

## Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy

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**Abstract** Phylogenetic relationships among 40 of the 43 recognized species of *Fraxinus* L. (Oleaceae) were estimated on the basis of 106 nuclear ribosomal ITS sequences. ITS trees resulting from maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) are congruent and identify six distinct lineages. These clades allow establishing sections with high molecular and morphological support. The basal resolution generally has low ML bootstrap and MP jackknife support, but the Bayesian posterior probabilities are high for certain relationships. An independent data set of combined sequences from the chloroplast *rps16* and *trnL-F* regions contains few informative sites but corroborate most of the relationships in the ITS tree. The molecular phylogeny is discussed in the light of morphological and other data and a revised infrageneric classification with six sections are presented. The subgenera and subsections are abandoned and the section *Pauciflorae* is a new combination. *Fraxinus quadrangulata* and *Fraxinus anomala* are united with *Fraxinus dipetala* in the section *Dipetatae* and *Fraxinus platypoda* is transferred to the section *Fraxinus*. *Fraxinus chiisanensis*, *Fraxinus spaethiana* and *Fraxinus cuspidata* are treated as *incertae sedis*. A sectional key is given, together with a systematic list of the 43 recognized species, with common synonyms and distribution. Breeding system and other traits mapped on the phylogeny show that dioecy has three separate origins, and in each case followed after the transition from insect to wind pollination. In one instance

dioecy evolved from hermaphroditism via androdioecy and twice via polygamy.

**Keywords** *Fraxinus* · Oleaceae · ITS · Phylogeny · Key · Classification · Taxonomy · Section *Pauciflorae*

### Introduction

The genus *Fraxinus* L., the ashes, comprises 43 species occurring in temperate and subtropical regions of the northern hemisphere. The two main distribution areas are North America (20 species) and eastern Asia (20 species). Three species occur in Europe and western Asia. *Fraxinus* is one of 24 extant genera of Oleaceae (the olive family) and sole member of the subtribe Fraxininae, which is sister group to the subtribe Oleinae in the tribe Oleaceae (Wallander and Albert 2000). The genus was described by Linnaeus in 1753 and since then over 450 taxa have been described, most of which are regarded as synonyms today. The latest and most thorough monograph of the entire genus includes 64 species (Lingelsheim 1920), and more taxa have been described since then.

The genus is monophyletic (Wallander and Albert 2000) and unique in the Oleaceae by mostly having relatively large imparipinnate leaves and one-seeded samaras. Most of the species are large or medium-sized trees, but some are shrubs in dry areas. There is much variation in leaf morphology (shape, texture, number of leaflets, leaflet margin, petiolule length, indumentum, epidermal papillae, rachis wings, etc.) and intraspecific variations in these features have been the cause of most synonyms. As it is the characteristic of nearly all taxa of Oleaceae, the small flowers have only one pistil and two stamens. The corolla may be lacking or consists of four (rarely two), white, linear, and

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free (rarely fused) petals. The synsepalous calyx is small, cup-shaped, and usually dentate, or lacking. The petaliferous and insect-pollinated flowers are (with two exceptions) borne in large showy panicles that emerge together with the leaves from terminal buds. The apetalous flowers, which are wind-pollinated, occur in lateral or terminal inflorescences and emerge before the leaves unfold. The syncarpous ovary contains four ovules, two in each locule, but normally develops into a one-seeded samara.

The genus has traditionally been divided into two sections or subgenera on the basis of morphology (Table 1). The section or subgenus *Fraxinus* (*Fraxinaster* is an invalid name according to ICBN 22.1) comprised all taxa with lateral inflorescences, whereas the section or subgenus *Ornus* comprised taxa where the flowers are borne in terminal panicles together with the leaves. The section *Fraxinus* was further divided into five subsections on the basis of presence or absence of calyx, number of petals, and winged or unwinged leaf petioles. The section *Ornus* was subdivided into two subsections, *Ornus* (*Euornus* is an invalid name according to ICBN 21.3) with petals and *Ornaster* without petals. This classification has remained relatively stable, although some authors after Lingelsheim (1920) have chosen other ranks and corrected the names for some infrageneric groups. The classification of a few taxa has been uncertain, e.g. *Fraxinus chiisanensis*, *Fraxinus cuspidata*, *Fraxinus platypoda*, and *Fraxinus anomala*, but most of the differences between the classifications have concerned the number of included species and different opinions about the synonymy.

There is a variety of pollination and breeding systems within the genus *Fraxinus*. This situation offers an interesting case for studying the evolution of traits related to reproductive biology. About one-third of the species are entomophilous and two-thirds are anemophilous. Most of the anemophilous species are dioecious or polygamous. A few of the entomophilous species are hermaphrodites, but the majority are androdioecious. This is considered to be a very rare breeding system (e.g. Charlesworth 1984; Pannell 2002), but in Oleaceae, and particularly in *Fraxinus*, there are many species that are morphologically androdioecious.

Jeandroz et al. (1997) published the first molecular phylogeny of *Fraxinus*, which was based on ITS-1 and ITS-2 sequences of the nuclear ribosomal DNA from 20 species. Because they only included less than half of the number of species, and left out representatives of the two sections *Pauciflorae* and *Sciadhanthus*, their phylogeny could not be used for the purpose of this study. Although they used their phylogenetic tree to map some floral characters (presence or absence of calyx and/or corolla), a

**Table 1** Different classification schemes of *Fraxinus*, including the revised one proposed in this study

Lingelsheim (1920), Rehder (1940), Dayton (1954), Miller (1955)
Sect. <i>Fraxinaster</i> DC.
Subsect. <i>Bumelioides</i> (Endl.) Lingelsh.
Subsect. <i>Melioides</i> (Endl.) Lingelsh.
Subsect. <i>Sciadhanthus</i> (Coss. et Dur.) Lingelsh.
Subsect. <i>Dipetalae</i> Lingelsh.
Subsect. <i>Pauciflorae</i> Lingelsh.
Sect. <i>Ornus</i> (Neck.) DC.
Subsect. <i>Euornus</i> Lingelsh.
Subsect. <i>Ornaster</i> (Koehne et Lingelsh.) Lingelsh.
Vassiljev (1952) <sup>a</sup>
Subgenus <i>Fraxinaster</i> (DC.) V. Vassil.
Sect. <i>Melioides</i> (Endl.) Pfeiff.
Sect. <i>Bumelioides</i> (Endl.) Pfeiff.
Subgenus <i>Ornus</i> (Boehm.) Pers.
Sect. <i>Euornus</i> Koehne et Lingelsh.
Sect. <i>Ornaster</i> Koehne et Lingelsh.
Nikolaev (1981)
Subgenus <i>Fraxinus</i>
Sect. <i>Fraxinus</i>
Subsect. <i>Paniculatae</i> E. Nikolaev
Subsect. <i>Racemosae</i> E. Nikolaev
Sect. <i>Melioides</i> (Endl.) Pfeiff.
subsect. <i>Melioides</i> (Endl.) Lingelsh.
Subsect. <i>Sciadhanthus</i> (Coss. et Dur.) Lingelsh (incl. subsect. <i>Pauciflorae</i> Lingelsh.)
Sect. <i>Dipetalae</i> (Lingelsh.) E. Nikolaev
Subgenus <i>Ornus</i> (Boehm.) Pers.
Sect. <i>Ornus</i> (Boehm.) DC.
Sect. <i>Ornaster</i> Koehne et Lingelsh.
Wei (1992) <sup>a</sup>
Subgenus <i>Fraxinus</i>
Sect. <i>Fraxinus</i>
Sect. <i>Melioides</i> (Endl.) Pfeiff.
Sect. <i>Sciadhanthus</i> Coss. et Dur.
Subgenus <i>Ornus</i> (Boehm.) Pers.
Sect. <i>Ornus</i> (Boehm.) DC.
Sect. <i>Ornaster</i> Koehne et Lingelsh.
Wallander (this study)
Sect. <i>Dipetalae</i> (Lingelsh.) E. Nikolaev
Sect. <i>Fraxinus</i>
Sect. <i>Melioides</i> (Endl.) Pfeiff.
Sect. <i>Ornus</i> (Boehm.) DC.
Sect. <i>Pauciflorae</i> (Lingelsh.) E. Wallander
Sect. <i>Sciadhanthus</i> (Coss. et Dur.) Lingelsh.

<sup>a</sup> Vassiljev (1952) and Wei (1992) did not include *Dipetalae* or *Pauciflorae* because these sections are not native to the regions they covered. Earlier classifications are detailed by Miller (1955). Several author citations have been corrected due to errors in original publications

number of coding errors made their interpretation of character evolution incorrect.

The main objective of this study in the genus *Fraxinus* was twofold: first, to estimate the phylogeny of the entire genus *Fraxinus* on the basis of molecular data and; second, to use this phylogenetic estimate to study the evolution of wind pollination and related traits in the genus. In particular, I have been interested in how the evolution of unisexual flowers correlates with that of wind pollination. During the course of the phylogenetic work, new relationships among taxa were discovered which together with a deeper morphological study led to a revised infrageneric classification of *Fraxinus*. This article presents a well-supported phylogeny of the genus *Fraxinus* on the basis of DNA sequences from the nuclear ribosomal ITS and two chloroplast regions, and a revised classification. Some traits relating to pollination systems are mapped on the tree and briefly commented, but the interpretations of the floral evolution stemming from this work, in relation to transitions between pollination systems, are discussed in more detail elsewhere (Wallander 2001).

## Materials and methods

### Materials

First, a thorough inventory of the *Fraxinus* taxa accepted in various recent treatments and their putative synonyms was undertaken to come up with a reasonable representation of taxa to include in this study. This inventory was based on original descriptions, floristic treatments (Sargent 1949; Little 1952; Vassiljev 1952; Dayton 1954; Murray 1968; Franco and Rocha Afonso 1972; Nakaike 1972; Grohmann 1974; Scheller 1977; Yaltirik 1978; Nikolaev 1981; Vines 1984; Yamazaki 1993; Wei and Green 1996), regional monographs (mainly Standley 1924; Miller 1955; Hara 1956, 1982; Kitagawa 1979; Sun 1985; Green 1991), and the only monograph of the genus (Lingelsheim 1920). Many taxa were studied in the field and in botanical gardens and arboreta (Göteborg Botanical Garden and Arboretum, New York Botanical Garden, Missouri Botanical Garden and Shaw Arboretum, Palermo Botanical Garden, Kyoto Botanical Garden, and the Royal Botanic Gardens at Kew). Over 1,000 herbarium specimens from BM, C, E, GB, K, MO, NY, S, and UPS (acronyms according to Index Herbariorum) were also studied. This work resulted in a provisional list of about 50 species, 43 of which I eventually accepted (on the basis of morphology, molecular data, and opinions of other authors, as explained in “Discussion”) (Table 2). On the basis of this list, at least two representatives of

each recognized species, and in some cases subspecies, were chosen for DNA sequencing. In addition, some taxa of uncertain status (putative synonyms) were chosen to estimate their relationships.

Fresh or silica-gel dried leaf or seed material from many taxa cultivated in botanical gardens, and from field collections made in Spain, Italy, the USA, Mexico, China, and Japan, were used for the molecular work. Vouchers for these are deposited at GB. Materials from the aforementioned herbaria were also used. The final number of ingroup specimens sequenced for this study was 89, which represents 40 of the 43 recognized species. Amplification failed for several specimens each of the three missing species (*Fraxinus griffithii*, *Fraxinus malacophylla*, and *Fraxinus baroniana*). Seventeen ITS-1 and ITS-2 sequences from the study by Jeandroz et al. (1997) were taken from GenBank and included in the analysis. Five outgroup taxa were chosen from the closely related subtribes Ligustrinae and Oleinae, on the basis of the Oleaceae phylogeny of Wallander and Albert (2000). All vouchers and GenBank accession numbers are listed in the “Appendix”, and all accepted species and relevant synonyms are given with authors in Table 2.

### Molecular methods

DNA extraction, PCR amplification, and automated sequencing were mainly done using methods and equipment described by Wallander and Albert (2000). In addition, a few samples were extracted using the DNeasy Plant Mini kit (QIAGEN) and PCR performed using the HotStarTaq<sup>®</sup> Master Mix kit (QIAGEN) without modifications. Amplifications were carried out in a GeneAmp<sup>®</sup> PCR System 9700 (Perkin-Elmer Applied Biosystems) and PCR products sequenced on a CEQ<sup>™</sup> 8000 Genetic Analysis System (Beckman Coulter). The ITS4 and ITS5 primers designed by Nickrent et al. (1994) and Wojciechowski et al. (1993) were used to amplify the entire ITS region of the nuclear ribosomal DNA. In some difficult cases, the ITS-1 and ITS-2 regions were amplified and sequenced separately using the internal primers ITS2 and ITS3 of Wojciechowski et al. (1993).

The forward and reverse ITS sequences were assembled and edited using Sequencher<sup>™</sup> 4.1.2 (Gene Codes Corporation, Ann Arbor, MI, USA). Consensus sequences were aligned using the alignment feature in Sequencher and then manually adjusted. Furthermore, the *rps16* and *trnL-F* intron sequences of ten *Fraxinus* species from the study by Wallander and Albert (2000) were combined with five new ones (obtained in the same way) and two outgroup species into one data matrix, representing all sections. The alignments can be obtained from GenBank.

**Table 2** Revised infrageneric classification of *Fraxinus* (Oleaceae) listing the 43 accepted species and their geographical distribution, together with common synonyms or those mentioned in this study

Sections and species	Geographic distribution	Synonyms
Section <i>Dipetalae</i> (Lingelsh.) E. Nikolaev		
<i>F. anomala</i> Torr. ex S. Wats.	SW USA	<i>F. lowelli</i> Sarg., <i>F. potosina</i> T. S. Brandeg.
<i>F. dipetala</i> Hook. and Arn.	SW USA	<i>F. jonesii</i> Lingelsh., <i>F. parryi</i> Moran, <i>F. trifoliata</i> (Torr.) Lewis and Epling
<i>F. quadrangulata</i> Michx.	C and E USA, C Canada	
Section <i>Fraxinus</i>		
<i>F. angustifolia</i> Vahl	S and C Europe to Central Asia	<i>F. oxycarpa</i> Willd., <i>F. oxyphylla</i> M. Bieb. (nom. illeg.), <i>F. pallisiae</i> A.J. Willmott, <i>F. potamophila</i> Herder, <i>F. sogdiana</i> Bunge, <i>F. syriaca</i> Boiss.
<i>F. excelsior</i> L.	N and C Europe to W Russia	<i>F. coriariifolia</i> Scheele
<i>F. mandshurica</i> Rupr.	China, Japan, Korea, E Russia	<i>F. nigra</i> ssp. <i>mandshurica</i> (Rupr.) S. S. Sun
<i>F. nigra</i> Marsh.	E USA, E Canada	
<i>F. platypoda</i> Oliv.	China	
Section <i>Melioides</i> (Endl.) Lingelsh.		
<i>F. americana</i> L.	E USA and E Canada	<i>F. biltmoreana</i> Beadle
<i>F. berlandieriana</i> DC.	SW USA, Mexico	
<i>F. caroliniana</i> Mill.	SE USA	<i>F. cubensis</i> Griseb.
<i>F. latifolia</i> Benth.	W USA	<i>F. oregona</i> Nutt.
<i>F. papillosa</i> Lingelsh.	SW USA, Mexico	
<i>F. pennsylvanica</i> Marsh.	C and E USA, Canada	
<i>F. profunda</i> (Bush) Bush	SE USA	<i>F. tomentosa</i> Michx. f. (nom. rej.)
<i>F. texensis</i> (Gray) Sarg.	SW USA (Texas)	
<i>F. uhdei</i> (Wenzig) Lingelsh.	C America, Hawaii	<i>F. cavekiana</i> Standley and Steyerf., <i>F. chiapensis</i> Lundell, <i>F. hondurensis</i> Standley
<i>F. velutina</i> Torr.	SW USA, Mexico	<i>F. attenuata</i> M. E. Jones, <i>F. pistaciaefolia</i> Torr., <i>F. toumeyii</i> Britt.
Section <i>Ornus</i> (Boehm.) DC.		
<i>F. apertisquamifera</i> Hara	Japan	
<i>F. bungeana</i> DC.	China	
<i>F. floribunda</i> Wall.	Himalaya, E Asia	<i>F. insularis</i> Hemsl., <i>F. retusa</i> Champ. ex Benth.
<i>F. griffithii</i> C. B. Clarke	SE Asia	<i>F. ferruginea</i> Lingelsh., <i>F. formosana</i> Hayata, <i>F. philippinensis</i> Merr.
<i>F. lanuginosa</i> Koidz.	Japan	
<i>F. malacophylla</i> Hemsl.	China, Thailand	<i>F. retusifoliolata</i> Feng ex P. Y. Bai
<i>F. ornus</i> L.	C and E Mediterranean	
<i>F. paxiana</i> Lingelsh.	Himalaya, China	<i>F. sikkimensis</i> (Lingelsh.) Hand.-Mazz., <i>F. suaveolens</i> W.W. Smith
<i>F. raibocarpa</i> Regel	C Asia	
<i>F. sieboldiana</i> Blume	China, Japan, Korea	
<i>F. trifoliolata</i> W. W. Smith	China	
<i>F. baroniana</i> Diels	China	
<i>F. chinensis</i> Roxb.	E Asia	<i>F. japonica</i> Blume ex K. Koch, <i>F. rhynchophylla</i> Hance
<i>F. longicuspis</i> Sieb. and Zucc.	Japan	
<i>F. micrantha</i> Lingelsh.	Himalaya	
Section <i>Pauciflorae</i> (Lingelsh.) E. Wallander, stat. nov.		
<i>F. dubia</i> (Willd. ex Schult. and Schult. f.) P. S. Green and M. Nee	Mexico, Guatemala	<i>F. petenensis</i> Lundell, <i>F. schiedeana</i> Schlecht. and Cham.
<i>F. gooddingii</i> Little	SW USA, N Mexico	
<i>F. greggii</i> A. Gray	SW USA, Mexico	
<i>F. purpusii</i> Brandege	Mexico, Guatemala	<i>F. bicolor</i> Standley and Steyerf., <i>F. vellerea</i> Standley and Steyerf.

**Table 2** continued

Sections and species	Geographic distribution	Synonyms
<i>F. rufescens</i> Lingelsh.	Mexico	
Section <i>Sciadanthus</i> (Coss. et Dur.) Lingelsh.		
<i>F. hubeiensis</i> S. Z. Qu, C. B. Shang and P. L. Su	China	
<i>F. xanthoxyloides</i> (G. Don) DC.	N Africa to China	<i>F. dimorpha</i> Coss. and Dur.
Incertae sedis		
<i>F. cuspidata</i> Torr.	SW USA, Mexico	
<i>F. chiisanensis</i> Nakai	Korea	
<i>F. spaethiana</i> Lingelsh.	Japan	

### Phylogenetic analyses

The final ITS data matrix contained 106 ingroup sequences, representing all but three of the 43 recognized species and some of their putative synonyms, plus five outgroup sequences. A parsimony analysis was performed using heuristic searches in PAUP\* 4.0b10 (Swofford 2002). The search consisted of TBR branch-swapping of 99 random addition sequence replicates limited to a maximum of 1,000 trees saved per replicate. All characters were considered to be unordered and given equal weight. Although some indels appeared to be phylogenetically informative, they were not separately coded. Gaps were treated as missing data. Multistates were interpreted as uncertainties (even if many appeared to be true polymorphisms). Branch support was evaluated through parsimony jackknifing using XAC (James S. Farris, Swedish Museum of Natural History, Stockholm) with 1,000 replicates, each with ten random addition sequence replicates and non-rotational branch-swapping. An additional output with GC values (Goloboff et al. 2003) provided a measure of group frequency minus greatest frequency of a conflicting group.

In addition to the parsimony analyses, a maximum likelihood analysis was performed as implemented in the PhyML online web server (Guindon et al. 2005). Because of computational limitations, the 111-taxon dataset had to be reduced to 108. The three OTUs removed were all sequences that were nearly identical duplicates of another conspecific sequence. Although the GTR + G nucleotide substitution model was selected by MrModeltest 2.2 (Nylander 2004), the GTR model and other parameters estimated were used as input values. The reliability of the internal nodes was estimated through 100 parametric bootstrap replicates.

Node support was also established using Bayesian inference, as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). First, the partition homogeneity test (Farris et al. 1994), as implemented in PAUP\* version 4.0b10 (Swofford 2002), was applied to test the congruence of results produced

independently from the three partitions defined for the data set (ITS1: matrix positions 1–269, 5.8S: 270–428, and ITS2: 429–663). For this purpose, a heuristic search was performed with 1,000 replicates, 100 random addition sequences, TBR branch swapping, and saving up to 50 trees per replicate. MrModeltest 2.2 (Nylander 2004) was used to determine the optimal nucleotide substitution model for each of the three regions analyzed. Following the recommendations of recent works (Pol 2004; Posada and Buckley 2004), the evolutionary models chosen by the Akaike information criterion, the GTR + G model for ITS1 and ITS2 and the k80 + I model for the nearly invariable 5.8S region, were then incorporated into a MrBayes block in the input file. The program performed two simultaneous runs until the average standard deviation of split frequencies became lower than 0.01. For each run, eight Metropolis-coupled Markov chain Monte Carlo (MCMCMC) chains were initiated, sampling every 500 generations, saving branch lengths, and using other default settings.

The chloroplast data set (15 ingroup and two outgroup sequences of the *rps16* intron and the *trnL-F* region combined) was analyzed separately to provide an independent estimate of the relationships between the sections. The parsimony analysis consisted of 1,000 random addition sequence replicates and the number of saved trees per replicate was not limited. A maximum likelihood analysis was also carried out using the PhyML online web server. The HKY nucleotide substitution model was selected by MrModeltest 2.2 (Nylander 2004) and the parameters estimated were used as input values. ITS sequences for the same 17 species were analyzed in the same way (but with the GTR model in the ML analysis) and the variable characters of the chloroplast data set were then optimized onto the ML tree using the software MacClade 4.08.

### Morphological data

Herbarium material was studied for all species and some were also studied in the field. Some information was also

gathered from the literature (see references for materials). Characters considered important in previous classifications (inflorescence position, number of petals, presence or absence of calyx, and presence or absence of leaf rachis wings) are mapped on a summary of the phylogenetic trees. Pollination and breeding system are also mapped to show how breeding systems have evolved in relation to pollination system and related floral traits. MacClade 4.08 was used to optimize the traits on the tree. Both ACCTRAN and DELTRAN resolving options were tested, but since there were no differences only one tree is shown.

## Results

### Phylogenetic analyses and congruence between inference methods

The sequence characteristics for all data sets and partitions are presented in Table 3. The partition homogeneity test did not reach significance for the rejection of congruency among the data partitions ( $P = 0.50$ ). The Bayesian analysis was stopped after ten million generations, i.e. long after the standard deviation of split frequencies of the two parallel runs had reached the critical value (0.01). The burn-in phase was defined as the first 1 million generations, long after the standard deviation of split frequencies had reached 0.05. Figure 1 shows the majority-rule consensus tree of 36,002 trees (sampled from a total of 40,002 trees from both parallel runs) estimated using Bayesian inference (BI).

The maximum likelihood (ML) analysis yielded a tree that is largely identical to the BI tree. The maximum parsimony (MP) analysis resulted in 47,863 most parsimonious trees. The strict consensus of these trees

**Fig. 1** Majority rule consensus tree resulting from the Bayesian analysis of 111 ITS sequences representing 40 *Fraxinus* species and five Oleaceae outgroup species. For relevant nodes only, Bayesian posterior probabilities are shown above the branches and parsimony jackknife/maximum likelihood bootstrap values for the same data set below the branches. A dash is shown instead of a jackknife value for those branches that are collapsed in the strict consensus of the most parsimonious trees. Asterisks denote clades that have additional support from indels (that were not separately coded). In the few cases where multiple sequences representing the same species do not group together, they have been assigned numbers (1–3) to provide an option to trace their vouchers in the “Appendix”. GB after taxon names indicates that those sequences were taken from GenBank (study by Jeandroz et al. 1997). Names within parentheses are original determinations of some vouchers, which are here regarded as synonyms. Sectional assignments are according to the revised classification in this study

contains the same major clades (=sections) as obtained by BI and ML, but with poor resolution between them. The MP and ML trees are not shown, but their jackknife and bootstrap support values, respectively, are shown along with the posterior probabilities on the BI tree (Fig. 1). There is no conflict between the trees yielded by MP compared to BI and ML, in the sense that there are no major clades that are strongly supported in one tree but contradicted in the other. All three inference methods find the same major clades, which are strongly supported by bootstrap, jackknife as well as posterior probability values, but the difference between the results lies in that the strict consensus of the MP trees is unresolved at the base whereas most of the basal resolution of the Bayesian tree (Fig. 1) is strongly supported.

In the following, support will be referred to as strong for a posterior probability >0.91 or a jackknife and bootstrap value >88%. These values have been shown to represent

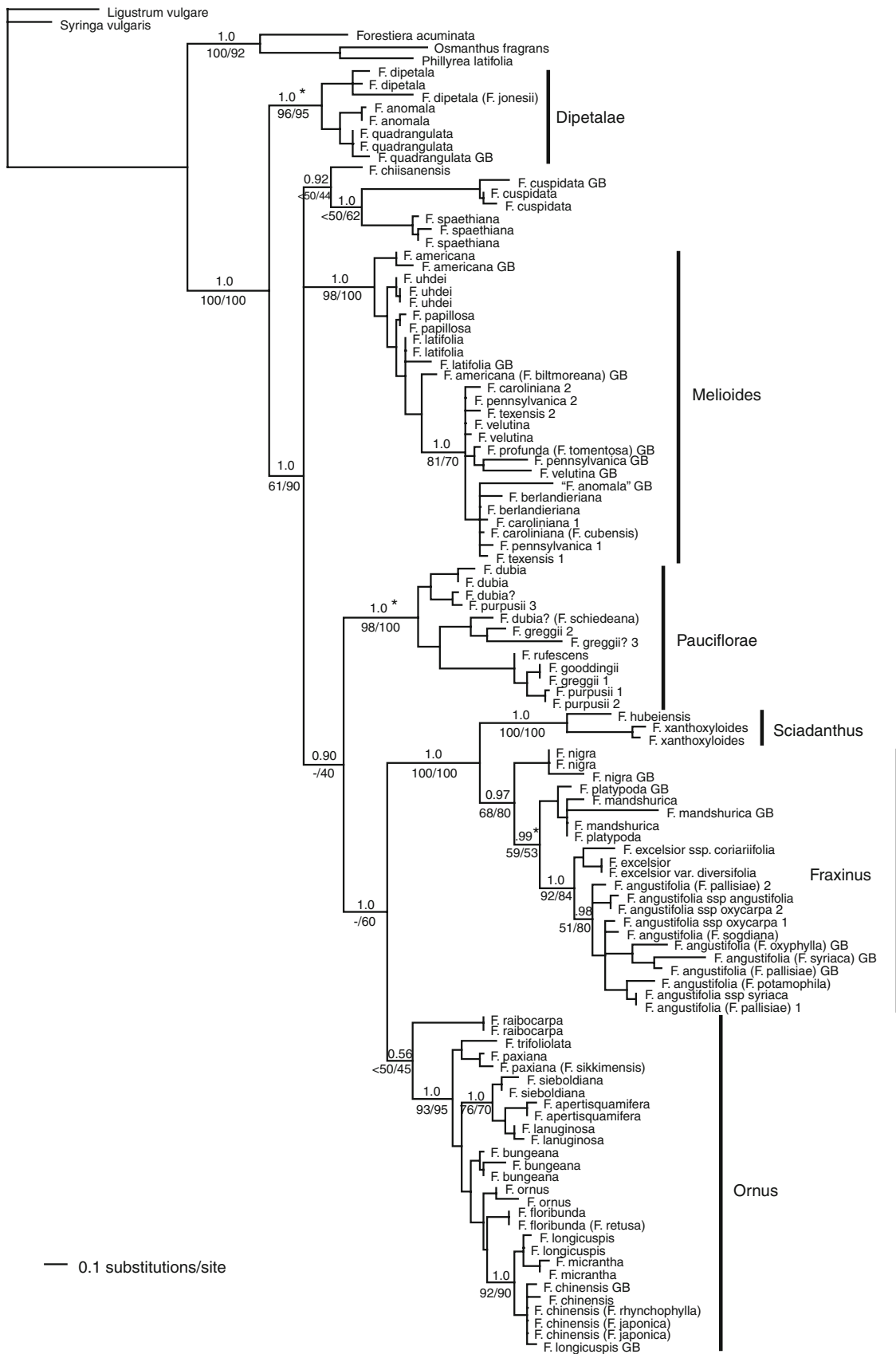
**Table 3** Sequence characteristics of the ITS data (106 ingroup and five outgroup taxa) and the combined chloroplast data set of the *trnL-F* region and the *rps16* intron (15 ingroup and 2 outgroup taxa)

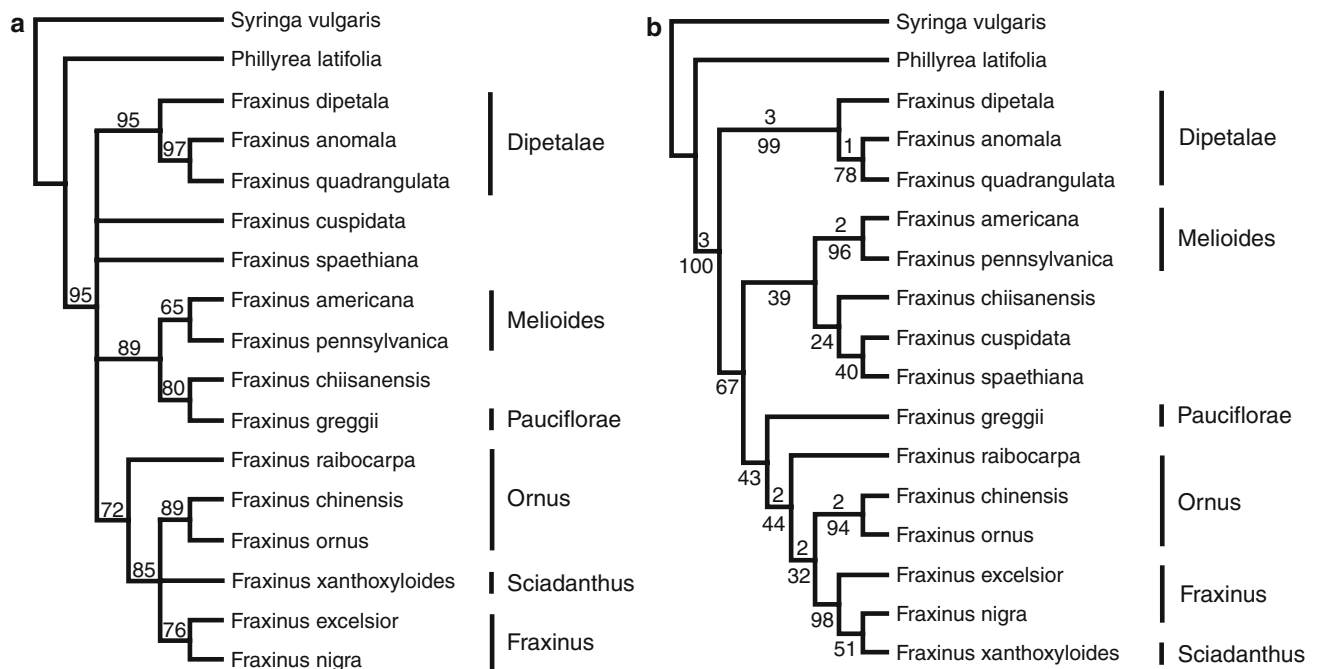
Data parameters	ITS region	ITS1	ITS2	5.8S	<i>trnL-F</i> + <i>rps16</i>
Sequence length range (bp) <sup>a</sup>	611–638	238–252	205–228	159	1677–1690
Aligned length (bp)	663	269	235	159	859 + 831
Mean GC content (%) (range)	61 (54–67)	63 (54–74)	63 (53–71)	53 (50–55)	35 (34–36)
Number of informative sites (within ingroup)	220 (185)	114 (99)	99 (80)	7 (6)	17 (14)
Parsimony result					
Number of MP trees	47863				18
Length of MP trees	918				70
RI (retention Index)	0.86				0.97
Likelihood result					
Likelihood	–6005 (–2801 <sup>b</sup> )				–2826

Some results from the parsimony and maximum likelihood analyses of the data sets are also listed

<sup>a</sup> Sequences that are not full length (i.e. a stretch missing in the beginning and/or end) are excluded from the count

<sup>b</sup> Likelihood value for the ITS tree with the same 17 taxa as in the chloroplast data set





**Fig. 2** **a** Maximum likelihood tree of the chloroplast data set (*rps16* intron and *trnL-F* region) from 15 *Fraxinus* and two Oleaceae outgroup species. Bootstrap values are shown above the branches. **b** Maximum likelihood tree of a reduced set of the ITS data with the same taxon sampling as the chloroplast data set. Bootstrap values are

shown below the branches. Numbers above branches are number of unambiguous changes in the chloroplast data supporting that branch (not shown at tips). Sectional assignments are according to the revised classification in this study

minimal values required for a 95% confidence interval of a node under certain circumstances (Zander 2004).

Congruence between the two independent data sets

The ML tree of the combined *rps16* and *trnL-F* chloroplast data set is shown in Fig. 2a. The MP analysis of the same data set resulted in 18 MP trees (data characteristics in Table 3) and the strict consensus of these trees (not shown) is largely congruent with the ML tree. The only difference in topology between them is that *Fraxinus greggii* and *F. chiisanensis* are resolved as a sister group to the section *Melioides* in the ML tree whereas they are unresolved in the MP tree.

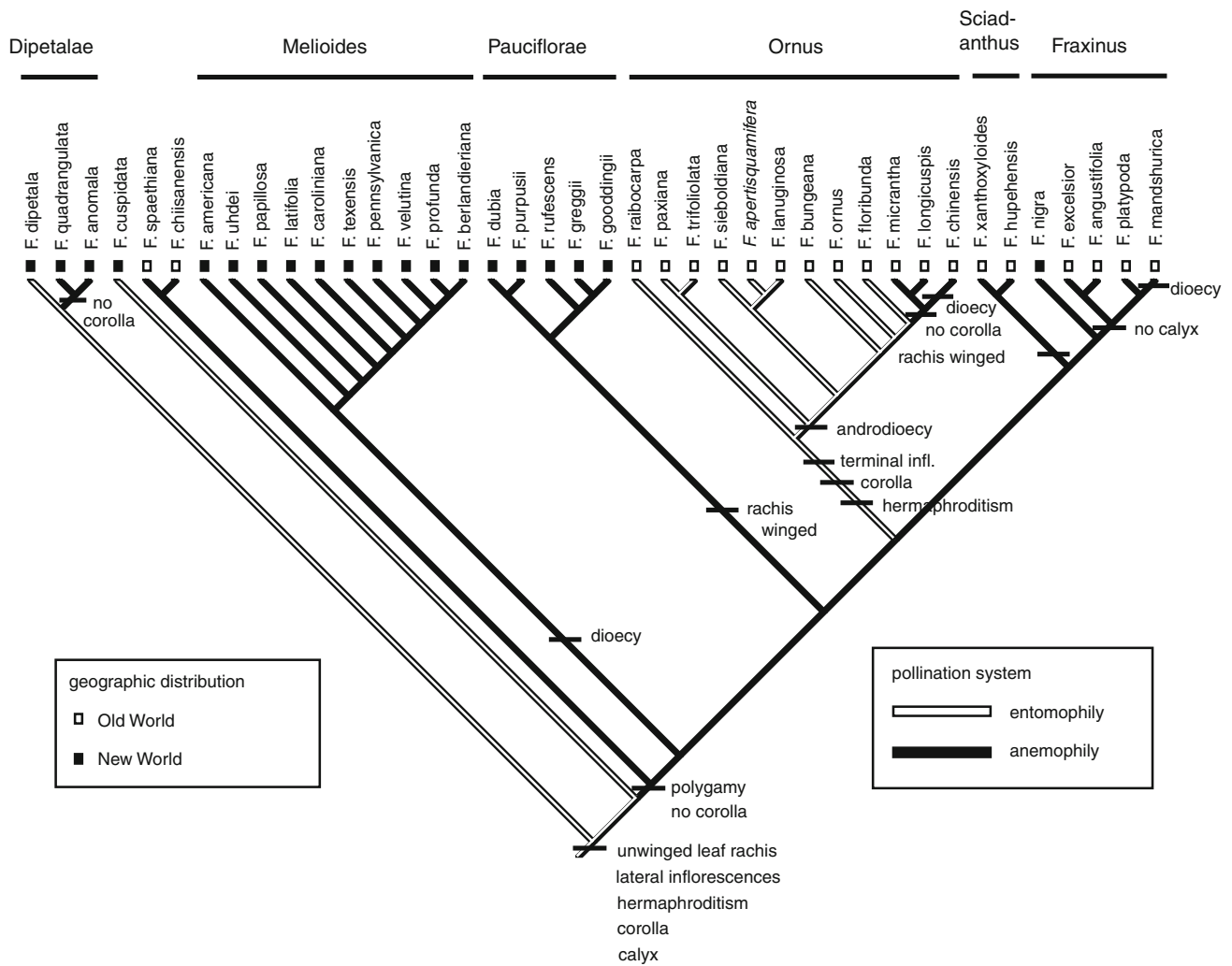
The ML chloroplast tree is largely congruent with, but less resolved than, the ITS tree produced by ML (Fig. 2b) based on the same taxon sampling. Here the variable sites of the chloroplast data are mapped on the internal branches to visualize the congruence between the data sets in another way. Of the 17 informative sites of the cp data, 15 are unambiguously changing on the internal branches of the ML ITS tree. Only *F. greggii* has a position that is not congruent in the chloroplast compared to the ITS tree. In both trees, *Fraxinus raibocarpa* (which is classified under the section *Ornus* on the basis of morphology) is resolved as sister to the three sections *Fraxinus*, *Sciadanthus* and

*Ornus*, instead of sister to only the rest of section *Ornus* (as in the complete ITS tree). The positions of *Fraxinus spaethiana* and *F. cuspidata* are not resolved in the chloroplast tree, but the section *Dipetalae*, in particular, receives strong support in both trees.

Congruence between the phylogenetic estimate and other data

The ITS trees produced by all three methods of phylogenetic inference identify six major clades (the sections indicated in Fig. 1). These clades are all well supported by MP jackknife, ML bootstrap values as well as Bayesian posterior probabilities, except for the low support for the inclusion of *F. raibocarpa* in the section *Ornus*. These clades also agree well with morphological data, which are given for each section in the taxonomic discussion below, and some characters are mapped on the tree in Fig. 3. A few unexpected relationships were discovered in the analyses, such as the three species in section *Dipetalae* and the positions of *F. cuspidata* and *F. platypoda*. However, when viewing morphology and other data such as leaf flavonoid patterns, ecology, and geographical distribution in the light of molecular data, most of the previously unreliably placed taxa could be classified as discussed below.





**Fig. 3** Summary of the *Fraxinus* phylogeny with some morphological characters mapped. It is based on the tree in Fig. 1, but *F. cuspidata* is here placed in a more parsimonious position based on morphological data (see “Discussion”). Some species relationships within the sections *Melioides* and *Pauciflorae* were arbitrarily resolved. The outgroup is not shown here. Characters mapped were considered important in previous classifications (inflorescence position, presence or absence of corolla, presence or absence of calyx, and

presence or absence of leaf rachis wings). Breeding systems are also mapped to show how they have evolved in relation to pollination mode and related floral traits. Pollination mode is shown as black (anemophily) or white (entomophily) lines. Geographic distribution is shown with black (New World) or white (Old World) boxes below the taxon names. Sectional assignments are according to the revised classification in this study

There are two different 8 bp indels in the ITS data set and one in the *trnL-F* data set. These indels were not separately coded in the data matrices, but an a posteriori mapping of them on the tree shown in Fig. 1 gives additional support for the following clades. The section *Dipetalae* is supported by an 8 bp deletion in the *trnL-F* data, all taxa of the section *Pauciflorae* share an 8 bp deletion in the ITS data, and the four Eurasian species of the section *Fraxinus* are also supported by another 8 bp deletion in the ITS data.

Two or more accessions of the same species did not group together in six cases, three in the section *Melioides* and three in *Pauciflorae* (not counting some sequences

from the study by Jeandroz et al. (1997) that may be misidentified). This includes the ITS sequence of an additional specimen of *Fraxinus americana* (added at the last minute and not included in the Bayesian analyses presented in Fig. 1) that does not group with the other two *F. americana* in the tree. Instead, it appears in the *Fraxinus pennsylvanica* clade.

**New classification**

On the basis of the morphological study and information from the molecular phylogeny, I recognise 43 species of *Fraxinus* (Table 2). Forty of these are classified into six

**Fig. 4** Key to the sections of *Fraxinus*. Two of the three species incertae sedis, *F. chiisanensis* (Korea) and *F. spaethiana* (Japan), are not covered in this sectional key because some of their traits are found in both the sections *Fraxinus* and *Melioides*

1. Inflorescences emerging with the leaves on current year shoots from terminal buds; flowers with four, free, white petals, or apetalous, always with calyx; hermaphrodites or androdioecious; 15 Old World species ..... section *Ornus*
1. Inflorescences emerging from lateral buds on previous year's shoot, before or at the same time as the leaves emerge from terminal buds; flowers with 2-4 united petals or apetalous, with or without calyx; hermaphrodites, polygamous, or dioecious; New World or Old World species
  2. Stems quadrangular; flowers mostly bisexual, with 2 united petals or without petals; 3 New World species ..... section *Dipetalae*
  2. Stems terete; flowers uni- or bisexual, with 4 united petals or without petals; New World or Old World species
    3. Corolla with 4 united petals; 1 New World species ..... *F. cuspidata*
    3. Flowers without petals; New World or Old World species
      4. Shrubs or small trees; leaf rachis winged; flowers with calyx; polygamous
        5. Few-flowered, cymose panicles; samaras small (1.5-2.5 cm); 5 New World species ..... section *Pauciflorae*
        5. Many-flowered and dense, cymose panicles; samaras large (3-5 cm); 2 Old World species ..... section *Sciadanthus*
      4. Mostly large trees; leaf rachis not winged; flowers with or without calyx; dioecious or polygamous
        5. Flowers without calyx or with small and/or deciduous calyx; polygamous or dioecious with rudimentary stamens; seed cavity of samara flattened; 4 Old World species and 1 New World ..... section *Fraxinus*
        5. Flowers with calyx; strictly dioecious; seed cavity of samara terete; 10 New World species ..... section *Melioides*

sections (*Dipetalae*, *Fraxinus*, *Melioides*, *Ornus*, *Pauciflorae* and *Sciadanthus*), and three species are left unplaced (*F. chiisanensis*, *F. spaethiana*, and *F. cuspidata*). Although the sectional circumscription mostly is the same as in previous classifications (Table 1), the subgenera are abandoned and three species (*F. anomala*, *Fraxinus quadrangulata*, and *F. platypoda*) need to be transferred to other sections as discussed further below. Some diagnostic

characters of the sections are found in the key (Fig. 4) and in Fig. 3.

#### Floral evolution

Some morphological characters presumed to be taxonomically informative in previous classifications and those found interesting for the evolution of pollination

systems are mapped on the summary tree in Fig. 3. There are no differences between the ACCTRAN or DELTRAN optimizations. The ancestral states (determined through outgroup comparison) are unwinged leaf rachis, lateral inflorescences, hermaphrodite flowers with corolla and calyx. The calyx has been lost once (in section *Fraxinus*) and the corolla three times (near the base of the tree and within each of the sections *Dipetalae* and *Ornus*). Corolla also appears to have been regained once in the section *Ornus*, coinciding with a shift from lateral to terminal inflorescences, but later lost again in the wind pollinated species. Dioecy has evolved via polygamy in the sections *Melioides* and *Fraxinus* and via androdioecy in the section *Ornus*. In all cases, dioecy followed after the transition from insect to wind pollination.

## Discussion

### The phylogenetic estimate and its support

The ITS phylogeny identifies six major clades (the sections *Dipetalae*, *Melioides*, *Pauciflorae*, *Fraxinus*, *Sciadhanthus* and *Ornus*), which mostly receive strong support from bootstrapping, jackknifing as well as Bayesian posterior probabilities. But the relationships between the sections are less clear. The resolution of the ITS tree receives high support from posterior probabilities but much less so from ML bootstrapping and MP jackknifing. Typically, Bayesian posterior probabilities are higher than jackknife or bootstrap values (Cummings et al. 2003; Erixon et al. 2003; Zander 2004), but in the present case the Bayesian support values are between 0.9 and 1.0 for clades that have around 50% or less jackknife/bootstrap support. This appears not to be uncommon, for example in the study by Martins (2006). The lack of basal resolution and/or support for the resolution also seems to be common in comparable ITS studies (Barker et al. 2004; Plunkett et al. 2004; Denk et al. 2005; Tate et al. 2005; Galicia-Herbada 2006; Martins 2006).

Several workers acknowledge the problems (paralogy, pseudogenes, etc.) with using the multi-copy nuclear ITS region for phylogenetic analyses (see review by Álvarez and Wendel 2003; Bailey et al. 2003, but also Baldwin et al. 1995; Barker et al. 2004; Denk et al. 2005; Tate et al. 2005). Especially when polyploidization and hybridization events are relatively recent, the problems may be accentuated. This is the case in the section *Melioides*, where several species are polyploid and hybridization appears to confound the phylogenetic result. For example, an additional sequence of *F. americana* did not group with the other but instead in the *F. pennsylvanica* clade. Both these specimens (i.e. not counting the sequence from Jeandroz et al. 1997) have been verified as *F. americana* and they

even originate from the same locality (Shaw Arboretum, MO, USA). However, both *F. americana* and *F. pennsylvanica* occur in that locality. It is thus possible that one of them represents a polyploid hybrid with *F. pennsylvanica* and therefore ends up in the *F. pennsylvanica* group. This demonstrates the likelihood that there is intraspecific variability of the ITS repeat in the genome, that have not yet homogenized through concerted evolution, and chance may decide which copy amplify in the PCR (Álvarez and Wendel 2003). To verify this, cloning the PCR products is necessary. This was not done, however, because it was not an aim of this study to go deeper into the relationships within the section *Melioides*. Heterogeneity of paralogous sequences and selective amplification might also be causing the pattern found within the section *Pauciflorae*, where two or more accessions of some species do not group together, although polyploidy is unknown here.

It is strongly advised to complement the ITS data with other sources of data, such as low-copy nuclear genes and/or single-copy chloroplast introns (Barker et al. 2004). Thus, in an attempt to corroborate the results from nuclear ITS data with chloroplast data, I analyzed a combined data set with *trnL-F* and *rps16* intron sequences from 15 ‘backbone’ species that represented all sections and the incertae sedis. Previous works (Gielly and Taberlet 1994; Wallander and Albert 2000) indicated that there was too little variation in these chloroplast regions to be useful for phylogenetic studies in the genus *Fraxinus*. After adding sequences from five more species, in addition to the ten already published (Wallander and Albert 2000), the number of informative characters increased from 12 to 14. There is not enough phylogenetic information in the two slow evolving chloroplast regions to shed any light on the basal resolution of *Fraxinus*. Even so, the chloroplast data could corroborate the shared ancestry of the three species in the section *Dipetalae* as well as the relationship between the section *Ornus* and the other European sections (Fig. 2a). The slight difference in topology between the ITS trees in Fig. 1 and 2b (where the section *Fraxinus* appears paraphyletic in the latter) may be an artefact of the low taxon sampling. The incongruent position of *F. greggii* in the chloroplast tree compared to the ITS tree may also be due to this, or due to the paralogy problem described earlier.

The ITS phylogeny generally shows very good congruence with morphological data and together they provide a reliable estimate of the relationships within the genus *Fraxinus*. The close relationship between the three species in the section *Dipetalae* was unexpected but is strongly supported, not only by the ITS and chloroplast data (including an 8 bp long deletion in the *trnL-F* data set) but also because a closer investigation revealed that the three species share two unique morphological characters (quadrangular stems and hermaphrodite flowers in leafless

lateral inflorescences). There is weak molecular support for the inclusion of *F. raibocarpa* in the section *Ornus*, but there is high morphological support for this group in that all species share terminal inflorescences and a corolla with four free petals (except for the secondary loss of petals in a few species). On the basis of the GC value (Goloboff et al. 2003), the low molecular support in this case appears not to be due to a conflicting position. The section *Pauciflorae* is supported by an 8 bp long deletion in the ITS sequences and several unifying morphological characteristics (the most distinctive being the small leaves with winged rachises, in combination with small fruits). Within the section *Fraxinus*, which is strongly supported by posterior probability and bootstrap values but only weakly supported by jackknife values, the Eurasian species also share an indel. The section *Sciadhanthus* is a strongly supported sister to the section *Fraxinus* in all analyses. The section *Melioides* is also a strongly supported monophyletic group. The relation between the three species incertae sedis, which form a clade in some analyses and is strongly supported by posterior probability values but ambiguously by bootstrap and jackknife values, is difficult to interpret since *F. cuspidata* is spuriously placed here. In this case, I speculate that none of the inference methods have been able to accurately resolve the true relationships because of their relatively divergent DNA sequences. It is possible that this may be attributable to the phenomenon known as long-branch attraction (Felsenstein 1978), which may affect parsimony as well as likelihood based methods such as Bayesian inference (e.g. Archibald et al. 2003; Zhang et al. 2006). Therefore, in Fig. 3, *F. cuspidata* has been placed in a more parsimonious position on the basis of morphological and other evidence. These relationships are all discussed in detail in “Taxonomy and morphology in *Fraxinus*”.

Jeandroz et al. (1997) presented a phylogeny on the basis of maximum parsimony and neighbour-joining analyses of ITS data. They did not include any representatives from the sections *Pauciflorae* or *Sciadhanthus*, and only one representative of the petaliferous taxa in section *Ornus*, and the tree has a poorly supported basal resolution. The topology is not congruent with that obtained by Bayesian inference (Fig. 1). Other major discrepancies include the misplacement of *F. anomala* in their study, which is probably because of a misidentification, as evidenced by the position of their sequence obtained from GenBank in Fig. 1.

#### Systematics and new classification

There is a remarkable congruence between the lineages identified in the molecular phylogeny and traditional ideas of relationships within the genus. A few discrepancies exist and I therefore present a revised infrageneric classification

of the genus *Fraxinus* (Table 2) consisting of six sections: *Dipetalae*, *Fraxinus*, *Melioides*, *Ornus*, *Pauciflorae*, and *Sciadhanthus*. In previous classifications (Table 1), the genus *Fraxinus* has been divided into two subgenera or sections, *Ornus* and *Fraxinus*, on the basis of inflorescence position. The section *Ornus*, with terminal inflorescences, is clearly monophyletic and derived from taxa with lateral inflorescences. These latter taxa, on the other hand, do not form a monophyletic group. Thus, a more natural infrageneric classification is achieved by having only six sections. It may seem unnecessary to recognize six sections within such a relatively small genus as *Fraxinus*. However, the six sections are each clearly monophyletic and well separated from each other morphologically, as well as forming molecularly distinct lineages. A case could be made for including the small section *Sciadhanthus* with the section *Fraxinus*, although I have kept them separate here for traditional reasons.

*Fraxinus cuspidata* forms an ambiguously supported clade together with *F. chiisanensis* and *F. spaethiana* in some analyses. Because of the present uncertainties and pending further data to elucidate their positions, I leave all three species as incertae sedis. Possible affinities on the basis of non-molecular data are discussed further below.

Previously, the section *Ornus* comprised two subsections (sensu Lingelsheim 1920): *Ornus* with petals and *Ornaster* without. Jeandroz et al. (1997) concluded, on the basis of their limited analysis, that the two subsections were monophyletic. However, that conclusion was unjustified when only *F. ornus* represented the subsection *Ornus*. In the present study, where nearly all species are included, subsection *Ornus* is shown to be paraphyletic with respect to subsection *Ornaster*. I have therefore chosen not to recognize the subsections here, as the apetalous taxa are derived from petalous ones (discussed more under section *Ornus* below).

In summary, the differences compared to previous classifications are: (1) abandoning the subgeneric and subsectional levels and only recognizing six sections, of which the section *Pauciflorae* is a new combination, (2) moving *F. platypoda* from the section *Melioides* to the section *Fraxinus*, (3) moving both *F. quadrangulata* from the section *Fraxinus* and *F. anomala* from the section *Melioides* to the section *Dipetalae*, and (4) treating *F. chiisanensis*, *F. spaethiana* and *F. cuspidata* as incertae sedis. All taxonomic changes and morphological support are discussed below.

#### Taxonomy and morphology in *Fraxinus*

The genus *Fraxinus* comprises 43 species in six sections: *Dipetalae*, *Melioides*, *Pauciflorae*, *Sciadhanthus*, *Fraxinus*,

and *Ornus*. The first five sections, excluding *Fraxinus dipetala* but including the incertae sedis *F. spaethiana* and *F. chiisanensis*, comprise 26 wind-pollinated species, which are characterized by apetalous flowers in inflorescences that emerge from lateral buds before the terminal leaf buds open. *F. dipetala* and the incertae sedis *F. cuspidata* also have lateral inflorescences, but sympetalous, hermaphrodite and insect-pollinated flowers. The 15 species in the Eurasian section *Ornus* have terminal inflorescences, with four free petals or without, and this section appears to be derived from a common ancestor shared with the other Eurasian sections *Fraxinus* and *Sciadanthus*. A taxonomic discussion for each section is given below.

#### The section *Dipetalae*

The section *Dipetalae* now comprises three American species, previously scattered in different sections. *F. quadrangulata* belonged to the section *Fraxinus*, *F. anomala* to *Melioides*, and *F. dipetala* used to be the sole member of *Dipetalae*. Having found strong molecular support (from both chloroplast and nuclear sequences) for the relationship between these three species, I investigated their morphological characteristics more closely. In *Fraxinus*, they are unique in having quadrangular twigs (due to development of corky ridges) and hermaphrodite flowers occurring in leafless lateral inflorescences. Other morphological similarities include oval to ovate shaped wings of the samaras, contrasting to the more elongated wings characteristic of the samaras of the other sections. Although the three species are seemingly quite different in other morphological characters, such as number of petals, number of leaflets, and life form, they are clearly most closely related to each other. In addition to the high support from ITS and chloroplast data, they also share an 8 bp long deletion in the *trnL-F* data set.

*Fraxinus dipetala* (Two-petal ash) is a shrub or small tree restricted to southwestern USA. It is the only *Fraxinus* species having two petals, which are united and tubular by fusion with the filaments. Sometimes, the petals are lacking and the two forms may occur in the same panicle (Vines 1984). The flowers are fragrant and occur in many-flowered and showy inflorescences, which probably attract insects. The anthers are relatively large and protrude from the corolla, an indication that the flowers might be both wind- and insect-pollinated. It shows some morphological affinity to *F. cuspidata* in the united petals.

*Fraxinus anomala* (Single-leaf ash) is also a shrub or small tree, predominantly occurring in southwestern USA. It is the only *Fraxinus* species with simple leaves, or occasionally 3–5 leaflets (*F. anomala* Torr. var. *lowelli* (Sarg.) Little). It differs from the species in the section *Melioides*, where it was formerly classified by Lingelsheim

(1920), Miller (1955), and Nikolaev (1981), in having quadrangular twigs and bisexual or sometimes unisexual flowers (Sargent 1949; Vines 1984). The flowers have a persistent calyx, but lack corolla, and appear in lateral panicles before or with the young leaves. They are apparently wind-pollinated.

*Fraxinus quadrangulata* (Blue ash) is a large tree with conspicuously quadrangular twigs, occurring in eastern and central North America. It was previously classified in the section *Fraxinus* (*Bumelioides*). The flowers are mostly hermaphrodite (Sargent 1949; Miller 1955; Vines 1984), apetalous, and presumably wind-pollinated.

#### The section *Melioides*

The section *Melioides* has strong molecular support. They are all medium-sized to large trees, deciduous and dioecious. The unisexual flowers are apetalous and wind-pollinated. The female flowers consist of a calyx and one pistil, and the male flowers of two stamens with elongated anthers and a small calyx. There are no rudimental organs of the opposite sex in the flowers (a unique synapomorphy for this section). The calyx is persistent in the samaras, which have a distinctly terete seed cavity (except *Fraxinus caroliniana*). The wing may be decurrent along the seed cavity or not. In addition, the presence of flavones in the leaves (besides the plesiomorphic flavonols) is a synapomorphy for these species (see further below about flavones in *F. chiisanensis*).

Many taxa have been described in the section *Melioides* and different authors accept different numbers of species (Sargent 1949; Little 1952; Miller 1955; Kartesz 1994; USDA, NRCS 2007). On the basis of examinations of over 300 herbarium specimens, and studies of some species in the field, I accept ten species (Table 2) with a wide distribution in North America. A few species occur in Mexico and Central America as well. Identification of the species in this section is difficult because of both morphological and ecological variation, and delimitation of taxa is also complicated by extensive hybridization and polyploidy (Miller 1955). As stated earlier, the ITS is not suitable for elucidating relationships within this section because of this. Some species may also be poorly defined but it is beyond the scope of the present study to go deeper into this matter.

Traditionally, the species have been divided into two main complexes, distinguished primarily by the presence of papillae on the lower epidermis of the leaflets ('the white ash complex') or by the absence of papillae ('the red ash complex') (Wright 1944c; Wilson and Wood 1959; Hardin and Beckmann 1982). The white ash complex comprises *F. americana* (and *Fraxinus biltmoreana*), *Fraxinus papillosa*, and *Fraxinus texensis*, and the red ash complex comprises *Fraxinus berlandieriana*, *F. caroliniana*,

*Fraxinus latifolia*, *F. pennsylvanica*, *Fraxinus profunda*, and *Fraxinus velutina* (Miller 1955). To the extent that ITS data can say anything reliable about this group, there is no clear-cut division in the molecular data that supports these complexes (Fig. 1). There is one monophyletic group, however, but it does not entirely correspond to the red ash complex, as circumscribed by Miller (1955), since *F. texensis* is found within this clade and *F. latifolia* is found outside. Thus, presence or absence of epidermal papillae appears not to be phylogenetically informative in this section.

*Fraxinus americana* (White ash) is a large tree distributed in eastern North America. This species complex is composed of three ecotypes (Wright 1944a; Wilson and Wood 1959) with different ploidy levels. It is diploid ( $2n = 46$ ) in the northern parts of its range and diploid, tetraploid ( $2n = 92$ ), or hexaploid ( $2n = 138$ ) in the southern parts (Wright 1944a). Black and Beckmann (1983) found trees of all ploidy levels within immediate vicinity of each other in North Carolina. This might explain why the two accessions of *F. americana* that came from the same locality (Shaw Arboretum, MO, USA) did not group together. It is hypothesized that *F. biltmoreana*, which is not recognized here, is an allopolyploid hybrid between a tetraploid *F. americana* and a diploid *F. pennsylvanica* (Miller 1955; Santamour 1962, but see Hardin and Beckmann 1982).

Miller (1955) treated *F. texensis* (Texas ash) as a subspecies of *F. americana*, noting that *F. texensis* is separated ecologically, physiologically, and morphologically from *F. americana*. The most distinctive characters of *F. texensis*, which only occurs in Texas and northern Oklahoma, are that it has fewer leaflets and smaller samaras than those of *F. americana*.

*Fraxinus papillosa* (Chihuahuan ash) is a small tree that occurs in southwestern USA and Mexico. Like the eastern and much larger *F. americana* it has a distinctly papillose lower leaf epidermis, but in contrast the leaflets are sessile.

*Fraxinus uhdei* (Shamel ash or Tropical ash) is distributed in Mexico, Guatemala, and Honduras. It is also cultivated in Hawaii and naturalized in Puerto Rico and some other tropical areas. It was first described as a variety of *F. americana*, but later raised to species level by Lingelsheim (1907). It is distinguished by its long-petiolulate and long-acuminate leaflets without papillae on the lower epidermis, and tropical distribution, and therefore treated as a separate species.

*Fraxinus pennsylvanica* (Red or Green ash) is distributed in the central and eastern USA and Canada. It also consists of at least three different ecotypes, but in contrast to *F. americana* they are all diploid (Wright 1944b).

Miller (1955) treated both *F. velutina* (Velvet ash) and *F. latifolia* (Oregon ash, synonym *F. oregona*) as subspecies of *F. pennsylvanica*. There is a report of polyploidy in *F. velutina* ( $2n = 92$ , Taylor 1945) and it may hybridize

with *F. pennsylvanica* (Wright in Little 1952). Both *F. velutina* and *F. latifolia* are western species, but *F. velutina* occurs only in southwestern USA and northern Mexico and *F. latifolia* is restricted to N California, Oregon, and Washington. Both species have pubescent leaves, but *F. latifolia* is distinguished by its sessile leaflets whereas those of *F. velutina* are petiolulate.

*Fraxinus profunda* (Pumpkin ash, synonym *F. tomentosa*) is hexaploid ( $2n = 138$ ) and thought to be an autopolyploid of *F. pennsylvanica* or, like *F. biltmoreana*, another hybrid of a tetraploid *F. americana* and a diploid *F. pennsylvanica* (Miller 1955; Wilson and Wood 1959; Wright 1962, 1965, but see Hardin and Beckmann 1982). Its leaves, twigs, flowers, and samaras are all larger than those of *F. pennsylvanica* and *F. americana*, but qualitatively similar to one or the other of these two species. According to Miller (1955), it is doubtful whether *F. profunda* should be recognized, as it is not clearly separated from *F. pennsylvanica* other than by its 'gigas' characters due to polyploidy. However, these characters make it morphologically distinct and therefore *F. profunda* is generally regarded as a separate species.

*Fraxinus berlandieriana* (Mexican ash) occurs in the southwestern USA and northern Mexico. Miller (1955) treated it as a synonym of *F. pennsylvanica*. But apart from its southwestern distribution, it also differs from *F. pennsylvanica* in being a much smaller tree (about 10 m), having fewer pairs of leaflets (3–5 vs. 5–9), and a wing of the samara that is decurrent to the base (Sargent 1949; Vines 1984).

*Fraxinus caroliniana* (Carolina ash or Water ash) occurs in the southeastern swamps of the USA and is an extremely variable species (Little 1952; Hardin 1974). It differs from the other species of this section in not having a terete seed cavity. *Fraxinus cubensis* in Cuba is treated as part of the *F. caroliniana* complex. *F. caroliniana* reportedly forms hybrids with *F. americana* (Miller 1955).

#### The section *Pauciflorae*

*Fraxinus* section *Pauciflorae* (Lingelsh.) E. Wallander, comb. et stat. nov. (basonym: *Fraxinus* section *Fraxinaster* subsection *Pauciflorae* Lingelsh., Engler's Bot. Jahrb. 40:218. 1907), is a monophyletic group consisting of five New World species that all occur in arid regions of the southwestern USA, Mexico, and Guatemala. They are shrubs or small trees with small coriaceous leaves. In common with the two species of section *Sciadanthus*, the leaves have winged rachises, but in contrast they have few-flowered panicles. The flowers are polygamous, apetalous, and wind-pollinated. The samaras have a persistent calyx.

Two species occur in the southwestern USA and Mexico: *F. greggii* (Gregg's ash) in Texas, Arizona, New

Mexico, and Mexico, and *F. gooddingii* (Goodding's ash) restricted to Arizona in the USA and Sonora in Mexico. The latter was described by Little (1952), but later referred to as a subspecies of *F. greggii* by Murray (1982), and has an ITS sequence identical to one of the specimens of *F. greggii*. It is doubtful whether *F. gooddingii* should be retained as a separate species. Nevertheless, I have tentatively accepted *F. gooddingii* because I have not seen more than one herbarium specimen of it. *Fraxinus rufescens*, occurring only in Mexico, is also related to these two species but distinguished by its ferruginous-tomentose shoots and inflorescences.

*Fraxinus dubia* and *Fraxinus purpusii* both occur in Mexico and Guatemala and should not be so difficult to distinguish on the basis of morphology. The leaflets of *F. purpusii* have coarsely serrate margins whereas the leaflets of *F. dubia* have entire margins. Nevertheless, the analysis of several specimens each of *F. dubia*, including its supposed synonym *F. schiedeana* (Green 1991), *F. purpusii*, and *F. greggii*, indicates that the species boundaries between them are not so clear (Fig. 1). An alternative explanation for the pattern found may be, although polyploidy and hybridization is unknown in this section, that heterogenous paralogs of the ITS may have been sequenced. Except for *F. greggii*, the Mexican species are not well described or illustrated in the literature (Standley 1924; but see the recent treatment by Rzedowski 2004) and I have only seen the type of *F. purpusii*. This section is in need of a more thorough revision.

#### The section *Sciadhanthus*

The section *Sciadhanthus* consists of only two Old World species: *Fraxinus xanthoxyloides* (Afghan or Algerian ash) distributed from Morocco and Algeria in north Africa through the Middle East to the Himalaya and China, and *Fraxinus hubeiensis* which is a threatened species endemic to the Hubei province in China (Ming and Liao 1998). They are both small trees or shrubs, and characterized by apetalous flowers with calyx, except that the male flowers of *F. xanthoxyloides* lack calyx. The flowers are polygamous and wind-pollinated. They resemble the New World *Pauciflorae*, but have many more flowers in their congested, cymose panicles, and larger samaras. The leaves are relatively small (7–15 cm) and possess a winged rachis. They form a well-supported sister group to the section *Fraxinus* and share the same main geographical distribution area.

#### The section *Fraxinus*

The section *Fraxinus* (invalid name *Bumelioides*) comprises five species, and all except *F. nigra* are distributed in Eurasia. In common with the species of the section

*Melioides* they are all relatively large and wind-pollinated trees. In previous classifications, this group was characterized by polygamous flowers without calyx, but now *F. platypoda*, which has a reduced calyx, is included as well. *Fraxinus nigra* has a deciduous calyx, but the other three species have asepalous flowers. The male flowers consist of two stamens, the hermaphrodite flowers of one pistil and two stamens, and the female flowers of one pistil and sometimes rudimentary stamens. They are distinguished from the strictly dioecious species of the section *Melioides* in also having a flattened seed cavity of the samara (vs. terete in the section *Melioides*) and foliar terminal bud scales (vs. entire in the section *Melioides*) (Whelden 1934). Although not all species have been investigated, the two sections also differ from each other in RFLP pattern (Morand et al. 2001) and foliar flavonoid content (Harborne and Green 1980; Min et al. 2001).

*Fraxinus excelsior* (Common ash or European ash) is distributed in the northern and central Europe and eastwards to Volga river basin in western Russia. It displays a polygamous breeding system, but recent studies indicate that it might be subdioecious or functionally dioecious (Wallander 2001; FRAXIGEN 2005). *Fraxinus coriariifolia*, distinguished only by its variably pubescent shoots and leaves, is found in Romania, Turkey, Caucasus, and northern Iran. It may deserve recognition as a subspecies of *F. excelsior*, as treated by Murray (1968) and Yaltirik (1978).

*Fraxinus angustifolia* (Narrow-leaved ash) includes a complex of taxa which have not been fully clarified due to extreme variation in morphology. After considering the opinions of several authors (Anderson and Turrill 1938; Metcalfe 1938; Vassiljev 1952; Franco and Rocha Afonso 1972; Scheller 1977; Yaltirik 1978; de Jong 1990), studying herbarium material and some populations in the field, and having seen the minimal variation among the ITS sequences of these taxa, I have come to the conclusion that *Fraxinus oxycarpa*, *Fraxinus syriaca*, *Fraxinus pallisiae*, *Fraxinus potamophila*, and *Fraxinus sogdiana* should be synonymized under *F. angustifolia* (see Yaltirik 1978, for additional synonyms). The first two taxa may be retained as subspecies together with the autonym, as they have been treated by Franco and Rocha Afonso (1972) and Yaltirik (1978). The leaf and shoot pubescence and leaflet morphology within this complex are too variable to deserve specific recognition. A more detailed analysis of the *F. angustifolia* complex (unpublished ITS data involving more specimens) reveals a nested relationship among the taxa and there appears to be no monophyletic groups that could be regarded as separate species. Following this view, *F. angustifolia* s. l. has a wide distribution in the Mediterranean area and southeast Europe, through Turkey and the Caucasus region, Southwest Asia, and east to the Turkestan region (*F. sogdiana*).

*Fraxinus angustifolia* is closely related to *F. excelsior* and they have also been shown to hybridize (Jeandroz et al. 1996; Raquin et al. 2002; Fernandez-Manjarres et al. 2006; Heuertz et al. 2006). *Fraxinus angustifolia* differs morphologically from *F. excelsior* (Fukarek 1960) and is andromonoecious (Grunwald and Karschon 1984; Gyenova 1993; FRAXIGEN 2005). In contrast to all other taxa of the genus, which have compound paniculate inflorescences, *F. angustifolia* (including all its synonyms) has simple racemes. Additional characters for distinguishing between *F. angustifolia* and *F. excelsior* are listed by FRAXIGEN (2005).

*Fraxinus mandshurica* (Manchurian ash) is most closely related to *F. platypoda* and both species occur in China, although *F. mandshurica* has a wider distribution into eastern Russia, Korea, and Japan. *F. mandshurica* is dioecious with rudimentary stamens in the pistillate flowers (Yamazaki 1993, personal observation).

*Fraxinus platypoda* was previously placed in the section *Melioides* (Lingelsheim 1920; Nikolaev 1981), despite its Asian distribution, because of the presence of a small calyx, at least in the bisexual flowers (Wei and Green 1996). Nevertheless, it exhibits all other characters shared by the taxa in section *Fraxinus*, including the distinctly flattened seed cavity of the samara, and molecular data give strong support for its placement here. In fact, the ITS sequences of *F. platypoda* and *F. mandshurica* are nearly identical. Many authors (e.g., Nakaike 1972; Wei and Green 1996) place *F. spaethiana* (endemic to Japan) in synonymy under *F. platypoda*. Both of them have a distinctly swollen base of the leaf petiole clasping the shoot, but *F. platypoda* differs from *F. spaethiana* in not having a papillose epidermis on the abaxial side of the leaves. The ITS sequences of *F. platypoda* and *F. spaethiana* are also quite dissimilar and I recognize them as separate species. It is possible that the previous placement of *F. platypoda* was based on a mix-up with *F. spaethiana* (which appears to be related to *Melioides*).

*Fraxinus nigra* (Black ash) is an eastern North American species, but there has never been any disagreement over whether it belongs to this section or to the American *Melioides*. It is morphologically similar to *F. mandshurica*, and the latter has been referred to as a subspecies of *F. nigra* by Sun (1985) and Green in Wei and Green (1996). The flowers are polygamous and the calyx is small and deciduous, or absent. The seed cavity of the samara is flattened, similar to the other species in this section.

#### Section *Ornus*

The molecular support for the section *Ornus* is high, although with low support for the inclusion of

*F. raibocarpa*, and there is no doubt that it is a monophyletic group. Within *Fraxinus*, the species are unique in that the inflorescences are borne on current year shoots together with the leaves, which emerge from terminal buds, in contrast to lateral inflorescences on previous year's shoots in all the other sections.

The section *Ornus* as circumscribed here comprises 15 species, all distributed in Eurasia and with a concentration in eastern Asia. Eleven of these species have four, essentially free, petals that are only united at the very base (compared to *F. cuspidata*). The flowers are fragrant and produce a lot of pollen. Apparently, they are insect-pollinated or both wind- and insect-pollinated (personal observation). These 11 species constitute subsection *Ornus* sensu Lingelsheim (1920). I could not obtain ITS sequences from two petaliferous species of this section, *F. griffithii* and *F. malacophylla*, but they undoubtedly belong here. *F. griffithii* occurs in Japan, Taiwan, and the Philippines and is an evergreen or semi-evergreen small tree with large showy panicles. *F. malacophylla* is a relatively large tree, distributed in southern China and northern Thailand, and characterized by brown tomentose leaflets. In these two species, the flowers are always hermaphrodite (Yamazaki 1993; Wei and Green 1996). This is also the case for *F. raibocarpa*, a shrub or small tree in Central Asia with characteristically falcate samaras. The remaining eight petaliferous species are all androdioecious (Yamazaki 1993; Wei and Green 1996, personal observation) and occur mostly in the Himalayan mountains, China, and Japan. Of them, *Fraxinus sieboldiana* (China and Japan), *Fraxinus lanuginosa* (Japan), and *Fraxinus apertisquamifera* (Japan) form a strongly supported group. The rest, including the European species *F. ornus* (Manna ash), have no supported resolution among them.

Four species are apetalous (previously subsection *Ornaster*): *F. micrantha* in the Himalaya, *F. longicuspis* in Japan, *F. baroniana* in China, and the more widespread southeast Asian *F. chinensis* s. l. These species form a well-supported monophyletic group, distinguished morphologically from the rest of section *Ornus* in having no corolla. They are mostly relatively large trees and although the flowers and leaves emerge together, they flower before the leaves are expanded and are wind-pollinated. Unfortunately, I could not obtain any ITS sequence from *F. baroniana*, but on the basis of morphology it undoubtedly belongs to this group. It is also apetalous, but differs morphologically from the other species in being a smaller tree and in having relatively narrow leaflets. *Fraxinus micrantha* and *F. longicuspis* are androdioecious (Nakaike 1972; Yamazaki 1993, personal observation) and *F. baroniana* and *F. chinensis* are dioecious (Wei and Green 1996). Some individuals of *F. chinensis* have rudimentary



stamens in their female flowers (Wei and Green 1996, personal observation).

The ITS sequences of both *Fraxinus japonica* (Japan) and *Fraxinus rhynchophylla* (Korea and northern China) are practically identical to *F. chinensis*, and they are here treated as synonyms to the latter species. Wei and Green (1996) also treat *F. japonica* as a synonym of *F. chinensis* Roxb. ssp. *rhynchophylla* (Hance) E. Murray. There are no clear morphological boundaries between the taxa of the *F. chinensis* complex (Nakaike 1972, personal observation, but see Kang et al. 2002). *F. chinensis* has  $2n = 46, 92,$  or  $138$  (Wright 1962; Nikolaev 1981) and the ploidy levels in this species complex may explain some of the morphological variation.

The section *Ornus*, as circumscribed by Lingelsheim (1920) and others, encompassed two subsections. Although subsection *Ornaster* is clearly monophyletic based on strong molecular support and floral synapomorphies, in the present phylogenetic classification there is no support for recognising the subsection *Ornus*, as the former is derived from the latter. I have therefore abandoned the subsections within section *Ornus*, despite the fact that the two groups are morphologically well distinguished (with or without petals).

#### Species incertae sedis

The odd American species *F. cuspidata* (Fragrant ash, Fresno) is a small entomophilous tree occurring in Mexico and the southwestern USA. On the basis of its possession of corolla it was included in the otherwise Eurasian section *Ornus* (Lingelsheim 1920). Although having four petals, its flowers are not similar to those of the species in this section. The petals are united and form a tube about one-third of the length of the corolla, not free as in the section *Ornus*. The two stamens are united with the corolla tube and shorter than the petals. The fragrant flowers are borne terminally in lateral, leafy panicles developed from the axils of the leaves of the previous year, not in terminal panicles on current year shoots as in the section *Ornus*. Nikolaev (1981) included *F. cuspidata* with *F. dipetala* in the section *Dipetalae*. Although differing in petal number (*F. dipetala* has two and *F. cuspidata* four petals), they are both hermaphrodites with the plesiomorphic sympetalous corolla fused with the filaments. They both have inflorescences on lateral shoots (which are not leafy in *F. dipetala*) and are also the only two petaliferous species of *Fraxinus* in America. The results from molecular data do not support a close relationship with the section *Ornus* or the section *Dipetalae*, but instead *F. cuspidata* is found in a poorly supported clade with the other two unclassified species *F. chiisanensis* and *F. spaethiana*. Although this is a highly dubious position, *F. cuspidata* appears to not belong within

any of the previously described sections. On the basis of its unique morphology within the genus, such as the four united petals and inflorescence position, a case could be made for placing it in a section of its own. However, pending further data to elucidate its position I have tentatively left it as incertae sedis.

The ITS sequences of *F. chiisanensis* and *F. spaethiana* are quite divergent compared to the other taxa and are only similar to the odd *F. cuspidata*. These three species form a clade that is moderately supported by Bayesian posterior probabilities but with less than 50% bootstrap and jackknife support. In the taxon-reduced chloroplast and ITS trees (Fig. 2a, b), one or all three of them appear as closely related to the section *Melioides*. The GC output of the complete ITS jackknife tree gives weak support for this position due to grouping conflicts and in Fig. 1 the clade occurs in a basal trichotomy. Possible affinities on the basis of non-molecular data are discussed below.

*Fraxinus chiisanensis* is a wind-pollinated tree, endemic to Korea. The samaras have a persistent calyx and the leaf rachis is not winged. Surprisingly, these features led the auctor Nakai (1929) to conclude that it belonged to section *Dipetalae*, which previously comprised only the American *F. dipetala*. However, the combination of a persistent calyx and non-winged rachis is also a feature of the section *Melioides*, where it would fit much better. The relationships of *F. chiisanensis* were discussed by Nakaike (1972), who believed that the section to which this species belongs could not be determined without flowers (which he had not seen). Hypotheses on the putative hybrid origin of *F. chiisanensis* from *F. mandshurica* (section *Fraxinus*) and *F. chinensis* (section *Ornus*) have been refuted by Noh et al. (1999) on the basis of RFLP patterns and by Min et al. (2001) on the basis of foliar flavonoids. Although the apetalous flowers of *F. chiisanensis* appear to be intermediate in morphology between *F. mandshurica* and *F. chinensis* (Min et al. 2001), these species have no other close similarities. I also find it unlikely that two distantly related species from such morphologically different sections could hybridize. On the basis of examinations of flowers of *F. chiisanensis*, and photos by Min et al. (2001), I noted that the lateral inflorescences have either apetalous male flowers or hermaphrodite flowers with elongated anthers, similar to those of section *Melioides*. Although polygamous or androdioecious in appearance, the anthers in the hermaphrodite flowers seem to be smaller than in the male ones, suggesting functional dioecy. The samaras of *F. chiisanensis* are similar to those of *Melioides*, with a terete seed cavity. The geographical distribution and presence of bisexual flowers, on the other hand, suggest a relationship with *F. platypoda* and *F. mandshurica* in the section *Fraxinus*. Recently, chemical support for a shared

ancestry of *F. chiisanensis* and the section *Melioides* has come from studies of foliar flavonoids. A study by Min et al. (2001) found the presence of advanced flavones in *F. chiisanensis*, in common with *F. americana* and *F. pennsylvanica* of the section *Melioides* (Harborne and Green 1980; Black-Schaefer and Beckmann 1989). An expanded study by Chang et al. (2002) confirmed that *F. chiisanensis* shares the apomorphic flavones with several other species of the section *Melioides*. Other taxa of *Fraxinus* that have been investigated have only the plesiomorphic flavonols.

*Fraxinus spaethiana* is a large wind-pollinated tree, endemic to Japan. Lingelsheim (1907, 1920) classified it in the section *Ornus*, on the basis of a mistake that it had four petals. It has lateral inflorescences with polygamous and apetalous flowers. Calyx is present only in pistillate flowers (Yamazaki 1993) and the samaras have a flattened seed cavity. As mentioned earlier, it is morphologically similar to the Chinese *F. platypoda*, and has been suggested to be a synonym of this species (Nakaike 1972; Wei and Green 1996). The molecular data, however, places these taxa in widely separated positions. The flavonoid study by Min et al. (2001) only found flavonols in *F. spaethiana*. Thus, only the ITS sequence is similar to *F. chiisanensis*.

The molecular data are not conclusive, but they indicate a relationship between the three species and the section *Melioides*. Some morphological and chemical data also suggest a close relationship for at least *F. chiisanensis* with the species in the section *Melioides*. Min et al. (2001) proposed that *F. chiisanensis* should be included in the section *Melioides* due to its chemical affinity with this section. Chang et al. (2002) found the same chemical pattern, but on the other hand noted a strong discontinuity between *F. chiisanensis* and the species in the section *Melioides* in the shape of the terminal bud scales, some leaf characters, and floral sexuality. They also compared ITS sequences and concluded that “*F. chiisanensis* seems to be a highly primitive species within the section *Melioides*” and that “it was probably differentiated from the ancestor of this group a long time ago”. The results for *F. spaethiana* are unclear due to the non-finding of flavones and its flattened seed cavity. Because of these present uncertainties, I leave both species as *incertae sedis*.

## Evolutionary comments

### Floral evolution

Jeandroz et al. (1997) traced the evolution of corolla and calyx, and concluded that the floral evolution had been homoplasious. However, owing to an incomplete

phylogeny as well as several errors in character state coding (*F. quadrangulata* and *F. nigra* have a small and deciduous calyx, and all included species of previous subsection *Ornaster* have a calyx but no corolla), the number of inferred character state changes are incorrect. On the basis of my nearly complete phylogeny, as depicted in Fig. 3, the presence of calyx and corolla is ancestral in the genus. The corolla has been lost three times, but it also appears to have been regained from an apetalous state in the ancestor of section *Ornus* (before lost again). This floral trait is correlated with pollination mode and loss of corolla is one of a suite of characters coupled to the anemophilous syndrome (Faegri and van der Pijl 1979). The calyx has been completely lost only once (within the section *Fraxinus*). In a few taxa of some other sections, the calyx is only reduced. Simultaneously with regaining the corolla, terminal inflorescences appear to have evolved from lateral ones in the ancestor of section *Ornus*.

### Breeding system evolution

The species of *Fraxinus* display several, to some extent intergrading, breeding systems. Hermaphroditism is ancestral in the family Oleaceae (Wallander and Albert 2000) and appears to be the ancestral state in *Fraxinus* as well. The three species of section *Dipetalae*, plus *F. malacophylla*, *F. griffithii* and *F. raibocarpa* of the section *Ornus*, and *F. cuspidata*, are the only hermaphroditic species. No less than ten species in section *Ornus* (eight with and two without corolla) are androdioecious, at least phenotypically (Yamazaki 1993; Wei and Green 1996, personal observation). Androdioecy (separate male and hermaphrodite individuals) is an extremely rare breeding system (e.g. Charlesworth 1984; Pannell 2002). The genus *Fraxinus* is therefore unusual among angiosperms in having several androdioecious species. Several other genera of the Oleaceae also have androdioecious species, e.g. *Chionanthus* (Ueda 1996), *Osmanthus* (e.g. Wei and Green 1996), and *Phillyrea* (e.g. Vassiliadis et al. 2002), and this high incidence is probably tied to their common phylogenetic history (Wallander 2001). Dioecy occurs in all ten species of the section *Melioides*, the two apetalous *F. chinensis* and *F. baroniana* of the section *Ornus*, and in *F. mandshurica* of the section *Fraxinus*. The remaining four species of the section *Fraxinus* are morphologically polygamous, as well as the five species of the section *Pauciflorae* and the two species of the section *Sciadhanthus*. The conclusion drawn from the phylogenetic work is that several of the wind-pollinated species have evolved dioecy independently. Dioecy evolved once from hermaphroditism via androdioecy in the section *Ornus*, once via polygamy in the section *Fraxinus*, and once in the ancestor

of the section *Melioides*. This gradual loss of first female function resulting in male flowers and later loss of male function resulting in functionally female flowers has happened repeatedly following the transition from insect to wind pollination. This interesting trend in the evolution of unisexual flowers is discussed in more detail elsewhere (Wallander 2001).

### Biogeography

It is beyond the purpose of the present paper to do a thorough biogeographical analysis. However, on the basis of the phylogenetic result and morphological studies, the following biogeographical interpretation is made. Supported by fossil evidence (Call and Dilcher 1992), *Fraxinus* is hypothesized to have originated in the North America during the Eocene with two subsequent dispersal events across one or both of the two land bridges into Eurasia (the ancestor of *F. chiisanensis* and *F. spaethiana*, and the ancestor of the sections *Ornus*, *Fraxinus*, and *Sciadanthus*) and one dispersal event back to North America (*F. nigra*). This hypothesis differs from scenario A proposed by Jeandroz et al. (1997) because the dispersal of the ancestor of *F. chiisanensis* and *F. spaethiana* was not accounted for in their study.

### Conclusions

The reliability of the phylogenetic hypothesis was assessed in several ways. The ITS data were analyzed using three different methods of phylogenetic inference, maximum parsimony, maximum likelihood, and Bayesian analyses, and the clade support in the resulting trees was evaluated through jackknife, bootstrap, and posterior probability values, respectively. Although with variable support for the basal resolution, no highly supported clades were contradicted. An independent data set with a smaller number of representative taxa, consisting of combined chloroplast sequences from the *rps16* and *trnL-F* regions, was found to be largely congruent with the ITS phylogeny. In addition, several morphological characters support the major clades, which correspond well to the sections in previous classifications. Thus, I conclude that the result obtained here most likely represents a reliable estimate of the phylogenetic relationships within the genus *Fraxinus*.

The subgenus *Fraxinus* was found to be paraphyletic, because subgenus *Ornus* is derived from it, and in the

revised classification I have abandoned the subgeneric rank. I recognize six sections only and a total of 43 species. Three species have been transferred to other sections to accord with the phylogenetic results and three species are treated as *incertae sedis* because their phylogenetic positions are still uncertain. Further data, including other DNA regions and additional morphological and biochemical data, may be able to elucidate their phylogenetic relationships.

Morphological and chemical data were interpreted in the light of the molecular phylogeny. Most of the traditional taxonomical characters appeared to be informative. However, traits such as absence of corolla and/or calyx, which are correlated with the evolution of wind pollination, constitute parallel losses in some groups and do not reflect a common phylogenetic history. Unisexual and bisexual flowers occur in different combinations in most species and the evolution of breeding systems shows a trend from hermaphroditism via androdioecy or polygamy to dioecy. The interpretation of the floral evolution, in relation to transitions between pollination systems, is discussed in more detail elsewhere (Wallander 2001).

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### Appendix

See Table 4

**Table 4** Voucher information and GenBank accession numbers for 90 specimens of *Fraxinus* and five outgroup taxa used for ITS sequencing (the *F. americana* sequence marked asterisk was not included in the Bayesian analysis)

Taxon	Voucher	Origin	GenBank accession number (ITS)	GenBank accession number (trnL-F + rps16)
<b>Ingroup</b>				
<i>F. americana</i>			U82906 + U82907	
<i>F. americana</i> *	Wallander 99 (GB)	USA (Missouri, Shaw Arb.)	EU314811	
<i>F. americana</i>	Wallander 101 (GB)	USA (Missouri, Shaw Arb.)	EU314812	AF231825 + AF225233
<i>F. americana</i> ( <i>F. biltmoreana</i> )			U82910 + U82911	
<i>F. angustifolia</i> ssp. <i>angustifolia</i>	Wallander 135 (GB)	Tenerife (cult. Puerto de la Cruz)	EU314813	
<i>F. angustifolia</i> ssp. <i>oxycarpa</i> 1	Paule 44a18 (ZV)	Turkey	EU314814	
<i>F. angustifolia</i> ssp. <i>oxycarpa</i> 2	Wallander 2 (GB)	Italy (cult. Göteborg Bot. Garden)	EU314815	
<i>F. angustifolia</i> ssp. <i>syriaca</i>	Samuelsson 1 (GB)	Israel (Acco)	EU314816	
<i>F. angustifolia</i> ( <i>F. syriaca</i> )			U82872 + U82873	
<i>F. angustifolia</i> ( <i>F. oxyphylla</i> )			U82868 + U82869	
<i>F. angustifolia</i> ( <i>F. pallisiae</i> )			U82870 + U82871	
<i>F. angustifolia</i> ( <i>F. pallisiae</i> ) 1	Paule 43a29 (ZV)	Turkey	EU314817	
<i>F. angustifolia</i> ( <i>F. pallisiae</i> ) 2	Rechinger 10066 (S)	Greece	EU314818	
<i>F. angustifolia</i> ( <i>F. potamophila</i> )	Wallander 88 (GB)	Uzbekistan (cult. Missouri Bot. Garden)	EU314819	
<i>F. angustifolia</i> ( <i>F. sogdiana</i> )	Elias 10008 (C)	Tajikistan	EU314820	
<i>F. anomala</i> (wrong ID)			U82914 + U82915	
<i>F. anomala</i>	Pinzl 10931 (NY)	USA (Nevada)	EU314821	
<i>F. anomala</i>	Rollins 1899 (GB)	USA (Colorado)	EU314822	AF231826 + AF225234
<i>F. apertisquamifera</i>	Kinoshita sn 1999-07-14 (GB)	Japan (Fukui)	EU314823	
<i>F. apertisquamifera</i>	Wallander 274 (GB)	Japan (Saitama)	EU314824	
<i>F. berlandieriana</i>	Jones 3595 (NY)	USA (Texas)	EU314825	
<i>F. berlandieriana</i>	Pringle 13584 (S)	Mexico (Hidalgo)	EU314826	
<i>F. bungeana</i>	King 168 (S)	China (Hupei)	EU314827	
<i>F. bungeana</i>	Tianwei & Zhaofen 228 (MO)	China	EU314828	
<i>F. bungeana</i>	Wallander 406 (GB)	China (Beijing)	EU314829	
<i>F. caroliniana</i> 1	Hill 11048 (NY)	USA (Florida)	EU314830	
<i>F. caroliniana</i> 2	Massey & Boufford 4500 (MO)	USA (North Carolina)	EU314831	
<i>F. caroliniana</i> ( <i>F. cubensis</i> )	Rova 2261 (GB)	Cuba	EU314832	
<i>F. chiisanensis</i>	Min 264 + Min 304 (SNUA) (identical ITS sequences)	South Korea	EU314833	EU284157 + EU284157
<i>F. chinensis</i>			U82884 + U82885	
<i>F. chinensis</i>	Wallander 116 (GB)	China (cult. Göteborg Bot. Garden)	EU314834	AF231827 + AF225235
<i>F. chinensis</i> ( <i>F. japonica</i> )	Wallander 235 (GB)	Japan (cult. Kyoto)	EU314835	
<i>F. chinensis</i> ( <i>F. japonica</i> )	Wallander 245 (GB)	Japan (cult. Kyoto)	EU314836	
<i>F. chinensis</i> ( <i>F. rhynchophylla</i> )	Wallander 400 (GB)	China (Heilongjiang)	EU314837	
<i>F. cuspidata</i>			U82916 + U82917	

**Table 4** continued

Taxon	Voucher	Origin	GenBank accession number (ITS)	GenBank accession number (trnL-F + rps16)
<i>F. cuspidata</i>	Barneby 18368 (NY)	USA (Arizona)	EU314838	
<i>F. cuspidata</i>	Reichenbacher 1716 (MO)	USA (Arizona)	EU314839	AF231828 + AF225236
<i>F. dipetala</i>	Walker 1287 (NY)	USA (California)	EU314840	
<i>F. dipetala</i>	Wallander 180 (GB)	USA (California)	EU314841	AF231829 + AF225237
<i>F. dipetala</i> ( <i>F. jonesii</i> )	Thorne 58757 (NY)	Mexico (Baja California)	EU314842	
<i>F. dubia</i>	Breedlove 32784 (MO)	Mexico (Chiapas)	EU314843	
<i>F. dubia</i>	García 1456 (MO)	Mexico (Chiapas)	EU314844	
<i>F. dubia?</i>	Martínez & Soto 3718 (MO)	Mexico (Guerrero)	EU314845	
<i>F. dubia?</i> ( <i>F. schiedeana</i> )	Villanueva 274 (NY)	Mexico (Veracruz)	EU314846	
<i>F. excelsior</i>	Wallander 159 (GB)	Sweden (Göteborg)	EU314847	AF231830
<i>F. excelsior</i> L. var. <i>diversifolia</i> Ait.	Wallander 1 (GB)	Sweden (cult. Göteborg Bot. Garden)	EU314848	+ AF225238
<i>F. excelsior</i> ssp. <i>coriariifolia</i>	Wallander 353 (GB)	Romania (Tulcea)	EU314849	
<i>F. floribunda</i>	Wallander 240 (GB)	Japan (cult. Kyoto)	EU314850	
<i>F. floribunda</i> ( <i>F. retusa</i> )	Wallander 249 (GB)	Japan (Okinawa)	EU314851	
<i>F. gooddingii</i>	McGill & Lehto 20365 (NY)	USA (Arizona)	EU314852	
<i>F. greggii</i> 1	Annable 2379 (NY)	USA (Arizona)	EU314853	
<i>F. greggii</i> 2	Johnston 7214 (S)	Mexico (Coahuila)	EU314854	
<i>F. greggii?</i> 3	Diaz 406 (MO)	Mexico (Tamaulipas)	EU314855	AF231831 + AF225239
<i>F. hubeiensis</i>	Xu Youming s.n. 2001–07 (WH)	China (Hubei)	EU314856	
<i>F. lanuginosa</i>	Seino 2 (GB)	Japan (Hokkaido)	EU314857	
<i>F. lanuginosa</i>	Wallander 266 (GB)	Japan (Saitama)	EU314858	
<i>F. latifolia</i>			U82912 + U82913	
<i>F. latifolia</i>	Wallander 182 (GB)	USA (California)	EU314859	
<i>F. latifolia</i>	Wallander 322 (GB)	USA (Washington)	EU314860	
<i>F. longicuspis</i>			U82888 + U82889	
<i>F. longicuspis</i>	Im 10518 (NY)	Japan (Shiga)	EU314861	
<i>F. longicuspis</i>	Wallander 256 (GB)	Japan (Saitama)	EU314862	
<i>F. mandshurica</i>			U82874 + U82875	
<i>F. mandshurica</i>	Seino 1 (GB)	Japan (Hokkaido)	EU314863	
<i>F. mandshurica</i>	Wallander 396 (GB)	China (Heilongjiang)	EU314864	
<i>F. micrantha</i>	Bist 96 (S)	India	EU314865	
<i>F. micrantha</i>	Polunin et al. 4299 (UPS)	Nepal	EU314866	
<i>F. nigra</i>			U82878 + U82879	
<i>F. nigra</i>	Rickson 239 (GB)	USA (cult. Miami University Campus)	EU314867	
<i>F. nigra</i>	Wallander 105 (GB)	USA (cult. Missouri Bot. Garden)	EU314868	EU284158 + EU284163
<i>F. ormus</i>	Wallander 38 (GB)	Italy (Sicily)	EU314869	AF231832 + AF225240

**Table 4** continued

Taxon	Voucher	Origin	GenBank accession number (ITS)	GenBank accession number (trnL-F + rps16)
<i>F. ornus</i>	Wallander 216 (GB)	Cult. NY Bot. Garden	EU314870	
<i>F. papillosa</i>	Felger 94–288 (MO)	Mexico (Chihuahua)	EU314871	
<i>F. papillosa</i>	Tucker 2597 (S)	Mexico (Chihuahua)	EU314872	
<i>F. paxiana</i>	Wallander 187 (GB)	China (cult. Göteborg Bot. Garden)	EU314873	
<i>F. paxiana (F. sikkimensis)</i>	Wallander 188 (GB)	China (cult. Göteborg Bot. Garden)	EU314874	
<i>F. pennsylvanica</i>			U82902 + U82903	
<i>F. pennsylvanica</i> 1	Wallander 83 (GB)	USA (cult. Missouri Bot. Garden)	EU314875	
<i>F. pennsylvanica</i> 2	Wallander 103 (GB)	USA (Missouri, Shaw Arb.)	EU314876	EU284159 + EU284164
<i>F. platypoda</i>			U82876 + U82877	
<i>F. platypoda</i>	Wallander 114 (GB)	China (cult. Göteborg Bot. Garden)	EU314877	
<i>F. profunda (F. tomentosa)</i>			U82896 + U82897	
<i>F. purpusii</i> 1	Breedlove & Thorne 30445 (NY)	Mexico (Chiapas)	EU314878	
<i>F. purpusii</i> 2	Breedlove 42154 (MO)	Mexico (Chiapas)	EU314879	
<i>F. purpusii</i> 3	Medrano et al. 11420 (MO)	Mexico (Oaxaca)	EU314880	
<i>F. quadrangulata</i>			U82880 + U82881	
<i>F. quadrangulata</i>	Wallander 94 (GB)	USA (Missouri, Shaw Arb.)	EU314881	
<i>F. quadrangulata</i>	Wallander 98 (GB)	USA (Missouri, Shaw Arb.)	EU314882	AF231833 + AF225241
<i>F. raibocarpa</i>	Regel s.n. July 1982 (S)	Tajikistan (Hissar)	EU314883	EU284160 + EU284165
<i>F. raibocarpa</i>	Sabirov s.n. 1955-08-17 (MO)	Uzbekistan	EU314884	
<i>F. rufescens</i>	Zamudio 3673 (MO)	Mexico (Queretaro)	EU314885	
<i>F. sieboldiana</i>	Takahashi et al. 1708 (MO)	Japan (Honshu)	EU314886	
<i>F. sieboldiana</i>	Wallander 265 (GB)	Japan (Saitama)	EU314887	
<i>F. spaethiana</i>	Wallander 142 (GB)	Japan (cult. Göteborg Bot. Garden)	EU314888	
<i>F. spaethiana</i>	Wallander 259 (GB)	Japan (Saitama)	EU314889	EU284161 + EU284166
<i>F. spaethiana</i>	Wallander 320 (GB)	Japan (cult. Göteborg Bot. Garden)	EU314890	
<i>F. texensis</i> 1	Chase 3887 (K)	USA (cult. Kew Garden)	EU314891	
<i>F. texensis</i> 2	Walker 1692 (NY)	USA (Texas)	EU314892	
<i>F. trifoliolata</i>	Forrest 15313 (E)	China (Yunnan)	EU314893	
<i>F. uhdei</i>	Boa s.n. 2001–08 (GB)	Colombia (cult.)	EU314894	

**Table 4** continued

Taxon	Voucher	Origin	GenBank accession number (ITS)	GenBank accession number (trnL-F + rps16)
<i>F. uhdei</i>	Wallander 192 (GB)	USA California (cult. Fairchild Tropical Garden)	EU314895	
<i>F. uhdei</i>	Wallander 389 (GB)	Mexico (Chiapas)	EU314896	
<i>F. velutina</i>	Broome et al. 1829 (NY)	USA (Arizona)	EU314897	
<i>F. velutina</i>	Chase 3886 (K)	USA (cult. Kew Garden)	EU314898	
<i>F. velutina</i>			U82904 + U82905	
<i>F. xanthoxyloides</i>	Dubuis 12424 (C)	Algeria	EU314899	
<i>F. xanthoxyloides</i>	Wallander 141 (GB)	Morocco (cult. Göteborg Bot. Garden)	EU314900	AF231834 + AF225242
Outgroup				
<i>Ligustrum vulgare</i> L.	Wallander 168 (GB)	Sweden (cult. Göteborg Bot. Garden)	EU314901	AF231848 + AF225257
<i>Syringa vulgaris</i> L.	Wallander 111 (GB)	Sweden (cult. Göteborg Bot. Garden)	EU314902	AF231882 + AF225292
<i>Forestiera acuminata</i> Poir.	Wallander 100 (GB)	USA (Missouri)	EU314903	AF231819 + AF225227
<i>Osmanthus fragrans</i> Lour.	Wallander 28 (GB)	China (cult. Palermo Bot. Garden, Sicily)	EU314904	AF231869 + AF225278
<i>Phillyrea latifolia</i> L.	Chase 3880 (K)	Cult. Kew Garden, UK	EU314905	AF231874 + AF225283

In addition, 17 *Fraxinus* ITS1 + ITS2 sequences from the study by Jeandroz et al. (1997) are included. These GenBank accession numbers begin with the letter U and no vouchers are given. Authors of taxa are only given if not found in Table 2. Species regarded as synonyms in this study are listed within parentheses after their accepted name. In cases where multiple sequences representing the same species do not group together (Fig. 1), numbers after the names identify them. GenBank accession numbers are also given for the 15 chloroplast DNA sequences (*rps16* and *trnL-F* separately), of which five are new for this study and ten from Wallander and Albert (2000)

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