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 (Kay 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 tuberculata 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 used 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. Collenette 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*.**

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

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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References
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