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MOLECULAR SYSTEMATICS OF SOME BIFURCATE HAIRY SECTIONS IN *ASTRAGALUS* L. (FABACEAE) AS INFERRED FROM NUCLEAR AND CHLOROPLAST DNA SEQUENCES

REZA SHEIKHAKBARI-MEHR[1], ALI ASGHAR MAASSOUMI[2] AND SHAHROKH KAZEMPOUR OSALOO[3]

Department of Biology, Faculty of Science, University of Qom, Qom, Iran

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Abstract

In this study, 38 species belonging to some bifurcate hairy sections of *Astragalus* L. were analyzed phylogenetically, using nuclear and plastid DNA sequences. Based on our results, *Astragalus* sect. *Dissitiflori* DC. with the inclusion of the members of section *Erioceras* Bunge, formed a monophyletic group. The members of sect. *Ornithopodium* Bunge and *Onobrychoidei* DC. were located together within a highly supported monophyletic clade, apart from other sections studied, on the basis of the present molecular data.The positioning of the enigmatic, recently established species, *A. juladakensis* Maassoumi, within the sect. *Dissitiflori* was verified. In addition, our results showed that *A. pravitzii* Podl., which had been already transferred to sect. *Ornithopodium*, belongs to the section *Dissitiflori*.

Introduction

Astragalus L. (family Fabaceae, subfamily Faboideae) is among the largest genera of the flowering plants containing up to 3000 species of herbs and small shrubs (Maassoumi, 2005; Lewis *et al.,* 2005).The south-western and central Asia are considered as the main centers of biodiversity for the Old World *Astragalus* (Lock and Simpson, 1991). Infrageneric and sectional classification of *Astragalus* was first carried out by De Candolle (1825) with the description of 14 sections, a number then increased by Boissier (1843).However, the first comprehensive classification of the Old World *Astragalus* was presented by Bunge (1868), with the description of 150 sections in 10 subgenera. The current distinction of 150 and 93 sections belonging to the Old World and New World *Astragalus* respectively indicates that *Astragalus* is a complex genus within Angiosperms (Barneby, 1964; Podlech, 1986). These sections are distinguished based on some morphological characters such as stem features, stipules connation, leaf shape, inflorescence and legume features (Maassoumi, 2000). There are more than 800 species of *Astragalus* in Iran, which has a high endemism rate of 65% (Podlech, 1999; Maassoumi, 2005).

Astragalus sect. *Dissitiflori* DC is one of the largest sections among bifurcate hairy *Astragalus*, with more than 150 species in the world (Ranjbar, 2004) and about 20 species in Iran (Podlech *et al.,* 2010). Ghahreman *et al.,* (1996) transferred *A. viridis* Bunge and *A. dendroproselius* Rech. f. from *Dissitiflori* to the section *Cystodes* Bunge. Later on, these two species along with *A. aestimabilis* Podlech were moved to sect. *Corethrum* Bunge (Maassoumi, 2005). According to Maassoumi (2005), sect. *Corethrum* Bunge is closely related to sect. *Dissitiflori* but differs from that especially in having oblong elliptic pods and long spreading hairs

[1] Corresponding author. Email: r.sheikhakbari@qom.ac.ir; reza.sheikhakbari@gmail.com
[2] Botany Division, Research Institute of Forests and Rangelands, Tehran, Iran.
[3] Department of Botany, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

on fruit. Therefore, this section was recorded for Iran by transferring three aforementioned species from sect. *Dissitiflori* based on their fruit characteristics (Maassoumi, 2005).

Astragalus sect. *Erioceras* Bunge is closely related to the *Dissitiflori* and has been probably evolved by shortening of stem in the latter (Ranjbar and Karamian, 2002). The species of sect. *Erioceras* are xerophytes and more or less caespitose in contrast to many other bifurcate hairy sections.

Sect. *Cytisodes* Bunge which was originally established by Bunge (1868) with one species is now presented by 17 species (Podlech, 2010). This section was included in Flora of Iran after discovery of a new species, *A. gigantirostratus* Maassoumi *et al.*, (1999). Later on, Podlech (2004) published another new species belonging to sect. *Cytisodes* in Iran. Recently Maassoumi (2005) transferred *A. zoshkensis* Ghahremani, from section *Dissitiflori* to the *Cytisodes*. However, according to the latest revision of *Astragalus* in Flora Iranica, section *Cytisodes* has only two species in Iran (Podlech *et al.*, 2010).

The only inclusive molecular phylogenetic analyses of the Old World *Astragalus*, using nrDNA ITS and in part plastid gene *ndh*F sequences are those of Kazempour Osaloo *et al.*, (2003, 2005). Based on these studies, large sections of *Astragalus* such as *Incani* DC., *Cenanthrum* Bunge and *Ammodendron* Bunge formed monophyletic groups. In contrast, sections *Chlorostachys* Bunge, *Hystrix* Bunge, *Heterodonthus* Bunge, *Hymenostegis* Bunge, *Acidodes* Bunge, *Rhacophorus* Bunge and Iranian endemic section *Leucocercis* Bunge are not monophyletic. Moreover, monophyly of sections *Dissitiflori* DC., *Erioceras* Bunge, *Laguropsis* Bunge, *Macrocystis* Popov, *Stenonychium* Bunge, and *Onobrychoidei* DC. remained unresolved (Kazempour Osaloo *et al.*, 2005).

The aims of this study were: 1) to evaluate the phylogenetic status of sections *Dissitiflori* and *Erioceras* in Iran, on the basis of nrDNA and cpDNA sequences, and 2) to find the correct position of some problematic species i.e. *A. juladakensis* Maassoumi (2007), *A. pravitzii* Podlech (2001), and *A. zoshkensis* Ghahremani-nejad (2003)) related to these sections.

Materials and Methods

Taxon sampling

A total of 38 taxa were chosen as in-group for nrDNA ITS, and cpDNA *trn*H-*psb*A, *mat*K (as partial), and *trn*T-*trn*Y sequence analyses (Table 1). The in-group mainly belonged to sections *Dissitiflori* and *Erioceras*. In order to determine the situation of some controversial species, a number of representatives pertaining to the closely related sections such as *Ornithopodium* Bunge, *Onobrychoidei*, and *Cytisodes* were introduced in the analyses. *Astragalus stocksii* Bunge and *A. frigidus* (L.) A. Gray was chosen as outgroups following previous molecular phylogenetic studies in the Old World *Astragalus* (Kazempour Osaloo *et al.*, 2003, 2005; Sheikh Akbari-Mehr *et al.*, 2012a, 2012b). The cpDNA sequences for majority of in-group and ITS for 16 species (marked with an asterisk at Table 1) are published here for the first time.

DNA extraction, PCR and Sequencing

Total genomic DNA was extracted from dry leaves of individual plants, deposited in Central Herbarium of Iran (TARI) and Ferdowsi University of Mashhad Herbarium (FUMH), following the modified CTAB procedure of Doyle and Doyle (1987). The complete nrDNAITS+5.8S region was amplified using primers ITS4 of White *et al.*, (1990) and ITS5m of Sang *et al.*, (1997). The cpDNA *mat*K (partial), *trn*H-*psb*A and *trn*T-*trn*Y regions were amplified using primers trnK-F and matK-R (Wojciechowski *et al.*, 2004), trnH and psbA (Tate and Simpson, 2003) and trnT and

Table 1. Taxa included in the molecular analyses and their voucher specimens. Sequences obtained from GenBank marked with an asterisk.

Species	Voucher no.	GenBank accession no.			
		ITS	*trn*T/Y	*trn*H/*psb*A	*mat*K
Astragalus argyroides Beck.	Mozaffarian & Freitag, 28538(TARI)	*AB721936	LC129368	LC129321	*AB727543
A. aucheri Boiss.	Mottaghi, 1061(TARI)	*AB721937	-	LC129319	-
A. argentocalyx Ali *ex* Podl.	Ghahremaninejad & Joharchi, 34738(TARI)	LC129287	-	LC129323	LC129310
*A. eburneus*Born. & Gauba	Mozaffarian, 44936(TARI)	*AB721938	LC129353	LC129318	LC129299
A. husseinovii Rezazade	Maassoumi & Safavi, 8721(TARI)	*AB721939	-	LC129341	LC129308
A. juratzkanus Freyn & Sint.	Maassoumi & Pakravan, 72351(TARI)	*AB721940	LC129366	LC129347	LC129306
A. melanocalyx Boiss. & Buhse	Noruzi & Feizi, 5860(TARI)	*AB721941	LC129357	LC129335	LC129298
A. baraftabensis Maass.& Podl.	Tayebi, 4458(TARI)	*AB721942	LC129352	LC129317	LC129307
A. nigrolineatus Sirj. & Rech.f.	Faghihnia & Zangooee,29042(FMUH)	*AB721943	LC129367	LC129324	LC129297
A. pravitzii Podl.	Foroughi,2183(TARI)	*AB721944	LC129358	LC129332	*AB727544
A. ruscifolius Boiss.	Mozaffarian & Freitag, 28640(TARI)	*AB721945	LC129369	LC129320	*AB727545
A. sitiens Bge.	Wendelbo & Foroughi, 11270(TARI)	*AB721947	LC129362	LC129333	LC129305
A. saadatabadensis Podl.	Grant, 15784(TARI)	*AB721946	-	LC129330	LC129292
A. sumbari Popov	Wendelbo & Foroughi, 11063(TARI)	*AB721948	LC129370	LC129316	-
A. xiphidium Bge.	Youssefi, 7611(TARI)	*AB721949	-	LC129336	LC129296
A. juladakensis Maassoumi	Maassoumi, 39383 (TARI)	*AB721950	-	LC129340	LC129295
A. aestimabilis Podl.	Dehshiri, 38523(TARI)	*AB721951	-	-	-
A. dendroproselius Rech.f.	Dehshiri, 30231(TARI)	*AB721952	-	LC129322	LC129293
A. viridis Bunge.	Moussavi, 1152(TARI)	*AB721953	-	LC129345	-
A. zoshkensis F. Ghahremani	Mozaffarian, 77059(TARI)	*AB721954	LC129360	LC129331	LC129294
A. gigantirostratus Maassoumi *et al.,*	Maassoumi & al., 72339(TARI)	*AB721955	-	LC129338	-
A. anacamptus Bunge.	Emadzadeh & al., 35908(FUMH)	* AB721956	LC129365	LC129327	LC129311
A. djenarensis Sirj. & Rech.f.	Joharchi & Zangooee, 1100(TARI)	*AB721957	LC129355	LC129342	LC129303
A. stocksii Bunge.	Foroughi, 10802(TARI)	*AB051966	*AB741437	-	*AB741345
A. frigidus(L.) A. Gray	5732(TARI)	*AM943381	*AB741412	-	*AB741320

Table 1 contd.

Species	Voucher no.	GenBank accession no.			
		ITS	*trn*T/Y	*trn*H/*psb*A	*mat*K
A. bifoliolatus Sirj. & Rech.f.	Asadi & Amirabadi, 9342(TARI)	LC129283	LC129361	-	LC129309
A. alamliensis Rech.f.	Asadi, 84461(TARI)	LC129284	-	LC129334	-
A. catacamptus Bunge	Dini & bazargan, 5328(TARI)	LC129288	-	LC129329	LC129312
A. keredjensis Podl.	Asadi, 82404(TARI)	LC129291	LC129355	LC129328	-
A. neosytinii Ranjbar	Asadi, 84571(TARI)	LC129280	LC129354	LC129343	LC129301
A. nubicola Podl.	Wendelbo, 11165(TARI)	LC129289	-	LC129339	-
A. pakravaniae Podlech & Maassoumi	Asadi & Maassoumi, 55534(TARI)	LC129286	-	LC129337	-
A. pentanthus Boiss.	Maroofi, 1917(TARI)	LC129290	LC129363	LC129325	LC129302
A. sympiliecarpus Rech.f.	Asadi & Maassoumi, 83362(TARI)	LC129285	LC129351	LC129344	LC129300
A. versipilus Rech. f. & Koie	Asadi & Amirabadi, 84615(TARI)	LC129281	LC129356	LC129346	LC129313
A. brachyodontus Boiss.	Asadi & Wendelbo, 27666(TARI)	*AB727530	-	-	*AB727537
A. jodostachys Boiss. & Buhse	Abuhamzeh & Maassoumi, 45496(TARI)	*AB727532	-	-	*AB727539
A. gotkschaicus Grossh.	Asadi & Foroughi, 13756(TARI)	*AB727515	LC129372	LC129350	LC129315
A. teheranicus Boiss. & Hohen.	Babakhanlou & Amin, 15069(TARI)	*AB727523	LC129371	LC129349	LC129314
A. ahangarensis Zarre & Podl.	Abbasi & Amirabadi, 4416(TARI)	LC129282	LC129359	LC129326	LC129304

trnY (Demesure *et al.*, 1995), respectively. The total volume of amplification reaction was 25 µl, made up of 18 µl deionized water, 2.5 µl of 10× PCR buffer, 2.5 µl of 2.5 mM dNTPs, 0.5 µl of each primer (5 pmol µl-1), 0.25 µl (5 units per µl) of *Taq*DNA polymerase and0.75 µl of template DNA. The PCR profile for ITS consisted of 2.5 min at 95°C for pre-denaturation followed by 27cycles of 1 min at 95°C for denaturation, 45 sec at53.7°C for primer annealing and 50 sec at 72°C for primer extension, and a final primer extension of 7 min at 72°C.PCR procedure for amplification of three cpDNA regions was as follows: 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 51–64°C, 1.5 min at72°C, and terminal elongation of 7 min at 72°C.PCR products were directly used for sequencing reactions. Sequencing of the nrDNA ITS and cpDNA fragments were performed using an ABI 3130Genetic DNA Analyzer (Applied Biosystems, USA).

Sequence alignment

Sequences of nuclear and plastid DNA were edited by BioEdit package version7 (Hall 1999). The sequence alignment was carried out using ClustalX (Larkin *et al.*, 2007) and adjusted manually. Indel positions were treated as missing data.

Phylogenetic analyses

Maximum parsimony

Sequenced nuclear and plastid fragments were analyzed separately and in combination, using maximum parsimony method (MP) as implemented in the PAUP* version 4.0b10 (Swofford, 2002). Multiple tree searches were conducted using heuristic search options that included random addition sequences (100 replicates), holding five trees per replicate, and tree bisection-reconnection (TBR) branch swapping with retention of multiple parsimonious trees (Maxtrees = 25000). Bootstrap (BP) support values (Felsenstein, 1985) were calculated using a full heuristic search with 1000 replicates, each with a simple addition sequence and TBR branch swapping. Uninformative characters were excluded from analyses. Parsimony trees were not shown here.

Bayesian analyses

All datasets separately and in combination, were analyzed using Bayesian inference (BI) as implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). The incongruent length difference (ILD) test was performed to evaluate the combinability of the all DNA regions studied (Farris *et al.,* 1995). Appropriate evolutionary models for analyzing sequences were selected using the MrModeltest2 (Nylander, 2004) based on the Akaike information criterion (AIC) (Posada and Buckley 2004). K80+I+G, GTR+I+G, GTR+I, and F81+G were chosen as the models that best fit the datasets of nrDNA ITS, *trn*H-*psb*A, *mat*K and *trn*T-*trn*Y respectively. In combined dataset, various sequences were included as separate partitions. BI analyses were run for two million generations, using Markov chain Monte Carlo search. MrBayes performed two simultaneous analyses starting from different random trees (N runs=2) each with four Markov chains and trees sampled at every 100 generations. In all analyses average standard deviation of split frequencies had dropped significantly below 0.01 after completion of the generations. Once reaching the stationary phase, trees were collected and after burning in one fourth of them, used to build a 50% majority rule consensus tree accompanied with posterior probability (PP) values. Trees were showed using TreeGraph2 (Stöver and Müller, 2010).

Results and Discussion

nrDNA ITS dataset analyses

The average length of aligned nrDNA ITS fragment was 596. Three nucleotide sites, of which 60 sites were parsimony informative. The Bayesian tree with posterior probabilities (PP) and bootstrap values is similar to that of MP analysis (Fig. 1). Based on these analyses, four species belonging to the sections *Ornithopodium* and *Onobrychoidei* were located at the base of tree as a sister group to a large assemblage of five subclades. *Astragalus juladakensis* was placed at the base of this group. Members of sections *Dissitiflori* and *Erioceras* plus *Cytisodes* were well intermixed and formed several subclades within a large monophyletic group (Fig. 1). Although relationships among these subclades were not resolved, each one is supported with moderately to highly bootstrap or PP values.

cpDNA and combined datasets analyses

Parsimony trees obtained from three single cpDNA and the combined cpDNA plus ITS datasets, were topologically identical to those of Bayesian analyses. The length and composition of DNA sequences as well as the tree statistics from the single and combined analyses have been summarized in Table 2. In *trn*H-*psb*A tree, *A. tehranicus* Boiss. & Hohen. and *A. goktschaicus* Grossh. belonging to the sect. *Onobrychoidei* were united in a highly supported subclade (PP= 1) and placed at base of the tree as a sister to the remaining species (Fig. 2). Again, the members of

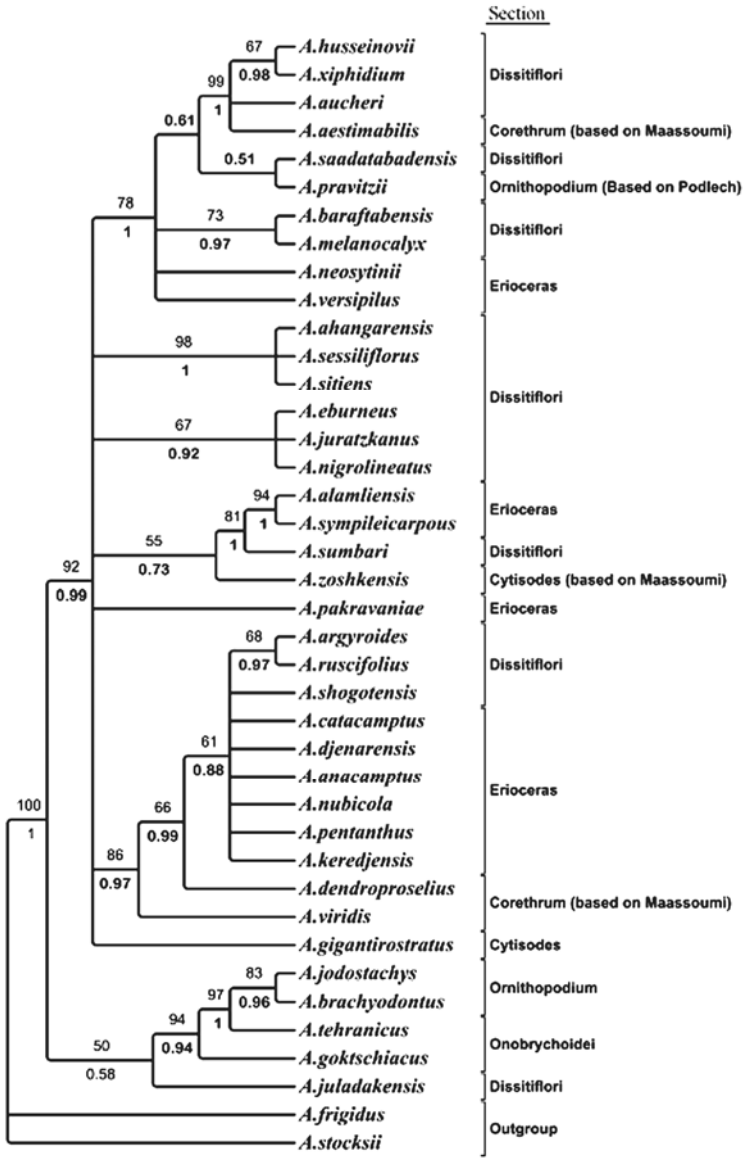

Fig.1. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the nrDNA ITS dataset. Numbers above and below branches are bootstrap values and posterior probabilities, respectively.

sections *Dissitiflori* and *Erioceras* plus some controversial species (i.e. *A. zoshkensis*, *A. aestimabilis* Podl., *A. dendroproselius* Rech. f. and *A. viridis* Bunge) were intermixed within a large polytomic assemblage (Fig. 2). In the *mat*K tree, species sampled from two sections *Onobrychoidei* and *Ornithopodium* revealed a highly supported group (BS= 80%, PP= 0.95) and placed as a sister to the members of other sections. The remaining species, in this tree as well as two other cpDNA trees, placed together within a polytomic large clade (Fig. 3). *trn*T-*trn*Y region was not amplified in some of in-groups due to difficulties with the PCR. However, the topology of the tree obtained from this sequence was similar to the other trees in general (tree not shown here).

Table 2. Dataset and tree statistics from separate and combined analyses of the nuclear and three chloroplast regions.

Data sets	ITS	*trn*T/*trn*Y	*trn*H/*psb*A	*mat*K	combined
Nucleotide sites (average)	596.3	629	397.7	931	2554
Variable sites	120	76	82	61	337
Informative characters	60	58	44	18	178
Number of MPTs	10	39	6494	68	398
Length of MPTs	86	74	80	29	335
CI of MPTs	0.756	0.824	0.637	0.828	0.670
RI of MPTs	0.882	0.911	0.839	0.891	0.719

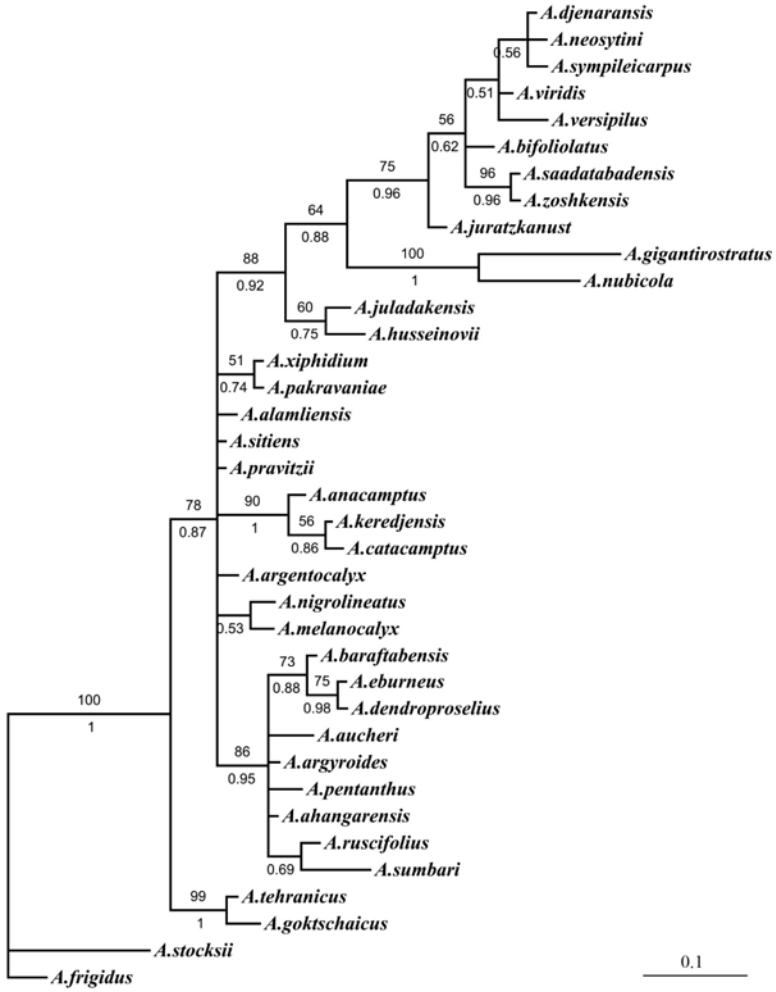

Fig. 2. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the *trn*H/*psb*A dataset. Numbers above and below branches are bootstrap values and posterior probabilities, respectively.

ILD test suggested that the four datasets were slightly incongruent (P=0.01). Following the suggestions of several authors that the ILD test may be unreliable (Seelanan *et al.*, 1997; Wiens, 1998; Yoder *et al.*, 2001), we decided to combine these datasets. The DNA fragments which had not been sequenced for some species in this study were treated as missing data in the combined dataset. The topology of the resulted tree (Fig. 4) was roughly the same as those of single dataset trees, with the exception that resolution, bootstrap and PP values were higher. The combined tree

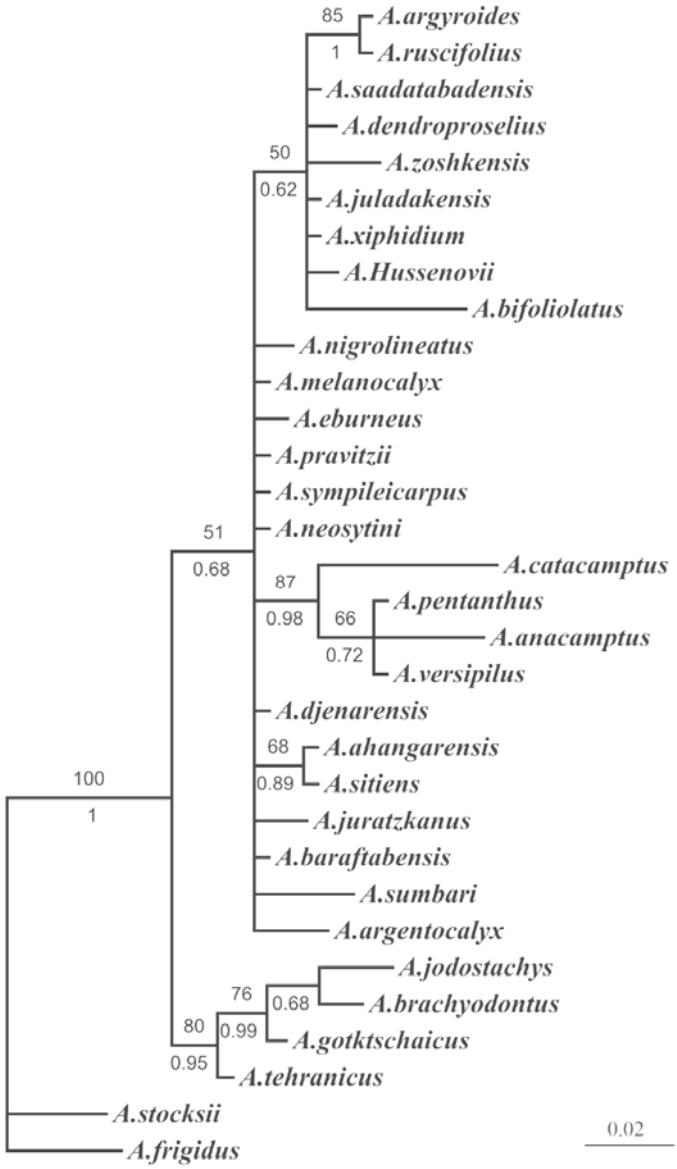

Fig. 3. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the cpDNA *mat*K dataset. Numbers above and below branches are bootstrap values and posterior probabilities, respectively.

was composed of two obvious clades among in-groups studied. At base of the tree, four species belonging to the sections *Onobrychoidei* and *Ornithopodium* were separated from other in groups and formed a highly supported clade as a sister group to the remaining species (Fig. 4). The next main clade was composed of two clades, each of successive subclades including the members of sections *Dissitiflori* and *Erioceras* and their closely related taxa. The relationships of these subclades were well resolved (Fig. 4).

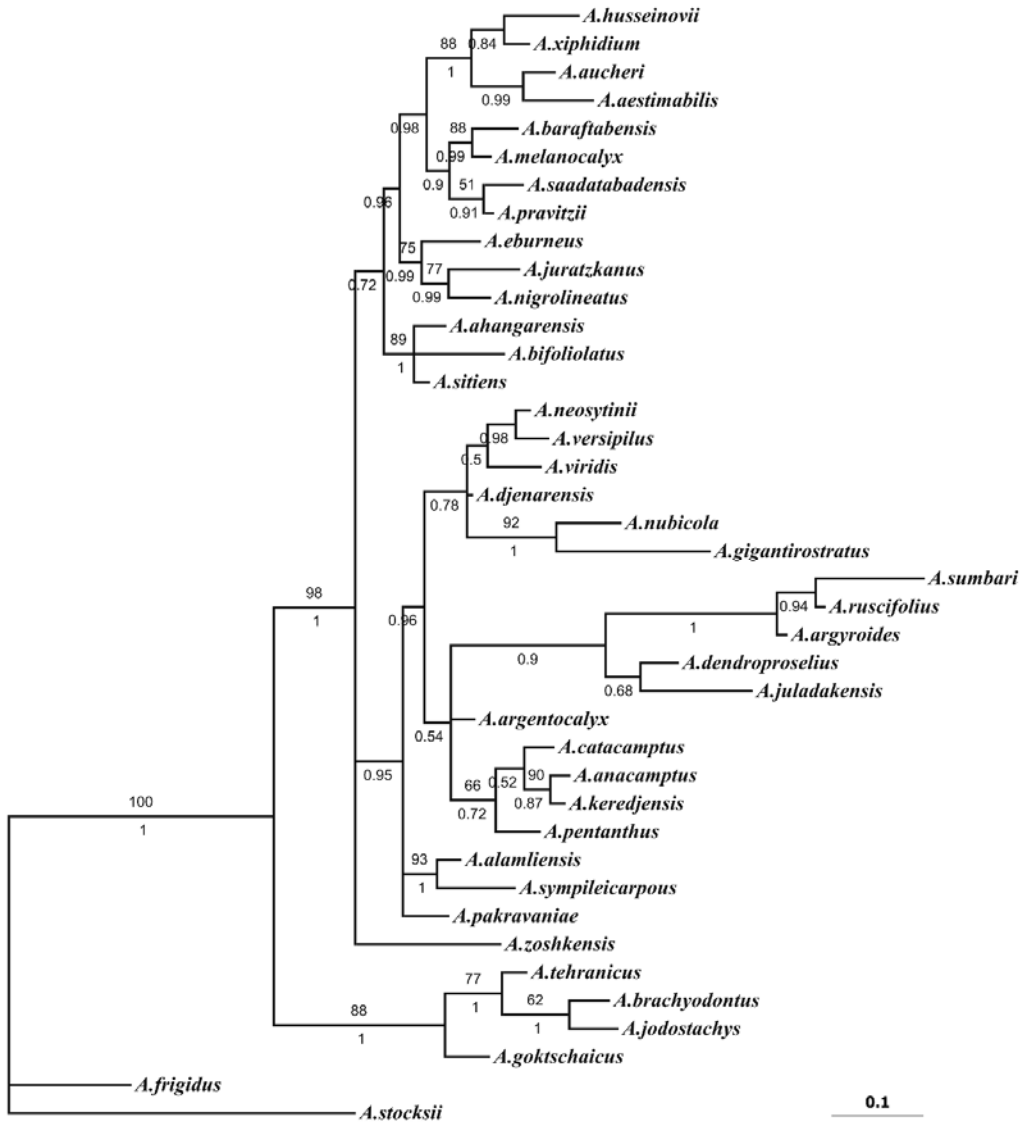

Fig. 4. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the nrDNA and cpDNA combined dataset. Numbers above and below branches are bootstrap values and posterior probabilities, respectively.

Among different datasets analyzed here, relationships of species were well resolved on the ITS and combined trees. *Astragalus* sect. *Dissitiflori* is one of the largest sections of the genus including more than 40 species in the Iranian Plateau (Podlech *et al.,* 2010). Among bifurcate hairy *Astragalus*, the members of *Dissitiflori* are distinguished by some features including stem with long internodes, linear pod and asymmetrical and gibbous calyx at the base (Ghahremaninejad, 2004; Sheikh Akbari *et al.,* 2012a). It seems that this section belongs to a group of medifixed hairy *Astragalus* including *A.* sect. *Cystodes*, *A.* sect. *Erioceras*, *A.* sect. *Cystium* Bunge, *A.* sect. *Cremoceras* Bunge and *A.* sect. *Trachycercis* Bunge (Ranjbar 2004). This idea is also supported partially with molecular evidences (Kazempour Osaloo *et al.,* 2005; Sheikh Akbari *et al.,* 2012b). Molecular phylogenetic analyses of the present study showed that the members of sections *Erioceras* and *Cytisodes* in Iran, were intermixed with those of section *Dissitiflori* and located within a large assemblage (Fig. 4).

A. juladakensis, which was recently introduced as a new species belonging to the section *Dissitiflori* (Maassoumi, 2007), revealed some affinity to the members of *Onobrychoidei* based on ITS sequences and nested at the base of ITS tree, as a sister to the remaining species (Fig. 1). Based on our previous phylogenetic study on the sect. *Dissitiflori* (based on ITS), this species revealed a separated position among other members of the section and its affinity to the sect. *Dissitiflori* remained questionable (Sheikh Akbari Mehr *et al.,* 2012b). Despite these results, *A. juladakensis*, was placed beside the other members of sect. *Dissitiflori* on the basis of our cpDNA and combined datasets analyses (Fig. 4). On the other hand, this species along with *A. husseiovii* Rezazade was united within a moderately supported subclade within sect. *Dissitiflori*, based upon morphological features (Sheikh Akbari *et al.,* 2012a); hence, the positioning of this species within the section *Dissitiflori* is verified.

A. pravitzii Podl. and *A. saadatabadensis* Podl. formed a sister subclade within section *Dissitiflori*, on the basis of ITS and combined trees. After introducing *A. pravitzii* as a new species from sect. *Dissitiflori* (Podlech, 2001), Podlech and Sytin (2010) moved it to the sect. *Ornithopodium*. In accordance with previous morphological data analysis (Sheikh Akbari Mehr *et al.,* 2012a), our present molecular data revealed that this taxon is a member of sect. *Dissitiflori* (Figs 1, 4).

According to Gontscharov *et al.* (1946) and Maassoumi (2005), A. sect. *Corethrum* is closely related to the sect. *Dissitiflori* but differs with that in having asymmetrical long hairs on calyx and pod shape. Three species (*A. aestimabilis*, *A. dendroproselius* and *A. viridis*) belonging to the sect. *Dissitiflori* were separated from the section and introduced as the members of newly recorded section *Corethrum* for Iran, based on having ovate-elliptic pods and asymmetrical standing indumentum on calyx (Maassoumi, 2005). However, in accordance with Podlech and Zarre (2013), our present molecular dataset analyses revealed that these taxa belong to the sect. *Dissitiflori*. sect. *Erioceras* is characterized by a short stem, prostrate habit, asymmetrical long hairs, oblong elliptic pods and rupturing of calyx (Maassoumi, 2005). It seems that sect. *Erioceras* has been evolved by reducing of stem length in sect. *Dissitiflori* (Ranjbar and Karamian, 2002). However, our results obtained from single and combined molecular datasets revealed no distinction between two sections. The members of sect. *Erioceras* have adapted to arid and windy sub-mountainous regions. They are distributed in arid central and north-eastern of Iran. The evolution of prostrate habit and dense and long hairs within section *Erioceras* is likely an adaptive behaviour due to its environmental conditions.

Section *Cytisodes* is a small section among bifurcate hairy *Astragalus* and is distinguished by their short stem internodes, calyx with standing hairs and long beak on the pod (Bunge, 1868). Maassoumi *et al.* (1999) introduced a new species from eastern part of Elburz Mountains, showing the features of sect. *Cytisodes*, and named *A. gigantirostratus*. Occurrence of this species in the

Hyrcanian province astonished the authors, because known species of the section are all confined to the Turkestanian floristic province of the Irano-Turanian region. Later on, Podlech (1999) introduced *A. neyshaburensis* Podl. as a new species from sect. *Cytisodes* in Iran. Maassoumi (2005) moved *A. zoshkensis* from section *Dissitiflori* to the *Cytisodes* based on calyx hairs and pod features. However, in agreement with a recent morphological study (Sheikh Akbari Mehr *et al.*, 2012a), our present molecular results revealed that these species are placed within section *Dissitiflori* and it is recommended that section *Cytisodes* is best to be retreated after complementary studies.

In summary, different genomic sequences revealed that the sect. *Dissitiflori* with the inclusion of the members of section *Erioceras* as well as members of *Cytisodes* in Iran, formed a monophyletic group. The present results indicated that taxa which had been transferred from sect. *Dissitiflori* have to be returned to the section, and from this point of view, sect. *Corethrum* has no representative in Iran and this result is in accordance with Podlech *et al.*, (2010) and Podlech and Zarre (2013) classifications. Our findings showed that delimitation of sect. *Dissitiflori* needs to be revised. Indeed, beside the increase of samples, the analysis of type specimen of aforementioned sections seems to be necessary to assess exact taxonomic situation of taxa discussed above.

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References

Barneby, R. 1964. Atlas of North American *Astragalus*. Memoirs of the New York Botanical Garden **13**: 1-1188.

Boissier, E. 1843. Diagnoses Plantarum Orientalium Novarum, Ser. I, Part. 2. Typographia Ferd Ramboz, Genevae, pp. 1-115.

Bunge, A. 1868. Generis Astragali Species Gerontogeae. Académie Impériale des Sciences, St. Pétersburg, pp. 1-254.

De Candolle, A.P. 1825. Prodromus Systematis Naturalis, Regni Vegetabilis, Vol **2.** *Astragaleae*. Argentorati et Londii, Parisiis, pp. 1-644.

Demesure, B., Sodzi, N. and Petit, R.J. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology **4**: 129-131.

Doyle, J.J. and Doyle ,J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin **19**: 11-15.

Ekici, M., Akan, H. and Aytac, Z. 2011.Taxonomic revision of *Astragalus* section Onobrychoidei DC. (Fabaceae) in Turkey. Turkish Journal of Botany **35**: 1-73.

Farris, J.S., Kallersjo, M., Kluge, A.G. and Bult, C. 1995. Testing significance of incongruence. Cladistics **10**: 315-319.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783-791.

Ghahreman, A., Pakravan, M. and Maassoumi, A. A. 1996. Note on the genus *Astragalus* (sect. *Xiphidium*) in Iran. Iranian Journal Botany **7**(1): 45-50.

Ghahremani-nejad, F. 2003. *Astragalus zoshkensis* (Fabaceae), a new species from Iran. Annales Botanici Fennici **40**: 117-121.

Ghahremani-nejad, F. 2004. The sections of *Astragalus* L. with bifurcating hairs in Iran. Turkish Journal of Botany **28**: 101-117.

Gontscharov, N.F., Borissova, A.G., Gorschkova, S.G., Popov, M.G. andVassilczenko, I.T. 1946. *Astragalus* L. *In*: Komarov, V.L. and Shishkin,B.K. (Eds), Flora USSR. Vol. **12**. Editio Academiae Scientiarum, Leningrad, Russia, pp. 1-681.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**: 95-98.

Kazempour Osaloo, S., Maassoumi, A.A. and Murakami, N. 2003. Molecular systematics of the genus *Astragalus* L. (Fabaceae): phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacers and chloroplast gene *ndh*F sequences. Plant Systematics and Evolution **242**: 1-32.

Kazempour Osaloo, S., Maassoumi, A.A. and Murakami, N. 2005. Molecular systematics of the Old World *Astragalus* (Fabaceae) as inferred from nrDNA ITS sequence data. Brittonia **57**: 367-381.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace ,I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G.2007. Clustal Wand Clustal X version 2.0. Bioinformatics **23**:2947-2948.

Lewis, G.P., Schrire, B.D., Mackinder, B.A. and Lock, M. 2005. Legumes of the world. Kew Publishing, London, UK, pp. 577.

Lock, J.M. and Simpson, K. 1991. Legumes of West Asia: A Check-List. Kew Publishing.

Maassoumi, A. A., Ghahreman, A., Ghahremani-nejad, F. and Matin, F. 1999.*Astragalus gigantirostratus* (Fabaceae), a remarkable new species from N Iran and supplementary notes on *A.* sect. *Cytisodes* Bunge. Willdenowia **29**: 221-225.

Maassoumi, A.A. 2000. The genus *Astragalus* in Iran, Perennials. Research Institute of Forests and Rangeland Publications, Tehran, Iran, pp. 441.

Maassoumi, A.A. 2005. The Genus *Astragalus* in Iran. Research Institute of Forests and Rangeland Publications, Tehran, Iran, pp. 786.

Maassoumi, A.A.2007. Two new species of the genus *Astragalus* L. (Fabaceae) from Iran. Iranian Journal of Botany **13**(2): 78-81.

Nylander, J.A.A. 2004.MrModeltest, ver. 2. Program distributed by the author. Uppsala University Press, Uppsala, Sweden.

Podlech, D. 1986. Taxonomic and phytogeographical problems in *Astragalus* of the Old World and south-west Asia. Proceedings of the Royal Society of Edinburgh **89**: 37-43.

Podlech, D. 1999. New *Astragali* and *Oxytropis* from North Africa and Asia, including some new combinations and remarks on some species. Sendtnera **6**: 135-174.

Podlech, D. 2001. Contributions to the knowledge of the genus *Astragalus* L. (Leguminosae). Sendtnera **7**: 163-201.

Podlech, D. 2004. New species of *Astragalus* L. (Leguminosae), mainly from Iran. Annalen des Naturhistorischen Museums in Wien **105**: 565-596.

Podlech, D. and Sytin, A. 2010. Papilionaceae VI: *Astragalus* section *Ornithopodium*. *In*: Rechinger, K.H. (Ed.), Flora Iranica. Vol. **178**. Akademische Druck-u Verlagsanstalt, Wien, Austria, pp. 173-184.

Podlech, D., Zarre, SH., Maassoumi, A.A., Ekici, M. and Sytin, A. 2010. Papilionaceae VI: Astragaleae, *Astragalus* L. IV. *In*: Rechinger, K.H. (Ed.). Flora Iranica. Vol. **178**. Akademische druck-u verlagsanstalt, Wien, Austria, pp. 1-430.

Podlech, D. and Zarre, SH. 2013. A taxonomic revision of the genus *Astragalus* L. (Leguminosae) in the Old World. Naturhistorisches Museum, Wien, Austria, pp. 2439.

Posada, D. and Buckley, T. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio rates. Systematic Biology **53**: 793-808.

Ranjbar, M. 2004. *Astragalus* sect. *Dissitiflori* (Fabaceae) in Iran. Nordic Journal of Botany **24** (5): 523-531.

Ranjbar, M. and Karamian, R.2002. Taxonomic study of *Astragalus* sect. *Erioceras* (Fabaceae) in Iran, additional notes and key to the species. Nordic Journal of Botany **22**(6): 713-717.

Ronquist, F. and Huelsenbeck, J.P. 2003. MrBayes 3, Bayesian phylogenetic inference under mixed models. Bioinformatics **19**: 1572-1574.

Sanderson, M.J. and Liston, A. 1995. Molecularphylogenetic systematics of Galegeae, with special reference to *Astragalus*. *In*: Crisp, M. and Doyle, J.J.(Eds.). Advances in legume systematics. Vol. **7**. Kew Publishing, London, UK, pp. 331-350.

Sanderson, M.J. and Wojciechowski, M.F. 1996. Diversification rates in a temperate legume clade: are there ''so many species'' of *Astragalus* (Fabaceae)? American Journal of Botany **83**: 1488-1502.

Sang, T., Crawford, D.J. and Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). American Journal of Botany **84**: 1120-1136.

Seelanan, T., Schnabel, A. and Wendel, J.F. 1997. Congruence and consensus in the cotton tribe (Malvaceae). Systematic Botany **22**: 259-290.

Sheikh Akbari Mehr, R., Maassoumi, A. A., Saidi, A., Kazempour Osaloo, S. and Ghorbani Nohooji, M. 2012a.Morphological cladistic analysis of some bifurcate hairy sections of *Astragalus* (Fabaceae) in Iran. Turkish Journal of Botany **36**: 434-442.

Sheikh Akbari Mehr, R., Saidi, A., Kazempour Osaloo, S. and Maassoumi, A. A. 2012b. Phylogeny of *Astragalus* section *Dissitiflori* based on nrDNAITS and morphological data in Iran. Iranian Journal of Botany **18**: 1-9.

Stöver, B.C. and Müller, K.F. 2010. TreeGraph 2 Combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics **11**: 7-15.

Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods). Version. 4.0b10. Sinauer Associates Inc., Sunderland, Massachusetts, USA.

Tate, J.A. and Simpson, B.B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species.Systematic Botany **28**: 723-737.

Vural, C., Ekici, M., Akan, H. and Aytac, Z. 2008.Seed morphology and its systematic implications for genus *Astragalus* L. sections *Onobrychoidei* DC., *Uliginosi* Gray and *Ornithopodium* Bunge (Fabaceae). Plant Systematics and Evolution **274**: 255-263.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M., Gelfand, D., Sninsky, J. and White, T. (Eds). PCR protocols: A guide to methods and applications. Academic Press, San Diego, California, USA, pp. 315-322.

Wiens, J.J. 1998.Combining data sets with different phylogenetic histories. Systematic Biology **47**: 568-581.

Wojciechowski, M.F., Lavin, M. and Sanderson, M.J. 2004. A phylogeny of Legums (Leguminosae) based on analysis of the plastid *mat*K gene resolves many well-supported subclades within the family. American Journal of Botany **91**: 1846-1862.

Yoder, A.D., Irwin, J.A. and Payseur, B.A. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Systematic Biology **50**(3): 408-424.

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