Kastamonu Uni., Orman Fakültesi Dergisi, 2025, 25(3): 384-391 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1845395

Molecular Phylogeny of Medicinal-Aromatic Plant *Osmanthus decorus* from Artvin, Türkiye

Hayal AKYILDIRIM BEĞEN*[1,4], Özgür EMİNAĞAOĞLU[1], Perinçek Seçkinozan ŞEKER[2], Ahmet Yesari SELÇUK[2], Batuhan Yaman YAKIN[3], Nasibe TEKİNER AYDIN[1], Ayşegül SARAL SARIYER[4], Emrah SARIYER[5]

[1]Artvin Çoruh University, Ali Nihat Gökyiğit Botanical Garden Application and Research Center, Artvin, TÜRKİYE
[2]Artvin Çoruh University, Artvin Vocational School, Department of Forestry, Artvin, TÜRKİYE
[3]Avrasya University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Trabzon, Türkiye.
[4]Artvin Çoruh University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Artvin, TÜRKİYE
[5]Artvin Çoruh University, Vocational School of Health Services, Department of Medical Laboratory Techniques, Artvin, TÜRKİYE
*Corresponding Author: h.akyildirim@artvin.edu.tr

Received Date: 27.12.2024 Accepted Date: 12.06.2025

Abstract

Aim of study: The white flowers of *Osmanthus* species have been valued for centuries in medicinal and aromatic applications due to their distinct fragrance and chemical composition. Among the 27 species distributed globally, *Osmanthus decorus* is the only species endemic to the Caucasus region, found in Russia, Georgia, and Türkiye. *O. decorus* has a distinct distribution area around Artvin but has not been sufficiently studied at the molecular level regarding its differentiation from other species.

Material and method: This study focuses on identifying the molecular differences between Turkish *O. decorus* samples and other species while constructing their phylogenies. Phylogenetic relationships were analyzed using Bayesian inference with universal DNA barcode markers (*nrDNA ITS* and cpDNA *matK*).

Main results: The nuclear and chloroplast data were consistent, and all Caucasian *Osmanthus* specimens clustered into a strongly supported monophyletic group. As a result of the Bayesian analysis, it was determined that *O. decorus* does not clearly separate from other species within the genus.

Research highlights: According to the results of BLAST analysis, it was determined that the samples from Russia and Georgia, which are geographically close to the samples from Türkiye, were 100% matched.

Keywords*:* Artvin, DNA Barcod, Molecular, *Osmanthus decorus,* Türkiye

Artvin (Türkiye) Kökenli Tıbbi ve Aromatik Bitki *Osmanthus decorus*'un Moleküler Filogenetik Analizi

Öz

Çalışmanın amacı: Osmanthus türlerinin beyaz çiçekleri, kendine özgü kokuları ve kimyasal bileşimleri nedeniyle yüzyıllardır tıbbi ve aromatik amaçlarla kullanılmaktadır. Dünya üzerinde yayılış gösteren 27 türlerden sadece *Osmanthus decorus,* Kafkas endemiği olup, Rusya, Gürcistan ve Türkiye'de yayılış göstermektedir. Türkiye'de *O. decorus,* özellikle Artvin çevresinde yayılım göstermektedir; ancak, cinsin diğer türlerinden moleküler farkları yeterince araştırılmamıştır.

Materyal ve yöntem: Bu çalışmada, *O. decorus*'un Türkiye örneklerinin diğer türlerinden moleküler düzeyde farklılıklarının ortaya konulması ve filogenilerinin oluşturulması amaçlanmıştır. Evrensel DNA barkod işaretleyicileri (nrDNA ITS ve cpDNA matK) kullanılarak filogenetik ilişkiler Bayesian analizi ile değerlendirilmiştir.

Temel sonuçlar: Çekirdek ve kloroplast verileri tutarlılık göstermiş ve tüm Kafkas *Osmanthus* örnekleri güçlü bir şekilde desteklenen monofiletik bir grup içinde toplanmıştır. Bayesian analizi sonucunda, *O. decorus*'un cins içerisindeki diğer türlerden net bir şekilde ayrılmadığı belirlenmiştir.

Araştırma vurguları: BLAST analizi sonuçlarına göre, Türkiye örnekleri ile coğrafi olarak yakın olduğu rusya ve Gürcistan örnekleri ile %100 eşleştiği belirlenmiştir.

Anahtar Kelimeler: Artvin, DNA Barkod, Moleküler, *Osmanthus decorus,* Türkiye

Citation (Atıf): Akyildirim Begen, H., Eminagaoglu, O., Seckinozan Seker, P., Selcuk, A. Y., Yakin, B. Y., Tekiner Aydin, N., Saral Sariyer, A., & Sariyer, E. (2025). Molecular Phylogeny of Medicinal-Aromatic Plant *Osmanthus decorus* from Artvin, Türkiye. *Kastamonu University Journal of Forestry Faculty*, 25 (3), 384-391.

Introduction

The Oleaceae family is a medium-sized plant family comprising approximately 600 species and 25 genera. This family is distributed across all continents except Antarctica, ranging from the temperate regions of the north to the subtropical regions of the south, and from low to high altitudes (IPNI, 2024). Many plants belonging to the Oleaceae family are notable for their medicinal and aromatic properties. These plants are widely used in the pharmaceutical, cosmetic, food, and aromatherapy industries (Huang et al., 2019).

Osmanthus Lour. is a plant genus belonging to the Oleaceae family, known for its fragrant flowers. This genus is primarily distributed in East Asia, especially in China, Japan, and Southeast Asia (Figure 1). The most well-known species is *Osmanthus fragrans* Lour. (sweet olive), which is used as an ornamental plant, an aromatherapy product, and for medicinal purposes. The *Osmanthus* plant can take the form of a shrub or a tree and bears small, pleasantly scented flowers that are white, yellow, or orange in color. *Osmanthus* essential oil, extracted from its flowers, is used in aromatherapy due to its calming and soothing effects on the nervous system. This oil supports emotional balance and promotes mental relaxation. In China, *Osmanthus* tea is particularly popular as a herbal beverage that supports health. Additionally, it is used to add flavor and aroma to fruit syrups, desserts, and non-alcoholic beverages (Li and Huang, 2020; Eminağaoğlu, 2023a, Eminağaoğlu et al., 2023b). The genus *Osmanthus* is part of the subfamily Oleinae (Oleaceae) and includes 27 evergreen tree and shrub species (Banki et al., 2024; IPNI, 2024) (Green, 2004). Based on Green's contributions (1958, 1963), these taxa are divided into five sections: O. sect. *Leiolea* (Spach) P.S. Green, O. sect. *Linocieroides* P.S. Green, O. sect. *Osmanthus*, O. sect. *Siphosmanthus* Franch. and O. sect. *Notosmanthus* P.S. Green. *O. heterophyllus* (G. Don) P.S. Green (Xiang & Liu, 2008), *O. fragrans* (Thunb.) Lour., and *O. delavayi* Franch. taxon are commonly known and used. Some species are 'endangered' or 'vulnerable' status because of decreasing population size (Melia et al., 2012; Qin et al., 2017; Tamar, 2020).

Molecular approaches have facilitated the delimitation of intergeneric boundaries (Dupin et al., 2022; Li et al., 2022). Two of the most commonly used gene regions for molecular-level species identification and phylogenetic analysis in plants are nrDNA ITS (the Internal Transcribed Spacer region of nuclear ribosomal DNA) and cpDNA matK (the maturase K gene of chloroplast DNA). The matK gene, located within the chloroplast genome, plays a role in the splicing of introns from certain genes involved in photosynthesis. The ITS region is a rapidly evolving, non-coding segment of nuclear ribosomal DNA, and it generally provides high resolution for distinguishing species at the taxonomic level (Aoki & Ito, 2000; Sevindik et al., 2024; Zhang et al., 2022). However, within the genus, conflicts persist between nuclear and plastid gene trees, indicating the presence of reticulate evolution (Guo et al., 2011; Li et al., 2020; Yuan et al., 2010). Morphologically, species lack distinct traits for clear differentiation, and there is significant trait overlap. For instance, the leaf blades of *O. urceolatus* P.S.Green resemble those of *O. cooperi* Hemsl., while its floral traits are similar to those of *O. armatus* Diels. Moreover, geographically distant species such as *O. decorus* and *O. delavayi* can produce hybrids like *O. burkwoodii* (Burkwood & Skipwith) P.S.Green (Green, 1972). This suggests that hybridization and introgression may influence species relationships within the genus *Osmanthus.* Recently, DNA barcoding has become a popular diagnostic technique aimed at identifying species by uncovering DNA sequences. This technique also helps reveal phylogenetic relationships among living groups (Lahaye et al., 2008). Many studies have been conducted in which species identifications were made using the DNA barcoding method (Lahaye et al., 2008).

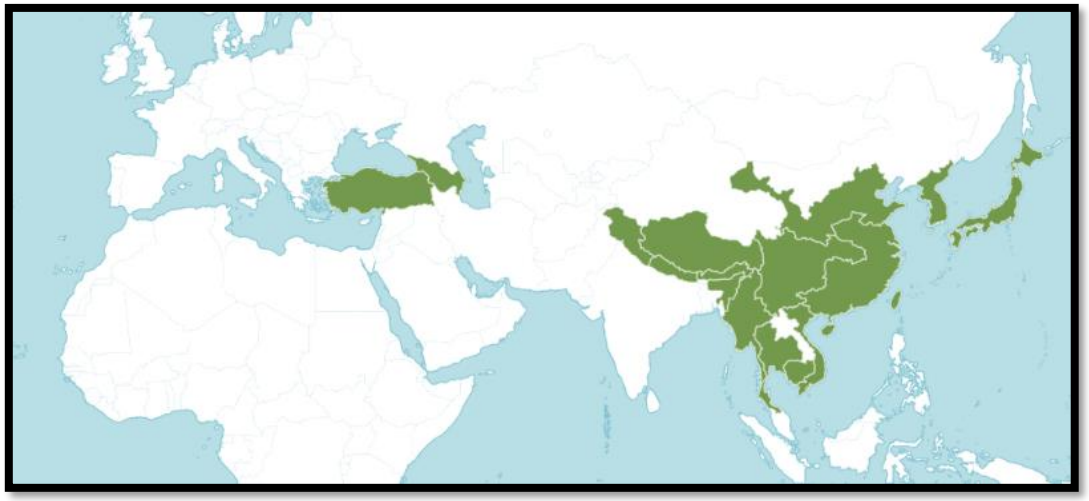

Figure 1. Geographical distributions of *Osmanthus* species throughout the World (IPNI, 2024)

O. decorus Kasapligil is distributed in the Caucasus regions of Asia and others in East Asia, from Tibet to Japan, Korea and southeastern China (Green, 1958; Chang et al., 1996; Eminağaoğlu, 2015; Eminağaoğlu, 2023a; POWO, 2024). The lack of obvious distinctive traits and synapomorphies shared between or within genera has led to a lack of consensus in delimiting the genus *Osmanthus* based solely on morphological data (Ji, 2004; Lu et al., 2008; Shang et al., 2012). However, recent molecular phylogenetic studies (Wallander & Albert, 2000; Yuan et al., 2010; Guo et al., 2011; Lu et al., 2011) have shown that *Osmanthus* is not a monophyletic group, challenging previous taxonomic views based on morphology. These studies have contributed significantly to the understanding of the phylogenetic relationships of *Osmanthus*.

Osmanthus decorus has a district distribution area around Artvin, but has not been sufficiently addressed in molecular studies on differentiation from other species. In this study, it was aimed to reveal the gene sequences of *O. decorus*, which is distributed in Türkiye, by studying the two barcoding gene regions (chloroplast matK and nucleus ITS DNAs) and to highlight the phylogenetic distance of the genus from other species.

Material and Methods

Collecting Plant Specimens and DNA extraction

Osmanthus decorus specimens were sampled from Borçka (Artvin, Türkiye) in northeastern Türkiye (Figure 2). In 2023, the specimens collected from 800-1500 m a.s.l. and preserved in Artvin Çoruh University Herbarium (ARTH) numbered as *AÇÜ-18477*.

Figure 2. *Osmanthus decorus* locations from Artvin, Türkiye

Species keys in Flora of Turkey and the East Aegean Islands were used for the identification of the specimens (Davis et al., 1988). One to three leaves (40-50 g) were taken for identification and finalized, and dried using silica gel at room temperature to facilitate DNA extraction. The DNA barcoding study was conducted in three stages: DNA extraction, partial amplification of matK-ITS, and data collection followed by phylogenetic analysis. Leaf tissues were processed for DNA extraction using the DNeasy Plant Mini Kit (Qiagen), adhering to

the instructions provided by the manufacturer. Total DNA concentrations were assessed using a NanoDrop (Thermo Microvolume UV-Vis) and verified through 2% agarose gel electrophoresis. PCR amplification was performed with the DNA obtained.

Table 1. Universal primers used in this study

Gen region	Primer	Sequences	References
ITS	ITS-5-R	GGAAGTAAAAGTCGTAACAA	Källersjö et al., 2005
	ITS-4-F	TCCTCCGCTTATTGATATGC	Oceguera-Figueroa, 2012
matK	1326-R	TCTAGCACACGAAAGTCGAA	Shaw et al., 2007
	390-F	CGATCTATTCATTCAATATT	Shaw et al., 2007

The partial matK region (Shaw, Lickey, Schilling, & Small, 2007) and partial ITS region (Källersjö, Von Proschwitz, Lundberg, Eldenäs, & Erséus, 2005; Oceguera-Figueroa, 2012) gene regions of *O. decorus* were studied (Table 1). PCR reaction mixture and PCR condution are given in Table 2 and 3. Sequence analysis in both directions (Forward and Reverse, Tablo 1) was performed by ABI 3100 Genetic Analyzer, Macrogene.

Table 2. PCR reaction mixture

Compenents	Quantity	Final concentration
10X Taq Buffer	5.0 µl	1X
10 mM dNTPmix	1.3 µl	0.27 mM
10 µM Primer (Foward)	0.5 µl	0.1 µM
10 µM Primer (Reverse)	0.5 µl	0.1 µM
25 mM MgCl2	3 µl	1.5 mM
BSA (Bovine Serum Albumin) 10mg/ml	4 µl	0.8 mg/ml
sdH2O	33.45 µl	---
Taq DNA polymerase Polymerase (5u/µl)	0.25 µl	1.25 u
Total DNA	2 µl	---
Total	50 µl	

Table 3. The PCR condition of chloroplast (matK) and nuclear gene (ITS) regions

PCR Steps	°C	Time	Cycle
Pre-denaturation	95°C	5 minute	
Denaturation	94°C	30 second	
Anneling of primers	48-52 °C	1.30 minute	40 cycles
Extension	72°C	1 minute	
Final extension	72°C	10 minute	
Hold	+4	Infinite	

Phylogenetic Analyses

The matK and ITS gene region of the taxon was aligned using MAFFT (Multiple sequence alignment) in Geneious prime 2025.0.3. Other sequence data of the studied taxa were obtained from GenBank database (Table 4). In phylogenetic analyses, *Olea parvilimba* (EU409441) and *Picconia excels* (LR031501) species were selected as outgroups. BI methods analyses were performed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001).

Results and Discussion

In this study, partial *ITS* (621 bp) and partial *matK* (837 bp) gene regions of the *O. decorus* sample (sample no: TAB-50) were obtained. The sequences of these gene regions were deposited in GenBank under the accession number PX764321. The BLAST analysis of the obtained gene regions with the sequences stored in GenBank revealed that the *ITS* data obtained in this study had the same sequence as those in GenBank under accession numbers OR544235/39 - LR031496 - KJ188968, and the *matK* gene region showed the same sequence as the GenBank entry MK299398 (Table 4).

Table 4. Blast results of the *ITS* and *matK* sequences of *Osmantus decorus (*sample no: TAB-50)

Sample no	Organism (current study)	Gen region	Accession	Location	Pairwise	References
TAB-50	*Osmantus decorus*	*ITS*	OR544235	China	%100	Li et al., (2024)
			OR544236	China	%100	Li et al., (2024)
			OR544237	China	%100	Li et al., (2024)
			OR544238	China	%100	Li et al., (2024)
			OR544239	China	%100	Li et al., (2024)
			LR031496	Russia	%100	Besnard (2019) (Unpublished data)
			KJ188968	Georgia	%100	Kondraskov et al., (2015)
		maturase K	JX862650	Georgia	%99.837	Hong and Besnard, (2013)
			MK299398	Russia	%100	Olofsson et al., (2019)

In this study, the *ITS* sequence obtained showed 100% similarity with the sample from Georgia (KJ188968), which is geographically close. However, a single nucleotide difference was found with another Georgia sample (JX862650), and this difference is attributed to a deletion in the Georgia sample. A similar situation applies to the matK gene region. The *matK* gene region obtained in this study shows 100% similarity with the sample from Russia (MK299398), which is geographically close. However, due to the short lengths of the partial *ITS* and *matK* gene sequences, phylogenetic relationships might be blurred (Rosenberg and Kumar, 2003). Therefore, conducting phylogenetic analyses with longer gene regions (more nucleotides) could lead to more accurate taxonomic relationships (Dai et al., 2024).

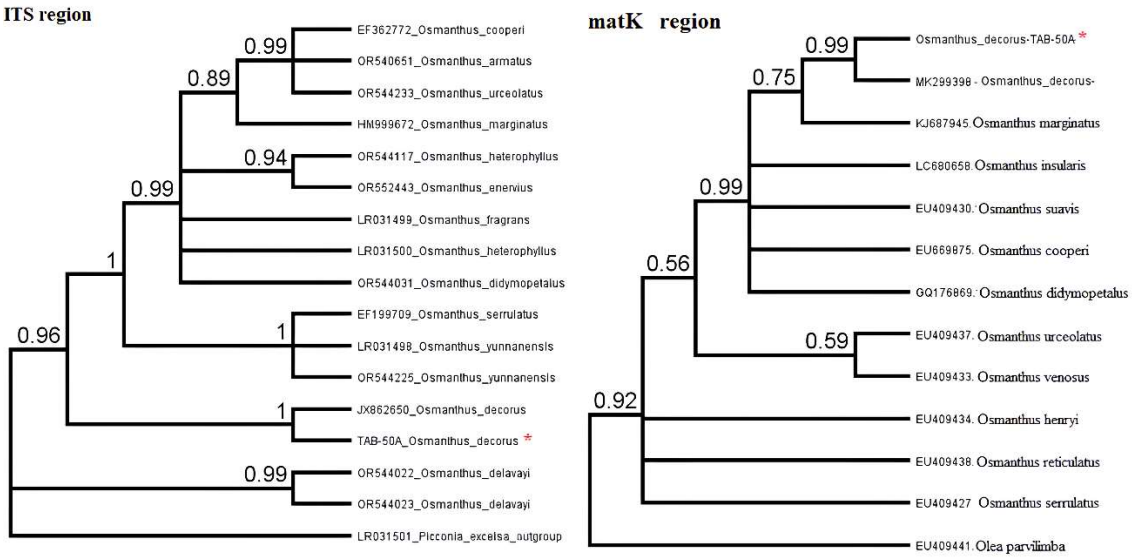

Figure 3. *Osmanthus decorus* phylogenetic relationships for the partial *ITS* and *matK* gene regions, based on the Bayesian Inference (BI) tree (3000000 generation, model: GTR+I+G)

The phylogenetic tree constructed from *matK, ITS* gene regions sequences were clustered into the one main groups. *O. decorus* wasn't separated from other *Osmanthus* species clearly. As a result of phylogenetic analysis, it was determined that *O. decorus* was close to other *Osmanthus* (Figure 3). The resulting trees do not align with previous studies (Li et al., 2024).

While *O. decorus* is expected to be the earliest diverging group, the *ITS* gene region failed to show this separation, and the *matK* gene region produced a result different from the literature (Olofsson et al., 2019) (Figure 3). Li et al. (2020) revealed genetic differences and evolutionary relationships among species using mainly nrDNA *ITS* and several chloroplast DNA regions (e.g., *matK*, *rbcL*). Their study found that the genus *Osmanthus* is paraphyletic, and they proposed reclassifying some species into a new genus, *Chengiodendron.*

Conclusions

The findings of this study suggest that using only a few universal primers (ITS + matK) is not a completely effective method for amplifying, identifying, and distinguishing plant species. It indicates that studying additional gene regions or the entire genome would yield clearer results. *O. decorus* species, in addition to being medicinal and aromatic, has a limited distribution in the Caucasus region. It is important to reveal the genetic differentiation of this species, as providing molecular-level differentiation from other species is crucial for its identification and conservation. Analyses were conducted with samples collected for the first time from Türkiye, and a reference genetic data has been established. In conclusion, additional sequence data from highly variable gene regions and broader sampling across a wider geographic range are required to thoroughly investigate the phylogenetic relationships within *Osmanthus.*

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: H.A.B., B.Y.Y., Ö.E.; Investigation: H.A.B., B.Y.Y., A.Y.S., P.S.Ş.; Material and Methodology: H.A.B., Ö.E., B.Y.Y., A.Y.S., P.S.Ş., E.S., A.S., N.T.A.; Visualization: Y.B.Ö.; H.A.B., A.Y.S., B.Y.Y.; Writing-Original Draft: H.A.B., A.Y.S., B.Y.Y.; Writing-review & Editing: H.A.B. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This study is being conducted as part of the project numbered 2021-TAB-04 under the Coordination Office for the Specialization in Medicinal and Aromatic Plants at Artvin Çoruh University, Türkiye.

References

Aoki, S. & Ito, M. (2000). Molecular phylogeny of *Nicotiana* (Solanaceae) based on the nucleotide sequence of the matK gene. *Plant biology*, 2(03), 316-324.

Bánki, O., Roskov, Y., Döring, M., Ower, G., Hernández Robles, D. R., Plata Corredor, C. A., Stjernegaard Jeppesen, T., Örn, A., Pape, T., Hobern, D., Garnett, S., Little, H., DeWalt, R. E., Ma, K., Miller, J., Orrell, T., Aalbu, R., Abbott, J. Aedo, C. & et al. (2024). *Catalogue of Life* (Version 2024-12-19). Catalogue of Life, Amsterdam, Netherlands. https://doi.org/10.48580/dglq4

Chang, M. C., Qiu, L. Q. & Green, P. S. (1996). Oleaceae. *Flora of China*, 272-319. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis.

Dai, J., Liu, Q., Tan, Z., Cai, Y., Gao, X. & Zhu, S. (2024). Comparative and phylogenetic analysis of the complete chloroplast genomes of *Osmanthus* (Oleaceae) species. *Authorea Preprints*, 1-31.

Davis, P. H., Chamberlain, D. F. & Matthews, V. A. (1972). *Flora of Turkey* (Vol. 4, pp. xviii+-657).

Dupin, J., Hong-Wa, C., Pillon, Y. & Besnard, G. (2022). From the Mediterranean to the Pacific: Re-circumscription towards *Notelaea* s.l. and historical biogeography of a generic complex in Oleinae (Oleaceae). *Botanical Journal of the Linnean Society*, 200, 360-377.

Eminağaoğlu, Ö. (Ed.). (2015). *Artvin'in Doğal Bitkileri*. İstanbul: Promat.

Eminağaoğlu, Ö. (Ed). (2023a). *Artvin'in Tıbbi-Aromatik Bitkileri*. Artvin: Zafer Medya.

Eminağaoğlu, Ö., Akyıldırım Beğen, H., Salioğlu, Ş., Açıkgöz Harşıt, C., Aslan, B., Tekiner Aydın, N., Yazıcıoğlu, E., Özcan, M., Erşen Bak, F. & Durmaz, A. (2023b). Artvin'in Bazı Tıbbi Aromatik Bitkilerinin İçerik ve Kullanım

Alanları, s. 317-356. In Eminağaoğlu, Ö. (Ed.), *Artvin'in Tıbbi-Aromatik Bitkileri*, Artvin: Zafer Medya.

Green, P. S. (1958). A monographic revision of *Osmanthus* in Asia and America. *Notes from the Royal Botanical Garden Edinburgh*, 22, 439-542.

Green, P.S. (1963). A revision of the New Caledonian species of *Osmanthus*. *Journal of the Arnold Arboretum*, 44(2), 268-283.

Green, P.S. (1972). *Osmanthus decorus* and disjunct Asiatic-European distributions in the Oleaceae. *Kew Bulletin*, 26, 487-490.

Green, P.S. (2004). Oleaceae. In *Flowering plants, dicotyledons: Lamiales (except Acanthaceae including Avicenniaceae)*, 296-306. New York: Springer.

Guo, S.Q., Xiong, M., Ji, C.F., Zhang, Z.R., Li, D.Z. & Zhang, Z.Y. (2011). Molecular phylogenetic reconstruction of *Osmanthus* Lour. (Oleaceae) and related genera based on three chloroplast intergenic spacers. *Plant Systematics and Evolution*, 294, 57-64.

Huang, Y. L., Oppong, M. B., Guo, Y., Wang, L. Z., Fang, S. M., Deng, Y. R. & Gao, X. M. (2019). The Oleaceae family: A source of secoiridoids with multiple biological activities. *Fitoterapia*, 136, 104155.

Hong-Wa, C. & Besnard, G. (2013). Intricate patterns of phylogenetic relationships in the olive family as inferred from multi-locus plastid and nuclear DNA sequence analyses: a close-up on *Chionanthus* and *Noronhia* (Oleaceae). *Molecular phylogenetics and Evolution*, 67(2), 367-378.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8), 754-755.

IPNI. (2024). *International Plant Names Index*. The Royal Botanic Gardens, Kew; Harvard University Herbaria & Libraries; Australian National Herbarium. http://www.ipni.org (Accessed 20-december-2024).

Ji, C. F., Xiang, Q. B. & Xu, B. S. (2004). Characters of leaf micromorphology of the genus *Osmanthus* (Oleaceae) and its application in systematic taxonomy. *Journal of Nanjing Forestry University*, 47(04), 54.

Källersjö, M., Von Proschwitz, T., Lundberg, S., Eldenäs, P. & Erséus, C. (2005). Evaluation of ITS rDNA as a complement to mitochondrial gene sequences for phylogenetic studies in freshwater mussels: an example using Unionidae from north-western Europe. *Zoologica Scripta*, 34(4), 415-424.

Kondraskov, P., Schütz, N., Schüßler, C., de Sequeira, M. M., Guerra, A. S., Caujapé-Castells, J., ... & Thiv, M. (2015). Biogeography of mediterranean hotspot biodiversity: re-evaluating the'tertiary relict'hypothesis of macaronesian laurel forests. *PloS one*, 10(7), e0132091.

Lahaye, R., Van Der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T. G. & Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 2923-2928.

Li, Y. F., Zhang, M., Wang, X. R., Sylvester, S. P., Xiang, Q. B., Li, X. & et al. (2020) Revisiting the phylogeny and taxonomy of *Osmanthus* (Oleaceae) including description of the new genus Chengiodendron. *Phytotaxa*, 436, 283-292

Li, Y. F., Li, X., Sylvester, S. P., Zhang, M., Wang, X. R. & Duan, Y. F. (2022) Plastid genomes reveal evolutionary shifts in elevational range and flowering time of *Osmanthus* (Oleaceae). *Ecology and Evolution*, 12, e8777.

Li, Y., Li, X., Nie, S., Zhang, M., Yang, Q., Xu, W., ... & Wang, X. (2024). Reticulate evolution of the tertiary relict *Osmanthus*. *The Plant Journal*, 117(1), 145-160.

Lu, L. D., Chang, S. H. & GAO, W. J. (2008) The structure of leaf micromorphology of *Osmanthus*. *Journal of Nanjing Forestry University*, 32, 52-56.

Lu, L. D., Duan, H. Y., Lu, Z. Y., Li, X. W., Duan, Z. Q. & Sun, X. (2011). Studies on ITS sequences and systematic classification of *Osmanthus*. *Pakistan Journal of Botany*, 43(2), 759-771.

Melia, N., Gabedava, L., Barblishvili, T. & Jgenti, L. (2012). Reproductive biology studies towards the conservation of two rare species of Colchic flora, *Arbutus andrachne* and *Osmanthus decorus*. *Turkish Journal of Botany*, 36(1), 55-62.

Oceguera-Figueroa, A. (2012). Molecular phylogeny of the New World bloodfeeding leeches of the genus Haementeria and reconsideration of the biannulate genus Oligobdella. *Molecular Phylogenetics and Evolution*, *62*(1), 508-514.

Olofsson, J. K., Cantera, I., Van de Paer, C., Hong-Wa, C., Zedane, L., Dunning, L. T., ... & Besnard, G. (2019). Phylogenomics using low-depth whole genome sequencing: A case study with the olive tribe. *Molecular ecology resources*, *19*(4), 877-892.

POWO (2024). Plants of the world online. Kew: Royal Botanic Gardens.

Tamar, N. (2020). *Growth-development peculiarities of some rare and endangered*

plants in nature and culture. *International Journal of Life Sciences*, 4, 37-41.

Rosenberg, M. S., & Kumar, S. (2003). Taxon sampling, bioinformatics, and phylogenomics. *Systematic Biology*, 52(1), 119.

Sevindik, E., Korkom, Y., & Murathan, Z. T. (2024). Evaluating DNA barcoding using cpDNA mat K and rbc L for species identification and phylogenetic analysis of *Prunus armeniaca* L.(Rosaceae) genotypes. Genetic *Resources and Crop Evolution*, 71(5), 1825-1835.

Shaw, J., Lickey, E. B., Schilling, E. E., Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, 94, 275-288.

Shang, F. D., Han, Y. J., Yuan, W. J. & Dong, M. F. (2012). Review on classification of Osmanthus and *Osmanthus fragrans* Cultivars. *Journal of Henan University*, 42, 608-612. https://doi.org/10.15991/j.cnki.411100.2012.05.015

Qin, H. N., Yang, Y., Dong, S. Y., He, Q., Jia, Y., Zhao, L., & et al. (2017). Threatened species list of China's higher plants. *Biodiversity Science*, 25, 696-744.

Wallander, E., & Albert, V. A. (2000). Phylogeny and classification of Oleaceae based on rps16 and trnL-F sequence data. *American Journal of botany*, 87(12), 1827-1841.

Wu, L., Liu, J., Huang, W., Wang, Y., Chen, Q., & Lu, B. (2022). Exploration of *Osmanthus fragrans* Lour.'s composition, nutraceutical functions and applications. *Food Chemistry*, 377, 131853.

Xiang, Q.B., & Liu, Y.L. (2008). *An illustrated monograph of the sweet Osmanthus cultivars in China*. Hangzhou: Zhejiang Science and Technol-ogy Press.

Yuan, W. J., Zhang, W. R., Han, Y. J., Dong, M. F., & Shang, F. D. (2010). Molecular phylogeny of Osmanthus (Oleaceae) based on non-coding chloroplast and nuclear ribosomal internal transcribed spacer regions. *Journal of Systematics and Evolution*, 48, 482-489.

Zhang, J., Chi, X., Zhong, J., Fernie, A., Alseekh, S., Huang, L., & Qian, D. (2022). Extensive nrDNA ITS polymorphism in Lycium: Non-concerted evolution and the identification of pseudogenes. *Frontiers in plant science*, 13, 984579.