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Original scientific article

MOLECULAR SYSTEMATIC STUDIES IN THE GENUS *Glaucium* (Papaveraceae)

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Glaucium is mainly distributed from Atlantic Europe to Central Asia. The genus comprises two sections and 23 species: sect. *Acropetala* Mory with four species, four subspecies and two varieties and sect. *Glaucium* with 19 species, eight subspecies and 16 varieties. Species identification is fundamentally important within the fields of biology, biogeography, ecology and conservation. There are 10 species including 4 subspecies and 14 varieties of *Glaucium* in Iran. Taxonomy and phylogeny of the genus is highly complicated and controversial. The present study was done by use of phenetic analyses of morphological characters as well as Bayesian analyses of molecular data (ITS sequences) to illustrate the species relationships, taxonomic classification, monophyly versus paraphyly of the species in the genus *Glaucium.* We used ten *Glaucium* species for molecular studies, of which, nrDNA, ITS sequences were newly obtained for 7 species. The molecular analysis, based on successive reweighting by rescaled consistency index, revealed that Maximum parsimony, maximum likelihood and Bayesian methods gave very similar results based on ITS dataset. In general, the present study revealed that the species could be differentiated by morphological characters. PCA and cluster analysis (Ward's method) carried out for morphological traits divided the *Glaucium* species in two type cluster. Phylogenetic relationships within *Glaucium* are known and ITS-based phylogenetic trees and morphological characters were in concordance.

Keywords: Cladistics, *Glaucium*, ITS, Phenetic, Phylogeny

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INTRODUCTION

Biosystematics investigations in plants comprise different tasks as species delineation, population divergence, species relationships, date of divergence determination, etc. Such data are not completed in majority of plant groups and one or few of these types of investigations have been performed in them. This holds true for the genus *Glaucium* (Papaveraceae)**.**

Papaveraceae s. str. was formerly divided into four subfamilies: Chelidoniodeae Ernest, Eschscholzioideae Ernest, Papaveroideae Ernest and Platystemonoideae Ernest (ERNEST, 1962-KADEREIT, 1993). Later on, KADEREIT *et al.* (1994) included the subfamily of Platystemonoideae in Papaveroideae as well. The monophyly of two groups, Eschscholzioideae and Papaveroideae (including Platystemonoideae), was supported in phylogenetic analyses of Papaveraceae based on a combination of molecular and morphological data by HOOT *et al.* (1997). *Glaucium* Mill. and *Dicranostigma* Hook. f. et Thoms were also included as a part of Chelidonoideae while the morphological analysis of KADEREIT *et al.* (1994) represented *Glaucium* and *Dicranostigma* as a sister group to Papaveroideae.

Glaucium is mainly distributed from Atlantic Europe to Central Asia (KADEREIT, 1993). The genus comprises two sections and 23 species: sect. *Acropetala* Mory with four species, four subspecies and two varieties and sect. *Glaucium* with 19 species, eight subspecies and 16 varieties (MORY, 1979). In Iran, it was represented by 11 (CULLEN, 1966) to 13 (MOBAYEN, 1985; GRAN and SHARIFNIA, 2008) species, of these, five are endemics: *G. calycinum* Boiss., *G. contortuplicatum* Boiss., *G. elegantissimum* Mobayen, *G. matthiifolium* Mobayen and *G. golestanicum* Gran & Sharifnia. *G. vitellinum* Boiss. & Buhse was reduced to the synonymy of *G. oxylobum* Boiss. & Buhse and *G. calycinum* by CULLEN (1966) and MORY (1979), respectively. Mobayen reduced this species to the ranks of variety and subspecies (1985): *G. flavum* Crantz var. *vitellinum* (Boiss. & Buhse) Mobayen and *G. flavum* subsp. *vitellinum* (Boiss. & Buhse) Mobayen, while it was introduced as a distinct species by GRAN and SHARIFNIA (2008). *G. leiocarpum* Boiss. and *G. elegans* Fisch. & C.A. Mey. var. *integerrima* Mobayen were placed as synonymous with *G. flavum* and *G. elegans* var. *elegans*, respectively (TAVAKKOLI, 2016).

Trichomes morphology and their frequency and distribution have shown that they provide valuable characters for delimitation of taxa at sectional level (VAFADAR *et al.*, 2010). The structure and shape of trichomes are taxonomically significant for identification of *Glaucium* species as demonstrated by MORY (1979). Morphological description of the genus: Annual, biennial or perennial herbs. Stems with corymbose branches. Leaves lobed, pinnatifid or pinnatisect to pinnapartite and sometimes entire. Flowers solitary, axillary or terminal. Sepals 2, caduceus, with or without hairs. Petals 4, yellow or orange, reddish orange, dark red, with or without spots. Stamens yellow or orange and numerous; anthers linear to oblong; filaments variable. Ovary linear, tuberculate, hairy or glabrous. Capsules siliquiform, linear to cylindrical, up to 38 cm long, with spongy septum, dehiscing by two valves. Stigma two lobed; lobes divergent, horizontal or curved. Seeds numerous, without appendages. The taxonomically most valuable characters in the genus are: the shape and cellular structure of trichomes on young fruits and sepals, the shape of stem leaves and filaments, the length of siliquae, anther and buds.

Several taxonomic studies have shown that seed and trichome micromorphology can be useful for classification and delimitation of taxa at all taxonomic levels and in different plant families

(BARTHLOTT, 1981; KRAK and MRAZ, 2008; SALMAKI *et al*., 2009; SATIL *et al*., 2011; SALIMI MOGHADAM *et al*., 2015; TAVAKKOLI and ASSADI, 2016; ARABI *et al*., 2017). Furthermore, the seed ornamentations of 14 *Glaucium* taxa in Iran were investigated by GRAN and SHARIFNIA (2008). Seeds and trichomes of 15 taxa of the genus *Glaucium* distributed in Iran were examined by light microscopy (LM) and scanning electron microscopy (SEM) (TAVAKKOLI and ASSADI, 2019). According to ARABI *et al*. (2017) *Glaucium* taxa were investigated in terms of their morphological, palynological and phylogenetical characteristic. Their results show differences between the taxa in some of these characteristics, especially in micromorphology and formation of clades in phylogenetic trees based on the *matK* and ITS3-6 DNA sequence data. Based on the findings of the molecular analyses supported by morphological data (stem's trichomes), the genus *Glaucium* of Turkey was divided into subsections *Glabrousae* and *Pubescentae*. In a micromacromorphological study performed by GRAN and SHARIFNIA (2008) of 18 *Glaucium* taxa, the species *G. haussknechtii* has been recognized as synonymous with *G. grandiflorum* based on the analyses of 28 qualitative and 37 quantitative characters. Taxa with pubescence stems were *G. corniculatum* subsp. *corniculatum* and *G. corniculatum* subsp. *refractum*, *G. grandiflorum* var. *grandiflorum*, *G. grandiflorum* var. *torquatum*, *G. grandiflorum* var. *haussknechtii* and *G. secmenii*, while the taxa with hairless stems were *G. flavum*, *G. leiocarpum*, *G. acutidentatum* and *G. cappadocicum*. The petals of the taxa included in the hairy group were red, or reddish-orange, while those with hairless group were yellow or yellowish-orangethe seeds were separated by thin prominent sections. Molecular data and advanced bioinformatics analyses are extensively used to answer the existing questions on systematic of the plant groups, the species relationships, and their mod of divergence. The Molecular data are gathered from various molecular markers and gene sequences. Multilocus molecular markers are non-selective (neutral) in nature and are of numerous kinds for example, AFLP (Amplified fragments length polymorphism), SSRs (Simple sequence repeats), ISSRs (Inter-simple sequence repeats), and retrotrasposon (REMAP) (ESFANDANI-BOZCHALOYI *et al*. 2017a; 2017b). The gene sequences most often used in plant molecular systematic and phylogenetic investigations are mainly nuclear ribosomal DNA and chloroplast genes and spacers (OLMSTEAD and PALMER, 1994; BOGLER *et al*., 2004; GOBERT *et al*., 2006; ZHANG *et al*., 2015; ESFANDANI-BOZCHALOYI *et al*., 2017c; 2017d; 2018). There is a wide acceptance of combination and simultaneous analysis of all available data sets (BAKKER *et al*., 2004).

There has been no detailed molecular systematic study on *Glaucium* species in Iran. Moreover, there are few species in the country, which have overlapping distribution with the possibility of forming interspecific hybrids. Therefore, the present study was carried out to clarify the indigenous *Glaucium* species relationships of Iran.

MATERIAL AND METHODS

Plant materials

For morphometric studies (phonetic analyses) we used 15 plant specimens of 9 *Glaucium* species growing in Iran (Table 1, Fig1) and for nrDNA ITS phylogenetic tree 11 species (including 1 out-group species *Papaver somniferum* L.) were used. One of the collected samples did not match with descriptions in Flora of Iran and Flora of USSR, and it seems that the sample belongs to a new species of *Glaucium* from Iran. Therefore, we decide to definite it as G.

new, until its legal name is validated. Voucher specimens were deposited at Herbarium of Islamic Azad University Tehran (IAUNT). nrDNA, ITS sequences were obtained for 9 species in this study. The remaining sequences were obtained from GenBank. Information concerning voucher specimens and previously published sequences are presented in the Appendix.

Table 1. Collection data and geographical location of selected Glaucium specimens in Iran

Taxa	Locality
G. elegans var *elegans* Fisch. & C.A.Mey. 97	Mazandaran, Chalous Road of Taghistan Mountains
G. x(new) 114	Tehran, Kan
G. grandiflorum subsp. *grandiflorum* var. *grandiflorum* Boiss. & A.Huet *38*	Hamedan, Heidreh Balashahr Road
G. grandiflorum subsp. *refractum* (Nábělek) Mory 33	Markazi, Nobaran
G. grandiflorum subsp. *grandiflorum* var. *iranicum100*	Tehran, Sorkheh Hesar
G. corniculatum var. *flaviflorum* DC. 108	Tehran, Damavand
G. corniculatum var. *corniculatum* (L.) Curtis 63	East Azerbaijan,South[illegible]st Azarshahr
G. flavum var. *serpieri* (Heldr.) Halácsy 93	Shahrekord, Tele[illegible]an
G. flavum var. *flavum* (Sm.) Fedde 55	East Azerbai[illegible], South[illegible]ast Az[illegible]hr
G. calycinum var. *calycinum* Boiss. 103	Yazd
G. oxylobum var. *oxylobum* Boiss. & Buhse 79	Maza[illegible]aran, He[illegible]riarib
G. contortuplicatum var. *cantortuplicatum* Boiss. 89	G[illegible]est[illegible] Gorgan
G. golestanicum A.Gran & Sharifnia 87	Golestan, G[illegible]rgan
G. fimbrilligerum Boiss. 31	Qazvin to Rasht
G. squamigerum Kar. & Kir.	NCBI
Papaver somniferum L.	Outgr[illegible]up NCBI

Fig 1. *G. grandiflorum* subsp. *grandiflorum* var. *iranicum* general shape, a,b) Habit; c,d) flower and fruit

Morphological studies

Four-five samples from each species were used for morphometry. In total 42 morphological (17 qualitative, and 25 quantitative) characters were studied (Table 2). Data obtained were standardized (Mean= 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (PODANI, 2000).

Table 2. Morphological characters in studied species

No	Characters	No	Characters
1	Plant height (mm)	19	Seed length (mm)
2	Length of stem leaves petiole (mm)	20	Seed width (mm)
3	Length of stem leaves (mm)	21	Seed length/ width (mm)
4	Width of stem leaves (mm)	22	Pedicel length (mm)
5	Length / Width of stem leaves (mm)	23	Peduncle length (mm)
6	Number of segment stem leaves (mm)	24	Stamen filament length (mm)
7	Length of basal leaves petiole (mm)	25	Style length (mm)
8	Length of basal leaves (mm)	26	Vegetation-forms
9	Width of basal leaves (mm)	27	State of plant strength
10	Length / Width of basal leaves (mm)	28	Petal shape
11	Number of segment basal leaves	29	Leaf outline
12	Calyx length (mm)	30	Seed color
13	Calyx width (mm)	31	Leaf tips:
14	Calyx length/ width (mm)	32	Shape of segments basal leaves
15	Petal length (mm)	33	Stamen filament color:
16	Petal width (mm)	34	Stigma hair
17	Petal length / width (mm)	35	Stem hair
18	Fruit length (mm)	36	Petioles and Leaf hair
37	Anthers color	40	Sepal hair
38	Stamen filament hair	41	Peduncle and pedicel hair
39	Shape of calyx	42	Shape of segments cauline leaves

ITS- sequences

The nrDNA ITS region was amplified using as primers both ITS4 and ITS5 (WHITE *et al.*, 1990). PCR reactions were carried out in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 1 U of *Taq* DNA polymerase (Bioron, Germany). The amplification reactions were performed in Techne thermo cycler (Germany) with the following program: 5min initial denaturation step 94°C, followed by 35 cycles of 1 min at 94°C; 45s at 57°C and 2 min at 72°C. The reaction was completed by final extension step of 7-10 min at

72°C. The amplification products were observed by running on 1% agarose gel, followed by theethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany). The ITS and *trn*L-F regions were amplified using primers reported as universal primers by WHITE *et al*. (1990) and TABERLET *et al.* (1991), respectively, for flowering plants.

Data analysis

Morphological studies

We used phenetic analyses for morphological data. For grouping of the plant specimens, UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and Ward (Minimum spherical variance) as well as PCoA plot (Principal coordinate analysis) were used (PODANI, 2000). All morphological characters contained 17 qualitative and 25 quantitative were used (Table 2).

PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI, 2000). Maximum parsimony (MP was used for cladistics analysis, followed by bootstrapping (100 times). PAST version 2.17 (HAMMER *et al.*, 2012) and PAUP (SWOFFORD, 2002) were used for these analyses. Both qualitative and quantitative features were applied for maximum parsimony analyses. For this purpose, quantitative features were coded.

Molecular analyses

Species relationship by ITS sequences

Different phylogenetic methods were used to study the species relationship like Maximum parsimony (MP), Maximum likelihood (ML), and Bayesian approach. Maximum parsimony (MP) analysis was conducted using PAUP* (SWOFFORD, 2002). The heuristic search option was used for each of the two single region datasets using tree bisection–reconnection (TBR) branch swapping, with 1,000 replicates of the random addition sequence. Uninformative characters were excluded from the analysis. Branch support values were calculated using a full heuristic search with 1,000 bootstrap replicates (FELSENSTEIN, 1985) each with a simple addition sequence. Combinability of these two datasets was assessed by use of the partition homogeneity test (the incongruence length difference test (ILD) of FARRIS *et al.* (1995) as implemented in PAUP* (SWOFFORD, 2002). The test was conducted with invariant characters excluded (CUNNINGHAM, 1997) using the heuristic search option involving 100 replicates of the random addition sequence and TBR branch swapping with 1,000 homogeneity replicates. The maximum number of trees was set to 5000. The model of sequence evolution for each dataset was selected by use of the software MrModeltest v. 2.3 (NYLANDER, 2004) as implemented in MrMTgui (NUIN, 2005) based on the Akaike information criterion (AIC) (POSADA and BACKLEY, 2004). All datasets were analyzed as a single partition with the Kimura 2-parameters + G model by Bayesian inference (BI) using the software MrBayes version 3.12 (RONQUIST and HUELSENBECK, 2003). Posteriors on the model parameters were estimated from the data, using the default priors. The analysis was performed with 4 million generations, using Markov chain Monte Carlo search. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2)

each with four Markov Chains trees sampled every 100 generations. The first 25 % of trees were discarded as the burn-in. The remaining trees were then used to build a 50 % majority rule consensus tree accompanied by posterior probability (PP) values. Tree visualization was performed by use of Tree View version 1.6.6 (Page 2001).

RESULTS

Morphometry

Species delimitation and inter-relationship

WARD clustering based on all collected sample (only few sample were used for drawing the figures to be brief and readable) separated plants of each species in a distinct cluster or groups (Fig. 2). Therefore, *Glaucium* species indigenous to Iran can be differentiated based on the studied morphological characters. Morphological characters used not only separated each subspecies but also could delineate the presumed species.

PCA analysis revealed that the first three factors comprised over 70% of the total variation. In the first PCA axis with 45% of total variance, morphological characters like peduncle and pedicel hair, stem hair, leaf hair, petiole hair, width of petal, had the highest correlation (>0.7).

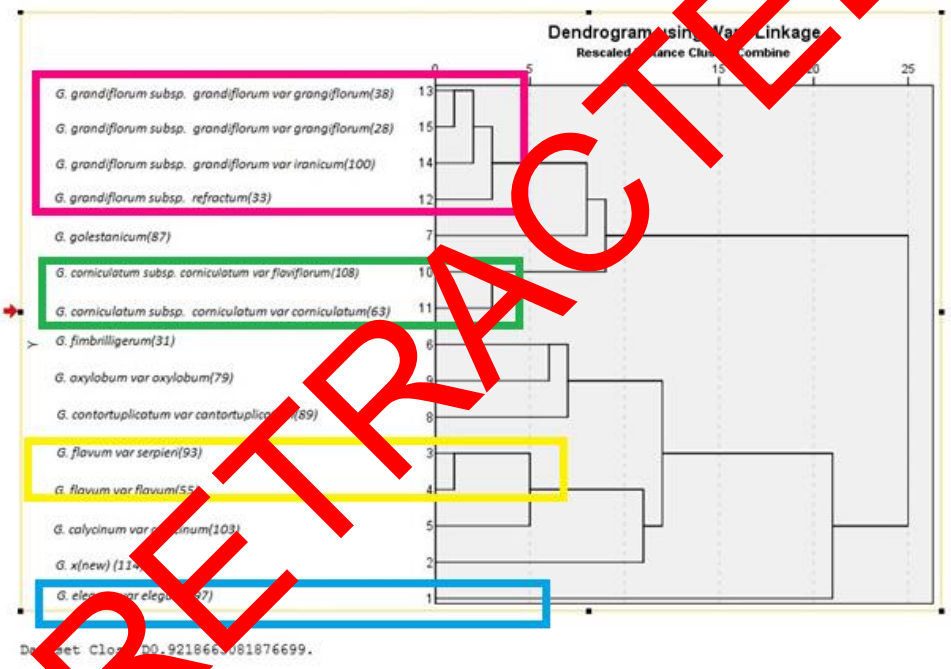

Fig. 2. WARD clustering of morphological characters revealing species delimitation in *Glaucium*

Two major clusters were formed in WARD tree (Fig. 2). The first major cluster contained two sub-clusters: plants of *G. grandiflorum* subsp. *grandiflorum* var. *grandiflorum; G. grandiflorum* subsp. *grandiflorum* var. *iranicum; G. grandiflorum* subsp. *refractum; G. corniculatum* var. *corniculatum; G. golestanicum* and *G. corniculatum* var. *flaviflorum* comprised the first cluster. The second major cluster also contained two sub-clusters, *G. fimbrilligerum; G. flavum* var. *flavum; G. oxylobum* var. *oxylobum; G. contortuplicatum* var. *cantortuplicatum; G. flavum* var. *serpieri; G. elegans* var *elegans; G. calycinum* var. *calycinum* and *G.* x(new) were placed close to each other due to morphological similarity. Morphological characters used not only separated each subspecies but also could delineate the presumed species.

Molecular studies

ITS sequence based phylogeny

An image of the ITS generated by ITS4 primer is shown in Figure 3. Maximum parsimony, maximum likelihood and Bayesian methods gave very similar results based on ITS dataset. However, manual comparison showed a higher degree of similarity between ITS and morphological characters' trees (Fig. 4). We here show only Bayesian trees along with posterior probability (PP) and bootstrap based on ITS. In both ITS and Morphological characters trees, the species *G. grandiflorum* subsp. *grandiflorum* var. *grandiflorum; G. grandiflorum* subsp. *grandiflorum* var. *iranicum; G. grandiflorum* subsp. *refractum;* show close affinity, similarly, species *G. corniculatum* var. *corniculatum* and *G. corniculatum* var. *flaviflorum*, as well as *G. flavum* var. *flavum* and *G. flavum* var. *serpieri*, are closely related.

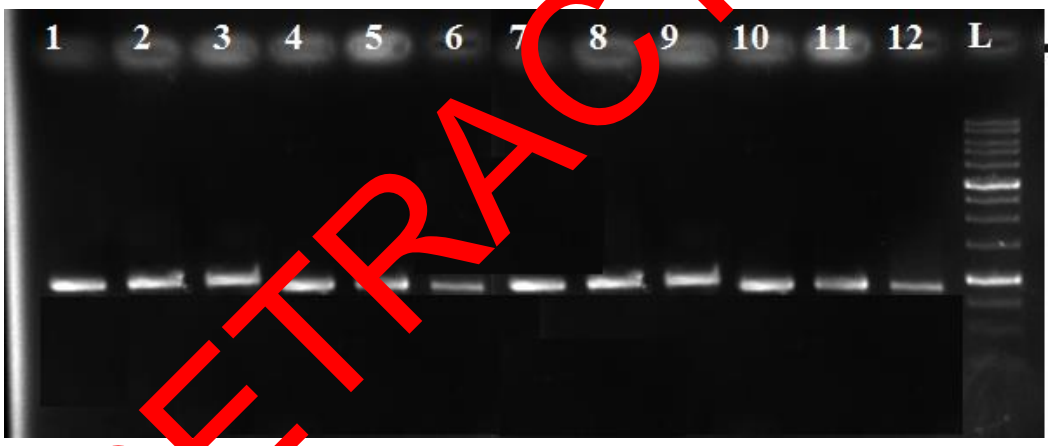

Figure 3. Results of amplification with primer ITS on agarose 1.8% with 14 lanes gel tray. 1-12 individuals of *Gladiolus* L. 1: *G. grandiflorum* subsp. *grandiflorum* var. *grandiflorum;* 2: *G. fimbrilligerum;* 3: *G. grandiflorum* subsp. *refractum;* 4: *G. grandiflorum* subsp. *grandiflorum* var. *grandiflorum;* 5: *G. flavum* var *flavum;* 6: *G. corniculatum* var *corniculatum7: G. oxylobum var oxylobum; 8: G. golestanicum; 9: G. contortuplicatum var cantortuplicatum; 10: G. flavum var serpieri; 11: G. elegans var elegans; 12: G. grandiflorum subsp. grandiflorum var. iranicum*

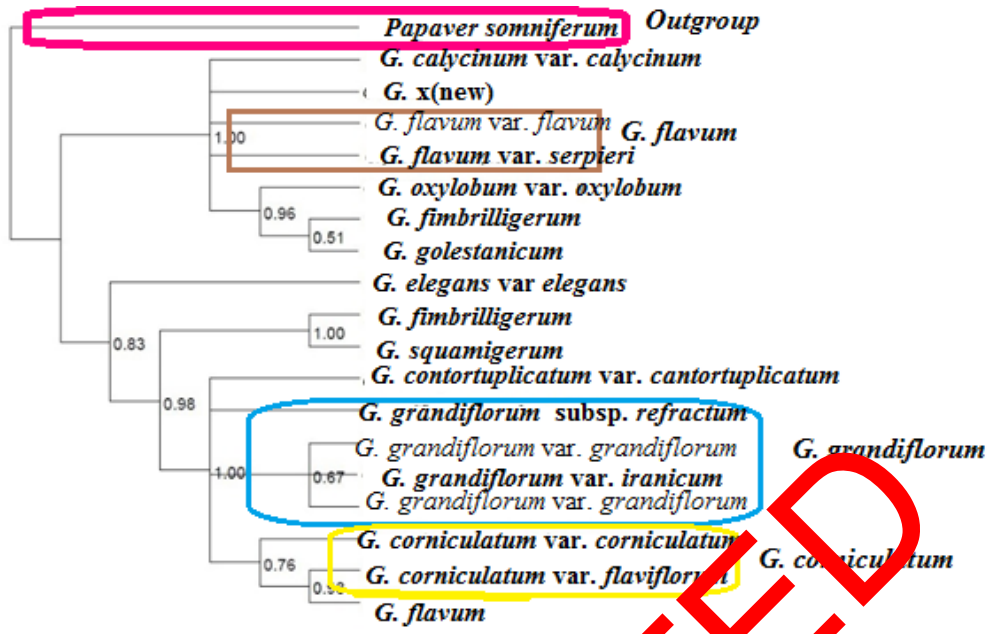

Fig. 4. Bayesian tree for the species phylogeny for 16 *Glaucium* species and *Papaver somniferum* as outgroups, inferred by joint analysis of nrDNA ITS data, Branch support values are given as bootstrap (BP) value above branches.

DISCUSSION

Plant molecular studies enormously advanced in the recent years and molecular phylogenetic has dramatically reshaped our views of organismal relationships and evolution at all taxonomic levels of the hierarchy of life, from the species level (and below) to kingdoms (and above). This revised concepts of relationship based on phylogenetic analyses are also resulting in revised classifications in many groups of plants. However, we are aware that reliance on a single data set may result in insufficient resolution or an erroneous picture of phylogenetic relationships. As a result, it is now common practice to use multiple data sets (preferably both molecular and non-molecular) for phylogenetic inference (SOLTIS and SOLTIS, 2000; GHOLAMIN and KHAYATNEZHAD, 2020a; 2020b, 2020c; JI et al, 2020a; 2020b; WANG *et al*, 2020). However, though multiple data sets are needed for estimating phylogenetic relationships reliably, different genes may possess different histories and the phylogenetic trees they construct may not picture the true relationships and different orthologous genes may often yield strongly supported but incompatible tree topologies.

The three primary causes of incongruence in tree topologies are gene duplication, horizontal gene transfer, and deep coalescence, which have varying levels of importance depending on the

taxa and genes under study. Moreover, deep coalescence or incomplete lineage sorting, chloroplast capture and branch length heterogeneity due to the coalescent process are additional source of gene tree heterogeneity (SOLTIS and SOLTIS, 2000; EDWARDS, 2008; BI *et al*, 2021; CHENG *et al*, 2021; KHAYATNEZHAD, and GHOLAMIN , 2020, 2021).

Three alternatives have been proposed for handling multiple data sets in phylogenetic analyses: the *combined* approach, the *consensus* approach, and the *conditional combination* approach. *Conditional combination* involves combining data except in those instances in which significant heterogeneity exists between data sets and that heterogeneity appears to be attributable to different branching histories (SOLTIS and SOLTIS, 2000; MA *et al*, 2021; PENG *et al*,, 2021; SI *et al*., 2021; SUN *et al*., 2021). For this reason, many have suggested different statistical test for phylogenetic trees congruence (see for example, FOULDS and ROBINSON, 1981). However, several authors believe that statistical tests for congruence may not provide a definitive answer as to whether it is appropriate to combine data sets. That is, even if congruence tests reveal some low level of heterogeneity between data sets, the investigator may be justified in combining data sets (SUN and KHAYATNEZHAD 2021; TAO *et al*, 2021; WANG *et al*, 2021; XU *et al*., 2021; YIN *et al*., 2021; ZHANG *et al*, 2021).

Multispecies coalescent (MSC) approaches (e.g. DEGNAN and ROSENBERG, 2009; KUBATKO and DEGNAN, 2007; ZHENG *et al*., 2021; ZHU *et al*, 2021) are now considered state-of-the-art in estimating a species tree from a set of gene alignments. Recent advances now allow for the simultaneous estimation of gene phylogenies, the MSC species phylogeny, and ancestral state reconstruction (ASR) of particular characters of interest, such as geographical of morphological evolution (BOUCKAERT *et al*., 2014).

We found morphological taxonomic identification was often congruent with nrDNA markers. The species relationships obtained in ITS-based tree are also in agreement with morphological tree.

Systematic implications and evolutionary aspects of morphological characters

WARD clustering of morphological characters separated each species, this is in agreement with phylogenetic analysis by using *ITS* sequences. This study documents the occurrence of 9 species and 11 varieties belonging to the genus *Glaucium* that have been found in Iran. The taxonomically most valuable characters in the genus are: the shape and cellular structure of trichomes on young fruits and sepals, the shape of stem leaves and filaments, the length of siliquae, anther and beaks (Tab. 2).

G. corniculatum subsp. *refractum* (Nab.) Cullen with refracted fruiting pedicels and contorted siliquae was reported from western Iran (CULLEN, 1966). One of the diagnostic characteristics of *G. corniculatum* is fruiting pedicels that are shorter than their subtended leaves while a reported specimen in Flora Iranica (Farahbakhsh 5956; deposited in IRAN herbarium) has fruiting pedicels longer than their subtended leaves as *G. grandiflorum*. Hence, similar to Mory's view *G. grandiflorum* subsp. *refractum* (Nab.) Mory is used.

The main characteristics of *G. oxylobum* have been reported to be: ovaries tuberculate–papilate and petals yellow (CULLEN, 1966); ovaries (young siliquae) with tubercles narrowed at the apex and petals dark red to violet (BOISSIER, 1867); ovaries with conical hairs and petals pale red with black basal spot (MORY, 1979).

G. elegantissimum with glabrous siliquae and dilute red petals and *G. golestanicum* possessing hairy siliquae and red petals with a black basal spot and maculate leaves were accepted as new species by MOBAYEN (1985) and GRAN and SHARIFNIA (2008), respectively. According to (TAVAKKOLI and ASSADI, 2019) showed that both species have tuberculate young siliquae and orange red petals with a black middle spot.

Based on the mentioned variations, the specimens of *G. oxylobum* fall into two groups with respect to young siliqua indumentum, which is either glabrous or hairy with petal color variations in each group. the separation of the species *G. pulchrum*, *G. elegantissimum* and *G. golestanicum* from *G. oxylobum* is not possible and they suggest their synonymy with *G. oxylobum* (TAVAKKOLI and ASSADI, 2019). We agree with PARSA (1986) regard *G. paucilobum* as a variety of *G. oxylobum*.

Diagnostic characteristics of *G. fimbrilligerum* Traut & Buhse are maculate orange petals and sepals with the length of 20 mm long and they possess long and adpressed hairs on the siliquae (CULLEN, 1966; Khayatnezhad, and Gholamin , 2021a, 2021b). A detailed examination of the species *G. fimbrilligerum* (deposited in W herbarium-No. ooo4645 Afghanistan: Chord Kabol, 2070 m, 6. 8. 1951, Gilli 866) with a particular focus on the trichome cellular structure of young siliquae, provides strong support for the proposal that this species was not been found in Iran so far (TAVAKKOLI and ASSADI, 2019).

G. elegans var. *integerrima* Mobayen was repo-rted from NW Iran based on dentate or lobed leaves and yellow and pink petals (MOBAYEN, 1985; KAR AKAL *et al*, 2020a, 2020b; HUANG *et al*, 2021; HOU *et al*, 2021, GUO *et al*, 2021). *G. elegans* was reported by CULLEN (1966), POPOV (1937) and FEDDE (1909) for having pinatifid or pinnatisect radical leaves; the stem leaves nearly ovate, sinuate to dentate or rarely entire and having yellow petals, red in the middle part and with black basal spot. Among specimens studied, some were observed which had lobed or pinnatisect to pinnapartite basal and stem leaves. Some specimens also had lobed or dentate stem leaves and pinnatisect basal leaves. Their petals were yellow to orange (with black basal spot) in fresh stage but pinkish in dry stage. Therefore, the separation of var. *integerrima* from the type variety cannot be justified.

During the taxonomic revision of the genus *Glaucium*, *G. contortuplicatum* var. *hirsutum* is described as a new variety (TAVAKKOLI and ASSADI, 2019; Ren and Khayatnezhad 2021; KHAYATNEZHAD and NASEHI, 2021; *et al*., 2021; JIA *et al*, 2021). This taxon differs from the type variety by having dense appressed trichomes all along ovary (or junior silique). Leaf epidermis micro-characters of these two taxa are also compared. *G. leiocarpum* and *G. elegans* var. *integerrima* are reduced to synonymy of *G. flavum* and *G. elegans* var. *elegans*, respectively. Additionally, morphological characters and geographical distribution of the taxa studied in the world and in Iran are presented.

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MOLEKULARNO PROUČAVANJE RODA *Glaucium* (Papaveraceae)

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Izvod

Glaucium uglavnom je distribuiran iz Atlantske Evrope u centralnu Aziju. Rod se sastoji od dva dela i 23 vrste: sect. *Acropetala* Mori sa četiri vrste, četiri podvrste i dve sorte i sec. *Glaucium* sa 19 vrsta, osam podvrsta i 16 sorti. Identifikacija vrsta je fundamentalno važna u oblastima biologije, biogeografije, ekologije i konzervacije. U Iranu postoji 10 vrsta, uključujući 4 podvrste i 14 sorti *Glaucium*. Taksonomija i filogenija roda veoma su komplikovane i kontroverzne. Ovo istraživanje je urađeno korišćenjem morfoloških karakteristika, kao i Baiesovim analizama molekularnih podataka (ITS sekvence) radi ilustracije odnosa vrsta, taksonomske klasifikacije, monofilije naspram parafilije vrste u rodu *Glaucium*. Za molekularna istraživanja koristili smo deset vrsta *Glaucium*, od kojih su nrDNA, ITS sekvence prvobitno dobijene za 7 vrsta. Molekularna analiza, zasnovana na uzastopnom ponderisanju indeksom konzistentne promene, otkrila je da su maksimalna oskudnost, maksimalna verovatnoća i Baiesove metode dale vrlo slične rezultate zasnovane na ITS skupu podataka. Generalno, ova studija je otkrila da se vrste mogu razlikovati po morfološkim karakteristikama. PCA i klaster analiza (Ward -ova metoda) sprovedena za morfološke osobine podelila je vrste *Glaucium* u dve grupe. Filogenetski odnosi unutar *Glaucium* su poznati, a filogenetska stabla zasnovana na ITS-u i morfološki karakteri bili su u skladu.

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