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A Taxonomic Reassessment of *Consolida* (Ranunculaceae) Species: Insight from Morphological and Molecular Data

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ABSTRACT

In order to compare the efficiency of morphological traits and molecular markers in *distinguishing the Consolida* species, molecular analysis using nrDNA ITS and cpDNA *trn*L-*trn*F with maximum parsimony and Bayesian methods were done in a total of 34 species and forma representing 28 species of *Consolida*, 6 species of *Aconitella,* plus two species of *Delphinium* and two species of *Aconitum* as out groups. Beside phenetic analysis for 20 quantitative morphological traits in 17 species of *Consolida* in Iran are performed. The molecular analysis, based on successive reweighting by rescaled consistency index, revealed that Maximum parsimony method and Bayesian analysis gave very similar results based on individual and combine data sets. In the combined analysis (chloroplast and nuclear DNA) recovered most parsimonious trees (L= 558 steps, CI=0.695, RI=0.827). The ITS results revealed that *Consolida* is not monophyletic and the genus *Aconitella* is clearly nested within *Consolida.* Our results confirm the decrease of *C. paradoxa* Bunge to a forma of *C. rugulosa* also confirmed the decrease of *C. kabulica* as a variety of *C. stokciana*. One way ANOVA, principal component analysis (PCA) and cluster analysis were used in phenetic analysis to visualize the species among different traits. Most of the quantitative morphological traits which showed significant differences between populations were deleted. PCA and cluster analysis carried out for morphological traits divided the *Consolida* species in to two cluster and *A. barbata* has separated from other species. *Aconitella* species are located in separate cluster and location of other species are almost similar to molecular results.

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Introduction

The genus *Consolida* S.F. Gray was considered as a separate genus based on one species (*C. regalis*) by Gray (1821), who worked on British flora. But some researchers considered *Consolida* as a section of *Delphinium* (De Candole, 1824; Boissier, 1867; Huth, 1895; Nevskii, 1937). Unlike the others based on annual life form, single spured petal, single follicle compared to 3 or 5 sessile follicles of *Delphinium* recognized *Consolida* as a separate genus (Tutin *et al.*, 1964; Davis, 1965; Munz, 1967, a.b., Hayek, 1970; Iranshahr, 1992; Styrid and Tan, 2002; Ertugrul *et al.*, 2016; Khalaj, 2013).

Kemularia-Nathades (1939) recognized a new genus *Aconitopsis* from species of *Consolida* based on peculiar formation of the petal, upper sepal, and spur. The name *Aconitopsis* was later rejected by Sojak (1969) and being replaced by *Aconitella* because of nomenclature priority. Some researchers have studied these genera taxonomically (Soo, 1922; Munz, 1967 a.b.; Davis 1965; Iranshahr *et al.*, 1992; Constantinidis *et al.*, 2001). *Consolida* has about 40 species, of which 20 have been recorded from Iran. *Aconitella* with ca. 10 species (4 species in Iran) and 31 species of *Delphinium* (species in Iran) are centred in Irano-Turanian and Mediterranean

phytogeographic regions (Trifonova, 1990; Hasanzadeh *et al.* 2017).

Some biosystematic studies have carried out in various field such as chromosomal studies (Trifonova, 1990; Koeva, 1992; Hong, 1986; Tavassoli *et al.,* 2012) chemical studies (Aitzetmuller *et al.*, 1999), palynological studies (Khalaj *et al.*, 2016) and phylogenetic investigations by using DNA sequence data (Johansson 1995; RO *et al.*, 1997; Jabbour & Renner 2011; 2012; Yosefzadeh *et al.*, 2012). In the recent molecular studies (Jabbour and Renner 2001; 2012) it was showed that *Consolida* and *Aconitella* form a clade embeded in *Delphinium* and also *Aconitella* is embedded within *Consolida*. The Jabbour and Renner (2011) results showed that *Consolida* diverged from *Delphinium* relatives at least in the early of middle Miocene. Although the phylogenetic relationships of the tribe *Delphinieae* have described (Jabbour & Renner, 2011) but we used of plastid and nuclear DNA sequences data from herbarium materials to show the relationship of *Consolida* and *Aconitella* with using species of Iran and GenBank data Also morphological traits used to compare the efficiency of morphological traits and molecular markers in *distinguishing the Consolida* species.

Materials and methods

Plant materials.

Forty taxon (28 species of *Consolida* and 6 species of *Aconitella*) were included for molecular analyses. Four species (two species of *Delphinium* and two species of *Aconitum*) were used as out-groups. Sequence of nrDNA ITS and *trn*L-F were retrieved from GenBank (Table 1). For phenetic analysis, 17 species were used (Table 2).

Table 1. GenBank accession number and source for sample used in the study

Species	nrDNA ITS	*trn*L-F	Source
Aconitella anthoroidea	JF331875	JF331680	Iran
Aconitella hohenackeri	JF331877	JF331682	Turkey
Aconitella saccata	-	JF331683	Germany
Aconitella scleroclada	-	JF331684	Germany
Aconitella thirkeana	JF331879	JF331686	Turkey
Aconitella barbata	JF331876	JF331681	Afghanistan
Consolida ajacis	JF331880	JF331687	Germany
Consolida ambigua	LC413716	-	Iran
Consolida aucheri	JF331884	JF331691	Afghanistan
Consolida axilliflora	JF331885.	JF331692	Turkey
Consolida brevicornis	-	JF331693	Germany
Consolida camptocarpa	JF331886	JF331694	Kazakhstan
Consolida camptocarpa	LC413717	LC413710	Iran
Consolida flava	JF331887	JF331695	Iraq
Consolida glandulosa	JF331888	JF331696	Turkey
Consolida hellespontica	JF331889	JF331697	Turkey
Consolida hispanica	JF331890	JF331698	Germany
Consolida incana	-	JF331699	Germany
Consolida kabuliana	JF331891	JF331700	Afghanistan
Consolida leptocarpa	-	JF331702	Afghanistan
Consolida leptocarpa	LC413718	LC413711	Iran
Consolida mauritanica	JF331894	JF331704	Morocco
Consolida oliveriana	-	JF331705	Turkey
Consolida oliveriana	-	LC413712	Iran
Consolida orientalis	JF331896	JF331707	Iran
Consolida persica	JF331897	JF331708	Iran
Consolida pubescens	JF331898	JF331709	Spain
Consolida raveyi	-	JF331711	Germany
Consolida regalis	JF331900	JF331712	Germany
Consolida rugulosa	-	JF331718	Afghanistan
Consolida rugulosa	LC413719	LC413713	Iran
Consolida songorica	JF331902	JF331719	Kazakhstan
Consolida stocksiana	JF331903	JF331720	Afghanistan
Consolida olopetala	JF331895	JF331706	Turkey
Consolida kandaharica	JF331892	JF331701	Afghanistan
Consolida ambigua	AF258682	-	Egypt
Consolida trigonelloides	LC413720	LC413714	Iran
Consolida tehranica	LC413721	LC413715	Iran
Delphinium requienii	JF332021	JF331742	Italy
Delphinium staphisagria	JF332023	JF331743	Egypt
Aconitum delphinifoliom	AF258681	JF331725	Kenai
Aconitum pentheri	JF331915	JF331729	Serbia

Hyphens (-) indicate that ITS or *trn*L-F regions for those taxa were not determined.

Table 2. List of species studied for phenetic, localities and voucher specimens.

Species	Collector	Voucher	Locality
C. camptocarpa (Fisch. &C.A.Mey.) Nevski	Poorhabibian	ALUH 1599	Khorassan: Jajarm road
C. camptocarpa (Fisch. &C.A.Mey.) Nevski	Poorhabibian	ALUH 35379	Semnan: 58 km of Shahrud to Sabzevar
C. leptocarpa Nevski	Poorhabibian	ALUH 1603	Khorassan: Sarakhs, 12 km to Mozduran
C. leptocarpa Nevski	Poorhabibian	ALUH 1590	Golestan: Golestan national park, Mirzabailoo
C. leptocarpa Nevski	Poorhabibian	ALUH 1605	Khorassan: Sarakhs road
C. persica (Boiss.) Grossh.	Poorhabibian	ALUH 1600	Khorassan: Sarakhs, 14 km to Mozduran
C. persica (Boiss.) Grossh.	Poorhabibian	ALUH 1555	Hamedan: Khan Abad
C. persica (Boiss.) Grossh.	Poorhabibian	ALUH 1556	Tehran: Firuzkuh
C. rugulosa Schrödinger	Poorhabibian	ALUH 1606	Azarbayejan: Tabgriz, Ahar road
C. rugulosa (Boiss.) Schrödinger	Poorhabibian	ALUH 1597	Golestan: Golestan national park, Mirzabailoo
C. rugulosa (Boiss.) Schrödinger	Poorhabibian	ALUH 1557	Khorassan: Mashhad
C. paradoxa Nevski	Poorhabibian	ALUH 1558	Hamedan: Khan Abad
C. paradoxa Nevski	Poorhabibian	ALUH 1598	Khorassan: Neyshabur, Sharif Abad village
A. anthoroidea (Boiss.) Schrödinger	Poorhabibian	ALUH 18570	Khorassan: Ferdowsi University Campus
A. anthoroidea (Boiss.) Schrödinger	Poorhabibian	ALUH 1586	Hamedan: Almaghlagh village
A. anthoroidea (Boiss.) Schrödinger	Pakravan	ALUH 1595	Hamedan: Nahavand road, Garo Mt.
A. tehranica (Boiss.) Rech.f.	Mahdavii	ALUH 2783	Markazi: Kuhe Chepeghli
A. tehranica (Boiss.) Rech.f.	Assadi & Maassoumi	TARI 1701	Tehran: Between Karaj and Eshtehard
C. stocksiana Nevski	Zarre & Amini	HNBG 5077	Mazandaran: Pol Sefid
A. hohenackeri (Boiss.) Grossh.	Poorhabibian	ALUH 1598a	Khorassan: Neyshabur
A. hohenackeri (Boiss.) Grossh.	Poorhabibian	ALUH 1587	Hamedan: Kuhe Garo
C. aucheri (Boiss.) Iranshahr	Mozaffarian	TARI 71498	Fars: Bamo national park
C. ambigua (L.) Ball & Heywood	Poorhabibian	ALUH 1600a	Khorassan: Sarakhs, 14 km to Mozduran
C. ambigua (L.) Ball & Heywood	Seraj	TARI 24663	Kermanshah: Ghasreshirin
C. orientalis (Gray) Schrödinger	Poorhabibian	ALUH 1580	Tehran: Rudehen
C. orientalis (Gray) Schrödinger	Poorhabibian	ALUH 27543	Mazandaran: Sari
C. regalis S.F. Gray	Assadi & Mozaffarian	TARI- 30036	Azarbaijan: 20 km from Jolfa to Marand
C. regalis S.F. Gray	Assadi & Musavi	TARI-20531	Azarbaijan: Arasbaran
C. regalis S.F. Gray	Zarre	ALUH-1606	Azarbaijan: Tabriz
C. oliveriana (DC.)Schrod.	Assadi & Wendelbo	TARI-16616	Lorestan: 110 km Khorram abad
C. oliveriana (DC.)Schrod.	Assadi	TARI-24900	Kermanshah: 31 km to Ghasre-shirin
C. oliveriana (DC.)Schrod.	Riazi	TARI-9422	Khuzestan: Do-gonbadan
C. flava (DC.)Schrod.	Mozaffarian	TARI-53570	Khuzestan: 20 km from Ramhormoz
C. flava (DC.)Schrod.	Mozaffarian	TARI-63218	Khuzestan: W of Bostan
C. trigonelloides (Boiss.) Munz	Forughi & Assadi	TARI-17896	Kerman: Laleh zar Mt.
C. trigonelloides (Boiss.) Munz	Mozaffarian	TARI-71262	Esfahan: Semirom to Keikha
C. trigonelloides (Boiss.) Munz	Yusefi	TARI-1376	Esfahan: Ghamishloo protected area
C. oligantha (Boiss.) Schrod.	Pabo	TARI-29377	Kermanshah: Hersin

Abbreviations used in accession information: ALUH = Alzahra University Herbarium, Tehran, Iran; TARI= Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran.

Morphological traits

The 25 quantitative and qualitative trait were access to characterized and estimate genetic distance. But 20 quantitative morphological traits were used because other traits had polymorphism and overlapping in different species (Table 3).

DNA extraction, PCR amplification, and sequencing

Previously collected herbarium specimens, as well as field-collected material dried and stored in silica gel, were used for DNA extraction. DNA isolation and sequencing relied on commercial kits (Plant BioFlux, Bioer Co. China). The complete nrDNA ITS region was amplified using primers ITS4 and ITS5 of White *et al.* (1990) and for amplifying and sequencing the *trnL* intron and adjacent *trnL-trnF* intergenic spacer we used of two primers *trn*L-F (Jabbour & Renner, 2011).

Amplification was done in a DNA thermal cycler (Primus 96, MWG, Germany).
All samples were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit with the same PCR primers in an ABI Prism 377 DNA Sequencer.
The sequences were edited using Bioedit Sequence Alignment Editor Version 7.0.9.0 (Hall, 1999)

Table 3. Characters used in phenetic analysis

Character	Character states
Presence of petiole in caulin leaves	0: present 1: absent
Presence of hair on the leaf surface	0: present 1: absent
Overtopping the bract from flower	0: yes 1: no
Overtopping the bract from fruit	0: yes 1: no
Position of bract	0: near the flower … 1: far from the flower
spure	0: present . 1: absent
Shape of spure	0: curved 1: erect
Position of hair on lateral sepal	0: scattered . 1: on the middle vein
number of petal lobes	0: 5 1: 3
Proportion of petal middle lobes to lateral lobes	0: equal 1: shorter 2: longer
Presence of hair on the filament	0: absent 1: present
Position of hair on filament	0: wing 1: total of filament
Colour of anther	0: brown 2: yellow
Shape of follicle beak	0: erect 1: curved
Shape of follicle	0: falciform 1: erect
Presence of hair on the follicle surface	0: absent 1: present
Shape of fruit stalk	0: antrorse 1: erect 2: decurved
Proportion of pedicle to flower	0: shorter 1: longer
Proportion of pedicle to fruit	0: shorter 1: longer
Length of basal leaves	0: 50 mm 1: 50mm
Number of bracts	0: 0 1: 1 2: 2
Broad of petal	0:2-8 mm 1: 9-18 mm
Number of bracteole	0: variable 1: constant
Length of bracteole	0: ≤ 7mm 1: ≥10 mm
Length of spure	0: ≤ 20 mm 1: ≥ 22 mm

Phylogenetic analyses

The phylogenetic analyses employed for the data sets included maximum parsimony (MP) and Bayesian inference (BI).
Maximum parsimony analyses (MP) were run in PAUP*ver. 4.0b10 (Swofford, 2002). The heuristic search option was selected using 1000 replications of random addition sequence and TBR branch-swapping with MULTREES on and steepest descent off. Confidence limits for trees were assessed by performing 1000 replicates of bootstrapping (Felsenstein, 1985). The consensus trees from two independent runs were compared with one another and with the consensus tree from the parsimony analysis.
Bayesian inference was performed using MrBayes ver. 3.1.1 (Nylander, 2004) based on Akakia information criterion (Posada & Buckley, 2004). Bayesian analysis was performed on the data sets with GTR+G model. The analysis involved two simultaneous runs of 10 million generations of Monte Carlo Markov chains by saving every 100th tree. Mr. Bayes performed two simultaneous analyses starting from different random trees sampled at every 100 generations. The first 25% of trees were discarded as burn-in. The remaining trees were then used to build a 50% majority rule consensus tree accompanied with posterior probabilities values. Tree visualization was carried out using Tree View X ver.0.5.0 (Page, 2005).

Genetic similarity, cluster and data analysis

Morphological descriptors were analysed using principal component analysis (PCA). The number of principal components to retain in the analysis was determined using the minimum eigenvector criterion proposed by

Kaiser (1960). Genetic similarity/distances carried out on the matrix of Euclidean distances were assessed using cluster analysis (Ward) method. The statistical treatment of morphological traits was performed using SPSS software (ver. 20)**.**

Results

Sequence analyses

The nrDNA ITS alignment matrix comparies 34 sequences and 643 characters. Including 207 potentially parsimony-informative sites and 97 parsimony-uninformative ones. For *trn*L-F region, the matrix of 40 sequences contains 1175 characters, of which 123 are potentially parsimony-informative sites and 101 are parsimony-uninformative. More information about data sets and tree statistics is summarized in Table 4.

Phylogenetic analyses

Phylogenetic analyses of individual data sets

Bayesian analyses of two single data sets were topologically identical to those of parsimony analyses (tree not shown). The *trn*L-F tree of 31 species included a polytomy which species of *Aconitella* were united among of *Consolida* species (Fig. 1). Just one subclade contains three species of *Aconitella* (*A. hohenackeri, A. scleroclada, and A. anthoroideae;* Bp= 67%). We show only Bayesian trees along with posterior probability (PP) and bootstrap based on ITS, *trn*L-F *data set* (Fig. 1 & 2).
In Bayesian nrDNA ITS tree *Aconitella* species are completely nested in the *Consolida* species (Fig. 2). Two *Delphinium* and *Aconitum* species occur as outgroups in a separate clade (PP= 1, BP=100%). *C. olopetala* and *C. Trigonelloides*, as sister taxa (A), were the first diverging species. Clade B included all species of *Consolida* and *Aconitella*. This large clade comprises of two main clades (C and D). Clade C included two subclades of two species each. One subclade contains *C. hellespontica* and *C. glandlosa* (PP= 1, Bp= 100%) and the other one comprised *C. mauritanica* and *C. pubescens* (pp= 1, Bp= 88). In clade D, the first diverging species was *A. barbata*, followed by two subclades with good support (E and F) that consisted of all other *Cosolida* species that consist of several subclades and species with resolved positions.

Combined phylogenetic analyses

The topology observed in BI analysis of the combine data sets was similar to MP trees. In BI tree, some species of *Consolida* separated with high support but *Aconitella* species occupied unresolved position and *A. barbata* nested in other subclade (Fig. 3).

Genetic similarity assessed by morphological data.

Genetic similarity evaluated by using of quantitative morphological traits using cluster analysis (Ward) method (Fig. 5) show the presence of similarity and distances between *Consolida* species. Comparisons of data and cluster analysis generate a dendrogram where 17 species were grouped into two main clusters (Fig. 5).

Table 4. Statistics of *trn*L-F, ITS, and combined nuclear and chloroplast region analyses of *Consolida* species

Data set	*trn*L-F	nrDNA ITS	Combined data (*trn*L-F, ITS)
Alignment length	1175	643	1818
Number of uninformative characters	101	97	119
Number of parsimony informative characters	123	207	281
Consistency Index	0.917	0.638	0.695
Retention Index	0.950	0.790	0.827

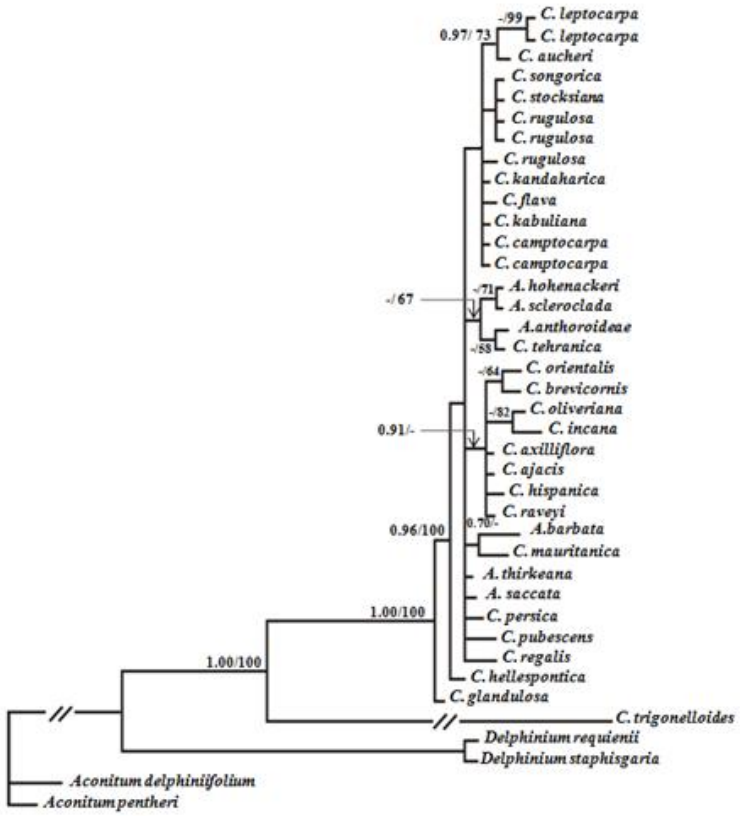

Fig. 1. Bayesian inference tree of *trn* L-F data set in *Consolida* species: Numbers above the branches or arrows indicate Bayesian posterior probabilities (PP) and maximum parsimony bootstrap (MP). Values < 50% not shown. (*C. tehranica =A. tehranica)*

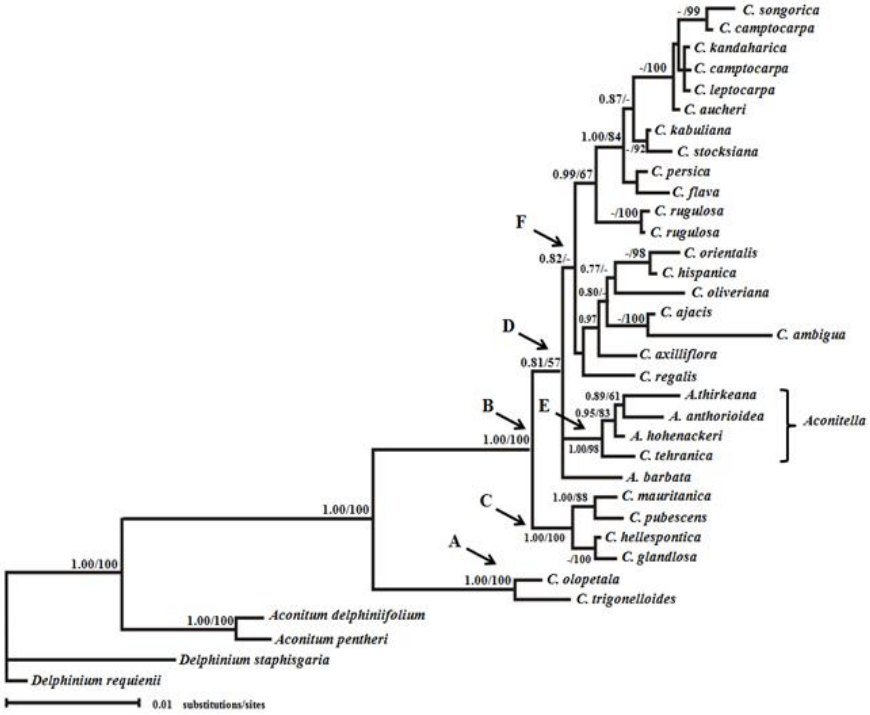

Fig. 2. Bayesian inference tree of data set nrDNA ITS in *Consolida* species: Numbers above the branches or arrows indicate Bayesian posterior probabilities (PP) and maximum parsimony bootstrap (MP). Values < 50% not shown. (*C. tehranica =A. tehranica)*

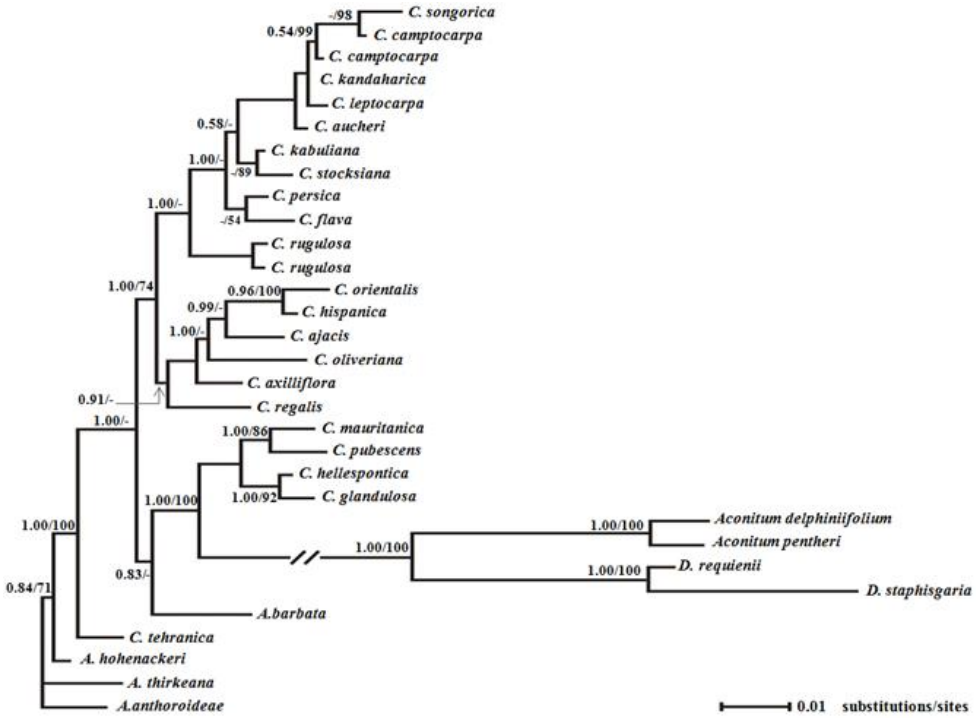

Fig. 3. Majority- rule (50%) consensus tree resulting from Bayesian analysis of the combined data set (*trn*L-F and nr DNA ITS) in *Consolida* species. Support values are indicated above the branches (Bayesian posterior probabilities (PP) and maximum parsimony bootstrap (MP), respectively). Values < 50% not shown.

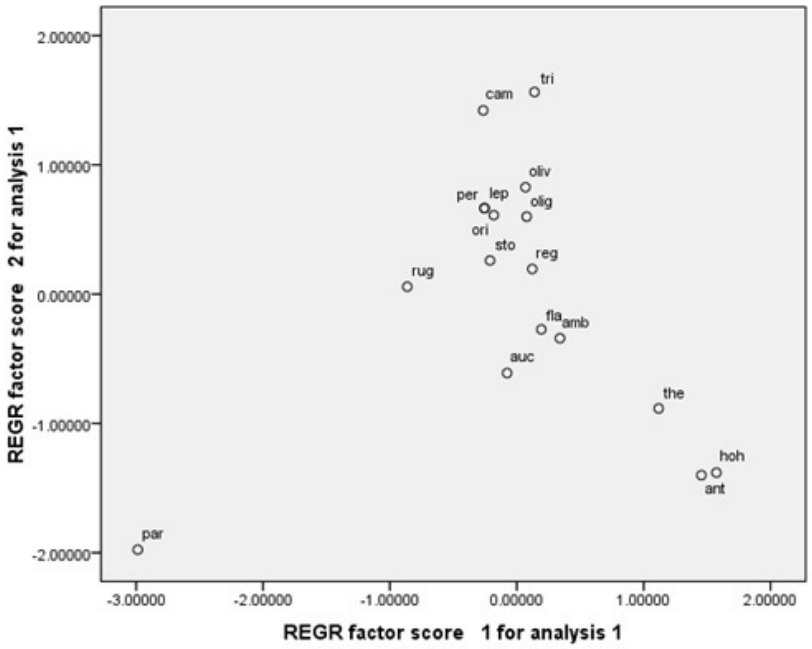

Fig. 4. PCA analysis of qualitative characters based on factor 1 and 2.

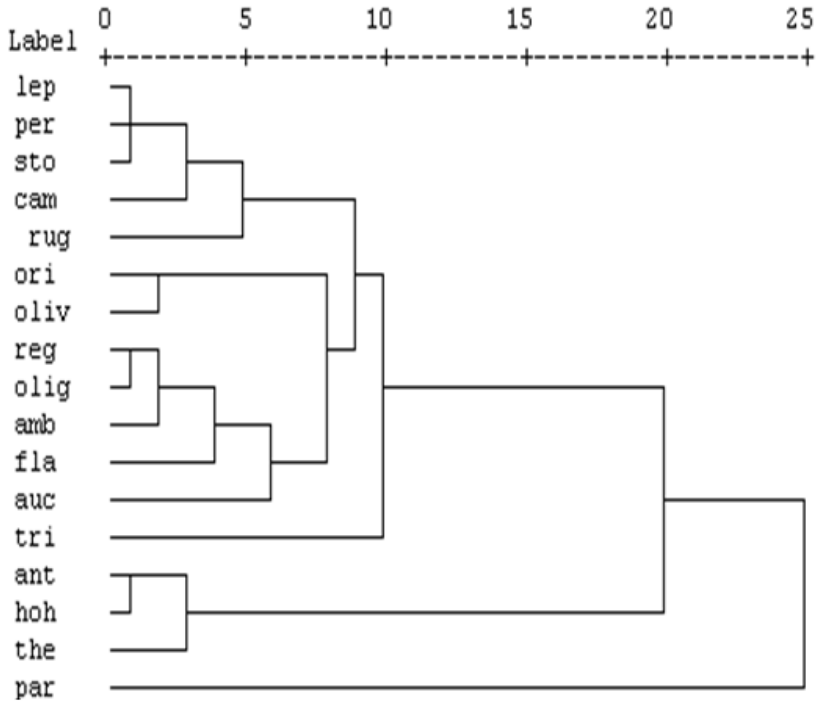

Fig. 5. Phenogram based on morphological analysing data of 17 taxa species by Ward method.
(*ant=A. anthoroidea, ori=C. orientalis, per= C. persica, oliv=C. oliveriana, rug=C. rugulosa f.rugulosa, fla=C. flava,olig= C. olighantha, hoh= A. hohenackeri, cam=C. camptocarpa, lep=C. leptocarpa, sto=C. stocksiana, the=A. tehranica f.tehranica, amb=C. ambigua, tri=C. trigonelloides, reg=C. regalis subsp. Divericata, par= C. paradoxa, auc= C.aucheri*)

In the dendrogram, 14 species cluster I were grouped into three main subclusters consisting of 7, 2 and 5 species, respectively. Cluster II consists of 3 species (Fig. 5). In this dendrogram, *C. paradoxa* has separated from the other species. Study results show presence of similarity between *C. leptocarpa, C. persica, C. stocksiana, C. camptocarpa* and *C. rugulosa.* There were also two other species (*C. orientalis, C. oliveriana*) that show similarity with *C. regalis, C. oliganta, C. ambigua, C. flava, C. aucheri*. The last cluster contains three species: *A. anthoroidea, A. hohenackerii* and *A. tehranica* (which could write *Consolida anthoroidea*, *C. hohenackeri*, *C. tehranica)* which show the higher estimated genetic distance with other species.

PCA analysis of morphological data revealed that the first 3 components comprise about 65.8% of total variance. In the first component with about 35.85% of total variance, morphological characters including bract exerting from fruit, presence of spore, shape of spore apex, the number of petal, the number of petal lobes showed the highest positive correlation. In the second component with about 17.90% of total variance apex of follicle, beak showed the highest positive correlation. In the third component with about 12.04% of total variance, position of fruit stalk and bract shape showed the highest positive correlation. Therefore, there are the most variable morphological characters among the species studied. (Table 6). In the present study, the cluster results were similar to those of PCA analysis. (Figs. 4 &5).

Discussion

Jabbour and Renner (2011) were the last worker to consider *Consolida* as part of *Delphinium* based on DNA sequences data. In this research, the combined tree by Maximum likelihood method confirms the closed relationships between *Delphinium* and *Consolida* and *Aconitum*. Jabbour and Renner (2011) also showed that *Aconitella* is part of *Consolida* which some previous authors have agree to such relationship (Constantinidis *et al.* 2001). The tree by Bayesian method based on ITS and *trn*L-F data confirm that *Aconitella* is embedded in *Consolida* (BP=100%) while

some anatomical study on petiole has separated *Consolida* and *Aconitella* species (Trifonova, 1990). Some researcher such as Sojak (1969) and Trifonova (1990) suggested *Consolida* and *Aconitella* might be sister groups while this hypothesis rejected by Jabbour and Renner (2011) and also in this research.

Species relationships within *Consolida*

Our phylogenetic results, coupled with evidence from morphology, distribution, and chromosome, represent a useful first step towards addressing the issue of species circumscription and identity in *Consolida*.
Aconitella tehranica, A. hohenackeri, A. thirkeana and *A. anthoroidea* form a clade. These species in phenetic analysis located in a distinct cluster and separated from other species. While *A. barbata* form a sister clade to species of *Aconitella*. This species is only representative of the genus in Middle Asia (Jabbour, 2011). The form of its upper unpaired sepal spur and of the petal is intermediate between the genera *Consolida* and *Aconitella* (Constantinidis *et al.*, 2001). Anatomical study of the petiole structure showed that this species is identical to the representatives of the genus *Aconitella* and should definitely be regarded as within the limits of the genus (Trifinova, 1990). *A. barbata* traditionally placed in sect. *parviflorae* but Constanidine *et al.* (2001) transfered *A. barbata* to sect. *Involutae* based on seed morphology, this opinion already proposed by previous researchers (Kemularia-Nathadase, 1939; Sojak, 1960; Trifinova, 1990).
Members of the Sect. *Brevipedunculatae* are placed in the K clade. The situation of *C. rugulosa* forma *paradsoxa* (Bunge) Iranshahr (with spureless calyx) alongside to *C. rugulosa* forma *rugulosa* in one subclae (100%) confirms the decrease of *C. paradoxa* Bunge to a forma of *C. rugulosa* as Iranshahr has believed (Iranshahr *et al.*, 1992). But this species located as a separate branch from all of other studied species in phenetic analysis (Fig. 5). It is a good evidence that presence of spure isn't a good character for delimiting the species of *Consolida*. The *C. flava* together with *C. barbata* traditionally placed in Sect. *parviflora*. Constantinidis and Renner's (2001) research on the seed coat micromorphology showed that *C. flava* had hilum zone in acrateri form cavity, surrounded by fringe-like projections as in species of Sect. *Brevipedunculare* (Constantinidis *et al.* 2001). In this clade *C. flava* placed near the other member of the sect. *Brevipedunculare* (100 %). *C. flava* position in ward analysis is separate from other section members but only near to *C. aucheri*.
Two accession of *C. camptocarpa* place somewhat far from each other because of morphological polymorphism in the follicle stripe (erect and curve). There are a few differences between *C. camptocarpa* and *C. leptocarpa* in morphological characters (Tavassoli *et al.* 2012) and there are many specimens with intermediate characters. Also, karyotype analysis of *C. camptocarpa* and *C. leptocarpa* showed many similarities between them (both have 1 pair of long *m*-chromosomes with satellite, 1 pair of long *m*-chromosomes, 1 pair of st-chromosomes and 5 pairs of t-chromosomes) (Tavassoli *et al.* 2011). They are differing in nrDNA in 9 nucleotids and in cp DNA in 5 nucleotids. Our studies confirm Tavassoli *et al.* (2011) results that consider *C. camptocarpa* and *C. leptocarpa* as a complex species. Position of these species in ward cluster are in separate cluster.
The *C. kabuliana* is endemic to Afghanistan that has decreased to variety level of *C. stokciana* by Tamura (1960). They are much closed species morphologically and are different only in length of petal, spure, and anther. They are differing in nrDNA in 8 nucleotids and in cpDNA in 2 nucleotids. Also in Bayesian and combined trees, they are placed in one subclade. Our results confirmed the decrease of *C. kabulica* as a variety of *C. stokciana*.
C. aucheri was made by Boissier as a variety of *Delphinium* (1841) and again as a variety of *D. persica* introduced by the same author (Boiss. 1877). Iranshahr *et al.* (1992) considered *C. aucheri* as a new combination. Our results showed they are placed in separate clades. They are differing in nrDNA in 18 nucleotids and in cpDNA in 4 nucleotids. Therefore, these results are in agreement to Iranshahr *et al.* (1992) and Boissier (1877) that considered *C. aucheri* as a valid species.
C. regalis, *C. axilliflora*, *C. ajacis*, *C. oliveriana*, *C. hespanica*, *C. orientalis* situated in G clade. Except *C. axilliflora* the others belong to both sect. *Consolida* and sect. *Macrocarpa.* In this clade, *C. ambiguae* that distributed in Iran and Mediterranean region is

very close to *C. orientaslis* morphologically. Both have large fruit.

C. mauritiana, *C. pubescens*, *C. hellespontica* and *C. glandulosa* situate in E clade. Except *C. hellespontica* the three other species belong to sect. *Consolida*. These species have some characteristic that separate them from other member of the genus. *C. mauritiana* and *C. pubescens* share three metacentric chromosome pairs in their complement, in opposite to other member of *Consolida* that have only two metacentric chromosomes pairs (Constantinidis *et al.*, 2001).

In *C. hellespontica* the central part of the hilum area may form a characteristic shape that is less apparent in other species (Constandnidin *et al.*, 2001).

C. trigonelloides in the combined tree occur in a separate clade and in the Bayesian tree, together with *C. olopetala* also occur in the separate clade. Based on the flower morphology it could place in the sect. *Consolida* but because of seed characteristic which is penta hedral (in other species globose, pyramidal and tetrahdral shape are seen) that do not find in other species, it places in a separate clade.

The relationship between morphological traits and molecular markers results is 58%. Results of this study were congruent with results of Baranger *et al.* (2004); Simioniuc *et al.* (2002); Hoey *et al.* (1996); Tar'an *et al.* (2005), who suggested low to medium correlations among molecular and morphological data.

Molecular data again illustrate the great potential of nrDNA ITS and *trn*L-F sequences for resolving relationship at a range of taxonomic levels, from closely related species to sectional level. However, more taxon sampling and another source of DNA sequence, like chloroplast coding (e.g., *matK*, or *ndh*F) regions, are definitely necessary to be analyzed in order to comparing and combination of produced gene phylogenies for the *Consolida* species.

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References

Aitzetmuller K, Tsevegsuren N, Werner F. 1999. Seed oil fatty acid patterns of *Aconitum*- *Delphinium*- Helleborous complex (Ranunculaceae). *Plant Syst Evol* 213: 37-47.

Baranger A, Aubert G, Arnau G, Laine AL, Deniot G, Potier J, Weinachter C, Lejeune-Henaut I, Lallemand J, Burstin J. 2004. Genetic diversity within *Pisum sativum* using protein- and PCR-based markers. *Theor Appl Genet* 108: 1309-1321.

Boissier E. 1841. Voyage botanique dans le midi de l'Espagne, Livraison 16, Vol. 2. Paris: Gide & Cie.

Boissier E. 1867. *Flora Orientalis*, vol. 1. Basileae: H. Georg.

Constantinidis T, Psaras GK, Kamari G. 2001. Seed morphology in relation to infrageneric classification of *Consolida* (DC.) Gray (Ranunculaceae). *Flora* 196: 81-100.

Davis PH. 1965. *Consolida* (DC.) S.F. Gray. Pp. 119-134 in: Davis PH. (ed.), *Flora of Turkey and the East Aegean islands*, vol. 1, Edinburgh: Edinburgh University Press.

De Candolle AP. 1825. Prodromus Systematis Naturalis Regni Vegetabilis. Vol. 2. Paris, Strasbourg, London.

Ertuğrul K, Arslan E, Tugay O. 2010. Characterization of *Consolida* S.F. Gray (Ranunculaceae) taxa in Turkey by seed storage protein electrophoresis. *Turk J Biochem* 35: 99-104.

Felsentien J. 1985. Confidence limits n phylogenies. An approach using the bootstrap. *Evolution* 39: 783-791.

Gray SF. 1821. A *natural arrangement of British plants*. vol. 2. London.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 14: 68-73.

Hasanzadeh F, Kharazian N, Parishani M. 2017. Floristic, Life Form, and Chorological Studies of Saldaran Protected Region,

Chaharmahal and Bakhtiari Province, Iran. *J Genet Resour* 3(2): 113-129.

Hayek A. 1970. Prodromus Florae Peninsulae Balcanicae: Im Verlag von Otto Koeltz, Koenigstein, Taunus, 576 pp.

Hoey BK, Crowe KR, Jones VM, Polans NO. 1996. A phylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers. *Theor Appl Genet* 92: 92-100.

Hong De-Y. 1986. Biosystematic observation on 5 species of *Consolida* (Ranunculaceae). *Acta Bot Sin* 28: 1-10.

Huth E. 1895. Monographie der Gattung *Delphinium*. *Bot Jahrb Syst* 20: 322-499.

Iranshahr M, Rechinger, f., KH, Riedl H. 1992. *Delphinium* L. (Ranunculaceae). In: Flora Iranica. No. 171, Rechinger KH (ed.) Akademische Druckund-Verlagsanstalt, Graz, pp. 89-114.

Jabbour F, Renner S. 2012. A phylogeny of Delphinieae (Ranunculaceae) shows that *Aconitum* is nested within *Delphinium* and that Late Miocene transitions to long life cycles in the Himalayas and Southwest China coincide with bursts in diversification. *Mol Phylogenet Evol* 62: 928-942

Jabbour F, Renner SS. 2011a. *Consolida* and *Aconitella* are an annual clade of *Delphinium* (Ranunculaceae) that diversified in the Mediterranean basin and the Irano-Turanian region. *Taxon* 60:1029-1040.

Johansson JT. 1995. A revised chloroplast DNA phylogeny of the Ranunculaceae. *Pl Syst Evol (suppl.)* 9: 253-261

Kemularia-Nathadze L. 1939. Eine neue Ranunculaceen gattung *Aconitopsis* Kem. Nath *Trudy Tbilissk Bot Inst* 7: 115-134.

Khalaj Z. 2013. Phylogenetic study of the genus *Consolida* in Iran. Unpublished MSc thesis, Alzahra University, Tehran [In Farsi].

Koeva TJ. 1992. Comparative karyotype treatment of representatives from tribes *Delphinieae* of subfam. Delphinioideae, Family Ranunculaceae. *Ann Univ Sofia* 82: 51-61.

Munz PA. 1967a. A synopsis of African species of *Delphinium* and *Consolida*. *J Arnold Arbor* 48: 30-55.

Munz PA. 1967b. A synopsis of the Asian species of *Consolida* (Ranunculaceae).

Nevskii SA. 1937. *Delphinium* L. 79-143 in: Shishkin BK, (ed.), *Flora of the U.S.S.R.* vol. 7. Moscow, Leningrad: Akademii Nauk SSSR. Translated from Russian.

Nylander JAA. 2004. MrModeltest v2. Evolutionary Biology Center, Uppsala University.

Page DM. 2005. Treeview X: Tree drawing software for Apple Macintosh and Microsoft windows, ver 0.5.0. Available at http://drawin.zoology.gla.ac.uk/rpage/treeviewx/download.html.

Pakravan M. 2016**.** Palynological study of the genus *Consolida* in Iran. *Nov Biol Repert* 3(2): 177-183.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of akakia information criterion and Bayesian approaches over likelihood ratio tests. *Syst Biol* 53: 793-808.

Ro K-E, Keener CS, Mc Pheron BA. 1997. Molecular phylogenetic study of the Ranunculaceae: Utility of the nuclear 26S ribosomal DNA in inferring intrafamilial relationships. *Mol Phylogenet Evol* 8: 117-127.

Simioniuc D, Uptmoor R, Friedt W, Ordon F. 2002. Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs. *Plant Breed* 121: 429-435.

Sojac J, 1969. Aconitel; la Spach, eine vergessene Gattung der Familie Ranunculaceae. *Folia Geobot Phytota (Praha)* 4: 447-449.

So□ R. von. 1922. Über die mitteleuropäischen Arten und Formen der Gattung *Consolida* (DC.) S.F. Gray. *Oesterr Bot Z* 71: 233-246.

Styrid A & Tan K. 2002. Flora Hellenica. Gantner ARG, Verlag Königstein: Koeltz, pp 21-25.

Swofford DL. 2002. PAUP: Phylogenetic analysis using parsimony (and other methods), versin 4.0b10. Sinauer Associates. Sunderland, MA.

Tamura M, 1966. Morphology, ecology and phylogeny of the Ranunculaceae VI. *Sci Rep S Coll N Coll Osaka Univ* 15: 13-35.

Tar'an B, Zhang C, Warkentin T, Tullu A, Vanderberg A. 2005. Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum*) based on molecular markers, and morphological and physiological characters. *Genome* 48: 257-272.

Tavassoli A, Pakravan M, Kiarostami K, Poorhabibian R. 2012. Karyotype analysis in some species of *Consolida* (Ranunculaceae) from Iran. *Fedd Repert* 123 (4): 257- 263

Trifonova VI. 1990. Comparative biomorphological study of the taxonomy and phylogeny of the genera *Consolida* (DC.) S.F. Gray and *Aconitella* Spach. *Collect Bot (Barcelona)* 19: 97-110.

Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, and Webb DA. 1964. Flora Europaea vol. 1-5: Cambridge University Press, Cambridge.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics in Innis DH. Gelfand JJ. Sninsky *et al.* (eds.) PCR protocols: a guide to methods and applications. Academic Press, San Diego. pp. 315-322.

Whitten WM, Williams NH, Chase MW. 2000. Subtribal and generic relationship of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. *Am J Bot* 87:1842-1856.

Yosefzadeh H, Hosseinzadeh Colagar A, Tabari M, Sattarian A, and Assadi M. 2012 Utility of ITS region sequence and structure for molecular identification of *Tilia* species from Hyrcanian forests, Iran. *Plant Syst Evol* 298: 947-961.