

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

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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 Herbarium, 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 mini kit (Valencia, CA, USA). The internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) were amplified using forward primer ITS1 (5'-GTCCACTGAACCTTATCATTAG-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-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 1. GenBank Accession number of Plant species used for molecular phylogenetic analyses.

Group	Species	GenBank Acc. number
Ingroup	1. <i>Euryops annuus</i> Compt.	EU667487
	2. <i>Euryops anthemoides</i> B. Nord.	EU667501
	3. <i>Euryops arabicus</i> Steud.	EU667464
	4. <i>Euryops brachypodus</i> (DC.) B. Nord.	EU667485
	5. <i>Euryops brevilobus</i> Compt.	EU667488
	6. <i>Euryops dacrydioides</i> Oliv.	EU667529
	7. <i>Euryops decumbens</i> B. Nord.	EU667474
	8. <i>Euryops ericifolius</i> (Bel.) B. Nord.	EU667519
	9. <i>Euryops ericoides</i> (L.f.) B. Nord.	EU667509
	10. <i>Euryops evansii</i> Schltr.	EU667471
	11. <i>Euryops hypnoides</i> B. Nord.	EU667527
	12. <i>Euryops jaberiana</i> Abedin & Chaudhary	KU577443
	13. <i>Euryops montanus</i> Schltr.	EU667462
	14. <i>Euryops othonnoides</i> (DC.) B. Nord.	EU667503
	15. <i>Euryops pectinatus</i> (L.) Cass.	EU667514
	16. <i>Euryops pinifolius</i> A. Rich.	EU667530
	17. <i>Euryops speciosissimus</i> DC.	EU667717
Outgroup	18. <i>Euryops trilobus</i> Harv.	EU667469
	19. <i>Gymnodiscus capillaris</i> (L. f.) Less.	EU667515

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*.

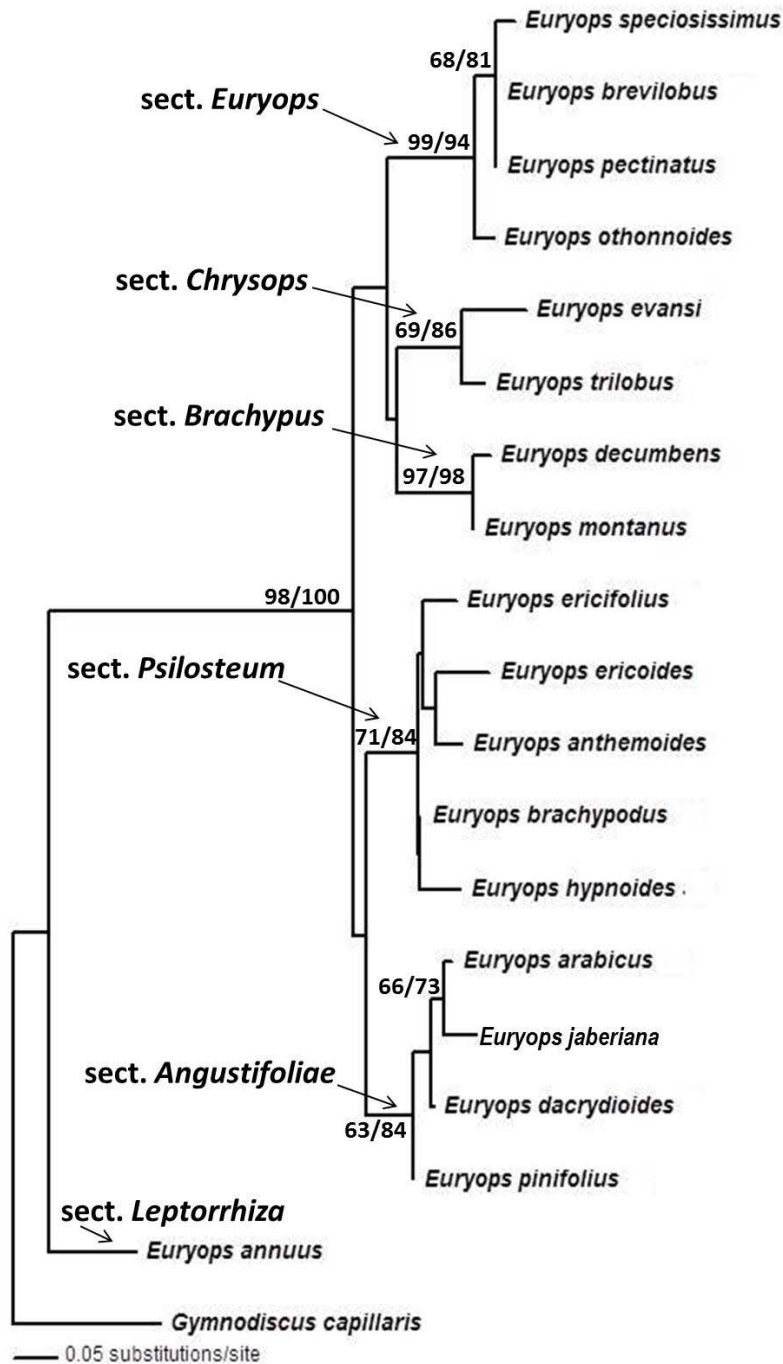


Fig. 1. The NJ tree inferred from Neighbour Joining analysis of ITS sequence of nuclear ribosomal DNA of 18 species of *Euryops*. The bootstrap (MP/ML) support greater than 50% in 1000 bootstrap replicates shown on the branch.

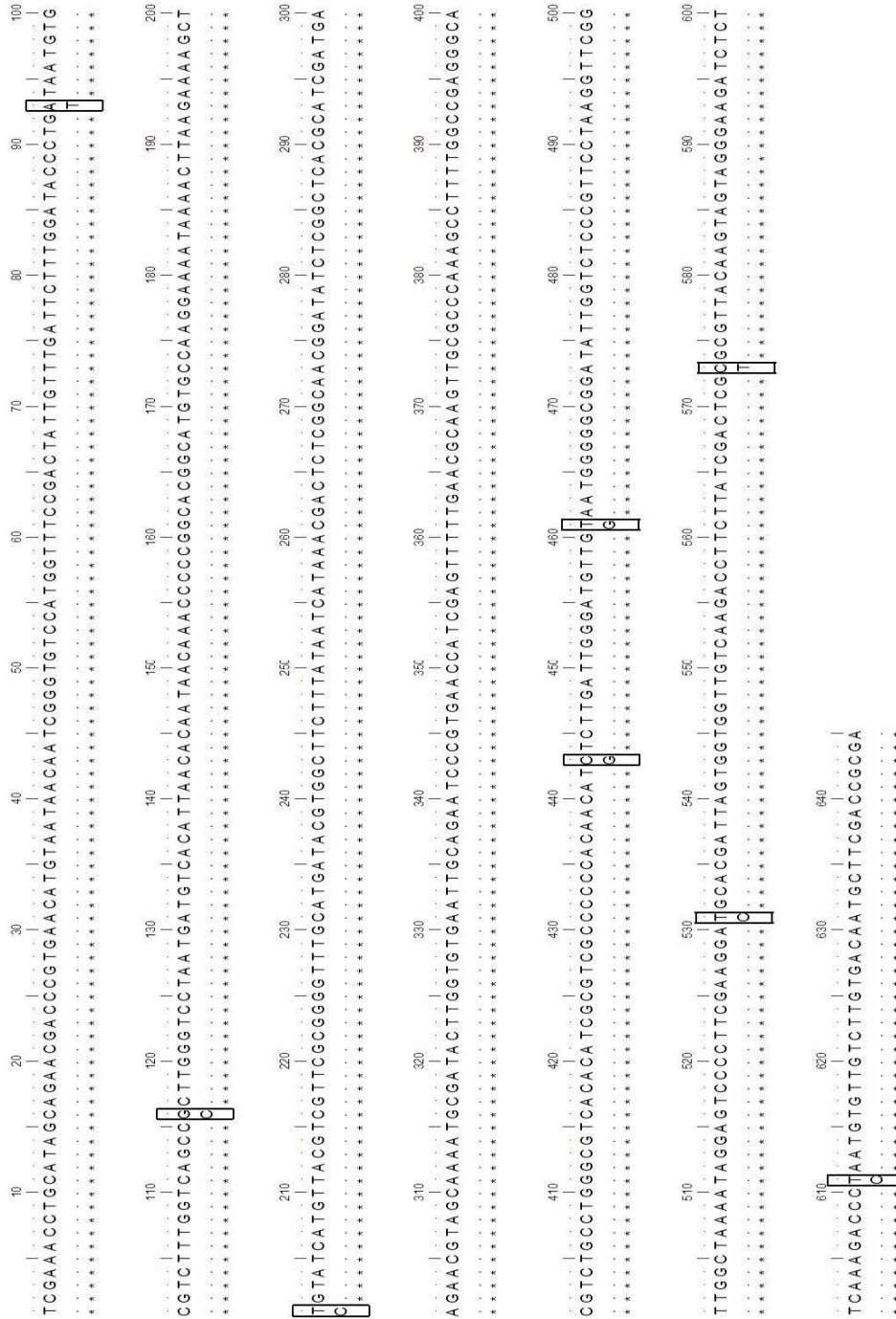


Fig. 2. Differences in the nucleotide base pairs position marked with box. Lane 1: *E. jabriana*, Lane 2: *E. arabicus*, and Lane 3: Clustal consensus.

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