

# Molecular phylogenetic reconstruction of *Osmanthus* Lour. (Oleaceae) and related genera based on three chloroplast intergenic spacers

Shi-Quan Guo · Min Xiong · Chun-Feng Ji ·  
Zhi-Rong Zhang · De-Zhu Li · Zhi-Yong Zhang

Received: 11 May 2010/Accepted: 25 March 2011/Published online: 29 April 2011  
© Springer-Verlag 2011

**Abstract** Although polyphyly of *Osmanthus* has been suggested by different authors, the conclusions of previous studies have lacked robust support due to limited sampling or a paucity of phylogenetic characters. In this study, the phylogeny of *Osmanthus* was explored using sequences of three informative chloroplast regions (*psbJ-petA*, *rpl32-trnL* and *rps16-trnQ*), including all the five sections of the genus and eight closely related genera. The results confirm that *Osmanthus*, as presently circumscribed, is a polyphyletic group, containing three or four distinct lineages, i.e. sect. *Leiolea* (lineage I), sect. *Notosmanthus* (lineage III), sects. *Osmanthus* (excluding *O. decorus*), *Siphosmanthus* and *Linocieroides* (lineage IV), and an uncertain lineage including only *O. decorus* (lineage II). These results emphasize that the generic delimitation within subtribe Oleinae is in need of revision. In addition, this study found that the four cultivar groups of sweet osmanthus formed a

paraphyletic clade, implying that cultivated sweet osmanthus might originate from several species.

**Keywords** Phylogeny · *Osmanthus* · Oleaceae *rps16-trnQ* · *rpl32-trnL*<sup>(UAG)</sup> · *psbJ-petA*

## Introduction

The genus *Osmanthus* was founded by Loureiro in his *Flora Cochinchinensis* in 1790, using *Osmanthus fragrans* (Thunb.) Lour. as type species, which was also the only species of the genus at that time. After the establishment of *Osmanthus*, it took a considerable time for its acceptance by taxonomists. Until the last two decades of the nineteenth century only five species were known. Later, other species were gradually described. Presently this genus is composed of about 35 species in five sections (Green 1958, 1963; Xiang and Liu 2008). The species in *Osmanthus* are evergreen shrubs or trees and most of them occur in temperate to subtropical parts of eastern Asia, mainly China, and New Caledonia. One species, *O. americanus*, is distributed in subtropical parts of the USA and Mexico (Green 1958). Another species, *O. decorus*, occurs in western Asia, Turkey and the Mediterranean region (Xiang and Liu 2008). China is inhabited by 24 species of *Osmanthus* and can be considered as the center of diversity of the genus (Xiang and Liu 2008).

The flowers of most species of *Osmanthus* are scented and beautiful. Therefore, a few species of *Osmanthus* have been brought into cultivation as ornamentals. Among them, *Osmanthus fragrans* (sweet osmanthus) is the most well-known. It has been recognized and cultivated for about 2,500 years and enjoys a reputation among the top ten

---

**Electronic supplementary material** The online version of this article (doi:[10.1007/s00606-011-0445-z](https://doi.org/10.1007/s00606-011-0445-z)) contains supplementary material, which is available to authorized users.

S.-Q. Guo · M. Xiong · Z.-R. Zhang · Z.-Y. Zhang (✉)  
Laboratory of Subtropical Biodiversity, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, China  
e-mail: pinus-rubus@163.com

C.-F. Ji  
College of Forestry, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, China

Z.-R. Zhang · D.-Z. Li  
Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species and Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China

traditional flowers in China. A total of 166 cultivars, belonging to four cultivar groups, are recognized (Xiang and Liu 2008). *Osmanthus heterophyllus* is another ornamental species with a long cultivation history, but mainly planted in Japan (Green 1958). During the last 100 or so years, several species from the Sino-Himalayan regions have also been introduced into cultivation (Green 1958). For example, *O. delavayi* was introduced into England early in the nineteenth century and is now widely cultivated in the gardens of England (Xiang and Liu 2008). In total, at least ten species of *Osmanthus* are cultivated, mainly from China (Xiang and Liu 2008).

The genus *Osmanthus* belongs to the subtribe *Oleinae* (sensu Wallander and Albert 2000) characterized by drupes. This subtribe includes a complex of five supposedly more closely related genera, distributed mainly in the subtropics: *Osmanthus*, *Phillyrea*, *Picconia*, *Nestegis*, and *Notelaea*. Woody anatomical characters (dendritic vessel distribution and vascular tracheids; Baas et al. 1988) and molecular evidence (Wallander and Albert 2000) suggest that this generic complex might be monophyletic. However, generic delimitations in this complex are very difficult morphologically and cytogenetically (Taylor 1945; Green 1958, 1963). Molecular phylogenetics also provided little information concerning the relationships within the complex due to limited informative characters of sequences (Wallander and Albert 2000). In addition, recent studies have suggested that other genera, such as *Chionanthus* and *Olea*, might be closely related to *Osmanthus* (Besnard et al. 2009). Therefore, the phylogenetic relationships between *Osmanthus* and other closely related genera remain open.

Green (1958, 1963) divided the genus *Osmanthus* into five sections: *Leiolea* (Spach) P. S. Green, *Siphosmanthus* Franch., *Linocieroides* P. S. Green, *Notosmanthus* P. S. Green, and *Osmanthus*. This classification is widely used (Xiang and Liu 2008), but the intersectional relationships are a matter of controversy. In 1929, Staph considered that the cylindrical corolla tube of *O. suavis* and *O. delavayi* (sect. *Siphosmanthus*) was sufficiently distinct to warrant generic recognition, separating them as genus *Siphosmanthus*. The North American *O. americanus* (sect. *Leiolea*) has also been treated as a distinct genus, first by Rafinesque in 1838 as the genus *Cartrema* and then by Small, nearly 100 years later, as the genus *Amarolea*. Two members of sect. *Notosmanthus* in the Southern Hemisphere, differing in their inflorescence characters from other sections, were once segregated in a resurrected genus *Nestegis* Raf. [*Gymnelaea* (Endl.) Spach] (Johnson 1957). Johnson (1957) suggested that *Osmanthus* might be a polyphyletic group, which was supported by woody anatomy and phytochemical constituents (Baas et al. 1988; Jensen et al. 2002). By using two chloroplast genomic

regions, Wallander and Albert (2000) also indicated that *Osmanthus* might be a polyphyletic group. However, the notion that *Osmanthus* might be polyphyletic lacked robust support as the number of *Osmanthus* species included was limited in these studies and the results of Wallander and Albert (2000) were complicated by the low resolution of DNA sequences. In contrast, based on chloroplast *matK* sequences, Yang et al. (2009) suggested that *Osmanthus* could be a monophyletic group. But this result is of limited value because only three sections of *Osmanthus* and two related genera (*Olea* and *Chionanthus*) were sampled and the *matK* sequences have been suggested to be of limited resolution in inferring phylogenetic relationships of woody genera (e.g. Zhang and Li 2004).

Because of the general constancy within sections, particularly within sect. *Osmanthus*, with very little floral variation and the most pronounced differences lying in the leaf shape, it has been difficult to arrive at any decisions about affinities between the species (Green 1958). Furthermore, as pointed out by Heuertz et al. (2004) and indicated by Besnard et al. (2002), a low chloroplast DNA mutation rate is a feature of Oleaceae. Additional investigations, including both extended sampling and more informative markers are thus needed to reconstruct a robust phylogeny to test for polyphyly and infer the intersectional and interspecific relationships of *Osmanthus*.

In the present study, we screened three informative chloroplast intergenic spacers for phylogenetic reconstruction of *Osmanthus*. Our specific objectives were: (1) to test the polyphyly of *Osmanthus* in the context of subtribe *Oleinae* (sensu Wallander and Albert 2000), with special focus on the affinities of *Osmanthus* with other closely related genera; (2) to infer the intersectional and interspecific relationships within *Osmanthus*.

## Materials and methods

### Plant materials sampled

Sampled materials include 21 *Osmanthus* species, covering all the five sections and most of the distribution range of *Osmanthus*. *O. fragrans* was represented by four accessions of different cultivar groups. Of 12 genera within subtribe *Oleinae*, 8 were also included. Among them, all of the putatively closely related genera (i.e. *Phillyrea*, *Picconia*, *Nestegis*, *Notelaea*, *Olea*, *Linociera*, and *Chionanthus*) were contained. One accession of the sister group of subtribe *Oleinae* (*Fraxinus pennsylvanica*) was sampled as outgroup. The chloroplast genome sequence of *Jasminum nudiflorum* had been published (Lee et al. 2007); therefore this species was also included. All the plant accessions used in this study are presented in supplementary Table 1.

## DNA extraction, PCR amplification, and sequencing

Total genomic DNA from each accession was extracted using a 2× CTAB method (Doyle and Doyle 1987), except for *Nestegis sandwicensis*, *Noronhia emarginata* and *Jasminum nudiflorum* (see supplementary Table 1). Because of the low chloroplast DNA mutation rate within Oleaceae, we comprehensively screened suitable chloroplast genomic regions for phylogenetic reconstruction. Finally, three intergenic spacers (*rps16-trnQ*, *rpl32-trnL<sup>(UAG)</sup>*, and *psbJ-petA*) with relatively high sequence variation were selected. The amplification primer sequences of the three regions were described by Shaw et al. (2007). PCR amplifications were performed in a Bioer XP cycler (Bioer, Hangzhou, China) programmed for an initial 240 s at 94°C, followed by 30 cycles of 60 s at 94°C, 45 s at 55°C (*rps16-trnQ*), 55°C (*rpl32-trnL<sup>(UAG)</sup>*) or 50°C (*psbJ-petA*), 120 s at 72°C, and a final 600 s at 72°C. Reactions were carried out in a volume of 30 µl containing 1.6 mM/l MgCl<sub>2</sub>, 0.5 µM/l dNTP, 10× buffer, 2 µM/l primer, 1.5 U *Taq* DNA and 30 ng DNA template. Sequencing reactions were conducted using the PCR primers (Sangon Biotech, Shanghai, China).

## Phylogenetic reconstructions

Sequences were checked and edited with CONTIG and sequence alignments were made with CLUSTAL W (Thompson et al. 1997) and refined manually. Phylogenetic analyses of the sequence data, separately and all three combined, were performed using the parsimony and Bayesian Markov chain Monte Carlo (MCMC) methods. Before analyzing all data together, the partition homogeneity test, as implemented in PAUP version 4.0b10 (Swofford 2001), was used to evaluate the topological congruence between gene trees (Farris et al. 1995). Maximum parsimony (MP) analyses were conducted using PAUP version 4.0b10 (Swofford 2001). All characters were equally weighted, gaps were treated as missing, and character states were treated as unordered. A heuristic search was performed with the MULPARS option, tree-bisection-reconnection (TBR) branch swapping, and RANDOM stepwise addition with 100 replicates. Topological robustness was assessed by bootstrap analysis with 1,000 replicates using simple taxon addition (Felsenstein 1985).

Appropriate nucleotide substitution models were determined using MrModeltest 2.3 (Nylander 2004) for all datasets and combined sequences following the Akaike information criterion and a model (GTR + G) was used for subsequent Bayesian analysis of the combined sequence data. Bayesian inference was conducted using MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003). One

cold and three heated MCMC chains were run for 10<sup>6</sup> generations, with trees sampled every ten generations using the random tree as the starting point and a temperature parameter value of 0.2 (the default setting of MrBayes). MCMC runs were repeated twice as a safeguard against spurious results. The first 10<sup>4</sup> trees were discarded as burn-in, and the remaining trees were used to construct Bayesian trees. Examination of the log-likelihoods and the observed consistency between runs suggested that the burn-in periods were sufficiently long for chains to have become stationary.

## Results

### Amplification, sequencing, and sequence characteristics

PCR amplifications of all three chloroplast regions for all the accessions included in this study were successful except for the sequences of *psbJ-petA* in *Chionanthus brachystachys* and *C. retusus*. Two length polymorphisms of *rpl32-trnL<sup>(UAG)</sup>* were observed within *Osmanthus*: sects. *Leiolea* and *Notosmanthus* had a PCR product of about 1,100 bp, but a product of about 750 bp in other sections. Due to problems in the sequencing of *rpl32-trnL<sup>(UAG)</sup>*, only 1,099 bp of aligned sequences were obtained. Poly-A/T regions were very frequent within *rps16-trnQ*, so only partial sequences of the region were obtained. GenBank accession numbers and information of the three chloroplast regions used are shown in supplementary Table 1 and in Table 1, respectively.

### Phylogenetic reconstructions

Phylogenetic analyses of *trnQ-rps16* and *rpl32-trnL* spacers provided similar topologies, in which *Osmanthus* formed three or four distinct lineages (supplementary Figs. 2 and 3). The dataset of *psbJ-petA* produced a tree of low resolution, but the polyphyly of *Osmanthus* was also obvious (supplementary Fig. 1). In the partition homogeneity test, there was significant incongruence among the three datasets ( $P = 0.024$ ), which might be a result of the uncertain position of *O. decorus* and *Phillyrea latifolia* (see supplementary Figs. 2 and 3). However, we still chose to combine all three regions for further phylogenetic analyses. The combined sequences (a total length of 3,289 bp) generated 14 most parsimonious (MP) trees (1,513 steps, consistency index, CI = 0.835; retention index, RI = 0.841). The 50% majority-rule tree (Fig. 1) clearly showed that *Osmanthus* was a polyphyletic group comprising four distinct lineages. The first lineage (lineage I) consisted of members of sect. *Leiolea* (bootstrap value, BP, 99%), which were closely related to members of *Olea* sect.

**Table 1** Summary of phylogenetic characteristics obtained from the analyses of three chloroplast intergenic spacers

	<i>rps16-trnQ</i>	<i>rpl32-trnL<sup>(UAG)</sup></i>	<i>psbJ-petA</i>	Combined
Substitution model	GTR + G	GTR + G	GTR + I + G	GTR + G
Within <i>Osmanthus</i> s. str.	18	18	18	18
Length range (bp)	2,000–2,200	500–700	1,000–1,200	3,500–4,000
Aligned length (bp)	1,048	590	862	2,500
Potentially informative characters (%)	7 (0.67)	6 (1.02)	1 (0.12)	14 (0.56)
Mean sequence divergence (K-2-p)	0.003	0.005	0.001	0.003
Mean G + C content (%)	29.6	27.6	32.5	30.1
Within <i>Osmanthus</i>	24	24	24	24
Length range (bp)	2,000–2,200	700–1,100	1,000–1,200	3,700–4,500
Aligned length (bp)	1,099	943	933	2,975
Potentially informative characters (%)	68 (6.19)	90 (9.54)	12 (1.29)	170 (5.71)
Mean sequence divergence (K-2-p)	0.011	0.052	0.004	0.018
Mean G + C content (%)	29.6	27.6	32.4	30.1
Within subtribe Oleinae	38	38	36	38
Length range (bp)	2,000–2,200	700–1,100	1,000–1,200	3,700–4,500
Aligned length (bp)	1,099	943	933	2,975
Potentially informative characters (%)	225 (20.47)	100 (10.60)	41 (4.39)	366 (12.30)
Mean sequence divergence (K-2-p)	0.033	0.056	0.011	0.037
All taxa	40	40	39	40
Length range (bp)	2,000–2,200	700–1,100	1,000–1,200	3,700–4,500
Aligned length (bp)	1,210	964	1,125	3,289
Potentially informative characters (%)	225 (18.60)	118 (12.24)	47 (4.18)	390 (11.86)
Mean sequence divergence (K-2-p)	0.035	0.067	0.013	0.051

K-2-p Kimura-2 parameter.

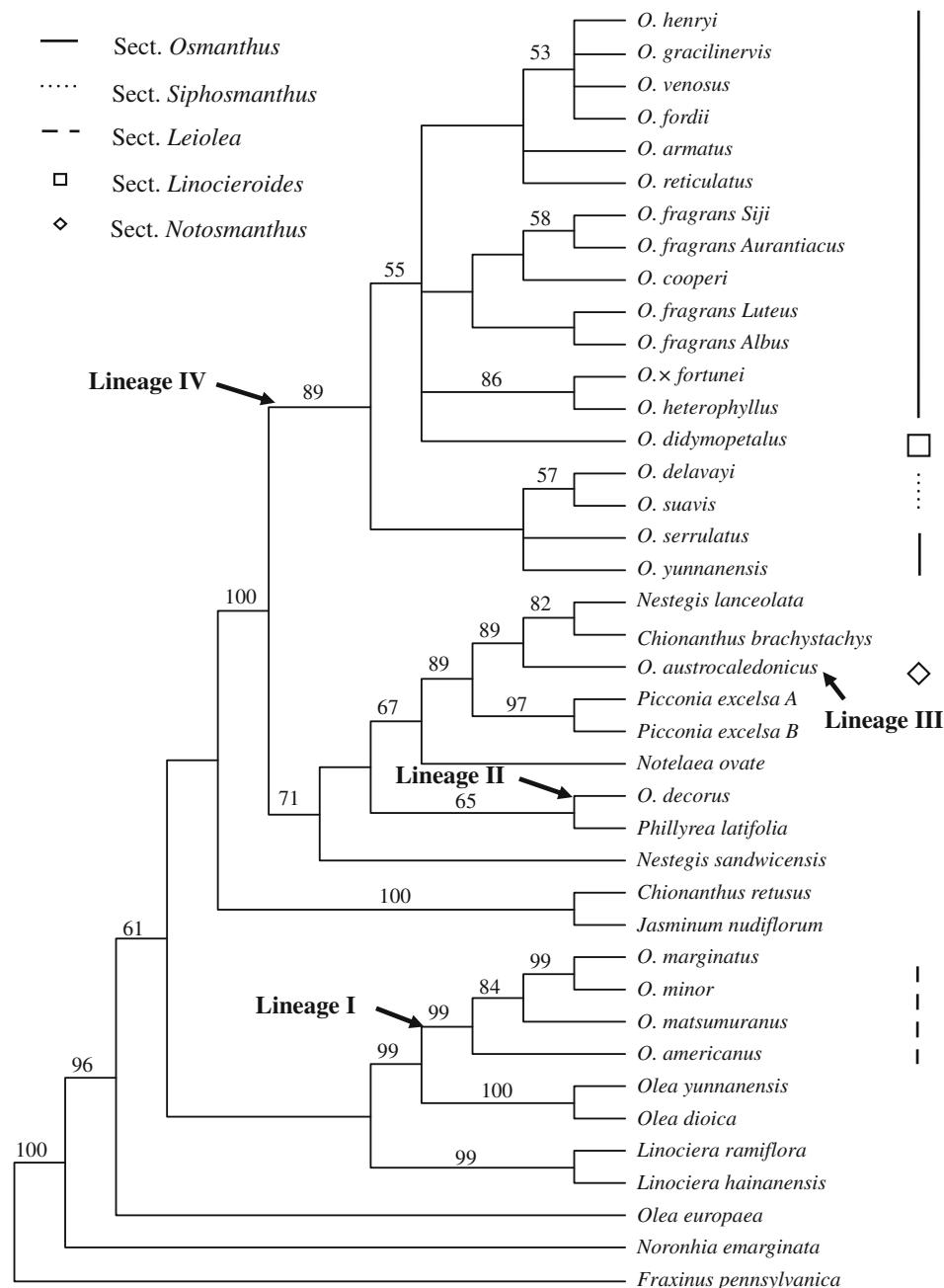
*Tetrapilus*. The second lineage (lineage II) was one member of sect. *Osmanthus*, *O. decorus*, forming a clade with *P. latifolia* (65% BP). The third lineage (lineage III) comprised one representative of sect. *Notosmanthus*, sister to a clade comprising *Nestegis lanceolata* and *C. brachystachys* (89% BP). The fourth lineage (lineage IV) consisted of members of the other three sections, i.e. sects. *Osmanthus*, *Siphosmanthus*, and *Linocieroides*, except for *O. decorus*, and received 89% bootstrap support.

Within lineage I, the American representative, *O. americanus* was sister to the three species distributed in Asia. Within lineage IV, four species, *O. serrulatus*, *O. yunnanensis*, *O. suavis*, and *O. delavayi*, which are characterized by blooming in spring (to early summer), formed a polytomy sister to a clade containing all other accessions of lineage IV. Excluding *O. serrulatus*, *O. yunnanensis*, *O. suavis*, and *O. delavayi*, other members within lineage IV were mostly unresolved, except for several subclades. For example, *O. × fortunei* and *O. heterophyllus* formed a subclade (86% BP), *O. henryi* and *O. gracilinervis*, *O. venosus*, and *O. fordii* grouped together with 53% BP support, and two cultivars of *O. fragrans*, *Siji* and *Aurantiacus*, clustered together (58% BP).

The Bayesian analysis of the combined dataset under the GTR + G model generated a similar topology to that of the MP tree, but with higher Bayesian posterior probability (PP) for most clades (Fig. 2). The main discrepancy between the two trees (Figs. 1 and 2) was the position of *O. decorus*. On the Bayesian tree, *O. decorus* was sister to all members of lineage IV (Fig. 2), but with low posterior probability (0.54 PP). Therefore, *O. decorus* might not represent a distinct lineage, and only three lineages were characterized by Bayesian analysis within *Osmanthus*.

## Discussion

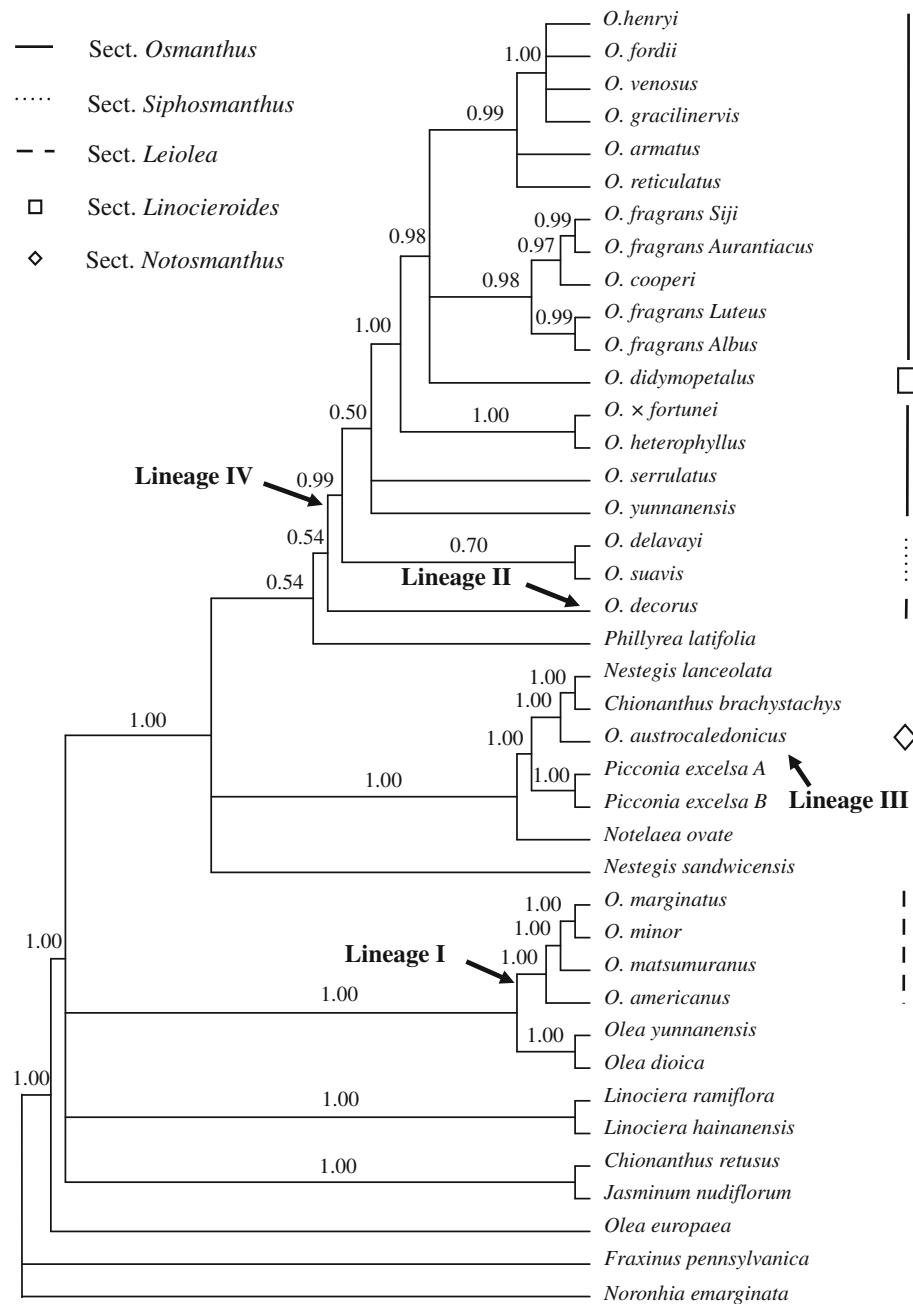
The monophyly of *Osmanthus* has been questioned by different authors (Baas et al. 1988; Jensen et al. 2002; Johnson 1957; Wallander and Albert 2000; Wallander 2001). However, Yang et al. (2009) concluded that *Osmanthus* is a monophyletic group based on chloroplast *matK* sequences. In this study, an extensive sampling of all the sections of *Osmanthus* and eight closely related genera convincingly shows that *Osmanthus* is a polyphyletic group containing three or four distinct lineages (Figs. 1 and 2).



**Fig. 1** The 50% majority-rule tree of 14 most parsimonious (MP) trees based on the combined dataset of three chloroplast intergenic spacers, *rps16-trnQ*, *rpl32-trnL<sup>(UAG)</sup>* and *psbJ-petA* (1,513 steps; consistency index, CI = 0.835, retention index, RI = 0.841). Clades with >50% bootstrap values are shown above the branches

The first distinct lineage found in our study is sect. *Leiolea* (lineage I in Figs. 1 and 2). The uniqueness of sect. *Leiolea* within *Osmanthus* can be easily observed in the majority of herbarium specimens for their paniculate inflorescence. For this reason, the North American member of sect. *Leiolea*, *O. americanus* has been treated as a distinct genus. In the phylogenetic reconstruction of Oleaceae, Wallander and Albert (2000) also found that *O. americanus* was distant from four members of sect. *Osmanthus*.

However, due to limited sampling and low resolution of DNA sequences in their study, the monophyly and affinities of sect. *Leiolea* with other genera had not been resolved. In this study, the monophyly of sect. *Leiolea* was well determined: all the four representatives from East Asia and North America formed a well-supported clade (99% BP and 1.0 PP). This finding agrees with the notion of Green (1958) that all the species of sect. *Leiolea* show remarkable affinity. This study also found that the most



**Fig. 2** The Bayesian tree based on the combined dataset of three chloroplast intergenic spacers, *rps16-trnQ*, *rpl32-trnL<sup>(UAG)</sup>* and *psbJ-petA*. Bayesian posterior probabilities are shown above the branches

closely related group of sect. *Leiolea* may be *Olea* sect. *Tetrapilus*, because two Asian *Olea* species from sect. *Tetrapilus* (*Olea yunnanensis* and *O. dioica*) form a sister clade to sect. *Leiolea* on both MP and Bayesian trees with high support (99% BP and 1.0 PP). This topology largely agrees with the ITS tree of Wallander (2001) and evidence can be found from their morphological similarities, i.e. sect. *Leiolea* and the genera of *Olea* and *Notelaea* (Green 1958), were all characterized by small, oblong, rounded stamens without the terminal appendage.

Jensen et al. (2002) have pointed out that *O. austrocaledonicus* contains different iridoid constituents from other members of *Osmanthus*, indicating that sect. *Notosmanthus* is a unique group within *Osmanthus*. On our MP and Bayesian trees, *O. austrocaledonicus* being nested within a clade containing members of *Nestegis*, *Chionanthus*, *Picconia*, and *Notelaea*, supports the idea of Jensen et al. (2002). Some evidence for this topology can also be found in the classification history of *O. austrocaledonicus*, along with the other two members of sect. *Notosmanthus*,

*O. monticola* and *O. cymosus*. They differ from the other members of *Osmanthus* by having a racemosus inflorescence and two of them were once segregated into the resurrected genus *Nestegis* Raf., along with some other Australasian species (Green 1963). In addition to being considered in the genus *Nestegis*, these species have at times been classified in the genus *Notelaea* (Green 1963). The results of this study agree with the traditional convention that sect. *Notosmanthus* may have closer affinity with *Nestegis* (Wallander 2001) than with other members of *Osmanthus*.

Excluding sects. *Leolea* and *Notosmanthus*, the other members of *Osmanthus* form a monophyletic clade on the Bayesian tree (Fig. 2). However, on the MP tree, *O. decorus* and *P. latifolia* form a distinct clade, sister to a clade containing members of *Notelaea*, *Picconia*, *Nestegis*, *Chionanthus*, and sect. *Notosmanthus* (Fig. 1), suggesting that the systematic position of *O. decorus* is uncertain. This species was first put into the genus *Phillyrea* but then transferred to *Osmanthus* (Green 1972). A hybrid species, *O. × burkwoodii*, derived from crossing between *O. decorus* and *O. delavayi*, suggests that *O. decorus* should be incorporated into *Osmanthus*. However, given the low support on the Bayesian tree for the grouping of *O. decorus* with lineage IV, drawing a conclusion as to the phylogenetic position of *O. decorus* should be done with caution until robust phylogenetic trees are available.

Another well-characterized lineage on both the MP and the Bayesian trees is lineage IV (hereafter referred to as *Osmanthus* s. str.), which contains sects. *Osmanthus* (except for *O. decorus*), *Siphosmanthus* and *Linocieroides*. Although this lineage is largely unresolved, several subclades are worthy of being noted. First, on the Bayesian tree, two members of sect. *Siphosmanthus* (*O. suavis* and *O. delavayi*) form a sister group to all other *Osmanthus* sens. str. members. Due to possessing several distinct characters within *Osmanthus*, such as long corolla tubes (7–11 mm), small leaves (less than 5 cm long), and thin endocarps (Green 1958; Xiang and Liu 2008), sect. *Siphosmanthus* was once recognized as a separate genus, *Siphonosmanthus* (Green 1958). Both molecular and morphological evidence suggests that this section may represent a distinct lineage with *Osmanthus* s. str. Second, *O. × fortunei*, a putative hybrid between *O. fragrans* and *O. heterophyllus*, groups with *O. heterophyllus* (86 BP and 1.0 PP), supporting the notion that *O. heterophyllus* is one of the parents (maternal parent here) of *O. × fortunei*. Third, the accessions of four cultivars do not form a monophyly, but group with *O. cooperi* (0.98 PP), implying multiple origins of cultivated sweet osmanthus. Multiple origins are not uncommon for cultivated plants (Olsen and Schaal 1999; Miller and Schaal 2005; Besnard et al. 2007). *O. fragrans* used to be widespread across southern China, as depicted in much

classical literature (e.g. *The Book of Songs*, Shi Jing), and several closely related species, such as *O. cooperi* sampled here, are codistributed with *O. fragrans*. Therefore, bringing several closely related species along with *O. fragrans* into cultivation is most likely and all of them could be treated as sweet osmanthus equally.

The flowers of *O. didymopetalus* in sect. *Linocieroides* are unique within *Osmanthus* in possessing a deeply divided corolla with the four almost free petals united in pairs at their very base. In this respect, it closely resembles the genus *Linociera* (Green 1958) rather than other members of *Osmanthus*. A cladistic analysis of *Osmanthus* also suggested that *O. didymopetalus* is unique within the genus, sister to all other species except sect. *Leolea* (Lu et al. 2007). However, *O. didymopetalus* clusters with members of sect. *Osmanthus*, indicative of close relationships among them. Indeed, besides the unique corolla in sect. *Linocieroides*, there are few diagnostic characters for recognizing sect. *Linocieroides*. Green (1958) claimed that the inflorescence and fruit characters of sect. *Linocieroides* agree with other species of *Osmanthus*, and might represent the furthest condition in floral reduction within *Osmanthus* that is also observed in *Olea*, *Notelaea* and other related genera.

Taken together, by using three informative chloroplast regions, this study confirms that *Osmanthus* is a polyphyletic group, containing three or four distinct lineages. This result may contribute significantly to the phylogenetic reconstruction of *Osmanthus*, as well as the taxonomic delimitation of genera within subtribe Oleinae. Additionally, this study provides valuable information concerning the origin of cultivated sweet osmanthus, which is of great importance for future management of the genetic resources of sweet osmanthus (Forest et al. 2007).

**Acknowledgments** The authors are indebted to Drs. Guillaume Besnard of the Department of Ecology and Evolution, University of Lausanne, Jeremy J. Brühl of NCW Beadle Herbarium (NE), James C. Solomon of the Herbarium Missouri Botanical Garden, Michael S. Dosmann and Kathryn Richardson of the Arnold Arboretum of Harvard University, Jordi López-Pujol of the Botanic Institute of Barcelona, and Philip Thomas of the Royal Botanic Garden Edinburgh, for their kind help in collecting DNA samples. Special appreciation goes to Dr. Eva Wallander for her valuable comments and linguistic help. This study is supported by the Natural Science Foundation of Jiangxi Province (0630047), the National Natural Science Foundation of China (30760016) and the Cultivation Program for Young Scientist of Jiangxi Province (2008DQ01500).

## References

- Baas P, Esser PM, van der Westen MET, Zandee M (1988) Wood anatomy of the Oleaceae. IAWA Bull 9:103–182
- Besnard G, Khadari B, Baradt P, Bervillé A (2002) *Olea europaea* (Oleaceae) phylogeography based on chloroplast DNA polymorphism. Theor Appl Genet 104:1353–1361

- Besnard G, Rubio de Casas R, Vargas P (2007) Plastid and nuclear DNA polymorphism reveals historical processes of isolation and reticulation in the olive tree complex (*Olea europaea*). *J Biogeogr* 34:736–752
- Besnard G, Rubio de Casas R, Christin PA, Vargas P (2009) Phylogenetics of *Olea* (Oleaceae) based on plastid and nuclear ribosomal DNA sequences: tertiary climatic shifts and lineage differentiation times. *Ann Bot* 104:143–160
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
- Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of incongruence. *Cladistics* 10:315–319
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Forest F, Grenyer R, Rouget M, Davies TJ, Cowling RM, Faith DP, Balmford A, Manning JC, Proches S, van der Bank M, Reeves G, Hedderson TA, Savolainen V (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature* 445:757–760
- Green PS (1958) A monographic revision of *Osmanthus* in Asia and America. *Notes Roy Bot Gard Edinb* 22:435–542
- Green PS (1963) A revision of the new Caledonian species of *Osmanthus*. *J Arnold Arb* 44:268–283
- Green PS (1972) *Osmanthus decorus* and disjunct Asiatic-European distributions in the Oleaceae. *Kew Bull* 26:487–790
- Heuertz M, Fineschi S, Anzidei M, Pastorelli R, Salvini D, Paule L, Frascaria-Lacoste N, Hardy OJ, Vekemans X, Vendramin GG (2004) Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Mol Ecol* 13:3437–3452
- Jensen SR, Franzky H, Wallander E (2002) Chemotaxonomy of the Oleaceae: iridoids as taxonomic markers. *Phytochemistry* 60:213–231
- Johnson LAS (1957) A review of the family Oleaceae. *Contrib N S W Natl Herb* 2:395–418
- Lee HL, Jansen RK, Chumley TW, Kim KJ (2007) Gene relocations within chloroplast genomes of *Jasminum* and *Menodora* (Oleaceae) are due to multiple, overlapping inversions. *Mol Biol Evol* 24:1161–1180
- Lu LD, Deng CL, Chang SH, Gao WJ, Qin RY, Ji CF, Xiang QB (2007) Studies on cladistic analysis of *Osmanthus*. *J Henan Norm Univ (Nat Sci)* 35:144–149
- Miller A, Schaal B (2005) Domestication of a Mesoamerican cultivated fruit tree, *Spondias purpurea*. *Proc Natl Acad Sci USA* 102:12801–12806
- Nylander JAA (2004) MrModeltest 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University
- Olsen KM, Schaal BA (1999) Evidence on the origin of cassava: phylogeography of *Manihot esculenta*. *Proc Natl Acad Sci USA* 96:5586–5591
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Shaw J, Lickey EB, Schiling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *Am J Bot* 94:275–288
- Swofford DL (2001) PAUP\*: phylogenetic analysis using parsimony (\*and other methods), version 4.0b10. Sinauer, Sunderland
- Taylor H (1945) Cyto-taxonomy and phylogeny of the Oleaceae. *Brittonia* 5:337–367
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882
- Wallander E (2001) Evolution of wind-pollination in *Fraxinus* (Oleaceae)—an ecophylogenetic approach. Dissertation, Göteborg University, Sweden
- Wallander E, Albert VA (2000) Phylogeny and classification of Oleaceae based on *rps16* and *trnL-F* sequence data. *Am J Bot* 87:1827–1841
- Xiang Q, Liu Y (2008) An illustrated monograph of the sweet *Osmanthus* cultivars in China. Zhejiang Science and Technology Press, Hangzhou
- Yang XQ, Deng CL, Liu LY, Sha T, Yu ML, Lu LD (2009) Phylogenetic analysis of genus *Osmanthus* (Oleaceae) based on *matK* sequence. *J Beijing Forest Univ* 31:9–14
- Zhang ZY, Li DZ (2004) Molecular phylogeny of section *Parrya* of *Pinus* (Pinaceae) based on chloroplast *matK* gene sequence data. *Acta Bot Sin* 46:171–179