

SABRAO Journal of Breeding and Genetics  
 58 (5) 2222-2232, 2026  
<http://doi.org/10.54910/sabrao2026.58.5.22>  
<http://sabraojournal.org/>  
 pISSN 1029-7073; eISSN 2224-8978



## GEOGRAPHICAL, MORPHOLOGICAL, AND MOLECULAR ANALYSES OF SIX WILD ORCHID TAXA IN THE KURDISTAN REGION, IRAQ

A.M. MAHMOOD<sup>1</sup>, L.H. KHAL<sup>2\*</sup>, K.S. RASUL<sup>2</sup>, and T.H. HALABJAI<sup>3</sup>

<sup>1</sup>Department of Biology, College of Education, University of Garmian, Kalar, Kurdistan Region, Iraq

<sup>2</sup>Horticulture and Landscape Design Department, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq

<sup>3</sup>Swren High School for Boys, Ministry of Education, Halabja, Kurdistan Region, Iraq

\*Corresponding author's email: [lanja.khall@univsul.edu.iq](mailto:lanja.khall@univsul.edu.iq)

Email addresses of co-authors: [asaad@garmian.edu.krd](mailto:asaad@garmian.edu.krd), [kamaraman.rasul@univsul.edu.iq](mailto:kamaraman.rasul@univsul.edu.iq), [teymurhesen@gmail.com](mailto:teymurhesen@gmail.com)

### SUMMARY

The family Orchidaceae has an extremely diverse and complex evolutionary relationship. Despite global studies on orchid genetic diversity and evolution, the local wild orchids remain less characterized for genetic diversity in the Kurdistan Region, Iraq. Consequently, the genetic diversity of this local ecotype remains unknown. This study aimed to examine the morphometric patterns and molecular genotyping of six species (*Anacamptis papilionacea*, *Himantoglossum caprinum*, *Ophrys reinholdii*, *Ophrys schulzei*, *Ophrys sphegodes* subsp. *taurica*, and *Orchis mascula*) of wild orchids collected in the Kurdistan Region, Iraq. A morphological study revealed that the genus *Ophrys* taxa had remarkable phenotypic differences, particularly in the speculum colors and labellum patterns. Species *H. caprinum* was distinctive because of the long and ribbon-like central labellum lobe. The morphological characterization through Iraqi flora and molecular analysis confirmed the species *O. mascula* as an *Orchis* clade component. Molecular analysis involved amplification and sequencing of the ITS1-5.8S-ITS4 region, and the phylogenetic tree showed four major clades corresponding to the four genera *Ophrys*, *Anacamptis*, *Himantoglossum*, and *Orchis*. Consequently, integrating morphological traits with ITS-based phylogeny supported the current taxonomic placement of the studied taxa and highlighted the values of integrative approaches in plant taxonomy.

**Keywords:** Orchidaceae, genetic diversity, morphological traits, ITS markers, *Ophrys*, *Anacamptis*, *Himantoglossum*, *Orchis*

**Key findings:** The results revealed the ITS marker provides a reliable and efficient approach for species identification among six orchid taxa, hence enhancing integrative taxonomy and facilitating species differentiation within the family Orchidaceae.

Communicating Editor: Dr. Gwen Iris Descalsota-Empleo

Manuscript received: March 18, 2026; Accepted: May 28, 2026.

© Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2026

**Citation:** Mahmood AM, Khal LH, Rasul KS, Halabjai TH (2026). Geographical, morphological, and molecular analyses of six wild orchid taxa in the Kurdistan Region, Iraq. *SABRAO J. Breed. Genet.* 58 (5) 2222-2232. <http://doi.org/10.54910/sabrao2026.58.5.22>.

## INTRODUCTION

The Orchidaceae is one of the most evolutionarily diverse flowering plant families, comprising around 31,000 species distributed worldwide (Freudenstein, 2025), with 22 species identified in Iraq (Townsend, 1966). Orchids exhibit unique biological traits that differentiate them from other angiosperms, including diverse flowers and seeds, with close interactions with mycorrhizal fungi, crassulacean acid metabolism, and unmatched reproductive strategies that facilitate wide ecological specialization (Vitt *et al.*, 2023). Beyond their ecological and evolutionary significance, orchids hold considerable economic value in the floriculture industry and have long been beneficial for medicinal and nutritional purposes worldwide. Considering these vital traits, orchids represent valuable model systems for studying plant evolution and diversification (Fay *et al.*, 2025). Recent advancements in molecular and genomics analyses have basically reformed the understanding of orchid phylogeny and lineage diversification (Zhang *et al.*, 2022). Despite recent advances in molecular systematics, orchid taxa have experienced delimitation based on diagnostic parameters, such as labellum morphology, spur length, inflorescence architecture, floral coloration, and vegetative traits (Laface and Torino, 2025).

Terrestrial orchids in the Mediterranean and West Asian regions significantly contribute to the overall diversity in the family Orchidaceae. However, in these regions, several genera remain taxonomically challenging, including *Orchis*, *Ophrys*, *Anacamptis*, and *Himantoglossum* (Turco *et al.*, 2023). In particular, within the genus *Ophrys*, the pollinator-driven selection has further promoted the rapid floral diversification, often resulting in slight morphological differences in reproductive structures with no obvious vegetative distinctions. Consequently, the intimately related taxa may exhibit coincident morphological traits, whereas distantly related species may converge in floral traits due to similar pollination strategies (Baguette *et al.*,

2020). These complexities further limited the reliability of morphology-based identifications and crucially highlighted the need for harmonizing molecular approaches.

Molecular systematics has reshaped orchid taxonomy and phylogenetic research. Among the widely employed genetic markers, the nuclear ribosomal internal transcribed spacer (ITS) region has proven to be particularly informative for species-level identification and phylogenetic presumptions within the family Orchidaceae (Al-Taie *et al.*, 2025; Tsaballa *et al.*, 2023). The ITS region combines relatively rapid evolutionary rates, facilitating discrimination among the closely related taxa, with conserved contiguous sequences that enable reliable alignment across the genera. Owing to its high copy number and biparental inheritance, ITS has become a standard marker in orchid systematics. Phylogenetic analysis based on ITS data provides crucial insights into lineage relationships, patterns of speciation, and evolutionary variances (Karim *et al.*, 2025). Particularly, the genetically similar taxa may exhibit considerable morphological divergence due to ecological differences in pollination methods. Accordingly, the integrative approaches that combine morphological evidence with molecular data provide a reliable method for determining taxonomic identification and evolutionary relationships (Johnson, 2025).

Geographic and altitudinal distribution patterns considerably influence the orchid diversity and genetic structures, with mountainous regions often acting as centers of endemism and refugia that promote population isolation and local adaptation (Liu *et al.*, 2024). The Kurdistan Region of Iraq has a location within the Irano-Anatolian phytogeographical zone, characterized by complex topography, broad altitudinal gradients, and diverse habitats suitable for terrestrial orchids. Despite this ecological potential, orchid genetic diversity has not received thorough examination at the genetic levels, with most previous studies relying solely on morphological identifications in the Kurdistan Region. Recent research highlighted the importance of integrating genetic data with

spatial distribution analysis to accurately document orchid diversity and explain the biogeographical patterns in understudied regions (Pérez-Escobar *et al.*, 2021; Evans *et al.*, 2023). Addressing this gap enabled the following study to integrate morphological characterization, ITS-based molecular identification, phylogenetic reconstruction, and geographic distribution analyses to provide the first comprehensive assessment of selected wild orchid taxa in the Kurdistan Region, Iraq.

## MATERIALS AND METHODS

### Taxon sampling

In the presented study, dividing the Kurdistan Region, Iraq, resulted in three districts: Amadya (MAM), Rawanduz (MRO), and Sulaymaniyah (MSU). During 2024–2025, the collection of six terrestrial orchid taxa succeeded from the different locations of these three districts (Figure 1). These comprised *Anacamptis papilionacea* (L.) R. M. Bateman, Pridgeon & M. W. Chase, *Himantoglossum caprinum* (M. Bieb.) Spreng, *Ophrys reinholdii* Spruner ex Boiss., *Ophrys schulzei* Bornm. & Fleischm., *Ophrys sphegodes* subsp. *taurica* (Aggeenko) Soó ex Niketic & Djordjevic, and *Orchis mascula* (L.), with all taxa deposited at the University of Sulaimani, College of Agricultural Engineering Science Herbarium (SUFA). The confirmation of their identification used both morphological keys and ITS molecular markers.

### Morphological description

In the field, all the samples collected were according to their morphological traits, including roots, stems, leaves, flowers, fruits, and seeds. The morphological traits especially included the flower arrangement and the unique structure of the flower lip. The flower lip (labellum) was the spatula-shaped with scalloped edges and faint spots, which divided into three deep lobes, and the middle lobe appearing brown-ish-green. Following morphological descriptions, the transfer of

samples to the laboratory enabled examination by taxonomists, further comparing them with specimens placed in the Sulaimani University, Iraq. The studied samples also sustained comparison with the flora of Iraq and neighboring countries such as Iran and Turkey. The vouchers received designations with numbers SUFA-61 to SUFA-66. Fresh leaf tissues immediately underwent flash-freezing in liquid nitrogen before storage at -20 °C until DNA extraction for molecular analyses.

### DNA extraction

The DNA extraction procedure proceeded according to previous studies, with some modifications (Khal *et al.*, 2023). These modifications included increasing the lysis time from 75 to 90 min and incubating at 65 °C instead of 63 °C. DNA quality and concentrations underwent assessment using 1% agarose gel electrophoresis and a Nano Drop spectrophotometer (Nano PLUS, MAANLAB AB, Växjö, Sweden).

### PCR assay, purification, and sequencing

The nuclear ribosomal internal transcribed spacer (nrDNA ITS) region entailed amplification using the universal primers ITS1 (5' TCCGTAGGTGAAC0CTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (Jawad *et al.*, 2024). The conduct of PCR amplification continued in a final volume of 26 µL containing 5 µL of genomic DNA (50 ng), 10 µL of master mix (AddStart Taq Master, AddBio, Daejeon, Republic of Korea), 2 µL (10 µM) of each ITS primer, and 7 µL of nuclease-free water. The thermal cycling conditions comprised an initial denaturation at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 2 min, with a final extension at 72 °C for 10 min. Using a negative control (without template DNA) served to monitor each reaction set. Following PCR amplification, the product's separation ensued on 1.5% agarose gels with 1X TBE buffer, stained with ethidium bromide, and visualized under UV light (ENDURO GDS Touch, Labnet, USA) (Rasul *et al.*, 2022).

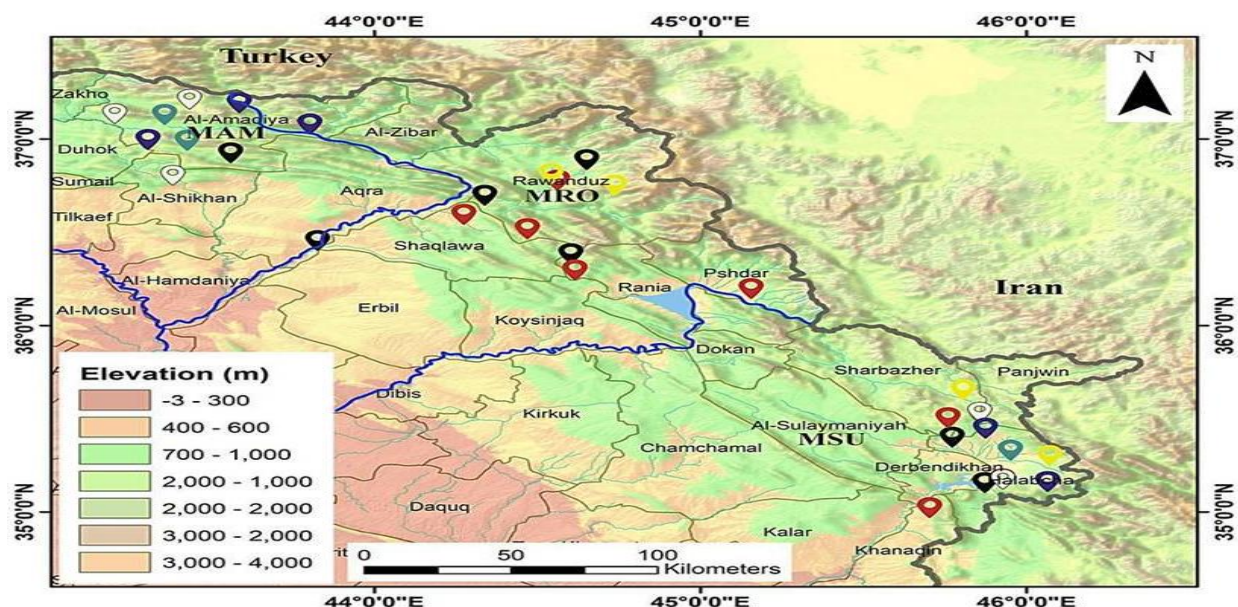
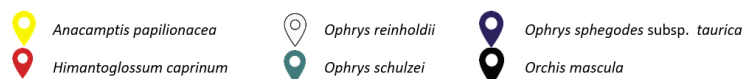


**Figure 1.** Locations of the six wild Orchids collected from different areas of Kurdistan Region, Iraq.



PCR product sequencing excised ITS bands, followed by DNA amplicon extraction from the agarose using a Gel Extraction Kit (AddBio, Daejeon, Republic of Korea). The DNA sequencing continued at Microgen Company in South Korea (Mahmood *et al.*, 2020). The purified amplicons, as sequenced, had the obtained sequences compared with reference sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLASTn algorithm. Species identity assignment depended on the highest sequence similarity ( $\geq 99\%$ ) and query coverage.

### Phylogenetic tree and statistical analysis

The research submitted obtained ITS sequences in the GenBank database of the NCBI, with accession numbers being assigned (<https://www.ncbi.nlm.nih.gov/WebSub>). Closely related reference sequences entailed retrieval from GenBank for comparative analysis. Multiple sequence alignment, as performed, used ClustalX version 2.1 with default parameters (Larkin *et al.*, 2007).

Phylogenetic analysis continued using the Neighbor-Joining (NJ) method in MEGA version 11. The robustness of the inferred tree topology received assessment with 1000 bootstrap replicates (Kumar *et al.*, 2016). All molecular work, including DNA extraction, PCR, and gel purification, but excluding sequencing, took place at the Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani.

## RESULTS AND DISCUSSION

### Geographical distributions

The orchid taxa distribution map comprising various districts of the Kurdistan Region, Iraq, appears in Figure 1. The results revealed the species *A. papilionacea* was dominant in the districts of Sulaymaniyah and Rawanduz at altitudes between 758 and 1392 meters above sea level (masl). The species *H. caprinum* also had distribution in the districts of Sulaymaniyah and Rawanduz at the elevations ranging from 947 to 1606 masl. Additionally,

both species, *O. reinholdii* and *O. schulzei*, were evident in the latter districts too, but at elevations of 1428–1611 and 1398–1481 masl, respectively. The species *O. sphegodes* subsp. *taurica* showed the widest distribution across the districts of Sulaymaniyah, Rawanduz, and Amadya at the elevation of 1351–1772 masl. Furthermore, the species *Orchis mascula* was available in the districts of Sulaymaniyah and Rawanduz within the altitudinal range of 893–1752 masl. Despite the selected taxa sharing a common ancestor, their procurement was from different locations of the Kurdistan Region, Iraq. These results indicate genetic variation among the studied taxa and suggest their adaptation to the studied environments (Baumann et al., 2006).

### Morphological description

The presented study evaluated the similarity of the morphological trait differentiations among the six terrestrial orchid taxa obtained from the Kurdistan Region, Iraq. Qualitative and quantitative morphological traits of the six orchid taxa underwent observations as below, with discussions herein in the Figures 2 A-F.

### *Anacamptis papilionacea*

The species *A. papilionacea* showed characteristics of an upright stem measuring 15–30 cm, often with 2–8 lanceolate, unspotted leaves (Figure 2A). This subspecies was predominantly quite stocky, with spike-like inflorescences bearing numerous flowers. Its flowers represent a spatula-shaped, discreetly spotted, crenellated lip, which are the distinctive features of the said taxon (Townsend, 1966; Reching, 1970).

### *Himantoglossum caprinum*

The morphology of the species *H. caprinum* has a tuberous rootstock with an erect, robust, leafy stem measuring 40–70 cm and has one or two membranous sheaths at the base (Figure 2B). Leaves are dull bluish-green, the lowermost 7–10 grouped at the base, while the uppermost (2–3 cauline) are bract-like, often reaching the base of the inflorescence. The inflorescence was elongated and lax (10–35), bearing (15–40) grayish-green and brown flowers. Sepals were green, often observed with bordered dull purple and brownish-violet;

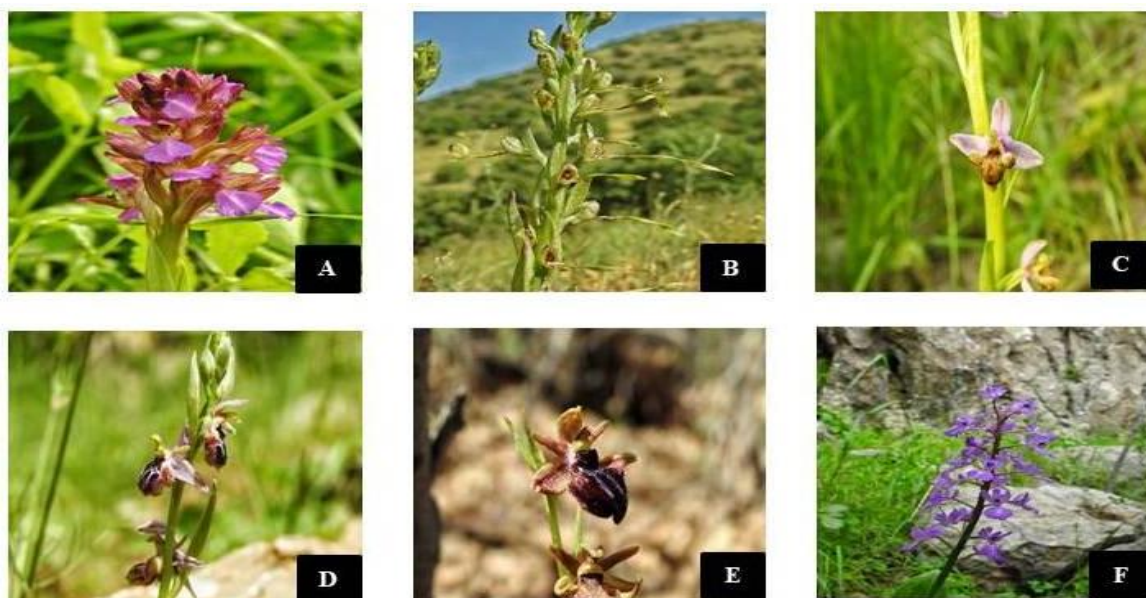


**Figure 2.** Qualitative and quantitative characteristics of inflorescences of the studied taxa. **A:** *Anacamptis papilionacea*, **B:** *Himantoglossum caprinum*, **C:** *Ophrys reinholdii*, **D:** *Ophrys schulzei*, **E:** *Ophrys sphegodes* subsp. *taurica*, and **F:** *Orchis mascula*.

the veins on the inner surface were usually deep purple, conniving to form an obtuse helmet. Petals were pale green and linear-lanceolate. Loki *et al.* (2015) also reported the labellum was deeply three-lobed, with a middle lobe brownish green, more or less spirally twisted, and more or less divided at the apex into two segments (2–15 mm); the lateral lobes were falcately lanceolate, with an undulate margin.

In terms of taxonomy, the most important point about the said species was that how the labellum stretches and twists. This taxon's description of the middle lobe as 'spirally twisted' and severely split is unusual. This unique feature, along with the 'lizard-like' or 'goat-like' appearance (suggested by the twisting and dividing apex), sets it apart from the compact and helmeted blossoms of *Orchis* and *Anacamptis*.

### **Ophrys species**

#### ***Ophrys reinholdii***

The species *O. reinholdii* is a tuberous plant with an erect, leafy (30–60 cm) stem, featuring 1–2 sheaths above and remaining bare below the inflorescence (Figure 2C). It has 3–6 lanceolate and rosulate leaves. The inflorescence is elongate, with 3–10 lax flowers. Sepals are often rose, sometimes whitish and greenish-rose, with a dark green midnerve, spreading to reflex; petals were ligulate and pinkish-brown to dark violet-rose. The labellum was deeply three-lobed, 12–15 mm long, and slightly convex downward, with a broad greenish border; the mid-lobe is dark brown and the speculum snowy-white, comprising two spots or parallel lines sometimes joined at the base (like the Greek letter Pi: Π). The lateral lobes are purple-brown with smoothed gibbosities, mostly whitish outside (var. *leucotaenia*), and sometimes entirely brownish. Baumann *et al.* (2006) also reported that the apical appendage was small and often reduced to a triangular mucro.

#### ***Ophrys schulzei***

The species *O. schulzei* is a tuberous plant with a slender, erect, leafy stem measuring 25–65 cm (Figure 2D). Leaves are 4–5 and narrowly elliptic to lanceolate. It has a loose arrangement and elongation, reaching 20–30 cm in length, bearing many spreading, small, and lax flowers; sepals were reflex, pink or violet-rose with a green mid-vein, while petals were minute, elongated, triangular, rose-violet, velvety near the base, and papillose towards the apex. Rechinger (1970) reported the labellum as distinctly three-lobed and robustly convex; the lateral lobes are large, forming obtuse, hairy cones outside. Meanwhile, the mid-lobe is dark brown, strongly convex, with a revolute margin and apex; the speculum is reddish-brown, irregular, extending from the base to almost the apex of the mid-lobe and diverging in varying ways toward the sides and enclosing a central dark brown area. The apical appendage is greenish-yellow, large, entirely or slightly tridentate, and recurved downwards.

#### ***Ophrys sphegodes* subsp. *Taurica***

The species *O. sphegodes* has an erect, slender stem measuring 15–40 cm, with 2–5 leaves crowded near the base (Figure 2E). The inflorescence was lax to dense, comprising 3–12 flowers. The dorsal sepal was greenish, while the lateral sepals were half green at the top and half purplish at the bottom. Petals were ribbon-like, either glabrous or subglabrous, and greenish with brownish-rose suffusion. The labellum was slightly three-lobed, which narrowed toward the base, with a spiculate apex and no prominent appendix; it was partially convex, brown to purplish-brown, and velvety (Rechinger, 1970). The speculum was grayish-blue, sometimes iridescent, and H-shaped.

Collectively, the three studied *Ophrys* species (*O. reinholdii*, *O. schulzei*, and *O. sphegodes* subsp. *taurica*) exhibited the highest degree of floral specialization traits of sexually deceptive orchids. For instance, the

*Ophrys* species, in contrast with other genera, rely more on intricate labellum patterning (speculum) and texture rather than on nectar incentives and spurred forms. The speculum can considerably be an important morphological feature in distinguishing *Ophrys* species from others. In several cases, such specialization had the interpretation as a response to pollinator-mediated selection, a mechanism implicated in rapid genus divergence (ref.). The speculum of the species *O. reinholdii* displayed a shape like that of a 'Pi' (Π), which makes it different from the H-shaped iridescence of the species *O. sphegodes* subsp. *taurica* and the uneven, diverging reddish-brown markings of the species *O. schulzei*. All three studied taxa have rounded forms and textures that feel like velvet (the species *O. schulzei* has hairy lobes, while *O. sphegodes* has velvety lobes). There existed reports of sexually deceptive orchids showing this pattern due to recent radiation, incomplete lineage sorting, and ongoing gene flow. The species *O. schulzei* appeared to be considerably different from other plants because its edge was highly revolute and its side lobes were cone-shaped and obtuse. On the said flower, the petals were not like those on the species *O. sphegodes*, which were flat and ribbon-like (Devey *et al.*, 2009).

### ***Orchis mascula***

The species *O. mascula* is a tuberous rootstock plant with an erect and stout stem (20–45 cm), pale green and often reddish-violet at the top, marked at the base with dark spots (Figure 2F). The leaves were glossy green, oblong-lanceolate, and unspotted. The inflorescence was long and lax, rose-purple, with many flowers (25–50). Sepals and petals were entirely reddish to mauve, with the lateral sepal and the petals forming an incomplete helmet. The labellum has marks with only a few spots toward the center. The spur, 1.5 or two times the height of the labellum, was generally straight and rounded at the apex (Govaerts *et al.*, 2017).

The comparison between the species *A. papilionacea* and *Orchis mascula* revealed the

'classic' orchid flower plant, in which the sepals and petals work together to form a protective hood. The species *Orchis mascula* has a large spur (1.5 to two times the height of the labellum) and a very lobed lip. The vegetative description revealed unspotted leaves, a significant finding, as some populations of the species *O. mascula* in different areas have spotted leaves. The results suggested the specimens being studied belong to a different morphotype or subspecies. Moreover, the species *Anacamptis papilionacea* was thicker; however, it grows in the same way. The 'spatula-shaped' lip with crenellated edges and prominent veining (crenellation) makes it easy to describe the differences between this and the three-lobed structure of the *Orchis* and *Himantoglossum* species.

### **Phylogenetic analysis**

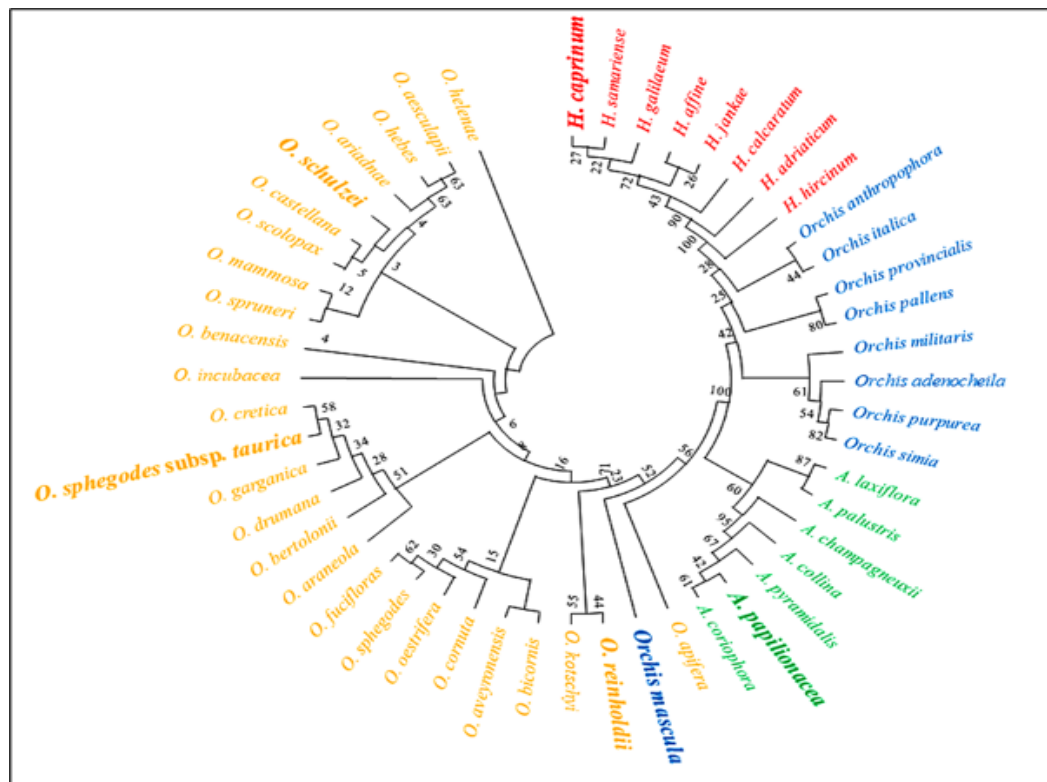
The sequenced ITS rDNA PCR products of the six orchid species appear in Table 1. The ITS sequences succeeded their checking through ITS sequencing (the sequences comprising 18S rRNA, the entire ITS1 region, and the 5.8S rRNA region) with the universal primer pairs ITS1 and ITS4. The sequences of these taxa reached uploading into the NCBI database with the six species names, viz., *A. papilionacea*, *H. caprinum*, *O. reinholdii*, *O. schulzei*, *O. sphegodes* subsp. *taurica*, and *O. mascula*. The accession numbers for each taxon were PX794516, PX794522, PX794518, PX810173, PX803112, and PX794523, respectively.

The sequences of the studied taxa revealed alignments with those of 44 orchid species that showed more close relations to construct the phylogenetic tree and consequently generated four clusters (Figure 3). The first clade (orange) comprised 27 species. Three of the studied taxa (including *O. schulzei*, *O. sphegodes* subsp. *taurica*, and *O. reinholdii*) had their location in this clade. Moreover, the species *O. schulzei* lay within a separate sub-clade of seven *Ophrys* taxa, which was in close association with the species *O. castellana* and *O. scolopax*. However, the species *O. sphegodes* subsp. *taurica* had its nesting within a subclade with five other taxa

**Table 1.** Sampled terrestrial orchid taxa from the Kurdistan Region, Iraq, with voucher information, sampling districts, altitudinal ranges, and GenBank accession numbers based on rDNA ITS sequences.

| Genus                 | Taxa                                          | Voucher No. | Sampling districts | Altitude range (m asl) | GenBank accession No. |
|-----------------------|-----------------------------------------------|-------------|--------------------|------------------------|-----------------------|
| <i>Anacamptis</i>     | <i>Anacamptis papilionacea</i>                | SUFA -61    | MSU and MRO        | 758–1392               | PX794516              |
| <i>Himantoglossum</i> | <i>Himantoglossum caprinum</i>                | SUFA-62     | MSU and MRO        | 947–1606               | PX794522              |
| <i>Ophrys</i>         | <i>Ophrys reinholdii</i>                      | SUFA-63     | MSU and MAM        | 1428–1611              | PX794518              |
|                       | <i>Ophrys schulzei</i>                        | SUFA-64     | MSU and MAM        | 1398–1481              | PX810173              |
|                       | <i>Ophrys sphegodes</i> subsp. <i>taurica</i> | SUFA-65     | MSU, MRO, and MAM  | 1351–1772              | PX803112              |
| <i>Orchis</i>         | <i>Orchis mascula</i>                         | SUFA-66     | MSU and MRO        | 893–1752               | PX794523              |

MAM = Amadya District, MRO = Rawanduz District, and MSU = Sulaymaniyah District.

**Figure 3.** Neighbor-joining tree for six wild Orchid taxa using MEGA 11 as the nucleotide substitution model. The branch lengths are relative to the number of predicted nucleotide substitutions. The numbers represent the bootstrap values. Different colors signify different clades. The bold taxon represents the studied samples.

of *Ophrys*, showing the highest similarity to the species *O. cretica* and *O. garganica*. Additionally, the species *O. reinholdii* taxon revealed a close correlation to *O. kotschyi*.

The genus *Himantoglossum* generated a separate clade (red) and comprised eight taxa. The studied taxon *H. caprinum* sat in this clade and shared a common ancestor with *H.*

*samariense* and *H. galilaeum*, though its placement on the relatively long branch suggested the highest dissimilarity between the species *H. caprinum* and *H. hircinum*. According to such molecular studies, species *H. caprinum* belongs to the genus *Himantoglossum* clade, supporting the taxonomic validity of these distinctive floral

traits and its generic placement (Harzli *et al.*, 2025).

Interestingly, the taxon *Orchis mascula* was split from the third clade (blue) of the genus *Orchis*, which includes eight taxa. However, the species *O. mascula* showed a recovery within the *Orchis* clade but formed a distinct lineage separated from other *Orchis* taxa, with moderate bootstrap support. It appeared to exhibit the significant diversity within the main *Orchis* clade. Geographic structure and intraspecific divergence across its distribution range may explain the observed genetic differentiation (Mafakheri *et al.*, 2022). The results suggested a polyphyletic and highly diverse nature within the genus *Orchis* in the studied region. Moreover, the fourth clade (green), as generated by the genus *Anacamptis*, comprised seven taxa. The species *A. papilionacea* revealed obvious clustering with the other genus, *Anacamptis* species. It appeared that this taxon had mostly a close relation to the species *A. pyramidalis* and *A. coriophora* (Scopece *et al.*, 2013).

The nuclear ribosomal internal transcribed spacer (ITS) region proved to be the best single-locus marker for assessing orchid taxa (Górecka *et al.*, 2025). Numerous studies enunciated that the ITS was useful for more than just identifying objects; its biparental inheritance pattern proves it essential for detecting hybrids, which are common in orchid biology. The ITS may reveal the additive polymorphisms that suggest a hybrid origin, but this more complicated the sequence analysis (Vu *et al.*, 2017). Despite the lack of obvious evidence of hybrid origin in the presented study, it may be essential to rely on more multi-locus approaches to fully understand the species boundaries. Moreover, ITS was well documented to effectively recognize the early developmental stages, such as protocorms, which had complete characterization by the lack of distinctive morphological traits (Harzli *et al.*, 2025).

In the current study, the use of ITS markers sought to expose the similarities among the studied taxa and to compare them with other taxa documented in NCBI. The results showed these six taxa were genetically consistent with their current botanical names;

however, they also showed interesting evolutionary distances. The location of the species *O. sphegodes* subsp. *taurica*'s in the *Ophrys* cluster confirmed that it was a member of the *sphegodes* aggregate. The high bootstrap support in this area suggested that these species have numerous varying shapes but emerged diversified from the same ancestor very recently. A big part of the conversation was about how the species *Orchis mascula* differed from the main *Orchis* cluster. *Orchis* is a complicated genus that has received studies at the molecular level. Some species, such as *O. mascula*, occurred more closely related to *Ophrys* than to other *Orchis* species (Scopece *et al.*, 2013; Mafakheri *et al.*, 2022). These results revealed that morphological traits were insufficient to significantly distinguish the *Orchis* species. The findings proposed that morphological traits alone do not provide sufficient resolution to differentiate the *Orchis* species. Nevertheless, the integration of morphological traits with molecular approaches offered an accurate framework for species identification and classification (Górecka *et al.*, 2025). However, it is vital to note that the use of a single molecular marker (ITS) may limit the resolution of phylogenetic relationships (Al-Taie *et al.*, 2025), hence recommending future studies incorporating additional loci.

## CONCLUSIONS

The morphological characterization successfully distinguished orchid taxa in the Kurdistan Region, Iraq, particularly within *Ophrys*, where distinct patterns of the labellum and speculum were noteworthy. Based on ITS-based phylogenetics, the studied taxa entailed groupings within *Anacamptis*, *Himantoglossum*, *Orchis*, and *Ophrys*. Despite this, relationships between closely associated taxa only reached partial resolution at the species level, especially among *Ophrys*. Overall, the concordance between morphological evidence and ITS phylogeny supports the current taxonomic treatment of these orchids in the Kurdistan Region, Iraq.

## ACKNOWLEDGMENTS

The authors are grateful to the staff of the College of Agricultural Engineering Sciences at the University of Sulaimani, Sulaymaniyah, Iraq, for their assistance and cooperation throughout the research process.

## REFERENCES

- Al-Taie AT, Golparvar AR, Hasan MA, Darbaghshahi MRN (2025). Molecular taxonomy of *Vicia* L. species (Fabaceae) from the mountainous regions of Iraqi Kurdistan based on ITS barcoding. *Phytotaxa* 726(4): 259–271.
- Baguette M, Bertrand JA, Stevens VM, Schatz B (2020). Why are there so many bee-orchid species? Adaptive radiation by intra-specific competition for mnesic pollinators. *Biol. Rev.* 95: 1630–1663.
- Baumann H, Kunkele S, Lorenz R (2006). Orchideen Europas: Mit Angrenzenden Gebieten. Eugen Ulmer, Stuttgart.
- Devey DS, Bateman RM, Fay MF, Hawkins JA (2009). Genetic structure and systematic relationships within the *Ophrys fuciflora* aggregate (Orchidaceae: Orchidinae): High diversity in Kent and a wind-induced discontinuity bisecting the Adriatic. *Ann. Bot.* 104: 483–495.
- Evans A, De Kort H, Brys R, Duffy KJ, Jersáková J, Kull T, Selosse MA, Tsiftsis S, Minasiewicz J, Jacquemyn H (2023). Historical biogeography and local adaptation explain population genetic structure in a widespread terrestrial orchid. *Ann. Bot.* 131(4): 623–634.
- Fay MF, Andriamahefarivo L, Bachman S, Brown M, Calevo J, Campbell T, Charitonidou M, Dixon K, Droissart V, Forest F (2025). How threatened are orchids? A review of the state of play and identification of gaps and priorities. *Biodivers. Conserv.* 34: 5075–5115.
- Freudenstein JV (2025). Orchid phylogenetics and evolution: History, current status and prospects. *Ann. Bot.* 135(5): 805–822.
- Górecka M, Rewicz A, Kolanowska M (2025). DNA barcoding protocol for Masdevallia orchids: A tool for CITES-based identification and trade control. *Genes* 16: 1423.
- Govaerts R, Bernet P, Kratochvil K, Gerlach G, Carr G, Alrich P, Pridgeon AM, Pfahl J, Campacci MA, HollandBaptista D, Tigges H, Shaw J, Cribb P, George A, Kreutz K, Wood J (2017). World Checklist of Orchidaceae. Facilitated by the Royal Botanic Gardens, Kew.
- Harzli I, Mutlu VA, Özdener Kömpe Y (2025). Evaluating ITS barcode as a molecular tool for determining orchid protocorms genetic identity. *J. Oasis. Agric. Sustain. Dev.* 7(1): 1–7.
- Jawad MM, Al-Taie AT, Al-Hadeethi MAH, Khal LH (2024). Molecular analysis of wildy grown genus *Veronica* species (Plantaginaceae) in Iraq. *SABRAO J. Breed. Genet.* 56(4): 1473–1483.
- Johnson SD (2025). Pollination ecotypes and the origin of plant species. *Proc. Biol. Sci.* 292(2039): 20242787.
- Karim HR, Rasul KS, Halshoy H, Mohammed AA (2025). Molecular identification, chemical profiling, and bioactive potential of *Eryngium thyrsoideum* Boiss.: Ethnopharmacological perspectives. *Sci. Nat.* 112: 96.
- Khal LH, Tahir NA-R, Abdul-Razaq RT (2023). Molecular variation in some taxa of genus *Astragalus* L. (Fabaceae) in the Iraqi Kurdistan Region. *Horticulturae* 9(10): 1110.
- Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33: 1870–1874.
- Laface VLA, Torino L (2025). New reports of Orchidaceae family in Southern Calabria (Italy): Distribution and conservation. *Conservation* 5: 85.
- Larkin M, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson J, Gibso TJ, Higgins DG (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.
- Liu Y, Wang H, Yang J, Dao Z, Sun W (2024). Conservation genetics and potential geographic distribution modeling of *Corybas taliensis*, a small 'sky island' orchid species in China. *BMC Plant Biol.* 24: 11.
- Loki V, Tokolyi J, Suveges K, Lovas-Kiss A, Hurkan K, Sramko G, Molnar VA (2015). The orchid flora of Turkish graveyards: A comprehensive field survey. *Willdenowia* 45: 231–243.
- Mafakheri M, Bakhshipour M, Omrani M, Gholizadeh H, Rahimi N, Mobaraki A, Rahimi M (2022). The impact of environmental and climatic variables on genetic diversity and plant functional traits of the endangered tuberous orchid (*Orchis mascula* L.). *Sci. Rep.* 12: 19765.
- Mahmood AM, Khalil MI, Darweesh KF (2020). Morphological and molecular phylogenetic analyses reveal a new record to the flora of

- Iraq: *Azolla filiculoides*. *Zanco J. Pure Appl. Sci.* 32(1): 95–103.
- Pérez-Escobar OA, Dodsworth S, Bogarín D, Bellot S, Balbuena JA, Schley RJ, Kikuchi IA, Morris SK, Epitawalage N, Cowan R, Maurin O, Zuntini A, Arias T, Serna-Sánchez A, Gravendeel B, Jimenez MFE, Nargar K, Chomicki G, Chase MW, Leitch IJ, Forest F, Baker WJ (2021). Hundreds of nuclear and plastid loci yield novel insights into orchid relationships. *Am. J. Bot.* 108: 1166–1180.
- Rasul KS, Grundler FMW, Tahir NA (2022). Genetic diversity and population structure assessment of Iraqi tomato accessions using fruit characteristics and molecular markers. *Hort. Environ. Biotechnol.* 63: 523–538.
- Rechinger KH (1970). Flora Iranica. Flora des Iranischen Hochlandes und der umrahmenden Gebirge, Persien, Afghanistan, Teile von West Pakistan, Nord Iraq, Azerbaidjan, Turkmenistan. *Taxon* 19(2): 285.
- Scopece G, Croce A, Lexer C, Cozzolino S (2013). Components of reproductive isolation between *Orchis mascula* and *Orchis pauciflora*. *Evolution* 67(7): 2083–2093.
- Townsend CC (1966). Flora of Iraq. In: E. Guest (Ed.). Ministry of Agriculture of the Republic of Iraq, Baghdad.
- Tsaballa A, Kelesidis G, Krigas N, Sarropoulou V, Bagatzounis P, Grigoriadou K (2023). Taxonomic identification and molecular DNA barcoding of collected wild-growing orchids used traditionally for salep production. *Plants* 12: 3038.
- Turco A, Albano A, Medagli P, Wagensommer RP, D'Emerico S (2023). Comparative cytogenetic of the 36-chromosomes genera of Orchidinae subtribe (Orchidaceae) in the Mediterranean region: A summary and new data. *Plants* 12: 2798.
- Vitt P, Taylor A, Rakosy D, Kreft H, Meyer A, Weigelt P, Knight TM (2023). Global conservation prioritization for the Orchidaceae. *Sci. Rep.* 13: 6718.
- Vu THT, Le TL, Nguyen TK, Tran DD, Tran HD (2017). Review on molecular markers for identification of orchids. *Vietnam. J. Sci. Technol. Eng.* 59: 62–75.
- Zhang D, Zhao XW, Li YY, Ke SJ, Yin WL, Lan S, Liu ZJ (2022). Advances and prospects of orchid research and industrialization. *Hort. Res.* 9: uhac220.