

## PHYLOGENY AND CLASSIFICATION OF OLEACEAE BASED ON *RPS16* AND *TRNL-F* SEQUENCE DATA<sup>1</sup>

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Phylogenetic relationships among 76 species of Oleaceae, representing all 25 recognized genera of the family, were assessed by a cladistic analysis of DNA sequences from two noncoding chloroplast loci, the *rps16* intron and the *trnL-F* region. Consensus trees from separate and combined analyses are congruent and agree well with nonmolecular data (chromosome numbers, fruit and wood anatomy, leaf glycosides, and iridoids). The two debated genera *Dimetra* and *Nyctanthes*, previously suggested to belong to Verbenaceae (sensu lato) or Nyctanthaceae, are shown to belong to Oleaceae, sister to the hitherto genus incertae sedis *Myxopyrum*. This clade is also supported by anatomical and chemical data. The subfamily Jasminoideae is paraphyletic, and a new classification is presented. The subfamily level is abandoned, and the former Jasminoideae is split into four tribes: Myxopyreae (*Myxopyrum*, *Nyctanthes*, and *Dimetra*), Fontanesieae (*Fontanesia*), Forsythieae (*Abeliophyllum* and *Forsythia*), and Jasmineae (*Jasminum* and *Menodora*). The tribe Oleaceae (previous subfamily Oleoideae) is clearly monophyletic, comprising the subtribes Ligustrinae (*Syringa* and *Ligustrum*), Schreberinae status novus (*Schrebera* and *Comoranthus*), Fraxininae status novus (*Fraxinus*), and Oleinae (12 drupaceous genera). An *rps16* sequence obtained from *Hesperelaea*, known only from the type specimen collected in 1875, confirmed the placement of this extinct taxon in the subtribe Oleinae.

**Key words:** cpDNA; *Dimetra*; *Myxopyrum*; *Nyctanthes*; phylogeny; Oleaceae; *rps16*; *trnL-F*.

The Oleaceae is a medium-sized family of ~600 species in 25 genera (Table 1). The family is distributed on all continents except the Antarctic, from northern temperate to southern subtropical regions and from low to high elevations. Some genera are widespread and occur on more than one continent, e.g., *Chionanthus*, *Menodora*, and *Fraxinus* (authors of names are given only if not listed in the Appendix or Table 3, and only the first time they are mentioned). The genus *Jasminum* is the largest with over 200 species. Many of the genera are economically important, e.g., the olive (*Olea europaea*) is cultivated for its fruit and oil, species of *Fraxinus* are grown for timber, and *Jasminum*, *Forsythia*, *Syringa*, and *Ligustrum* are planted as ornamentals.

The family is considered monophyletic on the basis of several morphological synapomorphies and is easily circumscribed. The members of the family are trees, shrubs, or woody climbers with opposite, simple, or compound leaves without stipules. The flowers are hypogynous and four-merous, generally with two stamens, but with four stamens in some species. The corolla is actinomorphic and usually sympetalous. Free petals occur in *Chionanthus* and *Fraxinus*, and apetalous

flowers are known in *Nestegis*, *Forestiera*, and wind-pollinated species of *Fraxinus*. The ovary is syncarpous, consisting of two carpels. Fruit types range from loculicidal capsules, woody schizocarps, and samaras to berries and drupes.

The Oleaceae have by recent molecular studies been placed in Lamiales, sister group to the rest of the order (Wagstaff and Olmstead, 1997), and APG (1998) classified it in this order. The family has also been treated in an order of its own, Oleales, by, e.g., Takhtajan (1997). Most classifications of Oleaceae divide the family into two subfamilies, Jasminoideae and Oleoideae (Table 2). Knoblauch (1895) based his division on the point of attachment of the ovules and the presence of a constriction through the apex of the fruit. Taylor (1945) rearranged some genera on the basis of chromosomal data and fruit morphology. The most recent review of the entire family is that of Johnson (1957). His division of Oleaceae into subfamilies and tribes follows Taylor (1945), with a few exceptions. The members of the subfamily Oleoideae apparently form a monophyletic group. They all have  $x = 23$  and are thought to be of an allopolyploid origin (Taylor, 1945). In addition, they share a number of anatomical, morphological, and chemical apomorphies. In contrast, the Jasminoideae are a heterogeneous assemblage of those genera that do not fit in the Oleoideae. Except for the family-wide characters, the tribes of Jasminoideae share no apomorphies, but they are well distinguished from the Oleoideae. Therefore, most authors have placed them in a separate subfamily.

The phylogenetic position of the genus *Nyctanthes* and its close relative *Dimetra* has been much debated. *Nyctanthes* was placed in Oleaceae by Bentham (1876) and *Dimetra* next to *Nyctanthes* by Kerr (1938). Later, both genera were suggested to belong to the Verbenaceae (Airy Shaw, 1952; Stant, 1952), or in a family of their own, Nyctanthaceae (Kundu and De, 1968). The exclusion from the Oleaceae was based mainly on the plants' "Verbenaceous appearance" (Airy Shaw, 1952). Since then, the morphology of these genera has been investigated and compared to Oleaceae and Verbenaceae in a number

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TABLE 1. The 25 genera recognized for this study, the number of representatives sequenced, the approximate number of species in the genus, and their world distribution. \* denotes two individuals of the only species in that genus.

Genus	No. representatives sequenced	No. of species	Distribution
<i>Abeliophyllum</i>	2*	1	Korea
<i>Chionanthus</i>	4	ca 100	Tropical and subtropical Africa, America, Asia, and Australia
<i>Comoranthus</i>	2	3	Madagascar and the Comores
<i>Dimetra</i>	2*	1	Thailand
<i>Fontanesia</i>	2	1–2	SW Asia (and Sicily) and China
<i>Forestiera</i>	4	ca 15	Subtropical North America, West Indies, and N South America
<i>Forsythia</i>	2	11	E Asia and SE Europe (one sp.)
<i>Fraxinus</i>	10	40–50	Mainly temperate and subtropical regions of the Northern Hemisphere
<i>Haenianthus</i>	2	3	West Indies
<i>Hesperelaea</i>	1	1 (extinct)	Mexico (was endemic to Guadalupe Island)
<i>Jasminum</i>	8	200+	Tropical and subtropical parts of the Old World
<i>Ligustrum</i>	4	45	Temperate to tropical parts of the Old World, except Africa
<i>Menodora</i>	2	24	Subtropical North and South America and S Africa
<i>Myxopyrum</i>	3	4	Tropical SE Asia
<i>Nestegis</i>	4	5	New Zealand and Hawaii (one sp.)
<i>Noronhia</i>	1	41	Madagascar
<i>Notelaea</i>	3	12	Australia and Tasmania
<i>Nyctanthes</i>	2	2	Tropical and subtropical SE Asia
<i>Olea</i>	4	40+	Tropical and subtropical parts of the Old World
<i>Osmanthus</i>	5	30	Subtropical parts of E Asia and North America (1–2 spp.)
<i>Phillyrea</i>	3	2	Mediterranean region to W Asia
<i>Picconia</i>	2*	1(–2)	Macaronesia
<i>Priogymnanthus</i>	2	2	South America (Bolivia, Brazil, Paraguay, Ecuador)
<i>Schrebera</i>	2	4	Tropical parts of Africa and India
<i>Syringa</i>	4	20	Mainly subtropical parts of Eurasia
Sum	80	600+	

of studies (cf. Kiew and Baas, 1984), all reaching the conclusion that they should belong to Oleaceae. In an attempt to test this hypothesis with molecular data, the two genera were included in this study.

The position of the distinct genus *Myxopyrum* within Oleaceae has also been uncertain. It was first assigned to the Oleineae by Bentham (1876), kept in the Oleoideae-Oleineae by Knoblauch (1895), then Taylor (1945) thought that it was “not Oleineae,” and Johnson (1957) put it in a tribe of its own in the Jasminoideae. Later, arguments for placement in the subfamily Oleoideae have come from Kiew (1983, 1984), but Baas et al. (1988) and Rohwer (1996) have doubted this. One of the aims of the present study has been to let molecular data shed new light on possible relationships for this genus incertae sedis.

Despite containing such well-known and economically important genera, no recent classification of the entire family based on an explicit phylogeny has been published. The first author to present a “phylogeny” for the Oleaceae was Taylor (1945), who drew a phylogenetic chart based on cytological data. Later, Johnson (1957) made an important contribution to the systematics of the family by reviewing its taxonomy and classification. So far, only two studies have employed cladistic methods in evaluating phylogenetic hypotheses for Oleaceae. Baas et al. (1988) studied wood anatomy of the whole family and based cladistic and phenetic analyses on wood anatomical characters. Rohwer (1996) based his cladistic analyses mainly on fruit and seed characters. Our molecular phylogeny is the first to be documented [see also Kim and Jansen (1993) and Kim (1999)] and contributes new insights towards a revised classification of the family.

## MATERIALS AND METHODS

**Material**—Table 1 lists the 25 genera we recognize and used in this study (based on previous classifications; mainly Johnson, 1957), the approximate number of species in each genus, the number of species sequenced, and the world distribution of the genera. At least two representatives from each genus in the family were sequenced, including *Nyctanthes* and *Dimetra*. Where possible we tried to use material from the type-bearing species of the genus. In the monotypic genera *Abeliophyllum*, *Dimetra*, and *Picconia* two different individuals of each species were sequenced. The genus *Hesperelaea* is also monotypic, but because it is extinct and known solely from the type collection, only a sample from this could be used. About a third of the material studied was silica-gel dried plant material, and a few fresh samples from Göteborg Botanical Garden and the New York Botanical Garden, collected by Eva Wallander. Silica-gel dried material of three Australian and New Zealand taxa were received from Wayne K. Harris (BRI), a sample of *Nestegis sandwicensis* from Timothy J. Motley (NY), and a recent collection of *Dimetra craibiana* from S. Suddee (by courtesy of the Bangkok Forestry Department, Thailand). Another third of the DNA was isolated from herbarium specimens held at BM, C, GB, MO, and NY. DNA extracts from plants cultivated at the Royal Botanic Gardens at Kew and herbarium specimens held at K were received from Mark W. Chase. Vouchers for all sequenced taxa are listed in Table 3 along with their GenBank accession numbers. As outgroup taxa we chose species of Verbenaceae and Myoporaceae (Lamiales) to test the position of *Dimetra* and *Nyctanthes*, and members of Rubiaceae, Loganiaceae, Strychnaceae, and Gelsemiaceae (Gentianales) were included to provide a root hypothesis for Oleaceae. Except for two Verbenaceae sequences, outgroup sequences were received from various authors, which are also listed in Table 3.

**DNA extraction**—Fresh leaf tissue was manually ground with a pestle in an Eppendorf tube immersed in liquid nitrogen, and dried tissue was homogenized using the FastPrep® instrument (BIO 101, Vista, California, USA). Total DNA was extracted using a lysis buffer consisting of 2% CTAB (cetyltrimethylammonium bromide), 1% PEG 6000 (polyethylene glycol, molec-

TABLE 2. Previous classifications of recognized genera of Oleaceae into subfamilies and tribes by different authors. The genera are arranged in order based on our new classification into tribes and subtribes.

Genus	Bentham (1876)	Knoblauch (1895)	Taylor (1945)	Johnson (1957)	This study
<i>Fontanesia</i>	Fraxineae	Oleoideae-Fraxineae	Jasminoideae-Fontanesaeae	Jasminoideae-Fontanesieae	Fontanesieae
<i>Abelophyllum</i>			Jasminoideae-Fontanesaeae	Jasminoideae-Forsythieae	Forsythieae
<i>Forsythia</i>	Syringaeae	Oleoideae-Syringaeae	Jasminoideae-Forsythieae	Jasminoideae-Forsythieae	Forsythieae
<i>Jasminum</i>	Jasmineae	Jasminoideae-Jasmineae	Jasminoideae-Jasmineae	Jasminoideae-Jasmineae	Jasmineae
<i>Menodora</i>	Jasmineae	Jasminoideae-Jasmineae	Jasminoideae-Jasmineae	Jasminoideae-Jasmineae	Jasmineae
<i>Myxopyrum</i>	Oleineae	Oleoideae-Oleineae	incertae sedis ("not Oleineae") <sup>d</sup>	Jasminoideae-Myxopyreae	Myxopyreae
<i>Nyctanthes</i>	Jasmineae	Jasminoideae-Jasmineae	Jasminoideae-incertae sedis <sup>d</sup>	Verbenaceae	Myxopyreae
<i>Dimetia</i>				Verbenaceae	Myxopyreae
<i>Ligustrum</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae	Oleoideae-Oleeeae	Oleoideae-Ligustrinae
<i>Syringa</i>	Syringaeae	Oleoideae-Syringaeae	Oleoideae-Syringaeae	Oleoideae-Oleeeae	Oleoideae-Ligustrinae
<i>Comoranthus</i>			Oleoideae-Oleineae <sup>d</sup>	Jasminoideae-Schreberaeae	Oleoideae-Schreberinae
<i>Schrebera</i>	Syringaeae	Oleoideae-Syringaeae <sup>b</sup>	Oleoideae-Syringaeae <sup>d</sup>	Jasminoideae-Schreberaeae	Oleoideae-Schreberinae
<i>Fraxinus</i>	Fraxineae	Oleoideae-Fraxineae	Oleoideae-Fraxineae	Jasminoideae-Schreberaeae	Oleoideae-Schreberinae
<i>Chionanthus</i>	Oleineae <sup>a</sup>	Oleoideae-Oleineae <sup>c</sup>	Oleoideae-Oleineae	Oleoideae-Fraxineae	Oleoideae-Fraxininae
<i>Forestiera</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Haemianthus</i>			Oleoideae-Oleineae <sup>d</sup>	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Hesperelaea</i>			Oleoideae-Oleineae	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Nestegis</i>				Oleoideae-Oleeeae <sup>f</sup>	Oleoideae-Oleineae
<i>Noronhia</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae <sup>d</sup>	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Notelaea</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae <sup>d</sup>	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Olea</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae	Oleoideae-Oleeeae <sup>g</sup>	Oleoideae-Oleineae
<i>Osmanthus</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae	Oleoideae-Oleeeae <sup>h</sup>	Oleoideae-Oleineae
<i>Phillyrea</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Picconia</i>	Oleineae	Oleoideae-Oleineae	Oleoideae-Oleineae	Oleoideae-Oleeeae	Oleoideae-Oleineae
<i>Prigymnanthus</i>				Oleoideae-Oleeeae	Oleoideae-Oleineae

<sup>a</sup> Including *Linociera*.

<sup>b</sup> as *Nathusia*.

<sup>c</sup> incl. *Mayepea* and *Tessarandra*.

<sup>d</sup> genera not in Taylor's classification based on cytological studies, but relationships were discussed in the text.

<sup>e</sup> incl. *Linociera* and *Tessarandra*.

<sup>f</sup> as *Gymnelaea*.

<sup>g</sup> incl. *Tetrapilus*.

<sup>h</sup> incl. *Amarolea* and *Siphonosmanthus*.

TABLE 3. Vouchers, or references, and GenBank accession numbers for taxa sequenced. \* indicate type species of a genus. Herbaria acronyms follow Index Herbariorum (Holmgren, Holmgren, and Barrett, 1990).

Taxon	Source of DNA/voucher	GenBank accession number*
	<i>trnL-F</i>	<i>rps16</i>
<b>Ingroup</b>		
<i>Abelophyllum distichum</i> Nakai*	M. W. Chase 3881 (K)	GBAN-AF225216
<i>Chionanthus filiformis</i> (Vell.) P. S. Green	P. I. Oliveira 659 (GB)	GBAN-AF231809
<i>Chionanthus ramiflorus</i> Roxb.	Collected in Australia, without voucher	GBAN-AF225217
<i>Chionanthus retusus</i> Lindley & Paxton	E. Wallander 82 (GB)	GBAN-AF225218
<i>Chionanthus virginicus</i> L.*	E. Wallander 81 (GB)	GBAN-AF225219
<i>Comoranthus madagascariensis</i> H. Perrier	Capuron 20913 (K)	GBAN-AF225220
<i>Comoranthus minor</i> H. Perrier	L. J. Dorr 4135 (K)	GBAN-AF225221
<i>Dimetra crabiiana</i> Kerr*	A. F. G. Kerr 20476 (BM)	GBAN-AF225222
<i>Dimetra crabiiana</i> Kerr*	S. Suddee et al. 1000 (BKF, K, TCD)	GBAN-AF225223
<i>Fontanesia phyllyroides</i> Labill. ssp. <i>fortunei</i> (Carr.) Yait	M. W. Chase 3878 (K)	GBAN-AF225224
<i>Fontanesia phyllyroides</i> Labill.*	E. Wallander 20 (GB)	GBAN-AF225225
<i>Forestiera acuminata</i> (Michx.) Poir.	E. Wallander 100 (GB)	GBAN-AF225226
<i>Forestiera eggersiana</i> Krug & Urban	E. Wallander 20 (GB)	GBAN-AF225227
<i>Forestiera neo-mexicana</i> A. Gray	W. G. D'Arcy 5135A (C)	GBAN-AF225228
<i>Forestiera segregata</i> (Jacq.) Krug & Urban var. <i>pinetorum</i> (Small) E. Murray	A. Carter 1045 (GB)	GBAN-AF225229
<i>Forsythia x intermedia</i> Zabel	E. Wallander 191 (GB)	GBAN-AF225230
<i>Fraxinus americana</i> L.	J. Jutila 556 (NY)	GBAN-AF225231
<i>Fraxinus anomala</i> Torr.	Å. Dahl 702 (GB)	GBAN-AF225232
<i>Fraxinus chinensis</i> Roxb. var. <i>rhyncophylla</i> (Hance) E. Murray	E. Wallander 101 (GB)	GBAN-AF225233
<i>Fraxinus cuspidata</i> Torr. var. <i>macroptala</i> (Eastw.) Rehd.	R. C. Rollins 1899 (GB)	GBAN-AF225234
<i>Fraxinus dipetala</i> Hook. & Arn.	E. Wallander 116 (GB)	GBAN-AF225235
<i>Fraxinus excelsior</i> L.*	F. W. Reichenbacher 1716 (MO)	GBAN-AF225236
<i>Fraxinus excelsior</i> L. var. <i>diversifolia</i>	E. Wallander 180 (GB)	GBAN-AF225237
<i>Fraxinus greggii</i> A. Gray	E. Wallander 159 (GB)	GBAN-AF225238
<i>Fraxinus ornus</i> L.	E. Wallander 1 (GB)	GBAN-AF225239
<i>Fraxinus quadrangulata</i> Michx.	Rafael Diaz 406 (MO)	GBAN-AF225240
<i>Fraxinus xanthoxyloides</i> (G. Don) DC. var. <i>dimorpha</i> (Coss. & Dur.) Lingelsh.	E. Wallander 31 (GB)	GBAN-AF225241
	E. Wallander 98 (GB)	GBAN-AF225242
	E. Wallander 141 (GB)	GBAN-AF225243
<i>Haenianthus incrassatus</i> Griseb.*	R. F. Thome & G. R. Proctor 48278 (NY)	GBAN-AF225244
<i>Haenianthus salicifolius</i> Griseb. var. <i>obovatus</i> (Krug & Urban) Knob.	B. Ståhl & J. Knudsen 2302 (GB)	GBAN-AF225245
<i>Hesperaloe palmieri</i> A. Gray	Palmier 81 (K)	GBAN-AF225246
<i>Jasminum fluminense</i> Vell.	L. Struwe 1098 (NY)	GBAN-AF225247
<i>Jasminum humile</i> L.	K. Å. Dahlstrand 2073 (GB)	GBAN-AF225248
<i>Jasminum mesnyi</i> Hance	K. Å. Dahlstrand 37 (GB)	GBAN-AF225249
<i>Jasminum nitidum</i> Skan	E. Wallander 195 (GB)	GBAN-AF225250
<i>Jasminum nudiflorum</i> Lindl.	E. Wallander 193 (GB)	GBAN-AF225251
<i>Jasminum odoratissimum</i> L.	E. Wallander 130 (GB)	GBAN-AF225252
<i>Jasminum officinale</i> L.*	E. Wallander 194 (GB)	GBAN-AF225253
<i>Jasminum sinense</i> Hemsl.	Sino-American Guizhou Bot. Exp. no. 228 (NY)	GBAN-AF225254
<i>Ligustrum ovalifolium</i> Hassk.	E. Wallander 197 (GB)	GBAN-AF225255
<i>Ligustrum sempervirens</i> (Franch.) Lingelsh.	cult. Kew, voucher # 000.73.10104 (K)	GBAN-AF225256
<i>Ligustrum sinense</i> Lour.	E. Wallander 198 (GB)	GBAN-AF225257
<i>Ligustrum vulgare</i> L.*	E. Wallander 168 (GB)	GBAN-AF225258
<i>Menodora africana</i> Hook.	K. Å. Dahlstrand 1081 (GB)	GBAN-AF225259
<i>Menodora integrifolia</i> (Cham. & Schltdl.) Steud.	S. G. Tressens et al. 546 (GB)	GBAN-AF225260
<i>Myxopyrum nervosum</i> Blume*	M. J. E. Coode 6845 (K)	GBAN-AF225261
<i>Myxopyrum smilacifolium</i> Blume var. <i>confertum</i> (Kerr) Kiew	C. F. van Beusekom & T. Santisuk 2859 (C)	GBAN-AF225262
<i>Myxopyrum smilacifolium</i> Blume var. <i>confertum</i> (Kerr) Kiew	C. Wang 34636 (NY)	GBAN-AF225263
<i>Nesstegis apetala</i> (Vahl) L. Johnson*	M. W. Chase 3940 (K)	GBAN-AF225264
<i>Nesstegis cunninghamii</i> (Hook. f.) L. Johnson	M. W. Chase 3884 (K)	GBAN-AF225264

TABLE 3. Continued.

Taxon	Source of DNA/voucher	<i>rnl-F</i>	GenBank accession number <sup>a</sup> <i>rps16</i>
<i>Nestegis lanceolata</i> (Hook. f.) L. Johnson	M. Pole s.n. (BRI)	GBAN-AF231856	GBAN-AF225265
<i>Nestegis sandwicensis</i> (Gray) O. & I. Deg. & L. Johnson	T. Motley 2014 (NY)	GBAN-AF231857	GBAN-AF225266
<i>Noronhia emarginata</i> (Lam.) Thouars*	J. Pruski, B. Stein & S. Zona 2885 (NY)	GBAN-AF231858	GBAN-AF225267
<i>Notelaea longifolia</i> Vent.*	K. Egeröd 9264 (GB)	GBAN-AF231859	GBAN-AF225268
<i>Notelaea microcarpa</i> R. Brown	W. K. Harris 91 (BRI)	GBAN-AF231860	GBAN-AF225269
<i>Notelaea punctata</i> R. Brown	W. K. Harris 89 (BRI)	GBAN-AF231861	GBAN-AF225270
<i>Nyctanthes aculeata</i> Craib	A. F. G. Kerr 13501 (K)	GBAN-AF231862	GBAN-AF225271
<i>Nyctanthes arbor-tristis</i> L.*	K. A. Dahlstrand s.n., 2 Oct. 1952 (GB)	GBAN-AF231863	GBAN-AF225272
<i>Olea brachiata</i> (Lour.) Merrill	H. Y. Liang 66470 (GB)	GBAN-AF231864	GBAN-AF225273
<i>Olea capensis</i> L.	E. Wallander 26 (GB)	GBAN-AF231865	GBAN-AF225274
<i>Olea europaea</i> L.*	Å. Dahl 703 (GB)	GBAN-AF231866	GBAN-AF225275
<i>Olea paniculata</i> R. Brown	M. W. Chase 3882 (K)	GBAN-AF231867	GBAN-AF225276
<i>Osmanthus americanus</i> (L.) A. Gray	S. A. Spongberg et al. 17159 (NY)	GBAN-AF231868	GBAN-AF225277
<i>Osmanthus fragrans</i> Lour.*	E. Wallander 28 (GB)	GBAN-AF231869	GBAN-AF225278
<i>Osmanthus heterophyllus</i> (G. Don) P. S. Green	E. Wallander 223 (GB)	GBAN-AF231870	GBAN-AF225279
<i>Osmanthus rigidus</i> Nakai	E. Wallander 231 (GB)	GBAN-AF231871	GBAN-AF225280
<i>Phillyrea angustifolia</i> L.	E. Wallander 230 (GB)	GBAN-AF231872	GBAN-AF225281
<i>Phillyrea latifolia</i> L.*	J. Lewalle 10113 (C)	GBAN-AF231873	GBAN-AF225282
<i>Phillyrea media</i> L.	M. W. Chase 3880 (K)	GBAN-AF231874	GBAN-AF225283
<i>Picconia excelsa</i> (Aiton) DC.*	H. C. Stutz 3140 (NY)	GBAN-AF231875	GBAN-AF225284
<i>Priogymnanthus apertus</i> (B. Stühl) P. S. Green	E. Wallander 132 (GB)	GBAN-AF231876	GBAN-AF225285
<i>Priogymnanthus hasslerianus</i> (Chodat) P. S. Green*	X. Cornejo & C. Bonifaz 5303-B (GB)		GBAN-AF225286
<i>Schrebera alata</i> (Hochst.) Welw.	E. Hasler 11889 (C)	GBAN-AF231877	GBAN-AF225287
<i>Schrebera mazoensis</i> S. Moore	M. W. Chase 3883 (K)	GBAN-AF231878	GBAN-AF225288
<i>Syringa pekinensis</i> Rupr.	D. C. Plowes 1303 (NY)	GBAN-AF231879	GBAN-AF225289
<i>Syringa reticulata</i> (Blume) H. Hara	Lancaster 1623 (K)	GBAN-AF231880	GBAN-AF225290
<i>Syringa vulgaris</i> L.*	E. Wallander 228 (GB)	GBAN-AF231881	GBAN-AF225291
<i>Syringa yunnanensis</i> Franchet	E. Wallander 111 (GB)	GBAN-AF231882	GBAN-AF225292
	E. Wallander 170 (GB)	GBAN-AF231883	GBAN-AF225293
<b>Outgroup</b>			
<i>Lantana camara</i> L. (Verbenaceae)	C. Vazquez Yanes 526 (GB)	GBAN-AF231884	GBAN-AF225294
<i>Stachytarpheta dichotoma</i> (Ruiz & Pav.) Vahl (Verbenaceae)	Olmstead and Reeves (1995)	GBAN-AJ299260	GBAN-AJ299259
<i>Verbena officinalis</i> L. (Verbenaceae)	H. Kalheber 78–506 (GB)	GBAN-AF231885	GBAN-AF225295
<i>Myoporum insulare</i> R. Br. (Myoporaceae)	B. Oxelman (unpublished)		GBAN-AJ299258
<i>Myoporum mauritianum</i> DC.	Olmstead and Reeves (1995)	GBAN-AJ299257	
<i>Gelsemium sempervirens</i> (L.) Aiton (Gelsemiaceae)	Rova et al. (unpubl.); Andersson and Rova (1999)	GBAN-AFI02428 +	GBAN-AF004092
<i>Antonia ovata</i> Pohl. (Loganiaceae)	Rova et al. (unpubl.); Andersson and Rova (1999)	GBAN-AFI159696	GBAN-AF004091
<i>Strychnos tomentosa</i> Benth. (Strychnaceae)	Rova et al. (unpubl.)	GBAN-AFI02379	GBAN-AF004094
<i>Strychnos nux-vomica</i> L. (Strychnaceae)	Andersson and Rova (1999)	GBAN-AFI02484	
<i>Cinchona pitayensis</i> Wedd. (Rubiaceae)	Rova et al. (unpubl.)	GBAN-AFI152684	
<i>Cinchona pubescens</i> Vahl (Rubiaceae)	Andersson and Rova (1999)		GBAN-AF004035
<i>Coffea arabica</i> L. (Rubiaceae)	Rova et al. (unpubl.); Andersson and Rova (1999)	GBAN-AFI02405	GBAN-AF004038
<i>Luculia gratissima</i> Sweet x <i>tsetensis</i> (Rubiaceae)	Rova et al. (unpubl.)	GBAN-AFI02453	GBAN-AF242974

<sup>a</sup> The prefix GBAN- has been added to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.

TABLE 4. Primer sequences used for PCR and sequencing.

Name	Primer sequence (5'–3')	Position	Reference
tRNc	CGAAATCGGTAGACGCTACG	trnL fwd	Taberlet et al. (1991)
tRNd	GGGATAGAGGGACTTGAACC	trnL rev	Taberlet et al. (1991)
tRNe	GGTTCAAGTCCCTCTATCCC	trnL-F igs fwd	Taberlet et al. (1991)
tRNf	ATTTGAACTGGTGACACGAG	trnL-F igs rev	Taberlet et al. (1991)
rpsF	GTGGTAGAAAGCAACGTGCGACTT	rps 16 end fwd	Oxelman, Lidén, and Berglund (1997)
rpsMRP	GGATCCCAAAACAAGGAAACACC	rps 16 internal rev	Persson (2000)
rpsMF2	GGGTATGTTGCTGCCATTTTGAAA	rps 16 internal fwd	this study
rpsR2	TGCGGATCGAACATCAATTGCAAC	rps 16 end rev	Oxelman, Lidén, and Berglund (1997)

ular weight 6000), 1.4 mol/L NaCl, 10 mmol/L Tris-HCl, and 20 mmol/L EDTA. Lysis was performed at 74°C with 2% mercaptoethanol added, followed by cleaning with the GeneClean® II kit (BIO 101). Cleaned DNA was transferred to 10 mmol/L Tris and kept in freezer.

**cpDNA regions and primers**—For our study, we chose two noncoding chloroplast regions, the *trnL-F* region and the intron of *rps16*. The *trnL-F* region consists of the *trnL* intron and the *trnL-trnF* intergenic spacer (Taberlet et al., 1991). The primer pair tRNc/tRNf (Table 4) was used to amplify the entire region of ~900 bp in one PCR (polymerase chain reaction). In some cases, the tRNc/tRNd and the tRNe/tRNf primer pairs were used to amplify the intron and the spacer, respectively. The intron of *rps16* is a group II intron that was first used for phylogenetic studies by Oxelman, Lidén, and Berglund (1997). The primer pair rpsF/rpsR2 was used to amplify the entire 800–900 bp region. For DNA of low quality, internal primers were used with each of the end primers to split that region into two approximately equal halves. The position of the internal forward primer (rpsMF2) is located ~50 base pairs downstream of the internal reverse primer (rpsMRP), giving sufficient overlap for determining a full sequence.

**Amplification**—Most PCRs were performed in a 25- $\mu$ L reaction volume using the Taq kit from Boehringer Mannheim (now Roche Molecular Biochemicals, Indianapolis, Indiana, USA). These amplifying reactions were run on a Perkin Elmer GeneAmp® PCR System 9600 version 2.01, using the same program for both chloroplast regions (30 cycles of [95°C 50 sec, 60°C 50 sec, 72°C 1 min 50 sec]). A second round of PCR was sometimes performed using the first PCR product as template. In this case, the first PCR products were run out on a low-melting-point agarose gel, stained with ethidium bromide to visualize the bands, then cut out and dissolved in water. No further cleaning was done before the second PCR. The PCR products (from first or second PCR) were purified before sequencing using the GeneClean® II kit (BIO 101). Some PCR reactions were performed in 50- $\mu$ L volumes using polymerase and buffer of the Thermoprime + kit (Advanced Biotechnologies Ltd., Surrey, UK), or in 25- $\mu$ L reactions using Ready-To-Go® PCR beads (Amersham Pharmacia Biotech AB, Uppsala, Sweden), following the manufacturer's instructions. In these cases, both chloroplast regions were amplified on a Perkin Elmer Cetus 480 version 1.1, using the same cycling program (27 cycles of [94°C 1 min, 60°C 1 min, 72°C 1 min]). The PCR products were purified before sequencing using the QIAquick® PCR Purification Kit (QIAGEN® GmbH, Hilden, Germany).

**Sequencing**—Sequencing reactions, using the same primer sequences as in the PCR, were performed on a Perkin Elmer GeneAmp® PCR System 9600 version 2.01 (1 min at 95°C, followed by 32 cycles of [95°C 10 sec, 50°C 5 sec, 60°C 3 min]), using the dRhodamine Terminator Cycle Sequencing Ready Reaction DNA sequencing kit with AmpliTaq® DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, California, USA) and HT1000 *half-TERM* Dye Terminator Reagent (GENPAK Inc., Stony Brook, New York, USA). Before gel separation, the sequence reaction products were cleaned using Sephadex® G-50 Fine DNA Grade (Amersham Pharmacia Biotech AB) in Centrisep Spin Columns (Princeton Separations, Philadelphia, Pennsylvania, USA). Separation of the fragments was done on a 5% Long Ranger® gel (FMC BioProducts, Rockland, Maine, USA) on an ABI Prism® 377 DNA

Sequencer (Perkin Elmer Applied Biosystems). The ABI Prism® 377 Collection software version 2.1 was used to evaluate the sequences. Some sequencing reactions were also performed on an ALFexpress® DNA Sequencer (Amersham Pharmacia Biotech AB). Reactions were then performed using the ThermoSequenase fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech AB) and Cy5-labeled primers. Cycle sequencing reactions were performed on a Perkin Elmer Cetus 480 version 1.1 (2 min at 96°C, followed by 18 cycles of [95°C 30 sec, 60°C 40 sec]). The reaction products were loaded without further cleaning on a 0.5 mm 5.28% Page-Plus gel (Amresco®, Solon, Ohio, USA). Sequences were evaluated with the ALFwin® software version 1.10.

**Alignment and indel coding**—The forward and reverse sequences were checked and edited using the Sequencher® software version 3.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Consensus sequences from each of the two chloroplast loci were aligned separately. The two sequences of each of *Abeliophyllum* and *Picconia* were found to be identical so only one of them was included. Alignment was done using the assembly feature in Sequencher, and then manually adjusted using criteria described in Andersson and Rova (1999). Adding new sequences to the alignment was relatively easy because of conserved regions and shared indels. Twenty-two indels in the *rps16* matrix and 20 in the *trnL-F* were considered informative, and indel characters were added to the combined matrix (using A/T for present/absent). A few insertions, which did not contain informative characters, were then deleted. Autapomorphic insertions were also removed. The alignment is available from the corresponding author upon request.

**Cladistic analyses**—The combined matrix consisted of 78 ingroup taxa and ten outgroup taxa. Two taxa in this matrix, *Hesperelaea palmeri* and *Priogymnanthus apertus*, were represented by the *rps16* sequence only, and in the case of *Hesperelaea*, only the first 423 bp of the sequence were included (because of sequencing problems with the second part). The two data sets were subjected to parsimony analyses separately, and in combination, and in the latter case with and without indel characters, using PAUP\* version 4.0b4a (Swofford, 2000) on a Power Macintosh. All characters were analyzed using equal weights (=1), and gaps were treated as missing data.

Initial rounds of PAUP analyses yielded tree overflow with maximum memory settings so the following search strategy was adopted: first a search for multiple tree islands was conducted by doing 100 random addition sequence replicates, limited to only ten saved trees from each. The resulting most parsimonious trees were then used as starting trees for TBR (tree bisection-reconnection) branch swapping in an additional heuristic search for shorter trees. Up to 5000 additional trees of equal or shorter length were allowed to be saved and were then compared to the starting trees as consensus trees.

Another strategy was also adopted, namely excluding some taxa thought to cause most of the problems with thousands of equally parsimonious trees. By starting out with only two of the closest outgroup taxa (Verbenaceae) and 16 ingroup “backbone taxa,” and then restoring a few taxa in successive runs, we were able to determine which were causing the problem. It was also evident from the first runs, and from inspecting the alignment, that the taxa of Johnson's tribe Oleae are very closely related and not many characters are available to support any particular interrelationship. By only representing each genus in this tribe with one sequence, but excluding taxa with an in-

complete sequence (e.g., *Hesperelaea palmeri*), the number of trees obtained drastically decreased. In an analysis that made the best compromise between computational time and fewest excluded taxa, 64 of the 78 ingroup taxa were included, and 4312 trees were found with complete TBR branch-swapping.

Parsimony jackknifing (Farris et al., 1996) was performed on the combined matrix, with and without indel characters added, using XAC (J. S. Farris, Swedish Museum of Natural History, Stockholm, Sweden). One thousand jackknife replicates, each with ten random addition sequences and nonrotational branch-swapping (J. S. Farris, Swedish Museum of Natural History, Stockholm, Sweden, personal communication), were conducted.

## RESULTS

The *rps16* data set contained 1212 characters, of which 265 were informative, and the *trnL-F* 1211 characters, of which 240 were informative. The combined matrix with the indel characters included (and autapomorphic and other uninformative indels removed) contained 1890 characters, of which 524 were informative. The resulting consensus trees of the most parsimonious trees from the separate analyses were compatible, although not equally well resolved (not shown). The limited analysis of the combined matrix with indel characters resulted in 810 most parsimonious trees of length 1509. In the additional analysis, no shorter trees were found. Strict consensus trees computed for the first 810 trees and for the 5000 extra trees were identical, shown in Fig. 1 with jackknife support values exceeding 50%. In Fig. 2, one of the most parsimonious trees (randomly chosen) from this analysis is shown as a phylogram. The strict consensus trees from the analyses of the combined data sets, compared to those from the separate analyses, are resolved to a higher degree, but there were no differences in topology between the strict consensus trees from the analyses with and without indel characters. The only difference was in the amount of jackknife support, i.e., clades that shared informative indels received slightly higher support values. The trees from the alternative search strategy were, although containing fewer taxa, congruent with the trees from the full analyses. The RI of the trees from all analyses varied between 82 and 84%.

## DISCUSSION

**The molecular result**—Although the Oleaceae traditionally are divided into two subfamilies, this may not be a phylogenetically natural representation. In the consensus tree (Fig. 1), the Jasminoideae are paraphyletic because the tribe Jasmineae is sister to the Oleoideae. The jackknife analysis gives 76% support for this resolution, and all tribal clades are given strong support (95–100%). The phylogram (Fig. 2) shows that within *Fraxinus* and its sister group, branch lengths are very short, explaining the relatively low support in this group. Like many other noncoding chloroplast regions, *rps16* and *trnL-F* have too little variation to resolve phylogenies at an infrageneric level, at least for relatively recently diverged groups (cf. Small et al., 1998). Previously, Gielly and Taberlet (1994) have shown that the variation in the *trnL-F* region is too low to resolve relationships within *Fraxinus*. For the Oleaceae family, the *rps16* intron is more informative than the *trnL-F* region, but still, the combined data set does not contain enough informative characters to resolve the inter-tribal relationships outside Jasmineae and Oleaeae (i.e., branch lengths between the basal tribes are almost zero).

**Congruence between molecular data and other characters**—The result of the molecular analyses agrees well with nonmolecular data, e.g., chromosomal data (Taylor, 1945), wood anatomy (Baas et al., 1988), ovule number and position (Taylor, 1945; Rohwer, 1996), fruit anatomy (Rohwer, 1996), flavonoid glycosides (Harborne and Green, 1980), and iridoids (Jensen, 1992). The monophyly of the Oleoideae is not only supported by the present molecular analysis, but also by numerous morphological, anatomical, chemical, and chromosomal synapomorphies. The subfamily Jasminoideae, on the other hand, is not supported by this study, nor by any nonmolecular synapomorphies. Some of the many characters supporting the results from this study are shown on a summary tree (Fig. 3) and are discussed under the tribal and subtribal sections further below. Fruit type, which varies considerably in the Oleaceae (Rohwer, 1996), is also discussed.

**A new classification**—Based on the molecular phylogeny, supported by nonmolecular data, some changes in the classification are necessary to accord with these results. The subfamily Jasminoideae is clearly paraphyletic, and it is now time to abandon the subfamilial classification. It has been convenient to put all genera excluded from the Oleoideae in another subfamily, even though they do not share any apomorphies. Already Johnson (1957) saw the need to “ultimately abandon the subfamilies and to treat the allotetraploid Oleoideae as equivalent to the other tribes.” Rohwer (1996) stated that “the Jasminoideae is so heterogeneous in its present circumscription that it seems advisable to dismember it as a taxonomic unit,” and Qin (1996), who based his conclusions on leaf peroxidases and morphology of a few genera of the family, revoked “the subfamily rank because the tribes in subfamily Jasminoideae have no points in common.” Kiew and Baas (1984) proposed to abandon the use of subfamilies and revert to the old tribes sensu Bentham (1876). In this case, the Oleoideae would fall apart to Fraxineae, Syringaeae, and Oleaeae, and the jasminoids would stay in their assigned tribes (sensu Johnson). However, our present findings suggest that only dropping the subfamily rank and keeping the tribes unaltered is unsatisfactory. Because chromosomal data and a number of morphological characters support the monophyly of Oleoideae, we think it is important to recognize this. We suggest that changing rank of the subfamily Oleoideae to tribe Oleaeae, and changing all previous tribes of Oleoideae to subtribes, is a better solution. In this way the monophyly of this group is shown, equal in status to the jasminoid tribes.

Therefore, we present a revised classification of Oleaceae (Table 2; Appendix), shown on a summary tree in Fig. 3. We recognize five tribes: Myxopyreae (*Myxopyrum*, *Nyctanthes*, and *Dimetra*), Fontanesieae (*Fontanesia*), Forsythieae (*Forsythia* and *Abeliophyllum*), Jasmineae (*Jasminum* and *Menodora*) and Oleaeae. The Oleaeae now contain four subtribes: Ligustrinae (*Syringa* and *Ligustrum*), Schreberinae (*Schrebera* and *Comoranthus*), Fraxininae (*Fraxinus*), and Oleinae (the remaining 12 genera). The subtribes Schreberinae and Fraxininae are new (Appendix).

From this point forward, when we discuss and compare our results with those of other studies, there are cases where it is simpler to refer to the old taxonomy, i.e., subfamilial groupings. In order not to cause confusion when using the tribal name Oleaeae, we will state whether it is our new tribe Oleaeae (former subfamily Oleoideae) or subtribe Oleinae (former tribe Oleaeae). The term “jasminoids” is used to refer to the tribes

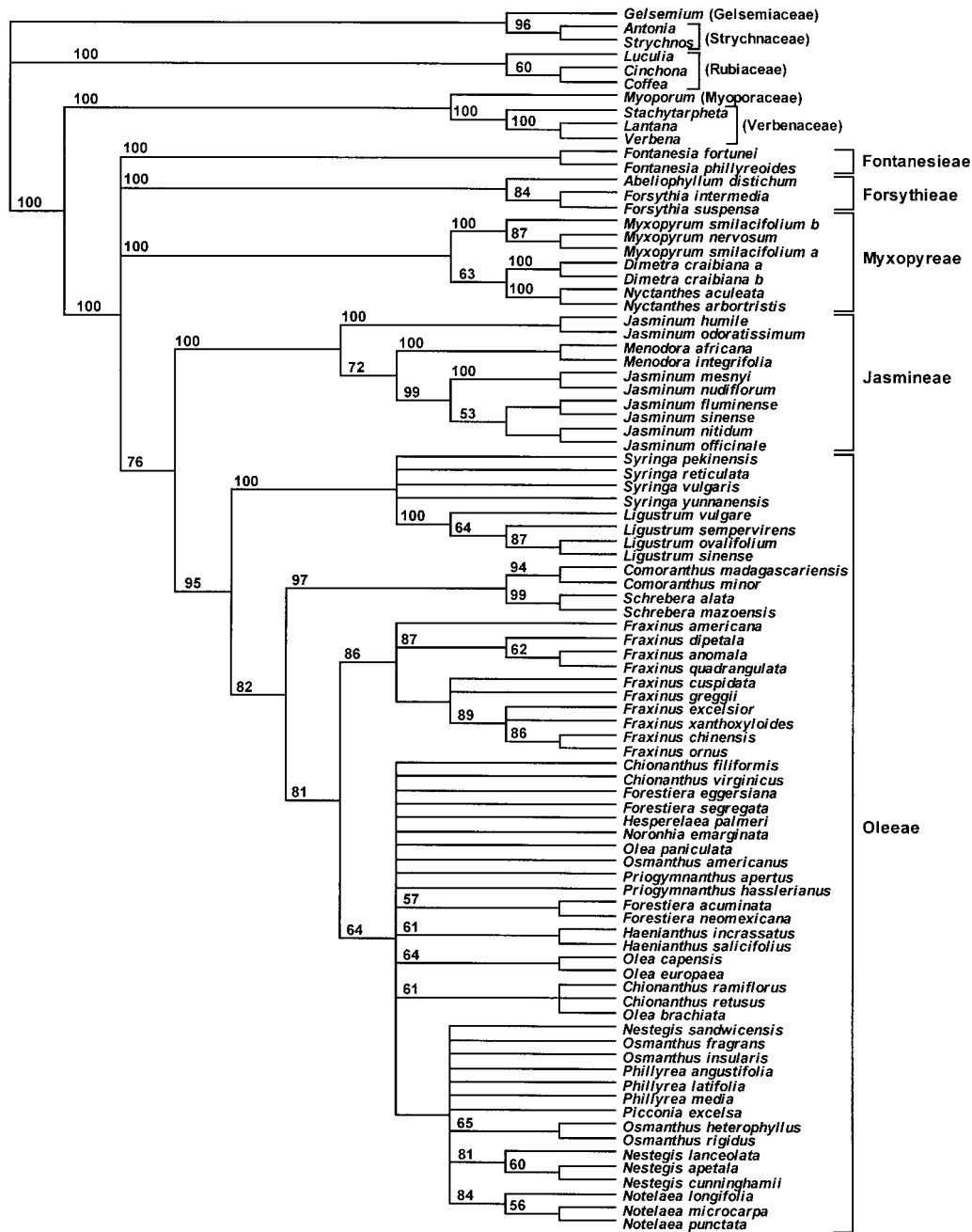


Fig. 1. Strict consensus tree of the most parsimonious trees from the analyses of the combined data set with indels coded as separate characters. Jackknife support values over 50% are shown above the branches. Tribal delimitations follow this study.

Myxopyreae, Fontanesieae, Forsythieae, and Jasmineae, i.e., the former subfamily Jasminoideae.

As can be seen by comparing our classification with that of Johnson (1957), apart from alterations in ranks, the changes are: (1) transfer of the tribe Schreberae (as subtribe Schreberinae) back to our tribe Oleaceae, (2) reinstatement of the subtribe Ligustrinae (with *Syringa* and *Ligustrum*) in tribe Oleaceae, and (3) inclusion of the formerly incertae sedis *Nyctanthes* and *Dimetra* with *Myxopyrum* in Myxopyreae.

**The new tribe Oleaceae**—The Oleaceae are clearly a monophyletic group, supported by numerous data (Fig. 3). The haploid

chromosome number  $n = 23$  is basic in all genera of the new tribe Oleaceae. In contrast, the basic number of the jasminoids is either  $x = 11$ , (12), 13, or 14. Chromosome numbers served as one of the fundamental characters upon which Taylor (1945) based his division of Oleaceae into subfamilies and tribes. It was suggested by Taylor that the  $x = 23$  group has an allopolyploid origin (from two unknown and now probably extinct jasminoids with  $x = 11$  and 12).

The ovaries of all Oleaceae are bilocular and the number of ovules in each locule vary from one to many. All genera of Oleaceae have pendulous ovules, except Myxopyreae, which has ascending ovules (see below). The synapomorphy for the



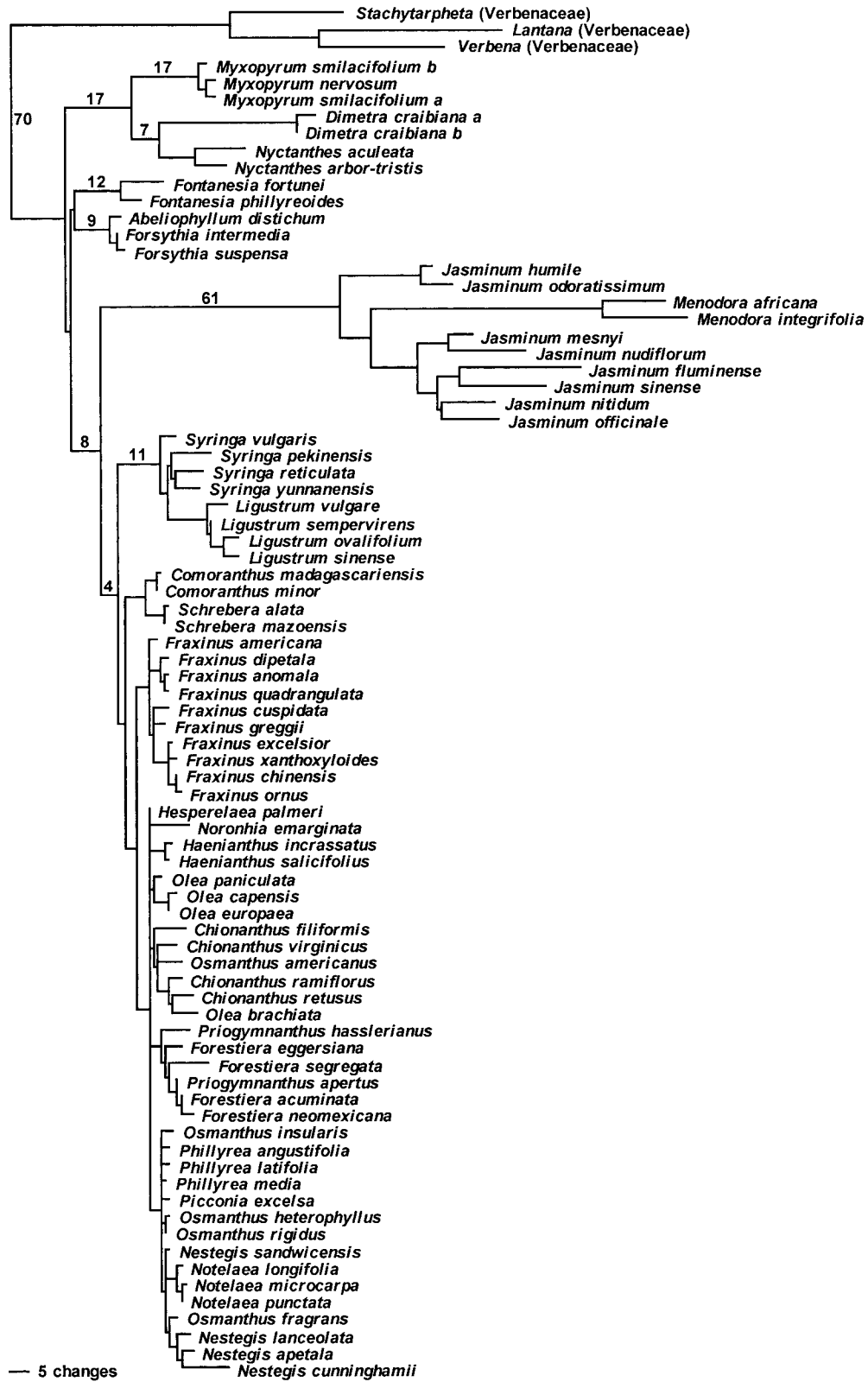


Fig. 2. A randomly selected phylogram from the analyses of the combined data set with indel characters. Because branches are quite long in the outgroup, only the closest outgroup (Verbenaceae) is shown. Numbers above branches indicate number of changes. The scale bar represents five changes.

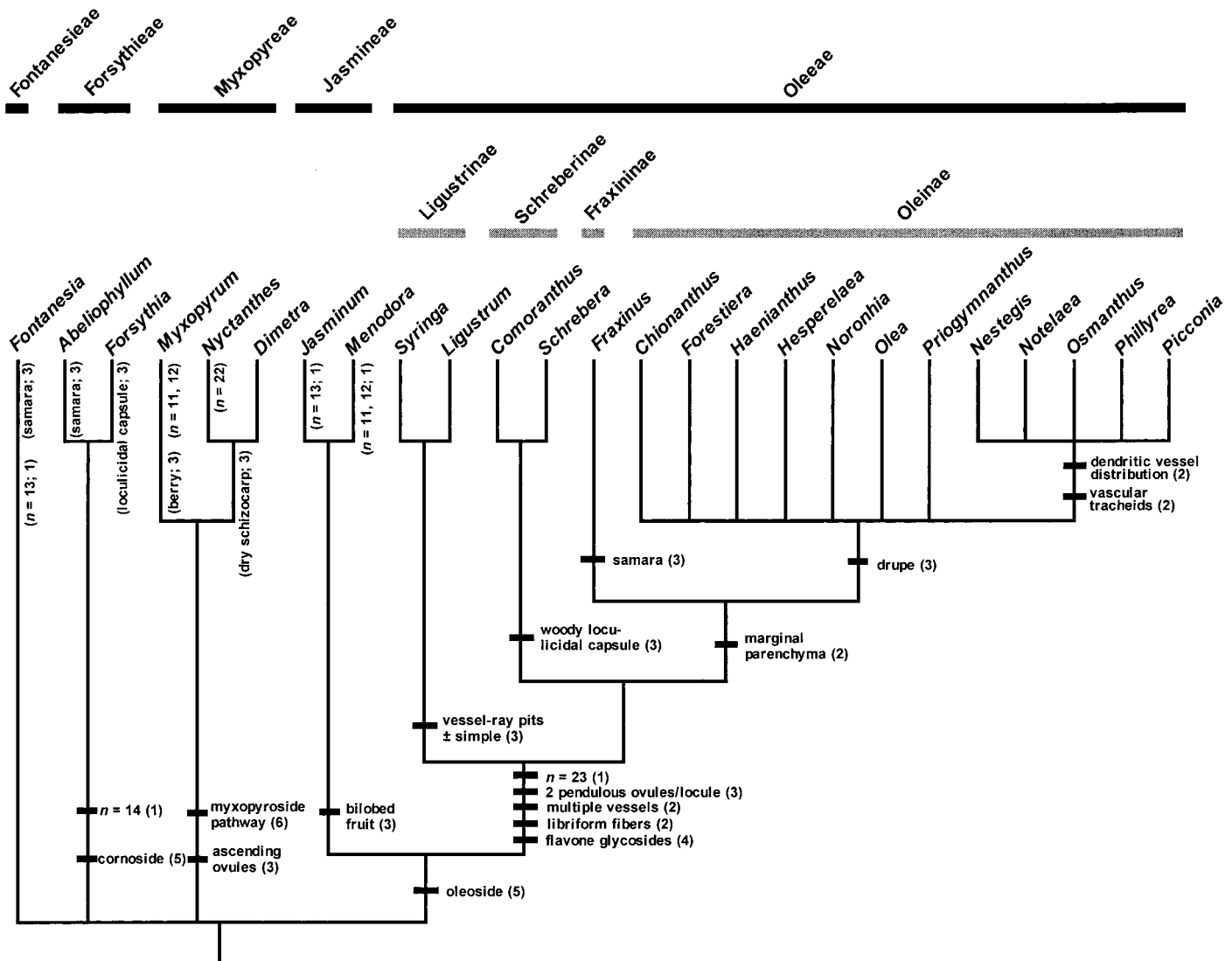


Fig. 3. Summary of the molecular phylogeny of Oleaceae, with the new classification shown on top. Some nonmolecular characters that support this phylogeny are plotted onto the tree and discussed in the text. Synapomorphies for clades are shown with a bar. Some plesiomorphic characters and others with uncertain polarity are shown within parentheses for comparison. A number after each character corresponds to data from the following authors: (1) karyology (Taylor, 1945), (2) wood anatomy (Baas et al., 1998), (3) fruit anatomy (Rohwer, 1996), (4) flavonoid glycosides (Harborne and Green, 1980), (5) verbascoside and iridoid glucosides (Jensen, 1992), and (6) iridoid glucosides (H. Franzyk, S. R. Jensen, and C. E. Olsen, Technical University of Denmark, unpublished data).

new tribe Oleaceae is two pendulous ovules per locule (except *Schrebera*, which has four; Taylor, 1945). In contrast, the tribes Fontanesieae and Forsythieae have varying numbers of ovules per locule, but never two. *Jasminum* and *Menodora* in the Jasmineae have 1–2 and 2–4 ovules per locule, respectively, but their position is more horizontal.

Harborne and Green (1980) carried out an investigation of flavonoid glycosides in leaves of all genera of Oleaceae. The pattern they found was clear: all the jasminoid genera have only the plesiomorphic flavonols present, but more complex flavonoids, including flavones and flavanones, were found to be a synapomorphy for taxa with  $x = 23$ , i.e., the new tribe Oleaceae. Unfortunately, they did not recognize *Nyctanthes* and *Dimetra* in Oleaceae, which were excluded from the study despite containing two common flavonols. Harborne and Green, also investigating flavonoid patterns in other closely

related families came to the conclusion that keeping Oleaceae in an order of its own (Oleales) was justified based on the fact that the flavonoid pattern in this family differed from other sympetalous families.

Baas et al. (1988) studied wood characters for the whole family (including *Nyctanthes*), and made both phenetic and cladistic analyses of the data. Trees from both analyses agree in principal with our results. The distribution of fiber and vessel characters especially agrees with the molecular phylogeny presented here, and libriform fibers and multiple vessels form synapomorphies for the new tribe Oleaceae (exceptions in Ligustrinae, see below).

**The jasminoids**—The tribes of the former subfamily Jasminoideae, viz. Fontanesieae, Forsythieae, Myxopyreae, and Jasmineae, share no apparent morphological apomorphies with

each other, nor with the new tribe Oleae (except Jasmineae, see under this subheading below). Taylor (1945), in his cytological study of the family, found varying low basic chromosome numbers ( $x = 11, 12, 13,$  or  $14$ ) in the jasminoids. George, Geethamma, and Ninan (1989) have proposed that  $x = 11$  (found in *Menodora* and *Myxopyrum*) is the basic chromosome number of the family, and that all other low numbers have originated by aneuploidy. In the study of flavonoid glycosides by Harborne and Green (1980), the jasminoids were shown to contain only the plesiomorphic flavonols (except *Myxopyrum* that also contained advanced flavones, but not of the same type as in Oleoideae). In the wood anatomical study by Baas et al. (1988), fiber-tracheids and solitary vessels were shown to be plesiomorphic characters in common for the jasminoids.

**Tribe Jasmineae**—In the strict consensus tree (Fig. 1), Jasmineae are resolved as sister group to Oleae, supported by eight steps and a jackknife value of 76%. Nonmolecular support has also come from Jensen (1992), who investigated iridoids in a number of species of Oleaceae, and the results fit very well with the molecular phylogeny. The tribes Jasmineae and Oleae both contain oleoside, whereas in Fontanesieae (Damtoft, Franzyk, and Jensen, 1995), Forsythieae (Damtoft, Franzyk, and Jensen, 1994), and Myxopyreae (S. R. Jensen, Technical University of Denmark, unpublished data) this compound is absent. Our results also indicate that *Jasminum* is paraphyletic, as Kim and Jansen (1993) and Rohwer (1996) have suggested, because *Menodora* is nested within it. There is, however, no doubt that the tribe is monophyletic. The phylogram (Fig. 2) shows that the clade is supported by 61 steps, and based on fruit anatomy the Jasmineae are unique in the family in having bilobed fruits. *Jasminum* has a bilobed berry (each lobe one-to-two-seeded, one lobe frequently aborted) and *Menodora*, the New World counterpart of *Jasminum*, has a bilobed circumscissile capsule. The development of these two seemingly different fruit types is in fact very similar except for the final stages (Rohwer, 1995, 1997).

**The position of *Nyctanthes* and *Dimetra***—The molecular results presented here clearly show that both *Nyctanthes* and *Dimetra* belong to Oleaceae. Their inclusion in the family is supported by a jackknife value of 100%. *Nyctanthes arbor-tristis* L. was placed in Oleaceae by Bentham (1876), Knoblauch (1895), and Taylor (1945) (Table 2). Takhtajan (1997) placed *Nyctanthes* in its own subfamily in Oleaceae (Nyctanthoideae). The second species of *Nyctanthes*, *N. aculeata* Craib, was described in 1916 and placed by the author in Oleaceae-Jasmineae. When Kerr (1938) described the new monotypic genus *Dimetra*, he assigned it to Oleaceae without hesitation. He stated that its closest alliance clearly was with *Nyctanthes*. Later, Airy Shaw (1952) transferred both of them to Verbenaceae (in subfam. Nyctanthoideae) because “the Verbenaceae facies of *Nyctanthes* almost hits one in the eye.” Stant (1952) supported this view with a study of some anatomical characters, and Johnson (1957) agreed. This transfer generated a number of papers investigating various morphological aspects of *Nyctanthes* and *Dimetra*. Kundu and De (1968) investigated cytology, palynology, and leaf, wood, and floral anatomy of *Nyctanthes* and compared it with members of Oleaceae, Verbenaceae, and Loganiaceae. They came to the conclusion that it should be placed in a family of its own, Nyctanthaceae, because of differences with both Oleaceae and

Verbenaceae. They described Nyctanthaceae as a new family, not knowing that it had already been described by Agardh in 1858 (as Nyctanthaeae). Support for placing *Nyctanthes* in Oleaceae has come from studies of embryology (Kapil and Vani, 1966), structure and vascular anatomy of the gynoecium (Kshetrapal and Tiagi, 1970), vessel anatomy (Murthy et al., 1978), leaf morphology (Mohan and Inamdar, 1983), wood anatomy (Baas et al., 1988), ultrastructure and morphology of intranuclear proteinic inclusions in the mesophyll parenchymatic cells (Bigazzi, 1989), and fruit anatomy (Kuriachen and Dave, 1989; Rohwer, 1994, 1996). These and other studies are reviewed in detail by Kiew and Baas (1984), who summarized the overwhelming evidence that *Nyctanthes* belongs to Oleaceae. Because *Nyctanthes* shares a number of characters with *Jasminum* and *Menodora* (Kiew and Baas, 1984), and because they did not want to erect a monogeneric tribe, they proposed that *Nyctanthes* should be kept in Jasmineae sensu Bentham. Although no one has disputed a close relationship between *Dimetra* and *Nyctanthes*, *Dimetra* was not included in most of the studies and was not mentioned in the review by Kiew and Baas (1984). Since our results clearly point to the close relationship between *Nyctanthes* and *Dimetra*, grouped with *Myxopyrum* rather than with Jasmineae, we argue for placing them in the tribe Myxopyreae. The pertinent node is supported by a jackknife value of 100%.

**The position of *Myxopyrum***—The genus *Myxopyrum* consists of four species distributed in subtropical and tropical east Asia (Kiew, 1984). They are scandent shrubs with quadrangular stems and conspicuously triplinerved leaves. They share the common basic characters with other Oleaceae, but some divergent features have made the genus difficult to place, and there has therefore been different opinions on where it belongs (Table 2). Bentham (1876) and Knoblauch (1895) put it in the Oleineae (sensu Bentham), but according to Taylor (1945) it differed in so many characters that it should probably be separated from the Oleineae. Johnson (1957) erected a new tribe for it, Myxopyreae, and placed it in the heterogeneous Jasminoideae. The results from this study strongly support the placement of *Myxopyrum* as sister to *Nyctanthes* and *Dimetra*, as discussed above, even though there are no apparent outer morphological similarities between them. However, the three genera share the apomorphic character of ascending ovules (Fig. 3), and *Nyctanthes* and *Myxopyrum* both have quadrangular stems with cortical bundles in the corners (Kiew, 1984; Kiew and Baas, 1984). Rohwer's (1996) investigation of fruit and seed characters of the Oleaceae showed that *Myxopyrum* and *Nyctanthes*, apart from ascending ovules, also share a deep stylar canal and the presence of a distinctive tissue in the center of the ovary septum. In contrast to the ovary, the fruit of *Myxopyrum* (a one-to-four-seeded berry) is not similar to that of *Nyctanthes* (a dry schizocarp that splits into two one-seeded mericarps), and *Myxopyrum* has varying one to three ovules per locule, whereas *Dimetra* and *Nyctanthes* have only one. Apart from the above synapomorphies, it is difficult to find morphological characters that unite these quite distinct genera. Most characters are either plesiomorphic and found in other jasminoid genera as well, or autapomorphic. For example, the wood anatomical study by Baas et al. (1988) showed that *Myxopyrum* only shared plesiomorphies with the other jasminoids, and the phytochemical study by Harborne and Green (1980) showed that *Myxopyrum* contains three apigenin glycosides that are not found in any of the other genera of Ole-

aceae. This finding of advanced glycosides in *Myxopyrum* led Kiew (1984), together with her own investigation of the morphology, to conclude that *Myxopyrum* should be retained within the Oleoideae. The chromosome number of *Myxopyrum* was unknown at that time, but now there are two reports:  $2n = 22$  for *M. hainanense* Chia (synonym to *M. pierrei* Gagnep.) (Weng and Zhang, 1992) and  $2n = 24$  for *M. smilacifolium* Blume (George and Geethamma, 1983). At least the former fit well with  $2n = 44$  reported for *Nyctanthes arbor-tristis* (George and Geethamma, 1984), which would suggest that the ancestor of *Nyctanthes* (and *Dimetra*) arose by polyploidy from the ancestor in common with *Myxopyrum*. The chromosome number of *Dimetra* is not known. Chromosome counts in *Nyctanthes* are notoriously variable (Rohwer, 1996), however, so one should not draw any conclusions based on chromosome number alone.

New chemical evidence (S. R. Jensen, Technical University of Denmark, unpublished data) on two new carbocyclic iridoid glucosides in *Myxopyrum smilacifolium* shows that these are very similar to the compounds found in *Nyctanthes* and structurally represent the same biosynthetic pathway (myxopyroside). Also, the three genera do not contain oleoside, a compound that only occurs in the two tribes Jasmineae and Oleaceae (Jensen, 1992). These findings, together with chromosome numbers, further strengthens the conclusion that *Myxopyrum* does not belong in the former Oleoideae. To conclude, a number of nonmolecular synapomorphies do support the Myxopyreae clade, despite no obvious outer morphological similarities.

**Tribes Fontanesiae and Forsythiae**—The molecular result shows a closer relationship between *Forsythia* and *Abeliophyllum* than between *Fontanesia* and *Abeliophyllum*, as might have been expected on the basis of fruit morphology (Taylor, 1945; Rohwer, 1996). *Fontanesia* and *Abeliophyllum* both have the same type of samara (differing from the one in *Fraxinus*, see below), but *Forsythia* has loculicidal capsules. Other characters, e.g., karyology (*Fontanesia* has  $x = 13$  and Forsythiae  $x = 14$ ; Taylor, 1945) and chemical data (only Forsythiae contains cornoside; Damtoft, Franzyk, and Jensen, 1994), also support the close relationship between *Forsythia* and *Abeliophyllum*. But as can be seen in Fig. 2, *Fontanesia* is resolved as sister group to the Forsythiae clade. This is the fact in most of the equally parsimonious trees and, although the branch length is extremely short (one step!), this relationship can be expected to be phylogenetically most probable, because *Fontanesia* and *Abeliophyllum* share fruit characters that are much easier interpreted as synapomorphies than parallelisms (J. G. Rohwer, University of Hamburg, Germany, personal communication). Because the strict consensus tree does not resolve the position of *Fontanesia* and because of the conflict between characters, we continue to leave *Fontanesia* alone in its own tribe.

**Subtribe Ligustrinae**—*Syringa* and *Ligustrum* form a well-supported basal clade within the new tribe Oleaceae. They have dry bilocular capsules and one-to-four-seeded berries (except *Ligustrum sempervirens* that has dehiscent drupes), respectively. Their fruits are quite similar in development, the only differences being in the development of the mesocarp and fruit dehiscence (Taylor, 1945). Johnson (1957) also stated that *Ligustrum* is undoubtedly more closely related to *Syringa* than to the rest of Oleaceae, but instead of including *Ligustrum* in the

Syringaceae (sensu Taylor), he placed both of them in the Oleaceae. Because they form a distinct and well-supported clade in our tribe Oleaceae, we have reinstated subtribe Ligustrinae Koehne to accommodate them.

In Fig. 3, libriform fibers and multiple vessels are plotted as synapomorphies for the tribe Oleaceae (with the plesiomorphic states fiber-tracheids and solitary vessels). This is true in the sense that no taxa outside this clade have this type of wood anatomy, but these features are poorly developed in some taxa of both *Ligustrum* and *Syringa*. They are unique in having both fiber types and have most of their vessels solitary rather than in multiples (Baas et al., 1988), i.e., the plesiomorphic states are retained alongside with the apomorphic. All taxa in the Oleaceae clade, excluding *Ligustrum* and *Syringa*, always have vessel multiples and exclusively libriform fibers. This condition supports the position of Ligustrinae as basal in the Oleaceae. The molecular results also indicate that *Syringa* might be paraphyletic (Fig. 2).

**Subtribe Schreberinae**—The genus *Schrebera* has a disjunct distribution in Africa and India, but *Comoranthus* occurs only on Madagascar and the Comores. There is also a report of *Schrebera americana* Gilg. from Peru. Both genera have bivalved woody capsules (Rohwer, 1996), and, based on overall morphology, it is obvious that they are closely related, if not congeneric (P. S. Green, Royal Botanic Gardens, Kew, personal communication). Johnson (1957) grouped *Schrebera* and *Comoranthus* in the new tribe Schreberae and, pending chromosomal data, provisionally referred it to his subfamily Jasminoideae. Briggs (1970) determined the haploid chromosome number of *Schrebera* to be 23, but the chromosome number of *Comoranthus* is still unknown. The present study clearly shows that these genera form a distinct clade that belongs to the same group as the other genera with  $x = 23$ , and we have therefore placed them in the subtribe Schreberinae status novus in the new tribe Oleaceae. The chemotaxonomic survey by Harborne and Green (1980) and the wood anatomical study by Baas et al. (1988) also give support to this placement.

**Subtribe Fraxininae**—This new subtribe contains only the genus *Fraxinus*. It is a circumpolar genus of the northern hemisphere, comprising ~50 species of mainly trees. The genus is characterized by large pinnate leaves and samaras, and there is no doubt that it represents a monophyletic group. Because of the fruit type, *Fontanesia* was included in the Fraxineae by Bentham (1876) and Knoblauch (1895). However, the samaras in *Fontanesia* and *Abeliophyllum*, compared with those of *Fraxinus*, are neither morphologically nor developmentally similar. Instead, the samara of *Fraxinus* shows an internal structure very similar to that of the loculicidal capsule of *Syringa* (Rohwer, 1996). The fruit of *Fraxinus* has two ovules per locule but usually only one ovule develops, making the samara one-seeded. In contrast, *Fontanesia* and *Abeliophyllum* have only one ovule per locule, and although both ovules start to develop, the mature fruit is usually one-seeded (Rohwer, 1996). There are also differences in the morphology of the wing. In the long terminal wing of *Fraxinus* the fibers run longitudinally, and in *Fontanesia*'s short lateral wings, they run obliquely perpendicular (Rohwer, 1993).

**Subtribe Oleinae**—The subtribe Oleinae, former tribe Oleaceae, is characterized by drupes. Although this group does

not receive strong jackknife support, there is nevertheless no doubt that this is a monophyletic group. Relationships between the genera in this subtribe are difficult to elucidate, with neither the cpDNA data nor morphology giving a clear answer. Several studies have found that some genera in this group may be polyphyletic as presently circumscribed, e.g., *Olea* (Altamura, Altamura, and Mazzolani, 1985, 1987; Kiew, 1979) and *Osmanthus* (Johnson, 1957), and our study can only confirm this suspicion. For example, *Olea brachiata* (formerly placed in the separate genus *Tetrapilus* Lour.; Johnson, 1957) seems to be more related to *Chionanthus*, and so does *Osmanthus americanus*, the only New World species of *Osmanthus* (once treated separately in *Amarolea* Small; Johnson, 1957). These results are also supported by wood anatomy (Baas et al., 1988).

Within this subtribe lies a complex of five supposedly more closely related Old World genera, distributed mainly in the subtropics: *Osmanthus* (except *O. americanus*), *Phillyrea*, *Picconia*, *Nestegis*, and *Notelaea*. There is no jackknife support for this grouping, but it is shown in the strict consensus tree (Fig. 1). Green (1958) mentioned this generic complex and Baas et al. (1988) found some support for its monophyly in wood anatomical characters. The synapomorphies are dendritic vessel distribution and vascular tracheids. The similarity in fruit morphology between *Phillyrea* and *Picconia* has been pointed out by Taylor (1945), and other characters by Johnson (1957). W. K. Harris (University of Queensland, personal communication) has found that, based on nuclear ITS sequences, the Australian, New Zealand, and New Caledonian taxa of *Osmanthus* and *Nestegis* should be included in *Notelaea*. Generic delimitations in this complex are admittedly difficult (P. S. Green, Royal Botanic Gardens, Kew, personal communication) and further studies, using loci with more variation than in the present study (e.g., ITS), are needed to clarify relationships within the entire subtribe Oleinae.

*Hesperelaea*—The genus *Hesperelaea* is now extinct (Moran, 1996), but we were successful in obtaining an *rps16* intron sequence from the type specimen, the one and only collection from 1875. It is only known from its type locality on Guadalupe, a Mexican island off Baja California. *Hesperelaea* was collected by Edward Palmer and described by Asa Gray (Watson, 1876) as *H. palmeri*, a new monotypic genus of Oleaceae. When collected, Palmer found only three old trees alive, no young trees, but several dead ones. The area was heavily grazed by goats, which presumably led to *Hesperelaea*'s extinction (Moran, 1996). Not much is known about the genus; Gray's description was rather short, but noteworthy was that its flowers had four stamens. In other genera of Oleaceae, the most common condition is two stamens, but four stamens occasionally occur, e.g., in *Chionanthus*, *Osmanthus*, *Noronhia*, *Schrebera*, and *Forestiera*. The fruit was a drupe and so it was placed by Johnson (1957) among the other genera with drupes in his tribe Oleae (the new Oleinae). Despite having only part of the *rps16* intron sequence to confirm this placement, we feel sure that this is correct.

**Conclusions**—This study has presented molecular evidence, congruent with other data, that requires a revised classification of the Oleaceae. (1) The subfamily level is abandoned because Jasminoideae is paraphyletic. (2) The monophyly of the former Oleoideae—here recognized as tribe Oleae—is strongly supported and treated equal in status to the former jasminoid

tribes Fontanesieae, Forsythieae, Myxopyreae, and Jasmineae. (3) The tribe Jasmineae is sister to Oleae. This relationship is supported by chemical data. (4) The long-debated genera *Nyctanthes* and *Dimetra* clearly belong to the Oleaceae. (5) The position of the hitherto genus incertae sedis *Myxopyrum* is supported as sister to *Nyctanthes* and *Dimetra*. All three genera are placed in Myxopyreae. (6) The monophyly of the subtribe Oleinae, characterized by drupes, is supported. (7) The *rps16* sequence of *Hesperelaea palmeri*, known only from the type specimen collected in 1875, confirms the placement of this extinct taxon in the subtribe Oleinae. (8) A closer relationship between a group of five genera in the Oleinae, viz. *Osmanthus*, *Picconia*, *Phillyrea*, *Nestegis*, and *Notelaea*, is suggested by molecular data and has morphological and wood anatomical support. (9) The two noncoding chloroplast loci, the *rps16* intron and the *trnL-F* region, have proven useful for this infrafamilial study, in combination giving over 500 informative sites. In contrast, the variation at infra- and intergeneric level in the Oleae, especially in the genus *Fraxinus* and in the subtribe Oleinae, is too low to be useful.

#### LITERATURE CITED

- AGARDH, J. 1858. Nyctantheae. *Theoria Systematis Plantarum*: 284.
- AIRY SHAW, H. K. 1952. Note on the taxonomic position of *Nyctanthes* L. and *Dimetra* Kerr. *Kew Bulletin* (1952): 271–272.
- ALTAMURA, L., M. M. ALTAMURA, AND G. MAZZOLANI. 1987. Elements for the revision of the genus *Olea* (Tourn.) L. VII. The taxa present in Asia which can be ascribed to *Olea* and allied genera. *Annali di Botanica* 45: 119–134.
- ALTAMURA, M. M., L. ALTAMURA, AND G. MAZZOLANI. 1985. Elements for the revision of the genus *Olea* (Tourn.) L. VI. The taxa present in Oceania which can be ascribed to *Olea* and allied genera. *Annali di Botanica* 43: 45–52.
- ANDERSSON, L., AND J. H. E. ROVA. 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). *Plant Systematics and Evolution* 214: 161–186.
- APG (ANGIOSPERM PHYLOGENY GROUP). 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* 85: 531–553.
- BAAS, P., P. M. ESSER, M. E. T. VAN DER WESTEN, AND M. ZANDEE. 1988. Wood anatomy of the Oleaceae. *IAWA Bulletin* 9: 103–182.
- BENTHAM, G. 1876. Oleaceae. In G. Bentham and J. D. Hooker [eds.], *Genera Plantarum* 2: 672–680.
- BIGAZZI, M. 1989. Ultrastructure of nuclear inclusions and the separation of Verbenaceae and Oleaceae (including *Nyctanthes*). *Plant Systematics and Evolution* 163: 1–12.
- BRIGGS, B. G. 1970. Some chromosome numbers in the Oleaceae. *Contributions from the New South Wales National Herbarium* 4: 126–129.
- CRAIB, W. G. 1916. Contributions to the flora of Siam. *Additamentum IX. Kew Bulletin* (1916): 265.
- DAMTOFT, S., H. FRANZYK, AND S. R. JENSEN. 1994. Biosynthesis of iridoids in *Forsythia* spp. *Phytochemistry* 37: 173–178.
- , ———, AND ———. 1995. Biosynthesis of secoiridoids in *Fontanesia*. *Phytochemistry* 38: 615–621.
- FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.
- GEORGE, K., AND S. GEETHAMMA. 1983. Lactopropionic orcein as a suitable stain for chromosomes of Oleaceae. *Current Science* 52: 733–734.
- , AND ———. 1984. Cytological and other evidences for the taxonomic position of *Nyctanthes arbor-tristis* L. *Current Science* 53: 439–441.
- , ———, AND C. A. NINAN. 1989. Chromosome evolution in Oleaceae. *Journal of Cytology and Genetics* 24: 71–77.
- GIELLY, L., AND P. TABERLET. 1994. Chloroplast DNA polymorphism at the intrageneric level and plant phylogenies. *Comptes Rendus de l'Académie des Sciences, Serie III, Sciences de la Vie* 317: 685–692.
- GREEN, P. S. 1958. A monographic revision of *Osmanthus* in Asia and America. *Notes from the Royal Botanical Garden Edinburgh* 22: 439–542.

- HARBORNE, J. B., AND P. S. GREEN. 1980. A chemotaxonomic survey of flavonoids in leaves of the Oleaceae. *Botanical Journal of the Linnean Society* 81: 155–167.
- HOLMGREN, P. K., N. H. HOLMGREN, AND L. C. BARRETT. 1990. Index Herbariorum, part I, The herbaria of the world. New York Botanical Garden Press, Bronx, New York, USA.
- JENSEN, S. R. 1992. Systematic implications of the distribution of iridoids and other chemical compounds in the Loganiaceae and other families of the Asteridae. *Annals of the Missouri Botanical Garden* 79: 284–302.
- JOHNSON, L. A. S. 1957. A review of the family Oleaceae. *Contributions from the New South Wales National Herbarium* 2: 395–418.
- KAPIL, R. N., AND R. S. VANI. 1966. *Nyctanthes arbor-tristis* Linn.: embryology and relationships. *Phytomorphology* 16: 553–563.
- KERR, A. F. G. 1938. XIX Contributions to the flora of Siam. *Kew Bulletin* (1938): 127–133.
- KIEW, R. 1979. Florae Malesianae praecursores LX. The Oleaceae of Malesia. II. The genus *Olea*. *Blumea* 25: 305–313.
- . 1983. Two unusual *Chionanthus* species from Borneo and the position of *Myxopyrum* in the Oleaceae. *Journal of the Arnold Arboretum* 64: 619–626.
- . 1984. The genus *Myxopyrum* L. (Oleaceae). *Blumea* 29: 499–512.
- , AND P. BAAS. 1984. *Nyctanthes* is a member of Oleaceae. *Proceedings of the Indian Academy of Science* 93: 349–358.
- KIM, K.-J. 1999. Phylogeny of the olive family (Oleaceae). In Abstracts. XVI International Botanical Congress, St. Louis. (abstract number 19.2.6, p. 227).
- , AND R. K. JANSEN. 1993. Phylogeny of Oleaceae based on *ndhF* sequence variation and chloroplast genome rearrangements. In Abstracts. XV International Botanical Congress, Tokyo. (abstract number 1065, p. 209).
- KNOBLAUCH, E. 1895. Oleaceae. In A. Engler [ed.], *Die Natürlichen Pflanzenfamilien* IV, 2: 1–16.
- KSHETRAPAL, S., AND Y. D. TIAGI. 1970. Structure, vascular anatomy and evolution of the gynoeceum in the family Oleaceae and their bearing on the systematic position of genus *Nyctanthes* L. *Acta Botanica Academiae Scientiarum Hungaricae* 16: 143–151.
- KUNDU, B. C., AND A. DE. 1968. Taxonomic position of the genus *Nyctanthes*. *Bulletin of the Botanical survey of India* 10: 397–408.
- KURIACHEN, P. M., AND Y. S. DAVE. 1989. Structural studies in the fruits of Oleaceae with discussion on the systematic position of *Nyctanthes* L. *Phytomorphology* 39: 51–60.
- MOHAN, J. S. S., AND J. A. INAMDAR. 1983. Studies of the leaf architecture of the Oleaceae with a note on the systematic position of the genus *Nyctanthes*. *Feddes Repertorium* 94: 201–211.
- MORAN, R. 1996. The Flora of the Guadalupe Island, Mexico. *Memoirs of the California Academy of Sciences* 19: 28, 40–43, 128–129 (pages about *Hesperelaea*).
- MURTHY, G. S. R., K. M. ALEYKUTTY, V. S. RAO, AND J. A. INAMDAR. 1978. Vessels of Oleaceae and Verbenaceae. *Feddes Repertorium* 89: 359–368.
- OLMSTEAD, R. G., AND P. A. REEVES. 1995. Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbcL* and *ndhF* sequences. *Annals of the Missouri Botanical Garden* 82: 176–193.
- OXELMAN, B., M. LIDÉN, AND D. BERGLUND. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.
- PERSSON, C. 2000. Phylogeny of Gardenieae (Rubiaceae) based on chloroplast DNA sequences from the *rps16* intron and *trnL* (UAA)-F(GAA) intergenic spacer. *Nordic Journal of Botany* 20: 257–269.
- QIN, X.-K. 1996. The use of peroxidases in the systematics of Oleaceae. *Acta Botanica Yunnanica* 18: 159–166.
- ROHWER, J. G. 1993. A preliminary survey of the fruits and seeds of the Oleaceae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 115: 271–291.
- . 1994. Fruits and seeds of *Nyctanthes arbor-tristis* L. (Oleaceae): a comparison with some Verbenaceae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 115: 461–473.
- . 1995. Fruit and seed structures in *Menodora* (Oleaceae): a comparison with *Jasminum*. *Botanica Acta* 108: 163–168.
- . 1996. Die Frucht- und Samenstrukturen der Oleaceae. *Bibliotheca Botanica* 148: 1–177.
- . 1997. The fruits of *Jasminum mesnyi* (Oleaceae), and the distinction between *Jasminum* and *Menodora*. *Annals of the Missouri Botanical Garden* 84: 848–856.
- SMALL, R. L., J. A. RYBURN, R. C. CRONN, T. SEELANAN, AND J. F. WENDEL. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear ADH sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany* 85: 1301–1315.
- STANT, M. Y. 1952. Anatomical evidence for including *Nyctanthes* and *Diometra* in the Verbenaceae. *Kew Bulletin* 7: 273–276.
- SWOFFORD, D. L. 2000. PAUP\*: phylogenetic analysis using parsimony (\* and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.
- TABERLET, P., L. GIELLY, G. PANTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- TAKHTAJAN, A. 1997. Oleanae—Oleales—Oleaceae. In *Diversity and classification of flowering plants*, 449–451. Columbia University Press, New York, New York, USA.
- TAYLOR, H. 1945. Cyto-taxonomy and phylogeny of the Oleaceae. *Brittonia* 5: 337–367.
- WAGSTAFF, S. J., AND R. G. OLMSTEAD. 1997. Phylogeny of Labiatae and Verbenaceae inferred from *rbcL* sequences. *Systematic Botany* 22: 165–179.
- WATSON, S. 1876. Botanical contributions. I. On the flora of Guadalupe Island, Lower California. *Proceedings of the American Academy of Arts and Sciences* XI: 105–112.
- WENG, R.-F., AND M.-Z. ZHANG. 1992. Chromosome numbers in Chinese Oleaceae I. *Investigatio et Studium Naturae* 12: 66–77.

## APPENDIX

A new generic and suprageneric classification of Oleaceae. Source for most of the nomenclatural information is the Indices Nominum Supragenericorum Plantarum Vascularium Project Database (<http://matrix.nal.usda.gov:8080/star/supragenericname.html>).

**Fam. Oleaceae** Hoffmanns. & Link, Fl. Portug. 1: 385. 1813–1820 (Oleinae), *nom. cons.*

## Synonyms:

- Bolivariaceae Griseb., Gen. Sp. Gent.: 20. Oct 1838.
- Forestieraceae Endl., Ench. Bot.: 174. 15–21 Aug 1841 (Forestiereae).
- Fraxinaceae Vest, Anleit. Stud. Bot.: 269, 288. 1818 (Fraxinoideae).
- Jasminaceae Adans., Fam. Pl. 2: 220. Jul–Aug 1763 (Jasmina).
- Ligustraceae G. Mey., Chloris Han.: 245, 254. Jul–Aug 1836 (Ligunstrinae).
- Lilacaceae Vent., Tabl. Règne Vég. 2: 307. 5 Mai 1799 (Lilaceae), *nom. illeg.*
- Nyctanthaceae J. Agardh, Theoria Syst. Pl.: 284. Apr–Sep 1858 (Nyctantheae).
- Schreberaceae (Wight) Schnizl., Iconogr. Fam. Regni Veg. 2: ad t. 151\*. 1857–1870.
- Syringaceae Horan., Char. Ess. Fam.: 115. 1847.

**Tribe Oleaeae** (Hoffmanns. & Link ex R. Br.) Dumort., Fl. Belg.: 52. 1827 (Oleinae).

**Subtribe Oleinae**

## Synonyms:

- Tribe Chionantheae DC., Prodr. 8: 294. mid Mar 1844.
- Tribe Forestiereae Horan., Char. Ess. Fam.: 80. 1847 (Forrestiereae).
- Subtribe Forestierinae Koehne, Deut. Dendrol.: 500. Mai 1893 (Forestiereae).
- Tribe Hesperelaeae Baill., Hist. Pl. 11: 242, 249. Sep–Oct 1891.
- Tribe Notelaeae G. Don, Gen. Hist. 4: 44, 51. 1837–8 Apr 1838 (Notelaeiae).

## Accepted genera:

- Chionanthus* L.
- Forestiera* Poir.
- Haenianthus* Griseb.
- Hesperelaea* A. Gray
- Nestegis* Rafin.
- Noronhia* Stadmann ex Thouars
- Notelaea* Vent.
- Olea* L.
- Osmanthus* Lour.
- Phillyrea* L.
- Picconia* DC.
- Priogymnanthus* P.S. Green

**Subtribe Fraxininae** (Vest) E. Wallander & V. A. Albert, *stat. nov.*

Basionym: Fam. Fraxinaceae Vest, *Anleit. Stud. Bot.*: 269, 288. 1818 (Fraxinoideae).

## Synonyms:

Subfam. Fraxinoideae Kostel., *Allg. Med.-Pharm. Fl.* 3: 998. Apr–Dec 1834 (Fraxineae).

Tribe Fraxineae Bartl., *Ord. Nat. Pl.*: 218. Sep 1830 (Fraxinea).

## Accepted genus:

*Fraxinus* L.

**Subtribe Schreberinae** (Wight) E. Wallander & V. A. Albert, *stat. nov.*

Basionym: Subfam. Schreberoideae Wight, *Ill. Ind. Bot.* 2: 185. 1850 (Schreberaceae).

## Accepted genera:

*Schrebera* Roxb.

*Comoranthus* Knobl.

**Subtribe Ligustrinae** Koehne, *Deut. Dendrol.*: 500. Mai 1893 (Ligustreae).

## Synonyms:

Tribe Ligustreae Burnett, *Outl. Bot.*: 1022, 1102. Jun 1835.

Subfam. Syringoideae Leurss., *Handb. Syst. Bot.* 2: 1041. Nov 1882 (Syringae).

Tribe Syringae Burnett, *Outl. Bot.*: 1022, 1103. Jun 1835.

## Accepted genera:

*Syringa* L.

*Ligustrum* L.

**Tribe Jasmineae** Lam. & DC., *Syn. Pl. Fl. Gall.*: 216. 30 Jun 1806.

## Synonym:

Tribe Bolivarieae Horan., *Char. Ess. Fam.*: 116. 1847.

## Accepted genera:

*Jasminum* L.

*Menodora* Humb. & Bonpl.

**Tribe Myxopyreae** Boerl., *Handl. Fl. Nederl. Ind.* 2: 324. 1 Jan 1899.

## Synonym:

Subfam. Myxopyroideae Boerl., *Handl. Fl. Nederl. Ind.* 2: 324. 1 Jan 1899 (Myxopyreae).

## Accepted genera:

*Myxopyrum* Blume

*Nyctanthes* L.

*Dimetra* Kerr

**Tribe Forsythieae** H. Taylor ex L. Johnson, *Contrib. N.S.W. Natl. Herb.* 2: 397. 1957.

## Accepted genera:

*Abeliophyllum* Nakai

*Forsythia* Vahl

**Tribe Fontanesieae** H. Taylor ex L. Johnson, *Contrib. N.S.W. Natl. Herb.* 2: 397. 1957.

## Accepted genus:

*Fontanesia* Labill.