PHYLOGENETIC IMPLICATION OF MOLECULAR GENOTYPING OF
EURYOPS JABERIANA ABEDIN & CHAUDHARY (ASTERACEAE)

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Keywords: Euryops jaberiana; Asteraceae; nrDNA ITS; Genotyping; Saudi Arabia.

Abstract

The taxonomic status of Euryops jaberiana Abedin & Chaudhary (tribe Senecioneae, family Asteraceae), endemic to northern Saudi Arabia was evaluated based on molecular phylogenetic analyses of internal transcribed spacer sequence (ITS) of nuclear ribosomal DNA (nrDNA) in order to ascertain its position within the genus. The phylogenetic tree constructed by the Neighbour Joining, Maximum Parsimony and Maximum Likelihood analyses showed a clear resolution of taxon included in the analyses at the level of sections, and E. jaberiana nested within the clade of the section Angustifoliae. E. jaberiana showed proximity with the allied species E. arabicus; however, a total number of eight nucleotide differences were evident between E. jaberiana and E. arabicus, indicating E. jaberiana as distinct from its allied species.

Introduction

The genus Euryops (Cass.) Cass. belonging to the tribe Senecioneae of the family Asteraceae comprises approximately 100 species and displays a restricted distribution in Africa to Arabia and Socotra (Devos et al., 2010). Euryops is characterized by perennial shrubs (except E. annuus Compt.), coriaceous leaves and yellow or orange-flowered capitula on simple peduncles, usually devoid of leaves or bracts. Despite the genus was divided into six sections Angustifoliae, Brachypus, Chrysops, Euryops, Leptorrhiza and Psilosteum based on morphology (Nordenstam, 1968), its phylogeny and phytogeography based on molecular data remains poorly understood (Nordenstam, 1969; Nordenstam et al., 2009; Devos et al., 2010). In Saudi Arabia, the genus Euryops is represented by only two species, viz. E. arabicus Steud. ex Jaub. & Spach, and E. jaberiana Abedin & Chaudhary. E. arabicus is the only species found outside of Africa and is endemic to Arabian Peninsula, while E. jaberiana is endemic to northern Saudi Arabia. Morphologically E. jaberiana very closely resembles with E. arabicus (Abedin and Chaudhary, 2000). Therefore, the main objectives of the present study are two-folds: i) to assess the

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phylogenetic relationships of *E. jaberiana* within the genus, and ii) to shed light on the molecular authentication of *E. jaberiana*.

**Materials and Methods**

*Plant material:*

Leaf materials of *Euryops jaberiana* were collected from the herbarium specimens [voucher-Saudi Arabia, Jabal Shaar near Al-Muwaylih, N. Hijaz, Alt. 1400-1500 m, 03 March 1988, S. Chaudhary and J. Thomas 16873, Isotype: (RIY)] housed at National Herbaium, Riyadh, Saudi Arabia (RIY).

*Total genomic DNA extraction, amplification of ITS region and DNA sequencing:*

The total genomic DNA was isolated using Qiagen DNeasy plant minikit (Valencia, CA, USA). The internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) were amplified using forward primer ITS1 (5'-GTCCACTGAACCTTATCATTTAG-3') and reverse primer ITS4 (5'-TCTCCGCTTATTGATATGC-3') of White *et al.* (1990). The amplified product was sequenced on the ABI 3730 XL sequencing platforms by following methods described by Al-Hemaid *et al.* (2014) and Ali *et al.* (2015a).

*Phylogenetic analysis:*

The sequence of *E. jaberiana* (GenBank accession Number KU577443) was aligned with a total number of 17 representative sequences belongs to each section of the genus *Euryops* and an outgroup sequence of *Gymnodiscus capillaris* retrieved from GenBank (Table 1). The alignment was performed using CLUSTAL X version 1.81 (Thompson *et al.*, 1997). The alignment was manually adjusted using the software BioEdit (Hall, 1999). The Neighbour Joining (NJ) and also

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
<th>GenBank Acc. number</th>
</tr>
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<tbody>
<tr>
<td>Ingroup</td>
<td>1. <em>Euryops annuus</em> Compt.</td>
<td>EU667487</td>
</tr>
<tr>
<td></td>
<td>2. <em>Euryops anthemoides</em> B. Nord.</td>
<td>EU667501</td>
</tr>
<tr>
<td></td>
<td>3. <em>Euryops arabicus</em> Steud.</td>
<td>EU667464</td>
</tr>
<tr>
<td></td>
<td>4. <em>Euryops brachypodus</em> (DC.) B. Nord.</td>
<td>EU667485</td>
</tr>
<tr>
<td></td>
<td>5. <em>Euryops brevilobus</em> Compt.</td>
<td>EU667488</td>
</tr>
<tr>
<td></td>
<td>6. <em>Euryops dacrydioides</em> Oliv.</td>
<td>EU667529</td>
</tr>
<tr>
<td></td>
<td>7. <em>Euryops decumbens</em> B. Nord.</td>
<td>EU667474</td>
</tr>
<tr>
<td></td>
<td>8. <em>Euryops ericifolius</em> (Bel.) B. Nord.</td>
<td>EU667519</td>
</tr>
<tr>
<td></td>
<td>9. <em>Euryops ericoides</em> (L.f.) B. Nord.</td>
<td>EU667509</td>
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<td></td>
<td>10. <em>Euryops evansii</em> Schltr.</td>
<td>EU667471</td>
</tr>
<tr>
<td></td>
<td>11. <em>Euryops hypnoides</em> B. Nord.</td>
<td>EU667527</td>
</tr>
<tr>
<td></td>
<td>12. <em>Euryops jaberiana</em> Abedin &amp; Chaudhary</td>
<td>KU577443</td>
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<td></td>
<td>13. <em>Euryops montanus</em> Schltr.</td>
<td>EU667462</td>
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<tr>
<td></td>
<td>14. <em>Euryops othonnoides</em> (DC.) B. Nord.</td>
<td>EU667503</td>
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<tr>
<td></td>
<td>15. <em>Euryops pectinatus</em> (L.) Cass.</td>
<td>EU667514</td>
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<td></td>
<td>17. <em>Euryops speciosissimus</em> DC.</td>
<td>EU667717</td>
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<tr>
<td></td>
<td>18. <em>Euryops trilobus</em> Harv.</td>
<td>EU667469</td>
</tr>
<tr>
<td>Outgroup</td>
<td>19. <em>Gymnodiscus capillaris</em> (L. f.) Less.</td>
<td>EU667515</td>
</tr>
</tbody>
</table>
the Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were carried out using PAUP (Swofford, 2002) and MEGA5 (Tamura et al., 2011) respectively by the methods as described by Pandey and Ali (2012), Ali et al. (2013, 2015b), and Lee et al. (2013).

Results and Discussion

The present study revealed that the combined length of ITS region (ITS1-5.8S-ITS2) in *E. jaberiana* was 645 nucleotide base pair (bp). The ITS1 region was 260 bp (with GC content 43%), the 5.8S gene was 154 bp long (GC content 54%), and the ITS2 region was 231 bp (GC content 50%). The nrDNA in eukaryotes encodes for ribosome subunits, which occurs in thousands of copies (Prokopovich et al., 2003) that simplify the amplification by polymerase chain reaction (PCR). The nrDNA consist of both highly variable parts of ITS region (i.e. ITS1 and ITS2) and the conserved 5.8S gene between ITS1 and ITS2 (Baldwin et al., 1995). Although reliance on the use of ITS sequence of nrDNA as the sole source of phylogenetic evidence has come under serious criticism (Alvarez and Wendel, 2003); even then, it is one of the most common molecular markers used for generating species-specific phylogenetic inferences in most groups of plants, fungi and animals (Poczai and Hyvönen, 2010; Ali et al., 2014) and DNA barcoding (Chen et al., 2010; Yao et al., 2010; Ali et al., 2014, 2015c) owing to the patterns of polymorphism and ITS types which are specific to particular taxon and population (Baldwin et al., 1995; Feliner et al., 2004; Szabo et al., 2005). The ITS sequence of nrDNA has gained much attention as smartest gene available for the genotyping of taxon and the epitome of species identification has thus now been changed due to application of genotyping in systematics (Ali et al., 2013, 2014).

The BLAST search (Altschul et al., 1990) of the generated nrDNA ITS sequence of *E. jaberiana* showed 99% identity with *E. arabicus*. The phylogenetic analyses revealed a total number of 610 positions in the final aligned dataset, of which 35 were parsimony informative. The MP analysis of the entire ITS region resulted in 82 maximally parsimonious trees (MPTs), the consistency index was 0.671, the retention index was 0.727, the composite index was 0.488 and homoplasy index 0.354. The phylogenetic tree recovered by the analyses provided a clear resolution of taxon at the section level which is consistent with previous study (Devos et al., 2010).

Neighbour Joining (NJ) tree inferred from ITS sequence of nuclear ribosomal DNA of 18 species of *Euryops* revealed that *E. jaberiana* is phylogenetically most closely related to *E. arabicus* (Fig. 1). The NJ analysis recovered tree topology similar to MPT and MLT, and therefore, only the NJ topology with bootstrap support at the node is presented in Fig. 1.

The key morphological features which differentiate *E. jaberiana* from *E. arabicus* are: leaves 3-lobed at the tips, pappus hairs transparent or rarely dull white, and achenes glabrescent, while in *E. arabicus*, the leaves are unlobed, pappus hairs are dull white and achene densely lanate hairy (Abedin and Chaudhary, 2000). In both the MP and ML analyses, *E. jaberiana* nested within the clade of the section Angustifoliae. *E. jaberiana* shows proximity with *E. arabicus* (66% bootstrap support in MPT and 73% bootstrap support in MLT). A total of eight specific nucleotide differences i.e. at the alignment position 93 (A → T), 116 (G → C), 201 (T → C), 443 (C → G), 461 (T → G), 531 (T → C), 573 (C→T) and 611 (T→C) were detected between *E. jaberiana* and *E. arabicus* (Fig. 2). Thus on the basis of phylogenetic relationships of *E. jaberiana* within the genus and nucleotide differences, we herein recognized *E. jaberiana* as a distinct species and different from *E. arabicus*.
Fig. 1. The NJ tree inferred from Neighbour Joining analysis of ITS sequence of nuclear ribosomal DNA of 18 species of *Euryops*. The bootstrap (MP/ML) support greater than 50% in 1000 bootstrap replicates shown on the branch.
Fig. 2. Differences in the nucleotide base pairs position marked with box. Lane 1: *E. jabriana*, Lane 2: *E. arabicus*, and Lane 3: Clustal consensus.
Acknowledgement

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding of this research through the Research Group Project No. RGP-195.

References


(Manuscript received on 11 February 2016; revised on 20 April 2016)