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Phylogenetic study of the genus *Eryngium* L. (Apiaceae) based on chloroplast *mat*K & *trn*L-F gene

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***Abstract*---**The current research deals with molecular systematics study of the genus *Eryngium* (Apiaceae) in Iraq. The study included molecular characteristics traits of chloroplast *mat*K & *trn*L-F gene that have reliable value in separate and determinate the genus from others genera in Apiaceae family. The result of molecular study reaches to investigated the phylogenetic relation of the species belongs to *Erymgium* using five in-group species *E. campestre* L., *E. creticum* Lam., *E. billardieri* Del., *E. glomerutum* Lam. and *E. thyrosoideum* Boiss and one out-group *pycnocycla aucheriana* were used as related species. Based on *trn*L-F & *mat*K intergeneric chloroplast gene.

***Keywords*---**Phylogenetic, *trn*L-F & *mat*K, *Eryngium*, Iraq.

Introduction

Eryngium L. is an important plant belongs to the family Apiaceae compromising more than (2500-3000) species (Al -Musawi, 1987). Distributed on 440 genera (Singh,2010). In iraq, The *Eryngium* has not been dealt with extensive molecular studies as other genera in the flora of Iraq which need to investigate phylogenetic relations studies and the flora of Iraq is remains incomplete from such phylogenetic studies. The population of the *Eryngium* species grow naturally and mainly in north of Iraq are *E. campestre* L., *E. creticum* Lam., *E. billardieri* Del., *E. glomerutum* Lam. and *E. thyrosoideum* Boiss. The advanced in molecular & cytogenetics studies in past decade play a crucial role in understand Evolutionary & phylogenetic processes in all living organism (Baumung *et al.,* 2004). Recently among the molecular technique have been developed and making a great scientific revolution is the uses DNA sequences data. (Determination of Nucleotides Adenine, Guanine, Thymine and Cytosine for the desired gene or region) in taxon. Other technique in molecular diagnostic DNA restriction sites,

Alloenzyme, Microsatellites, AFLPS (Amplified Fragment Length Polymerase) and RAPDS (Random `Amplified Polymerase DNA) which is reliable methods used in distinguish between related taxa (Simpson, 2006; Medhat and Aljanabay, 2022). Now the application of modern computer program and mathematical equations in analysis of different molecular data and to infer evolutionary changes between living organism to establish phylogenetics relationships become one of the basis principals in biosystematics (Singh, 2010; Hadi and Aljanaby, 2022; Abdulla *et al.,* 2022). The study aimed to determine phylogenetic relation between *Eryngium* species based on the sequencing of chloroplast *mat*K & *trn*L-F gene to add a small part to the information about the taxonomy of genus *Eryngium* in Iraq.

Materials & Methods

Taxon Sampling

The plant taxa used in the present study were collected from the different districts of region-Iraq that preserved in the Herbarium of College of Education, University of Salahaddin-Erbil. Five distinct taxa consist of Five ingroup 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).with 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 ddH2O), the washing process of the DNA pellet has been conducted twice with 0.5 mL of 80% ethanol, then DNA was dissolved in 25 µl TE-buffer.

PCR and DNA Sequencing

The Chloroplast gene *mat*K & *trn*L-F was amplified by using the primers trn-C and trn-F of (Chen *et al .,* 2010) and *mat*K 7B and MG1 (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 made up of 10.8 µl of ddH2O, 2.5 µl ThermoPol reaction buffer, 2.5 µl MgCl2, 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 EtBr and photographed under a UV transilluminator.

Table 1
List of primers and their sequences that have been used in the study

Primer	Product size	Direction	Sequence 5'---- 3'	Resources
trn-C	600 bp	Forward	ATT TGA ACT GGT GAC ACG AG	(Chen *et al.*, 2010)
trn-F		Reverse	CGA AAT CGG TAG ACG CTA CG	(Chen *et al.*, 2010)
7B	400 bp	Forward	GGATCGGGCATCCTATT	(White *et al.*, 1991)

MG1	Reverse	GACTCGAACCGGAACTAG	(white *et al.*, 1991)

PCR products were purified by using Kits (Promega company-Madison-USA). The purified PCR products were sent to the macrogen for sequencing.

Sequence Alignment

All the DNA sequences were edited and aligned with the ClastalW option available in BioEdit , 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 Maximum Parsimony (MP) methods. The analysis was carried out for separate regions. MP analysis was performed by using PAUP* version 4.0a164 (Swofford, 2000). Using heuristic search with 100 replicates of random taxon additions, Tree-Bisection-Reconnection (TBR) branch swapping, MulTrees on, and steepest decent off was performed. The maximum numbers of saved trees were 100 for each replicate. The bootstrap values were calculated from 100 replicates, the consistency index (CI), retention index (RI), rescaled consistency (RC), and homoplasy index (HI) were measured (Felsenstein, 1985).

Bayesian analysis

Bayesian analysis was carried out by using MrBayes version. 3.2 (Ronquist and Huelsenbeck, 2003). The parameters and evolutionary models were selected by assistant of MrModeltest2 version 2.3 (Nylander *et al.*, 2004), based on Akaike Information Criterion (AIC), which selected GTR+G model for ITS region, while GTR+G+I was selected for *trn*L-F. Two independent analyses were run 1000000 generations with four chains (one cold and three heated) for each generation and the temperature parameter set to 0.1. Trees were sampled every 100th generations. After that (25% of initial tree sampled) were removed by burn-in period samples, a tree with maximum 50% (majority rule consensus tree) was plotted. The value of posterior probability (PP) was calculated and the final tree was plotted by using FigTree software version 1.4.3 (Rambaut, 2016).

Results and Discussion

Data matrix, tree statistics for Phylogeny analysis

The characteristics of each data matrix and tree statistics of *trn*L-F and *mat*K gene are summarized in (Tables 2).

Table 2
A summary of alignment and tree statistics of trnL-F and matK

Parameters/ Regions	*matK*	*trn*L-F	Combined
Aligned length	1280	756	2036
Number of parsimony informative characters	5	25	30
Number of variable parsimony uninformative	33	94	127

characters			
Number of constant characters	1242	637	1879
Tree length (steps)	38	138	181
CI (Consistency Index)	1.000	0.957	0.939
RI (Retention Index)	1.000	0.786	0.667
RC (Rescaled Index)	1.000	0.752	0.626
HI (Homoplasy index)	0.000	0.043	0.061
Model	GTR	GTR+G	GTR+G

Phylogenetic relationships within Eryngium species

Only two major clade was recovered within *Eryngium* in *trn*L-F and *mat*K tree (Figure 1 and 2). The analyses were carried out for separate regions, consisted of five ingroups and one outgroup taxa. The tree topology of the maximum parsimony showed same results with bayesian analysis. The clade of *trn*L-F gene is monophyletic and bootstrap support was (bs=98%, pp=1.00) for the first major clade , clade A for *E.thyrosoideum* with the second major clade that gathered other *Eryngium* clade, Clade B that subdivided in to three secondary clade , *E.creticum* in the basal lineage (bs 62%,pp=0.97) with the *E.glomerutum* which finally consider sister clade gathered *E.campertre* and *E.billardieri* , (bs 74%,pp=0.73) Figure1. The Clade of *mat*K gene are as follow: Clade A for *E.thyrosoideum* consider the first major clade which is the first main clade in the phylogenetic tree of the *Eryngium* with full bootstrap value (98%) as the sister to the second main clade which included the other *Eryngium* species. *E.campestre* in the basal of the second major clade as sister to *E.creticum* with medium bootstrap value (62%). Finally, *E.creticum* sister clade to the *E.billardieri and E.glomerutum* with good bootstrap (74%).

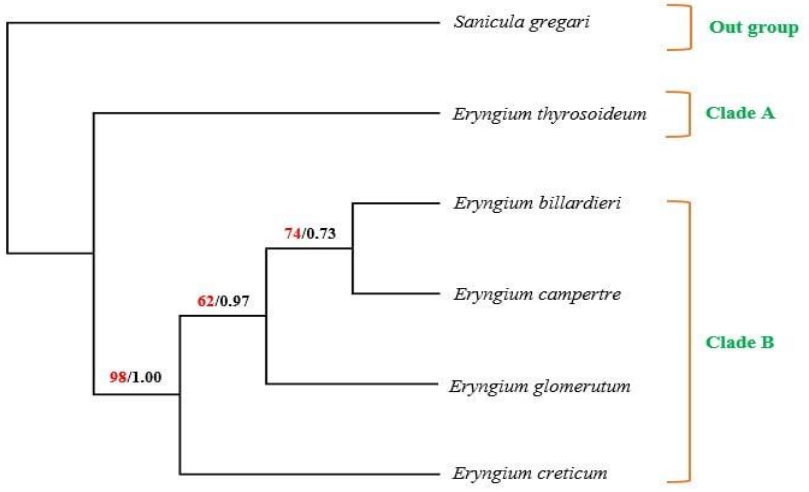

Figure 1: Strict consensus tree of most parsimonious tree resulting from phylogenetic analysis of the cpDNA trnL-F sequences with heuristic search using maximum parsimony analysis. (Tree length of 138 steps, CI = 0.957, RI = 0.786, RC = 0.752 and HI =0.043). Numbers in red color indicate bootstrap support and numbers in black color are Bayesian posterior probability values.

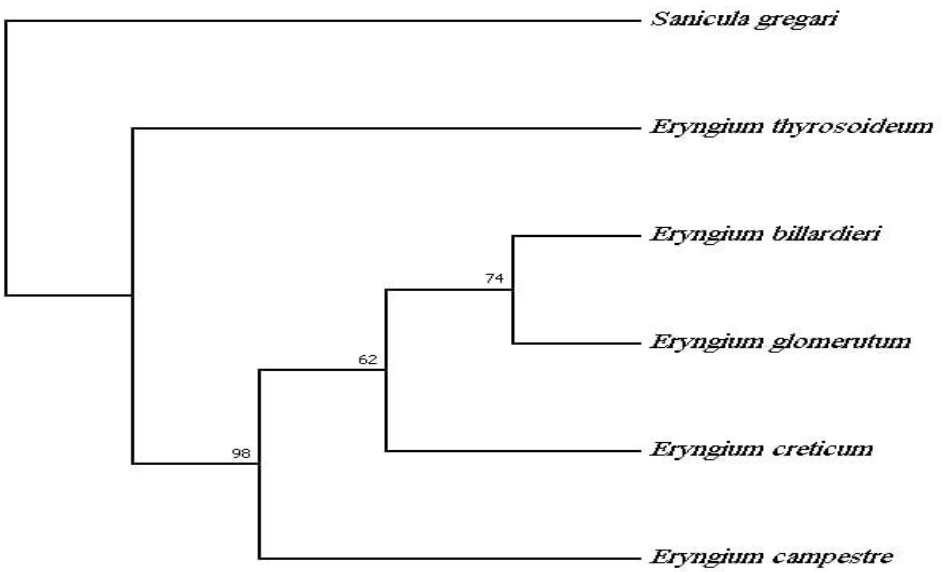

Figure 2: Strict consensus tree of most parsimonious tree resulting from phylogenetic analysis of the cpDNA matK sequences with heuristic search using maximum parsimony analysis. (Tree length of 38 steps, CI = 1.000, RI = 1.000, RC = 1.000 and HI =0.000). Numbers in rblack color indicate bootstrapping support

The importance of molecular sequencing technique for chloroplast DNA as modern evidence in plant taxonomy in general and especially *trn*L-F and *mat*K gene and efficiency of these gene in the molecular taxonomy of plant families in the world and especially in *Egyngium* genus in Iraq. and analysis of these data by modern program display authenticity study particularly Apiaceae systematic and other families agrees with the finding of Hasan (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 uses a preliminary marker of molecular systematic like IRAP and RAPD in their study of *the Eryngium* genus in Syria and differentiate between species of the genus depend on the cluster analysis of molecular and ecological character. According to our findings, the current study provides the first molecular data regarding *Eryngium* species in Iraq and phylogenetic analysis based on the nrDNA indicates a strong relationship and the monophyletic of the *Eryngium* species which support its mountainous habitat in the Kurdistan region in Iraq.

References

Ahya Abdulla, N., Abduljabbar Jaloob Aljanaby, I., T Hasan, H., & Abduljabbar Jaloob Aljanaby, A. (2022). Assessment of ß-lactams and Carbapenems Antimicrobials Resistance in Klebsiella Oxytoca Isolated from Patients with Urinary Tract Infections in Najaf, Iraq. Archives of Razi Institute, 77(2), 669-673.

Al- Musawi, A. H. (1987). Plant Taxonomy. Univ. Baghdad. 379- (in Arabic).

Baumung, R., Simianer,H.& Hoffmann,I.(2004). Genetic diversity studies in farm animals-a survey Journal *of Animal Breeding and Genetics*,121,361-373.

CHEN, S., YAO, H., HAN, J., LIU, C., SONG, J., SHI, L., ZHU, Y. MA, X., GAO, T. & PANG, X. (2010) . Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species.

Doyle, J. J. & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12, 13-15.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783-791.

Hadi, H. I., & Aljanaby, A. A. J. (2022). Correlation between CD14 and CD163 in duodenal ulcer and gastric cancer patients infected with Helicobacter Pylori. International Journal of Health Sciences, 6(S1), 4211–4217. https://doi.org/10.53730/ijhs.v6nS1.5593

Hall, R. E. (2001). The Stock Market and Capital Accumulation. American Economic Review, 91, 1185-1202.

Hasan, H. H. (2019). A Comparative Systematic Study of the Genus *Potentilla* L. (Rosaceae) in Kurdistan Region Iraq. M. Sc. Thesis, University of Salahuddin - Erbil, Iraq.

Medhat, A. R., & Aljanabay, A. A. J. (2022). Assessment of some immune markers in typhoid-patients: A case control study . International Journal of Health Sciences, 6(S1), 4199–4210. https://doi.org/10.53730/ijhs.v6nS1.5592

Nylander, J. A., Ronquist, F., Huelsenbeck, J. P. & Nieves-Aldrey, J. L. (2004). Bayesian phylogenetic analysis of combined data. Syst Biol, 53, 47-67. Review of Palaeobotany and Palynology, 64, 103-108.

Primatanti, P. A., & Jawi, I. M. (2019). Anthocyanin as neuroprotector for methamphetamine-induced neurotoxicity. International Journal of Health & Medical Sciences, 3(1), 11-16. https://doi.org/10.31295/ijhms.v3n1.101

Rambaut, A. (2016). ⟨http://tree.bio.ed.ac.uk/software/figtree)/ Accessed 6 January 2016.

Simpson, M.G.(2006). Plant Systematics. Elsiver *Academic press.New* York,477.

Singh, G. (2010). Plant Systematics, An integrated approach. Third edition, Science Publishers, Enfield, NH, USA, p. 644.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. International Journal of Health Sciences, 5(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Swofford, D. L. (2000). PAUP*. Phylogenetic Analysis Using Parsimony, Version 4, Sunderland, Massachusetts, Sinauer Associates: 6-10.

White, T. J., Bruns, T., Lee, S. J. & Taylor, W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (eds.) PCR Protocols: a Guide to Methods and Applications. New York, NY, USA: Academic Press: 317-319.