

**ITS GENE BASED MOLECULAR GENOTYPING OF *NEPETA SHEILAE*
HEDGE & R.A. KING (LAMIACEAE) ENDEMIC TO SAUDI ARABIA**

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The genus *Nepeta* L. (family Lamiaceae), commonly known as ‘catmint’ or ‘catnip’, is represented by c. 300 species (Kaya and Dirmenci, 2008), distributed in Asia, Europe, North Africa and America (Jamzad *et al.*, 2000), morphologically characterized by herbaceous, perennial or annuals, sturdy stem and green to greyish-green cordate leaves (Jamzad *et al.*, 2003). In the flora of Saudi Arabia, the genus *Nepeta* is represented by two species i.e. *N. deflersiana* Schweinf. and *N. sheilae* Hedge & R.A. King. *N. sheilae* is endemic to Saudi Arabia, mainly distributed in northern Hizaz mountains (Chaudhary, 2000). The morphological characters of *N. sheilae* i.e. woody-based, lamina triangular ovate, inflorescence verticillaster, many-flowered, bracteoles narrowly linear-lanceolate, corolla exerted, curved, nutlets brown, apically verrucose or tuberculate etc. overlap with *N. deflersiana* (Chaudhary, 2000). The morphology of *N. sheilae* (Chaudhary, 2000) resembles with section *Oxynepeta*, and the section *Oxynepeta* is consistent in the generic classification of *Nepeta* proposed by Bentham (1848), Briquet (1896) and Budantsev (1993), which are characterized by herbaceous habit; bracts green, inconspicuous; inflorescence interrupted, verticillaster or lax, pedunculate cymes; middle lobe of the lower lip of corolla concave with dentate margin; pollen bi-reticulate, rarely perforate reticulate; and pollen primary muri well-defined, prominent, while secondary muri inconspicuous (Jamzad *et al.*, 2000). Though the phylogenetic relationships in the genus *Nepeta* and other related genera of Lamiaceae have previously been inferred using ITS sequences of nrDNA, the taxonomic status of *N. sheilae* is unresolved (Jamzad *et al.*, 2003). The nrDNA ITS sequence is well known plant DNA barcoding gene widely applied to represent evolutionary relationships at lower taxonomic ranks, notably at the intrageneric ones (Ali, 2019); hence, the present study aims to resolve the taxonomic status of *N. sheilae* using molecular genotyping of ITS sequence of nrDNA.

The leaves of *N. sheilae* for sequencing were collected from the herbarium specimen [Voucher information: Jabal Lakus Lauz area, South of Haql NW side, 20.5.1990, I.S. Collette 13417 (RIY)]. The total genomic DNA was isolated using Qiagen DNeasy Plant Mini Kit (Valencia, CA, USA). The nrDNA ITS sequence was amplified using ITS primer (White *et al.*, 1990), and sequenced using ABI PRISM 3100 DNA Analyzer (Perkin-Elmer, Applied Biosystems). In order to unravel the proximity of *N. sheilae* with the members of Nepetoideae, the

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nrDNA ITS sequence of *N. sheilae* was analyzed together with the highly similar sequence revealed from the BLAST search i.e. *N. heliotropifolia*, morphologically similar to *N. deflersiana*, and the representative from six different genera belongs to the family Lamiaceae, subfamily Nepetoideae, and outgroup *Paulownia tomentosa* (Thunb.) Steud. (family Paulowniaceae) and *Pedicularis groenlandica* Retz. (family Orobanchaceae) following previous studies (Li *et al.*, 2016) retrieved from the GenBank.

The present study revealed that the length of nrDNA ITS region (ITS1, 5.8S and ITS2) in *N. sheilae* sequenced was found to be 640 nucleotides. The generated sequence of *N. sheilae* was submitted to GenBank (accession number MN907379). Among all the *Nepeta* taxa included in the study, *N. sheilae* showed the highest sequence similarity of 97.66% with *N. heliotropifolia* (Table 1) in the BLAST-searched (Altschul *et al.*, 1990).

Table 1. Results of BLAST search of nrDNA ITS sequence of *Nepeta sheilae*.

Sl. No.	Taxa	Max. Score	Total Score	Query cover (%)	Percent identity (%)	GenBank accession number
1	<i>Nepeta heliotropifolia</i> Lam.	1099	1099	100	97.66	AJ515312.1
2.	<i>Nepeta congesta</i> Fisch. & C.A. Mey.	1096	1096	100	97.51	AJ515161.1
3.	<i>Nepeta scrophularioides</i> Rech.f.	1077	1077	100	97.04	AJ515319.1
4.	<i>Nepeta cataria</i> L.	1077	1077	100	97.04	AJ515313.1
5.	<i>Nepeta kurdica</i> Hausskn. & Bornm.	1074	1074	100	96.88	AJ515320.1
6.	<i>Nepeta isaurica</i> Boiss. & Heldr. <i>ex</i> Benth.	1064	1064	100	96.72	AJ515306.1
7.	<i>Nepeta deflersiana</i> Schweinf.	1053	1053	100	96.41	KF765442.1

The phylogenetic analyses of the aligned dataset [CLUSTALX v.1.81 (Thompson *et al.*, 1997)] using Minimum Evolution method (Rzhetsky and Nei, 1992) in MEGA4 (Tamura *et al.*, 2007) were performed. The positions containing gaps and missing data were eliminated from the aligned dataset. There were a total number of 470 positions in the final dataset, out of which 93 were parsimony informative. Variation in the base pair between the sequence of *N. sheilae* and *N. deflersiana* is shown in Fig. 1. The molecular phylogenetic relationships of *N. sheilae* with its closely related *N. heliotropifolia* and *N. deflersiana*, and other members of the subfamily Nepetoideae are illustrated in Fig. 2.

The base pair differences between the sequence of *N. sheilae* and *N. deflersiana*, and the close proximity of *N. sheilae* with *N. heliotropifolia* (bootstrap 47%) in the phylogenetic tree revealed harmony with the BLAST search result; thus, we herein recognized *N. sheilae* as a distinct species and is different from morphologically similar *N. deflersiana*.

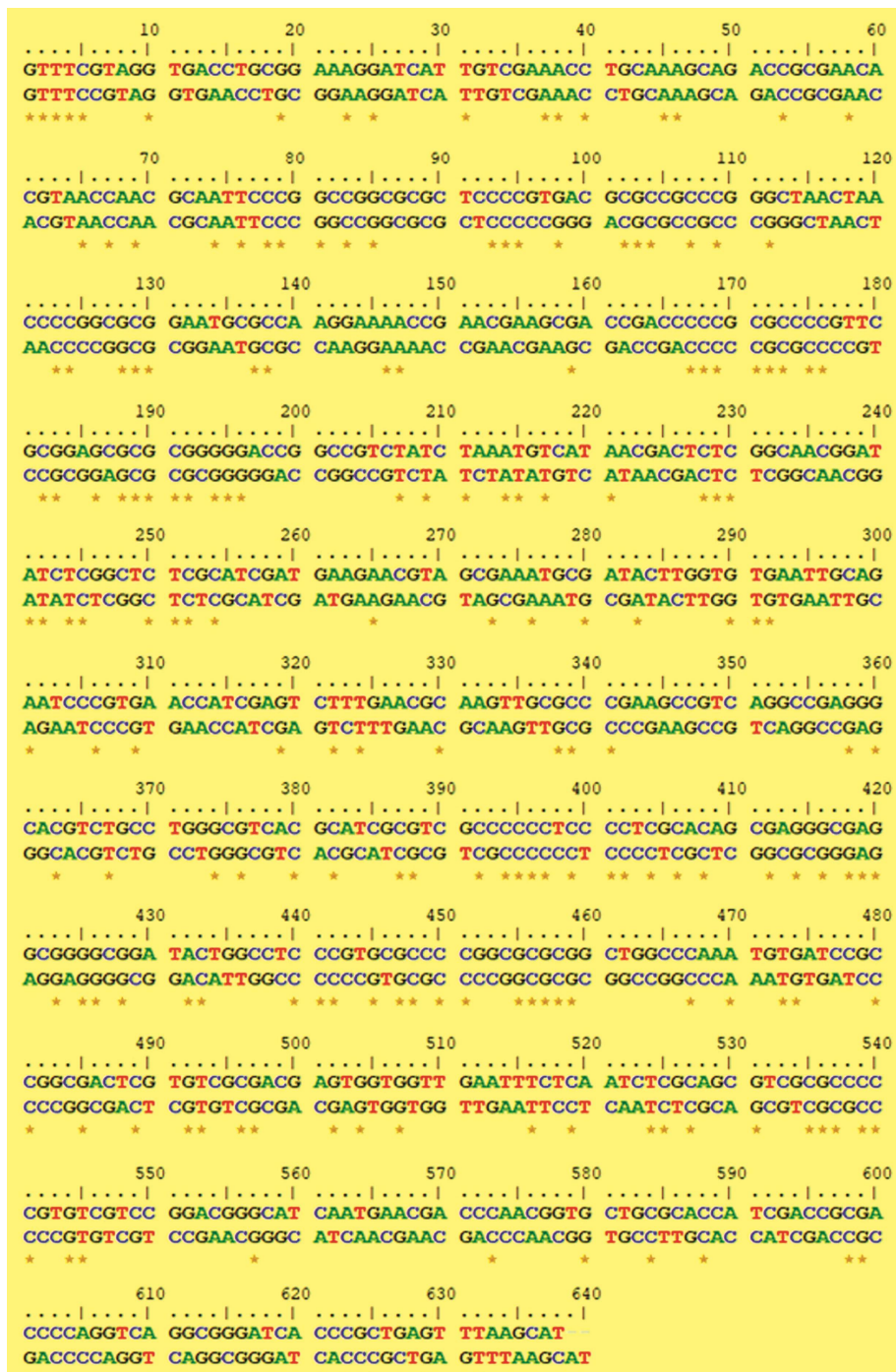


Fig. 1. The differences in the nucleotide base pairs in the alignment (sequence lane 1: *Nepeta deflersiana*; sequence lane 2: *N. sheilae*; lane 3: Clustal consensus).

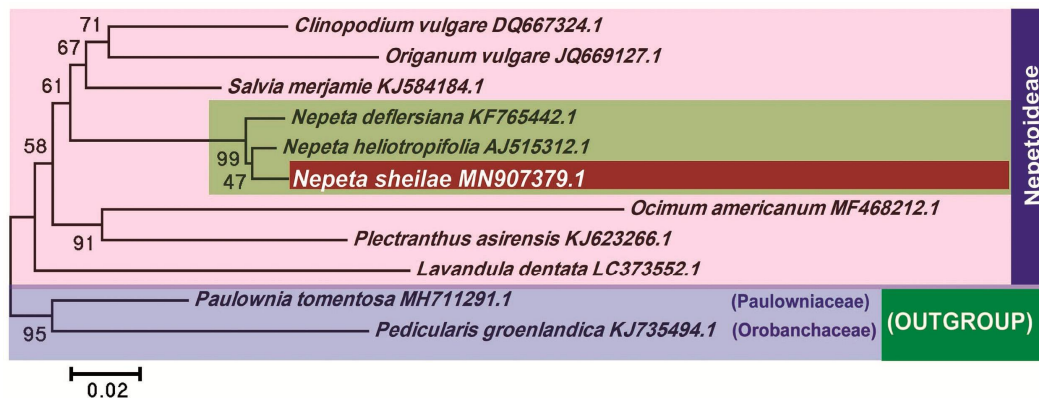


Fig. 2. The phylogenetic tree showing relationships of *Nepeta sheilae* with its closely related species based on nrDNA ITS sequence data inferred using minimum evolution method. The GenBank accession number shown next to taxon. The bootstrap support in 500 bootstrap replicates shown on the branch.

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