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ORIGINAL ARTICLE

MYOSOTIS ARVENSIS SUBSP. ARVENSIS (L.) HILL, 1764 (BORAGINALES, BORAGINACEAE): A NEW RECORD FOR THE FLORA OF IRAQ

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ABSTRACT

Myosotis L., 1753 is one of the biggest genera in the family Boraginaceae, subfamily Cynoglossoideae, and tribe Myosotideae, numerous pollen grain features, including shape, color, size, sculpture and number have been examined for the species *M. arvensis*, along with a thorough sampling analysis of macro- and micro-morphology. Molecular investigation has been conducted to confirm *M. arvensis* subsp. *arvensis* taxonomic rank within Boraginaceae. In contrast to the within the same genus that are already grow in Iraq, the collected specimens of *M. arvensis* subsp. *arvensis* are differentiated by a saucer-shaped corolla limb reaching 3 mm in diameter, reaching 3 mm in diameter, and the lobes that are connivent in fruit with many patent hooked hairs. Pollens were small, yellow, monad, tricolporate, ellipsoid in equatorial view, and spheroid in polar view. The molecular study has firmly established the classification and evolutionary ancestry of this recently discovered subspecies by confirming its genetic link and location within the larger family framework. This study aims to prove and describe this subspecies for the first time for the flora of Iraq.

Keywords: Boraginaceae, Iraq, ITS, *Myosotis arvensis*, Phylogeny.

INTRODUCTION

Myosotis L. is a substantial genus within the family Boraginaceae, subfamily Cynoglossoideae, encompassing approximately 100 species, commonly known as forget-me-nots. This genus exhibits a widespread distribution, predominantly inhabiting the temperate regions of the Northern and Southern hemispheres (Hao *et al.*, 2017). However, it is noteworthy that certain taxa within this genus can also be found in the alpine areas within the tropics (Winkworth *et al.*, 2002). With over 60 species, Western Eurasia is one of the two primary centers of *Myosotis* L. 1753 diversity (Al-Shehbaz, 1991). With around 35 species, New Zealand is the second largest center of biodiversity (Mabberley, 2017). According to Al-Rawi (1964), there are seven species of *Myosotis* in Iraq. Regarding this genus in Turkey,

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Davis *et al.* (1978) documented the 23 species, including *M. arvensis*. Before this, Riedl (1967) had reported the occurrence of 18 species of *Myosotis* in Iran.

According to the previous literature, none of the researchers who conducted a comprehensive survey of Iraqi districts, including the Kurdistan Region, have mentioned the presence of *M. arvensis* subsp. *arvensis* in their his findings (Khalaf, 1980; Ridda and Daood, 1982; Faris, 1983; Fatah, 2003; Ahmad, 2013; Hameed *et al.*, 2016; Darwesh, 2017; Abbas and Saeed, 2021; Mrad and Saeed, 2023). These plants exhibit a broad geographical distribution, flourishing in rainforest ecosystems across both the Northern and the Southern hemispheres, as well as they occur as a ruderal in dry or moist places and prefers a slightly small amount of light. It occurs in wet meadows, at the edge of waters, wetlands on fresh and dry soils such as fields, fallows, roadsides, grasslands and forest edges (Jager, 2011). The taxonomic classification of the genus *Myosotis* presents a complex challenge despite its well-defined vegetative and reproductive characteristics, while the genus is distinct, researchers continue to debate its formal boundaries, hierarchical rankings and infrageneric classifications (Nazaire and Hufford, 2012). Traditional taxonomic approaches emphasized corolla scale features and anther positioning (de Candolle, 1846), though subsequent research by Moore (1961) and Grau and Leins (1968) cast doubt on the reliability of these traits, particularly regarding anther exertion.

Recent researches by Grau and Leins (1968) and later Groove and Schwab (1982) included a taxonomic revision, which led to the proposal of a new classification criterion based on pollen grain morphology, microscopic analysis of stigma and corolla scale characteristics. They reached the decision to divide this genus into two distinct sections, *Myosotis* and *Exarrhena*. Many researchers support that the Boraginaceae family is a monophyletic group based on morphological, molecular and phytochemical traits. While, other researchers proposed a new phylogenetic analysis and put Boraginaceae as a paraphyletic with Hoplestigmataceae, Hydrophyllaceae, and Lennoaceae (Nazaire and Hufford, 2012).

Despite the availability of abundant statistics and information on plants in Iraq, Iraq still needs field exploration to fully document the plant resources. For this purpose, the main objective of this study is to record *Myosotis arvensis* subsp. *arvensis* as a new subspecies record for the first time in the flora of Iraq, as well as to protect all the endangered plants by documenting and protecting them in our herbaria.

MATERIALS AND METHODS

Specimens collection and morphological study: Large-scale field surveys were carried out in Iraqi Kurdistan MAM, MRO, MSU, FPF, FKI, FAR and FNI districts (spring-summer season 2024). All botanical examples were specifically gathered from the MRO and MAM districts. The recognition of the taxa used a few specific taxonomical sources, and mainly flora of Turkey. The specimens of each voucher were then subject to procedures of herbarium preparation to become formal herbarium vouchers. Associated ecological information was carefully recorded at the time of sample collection, and a detailed 'holistic map' (Map 1) was constructed to provide an overview of the geographical scope of this research. Morphological

features were observed under Kruss dissecting microscope and a Zeiss binocular microscope using micrometer-calibrated lenses in order to measure small structures accurately. Altitudes were determined by altimeter and GPS, respectively.

Pollen grain study

Light Microscopy (LM): Pollen grains from anthers were studied at the early flowering or large bud stage of samples that are preserved in 70% ethyl alcohol. For species not obtained directly from the field, dried specimens deposited in Iraqi herbaria were used after confirming their classification. The anther was placed in a watch glass bottle, and drops of safranin-glycerin stain were added (Sardar, 2017). The anther was opened with two fine dissections and crushed to extract the pollen grains and expose them to the dye. The pollen grains with the dye were drawn off using a special dropper for each species and poured onto a clean glass slide, then the slide was gently covered and examined under a Zeiss binocular light microscope, and images were captured with a Sony digital camera at the College of Education, Salahaddin University-Erbil. The results used in this study were based on samples previously collected from different locations in the country to account for variations resulting from environmental differences. Measurements were taken from 40 pollen grains in the equatorial view and 40 grains in the polar view. The study included aspects of taxonomic importance.

Scanning Electron Microscopy (SEM): Initially, the anther samples containing pollen grain were washed in an Eppendorf tube using sterile distilled water, this technique was proposed by Aziza *et al.* (2024). The samples were then dehydrated with a series of increasing concentrations of alcohol as follows: 50, 70, 80, 85, 90, 95% and three times at 100%. This was followed by three times of 100% acetone for 30 minutes each time. The specimens were then placed at room temperature to dry until no impurities remained on the wall that would cause ambiguity of the walls. Subsequently, the pollen grains were mounted on metal stubs using double-sided adhesive tape and sputter-coated with a gold-palladium film. The prepared samples were examined using an INSPECTS50 scanning electron microscope at the College of Science, University of Kufa. All pollen terminology follows the standardized nomenclature established by Erdtman (1952).

DNA extraction, marker amplification and sequencing: Dried leaf tissue samples were used to extract a whole genome by using a modified cetyltrimethylammonium bromide (CTAB) protocol based on Doyle and Doyle (1990). The modified CTAB procedure unified 2% PVP 40 (polyvinylpyrrolidone). To preserve the highest DNA quality and yield, tissue samples were limited to 100 mg each extraction. The Internal Transcribed Spacer (ITS) region was selected and amplified using primers listed in Table (1). Those primers were ordered from Macrogen Company, Seoul, Korea. The total volume of amplification reaction was 25 μ L, which consisted of 12.5 μ L master mix (Amplicon, Odense Denmark), 3 μ L genomic DNA extract (10 ng/ μ L concentration), 2 μ L of each primer, and 5.5 μ L nuclease-free water. The master mix contained 3 mM MgCl₂, 0.2% Tween® 20, 20 mM Tris-HCl pH 8.5, (NH₄)₂SO₄, 0.2 units/ μ L Ampliqon Taq DNA polymerase, 0.4 μ M of each primer, and 0.4 mM of each dNTP.

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The Polymerase Chain Reaction (PCR) thermal cycling protocol for the ITS gene began with initial denaturation at 94°C for 5 minutes, followed by 35 cycles of: denaturation at 94°C (60 seconds), annealing at 58°C (60 seconds), and extension at 72°C (120 seconds). A final extension step was performed at 72°C for 5 minutes. PCR products were visualized on 1.5% agarose gel electrophoresis in TAE buffer, stained with Safe Red dye, and documented under UV transillumination.

The PCR product was quantified by using NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, USA), the ratio of DNA to protein (1.8) was conducted by measuring A_{260}/A_{280} (1.8) and the ratio of DNA to (2.2) from phenolic compounds and polysaccharides by measuring A_{260}/A_{230} . Amplification products underwent sequencing at Thailand's National Science and Technology Development Agency (NSTDA). Forward and reverse forward and reverse primers were manually edited and consolidated into consensus by using Geneious v5.4 software (Biomatters). The resulting sequence was deposited in the National Center for Biotechnology Information GenBank database under accession number PQ869645.

Sequence Alignment and Phylogenetic Tree Construction: The DNA sequences underwent editing and alignment using the ClustalW feature in BioEdit Version 7.0.4.1 (Hall, 2001) followed by manual adjustments. The ITS region analysis incorporated 23 accessions as shown in Table (2), including the outgroup species. Independent Bayesian inference (BI) and Maximum parsimony (MP) analyses were performed on individual datasets comprising 23 terminal taxa with complete sequence availability. Maximum Parsimony (MP) analysis was applied on PAUP_ 4.0a164 (Swofford, 2002) with heuristic search parameters including 100 random taxon addition replicates, Tree-Bisection-Reconnection (TBR) branch swapping, Mul-Trees were enabled, and disabled steepest descent. Each replicate was restricted to 100 saved trees per analysis. The analysis assessed consistency index (CI), retention index (RI), rescaled consistency (RC), and homoplasy index (HI), with bootstrap values intended from 100 repetitions (Felsenstein, 1985).

For (BI) analysis, the ideal substitution model was selected by performing MrModeltest2 version 2.3 and Akaike information criterion (AIC) (Nylander *et al.*, 2004). The ITS region best-fit model was recognized as the general time reversible model with gamma-shaped rate variation (GTR+I+G). The analysis employed MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003), which generated automated prior estimates for state frequencies, rates and inter-site variance. Two independent analyses were conducted over two million generations for the ITS dataset, utilizing four Markov chains (one cold and three heated) per generation with a temperature parameter of 0.1. Tree samples were collected every 100 generations. Following the removal of 25% burn-in phase samples, a majority rule consensus tree (maximum 50%) was generated. Posterior probability (PP) values were calculated, and the final tree was visualized using FigTree software version 1.4.3 (Rambaut, 2016).

Table (1): List of primers and their sequences used in the study.

Primer for region	Product size	Sequence 5'---- 3'	Reference
ITS	400-600 bp	Foreword ATGCGATACTTGGTGTGAAT	(White <i>et al.</i> , 1990)
		Reverse TCCTCCGCTTATTGATATGC	

Table (2): Accessions involved in nrDNA ITS phylogenetic analyses obtained from NCBI GenBank.

Taxa	DNA source (location and Voucher)	Accession no.
<i>Myosotis laxa</i>	Sherafati <i>et al.</i> (2014), Iran	AB989077
<i>M. sylvatica</i>	Mu <i>et al.</i> (2014), Germany	PP763526
<i>M. discolor</i>	Meudt <i>et al.</i> (2015), New Zealand	JX128891
<i>M. ramosissima</i>	Hasan and Sardar (2025), Iraq	PV418156.1
<i>M. sparsiflora</i>	Hasan and Sardar (2025), Iraq	PV418155.1
<i>M. incrasata</i>	Hasan and Sardar (2025), Iraq	PQ640283
<i>M. propinqua</i>	Hasan and Sardar (2025), Iraq	AB989074
<i>M. refracta</i>	Hasan and Sardar (2025), Iraq	PV418158.1
<i>M. olympica</i>	Hasan and Sardar (2025), Iraq	PV418154.1
<i>M. stricta</i>	Sherafati <i>et al.</i> (2014), Iran	AB989059
<i>M. minutiflora</i>	Hasan and Sardar (2025), Iraq	PV418153.1
<i>Paracaryum rugulosum</i>	Hasan and Sardar (2025), Iraq	PV418160.1
<i>P. sintenisii</i>	Hasan and Sardar (2025), Iraq	PV418157.1
<i>P. strictum</i>	Hasan and Sardar (2025), Iraq	PV418161.1
<i>P. cristatum</i>	Hasan and Sardar (2025), Iraq	PV418159.1
<i>Rochelia disperma</i>	Hasan and Sardar (2025), Iraq	PV424063.1
<i>R. cardiosepala</i>	Khoshokhan <i>et al.</i> 2010, Iran	AB564701
<i>R. bungei</i>	Khoshokhan <i>et al.</i> 2010, Iran	AB564695
<i>R. persica</i>	Khoshokhan <i>et al.</i> 2010, Iran	AB564697
<i>Mertensia davurica</i>	Chacon <i>et al.</i> 2016, Germany	KU927736
<i>M. sibirica</i>	Chacon <i>et al.</i> 2016, UK	KU927739
<i>Brachybotrys paridiformis</i>	Chacon <i>et al.</i> 2016, Russia	KU927662

RESULTS AND DISCUSSION

Morphological Study

Myosotis arvensis (L.) Hill, Veg. Syst. 7:55 (1764) Link in (1821) nec Reichb. (1830).
subsp. *arvensis*. Syn: *M. scorpioides* L. var. *arvensis* L., Sp. Pl. 131 (1753); *M. intermedia*
Link, Enum. Hort. Berol. Alt. 1:164 (1821). Ic: Bonnier, Fl. Compl. Fr., Suisse et Belg. 8: t.
425 f. 1991 (1926). Figure 12, p. 267.

Annual or biennial herb, 10 – 66 cm. Stems ascending-erect, green or green-yellow, 15–62×0.07–0.15 cm, much branched at base, patent-hairy at base and hairs antrorse above. Leaves simple, alternate-spiral; basal leaves spatulate or oblanceolate, margin entire, apex obtuse-acute, base attenuate, green-yellow, 17–25×3.5–5.0 mm, sessile; lower cauline leaves lanceolate, margin entire, apex acute-obtuse, base acute or subamplexual, green, 17–22×4.0–6.5 mm; upper cauline leaves narrowly lanceolate, margin entire, apex acuminate,

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base acute, green, 10 – 12×2.5 – 3.0 mm. Inflorescence terminal and rarely axial cyme, ebracteate, peduncle terete, green, 1.6–1.9×0.4–0.6 mm. Flowers actinomorphic, pentamerous, 2.0–2.5×1.3–1.5 mm, pedicel terete, green, 0.5–0.7×0.2–0.3 mm. Calyx gamosepalous, 5-lobed ± to base, narrowly lanceolate, margin entire, apex acuminate, base obtuse, green, 1.5 – 1.8×0.8 – 1.2 mm, persistent calyx, accrescent in fruit, bigger than calyx, 2.8–3.15×2.0–2.25, apex acute, base ½ open, margin entire, with hooked hair. Corolla rotate, bright blue, glabrous, with tube and limb, tube 1.0 – 1.5×0.5 – 0.7 mm; limb 5-lobed, 0.5–0.9×1.0–1.3 mm, lobes saucer-shaped, margin undulate, apex obtuse, lobes width 0.8–1.0 mm; throat of 5, violet, semicircular, antipetalous scales (appendages), 0.1–0.2×0.2–0.3 mm (broader than long, where base of anther is below scale base), apex round. Stamens 5, epipetalous, alternating with corolla lobes, inserted on the upper half of the corolla tube; filaments filiform, yellow, 0.2–0.8×0.05–0.1 mm, anthers oblong-ellipsoid, basifixed, dark yellow, 0.5–0.7×0.2–0.25 mm. Pistil one, ovary superior, 4-locular, pyramidal, dark yellow, 0.5–0.8×0.3–0.45 mm; style gynobasic, included, filiform, yellow, 0.5–0.65×0.05–0.15 mm; stigma entire, discoid, yellow, 0.05 – 0.08×0.08–0.1 mm. Fruit schizocarpic with 4 nutlets without a rim, nutlets, 1.0–1.15×0.65–0.75 mm, ovate-ellipsoid, brown, attachment scar with two marginal grooves, rounded small at base (Pls. 1, 2, 3 A, C, D).

The present study explores *Myosotis arvensis* subsp. *arvensis*, which represents a new record of the family Boraginaceae in Iraq (Fig. 1). The research adopts a multifaceted approach, including morphological characterization, palynological investigation, phylogenetic analysis, and an assessment of the environmental conditions associated with the species' occurrence. A comprehensive literature review was done by the authors on the genus *Myosotis*. Although examination of specimens from different herbaria, especially the Herbarium of the College of Science at the University of Salahaddin-Erbil, Iraq (ARB), the National Herbarium of Iraq (BAG) and the Herbarium of the College of Education at the University of Salahaddin-Erbil, Iraq (ESUH) the researchers did not find any specimens related to the genus. Therefore, this research presents the new record of *M. arvensis* subsp. *arvensis* for the first time in the flora of Iraq, where the subspecies was found within MRO districts near Bekhma Dam.

Myosotis arvensis subsp. *arvensis* is a newly identified species in Iraq that differs from other species in the area due to its unique morphological traits. The appearance of its calyces during the fruiting stage regards the most distinctive feature. In contrast to the calyces of other species, *M. arvensis* subsp. *arvensis* rarely are appressed, which means they don't lie flat or near the stem. Furthermore, the calyx lobe is connivent in fruit and the corolla limb is 2-4 mm wide and these calyces are less than 2 millimeters long and are either densely clustered or crowded together. The non-appressed, sparsely distributed, and small calyces in particular are significant diagnostic features that set *M. arvensis* subsp. *arvensis* apart from other species within Iraqi flora. This finding was congruent with the result of Davis *et al.* (1978) who the first-time recorded *M. arvensis* in Turkey. Identifying species from fresh material is typically straightforward in most cases. However, many morphological characteristics employed in identification keys (Fischer *et al.*, 2008; Rottensteiner, 2014), such as calyx openness and pedicel growth direction during fruiting, are often unreliable on herbarium specimens due to

deformation of plant material during the preservation process. Moreover, certain reliable identification features are found in nutlets frequently absent from herbarium specimens.

Molecular study

Table (3) provides a comprehensive overview of the alignment characteristics and statistical data derived from Maximum Parsimony (MP) analyses. The phylogenetic trees generated through Bayesian Inference (BI) and MP analyses of the Internal Transcribed Spacer (ITS) region demonstrated consistent topologies, though with notable well-supported conflicting elements, tree topologies resulting from BI and MP (Diag. 1). According to the phylogenetic hypothesis based on the ITS data set was constructed five clades (Goremykin *et al.*, 2010) containing 23 specimens including the outgroup. The matrix consisted of 663 nuclear ribosomal DNA (nrDNA) characters of the Internal Transcribed Spacer ITS region. The model of molecular evolution selected by AIC was applied to both analyses. The systematic study revealed that all genera with more than one species formed highly supported monophyletic clades. The genus *Myosotis* including *M. arvensis* formed two clades that were sister to the clade comprising *Paracaryium* and *Mertensia*, while *Rochelia* occupied a basal position relative to all other genera (Diag. 1). Within *Myosotis*, two major clades emerged. Clade A contained most species and Clade B encompassed other species including *M. arvensis*. Both clades exhibited high support values. According to the molecular study, five distinct phylogenetic clades were identified within the *Myosotis* genus. While these primary groups demonstrate clear genetic differentiation, the phylogenetic relationships among them exhibit robust resolution. Future molecular studies incorporating additional species may potentially reveal additional clades within this taxonomic group. This topology aligns with previous studies on *Myosotis* (Winkworth *et al.*, 2002; Meudt *et al.*, 2015; Hao *et al.*, 2017). The newly recorded species was nested within clade A (Diag. 1). Furthermore, *M. arvensis* was a sister clade with *M. sylvatica*; also, this finding was congruent with the finding of Cohen (2013). The primary objective of our endeavor was to ascertain that the genus we encountered aligned with the taxonomic classification of *Myosotis*, and our findings have successfully corroborated this hypothesis.

Palynology

Pollens are small, yellow in color, single, tri-colporate, circular in equatorial view, spheroid in polar view, small-sized, equatorial axis (6.5-8.5) μm , polar axis (11.5-13.0) μm , numerous and pollen shape prolate. The mature pollen grains possess diminutive dimensions. When observed from a lateral perspective, they exhibit an elliptical shape with two shallow indentations on either side. This configuration is also apparent when viewed from above. In the latter orientation, the pollen grains make three small, spherical entities adhering together in a tetrad arrangement (Pls. 3B, 4).

M. arvensis has a small size, monad, tricolporate and prolate, and has a psilate surface, with a heteroaperturate pollen grain. This feature was congruent with (Meudt, 2016). The importance of this study is that it provides us with broad and comprehensive information about the diversity of plants in Iraq by documenting *M. arvensis* subsp. *arvensis*, which had not been found before in this country. The researchers also provided valuable insight into the

Myosotis arvensis subsp. *arvensis*

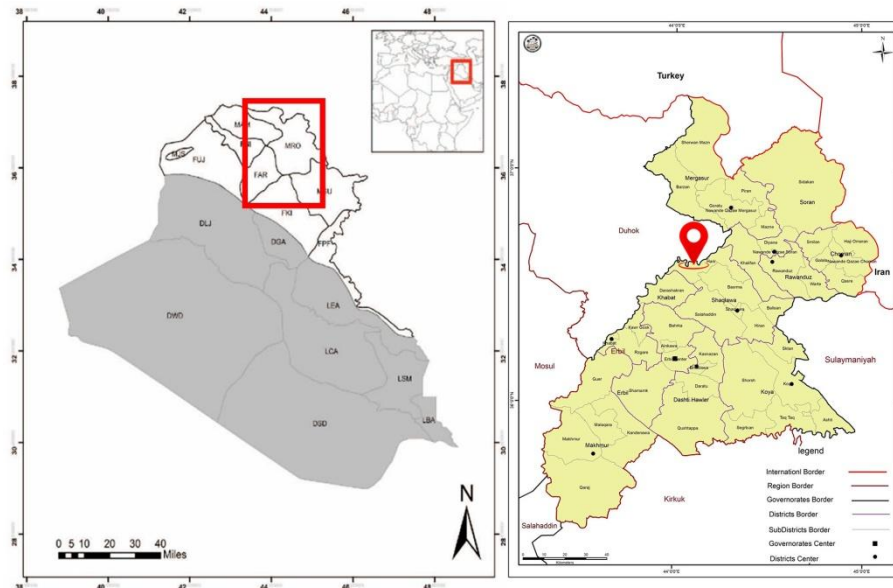
distribution patterns and ecological niches of the subspecies that occupied various range sites. In addition, comprehensive studies of palynology, morphology and phylogenetic characteristics have not only aided in accurately identify and classifying these subspecies, but will also support us in the future to study their evolutionary history and application in various aspects.

Materials examined

MRO: ESUH/ Bekhma dam. (north-east of Erbil), 500-700 m, 26.5.2024, H. Hasan and A. Sardar, 9012, Coordination: 36.703854, 44.279787.

Environment and Presence

This subspecies is isolated and adapted to grow in dry areas, especially in rocky soils and rocks, as well as growing at altitudes between 1600 and 1800 meters above sea level. Their flowering period falls in mid-summer in July and August. The species occurs as isolated individuals distributed throughout the region, specifically adapted to arid environments characterized by sandy substrates and rocky terrain. The population has been documented within the Bekhma Dam complex, situated in the Rouanduz district of the Mountain Region (MRO) (Map 1). This habitat preference suggests that the species has adapted to mesophytic conditions as well as growing at high altitudes in such environments.



Map (1): Physiographic districts of Iraq modified from Guest (1966), the area in white shows the districts of Kurdistan Region.



Plate (1): Herbarium specimen of *M. arvensis* subsp. *arvensis*.

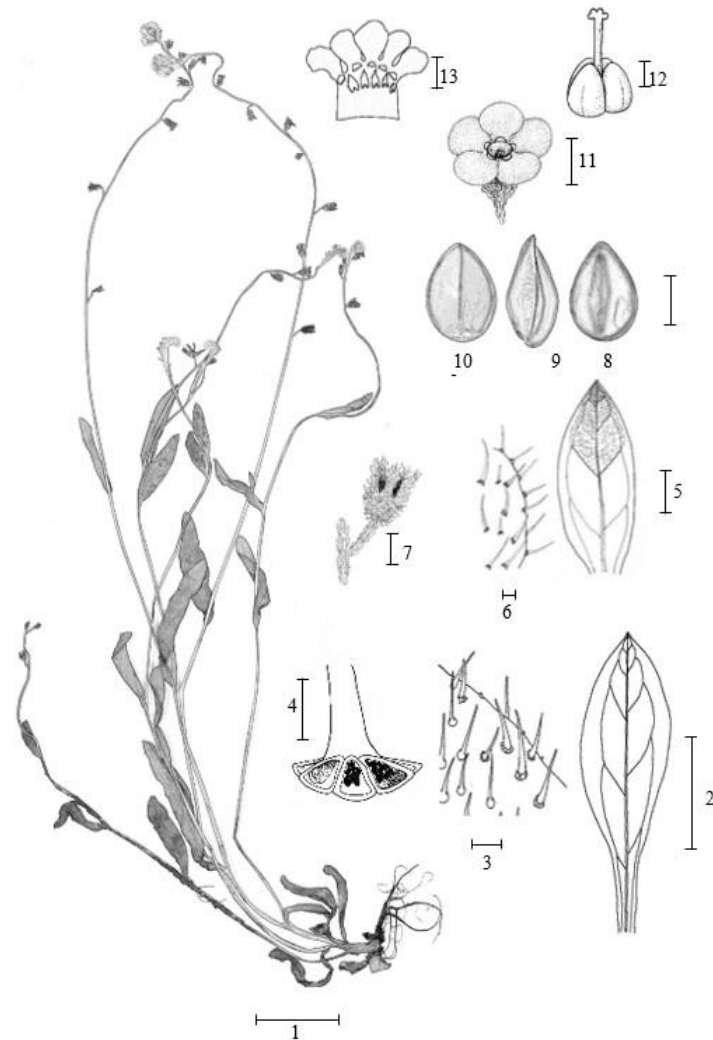
Myosotis arvensis subsp. *arvensis*

Figure (1): *Myosotis arvensis* subsp. *arvensis*. 1: whole plant body, scale bar: 2 cm; 2: Basal leaf, scale bar: 10 mm; 3: trichome, scale bar: 0.2 mm; 4: trichome base, scale bar= 0.1 mm; 5: Cauline leaf, scale bar: 5 mm; 6: trichome, scale bar: 0.1 mm; 7: Fruiting calyx, scale bar:1 mm; 8: Fruit upper surface view; 9: lateral side view; 10: lower surface view, scale bar: 0.2 mm; 11: Flower, scale bar:1mm; 12: Pistil, scale bar: 0.2 mm and 13: Opened corolla, scale bar:1 mm.

Hasan and Sardar



Basal leaf



Lower cauline leaf



Upper cauline leaf



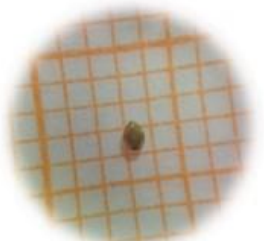
Flower



Inflorescence



Fruiting calyx




Fruit



Pistil



Opened corolla showing
scales & stamens

Plate (2): Plant parts of *M. arvensis* subsp. *arvensis* [ 3 mm]

Myosotis arvensis subsp. *arvensis*

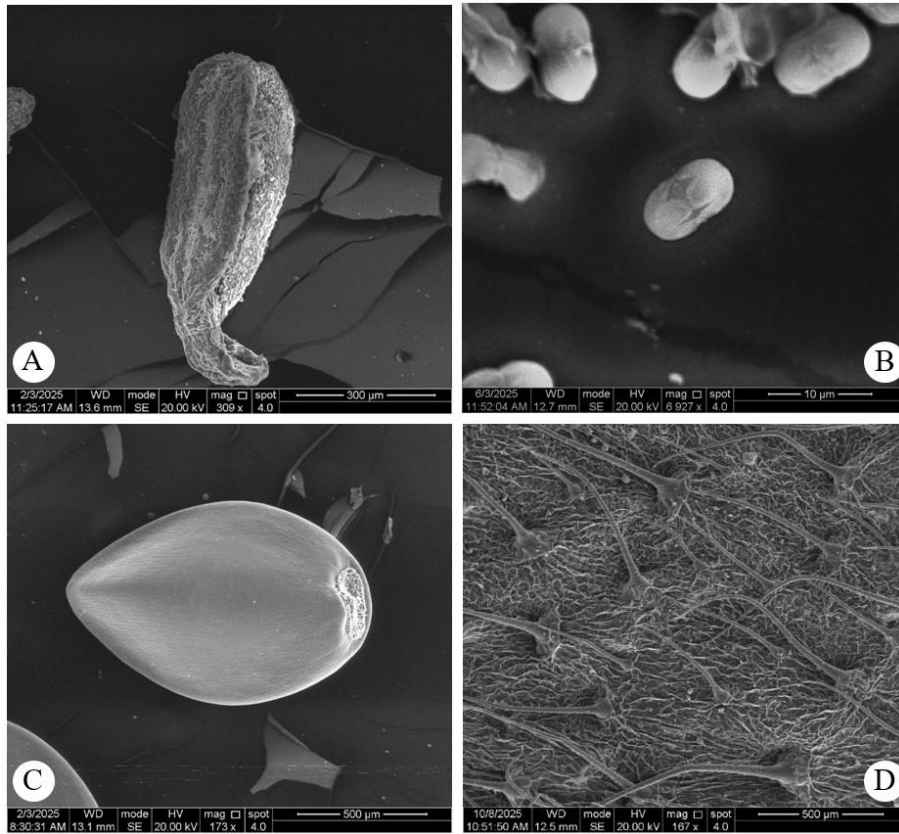


Plate (3): SEM of *Myosotis arvensis* subsp. *arvensis*; (A) Anther, (B) Pollen grains, (C) Fruit, (D) Leaf surface showing trichomes.

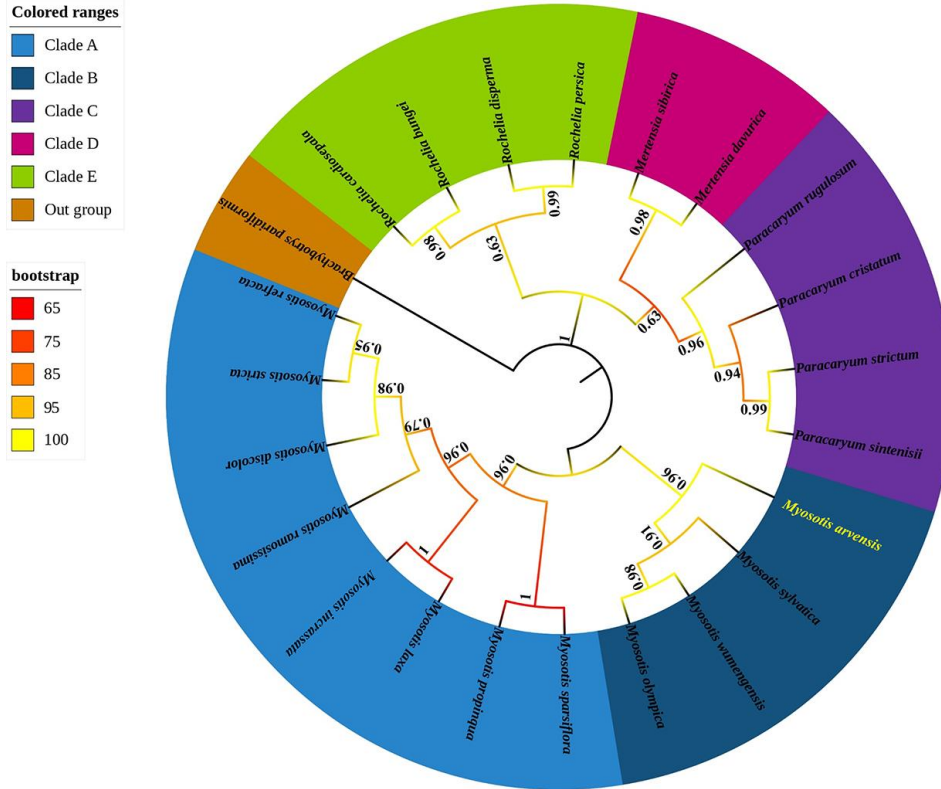


Diagram (1): Strict consensus of most parsimonious tree of selected *M. arvensis* based on maximum parsimony and Bayesian inference analysis of ITS region. Bootstrap proportions are represented by color of the maximum parsimony study and numbers above the branches indicate posterior probability of the Bayesian inference study and clades are denoted by color.

Table (3): Summary of alignment and tree statistics of nrDNA ITS region analyses.

Parameters/Regions	ITS
Aligned length	663
Number of parsimony informative characters	214
Number of variable parsimony uninformative characters	335
Number of constant characters	114
Tree length (steps)	1033
CI (Consistency Index)	0.797
RI (Retention Index)	0.740
RC (Rescaled Index)	0.590
HI (Homoplasy index)	0.203
Model	GTR+G

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10 µm

Plate (4): Pollen grains of *M. arvensis*, showing equatorial view by LM - X100

CONCLUSIONS

The present study has made a significant contribution to the field of botany in Iraq by confirming the occurrence of *Myosotis arvensis* subsp. *arvensis*, representing a new record for the country's flora. The morphologically newly documented species is distinguished from others by its distinct calyx dimensions and the configuration of calyx lobes during fruiting, along with differences in the corolla limb. However, molecular evidence confirmed that the position of *M. arvensis* shows as a sister clade to *M. sylvatica*. Furthermore, the characteristics like tricolpate pollen grain, porolate shape, psilate surface texture and heteroaperturate structure approve the new subspecies record.

A comprehensive literature related to the genus demonstrates that the total number of *Myosotis* species in Iraq that were grown in mountainous regions is eight species. These species are *M. alpestris*, *M. discolor*, *M. olympica*, *M. ramosissima*, *M. laxa*, *M. refracta*, *M. silvatica*, and the newly recorded *M. arvensis*. This finding highlights the importance of continuous efforts to document and protect Iraq's plant diversity, and it also highlights the importance of various strategies to manage and protect this valuable natural resource for future generations.

CONFLICT OF INTEREST STATEMENT

"The authors have no conflicts of interest to declare".

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تسجيل جديد للنوع *Myosotis arvensis* subsp. *arvensis* (L.) Hill, 1764

(Boraginales, Boraginaceae)

للمجموعة النباتية العراقية

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الخلاصة

يعتبر جنس النبات *Myosotis* L., 1753 أحد أكبر اجناس العائلة Boraginaceae التي تعود الى عويلة Cynoglossoideae و قبيلة Myosotideae، فحصت العديد من خصائص حبوب اللقاح، بما في ذلك الشكل واللون والحجم والزخرفة السطحية والعدد لتحت نوع المظهرية الكبيرة والدقيقة. كما أجريت دراسة جزيئية لتأكيد المكانة التصنيفية للنبات ضمن العائلة. بالمقارنة مع الأنواع الأخرى ضمن نفس الجنس والتي تنمو في العراق، تتميز النماذج التي جمعت لهذا النوع بأن طرف التويج فيها يكون طبقي الشكل ويصل قطره إلى 3 ملم، كما ان فصوص الكاس تكون متقاربة في الثمرة وتُغطى بالعديد من الشعيرات الخطافية المنتشرة. كانت حبوب اللقاح صغيرة وصفراء وأحادية وثلاثية الأضداد المثقبة وظهرت إهليلجيه في المنظر الاستوائي وكروية في المنظر القطبي.

أكدت الدراسة الجزيئية التصنيف والانحدار التطوري لهذا النوع المكتشف حديثاً من خلال تأكيد ارتباطه الوراثي وموقعه ضمن الإطار العام للعائلة النباتية. هدفت الدراسة الى إثبات ووصف التحت نُميط للمرة الاولى ضمن الموسوعة النباتية العراقية.