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A MOLECULAR PHYLOGENETIC STUDY ON SOME TURKISH *FERULA* L. (*APIACEAE*) SPECIES USING nrDNA ITS SEQUENCES

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Abstract

nrDNA ITS sequence variation of *Ferula* species were studied by comparing with the other species of *Ferula*, *Leutea* and *Dorema* to clarify relationships amongst the taxa and infrageneric delimitation of the genus *Ferula*. In total, 35 nrDNA ITS sequence accessions (34 species) of *Ferula*, *Leutea* and *Dorema* were included in the analyses. 10 of these accessions were newly sequenced and belonged to Turkish species. Possible phylogenetic relationships amongst the species were determined using Maximum parsimony and neighbour joining tree methods. The analyses showed that the genus might be monophyletic upon the addition of *Leutea* and *Dorema*, but did not support the classification of the genus dividing into the subgenera.

Introduction

Ferula L. is the third largest genus in the family *Apiaceae*. The genus contains 180–185 species (Pimenov & Leonov, 2004) and distributed mainly central and south-west Asia, but also occur as far east as North India and in the Mediterranean basin (Ajani & Ajani, 2008). The first revision of the genus *Ferula* in Turkey was performed by Peşmen (1972). He recognized 18 species, of which one was incompletely known and nine were endemic. Two new species have since been added to the Flora of Turkey (Duman & Sağıroğlu 2005; Sağıroğlu & Duman 2007a), and also the one incompletely known species was collected and redescribed by Sağıroğlu & Duman (2007b). 55 % of Turkish species (11 of 20 in total) are endemic. This endemism ratio shows that Turkey is a sericeous gene centre of the genus.

Turkish *Ferula* species are monocarpic or polycarpic herbaceous perennial plants growing to 20–500 cm tall and hollow. The leaves are tri-seven pinnate, with generally a stout basal sheath clasping the stem. The flowers are yellow, without or with reduced sepals in large umbels. Mericarps are dorsally compressed with generally 1-3 vittae per vallecula on dorsal surface.

Boissier (1872) recognised 29 *Ferula* species, dividing the genus into three sections based on vittae number and petal shape. These sections are: I. *Peucedanoides* Boiss., *Euferula* Boiss. and. *Scorodosma* Bunge. The most comprehensive study on the genus *Ferula* was proposed by Korovin (1947) in his monograph, in which he recognised six subgenera and sections. Although Korovin's monograph was subsequently modified by himself (1951), his taxonomical treatment of the genus has not been commonly followed by the other taxonomists. Peşmen (1972) in *Flora of Turkey* did not recognise any of the subgenus and section. In *Flora Iranica* Chamberlain and Rechinger (1987a,b) retained Korovin's subgenera.

There have been some studies on the phylogeny of the family *Apiaceae* (Downie & Katz-Downie, 1996; Downie *et al*., 1998; Valiejo-Roman *et al*., 1998; Katz-Downie *et al*., 1999; Downie *et al*., 2000a,b,c; Lee & Downie 2000; Downie *et al*., 2001; Ajani *et al*., 2008; Kurzyna-Młynik *et al*., 2008). The genus *Ferula* has long been regarded as a monophyletic genus because its members are similar in habit and morphology (Kurzyna-Młynik *et al*., 2008), but recent molecular studies stated that there is a controversy on both upper and lower level classification of the genus *Ferula*. Pimenov & Leonov (1993) recognised the genus in the tribe *Peucedaneae*. Downie *et al*., (2001) in their comprehensive molecular phlogenetic work, stated that the genus *Ferula,* based on three species they studied could not be placed into any tribe or clade, but the apioid superclade. Ajani *et al*., (2008) concluded that *Ferula* group including *Dorema, Leutea* and *Ferula* is in the tribe *Scandiceae*, based on ITS sequence analysis.

Kurzyna-Młnik *et al*., (2008) stated that in recent molecular systematic investigations, *Ferula* appeared as polyphyletic, and their study based on the data of nrDNA ITS variation supported the monophyly of the genus upon the addition of *Dorema* and *Leutea* (as *Ferula* sensu lato). Ajani *et al*., (2008) investigated the relationships within five genus groups of of *Apiaceae* subfamily *Apioideae* native to the *Flora Iranica* region using nrDNA ITS sequences with suplemantary data especially from morphology. They redefined five groups, in which *Ferula* alongside *Dorema* and *Leutea* was placed in *Ferula* group, stated that *Dorema* and *Leutea* arise from within a paraphyletic *Ferula* and suggested nomenclatural changes.

The aim of this study is to investigate the molecular data obtained from nrDNA ITS sequences of the Turkish *Ferula* species and to understand their contribution to species and infrageneric delimitation of the genus and to find out their possible phylogenetic position within the species of the genus *Ferula*.

Materials and Methods

The materials, used for isolating DNA extracts in this study were seeds of 10 *Ferula* species (Table 1), of which one is probably a new species, collected and identified by the third author from Turkey. Four, probably five species studied are endemic to the country. Voucher specimens are kept in GAZI herbarium (Table 2).

Table 1. The Turkish species studied and their probable infrageneric placement is as follows:

1	Subgenus: *Merwia* (B. Fedtsch.) Drude	*Ferula szowitsiana* DC.
2	Subgenus: Narthex (Falc.) Drude	*Ferula coskunii* H. Duman & M.Sağıroğlu *Ferula mervynii* M. Sağıroğlu & H. Duman
2	Subgenus: *Peucenadoides* (Boiss.) Korovin	*Ferula hermonis* Boiss. *Ferula orientalis* L. *Ferula halophila* Peşmen *Ferula haussknechtii* Wolf ex Rech. *Ferula elaeochytris* Korovin *Ferula longipedunculata* Peşmen

Table 2. Voucher specimens of complete nrDNA ITS sequences of Turkish *Ferula* species used in this study. All specimens are deposited in GAZI herbarium, Ankara.

Species	Voucher numbers and herbaria
F. halophila	M. Sağıroğlu 2146, GAZI
F. elaeochytris	M. Sağıroğlu 2227, GAZI
F.longipedunculata	M. Sağıroğlu 2235, GAZI
Ferula. sp.	M. Sağıroğlu 2181, GAZI
F. haussknechtii	M. Sağıroğlu 2255, GAZI
F. szowitsiana	M. Sağıroğlu 2147, GAZI
F. mervynii	M. Sağıroğlu 2262, GAZI
F. coskunii	M. Sağıroğlu 2270, GAZI
F. orientalis	M. Sağıroğlu 2170, GAZI
F. hermonis	M. Sağıroğlu 2246, GAZI

Laboratory Procedures

DNA Isolation: Total genomic DNA was isolated from endosperms of three dried mericarps of each *Ferula* species studied, powdered after peeling the rest, according to McDonald *et al*., (1994) with slight modifications (see Fig. 1 for electrophoresis pictures).

Amplification of ITS Region: Complete nrDNA ITS regions in each genomic DNA of the species were PCR amplified using universal primers ITS5 and ITS4 (White *et al*., 1990). Each PCR reaction cycle included 1 min. at 94ºC for denaturation of the template DNA, 1 min. at 53ºC for annealing primers and 1 min. at 72ºC for extention of primers. PCR reaction was achieved as 35 thermal cycles and followed 10 min. at 72ºC extension for completion. Each amplified DNA fragment was run in a 1.5% agarose gel (see Fig. 2 for electrophoresis pictures). Although DNA of almost all Turkish *Ferula* species was succesfully extracted, only 10 gave good results in PCR amplification.

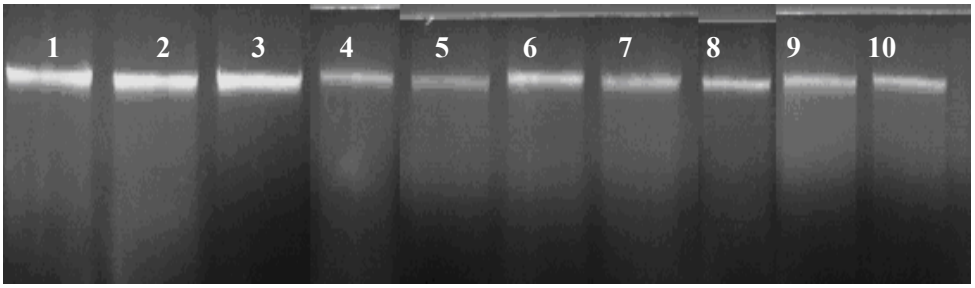

Fig. 1. Genomic DNA of *Ferula halophila* (1) *Ferula elaeochytris* (2), *Ferula longipedunculata* (3), *Ferula* sp. (4), *Ferula haussknechtii* (5), *Ferula szowitsiana* (6), *Ferula mervynii* (7), *Ferula coskunii* (8), *Ferula orientalis* (9), *Ferula hermonis* (10) run on 1 % gel electrophoresis.

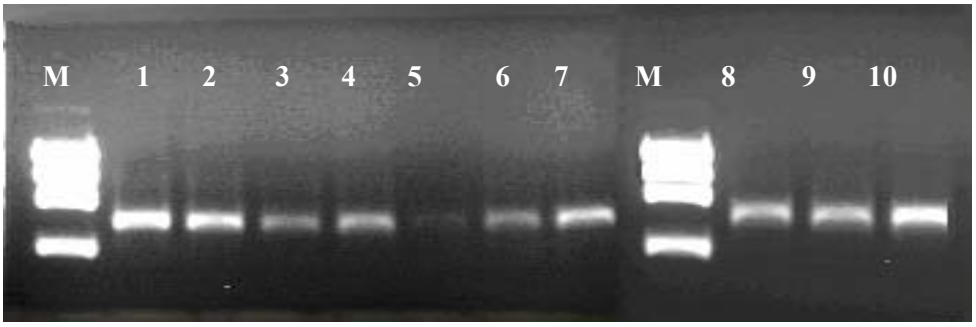

Fig. 2. nrDNA ITS PCR region amplication results of Ferula halophila (1) Ferula elaeochytris (2), Ferula longipedunculata (3), Ferula sp. (4), Ferula orientalis (5), Ferula hermonis (6), Ferula haussknechtii (7), Ferula szowitsiana (8), Ferula mervynii (9), Ferula coskunii (10) run on 1.5 % gel electrophoresis

Sequencing and pyhylogenetic analyses: nrDNA ITS base sequences of 10 Turkish *Ferula* species were obtained using the ABI 310 DNA sequence of Middle East Technical University Central Laboratory. ITS sequences of 25 species belonging to *Ferula*, *Leutea* and *Dorema*, and also the species of *Orlaya grandiflora, Torilis japonica, Chaerophyllum temulum, Smyrnium olusatrum* and *Aciphylla squarrosa* used as outgroups in the analyses were obtained from the gene bank (see Appendix). The sequences were aligned using CLUSTALW. nrDNA ITS data matrix was produced with MacClade 4. 03 (Maddison & Maddison 1992). Phylogenetic analyses included maximum parsimony (MP) and neighbour joining (NJ) methods and were performed using PAUP 4.0b10 (Swafford 1998). The data matrix was analysed for MP by assuming unordered character states. Heuristic searches were replicated 1000 times with random addition sequence, tree bisection-reconnenction (TBR) branch swapping. Pairwise nucleotid distances of unambiguously aligned sequences were determined using distance matrix options in PAUP 4.0b10 (Swafford, 1998). Maximum parsimony trees were performed to produce a majority rule consensus tree (Fig. 3). NJ analysis (Saitou & Nei, 1987) was also performed and distance trees were calculated using the maximumum likelihood method in PAUP 4.0b.10 program (see Fig. 4).

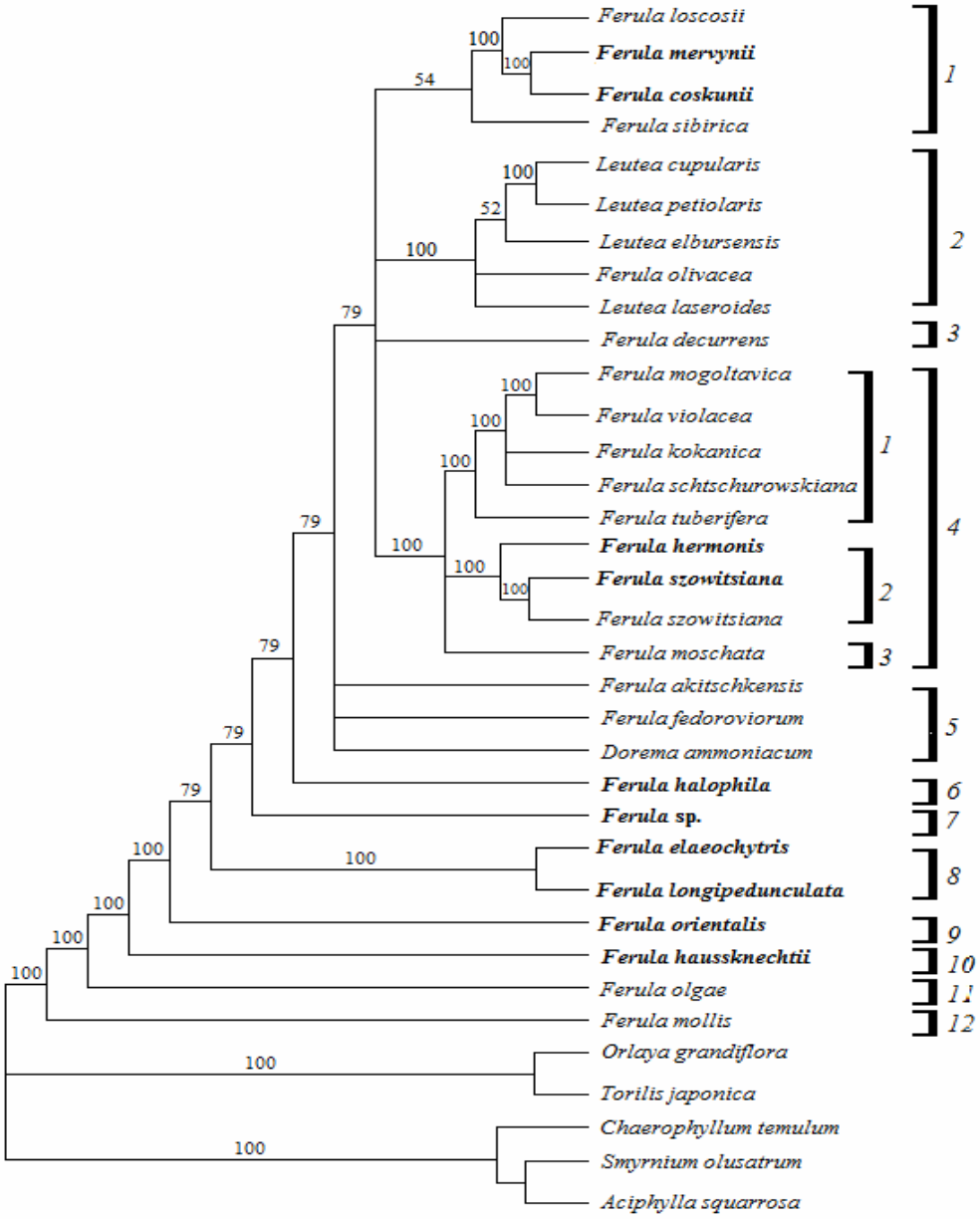

Fig. 3. Majority rule consensus of 71 trees with the length of 525 steps derived from maximum parsimony analysis of nrDNA ITS sequences of 34 species (35 accessions) (consistency indices (CI) = 0.7105, homoplasy indices of (HI) = 0.2895 with uninformative characters and CI = 0.5836 and HI = 0.4164 with exluding uninformative characters and retention index, RI = 0.5824). Numbers above branches are majority rule consensus values. The species written in bold are newly sequenced Turkish species.

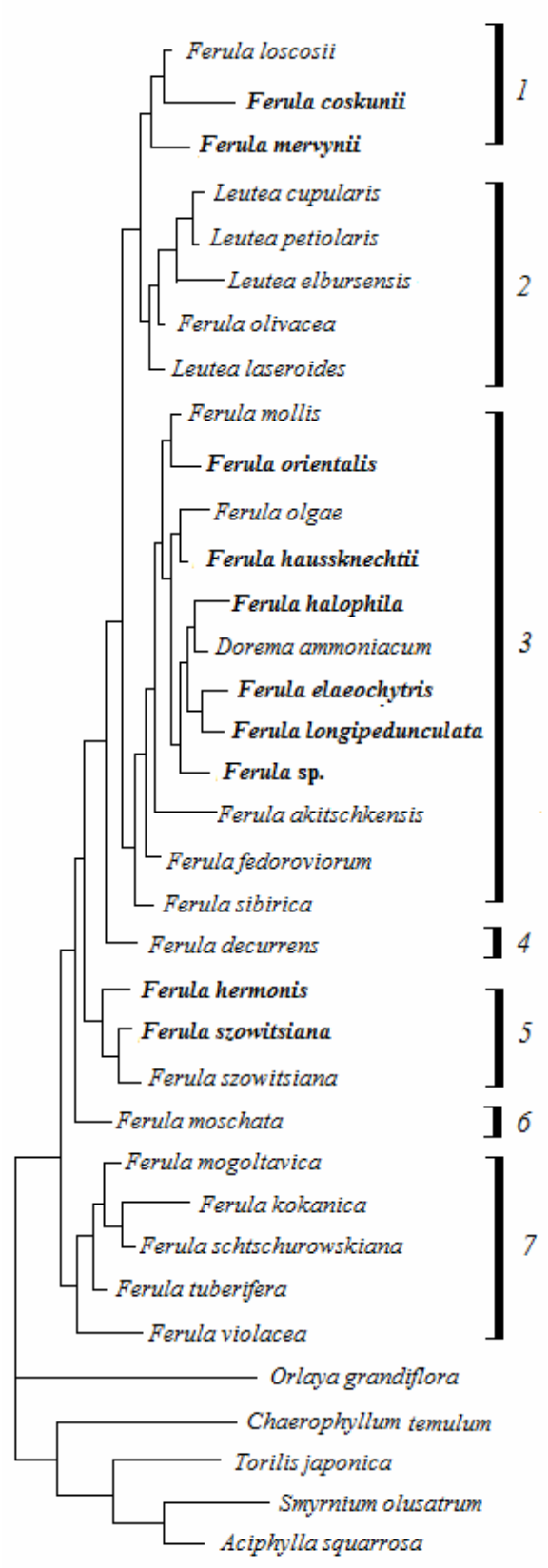

Fig. 4. Neighbor-joining tree inferred from aligned ITS sequences of nrDNA ITS sequences 34 species (35 accessions). The species written in bold are newly sequenced Turkish species.

Results and Discussion

DNA sequencing and alignment: Complete length of the nrDNA ITS region (including ITS1, 5.8S and ITS2) in Turkish *Ferula* species studied, varies from 692 to 700 bp. The length of the ITS is 692 bp in *F. halophila* and *Ferula* sp., 693 bp in *F. mervynii*, 694 bp in *F. szowitsiana* and *F. coskunii*, 696 bp in *F. orientalis*, 697 bp in *F. longipedunculata*, 698 bp in *F. elaeochytris* and 700 bp in *F. haussknechtii*. GenBank reference numbers of the ITS regions for the specimens used in the analyses are given in Appendix. Complete ITS sequences were potentially informative to perform analyses for producing possible phylogenetic trees. The result of aligned ITS sequence of the species using CLUSTALW produced 738 characters, of which 456 nucleotide of them constant, 137 nukleotide variable. In direct pairwise comparisons of sequences among all Turkish *Ferula* species studied, base differences ranged from 0.00574 to 0.06237.

Phylogenetic analysis: Maximum parimony searches resulted in 71 maximally parsimonious trees. Majority rule consensus of these trees with the values is presented in Fig. 3. These trees have a length of 525 steps, consistency indices (CI) of 0.7105, homoplasy indices of (HI) 0.2895 with uninformative characters and CI of 0.5836 and HI of 0.4164 with exluding uninformative characters and retention index, RI of 0.5824.

In the analyses of maximum parsimony and neighbour-joining of *Ferula, Dorema* and *Leutea* species, *Dorema* and *Leutea* come alongside *Ferula* species as in the studies of of Kurzyna-Młnik *et al*., (2008) and Ajani *et al*., (2008). The analyses supported very little the classification of the genus dividing into the subgenera. In maximum parsimony analyses the majority consensus tree produced 12 lineages within a *Ferula* group (Ferula sensu lato). The first lineage consists of four species, *F. loscosii*, *F. mervynii*, *F. coskunii* and *F. sibirica*, of which the two are turkish species forming a small subclade. *F. mervynii* and *F. coskunii* are very closely related species (Sağıroğlu & Duman, 2007 b) and share some morphologically important characters used at the level of species and sometimes subgenus within the genus. Both species have glabrous and membranous sheaths; lax paniculate-corymbose inflorescence; umbellules with 20-55 flowers; 1-2mm long, eliptic-oblong petals and oblong-orbicular mericarps with 0.1-0.5mm lateral wings (Duman & Sağıroğlu 2005; Sagiroglu & Duman, 2007). This subclade seems congruent with the subgenus *Narthex*, of which inflorescence is lax paniculate; sheath membranous and petal 1-2mm long (Chamberlain 1987 a-b, Korovin 1951). However, the other species *F. loscosii* and *F. sibirica* belong to the subgenera *Ferula* and *Peucenadoides*. *F. sibirica* and *F. loscosii* were taken place in group B and sister group C respectively in the study of Kurzyna-Młnik *et al*., (2008). Four species of *Leutea* (*L. cupularis, L. petiolaris, L. elbursensis and L. laseroides*) and *Ferula olivacea* formed the second clade. Third lineage constitutes a single species, *F. decurrens* of

the subgenus *Merwia.* Fourth lineages includes three subclades and the first one constitutes of 5 *Ferula* species, *F. mogoltavica, F. violacea, F. kokanica, F. schtschurowskiana* and *F. tuberifera* belonging to the sugenera *Merwia, Narthex, Ferula, Dorematoides* and *Ferula,* respectively. The second subclade consists of only two Turkish species, *F. hermonis and F. szowitsiana*. In this subclade Turkish *F. szowitsiana* came together with the *F. szowitsiana* sequence accession obtained from the gene bank. Third subclade constitutes a single species of *Peucenadoides, F. moschata. F. akitschkensis, F. fedoroviorum* and *Dorema ammoniacum* formed a politomies. Two Turkish species *F. elaeochytris* and *F. longipedunculata* both formed a dichotomic clade. Cladogram placed two Turkish Ferulas, *F. halophila* and *Ferula* sp. between the dichotomic and politomic clades. Ninth, tenth, eleventh and twelfth lineages each constitues a single species, *F. orientalis, F. haussknechtii* (these 2 are Turkish species), *F. olgae* and *F. mollis* respectively.

Neighbour joining tree is partly similar to the majority rule consensus tree. Seven groups are distinguished in the three, which are evident in majority rule consensus tree. First and second groups are identical with the the first and second clade in the absence of *F. sibirica* from the first clade. Third group in NJ tree consists of the lineages with only one and two species near to base of the cladogram except *F. sibirica*. Fourth group constitues a single species, *F. decurrens*. Fifth, sixth and seventh groups are identical with the subclades of the third clade, the second, third and first subclade respectively.

As a conlusion this study showed similarities with some earlier studies. According to the results of the analyses, it might be said that the genus *Ferula* is monophyletic upon the addition of *Leutea* and *Dorema* (as *Ferula* sensu lato) (Kurzyna-Młnik *et al*., 2008). Clades on the cladogram are mostly not congruent with the subgenera of the genus *Ferula*. Therefore it is said that this study does not strongly support the subdivision of the genus into subgenera.

References

Ajani, Y. and M. Ajani. 2008. A new species of *Ferula* (*Umbellıferae*) from southern Iran. *Edinb. J. Bot.,* 65: 425-431.

Ajani, Y., A. Ajani, J.M. Cordes, M.F. Watson and S.R. Downie. 2008. Phylogenetic analysis of nrDNA ITS sequences reveals relationships within five groups of Iranian *Apiaceae* subfamily *Apioideae*. *Taxon,* 57(2): 383-401.

Boissier, E. 1872. *Umbelliferae*. In: (Ed.): E. Boissier, *Flora Orientalis.* H. Georg., Geneva, 2: 819-1090.

Chamberlain, D.F. and K.H. Rechinger. 1987a. *Ferula* L. In: (Ed.): K.H. Rechinger, *Flora Iranica.* Akad Druk-u Verlagsanst, Graz and Wien, 162: 387-426.

Chamberlain, D.F. and K.H. Rechinger. 1987b. *Ferula* L. In: (Ed.): K.H. Rechinger, *Flora Iranica.* Akad Druk-u Verlagsanst, Graz and Wien, 162: 317–384.

Downie, R. and D.S. Katz-Downie. 1996. A molecular phylogeny of *Apiaceae* subfamily *Apioideae*: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Am. J. Bot.,* 83(2): 234-251.

Downie, S.R., D.S. Katz-Downie and K. Spalik. 2000b. A phylogeny of *Apiaceae* tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Am. J. Bot.,* 87(1): 76-95.

Downie, S.R.**,** D.S. Katz-Downie and M.F. Watson. 2000a. A phylogeny of the flowering plant family *Apiaceae* based on chloroplast DNA rpl16 and rpoC1 intron sequences: towards a suprageneric classification of subfamily *Apioideae*. *Am. J. Bot.,* 87: 273-292.

Downie, S.R., G.M. Plunkett, M.F. Watson, K. Spalik, D.S. Katz-Dawnie, C.M. Valiejo-Roman, E.I. Terentieva, A.V. Troitsky, B.Y. Lee, J. Lahham and A. El-Oqlah. 2001.Tribes and clades within *Apiaceae* subfamily *Apioideae*: the contribution of molecular data. *Edinb. J. Bot.,* 58(2): 301-330.

Downie, S.R., S. Ramanath, D.S. Katz-Downie and E. Llanas. 1998. Molecular systematics of *Apiaceae* subfamily *Apioideae*: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid rpoC1 intron sequences. *Am. J. Bot.,* 85: 563-591.

Downie, S.R..**,** M.F. Watson, K. Spalik and D.S. Katz-Downie. 2000c. Molecular systematics of Old World *Apioideae* (*Apiaceae*): relationships among some members of tribe *Peucedaneae* sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade. *Can. J. Bot.,* 78: 506-528.

Duman, H. And M. Sağıroğlu. 2005. A new species of *Ferula* (*Apiaceae*) from South Anatolia, Turkey. *Bot. J. Linn. Soc.,* 147: 357-361.

Katz-Downie, D.S., C.M. Valiejo-Roman, E.I. Terentieva, A.V. Troitsky, M.G. Pimenov, B. Lee and S.R. Downie. 1999. Towards a molecular phylogeny of *Apiaceae* subfamily *Apioideae*: additional information from nuclear ribosomal DNA ITS sequences. *Pl. Syst. Evol.,* 216**:** 167-195.

Korovin, EP. (1951). *Ferula* L. In: (Ed.): B.K. Schisckin, *Flora of the USSR*. XVII (*Umbelliflorae*). Akademii Nauk SSSR, Moscow, Leningrad, pp. 44-3101.

Korovin, E.P. 1947. *Generis Ferula (Tourn.). L. monographia illustrata*. Academiae Scientiarum UzRSS, Tashkent.

Kurzyna-Młynik, R., A.A. Oskolski, S.R. Downie, R. Kopacz, A. Wojewódzka and K. Spalik. .2008. Phylogenetic position of the genus *Ferula* (*Apiaceae*) and its placement in tribe *Scandiceae* as inferred from nrDNA ITS sequence variation. *Plant Syst. Evol.,* 274: 47.

Lee, B.Y. and S.R. Downie. 2000**.** Phylogenetic analysis of cpDNA restriction sites and rps16 intron sequences reveals relationships among *Apiaceae* tribes *Caucalideae*, Scandiceae and related taxa. *Pl. Syst. Evol.,* 221: 35-60.

Madison, W.P. and D.R. Madison. 1992. MacClade, Analysis of phylogeny and character evolution, version 3 edition, Sinauer Associates, Sunderland, Mass.

McDonald, M., L. Eliot and P. Sweeney. 1994. DNA extraction from dry seeds for RAPD analyses in varietal identification studies. *Seed. Sci. Tech.* 22: 171-176.

Peşmen, H. 1972. *Ferula* L. In: (Ed.): P.H. Davis, *Flora of Turkey and the East Aegean Islands.* Edinburg University Pres, Edinburg, 4: 440-453.

Pimenov, M.G. and M.V. Leonov. 1993. *The Genera of the Umbelliferae*. Whitestable Litho, Royal Botanic Gardens, Kew.

Pimenov, M.G. and M.V. Leonov. 2004. The Asian *Apiaceae* biodiversity database (ASIUM) with particular reference to south-west Asian taxa. *Turk. J. Bot.,* 6: 139-145.

Sağıroğlu, M. and H. Duman. 2007a. *Ferula mervynii* (*Apiaceae*), a distinct new species from north-east Anatolia, Turkey. *Bot. J. Linn. Soc.,* 153: 357-362.

Sağıroğlu, M. and H. Duman. 2007b. *Ferula parva* Freyn & Bornm. (*Apiaceae*): some contribution to an enigmatic species from Turkey. *Turk. J. Bot.,* 30: 399-404.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.,* 4: 406-425.

Swafford, D.L. 1998. *PAUP. Phylogenetic analysis using parsimony (and other methods) vers. 4.0b 10.* Sinaver, Sunderland, Massachusetts.

Valiejo-Roman, C.M., E.I. Terentieva, T.H. Samigullin, M.G. Pimenov, F. Ghahremani-Nejad, V. Mozaffarian. 2006. Molecular data (nrITS-sequencing) reveal relationships among Iranian endemic taxa of Umbelliferae. *Feddes Repert.,* 117: 367-388

Valiejo-Roman, C.M., M.G. Pimenov, E.I. Terentieva, S.R. Downie, D.S. Katz-Downie and A.V. Troitsky. 1998. Molecular systematics of the *Umbelliferae*: using nuclear rDNA internal transcribed spacer sequences to resolve issues of evolutionary relationships. *Bot. Zhurnal.,* 83: 1-22.

White, T.J., T.D. Bruns, S.B. Lee and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR-Protocols and Applications-A Laboratory Manual*. (Eds.): N. Innis, D. Gelfand, J. Sninsky and J. White. Academic Pres, New York, pp 315-322.

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