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Molecular insights on some Iranian species of *Delphinium* L. and *Aconitum* L. (Ranunculaceae)

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Abstract. To be precise, 29 taxa of *Delphinium* and 2 species of *Aconitum* belonging to Iran have been documented in Flora Iranica. In this research, with regard to both mentioned genera, a total of 25 species for the chloroplast trnl-f region and 11 species for the Internal Transcribed Spacer (ITS) were investigated. After genome extraction, PCR and the sequencing of samples, the sequences were edited, and phylogenetic trees were prepared using Bayesian methods. The phylogenetic study of this genera led us to the monophyletic aspect of them despite the segregation of *Aconitum* and *Delphinium* in their related classic taxonomy. It has been observed that there are some complicated species in the genus *Delphinium*. The results of molecular analysis confirmed the separation of *Delphinium elbursense*, *Delphinium speciosum*, *Delphinium crispulum* and *Delphinium dasycarpum* (the complicated species of northern and northwestern Iran). Furthermore, based on the molecular results, it is suggested for *D. elbursense var. gymnobotrys* to have a higher taxonomic level as a distinct species. Meanwhile, *Delphinium tuberosum*, *Delphinium cyphoplectrum*, *Delphinium quercetorum*, *Delphinium pallidiflorum*, and *Delphinium laxiusculum* (western and northwestern species of Iran), which are regarded as complex species, were placed in a distinct molecular tree. At the end, *Delphinium dolichostachyum* was reported as a new record for Iran, and the species has been compared to the related species *Delphinium carduchorum*.

Keywords: PCR, Bayesian, monophyletic, ITS, new record.

INTRODUCTION

It has been reported that the family Ranunculaceae contains five subfamilies, 43 genera and 2346 species at the present time (Christenhusz & Byng, 2016). The tribe Delphinieae (*Aconitum* L., *Delphinium* L., *Consolida* (DC.) Gray, *Aconitella* Spach) comprises 650-700 species, which amounts to some 25% of all Ranunculaceae (Jabbur & Renner, 2012), and is distributed in the temperate regions of the northern hemisphere (Tamura 1990; Stevens 2001). The key feature of this tribe is the nectar placed in inner

tepal (Jabbour & Renner 2012; Ilarslan et al. 1997; Espinosa et al. 2017). Based on Flora Iranica (Iranshahr 1992), 29 species of *Delphinium* and 2 species of *Aconitum* are reported from Iran. Iranian species of *Delphinium* are divided into two subgenera (differences between subgenera are in the form of seed and vegetative period): *Olighophyllon* Dimitrova and *Delphinium*, which are perennial and annual species, respectively (Iranshahr 1992; Beltran et al., 2021; Cabusora et al., 2020; Fikirie et al., 2020). Iranian *Aconitum* species are also divided into two subgenera, which are *Aconitum* and *Lycoctonum* DC. (the difference between subgenera is the shape of galea) (Iranshahr 1992). Mobayen (1985) reported for the flora of Iran; *D. dasycarpum* Stev. ex DC., *D. venulosum* Boiss. and *D. micranthum* Boiss. & Hohen. Sharifnia et al. (2013) recorded *D. kurdicum* Boiss. & Hohen. for the first time for the flora of Iran. Recently, *D. khorasanicum* Sharifnia & Hasanbarani was reported as a new species from Khorasan province (Hasanbarani et al. 2017). In general, several studies have been carried out on the Delphinieae in the world; for instance, Seed morphology of 28 *Delphinium* L. species has been studied (Ilarslan et al. 1997; Mieso & Befa 2020; Mustafa 2020; Varamesh et al., 2014; Rajaei et al., 2020; Fataei et al., 2013). Ozpelic & Uztiirk (2000) worked on the morphology and ecology of 12 populations of *D. cyphoplectrum* Boiss. in Turkey. Palynology study of 21 taxa from *Delphinium* has also been performed (Bursali & Dogan 2005). The molecular analysis of nuclear and chloroplast sequences of Delphinieae were studied in the geographical range of Asia, the Mediteraneaen, North America and the mountains of east Africa; the monophyly of the genus *Consolida* DC, *Aconitum* L. and *Delphinium* L. was proved (Jabbour & Renner 2011, 2012). Wang et al. (2013) reported that based on molecular markers *Gymnoaconitum* (Stapf) Wei Wang & Z.D Chen differs from the other species of *Aconitum* and other genera of the tribe Delphinieae. Xiang et al. (2017) conducted a broad phylogenetic analysis within Ranunculaceae using *matk* sequence and performed a series of analysis using four molecular markers focused on the tribe. Micromorphological characters of the genus *Delphinium* L. (sensu lato) seeds and fruits were studied using microscopic techniques (Hadidchi et al. 2019). In China based on observations on living plants in the field, together with examination of herbarium specimens, demonstrated that *Delphinium iliense* (Ranunculaceae) is highly variable in the indumentum of peduncles, pedicels, bracteoles, sepals and also in the shape of bracteoles and their position on pedicels (Li et al. 2019).

Table 1. *Delphinium* species in Iran (following taxonomic studies of this genus in 2013-2017, endemic species are bold).

Species	root form
***D. aquilegifolium* (Boiss.) Bornm.**	tuberiformis
D. biternatum Huth.	tuberiformis
D. carduchorum Chowdhuri & Davis	tuberiformis
D. cyphoplectrum Boiss.	tuberiformis
D. crispulum Rupr.	non-tuberiformis
D. dasycarpum Stev. ex DC.	non-tuberiformis
D. dasystachyum Boiss. & Bal.	tuberiformis
D. dolichostachyum Chowdhuri & Davis	tuberiformis
***D. elbursense* var. *elbursense* Rech.f.**	non-tuberiformis
***D. elbursense* var. *gymnobotrys* Rech.f**	non-tuberiformis
D. ilgazense P.H. Davis	tuberiformis
***D. jacobsii* Iranshahr**	tuberiformis
***D. khorasanicum* Sharifnia & HasanBarani**	tuberiformis
D. kurdicum Boiss. & Hohen.	tuberiformis
Delphinium lanigerum Boiss. & Hohen	tuberiformis
D. laxiusculum (Boiss.) Rouy	tuberiformis
D. macropogon Prokhanov	tuberiformis
D. micranthum Boiss. & Hohen.	tuberiformis
D.ochrolecum Stev. ex DC.	tuberiformis
D. pallidiflorum Freyn	tuberiformis
D. peregrinum L.	tuberiformis
D. quercetorum Boiss. & Hausskn	tuberiformis
D. szowitsianum Boiss.	tuberiformis
D. speciosum M.B.	tuberiformis
D. semibarbatum Bienert ex Boiss.	tuberiformis
D. saniculifolium Boiss.	tuberiformis
D. schmalhausenii Alboff	tuberiformis
D. tuberosum Auch. ex Boiss.	tuberiformis
D. turkmenum Lipsky	tuberiformis
D. venulosum Boiss.	tuberiformis
D. zalil Aitch. & Hemsl.	tuberiformis

During a taxonomic study on *Delphinium* species in 2013-2018 based on herbarium specimens (TARI) and also taking into account the descriptions and images of types, 31 species of *Delphinium* were detected (Table 1); among them, the subgen. *Delphinium* includes the annual species: *D. venulosum* Boiss. and *D. peregrinum* L., whereas the subgen. *Oligophyllon* comprises perennial species, which have either tuberiformis or non-tuberiformis roots (root form is one of the characters that is used in flora iranica *Delphinium* key). *D. speciosum* M.B., *D. lanigerum* Boiss. & Hohen., *D. elbursense* Rech.f., *D. crispulum* Rupr. and *D. dasycarpum* Stev. ex DC. are characterized by a non-tuberiformis root. These species have a similar distribution, and they are morphologically very closely related. The other species in the

genus *Delphinium* (*D. cyphoplectrum* Boiss., *D. tuberosum* Auch. ex Boiss, *D. laxiusculum* (Boiss.) Rouy, *D. pallidiflorum* Freyn, and *D. quercetorum* Boiss. & Hausskn) have a tuberiformis root and non-yellow flower; they form a complex morphologically related species in this genus (Iranshahr 1992).

Due to the large number of species distributed in Iran and the controversies in taxonomical ideas among researchers, a taxonomic review of these species is required. Moreover, we reported in our previous research that for the biosystematic study of *Delphinium* species in IRAN, there is a strict necessity to have the help of molecular analysis methods to more confidently classify this genus.

MATERIALS AND METHODS

Plant materials

In this research, in order to conduct molecular study, the plant materials were taken from Central Herbarium of Iran (TARI), and the samples were collected from the field dried on silica gel (this species is available in IAUNT herbarium). It must also be mentioned that 25 species for the chloroplast marker (two species of *Aconitum*) and 11 species for the ITS marker (one species of *Aconitum*; *Aconitum iranshahrii* endemic of Iran and the sequences available in Genbank) were investigated (table 2).

DNA extraction and PCR amplification

Total DNA was extracted using the MBST kit (Shayan et al. 2007). The amplification of DNA fragments was carried out for ITS sequence and *trnL-F* region. The entire ribosomal ITS region was amplified using primers pairs AB101 (forward, 5 -ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3) and AB 102 (reverse, 5-TAG AAT TCC CCG GTT CGC TCG CCG TTA C-3) (Douzery et al. 1999), and the PCR reaction for nuclear marker was executed using a denaturation step of 5 min at 95C followed by 35 cycles of 30 S denaturation at 95C, 30 S of annealing at 56C, and 90 S extension at 72C, followed by a final extension step of 7 min at 72C.

The *trnL-F* region was amplified using primers C (Forward, 5-TAC GAC GAT CTY TCT AAA CAA GC-3) and F (reverse, 5- GGA AAG ATT GCT CAA ATA CCA G-3) (Taberlet & Gielly 1991). The PCR reaction for chloroplast marker was carried out with a denaturation step of 5 min at 95C, followed by 35 cycles of 30 S denaturation at 95C, 30 S annealing at 54.4C, and 1 min extension at 72C, followed by a final extension step of 7 min at 72C. The PCR products were migrated on 1% agarose gel and were visualized by ethidium bromide.

Sequence alignment and phylogenetic analyses

After sequencing, the sequences were edited using BioEdit software ver. 7.0.9.0 (Hall 1999) and then were aligned using the Mesquite software (Maddison & Mad-

Table 2. *Delphinium* and *Aconitum* species included in the molecular study (species used in ITS marker are shown with stars).

Species	Locality
Aconitum Iranshahrii*	Mazandaran: Polsefid, forest above village Sangdeh, 1500-2500 m, Assadi 73445.
Aconitum nasatum	Eeast Azarbaijan: Arasbaran protected area, Doghrun mountain, 2500 m, Assadi & Sardabi 23945.
***Delphinium aquilegifolium* (Boiss.) Bornm.**	Mazandaran: Lar valley, 2450-2550m, Wendelbo & Assadi, 13264-TARI. Tehran: W of Tehran, Suleghun valley, 1500-2000m, Assadi & Mozaffarian 32699-TARI. Tehran:10 Km from Karaj, On Chalus road, 1750m Babakhanlu & Amin 20004-TARI.
D. cyphoplectrum* Boiss.	Fars: Kazerun, Komaraj,980m, Forughi 7930-TARI. Khusestan: 74128-TARI. Khuzestan: 47 Km to Masjedsoleiman from Haftgel, Assadi & Abohamzeh 38933-TARI.
D. crispulum* Rupr	Ardabil: Ca 9 Km from Khalkhal on the road to Asalem, 2050m, Assadi & Shahsavari 66000-TARI. West Azerbaijan: Khoy, Hasan Deh- e-Kan, 2500m, Amini, 1716-TARI. East Azerbaijan: 35 Km. NE of Marand, KiamakiDagh Mt., Assadi & Olfat 68603, TARI. East Azerbaijan:23 km SE of Jolfa, Near the Geshlagh village, Miaran, Assadi & Shahsavari 65786, TARI.
***D. carduchorum* Chowdhuri & Davis**	West Azerbaijan: Urumieh, Mavana, Kuhe dare rash, 2100-2700m, Mozaffarian 74872-TARI.
D. dolichostachyum*	Kurdestan: Baneh, 1650m, Maroofi & Fani 6959-TARI.
***D. dasycarpum* Stev. ex DC.**	East Azerbaijan: Sahand Mt., 2200m Assadi & Mozaffarian, 30641- TARI.
D. dasycris= D. dasycarpum* × *D. crispulum	East Azerbaijan: 60 km N.E of Maragheh, Chagh-Chagh Pasture, 1850m, Benvan 25028-TARI.

Species	Locality
D. elbursense* var. *elbursense* Rech.	Mazandaran: Polesefid, forest above village Sangdeh, 1500-2500m, Assadi 73521& 73451-TARI Golestan: Kurdkuy, 5-10 Km from Radkan to Kurdkuy, 2200m, Mozaffarian 78137-TARI. Mazandaran: Polesefid, forest above village Sangdeh, 1500-2500m, Assadi 73521-TARI.
***D. elbursense* var. *gymnobotrys* Rech.**	Mazandaran: Ramsar, S of Javaherdeh, 2600-3200m, Masassumi 56821-TARI. Mazandaran: Siahbisheh, Chalus Valley, 2120m, Sabeti 2056-TARI. Mazandaran: Siahbisheh, Chalus Valley, 2100m, Sabeti 1785-TARI. Mazandaran: Siahbisheh.Chalus Valley, 2300m, Sabeti 7964-TARI.
D. ilgazense* P.H. Davis	Azerbaijan: Tabriz, Ahar road, 22 km to Ahar, 1900-2000m, Mozaffarian & Mohammadi 37587-TARI.
***D. khorasanicum* Sharifnia & HasanBarani**	Khorassan: North west of Neyshabur, Bar fall, 2004 M, Sharifnia and HasanBarani 16155 IAUNT.
***D. laxiusculum* (Boiss.) Rouy**	West Azerbaijan: Gooshchi Pass, 1800m, Siami & Zehzad 7019-TARI. Ardabil: 45km from Namin to Germi, 220m, Mozaffarian & Nowrozi 34598-TARI. Ardabil:40 km from Razi to Germi, 1700m, Mozaffarian & Nowrozi 34762-TARI. Azerbaijan: Kaleybar to Jananloo, kiaragh, 1200m, Hasanbarani 16785-IAUNT.
***D. lanigerum* Boiss. & Hohen.**	Hamedan: Alvand Mt., 2700m, Assadi & Mozaffarian, 2700m 36809-TARI. Hamedan: near Ganjnameh, 2100m, Assadi & Mozaffarian 36784-TARI. Tehran: Shemiran, Darband & Passghale, 2000-2500m, Mozaffarian & Jamzad 43742-TARI.
***D. micranthum* Boiss. & Hohen.**	Kurdestan: From Baneh to Saghez, Kalawarash, 1900m, Fattahi & Hatami 2539-TARI. Kurdestan: Saghez to Baneh, Nacarouz Mt., 2500m, Maroofi & Mohammadi 6590-TARI. 85470-TARI.
***D. ochrolecum* Stev. ex DC.**	Ardabil: 9km from diviation of Kivi to Ardebil road, above Meresht village, 2000m, Mozaffarian & Nowrozi 34391-TARI. West Azerbaijan: Urumieh, Marmishu vally, 1737m, Mozaffarian 87255-TARI.
D. pallidiflorum* Fyen	Esfahan: Fereydunshahr, near the village Sibak, 2800m, Assadi & Khatamsaz 76521-TARI.
D. peregrinum* L.	Fars: Nurabad, 22 km from Fahilan to Rashk, 900-1200m, Mozaffarian 45975-TARI. Fars: 15 to 20 km from Shiraz to Esfahan, 1600-1900m, Assadi & Ranjbar 82991-TARI.
***D. quercetorum* Boiss. & Hausskn.**	East Azerbaijan: Ca. 20Km W of Marand, Mountain above the village Orlan, Mishoudagh, 2000-2500, Assadi & Shahsavari 65472-TARI. Kurdistan: Marivan, dizil,expose to Iraq frontier, 2350m, Maassumi & Nickchehre, 80189-TARI. Kurdistan: 34Km from Chenareh to Baneh, 1922m, Assadi 85087-TARI.
***D. schmalhausenii* Alboff**	Kurdistan:Kurdestan, Ca. 17 Km from Baneh to Marivan, 1740m, Mozaffarian 87400-TARI.
D. speciosum* M. B.	Semnan: between Shahrud and Azadshahr, Kuhe abr, 2600m, Assadi & Maassumi 21523-TARI. Golestan: N Gorgan, Ca 20 Km Charbagh toward Gorgan, 1550m, Assadi
***D. turkmenum* Lipsky**	Semnan: Touran protected area. 22 km from Ghazaran to Miandasht, 1240m, Feritagh & Jadidi 28987-TARI. Khorassan: North west of Neyshabur, Bar fall, 2004 M, Sharifnia and HasanBarani 17003- IAUNT.
***D. tuberosum* Auch. ex Boiss. ***	West Azerbaijan: Ca. 15 Km to Maku on Road from Marand, 1200-1400m, Assadi & Mozaffarian 30110-TARI. Hamedan 64503-TARI. Zanjan 29393-TARI. East Azerbaijan: Kaleybar to Jananloo, kiaragh, 1200m, Hasanbarani 16798-IAUNT.
***D. ursinum* Rech.**	Gorgan: Tanghegol Forest, 700-1000m, Wendelbo & Forughi 12766-TARI. Mazandaran: 32592-TARI. Tehran: Between Ushan & Tehran, 1730m, Assadi & Shahsavari 69764-TARI.
D. venulosum* Boiss.	Lorestan: Nowjian, (Between Khoramabad & Keshvar) 1850m, Runemark & Lazari 26112-TARI. Ilam: 10 km N.W. of Islam Abad, Ilam road, 1550m, Seraj 24666-TARI.

dion 2010). Some sequences were obtained from the GenBank (Table 3). The basis for the selection of taxon from the gene bank was the geographical distribution. Phylogenetic relationships were assessed using Bayesian Inference (BI). The substitution model was obtained using the program Mrrmodeltest ver. 2.3 (Nylander 2004). GTR + G + I for nuclear DNA and GTR + G for *trnL-F* region were identified as the best model for the dataset. The program Mrbayes version 3.2 (Ronquist & Huelsenbeck 2003) was used for the Bayesian reconstruction. After drawing several trees with different outgroups from Ranunculaceae, the best results were obtained from these outgroups (*Nigella damascena* for ITS marker and *Helleborus niger* for *trnL-F* marker).

Table 3. GenBank accession number taken from NCBI.

Species	*trnL-F* GenBank	ITS *GenBank*
Delphinium halteratum	JF331737	-
Delphinium leroyi	JN73564	-
Aconitum baicalense	JF331723	-
Aconitum ciliare	JF331724	AB004952
Aconitum delphinifollium	JF331725	AF258681
Aconitum ferox	JF331726	AB004961–2
Aconitum pendulum	JF331728	AY150235
Aconitum pentheri	JF331729	JF331905-18
Aconitum racemolusom	AF258652	AY150233 2
Aconitum septentrionale	JF331730	AF216552
Aconitum tanguticum	JN573573	AY15023
Consolida ajacis	JF331687	JF33188
Consolida axilliflora	JF331692	-
Consolida flava	JF331695	JF331887
Consolida orientalis	JF331707	JF331896
Delphinium pyramidale	JN573581	-
Delphinium afgahnicum	JN573529	-
Delphinium albocoeruleum	JN573530	-
Delphinium bakeri	AF258652	AF258697
Delphinium balansae	JF331732	-
Delphinium bicolor	-	AF258711
Delphinium brachycentrum	-	JN573515
Delphinium cardinale	-	AF258740
Delphinium crassifolium	JN573540	-
Delphinium cuneatum	JN573542	-
Delphinium dasycaulon	JN573544	-
Delphinium decorum	-	AF258744
Delphinium delavayi	-	AF258705
Delphinium dubium	JN573568	-
Delphinium elatum	JN573549	-
Delphinium favargeri	JF331679	-
Delphinium fissum	JN573552	-
Delphinium flexosum	JN573553	-
Delphinium gracile	JF331736	AF258763
Delphinium gypsophilum	-	AF258721
Delphinium hesperium	-	AF258772
Delphinium hirschefeldianum	-	JF331988-95
Delphinium incisum	JN573558	-
Delphinium kohatense	JN573561	-
Delphinium maakianum	JN573573	-
Delphinium macropetalum	-	JF331996-2000
Delphinium muscosum	JN573572	-
Delphinium oreophilum	JN573576	-
Delphinium suave	JN573596	-
Delphinium verdunanse	JN573596	-
Delphinium virgatum	-	JF332030-1
Delphinium viscosum	JN573597	-
Delphinium wendelboie	JN573598	-
Delphinium staphisagria		JF332022
Helleborus niger	AJ413290	-
Nigella damascene	-	AY150260

RESULTS AND DISCUSSION

The Bayesian analysis result for the *trnL-F* region with posterior probabilities (PP) is shown as consensus tree in Fig. 1. The length of the *trnL-F* sequences included in the final matrix ranged from 950 to 1050 base pair. *Helleborus niger* is taken as an outgroup. This cladogram has several groups: species of annual *Delphinium* (clade d), perennial *Delphinium* (clade e), *Consolida* (clade c) and *Aconitum* (clade b). This result is congruent with the achievement of the study of Jabbour & Renner (2011). Clade (a) includes the *Aconitum*, *Delphinium* and *Consolida* (Delphinieae tribe); Jabbour & Renner (2012) have revealed the monophyly of *Delphinium* and *Aconitum*. *Delphinium* species (both annual and perennial species) make a clade with a pp= 0.63 in which the annual and perennial species create two distinct groups as subgenus *Delphinium* (d) and subgenus *Delphiniastrum* (e).

The Bayesian analysis result for the ITS region is shown in Fig. 2. *Nigella damascena* was considered as an outgroup. The length of the ITS sequences included in the final matrix ranged from 600 to 700. There were several groups in consensus tree, similar to the results of *trnL-F* marker: annual *Delphinium* (clade e), perennial *Delphinium* (clade d), *Aconitum* (clade b), and *Consolida* (clade c).

By examining the results of chloroplast and nuclear marker, *D. dasycarpum* (only in chloroplast tree), *D. speciosum*, *D. crispulum*, *D. elbursense* var. *elbursense* and *D. elbursense* var. *gymnobotrys* (only in chloroplast tree) were close to each other, in spite of the fact that they are distinct species. In the USSR flora, there are two subgenera: *Consolida* and *Eudelphinium*,. *Eudelphinium* includes 3 sections: *Kolobopetala*, *Elaptosis* and *Diedropetala* (Komarov 1970). According to USSR flora, *D. speciosum*, *D. crispulum* and *D. dasycarpum* belong to the *Elaptosis* section similar to our molecular study (chloroplast tree) which are all in the same group. These species have cylindrical root, dark blue flowers, black anther and lower petals which are black with yellow barbate. *Delphinium turkmenum*, *D. laxiusculum*, *D. quercetorum*, *D. schmalhausenii*, *D. szowitsianum*, *D. ochrolecum*,

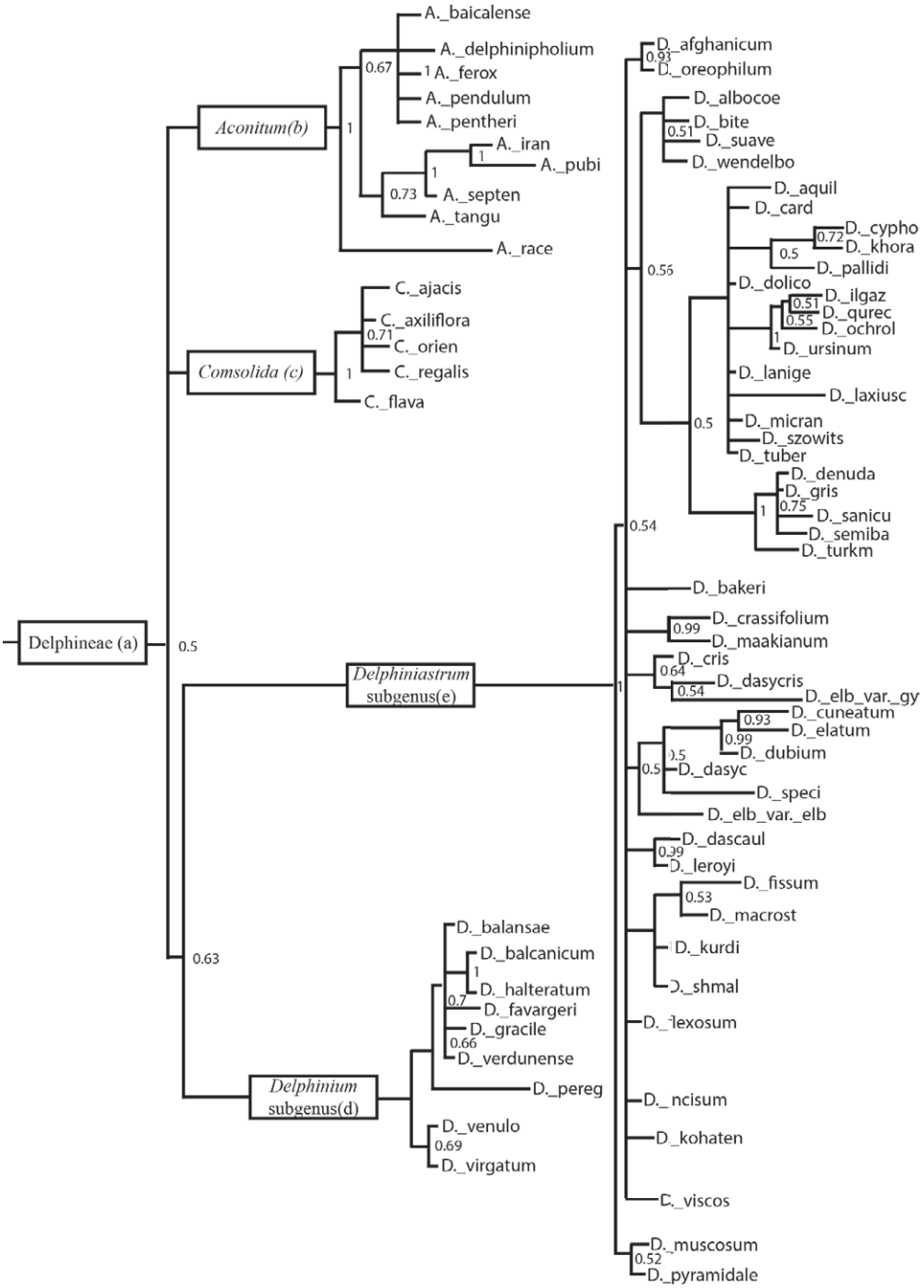

Figure 1. Bayesian tree for chloroplast DNA (*trnL-F* region). Abbreviations*: H. niger= Heleborus niger; A. iran= A. iranshahrii; A. pubi= A. pubiceps* ***Aconitum pubiceps (Rupr.) Trautv. is a synonym of Aconitum nasutum Fisch. ex Rchb****. ; A. septen= A. septentrionale; A. tangu= A. tanguaticum; A. race= A. racemolusom; C. orien= C. orientalis; D. cris= D. crispulum; D. dasyc=D. dasycarpum;D. elb var. elb= D. elbursens var. elbursense; D. speci= D. speciosum; D. elb var. gy= D. elbursense var. gymnobotrys; D. lanige= D. lanigerum; D. ilgaz= D. ilgazense; D. dolico= D. dolichostachyum; D. card= D. carduchorum; D. micran: D. micranthum; D. schmal= D. schmalhausenii; D. bite= D. biternatum; D. semiba; semibarbatum; D. ochrol= D. ochrolecum; D. szowits= D. szowitsianum; D. turkm= D. turkmenum; D.cypho= D. cyphoplectrum; D. tuber= D. tuberosum; D. laxiusc=D. laxiusculum; D. pallidi= D. pallidiflorum; D. querc= D. quercetorum; D. aquil= D. aquilegifolium; D. khora= D. khorasanicum; D.pereg= D. peregrinum; D. venulo= D. venulosum, D. virga= D. virgatum, D. albocoe=D. albocoeruleum; D. viscos= D. viscosum; D. gris= D. griseum; D. sanicu= D. saniculifolium; D. dasycaul= D. daycaulon; D. macrost= D. macrostachyum; D. kurdi= D. kurdicum; D. shmal= D. schmalhausenii; D. kohaten= D. kohatense; D. dasycris= D. dasycarpum × D. crispulum.*

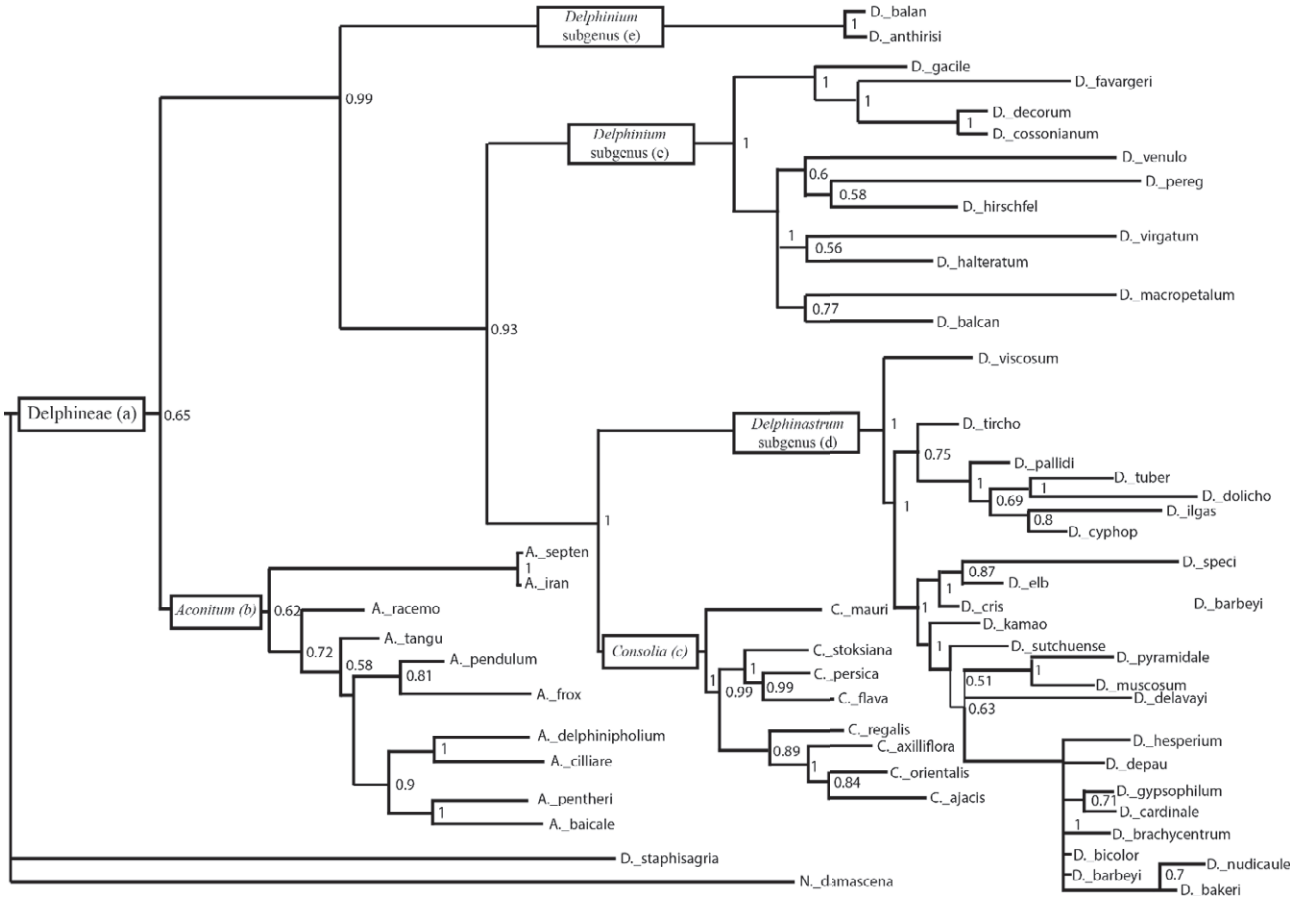

Figure 2. Bayesian tree for nuclear DNA (ITS marker). Abbreviations*: A. iran= A. iranshahrii; A. septen= A. septentrionale; D. kamao= D. kamaoense; D. cris= D. crispulum*; *D. elb= D. elbursene var. elbursense; D. speci= D. speciosum; D. tricho= D. trichoporum; D. ilgaz= D. ilgazense; D. cypho= D. cyphoplectrum; D. pallidi= D. pallidiflorum; D. dolicho= D. dolichostachyum; D. tuber= D. tuberosum; D.pereg= D. peregrinum; D. venulo= D. venulosum; D. balcan= D. balcanicum; D. hirschfel= D. hirschfeldianum; D. anthirisi= D. anthriscifolium; D. balan= D. balansae.*

D. biternatum, and *D. semibarbatum* are placed in the *Diedropetala* section (Komarov 1970) and in Fig. 1, and except for *D. schmalhausenii* the other species are placed in one group. *Delphinium schmalhausenii* is very similar to *D. kurdicum* and *D. fissum* but differs in flower color (*D. shmalhausenii* is brown-violet), and there seems to be a new position for *D. schmalhausenii* as a variety of *D. Kurdicum* instead of a being a species. Also in *Diedropetala* section, *D. cyphoplectrum*, *D. pallidiflorum*, *D. laxiusculum*, *D. quercetorum* and *D. tuberosum* (complex species) are closely related to each other (Iranshahr 1992). In flora of Iraq, *D. tuberosum* is synonymous with *D. cyphoplectrum*, *D. quercetorum*, *D. pallidiflorum* and *D. laxiusculum* (Townsend & Evan 1974). Based on the molecular study (*trnL-F marker*), the separation of these species is confirmed. *D. elbursense* is an endemic species in Iran and Rechinger has announced two varieties for this species that were distributed in Azerbaijan and Hyrcanian region (Iranshahr 1992). In our research, the separation of these two varieties based on Chloroplast marker was approved (Fig 1). Based on the molecular result, it is suggested that the taxonomic level of *D. elbursense* var. *gymnobotrys* be elevated to a higher level. Moreover, the results of micromorphological tepal epidermal patterns study confirmed that *D. elbursense* var. *elbursense* and *D. elbursense* var. *gymnobotrys* are different in the tepal epidermal patterns (Hasanbarani et al. 2016). Annual taxa in the genus *Delphinium* are arranged in *Delphinium* subgenus and from the morphology point of view they are different from perennial species (lower petals in this subgenus are without lobe, whereas they are accompanied by lobe and barbate in perennial species), and based on ITS and *trnL-F* trees they are classified as clade e and clade d, respectively. Subgenus *Delphinium* is divided into two section: sect. *Anthriscifolium* W.T Wang and sect. *Delphinium*. The

geogeraphic distribution of the two sections of subg. *Delphinium* is disjunct; *Delphinium* section is distributed in the Irano-Turanian region, whereas *Anthriscfolium* section is distributed in the warm zone of central and southern China and northern Vietnam (Xiang et al. 2017); the same results are confirmed in Fig. 2. In Iran, only *D. venulosum* and *D. peregrinum* are in the subgenus *Delphinium* and their morphological differences are in the form of lower petal; their separation is clearly evident in the molecular tree.

Our other research on Delphinieae tribe has shown that the genus *Delphinium*, *Aconitum* and *Consolida* are distinct base on morphological features (Hasanbarani et al. 2020). Pollen studies in Iranian species of the genus *Delphinium* prove that if the two species are morphologically similar, it does not mean that the two species are close pollen type (Hasanbarani et al. 2019). For example, the *D. venulosum* and *D. pregrinum*, which form a clade in molecular studies, differ in shape of the pollen. *D. cyphoplectrum* and *D. tuberosum* which are separate in molecular studies were also different in pollen studies. In the study of the flower morphology in *Delphinium*, annual taxa like morphological studies were placed in separate morphological phenogram (Hasanbarani et al. 2018). Some species that are similar in flower morphology studies were included in a separate phylogenetic study.

New record for Iran

D. dolichostachyum Chowdhuri & P. H. Davis in Notes R.B.G. Edinb. 22: 408 (1958). Locality: Iran. Kurdistan: Baneh, Kochar cemetery, 1650 m, Maroofi & Fani, 6959 TARI.

D. dolichostachyum was originally described from Turkey (Davis 1965). This species was collected from Kurdistan (Baneh) and is morphologically related to *D. carduchorum*, but differs from it mainly considering the following characters: bract length, spur length, flower color and plant length (Table 4). According to the distribution area and morphological character, it may seem that this species is *D. carduchorum* at first sight. *Delphinium dolichostachyum* image and the type specimen are presented in Fig. 3 and 4.

Table 4. Morphological characters useful in separating *Delphinium carduchorum* and *Delphinium dolicostachyum*.

Characters	*D. dolicostachyum*	*D. carduchorum*
Plant length	60 cm	100 cm
Bract length	5 mm	40 mm
Bract form	linear	Trisect
Inflorescence	Panicle	Raceme
Spur form	Cylindrical	attenuate
Spur length	9-10mm	15-16mm
Color of sepal	pale blue	dark blue
Color of petal	White	Yellow

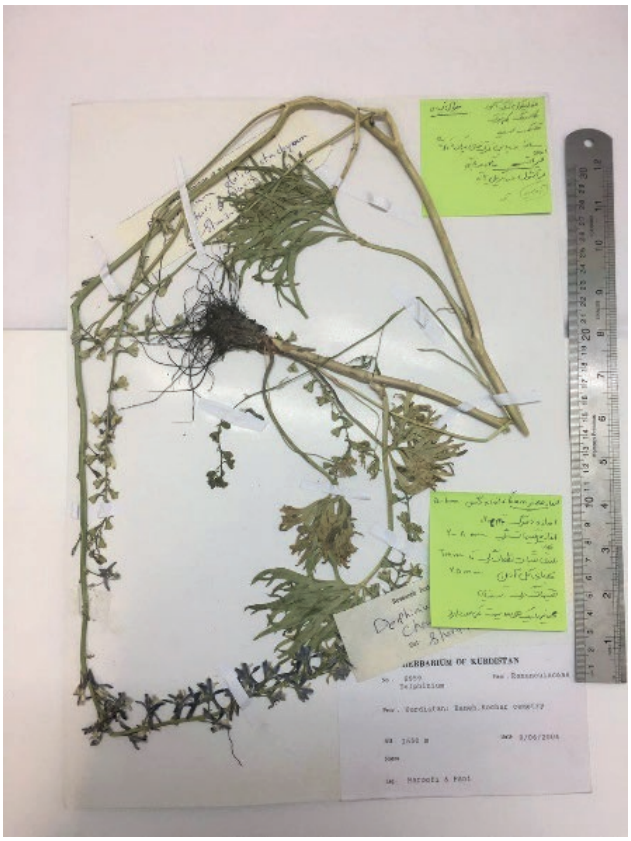

Figure 3. Image of *D. dolichostachyum* (This species is available in TARI).

CONCLUSION

The present molecular data provide strong support for the monophyly of *Delphinium*, *Aconitum* and *Consolida*, and therefore *D. elbursense* var. *gymnobotrys* could be at high taxonomic level as distinct species. *D. dolichostachyum* is newly recorded for the flora of Iran. The separation of *D. tuberosum* and *D. cyphoplectrum* (controversial species) is confirmed by molecular results.

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Figure 4. Type specimens of *D. dolichostachyum* (Image taken from Kew) https://www.gbif.org/occurrence/912539463.

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