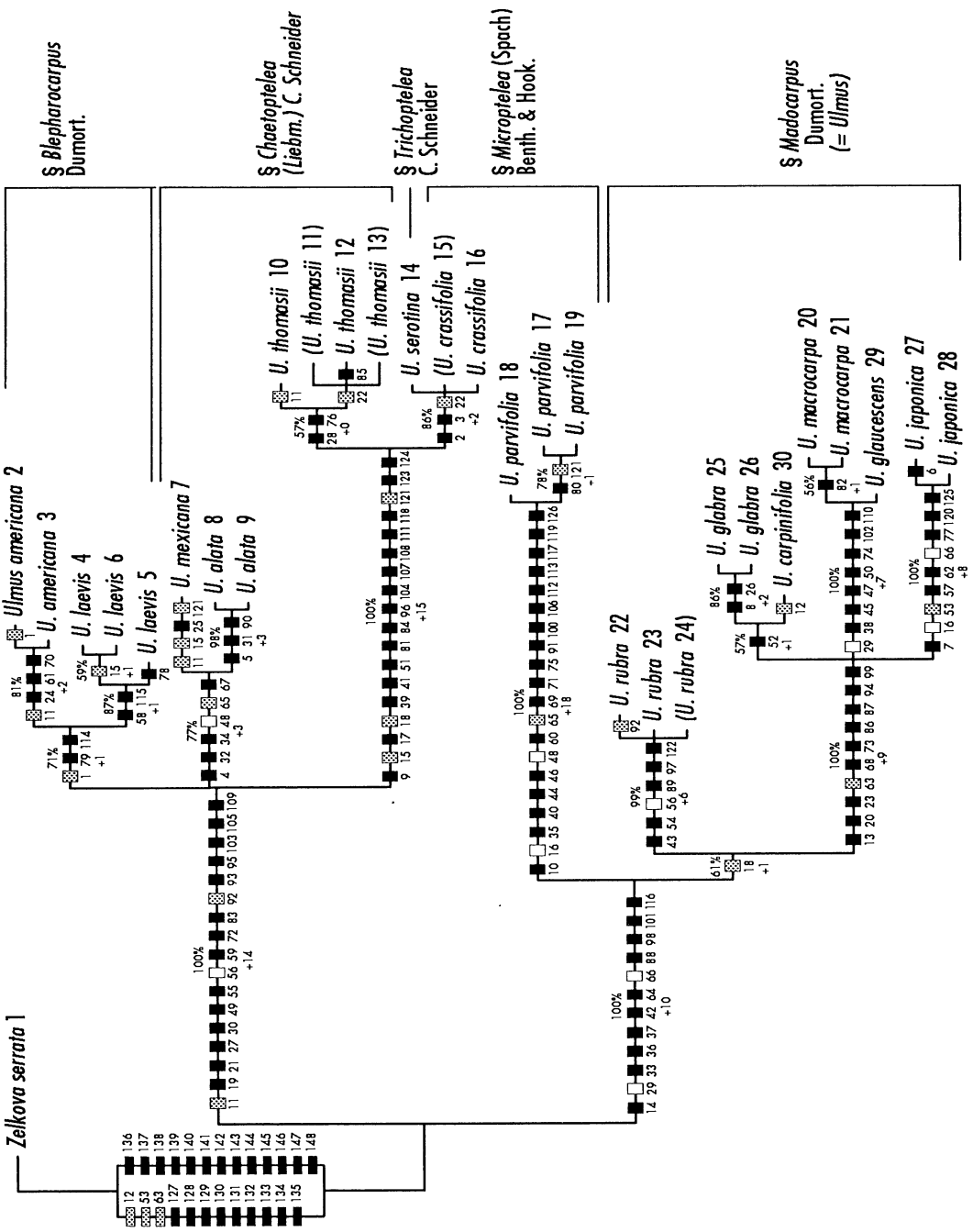

FIG. 1. Phylogram depicting one of five most parsimonious Wagner trees (168 steps) and the single weighted parsimony tree (weights 1.05 to 1.3:1 of gains : losses) based on cpDNA restriction site data. Taxa in parentheses were not included in the phylogenetic analyses because of excessive missing data and were placed *a posteriori* on the phylogram using diagnostic site mutations for the different lineages. Sectional designations follow Schneider (1916). Mutation distribution (numbering follows Appendix 1), estimated bootstrap values, and decay indices (extra number of steps necessary in relaxation of parsimony for collapse of clade) are shown for each branch. Character state transformations were placed onto the phylogram using "ACCTRAN opti-

tations that separate the clade comprising *Ulmus alata, U. americana, U. crassifolia, U. laevis, U. mexicana, U. serotina,* and *U. thomasii* from the clade including the remainder of the genus. These clades are supported not only by a large number of cpDNA restriction site synapomorphies (Fig. 1), but also by nuclear rDNA restriction site data (Wiegrefe 1992; Wiegrefe et al., in mss.). The two clades exhibit a pronounced biogeographic division. The first clade is the "western" group, referring to the hemisphere in which all but *U. laevis* occur, and the second clade is the "eastern" (hemisphere) group, in which the North American *U. rubra* is the only exception. However, the extant distribution of sections is apparently relictual, with a number of fossils reported in areas currently devoid of representatives for each section. For example, representatives of the "western" sect. *Chaetoptelea* (e.g., *U. komarovii* Shaparenko) have been reported in Miocene deposits in Switzerland (Shaparenko 1939; Hantke 1954). Representatives of "western" sect. *Blepharocarpus* also have been found in Kazakhstan and Japan (Grudzinskaya 1967). In addition, fossil taxa referred to either sects. *Madocarpus/Ulmus* or *Blepharocarpus* (e.g., *U. tenuinervis* Lesq.) have been discovered in western United States (MacGinitie 1953) where no extant, native elm species now occurs. Moreover, not all extant species were sampled in this study. In particular, *U. elongata* Fu & Ding and *U. villosa* Brandis ex Gamble, which occur in southeastern China and the western Himalayas respectively, could not be obtained for this study. Both species have been placed in "western" sect. *Chaetoptelea* by various taxonomists (Grudzinskaya 1974, 1980; Fu 1980; Richens 1983).

An appropriate name for the group containing the "western" species, given the cpDNA differentiation shown here, is subg. *Oreoptelea.* This is the name, adopted from the sectional name of Spach (1841), originally given to some members of this group by Planchon (1848). The second major clade (the "eastern" species) would then be subg. *Ulmus.* This dichotomy is supported by other lines of evidence. Morphological characters that differentiate these two groups are pedicel length and fruit ciliation (Fig. 2). Elongated pedicels are clearly a synapomorphy of subg. *Oreoptelea* (with secondary reduction in length in *U. crassifolia*). Ciliation on the samara margins in subg. *Oreoptelea* is another shared characteristic, but this feature has been independently derived in certain species within sect. *Ulmus.* Polarity within the latter character is unknown based on outgroup comparisons to *Zelkova* and *Planera.* Neither of these genera has the samara fruit, but they are the extant genera closest to *Ulmus* based on restriction site mapping and *rbc*L sequencing (Wiegrefe 1992; Wiegrefe et al., in mss.). Fossil evidence has not been examined in a phylogenetic fashion, an analysis that will be critical for understanding relationships within *Ulmus* based on morphological and anatomical characters. Although no known morphological synapomorphy presently unites subg. *Ulmus* (Fig. 2), nuclear encoded ribosomal restriction site data provide additional evidence for its monophyly (Wiegrefe 1992; Wiegrefe et al., in mss.). In light of the findings of this study, we strongly recommend recognition and use of subg. *Oreoptelea* and subg. *Ulmus* as names for these two major clades.

Differences in Subgeneric Divisions. The recognition of two subgenera within the genus *Ulmus* was also recommended by Grudzinskaya (1980). However, because she considered *U. americana* the type for the genus, her subg. *Ulmus* and subg. *Dryoptelea* correspond to subg. *Oreoptelea* and subg. *Ulmus,* respectively, recommended in this paper. The species composition of the two subgenera, as determined by this cpDNA study, is somewhat similar to that proposed by her. However, they diverge noticeably in her alignment of sects. *Microptelea* and *Lanceifolia* with the taxa we place in subg. *Oreoptelea.* Grudzinskaya's subgeneric division was based on three characters: the presence of an articulated pedicel, the shape of the adjacent perigynous tube, and the venation pattern of the samaras (Grudzinskaya 1980; pers. comm.). She defined subg. *Ulmus* (Table 1) as those taxa possessing broader, shallower perigynous tubes tapering abruptly to the receptacle, a distinct constriction where the fruit stalk meets the ped-

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←

mization" in PAUP. Black boxes represent unique mutations, stippled boxes indicate parallel/convergent losses or gain/losses, and white boxes represent parallel/convergent gains or loss/gains.

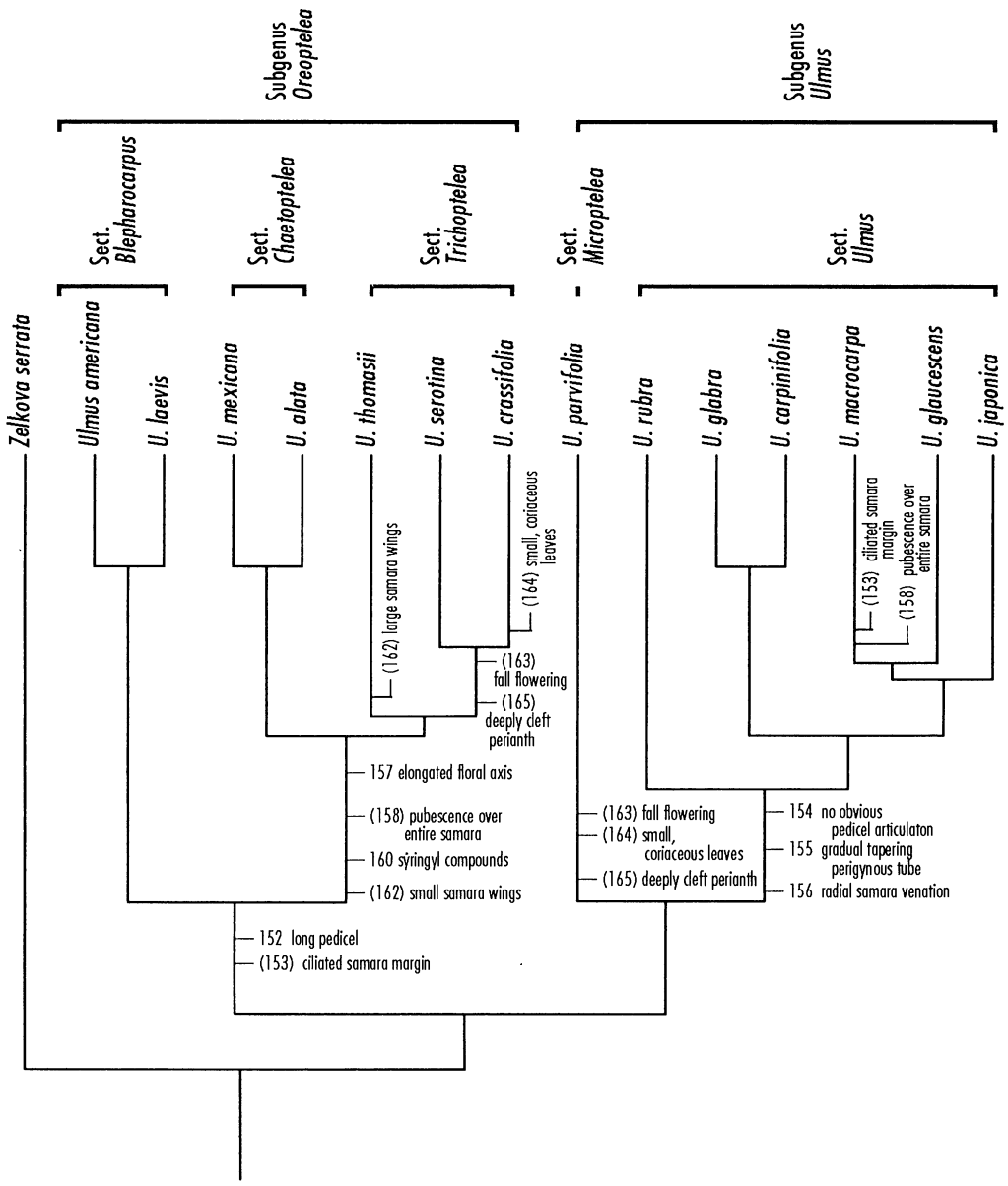

FIG. 2. Proposed classification of *Ulmus* based on the consensus of the two trees from the combined cpDNA and morphological/chemical data set (*U. thomasii* forms either a monophyletic species or a basal and paraphyletic species within sect. *Trichoptelea*). Selected morphological character state changes are mapped onto the cladogram using "ACCTRAN optimization" in PAUP. "DELTRAN optimization" affects the placement of characters 157 and 162. Character state changes involving polymorphic taxa are not shown.

icel and abscises (i.e., an "articulated" pedicel), and reticulate venation in the samara wings. By contrast, members of her subg. *Dryoptelea* have narrower, deeper perigynous tubes that taper gradually to meet the pedicel. She found that the abscission zone in the latter group was discernable only with careful ontogenetic study. The venation of the samaras of this group was described as radial.

The discrepancy of Grudzinskaya's (1980)

subgeneric division relative to that suggested by cpDNA is a result of Grudzinskaya's "traditional" analysis of morphological variation [e.g., assuming that the group united by the plesiomorphic character state has validity equal to the group sharing the apomorphic state, contrary to the basic tenets of cladistic analysis (Hennig 1966)]. Although the polarity of the character state transformations in question can be inferred indirectly by referring to the molecular and combined trees (Figs. 1, 2), they can also be inferred from Grudzinskaya's analysis. Grudzinskaya (1980) identified the genera *Holoptelea* and *Phyllostylon*, both members of Ulmaceae s. str. and thus potential outgroups for *Ulmus*, as having the articulated pedicel, suggesting it is the plesiomorphic state. This information is consistent with the topology of our phylogeny (Figs. 1, 2) with the loss of the articulated pedicel and radial venation in the samaras being the apomorphic states uniting members of Schneider's section *Madocarpus/Ulmus*, excluding *U. lanceifolia* Roxb. (sect. *Lanceifolia*).

Relationships within subg. Oreoptelea. Although the strict Wagner consensus tree and the character state weighted trees for cpDNA data (Fig. 1) depict the basal node of subg. *Oreoptelea* as unresolved, there is only one full resolution of this polytomy. Three of the five Wagner trees have a true polytomy with zero-length branches, but two trees place sect. *Blepharocarpus* as the sister group to the remainder of the subgenus (*Ulmus alata, U. mexicana, U. crassifolia, U. serotina,* and *U. thomasii*). The clade formed by *U. alata, U. crassifolia, U. mexicana, U. serotina,* and *U. thomasii* is known in the forest products industry as the "hard" or "rock" elms (Rowe et al. 1972). The morphological/chemical data support this latter clade when combined with the cpDNA data (Fig. 2). Characters uniting the rock elms include: expanded floral axes (thyrsoid racemes; Grudzinskaya 1966), pubescence on all fruit surfaces (parallel gain in *U. macrocarpa*), small wings on samaras (reversal in *U. thomasii*), the presence of syringyl compounds in heartwood extracts (Rowe et al. 1972), earlywood composed of a single discontinuous row of widely-spaced, small diameter pores (vs. a single near-continuous row of solitary pores of larger diameter in *U. americana* or multiple rows of large diameter pores in *U. rubra*; Wheeler et al. 1988), and the usual presence of myricetin glycosides in the leaves (Sherman and Giannasi 1988). [Although the local polarity of the presence of myricetin (Harborne 1977; Gornall and Bohm 1978), small wing size (Manchester 1989), and the ring diffuse condition (Gilbert 1940; Cox 1941; Sweitzer 1971) that the wood of the hard elms closely resembles (Jane 1970) is uncertain, these traits are potentially plesiomorphic.] The rock elms are an example in which the addition of morphological/chemical evidence clearly resolves a clade not fully supported by cpDNA evidence. In addition, the rock elms are united by a syn-

TABLE 3. Characters and character states used in the morphology and combined cladistic analyses of *Ulmus*. The 18 characters are numbered consecutively following the 148 cpDNA restriction site characters (Appendix 1). All characters are treated as unordered except character number 151.

149. Fruit type: 0 = nutlet, 1 = samara (flattened with peripheral wing or hairs)
150. Leaf margins: 0 = singly serrate, 1 = mostly doubly serrate
151. Leaf bases: 0 = equal, 1 = nearly equal, 2 = strongly oblique
152. Pedicel length: 0 = <2× perianth length, 1 = >2× perianth length
153. Fruit margins: 0 = eciliate, 1 = ciliate
154. Pedicel articulation: 0 = visible, 1 = not obviously visible
155. Perigynous tube shape: 0 = wide, shallow, abrupt taper, 1 = narrow, deep, gradual taper
156. Samara venation: 0 = reticulate, 1 = radial
157. Floral axis: 0 = compressed, 1 = elongate
158. Mature samara surface: 0 = glabrous or sparsely pubescent, 1 = densely pubescent over seed cavity, 2 = densely pubescent over entire surface
159. Maximal earlywood pore diameter: 0 = <200 μm, 1 = >200 μm
160. Heartwood extracts: 0 = syringyl compounds absent, 1 = syringyl compounds present
161. Foliar flavonoids: 0 = myricetin glycosides present, 1 = myricetin glycosides absent
162. Samara wing width: 0 = < seed width, 1 = > seed width
163. Time of flowering: 0 = early spring, 1 = early to mid summer, 2 = late summer to fall
164. Leaf blade length and texture: 0 = large (>5 cm) and chartaceous, 1 = small (<5 cm) and usually coriaceous or subcoriaceous
165. Perianth lobe length: 0 = <½ of perianth length, 1 = >½ of perianth length
166. Seed position in samara: 0 = medial, 1 = distal, adjacent to notch

apomorphic restriction site in the nuclear ribosomal gene (Wiegrefe 1992; Wiegrefe et al., in mss.). The exclusion of *U. parvifolia* from the rock elm clade by both cpDNA and rDNA data suggests that three morphological traits have evolved in parallel between portions of the two groups (Fig. 2). The three traits (fall flowering, small and coriaceous leaves, and deeply cleft perianth) could have arisen independently in the two clades as these traits could be viewed as convergences for specialized ecological habitats of these species.

The inclusion of *Ulmus mexicana* (*Chaetoptelea mexicana* Liebm.) within *Ulmus* is strongly supported by the findings of this study. An additional 17 steps are required to place the taxon as the sister group to the genus *Ulmus*. In all phylogenetic analyses, this species is shown to be closely related to *U. alata*. This affinity was previously noted by Fu (1980) who constructed the series *Mexicanae* for these two taxa, separating them from the less closely related *U. thomasii* (ser. *Thomasianae*) (Table 1). There is also no strong evidence for considering *U. mexicana* to be highly derived morphologically and worthy of generic status (thereby leaving *Ulmus* paraphyletic). The characters used by Sweitzer (1971) to uphold the exclusion of this species are: 1) mostly solitary and radial pore multiples (vs. mostly solitary and clustered pores); 2) lack of spiral thickenings in the vessel elements (vs. spiral thickenings in smallest vessel elements); 3) unspecified differences in leaf venation, and 4) lack of vestiges of wings on fruits (Standley 1922). The lack of spiral thickenings (Cox 1941; Sweitzer 1971; Baas 1973; van der Graaf and Baas 1974) are most likely plesiomorphic character states for the genus *Ulmus*. In addition, as Grudzinskaya (1974) noted, the trend in pore distribution and wing size are traits shared by various other rock elms, especially *U. alata* (see Fig. 2).

The cpDNA cladogram (Fig. 1) does not support Schneider's (1916) separate sectional placements of *U. crassifolia*, *U. serotina*, and *U. thomasii* (syn. *U. racemosa* Thomas; for a complete list of synonymy for the North American elms studied, excluding *U. mexicana*, see Sherman 1987). The evidence from this cpDNA study indicates that these three taxa should be placed in one section, which, according to rules of priority, is named *Trichoptelea*. Fifteen additional steps are required to place *U. thomasii* as the sister species to *U. alata* and *U. mexicana* in order to maintain monophyly of sect. *Chaetoptelea* sensu Schneider. An additional 49 steps are necessary to unite *U. crassifolia* and *U. parvifolia* as sister species and maintain monophyly of sect. *Microptelea* sensu Schneider. Based on morphological characters, Grudzinskaya (1974, 1975, 1980) suggested that *U. thomasii* and *U. crassifolia* were misplaced taxonomically. She thus transferred *U. thomasii* to sect. *Trichoptelea* with *U. serotina* and erected a new section, *Anisoptelea*, for *U. crassifolia*. As noted earlier, the cpDNA restriction site data support the former action but not the latter. None of the morphological/chemical characters presented here unite only *U. crassifolia*, *U. serotina*, and *U. thomasii* (Fig. 2). However, nuclear rDNA evidence supports the cpDNA results in uniting *U. crassifolia*, *U. serotina*, and *U. thomasii* in a clade with *U. alata* and *U. mexicana* (Wiegrefe 1992; Wiegrefe et al., in mss.).

Hybridization within subg. Oreoptelea. Grudzinskaya (1975) invoked hybridization in *Ulmus* to explain the combination of characters from two different taxa occurring in a third taxon. She considered *U. thomasii* to be a hybrid species that combined the traits of sects. *Blepharocarpus* and *Chaetoptelea*. This conclusion was based on the apparent additivity of a number of vegetative characters and the distribution of *U. thomasii* in the region of sympatry of *U. americana* and *U. alata*. Likewise, *U. serotina* was suggested to be a hybrid between *U. crassifolia* and either *U. thomasii* or *U. mexicana*. She also considered *U. crassifolia* to be a very ancient and specialized species possibly arising as a hybrid in the early Paleocene, which "unites characteristics of the (modern) sects. *Microptelea*, *Chaetoptelea*, and *Blepharocarpus*" (Grudzinskaya 1975).

Aside from sect. *Blepharocarpus* (Ager and Guries 1982), elms are relatively free of barriers to interspecific hybridizations (Townsend 1975; Mittempergher and La Porta 1991). There is, however, no molecular evidence to support the hypothesis of hybrid species origin for *Ulmus thomasii*, *U. serotina*, or *U. crassifolia*, but much evidence that discounts the hypothesis. *Ulmus thomasii* does not possess a chloroplast genome resembling that of *U. americana*, *U. alata*, or *U. mexicana* (possible maternal progenitors), nor does *U. crassifolia* share a similar chloroplast genome with *U. parvifolia* (sect. *Microptelea*), *U.*

americana (sect. *Blepharocarpus*), or *U. mexicana* and *U. alata* (sect. *Chaetoptelea*). There is, furthermore, no flavonoid evidence for the hybrid origin of *U. thomasii* and *U. crassifolia* (Sherman and Giannasi 1988). Unfortunately, there are no flavonoid or nuclear encoded ribosomal DNA markers (Wiegrefe 1992; Wiegrefe et al., in mss.) to provide evidence for or against the hybridization hypothesis as it is applied to the origin of *U. serotina*. In this case, the taxon has a chloroplast genome identical to one of its putative parental species, *U. crassifolia*. There are also no data on any artificial hybrids between *U. crassifolia* and *U. thomasii* with which to compare the morphology of *U. serotina*.

An alternative explanation, however, for such a distribution of character states is that of an evolutionary series where the third or "intermediate" taxon possesses some derived states in common with one taxon and has retained some primitive states that are also possessed by the second taxon. That is, Grudzinskaya's (1975) traditional analysis did not distinguish between symplesiomorphic and synapomorphic character states in assessing relationships among these taxa. An extension of the cladistic analysis to include the morphological characters used by Grudzinskaya and of nuclear encoded molecular evidence is needed to clarify the possible hybrid origin of *Ulmus serotina*.

Relationships within sect. Madocarpus/Ulmus. Section *Madocarpus/Ulmus* is the most diverse in the genus, encompassing over 30 species (Schneider 1916; Fu 1980), the great majority of which occur in Asia. Thus, the species included in this study are a subsample chosen to represent as many as possible of the sections proposed by Cheng et al. (1963) and Grudzinskaya (1975, 1980), and both of Schneider's (1916) subsections. The cpDNA data provide significant evidence at both of these levels and on an unexpected third matter as well. These results are not changed in a larger sampling of the section using 44 accessions of 21 species (Wiegrefe 1992; Wiegrefe et al., in mss.).

SECTION FOLIACEAE. This study provides some evidence that Grudzinskaya's sect. *Foliaceae* is not monophyletic, but the data are equivocal. Only one additional step is required to place *Ulmus carpinifolia* as the sister species to *U. japonica* with the cpDNA data. In this instance the conservative rate of chloroplast evolution results in insufficient data at this relatively low taxonomic rank. These two species do share (although independently in the combined tree of Fig. 2) the positioning of the seed at the distal end of the samara.

SECTION TRICHOCARPUS. Cheng et al. (1963) erected sect. *Trichocarpus*, which includes species previously contained in sect. *Ulmus*. Section *Trichocarpus* was described as containing those species with flowers in short racemes that arise from mixed buds at the base of the current season's growth (vs. fasciculate inflorescences arising from flower buds on one-year-old branches). Cheng et al. (1963) placed two species in this section, *U. glaucescens* and *U. kunmingensis* Cheng. Subsequent workers are divided into three camps on this issue: those who do not consider these changes valid (Townsend 1975; Fu 1980; Richens 1983); those who have adopted the sectional designation and included in this section both *U. davidiana* Planchon and *U. macrocarpa* (Santamour 1972a, 1972b; Heybroek 1976); and those who have excluded *U. kunmingensis* from the section and included *U. macrocarpa* and *U. gaussenii* Cheng in it (Grudzinskaya 1980).

According to Fu's (1980) key to the Chinese elms, *Ulmus macrocarpa* has fascicled inflorescences, the flowers of *U. davidiana* occur in flower buds on previous year's growth, and the flowers of *U. glaucescens* occur on previous year's twigs and are often solitary, i.e., not in a raceme. Thus, the morphological characters defining this group are sufficiently inconsistent to be useless. *Ulmus davidiana* is considered by some taxonomists (Fu 1980) to be conspecific with *U. japonica*, shown here to be very distinct from *U. glaucescens* and *U. macrocarpa* based on the molecular evidence (Fig. 1). Although this study indicates a close relationship between *U. glaucescens* and *U. macrocarpa*, separately or together they do not exhibit the degree of differentiation of the chloroplast genome nor morphology comparable to that found among the other sections. The lineages represented in this study by *U. glabra* and *U. carpinifolia*, *U. japonica*, and *U. macrocarpa* and *U. glaucescens* are more appropriately recognized at the rank of series based on evidence from this study. Further study is needed to provide better characterization of each of the lineages, establish their component species, and determine their relationship to each other within sect. *Ulmus*.

SUBSECTIONAL CLASSIFICATION. Schneider's

(1916) subsectional classification system within sect. *Madocarpus/Ulmus* has not been generally accepted by later elm taxonomists (Table 1). His system was primarily based on the seed position within the samara and its proximity to the apical notch. The results of this study confirm that his subsectional classification system does not produce a natural grouping. *Ulmus macrocarpa* (with its centrally located seed) is shown to be much more closely related to *U. glaucescens* (placed in subsect. *Nitentes* by Schneider due to proximity of the centrally located seed to the apical notch) than it is to *U. glabra* or *U. rubra*, the latter two placed in the same subsection as *U. macrocarpa*. Also, *U. japonica* is quite distinct from *U. glaucescens*, although they had been placed in the same subsection. A more comprehensive survey of the morphological and various types of molecular variation in this group is needed before restructuring the subsectional classifications. This is especially important because a number of the species involved have sympatric distributions (Fu 1980), a condition that makes possible introgression and chloroplast capture (Rieseberg and Brunsfeld 1992).

One species, however, is clearly distinct from all members of sect. *Madocarpus/Ulmus*. The amount of differentiation between *Ulmus rubra* and the other accessions traditionally placed in sect. *Madocarpus/Ulmus* approaches that differentiating them from *U. parvifolia* (sect. *Microptelea*). This separation of *U. rubra* is maintained in the larger sampling of sect. *Madocarpus/Ulmus* (Wiegrefe 1992; Wiegrefe et al., in mss.). This divergence has not been detected by morphological or flavonoid data, but is consistent with nuclear rDNA data that generate several clades within subg. *Ulmus* (including one containing *U. parvifolia* and one containing *U. rubra*; Wiegrefe 1992; Wiegrefe et al., in mss.). Only one homoplastic cpDNA synapomorphy was detected that unites *U. rubra* and sect. *Ulmus* (Fig. 1), and this branch collapsed in the strict consensus tree of all trees one step longer than the most parsimonious trees. Three morphological characters (no obvious pedicel articulation, gradual tapering perigynous tube, radial samara venation), however, do unite *U. rubra* and sect. *Ulmus* (Fig. 2). Elevation of *U. rubra* to its own section (sister to sect. *Ulmus*) might be merited based on these results. The fact that *U. rubra* occurs in North America, whereas the other extant members of sect. *Ulmus* are exclusively Eurasian, gives biogeographic credence to any sectional change.

Taxonomic changes

Considerable variation exists among the cpDNAs of the species in the genus *Ulmus* and cladistic analysis of this variation produces cladograms that are, with a few minor exceptions, well resolved and have well supported branches (Fig. 1). The inclusion of the more limited morphological/chemical data set into a combined data set permits even more resolution in certain regions of the tree (Fig. 2). This study provides strong evidence regarding each of the four areas of contention that have arisen concerning Schneider's (1916) classification system for the genus. First, it supports his inclusion of *U. mexicana* (syn. *Chaetoptelea mexicana*) in the genus *Ulmus*. Second, it identifies two major clades within the genus, subgenera *Oreoptelea* and *Ulmus*, with species compositions similar but not identical to previously constructed subgenera (Grudzinskaya 1980). Third, it provides evidence that Schneider's system does not accurately reflect the phylogeny of the rock or hard elms. Molecular and morphological/chemical data suggest instead that *U. thomasii* (syn. *U. racemosa*) and *U. crassifolia* be placed in sect. *Trichoptelea* with *U. serotina*. Grudzinskaya's (1975) view that *U. crassifolia* merits its own section, *Anisoptelea*, is not supported. Fourth, sect. *Trichocarpus*, erected by Cheng et al. (1963) based on species included in sect. *Madocarpus/Ulmus* by Schneider, does not form a taxonomic unit of molecular or morphological differentiation comparable to the other sections of the genus and thus the sectional rank should not be maintained. The monophyly of sect. *Foliaceae* was not supported, but additional data are required to clarify the relationships in this group. In addition, Schneider's subsectional classification does not appear to represent a natural system; further study of sect. *Ulmus* is recommended to determine the evolutionary relationships within this group and to enable the construction of a classification system that reflects them. As noted above, a revision of Schneider's sect. *Ulmus*, for which cpDNA restriction site analysis does provide support, might involve the transfer of *U. rubra* to a new

section. Nomenclatural issues involving *U. rubra*, however, preclude any formal change at this time.

A hierarchical classification of *Ulmus* is presented here with the placement of species examined in this study. Although the cpDNA results are used extensively in the classification, interpretation of morphological, anatomical, and phytochemical characters are largely consistent with changes proposed here (Fig. 2). Additionally, nuclear rDNA data do not contradict the groupings presented here (Wiegrefe 1992; Wiegrefe et al., in mss.), indicating that hybridization and/or introgression are not confounding the classification at this level, although within sect. *Ulmus*, for example, they might well be important.

subg. *Oreoptelea* (Spach) Planchon

- sect. *Blepharocarpus* Dumort. (*U. americana* and *U. laevis*)
- sect. *Chaetoptelea* (Liebm.) C. Schneider (including *U. alata* and *U. mexicana*)
- sect. *Trichoptelea* C. Schneider (*U. crassifolia, U. serotina,* and *U. thomasii*)

subg. *Ulmus*

- sect. *Lanceifolia* (C. Schneider) Grudz. (including *U. lanceifolia*; tentatively recognized pending further investigation)
- sect. *Microptelea* (Spach) Benth. & Hook. (*U. parvifolia*)
- sect. *Ulmus* (syn. *Madocarpus* Dumort) (including *U. carpinifolia, U. glabra, U. glaucescens, U. japonica,* and *U. macrocarpa*; tentatively including *U. rubra* pending further investigation)

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APPENDIX 1. Chloroplast DNA restriction site mutations detected: their code number, the enzyme exhibiting cleavage site mutation, the probe location of the site (following Sytsma and Gottlieb 1986b, and Jansen and Palmer 1988; the "IR" region was probed with a combination of P12, P14, *Pst*I/*Sst*I 6.2, *Sst*I 3.5, and *Sst*I 1.8), the restriction fragments involved (with the derived condition listed after the equals sign; the sizes of fragments whose existence was inferred but not visually detected are listed in parentheses), and the accessions with the derived character state using the enumeration of accessions in Table 2 (with accessions whose character state is unknown in parentheses). The final 22 mutations are unpolarized because they differ between *Ulmus* and *Zelkova.*

No.	Enzyme	Probe Region	Fragments (in kb)	Taxa (Table 2) with derived state (taxa with character state unknown)
1.	*Eco*RI	S8/S6	2.8 = 1.6 + 1.2	2–6 (15,24)
2.	*Eco*RI	S6	1.1 + 1.6 = 2.7	4,16 (9,15,20,24)
3.	*Eco*RI	S6	2.7 = 1.5 + 1.3	14,16 (9,15,20,24)
4.	*Eco*RI	S6	1.5 + (.7) = 2.2	8 (3,4,6,7,9,12,13,15,20,23,24)
5.	*Eco*RI	P3	4.7 = 3.6 + 1.1	8,9 (13,15,24)
6.	*Eco*RI	P3	4.5 = 3.7 + 0.9	27 (8,13,15,21,24)
7.	*Eco*RI	P3	4.3 = 2.3 + 2.0	27,28 (1,7,9,13,15,24)
8.	*Eco*RI	P10	5.2 = 4.1 + 1.4	25,26 (1,11,15,24)
9.	*Eco*RI	P10	5.2 = 3.3 + (1.9)	10,12–14,16 (1,11,15,24)
10.	*Eco*RI	P10	5.2 = 3.1 + 2.2	17–19 (1,11,15,24)
11.	*Eco*RI	P19	4.6 = 2.2 + 2.3	4–6,8,9,11–14,16 (15,20,24)

APPENDIX 1. Continued.

No.	Enzyme	Probe Region	Fragments (in kb)	Taxa (Table 2) with derived state (taxa with character state unknown)
12.	*Eco*RI	L1	1.4 + (0.7) = 2.1	2–14,16–23,25–29 (15,24)
13.	*Eco*RV	L1	13.6 + 3.7 = 17.5	20,21,25–30 (3,4,6,7,9,12,13,15,23,24)
14.	*Dra*I	S8	1.3 + (0.4) = 1.7	17–19,21,22,25,27,30 2–7,10–16,20,23,24,26,28,29)
15.	*Dra*I	S6	1.7 + (0.2) = 1.9	4,6,7,10–14,16 (15,23,24)
16.	*Dra*I	P3	7.7 = 4.3 + 3.4	17–19,27,28 (1,13,15,24)
17.	*Dra*I	P3	7.7 + 2.5 = 10.2	10–12,14,16 (1,13,15,19,24)
18.	*Dra*I	P3	2.3 + (0.5) = 2.8	10–14,16,20–22,25–30 (15,23,24)
19.	*Dra*I	P6	5.0 = 4.0 + (1.0)	2–16 (23,24)
20.	*Dra*I	P6	5.0 = 3.0 + 2.1	20,21,25–30 (24)
21.	*Dra*I	P8	3.7 = 1.85 + 1.85	2–7,9–12,14,16 (8,13,15,24)
22.	*Dra*I	P8	0.9 + 1.85 = 2.8	11–16 (24)
23.	*Dra*I	P18	3.2 = 2.9 + (0.3)	20,21,25–30 (15,24)
24.	*Dra*I	P18	3.2 = 1.8 + 1.5	2,3 (15,24)
25.	*Dra*I	P19	7.5 + 2.1 = 9.6	7 (15,24,26,29)
26.	*Dra*I	IR	5.5 + 1.8 = 7.3	25,26 (4,16)
27.	*Dra*I	L1	1.6 + 2.9 = 4.5	2–6,8–14,16 (7,15,24)
28.	*Dra*I	L1	4.5 = 3.2 + 1.3	10–13 (15,24)
29.	*Dra*I	L1	1.4 + 2.9 = 4.3	17–19,22,23,25–28,30 (10,15,24)
30.	*Dra*I	L1	1.4 + (0.6) = 2.0	2–14,16 (15,17,18,23,24,30)
31.	*Bam*HI	S6	11.8 = 8.7 + 3.1	8,9 (13,15)
32.	*Bam*HI	S8	2.3 + (0.3) = 2.6	7,8,9 (13,15,24)
33.	*Bam*HI	P3	2.7 = 2.6 + (0.1)	17–23,25–30 (9,13,15,24)
34.	*Bam*HI	P6	4.5 = 4.3 + (0.2)	7–9 (24)
35.	*Bam*HI	P6	2.1 + (0.2) = 2.3	17,19 (15,18,24)
36.	*Bam*HI	L1	1.8 + 5.3 = 7.1	17,19,21–25,27,30 (11,13,18,20,26,28,29)
37.	*Xba*I	S8/P18	3.6 = 3.0 + 0.6	11,17–23,25–30 (1,10,15,24)
38.	*Xba*I	S8	3.0 + (0.1) = 3.1	20,21,29 (2–18,23,24)
39.	*Xba*I	P3	12 = 9 + 2.9	10–12,14,16 (13,15,24)
40.	*Xba*I	P10	7.5 + 2.2 = 9.7	17–19 (13,15,24)
41.	*Xba*I	P10	2.2 + (0.2) = 2.4	10–16 (1,9,17–19,24)
42.	*Xba*I	L1	4.5 + 0.9 = 5.5	17–19,21–23,25,27,30 (11,13,15,20,24,26,28,29)
43.	*Xba*I	L1	6.0 = 5.3 + 0.7	22,23 (11,13,15,20,24,26,28,29)
44.	*Sst*II	S8	2.8 + 50 = ++	17–19 (4,24)
45.	*Bgl*II	S6	2.9 = 2.2 + 0.7	20,21,29 (15,24)
46.	*Bgl*II	P8	9.9 = 9.2 + 0.7	17–19 (15,24)
47.	*Bgl*II	P10	2.6 = 1.3 + 1.3	20,21,29 (1,15)
48.	*Bgl*II	P19	6.8 = 3.9 + 3.0	7–9,17–19 (15,24)
49.	*Bgl*II	IR	2.6 = 1.8 + (0.8)	2–7,9,10,12–16 (8,11,24)
50.	*Bgl*II	L1	9.8 = 7.0 + 2.8	21 (11,13,15,20,26,28,29)
51.	*Bgl*II	L1	2.0 + (0.3) = 2.3	10,14,16 (3,4,6,7,9,11–13,15,20,23,24,26,28,29)
52.	*Bgl*II	L1	2.2 = 2.0 + (0.2)	25,30 (6,11,15,20,24,26,28,29)
53.	*Bgl*II	L1	12.9 = 10.3 + 1.6	2–10,12–14,16,17,19,21–23,25,30 (11,15,18,20,24,26,28,29)
54.	*Hin*dIII	S8	6.2 + 4.5 = 10.7	22–24
55.	*Hin*dIII	P10	10.9 = 6.6 + 4.3	2–12,14–16 (13,24)
56.	*Cla*I	S8	6.4 = 4.2 + 2.2	2–12,14–16,22,23 (13,24)
57.	*Cla*I	S6	4.3 + 1.2 = 5.5	27,28 (13,24)
58.	*Cla*I	P3	3.4 = 2.5 + 0.9	4–6 (13,24)
59.	*Cla*I	P3	3.1 + (0.3) = 3.4	2–12,14–16 (13,24,27,28)
60.	*Cla*I	P3	2.2 = (0.3) + 1.9	17–19 (13,24)
61.	*Cla*I	P3	3.4 = 2.2 + 1.2	2,3 (13,24)
62.	*Cla*I	P3	3.1 = 2.4 + 0.7	27,28 (13,24)

APPENDIX 1. Continued.

No.	Enzyme	Probe Region	Fragments (in kb)	Taxa (Table 2) with derived state (taxa with character state unknown)
63.	*Cla*I	P6	2.1 = 1.5 + (0.6)	2–19,22,23 (24)
64.	*Cla*I	P10	15 = 14 + 0.9	17–23,25–30 (13,24)
65.	*Cla*I	P18	1.8 + (0.4) = 2.2	7–9,17–19
66.	*Cla*I	L1	5.9 + 2.1 = 8.2	17–23,25,26,29,30 (8,13,24)
67.	*Cla*I	L1	7.0 = 6.2 + 0.8	7,9 (1,8,13,15,24)
68.	*Xmn*I	S8	3.1 = 2.6 + 0.5	20,21,25–30 (24)
69.	*Xmn*I	S6	3.1 = 2.5 + 0.6	18,19 (11,17,20,24,26,28,29)
70.	*Xmn*I	P3	5.9 = 3.3 + 2.6	2,3 (17,24)
71.	*Xmn*I	P3	1.9 + 1.9 = 3.7	18,19 (17,24)
72.	*Xmn*I	P6	2.8 + 0.8 = 3.6	2–16 (17,18,24)
73.	*Xmn*I	P6	2.8 = 2.6 + (0.2)	20,21,25–30 (17,24)
74.	*Xmn*I	P8	1.6 = 1.0 + 0.6	20,21,29 (15,17,24)
75.	*Xmn*I	P10	6.4 = 3.9 + 2.6	18,19 (13,15,17,24)
76.	*Xmn*I	P10	2.1 + (0.3) = 2.4	10,12,13 (1,11,15,17,18,20,24,26,28,29)
77.	*Xmn*I	P18	2.2 = 1.8 + (0.4)	27,28 (3,4,6,7,9,12,13,15,23,24)
78.	*Xmn*I	IR	1.7 + 1.1 = 2.7	5
79.	*Xmn*I	L1	4.0 = 2.8 + 1.2	2–6 (13,15,17,20,24,26,28,29)
80.	*Xmn*I	L1	1.7 = 1.5 + (0.2)	19 (11,13,15,17,20,24,26,28,29)
81.	*Ava* I	P3	12.6 = 6.3 + 6.3	10–14,16 (15,24)
82.	*Ava* I	P3	4.1 + 0.5 = 4.6	20,21 (1,15,24)
83.	*Ava* I	P6	6.5 = 4.1 + 2.4	2–14,16 (15,24)
84.	*Bcl*I	S6	2.7 + 0.6 = 3.3	10,11,13–16 (12,24)
85.	*Bcl*I	S6	3.3 + 1.1 = 4.4	12 (15)
86.	*Bcl*I	P3	2.0 + (1.7) = 3.7	20,21,25–30 (15,24)
87.	*Eco*NI	S6	13.5 = 10.2 + 3.3	20,21,25–30 (13,17,24)
88.	*Eco*O1090	IR	5.4 + 1.6 = 7.0 10.0 + 1.6 = 11.6	17–23,25–30 (24)
89.	*Eco*O1090	P3	6.1 + 1.0 = 7.1	22,23 (13,15,24)
90.	*Eco*O1090	P8	5.4 + 4.0 = 9.4	8,9 (24)
91.	*Pst*I	P6	17 = 12.2 + 4.7	17–19 (13,15,24)
92.	*Pst*I	L1	16.4 + 2.9 = 20	2–12,14,16,22 (13,15,24)
93.	*Sma*I	P6	ca. 40 = 25 + 12.3	2–8,10–12,14,16 (1,9,13,15,24)
94.	*Sst*I	L1	22 = 18 + 3.6	20,21,25–30 (13,15,24)
95.	*Bgl*I	S8/S6	34 = 17 + 17	2–12,14–16 (13,24)
96.	*Apa*I	P6	12.2 = 4.0 + 8.2	10–14,16 (15,24)
97.	*Apa*I	P10	16 = 4.0 + 11.6	22,23 (24)
98.	*Apa*I	P19	14 + 2.1 = 16	17–23,25–30 (4,24)
99.	*Apa*LI	P3	18 = 12 + 6.2	20,21,25–30 (13,15,24)
100.	*Bst*BI	S8	3.3 = 2.8 + 0.5	17–19 (13,15,24)
101.	*Bst*BI	S6	5.4 = 2.8 + 2.6	17–23,25–30 (15,24)
102.	*Bst*BI	S6	2.6 = 1.8 + (0.8)	20,21,29 (13,24,30)
103.	*Bst*BI	P3/P6	1.9 + 4.1 = 6.0	2–12,14–16 (13)
104.	*Bst*BI	P3	6.0 = 3.6 + 2.5	10–12,14,16 (13,15,18,24)
105.	*Bst*BI	P3	2.5 + (0.4) = 2.9	2–14,16 (15,18,24)
106.	*Bst*BI	P3	2.0 + 2.2 = 4.1	17–19 (15,24)
107.	*Bst*BI	P6	3.5 + (0.4) = 3.9	10–12,14,16 (13,15,24)
108.	*Bst*BI	P6	2.4 + (0.5) = 2.9	10,11,14,16 (1,4,6,7,9,12,13,15,23,24)
109.	*Bst*BI	P8	2.5 = 1.4 + 0.9	2,5,8,10,11,14,16 (3,4,6,7,9,12,13,15,24)
110.	*Bst*BI	P10	3.9 = 3.1 + 0.8	20,21,29 (3,4,6,7,9,12,13,15,23,24)
111.	*Bst*BI	P10	1.9 + 2.3 = 4.2	10–14,16 (1,15,24)
112.	*Bst*BI	IR	4.1 + (1.0) = 5.1 3.7 + (1.0) = 4.7	17–19 (13,15,24)
113.	*Mlu*I	P3	ca. 60 = 20 + ca. 40	17–19 (1,13,15)
114.	*Sca*I	S8	4.9 = 3.7 + 1.3	2–6 (24)

APPENDIX 1. Continued.

No.	Enzyme	Probe Region	Fragments (in kb)	Taxa (Table 2) with derived state (taxa with character state unknown)
115.	*Sca*I	S8	3.7 = 3.3 + (0.4)	4–6
116.	*Sca*I	S6	2.3 + 1.8 = 4.1	17–23,25–30 (24)
117.	*Sca*I	P6	11.0 = 5.7 + 5.3	17–19 (24)
118.	*Sca*I	P8	12.2 = 8.8 + 3.8	10–14,16 (15,24)
119.	*Sca*I	P19	5.0 = 4.0 + 1.0	17–19 (24)
120.	*Sca*I	L1	6.6 + 4.7 = 11.3	27,28 (13,15,24,30)
121.	*Stu*I	P3	5.4 + 15.6 = 21	7,10–12,14,16,17,19 (9,13,15,23,24)
122.	*Stu*I	P3	4.9 = 4.2 + (0.7)	22–24 (13,15)
123.	*Stu*I	L1	18 = 15.3 + 3.1	10–12,14,16 (9,13,15,22,24,30)
124.	*Sty*I	S6	6.1 = 3.1 + 3.0	10,12–14,16 (8,11,15,24)
125.	*Sty*I	P3	6.4 = 5.2 + 1.2	27,28 (6,13,15,24)
126.	*Sty*I	P8	1.2 + (0.2) = 1.4	17–19 (11,24)
127.	*Eco*RI	P3	13.5 = 9.4 + 4.1	Outgroup (13,15,24)
128.	*Eco*RI	P10	5.1 = 3.8 + 1.3	Outgroup (15,24)
129.	*Eco*RV	P6	4.3 = 3.8 + (0.5)	Outgroup (3,4,6,7,9,12,13,15,24)
130.	*Dra*I	S6	3.4 + 1.5 = 4.9	Outgroup (15,24)
131.	*Dra*I	P3	7.3 = 5.4 + 1.9	Outgroup (13,15,17–19,24)
132.	*Dra*I	IR	11.4 + 1.9 = 13.3	Outgroup (15,24)
133.	*Bam*HI	P6	5.4 = 4.8 + (0.6)	Outgroup (13,15,24)
134.	*Xho*I	P3	20 = 3.0 + 17	Outgroup (3,4,6,7,9,11–13,15,24)
135.	*Xba*I	P18 /P19	3.5 + (0.4) = 3.9	Outgroup (24)
136.	*Sst*II	P6	7.6 = 7.4 + (0.2)	Outgroup (13,15,24)
137.	*Bgl*II	P10	9.7 + 2.6 = 12.4	Outgroup (13,15,24)
138.	*Cla*I	P6	7.0 + 3.6 = 10.6	Outgroup (24)
139.	*Xmn*I	P10	6.3 = 3.8 + 2.4	Outgroup (24)
140.	*Ava*I	P3	4.1 + 3.6 = 7.7	Outgroup
141.	*Ava*I	P3	7.7 = 4.6 + 3.2	Outgroup (13,15,24)
142.	*Ava*I	P6	6.5 = 5.9 + 0.6	Outgroup (13,15,24)
143.	*Bcl*I	P6	3.5 = 2.9 + (0.6)	Outgroup (13,15,24)
144.	*Eco*NI	P10	38 = 33 + 4.9	Outgroup (13,15,20,24,26,28,29)
145.	*Eco*O1090	P3	6.2 = 3.2 + 3.0	Outgroup (13,15,24)
146.	*Sst*I	S6/P3	9.4 = 7.6 + 1.8	Outgroup (13,15,24)
147.	*Apa*LI	P8	18 = 16 + 2.0	Outgroup (13,15,24,30)
148.	*Sty*I	S6	5.4 = 4.9 + (0.5)	Outgroup

APPENDIX 2. Matrix indicating distribution of character states used in the phylogenetic analysis based on 18 morphology/chemical data. Character numbers start at 149 (following 148 cpDNA restriction site characters). See Table 3 for the characters and their states. Accessions are listed as in Table 2. Missing data are designated by "9." Parentheses around character states indicate taxa polymorphic for the character. References for character states are indicated by superscripts: 1 = Anonymous (1957), 2 = Bate-Smith and Reichens (1973), 3 = Chun (1921), 4 = Elias (1970), 5 = Elias (1980), 6 = Fu (1980), 7 = Giannasi (1978), 8 = Grudzinskaya (personal communication), 9 = Grudzinskaya and Chernik (1976), 10 = Li (1976), 11 = Manchester (1989), 12 = Nee (1984), 13 = Pennington and Sarukhan (1968), 14 = P'Ei (1947), 15 = Rowe et al. (1972), 16 = Schneider (1916), 17 = Schreiber (1981), 18 = Sherman (1987), 19 = Sherman and Giannasi (1988), 20 = Sweitzer (1971), 21 = Wheeler et al. (1988), 22 = Wiegrefe and Sytsma (personal observation), 23 = Yarmolenko (1936), 24 = Nee (1984) depicts as distal but maturity of fruit is suspect because mature seed fills cavity and is medial, 25 = Schneider (1916) erroneously characterizes as spring flowering, 26 = Bate-Smith and Reichens (1973) detected myricetin in *U. davidiana* that may be conspecific.

	Character Number								
Taxon	149	150	151	152	153	154	155	156	157
1	0^{11}	0^{14}	0^{14}	0^{14}	9	0^{22}	0^{14}	9	$0^{14,24}$
2–3	$1^{11,16}$	$1^{5,18}$	$2^{16,18}$	1^{5}	$1^{5,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$(0,1)^{16}$
4–6	1^{16}	$1^{17,23}$	$2^{16,17,23}$	1^{17}	$1^{16,17}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$(0,1)^{16}$
7	$1^{11,16}$	$(0,1)^{12,16}$	$(0,1)^{12,13}$	$(0,1)^{12,22}$	$1^{12,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$1^{12,16}$
8–9	$1^{11,16}$	$1^{5,18}$	$(0,1)^{18}$	1^{5}	$1^{5,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$1^{5,16}$
10–13	1^{16}	$1^{5,18}$	2^{18}	1^{5}	$1^{5,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$1^{5,16}$
14	1^{16}	$1^{5,18}$	1^{18}	1^{22}	$1^{5,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$1^{5,16}$
15–16	1^{16}	$1^{5,18}$	$(0,1)^{18}$	$(0,1)^{22}$	$1^{5,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$(0,1)^{5,16}$
17–19	1^{16}	$1^{6,10,14,21}$	$1^{14,17}$	0^{6}	$0^{6,14,16}$	$0^{8,9}$	$0^{8,9}$	$0^{8,9}$	$0^{6,16}$
20–21	1^{16}	$(0,1)^{6,24}$	$(0,1)^{10,14}$	0^{6}	$1^{6,16,23}$	$1^{8,9}$	$1^{8,9}$	$1^{8,9}$	$0^{6,16}$
22–24	$1^{11,16}$	$1^{5,18}$	$2^{17,18}$	$0^{5,17}$	$0^{4,16}$	1^{8}	1^{8}	$1^{8,22}$	$0^{5,16}$
25–26	1^{16}	$1^{17,23}$	$2^{5,17}$	0^{17}	$0^{16,17}$	$1^{8,9}$	$1^{8,9}$	$1^{8,9}$	$0^{16,17}$
27–28	1^{16}	1^{6}	$(0,1)^{14,16}$	0^{17}	$0^{6,16,23}$	$1^{8,9}$	$1^{8,9}$	$1^{8,9}$	$0^{6,16}$
29	1^{16}	$(0,1)^{6,16}$	0^{6}	0^{6}	$0^{6,16}$	1^{8}	1^{8}	1^{8}	$0^{6,16}$
30	1^{16}	$1^{17,23}$	$2^{16,17}$	0^{17}	$0^{16,17}$	$1^{8,9}$	$1^{8,9}$	$1^{8,9}$	$0^{16,17}$

	Character Number								
Taxon	158	159	160	161	162	163	164	165	166
1	9	0^{20}	9	$(0,1)^{2,7}$	9	$1^{22,23}$	0^{14}	0^{3}	9
2–3	$0^{5,16,17}$	1^{21}	0^{15}	$1^{2,19}$	$1^{5,6}$	$0^{5,16}$	$0^{5,17,18}$	$0^{5,16}$	0^{6}
4–6	0^{16}	9	9	1^{2}	1^{6}	$0^{16,23}$	0^{17}	0^{16}	$0^{6,17,23}$
7	$2^{13,16}$	9	9	$(0,1)^{2,7}$	0^{5}	$0^{12,13,16}$	0^{12}	0^{16}	$0^{12,13,24}$
8–9	$2^{5,16}$	0^{21}	1^{15}	$(0,1)^{2,19}$	0^{5}	$0^{5,16}$	$(0,1)^{4,18}$	$0^{5,16}$	0^{23}
10–13	$2^{5,16,17}$	0^{21}	1^{15}	$(0,1)^{2,19}$	1^{5}	$0^{5,16}$	$0^{4,17,18}$	$0^{5,16}$	0^{1}
14	2^{16}	0^{21}	1^{15}	$0^{2,19}$	0^{5}	$2^{5,16}$	$0^{5,18}$	$1^{5,16}$	0^{5}
15–16	$2^{5,16}$	0^{21}	1^{15}	$0^{2,19}$	0^{5}	$2^{5,25}$	$1^{5,18}$	$1^{5,16}$	0^{5}
17–19	$0^{6,14,16}$	9	9	$1^{2,19}$	$(0,1)^{6,10}$	$2^{6,16}$	$1^{3,17}$	$1^{10,16}$	$0^{3,6}$
20–21	$2^{6,16}$	9	9	$(0,1)^{2}$	1^{6}	$0^{6,16,23}$	0^{23}	0^{16}	$0^{3,6,16}$
22–24	$1^{16,17}$	$1^{20,21}$	0^{15}	$1^{2,19}$	$1^{1,5}$	$0^{5,16}$	$0^{5,17,18}$	0^{16}	$0^{1,16}$
25–26	$0^{5,16,17}$	1^{20}	9	1^{2}	$1^{5,17}$	$0^{16,23}$	$0^{5,17,23}$	0^{16}	$0^{16,17}$
27–28	$0^{6,16}$	9	9	$(0,1)^{2,19}$	1^{6}	$0^{6,16}$	$0^{3,17}$	0^{16}	$1^{6,16}$
29	$0^{6,16}$	9	9	9	1^{6}	$0^{6,16}$	$0^{6,16}$	0^{16}	$0^{6,16}$
30	$0^{16,17}$	9	9	$(0,1)^{2,19,26}$	1^{17}	$0^{16,23}$	$0^{17,23}$	0^{16}	$1^{16,17}$