Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Molecular study of the genus *Eryngium* L. (Apiaceae) in Iraq

Aboothar Ha. Al Edhari[1]*, Shayma M. Al Rahbawi[1], Abdullah Sh. Sardar[2]

1. Department of Ecology, College of Science, University of Kufa, Najaf, Iraq
2. Department of Biology, College of Education, University of Salahaddin, Erbil, Erbil, Iraq

** Corresponding author's E-mail: abothar.alathari@uokufa.edu.iq*

ABSTRACT

This study was conducted to investigate the complete sequences of nuclear ITS region, which determined five species of *Eryngium* L. Apiaceae in Iraq to inference phylogenetic relationships. *Pycnocycla aucheriana* was used as outgroups. Phylogenetic analysis was performed using ClastalW in MEGA6 version 7.0.4.1 with high supported (bootstrapping) value for each clade in a dendrogram. The phylogenetic trees showed *Eryngium glomerutum* as the basal lineage in a dendrogram. The sister clade to *E. glomerutum* was *E. billardieri* which form the second clade in phylogenetic trees. The third clade included only *E. creticum* which is considered as a sister clade to the *E. campestre* and *E. thyrosoideum* in the dendrogram. Finally, the monophylly of each clade was well supported, and phylogenetic relationships between *Enyngium* species was cleared based on DNA Sequencing character.

Keywords: Molecular study, Plant, *Eryngium*, Apiaceae, Iraq.
Article type: Short Communication.

INTRODUCTION

Apiaceae is from the families present in Iraq which involves 3590 species in the world, distributed on 440 genera (Singh 2010). Iraq has 130 species distributed on 59 genera (Al Rawi 1964). In Europe, Chater (1968) reported 26 species of the genus *Eryngium.* In Turkey, Davis (1972) identified 19 species of the genus, while in Saudi Arabia, Migahid & Hammouda (1978) did not recognize any species. Ghahreman & Attar (1999) pointed out that 9 species of the genus are present in Iran. Rechinger (1964) in the Flora of low land in Iraq reported 1 species. Al Rawi (1964) described 6 species found in Iraq mentioning the districts in which the species distribute. In addition, Chakravarty (1976) pointed out the presence of 4 species of the genus in Iraq, while Ridda & Daood (1982) and also Ghazanfer & Edmondson (2013) described 8 species found in Iraq. Khalaf (1980) and Ahmad (2013) pointed out the presence of 4 species in Sinjar mountain and Hawraman region separately. Faris (1983) and Darwesh (2017) described 1 species in Piramagrun Mountain and Choman respectively. Moreover, Fatah (2003) and Hameed (2016) did not report any species in Haybat Sultan and Hujran Basin respectively. There are also many reports about identification of plants in the world (Milani *et al.* 2017; Aliyeva *et al.* 2021; Al- Abbasi *et al.* 2022) The dispersals coincide with the polytomies played key roles in the diversification of the genus *Eryngium*. The evolution of *Eryngium* combines a history of long-distance dispersals, rapid radiations, and hybridization, culminating in the taxonomic complexity observed today in the genus (Carolina *et al*. 2008). A cost-benefit examination indicated that maximum parsimony analysis of *trnQ-trnK* plus three regions (*trnG-trnS, rpl32-trnL*, and *3'trnV-ndhC)* results in the same number of clades and similar bootstrap support values as in the combined analysis of all cpDNA regions in the genus *Eryngium* (Carolina *et al*. 2010). On average, 20.4% of SSAP (sensitive retrotransposon-sequence-specific amplification polymorphism bands) were polymorphic for the five most informative primer combinations in a set of 150 Northern European *E. maritimum* from 13 locations, providing a

Caspian Journal of Environmental Sciences, Vol. 20 No. 4 pp. 845-848 Received: March 26, 2022 Revised: May 14, 2022 Accepted: July 16, 2022
DOI: 10.22124/CJES.2022.5777

Publisher: University of Guilan, Iran

useful tool for assessment of genetic diversity in this endangered species (Ievina *et al*. 2010). The present study aimed to study some molecular characters of the species *E. campestre* L., *E. creticum* Lam., *E. billardieri* Del., *E. glomerutum* Lam. and *E. thyrosoideum* Boiss. to add a small part to the information about the genus *Eryngium* in Iraq.

MATERIALS AND METHODS

Taxon Sampling

The plant taxa used in the present study were collected from the different districts of region in Iraq preserved in the Herbarium of College of Education, University of Salahaddin, Erbil, Iraq. Five distinct taxa consist of five in-group taxa and one outgroup *Pycnocycla aucheriana* were used in the analysis.

DNA Extraction

Total DNA was extracted from the collected specimens. The extraction method was based on the CTAB protocol of (Doyle & Doyle 1990) by some modification (1X CTAB: 10 mL of 1.0 M Tris-HCl, PH 8; 4 mL of 0.5 M EDTA, PH 8; 28 mL of 5 M NaCl; 2% CTAB; 2 g PVP; and 158 mL ddH_2O). The washing process of the DNA pellet was conducted twice with 0.5 mL of 80% ethanol, then DNA was dissolved in 25 µL TE-buffer.

PCR and DNA sequencing

The nuclear region ITS was amplified using the primers ITS A and ITS B of White *et al*. (1990; Table 1). The primers were ordered from (IDT) Company-Skokie, Illinois-USA. The total volume of amplification reactions was 25 µL and Master Mix was made up of 10.8 µL ddH_2O, 2.5 µL ThermoPol reaction buffer, 2.5 µL $MgCl_2$, 5 µL dNTPs, 2 µL template, 1 µL from each primer, 0.2 µL DNA polymerase (Taq polymerase). The PCR-Thermal cycler started with 2 min for initial denaturation at 94 °C followed by 39 cycles: 30 sec. ITS1 for denaturation at 94 °C; 60 sec. for annealing at 52 °C, Extension 90 sec at 72 °C. The resultant PCR products were checked on 1.5% agarose gel run in TAE buffer. The gel was stained with ethidium bromide and photographed under a UV trans illuminator.

Table 1. List of primers and their sequences used in this study.

Primer	Direction	Sequence 5'---- 3'	Resources
ITS A	Forward	5'-GGAAGGAGAAGTCGTAACAAGG-3	(white *et al*. 1990)
ITS B	Reverse	5'-CTTTTCCTCCGCTTATTGATATG-3	(white *et al.* 1990)

PCR products were purified using kits (Promega Company, Madison, USA). The purified PCR products were sent to the National Science and Technology Development Agency (NSTDA) in Thailand for sequencing.

Sequence alignment

All the DNA sequences were edited and aligned with the ClastalW option available in MEGA6, Version 7.0.4.1 (Hall 2001) and manual adjustment. There are 6 accessions for each ITS1, including the out-group species.

Phylogenetic analyses

Maximum parsimony analysis

The reconstruction of the phylogenetic relationships was based on UPGMA methods. UPGMA analysis was performed using Bio Editor (Swofford 2000). Using heuristic search with 100 replicates of random taxon additions, Tree-Bisection-Reconnection (TBR) branch swapping, MulTrees on, and steepest descent off were performed. The maximum numbers of saved trees were 100 for each replicate. The bootstrap values were calculated from 100 replicates.

RESULTS AND DISCUSSION

Phylogenetic relationships within *Eryngium* species Three major clades were recovered within *Eryngium* based on a nuclear ribosomal tree, although the positions of these clades are varied (Fig. 1). The analyses were carried out by UPGMA methods, consisted of six in-groups and one outgroup taxa. The tree topology of the UPGMA showed that the species are closely related to each other with a difference in bootstrapping value. The clades of the ITS region in the UPGMA tree are as follows: the first clade consists of only *E. glomerutum* with bootstrap support (bs = 95%); the sister clades to *E. glomerutum* include *E. billardieri* alone with full bootstrap support (bs

= 100%). The same thing occurs with *E. creticum* in insulation in one clade alone and the final clade are excellent supported (bs = 87%) consists of *E. campestre* and *E. thyrosoideum* (Fig. 1). The importance of molecular sequencing for DNA is obvious in modern plant systematics in general and in particular nuclear ITS region and efficiency of this region in the molecular taxonomy of plant families is evident in the world and especially in *Egyngium* genus in Iraq. Moreover, the analysis of these data by modern and different software display authenticity study particularly in Apiaceae systematic and other families agrees with the finding of Hasan (2019) on his study on *potentialla* (Rosaceae) in Kurdistan region, Iraq. Support for this finding comes from the work of Dana *et al.* (2010) who used a preliminary marker of molecular systematic like IRAP and RAPD in their study on *Eryngium* genus in Syria and differentiated between species of the genus depending on the cluster analysis of molecular and ecological character. According to our knowledge and findings, the current study provides the first molecular data regarding *Eryngium* species in Iraq and phylogenetic analysis based on the nrDNA indicating a strong relationship and the monophyletic of the *Eryngium* species which support its mountainous habitat in the Kurdistan region, Iraq.

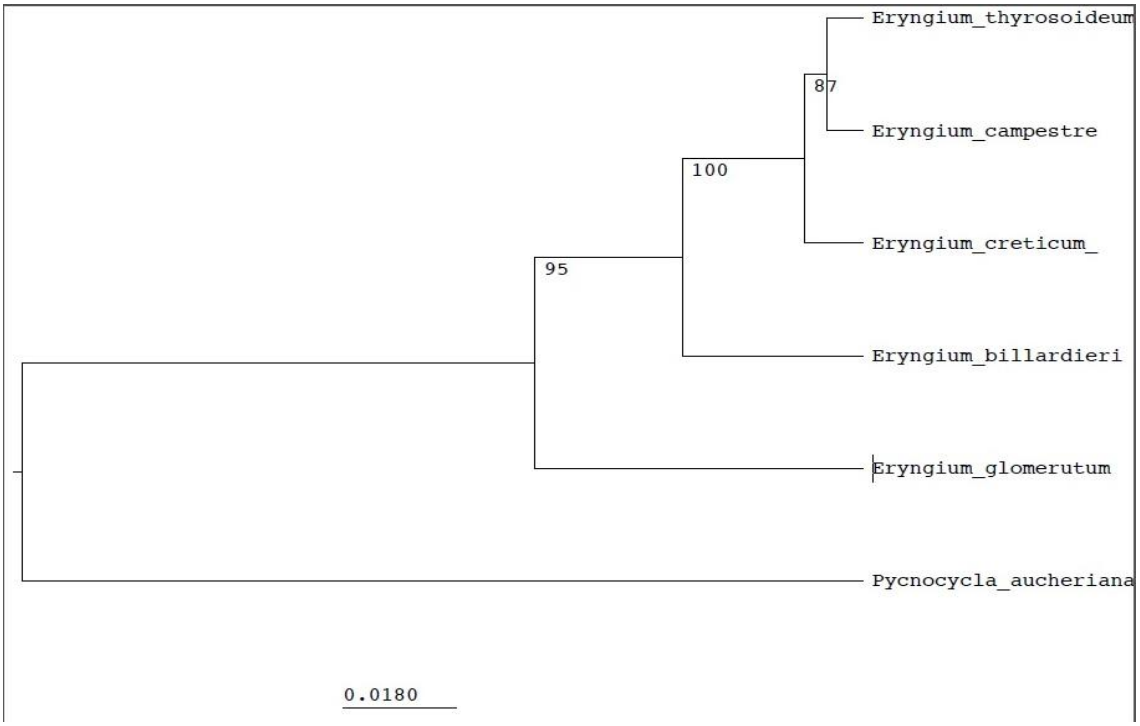

Fig 1. Strict consensus tree of most UPGMA trees resulting from phylogenetic analysis of the nr DNA ITS. The number of branches indicates bootstrap support.

REFERENCES

Ahmad, SA 2013, Vascular Plants of Hawraman Region in Kurdistan Iraq. PhD Dissertation, University of Sulaimani, Sulaimaniya, Iraq, pp. 28-29.

Al- Abbasi, SHAH, Altaef, AQ, AL-Samarraie, M, Al-Naqib, ATH, Al-Majmaei, AAM 2022, Collection and diagnosis of some wild plants in the vicinity of Samarra University, Iraq. *Caspian Journal of Environmental Sciences*, 20: 437-440.

Aliyeva, G, Ojaghi, J, Rustamova, S 2021, Molecular diversity and phylogenetic analysis of Azerbaijan oaks (*Quercus* spp.) revealed by RAPD markers. *Caspian Journal of Environmental Sciences*, 19: 457-468.

Al Rawi, A 1964, Wild plants of Iraq with their distribution. State Board for Agricultural & Water Resources Research, National Herbarium of Iraq, Ministry of Agriculture & Irrigation, Baghdad, pp. 88-97.

Baiba, I, Naeem, HS, Andrew, JF, Gederts, I & Nils, R 2010, Development of retrotransposon-based SSAP molecular marker system for the study of genetic diversity in sea holly (*Eryngium maritimum* L). *Plant Genetic Resources*, 8: 258–266.

Carolina, IC, Susana, GM & Stephen, RD 2008, The evolutionary history of Eryngium (Apiaceae, Saniculoideae): Rapid radiations, long-distance dispersals, and hybridizations. *Molecular Phylogenetics and Evolution*, 46: 1129-1150.

Carolina, IC, Susana, GM & Stephen, RD 2010, Unravelling the taxonomic complexity of *Eryngium* L. (Apiaceae, Saniculoideae): Phylogenetic analysis of 11 non-coding cpDNA loci corroborates rapid radiations. *Plant Diversity and Evolution*, 128: 137-149.

Chakravarty, HL 1976, Plant wealth of Iraq. A Dictionary of Economic Plants, Vol. 1, Botany Directorate, Ministry of Agriculture & Agrarian Reform, Baghdad, Iraq.

Chater, AO 1968, In Flora Europaea.Vol.2. Cambridge University Press, pp. 320-324.

Dana, J, Al Faoury, H, Ayyoubi, Z & Al Safadi, B 2010, A molecular and ecological study of Eryngium species in Syria. *Biologia*, 65: 796-804.

Darwesh, DTD 2017, Plant Biodiversity and Ethnobotanical Properties of Various Plants in Choman (Erbil-Iraq). M. Sc. Thesis, Kahramanmaraş Sütçü İmam University, Graduate School of Natural and Applied Sciences University, Kahramanmaraş, Turkey: 1

Davis, PH 1972, In: Flora of Turkey. Vol. 4. Edinburgh at the University Press, pp. 292-304.

Doyle, JJ & Doyle, JL 1990), Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Faris, YS 1983, The vascular plants of Pira Magrun Mountain. MSc. Dissertation, Salahaddin University, Erbil, Iraq, 111 p.

Fatah, HU 2003, The vascular plants of Haibat Sultan Mountain and the adjacent areas. MSc. Dissertation, University of Sulaimani, Sulaimaniya, Iraq, pp. 96-97.

Ghahreman, A & Attar, F 1999, Biodiversity of plant species in Iran. Central Herbarium, Tehran University, Tehran, Iran, 121 p.

Ghazanfer, ShA & Edmondson, JR 2013, Flora of Iraq. Vol. 5, Part 2, Royal Botanic Gardens, KEW, Ministry of Agriculture, Iraq, pp. 113-119.

Hall, RE 2001, The stock market and capital accumulation. *American Economic Review*, 91: 1185-1202.

Hameed, MAH 2016, Vascular plant taxa of Hujran Basin, Erbil, Iraq. MSc. Dissertation, Kahramanmaraş Sütçü İmam University, Graduate School of Natural and Applied Sciences University, Kahramanmaraş, Turkey, pp. 59- 62

Hasan, HH 2019, A comparative systematic study of the genus *Potentilla* L. (Rosaceae) in Kurdistan Region, Iraq. MSc. Dissertation, University of Salahuddin -Erbil, Iraq.

Khalaf, MK 1980, The vascular plants of Jabal Sinjar. MSc. Dissertation, Baghdad University, Baghdad, Iraq, 149 p.

Migahid, AM & Hammouda, MA 1978, Flora of Saudi Arabia. Vol. 1, Riyadh University Publication, pp. 375-386.

Milani, E, Saeidi Mehrvarz, Sh & Gholizadeh, H 2017, Floristic, life form and chorological studies of the Abshar protected area, Shirgah, Mazandaran Province, north of Iran *Caspian Journal of Environmental Sciences*, 15: 165-180.

Rechinger, KH 1964, Flora of low land Iraq. Weinheim J. Cramer, OCLC Number: 912422, pp. 446-457.

Ridda, TJ & Daood, WH 1982, Geographical distribution of wild vascular plants of Iraq. National Herbarium of Iraq, 78 p.

Singh, G 2010, Plant systematics, An integrated approach. 3rd Edition, Science Publishers, Enfield, NH, USA, 644 p.

Swofford, DL 2000, PAUP: Phylogenetic Analysis Using Parsimony, Version 4, Sunderland, Massachusetts, Sinauer Associates, pp. 6-10.

White, TJ, Bruns, T, Lee, SJ & Taylor, W 1990, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In: MA, Innis, DH, Gelfand, JJ, Sninsky & TJ White (Eds.) PCR protocols: A guide to methods and applications. Academic Press, New York, NY, USA, pp. 317.

Bibliographic information of this paper for citing:

Al Edhari, A,H, Al Rahbawi, S,M, Sardar, A,S 2022, Molecular study of the genus *Eryngium* L. (Apiaceae) in Iraq. Caspian Journal of Environmental Sciences, 20: 845-848.