

## INTER-SIMPLE SEQUENCE REPEATS GENETIC RELATIONSHIPS BETWEEN POPULATIONS OF *Consolida* (RANUNCULACEAE)

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In fields like ecology, biogeography, conservation biology, and macroevolutionary theory, species are considered as the fundamental unit of study. Gray (1821), an expert on British flora, regarded the genus *Consolida* to be a distinct genus based on a single species (*C. regalis*). *Consolida*, on the other hand, is believed by some scholars to be a part of *Delphinium*. There are no reports on the genetic diversity, modes of divergence, or dispersion patterns of the various *Consolida* species that grow in Iran despite their widespread distribution. Hence, a combination of atomic (ISSR markers) and morphological examinations of 78 increases from seven species of *Consolida* were conducted. One goal of this work is to determine the diagnostic usefulness of ISSR markers in the delimitation of *Consolida* species, another is to determine the genetic structure of these taxa, and a third is to look at the relationships between species within these groups. It was discovered in the current research that combining morphological and ISSR data may help identify species. Analysis using AMOVA and STRUCTURE found that the species of *Consolida* are genetically distinct, yet have some common genes.

*Key words:* *Consolida*, Inter-simple sequence repeat, Morphology, Species delimitation

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## INTRODUCTION

Species delimitation is imperative in a assortment of natural areas, counting biology, biogeography, and plant preservation (ALIZADEH, 2020). Species delimitation is accomplished using both tree-based and non-tree-based methods. Species form differentiating clades in the first way (phylogenetic species concept), but in the non-tree-based method, species are classified based on gene flow analyses (biological species concept; MA *et al.*, 2021; PENG *et al.*, 2021; SI *et al.*, 2021; Я *et al.*, 2021).

*Consolida* (DC.) Gray (Ranunculaceae) is a genus of the *Delphinieae* tribe. It is made up of around 52 species, including members of the genus *Aconitella* Spach. Iran is one of the wealthiest nations in South-West Asia for the genus, with 24 species (IRANSHAHR *et al.*, 1992). De Candolle distinguished *Consolida* from *Delphinium* based on single impelled petals, one follicle, and an yearly life cycle, and it has happened in a distinctive section. Gray was the primary to classify it as a particular sort in 1821. (TRIFFONOVA, 1990).

Concurring to the developmental examinations of JABBOUR and RENNER (2011), *Aconitella* could be a part of *Consolida*, both of which are implanted in *Delphinium*. According to the discoveries of JABBOUR and RENNER (2011), *Consolida* wandered from *Delphinium* relatives amid the Early to Center Miocene, a period of increasing aridity caused basically by sea-level decrease within the Mediterranean (HAYEK, 1970; IRANSHAHR, 1992; ERTUGRUL *et al.*, 2010) and desertification in Asia (TRIFFONOVA, 2010).

GRAY (1821), who worked on British flora, regarded the genus *Consolida* S.F. Gray to be a distinct genus based on one species (*C. regalis*). However, some experts regarded *Consolida* to be a subspecies of *Delphinium* (DE CANDOLE, 1824; BOISSIER, 1867). Unlike the others, *Consolida* was designated as an unique genus based on its annual life form, single spurred petal, and solitary follicle contrasted to *Delphinium*'s 3 or 5 sessile follicles (DAVIS, 1965; MUNZ, 1967; IRANSHAHR, 1992; ERTUGRUL *et al.*, 2010; KHALAJ, 2013). Based on the unusual morphology of the petal, upper sepal, and spur, KEMULARIA-NATHADES (1939) identified a new genus *Aconitopsis* from *Consolida* species.

Because of nomenclature priority, SOJAK (1969) rejected the name *Aconitopsis* and eplaced it with *Aconitella*. Some academics have looked at the taxonomy of these genera MUNZ, 1967; DAVIS, 1965; IRANSHAHR *et al.*, 1992; CONSTANTINIDIS *et al.*, 2001).

*Consolida* contains around 40 species, 19 of which have been documented from Iran. The Irano-Turanian and Mediterranean phytogeographic zones are dominated by *Aconitella*, which has around 10 species (3 of which are found in Iran), and *Delphinium*, which has 31 species (3 of which are found in Iran) (TRIFONOVA, 1990; HASANZADEH *et al.*, 2017).

Biosystematic studies have been conducted in a variety of domains, including chromosomal studies (TRIFONOVA, 1990), chemical research (AITZETMULLER *et al.*, 1999), palynological studies (MUNZ, 1967), and phylogenetic investigations employing DNA sequence data (JABBOUR and RENNER, 2011; 2012; YOSEFZADEH *et al.*, 2012).

According to recent genetic research (JABBOUR and RENNER, 2001; 2012), *Consolida* and *Aconitella* constitute a clade embedded in *Delphinium*, and *Aconitella* is also embedded inside *Consolida*. According to the findings of JABBOUR and RENNER (2011), *Consolida* split from *Delphinium* cousins at least in the early to middle Miocene.

As a result, we conducted morphological and hereditary examinations on seven *Consolida* species that were gathered. The extend looks for to address the taking after issues:

- 1) Is there intra- and inter-specific hereditary variety among the species beneath study?
- 2) Is hereditary difference between these species associated to their geological dissimilarity?
- 3) How are populations and taxa genetically structured?
- 4) Is there any gene exchange occurring in Iran amongst *Consolida* species?

As a result, it is critical to delimit the discovered species in order to conduct extensive molecular research.

## MATERIALS AND METHODS

### *Plant materials*

The current research gathered 78 plant samples from ten regional groups belonging to seven *Consolida* species. Numerous sources were consulted to ensure that species were correctly identified (IRANSHAHR, 1992; ERTUGRUL *et al.*, 2010; KHALAJ, 2013).

The locations of the sample sites are specified (Table 1). Vouchers were placed in Islamic Azad University's Science and Research Branch's herbarium in Tehran, Iran (IAUH).

Table 1. Voucher details of *Consolida* species in this study from Iran

No	Sp.	Locality	Latitude	Longitude	Altitude (m)
Sp1	<i>C. tehranica</i> (Boiss.) Rech.f.	Tehran: Damavand	38 ° 52'37"	47 ° 23' 92"	1144
Sp2	<i>C. camptocarpa</i> (Fisch. &C.A.Mey.) Nevski	Khorassan: Sarakhs, 14 km to Mozduran	32°50'03"	51°24'28"	1990
Sp3	<i>C. lorestanica</i> IRANSHAHR,	Lorestan: 110 km Khorram abad	29°20'07"	51 ° 52'08"	1610
Sp4	<i>C. leptocarpa</i> Nevski	Golestan: Golestan national park, Mirzabailoo Tehran: Rodehen Golestan, Ramian	38 ° 52'373"	47 ° 23' 92"	1144
Sp5	<i>C. persica</i> (Boiss.) Grossh.	Fars: Bamo national park	33° 57'12"	47° 57'32"	2500
Sp6	<i>C. aucheri</i> (Boiss.) Iranshahr	Khorassan: Neyshabur	34 ° 52'373"	48 ° 23' 92"	2200
Sp7	<i>C. anthoroidea</i> (Boiss.) Schrödinger	East Azerbaijan: kaleybar, Cheshme Ali Akbar Markazi: Arak	38 ° 52'373"	47 ° 23' 92"	1144

### *Morphological studies*

Morphometry was performed on five to ten samples from each species. In all, 22 morphological features (10 qualitative, 12 quantitative) were examined. The data were

normalized (Mean=0, variance=1) and used to calculate the Euclidean distance for clustering and ordination analysis (PODANI, 2000).

#### *DNA extraction and ISSR assay*

Fresh leaves were taken at random from five to ten plants in each of the populations investigated. Silica gel powder was used to dry them. Genomic DNA was extracted using the CTAB activated charcoal technique (ESFANDANI-BOZCHALOYI *et al.*, 2019).

#### *Data analyses*

##### *Morphological studies*

To begin, morphological features were normalized (Mean = 0, Variance = 1) and used to calculate Euclidean distances between taxon pairs (PODANI, 2000).

The ISSR bands collected were binary coded (presence = 1, absence = 0) and utilized to analyze genetic diversity.

A assortment of parameters were calculated, counting Nei's quality differences (H), the Shannon data file (I), the number of viable alleles, and the rate of polymorphism (ESFANDANI, 2021).

## RESULTS

#### *Species delimitation and hereditary diversity*

##### *None of the ISSR preliminaries created polymorphic bands.*

All three primers (AGC) 5GT, UBC 810, and (GA) 9T generated 98 bands, with (AGC) 5GG producing the fewest bands at 23. Genetic diversity characteristics assessed in the examined species (Table 2) indicated that *C. tehranica* (sp1) exhibited the greatest amount of genetic polymorphism (57.88%), while *C. persica* exhibited the lowest level (15.59%). (sp5). Additionally, *C. tehranica* exhibited the largest effective number of alleles ( $N_e = 1.610$ ) and Shannon information index ( $I = 0.398$ ).

The AMOVA test revealed a substantial genetic difference between the examined species ( $P = 0.001$ ). It indicated that 51% of overall variance occurred across species and 49% occurred within species (Table 3). The  $F_{ST}$  scores for pairs of species revealed a considerable difference between them (Table 4). Additionally, these species' genetic distinction was revealed by substantial Nei's  $G_{ST}$  (0.876,  $P = 0.0001$ ) and  $D_{est}$  (0.324,  $P = 0.001$ ) values.

Non-metric MDS plots of ISSR data (Figure 1) revealed that species number one (*C. tehranica*) exhibited more within-species genetic diversity, corroborated by genetic diversity metrics collected (Table 2). The MDS plot identified various groupings of species. This demonstrates that ISSR molecular markers may be utilized to delimit *Consolida* species.

This is consistent with the ANOVA and genetic diversity metrics previously reported.

The species are genetically distinct from one another. Additionally, the  $N_m$  study performed by Popgene software yielded a mean  $N_m = 0.66$ , which is regarded a very low amount of gene flow between the analyzed species.

Table 2. Genetic diversity parameters in the studied *Consolida* species.

Pop	N	Na	Ne	I	He	UHe	%P
sp1	12.000	1.247	1.610	0.398	0.232	0.144	57.88%
sp2	8.000	0.419	1.097	0.184	0.156	0.505	36.13%
sp3	6.000	0.258	1.029	0.133	0.114	0.305	20.38%
sp4	12.000	0.925	1.259	0.233	0.155	0.152	48.09%
sp5	11.000	0.774	1.166	0.112	0.104	0.098	15.59%
sp6	14.000	0.344	1.069	0.264	0.215	0.436	28.98%
sp7	14.000	0.570	1.106	0.298	0.164	0.696	41.51%

(N = number of samples, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations)

Table 3. Analysis of molecular variance (AMOVA) of the studied species

Source	df	SS	MS	Est. Var.	%	$\Phi_{PT}$	
Among Pops		29	1651.364	85.559	10.133	51%	
Within Pops		140	354.443	2.905	4.85	49%	51%
Total		169	2005.807		14.983	100%	

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance;  $\Phi_{PT}$ : proportion of the total genetic variance among individuals within an accession, ( $P < 0.001$ ).

Table 4. Pair-wise  $F_{ST}$  values among the studied *Consolida* species.

	sp1	sp2	sp3	sp4	sp5	sp6	sp7
sp1	-	0.010	0.010	0.010	0.010	0.010	0.010
sp2	0.552	-	0.010	0.010	0.010	0.010	0.010
sp3	0.597	0.643	-	0.020	0.010	0.010	0.010
sp4	0.429	0.513	0.915	-	0.010	0.010	0.010
sp5	0.520	0.300	0.443	0.359	-	0.010	0.010
sp6	0.546	0.899	0.885	0.576	0.598	-	0.010
sp7	0.598	0.568	0.709	0.554	0.631	0.390	-

(Above diagonal =  $F_{ST}$  value, below diagonal = P value). sp1= *C. tehranica*; sp2= *C. camptocarpa*; sp3= *C. lorestanica*; sp4= *C. leptocarpa*; sp5= *C. persica*; sp 6= *C. aucheri*; sp7= *C. anthoroidea*.

The Mantel test with 5000 permutations revealed a substantial association between genetic and geographical distance ( $r = 0.76$ ,  $p=0.0002$ ), indicating that isolation by distance (IBD) occurred among the *Consolida* species tested.

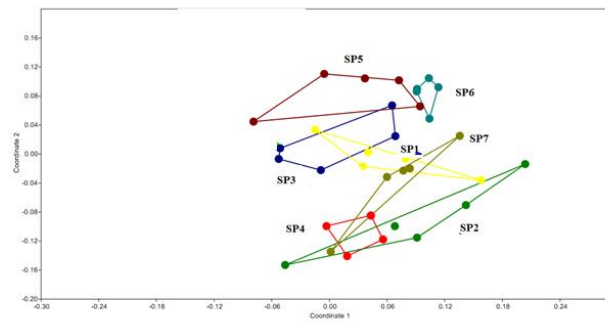


Fig 1. MDS plot of *Consolida* species based on ISSR data; sp1= *C. tehranica*; sp2= *C. camptocarpa*; sp3= *C. lorestanica*; sp4= *C. leptocarpa*; sp5= *C. persica*; sp 6= *C. aucheri*; sp7= *C. anthoroidea*.

Nei's genetic identity and the genetic distance between the analyzed species were established (Table is not included). The findings indicated that *C. tehranica* and *C. leptocarpa* had the greatest degree of genetic similarity (0.94). *C. camptocarpa* and *C. anthoroidea* had the least genetic similarity (0.77).

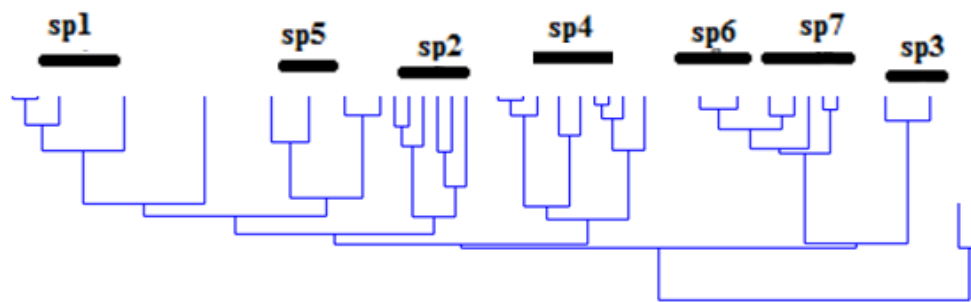


Fig. 2. Neighbor joining tree of inter simple sequence repeats data in the studied *Consolida* species; sp1= *C. tehranica*; sp2= *C. camptocarpa*; sp3= *C. lorestanica*; sp4= *C. leptocarpa*; sp5= *C. persica*; sp 6= *C. aucheri*; sp7= *C. anthoroidea*.

The NJ tree constructed using Nei's genetic distance (Figure 2) revealed that *C. lorestanica* was genetically distinct from the other species tested, owing to its geographic isolation. This dendrogram demonstrated close genetic affinity between *C. tehranica* and *C. leptocarpa*, corroborating our previous morphological findings (Figure. 2). The remaining *Consolida* species were clustered closer together using ISSR data, although their genetic affinity is not apparent in the morphological tree.

### *The species genetic STRUCTURE*

To decide the perfect number of hereditary bunches, we utilized STRUCTURE investigation taken after by the Evanno test. The admixture demonstrate was utilized to portray interspecific quality stream or/and ancestrally shared alleles within the species beneath *ponder*. The STRUCTURE analysis was followed by the Evanno test, which resulted in  $\Delta K = 6$ .

The Organization plot (Figure 3) revealed further information about the genetic structure of the species under investigation, as well as shared ancestral alleles and/or gene flow amongst *Consolida* species. Due to shared common alleles, this plot demonstrated genetic similarity between *C. tehranica* and *C. lorestanica* (similarly colored, No. 1, 3). This is consistent with the previous Neighbor Joining Dendrogram. The allele makeup of the other species differs.

The  $N_m$  esteem (0.66) infers negligible quality stream or ancestrally shared alleles among the species explored, whereas K-Means and STRUCTURE ponders affirm hereditary stratification. The comes about of the populace task test supported with  $N_m$ 's discoveries, demonstrating that there was no significant quality stream among people of the explored species. However, a reticulogram based on the slightest square approach (not appeared) demonstrated a few common alleles between species 1 and 3, 4 and 7, and 2 and 3, as well as between 2 and 3. As appeared by the STRUCTURE plot based on the admixture show, these common alleles as it were make up a little parcel of the genomes within the species tried, and all of these discoveries point to a noteworthy degree of hereditary stratification within the species explored.

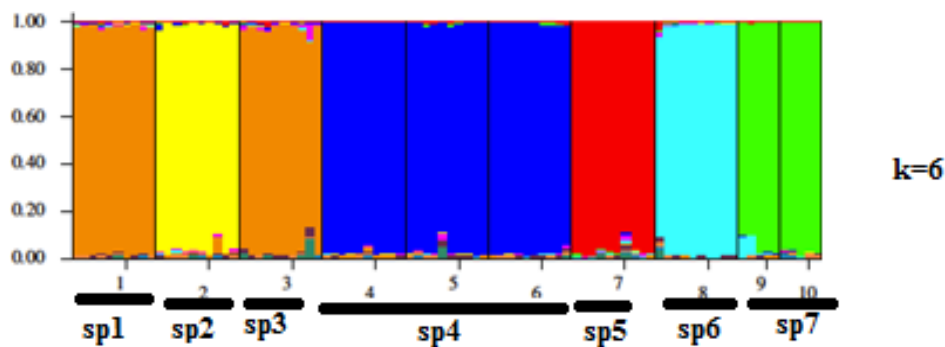


Fig. 3. STRUCTURE plot of *Consolida* species based on ISSR data; sp1= *C. tehranica*; sp2= *C. camptocarpa*; sp3= *C. lorestanica*; sp4= *C. leptocarpa*; sp5= *C. persica*; sp 6= *C. aucheri*; sp7= *C. anthoroidea*.

## DISCUSSION

### *Genetic structure and gene flow*

The hereditary structure of plants, stratification vs quality stream inside species populaces, hereditary uniqueness of populaces, and other subjects are examined in populace hereditary qualities (ESFANDANI-BOZCHALOYI *et al.*, 2018a; 2018b; 2018c; 2018d; JIA *et al.*,

2021). This data may be utilized for a assortment of purposes, counting understanding the science of the species, preservation of imperiled species, selecting fitting guardians for hybridization and breeding, and phylogeography and attack instruments (BI *et al.*, 2021; CHENG *et al.*, 2021).

The AMOVA and STRUCTURE analyses indicated that although the species in this genus are genetically distinct, they share some common genes. In *Consolida* species, there are many pollination mechanisms with a slow transition between them. The techniques we utilized, according to PHILIPP (1985), are indirect estimations of gene flow, and if it is shown to exist across species, it might be owing to ancestral common alleles or continuing gene flow. The Nm value derived from ISSR data suggested relatively little gene flow across the examined species, which was further validated by STRUCTURE analysis, since *Consolida* species tended to have different genetic structures. ISSR gene flow was also discovered by reticulation analysis.

We didn't find any intermediate forms in our large plant sample, although there was considerable morphological variety within each species. As a result, the low degree of gene flow seen by indirect approaches might be owing to a lack of gene flow between ancestral common alleles and continuing genes.

Nm is the movement of genes across and between populations, and it is inversely associated to genetic differentiation (GRANT, 1991). It is critical for plant population movement and development and may be computed using the following formula  $Nm = 0.5 (1 - GST)/GST$

In the present study, Nm among varietal populations of *Consolida* species was 0.66, indicating a low level of Nm and a high degree of genetic differentiation. Nm among bud-type varieties populations was even lower, at 0.110. To conclude, the present study revealed the use of ISSR molecular markers along with morphological characters in *Consolida* species delimitation. Some degrees of interspecific genetic admixture occur in *Consolida* species, but the studied species are strongly differentiated during the speciation process and invasion in new habitats. Genetic drift, strong inbreeding, and local adaptation are effective evolutionary forces operating in *Consolida* species and population divergence and adaptation.

Plant species delimitation is basic in phylogenetic systematics, advancement, biogeography, and biodiversity research. It is imperative to get it the designs and forms of speciation and hybridization, the developmental prepare through which unused organic species rise and quality stream between closely related phylogenetic species may happen (ZHENG *et al.*, 2021; ZHU *et al.*, 2021; YIN *et al.*, 2021). Distance segregation, neighborhood adaption, and quality stream are among methodologies that contribute to species separation and hereditary differing qualities.

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## GENETIČKA POVEZANOST IZMEĐU POPULACIJA *Consolida* (RANUNCULACEAE) NA OSNOVU ISSR MARKERA

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### Izvod

Razgraničenje vrsta je od suštinskog značaja jer se vrsta smatra osnovnom jedinicom analize u skoro svim biološkim disciplinama, kao što su ekologija, biogeografija, biologija očuvanja i makroevolucija. Grej (1821), koji je radio na britanskoj flori, smatrao je rod *Consolida* kao poseban rod zasnovan na jednoj vrsti (*C. regalis*). Ali neki istraživači su smatrali *Consolida* kao deo *Delphiniuma*. Uprkos velikoj rasprostranjenosti mnogih vrsta *Consolida* koje rastu u Iranu, nema dostupnih izveštaja o njihovoj genetskoj raznolikosti, načinu divergentnosti i obrascima širenja. Zbog toga su urađene molekularne (ISSR markeri) i morfološke analize 78 uzoraka iz 7 vrsta *Consolida*, koje su prikupljene sa različitih staništa. Ciljevi ove studije su: 1) da se pronađe dijagnostička vrednost ISSR markera u razgraničenju vrsta *Consolida*, 2) da se pronađe genetička struktura ovih taksona i 3) da se istraži međuodnos vrsta. Ova studija je otkrila da kombinacija morfoloških i ISSR podataka može razgraničiti vrstu. Analiza AMOVA i STRUKTURA otkrila je da su vrste *Consolida* genetski diferencirane, ali imaju određeni stepen zajedničkih alela.

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