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مطالعه موقعیت تاکسونومیک گونه *Medicago siniskiae*: شواهدی از اطلاعات ریخت‌شناسی و مولکولی

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چکیده

براساس برخی منابع، درباره موقعیت تاکسونومیک گونه *Medicago siniskiae* تردید وجود دارد. هدف از این پژوهش، مشخص کردن موقعیت این گونه در کمپلکس گونه‌ای *M. rigidula* شامل *M. siniskiae*، *M. constricta* و *M. rigidula* s.l. (از جمله *M. rigiduloides*) بود. آنالیز تجزیه به عوامل و خوشه‌بندی براساس ۲۹ صفت ریخت‌شناسی انجام شد؛ همچنین از توالی ناحیه ITS، برای بررسی ارتباط بین گونه‌ها استفاده شد. نتایج مطالعات ریخت‌شناسی و مولکولی نشان داد *M. siniskiae* یک گونه مجزا است و گونه‌های *M. rigidula* و *M. rigiduloides* نزدیک‌ترین گونه‌ها به آن هستند؛ همچنین طبق نتایج، نیام این گونه دارای تنوع‌های بدون خار و خاردار است. با توجه به الگوی پراکندگی و حضور آن در نمونه‌های جمع‌آوری شده، گونه *M. siniskiae*، گونه‌ای با گسترش سریع در غرب و جنوب غرب ایران در نظر گرفته می‌شود.

واژه‌های کلیدی: آنالیز خوشه‌بندی، آنالیز تجزیه به مؤلفه‌ها، گیاهان مهاجم، یونجه‌های یک‌ساله، ایران.



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The Taxonomic Status of *Medicago Sinskieae*: Insights from Morphological and Molecular Data

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Abstract

Medicago sinskieae is a poorly understood but fast-expanding species in western and southwestern Iran. The present study was performed to investigate its relationships with other taxa in the *M. rigidula* complex including *M. constricta*, and *M. rigidula* s.l. (including *M. rigiduloides*). The Principal Component Analysis (PCA) and cluster analysis were conducted on 29 morphological characters. The group was also examined based on the Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA. The morphological and genetic analyses indicated *M. sinskieae* (including both spiny and spineless variants) was a separate species, most closely related to *M. rigidula* and *M. rigiduloides*. The results also showed that the pod of this species had thornless and thorny varieties. Due to the distribution pattern and its presence in the collected samples, *M. sinskieae* is considered as a species with rapid expansion in the west and southwest of Iran.

Keywords: Cluster Analysis, Principal Component Analysis, Invasive Species, Medics, Iran.

Introduction

The genus *Medicago* L. (Fabaceae) has ca. 85 annual and perennial species worldwide, centered mainly in the Mediterranean basin (Small, 2011). The genus has been divided into 14 sections with two transferred from the genus *Trigonella* section *Buceras* (Ser.) E. Small, and *Lunatae* (Boiss.) E. Small) and one (section *Geocarpa* E. Small) from the genus *Factorovskya*. Species of the annual section *Spirocarpos* Ser. are generally known as medics. The diagnostic characters of the medics are primarily fruit (pod) morphological features (Small, 2011). *Medicago rigidula* (L.) All. and its closely related species *M. rigiduloides* E. Small, *M. sinskiae* Uljanova, and *M. constricta* Durieu, have been termed the *M. rigidula* complex by some authors (Mehregan *et al.*, 2002). All members of this complex belongs to *M. sect. Spinocarpos* subsect. *Pachyspirae* (Urb.) Heyn, a group which is taxonomically difficult. Indeed, *M. sinskiae* may currently be the most poorly understood annual species of the entire genus. *Medicago sinskiae* is particularly closely related to *M. rigidula* s.l. (including *M. rigiduloides* Small) (Downie *et al.*, 1998; Bena *et al.*, 1998a and 1998b; Mehregan *et al.* 2002). *Medicago sinskiae* has small trifoliolate leaves, yellow flowers and coiled and usually spiny pods. Its flowering starts in early April and the fruits mature in May and June (Small, 2011).

The annual *Medicago* species show a great variation in pod morphology, especially in size and shape of spines on the pods (Bena *et al.*, 1998a, 1998b; Small, 2011). *Medicago sinskiae* was first recognized by Uljanova (1964) from the western Kopet-Dagh in Turkmenistan based on some individuals with spineless fruits. The type specimens of *M. sinskiae* includes individuals with only spineless pods (Uljanova, 1964; Small and Brooks, 1991). The species was long neglected until Small and Brooks (1991) deduced that *M. sinskiae* deserved identification as a separate species. Downie *et al* (1998) showed that at least the type gathering of *M. sinskiae* is distant from *M. rigidula* and *M. rigiduloides*. Mehregan *et al* (2002 and 2003) reported several samples of *M. sinskiae* from western Iran with both spineless pods and spines up to 3.5 mm long usually hooked at the apex. Based on the variation observed in the material collected from Iran, Mehregan *et al* (2002) suggested that *M. sinskiae*, *M. constricta*, *M. rigidulla*, and *M. rigiduloides* should represent a single species, with *M. sinskiae* reduced as *M. rigidula* subsp. *sinskiae* (Uljanova) Mehregan & Rahiminejad.

Unlike other annual species of *Medicago*, *M. sinskiae* has been poorly understood with no germplasm sources available (Small, 2011). Its unusual geographical distribution, lack of morphological and molecular documentation, and confusion regarding its taxonomy encouraged us to perform a full scale study. We used morphological traits and internal transcribed sequence (ITS) data to clarify the phylogenetic and taxonomic status of *M. sinskiae*.

Material and Methods

Plant material

Samples of *M. sinskiae*, *M. constricta*, and *M. rigidula* were collected from 10 different sites in western and southwestern Iran in July 2017 (Table 1; Fig 1). We sampled the pods of eight populations of *M. sinskiae*, a single population of *M. rigidula*, and a single population of *M.*

constricta. All collected samples were identified and labeled based on authoritative identification keys (Heyn, 1984; Small and Jomphe, 1989; Mehregan *et al.*, 2002; Small, 2011). Six to eight seeds of each individual were planted in separate pots. The plants were cultivated on a research farm 48 km SE of Gachsaran, SW Iran, under common conditions similar to the plants' natural habitats. Vouchers are preserved in the central herbarium of the Islamic Azad University, Science and research branch (IAUH). We examined characters in two successive generations: 1) original samples from the field, and 2) samples grown from the cultivated seeds.

Table 1. List of populations (Pop) of *Medicago* studied in this paper.

Species	Pop	Locality	Elevation, Coordinates	Herbarium number	Genbank Accession no.
<i>M. sinskieae</i>	ABD	Iran. Ilam: Abdanan, Kabir kuh	1000 m; 49° 25.531' E; 33° 0.263' N	IAUH-14972	MW440557
<i>M. sinskieae</i>	BSN	Iran. Kohgiluyeh and Boyer-Ahmad: 50 km from Gachsaran toward Shiraz	900 m; 51° 13.92' E; 30° 19.80' N	IAUH-15012	MW440558
<i>M. sinskieae</i>	FTH	Iran. Kohgiluyeh and Boyer-Ahmad: Gachsaran, 30 km from Basht towards Choram, the road to the village Fath	1100 m; 51° 51.54' E; 30° 35.04' N	IAUH-15013	MW440559
<i>M. sinskieae</i>	SPD	Iran. Lurestan: Sepid-Dasht, 5 km from Sepid-Dasht to Khorram-Abad	1300 m; 48° 51.778' E; 33° 13.175' N	IAUH-14965	MW440560
<i>M. sinskieae</i>	KHR	Iran. Lurestan: Khorram-Abad, 35 km from Khorram-Abad to Pol-Dokhtar	940 m; 47° 57.328' E; 33° 57.121' N	IAUH-14958	MW440561
<i>M. sinskieae</i>	KHW	Iran. Lurestan: Khorram-Abad, 5 km from Khorram-Abad to Kohdasht	1220 m; 48° 15.164' E; 33° 28.917' N	IAUH-15016	MW440562
<i>M. sinskieae</i>	PLS	Iran. Lurestan: Pol-Dokhtar, 5 km from Pol-Dokhtar to Andimeshk	800 m; 47° 42.448' E; 33° 6.480' N	IAUH-15003	MW440563
<i>M. sinskieae</i>	SPC	Iran. Lurestan: Sepid-Dasht, 15 km from Sepid-Dasht to Khorram-Abad	1280 m; 48° 50.649' E; 33° 13.292' N	IAUH-14971	MW440564
<i>M. constricta</i>	BSC	Iran. Kohgiluyeh and Boyer-Ahmad: 50 km from Gachsaran toward Shiraz	900 m; 51° 13.92' E; 30° 19.80' N	IAUH-15012-C	MW440556
<i>M. rigidula</i>	SPN	Iran. Lurestan: Sepid-Dasht, 30 km from Sepid-Dasht to Khorram-Abad	1940 m, 48 ° 44.719' E; 33 ° 16.024' N	IAUH-14962	-

Morphological traits

Morphological measurements were made on 30 individuals of each of eight populations of *M. sinskieae*, one population of *M. constricta*, and one population of *M. rigidula*. Nineteen morphological fruit characters were examined in the original collections. Additional vegetative characters of leaves, stipule, petiole, inflorescence, flower, and calyx were also studied on the plants grown from the seeds (Table 2). Although the data are not reported here, additional herbarium specimens were also examined. Statistical analyses were carried out using the Statistical Package

for the Social Sciences (SPSS 16) software (IBM Corp., Armonk, NY). The same software was also used to calculate the correlation between the common quantitative traits of the original samples and and also of the second (planted) generation. Principal component analysis (PCA) with varimax rotation was used to determine the most differentiating characteristics of the pods (Ebrahimi *et al*, 2017). Cluster analysis using Ward's method was performed to examine pod variation between populations. Data analysis was performed using SPSS 16 (IBM Corp., Armonk, NY).

Table 2. List of morphological traits investigated in this study and their abbreviations.

	Traits	Abbreviations
1	Length of fruit	FLT
2	Fruit diameter	FDM
3	Number of coils	NMC
4	Number of spines on middle coil	SMC
5	Length of longest spines	LSL
6	Adpression of fruits (width of gaps between coils)	FAP
7	Angel of spine insertion	ASP
8	Length of seeds	SLT
9	Width of seeds	SWT
10	Number of seeds in middle coils	SDN
11	Number of seeds	STN
12	Thickness of spine base	SBT
13	Thickness of middle coil	MCT
14	Pubescence simple hairs	PSH
15	Glandular hairs	PGH
16	Shape of end of fruit	FESH
17	Dorsal to middle vein protrusion	DSP
18	Percentage of lateral grooves	PGD
19	Length of petiole	LPT
20	Length of middle internode	LMI
21	Length of middle petiole	LMP
22	Length of leaflet	LLL
23	Width of leaflet	WLE
24	Leaflet shape	LSH
25	Length of inflorescence	LIN
26	Number of marginal serrations on leaflet	NSL
27	Number of floret	NFI
28	Total length of calyx	TLC
29	Density of hairs of leaf	DHL

DNA extraction PCR and ITS sequencing

One sample from each population of *M. siniskiae* and one from *M. constricta* was taken for DNA extraction. Total genomic DNA was extracted from silica gel dried leaves according to the CTAB (cetyltrimethylammonium bromide) protocol of Doyle and Doyle (1987) using the Nucleospin® Plants kits (Machery-Nagel, Germany) following the manufacturer's instructions. The concentration of DNA extraction was initially checked on 1% agarose gel. The complete ITS region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA was amplified using the forward primer AB101 (5'- ACG AAT TCA TGG TCC GGT GAA GTG TTC G -3') and the reverse primer AB102 (5'-TAG AAT TCC CCG GTT CGC TCG CCG TTA C -3') (Douzery *et al.*, 1999). PCR reactions were performed in a 25µL volume containing 10Mm Tris-HCl buffer at pH 8, 50Mm KLC, 1.5Mm MgCl₂, 0.2Mm of each dNTP, 0.2 µL of a single primer, 20 ng genomic DNA, and 1 U of Taq DNA polymerase under the following conditions: a pretreatment of 5 min at 95°C, 35 cycles of 30 sec at 95°C, 30 sec at 50°C and 90 sec at 72°C, and a final extension of 7 min at 72°C. The quality of PCR products was assessed by electrophoresis in 1% agarose gel and then visualized under UV light. Amplicons were sequenced on an ABI 3730 DNA Analyzer (Hitachi-Applied Biosystems, Waltham, Massachusetts, USA).

Phylogenetic analysis

Forward and reverse sequences were visually checked and edited, and then initially aligned using Sequencher 4 software (Gene Codes Corporation, Ann Arbor, Michigan, USA). In addition to our sequences, ITS sequences from 62 additional accessions were taken from the Genbank (see Fig 3). In order to save space, accession numbers of those samples are added to Fig 3. *Medicago radiata* L. was selected as outgroup following Bena *et al.* (1998a and 1998b). Sequences were assembled and aligned using MacClade 4 (Maddison & Maddison 2000). Maximum Parsimony analysis (MP) was performed using PAUP* software (Swofford, 2002) with the following criteria: 100 heuristic search, 100 replicates, swapping method: TBR. The strict consensus tree was formed by combining the shortest trees recovered under MP. Bootstrap support (BS) for each branch was calculated using full heuristic with 100 replicates and a similar setting as explicated above (Felsenstein, 1985). The Bayesian analysis (BA) of the ITS dataset was performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). In order to find the appropriate model of DNA substitution, the Maximum Likelihood criteria for the dataset was determined by the Akaike information criterion (AIC; Akaike, 1974) as implemented in ModelTest v3.7 (Posada & Crandall, 1998). "TIMef" was selected as the best fit model for our dataset by ModelTest v3.7. The MCMC (Markov Chain Monte Carlo) process was set so that four chains ran simultaneously for 5,000,000 generations. With 25 % of initial trees being discarded, the remained trees sampled from each generation were then combined into a 50 % majority rule tree.

Results

Cluster (Fig 1) and PCA analyses (Fig 2) of the morphological characters showed three distinct groups representing *Medicago sinskiae*, *M. rigidula*, and *M. constricta*. In both analyses, all populations of *M. sinskiae* were grouped together. As can be seen in Fig. 1, populations that are geographically close were also morphologically close in the UPGMA dendrogram.

High variability in the pod characteristics of the three *Medicago* species was observed (Table 2: characters 1-18, Fig. 4). The number of coils ranged from 6-8 in *M. constricta*, 4-7.5 in *M. rigidula* (and *M. rigiduloides*), and 2.5-5 in *M. sinskiae*. Results of morphometrical measurements are given in table 3. Pods shape and size tended to be different in the three studied species. The results show that pods of *M. sinskiae* have less depression compared to *M. rigidula* and *M. constricta*. Pods were more compressed in *M. constricta* with no obvious gaps between the coils, and less compressed in *M. sinskiae* with visible gaps between the coils (Fig 4).

Pod morphology of *M. sinskiae* showed great variability within and between populations (Table 3). According to our results, mean FLT (length of pods) ranged from 3.68 mm (in KHR) to 4.15 mm (in PLS), mean of FDM (fruit diameter) ranged from 6.08 mm (in SPD) to 6.81 mm in ABD, mean of NMC (Number of coils) ranged from 3.22 (in FTH) to 3.73 (in ABD), mean of FAP (adpression of fruit, width of the gaps between coils) ranged from 0.54 mm (in BSN) to 0.97 mm (in SPC), mean of MCT (thickness of middle coil) ranged from 0.93 mm (in BSN) to 1.63 mm (in KHW), mean of LSL (length of longest spines) ranged from 0.93 mm (in SPC) to 2.25 mm (in PLS), mean of SBT (thickness of spine base) ranged from 0.32 mm (in BSN) to 0.59 mm (in KHR), mean of SMC (number of spines on middle coil) ranged from 22.27 (in SPD) to 23.97 (in KHW), mean of ASP (angel of spine insertion) ranged from 4.33 degrees (in BSN) to 8.6 degrees (in KHR), mean of SLT (length of seeds) ranged from 3.1 mm (in FTH) to 3.36 mm (in KHR and KHW), mean of SWT (width of seeds) ranged from 1.85 mm (in ABD) to 1.98 mm (in KHW), mean of SDN (number of seeds in middle coil) ranged from 1.68 (in PLS) to 2.4 (in FTH), and mean of STN (number of seeds per pod) ranged from 3.68 (in KHW) to 5.46 (in BSN). Data distribution diagram of the length of longest spine (LSL) of studied populations of *M. sinskiae* is presented in Fig 5. This diagram shows a normal distribution with no obvious disjunction between measurements. The fruit diameter (FDM) and length of the longest spine (LSL) in the first generation were positively correlated with the same traits in the second generation and showed that these morphological traits are not affected by environmental conditions (data not shown). We also observed negative correlations between the length of fruit (FLT), the number of coils (NMC), and the number of spines on the middle coil (SMC) in the pods collected from the field and those pods on the individuals grown from the same pods (second generation; data not shown). Those characters are greatly influenced by environmental conditions.

The aligned ITS (ITS1 + ITS2) dataset included 458 characters, of which 66 variable characters (14.4%) were parsimony-uninformative and 85 variable characters (18.5%) were potentially parsimony informative. MP analysis resulted in 176 shortest trees with length = 260 steps, consistency index (CI) = 0.685 and retention index (RI) = 0.837. All shortest trees were combined into a strict consensus tree with length = 268 steps, CI = 0.664 and RI = 0.821. Topology of the strict consensus tree (partly shown in Fig 3) obtained from MP analysis is highly similar to that of the 50% majority rule tree obtained from the Bayesian Analysis (Fig. 3). In both MP and BA trees ITS sequences of seven accessions of *M. sinskiae* were grouped in a monophyletic clade (BS: 80%, PP: 1.00; Fig 3). This clade was sister to the clade containing *M. rigidula* and *M. rigiduloides* (BS: 87%, PP: 1.00). In both trees *M. constricta* was sister to clade mentioned above.

Discussion

Congruency of morphological and molecular data

Our morphological results were highly compatible with the molecular ones. Both data sets indicated that *Medicago sinskieae* is closely related to *M. rigidula* s.l. (including *M. rigiduloides*) (Fig 3). Close relationship between *M. rigidula* s.l. and *M. sinskieae* was also observed by Downie *et al.* (1998). Bena *et al.*, (1998) found that *M. rigidula* and *M. constricta* are closely related and share the same unusual basic chromosome number (seven; the majority of *Medicago* species have eight). We also found that despite great morphological variation in pod morphology (especially size, number of coils, and spine length), no variability was found in ITS sequences of *M. sinskieae* populations. Our results clearly showed that the samples from Turkmenistan and the populations collected from Iran belong to the same species.

The annual *Medicago* species show a great variation in pod morphology, especially in size and shape of spines on the pods (Bena *et al.*, 1998; Small, 2011). The type specimens of *M. sinskieae* includes individuals with only spineless (very short) pods (Uljanova, 1964; Small and Brooks, 1991). The original collection of *M. sinskieae* from Kopet-Dag of Turkmenistan is characterized by lack of spines and presence of only barely visible tubercles (Uljanova, 1964). Mehregan *et al.* (2002) reported both spineless and spiny forms of *M. sinskieae* in Iran. We observed that pods had spines with different length (Table 3, Figs 4 and 5).

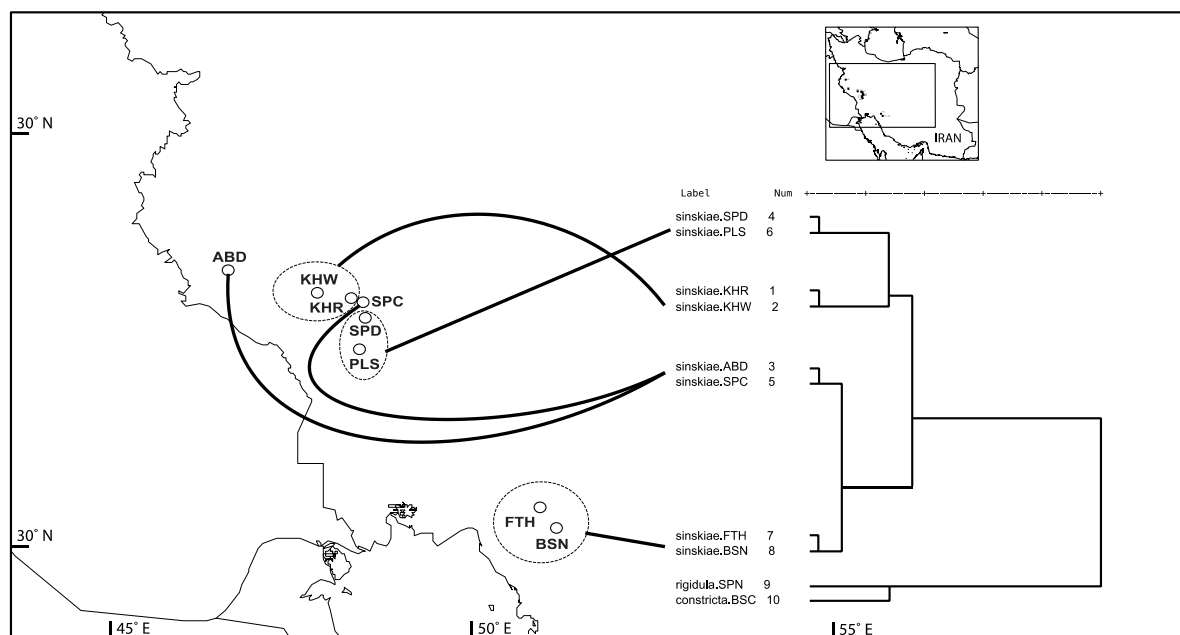


Fig 1. Locality of populations studied in this paper. UPGMA dendrogram based on the cluster analysis of morphological characteristics of three species *M. sinskieae*, *M. rigidula*, and *M. constricta* is also shown on the map.

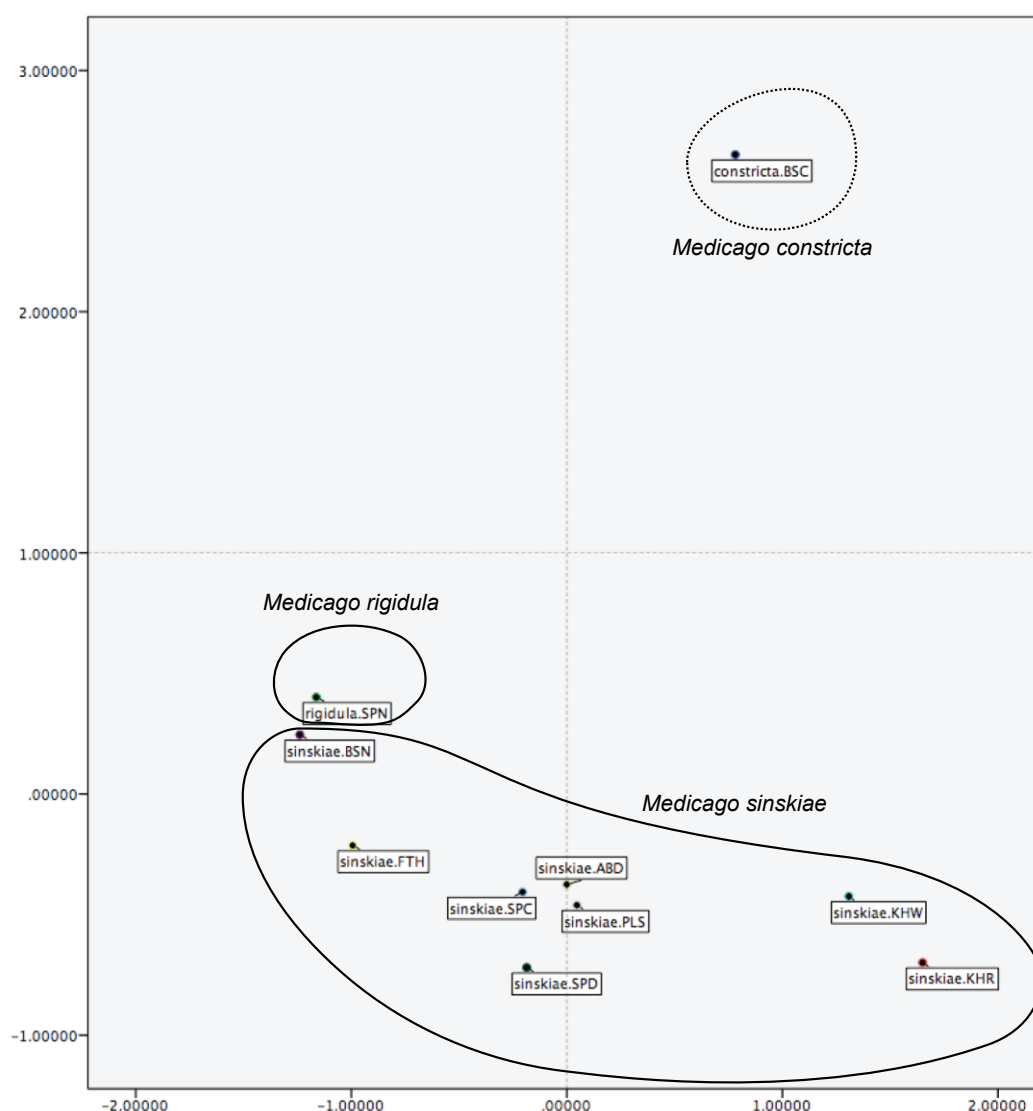


Fig 2. Two dimensional principal component analysis (PCA) of 14 quantitative morphological characters of three species *M. sinskiae*, *M. rigidula*, and *M. constricta* based on the first two components.

Among the annual species of *Medicago*, pods of *M. orbicularis*, *M. scutellata*, *M. soleirolii*, *M. shepardii*, *M. noeana*, *M. secundiflora*, and *M. rugosa* are always spineless. Pods of other species are spiny, but some have both spiny and spineless pods depending on the population (Lesins and Lesins, 1979; Small and Jomph, 1988). All three species of *M. sinskiae*, *M. rigidula*, and *M. constricta* had spiny and spineless pods (Small, 2011; Mehregan *et al.*, 2002). In polymorphic species, spiny populations are the most frequent whereas spineless populations are rare (Bena *et al.*, 1998b). Bena *et al.*, (1998b) suggested that spiny pods are ancestral state in the genus *Medicago*, and in *M. rigidula* have occurred a transition from an ancestral spiny condition to a polymorphic state. Lesins and Lesins (1979) suggested that in some species just a single mutation can transform the spiny into a spineless state.

Biogeography of *M. sinskieae*

Uljanova (1964) first introduced *M. sinskieae* based on a single collection from Turkmenistan. Khassanov (1972) stated that the presence of *M. sinskieae* in neighboring areas of the Iranian Plateau may be presumed. It was ignored for decades until Small and Brookes (1991) reintroduced *M. sinskieae*. Mehregan *et al.* (2002) reported the first presence of *M. sinskieae* outside Turkmenistan. Western and southwestern Iran was intensively searched for annual medics between 1994 and 1997 by the last author and the samples of *M. sinskieae* were spotted only in the western part (provinces Kermanshah and Luristan), represented in this study by populations ABD, KHW, KHR, SPC, SPD. After two decades (2016-2107) and for the first time, *M. sinskieae* was collected from southwestern Iran, represent here by BSN and FTH populations. This indicates that distribution of the species is rapidly expanding further south. Fast expansion of the species is also confirmed by SSR data (unpublished data). Iran and Turkmenistan share a common border of more than 1000 km. We observed no samples of *M. sinskieae* from the northeastern Iran, adjacent to Turkmenistan. The historical relevance between the two countries' populations is unknown. They could be the remainder of a species that was once widely distributed or a relatively newly generated species that is now spreading its range.

Taxonomy and morphology

Medicago sinskieae Uljanova, Novosti Sist. Vyssh. Rast. (Novist. Syst. Pl. Vasc. Acad. Sci. URSS) 1: 175 (1964).

Type: Turkmenistan, Southwestern Turkmenia, western Kopetdag, Sumbar-Chan-dyrskii ridge, the Kuraty Canyon, Altitude 700 m, debris cone. 1961. Collector: T. N. Uljanova (LE!). The holotype was examined by the ?author in LE in 1988.

Syn. *M. rigidula* (L.) All. Subsp. *sinskieae* (Uljanova) Mehregan & Rahiminejad, Iran. J. Bot. 9: 214 (2002).

Description: Annual plant covered by simple hairs. Stems (5)15–25(40) cm long, prostrate to ascending, branched from base. Stipules 2(3)–4(5) mm long, dentate to laciniate. Peduncles with 1–3(5) flowers, 3–6 mm long. Calyx pubescent, with teeth longer than tube. Corolla yellow or orange-yellow. Mature pods ovoid, cylindrical, or discoid, pubescent with both simple and gland-tipped hairs, 4–8 mm long, 4(5) –6 mm wide, with 2.5–5 coils, spineless or with spines up to 4 mm long, hardened at maturity, some gaps often present between mature coils. Seeds 1.75–2.5 mm long, 1–2 per coil, separated by spongy fruit partitions, smooth, yellow to yellow-brown, the radicle about half as long as the length of the seed.

Distribution: W & SW Iran, Turkmenistan (only type specimens).

Conclusion

Based on morphological and molecular evidence, *M. sinskieae* should be accepted as a separate species. It is closely related to *M. rigidula* s.l. (including *M. rigiduloides*). The indehiscent spiny fruits of the annuals medics are well adapted to distribution in animal fur (Small, 2011). *Medicago sinskieae* is an inbreeding fairly homozygous species (Small, 2011), which is often the case in narrowly endemic species. Nevertheless, *M. sinskieae* is expanding its range quickly in Iran and could potentially be invasive. Inbreeding species such as annual medics often have increased ability to instantly spread because they do not need pollinators (Barrett *et al.*, 2008; Kalisz *et al.*, 2004). Wind, water, and human activities are potential seed distributors for the *M. sinskieae* and the spines

on the pods may assist in distribution by becoming attached to the fur of an animal. *Medicago sinskiae* is spreading rapidly in the western part of the Zagrosian regions of Iran covered with oak forests (Zohary, 1973). Zagrosian regions show the presence of domestic and wild animals such as sheep and goats and their life depend on grazing of the region. *Medicago sinskiae* is an excellent plant for feeding livestock (Khassanov, 1972).

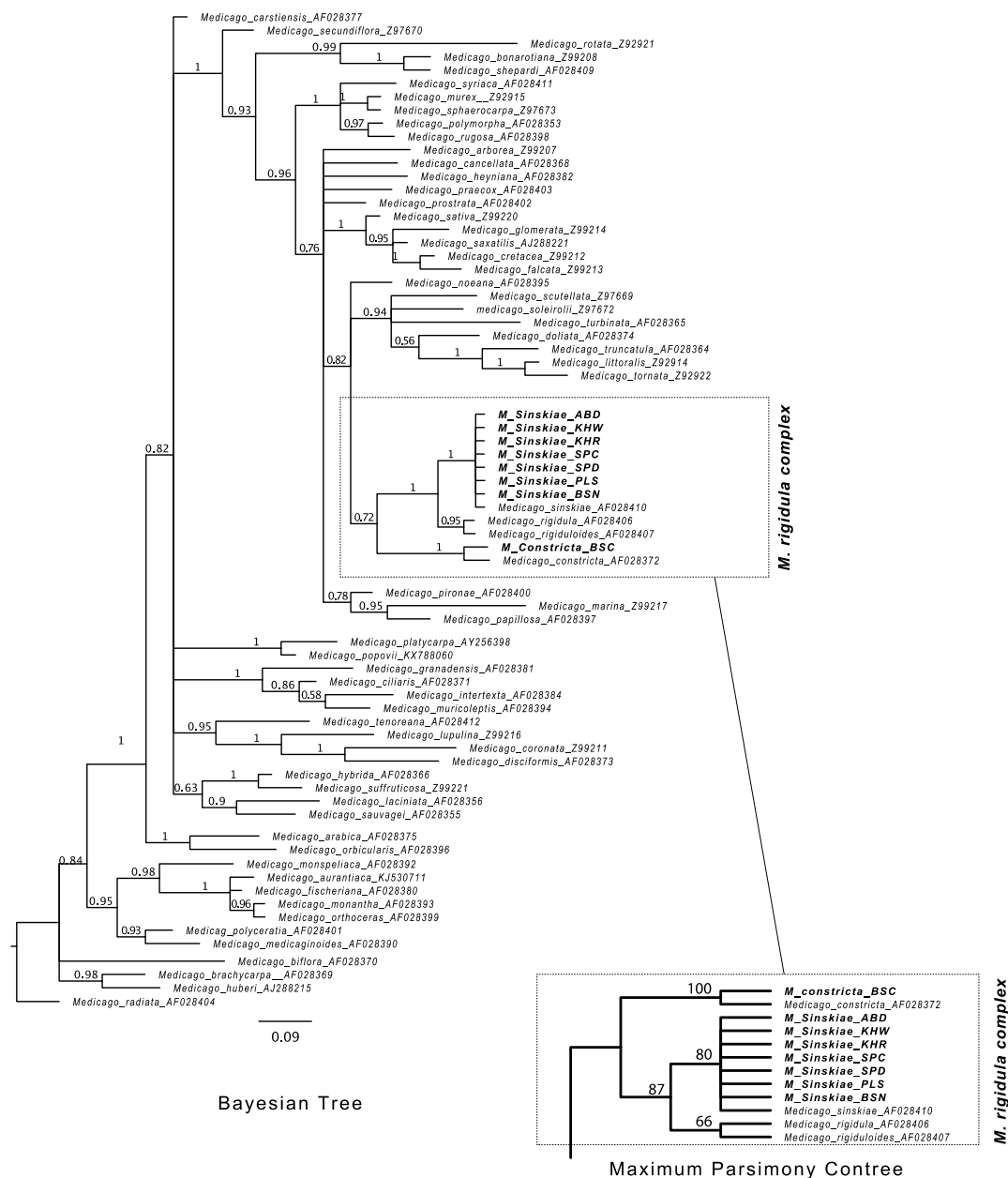


Fig 3. Phylogram obtained from the Bayesian analysis of ITS DNA sequences. The number above the branches are Posterior Probabilities (PP). Our samples are typed **boldfaced**. Codes after each species taken from the Genbank are accession numbers. A portion of the strict consensus tree composed of 176 shortest trees obtained from the Maximum Parsimony (MP) analysis of the same data is shown below right. It shows the *M. rigidula* complex. Number above each clade is the Bootstrap value.

Table 3. List of 13 morphological characters of *M. sinskaiae* measured in this study. Abbreviations: FLT: Pod length, FDM: fruit diameter, NMC: Number of coils, FAP: Adpression of fruit, width of the gaps between coils, MCT: Thickness of middle coil, LSL: length of longest spines, SBT: Thickness of spine base, SMC: Number of spines on middle coil, ASP: Angel of spine insertion, SLT: Length of seeds, SWT: Width of seeds, SDN: Number of seeds in middle coil, STN: Number of seeds per pod.

Trait popul ation	FLT (mm)	FDM (mm)	NMC	FAP (mm)	MCT (mm)	LSL (mm)	SBT (mm)	SMC	ASP (°)	SLT (mm)	SWT (mm)	SDN	STN
KHR	3.68±0.86	6.45±0.66	3.63±0.48	0.86±0.48	1.52±0.35	1.09±0.37	0.59±0.13	23.48±2.12	8.6±2.12	3.36±0.362	1.97±0.16	2.14±0.79	3.8±1.53
KHW	4.09±0.7	6.71±0.48	3.6±0.4	0.61±0.27	1.63±0.34	1.28±0.87	0.47±0.18	23.97±2.93	7.66±3.133	3.36±0.39	1.98±0.14	2.13±0.69	3.68±1.28
ABD	3.91±0.65	6.81±0.50	3.73±0.41	0.61±0.14	1.31±0.39	1.20±0.59	0.41±0.12	23.54±2.37	7.56±2.35	3.29±0.46	1.85±0.341	2.19±0.78	4.66±1.41
SPD	3.9±0.56	6.08±0.69	3.68±0.30	0.69±0.25	1.12±0.25	1.21±0.43	0.42±0.14	22.27±1.98	7.23±2.58	3.21±0.40	1.90±0.21	1.79±0.72	4.06±1.16
SPC	3.93±0.56	6.63±0.49	3.64±0.57	0.97±0.17	1.21±0.24	0.93±0.4	0.37±0.11	23.84±2.89	6.87±2.63	3.24±0.34	1.9±0.24	2.32±0.74	4.87±1.45
PLS	4.15±0.85	6.37±0.4	3.4±0.27	0.71±0.24	1.07±0.27	2.25±0.4	0.46±0.14	22.75±1.8	7.18±3.35	3.24±0.48	1.91±0.27	1.68±0.7	4.25±2.04
FTH	3.71±0.41	6.69±0.44	3.22±0.28	0.66±0.14	1.03±0.2	1.67±0.52	0.34±0.11	23.7±2.68	5.75±2.48	3.1±0.32	1.89±0.18	2.4±0.71	5.03±1.42
BSN	3.76±0.54	6.73±0.41	3.36±0.45	0.54±0.2	0.93±0.2	1.81±0.5	0.32±0.11	23.57±2.68	4.33±2.8	3.22±0.34	1.89±0.23	2.16±0.46	5.46±1.45

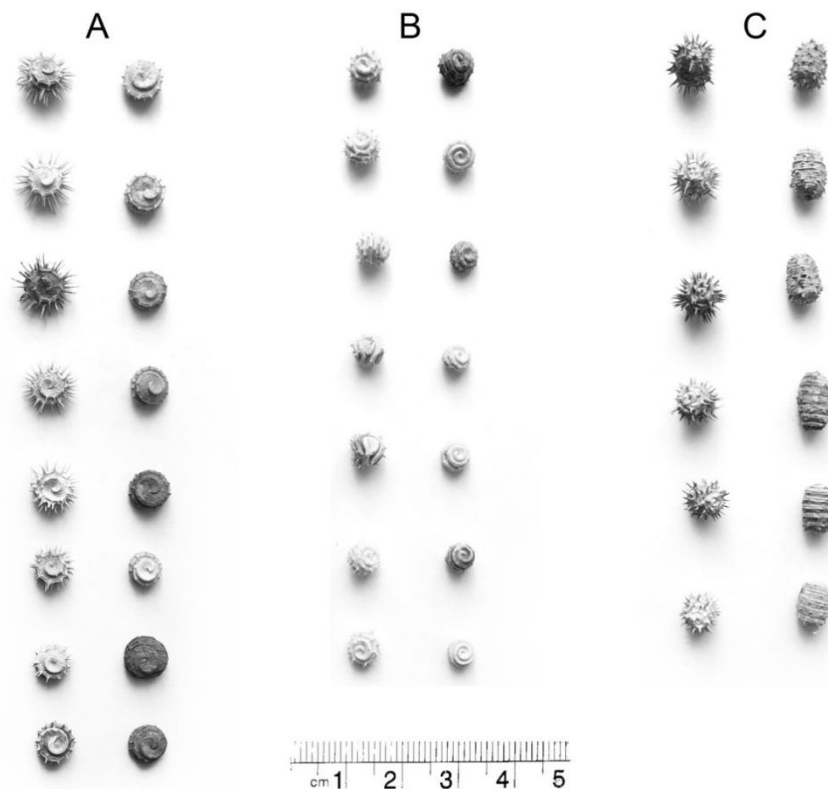


Fig 4. Morphological variation in pods of A: *Medicago sinskaiae*, B: *M. rigidula*, and C: *M. constricta*.

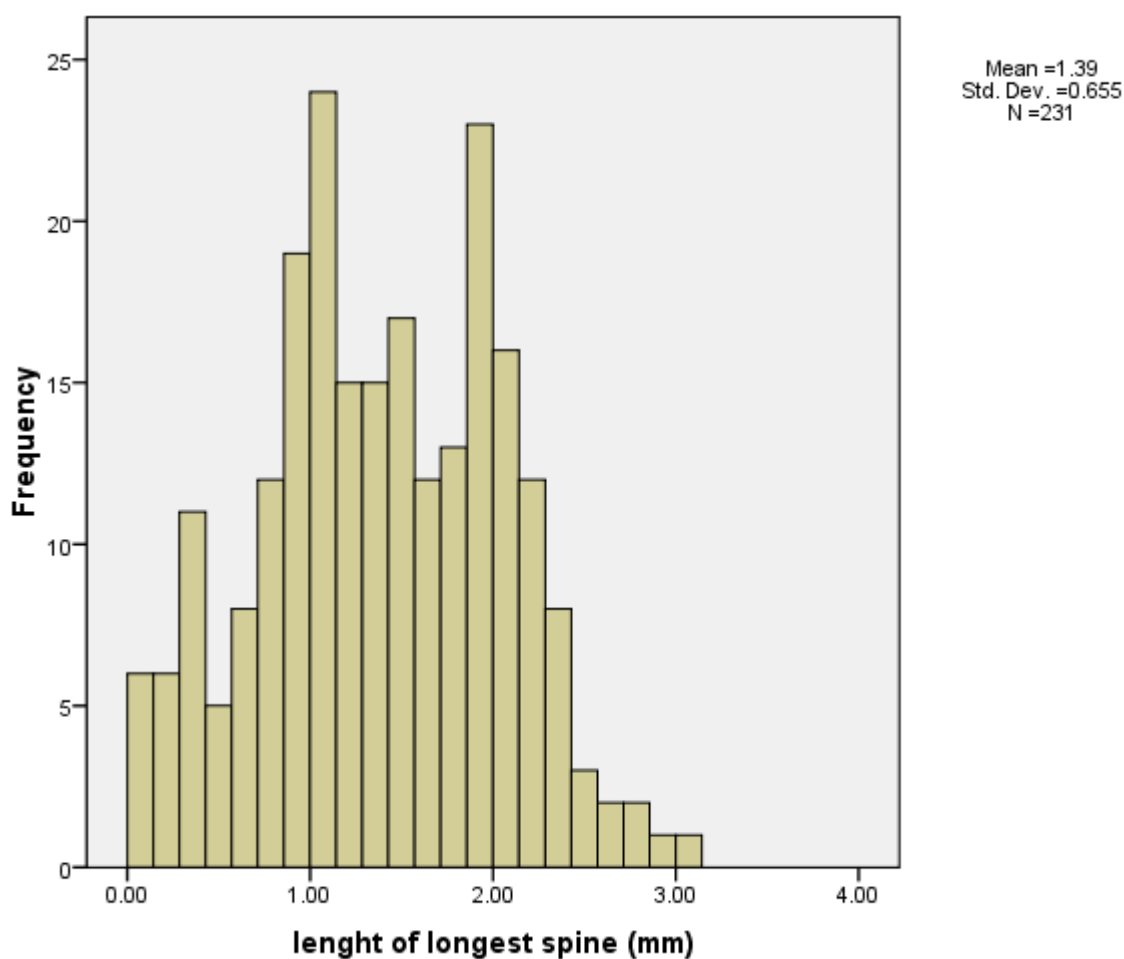


Fig 5. Data distribution diagram of length of longest spine in *M. sinskiae*.

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References

- Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6): 716–723.
- Barret, S.C., Colautti, R.I. and Eckert, C.G. (2008) Plant reproductive system and evolution during biological invasion. *Molecular Ecology* 17(1): 373–383.
- Bena, B., Prosperi, J.M., Lejeune, B. and Olivieri, I. (1998) Evolution of annual species of the Genus *Medicago*: A molecular phylogenetic Approach. *Journal of Molecular Evolution* 9(3): 552–559.

- Bena, B., Prosperi, J.M., Olivieri, I. and Lejeune, B. (1998) Ribosomal External and Internal Transcribed Spacer: combined use in the phylogenetic analysis of *Medicago* (Leguminosae). *Journal of Molecular Evolution* 46: 299–306.
- Douzery, E.J.P., Pridgeon, A.M., Kores, P., Linder, H.P., Kurzweil, H. and Chase, M.W. (1999) Molecular phylogenetics of *Diseae* (Orchidaceae): a contribution from nuclear ribosomal ITS sequence. *American Journal of Botany* 86(6): 887–899.
- Downie, S.R., Katz-Downie, D.S., Rogers, E.J., Zujewski, H.L. and Small, E. (1998) Multiple independent losses of the plastid *rpoCl* intron in *Medicago* (Fabaceae) as inferred from phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer sequences. *Canadian Journal of Botany* 76(5): 791–803.
- Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bull* 19(1): 19–21.
- Ebrahimi, A., Nejadshattari, T., Assadi, M., Larijani, K. and Mehregan, I. (2017). Morphological and Molecular differentiation in populations of Persian Oak (*Quercus Brantii* Lindl.) in southwestern Iran. *Egyptian Journal of Botany* 57(2): 1–15.
- Felsenstein, J. (1985) Phylogenies and comparative method. *American Naturalist* 125(1):1–15.
- Huelsenbeck, J.P. and Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754–755.
- Heyn, CC. (1984) Papilionaceae. In: *Flora Iranica*. (Ed. Rechinger, K. H.) 253–271. Akademische Druck-u., Verlagsanstalt, Graz, Austria.
- Kalisz, S., Vogler, D.W. and Hanley, K.M. (2004) Context dependent autonomous self-fertilization yield reproductive assurance and mixed mating. *Nature* 430: 884–887.
- Khassanov, O.Kh. (1972) Wild alfalfas of central Asia, Tashkent, Uzbekistan.
- Lesins, K.A. and Lesins, I. (1979) Genus *Medicago* (Leguminosae). A taxogenetic study. Dr. W.Junk bv, The Hague, The Netherlands.
- Maddison, D.R. and Maddison, W.P. (2005) MacClade 4: Analysis of phylogeny and character evolution. Version 4.08a.
- Mehregan, I., Rahiminejad, M.R. and Azizian, D. (2002) A taxonomic revision of the genus *Medicago* L. (Fabaceae) in Iran. *Iranian Journal Botany* 9(2): 207–221.
- Mehregan, I., Moussavi, M. and Nasrabadi, N. (2003) The genus *Medicago* in Iran: Biodiversity and variation centres. *Rostaniha (Botanical Journal of Iran)* 4(1): 5–18.
- Posada, D. and Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14(9): 817–818.
- Small, E. and Brookes, B. (1991) A clarification of *Medicago sinskiae* Uljan. *Canadian Journal of Botany* 69: 100–106.

- Small, E. and Jomphe, M. (1989) A synopsis of the genus *Medicago* (Leguminosae). Canadian Journal of Botany 67: 3260–3294.
- Small, E. (2011) Alfalfa and relatives, Evolution and classification of *Medicago*, NRC Research Press, Ottawa, Ontario, Canada.
- Swofford, D.L. (2002) PAUP. Phylogenetic analysis using parsimony. Sunderland: Sinauer associates.
- Uljanova, T. (1964) A new species of medic from Turkmenia. Novit Syst Plant Vasc Acad Sci URSS 1, 175–177.
- Zohary, M. (1973) Geobotanical foundations of the Middle East, Gustav Fischer, Verlag, Stuttgart.